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Research paper

# Polyvalent effect enhances diglycosidic antiplasmodial activity

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ABSTRACT

An efficient and facile total synthesis of diglycoside Matayoside D isolated from the root bark of *Matayba guianensis* with antiplasmodial activity have been accomplished in 11 steps with 5% overall yields starting from commercially available glucose and rhamnose. Furthermore, a class of the diglycosidic derivatives with different lengths of the linker and valences were also prepared and evaluated for their antiplasmodial activities against chloroquine-susceptible (3D7) and chloroquine-resistant (W2) strains of *Plasmodium falciparum*. Low valent and short linker attached diglycoside show no enhancement of the antiplasmodial activity while polyvalent conjugates showed enhanced antiplasmodial activity with $IC_{50}$ value at least 20 fold better than that of the corresponding diglycosidic monomer. The polyvalent diglycoside were non-cytotoxic against normal mammalian cells under 50,000 μg/L.



## 1. Introduction

Malaria parasites, particularly *Plasmodium falciparum* (*P. falciparum*), accounts for over 214 million infections alone in 2015 and estimated 438,000 deaths [1]. During the last decade, with increasing effort in malaria control worldwide, the estimated number of mortality has been decreased by 45% [2]. However, malaria control and prevention is still not optimistic, especially in resource scarce countries in tropical and subtropical regions [3]. On the other hand, as international travel increases, more imported cases were reported among people returning from malaria endemic areas [4]. Therefore, effective prophylaxis and treatment of malaria is essential to global public health.

*P. falciparum* parasites resistant to cheap antimalarial drugs [5] such as chloroquine have been widely observed for decades [6]. In recent years, artemisinin-resistance among *P. falciparum* population is spreading in Southeast Asia [7]. Thus, the search for new antiplasmodial drugs is in urgent need [8]. It has been widely accepted that natural products are rich source for antiplasmodial drug discovery [9]. For example, artemisinin, the main component of artemisinin combination therapy (ACT), was isolated from *Artemisia annua* and the oldest drug Quinine was isolated from *Cinchona ledgeriana*. Recently, Matayoside D, **1** (Scheme 1), a new structure of diglycoside extracted from the root of *Matayba guianensis* in Brazilian Cerrado was reported to show a moderate antiplasmodial activity with $IC_{50}$ around 2.5 μg/mL (3.4 μM) against chloroquine resistant strain FcB1/Colombia [10]. However, due to its limited supply from natural source, as well as the complication of extraction and purification, total synthesis of Matayoside D is required for further antimalarial studies. Furthermore, designed analogs of Matayoside D with enhanced antimalarial activity may become lead compounds for the development of novel antiplasmodial agents.

Usually, two strategies are widely used to increase the carbohydrate–protein interaction, which leads to the enhancement of

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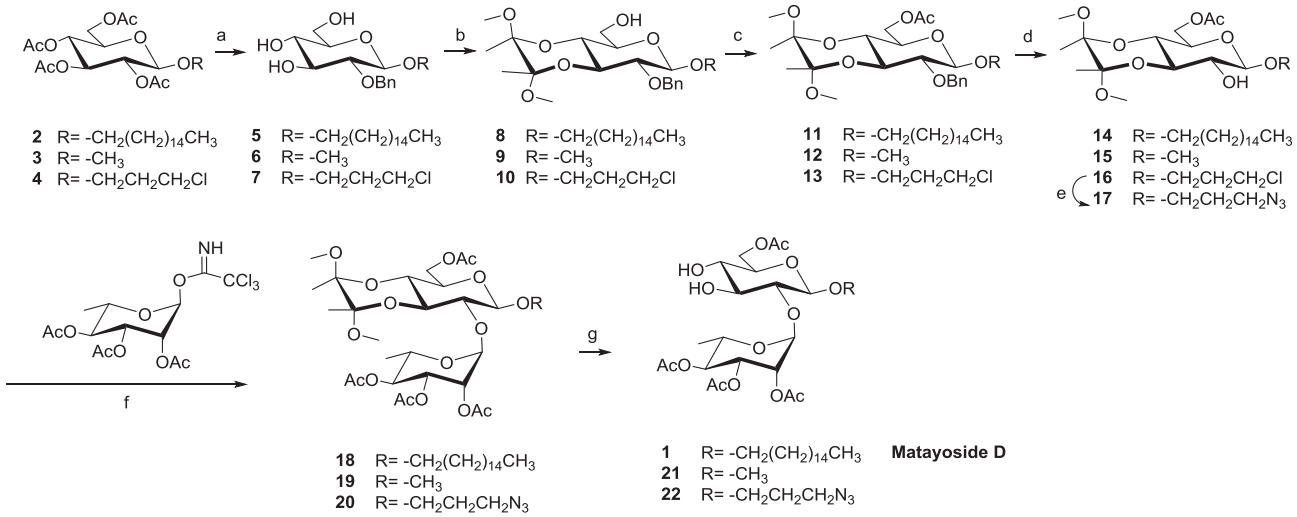


**Scheme 1.** Synthesis of Matayoside D, **1** and its analogs. Reagents and conditions: (a) (i) MeONa/MeOH; (ii) $Bu_2SnO$/MeOH; (iii) Tetrabutylammonium bromide (TBAB), BnBr; (b) 2, 3-butanedione, trimethyl orthoformate, $BF_3{\cdot}Et_2O$; (c) $Ac_2O$/Py; (d) $H_2$, Pd/C; (e) $NaN_3$/DMF, 80 °C; (f) $BF_3{\cdot}Et_2O/CH_2Cl_2$, 0 °C; (g) TFA/$CH_2Cl_2$.

biological activity of oligosaccharides. The first method is to modify glycan structures based on the Quantitative Structure - Activity Relationship (QSAR) [11]. However, since the receptor of Matayoside D is undefined, it is difficult for rational structural design. The other method is inspired by the natural principle, called 'Cluster Effect' [12,13]. Numeric researches have shown that when attaching the monomeric saccharides onto multivalent scaffolds, the carbohydrate–protein interaction is dramatically enhanced, therefore higher activity of the glycan ligand can be achieved [14].

Herein, we report the full chemical synthesis of natural diglycoside Matayoside D, **1** and its analogs with a different linker on the glucosyl anomeric center. 'Click Chemistry' is used for the construction of multi- and polyvalent glycoconjugates. We have tested the antimalarial activity of the glycoclusters with various valencies and hope to get a panel of potent antimalarial glycoconjuagtes.

## 2. Results and discussion

### 2.1. Chemistry

#### 2.1.1. Synthesis of diglycosidic monomer

At first, the synthesis of the diglycoside **1** started from peracetylated hexadecyl glucopyranoside **2**, which was obtained with a reported procedure [15] (Scheme 1). Deacetylation and then regioselective benzylation of the dibutyltin complex of hexadecyl glucopyranoside, gave the corresponding 2-*O*-benzyl glucopyranoside **5** in 85% yield [16]. The acetylation of **5** was used to confirm the position of the benzyl group on the C-2 hydroxy, along with 2D NMR experiments, because it shifted H-3, H-4 and H-6 downfield (see Supporting Information). **5** was transacetalized with butadiene, trimethyl orthoformate and $BF_3{\cdot}Et_2O$ at C-3 and C-4 hydroxyl group to give diacetal **8** in 53% yield [17]. After acetylation on the C-6 and hydrogenation to remove the benzyl group, the acceptor **14** with the free C-2 hydroxyl group of the diglycoside was prepared. Union of **14** and rhamnopyranosyl imidate using standard $BF_3{\cdot}Et_2O$ catalyzed glycosylation methodology [18] furnished disaccharide **18** in 75% yield. The diacetal protecting group was successfully removed with trifluoroacetic acid (TFA) in $CH_2Cl_2$ at room temperature gave Matayoside D, **1**. The two anomeric protons at δ 4.29 (d, $J$ = 7.7 Hz) and 5.18 (d, $J$ = 1.6 Hz) were in good agreement with that reported in the literature [10] at δ 4.32 (d, $J$ = 7.7 Hz) and 5.24 (d, $J$ = 1.8 Hz).

Using the similar procedure, methyl **19** and azidopropanyl glycoside **20** were also prepared. **19** and **20** differ in the length of the linker attached on the glucosyl anomeric center, which can be used to study the influence of linker structure on antimalarial activity.

#### 2.1.2. Synthesis of diglycosidic multi- and polyvalent conjugates

We then changed our attention to conjugate the diglycosidic monomer **20** onto various scaffolds to produce multivalent glycoconjugates in a controllable fashion by adopting the general propargylation method with NaH/propargyl bromide and different commercial valuable alcohol. It has been reported that the carbohydrate-protein interactions was greatly influenced by the valency, shape, topological aspects [19,20] and spacer rigidification [21] of the scaffolds. By adopting various scaffolds, we can determine the optimal scaffold structure. The scaffold structures and corresponding multivalent diglycosides were shown in Scheme 2.

Cu (I)-catalyzed azide–alkyne cycloaddition (CuAAC reaction, also called Click Chemistry) [22] was applied to conjugate the azido saccharide with the alkynlated scaffolds. Typically, fully protected azido glycan **20** was dissolved with the alkyne in a 1:1 mixture of $H_2O$ and THF, catalytic quantity of $CuSO_4{\cdot}5H_2O$ was added followed by sodium ascorbate. The mixture was stirred at room temperature overnight, and then fully protected glycoconjugates were obtained by purification with chromatography. After deprotection of the diacetal groups with TFA in $CH_2Cl_2$ at room temperature, di- (**DiD**), tri- (**TrD**), tetra-(**TeD**), penta-(**PeD**) and octavalent (**OcD**) disaccharides were obtained. The copper complex was absorbed by CupriSorb® if needed and the crude products were purified by Sephendex LH-20. All of the final products were characterized by NMR and MALDI-TOF.

In order to attach more saccharidic copies on the scaffold, we chose poly alkyne **29** prepared from poly (methyl vinyl ether-*alt*-maleic anhydride, MW~130,000) **28** reacted with propargylamine. After purification by dialysis, and lyophilization, the polyvalent backbone **29** was obtained. The disaccharide **22** was conjugated on the scaffold using Click Chemistry described above. After dialysis and lyophilization again, the polyvalent disaccharide **PoD** was prepared as a white powder.