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Synthesis of novel mannopyranosyl betulinic acid phosphoniohexyl ester

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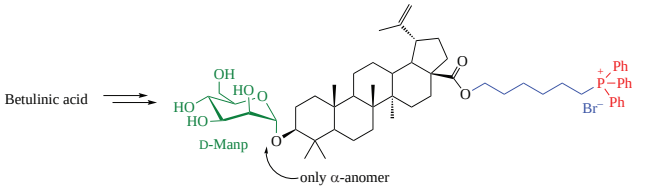
A general approach to lupane monodesmosides containing a C-28 phosphonioalkyl group is based on the initial glycosylation of triterpene acid ω-haloalkyl esters at the C-3 position. The subsequent phosphorylation with triphenylphosphine and deprotection in the carbohydrate moiety finalize the synthesis.



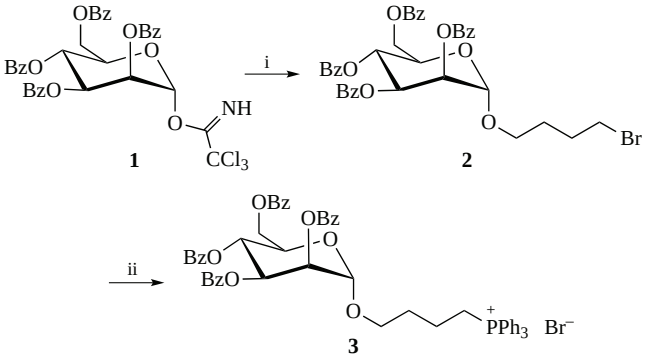
Keywords: lupane, triterpenes, betulinic acid, saponins, glycoside, mannopyranose, glycosylation, phosphonium salts.

Triterpenoids and their seco/nor derivatives are among the most important representatives of isoprenoids,[1] which play a significant role in living organisms.[2] Some of pentacyclic triterpenoids are part of clinically approved complex medicines and food supplements. The availability of triterpenoids combined with a rich spectrum of biological activity allows them to be considered as a universal platform for creating modern multi-purpose medicines.[3] Pentacyclic triterpenoids exhibit anti-inflammatory,[4] antioxidant,[5] antiviral,[6] antidiabetic,[7] antitumor,[8] hepatoprotective,[9] cardioprotective[10] and anti-ulcer[11] activity. Lupane-type betulin and betulinic acid revealed recently high potential in the treatment of tumor diseases,[12] while betulonic and betulinic acids exhibit cytotoxic activity against various tumor cell lines.[13] These acids cause apoptosis of tumor cells along the internal mitochondrial way.[14]

Conjugation of natural compounds with vector fragments, *e.g.*, with lipophilic delocalized cations (rhodamine, triphenylphosphonium) can provide targeted delivery of the drug agent to required organs (tissues) and/or selective interaction with a certain type of transformed cells. This approach allows for achieving an increase in the concentration of the active agent in the mitochondrial matrix by more than 1000 times.[15] The inclusion of a phosphonium fragment into the structure of terpenoids leads to a significant increase in their cytotoxicity against human cancer cell lines.[16]

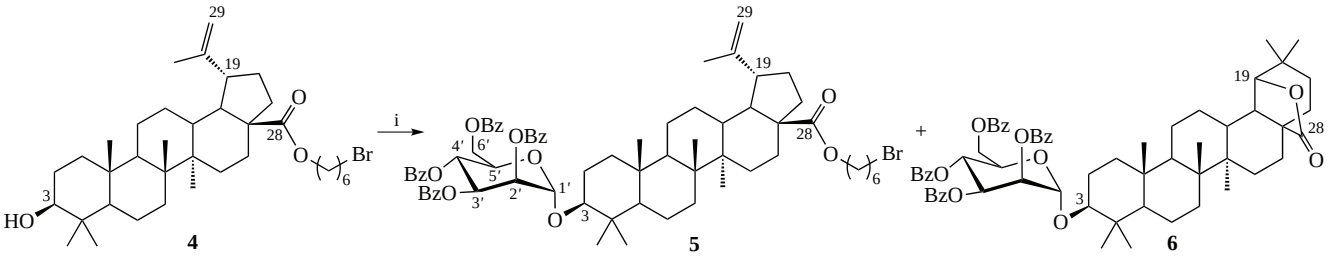
Some limitation to the widespread use of triterpenoids as drug agents is their low solubility in water and physiological media.[17] This problem can be sometimes overcome by introducing hydrophilic carbohydrate moieties into the triterpenoid structure thus resulting in triterpene glycosides or saponins.[18] These glycosides possess a specified sequence of monosaccharide fragments, so the carbohydrate fragments act not only as hydrophilic components but also can impart to the molecule the selectivity in action on tumor cells. The results on the relationship between the nature of sugar fragment in conjugates with lupane and oleanane triterpenoids and their antitumor activity were reported.[19] In the lupane series, the greatest activity was shown by L-rhamnopyranoside and D-mannopyranoside.

Here we present the synthesis of the first representative of phosphonioalkylated triterpene monodesmosides, namely, [6-(3-α-D-mannopyranos-1-yloxy-28-oxolup-20(29)en-28-yloxy)-hexyl]triphenylphosphonium bromide. Since functionalized terpenoids containing both phosphonium group and sugar residue are not documented, it seemed necessary to find the most optimal sequence of synthetic stages. For the model system test (Scheme 1), phosphonium derivative **3** was chosen as the target. Its precursor, 4-bromobutyl D-mannopyranoside **2**, was obtained herein by the Schmidt's acetimidate method which is well-approved in the synthesis of triterpene glycosides[20] providing good stereoselectivity and high yields under relatively mild conditions. In our hands, trichloroacetimidate derivative of tetra-*O*-benzoyl-D-mannopyranose **1** was reacted with 4-bromobutanol in the presence of trimethylsilyl triflate at room temperature (see Scheme 1).† In the second stage, quaternization of triphenylphosphine with bromo ether **2** under prolonged heating in acetonitrile was accompanied neither by the cleavage of the glycosidic bond nor deprotection, and the yield of phosphonium salt **3** amounted to 95%. The $^{1}J_{PC}$ value of 88 Hz for the *ipso*-carbon multiplet (δ_C 118 ppm) in the ^{13}C-{^{1}H}



Scheme 1 *Reagents and conditions*: i, 4-bromobutanol, TMSOTf, CH_2Cl_2, room temperature; ii, PPh_3, MeCN, reflux.

† For the synthesis and characteristics of compounds obtained, see Online Supplementary Materials.



Scheme 2 *Reagents and conditions*: i, compound **1**, TMSOTf, CH_2Cl_2, –40 °C.

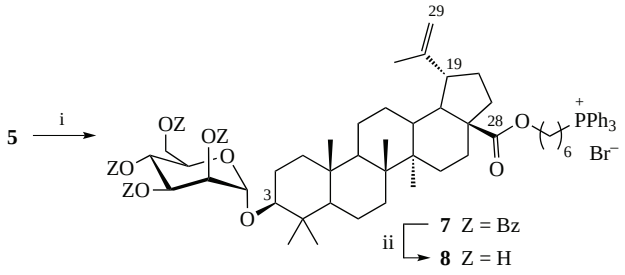
NMR spectrum of salt **3** is the evidence for its phosphonic nature.

The results obtained during the study of the model system (see Scheme 1) were applied for the synthesis of phosphonio-alkylated triterpene glycosides. In the first stage, betulinic acid 6-bromohexyl ester **4** was glycosylated with protected (mannopyranosyl)acetimidate (Scheme 2). The reaction in absolute CH_2Cl_2 at –40 °C in the presence of trimethylsilyl triflate afforded two products which were separated by column chromatography. The predominant product was the target betulinic acid glycoside **5** (~70% yield). The structure of the minor product determined by NMR and IR spectroscopy turned to be 28-oxoallobetulin **6** glycoside (15% yield). In the IR spectrum of compound **6**, the absorption band at 1769 cm^{-1} is typical of lactone carbonyl while no absorption band was observed at 1640 cm^{-1} typical of non-cyclic esters. In the ^1H NMR spectrum of compound **6**, seven upfield singlets for methyl groups are observed while the H-19 proton signal is shifted downfield (δ 3.5 ppm) compared to those of typical lupanes. Also, no signals for isopropenyl and bromohexyl groups were present. The obtained data indicate the implementation of the Wagner–Meerwein skeletal rearrangement, according to which lupane-type triterpenoids are isomerized into germanican derivatives. Acidic agents that are usually used in glycosylation reactions (in this case, trimethylsilyl triflate) can provoke this type of rearrangement (*cf.* ref. 21). Additional studies have shown that when the reaction time is reduced, the rearrangement product **6** is not formed, however the content of the target product **5** is reduced to 30–40% as well.

Glycoside **5** is formed as a single α-anomeric form, which largely depends on the nature of the protective groups in the carbohydrate component.[22] During glycosylation with benzoyl derivatives of D-mannopyranose, an exclusively 1,2-*trans*-glycosidic bond is formed, which is confirmed by the chemical shift of the anomeric proton $H^{1'}$ (δ 5.29 ppm) as well as the $^3J_{H^{2'}H^{1'}}$ value of 1.2 Hz. In the upfield region of the ^1H NMR spectrum of compound **5**, six singlets for methyl protons are observed, the H^3 signal is somewhat shifted downfield (δ 3.4 ppm) relative to the position of the same signal (δ 3.17 ppm) in the spectrum of betulinic acid unsubstituted at the 3-positioned OH group. The glycoside residue protons signals resonate in the region of δ 4.5–6.0 ppm.

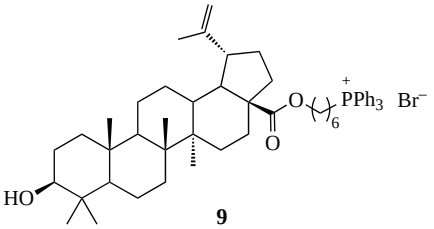
The reaction of compound **5** with an excess of PPh_3 in acetonitrile leads to phosphonium salt **7** in a yield of 90% (Scheme 3). The formation of phosphonium salt **7** was proved by ^1H and ^{13}C NMR spectroscopy and mass spectrometry. In the ^{13}C NMR spectrum, a multiplet for the CH_2–P^+ carbon with $^1J_{PC}$ 49.7 Hz is observed at δ_C 22.8 ppm.

At the final stage, the task was to remove the benzoyl protective groups from the glycoside fragment. Most of the described procedures deal with basic (alkaline) agents in a suitable solvent, for example, NaOH/THF/H_2O/MeOH system. In our hands with this method, a complex mixture was formed, some products being phosphorus-deprived. The target compound **8** was obtained in a 70% yield in the K_2CO_3/MeOH system. As the reaction time increased, the formation of significant amounts



Scheme 3 *Reagents and conditions*: i, PPh_3, MeCN, reflux; ii, K_2CO_3, MeOH, room temperature.

of phosphonium salt **9**, a product of glycosidic bond hydrolysis, was observed. Additional experiments have shown that phosphonium salt **7** cannot be obtained by glycosylation of compound **9** with an imidate derivative of mannose **1** even under the excessive action of trimethylsilyl triflate. This is apparently due to competitive processes involving a halogenide ion as a nucleophile in the formation of an acylonium ion at the intermediate stage of the glycosylation reaction.



The structure of compound **8** was reliably confirmed by the data from ^1H and ^{13}C NMR spectroscopy and mass spectrometry. Synthesis of its analogues as well as their biological studies are planned to be performed.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2021.01.034.

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