Metal Free Activation of Alkynyl Glycosyl Carbonate Donors

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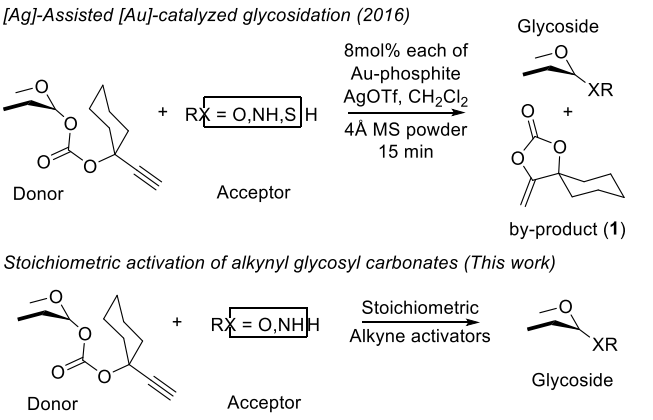
Abstract: Accessing homogenous glycoconjugates is of significance for assessing their biophysical activities and sometimes as vaccine candidate. Synthesis of glycoconjugates involves two species of which, glycosyl donor plays pivotal role in controlling the outcome of the glycosylation. Recently discovered alkynyl glycosyl carbonate donors possess self-stability, high reactivity, and fast reaction time (15-30 min) when activated with [Au]/[Ag]-catalysts. Herein, we report a new metal free condition for the activation of the same alkynyl glycosyl carbonate donors using I_2/TMSOTf in stoichiometric quantities. Extrusion product was characterized to be the vinylidene iodide by single crystal X-ray analysis. The reaction conditions were illustrated to be suitable for synthesis of various glycosides, purine/pyrimidine nucleosides and a pentasaccharide repeating unit of *Klebsiella pneumoniae* (O-3-antigen).

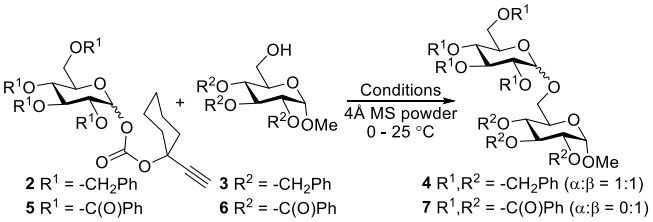


Scheme 1. General representation of the synthesis of glycosides by the activation of alkynyl glycosyl donors. Au-phosphite: chloro [tris(2,4-di-tert-butylphenyl)phosphite] gold. MS: molecular sieves.

Most of the living organisms require glycoconjugates and/or carbohydrates for energy storage, cell-cell communication, embryogenesis, protein folding, fertilization, structural support.[1] Many pioneering studies culminated in the development of excellent strategies for the synthesis of glycosides, nucleosides and assembly of complex oligosaccharides.[2] Glycosylation is one of the key reactions that enables rapid synthesis of glycoconjugates. In spite of developing excellent glycosylation methods, the synthesis of all glycosides is still a major challenge.[3] Discovery of novel glycosylation methods can be augmented by the identification of new reactivity patterns and reaction conditions for already existing glycosylation protocols.[4]

Glycosylation by silver-assisted gold-catalyzed activation of alkynyl glycosyl carbonates has emerged as one of the versatile alternatives for the rapid, stereoselective and catalytic synthesis of *O*-glycosides,[5a] nucleosides,[5a] *N*-glycosides[5b] and oligosaccharides (Scheme 1).[5] Whereas alkynyl glycosyl carbonate donors can be synthesized easily, [Au]/[Ag]-catalysts are not routinely available in all organic chemistry laboratories though the glycosylation is quite efficient, high yielding and catalytic. In addition, access to gold and silver salts has become difficult during pandemic; therefore, an alternate mode of activation of carbonates was investigated. Herein, we present activation of alkynyl glycosyl carbonates donors by other routinely available alkynophilic reagents drawing inspiration from the exceptional affinity of iodonium reagents towards unsaturated compounds.[6]

We commenced our studies with the alkynyl glycosyl carbonate **2** and the acceptor **3** as model substrates for our exploration (Scheme 2). Glycosylation with the easily available alkynophilic reagent *N*-iodosuccinimide or Lewis acid TMSOTf failed to produce the desired disaccharide **4** (entry 1,2). However, combination of 1.5 equiv of NIS and 0.15 equiv of TMSOTf afforded excellent yield (91%) of the disaccharide **4** (entry 3). In general, per-*O*-benzoyl glucopyranosides (**5**) are less reactive (disarmed) compared to the corresponding benzylated substrates (**2**).[7] The glycosyl donor **5** and acceptor **6** gave poor yield (43%) of the disaccharide **7** when 0.15 equiv of TMSOTf was employed (entry 2); pleasingly, addition of 0.5 equivalents of TMSOTf improved the performance to 86% (Scheme 2, entry 3).



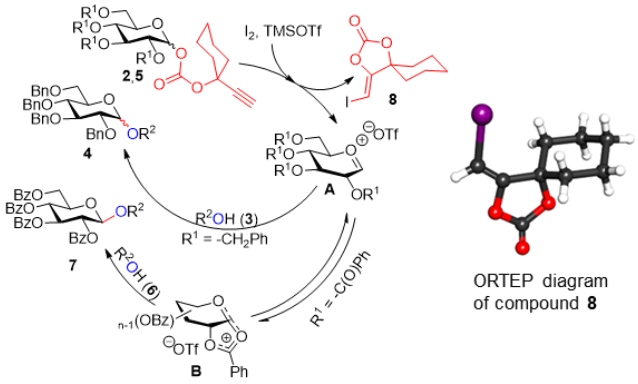
Entry	Reaction Conditions	% Yield	
		4	**7**
1	NIS (1.5 equiv.)	0	0
2	TMSOTf (0.15 equiv.)	0	0
3	NIS (1.5 equiv.) + TMSOTf (0.15 equiv.)	91	43
4	NIS (1.5 equiv.) + TMSOTf (0.50 equiv.)	93	86
5	I_2 (1.4 equiv.)	50	10
6	I_2 (1.5 equiv.) + TMSOTf (0.15 equiv.)	96	56
7	I_2 (1.5 equiv.) + TMSOTf (0.50 equiv.)	95	94

Scheme 2. Screening of reaction conditions for the model glycosidation reaction. NIS: *N*-Iodosuccinimide. TMSOTf: trimethylsilyl trifluoromethanesulfonate. Bn: benzyl.

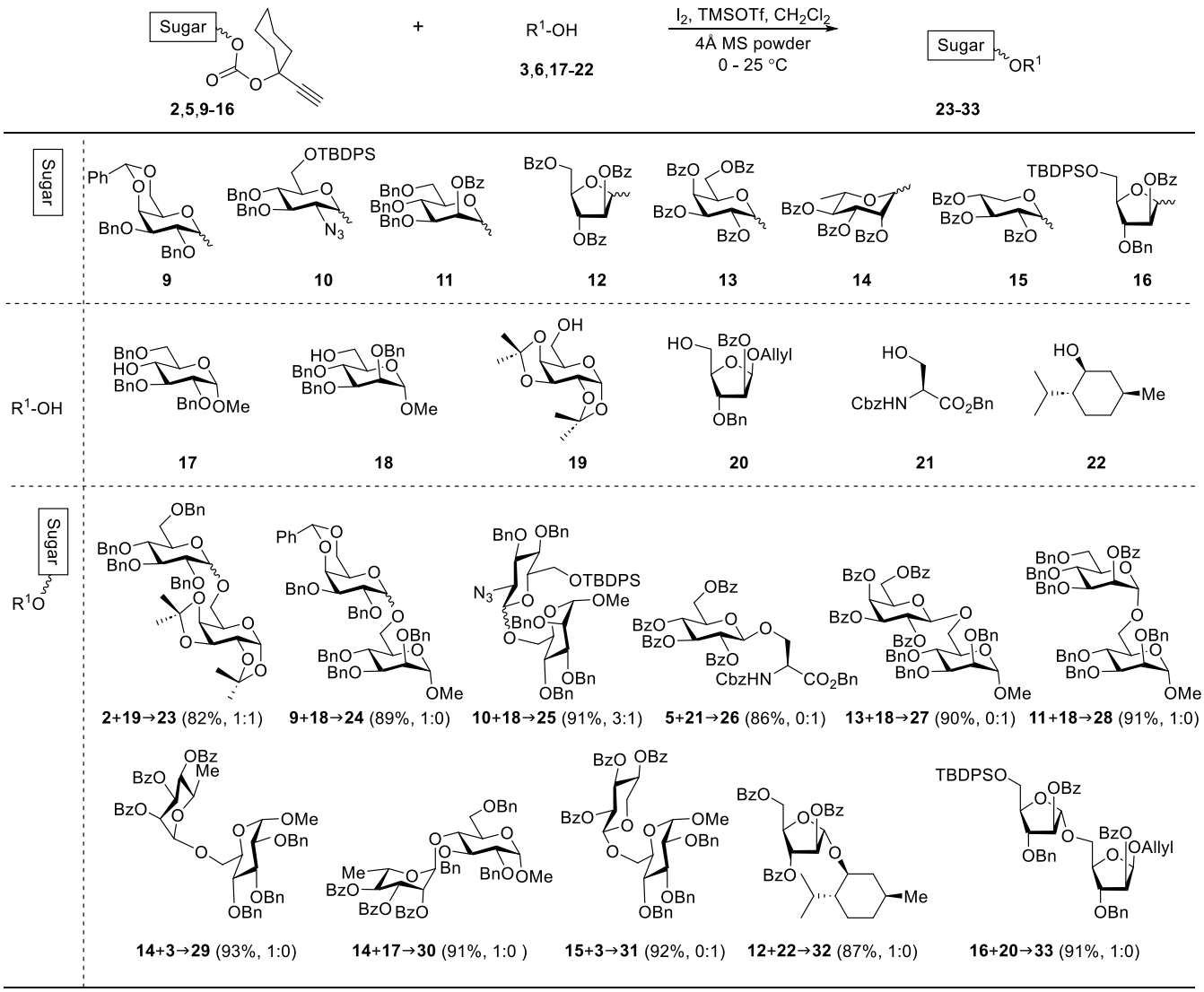
Hygroscopicity of NIS has posed a major concern and thus, the same glycosylations (**2**+**3**→**4** and **5**+**6**→**7**) were performed with

cheap and readily available molecular iodine to notice the formation of disaccharides **4** and **7** in 50% and 10% respectively (entry 5); performance of the reaction (**2**+**3**→**4**) is enhanced by adding TMSOTf (0.15 equivalent) for the disaccharide **4** (entry 6) and addition of 0.5 equivalents of TMSOTf has been identified to be suitable for both the reactions (Scheme 2, entry 7). Control experiments with the simple Lewis acid TMSOTf (0.15 equivalents) alone did not provide the disaccharides under these optimized conditions. Glycosidations performed in CH_3CN and Et_2O proceeded slowly (>24 h) to afford glycosides in <10% yields.

Mechanistically, the alkyne activation by the molecular iodine would activate the alkynyl carbonate (**2**,**5**) producing iodonium species that would be attacked by the carbonyl resulting in the extrusion of the compound **8** and the oxacarbenium ion intermediate **A** (Scheme 3). The latter would be available for the attack of the acceptor **3** in a non-stereoselective fashion to afford anomeric mixture of disaccharides **4**. However, the intermediate **A** resulting from the C2-OBz containing donor **5** would be in equilibrium to produce the trioxalenium ion intermediate **B** that would be amenable for the attack by the acceptor **6** in a 1,2-*trans* selective fashion to produce disaccharide **7**. The stereochemical outcome of the glycosylation was dependent entirely on the C2-substituent; C2-esters giving the 1,2-*trans* selectivity whereas C2-ethers resulted in anomeric mixtures. Additionally, the anomeric ratio or the time taken for the reaction did not alter when glycosylation was conducted with homogenous **2α** or **2β**. The protodeauration noticed previously for the [Au]/[Ag/-catalyzed



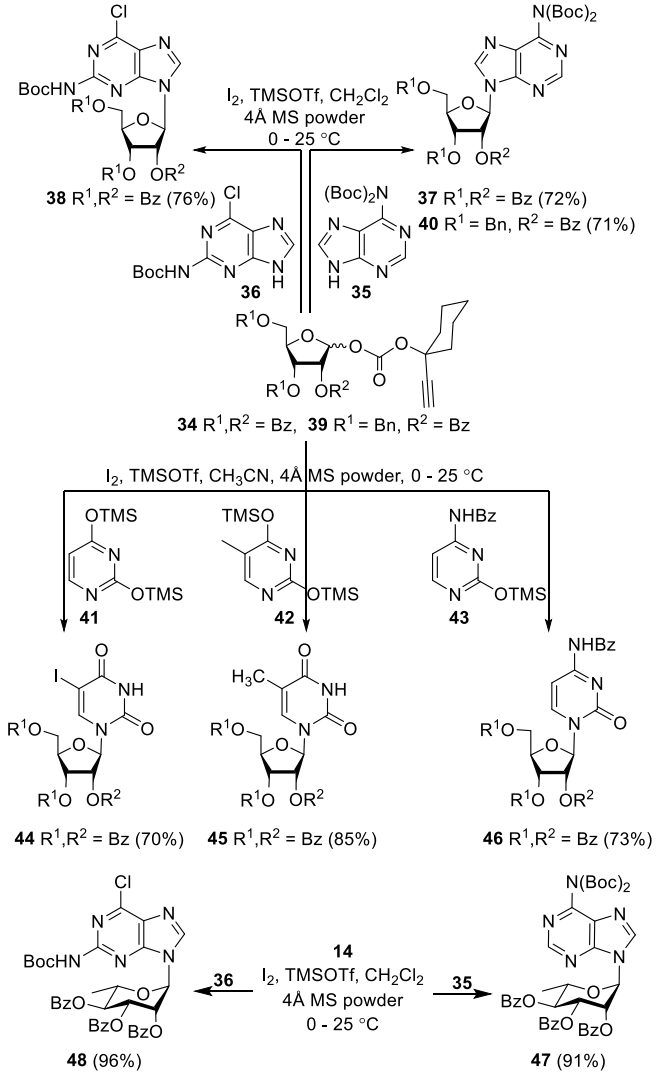
Scheme 3. Plausible mechanism for the activation of ethynylcyclohexyl carbonate glycosides by I_2/TMSOTf. Bz: benzoyl. Bn: benzyl. TMSOTf: trimethylsilyl trifluoromethanesulfonate. Tf: trifluoromethanesulfonyl.



Scheme 4. Substrate scope of the glycosidation by stoichiometric quantities of I_2/TMSOTf. MS: molecular sieves. Cbz: benzyloxycarbonyl. TBDPS: *tert*-butyldiphenylsilyl. TMSOTf: trimethylsilyl trifluoromethanesulfonate

glycosylation was absent and hence, this metal free activation requires stoichiometric quantities of molecular iodine. Gratifyingly, the extrusion product **8** from the carbonate moiety could be crystallized so that its structure could be established by the SCXRD analysis (Scheme 3).[13]

A significant consequence is that the reaction is well-suited with many different reacting partners. For example, the utility of this metal-free activation of glycosyl carbonates was evaluated with a panel of glycosyl donors (**9**-**16**) comprising pyranosyl (**9**-**11, 13**-**15**), furanosyl (**12**, **16**) derived from pentoses and hexoses. In all the glycosylations, carbonate donors having C2-ethers afforded mixtures of 1,2-*trans* and 1,2-*cis* glycosides (**23**-**25**) whereas those that were embedded with C2-benzoates produced 1,2-*trans* glycosides (**26**-**33**) in a stereoselective fashion. Benzylidene acetal, silyl ethers, isopropylidenes survived during the metal-free activation (e.g. **23**-**25** and **33**) (Scheme 4).[8]



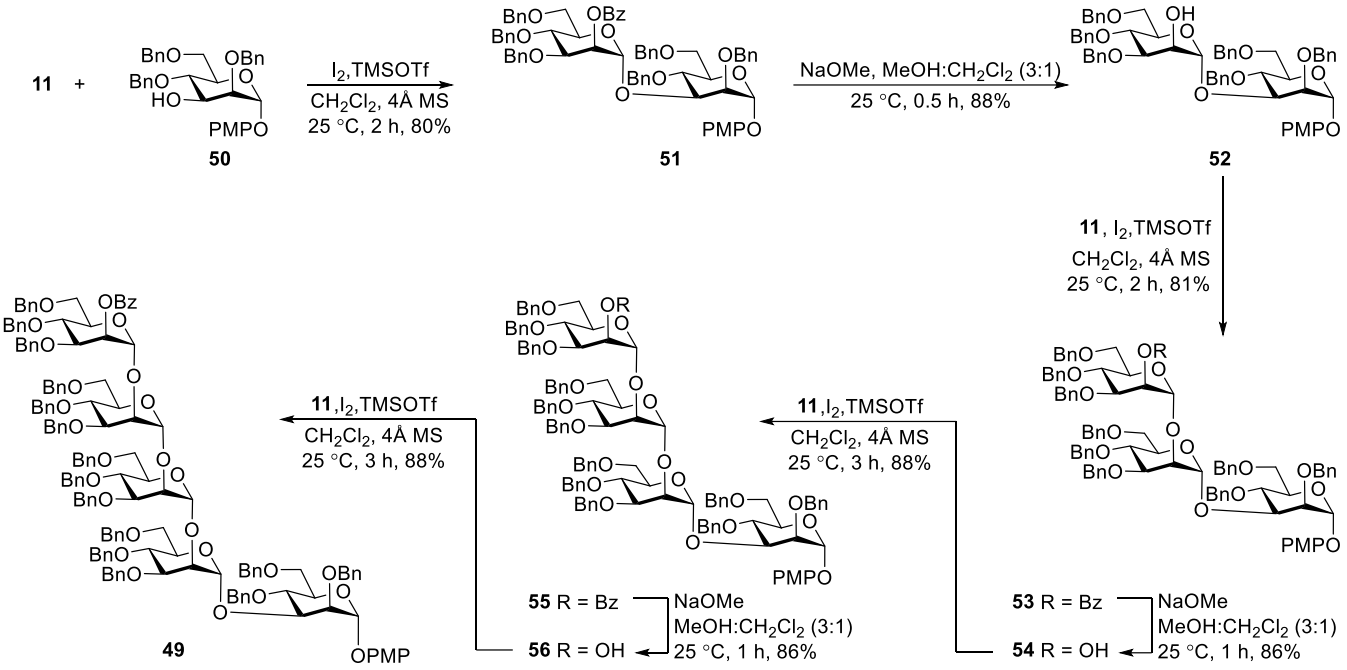
Scheme 5. Synthesis of nucleosides. Boc: tert-butoxycarbonyl. Bz: benzoyl. Bn: benzyl. Tf: trifluoromethanesufonyl. MS: molecular sieves. TMS: trimethylsilyl.

To demonstrate the utility of the metal-free activation, we have investigated the synthesis of purine and pyrimidine nucleosides. Accordingly, Rib*f* (D-ribofuranose)-derived carbonate **34** was treated with the Bis-Boc protected adenine derivative (**35**) to afford the adenosine **37** in 72% yield. The presence of bis-Boc moiety serves the dual role of increasing the solubility of the nucleobase (**35**) in dichloromethane and N^7/N^9-regioselectivity in favour of N^9-site.[5b] Guanosines are conveniently prepared from chloropurine derivative (**36**).[9] Metal-free activation of alkynyl glycosyl carbonate donors **34** produced the chloropurine derivative **38** in 76% yield. Furthermore, nucleosides containing 2'-modifications are important for siRNA therapeutics and probes.[10] In a separate set of experiments, easily synthesized super-armed ribofuranosyl carbonate **39** produced the adenosine derivative **40** in 71% yield. The silyl-modified pyrimidine base **41** resulted in the formation of 5-iodouridine derivative **44** in 70% yield that can be further extrapolated to many important chemical probes through the Sonogashira coupling.[11] Similar glycosylation between the donor **34** and silylated- thymine (**42**) or cytosine (**43**) resulted in the formation of nucleosides **45** and **46** in high yields. The reaction conditions were found to be excellent for the synthesis of non-natural nucleosides **47** and **48** by glycosylating donor **14** with nucleobases **35** and **36** respectively (Scheme 5).

Experience and successful synthesis of the above glycosides, disaccharides and nucleosides has encouraged us to apply the metal-free activation of alkynyl glycosyl carbonate donor to the synthesis of a pentasaccharide motif reminiscent of *Klebsiella pneumoniae* (O-3-antigen).[12] Pneumoniae that is caused by Klebsiella sp. is one of the leading causes of death in patients whose care requires devices such as ventilators or intravenous catheters. Understanding the biochemical pathways leading to the disease is highly desirable for improved prognosis with an overarching goal of a candidate vaccine. O3-Antigen containing polymeric pentamannan is one of the significant carbohydrate epitopes of K. pneumoniae.

A practical synthesis of the pentamannan (**49**) is next targeted. The synthesis effort commenced with the metal-free glycosylation between the glycosyl donor **11** and the easily accessible acceptor **50** gave the disaccharide **51** in 80% yield. Saponification under Zemplén conditions afforded the alcohol **52** that was glycosylated again with the donor **11** under aforementioned conditions to obtain the trimannoside **53** which was again saponified to give the alcohol **54**. One more cycle of glycosylation→saponification →glycosylation afforded the desired pentamannan **49** in a very facile manner with an overall yield of 37% from monosaccharides **11** and **50** (Scheme 6).

In summary, stable alkynyl glycosyl carbonate donors were activated under metal-free conditions using stoichiometric quantity of molecular iodine and TMSOTf. The extrusion product resulting from the carbonate moiety was characterized by the SCXRD to be the vinylidene cyclic carbonate. Apart from the use of stoichiometric quantities, glycosylations afford glycosides, disaccharides and nucleosides in a very simple fashion. The reaction conditions are tolerated well by most of the protecting groups (benzyl, benzoyl, TBDPS, benzylidene acetal, isopropylidene). The glycosylations produced anomeric mixtures when C2-position of the donor was occupied by the benzyl ethers or azide; whereas, 1,2-*trans* glycosides were stereoselectively obtained when the C2-position was equipped with the participating benzoates due to the well-known trioxolenium ion intermediate formed due to the anchimeric assistance. Finally, the utility of the metal-free stoichiometric activation of alkynyl glycosyl carbonates method was illustrated with the successful synthesis of a pentamannan moiety of the *Klebsiella pneumoniae* O3-antigen. These results demonstrate that the alkynyl glycosyl carbonates can be activated by I_2 (1.5 equivalents)/TMSOTf(0.5 equivalents) for the synthesis of glycosides and oligosaccharides.



Scheme 6. Synthesis of the pentasaccharide repeating motif reminiscent of *Klebsiella pneumoniae* (O-3-antigen). Bz: benzoyl. Bn: benzyl. Tf: trifluoromethanesufonyl. MS: molecular sieves. PMP: *p*-methoxyphenyl

Experimental Section

General Methods. Unless otherwise noted, materials were obtained from commercial suppliers and were used without purification. 1-ethynylcyclohexanol, *p*-nitrophenyl chloroformate, purine & pyridine bases, *N*-iodosuccinimide, iodine (I_2), trimethylsilyl trifluoromethanesulfonate, 4Å molecular sieves and *N*, *O*-bistrifluoroacetamide (BSTFA) were purchased from Sigma-Aldrich and used as they were received. Unless otherwise noted, all reactions were performed under nitrogen atmosphere. Solvent was removed under vacuum. Products obtained as solids or syrups or gums were further dried under high vacuum. Analytical thin layer chromatography was performed for monitoring of reactions on pre-coated silica plates (F_{254}, 0.25 mm thickness), compounds were visualised by UV light or by staining with anisaldehyde spray. Specific rotations were measured by using digital polarimeter. IR spectra were recorded on FT-IR spectrometer. NMR spectra were recorded either on Bruker 400MHz or 600MHz NMR spectrometer using $CDCl_3$ as a solvent and TMS as internal standard. All the *J*-values are in Hz. High resolution mass spectroscopy (HRMS) was performed using ESI-TOF mass analyser. Single crystal data was obtained on Bruker APEX(II) DUO CCD diffractometer using Mo Kα radiation (λ=0.71073Å) at 150K.

Synthesis of 1-ethynylcyclohexyl carbonate donors (**2**, **5**, **9**-**16**, **34**, **39**): To a dichloromethane (6 mL/g) solution of hemiacetals (1.0 mmol), 1-ethynylcyclohexyl (4-nitrophenyl) carbonate (1.2 mmol) and DMAP (1.2 mmol) were added and stirred for 4 h at 25 °C. After complete conversion of the hemiacetals as adjudged by the TLC analysis, the reaction mixture was diluted by adding CH_2Cl_2 and washed with aqueous saturated $NaHCO_3$ to remove the 4-nitrophenol. Combined organic layers were dried over anhydrous sodium sulphate, filtered and the filtrate was concentrated *in vacuo* to obtain an oily residue that was purified by silica gel column chromatography.

General procedure for the synthesis of *O*-glycosides (**4**, **7**, **23**-**33**, **51**, **53**, **55**, **49**): To a solution of glycosyl donor (1.0 mmol) and glycosyl acceptor (1.0 mmol) in anhydrous CH_2Cl_2 (3 mL/g of donor), activated 4Å MS powder was added and stirred for 15 min, molecular iodine (1.5 mmol) and TMSOTf (0.1 mmol for armed donors, 0.5 mmol for disarmed donor) were added and stirred at 25 °C. Triethylamine was added to quench the reaction, filtered through a bed of Celite®, the filtrate was concentrated *in vacuo*, and purified by silica gel column chromatography.

General procedure for the synthesis of purine nucleosides (**37**, **38**, **40**, **47**,**48**): Glycosyl donor (1.0 mmol) and protected purine base (1.0 mmol) were dissolved in anhydrous CH_2Cl_2 (6 mL/g of donor) containing freshly activated 4Å MS powder. After stirring for 15 min, molecular iodine (1.5 mmol) and TMSOTf (0.5 mmol) were introduced into the reaction mixture, continued stirring for 3 h. Triethylamine was added to arrest the reaction, filtered through a bed of Celite®, the filtrate was concentrated *in vacuo* and the resulting residue was purified by silica gel column chromatography.

General procedure for the synthesis of pyrimidine nucleosides (**44**, **45**, **46**): To a suspension of pyrimidine base (1.5 mmol) in acetonitrile (10 mL/g of donor), *N*,*O*-bistrifluoroacetamide (BSTFA) (4.0 mmol) was added and stirred vigorously at 25 °C for 30 min or until a clear solution was noticed. In a separate round bottom flask, glycosyl donor (1.0 mmol) and activated 4Å MS powder in acetonitrile were stirred for 15 mins under nitrogen atmosphere and the above prepared silylated nucleobase was cannulated into this flask. The reaction vessel was cooled to 0 °C, I_2 (1.5 mmol) and TMSOTf (0.5 mmol) were added, reaction temperature was warmed to 25 °C and stirred for 3 h. Triethylamine was added to arrest the reaction, passed through a pad of Celite®, the filtrate was concentrated *in vacuo*, and purified by silica gel column chromatography.

Acknowledgements

KP acknowledges fellowship from CSIR-NET. SH, SP, FJ, MT thank CEFIPRA-New Delhi (6305-1) for the financial support.

Keywords: Glycosides • Glycosylation • Glycoconjugates • Nucleosides • Oligosaccharides

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[13] CCDC-2246866 contains the supplementary crystallographic data of compound **8**. The data can be obtained free of charge from The Cambridge Crystallographic Data via https://www.ccdc.cam.ac.uk/structures/

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