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SYNTHESIS OF PROTOESCIGENIN GLYCOCONJUGATES WITH O-28 TRIAZOLE LINKER

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Abstract: New triazole linked conjugates were obtained from protoescigenin monopropargyl ethers and sugar azides, under Cu(II) salt promotion in good yield, without losing isopropylidene protection.

Keywords: protoescigenin propargyl ether, sugar azides, click reaction, copper acetate

Over 50 years ago, protoescigenin (PES, **1**; Scheme 1) was identified as the main aglycone of horse chestnut saponins (escins) and its structure has been firmly established (1–3). Unlike many other pentacyclic triterpene genins (particularly oleanolic, ursolic and lupeolic acids), which evoke continuous interest as prospective lead compounds for medicinal chemistry, this compound was completely absent from the life sciences research area until recent time. Although clinical position of escin is well established as venotonic and chronic venous insufficiency therapeutic (4), a challenge has been undertaken to examine single chemical entities – either constituents of the escin saponin complex or their new semi-synthetic derivatives, particularly in respect to their anti-inflammatory and endothelial activity (5). Consequently, **1** has emerged as the key chemical entity of the project, its isolation and purification had to be elaborated into technically viable and validated process (6) and its reactivity in terms of selective protection had to be re-examined (7). It turned out that selective ketalization is the most feasible, scalable process for effective partial protection of **1**, affording facile access to derivatives functionalized exclusively at C28-OH, such as the propargyl ether **2**.

This finding offers a chance to overcome persistent problems with semi-synthesis of triterpene saponin mimetics based on saccharide connection at C3-OH. Glycosylating reactions starting from **1** or its partially protected congeners, including Koenigs-Knorr and Schmidt protocols, were not successful and similarly, acid catalyzed procedures (e.g., involving thioglycosides or glycals) failed, indicating substrate inertness or instability.

Therefore, it has been decided to continue exploration of derivative **2** reactivity, in the format of click chemistry using custom synthesized novel sugar azide synthons. This decision was supported by recent findings that 1,2,3-triazoles, earlier considered pharmacologically and metabolically inert, may actually contribute some inherent biological activity (8). Since its introduction by Sharpless and coworkers at the turn of the century, *Click-Chemistry* have been applied in thousands of laboratories, in all fields of life sciences (9, 10). Azide – alkyne cycloadditions have been performed under variety of protocols, involving Cu(I) salt catalysis, Cu(II) salts in the presence or absence of a reducing agent, or metallic copper. Non-catalyzed versions have been recommended for application in biological chemistry (11, 12). Application of *Click-Chemistry* to carbohydrate chemistry and glycoscience has been so extensive that it has recently resulted in publishing an extensive monograph, which summarized over a decade of research (13).

Our first experiments on condensation of 28-*O*-propargyl PES ether **2** with monosaccharide derived azides were carried out according to well established procedure assuming CuAAC protocol –

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Cu(I) species were generating from Cu(II) by reduction with sodium ascorbate (7, 14, 15). Meanwhile, reports have appeared that use of Cu(II) salts (or in fact skipping additives acting as reducing agents) in an alkyne – azide click reaction protocols give just as satisfactory results as using ascorbate catalyzed version (16–20). We have confirmed these reports by obtaining new triazoles in reaction of sugar derived azides with **2** in the presence of copper(II) acetate without reducing agent, as depicted in Scheme 2. Azido-sugar substrates for these reactions fall in two distinct structural categories: 1) 1,6-anhydro-2-azido-2-deoxy-β-D-glucopyranoside **3** and 2) 3-azidopropyl 3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranoside **5** and 3-azidopropyl-4-*O*-benzyl-3-*O*-[4'-*O*-benzyl-2',3',6'-trideoxy-α-D-*erythro*-hex-2'-enepyranosyl]-2,6-dideoxy-D-glucopyranoside **8**, which may be reflected by different chemical (primary and secondary azides) and metabolic stability (susceptibility to *in vivo* carbon – heteroatom cleavage). Compound **3** was obtained from well known levoglucosane epoxide, as described in the literature (21). The remaining azido-sugars **5** and **8** bearing terminal azido group were obtained from corresponding glycals **4** and **7** by addition of 3-azidopropanol to unsaturated bond under TPHB catalysis as described in Schemes 3 and 4.

EXPERIMENTAL

General

Chemical materials like solvents, sorbents, inorganic salts and organic reagents were of commercial origin, certified for laboratory use, and applied without purification. Protoescigenin derivative **2** was prepared as described (7). 1,6-Anhydro-2-azido-2-deoxy-β-D-glucopyranoside **3,** 3,4,6-tri-*O*-acetyl-D-galactal **4** and 4-*O*-benzyl-L-rhamnal **6** and **7** were prepared according to the published procedures (21–25).

Reactions were monitored by TLC on precoated plates of silica gel 60 F_{254} (Merck). Visualization was performed by UV light (λ = 254 and/or 365 nm), and by spraying with 10% sulfuric acid in ethanol or cerium molybdate stain (26, 27). Crude products were purified using column chromatography performed on silica gel 60 (70–230 mesh, Fluka).

Organic solvents were evaporated on a rotary evaporator under diminished pressure at 40°C.

The ^{1}H and ^{13}C-NMR NMR spectra were recorded in $CDCl_3$ and DMSO-d_6 solutions using TMS as an internal standard with an Agilent spectrometer 400 MHz, Varian spectrometer 300 MHz and 600 MHz, and Varian-NMR-vnmrs-500. NMR solvent was purchased from ACROS Organics (Geel, Belgium). The ^{1}H and ^{13}C-NMR chemical shifts are given relative to the TMS signal at 0.0 ppm (^{1}H) and DMSO-d_6 signal at 39.5 ppm (^{13}C). Chemical shifts (δ) are expressed in ppm and coupling constants (*J*) in Hz. Used abbreviations: s – singlet, d – doublet, t – triplet, q – quartet, q_{AB} – AB quartet, m – multiplet, ov – signals overlapped. Recorded signals were compared to 2D (HMBC, HSQC) solved spectra of protoescigenin and its derivatives (6, 7).

Optical rotations were measured on a JASCO 2000 polarimeter or a PERKIN ELMER 341 Polarimeter using a sodium lamp (589.3 nm).

Mass spectra were recorded with a WATERS LCT Premier XE system and 4000 QTrap (Applied Biosystems/MDS Sciex) using electrospray ionization (ESI) technique or on a MaldiSYNAPT G2-S HDMS (Waters) Spectrometer *via* electrospray ionization (ESI-MS).

Melting points were determined by differential scanning calorimetry (DSC) carried out by means of the DSC822 with IntraCooler (Mettler Toledo).

Synthesis of sugar azides 5 and 8

3-Azidopropyl 3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranoside, 5

To a solution of 3,4,6-tri-*O*-acetyl-D-galactal **4** (1 mmol, 272.2 mg) in dry CH_2Cl_2 (5 mL) 3-azidopropanol (0.12 mL, 131.4 mg, 1.3 mmol) and molecular sieves 5Ĺ were added. Reaction mixture was cooled in water bath (0°C), then TPHB (triphenylphosphine hydrobromide, 36.3 mg, 0.1 mmol) was added. Reaction was monitored by TLC (hexane : ethyl acetate, 3 : 1, v/v). After 5 h, triethylamine (0.1 mL) was added, reaction mixture was filtered and then solvent was evaporated under reduced pressure. Crude product was purified by column chromatography on silica gel with hexane – acetone (20 : 1, v/v) elution system. Product **5** was obtained as a colorless syrup with 52% yield. $[a]_D^{20} = +113.8$ (c 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 5.33 (d, *J* = 3.0 Hz, 1H, H-4), 5.27 (ddd, *J* = 12.4, 5.0, 3.1 Hz, 1H, H-3), 5.01 (d, *J* = 3.0 Hz, 1H, H-1), 4.18–4.05 (m, 3H, H-5, H-$6_{a,b}$), 3.76 (dt, *J* = 9.9, 6.0 Hz, 1H, -$OCH_{2(a)}$-), 3.48 (dt, *J* = 9.9, 6.1 Hz, 1H, -$OCH_{2(b)}$-), 3.41 (dt, *J* = 6.5, 3.2 Hz, 2H, -CH_2N_3), 2.09 (m, 1H H-2_e), 2.14, 2.06, 1.99 (3s, 9H, 3 × -OAc), 1.91–1.81 (m, 3H, -CH_2-, H-2_e). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 170.5, 170.3, 170.0 (3 × C=O), 97.6 (C1), 66.8 (C3, C4, C5), 66.6, 66.1, 64.3 (-OCH_2-), 62.5 (C6), 48.3 (-CH_2N_3), 30.1 (C2, CH_2), 28.8, 20.8 (-CH_3), 20.7. LRMS (ESI): calcd. for

$C_{15}H_{23}N_3O_8Na$ ([M + Na]$^+$): m/z 396.3482; found: m/z 396.3.

3-Azidopropyl-4-*O*-benzyl-3-*O*-[4'-*O*-benzyl-2',3',6'-trideoxy-α-D-*erythro*-hex-2'-enopyranosyl]-2,6-dideoxy-D-glucopyranoside, 8

To a solution of disaccharide **7** (450 mg, 1.06 mmol) in dry CH_2Cl_2 (5 mL) 3-azidopropanol (0.09 mL, 1.28 mmol) and molecular sieves 5Ĺ were added. Reaction mixture was cooled in water bath (0^{o}C), then TPHB (triphenylphosphine hydrobromide, 36 mg, 0.1 mmol) was added. Reaction was monitored by TLC (hexane : ethyl acetate, 3 : 1, v/v). After 5 h, trietylamine was added, reaction mixture was filtered and then solvent was evaporated under reduced pressure. Crude product was purified by column chromatography on silica gel with hexane : EtOAc (20 : 1, v/v) elution system. Product **8** (α:β 4:1) was obtained as a colorless syrup with 52% yield. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.42–7.23 (m, 10H, H_{Ar}), 6.04 (d, *J* = 10.2 Hz, 1H, H-3'), 5.67 (ddd, *J* = 10.2, 2.6, 2.0 Hz, 1H, H-2'), 5.19–5.15 (m, 1H, H-1'), 4.82 (1/2 q_{AB}, *J* = 11.1 Hz, 1H, -C$\underline{H}_2$Ph), 4.78 (d, *J* = 2.7 Hz, 1H, H-1$_\alpha$), 4.67 (1/2 q_{AB}, *J* = 11.7 Hz, 2H, -C$\underline{H}_2$Ph), 4.65 (1/2 q_{AB}, *J* = 11.1 Hz, 1H, -C$\underline{H}_2$Ph), 4.54 (1/2q_{AB}, *J* = 11.7 Hz, 2H, -C$\underline{H}_2$Ph), 4.46 (dd, *J* = 9.8, 2.0 Hz, 1H, H-1$_\beta$), 4.07 (ddd, *J* = 11.5, 8.9, 5.3 Hz, 1H, H-3), 3.92 (dq, *J* = 8.8, 6.4 Hz, 1H, H-5'), 3.74 – 3.65 (m, 3H, H-4', H-5, H-1''$_a$); 3.44–3.31 (m, 3H, H-1''$_b$, H-3''$_{ab}$), 3.06 (dd~t, *J* = 9.2 Hz, 1H, H-4), 2.25 (ddd, *J* = 13.1, 5.3, 1.0 Hz, 1H, H-2$_{eq}$), 1.89–1.79 (m, 2H, H-2''$_{ab}$), 1.76 (ddd, *J* = 13.0, 11.5, 3.8 Hz, 1H, H-2$_{ax}$), 1.28 (d, *J* = 6.4 Hz, 3H, 6-CH_3), 1.27 (s, *J* = 6.4 Hz, 3H, 6'-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 138.5, 138.1 (C-1'', C-1''''', C_{Ar}), 130.60 (C-3'), 128.43, 128.41, 127.81, 127.77, 127.69 (10 × C_{Ar}), 126.84 (C-2'), 99.67 (C-1β), 97.27 (C-1α), 96.17 (C-1'α), 84.44 (C-4), 77.48 (C-3), 76.41 (C-4'), 75.31, 71.02 (2 × -C$\underline{H}_2$Ph), 67.31 (C-5), 65.62 (C-5), 63.76 (CH_2O), 48.51 (-CH_2N_3), 37.99 (C-2), 28.97 (CH_2), 18.25 (C-6'), 18.17 (C-6).

General procedure for PES triazoles synthesis

Without sodium ascorbate

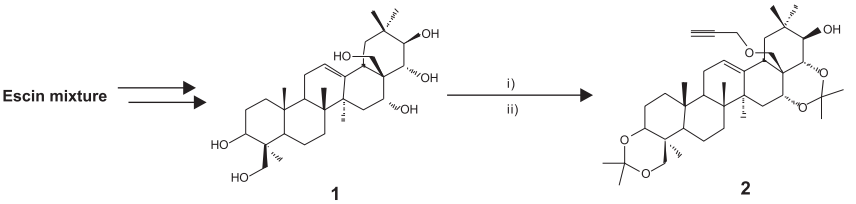
To the solution of azidosugar (0.2 mmol) in 0.1 mL CH_2Cl_2 protected protoescigenin 28-*O*-propargyl ether **2** (0.2 mmol) in methanol (4 mL) and aquous solution of $Cu(OAc)_2$ (0.4 M, 0.025 mL) were added. Reaction mixture was stirred overnight in the room temperature. After that, solvents were evaporated under vacuum and product was separated by column chromatography (SiO_2).

PES triazole 9

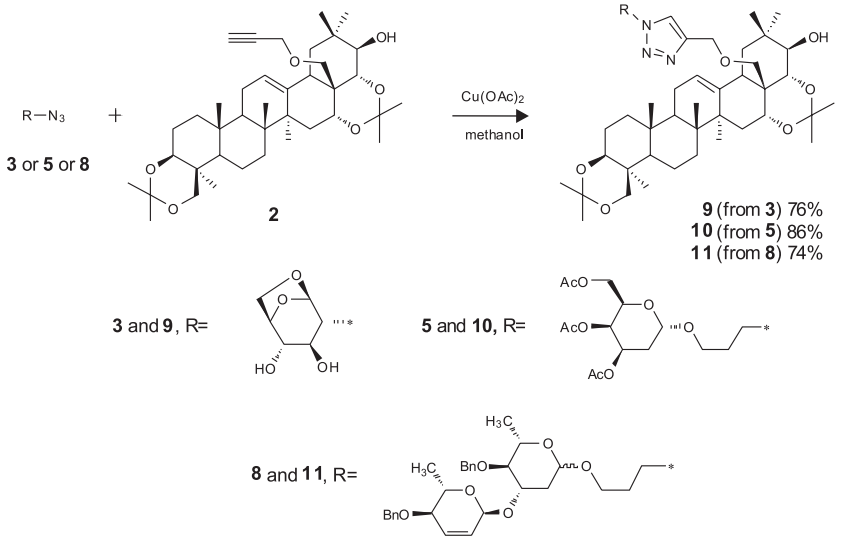
From sugar **3.** Reaction mixture was stirred 10 days at room temperature. The crude reaction product was purified by column chromatography (SiO_2, CH_2Cl_2 : acetone, 6:1 → 2:1) with 76% yield as white solid. M.p. 223-232^{o}C (dec.), $[a]_D^{20}$ = +38.2^o (*c* 0.50, DMSO). ^{1}H NMR (600 MHz, DMSO, δ, ppm): 8.36 (s, 1H, H3'), 5.7 (d, *J* = 3.75 Hz, 1H, C3''OH, C4''OH), 5.68 (d, *J* = 4.2 Hz, 1H, C3''OH,C4''OH), 5.34 (s, 1H, H12), 5.03 (bs, 1H, H1''), 4.59 (d, *J* = 6.1 Hz, 1H), 4.56 (d, *J* = 16.1 Hz, 2H), 4.43 (d, *J* = 12.5 Hz, 1H), 4.24 (d, *J* = 4.7 Hz, 1H, C21-OH), 4.12 (d, *J* = 6.4 Hz, 1H), 3.93 (d, *J* = 11.6 Hz, 1H, H24), 3.8 (d, *J* = 9.2 Hz, 1H, H22), 3.74–3.69 (m, 1H), 3.69–3.62 (m, 2H), 3.63–3.58 (m, 1H), 3.44 (qd, *J* = 7.0,5.1 Hz, 2H, H16), 3.39–3.33 (m, 2H), 3.32 (s, 2H), 3.26 (d, *J* = 8.5 Hz, 1H), 3.14 (d, *J* = 11.5 Hz, 1H, H24), 2.41 (d, *J* = 11.3 Hz, 1H, H18), 1.98–1.84 (m, 3H), 1.84–1.74 (m, 1H), 1.69–1.58 (m, 2H), 1.57–1.40 (m, 5H), 1.40 (m, ov, 1H), 1.35 (s, 3H, CH_3), 1.33 (s, ov, 3H, CH_3), 1.33 (m, ov, 1H), 1.27 (s, 3H, CH_3), 1.25 (s, 3H, CH_3), 1.18 (dd, *J* = 13.0,3.2 Hz, 1H), 1.12 (s, 3H, CH_3), 1.11 (s, 3H, CH_3), 1.09–1.04 (m, 4H), 1.02 (m, 1H), 0.86 (d, *J* = 11.9 Hz, 1H), 0.75 (s, 3H, CH_3), 0.71 (s, 3H, CH_3). ^{13}C NMR (150 MHz, DMSO, δ, ppm): 143.9 (C2'), 140.3 (C13), 124.0 (C3'), 122.9 (C12), 99.8 (C1''), 98.03, 97.97, 76.3, 76.1, 75.6, 72.5, 71.3, 70.7, 70.5, 68.1 (C16), 64.9, 63.5, 62.9, 62.8, 53.1 (C5), 46.8, 44.4, 41.10, 41.06, 40.8, 36.8, 36.1, 35.8, 35.3, 34.8, 32.1 (C7), 30.32, 30.27, 27.9, 25.7, 25.4, 24.5, 23.6, 22.7, 18.5, 18.0, 17.4, 16.5. LRMS (ESI): calcd. for $C_{45}H_{69}N_3O_{12}Na$ ([M + Na]$^+$): *m*/*z* 835.0332; found: *m*/*z* 835.0.

PES triazole 10

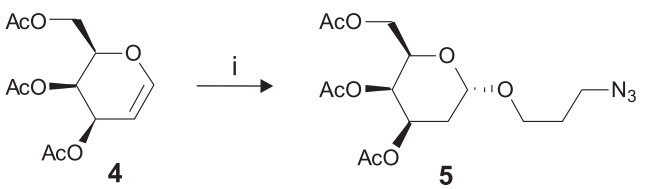
The crude reaction product obtained from glycoside **5** was purified by column chromatography with gradient elution system CH_2Cl_2 : acetone (6 : 1 → 2 : 1) yielding colorless syrup with 74% yield. $[a]_D^{20}$ = +38.2 (*c* 1.00, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 7.53 (s, 1H, H3'), 5.36–5.20 (m, 3H), 4.99 (d, *J* = 2.7 Hz, 1H, H1''), 4.65 (s, 2H, H1'), 4.58–4.38 (m, 2H), 4.20–4.00 (m, 4H), 3.98–3.80 (m, 2H), 3.76–3.64 (m, 1H), 3.60–3.18 (m, 6H), 2.63 (dd, *J* = 14.3, 3.9 Hz, 1H), 2.37–2.18 (m, 1H), 3.25–2.18 (m, 2H), 2.14 (s, 3H, OAc), 2.12–2.04 (m, 1H), 2.04 (s, 3H, OAc), 2.00 (s, 3H, OAc), 1.99–1.48 (m, 12H), 1.46 (s, 3H, CH_3), 1.44 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.33 (s, 3H, CH_3), 1.22 (s, 3H, CH_3), 1.20^o–1.19 (m, 1H), 1.18 (s, 3H, CH_3), 1.12 (s, 3H, CH_3), 0.96–0.85 (m, 2H), 1.04 (s, 3H, CH_3), 0.85 (s, 3H, CH_3), 0.79 (s, 3H, CH_3). ^{13}C NMR (150 MHz, $CDCl_3$, δ, ppm): 170.48, 170.26,



Scheme 1. Protoescigenin **1**, the main product of controlled hydrolysis of escin saponins and its partially protected 28-*O*-propargyl ether **2**. Conditions: i) 2,2-dimethoxypropane, acetone, *cat*. *p*-TSA, *RT*, overnight, ii) $BrCH_2C{\equiv}CH$, TBAB, KOH, THF, room temp., overnight



Scheme 2. General procedure of synthesis PES triazoles **9–11**

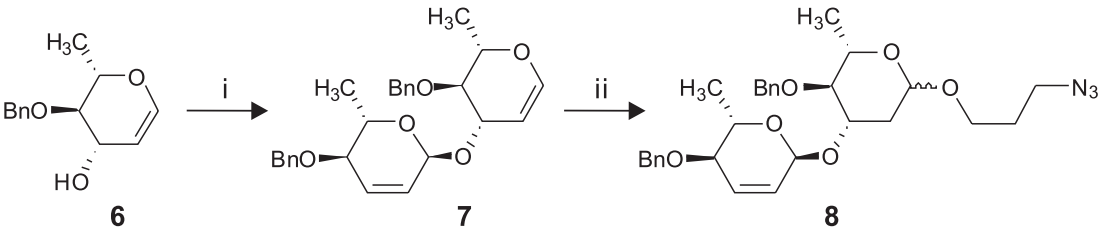


Scheme 3. Synthesis of 3-azidopropyl galactopyranoside **5**; i) 3-azidopropanol, TPHB, CH_2Cl_2, 0°C, 5 h

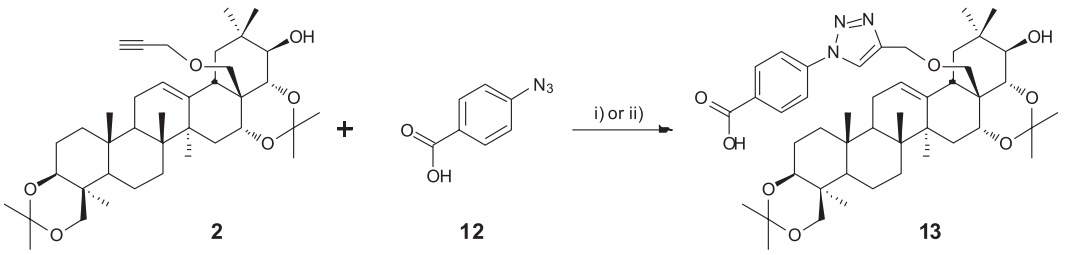
170.06 (3 × C=O); 140.12, 122.88, 99.2, 98.81, 97.71 (C1''), 77.19, 74.17, 71.27, 69.24 (C28), 66.83 (C16), 66.46 (C4''), 66.43 (C3''), 66.03 (C5''), 63.99, 63.67, 62.24, 53.74 (C5), 47.53, 44.25, 43.9, 41.8, 41.69, 40.15, 37.44, 36.58, 36.3, 36.26, 35.13, 32.41 (C7), 30.13, 30.06, 29.38, 28.83, 27.77, 26.06, 25.55, 25.53, 24.77, 24.53, 23.17, 20.94, 20.7, 20.68 (3 × OAc), 18.88, 17.93, 17.07, 16.69. LRMS (ESI): calcd. for $C_{54}H_{83}N_3O_{14}Na$ ($[M + Na]^+$): *m*/*z* 1021.2383; found: *m*/*z* 1021.0.

PES triazole 11

The crude reaction product obtained from sugar azide **8** was purified by column chromatography with gradient elution system CH_2Cl_2 : acetone (10 : 1›5 : 1) with 74% yield as light yellow syrup. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.5 (s, 1H, H3'), 7.43–7.20 (m, 10H, H_{Ar}), 6.05 (d, J = 10.2 Hz, 1H, H3'''), 5.70–5.65 (m, 1H, H2'''), 5.25–5.20 (m, 1H, H12), 5.19 (bs, 1H, H1'''), 4.82 (1/2 q_{AB}, J = 11.9 Hz, 1H, -CH_2Ph), 4.78 (d, J = 3.1 Hz, 1H,



Scheme 4. Synthesis of 3-azidopropyl disaccharide **8**; i) *p*-TsCl, $NaOH_{aq}$, TBAI, toluene, room temp., 24 h; i) 3-azidopropanol, TPHB, CH_2Cl_2, 0°C, 5 h



Scheme 5. Synthesis of PES triazoles **13**. Conditions: i) $Cu(OAc)_2$, AscNa, *t*-BuOH, water, room temp.; ii) $Cu(OAc)_2$, MeOH, room temp.

H1''), 4.67 (1/2 q_{AB}, J = 11.6 Hz, 1H, -CH_2Ph), 4.62 (1/2 q_{AB}, J = 11.9 Hz, 1H, -CH_2Ph), 4.67–4.63 (m, 2H, H1'), 4.54 (1/2 q_{AB}, J = 11.6 Hz, 1H, -CH_2Ph), 4.44 (ddd~dt, J = 13.5, 6.8 Hz, 2H, H4'), 4.08 (ddd, J = 11.3, 8.9, 5.0 Hz, 1H, H3''), 4.04 (d, J = 11.8 Hz, 1H), 3.96–3.90 (m, 1H, H5'''), 3.91 (d, J = 10.2 Hz, 1H), 3.86 (d, J = 10.2 Hz, 1H), 3.73–3.62 (m, 3H), 3.53 (d, J = 8.4 Hz, 1H), 3.45 (dd, J = 9.2, 4.5 Hz, 1H), 3.39–3.19 (m, 3H), 3.08 (dd~t, J = 9.2 Hz, 1H, H4''), 2.67–2.59 (m, 1H), 2.27 (dd, J = 12.5, 5.4 Hz, 1H, $H2''_{eq}$), 2.20–2.12 (m, 2H, H5'), 2.19–1.80 (m, 6H), 1.82–1.74 (m, 1H, $H2''_{ax}$), 1.74–1.3 (m, 20H), 1.3 (d, J = 6.2 Hz, 3H, C6'''-CH_3), 1.26 (d, J = 6.2 Hz, 3H, C6''-CH_3), 1.24–0.98 (m, 15H), 0.91–0.77 (m, 7H). ^{13}C NMR (100 MHz, $CDCl_3$), δ, ppm): 145.4 (C2'), 140.1 (C13), 138.4 (-Ph), 138.1 (-Ph), 130.6 (C3'''), 128.43 (-Ph), 128.41 (-Ph), 127.8 (-Ph), 127.7 (-Ph), 126.8 (C2'''), 122.9, 122.4, 99.2, 98.8, 97.3, 96.2 ($C1'''_a$), 84.3 (C4''), 77.5, 77.22, 77.06, 76.4, 75.3, 74.2, 71.1, 71.0, 69.3, 67.4, 65.7, 64.6, 63.8, 63.4, 53.8 (C5), 47.6, 47.4, 44.3, 43.9, 41.8, 41.7, 40.2, 37.9, 37.5, 36.6, 36.3, 35.1, 32.5 (C7), 30.9, 30.3, 29.7, 29.7, 28.9, 27.9, 26.1, 25.6, 24.6, 18.9, 18.3, 18.2, 18.0, 17.1, 16.7. LRMS (ESI): calcd. for $C_{68}H_{97}N_3O_{12}Na$ ($[M + Na]^+$): m/z 1171.5005; found: m/z 1171.4.

Reactions of 2 with 4-azidobenzoic acid 12

With sodium ascorbate

From azide **12**. Reaction mixture was stirred 8 days at room temperature. The crude reaction product was purified by column chromatography (SiO_2, $CH_2Cl_2 \rightarrow CH_2Cl_2$: MeOH 85 : 15) yielding white solid with 91% yield.

With sodium ascorbate

Ether **2** (110 mg, 0.17 mmol) and 4-azidobenzoic acid **12** (64 mg, 0.21 mmol) were dissolved in *t*-BuOH (7.5 mL) and water (4 mL) was added. Then was added solution of $Cu(AcO)_2$ (1.5 mL of soln. 280 mg of $Cu(AcO)_2$ in 10 mL of water) and solution of sodium ascorbate (AscNa, 1.5 mL soln. of 260 mg AscNa in 10 mL of water). Reaction mixture became brown immediately and then turbid. After two days, TLC control ($CHCl_3$: MeOH, 9 : 1, v/v, product R_F = 0.34) indicated complete depletion of the substrate. To the reaction mixture water (70 mL) was added and product was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phases were washed with 5% aqueous $NaHCO_3$ and dried over anhydrous Na_2SO_4. After filtration and concentration crude **13** was obtained, and purified by col-

umn chromatography with gradient elution system (SiO_2, $CH_2Cl_2 \rightarrow CH_2Cl_2$: MeOH 85 : 15) to give pure triazol **13** with yield 85% as a colorless solid. TLC: R_F = 0.34 ($CHCl_3$: MeOH 9 : 1, v/v); 0.40 (CH_2Cl_2 : MeOH 85 : 15, v/v); $[a]_D^{20}$ = +8.55 (*c* 1.0, THF). M.p. (DSC) 184.4°C (crystallized from THF). ^{1}H NMR (600 Hz, DMSO-d_6, d, ppm): 13.20 (bs, 1H, -CO_2H), 9.04 (s, 1H, H3'), 8.14 (d_{AB}, *J* = 8.8 Hz, 2H, Ar), 8.10 (d_{AB}, *J* = 8.7 Hz, 2H, Ar), 4.85 (bs, 1H, H12), 4.77 (d, *J* = 12.8 Hz, 1H, H1'), 4.47 (d, *J* = 12.9 Hz, 1H, H1'), 4.23 (d, *J* = 4.4 Hz, 1H, C21-OH), 3.82 (d, *J* = 9.6 Hz, 1H), 3.79 (d, *J* = 12.3 Hz, 1H), 3.68 (dd, *J* = 4.6, 9.2 Hz, 1H), 3.36–3.26 (m, ov, 4H), 3.14 (d, *J* = 8.3 Hz, 1H), 3.07 (d, *J* = 11.4 Hz, 1H), 2.40 (dd, *J* = 3.1, 14.2 Hz, 1H, H18), 1.89–1.75 (m, ov, 5H), 1.56–1.38 (m, ov, 7H), 1.35 (s, 3H, CH_3), 1.30 (s, 3H, CH_3), 1.28 (s, 3H, CH_3), 1.23 (s, 3H, CH_3), 1.18 (d, *J* = 11.0 Hz, 1H), 1.06 (s, 3H, CH_3), 1.04 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 0.88–0.85 (m, 1H), 0.77 (s, ov, 3H, CH_3), 0.75 (m, ov, 1H), 0.61 (s, 3H, CH_3), 0.53 (s, 3H, CH_3). ^{13}C NMR (150 Hz, DMSO-d_6, δ, ppm): 166.3 (C=O), 144.8 (C2'), 139.9 (C13), 139.5 (Ar), 131.1 (Ar), 130.5 (Ar), 122.7 (C3'), 121.8 (C12), 119.3 (Ar), 98.3, 97.7, 75.9, 75.6, 72.7, 69.7 (C28), 68.4 (C16), 62.7, 62.6, 52.8 (C5), 46.6, 44.2, 43.0, 41.0, 40.8, 39.6, 36.7, 35.7, 35.5, 35.4, 35.1, 31.9 (C7), 30.33, 30.16, 28.1, 27.8, 25.6, 25.2, 24.2, 24.0, 22.4, 18.0, 17.8, 17.4, 16.2. HRMS (ESI): calcd. for $C_{46}H_{65}N_3O_8Na$ $[M + Na]^+$ *m*/*z*: 810.4669; found: *m*/*z*: 810.4673.

RESULTS AND CONCLUSIONS

The four new triazole derivatives of protoescigenin were obtained – three of them containing a glycoside moiety. In all cases to perform the cycloaddition reaction there was no need of addition a reductant (e.g., sodium ascorbate). However, for the secondary azide, the reaction time was highly elongated.

It has been suggested that in Cu(II) catalyzed click reactions an alcohol used as the reaction solvent gets oxidized by copper salt to a sufficient degree to provide Cu(I) catalyst, which binds terminal acetylenic substrate by a covalent bond. Without entering a mechanistic dispute, it seems useful to sort out a situation in which Cu(I) and Cu(II) as well as non catalytic reaction pathways have been claimed, for purely practical purposes.

Thus, PES propargylic ether **13** has been subjected to click reactions with 4-azidobenzoic acid **12** (Scheme 5), side by side, under catalytic and non-catalytic conditions. It turned out that copper catalyzed reactions proceeded with comparable yield, while lack of reductant (sodium ascorbate, AscNa) resulted in significant prolongation of reaction time (from 1 day in the presence to 8 days in the absence of AscNa). Similar observation was done for the reaction of **2** with azidosugar **3**: in absence (presence) of AscNa product **9** was obtained with 76% (71%) yield after 10 days (1 day) of reaction time, respectively.

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