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Carbohydrate Synthesis for Biomedical Applications

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Abstract

Glycoconjugates are carbohydrates covalently attached to non-sugar components such as proteins and lipids. They mediate cell growth, cell-cell communication, cellular differentiation and immune defenses in a living organism. Due to diverse patterns of glycan, glycoconjugates have various applications for biomedical purposes. In this thesis, ‘protein cage –based nanoparticles for the recognition and binding of cell-surface lectins’, ‘oligosaccharide synthesis for HIV-1 vaccine’, ‘graphene-based biosensor for specific lectin detection’, and ‘protein cage-based nanoparticle for delivery vehicles’ are presented.

Carbohydrates selectively recognize and target endogenous cell-surface lectins. Human ferritin protein cage nanoparticles (HFPCNs) are used as targeted-delivery platform for carbohydrate derivatives. Maleimide-terminated mannosyl and galactosyl derivatives were synthesized for binding to the surface of HFPCNs via thiol-maleimide Michael-type addition. Through fluorescent cell imaging, we confirmed specific interaction between polyvalently displayed mannosyl and galactosyl derivatives to DCEK and HepG cells, respectively.

DC-SIGN specifically interact to HIV-1 viruses. DC-SIGN recognizes gp120, a glycan shield on HIV-1 viruses. To mimic the function of gp120, oligomannosyl derivatives were synthesized. Di-mannosyl derivatives were synthesized by glycosylation between glycosyl donor and acceptor. The chemical structure of di-mannosyl derivatives were characterized by thin layer chromatography (TLC), nuclear magnetic resonance spectroscopy (NMR), and electrospray ionization mass spectrometry (ESI-MS).

Due to their novel mechanical, electrical, and chemical properties, graphene-based nanomaterials have potential for application of biosensors. Graphene-based FET-style biosensor was prepared for the recognition of lectin. Pyrene-mannosyl derivatives were synthesized for binding to graphene device and recognition of Concanavalin A. Non-covalent π-π stacking interaction was used in binding between graphene device and pyrene-mannosyl derivatives. Through UV-vis, Raman, and atomic force microscopic analysis, non-covalent binding was investigated. Graphene-based sensor recognized current-voltage characteristic of graphene-based biosensor showed selective recognition of Con A.

Human heavy-chain ferritin (HHFn) is protein cage-based nanoplatform for delivery of biomolecules. The outer surface of HHFn is modified with β-cyclodextrin (β-CD) through thiol-maleimide Michael addition followed by copper (I)-catalyzed azide-alkyne cycloaddition (CuAAC). The hydrophobic central cavity of β-CD non-covalently interacts to FTIC-AD to form β -CD-C90 HHFn / FITC-AD inclusion complexes. FITC-AD is reversibly released from protein cage-based inclusion complexes.
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Chapter 1. Introduction

1.1 Biological Importance of Carbohydrate

Carbohydrate is one of the four major macromolecules in the biological system. Each unit of monomers can be combined to one another by various linkage types, and it is possible to make a large number of branches. These characteristics allow numerous combinations of glycans. Glycoconjugates are carbohydrates covalently attached to non-sugar components such as proteins and lipids. In a living organism, carbohydrates usually exist as a form of glycoconjugates that mediates cell growth, cell-cell communication, cellular differentiation and immune defenses. Glycan patterns in glycoconjugates determine biological, Chemical and physical characteristics of glycoconjugates.

1.2 Protein Cage-Based Glyco-Nanomaterials

With the development of nanotechnology, it is possible for nanomaterials, to control sizes, shapes, size distributions, and chemical compositions. Colloidal nanoparticles can carry a large amount of materials due to a large surface area and polyvalency. Modifying the surface of nanoparticles with varied ligand nanoparticles and enable them to recognize several biomolecules and enhance chemical stabilities. Relative large size of Colloidal nanoparticles to free molecules prolong the circulation time of drugs. For these characteristics, Colloidal nanoparticles are promise candidates for targeted delivery nanoplatforms. But it is still hard to reproduce nanoparticles with homogenous characteristics due to complicated passivation process and its chemical diversity. Protein cage structure is the solution to these problems. They tend to self-assemble to form nanoparticles with uniform sizes and shapes. Additionally, high order of symmetry and polyvalency of protein cage structure allow modifying surface of protein-caged nanoparticles (PCNs) with various ligands. PCNs have exterior and interior surfaces. Exterior surfaces are used for polyvalent displaying biomolecules like imaging probes and affinity tags. Interior surfaces are used for encapsulating and carrying organic materials such as drugs. These surface modifications make PCNs act as cargo loading nanoplatforms for targeted delivery.
Chapter 2. Protein Cage-based Nanoparticles for the Recognition and Binding of Cell-Surface Lectins

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Collaboration: All the biological analysis of HFPCNs was done by Young Ji Kang (Prof. Sebuyng Kang group)

2.1 Introduction & Background

2.1.1 Polyvalent Glycan-Displaying Nanoplatforms

Glycans on glycoconjugates specifically interact with lectins on the cell surfaces. These carbohydrate-protein interaction mediates cell growth, cell-cell communication, cellular differentiation and immune defenses. Glycans act as definite signs in glycoconjugate for binding to cell-surface lectins. But the binding affinity between carbohydrate and lectin is quite weak. Inspired by nature, polyvalent glycan interaction was used to overcome this problem. Previously, many researchers have studied polyvalent glycan displays on various platforms. Polyvalent platforms such as dendrimers, polymers, and nanomaterials allow high affinity and specificity to carbohydrate-lectin interactions.

2.1.2 Research objectives

In this study, we synthesize two monosaccharide-derivatives to conjugate human heavy-chain ferritin protein cage nanoparticles (HFPCNs) for multimeric glycoconjugate nanoplatforms. These monosaccharide-derivatives were attached to the surface of HFPCNs through Michael addition. (Scheme 2.1) To synthesize these carbohydrate derivatives, we select thio-glycosyl derivatives due to easy formation and chemical stability during reaction process. We investigated chemical structures of glycosyl derivatives by $^1$H and $^{13}$C nuclear magnetic resonance (NMR) and mass spectrometry (MS) and confirmed conjugation of glycosyl derivatives to HFPCNs by mass spectrometry (MS). Finally, we presented specific binding of monosaccharide-conjugated HFPCNs by fluorescent cell imaging.
Scheme 2.1. Synthetic pathway of Gal- and Man- fHFPCNs and their specific recognition to cell surface lectins. Reproduced from [35].
2.2 AMM- and AGM- modified HFPCNs

2.2.1 Synthesis of AMM and AGM

Glycosyl bromide was treated by thiourea and hydrolyzed to produce 2,3,4,6-tetra-O-acetyl-1-thio-α-D-mannopyranoside 1 and 2,3,4,6-tetra-O-acetyl-1-thio-β-D-galactopyranoside 5. The thiosugars 1, 5 were reacted with nitrobenzyl bromide in the existence of TEA. The nitrobenzyl groups were reduced by tin chloride to generate (p-aminobenzyl)-2,3,4,6-tetra-O-acetyl-1-thio-α-D-mannopyranoside 3, and (p-aminobenzyl)-2,3,4,6-tetra-O-acetyl-1-thio-β-D-galactopyranose 7. N-succinimidyl-3-maleimido-propionate were added to p-anilinyl-linked sugar derivatives to produce maleimido group terminated per-acetylated-mannopyranoside (AMM) 4 and maleimido group terminated per-acetylated-galactopyranoside (AGM) 8 (Scheme 2.2).

Scheme 2.2. Synthetic scheme of AMM and AGM: a) 4-nitrobenzyl bromide, DMF, TEA, r.t., Man 81%, Gal 90%; b) SnCl2–2H2O, EtOH, 70 °C, Man 44%, Gal 50%; c) N-succinimidyl 3-maleimido-propionoate, DMF/(DCM), DIPEA, Man 73%, Gal 52%. DMF, N,N-dimethylformamide; DCM, dichloromethane; DIPEA, N,N-diisopropylethylamine; TEA, triethylamine. Reproduced from [35].
2.2.2 Conjugation of AGM and AMM to HFPCNs

We used human ferritin as a template for the synthesis of protein-cage based nanoplatform for targeted-delivery.\textsuperscript{41-46} 24 subunits of Human heavy-chain ferritin protein cage nanoparticles (HFPCNs) have cysteine residue (C90) on their exterior surface.\textsuperscript{39} These cysteines can be modified by other functional moieties. We conjugated maleimido group terminated per-acetylated-mannopyranoside (AMM, AGM) to the cysteine moieties on the surface of HFPCNs (Scheme 2.1). AMM and AGM have acetyl groups that protect hydroxyl groups. To deprotect the acetyl groups to hydroxyl groups, we dialyzed AMM- or AGM-modified HFPCNs overnight at pH 11.\textsuperscript{47}

2.3 Characterization of Man-HFPCNs and Gal-HFPCNs

We investigated the presence of hydroxyl groups on HFPCNs using electrospray ionization-time of flight mass spectrometry (ESI-TOF MS) to investigate the completion of deacetylation.\textsuperscript{18,22} The molecular masses of each subunit of AMM- or AGM-modified HFPCNs was 21,683.0 Da, which corresponded to the calculated value (21,682.5 Da) (Figure 2.1). Both AMM- and AGM-modified HFPCNs showed same molecular mass because they have the same composition of the atoms. From this data, we found that each subunit of HFPCNs has one AMM or AGM on their surface (24 AMMs or AGMs per cage). During reaction, we collected each modified HFPCN samples at several points and measured the molecular masses of the samples to check the process of deacetylation. As one acetyl group in AMM or AGM moiety on HFPCNs is deprotected to the hydroxyl group, they have mass decreases of 42 Da. The molecular mass data showed the gradual mass decrease of modified HFPCNs as reaction proceeds (Figure. 2.2). After end of deacetylation, modified HFPCNs had final molecular mass of 21,533.0 Da, which had a minor error compared with the calculated value (21,514.5 Da) (Figure 2.1 and Figure 2.2). Previous article reported that oxidation of thioether groups caused this error.\textsuperscript{48}
Figure 2.1. Electrospray ionization-time of flight mass spectrometry of each subunit of (A) AMM-HFPCN (calcd. 21 682.5 Da, obs. 21 683.0 Da), (B) Man-HFPCNs (calcd., 21 514.5 Da, obs. 21 533.0 Da), (C) AGM-HFPCN (Calcd, 21682.5 Da, obs, 21683.0 Da) and (D) Gal-HFPCNs (Calcd, 21514.5 Da, obs, 21533.0 Da). Reproduced from [35].
Figure 2.2 Gradual mass decrease of (A) Man-HFPCN, (B) AMM-HFPCN, (C) Gal-HFPCN (D) AGM-HFPCN at various point (0hr, 1hr, 6hr, 15hr, 24hr, and 48hr). AGM-HFPCN incubated at pH 11 for different time. Observed masses were shown in a below table. Reproduced from [35].
2.4 Targeted Delivery of Modified HFPCNs

2.4.1 Cell-Surface Lectins

In targeted delivery of biomolecules to particular cells, cell-surface lectins act as a marker due to their high specificity in binding to glycans.\textsuperscript{27-29} we selected DC-SIGN and asialoglycoprotein receptor (ASGP-R) as target lectins for delivery of Man-HFCPCNs and Gal-HFPCNs to specific target cells. Dendritic cells (DCs) has DC-SIGN, a C-type lectin on their surface. DC-SIGN recognizes mannose- and fucose-type glycans on glycoproteins.\textsuperscript{28, 49} As a pattern recognition receptor, DC-SIGN binds to carbohydrate a moiety on HIV-1 and Mycobacterium tuberculosis, inducing immune responses in human immune system.\textsuperscript{49} ASGP-R is an endocytotic receptor present on the surface of hepatocytes. It explicitly identifies galactose-containing glycoproteins and mediates endocytosis for targeted delivery of genes and drugs.\textsuperscript{50}

2.4.2 Fluorescent Cell Imaging Analyses of Modified HFPCNs

We treated Man-HFPCNs and Gal-HFPCNs with N-hydroxysuccinimide (NHS)-ester-fluorescein to observe lectin-targeted delivery of nanoparticles to specific cells. We prepared DCEK cells, and HepG2 cells, which have DC-SIGN and ASGP-R on their surface, respectively.\textsuperscript{51, 52} Fluorescent labeled Man-HFPCNs and Gal-HFPCNs (fMan-HFPCNs and fGal-HFPCNs) were detected by fluorescence microscopy to confirm specific recognition between mannose- and galactose-modified HFPCNs and target lectin. For negative controls, we also labeled AMM-HFPCNs and AGM-HFPCNs with NHS-ester-fluorescein (fAMM-HFPCNs and fAGM-HFPCNs) because AMM and AGM don’t interact to cell-surface lectins.

We investigated specific carbohydrate-lectin binding between fMan-HFPCNs and DCEK cells, and fGal-HFPCNs and HepG2 cells through fluorescent microscopy. (Figure 2.3A, B bottoms). However, in cases of fAMM-HFPCNs and fAGM-HFPCNs, there were no carbohydrate-lectin interaction (Figure 2.3 A, B tops). From these data, we demonstrated that mannose and galactose moieties on HFPCNs selectively recognize target lectins on the cell surfaces, DC-SIGN and ASGP-R, respectively.
Figure 2.3. Fluorescent microscopic images of (A) DCEK cells and (B) HepG2 cells. DCEK cells were stained with fMal-HFPCN (bottom, left) and fAMM-HFPCN (top, left). HepG2 cells were stained with fGal-HFPCN (bottom, left) and fAGM-HFPCN (top, left) Nucleus was treated with DAPI (top and bottom, right). Reproduced from [35].
2.5 Conclusions

We conjugated AMM and AGM to HFPCNs, which had 24 cysteine residues on their surface. From mass spectrometry analysis, we confirmed uniform and polyvalent displays of monosaccharide derivatives on the surface of HFPCNs. Fluorescent cell imaging data demonstrate specific carbohydrate-lectin binding and targeted delivery of modified HFPCNs. Man-HFPCNs and Gal-HFPCNs specifically bound to DC-SIGN on DCEK cells and ASGP-R on HepG2 cells, respectively. It is also possible to modify the interior cavity of HFPCNs. Biomolecules such as medicinal drugs and imaging probes can be conjugated on the interior surface of HFPCNs. Through both exterior and interior surface modification, we can advance multifunctional protein cage nanoparticles for targeted drug delivery.
Chapter 3. Oligosaccharide synthesis for HIV-1 vaccine

3.1 Introduction & Background

3.1.1 DC-SIGN: Receptor for HIV-1

HIV-1 is one of the fatal disease which infect about 60 million and killed over 20 million people.\textsuperscript{54, 55} This virus attacks an immune system in the human body, making them immune deficiency.\textsuperscript{56} HIV-1 viruses have glycan shield on their surfaces, so-called gp120, which help to invade target T-cells.\textsuperscript{57, 58} The interaction between gp120 and T-cell is key for viral invasion of HIV-1.\textsuperscript{59} DC-SIGN (Dendritic Cell-specific Intercellular adhesion molecule-3-Grabbing Non-integrin) is one of the HIV-1 receptor recognizing mannose-containing glycan shield of HIV-1 presented on the surface of dendritic cells.\textsuperscript{60} When a complex between HIV-1 and DC-SIGN formed, these complex attacks the CD4+T cell and led productive T cell infection.\textsuperscript{28}

3.1.2 Research Objectives

In this research, we observed immunogenic effect of oligomannose-modified protein cage nanoparticles, specifically interaction to DC-SIGN. We synthesized oligomannose which mimic gp120, glycan shield on HIV-1 virus. For the oligosaccharide synthesis, we prepared glycosyl donor and acceptor first and linked them through glycosylation. The chemical structures of glycosyl donor, acceptor, and oligomannose were confirmed through $^1$H nuclear magnetic resonance (NMR) and mass spectrometry (MS) analysis.
3.2 Synthesis of Mannosyl Derivatives

3.2.1 Synthesis of Glycosyl Donor and Acceptor

To build up disaccharide derivatives, 2-\(O\)-Acetyl-3,4,6-tri-\(O\)-benzyl-\(\alpha\)-D-mannopyranosyl trichloroacetimidate 8 and 2’-azidoethyl-3,4,6-tri-\(O\)-benzyl-\(\alpha\)-D-mannopyranoside 10 were selected as glycosyl donor and acceptor, respectively. They were prepared from D-mannose through several processes (Scheme 3.1). First, hydroxyl groups of D-mannose were acetylated by acetic anhydride and iodine. Acetylated mannose was treated with hydrogen bromide solution for substitution of the acetyl group in anomeric position with bromine. 2,3,4,6-tetra-\(O\)-acetyl-\(\alpha\)-D-mannopyranosyl bromide 2 was reacted with methanol to generate Exo-3,4,6-tri-\(O\)-acetyl-1,2-\(O\)-(1-methoxyethylidene)-\(\beta\)-D-mannopyranoside 3. Mannosyl orthoester derivative 3 was deacetylated under Zemplen condition, followed by the benzylation with BnBr. Exo-3,4,6-tri-\(O\)-benzyl-1,2-\(O\)-(1-methoxyethylidene)-\(\beta\)-D-mannopyranoside 5 was treated with acetic acid and acetic anhydride to produce 1,2-Di-\(O\)-acetyl-3,4,6-tri-\(O\)-benzyl-D-mannopyranose 6. 2-\(O\)-Acetyl-3,4,6-tri-\(O\)-benzyl-\(\alpha\)-D-mannopyranoside 7 was generated by selective anomeric deacetylation of 6 with benzylamine. 2-\(O\)-Acetyl-3,4,6-tri-\(O\)-benzyl-\(\alpha\)-D-mannopyranoside 7 was reacted to trichloroacetimide to generate glycosyl donor, 2-\(O\)-Acetyl-3,4,6-tri-\(O\)-benzyl-\(\alpha\)-D-mannopyranosyl trichloroacetimidate 8. Glycosyl donor 8, was reacted with 2-azidoethane and subsequently deacetylated to produce glycosyl acceptor, 2’-azidoethyl-3,4,6-tri-\(O\)-benzyl-\(\alpha\)-D-mannopyranoside 10.
Scheme 3.1. Synthetic scheme of glycosyl donor 8 and glycosyl acceptor 10. (a) Ac₂O, RT, quantitative yield; (b) HBr, RT, 91%; (c) MeOH, DCM, 40 °C, 91%; (d) NaOMe, MeOH, RT, 75%; (e) BnBr, NaH, DMF, RT, 63%; (f) H₂O, AcCOOH, pyridine, Ac₂O, RT, 80%; (g) BnNH₂, THF, RT, 78.5%; (h) Trichloroacetonitrile, DCM, 1,8-Diazabicyclo[5,4,0]undec-7-ene, RT, 64%; (i) 2-azidoethanol, TMSOTf DCM, RT, 80.5%; (j) NaOMe, MeOH, RT, 100%, quantitative yield.
3.2.2 Glycosylation

Disaccharide is synthesized by linking of two monosaccharide precursors by formation of glycosidic linkage. Glycosidic bond is formed by displacement of a leaving group at the anomeric position of glycosyl donor 8 with a free hydroxyl group of glycosyl acceptor 10. Activated with Lewis acid, they provide disaccharide in good yields (48%). After glycosylation, disaccharide was deprotected under Zemplen condition followed by a debenzylation with Pd/C and hydrogen gas. (Scheme 3.2).

Scheme 3.2 Glycosylation between glycosyl donor 8 and acceptor 10. (a) TMSOTf, DCM, RT, 48.3%; (b) i) NaOMe, MeOH, RT; ii) Pd/C, H₂(g), MeOH, RT, 89%.

3.3 Characterization of Mannosyl Derivatives

We confirmed the chemical structure of 2-aminoethyl-α-D-mannopyranosyl-(1→2)-α-D-mannopyranoside 12 by ¹H nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) (Figure 6.14). Anomeric proton peak of each mannosyl unit were observed at 5.0 ppm and 5.1 ppm to indicate glycosyl donor and acceptor were connected successfully. Broaden peak at 3.15-3.25 ppm shows characteristics of amine peak. This peak suggested that azide group in 2’-azidoethyl-2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranoside 11 was reduced by hydrogen gas purging. The observed molecular mass data of dimannosyl amine derivative was 386.21 Da, which corresponded to the calculated value (386.17 Da). These data demonstrated the presence and high purity of our product.
3.4 Conclusions

We synthesized glycosyl donor and acceptor for glycosylation of dimannosyl amine derivative. Dimannosyl amine derivative was characterized by NMR, MS analysis. In the future, we should convert amino group to azide group and synthesize maleimide-alkyne linker for conjugation to the virus-like particles. The complex of disaccharide and virus-like particles will be used to test an immune response against DC-SIGN.
Chapter 4. Graphene-based Biosensor for Specific Lectin Detection

Collaborations: Preparation of graphene device and electrical analysis was done by Mi-Sun Lee (Prof. Jang-Ung Park group)

4.1 Introduction & Background

4.1.1 Graphene-Based Biosensor

Graphene is a single atom-thick, two-dimensional lattice of sp²-hybridized carbon atoms. Owing to its excellent mechanical, electronic, optical and thermal properties, graphene has been attracting considerable interest in various scientific fields. In specific, graphene has potential for applications in FET (Field effect transistor)-style biosensors due to its high-sensitivity, high-flexibility, cost effectiveness and easy surface modification. Graphene-based biosensors detect biomolecules with high-selectivity and low-noise levels. Recently, carbohydrate has been used as the components of graphene biosensor. In a living organism, carbohydrate mediates cell growth, cell-cell communications, cellular differentiations and immune responses. Modified by carbohydrate moieties, graphene-based biosensor can detect specific lectins.

4.1.2 Covalent and Non-covalent Modification

Both covalent and non-covalent modification has been used to attach biomolecules to graphene-based FET. Covalent modifications, such as oxidation, give strong bonding between biomolecules and graphene. But it breaks down sp² planar structures of graphene to sp³ structures. On the contrary, non-covalent modification preserves structure of graphene and its intrinsic properties. Recently, non-covalent π-π stacking interaction has been widely used in chemical modification of graphene.
4.1.3 Research Objectives

In this research, we designed a graphene-based FET biosensor to detect Concanavalin A (Con A), which specifically interacts to mannose. We conjugated glyco-pyrene derivatives to FET-style graphene devices via π-π stacking interaction. This glyco-pyrene graphene complex was expected to monitor the reversible interaction between Concanavalin A and mannosyl moiety as graphene-based biosensor.

4.2 Synthesis of Graphene-Based Biosensor

4.2.1 Synthesis of Glyco-Pyrene Derivatives

We selected thio-glycosyl derivative 1 and 4 as starting compound for the synthesis of glycol-pyrene derivatives. (Scheme 1) Thio-glycosyl derivatives are chemically stable, so they don’t break during organic reactions. p-Aniliyl type termini in thio-glycosyl derivatives helps π-π stacking interaction because of its aromatic ring. We conjugated 1-pyrenebutyric acid to aniliyl termini through amide bond formation. Then, we deprotected acetyl groups in 2 and 5 under the Zemplen condition to generate glyco-pyrene derivatives 3 and 6.

4.2.2 Conjugation of Glyco-Pyrene Derivatives to Graphene-Based FET

Graphene-based FET was incubated by solution of in methanol (2 g/L) at RT. After 12 h, excess glyco-pyrene derivatives were washed by methanol and graphene-based FET was dried.

4.3 Characterization of Graphene-Based Biosensor

4.3.1 Characterization of Glyco-Pyrene Derivatives

We investigated the chemical structure of glyco-pyrene derivatives 3 and 6 through $^1$H and $^{13}$C nuclear magnetic resonance (NMR) and mass spectrometry (MS) analysis (Figure 5.1). We confirmed the presence of pyrene moiety by observing aromatic C-H peaks at 7.5-8.4 ppm. Additionally, we found characteristic mannosyl and galactosyl peaks at 3.0-5.0ppm. The detailed chemical structure of glyco-pyrene derivatives 3 and 6 were characterized by two-dimensional NMR spectroscopy such as correlation spectroscopy (COSY) and heteronuclear single quantum coherence spectroscopy (HSQC) (figure 6.15-6.20 in Experimental 6.4).
Figure 4.1. $^1$H NMR (600 MHz, DMSO-$d_6$) spectra of glyco-pyrene derivatives 3 (a) and 6 (b).
4.3.2 Characterization of Non-Covalent $\pi-\pi$ Stacking Interaction

4.3.2.1 UV-Vis Spectroscopy

We confirmed non-covalent $\pi-\pi$ stacking interaction between mannosyl-pyrene derivatives 3 and graphene-based FET by UV-Vis spectroscopy (Figure 5.2a). UV-Vis spectra of mannosyl pyrene 3 shows characteristic pyrene peaks at 335 and 351 nm. However, after non-covalent modification of graphene with mannosyl pyrene 3, each pyrene peaks were shifted to 332 and 348 nm, respectively. These data indicate that the non-covalent interaction between pyrene moieties and graphene affects UV-Vis spectra of mannosyl pyrene-graphene.

4.3.2.2 Raman Spectroscopy

We investigated Raman spectra of pristine graphene and graphene non-covalently modified with mannosyl pyrene 3. The G band in pristine graphene was observed at 1588 cm$^{-1}$. After incubating the graphene with mannosyl-pyrene derivatives, the G band was found at 1592 cm$^{-1}$ with slight blue-shift. This shift suggests that charge-transfer interaction occur between graphene and pyrene moieties as previous researches demonstrated.$^{67, 68}$

![Figure 4.2](image.png)

*Figure 4.2.* UV-Vis spectra (c) and Raman spectra (d) of mannosyl-pyrene derivative 3.
4.3.2.3 Atomic Force Microscopy

Atomic force microscopy (AFM) was used to confirm the non-covalent binding between graphene and mannosyl-pyrene derivatives. AFM images of pristine graphene film and graphene film incubated with mannosyl-pyrene derivative 3 reveal that the thickness of pristine graphene film was ~4 nm while that of graphene film incubated with mannosyl-pyrene derivative 3 was ~5 nm with some noises (figure 4.2).

![AFM images](image1)

**Figure 4.3.** AFM images and the height profiles of pristine graphene (a, c) and graphene incubated with mannosyl-pyrene derivative 3 (b, d), respectively.
4.4 Characterization of Lectin Recognition

We investigated current-voltage characteristics of graphene device incubated by glycol-pyrene derivatives followed by Concanavalin A (Con A) to confirm selective interaction between mannose and Con A. When Con A was incubated on mannosyl-pyrene-coated graphene device, $I_D$-$V_G$ curve shifted (Figure 4.3 a) while no change occurred in $I_D$-$V_G$ curve of galactosyl-pyrene-coated graphene device (Figure 4.3 b). To demonstrate reversible binding of con A and mannose, we flew con A and excess mannose alternately to graphene device. In the case of the mannosyl-pyrene-coated graphene device, there was $I_D$-$V_G$ curve shift as con A was bound and repeatedly detached, but there was no change in the galactosyl-pyrene-coated graphene device (Figure 4.3 c). These data suggest that Con A interacts to only mannose specifically.

![Figure 4.4](image-url)

**Figure 4.4.** $I_D$-$V_G$ curve of graphene-based biosensor. (a) After incubation of mannosyl-pyrene derivative 3 and Concanavalin A on graphene. (b) After incubation of galactosyl-pyrene derivative 6 and Concanavalin A on graphene (negative control). (c) Repeated binding and detaching of Concanavalin A on graphene device by alternate flow of Concanavalin A solution and excess amount of D-mannose.
4.5 Conclusions

In this research, we synthesize glyco-pyrene derivatives to conjugate graphene device for graphene-based FET style biosensor. Glyco-pyrene derivatives were bound to graphene through non-covalent π-π stacking interaction. Chemical structure of glycol-pyrene derivatives were confirmed by \(^1\)H and \(^{13}\)C nuclear magnetic resonance (NMR) and mass spectrometry (MS). UV-Vis, Raman, and atomic force microscopic analysis (AFM) demonstrate non-covalent binding between mannosyl-pyrene derivatives and graphene device. But, it is needed to investigate loading concentration of glycol-derivatives and concanavalin A on graphene. From controlling loading concentration of carbohydrate and protein, we can investigate how concentration affects the electrical characteristic of graphene-based biosensor.
Chapter 5. Protein Cage-based Nanoparticles for delivery vehicles

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Collaborations: All the biological analysis of HHFns was done by Young Ji Kang (Prof. Sebuyng Kang group)

5.1 Introduction & Background

5.1.1 HHFn: Protein-Caged Nanoplatforms

Human heavy chain ferritin (HHFn) is protein-caged nanoparticles consist composed of 24 identical subunits. Self-assembly of 24 subunits give hollow cage structure to HHFn with outer diameter 12 mm and inner diameter 8 mm.\(^6^9\) Previously many researchers have studied an application of HHFn as biomimetic nanoscale vehicles for targeted delivery.\(^4^1\)\(^-\)\(^4^6\) HHFn with uniform characteristics is easily reproduced due to simple control of sizes, and shapes.\(^6^9\) Additionally, inner and outer surface modification give HHFn many advantageous characteristics for targeted delivery.\(^1^3\)\(^-\)\(^2^4\)

5.1.2 β-Cyclodextrins

β-Cyclodextrins (β-CDs) is 7-membered ring carbohydrates. β-Cyclodextrin is used for encapsulating and delivering non-polar molecules in aqueous condition due to different polarity of exterior and interior surfaces. On the exterior surface of β-CD, a number of hydroxyl groups exist. These hydroxyl groups make β-CD soluble in water. On that contrary, the interior surface of β-CD is hydrophobic. These characteristics make reversible binding and releasing non-covalently between β-CD and non-polar molecules.\(^7^0\),\(^7^1\)
5.1.3 Click Chemistry

Protein cage-based nanoparticles (PCNs) are used for targeted delivery of biomolecules. These biomolecules are attached to surface of PCNs via covalent linkages. But most of these linkages are easily broken down in living organisms.\textsuperscript{13,21} Copper (I) catalyzed azide-alkyne cycloaddition (CuAAC) so-called click chemistry is widely used in a biological system because of its bioorthogonality and chemical stability in aqueous condition. Azide and alkyne pairs react each other in mild and aqueous conditions, giving high yield of products.\textsuperscript{72,73} For these novel characteristics of click chemistry, it has the potential for application of drug delivery and discovery in medicinal chemistry.\textsuperscript{74}

5.1.4 Research Objectives

In this research, we utilized human heavy chain ferritin (HHFn) as a nanoplatform for targeted delivery. Each subunit of HHFn has one cysteine moiety on their surface. We conjugate β-cyclodextrins (β-CDs) to thiol groups in cysteines through CuAAC and subsequent Michael addition. (Scheme 5.1) Finally, 24 β-cyclodextrins are adhered on the surface of single cage structure of HHFn. We investigate the chemical structure of β-CD-modified HHFns by mass spectrometry. We demonstrate reversible inclusion and release of fluorescein isothiocyanate-conjugated adamantane (FITC-AD) in β-CDs through UV-Vis spectrometry.
Scheme 5.1. Synthetic process of β-CD-C90 HHFn/FITC-AD Inclusion Complex. β-CDs are uniformly presented on the surface of C90 HHFn through Michael addition and CuAAC. The inclusion complex is formed with FITC-AD. Reproduced from [39].
5.2 Synthesis of β-CD-C90 HHFn/FITC-AD Inclusion Complex

5.2.1 Synthesis of β-CD-N₃

β-Cyclodextrin-hydrate was treated with 1-(p-toluenesulfonyl)imidazole for selective tosylation of hydroxyl groups. Subsequently, sodium methoxide was added to the reaction mixture to generate Mono-6-(p-toluenesulfonyl)-6-deoxy-β-cyclodextrin (β-CD-Ts) 1. After completion of the reactants, ammonium chloride was added to quench the reaction. To substitute tosyl group with the azide group, β-CD-Ts 1 was reacted with sodium azide. The reaction mixture was concentrated and treated with 1,1,2,2-tetrachloroethane. Centrifugation and recrystallization were used to purify mono-6-azide-deoxy-6-β-cyclodextrins (β-CD-N₃) 2. We confirmed the molecular structure of β-CD-Ts and β-CD-N₃ by ¹H and ¹³C nuclear magnetic resonance (NMR) spectrometry and mass spectrometry (Figure 6.22 in Experimental 6.5). Purity of β-CD-Ts and β-CD-N₃ were investigated by thin layer chromatography (TLC). Each sample of β-CD, β-CD-Ts, and β-CD-N₃ showed quite pure spot in silica plate with a few impurity. (Figure 5.1)

Scheme 5.2. Synthesis of Mono-6-azide-deoxy-6-β-cyclodextrin (β-CD-N₃) 2. (a) 1-(p-toluenesulfonyl)imidazole, NaOH, 60 °C; (b) NaN₃, 100 °C. Reproduced from [39].
Figure 5.1. Thin layer chromatography (TLC) analysis of β-CD, β-CD-Ts, and β-CD-N₃. Running solvent consists of iso-propanol, water, ethanol, ammonium hydroxide with the ratio of 5:3:1:1. Reproduced from [39].
5.2.2 Synthesis of N-Propargyl 3-maleimido-propionamide (PMP)

Maleic anhydride was treated with β-alanine at RT to generate 3-Maleimido propionic acid 1. 3-Maleimido propionic acid 3 was reacted to N-hydroxysuccinimide (NHS) ester with DCC. In this process, carboxyl acid in Maleimido propionic acid 3 reacted to the primary amine in NHS ester to form amide bond in succinimido 3-maleimido-propionate 4. The NHS group in succinimido 3-maleimido-propionate 4 was substituted with propargylamine to generate N-Propargyl 3-maleimido-propionamide (PMP) 5. The structure of PMP was confirmed by $^1$H and $^{13}$C nuclear magnetic resonance (NMR) spectrometry and mass spectrometry. (Figure 6.21)

Scheme 5.3. Synthesis of N-Propargyl 3-maleimido-propionamide (PMP) 5. (a) β-alanine, RT ; (b) DCC, NHS, 0°C→RT, 50%; (c) propargylamine, 0°C→RT, 6%. Reproduced from [39].

5.2.3 Synthesis of β-CD-C90 HHFn

Originally, each subunit of wild-type HHFn had three cysteine moieties (C90, C102, and C130) on the exterior surface. Two cysteines (C102, C130) were substituted by serine moieties (C102S, C130S) to prevent steric hindrance in conjugation between HHFn and β-CDs. Therefore, only one cysteine moiety (C90) reacted to β-CDs. In order to conjugate β-CD to C90 HHFn on the precise position, C90 HHFn was treated with N-propargyl-3-maleimidopropionamide (PMP). Maleimide group in PMP reacted to C90 cysteine group through maleimide-thiol Michael-type addition. Finally, Alkyne group in C90 HHFn was reacted to β-CD-N$_3$ with tris(3hydroxypropyltriazolylmethyl)amine (THPTA), a Cu ligand by copper(I) catalyzed azide-alkyne cycloaddition (CuAAC) to form β-CD-C90 HHFn. (Scheme 5.1)
5.2.4 Synthesis of β-CD-C90 HHFn/FITC-AD Inclusion Complex

Since β-CD can encapsulate small hydrophobic molecules in its interior cavity, β-CD-C90 HHFn has a potential for application in targeted delivery nanoplatform.\textsuperscript{70,71} We synthesized inclusion complex of β-CD-C90 with guest molecules. FITC-AD was chosen as guest molecules due to its fluorescence and non-covalent interaction to β-CD. To synthesize FITC-AD, we conjugate 1- adamantylamine to fluorescein isothiocyanate (FITC) with a mild base. (Scheme 5.4).\textsuperscript{78} In FTIC-AD, adamantyl group non-covalently interacts to β-CD with a high association constant (105L mol\textsuperscript{-1}) and FITC group emits green fluorescent light to track the guest molecules.\textsuperscript{78} β-CD-C90 HHFn was treated with FITC-AD to form FITC-AD/ β-CD/C90 HHFn inclusion complexes (Scheme 5.1).

![Scheme 5.4. Synthesis of FITC-AD conjugate. Reproduced from [39].]
5.3 Characterization of β-CD-C90 HHFn/FITC-AD Inclusion Complex

5.3.1 Characterization of β-CD-C90 HHFn

The formation of 1,2,3-triazole ring between HHFn subunit and β-CD-N₃ was investigated by electrospray ionization-time of flight mass spectrometry (ESI-MS TOF) (Figure 5.2A). The charge state affects to measure molecular mass of proteins. But in this case, the change of charge state was small enough to ignore. We assume that the charge distribution of chemically modified HHFn and unmodified HHFn were nearly same. The molecular masses of each subunit of unmodified C90 HHFn was 21064.0 Da, which corresponded to the calculated value (21062.3 Da). After treating PMP 5 to C90 HHFn, the molecular mass increased by 206.0 Da. (Figure 5.2 middle). Subsequently, after β-CD addition, the molecular mass increased by 159.0 Da. These data showed that all the C90 HHFn subunits were conjugated to PMP 5, and reacted to β-CD-N₃ to form 1,2,3-triazole ring via CuAAC. The cage-like structure of β-CD-C90 HHFn were confirmed by transmission electron microscopy (TEM) (Figure 5.3A). TEM image showed outer diameter of β-CD-C90 HHFn was 13 nm. We investigate the mean hydrodynamic diameter of β-CD-C90 HHFn by dynamic light scattering (DLS). The mean hydrodynamic diameter of β-CD-C90 HHFn was 15.6 mm, which is longer than that of unmodified C90 HHFn (12 mm). From these TEM images and dynamic light scattering data, it seems that β-CD-C90 HHFn retain cage-like structure after conjugation of maleimide linker and CuAAC reactions.
Figure 5.2. Molecular mass analyses of subunits of β-CD-N₃-clicked C90 HHFn (top), PMP-treated (middle), and C90 HHFn (bottom). Reproduced from [39].

Figure 5.3. (A) TEM image of β-CD-C90 HHFn stained with 2% uranyl acetate. (B) DLS measurement of β-CD-C90 HHFn (mean diameter: 15.6 nm). Reproduced from [39].
5.3.2 Characterization of β-CD-C90 HHFn/FITC-AD Inclusion Complex

We investigated non-covalent interaction between FITC-AD and β-CD-C90 HHFn by UV-Vis spectrometry. We treated FITC-AD to β-CD-C90 HHFn and C90 HHFn at RT and remove the excess FITC-AD by PD-10 desalting columns. C90 HHFn was used as negative controls because they do not interact to FITC-AD. After purification, FITC-AD-treated β-CD-C90 HHFn shows green color, while FITC-AD-treated C90 HHFn shows no color (figure 5.4B). In the UV/Vis spectra of FITC-AD-treated β-CD-C90 HHFn, characteristic fluorescein peaks were observed at 495 nm. (Figure 5.4A solid line) But, in case of C90 HHFn, there was no peak observed at 495 nm. (Figure 5.4A dashed line). These data indicates that β-CDs on the surface of C90 HHFn encapsulate FITC-AD and β-CD-C90 HHFn/FITC-AD inclusion complex are successfully formed. To investigate the deformation of FITC-AD/β-CD-C90 HHFn inclusion complexes, we checked the change of absorbance of FITC-AD peak by UV/Vis spectrophotometer during dialysis. FITC-AD/β-CD-C90 HHFn inclusion complex was dialyzed at RT. We collected and analyzed the sample at varying times. As FITC-AD released, absorbance of FITC-AD decreases indicating that FITC-AD/β-CD-C90 HHFn inclusion complex was decomposed (Figure 5.5A). The release of FITC-AD was getting slower showing exponential decay curve. (Figure 5.5B) These results indicate that β-CD-C90 HHFn can release FITC-AD reversibly as well as encapsulate FITC-AD and FITC-AD/β-CD-C90 HHFn have a potential for application of nanoplatform for targeted delivery.
Figure 5.4. (A) UV/Vis spectra of FITC-AD-treated β-CD-C90 HHFn (solid line) and C90 HHFn (dashed line) (B) PD-10 desalting column elutes of FITC-AD treated C90 HHFn (left) and β-CD-C90 HHFn (right). Reproduced from [39].

Figure 5.5. (A) UV/Vis spectra FITC-AD from the FITC-AD/β-CD-C90 HHFn inclusion complexes during dialysis. Samples were measured at 0, 10, 20, and 40 min, 1, 2, 4, 9, 13, and 22 h. (B) Release profile of FITC-AD. Reproduced from [39].
5.4 Conclusions

We used β-CD-C90 HHFn as a nanoplatform for delivery of insoluble molecules. β-CD was conjugated to C90 HHFn through Michael-type addition followed by CuAAC. β-CD-C90 HHFn formed inclusion complex with FITC-AD, which was released reversibly. FITC-AD/β-CD-C90 HHFn inclusion complex showed capability of delivering FITC-AD for only short time. Since β-CD-C90 HHFn has additional site in the interior cavity, modifying internal cavity will be the solution to the time problem. In the future, we can conjugate β-CD to other types of protein cages to develop multifunctional protein cage-based nanoplatfroms for targeted delivery.
Chapter 6. Experimental

6.1 General

Chemical Characterizations

Proton nuclear magnetic resonance ($\delta_H$) spectra and carbon nuclear magnetic resonance ($\delta_C$) spectra were recorded by using an Agilent 400-MR DD2 spectrometer. Low resolution mass spectra were recorded on a Bruker HCT Basic System using electrospray ionization (ESI). High resolution mass spectra were recorded on a ABI API-3000 electrospray ionization mass spectrometer. Optical rotations were measured on a ATAGO AP-300 automatic polarimeter with a path length of 1 with expressing concentrations in g/100 mL. Thin layer chromatography (TLC) was carried out on Merck silica gel 60F$_{254}$. Visualization of the plates was achieved using an ultraviolet lamp (254 nm) and/or potassium permanganate (0.5% in 1M NaOH). Flash column chromatography was carried out using Merck silica gel 60 (0.040-0.063 mm).
6.2 Experimental for Chapter 2

6.2.1 Procedures

\((p\text{-Nitrobenzyl})\)-2,3,4,6-tetra-O-acetyl-1-thio-\(\alpha\)-D-mannopyranoside 2

To a solution of 2,3,4,6-tetra-O-acetyl-1-thio-\(\alpha\)-D-mannopyranose 1 (4.0 g, 11.0 mmol) and 4-nitrobenzyl bromide (4.7 g, 22.0 mmol) in DMF (anhydrous, 50 mL) was added TEA (2.3 mL). After stirring overnight at r.t., the reaction mixture was diluted with ethyl acetate (100 mL) and completely washed with 1M HCl (70 mL), DI water (70 mL), and brine (70 mL). The organic phase was dried over Na\(_2\)SO\(_4\), filtered. Filtrate was concentrated and purified by flash column chromatography (3:1 → 0:1, n-hexane: ethyl acetate) to afford the product as an amorphous solid (4.4 g, 81%). TLC (1:2, n-hexane: ethyl acetate) (\(R_f\) 0.7); \([\alpha]_D^{25} = +101.9^\circ\) (c = 0.5 in CHCl\(_3\)); \(^1\)H NMR (600 MHz, CDCl\(_3\)) \(\delta\) 8.19 (d, \(J = 8.6\) Hz, 2H), 7.49 (d, 2H), 5.32-5.25 (m, 3H), 5.01 (brs, 1H), 4.35-4.28 (m, 2H), 4.06 (dd, \(J = 12.2\) Hz, \(J = 2.3\)Hz, 1H), 3.86 (d, \(J = 13.9\) Hz, 1H), 3.80 (d, 1H) 2.12, 2.11, 2.05, 1.98 (4×s, 12H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 170.5, 169.8, 169.7, 147.3, 144.5, 130.0, 124.0, 81.0, 70.3, 69.5, 69.4, 66.2, 33.8, 20.9, 20.8, 20.7, 20.6; m/z (ESI+) calcd. for C\(_{21}\)H\(_{25}\)NO\(_{11}\)SNa [M+Na\(^+\)] 522.1, measured 522.2; HRMS (ESI+) calcd. for C\(_{21}\)H\(_{25}\)NO\(_{11}\)SNa [M+Na\(^+\)] 522.1046, measured 522.1041.

\((p\text{-Aminobenzyl})\)-2,3,4,6-tetra-O-acetyl-1-thio-\(\alpha\)-D-mannopyranoside 3

\((p\text{-Nitrobenzyl})\)-2,3,4,6-tetra-O-acetyl-1-thio-\(\alpha\)-D-mannopyranoside 2 (4.0 g, 8.0 mmol) and SnCl\(_2\)-2H\(_2\)O (9.7 g, 40.1 mmol) were dissolved in EtOH (160 mL). After stirring overnight at 70 °C under Ar, the reaction mixture was poured into ice/water (160 mL), and then pH was adjusted by sat. NaHCO\(_3\). The residue was extracted with ethyl acetate (200 mL ×2). The organic phase was dried over MgSO\(_4\), filtered. Filtrate was concentrated and purified by flash column chromatography (1:1 → 2:3, nhexane: ethyl acetate) to afford the product (1.6 g, 44%). TLC (1:2, n-hexane:ethyl acetate) (Rf 0.6); \([\alpha]_D^{25} = +51.0^\circ\) (c = 1.0 in CHCl\(_3\)); \(^1\)H NMR (600 MHz, CDCl\(_3\)) \(\delta\) 7.08 (d, \(J = 8.3\) Hz, 2H), 6.61 (d, 2H), 5.29-5.27 (m, 3H), 5.08 (brs, 1H), 4.40-4.37 (m, 1H), 4.31 (dd, \(J = 5.4\) Hz, \(J = 12.2\) Hz, 1H), 4.02 (dd, \(J = 2.2\) Hz, 1H), 3.71-3.64 (m, 4H), 2.11, 2.10, 2.03, 1.95 (4×s, 12H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 170.5, 169.9, 169.8, 144.9, 130.2, 127.0, 115.8, 81.2, 70.8, 69.9, 69.0, 66.5, 62.5, 34.4, 21.0, 20.9, 20.8, 20.7; m/z (ESI+) calcd. for C\(_{21}\)H\(_{25}\)NO\(_{9}\)SNa [M+Na\(^+\)] 492.1, measured 492.3; HRMS (ESI+) calcd. for C\(_{21}\)H\(_{25}\)NO\(_{9}\)SNa [M+Na\(^+\)] 492.1304, measured 492.1299.
(3-Maleimido-propionyl) (p-aminobenzyl)-2,3,4,6-tetra-O-acetyl-1-thio-α-D-mannopyranoside 4

Mannopyranoside 3 (50 mg, 0.11 mmol) and succinimido 3-maleimido-propionoate (34 mg, 0.13 mmol) were dissolved in DMF (anhydrous, 5 mL) under Ar. After stirring overnight at r.t, the reaction mixture was concentrated in vacuo and purified by flash column chromatography (1:1, n-hexane:ethyl acetate) to afford the product as a light yellow solid (50 mg, 73%). TLC (1:2, n-hexane:ethyl acetate) (Rf 0.3); \([\alpha]_D^{25} = +10.0^\circ (c = 1.0 \text{ in CHCl}_3); ^1H \text{ NMR} (600 MHz, CDCl}_3 \delta H 7.62 (s, 1H), 7.44 (d, 2H), 7.23 (d, 2H), 6.70 (s, 2H), 5.27 (m, 3H), 5.05 (brs, 1H), 4.33-4.36 (m, 1H), 4.27 (dd, J 5.3 Hz, J 12.2 Hz, 1H), 4.00 (dd, J 2.2 Hz, 1H), 3.91 (t, J 7.0 Hz, 2H), 3.74 (d, J 13.6 Hz, 1H), 3.68 (d, 1H), 3.65 (ddd, J 2.3 Hz, 5.0 Hz, 10.0 Hz, 1H), 2.10, 2.04, 2.02, 2.00 (4×s, 12H); ^13C \text{ NMR} (150 MHz, CDCl}_3 \delta C 170.7, 170.6, 169.9, 169.8, 137.3, 134.3, 132.4, 129.6, 120.3, 81.0, 70.6, 69.8, 69.0, 66.4, 62.5, 34.2, 34.1, 29.7, 20.9, 20.8, 20.7; m/z (ESI+) calcd. for C28H32N2O12SNa [M+Na]+ 643.2, measured 643.6.

(p-Nitrobenzyl)-2,3,4,6-tetra-O-acetyl-1-thio-β-D-galactopyranose 6

To a solution of 2,3,4,6-tetra-O-acetyl-1-thio-β-D-galactopyranose 5 (2.0 g, 5.5 mmol) and 4-nitrobenzyl bromide (2.4 g, 11.0 mmol) in DMF (anhydrous, 15 mL) was added TEA (1.2 mL). After stirring overnight at r.t, the reaction mixture was diluted with ethyl acetate (50 mL) and completely washed with 1M HCl (35 mL), D.I water (35 mL), brine (35 mL). The organic phase was dried over Na2SO4, filtered. The solution part was concentrated and purified by flash column chromatography (3:1, n-hexane:ethyl acetate) to afford the product as an amorphous solid (2.5 g, 90%). TLC (1:2, n-hexane:ethyl acetate) (Rf 0.7); \([\alpha]_D^{25} = -88.0^\circ (c = 1.0 \text{ in CHCl}_3); ^1H \text{ NMR} (600 MHz, CDCl}_3 \delta H 8.18 (d, J 8.6 Hz, 2H), 7.49 (d, 2H), 5.17 (at, J 9.4 Hz, 1H), 5.12-5.05 (m, 2H), 4.33 (d, 1H), 4.25-4.22 (m, 1H), 4.16-4.10 (m, 1H), 4.02 (d, J 13.1 Hz, 1H), 3.90 (d, 1H), 3.65 (ddd, J 2.3 Hz, 5.0 Hz, 10.0 Hz, 1H), 2.10, 2.04, 2.02, 2.00 (4×s, 12H); ^13C \text{ NMR} (100 MHz, CDCl}_3 \delta C 170.3, 169.9, 169.3, 147.1, 144.9, 129.9, 123.7, 81.7, 75.9, 73.5, 69.5, 68.2, 62.0, 32.7, 20.6, 20.5, 20.4; m/z (ESI+) calcd. for C21H23NO11SNa [M+Na]+ 522.1, measured 522.2; HRMS (ESI+) calcd. for C21H23NO11SNa [M+Na]+ 522.1046, measured 522.1045.
(p-Aminobenzyl)-2,3,4,6-tetra-O-acetyl-1-thio-β-D-galactopyranose 7

(p-Nitrobenzyl)-2,3,4,6-tetra-O-acetyl-1-thio-β-D-galactopyranose 6 (1.1 g, 2.1 mmol) and SnCl2· 2H2O (2.3 g, 10.3 mmol) were dissolved in EtOH (80 mL).

After stirring overnight at 70 °C under Ar, the reaction mixture was poured into ice/water (160 mL), and then pH was adjusted by sat. NaHCO3. The residue was extracted with ethyl acetate (250 mL × 2). The organic phase was dried over MgSO4, filtered. The solution part was concentrated and purified by flash column chromatography (1:1 → 2:3, n-hexane:ethyl acetate) to afford the product as a light yellow solid (499 mg, 50%).

TLC (1:2, n-hexane:ethyl acetate) (Rf 0.5); [α]25D = -53.3° (c = 1.2 in CHCl3);

1H NMR (600 MHz, CDCl3) δ H 7.07 (d, J 8.3 Hz, 2H), 6.62 (d, 2H), 5.39 (dd, J 3.5 Hz, J 1.2 Hz, 1H), 5.25 (at, J 10.0 Hz, 1H), 4.95 (dd, J 10.0 Hz, J 3.4 Hz, 1H), 4.26 (d, J 10.0 Hz, 1H), 4.16 (dd, J 6.7 Hz, J 11.4 Hz, 1H), 4.09-4.12 (m, 1H), 3.86 (d, J 12.8 Hz, 1H), 3.80-3.82 (m, 1H), 3.75 (d, 1H), 3.70 (s, 2H), 1.96, 2.01, 2.06, 2.14 (4×s, 12H);

13C NMR (100 MHz, CDCl3) δ C 170.6, 170.1, 169.4, 145.8, 130.0, 125.9, 115.1, 81.8, 75.6, 73.8, 69.8, 68.4, 62.2, 33.4, 20.7, 20.6, 20.5; m/z (ESI+) calcd. for C21H27NO9SNa [M+Na]+ 492.1, measured 492.3; HRMS (ESI+) calcd. for C21H25NO11SNa [M+H]+ 470.1485, measured 470.1482.

(3-Maleimido-propionyl) (p-aminobenzyl)-2,3,4,6-tetra-O-acetyl-1-thio-β-D-galactopyranose 8 (AGM)

Galactopyranose 7 (60 mg, 0.13 mmol) and succinimido 3-maleimido-propionoate (102 mg, 0.38 mmol) were dissolved in DMF (anhydrous, 1 mL) under Ar. To the reaction mixture were added DCM (0.5 mL) and DIPEA (262 μL). After stirring overnight at r.t, the solution part was concentrated in vacuo and purified by flash column chromatography (1:2, n-hexane:ethyl acetate) to afford the product (42 mg, 52%). TLC (1:2, n-hexane:ethyl acetate) (Rf 0.3); [α]25D = -266.0° (c = 1.0 in CHCl3);

1H NMR (600 MHz, CDCl3) δ H 7.53 (s, 1H), 7.46 (d, J 8.3 Hz, 2H), 7.25 (d, 2H), 6.72 (s, 2H), 5.41-5.40 (m, 1H), 5.26 (at, J 10.0 Hz, 1H), 4.96 (dd, J 3.3 Hz, J 10.0 Hz, 1H), 4.27 (d, 1H), 4.14 (dd, J 6.7 Hz, J 11.4 Hz, 1H), 4.08 (dd, J 6.5 Hz, 1H), 3.94-3.91 (m, 3H), 3.82-3.79 (m, 2H), 2.73 (t, J 7.0 Hz, 2H), 2.15, 2.07, 2.02, 1.96 (4×s, 12H);

13C NMR (100 MHz, CDCl3) δ C 170.6, 170.5, 170.3, 170.1, 169.7, 137.4, 134.3, 132.8, 129.7, 120.1, 82.4, 74.4, 71.9, 67.4, 67.2, 61.7, 34.2, 33.4, 29.7, 20.8, 20.7, 20.6; m/z (ESI+) calcd. for C21H25NO13Na [M+Na]+ 643.2, measured 643.4.
Figure 6.1. \(^1\)H (A) and \(^{13}\)C (B) NMR spectra of \((p\text{-Nitrobenzyl})\)\text{-2,3,4,6-tetra-O-acetyl-1-thio-} \alpha\text{-D-mannopyranoside 2.}
Figure 6.2. $^1$H (A) and $^{13}$C (B) NMR spectra of (p-Aminobenzyl)-2,3,4,6-tetra-O-acetyl-1-thio-α-D-mannopyranoside 3.
Figure 6.3. $^1$H (A) and $^{13}$C (B) NMR spectra of (3-Maleimido-propionyl) (p-aminobenzyl)-2,3,4,6-tetra-O-acetyl-1-thio-α-D-mannopyranoside 4 (AMM).
Figure 6.4. $^1$H (A) and $^{13}$C (B) NMR spectra of (p-Nitrobenzyl)-2,3,4,6-tetra-O-acetyl-1-thio-β-D-galactopyranose 6.
Figure 6.5. $^1$H (A) and $^{13}$C (B) NMR spectra of (p-Aminobenzyl)-2,3,4,6-tetra-O-acetyl-1-thio-β-D-galactopyranose 7.
Figure 6.6. $^1$H (A) and $^{13}$C (B) NMR spectra of (3-Maleimido-propionyl) (p-aminobenzyl)-2,3,4,6-tetra-O-acetyl-1-thio-β-D-galactopyranose 8 (AGM).
6.3 Experimental for Chapter 3

6.3.1 Procedures

*Exo*-3,4,6-tri-*O*-acetyl-1,2-*O*-(1-methoxyethylidene)-β-D-mannopyanoside 3

2,6-Lutidine (4.2 mL, 36.5 mmol) and anhydrous methanol (2.47 mL, 60.8 mmol) were added to 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide (5g, 12.1 mmol) in anhydrous DCM (20 mL) and the reaction mixture was heated to reflux at 40°C. After stirring for 16 h, t.l.c. (1:1, n-hexane:ethyl acetate) indicated formation of a product (R<sub>f</sub> 0.6) with complete consumption of the starting material (R<sub>f</sub> 0.7). Water (150 mL) was added to the reaction mixture which was then extracted with DCM (150 mL × 2). The organic layer was washed with brine (100 mL × 2), dried over MgSO<sub>4</sub>, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (1:2, n-hexane:ethyl acetate) to afford the product as a white crystalline solid (2.8 g, 63%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400MHz) δ<sub>H</sub> 1.72 (s, 3H), 2.03 (s, 3H), 2.05 (s, 3H), 2.10 (s, 3H), 3.26 (s, 3H), 3.67 (ddd, J = 9.5, 4.9, 2.7 Hz, 1H), 4.12 (dd, J = 12.1, 2.7 Hz, 1H), 4.22 (dd, J = 12.1, 4.9 Hz, 1H), 460 (dd, J = 4.0, 2.6 Hz, 1H), 5.13 (dd, J = 9.9, 4.0 Hz, 1H), 5.28 (t, J = 9.7 Hz, 1H), 5.48 (d, J = 2.6 Hz, 1H).

*Exo*-1,2-*O*-(1-methoxyethylidene)-β-D-mannopyanoside 4

*Exo*-3,4,6-tri-*O*-acetyl-1,2-*O*-(1-methoxyethylidene)-β-D-mannopyanoside 3 (1 g, 2.8 mmol) and sodium methoxide (catalytic amount, in situ) were added to dry methanol (20 mL). The reaction mixture was stirred under argon at RT. After 12 h, Amberlite<sup>®</sup> IR 120 hydrogen form washed by MeOH was added to the reaction mixture to adjust pH to 7. Then the reaction mixture was filtered, and concentrated in vacuo to afford the product as a white solid (490 mg, 75%). m/z (ESI+) calcd. for C<sub>9</sub>H<sub>16</sub>O<sub>5</sub>Na [M+Na]<sup>+</sup> 259.1, measured 259.1.
Exo-3,4,6-tri-O-benzyl-1,2-O-(1-methoxyethylidene)-β-D-mannopyranoside 5

Benzylbromide (16.1 mL, 135.5 mmol) was added dropwise to a solution of Exo-1,2-O-(1-methoxyethylidene)-β-D-mannopyranoside (3.2 g, 13.6 mmol) 4 and sodium hydride (60% dispersion in mineral oil, 227 mg) in anhydrous DMF (100 mL) and stirred under argon. After 16 h, t.l.c (1:1, n-hexane:ethyl acetate) indicated formation of a product (R_f 0.8) with complete consumption of the starting material (R_f 0.0). Methanol (100 mL) was added to quench the reaction mixture, which was then concentrated in vacuo. The residue was suspended in water (200 mL) and extracted with diethyl ether (150 mL × 2). The combined organic layers were washed with brine (100 mL), dried over MgSO_4, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (3:1, n-hexane:ethyl acetate) to afford Exo-3,4,6-tri-O-benzyl-1,2-O-(1-methoxyethylidene)-β-D-mannopyranoside 5 (4.3 g, 63%).

1H NMR (400 MHz, CDCl_3) δ_H 1.78 (s, 3H), 3.32 (s, 3H), 3.45 (ddd, J = 9.4, 4.4, 2.3 Hz, 1H), 3.72 – 3.79 (m, 3H), 3.97 (t, J = 9.3 Hz, 1H), 4.43 (dd, J = 4.0, 2.6 Hz, 1H), 4.54 – 4.67 (m, 3H), 4.82 (d, J = 5.6 Hz, 2H), 4.94 (d, J = 10.8 Hz, 1H), 5.37 (d, J = 2.5 Hz, 1H), 7.24 – 7.47 (m, 15H) ; m/z (ESI+) calcd. for C_30H_34O_7Na [M+Na]^+ 529.2, measured 529.3.

1,2-Di-O-acetyl-3,4,6-tri-O-benzyl-D-mannopyranose 6

Exo-3,4,6-tri-O-benzyl-1,2-O-(1-methoxyethylidene)-β-D-mannopyranoside 5 (2 g, 3.9 mmol) was stirred in water (20 mL) and acetic acid (10 mL). After 4 h, t.l.c (1:1, ethyl acetate: n-hexane) indicated formation of two products (R_f 0.5, 0.6) with complete consumption of the starting material (R_f 0.8). The reaction mixture was diluted with ethyl acetate (100 mL) and the layers separated. The aqueous layer was extracted with ethyl acetate (50 mL × 2), and the combined organic layers washed with sodium hydrogen carbonate (100 ML of a saturated aqueous solution), dried over MgSO_4, filtered and concentrated in vacuo. The residue was coevaporated with toluene and dried in vacuo. The residue was suspended in pyridine (20 mL), cooled to 0°C, and acetic anhydride (10 mL) was added dropwise. The reaction mixture was allowed to warm to room temperature. After 16 h, t.l.c (1:1, ethyl acetate: n-hexane) indicated formation of a product (R_f 0.8) with complete consumption of the starting materials (R_f 0.5, 0.6). The reaction mixture was concentrated in vacuo to afford 1,2-Di-O-acetyl-3,4,6-tri-O-benzyl-D-mannopyranose 6 (α: β, 1:2) (1.7 g, 80%).

1H NMR (400 MHz, CDCl_3) δ_H 2.10 (s, 3H), 2.12 (s, 3H), 2.20 (s, 3H), 2.25 (s, 3H), 3.61 (ddd, J = 9.7, 4.1, 2.2 Hz, 1H), 3.70 – 3.94 (m, 7H), 4.00 – 4.05 (m, 2H), 4.50 – 4.62 (m, 6H), 4.68 – 4.79 (m, 4H), 4.88 – 4.93 (m, 2H), 5.41 (t, J = 2.4 Hz, 1H), 5.66 (dd, J = 3.2, 1.0 Hz, 1H), 5.78 (d, J = 1.1 Hz, 1H), 6.17 (d, J = 2.0 Hz, 1H), 7.30 – 7.40 (m, 15H) ; m/z (ESI+) calcd. for C_{31}H_{35}O_8Na [M+Na]^+ 557.2, measured 557.3.
2-\textit{O}-Acetyl-3,4,6-tri-\textit{O}-benzyl-\textalpha-D-mannopyranose 7

Benzylamine (0.7 mL, 6.4 mmol) was added to a solution of 1,2-di-\textit{O}-acetyl-3,4,6-tri-\textit{O}-benzyl-D-mannopyranose 6 (1.7 g, 3.2 mmol) in THF (30 mL). After 24 h, t.l.c. (2:1, n-hexane:ethyl acetate) indicated formation of a product (R<sub>f</sub> 0.3) with complete consumption of the starting material (R<sub>f</sub> 0.6). The reaction mixture was concentrated in vacuo, dissolved in ethyl acetate (300 mL) and washed with ice cold hydrochloric acid (100 mL of a 1 M aqueous solution) and sodium hydrogen carbonate (100 mL of a saturated aqueous solution). The organic layer was dried over MgSO<sub>4</sub>, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (1:2,ethyl acetate: n-hexane) to afford 2-\textit{O}-Acetyl-3,4,6-tri-\textit{O}-benzyl-\textalpha-D-mannopyranose 7 (1.2 g, 78.5%)<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ<sub>H</sub> 2.15 (s, 3H), 3.74 – 3.68 (m, 3H), 3.76 (d, <i>J</i> = 6.3 Hz, 1H), 4.04 (dd, <i>J</i> = 9.3, 3.3 Hz, 2H), 4.47 (d, <i>J</i> = 10.9 Hz, 1H), 4.51 (d, <i>J</i> = 9.1 Hz, 1H), 4.54 (d, <i>J</i> = 8.1 Hz, 1H), 4.61 (d, <i>J</i> = 12.1 Hz, 1H), 4.71 (d, <i>J</i> = 11.2 Hz, 1H), 4.87 (d, <i>J</i> = 10.9 Hz, 1H), 5.21 (d, <i>J</i> = 1.0 Hz, 1H), 5.37 (dd, <i>J</i> = 3.3, 1.9 Hz, 1H), 7.24 – 7.39 (m, 15H) ; m/z (ESI+) calcd. for C<sub>29</sub>H<sub>32</sub>O<sub>7</sub>Na [M+Na]<sup>+</sup> 515.2, measured 515.4

2-\textit{O}-Acetyl-3,4,6-tri-\textit{O}-benzyl-\textalpha-D-mannopyranosyl trichloroacetimidate 8

2-\textit{O}-Acetyl-3,4,6-tri-\textit{O}-benzyl-\textalpha-D-mannopyranose 7 (400 mg, 0.81 mmol) and trichloroacetonitrile (812 μL, 8.1 mmol) were stirred in anhydrous DCM (20 mL) with 4 Å molecular sieves for 30 min. 1,8-Diazabicyclo[5,4,0]undec-7ene (6 μL, 0.04 mmol) was added and the reaction mixture stirred under argon at RT. After 16 h, t.l.c. (1:1, ethyl acetate: n-hexane), indicated formation of a product (R<sub>f</sub> 0.7) with complete consumption of the starting material (R<sub>f</sub> 0.4). Triethylamine (1 mL) was added and the reaction mixture filtered through celite and concentrated in vacuo. The residue was purified by flash column chromatography (1:1, ethyl acetate: n-hexane) to afford 2-\textit{O}-Acetyl-3,4,6-tri-\textit{O}-benzyl-\textalpha-D-mannopyranosyl trichloroacetimidate 8 (330 mg, 64%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ<sub>H</sub> 2.20 (s, 3H), 3.73 (dd, <i>J</i> = 11.2, 1.7 Hz, 1H), 3.86 (dd, <i>J</i> = 11.2, 3.6 Hz, 1H), 3.97 – 4.16 (m, 3H), 4.53 (dd, <i>J</i> = 12.3, 9.7 Hz, 2H), 4.60 (d, <i>J</i> = 11.2 Hz, 1H), 4.70 (d, <i>J</i> = 12.0 Hz, 1H), 4.75 (d, <i>J</i> = 11.2 Hz, 1H), 4.89 (d, <i>J</i> = 10.6 Hz, 1H), 5.51 (t, <i>J</i> = 2.3 Hz, 1H), 6.32 (d, <i>J</i> = 1.9 Hz, 1H), 7.15 – 7.43 (m, 15H), 8.70 (s, 1H).
2’-azidoethyl-2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranoside 9

2'-azidoethyl-2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl trichloroacetimidate 8 (1 g, 1.57 mmol) and 2-azidoethanol (150 mg, 1.73 mmol) were stirred in anhydrous DCM (20 mL) with 4 Å molecular sieves for 30 min. Trimethylsilyl trifluoromethanesulfonate (100 μL) was added dropwise and the reaction mixture stirred under argon at RT. After 8 h, the reaction mixture was quenched by Triethylamine (100 μL) for 30 min. The reaction mixture was filtered and concentrated in vacuo. The residue was purified by flash column chromatography (1:4, ethyl acetate: n-hexane) to afford 2’-azidoethyl-2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranoside 9 (710 mg, 80.5%). 1H NMR (400 MHz, CDCl3) δH 2.16 (s, 3H), 3.39 (ddd, J = 5.7, 4.3, 1.3 Hz, 2H), 3.62 (ddd, J = 10.5, 5.8, 4.6 Hz, 1H), 3.69 – 3.74 (m, 1H), 3.77 – 3.82 (m, 1H), 3.82 – 3.85 (m, 1H), 3.85 – 3.88 (m, 1H), 3.90 (dd, J = 7.5, 3.3 Hz, 1H), 4.02 (dd, J = 8.9, 3.4 Hz, 1H), 4.48 (d, J = 10.8 Hz, 1H), 4.52 (d, 8.0 Hz, 1H), 4.54 (d, J = 7.1 Hz, 1H), 4.67 (d, J = 10.4 Hz, 1H), 4.70 (d, J = 9.5 Hz, 1H), 4.87 (d, J = 10.8 Hz, 1H), 4.90 (d, J = 1.8 Hz, 1H), 5.39 (dd, J = 3.4, 1.9 Hz 1H), 7.08 – 7.47 (m, 15H) ; m/z (ESI+) calcd. for C31H35O7N3Na [M+Na]+ 584.2, measured 584.4.

2'-azidoethyl-3,4,6-tri-O-benzyl-α-D-mannopyranoside 10

2’-azidoethyl-2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranoside 9 (380 mg, 0.67 mmol) and sodium methoxide (catalytic amount, in situ) were added to anhydrous methanol (10 mL). The reaction mixture was stirred under argon at RT. After 12 h, Amberlite® IR 120 hydrogen form washed by MeOH was added to the reaction mixture to adjust pH to 7. Then the reaction mixture was filtered, and concentrated in vacuo to afford 2’-azidoethyl-3,4,6-tri-O-benzyl-α-D-mannopyranoside 10 (352 mg, 100%, quantitative yield). 1H NMR (400 MHz, CD3OD) δH 3.29 (dt, J = 3.3, 1.6 Hz, 1H), 3.36 (dt, J = 5.8, 3.6 Hz, 2H), 3.59 (ddd, J = 8.7, 6.0, 4.0 Hz, 1H), 3.66 (t, J = 3.1 Hz, 2H), 3.75 (ddd, J = 9.7, 6.4, 3.7 Hz, 1H), 3.88 – 3.79 (m, 3H), 4.04 (t, J = 2.0 Hz, 1H), 4.47 (dd, J = 11.4, 9.7 Hz, 2H), 4.55 (dd, J = 11.8, 4.4 Hz, 2H), 4.70 (d, J = 11.7 Hz, 1H), 4.79 (d, J = 11.0 Hz, 1H), 7.41 – 7.13 (m, 15H) ; m/z (ESI+) calcd. for C29H33O6N3Na [M+Na]+ 542.2, measured 542.5.
2'-azidoethyl-2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-manno-pyranoside 11

2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl trichloroacetimidate 8 (1.9 g, 3.00 mmol) and 2'-azidoethyl-3,4,6-tri-O-benzyl-α-D-mannopyranoside 10 (1.3 g, 2.5 mmol) were stirred in anhydrous DCM (20 mL) with 4 Å molecular sieves for 30 min. Trimethylsilyl trifluromethanesulfonate (200 μL) was added dropwise and the reaction mixture stirred under argon at RT. After 9 h, the reaction mixture was quenched with triethylamine (200 μL). After 30 min, the reaction mixture was filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (1:4, ethyl acetate: n-hexane) to afford 2'-azidoethyl-2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-manno-pyranoside 11 (1.2 g, 48.3%); m/z (ESI+) calcd. for C₅₈H₆₃O₁₂Na [M+Na]+ 1016.4, measured 1016.5.

2-aminoethyl-α-D-mannopyranosyl-(1→2)α-D-mannopyranoside 12

2'-azidoethyl-2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-manno-pyranoside 11 (300 mg, 0.30 mmol) and sodium methoxide (catalytic amount, in situ) were added to anhydrous methanol (10 mL). The reaction mixture was stirred under argon at RT. After 12 h, Amberlite® IR 120 hydrogen form washed by MeOH was added to the reaction mixture to adjust pH to 7. Then the reaction mixture was filtered, and concentrated in vacuo. The residue was dissolved in MeOH (20 mL). Palladium on carbon (5 wt. %) (500 mg) was added to the solution. The reaction mixture was flushed with hydrogen five times and stirred at RT under an atmosphere of hydrogen. The reaction mixture was filtered through celite® and concentrated in vacuo to afford 2-aminoethyl-α-D-mannopyranosyl-(1→2)α-D-mannopyranoside 12 (89%). ¹H NMR (400 MHz, CD₃OD) δ 5.13 (s, 1H), 4.99 (s, 1H), 4.01 – 3.83 (m, 6H), 3.76 – 3.60 (m, 6H), 3.60 – 3.49 (m, 3H), 3.32 – 3.29 (m, 1H), 3.19 (s, 2H); m/z (ESI+) calcd. for C₁₄H₂₈O₁₁N [M+H]+ 386.2, measured 386.2.
6.3.2 Supporting Figures

Figure 6.7. $^1$H NMR spectra of Exo-3,4,6-tri-O-acetyl-1,2-O-(1-methoxyethylidene)-β-D-mannopyranoside 3.

Figure 6.8. $^1$H NMR spectra of Exo-3,4,6-tri-O-benzyl-1,2-O-(1-methoxyethylidene)-β-D-mannopyranoside 5.
Figure 6.9. $^1$H NMR spectra of 1,2-Di-$O$-acetyl-3,4,6-tri-$O$-benzyl-D-mannopyranose 6.

Figure 6.10. $^1$H NMR spectra of 2-$O$-Acetyl-3,4,6-tri-$O$-benzyl-$\alpha$-D-mannopyranose 7.
Figure 6.11. $^1$H NMR spectra of 2-$O$-Acetyl-3,4,6-tri-$O$-benzyl-$\alpha$-D-mannopyranosyl trichloroacetimidate 8.

Figure 6.12. $^1$H NMR spectra of 2'-azidoethyl-2-$O$-acetyl-3,4,6-tri-$O$-benzyl-$\alpha$-D-mannopyranoside 9.
Figure 6.13. $^1$H NMR spectra of 2'-azidoethyl-3,4,6-tri-$O$-benzyl-$\alpha$-D-mannopyranoside 10.

Figure 6.14. $^1$H NMR spectra of 2'-aminoethyl-$\alpha$-D-mannopyranosyl-(1$\rightarrow$2)$\alpha$-D-mannopyranoside 12.
6.4 Experimental for Chapter 4

6.4.1 Procedures

(4-Pyrenebutanoyl)(p-Aminobenzyl)-2,3,4,6-tetra-O-acetyl-1-thio-α-D-mannopyranoside 2

(p-Aminobenzyl)-2,3,4,6-tetra-O-acetyl-1-thio-α-D-mannopyranoside 1 (1000 mg, 2.1 mmol) and 1-pyrenebutyric acid (917 mg, 3.2 mmol) were added to dry DMF (30 mL). TBTU (1200 mg, 3.2 mmol) and triethylamine (590 µL, 4.2 mmol) were added to the mixture. The reaction mixture was stirred under argon at RT. After stirring for 12 h, the reaction mixture was diluted with DCM (100 mL). The mixture was washed with 1M HCl (50 mL) and brine (50 mL). The aqueous layer was extracted with dichloromethane (50 mL × 3). The combined organic layer was dried over MgSO₄, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (3:4, n-hexane:ethyl acetate) to afford the product as a light brown solid (1289 mg, 82%). m.p.: 105 °C; [α]D²⁵ = +98.5° (c = 1.0 in CH₂Cl₂); ¹H NMR (CDCl₃, 400 MHz) δ H 1.97, 2.04, 2.10 (3×s, 12H), 2.24-2.27 (m, 2H), 2.36 (t, J = 6.9 Hz, 2H), 3.39 (t, J = 7.3 Hz, 2H), 3.68-3.74 (m, 2H), 4.02 (dd, J = 12.1, 2.2 Hz, 1H), 4.30 (dd, J = 12.1, 5.3 Hz, 1H), 4.37 (m, 1H), 5.07 (brs, 1H), 5.29-5.31 (m, 3H), 7.20 (d, J = 8.4 Hz, 2H), 7.27 (s, 1H), 7.40 (d, 2H), 7.82 (d, J = 7.8 Hz, 1H), 7.96-8.00 (m, 3H), 8.06 (m, 2H), 8.14 (d, J = 7.6 Hz, 2H), 8.26 (d, J = 9.3 Hz, 1H); ¹³C NMR (100 MHz, CDCl₃) δ C 20.7, 20.8, 20.9, 27.1, 32.6, 34.1, 36.8, 62.5, 66.4, 69.0, 69.8, 70.6, 81.1, 120.0, 123.4, 124.9, 125.0, 125.1, 125.9, 126.8, 127.4, 127.5, 128.8, 129.6, 130.0, 130.9, 131.4, 132.4, 135.7, 137.3, 169.8, 169.9, 170.7, 171.0; m/z (ESI+) calcd. for C₄₁H₄₁NO₁₀SnNa [M+Na]⁺ 762.2, measured 762.4; HRMS (ESI+) calcd. for C₄₁H₄₁NO₁₀SnNa [M+Na]⁺ 762.2343, measured 762.2338.
(4-Pyrenebutanoyl)(p-Aminobenzyl)-1-thio-α-D-mannopyranoside 3

(4-Pyrenebutanoyl)(p-Aminobenzyl)-2,3,4,6-tetra-O-acetyl-1-thio-α-D-mannopyranoside 2 (350 mg, 0.5 mmol) and sodium methoxide (catalytic amount, in situ) were added to dry methanol (10 mL). The reaction mixture was stirred under argon at RT. After 12 h, Amberlite® IR 120 hydrogen form washed by MeOH was added to the reaction mixture to adjust pH to 7. Then the reaction mixture was filtered, and concentrated in vacuo to afford the product as a light brown solid (270 mg, 99%).

m.p.: 215 °C; [α]25D = +183° (c = 1.0 in DMF); 1H NMR (400 MHz, DMSO-d6, aliquot amount of D2O) δH 2.05-2.09 (m, 2H), 2.43 (t, J = 7.1 Hz, 2H), 3.32-3.36 (m, 2H), 3.39-3.45 (m, 2H), 3.47-3.51 (m, 1H), 3.60 (m, 1H), 3.63-3.71 (m, 4H), 4.89 (brs, 1H), 7.23 (d, J = 8.5 Hz, 2H), 7.48 (d, 2H), 7.94 (d, J = 7.8 Hz, 1H), 8.03 (at, J = 7.6 Hz, 1H), 8.10 (at, J = 8.7 Hz, 2H), 8.23-8.26 (m, 2H), δ 8.35 (d, J = 9.3 Hz, 1H); 13C NMR (100 MHz, DMSO-d6) δC 27.3, 32.2, 32.5, 35.9, 61.1, 67.3, 71.5, 71.7, 74.6, 83.0, 119.1, 123.5, 124.1, 124.2, 124.8, 125.0, 126.1, 126.5, 127.2, 127.4, 127.6, 128.2, 129.3, 130.4, 130.9, 132.6, 136.4, 138.0, 170.9; m/z (ESI+) calcd. for C33H33NO6SNa [M+Na]+ 594.2, measured 594.4; HRMS (ESI+) calcd. for C33H33NO6SNa [M+Na]+ 594.1921, measured 594.1914.

(4-Pyrenebutanoyl)(p-Aminobenzyl)-2,3,4,6-tetra-O-acetyl-1-thio-β-D-galactopyranoside 5

(p-Aminobenzyl)-2,3,4,6-tetra-O-acetyl-1-thio-β-D-galactopyranoside 4 (1000 mg, 2.1 mmol) and 1-pyrenebutyric acid (730 mg, 2.5 mmol) were added to dry DMF (30 mL). TBTU (815 mg, 2.5 mmol) and triethylamine (590 µL, 4.2 mmol) were added to the mixture. The reaction mixture was stirred under Argon at RT. After 12 h, the reaction mixture was diluted with DCM (100 mL). The mixture was washed with 1M HCl (50 mL) and brine (50 mL). The aqueous layer was extracted with DCM (50 mL×3). The combined organic layer was dried over MgSO4, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (3:4, n-hexane:ethyl acetate) to afford the product as a light brown solid (900 mg, 57%). m.p.: 95 °C; [α]25D = -42.1° (c = 1.0 in CH2Cl2); 1H NMR (CDCl3, 400 MHz) δH 1.97, 2.00, 2.11 (4×s, 12H), 2.16-2.22 (M, 2H), 2.31 (t, J = 7.1 Hz, 2H), 3.30 (t, J = 7.3 Hz, 2H), 3.72-3.76 (m, 2H), 3.85-3.88 (m, 1H), 4.00-4.13 (m, 2H), 4.24 (d, J = 10.0 Hz, 1H), 4.97 (dd, J = 10.0, 3.3 Hz, 1H), 5.28 (at, J = 10.0 Hz, 1H), 5.38-5.39 (m, 1H), 7.18 (d, J = 8.3 Hz, 2H), 7.43 (d, 2H), 7.74-7.75 (m, 2H), 7.91-8.01 (m, 5H), 8.07-8.10 (m, 2H), 8.18 (d, J = 9.3 Hz, 1H); 13C NMR (100 MHz, CDCl3) δC 20.5, 20.6, 20.7, 27.0, 32.4, 33.2, 36.6, 61.5, 67.1, 67.3, 71.7, 74.3, 82.3, 119.8, 123.2, 124.7, 124.8, 124.9, 125.8, 126.6, 127.2, 127.3, 128.6, 129.5, 129.8, 130.7, 131.2, 132.3, 135.6, 137.3, 169.6, 169.8, 170.1, 170.2, 170.3, 171.1, 171.2; m/z (ESI+) calcd. for C41H33NO10SNa [M+Na]+ 762.2, measured 762.3; HRMS (ESI+) calcd. for C41H33NO10SNa [M+Na]+ 762.2343, measured 762.2336.
(4-Pyrenebutanoyl)(p-Aminobenzyl)-1-thio-β-D-galactopyranoside 6

(p-Aminobenzyl)-2,3,4,6-tetra-O-acetyl-1-thio-β-D-galactopyranosyl-pyrenebutanamide 5 (200 mg, 0.3 mmol) and sodium methoxide (catalytic amount, in situ) were added to dry methanol (10 mL). The reaction mixture was stirred under argon at RT. After 12h, Amberlite® IR 120 hydrogen form washed by MeOH was added to the reaction mixture to adjust pH to 7. Then the reaction mixture was filtered, and concentrated in vacuo to afford the product as a light brown solid (154 mg, 99%). m.p.: 205 °C; [α]D25 = -70° (c = 0.2 in DMSO); 1H NMR (400 MHz, DMSO-d6, aliquot amount of D2O) δ H 2.02-2.10 (m, 2H), 2.44 (t, 2H), 3.22 (dd, J = 9.1, 3.3 Hz, 1H), 3.29-3.33 (m, 3H), 3.41 (at, J = 9.3 Hz, 1H), 3.50–3.59 (m, 2H), 3.69-3.72 (m, 2H), 3.86 (d, J = 12.9 Hz, 1H), 4.02 (d, J = 9.6 Hz, 1H), 7.24 (d, J = 8.6 Hz, 2H), 7.53 (d, 2H), 7.88 (d, J = 7.9 Hz, 1H), 7.98 (t, J = 7.6 Hz, 1H), 8.02-8.07 (m, 2H), 8.13 (dd, J = 8.4, 7.4 Hz, 2H), 8.19 (dd, J = 7.7, 3.0 Hz, 2H), 8.32 (d, J = 9.3 Hz, 1H); 13C NMR (100 MHz, DMSO-d6, aliquot amount of D2O) δ C 27.3, 31.9, 32.2, 35.9, 48.6, 60.7, 68.5, 70.0, 74.8, 79.4, 83.6, 119.0, 123.5, 124.1, 124.2, 124.8, 124.9, 125.0, 126.1, 126.5, 127.3, 127.4, 127.6, 128.2, 129.3, 130.4, 130.9, 132.8, 136.5, 137.9, 170.9; m/z (ESI+) calcd. for C33H33NO6SNa [M+Na]+ 594.2, measured 594.8; HRMS (ESI+) calcd. for for C33H33NO6SNa [M+Na]+ 594.1921, measured 594.1918.

Incubation of Glyco-Pyrene Derivatives on Graphene Device and Lectin Binding.

Graphene film was deposited to the device, Si wafer and glass wafer. For the Raman spectroscopy and atomic force microscopy, Si wafer was used. And glass wafer was used for the UV-Vis spectroscopy. In a typical preparation experiment, graphene film on wafer was incubated in 2 g/L of (4-Pyrenebutanoyl)(p-Aminobenzyl)-1-thio-α-D-mannopyranoside 3 solution in MeOH for 12h at RT. For the protein binding sensing, Concanavalin A (ConA) in 10 mM HEPES, 150 mM NaCl, 5 mM CaCl2 buffer solution (0.1 mL/mg, pH 7.5) was used. Each sample was thoroughly rinsed with MeOH before analysis to remove excess amount of 3 on the sample. Finally, rinsed sample was dried over the air.
6.4.2 Supporting Figures

(a)
Figure 6.15. (a) $^1$H and (b) $^{13}$C NMR spectra of manno-pyranoside 2.
Figure 6.16. (a) $^1$H and (b) $^{13}$C NMR spectra of mannopyranoside 3.

Figure 6.17. (a) COSY and (b) HSQC spectra of mannopyranoside 3.
Figure 6.18. (a) $^1$H and (b) $^{13}$C NMR spectra of galactopyranoside 5.
Figure 6.19. (a) $^1$H and (b) $^{13}$C NMR spectra of galactopyranoside 6.

Figure 6.20. (a) COSY and (b) HSQC spectra of galactopyranoside 6.
6.5 Experimental for Chapter 5

6.5.1 Procedures

Mono-6-(p-toluenesulfonyl)-6-deoxy-\(\beta\)-cyclodextrin (\(\beta\)-CD-Ts) 1

In a 1-L double-necked, round-bottom flask, \(\beta\)-CD hydrate (20.0 g, 35.2 mmol) was dissolved in deionized water (450 mL) by heating to 60°C with vigorous stirring. To the resulting milky suspension was added 1-(p toluenesulfonyl) imidazole (12 g, 54mmol). After 2 h, a solution of sodium hydroxide (9 g, 225 mmol) of 25mL of water was added over 20 min. After 10 min, 1-(p-toluenesulfonyl)-imidazole was separated by filtration. Filtrant was concentrated and dried overnight.

\(^{1}\)HNMR (500MHz, DMSO-\(d_6\), d): 2.42 (3H, s), 3.20–3.65 (40H, overlap with HDO, m), 4.15–4.23 (1H, m), 4.30–4.38 (2H, m), 4.40–4.54 (2H, m), 4.50 (3H, br s), 4.76 (2H, br s), 4.84 (4H, br s), 5.62–5.84 (14H,m), 7.43 (2H, d, J = 7.75Hz), 7.73 (2H, d, J = 7.75Hz); MALDI-TOF MS (m/z) calculated for C\(_{49}\)H\(_{76}\)O\(_{37}\)SNa, 1311.4; found 1311.5 for [M+Na]+.

Mono-6-azide-deoxy-6-\(\beta\)-cyclodextrin (\(\beta\)-CD-N\(_3\)) 2

In a 1-L-double-necked, round-bottomed flask, \(\beta\)-Ts-CD (5 g, 3.9 mmol) was dissolved in deionized water (500mL) and added sodium azide (5 g, 76.9 mmol) into the flask by heating to100°C with oil bath. The reaction mixture was refluxed overnight in the oil bath and cool down the reaction to room temperature. The filtered reaction solution was concentrated at least 95% of water under reduced pressure using a rotary evaporator. To the concentrated filtrate was added 1,1,2,2-tetrachloroethane dropwise (5 mL) and the resultant mixture was stirred for 30 min. The residue was extracted, washed and concentrated in vacuo. The powder was recrystallized and dried at 60 °C in vacuo to generate \(\beta\)-CD-N\(_3\). \(^{13}\)CNMR (125MHz, D\(_2\)O, d): 53.2, 62.3, 74.3, 74.4, 75.4, 83.4, 104.4; ESI-MS (m/z): calculated for C\(_{42}\)H\(_{69}\)O\(_{34}\)N\(_3\), 1182.4; found, 1182.5 for [M+Na]+.
Succinimido 3-maleimido-propionate 4

β-Alanine (2.7 g, 30 mmol) and maleic anhydride (2.9 g, 30 mmol) was dissolved in DMF (anhydrous, 30 mL) at RT under Ar. After stirring for 2 h, the reaction mixture was cooled down to 0 °C and NHS (4.2 g, 37 mmol) was added followed by DCC (12.9 g, 63 mmol). After 10 min, the ice bath was removed and the mixture was vigorously stirred for overnight. White urea precipitate was filtered out and filtrate was concentrated, then poured into ice/water. The precipitate was rinsed with water, and the residue was purified by flash column chromatography (n-hexane:EtOAc, 1:1→0:1) to afford the product as a white solid (4.0 g, 50%). \(^1\)H NMR (600 MHz, DMSO-d\(_6\)) δ 2.79 (4H, s), 3.04 (2H, t, \(J = 6.8\) Hz), 3.74 (2H, t, \(J = 6.8\) Hz), 7.04 (2H, s).

N-Propargyl 3-maleimido-propionamide (PMP) 5

To a solution of succinimido 3-maleimido-propionoate (400 mg, 1.50 mmol) in THF (10 mL) was added propargylamine (106 mL, 4.95 mmol) at 0 °C. After stirring overnight at RT, the reaction mixture was diluted in DCM (100 mL) and extracted with water (100 mL). The organic phase was dried under Na\(_2\)SO\(_4\), filtered, and concentrated in vacuo. The residue was filtered and rinsed with DCM. The solution part was concentrated and purified by flash column chromatography (n-hexane:EtOAc, 1:2→0:1) to afford the titled product as a white solid (17 mg, 6%). \(^1\)H NMR (600 MHz, CDCl\(_3\)) δ 2.22 (1H, t, \(J = 2.5\) Hz), 2.55 (2H, t, \(J = 7.2\) Hz), 3.84 (2H, t, \(J = 7.2\) Hz), 4.01 (2H, dd, \(J = 2.5, 5.2\) Hz), 6.02 (1H, s), 6.70 (2H, s); \(^{13}\)C NMR (150 MHz, CDCl\(_3\)) δ 29.3, 34.2, 34.5, 17.8, 79.5, 134.4, 169.4, 170.6; ESI-MS (m/z): calculated for C\(_{10}\)H\(_{10}\)N\(_2\)O\(_3\)S, 229.1; found 228.8 [M+H]\(^+\).

FITC-AD Conjugate

To a solution of FITC-isothiocyanate (52 mg, 0.134 mmol) in DMF (anhydrous, 2 mL) was added 1-adamantylamine (20 mg, 0.134 mmol) and DIPEA (50 mL, 0.302 mmol). After stirring for 16 h under Ar, the reaction mixture was concentrated in vacuo and purified by flash column chromatography (n-hexane:EtOAc, 2:1→1:5) to afford the product as an orange colored solid (quantitative). \(^1\)H NMR (600 MHz, acetone-d\(_6\)) δ 1.73 (6H, m), 2.10 (3H, s), 2.36-2.37 (6H, m), 6.63 (2H, dd, \(J = 2.4, 8.7\) Hz), 6.74 (2H, d, \(J = 8.7\) Hz), 6.70 (2H, d, \(J = 2.4\) Hz), 7.18 (1H, d, \(J = 8.2\) Hz), 7.83 (1H, dd, \(J = 1.8, 8.2\) Hz), 8.27 (1H, d, \(J = 1.8\) Hz), 8.97 (1H, s), 8.99 (2H, s); ESI-MS (m/z): calculated for C\(_{31}\)H\(_{28}\)N\(_2\)O\(_5\)S, 540.2; found 540.7 [M+H]\(^+\).
6.5.2 Supporting Figures

Figure 6.21 (A) $^1$H NMR spectrum of $N$-Propargyl 3-maleimidopropionamide (PMP). (B) $^{13}$C NMR spectrum of PMP.
Figure 6.22. $^1$H NMR spectrum of $\beta$-CD-Ts. (B) $^{13}$C NMR spectrum of $\beta$-CD-N$_3$. 
Figure 6.23. $^1$H NMR of FITC-AD conjugate.
References


감사의 글

2011년, 연구실의 첫 학생으로서 교수님께 실험을 배우던 시간이 옛그제 같은데, 어느새 석사과정의 끝에 다다랐습니다. 지난 4년간, 유기 및 무기 합성 연구실에서 수학하면서 너무나도 많은 사람들의 도움을 받았습니다. 이 지면을 통해 감사의 말씀을 전하고 싶습니다.

먼저 부족한 저를 지도해주신 교수님께 감사의 말씀을 드리고자 합니다. 석사과정 동안 교수님의 지도를 받은 것은 저에게 큰 행운이었습니다. 또한 저의 석사논문심사를 도와주신 강세병, 양창덕 교수님께도 감사의 말씀을 드립니다.

4년간 함께 실험실에서 동고동락한 유기 및 무기 합성 연구실 학생들에게도 감사의 말씀을 전합니다. 연구를 진행하는데 많은 도움을 준 BIJOY씨, 항상 밝은 얼굴로 맞아주는 현지 누나, 함께 디펜스를 준비하면서 고생한 현호형, 동갑내기 친구들에 도와주신, 몇 년째 막내를 맡아 고생하는 민호, 그리고 우리 학과를 거쳐간 여러 학부인턴 학생들까지 모두들 감사합니다. 앞으로도 열심히 연구하고 노력하여 훌륭한 업적으로 우리 연구실을 빛내주기 바랍니다. 유니스의 1기 입학생으로 함께 좋은 추억을 쌓은 09학번 친구들, 그리고 후배들에게도 감사의 말씀을 전합니다.

마지막으로 항상 뒤에서 든든하게 저를 지지해준 가족들에게 감사의 말씀을 전합니다. 제가 무엇을 하든 제 선택을 지지해주신 학업에 전념할 수 있게 도와주신 아버지와 어머니, 물심양면으로 도와주신 누나와 매형, 그리고 일가 친척 여러분들에게 감사의 말씀을 전합니다. 앞으로 무엇을 하든지 지금까지 받은 도움을 잊지 않겠습니다.

모든 분들께 다시 한번 감사 드립니다.

전상빈 올림