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SYNTHESIS OF CONJUGATES OF LUPANE-TYPE PENTACYCLIC TRITERPENOIDS WITH 2-AMINOETHANE- AND *N*-METHYL-2-AMINOETHANESULFONIC ACIDS. ASSESSMENT OF *in vitro* TOXICITY

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Conjugates of betulin and betulinic and betulonic acids with 2-aminoethane- and N*-methyl-2-aminoethanesulfonic acids were synthesized for the first time and were interesting as potential biologically active compounds. Experiments in vitro in MDCK cell culture using the MTT assay found that betulin and betulinic-acid derivatives with aminoethanesulfonic acid bound to triterpene C-3 or C-28 through an ester linker were less toxic than the native compounds.*

Keywords: betulin, betulinic acid, betulonic acid, 2-aminoethanesulfonic acid, *N*-methyl-2-aminoethanesulfonic acid, toxicity *in vitro*.

Lupane-type pentacyclic triterpenoids are interesting as platforms for developing new drugs with various biological activities [1–4]. In continuation of research on the modification of lupane triterpenoids, we developed approached to conjugates containing aminoalkanesulfonic acids. The endogenous metabolite taurine (2-aminoethanesulfonic acid), its derivatives, and natural compounds containing taurine possess broad spectra of biological activity [5–7] including antiviral [8–12], antibiotic [13], and antitumor [14, 15]. Introduction of taurine or its homologs into lupane-type pentacyclic triterpenoids would expand the spectrum of biologically active compounds in this series and provide new information about the structure–activity (biological) relationship.

Herein, conjugates containing C-28/C-3 2-aminoethane- or *N*-methyl-2-aminoethanesulfonic acid (*N*-methyltaurine) as the ammonium or sodium salt or the free aminoethanesulfonic acids bound to the triterpene skeleton via an ester linker were synthesized from betulin (**1**) chloroacetates, betulonic acid (**2**) bromoalkyl esters, and betulinic acid (**3a**) methyl chloroacetates or benzyl esters. Experiments *in vitro* assessed the toxicity of the synthesized compounds in MDCK cell culture (canine kidney cell line).

Starting chloroacetates **4** and **5a**,**b** were obtained from **1** or **3b**,**c** and chloroacetic acid using the carbodiimide method. Betulonic acid 2-bromoethyl and 3-bromopropyl esters **6a**,**b** were synthesized via *O*-alkylation of acid **2** with the appropriate dibromoalkane (DMF, K_2CO_3). Acid **2** was prepared by a modified literature method [16] using sequential oxidation of **1** by pyridinium chlorochromate (PCC) (2 h) and treatment of the obtained betulone aldehyde with $NaClO_2$–NaH_2PO_4 in the presence of 2-methyl-2-butene for 15 min with an aldehyde–$NaClO_2$–NaH_2PO_4 ratio of 1:6:6. The yield of acid **2** after purification through the potassium salt was 75%. Acid **3a** and methyl **3b** and benzyl **3c** esters were synthesized by the reported methods [17–19]. *N*-Methyltaurine was prepared by *N*-alkylation of methylamine with sodium 2-bromoethanesulfonate in 73% yield [20].

Conjugates of taurine **7** and **8** or *N*-methyltaurine **9** and **10a**,**b** were synthesized by *N*-alkylation of the tetrabutylammonium salt of taurine [21] or *N*-methyltaurine chloroacetates **4** and **5a** or **5b** in DMF in the presence of K_2CO_3 at 95°C.

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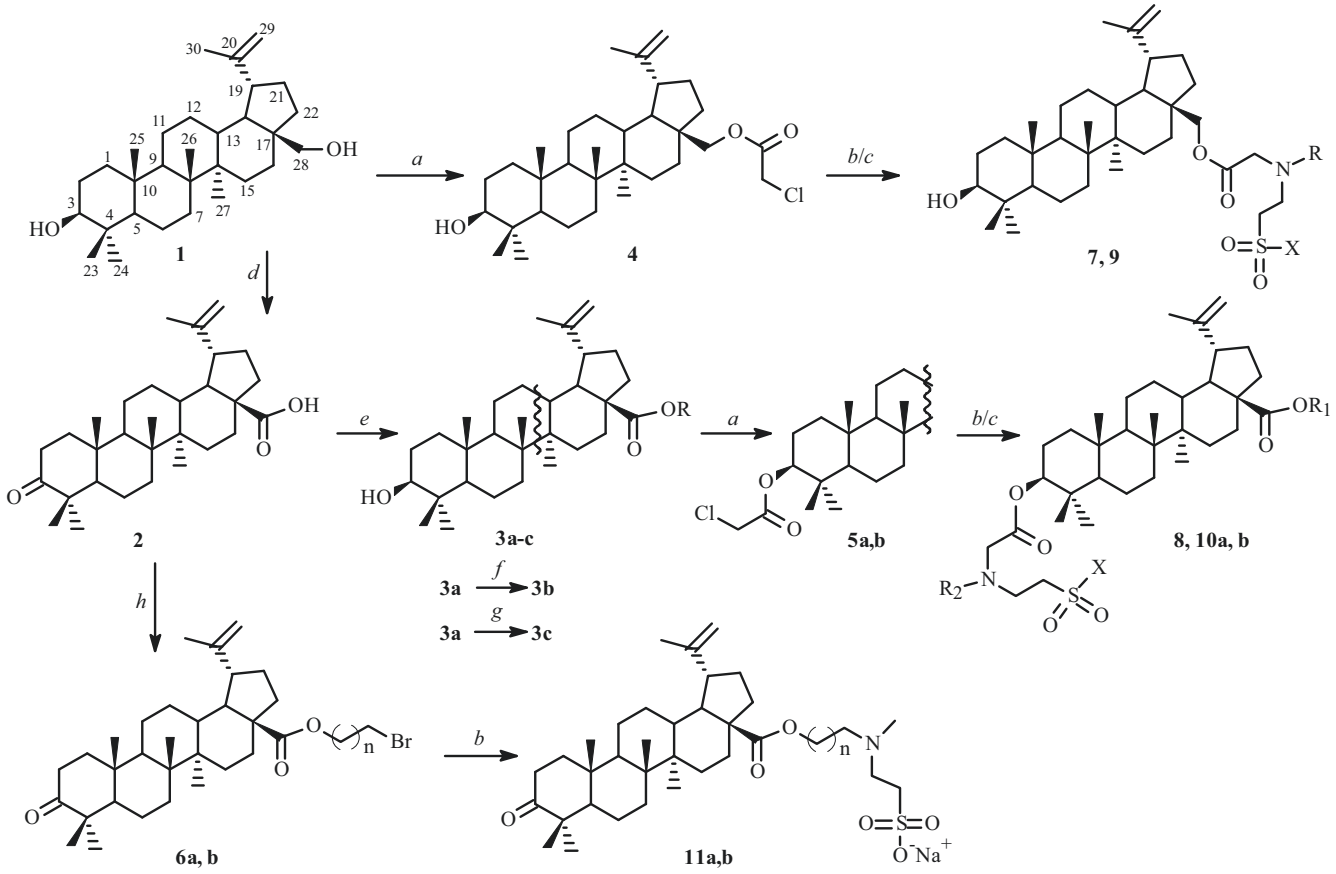

7: R = H, X = $O^-N^+Bu_4$; **9:** R = Me, X = OH; **3a:** R = H; **3b,5a:** R = Me; **3c,5b:** R = Bn;
8: R_1 = Me, R_2 = H, X = $O^-N^+Bu_4$; **10a:** R_1 = R_2 = Me, X = OH; **10b:** R_1 = Bn, R_2 = Me, X = OH
6a, 11a: n = 1; **6b,11b:** n = 2

a. $ClCH_2CO_2H$, DCC, DMAP, CH_2Cl_2; *b*. $NH_2CH_2CH_2SO_3^-N^+Bu_4$, DMF, K_2CO_3, 95°C, 3 h; *c*. $MeNHCH_2CH_2SO_3H$, DMF, K_2CO_3, 95°C, 50 h (for **9**), 6 h (for **10a,b**); *d*. 1. PCC, CH_2Cl_2, 2 h, 2. $NaClO_2/NaH_2PO_4$/2-methyl-2-buten, *t*-BuOH, 15 min; *e*. $NaBH_4$, THF; *f*. CH_2N_2, Et_2O; *g*. BnCl, DMF, K_2CO_3; *h*. $BrCH_2CH_2Br$ or $BrCH_2CH_2CH_2Br$, K_2CO_3, DMF, 4 h

Betulonic acid derivatives with sodium *N*-methyl-2-aminoethanesulfonate **11a** and **11b** were prepared by reacting bromoalkyl esters **6a** and **6b** with the sodium salt of *N*-methyltaurine [22] in the presence of K_2CO_3 in DMF at 95°C for 50 h.

The yields of conjugates **7**–**9**, **10a**,**b**, and **11a**,**b** were 64–90%. The structures of these compounds were established using PMR and ^{13}C NMR spectroscopy and mass spectrometry. PMR and ^{13}C NMR spectra of conjugates **8**, **9**, **10b**, and **11a** were fully assigned using 2D experiments (Table 1).

Toxicity *in vitro* of 1, 2, 3a, 7–9, 10a, and 11a. Assessment of the toxicity of the compounds was an important step in preclinical studies of their biological activity. The toxicity of synthesized **7**–**9**, **10a**, and **11a** was screened *in vitro* using MDCK cell culture and the MTT assay. The median cytotoxic dose (CTD_{50}, dose leading to the death of 50% of the cells) and minimum toxic dose (MTD, dose causing toxic effects to appear in the cells) were determined. The MTD became an independent important toxicity characteristic if the CTD_{50} value exceeded the upper limit of the tested concentrations. According to this criterion, conjugates **7**, **9**, and **10a** were slightly toxic compounds (Table 2). A criterion reflecting the rate of toxicity increase with increasing concentration (CTD_{50}/MTD) served as a guide for comparing the toxicity of compounds with similar CTD_{50} values, e.g., **8** (CTD_{50} 30 μg/mL) and **11a** (CTD_{50} 37.5 μg/mL). According to this criterion, **11a** (CTD_{50}/MTD = 1.3; for **8**, 4.8) was the most toxic compound.

The toxicity results for **7**–**9** and **10a** showed that adding aminoethanesulfonic acids through ester linkers to triterpene C-3 or C-28 reduced considerably the toxicity of the conjugates as compared with native precursors **1** and **3a** (Table 2, compounds **1**, **7**, **9**, **3a**, **8**, **10a**). The exception was betulonic acid derivative **11a**, the toxicity of which was the same as acid **2** itself (CTD_{50}/MTD = 1.5 and 1.6, respectively). An analysis of the toxicities of betulin and betulinic acid derivatives with the same substituents (pairs **7**,**8** and **9**,**10a**) (Table 2) or derivatives within each series **7**,**9** and **8**,**11a** with different substituents showed that the toxicity depended on both the triterpenoid structure and the fine differences in the substituents.

TABLE 1. ^{13}C NMR Spectra of **6a**,**b**–**9**, **10a**,**b**, and **11a**,**b** (δ, ppm)*

C atom	**6a**	**6b**	**7**	**8**	**9**	**10a**	**10b**	**11a**	**11b**
1	39.62	39.55	38.63	38.34	38.27	37.57	38.21	40.64	40.64
2	34.13	34.06	26.96	23.64	27.18	23.38	23.92	35.26	35.10
3	218.01	218.03	78.75	81.40	76.79	79.98	80.49	221.43	220.08
4	47.32	47.24	38.77	37.78	36.69	37.28	37.81	47.81	47.66
5	54.96	54.87	55.20	55.40	54.86	54.55	55.06	56.11	56.08
6	19.63	19.56	18.21	18.09	17.99	17.64	18.17	20.76	20.71
7	33.60	33.54	34.08	34.17	33.78	33.59	34.12	34.77	34.74
8	40.67	40.56	40.78	40.62	41.11	40.09	40.36	41.89	41.84
9	49.89	49.82	50.26	50.40	49.77	49.55	50.07	51.21	51.15
10	36.90	36.82	37.05	37.03	38.53	36.53	37.04	38.05	38.01
11	21.41	21.36	20.70	20.83	20.29	20.36	20.88	22.60	22.61
12	25.52	25.44	25.09	25.40	24.74	24.89	25.44	26.58	26.84
13	38.41	38.31	37.50	38.17	37.09	37.57	38.05	39.72	39.81
14	42.46	42.39	42.60	42.32	42.26	41.89	42.42	43.64	43.61
15	29.65	29.51	27.31	29.59	26.62	29.91	29.48	30.87	30.81
16	31.99	31.58	29.49	32.09	29.12	31.37	31.89	31.64	31.65
17	56.54	56.49	46.31	56.49	46.10	55.79	56.31	57.87	57.90
18	49.34	49.25	48.71	49.39	48.22	48.65	49.15	50.27	50.49
19	46.90	47.24	47.62	46.93	46.99	46.56	47.08	49.34	**
20	150.32	150.28	150.10	150.50	149.78	149.97	150.47	151.82	151.72
21	30.57	30.49	29.60	30.53	28.96	31.20	30.42	33.02	33.11
22	36.90	37.00	34.45	36.90	33.99	36.07	36.62	37.89	37.80
23	26.61	26.44	27.94	37.99	28.13	27.63	28.16	27.18	27.10
24	21.03	20.95	15.34	15.88	15.68	15.51	15.92	21.41	21.40
25	15.95	15.90	16.02	16.09	15.93	15.74	16.30	16.64	16.55
26	15.80	16.76	15.92	16.45	15.84	16.43	16.97	16.47	16.44
27	14.63	14.55	14.68	14.60	14.53	14.29	14.78	15.08	15.02
28	175.68	175.77	62.85	175.50	64.96	175.56	175.27	177.50	177.62
29	109.75	109.67	109.74	109.55	110.07	109.70	110.24	110.37	110.35
30	19.39	19.29	19.03	19.29	18.79	18.82	19.34	19.60	19.55
$OC(O)\underline{C}H_2$			50.75	50.87	55.98	57.88	58.41		
$O\underline{C}(O)CH_2$			172.36	170.50	167.90	170.18	170.68		
$NCH_2\underline{C}H_2SO_3$			50.66	50.40	46.55	49.30	49.83	48.55	48.70
$N\underline{C}H_2CH_2SO_3$			45.69	45.70	53.07	52.35	52.88	54.24	53.67
NCH_3					40.44	41.47	42.00	42.67	42.55
$C(O)OCH_3$				51.20		51.12			
CH_2Ph							65.57		
Ph							128.43		
							128.50		
							128.83		
							136.90		
$C(O)OCH_2\underline{C}H_2$	29.18							56.56	
$C(O)O\underline{C}H_2CH_2$	63.37							62.66	
$C(O)OCH_2\underline{C}H_2CH_2$		29.60							27.41
$C(O)OCH_2CH_2\underline{C}H_2$		32.02							55.08
$C(O)O\underline{C}H_2CH_2CH_2$		61.48							63.41
$CH_2CH_2CH_2\underline{C}H_3$			13.64	13.67					
$CH_2CH_2\underline{C}H_2CH_3$			19.66	19.68					
$CH_2\underline{C}H_2CH_2CH_3$			23.94	23.97					
$\underline{C}H_2CH_2CH_2CH_3$			58.69	58.68					

*__6a__, __6b__ (75.47 MHz, $CDCl_3$); __7__, __8__ (125.47 MHz, $CDCl_3$, TMS); __9__, __10b__ (125.47 MHz, DMSO-d_6, TMS); __10a__ (75.47 MHz, DMSO-d_6), __11a__ (125.47 MHz, CD_3OD), __11b__ (75.47 MHz, CD_3OD); **C-19 resonance overlapped solvent resonances.

TABLE 2. Toxicity Screening of **1**, **2**, **3a**, **7**–**9**, **10a**, and **11a**

Compound	CTD_{50},* μg/mL	MTD,** μg/mL	CTD_{50}/MTD
9	> 200	50	–
7	> 200	50	–
10a	> 100	100	–
11a	37.5	25	1.5
8	30	6.3	4.8
1	40	6.3	6.3
2	10	6.3	1.6
3a	9.4	3.1	3.0

*Median cytotoxic dose from MTT assay; **minimum toxic dose.

Thus, methods for preparing conjugates of betulin and betulinic and betulonic acids with aminoethanesulfonic acids that were interesting as potential biologically active compounds were developed. They were also intermediates for synthesizing various sulfonamide and peptide derivatives of lupane-type pentacyclic triterpenoids. The *in vitro* experiments provided seminal information on the effects of aminoethanesulfonic acid substituents on the toxicity of these compounds that was valuable for planning further syntheses of biologically active compounds based on aminoethanesulfonic acids.

EXPERIMENTAL

IR spectra were taken in mineral oil on a Shimadzu Prestige-21 IR spectrophotometer. PMR and ^{13}C NMR spectra were recorded on a Bruker Avance III pulsed spectrometer at operating frequencies 500.13 MHz for ^{1}H and 125.47 MHz for ^{13}C using a *Z*-gradient PABBO probe at 298 K or a Bruker AM-300 spectrometer at operating frequencies 300.13 MHz for ^{1}H and 75.47 MHz for ^{13}C. Chemical shifts in PMR and ^{13}C NMR spectra were given in ppm relative to solvent ($CDCl_3$) resonances (δ_H 7.27 and δ_C 77.1) or TMS internal standard. 2D spectra were recorded using standard multi-pulse sequences of the Bruker Avance III spectrometer software. Mass spectra were obtained using electrospray ionization (ESI) or chemical ionization at atmospheric pressure (APCI) on an LCMS-2010EV liquid chromatograph-mass spectrometer (Shimadzu) (sample injection via syringe, 0.1 mL/min, MeCN–H_2O eluent, 95:5) in positive- and negative-ion modes at capillary potential 4.5 and –3.5 kV. The APCI interface temperature was 250°C, heater 200°C, vaporizer 230°C. The spray-gas (N_2) flow rate was 1.5 and 2.5 L/min for ESI and APCI, respectively. Rotation angles were measured on a PerkinElmer 341C instrument. Column chromatography used SiO_2 (PTSKh-AF-A, Imid, Krasnodar, Russia). Melting points were determined on a Kofler apparatus. Betulin (**1**) was isolated from birch bark by the literature method [23]. Spectral characteristics (PMR and ^{13}C NMR) of betulinic acid **3a** and its esters **3b** and **3c** that were prepared according to the literature [17–19] agreed with those reported. Commercial 2-aminoethanesulfonic acid was used. DMF was stored over $MgSO_4$ and vacuum distilled under Ar from 4Å molecular sieves. CH_2Cl_2 was distilled from CaH_2. K_2CO_3 was calcined before the reactions.

3-Oxolup-20(29)-en-28-oic Acid (betulonic acid) (2). A solution of **1** (5.00 g, 11.29 mmol) in CH_2Cl_2 (500 mL) was treated with PCC (12.17 g, 56.47 mmol), stirred for 2 h, diluted with Et_2O (500 mL), stirred for 15 min, and filtered through a layer of Al_2O_3. The filtrate was concentrated. The residue was purified by flash chromatography over SiO_2 [C_6H_6–methyl-*tert*-butylether (MTBE), 4:1] to afford betulonic aldehyde (4.20 g, 85%) (PMR and ^{13}C NMR spectra agreed with the literature [16]). A solution of the aldehyde (4.20 g, 9.57 mmol) in *t*-BuOH (200 mL) was treated with 2-methyl-2-butene (4 mL, 37.75 mmol) and simultaneously dropwise with $NaClO_2$ (5.20 g, 57.44 mmol) in H_2O (22 mL) and NaH_2PO_4 (6.89 g, 57.44 mmol) in H_2O (21 mL). The reaction mixture was stirred for 15 min, diluted with H_2O (200 mL), and extracted with $CHCl_3$. The organic layer was separated, washed with H_2O, dried over Na_2SO_4, and evaporated. The resulting residue was dissolved in C_6H_6 (150 mL), treated with KOH (0.58 g, 10.36 mmol, 15% solution in H_2O), and refluxed for 1 h. The resulting precipitate of the potassium salt of betulonic acid was filtered off and dissolved in a mixture of MTBE (200 mL) and HCl (10%, 50 mL). The organic layer was separated, washed with H_2O, dried over Na_2SO_4, and evaporated to afford **2** (3.26 g, 75%). The PMR and ^{13}C NMR spectra agreed with the literature data [16].

3*β*-Hydroxylup-20(29)-en-28-yl 2-Chloroacetate (4). A solution of **1** (3.00 g, 6.78 mmol) and Et_3N (2.83 mL, 20.33 mmol) in CH_2Cl_2 (300 mL) was stirred, treated dropwise with a solution of chloroacetic chloride (1.3 mL, 16.26 mmol) in CH_2Cl_2 (50 mL), stirred at 20°C for 30 min, washed with HCl (10%, 100 mL) and H_2O, dried over Na_2SO_4, and evaporated.

The residue was chromatographed over SiO_2 (C_6H_6–MTBE, 8:1) to afford chloroacetate **4** (2.18 g, 63%), mp 152–154°C (lit. [24]: 154–156°C). IR spectrum (ν, cm^{-1}): 1660, 1740, 3325. ^1H NMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.67 (1H, d, J = 8.9, H-5), 0.76 (3H, s, CH_3-24), 0.82 (3H, s, CH_3-25), 0.96 (3H, s, CH_3-23), 0.98 (3H, s, CH_3-26), 1.03 (3H, s, CH_3-27), 1.68 (3H, s, CH_3-30), 2.43 (1H, td, J = 10.5, 5.5, H-19), 3.18 (1H, dd, J = 11.0, 5.3, H-3), 3.86 (2H, s, $OC(O)CH_2$), 3.94, 4.37 (each 1H, d, J = 11.0, H-28), 4.60, 4.70 (each1H, br.s, H-29) (only characteristic resonances are given). The ^{13}C NMR spectrum agreed with the literature data [24].

Preparation of 5a and 5b. A solution of ester **3b** or **3c** (1.06 mmol) in CH_2Cl_2 (60 mL) was treated under Ar with chloroacetic chloride (2.13 mmol) and DMAP (2.13 mmol), stirred for 5 min, treated with DCC (2.13 mmol), and stirred for 20 min. The precipitate of *N*,*N*-dicyclohexylurea was filtered off. The organic layer was washed with HCl (10%, 20 mL) and H_2O, dried over Na_2SO_4, and evaporated. The residue was purified by flash chromatography over SiO_2 (C_6H_6–MTBE, 4:1).

Methyl Ester of 3β-(2-Chloroacetoxy)lup-20(29)-en-28-oic Acid (5a). Yield 0.54 g (94%), amorph., $[\alpha]_D^{20}$ +4° (*c* 0.15, $CHCl_3$). IR spectrum (ν, cm^{-1}): 1650, 1734. ^1H NMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.68 (1H, d, J = 10.0, H-5), 0.84 (3H, s, CH_3-24), 0.85 (3H, s, CH_3-25), 0.86 (3H, s, CH_3-23), 0.91 (3H, s, CH_3-27), 0.95 (3H, s, CH_3-26), 1.68 (3H, s, CH_3-30), 1.87 (2H, m, H-21, 22), 2.19 (1H, m, H-13), 2.22 (1H, m, H-16), 3.00 (1H, td, J = 11.0, 4.5, H-19), 3.66 (3H, s, $C(O)OCH_3$), 4.05 (2H, s, CH_2Cl), 4.56 (1H, dd, J = 10.0, 6.0, H-3), 4.60, 4.73 (each 1H, s, H-29). ^{13}C NMR spectrum (75.47 MHz, $CDCl_3$, δ, ppm): 14.64 (C-27), 16.13 (C-25), 16.40 (C-26), 15.92 (C-24), 18.10 (C-6), 19.31 (C-30), 20.86 (C-11), 23.52 (C-2), 25.41 (C-12), 27.88 (C-23), 29.63 (C-15), 30.55 (C-21), 32.12 (C-16), 34.17 (C-7), 36.92 (C-22), 37.06 (C-10), 37.97 (C-4), 38.18 (C-13), 38.28 (C-1), 40.65 (C-8), 41.23 (CH_2Cl), 42.37 (C-14), 49.41 (C-19), 51.26 ($C(O)O\underline{C}H_3$), 46.95 (C-18), 50.40 (C-9), 55.35 (C-5), 56.51 (C-17), 83.35 (C-3), 109.62 (C-29), 150.52 (C-20), 167.14 (C=O), 176.64 (C-28). Mass spectrum (ESI), *m/z* 453 $[M+H-ClCH_2CO_2H]^+$ (calcd for $C_{33}H_{51}ClO_4$, 546.5).

Benzyl Ester of 3β-(2-Chloroacetoxy)lup-20(29)-en-28-oic Acid (5b). Yield 0.61 g (92%), mp 57–59°C, $[\alpha]_D^{20}$ –9° (*c* 0.22, $CHCl_3$). IR spectrum (ν, cm^{-1}): 700, 1650, 1730, 1762. ^1H NMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.76 (3H, s, CH_3-24), 0.83 (3H, s, CH_3-25), 0.84 (3H, s, CH_3-26), 0.85 (3H, s, CH_3-23), 0.94 (3H, s, CH_3-27), 1.68 (3H, s, CH_3-30), 1.88 (2H, m, H-21, 22), 2.18 (1H, td, J = 11.0, 5.4, H-13), 2.28 (1H, d, J = 11.8, H-16), 3.02 (1H, td, J = 10.5, 4.2, H-19), 4.02, 4.05 (each1H, d, J = 18.5, CH_2Cl), 4.55 (1H, dd, J = 10.5, 4.3, H-3), 4.60, 4.72 (each 1H, s, H-29), 5.08, 5.18 (each 1H, d, J = 19.6, $C\underline{H}_2Ph$), 7.36 (5H, m, Ph). ^{13}C NMR spectrum (75.47 MHz, $CDCl_3$, δ, ppm): 14.64 (C-27), 15.83 (C-25), 16.19 (C-26), 16.43 (C-24), 18.14 (C-6), 19.35 (C-30), 20.90 (C-11), 23.56 (C-2), 25.46 (C-12), 27.94 (C-23), 29.55 (C-15), 30.55 (C-21), 32.10 (C-16), 34.18 (C-7), 36.94 (C-22), 37.07 (C-10), 38.00 (C-4), 38.15 (C-13), 38.31 (C-1), 40.65 (C-8), 41.28 (CH_2Cl), 42.40 (C-14), 46.96 (C-19), 49.41 (C-18), 50.43 (C-9), 55.37 (C-5), 56.53 (C-17), 65.74 ($\underline{C}H_2Ph$), 83.35 (C-3), 109.68 (C-29), 128.08, 128.26, 128.50 (Ph), 136.50 (Ph: C-1), 150.55 (C-20), 167.17 (C=O), 175.83 (C-28). Mass spectrum (ESI), *m/z* 529 $[M + H - ClCH_2CO_2H]^+$ (calcd for $C_{39}H_{55}ClO_4$, 622).

Preparation of 6a and 6b. A suspension of acid **3** (4.40 mmol) and K_2CO_3 (4.40 mmol) in DMF (18 mL) was treated dropwise with 1,2-dibromoethane or 1,3-dibromopropane (4.40 mmol) in DMF (2 mL) and stirred for 4 h. The K_2CO_3 was filtered off. The filtrate was evaporated. The residue was filtered through a layer of Al_2O_3. The filtrate was evaporated.

2-Bromoethyl Ester of 3-Oxolup-20(29)-en-28-oic Acid (6a). Yield 1.9 g (77%), amorph., $[\alpha]_D^{20}$ +4° (*c* 0.25, $CHCl_3$). IR spectrum (ν, cm^{-1}): 1650, 1726. ^1H NMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.92 (3H, s, CH_3-25), 0.97 (3H, s, CH_3-26), 0.98 (3H, s, CH_3-27), 1.02 (3H, s, CH_3-24), 1.07 (3H, s, CH_3-23), 1.69 (5H, s, CH_3-30, m, H-15, H-18), 1.91 (3H, m, H-1, 21, 22), 2.24 (1H, td, J = 12.0, 4.8, H-13), 2.28 (1H, m, H-16), 2.44 (H, m, H-2), 3.02 (1H, td, J = 11.5, 5.3, H-19), 3.54 (2H, t, J = 6.0, CH_2Br), 4.40, 4.42 (each 1H, dt, J = 12.0, 6.0, $C(O)OC\underline{H}_2CH_2$), 4.61, 4.74 (each 1H, s, H-29). ^{13}C NMR spectrum (Table 1). Mass spectrum (APCI), *m/z* 561 $[M + H]^+$ (calcd for $C_{32}H_{49}BrO_3$, 560).

3-Bromopropyl Ester of 3-Oxolup-20(29)-en-28-oic Acid (6b). Yield 1.9 g (76%), mp 80–83°C, $[\alpha]_D^{20}$ –6° (*c* 0.40, $CHCl_3$). IR spectrum (ν, cm^{-1}): 1630, 1730. ^1H NMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.92 (3H, s, CH_3-25), 0.96 (3H, s, CH_3-26), 0.97 (3H, s, CH_3-27), 1.01 (3H, s, CH_3-24), 1.06 (3H, s, CH_3-23), 1.68 (5H, s, CH_3-30, m, H_b-15, H-18), 1.88 (3H, m, H-1, 21, 22), 2.20 (2H, m, H-13, 16), 2.43 (2H, m, H-2), 3.00 (1H, m, H-19), 3.48 (2H, t, J = 6.5, CH_2Br), 4.21 (2H, m, $C(O)OC\underline{H}_2CH_2$), 4.64, 4.73 (each 1H, s, H-29) (only characteristic resonances are given). Table 1 lists the ^{13}C NMR spectrum. Mass spectrum (APCI), *m/z* 575 $[M + H]^+$ (calcd for $C_{33}H_{51}BrO_3$, 574).

Tetrabutylammonium 2-{2-[3β-Hydroxylup-20(29)-en-28-yloxy]-2-oxoethylamino}ethanesulfonate (7). A solution of $NH_2CH_2CH_2SO_3^-Bu_4N^+$ (0.16 g, 0.44 mmol) in DMF (4 mL) was treated with K_2CO_3 (0.06 g, 0.44 mmol), stirred for 10 min, treated dropwise with betulin chloroacetate (**4**, 0.23 g, 0.44 mmol) in DMF (5 mL), and stirred at 95°C for 3 h. The K_2CO_3 was filtered off. The filtrate was evaporated. The residue was chromatographed over SiO_2 (C_6H_6–MTBE, 4:1; $CHCl_3$–MeOH, 10:1) to afford **7** (0.25 g, 68%), amorph. IR spectrum (ν, cm^{-1}): 1035, 1192, 1640, 1740, 3378. ^1H NMR

spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.67 (1H, d, J = 9.1, H-5), 0.76 (3H, s, CH_3-24), 0.82 (3H, s, CH_3-25), 0.96 (6H, s, CH_3-23, 27), 1.01 (12H, t, J = 7.3, butyl: $CH_2CH_2CH_2C\underline{H}_3$), 1.03 (3H, s, CH_3-26), 1.45 (10H, m, butyl: $CH_2CH_2C\underline{H}_2CH_3$, 2H-2), 1.67 (8H, m, butyl: $CH_2C\underline{H}_2CH_2CH_3$), 1.69 (3H, s, CH_3-30), 1.76 (1H, dd, J = 12.2, 8.8, H-22), 1.83 (1H, d, J = 12.4, H-16), 1.95 (1H, m, H-21), 2.42 (1H, td, J = 10.5, 5.5, H-19), 3.03 (2H, t, J = 6.3, $NCH_2C\underline{H}_2SO_3$), 3.13 (2H, t, J = 6.3, $NC\underline{H}_2CH_2SO_3$), 3.18 (1H, dd, J = 11.2, 4.5, H-3), 3.32 (8H, t, J = 6.5, butyl: $C\underline{H}_2CH_2CH_2CH_3$), 3.48 (2H, s, $OC(O)CH_2$), 3.88, 4.29 (each 1H, d, J = 11.0, H-28), 4.58, 4.68 (1H, s, H-29). Table 1 lists the ^{13}C NMR spectrum. Mass spectrum (ESI), *m/z* 606 $[M - N^+Bu_4]^-$ (calcd for $C_{50}H_{92}N_2O_6S$, 848).

Tetrabutylammonium 2-{2-[17β-Methoxycarbonyllup-20(29)-en-3β-yloxy]-2-oxoethylamino}ethanesulfonate (8) was prepared analogously to **7**. Yield 0.30 g (82%), mp 78–80°C, $[\alpha]_D^{20}$ –8.5° (*c* 0.43, $CHCl_3$). IR spectrum (ν, cm^{-1}): 1034, 1166, 1190, 1200, 1640, 1729, 3420. ^1H NMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.76 (1H, d, J = 9.5, H-5), 0.82 (3H, s, CH_3-25), 0.83 (3H, s, CH_3-26), 0.91 (3H, s, CH_3-24), 0.82 (3H, s, CH_3-23), 0.95 (3H, s, CH_3-27), 0.96 (1H, m, H-1), 1.01 (12H, t, J = 7.3, butyl: $CH_2CH_2CH_2C\underline{H}_3$), 1.14 (1H, m, H-15), 1.28 (1H, m, H-11), 1.36 (4H, m, 1H-6, 2H-7, 1H-21), 1.45 (4H, m, 1H-11, 15, 16, 22), 1.46 (2H, m, H-2), 1.47 (8H, sextet, J = 7.3, butyl: $CH_2CH_2C\underline{H}_2CH_3$), 1.49 (1H, m, H-6), 1.58 (1H, t, J = 11.3, H-18), 1.65 (9H, m, butyl: $CH_2C\underline{H}_2CH_2CH_3$, H-1), 1.65 (8H, m, butyl: $CH_2C\underline{H}_2CH_2CH_3$), 1.68 (3H, s, CH_3-30), 1.88 (2H, m, H-21, 22), 2.21 (1H, td, J = 12.0, 5.5, H-13), 2.23 (1H, dd, J = 10.0, 3.3, H-16), 2.90 (1H, br.s, NH), 3.00 (1H, m, H-19), 3.01 (2H, t, J = 6.2, $NCH_2C\underline{H}_2SO_3$), 3.12 (2H, t, J = 6.2, $NC\underline{H}_2CH_2SO_3$), 3.31 (8H, t, J = 6.5, butyl: $C\underline{H}_2CH_2CH_2CH_3$), 3.45 (2H, s, $OC(O)CH_2$), 3.67 (3H, s, $C(O)OCH_3$), 4.50 (1H, dd, J = 11.0, 5.5, H-3), 4.60, 4.73 (each 1H, s, H-29). Table 1 lists the ^{13}C NMR spectrum. Mass spectrum (ESI), *m/z* 634 $[M - N^+Bu_4]^-$ (calcd for $C_{51}H_{92}N_2O_7S$, 876).

Preparation of 9, 10a, and 10b. General Method. A suspension of *N*-methyltaurine (0.19 mmol) in DMF (3 mL) was treated with K_2CO_3 (0.19 mmol), stirred for 15 min, treated dropwise with chloroacetate **4**, **5a**, or **5b** (0.19 mmol) in DMF (2 mL), and stirred at 95°C for 50 h (for **9**) or 6 h (for **10a** and **10b**). The K_2CO_3 was filtered off. The filtrate was evaporated to dryness. The resulting oily residue was triturated with hexane. The resulting solid was filtered off. Solid compound **9** was rinsed with MTBE and dried in vacuo (KOH, 80°C). Compounds **10a** and **10b** that were obtained after hexane work-up were chromatographed over SiO_2 (C_6H_6–MTBE, 4:1; $CHCl_3$–MeOH, 4:1).

2-({2-[3β-Hydroxylup-20(29)-en-28-yloxy]-2-oxoethyl}(methyl)amino)ethanesulfonic Acid (9). Yield 0.11 g (90%), amorph., $[\alpha]_D^{20}$ +9.5° (*c* 0.64, CH_3OH). IR spectrum (ν, cm^{-1}): 1038, 1192, 1213, 1637, 1653, 1748, 3420. ^1H NMR spectrum (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 0.62 (1H, d, J = 9.8, H-5), 0.65 (3H, s, CH_3-24), 0.76 (3H, s, CH_3-25), 0.83 (1H, m, H_a-1), 0.87 (3H, s, CH_3-23), 0.94 (3H, s, CH_3-27), 0.99 (5H, s, CH_3-26, m, H_a-12, 15), 1.09 (1H, m, H_a-22), 1.20 (1H, m, H_a-11), 1.26 (2H, m, H-9, H_a-16), 1.34 (5H, m, H_a-6, 21, 2H-7, H_b-11), 1.44 (3H, m, 2H-2, H_b-6), 1.54 (1H, m, H_b-1), 1.57 (1H, m, H-18), 1.60 (2H, m, H-13, H_b-12), 1.65 (4H, s, CH_3-30, m, H_b-15), 1.72 (1H, m, H_b-22), 1.74 (1H, m, H_b-16), 1.91 (1H, m, H_b-21), 2.48 (1H, m, H-19), 2.66 (3H, s, NCH_3), 2.84 (2H, t, J = 7.5, $NCH_2C\underline{H}_2SO_3$), 2.96 (1H, dd, J = 10.0, 5.3, H-3), 3.21 (2H, m, $NC\underline{H}_2CH_2SO_3$), 3.28 (1H, d, J = 10.8, H_a-28), 3.98 (2H, s, $OC(O)CH_2$), 4.37 (1H, d, J = 10.8, H_b-28), 4.57, 4.71 (each 1H, s, H-29), 8.73 (1H, br.s, SO_3H). Table 1 lists the ^{13}C NMR spectrum. Mass spectrum (ESI), *m/z* 620 $[M - H]^-$ (calcd for $C_{35}H_{59}NO_6S$, 621).

2-({2-[17β-Methoxycarbonyllup-20(29)-en-3β-yloxy]-2-oxoethyl}(methyl)amino)ethanesulfonic Acid (10a). Yield 0.08 g (66%), amorph., $[\alpha]_D^{20}$ –8° (*c* 0.65, CH_3OH). IR spectrum (ν, cm^{-1}): 1045, 1174, 1188, 1201, 1640, 1729, 3446. ^1H NMR spectrum (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 0.79 (10H, m, H-5, s, CH_3-23, 24, 25), 0.83 (3H, s, CH_3-26), 0.94 (3H, s, CH_3-27), 1.64 (3H, s, CH_3-30), 2.12 (1H, m, H-16), 2.24 (3H, s, NCH_3), 2.63 (2H, m, $NCH_2C\underline{H}_2SO_3$), 2.75 (2H, m, $NC\underline{H}_2CH_2SO_3$), 2.90 (1H, m, H-19), 3.23 (2H, s, $OC(O)CH_2$), 3.58 (3H, s, $C(O)OCH_3$), 4.40 (1H, dd, J = 10.5, 5.3, H-3), 4.56, 4.69 (each 1H, s, H-29). Table 1 lists the ^{13}C NMR spectrum. Mass spectrum (ESI), *m/z* 648 $[M - H]^-$ (calcd for $C_{36}H_{59}NO_7S$, 649).

2-({2-[17β-Benzyloxycarbonyllup-20(29)-en-3β-yloxy]-2-oxoethyl}(methyl)amino)ethanesulfonic Acid (10b). Yield 0.09 g (64%), amorph., $[\alpha]_D^{20}$ +11° (*c* 0.38, CH_3OH). IR spectrum (ν, cm^{-1}): 1041, 1129, 1149, 1191, 1231, 1640, 1719, 1724, 3300. ^1H NMR spectrum (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 0.69 (3H, s, CH_3-24), 0.78 (3H, s, CH_3-25), 0.79 (7H, m, H-5, s, CH_3-23, 26), 0.92 (4H, m, H_a-1, s, CH_3-27), 0.97 (1H, m, H_a-12), 1.03 (1H, m, H_a-15), 1.12 (1H, m, H_a-11), 1.22 (2H, m, H_a-7, H_b-15), 1.27 (2H, m, H_a-6, H-9), 1.32 (3H, m, H_b-7, 11, H_a-21), 1.42 (2H, m, H_b-6, H_a-16), 1.47 (1H, m, H_a-22), 1.55 (1H, m, H_a-2), 1.57 (2H, m, H_b-2, H-18), 1.60 (2H, m, H_b-1, 12), 1.64 (3H, s, CH_3-30), 1.75 (1H, m, H_b-21), 1.80 (1H, m, H_b-22), 2.11 (1H, td, J = 11.0, 4.0, H-13), 2.17 (1H, d, J = 13.0, H_b-16), 2.26 (3H, s, NCH_3), 2.62 (2H, m, $NCH_2C\underline{H}_2SO_3$), 2.78 (2H, m, $NC\underline{H}_2CH_2SO_3$), 2.94 (1H, td, J = 11.0, 5.5, H-19), 3.22, 3.26 (each 1H, d, J = 17.3, $OC(O)CH_2$), 4.41 (1H, dd, J = 10.5, 5.3, H-3), 4.56, 4.68 (each 1H, s, H-29), 5.08, 5.12 (each 1H, d, J = 12.3, $C\underline{H}_2Ph$), 7.35 (5H, s, Ph). Table 1 lists the ^{13}C NMR spectrum. Mass spectrum (ESI), *m/z* 724 $[M - H]^-$ (calcd for $C_{42}H_{63}NO_7S$ 725).

Preparation of 11a and 11b. A suspension of the Na-salt of *N*-methyltaurine (0.14 g, 0.89 mmol) and K_2CO_3 (0.12 g, 0.89 mmol) in DMF (8 mL) was stirred under Ar for 15 min, treated dropwise with the appropriate bromoalkyl ester **6a** or **6b** (0.50 g, 0.89 mmol) in DMF (12 mL), and stirred for 50 h at 95°C. The K_2CO_3 was filtered off. The filtrate was evaporated. The oil residue was triturated with hexane. The solution was decanted. The solid was rinsed with MTBE (TLC monitoring) and dried (KOH, 80°C).

Sodium 2-({2-[3-Oxolup-20(29)-en-17β-carbonyloxy]ethyl}(methyl)amino)ethanesulfonate (11a). Yield 0.42 g (74%), mp 170–172°C, $[\alpha]_D^{20}$ +15.3° (*c* 0.45, CH_3OH). IR spectrum (ν, cm^{-1}): 1200, 1720, 3420. ^1H NMR spectrum (500 MHz, CD_3OD, δ, ppm, J/Hz): 0.93 (3H, s, CH_3-25), 0.98 (3H, s, CH_3-26), 1.01 (6H, s, CH_3-24, 27), 1.05 (3H, s, CH_3-23), 1.10 (1H, qd, J = 12.5, 5.0, H-12), 1.20 (1H, m, H_a-15), 1.30 (1H, dt, J = 12.5, 5.0, H_a-11), 1.40 (1H, m, H-5), 1.46 (10H, m, 1H-1, 6, 16, 21, 22, 2H-7, H-9, 1H-11, 15), 1.50 (1H, m, H-6), 1.66 (1H, t, J = 12.5, H-18), 1.68 (3H, s, CH_3-30), 1.73 (1H, m, H-12), 1.91 (2H, m, H-1, 22), 2.28 (2H, m, H-16, 21), 2.30 (1H, m, H-13), 2.33 (3H, s, NCH_3), 2.48 (2H, m, H-2), 2.70 (2H, m, $C(O)OCH_2C\underline{H}_2$)), 2.93 (2H, m, $NC\underline{H}_2CH_2SO_3$), 3.00 (1H, m, H-19), 3.03 (2H, m, $NCH_2C\underline{H}_2SO_3$), 4.18, 4.24 (each 1H, dt, J = 11.5, 5.3, $C(O)OC\underline{H}_2CH_2$), 4.59, 4.71 (each 1H, s, H-29). Table 1 lists the ^{13}C NMR spectrum. Mass spectrum (ESI), *m/z* 618 $[M - Na]^-$ (calcd for $C_{35}H_{56}NNaO_6S$, 641).

Sodium 2-({2-[3-Oxolup-20(29)-en-17β-carbonyloxy]propyl}(methyl)amino)ethanesulfonate (11b). Yield 0.40 g (69%), mp 162–164°C, $[\alpha]_D^{20}$ +15.5° (*c* 0.54, CH_3OH). ^1H NMR spectrum (300 MHz, CD_3OD, δ, ppm, J/Hz): 0.95 (3H, s, CH_3-25), 0.99 (3H, s, CH_3-26), 1.02 (3H, s, CH_3-24), 1.03 (3H, s, CH_3-27), 1.06 (3H, s, CH_3-23), 1.70 (5H, s, CH_3-30, m, 1H-12, H-18), 1.88 (4H, m, 1H-1, 22, $C(O)OCH_2C\underline{H}_2CH_2$), 2.29 (3H, m, H-13, 1H-16, 21), 2.38 (3H, s, NCH_3), 2.51 (4H, m, 2H-2, $C(O)OCH_2CH_2C\underline{H}_2$), 2.92 (2H, m, $NC\underline{H}_2CH_2SO_3$), 3.00 (3H, m, $NCH_2C\underline{H}_2SO_3$, H-19), 4.12 (2H, m, $C(O)OC\underline{H}_2CH_2CH_2$), 4.61, 4.73 (each 1H, s, H-29) (only characteristic resonances are given). Table 1 lists the ^{13}C NMR spectrum. Mass spectrum (ESI), *m/z* 632 $[M - Na]^-$ (calcd for $C_{36}H_{58}NNaO_6S$, 655).

Screening for Toxicity *in vitro*. The toxicity of the compounds was studied using a continuous culture of Madin-Darby canine kidney cells (MDCK) that was obtained initially from the Centers for Disease Control and Prevention (CDC, Atlanta, USA). Toxicity *in vitro* was assessed using the MTT assay, i.e., reduction of the dye thiazolyl blue tetrazolium bromide (MTT) by cells in culture. The experiments with the cell culture were conducted as follows. A weighed portion (5 mg) of compound in a sterile tube was diluted with DMSO to a concentration of 1 mg/mL (stock solution). Then, six sequential two-fold dilutions (100, 50, 25, 12.5, 6, and 3 μg/mL) with MDCK growth medium (Biolot, St. Petersburg) produced the solutions used to determine the toxicity. The test was performed in triplicate for each concentration. A 1-d culture of MDCK cells grown in 96-well plates was inspected visually using an inverted microscope for the integrity of the monolayer. The plates were rinsed twice with medium without serum. The test compound (100 μL per well) of the appropriate concentration was placed into each well. The plates were incubated for 72 h at 37°C with 5% CO_2. Test results were recorded visually by evaluating the integrity of the cell monolayer as compared with a control.

The MTT assay used 96-well plates and the standard protocol [25]. Optical density was recorded at 550 nm on a Varioscan microplate reader (Thermo Fisher). The median cytotoxic dose (CTD_{50}) was determined by linear regression of the photometric data using the Excel 2010 program. The regression equations were valid because $R^2 > 0.9$.

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