



Efficient synthesis of ethisterone glycoconjugate via bis-triazole linkage



Kunj B. Mishra, Bhuwan B. Mishra, Vinod K. Tiwari *

Department of Chemistry, Centre of Advanced Study, Faculty of Science, Banaras Hindu University, Varanasi 5, India

ARTICLE INFO

Article history:

Received 4 June 2014

Received in revised form 30 August 2014

Accepted 5 September 2014

Available online 16 September 2014

Keywords:

Click chemistry

Carbohydrates

Steroids

Glycoconjugates

ABSTRACT

Synthesis of sugar based triazolyl azido-alcohols was accomplished via one pot click reaction of glycosyl alkynes with epichlorohydrin in aqueous medium. All the developed triazolyl azido-alcohols were further utilized for the synthesis of bis-triazolyl ethisterone glycoconjugates using CuAAC reaction. The developed triazole-linked ethisterone glycoconjugates would be crucial in androgen receptor pharmacology and chemical biology.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Carbohydrates covalently linked with proteins, peptides, lipids, saccharides etc. have lately attracted considerable interest due to involvement in complex biological processes such as catalysis and highly selective molecular recognition.¹ They are crucial to cellular recognition events, including signal transduction,² cell adhesion and inflammation,³ immune response,⁴ tumor metastasis,⁵ and viral & bacterial infections.^{6,7} Many microbes, including viruses, bacteria, and their toxins, have evolved to bind cell surface carbohydrates, a binding that is a prerequisite for infection to occur.⁸ The multivalent nature of these molecules is frequently used to increase the affinities to the targets in different biological processes, such as the binding of bacteria, bacterial toxins, galectins, and other lectins.⁹ The several chiral hydroxyl groups in carbohydrates, upon appropriate modifications and utilization, would stimulate chemical diversities, that is, glycoconjugates/glycohybrids are of great significance in drug discovery and development.^{10,11}

Steroids, due to their rigid framework, broad spectrum activity, and ability of binding to the specific hormonal receptors, have become preferred chiral synthons for the development of diverse bioconjugates.¹² Many steroidal framework conjugates are known to literature, for example, steroid–polyamine conjugates, steroid–anthraquinone hybrids, steroid–carbohydrate conjugates etc.¹³ The medicinal applications of these are based on the fact that several new conjugates arising through such bioconjugation have

been found to exhibit unusual biological properties and activities as the different molecular segments act cooperatively. In literature reports, ethisterone is known to compete for androgen receptor (AR) binding, and suppresses the levels of AR transcriptional activation relative to dihydrotestosterone (DHT).¹⁴ Earlier, Kirshenbaum group reported the conjugation of ethisterone to peptoid side chains using copper-catalyzed azide-alkyne cycloaddition (CuAAC) to afford a family of multivalent conjugates that exhibit potent anti-proliferative activity in LNCaP-abl cells, a model of therapy-resistant prostate cancer.^{15,16} Thus, the development of numerous triazole-linked ethisterone glycoconjugates would be crucial in AR pharmacology and chemical biology.

The interest on conjugating lipophilic scaffolds like steroids to sugars also derives from the recognized capability of the resulting amphipathic hybrids to interact with phospholipid membranes and liposomes.¹⁷ Numerous facially amphiphilic steroid-disaccharide hybrids have proven success on the solubilization and stabilization of membrane proteins, thus opened up new perspectives for the extensive manipulation and characterization of membrane proteins.¹⁸ Alternatively, the growing development of 'click chemistry' has also have an impact on the development of novel sugar/steroid hybrid architectures. The triazole unit in these is more than just a passive linker; it readily associates with biological targets through hydrogen bonding and dipole interactions, and shows interesting biological properties such as anti-allergic, anti-bacterial, and anti-HIV activities. Numerous lipophilic steroids have been ligated to sugars by the CuAAC reaction, thus producing amphipathic hybrids useful to biodynamic applications.^{19,20} Thus, considering the growing importance of sugar/steroid hybrids in

* Corresponding author. Tel.: +91 542 6702466; fax: +91 542 2368174.

E-mail address: tiwari_chem@yahoo.co.in (V.K. Tiwari).

drug discovery and biological chemistry, we were prompted to pursue a novel carbohydrate-steroid conjugation approach alternative to the traditional glycosylation and capable to produce unique types of molecular chimeras of value to androgen receptor (AR) pharmacology and chemical biology.

2. Results and Discussion

Our synthetic strategy begins with cheap and readily available carbohydrates (D-glucose, D-galactose, D-mannose, D-ribose, and D-xylose), which after processing through a number of high-yielding protection, and modification steps afforded sugar based terminal alkynes **1**.²¹ In continuation to our recent report on the synthesis of diverse triazolyl azido alcohols **2** from terminal alkynes via oxirane ring opening of epichlorohydrin followed by CuAAC reaction with alkynes, and their utilization in the synthesis of morpholine fused triazoles,²² this time we disclose our results on click reaction of **2** with a naturally occurring steroid alkyne ethisterone **3** to afford bis-triazolyl ethisterone glycoconjugates **4** regioselectively in good yields (Scheme 1).

Earlier, several reports have established dichloromethane (DCM) as a solvent of choice for CuAAC click transformations, hence the click reaction of compound **2a** (0.33 mmol) with **3** (0.33 mmol) in the presence of CuI (5 mol%) and *N,N*-diisopropylethylamine (DIPEA, 0.33 mmol) was carried out in anhydrous DCM under inert atmosphere at rt to afford bis-triazolyl ethisterone glycoconjugate **4a** regioselectively in 85% yield.

The formation of compound **4a** was further confirmed by comparison of ¹H NMR spectrum with compound **2a** (Fig. 1). In 300 MHz ¹H NMR spectrum, the signal for characteristic triazole-*H* proton resonated at δ 7.74. The carbon resonances observed at δ 144.9 and δ 124.2 in ¹³C NMR spectrum were attributed for the triazole ring in compound **2a**. In 300 MHz ¹H NMR spectrum of **4a**, both the triazolyl protons resonated as singlets one proton each at δ 7.79 and δ 7.56. The anomeric proton of furanose sugar (H-1) appeared as doublet at δ 5.85 (*J* = 3.3 Hz). A singlet observed at δ 5.67 was identified for the methyne resonance of ethisterone while a two proton multiplet observed in the range between δ 4.81 and 4.71 was established for corresponding oxymethylene (–OCH₂–) resonance in compound **4a**. A multiplet integrated to four protons resonated between δ 4.58 and 4.48 was attributed to triazolyl methylene, >CH–OH and furanosyl sugar protons H-2 & H-4. Likewise, a four proton multiplet observed between δ 4.11 and 3.95 was established for sugar protons H-5, H-3, and H-6. The rest of steroid protons in addition to twelve protons of isopropylidene moiety resonated between δ 2.41 and 0.43 ppm.

In the ¹³C NMR spectrum of compound **4a**, a carbon resonance signal observed at δ 199.5 was evidenced for carbonyl carbon of ethisterone while the anomeric carbon of furanose sugar resonated

at δ 105.1. Thus, all the developed triazolyl azido-alcohols **2a–i** were reacted readily with **3** in the presence of CuI and DIPEA in anhydrous DCM under argon atmosphere at rt to afford respective bis-triazolyl ethisterone glycoconjugates **4a–i** in good yields (Table 1). Using spectral studies (FTIR, NMR, and HRMS) the structures of compounds **4a–i** were elucidated.

The targeted compounds **4a** were also synthesized successfully by another pathway outlined in Scheme 2, where ethisterone linked triazolyl azido-alcohol **2j** was synthesized from **3** under one-pot method using CuAAC reaction. However, the synthesis of precursor compound **2j** via this route took longer reaction time (15 h) giving products in low yields after consuming excess of compound **3**. A subsequent click reaction of **2j** with compound **1a** in the presence of CuI and DIPEA using DCM as a reaction medium furnished compound **4a** in good yields.

In conclusion, a number of triazole containing glycosyl azido-alcohols were prepared by multicomponent click reaction of glycosyl alkynes with epichlorohydrin and NaN₃ in the presence of CuSO₄·5H₂O/NaAsc. These azido-alcohols were further subjected to Cu-catalyzed click reaction with ethisterone, a naturally occurring steroid alkyne to afford rare bis-triazolyl ethisterone glycoconjugates for potential application in androgen receptor (AR) pharmacology and chemical biology.

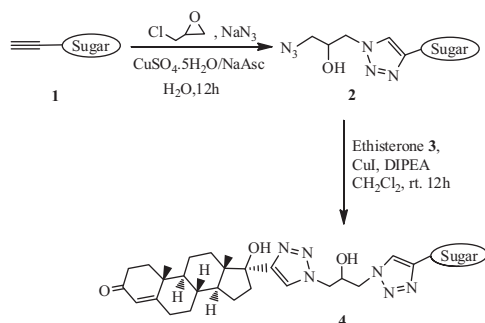
3. Experimental

3.1. General methods

All of the reactions were carried out using anhydrous solvents under an argon atmosphere in one-hour oven-dried glassware at 100 °C. For the reactions in aqueous condition, normal water was used. All reagents and solvents were of pure analytical grade. Thin-layer chromatography (TLC) was performed on 60 F₂₅₄ silica gel, pre-coated on aluminum plates, and revealed with either a UV lamp (λ_{max} = 254 nm) or a specific color reagent (iodine vapors) or by spraying with methanolic H₂SO₄ solution and subsequent charring by heating at 100 °C. ¹H and ¹³C NMR were recorded at 300 and 75 MHz, respectively. Chemical shifts were given in ppm downfield from internal TMS; *J* values in Hz. Elemental analysis was performed using a C, H, N analyzer and results were found to be within $\pm 0.4\%$ of the calculated values. Mass spectra were recorded using electrospray ionization mass spectrometry (ESI-MS). HR-MS were recorded using TOF MS ES+ 1.74e3. Infrared spectra were recorded as Nujol mulls in KBr palates.

3.1.1. 3-Azido-1-(4-(methyl-2,3,4-tri-O-benzyl- α -D-galucopyranose-5-yl)oxy)methyl]-1H-1,2,3-triazol-1-yl propan-2-ol (**2i**)

In a mixture of epichlorohydrin (2.0 mmol) and methyl-2,3,4-tri-O-benzyl-5-O-(prop-2-ynyl)- α -D-glucopyranose²³ (1.0 mmol), a solution of NaN₃ (4.0 mmol), CuSO₄·5H₂O (0.1 mmol), and sodium ascorbate (0.2 mmol) in water was added. The resulting solution was stirred for 10–12 h at room temperature. After consumption of starting material (monitored by TLC), the reaction mixture was extracted with ethyl acetate (3 $\times$ 15 mL), combined organic layers were dried over anhydrous Na₂SO₄, and concentrated in vacuo. The resulting residue was purified by flash chromatography (SiO₂) using gradient mixtures of *n*-hexane/ethyl acetate (3:7) as eluent to afford **2i** (0.425 g, 50%) as a viscous liquid; IR (KBr) cm^{−1}: 3402, 2920, 2104, 1715, 1445, 1075; MS: *m/z* 667 [M+Na]⁺; ¹H NMR (300 MHz, CDCl₃): δ 7.56 (s, 1H), 7.33–7.21 (m, 15H), 4.98–4.21 (m, 11H), 4.00–3.94 (m, 2H), 3.80–3.71 (m, 3H), 3.57–3.46 (m, 2H), 3.37–3.21 (m, 5H); ¹³C NMR (75 MHz, CDCl₃): δ 144.9, 138.6, 138.2, 138.1, 128.4, 128.1, 127.9, 127.9, 127.7, 124.1, 98.2, 81.9, 79.7, 75.7, 74.8, 73.3, 69.9, 69.2, 64.7, 55.1, 53.7, 52.9.



Scheme 1. Synthesis of bis-triazolyl ethisterone glycoconjugates **4** from triazolyl azido alcohols **2**.

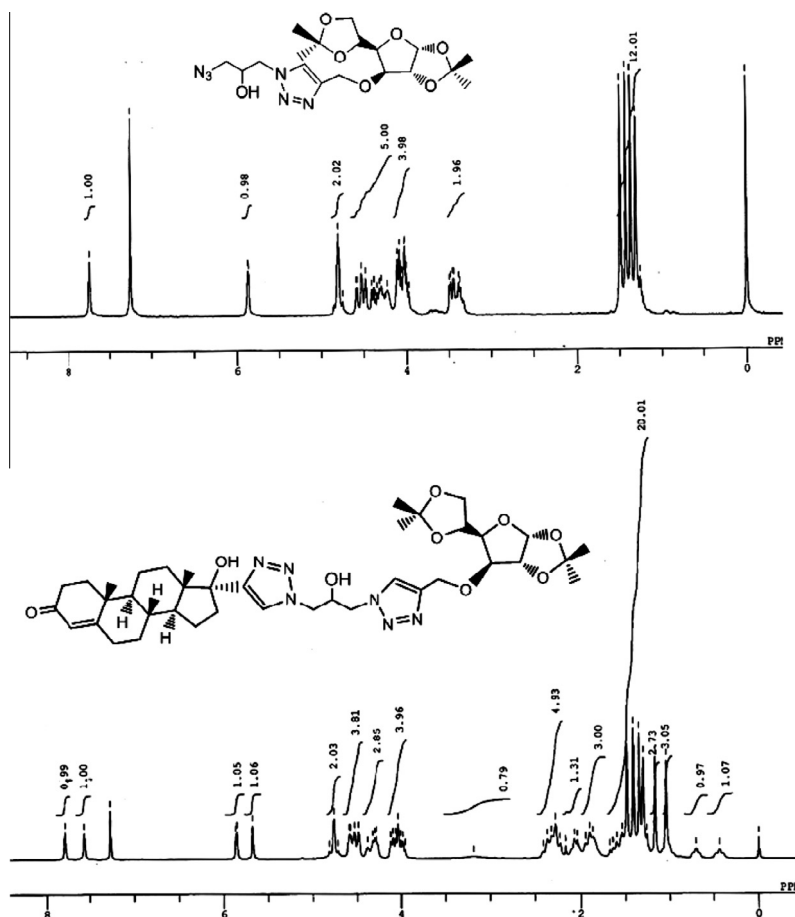


Figure 1. Comparison between ^1H NMR spectra of compounds **2a** and **4a**.

3.1.2. General procedure for the synthesis of bis-triazolyl glycoconjugate ethisterone

3.1.2.1. Bis-triazolyl ethisterone glycoconjugate (Table 1, entry 1). In a stirring solution of compound **2a** (0.134 g, 0.3 mmol) and ethisterone (0.103 g, 0.33 mmol) in dry DCM (5 mL), were added DIPEA (0.056 mL, 0.3 mmol) and CuI (0.028 g, 0.15 mmol). The reaction mixture was allowed to stir for 10–12 h under inert atmosphere at room temperature. After completion of reaction (monitored by TLC), the reaction mixture was extracted with dichloromethane (3×15 mL), combined organic layers were dried over anhydrous Na_2SO_4 , and concentrated in vacuo. The resulting residue was purified by flash chromatography (SiO_2) using chloroform/methanol (9:1) as eluent to afford compound **4a** (0.192 g, 85%) as white solid; mp = 110–112 °C; IR (KBr) cm^{-1} : 3432, 2983, 2940, 1737, 1662, 1374, 1281, 1074; ^1H NMR (300 MHz, CDCl_3): δ 7.79 (s, 1H), 7.56 (s, 1H), 5.85 (d, $J = 3.3$ Hz, 1H), 5.67 (s, 1H), 4.81–4.71 (m, 2H), 4.58–4.48 (m, 4H), 4.38–4.27 (m, 3H), 4.11–3.95 (m, 4H), 3.18 (broad s, 1H), 2.41–2.23 (m, 5H), 2.17–2.04 (m, 1H), 1.94–1.86 (m, 3H), 1.67–1.25 (m, 20H), 1.16 (s, 3H), 1.04 (s, 3H), 0.70 (m, 1H), 0.43 (m, 1H); ^{13}C NMR (75 MHz, CDCl_3): δ 199.5, 171.3, 152.3, 144.7, 124.5, 123.7, 111.8, 109.1, 105.1, 82.5, 82.0, 81.8, 80.9, 72.3, 68.7, 67.2, 63.8, 53.0, 52.9, 48.8, 46.7, 38.5, 37.5, 36.1, 35.5, 33.8, 32.7, 32.6, 31.5, 26.8, 26.7, 26.2, 25.4, 23.6, 20.5, 17.3, 14.1; HRMS: Calcd for $\text{C}_{39}\text{H}_{56}\text{N}_6\text{O}_9$ $[\text{M}+\text{Na}]^+$ 775.4006; found 775.4006.

3.1.2.2. Bistriazolyl ethisterone glycoconjugate (Table 1, entry 2). Compound **2b** (0.120 g, 0.26 mmol), ethisterone (0.081 g, 0.26 mmol), DIPEA (0.044 g, 0.26 mmol), and CuI (0.018 g, 0.13 mmol) were reacted in dry DCM (5 mL) to afford **4b** (0.177 g,

88%) after flash column chromatography (SiO_2) using chloroform/methanol (9:1) as eluent. White solid; mp = 72–74 °C; IR (KBr) cm^{-1} : 3325, 3156, 2988, 2924, 2840, 1670, 1440, 1350, 1256, 1080; ^1H NMR (300 MHz, CDCl_3): δ 7.63 (s, 1H), 7.55 (s, 1H), 7.30 (m, 5H), 5.87 (d, $J = 3.3$ Hz, 1H), 5.66 (s, 1H), 4.74–4.19 (m, 11H), 4.00 (d, $J = 2.7$ Hz, 1H), 3.74–3.65 (m, 2H), 2.36–1.62 (m, 12H), 1.47–1.37 (m, 5H), 1.29–1.15 (m, 9H), 1.03–0.85 (m, 4H), 0.69 (m, 1H), 0.44 (m, 1H); ^{13}C NMR (75 MHz, CDCl_3): δ 199.6, 171.4, 153.3, 144.4, 137.8, 128.4, 127.7, 124.4, 123.7, 123.3, 111.7, 104.8, 82.2, 81.9, 81.7, 78.6, 73.3, 68.5, 67.1, 63.4, 53.1, 52.8, 48.7, 46.7, 38.5, 37.6, 36.1, 35.4, 33.8, 32.7, 32.6, 31.5, 26.7, 26.1, 23.6, 20.5, 17.3, 14.1; MS: m/z 773 $[\text{M}+\text{H}]^+$. Anal. Calcd for $\text{C}_{42}\text{H}_{56}\text{N}_6\text{O}_8$: C, 65.25; H, 7.31; N, 10.88. Found: C, 65.62; H, 7.02; N, 11.19.

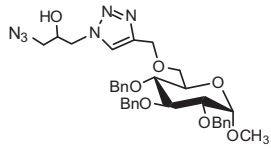
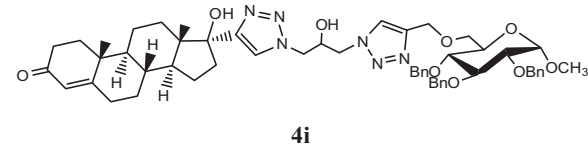
3.1.2.3. Bis-triazolyl ethisterone glycoconjugate (Table 1, entry 3). Compound **2c** (0.091 g, 0.2 mmol), ethisterone (0.61 g, 0.2 mmol), DIPEA (0.033 mL, 0.2 mmol), and CuI (0.018 g, 0.01 mmol) were reacted in dry DCM (5 mL) to afford **4c** (0.144 g, 95%) after flash column chromatography (SiO_2) using chloroform/methanol (9:1) as eluent. White solid; mp = 90–92 °C; IR (KBr) cm^{-1} : 3417, 3145, 2937, 2870, 1666, 1454, 1375, 1073; ^1H NMR (300 MHz, CDCl_3): δ 7.68 (s, 1H), 7.58 (s, 1H), 7.33–7.29 (m, 5H), 5.91 (d, $J = 3.6$ Hz, 1H), 5.67 (s, 1H), 4.67–4.26 (m, 11H), 3.94 (d, $J = 2.4$ Hz, 1H), 3.77–3.76 (m, 2H), 2.36–2.22 (m, 6H), 2.06–1.89 (m, 6H), 1.58–1.25 (m, 12H), 1.15 (s, 3H), 1.03 (s, 3H), 0.69 (m, 1H), 0.44 (m, 1H); ^{13}C NMR (75 MHz, CDCl_3): δ 199.6, 171.4, 153.3, 144.5, 137.2, 128.4, 127.9, 127.6, 124.6, 123.6, 111.6, 105.0, 82.0, 81.9, 81.7, 79.1, 71.8, 68.5, 68.4, 64.5, 53.0, 52.9, 48.7, 46.7, 38.5, 37.4, 36.1, 35.4, 33.8, 32.7, 32.6, 31.4, 26.6, 26.1,

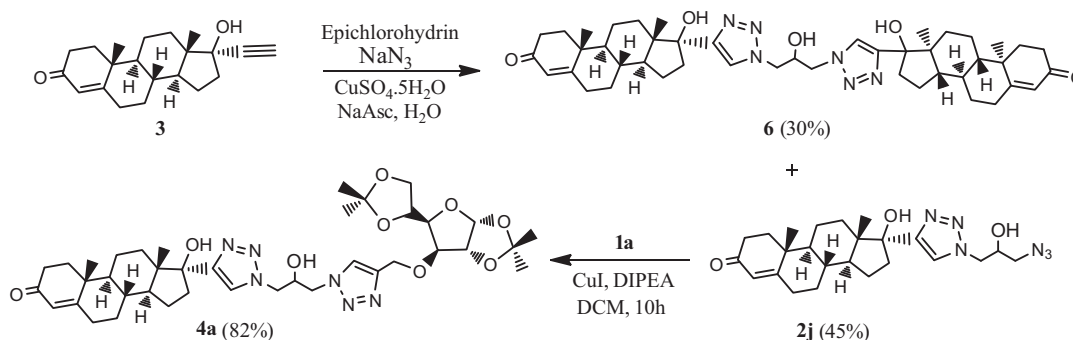
Table 1
Synthesis of bis-triazolyl ethisterone glycoconjugates **4a–i**

Entry ^a	Azide ^b	Click product ^c	Time ^d	Yield ^e
1			12	85
2			10	88
3			12	95
4			10	92
5			11	82
6			12	86
7			10	88
8			12	88

(continued on next page)

Table 1 (continued)

Entry ^a	Azide ^b	Click product ^c	Time ^d	Yield ^e
9	 2i	 4i	12	80

^a Molar ratios: sugar azide (1.0 equiv), ethisterone (1.0 equiv), CuI (0.5 equiv), and DIPEA (1 equiv).^b Sugar based triazolyl azido-alcohols.^c Bis-triazolyl ethisterone glycoconjugate.^d Time in hours.^e Isolated yield.Scheme 2. Synthesis of bistriazolyl ethisterone glycoconjugate **4a** via ethisterone azido-alcohol **2j**.

23.6, 20.5, 17.3, 14.2; HRMS: Calcd for $C_{42}H_{56}N_6O_8$ [M+Na] 795.4057; found 795.4052.

3.1.2.4. Bis-triazolyl ethisterone glycoconjugate (Table 1, entry 4).

Compound **2d** (0.265 g, 0.7 mmol), ethisterone (0.24 g, 0.7 mmol), DIPEA (0.129 mL, 0.7 mmol), and CuI (0.065 g, 0.3 mmol) were reacted in dry DCM (10 mL) to afford **4d** (0.441 g, 92%) after flash column chromatography (SiO_2) using chloroform/methanol (9:1) as eluent. White solid; mp = 132–134 °C; IR (KBr) cm^{-1} : 3426, 3135, 2976, 2920, 1666, 1465, 1371, 1093; 1H NMR (300 MHz, $CDCl_3$): δ 7.73 (s, 1H), 7.59 (s, 1H), 5.67 (s, 1H), 4.94 (s, 1H), 4.64–4.48 (m, 7H), 4.36–4.26 (m, 3H), 3.60–3.48 (m, 2H), 3.29 (s, 3H), 2.41–1.86 (m, 12H), 1.63–1.25 (m, 12H), 1.16 (s, 3H), 1.04 (s, 3H), 0.73–0.65 (m, 1H), 0.48–0.40 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 199.5, 171.3, 153.3, 144.7, 123.7, 112.4, 109.2, 85.0, 84.9, 81.9, 81.9, 71.6, 68.6, 64.5, 54.8, 53.1, 48.7, 46.7, 38.5, 37.6, 36.1, 35.5, 33.8, 32.7, 32.6, 31.5, 26.4, 24.9, 23.6, 20.5, 17.3, 14.2; HRMS: Calcd for $C_{36}H_{52}N_6O_8$ [M+Na] 719.3744; found 719.3748.

3.1.2.5. Bis-triazolyl ethisterone glycoconjugate (Table 1, entry 5).

Compound **2e** (0.069 g, 0.13 mmol), ethisterone (0.041 g, 0.13 mmol), DIPEA (0.022 mL, 0.13 mmol), and CuI (0.012 g, 0.06 mmol) were reacted in dry DCM (5 mL) to afford **4e** (0.093 g, 86%) after flash column chromatography (SiO_2) using chloroform/methanol (9:1) as eluent. White solid; mp = 68–70 °C; IR (KBr) cm^{-1} : 3406, 2922, 2851, 1662, 1454, 1231, 1058; 1H NMR (300 MHz, $CDCl_3$): δ 7.64 (s, 1H), 7.53 (s, 1H), 7.31–7.29 (m, 10H), 5.67 (s, 1H), 4.87 (s, 1H), 4.62–4.28 (m, 12H), 4.04–4.00 (m, 2H), 3.75–3.66 (m, 2H), 3.38 (s, 3H), 2.36–2.28 (m, 6H), 2.04–1.1.89 (m, 6H), 1.59–1.41 (m, 3H), 1.33–1.25 (m, 3H), 1.15 (s, 3H), 1.03 (s, 3H), 0.69 (m, 1H), 0.42 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 199.5, 171.2, 153.4, 144.5, 137.6, 128.4, 128.3, 127.8, 127.6, 124.5, 123.7, 107.7, 86.9, 81.2, 79.9, 73.4, 72.3, 69.6, 68.6,

63.2, 55.6, 53.1, 52.9, 48.8, 44.9, 38.5, 37.6, 36.1, 35.5, 33.8, 32.7, 32.6, 31.5, 23.6, 20.5, 17.3, 14.1; HRMS: Calcd for $C_{47}H_{60}N_6O_8$ [M+Na] 859.4370; found 859.4369.

3.1.2.6. Bis-triazolyl ethisterone glycoconjugate (Table 1, entry 6).

Compound **2f** (0.138 g, 0.26 mmol), ethisterone (0.082 g, 0.26 mmol), DIPEA (0.044 mL, 0.26 mmol), and CuI (0.024 g, 0.13 mmol) were reacted in dry DCM (10 mL) to afford **4f** (0.180 g, 82%) after flash column chromatography (SiO_2) using chloroform/methanol (9:1) as eluent. Viscous liquid; IR (KBr) cm^{-1} : 3403, 3260, 3030, 2923, 2853, 1731, 1659, 1496, 1454, 1210, 1070; 1H NMR (300 MHz, $CDCl_3$): δ 7.68 (s, 1H), 7.53 (s, 1H), 7.30–7.25 (m, 10H), 5.66 (s, 1H), 4.94 (d, J = 4.2 Hz, 1H), 4.74–4.08 (m, 14H), 3.71–3.56 (m, 2H), 3.39 (s, 3H), 2.35–1.88 (m, 12H), 1.58–1.48 (m, 3H), 1.41–1.25 (m, 3H), 1.15 (s, 3H), 1.03 (s, 3H), 0.68 (m, 1H), 0.42 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 199.5, 171.2, 153.8, 144.6, 138.0, 137.9, 128.3, 127.7, 127.5, 124.7, 123.7, 100.2, 83.9, 81.3, 81.2, 75.9, 73.4, 72.6, 69.2, 68.5, 63.7, 55.2, 53.0, 52.9, 48.8, 46.7, 38.5, 37.6, 36.1, 35.5, 33.8, 32.7, 32.6, 31.4, 23.6, 20.5, 17.3, 14.1; HRMS: Calcd for $C_{47}H_{60}N_6O_8$ [M+Na] 859.4370; found 859.4374.

3.1.2.7. Bis-triazolyl ethisterone glycoconjugate (Table 1, entry 7).

Compound **2g** (0.125 g, 0.28 mmol), ethisterone (0.80 g, 0.28 mmol), DIPEA (0.047 mL, 0.28 mmol), and CuI (0.026 g, 0.13 mmol) were reacted in dry DCM (10 mL) to afford **4g** (0.185 g, 88%) after flash column chromatography (SiO_2) using chloroform/methanol (9:1) as eluent. White solid; mp = 115–117 °C; IR (KBr) cm^{-1} : 3444, 2975, 2936, 1745, 1668, 1365, 1283, 1068; 1H NMR (300 MHz, $CDCl_3$): δ 7.77 (s, 1H), 7.62 (s, 1H), 5.60 (s, 1H), 5.08 (s, 1H), 4.75–4.48 (m, 7H), 4.36 (m, 2H), 4.07–3.93 (m, 4H), 2.27–1.89 (m, 12H), 1.59–1.35 (m, 7H), 1.30–1.25 (12H), 1.16–0.87 (m, 5H), 0.68 (m, 1H), 0.48 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 199.5, 171.5, 153.3, 144.0, 124.7, 123.4, 112.5, 109.0,

105.8, 84.8, 81.8, 80.2, 79.2, 72.9, 68.5, 66.5, 60.2, 53.0, 48.7, 46.6, 38.4, 37.4, 36.0, 35.3, 33.7, 32.5, 31.4, 26.6, 25.6, 24.9, 24.3, 23.6, 20.4, 17.1, 14.0; MS: m/z 753 $[M+H]^+$; Anal. Calcd for $C_{39}H_{56}N_6O_9$: C, 62.20; H, 7.50; N, 11.17. Found: C, 61.75; H, 7.12; N, 11.40.

3.1.2.8. Bis-triazolyl ethisterone glycoconjugate (Table 1, entry 8).

Compound **2h** (0.216 g, 0.5 mmol), ethisterone (0.153 g, 0.5 mmol), DIPEA (0.083 mL, 0.5 mmol), and CuI (0.046 g, 0.24 mmol) were reacted in dry DCM (10 mL) to afford **4h** (0.326 g, 88%) after flash column chromatography (SiO_2) using chloroform/methanol (9:1) as eluent. White solid; mp = 102–104 °C; IR (KBr) cm^{-1} : 3417, 3146, 2979, 2938, 1667, 1454, 1382, 1256, 1069; 1H NMR (300 MHz, $CDCl_3$): δ 7.74 (s, 1H), 7.59 (s, 1H), 5.67 (s, 1H), 5.52 (d, J = 4.5 Hz, 1H), 4.64–4.47 (m, 6H), 4.31–4.21 (m, 4H), 3.98 (s, 1H), 3.71–3.68 (m, 2H), 2.37–2.23 (m, 5H), 2.07–2.04 (m, 3H), 1.94–1.85 (m, 3H), 1.63–1.25 (m, 18H), 1.16 (s, 3H), 1.04 (s, 3H), 0.63 (m, 1H), 0.46 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 199.6, 171.5, 153.3, 144.7, 124.7, 123.6, 109.2, 108.5, 96.2, 81.9, 71.0, 70.5, 70.3, 69.7, 68.5, 66.8, 64.4, 53.0, 53.0, 48.7, 46.8, 38.5, 37.4, 36.1, 35.4, 33.8, 32.7, 32.5, 31.4, 25.9, 25.9, 24.8, 24.3, 23.6, 20.7, 17.3, 14.1; HRMS: Calcd for $C_{39}H_{56}N_6O_9$ $[M+Na]$ 775.4006; found 775.4003.

3.1.2.9. Bis-triazolyl ethisterone glycoconjugate (Table 1, entry 9).

Compound **2i** (0.170 g, 0.26 mmol), ethisterone (0.081 g, 0.26 mmol), DIPEA (0.044 mL, 0.26 mmol), and CuI (0.024 g, 0.13 mmol) were reacted in dry DCM (10 mL) to afford **4i** (201 mg, 80%) after flash column chromatography (SiO_2) using chloroform/methanol (9:1) as eluent. White solid; mp = 76–78 °C; IR (KBr) cm^{-1} : 3407, 3144, 3030, 2923, 2853, 1711, 1667, 1454, 1361, 1211, 1070; 1H NMR (300 MHz, $CDCl_3$): δ 7.61 (s, 1H), 7.50 (s, 1H), 7.31–7.21 (m, 15H), 5.67 (s, 1H), 5.01–4.75 (m, 5H), 4.66–4.59 (m, 3H), 4.52–4.38 (m, 4H), 4.26 (m, 2H), 3.96 (t, J = 9.0 Hz, 1H), 3.75–3.71 (m, 3H), 3.58–3.52 (m, 2H), 3.36 (s, 3H), 2.36–2.28 (m, 6H), 2.05–1.84 (m, 6H), 1.58–1.37 (m, 4H), 1.15–1.02 (m, 4H), 0.87–0.85 (m, 2H), 0.69 (m, 1H), 0.49 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 199.4, 171.1, 153.3, 144.8, 138.6, 138.4, 138.0, 128.4, 128.3, 128.0, 127.9, 127.8, 127.7, 126.5, 124.4, 123.7, 104.3, 98.1, 89.9, 89.7, 87.9, 86.9, 81.8, 79.7, 75.7, 74.8, 73.3, 69.9, 69.0, 55.2, 53.1, 52.8, 48.8, 42.5, 38.5, 35.6, 35.5, 33.7, 32.6, 31.8, 31.5, 23.6, 20.5, 17.3, 14.1; HRMS: Calcd for $C_{55}H_{68}N_6O_9$ $[M+Na]$ 979.4945; found 979.4945.

Acknowledgements

Authors gratefully acknowledge the Council of Scientific & Industrial Research (CSIR), India for the funding (Grant No. 02(0173)/13/EMR-II). KBM thanks UGC, New Delhi for J.R.F., and BBM thanks the Department of Science and Technology (DST) for Fast Track Young Scientist award (SB/FT/CS-067/2012). Authors sincerely thanks CISC, Department of Chemistry, Banaras Hindu University and Indian Institute of Science, Bangalore for providing spectroscopic data of synthesized compounds.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.carres.2014.09.001>.

References

- (a) Varki, A. *Nature* **2007**, 446, 1023–1029; (b) Varki, A. *Glycobiology* **1993**, 3, 97–130; (c) Dwek, R. A. *Chem. Rev.* **1996**, 96, 683–720; (d) Cummings, R. D.; Liu, F. T. Galectins. In *Essentials of Glycobiology*; Varki, A., Cummings, R. D., Esko, J. D., Freeze, H. H., Stanley, P., Bertozzi, C. R., Hart, G. W., Etzler, M. D., Eds., 2nd ed.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor (NY), 2009. Chapter 33; (e) Tiwari, V. K.; Mishra, R. C.; Sharma, A.; Tripathi, R. P. *Mini-Rev. Med. Chem.* **2012**, 12, 1497–1519; (f) Kushwaha, D.; Dwivedi, P.; Kuanar, S. K.; Tiwari, V. K. *Curr. Org. Synth.* **2013**, 10, 90–135; (g) Cao, H.; Jeol, H.; Chen, X. Carbohydrate-Containing Natural Products in Medicinal Chemistry. In *Opportunity, Challenges and Scope of Natural Products in Medicinal Chemistry*; Tiwari, V. K., Mishra, B. B., Eds.; Research Signpost Publications, 2011; pp 411–431.
- Haltiwanger, R. S. *Curr. Opin. Struct. Biol.* **2002**, 12, 593–598.
- Kitajima, K.; Sato, C. The Roles of Carbohydrate Binding in Cell Adhesion and Inflammation. In *Carbohydrate Recognition: Biological Problems, Methods, and Applications*; Wang, B., Boons, G.-J., Eds.; John Wiley & Sons: Hoboken, NJ, USA, 2011; pp 33–63.
- Cobb, B. A.; Kasper, D. L. *Eur. J. Immunol.* **2005**, 35, 352–356.
- Kannagi, R.; Izawa, M.; Koike, T.; Miyazaki, K.; Kimura, N. *Cancer Sci.* **2004**, 95, 377–384.
- Air, G. M. The Role of Carbohydrates in Viral Infections. In *Carbohydrate Recognition: Biological Problems, Methods, and Applications*; Wang, B., Boons, G.-J., Eds.; John Wiley & Sons: Hoboken, NJ, USA, 2011; pp 65–91.
- Backhed, F.; Ley, R. E.; Sonnenburg, J. L.; Peterson, D. A.; Gordon, J. I. *Science* **2005**, 307, 1915–1920.
- Holgersson, J.; Gustafsson, A.; Breimer, M. E. *Immunol. Cell Biol.* **2005**, 83, 694–708.
- (a) Dam, T. K.; Brewer, C. F. *Glycobiology* **2010**, 20, 270–279; (b) Imberty, A.; Mitchell, E. P.; Wimmerova, M. *Curr. Opin. Struct. Biol.* **2005**, 15, 525–534.
- Lindhorst, T. K.; Dubber, M.; Krallmann-Wenzel, U.; Ehlers, S. *Eur. J. Org. Chem.* **2000**, 2027–2034.
- Joosten, J. A. F.; Loimaranta, V.; Appeldoorn, C. C. M.; Haataja, S.; Ait El Maate, F.; Liskamp, R. M. J.; Finne, J.; Pieters, R. J. *J. Med. Chem.* **2004**, 47, 6499–6508.
- (a) Shah, S. A. A.; Sultan, S.; Hassan, N. B.; Muhammad, F. K. B.; Faridz, M. A. B. M.; Hussain, F. B. M.; Hussain, M.; Adnan, H. S. *Steroids* **2013**, 78, 1312–1324; (b) Novak, I.; Kovac, B. *Biophys. Chem.* **1999**, 78, 233–240; (c) Mishra, B. B.; Tiwari, V. K. *Eur. J. Med. Chem.* **2011**, 46, 4769–4807.
- (a) Chitkul, K.; Arash, B.; Bradley, M. *Tetrahedron Lett.* **2001**, 42, 6211–6214; (b) De Riccardis, F.; Izzo, I.; Di Filippo, M.; Sodano, G.; D'Acquisto, F.; Carnuccio, R. *Tetrahedron* **1997**, 53, 10871–10882; (c) Gargiulo, D.; Blizzard, T. A.; Nakanishi, K. *Tetrahedron* **1989**, 45, 5423–5432; (d) Mehta, G.; Singh, V. *Chem. Soc. Rev.* **2002**, 31, 324–334; (e) Kumar, D.; Mishra, K. B.; Mishra, B. B.; Mondal, S.; Tiwari, V. K. *Steroids* **2014**, 80, 71–79.
- Lemus, A. E.; Enriquez, J.; Garcia, G. A.; Grillasca, I.; Perez-Palacios, G. *J. Steroid Biochem.* **1997**, 60, 121–129.
- Levine, P. M.; Lee, E.; Greenfield, A.; Bonneau, R.; Logan, S. K.; Garabedian, M. J.; Kirshenbaum, K. *ACS Chem. Biol.* **2012**, 7, 1693–1701.
- Levine, P. M.; Imberg, K.; Garabedian, M. J.; Kirshenbaum, K. *J. Am. Chem. Soc.* **2002**, 124, 6912–6915.
- (a) Faivre, V.; Rosilio, V. *Expert Opin. Drug Del.* **2010**, 7, 1031–1048; (b) Faivre, V.; Costa Mde, L.; Boullanger, P.; Baszkin, A.; Rosilio, V. *Chem. Phys. Lipids* **2003**, 125, 147–159; (c) Faivre, V.; Rosilio, V.; Boullanger, P.; Almeida, L. M.; Baszkin, A. *Chem. Phys. Lipids* **2001**, 109, 91–101; (d) Chae, P. S.; Laible, P. D.; Gellmann, S. H. *Mol. Biosyst.* **2010**, 6, 89–94; (e) Mbadugha, B. N. A.; Menger, F. M. *Org. Lett.* **2003**, 5, 4041–4044; (f) Slidregt, L. A. J. M.; Rensen, P. C. N.; Rump, E. T.; van Santbrink, P. J.; Bijsterbosch, M. K. A.; Valentijn, R. P. M.; van der Marel, G. A.; van Boom, J. H.; van Berkel, T. J. C.; Biessen, E. A. L. *J. Med. Chem.* **1999**, 42, 609–618; (g) Cheng, Y.; Ho, D. M.; Gottlieb, C. R.; Kahne, D.; Bruck, M. A. *J. Am. Chem. Soc.* **1992**, 114, 7319–7320.
- (a) Chae, P. S.; Gotfryd, K.; Pacyna, J.; Miercke, L. J. W.; Rasmussen, S. G. F.; Robbins, R. A.; Rana, R. R.; Loland, C. J.; Kobilka, B.; Stroud, R.; Byrne, B.; Gether, U.; Gellmann, S. H. *J. Am. Chem. Soc.* **2010**, 132, 16750–16752; (b) Zhang, Q.; Ma, X.; Ward, A.; Hong, W.-X.; Jaakola, V.-P.; Stevens, R. C.; Finn, M. G.; Chang, G. *Angew. Chem., Int. Ed.* **2007**, 119, 7153–7155.
- Ferro, V.; Karoli, T.; Liu, L.; Handley, P. N.; Johnstone, K. D.; Wimmer, N.; Hammond, E. T. *WO 2009/049370*, 2009.
- Quader, S.; Boyd, S. E.; Jenkins, I. D.; Houston, T. A. *J. Org. Chem.* **2007**, 72, 1962–1979.
- (a) Kumar, D.; Mishra, A.; Mishra, B. B.; Tiwari, V. K. *J. Org. Chem.* **2013**, 78, 899–909; (b) Kushwaha, D.; Tiwari, V. K. *J. Org. Chem.* **2013**, 78, 8184–8190; (c) Kushwaha, D.; Singh, R. S.; Tiwari, V. K. *Tetrahedron Lett.* **2014**, 55, 4532–4536; (d) Kushwaha, D.; Pandey, P.; Kale, R. R.; Tiwari, V. K. *Trends Carbohydr. Res.* **2012**, 4, 45–53.
- Mishra, K. B.; Tiwari, V. K. *J. Org. Chem.* **2014**, 79, 5752–5762.
- Mereyala, H. B.; Gurralla, S. R.; Mohan, S. K. *Tetrahedron* **1999**, 55, 11331–11342.