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β-11-Keto-boswellic acid derived amides: synthesis and cytotoxicity

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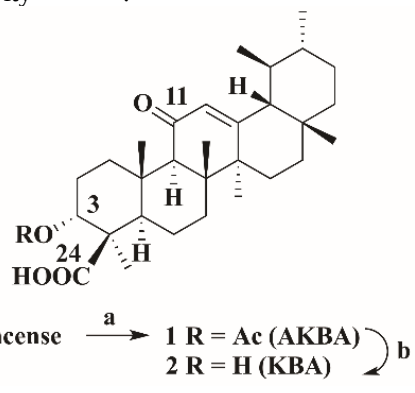
Abstract: The aim of this study was to prepare 11-keto-β-boswellic acid derivatives modified at C-24 and to evaluate their *in vitro* cytotoxicity. Acetyl-11-keto-β-boswellic acid (AKBA) was isolated from frankincense and transformed into 11-keto−β-boswellic acid (KBA). Both compounds served as starting materials for the synthesis of several amides or hydrazides. The derivatives were fully characterized, and their cytotoxicity was evaluated *in vitro* using sulforhodamine B (SRB) assays employing two human tumor cell lines (A2780 and MCF7) as well as nonmalignant mouse fibroblasts (NIH 3T3). Nearly all of the compounds were more cytotoxic than their parent compounds. The highest cytotoxicity was observed for (3α, 4β) 3-acetyloxy-N-(3-aminopropyl)-11-oxo-urs-12-en-24-amide (15) and (3α, 4β) 3-acetyloxy-N-[4-(3-aminopropyl)piperazin-1-yl]-propyl-11-oxo-urs-12-en-24-amide (16) and the ovarian carcinoma cell line A2780. These compounds showed EC_{50} = 1.0-1.7 μM while being significantly less toxic for the mouse fibroblasts NIH 3T3 (EC_{50} = 9.3-16.3 μM). Thus, compounds 15 and 16 have good antitumor effects and may serve as starting points for developing potential and selective antitumor agents.

Keywords: Frankincense; Boswellic acids; Cytotoxicity.

Introduction

Frankincense was one of the three gifts from the Magi (three Kings) [1, 2] but its use is much older. One of the oldest records of its application is found in an inscription on the tomb of the Egyptian queen Hatshepsut's dated 15th century BCE. The usage of frankincense is heavily associated with cults and (religious) rituals: It was eaten, extracts were drunk, it was burned but also used in the process of mummification [3]. In those days, it was one of the costliest substances of the ancient world and valuable as gold.

Frankincense is a resin obtained from Boswellia trees. This oleo-gum-like resin contains compounds of many different structures and a broad variety of activities have been reported or at least assumed to be associated with frankincense [4, 5]. β-Boswellic acids like 3-*O*-acetyl-11-keto-β-boswellic acid (AKBA, 1, Scheme 1) and 11-keto-β-boswellic acid (KBA, 2) are those constituents of frankincense that gained much attraction during the last decades because of their interesting pharmaceutical properties [3, 6-9]. Several studies revealed these pentacyclic triterpenoids to have antitumor activity [10-12]. While the cytotoxicity of 1 or 2 is low, derivatives of AKBA or KBA showed significant higher activity [5, 11, 13-20].



Scheme 1. Structure of AKBA (1) and KBA (2); a) isolation from frankincense [21];
b) NaOH, EtOH, 25 °C, 12 h, 98%.[20]

In continuity of our search for natural product derived antitumor active compounds, we prepared several derivatives of 1 and 2 and screened them in

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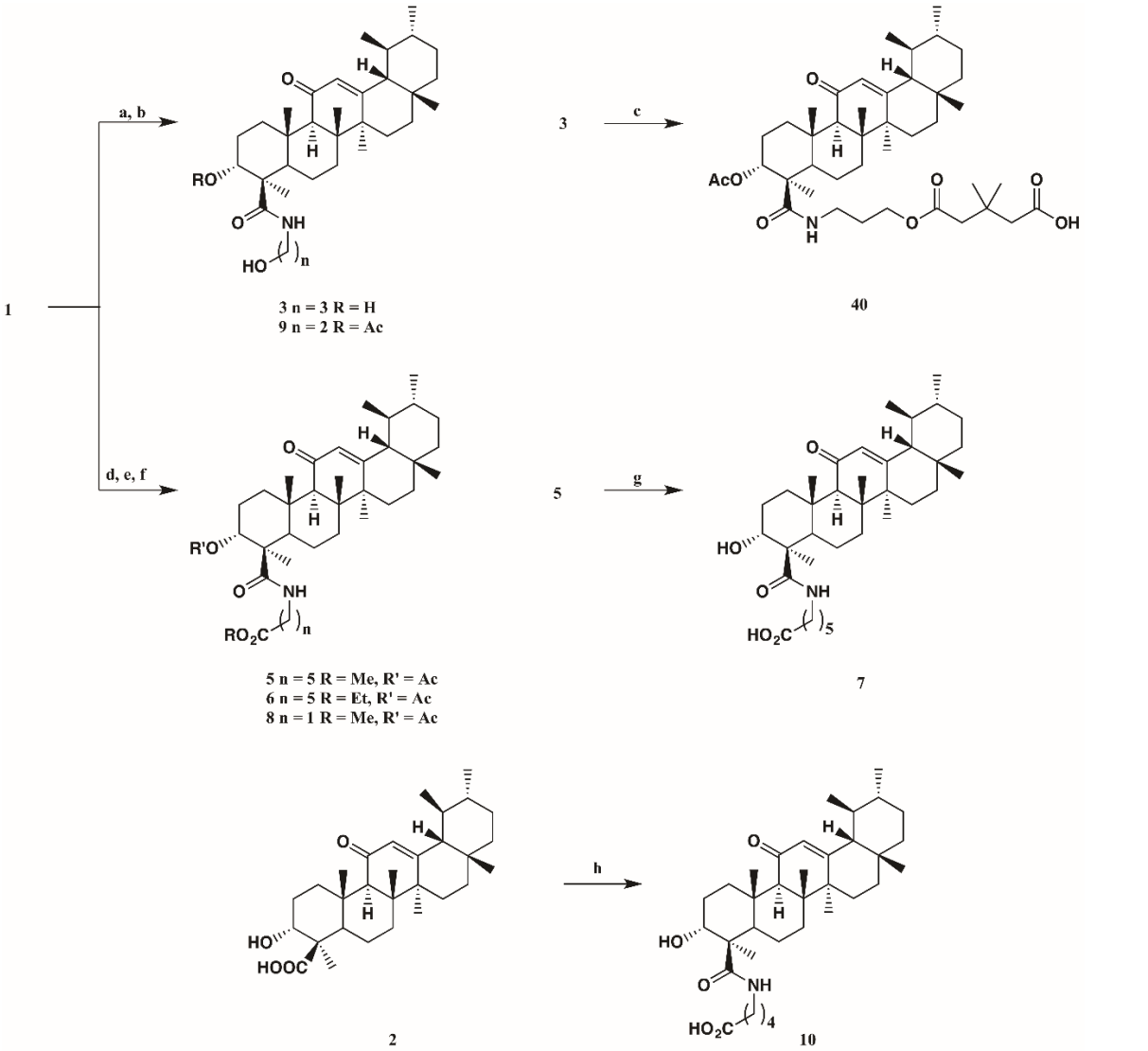
sulforhodamine B assays for their cytotoxicity employing several human tumor cell lines.

Results and discussion

The starting material for this investigation, AKBA (1, Scheme 1) was extracted from frankincense applying a modification of Jauch's procedure [21]. Deacetylation of 1 with an aqueous solution of sodium hydroxide in ethanol for 12 h, gave KBA (2) in almost quantitative yield [20]. For both compounds, a reduced bioavailability can be expected because of a reduced solubility in water and unfavorable lipophilicity (cf. log P of 1 10.27 ± 0.40; log P of 2: 9.38 ± 0.39). Therefore, several amides of 2 were prepared; esters might be easily cleaved under physiological conditions by (un)-enzymatic hydrolysis.

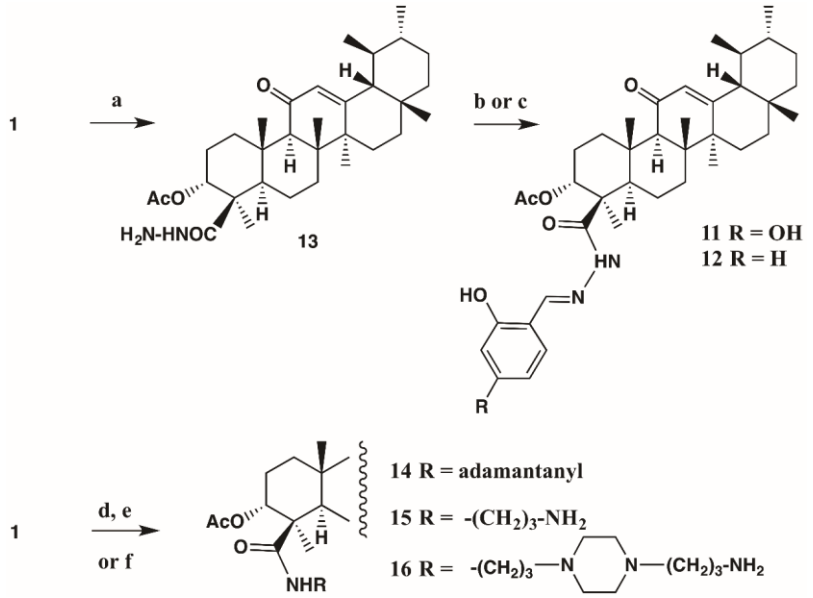
Thus, treatment of AKBA (Scheme 2) with thionyl chloride and reaction of the intermediate boswellic acid chloride with 3-aminopropanol/*n*-BuLi gave the corresponding *N*-(3-hydroxypropyl) derivative 3 which is also deacetylated under these conditions; treatment of KBA under the same conditions led to significant lower yields of 3.



Scheme 2. Synthesis of amides 3-10: a) $SOCl_2$, Δ, 3 h, then HO-$(CH_2)_3NH_2$, *n*-BuLi, THF, 25 °C, 30 min, 86%; b) $SOCl_2$, Δ, 3 h, then H_2N-$(CH_2)_2ONa$, DCM, 25 °C, 12 h, 67%; c) 3,3-dimethylglutaric anhydride, pyridine, 50 °C, 5 d, 64%; d) $(COCl)_2$, DCM, 25 °C, 5 d, then H_2N-$(CH_2)_5CO_2Me$.HCl, NEt_3, 5 °C, 2 d, 54%; e) $SOCl_2$, Δ, 3 h, then H_2N-$(CH_2)_5$-CO_2Et . HCl, pyridine, 25 °C, 12 h, 46%; f) $(COCl)_2$, 3 d, 25 °C, then NH_2-CH_2-CO_2Me.HCl, NEt_3, DCM, 25 °C, 12 h, 68%; g) THF, aq. NaOH, 25 °C, 12 h, 83%, h) $SOCl_2$, Δ, 3 h, then H_2N-$(CH_2)_4$-OH, pyridine, 25 °C, 12 h, 78%.

Reaction of 3 with 3,3-dimethylglutaric anhydride furnished 4. This reaction went sluggish and only 64% of the product 4 were isolated after chromatography. From the reaction of AKBA/oxalyl chloride and methyl aminocaproate or ethyl aminocaproate 5 or 6 were obtained, respectively. Deacetylation of the former afforded 7. Activation of AKBA with oxalyl chloride followed by the addition of methyl glycinate gave 8; the corresponding *N*-(2-hydroxyethyl) analogue 9 was obtained from AKBA/thionyl chloride and 2-aminoethanolate while a chain elongated *N*-(4-hydroxybutyl)-amide 10 was prepared from KBA/thionyl chloride and 4-aminobutanolate in 78% isolated yield.

Hydrazides and hydrazones are potential substrates for drug design, and – among other activities – several of them showing antitumor activity have been described so far.[22-26] The hydrazides 11 and 12 (Scheme 3) were easily accessed from the reaction of the hydrazide 13 [27] with 2,4-dihydroxybenzaldehyde or salicylaldehyde in refluxing ethanol, respectively.



Scheme 3. Synthesis of AKBA-derived derivatives 11-16: a) $SOCl_2$, Δ, 3 h, then H_2N-NH_2.H_2O, THF, 25 °C, 1 h, 88%; b) 2,4-dihydroxybenzaldehyde, EtOH, Δ, 3 h, 80%; c) salicylaldehyde, EtOH, Δ, 3 h, 58%; d) $SOCl_2$, Δ, 3 h, then 1-aminoadamantane.HCl, NEt_3, 25 °C, 12 h, 58%; e) $SOCl_2$, Δ, 3 h, then H_2N-$(CH_2)_3$-NH_2, THF, pyridine, 25 °C, 12 h, 88%; f) $SOCl_2$, Δ, 3 h, then H_2N-$(CH_2)_3$-N-[$(CH_2)_2$]$_2$-N-$(CH_2)_3$-NH_2, THF, pyridine, 25 °C, 12 h, 78%.

The starting material of this reaction, hydrazide 13 was obtained from the reaction of AKBA with thionyl chloride followed by adding hydrazine hydrate in 88% yield. Hydrazides have only hardly been prepared from triterpenoids and their potential remains to be explored in more detail. The configuration of the hydrazides 11 and 12 was determined from their NOESY spectra. Extra MOPAC/PM3 (closed shell) calculations were performed. These calculations were in good agreement with the NOESY experiments showing the (*E*) isomer of lower energy than the corresponding (*Z*) isomer. The NOESY spectra revealed, for example, cross peaks between 33-H (N-N=CH-) and the *ortho* hydrogen of the aromatic system. As a result, the absolute configuration of the hydrazides was established as (*E*).

Yields dropped for the reaction of AKBA/thionyl chloride and 1-aminoadamantane, and 14 was obtained after chromatographic work-up in moderate 58% yield. Compound 14 is characterized in its ^{1}H NMR spectrum by the presence of the signals for the boswellic acid skeleton and, in addition, the signals of the adamantanyl moiety. Thus, the methylene groups were detected at δ = 1.96 and 1.64 ppm, while the methine protons were observed at δ = 2.01 ppm, respectively.

Finally, to explore the influence of additional amino groups, compounds 15 and 16 were prepared from AKBA by its reaction with thionyl chloride and 1,3-diaminopropane (→ 15, 88%) or 1,4-bis (3-aminopropyl)piperazine (→ 16, 78%).

The cytotoxicity of the compounds was determined in sulforhodamine B assays (SRB), and the results from these assays are compiled in Table 1. As a result, most of the esters and amides gave only moderate EC_{50} values while hydroxyalkyl substituents improved the cytotoxicity of the compounds. The hydrazide 13 (log P = 8.02 ± 0.38) showed a low EC_{50} value being comparable with the

values obtained for the substituted hydrazides. No EC_{50} values could be determined for adamantanyl-substituted 14 due to its low solubility. The best results, however, were obtained for amino substituted compounds 15 (log P = 8.26 ± 0.48) and 16 (log P = 7.90 ± 0.59), and EC_{50} values as low as 1.0 μM were observed for 16 and A 2780 human ovarian carcinoma cells.

Table 1. Cytotoxicity of selected compounds (EC_{50} values in μM from SRB assays after 96 hours of treatment; the values are averaged from three independent experiments performed each in triplicate; confidence interval CI = 95 %, cut-off of the assay 30 μM; n.d. not detected); standard: betulinic acid). Human cancer cell lines: A2780 (ovarian carcinoma), MCF7 (breast adenocarcinoma), and nonmalignant mouse fibroblasts NIH 3T3; betulinic acid (BA) was used as a standard.

Compound	A 2780	MCF7	NIH 3T3
1	21.1 ± 1.9	24.3 ± 1.7	> 30
2	> 30	> 30	> 30
3	5.9 ± 0.9	4.5 ± 0.7	13.3 ± 1.6
4	> 30	17.4 ± 2.6	> 30
5	11.4 ± 1.2	17.2 ± 1.9	25.3 ± 1.1
6	14.1 ± 1.7	19.3 ± 1.6	22.2 ± 1.2
7	25.1 ± 1.9	29.0 ± 3.0	> 30
8	19.4 ± 1.8	17.7 ± 1.72	24.4 ± 2.0
9	18.2 ± 1.7	> 30	> 30
10	13.7 ± 2.4	18.4 ± 1.3	27.3 ± 3.0
11	3.4 ± 1.4	7.1 ± 1.7	16.3 ± 1.1
12	5.3 ± 0.7	8.9 ± 1.4	11.4 ± 1.6
13	3.8 ± 0.7	5.2 ± 1.1	11.7 ± 0.8
14	n.d.	n.d.	n.d.
15	1.7 ± 0.6	1.9 ± 0.4	16.3 ± 1.7
16	1.0 ± 0.2	3.8 ± 0.2	9.3 ± 1.1
BA	12.7 ± 0.8	11.4 ± 0.4	13.1 ± 1.1

Conclusion

Acetyl-11-keto-β-boswellic acid (AKBA) was isolated from frankincense and transformed into 11-keto-β-boswellic acid (KBA). Both compounds served as starting materials for the synthesis of several amides or hydrazides. These compounds were fully characterized, and their cytotoxicity was evaluated *in vitro* using sulforhodamine B (SRB) assays employing two human tumor cell lines

(A 2780 and MCF7) as well as nonmalignant mouse fibroblasts (NIH 3T3). The highest cytotoxicity was observed for two amides 15 and 16 and the ovarian carcinoma cell line A 2780. These compounds showed EC_{50} = 1.0-1.7 μM while being significantly less toxic for the mouse fibroblasts NIH 3T3 (EC_{50} = 9.3-16.3 μM). Thus, compounds 15 and 16 may serve as starting points for developing potential and selective antitumor agents.

Experimental

The equipment used in this study has already been described.[20] Frankincense was bought from different commercial suppliers in bulk quantities, and the SRB assays were performed as previously reported [15, 16]. 3-*O*-Acetyl-11-keto-β-boswellic acid (1, AKBA) was isolated following a modified Jauch's procedure [21], and (3α, 4β) 3-hydroxy-11-oxo-urs-12-en-24-oic acid (= 11-keto–β-boswellic acid, 2, KBA) was prepared as reported.[20]

(3α, 4β) 3-Hydroxy-N-(3-hydroxypropyl)-11-oxo-urs-12-en-24 amide (3)

A solution of AKBA (200 mg, 0.4 mmol) in thionyl chloride (1 mL) was stirred at 90 °C for 3 h. The volatiles were removed under diminished pressure, and the residue was dissolved in dry THF (5 mL). A solution of 3-aminopropan-1-ol (1.0 g, 13.5 mmol) and *n*-BuLi (1.6 M in hexane, 8.12 mL) was slowly added, and the mixture was stirred at 25 °C for 30 min. Usual aqueous work-up, extraction with diethyl ether (2 x 20 mL) followed by chromatography (silica gel, ethyl acetate) gave 3 (182 mg, 86%) as a colorless solid; m.p. = 235–237 °C; R_F = 0.14 (ethyl acetate); $[\alpha]_D$ = +128.2° (*c* = 4.0, $CHCl_3$); UV-vis (MeOH): λ_{max} (log ε) = 267 nm (4.01); IR (KBr): $\tilde{\nu}$ = 3465*s*, 3405*s*, 2969*m*, 2921*m*, 2845*m*, 1657*s*, 1605*m*, 1537*m*, 1452*m*, 1382*m*, 1369*w*, 1354*w*, 1308*w*, 1293*m*, 1229*w*, 1200*m*, 1067*m*, 1023*w* cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 6.00 (*t*, 1H, N*H*, ^3J = 5.4 Hz), 5.52 (*s*, 1H, 12-H), 4.08 (*dd*, 1H, 3-H, ^3J = 2.5, 2.9 Hz), 3.64 (*m*, 2H, 33-H), 3.45 (*m*, 1H, 31-H_a), 3.32 (*m*, 1H, 31-H_b), 2.47 (*ddd*, 1H, 1-H_a, ^2J = 13.3 Hz, ^3J = 3.3, 3.7 Hz), 2.41 (*s*, 1H, 9-H),

2.34 (*m*, 1H, 2-H_a), 2.07 (*ddd*, 1H, 16-H_a, ^2J = 13.7 Hz, ^3J = 5.0, 13.6 Hz), 1.85 (*ddd*, 1H, 15-H_a, ^2J = 13.7 Hz, ^3J = 5.0, 13.6 Hz), 1.74 (*m*, 2H, 6-H), 1.70 (*m*, 3H, 7-H_a, 32-H), 1.54 (*m*, 1H, 2-H_b), 1.51 (*dd*, 1H, 18-H, ^3J = 11.2 Hz, ^4J = 1.7 Hz), 1.46 (*m*, 3H, 5-H, 7-H_b, 22-H_a), 1.42 (*m*, 1H, 21-H_a), 1.38 (*m*, 1H, 19-H), 1.35 (*m*, 2H, 1-H_b, 21-H_b), 1.32 (*m*, 1H, 22-H_b), 1.29 (*s*, 3H, 27-H), 1.25 (*s*, 3H, 23-H), 1.18 (*m*, 1H, 15-H_b), 1.16 (*s*, 3H, 26-H), 1.10 (*s*, 3H, 25-H), 0.99 (*ddd*, 1H, 16-H_b, ^2J = 13.3 Hz, ^3J = 2.1, 2.5 Hz), 0.93 (*m*, 1H, 20-H), 0.92 (*s*, 3H, 30-H), 0.80 (*s*, 3H, 28-H), 0.77 (*d*, 3H, 29-H, ^3J = 6.2 Hz) ppm;
^{13}C NMR (125 MHz, $CDCl_3$): δ = 198.3 (C-11), 176.6 (C-24), 163.9 (C-13), 129.5 (C-12), 69.7 (C-3), 59.5 (C-9), 59.2 (C-33), 58.0 (C-18), 47.8 (C-5), 46.4 (C-4), 44.1 (C-8), 42.8 (C-14), 39.9 (C-22), 38.3 (C-19), 38.3 (C-20), 36.5 (C-10), 35.8 (C-31), 33.3 (C-1), 33.0 (C-7), 32.2 (C-17), 31.0 (C-32), 29.9 (C-21), 27.9 (C-28), 26.5 (C-16), 26.2 (C-15), 25.6 (C-2), 24.4 (C-23), 20.1 (C-30), 19.5 (C-27), 18.6 (C-6), 17.4 (C-26), 16.4 (C-29), 12.5 (C-25) ppm;
MS (ESI, MeOH): *m/z* = 528.4 ($[M+H]^+$, 88%), 550.5 ($[M+Na]^+$, 12%);
Analysis calcd for $C_{33}H_{53}NO_4$ (527.78): C 75.10, H 10.12, N 2.65; found: C, 74.84, H 10.31, N 2.78.

5-[(3-{[(3α, 4β) 3-Hydroxy-11,24-dioxo-urs-12-en-24-yl]amino}propyl)oxy]-3,3-dimetyl-5-oxo-pentanoic acid (4)
A solution of 3 (100 mg, 0.19 mmol) and 3,3-dimethylglutaric anhydride (320 mg, 2.25 mmol) and DMAP (23 mg, 0.19 mmol) in dry pyridine (5 mL) was stirred at 50 °C for 5 d. The mixture was diluted with DCM (50 mL), followed by the addition of aq. HCl (0.1 N), and extraction with DCM (3 x 50 mL). After chromatography (silica gel, DCM/MeOH, 95:5) 4 (82 mg, 64%) was obtained as an off-white solid; m.p. = 168–171 °C; R_F = 0.10 (hexane/ethyl acetate/HOAc, 70:30:1); $[\alpha]_D$ = +80.8° (*c* = 3.98, $CHCl_3$); UV-vis (MeOH): λ_{max} (log ε) = 267 nm (3.95); IR (KBr): $\tilde{\nu}$ = 3427*m*, 2927*s*, 1732*s*, 1654*s*, 1521*m*, 1457*m*, 1384*m*, 1230*m*, 1149*m*, 1081*w*, 1057*m*, 1001*m* cm^{-1};
^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.93 (*t*, 1H, N*H*, ^3J = 5.4 Hz), 5.51 (*s*, 1H, 12-H), 4.13 (*m*, 2H, 33-H), 4.06 (*dd*, 1H, 3-H, ^3J = 2.5, 2.9 Hz), 3.30 (*m*, 1H, 31-H_a), 3.24 (*m*, 1H, 31-H_b), 2.46 (*m*, 1H, 1-H_a), 2.43, 2.40 (2 x *s*, 4H, 35-H, 37-H), 2.41 (*s*, 1H, 9-H), 2.31 (*m*, 1H, 2-H_a), 2.06 (*ddd*, 1H, 16-H_a, ^2J = 13.7 Hz, ^3J = 5.0, 13.6 Hz), 1.85 (*ddd*, 1H, 15-H_a, ^2J = 13.7 Hz, ^3J = 5.0, 13.6 Hz), 1.81 (*m*, 2H, 32-H), 1.76 (*m*, 2H, 6-H), 1.54 (*m*, 1H, 2-H_b), 1.51 (*dd*, 1H, 18-H, ^3J = 11.2 Hz, ^4J = 1.2 Hz), 1.45 (*m*, 4H, 5-H, 7-H, 1H, 22-H_a), 1.40 (*m*, 2H, 21-H), 1.38 (*m*, 1H, 19-H), 1.34 (*m*, 1H, 1-H_b), 1.30 (*m*, 1H, 22-H_b), 1.28 (*s*, 3H, 27-H), 1.23 (*s*, 3H, 23-H), 1.18 (*m*, 1H, 15-H_b), 1.15 (*s*, 3H, 26-H), 1.11 (s, 6H, 39-H, 40-H), 1.07 (*s*, 3H, 25-H), 0.98 (*m*, 1H, 16-H_b), 0.91 (*s*, 3H, 30-H), 0.90 (*m*, 1H, 20-H), 0.79 (*s*, 3H, 28-H), 0.76 (*d*, 3H, 29-H, ^3J = 6.2 Hz) ppm;
^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.5 (C-11), 176.9 (C-24), 174.9, 172.4 (C-34, C-38), 165.1 (C-13), 130.4 (C-12), 70.6 (C-3), 61.7 (C-33), 60.4 (C-9), 58.4 (C-18), 48.8 (C-5), 47.5 (C-4), 45.1, 44.8 (C-35, C-37), 44.6 (C-8), 43.8 (C-14), 40.9 (C-22), 39.3 (C-19), 39.2 (C-20), 37.5 (C-10), 36.3 (C-31), 34.2 (C-1), 33.9 (C-36), 33.2 (C-17), 32.4 (C-7), 30.9 (C-21), 28.8 (C-28), 28.4 (C-32), 28.0, 28.1 (C-39, C-40), 27.5 (C-16), 27.1 (C-15), 26.5 (C-2), 25.1 (C-23), 21.1 (C-30), 20.5 (C-27), 19.5 (C-6), 18.3 (C-26), 17.4 (C-29), 13.5 (C-25) ppm;
MS (ESI, MeOH): *m/z* = 670.3 ($[M+H]^+$, 100%), 692.5 ($[M+Na]^+$, 65%);
Analysis calcd for $C_{40}H_{63}NO_7$ (669.93): C 71.71, H 9.48, N 2.09; found: C 71.43, H 9.71, N 2.25.

Methyl 6-{[(3α, 4β) 3-acetyloxy-11,24-dioxo-urs-12-en-24-yl]amino}hexanoate (5)
A solution of AKBA (150 mg, 0.3 mmol) in dry DCM (20 mL) and oxalyl chloride (76 mg, 0.6 mmol) was stirred for 3 h at 25 °C. The volatiles were removed under reduced pressure, and residue was dissolved in dry DCM (15 mL). At 0 °C methyl 6-aminocaproate hydrochloride (43 mg, 0.23 mmol) and dry triethylamine (0.2 mL) were added, and the mixture was kept for 2 d at 5 °C. Usual aqueous work-up followed by chromatography (silica gel, hexane/ethyl acetate, 9:1) furnished 5 (80 mg, 54%) as an amorphous colorless solid; R_F = 0.38 (hexane/ethyl acetate/HOAc, 70:30:1); $[\alpha]_D$ = +81.5° (*c* = 3.80, $CHCl_3$); UV-vis (MeOH): λ_{max} (log ε) = 266 nm (4.02); IR (KBr): $\tilde{\nu}$ = 3424*m*, 2928*s*, 2868*s*, 1739*s*, 1659*s*, 1524*m*, 1458*m*, 1372*m*, 1320*m*, 1248*s*, 1201*s*, 1107*w*, 1052*w*, 1025*m* cm^{-1};
^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.52 (*s*, 1H, 12-H), 5.52 (*t*, N*H*, ^3J = 5.8 Hz), 5.26 (*dd*, 1H, 3-H, ^3J = 2.4, 2.9 Hz), 3.62 (*s*, 3H, 39-H), 3.20 (*m*, 2H, 33-H), 2.51 (*ddd*, 1H, 1-H_a, ^2J = 13.3 Hz, ^3J = 3.3 Hz, 3.2 Hz), 2.38 (*s*, 1H, 9-H), 2.27 (*t*, 2H, 37-H, ^3J = 7.5 Hz), 2.24 (*m*, 1H, 2-H_a), 2.07 (*ddd*, 1H, 16-H_a, ^2J = 13.7 Hz, ^3J = 5.0, 13.7 Hz), 2.05 (*s*, 3H, 32-H), 1.85 (*ddd*, 1H, 15-H_a, ^2J = 13.7 Hz, ^3J = 5.0, 13.7 Hz), 1.74 (*m*, 2H, 6-H), 1.70 (*ddd*, 1H, 7-H_a, ^2J = 12.9 Hz, ^3J = 3.7, 12.8 Hz), 1.66 (*m*, 1H, 2-H_b), 1.61 (*m*, 4H, 35-H; 36-H), 1.51 (*dd*, 1H, 18-H, ^3J = 11.2 Hz, ^4J = 1.2 Hz), 1.50 (*m*, 2H, 34-H), 1.47 (*m*, 3H, 22-H, 7-H_b), 1.44 (*m*, 2H, 21-H), 1.39 (*m*, 1H, 19-H), 1.35 (*dd*, 1H, 5-H, ^3J = 2.5 Hz, 12.5 Hz), 1.31 (*s*, 3H, 27-H), 1.20 (*m*, 2H, 1-H_b, 15-H_b), 1.17 (*s*, 3H, 26-H), 1.11 (*s*, 3H, 23-H), 1.09 (*s*, 3H, 25-H), 0.99 (*ddd*, 1H, 16-H_b, ^2J = 13.7 Hz, ^3J = 2.1, 2.5 Hz), 0.91 (*s*, 3H, 30-H), 0.90 (*m*, 1H, 20-H), 0.79 (*s*, 3H, 28-H), 0.77 (*d*, 3H, 29-H, ^3J = 6.6 Hz) ppm;
^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.0 (C-11), 175.1 (C-24), 173.9 (C-38), 170.1 (C-31), 164.6 (C-13), 130.5 (C-12), 73.5 (C-3), 60.3 (C-9), 59.0 (C-18), 51.4 (C-39), 50.4 (C-5), 46.6 (C-4), 45.0 (C-8), 43.7 (C-14), 40.9 (C-22), 39.3 (C-19), 39.3 (C-20), 39.2 (C-33), 37.4 (C-10), 34.9 (C-1), 33.9 (C-17), 33.1 (C-37), 33.0 (C-7), 30.9 (C-21), 28.9 (C-28), 28.8 (C-34), 27.5 (C-16), 27.2 (C-15), 24.7 (C-23), 24.4 (C-35), 24.3 (C-36), 23.5 (C-2), 21.3

(C-30), 21.1 (C-32), 20.5 (C-27), 19.5 (C-6), 18.3 (C-26), 17.4 (C-29), 13.4 (C-25) ppm;
MS (ESI, MeOH): *m/z* = 640.4 ($[M+H]^+$, 95%), 662.5 ($[M+Na]^+$, 100%);
Analysis calcd for $C_{39}H_{61}NO_6$ (639.90): C 73.20, H 9.61, N 2.19; found: C 73.01, H 9.84, N 2.32.

Ethyl 6-{[(3α, 4β) 3-acetyloxy-11,24-dioxo-urs-12-en-24-yl]amino}hexanoate (6)
Following the procedure given for 3, from AKBA (150 mg, 0.3 mmol), thionyl chloride and ethyl 6-aminocapronoate hydrochloride (586 mg, 3.2 mmol) in dry pyridine, followed by chromatography (silica gel, hexane/ethyl acetate, 9:1 → 7:3) 6 (128 mg, 46%) was obtained as an off-white solid; m.p. = 127–129 °C; R_F = 0.24 (hexane/ethyl acetate/HOAc, 70:30:1); $[\alpha]_D$ = +73.6° (*c* = 9.1, $CHCl_3$); UV-vis (MeOH): λ_{max} (log ε) = 267 nm (4.20); IR (KBr): $\tilde{\nu}$ = 3426*m*, 2928*s*, 2868*s*, 1737*s*, 1661*s*, 1521*m*, 1458*m*, 1372*m*, 1320*m*, 1248*s*, 1201*s*, 1128*w*, 1105*w*, 1051*m*, 1026*m* cm^{-1};
^1H NMR (400 MHz, $CDCl_3$): δ = 5.53 (*s*, 1H, 12-H), 5.51 (*t*, N*H*, ^3J = 5.8 Hz), 5.28 (*dd*, 1H, 3-H, ^3J = 2.5, 2.9 Hz), 4.10 (*q*, 2H, 39-H, ^3J = 7.1 Hz), 3.22 (*m*, 2H, 33-H), 2.53 (*ddd*, 1H, 1-H_a, ^2J = 13.3 Hz, ^3J = 3.3, 3.2 Hz), 2.39 (*s*, 1H, 9-H), 2.27 (*t*, 2H, 37-H), ^3J = 7.1 Hz), 2.23 (*m*, 1H, 2-H_a), 2.08 (*ddd*, 1H, 16-H_a, ^2J = 13.3 Hz, ^3J = 5.0, 13.2 Hz), 2.06 (*s*, 3H, 32-H), 1.88 (*ddd*, 1H, 15-H_a, ^2J = 13.3 Hz, ^3J = 5.0, 13.2 Hz), 1.76 (*m*, 2H, 6-H), 1.72 (*ddd*, 1H, 7-H_a, ^2J = 12.9 Hz, ^3J = 3.7, 12.8 Hz), 1.61 (*m*, 3H, 2-H_b; 36-H), 1.53 (*d*, 1H, 18-H, ^3J = 11.2 Hz), 1.48 (*m*, 2H, 34-H), 1.46 (*m*, 3H, 7-H, 22-H_a), 1.44 (*m*, 2H, 21-H), 1.39 (*m*, 1H, 19-H), 1.36 (*dd*, 1H, 5-H, ^3J = 2.5, 11.6 Hz), 1.35 (*m*, 2H, 35-H), 1.33 (*s*, 3H, 27-H), 1.29 (*m*, 1H, 22-H_b), 1.23 (*t*, 3H, 40-H, ^3J = 7.1 Hz), 1.22 (*s*, 3H, 23-H), 1.18 (*s*, 3H, 26-H), 1.17 (*m*, 2H, 1-H_b, 15-H_b), 1.12 (*s*, 3H, 25-H), 0.99 (*ddd*, 1H, 16-H_b, ^2J = 14.1 Hz, ^3J = 2.1, 2.5 Hz), 0.94 (*m*, 1H, 20-H), 0.93 (*s*, 3H, 30-H), 0.81 (*s*, 3H, 28-H), 0.78 (*d*, 3H, 29-H, ^3J = 6.2 Hz) ppm;
^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.0 (C-11), 175.1 (C-24), 173.5 (C-38), 170.2 (C-31), 164.6 (C-13), 130.5 (C-12), 73.5 (C-3), 60.3 (C-9), 60.2 (C-39), 59.0 (C-18), 50.4 (C-5), 46.6 (C-4), 45.0 (C-8), 43.7 (C-14), 40.9 (C-22), 39.4 (C-33), 39.3 (C-19), 39.2 (C-20), 37.4 (C-10), 34.9 (C-1), 34.1 (C37, *C*H_2), 33.9 (C-17), 33.1 (C-7), 30.9 (C-21), 28.9 (C-34), 28.8 (C-28), 27.5 (C-16), 27.2 (C-15), 26.5 (C-35), 24.4 (C-36), 23.8 (C-23), 23.5 (C-2), 21.3 (C-30), 21.1 (C-32), 20.5 (C-27), 18.7 (C-6), 18.3 (C-26), 17.4 (C-29), 14.2 (C-40), 13.4 (C-25) ppm;
MS (ESI, MeOH): *m/z* = 654.3 ($[M+H]^+$, 100%), 676.4 ($[M+Na]^+$, 33%);
Analysis calcd for $C_{40}H_{63}NO_6$ (653.93): C 73.47, H 9.71, N 2.14; found: C 73.22, H 9.98, N 1.96.

6-{[(3α, 4β) 3-Hydroxy-11,24-dioxo-urs-12-en-24-yl]amino}hexanoic acid (7)
To a solution of 6 (65 mg, 0.097 mmol) in THF (6 mL) and methanol (1 mL) an aq. solution of NaOH (4 N, 0.5 mL) was added, and the mixture was stirred overnight. Usual aq. work-up followed by chromatography (silica gel, hexane/ethyl acetate, 9:1) gave 7 (50 mg, 83%) as an amorphous, colorless solid; R_F = 0.10 (hexane/ethyl acetate/HOAc, 70:30:1); $[\alpha]_D$ = +110.8° (*c* = 3.26, $CHCl_3$); UV-vis (MeOH): λ_{max} (log ε) = 214 nm (4.10); IR (KBr): $\tilde{\nu}$ = 3425*m*, 2926*s*, 2858*m*, 1729*m*, 1656*s*, 1524*w*, 1057*w*, 1384*m*, 1231*w*, 1200*m*, 1081*w*, 1057*w*, 1001*w* cm^{-1};
^1H NMR (400 MHz, $CDCl_3$): δ = 5.55 (*br*, 1H, N*H*), 5.52 (*s*, 1H, 12-H), 4.07 (*dd*, 1H, 3-H, ^3J = 2.1, 2.5 Hz), 3.20 (*m*, 2H, 31-H), 2.45 (*ddd*, 1H, 1-H_a, ^2J = 13.3 Hz, ^3J = 3.3, 3.2 Hz), 2.40 (*s*, 1H, 9-H), 2.35 (*m*, 1H, 2-H_a), 2.31 (*t*, 2H, 35-H, ^3J = 7.3 Hz), 2.05 (*m*, 1H, 16-H_a), 1.84 (*ddd*, 1H, 15-H_a, ^2J = 13.3 Hz, ^3J = 5.0, 13.3 Hz), 1.71 (*m*, 2H, 6-H), 1.65 (*m*, 1H, 2-H_b), 1.60 (*m*, 1H, 7-H_a), 1.50 (*d*, 1H, 18-H, ^3J = 10.9 Hz), 1.49 (*m*, 2H, 32-H), 1.44 (*m*, 4H, 5-H, 21-H, 22-H_a), 1.39 (*m*, 1H, 19-H), 1.38 (*m*, 2H, 34-H), 1.36 (*m*, 2H, 33-H), 1.30 (*m*, 1H, 1-H_b), 1.28 (*s*, 3H, 27-H), 1.26 (*m*, 1H, 22-H_b), 1.22 (*m*, 1H, 7-H_b), 1.21 (*s*, 3H, 23-H), 1.18 (*m*, 1H, 15-H_b), 1.16 (*s*, 3H, 26-H), 1.07 (*s*, 3H, 25-H), 0.97 (*ddd*, 1H, 16-H_b, ^2J = 13.7 Hz, ^3J = 2.1, 2.5 Hz), 0.91 (*s*, 3H, 30-H), 0.90 (*m*, 1H, 20-H), 0.78 (*s*, 3H, 28-H), 0.75 (*d*, 3H, 29-H, ^3J = 6.6 Hz) ppm;
^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.4 (C-11), 178.6 (C-24), 176.6 (C-36), 164.9 (C-13), 130.4 (C-12), 70.7 (C-3), 60.4 (C-9), 59.0 (C-18), 48.7 (C-5), 47.3 (C-4), 45.0 (C-8), 43.8 (C-14), 40.9 (C-22), 39.3 (C-19), 39.2 (C-31), 39.2 (C-20), 37.5 (C-10), 34.2 (C-1), 33.9 (C-17), 33.7 (C-35), 33.2 (C-2), 30.9 (C-21), 29.7 (C-34), 28.9 (C-32), 28.8 (C-28), 27.5 (C-16), 27.1 (C-15), 26.4 (C-33), 25.2 (C-23), 24.2 (C-7), 21.1 (C-30), 20.4 (C-27), 19.6 (C-6), 18.3 (C-26), 17.4 (C-29), 13.5 (C-25) ppm;
MS (ESI, MeOH): *m/z* = 584.4 ($[M+H]^+$, 82%), 606.5 ($[M+Na]^+$, 25%);
Analysis calcd for $C_{36}H_{57}NO_5$ (583.85): C 71.71, H 9.48, N 2.09; found C 71.53, H 9.62, N 2.17.

N-[(3α, 4β) 3-Acetyloxy-11, 24-dioxo-urs-12-en-24-yl]glycine methyl ester (8)
Following the procedure given for 5, from AKBA (200 mg, 0.4 mmol), oxalyl chloride (101 mg, 0.8 mmol), methyl glycinate hydrochloride (50 mg, 0.4 mmol) and dry triethylamine (101 mg, 1.0 mmol) followed by chromatography (silica gel, hexane/ethyl acetate, 98:2 → 4:1) 8 (160 mg, 68%) was obtained as a colorless solid; m.p. = 250–255 °C; R_F = 0.37 (hexanes/ethyl acetate/HOAc, 70:30:1); $[\alpha]_D$ = +76.7° (*c* = 4.66, $CHCl_3$); UV-vis (MeOH): λ_{max} (log ε) = 267 nm (3.96); IR (KBr): $\tilde{\nu}$ = 3503*s*, 3413*s*, 2931*s*, 2870*m*, 1764*s*, 1733*s*, 1670*s*, 1648*s*, 1611*w*, 1523*s*, 1458*m*, 1404*m*, 1373*m*, 1318*m*, 1244*s*, 1202*s*, 1175*s*, 1130*w*, 1025*m* cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.07 (*t*, 1H, N*H*, ^3J = 4.6 Hz), 5.53 (*s*, 1H, 12-H), 5.33 (*dd*, 1H, 3-H, ^3J = 2.5 Hz, 2.4 Hz), 4.03 (*d*, 2H, 33-H, ^3J = 4.6 Hz), 3.74 (*s*, 3H, 35-H), 2.52 (*ddd*, 1H, 1-H_a, ^2J = 13.3 Hz, ^3J = 3.3, 3.7 Hz), 2.40 (*s*, 1H, 9-H), 2.29 (*m*, 1H, 2-H_a), 2.08 (*ddd*, 1H, 16-H_a, ^2J = 13.3 Hz, ^3J = 4.6, 13.2 Hz), 2.06 (*s*, 3H, 32-H), 1.88 (*ddd*, 1H, 15-H_a, ^2J = 13.3 Hz, ^3J = 4.6, 13.2 Hz), 1.80 (*m*, 1H, 6-H_a), 1.75 (*m*, 1H, 6-H_b), 1.72 (*ddd*, 1H, 7-H_a, ^2J = 12.9 Hz, ^3J = 3.7, 12.8 Hz), 1.61 (*m*, 1H, 2-H_b), 1.52 (*dd*, 1H, 18-H, ^3J = 11.2 Hz, ^4J = 1.2 Hz), 1.50 (*m*, 1H, 7-H_b), 1.48 (*m*, 1H, 22-H_a), 1.45 (*m*, 2H, 21-H), 1.41 (*dd*, 1H, 5-H, ^3J = 1.7 Hz, 12.0 Hz), 1.39 (*m*, 1H, 19-H), 1.33 (*s*, 3H, 27-H), 1.30 (*m*, 1H, 22-H_b), 1.21 (*m*, 2H, 1-H_b, 15-H_b), 1.19 (*s*, 3H, 26-H), 1.16 (*s*, 3H, 23-H), 1.08 (*s*, 3H, 25-H), 1.00 (*ddd*, 1H, 16-H_b, ^2J = 13.7 Hz, ^3J = 2.1, 2.5 Hz), 0.94 (*m*, 1H, 20-H), 0.93 (*s*, 3H, 30-H), 0.80 (*s*, 3H, 28-H), 0.78 (*d*, 3H, 29-H, ^3J = 6.2 Hz) ppm;
^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.0 (C-11), 175.5 (C-24), 170.7 (C-34), 170.1 (C-31), 164.7 (C-13), 130.5 (C-12), 73.5 (C-3), 60.4 (C-9), 59.0 (C-18), 52.4 (C-35), 50.3 (C-5), 46.6 (C-4), 45.0 (C-8), 43.7 (C-14), 40.9 (C-22), 39.3 (C-19), 39.3 (C-20), 37.4 (C-10), 34.9 (C-1), 34.6 (C-33), 34.0 (C-17), 33.1 (C-7), 30.9 (C-21), 28.8 (C-28), 27.5 (C-16), 27.2 (C-15), 24.6 (C-23), 23.8 (C-2), 21.3 (C-30), 21.1 (C-32), 20.5 (C-27), 19.3 (C-6), 18.3 (C-26), 17.4 (C-29), 13.2 (C-25) ppm;
MS (ESI, MeOH): *m/z* = 584.2 ($[M+H]^+$, 100%), 606.5 ($[M+Na]^+$, 45%);
Analysis calcd for $C_{35}H_{53}NO_6$ (583.80): C 72.00, H 9.15, N 2.40; found: C 71.76, H 9.31, N 2.53.

(3α, 4β) 3-Acetyloxy-N-(2-hydroxyethyl)-11-oxo-urs-12-en-24-amide (9)

Following the procedure given for the synthesis of 3, from the reaction of AKBA (200 mg, 0.4 mmol), thionyl chloride and sodium 2-aminoethanolat [from 2-amino-ethanol (0.1 mL) and sodium (100 mg)] followed by chromatography (silica gel, ethyl acetate) 9 (150 mg, 67%) was obtained as a colorless solid; m.p. = 239–243 °C (lit.: 284–290 °C [27]); R_F = 0.44 (ethyl acetate); $[\alpha]_D$ = +84.1° (*c* = 4.32, $CHCl_3$); UV-vis (MeOH): λ_{max} (log ε) = 267 nm (4.05); IR (KBr): $\tilde{\nu}$ = 3465*s*, 3429*s*, 2925*m*, 1726*m*, 1664*m*, 1630*m*, 1528*w*, 1455*w*, 1369*w*, 1318*w*, 1248*m*, 1202*w*, 1101*w*, 1072*w*, 1026*w* cm^{-1};
^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.00 (*t*, N*H*, ^3J = 5.0 Hz), 5.52 (*s*, 1H, 12-H), 5.30 (*dd*, 1H, 3-H, ^3J = 2.5, 2.9 Hz), 3.70 (*t*, 2H, 34-H, ^3J = 5.4 Hz), 3.40 (*dt*, 2H, 33-H, ^3J = 5.0 Hz, 5.4 Hz), 2.52 (*ddd*, 1H, 1-H_a, ^2J = 13.3 Hz, ^3J = 3.3 Hz, 3.4 Hz), 2.40 (*s*, 1H, 9-H), 2.28 (*m*, 1H, 2-H_a), 2.08 (*ddd*, 1H, 16-H_a, ^2J = 13.7 Hz, ^3J = 5.0, 13.6 Hz), 2.07 (*s*, 3H, 32-H), 1.87 (*ddd*, 1H, 15-H_a, ^2J = 13.7 Hz, ^3J = 5.0, 13.6 Hz), 1.80 (*m*, 1H, 6-H_a), 1.72 (*m*, 1H, 6-H_b), 1.67 (*m*, 1H, 7-H_a), 1.60 (*m*, 1H, 2-H_b), 1.52 (*dd*, 1H, 18-H, ^3J = 11.2 Hz, ^4J = 1.2 Hz), 1.49 (*m*, 1H, 7-H_b), 1.46 (*m*, 1H, 22-H_a), 1.42 (*m*, 2H, 21-H), 1.39 (*m*, 1H, 19-H), 1.38 (*dd*, 1H, 5-H, ^3J = 2.5 Hz, 11.7 Hz), 1.33 (*s*, 3H, 27-H), 1.30 (*m*, 1H, 22-H_b), 1.20 (*m*, 2H, 1-H_b, 15-H_b), 1.17 (*s*, 3H, 26-H), 1.14 (*s*, 3H, 23-H), 1.12 (*s*, 3H, 25-H), 1.00 (*ddd*, 1H, 16-H_b, ^2J = 13.7 Hz, ^3J = 2.1, 2.7 Hz), 0.93 (*m*, 1H, 20-H), 0.92 (*s*, 3H, 30-H), 0.80 (*s*, 3H, 28-H), 0.78 (*d*, 3H, 29-H, ^3J = 6.6 Hz) ppm;
^{13}C NMR (125 MHz, $CDCl_3$): δ = 198.1 (C-11), 175.4 (C-24), 169.2 (C-31), 163.8 (C-13), 129.5 (C-12), 72.5 (C-3), 61.0 (C-34), 59.4 (C-9), 58.0 (C-18), 49.4 (C-5), 45.7 (C-4), 44.0 (C-8), 42.8 (C-14), 41.3 (C-33), 39.9 (C-22), 38.3 (C-19), 38.3 (C-20), 36.5 (C-10), 33.9 (C-1), 33.0 (C-17), 32.1 (C-7), 29.9 (C-21), 27.9 (C-28), 26.5 (C-16), 26.2 (C-15), 23.8 (C-23), 22.9 (C-2), 20.3 (C-30), 20.1 (C-32), 19.5 (C-27), 18.4 (C-6), 17.3 (C-26), 16.4 (C-29), 12.3 (C-25) ppm;
MS (ESI, MeOH): *m/z* = 556.3 ($[M+H]^+$, 100%), 578.4 ($[M+Na]^+$, 30%);
Analysis calcd for $C_{34}H_{53}NO_5$ (555.79): C 73.47, H 9.61, N 2.52; found: C 73.24, H 9.80, N 2.71.

(3α, 4β) 3-Hydroxy-N-(4-hydroxybutyl-11-oxo-urs-12-en-24-amide (10)

Following the procedure given for the synthesis of 3, from KBA (188 mg, 0.4 mmol), thionyl chloride (1 mL) and 4-aminobutanol (360 mg, 4.0 mmol) in dry pyridine (3 mL), followed by chromatography (silica gel, ethyl acetate/MeOH 95:5) 10 (168 mg, 78%) was obtained as a colorless solid; m.p. = 234–240 °C; R_F = 0.26 (ethyl acetate/MeOH 95:5); $[\alpha]_D$ = +123.5° (*c* = 5.46, $CHCl_3$); UV-vis (MeOH): λ_{max} (log ε) = 267 nm (4.05); IR (KBr): $\tilde{\nu}$ = 3429*s*, 3385*s*, 2922*s*, 1660*s*, 1522*m*, 1457*m*, 1381*m*, 1321*w*, 1264*w*, 1231*m*, 1199*m*, 1083*m*, 1058*m*, 1002*w* cm^{-1};
^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.68 (*t*, 1H, N*H*, ^3J = 5.8 Hz), 5.52 (*s*, 1H, 12-H), 4.08 (*dd*, 1H, 3-H, ^3J = 2.5, 2.9 Hz), 3.65 (*t*, 2H, 34-H, ^3J = 6.0 Hz), 3.25 (*m*, 2H, 31-H), 2.47 (*ddd*, 1H, 1-H_a, ^2J = 13.3 Hz, ^3J = 3.3, 4.2 Hz), 2.41 (*s*, 1H, 9-H), 2.37 (*m*, 1H, 2-H_a), 2.07 (*ddd*, 1H, 16-H_a, ^2J = 13.7 Hz, ^3J = 5.0, 13.6 Hz), 1.85 (*ddd*, 1H, 15-H_a, ^2J = 13.7 Hz, ^3J = 5.0, 13.6 Hz), 1.76 (*m*, 2H, 6-H), 1.72 (*m*, 1H, 7-H_a), 1.58 (*m*, 4H, 21-H, 32-H), 1.55 (*m*, 1H, 2-H_b), 1.51 (*dd*, 1H, 18-H, ^3J = 10.8 Hz, ^4J = 1.2 Hz), 1.48 (*m*, 2H, 7-H_b, 22-H_a), 1.46 (*dd*, 1H, 5-H, ^3J = 2.1, 9.1 Hz), 1.44 (*m*, 2H, 33-H), 1.39(*m*, 1H, 19-H), 1.31 (*m*, 1H, 1-H_b), 1.29 (*s*, 3H, 27-H), 1.28 (*m*, 1H, 22-H_b), 1.23 (*s*, 3H, 23-H), 1.19 (*m*, 1H, 15-H_b), 1.16 (*s*, 3H, 26-H), 1.10 (*s*, 3H, 25-H), 1.00 (*m*, 1H, 16-H_b), 0.92 (*s*, 3H, 30-H), 0.91 (*m*, 1H, 20-H), 0.80 (*s*, 3H, 28-H), 0.77 (*d*, 3H, 29-H, ^3J = 6.6 Hz) ppm;
^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.3 (C-11), 176.5 (C-24), 164.7 (C-13), 130.5 (C-12), 70.7 (C-3), 62.3 (C-34), 60.4 (C-9), 59.0 (C-18), 48.7 (C-5), 47.3 (C-4), 45.0 (C-8), 43.8 (C-14), 40.9 (C-22), 39.3 (C-19), 39.2 (C-20), 39.2 (C-31), 37.5 (C-10), 34.2 (C-1), 33.9 (C-17), 33.2 (C-7), 32.8 (C-33), 30.9 (C-21), 28.8 (C-28), 27.5 (C-16), 27.1 (C-15), 26.6 (C-2), 25.9 (C-32), 25.2 (C-23), 21.1 (C-30), 20.5 (C-27), 19.6 (C-6), 18.3 (C-26), 17.4 (C-29), 13.5 (C-25) ppm;

MS (ESI, MeOH): *m/z* = 542.4 ($[M+H]^+$, 100%);
Analysis calcd for $C_{34}H_{55}NO_4$ (541.80): C 75.35, H 10.23, N 2.58; found: C 75.11, H 10.41, N 2.38.

(3α,4β) 3-Acetyloxy-11-oxo-urs-12-en-24-oic acid (2,4-dihydroxyphenyl)methylene-hydrazide (11)

A solution of 13 (200 mg, 0.38 mmol) and 2,4-dihydroxybenzaldehyde (53 mg, 0.38 mmol) in ethanol (20 mL) was heated for 3 h under reflux. The solvent was removed under reduced pressure, and the residue subjected to chromatography (silica gel, $CHCl_3/Et_2O$, 3:2) to afford 11 (198 mg, 80%) as a colorless solid; m.p. = 290 °C; R_F = 0.21 ($CHCl_3/Et_2O$, 1:1); $[\alpha]_D$ = +44.3° (*c* = 4.08, MeOH); UV-vis (MeOH): λ_{max} (log ε) = 217 nm (4.19); IR (KBr): $\tilde{\nu}$ = 3446*m*, 3345*m*, 2928*m*, 2862*m*, 1709*s*, 1658*s*, 1631*m*, 1512*m*, 1458*m*, 1377*m*, 1353*w*, 1261*m*, 1234*m*, 1186*m*, 1124*m*, 1050*w*, 1027*w* cm^{-1};
^{1}H NMR (400 MHz, DMSO): δ = 11.54 (*s*, 1H, O*H*), 10.56 (*s*, 1H, N*H*), 9.88 (*s*, 1H, O*H*), 8.48 (*s*, 1H, 33-H), 7.22 (*d*, 1H, 39-H, ^3J = 8.7 Hz), 6.33 (*dd*, 1H, 38-H, ^3J = 8.7 Hz, ^4J = 2.5 Hz), 6.26 (*d*, 1H, 36-H, ^4J = 2.5 Hz), 5.42 (*s*, 1H, 12-H), 5.20 (*dd*, 1H, 3-H, ^3J = 2.5, 2.9 Hz), 2.39 (*s*, 1H, 9-H), 2.38 (*m*, 1H, 1-H_a), 2.29 (*m*, 1H, 2-H_a), 2.08 (*ddd*, 1H, 16-H_a, ^2J = 13.7 Hz, ^3J = 5.0, 13.6 Hz), 2.05 (*s*, 3H, 32-H), 1.90 (*m*, 1H, 6-H_a), 1.86 (*ddd*, 1H, 15-H_a, ^2J = 13.7 Hz, ^3J = 5.0, 13.6 Hz), 1.70 (*m*, 1H, 6-H_b), 1.65 (*m*, 1H, 7-H_a), 1.54 (*dd*, 1H, 18-H, ^3J = 11.2 Hz, ^4J = 1.2 Hz), 1.50 (*m*, 1H, 2-H_b), 1.48 (*m*, 1H, 22-H_a), 1.45 (*m*, 3H, 7-H_b; 21-H), 1.39 (*m*, 1H, 19-H), 1.36 (*m*, 1H, 5-H), 1.30 (*s*, 3H, 27-H), 1.28 (*m*, 1H, 22-H_b), 1.21 (*m*, 2H, 1-H_b, 15-H_b), 1.16 (*s*, 3H, 26-H), 1.08 (*s*, 6H, 23H, 25-H), 0.99 (*m*, 1H, 16-H_b), 0.91 (*s*, 3H, 30-H), 0.89 (*m*, 1H, 20-H), 0.78 (*s*, 3H, 28-H), 0.75 (*d*, 3H, 29-H, ^3J = 6.2 Hz) ppm; ^{13}C NMR (125 MHz, DMSO):
δ = 198.0 (C-11), 170.7 (C-24), 169.7 (C-31), 166.9 (C-37), 164.3 (C-13), 160.5 (C-35), 149.3 (C-33), 131.5 (C-39), 129.6 (C-12), 110.4 (C-34), 107.5 (C-38), 102.6 (C-36), 72.5 (C-3), 59.7 (C-9), 58.1 (C-18), 49.9 (C-5), 45.9 (C-4), 44.4 (C-8), 43.3 (C-14), 40.0 (C-22), 38.6 (C-19), 38.5 (C-20), 36.8 (C-10), 33.5 (C-1), 33.0 (C-17), 31.5 (C-7), 30.6 (C-21), 28.4 (C-28), 26.9 (C-16), 26.6 (C-15), 22.7 (C-26), 22.0 (C-2), 21.1 (C-32), 21.0 (C-30), 20.0 (C-27), 19.3 (C-6), 17.0 (C-23, C-25), 14.2 (C-29) ppm; MS (ESI, MeOH): *m/z* = 647.4 ($[M+H]^+$, 64%), 669.5 ($[M+Na]^+$, 60%);
Analysis calcd for $C_{39}H_{54}N_2O_6$ (646.86): C 72.41, H 8.41, N 4.33; found: C 72.13, H 8.62, N 4.06.

(3α,4β) 3-Acetyloxy-11-oxo-urs-12-en-24-oic acid (2-hydroxyphenyl)methylene-hydrazide (12)

A solution of 13 (210 mg, 0.4 mmol) and salicylaldehyde (49 mg, 0.4 mmol) in ethanol (20 mL) was heated under reflux for 3 h. The mixture was cooled, and the product 12 (145 mg, 58%) was filtered off and obtained as a colorless solid; an analytical sample showed m.p. = 275 °C; R_F = 0.50 ($CHCl_3/Et_2O$ 8:2); $[\alpha]_D$ = +148.9° (*c* = 4.42, $CHCl_3$); UV-vis (MeOH): λ_{max} (log ε) = 299 nm (4.10); IR (KBr): $\tilde{\nu}$ = 3465*m*, 3406*m*, 3344*m*, 2980*m*, 2920*m*, 2863*m*, 2361*w*, 1708*s*, 1674*s*, 1614*m*, 1576*w*, 1522*m*, 1490*m*, 1457*m*, 1382*m*, 1355*m*, 1322*m*, 1272*s*, 1226*m*, 1201*m*, 1184*m*, 1144*m*, 1110*m*, 1082*w*, 1049*m*, 1026*m* cm^{-1};
^{1}H NMR (400 MHz, DMSO): δ = 11.34 (*s*, 1H, N*H*), 10.74 (*s*, 1H, O*H*), 8.60 (*s*, 1H, 33-H), 7.42 (*dd*, 1H, 39-H), ^3J = 8.3 Hz, ^4J = 1.7 Hz), 7.24 (*ddd*, 1H, 37-H, ^3J = 8.3, 10.0 Hz, ^4J = 1.7 Hz), 6.87 (*m*, 1H, 38-H), 6.85 (*m*, 1H, 36-H), 5.40 (*s*, 1H, 12-H), 5.18 (*dd*, 1H, 3-H, ^3J = 2.5 Hz, 2.4 Hz), 2.37 (*s*, 1H, 9-H), 2.35 (*m*, 1H, 1-H_a), 2.27 (*m*, 1H, 2-H_a), 2.06 (*ddd*, 1H, 16-H_a, ^2J = 13.7 Hz, ^3J = 5.0, 13.6 Hz), 2.03 (*s*, 3H, 32-H), 1.88 (*m*, 1H, 6-H_a), 1.80 (*ddd*, 1H, 15-H_a, ^2J = 13.7 Hz, ^3J = 5.0, 13.6 Hz), 1.68 (*m*, 1H, 6-H_b), 1.62 (*m*, 1H, 7-H_a), 1.51 (*d*, 1H, 18-H, ^3J = 11.2 Hz), 1.50 (*m*, 1H, 2-H_b), 1.48 (*m*, 1H, 21-H_a), 1.42 (*m*, 1H, 7-H_b; 1H, 22-H_a), 1.38 (*m*, 1H, 19-H), 1.36 (*d*, 1H, 5-H, ^3J = 10.8 Hz), 1.30 (*m*, 1H, 21-H_b; 1H, 22-H_b), 1.28 (*s*, 3H, 27-H), 1.25 (*m*, 1H, 1-H_b), 1.20 (*m*, 1H, 15-H_b), 1.15 (*s*, 3H, 26-H), 1.06 (*s*, 6H, 23-H, 25-H), 0.99 (*m*, 1H, 16-H_b), 0.91 (*m*, 1H, 20-H), 0.89 (*s*, 3H, 30-H), 0.75 (*s*, 3H, 28-H), 0.72 (*d*, 3H, 29-H, ^3J = 6.2 Hz) ppm;
^{13}C NMR (125 MHz, DMSO): δ = 198.2 (C-11), 171.3 (C-24), 169.9 (C-31), 164.5 (C-13), 157.6 (C-35), 148.6 (C-33), 131.3 (C-37), 129.9 (C-39), 129.8 (C-12), 119.4 (C-38), 118.6 (C-34), 116.5 (C-36), 72.6 (C-3), 59.9 (C-9), 58.3 (C-18), 50.1 (C-5), 46.1 (C-4), 44.6 (C-8), 43.5 (C-14), 40.0 (C-22), 38.8 (C-19), 38.6 (C-20), 36.9 (C-10), 34.4 (C-17), 33.7 (C-1), 32.4 (C-7), 30.5 (C-21), 28.6 (C-28), 27.0 (C-16), 26.8 (C-15), 23.7 (C-2), 22.8 (C-26), 21.2 (C-32), 21.0 (C-30), 20.2 (C-27), 18.9 (C-6), 17.2 (C-23, C-25), 13.3 (C-29) ppm;
MS (ESI, MeOH) $C_{39}H_{54}N_2O_5$: *m/z* = 631.3 ($[M+H]^+$, 100%), 653.5 ($[M+Na]^+$, 80%);
Analysis calcd for $C_{39}H_{54}N_2O_5$ (630.86): C 74.25, H 8.63, N 4.44; found: C 74.00, H 8.83, N 4.27.

(3α, 4β) 3-Acetyloxy-11-oxo-12-en-24-oic acid hydrazide (13)

Acid activation of AKBA (400 mg, 0.8 mmol) with thionyl chloride (2 mL) as described above, and reaction of the residue in dry THF (20 mL) with hydrazine hydrate (10 mL) for 1 h at 25 °C followed by usual work-up and chromatography (silica gel,ethyl acetate/0.1% NH_4OH) gave 13 (370 mg, 88%) as an off-white solid; m.p. = 186–189 °C (lit.: 190–192 °C) [27]; R_F = 0.58 (ethyl acetate/0.1% NH_4OH); $[\alpha]_D$ = +86.3° (*c* = 4.66, $CHCl_3$); UV-vis (MeOH): λ_{max} (log ε) = 268 nm (4.03); IR (KBr): $\tilde{\nu}$ = 3405*m*, 3340*m*, 2926*s*, 2869*m*, 1739*s*, 1662*s*, 1620*m*, 1500*w*, 1458*m*, 1370*m*, 1320*w*, 1248*s*, 1201*m*, 1178*m*, 1128*w*, 1051*w*, 1026*m*, 997*m* cm^{-1};
^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.85 (*s*, 1H, N*H*), 5.53 (*s*, 1H, 12-H), 5.31 (*dd*, 1H, 3-H, ^3J = 2.5, 2.9 Hz), 2.52 (*ddd*, 1H, 1-H_a, ^2J = 12.9 Hz, ^3J = 3.3 Hz, 3.2 Hz), 2.39 (*s*, 1H, 9-H), 2.27 (*m*, 1H, 2-H_a), 2.08 (*m*, 1H, 16-H_a), 2.06 (*s*, 3H, 32-H), 1.87 (*ddd*, 1H, 15-H_a, ^2J = 13.7 Hz, ^3J = 5.0, 13.6 Hz), 1.80 (*m*, 1H, 6-H_a), 1.72 (*m*, 2H, 6-H_b), 1.70 (*ddd*,

1H, 7-H_a, ^2J = 11.6 Hz, ^3J = 4.1, 11.7 Hz), 1.61 (*m*, 1H, 2-H_b), 1.52 (*dd*, 1H, 18-H, ^3J = 11.2 Hz, ^4J = 1.2 Hz), 1.48 (*m*, 1H, 7-H_b), 1.46 (*m*, 1H, 22-H_a), 1.44 (*m*, 1H, 21-H_a), 1.39 (*m*, 1H, 19-H), 1.38 (*dd*, 1H, 5-H, ^3J = 3.3, 10.4 Hz), 1.32 (*s*, 3H, 27-H), 1.28 (*m*, 2H, 21-H_b, 22-H_b), 1.20 (*m*, 2H, 1-H_b, 15-H_b), 1.17 (*s*, 3H, 26-H), 1.12 (*s*, 3H, 23-H), 1.07 (*s*, 3H, 25-H), 1.00 (*ddd*, 1H, 16-H_b, ^2J = 13.3 Hz, ^3J = 2.5, 2.4 Hz), 0.92 (*s*, 3H, 30-H), 0.90 (*m*, 1H, 20-H), 0.80 (*s*, 3H, 28-H), 0.78 (*d*, 3H, 29-H, ^3J = 6.2 Hz) ppm;
^{13}C NMR (125 MHz, $CDCl_3$): δ = 198.8 (C-11), 176.5 (C-24), 170.1 (C-31), 164.7 (C-13), 130.5 (C-12), 73.1 (C-3), 60.3 (C-9), 59.0 (C-18), 50.2 (C-5), 46.2 (C-4), 45.0 (C-8), 43.8 (C-14), 40.9 (C-22), 39.3 (C-19), 39.3 (C-20), 37.3 (C-10), 34.8 (C-1), 34.0 (C-17), 33.1 (C-7), 30.9 (C-21), 28.8 (C-28), 27.5 (C-16), 27.2 (C-15), 24.5 (C-23), 23.9 (C-2), 21.3 (C-30), 21.1 (C-32), 20.5 (C-27), 19.5 (C-6), 18.3 (C-26), 17.4 (C-29), 14.2 (C-25) ppm;
MS (ESI, MeOH): *m/z* = 527.3 ($[M+H]^+$, 100%).

(3α, 4β) 3-Acetyloxy-N-(1-adamantanyl)-11-oxo-urs-12-en-24-amide (14)

Following the procedure given for the synthesis of 3, from AKBA (150 mg, 0.3 mmol), thionyl chloride (1 mL), dry triethylamine (0.2 mL) and 1-aminoadamantan hydrochloride (71 mg, 0.375 mmol) followed by chromatography (silica gel, hexane/ethyl acetate 95:5) 14 (106 mg, 58%) was obtained as an amorphous colorless solid;R_F = 0.87 (hexane/ethyl acetate/HOAc 70:30:1);$[\alpha]_D$ = +57.1° (*c* = 3.84, $CHCl_3$); UV-vis (MeOH): λ_{max} (log ε) = 266 nm (3.91); IR (KBr): $\tilde{\nu}$ = 3458*m*, 3423*m*, 2911*s*, 2852*s*, 1739*s*, 1664*s*, 1510*s*, 1456*s*, 1370*m*, 1345*m*, 1312*m*, 1292*m*, 1248*s*, 1201*m*, 1104*m*, 1091*m*, 1050*m*, 1026*m*, 998*m* cm^{-1};
^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.52 (*s*, 1H, 12-H), 5.22 (*dd*, 1H, 3-H, ^3J = 2.5 Hz, 2.9 Hz), 5.08 (s, 1H, N*H*), 2.51 (*ddd*, 1H, 1-H_a, ^2J = 13.3 Hz, ^3J = 3.2 Hz, 3.3 Hz), 2.37 (*s*, 1H, 9-H), 2.25 (*m*, 1H, 2-H_a), 2.07 (*ddd*, 1H, 16-H_a, ^2J = 14.1 Hz, ^3J = 5.0, 14.1 Hz), 2.04 (*s*, 3H, 32-H), 2.01 (*m*, 3H, 35-H, 37-H, 40-H), 1.96 (*m*, 6H, 34-H, 38-H, 39-H), 1.87 (*ddd*, 1H, 15-H_a, ^2J = 14.1 Hz, ^3J = 5.0, 14.1 Hz), 1.81 (*m*, 1H, 6-H_a), 1.68 (*m*, 1H, 6-H_b), 1.66 (*m*, 1H, 7-H_a), 1.64 (*m*, 6H, 36-H, 41-H, 42-H), 1.57 (*m*, 1H, 2-H_b), 1.51 (*dd*, 1H, 18-H, ^3J = 11.2 Hz, ^4J = 0.8 Hz), 1.46 (*m*, 1H, 7-H_b), 1.41 (*m*, 2H, 21-H), 1.39 (*m*, 1H, 19-H), 1.33 (*m*, 1H, 5-H), 1.31 (*s*, 3H, 27-H), 1.28 (*m*, 2H, 22-H), 1.19 (*m*, 2H, 1-H_b, 15-H_b), 1.18 (*s*, 3H, 26-H), 1.17 (*s*, 3H, 25-H), 1.09 (*s*, 3H, 23-H), 0.99 (*m*, 1H, 16-H_b), 0.92 (*m*, 1H, 20-H), 0.91 (*s*, 3H, 30-H), 0.79 (*s*, 3H, 28-H), 0.77 (*d*, 3H, 29-H, ^3J = 6.6 Hz) ppm;
^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.0 (C-11), 173.9 (C-24), 170.1 (C-31), 164.5 (C-13), 130.5 (C-12), 73.6 (C-3), 60.4 (C-9), 59.0 (C-18), 52.0 (C-33), 50.3 (C-5), 46.9 (C-4), 45.0 (C-8), 43.7 (C-14), 41.5 (C-34, C-38, C-39), 40.9 (C-22), 39.3 (C-19), 39.2 (C-20), 37.5 (C-10), 36.4 (C-36, C-41, C-42), 34.9 (C-1), 33.9 (C-7), 33.2 (C-17), 30.9 (C-21), 29.4 (C-35, C-37, C-40), 28.8 (C-28), 27.5 (C-16), 27.2 (C-15), 25.0 (C-23), 23.9 (C-2), 21.3 (C-30), 21.1 (C-32), 20.4 (C-27), 19.4 (C-6), 18.4 (C-26), 17.4 (C-29), 13.1 (C-25) ppm;
MS (ESI, MeOH): *m/z* = 646.4 ($[M+H]^+$, 100%), 668.5 ($[M+Na]^+$, 45%);
Analysis calcd for $C_{42}H_{63}NO_4$ (645.95): C 78.09, H 9.83, N 2.17; found: C 77.83, H 9.91, N 2.27.

(3α, 4β) 3-Acetyloxy-N-(3-aminopropyl)-11-oxo-urs-12-en-24-amide (15)

Following the procedure given for the synthesis of 3, from AKBA (200 mg, 0.4 mmol), thionyl chloride (1 mL), 1,3-diaminopropane (300 mg, 4.0 mmol), dry THF (15 mL) and dry pyridine (5 mL), followed by chromatography (silica gel, DCM/MeOH/NEt_3, 80:20:1) 15 (200 mg, 88%) was obtained as an off-white, amorphous solid; R_F = 0.32 (DCM/MeOH/NEt_3, 80:20:1); $[\alpha]_D$ = +75.4°(*c* = 4.32, $CHCl_3$); UV-vis (MeOH): λ_{max} (log ε) = 267 nm (3.97); IR (KBr): $\tilde{\nu}$ = 3417*m*, 3372*m*, 2926*s*, 2829*m*, 1737*s*, 1661*s*, 1523*m*, 1457*m*, 1371*m*, 1320*m*, 1249*s*, 1201*m*, 1176*m*, 1129*m*, 1052*m*, 1025*m* cm^{-1};
^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.81 (*t*, N*H*, ^3J = 5.0 Hz), 5.52 (*s*, 1H, 12-H), 5.28 (*dd*, 1H, 3-H, ^3J = 2.5, 2.9 Hz), 3.35 (*m*, 1H, 33-H_a), 3.31 (*m*, 2H, 33-H_b), 2.82 (*dd*, 2H, 35-H, ^3J = 5.8, 6.2 Hz), 2.52 (*ddd*, 1H, 1-H_a, ^2J = 13.7 Hz, ^3J = 3.2, 3.3 Hz), 2.39 (*s*, 1H, 9-H), 2.29 (*m*, 1H, 2-H_a), 2.08 (*ddd*, 1H, 16-H_a, ^2J = 13.3 Hz, ^3J = 4.6, 13.2 Hz), 2.06 (*s*, 3H, 32-H), 1.87 (*ddd*, 1H, 15-H_a, ^2J = 13.3 Hz, ^3J = 4.6, 13.2 Hz), 1.83 (*m*, 1H, 6-H_a), 1.75 (*m*, 1H, 6-H_b), 1.66 (*ddd*, 1H, 7-H_a, ^2J = 12.5 Hz, ^3J = 3.7, 12.6 Hz), 1.58(*m*, 1H, 2-H_b), 1.52 (*dd*, 1H, 18-H, ^3J = 10.8 Hz, ^4J = 1.2 Hz), 1.48 (*m*, 2H, 7-H_b, 22-H_a), 1.42 (*m*, 2H, 21-H), 1.39 (*m*, 1H, 19-H), 1.35 (*m*, 1H, 5-H), 1.32(*s*, 3H, 27-H), 1.30 (*m*, 1H, 22-H_b), 1.23 (*m*, 2H, 34-H), 1.20 (*m*, 2H, 1-H_b, 15-H_b), 1.17 (*s*, 3H, 26-H), 1.12 (*s*, 3H, 23-H), 1.11 (*s*, 3H, 25-H), 0.98 (*m*, 1H, 16-H_b), 0.92 (*s*, 3H, 30-H), 0.91 (*m*, 1H, 20-H), 0.80 (*s*, 3H, 28-H), 0.77 (*d*, 3H, 29-H, ^3J = 6.2 Hz) ppm;
^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.2 (C-11), 175.2 (C-24), 170.2 (C-31), 164.7 (C-13), 130.5 (C-12), 73.5 (C-3), 60.4 (C-9), 59.0 (C-18), 50.5 (C-5), 46.6 (C-4), 45.0 (C-8), 43.7 (C-14), 40.9 (C-22), 40.6 (C-35), 39.3 (C-19), 39.2 (C-20), 38.8 (C-33), 37.4 (C-10), 34.9 (C-1), 33.9 (C-17), 33.1 (C-7), 31.1 (C-21), 30.9 (C-34), 28.8 (C-28), 27.5 (C-16), 27.2 (C-15), 24.7 (C-23), 24.0 (C-2), 21.3 (C-30), 21.1 (C-32), 20.5 (C-27), 19.4 (C-6), 18.3 (C-26), 17.4 (C-29), 13.4 (C-25) ppm;
MS (ESI, MeOH): *m/z* = 569.4 ($[M+H]^+$, 100%);
Analysis calcd for $C_{35}H_{56}N_2O_4$ (568.83): C 73.90, H 9.92, N 4.92; found: C 73.77, H 10.15, N 4.61.

(3α, 4β)3-Acetyloxy-N-[4-(3-aminopropyl)-piperazin-1-yl]-propyl -11-oxo-urs-12-en-24-amide (16)

Following the procedure given for the synthesis of 3, from AKBA (200 mg, 0.4 mmol), thionyl chloride (1 mL), 1,4-bis(3-aminopropyl)piperazin (810 mg,

4.0 mmol) and dry pyridine (5 mL) followed by chromatography (silica gel, DCM/MeOH, NEt_3, 80:20:1) 16 (216 mg, 78%) was obtained as an off-white solid; m.p. = 151–155 °C; R_F = 0.10 (DCM/MeOH/NEt_3, 80:20:1); $[\alpha]_D$ = +51.3° (*c* = 4.0, $CHCl_3$); UV-vis (MeOH): λ_{max} (log ε) = 267 nm (3.89); IR (KBr): $\tilde{\nu}$ = 3422*m*, 2927*s*, 2814*m*, 1737*s*, 1661*s*, 1518*m*, 1458*m*, 1371*m*, 1318*m*, 1248*s*, 1201*m*, 1130*m*, 1052*m*, 1024*m* cm^{-1};
^1H NMR (400 MHz, $CDCl_3$): δ = 6.54 (*t*, 1H, N*H*, ^3J = 4.6 Hz), 5.53 (*s*, 1H, 12-H), 5.24 (*dd*, 1H, 3-H, ^3J = 2.5 Hz, 2.9 Hz), 3.28 (*m*, 2H, 33-H), 2.83 (*t*, 2H, 35-H ^3J = 6.4 Hz), 2.55–2.37 (*m*, 14H, 1-H_a, 9-H, 36-H, 37-H, 38-H, 39-H, 40-H, 42-H), 2.27 (*m*, 1H, 2-H_a), 2.08 (*ddd*, 1H, 16-H_a, ^2J = 13.7 Hz, ^3J = 5.0, 13.6 Hz), 2.06 (*s*, 3H, 32-H), 1.87 (*ddd*, 1H, 15-H_a, ^2J = 13.7 Hz, ^3J = 5.0, 13.6 Hz), 1.84 (*m*, 1H, 6-H_a), 1.76 (*m*, 1H, 6-H_b), 1.67 (*m*, 5H, 7-H_a; 34-H, 41-H), 1.60 (*m*, 1H, 2-H_b), 1.52 (*d*, 1H, 18-H, ^3J = 11.2 Hz), 1.48 (*m*, 2H, 7-H_b, 22-H_a), 1.42 (*m*, 2H, 21-H), 1.39 (*m*, 1H, 19-H), 1.35 (*dd*, 1H, 5-H, ^3J = 2.1, 12.0 Hz), 1.32 (*s*, 3H, 27-H), 1.29 (*m*, 1H, 22-H_b), 1.20 (*m*, 2H, 1-H_a, 15-H_b), 1.17 (*s*, 3H, 26-H), 1.12 (*s*, 3H, 23-H), 1.09 (*s*, 3H, 25-H), 0.99 (*m*, 1H, 16-H_b), 0.92 (*s*, 3H, 30-H), 0.91 (*m*, 1H, 20-H), 0.80 (*s*, 3H, 28-H), 0.78 (*d*, 3H, 29-H, ^3J = 6.2 Hz) ppm;
^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.1 (C-11), 175.1 (C-24), 170.0 (C-31), 164.7 (C-13), 130.6 (C-12), 73.5 (C-3), 60.4 (C-9), 59.0 (C-18), 57.8, 56.8 (C-36, C-37, C-38, C-39), 53.6, 52.9 (C-40, C-42), 50.6 (C-5), 46.6 (C-4), 45.0 (C-8), 43.7 (C-14), 41.0 (C-35), 40.9 (C-22), 39.8 (C-33), 39.3 (C-19), 39.3 (C-20), 37.3 (C-10), 34.9 (C-1), 34.0 (C-17), 33.1 (C-7), 30.9 (C-21), 28.8 (C-28), 28.2 (C-41), 27.5 (C-16), 27.2 (C-15), 24.8 (C-34), 24.7 (C-23), 24.1 (C-2), 21.3 (C-30), 21.1 (C-32), 20.5 (C-27), 19.6 (C-6), 18.3 (C-26), 17.4 (C-29), 13.4 (C-25) ppm;
MS (ESI, MeOH): *m/z* = 695.5 ($[M+H]^+$, 85%);
Analysis calcd for $C_{42}H_{70}N_4O_4$ (695.03): C 72.58, H 10.15, N 8.06; found: C 72.31, H 10.23, N 8.29.

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