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ScienceDirect
Mendeleev Commun., 2009, **19**, 32–33

Mendeleev Communications

Synthesis of squalamine analogues on the basis of lupane triterpenoids

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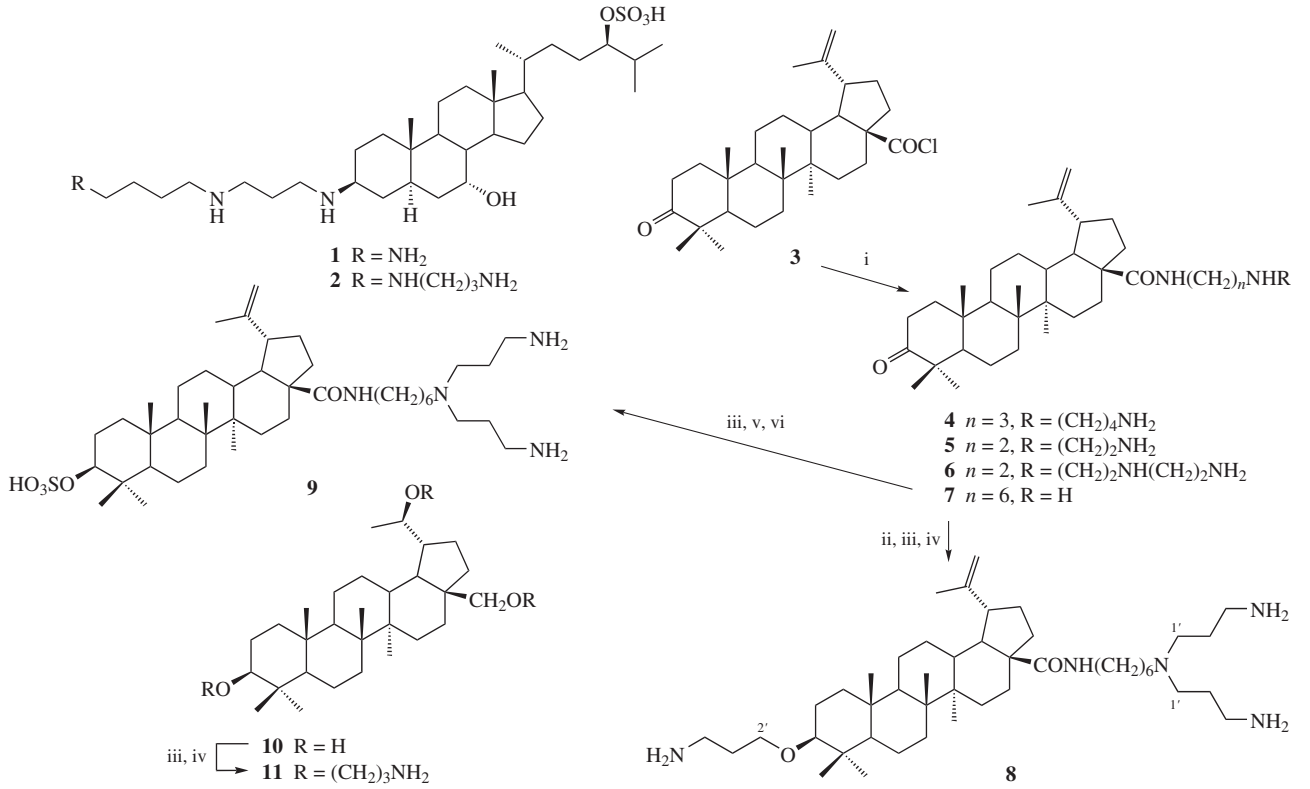
DOI: 10.1016/j.mencom.2009.01.013

The first synthesis of a family of betulin-based triterpene–polyamine conjugates, which are structurally related to the potential antibiotic squalamine, is demonstrated.

Sterol–spermidine conjugate squalamine **1**, isolated from the dogfish shark *Squalus acanthias*, is unusual natural product that has attracted considerable attention because of its broad-spectrum activity against bacteria and fungi.[1,2] Squalamine has perspectives for treatment of serious diseases such as cancer, age-related macular degeneration and in the control of body weight.[3] Actually, squalamine lactate is in phase III clinical trial for the treatment of non mall cell lung cancer.[4] Sulfated spermine-sterol trodusquemine **2** suppresses mammalian appetite through inhibition of protein tyrosine phosphatase 1B and is being evaluated in a second phase I trial in overweight type 2 diabetics.[5] At present, the feasibility of obtaining large quantities of squalamine and other aminosterols from natural sources appears questionable since only trace amounts of **1**, **2** are present in the liver and gall-bladder of the shark.[6] The short route to squalamine was achieved in eleven steps with an overall yield of 19% and 99% *ee* using methyl-3-keto-5α-chenodeoxycholanate as a starting material.[7] A series of 3-amino and polyaminosterol analogues of squalamine and trodusquemine were synthesized using different synthetic approaches.[8]

On the other hand, pentacyclic triterpenoids, especially betulin, ursolic and oleanolic acids exist abundantly in the plant kingdom and are used in the prevention and treatment of hepatitis, parasitic and protozoal infections.[9,10] Birch bark triterpenoids represent a new class of anti-cancer and anti-HIV agents with a novel mechanism of action.[11] We propose lupane triterpenoids as efficient platforms for the design of squalamine analogues.

For introduction of polyamine moieties into the structure of triterpenoid the direct condensation of betulonic acid chloride[12] **3** with commercially available alkane polyamines (spermidine, diethylenetriamine and triethylenetetramine) was used to produce compounds **4**–**6**† with an average yield of 48–54% after purification by column chromatography (Scheme 1).



Scheme 1 *Reagents and conditions*: i, $NH_2(CH_2)_2NH(CH_2)_2NH_2$ or $NH_2(CH_2)_2NH(CH_2)_2NH(CH_2)_2NH_2$ or $NH_2(CH_2)_3NH(CH_2)_4NH_2$ or $NH_2(CH_2)_6NH_2$, Et_3N, $CHCl_3$, 60 °C, 3 h; ii, $NaBH_4$, Pr^iOH, 0 °C, 2 h; iii, CH_2CHCN, 1,4-dioxane, 40% KOH, TEBAH, room temperature, 14 h; iv, H_2, Raney Ni, MeOH, 100 °C, 100 atm, 8 h; v, $LiAlH_4$, THF, 60 °C, 3 h; vi, H_2SO_4, Ac_2O, pyridine, 55 °C, 1 h, then 0 °C, 15 min.

Another approach is based on the successive transformations of betulonic acid amide with 1,6-diaminohexane **7**‡ as a starting material using known procedures of polyamine synthesis.[3,13,14] Reduction of **7** with $NaBH_4$ followed by cyanoethylation of both hydroxyl (C-3) and amino groups in the presence of the phase transfer catalyst benzyltriethylammonium chloride in dioxane with good yield gave 3β-*O*-(2′-cyanoethyl)-*N*-(2,2′-dicyanoethyl)-diaminohexane derivative, which after the catalytic hydrogenation produced designed compound **8**‡ in 72% yield. The synthesis of partially water soluble 28-[diaminohexyl-*N*-(3,3′-dipropylamino)]betulinic acid 3β-*O*-sulfate **9**‡ was carried out using the same strategies.

A similar sequence of reactions (cyanoethylation, catalytic hydrogenation) was applied to prepare branched 3β,20*R*,28-tri-*O*-(3-aminopropyl)-29-norlupane **11**‡ from triol **10**[15] in 70% yield.

The nitrile bonds in IR spectra of cyanoethylated triterpenoids were detected according to the intense absorption bands at 2249 cm^{-1}. The carbon signal of C≡N groups appeared at 117–118 ppm in the ^{13}C NMR spectra. The evidence for the formation of polyamine chains in the triterpene derivatives **4**–**6**, **8**, **9**, **11** was also given by the signals of polymethylene fragments at 25.5–51.6 ppm in the ^{13}C NMR spectra and as multiplets at 2.14–2.88 and 3.33–3.47 ppm in the ^1H NMR spectra.

In conclusion, we have synthesized a family of betulin-based triterpene–polyamine conjugates, which are structurally related to the potential antibiotics squalamine and its aminosterol analogues.

This work was supported by the Russian Foundation for Basic Research (grant no. 08-03-00868).

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Received: 16th July 2008; Com. 08/3183

† *3-Oxo-28-(spermidino)carbonyl-20(29)-lupene* **4**: mp 154–157 °C, $[\alpha]_D^{20}$ +29 (*c* 0.6, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$) δ: 0.86, 0.92, 0.96, 1.04, 1.13 (5s, 15H, 5Me), 1.10–2.18 (m, 30H, CH_2, CH, NH_2, NH), 1.69 (s, 3H, Me), 2.35–2.56 (m, 3H, 13-H, 16-H), 2.74–2.89 (m, 6H), 3.03–3.17 (m, 1H, 19-H), 3.33–3.47 (m, 2H), 4.62 and 4.75 (2s, 2H, 29-H), 6.26–6.37 (m, 1H, CONH). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 217.9 (3-C), 176.2 (28-C), 150.7 (20-C), 109.3 (29-C), 55.7, 55.4, 50.0, 49.9, 48.9, 47.2, 46.6, 42.4, 40.6, 39.5, 38.6, 38.3, 37.6, 37.4, 3×36.8, 34.0, 33.6, 30.8, 29.3, 27.2, 26.5, 26.1, 25.5, 21.4, 20.9, 19.5, 19.3, 16.0, 15.9, 15.1, 14.4. Found (%): C, 76.37; H, 10.91; N, 7.22. Calc. for $C_{37}H_{63}N_3O_2$ (%): C, 75.87; H, 10.41; N, 6.72.

3-Oxo-28-(diethylenetriamino)carbonyl-20(29)-lupene **5**: mp 158–160 °C, $[\alpha]_D^{20}$ +21.5 (*c* 0.23, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$) δ: 0.86, 0.92, 0.96, 1.04, 1.13 (5s, 15H, 5Me), 1.10–2.18 (m, 24H, CH_2, CH, NH_2, NH), 1.69 (s, 3H, Me), 2.35–2.56 (m, 3H, 13-H, 16-H), 2.74–2.89 (m, 6H), 3.03–3.17 (m, 1H, 19-H), 3.33–3.47 (m, 2H), 4.62 and 4.75 (2s, 2H, 29-H), 6.26–6.37 (m, 1H, CONH). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 217.7 (3-C), 176.7 (28-C), 150.7 (20-C), 109.4 (29-C), 55.7, 55.0, 50.1, 50.0, 49.3, 47.2, 46.6, 42.5, 40.7, 39.6, 38.7, 38.3, 37.8, 36.9, 34.0, 33.7, 33.4, 30.9, 29.4, 27.4, 26.6, 25.6, 21.4, 20.9, 19.6, 19.4, 16.1, 15.9, 15.8, 14.5. Found (%): C, 75.65; H, 10.64; N, 7.78. Calc. for $C_{34}H_{57}N_3O_2$ (%): C, 75.15; H, 10.14; N, 7.28.

3-Oxo-28-(triethylenetetramino)carbonyl-20(29)-lupene **6**: mp 173–175 °C, $[\alpha]_D^{20}$ +19.2 (*c* 0.35, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$) δ: 0.86, 0.92, 0.96, 1.04, 1.13 (5s, 15H, 5Me), 1.10–2.18 (m, 25H, CH_2, CH, NH_2, 2NH), 1.69 (s, 3H, Me), 2.35–2.56 (m, 3H, 13-H, 16-H), 2.71–2.85 (m, 4H), 2.89–2.98 (m, 4H), 3.03–3.17 (m, 1H, 19-H), 3.25–3.62 (m, 4H), 4.62 and 4.75 (2s, 2H, 29-H), 6.28–6.41 (m, 1H, CONH). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 218.0 (3-C), 176.6 (28-C), 150.9 (20-C), 109.4 (29-C), 55.7, 55.0, 53.9, 53.1, 50.0, 49.0, 2×47.3, 46.7, 45.7, 42.5, 40.7, 40.5, 40.2, 39.6, 38.4, 37.8, 36.9, 34.1, 33.7, 30.9, 29.4, 29.3, 26.6, 25.7, 21.5, 21.0, 19.7, 19.4, 16.0, 15.9, 14.6. Found (%): C, 74.18; H, 10.72; N, 9.61. Calc. for $C_{36}H_{62}N_4O_2$ (%): C, 73.68; H, 10.22; N, 9.11.

‡ *3-Oxo-28-(6-aminohexylamino)carbonyl-20(29)-lupene* **7**: mp 188–190 °C, $[\alpha]_D^{20}$ –36.3 (*c* 0.07, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$) δ: 0.86, 0.92, 0.96, 1.04, 1.13 (5s, 15H, 5Me), 1.20–2.13 (m, 31H, CH_2, CH, NH_2), 1.67 (s, 3H, Me), 2.32–2.58 (m, 5H, 13-H, 16-H), 3.03–3.32 (m, 3H, 19-H), 4.58 and 4.72 (2s, 2H, 29-H), 5.75–5.88 (m, 1H, CONH). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 176.1 (28-C), 150.9 (20-C), 109.3 (29-C), 55.5, 54.9, 50.0, 49.9, 47.3, 46.6, 45.8, 42.4, 40.6, 39.6, 38.4, 37.7, 36.8, 34.1, 33.7, 30.8, 29.7, 29.3, 28.9, 26.5, 26.4, 25.9, 25.5, 23.5, 22.9, 21.4, 20.9, 19.6, 19.4, 16.1, 15.9, 14.5, 14.2. Found (%): C, 77.72; H, 11.18; N, 5.18. Calc. for $C_{36}H_{60}N_2O_2$ (%): C, 77.22; H, 10.68; N, 4.68.

(3β)-3-(3-Aminopropyloxy)-28-{6-[bis(3-aminopropyl)amino]hexylamino}-carbonyl-20(29)-lupene **8**: mp 208–210 °C, $[\alpha]_D^{20}$ –5.5 (*c* 0.45, MeOH). ^1H NMR (300 MHz, CD_3OD) δ: 0.82, 0.91, 0.92, 0.96, 1.04 (5s, 15H, 5Me), 1.12–2.03 (m, 44H, CH_2, CH, $3NH_2$), 1.67 (s, 3H, Me), 2.14–2.19 (m, 4H, 2×1′-H), 2.39–2.51 (m, 5H, 13-H, 16-H), 2.64–2.88 (m, 6H), 3.08–3.34 (m, 5H, 19-H, 2′-H), 4.62 and 4.78 (2s, 2H, 29-H), 5.75–5.88 (m, 1H, CONH). ^{13}C NMR (300 MHz, CD_3OD) δ: 176.1 (28-C), 86.2 (3-C), 65.4 (2′-C), 58.6, 2×55.7 (1′-C), 54.6, 54.3, 49.3, 48.6, 48.5, 46.7, 42.8, 41.3, 39.6, 4×38.6, 37.6, 37.4, 37.1, 36.0, 33.4, 2×31.9, 28.7 (20-C), 3×27.9, 26.7, 26.1, 25.9, 24.7, 22.4, 22.2, 21.7, 21.5, 19.8, 17.5 (29-C), 17.1 (30-C), 16.9, 14.8, 14.7, 14.2. Found (%): C, 74.22; H, 11.76; N, 9.62. Calc. for $C_{45}H_{85}N_5O_2$ (%): C, 73.82; H, 11.26; N, 9.12.

(3β)-3-Hydroxysulfonyloxy-28-{6-[bis(3-aminopropyl)amino]hexylamino}-carbonyl-20(29)-lupene **9**: mp 184–186 °C, $[\alpha]_D^{20}$ +14 (*c* 0.8, MeOH). ^1H NMR (300 MHz, CD_3OD) δ: 0.86, 0.92, 0.96, 1.04, 1.13 (5s, 15H, Me), 1.18–2.03 (m, 37H, CH_2, CH, $2NH_2$), 1.69 (s, 3H, Me), 2.18–2.48 (m, 5H, 13-H, 16-H), 2.46–2.67 (m, 4H, 2×1′-H), 3.05–3.28 (m, 2H, 19-H, OH), 3.31–3.48 (m, 6H), 4.31–4.38 (m, 1H, 3-H), 4.62 and 4.78 (2s, 2H, 29-H), 5.52–5.73 (m, 1H, CONH). ^{13}C NMR (300 MHz, CD_3OD) δ: 176.8 (28-C), 150.7 (20-C), 109.8 (29-C), 87.3 (3-C), 55.7, 51.7, 51.1, 2×49.7 (1′-C), 48.1, 46.5, 46.4, 42.6, 40.5, 2×39.2, 38.3, 37.7, 37.3, 36.7, 35.2, 2×34.2, 33.5, 32.7, 32.3, 30.7, 30.3, 30.1, 29.9, 29.2, 28.9, 25.6, 25.4, 23.6, 22.9, 21.8, 20.1, 19.3, 17.6, 15.4, 14.4. Found (%): C, 67.34; H, 10.23; N, 7.48; S, 4.28. Calc. for $C_{42}H_{76}N_4O_5S$ (%): C, 66.84; H, 9.73; N, 6.98; S, 3.78.

*3β,20*R*,28-Tri-*O*-(3-aminopropyl)-29-norlupane* **11**: mp 202–204 °C, $[\alpha]_D^{20}$ +18.2 (*c* 0.05, MeOH). ^1H NMR (300 MHz, CD_3OD) δ: 0.74, 0.81, 0.87, 0.96, 0.98, 1.04 (6s, 18H, 6Me), 1.06–2.00 (m, 37H, CH_2, CH, $3NH_2$), 2.03–2.11 (m, 1H, 19-H), 2.74–2.83 (m, 6H), 3.29–3.37 (m, 7H, 29-H, 2′-H, 3′-H, 4′-H), 3.27 and 3.81 (d, 2H, 28-H, *J* 11 Hz). ^{13}C NMR (300 MHz, CD_3OD) δ: 87.2 (3-C), 69.4 (20-C), 65.8 (28-C), 65.4 (1′-C), 63.8 (2′-C), 62.5 (3′-C), 55.2, 49.8, 49.2, 47.7, 46.8, 42.5, 40.7, 39.9, 2×39.7, 38.5, 36.8, 36.4, 34.0, 33.4, 29.3, 29.0, 27.6, 26.7, 26.6, 22.4, 21.3, 20.8, 20.6, 18.9, 18.5, 18.4, 17.8, 15.7, 15.6, 15.1, 14.2. Found (%): C, 73.85; H, 11.58; N, 6.80. Calc. for $C_{38}H_{71}N_3O_3$ (%): C, 73.35; H, 11.08; N, 6.50.