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An approach to C-glycosidic conjugates of isoflavones

Abstract: A novel approach to isoflavone glycoconjugates, designed as less biodegradable congeners of natural glycosides, is presented on example of daidzein linkage to a C-glycosidic synthon derived from L-rhamnose. 1,3-Dipolar cycloaddition was employed for chemical ligation of daidzein 7-*O*-propargyl ester and alkyl azide containing 2,3-unsaturated pyranoside moiety. The obtained constructs with opposite anomeric configuration both exhibited a considerable increase in cytotoxic activity against the HTC 116 cell line, in comparison to the parent isoflavone.

Keywords: click-chemistry; cytotoxic activity; daidzein; glycoconjugates.

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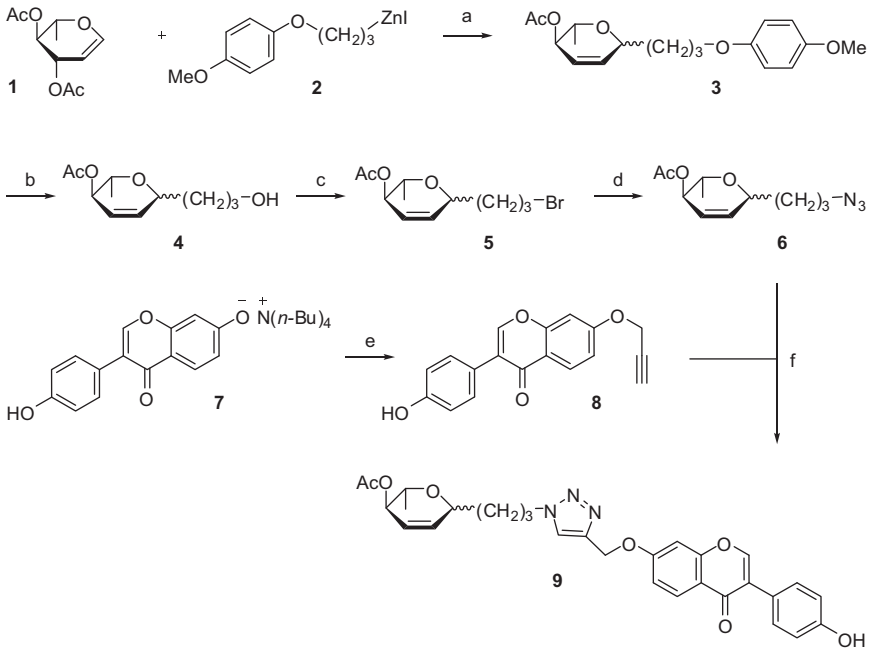
Introduction

Natural products are often sources of new drugs [1, 2]. Natural isoflavones (genistein, daidzein; their glycosides and biogenetic esters) are present in soy and soy-derived food and, therefore, constitute a significant non-nutritional component of animal feed and human diet. Numerous biological and preclinical studies have revealed pleiotropic action of isoflavones, with at least two types of biological activity, important for human health: hormonal (e.g., affinity to estrogen receptor β) [3, 4] and modulatory, influencing intracellular signaling and gene expression [5, 6]. Although epidemiological studies have indicated considerable cancer preventive potential of soy-derived isoflavones, the correlation between *in vitro* activity and clinical results remains elusive. There is long-standing controversy concerning bioavailability of isoflavones and their glycosides, and it may prove crucial to the problem of future drug design, and an effective pro-drug strategy, involving a covalently bound derivative of an isoflavone pharmacophore [7, 8]. Such problems have to be resolved by means of chemical synthesis, providing a variety of glycoconjugates with different susceptibility for biodegradation *in vivo*. In this communication, we present synthesis of a daidzein conjugate, which links the isoflavone, with particularly promising hex-2-enopyranoside moiety as a pharmacological activity modifier [9, 10], by means of an alkyltriazole linker. The presented design compromises stereoselectivity introducing some stereochemical ambiguity at the sugar coupling step, which is allowed to be addressed later by separation of anomers. By contrast, this approach secures biostability of obtained compounds under pharmacological test conditions, in particular rendering them resistant towards glycoside splitting enzymes such as abundant and relatively nonselective glycosyl transferases. As the principal soy isoflavone, genistein, has passed modern tests towards multitargeted therapy of cancer [11], more studies on its close structural analogs are warranted. Isoflavone glycoconjugates appear to be especially a prospective class of new derivatives and among them C-glycosidic constructs seem particularly desirable.

Results and discussion

Compounds designed as isoflavone derivatives containing some pyranose moiety but devoid of O-glycosidic bond could, in principle, be obtained on various routes, as indicated by the rich literature devoted to synthesis of C-glycosides [12–14]. Our approach to a C-glycosidic synthon focusing on 2,3-unsaturated hexose moiety, presented below in Scheme 1, stems from lasting involvement in unsaturated sugar chemistry, which has already proven its utility in medicinal chemistry projects [9, 10]. As the idea of linking two principal synthons, namely a sugar moiety and an isoflavone derivative, by exploiting chemical ligation based on 1,3-dipolar cycloaddition seemed most feasible from the point of view of synthetic accessibility [15, 16], we decided to place the azido function on an alkyl chain attached to anomeric position of the



Scheme 1 Synthesis of daidzein C-glycosidic conjugate using 1,3-dipolar cycloaddition for linking 1-alkyl-hex-2-enopyranosyl moiety and 7-*O*-propargyl ether of the soflavone: (A) BF_3·OEt_2, CH_2Cl_2, -30°C overnight, -15°C overnight; (B) CAN (cerric ammonium nitrate), 10 min, 0°C, H_2O/CH_3CN(1/4); (C) Ph_3PBr_2, 5 min, CH_2Cl_2, rt; (D) H_2O/CH_2Cl_2, TBAB, NaN_3, rt; (E) DMF, propargyl bromide, 45–50°C, 1 h; (F) $Cu(OAc)_2$, MeOH, overnight, rt.

pyranose ring (**6**) and the acetylene group in the form of propargyl phenyl ether at the isoflavone more acidic 7-OH group (**8**). To our knowledge, 1-C- hydroxypropylation of L-rhamnal has not been described before, despite obvious utility of such synthon. Choice of reagent **2** for this particular purpose results from trying several alkylzinc iodides, which need to balance low reactivity securing protecting group tolerance, with the ability to perform C-C coupling and effective deprotection of the terminal hydroxyl function [17]. Formation of the azide function in two steps and cyclization to the triazole were carried out according to well-known standard procedures [18]. Alkylation of daidzein with propargyl bromide is expected to be selective, as pK_a values for 7-OH and 4′-OH phenolic functions are two orders of magnitude apart but, in fact, most of the alkylating procedures, including phase-transfer catalysis, afford a mixture of regioisomeric products. Our experience indicates that formation of the stoichiometric tetraalkylammonium salt prior to the propargylation step is advantageous for regioselectivity and efficiency.

The intermediate sugar synthons are poorly separable on TLC but conjugates **9** can be obtained as individual anomers by using silica gel column chromatography. Both new compounds performed considerably better in the cytotoxicity test carried out on HCT 116 cancer cell lines than daidzein, the parent isoflavone, as can be seen from Table 1 which compares the efficacy of effective concentrations of all three compounds. This result supports an earlier advanced hypothesis that hex-2-enopyranoside moiety is particularly effective in enhancing cytotoxic effects of isoflavones, while the mode of its attachment becomes an important factor for efficacy [9, 19].

Table 1 Anticancer activity of daidzein glycoconjugates.

	HCT 116 [IC_{50}] µmol
Daidzein	144.3
RamC3TRIAZαDAI (**9a**)	24.8
RamC3TRIAZβDAI (**9b**)	26.2

Experimental

General

Daidzein was purchased from LC Laboratories, Woburn, MA, USA; 3,4-di-*O*-acetyl-L-Rhamnal was a commercial reagent. Tetra-*n*-butylammonium salt of daidzein (**7**) was prepared as described for tetra-*n*-butylammonium salt of genistein [7]. The remaining

reagents, solvents and sorbents were of commercial origin, certified for research use. Solvents were additionally dried before use as recommended in the literature [20]. Products of the sugar reactions were recognized based on silica gel TLC R_F values (in toluene/ethyl acetate, 2:1), These products were purified by column chromatography performed on silica gel 60 (70–230 mesh, E. Merck) and characterized by HRMS [positive mode, a Mariner Per Septive Biosystem detector, electrospray-ionization (ESI)], ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectra [recorded in $CDCl_3$ solution (unless stated otherwise) with TMS internal standard] on a Varian Inova 600 MHz apparatus. Optical rotations were recorded using a Perkin-Elmer 141 polarimeter. Melting points were determined on a Koefler apparatus and are not corrected. All evaporations were performed under reduced pressure at 50°C.

Cancer cell lines

HCT 116 colorectal cancer prostate cancer cell lines were obtained from ATCC (American Type Culture Collection, Rockville, MD, USA) and cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS, ICN Pharmaceuticals) and 1 μg/mL gentamicin (KRKA, Novo Mesto, Slovenia), at 37°C in a humidified atmosphere containing 5% CO_2 in the air. Cells were split at 90% confluence. The grown cells were detached by rinsing with 0.02% ethylenediaminetetraacetic acid (EDTA) followed by 0.25% trypsin.

Cytotoxicity assay

Cell viability was estimated using an MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay (Sigma-Aldrich, Germany), according to the supplier's protocol. After the 72-h treatment with tested agents, the medium was aspirated and cells were incubated for 3 h at 37°C with 0.5 mg/mL MTT solution (50 μL) in Dulbecco's modified essential medium (DMEM) (Sigma-Aldrich, Germany) without phenol red. Then the medium was aspired, insoluble crystals of formazan were solubilized in 2-propanol:HCl solution, and optical density (λ = 570 nm) was determined in a microplate reader BioTek Synergy II (BioTek Instruments, USA). IC_{50} was estimated using CalcuSyn software using the four-parameter nonlinear regression model for curve-fitting analysis. The experiments were repeated at least three times.

Synthesis of 1-[3-(4-methoxyphenoxy)]propylzinc iodide (2)

A mixture of zinc dust (65 mg, 1 mmol), THF (5 mL) and 1,2-dibromoethane (0.1 mL) was heated at 56°C for 10 min. After addition of chlorotrimethylsilane (13 μL, 0.15 equiv.), the mixture was sonicated at room temperature for 15 min, then treated with 1-[3-(4-methoxyphenoxy)]propyl iodide and sonicated for an additional 45 min at room temperature. Next, the mixture was heated to 50°C and stirred on a magnetic stirrer for a few hours, cooled to room temperature and concentrated under reduced pressure. The gel-like residue of the reagent **2** was used as such in the unsaturated sugar coupling step.

Synthesis of 1-(4-*O*-acetyl-2,3,6-trideoxy-α-L-*erythro*-hex-2-enopyranose)-3-(4-methoxyphenoxy)propane (3a) and 1-(4-*O*-acetyl-2,3,6-trideoxy-β-L-*erythro*-hex-2-enopyranose)-3-(4-methoxyphenoxy) propane (3b)

A solution of 3,4-di-*O*-acetyl L-rhamnal (200 mg, 0.93 mmol) in dry dichloromethane (DCM, 2.0 mL) was added to alkylzinc iodide **2** (the gel-like residue, 1 mmol) placed in a 50-mL round bottom flask and the mixture was cooled to -30°C before addition of boron trifluoride etherate (200 μL, 1.6 mmol) diluted with DCM (1.6 mL). The tightly stoppered reaction flask was left at -30°C for 24 h and then at 15°C for another 24 h, after which time the mixture was diluted with DCM (5.0 mL), transferred to a separation funnel and washed with chilled water. After phase separation the aqueous layer was extracted once with DCM and the combined organic layers were dried with anhydrous sodium sulfate, filtered and concentrated under reduced pressure to afford an oily residue of **3a/3b**. This mixture was separated on a silica gel column eluting with hexane/acetone (20:1) with TLC control of individual fractions.

Compound 3a Yellow oil; yield 181 mg (61%) $[\alpha]_D^{20}$-57.0° (c 1.366, $CHCl_3$); ^{1}H NMR: δ 1.24 (d, *J* = 6.6 Hz, 3H, CH_3), 1.70–2.00 (m, 4H, CH_2CH_2), 2.08 (s, 3H, CH_3CO), 3.76 (s, 3H, OCH_3), 3.91 (dq, *J* = 6.6 Hz and 4.9 Hz, 1H, H-5), 3.95 (t, *J* = 6.3 Hz, 2H, OCH_2), 4.21 (m, 1H, H-1), 4.89 (m, 1H, H-4), 5.78 (ddd, *J* = 10.3 Hz, 3.5 Hz and 2.1 Hz, 1H, H-3), 5.92 (ddd, 1H *J* = 10.4 Hz, 2.2 Hz and 1.3 Hz, 1H, H-2), 6.83 (s, 4H, H-2_{ar} H-3_{ar} H-5_{ar} H-6_{ar}); ^{13}C NMR: δ 17.2 (CH_3), 21.5 ($\underline{CH_3}CO$), 26.0 ($CH_2\underline{CH_2}CH_2$), 30.4 ($\underline{CH_2}CH_2CH_2$), 56.0 (OCH_3), 68.5 (C-5), 68.7 (CH_2O), 69.9 (C-4), 70.1 (C-1), 114.6 (C-3_{ar}; C-5_{ar}), 115.7 (C-2_{ar}; C-6_{ar}), 123.1 (C-3), 134.2 (C-2), 153.4 (C-1_{ar}), 154.0 (C-4_{ar}), 171.0 (C=O). HRMS Calcd for $[M+Na]^+$: m/z 359.1471. Found: m/z 359.1473.

Compound 3b Yellow oil; yield 60 mg (20%); $[\alpha]_D^{20}$-65.60° (c 1.744, $CHCl_3$); ^{1}H NMR: δ 1.24 (d, *J* = 6.6 Hz, 3H, CH_3), 1.50–1.90 (m, 6H, $CH_2CH_2CH_2$), 2.08 (s, 3H, CH_3CO), 3.76 (s, 3H, OCH_3), 3.88–3.96 (m, 3H, OCH_2; H-5), 4.16 (m, 1H, H-1), 4.89 (m, 1H, H-4), 5.76 (ddd, *J* = 10.3 Hz, 3.5 Hz and 2.1 Hz, 1H, H-3), 5.92 (ddd, *J* = 10.4 Hz, 2.1 Hz and 1.3 Hz, H, H-2), 6.83 (s, 4H, H-2_{ar} H-3_{ar} H-5_{ar} H-6_{ar}); ^{13}C NMR: δ 17.2 (CH_3), 21.2 ($\underline{CH_3}CO$), 22.4 ($CH_2\underline{CH_2}CH_2O$), 29.2 ($CH_2CH_2\underline{CH_2}O$), 33.4 ($\underline{CH_2}CH_2CH_2O$), 55.7 ($OCH_3$), 68.3 (C-5), 69.6 (C-4), 69.9 (C-1), 114.5 (C-3_{ar}; C-5_{ar}), 115.4 (C-2_{ar}; C-6_{ar}), 122.6 (C-3), 134.1 (C-2), 153.1 (C-1_{ar}), 153.67 (C-4_{ar}), 170.7 (C=O). HRMS Calcd for $[M+Na]^+$: m/z 359.1471. Found: m/z 359.1472.

Synthesis of 1-(4-*O*-acetyl-2,3,6-trideoxy-α-L-*erytro*-hex-2-enopyranose)-3-hydroxypropane (4a) and 1-(4-*O*-acetyl-2,3,6-trideoxy-β-L-*erytro*-hex-2-enopyranose)-3-hydroxypropane (4b)

A solution of compound **3a** or **3b** (230 mg, 1 mmol) in a mixture of acetonitrile and water (4:1, 8.0 mL) was cooled to 0°C and treated with cerric ammonium nitrate (CAN, 1.20 g, 2.2 mmol) with stirring. After 10 min a cooling bath was removed and the mixture was allowed to

reach ambient temperature. Saturated brine was added (20 mL) and the resulting solution was extracted with ethyl acetate (3 × 20 mL). Combined organic layers were dried with anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The oily residue was purified by column chromatography on silica gel, eluting with a mixture of hexane/acetone mixture (8:1). Deprotected compounds **4a** and **4b** were obtained in 82% and 81%, respectively.

Compound 4a Yellow oil; yield 82%; $[\alpha]_D^{20}$-101.13° (c 1.147, $CHCl_3$); ^{1}H NMR: δ 1.24 (d, *J* = 6.6 Hz, 3H, CH_3), 1.60–1.80 (m, 4H, CH_2CH_2), 2.03 (s, 3H, CH_3CO), 2.32 (s 1H, OH), 3.66 (m, 2H, $\underline{CH_2}OH$), 3.92 (dq, *J* = 6.5 Hz and 4,5 Hz, 1H, H-5), 4.18 (m, 1H, H-1), 4.87 (m, 1H, H-4), 5.79 (ddd, *J* = 10.3 Hz, 3.6 Hz and 2.1 Hz, 1H, H-3), 5.89 (ddd, *J* = 10.3 Hz, J=2.1 Hz and 1.2 Hz, 1H, H-2); ^{13}C NMR: δ 16.76 (CH_3), 21.2 ($\underline{CH_3}CO$), 29.2 ($CH_2\underline{CH_2}CH_2OH$), 30.4 ($\underline{CH_2}CH_2CH_2OH$), 62.6 ($CH_2OH$), 68.7 (C-5), 69.4 (C-4), 69.9 (C-1), 122.7 (C-3), 134.8 (C-2), 170.7 (C=O). HRMS Calcd for $[M+Na]^+$: m/z 253.1052. Found: m/z 253.1050.

Compound 4b Yellow oil; yield 81%; $[\alpha]_D^{20}$-126.81° (c 0.817, $CHCl_3$); ^{1}H NMR: δ 1.24 (d, *J* = 6.0 Hz, 3H, CH_3), 1.54–1.63 (m, 1H, $\underline{CH}_{2(a)}CH_2$) 1.65–1.75 (m, 3H, $\underline{CH}_{2(b)}\underline{CH_2}$), 2.08 (s, 3H, CH_3CO), 2.42 (s 1H, OH), 3.60 (dq, 1H, *J* = 8.7 Hz and 6.2 Hz, H-5), 3.64 (m, 2H, $\underline{CH_2}OH$), 4.19 (m, 1H, H-1), 5.05 (m, 1H, H-4), 5.70 (ddd, *J* = 10.3 Hz, 2.1 Hz and 2.1 Hz, 1H, H-2), 5.77 (ddd, *J* = 10.3 Hz, 1.6 Hz and 1.6 Hz, 1H, H-3); ^{13}C NMR ($CDCl_3$): δ (ppm) 18.4 (CH_3), 21.07 ($\underline{CH_3}CO$), 28.3 ($CH_2\underline{CH_2}CH_2OH$), 32.0 ($\underline{CH_2}CH_2CH_2OH$), 62.6 ($CH_2OH$), 71.1 (C-5), 72.43 (C-4), 74.5 (C-1), 125.6 (C-3), 132.7 (C-2), 170.5 (C=O). HRMS Calcd for $[M+Na]^+$: m/z 253.1052. Found: m/z 253.1056.

Synthesis of 1-(4-*O*-acetyl-2,3,6-trideoxy-α-L-*erythro*-hex-2-enopyranose)-3-bromopropane (5a) and 1-(4-*O*-acetyl-2,3,6-trideoxy-β-L-*erythro*-hex-2-enopyranose)-3-bromopropane (5b)

A solution of triphenylphosphine (TPP; 289 mg, 1.1 mmol) in DCM (5.0 mL) was stirred at room temperature and treated with bromine (54 μL, 1.05 mmol). After disappearance of the bromine color, a solution of compound **4a** or **4b** (230 mg, 1 mmol) in DCM (4.0 mL) was added in one portion and stirring was continued for 15 min, after which time the sugar hydroxypropyl derivative was consumed completely (TLC). The mixture was concentrated and the residue was chromatographed on a silica gel column. Elution with hexane/acetone (20:1) afforded bromopropane derivative **5a** or **5b**.

Compound 5a Yellow oil; yield 88%; $[\alpha]_D^{20}$-62.20° (c 0.947, $CHCl_3$); ^{1}H NMR: δ 1.24 (d, *J* = 6.6 Hz, 3H, CH_3), 1.62–2.15 (m, 4H, CH_2CH_2), 2.09 (s, 3H, CH_3CO), 3.48 (m, 2H, CH_2Br), 3.90 (dq, *J* = 6.5 Hz and *J* = 4.7 Hz, 1H, H-5), 4.18 (m, 1H, H-1), 4.89 (m, 1H, H-4), 5.79 (ddd, *J* = 10.3 Hz, 3.5 Hz and 2.0 Hz, 1H, H-3), 5.89 (ddd, *J* = 10.3 Hz, 2.1 Hz and 1.1 Hz, 1H, H-2); ^{13}C NMR: δ 16.9 (CH_3), 21.2 ($\underline{CH_3}CO$), 29.0 ($CH_2\underline{CH_2}CH_2Br$), 31.9 ($\underline{CH_2}CH_2CH_2Br$), 33.8 ($CH_2Br$), 68.5 (C-5), 69.4 (C-4), 69.5 (C-1), 123.1 (C-3), 134.7 (C-2), 170.7 (C=O). HRMS Calcd for $[M+Na]^+$: m/z 315.0208. Found: m/z 315.0205.

Compound 5b Yellow oil, yield 92%; $[\alpha]_D^{20}$-112.58° (c 1.272, $CHCl_3$); ^{1}H NMR: δ 1.23 (d, *J* = 6.3 Hz, 3H, CH_3), 1.54–1.81 (m, 2H, $\underline{CH_2}CH_2CH_2Br$), 1.91–2.05 (m, 2H, $CH_2\underline{CH_2}CH_2Br$), 2.08 (s, 3H, CH_3CO), 3.44 (t, 2H, *J* = 6.7 Hz, CH_2Br), 3.56 (dq, 1H *J* = 8.7 Hz and 6.2 Hz, H-5), 4.18 (m, 1H, H-1), 5.03 (m, 1H, H-4), 5.71 (ddd, *J* = 10.4 Hz, 1.7 Hz and 1.7 Hz, 1H, H-2), 5.77 (ddd, *J* = 10.3 Hz, 1.2 Hz and 1.2 Hz, 1H, H-3); ^{13}C NMR: δ 18.4 (CH_3), 21.1 ($\underline{CH_3}CO$), 28.1 ($CH_2\underline{CH_2}CH_2Br$), 33.6 ($\underline{CH_2}CH_2CH_2Br$), 33.9 ($CH_2Br$), 71.1 (C-5), 72.34 (C-4), 74.0 (C-1), 126.0 (C-3), 132.5 (C-2), 170.5 (C=O). HRMS Calcd for $[M+Na]^+$: m/z 315.0208. Found: m/z 315.0204.

Synthesis of 1-(4-*O*-acetyl-2,3,6-trideoxy-α-L-*erythro*-hex-2-enopyranose)-3-azidopropane (6a) and 1-(4-*O*-acetyl-2,3,6-trideoxy-β-L-*erythro*-hex-2-enopyranose)-3-azidopropane (6b)

The 3-bromopropyl C-glycoside **5a** or **5b** (292 mg, 1.0 mmol) was added with stirring to a mixture of DCM and water (10.0 mL each), containing tetra-*n*-butylammonium bromide (64 mg, 0.2 mmol) and sodium azide (260 mg, 4.0 mmol). Stirring was continued at room temperature for another 72 h, after which time the sugar substrate was completely exhausted (TLC in hexane/acetone, 3:1). Then, the lower layer was separated and the aqueous layer was extracted with an additional portion of DCM (10 mL). Combined organic layers were dried with anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was taken up into hexane (20 mL) and the solution was concentrated again to afford pure azide **6a** or **6b** (240 mg, 94% yield).

Compound 6a Yellow oil; yield 240 mg (94%); $[\alpha]_D^{20}$-88.38° (c 0.835, $CHCl_3$); ^{1}H NMR: δ 1.24 (d, *J* = 6.6 Hz, 3H, CH_3), 1.55–1.91 (m, 4H, CH_2CH_2), 2.09 (s, 3H, CH_3CO), 3.34 (t, *J* = 6.5 Hz, 2H, CH_2N_3), 3.91 (dq, *J* = 6.5 Hz and 4.8 Hz, 1H, H-5), 4.17 (m, 1H, H-1), 4.88 (m, 1H, H-4), 5.79 (ddd, *J* = 10.4 Hz, 3.4 Hz and 2.0 Hz, 1H, H-3), 5.89 (ddd, *J* = 10.3 Hz, 2.1 Hz and 1.0 Hz, 1H, H-2); ^{13}C NMR: δ 16.8 (CH_3), 21.1 ($\underline{CH_3}CO$), 25.1 ($CH_2\underline{CH_2}CH_2N_3$), 30.6 ($\underline{CH_2}CH_2CH_2N_3$), 33.8 ($CH_2N_3$), 68.5 (C-5), 69.4 (C-4, C-1), 123.0 (C-3), 133.7 (C-2), 170.7 (C=O). HRMS Calcd for $[M+Na]^+$: m/z 301.1015. Found: m/z 301.1011.

Compound 6b Yellow oil; yield 240 mg (94%); $[\alpha]_D^{20}$-125.96° (c 0.497, $CHCl_3$); ^{1}H NMR: δ 1.23 (d, *J* = 6.2 Hz, 3H, CH_3), 1.52–1.60 (m, 1H, $\underline{CH}_{2(a)}CH_2CH_2N_3$), 1.62–1.78 (m, 3H, $\underline{CH}_{2(b)}\underline{CH_2}CH_2N_3$), 2.08 (s, 3H, CH_3CO), 3.31 (t, *J* = 6.8 Hz, 2H, CH_2N_3), 3.56 (dq, *J* = 8.7 Hz and 6.2 Hz, 1H, H-5), 4.18 (m, 1H, H-1), 5.03 (m, 1H, H-4), 5.71 (ddd, *J* = 10.3 Hz, 1.9 Hz and 1.9 Hz, 1H, H-2), 5.75 (ddd, *J* = 10.3 Hz, 1.5 Hz and 1.5 Hz, 1H, H-3); ^{13}C NMR: δ 18.48 (CH_3 ram.), 21.1 ($\underline{CH_3}CO$), 24.3 ($CH_2\underline{CH_2}CH_2N_3$), 32.2 ($\underline{CH_2}CH_2CH_2N_3$), 51.5 ($CH_2N_3$), 71.2 (C-5), 72.4 (C-4), 74.0 (C-1), 126.0 (C-3), 132.5 (C-2), 170.6 (C=O). HRMS Calcd for $[M+Na]^+$: m/z 301.1015. Found: m/z 301.1012.

7-*O*-propargyl daidzein (8)

Dry 7-*O*-tetra-*n*-butylammonium salt of daidzein (**7**) (248 mg 0.50 mmol) was suspended in DMF (1.0 mL) with stirring and propargyl bromide (60 μL of 80% solution in toluene, 0.50 mmol) was added. The mixture was heated at 45–50°C until a clear solution was obtained (ca. 1 h). The solvent was co-evaporated with toluene on

a rotary evaporator, and the residue was taken up into chloroform (20 mL) and repeatedly washed with cold water. The chloroform solution was dried with anhydrous sodium sulfate, filtered and concentrated. The residue was triturated with toluene (2.0 mL) and left in a refrigerator overnight. The resultant precipitate of **8** was collected by filtration (105 mg, 72% yield) and crystallized from isopropanol for analytical purposes: mp 218–221°C; ^{1}H NMR (DMSO-d_6): 3.68 (t, J = 2.4 Hz, 1H, CH), 5.00 (d, J = 2.1 Hz, 2H, OCH_2), 6.83 (d′, J = 8.7 Hz, 2H, H-3′g, H-5′g), 7.12 (dd, J = 8.9 Hz and 2.4 Hz, 1H, H-6d), 7.22 (d, J = 2.4 Hz, 1H, H-8d), 7.41 (d, J = 8.7 Hz, 2H, H-2′d, H-6′d), 8.06 (d, J = 8.9 Hz, 1H, H-5d), 8.38 (s, 1H, H-2d), 9.58 (s, 1H, 4′-OH); ^{13}C NMR (DMSO-d_6): 56.2 (OCH_2), 78.3 (CCH), 78.9 (CH), 101.7 (C-8d), 114.9 (C-3′d, C-5′d, C-6d), 118.0 (C-4a), 122.2 (C-1′d), 123.7 (C-3d), 127.0 (C-5d), 130.0 (C-2′d, C-6′d), 153.1 (C-2d), 157.0 (C-4′d), 157.2 (C-8a-d), 161.3 (C-7d), 174.61 (C-4d). HRMS Calcd for [M+Na]$^+$: m/z 315.0634. Found: m/z 315.0630.

Synthesis of 7-(1-[3-(4-*O*-acetyl-2,3,6-trideoxy-α-L-*erythro*-hex-2-enopyranose) propyl]-1*H*-1,2,3-triazol-4-yl)methoxy-3-(4-hydroxyphenyl)-4*H*-chromen-4-one (9a) [RamC3TRIAZαDAI], and 7-(1-[3-(4-*O*-acetyl-2,3,6-trideoxy-β-L-*erythro*-hex-2-enopyranose)propane]-1*H*-1,2,3-triazol-4-yl) methoxy-3-(4-hydroxyphenyl)-4*H*-chromen-4-one (9a) [RamC3TRIAZβDAI]

The C-glycosyl azide **6** (140 mg, 0.55 mmol) and isoflavone derivative **8** (160 mg, 0.55 mmol) were dissolved in methanol (4.0 mL) with stirring and the solution was treated with 60 μL of saturated aqueous solution of Cu (II) acetate. The mixture was stirred at room temperature overnight and then concentrated under reduced pressure. The residue was taken up into a minimal amount of DCM and applied onto a silica gel column. Elution with a gradient of DCM/acetone (25:1 → 10:1) gave the triazoles **9a** and **9b.**

Compound 9a This compound was obtained as white needles (toluene); yield 199 mg (75%); mp 145–151°C; $[\alpha]_D^{20}$-41.79° (c 0.67, $CHCl_3$); ^{1}H NMR: δ 1.24 (d, J = 6.6 Hz, 3H, CH_3), 1.55–1.63 (m, 1H, $\underline{CH}_{(a)}CH_2CH_2N$), 1.63–1.71 (m, 1H, $\underline{CH}_{(b)}CH_2CH_2N$), 2.00–2.08 (m, 2H, $CH_2\underline{CH}_2CH_2N$), 2.07 (s, 3H, CH_3CO), 3.93 (dq, J = 6.6 Hz and 4.0 Hz, 1H, H-5), 4.20 (m, 1H, H-1), 4.43 (t, J = 7.2 Hz, 2H, CH_2N), 4.87 (m, 1H, H-4), 5.23 (s, 2H, OCH_2), 5.81 (ddd, J = 10.4 Hz, 3.6 Hz and 1.8 Hz, 1H, H-3), 5.95 (ddd, J = 10.3 Hz, 1.9 Hz and 0.8 Hz, 1H, H-2), 6.87 (d, J = 8.6 Hz, 2H, H-3′d, H-5′d), 6.93 (d, J = 2.4 Hz, 1H, H-8d), 6.98 (dd, J = 8.9 Hz and 2.4 Hz, 1H, H-6d), 7.32 (d, J = 8.7Hz, 2H, H-2′d, H-6′), 7.61 (s, 1H, N-$\underline{CH}$=C) 7.78 (s, 1H, 4′-OH), 7.86 (s, 1H, H-2d), 8.13 (d, 1H, J = 8.9, H-5d); ^{13}C NMR: δ 16.6 (CH_3), 21.21 ($\underline{CH}_3CO$), 26.5 ($CH_2\underline{CH}_2CH_2N$), 30.3 ($\underline{CH}_2CH_2CH_2N$), 50.3 ($CH_2N$), 62.2 ($CH_2O$), 68.9 (C-5), 69.2 (C-4, C-1), 101.2 (C-8d) 115.0 (C-6d), 115.8 (C-3′d, C-5′d), 118.5 (C-4a–d), 123.0 (C-3), 123.2 (C-1′d), 125.1 (C-3d), 127.8 (C-5d, N-$\underline{CH}$=C), 130.2 (C-2′d, C-6′d), 133.6 (C-2), 142.8 (N-CH=$\underline{C}$), 152.4 (C-2d), 156.7 (C-4′d), 157.8 (C-8a–d), 162.5 (C-7d), 170.8 (C=O), 176.2 (C-4d). HRMS Calcd for [M+Na]$^+$: m/z 570.1853. Found: m/z 570,1849.

Compound 9b This compound was obtained as white needles (toluene); yield 178 mg (68%); $[\alpha]_D^{20}$-57.70° (c 0.67, $CHCl_3$); mp 125–135°C; ^{1}H NMR: δ 1.23 (d, 3H, J = 6.0 Hz, CH_3), 1.47–1.56 (m, 1H, $\underline{CH}_{(a)}CH_2CH_2N$), 1.58–1.66 (m, 1H, $\underline{CH}_{(b)}CH_2CH_2N$) 2.00–2.06 (m, 2H, $CH_2\underline{CH}_2CH_2N$), 2.08 (s, 3H, CH_3CO), 3.57 (dq, J = 6.2 Hz and 8.7 Hz, 1H, H-5), 4.20 (m, 1H, H-1), 4.36–4.46 (m, 2H, CH_2N), 5.01–5.05 (m, 1H, H-4), 5.25 (s, 2H, OCH_2), 5.67–5.74 (m, 2H, H-2, H-3), 6.87 (d, J = 8.6 Hz, 2H, H-3′d, H-5′d) 6.95 (d, J = 2.4 Hz, 1H, H-8d), 7.00 (dd, J = 8.9 Hz and 2.4 Hz, 1H, H-6d), 7.32 (d, J = 8.6 Hz, 2H, H-2′d, H-6′), 7.50 (bs, 1H, 4′-OH), 7.64 (s, 1H, N-$\underline{CH}$=C), 7.87 (s, 1H, H-2d), 8.15 (d, 1H, J = 8.9, H-5d); ^{13}C NMR: δ 18.45 (CH_3), 21.1 ($\underline{CH}_3CO$), 25.7 ($CH_2\underline{CH}_2CH_2N$), 31.7 ($\underline{CH}_2CH_2CH_2N$), 50.5 ($CH_2N$), 62.3 ($CH_2O$), 71.1 (C-5), 72.5 (C-4), 73.9 (C-1), 101.3 (C-8d), 115.1 (C-6d), 115.8 (C-3′d, C-5′d), 118.6 (C-4a′d), 123.2 (C-1′d), 123.4 (N-$\underline{CH}$=C), 125.1 (C-3d), 126.4 (C-3), 127.8 (C-5d), 130.3 (C-2′d, C-6′d), 132.6 (C-2), 142.8 (N-CH=$\underline{C}$), 152.4 (C-2d), 156.1 (C-4′d), 157.8 (C-8a–d), 162.5 (C-7d), 170.6 (C=O), 176.2 (C-4). HRMS Calcd for [M+Na]$^+$: m/z 570.1853. Found: m/z 570.1850.

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