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Hydroxyethylamide substituted triterpenoic acids hold good cytotoxicity for human tumor cells

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ABSTRACT

Pentacyclic triterpenoic acids, betulinic acid, platanic acid, oleanolic acid and ursolic acid, were acetylated and subsequently converted into mono-, bis- and tris(hydroxyl)ethyl amides. While parent compounds are of none or minor cytotoxicity, these amides showed EC_{50} values even in low μM concentration for a variety of different human tumor cell lines. Especially a bis(hydroxyethyl)amide **12** derived from oleanolic acid held an $EC_{50} = 7.7$ μM for A375 melanoma cells while being less cytotoxic for non-malignant fibroblasts NIH 3 T3 ($EC_{50} > 30$ μM). Several of these amides were converted into their corresponding sulfamates. While these sulfamates held no inhibitory activity for the enzyme carbonic anhydrase II, the highest cytotoxicity was observed for a betulinic acid derived sulfamate **17** with an $EC_{50} = 4.9$ μM for A375 melanoma cells.

Introduction

NVX-207 (Fig. 1) is a 3-*O*-acetyl-betulinic acid derivative that has shown good cytotoxicity for a variety of different human tumor cell lines.[1–7] Most recently, its application to treat melanoma [8], for example of aged horses, has been reported, too. This compound overcomes at least partially the notorious problem usually associated with betulinic acid (**BA**) [9–15] and its derivatives: a diminished solubility in aqueous solutions and biological fluids hence limiting its applications. [16–24] To improve solubility, we became interested in investigating hydroxyethyl substituted analogs of **BA**, and – for comparison – analogs derived from platanic acid (**PA**), oleanolic acid (**OA**) and ursolic acid (**UA**) holding one, two or three of these moieties. Furthermore, sulfamates thereof might be of interest as inhibitors of carbonic anhydrases. [25–31] The latter compounds have gained much interest recently as new scaffolds, especially to treat tumors [32–35] under hypoxic conditions, for example glioblastoma.[36–42] Hence, some of these compounds were prepared, too, and included into this investigation.

Results and discussion

Triterpenoic aids **BA**, **PA**, **UA**, and **OA** were acetylated to yield well-known acetates **1**–**4** in good to excellent yields (Scheme 1). Reaction of these acetates with oxalyl chloride in the presence of catalytic amounts of DMF followed by the addition of ethanolamine furnished amides **5**–**8** in 70–86% yield, respectively. From the reaction with diethanolamine, amides **9**–**12** were obtained. Compound **9** could not be isolated in pure form from these reactions, while no such problems occurred for the synthesis of **10**–**12**. As an alternative to this classical approach, the PyBOB [43,44] mediated coupling was investigated, too. Application of this method gave complete reactions within a short period of time (usually one hour or even less), and the yields, especially concerning compounds **5**–**8** were higher than for the corresponding reaction employing oxalyl chloride. Yields dropped significantly, however, for the PyBOB mediated synthesis of compounds **9**–**12** and **13**–**16** – probably due to steric hindrance. Thus, tris analogues **13**–**16** were synthesized from the acetates **1**–**4** using oxalyl chloride followed by adding tris; **14** was only obtained in traces. For the synthesis of sulfamates, compounds **5**–**8**, **11** and **12** were deprotonated with NaH followed by adding sulfamoyl chloride [45–49] to yield compounds **17**–**22**. The latter reagent was synthesized from the reaction of chlorosulfonylisocyanate with formic acid.

Photometric sulforhodamine B assays (SRB, Table 1) were used to assess the cytotoxicity of the compounds. While compounds **5**–**8** as well

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as compounds **16** and **18** were not soluble under the conditions of the assay, many compounds of this study exhibited good cytotoxicity, and in some cases selectivity so not expected. Thus, platanic acid derived **10** was not cytotoxicity for all cell lines and also not for non-malignant fibroblasts NIH 3 T3 but showed an EC_{50} of 17.6 ± 1.5 μM for the human melanoma cell line A375. All of the compounds exhibited a higher cytotoxicity for the cancer cell line than for the non-malignant fibroblasts. The highest cytotoxicity was observed for betulinic acid derived **17** (EC_{50} = 4.9 ± 0.2 μM) and A375 cells, while being non-cytotoxic for human colon cancer cells HT29 and non-malignant fibroblasts NIH 3 T3. A similar selectivity was observed for oleanolic acid derived **12**.

Calculation of the ADME relevant parameters (swissadme.ch) showed **NVX-207** and compound **17** of this study to hold a similar profile (Fig. 2); this parallels a similar biological activity concerning cytotoxicity and cell selectivity.

Previously, several sulfamates of triterpenoids have been shown to act as inhibitors of carbonic anhydrases II and IX.[35,50] Hence, the compounds were screened for their inhibitory activity for the former enzyme. The results of these assays are summarized in Table 2.

These results indicate that the possible mode of action of the compounds concerning their cytotoxicity is unlikely to be caused by an inhibition of CA.

Conclusion

Pentacyclic triterpenoic acids, betulinic acid, platanic acid, oleanolic acid and ursolic acid, were acetylated and subsequently converted into mono-, bis- and tris(hydroxyethyl)amides. These amides showed EC_{50} values even in low μM concentration for a variety of different human tumor cell lines. A bis(hydroxyethyl)amide **12** derived from oleanolic acid held an EC_{50} = 7.7 μM for A375 melanoma cells; this compound was less cytotoxic for non-malignant fibroblasts NIH 3 T3 (EC_{50} > 30 μM). Several of these amides were converted into their corresponding sulfamates and screened for cytotoxicity as well as their activity to act as an inhibitor for the enzyme carbonic anhydrase II. While these sulfamates held no inhibitory activity for this enzyme, the highest cytotoxicity was observed for a betulinic acid derived sulfamate **17** with an EC_{50} = 4.9 μM for A375 melanoma cells.

Experimental

The equipment and the conditions for the cytotoxic evaluation as well as for the CA II assay have been described in the supplementary materials file.

General procedures

*General procedure for the synthesis of triterpenoic acid acetates **1**–**4** (GP A)*

To a solution of the triterpene carboxylic acid (5.0 g, 0.01 mol) in dry DCM (150 mL) and triethylamine (4.5 mL, 0.03 mol, 3 eq.), acetic anhydride (3.2 mL, 0.03 mol, 3 eq.) and DMAP (catal.) were added, and stirring at 25 °C was continued for 1 day. For work-up, a solution of ammonia in MeOH (7 M, 5 mL) was added. After 30 min of stirring, an aq. solution of HCl (2 M, 50 mL) was added, the phases were separated, and the aqueous phase was extracted with DCM (2 × 10 mL). The combined organic phases were washed with brine (1 × 20 mL) and dried (Na_2SO_4). The solvent was removed under reduced pressure, and the residue purified by re-crystallization from ethanol to afford **1**–**4**. Crude material was re-crystallized from ethanol.

*General procedure for the synthesis of amides **5**–**8** using oxalyl chloride (GP B1)*

To a solution of acetate **1**–**4** (0.8 g, 1.6 mmol) in dry DCM (25 mL), oxalyl chloride (0.6 mL, 7.2 mmol, 4.5 eq.) and a catalytic amount of DMF were slowly added, and the mixture was stirred until the evolution of gases had ceased (approx. 30 min). The volatiles were removed under reduced pressure, the residue re-dissolved in dry THF (3 × 10 mL), and the volatiles were again removed under reduced pressure. Subsequently, the residue was dissolved in dry DCM (20 mL) and added dropwise to a solution of ethanolamine (0.48 mL, 5.33 mmol, 5 eq.) in dry DCM (8 mL). The reaction mixture was stirred for 24 h at 23 °C. Usual aqueous work-up followed by chromatography gave products **5**–**8**, respectively.

*General procedure for the synthesis of amides **5**–**8** using PyBOP (GP B2)*

To a solution of acetate **1**–**4** (1.0 g, 2 mmol) in dry DCM (15 mL), PyBOP (1.25 g, 2.4 mmol, 1.2 eq.) and triethylamine (0.56 mL, 4 mmol, 2 eq.) were added. After stirring for an additional 15 min, the respective amine (2.4 mmol, 1.2 eq.) was added, and stirring was continued for

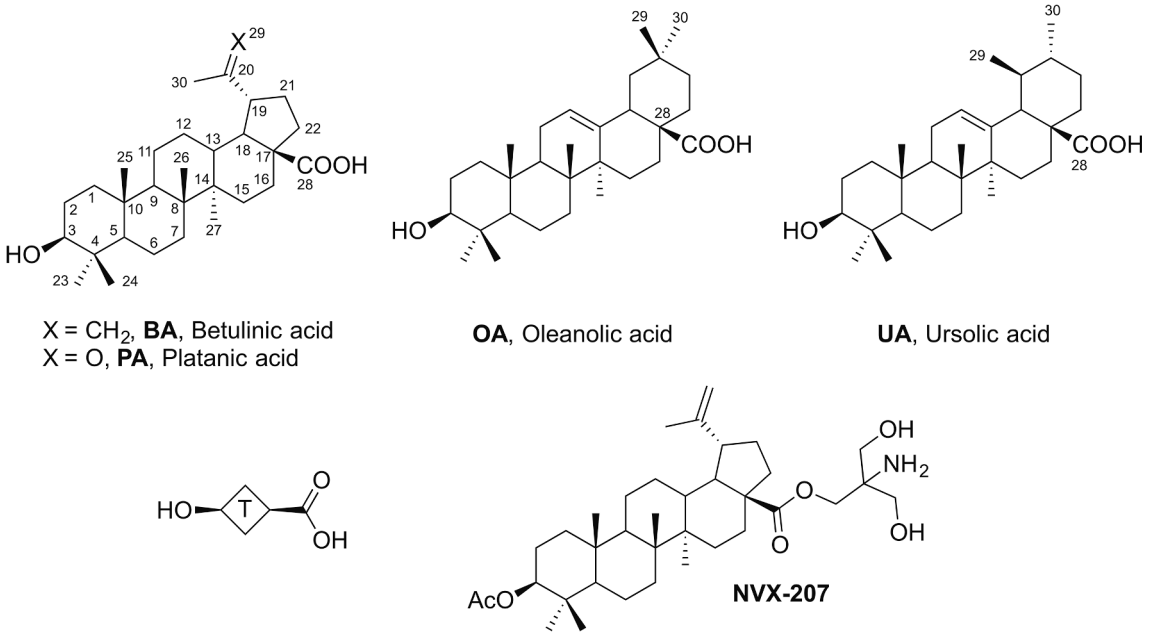


Fig. 1. Structure of triterpenoic acids betulinic acid (**BA**), platanic acid (**PA**), oleanolic acid (**OA**) and ursolic acid (**UA**), a generalized formula representing these triterpenoic acids as well as the structure of cytotoxic agents **NVX-207**.

another 2 h. Usual aqueous work-up followed by chromatography furnished products **5**–**8**, respectively.

*General procedure for the synthesis of amides **9**–**12** (GP C)*

Acetates **1**–**4** were converted with oxalyl chloride into their respective acid chlorides as described above. A solution of the acid chloride acid (0.8 g, 1.6 mmol) in dry DCM (18 mL) was slowly added to a solution of diethanolamine (0.51 mL, 5.33 mmol, 5 eq.) in dry DCM (7 mL), and the mixture was stirred for 72 h. Work-up as described above followed by chromatography gave products **9**–**12**, respectively.

*General procedure for the synthesis of amides **13**–**16** (GP D)*

The acid chloride was prepared as described above (GP B). It (0.8 g, 1.6 mmol) was dissolved in a mixture of dry pyridine (9 mL) and dry DCM (3 mL), and this solution was added slowly to a solution of TRIS (0.38 g, 1.6 mmol, 2 eq.) in dry pyridine (2 mL) in the presence of DMAP (catal.). After stirring for 72 h at 23 °C, usual aqueous work-up and chromatography, products **13**–**16** were obtained.

*General procedure for the synthesis of sulfamates **17**–**22** (GP E)*

NaH (21.7 mg, 0.64 mmol, 1 eq. per OH group) was washed under argon with *n*-heptane (3x 15 mL) and then suspended in dry DMF (10 mL). At 0 °C, the corresponding amide (0.64 mmol) was added. The mixture was stirred at 0 °C for 10 min, a solution of sulfamoyl chloride (150 mg, 1.3 mmol, 2 eq. per OH group) in dry DMF (3 mL) was slowly added, and the mixture was stirred at 23 °C for 2 days. The reaction mixture was diluted with ethyl acetate (30 mL); usual aqueous work-up followed by chromatography furnished products **17**–**22**, respectively.

Synthesis of sulfamoyl chloride

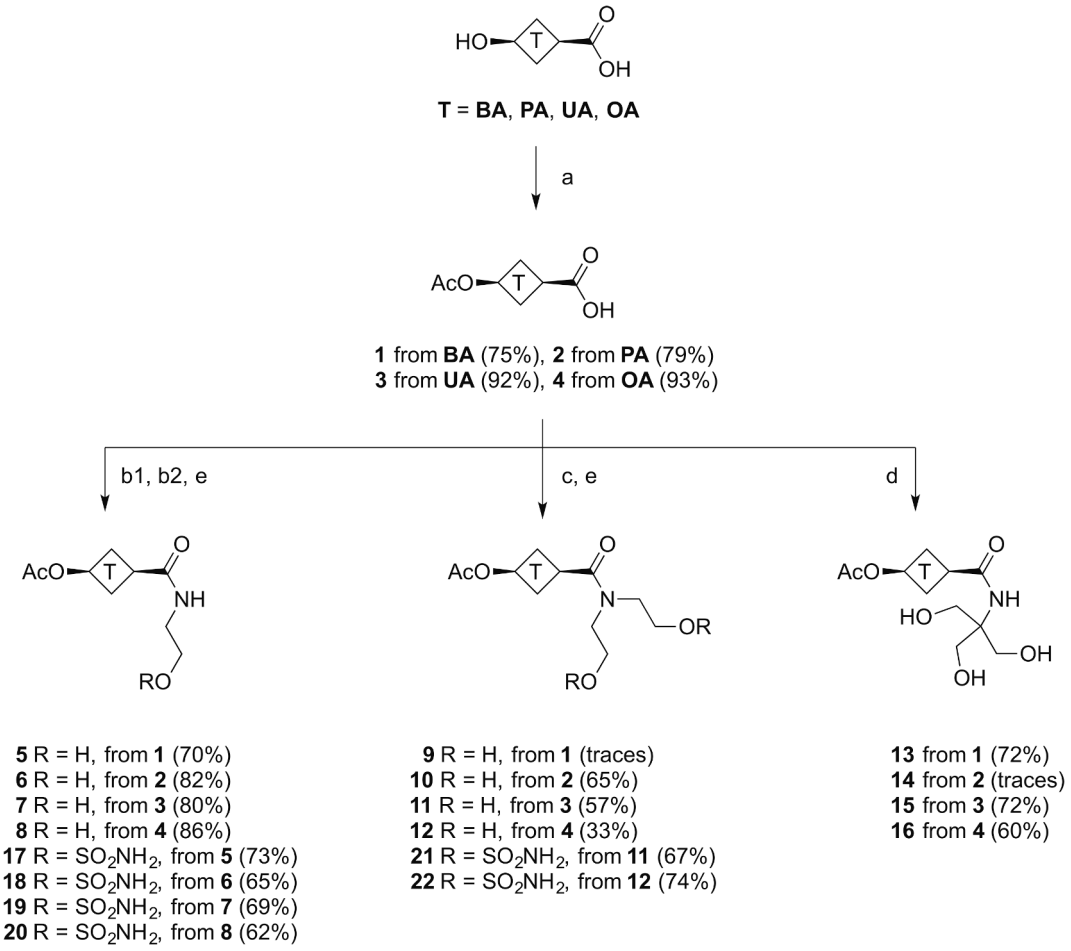
Formic acid (0.95 mL, 0.025 mol) was added slowly at 0 °C to chlorosulfonyl isocyanate (2.2 mL, 0.025 mol). After standing at 23 °C for 2 h, the crystalline product was filtered off and used in the reactions to follow; yield: 2.7 g (92%); m.p. 39–41 °C.

(3β) 3-Acetyloxy-lup-20(29)-en-28-oic acid (1)

Following GP A, from betulinic acid (**BA**, 5.0 g, 0.011 mol) **1** (4.1 g, 75%) was obtained as a colorless solid; $R_f = 0.72$ (toluene/ethyl acetate/*n*-heptane/formic acid, 80:26:10:5); m.p. 273–276 °C (lit.:[51] 280–282 °C); $[\alpha]_D^{20} = +20.6°$ ($c = 0.350$, $CHCl_3$), lit.:[52] $[\alpha]_D^{20} = +26.4°$ ($c = 0.540$, $CHCl_3$); MS (ESI): *m/z* (%) = 497 ([M−H]$^-$, 20), 995 ([2 M−H]$^-$, 100), 1018 ([2 M−2H + Na]$^-$, 32).

(3β) 3-Acetyloxy-20-oxo-30-norlupan-28-oic acid (2)

Following GP A, from platanic acid (**PA**, 5.0 g, 0.011 mol) **2** (4.3 g, 79%) was obtained as a colorless solid; $R_f = 0.50$ (toluene/ethyl acetate/*n*-heptane/formic acid, 80:26:10:5); m.p. 260–262 °C (lit.:[53] 252–255 °C); $[\alpha]_D^{20} = -9.1°$ ($c = 0.340$, $CHCl_3$), lit.:[53] $[\alpha]_D^{20} = -9.5°$ ($c = 0.800$, $CHCl_3$); MS (ESI): *m/z* (%) = 499 ([M−H]$^-$, 14), 545 ([M + HCO_2]$^-$, 15), 999 ([2 M−H]$^-$, 100).



Scheme 1. Synthesis of the target compounds; reactions and conditions: a) DCM, Ac_2O, TEA, DMAP (cat.), 21 °C, 12 h; b1, c) 1. $(COCl)_2$, DCM, DMF, 21 °C, 1 h 2. then amine, DCM, DMAP (cat.), 21 °C, 24 h; b2) DCM, PyBOP, TEA, 21 °C, 15 min, 2. then amine, 2 h, 21 °C; d) 1. $(COCl)_2$, DCM, DMF, 21 °C, 1 h 2. then tris, pyridine, DMAP (cat.), 21 °C, 24 h; e) DMF, NaH, sulfamoyl chloride, 0 °C, 48 h.

Table 1
SRB assay EC_{50} values [μM] after 72 h of treatment; averaged from three independent experiments performed each in triplicate; confidence interval CI = 95%. Human cancer cell lines: A375 (melanoma), HT29 (colorectal carcinoma), MCF-7 (breast adenocarcinoma), A2780 (ovarian carcinoma), FaDu (pharynx carcinoma), NIH 3 T3 (non-malignant fibroblasts); cut-off 30 μM, n.d. not determined; n.s. not soluble under the conditions of the assay; doxorubicin (**DX**) has been used as a positive standard.

Compound	A375	HT29	MCF-7	A2780	FaDu	NIH 3 T3
5–8	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
10	17.6 ± 1.5	> 30	> 30	> 30	> 30	> 30
11	7.5 ± 0.7	14.9 ± 0.8	7.0 ± 0.5	6.9 ± 1.3	9.5 ± 0.5	20.5 ± 0.3
12	7.7 ± 1.8	> 30	8.1 ± 1.3	13.6 ± 1.5	7.8 ± 1.2	> 30
13	12.7 ± 0.8	12.7 ± 0.8	17.2 ± 1.6	10.2 ± 0.8	12.3 ± 1.3	15.6 ± 1.1
15	9.7 ± 0.8	15.3 ± 0.7	8.2 ± 0.5	8.8 ± 1.5	11.7 ± 0.4	14.5 ± 1.2
16	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
17	4.9 ± 0.2	> 30	11.6 ± 1.6	11.1 ± 0.6	12.6 ± 0.9	> 30
18	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
19	10.4 ± 0.6	24.2 ± 2.0	13.0 ± 0.8	14.1 ± 1.1	14.6 ± 0.5	22.7 ± 1.5
20	22.5 ± 0.9	> 30	19.3 ± 1.3	24.2 ± 1.7	25.5 ± 2.2	> 30
21	8.6 ± 0.8	13.9 ± 0.8	9.2 ± 0.9	8.4 ± 0.8	12.1 ± 0.7	15.4 ± 1.8
22	10.5 ± 0.8	15.1 ± 1.1	10.2 ± 1.8	9.3 ± 0.8	13.9 ± 0.6	25.8 ± 2.2
DX	n.d.	0.9 ± 0.01	1.1 ± 0.3	0.01 ± 0.01	n.d.	0.4 ± 0.07

(3β) 3-Acetyloxy-urs-12-en-28-oic acid (3)

Following GP A, from ursolic acid (**UA**, 5.0 g, 0.011 mol) **3** (5.0 g, 92%) was obtained as a colorless solid; $R_f = 0.71$ (toluene/ethyl acetate/*n*-heptane/formic acid, 80:26:10:5); m.p. 268–271 °C (lit.:[54] 289–290 °C); $[\alpha]_D^{20} = +68.9°$ ($c = 0.315$, $CHCl_3$), lit.: [55] $[\alpha]_D^{20} = +71.2°$ ($c = 1.0$, $CHCl_3$); MS (ESI): *m/z* (%) = 499 ($[M + H]^+$, 74), 516 ($[M + NH_4]^+$, 36), 521 ($[M + Na]^+$, 34).

(3β) 3-Acetyloxy-olean-12-en-28-oic acid (4)

Following GP A, from oleanolic acid (**OA**, 5.0 g, 0.011 mol) **4** (5.1 g, 93%) was obtained as a colorless solid; $R_f = 0.75$ (toluene/ethyl acetate/*n*-heptane/formic acid, 80:26:10:5); m.p. 263–265 °C (lit.:[56] 260–261 °C); $[\alpha]_D^{20} = +71.0°$ ($c = 0.64$, $CHCl_3$), lit.:[57] $[\alpha]_D^{20} = +73.8°$ ($c = 0.8$, $CHCl_3$); MS (ESI): *m/z* (%) = 499 ($[M + H]^+$, 9), 521 ($[M + Na]^+$, 38), 1019 ($[2\ M + Na]^+$, 100).

(3β) 3-Acetyloxy-N-(2-hydroxyethyl)-lup-20(29)-en-28-amide (5)

According to GP B1, from **1** (800 mg, 1.6 mmol) followed by chromatography (SiO_2, *n*-hexane/ethyl acetate, 8:2) **5** (612 mg, 70%) was obtained as a colorless solid; $R_f = 0.29$ (toluene/ethyl acetate/*n*-heptane/formic acid, 80:26:10:5); m.p. 210–214 °C; $[\alpha]_D^{20} = +11.6°$ ($c = 0.315$, $CHCl_3$); IR (ATR): $\nu = 2940$ *m*, 1738 *s*, 1631 *s*, 1543 *m*, 1448*w*, 1391*w*, 1371 *m*, 1247 *s*, 1090 *m*, 1032 *m* cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) $\delta = 6.16–5.89$ (*m*, 1H, NH), 4.80–4.68 (*m*, 1H, 29-H_a), 4.65–4.54 (*m*, 1H, 29-H_b), 4.53–4.41 (*m*, 1H, 3-H), 3.72 (*dd*, $J = 4.9$ Hz, 2H, 34-H), 3.54–3.44 (*m*, 1H, 33-H_a), 3.43–3,30 (*m*, 1H, 33-H_b), 3.09 (*ddd*, $J = 11.1$, 4.3 Hz, 1H, 19-H), 2.52–2.33 (*m*, 1H, 13-H), 2.03 (*s*, 3H, 32-H), 2.01–1.89 (*m*, 2H, 16-H_a, 21-H_a), 1.83–1.07 (*m*, 18H, 1-H_a, 22-H_a, 12-H_a, 2-H_a, 2-H_b, 16-H_b, 18-H, 15-H_a, 22-H_b, 6-H_a, 6-H_b, 11-H_a, 7-H_a, 7-H_b, 21-H_b, 11-H_b, 9-H, 15-H_b), 1.68 (*s*, 3H, 30-H), 1.08–0.88 (*m*, 2H, 12-H_b, 1-H_b), 0.96 (*s*, 3H, 27-H), 0.94 (*s*, 3H, 26-H), 0.83 (*s*, 6H, 23-H, 25-H), 0.82 (*s*, 3H, 24-H), 0.80–0.75 (*m*, 1H, 5-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 178.2$ (C-28), 171.2 (C-31), 150.9 (C-20), 109.6 (C-29), 81.1 (C-3), 63.4 (C-34), 55.9 (C-17), 55.6 (C-5), 50.7 (C-9), 50.3 (C-18), 46.9 (C-19), 42.7 (C-14), 42.6 (C-33), 40.9 (C-8), 38.6 (C-1), 38.6 (C-22), 38.0 (C-4), 37.9 (C-13), 37.3 (C-10), 34.5 (C-7), 34.0 (C-16), 31.0 (C-21), 29.6 (C-15), 28.1 (C-23), 25.7 (C-12), 23.9 (C-2), 21.5 (C-32), 21.1 (C-11), 19.6 (C-30), 18.3 (C-6), 16.6 (C-24), 16.3 (C-26), 16.2 (C-25), 14.8 (C-27) ppm; MS (ESI): *m/z* (%) = 542 ($[M + H]^+$, 58), 564 ($[M + Na]^+$, 32), 1083 ($[2\ M + H]^+$, 12), 1105 ($[2\ M + Na]^+$, 100); analysis calcd for $C_{34}H_{55}NO_4$ (541.82): C 75.37, H 10.23, N 2.59; found: C 75.18, H 10.49, N 2.35.

(3β) 3-Acetyloxy-N-(2-hydroxyethyl)-20-oxo-30-norlupan-28-amide (6)

According to GP B1, from **2** (800 mg, 1.6 mmol) followed by chromatography (SiO_2, *n*-hexane/ethyl acetate, 8:2) **6** (712 mg, 82%) was

Table 2
Inhibitory percentage for compounds **10**–**22** as determined in a photometric assay with 4-nitrophenyl acetate and carbonic anhydrase II (from bovine erythrocytes); each experiment was performed in duplicate; compounds **5**–**8**, **16** and **18** were not soluble under the conditions of the assay.

Compound	Inhibition %	Compound	Inhibition %
10	14 ± 3	**17**	20 ± 3
11	12 ± 1	**19**	< 5
12	8 ± 0.6	**20**	< 5
13	< 5	**21**	16 ± 2
14	8 ± 3	**22**	< 5
15	< 5		

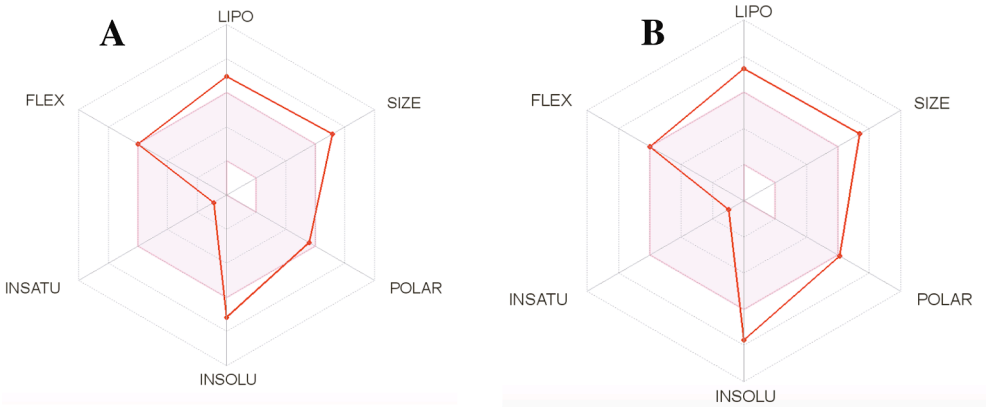


Fig. 2. Calculated ADME parameters for **NVX-207** (A) and compound **17** (B).

obtained as a colorless solid; R_f = 0.15 (toluene/ethyl acetate/*n*-heptane/formic acid, 80:26:10:5); m.p. 300–303 °C; $[\alpha]_D^{20}$ = +30.8° (*c* = 0.327, $CHCl_3$); IR (ATR): ν = 3365*w*, 2948 *m*, 1726 *m*, 1694 *m*, 1642 *m*, 1548 *m*, 1450*w*, 1392*w*, 1365 *m*, 1314*w*, 1240 *s*, 1196*w*, 1081 *m*, 1021 *m* cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ = 6.15 (*t*, *J* = 5.7 Hz, 1H, NH), 5.29 (*s*, 1H, OH), 4.52–4.36 (*m*, 1H, 3-H), 3.72 (*dd*, *J* = 4.8 Hz, 2H, 33-H), 3.55–3.18 (*m*, 3H, 19-H, 32-H), 2.26–2.18 (*m*, 1H, 13-H), 2.16 (*s*, 3H, 29-H), 2.12–2.04 (*m*, 2H, 18-H, 21-H_a), 2.03 (*s*, 3H, 31-H), 1.96–1.88 (*m*, 1H, 16-H_a), 1.81–1.72 (*m*, 1H, 22-H_a), 1.71–1.03 (*m*, 17H, 1-H_a, 2-H_a, 2-H_b, 6-H_a, 6-H_b, 7-H_a, 7-H_b, 9-H, 11-H_a, 11-H_b, 12-H_a, 12-H_b, 15-H_a, 15-H_b, 16-H_b, 21-H_b, 22-H_b), 0.99 (*s*, 3H, 27-H), 1.04–0.91 (*m*, 1H, 1-H_b), 0.91 (*s*, 3H, 26-H), 0.83 (*s*, 6H, 23-H, 25-H), 0.82 (*s*, 3H, 24-H), 0.80–0.75 (*m*, 1H, 5-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ = 213.0 (C-20), 178.0 (C-28), 171.2 (C-30), 81.0 (C-3), 63.2 (C-33), 55.7 (C-17), 55.5 (C-5), 51.2 (C-19), 50.5 (C-9), 50.1 (C-18), 42.5 (C-32), 42.4 (C-14), 40.8 (C-8), 38.5 (C-1), 38.1 (C-22), 37.9 (C-4), 37.3 (C-10), 37.0 (C-13), 34.3 (C-7), 33.2 (C-16), 30.4 (C-29), 29.6 (C-15), 28.7 (C-21), 28.1 (C-23), 27.3 (C-12), 23.8 (C-2), 21.4 (C-31), 21.1 (C-11), 18.3 (C-6), 16.6 (C-24), 16.3 (C-25), 16.1 (C-26), 14.8 (C-27) ppm; MS (ESI): *m/z* (%) = 544 ($[M + H]^+$, 45), 566 ($[M + Na]^+$, 25), 1087 ($[2\,M + H]^+$, 12), 1109 ($[2\,M + Na]^+$, 100); analysis calcd for $C_{33}H_{53}NO_5$ (543.79): C 72.89, H 9.82, N 2.58; found: C 72.50, H 10.03, N 2.41.

(3β) 3-Acetyloxy-N-(2-hydroxyethyl)-urs-12-en-28-amide (7)

According to GP B1, from **3** (800 mg, 1.6 mmol) followed by chromatography (SiO_2, *n*-hexane/ethyl acetate, 8:2) **7** [58] (691 mg, 80%) was obtained as a colorless solid; R_f = 0.29 (*n*-hexane/ethyl acetate, 1:2); m.p. 222–224 °C (lit.:[59] 184–186 °C); $[\alpha]_D^{20}$ = +42.2° (*c* = 0.335, $CHCl_3$); IR (ATR): ν = 2924 *m*, 1733 *s*, 1631 *s*, 1529 *m*, 1454*w*, 1369 *m*, 1250 *s*, 1203*w*, 1080*w*, 1025 *m* cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ = 6.36 (*t*, *J* = 5.5 Hz, 1H, NH), 5.33 (*dd*, *J* = 3.6, 3.6 Hz, 1H, 12-H), 4.54–4.44 (*m*, 1H, 3-H), 3.68 (*dd*, *J* = 5.7, 4.2 Hz, 2H, 34-H), 3.51–3.40 (*m*, 1H, 33-H_a), 3.29–3.17 (*m*, 1H, 33-H_b), 2.04 (*s*, 3H, 32-H), 2.01–1.94 (*m*, 3H, 16-H_a, 11-H_a, 11-H_b), 1.93–1.83 (*m*, 2H, 18-H, 22-H_a), 1.75–1.61 (*m*, 5H, 16-H_b, 15-H_a, 1-H_a, 2-H_a, 2-H_b), 1.58–1.20 (*m*, 9H, 9-H, 6-H_a, 21-H_a, 7-H_a, 22-H_b, 19-H, 6H_b, 21-H_b, 7-H_b), 1.09 (*s*, 3H, 27-H), 1.09–1.02 (*m*, 2H, 1-Hb, 15-H_b), 0.99–0.90 (*m*, 7H, 20-H, 30-H, 25-H), 0.88 (*s*, 3H, 29-H), 0.86 (*s*, 3H, 23-H), 0.85 (*s*, 3H, 24-H), 0.85–0.81 (*m*, 1H, 5-H), 0.81 (*s*, 3H, 26-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ = 180.1 (C-28), 171.1 (C-31), 139.8 (C-13), 125.9 (C-12), 80.9 (C-3), 62.9 (C-34), 55.4 (C-5), 54.0 (C-18), 48.0 (C-17), 47.6 (C-9), 43.1 (C-33), 42.6 (C-14), 39.9 (C-19), 39.8 (C-8), 39.2 (C-20), 38.5 (C-1), 37.8 (C-4), 37.4 (C-10), 37.0 (C-22), 32.8 (C-7), 31.0 (C-21), 28.2 (C-23), 28.0 (C-15), 25.1 (C-16), 23.7 (C-2), 23.6 (C-11), 23.4 (C-27), 21.4 (C-32), 21.4 (C-30), 18.3 (C-6), 17.4 (C-29), 17.0 (C-26), 16.9 (C-24), 15.7 (C-25) ppm; MS (ESI): *m/z* (%) = 542 ($[M + H]^+$, 50), 564 ($[M + Na]^+$, 20), 1083 ($[2\,M + H]^+$, 12), 1105 ($[2\,M + Na]^+$, 100); analysis calcd for $C_{34}H_{55}NO_4$ (541.82): C 75.37, H 10.23, N 2.59; found: C 75.16, H 10.31, N 2.38.

(3β) 3-Acetyloxy-N-(2-hydroxyethyl)-olean-12-en-28-amide (8)

According to GP B1, from **4** (800 mg, 1.6 mmol) followed by chromatography (SiO_2, *n*-hexane/ethyl acetate, 8:2) **8** (744 mg, 86%) was obtained as a colorless solid; R_f = 0.23 (*n*-hexane/ethyl acetate, 1:2); m.p. 170–173 °C (decomp.); $[\alpha]_D^{20}$ = +53.0° (*c* = 0.327, $CHCl_3$); IR (ATR): ν = 2943 *m*, 1732 *m*, 1627 *m*, 1520*w*, 1463*w*, 1370*w*, 1245 *s*, 1087*w*, 1026 *m* cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ = 6.43 (*t*, 1H, NH), 5.38 (*dd*, *J* = 7, 7 Hz, 1H, 12-H), 4.54–4.43 (*m*, 1H, 3-H), 3.68 (*dd*, *J* = 4.9 Hz, 2H, 34-H), 3.53–3.41 (*m*, 1H, 33-H_a), 3.31–3.21 (*m*, 1H, 33-H_b), 2.54 (*dd*, *J* = 13.1, 4.6 Hz, 1H, 18-H), 2.03 (*s*, 3H, 32-H), 2.02–1.94 (*m*, 1H, 16-H_a), 1.93–1.87 (*m*, 2H, 11-H), 1.79–1.71 (*m*, 1H, 19-H_a), 1.81–1.31 (*m*, 12H, 7-H_a, 16-H_b, 2-H_a, 2-H_b, 1-H_a, 7-H_b, 9-H, 15-H_a, 6-H_a, 22-H_a, 6-H_b, 21-H_a), 1.31–1.17 (*m*, 3H, 22-H_b, 21-H_b, 19-H_b), 1.16 (*s*, 3H, 27-H), 1.07–1.00 (*m*, 2H, 15-H_b, 1-H_b), 0.93 (*s*, 3H, 25-H), 0.90 (*s*, 6H, 29-H, 30-H), 0.86 (*s*, 3H, 23-H), 0.85 (*s*, 3H, 24-H), 0.83–0.80 (*m*, 1H, 5-H), 0.79 (*s*, 3H, 26-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ = 180.2 (C-28), 171.0 (C-31), 144.6 (C-13), 122.9 (C-12), 80.8 (C-3), 62.8 (C-34), 55.2 (C-5), 47.5 (C-9), 46.7 (C-19), 46.3 (C-17), 43.0 (C-33),42.2 (C-18), 42.0 (C-14), 39.4 (C-8), 38.2 (C-1), 37.7 (C-4), 36.8 (C-10), 34.1 (C-21), 32.9 (C-29), 32.5 (C-7), 32.2 (C-22), 30.7 (C-20), 28.0 (C-23), 27.2 (C-15), 25.7 (C-27), 23.8 (C-30), 23.6 (C-2), 23.5 (C-11), 23.5 (C-16), 21.3 (C-32), 18.1 (C-6), 16.8 (C-26), 16.6 (C-24), 15.4 (C-25) ppm; MS (ESI): *m/z* (%) = 542 ($[M + H]^+$, 67), 564 ($[M + Na]^+$, 22), 1083 ($[2\,M + H]^+$, 14), 1105 ($[2\,M + Na]^+$, 100); analysis calcd for $C_{34}H_{55}NO_4$ (541.82): C 75.37, H 10.23, N 2.59; found: C 75.02, H 10.51, N 2.26.

(3β) 3-Acetyloxy-N, N-bis(2-hydroxyethyl)-20-oxo-30-norlupan-28-amide (10)

According to GP C, from **2** (800 mg, 1.6 mmol) followed by chromatography (SiO_2, *n*-hexane/ethyl acetate, 8:2) **10** (650 mg, 65%) was obtained as a colorless solid; R_f = 0.13 ($CHCl_3$/MeOH, 9:1); m.p. 264–267 °C; $[\alpha]_D^{20}$ = -12.9° (*c* = 0.323, $CHCl_3$); IR (ATR): ν = 2942 *m*, 1799*w*, 1712 *s*, 1451*w*, 1368 *m*, 1243 *s*, 1132 *m*, 1104 *m*, 1020 *s* cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ = 4.46 (*dd*, *J* = 10.8, 5.2 Hz, 1H, 3-H), 4.27–4.13 (*m*, 4H, 33-H, 35-H), 3.93–3.34 (*m*, 4H, 32-H, 34-H), 3.21 (*ddd*, *J* = 21.6, 10.8, 10.8 Hz, 1H, 19-H), 2.23–2.18 (*m*, 1H, 16-H_a), 2.16 (*s*, 3H, 29-H), 2.13–2.06 (*m*, 1H, 18-H), 2.03 (*s*, 3H, 31-H), 2.01–1.94 (*m*, 1H, 13-H), 1.94–1.78 (*m*, 2H, 21-H_a, 22-H_a), 1.71–1.54 (*m*, 5H, 1-H_a, 16-H_b, 2-H_a, 2-H_b, 22-H_b), 1.54–1.10 (*m*, 10*H*, 6-H_a, 6-H_b, 21-H_b, 11-H_a, 7-H_a, 7-H_b, 9-H, 15-H_a, 15-H_b, 11-H_b), 1.10–1.01 (*m*, 2H, 12-H_a, 12-H_b), 1.01–0.95 (*m*, 1H, 1-H_b), 0.98 (*s*, 3H, 27-H), 0.91 (*s*, 3H, 25-H), 0.88 (*s*, 3H, 26-H), 0.83 (*s*, 3H, 23-H), 0.82 (*s*, 3H, 24-H), 0.80–0.75 (*m*, 1H, 5-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ = 212.9 (C-20), 175.8 (C-28), 171.1 (C-30), 81.0 (C-3), 62.8 (C-32, C-34), 61.7 (C-33, C-35), 56.6 (C-17), 55.5 (C-5), 51.1 (C-19), 50.8 (C-9), 49.5 (C-18), 42.3 (C-14), 40.8 (C-8), 38.5 (C-1), 38.0 (C-4), 37.4 (C-13), 37.4 (C-10), 36.7 (C-22), 34.3 (C-7), 32.1 (C-16), 30.2 (C-29), 29.9 (C-15), 29.0 (C-21), 28.1 (C-23), 27.3 (C-12), 23.8 (C-2), 21.4 (C-31), 21.3 (C-11), 18.3 (C-6), 16.3 (C-24), 16.2 (C-26), 16.1 (C-25), 14.8 (C-27) ppm; MS (ESI): *m/z* (%) = 588 ($[M + H]^+$, 100), 610 ($[M + Na]^+$, 12), 1175 ($[2\,M + H]^+$, 22), 1198 ($[2\,M + Na]^+$, 28); analysis calcd for $C_{35}H_{57}NO_6$ (587.84): C 71.51, H 9.77, N 2.38; found: C 71.24, H 9.98, N 2.03.

(3β) 3-Acetyloxy-N, N-bis(2-hydroxyethyl)-urs-12-en-28-amide (11)

According to GP C, from **3** (800 mg, 1.6 mmol) followed by chromatography (SiO_2, *n*-hexane/ethyl acetate, 2:8) **11** (539 mg, 57%) was obtained as a colorless solid; R_f = 0.14 (toluene/ethyl acetate/*n*-heptane/formic acid, 80:26:10:5); m.p. 252–254 °C (decomp.) (lit.:[60] 226–228 °C); $[\alpha]_D^{20}$ = 19.6° (*c* = 0.251, $CHCl_3$); IR (ATR): ν = 3267*w*, 2923 *m*, 1732 *s*, 1590 *s*, 1463 *m*, 1411 *m*, 1393 *m*, 1376 *m*, 1247 *s*, 1203*w*, 1047 *s*, 1032 *s*, 1004 *m* cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ = 5.27 (*dd*, *J* = 3.7, 3.7 Hz, 1H, 12-H), 4.48 (*dd*, *J* = 10.5, 5.5 Hz, 1H, 3-H), 4.13–4.02 (*m*, 4H, 34-H, 36-H), 4.02–3.91 (*m*, 4H, 33-H, 35-H), 2.40 (*ddt*, *J* = 14.8, 3.7 Hz, 1H, 16-H_a), 2.36–2.28 (*m*, 1H, 18-H), 2.19–2.07 (*m*, 1H, 22-H_a), 2.04 (*s*, 3H, 32-H), 2.01–1.74 (*m*, 4H, 22-H_b, 11-H_a, 16-H_b, 2-H_a), 1.73–1.40 (*m*, 9H, 11-H_b, 2-H_a, 21-H_a, 1-H_a, 6-H_a, 15-H_a, 9-H, 7-H_a, 19-H), 1.40–1.16 (*m*, 4H, 6-H_b, 21-H_b, 7-H_b, 15-H_b), 1.12 (*s*, 3H, 27-H), 1.10–1.02 (*m*, 2H, 1-H_b, 20-H), 0.96 (*s*, 3H, 30-H), 0.94 (*s*, 3H, 25-H), 0.88 (*s*, 3H, 24-H), 0.86 (*s*, 3H, 23-H), 0.86 (*d*, *J* = 5.3 Hz, 3H, 29-H), 0.83–0.79 (*m*, 1H, 5-H), 0.75 (*s*, 3H, 26-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ = 182.6 (C-28), 171.1 (C-31), 136.9 (C-13), 127.8 (C-12), 80.8 (C-3), 57.1 (C-34, C-36), 55.3 (C-5), 53.6 (C-18), 51.6 (C-33, C- 35), 47.5 (C-17), 47.3 (C-9), 42.2 (C-14), 39.7 (C-8), 39.2 (C-19), 38.3 (C-1), 38.2 (C-20), 37.8 (C-4), 37.0 (C-10), 35.4 (C-22), 32.6 (C-7), 30.0 (C-21), 28.2 (C-23), 28.1 (C-15), 24.3 (C-16), 24.1 (C-27), 23.6 (C-2), 23.5 (C-11), 21.4 (C-32), 21.0 (C-30), 18.2 (C-6), 17.1 (C-26), 16.9 (C-24), 16.8

(C-29), 15.6 (C-25) ppm; MS (ESI): *m/z* (%) = 586 ($[M + H]^+$, 50), 608 ($[M + Na]^+$, 18), 1171 ($[2\ M + H]^+$, 5), 1193 ($[2\ M + Na]^+$, 100); analysis calcd for $C_{36}H_{59}NO_5$ (585.87): C 73.80, H 10.15, N 2.39; found: C 73.55, H 10.38, N 2.15.

(3β) 3-Acetyloxy-N,N-bis(2-hydroxyethyl)-olean-12-en-28-amide (12)

According to GP C from **4** (800 mg, 1.6 mmol) followed by chromatography (SiO_2, *n*-hexane/ethyl acetate, 2:8) **12** (307 mg, 33%) was obtained as a colorless solid; R_f = 0.14 (toluene/ethyl acetate/*n*-heptane/formic acid, 80:26:10:5); m.p. 278–280 °C; $[\alpha]_D^{20}$ = +29.8° (*c* = 0.305, $CHCl_3$); IR (ATR): ν = 3366*w*, 2946 *m*, 1720 *s*, 1594 *s*, 1464 *m*, 1393 *m*, 1373 *m*, 1259 *s*, 1036 *m*, 1007 *s* cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ = 5.21 (*dd*, *J* = 3.7, 3.7 Hz, 1H, 12-H), 4.46 (*dd*, *J* = 11.1, 5.0 Hz, 1H, 3-H), 3.74–3.67 (*m*, 4H, 34-H, 36-H), 3.58–3.47 (*m*, 4H, 33-H, 35-H), 3.07–2.98 (*m*, 1H, 18-H), 2.25–2.11 (*m*, 1H, 16-H_a), 2.03 (*s*, 3H, 32-H), 1.96–1.72 (*m*, 5H, 2-H_a, 22-H_a, 11-H_a, 16-H_b, 19-H_a), 1.70–1.36 (*m*, 10*H*, 11-H_b, 1-H_a, 2-H_b, 15-H_a, 22-H_b, 9-H, 6-H_a, 7-H_a, 6-H_b, 21-H_a), 1.35–1.27 (*m*, 1H, 7-H_b), 1.27–1.20 (*m*, 1H, 21-H_b), 1.18 (*s*, 3H, 27-H), 1.16–1.00 (*m*, 3H, 19-H_b, 15-H_b, 1-H_b), 0.99 (*s*, 3H, 25-H), 0.94 (*s*, 3H, 29-H), 0.91 (*s*, 3H, 30-H), 0.89 (*s*, 3H, 23-H), 0.88 (*s*, 3H, 24-H), 0.87–0.83 (*m*, 1H, 5-H), 0.81 (*s*, 3H, 26-H) ppm; ^{13}C NMR (100 MHz, CD_3OD) δ = 179.0 (C-28), 172.9 (C-31), 146.1 (C-13), 122.8 (C-12), 82.5 (C-3), 60.9 (C-34, C-36), 56.8 (C-5), 52.6 (C-33, C-35), 49.4 (C-17), 49.1 (C-9), 48.1 (C-19), 45.2 (C-18), 43.2 (C-14), 40.6 (C-8), 39.4 (C-1), 38.8 (C-4), 38.2 (C-10), 35.1 (C-21), 34.0 (C-7), 33.4 (C-29), 31.2 (C-22), 31.0 (C-20), 29.4 (C-15), 28.6 (C-23), 26.3 (C-27), 24.5 (C-2), 24.5 (C-11), 24.5 (C-30), 23.5 (C-16), 21.1 (C-32), 19.4 (C-6), 17.8 (C-26), 17.2 (C-24), 16.0 (C-25) ppm; MS (ESI): *m/z* (%) = 586 ($[M + H]^+$, 60), 608 ($[M + Na]^+$, 20), 1193 ($[2\ M + Na]^+$, 100); analysis calcd for $C_{36}H_{59}NO_5$ (585.87): C 73.80, H 10.15, N 2.39; found: C 73.59, H 10.39, N 2.08.

(3β) 3-Acetyloxy-N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]-lup-20 (29)-en-28-amide (13)

According to GP D from **1** (800 mg, 1.6 mmol) followed by chromatography (SiO_2, *n*-hexane/ethyl acetate, 2:8) **13** [61] (692 mg, 72%) was obtained as a colorless solid; R_f = 0.19 (toluene/ethyl acetate/*n*-heptane/formic acid, 80:26:10:5); m.p. 218–222 °C (decomp.) (lit.:[62] 184 °C); $[\alpha]_D^{20}$ = +4.9° (*c* = 0.298, $CHCl_3$); IR (ATR): ν = 3403*w*, 2942 *s*, 2870 *m*, 1732 *s*, 1637 *m*, 1507 *m*, 1454 *m*, 1368 *s*, 1243 *s*, 1108*w*, 1023 *s* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ = 6.71 (*s*, 1H, NH), 4.73 (*s*, 1H, 29-H_a), 4.63–4.59 (*m*, 1H, 29-H_b), 4.46 (*dd*, *J* = 10.4, 5.8 Hz, 1H, 3-H), 3.64 (*s*, 6H, 34-H, 35-H, 36-H), 3.03 (*ddt*, *J* = 11.1, 4.2 Hz, 1H, 19-H), 2.37 (*ddt*, *J* = 12.6, 12.2, 3.7 Hz, 1H, 13-H), 2.04 (*s*, 3H, 32-H), 2.02–1.80 (*m*, 2H, 22-H_a, 21-H_a), 1.74–1.12 (*m*, 18H, 1-H_a, 12-H_a, 2-H_a, 2-H_b, 22-H_b, 18-H, 6-H_a, 11-H_a, 15-H_a, 21-H_b, 7-H_a, 7-H_b, 6-H_b, 9-H, 16-H_a, 16-H_b, 11-H_b, 15-H_b), 1.68 (*s*, 3H, 30-H), 1.03–0.97 (*m*, 2H, 12-H_b, 1-H_b), 0.96 (*s*, 3H, 27-H), 0.94 (*s*, 3H, 26-H), 0.85 (*s*, 3H, 25-H), 0.84 (*s*, 3H, 23-H), 0.83 (*s*, 3H, 24-H), 0.80–0.76 (*m*, 1H, 5-H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ = 179.0 (C-28), 171.2 (C-31), 150.7 (C-20), 109.8 (C-29), 80.9 (C-3), 62.6 (C-34, C-35, C-36), 61.2 (C-33), 56.5 (C-17), 55.6 (C-5), 50.7 (C-9), 49.9 (C-18), 46.9 (C-19), 42.7 (C-14), 41.0 (C-8), 38.6 (C-1), 38.0 (C-13), 38.0 (C-4), 37.3 (C-10), 34.4 (C-7), 34.2 (C-22), 32.0 (C-16), 31.0 (C-21), 29.6 (C-15), 28.1 (C-23), 25.7 (C-12), 23.8 (C-2), 21.5 (C-32), 21.1 (C-11), 19.6 (C-30), 18.3 (C-6), 16.6 (C-24), 16.4 (C-25), 16.2 (C-26), 14.8 (C-27) ppm; MS (ESI): *m/z* (%) = 602 ($[M + H]^+$, 100), 624 ($[M + Na]^+$, 52), 1225 ($[2\ M + Na]^+$, 13); analysis calcd for $C_{36}H_{59}NO_6$ (601.87): C 71.84, H 9.88, N 2.33; found: C 71.63, H 10.07, N 1.96.

(3β) 3-Acetyloxy-N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]-urs-12-en-28-amide (15)

According to GP D, from **3** (800 mg, 1.6 mmol) followed by chromatography (SiO_2, *n*-hexane/ethyl acetate, 2:8) **15** (680 mg, 72%) was obtained as a colorless solid; R_f = 0.16 (toluene/ethyl acetate/*n*-heptane/formic acid, 80:26:10:5); m.p. 214–217 °C (decomp.); $[\alpha]_D^{20}$ = +31.7° (*c* = 0.137, $CHCl_3$); IR (ATR): ν = 3304*w*, 2923 *m*, 1737 *m*, 1622 *m*, 1514 *m*, 1454 *m*, 1371 *m*, 1240 *s*, 1051 *m*, 1029 *s* cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ = 7.14 (*s*, 1H, NH), 5.35 (*dd*, *J* = 3.5, 3.5 Hz, 1H, 12-H), 5.30 (*s*, 3H, OH), 4.49 (*m*, 1H, 3-H), 3.77–3.36 (*m*, 6H, 34-H, 35-H, 36-H), 2.08–2.01 (*m*, 1H, 11-H_a), 2.04 (*s*, 3H, 32-H), 1.99–1.74 (*m*, 3H, 2-H_a, 18-H, 22-H_a), 1.74–1.46 (*m*, 9H, 11-H_b, 1-H_a, 16-H_a, 16-H_b,15-H_a, 6-H_a, 21-H_a, 9-H, 7-H_a), 1.45–1.28 (*m*, 6H, 22-H_b, 19-H, 6-H_b, 7-H_b, 21-H_b, 2-H_b), 1.17–1.04 (*m*, 2H, 15-H_b, 1-H_b), 1.10 (*s*, 3H, 27-H), 1.03–0.98 (*m*, 1H, 20-H), 0.96 (*s*, 3H, 25-H), 0.95 (*s*, 3H, 30-H), 0.88 (*d*, *J* = 8.1 Hz, 3H, 29-H), 0.87 (*s*, 3H, 23-H), 0.86 (*s*, 3H, 24-H), 0.85 (*s*, 3H, 26-H), 0.82–0.79 (*m*, 1H, 5-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ = 180.8 (C-28), 171.2 (C-31), 137.7 (C-13), 127.0 (C-12), 81.0 (C-3), 62.4 (C-33), 61.3 (C-34, C-35, C-36), 55.4 (C-5), 54.1 (C-18), 48.8 (C-17), 47.6 (C-9), 42.6 (C-14), 40.0 (C-19), 39.9 (C-8), 39.0 (C-20), 38.6 (C-1), 38.3 (C-4), 37.8 (C-22), 37.0 (C-10), 33.0 (C-7), 31.0 (C-21), 28.2 (C-23), 28.0 (C-15), 24.9 (C-11), 23.7 (C-16), 23.6 (C-2), 23.3 (C-27), 21.5 (C-32), 21.3 (C-30), 18.3 (C-6), 17.6 (C-26), 17.2 (C-29), 16.9 (C-24), 15.8 (C-25) ppm; MS (ESI): *m/z* (%) = 602 ($[M + H]^+$, 82), 624 ($[M + Na]^+$, 50), 1225 ($[2\ M + Na]^+$, 100); analysis calcd for $C_{36}H_{59}NO_6$ (601.87): C 71.84, H 9.88, N 2.33; found: C 71.55, H 10.03, N 2.19.

(3β) 3-Acetyloxy-N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]-olean-12-en-28-amide (16)

According to GP D from **4** (800 mg, 1.6 mmol) followed by chromatography (SiO_2, *n*-hexane/ethyl acetate, 2:8) **16** (586 mg, 60%) was obtained as a colorless solid; R_f = 0.16 (toluene/ethyl acetate/*n*-heptane/formic acid, 80:26:10:5); m.p. 234–238 °C (decomp.); $[\alpha]_D^{20}$ = +44.4° (*c* = 0.253, $CHCl_3$); IR (ATR): ν = 3341*w*, 2938 *m*, 1727 *s*, 1616 *m*, 1521 *m*, 1459 *m*, 1363 *m*, 1242 *s*, 1059 *m*, 1028 *s* cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ = 7.03 (*s*, 1H, NH), 5.37 (dd, *J* = 13.2, 7.2 Hz, 1H, 12-H), 4.52–4.45 (*m*, 1H, 3-H), 3.66–3.45 (*m*, 6H, 34-H, 35-H, 36-H), 2.56 (*dd*, *J* = 12.9, 4.3 Hz, 1H, 18-H), 2.04 (*s*, 3H, 32-H), 2.03–1.94 (*m*, 1H, 16-H_a), 1.94–1.79 (*m*, 2H, 11-H_a, 11-H_b), 1.79–1.25 (*m*, 14H, 19-H_a, 2-H_a, 1-H_a, 2-H_b, 22-H_a, 22-H_b, 16-H_b, 6-H_a, 9-H, 15-H_a, 7-H_a, 21-H_a, 6-H_b, 7-H_b), 1.25–1.03 (*m*, 4H, 21-H_b, 19-H_b, 15-H_b, 1-H_b), 1.16 (*s*, 3H, 27-H), 0.94 (*s*, 3H, 25-H), 0.91 (*s*, 6H, 29-H, 30-H), 0.88–0.78 (*m*, 1H, 5-H), 0.86 (*s*, 3H, 23-H), 0.85 (*s*, 3H, 24-H), 0.82 (*s*, 3H, 26-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ = 180.4 (C-28), 171.2 (C-31), 143.1 (C-13), 123.6 (C-12), 81.0 (C-3), 61.9 (C-33), 61.4 (C-34, C-36, C-35), 55.4 (C-5), 47.6 (C-9), 47.3 (C-17), 46.7 (C-19), 42.5 (C-18), 42.2 (C-14), 39.7 (C-8), 38.4 (C-1), 37.8 (C-4), 37.0 (C-10), 34.3 (C-21), 33.5 (C-22), 33.1 (C-30), 32.7 (C-7), 30.8 (C-20), 28.2 (C-23), 27.5 (C-15), 25.8 (C-27), 23.8 (C-16), 23.8 (C-29), 23.7 (C-2), 23.7 (C-11), 21.5 (C-32), 18.3 (C-6), 17.5 (C-26), 16.8 (C-24), 15.7 (C-25) ppm; MS (ESI): *m/z* (%) = 602 ($[M + H]^+$, 82), 624 ($[M + Na]^+$, 50), 1225 ($[2\ M + Na]^+$, 100); analysis calcd for $C_{36}H_{59}NO_6$ (601.87): C 71.84, H 9.88, N 2.33; found: C 71.64, H 10.05, N 2.08.

(3β) 3-Acetyloxy-N-(2-aminosulfonyloxyethyl)-lup-20(29)-en-28-amide (17)

According to GP E, from **5** (350 mg, 0.65 mmol) followed by chromatography (SiO_2, $CHCl_3$/MeOH, 9:1) **17** (294 mg, 73%) was obtained as a colorless solid; R_f = 0.29 (toluene/ethyl acetate/*n*-heptane/formic acid, 80:26:10:5); m.p. 170–173 °C; $[\alpha]_D^{20}$ = +19.7° (*c* = 0.340, $CHCl_3$); IR (ATR): ν = 1731 *m*, 1631 *m*, 1248 *s* cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ = 6.03 (*t*, *J* = 4.6 Hz, 1H, NH), 4.73 (*d*, *J* = 2.3 Hz, 1H, 29-H_a), 4.62–4.57 (*m*, 1H, 29-H_b), 4.49–4.42 (*m*, 1H, 3-H), 3.79–3.38 (*m*, 4H, 33-H, 34-H), 3.09 (*ddd*, *J* = 11.1, 4.3 Hz, 1H, 19-H), 2.42 (*ddd*, *J* = 12.9, 11.4, 3.7 Hz, 1H, 13-H), 2.03 (*s*, 3H, 32-H), 2.00–1.90 (*m*, 2H, 16-H_a, 21-H_a), 1.80–1.61 (*m*, 3H, 22-H_a, 12-H_a, 1-H_a), 1.68 (*s*, 3H, 30-H),

1.61–1.28 (*m*, 11*H*, 2-H, 18-H, 16-H_b, 15-H_a, 6-H_a, 22-H_b, 11-H_a, 21-H_b, 6-H_b, 7-H), 1.28–1.19 (*m*, 2H, 9-H, 11-H_b), 1.18–1.10 (*m*, 1H, 15-H_b), 1.04–0.87 (*m*, 2H, 12-H_b, 1-H_b), 0.96 (*s*, 3H, 27-H), 0.93 (*s*, 3H, 26-H), 0.83 (*s*, 3H, 23-H), 0.83 (*s*, 3H, 25-H), 0.82 (*s*, 3H, 24-H), 0.80–0.74 (*m*, 1H, 5-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ = 176.7 (C-28), 171.2 (C-31), 150.9 (C-20), 109.6 (C-29), 81.1 (C-3), 56.0 (C-17), 55.6 (C-5), 50.7 (C-9), 50.2 (C-18), 47.0 (C-19), 44.5 (C-34), 42.6 (C-14), 41.2 (C-33), 40.9 (C-8), 38.6 (C-1), 38.5 (C-22), 37.9 (C-4), 37.9 (C-13), 37.3 (C-10), 34.5 (C-7), 33.9 (C-16), 31.0 (C-21), 29.6 (C-15), 28.1 (C-23), 25.7 (C-12), 23.8 (C-2), 21.5 (C-32), 21.1 (C-11), 19.6 (C-30), 18.3 (C-6), 16.6 (C-24), 16.3 (C-26), 16.2 (C-25), 14.8 (C-27) ppm; MS (ESI): *m/z* (%) = 239 ([M−$H_2N(CH_2)_2SO_3NH_2$-AcO + H + K]$^{2+}$, 43), 524 ([M−SO_3NH_2 + H]$^+$, 43); analysis calcd for $C_{34}H_56N_2O_6S$ (620.89): C 65.77, H 9.09, N 4.51; found: C 65.47, H 9.24, N 4.35.

(3β) 3-Acetyloxy-N-(2-aminosulfonyloxyethyl)-20-oxo-30-norlupan-28-amide (18)

According to GP E, from **6** (350 mg, 0.64 mmol) followed by chromatography (SiO_2, $CHCl_3$/MeOH, 9:1) **18** (257 mg, 65%) was obtained as a colorless solid; R_f = 0.25 ($CHCl_3$/MeOH, 8:2); m.p. 208–212 °C; $[\alpha]_D^{20}$ = -17.8° (*c* = 0.226, $CHCl_3$); IR (ATR): ν = 2941 *s*, 2870 *m*, 1732 *m*, 1709 *s*, 1651 *m*, 1449 *m*, 1358 *s*, 1244 *s*, 1197*w*, 1164*w*, 1123 *m*, 1037 *m*, 1027 *m* cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.05 (*t*, *J* = 5.3 Hz, 1H, NH), 4.46 (*dd*, *J* = 10.8, 5.2 Hz, 1H, 3-H), 4.24–4.09 (*m*, 2H, 33-H), 3.92–3.82 (*m*, 2H, 32-H), 3.47–3.35 (*m*, 1H, 19-H), 2.30–2.19 (*m*, 2H, 13-H, 16-H_a), 2.18 (*s*, 3H, 29-H), 2.14–2.07 (*m*, 1H, 18-H), 2.06–2.04 (*m*, 1H, 21-H_a), 2.03 (*s*, 3H, 31-H), 1.98–1.72 (*m*, 2H, 22-H_a), 1.71–1.23 (*m*, 17H, 1-H_a, 16-H_b, 2-H_a, 2-H_b, 12-H_a, 22-H_b, 21-H_b, 6-H_a, 6-H_b, 15-H_a, 11-H_a, 7-H_a, 7-H_b, 9-H, 11-H_b), 1.21–1.02 (*m*, 2H, 15-H_b, 12-H_b), 0.99–0.85 (*m*, 1H, 1-H_b), 0.98 (*s*, 3H, 25-H), 0.95 (*s*, 3H, 27-H), 0.90 (*s*, 3H, 24-H), 0.83 (*s*, 3H, 23-H), 0.80 (*s*, 3H, 26-H), 0.71–0.65 (*m*, 1H, 5-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ = 212.6 (C-20), 176.5 (C-28), 171.1 (C-30), 81.0 (C-3), 66.4 (C-33), 55.8 (C-17), 55.5 (C-5), 54.7 (C-32), 51.2 (C-19), 50.5 (C-9), 49.7 (C-18), 42.4 (C-14), 41.2 (C-8), 40.8 (C-10), 38.8 (C-1), 38.1 (C-22), 37.94 (C-4), 36.8 (C-13), 34.3 (C-7), 32.2 (C-16), 30.2 (C-29), 29.4 (C-15), 28.6 (C-21), 28.1 (C-27), 28.1 (C-23), 27.5 (C-12), 23.8 (C-2), 21.5 (C-31), 21.1 (C-11), 18.3 (C-6), 16.3 (C-26), 16.0 (C-24), 14.9 (C-25) ppm; MS (ESI): *m/z* (%) = 484 ([M−SO_3NH_2−Ac + H]$^+$, 48), 526 ([M−SO_3NH_2 + H]$^+$, 100); analysis calcd for $C_{33}H_{54}N_2O_7S$ (622.86): C 63.64, H 8.74, N 4.50; found: C 63.52, H 8.91, N 4.39.

(3β) 3-Acetyloxy-N-(2-aminosulfonyloxyethyl)-urs-12-en-28-amide (19)

According to GP E, from **7** (350 mg, 0.65 mmol) followed by chromatography (SiO_2, $CHCl_3$/MeOH, 9:1) **19** (282 mg, 69%) was obtained as a colorless solid; R_f = 0.38 ($CHCl_3$/MeOH, 8:2); m.p. 114–118 °C; $[\alpha]_D^{20}$ = +45.8° (*c* = 0.298, $CHCl_3$); IR (ATR): ν = 3406*w*, 2925 *s*, 1733 *m*, 1651 *m*, 1454 *m*, 1367 *m*, 1244 *s*, 1137*w*, 1097*w*, 1037 *m*, 1027 *m* cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.34 (*t*, *J* = 5.5 Hz, 1H, NH), 5.24 (*dd*, *J* = 14.8, 14.8 Hz, 1H, 12-H), 4.52–4.44 (*m*, 1H, 3-H), 4.19 (*t*, *J* = 9.2 Hz, 2H, 34-H), 3.81 (*t*, *J* = 9.4 Hz, 2H, 33-H), 2.22 (*d*, *J* = 28.6 Hz, 1H, 18-H), 2.08 (*ddd*, *J* = 4.5 Hz, 1H, 16-H_a), 2.04 (*s*, 3H, 32-H), 2.00–1.79 (*m*, 4H, 2-H_a, 11-H_a, 11-H_b, 15-H_a), 1.79–1.39 (*m*, 9H, 22-H_a, 22-H_b, 16-H_b, 1-H_a, 2-H_b, 6-H_a, 9-H, 21-H_a, 7-H_a), 1.39–1.14 (*m*, 4H, 6-H_b, 19-H, 21-H_b, 7-H_b), 1.15–0.98 (*m*, 3H, 15-H_b, 1-H_b, 20-H), 1.07 (*s*, 3H, 27-H), 0.99 (*s*, 3H, 23-H), 0.95 (*d*, *J* = 2.8 Hz, 3H, 30-H), 0.94 (*s*, 3H, 25-H), 0.86 (*d*, *J* = 4.2 Hz, 3H, 29-H), 0.85 (*s*, 3H, 24-H), 0.83–0.80 (m, 1H, 5-H), 0.78 (*s*, 3H, 26-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ = 174.4 (C-28), 171.1 (C-31), 138.3 (C-13), 125.7 (C-12), 81.1 (C-3), 67.4 (C-34), 55.4 (C-5), 53.8 (C-33), 53.4 (C-18), 47.7 (C-17), 47.6 (C-9), 42.6 (C-14), 39.2 (C-8), 39.1 (C-19), 39.0 (C-20), 38.4 (C-1), 37.8 (C-4), 37.5 (C-22), 32.9 (C-7), 30.7 (C-21), 28.2 (C-23), 27.8 (C-15), 24.7 (C-16), 23.9 (C-27), 23.7 (C-2), 23.5 (C-11), 21.5 (C-32), 21.4 (C-30), 18.4 (C-6), 17.3 (C-24), 16.9 (C-29), 16.8 (C-26), 15.6 (C-25) ppm; MS (ESI): *m/z* (%) = 524 ([M−SO_3NH_2 + H]$^+$, 100), 561 ([M−AcO−H_2O + H]$^+$, 13); analysis calcd for $C_{34}H_{56}N_2O_6S$ (620.89): C 65.77, H 9.09, N 4.51; found: C 65.56, H 10.24, N 4.31.

(3β) 3-Acetyloxy-N-(2-aminosulfonyloxyethyl)-olean-12-en-28-amide (20)

According to GP E, from **8** (350 mg, 0.59 mmol) followed by chromatography (SiO_2 $CHCl_3$/MeOH, 9:1) **20** (250 mg, 62%) was obtained as a colorless solid; R_f = 0.42 ($CHCl_3$/MeOH, 8:2); m.p. 106–109 °C; $[\alpha]_D^{20}$ = +25.7° (*c* = 0.291, $CHCl_3$); IR (ATR): ν = 2943 *m*, 1732 *m*, 1654 *m*, 1460 *m*, 1364 *m*, 1243 *s*, 1025 *s* cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.12 (*t*, *J* = 5.6 Hz, 1H, NH), 5.13 (*dd*, *J* = 4.0, 4.0 Hz, 1H, 12-H), 4.36 (*dd*, *J* = 11.4, 4.4 Hz, 1H, 3-H), 3.40 (*d*, *J* = 7.7 Hz, 2H, 34-H), 3.04–2.90 (*m*, 1H, 33-H_a), 2.75 (*td*, *J* = 17.7, 13.1, 4.5 Hz, 1H, 33-H_b), 2.48 (*dd*, *J* = 13.1, 4.6 Hz, 1H, 18-H), 2.00–1.97 (*m*, 1H, 16-H_a), 1.97 (*s*, 3H, 32-H), 1.93–1.73 (*m*, 2H, 2-H_a, 16-H_b), 1.73–1.26 (*m*, 13H, 15-H_a, 19-H_a, 7-H_a, 11-H_a, 11-H_b, 2-H_b, 1-H_a, 9-H, 6-H_a, 6-H_b, 7-H_b, 22-H_a, 21-H_a), 1.28–1.02 (*m*, 2H, 22-H_b, 21-H_b), 1.08 (*s*, 3H, 27-H), 1.06–0.87 (*m*, 3H, 19-H_b, 1-H_b, 15-H_b), 0.86 (*s*, 3H, 25-H), 0.85 (*s*, 6H, 30-H, 29-H), 0.83–0.80 (*m*, 1H, 5-H), 0.79 (*s*, 6H, 23-H, 24-H), 0.67 (*s*, 3H, 26-H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ = 176.6 (C-28), 170.1 (C-31), 143.9 (C-13), 121.4 (C-12), 79.9 (C-3), 59.9 (C-34), 54.6 (C-5), 46.9 (C-9), 45.8 (C-19), 45.4 (C-17), 41.6 (C-33), 41.1 (C-14), 39.8 (C-18), 39.0 (C-8), 37.6 (C-1), 37.3 (C-4), 36.5 (C-10), 33.2 (C-21), 32.8 (C-29), 32.7 (C-7), 32.1 (C-22), 30.4 (C-20), 27.8 (C-23), 26.9 (C-15), 25.7 (C-27), 23.4 (C-30), 23.2 (C-11), 22.9 (C-16), 22.4 (C-2), 21.0 (C-32), 17.8 (C-6), 16.7 (C-24), 16.6 (C-26), 15.1 (C-25) ppm; MS (ESI): *m/z* (%) = 620 ([M−H]$^-$, 100), 1240 ([2 M−H]$^-$, 18); analysis calcd for $C_{34}H_{56}N_2O_6S$ (620.89): C 65.77, H 9.09, N 4.51; found: C 65.50, H 9.27, N 4.18.

(3β) 3-Acetyloxy-N, N-bis(2-aminosulfonyloxyethyl)-urs-12-en-28-amide (21)

According to GP E, from **11** (350 mg, 0.6 mmol) followed by chromatography (SiO_2, $CHCl_3$/MeOH, 9:1) **21** (298 mg, 67%) was obtained as a colorless solid; R_f = 0.34 ($CHCl_3$/MeOH, 8:2); m.p. 106–110 °C; $[\alpha]_D^{20}$ = -45.8° (*c* = 0.335, $CHCl_3$); IR (ATR): ν = 3467 *m*, 2939*w*, 1656 *s*, 1419 *s*, 1326 *s*, 1248 *m*, 1148*w*, 1109 *m*, 1036 *m* cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ = 5.35 (*dd*, *J* = 3.7, 3.7 Hz, 1H, 12-H), 4.46 (*dd*, *J* = 10.6, 5.4 Hz, 1H, 3-H), 4.34–4.29 (*m*, 2H,36-H), 4.23–4.14 (*m*, 2H,34-H), 3.61–3.56 (*m*, 2H, 33-H), 3.53–3.43 (*m*, 2H, 35-H), 2.28–2.22 (*m*, 1H, 18-H), 2.10–2.04 (*m*, 2H, 22-H_a, 11-H_b), 2.03 (*s*, 3H, 32-H), 1.98–1.92 (*m*, 3H, 11-H_a, 2-H_a, 2-H_b), 1.90–1.50 (*m*, 10*H*, 22-H_b, 15-H_a, 1-H_a, 16-H_a, 16-H_b, 9-H, 21-H_a, 6-H_a, 7-H_a, 19-H), 1.50–1.23 (*m*, 4H, 6-H_b, 21-H_b, 7-H_b, 15-H_b), 1.19 (*s*, 3H, 27-H), 1.14–1.04 (*m*, 1H, 1-H_b, 20-H), 1.02 (*s*, 3H, 25-H), 1.00 (*s*, 3H, 30-H), 0.93 (*s*, 3H, 29-H), 0.90 (*s*, 3H, 24-H), 0.89 (*s*, 3H, 23-H), 0.88–0.87 (*m*, 1H, 5-H), 0.86 (*s*, 3H, 26-H) ppm; ^{13}C NMR (126 MHz, CD_3OD) δ = 180.0 (C-28), 172.9 (C-31), 138.2 (C-13), 128.9 (C-12), 82.4 (C-3), 58.2 (C-34, C-36), 56.6 (C-5), 54.9 (C-18), 53.0 (C-33, C-35), 49.3 (C-17), 48.6 (C-9), 43.2 (C-8), 40.9 (C-14), 40.2 (C-19), 39.5 (C-20), 39.3 (C-1), 38.7 (C-4), 38.0 (C-10), 35.2 (C-22), 33.6 (C-7), 30.9 (C-21), 29.2 (C-15), 28.5 (C-23), 24.8 (C-11), 24.5 (C-16), 24.4 (C-2), 24.3 (C-27), 21.1 (C-30), 21.1 (C-32), 19.2 (C-6), 17.5 (C-29), 17.4 (C-26), 17.2 (C-24), 16.0 (C-25) ppm; MS (ESI): *m/z* (%) = 568 ([M−AcO−SO_3NH_2−H_2O + H]$^+$, 100), 586 ([M−AcO−SO_3NH_2 + H]$^+$, 36); analysis calcd for $C_{36}H_{61}N_3O_9S_2$ (744.02): C 58.12, H 8.26, N 5.65; found: C 57.85, H 8.53, N 5.31.

(3β) 3-Acetyloxy-N, N-bis(2-aminosulfonyloxyethyl)-olean-12-en-28-amide (22)

According to GP E, from **12** (350 mg, 0.59 mmol) followed by

chromatography (SiO_2 $CHCl_3$/MeOH, 9:1) **22** (332 mg, 74%) was obtained as a colorless solid; $R_f = 0.08$ (toluene/ethyl acetate/*n*-heptane/formic acid, 80:26:10:5); m.p. 236–239 °C; $[\alpha]_D^{20} = +23.3°$ ($c = 0.255$, $CHCl_3$); IR (ATR): ν = 3440*w*, 2945 *m*, 1730*w*, 1662*w*, 1611*w*, 1430 *m*, 1316 *s*, 1244 *s*, 1027 *s* cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) $\delta = 5.26$ (*dd*, $J = 3.7$, 3.7 Hz, 1H, 12-H), 4.51–4.45 (*m*, 1H, 3-H), 3.86–3.75 (*m*, 4H, 34-H, 36-H), 3.62–3.49 (*m*, 4H, 33-H, 35-H), 3.08–3.01 (*m*, 1H, 18-H), 2.11 (*dd*, $J = 14.3$, 3.0 Hz, 1H, 16-H_a), 2.03 (*s*, 3H, 32-H), 1.97–1.55 (*m*, 11*H*, 11-H_a, 22-H_a, 16-H_b, 19-H_a, 22-H_b, 11-H_b, 2-H_a, 2-H_b, 1-H_a, 15-H_a, 9-H), 1.55–1.15 (*m*, 7H, 6-H_a, 7-H_a, 6-H_b, 21-H_a, 7-H_b, 21-H_b, 19-H_b), 1.13 (*s*, 3H, 27-H), 1.11–0.98 (*m*, 2H, 15-H_b, 1-H_b), 0.92 (*s*, 3H, 25-H), 0.92 (*s*, 3H, 30-H), 0.89 (*s*, 3H, 29-H), 0.85 (*s*, 3H, 23-H), 0.84 (*s*, 3H, 24-H), 0.77 (*s*, 3H, 26-H), 0.74–0.69 (*m*, 1H, 5-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ = 179.0 (C-28), 171.2 (C-31), 144.9 (C-13), 121.6 (C-12), 81.1 (C-3), 61.4 (C-34, C-36), 55.5 (C-5), 52.2 (C-33, C-35), 48.3 (C-17), 47.8 (C-9), 46.9 (C-19), 43.9 (C-18), 42.1 (C-14), 39.4 (C-8), 38.3 (C-1), 37.8 (C-4), 37.1 (C-10), 34.3 (C-21), 33.1 (C-29), 33.0 (C-7), 30.5 (C-20), 30.2 (C-22), 28.2 (C-15), 28.2 (C-23), 25.9 (C-27), 24.2 (C-30), 23.7 (C-2), 23.5 (C-11), 22.8 (C-16), 21.5 (C-32), 18.4 (C-6), 17.1 (C-26), 16.8 (C-24), 15.6 (C-25) ppm; MS (ESI): *m/z* (%) = 568 ([M−AcO−SO_3NH_2−H_2O + H]$^+$, 100), 586 ([M−AcO−SO_3NH_2−H]$^+$, 52); analysis calcd for $C_{36}H_{61}N_3O_9S_2$ (744.02): C 58.12, H 8.26, N 5.65; found: C 57.86, H 8.41, N 5.54.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rechem.2022.100371.

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