Chromatography Free Synthesis of Reversed *N*-Triazole Nucleosides Starting from α–D-Galactopyranose using 1,3-Dipolar Cycloaddition Reactions

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Summary: A chromatography free, four-step strategy has been developed to synthesize reversed *N*-nucleosides (**4A-D**) starting from α-D-galactopyranose. The triaozle moiety served as heterocyclic part of nucleoside was created *via* 1,3 dipolar cycloaddition reaction between sugar azide (*1,2:3,4-di-O-isopropylidene-6-deoxy-6-azido-*α*-Dgalactopyranoside,* **3**) and terminal alkyne. Sugar azide **3** was obtained from *1,2:3,4-di-O-isopropylidene-6-tosyl-*α *-Dgalactopyranoside* (**2**). Purification of products was carried out through solvent-solvent extraction and/or crystallization techniques. This is an example of click reaction. Some advantages of click reaction include mild reaction conditions, selectivity, efficiency and high yields.

Key words: Chromatography free; Reversed N-nucleosides; Click reaction; Galactopyranose.

Introduction

Carbohydrates are a well-known class of naturally compounds. Carbohydrates can easily be subjected to chemical modification by means of nucleophilic displacement reactions due to multiple chiral centres [1]. Nucleosides are one of the modified classes of carbohydrates that deserve the attention of synthetic chemists due to their extra-ordinary efficiency against specific microbes. The biological properties of nucleoside analogues rang from antiviral to antineoplastic [2-6]. Nitrogen containing sugar moieties are always remained as a profound target for synthesis due to their natural abundance and therapeutic characteristics [7-9]. Although nucleosides play a significant role in medicinal chemistry, the current limitation in this field stems from the challenging synthetic process they undergo [10-12]. Modifying carbohydrates continues to pose a challenge, particularly due to the multiple free hydroxyl groups and their varied behaviour in different carbohydrate moieties [13].

Despite the challenges encountered in synthetic approaches to carbohydrate analogues, it is undeniable that nucleosides hold a crucial value as active antimicrobials [4]. Based on the connectivity of nitrogenous base to the carbohydrate moiety, reversed nucleosides are categorized as a class of compounds in which nitrogenous base is connected to any carbon of carbohydrate moiety other than anomeric carbon and the absence of glycosidic linkage makes this a stable class of compound towards hydrolysis [14]. Analogues of nucleosides are recognized for their broad-spectrum antiviral activities in their cyclic / acyclic forms [15]. Since carbohydrates function as chiral auxiliaries, therefore reversed acyclic nucleosides can also serve as the chiral pool for the synthesis of respective acyclic nucleosides [14, 16]. Beside the significance of nucleosides, carbohydrate derivatives are recognized as potent drug metabolites, ranging their activities from antimycotic to anticarcinogenetic [17]. The incorporation of carbohydrates with other biomolecules, along with consideration of their stereochemistry, contributes to the impactful modulation of properties in newly developed compounds [18].

The primary synthetic challenges in carbohydrate derivatization include; a) the protection and de-protection of carbohydrate hydroxyl groups, and b) purification, particularly when it comes to chromatography. Chromatography based purification method involves excess solvent runoff, extended run time and risk of decomposition or breakdown of carbohydrate moieties specially if left on silica for a longer period of time. Hence, chromatography is an expensive and time-consuming technique specially, when synthesis needs to be carried out at industrial scale [19]. In view of chromatography-associated challenges, there is a need of chromatography free protocols. The click reaction serves that purpose.

In the present study, we have implemented a strategy for the synthesis of reversed N-nucleosides that is entirely free from chromatography. The approach begins with conversion of α-*D*-galactopyranose into azide sugar **3** followed by its coupling with terminal

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acetylenes to furnish triazoles. Our primitive objective of present study is to substitute a hydroxyl group of *α-D*-galactopyranose by nitrogen atom at other than anomoric carbon. For this purpose, the traditional bimolecular substitution reaction (S_N2) was carried out to introduce azide following a cascade of reactions in chemoselective manner. Incorporation of heterocyclic moiety carried out via Copper (I) catalyzed click reaction that is well known for the chromatography free extraction and easy optimization [20].

Experimental

Reagents and chemicals

α-D-galactose, crystalline iodine, 17-*α*-ethynylestradiol, 1-heptyne, 1-octyne, 1-noyne, sodium azide and tosyl chloride were purchased from Sigma-Aldrich and used as purchased without further purification. All solvents were of analytical grade and used as purchased without further purification.

Instrumentation

IR spectra were recorded on SHIMADZU FTIR instrument. For 1D and 2D NMR spectroscopy, AVANCE AV-700 BRUKER was used. Melting points of products were recorded on Stuart Digital Melting Apparatus SMP 10. Mass spectra were recorded on JEOL JMS 600H-1, Inlet: direct probe, Ionization mode: EI^+.

Synthesis of 1,2:3,4-di-O-isopropylidene-α-Dgalactopyranoside (**1**)

Compound **1** was obtained as viscous liquid **1**, applying previously reported method [1].

Synthesis of 1,2:3,4-di-O-isopropylidene-6-tosyl-α -Dgalactopyranoside (**2**)

Tosyl **2** was prepared as white crystalline compound applying previously reported method [22].

Synthesis of 1,2:3,4-di-O-isopropylidene-6-deoxy-6-azido-α -Dgalactopyranoside (**3**)

In 190 mL DMSO, 11 g of **2** with 16 equivalents of NaN_3 was added. The reaction mixture was refluxed for 4 hours with constant stirring. Reaction progress was monitored through TLC (hexane-ethyl acetate; 7:3). The presence of the product was visualized and confirmed by the charring [23]. Cooling of the reaction mixture to room temperature was followed by addition of water. Extraction of the product carried out through solvent-solvent extraction using DCM. The crude azide **3** was purified by adding hexane to DCM solution followed by refrigerating below 0°C overnight. Excess NaN_3 was crystalized out and the organic layer contained pure **3** was decanted and evaporated. Azide **3** was obtained as a colourless, viscous liquid, % yield; 45.32, R_f; 0.43 [24].

Click reaction for the synthesis of triazole based reversed N-nucleosides **4A-D**

Click reaction/ 1,3-dipolar cycloaddition reaction was carried out according to reported method [27]. 1 mmol of each sugar azide **3** and the respective terminal alkynes were mixed in a 4 mL 1:1 solution of H_2O and tertiary butanol (t.BuOH) and continuously stirred at room temperature for about 2-4 hours. A freshly prepared IM aqueous solution of sodium ascorbate (0.1 mmol, 100 μL) and 0.01mmolar aqueous solution of $CuSO_4.5H_2O$ (2.5g in 35μL water) were added to the reaction mixture. A heterogeneous reaction mixture was formed. The reaction progress was monitored through TLC (hexane-ethyl acetate; 8:2). Formation of products was visualized by UV 254 nm and charring with 10% sulfuric acid in ethanol. Generally, after the confirmation of reaction completion ice is added to cease the reaction, [27] so, cold water was added to the crude product for the same purpose and reaction mixture was refrigerated. In the heterogeneous reaction mixture, the product coagulated around the magnetic bead. The solvent was easily decanted and the product was taken out by dissolving the solid in DCM. Anhydrous Na_2SO_4 was used to absorb the remaining moisture and the organic phase was filtered and evaporated on rotary evaporator to obtain pure reverse N-Nucleoside derivative (**4A-D**) as off-white solids. The complete conversion of starting material and purity of newly synthesized nucleosides were monitored by TLC (DCM: MeOH; 9:1), visualizing through UV using wavelength 254 nm followed by charring of compounds with an 10% ethanolic solution of sulfuric acid (H_2SO_4) using aluminum thin sheets loaded with Merck silica 60 F254. NMR data of compounds **4A-D** see Table-2 and Table-3. Mass Spectral data of compounds **4A-D** is presented in Table-4.

IR spectral data is presented in Table-5.

Table-1: Summary of Click Reactions.

Reverse N-Nucleosides	Terminal Alkyne	Physical State	Colour	Melting point	R_f Value	% Yield	Time hrs
4A	17-*α*-ethynylestradiol	Solid	Off-white	145°C	0.69	64	2
4B	1-Heptyne	Solid	Off-white	98°C	0.22	75	1
4C	1-Octyne	Solid	Off-white	89°C	0.31	80	1
4D	1-Nonyne	Solid	Off-white	87°C	0.55	82	1

Results and Discussion

Synthesizing reverse N-nucleoside as a derivative of carbohydrate, along with their purification, always remained as a challenge for synthetic organic chemists. To choose the appropriate hydroxyl group on a parent carbohydrate and make it available for sequential reactions through specific protections and timely de-protections is an art of synthesis.

Starting with the commercially available α-D-galactopyranose, we have proposed a synthetic scheme (Scheme 01). Purification of products (**1-4D**) involves no chromatographic technique. Primarily, α-D-galactopyranose was subjected to protection of hydroxyl group at C_1-C_4 through di-*O*-isopropylidene formation with acetone using iodine as catalyst at room temperature followed by simple solvent-solvent extraction to afford the 1,2:3,4-di-*O*-isopropylidine-*α*-D-galactopyranoside **1** as colorless, viscous pure oil [21]. The remaining free primary hydroxyl group at C-6 was activated for nucleophilic displacement by tosylation in the presence of triethylamine (Et_3N, TEA) and 4-(dimethylamino)-pyridine (DMAP), where dichloromethane (DCM) served as a solvent [22-23]. The crude product was dissolved in methanol and refrigerated overnight. Tosylate **2** obtained as colourless crystalline solid by decantation of cold methanol/CH_2Cl_2 showing a single spot on TLC [23]. Heading towards the nitrogen addition, the tosylate **2** was imperiled to S_N2 reaction with sodium azide in the presence of polar aprotic solvent dimethylsulfoxide (DMSO). Since this reaction occurs with the inversion of the configuration [24], so the C-6 was found to be an appropriate position on a pyranose ring that helps to maintain initial stereochemistry of the sugar intact. Complete conversion of tosylate **2** into azide **3** observed after 4 hours reflux at 80°C with continuous stirring. The color of reaction mixture transformed from blue to green to dark brown i.e. original appearance. Since, at gram scale (5g of tosylate was used) the reaction was found to be exothermic without any change in colour of the reaction mixture. The remaining sodium azide was precipitated out after the addition of hexane to reaction mixture. Insoluble inorganic impurities separated via filtration. The organic phase was evaporated to furnish the colourless, viscous organic azide in the purest form [25].

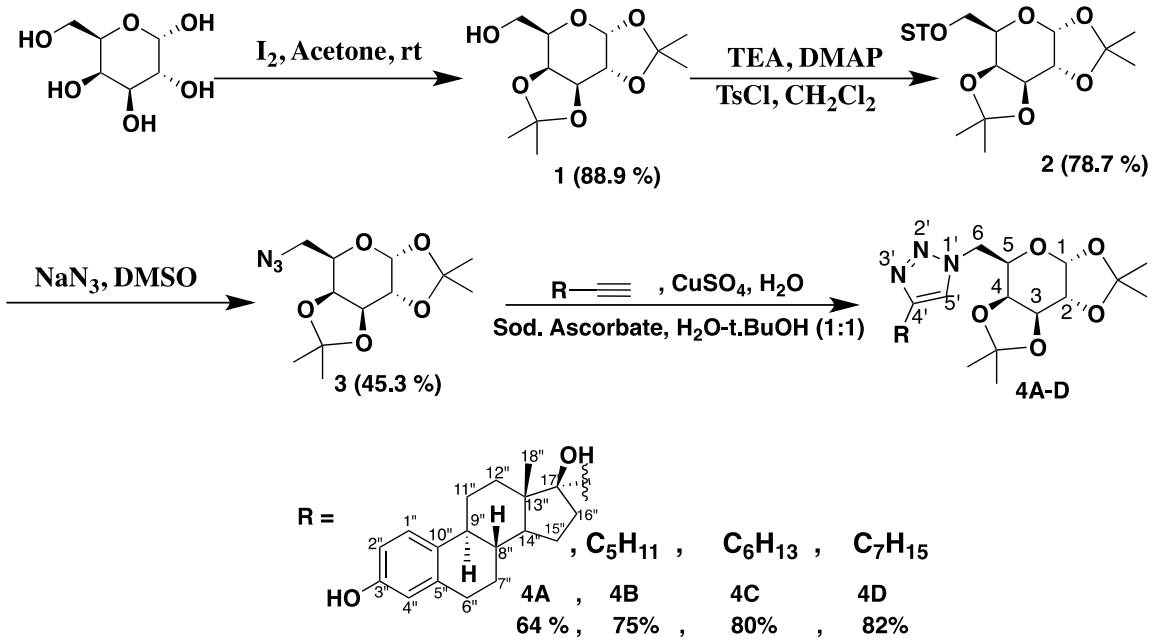
Nitrogen and oxygen containing heterocyclic rings, if attached, on varying position at any carbohydrate as a parent molecule, are recognized as a potent drug candidates [26]. This significance was worth the attention towards the synthesis of triazole-based reverse N-nucleosides employing 1,3-dipolar Husigen cycloaddition, also known as the click reaction. The choice of click reaction as the final step makes the entire synthetic scheme chromatography free as rapid conversion in click reaction supports easy extraction and high yield of pure compounds without using lengthy or tedious purification techniques [20]. Compound **3,** terminal azide was reacted with four different terminal alkynes in the presence of freshly prepared sodium ascorbate [27] and a catalytic amount of pentahydrated copper (II) sulphate mainly responsible to generate copper(I) ion in-situ. Compounds **4A-D** were easily obtained through solvent-solvent extraction as stable products at room temperature. This category of 1,4-disubstituted triazoles based reverse N-nucleosides are known for their cytotoxic and antiviral activities [28].

Since thin layer Chromatography (TLC) remained a hands-on technique to monitor the reaction progress and degree of purification of products on the basis of their R_f values. Scheme 01 shows the general synthesis of reversed N-nucleosides **4A-D**. Terminal alkynes that were used in click reaction given in Table-1.

The identification of all newly developed reverse N-nucleosides **4A-4D** was mainly approved by mass spectroscopy and 1D and 2D NMR spectroscopic techniques.

The mass spectra showed molecular ion peaks for compounds **4A-D** at *m/z* 581.3, 381.2, 396.2, and 409.2 corresponding to molecular formula $C_{32}H_{43}N_3O_7$, $C_{19}H_{31}N_3O_5$, $C_{20}H_{34}N_3O_5$ and $C_{21}H_{35}N_3O_5$ respectively (For details *see*; Table-4, supplementary material Fig S19-S-22). The distinct peaks of parent carbohydrate moiety (*α*-D-Galactopyranoside) observed around δ 4.0-5.6 ppm (Fig-1). The stereochemistry of anomeric proton at equatorial position established on the basis of a downfield doublet around δ 5.4 ppm ($J \cong 4.8$ Hz). Beside that four methine and one methylene groups were observed around δ 4.5-4.3 ppm. In the ^{13}C-NMR spectrum (BB and DEPT) significant resonances for carbohydrate moiety were observed at δ 96.0, 71.0, 70.5, 70.2 and 67.7 ppm for five methine and for methylene at δ 50.4 ppm.

A singlet of one proton appeared at δ 7.8 ppm endorsed the proton at position C-5' (in triazole). The IR spectra of all these nucleosides showed distinctive peaks for C-O (around 1080) cm^{-1}), C-N (around 1220 cm^{-1}), C=N (around 1630 cm^{-1}) and N=N (around 1450 cm^{-1}) (Table-5).



Scheme-1: Synthesis of Reversed *N*-nucleosides **4A-D.**

Table-2: NMR spectral data of **4A** (700 MHz ^{1}H-NMR; 175 Hz ^{13}C-NMR), (DMSO$_{d6}$, δin ppm, *J* in Hz), COSY correlation and HMBC correlation.

C/H No.	4A			
	δ_C	δ_H	COSY Correlation	HMBC Correlation
1	96.1	5.48, d (4.8)	H-2, CH_3 (isopropylidene)	C-7, CH_3 (isopropylidene),
2	70.2	4.53, dd (14.0, 4.8)	H-1, H-3	
3	70.6	4.30, dd (14.0, 4.8)	H-2, H-4	
4	71.1	4.29, dd (9.3, 8.5)	H-3	C-4’
5	67.7	3.69, m	H-6	C-4’
6	50.4	3.69, m	H-5	
7	109.3	-	-	
8	108.4	-	-	
CH_3 x 4	26.4	1.43-1.20 br s	H-1	C-6, C-7
	26.1			
	25.3			
	24.7			
4’	154.0	-		
5’	123.6	7.78, s		C-17”
1”	126.3	6.95, d (8.4)	H-2”	C-3”, C-4”, C-9”
2”	113.1	6.47, d (8.4)	H-1”	C-1”, C-3”, C-4”
3”	155.3	-		
4”	115.3	6.41		C-2”, C-3”, C-5”,
5”	137.6	-		
6”	32.9	2.40, t (7.7)		C-9”
7”	27.7	-		
8”	n.o			
9”	43.6	2.72, m		C-5”
10”	130.8	-		
11”	29.7			
12”	23.9			
13”	47.1	-		
14”	47.9			
15”	26.6			
16”	37.3			
17”	81.4	-		
18”	14.8	0.92, s		C-17”, C-14”, C-11”

n.o = not observed

Table-3: NMR spectral data of **4B-D** (700 MHz ^{1}H-NMR; 175 Hz ^{13}C-NMR), (DMSO$_{d6}$, δ in ppm, *J* in Hz), COSY correlation and HMBC correlation.

C/H No.	4B				4C				4D			
	δ_C	δ_H	COSY Correlation	HMBC Correlation	δ_C	δ_H	COSY Correlation	HMBC Correlation	δ_C	δ_H	COSY Correlation	HMBC Correlation
1	95.9	5.42, d (4.7)	H-2		95.9	5.41, d (4.9)	H-2		95.9	5.42, d (4.9)	H-2	
2	70.2	4.30, dd (14.8, 4.7)	H-1, H-3	C-5	70.2	4.30, dd (14.0, 4.9)	H-1, H-3	C-5	70.1	4.30, dd (14.8, 4.9)	H-1, H-3	C-5
3	70.6	4.53, dd (14.0, 4.8)	H-2	C-1	70.6	4.53, dd (14.0, 4.8)	H-2	C-1	70.5	4.53, dd (14.0, 4.8)	H-2	C-1
4	71.0	4.30, dd (9.3, 8.5)			71.0	4.30, dd (9.3, 8.5)			71.0	4.30, dd (9.3, 8.5)		
5	67.4	4.37, m		C-2, C-3	67.4	4.37, m		C-2, C-3	67.3	4.37, m		C-2 , C-3
6	50.3	4.37, m			50.3	4.37, m			50.3	4.37, m		
7	109.2	-		CH_3 (isopropylidene)	109.2	-		CH_3 (isopropylidene)	109.2	-		CH_3 (isopropylidene)
8	108.5	-		CH_3 (isopropylidene)	108.5	-		CH_3 (isopropylidene)	108.5	-		CH_3 (isopropylidene)
CH_3 x 4	26.1 25.4 25.3 24.7	 1.25, 1.31, 1.33, 1.36 all singlets			26.1 25.4 25.3 24.7	 1.24, 1.29, 1.30, 1.33 all singlets			26.1 25.4 25.3 24.7	 1.24, 1.33, 1.35, 1.42 all singlets		
4’	147.0	-			147.0				147.0			
5’	122.6	7.81, s		C-4’	122.6	7.80, s		C-4’	122.6	7.80, s		C-4’
1”	31.1	2.61, t (7.3)	H-2”	C-4’, C-5’	31.5	2.61, t (7.5)	H-2”		31.7	2.61, t (7.0)	H-2”	C-4’, C-5’
2”	29.1	1.58, p (7.0)	H-1”	C-4’	26.4	1.58, p (7.0)	H-1”		26.4	1.58, p (6.3)	H-1”	C-4’
3”	29.1	1.58, p (7.0)			28.6	1.58, p (7.0)			28.8	1.58, p (6.3)		
4”	22.6	1.58, p (7.0)			29.3	1.58, p (7.0)			29.4	1.58, p (6.3)		
5”	14.4	0.87, t (6.7)			22.5	1.58, p (7.0)	H-6”		24.7	1.58, p (6.3)		
6”					14.4	0.87, t (6.3)	H-5”		22.5	1.58, p (6.3)		
7”									14.4	0.86, t (6.3)		C-1”, C-6”

Table-4: Mass spectral data of compounds **4A-D.**

	EIMS: *m/z* (rel. int)
4A	**581.3 [M^+; $C_{32}H_{43}N_3O_7$] (75.9); 566.3 [M^+-CH_3; $C_{31}H_{41}N_3O_7$] (63.0); 553.3 [M^+-C_2H_4; $C_{30}H_{39}N_3O_7$] (77.0); 366.2 [$C_{18}H_{29}N_3O_5$] (27.4); 354.2 [$C_{17}H_{28}N_3O_5$] (100.0). OH groups could not identify due to moisture in instrument.**
4B	**381.2 [M^+; $C_{19}H_{31}N_3O_5$] (12.5); 366.1 [M^+- CH_3; $C_{18}H_{29}N_3O_5$] (70.1); 352.2 [M^+- C_2H_4; $C_{17}H_{27}N_3O_5$] (4.3); 124.1 [$C_6H_{11}N_3$] (100)**
4C	**396.2 [M^+; $C_{20}H_{34}N_3O_5$] (63.7); 380.2 [M^+-H-CH_3; $C_{20}H_{34}N_3O_5$] (100); 366.2 [M^+- C_2H_5; $C_{18}H_{29}N_3O_5$] (8.1); 352.1 [M^+- C_3H_7; $C_{17}H_{27}N_3O_5$] (22.6)**
4D	**409.2 [M^+; $C_{21}H_{35}N_3O_5$] (9.3); 394.3 [M^+- CH_3; $C_{20}H_{32}N_3O_5$] (47.8); 366.3 [M^+- C_3H_6; $C_{18}H_{29}N_3O_5$] (4.8); 352.2 [M^+- C_2H_4; $C_{17}H_{27}N_3O_5$] (16.2); 43.0 [C_3H_7] (100)**

Table-5: IR spectral data of compounds 4A-D.

	ν_{max} (KBr) cm^{-1}
4A	**2926.01 (C-H Stretching); 1622.13 (C=N); 1442.75 (N=N); 1386.82; 1130.29 (C-N); 1089.78 (C-O)**
4B	**2933.73 (C-H Stretching); 1633.71(C=N); 1450.47 (N=N); 1382.96; 1219.01(C-N); 1153.43; 1091.71(C-O)**
4C	**2929.87 and 2860.43(C-H Stretching); 1633.71 (C=N); 1458.18 (N=N); 1381.03; 1219.01(C-N); 1159.22; 1082.07 (C-O)**
4D	**2926.01 and 2858.51(C-H Stretching); 1631.78(C=N); 1458.18 (N=N); 1386.82 (C-N); 1128.36; 1085.92 (C-O)**

We selected Rostovtsev *et. al*. [29] method for 1,4-disubstituted-1,2,3-triazole formation due to its regiospecificity, chemoselectivity, improved product purity and ease of workup. The regiospecificity in newly formed products **4A-D** was confirmed through 2D-NMR spectroscopic data. The placement of the alkyl chain at C-4' position of triazole moiety was established on the basis of COSY-45 and HMBC interactions. The HMBC spectra clearly displayed 4-5 bond C-H correlations for C-4' (quaternary carbon of triazole) with both sugar and alkyl chain while C-5' (triazole methine) exhibits interactions only with alkyl chain. Fig-S9 represents HMBC spectrum of nucleoside **4B** showing pertinent interacti

Conclusion

In conclusion, we have successfully developed a practical chromatography free synthesis of reversed N-nucleosides employing click chemistry. Furthermore, the gram scale synthesis of azide sugar (**3**) was demonstrated. The protocol presents an economical, efficient, clean and high yielding method.

Conflict of interest

No hidden conflict of interest was found among the authors of this paper.

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