

Novel 18 β -glycyrrhetic acid analogues as potent and selective inhibitors of 11 β -hydroxysteroid dehydrogenases

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Abstract—Extensive structural modifications to the 18 β -glycyrrhetic acid template are described and their effects on the SAR of the 11 β -hydroxysteroid dehydrogenase isozymes type 1 and 2 from the rat are investigated. Isoform selective inhibitors have been discovered and compound 7 *N*-(2-hydroxyethyl)-3 β -hydroxy-11-oxo-18 β -olean-12-en-30-oic acid amide is highlighted as a very potent selective inhibitor of 11 β -hydroxysteroid dehydrogenase 2 with an IC₅₀ = 4 pM.

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1. Introduction

The short-chain dehydrogenases/reductases (SDR) are a large well-established family of functionally heterogeneous enzymes present in all forms of life.¹ The 11 β -hydroxysteroid dehydrogenases (11 β -HSDs) are members of this family and are microsomal enzymes catalyzing the conversion of active glucocorticoids to their 11-dehydro products and vice versa.² The 11 β -hydroxysteroid dehydrogenase isozymes type 1 and 2 are responsible for the interconversion of cortisone (E) and cortisol (F) as shown in Figure 1.

The 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1) isoform converts cortisone to the active gluco-

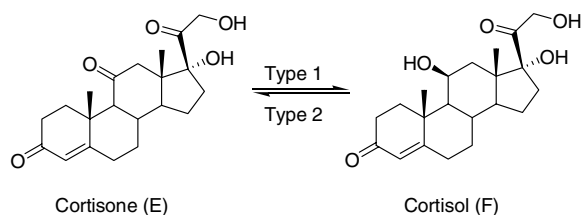


Figure 1. Interconversion of cortisone (E) and cortisol (F) by 11 β -HSD type 1 and 2.

corticoid cortisol and is present in liver, muscle and adipose tissue.³ Cortisol has a central role in regulating carbohydrate metabolism and its main action in this respect is to oppose the action of insulin and 11 β -HSD1 has been shown to mediate glucocorticoid action and insulin release in pancreatic islets.⁴ In the liver, while insulin stimulates glucose uptake and inhibits gluconeogenesis, cortisol acts to inhibit glucose uptake and stimulate gluconeogenesis. Gluconeogenesis in this organ is reduced in knockout mice without the 11 β -HSD1 gene resulting in lower blood sugar levels.⁵ Recently, it has been shown that selective inhibition of 11 β -HSD1 decreases blood glucose concentrations in hyperglycaemic mice.⁶ Inhibition of 11 β -HSD1 in subjects suffering from impaired glucose tolerance or diabetes mellitus could increase glucose uptake by the liver and inhibit gluconeogenesis. Thus, a selective inhibitor of 11 β -HSD1 has considerable potential as an antidiabetic agent. The effects of the non-selective 11 β -HSD inhibitor carbenoxolone (CBX 2) on insulin sensitivity in non-obese men with type 2 diabetes has recently been shown to support this hypothesis.⁷ The choice of lean patients for this study was an important consideration as these patients had no change in the 11 β -HSD1 levels in adipose tissue.⁷ Several groups have programmes targeted at the development of selective 11 β -HSD1 inhibitors for the treatment of metabolic disorders such as insulin resistance, obesity, hyperlipidemia and arterial hypertension.^{4,8–11} Although it has been known for some

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time that 18 β -glycyrrhetic acid (18 β -GA **1**), a principal active ingredient of liquorice root, and its hemisuccinate derivative carbenoxolone (CBX **2**) (Fig. 2) are potent non-selective inhibitors of both 11 β -HSD1 and 11 β -HSD2,¹² only recently have selective inhibitors of 11 β -HSD1 with nanomolar potency been reported.⁸

The 11 β -HSD type 2 isoform inactivates cortisol to cortisone and is a unidirectional enzyme and exclusively acts as a NAD⁺ dependent dehydrogenase of adrenal glucocorticoids. This isoform is found primarily in mineralocorticoid (MC) tissues such as the kidney, colon, salivary glands and the sodium-transporting epithelia of the lung a nonclassical MC target organ.¹³ Inhibition of 11 β -HSD2 could be used to potentiate the anti-inflammatory effects of glucocorticoids, leading to a reduction in the amount of glucocorticoid required for anti-inflammatory therapy and in the severity of very significant side effects that can result from this type of medication. Inhibition of 11 β -HSD2 with liquorice derivatives results in cortisol-dependent mineralocorticoid excess with hypertension and hypokalemic alkalosis.^{14,15} Disruption or mutations in the 11 β -HSD2 gene result in sodium retention, hypokalemia and hypertension because of inappropriate glucocorticoid occupation of the MC receptor in the kidney, where 11 β -HSD2 is principally expressed in the distal nephron.^{5,16} An excellent recent review on the 11 β -HSDs highlighted the link between 11 β -HSD2 and cancer.¹⁷ Adrenal cortical adenomas and carcinomas synthesize 11 β -HSD2,¹⁸ and 11 β -HSD2 levels are high in pituitary tumours.^{19,20} Ductal and lobular breast epithelial cells have also been shown to synthesize 11 β -HSD2, with increased synthesis of 11 β -HSD2 seen in invasive carcinomas.²¹ Transfection experiments to study the role of 11 β -HSDs showed that in stably transfected cells overexpressing *HSD11B2* cell proliferation was increased.²² In addition, the observation that 11 β -HSD2 potentiates the antiproliferative effects of glucocorticoids in endometrial, and some breast cancer cell lines highlights a putative role for 11 β -HSD2 activity in tumourigenesis.²³ These observations highlight the need for selective inhibitors of 11 β -HSD1 and 11 β -HSD2 as therapeutic agents or tools for further mechanistic studies.

A number of natural products have been reported to inhibit the 11 β -HSDs and these include xenobiotics, flavonoids, polyphenolic compounds from tea, gossypol and the constituents of Saiboku-To, a Chinese herbal remedy.^{24,25} In general, these compounds are non-selective inhibitors of the 11 β -HSDs and are much less potent than 18 β -GA **1**. Some selectivity for 11 β -HSD1 has been reported for weakly inhibiting synthetic steroids and chenodeoxycholic acid; the diuretic furosemide has also been reported as a non-selective inhibitor.^{24,25}

As 18 β -GA **1** and CBX **2** are potent inhibitors of 11 β -HSD lacking selectivity a programme of work was initiated to evaluate the effects on SAR by modifying the 18 β -GA template on 11 β -HSD activity and selectivity. The use of a number of 18 β -GA derivatives was the topic of a recent patent by our group.²⁶ Here we sub-

stantially extend the structural modifications of the 18 β -GA template and report the effects of these novel compounds on the SAR of 11 β -HSD1 and 11 β -HSD2 isoforms from the rat.

2. Chemistry

The synthesis of compounds from the 18 β -GA template **1** was targeted at three key areas of the molecule where functionality is present for further modification. The template was modified in the A, C and E rings at positions 3, 11 and 30, respectively, as indicated in Figure 3.

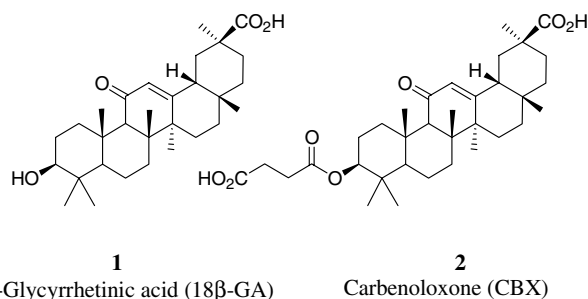


Figure 2. Structures of 18 β -glycyrrhetic acid and carbenoxolone.

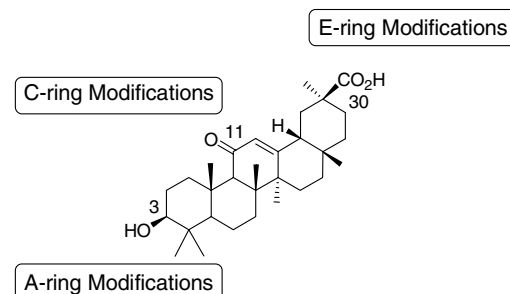
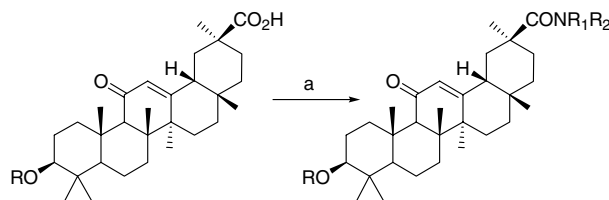


Figure 3. Structural modification of 18 β -glycyrrhetic acid template.

2.1. Modifications to the 30-position

2.1.1. Amides. A diverse set of amides **3–33** was synthesized by activation of the carboxylic acid moiety at the 30-position and reaction with a range of amines. Syntheses were performed classically and in parallel using the water soluble EDCI as the activating agent under a variety of conditions (Scheme 1).²⁷ The compounds listed in Tables 1 and 2 were synthesized by these methods.



Scheme 1. Synthesis of 18 β -glycyrrhetic acid amides. Reagents and conditions: (a) EDCI, R₁R₂NH, CH₂Cl₂ or CHCl₃, DMAP, HOBT (optional), rt.

Table 1. Secondary amide derivatives of 18 β -GA (**1**) at the 30-position and their inhibition of 11 β -HSD1 and 11 β -HSD2

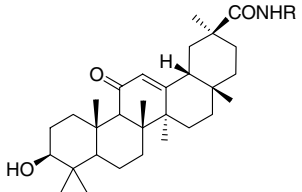
			
Compd	R	% Inhibition of 11 β -HSD1 at 10 μ M ^a	% Inhibition of 11 β -HSD2 at 10 μ M ^a
1		85	101
2		52	100
3	CH ₂ CH ₂ CH ₂ CH ₃	13	100
4		90	103
5		25	35
6		58	39
7	CH ₂ CH ₂ OH	36	92
8	CH ₂ (CH ₂) ₄ OH	88	98
9	CH ₂ CH ₂ NHCH ₃	36	101
10		71	25
11	CH(CH ₂ CH ₂ OH)CO ₂ H	50	6
12		19	66
13		34	20
14		7	92
15		10	38
16		23	100
17		36	23
18		29	18
19		66	99
20	CH ₂ C ₆ H ₅	85	74
21		35	96
22		21	44
23		52	77
24		18	99

Table 1 (continued)

Compd	R	% Inhibition of 11 β -HSD1 at 10 μ M ^a	% Inhibition of 11 β -HSD2 at 10 μ M ^a
25		38	15
26		58	93
27		55	94

*Indicates point of attachment.

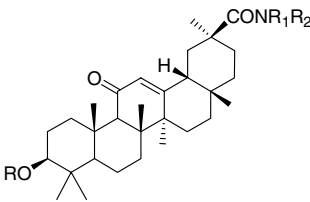
^a Mean of at least two measurements with typically $\pm 5\%$ variation.

2.1.2. Ethers and esters. A number of chemical modifications to the 30-position of the 18 β -GA template have been published. However, the effects of these structural changes on 11 β -HSD activity and selectivity have not been reported.^{26,28,29} Schemes 2 and 4 illustrate the introduction of alcohol and ether functionalities into the 18 β -GA template. The C-30 esters were prepared from 18 β -GA **1** using the standard methods with the requisite alkyl halides as previously described to give **35–38**.²⁶ Compound **34** had previously been prepared by the above method,²⁶ and also by treatment of 18 β -GA **1** with diazomethane;³⁰ in this instance *p*-TSA in methanol was used.³¹

The C-30 hydroxymethyl analogue of 18 β -GA **39** was prepared from 18 β -GA **1** by reduction with LAH followed by oxidation of the allylic alcohol with manganese dioxide (Scheme 2). Esterification of the C-30 acid of 18 β -GA **1** using MeOH/*p*-TSA followed by protection of the 3 β -OH as its THP ether using THP/PPTS gave **45** as an intermediate for further modification. Etherification of the C-30 hydroxymethyl moiety using sodium hydride and the requisite alkyl halide under standard conditions followed by deprotection of the 3-position using PPTS gave the ether derivatives **40–43**. The tertiary *gem* dimethyl alcohol **44** was one of the minor products isolated by treatment of the C-30 methyl ester of 18 β -GA **34** with methyl lithium (Scheme 4). Compounds synthesized in Schemes 2 and 4 and C-30 esters where the 11-position is unchanged are listed in Table 3.

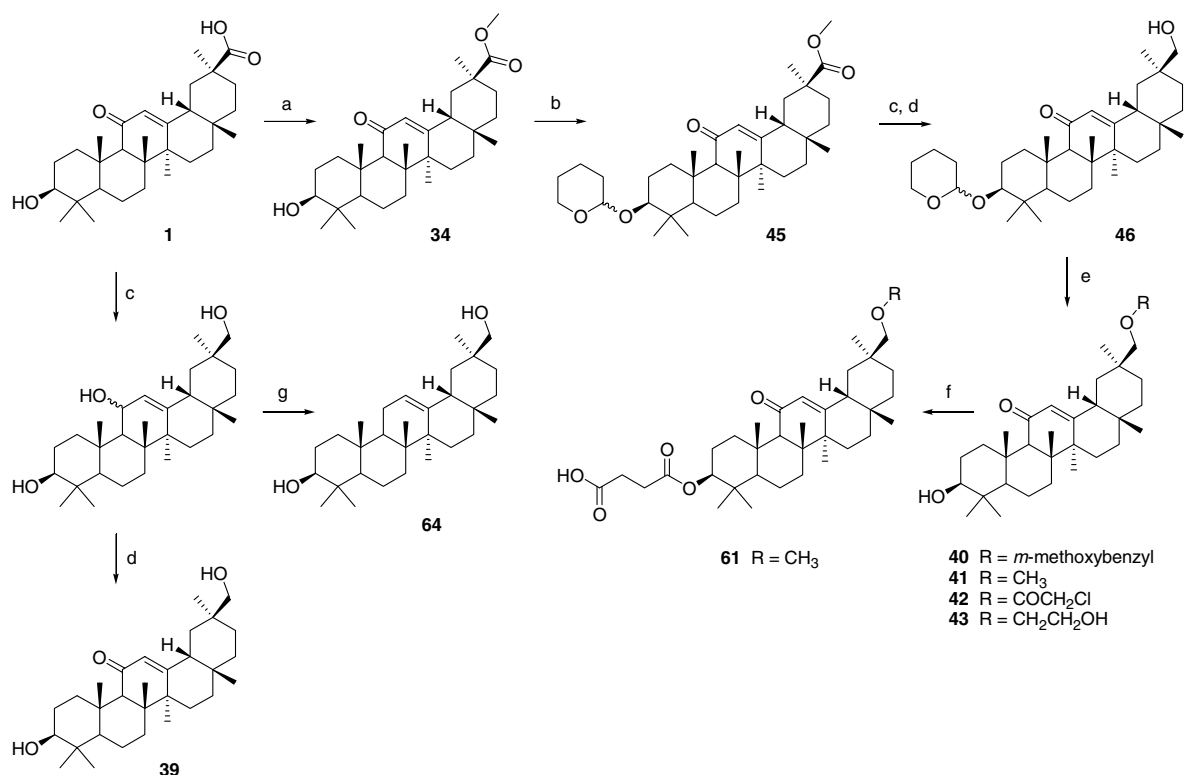
2.2. Modifications to the C-3 position

A number of modifications have been examined at the 3-position, these include the synthesis of ether and ester derivatives and oxidation of the C-3 hydroxyl group to give 3-oxo compounds. The C-3 acetate **58** was prepared as previously described,³¹ and the C-3 ethers **47–57** were prepared as outlined in Scheme 3 using standard procedures these compounds are listed in Table 4.

Table 2. Tertiary and other amide modifications of (**1**) coupled with changes to the 3-position


Compd	R	R ₁	R ₂	% Inhibition of 11β-HSD1 at 10 μM ^a	% Inhibition of 11β-HSD2 at 10 μM ^a
28	COCH ₃	H	CH ₂ C ₆ H ₅	36	27
29	COCH ₃	H	OH	32	89
30	COCH ₃	H	(CH ₂) ₂ OH	39	4
31	CH ₃	H	(CH ₂) ₂ OH	32	0
32	H	R ₁ R ₂ = -(CH ₂) ₅ -		59	23
33	CO(CH ₂) ₂ COOH	H		40	23

*Indicates point of attachment.

^a Mean of at least two measurements with typically ±5% variation.**Scheme 2.** Modifications to the 30 and 11 positions of 18β-GA. Reagents and conditions: (a) CH₃OH, *p*-TSA, reflux; (b) THP, PPTS, DCM, rt; (c) LiAlH₄, THF, 60 °C; (d) MnO₂, CHCl₃, rt; (e) (i) NaH, RX or ClCH₂CO₂C₂H₅ for **42**, THF or toluene, 60 °C; (ii) PPTS, CH₃OH, 50 °C; (f) succinic anhydride, pyridine, DMAP, 90 °C; (g) PtO₂, H₂, C₂H₅OH–THF, rt.

2.3. 3-Oxo-compounds and carbenoxolone (CBX) analogues

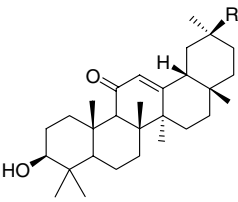
The 3-oxo analogues of 18β-GA **1** were prepared by oxidation of the 3-position of 18β-GA **1** and the corresponding C-30 methyl ester **34** using Jones reagent as previously described to give **59** and **60** (Scheme 3).^{26,30}

The succinate ester derivatives **33** and **61** related to CBX **2** were prepared from the corresponding 3β-hydroxy-

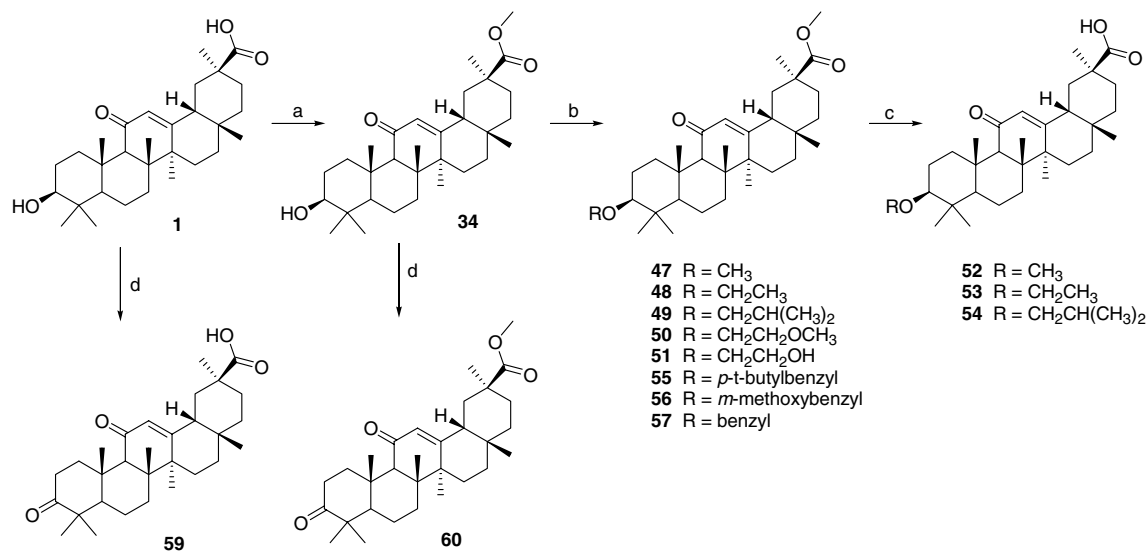
GA compounds using succinic anhydride and DMAP in pyridine using standard methods.

2.4. Modifications to the 11-position of the C-ring

The reduced compound **64** was prepared by treatment of 18β-GA **1** with LAH followed by reduction of the allylic alcohol at the 11-position with hydrogen using PtO₂ as the catalyst (Scheme 2).²⁹ Reduction of 18β-GA **1** with hydrogen over PtO₂ gave the 11-deoxo-18β-GA ana-

Table 3. Ester, ether and alcohol modifications of the 30-position of 18 β -GA (**1**)


Compd	R	% Inhibition of 11 β -HSD1 at 10 μ M ^a	% Inhibition of 11 β -HSD2 at 10 μ M ^a
34	COOCH ₃	85	105
35	COOCH ₂ CH ₃	81	98
36	COOC(CH ₃) ₃	51	23
37	COOCH ₂ C ₆ H ₁₁	38	34
38	COOCH ₂ C ₆ H ₅	53	60
39	CH ₂ OH	90	100
40	H ₂ COCH ₂ C ₆ H ₄ OCH ₃	6	14
41	CH ₂ OCH ₃	43	8
42	CH ₂ OCOCH ₂ Cl	6	92
43	CH ₂ OCH ₂ CH ₂ OH	88	19
44	C(CH ₃) ₂ OH	31	9

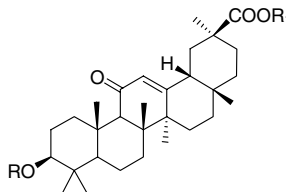
^a Mean of at least two measurements with typically $\pm 5\%$ variation.**Scheme 3.** Modifications to the 3-position of 18 β -GA. Reagents and conditions: (a) CH₃OH, *p*-TSA, reflux; (b) NaH, RX, THF, reflux; (c) NaOH, CH₃OH–THF, reflux; (d) Jones reagent.¹⁹

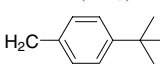
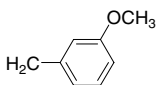
logue **62**, the corresponding C-30 methyl ester **63** was prepared in the same manner from 18 β -GA methyl ester **34** formed from 18 β -GA **1** by treatment with *p*-TSA in methanol (Scheme 4).^{26,31} Treatment of 18 β -GA methyl ester **34** with methyl lithium gave three products as indicated in Scheme 4 with the major products being the 1,2-addition product **65** and the *exo*-methylene derivative **66** resulting from the dehydration of **65**. The corresponding C-30 acid derivatives were formed by hydrolysis of the esters with potassium hydroxide to yield **67** and **68**. Alternatively, **68** was also synthesized in high yield from 18 β -GA **1** by treatment with methyl lithium

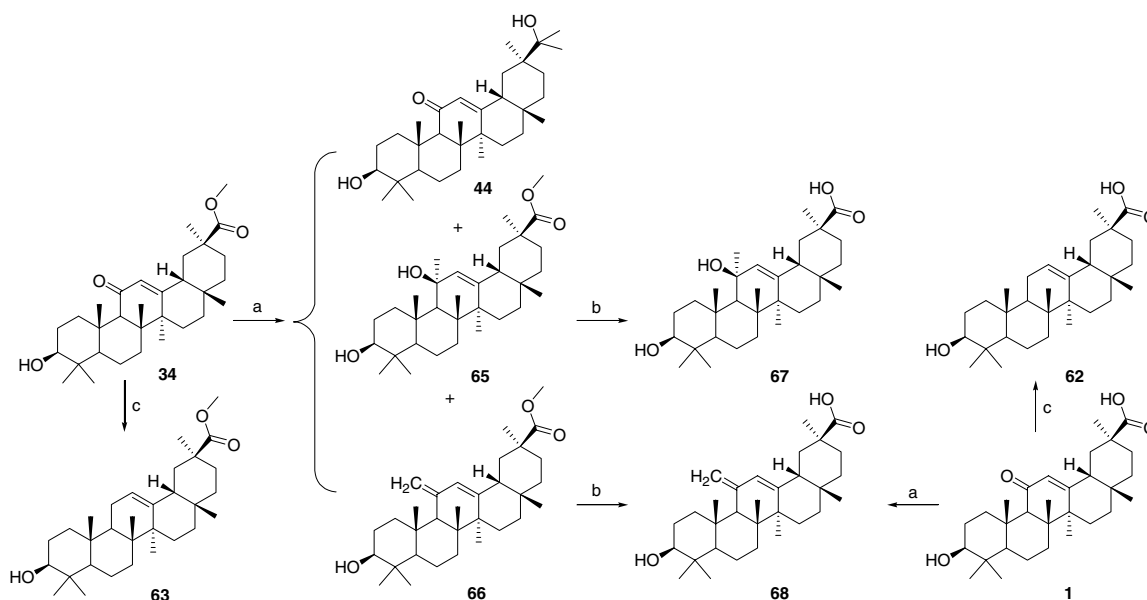
followed by dehydration under acidic conditions with hydrochloric acid during work up (Scheme 4, Table 5).

3. Results and discussion

On the rat 11 β -HSD type 1 and 2 isozymes 18 β -GA **1** and CBX **2** show potent and non-selective inhibition at 10 μ M. Both **1** and **2**, although being non-selective, inhibit the type 2 enzyme to a higher degree as evidenced by the greater inhibition of 11 β -HSD2 over 11 β -HSD1 at different concentrations.²⁶ As **1** and **2** show maximal

Table 4. 3-Ether and ester 18 β -GA analogues and their inhibitions for 11 β -HSD


Compd	R	R ₁	% Inhibition of 11 β -HSD1 at 10 μ M ^a	% Inhibition of 11 β -HSD2 at 10 μ M ^a
47	CH ₃	CH ₃	8	6
48	CH ₂ CH ₃	CH ₃	32	18
49	CH ₂ CH(CH ₃) ₂	CH ₃	33	19
50	CH ₂ CH ₂ OCH ₃	CH ₃	11	10
51	CH ₂ CH ₂ OH	CH ₃	38	5
52	CH ₃	H	28	99
53	CH ₂ CH ₃	H	74	89
54	CH ₂ CH(CH ₃) ₂	H	61	43
55		CH ₃	15	26
56		CH ₃	2	4
57	CH ₂ C ₆ H ₅	CH ₃	18	17
58	COCH ₃	H	24	99

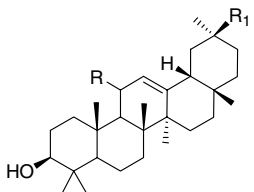
^a Mean of at least two measurements with typically $\pm 5\%$ variation.**Scheme 4.** Modifications to the 11 and 30 positions of 18 β -GA. Reagents and conditions: (a) CH₃Li-ether, THF, -78 to 25°C ; (b) KOH, CH₃OH–THF–H₂O; (c) PtO₂, H₂, C₂H₅OH–THF, rt.

inhibition of 11 β -HSD2 at 10 μ M, compounds synthesized by template modifications were screened at 10 μ M against both isozymes. Selected analogues were examined at lower concentrations and IC₅₀ values determined for a number of highly potent compounds. In general terms compounds have been classed as showing some selectivity if there is greater than 2.5-fold difference in % inhibition, provided $>50\%$ inhibition is observed for one isozyme. For the more potent selective inhibitors high-

lighted the percentage inhibition for one isozyme is $>90\%$.

3.1. Amide modifications

The secondary amide modifications at the 30-position (Table 1) gave a wide range of activities on both 11 β -HSD1 and 11 β -HSD2. In general, most compounds

Table 5. 11-Modified 18 β -GA analogues and their inhibition for 11 β -HSD1 and 11 β -HSD2


Compd	R	R ₁	% Inhibition of 11 β -HSD1 at 10 μ M ^a	% Inhibition of 11 β -HSD2 at 10 μ M ^a
62	H	COOH	93	109
63	H	COOCH ₃	20	20
64	H	CH ₂ OH	13	60
65	β -OH- α -CH ₃	COOCH ₃	30	5
66	=CH ₂	COOCH ₃	40	20
67	β -OH- α -CH ₃	COOH	88	14
68	=CH ₂	COOH	57	22

^a Mean of at least two measurements with typically $\pm 5\%$ variation.

showed a greater inhibition of 11 β -HSD2 over 11 β -HSD1 and were non-selective. A number of compounds showed >90% inhibition of 11 β -HSD2 with >2.5-fold % inhibition over 11 β -HSD1, indicating selectivity. These compounds included the neutral species **7** and **3** with hydroxyethyl and *n*-butyl side chains, respectively. Most compounds in this series that displayed the above criteria contained basic side chains. These derivatives included the 2-pyridinylethyl and 2-pyridinylmethyl analogues **24** and **21**, the 4-ethylpiperidinyl and piperazinylmethyl analogues **16** and **14**, and the ethylaminomethyl derivative **9**.

The 2-hydroxyethyl analogue **7** was designed to mimic the C-17 side chain of cortisol/cortisone and probe the hydrogen bonding characteristics in this region. Although being longer by two atoms than the side chain in cortisone the amide carbonyl in **7** is isosteric with the ketone at the 20-position of cortisone and the hydroxyl is in a similar spatial region to the C-21 hydroxyl in cortisone. This compound has an IC₅₀ of 4 pM against 11 β -HSD2 with only 36% inhibition against 11 β -HSD1 at 10 μ M, and is the most potent 11 β -HSD2 inhibitor reported against the rat isozyme. The position of the hydroxyl group in **7** is important for selectivity as the 5-hydroxypentyl analogue **8** is potent on 11 β -HSD1 and 11 β -HSD2.

The *n*-butyl analogue **3** is a hydrophobic derivative with a linear alkyl chain. The other hydrophobic derivatives **4** and **20** with cyclopropyl and benzyl side chains did not show selectivity for 11 β -HSD2. This observation indicates that the hydrophobic interactions in this region of 11 β -HSD1 are more restricted than in 11 β -HSD2 as **3**, with a hydrophobic side chain with more degrees of freedom, is poorly active against 11 β -HSD1.

The active and selective basic amides indicate that the position of the basic moiety is important for activity and selectivity on 11 β -HSD2. Both **16** and **14** have a secondary amine moiety in a ring on the basic side chain in a similar region of space, and as the *N*-ethylpiperidinyl, *N*-ethylpyrrolidinyl and *N*-ethylmorpholinyl analogues **17**–

18 and **12** are weakly active compared to **16** and **14** the importance of the basic interaction in this region is highlighted. As the 2-pyridinyl compounds **24** and **21** are both potent and selective the position of the basic nitrogen is further highlighted as both the 3-pyridinyl and 4-pyridinyl compounds **22** and **23** are less potent and non-selective. The ethylaminomethyl analogue **9** is potent and selective and has a secondary amine in the same region as the pyridyl nitrogen in **21**. As both pyridyl and secondary amine derivatives have been shown to be potent selective inhibitors of 11 β -HSD2 this suggests that a hydrogen bond acceptor interaction may be important in this region. Although the position of the basic group in the side chain is important for activity and selectivity it is likely that different interactions are being picked up by the sets **16**, **14**, **9**, and **21**, **24** as the basic group in these sets occupies a different spatial region.

The 4-methoxypyridin-3-yl analogue **25** was weakly active on both isozymes, however the 2,4-dimethoxypyridin-3-yl compound **26**, the thiazol-2-yl analogue **27** and the pyrrolidinyl hydrazide **19**, although non-selective, were potent 11 β -HSD2 inhibitors, highlighting the importance of electronic effects in this region.

A number of compounds in this series were weakly active and non-selective, these include the ethyl ester **5** and its corresponding carboxylic acid analogue **6**; the *N*-methylpiperazinyl, morpholinyl and derivatives **15**, and **13** also show low activity and poor selectivity. These results indicate that the steric and electronic effects of these groups in this region are not providing favourable interactions with either of the 11 β -HSD isozymes.

The cyclic lactone **10**, although not potent, does show some selectivity for 11 β -HSD1 as does the hydroxy acid **11**, which is the ring opened version of **10**. The possibility, although unlikely, that **10** is hydrolytically converted to **11** under the assay conditions cannot be ruled out.

Other secondary amide modifications (Table 2) include compounds with *O*-acetyl **28**, **30**, *O*-methyl **31** and succinate **33** groups at the 3-position. The 3-*O*-acetyl

benzyl amide **28** was weakly active and non-selective. The 3-acetyl hydroxamate analogue **29** showed selectivity for 11 β -HSD2 similar to the close structural analogue **58**, 3-*O*-acetyl 18 β -GA (Table 4). Interestingly, **30** the 3-*O*-acetyl analogue of the potent selective 11 β -HSD2 inhibitor **7** (Table 1) was inactive on 11 β -HSD2, indicating that the combination of these groups is not tolerated by the active site of the type 2 isozyme. This observation was also noted for the 3-*O*-methyl analogue **31**, which was also inactive on 11 β -HSD2 (Table 2) compared to **52** the 3-*O*-methyl analogue of 18 β -GA (Table 4), which was a potent inhibitor of 11 β -HSD2. This indicates that changes to the 3 and 30 positions to give **50** were not additive and probably modifications at both ends of the template are resulting in unfavourable steric effects. The tertiary amide **32** was a weak non-selective inhibitor of both isozymes, possibly indicating that a tertiary amide in this region does not have the required electronic effect, but as the similar secondary amide analogues **17** and **18** discussed above were also weakly active, an unfavourable hydrophobic interaction in the region is also possible.

In summary, amide modifications at the 30-position are generally non-selective but show greater inhibition of 11 β -HSD2 than 11 β -HSD1. The position of the basic nitrogen in amide side chains is important for activity and selectivity on 11 β -HSD2. The most potent compound in this series is the hydroxyethyl analogue **7** and elongation of the alkyl chain decreases activity.

3.2. Ester and ether modifications

Further modifications to the 30-position including esters, ethers and alcohols are listed in Table 3. Esters with small alkyl groups such as methyl and ethyl, **34** and **35**, are potent non-selective inhibitors of 11 β -HSD1 and 11 β -HSD2. The more bulky hydrophobic esters such as its *t*-butyl, cyclohexylmethyl and benzyl derivatives, **36**–**38** are less potent and non-selective, indicating that large hydrophobic groups in this region are not beneficial for activity.

The hydroxymethyl derivative **39** is potent on both 11 β -HSD1 and 11 β -HSD2 and has comparable activity to 18 β -GA **1**. This indicates that a carbonyl group at the 30-position is not a requirement for activity. However, the more bulky *gem*-dimethyl derivative **44** shows weak activity, indicating the poor tolerance of steric bulk and the importance of an interaction in this region. Interestingly, the 2-hydroxyethyloxymethyl compound **43** is selective for 11 β -HSD1 and this compound has the hydroxyl moiety in a similar region to **11** indicating an important interaction in this region for 11 β -HSD1 activity.

The bulky ether **40** is inactive on both 11 β -HSD1 and 11 β -HSD2, indicating that the tolerance for steric bulk for activity has been exceeded. The smaller methoxymethyl ether **41** although weakly active, shows some selectivity for 11 β -HSD1 and it is interesting to note this selectivity is also observed in **61** (Scheme 2), the corre-

sponding carbenoxolone analogue (with 75% and 19% inhibition of 11 β -HSD1 and 11 β -HSD2, respectively at 10 μ M, not shown in tables). Conversely, **42** is a potent and selective 11 β -HSD2 inhibitor; clearly, interactions in this area of space are key to determine selectivity with unfavourable hydrophobic effects being limiting for both isozymes.

The 3-ether analogues of the 18 β -GA template are listed in Table 4. The direct analogues of 18 β -GA **1** itself show that the 3-methoxy ether **52** is potent and selective for 11 β -HSD2. By increasing the steric bulk to ethyl and isopropyl selectivity for 11 β -HSD2 is lost, although moderate activity, decreasing with hydrophobicity is maintained. The 3-ether analogues of the C-30 methyl ester of 18 β -GA (**47**–**51**, **55**–**57**) are essentially inactive on both isozymes irrespective of the size and hydrophobicity of the ether. Clearly a combination of C-30 ester and C-3 ether moieties is sufficient to block key interactions on both 11 β -HSD1 and 11 β -HSD2.

The acetyl ester derivative of 18 β -GA, **58** was a potent selective inhibitor of 11 β -HSD2. The potency of **58** on 11 β -HSD2 is similar to that of the succinate ester CBX **2**, however hydrolytic instability of ester groups anywhere on the GA template cannot be ruled out and **58** may be slowly converted to 18 β -GA **1**.

The 3-oxo analogue of 18 β -GA, **60** is a non-selective and potent inhibitor of 11 β -HSD1 and 11 β -HSD2 and the corresponding C-30 methyl ester **59**, although losing some potency, is also non-selective (activities not shown, Scheme 3).²⁶ This indicates that a hydrogen bond donor at the 3-position is not essential for activity and a hydrogen bond acceptor interaction may be taking place in this region. This hypothesis is in agreement with the results observed above for the 3-ethers **52**–**53** and acetyl analogue of 18 β -GA **58** and also for CBX **2** where a hydrogen bond donor interaction close to C-3 may be important. The succinate ester side chain present in CBX **2** was incorporated into selected active compounds that had indicated selectivity for either 11 β -HSD1 or 11 β -HSD2; the synthetic ease of introduction of the succinate side chain was also taken into account when choosing the analogues. In the secondary amide series **21** was chosen as a selective 11 β -HSD2 inhibitor and, although only moderately active, **41** with a methoxymethyl ether at C-30 was chosen as a selective 11 β -HSD1 inhibitor. Activity and selectivity for 11 β -HSD1 was retained with **61**; however, for **33** selectivity was lost and only weak activity observed indicating the combination of structural changes at C-3 and C-30 was not tolerated in this case (activities not shown).²⁶

In summary, small alkyl esters such as methyl or ethyl at the 30-position are potent but not selective 11 β -HSD inhibitors and larger groups result in loss of potency. At the 3-position the acetyl compound is a potent and selective 11 β -HSD2 inhibitor however potency is lost with additional substitution at the 30-position. In the ether series the 3-methoxy compound is a potent and selective inhibitor of 11 β -HSD2 and a loss of selectivity is observed with larger substituents.

3.3. Modification of the 11-position

A number of modifications were made at the 11-position of the 18 β -GA **1** template, the region where the 11 β -HSD isozymes invoke their catalysis. The 11-*exo*-methylene compounds **66** and **68** were weakly active and non-selective showing that this carbonyl isostere, although having a similar size to a carbonyl, does not have the electronic and hydrogen bonding properties in this region likely to be required for inhibition of 11 β -HSD catalysis. The tertiary alcohol analogue **65** showed poor activity against both 11 β -HSD1 and 11 β -HSD2 although some selectivity for 11 β -HSD1 was indicated. This observation was strengthened by the corresponding C-30 acid derivative **67** showing reasonable potency and selectivity for the type 1 isozyme. Removal of the carbonyl at the 11-position to give the desoxo analogues **62–64** showed that with this change only the C-30 acid derivative **62** retained potency but was non-selective. The ester analogue **63** was weakly active and non-selective. The C-30 hydroxymethyl derivative **64**, although weakly active, demonstrated selectivity for 11 β -HSD2, an observation previously noted for small nonhindered C-30 side chain alcohols such as **7**. In summary, with hydrogen at the 11-position and the 30-position unsubstituted potency is maintained with lack of selectivity. Introduction of a methylene group at the 11-position gives weakly active non-selective compounds. Introduction of a tertiary alcohol giving **67** results in selectivity for 11 β -HSD1.

As the 11 β -HSD isozymes exert their catalytic action at the 11-position, it is not surprising to see dramatic changes in potency when this region of the molecule is modified.

From the extensive number of structural changes to the 18 β -GA template discussed a number of potent and selective inhibitors of 11 β -HSD2 have been identified and the IC₅₀ values of a selection these compounds are shown in Table 6 with 18 β -GA **1** included for comparison.

From the extensive modifications on the 18 β -GA template **1** discussed a number of potent and selective inhibitors of 11 β -HSD2 have been identified. A preliminary communication relating to some of this work has been published.³⁴ These compounds are currently being used in the construction of a pharmacophore for 11 β -HSD2, which will not only be used in the design of further selective inhibitors of 11 β -HSD2 but also in the

identification of novel nonsteroidal templates as 11 β -HSD inhibitors and as templates for further design.

4. Conclusions

Novel highly potent and selective inhibitors of rat 11 β -HSD2 have been identified and the knowledge of the SAR of glycyrrhetic acid analogues as inhibitors of the 11 β -hydroxysteroid dehydrogenases has been greatly extended. The selective 11 β -HSD2 described herein have potencies ranging from low picomolar to hundred nanomolar levels. Compound **7** is the most potent selective 11 β -HSD2 inhibitor reported to date with an IC₅₀ = 4 pM on the rat isozyme. Compounds such as **7** should prove useful as mechanistic tools for in vivo studies in rodent models to evaluate the biological effects of potent and selective 11 β -HSD2 inhibitors. In addition, they will serve as templates for further design of selective nonsteroidal inhibitors and the recent publication of a homology model of 11 β -HSD2 will be used in structure based drug design to accelerate this process.³⁵

5. Experimental

5.1. General

All chemicals were either purchased from the Aldrich Chemical Co. (Gillingham, UK), Lancaster Synthesis (Morecambe, UK) or ACROS Organics (Loughborough, UK). All organic solvents of A.R. grade were supplied by Fisher Scientific (Loughborough, UK).

Rat livers and rat kidneys from normal Wistar rats were obtained from Harlan Olac, Bicester, Oxon, UK. The PBS–sucrose buffer assay medium, Dulbecco's Phosphate Buffered Saline, 1 tablet/100 mL with 0.25 M sucrose, pH 7.4 was purchased from BDH Laboratory supplies, UK. Ecoscint A scintillation fluid was obtained from National Diagnostics, US. Radioactive cortisol solutions, [1,2,6,7-³H]-cortisol was obtained from American Radiolabelled Chemical Inc., (ARC) MD, USA with 2 × 10⁵ dpm and [4-¹⁴C]-cortisol was obtained from Perkin Elmer, MS, USA with 10,000 dpm. Radiolabelled cortisone was obtained from American Radiolabelled Chemical Inc., (ARC) MD, USA with 2 × 10⁵ dpm and [4-¹⁴C]-cortisone was bought from ARC (10,000 dpm). Cortisol, cortisone and cofactors NADPH and NADP were purchased from Sigma Chemical Co., Poole, UK.

Thin layer chromatography (TLC) was performed on pre-coated plates (Merck TLC aluminium sheets silica gel 60 F₂₅₄, Art. No 5554). Compounds were visualized by either viewing under UV light or treating with an ethanolic solution of phosphomolybdic acid (PMA) followed by heating. Flash chromatography was carried out using Sorbsil C60 silica gel or Isolute[®] pre-packed Flash Si columns from Argonaut Technologies. Parallel synthesis was performed on either Radleys Carousel reaction stations or Radleys GreenHouse parallel synthesizers. Solvent removal from parallel syntheses was

Table 6. IC₅₀ values for selective inhibitors of 11 β -HSD2

Compd	IC ₅₀ (nM) on 11 β -HSD2 ^a
1	360 ^b
7	0.004
42	164
52	184
58	0.02

^a Mean of at least two measurements with typically $\pm 5\%$ variation.

^b Reported value.³²

performed on a GeneVac DD4 evaporation system. NMR spectra were recorded with a JEOL GX-270 or Varian-Mercury-400 spectrometer, and chemical shifts are reported in parts per million (ppm, δ) relative to tetramethylsilane (TMS) as an internal standard. Only diagnostic proton signals are listed. Mass spectra were recorded at the Mass Spectrometry Service Centre, University of Bath. FAB-MS were carried out using *m*-nitrobenzyl alcohol (NBA) as the matrix. High performance liquid chromatography (HPLC) analysis was performed with a Waters Delta 600 liquid chromatograph with a Waters 996 photodiode Array Detector using a Waters Radialpack C18, 8×100 mm column. Melting points (mp) were measured with a Reichert-Jung ThermoGalen Kofler block or a Sanyo Gallenkamp melting point apparatus and are uncorrected.

Incubations were performed in a mechanically shaken water bath, SW 20, Germany. Evaporations were carried out using a Techne Driblock DB 3A, UK. Scintillation vials used were 20 mL polypropylene vials with caps, SARSTEDT, Germany, and scintillation counting was performed on a Beckman LS 6000 SC, Beckman Instruments Inc., USA.

5.2. Biology

Assays were performed essentially as described previously.²⁶ Kidneys and livers from normal Wistar rats were homogenized on ice in PBS–sucrose buffer (1 g/10 mL) using an Ultra-Turrax. After the livers and kidneys were homogenized the homogenate was centrifuged for 5 min at 4000 rpm. The supernatant obtained was removed and stored in glass vials at –20 °C. The amount of protein per μ L of rat liver and kidney centrifuged supernatants was determined using the Bradford method.³³

For the assay of 11 β -HSD type 1 activity rat centrifuged supernatant (76 μ g protein) was incubated for 90 min with [³H] cortisone and adjusted to a final substrate concentration of 0.5 μ M with cortisone. The assay was carried out in phosphate-buffered saline (PBS) with NADPH (1 mM) as the co-factor. At the end of the incubation period [4-¹⁴C] cortisol was added to monitor procedural losses together with 50 μ g of unlabelled cortisol to assist in the location of product steroid. Using this assay, conversion of cortisone to cortisol was linear with respect to time (0–120 min) and protein concentration (10–100 μ g) (data not shown).

The assay for 11 β -HSD type 2 activity employed rat kidney centrifuged supernatant (136 μ g protein) and [³H] cortisol adjusted to a final substrate concentration of 0.5 μ M with cortisol. PBS–sucrose was used as the buffer with NADP (1 mM) as the co-factor. [4-¹⁴C] Cortisone was added at the end of the incubation period (60 min) to monitor procedural losses with 50 μ g unlabelled cortisone being added to assist product location,

At the end of the incubation period and addition of [4-¹⁴C] and unlabelled product steroids, precursor and

product steroids were extracted with diethyl ether (4 mL). The organic phase was removed and taken to dryness under air. The resulting residue was dissolved in diethyl ether (50–100 μ L), transferred to a thin-layer chromatography plate, which contained a fluorescent dye, and developed using the system chloroform: methanol (9:1, v/v). Product steroids were visualized under UV light. In this system the R_f values for cortisol and cortisone were 0.5 and 0.8, respectively. The areas containing cortisol or cortisone were cut from the TLC plate and placed in a 20 mL scintillation vials. Steroids were eluted from the plate by the addition of methanol (0.5 mL) before the addition of scintillation fluid (10 mL) and determining the radioactivity using scintillation spectrometry. Enzyme activity was determined as the amount of product formed/mg protein/h after correction for procedural losses. Assays were carried out in the absence or presence of inhibitors. Initially, all compounds were tested at 10 μ M with IC₅₀ values being determined for compounds showing >90% inhibition when tested at this concentration.

5.3. Chemistry

5.3.1. General synthetic procedures

5.3.1.1. Method A: Synthesis of 18 β -GA 30-amides (4, 5, 7, 8, 10, 12, 30–32). To a solution of 18 β -GA (1) (0.5 mmol) in DCM (15 mL) were added the amine (1.0 mmol), HOBt (0.26 mmol), EDCI (0.55 mmol), DMAP (0.55 mmol) and triethylamine (0.55 mmol). The mixture was stirred under nitrogen at room temperature for 16–24 h. When TLC showed the completion of the reaction, the reaction mixture was poured into water and extracted with DCM. The organic phase was washed with 2% HCl and water, dried over MgSO₄. Evaporation of the solvent gave a residue that was purified by flash chromatography (SiO₂, ethyl acetate–hexane, gradient elution) to give the amide product.

5.3.1.2. Method B: Synthesis of 18 β -GA 30-amides (20, 28). To a solution of 18 β -GA (1) (0.2 mmol) in chloroform (5 mL) the requisite amine (0.4 mmol) and EDCI (0.4 mmol) were added. The mixture was stirred under nitrogen at room temperature for 6–16 h. When TLC showed the completion of the reaction, the reaction mixture was poured into water, extracted with DCM. The organic phase was washed with 2% HCl and water, dried over MgSO₄ and concentrated in vacuo to give a residue that was purified by flash chromatography (SiO₂, ethyl acetate–hexane, gradient elution) to give the amide products.

5.3.1.3. Method C: Parallel synthesis of 18 β -GA 30-amides using Radleys GreenHouse Parallel Synthesizer (3, 9, 13–19, 22–27). To a suspension of 18 β -GA (1) (50 mg, 0.106 mmol) in anhydrous DCM (4 mL, 10 mL tubes) under an argon atmosphere at room temperature, EDCI (1.1 M equiv), DMAP (cat.) were added followed by Et₃N (1.1 M equiv). The reaction mixture was stirred for 0.5 h at room temperature, added the amine (2 M equiv) and stirred overnight. The reactions were

monitored using TLC (DCM–MeOH, 95:5) and quenched by addition of water (1 mL). The resulting mixtures were stirred at rt for 10 min and the aqueous layer was separated. The organic phase was then washed subsequently with 1 M HCl (1 mL) and water (1 mL). The aqueous phase was removed and the bi-phasic mixture was evaporated using the *GeneVac*. The crude products were purified in a parallel manner using *Argonaut* pre-packed columns (5 g) on the *Vacuum Flash Master* using a gradient elution with hexane–ethyl acetate–methanol mixtures to give the products as white solids.

5.3.1.4. Method D: Synthesis of 3-alkylated 18 β -GA 30-methyl ester derivatives (47–51, 55–57). The intermediate methyl ester **34** was suspended in anhydrous THF under an argon atmosphere, NaH (60% dispersed in oil, 2 equiv) was added at rt over 5 min, followed by the requisite alkyl or aryl halide (2 equiv). The crude mixtures were heated at reflux under inert atmosphere. The reactions were monitored by TLC (EtOAc–hexane, 2:8). Once completed, the reactions were quenched by addition of water (dropwise) at rt. The crude mixtures were extracted with DCM, and the organic phase was washed with saturated NaCl solution. The organic extracts were dried (MgSO₄) and concentrated in vacuo to obtain the product as a solid. The crude product was then purified using flash chromatography (SiO₂, hexane–ethyl acetate, gradient elution) to obtain in general a colourless solid.

5.3.1.5. Method E: Synthesis of 3-alkylated 18 β -GA derivatives (52–54). The starting ester was suspended in THF–water (3:1, 4 mL), LiOH (5 M equiv) were added, and the mixture heated at reflux for 24 h. The crude reaction was cooled to rt, acidified with 2 M HCl (pH = 2) and extracted with DCM. The organic phase was dried (MgSO₄) and concentrated to obtain the crude product as a pale yellow solid. The crude product was then purified using flash chromatography eluting with ethyl acetate–methanol or DCM–methanol.

5.3.1.6. Method F: Synthesis of 18 β -GA 30-esters (35–38). A solution of **1** (1.0 g; 2.12 mmol) in anhydrous DMF (50 mL) containing anhydrous K₂CO₃ (2.9 g; 21.2 mmol) was stirred at rt under N₂ for 1 h. The requisite alkyl halide (11 equiv) was introduced to the reaction mixture followed by tetra-butyl ammonium iodide (200 mg). The mixture was stirred for 24 h at rt, poured into brine and extracted with ethyl acetate (3 $\times$ 200 mL). The organic phase was washed with water (3 $\times$ 100 mL), dried over MgSO₄, filtered and evaporated in vacuo to give a white solid. Purification by flash chromatography (SiO₂, EtOAc–Hexane gradient elution) and recrystallization from absolute ethanol gave the required product.

5.3.1.7. N-Cyclopropyl-3 β -hydroxy-11-oxo-18 β -olean-12-en-30-oic acid amide (4). **4** was synthesized with general method A. White crystalline solid (150 mg, 59%) was obtained. Mp 253–256 °C; HPLC purity 99% (*t*_R 3.9 min in 6% water–methanol); ¹H NMR (400 MHz, CDCl₃) δ 5.64 (s, 1H, 12-H), 3.23 (m, 1H, 3 α -H), 2.80

(dt, *J* = 13.3, 3.4 Hz, 1H, 1 β -H), 2.71 (m, 1H, –NHCH), 2.34 (s, 1H, 9 α -H), 2.15 (t, *J* = 8.2 Hz, 1H, 18 β -H), 1.37 (s, 3H, CH₃), 1.14 (s, 3H, CH₃), 1.13 (s, 3H, CH₃), 1.11 (s, 3H, CH₃), 1.01 (s, 3H, CH₃), 0.82 (s, 3H, CH₃), 0.81 (s, 3H, CH₃); FAB-MS *m/z*: 510 (100, MH⁺); FAB-HRMS calcd for C₃₃H₅₃NO₃ (MH⁺) 510.3947, found 510.3956.

5.3.1.8. N-(3 β -Hydroxy-11,30-dioxo-18 β -olean-12-en-30-yl)-L-alanine ethyl ester (5). **5** was synthesized with general method A starting from **1** (1.0 mmol). White amorphous powder (430 mg, 75%) was obtained. HPLC purity 99% (*t*_R 4.2 min in 6% water–methanol); ¹H NMR (400 MHz, CDCl₃) δ 6.14 (d, *J* = 8 Hz, 1H, NH), 5.76 (s, 1H, 12-H), 4.60 (m, 1H, NHCH), 4.23 (q, *J* = 7.3 Hz, 2H, –OCH₂CH₃), 3.23 (m, 1H, 3 α -H), 2.82 (dt, *J* = 13.3, 3.5 Hz, 1H, 1 β -H), 2.34 (s, 1H, 9 α -H), 2.28 (t, *J* = 8.5 Hz, 1H, 18 β -H), 1.40 (d, *J* = 7 Hz, 3H, CH₃), 1.39 (s, 3H, CH₃), 1.30 (t, *J* = 7.3 Hz, 3H, CH₃), 1.15 (s, 3H, CH₃), 1.14 (s, 3H, CH₃), 1.13 (s, 3H, CH₃), 1.01 (s, 3H, CH₃), 0.82 (s, 3H, CH₃), 0.81 (s, 3H, CH₃); FAB-MS *m/z*: 570 (100, MH⁺); FAB-HRMS calcd for C₃₅H₅₆NO₅ (MH⁺) 570.4158, found 570.4150.

5.3.1.9. N-(3 β -Hydroxy-11,30-dioxo-18 β -olean-12-en-30-yl)-L-alanine (6). To a solution of **5** (300 mg, 0.53 mmol) in methanol–THF (8 mL:4 mL) was added 1 N NaOH solution (1 mL). The mixture was stirred under nitrogen at rt for 2 h, adjusted to pH 3 with 10% HCl and extracted with DCM. The organic phase was washed with water, dried over MgSO₄ and concentrated to give a residue, which was purified with flash chromatography and recrystallized from ethyl ether. White crystalline solid (180 mg, 63%) was obtained. Mp 272–275 °C; HPLC purity 97% (*t*_R 2.6 min in methanol); ¹H NMR (400 MHz, CDCl₃) δ 6.67 (d, *J* = 7.8 Hz, 1H, NH), 5.64 (s, 1H, 12-H), 4.65 (m, 1H, –NHCH–), 3.18 (m, 1H, 3 α -H), 2.70 (dt, *J* = 13.3, 3.2 Hz, 1H, 1 β -H), 2.27 (s, 1H, 9 α -H), 1.33 (d, *J* = 7 Hz, 3H, CH₃), 1.28 (s, 3H, CH₃), 1.09 (s, 3H, CH₃), 1.05 (s, 3H, CH₃), 1.04 (s, 3H, CH₃), 0.94 (s, 3H, CH₃), 0.74 (s, 3H, CH₃), 0.73 (s, 3H, CH₃); FAB-MS *m/z*: 542 (100, MH⁺); FAB-HRMS calcd for C₃₅H₅₂NO₅ (MH⁺) 542.3845, found 542.3853.

5.3.1.10. N-(2-Hydroxyethyl)-3 β -hydroxy-11-oxo-18 β -olean-12-en-30-oic acid amide (7). **7** was synthesized with general method A. White crystalline solid (110 mg, 43%) was obtained. Mp 160–163 °C; HPLC purity 96% (*t*_R 6.2 min in 55% water–methanol); ¹H NMR (400 MHz, CDCl₃) δ 6.06 (t, *J* = 5.9 Hz, 1H, NH), 5.61 (s, 1H, 12-H), 3.67 (t, *J* = 5.1 Hz, 2H, CH₂), 3.39 (m, 2H, –NHCH₂–), 3.16 (m, 1H, 3 α -H), 2.72 (dt, *J* = 13.6, 3.6 Hz, 1H, 1 β -H), 2.27 (s, 1H, 9 α -H), 2.12 (t, *J* = 8.6 Hz, 1H, 18 β -H), 1.30 (s, 3H, CH₃), 1.08 (s, 3H, CH₃), 1.06 (s, 3H, CH₃), 1.05 (s, 3H, CH₃), 0.94 (s, 3H, CH₃), 0.75 (s, 3H, CH₃), 0.74 (s, 3H, CH₃); FAB-MS *m/z*: 542 (100, MH⁺); FAB-HRMS calcd for C₃₅H₅₂NO₅ (MH⁺) 542.3845, found 542.3853.

5.3.1.11. N-(5-Hydroxypentyl)-3 β -hydroxy-11-oxo-18 β -olean-12-en-30-oic acid amide (8). **8** was synthesized with general method A. White crystalline solid

(165 mg, 59%) was obtained. Mp 136–139 °C; HPLC purity 98% (t_R 5.1 min in 8% water–methanol); 1H NMR (400 MHz, $CDCl_3$) δ 5.67 (s, 1H, 12-H), 5.63 (m, 1H, NH), 3.68 (m, 2H, $-CH_2OH$), 3.42 (m, 1H, $-NHCH-$), 3.24 (m, 1H, $-NHCH-$), 3.23 (m, 1H, 3α -H), 2.80 (dt, J = 13.6, 3.5 Hz, 1H, 1β -H), 2.38 (s, 1H, 9α -H), 1.34 (s, 3H, CH_3), 1.14 (s, 6H, $2\times CH_3$), 1.13 (s, 3H, CH_3), 1.01 (s, 3H, CH_3), 0.82 (s, 3H, CH_3), 0.81 (s, 3H, CH_3); FAB-MS m/z : 556 (100, MH^+); FAB-HRMS calcd for $C_{35}H_{58}NO_4$ (MH^+) 556.4366, found 556.4376.

5.3.1.12. *N*-(2-Oxo-tetrahydrofuran-3-yl)- 3β -hydroxy-11-oxo-18 β -olean-12-en-30-oic acid amide (10). The diastereoisomers **10** were synthesized with general method A starting from **1** (0.75 mmol). White powder (305 mg, 74%) was obtained. HPLC purity 99% (as diastereoisomers, t_R = 3.9 min in 4% water–methanol); 1H NMR (400 MHz, $CDCl_3$) δ 6.37 (d, J = 6.2 Hz, 1H, NH), 6.32 (d, J = 6.2 Hz, 1H, NH), 5.75 (s, 1H, 12-H), 5.66 (s, 1H, 12-H), 4.58 (m, 2H, $2\times NHCH$), 4.49 (m, 2H, $-OCH_2$), 4.30 (m, 2H, $-OCH_2$), 3.23 (m, 2H, $2\times 3\alpha$ -H), 2.80 (m, 2H, $2\times 1\beta$ -H), 2.32 (s, 2H, $2\times 9\alpha$ -H), 1.35 (s, 6H, $2\times CH_3$), 1.16 (s, 6H, $2\times CH_3$), 1.11 (s, 12H, $4\times CH_3$), 0.98 (s, 6H, $2\times CH_3$), 0.81 (s, 6H, $2\times CH_3$), 0.79 (s, 6H, $2\times CH_3$); FAB-MS m/z : 554 (100, MH^+); FAB-HRMS calcd for $C_{34}H_{52}NO_5$ (MH^+) 554.3845, found 554.3832.

5.3.1.13. *N*-(3β -Hydroxy-11,30-dioxo-18 β -olean-12-en-30-yl)-2-amino-4-hydroxy-butyric acid (11). To a solution of **10** (110 mg, 0.20 mmol) in methanol (4 mL) was added 1N KOH solution (1 mL). The mixture was stirred under nitrogen at rt for 24 h, then adjusted to pH 2 with 10% HCl and extracted with DCM. The organic phase was washed with water, dried over $MgSO_4$ and concentrated to give a residue, which was purified with flash chromatography. White solid (65 mg, 57%) was obtained. Mp 220–229 °C; HPLC purity 95% (as diastereoisomers, t_R 2.0 min in 4% water–methanol); 1H NMR (400 MHz, $CDCl_3$) δ 7.0 (m, 1H, NH), 5.75 (s, 1H, 12-H), 4.40 (m, 1H, $-NHCH-$), 3.65 (m, 2H, $-OCH_2$), 3.18 (m, 1H, 3α -H), 1.31 (s, 3H, CH_3), 1.19 (s, 3H, CH_3), 1.05 (s, 3H, CH_3), 1.02 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 0.75 (s, 6H, $2\times CH_3$); FAB-MS m/z : 570 (30, $M-H^+$); FAB-HRMS calcd for $C_{34}H_{52}NO_6$ ($M-H^+$) 570.3795, found 570.3797.

5.3.1.14. *N*-[2-(Morpholin-4-yl)ethyl]- 3β -hydroxy-11-oxo-18 β -olean-12-en-30-oic acid amide (12). **12** was synthesized with general method A. White crystalline solid (200 mg, 69%) was obtained. Mp 135–138 °C; HPLC purity 97% (t_R 3.9 min in 6% water–methanol); 1H NMR (400 MHz, $CDCl_3$) δ 6.21 (m, 1H, NH), 5.71 (s, 1H, 12-H), 3.72 (m, 4H, $-(CH_2)_2O$ of morpholine), 3.38 (m, 2H, $-CONHCH_2$), 3.23 (m, 1H, 3α -H), 2.82 (dt, J = 13.5, 3.2 Hz, 1H, 1β -H), 2.52 (t, J = 5.9 Hz, 2H, $-CH_2N$), 2.47 (m, 4H, $-N(CH_2)_2$), 2.37 (s, 1H, 9-H), 1.40 (s, 3H, CH_3), 1.14 (s, 9H, $3\times CH_3$), 1.01 (s, 3H, CH_3), 0.82 (s, 3H, CH_3), 0.81 (s, 3H, CH_3); FAB-MS m/z : 583 (100, MH^+); FAB-HRMS calcd for $C_{36}H_{59}N_2O_4$ (MH^+) 583.4475, found 583.4492.

5.3.1.15. *N*-Benzyl- 3β -hydroxy-11-oxo-18 β -olean-12-en-30-oic acid amide (20). **20** was synthesized with general method B. Off-white crystalline powder (40 mg, 33%) was obtained. Mp 233–235 °C; HPLC purity 98% (t_R 2.6 min in 4% water–methanol); 1H NMR (400 MHz, $CDCl_3$) δ 7.32–7.36 (m, 2H, aromatic protons), 7.25–7.30 (m, 3H, aromatic protons), 5.83 (broad, 1H, NH), 5.57 (s, 1H, 12-H), 4.47 (m, 2H, $NHCH_2Ph$), 3.21 (dd, J = 10.5, 6.2 Hz, 1H, 3α -H), 2.78 (dt, J = 13.3, 4.5 Hz, 1H, 1β -H), 2.31 (s, 1H, 9α -H), 2.18 (dd, J = 9.8, 3.5 Hz, 1H, 18β -H), 1.36 (s, 3H, CH_3), 1.16 (s, 3H, CH_3), 1.13 (s, 3H, CH_3), 1.12 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 0.81 (s, 3H, CH_3), 0.80 (s, 3H, CH_3); FAB-MS m/z : 560 (100, MH^+); FAB-HRMS calcd for $C_{37}H_{54}NO_3$ (MH^+) 560.4104, found 560.4090.

5.3.1.16. *N*-Benzyl- 3β -acetyloxy-11-oxo-18 β -olean-12-en-30-oic acid amide (28). **28** was synthesized with general method B starting from 3β -acetyloxy glycyrrhetic acid (**58**).³¹ White crystalline solid (55 mg, 48%) was obtained. Mp 150–152 °C; HPLC purity 97.7% (t_R 3.6 min in 4% water–methanol); 1H NMR (400 MHz, $CDCl_3$) δ 7.32–7.37 (m, 2H, aromatic protons), 7.24–7.30 (m, 3H, aromatic protons), 5.83 (t, J = 5.5 Hz, 1H, NH), 5.56 (s, 1H, 12-H), 4.41–4.53 (m, 3H, 3α -H and $NHCH_2Ph$), 2.78 (dt, J = 13.6, 4.5 Hz, 1H, 1β -H), 2.33 (s, 1H, 9α -H), 2.16 (dd, J = 9.7, 3.2 Hz, 1H, 18β -H), 2.05 (s, 3H, $3-CH_2CO-$), 1.35 (s, 3H, CH_3), 1.16 (s, 6H, $2\times CH_3$), 1.15 (s, 3H, CH_3), 1.12 (s, 3H, CH_3), 0.88 (s, 3H, CH_3), 0.81 (s, 3H, CH_3); FAB-MS m/z : 602 (100, MH^+); FAB-HRMS calcd for $C_{39}H_{56}NO$ (MH^+) 602.4209, found 602.4211.

5.3.1.17. *N*-Hydroxy- 3β -acetyloxy-11-oxo-18 β -olean-12-en-30-oic acid amide (29). To a solution of **58** (235 mg, 0.45 mmol) in anhydrous THF (8 mL) was added DMAP (11 mg, 0.09 mmol), followed by carbonyldiimidazole (89 mg, 0.55 mmol). The mixture was stirred at rt under nitrogen for 18 h. A solution of hydroxylamine hydrochloride (64 mg, 0.92 mmol) in DMF (2 mL) was added. The mixture was stirred at 60 °C for 5 h, and poured into water, extracted with DCM. The organic phase was washed brine, dried over $MgSO_4$ and concentrated in vacuo to afford a residue that was purified with flash chromatography (EtOAc–hexane gradient elution). White crystalline solid (90 mg, 38%) was obtained. Mp 294–299 °C; HPLC purity 99% (t_R 7.2 min in 10% water–methanol); 1H NMR (400 MHz, $CDCl_3$) δ 6.37 (s, 1H, NH), 5.69 (s, 1H, 12-H), 4.52 (dd, J = 11.7, 7.1 Hz, 1H, 3α -H), 2.81 (dt, J = 13.6, 3.5 Hz, 1H, 1β -H), 2.28 (s, 1H, 9α -H), 2.08 (s, 3H, CH_3CO-), 1.36 (s, 3H, CH_3), 1.20 (s, 3H, CH_3), 1.16 (s, 3H, CH_3), 1.13 (s, 3H, CH_3), 0.88 (s, 6H, $2\times CH_3$), 0.81 (s, 3H, CH_3); FAB-MS m/z : 528 (100, MH^+); FAB-HRMS calcd for $C_{32}H_{50}NO_5$ (MH^+) 528.3689, found 528.3698.

5.3.1.18. *N*-(2-Hydroxyethyl)- 3β -acetyloxy-11-oxo-18 β -olean-12-en-30-oic acid amide (30). **30** was synthesized with general method A starting from **58** (872 mg, 1.70 mmol). White crystalline solid (688 mg, 73%) was obtained. Mp 236–238 °C; HPLC purity 99% (t_R 2.2 min

in methanol); ^1H NMR (400 MHz, CDCl_3) δ 6.22 (t, $J = 5.5$ Hz, 1H, NH), 5.68 (s, 1H, 12-H), 4.51 (dd, $J = 12.7, 5.1$ Hz, 1H, 3 α -H) 3.73 (m, 2H, $-\text{CH}_2\text{OH}$), 3.54 (m, 1H, $-\text{NHCH}-$), 3.39 (m, 1H, $-\text{NHCH}-$), 2.86 (broad, 1H, OH), 2.77 (dt, $J = 13.7, 3.5$ Hz, 1H, 1 β -H), 2.36 (s, 1H, 9 α -H), 2.18 (t, $J = 8.6$ Hz, 1H, 18 β -H), 2.06 (s, 3H, $\text{CH}_3\text{CO}-$), 1.37 (s, 3H, CH_3), 1.15 (s, 6H, $2\times\text{CH}_3$), 1.12 (s, 3H, CH_3), 0.88 (s, 6H, $2\times\text{CH}_3$), 0.81 (s, 3H, CH_3); ^{13}C NMR (270 MHz, CDCl_3) δ 200.2, 176.9, 171.0, 169.5, 128.4, 80.6, 62.5, 61.7, 55.0, 48.2, 45.4, 43.7, 43.2, 42.0, 41.9, 38.8, 38.0, 37.4, 36.9, 32.7, 31.9, 31.5, 29.5, 28.5, 28.0, 26.4, 26.3, 23.5, 23.3, 21.3, 18.7, 17.3, 16.7, 16.4; FAB-MS m/z : 556 (100, MH^+); FAB-HRMS calcd for $\text{C}_{34}\text{H}_{54}\text{NO}_5$ (MH^+) 556.4002, found 556.3991.

5.3.1.19. *N*-(2-Hydroxyethyl)-3 β -methoxy-11-oxo-18 β -olean-12-en-30-oic acid amide (31). 31 was synthesized with general method A starting from 52 (500 mg, 1.03 mmol). White crystalline solid (396 mg, 75%) was obtained. Mp 146–148 °C; HPLC purity 99% (t_R 2.6 min in methanol); ^1H NMR (400 MHz, CDCl_3) δ 6.18 (t, $J = 5.4$ Hz, 1H, NH), 5.68 (s, 1H, 12-H), 3.74 (m, 2H, $-\text{CH}_2\text{OH}$), 3.38–3.48 (m, 2H, $-\text{NHCH}_2-$), 3.28 (s, 3H, $\text{CH}_3\text{O}-$), 2.80 (dt, $J = 13.7, 3.5$ Hz, 1H, 1 β -H), 2.68 (dd, $J = 12.6, 5.0$ Hz, 1H, 3 α -H), 2.33 (s, 1H, 9 α -H), 2.17 (t, $J = 8.5$ Hz, 1H, 18 β -H), 1.37 (s, 3H, CH_3), 1.15 (s, 3H, CH_3), 1.13 (s, 3H, CH_3), 1.12 (s, 3H, CH_3), 0.99 (s, 3H, CH_3), 0.82 (s, 3H, CH_3), 0.79 (s, 3H, CH_3); FAB-MS m/z : 528 (100, MH^+); FAB-HRMS calcd for $\text{C}_{33}\text{H}_{54}\text{NO}_4$ (MH^+) 528.4053, found 528.4041.

5.3.1.20. *N*-(3 β -Hydroxy-11,30-dioxo-18 β -olean-12-en-30-yl)-piperidine (32). 32 was synthesized with general method A. White crystalline solid (110 mg, 41%) was obtained. Mp 276–280 °C; HPLC purity 98% (t_R 4.8 min in 4% water–methanol); ^1H NMR (400 MHz, CDCl_3) δ 5.71 (s, 1H, 12-H), 3.55 (m, 4H, $-\text{N}(\text{CH}_2)_2$ of piperidine), 3.23 (m, 1H, 3 α -H), 2.82 (dt, $J = 13.3, 3.5$ Hz, 1H, 1 β -H), 2.35 (s, 1H, 9 α -H), 2.30 (t, $J = 8.2$ Hz, 1H, 18 β -H), 1.37 (s, 3H, CH_3), 1.22 (s, 3H, CH_3), 1.14 (s, 3H, CH_3), 1.13 (s, 3H, CH_3), 1.01 (s, 3H, CH_3), 0.82 (s, 3H, CH_3), 0.81 (s, 3H, CH_3); FAB-MS m/z : 538 (100, MH^+); FAB-HRMS calcd for $\text{C}_{35}\text{H}_{56}\text{NO}_3$ (MH^+) 538.4260, found 538.4239.

5.3.1.21. *N*-(Pyridin-2-yl-methyl)-3 β -hydroxy-11-oxo-18 β -olean-12-en-30-oic acid amide 3 β -succinic semiesther (33). To a solution of 21 (122 mg, 0.218 mmol) in pyridine (5 mL) were added succinic anhydride (304 mg, 3.04 mmol) and DMAP (106 mg, 0.88 mmol). The mixture was stirred at 90 °C for 72 h, and poured over 10% HCl solution, extracted with DCM. The organic phase was washed brine, dried over MgSO_4 and concentrated in vacuo to afford a residue, which was purified with flash chromatography. Yellow solid (65 mg, 45%) was obtained. Mp 152–154 °C; HPLC purity 99% (t_R 3.4 min in methanol); ^1H NMR (400 MHz, CDCl_3) δ 8.50 (dd, $J = 7.9, 2.0$ Hz 1H, Ar-H), 7.66 (dt, $J = 7.8, 2.0$ Hz, 1H, Ar-H), 7.53 (m, 1H, NH), 7.31 (d, $J = 7.8, 1\text{H}$, Ar-H),

7.15–7.23 (m, 1H, Ar-H), 5.71 (s, 1H, 12-H), 4.40–4.57 (m, 3H, 3 α -H and $-\text{CH}_2\text{Py}$), 2.71 (dt, $J = 13.3, 3.5$ Hz, 1H, 1 β -H), 2.58–2.64 (m, 4H, $-\text{COCH}_2\text{CH}_2\text{CO}-$), 2.34 (s, 1H, 9 α -H), 1.34 (s, 3H, CH_3), 1.19 (s, 3H, CH_3), 1.04 (s, 6H, $2\times\text{CH}_3$), 0.82 (s, 3H, CH_3), 0.81 (s, 3H, CH_3), 0.66 (s, 3H, CH_3); FAB-MS m/z : 661 (60, MH^+); FAB-HRMS calcd for $\text{C}_{40}\text{H}_{57}\text{N}_2\text{O}_6$ (MH^+) 661.4217, found 661.4231.

The following amides (3, 9, 13–19, 21–27) were synthesized with general method C.

5.3.1.22. *N*-Butyl-3 β -hydroxy-18 β -olean-12-en-11-one-30-oic acid amide (3). 3: HPLC purity 90% (t_R 2.38 min in 100% MeOH); ^1H NMR (CDCl_3 , 400 MHz) δ 5.69 (s, 1H, olefinic), 3.50–3.21 (m, 3H), 2.34 (s, 1H, CH), 1.64–1.43 (broad m, 4H), 1.37, 1.25, 1.23, 1.14, 1.01, 0.83 and 0.81 (7 $\times$ s, 3H each, CH_3); APCI-MS m/z : 526 (50, MH^+), 567 (50, $\text{M}+\text{CH}_3\text{CN}^+$); FAB-HRMS calcd for $\text{C}_{35}\text{H}_{55}\text{NO}_3$ (MH^+) 526.4260, found 526.4267.

5.3.1.23. *N*-(2-Amino-*N*-methylethyl)-3 β -hydroxy-18 β -olean-12-en-11-one-30-oic acid amide (9). 9: mp 153–156 °C; HPLC purity 95% (t_R 2.38 min in 100% MeOH); ^1H NMR (CDCl_3 , 400 MHz) δ 5.70 (s, 1H, olefinic H), 3.25–3.21 (m, 2H), 3.13–2.78 (broad dt, 1H, 1 β -CH), 2.18 (s, 3H $\text{N}-\text{CH}_3$), 1.37, 1.35, 1.12, 1.10, 1.00, 0.80 and 0.79 (7 $\times$ s, 3H each, $7\times\text{CH}_3$); APCI-MS m/z : 526 (100, M^+); FAB-HRMS calcd for $\text{C}_{33}\text{H}_{54}\text{N}_2\text{O}_3$ (M^+) 526.4089, found 526.4049.

5.3.1.24. *N*-(Morpholin-4-amino)-3 β -hydroxy-18 β -olean-12-en-11-one-30-oic acid amide (13). 13: mp 183–186 °C; HPLC purity >99% (t_R 1.53 min in 100% MeOH); ^1H NMR (CDCl_3 , 270 MHz) δ 6.30 (broad s, 1H, NH), 5.60 (s, 1H, olefinic), 3.82–3.78 (broad t, 4H, CH_2), 3.22–3.20 (broad m, 1H, CH), 2.80–2.77 (broad m, 5H, CH_2), 2.34 (s, 1H, CH), 1.37, 1.25, 1.10, 1.12, 1.01, 0.81 and 0.75 (7 $\times$ s, 3H each, CH_3); APCI-MS m/z : 555 (40, MH^+), 577 (30, $\text{M}+\text{Na}^+$); FAB-HRMS calcd for $\text{C}_{37}\text{H}_{54}\text{N}_2\text{O}_4$ (MH^+) 555.4525, found 555.4512.

5.3.1.25. *N*-(1-Piperazinylethyl)-3 β -hydroxy-18 β -olean-12-en-11-one-30-oic acid amide (14). 14: HPLC purity >90% (t_R 2.45 min in 100% MeOH); ^1H NMR (CDCl_3 , 270 MHz) δ 5.71 (s, 1H, olefinic), 3.64 (broad t, 4H, CH_2), 3.20–3.10 (broad m, CH), 2.81–2.71 (broad dt, 1H, 1 β -CH), 2.38–2.32 (broad t, 4H, CH_2), 2.30 (s, 1H, CH), 1.35, 1.25, 1.13, 1.12, 1.00, 0.81 and 0.80 (7 $\times$ s, 3H each, CH_3); APCI-MS m/z : 582 (100, MH^+), 553 (40, $\text{M}-\text{CH}_3^+$).

5.3.1.26. *N*-(4-Methylpiperazinyl-1-amino)-3 β -hydroxy-18 β -olean-12-en-11-one-30-oic acid amide (15). 15: mp 190–191 °C; HPLC purity >99% (t_R 2.45 min in 100% MeOH); ^1H NMR (CDCl_3 , 270 MHz) δ 5.71 (s, 1H, olefinic), 3.64 (broad t, 4H, CH_2), 3.20–3.10 (broad m, CH), 2.81–2.71 (broad dt, 1H, 1 β -CH), 2.38–2.32 (broad t, 4H, CH_2), 2.30 (s, 1H, CH), 1.35, 1.25, 1.13, 1.12, 1.00, 0.81 and 0.80 (7 $\times$ s, 3H each, CH_3); APCI-MS m/z : 568 (30, MH^+), 553 (100, $\text{M}-\text{CH}_3^+$).

5.3.1.27. *N*-(4-Piperidinylmethyl)-3 β -hydroxy-11-oxo-18 β -olean-12-en-30-oic acid amide (16). 16: mp 198–200 °C; HPLC purity >99% (t_R 1.68 min in 100% MeOH); 1H NMR ($CDCl_3$, 400 MHz) δ 5.67 (s, 1H, olefinic), 3.47–3.16 (m, 3H), 2.80–2.72 (broad dt, 1H, 1 β -CH), 2.32 (s, 1H, CH), 1.34, 1.20, 1.11, 1.10, 0.98, 0.81 and 0.78 (7 $\times$ s, 3H each, CH_3); APCI-MS m/z : 547 (70, MH^+).

5.3.1.28. *N*-(Piperidinylethyl)-3 β -hydroxy-11-oxo-18 β -olean-12-en-30-oic acid amide (17). 17: HPLC purity 90% (t_R 2.46 min in 100% MeOH); 1H NMR ($CDCl_3$, 400 MHz) δ 5.66 (s, 1H, olefinic H), 3.97–3.54 (m, 2H, CH_2), 3.30–3.20 (m, 3H), 2.84–2.73 (m, 1H, CH), 2.34 (s, 1H, CH), 1.39, 1.35, 1.13, 1.12, 1.00 (5 $\times$ s, 3H each, 5 $\times$ CH_3), 0.80 (s, 6H, 2 $\times$ CH_3); APCI-MS m/z : 581 (85, MH^+); FAB-HRMS calcd for $C_{37}H_{60}N_2O_3$ (MH^+) 581.4682, found 581.4674.

5.3.1.29. *N*-(Pyrrolidinyldethyl)-3 β -hydroxy-11-oxo-18 β -olean-12-en-30-oic acid amide (18). 18: mp 156–159 °C; HPLC purity 95% (t_R 2.37 min in 100% MeOH); 1H NMR ($CDCl_3$, 400 MHz) δ 5.67 (s, 1H, olefinic H), 3.31–3.28 (m, 2H, CH_2), 3.13–2.78 (broad s, 1H, β -CH), 2.77–2.73 (m, 1H, CH), 2.34–2.18 (m, 5H), 1.38, 1.21, 1.12, 1.11 and 1.00 (5 $\times$ s, 3H each, 5 $\times$ CH_3), 0.80 (s, 6H, 2 $\times$ CH_3); FAB-MS m/z : 567 (100, M^+); APCI-MS m/z : 567 (70, MH^+); FAB-HRMS calcd for $C_{36}H_{59}N_2O_3$ (MH^+) 567.4525, found 567.4533.

5.3.1.30. *N*-(Pyrrolidinyl)-3 β -hydroxy-11-oxo-18 β -olean-12-en-30-oic acid amide (19). 19: mp 191–195 °C; HPLC purity >99% (t_R 1.79 min in 100% MeOH); 1H NMR ($CDCl_3$, 400 MHz) δ 5.68 (s, 1H, olefinic H), 3.25–3.21 (dd, J = 10.5, and 5.4 Hz, 1H, CH–OH), 2.94–2.81 (broad m, 4H), 2.26 (s, 1H, CH), 1.37, 1.23, 1.14, 1.13, 1.01, 0.83 and 0.81 (7 $\times$ s, 3H each, 7 $\times$ CH_3); APCI-MS m/z : 539 (75, MH^+), 580 (100, $M+41^+$); FAB-HRMS calcd for $C_{37}H_{60}N_2O_3$ (MH^+) 539.4212, found 539.4209.

5.3.1.31. *N*-(Pyridin-2-yl-methyl)-3 β -hydroxy-11-oxo-18 β -olean-12-en-30-oic acid amide (21). 21: mp 150–153 °C; HPLC purity >99% (t_R 1.48 min in 100% MeOH); 1H NMR ($CDCl_3$, 400 MHz) δ 8.66–8.67 (d, J = 4.6 Hz, 1H, aromatic), 7.69–7.65 (m, 1H, aromatic), 7.38 (broad t, 1H, NH), 7.25–7.22 (m, 2H, aromatic), 5.89 (s, 1H, olefinic), 4.68–4.47 (m, J = 16.7, 5.0 Hz, 1H, benzylic CH), 4.48–4.43 (m, J = 16.7, 5.0 Hz, 1H, benzylic CH), 3.26–3.22 (dd, J = 10.5, 5.4 Hz, 1H, CH), 2.84–2.79 (dt, J = 13.2, 3.9 Hz, 1H, 1 β -CH), 2.37 (s, 1H, CH), 1.41, 1.19, 1.14, 1.12, 0.88, 0.81 and 0.77 (7 $\times$ s, 3H each, CH_3); APCI-MS m/z : 561 (MH^+); FAB-HRMS calcd for $C_{36}H_{52}N_2O_3$ (MH^+) 561.4056, found 561.4045.

5.3.1.32. *N*-(Pyridin-3-yl-methyl)-3 β -hydroxy-11-oxo-18 β -olean-12-en-30-oic acid amide (22). 22: mp 236–237 °C; HPLC purity >99% (t_R 1.48 min in 100% MeOH); 1H NMR ($CDCl_3$, 400 MHz) δ 8.54–8.52 (m, 2H, aromatic), 7.63–7.61 (d, J = 7.8 Hz, 1H, aromatic), 7.29–7.28 (m, 1H, aromatic), 6.01 (broad t, 1H, NH), 5.59 (s, 1H, olefinic), 4.55–4.47 (m, 2H, benzylic CH_2),

3.23–3.22 (broad m, 1H, CH), 2.79–2.76 (dt, J = 13.2, 3.1 Hz, 1H, 1 β -CH), 2.32 (s, 1H, CH), 1.36, 1.15, 1.13, 1.12 and 1.00 (5 $\times$ s, 3H each, 5 $\times$ CH_3), 0.80 (s, 6H, 2 $\times$ CH_3); APCI-MS m/z : 561 (100, MH^+), 583 (50, $M+Na^+$); FAB-HRMS calcd for $C_{36}H_{52}N_2O_3$ (MH^+) 561.4056, found 561.4035.

5.3.1.33. *N*-(Pyridin-3-yl-methyl)-3 β -hydroxy-11-oxo-18 β -olean-12-en-30-oic acid amide (23). 23: mp 250–251 °C; HPLC purity >99% (t_R 1.48 min in 100% MeOH); 1H NMR ($CDCl_3$, 400 MHz) δ 8.57–8.55 and 7.18–7.17 (2 $\times$ d, J = 5.8 Hz each, 4H, aromatic); 6.03 (broad t, 1H, NH), 5.62 (s, 1H, olefinic), 4.50–4.46 (m, 2H, benzylic CH_2), 3.23 (broad m, 1H, CH), 2.80–2.70 (broad dt, 1H, 1 β -CH), 2.33 (s, 1H, CH), 1.37, 1.19, 1.13, 1.00, 0.88, 0.82 and 0.81 (7 $\times$ s, 3H each, 7 $\times$ CH_3); APCI-MS m/z : 561 (100, MH^+), 583 (30, $M+Na^+$).

5.3.1.34. *N*-(Pyridin-2-yl-ethyl)-3 β -hydroxy-11-oxo-18 β -olean-12-en-30-oic acid amide (24). 24: mp 150–153 °C; HPLC purity >99% (t_R 1.59 min in 100% MeOH); 1H NMR ($CDCl_3$, 400 MHz) δ 8.57–8.56 (m, 1H, aromatic), 7.65–7.61 (ddd, J = 7.8, 1.5 Hz, 1H, aromatic), 7.21–7.19 (m, 2H, aromatic), 7.05 (broad t, 1H, NH), 5.63 (s, 1H, olefinic), 3.67–3.73 (m, 2H, CH_2), 3.21–3.25 (dd, J = 10.9, 5.8 Hz, CH), 3.02–2.99 (t, J = 11.7 Hz, 2H, CH_2), 2.83–2.79 (dt, J = 10.1, 3.5 Hz, 1H, 1 β -CH), 2.34 (s, 1H, CH), 1.37, 1.13, 1.10, 1.07, 1.01, 0.81 and 0.75 (7 $\times$ s, 3H each, CH_3); APCI-MS m/z : 575 (100, MH^+); FAB-HRMS calcd for $C_{37}H_{54}N_2O_3$ (MH^+) 575.4167, found 575.4199.

5.3.1.35. *N*-(6-Methoxypyridin-3-yl-methyl)-3 β -hydroxy-11-oxo-18 β -olean-12-en-30-oic acid amide (25). 25: mp 180–183 °C; HPLC purity >99% (t_R 1.63 min in 100% MeOH); 1H NMR ($CDCl_3$, 400 MHz) δ 8.11–8.10 (s, 1H, aromatic), 7.88–7.85 (d, J = 8.5 Hz, 1H, aromatic), 7.24 (s, 1H, NH), 6.75–6.73 (d, J = 8.5 Hz, 1H, aromatic), 5.67 (s, 1H, olefinic), 3.91 (s, 3H, OCH_3), 3.24–3.04 (broad m, 1H, CH), 2.81–2.76 (dt, J = 13.6, 3.9 Hz, 1H, 1 β -CH), 2.34 (s, 1H, CH), 1.61, 1.39, 1.26, 1.12, 1.01, 0.83 and 0.80 (7 $\times$ s, 3H each, CH_3); APCI-MS m/z : 577 (40, $M-CH_3^+$); FAB-HRMS calcd for $C_{37}H_{54}N_2O_4$ (MH^+) 591.3627, found 591.3606.

5.3.1.36. *N*-(2,6-Dimethoxypyridin-3-yl-methyl)-3 β -hydroxy-11-oxo-18 β -olean-12-en-30-oic acid amide (26). 26: mp 210–215 °C; HPLC purity >99% (t_R 1.63 min in 100% MeOH); 1H NMR ($CDCl_3$, 400 MHz) δ 8.46–8.43 (d, J = 8.5 Hz, 1H, aromatic), 7.68 (broad s, 1H, NH), 6.33–6.32 (d, J = 8.5 Hz, 1H, aromatic), 5.78 (s, 1H, aromatic), 4.08–3.89 (s, 3H, OCH_3), 3.50–3.49 (s, 3H, OCH_3), 3.23–3.21 (broad m, 1H, CH), 2.80–2.37 (dt, J = 13.6, 3.5 Hz, 1H, 1 β -CH), 2.32 (s, 1H, CH), 2.20–2.16 (dd, J = 12.8, 3.5 Hz, 1H, CH), 1.37, 1.26, 1.00, 0.86 and 0.81 (5 $\times$ s, 3H each, CH_3), 1.13 (s, 6H, 2 $\times$ CH_3); FAB-MS m/z : 607 (100, MH^+); FAB-HRMS calcd for $C_{37}H_{54}N_2O_5$ (MH^+) 607.4110, found 607.4090.

5.3.1.37. *N*-(2-Thiazolyl)-3 β -hydroxy-11-oxo-18 β -olean-12-en-30-oic acid amide (27). 27: mp 224–227 °C; HPLC purity >99% (t_R 1.91 min in 100% MeOH); 1H

NMR (CDCl₃, 400 MHz) δ 9.24 (1H, s, NH), 7.36 (d, J = 3.5 Hz, 1H, aromatic), 6.91–6.90 (d, J = 3.5 Hz, 1H, aromatic), 5.67 (s, 1H, olefinic), 3.16–3.13 (broad m, 1H, CH), 2.72–2.69 (dt, J = 13.6, 3.9 Hz, 1H, 1 β -CH), 2.26 (s, 1H, CH), 1.18, 1.06, 1.05, 1.04, 0.94, 0.74 and 0.73 (7 $\times$ s, 3H each, CH₃); APCI-MS m/z 553 (25, MH⁺), 594 (100, M+CH₃CN⁺); FAB-HRMS calcd for C₃₃H₄₈N₂O₃S (MH⁺) 553.3430, found 553.3455.

5.3.1.38. 3 β -Hydroxy-11-oxo-18 β -olean-12-en-30-oic acid methyl ester (34). To a solution of **1** (15 g, 31.9 mmol) in methanol (200 mL) was added *p*-toluenesulfonic acid (2 g). The mixture was refluxed for 72 h, concentrated in vacuo, poured into water, extracted with DCM. The organic phase was washed with 5% sodium bicarbonate and brine, dried over magnesium sulfate and concentrated to yield white solid, which was recrystallized from methanol. White crystals (14 g, 91%) were obtained. Mp 254–258 °C (lit.²⁵ 248–256 °C); ¹H NMR (400 MHz, CDCl₃) δ 5.67 (s, 1H, 12-H), 3.70 (s, 3H, –OCH₃), 3.23 (m, 1H, 3 α -H), 2.80 (dt, J = 13.6, 4.5 Hz, 1H, 1 β -H), 2.37 (s, 1H, 9 α -H), 1.37 (s, 3H, CH₃), 1.15 (s, 3H, CH₃), 1.14 (s, 3H, CH₃), 1.13 (s, 3H, CH₃), 1.01 (s, 3H, CH₃), 0.81 (s, 6H, 2 $\times$ CH₃); FAB-MS m/z : 485 (100, MH⁺).

5.3.1.39. 3 β -Hydroxy-11-oxo-18 β -olean-12-en-30-oic acid ethyl ester (35). **35** was synthesized with general method F. Pale yellow crystals (592 mg; 56%) were obtained. Mp 93–94 °C; FAB-MS m/z : 499 (100, MH⁺); FAB-HRMS calcd for C₃₂H₅₁O₄ (MH⁺) 499.3787, found 499.3786.

5.3.1.40. 3 β -Hydroxy-11-oxo-18 β -olean-12-en-30-oic acid *t*-butyl ester (36). **36** was synthesized with general method F. Yellow crystals (201 mg; 18%) were obtained. Mp 171–174 °C; FAB-MS m/z : 527 (100, MH⁺); FAB-HRMS calcd for C₃₄H₅₅O₄ (MH⁺) 527.4100, found 527.4105.

5.3.1.41. 3 β -Hydroxy-11-oxo-18 β -olean-12-en-30-oic acid cyclohexylmethyl ester (37). **37** was synthesized with general method F. White crystals (703 mg; 59%) were obtained. Mp 98–99 °C; FAB-MS m/z : 567 (100, MH⁺); FAB-HRMS calcd for C₃₇H₅₉O₄ (MH⁺) 567.4413, found 567.4409.

5.3.1.42. 3 β -Hydroxy-11-oxo-18 β -olean-12-en-30-oic acid benzyl ester (38). **38** was synthesized with general method F. White solid (680 mg; 57%) were obtained. Mp 125–126 °C; FAB-MS m/z : 561 (100, MH⁺); FAB-HRMS calcd for C₃₇H₅₃O₄ (MH⁺) 561.3944, found 561.3933.

5.3.1.43. 11-Oxo-18 β -olean-12-ene-3 β ,30-diol (39). **39** was synthesized by the literature²⁸ method. Mp 275–277 °C (lit.²⁸ 274–277 °C); HPLC purity 99% (t_R 7.6 min in 15% water–methanol).

5.3.1.44. 3 β -Tetrahydropyran-2-yloxy-11-oxo-18 β -olean-12-en-30-oic acid methyl ester (45). To a solution of **34** (900 mg, 1.86 mmol) in DCM (10 mL) was added

3,4-dihydro-2H-pyran (250 μ L, 2.74 mmol), followed by *p*-toluenesulfonic acid (0.1 g). The mixture was stirred at rt for 6.5 h, poured into 5% sodium bicarbonate, extracted with DCM. The organic phase was washed with brine, dried over magnesium sulfate and concentrated to afford white solid (1.0 g, 95%). HPLC showed the purity of diastereoisomers 51% and 48%, respectively (t_R 23 and 24 min in 10% water–methanol); ¹H NMR (400 MHz, CDCl₃) δ 5.66 (s, 2H, 2 $\times$ 12-H), 4.73 (broad t, 1H, 2'-H of tetrahydropyranyl), 4.58 (dd, J = 4.7, 3.5 Hz, 1H, 2'-H of tetrahydropyranyl), 3.95 (m, 2H, 6'-H of tetrahydropyranyl), 3.68 (s, 3H, –OCH₃), 3.47 (m, 2H, 6'-H of tetrahydropyranyl), 3.25 (dd, J = 11.7, 4.7 Hz, 1H, 3 α -H), 3.06 (m, 1H, 3 α -H), 2.78 (m, 2H, 1 β -H), 2.33 (s, 2H, 9 α -H), 1.36 (s, 3H, CH₃), 1.35 (s, 3H, CH₃), 1.15 (s, 12H, 4 $\times$ CH₃), 1.12 (s, 6H, 2 $\times$ CH₃), 1.06 (s, 3H, CH₃), 0.92 (s, 3H, CH₃), 0.83 (s, 3H, CH₃), 0.82 (s, 3H, CH₃), 0.80 (s, 6H, 2 $\times$ CH₃); FAB-MS m/z : 569 (80, MH⁺); FAB-HRMS calcd for C₃₆H₅₇O₅ (MH⁺) 569.4206, found 569.4215.

5.3.1.45. 3 β -Tetrahydropyran-2-yloxy-11-oxo-18 β -olean-12-en-30-ol (46). To a solution of **45** (900 mg, 1.58 mmol) in THF (50 mL) was added LiAlH₄ (500 mg, 13 mmol) in portions. The mixture was stirred at 60 °C for 20 h, quenched with saturated NH₄Cl solution and extracted with DCM. The organic phase was washed with brine, dried over magnesium sulfate and concentrated to afford an oily residue, which was dissolved in CHCl₃ (100 mL). MnO₂ (8 g, 92 mmol) was added. The suspension was stirred at rt for 8 h, filtered. Evaporation of the solvent in vacuo gave a brown residue that was purified with flash chromatography (EtOAc–hexane gradient). White crystalline solid (500 mg, 58%) was obtained. HPLC purity 99% (as diastereoisomers, t_R 7.7 min in 4% water–methanol); ¹H NMR showed the diastereoisomers in 2:1 ratio (400 MHz, CDCl₃) δ 5.58 (s, 2H, 2 $\times$ 12-H), 4.73 and 4.58 (m, 2H, 2'-H of tetrahydropyranyl), 3.95 and 3.45 (m, 4H, 6'-H of tetrahydropyranyl), 3.50 (m, 4H, –OCH₂–), 3.22 and 3.05 (m, 2H, 3 α -H), 2.78 (m, 2H, 1 β -H), 2.35 (s, 2H, 9 α -H), 1.36 and 1.35 (s, 6H, 2 $\times$ CH₃), 1.15 (s, 6H, 2 $\times$ CH₃), 1.13 (s, 3H, CH₃), 1.06 (s, 3H, CH₃), 0.93 (s, 12H, 4 $\times$ CH₃), 0.92 (s, 6H, 2 $\times$ CH₃), 0.83 and 0.82 (s, 6H, 2 $\times$ CH₃); FAB-MS m/z : 541 (100, MH⁺); FAB-HRMS calcd for C₃₅H₅₇O₄ (MH⁺) 541.4257, found 541.4279.

5.3.1.46. 3 β ,30-Dihydroxy-18 β -olean-12-en-11-one 30-*m*-methoxybenzyl ether (40). To a solution of **46** (60 mg, 0.11 mmol) in THF (5 mL) was added 3-methoxybenzyl chloride (87 mg, 0.56 mmol), followed by NaH (60% dispersion, 22 mg, 0.56 mmol). The mixture was stirred at 60 °C under nitrogen for 3 h, quenched with water and extracted with DCM. The organic phase was washed with brine, dried over magnesium sulfate and concentrated to afford white solid, which was dissolved in CH₃OH (6 mL). Pyridium *p*-toluenesulfonate (50 mg) was added. The mixture was stirred at 50 °C for 7 h, concentrated in vacuo gave white solid that was purified with flash chromatography (EtOAc–hexane gradient). White powder (28 mg, 44%) was obtained; HPLC purity 99% (t_R 8.1 min in 4% water–methanol);

^1H NMR (400 MHz, CDCl_3) δ 6.92 (broad, 1H, Ar–H), 6.91 (broad, 1H, Ar–H), 6.89 (broad, 1H, Ar–H), 6.82 (dd, $J = 7.5, 2.8$ Hz, 1H, Ar–H), 5.56 (s, 1H, 12-H), 4.49 (s, 2H, $-\text{CH}_2\text{Ph}$), 3.82 (s, 3H, $-\text{OCH}_3$), 3.28 (AB, $J = 25$ Hz, 2H, 30- OCH_2-), 3.23 (m, 1H, 3 α -H), 2.79 (dt, $J = 13.6, 3.6$ Hz, 1H, 1 β -H), 2.34 (s, 1H, 9 α -H), 1.37 (s, 3H, CH_3), 1.14 (s, 3H, CH_3), 1.12 (s, 3H, CH_3), 1.01 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 0.81 (s, 6H, $2\times\text{CH}_3$); FAB-MS m/z : 577 (85, MH^+); FAB-HRMS calcd for $\text{C}_{38}\text{H}_{57}\text{O}_4$ (MH^+) 577.4257, found 577.4275.

5.3.1.47. 3 β ,30-Dihydroxy-18 β -olean-12-en-11-one 30-methyl ether (41). Starting from **46** (95 mg, 0.18 mmol) and CH_3I (50 μL , 0.80 mmol), **41** was synthesized as described for **40**. White crystalline solid (65 mg, 78%) was obtained. Mp 224–227 °C; HPLC purity 99% (t_R 4.3 min in methanol); ^1H NMR (400 MHz, CDCl_3) δ 5.59 (s, 1H, 12-H), 3.34 (s, 3H, $-\text{OCH}_3$), 3.23 (m, 1H, 3 α -H), 3.20 (AB, $J = 25$ Hz, 2H, 30- OCH_2-), 2.79 (dt, $J = 13.7, 3.5$ Hz, 1H, 1 β -H), 2.34 (s, 1H, 9 α -H), 1.37 (s, 3H, CH_3), 1.14 (s, 3H, CH_3), 1.13 (s, 3H, CH_3), 1.01 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 0.81 (s, 3H, CH_3); FAB-MS m/z : 471 (100, MH^+); FAB-HRMS calcd for $\text{C}_{31}\text{H}_{51}\text{O}_3$ (MH^+) 471.3838, found 471.3826.

5.3.1.48. 3 β ,30-Dihydroxy-18 β -olean-12-en-11-one 30-chloroacetyl ester (42). Starting from **46** (60 mg, 0.11 mmol) and chloroacetic acid ethyl ester (60 μL , 0.56 mmol), **42** was synthesized as described for **40**. White solid (30 mg, 52%) was obtained. Mp 178–179 °C; HPLC purity 99% (t_R 5.2 min in 4% water–methanol); ^1H NMR (400 MHz, CDCl_3) δ 5.58 (s, 1H, 12-H), 4.12 (s, 2H, $-\text{CH}_2\text{O}-$), 4.08 (s, 2H, $-\text{CH}_2\text{Cl}-$), 3.23 (m, 1H, 3 α -H), 2.79 (dt, $J = 13.2, 3.4$ Hz, 1H, 1 β -H), 2.33 (s, 1H, 9 α -H), 1.38 (s, 3H, CH_3), 1.14 (s, 3H, CH_3), 1.13 (s, 3H, CH_3), 1.01 (s, 3H, CH_3), 0.96 (s, 3H, CH_3), 0.88 (s, 3H, CH_3), 0.81 (s, 3H, CH_3); FAB-MS m/z : 533 (100, MH^+); FAB-HRMS calcd for $\text{C}_{32}\text{H}_{50}\text{ClO}_4$ (MH^+) 533.3398, found 533.3389.

5.3.1.49. 3 β ,30-Dihydroxy-18 β -olean-12-en-11-one 30-(2-hydroxyethyl) ether (43). To a solution of **46** (100 mg, 0.19 mmol) in toluene (6 mL) was added NaH (60% dispersion, 42 mg, 1.05 mmol). After stirring at 50 °C for 10 min, 2-(2-bromoethoxy)-tetrahydro-pyran (200 μL , 1.32 mmol) was added. The mixture was stirred at 90 °C under nitrogen for 8 h. More NaH (30 mg, 0.75 mmol) and 2-(2-bromoethoxy)-tetrahydro-pyran (100 μL , 0.66 mmol) were added. The solution was stirred at 100 °C for 24 h, quenched with water and extracted with DCM. The organic phase was washed with brine, dried over magnesium sulfate and concentrated in vacuo to afford an oily residue that was dissolved in CH_3OH (10 mL). Pyridium *p*-toluenesulfonate (50 mg) was added to the solution. The mixture was stirred at 50 °C for 7 h, diluted with 5% NaHCO_3 solution, extracted with DCM. The organic phase was washed with brine, dried and concentrated in vacuo gave a yellow residue that was purified with flash chromatography. Off-white solid (37 mg, 39%) was obtained. Mp 208–211 °C; HPLC purity 98% (t_R 3.5 min in 4% water–

methanol); ^1H NMR (400 MHz, CDCl_3) δ 5.57 (s, 1H, 12-H), 3.73 (m, 2H, $-\text{OCH}_2-$), 3.56 (m, 2H, $-\text{CH}_2\text{O}-$), 3.40 (m, 2H, 30- OCH_2-), 3.23 (m, 1H, 3 α -H), 2.79 (dt, $J = 13.6, 3.5$ Hz, 1H, 1 β -H), 2.34 (s, 1H, 9 α -H), 1.38 (s, 3H, CH_3), 1.14 (s, 3H, CH_3), 1.13 (s, 3H, CH_3), 1.01 (s, 3H, CH_3), 0.95 (s, 3H, CH_3), 0.87 (s, 3H, CH_3), 0.81 (s, 3H, CH_3); FAB-MS m/z : 471 (100, MH^+); FAB-HRMS calcd for $\text{C}_{32}\text{H}_{53}\text{O}_4$ (MH^+) 501.3944, found 501.3928.

The following compounds (**47–51**, **55–57**) were synthesized with general method D.

5.3.1.50. 3 β -Methoxy-18 β -olean-12-en-11-one-30-oic acid methyl ester (47). **47**: mp ≥ 300 °C; HPLC purity >95% (t_R 5.63 min in 100% MeOH); ^1H NMR (CDCl_3 , 400 MHz) δ 5.67 (s, 1H, olefinic), 3.69 (s, 3H, CH_3), 3.36 (s, 3H, CH_3), 2.95–2.81 (dt, $J = 13.2, 3.1$ Hz, 1H, 1 β -CH), 2.70–2.66 (dd, $J = 11.7, 4.6$ Hz, 1H, CH), 2.33 (s, 1H, CH), 2.09–1.66 (m, 6H), 1.36, 1.15, 1.14, 1.12, 0.99, 0.81 and 0.79 (7 $\times$ s, 3H each, CH_3); FAB-MS m/z : 499 (100, MH^+); FAB-HRMS calcd for $\text{C}_{32}\text{H}_{50}\text{O}_2$ (MH^+) 499.3787, found 499.3801.

5.3.1.51. 3 β -Ethoxy-18 β -olean-12-en-11-one-30-oic acid methyl ester (48). **48**: HPLC purity >90% (t_R 2.59 min in 100% MeOH); ^1H NMR (CDCl_3 , 400 MHz) δ 5.66 (s, 1H, olefinic), 4.47–4.17 (m, 2H, CH_2), 3.68 (s, 3H, CH_3), 2.84–2.81 (dt, $J = 13.6, 3.5$ Hz, 1H, 1 β -CH), 2.35 (s, 1H, CH), 2.09–2.01 (m, 1H, 18 β -CH), 1.36, 1.157, 1.150 and 1.12, 0.95, 0.90 and 0.80 (7 $\times$ s, 3H each, CH_3); FAB-MS m/z : 513 (30, MH^+), 557 (80, $\text{M}+\text{CH}_3\text{CN}^+$); FAB-HRMS calcd for $\text{C}_{33}\text{H}_{52}\text{O}_4$ (MH^+) 513.3943, found 513.3941.

5.3.1.52. 3 β -Isobutyloxy-18 β -olean-12-en-11-one-30-oic acid methyl ester (49). **49**: mp 298–300 °C; HPLC purity >99% (t_R 4.97 min in 100% MeOH); ^1H NMR (CDCl_3 , 400 MHz) δ 5.59 (s, 1H, olefinic), 3.61 (s, 3H, CH_3), 3.35–3.32 (dd, $J = 8.9, 6.2$ Hz, CH), 2.90–2.86 (dd, $J = 8.9, 6.2$ Hz, CH), 2.75–2.69 (dt, $J = 13.2, 3.5$ Hz, 1H, 1 β -CH), 2.68–2.64 (dd, $J = 12.1, 4.6$ Hz, 1H, CH), 2.25 (s, 1H, CH), 1.48 (s, 6H, $2\times\text{CH}_3$), 1.29, 1.071, 1.05, 0.91 (4 $\times$ s, 3H each, CH_3), 0.86–0.84 (d, $J = 5.8$ Hz, 3H, CH_3), 0.82–0.80 (d, $J = 6.6$ Hz, 3H, CH_3), 0.73 (s, 6H, $2\times\text{CH}_3$); FAB-MS m/z : 527 (100, MH^+); FAB-HRMS calcd for $\text{C}_{37}\text{H}_{52}\text{O}_4$ (MH^+) 527.3736, found 527.3734.

5.3.1.53. 3 β -(2-Methoxyethoxy)-18 β -olean-12-en-11-one-30-oic acid methyl ester (50). **50**: HPLC purity >90% (t_R 4.82 min in 100% MeOH); ^1H NMR (CDCl_3 , 400 MHz) δ 5.66 (s, 1H, olefinic), 4.23–4.09 (m, 1H, CH), 3.78–3.73 (m, 1H, CH), 3.69 (s, 3H, OCH_3), 3.58–3.44 (m, 2H, CH_2), 3.38 (s, 3H, OCH_3), 2.83–2.78 (m, 2H, $2\times\text{CH}$), 2.33 (s, 1H, CH), 1.36, 1.15, 1.14, 1.12, 1.00, 0.81 and 0.80 (7 $\times$ s, 3H each, CH_3); FAB-MS m/z : 543 (90, MH^+); FAB-HRMS calcd for $\text{C}_{34}\text{H}_{54}\text{O}_5$ (MH^+) 543.4049, found 543.4035.

5.3.1.54. 3 β -(2-Hydroxyethoxy)-18 β -olean-12-en-11-one-30-oic acid methyl ester (51). **51**: mp ≥ 300 °C; HPLC purity >99% (t_R 4.82 min in 100% MeOH); ^1H

NMR (CDCl₃, 400 MHz) δ 5.66 (s, 1H, olefinic), 3.74–3.70 (broad m, 2H, CH₂), 3.69 (s, 3H, OCH₃), 3.43–3.38 (m, 2H, CH₂), 2.87–2.80 (m, 2H, 2 $\times$ CH), 2.33 (s, 3H, CH), 1.61, 1.36, 1.15, 1.12, 1.00, 0.81 and 0.80 (7 $\times$ s, 3H each, CH₃); FAB-MS m/z : 529 (55, MH⁺), 467 (30, [M-61]⁺); FAB-HRMS calcd for C₃₃H₅₂O₅ (MH⁺) 529.3893, found 529.3873.

5.3.1.55. 3 β -(4-*t*-Butylbenzyloxy)-18 β -olean-12-en-11-one-30-oic acid methyl ester (55). 55: mp 234–236 °C; HPLC purity >95% (t_R 4.97 min in 100% MeOH); ¹H NMR (CDCl₃, 400 MHz) δ 7.41–7.28 (2 $\times$ d, J = 8.5 Hz each), 5.66 (s, 1H, olefinic), 4.66–4.63 (d, 1H, J = 11.7 Hz, benzylic CH), 4.40–4.37 (d, 1H, J = 11.7 Hz, benzylic CH) 3.69 (s, 3H, CH₃), 2.96–2.92 (dd, J = 11.3, 4.2 Hz, 1H, CH), 2.84–2.80 (dt, J = 13.2, 3.5 Hz, 1H, 1 β -CH), 2.33 (s, 1H, CH), 2.09–2.08 (m, 18 β -CH), 1.315 (s, 9H, *tert*-butyl), 1.360, 1.154, 1.150, 1.12, 1.01, 0.86 and 0.80 (7 $\times$ s, 3H each, CH₃); FAB-MS m/z : 631 (40, MH⁺); FAB-HRMS calcd for C₄₂H₆₂O₄ (MH⁺) 632.4759, found 632.4771.

5.3.1.56. 3 β -(3-Methoxybenzyloxy)-18 β -olean-12-en-11-one-30-oic acid methyl ester (56). 56: mp $\geq$ 300 °C; HPLC purity >99% (t_R 5.03 min in 100% MeOH); ¹H NMR (CDCl₃, 400 MHz) δ 7.24–7.22 (m, 1H, aromatic), 6.94 (appd, 1H, aromatic), 6.92 (s, 1H, aromatic), 6.82–6.80 (m, 1H, aromatic), 5.66 (s, 1H, olefinic), 4.68–4.65 (d, J = 12.1 Hz, 1H, benzylic CH), 4.42–4.39 (d, J = 12.1, 1H, benzylic CH), 3.81 (s, 3H, CH₃), 3.69 (s, 3H, CH₃), 2.96–2.92 (dd, J = 11.7, 4.2 Hz, 1H, CH), 2.84–2.80 (dt, J = 13.2, 3.1 Hz, 1H, 1 β -CH), 2.33 (s, 1H, CH), 1.36, 1.16, 1.15, 1.13, 1.01, 0.87 and 0.81 (7 $\times$ s, 3H each, CH₃), FAB-MS m/z : 605 (100, MH⁺); FAB-HRMS calcd for C₃₉H₅₆O₅ (MH⁺) 605.4222, found 605.4206.

5.3.1.57. 3 β -Benzyloxy-18 β -olean-12-en 11-one 30-oic acid methyl ester (57). 57: mp 302–304 °C; HPLC purity >95% (t_R 4.97 min in 100% MeOH); ¹H NMR (CDCl₃, 400 MHz) δ 7.36–7.30 (m, 5H, aromatic), 5.66 (s, 1H, olefinic), 4.70–4.67 (d, J = 12.1 Hz, 1H, benzylic CH), 4.42–4.39 (d, J = 11.7 Hz, 1H, benzylic CH), 3.69 (s, 3H, CH₃), 2.95–2.91 (dd, J = 11.7, 4.2 Hz, 1H, CH), 2.84–2.80 (dt, J = 13.6, 3.5 Hz, 1H, 1 β -CH), 2.32 (s, 1H, CH), 2.09–1.66 (m, 6H), 1.35, 1.15, 1.14, 1.12, 1.00, 0.86 and 0.80 (7 $\times$ s, 3H each, CH₃); FAB-MS m/z : 575 (30, MH⁺); FAB-HRMS calcd for C₃₇H₅₂O₄ (MH⁺) 575.4055, found 575.4106.

The following compounds (52–53) were synthesized with general method E.

5.3.1.58. 3 β -Methyloxy-18 β -olean-12-en 11-one 30-oic acid (52). 52: mp $\geq$ 300 °C; HPLC purity >95% (t_R 5.63 min in 100% MeOH); ¹H NMR (CDCl₃, 400 MHz) δ 5.62 (s, 1H, olefinic), 3.29 (s, 3H, CH₃), 2.95–2.81 (dt, J = 13.2, 3.5 Hz, 1H, 1 β -CH), 2.70–2.66 (dd, J = 11.7, 4.2 Hz, 1H, CH), 2.26 (s, 1H, CH), 2.09–1.66 (m, 6H), 1.29, 1.15, 1.07, 1.05, 0.91, 0.76 and 0.71 (7 $\times$ s, 3H each, CH₃), FAB-MS 485 (100, MH⁺).

5.3.1.59. 3 β -Ethyloxy-18 β -olean-12-en 11-one 30-oic acid (53). 53: mp 284 °C (dec.); HPLC purity >95% (t_R 2.59 min in 100% MeOH); ¹H NMR (CDCl₃) δ 5.70 (s, 1H, olefinic), 3.70–3.64 (m, 1H, CH), 3.38–3.31 (m, 1H, CH), 2.82–2.75 (m, 2H, 2 $\times$ CH), 2.34 (s, 1H, CH), 2.19–2.03 (m, 1H, 18 β -CH), 1.37, 1.23, 1.14, 1.13, 0.98, 0.84 and 0.80 (7 $\times$ s, 3H each, CH₃), 1.19–1.65 (t, J = 13.6, 3H, CH₃); FAB-MS m/z : 499 (80, MH⁺), 453 (40, M-45)⁺, FAB-HRMS calcd for C₃₂H₅₀O₄ (MH⁺) 499.3787, found 499.3772.

5.3.1.60. 3 β -Isobutyloxy-18 β -olean-12-en-11-one-30-oic acid (54). 54: mp 238 °C (dec.); HPLC purity >95% (t_R 5.14 min in 100% MeOH); ¹H NMR (CDCl₃, 400 MHz) δ 5.70 (s, 1H, olefinic), 3.43–3.39 (dd, J = 8.9, 6.2 Hz, 1H, CH), 2.98–2.94 (dd, J = 8.9, 7.4 Hz, 1H), 2.81–2.75 (dt, J = 13.7, 3.4 Hz, 1H, 1 β -CH), 2.75–2.71 (dd, J = 12.1, 4.6 Hz, 1H, CH), 2.33 (s, 1H, CH), 1.37, 1.23, 1.14, 1.13, 0.99, 0.83, 0.81 (7 $\times$ s, 3H each, CH₃), 0.93–0.88 (2 $\times$ d, J = 6.6 Hz each, 2 $\times$ CH₃); FAB-MS m/z : 527 (100, MH⁺); FAB-HRMS calcd for C₃₄H₅₄O₄ [MH⁺] 527.4100, found 527.4090.

5.3.1.61. 3,11-Dioxo-18 β -olean-12-en-30-oic acid (59). Jones reagent (1 mL) was added to a solution of **1** (100 mg; 0.21 mmol) in acetone (5 mL). The reaction mixture was stirred for 0.5 h at 0 °C, poured into ice-water and extracted with CHCl₃ (50 mL). The organic phase was washed with water (3 $\times$ 50 mL), dried (MgSO₄) and evaporated to get a white solid (108 mg). The crude product was purified by recrystallization from hot absolute ethanol to give **59** as white crystals (82 mg; 82%). Mp 302–303 °C (lit.²³ mp 298–302 °C); FAB-MS m/z : 469 (100, MH⁺); FAB-HRMS calcd for C₃₀H₄₅O₄ (MH⁺) 469.3318, found 469.3322.

5.3.1.62. 3,11-Dioxo-18 β -olean-12-en-30-oic acid methyl ester (60). Starting from **34**, **60** was synthesized as described for the preparation of **59**. White crystals (59 mg; 59%) was obtained. Mp 249–250 °C (lit.³⁰ mp 254–256 °C); FAB-MS m/z : 483 (100, MH⁺); FAB-HRMS calcd for C₃₁H₄₇O₄ (MH⁺) 483.3474, found 483.3472.

5.3.1.63. 3 β ,30-Dihydroxy-18 β -olean-12-en-11-one 30-methyl ether 3 β -succinic semiester (61). Starting from **41** (25 mg, 0.05 mmol) and succinic anhydride (27 mg, 0.27 mmol), **61** was synthesized as described for **33**. Off-white solid (17 mg, 60%) was obtained. Mp 237–239 °C; HPLC purity 99% (t_R 2.5 min in 2% water-methanol); ¹H NMR (400 MHz, CDCl₃) δ 5.58 (s, 1H, 12-H), 4.55 (dd, J = 11.7, 4.7 Hz, 1H, 3 α -H), 3.33 (s, 3H, -OCH₃), 3.22 (AB, J = 25 Hz, 2H, 30-OCH₂-), 2.78 (dt, J = 13.3, 3.5 Hz, 1H, 1 β -H), 2.62–2.72 (m, 4H, -COCH₂CH₂CO-), 2.35 (s, 1H, 9 α -H), 1.36 (s, 3H, CH₃), 1.16 (s, 3H, CH₃), 1.13 (s, 6H, 2 $\times$ CH₃), 0.92 (s, 3H, CH₃), 0.88 (s, 6H, 2 $\times$ CH₃), 0.86 (s, 3H, CH₃); FAB-MS m/z : 571 (100, MH⁺); FAB-HRMS calcd for C₃₅H₅₅O₆ (MH⁺) 571.3999, found 571.3999.

5.3.1.64. 18 β -Olean-12-ene-3 β ,30-diol (64). **64** was synthesized with literature²⁹ method. Mp 245–247

°C (lit.²⁹ 250–251 °C, lit.²⁸ 246–250 °C); ¹H NMR (400 MHz, CDCl₃) δ 5.18 (t, 1H, *J* = 3.9 Hz, 12-H), 3.52 (m, 2H, 30-CH₂–), 3.22 (dd, *J* = 11.3, 5.9 Hz, 1H, 3α-H), 1.15 (s, 3H, CH₃), 1.00 (s, 3H, CH₃), 0.96 (s, 3H, CH₃), 0.94 (s, 3H, CH₃), 0.90 (s, 3H, CH₃), 0.83 (s, 3H, CH₃), 0.81 (s, 3H, CH₃).

5.3.1.65. 3β,30-Dihydroxy-30,30-dimethyl-18β-olean-12-en-11-one (44), 3β, 11β-dihydroxy-11α-methyl-18β-olean-12-en-30-oic acid methyl ester (65), 3β-hydroxy-11-methylene-18β-olean-12-en-30-oic acid methyl ester (66). To a solution of **34** (2.37 g, 4.90 mmol) in THF (25 mL) was added CH₃Li–ether solution (1.6 M, 6.2 mL, 9.92 mmol) dropwise at –78 °C under nitrogen. The mixture was stirred at –78 °C for 30 min, and then brought to rt gradually. After stirring at rt for 1 h, the reaction was quenched with 1% HCl and extracted with DCM. The organic phase was washed with 5% NaHCO₃ solution, brine, dried over magnesium sulfate and concentrated to give a white solid that was subjected to flash chromatography (EtOAc–hexane gradient elution). Starting material **34** (820 mg) was recovered. **44** was obtained as white crystals (130 mg, 8%). Mp 241–246 °C; HPLC purity 99% (*t*_R 2.4 min in methanol); ¹H NMR (400 MHz, CDCl₃) δ 5.59 (s, 1H, 12-H), 3.23 (m, 1H, 3α-H), 2.70 (dt, *J* = 13.7, 3.5 Hz, 1H, 1β-H), 2.27 (s, 1H, 9α-H), 1.35 (s, 3H, CH₃), 1.22 (s, 3H, CH₃), 1.21 (s, 3H, CH₃), 1.20 (s, 3H, CH₃), 1.14 (s, 3H, CH₃), 1.00 (s, 3H, CH₃), 0.95 (s, 3H, CH₃), 0.81 (s, 3H, CH₃), 0.68 (s, H, CH₃); FAB-MS *m/z*: 500 (15, M⁺), 483 (MH⁺–H₂O, 100); FAB-HRMS calcd for C₃₂H₅₃O₃ (MH⁺) 485.3955, found 485.4000.

65 was obtained as white crystals (455 mg, 28%). Mp 251–257 °C; HPLC purity 91% (*t*_R 2.6 min in methanol); ¹H NMR (400 MHz, CDCl₃) δ 5.07 (s, 1H, 12-H), 3.70 (s, 3H, –OCH₃), 3.25 (broad, 1H, 3α-H), 2.20 (dt, *J* = 12.5, 3.5 Hz, 1H, 1β-H), 1.44 (s, 3H, CH₃), 1.35 (s, 3H, CH₃), 1.16 (s, 3H, CH₃), 1.14 (s, 3H, CH₃), 1.06 (s, 3H, CH₃), 1.00 (s, 3H, CH₃), 0.82 (s, 6H, 2×CH₃); FAB-MS *m/z*: 500 (15, M⁺), 483 (MH⁺–H₂O, 100); FAB-HRMS calcd for C₃₂H₅₃O₄ (MH⁺) 501.3944, found 501.3905.

66 was obtained as white crystals (350 mg, 22%). Mp 168–170 °C; HPLC purity 99% (*t*_R 4.2 min in methanol); ¹H NMR (270 MHz, CDCl₃) δ 5.67 (s, 1H, 12-H), 5.03 and 4.95 (s, 2H, 11-CH₂), 3.68 (s, 3H, –OCH₃), 3.24 (m, 1H, 3α-H), 2.45 (dt, *J* = 13.3, 3.5 Hz, 1H, 1β-H), 2.35 (s, 1H, 9α-H), 1.18 (s, 3H, CH₃), 1.17 (s, 3H, CH₃), 1.11 (s, 3H, CH₃), 0.99 (s, 3H, CH₃), 0.92 (s, 3H, CH₃), 0.80 (s, 3H, CH₃), 0.75 (s, 3H, CH₃); FAB-MS *m/z*: 483 (100, MH⁺); FAB-HRMS calcd for C₃₂H₅₁O₃ (MH⁺) 483.3838, found 483.3838.

5.3.1.66. 3β, 11β-Dihydroxy-11α-methyl-18β-olean-12-en-30-oic acid (67). To a solution of **65** (100 mg, 0.2 mmol) in methanol–THF–water (5:2:1 mL) was added KOH (88 mg, 1.56 mmol). The mixture was stirred under nitrogen at 70 °C for 48 h, poured into 2% HCl, extracted with DCM. The organic phase was washed with brine, dried over magnesium sulfate and concentrated in vacuo to give a white solid that was recrystallized from CH₃OH–DCM. White solid (80 mg, 82%)

was obtained. Mp 287–290 °C; HPLC purity 95% (*t*_R 2.6 min in methanol); ¹H NMR (400 MHz, CDCl₃) δ 5.10 (s, 1H, 12-H), 3.23 (m, 1H, 3α-H), 2.20 (dt, *J* = 12.5, 3.5 Hz, 1H, 1β-H), 1.43 (s, 3H, CH₃), 1.35 (s, 3H, CH₃), 1.22 (s, 3H, CH₃), 1.16 (s, 3H, CH₃), 1.07 (s, 3H, CH₃), 1.00 (s, 3H, CH₃), 0.86 (s, 3H, CH₃), 0.83 (s, 3H, CH₃); FAB-MS *m/z*: 486 (10, M⁺), 469 (MH⁺–H₂O, 100); FAB-HRMS calcd for C₃₁H₅₁O₄ (MH⁺) 487.3787, found 487.3730.

5.3.1.67. 3β-Hydroxy-11-methylene-18β-olean-12-en-30-oic acid (68). Method 1: Starting from **66** (150 mg, 0.31 mmol), **68** was synthesized as described for the preparation of **67**. White crystals (122 mg, 84%) were obtained. Mp 295–296 °C; HPLC purity 99% (*t*_R 3.1 min in methanol); ¹H NMR (400 MHz, CDCl₃) δ 5.72 (s, 1H, 12-H), 5.06 and 4.98 (s, 2H, 11-CH₂), 3.27 (m, 1H, 3α-H), 2.50 (dt, *J* = 13.7, 3.5 Hz, 1H, 1β-H), 2.31 (s, 1H, 9α-H), 1.23 (s, 3H, CH₃), 1.22 (s, 3H, CH₃), 1.21 (s, 3H, CH₃), 1.02 (s, 3H, CH₃), 0.96 (s, 3H, CH₃), 0.83 (s, 3H, CH₃), 0.82 (s, 3H, CH₃); FAB-MS *m/z*: 469 (100, MH⁺); FAB-HRMS calcd for C₃₁H₄₉O₃ (MH⁺) 469.3682, found 469.3629.

Method 2: To a solution of **1** (0.8 g, 1.7 mmol) in THF (50 mL) was added CH₃Li–ether solution (1.6 M, 6.2 mL, 9.92 mmol) dropwise at rt under nitrogen. The mixture was stirred at rt for 1 h, and then quenched with 1% HCl and extracted with ethyl acetate. The organic phase was washed with 5% NaHCO₃, brine, dried over magnesium sulfate and concentrated to give a white solid that was re-crystallized from CH₃OH–DCM. White crystals (760 mg, 95%) were obtained. Mp 293–296 °C; ¹H NMR was identical with the compound from method 1.

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