



J. Serb. Chem. Soc. 88 (7–8) 715–727 (2023)
JSCS–5657

Click mediated synthesis of functionalized glycolipids with peptide-peptoid linkages*

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(Received 1 February, revised 18 March, accepted 30 June 2023)

Abstract: The present work describes the synthesis of a new class of glycolipids with systematic variations in the linkage region, as well as in the aglycon part using Cu(I) catalyzed *click* reaction. The linkage region between sugar and the aglycon part was diversified using amide, amido-triazole and 5-benzoyl triazole moieties. The structural diversity of glycolipids was further amplified by incorporating several polar peptide foldamer groups such as triazole, amide, peptide, or *N*-aryl peptoid in the aglycon part. The newly designed glycolipids were derived from the amalgamation of different peptide bond mimics. This work reports the first use of *N*-aryl peptoid in the synthesis of glycolipids. The newly synthesized glycolipids were characterized using different spectroscopic and spectrometric analyses. The impact of the amide bond as well as the triazole ring in the linkage region on the morphology of the glycolipids was analysed by comparing their self-assemblies using SEM analysis. The geometries of the glycolipids were also optimized using density functional theory and the optimized structures were found to be minima in the potential energy surfaces.

Keywords: *click* reaction; [3+2] cycloaddition; triazole-amide; 5-benzoyl triazole; *N*-aryl peptoid; SEM study.

INTRODUCTION

Glycolipids, an important class of biomolecules on the cell surface, have found a wide range of potential biological applications in addition to their applic-

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• Dedicated to late Prof. Duraikkannu Loganathan.

<https://doi.org/10.2298/JSC230201030S>



ations in detergents, agro-food chemicals, and cosmetics.¹⁻⁶ The glycan component of glycolipids has the affinity and the selectivity for different peptides and proteins, by virtue of which, glycolipids have the potential to be used as non-toxic and non-ionic surfactants for the extraction and the crystallization of integral membrane proteins. The bioactivity of glycolipids and their application as surfactants depends on the glycan part, the lipid chain, and the type of linkage between them, which emphasizes the need for the development of new methodologies for the synthesis of a series of glycolipids with diverse structures.⁷ Among various methods reported for the synthesis of glycolipids, triazole ring containing glycolipids, synthesized by Cu(I) catalyzed *click* reaction of azide and alkyne⁸⁻¹¹ under aqueous conditions, has found the maximum acceptance in recent times, due to the easy synthetic method, higher yield and better proteolytic stability. Since the triazole ring is an isosteric replacement of the trans amide bond, it can act as a linker between the sugar and the aglycon part for the synthesis of *N*-linked glycoconjugates.¹²⁻¹⁹ The C-5 proton of the triazole ring can be replaced by a suitable electrophile by the *click* reaction of azide and alkyne using Cu(I) catalyst under anhydrous conditions. Based on these assumptions, the authors have synthesized several glycoconjugates such as glycopeptoids,²⁰ halogenated glycolipids,²¹ and aromatic glycoconjugates (Fig. 1).²²

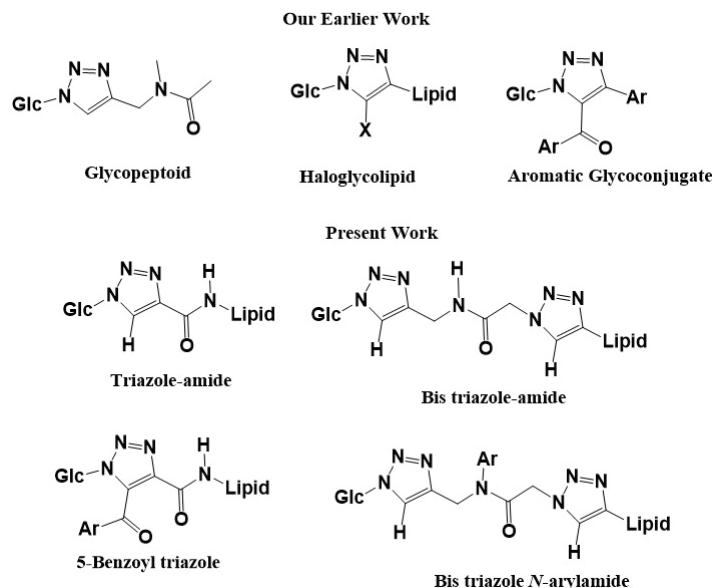


Fig. 1. Structural diversity in glycoconjugates using amide and its isosteres.

In addition to the rigid glycan part and the highly flexible aglycon part in glycolipid, the controlled flexibility of the linkage region by virtue of the res-

stricted rotation in the amide bond and its foldamers plays an important role in deciding its biological activity.^{23,24} The present work has explored the use of Cu(I) catalysed *click* reaction for the synthesis of diversely functionalized triazole-linked glycolipids, where the linkage between the sugar and the lipid part has been modified using amide-linked triazole, 5-benzoyl triazole and bis-triazole rings connected by either amide or *N*-aryl amide bond.²⁵ Given the crucial role exemplified by amide bonds in controlling the structures and activities of proteins, the amide bonds and their isosteres like triazole rings are paramount constituents in drug molecules. A combination of the amide bond and triazole ring (*i.e.*, triazole–amide) in the linkage between sugar and lipid will introduce different conformations and modes of interaction with other molecules. This is the first report on the synthesis of an *N*-aryl peptoid-linked glycolipid by modifying the linkage region between the glycan and the aglycon part. The present work aims at the impact of the modification of the linkage region on the morphology of the glycolipids.

EXPERIMENTAL

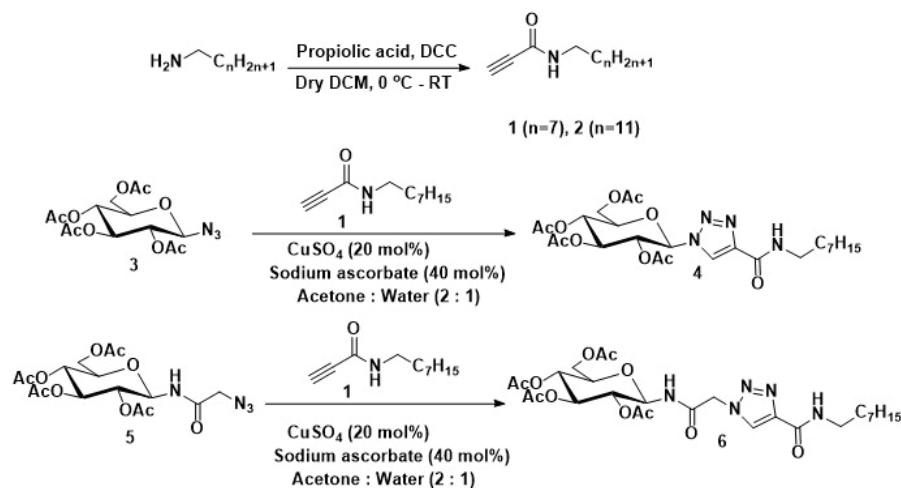
Experimental details and additional data are given in Supplementary material to this paper.

RESULTS AND DISCUSSION

In order to demonstrate the impact of amide and its isosteres on the morphological properties by virtue of noncovalent interactions, triazole-linked glycoconjugates were synthesized using Cu(I) catalysed *click* reaction with the systematic variation in the alkyne or reaction condition. For the synthesis of triazole–amide linked glycolipid **4**, per-*O*-acetylated β -D-glucopyranosyl azide **3** was reacted with propiolamide **1** in presence of Cu(I) as the catalyst, generated *in situ* by the reaction of CuSO₄ and sodium ascorbate in an aqueous acetone medium. The propiolamides **1** and **2** were previously synthesized by a reaction of the corresponding amine with dicyclohexylcarbodiimide (DCC)-activated propiolic acid (Scheme 1). The formation of compound **4** was confirmed by NMR spectroscopy and ESI-MS HRMS. In the ¹H-NMR spectrum of **4**, the triazole proton appeared as a singlet at 8.47 ppm whereas the –NH proton of the amide bond appeared as a triplet at 7.27 ppm. The anomeric proton appeared as a doublet at 5.98 ppm. The C-5 and C-4 carbon of the triazole ring appeared at 144.1 and 124.4 ppm, respectively, in the ¹³C-NMR spectrum.

To introduce more structural diversity in the glycolipid, azidoacetamide-based triazole–amide linked glycolipid **6**, where the sugar moiety is linked to the triazole ring with an extra amide group spacer, was synthesized using a similar procedure as that of **4**. Per-*O*-acetylated β -D-glucopyranosyl azidoacetamide²⁶ **5** was reacted with *n*-octyl propiolamide **1** in presence of Cu(I) as the catalyst (Scheme 1). The C–H proton of the triazole ring in **6** appeared as a singlet at 8.31

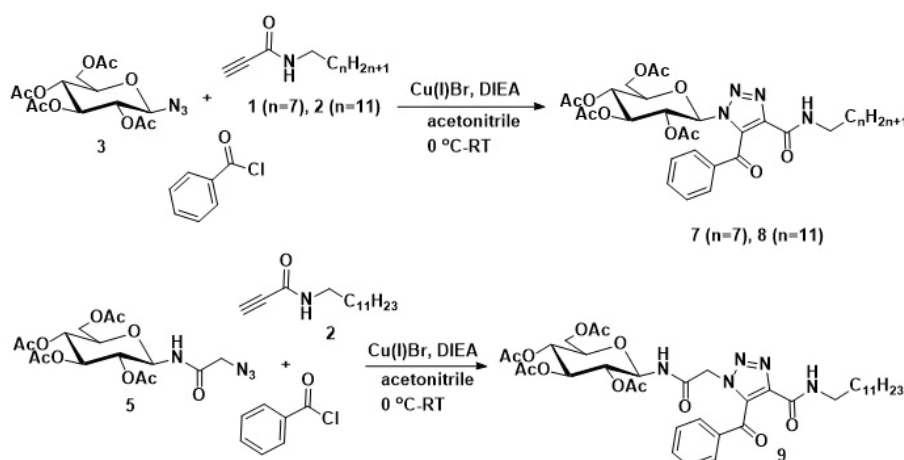
ppm in the $^1\text{H-NMR}$ spectrum. The NH proton of the anomeric amide bond appeared as a doublet at 7.56 ppm and the NH proton of the triazole–amide appeared as a triplet at 7.29 ppm.



Scheme 1. Synthesis of triazole-amide-linked glycolipids.

A crucial role is played by the aromatic proton of the triazole ring in controlling its structure and activity. These protons can act as participants in noncovalent interactions (*e.g.*, C–H \cdots O interactions) with other molecules. Replacement of the triazole proton with other functional groups, for instance, the benzoyl group will not only mutate the steric as well as the electronic atmosphere of the triazole ring but will also change its interaction with other molecules which is important for the activity of biomolecules like glycolipids. Keeping this in mind, glycolipids with 5-benzoyl triazole linkage were synthesized by the electrophilic addition of benzoyl chloride to the copper-triazolium intermediate, formed *in situ* by [3+2] cycloaddition reaction of azide functionalized sugar and alkyne functionalized long chain alkane under anhydrous condition. Azide **3** was reacted with propiolamide **1** and benzoyl chloride in presence of Cu(I)Br and diisopropylethylamine in dry acetonitrile (Scheme 2) which gave 5-benzoyl triazole-linked glycolipid **7** upon purification. In the $^1\text{H-NMR}$ spectrum of **7**, the absence of the triazole proton and the appearance of carbonyl carbon of the 5-benzoyl triazole group at 186.2 ppm in the $^{13}\text{C-NMR}$ spectrum confirmed the formation of the 5-benzoyl triazole ring. The formation of **7** was further confirmed by the ESI-MS HRMS spectrum. Glycolipid **8** with a longer lipid chain was synthesized by reacting protected glycosyl azide **3** with propiolamide **2** under similar reaction conditions as that of **7**.

For the introduction of another amide bond between the sugar and 5-benzoyl triazole ring, azidoacetamide **5** was reacted with propiolamide **2** and benzoyl chloride using Cu(I)Br and DIEA in dry acetonitrile (Scheme 2). The NH proton of the triazole–amide group appeared as a triplet at 7.20 ppm whereas the NH proton of the anomeric amide group appeared as a doublet at 7.06 ppm in the ^1H -NMR spectrum. In the ^{13}C -NMR spectrum of **9**, the carbonyl carbon of the 5-benzoyl triazole group appeared at 186.8 ppm.

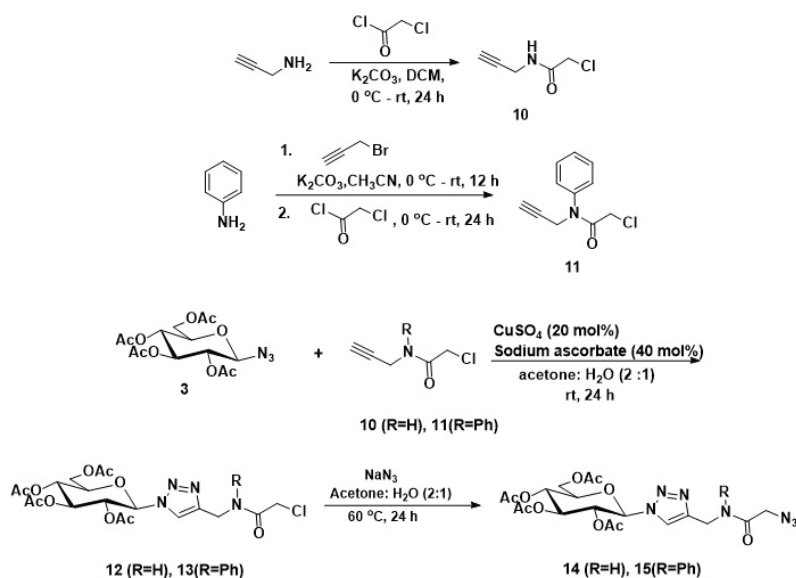


Scheme 2. Synthesis of 5-benzoyl triazole-linked glycolipids.

The reactivity of the triazole ring will be changed by the substitution of its proton by the benzoyl group, whereas on the other side, the presence of an additional triazole ring in the existing molecule may support its binding with other molecules or acquaint supplementary traits to its structure and activity. For the synthesis of bis-triazole linked glycolipid, azide functionalized triazole linked glycoconjugates **14** and **15** were synthesized for further reaction with alkyne functionalized long chain alkanes. Propargyl amine was reacted with chloroacetyl chloride in the presence of potassium carbonate to give the corresponding chloroacetamide **10** which upon reaction with per-*O*-acetylated β -D-glucopyranosyl azide **3** in presence of Cu(I) furnished triazole-linked chloroacetamide **12**. Further reaction of **12** with sodium azide in a mixture of acetone and water (2:1) at 60 °C resulted in the formation of the corresponding azidoacetamide **14** (Scheme 3).

To use the structural and conformational features of *N*-aryl peptoids as a linkage in glycolipids known in the literature of peptidomimetics, bis-triazole-linked glycolipid was synthesized with an *N*-aryl peptoid backbone. Azidoacetamide functionalized *N*-aryl peptoid glycoconjugate **15** was synthesized for further *click* reaction with alkyne functionalized lipid. The synthesis started with the monoalkylation of aniline using propargyl bromide (1.1 equiv.) in the presence of

potassium carbonate, followed by a reaction with chloroacetyl chloride, to give *N*-propargylated *N*-chloroacetamide of aniline **11** (Scheme 3). The reaction of **11** with per-*O*-acetylated β -D-glucopyranosyl azide **3** using copper sulphate and sodium ascorbate in an aqueous acetone medium gave triazole-linked *N*-aryl glycopeptoid chloroacetamide **13**. The chloroacetamide **13** was reacted with sodium azide in a mixture of acetone and water (2:1) at 60 °C to give the corresponding azidoacetamide **15** (Scheme 3). Unlike *N*-alkyl peptoids which exist as a mixture of *cis* and *trans* isomers, *N*-aryl peptoids prefer to exist as *trans* isomers. In the $^1\text{H-NMR}$ spectra of **14** and **15**, the triazole protons appeared as singlets at 7.84 and 7.97 ppm, respectively.

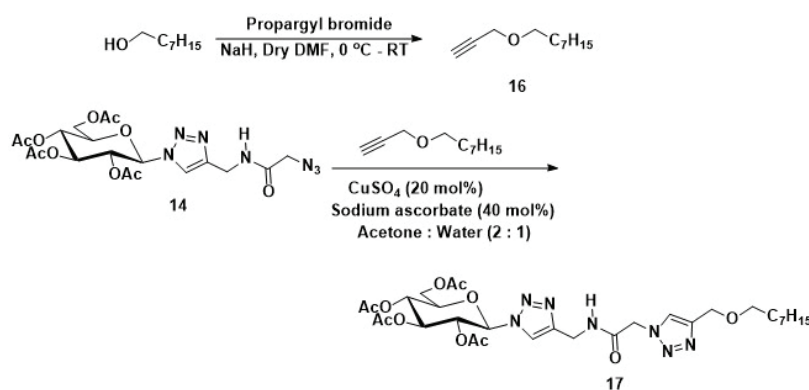


Scheme 3. Synthesis of azide functionalized triazole linked glycoconjugates.

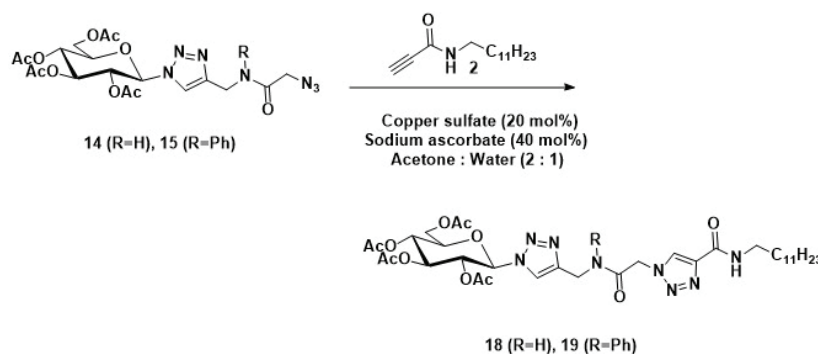
The triazole-linked azidoacetamide functionalized glycoconjugate **14** was reacted with *n*-octyl propargyl ether **16** using Cu(I) catalyzed *click* reaction to give bis-triazole linked glycolipid **17** after purification by column chromatography (Scheme 4). In the $^1\text{H-NMR}$ spectrum of **17**, two triazole protons appeared as singlets at 8.32 and 7.86 ppm, whereas the NH proton of amide appeared as a singlet at 7.33 ppm.

The reaction of **14** with propiolamide **2** by Cu(I) catalyzed *click* reaction gave bis-triazole-linked glycolipid **18** (Scheme 5). There are two types of triazole rings and two amide bonds in **18**, one of them is a triazole-amide. In the $^1\text{H-NMR}$ spectrum of **18**, two triazole protons appeared as singlets at 8.29 and 7.85 ppm whereas the two NH protons of two amide bonds appeared as multiplets in the range of 7.26–7.18 ppm.

A bis-triazole-linked glycolipid with an *N*-aryl peptoid backbone was designed by replacing the NH proton of the amide bond connecting two triazole rings with a phenyl group. The synthesis was done by Cu(I) catalysed *click* reaction of triazole-linked *N*-aryl glycopeptoid azidoacetamide **15** with propiolamide **2** to give bis-triazole linked glycolipid **19** after purification (Scheme 5). The two triazole protons of **19** appeared as singlets at 8.20 and 7.91 ppm, and the NH proton of the triazole-amide appeared as a triplet at 7.11 ppm in the ¹H-NMR spectrum.



Scheme 4. Synthesis of bis-triazole linked glycolipid **17**.



Scheme 5. Synthesis of bis-triazole-linked glycolipids.

The synthesized glycolipids contain aromatic triazole ring and other π -electron systems (*e.g.*, 5-benzoyl triazole and *N*-aryl amide) in the linkage region of sugar and lipid part, which can participate in noncovalent interactions with other bio-molecules and conjugated π -electron systems, thereby facilitating the study of these interactions by the spectroscopic methods like UV-Vis spectroscopy. To understand the diversity in the conjugated π -electron system, the UV-Vis spectra of the glycolipids were recorded. The UV-Vis spectra of triazole amide-linked

glycolipids **4** and **6** showed absorption maxima (λ_{\max}) at 210 and 223 nm, respectively (Fig. 2A and B). For the corresponding 5-benzoyl triazole-linked glycolipids **7** and **9**, there were additional peaks at λ_{\max} 256 and 261 nm, respectively (Fig. 2A and B). With the change in lipid chain length or changes in linkage region, there was no significant change in the absorption spectrum as shown in the case of **7**, **8** and **9** (Fig. 2C). Bis-triazole-linked glycolipids **17–19** showed absorption maxima (λ_{\max}) at 215, 213 and 211 nm, respectively, in the UV–Vis spectra (Fig. 2D).

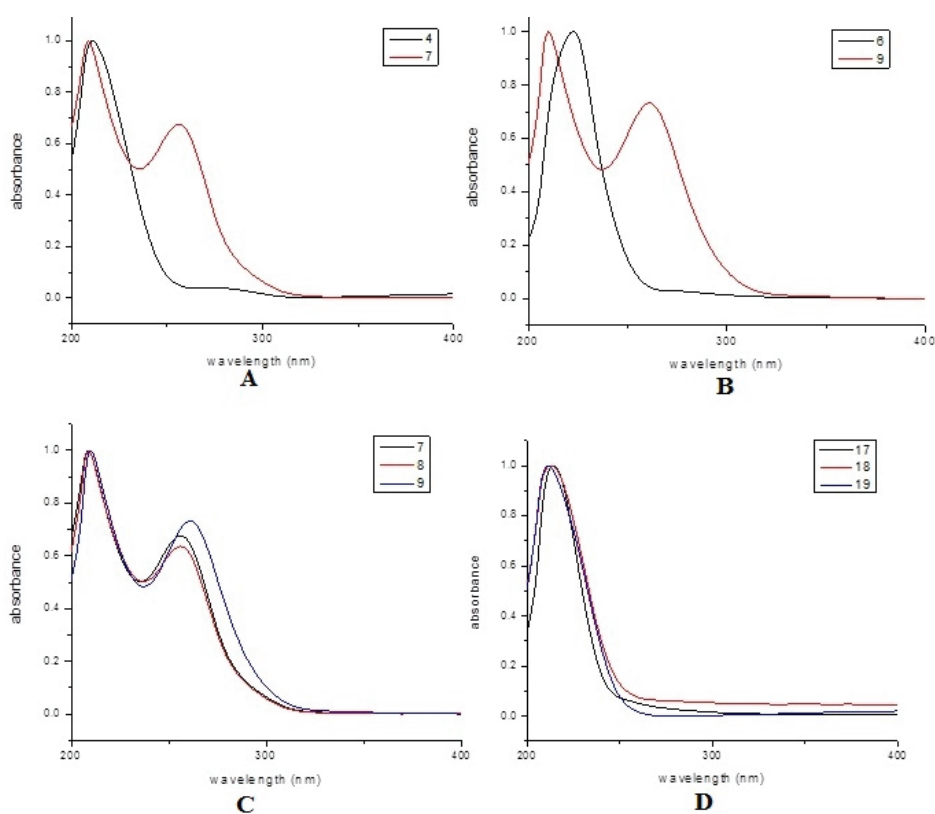


Fig. 2. UV–Vis spectra in methanol at 10^{-5} M concentration; A) glycolipids **4** and **7**; B) glycolipids **6** and **9**; C) glycolipids **7–9**; D) glycolipids **17–19**.

The ability of the functional groups present in the linkage region connecting the sugar and the lipid part to participate in non-covalent interaction plays an important role in controlling the physical properties of synthetic glycolipids. With a systematic variation in the sugar–lipid linkage region, twelve different glycolipids were selected from this work and our earlier work for the SEM study (Fig. 3). The synthesized glycolipids have different functional groups such as amide,

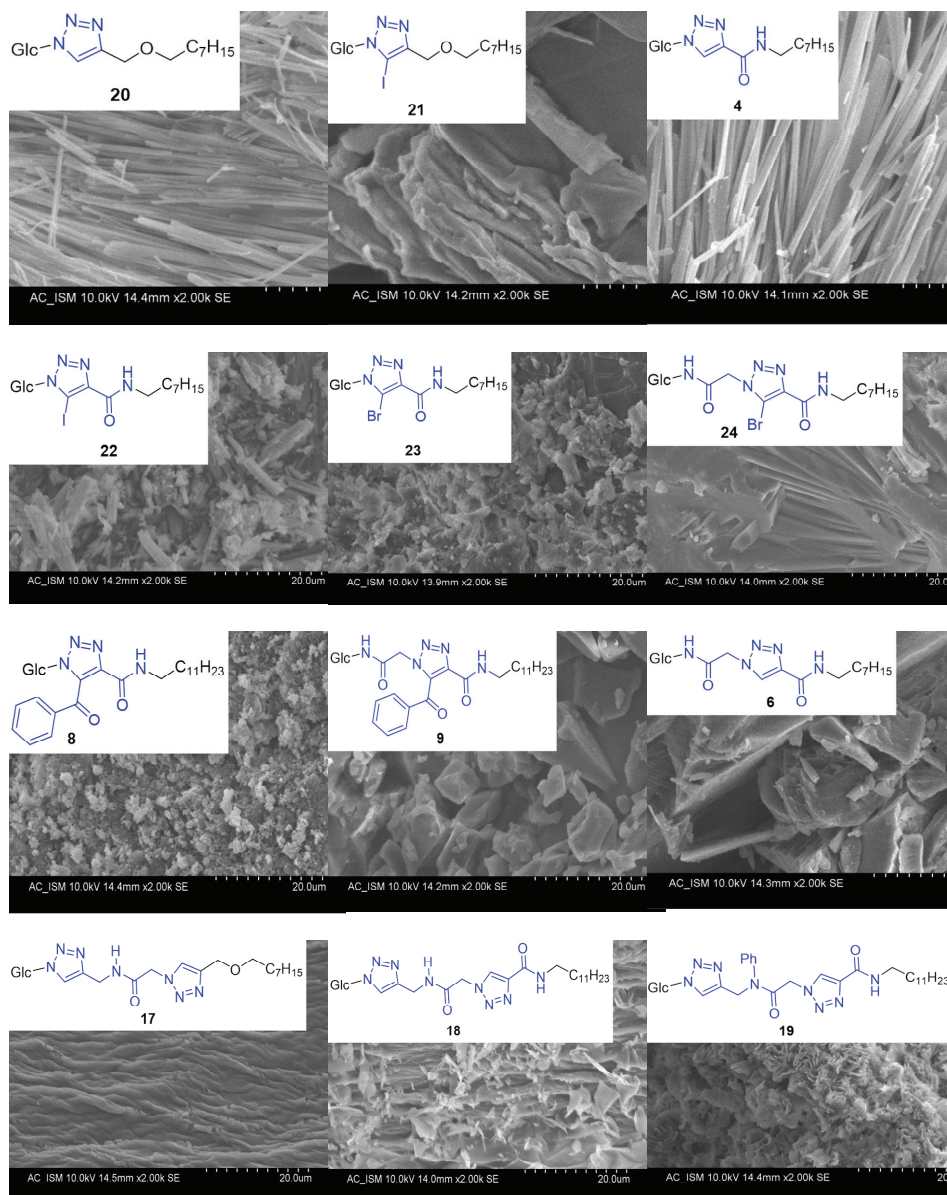


Fig. 3. SEM Images of the synthesized glycolipids.

triazole, amido triazole, halotriazole and bis-triazole in the sugar–lipid linkage region. To understand the influence of these functionalized triazole rings on the morphology of synthesized glycolipids, the self-assembled structures of the protected glycolipids were studied using a scanning electron microscope. Fig. 3 (A–L) displays the SEM images of the self-assembled structures of glycolipids in

a mixture of ethyl acetate and hexane. It was observed that a C-5 protonated triazole containing glycolipid exhibited fiber-like structures, probably due to an extended noncovalent interaction (*e.g.*, C–H···O interaction) as observed in **4** and **20**, whereas substitution of the C-5 proton by a halogen atom such as Br or I diminished such noncovalent interactions. This can be clearly visible from the SEM images of **21–24** where no regular arrangements were observed. In the case of an amido-triazole the effect of non-covalent interaction can be observed from the fibre-like arrangements in the SEM image of **4**, but replacing the triazole proton with a benzoyl group at the C-5 position of the triazole reduced the ability to form fibre-like arrangements as shown in the case of **8** and **9**.

The irregular arrangements of the glycolipids in the SEM images of **17–19** can be attributed to the replacement of an amide bond by either a triazole or *N*-aryl peptoid or ether linkage which reduced the noncovalent interactions. The above study clearly explained the impact of non-covalent interactions on the morphology of glycolipids.

To understand the stability of the glycolipids, the geometries of these molecules were optimized using B3LYP density functional theory. The optimized structures thus obtained were used for frequency calculations. In each case, positive frequencies were obtained, which implies that the optimized structures are minima in the potential energy surfaces and hence are stable. The optimized structures of **8** and **9** are given in Fig. 4.

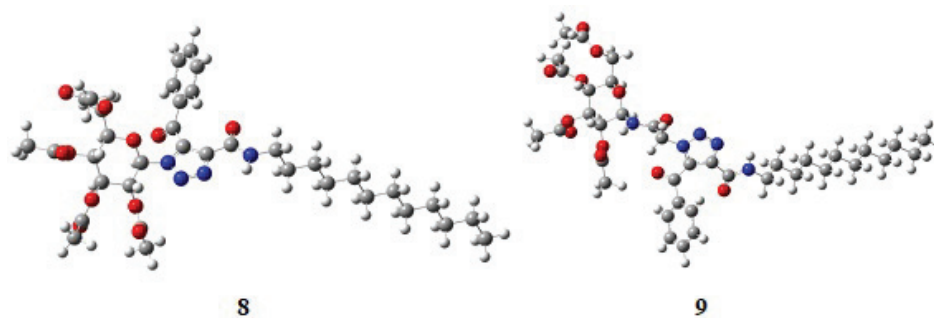


Fig. 4. DFT optimized geometries of synthesized glycolipids **8** and **9**.

CONCLUSION

Diversely functionalized triazole-linked glycolipids were synthesized with systematic variation in the linkage region between glycan and aglycon unit. These Cu(I) catalyzed [3+2] cycloaddition reactions not only gave higher yields under mild reaction conditions, but also have the advantage of functionalizing the triazole rings with modification in reaction condition (5-benzoyl triazole) or sequence of reactions (bis-triazole). The structure of the linkage region between sugar and lipid was systematically varied with different amide bonds and its iso-

steres like triazole-amide, 5-benzoyl triazole, bis-triazole with peptide or *N*-aryl peptoid backbone. The stability of the synthesized glycolipids was also determined based on DFT calculations. The scanning electron microscopy (SEM) study of self-assembled structures of the synthesized glycolipids showed the importance of the amide bond as well as the triazole ring in deciding the morphology of the glycolipids. Since these functional groups are important constituents in peptides or peptidomimics, synthetic glycolipids can be modified accordingly for the interactions with natural peptides, enzymes or other medically important biomolecules. This methodology can be extended to other monosaccharides and disaccharides, with variations in the length of the lipid chain, for the synthesis of a library of compounds. The application of synthesized glycolipids as nonionic surfactants as well as potential drug candidates is in progress.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: <https://www.shd-pub.org.rs/index.php/JSCS/article/view/12254>, or from the corresponding author on request.

Acknowledgements. The authors thank Indian Institute of Technology Madras, Indian Institute of Technology (ISM), Dhanbad and Berhampur University for the instrumentation facility. The authors are thankful to OSHEC, Government of Odisha for the seed grant under OURIP (1034/69/OSHEC) and SERB, New Delhi, for the TARE fellowship (Grant no. TAR/2018/000764).

ИЗВОД

СИНТЕЗА ГЛИКОЛИПИДА СА ПЕПТИД–ПЕПТОИД СПОЈЕМ ПРИМЕНОМ „CLICK” РЕАКЦИЈЕ

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У овом раду је описана синтеза нове класе гликолипида са системским променама споја између два дела структуре, као и агликонског дела молекула, применом „click” Cu(I)-катализоване реакције. Модификације споја између шећера и агликонског дела подразумевају амидне, амидо-триазолске и 5-бензоил триазолске структурне мотиве. Структурне разлике гликолипида су додатно истакнуте укључивањем неколико поларних завојних пептидних група са као што су триазоли, амиди, пептиди или *N*-арил пептоиди у агликонском делу. Нови гликолипиди су креирани комбиновањем различитих пептомиметичких спојева. У раду је први пут приказана примена *N*-арил пептоида у синтези гликолипида. Нова синтетисана једињења су окарактерисана применом спектроскопских и спектрометријских анализа. Утицај амидне везе као и триазолског прстена на морфологију гликолипида је упоређен са њиховом способношћу самоорганизовања, коришћењем SEM анализе. Геометрија гликолипида је оптимизована теоријом

функционалне густине и оптимизоване структуре се налазе у минимуму потенцијалне енергије површине.

(Примљено 1. фебуара, ревидирано 18. марта, прихваћено 30. јуна 2023)

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