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Electrochemical cholesterylation of sugars with cholesteryl diphenylphosphate

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ABSTRACT

Electrochemical cholesterylation of various sugars with cholesteryl diphenylphosphate was studied. The reaction afforded mono-, di-, tri-, and tetra-cholesterylated products using equivalent amounts of the reagent. The reactions turned out to be completely stereoselective with respect to both sugar and steroid but only partially regioselective – primary and anomeric hydroxyl groups in sugars were the most reactive ones while no substantial differences in reactivity was found for different secondary hydroxyl groups.

1. Introduction

Glycosylation is a process commonly occurring in Nature. In the reaction a carbohydrate, i.e. a glycosyl donor, is attached to a hydroxyl or other functional group of another compound (a glycosyl acceptor). In living organisms glycosylation is the enzymatic process that attaches glycans to proteins, lipids, or other organic molecules. In this way various biopolymers, including DNA, RNA, and glycoproteins, are formed. If the glycosyl acceptor is a steroid or triterpenoid, then the product is called a saponin. Saponins have usually one sugar chain but bisdesmosidic or trisdesmosidic natural compounds, containing two or three sugar chains, have also been isolated from plants [1]. Due to a presence of a sugar moiety in saponins the physical, chemical and biological properties of the aglycone are dramatically changed. The glycan chains control pharmacokinetics of a saponin drug, such as absorption, distribution, metabolism and excretion [2].

Lipid modifications of proteins are widespread and serve to increase protein hydrophobicity and association with cellular membranes [3]. Often, these modifications are absolutely essential for protein stability and localization, and play critical roles in dynamic regulation of protein function. Cholesterylation is known as a post-translational attachment of sterol to proteins [4,5]. However, this modification has been a characteristic of a single family of hedgehog proteins, which are well-established morphogenic molecules important in embryonic development. These proteins were also found to be involved in the progression of many cancer types. The C-terminally attached cholesterol via an ester bond is required for the formation of multimeric complexes and is thought to regulate the distribution of extracellular hedgehog protein ligand [6].

In sugar biotransformations only monolipidation (monocholesterylation) is observed, i.e. formation of glycosides. Enzymes catalyze formation of glycosidic (acetal) bonds only. Further cholesterylation would involve not only the anomeric hydroxyl bonds but also other hydroxyl groups present in sugar.

Chemical synthesis of some polycholesterylated materials have been carried out including various cholesterol-containing acrylate, methacrylate or crotonate polymers, copolymers and composites [7,8]. These materials have shown liquid-crystalline properties and may serve as platforms for biodegradable and biocompatible nanocarriers. Some non-viral vectors for targeted gene delivery, namely, mono-, di- and tricholesterol derivatives of oligoethyleneimine, glycolipids and chitosan derivatives have also been reported [9].

We have recently elaborated an electrochemical method for preparation of steroidal glycosides and glycoconjugates, in which

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steroid is covalently linked to sugar via an ether bond [10,11]. The method is applicable for glycosylation of sugars at either anomeric (glycoside synthesis) [12] or other positions bearing hydroxyl groups (synthesis of glycoconjugates). In the method non-activated sugars and cholesterol or active cholesteryl donors are used. We have tested a plethora of different cholesteryl donors and selected readily available cholesteryl diphenylphosphate as the most efficient and compatible with the electrochemical reaction conditions [13,14]. However, only monocholesterylated sugar derivatives have been obtained so far. Now, a synthesis of sugars containing two, three, or four cholesterol units is attempted.

2. Experimental

2.1. General

Melting points were determined on a Kofler bench (Böetius type) melting point apparatus. ^{1}H and ^{13}C NMR (400 and 100 MHz, respectively) spectra were recorded on a Bruker Avance II spectrometer in $CDCl_3$ solutions with TMS as the internal standard (only selected signals in the NMR spectra are reported; sugar protons are marked with index prime (′). Infrared spectra were recorded on a Nicolet series II Magna-IR 550 FTIR spectrometer in chloroform solutions. Mass spectra were recorded at 70 eV with a time-of-flight (TOF) AMD-604 spectrometer with electrospray ionization (ESI).

Merck Silica Gel 60, F 256 TLC aluminum sheets were applied for thin-layer chromatographic analysis. For a visualization of the products a 5% solution of phosphomolybdic acid in ethanol was used.

2.2. A general procedure for electrochemical reactions

The preparative electrolyses were performed with a potentiostat/galvanostat (Princeton Applied Research, model Parstat 2273) under galvanostatic conditions using a current that was equal in a typical experiment to 7.5 mA and a reaction time of 4000 s. The current applied was the maximum current available for the electrolysis set-up being used (power supply and ohmic resistance). During the electrolysis the potential of the anode was monitored and the process was stopped when the potential reached the value of +2.3 V vs Ag/0.1 M $AgNO_3$ to avoid the occurrence of undesired oxidation processes. The reactions were also monitored by TLC and stopped when no further increase in the concentration of the glycosylation products was observed. A divided H-cell was used in which the cathodic and anodic compartments (3.5 mL of electrolyte each) were separated by a glass frit. In all measurements, 0.1 M solution of tetrabutylammonium tetrafluoroborate ($TBABF_4$) from Aldrich in dichloromethane was used as a supporting electrolyte.

Alcohol substrate (e.g. sugar; 0.3 mmol) and cholesteryl diphenylphosphate (**1**; 0.3 mmol per each hydroxyl group) were introduced into the anodic compartment together with 0.3 g of 3 Å molecular sieves added to eliminate traces of water, whereas anionite (1.5–2 g, Dowex 2 × 8, 200–400 mesh, perchlorate form) was placed in the cathodic compartment to eliminate chloride ions that are formed by the reduction of dichloromethane. The solutions in both compartments were stirred during electrolysis and a continuous flow of argon was applied in the anodic compartment. A platinum mesh was used as a cathode and a platinum plate (2 × 1.5 cm) was used as an anode. All measurements were performed at 25 °C. When the electrolysis was completed the solvent was removed from the reaction mixture and products were separated by silica gel (J. T. Baker; 70–230 mesh) column chromatography.

2.3. Cholesterylation products

2.3.1. 2′,3′,4′,6′-Tetraacetyl-1′-(S-cholest-5-en-3β-yl)thio-β-D-glucopyranoside (3)

Compound **3** was eluted with a hexane-ethyl acetate 92:8 mixture. Colorless crystals, m.p. 130–133 °C (AcOEt-hexane); Rf = 0.35 (AcOEt-hexane 2:8); IR, ν_{max} (cm^{-1}): 1754, 1249, 1038; ^{1}H NMR, δ: 5.35 (m, 1H, H-6), 5.24 (t, 1H, J = 9.3 Hz, H-3′), 5.08 (t, 1H, J = 9.9 Hz, H-4′), 5.01 (dd, 1H, J = 10.0 Hz, J = 9.3 Hz, H-2′), 4.62 (d, 1H, J = 10.0 Hz, H-1′), 4.25 (dd, 1H, J = 12.3 Hz, J = 5.3 Hz, H-6a′), 4.13 (dd, 1H, J = 12.3 Hz, J = 2.3 Hz, H-6b′), 3.72 (ddd, 1H, J = 9.9 Hz, J = 5.3 Hz, J = 2.3 Hz, H-5′), 2.81 (m, 1H, H-3α), 2.08 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.04 (s, 3H, Ac), 2.02 (s, 3H, Ac), 1.00 (s, 3H, H-19), 0.92 (d, 3H, J = 6.4 Hz, H-21), 0.88 (d, 3H, J = 6.6 Hz, H-26 or H-27), 0.87 (d, 3H, J = 6.6 Hz, H-26 or H-27), 0.68 (s, 3H, H-18); ^{13}C NMR (ppm), δ: 170.6 (C), 170.2 (C), 169.4 (C), 169.3 (C), 141.5 (C), 121.4 (CH), 83.0 (CH), 75.79 (CH), 74.0 (CH), 70.3 (CH), 68.5 (CH), 62.3 (CH_2), 56.8 (CH), 56.2 (CH), 50.3 (CH), 44.6 (CH), 42.3 (C), 40.8 (CH_2), 39.8 (CH_2), 39.7 (CH_2), 39.5 (CH_2), 36.7 (C), 36.2 (CH_2), 35.8 (CH), 31.84 (CH_2), 31.81 (CH), 29.9 (CH_2), 28.2 (CH_2), 28.0 (CH), 24.3 (CH_2), 23.8 (CH_2), 22.8 (CH_3), 22.5 (CH_3), 20.9 (CH_2), 20.8 (CH_3), 20.7 (CH_3), 20.60 (CH_3), 20.57 (CH_3), 19.3 (CH_3), 18.7 (CH_3), 11.9 (CH_3); ESI MS, m/z: 755 [$(M+Na)^+$, 100%]; HRMS (ESI): m/z calcd for $C_{41}H_{64}O_9SNa^+$: 755.4169, found 755.4178.

2.3.2. 2′,3′,4′,6′-Tetraacetyl-1′-(S-3α,5α-cyclocholestan-6β-yl)thio-β-D-glucopyranoside (4)

Compound **4** was eluted with a hexane-ethyl acetate 93:7 mixture. Colorless crystals, m.p. 222–225 °C (AcOEt-hexane); Rf = 0.40 (AcOEt-hexane 2:8); IR, ν_{max} (cm^{-1}): 1746, 1249, 1040; ^{1}H NMR, δ: 5.22 (t, 1H, J = 9.3 Hz, H-3′), 5.06 (t, 1H, J = 9.9 Hz, H-4′), 4.98 (dd, 1H, J = 10.1 Hz, J = 9.3 Hz, H-2′), 4.41 (d, 1H, J = 10.1 Hz, H-1′), 4.24 (dd, 1H, J = 12.3 Hz, J = 5.2 Hz, H-6a′), 4.09 (dd, 1H, J = 12.3 Hz, J = 2.4 Hz, H-6b′), 3.64 (ddd, 1H, J = 9.9 Hz, J = 5.2 Hz, J = 2.4 Hz, H-5′), 2.75 (m, 1H, H-6), 2.07 (s, 3H, Ac), 2.05 (s, 3H, Ac), 2.02 (s, 3H, Ac), 2.01 (s, 3H, Ac), 0.97 (s, 3H, H-19), 0.92 (d, 3H, J = 6.5 Hz, H-21), 0.879 (d, 3H, J = 6.6 Hz, H-26 or H-27), 0.875 (d, 3H, J = 6.6 Hz, H-26 or H-27), 0.71 (s, 3H, H-18), 0.61 (dd, 1H, J = 5.1 Hz, J = 4.0 Hz, H-4a), 0.33 (dd, 1H, J = 7.9 Hz, J = 5.1 Hz, H-4b); ^{13}C NMR (ppm), δ: 170.6 (C), 170.2 (C), 169.4 (C), 169.3 (C), 83.9 (CH), 75.7 (CH), 74.1 (CH), 69.8 (CH), 68.5 (CH), 62.4 (CH_2), 56.3 (CH), 55.8 (CH), 49.3 (CH), 47.8 (CH), 43.1 (C), 42.8 (C), 40.1 (CH_2), 39.5 (CH_2), 36.8 (C), 36.6 (CH_2), 36.1 (CH_2), 35.8 (CH), 33.5 (CH_2), 30.6 (CH), 29.1 (CH), 28.2 (CH_2), 28.0 (CH), 25.3 (CH_2), 24.4 (CH_2), 23.8 (CH_2), 22.8 (CH_3), 22.6 (CH_2), 22.5 (CH_3), 20.73 (CH_3), 20.69 (CH_3), 20.60 (CH_3), 20.56 (CH_3), 19.9 (CH_3), 18.7 (CH_3), 14.3 (CH_2), 12.1 (CH_3); elemental analysis calcd (%) for $C_{41}H_{64}O_9S$: C 67.18, H 8.80, S 4.37; found: C 67.01, H 8.83, S 4.40.

2.3.3. 6′-(O-Cholest-5-en-3β-yl)-1′,2′-O-isopropylidene-α-D-glucofuranose (6)

Compound **6** was eluted with a hexane-ethyl acetate 3:1 mixture. Colorless crystals, m.p. 169–172 °C (AcOEt-hexane); Rf = 0.38 (AcOEt-hexane 1:1); IR, ν_{max} (cm^{-1}): 3408, 1104, 1083, 1065, 1011; ^{1}H NMR, δ: 5.98 (d, 1H, J = 3.6 Hz, H-1′), 5.36 (m, 1H, H-6), 4.55 (d, 1H, J = 3.6 Hz, H-2′), 4.36 (d, 1H, J = 2.5 Hz, H-3′), 4.17 (m, 1H, H-5′), 4.09 (dd, 1H, J = 5.8 Hz, J = 2.5 Hz, H-4′), 3.81 (dd, 1H, J = 9.8 Hz, J = 3.4 Hz, H-6a′), 3.59 (dd, 1H, J = 9.8 Hz, J = 5.6 Hz, H-6b′), 3.24 (m, 1H, H-3α), 1.50 (s, 3H, $-C(CH_3)_2$), 1.33 (s, 3H, $-C(CH_3)_2$), 1.01 (s, 3H, H-19), 0.92 (d, 3H, J = 6.5 Hz, H-21), 0.875 (d, 3H, J = 6.6 Hz, H-26 or H-27), 0.871 (d, 3H, J = 6.6 Hz, H-26 or H-27), 0.68 (s, 3H, H-18); ^{13}C NMR (ppm), δ: 140.4 (C), 122.0 (CH), 111.6 (C), 104.9 (CH), 85.1 (CH), 80.1 (CH), 79.9 (CH), 76.0 (CH), 69.7 (CH), 68.7 (CH_2), 56.7 (CH), 56.2 (CH), 50.1 (CH),