รายงานวิจัยฉบับสมบูรณ์

โครงการ
การเตรียมสาร amino-triazoleglycosidesชนิดใหม่
ด้วยวิธีที่ง่ายเพื่อตรวจสอบฤทธิ์ต้านมะเร็ง

Preparation of new amino-triazoles glycosides by simple method
for evaluation of anticancer activity

รุ่งนภา แซ่เอ็งและคณะ

โครงการวิจัยประเภทงบประมาณเงินรายได้
จากเงินอุดหนุนรัฐบาล (งบประมาณแผ่นดิน)
ประจำปีงบประมาณ 2560
มหาวิทยาลัยบูรพา

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29 มีนาคม 2560

กิตติกรรมประกาศ

งานวิจัยนี้ได้รับทุนสนับสนุนการวิจัยจากงบประมาณเงินรายได้จากเงินอุดหนุนรัฐบาล
(งบประมาณแผ่นดิน) ประจำปีงบประมาณพ.ศ. 2560 มหาวิทยาลัยบูรพา
ผ่านสำนักงานคณะกรรมการการวิจัยแห่งชาติเลขที่สัญญา100/2560

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คำนำ

โครงการวิจัย “การเตรียมสาร amino-triazoleglycosidesชนิดใหม่ด้วยวิธีที่ง่ายเพื่อตรวจสอบฤทธิ์ต้านมะเร็ง” ได้รับการสนับสนุนทุนการวิจัยงบประมาณแผ่นดินประจำปีงบประมาณ 2560 มหาวิทยาลัยบูรพา รายงานการวิจัยฉบับนี้เสนอรายละเอียดของการวิจัยซึ่งประกอบด้วยบทนำที่เสนอผลงานวิจัยที่เกี่ยวข้อง ผลการทดลองวิจัย การอภิปรายสรุปผล และการตรวจสอบโครงสร้างของสาร

การวิจัย “การเตรียมสาร amino-triazoleglycosidesชนิดใหม่ด้วยวิธีที่ง่ายเพื่อตรวจสอบฤทธิ์ต้านมะเร็ง”สำเร็จลุล่วงไปด้วยดีโดยผู้วิจัยต้องขอขอบคุณทีมวิจัยซึ่งประกอบด้วยที่ปรึกษาโครงการศ.ดร. อภิชาต สุขสำราญคณะวิทยาศาสตร์ มหาวิทยาลัยรามคำแหง ศ.ดร. ภาวิณีปิยะจตุรวัฒน์มหาวิทยาลัยมหิดล ผู้ร่วมโครงการดร. อุทัยวรรณศิริอ่อนรวมทั้งนิสิตปริญญาเอก สาขาวิชาเคมี นางสาววราภรณ์ สุขจริตรักษ์งานวิจัยนี้ได้รับการสนับสนุนจากภาควิชาเคมี คณะวิทยาศาสตร์และทุนเรียนดีวิทยาศาสตร์แห่งประเทศไทย

ผศ. ดร. รุ่งนภา แซ่เอ็ง
อาจารย์ประจำภาควิชาเคมี คณะวิทยาศาสตร์
หัวหน้าโครงการวิจัย

บทคัดย่อ

คาร์โบไฮเดรตเป็นสารที่มีประโยชน์ในธรรมชาติและได้รับการยอมรับว่าเป็นสารสำคัญในการใช้เป็นสารตั้งต้นเพื่อสังเคราะห์ผลิตภัณฑ์ธรรมชาติที่มีฤทธิ์ทางชีวภาพสำหรับอนุพันธ์1,2,3-triazoles ได้รับความสนใจเป็นอย่างมากในการประยุกต์ใช้จากฤทธิ์ทางชีวภาพที่หลากหลายนอกจากนี้สารประกอบเอมีนยังมีบทบาทสำคัญในการสังเคราะห์สารอินทรีย์เนื่องจากใช้เป็นสารสังเคราะห์สำหรับการผลิตยา เคมีภัณฑ์และสารออกฤทธิ์ทางชีวภาพการสังเคราะห์สารกลุ่มนี้ได้รับความสนใจอย่างมากสำหรับนักเคมีอินทรีย์งานวิจัยนี้มีวัตถุประสงค์เพื่อสังเคราะห์สารโดยประกอบรวมสารทั้งสารกลุ่มเหล่านี้เข้าด้วยกันในโมเลกุล "amino triazole glycoside" และพัฒนาวิธีการที่สะดวกในการสังเคราะห์สารชนิดนี้โดยผ่านการทำปฏิกิริยาสองขั้นตอนในหนึ่งหม้อปฏิกิริยาโดยวิธีalkylation ของอนุพันธ์ของ amine กับ propargyl bromide เพื่อให้propargylamineในขั้นตอนแรกตามมาด้วยปฏิกิริยา 'click' กับ azido-glycoside โดยใช้CuIในน้ำ ได้สารผลิตภัณฑ์amino-triazole glycosides ที่ให้ผลผลิตปานกลางถึงดี

Abstract

Carbohydrates are utility in nature and recognized as key substances using as starting materials in the synthesis of biologically active natural products.1,2,3-Triazole derivatives have received much attention for their application and wide variety of bioactivities. In addition, amines play an important role in organic synthesis due to their wide use as synthetic intermediates for the production of pharmacophores,fine chemicals, agrochemicals and bioactive compounds. All of their synthesis has received much interest for organic chemist. This work was aimed to combine these three classes of compounds in the same molecule “amino triazole glycoside”and to develop a convenient method for the synthesis of thistype of compounds*via* one-pot two steps reaction.The alkylation of amine derivatives with propargyl bromide to give propargylamine was performed in the first step subsequently followed by a ‘click’ reaction with various azido-glycoside in the presence of CuI in aqueous solution to provide amino-triazole glycosides in moderate to good yield.

Chapter 1 Introduction and Literature reviews

Introduction

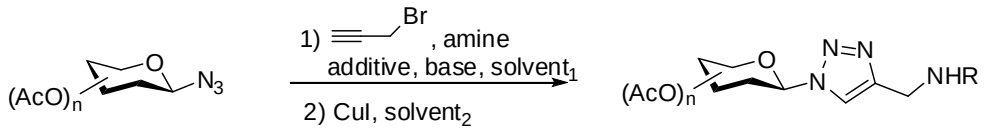
Carbohydrates are main energy source of biological in most cells provide energy to the body. Many carbohydrate-containing complex natural compounds are found in nature as important biological substances (Narayanaperumal et al, 2012) such asamygdalin (vitamin B17) and salicin (anti-inflammatory agent in the human body). Current definition is that "the carbohydrates" are a much large family of compounds, comprising monosaccharide, oligosaccharide and polysaccharide. Carbohydrate can also be covalently linked to other biopolymers, such as lipids and proteins (Boons and Hale, 2000). Carbohydrates are ultilized as substrates to synthesize useful complex bioactive compounds. In addition, they exist as oligo- and polysaccharides or included in aglycon structures as glycol-derivatives with roles in a broad range of biological processes (Filice and Palomo, 2012).

Amine in general are one of most common structural features of naturally and unnatural synthetic targets. Amine and their derivatives are the most widely and effective in biologically active compounds and used throughout the chemical industry (Salvatore, Nagle, & Jung, 2002; Singh,Kavala, Samal& Patel, 2007).

1,2,3-triazoles have found wide range of application and biological activities such as anti-HIV (Silva et al., 2009), antimicrobial (Banday et al., 2012; Sumangala et al., 2010), antibiotics (Agalave et al., 2011), anticancer (Salmon et al., 2012) and α-glucosidase inhibition (Ferreira et al., 2010; Senger et al., 2012).The popularity of 1,2,3-triazoles is largely driven by their facile and modular synthesis via click chemistry. Click chemistry was established by Sharpless and co-worker in 2001, which is addressing as a set of powerful, highly reliable,and selective reactions for the rapid synthesis of useful new compounds and combinatorial libraries. This reaction is wide in scopeof chemistry, including organic,medicinal, materials, surface, polymer chemistry and chemical biology (Sokolova and Nenajdenko, 2013). Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAC) has become the prime example of click reactions and that moreimportantly, many applications thereof not only aimed to linktwo units together but also to synthesize the triazole moiety in the complex molecules (Schulzeab and Schubert, 2014).

From the biological activities of carbohydrate, amine and triazole, we designed to combine all of these fragments together to study the biological activity.

In this work, we will develop a new synthetic methodologyfor the synthesis of Amino-Triazole Glycoside from sugar derivatives via one-pot reaction. All synthetic analogues will be further study for biological activity. 1-Azido-β-D-sugar will be prepared and used as a starting molecule. The one-pot of N-alkylation and click reaction for the synthesis of amino triazole-glycoside derivatives from commercial available amine will be carried out.



1 **2**

Scheme 1.1 The one-pot synthesis of amino triazole-glycoside from carbohydrate derivatives

Literature reviews

Carbohydrates are utility in nature forming complex and recognized as key substances to starting materials in the synthesis of biologically active natural products.

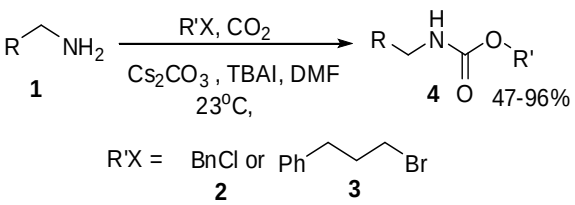
1,2,3-Triazole derivatives have received much attention for their application and wide variety of bioactivities. The most popular method for construction of 1,2,3-triazoles frameworks is the click reaction or 1,3-dipolar Huisgencycloaddition reaction of azide with alkynes. Therefore, it is desirable to develop a new, convenient and regiocontrolled synthetic approach for the formation of triazoles (Liang et al., 2005).

Amines play an important role in organic synthesis due to theirwide use as synthetic intermediates for the production of pharmacophores,fine chemicals, agrochemicals, bioactive compounds, polymers, and dyes (Cheng et al., 2014). N-Alkylated amines are very important intermediates in the organic synthesis chemistryand fine chemical industries.The most common method for the synthesis ofN-alkylated aminesis the nucleophilic substitution reaction of amines or ammoniawith alkyl halides, sulfonates, etc. The review of selected examples of various reactions using in this work is presented as follow.

2.1 Selected examples of preparation of amine derivatives by *N*-Alkylation

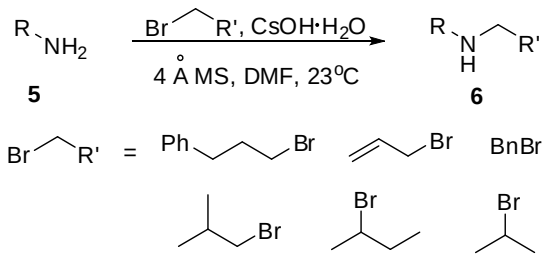
The *N*-alkylation of amines is important reaction in preparation of amine derivatives. There are a lot of applications in the field of pharmaceuticals, agriculture, popular in drug development and useful synthetic intermediates.

Salvatore, Shin, Nagle and Jung (2001) reported the synthesis of carbamates derivatives **4** in the present of three-components coupling which was performed using aliphatic, aromatic and heterocyclic amines contain electron donating and electron withdrawing group**1**, carbon dioxide and reactive and unreactive halides **2** or **3** by applying a similar procedure to carbonylation protocol. The reaction mixture was saturated with CO_2 in the presence of Cs_2CO_3 at room temperature in DMF as solvent. Tetrabutylammonium (TBAI) was found to be a crucial additive in averting direct N-alkylations and overalkylation of the produced carbamate. (Scheme **2.1**)



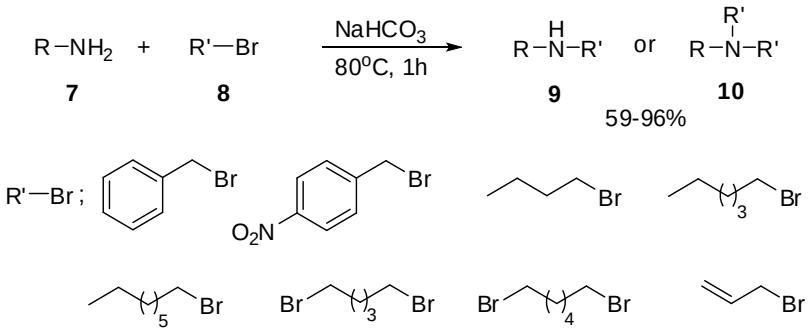
Scheme 2.1 Synthesis of carbamates derivatives **4**.

Salvatore, Shin, Nagle and Jung (2002) showed that the method using cesium hydroxide for the chemoselectiveN-alkylation of primary amines **5**, afforded secondary amines predominantly or exclusively. Observed selectivities between mono- and dialkylations were typically on the order of 9:1 respectively or higher chemoselectivity is "cesium effect". Reaction of amines united with various bromide produced the derivatives amines **6**in the presence of high yield using powdered dry 4 Å molecular sieves in anhydrous DMF. It was found that the inclusion molecular sieves accelerated the alkylation as well as improved the selectivity and yield of the secondary amine. This methodology proves to be a general protocol for the syntheses of various secondary amines, offering a wide variety of applications(Scheme **2.2**).



Scheme 2.2*N*-alkylation of amines **2.15**.

Singh, Kavala, Samal and Patel (2007) showed that the synthesis of secondary and tertiary amines **9** or **10**by direct N-alkylation of primary or secondary amines **7** with various benzylic halides or aliphatic halides **8** in an aqueous medium in the presence of a mild inorganic base such as $NaHCO_3$ and sodium dodecyl sulfate, the product were obtained in excellent yields. The advantage of this method is scalability, the absence of quaternary ammonium salt formation and operationally convenient conditions. (Scheme **2.3**)

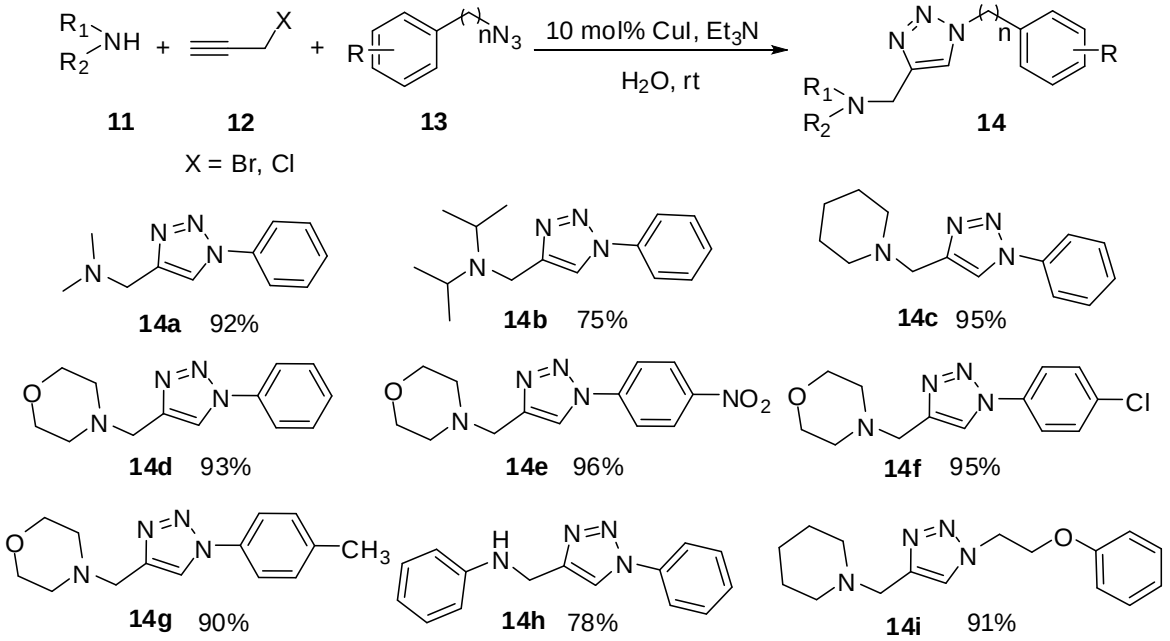


Scheme 2.3 Synthesis of secondary and tertiary amines **9** or **10**.

2.2 Selected examples of 1,4-disubstituted-1,2,3-triazoles formations by click chemistry

1,2,3-triazole derivatives have found application and biological activities such as agricultural, pharmaceutical, material, anti-HIV and anticancer. The synthesis of 1,2,3-triazole compounds employ easy method of Cu(I)-catalyze Huisgen 1,3-dipolar cycloaddition of azides and alkynes.

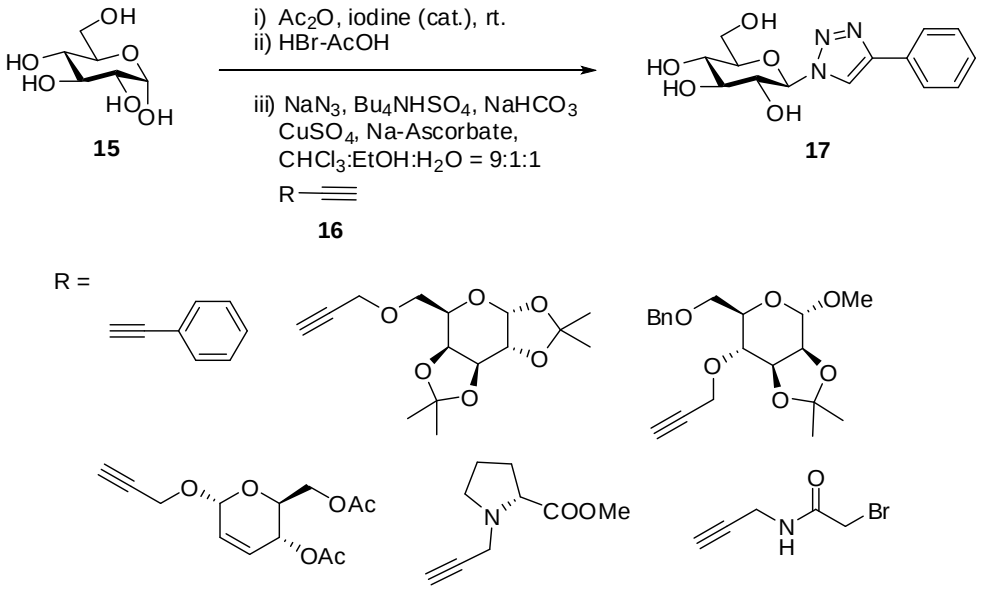
Yan, Zhao, Fan, Liu and Liang (2005) reported the synthesis of (1-substituted-1H-1,2,3-triazol-4-ylmethyl)-dialkylamines derivatives **14a-i** in the present of one-pot reaction of amines **11**, propargyl halides **12**, azides**13** and Cu(I) in water at room temperature. The substrates variety of substituents aromatic, benzyl and aliphatic with electron-withdrowing and electron-donation group were employed for this study. Twenty examples of triazole amine derivatives were afforded in 70-98% yield using this one pot procedure. Some examples are shown in Scheme **2.4**.



Scheme 2.4 Synthesis of (1-substituted-1*H*-1,2,3-triazol-4-ylmethyl)-dialkylamines**14a-h**.

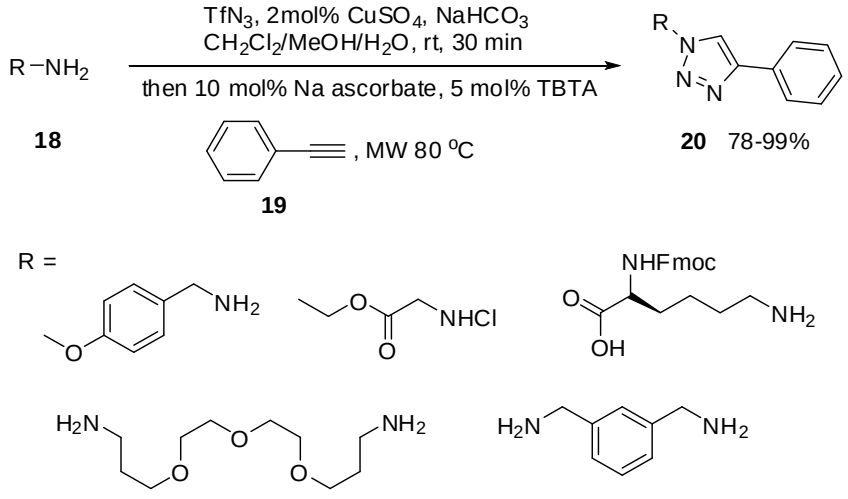
Chittaboina,Xie and Wang (2005) reported the highly efficient one-pot four steps synthesis of 1,2,3-triazole-linked glycoconjugates using unprotected saccharide **15**with acetic anhydride and trace amount of iodine, followed by brominolysis of anomeric acetate and subsequent azide conversion followed by click reaction with alkyne**16** in the presence of Cu(I) as a catalyst to give 1,2,3-triazoles-linked glycoconjugate**17** in excellent yield. By

executing several reaction steps in a single pot and purifying only at the final stage, this procedure excludes the isolation of all the intermadiates including labile glycosyl bromides, which significantly reduces the reaction time and improves the overall yield (Scheme **2.5**).



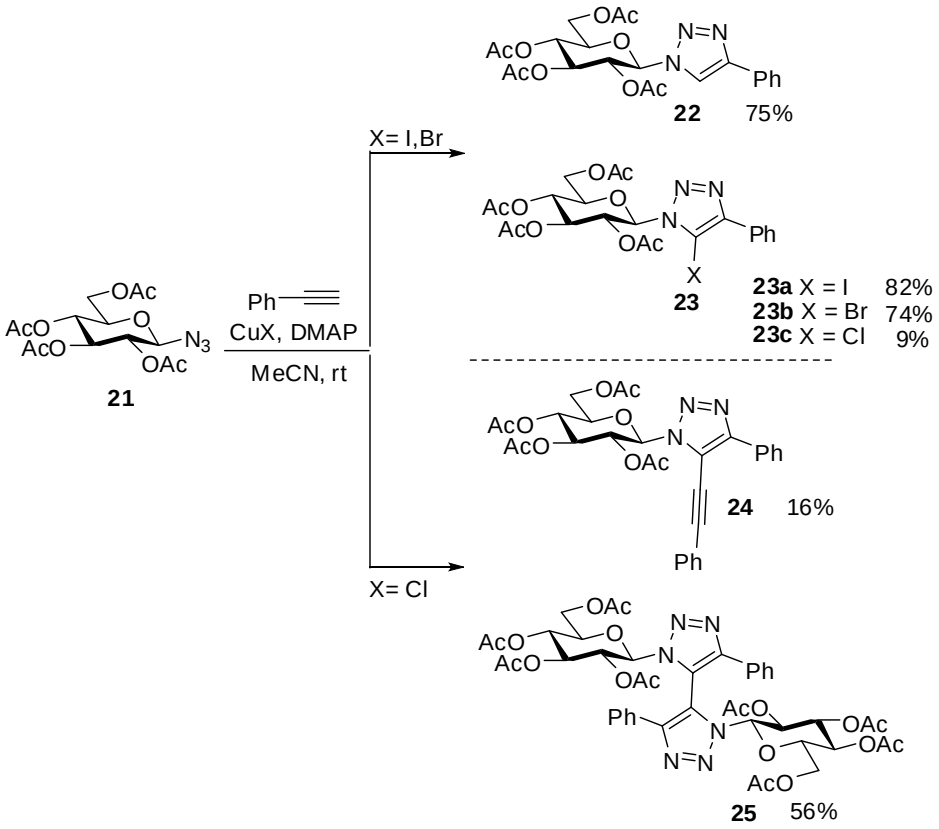
Scheme 2.5 Synthesis of 1,2,3-triazole-linked glycoconjugates**17**.

Beckmann and Wittmann (2007) reported the synthesis of 1,4-Disubstituted 1,2,3-triazoles **20** using amines **18** and alkyne **19**by one-pot reaction of Cu(II)-catalyzed diazo transfer at ambient temperature using sodium bicarbonate as base and Cu(I)-catalyzed azide-alkyne 1,3-dipolar cycloaddition by heating to 80^{O}C with microwave irradiation for avoiding the isolation of the azide intermediates to obtain high yield of products within reasonable reaction times. (Scheme **2.6**)



Scheme 2.6 Synthesis of 1,4-Disubstituted 1,2,3-triazoles **20**.

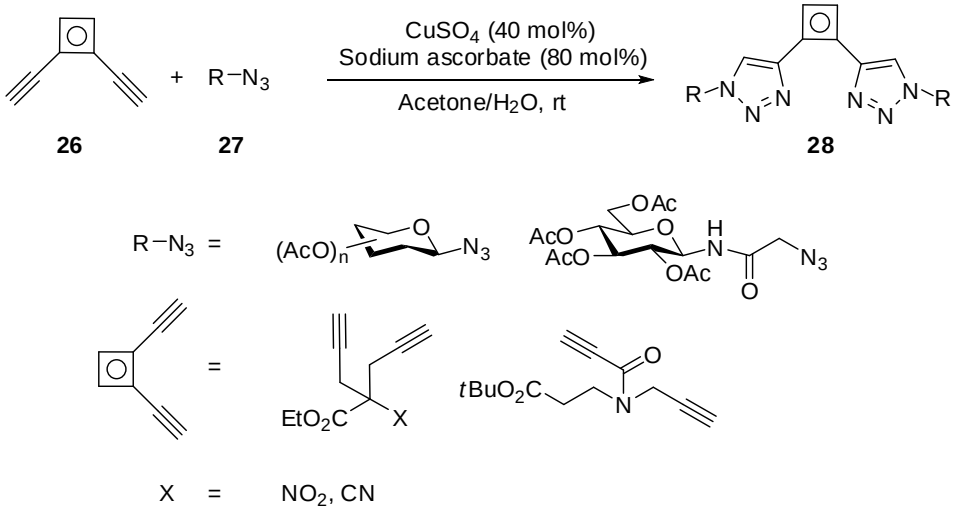
Goyard, Praly and vidal (2012) reported the synthesis of 5-halogenated 1,2,3-triazole under smooth condition using Cu(I)-halides (i.e., CuI, CuBr and CuCl), alkyne and DMAP to carry out the 1,3-dipolar cycloaddition and halogenation in one-pot reaction to obtain compound **24** (16% yield) and **25** (56% yield) in the presence of CuCl. Formation of the 5-proto-triazole **22** (75% yield) as a single product when using 0.1 equiv of CuI. The presence of iodinated derivative **23a**(82% yield) could be detected when using larger amounts of CuI. The 5-brominated 1,2,3-triazoles **23b**(74% yield) were prepared from excess CuBr 2 equiv. The introduction of a chlorine atom at the 5-position of the triazole ring of **23c** was more difficult and product was isolated in only 9% yield. The methodology reported herein appears as a valuable, no additiveand high yields. (Scheme **2.7**)



Scheme 2.7 Azide-alkyne 1,3-dipolar cycloaddition using of Cu(I)-halides and their corresponding 1,2,3-triazoles.

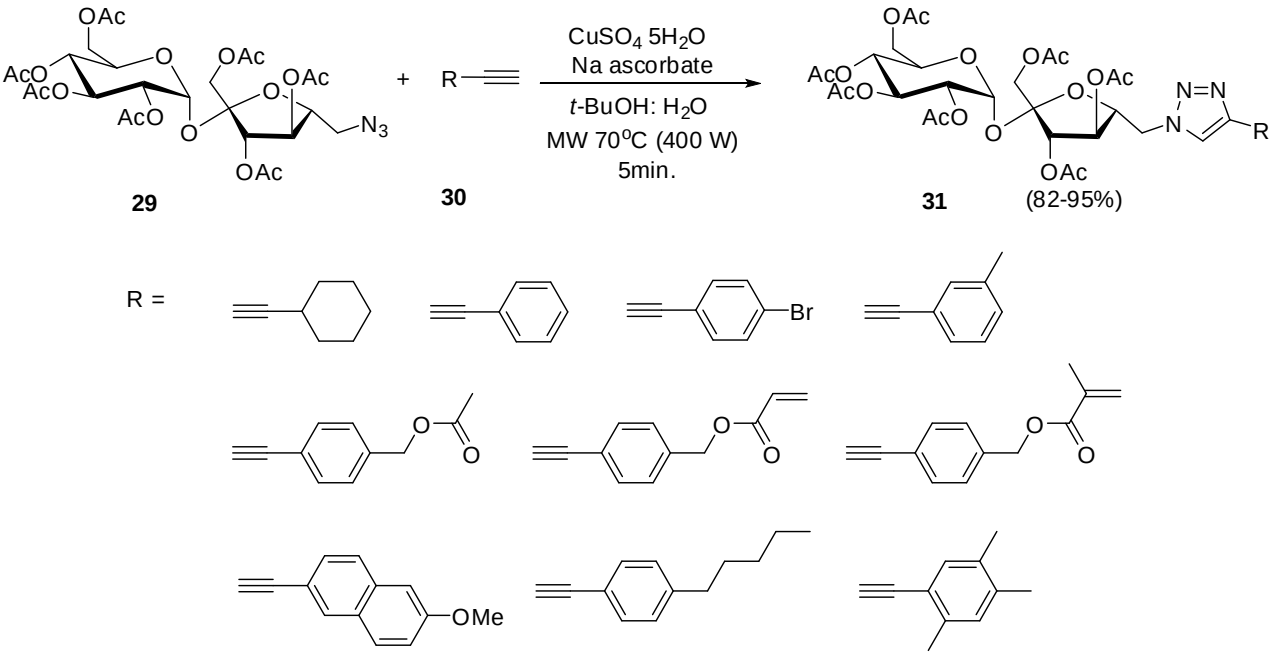
Sahoo, Singhamahapatra, Kumar and Loganathan (2013) reported the synthesis of triazole-linked divalent glycopeptide mimics **28**with conceptually new divalent linkers. The alkyne-functionalized divalent building block was used for the Cu(I)-catalyst [3+2] cycloadditionreacton with per-*O*-acetylated glycosylazides or azidoacetamide to synthesize

the triazole-linked divalent glycoconjugates with different linkers. The dialkyne-functionalized building blocks can be used for the synthesis of unsymmerical divalent compounds by sequential click reaction with different sugar azides and azidoaceamides. The peptide-based divalent glycoconjugates with two chemically non-equivalent triazolering have higher conformational flexibility and will be useful for biological analysis(Scheme **2.8**).



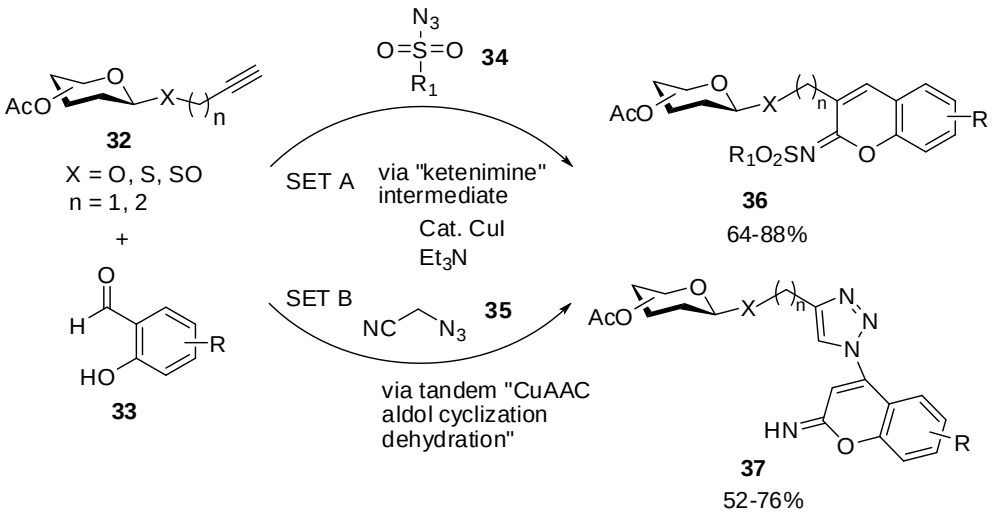
Scheme 2.8 Synthesis of triazole-linked divalent glycoamino acid mimics**28**.

Potewar, Petrova and Barros (2013)reported the click reaction of 1,2,3-triazole-sucrose derived **29**with various alkynes **30** in the presence of$CuSO_4$•$5H_2O$ catalyst, sodium ascorbate in *tert*-BuOH/H_2O (1:1) at 70^{o}Cusing microwave assisted to give 1-4-phenyl-1,2,3-triazole **31** in excellent yields in short reaction times (Scheme **2.9**).



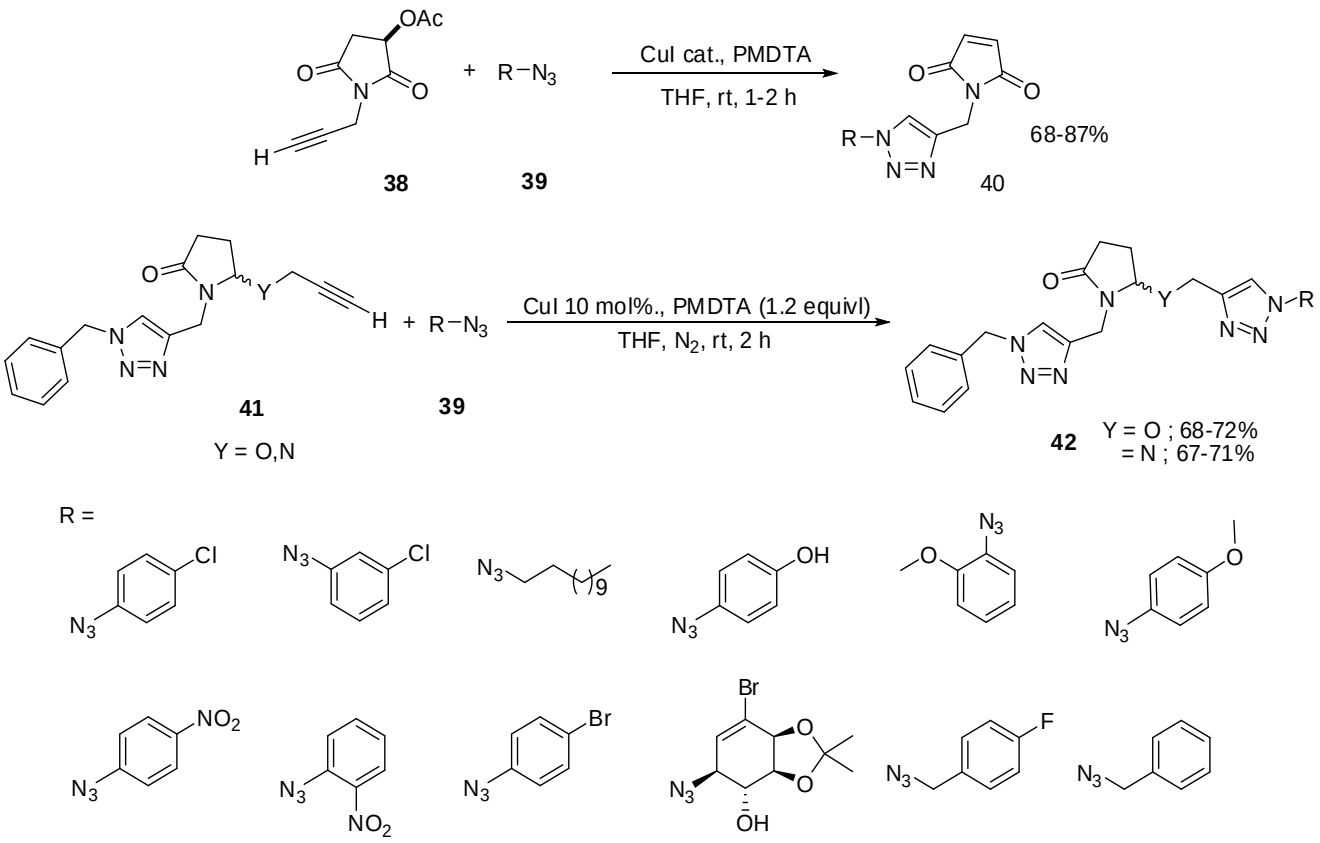
Scheme 2.9 Synthesis of 1-4-phenyl-1,2,3-triazole **31**.

Mandal (2014) reported the synthesis of glycosyl-iminocoumarins**36** in one-pot reaction fromsulfonylazides and salicylaldehydes in presence of CuI and triethlamine*via*ketenimine intermediate. Similarly, reaction of propargyl glycoside with slicylaldehyde and sulfonylazidegaveglycosyl 3-triazolyl-2-iminocoumarin derivative**37** in one-pot three component condensation via copper-catalyzed Huisgencycloaddition reaction. The method has been achieved to give product in good yields(Scheme **2.10**).



Scheme 2.10 Synthesis of glycosylated iminocoumarin**36**and 3-triazolyl-2-iminocounarinderivative**37**

Stefani, Ferreira, Ali and Pementa (2014) reported the synthesis offunctionalized*N*-triazolylmaleimides**40**and *N*-triazolyl-5-triazolyl pyrrolinones**42** in two steps one-pot elimination and click reaction. The reactions of *N*-propargyl imide with aromatic and alkyl azides were promoted by CuI using PMDTA as base and tetradydrofuran as a solvent at room temperatrue.The reaction was investigated with a variety of organic azides such as aromatic and non-aromatic organic azides which contained of electron-withdrawing and electron-donating to obtain products in moderate to good yields(Scheme **2.11**).

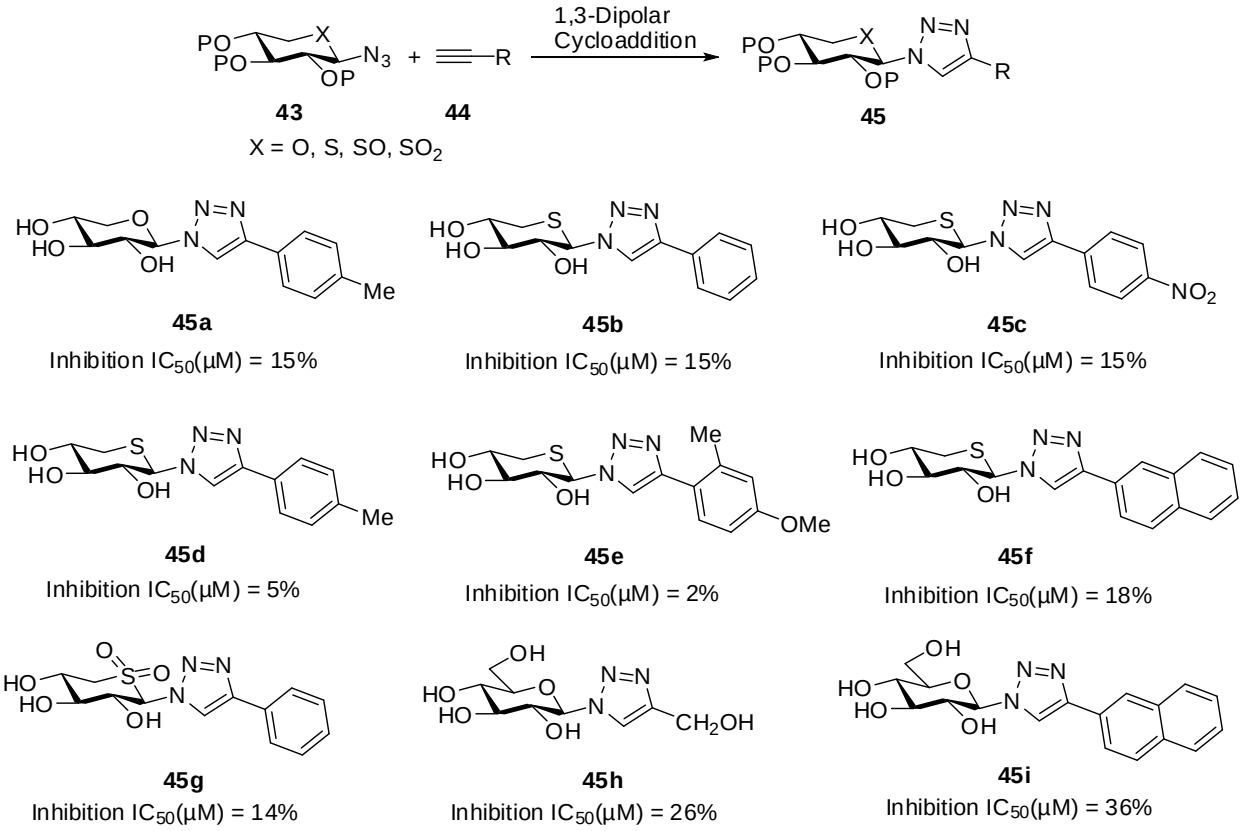


Scheme 2.11 Synthesis of *N*-tirazolylmaleimides**40**and *N*-triazolyl-5-triazolyl pyrrolinones**42**.

2.3 Application of 1,2,3-triazole compounds in medicinal chemistry.

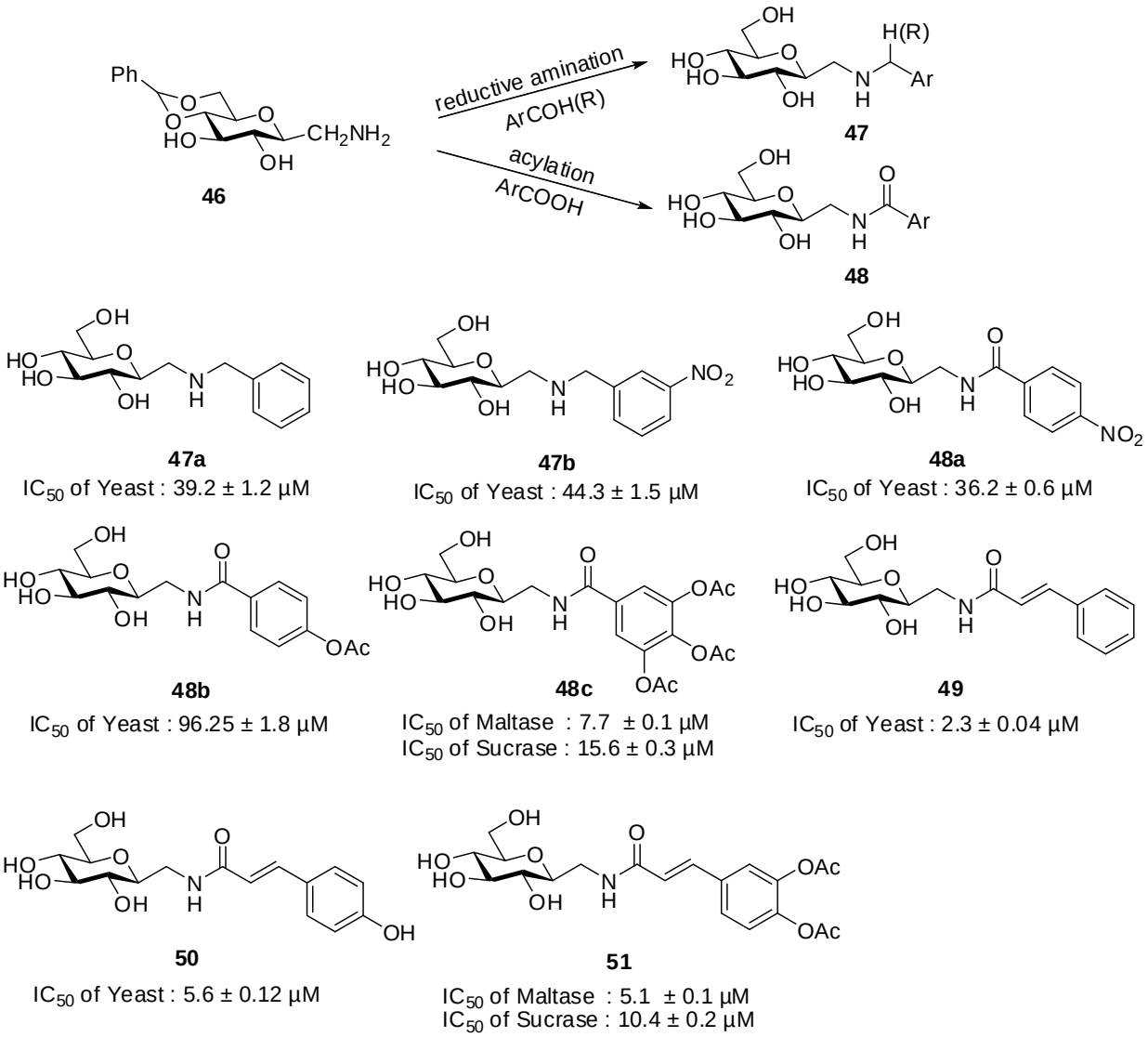
The 1,2,3-triazole ring derivatives are important building blocks used as antibacterial agent, pharmaceutical intermediates, anti-hyperglycemic, α-glucosidase inhibitors.

Goyard, Baron, Skourti, Chajistamatiou, Docsa,Gergely, Chrysina, Praly and Vidal (2012) reported the synthesis of 1,2,3-triazoles from xylosyl and 5-thioxylosyl azides. The reaction was coupled by Cu(I)-catalyzed of alkynes to the corresponding D-xylopyranosylazide. Oxidation of the sulfur atom afforded the sulfoxide and sulfone analogues in good yields. Deacetylation led to product which was evaluated as RMGP*b* inhibitors. Compared to glucose-based analogues, sulfoxide and sulfone analogues appeared to be much weaker inhibitors of glycogen phosphorylase, as the absence of a hydroxymethyl group weakens their binding at the enzyme active site(Scheme **2.12**).



Scheme 2.12 Synthesis of 1,2,3-triazoles from xylosyl and 5-thioxylosyl azides derivative **45a-i**.

Bian, Fan, Ke, Luan, Zhao and Zeng (2013) reported the synthesis ofN-substituted 1-amino methyl-β-D-glucopyranoside derivatives which were prepared from aminomethyl-4-6-*O*-benzylidene-β-D-glucopyranoside intermediate through reductive amination or acylation. These sugar mimetics were found to be α-glycosidase inhibitors from yeast and rat intestine (maltase and sucrase) with IC_{50}values covering a wide range from 2.3 μM to 2.0 μM in assay. Compound **50** and **51** were identified as the most potent inhibitors for yeast α-glucosidase of maltase and sucrase. The results suggest that the amino-methyl-β-D-glucopyranoside moiety can mimic the substates of α-glucosidase in the enzyme catalytic site leading to competitive enzyme inhibition. (Scheme **2.13**)



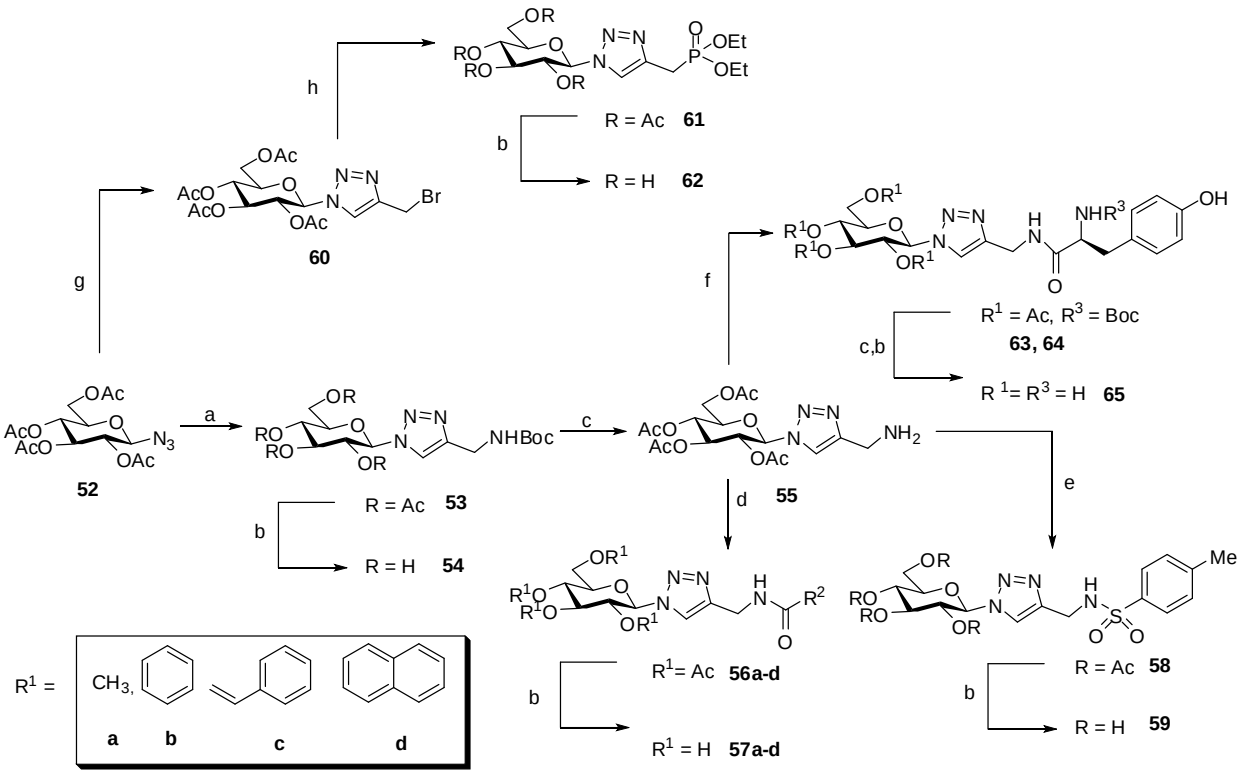
Scheme 2.13 Synthesis of N-substituted 1-amino methyl-β-D-glucopyranoside derivatives**.**

Goyard, Docsa, Gergely, Praly and Vidal (2015) reported the synthesis of4-amidomethyl-1-glucose-1,2,3-triazole derivatives via cycloaddition of Boc-propagylamide with peracetylatedglucosylazide and studies their potential of glycogen phosphorylase inhibitors toward this enzyme. The eight inhibitor candidates have been assayed *in vitro* for their inhibitory toward RMGP*b* as shown in Table1. Compound **54** showed the best inhibitor (IC_{50} = 620 µM) which unexpectedly slightly better than the 2-naphthylamido substituted analogue **57d** (IC_{50} = 650 µM). The evaluation of eight GP inhibitor candidates highlighted that the structural design was limited to only a small series of 4-amidomethyl-1-glucose-

1,2,3-triazoles while several other structural moieties (sulfamido, amino acid or phosphonate) proved detrimental. To surprise, the inhibitory properties of the Boc-amine protected intermediate **55** were the best in this study although with an IC_{50} value in the high μM range. (scheme **2.14**)

Table1 Enzyme kinetic studies of the eight inhibitor candidate against RMGP*b*.

Inhibitor	IC50 (μM)
54	620
57a	40% inhibition at 5000
57b	1400
57c	2100
57d	650
59	30% inhibition at 5000
65	No inhibition at 5000
62	No inhibition at 5000



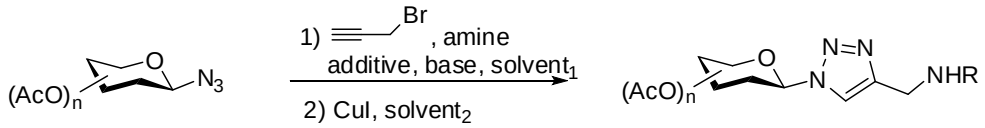
reagent and condition : (a) $HC{\equiv}CCH_2NHBoc$, CuI, *i*$PrNEt_2$, DMF, 70°C, 4h ; (b) MeONa, MeOH, rt, 16h ; (c) CH_2Cl_2/TFA (10:1),rt, 4h (d) R^2COCl, Et_3N, CH_2Cl_2, rt, 4h ; (e) TsCl, Et_3N, CH_2Cl_2, rt, 4h ; (f) BocTyrOH, EDCl, HOBt, CH_2Cl_2/DMF (2:1), -10°C (g) $HC{\equiv}CCH_2Br$, $CuSO_4$, sodium ascorbate, *t*$BuOH/H_2O$ (1:1), rt, 16h ; (h) $P(OEt)_3$, 140°C, 1h

Scheme 2.14 Synthesis of 4-amidomethyl-1-glucose-1,2,3-triazole derivatives.

According to the bioactivities of amine, glycoside and triazole, this project was aimed to studythemethodology for the synthesis of amino-triazoleglycosidederivatives.Thepurposeof our researchwas attempt to simplify by one-pot reactionas a safe, convenient and environmentally benign for eliminating the need for work-up and product isolation between successive synthetic steps.All synthetic analogues will be further study for biological activity

Chapter 2: Results and Discussions

In this work, we developed new synthetic methodology for the synthesis of amino-triazole glycoside from 1-Azido-β-D-sugar via one-pot two steps reaction. The one-pot of *N*-alkylation and click reaction from commercial available amine was carried out. The *N*-alkylation of amine derivatives with propargyl bromide to give propargylamine was performed in the first step subsequently followed by a 'click' reaction with various azido-glycosides in the presence of CuI in aqueous solution to provide amino-triazole glycosides.



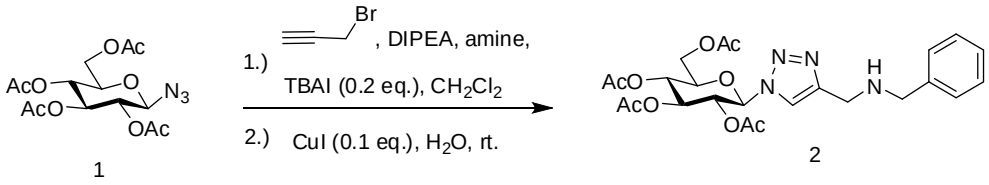
1 **2**

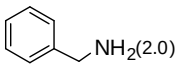
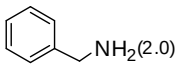
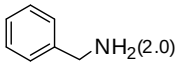
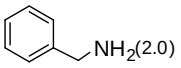
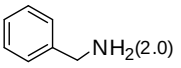
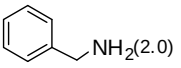
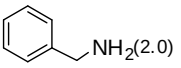
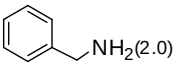
Scheme 2.1 The one-pot synthesis of amino triazole-glycoside from carbohydrate derivatives

To validate the strategy and investigation the condition for one pot synthesis of amino glycoside **2**, the reaction of glycosylazide with benzylamine followed by click reaction was examined as a model reaction under various conditions (Table 1). The starting material, azido-glucopyranoside 1 was easily prepared in high yield (85%) from the reaction of D-glucose pentaacetate with trimethylsilylazide ($TMSN_3$) at room temperature. The optimaization reaction was carried out by frist *N*-propargylation of benzylamine with propargyl bromide in the first step using DIPEA as base, with addition of TBAI as an additive followed by Huisgen 1,3-Dipolar Cycloaddition or copper (I) -catalyzed alkyne-azide cycloaddition (CuAAC) in second step.

When the reaction was conducted in THF, no reaction was occurred (entry 1). Low yields of product were observed when stirring the reaction in 1:1 mixture of water and THF and the mixture of water and CH_3CN. As shown in entries 2 and 3, the products were afforded in 10% and 9% respectively. Mixture of solvents H_2O:MeOH, H_2O:*t*-BUOH, H_2O:DMF, H_2O:CH_2Cl_2 and CH_2Cl_2 were investigated and were failed to obtain the product.

Table 1 Optimization conditions for one pot synthesis of amino glycoside **2**



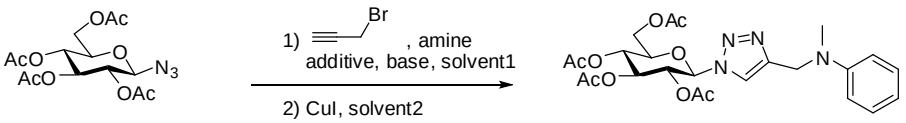
Entry	alkyne (eq.)	base (eq.)	amine (eq.)	T_1/T_2 (h)	solvent	yield
1	2.0	3.0		overnight	THF	-
2	2.0	3.0		1/23	THF:H_2O (1:1)	10 %
3	2.0	3.0		1/23	CH_3CN:H_2O (1:1)	9 %
4	2.0	3.0		1/3	MeOH:H_2O (1:1)	-
5	2.0	3.0		1/3	*t*-BuOH:H_2O (1:1)	-
6	2.0	3.0		1/3	DMF:H_2O (1:1)	-
7	2.0	3.0		1/23	CH_2Cl_2	-
8	2.0	3.0		1/3	CH_2Cl_2:H_2O (1:1)	-

Next, we investigated the reaction of glycosylazide and *N*-methylaniline as the model system for screening a variety of reaction parameters. As shown in Table 2, when the reaction was proceed in 2:1 mixture of water and dichloromethane and Et_3N was used as bases, no product was observed after stirring the reaction overnight (entry 1). The product was obtained in 52% yield in entry 2 when the reaction time was decreased to 11 h. These results are probably due to the decomposition of product in long reaction time.

To improve the yield of product, DIPEA and K_2CO_3 were employed as base together with additive such as TBAI and KI. In the presence of DIPEA and TBAI, the desired product was obtained in good yields (entries 3 and 4). Using K_2CO_3 as base in the present of KI as additives (entry 5), the reaction was carried out in 2:1 mixture of water and acetonitrile,

offering the greatest product in 92% yield. The conditions in entries 4 and 5 were employed as optimized conditions for synthesis a series of amino triazole glycoside.

Table 2 Optimization conditions for one pot synthesis of amino glycoside **2**



Entry	alkyne	amine	base	additive	solvent 1/2	Time 1/2(h)	yields[c](%)
1	Br (2.0eq.)	H N (2.0eq.)	Et_3N (2.0eq.)	-	CH_2Cl_2/H_2O	overnight	trace
2	Br (2.0eq.)	H N (2.0eq.)	Et_3N (1.0eq.)	-	CH_2Cl_2/H_2O	1/10	52%
3[d]	Br (2.0eq.)	H N (2.0eq.)	DIPEA (1.0eq.)	TBAI (0.2eq.)	CH_2Cl_2/H_2O	1/24	54%
4[d]	Br (2.0eq.)	H N (1.2eq.)	DIPEA (1.0eq.)	TBAI (0.2eq.)	CH_2Cl_2/H_2O	1/24	74%
5[c,*]	Br (2.0eq.)	H N (1.2eq.)	K_2CO_3 (1.0eq.)	KI (0.2eq.)	CH_3CN/H_2O	1/3	92%

[c] is isolated yields
[d] is recovery yields
* is in N-alkylation reaction have tempurature to 70^oC

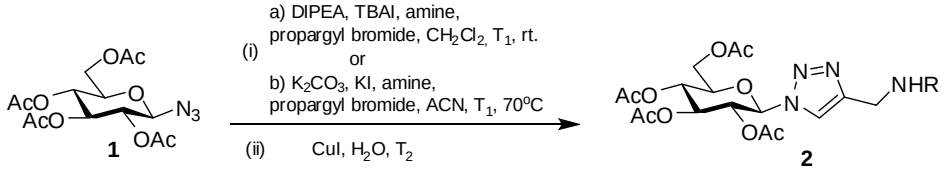
Under the optimized conditions in hand, a number of substrates were investigated (Table 3). D-glucosyl-azide was employed as starting material to perform click reaction with *in situ* generation of substituted amine. We examined the scope of D-glucosylazide and variety of substituents aromatic amine, aliphatic and heterocyclic amine. Both heterocyclic amine such as pyrrolidine and morpholine were reacted with propargyl bromide in the presence of DIPEA and TBAI to afford the N-alkylated compounds in 1 h and followed by click reaction to obtain the triazoleglycosides (entries 1-2). Morpholine provided an excellent yield of product while pyrrolidine gave good yield under the present reaction conditions.

Aniline derivatives provided the desired products in excellent yields under the condition b using K_2CO_3 as base in the present of KI as additives (entry 5). The reaction of *N*-butyl aniline gave 87% and *N*-methyl aniline provided 92%yields when the reactions were proceed in 2:1 mixture of water and acetonitrile. Moreover, this methodology could also be

extended to primary and secondary aliphatic amine (entries 5-7) led to the products in moderate yields. Using secondary aliphatic amines, the reaction was completed in 24 h in the click step (entries 5 and 7).

1-Naphthylamine could readily employed in this reactions condition, affording the desired products in 74%yield after performing *N*-alkylation step for 24 h followed by click reaction for 3 h (entry 8). The halogen substituted fluoro-, and bromo-aniline, which are useful for synthetic diversification, were proceed in the same condition and well tolerated in this reaction (entries 9-11) affording the product in good yields.

Table 3 Synthesis of amino triazole-D-glucopyranoside derivatives **2**



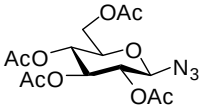
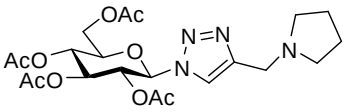
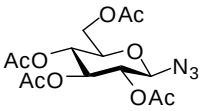
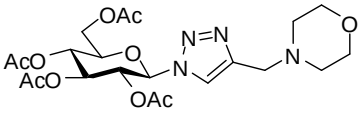
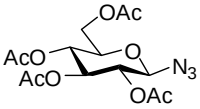
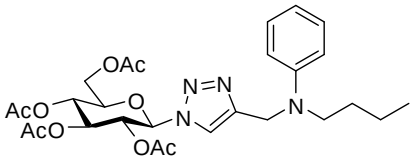
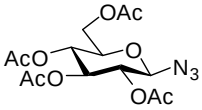
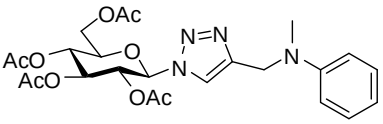
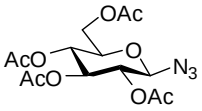
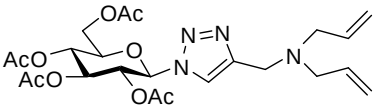
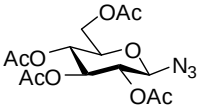
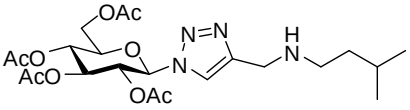
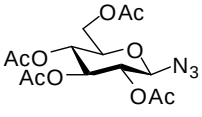
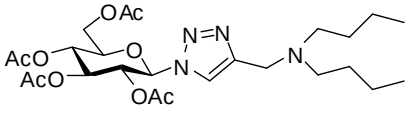
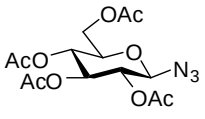
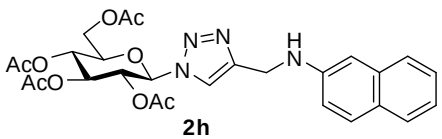
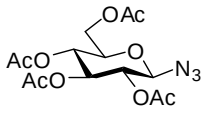
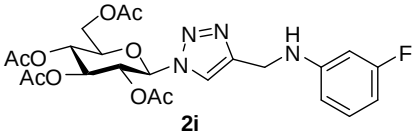
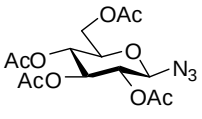
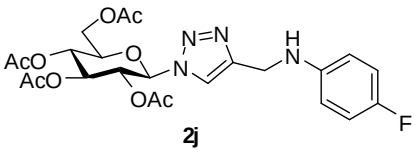
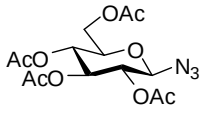
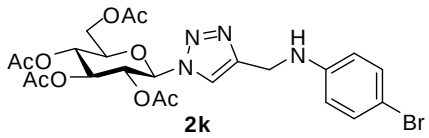
Entry	Glycosyl azide	Product	Time(h)	Yield(%)
1[a]		 **2a**	1/7	54
2[a]		 **2b**	1/3	96
3[b]		 **2c**	1/15	87
4[b]		 **2d**	1/3	92
5[b]		 **2e**	1/24	58
6[b]		 **2f**	2/2	51
7[b]		 **2g**	1/24	48

Table 3 (Continued)

Entry	Glycosyl azide	Product	Time(h)	Yield(%)
8[(b)]		 **2h**	24/3	74
9[(b)]		 **2i**	24/3	78
10[(b)]		 **2j**	24/3	78
11[(b)]		 **2k**	24/3	73

Next, we examined the scope of our finding methodology to employ D-galactosyl-azide as starting material (Table 4). The reaction conditions are compatible with amine derivatives as previous results in Table 3. Under the optimized conditions, D-galactosyl-azide was performed click reaction with *in situ* generation of substituted amine. We examined the scope of variety of substituents aromatic amine, aliphatic and heterocyclic amine.

Morpholine was reacted with propargyl bromide in the presence of DIPEA and TBAI to afford the *N*-alkylated compounds in 3 h and followed by click reaction for 3 h to obtain triazole glycoside in 90%yield (entries 1).

Pyrrolidine was reacted with propargyl bromide in the same condition as entry 1 to afford the *N*-alkylated compounds in 1 h and followed by click reaction for 7 h to obtain the triazole glycoside in moderate yield (entry 2).

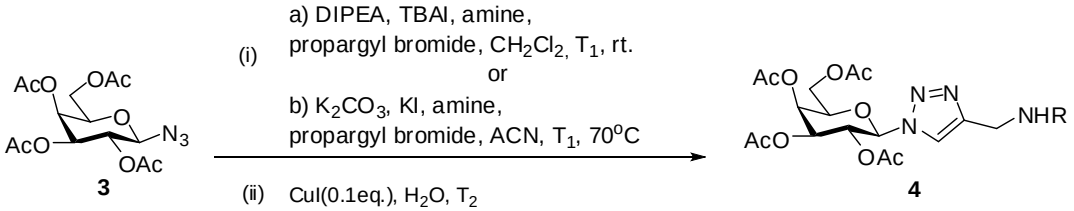
Secondary aliphatic amine provided the desired products in good yields under the condition b using K_2CO_3 as base in the present of KI as additives (entries 3-4). When the reactions were proceed in 2:1 mixture of water and acetonitrile, dibutyl amine gave 68% and

diallyl amine provided 75%yields. These reactions take 24 h to complete *N*-alkylation due to the steric hindrance of substituted groups and the click reaction was completed in 6 h.

Fluoro-anilines, which were carried out in the same condition (entries 5-6) affording the product in 75% for *m*-fluoro-aniline and 48% yield for *p*-fluoro-aniline. Bromo-aniline gave good yield of product by using the condition using DIPEA and TBAI (entry 7).

1-Naphthylamine was proceed N-alkylation step using K_2CO_3 as base in the present of KI as additives followed by click reaction affording the desired products in fair yield after performing *N*-alkylation step for 24 h followed by click reaction for 3 h (entry 8). The reaction of *N*-methylaniline in this one pot methodology gave excellent yield proving the efficiency of this methodology.

Table 4 Synthesis of amino triazole-D-galactopyranoside derivatives **4**



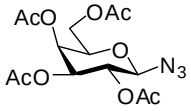
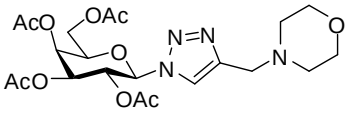
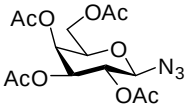
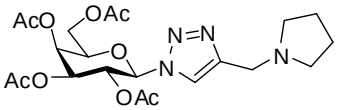
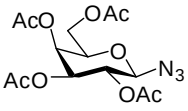
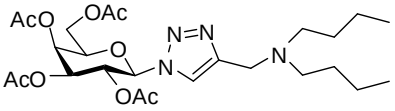
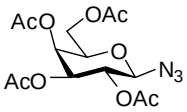
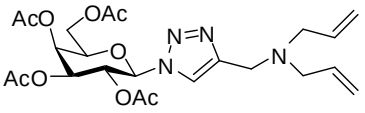
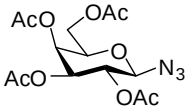
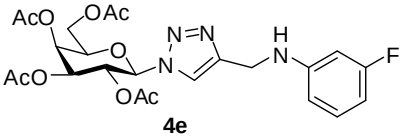
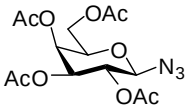
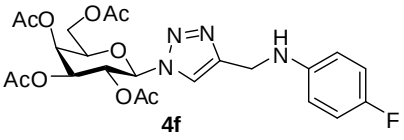
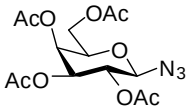
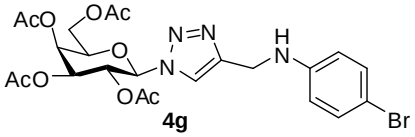
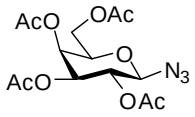
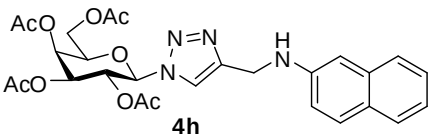
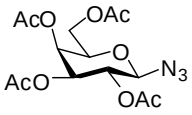
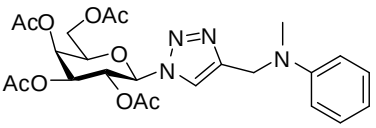
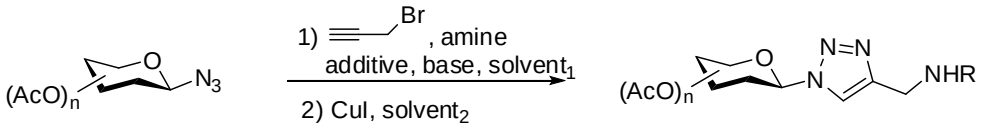
Entry	Glycosyl azide	Product	Time(h)	Yield(%)
1(a)		 **4a**	3/3	90
2(a)		 **4b**	1/7	51
3(b)		 **4c**	24/6	68
4(b)		 **4d**	24/6	75
5(b)		 **4e**	17/3	75
6(b)		 **4f**	17/3	48
7(a)		 **4g**	24/3	71

Table 4 (Continued)

Entry	Glycosyl azide	Product	Time(h)	Yield(%)
8(b)		 **4h**	24/3	48
9(b)		 **4i**	3/6	95

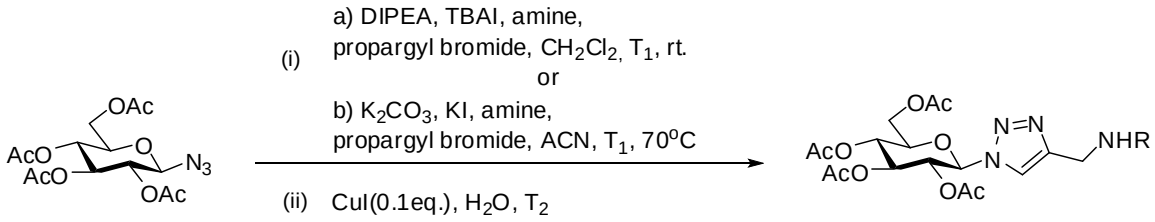
Chapter 3 Conclusion

In this work, we have developed new and convenient synthetic methodology for the synthesis of amino-triazole glycoside from 1-Azido-β-D-sugar using one-pot two steps procedure. The first step *N*-alkylation of amine derivatives with propargyl bromide to give propargylamine was performed subsequently followed by a 'click' reaction with azido-glycosides in the presence of CuI in aqueous solution to provide amino-triazole glycosides.

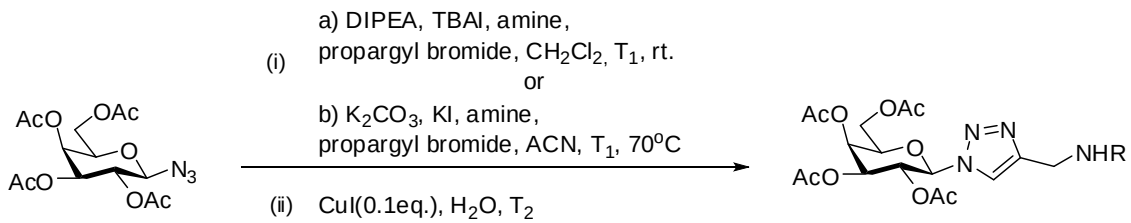


1 **2**

We studied and investigated the best conditions for *in situ* generation of substituted amine by *N*-alkylation which followed by click reaction. The two optimized conditions were found by using DIPEA and TBAI in dichloromethane:water as solvent system and K_2CO_3 and KI in 2:1 mixture of water and acetonitrile.



Eleven new analogues of desired product amino triazole glucopyranoside were prepared in fair to excellent yields using the one pot procedure, Moreover nine amino triazole galactoside were synthesize in fair to excellent yields proving the efficiency of our methodology.



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Output / Outcome

ผลงานที่ขอจดสิทธิบัตรการประดิษฐ์ จำนวน 1 เรื่อง

สารอนุพันธ์อะมิโน-ไตรอะโซล ไกลโคไซด์ชนิดใหม่ (New amino-triazole glycoside derivatives)

การนำเสนอผลงานวิจัยแบบโปสเตอร์ในงานประชุมระดับนานาชาติ

1. Waraporn Sutcharitruk, Uthaiwan Sirion, and Rungnapha Saeeng* One pot synthesis of amino triazole glycosides, The 12th International Symposium on Organic Reactions (ISOR-12) and the 6th German-Japanese Symposium on Electrosynthesis (GJSE-6), April 22-24, 2016, Shinmachi Kujo Minami-ku,Kyoto,Japan
2. Waraporn Sutcharitruk and Rungnapha Saeeng* Synthesis of Amino-triazole-glycoside, Scientific Frontiers in Natural Product Based Drugs Conference, July 6-7, 2017, Department of Pharmacology, National University of Singapore, Singapore

การนำเสนอผลงานวิจัยแบบโปสเตอร์ในงานประชุมระดับชาติ

1. Waraporn Sutcharitruk, Uthaiwan Sirion, and Rungnapha Saeeng* Synthesis of Amino-triazole-galactoside, Science RESEARCH conference 9th, May 25-26, 2017, Department of Science, Burapha University of Thailand

การผลิตบัณฑิต

นิสิตปริญญาเอก ภาคเคมี คณะวิทยาศาสตร์ ม.บูรพา จำนวน 1 คน (กำลังศึกษา)

นางสาววราภรณ์ สุจริตรักษ์