# 抗ウイルス治療薬の開発を目指して：エボラウイルス阻害薬としての糖ペプチドの有用性の検討

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## Antiviral Therapeutics: Glycans Attached to Peptides as Inhibitors of Ebola Virus

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In the quest to obtain specific novel antiviral drugs against infectious diseases it is required to focus on the essential elements of viral infection and inhibit the entry of the viral into the host cells. In here, we focus on inhibiting the internalization of lethal Ebola virus by using synthetic glycopeptides that mimic the viral glycoprotein. One of the entries of Ebola virus is through macrophage galactose lectin, then we plan to inhibit virus infection through inhibitors of the lectin, and further inhibition of infection and propagation. In this project we could synthesize this multivalent peptides attached to several glycans. Synthesis was done on solid-phase and the purification of the peptides was the bottleneck of the project. Finally, target compounds were obtained and ready for biological evaluations.

### 1) Introduction

Ebola virus causes a severe and often lethal hemorrhagic fever in humans and other mammals, and it has a mortality rate higher that 80% of infected persons. Ebola relies on viral envelope glycans (sugars) attached to proteins to infect host cells [1]. Ebola virus uses different routes to gain cellular entry depending on the cell type. One of this viral gates is the macrophage galactose lectin (MGL) receptor present on dendritic and macrophages cells on host body. To infect the cell, the viral glycoprotein attaches to MGL which allows entering into the cell and then progress to the viral replication [2]. All these evidences lead us to consider MGL as an attractive target to inhibit the infection of the lethal Ebola virus. The aim of this project was to create an inhibitor that attaches to MGL receptors and avoid the entrance of the Ebola virus and the consecutive infection (Fig. 1).

Currently, there is a vaccine against Ebola [3], but there is no specific therapeutics for treatment of Ebola infection. Until now, several potential small-molecule-based drugs have been developed with promising results [4]. Nevertheless, due to the likelihood of future outbreaks and generation of mutant viruses, the development of a variety of anti-Ebola therapeutics is urgent. In here, we developed the therapeutics that specifically target and inhibit the entry process of Ebola virus, which lead to the significant contribution for treatment and prevention of Ebola infection in the future.

In previous studies, we have already demonstrated that peptides attached to GalNAc (*N*-acetylgalactosamine) sugars are recognized by MGL mainly by sugar moiety with contribution of the peptidic sequence [5]. Then, in this project we thought that mimic and multivalency of natural biopolymers is an elegant approach to increase the binding avidity to receptors. Further, we could obtain a collection of novel candidates and provided key elements of molecular recognition. Then, in this project, we made the rational design and obtaining of a chemical library of GalNAc attached to peptides including one, two or multi sugars to verify the effect of multivalency.

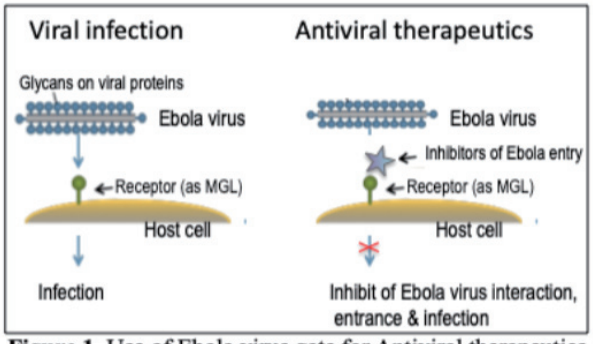


**Figure 1** Use of Ebola virus gate for Antiviral therapeutics.

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### 2) Results and Discussion

To get potential Ebola virus inhibitors, the following workflow was established as follows: 1) Design and synthesis of Ebola virus entry inhibitors; 2) Binding assays with Macrophage Galactose Lectin; 3) Biological Assays; 4) Lead optimisation.

We were able to successfully obtain the library of multi-glycosylated peptides by solid-phase peptide synthesis. An iterative approach was employed to allow multibranch compounds.
For the glycoamino acid coupling, the “double activation” method was chosen. Purification was done under RP-HPLC and HPLC. Characterization was done in RP-UPLC and MALDI- TOF MS.

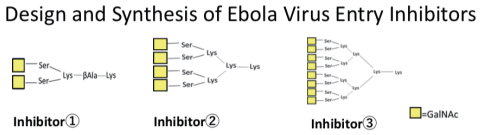


**Figure 2** Designed Ebola inhibitors.

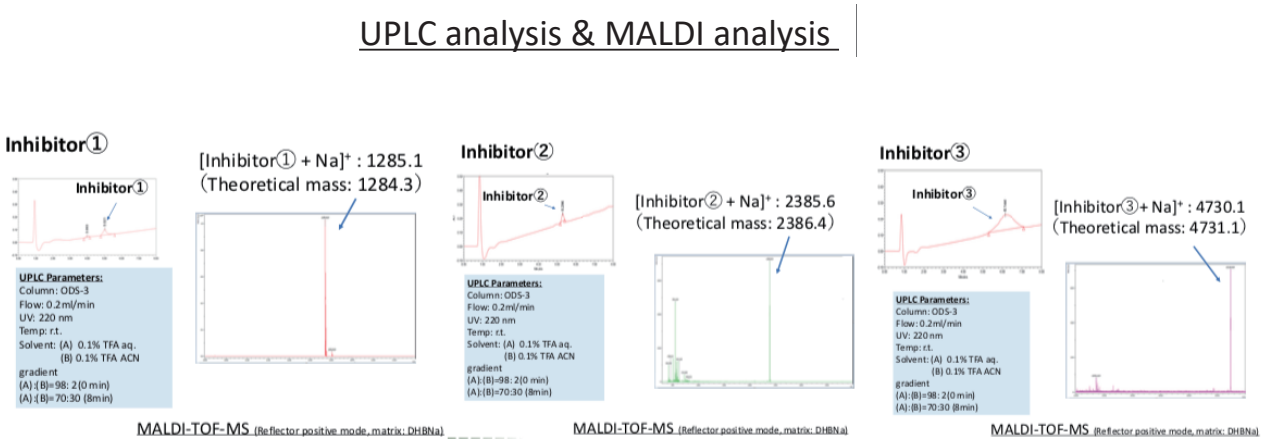


**Figure 3** Synthesized Ebola inhibitors and its characterization.

We also performed preliminary experiments on a microarray platform to confirm the binding affinity with Macrophage Galactose Lectin. Further, biological assays are currently ongoing by Professor Asuka Nanbo (Nagasaki University). Lead optimization was lastly performed during the research internship.

### 3) Conclusion

We designed a library of potential Ebola virus inhibitors based on multivalent GalNAc peptides. The inhibitors were successfully obtained by solid-phase glycopeptide synthesis. As the structure illustrates, the syntheses and purification of inhibitors **2** and **3** were tedious due to the complexity of the multivalent compounds. After several trials and optimization of synthesis and purification conditions, inhibitors were finally obtained in a high ( for inhibitor **1**) and moderate yield ( for inhibitors **2** and **3**). Preliminary microarray results are non-conclusive, then we decided to design and synthesize a new class of inhibitors based on **1**, **2** and **3**. In addition, biological assays of the inhibitors to establish the entrance of the Ebola virus, are actually undergoing by Prof. Asuka Nanbo (University of Nagasaki). We are very confident that the compounds and results of this project can serve to establish a new class of compounds that can prevent the entry and infection of the Ebola virus.

### 4) Acknowledgement

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