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# Synthesis and Cytotoxic Properties of N-Boc-phenylisoserinates of Sesquiterpenoic Alcohols from Mushrooms of *Lactarius* Genus, as Analogues of Taxotere®

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Sesquiterpenoic analogues of Taxotere® *i.e.*, N-BOC-phenylisoserinates of sesquiterpenoic alcohols, isolated from mushrooms of *Lactarius* genus, were synthesized. Cytotoxicity of compounds, thus obtained, was evaluated using Vero cells.

**Key words**: sesquiterpenoic alcohols isolated from mushrooms of *Lactarius* genus, (*2'R,3'S*)-N-BOC-phenylisoserinates, cytotoxicity

In the course of our study on biologically active components of mushrooms, belonging to *Lactarius* genus, we isolated and tested a large number of sesquiterpenoic alcohols, which exhibited antifeedant and antiviral properties [1–3]. Some time ago we started a new research on modification of these alcohols by esterification of the hydroxyl groups with N-acylphenylisoserine derivatives [4,5]. N-Acylphenylisoserine chains are fragments of crucial importance for the biological properties of antitumor drugs of taxane skeleton. In particular, Taxol® and Taxotere®, the two of the most successfully used substances in the cancer therapy, have N-benzoyl and N-*tert*-butoxycarbonylphenylisoserine moieties, respectively [6]. It was expected that combination of our sesquiterpenoic alcohols with N-acylphenylisoserines might shift the biological properties of sesquiterpenes toward the antitumor activity. Indeed, esterification of selected sesquiterpenoic alcohols with N-benzoylphenylisoserine afforded sesquiterpenoic analogues of Taxol®, which tested *in vitro* against A-549, PA-1, SKOV 3, U-373, PA cell cultures and *in vivo* against Mo-MSV exhibited in some cases very promising cytostatic properties [4]. In this paper we wish to present preparation and results of preliminary cytotoxicity assay of N-*tert*-butoxycarbonylphenylisoserinates of selected sesquiterpenoic alcohols, which are analogues of Taxotere®.

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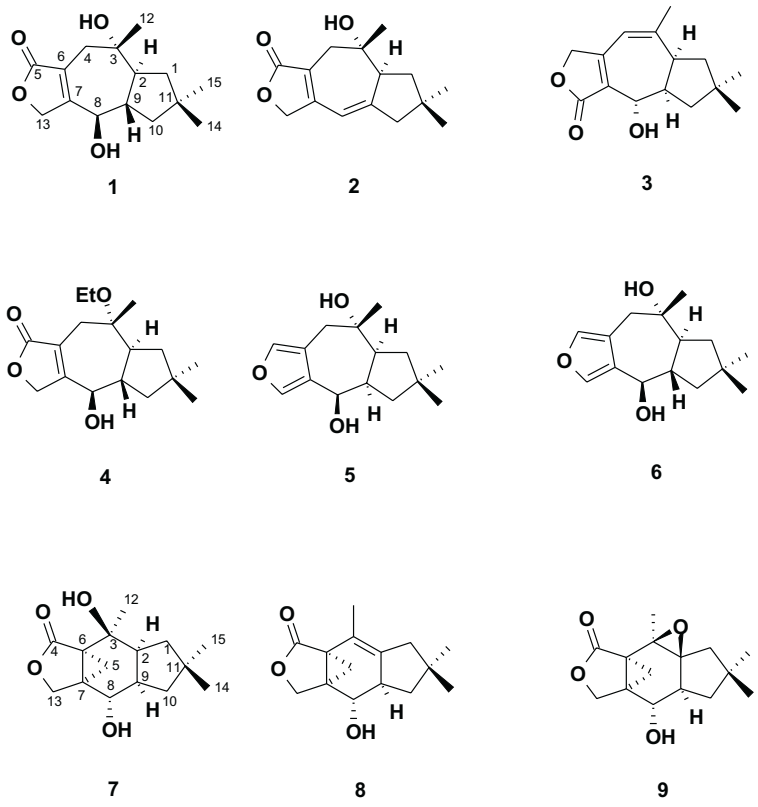
## RESULTS AND DISCUSSION

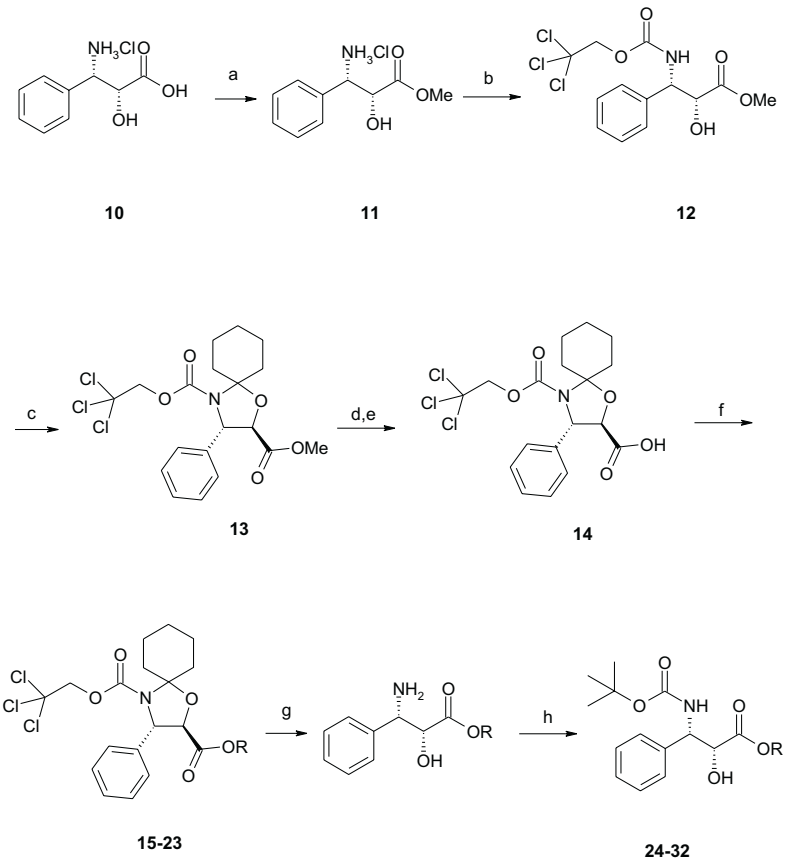
The sesquiterpenoic alcohols, which were used to the esterification, are shown in Scheme 1, comprise sesquiterpenoic alcohols of lactarane and isolactarane skeletons.

Scheme 1




The (2R,3S) phenylisoserine hydrochloride (**10**) was transformed into its methyl ester (**11**) in quantitative yield. Then, the ester was acylated with TrocCl to give the amide derivative (**12**). The hydroxyl group of the amide was protected with cyclohexanone dimethyl acetal by formation of oxazole (**13**). Alkaline hydrolysis of the ester group and then acidification gave the acid (**14**), which was used to esterification with various sesquiterpenoic alcohols in presence of DCC and DMAP. Esters (**15–23**) were deprotected by reduction with zinc in acetic acid to give amines. After acylation with $Boc_2O$, the required N-Boc-phenylisoserinates of various sesquiterpenoic alcohols **(24–32)** were obtained (Scheme 2).

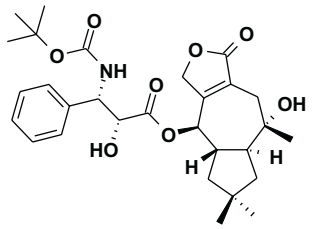
Scheme 2




a - $SOCl_2$, MeOH, $0^0$C, b - $Cl_3CH_2OCOCl$, $NaHCO_3$, $CH_2Cl_2$; c - 1,1-dimethoxycyclohexane, p-TsOH, toluene, 110 $^0$C; d - LiOH, $CH_3OH$; e - HCl; f - ROH (1-9), DCC, DMAP, toluene; g - Zn,$CH_3CO_2H$ - $CH_3OH$; h - $(^tBuOCO)_2O$, $Et_2O$

The sesquiterpene analogues of Taxotere® **(24–32)**, which were obtained using method described above, are shown in Scheme 3.

Scheme 3



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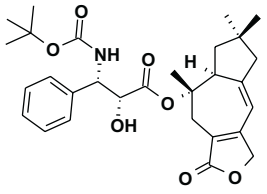

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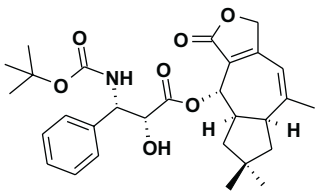

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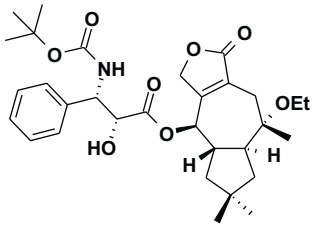

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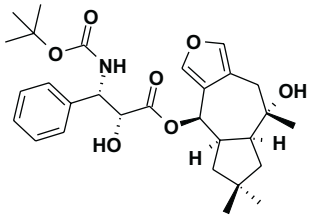

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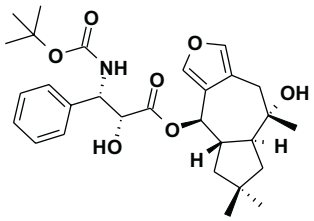

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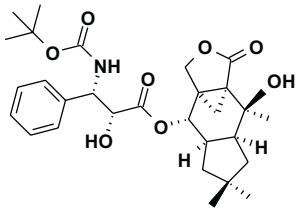

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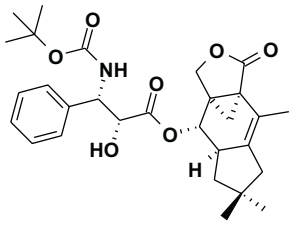

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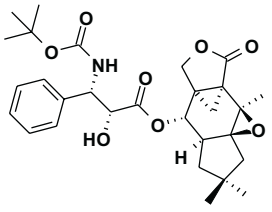

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**Cytotoxicity tests**. Preliminary estimation of cytotoxic activity is necessary for the investigation of biological activity such as antitumor activity antiviral activity *etc*., where subtoxic concentrations of evaluated compounds must be used. The cytotoxicity of synthesized N-BOC-phenylisoserinates of sesquiterpenoic alcohols (shown in Scheme 3) was tested using cell cultures of Vero cells. The results are presented in Table 1.

**Table 1.**

| Compound No | Concentration (μg/ml) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 256 | 128 | 64 | 32 | 16 | 8 | 4 | 2 |
| **24** | 46 ± 10* | 55 ± 9 | 82 ± 8 | **105 ± 8** | 98 ± 7 | 111 ± 10 | 103 ± 7 | 107 ± 4 |
| **25** | 57 ± 4 | 63 ± 5 | 85 ± 7 | 87 ±10 | **101 ± 4** | 105 ± 10 | 102 ± 5 | 108 ± 8 |
| **26** | 45 ± 6 | 51 ± 5 | 59 ± 7 | 62 ± 3 | 69 ± 8 | **95 ± 5** | 103 ± 8 | 99 ± 6 |
| **27** | 49 ± 3 | 68 ± 6 | **102 ± 4** | 106 ± 7 | 103 ± 7 | 105 ± 8 | 103 ± 4 | 105 ± 5 |
| **28** | 53 ± 5 | 73 ± 2 | 90 ± 8 | **103 ± 6** | 106 ± 7 | 104 ± 5 | 107 ± 6 | 100 ± 6 |
| **29** | 52 ± 6 | 57 ± 9 | 67 ±7 | 72 ± 4 | 78 ± 8 | **99 ± 10** | 104 ± 7 | 106 ± 8 |
| **30** | 43 ± 5 | 57 ± 9 | 64 ± 5 | 70 ± 3 | 75 ± 7 | **98 ± 5** | 99 ±10 | 107 ± 9 |
| **31** | 45 ± 6 | 49 ± 5 | 53 ±10 | 67 ±10 | 81 ± 7 | **100 ± 8** | 98 ± 3 | 104 ± 6 |
| **32** | 63 ± 2 | 89 ± 6 | **98 ± 7** | 104 ± 6 | 102 ± 3 | 105 ± 6 | 106 ± 5 | 101 ± 7 |

*Live cells (percentage of total).

The numbers shown in bold letters represent threshold of non toxic concentrations. In case of compounds **24** and **27** the change of hydroxyl group in position 3 of the sesquiterpene part into OEt group decreased toxicity. Change of stereochemistry of the ring junction between the five and seven member rings in compounds **28** and **29** caused increase of toxicity. The dehydration of the sesquiterpenoic part in compound **30** to produce compound **31** had no effect on toxicity. However, epoxidation of 2,3-double bond in **31** afforded compound **32** with much lower toxicity.

## EXPERIMENTAL

**Isolation and preparation of sesquiterpenoic alcohols**. The phenylisoserinates were prepared from sesquiterpenoic alcohols isolated from mushrooms (*Lactarius rufus* and *Lactarius vellereus*) or synthesized by transformation of natural products. The references of the procedures of isolation or preparation of the sesquiterpenes used for the preparation of esters (**15–23**) can be found in review [7].

**Phenylisoserine hydrochloride methyl ester (11)**. Phenylisoserine hydrochloride (**10**, 16.0 g, 74 mmole), prepared according to [8], was dissolved in methanol (120 ml) and the solution chilled to 0°C. Subsequently thionyl chloride (11.0 ml, 130 mmole) was added dropwise. The reaction mixture was stirred during 3 hrs and the volatiles were removed by evaporation leaving a residue, which contained the required ester (**11**) in quantitative yield. $[\alpha]_D^{20} = -19.7$ (c 1.0, $H_2O$); UV (EtOH) $\lambda_{max}$ 205 nm, $\varepsilon_{max}$ 9020; IR (film) $\nu_{max}$: 3429, 2955, 1742, 1615 $cm^{-1}$; $^1H$ NMR (400 MHz, $D_2O$) δ 3.68 (s, 3H); 4.70 (ABq, $J_{AB} = 6.9$ Hz, 2H); 7.45–7.49 (m, 2H); 7.50–7.54 (m, 3H); $^{13}C$ NMR (100 MHz, $D_2O$): 53.21; 57.12; 72.06; 127.52; 129.49; 130.06; 132.69; 172.40.

**N-2,2,2-Trichloroethoxycarbonyl phenylisoserine methyl ester** (**12**). Phenylisoserine methyl ester hydrochloride (**11**, 2 g, 8.7 mmole) was dissolved in $CH_2Cl_2$ (50 ml) and $NaHCO_3$ ground (20 g) and TrocCl (1.43 ml, 10.4 mmole) were added. Reaction was carried out in rt. and monitored with TLC ($CH_2Cl_2$:iPrOH, 98:2). After 0.5 hour the reaction mixture was filtered under reduced pressure to remove $NaHCO_3$ and then evaporated. The residue was crystallized from hexane-methylene chloride by precipitation and gave pure **12** (3.15 g, 98% yield). M.p. 131–133°C; $[\alpha]_D$ +5.5 (c 1.06, EtOH); UV (EtOH) $\lambda_{max}$

257 nm, $\varepsilon_{max}$ 150; IR (film) $\nu_{max}$: 3390; 2955; 1740; 1531 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) δ: 3.22 (d, J = 3.9 Hz, 1H); 3.84 (s, 3H); 4.52 (m, 1H); 4.71 (ABq, J = 12.0 Hz, 2H); 5.28 (d, J = 9.4 Hz, 1H); 5.90 (d, J = 9.4 Hz, 1H); 7.31 (tt, J = 6.9, 2.5 Hz, 1H); 7.33–7.41 (m, 4H); $^{13}C$ NMR (125 MHz, $CDCl_3$) δ: 53.24; 56.66; 73.17; 74.63; 95.45; 126.71; 128.10; 128.75; 138.30; 154.02; 172.98; ESI (MeOH) m/z: 391 $(M+Na)^+$; HR-MS 391.983 calculated for $C_{13}H_{14}NO_5NaCl_3$, found 391.9867.

**3-2,2,2-Trichloroethoxycarbonyl-2-cyclohexyl-4-phenyl-5-oxazolidinecarboxylic acid methyl ester (4*S*, 5*R*) (13)**. Compound **12** (1.5 g, 4 mmol) was dissolved in toluene (25 ml) and cyclohexanone dimethyl acetal (1.23 ml, 8 mmol) and p-toluenesulfonic acid (80 mg) were added. Reaction was carried out at 120°C and monitored by TLC (hexane:$CH_2Cl_2$:$Et_2O$, 45:45:10). After 3 hrs reaction was washed with $NaHCO_3$ solution, dried over $MgSO_4$ and the solvent was evaporated. The residue was purified by column chromatography on silica gel (hexane:diethyl ether, 93:7) and gave pure **13** (1.7 g, 93% yield). Oil; $[\alpha]_D$ +1.6 (c 1.16, EtOH); UV (EtOH) $\lambda_{max}$ 258 nm, $\varepsilon_{max}$ 307; IR (film) $\nu_{max}$: 2938; 2864; 1720 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) δ: 1.15–1.36 (m, 1H); 1.56–1.65 (m, 1H); 1.67–1.83 (m, 5H); 1.97 (br d, J = 13.7 Hz, 1H); 2.35–2.52 (m, 2H); 3.81 (s, 3H); 4.48–4.83 (m, 3H); 5.40 (d, J = 3.8 Hz, 1H); 7.24–7.40 (m, 5H); $^{13}C$ NMR (125 MHz, $CDCl_3$) δ: 23.05; 23.26; 24.53; 32.87; 34.72; 52.57; 63.27; 74.58; 81.53; 95.00; 99.99; 126.39; 127.86; 128.68; 140.48; 150.52; 171.06; ESI (MeOH) m/z: 472 $(M+Na)^+$; HR-MS 472.0456 calculated for $C_{19}H_{22}NO_5NaCl_3$, found 472.0484.

**3-2,2,2-Trichloroethoxycarbonyl-2-cyclohexyl-4-phenyl-5-oxazolidinecarboxylic acid (4*S*, 5*R*) (14)**. Compound **13** (1.5 g, 3.3 mmol) was dissolved in MeOH (25 ml) and 0.5 mol/l LiOH (7.3 ml, 3.6 mmole) was added dropwise. Reaction was carried out at rt. until **13** disappeared (TLC, hexane:$CH_2Cl_2$:$Et_2O$ 45:45:10), about 0.5 h. Then, reaction mixture was extracted with diethyl ether and the organic phase was removed. The aqueous phase was acidified by 0.1 mol/l HCl (40 ml) and extracted with methylene chloride. The extract was washed with water and dried over $MgSO_4$. After evaporating, pure **14** was obtained (1.42 g, 98% yield), which was immediately used for esterification.

**General method for esterification of alcohols**. The sesquiterpenoic alcohol (**1–9**, 1 mmole) and the acid **14** (1.2 mmole) were dissolved in toluene (25 ml) and DCC (1.4 mmole) and DMAP (0.15 mmole) were added. The reaction was carried out at rt. and was monitored by TLC. When the reaction was completed (5–20 hrs), the contents of the flask were filtered, the solution evaporated and the residue purified by chromatography to give the desired esters (**15–23**).

**Compound 15** was obtained from **1** according to the general method described above. Yield 78%; eluent: hexane-methylene chloride-acetone (46.5:46.5:7); oil; $[\alpha]_D$ –15.3(c 0.97, $CDCl_3$); UV (EtOH) $\lambda_{max}$ 209 nm; $\varepsilon_{max}$ 18702; IR (KBr) $\nu_{max}$: 3447; 2951; 1743; 1725 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) δ: 1.02 (s,3H); 1.09 (s, 3H); 1.14 (s, 3H); 1.20–1.35 (m, 1H); 1.39 (dd, J = 12.6, 10.7 Hz, 1H); 1.56 (dd, J = 13.2, 10.6 Hz, 1H); 1.68 (dd, J = 12.6, 7.0 Hz, 1H); 1.70–1.82 (m, 6H); 1.89 (dd, J = 13.2, 8.3 Hz, 1H); 1.98 (br d, J = 13.3 Hz, 1H); 2.19 (dtd, J = 10.8, 10.6, 7.0 Hz, 1H); 2.29 (td, J = 10.8, 8.4 Hz, 1H); 2.35–2.46 (m, 3H); 2.83 (d, J = 15.2 Hz, 1H); 4.51 (ddd, J = 17.6, 2.9, 1.1 Hz, 1H); 4.59 (br s, 1H); 4.70 (br d, J = 17.6 Hz, 1H); 4.42–4.74 (m, 2H); 5.28 (d, J = 4.7 Hz, 1H); 5.87 (d, J = 10.3 Hz, 1H); 7.30–7.40 (m, 5H); $^{13}C$ NMR (125 MHz, $CDCl_3$) δ: 20.91; 23.27; 23.31; 24.48; 30.96; 31.16; 33.31; 34.46; 34.67; 39.55; 43.38; 44.06; 46.39; 54.47; 63.75; 68.96; 72.23; 74.64; 76.82; 81.61; 94.95; 99.48; 124.63; 126.43; 128.36; 128.88; 150.48; 159.03; 169.46; 173.62; ESI (MeOH) m/z: 706 $(M+Na)^+$; HR-MS 706.1712 calculated for $C_{33}H_{40}NO_8NaCl_3$, found 706.1723.

**Compound 16** was obtained from **2** according to the general method described above. Yield 82%; eluent: hexane-methylene chloride-diethyl ether (48.5:48.5:3); M.p. 182–185°C; $[\alpha]_D$ +146.8 (c 0.96, EtOH); UV (EtOH) $\lambda_{max}$ 209 nm, 281 nm; $\varepsilon_{max}$ 15515; IR (KBr) $\nu_{max}$: 3441; 2953; 2867; 1759; 1728 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) δ: 0.93 (s, 3H); 1.13 (s, 3H); 1.28 (s, 3H); 1.49 (ddd, J = 12.2, 7.8, 1.7 Hz, 1H); 1.65 (t, J = 12.1 Hz, 1H); 1.68–1.85 (m, 6H); 1.97 (br d, J = 13.0 Hz, 1H); 2.30–2.40 (m, 2H); 2.41–2.53 (m, 2H); 2.95 (d, J = 18.2 Hz, 1H); 3.51 (d, J = 18.2 Hz, 1H); 3.85 (m, 1H); 4.45 (br s, 1H); 4.51 (d, J = 11.2 Hz, 1H); 4.62 (d, J = 11.2 Hz, 1H); 4.71 (br s, 2H); 5.32 (d, J = 3.9 Hz, 1H); 5.90 (s, 1H); 7.31–7.42 (m, 5H); $^{13}C$ NMR (125 MHz, $CDCl_3$) δ: 20.25; 23.32; 24.56; 26.59; 28.50; 33.10; 34.79; 36.85; 36.93; 43.17; 51.16; 51.41; 63.27; 70.84; 74.62; 81.97; 83.66; 95.04; 99.04; 112.37; 119.51; 125.28; 126.61; 127.89; 128.66; 150.50; 151.46; 159.01; 169.56; 174.59; ESI (MeOH) m/z: 688 $(M+Na)^+$; HR-MS 688.1606 calculated for $C_{33}H_{38}NO_7NaCl_3$, found 688.1611.

**Compound 17** was obtained from **3** according to the general method described above. Yield 74%; eluent: hexane-methylene chloride-diethyl ether (48:48:4); oil; $[\alpha]_D$ –103.2 (c 0.59, EtOH); UV (EtOH) $\lambda_{max}$ 286 nm; $\varepsilon_{max}$ 13364; IR (film) $\nu_{max}$: 3393; 2937; 2866; 1754; 1720 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) δ: 1.01 (s,3H); 1.05 (s, 3H); 1.10–1.37 (m, 2H); 1.44–1.51 (m, 1H); 1.60–1.80 (m, 7H); 1.91–1.96 (m, 2H); 1.97 (s, 3H); 2.31–2.51 (m, 3H); 2.87 (m, 2H); 4.45–4.65 (m, 3H); 4.68 (s, 2H); 5.40 (s, 1H); 5.66 (s, 1H); 5.77 (s, 1H); 7.28–7.40 (m, 5H); $^{13}C$ NMR (125 MHz, $CDCl_3$) δ: 23.17; 23.28; 24.59; 27.40; 29.37; 30.64; 32.89; 34.88; 35.87; 43.68; 47.50; 47.96; 48.58; 63.37; 68.46; 70.35; 74.54; 81.75; 95.08; 99.09; 114.07; 121.66; 126.37; 127.78; 128.59; 140.69; 150.47; 158.46; 159.36; 169.76; 172.67; ESI (MeOH) m/z: 688 $(M+Na)^+$; HR-MS 688.1606 calculated for $C_{33}H_{38}NO_7NaCl_3$, found 688.1589.

**Compound 18** was obtained from **4** according to the general method described above. Yield 93%; eluent: hexane-methylene chloride-diethyl ether (57:38:5); M.p. 151–154°C; $[\alpha]_D$ –10.9 (c 1.33, $CH_2Cl_2$); UV (EtOH) $\lambda_{max}$ 209 nm; $\varepsilon_{max}$ 16521; IR (KBr) $\nu_{max}$: 2952; 2867; 1763; 1723 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) δ: 1.00 (s,3H); 1.07 (s,6H); 1.13 (t, J = 6.9 Hz, 3H); 1.23–1.35 (m, 1H); 1.36 (dd, J = 12.6, 10.4 Hz, 1H); 1.53 (dd, J = 13.5, 10.5 Hz, 1H); 1.65 (dd, J = 12.6, 6.9 Hz, 1H); 1.66–1.80 (m, 6H); 1.80 (dd, J = 13.5, 8.2 Hz, 1H); 1.98 (br d, J = 12.9 Hz, 1H); 2.25 (dtd, J = 10.6, 10.4, 6.9 Hz, 1H); 2.34 (dt, J = 10.8, 8.4 Hz, 1H); 2.44 (d, J = 14.9 Hz, 1H); 2.46–2.60 (m, 2H); 2.80 (d, J = 14.9 Hz, 1H); 3.44 (m, 2H); 4.51 (dd, J = 17.5, 1.3 Hz, 1H); 4.41–4.72 (m, 2H); 4.59 (br s, 1H); 4.69 (d, J = 17.5 Hz, 1H); 5.29 (d, J = 4.6 Hz, 1H); 5.83 (d, J = 10.4 Hz, 1H); 7.30–7.40 (m, 5H); $^{13}C$ NMR (125 MHz, $CDCl_3$) δ: 15.95; 18.19; 23.27; 24.48; 30.90; 31.08; 33.28; 33.87; 34.48; 34.68; 43.29; 44.04; 46.19; 52.46; 56.37; 63.70; 69.04; 74.62; 75.97; 76.78; 81.64; 94.94; 99.43; 124.99; 126.41; 128.31; 128.91; 139.68; 150.40; 158.59; 169.51; 173.79; ESI (MeOH + $CHCl_3$) m/z: 734 $(M+Na)^+$; HR-MS 734.2025 calculated for $C_{35}H_{44}NO_8NaCl_3$, found 734.2047.

**Compound 19** was obtained from **5** according to the general method described above. Yield 66%; eluent: hexane-methylene chloride-diethyl ether (47.5:47.5:5); oil; $[\alpha]_D$ –17.9 (c 1.09, EtOH); UV (EtOH) $\lambda_{max}$ 212 nm; $\varepsilon_{max}$ 13506; IR (film) $\nu_{max}$: 3493; 2937; 2865; 1723 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) δ: 0.98 (s, 6H); 1.28 (s, 3H); 1.28–1.34 (m, 2H); 1.42–1.50 (m, 1H); 1.51–1.62 (m, 2H); 1.65–1.84 (m, 6H); 1.97 (br d, J = 12.3 Hz, 1H); 2.35–2.55 (m, 3H); 2.65 (d, J = 16.2 Hz, 1H); 2.75 (br s, 1H); 2.88 (d, J = 16.2 Hz, 1H); 4.35–4.80 (m, 2H); 4.55 (br s, 1H); 5.24 (s, 1H); 6.51 (br s, 1H); 7.22 (s, 2H); 7.27–7.37 (m, 5H); $^{13}C$ NMR (125 MHz, $CDCl_3$) δ: 23.30; 24.57; 29.22; 29.74; 31.96; 32.99; 33.10; 34.63; 36.77; 42.52; 42.82; 44.28; 51.75; 63.82; 73.23; 74.58; 77.19; 81.90; 95.00; 99.10; 118.68; 123.33; 126.52; 127.97; 128.75; 140.12; 140.42; 141.11; 150.48; 169.45; ESI (MeOH) m/z: 690 $(M+Na)^+$; HR-MS 690.1763 calculated for $C_{33}H_{40}NO_7NaCl_3$, found 690.1776.

**Compound 20** was obtained from **6** according to the general method described above. Yield 95%; eluent: hexane-methylene chloride-diethyl ether (47.5:47.5:5); oil; $[\alpha]_D$ –3.4 (c 0.62, EtOH); UV (EtOH) $\lambda_{max}$ 202 nm; $\varepsilon_{max}$ 17793; IR (film) $\nu_{max}$: 3510; 2939; 2865; 1720 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) δ: 1.00 (s, 3H); 1.04 (s, 3H); 1.06 (s, 3H); 1.20–1.35 (m, 1H); 1.39 (dd, J = 12.7, 10.1 Hz, 1H); 1.51 (dd, J = 12.8, 10.8 Hz, 1H); 1.69 (dd, J = 12.7, 7.1 Hz, 1H); 1.80 (dd, J = 12.8, 7.9 Hz, 1H); 1.67–1.85 (m, 6H); 2.00 (br d, J = 12.9 Hz, 1H); 2.03–2.19 (m, 2H); 2.46 (m, 2H); 2.59 (d, J = 14.1 Hz, 1H); 2.71 (d, J = 14.1 Hz, 1H); 4.61 (br s, 1H); 4.40–4.85 (m, 2H); 5.39 (d, J = 4.3 Hz, 1H); 5.84 (d, J = 10.0 Hz, 1H); 7.08 (s, 1H); 7.21 (s, 1H); 7.27–7.41 (m, 5H); $^{13}C$ NMR (125 MHz, $CDCl_3$) δ: 20.91; 23.35; 24.55; 31.02; 33.14; 34.80; 34.96; 40.07; 43.12; 46.28; 46.33; 55.00; 63.73; 73.41; 74.61; 75.90; 81.90; 95.03; 99.25; 119.13; 124.87; 126.48; 128.06; 128.80; 138.53; 140.89; 155.37; 172.54; LSIMS (NBA) m/z: 690 $(M+Na)^+$; HR-MS 690.1768 calculated for $C_{33}H_{40}NO_7NaCl_3$, found 690.1736.

**Compound 21** was obtained from **7** according to the general method described above. Yield 93%; eluent: hexane-methylene chloride-ethyl acetate (47.5:47.5:5); oil; $[\alpha]_D$ –33.2 (c 0.91, EtOH); UV (EtOH) $\lambda_{max}$ 209 nm; $\varepsilon_{max}$ 10536; IR ($CHCl_3$) $\nu_{max}$: 3609; 2940; 1767; 1719 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) δ: 0.93 (s, 3H); 1.09 (s, 3H); 1.09–1.11 (m, 1H); 1.26 (t, J = 7.1 Hz, 1H); 1.29 (t, J = 11.4 Hz, 1H); 1.46–1.54 (m, 1H); 1.60–1.66 (m, 2H); 1.64 (s, 3H); 1.67–1.83 (m, 6H); 1.97 (br d, J = 13.5 Hz, 1H); 2.07–2.15 (m, 2H); 2.39–2.55 (m, 2H); 4.10 (d, J = 9.4 Hz, 1H); 4.41 (d, J = 9.4 Hz, 1H); 4.56 (br s, 1H); 4.44–4.83 (m, 2H); 5.36 (d, J = 2.6 Hz, 1H); 5.62 (d, J = 7.7 Hz, 1H); 7.28–7.42 (m, 5H); $^{13}C$ NMR (125 MHz, $CDCl_3$) δ: 18.26; 23.28; 24.49; 24.70; 26.87; 28.73; 31.61; 32.93; 34.89; 38.44; 39.00; 39.97; 40.00; 45.82; 47.16; 63.39; 71.03; 71.47; 74.66; 79.31; 81.74; 95.07; 99.19; 126.52; 128.01; 128.75; 140.61; 150.49; 170.83; 175.00; ESI (MeOH) m/z: 706 $(M+Na)^+$; HR-MS 706.1712 calculated for $C_{33}H_{40}NO_8NaCl_3$, found 706.1801.

**Compound 22** was obtained from **8** according to the general method described above. Yield 99%; eluent: hexane-diethyl ether (80:20); oil; $[\alpha]_D$ –22.5 (c 0.57, EtOH); UV (EtOH) $\lambda_{max}$ 209 nm; $\varepsilon_{max}$ 17937; IR (film) $\nu_{max}$: 2937; 2866; 1775; 1721 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) δ: 0.99 (s, 3H); 1.07 (s, 3H); 1.26 (t, J = 11.6 Hz, 1H); 1.32 (d, J = 5.0 Hz, 1H); 1.53–1.65 (m, 1H); 1.70–1.83 (m, 7H); 1.82 (d, J = 5.0 Hz, 1H); 1.98 (br d, J = 13.5 Hz, 1H); 2.02 (t, J = 1.3 Hz, 3H); 2.00–2.15 (m, 2H); 2.40–2.60 (m, 3H); 4.16 (d, J = 9.7 Hz, 1H); 4.42 (d, J = 9.7 Hz, 1H); 4.45–4.83 (m, 2H); 4.60 (br s, 1H); 5.14 (d, J = 9.3 Hz, 1H); 5.40 (br s, 1H); 7.27–7.41 (m, 5H); $^{13}C$ NMR (125 MHz, $CDCl_3$) δ: 15.31; 23.28; 23.92; 24.47; 28.50; 29.58; 32.91; 33.69; 34.45; 34.88; 37.60; 42.04; 43.96; 45.96; 63.38; 71.43; 74.60; 77.92; 81.70; 95.04; 99.19; 120.97; 126.52; 128.04; 128.77; 134.28; 140.53; 150.47; 171.10; 173.41; ESI (MeOH +AcONa) m/z: 688 $(M+Na)^+$; HR-MS 688.1606 calculated for $C_{33}H_{38}NO_7NaCl_3$, found 688.1627.

**Compound 23** was obtained from **9** according to the general method described above. Yield 85%; eluent: hexane-methylene chloride-diethyl ether (65:33:2); M.p. 197–198°C ; $[\alpha]_D$ –36.9 (c 0.96, EtOH); UV (EtOH) $\lambda_{max}$ 210 nm; $\varepsilon_{max}$ 8718; IR (film) $\nu_{max}$: 2937; 2866; 1777; 1722 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) δ: 1.09 (s, 3H); 1.17 (s, 3H); 1.17–1.22 (m, 1H); 1.24–1.38 (m, 1H); 1.46 (d, J = 14.7 Hz, 1H); 1.61 (t, J = 12.4 Hz, 1H); 1.73 (s, 3H); 1.70–1.80 (m, 11H); 1.88 (d, J = 14.7 Hz, 1H); 1.96 (br d, J = 13.3 Hz, 1H); 2.16–2.26 (m,1H); 2.40–2.55 (m, 2H); 4.04 (d, J = 9.7 Hz, 1H); 4.38 (d, J = 9.7 Hz, 1H); 4.44–4.82 (m, 2H); 4.55 (br s, 1H); 5.26 (d, J = 9.7 Hz, 1H); 5.35 (br s, 1H); 7.28–7.40 (m, 5H); $^{13}C$ NMR (125 MHz, $CDCl_3$) δ: 15.08; 20.22; 23.31; 24.47; 31.11; 31.56; 32.17; 32.90; 34.87; 36.72; 36.83; 40.51; 43.52; 43.78; 58.24; 63.34; 71.74; 73.25; 74.60; 75.09; 81.57; 95.01; 99.17; 126.50; 128.08; 128.78; 140.32; 150.46; 170.82; 173.42; ESI (MeOH) m/z: 704 $(M+Na)^+$; HR-MS 704.1555 calculated for $C_{33}H_{38}NO_8NaCl_3$, found 704.5556.

**General method for the reduction of esters**. The ester (**15–23**, 200 mg) dissolved in MeOH (3 ml) was treated with powdered zinc (1 g) and concentrated AcOH (0.5 ml). The reaction was carried out at rt. until ester disappeared (TLC, $CH_2Cl_2$:iPrOH, 98:2), what took place in about 0.5 h. Subsequently, the excess of zinc was filtered off and the filtrate was diluted with water, extracted with methylene chloride and the extract was washed with aqueous $NaHCO_3$ solution. The aqueous phase was made alkaline with $NaHCO_3$ solution and extracted with methylene chloride. Combined methylene chloride extracts were dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography ($CH_2Cl_2$:iPrOH gradient) to give pure amine, which was immediately acylated.

**General method for the acylation**. To the amine (1 mmole) dissolved in diethyl ether (5 ml) $Boc_2O$ (5 mmole) was added. Reaction was carried out at rt. until amine disappeared (TLC, $CH_2Cl_2$:iPrOH 98:2), which took place within 5–20 hrs. Subsequently, the reaction mixture was evaporated and the residue was purified by column chromatography to give pure **24–32**.

**Compound 24** was obtained from **15** according to the general method described above. Yield: 42%; eluent: methylene chloride-isopropyl alcohol (98:2); $[\alpha]_D$ +28.5 (c 0.99, EtOH); UV (EtOH) $\lambda_{max}$ 209 nm; $\varepsilon_{max}$ 29113; IR (film) $\nu_{max}$: 3434; 2954; 1749; 1703 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) δ: 1.05 (s, 3H); 1.08 (s, 3H); 1.15 (s, 3H); 1.32 (m, 1H); 1.42 (br s, 9H); 1.58 (dd, J = 13.1, 10.1 Hz, 1H); 1.69 (dd, J = 12.7, 6.2 Hz, 1H); 1.89 (dd, J = 13.1, 7.8 Hz, 1H); 2.19–2.32 (m, 2H); 2.38 (d, J = 15.1 Hz, 1H); 2.84 (d, J = 15.1 Hz, 1H); 3.22 (br s, 1H); 4.52 (br s, 1H); 4.61 (d, J = 18.0 Hz, 1H); 4.80 (d, J = 18.0 Hz, 1H); 5.21 (d, J = 9.4 Hz, 1H); 5.44 (d, J = 9.4 Hz, 1H); 5.84 (d, J = 8.6 Hz, 1H); 7.30–7.42 (m, 5H); $^{13}C$ NMR (125 MHz, $CDCl_3$) δ: 20.83; 28.29; 31.02; 31.24; 34.37; 39.60; 43.46; 43.99; 46.46; 54.54; 55.85; 69.11; 72.25; 73.75; 77.81; 80.78; 124.27; 126.65; 128.08; 128.82; 138.97; 155.35; 159.44; 172.40; 173.96; ESI (MeOH) m/z: 552 $(M+Na)^+$; HR-MS 552.2568 calculated for $C_{29}H_{39}NO_8Na$, found 552.2587.

**Compound 25** was obtained from **16** according to the general method described above. Yield: 74%; eluent: hexane-methylene chloride-acetone (47.5:47.5:5); $[\alpha]_D$ +173.0 (c 1.23, EtOH); UV (EtOH) $\lambda_{max}$ 202 nm, $\lambda_2$ 281 nm; IR (film) $\nu_{max}$: 3440; 2958; 1746; 1633 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) δ: 0.97 (s, 3H); 1.16 (s, 3H): 1.35 (s, 3H); 1.42 (br s, 9H); 1.65–1.77 (m, 2H); 2.37 (ABq, J = 17.4 Hz, 2H); 3.14 (br s, 1H); 3.15–3.27 (m, 2H); 3.75 (br s, 1H); 4.37 (s, 1H); 4.70 (s, 2H); 5.16 (d, J = 7.1 Hz, 1H); 5.45 (d, J = 7.1 Hz, 1H); 5.91 (s, 1H); 7.34–7.40 (m, 5H); $^{13}C$ NMR (125 MHz, $CDCl_3$) δ: 19.56; 26.64; 28.39; 28.54; 36.80; 37.12; 42.97; 51.25; 52.26; 55.86; 70.78; 73.99; 80.00; 84.57; 112.43; 119.57; 126.63; 127.69; 128.60; 139.78; 151.49; 155.14; 158.85; 171.73; 174.50; ESI (MeOH) m/z: 534 $(M+Na)^+$; HR-MS 534.2462 calculated for $C_{29}H_{37}NO_7Na$, found 534.2447.

**Compound 26** was obtained from **17** according to the general method described above. Yield: 37%; eluent: hexane-methylene chloride-diethyl ether (45:45:10); oil; $[\alpha]_D$ –74.4 (c 0.40, EtOH; UV (EtOH) $\lambda_{max}$ 286 nm; $\varepsilon_{max}$ 12007; IR ($CHCl_3$) $\nu_{max}$: 3520, 3520, 3438, 2959, 1753, 1714, 1496 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) δ: 1.03 (s, 3H); 1.04 (s, 3H); 1.30–1.38 (m, 1H); 1.42 (br.s, 9H); 1.47 (dd, J = 12.7, 8.1 Hz, 1H); 1.66 (dd, J = 12.8, 7.0 Hz, 1H); 1.96 (dd, J = 12.7, 7.8 Hz, 1H); 2.07 (s, 3H); 2.80–2.93 (m, 1H); 3.03–3.11 (m, 1H); 4.50 (d, J = 1.7 Hz, 1H); 4.71 ABq, J = 17.1 Hz, 2H); 5.16 (d, J = 8.9 Hz, 1H); 5.48 (d, J = 8.9 Hz, 1H); 5.75 (d, J = 5.8 Hz, 1H); 5.80 (s, 1H); 7.26 (tt, J = 6.7, 1.7 Hz, 1H); 7.31–7.37 (m, 4H); $^{13}C$ NMR (125 MHz, $CDCl_3$) δ: 27.43; 28.32; 29.24; 30.62; 35.82; 43.62; 47.41; 47.76; 48.77; 55.70; 69.08; 70.47; 73.38; 79.69; 113.80; 121.18; 126.58; 127.56; 128.50; 139.62; 155.12; 159.19; 160.78; 172.20; 172.97; ESI (MeOH) m/z: 534 $(M+Na)^+$; HR-MS 534.2479 calculated for $C_{29}H_{37}NO_7Na$, found 534.2462.

**Compound 27** was obtained from **18** according to the general method described above. Yield: 70%; eluent: hexane-methylene chloride-acetone (47.5:47.5:5); $[\alpha]_D$ +32.8 (c 1.06, EtOH); UV (EtOH) $\lambda_{max}$ 208 nm, $\varepsilon_{max}$ 15791; IR (film) $\nu_{max}$: 3440; 2953; 2935; 1718 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) δ: 1.03 (s, 3H); 1.07 (s, 3H); 1.08 (s, 3H); 1.14 (t, J = 6.9 Hz, 1H); 1.26–1.34 (m, 1 H); 1.42 (br s, 9H); 1.55 (dd, J = 13.6, 10.2 Hz, 1H); 1.66 (dd, J = 12.7, 6.3 Hz, 1H); 1.81 (dd, J = 13.6, 7.8 Hz, 1H); 2.25–2.37 (m, 2H); 2.42 (d, J = 15.0 Hz, 1H); 2.84 (d, J = 15.0 Hz, 1H); 3.17 (br s, 1H); 3.44 (m, 2H); 4.51 (br s, 1H); 4.61 (d, J = 18.0 Hz, 1H); 4.79 (d, J = 18.0 Hz, 1H); 5.21 (d, J = 9.1 Hz, 1H); 5.42 (d, J = 9.1 Hz, 1H); 5.81 (d, J = 8.9 Hz, 1H); 7.30–7.40 (m, 5H); $^{13}C$ NMR (125 MHz, $CDCl_3$) δ: 15.99; 17.99; 28.29; 30.99; 31.23; 33.99; 34.34; 43.42; 44.05; 46.30; 52.69; 55.83; 56.37; 69.16; 73.72; 75.97; 77.80; 80.75; 124.61; 126.65; 128.07; 128.81; 139.00; 155.32; 159.07; 172.47; 174.15; ESI (MeOH) m/z: 580 $(M+Na)^+$; HR-MS 580.2881 calculated for $C_{31}H_{43}NO_8Na$, found 580.2920.

**Compound 28** was obtained from **19** according to the general method described above. Yield: 74%; eluent: hexane-methylene chloride-acetone (47.5:47.5:5); $[\alpha]_D$ –3.0 (c 1.14, EtOH); UV (EtOH) $\lambda_{max}$ 206 nm, $\varepsilon_{max}$ 12531; IR (film) $\nu_{max}$: 3440; 2953; 2935; 1718 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) δ: 1.03 (s, 3H); 1.07 (s, 3H); 1.33 (s, 3H); 1.38 (br s, 9H); 1.34–1.38 (m, 1H); 1.52–1.67 (m, 2H); 2.53 (q, J = 9.6 Hz, 1H); 2.68 (d, J = 16.2 Hz, 1H); 2.68–2.76 (m, 1H); 3.04 (d, J = 16.2 Hz, 1H); 3.20 (s, 1H); 4.49 (s, 1H); 5.19 (d, J = 7.3 Hz, 1H); 5.40 (d, J = 7.3 Hz, 1H); 6.48 (br s, 1H); 7.22 (s, 1H); 7.25–7.30 (m, 3H); 7.31–7.36 (m, 3H); $^{13}C$ NMR (125 MHz, $CDCl_3$) δ: 28.25; 29.68; 29.88; 32.15; 32.86; 36.74; 42.98; 43.12; 44.53; 52.05; 55.91; 73.29; 73.84; 74.37; 79.92; 118.54; 122.74; 126.59; 127.71; 128.54; 139.18; 141.17; 141.32; 154.94; 172.22; ESI (MeOH) m/z: 536 $(M+Na)^+$; HR-MS 536.2619 calculated for $C_{29}H_{39}NO_7Na$, found 536.2626.

**Compound 29** was obtained from **20** according to the general method described above. Yield: 74%; eluent: hexane-methylene chloride-acetone (47.5:47.5:5); oil; $[\alpha]_D$ +33.3 (c 0.67, EtOH); UV (EtOH) $\lambda_{max}$ 208 nm, $\varepsilon_{max}$ 16886; IR (film) $\nu_{max}$: 3434, 2953, 2866, 1737, 1700, 1498 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) δ: 1.03 (s, 6H); 1.07 (s, 3H); 1.28–1.35 (m, 1H); 1.42 (br.s, 9H); 1.54 (dd, J = 12.9, 10.5 Hz, 1H); 1.70 (dd, J = 12.7, 6.5 Hz, 1H); 1.84 (dd, J = 12.9, 7.6 Hz, 1H); 2.08–2.20 (m, 2H); 2.59 (d, J = 14.0 Hz, 1H); 2.71 (d, J = 14.0 Hz, 1H); 4.52 (br s, 1H); 5.32 (d, J = 8.8 Hz, 1H); 5.54 (d, J = 8.8 Hz, 1H); 5.83 (d, J = 9.1 Hz, 1H); 7.19 (s, 1H); 7.24 (s, 1H); 7.30 (tt, J = 7.3, 1.3 Hz, 1H); 7.35–7.44 (m, 4H); $^{13}C$ NMR (125 MHz, $CDCl_3$) δ: 20.58; 28.27; 31.17; 31.34; 34.52; 40.35; 43.36; 46.33; 46.62; 55.20; 55.69; 73.41; 73.79; 77.16; 80.28; 118.78; 124.10; 126.68; 127.78; 128.65; 139.03; 140.82; 155.20; 172.21; ESI (MeOH) m/z: 536 $(M+Na)^+$; HR-MS 536.2619 calculated for $C_{29}H_{39}NO_7Na$, found 536.2659.

**Compound 30** was obtained from **21** according to the general method described above. Yield: 36%; eluent: methylene chloride-isopropyl alcohol (98:2); oil; $[\alpha]_D$ –1.3 (c 0.59, EtOH); UV (EtOH) $\lambda_{max}$ 205 nm, $\varepsilon_{max}$ 10278; IR ($CHCl_3$) $\nu_{max}$: 3605, 3525, 3438, 2959, 1768, 1716, 1495 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) δ: 0.93 (s, 3H); 1.06 (s, 3H); 1.13 (dd, J = 6.2, 0.62 Hz, 1H); 1.17–1.31 (m, 1H); 1.40 (br.s, 9H); 1.48–1.52 (m, 1H); 1.63 (s, 3H); 1.60–1.75 (m, 3H); 2.00–2.22 (m, 2H); 4.09 (d, J = 9.4 Hz, 1H); 4.34 (d, J = 9.4 Hz, 1H); 4.48 (br.s, 1H); 5.17 (d, J = 8.0 Hz, 1H); 5.45 (d, J = 8.0 Hz, 1H); 5.63 (d, J = 8.3 Hz, 1H); 7.29 (tt, J = 6.9, 1.7 Hz, 1H); 7.33–7.39 (m, 4H); $^{13}C$ NMR (125 MHz, $CDCl_3$) δ: 18.33; 24.63; 26.76; 28.35; 28.67; 31.63; 38.38; 38.98; 39.81; 40.12; 46.01; 46.53; 56.05; 71.04; 71.30; 73.77; 79.94; 80.14; 126.64; 127.74; 128.60; 139.37; 155.12; 172.65; 175.11; ESI (MeOH) m/z: 552 $(M+Na)^+$; HR-MS 552.2573 calculated for $C_{29}H_{39}NO_8Na$, found 552.2570.

**Compound 31** was obtained from **22** according to the general method described above. Yield: 54%; eluent: hexane-methylene chloride-acetone (47.5:47.5:5); oil; $[\alpha]_D$ +10.8 (c 0.91, EtOH); UV (EtOH) $\lambda_{max}$ 206 nm, $\varepsilon_{max}$ 21023; IR ($CHCl_3$) $\nu_{max}$: 3387, 2956, 1774, 1719,1497 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) δ: 1.01 (s. 3H); 1.05 (s, 3H); 1.24 (t, J = 11.6 Hz, 1H); 1.36 (d, J = 5.0 Hz, 1H); 1.41 (br.s, 9H); 1.88 (d, J = 5.0 Hz, 1H); 5.86–5.91 (m, 1H); 2.03 (m, 3H); 2.09 (ABq, J = 16.9 Hz, 2H); 2.56–2.65 (m, 1H); 4.15 (d, J = 9.7 Hz, 1H); 4.38 (d, J = 9.7 Hz, 1H); 4.53 (br.s, 1H); 5.08 (d, J = 9.4 Hz, 1H); 5.19 (d, J = 9.4 Hz, 1H); 5.37 (d, J = 9.1 Hz, 1H); 7.30 (tt, J = 6.9, 1.7 Hz, 1H); 7.34–7.40 (m, 4H); $^{13}C$ NMR (125 MHz, $CDCl_3$) δ: 15.34; 23.93; 28.34; 28.46; 29.51; 33.76; 34.53; 37.62; 41.92; 44.17; 45.39; 56.21; 71.41; 73.77; 78.90; 80.04; 120.80; 126.69; 127.88; 128.68; 134.59; 139.07; 155.11; 173.14; 173.52; ESI (MeOH) m/z: 534 $(M+Na)^+$; HR-MS 534.2462 calculated for $C_{29}H_{37}NO_7Na$, found 534.2457.

**Compound 32** was obtained from **23** according to the general method described above. Yield: 71%; eluent: methylene chloride-isopropyl alcohol (98:2); $[\alpha]_D$ +10.0 (c 0.91, $CDCl_3$); UV (EtOH) $\lambda_{max}$ 206 nm, $\varepsilon_{max}$ 13014; IR (film) $\nu_{max}$: 3387; 2959; 1775; 1717 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) δ: 1.11 (s, 3H); 1.15 (s, 3H); 1.23 (d, J = 5.6 Hz, 1H); 1.41 (br s, 9H); 1.45 (d, J = 14.7 Hz, 1H); 1.57 (t, J = 14.7 Hz, 1H); 1.73 (s, 3H); 1.79 (d, J = 5.6 Hz, 1H); 1.87 (d, J = 14.7 Hz, 1H); 1.90–1.96 (m, 1H); 2.26 (ddd, J = 12.3, 9.6, 7.3 Hz, 1H); 3.10 (br s, 1H); 4.03 (d, J = 9.7 Hz, 1H); 4.34 (d, J = 9.7 Hz, 1H); 4.49 (br s, 1H); 5.14 (d, J = 8.6 Hz, 1H); 5.26 (d, J = 9.6 Hz, 1H); 5.34 (d, J = 8.6 Hz, 1H); 7.28–7.38 (m, 5H); $^{13}C$ NMR (125 MHz, $CDCl_3$) δ: 15.06; 20.26; 28.30; 31.07; 31.55; 32.23; 36.70; 36.84; 40.31; 42.92; 43.91; 56.13; 58.18; 71.62; 73.40; 73.65; 75.93; 80.09; 126.61; 127.87; 128.67; 138.99; 155.11; 172.74; 173.50; ESI (MeOH) m/z: 550 $(M+Na)^+$; HR-MS 550.2411 calculated for $C_{29}H_{37}NO_8Na$, found 550.2433.

**Determination of cytotoxicity**. In order to establish concentration level of newly synthesized compounds, determination of their cytotoxicity on Vero cell culture was performed. The cells were cultured in routine manner by the use of minimal essential medium /MEM, GIBCO/, supplemented with 10% of foetal bovine serum /FBS, GIBCO/. Antibiotics used in standard manner were added.

Tested compounds were dissolved in 50 μl of DMSO, initially, and then in MEM containg 2% FBS. For experiments, cell cultures were held in 96-well-plastic-plates /NUNC/. The concentration of cells was 20000 per well. After 24 hours of incubation at 37°C, solutions of each concentration of tested compounds were added respectively to wells. The plates were incubated 24 hours at 37°C.

Cytotoxicity was determined by observation of cell morphology [9,10]. Following decantation of liquids, (3-[4,5 dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide /MTT, Sigma/ was added, and the plates were incubated 4 hours at 37°C. After the cell lysis and centrifugation, the dye contents were estimated by the use of spectrophotometer Dynatech Mc 5000. The rate of the cultures vitality has been estimated by dividing the OD of cells under influence of the compound by the OD of control cells and given in percent. The results presented in Table 1 are the mean values obtained from experiments repeated three times.

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