รายงานวิจัยฉบับสมบูรณ์

โครงการวิจัยเรื่อง
การเตรียมสารสังเคราะห์ไตรเอโซลไกลโคไซด์เพื่อศึกษาสมบัติการต้านมะเร็งท่อน้ำดี
Preparation of synthetic triazoleglycosides
for cholangiocarcinoma tumor growth inhibitor

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สารบัญ

คำนำ

โครงการวิจัย “การเตรียมสารสังเคราะห์ไตรเอโซลไกลโคไซด์เพื่อศึกษาสมบัติการต้านมะเร็งท่อน้ำดี” ได้รับการสนับสนุนทุนการวิจัยโครงการต่อเนื่องงบประมาณแผ่นดินประจำปีงบประมาณ 2557 มหาวิทยาลัยบูรพา รายงานการวิจัยฉบับนี้เสนอรายละเอียดของการวิจัยซึ่งประกอบด้วยบทนำที่เสนอผลงานวิจัยที่เกี่ยวข้อง ผลการทดลองวิจัย การอภิปรายสรุปผล และวิธีการทดลองรวมถึงรายละเอียดของข้อมูลการวิเคราะห์ผลิตภัณฑ์

การวิจัย “การเตรียมสารสังเคราะห์ไตรเอโซลไกลโคไซด์เพื่อศึกษาสมบัติการต้านมะเร็งท่อน้ำดี” สำเร็จลุล่วงไปด้วยดี โดยผู้วิจัยต้องขอขอบคุณทีมวิจัยซึ่งประกอบด้วยที่ปรึกษาโครงการ ศ.ดร. อภิชาต สุขสำราญ มหาวิทยาลัยรามคำแหง ศ.ดร. ภาวิณี ปิยะจตุรวัฒน์ มหาวิทยาลัยมหิดล ศ. ดร.โสพิศ วงศ์คำ มหาวิทยาลัยขอนแก่น ผู้ร่วมโครงการการสังเคราะห์สาร ดร. อุทัยวรรณ ศิริอ่อน รวมทั้งนิสิตปริญญาโท ภาควิชาเคมี นายสุขสำราญ ไชยดำ ขอขอบคุณ คุณสุทธิพร พิกุลทอง มหาวิทยาลัยมหิดลที่ทำการตรวจสอบ High Resolution Mass ของสารสังเคราะห์ที่ได้ งานวิจัยนี้ได้รับการสนับสนุนจากภาควิชาเคมี คณะวิทยาศาสตร์และศูนย์นวัตกรรมความเป็นเลิศทางเคมี PERCH-CIC

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Abstract

A new series of 1,6-bis-triazole 2,3,4-tri-*O*-acetyl-α-D-galactopyranosyl derivatives were synthesized and evaluated for their in vitro cytotoxic activities against Thai human cholangiocarcinoma cells (KKU-M213, HUCCA-1, K-100). Thirty-two examples were prepared in high yields (70-99%) and the preliminary screening results indicated that some of the compounds demonstrated low to moderate cytotoxic activities. Compounds **12 ee** exhibited pronounced cytotoxicity against K-100 cell lines, comparable to the anticancer drug ellipticin. The chemical structures of all the newly synthesized compounds were characterized by means of spectral and elemental analyses.

Chapter 1
Introduction

Cholangiocarcinoma (CC) is a primary cancer of the bile duct epithelial cells with an incidence of about 3 per 100,000 per year.[1,2] The highest incidence of cholangiocarcinoma has been reported in the Northeastern area of Thailand. Cholangiocarcinoma has resulted in a high mortality rates and poor prognosis. At present, surgery is potentially curative approach however, no improvement with long term survival. In addition, the chemotherapy is ineffective. Therefore, searching for the effective drugs with less adverse effect and high sensitivity to cholangiocarcinoma are needed.

Prognosis for cholangiocarcinoma is poor. At the time of diagnosis only 30–50% of the patients with extrahepatic CC show local lymph node metastases and 10–20% show distantmetastases (especially in liver and peritoneum). Nevertheless, 70–80% of perihilar tumours are not resectable due to tumour extension to other adjacent anatomical structures.[3] Without treatment half of the patients die within three to four months due to the indirect consequences of local tumour progression, *i.e.* increasing bile duct obstruction, bacterial cholangitis, gallbladder empyema, liver abscesses, cholestasis and liver failure. Patients with extrahepatic CC usually present with painless icterus, pruritus, anorexia and rapid weight loss, or signs of cholangitis. So far, surgical removal of the tumour is the only curative approach. However, even after curative (R0) resection the 5-year survival rate is limited to 30–40%.[4] In most cases (70–80%) resection is precluded by local tumour extension - especially owing to the distinct anatomical features of the liver hilus comprising three neighbouring vessel systems (arterial, portal venous and biliary ductal). Liver transplantation may be considered in exceptional cases, although non-resectable CCs do not represent an assured indication.[5]

Tumours of the bile duct often show poor response to combination chemotherapy with median overall survival time up to 15 months at best with gemcitabine/oxaliplatin (or cisplatin) or gemcitabine/capecitabine. External beam radiation therapy does not improve the prognosis**;** nevertheless, CCs appear to respond moderately to combination radiochemotherapy. However, many of the patients with CC are never fit for aggressive chemo- or radiochemotherapy because of tumour complications like obstructive cholestasis or cholangitis and, furthermore, the survival benefit of this combined approach could not be demonstrated throughout all studies.

The incidence of CCA is low worldwide, however, it is very high in the northeastern part of Thailand especially in Khon Kaen province.[6] From population-based cancer registry in Khon Kaen, the incidence of CHCA is evidently high in area located near water reservoir with the liver fluke, *Opisthorchis viverrini*, contamination in the fish. The trend in incidence of CHCA in Khon Kaen has been slowly declining in both sexes with annual percent age changes of 0.7% and 0.4% in male and female, respectively. The survival rate of patient with CHCA is very low according to ineffective treatment modalities. At present, there is no effective chemotherapy regimen for treating patients with advanced cholangiocarcinoma. Therefore, novel and effective therapeutic agents and more effective medical treatment options are urgently needed.

1,2,3-Triazoles are potential targets for drug discovery as they exhibit a broad spectrum of biological properties and many efforts have been made to optimize methods for their preparation. One example is the 1,3-dipolar cycloaddition reaction between an azide and a terminal alkyne in the presence of a copper-based catalyst to give the triazole. This method, developed is potentially useful in the design and synthesis of new compounds with excellent regiocontrol. The triazole-linked glycosides have been extensively studied in research for the design of various functionalized glycoconjugates, facilitating a multitude of practical physical, chemical, biological studies and validated pharmaceutical properties. However, they are a few researches study on the synthesis of bis-triazoleglucosides *via* dual click chemistry and elucidation for their biological properties (Song et al., 2011; He et al, 2011).

In this work, we designed to synthesize a new class of 1,6-bis-triazole 2,3,4-tri-*O*-acetyl-α-D-galactopyranosyl analogues via click chemistry (Scheme 1). All the synthetic anologues of the bistriazole glycosides will be evaluated for anti-proliferative activity on human cholangiocarcinoma cells (KKU-M213, HUCCA-1, K-100), cancer cells derived from Thai patient.

Chapter 2

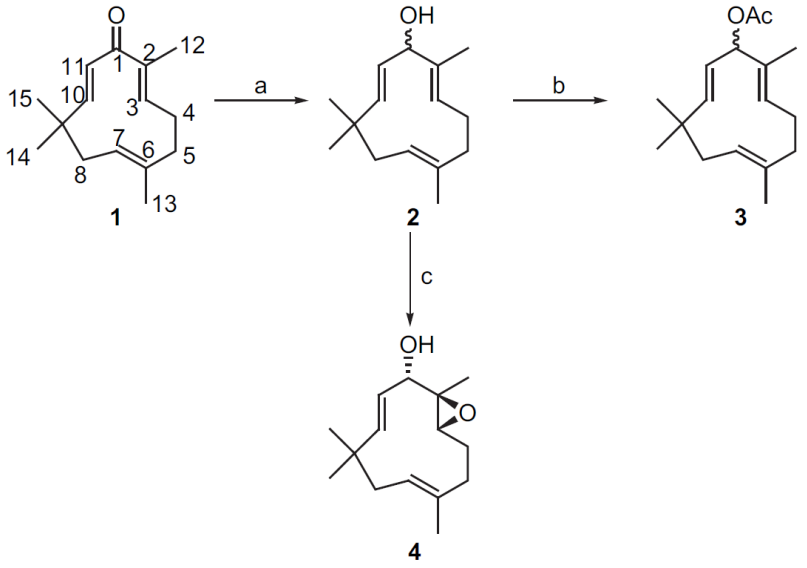
Literature reviews

During the past years, several compounds have been synthesized and study for their cytotoxicity against cholangiocarcinoma cell lines. Here are some recently reports.

2.1 Selected examples of synthetic compounds and their cytotoxicity against cholangiocarcinoma cell lines

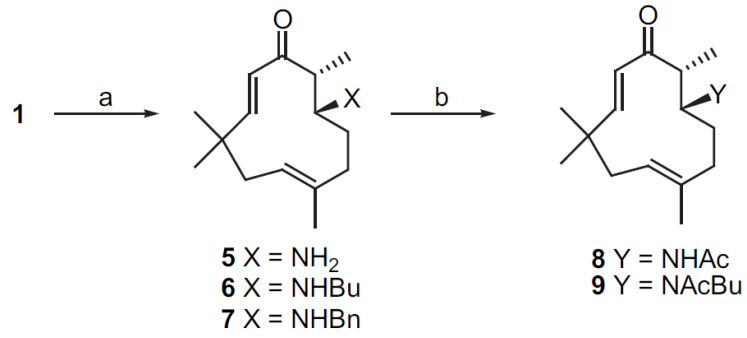
Zerumbone 1, a crystalline sesquiterpene, is the major component in the rhizomes of *Zingiber zerumbet Smith* and is readily available from a widespread natural source. A series of zerumbone derivatives were synthesized and their *in vitro* cytotoxicity against cholangiocarcinoma cell lines was evaluated.

Seventeen zerumbone derivatives were prepared by Yenjai and co-worker using organic reactions.[7] Reduction of zerumbone **1** using $LiAlH_4$ gave crystalline zerumbol **2** and acetylation of this compound using Ac_2O/pyridine afforded **3** while epoxidation of **2** with mCPBA yielded racemic **4** (Scheme 1).



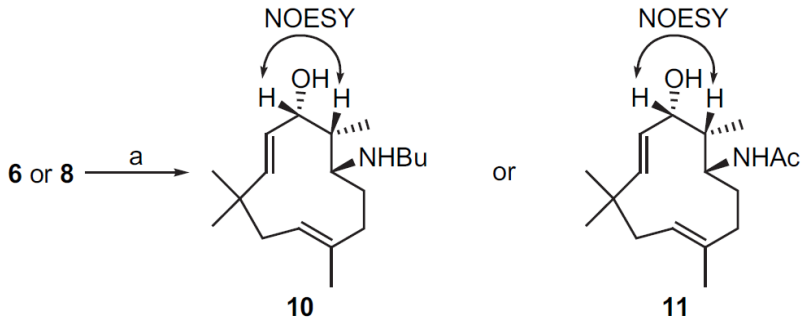
Scheme 1. Reagents and conditions: (a) $LiAlH_4$, THF, 0 °C, 1 h, 88%; (b) Ac_2O, pyridine, reflux, 30 min, 74%; (c) *m*CPBA, EtOAc, rt, 24 h, 15%. **Note**: The stereochemistry shown in all schemes are relative configuration.

Zerumbone **1** was stirred with excess ammonia, butylamine and benzylamine to provide monoamines **5**, **6** and **7**, respectively. Michael addition of amines occurred selectively at the less hindered conjugated double bond (C2-C3) of zerumbone. Acetylation of amine groups at C3 position of **5** and **6** using Ac_2O/pyridine provided the corresponding amides **8** and **9**, respectively.



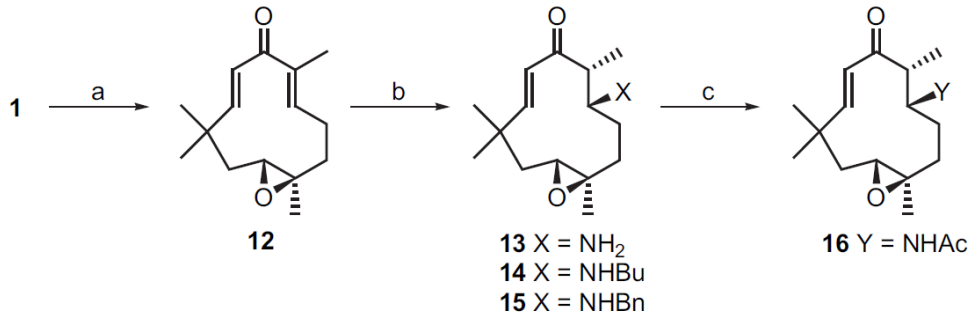
Scheme 2. Reagents and conditions: (a) NH_3 or $BuNH_2$ or $BnNH_2$, MeCN, rt, 5 days, 56% (**5**), 93% (**6**), 68% (**7**); (b) Ac_2O, pyridine, 0 °C, 8 h, 92% (**8**), 85% (**9**).

The reaction of butylamine **6** with $NaBH_4$ afforded hydroxyamine **10** in 81% yield. The reduction of amide **8** with $NaBH_4$ afforded a single diastereoisomer hydroxyamide **11** in the yield of 54% (Scheme 3).



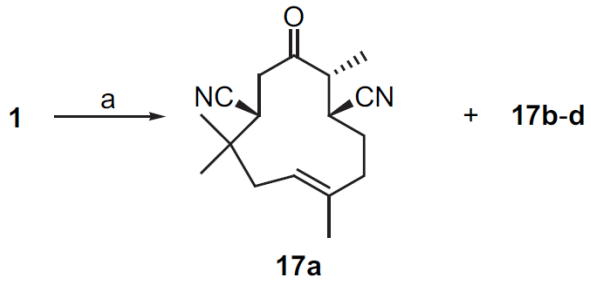
Scheme 3. Reagents and conditions: (a) $NaBH_4$, MeOH, 0 °C, 3 h, 81% (**10**), 54% (**11**).

Epoxidation of the isolated double bond in zerumbone at C6 and C7 using m-CPBA provided epoxide **12** in 97% yields and treated with an excess amount of various amines providing corresponding amines **13-15**. Further acetylation of **13** using Ac_2O in pyridine gave corresponding amide **16** in 66% yield (Scheme 4).



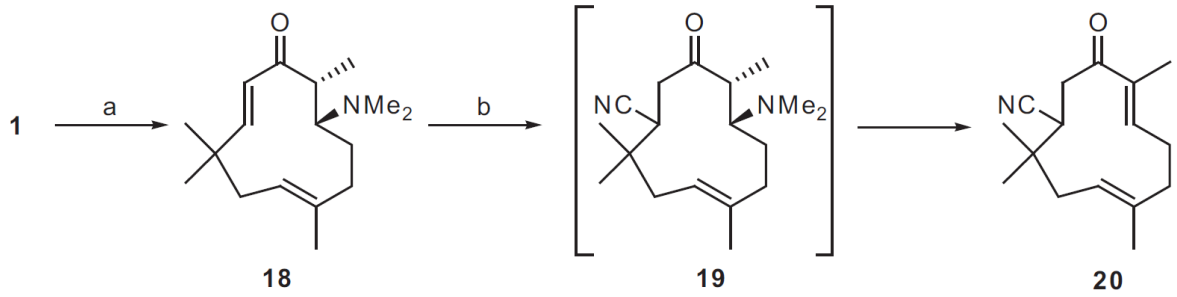
Scheme 4. Reagents and conditions: (a) *m*CPBA, EtOAc, rt, 24 h, 97%; (b) NH_3 or $BuNH_2$ or $BnNH_2$, MeCN, rt, 5 days, 51% (**13**), 15% (**14**), 18% (**15**); (c) Ac_2O, pyridine, 0 °C, 10 h, 66%.

The reaction of 1 with excess KCN at 40 ^{o}C for 3 days provided a mixture of four diastereoisomeric dicyano derivatives **17**. It was reported that in the major diastereoisomer (**17a**), two cyano groups were located on the same face of the ring while the two methyl groups at C2 and C6 lie on the opposite face (Scheme 5).



Scheme 5. Reagents and conditions: (a) excess KCN, MeCN-H_2O, 40 °C, 3 days, 42% (**17a**).

Treatment of **1** with dimethylamine in the presence of acetonitrile, followed by stirring with excess KCN, the nitrile derivative **20** was detected. This can be explained as that conjugate addition of dimethylamine at C3 gave intermediate **18**, while conjugate addition of the cyanide ion at C10 yielded intermediate **19**. After the easy elimination of the dimethylamino group, cyano **20** was observed as a sole product (Scheme 6).



Scheme 6. Reagents and conditions: (a) Me_2NH, MeCN, rt, 5 days; (b) KCN, MeCN-H_2O, 15 °C, 2 days, 30% in two steps.

Zerumbone (**1**) and its derivatives were tested for their cytotoxicity against CCA cell lines and their activities are shown in Table 1.

Compounds **5**, **10**, **14** and **20** exhibited cytotoxic activity against all CCA cell lines to different extents, indicating their broad spectrum of anti-CCA effects. The chemical structure diversity of the four compounds reflects the biological activities.

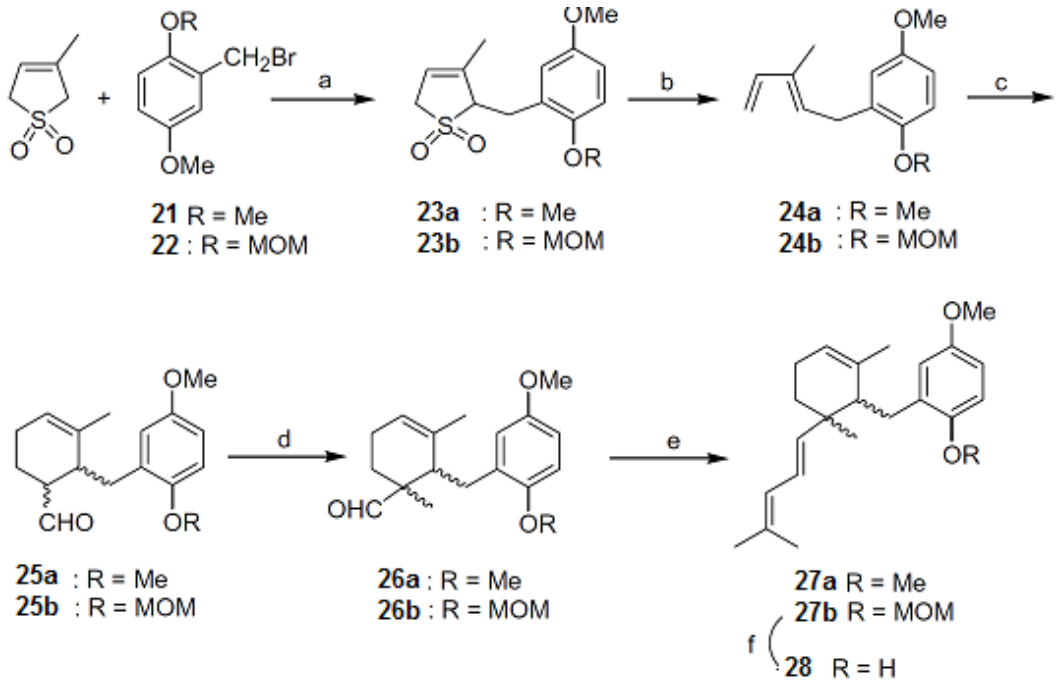
The presence of amine (**5**), hydroxylamine (**10**), epoxyamine (**14**), and nitrile (**20**) groups is believed to play an important role in potent anticancer activities. Among the tested compounds, **5**, which contained an amine group, exhibited higher potency. The docking result also indicates that **5** may inhibit the proliferation of cancer through EGFR inhibition.

Table 1
Cytotoxicity of all compounds against cholangiocarcinoma cell lines.[a]

Compound	IC_{50}(μM)				
	KKU-100	KKU-M139	KKU-M156	KKU-M213	KKU-M214
Zerumbone	NR	NR	NR	NR	NR
5	16.44 ± 0.59	63.26 ± 5.48	69.04 ± 5.40	51.88 ± 2.25	59.65 ± 7.39
6	NR	NR	NR	36.61 ± 0.65	41.55 ± 0.58
10	35.33 ± 5.89	19.63 ± 1.26	26.34 ± 4.0	27.19 ± 1.29	16.05 ± 6.06
12	NR	NR	NR	NR	52.61 ± 3.88
13	NR	NR	NR	NR	18.30 ± 0.39
14	16.52 ± 0.84	NR	48.20 ± 1.85	37.63 ± 2.4	51.00 ± 0.78
17a–d	NR	NR	NR	NR	66.52 ± 3.63
20	25.72 ± 2.60	55.88 ± 4.4	37.30 ± 3.58	45.12 ± 0.89	54.50 ± 1.5
Other	NR	NR	NR	NR	NR
Ellipticine	25.21 ± 0.2	4.5 ± 0.28	9.34 ± 1.66	1.62 ± 0.08	1.01 ± 0.02

NR = no response at > 75 μM.
[a] Data shown are from triplicate experiments.

Khan and co-worker reported a novel approach to synthesize riccardiphenol B analogs and have tested the cytotoxic activity against a variety of cancer cell lines including HuCCT-1 which was derived from an intrahepatic cholangiocarcinoma.[8]



Scheme 7
Reagents: (a) LiHMDS, HMPA, THF; (b) pyridine, reflux; (c) sealed tube, toluene; (d) NaH, MeI, THF; (e) prenyl phosphonate; (f) HCl,

In their synthesis, 3-methyl-3-sufolene was first alkylated with the substituted benzylic bromides in the presence of HMPA and LiHMDS as the base gave a colorless, viscous liquid, which was characterized as 2-(2-methoxymethoxy-5-methoxybenzyl)-3-methyl-3-sulfolene **23b**. The adduct **23b** was subjected to thermolysis by refluxing in pyridine gave compound **24b**. The Diels–Alder reaction was carried out between the diene **24b** and acrolein as the dienophile, in the presence of hydroquinone in a sealed tube at 90 ^{o}C for 2 h yielded the adduct **25b**. The compound **25b** was methylated using methyl iodide and NaH in dry THF yielded **26b** as a colorless, viscous liquid. The reaction of Prenyl

phosphonate with the LDA in THF gave the corresponding ylide, which was treated with aldehyde **26b** to give the required product **27b** in 17% yield. Further, the deprotection of MOM with HCl in THF gave compound **28** (Scheme 7). The synthesized compounds were characterized and assessed for its in vitro activity in a panel of human cancer cell lines of differing origin. The leading riccardiphenol analog, **28**, significantly inhibits the growth of different human cancer cells including HuCCT-1 which was derived from an intrahepatic cholangiocarcinoma (Figure 1).

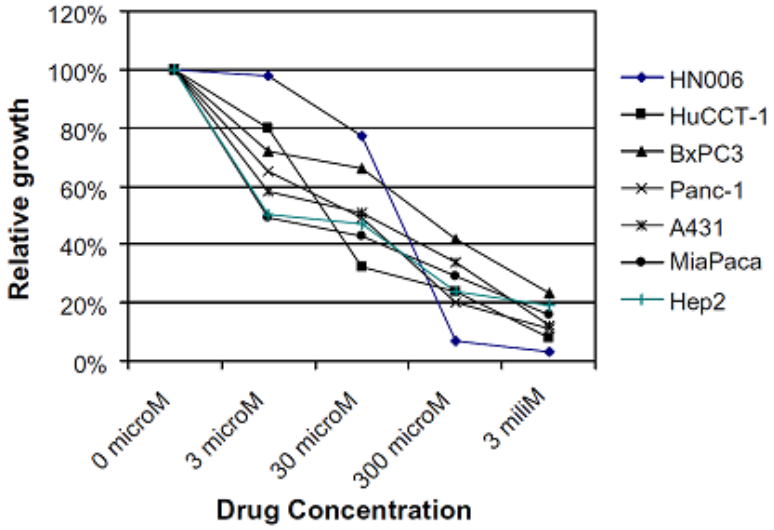
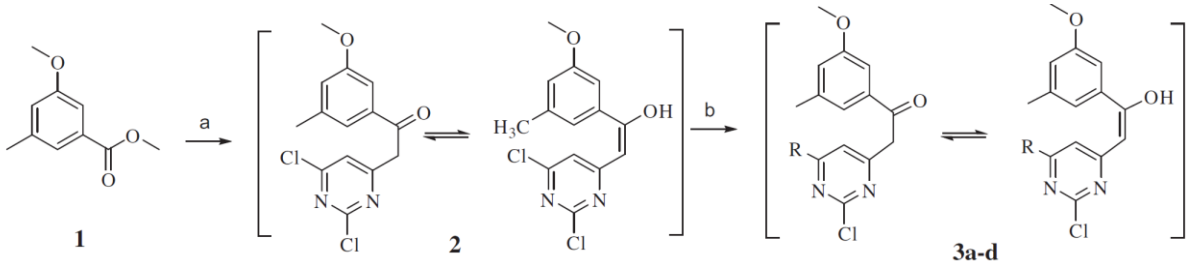
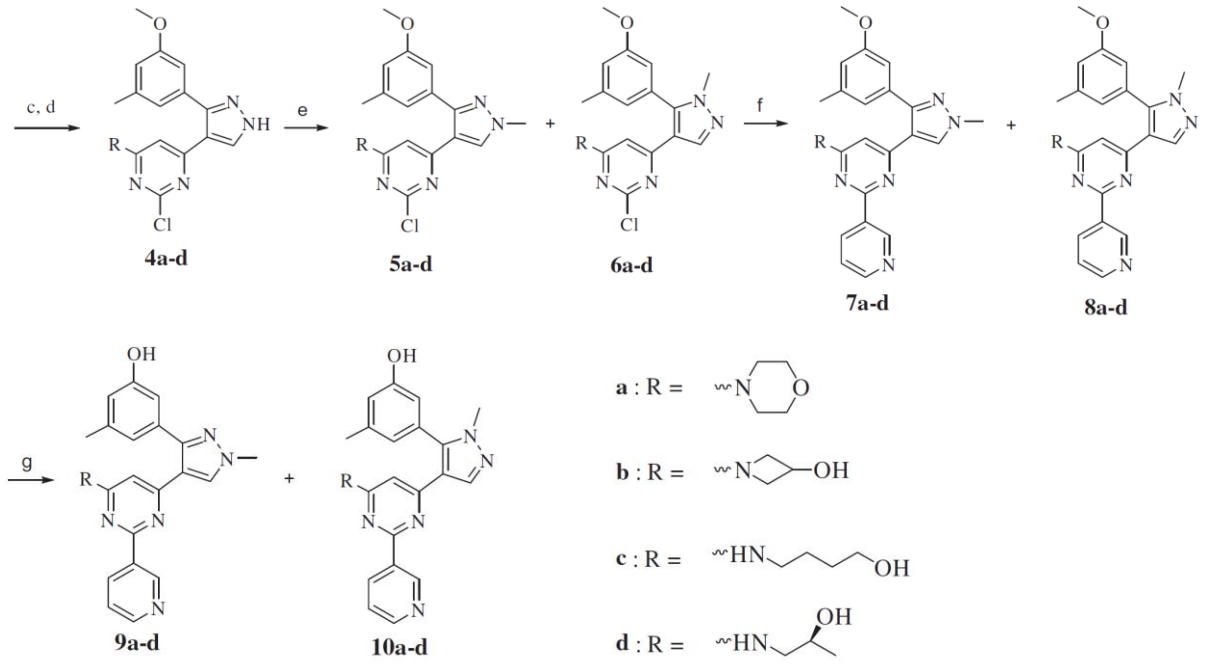


Figure 1. MTT assay in a panel of seven human-derived cancer cell lines from different origins. Relative growth after exposure to increasing concentrations of compound **28**

Recently, inhibition of ROS1 kinase has proven to be a promising strategy for several indications such as glioblastoma, non-small cell lung cancer (NSCLC), and cholangiocarcinoma. So Ha Lee and co-worker reported trisubstituted pyrazole-based ROS1 inhibitors by which two inhibitors showed good IC_{50} values in enzyme-based screening.[9]

Trisubstituted pyrazole-based scaffold has been built for study the SAR for as ROS1 inhibitors. Consequently, 16 compounds have been designed, synthesized and evaluated for ROS1 inhibition.





Scheme 8. Reagents and conditions: (a) LHMDS, 2,4-dichloro-6-methylpyrimidine, THF, N_2, rt, 24 h, 81%; (b) various amine, Hünig's base, EtOH, 50 °C, 5 h; (c) DMF-DMA, 90 °C, 12 h; (d) hydrazine hydrate, abs. EtOH, rt, 2 h; (e) K_2CO_3, iodomethane, DMF, rt, 2 h; (f) 3-pyridineboronic acid, $Pd(PPh_3)_2Cl_2$, K_2CO_3, N_2, CH_3CN/H_2O (4:1), 78 °C, 2 h; (g) $BF_3{\cdot}S(CH_3)_2$, dichloromethane, N_2, rt, 24 h.

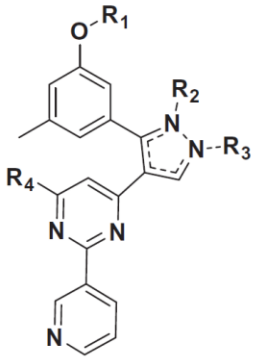
The synthesis of trisubstituted pyrazole compounds **7**–**10** is outlined in Scheme 8. Lithium hexamethyldisilazide (LHMDS) was selected for attacking a benzoate ester **1** by 2,4-dichloro-6-methylpyrimidine in dry THF to give the adduct with a mixture of keto and enol tautomers **2**. A SNAr reaction was used to substitute 4-chloro group on pyrimidine of the resulted tautomeric unsaturated ketone **2** with four amines, morpholine, 3-hydroxyazetidine, 4-hydroxybutylamine, and (S)-2-hydroxypropylamine to give **3**. The conversion of the resulted 6-substituted products **3a**–**d** to the required pyrazole derivative **4a**–**d** was achieved through two successive steps. In the first step, compound **3a**–**d** was heated with excess N,N-dimethylformamide dimethylacetal for 12 h, and was cyclized with hydrazine monohydrate in absolute ethanol into the pyrazole derivative **4a**–**d**. The reaction of the resulted pyrazole **4a**–**d** with iodomethane in the presence of excess potassium carbonate produced two different regioisomers, compounds **5a**–**d** and **6a**–**d**. Then Suzuki coupling of compound **5a**–**d** and **6a**–**d** with 3-pyridineboronic acid produced compounds **7a**–**d** and **8a**–**d** in the presence of dichlorobis(triphenylphosphine)Pd(II) and potassium carbonate in a mixed solvent of acetonitrile and water (4/1, v/v). The final hydroxyl products **9a**–**d** and **10a**–**d** were obtained by demethylation of the methoxy group of compounds **7a**–**d** and **8a**–**d** using 10 equiv of borontrifluoride–dimethylsulfide complex in dichloromethane (Scheme 8).

The synthesized compounds shown potent IC_{50} values in the enzymatic assay, which are from 13.6 to 283 nM (Table 2). Among these compounds, compound **9a** (IC50 = 13.6 nM) has exerted 5 fold potency than crizotinib and exhibited high degree of selectivity (selectivity score value = 0.028) representing the number of non-mutant kinases with

biological activity over 90% at 10 lM. The detailed SAR data demonstrates that pyrazoles having hydrophobic-disubstituted phenyl ring, small alkyl group, and disubstituted nucleus with solvent exposure and hinge region is very effective structure for ROS1 inhibition. All of the final potent compounds possess the essential distal pyridine group which interacts with Met2029 representing the key interaction with ROS1 and being responsible for their inhibitory activity.

Table 2

The IC_{50} values of compounds **7–10** against ROS1 kinase



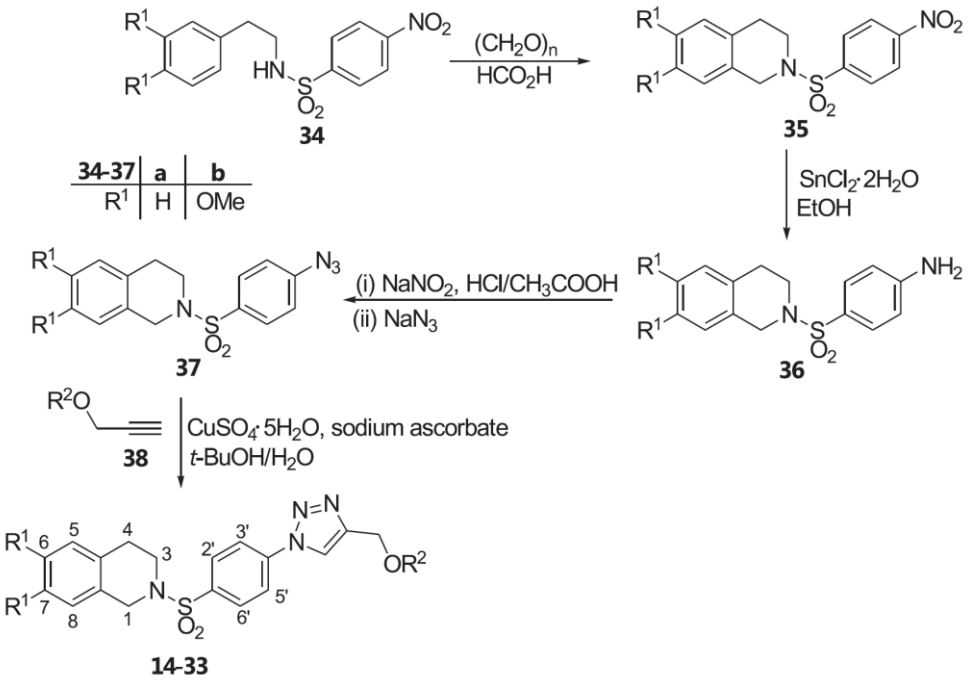
7-10

Compd	R_1	R_2	R_3	R_4	ROS1, (IC_{50}, nM)[a]
7a	CH_3	–	CH_3	Morpholino	59.1
7b	CH_3	–	CH_3	3-Hydroxyazetidin-1-yl	108
7c	CH_3	–	CH_3	4-Hydroxbutylamino	63.8
7d	CH_3	–	CH_3	(*S*)-2-Hydroxypropylamino	241
8a	CH_3	CH_3	–	Morpholino	104
8b	CH_3	CH_3	–	3-Hydroxyazetidin-1-yl	74.2
8c	CH_3	CH_3	–	4-Hydroxbutylamino	138
8d	CH_3	CH_3	–	(*S*)-2-Hydroxypropylamino	283
9a	H	–	CH_3	Morpholino	13.6
9b	H	–	CH_3	3-Hydroxyazetidin-1-yl	86.5
9c	H	–	CH_3	4-Hydroxbutylamino	133
9d	H	–	CH_3	(*S*)-2-Hydroxypropylamino	105
10a	H	CH_3	–	Morpholino	91.5
10b	H	CH_3	–	3-Hydroxyazetidin-1-yl	56.6
10c	H	CH_3	–	4-Hydroxbutylamino	25.4
10d	H	CH_3	–	(*S*)-2-Hydroxypropylamino	139
con[b]	–	–	–	–	60.0

[a] 10-dose IC_{50} mode with 3 fold serial dilutions starting at 20 μM concentration.
[b] Crizotinib.

Several new N-benzenesulfonyl-1,2,3,4-tetrahydroisoquinolines (**14**-**33**) were synthesized using the modified Pictete Spengler reaction by treatments of nitrobenzenesulfonamides **34** with paraformaldehyde in refluxing formic acid to furnish 1,2,3,4-tetrahydroisoquinolines **35** in good yields. Reduction of the nitro derivatives **35** was performed using stannous chloride in refluxing ethanol to give aminobenzenesulfonamides **36**. Conversion of the amino compounds **36** to the corresponding azidobenzensulfonamides **37** was readily achieved through diazotization reaction using sodium nitrite in a mixture of glacial acetic acid and concentrated hydrochloric acid in the presence of sodium azide.

Finally, cycloaddition reaction (the Click chemistry) of the azides **37** with various alkynes **38** obtaining from alkylation of the appropriate phenol derivatives with propargyl bromide afforded a variety of the desired triazoles **14-33** (Scheme 9) in moderate to good yields (45-94%).[10]



Scheme 9

Synthesis of *N*-benzenesulfonyl-1,2,3,4-tetrahydroisoquinoline based triazoles

A series of N-benzenesulfonyl-1,2,3,4-tetrahydroisoquinolines were preliminarily evaluated in vitro as antiproliferative agents against HuCCA-1 (cholangiocarcinoma) cell line. Results showed that substituents (R^1) on the isoquinoline ring and substituents (R^2) on the triazole core play important roles in governing their cytotoxicities. The ester analog **20** was shown to be the most potent compound against HuCCA-1 (IC_{50} 0.63 μM) (Figure 2).

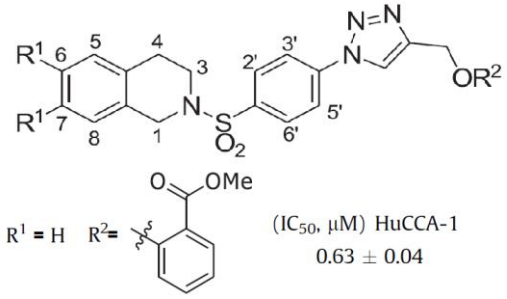
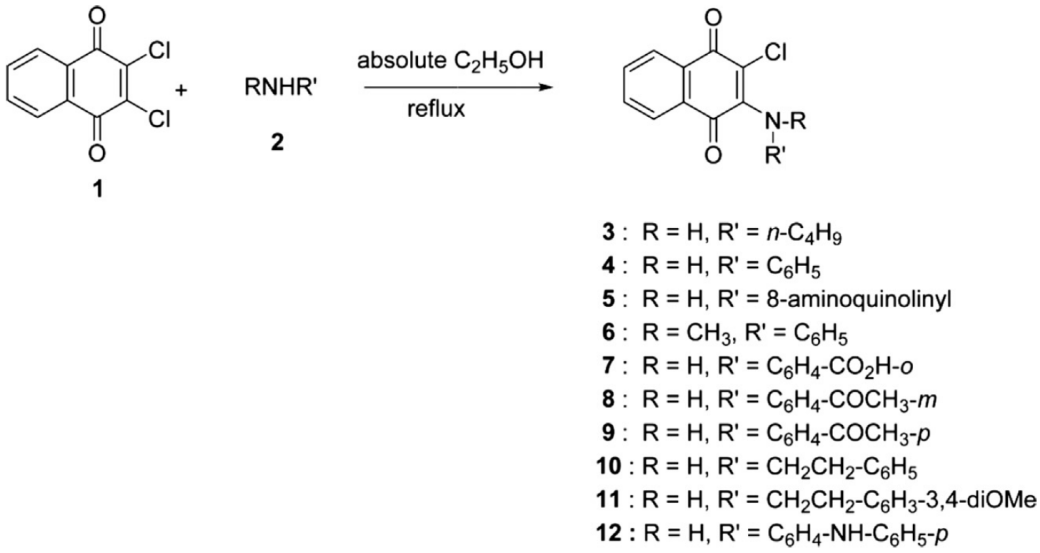


Figure 2 The most potent synthetic N-benzenesulfonyl-1,2,3,4-tetrahydroisoquinolines

A series of 2-substituted amino-3-chloro-1,4-naphthoquinone derivatives (**3-12**) were synthesized (Scheme 10) as anticancer agents and tested against HuCCA-1 (cholangiocarcinoma) cell line.



Scheme 10 Synthesis of aminonaphthoquinone derivatives

Cytotoxic activities of the synthesized aminoquinone compounds (**3**-**12**) and parent compound (**1**) were tested against HuCCA-1 (cholangiocarcinoma) cell line using etoposide and doxorubicin as reference drugs (Table 3).

Among all the tested compounds, acetylphenylaminoquinone compounds (**8** and **9**) were shown to be the most active compounds. Compounds **1**, **3**, **7**, **10** and **11** were inactive to weakly active compounds. In addition, compounds **4**, **5**, **6** and **12** displayed cytotoxic activity against HuCCA-1.

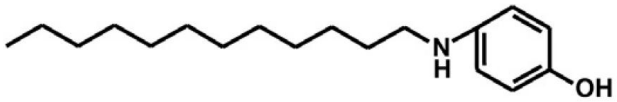
Significantly improved cytotoxic activities were found in compounds bearing acetylphenylamino substitutions (**8** and **9**). The enhanced effect of amino substituents is the following order: acetylphenylamino > quinolinylamino > alkylamino > phenylalkylamino. The most potent cytotoxic activity was found to be macetylphenylamino-1,4-naphthoquinone (**8**) affording IC_{50} values of 2.364 μM.[11]

Table 3
Cytotoxic activity of compounds **1** and **3**–**12**

Compound	IC_{50} (μM)
	HuCCA-1
1	19.204 ± 0.51[a]
3	61.163 ± 4.20[a]
4	8.636 ± 0.35[b]
5	7.916 ± 0.64[b]
6	5.206 ± 0.07[b]
7	50.134 ± 0.66[a]
8	2.364 ± 0.53[b]
9	3.285 ± 1.03[b]
10	inactive[c]
11	inactive[c]
12	10.672 ± 0.42[a]
Etoposide	–[d]
Doxorubicin	0.239 ± 0.02

[a] Weakly active compound.
[b] Moderately active compound.
[c] Inactive compound.
[d] Not tested.

p-Dodecylaminophenol was developed to be an effective anticancer agent without key side-effects of these agents.[12] This compound suppresses cell growth of pancreatic cancer (MIA Paca2) and cholangiocarcinoma (HuCCT1), potentially by inhibiting ras expression and signaling through ERK pathways in MIA Paca2 cells and both ERK and Akt pathways in HuCCT1 cells. p-Dodecylaminophenol may represent a potent and useful anti-cancer drug for use against pancreatic cancer and cholangiocarcinoma that lacks their key side-effects.

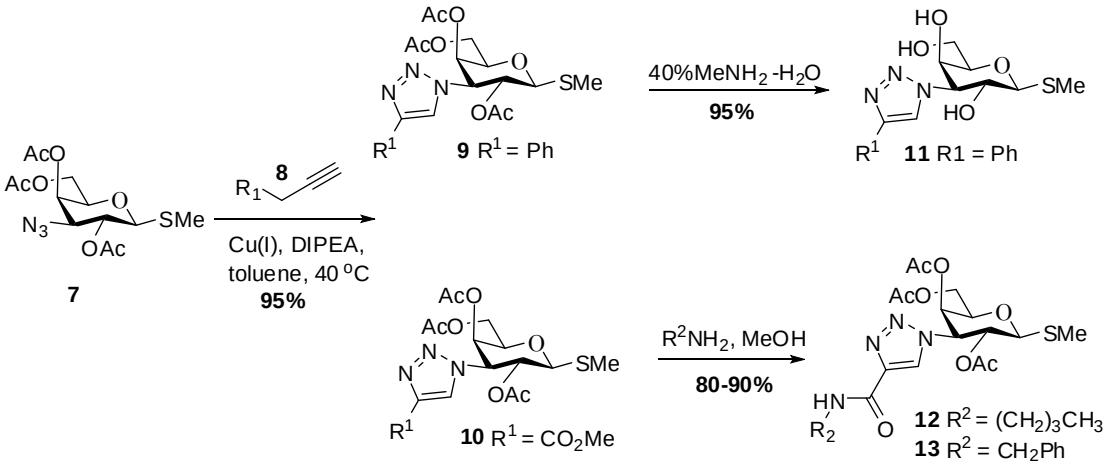


p-Dodecylaminophenol

Carbohydrates are the most abundant group of natural compounds commonly referred to sugars and starches. The glycoconjugates, are involved in important functions, as cell-cell recognition and communication, inflammation, immunological response, bacterial and viral infection, tumorigenesis and metastasis (Kumar,Seenivasan, Kumar V., &Das, 2011). Furthermore, carbohydrates linked to a heterocyclic moiety are important for bioactivity display a significant influence to the pharmacokinetics, drug targeting and mechanism of action (Kamenecka et al., 2009). Similarly, N-heterocyclic compounds, [1,2,3]-triazoles are use as a linker with various active functional moiety to improve the ability to drugs discovery and exhibit wide range of bioactivities (Brak et a., 2010).

Galectin is a growing family of beta-galactoside binding protein. More than 10 galectins have been characterized in mammals, of which galectin-1 (Gal-1) and galectin-3 (Gal-3) have been extensively studied. Galectin-3 is an intra and extra-cellular lectin with a correlation of expression and functional implication in inflammation and the aggressiveness and metastatic potential of cancer. Proteomic analysis of the cholangiocarcinoma cell line in Thai people has shown high expression levels of Gal-3 and 93% of the cholangiocarcinoma cells were positive for Gal-3 staining. Contrarily, there were decreased Gal-3 expressions in some tumors, such as prostate and uterine cancers. Natural saccharides have been proposed as inhibitors of galectins. However, they are difficult to synthesize, sensitive to hydrolysis, and they are typically too polar to be used as drugs. One approach to circumvent these disadvantages of glycosides is to prepare inhibitors of galectins in which the saccharide is replaced by simpler and less polar structures.

Salameh, Leffler, and Nilsson (2005) reported the three inhibitors **11-13** for the tumor and inflammation related galectin-3 with low K_d values as 107-147 μM. 3-deoxy-3-(1H-1,2,3-triazol-1-yl)-1-thio-galactosides contained with the phenyl-substituted **11**, and the butyl **12** and benzyl amides **13**, were synthesized *via* Cu(I)-catalyzed cycloaddition of methyl-3-azido-3-deoxy-1-thio-*β*-D-galactoside **7** with acetylene derivatives **8** in Cu(I), DIPEA and toluene at 40 ^{o}C. 1,4-Disubstituted triazoles **9** and **10** were obtained in high yield as single regioisomers. Finally, deprotection of **9** with methylamine in water gave **11** in 95% yields, whereas the reaction of methyl ester **10** with different amines gave a panel of 4-carbamoyltriazoles **12** and **13** in 80-90% yields (Scheme 5).



Scheme 5 Synthesis of 3-deoxy-3-(1H-1,2,3-triazol-1-yl)-1-thio-galactosides *via* Cu(I)-catalyzed cycloaddition.

Based on the literature reports of the importance of the triazole linked glycoside toward the tumor and inflammation related galectin-3, in this project, we designed to synthesize a new class of 1,4-disubstituted-1,2,3-bistriazole linked C-1, C-6 positions of α-D-glucopyranoside from commercial available *O*-methyl-D-glucopyranose. The synthetic compounds will be studied as the therapeutic agent for treatment of cholangiocarcinoma.

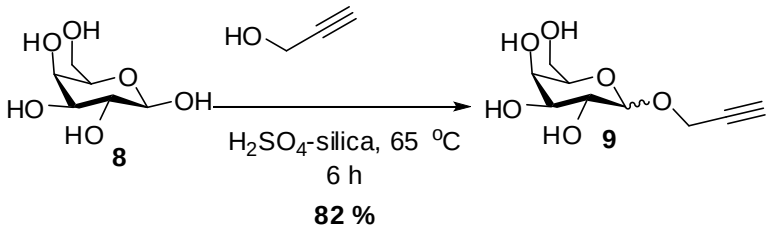
CHAPTER 3
RESEARCH METHODOLOGY

3.1 General Methods

All chemicals were purchased from commercial sources and used without further purification. Proton NMR spectra were recorded on a BRUKER AVANCE (400 MHz). All spectra were measured in $CDCl_3$ solvent and chemical shifts are reported as δ values in parts per million (ppm) relative to tetramethylsilane (δ 0.00) or $CDCl_3$ (δ 7.26) as an internal standard. Data are reported as follows; chemical shift (multiplicity, integrate intensity or assignment, coupling constants in Hz, assignment). Carbon NMR spectra were recorded on a BRUKER AVANCE (100 MHz). All spectra were measured in $CDCl_3$ solvent and chemical shifts are reported as δ values in parts per million (ppm) relative to $CDCl_3$ (δ 77.0) as internal standard. High-resolution mass spectra (HRMS) data were obtained with a Finnigan MAT 95. Infrared spectra (IR) were determined on a PERKIN ELMER FT/IR-2000S spectrophotometer and are reported in wave number (cm^{-1}). Analytical thin-layer chromatrography (TLC) was conducted on precoated TLC plates; silica gel 60F-254 (E. Merck, Darmstadt, Germany). Silica gel columns for open-column chromatrography utilized silica gel 60 (Grade 7729, < 230 mesh, 7734, 70-230 mesh and 9385, 230-400 mesh, E. Merck, Darmstadt, Germany). Melting points were recorded using GALLENKAMP Melting point apparatus Griffin.

3.2 Methodology for the synthesis of acetylene glycoside 10

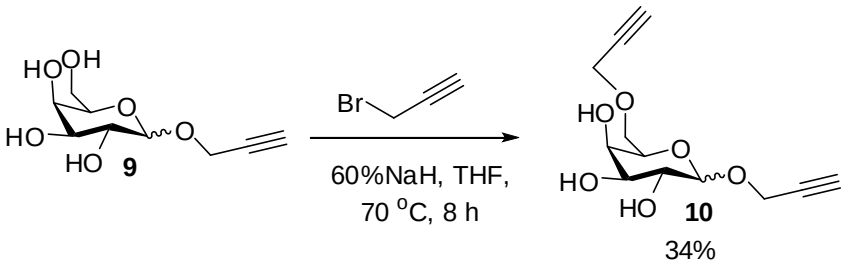
3.2.1 Preparation of 1-*O*-propargyl galactoside (**9)**



Scheme 1 Synthesis of *O*-propargyl galactoside **9**

D-galactose **8** (10.0 g, 0.056 mol) was suspended in propargyl alcohol (31 mL) and stirred at 65 ^{o}C. H_2SO_4–silica was added and stirring was continued until TLC showed complete consumption of the D-galactose. After cooling to room temperature, the reaction mixture was transferred to a short silica gel column to afford the desired 1-*O*-propargyl galactoside **9** as a pale yellow oil (10.0 g, 82 %).

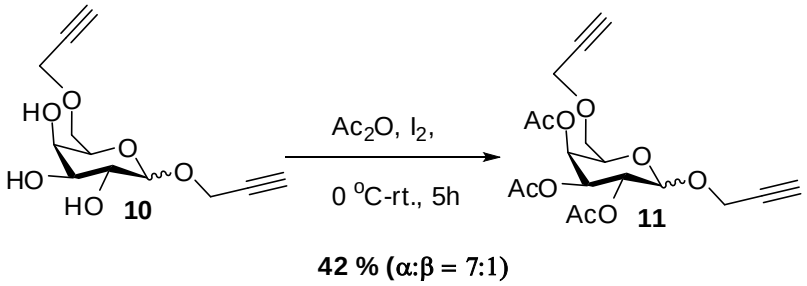
3.2.2 Preparation of 1,6-di-*O*-propargyl galactoside **(10)**



Scheme 2 Synthesis of 1-*O*-propargyl galactoside **10**

NaH (2.06 g, 52 mmol, 1.5 eq.) was added to the solution of **9** (7.5g, 34 mmol) in THF under N_2 atmosphere at 0 ^oC. Then, propargyl bromide (4.4 ml, 52 mmol, 1.5 eq) was added and the reaction was stirred at 70 ^oC. When the TLC showed the complete consumption of the D-galactose, the reaction was allowed to room temperature and extracted with EtOAc, washed with water and brine, then dried with Na_2SO_4 anhydrous, filtered, and evaporated in vacuo. The crude compound was purified by column chromatography to afford the desired 1,6-di-*O*-propargyl galactoside **10** in 34 **%** yield (3g).

3.2.3 Preparation of 2,3,4-tri-O-acetyl-1,6-di-*O*-propargylgalactoside **(11**)

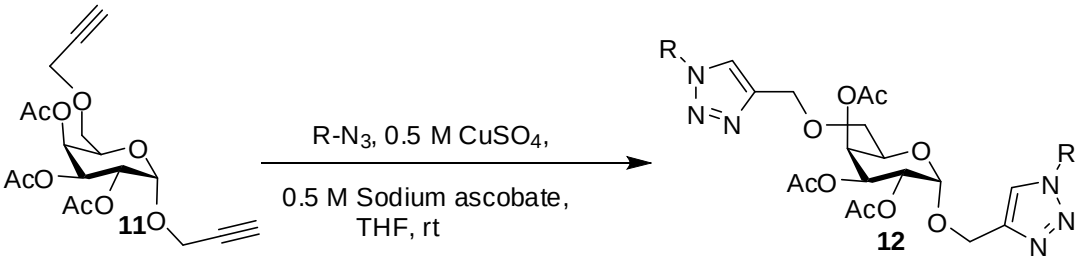


Scheme 3 Synthesis of 2,3,4-tri-O-acetyl-1,6-di-*O*-propargylgalactoside **11**

Molecular iodine (3%mol) was added to the solution of **10** (3 g, 11.7 mmol) in Ac_2O (5.6 ml) at 0 ^oC. The reaction was stirring at room temperature for 5h. The reaction mixture was transferred to a silica gel column to afford the desired 2,3,4-tri-*O*-acetyl-1,6-di-*O*-propargyl-α-glucoside**11** 2.87 in 42 **%** (α: β = 7:1).

3.3 Synthesis of 1,6-di-triazolyl-2,3,4-tri-*O*-acetyl-α-galactoside analogues

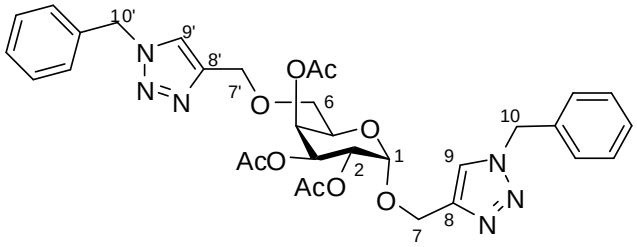
General procedure A



Scheme 4 Synthesis of 1,6-di-triazolyl-2,3,4-tri-*O*-acetyl-α-galactoside **12**

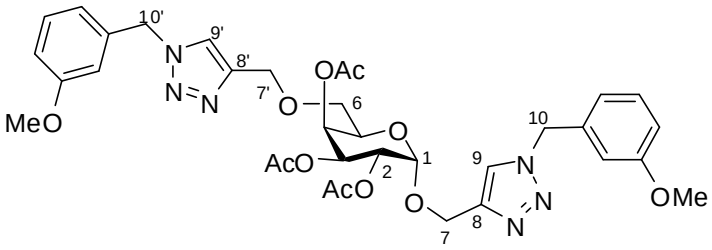
The solution of the bis-*O*-propargyl glycoside **11** (1 equivalent), azide (2.5 equivalent), 0.5 M aq. $CuSO_4 \cdot 5H_2O$ (20% mol) and 0.5 M aq. sodium ascorbate (20% mol) in THF was stirred at room temperature until TLC showed complete conversion. The reaction was quenched with H_2O, and extracted with 3xEtOAc. The organic phase was collected, dried with Na_2SO_4 anhydrous, filtered and evaporated in vacuo. The crude product was purified by column chromatography (SiO_2, 50-90% EtOAc/hexane as eluent) to afford the desired product **12**.

3.3.1 Synthesis of 1,6-di-triazolyl-2,3,4- *O*-tri-*O*-acetyl-α-galactoside product **12a.**



Following the general procedure A, bis-*O*-propargyl glycoside **11** (50 mg, 130.8 mmol) and benzyl azide (55.2mg) was used in click reaction. The reaction was stirred at room temperature for 10 minutes. The crude product was purified by silica gel column chromatography to afford bistriazole-linked glycoside **12a** as pale yellow oil (69.0 mg, 82%).

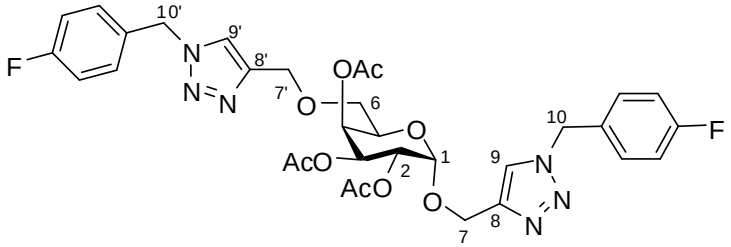
3.3.2 Synthesis of 1,6-di-triazolyl-2,3,4-*O*-tri-*O*-acetyl-α-galactoside product **12b.**



Following the general procedure A, bis-*O*-propargyl glycoside **11** (50 mg, 130.8 mmol) and 3-methoxybenzyl azide (68.5 mg) was used in click reaction. The reaction was

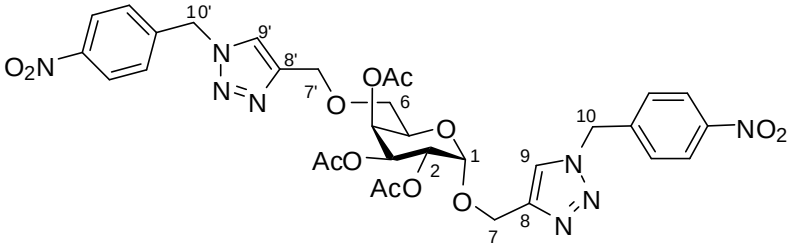
stirred at room temperature for 30 minutes. The crude product was purified by silica gel column chromatography to afford bistriazole-linked glycoside **12b** as pale yellow oil (86.0 mg, 86%).

3.3.3 Synthesis of 1,6-di-triazolyl-2,3,4-*O*-tri-*O*-acetyl-α-galactoside product **12c.**



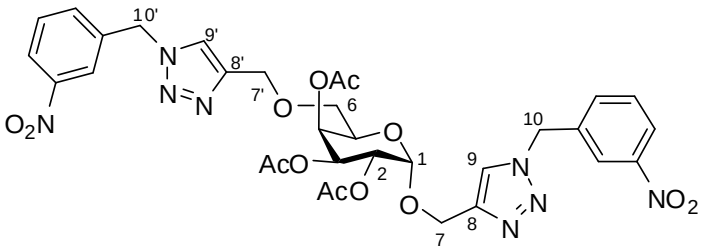
Following the general procedure A, bis-*O*-propargyl glycoside **11** (40 mg, 104.6 mmol) and 4-fluorobenzyl azide (39.3 mg) was used in click reaction. The reaction was stirred at room temperature for 5 mimutes. The crude product was purified by silica gel column chromatography to afford bistriazole-linked glycoside **12c** as a white solid (44.0 mg, 63%).

3.3.4 Synthesis of 1,6-di-triazolyl-2,3,4-*O*-tri-*O*-acetyl-α-galactoside product **12d.**



Following the general procedure A, bis-*O*-propargyl glycoside **11** (40 mg, 104.6 mmol) and 4-nitrobenzyl azide (71.3 mg) was used in click reaction. The reaction was stirred at room temperature for 5 minutes. The crude product was purified by silica gel column chromatography to afford bistriazole-linked glycoside **12d** as a white solid (52.0 mg, 70%).

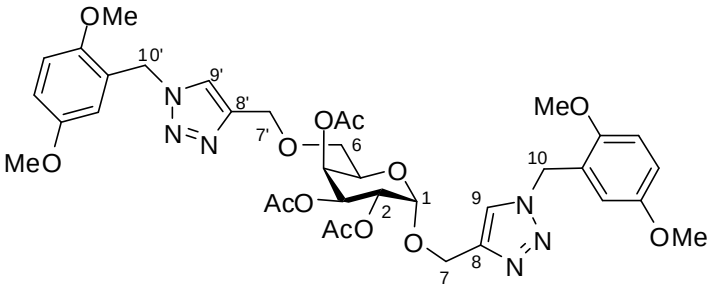
3.3.5 Synthesis of 1,6-di-triazolyl-2,3,4-*O*-tri-*O*-acetyl-α-galactoside product **12e.**



Following the general procedure A, bis-*O*-propargyl glycoside **11** (40 mg, 104.6 mmol) and 3-nitrobenzyl azide (71.3 mg) was used in click reaction. The reaction was stirred

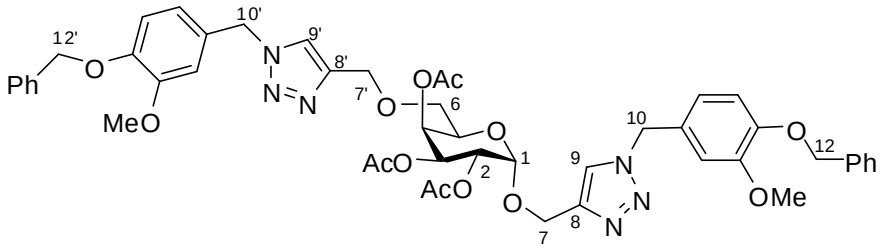
at room temperature for 5 minutes. The crude product was purified by silica gel column chromatography to afford bistriazole-linked glycoside **12e** as a yellow solid (42.0 mg, 56%).

3.3.6 Synthesis of 1,6-di-triazolyl-2,3,4-*O*-tri-*O*-acetyl-α-galactoside product **12f.**



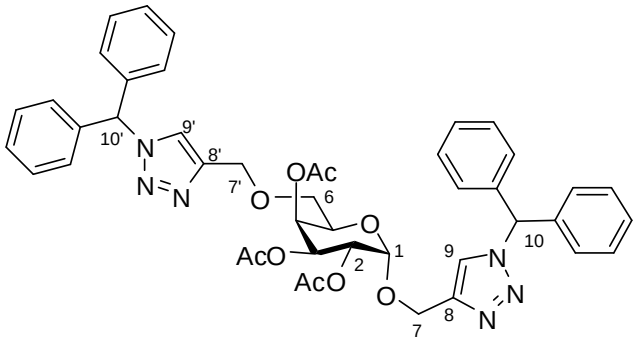
Following the general procedure A, bis-*O*-propargyl glycoside **11** (40 mg, 104.6 mmol) and 2,5-dimethoxybenzyl acetyl azide (76.2 mg) was used in click reaction. The reaction was stirred at room temperature for 5 minutes. The crude product was purified by silica gel column chromatography to afford bistriazole-linked glycoside **12f** as a white solid (72.0 mg, 93%).

3.3.7 Synthesis of 1,6-di-triazolyl-2,3,4-*O*-tri-*O*-acetyl-α-galactoside product **12g.**



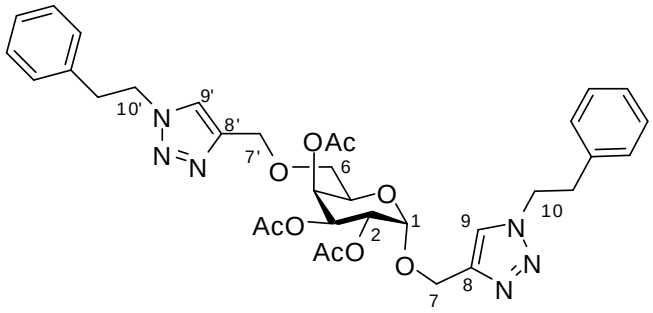
Following the general procedure A, bis-*O*-propargyl glycoside **11** (40 mg, 104.6 mmol) and 4-benzyloxy-3-methoxybenzyl acetyl azide (70.0 mg) was used in click reaction. The reaction was stirred at room temperature for 10 minutes. The crude product was purified by silica gel column chromatography to afford bistriazole-linked glycoside **12g** as a white solid (46.0 mg, 50%).

3.3.8 Synthesis of 1,6-di-triazolyl-2,3,4-*O*-tri-*O*-acetyl-α-galactoside product **12h.**



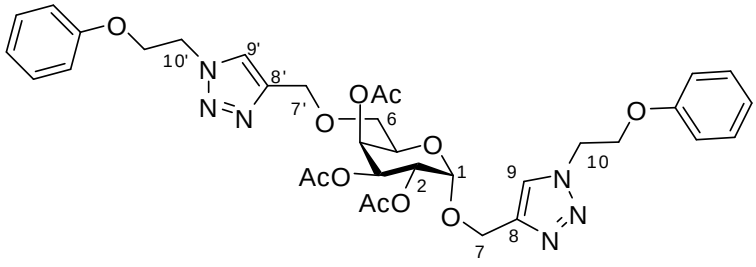
Following the general procedure A, bis-*O*-propargyl glycoside **11** (30 mg, 78.6 mmol) and diphenylmethyl acetyl azide (54.4 mg) was used in click reaction. The reaction was stirred at room temperature for 5 minutes. The crude product was purified by silica gel column chromatography to afford bistriazole-linked glycoside **12h** as a white solid (53.4 mg, 85 %).

3.3.9 Synthesis of 1,6-di-triazolyl-2,3,4-*O*-tri-*O*-acetyl-α-galactoside product **12i.**



Following the general procedure A, bis-*O*-propargyl glycoside **11** (50 mg, 130.8 mmol) and 2-phenylethyl azide (48.1 mg) was used in click reaction, the reaction was stirred at room temperature for 10 minutes. The crude product was purified by silica gel column chromatography to afford bistriazole-linked glycoside **12i** as a white solid (80.0 mg, 91%).

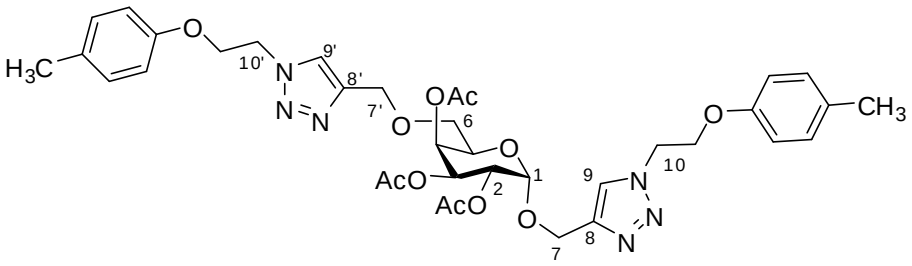
3.3.10 Synthesis of 1,6-di-triazolyl-2,3,4-*O*-tri-*O*-acetyl-α-galactoside product **12j.**



Following the general procedure A, bis-*O*-propargyl glycoside **11** (40 mg, 104.6 mmol) and 2-phenoxyethyl azide (42.4 mg) was used in click reaction, the reaction was

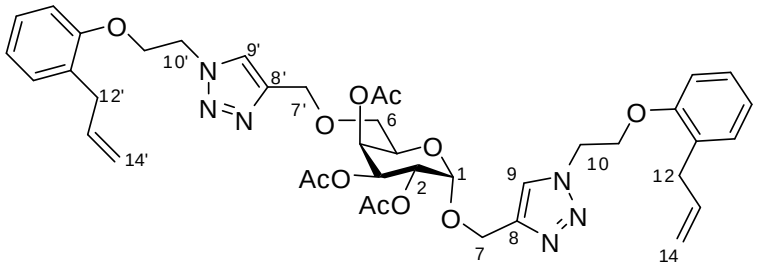
stirred at room temperature for 5 minutes. The crude product was purified by silica gel column chromatography (SiO_2, 80% EtOAc/hexane as eluent) to afford bistriazole-linked glycoside **12j** as colorless oil (54.0 mg, 73%).

3.3.11 Synthesis of 1,6-di-triazolyl-2,3,4-*O*-tri-*O*-acetyl-α-galactoside product **12k.**



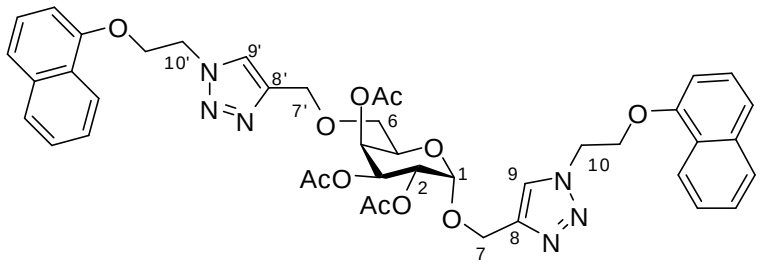
Following the general procedure A, bis-*O*-propargyl glycoside **11** (40 mg, 104.6 mmol) and 2-(4-methylphenoxy)-ethyl azide azide (46.1 mg) was used in click reaction. The reaction was stirred at room temperature for 5 minutes. The crude product was purified by silica gel column chromatography to afford bistriazole-linked glycoside **12k** as colorless oil (60.0 mg, 78%).

3.3.12 Synthesis of 1,6-di-triazolyl-2,3,4-*O*-tri-*O*-acetyl-α-galactoside product **12l.**



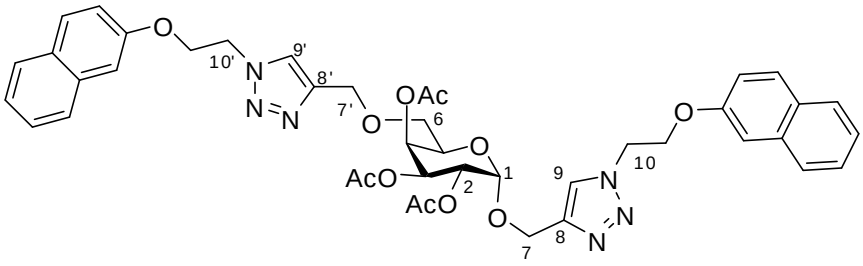
Following the general procedure A, bis-*O*-propargyl glycoside **11** (40 mg, 104.6 mmol) and 2-(2-allylphenoxy)-ethyl azide (52.8 mg) was used in click reaction. The reaction was stirred at room temperature for 5 minutes. The crude product was purified by silica gel column chromatography to afford bistriazole-linked glycoside **12l** as pale yellow oil (68.0 mg, 83%).

3.3.13 Synthesis of 1,6-di-triazolyl-2,3,4-*O*-tri-*O*-acetyl-α-galactoside product **12m.**



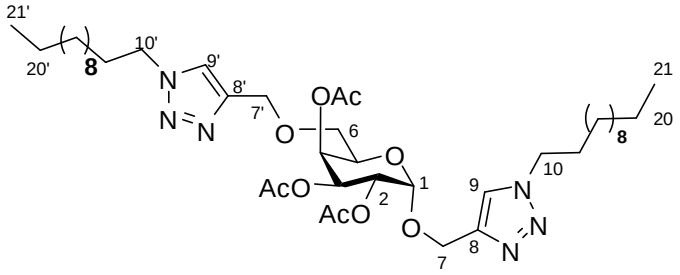
Following the general procedure A, bis-*O*-propargyl glycoside **11** (40 mg, 104.6 mmol) and 2-naphthalen-1-yl-ethyl azide (55.7 mg) was used in click reaction. The reaction was stirred at room temperature for 5 minutes. The crude product was purified by silica gel column chromatography to afford bistriazole-linked glycoside **12m** as pale yellow oil (62.0 mg, 73%).

3.3.14 Synthesis of 1,6-di-triazolyl-2,3,4-*O*-tri-*O*-acetyl-α-galactoside product **12n.**



Following the general procedure A, bis-*O*-propargyl glycoside **11** (40 mg, 104.6 mmol) and 2-naphthalen-2-yl-ethyl azide (55.7 mg) was used in click reaction. The reaction was stirred at room temperature for 5 minutes. The crude product was purified by silica gel column chromatography to afford bistriazole-linked glycoside **12n** as a white solid (54.0 mg, 67%).

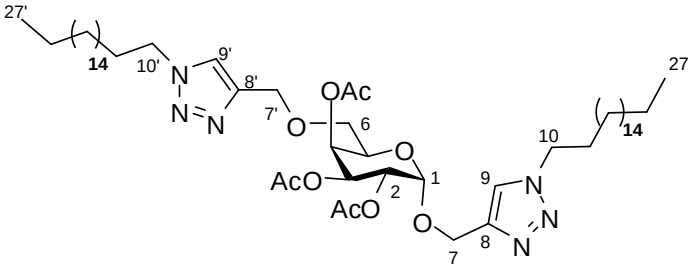
3.3.15 Synthesis of 1,6-di-triazolyl-2,3,4-*O*-tri-*O*-acetyl-α-galactoside product **12o.**



Following the general procedure A, bis-*O*-propargyl glycoside **11** (50 mg, 130.8 mmol) and lauryl azide (76.8 mg) was used in click reaction. The reaction was stirred at room

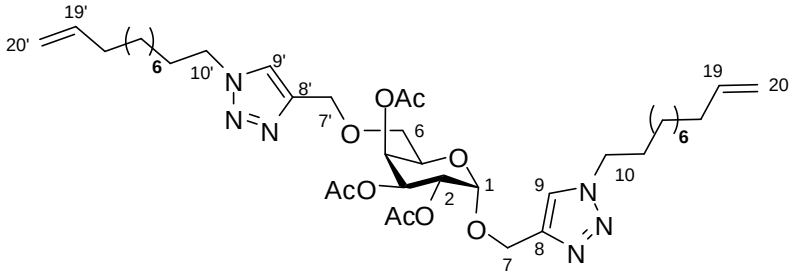
temperature for 1 h. The crude product was purified by silica gel column chromatography to afford bistriazole-linked glycoside **12o** as colorless oil (70.0 mg, 61%).

3.3.16 Synthesis of 1,6-di-triazolyl-2,3,4-*O*-tri-*O*-acetyl-α-galactoside product **12p.**



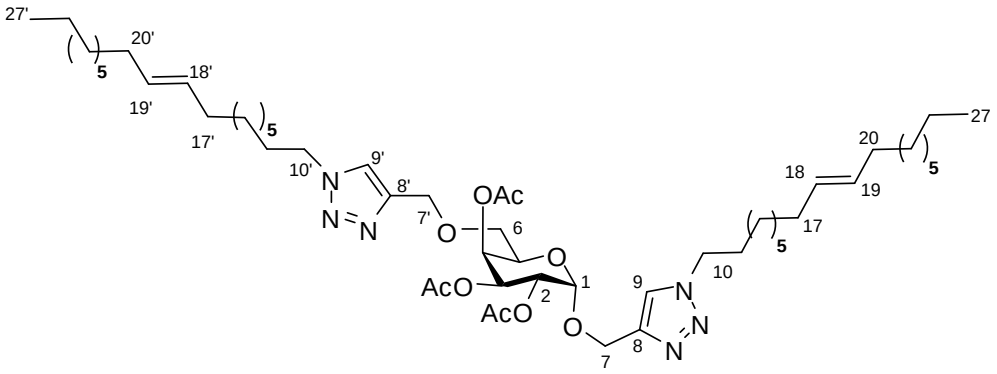
Following the general procedure A, bis-*O*-propargyl glycoside **11** (50 mg, 130.8 mmol) and octadecanyl azide (124.1 mg) was used in click reaction. The reaction was stirred at room temperature for 5 h. The crude product was purified by silica gel column chromatography to afford bistriazole-linked glycoside **12p** as a white solid (64.0 mg, 63%).

3.3.17 Synthesis of 1,6-di-triazolyl-2,3,4-*O*-tri-*O*-acetyl-α-galactoside product **12q.**



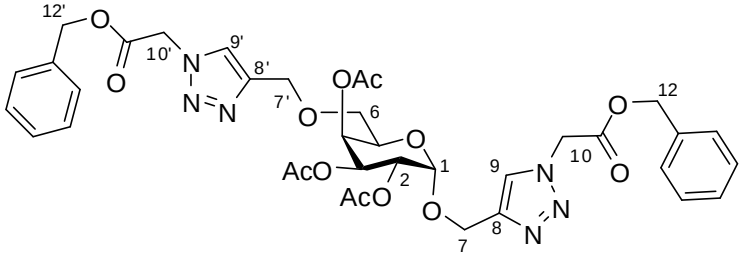
Following the general procedure A, bis-*O*-propargyl glycoside **11** (50 mg, 130.8 mmol) and omega-undecylenyl azide (82.0 mg) was used in click reaction. The reaction was stirred at room temperature for 1.5 h. The crude product was purified by silica gel column chromatography to afford bistriazole-linked glycoside **12q** as colorless oil (70.0 mg, 70 %).

3.3.18 Synthesis of 1,6-di-triazolyl-2,3,4-*O*-tri-*O*-acetyl-α-galactoside product **12r.**



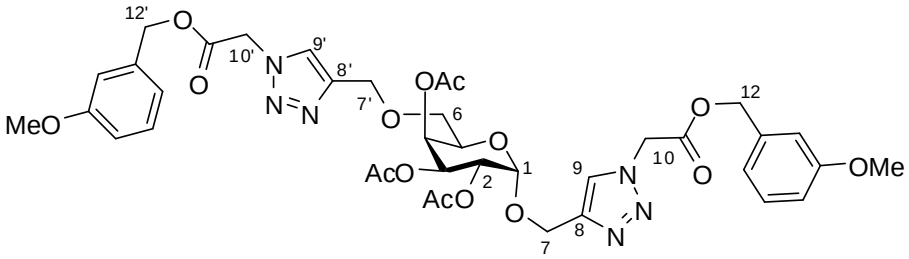
Following the general procedure A, bis-*O*-propargyl glycoside **11** (50 mg, 130.8 mmol) and olelyl azide (117.4 mg) was used in click reaction. The reaction was stirred at room temperature for 12 h. The crude product was purified by silica gel column chromatography to afford bistriazole-linked glycoside **12r** as pale yellow oil (83.6 mg, 67%).

3.3.19 Synthesis of 1,6-di-triazolyl-2,3,4-*O*-tri-*O*-acetyl-α-galactoside product **12s.**



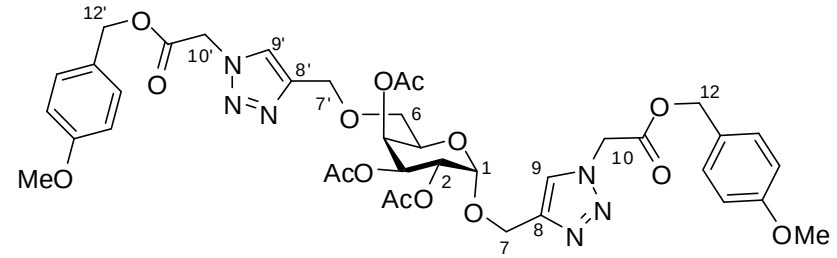
Following the general procedure A, bis-*O*-propargyl glycoside **11** (40 mg, 104.6 mmol) and benzyloxy acetyl azide (50.0 mg) was used in click reaction. The reaction was stirred at room temperature for 5 minutes. The crude product was purified by silica gel column chromatography to afford bistriazole-linked glycoside **12s** as colorless oil (80.0 mg, 99%).

3.3.20 Synthesis of 1,6-di-triazolyl-2,3,4-*O*-tri-*O*-acetyl-α-galactoside product **12t.**



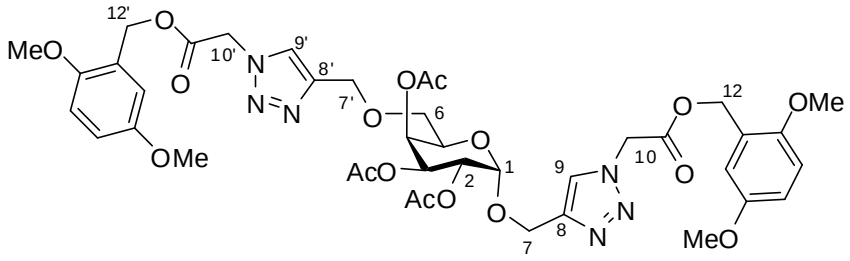
Following the general procedure A, bis-*O*-propargyl glycoside **11** (40 mg, 104.6 mmol) and 3-methoxybenzyloxy acetyl azide (58.0 mg) was used in click reaction. The reaction was stired at room temperature for 5 minutes. The crude product was purified by silica gel column chromatography (SiO_2, 100% EtOAc as eluent) to afford bistriazole-linked glycoside **12t** as pale yellow oil (81.0 mg, 95%).

3.3.21 Synthesis of 1,6-di-triazolyl-2,3,4-*O*-tri-*O*-acetyl-α-galactoside product **12u.**



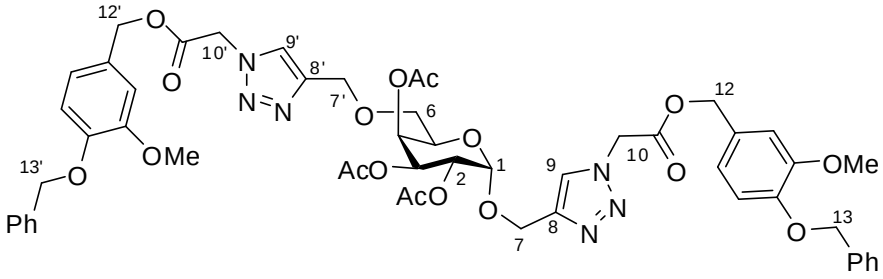
Following the general procedure A, bis-*O*-propargyl glycoside **11** (40 mg, 104.6 mmol) and 4-methoxybenzyloxy acetyl azide (58.0 mg) was used in click reaction. The reaction was stirred at room temperature for 5 minutes. The crude product was purified by silica gel column chromatography to afford bistriazole-linked glycoside **12u** as a pale yellow oil (87.0 mg, 99%).

3.3.22 Synthesis of 1,6-di-triazolyl-2,3,4-*O*-tri-*O*-acetyl-α-galactoside product **12v.**



Following the general procedure A, bis-*O*-propargyl glycoside **11** (40 mg, 104.6 mmol) and 2,5-dimethoxybenzyloxy acetyl azide (65.0 mg) was used in click reaction. The reaction was stirred at room temperature for 5 minutes. The crude product was purified by silica gel column chromatography to afford bistriazole-linked glycoside **12v** as pale yellow oil (45.0 mg, 49%).

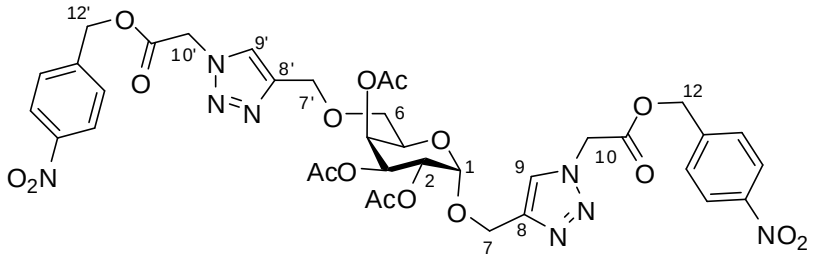
3.3.23 Synthesis of 1,6-di-triazolyl-2,3,4-*O*-tri-*O*-acetyl-α-galactoside product **12w.**



Following the general procedure A, bis-*O*-propargyl glycoside **11** (40 mg, 104.6 mmol) and 4-benzyloxy-3-methoxybenzyloxy acetyl azide (91.3 mg) was used in click reaction. The reaction was stirred at room temperature for 5 minutes. The crude product was

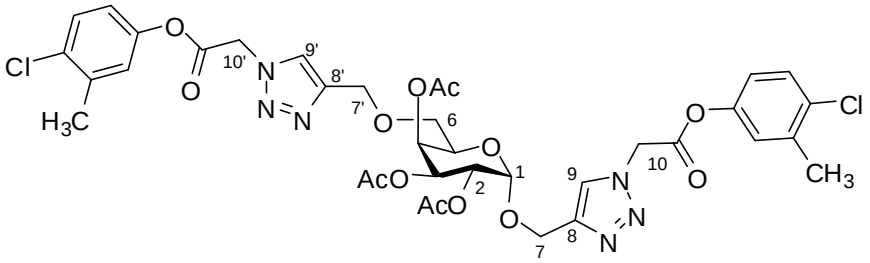
purified by silica gel column chromatography to afford bistriazole-linked glycoside **12w** as a white solid (67.5 mg, 62%).

3.3.24 Synthesis of 1,6-di-triazolyl-2,3,4-*O*-tri-*O*-acetyl-α-galactoside product **12x.**



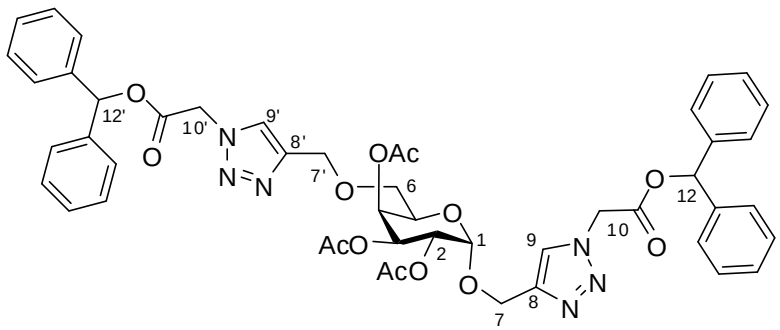
Following the general procedure C, bis-*O*-propargyl glycoside **11** (40 mg, 104.6 mmol) and 4-nitrobenzyloxy acetyl azide (71.3 mg) was used in click reaction. The reaction was stirred at room temperature for 5 minutes. The crude product was purified by silica gel column chromatography to afford bistriazole-linked glycoside **12x** as pale yellow oil (89.0 mg, 99%).

3.3.25 Synthesis of 1,6-di-triazolyl-2,3,4-*O*-tri-*O*-acetyl-α-galactoside product **12y.**



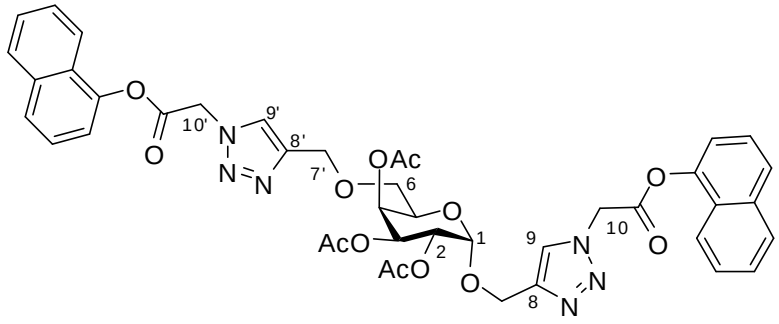
Following the general procedure A, bis-*O*-propargyl glycoside **11** (40 mg, 104.6 mmol) and 4-chloro-3-methylphenyl acetyl azide (69.0 mg) was used in click reaction. The reaction was stirred at room temperature for 5 minutes. The crude product was purified by silica gel column chromatography to afford bistriazole-linked glycoside **12y** as a white solid (37.2 mg, 43 %).

3.3.26 Synthesis of 1,6-di-triazolyl-2,3,4-*O*-tri-*O*-acetyl-α-galactoside product **12z.**



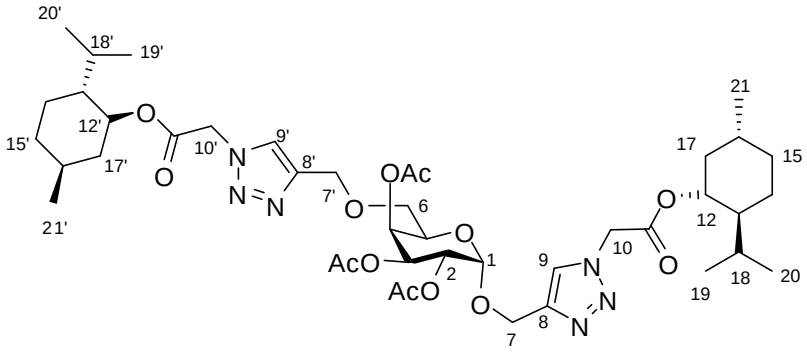
Following the general procedure A, bis-*O*-propargyl glycoside **11** (40 mg, 104.6 mmol) and diphenylmethoxy acetyl azide (79.3 mg) was used in click reaction. The reaction was stirred at room temperature for 2 h. The crude product was purified by silica gel column chromatography to afford bistriazole-linked glycoside **12z** as a white solid (72.0 mg, 75 %).

3.3.27 Synthesis of 1,6-di-triazolyl-2,3,4-*O*-tri-*O*-acetyl-α-galactoside product **12aa.**



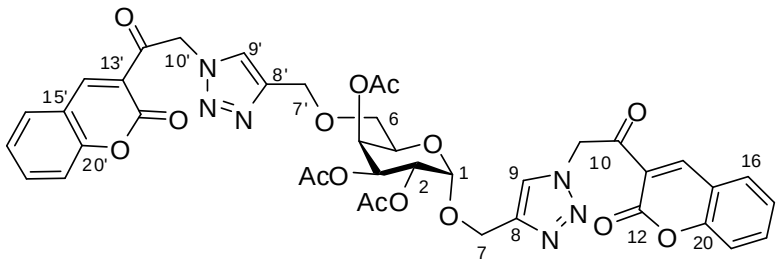
Following the general procedure A, bis-*O*-propargyl glycoside **11** (40 mg, 104.6 mmol) and naphthalen-1-yl acetyl azide (59.0 mg) was used in click reaction. The reaction was stirred at room temperature for 10 minutes. The crude product was purified by silica gel column chromatography to afford bistriazole-linked glycoside **12aa** as a colorless oil (66.0 mg, 76 %).

3.3.28 Synthesis of 1,6-di-triazolyl-2,3,4-*O*-tri-*O*-acetyl-α-galactoside product **12bb.**



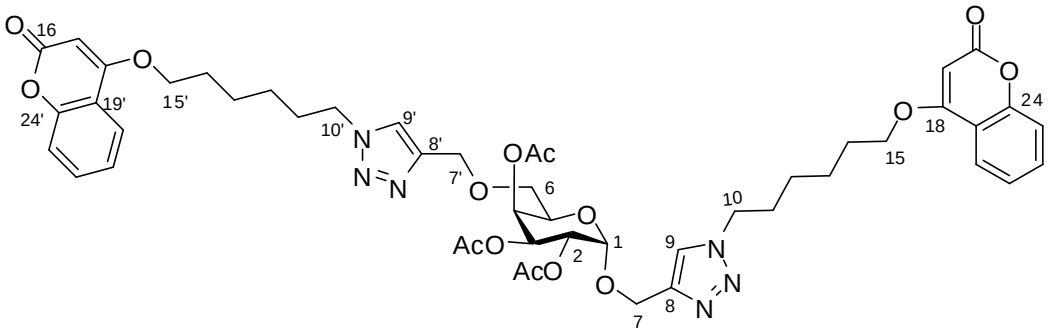
Following the general procedure A, bis-*O*-propargyl glycoside **11** (40 mg, 104.6 mmol) and (-) menthol acetyl azide (67.0 mg) was used in click reaction. The reaction was stirred at room temperature for 10 minutes. The crude product was purified by silica gel column chromatography to afford bistriazole-linked glycoside **12bb** as a white solid (91.0 mg, 99%).

3.3.29 Synthesis of 1,6-di-triazolyl-2,3,4-*O*-tri-*O*-acetyl-α-galactoside product **12cc.**



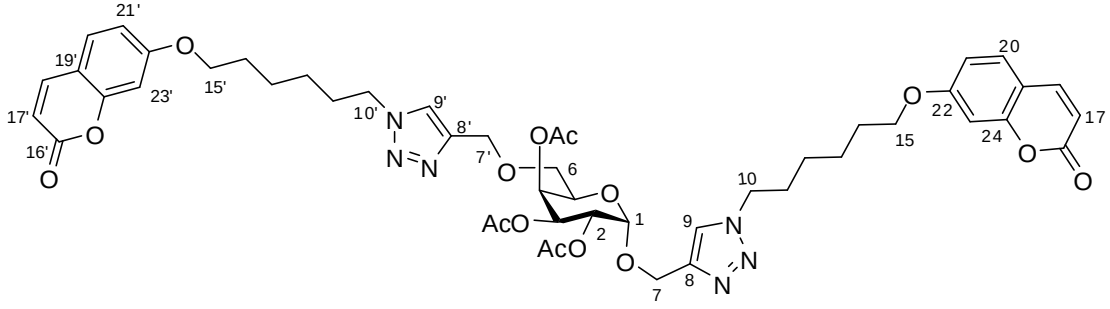
Following the general procedure A, bis-*O*-propargyl glycoside **11** (50 mg, 130.8 mmol) and 3-acetyl-coumarin azide (96.3 mg) was used in click reaction. The reaction was stirred at room temperature for 5 minutes. The crude product was purified by silica gel column chromatography to afford bistriazole-linked glycoside **12cc** as a yellow solid (40.0 mg, 37 %).

3.3.30 Synthesis of 1,6-di-triazolyl-2,3,4-*O*-tri-*O*-acetyl-α-galactoside product **12dd.**



Following the general procedure A, bis-*O*-propargyl glycoside **11** (40 mg, 104.6 mmol) and 4-hexyloxy-coumarin azide (75.0 mg) was used in click reaction. The reaction was stirred at room temperature for 30 minutes. The crude product was purified by silica gel column chromatography to afford bistriazole-linked glycoside **12dd** as pale yellow oil (98.0 mg, 98 %).

3.3.31 Synthesis of 1,6-di-triazolyl-2,3,4-*O*-tri-*O*-acetyl-α-galactoside product **12ee.**



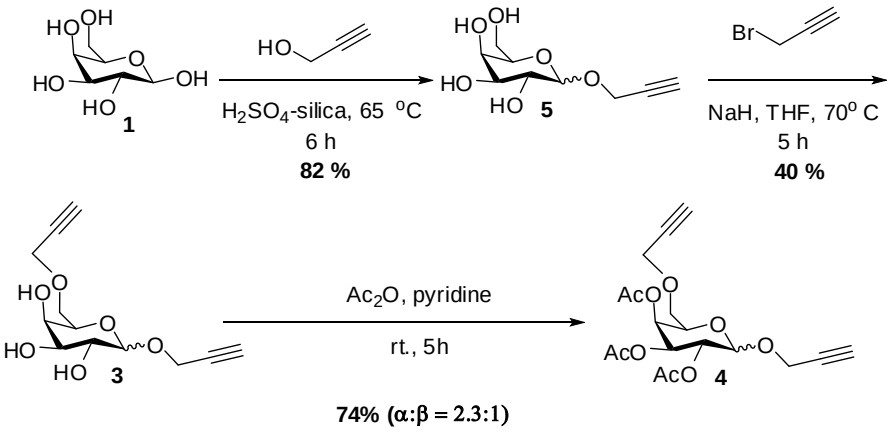
Following the general procedure A, bis-*O*-propargyl glycoside **11** (40 mg, 104.6 mmol) and 7-hexyloxy-coumarin azide (75.0 mg) was used in click reaction. The reaction was stirred at room temperature for 30 minutes. The crude product was purified by silica gel column chromatography to afford bistriazole-linked glycoside **12ee** as pale yellow oil (102.0 mg, 99 %).

CHAPTER 4
RESULTS, DISCUSSION & CONCLUSION

The triazole-linked glycosides have been extensively studied in research for the design of various functionalized glycoconjugates, facilitating a multitude of practical physical, chemical, biological studies and validated pharmaceutical properties. However, they are a few researches study on the synthesis of bis-triazoleglucosides *via* dual click chemistry and elucidation for their biological properties**.**

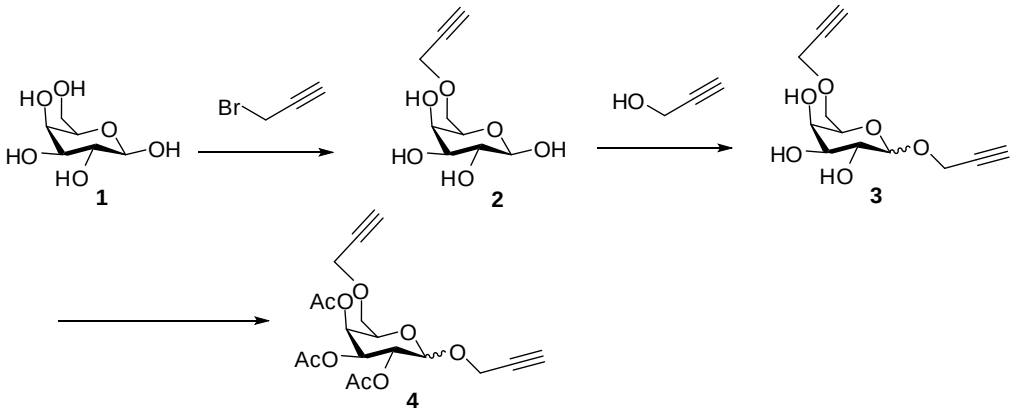
In this work, we designed to synthesize a new class of 1,6-bis-triazole 2,3,4-tri-*O*-acetyl-α-D-galactopyranosyl analogues via click chemistry (Scheme 1). All the synthetic anologues of the bistriazole glycosides will be evaluated for anti-proliferative activity on human cholangiocarcinoma cells (KKU-M213, HUCCA-1, K-100), cancer cells derived from Thai patient.

In the first part, bis-propargyl glycoside was prepared for using as precursor for synthesis of our target 1,6-bis-triazole 2,3,4-tri-*O*-acetyl-α-D-galactopyranosyl analogues. The reaction condition was optimized to obtain the product in high yields and good anomeric selectivity. Bis-*O*-propargyl-D-glucopyranosides was prepared *via* three-steps process using commercial available *O*-methyl-D-glucopyranose as a starting material. The reaction was shown in scheme **4.1**.



Scheme 4.1 Synthesis of bis-propargyl glycoside **12** from D-(+)-galactopyranoside **(9)**

4.1 The synthesis approach to 1,6-di-*O*-propargyl-2,3,4- di-*O*-acetylgalactoside 12

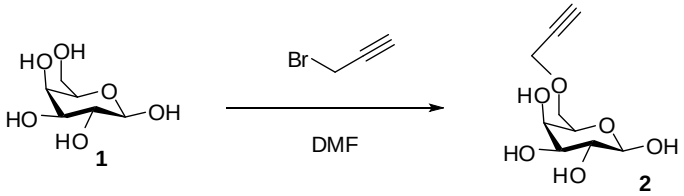


Scheme 4.2 1st Synthetic plan of 1,6-di-*O*-propargyl-2,3,4- di-*O*-acetylgalactoside **12**

First, we optimized the condition for synthesis of acetylene glycoside **4** from galactopyranoside **1** as shown in Scheme 4.2. Chemoselective propargylation at 6-positon of galactopyranoside **1** with propargyl bromide was performed followed by *O*-glycosidation by using propargly-alcohol and finally acetylaton of the remaining hydroxyl groups with acetic anhydride to give tri-acetyldiacetylene glycoside **4.**

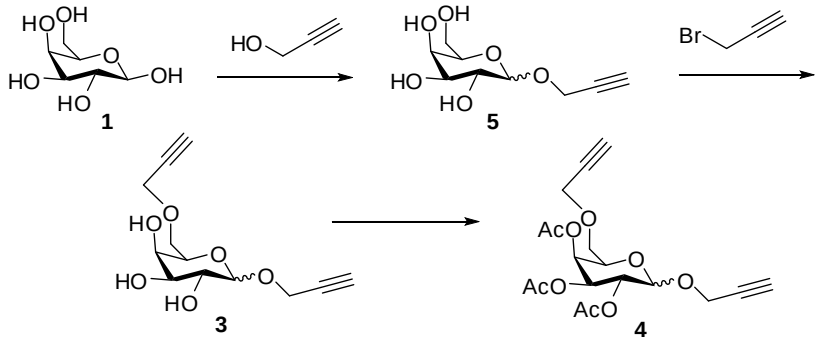
As showed in Table 4.1, optimized condition for the first step of synthesis of acetylene glycoside **2** was carried out by using various bases in DMF. Inorganic base Cs_2CO_3 and K_2CO_3 were employed in the reaction at room temperature or heating at 70 ^oC. The reactions were stirred 2-6 days to afford the desired compound **2** in fair yield by observation on TLC (60-70% yields) (entries 1-4). However, due to the high water solubility of compound **2**, purification by silica gel column chromatography led to the low yield of isolated product (1-9%). Moreover, no reaction was occurred when Et_3N and pyridine were employed as base (entry 5 and 6). Since these conditions could not generated compound **2** in short reaction time then we further optimized the new conditions for synthesis of glycoside **4.**

Table 4.1 Optimized condition for synthesis of acetylene glycoside **2**

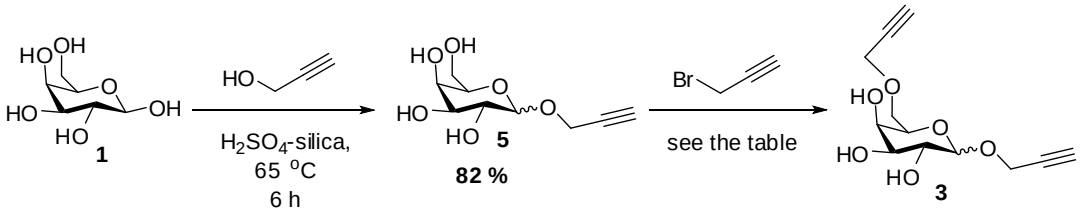


entry	base	T(^{o}C)	Time	%yield (TLC)	%yield (isolated)
1	Cs_2CO_3	rt	2 day	60	9
2	Cs_2CO_3	70	6 h	70	23
3	K_2CO_3	rt	2 day	50	1
4	K_2CO_3	70	2 day	70	23
5	Et_3N	70	2 day	No reaction	
6	pyridine	70	2 day	No reaction	

The condition for synthesis the precursor **4** was optimized by start with *O*-glycosylation of D-(+)-galactopyranoside **1** at anomeric position using Roy condition (Roy, 2007) and followed by chemoselective propargylation at 6-positon and acetylation of the remaining hydroxyl groups with acetic anhydride to give tri-acetyl-diacetylene glycoside **4** (Scheme 4.5).



Scheme 4.3 2nd Synthetic plan of 1,6-di-*O*-propargyl-2,3,4-di-*O*-acetylgalactoside **4**



Scheme 4.4 Synthesis of diacetylene glycoside **3** from galactopyranoside **5**

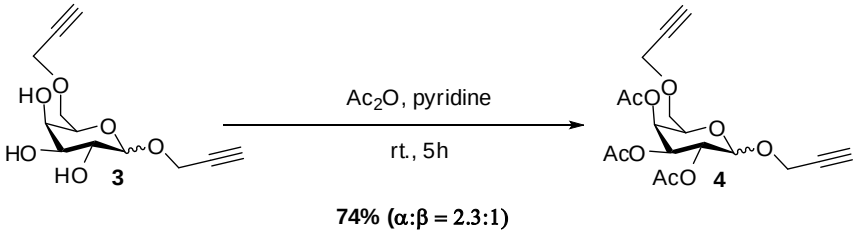
The synthesis of **4** in the 2nd plan was commenced with selective *O*-glycosylation of D-(+)-galactopyranoside **1** using propargyl alcohol as nucleophile and solvent. H_2SO_4-slilca was used as catalyst to generate soft acid, the reaction was heat at 65 ^oC for 6 h to afford the mixture isomer of galactopyranoside **5** in high yields (82%). Regioselective *O*-propargylation reaction of primary alcohol at C-6 position of **5** is a difficult and complicated. Therefore, the optimum conditions for the synthesis of compound **3** to give the best results were studied.

As shown in Table 4.2, first the reactions were proceeded smoothly using K_2CO_3 and Cs_2CO_3 as a base to generate alkoxide anions for nucleophilic substitution (S_N2) with propragyl bromide in CH_2CN and DMF, respectively (entry 1 and 2) affording **3** in a low yield. 60% NaH in mineral oil was used to generate alkoxide anions of C-6 position of **5** by using THF, a dilute of medium polar solvent. The reaction mixture was heated at 70 ^oC for 2 h to afford galactopyranoside **3** in low yields (entry 3). Prolong the reaction time led to the increasing of the yield of compound **3** to moderat yield (34-40%) (entries 4-5).

Table 4.2 Optimized condition for synthesis of 1,6-di-*O*-propargyl-2,3,4-tri-hydoxylgalactoside **3**

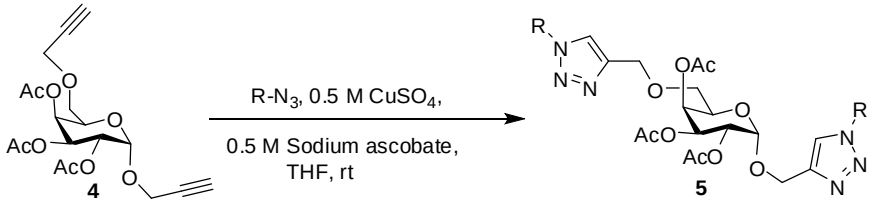
entry	base	solvent	T	Time	%yield
1	K_2CO_3	CH_3CN	60	24	18
2	Cs_2CO_3	DMF	rt	24	17
3	NaH	THF	70	2	17
4	NaH	THF	70	5	40
5	NaH	THF	70	8	34

With compound **3** in hand, acetylation of the remaining hydroxyl grups were performed by using Ac_2O in pyridine to afford 1,6-di-*O*-propargyl-2,3,4-di-*O*-acetylgalactoside **4** in high yield.



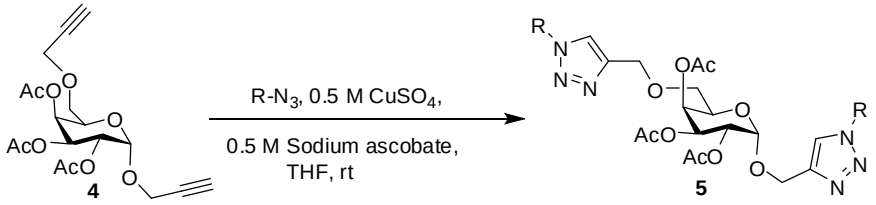
Scheme 4.5 Acetylation of 1,6-di-*O*-propargyl-2,3,4-tri-hydoxylgalactoside **3**

The new derivative of 1,6-di-*O*-propargyl-2,3,4-tri-*O*-acetylgalactoside **4** was prepared *via* three-steps process using commercial available D-(+)-galactopyranoside **(9)** as a starting material. Diacetylene glycoside **4** will be used as a precursor to prepare various bis-triazole glycosides as shown in Table 4.3 for study the cytotoxic activity on human cholangiocarcinoma cells (KKU-M213, HUCCA-1, K-100).



Scheme 4.6 Synthesis of 1,6-bis-triazole 2,3,4-tri-*O*-acetyl-α-D-galactopyranosyl derivatives

Table 4.3 Synthesis of 1,6-bis-triazole 2,3,4-tri-*O*-acetyl-α-D-galactopyranosyl derivatives



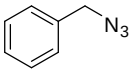
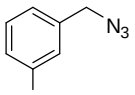
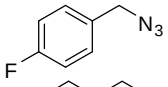
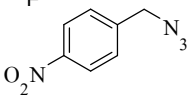
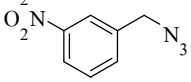
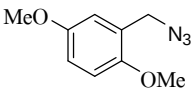
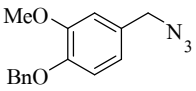
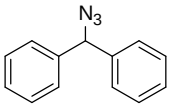
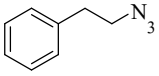
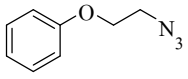
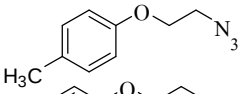
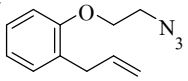
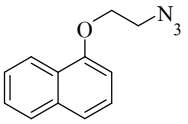
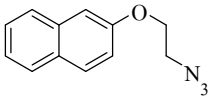
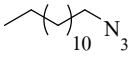
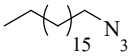
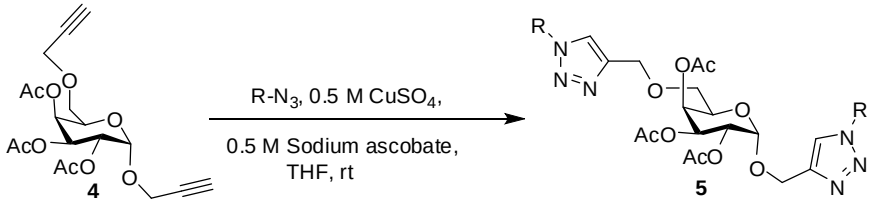
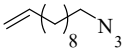
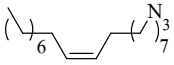
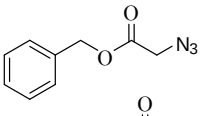
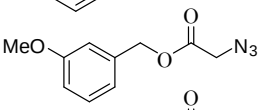
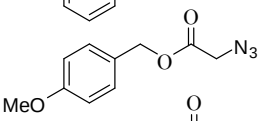
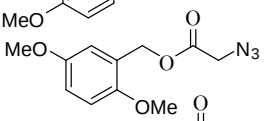
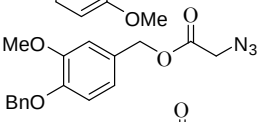
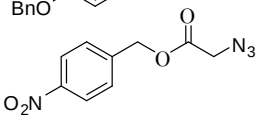
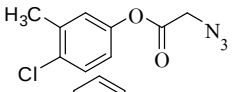
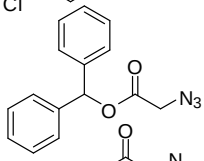
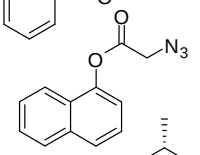
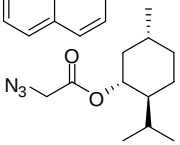
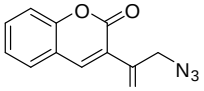
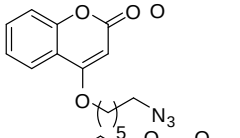
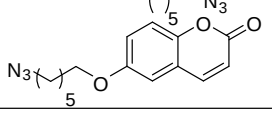
entry		R-N_3	12 t (min)	12 %yield
1	**a**		10	82
2	**b**		30	86
3	**c**		5	72
4	**d**		5	70
5	**e**		5	56
6	**f**		5	93
7	**g**		5	60
8	**h**		5	85
9	**i**		10	91
10	**j**		5	73
11	**k**		5	78
12	**l**		5	83
13	**m**		10	73
14	**n**		5	67
15	**o**		1h	61
16	**p**		5h	63

Table 4.3 Synthesis of 1,6-bis-triazole 2,3,4-tri-*O*-acetyl-α-D-galactopyranosyl derivatives



entry		R-N_3	11	
			t (min)	%yield
17	**q**		1.5h	70
18	**r**		12h	67
19	**s**		5	99
20	**t**		5	95
21	**u**		5	99
22	**v**		5	49
23	**w**		5	62
24	**x**		5	99
25	**y**		5	43
26	**z**		10	75
27	**aa**		10	76
28	**bb**		10	99
29	**cc**		5	37
30	**dd**		30	98
31	**ee**		30	99

Synthesis of 1,6-di-triazolyl-glycopyranoside analogues

A series of 1,6-bis-triazole 2,3,4-tri-*O*-acetyl-α-D-galactopyranosyl derivatives as were synthesized as shown in Scheme 4.6 and Table 4.3. The corresponding azides include with derivatives of benzyl, 2-ethoxybenzene, aliphatic, coumarin azides, derivatives of 2-azidoacetates with diacetylene glycosides were performed by dissolve in THF followed by the addition of catalytic sodium ascorbate and $CuSO_4 5H_2O$. The products were obtained in short reaction time and the results of quantitative conversions and purities were found from 37% to 99% yields.

Reaction of the benzylazide derivatives with diacetylene glucoside **11** (Table 4.3, entries 1-8) showed moderate to high conversions depending on the substituted on aromatic ring (56-93%yeilds). Click reactions of the phenylether azide derivatives were performed in 5-10 min. to give the product in 67-91%yields (Table 4.3, entries 10-14). Moreover, long chain aliphatic azides gave good yields of the desired products (Table 4.3, entries 15-18). Azide with substituted aromatic gave moderate to high yields of the desired bis-triazole glycoside depending on the electronic factor of substituents.

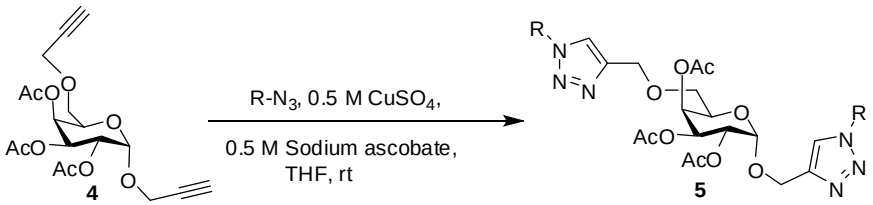
Furthermore, three derivertives of coumarin-glycoside were prepared with the high yields which were showed in the entries 30 and 31, but unstable 3-(2-(triazolyl)acetyl)-coumarin **ee** (entry 2**9)** can be decomposed in purification with silica gel column to give low yield of product.

Cytotoxic activity of 1,6-bis-triazole-tri-*O*-acetyl-α-D-galactopyranosyl derivatives

All the synthetic compounds were assayed for their cytotoxic activity against human cholangiocarcinoma cells (KKU-M213, HUCCA-1, K-100) cancer cells by MTT assay. The results were summarized in Table 3.

The structure activity relationship of the compounds showed that compound 5q with unsaturated aliphatic long chain on triazole rings exhibited moderate activity against all cancer cell lines compared to other tested compounds. Compounds 5k, 5l and 5aa were found to be active against KKU-M213 whereas compounds 4 and 5m were active against K-100 cell lines. Among the tested compounds, compound 5 ee exhibited pronounced cytotoxicity against K-100 cell lines with IC50 4.87 mM. Remaining compounds displayed no activity against the cell lines tested.

Table 4.4 Cytotoxic activity of 1,6-bis-triazole-tri-*O*-acetyl-α-D-galactopyranosyl derivatives

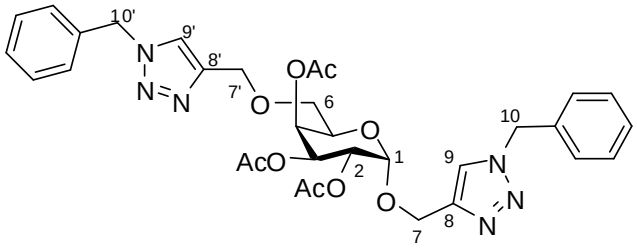


Entry	Compounds	ED_{50} (μM)[a] (SRB assay)		
		KKU-M213	HUCCA-1	K-100
1	**4**	>50	>50	28.36±1.93
2	**5a**	>50	>50	>50
3	**5b**	>50	>50	>50
4	**5c**	>50	>50	>50
5	**5d**	>50	>50	>50
6	**5e**	>50	>50	>50
7	**5f**	>50	>50	>50
8	**5g**	>50	>50	>50
9	**5h**	>50	>50	>50
10	**5i**	>50	>50	>50
11	**5j**	>50	>50	>50
12	**5k**	48.08±0.28	>50	>50
13	**5l**	37.62±0.96	>50	>50
14	**5m**	>50	>50	42.35±0.82
15	**5n**	>50	>50	>50
16	**5o**	>50	>50	>50
17	**5p**	>50	>50	>50
18	**5q**	22.58±4.53	28.71±1.17	14.87±1.52
19	**5r**	>50	>50	>50
20	**5s**	>50	>50	>50
21	**5t**	>50	>50	>50
22	**5u**	>50	>50	>50
23	**5v**	>50	>50	>50
24	**5w**	>50	>50	>50
25	**5x**	>50	>50	>50

Entry	Compounds	ED_{50} (μM)[a] (SRB assay)		
		KKU-M213	HUCCA-1	K-100
27	**5z**	>50	>50	>50
28	**5aa**	48.52±2.58	>50	>50
29	**5bb**	>50	>50	>50
30	**5cc**	>50	>50	>50
31	**5dd**	>50	>50	>50
32	**5ee**	>50	>50	4.87±0.37
ellipticine		3.42±0.74	3.15±1.63	4.51±0.23

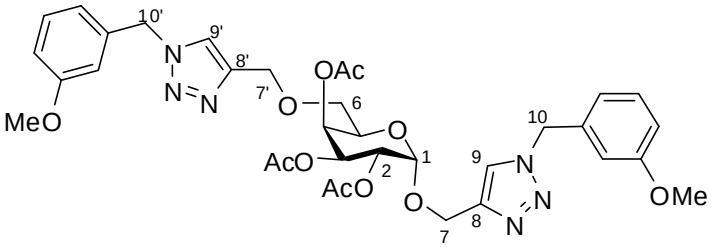
[a]Each value represents mean±SD from three different experiments performed in triplicate. Cell lines used are human cholangiocarcinoma cells (KKU-M213, HUCCA-1, K-100), cancer cells derived from Thai patient. Ellipticine (Ellipt) was used as a positive control. The results were expressed as ED_{50} values (drug concentration causing 50% growth inhibition) in μM.

4.4 Compounds characterization



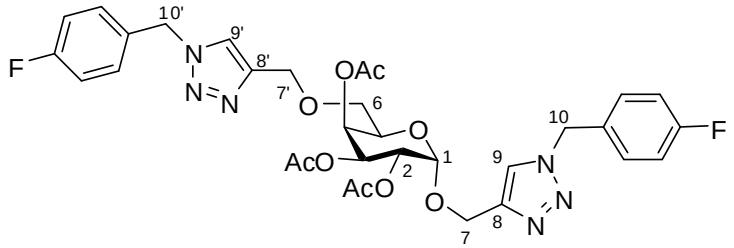
1-Benzyl-4-(((6-((1-benzyl-1H-1,2,3-triazol-4-yl)methoxy)-2,3,4-tri-*O*-acetyl-α-D-galactosyl)methoxy)methyl)-1*H*-1,2,3-triazole (**13-a**)

Compound 13-a: A colorless oil; R_f = 0.46 (4:1 EtOAc-hexane); $[\alpha]_D^{27}$ +77.41 (c 1.00, $CHCl_3$);^{1}H NMR (400 MHz, $CDCl_3$): δ 1.96 (3H, s, OAc), 1.97 (3H, s, OAc), 2.07 (3H, s, OAc), 3.49 (1H, dd, *J* = 10.0, 6.0 Hz, H-6a), 3.55 (1H, dd, *J* = 10.0, 6.5 Hz, H-6b), 4.17 (1H, t, *J* = 6.5 Hz, H-5), 4.55 (1H, d, *J* = 12.5 Hz, H-7a'), 4.68 (2H, d, *J* = 12.5 Hz, H-7b', H-7a), 4.78 (1H, d, *J* = 12.5 Hz, H-7b), 5.10 (1H, dd, *J* = 11.0, 3.5 Hz, H-2), 5.17 (1H, d, *J* = 3.5 Hz, H-1), 5.28 (1H, dd, *J* = 11.0, 3.5 Hz, H-3), 5.41 (1H, d, *J* = 3.0 Hz, H-4), 5.59 (4H, s, H-10, H-10'), 7.26-7.31 (5H, m, Ar), 7.33-7.40 (5H, m, Ar), 7.52 (1H, s, H-9'), 7.54 (1H, s, H-9).



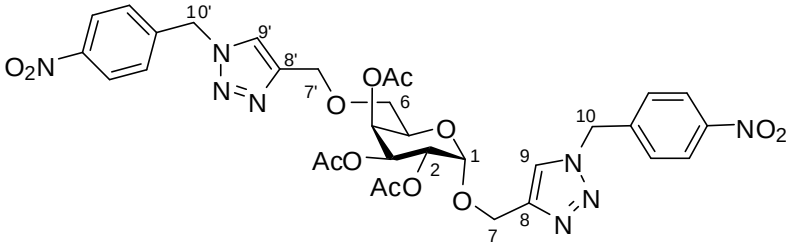
1-(3-Methoxybenzyl)-4-((3,4,5-tris(benzyloxy)-6-(((1-(3-methoxybenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-2,3,4-tri-*O*-acetyl-α-D-galactosyl) methoxy)methyl)-1*H*-1,2,3-triazole (**13-b**)

Compound 13-b: A pale yellow oil; R_f = 0.41 (4:1 EtOAc-hexane); $[\alpha]_D^{28}$ +34.01 (c 1.00, $CHCl_3$);^{1}H NMR (400 MHz, $CDCl_3$): δ 1.96 (3H, s, OAc), 1.98 (3H, s, OAc), 2.07 (3H, s, OAc), 3.49 (1H, dd, *J* = 9.5, 6.0 Hz, H-6a), 3.55 (1H, dd, *J* = 9.5, 6.0 Hz, H-6b), 3.77 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 4.17 (1H, t, *J* = 6.0 Hz, H-5), 4.55 (1H, d, *J* = 12.5 Hz, H-7a'), 4.62 (1H, d, *J* = 12.5 Hz, H-7b'), 4.64 (1H, d, *J* = 12.5 Hz, H-7a), 4.78 (1H, d, *J* = 12.5 Hz, H-7b), 5.10 (1H, dd, *J* = 11.0, 3.5 Hz, H-2), 5.18 (1H, d, *J* = 3.5 Hz, H-1), 5.28 (1H, dd, *J* = 11.0, 3.0 Hz, H-4), 5.42 (1H, d, *J* = 3.0 Hz, H-4), 5.48 (4H, s, H-10, H-10'), 6.79-6.91 (6H, m, Ar), 7.25-7.31 (2H, m, Ar), 7.53 (2H, s, H-9, H-9').



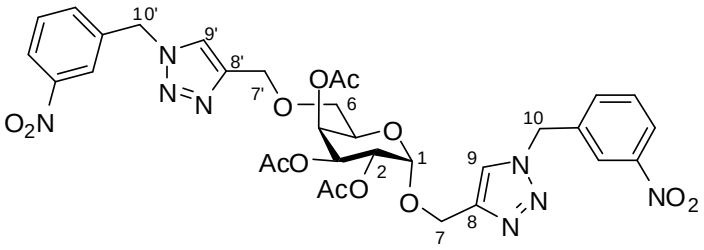
1-(4-Fluorobenzyl)-4-((6-(((1-(4-fluorobenzyl)-1*H*-1,2,3-triazol-4-yl)methoxy)methyl)-2,3,4-tri-*O*-acetyl-α-D-galactosyl)methyl)-1*H*-1,2,3-triazole (**13-c**)

Compound 13-c: A colourless oil; R_f = 0.44 (4:1 EtOAc-hexane); $[\alpha]_D^{28}$ +68.42 (c 0.50, $CHCl_3$);^{1}H NMR (400 MHz, $CDCl_3$): δ 1.96 (3H, s, OAc), 1.97 (3H, s, OAc), 2.08 (3H, s, OAc), 3.49 (1H, dd, *J* = 10.0, 6.0 Hz, H-6a), 3.55 (1H, dd, *J* = 10.0, 6.5 Hz, H-6b), 4.17 (1H, t, *J* = 6.0 Hz, H-5), 4.55 (1H, d, *J* = 12.5 Hz, H-7a'), 4.63 (2H, d, *J* = 12.5 Hz, H-7b', H-7a), 4.79 (1H, d, *J* = 12.5 Hz, H-7b), 5.10 (1H, dd, *J* = 11.0, 3.5 Hz, H-2), 5.18 (1H, d, *J* = 3.5 Hz, H-1), 5.27 (1H, dd, *J* = 11.0, 3.0 Hz, H-3), 5.42 (1H, d, *J* = 3.0 Hz, H-4), 5.49 (2H, s, H-10'), 5.50 (2H, s, H-10), 7.04 (2H, d, *J* = 8.5 Hz Ar), 7.08 (2H, d, *J* = 8.5 Hz Ar), 7.27-7.31 (4H, m, Ar), 7.53 (1H, s, H-9'), 7.54 (1H, s, H-9).



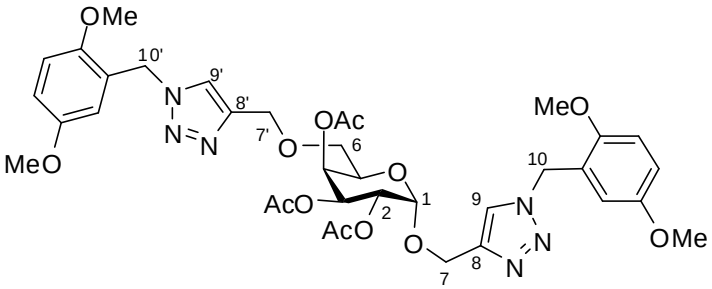
1-(4-Nitrobenzyl)-4-((6-(((1-(4-nitrobenzyl)-1*H*-1,2,3-triazol-4-yl)methoxy)methyl)- 2,3,4-tri-*O*-acetyl-α-D-galactosyl)methyl)-1*H*-1,2,3-triazole (**13-d**)

Compound 13-d: A white solid; R_f = 0.32 (4-1 EtOAc-hexane); mp 80 ^{o}C; $[\alpha]_D^{27}$ +73.94 (c 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.97 (3H, s, OAc), 2.01 (3H, s, OAc), 2.10 (3H, s, OAc), 3.52 (1H, dd, *J* = 10.0, 6.5 Hz, H-6a), 3.58 (1H, dd, *J* = 10.0, 6.5 Hz, H-6b), 4.15 (1H, t, *J* = 6.0 Hz, H-5), 4.58 (1H, d, *J* = 12.5 Hz, H-7a'), 4.66 (2H, d, *J* = 12.5 Hz, H-7b', H-7a), 4.82 (1H, d, *J* = 12.5 Hz, H-7b), 5.10 (1H, dd, *J* = 11.0, 3.5 Hz, H-2), 5.18 (1H, d, *J* = 3.5 Hz, H-1), 5.27 (1H, dd, *J* = 11.0, 3.0 Hz, H-3), 5.42 (1H, d, *J* = 3.0 Hz, H-4), 5.65 (2H, s, H-10'), 5.67 (2H, s, H-10), 7.42 (2H, d, *J* = 7.0 Hz Ar), 7.45 (2H, d, *J* = 7.0 Hz Ar), 7.27-7.31 (4H, m, Ar), 7.65 (1H, s, H-9, H-9'), 8.22 (2H, d, *J* = 7.0 Hz Ar), 8.24 (2H, d, *J* = 8.0 Hz Ar).



1-(3-Nitrobenzyl)-4-((6-(((1-(3-nitrobenzyl)-1*H*-1,2,3-triazol-4-yl)methoxy)methyl)-2,3,4-tri-*O*-acetyl-α-D-galactosyl)methyl)-1*H*-1,2,3-triazole (**13-e**)

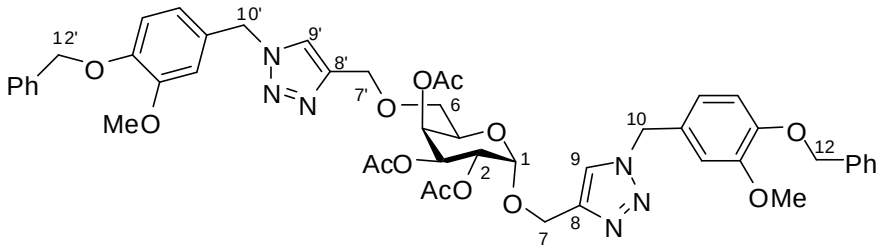
Compound 13-e: A yellow solid; R_f = 0.47 (100% EtOAc); mp 60 ^{o}C; $[\alpha]_D^{27}$ +73.62 (c 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.96 (3H, s, OAc), 2.02 (3H, s, OAc), 2.10 (3H, s, OAc), 3.50-3.62 (2H, m, H-6a, H-6b), 4.15-4.20 (1H, m, H-5), 4.57 (1H, d, *J* = 12.5 Hz, H-7a'), 4.67 (1H, d, *J* = 12.5 Hz, H-7b'), 4.68 (1H, d, *J* = 12.5 Hz, H-7a), 4.82 (1H, d, *J* = 12.5 Hz, H-7b), 5.11 (1H, dd, *J* = 11.0, 3.5 Hz, H-2), 5.20 (1H, d, *J* = 3.5 Hz, H-1), 5.25 (1H, dd, *J* = 11.0, 3.0 Hz, H-3), 5.41 (1H, d, *J* = 3.0 Hz, H-4), 5.67 (2H, s, H-10'), 5.69 (2H, s, H-10), 7.55-7.67 (4H, m, Ar), 7.74 (2H, s, H-9, H-9'), 8.18-8.22 (4H, m, Ar).



1-(2,5-Dimethoxybenzyl)-4-((6-(((1-(2,5-dimethoxybenzyl)-1*H*-1,2,3-triazol-4-yl) methoxy)methyl)-2,3,4-tri-*O*-acetyl-α-D-galactosyl) methyl)-1*H*-1,2,3-triazole (**13-f**)

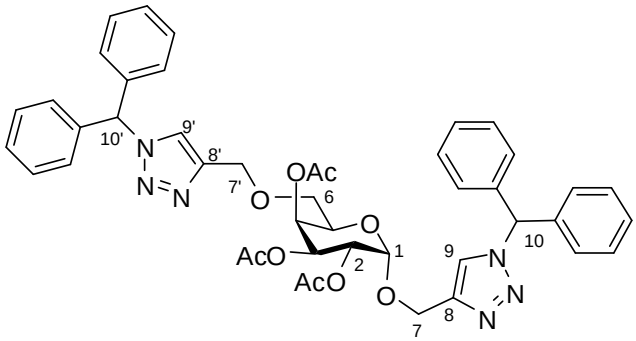
Compound 13-f: A colourless oil; R_f = 0.31 (4:1 EtOAc-hexane); $[\alpha]_D^{27}$ +34.24 (c 1.00, $CHCl_3$);^{1}H NMR (400 MHz, $CDCl_3$): δ 1.95 (3H, s, OAc), 1.97 (3H, s, OAc), 2.07 (3H, s, OAc), 3.49 (1H, dd, *J* = 10.0, 6.0 Hz, H-6a), 3.55 (1H, dd, *J* = 10.0, 6.0 Hz, H-6b), 3.72 (3H, s, OCH_3), 3.74 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 4.19 (1H, t, *J* = 6.0 Hz, H-5), 4.54 (1H, d, *J* = 12.5 Hz, H-7a'), 4.62 (2H, d, *J* = 12.5 Hz, H-7b', H-7a), 4.77 (1H, d, *J* = 12.5 Hz, H-7b), 5.10 (1H, dd, *J* = 11.0, 3.5 Hz, H-2), 5.18 (1H, d, *J* = 3.5 Hz, H-1), 5.29 (1H, dd, *J* = 11.0, 3.0 Hz, H-3), 5.43 (1H, d, *J* = 3.0 Hz, H-4), 5.49 (4H, s, H-10, H-

10'), 6.65 (2H, d, *J* = 5.0 Hz, Ar), 6.84 (2H, d, *J* = 6.0 Hz, Ar), 6.85 (2H, d, *J* = 6.0 Hz, Ar), 7.56 (1H, s, H-9'), 7.57 (1H, s, H-9).



1-(4-(Benzyloxy)-3-methoxybenzyl)-4-((6-(((1-(benzyloxy)-3-methoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methoxy)methyl)-2,3,4-tri-*O*-acetyl-α-D-galactosyl)methyl)-1*H*-1,2,3-triazole (**13-g**)

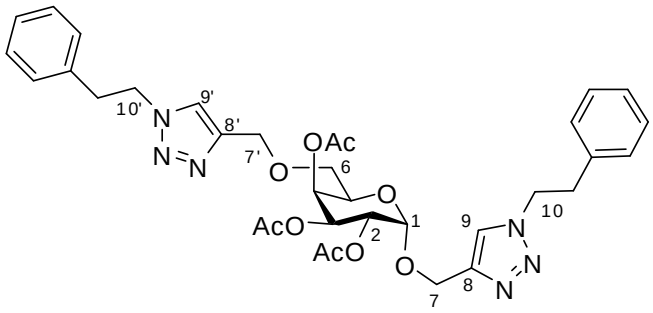
Compound 13-g: A white solid; R_f = 0.40 (4:1 EtOAc-hexane); mp 80 ^{o}C; $[\alpha]_D^{27}$ +77.41 (c 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.96 (6H, s, 2 x OAc), 2.06 (3H, s, OAc), 3.49 (1H, dd, *J* = 9.5, 6.0 Hz, H-6a), 3.55 (1H, dd, *J* = 9.5, 6.5 Hz, H-6b), 3.84 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 4.16 (1H, t, *J* = 6.0 Hz, H-5), 4.58 (1H, d, *J* = 12.5 Hz, H-7a'), 4.61 (2H, d, *J* = 12.5 Hz, H-7b', H-7a), 4.77 (1H, d, *J* = 12.5 Hz, H-7b), 5.09 (1H, dd, *J* = 11.0, 3.5 Hz, H-2), 5.13 (2H, s, H-12'), 5.14 (2H, s, H-12'), 5.17 (1H, d, *J* = 3.5 Hz, H-1), 5.27 (1H, dd, *J* = 11.0, 3.0 Hz, H-3), 5.42 (5H, brs, H-4, H-10, H-10'), 6.78-6.87 (6H, m, Ar), 7.28-7.43 (10H, m, Ar), 7.48 (1H, s, H-9), 7.49 (1H, s, H-9').



1-Benzhydryl-4-((6-(((1-benzhydryl-1*H*-1,2,3-triazol-4-yl)methoxy)methyl)-2,3,4-tri-*O*-acetyl-α-D-galactosyl)methyl)-1*H*-1,2,3-triazole (**13-h**)

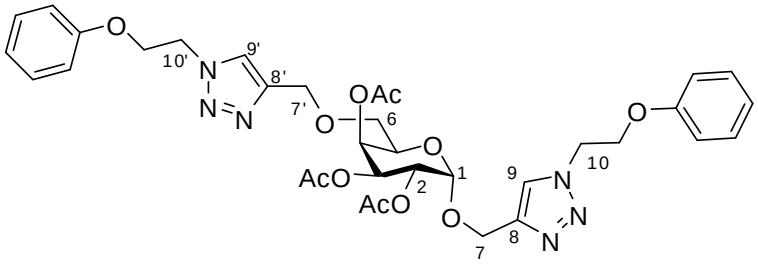
Compound 13-h: A white solid; R_f = 0.41 (1:1 EtOAc-hexane); mp 70 ^{o}C $[\alpha]_D^{28}$ +64.00 (c 1.00, $CHCl_3$);^{1}H NMR (400 MHz, $CDCl_3$): δ 1.93 (3H, s, OAc), 1.96 (3H, s, OAc), 2.06 (3H, s, OAc), 3.51 (1H, dd, *J* = 10.0, 6.0 Hz, H-6a), 3.56 (1H, dd, *J* = 10.0, 6.5 Hz, H-6b), 4.20 (1H, t, *J* = 6.0 Hz, H-5), 4.57 (1H, d, *J* = 12.5 Hz, H-7a'), 4.63 (2H, d, *J* = 12.5 Hz, H-7b', H-7a), 4.78 (1H, d, *J* = 12.5 Hz, H-7b), 5.09 (1H, dd, *J* = 11.0, 3.5 Hz, H-2), 5.18 (1H,

d, *J* = 3.5 Hz, H-1), 5.30 (1H, dd, *J* = 11.0, 3.0 Hz, H-3), 5.43 (1H, d, *J* = 3.0 Hz, H-4), 7.06-7.14 (10H, m, Ar), 7.33-7.37 (12H, m, H-10, H-10', Ar), 7.42 (1H, s, H-9), 7.46 (1H, s, H-9').



1-Phenethyl-4-((6-(((1-phenethyl-1*H*-1,2,3-triazol-4-yl)methoxy)methyl)-2,3,4-tri-*O*-acetyl-α-D-galactosyl)methyl)-1*H*-1,2,3-triazole (**13-i**)

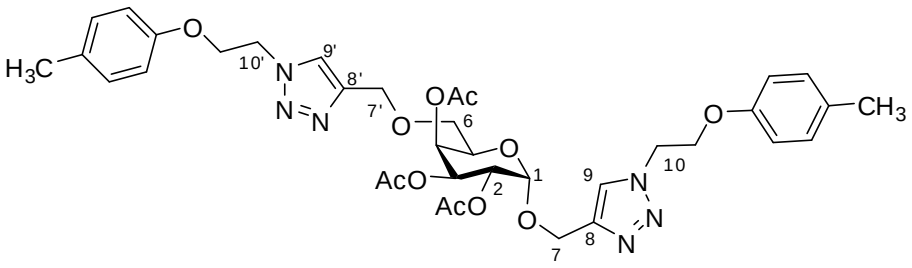
Compound 13-i: A colourless oil; R_f = 0.33 (4:1 EtOAc-hexane); $[\alpha]_D^{27}$ +78.57 (c 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.97 (3H, s, OAc), 2.03 (3H, s, OAc), 2.10 (3H, s, OAc), 3.19-3.25 (4H, m, H-11', H-11), 3.46 (1H, dd, *J* = 9.5, 6.0 Hz, H-6a), 3.54 (1H, dd, *J* = 9.5, 6.5 Hz, H-6b), 4.11 (1H, t, *J* = 6.0 Hz, H-5), 4.59 (1H, d, *J* = 12.5 Hz, H-7a'), 4.56-4.65 (6H, m, H-10, H-10', H-7b', H-7a), 4.78 (1H, d, *J* = 12.0 Hz, H-7b), 5.12 (1H, dd, *J* = 11.0, 3.5 Hz, H-2), 5.18 (1H, d, *J* = 3.5 Hz, H-1), 5.28 (1H, dd, *J* = 11.0, 3.0 Hz, H-3), 5.43 (1H, d, *J* = 3.0 Hz, H-4), 7.12 (4H, d, *J* = 7.0 Hz, Ar), 7.24-7.33 (6H, m, Ar), 7.38 (1H, s, H-9), 7.40 (1H, s, H-9').



1-(2-Phenoxyethyl)-4-((6-(((1-(2-phenoxyethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)methyl)-2,3,4-tri-*O*-acetyl-α-D-galactosyl)methyl)-1*H*-1,2,3-triazole (**13-j**)

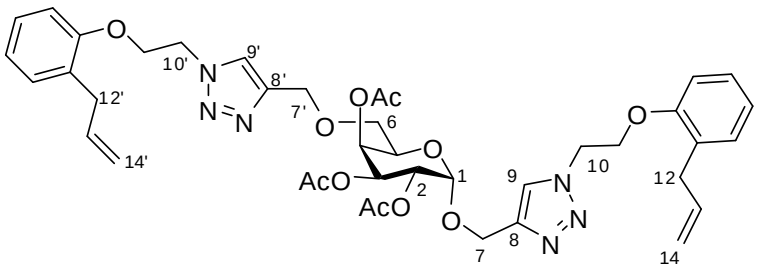
Compound 13-j: A colourless oil; R_f = 0.45 (4:1 EtOAc-hexane); $[\alpha]_D^{27}$ +71.77 (c 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.97 (3H, s, OAc), 2.01 (3H, s, OAc), 2.09 (3H, s, OAc), 3.48 (1H, dd, *J* = 10.0, 6.0 Hz, H-6a), 3.58 (1H, dd, *J* = 10.0, 6.5 Hz, H-6b), 4.19 (1H, t, *J* = 6.0 Hz, H-5), 4.35 (4H, t, *J* = 5.0 Hz, H-11', H-11), 4.57 (1H, d, *J* = 12.0 Hz, H-7a'), 4.66 (1H, d, *J* = 12.0 Hz, H-7b'), 4.78 (1H, d, *J* = 12.5 Hz, H-7a), 4.75 (4H, t, *J* = 5.0

Hz, H-10', H-10), 4.82 (1H, d, *J* = 12.5 Hz, H-7b), 5.13 (1H, dd, *J* = 11.0, 3.5 Hz, H-2), 5.22 (1H, d, *J* = 3.5 Hz, H-1), 5.31 (1H, dd, *J* = 11.0, 3.0 Hz, H-3), 5.43 (1H, d, *J* = 3.0 Hz, H-4), 6.87 (2H, d, *J* = 7.0 Hz, Ar), 6.95-7.00 (2H, m, Ar), 7.25-7.30 (6H, m, Ar),7.80 (1H, s, H-9), 7.81(1H, s, H-9').



1-(2-(p-Tolyloxy)ethyl)-4-((6-(((1-(2-(p-tolyloxy)ethyl)-1*H*-1,2,3-triazol-4-yl) methoxy)methyl)- 2,3,4-tri-*O*-acetyl-α-D-galactosyl)methyl)-1*H*-1,2,3-triazole (**13-k**)

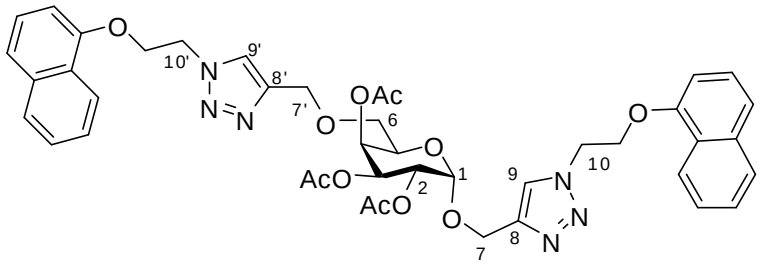
Compound 13-k: A colourless oil; R_f = 0.54 (4:1 EtOAc-hexane); $[\alpha]_D^{27}$ +64.14 (c 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.96 (3H, s, OAc), 2.01 (3H, s, OAc), 2.09 (3H, s, OAc), 2.27 (3H, s, 2 x CH_3), 3.48 (1H, dd, *J* = 10.0, 6.0 Hz, H-6a), 3.58 (1H, dd, *J* = 10.0, 6.5 Hz, H-6b), 4.19 (1H, t, *J* = 6.0 Hz, H-5), 4.31 (4H, t, *J* = 5.0 Hz, H-11', H-11), 4.57 (1H, d, *J* = 12.5 Hz, H-7a'), 4.65 (1H, d, *J* = 12.0 Hz, H-7b'), 4.68 (1H, d, *J* = 12.0 Hz, H-7a), 4.73 (4H, t, *J* = 5.0 Hz, H-10', H-10), 4.82 (1H, d, *J* = 12.5 Hz, H-7b), 5.13 (1H, dd, *J* = 11.0, 3.5 Hz, H-2), 5.22 (1H, d, *J* = 3.5 Hz, H-1), 5.31 (1H, dd, *J* = 11.0, 3.0 Hz, H-3), 5.43 (1H, d, *J* = 3.0 Hz, H-4), 6.76-6.78 (4H, m, Ar), 7.04-7.10 (4H, m, Ar), 7.78 (1H, s, H-9), 7.80 (1H, s, H-9').



1-(2-(2-Allylphenoxy)ethyl)-4-((6-(((1-(2-(2-allylphenoxy) ethyl)-1*H*-1,2,3-triazol-4-yl) methoxy)methyl)- 2,3,4-tri-*O*-acetyl-α-D-galactosyl) methyl)-1*H*-1,2,3-triazole (**13-l**)

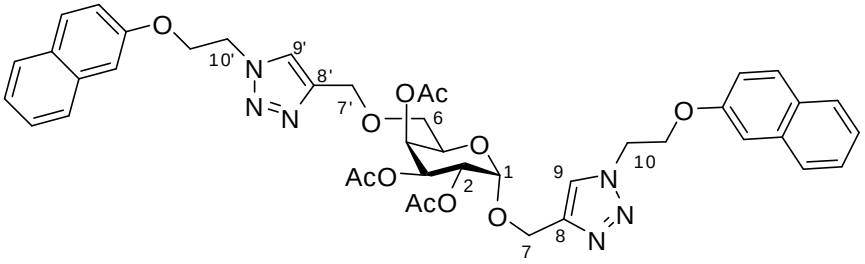
Compound 13-l: A pale yellow oil; R_f = 0.46 (3:2 EtOAc-hexane); $[\alpha]_D^{27}$ +62.02 (c 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.96 (3H, s, OAc), 2.00 (3H, s, OAc), 2.09 (3H,

s, OAc), 3.29-3.34 (4H, s, H-12, H-12’), 3.49 (1H, dd, *J* = 10.0, 6.0 Hz, H-6a), 3.58 (1H, dd, *J* = 10.0, 6.5 Hz, H-6b), 4.20 (1H, t, *J* = 6.0 Hz, H-5), 4.33-4.37 (4H, m, H-11, H-11’), 4.57 (1H, d, *J* = 12.5 Hz, H-7a’), 4.64 (1H, d, *J* = 12.5 Hz, H-7b’), 4.66 (1H, d, *J* = 12.5 Hz, H-7a), 4.76 (2H, t, *J* = 5.0 Hz, H-10’), 4.78 (2H, t, *J* = 4.0 Hz, H-10), 4.83 (1H, d, *J* = 12.5 Hz, H-7b), 5.13 4.95-5.02 (4H, m, H-14, H-14’), (1H, dd, *J* = 11.0, 3.5 Hz, H-2), 5.22 (1H, d, *J* = 3.5 Hz, H-1), 5.30 (1H, dd, *J* = 11.0, 3.0 Hz, H-3), 5.43 (1H, d, *J* = 3.0 Hz, H-4), 5.84-5.96 (2H, m, H-13, H-13’), 6.79 (2H, t, *J* = 7.0 Hz, Ar), 6.91-6.95 (2H, m, Ar), 7.12-7.19 (4H, m, Ar), 7.76 (1H, s, H-9), 7.78 (1H, s, H-9’).



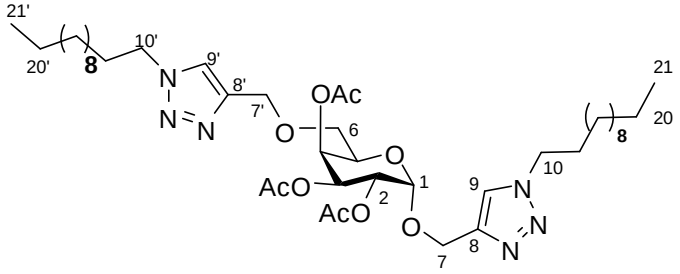
1-(2-(Naphthalen-1-yloxy)ethyl)-4-((6-(((1-(2-(naphthalen-1-yloxy)ethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)methyl)-2,3,4-tri-*O*-acetyl-α-D-galactosyl)methyl)-1*H*-1,2,3-triazole (**13-m**)

Compound 13-m: A white solid; R_f = 0.55 (4:1 EtOAc-hexane); mp 70 ^{o}C; $[\alpha]_D^{27}$ +61.65 (c 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.96 (3H, s, OAc), 1.99 (3H, s, OAc), 2.08 (3H, s, OAc), 3.48 (1H, dd, *J* = 10.0, 6.0 Hz, H-6a), 3.58 (1H, dd, *J* = 10.0, 6.5 Hz, H-6b), 4.20 (1H, t, *J* = 6.0 Hz, H-5), 4.44 (4H, t, *J* = 5.0 Hz, H-11’, H-11), 4.57 (1H, d, *J* = 12.5 Hz, H-7a’), 4.65 (1H, d, *J* = 12.0 Hz, H-7b’), 4.67 (1H, d, *J* = 12.0 Hz, H-7a), 4.79 (4H, t, *J* = 5.0 Hz, H-10’, H-10), 4.82 (1H, d, *J* = 12.5 Hz, H-7b), 5.13 (1H, dd, *J* = 11.0, 3.5 Hz, H-2), 5.22 (1H, d, *J* = 3.5 Hz, H-1), 5.31 (1H, dd, *J* = 11.0, 3.0 Hz, H-3), 5.44 (1H, d, *J* = 3.0 Hz, H-4), 7.07-7.12 (2H, m, Ar), 7.32-7.37 (2H, m, Ar), 7.41-7.46 (2H, m, Ar), 7.68-7.76 (6H, m, Ar), 7.82 (1H, s, H-9), 7.83 (1H, s, H-9’).



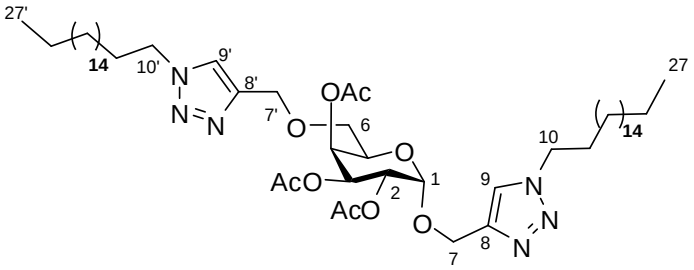
1-(2-(Naphthalen-2-yloxy)ethyl)-4-((6-(((1-(2-(naphthalen-2-yloxy)ethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)methyl)-2,3,4-tri-*O*-acetyl-α-D-galactosyl)methyl)-1*H*-1,2,3-triazole (**13-n**)

Compound 13-n: A white solid; R_f = 0.55 (4:1 EtOAc-hexane); mp 70 ^{o}C;$[\alpha]_D^{27}$ +61.00 (c 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.95 (3H, s, OAc), 1.96 (3H, s, OAc), 2.06 (3H, s, OAc), 3.34-3.39 (2H, m, H-6a), 3.47-3.54 (2H, m, H-6b), 4.04-4.14 (1H, m, H-5), 4.46-4.50 (4H, m, H-11', H-11), 4.57 (1H, d, *J* = 12.0 Hz, H-7a'), 4.64 (1H, d, *J* = 12.0 Hz, H-7b', H-7a), 4.75 (1H, d, *J* = 12.0 Hz, H-7b), 4.83-4.88 (4H, m, H-10', H-10), 5.10 (1H, dd, *J* = 11.0, 3.5 Hz, H-2), 5.19 (1H, d, *J* = 3.5 Hz, H-1), 5.27 (1H, dd, *J* = 11.0, 3.0 Hz, H-3), 5.33 (1H, d, *J* = 3.0 Hz, H-4), 6.70 (2H, d, *J* = 7.5 Hz, Ar), 7.30-7.35 (2H, m, Ar), 7.43-7.49 (6H, m, Ar), 7.77-7.80 (2H, m, Ar), 7.83 (1H, s, H-9), 7.86 (1H, s, H-9'), 8.12-8.14 (2H, m, Ar).



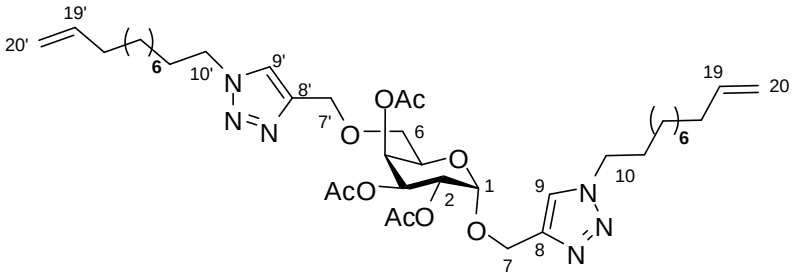
1-Lauryl-4-((6-(((lauryl-1*H*-1,2,3-triazol-4yl)methoxy)methyl) -2,3,4-tri-*O*-acetyl-α-D-galactosyl)methyl)-1*H*-1,2,3-triazole (**13-o**)

Compound 13-o: A white solid; R_f = 0.44 (1:1 EtOAc-hexane); mp 50 ^{o}C; $[\alpha]_D^{27}$ +29.58 (c 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (6H, t, *J* = 7.0 Hz, H-21', H-21), 1.22-1.36 (36H, m, H-12'-20', H-12-20), 1.86-1.94 (4H, m, H-11', H-11), 1.97 (3H, s, OAc), 2.04 (3H, s, OAc), 2.11 (3H, s, OAc), 3.53 (1H, dd, *J* = 10.0, 6.0 Hz, H-6a), 3.60 (1H, dd, *J* = 10.0, 6.5 Hz, H-6b), 4.23 (1H, t, *J* = 6.0 Hz, H-5), 4.34 (4H, t, *J* = 7.0 Hz, H-10, H-10'), 4.59 (1H, d, *J* = 12.5 Hz, H-7a'), 4.67 (1H, d, *J* = 12.5 Hz, H-7b'), 4.68 (1H, d, *J* = 12.5 Hz, H-7a), 4.84 (1H, d, *J* = 12.5 Hz, H-7b), 5.14 (1H, dd, *J* = 11.0, 3.5 Hz, H-2), 5.22 (1H, d, *J* = 3.5 Hz, H-1), 5.32 (1H, dd, *J* = 11.0, 3.0 Hz, H-3), 5.47 (1H, d, *J* = 3.0 Hz, H-4), 7.58 (1H, s, H-9), 7.59 (1H, s, H-9').



1-Octadecanyl-4-((6-(((octadecanyl-1*H*-1,2,3-triazol-4yl) methoxy)methyl)-2,3,4-tri-*O*-acetyl-α-D-galactosyl)methyl)-1*H*-1,2,3-triazole (**13-p**)

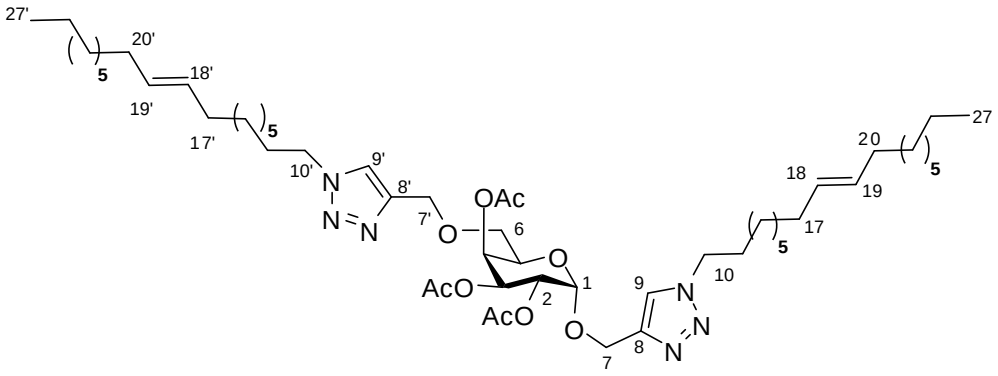
Compound 13-p: A white solid; R_f = 0.32 (1:1 EtOAc-hexane); mp 76 ^{o}C; $[\alpha]_D^{27}$ +51.88 (c 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (6H, t, *J* = 7.0 Hz, H-27', H-27), 1.23-1.35 (60H, m, H-12'-26', H-12-26), 1.85-1.94 (4H, m, H-11', H-11), 1.97 (3H, s, OAc), 2.04 (3H, s, OAc), 2.11 (3H, s, OAc), 4.35 (4H, d, *J* = 7.5, Hz, H-10, H-10'), 3.53 (1H, dd, *J* = 10.0, 6.0 Hz, H-6a), 3.60 (1H, dd, *J* = 10.0, 6.5 Hz, H-6b), 4.22 (1H, t, *J* = 6.0 Hz, H-5), 4.60 (1H, d, *J* = 12.5 Hz, H-7a'), 4.67 (1H, d, *J* = 12.0 Hz, H-7b'), 4.69 (1H, d, *J* = 12.0 Hz, H-7a), 4.84 (1H, d, *J* = 12.5 Hz, H-7b), 5.14 (1H, dd, *J* = 11.0, 3.5 Hz, H-2), 5.22 (1H, d, *J* = 3.5 Hz, H-1), 5.32 (1H, dd, *J* = 11.0, 3.0 Hz, H-3), 5.46 (1H, d, *J* = 3.0 Hz, H-4), 7.60 (1H, s, H-9), 7.62 (1H, s, H-9').



1-Omega-undecylenyl-4-((6-(((omega-undecylenyl-1*H*-1,2,3-triazol-4yl) methoxy)methyl)-2,3,4-tri-*O*-acetyl-α-D-galactosyl)methyl)-1*H*-1,2,3-triazole (**13-q**)

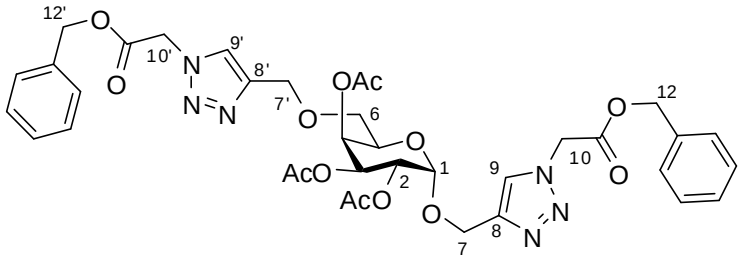
Compound 13-q: A pale yellow oil; R_f = 0.33 (1:1 EtOAc-hexane); $[\alpha]_D^{28}$ +30.08 (c 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.24-1.40 (24H, m, H-12'-17', H-12-17), 1.85-1.95 (4H, m, H-11', H-11), 1.97 (3H, s, OAc), 2.00-2.07 (4H, m, H-18', H-18), 2.04 (3H, s, OAc), 2.11 (3H, s, OAc), 3.53 (1H, dd, *J* = 10.0, 6.0 Hz, H-6a), 3.60 (1H, dd, *J* = 10.0, 6.5 Hz, H-6b), 4.23 (1H, t, *J* = 6.0 Hz, H-5), 4.32-4.36 (4H, m, H-10, H-10'), 4.59 (1H, d, *J* = 12.0 Hz, H-7a'), 4.67 (1H, d, *J* = 12.0 Hz, H-7b'), 4.68 (1H, d, *J* = 12.0 Hz, H-7a), 4.84 (1H, d, *J* = 12.0 Hz, H-7b), 5.14 (1H, dd, *J* = 11.0, 3.5 Hz, H-2), 5.22 (1H, d, *J* = 3.5 Hz, H-1), 5.32 (1H, dd, *J* = 11.0, 3.0 Hz, H-3), 5.47 (1H, d, *J* = 3.0 Hz, H-4), 4.93 (2H, dd, *J* = 10.0, 1.5

Hz, H-20’a, H-20a), 4.99 (2H, dd, *J* = 17.0, 1.5 Hz, H-20’b, H-20b), 5.81 (2H, ddt, *J* = 17.0, 10.0, 7.0 Hz, H-19’, H-19), 7.58 (1H, s, H-9), 7.59 (1H, s, H-9’).



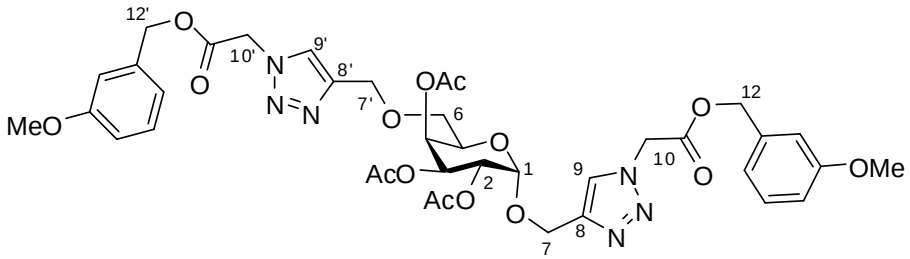
1-Olelyl-4-((6-(((olelyl-1*H*-1,2,3-triazol-4yl)methoxy)methyl) -2,3,4-tri-O-acetyl-α-D-galactosyl)methyl)-1*H*-1,2,3-triazole (**13-r**)

Compound 13-r: A pale yellow oil; R_f = 0.48 (1:1 EtOAc-hexane); $[\alpha]_D^{27}$ +25.42 (c 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (6H, t, *J* = 7.0 Hz, H-27’, H-27), 1.21-1.40 (44H, m, H-12’-16’, H-12-16, H-21’-26, H-21-26), 1.58-196 (4H, m, H-11’, H-11), 1.96-2.07 (8H, m, H-17’, H-17, H-20’, H-20), 1.97 (3H, s, OAc), 2.00-2.07 (4H, m, H-18’, H-18), 2.04 (3H, s, OAc), 2.11 (3H, s, OAc), 3.53 (1H, dd, *J* = 10.0, 6.0 Hz, H-6a), 3.60 (1H, dd, *J* = 10.0, 6.5 Hz, H-6b), 4.23 (1H, t, *J* = 6.0 Hz, H-5), 4.34 (4H, t, *J* = 7.0 Hz, H-10, H-10’), 4.59 (1H, d, *J* = 12.0 Hz, H-7a’), 4.67 (1H, d, *J* = 12.0 Hz, H-7b’), 4.68 (1H, d, *J* = 12.0 Hz, H-7a), 4.84 (1H, d, *J* = 12.0 Hz, H-7b), 5.14 (1H, dd, *J* = 11.0, 3.5 Hz, H-2), 5.22 (1H, d, *J* = 3.5 Hz, H-1), 5.27 (5H, ddd, m, H-3, H-18’, H-18, H-19’, H-19), 5.47 (1H, d, *J* = 3.0 Hz, H-4), 7.58 (1H, s, H-9’), 7.59 (1H, s, H-9).



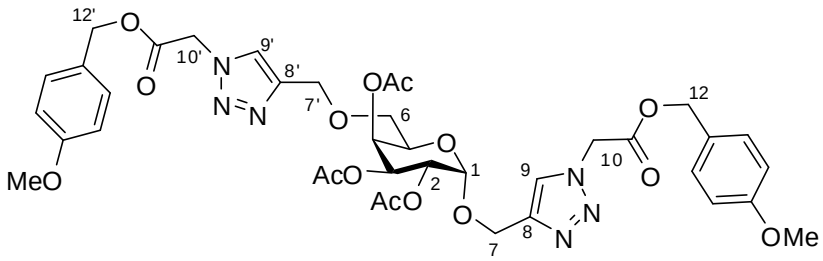
Benzyl 2-(4-((6-(((1-(2-(benzyloxy)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)-2,3,4-tri-O-acetyl-α-D-galactosyl)methyl)-1H-1,2,3-triazol-1-yl)acetate (**13-s**)

Compound 13-s: A colourless oil; R_f = 0.56 (4:1 EtOAc-hexane); $[\alpha]_D^{28}$ +64.40 (c 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.96 (3H, s, OAc), 2.05 (3H, s, OAc), 2.10 (3H, s, OAc), 3.49-3.64 (2H, m, H-6a, H-6b), 4.18 (1H, brs, H-5), 4.60 (1H, d, *J* = 12.0 Hz, H-7a'), 4.67 (2H, d, *J* = 12.0 Hz, H-7b'), 4.73 (1H, d, *J* = 11.5 Hz, H-7a), 4.81 (1H, d, *J* = 11.5 Hz, H-7b), 5.14 (1H, dd, *J* = 11.0, 3.0 Hz, H-2), 5.17 (1H, d, *J* = 3.0 Hz, H-1), 5.19-5.22 (7H, s, H-10, H-10', H-12, H-12'a), 5.19 (1H, d, *J* = 12.5 Hz, H-12'b), 5.30 (1H, dd, *J* = 11.0, 2.0 Hz, H-3), 5.44 (1H, d, *J* = 2.0 Hz, H-4), 7.32-7.40 (10H, m, Ar), 7.55 (1H, s, H-9'), 7.79 (1H, s, H-9).



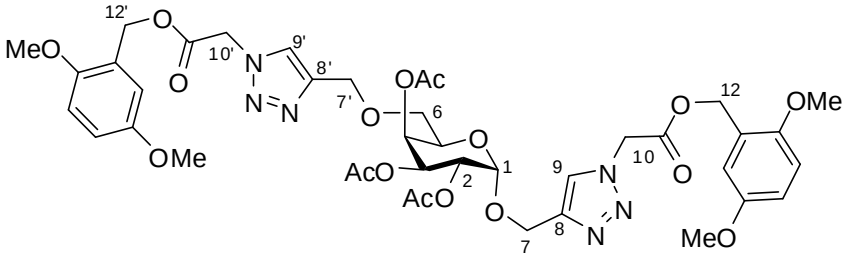
3-Methoxybenzyl 2-(4-((6-(((1-(2-(3-methoxybenzyl)- 2-oxoethyl)-1H-1,2,3-triazol-4-yl) methoxy)methyl)- 2,3,4-tri-O-acetyl-α-D-galactosyl)methyl)-1H-1,2,3-triazol-1-yl)acetate (**13-t**)

Compound 13-t: A pale yellow oil; R_f = 0.69 (100% EtOAc); $[\alpha]_D^{28}$ +56.63 (c 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.96 (3H, s, OAc), 2.04 (3H, s, OAc), 2.11 (3H, s, OAc), 3.52 (1H, dd, *J* = 10.0, 6.0 Hz, H-6a), 3.58 (1H, dd, *J* = 10.0, 6.5 Hz, H-6b), 3.80 (6H, s, 2 x CH_3), 4.18 (1H, t, *J* = 6.0 Hz, H-5), 4.60 (2H, d, *J* = 12.50 Hz, H-7a'), 4.67 (1H, d, *J* = 12.5 Hz, H-7b'), 4.72 (1H, d, *J* = 12.5 Hz, H-7a), 4.83 (1H, d, *J* = 12.5 Hz, H-7b), 5.14 (1H, dd, *J* = 11.0, 3.5 Hz, H-2), 5.19 (3H, d, *J* = 12.5 Hz, H-10a, 2 x H-10', 5.21 (4H, d, *J* = 11.0 Hz, H-12, H-12'a), 5.20-5.23 (1H, m, H-1), 5.24 (1H, d, *J* = 12.0 Hz, H-10b), 5.29 (1H, dd, *J* = 11.0, 3.0 Hz, H-3), 5.43 (1H, d, *J* = 3.0 Hz, H-4), 6.85-6.98 (6H, m, Ar), 7.280 (2H, t, *J* = 7.5 Hz, Ar), 7.74 (1H, s, H-9'), 7.79 (1H, s, H-9).



4-Methoxybenzyl 2-(4-((6-(((1-(2-(4-methoxybenzyl)- 2-oxoethyl)-1H-1,2,3-triazol-4-yl) methoxy)methyl)-2,3,4-tri-O-acetyl-α-D-galactosyl) methyl)-1H-1,2,3-triazol-1-yl)acetate (**13-u**)

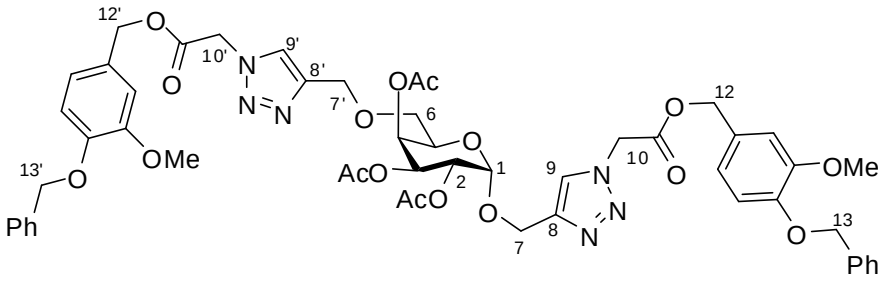
Compound 13-u: A pale yellow oil; R_f = 0.64 (100% EtOAc); $[\alpha]_D^{28}$ +70.78 (c 0.50, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.96 (3H, s, OAc), 2.05 (3H, s, OAc), 2.11 (3H, s, OAc), 3.52 (1H, dd, *J* = 10.0, 6.0 Hz, H-6a), 3.59 (1H, dd, *J* = 10.0, 6.5 Hz, H-6b), 3.81 (6H, s, 2 x CH_3), 4.18 (1H, t, *J* = 6.0 Hz, H-5), 4.61 (2H, d, *J* = 12.5 Hz, H-7a'), 4.68 (1H, d, *J* = 12.5 Hz, H-7b'), 4.74 (1H, d, *J* = 12.5 Hz, H-7a), 4.83 (1H, d, *J* = 12.5 Hz, H-7b), 5.14 (1H, dd, *J* = 11.0, 3.5 Hz, H-2), 5.15-5.18 (8H, m, H-10, H-10', H-12, H-12'), 5.23 (1H, d, *J* = 3.5 Hz, H-1), 5.29 (1H, dd, *J* = 11.0, 3.0 Hz, H-3), 5.43 (1H, d, *J* = 2.5 Hz, H-4), 6.89 (4H, d, *J* = 8.5 Hz, Ar), 7.28 (2H, d, *J* = 8.5 Hz, Ar), 7.29 (2H, d, *J* = 8.5 Hz, Ar), 7.71 (1H, s, H-9'), 7.77 (1H, s, H-9).



2,5-Dimethoxybenzyl 2-(4-((6-(((1-(2-(2,5-dimethoxybenzyl) -2-oxoethyl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)-2,3,4-tri-O-acetyl-α-D-galactosyl)methyl)-1H-1,2,3-triazol-1-yl) acetate (**13-v**)

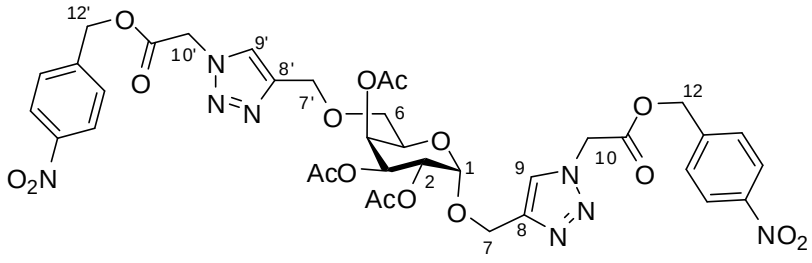
Compound 13-v: A pale yellow oil; R_f = 0.66 (100% EtOAc); $[\alpha]_D^{27}$ +59.64 (c 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.96 (3H, s, OAc), 2.05 (3H, s, OAc), 2.11 (3H, s, OAc), 3.57 (1H, dd, *J* = 10.0, 6.0 Hz, H-6a), 3.59 (1H, dd, *J* = 10.0, 6.5 Hz, H-6b), 3.76 (6H, s, 2 x CH_3), 3.79 (6H, s, 2 x CH_3), 4.19 (1H, t, *J* = 6.0 Hz, H-5), 4.61 (2H, d, *J* = 12.5 Hz, H-7a'), 4.68 (1H, d, *J* = 12.5 Hz, H-7b'), 4.74 (1H, d, *J* = 12.5 Hz, H-7a), 4.84 (1H, d, *J* = 12.5 Hz, H-7b), 5.14 (1H, dd, *J* = 11.0, 3.5 Hz, H-2), 5.20-5.25 (8H, m, H-10, H-10', H-12, H-

12'), 5.23 (1H, d, *J* = 3.5 Hz, H-1), 5.30 (1H, dd, *J* = 11.0, 3.0 Hz, H-3), 5.44 (1H, d, *J* = 2.5 Hz, H-4), 6.81-6.86 (6H, m, Ar), 7.74 (1H, s, H-9'), 7.80 (1H, s, H-9).



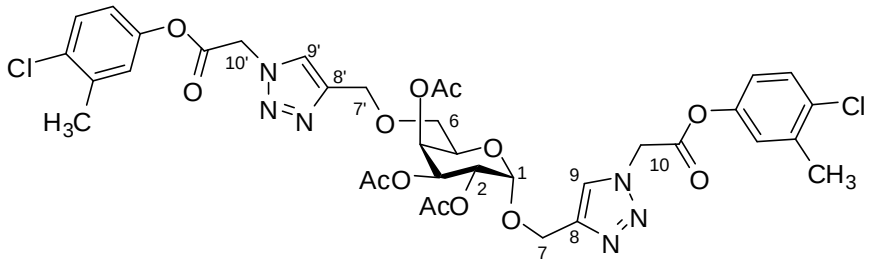
4-(Benzyloxy)-3-methoxybenzyl 2-(4-((6-(((1-(2-(4-(benzyloxy) -3-methoxybenzyl)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methoxy)methyl) -2,3,4-2,3,4-tri-O-acetyl-α-D-galactosyl) methyl)-1H-1,2,3-triazol-1-yl)acetate (**13-w**)

Compound 13-w: A white solid; R_f = 0.49 (4-1 EtOAc-hexane); mp 70 ^{o}C; $[\alpha]_D^{28}$ +51.49 (c 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.96 (3H, s, OAc), 2.04 (3H, s, OAc), 2.11 (3H, s, OAc), 3.52 (1H, dd, *J* = 10.0, 6.0 Hz, H-6a), 3.59 (1H, dd, *J* = 10.0, 6.5 Hz, H-6b), 3.89 (6H, s, 2 x CH_3), 4.18 (1H, t, *J* = 6.0 Hz, H-5), 4.64 (2H, d, *J* = 12.5 Hz, H-7a'), 4.67 (1H, d, *J* = 12.5 Hz, H-7b'), 4.73 (1H, d, *J* = 12.5 Hz, H-7a), 4.83 (1H, d, *J* = 12.5 Hz, H-7b), 5.13 (2H, s, H-13'), 5.14 (2H, s, H-13), 5.14-5.18 (1H, m, H-2), 5.14 (4H, d, *J* = 12.5 Hz, H-10, H-10'), 5.15 (4H, d, *J* = 12.0 Hz, H-12, H-12'), 5.23 (1H, d, *J* = 3.5 Hz, H-1), 5.29 (1H, dd, *J* = 11.0, 3.0 Hz, H-3), 5.43 (1H, d, *J* = 3.0 Hz, H-4), 6.83-6.90 (6H, m, Ar), 7.30 (4H, t, *J* = 7.0 Hz, Ar), 7.37 (4H, t, *J* = 7.5 Hz, Ar), 7.43 (4H, d, *J* = 7.0 Hz, Ar), 7.71 (1H, s, H-9'), 7.76 (1H, s, H-9).



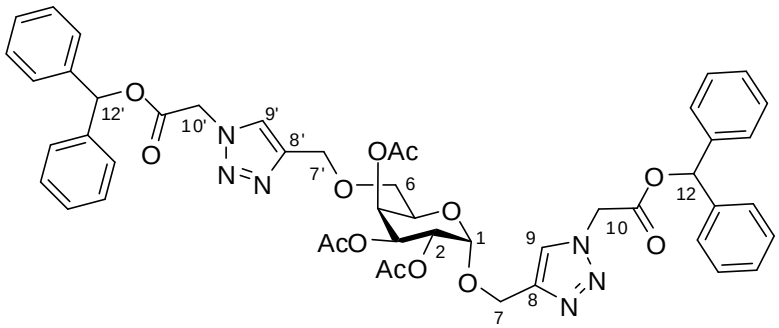
4-Nitrobenzyl 2-(4-((6-(((1-(2-(4-nitrobenzyl)- 2-oxoethyl)-1H-1,2,3-triazol-4-yl) methoxy)methyl)- 2,3,4-tri-O-acetyl-α-D-galactosyl) methyl)-1H-1,2,3-triazol-1-yl)acetate (**13-x**)

Compound 13-x: A yellow solid; R_f = 0.57 (100% EtOAc); mp 106 ^{o}C; $[\alpha]_D^{27}$ +56.28 (c 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.96 (3H, s, OAc), 2.05 (3H, s, OAc), 2.12 (3H, s, OAc), 3.52 (1H, dd, *J* = 10.0, 6.0 Hz, H-6a), 3.59 (1H, dd, *J* = 10.0, 6.5 Hz, H-6b), 4.17 (1H, t, *J* = 6.0 Hz, H-5), 4.60 (2H, d, *J* = 12.5 Hz, H-7a'), 4.68 (1H, d, *J* = 12.5 Hz, H-7b'), 4.73 (1H, d, *J* = 12.5 Hz, H-7a), 4.83 (1H, d, *J* = 12.5 Hz, H-7b), 5.14 (1H, dd, 11.0, *J* = 3.5 Hz, H-2), 5.23 (1H, d, *J* = 3.5 Hz, H-1), 5.23-5.32 (9H, m, H-3, H-10, H-10', H-12, H-12'), 5.43 (1H, d, *J* = 3.0 Hz, H-4), 7.50 (4H, d, *J* = 8.0 Hz, Ar), 7.75 (1H, s, H-9'), 7.81 (1H, s, H-9), 8.22 (4H, d, *J* = 8.0 Hz, Ar).



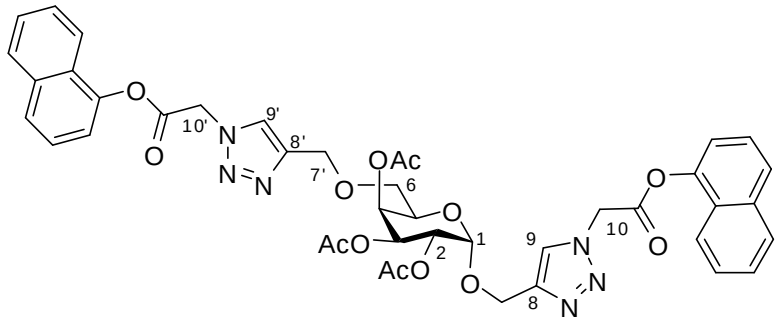
4-Chloro-3-methylphenyl 2-(4-((6-(((1-(2-(4-chloro-3-methyl phenyl)- 2-oxoethyl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)- 2,3,4-tri-O-acetyl-α-D-galactosyl) methyl)-1H-1,2,3-triazol-1-yl)acetate (**13-y**)

Compound 13-y: A colorless oil; R_f = 0.42 (4:1 EtOAc-hexane); $[\alpha]_D^{28}$ + (c 0.50, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.97 (3H, s, OAc), 2.05 (3H, s, OAc), 2.11 (3H, s, OAc), 2.35 (3H, s, CH_3), 2.36 (3H, s, CH_3), 3.51-3.64 (2H, m, H-6a, H-6b), 3.81 (6H, s, 2 x CH_3), 4.19 (1H, bs, H-5), 4.62 (2H, d, *J* = 12.5 Hz, H-7a'), 4.68 (1H, d, *J* = 12.5 Hz, H-7b'), 4.75 (1H, d, *J* = 12.0 Hz, H-7a), 4.83 (1H, d, *J* = 12.0 Hz, H-7b), 5.14 (1H, dd, *J* = 11.0, 3.5 Hz, H-2), 5.24 (1H, d, *J* = 3.5 Hz, H-1), 5.29 (1H, dd, *J* = 11.0, 3.0 Hz, H-3), 5.40 (1H, d, *J* = 11.0 Hz, H-10'), 5.41(1H, d, *J* = 11.0 Hz, H-10), 5.44 (1H, d, *J* = 2.5 Hz, H-4), 6.87-6.94 (2H, m, Ar), 7.02 (1H, d, *J* = 11.5 Hz, Ar), 7.03 (1H, d, *J* = 11.5 Hz, Ar), 7.32 (1H, d, *J* = 8.5 Hz, Ar), 7.34 (1H, d, *J* = 8.5 Hz, Ar), 7.81 (1H, s, H-9'), 7.86 (1H, s, H-9).



Benzhydryl 2-(4-((6-(((1-(2-(benzhydryl)- 2-oxoethyl)-1H-1,2,3-triazol-4-yl) methoxy)methyl)- 2,3,4-tri-O-acetyl-α-D-galactosyl) methyl)-1H-1,2,3-triazol-1-yl)acetate (**13-z**)

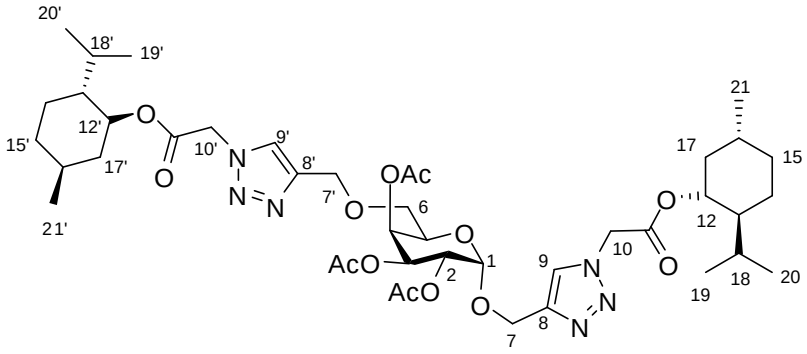
Compound 13-z: A white solid; R_f = 0.73 (100% EtOAc); mp 82 ^{o}C; $[\alpha]_D^{27}$ +56.46 (c 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.95 (3H, s, OAc), 2.02 (3H, s, OAc), 2.29 (3H, s, OAc), 3.54 (1H, dd, *J* = 10.0, 6.0 Hz, H-6a), 3.57 (1H, dd, *J* = 10.0, 6.5 Hz, H-6b), 4.15 (1H, t, *J* = 6.0 Hz, H-5), 4.57 (2H, d, *J* = 12.5 Hz, H-7a'), 4.69 (1H, d, *J* = 12.5 Hz, H-7b'), 4.71 (1H, d, *J* = 12.5 Hz, H-7a), 4.80 (1H, d, *J* = 12.5 Hz, H-7b), 5.13 (1H, dd, *J* = , 11.0, 3.5 Hz, H-2), 5.21 (1H, d, *J* = 3.5 Hz, H-1), 5.22 (2H, s, H-10'), 5.24 (1H, s, H-10'a), 5.26 (1H, s, H-10'b), 5.28 (1H, dd, *J* = 11.0, 3.5 Hz, H-3), 5.41 (1H, d, *J* = 3.0 Hz, H-4), 6.92 (3H, s, H-12'), 6.93 (3H, s, H-12'), 7.27-7.36 (20H, m, Ar), 7.68 (1H, s, H-9'), 7.74 (1H, s, H-9).



Naphthalen-1-yl 2-(4-((6-(((1-(2-(naphthalen-1-yl)-2-oxoethyl)-1H-1,2,3-triazol-4-yl) methoxy)methyl)- 2,3,4-tri-O-acetyl-α-D-galactosyl) methyl)-1H-1,2,3-triazol-1-yl)acetate (**13-q**)

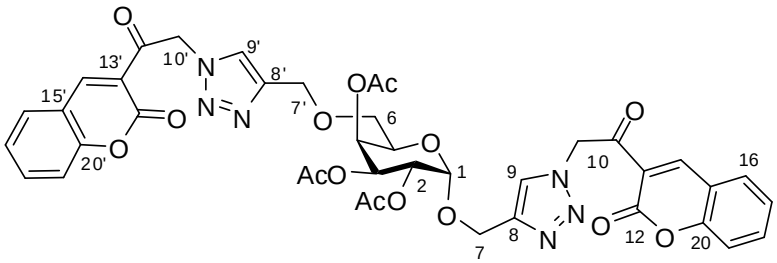
Compound 13-aa: A white solid; R_f = 0.30 (4:1 EtOAc-hexane); mp 90 ^{o}C; $[\alpha]_D^{27}$ +62.07 (c 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.96 (3H, s, OAc), 2.02 (3H, s, OAc), 2.09 (3H, s, OAc), 3.50 (1H, dd, *J* = 10.0, 5.5 Hz, H-6a), 3.58 (1H, dd, *J* = 10.0, 7.0 Hz, H-6b), 4.18 (1H, t, *J* = 6.0 Hz, H-5), 4.61 (1H, d, *J* = 12.5 Hz, H-7a'), 4.67 (1H, d, *J* = 12.5 Hz, H-7b'), 4.74 (1H, d, *J* = 12.5 Hz, H-7a), 4.82 (1H, d, *J* = 12.5 Hz, H-7b), 5.13 (1H, dd, *J* =

11.0, 3.5 Hz, H-2), 5.23 (1H, d, *J* = 3.5 Hz, H-1), 5.28 (1H, dd, *J* = 11.0, 3.0 Hz, H-3), 5.40 (1H, d, *J* = 3.0 Hz, H-4), 5.54 (2H, s, H-10'), 5.58 (1H, s, H-10a), 5.59 (1H, s, H-10b), 7.26 (1H, d, *J* = 7.5 Hz, Ar), 7.30 (1H, d, *J* = 7.5 Hz, Ar), 7.40-7.47 (2H, m, Ar), 7.49-7.52 (4H, m, Ar), 7.75-7.87 (6H, m, Ar), 7.87 (1H, s, H-9), 7.91 (1H, s, H-9').



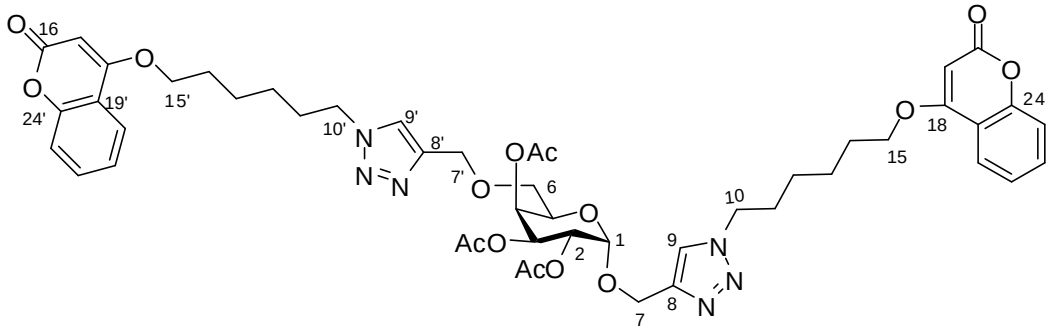
(*1R,2S,5R*)-2-Isopropyl-5-methylcyclohexyl 2-(4-((6-(((1-(2-((*1R,2S,5R*)-2-isopropyl-5-methylcyclohexyl)- 2-oxoethyl)-1H-1,2,3-triazol-4-yl) methoxy)methyl)-2,3,4-tri-O-acetyl-α-D-galactosyl) methyl)-1H-1,2,3-triazol-1-yl)acetate (**13-bb**)

Compound 13-bb: A white solid; R_f = 0.48 (3:2 EtOAc-hexane); mp 60 ^{o}C; $[\alpha]_D^{27}$ +18.12 (c 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.73 (3H, s, CH_3), 0.76 (3H, d, *J* = 2.0 Hz, CH_3), 0.86-0.94 (14H, m, 4 x CH3, H-14a, H-14a'), 0.99-1.08 (4H, m, H-15a, H-15a', H-17a, H-17a'), 1.36-1.50 (4H, m, H-13, H-13', H-16, H-16'), 1.69 (4H, d, *J* = 12.0 Hz, H-14b, H-14b', H-15b, H-15b'), 1.76-1.82 (2H, m, H-17b, H-17b'), 1.97 (3H, s, OAc), 1.99-2.01 (2H, m, H-18, H-18'), 2.05 (3H, s, OAc), 2.09 (3H, s, OAc), 3.57 (1H, dd, *J* = 10.0, 6.0 Hz, H-6a), 3.60 (1H, dd, *J* = 10.0, 6.5 Hz, H-6b), 4.21 (1H, t, *J* = 6.0 Hz, H-5), 4.62 (1H, d, *J* = 12.5 Hz, H-7a'), 4.70 (1H, d, *J* = 12.5 Hz, H-7b'), 4.76 (1H, d, *J* = 12.5 Hz, H-7a), 4.75-4.80 (2H, m, H-13, H-13'), 4.86 (1H, d, *J* = 12.5 Hz, H-7b), 5.14 (1H, dd, *J* = 11.0, 3.5 Hz, H-2), 5.16 (2H, s, H-10'), 5.17 (2H, s, H-10), 5.23 (1H, d, *J* = 3.5 Hz, H-1), 5.31 (1H, dd, *J* = 11.0, 3.0 Hz, H-3), 5.45 (1H, d, *J* = 3.0 Hz, H-4), 7.73 (1H, s, H-9), 7.78 (1H, s, H-9').



3-(2-(4-((6-(((1-(2-oxo-2-(2-oxo-2H-chromen-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)methoxy) methyl)-2,3,4-tri-O-acetyl-α-D-galactosyl)methyl)-1H-1,2,3-triazol-1-yl)acetyl)-2H chromen-2-one (**13-cc**)

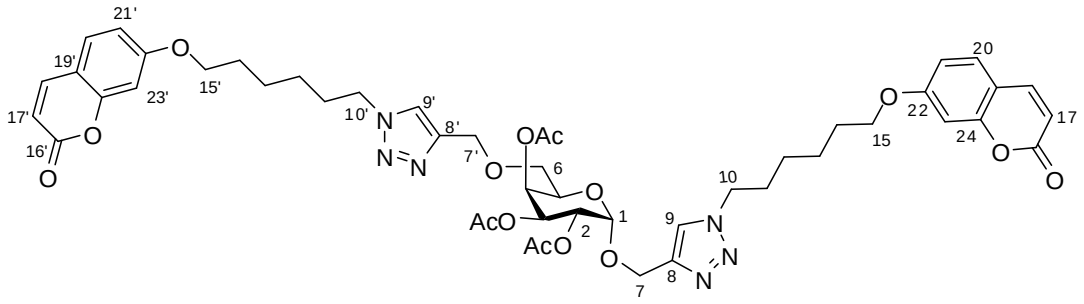
Compound 13-cc: A yellow solid; R_f = 0.34 (9-1 EtOAc-hexane); mp 130 ^{o}C; $[\alpha]_D^{27}$ +65.26 (c 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.97 (3H, s, OAc), 2.07 (3H, s, OAc), 2.14 (3H, s, OAc), 3.54-3.70 (2H, H-6a, H-6b), 4.24 (1H, brs, H-5), 4.63-4.92 (4H, m, H-7', H-7), 5.17 (1H, dd, *J* = 11.0, 3.0 Hz, H-2), 5.27 (1H, d, *J* = 3.0 Hz, H-1), 5.36 (1H, dd, *J* = 11.0, 2.0 Hz, H-3), 5.48 (1H, d, *J* = 2.0 Hz, H-4), 5.96 (4H, s, H-10', H-10), 7.22-7.45 (4H, m, H-17, H-17', H-19, H-19'), 7.69-7.77 (6H, m, H-9, H-9', H-16, H-16', H-18, H-18'), 8.67 (1H, s, H-14') 8.69 (1H, s, H-14).



4-(6-(4-((6-(((1-(6-(2-oxo-2H-chromen-4-yloxy)hexyl)-1H-1,2,3-triazol-4-yl)methoxy) methyl)-2,3,4-tri-O-acetyl-α-D-galactosylmethyl)-1H-1,2,3-triazol-1-yl)hexyloxy)-2H-chromen-2-one (**13-dd**)

Compound 13-dd: A pale yellow oil; R_f = 0.42 (100% EtOAc); $[\alpha]_D^{27}$ +47.84 (c 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.41-1.50 (4H, m, H-12, H-12'), 1.54-1.64 (4H, m, H-13, H-13'), 1.88-2.02 (8H, m, H-11, H-11', H-14, H-14'), 1.97 (3H, s, OAc), 2.05 (3H, s, OAc), 2.12 (3H, s, OAc), 3.54 (1H, dd, *J* = 9.5, 6.0 Hz, H-6a), 3.61 (1H, dd, *J* = 9.5, 6.5 Hz, H-6b), 4.12 (2H, t, *J* = 6.0 Hz, H-15'), 4.13 (2H, t, *J* = 6.0 Hz, H-15), 4.23 (1H, t, *J* = 6.0 Hz, H-5), 4.39 (2H, t, *J* = 7.0 Hz, H-10'), 4.40 (2H, t, *J* = 7.0 Hz, H-10), 4.59 (1H, d, *J* = 12.0 Hz, H-7a'), 4.68(2H, d, *J* = 12.0 Hz, H-7b', H-7a), 4.85 (1H, d, *J* = 12.0 Hz, H-7b), 5.14 (1H,

dd, *J* = 11.0, 3.5 Hz, H-2), 5.28 (1H, d, *J* = 3.5 Hz, H-1), 5.33 (1H, dd, *J* = 11.0, 3.0 Hz, H-3), 5.48 (1H, d, *J* = 3.0 Hz, H-4), 5.66 (2H, s, H-17, H-17'), 7.27 (2H, d, *J* = 7.0 Hz, H-23, H-23') 7.31 (2H, t, *J* = 8.5 Hz, H-21, H-21'), 7.56 (2H, t, *J* = 8.0 Hz, H-22, H-22'), 7.61 (1H, s, H-9'), 7.63 (1H, s, H-9), 7.81 (2H, d, *J* = 8.0 Hz, H-20, H-20').



4-(6-(4-((6-(((1-(6-(2-Oxo-2H-chromen-7-yloxy)hexyl)-1H-1,2,3-triazol-7-yl)methoxy) methyl)-2,3,4-tri-O-acetyl-α-D-galactosyl-methyl)-1H-1,2,3-triazol-1-yl)hexyloxy)-2H-chromen-2-one (**13-ee**)

Compound 13-ee: A pale yellow oil; R_f = 0.42 (100% EtOAc); $[\alpha]_D^{27}$ +55.45 (c 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.38-1.47 (4H, m, H-12, H-12'), 1.49-1.59 (4H, m, H-13, H-13'), 1.78-1.86 (4H, m, H-11, H-11'), 1.93-2.01 (4H, m, H-14, H-14'), 1.97 (3H, s, OAc), 2.05 (3H, s, OAc), 2.11 (3H, s, OAc), 3.54 (1H, dd, *J* = 10.0, 6.0 Hz, H-6a), 3.61 (1H, dd, *J* = 10.0, 6.5 Hz, H-6b), 3.99 (2H, t, *J* = 6.0 Hz, H-15'), 4.00 (2H, t, *J* = 6.0 Hz, H-15), 4.22 (1H, t, *J* = 6.0 Hz, H-5), 4.38 (2H, t, *J* = 7.0 Hz, H-10'), 4.39 (2H, t, *J* = 7.0 Hz, H-10), 4.59 (1H, d, *J* = 12.0 Hz, H-7a'), 4.67 (1H, d, *J* = 12.5 Hz, H-7b'), 4.67 (1H, d, *J* = 12.5 Hz, H-7a), 4.84 (1H, d, *J* = 12.5 Hz, H-7b), 5.14 (1H, dd, *J* = 11.0, 3.5 Hz, H-2), 5.22 (1H, d, *J* = 3.5 Hz, H-1), 5.32 (1H, dd, *J* = 11.0, 3.0 Hz, H-3), 5.47 (1H, d, *J* = 3.0 Hz, H-4), 6.24 (2H, d, *J* = 9.5 Hz, H-17, H-17'), 6.78 (2H, s, H-23, H-23'), 6.81 (1H, d, *J* = 8.5 Hz, H-21'), 6.82 (1H, d, *J* = 8.5 Hz, H-21), 7.37 (2H, d, *J* = 8.5 Hz, H-20, H-20'), 7.61 (1H, s, H-9'), 7.62 (1H, s, H-9), 7.64 (2H, d, *J* = 9.5 Hz, H-18, H-18').

Conclusion

A new series of 1,6-bis-triazole 2,3,4-tri-*O*-acetyl-α-D-galactopyranosyl derivatives were synthesized and evaluated for their in vitro cytotoxic activities against Thai human cholangiocarcinoma cells. The preliminary screening results indicated that some of the compounds demonstrated low to moderate cytotoxic activities, comparable to the anticancer drug ellipticin. Compounds 5 ee exhibited pronounced cytotoxicity against K-100 cell lines. The chemical structures of all the newly synthesized compounds were characterized by means of spectral and elemental analyses.

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