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# Click chemistry inspired highly facile synthesis of triazolyl ethisterone glycoconjugates

Dhananjay Kumar [1], Kunj B. Mishra [1], Bhuwan B. Mishra, Saheli Mondal [2], Vinod K. Tiwari *

*Department of Chemistry, Centre of Advanced Study, Faculty of Science, Banaras Hindu University, Varanasi 221005, India*

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ABSTRACT

Numerous deoxy-azido sugars **3** were prepared by the reaction of tosyl/bromo sugars with $NaN_3$ in dry DMF under heating condition. The 1,3-dipolar cycloaddition of deoxy-azido sugars **3** with ethisterone **4** to afford regioselective triazole-linked ethisterone glycoconjugates **5** was investigated in the presence of CuI and DIPEA in dichloromethane or $CuSO_4{\cdot}5H_2O$ and sodium ascorbate in aqueous medium. All the developed compounds were characterized by spectroscopic analysis (IR, $^1H$ & $^{13}C$ NMR, and MS spectra). Structure of triazolyl ethisterone glycoconjugate **5a** has been further confirmed by its Single Crystal X-ray analysis.



## 1. Introduction

Steroids, due to their rigid framework and potential for diverse range of functionalization, broad biological activity profile, and ability to penetrate the cell membrane and bind to specific hormonal receptors, have become preferred synthons for the development of diverse bioconjugates [1,2]. Many representatives of this group are widely used in medicine as essentials of anti-inflammatory, anabolic, and contraceptive drugs. For example, ethisterone is known to compete for androgen receptor (AR) binding, and suppresses levels of AR transcriptional activation relative to dihydrotestosterone (DHT) [3]. Several conjugates have been prepared from steroids through integration and/or linkage with other biomolecules and drugs for numerous pharmacological applications [4–7]. Recently, Levine et al. identified potential biomedical significance of multivalent peptoid conjugates for advanced prostate cancer [8]. Thus, development of triazole-linked ethisterone glycoconjugates would be crucial in AR pharmacology and chemical biology.

In medicinal chemistry, the triazoles are known to possess a number of desirable features including sufficient stability to acidic/basic hydrolysis and reductive/oxidative conditions, indicative of a high aromatic stabilization [9]. This moiety is relatively resistant to metabolic degradation. Tazobactam, a $\beta$-lactamase inhibitor is among the best-known examples of triazole containing structures with the broad-spectrum antibiotic piperacillin [10,11]. Also several members of the 1,2,3-triazole family have indeed shown interesting biological properties, such as anti-allergic, anti-bacterial and anti-HIV activity [12,13].

The coupling of two or more molecular entities with distinct properties to form novel conjugates with combined properties of parent components, has emerged as a fast growing technology in recent years [14–16]. Several new conjugates arising *via* such bioconjugation have been found to exhibit unusual biological properties and activities as the different molecular segments act cooperatively [17–19]. In this prospective, the 'Click'-chemistry [20] is a newer approach for the synthesis of drug-like molecules that can accelerate the drug discovery process by utilizing a few practical and reliable reactions [21–23].

Among the reactions comprising the click universe, the perfect example is the Huisgen 1,3-dipolar cycloaddition of terminal alkynes and organic azides to form 1,4-disubstituted-1,2,3-triazoles [20–25]. In addition, because of important role of carbohydrate in biological system [26], and their great chemotherapeutic potential [27], a wide variety of glycoconjugates so far has been synthesized using azide–alkyne cycloaddition approach [28–35]. However, the synthesis of steroid-glycoconjugates using 'click' chemistry has yet to be realized fully. Thus, in view of numerous medicinal effects of ethisterone, a 17$\alpha$-ethynyl analog of testosterone, and the utility of carbohydrates in numerous chemical, biological, medicinal, and pharmacological investigations, we herein report the high-yielding synthesis of novel triazole-linked ethisterone glycoconjugates (**5a–i**) using 1,3-dipolar cycloaddition

* Corresponding author. Tel.: +91 542 6702466; fax: +91 542 2368174.
*E-mail addresses:* vtiwari@ucdavis.edu, tiwari_chem@yahoo.co.in (V.K. Tiwari).
[1] Equal contribution.
[2] Present address: Chembiotek Research International, Salt Lake Electronics Complex, Sector V, Kolkata, WB 700091, India.

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reaction of azido-sugars (**3a–i**) with ethisterone (**4**) in the presence of Cu(I) catalyst at room temperature.

## 2. Experimental

### 2.1. General methods

All of the reactions were executed using anhydrous solvents under an argon atmosphere in one-hour oven-dried glassware at 100 °C. 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 ($\lambda_{max}$ = 254 nm) or a specific color reagent (iodine vapors) or by spraying with methanolic $H_2SO_4$ solution and subsequent heating at 60 °C. $^1H$ and $^{13}C$ NMR were recorded at 300 and 75 MHz, respectively. Chemical shifts given in ppm downfield from internal TMS; *J* values in Hz. Mass spectra recorded using electrospray ionization mass spectrometry (ESI–MS). Infrared spectra recorded as Nujol mulls in KBr plates. Elemental analysis was performed using a C, H, N analyzer, and results were found to be within ±0.4% of the calculated values. Reaction under microwave condition was carried out on Microwave CEM Discover R Lab Mate. Polarimeter with general tungsten lamp having tube of 2 dm and 20 ml capacity. Single-crystal X-ray data collected on Xcalibur Eos (Oxford) CCD-diffractometer.

### 2.2. Procedure for synthesis of protected sugars (1a–i)

The compounds **1a–i** were prepared from readily available carbohydrates (D-glucose, D-galactose, D-ribose, D-xylose, and D-lactose) using standard protection and modification methodologies [36–44].

### 2.3. General procedure for synthesis of tosyl-sugars (2a–e)

The stirring solutions of compounds **1a–e** in pyridine at 0 °C were added with *p*-toluene sulphonyl chloride under anhydrous condition. The reactions was allowed to come at room temperature and further stirred for 12 h. After completion (monitered by TLC), the reaction mixtures were *in vacuo* concentrated and the crude obtained were purified by flash column chromatography to afford tosyl-sugars **2a–e** in good yields.

### 2.4. General procedure for synthesis of bromo-sugars (2f-j)

The stirring solutions of compounds **1f–j** at 0 °C were treated with 33% HBr solution in glacial acetic acid under anhydous condition. The reation mixture was further stirred for 2 h at 0 °C. After completion of reaction (monitored by TLC), the reaction mixtures were neutralized with saturated $NaHCO_3$ solution followed by extraction in dichloromethane. The organic layers were dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure to afford compounds **2f–j**. Because of low stability at room temperature, all the developed bromo-sugars **2f–j** was subsequently utilized for the synthesis of sugar azides **3f–j** without further purification.

### 2.5. General procedure for synthesis of sugar azides (3a–j)

The stirring solutions of compounds **2a–j** in dry DMF were treated with $NaN_3$ [45–52]. The reaction mixtures were further heated at 80 °C under anhydrous condition followed by constant stirring over night. After completion of reaction (monitored by TLC), the reaction mixtures were *in vacuo* concentrated followed by silica gel column chromatography to afford compounds **3a–j** in good yields.

### 2.6. General procedure for synthesis of ethisterone glycoconjugates (5a–j)

#### 2.6.1. 1-(3-O-Benzyl-5-deoxy-1,2-O-isopropylidene-α-D-xylofuranos-5-yl)-4-ethisterone-1,2,3-triazole (5a)

A solution of **3a** (1.20 g, 3.9 mmol), and ethisterone **4** (1.35 g, 4.3 mmol) in presence of DIPEA (0.55 ml, 4.3 mmol) and CuI (0.37 g, 1.9 mmol) in dry $CH_2Cl_2$ was sttired at room temparature under argon atmosphenre for 12 h. After completion of reaction (monitored by TLC), the reaction mixture was *in vacuo* concentrated to obtain a crude residue which was further purified by silica gel (100–200 mesh) column chromatography to afford compound **5a**. Yellow crystalline solid (2.2 g, 90% yield); $[\alpha]_D$ = +20.0 (*c* 0.20 $CHCl_3$); IR (KBr) $\nu_{max}$: 3412, 3174, 2976, 2946, 1658, 1454, 1382, 1080 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 7.43 (s, 1H, triazolyl-H), 7.35–7.33 (m, 5H, Ar–H), 5.96 (d, *J* = 3.9 Hz, 1H, H-1), 5.69 (s, 1H, ethisterone-H), 4.73–4.65 (m, 3H, H-2 and OC$H_2$Ph), 4.54–4.47 (m, 3H, H-4 and H-5), 4.00 (m, H-3), 2.92 (s, 1H, ethisterone-H), 2.38–2.13 (m, 5H, ethisterone-H), 2.04 (m, 1H, ethisterone-H), 1.95–1.78 (m, 3H, ethisterone-H), 1.60–1.42 (m, 10H, 1 × $CH_3$ of >C($CH_3$)$_2$ and ethisterone-7H), 1.31 (m, 3H, 1 × $CH_3$ of >C($CH_3$)$_2$), 1.16 (s, 3H, ethisterone-C$H_3$), 1.05 (s, 3H, ethisterone-C$H_3$), 0.72–0.64 (m, 1H, ethisterone-H), 0.48–0.38 (m, 1H, ethisterone-H); $^{13}C$ NMR (75 MHz, $CDCl_3$): 199.4, 171.1, 153.3, 136.8, 128.6, 128.3, 127.7, 123.7, 122.1, 112.1, 105.1, 81.9, 81.8, 81.7, 78.8, 72.0, 53.2, 49.3, 49.0, 46.8, 38.5, 37.4, 36.2, 35.5, 33.8, 32.7, 32.6, 31.5, 26.7, 26.2, 23.4, 20.5, 17.3, 14.2; MS: *m/z* 618 $[M+H]^+$; Anal. Calcd for $C_{36}H_{47}N_3O_6$: C, 69.99; H, 7.67; N, 6.80. Found: C, 70.37; H, 7.98; N, 7.09.

Click reaction between glycosyl azide **3a** and ethisterone **4** using $CuSO_4{\cdot}5H_2O$/sodium ascorbate in aqueous medium at room temperature afford ethisterone triazolyl glycoconjugate **5a** in good yield. Furthermore, an equimolar mixture of glycosyl azide **3a** (35 mg) and ethisterone **4** (50 mg), in anhydrous toluene (10 ml) was added DIPEA (25 μL) and CuI (9 mg) as described above under inert atmosphere. The reaction was carried out under microwave heating condition (Microwave CEM Discover R Lab Mate) at 100 °C for 15 min. After completion of reaction (monitored by TLC), the reaction mixture was *in vacuo* concentrated, extracted with $CH_2Cl_2$ and washed with water. After drying over anhydrous $Na_2SO_4$, the organic layer was *in vacuo* concentrated. Purification using flash column chromatography afforded ethisterone triazolyl glycoconjugate **5a**. The physical data is closely matched with the developed molecule **5a**, where the reaction was carried out at room temperature.

#### 2.6.2. 4-Ethisterone-1-(methyl-5-azido-5-deoxy-2,3-O-isopropylidene-β-D-ribofuranosid-5-yl)-1,2,3-triazole (5b)

A solution of azide **3b** (1.0 g, 4.1 mmol), and ethisterone **4** (1.4 g, 4.6 mmol) in presence of DIPEA (0.75 ml, 4.6 mmol) and CuI (0.4 g, 2 mmol) in dry $CH_2Cl_2$ was sttired at room temparature under argon atmosphenre for 12 h followed purification described earlier afforded compound **5b**. Yellowish solid (2.1 g, 95% yield); $[\alpha]_D$ = −15.5 (*c* 0.19 $CHCl_3$); IR (KBr) $\nu_{max}$: 3453, 3140, 2939, 2856, 1667, 1330, 868 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 7.48 (s, 1H, triazolyl-H), 5.69 (s, 1H, ethisterone-H), 5.01 (s, 1H, H-1), 4.74–4.38 (m, 5H, H-2, H-3, H-4, H-5), 3.38 (s, 3H, –OC$H_3$), 2.82 (s, 1H, ethisterone-H), 2.37–2.29 (m, 5H, ethisterone-H), 2.14–2.10 (m, 1H, ethisterone-H), 1.91–1.86 (3H, ethisterone-H), 1.61–1.05 (m, 19H, ethisterone-H), 0.73 (m, 1H, ethisterone-H), 0.47 (m, 1H, ethisterone-H); $^{13}C$ NMR (75 MHz, $CDCl_3$): 199.4, 171.1, 153.6, 123.7, 121.4, 112.9, 110.0, 85.1, 84.9, 82.1, 81.7, 55.5, 53.1, 53.1, 48.8, 46.8, 38.5, 37.8, 36.2, 35.5, 33.8, 32.7, 32.6, 31.5, 26.3, 24.8, 23.6, 20.5, 17.3, 14.2; MS: *m/z* 542 $[M+H]^+$; Anal. Calcd for $C_{30}H_{43}N_3O_6$: C, 66.52; H, 8.00; N, 7.76. Found: C, 66.77; H, 8.33; N, 7.37.