Synthesis and Applications of Dynamic Multivalent Nanostructures

Kitjanit Neranon
กิจณิชญ์ เนรานนท์

Doctoral Thesis

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To my family
Abstract

This thesis focuses on the design, synthesis and development of dynamic multivalent nanostructures such as supramolecular dendrimers, liposomes and gold-functionalized nanostructures. These structures can be used for drug delivery and molecular sensing applications. This thesis is divided into three parts:

In part one, a general introduction to self-assembly, dynamic systems, metal-ligand exchange, nanostructured dendritic scaffolds, liposomes and gold nanostructures is given.

In part two, a microwave approach is presented as an efficient method for the regioselective deuteration of bipyridine scaffolds. Dynamic systems based on transition metal-bipyridine coordination complexes were investigated. The compositional self-adaptation and kinetics of these dynamic systems were successfully assessed by ESI-MS. Based on this amphiphilic dendrimers/metallodendrimers were also designed and synthesized via a convergent strategy. Their ability to self-assemble into supramolecular assemblies and their controlled disassembly was effectively demonstrated.

In part three, two types of drug delivery systems based on dynamic multivalent nanostructures of glycodendrimers/metaloglycodendrimers and drug-presenting liposomes were developed. The dynamic self-assembly of these architectures into supramolecular nanostructures with site-specific functionality through interacting carbohydrate or cholesterol moieties was assessed. The host-guest interaction/encapsulation and controlled release with external stimuli were studied using a fluorescent probe, as well as selected drug molecules. The antibacterial property of the drug delivery systems was also evaluated, demonstrating an enhanced bactericidal activity. A new, rapid and simple approach for the functionalization of plasmonic gold nanostructured surfaces was also developed. The optical performance and light-specific sensitivity of the fluorescent probe on the resulting nanostructures were also presented.

Keywords: multivalent nanostructures, self-assembly, metal-ligand exchange, dynamic covalent chemistry, bipyridine derivatives, dendrimers, liposomes, drug delivery, antimicrobial materials, fluorescent probe, plasmonic chemistry, gold surface functionalization.
Sammanfattning på svenska

Denna avhandling fokuserar på design, syntes och utveckling av dynamiska nanostrukturer såsom supramolekylära dendrimerer, liposomer och guldnastrukturer. Dessa strukturer kan användas för läkemedelstillförsel och molekylära analys- och analyslämpningar. Denna avhandling är indelad i tre delar:

I del ett, är en allmän introduktion till självorganisering, dynamiska system, metall-ligandutbyte, nanostrukturerade dendritiska strukturer, liposomer och guldnastrukturer ges.


Nyckelord: nanostrukturer, självmontering, metall-ligand-utbyte, dynamisk kovalent kemi, bipyridinderivat, dendrimerer, liposomer, drug delivery, antimikrobiella material, fluorescerande sond, plasmonisk kemi, guld, ytffunktionalisering.
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>Ac</td>
<td>Acetyl</td>
</tr>
<tr>
<td>AIBN</td>
<td>2,2′-Azobis(2-methylpropionitrile)</td>
</tr>
<tr>
<td>aq.</td>
<td>Aqueous</td>
</tr>
<tr>
<td>Boc</td>
<td>tert-Butyloxycarbonyl</td>
</tr>
<tr>
<td>BOP</td>
<td>(1H-Benzotriazol-1-yloxy)[tris(dimethylamino)]phosphonium</td>
</tr>
<tr>
<td>Bpy</td>
<td>Bipyridine</td>
</tr>
<tr>
<td>CDC</td>
<td>Constitutional dynamic chemistry</td>
</tr>
<tr>
<td>CHE</td>
<td>Cholesterol esterase</td>
</tr>
<tr>
<td>Chol</td>
<td>Cholesterol</td>
</tr>
<tr>
<td>CIP</td>
<td>Ciprofloxacin</td>
</tr>
<tr>
<td>CLSM</td>
<td>Confocal laser scanning microscopy</td>
</tr>
<tr>
<td>CPT</td>
<td>(S)-(+) Camptothecin</td>
</tr>
<tr>
<td>CT</td>
<td>Charge transfer</td>
</tr>
<tr>
<td>d</td>
<td>Day(s)</td>
</tr>
<tr>
<td>d</td>
<td>Deuterated</td>
</tr>
<tr>
<td>2D/3D</td>
<td>Two or three dimensional</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DIPEA</td>
<td>N,N-Diisopropylethylamine</td>
</tr>
<tr>
<td>DLS</td>
<td>Dynamic light scattering</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-Dimethylformamide</td>
</tr>
<tr>
<td>DPPC</td>
<td>Dipalmitoylphosphatidylcholine</td>
</tr>
<tr>
<td>E. coli</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>$\lambda_{em}$</td>
<td>Emission wavelength</td>
</tr>
<tr>
<td>eq.</td>
<td>Equivalent</td>
</tr>
<tr>
<td>ESI-MS</td>
<td>Electrospray ionization mass spectroscopy</td>
</tr>
<tr>
<td>$\lambda_{ex}$</td>
<td>Excitation wavelength</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>$^{19}$F qNMR</td>
<td>Quantitative fluorine nuclear magnetic resonance</td>
</tr>
<tr>
<td>Gal</td>
<td>D-Galactose</td>
</tr>
<tr>
<td>Glc</td>
<td>D-Glucose</td>
</tr>
<tr>
<td>h</td>
<td>Hour(s)</td>
</tr>
<tr>
<td>k</td>
<td>Reaction rate constant</td>
</tr>
<tr>
<td>K</td>
<td>Equilibrium constant</td>
</tr>
<tr>
<td>Man</td>
<td>D-Mannose</td>
</tr>
<tr>
<td>MIC</td>
<td>Minimum inhibitory concentration</td>
</tr>
<tr>
<td>min</td>
<td>Minute(s)</td>
</tr>
<tr>
<td>MLCT</td>
<td>Metal-ligand charge transfer</td>
</tr>
<tr>
<td>M. smegmatis</td>
<td><em>Mycobacterium smegmatis</em></td>
</tr>
<tr>
<td>NBS</td>
<td>N-Bromosuccinimide</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>NV</td>
<td>6-Nitroveratryl</td>
</tr>
<tr>
<td>OTf</td>
<td>Trifluoromethanesulfonate</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate-buffered saline</td>
</tr>
<tr>
<td>PDI</td>
<td>Polydispersity index</td>
</tr>
<tr>
<td>rt</td>
<td>Room temperature</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td><em>Staphylococcus epidermidis</em></td>
</tr>
<tr>
<td>TBABr</td>
<td>Tetrabutylammonium bromide</td>
</tr>
<tr>
<td>TEA</td>
<td>Triethylamine</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission electron microscopy</td>
</tr>
<tr>
<td>TFA</td>
<td>2,2,2-Trifluoroacetic acid</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>UV-Vis</td>
<td>Ultraviolet-Visible</td>
</tr>
<tr>
<td>wt%</td>
<td>Weight percentage</td>
</tr>
<tr>
<td>$\zeta$</td>
<td>Zeta potential</td>
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List of Publications

This thesis is based on the following papers, referred to in the text by their Roman numerals I-VI:

I. Rapid, Regioselective Deuteration of Dimethyl-2,2'-bipyridines via Microwave-assistance  
   Kitjanit Neranon and Olof Ramström  
   RSC Adv. 2015, 5, 2684-2688

II. Constitutional Dynamics of Bipyridine-Metal Complex Systems  
    Kitjanit Neranon and Olof Ramström  
    Manuscript

III. Design, Synthesis and Self-Assembly of Functional Amphiphilic Dendrimers and Metallo dendrimers  
     Kitjanit Neranon and Olof Ramström  
     Manuscript

IV. Stimuli-Responsive, Multivalent Glycodendrimer/Metalloglycodendrimer Assemblies for Targeted Delivery  
    Kitjanit Neranon and Olof Ramström  
    Manuscript

V. Ciprofloxacin-presenting Liposomes for Enhanced Antibacterial Activity  
   Kitjanit Neranon, Nanjing Hao, Xuan Chen, Joakim Romson, Sesha Manuguri, Mingdi Yan and Olof Ramström  
   Manuscript

VI. Laser-induced, Surface Plasmon-enhanced Two-photon Excitation for Efficient Chemical Functionalization of Nanostructured Gold Surfaces  
    Kitjanit Neranon, Mattias Åslund, Antanas Karalius, Min Yan, Hao Xu, Ying Fu, Ingemar Petermann, Per Björk, and Olof Ramström  
    Manuscript
Papers not included in this thesis:

VII. **Determination of Binding Affinity of Glyconanomaterials**
Juan Zhou, Sheng Xie, Kitjanit Neranon, Yang Zhang, Olof Ramström
and Mingdi Yan
*Manuscript*

VIII. **Glyconanomaterials for Sensing Applications**
Nanjing Hao, Kitjanit Neranon, Olof Ramström and Mingdi Yan
*Biosens. Bioelectron. 2015, 76, 113-130*
Table of Contents

Abstract
Abbreviations
List of publications

1. Introduction ............................................................................................................... 1
   1.1. Dynamic chemical systems .............................................................................. 1
       1.1.1. Reversible covalent chemistry ............................................................... 2
       1.1.2. Reversible non-covalent chemistry .......................................................... 2
   1.2. Metal coordination chemistry ............................................................................ 2
       1.2.1. Metal coordinative interaction ................................................................. 2
       1.2.2. Metal-ligand exchange reactions .............................................................. 3
       1.2.3. Design of building blocks ....................................................................... 3
   1.3. Dynamic supramolecular self-assembly ............................................................ 3
   1.4. Multivalent nanostructures based on supramolecular nanoscaffolds ........... 4
       1.4.1. Dendrimers/glycodendrimers/metalloglycodendrimers ................................ 5
           1.4.1.1. Introduction ....................................................................................... 5
           1.4.1.2. General synthetic approaches ............................................................ 7
           1.4.1.3. Self-assembly .................................................................................... 8
           1.4.1.4. Properties and applications ............................................................... 8
           1.4.2. Liposomes ............................................................................................ 9
           1.4.2.1. Introduction ....................................................................................... 9
           1.4.2.2. Preparation ...................................................................................... 9
           1.4.2.3. Properties and applications ............................................................... 10
           1.4.3. Gold-surface functionalization ............................................................... 10
           1.4.3.1. Introduction ....................................................................................... 10
           1.4.3.2. Preparation ...................................................................................... 10
           1.4.3.3. Properties and applications ............................................................... 11
   1.5. Aims of this thesis ............................................................................................. 11

2. Synthesis and dynamics of bipyridine-based dendrimers and their supramolecular assemblies ........................................................................... 13
   2.1. Microwave-assisted regioselective deuteration of dimethyl-2,2′-bipyridines .... 13
       2.1.1. Introduction ............................................................................................. 13
       2.1.2. Overview ............................................................................................... 13
       2.1.3. Deuteration under conventional heating .................................................... 14
       2.1.4. Deuteration under microwave irradiation .................................................... 15
       2.1.5. Characterization and optimization of reaction conditions ......................... 15
       2.1.6. Conclusions ............................................................................................ 17
   2.2. Dynamic systems based on bipyridine-metal complexes .............................. 18
       2.2.1. Introduction ............................................................................................. 18
## 2.2. Overview

- Identification of ligand systems ......................................................... 18
- Dynamics of mixed ligand systems ...................................................... 19
- Kinetics of the dynamic systems ......................................................... 21
- Conclusions ......................................................................................... 24

## 2.3.

Design and synthesis of amphiphilic dendrimers toward multifunctional supramolecular assemblies ......................................................... 26

- Introduction ......................................................................................... 26
- Overview ............................................................................................. 26
- Synthesis of dendrons/dendrimers ......................................................... 27
- Metal-dendritic ligand coordination ....................................................... 29
- Supramolecular self-assembly ............................................................... 31
- Biocompatible ligand competition for supramolecular disassembly ......... 34
- Conclusions ......................................................................................... 34

## 3.

Synthesis and applications of multivalent glycodendrimers, liposomes and gold nanoislands ................................................................. 37

### 3.1.

Self-assembly of supramolecular glycodendrimers ..................................... 38

- Introduction ......................................................................................... 38
- Overview ............................................................................................. 38
- Synthesis of glycodendrimers ............................................................... 39
- Supramolecular self-assembly ............................................................... 44
- Host-guest encapsulation capability and release profiles ....................... 47
- Conclusions ......................................................................................... 50

### 3.2.

Ciprofloxacin-presenting liposomes .......................................................... 51

- Introduction ......................................................................................... 51
- Overview ............................................................................................. 51
- Synthesis of CIP-Chol .......................................................................... 52
- Liposome formation ............................................................................. 54
- Drug-grafted liposome quantitation ....................................................... 56
- Enzyme-triggered drug release ............................................................. 57
- Antibacterial activity ............................................................................ 59
- Conclusions ......................................................................................... 60

### 3.3.

Plasmon-assisted surface functionalization of gold nanostructures .......... 60

- Introduction ......................................................................................... 60
- Overview ............................................................................................. 61
- Design and synthesis of fluorescent probe molecule .......................... 61
- Optical properties of fluorescent probe molecule .............................. 62
- Photolysis of fluorescent probe molecule ........................................... 63
- Probe fluorescence evaluation ............................................................. 64
- Gold surface functionalization via light-induced surface plasmon-enhanced two-photon excitation ......................................................... 65
- Conclusions ......................................................................................... 67

## 4.

Concluding Remarks ............................................................................. 69
Acknowledgements
Appendix
References
Life is originated from the dynamic self-organization of simple molecules. In nature, living organisms consist of well-ordered assemblies of an innumerable amount of molecules that spontaneously and simultaneously interact with each other.\textsuperscript{1} Examples of such behavior include the self-assembly of biological membrane in cells, and the folding of polypeptide chains into proteins and nucleic acids into their functional structures.\textsuperscript{2} Therefore, self-assembly is considered a fundamental principle to create structural assemblies at all levels from molecules to organisms and possibly even to galaxies (Figure 1.1). For this reason, understanding self-assembly could reveal the mystery behind the origin of life.\textsuperscript{3-6} To do this, one needs to look at the basis of the self-assembly process and the molecular interactions.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Complex systems from molecules to the living system.}
\end{figure}

1.1. Dynamic chemical systems

In spite of the fact that dynamic systems are omnipresent in nature, it has been challenging to realize in practice.\textsuperscript{7} Compelled and inspired by this, the field of systems chemistry has evolved rapidly with the emergence of dynamic chemical systems. Traditionally, supramolecular chemistry has been defined as chemistry beyond the molecule as it focuses on intermolecular interactions.\textsuperscript{8} Using the corresponding bonding properties of small components, highly complex, molecular structures with diverse functions can be designed and constructed. In this context, reversibility in covalent and non-covalent interactions/bonding is a hallmark governing the assembly and disassembly of these supramolecular architectures and the promotion of their useful diversity.\textsuperscript{9,10}
1.1.1. Reversible covalent chemistry
Control over reversible interactions by external stimuli is of primary importance in dynamic self-assembly processes. Reversible covalent linkages are promising tools to in this regard. A wide variety of covalent bonds have been shown to undergo reversible reactions in the presence of external stimuli such as light, pH, temperature, enzymes or applied electric fields. Examples of such bonds include esters, carbamates, imines, and disulfides.\textsuperscript{11-13} These reversible entities on molecular level can be used as potential building blocks for applications on the supramolecular level, controlling the self-assembly and disassembly in dynamic supramolecular materials.\textsuperscript{3}

1.1.2. Reversible non-covalent chemistry
Generally, non-covalent intermolecular interactions are weak and highly reversible. These reversible interactions can promote the self-assembly of multiple small components into the structures of higher complexity.\textsuperscript{3} Examples of non-covalent interactions include van der Waals forces, $\pi-\pi$ stacking, cation–π interaction, different types of dipole interactions, hydrogen bonding, ion-pairing and metal coordination. These interactions, as well as weak covalent bonds are frequently seen in self-assembly systems (Figure 1.2).\textsuperscript{1,6,9,10,14} These events give rise to spontaneous, controlled self-assembly into well-defined supramolecular architectures under pre-defined conditions for specific applications.\textsuperscript{11,15-19}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Figure12.png}
\caption{Molecular forces from reversible non-covalent interactions to bonds.}
\end{figure}

1.2. Metal coordination chemistry

1.2.1. Metal coordinative interaction
Another interesting reversible interaction is the metal coordination bond. This bond type has been used for building discrete and well-defined structures. Metals can provide a large variety of different coordination geometries, and the coordinative strength spreads the whole range of binding energies from
very labile coordination to almost covalent bonds. Therefore, metal coordination provides an excellent basis for self-assembly of supramolecular complexes. Importantly, those resulting supramolecular structures based on coordination complexes can be tuned and controlled. Thereby, supramolecular assemblies via metal coordination chemistry provide more tunability in size and shape compared to other reversible non-covalent chemistry. Furthermore, the disassembly process can be modified/controlled rendering such supramolecular structures useful as delivery systems.\textsuperscript{15,16,20}

1.2.2. Metal-ligand exchange reactions

Metal-ligand exchange reactions are one of the most widely used reactions in dynamic systems.\textsuperscript{21,22} However, the high cost of starting materials makes it difficult to screen a large variety of complexes for the optimization of selectivity. In this context, constitutional dynamic chemistry (CDC), providing systems of many different components, offer promises to overcome this obstacle.\textsuperscript{23-25} As mentioned before, the structural composition adapts to certain stimuli and the metal ions used.\textsuperscript{26} The ligand exchange or ligand substitution reactions occur via three different mechanisms: associative, dissociative and interchange mechanisms. It is noteworthy that the kinetic rates for these ligand exchange reactions are typically dependent on the metal ion used, and are more or less independent of the ligands exchanged. In general, metal ions that favor the formation of strongly covalent bonds (usually small ions) undergo exchange reactions slower than larger metal ions\textsuperscript{27-29}

1.2.3. Design of building blocks

Depending on the desired function of the supramolecular assembly, the ligand building blocks coordinated to the metal ions should have a specific design. The most important criterion is that the components are capable of reversibly coordinating with the metal preferred under certain stimuli. A structure capable of this preferred coordination with metals is the 2,2'-bipyridine motif, which has been widely used as N,N-chelate ligand building blocks. This motif has been studied in metal coordination chemistry for a long time,\textsuperscript{30} particularly in inorganic photochemistry and supramolecular chemistry.\textsuperscript{31}

1.3. Dynamic supramolecular self-assembly

Many dynamic self-assembly processes can be found in living systems based on the reversible interactions described for small molecular components, achieving potent functions of diverse actions. Examples of such organization processes are self-assembly of lipid bilayers and folding of proteins (Figure 1.3).\textsuperscript{32,33}
**Figure 1.3** Schematic structure of biological membrane consisting of self-assembled phospholipids.

Self-assembly in non-living systems can be inspired by biological systems. Dynamic self-assembly denotes an adaptive process, where the well-ordered structure can also change out of thermodynamic equilibrium based on input energy. Dynamic self-assembly is rarely found in chemical systems, but it has been demonstrated that such behavior can also be artificially generated. Achieving dynamic self-assembly remains challenging due to its sensitive nature; slight changes in the intermolecular interactions of the basic units and external stimuli can perturb the system.

Furthermore, the discovery of a variety of different nanostructured materials with unique properties feasibly emerges from supramolecular self-assembly, representing an essential part of nanoscience and nanotechnology. A prime example is the recent interest and development of nanomaterials for used in medical and biological applications.

### 1.4. Multivalent nanostructures based on supramolecular nanoscaffolds

The development of new classes of supramolecular nanostructures has been long researched for exploring their unique chemical and physical properties. Many properties of nanostructures such as size, shape, chemical composition, surface structure, charge, aggregation, agglomeration and solubility are of great importance for their specific applications. In particular, the nanoscale effects resulting from the increased specific surface area of nanomaterials have been continuously developed as promising tools for many applications in diagnostic biosensors, drug delivery, and biomedical imaging.

A nanostructure can be defined as a system with at least one dimension in the range of 1-100 nm. It can be categorized based on its basic structural configuration into four types: carbon-based nanostructures (fullerenes,
graphene and nanotubes), metal-based nanomaterials (nanogold, nanosilver and metal oxides like quantum dots), soft nanomaterials (micelles, liposomes, gels, dendrimers and copolymers) and multiphase composite materials (Figure 1.4). \cite{43,44} Nanomaterials can exhibit unique properties distinctively different from the bulk materials on macroscopic scale. \cite{45,46}

Figure 1.4 Examples of different structures of various sizes (left) and various nanomaterials (right).

1.4.1. Dendrimers/glycodendrimers/metalloglycodendrimers

1.4.1.1. Introduction

As one of the most interesting nanoscaffolds, dendrimers are in principle a type of hyperbranched functional macromolecules. It can be prepared via convergent or divergent iterative synthesis leading to a typically globular topology, and mono-dispersed and mostly nanoscale in size. Typically, the dendritic structure is a symmetrically well-defined architecture comprising of three structural regions: a central core or focal point; layers of highly branched repeat units, where each layer typically results from one stage of growth and is termed a generation; and the multiple functional groups at the peripheral layer (Figure 1.5). \cite{47-49}
Dendrimers possess multiple terminal functionalities that enable the enhancement of functions. The introduction of various biomolecular structures, for example, carbohydrate entities onto dendritic surfaces can be straightforwardly performed for various purposes in biological applications.\textsuperscript{3,50} The carbohydrate-presenting dendritic entities, so-called glycodendrimers, can be categorized according to the location of the carbohydrates in their architectures: on the dendrimer periphery or end groups (carbohydrate-coated); at the central core (carbohydrate-centered); or at the branching points (fully carbohydrate-based) as shown in Figure 1.6.

Apart from the purely organic glycodendrimers, metallodendrimers possess many advantages. Particularly, they could be used for the development of carbohydrate-functionalized dendrimers.\textsuperscript{51-53} Therefore, metalloglycodendrimers comprising synthetic metallodendrimers together with pendant carbohydrate molecules are of great interest for making potential glycoclusters in a variety of biomedical applications.\textsuperscript{42}
1.4.1.2. General synthetic approaches

The synthesis of dendrimers gives the possibility to create monodispersed and structural-controlled macromolecular architectures similar to those observed in biological systems. Two complementary synthetic approaches, divergent or convergent iterative methods, can be employed to prepare dendritic architectural motifs from dendron structures. Both methods are based on the repetition of a sequence of reactions, with each sequence creating a new dendritic generation (Figure 1.7). Consequently, they can yield targeted monodisperse macromolecules with primary structures on the same level of precision as small organic molecules.²⁵⁴

**Figure 1.7 Divergent and convergent synthetic routes.**

The divergent approach was first reported by Vögtle for the synthesis of poly(propyleneimine) dendrimers.⁵⁴ The synthesis was based on the growth of the dendritic structure outward from the core, in an exhaustive and stepwise fashion. This process provides dendrimers with increasing generation numbers. However, only one type of reaction can be performed at each step, providing a uniform display of only one functional group on the periphery. Because the growth occurs largely at the periphery of the dendritic structure, this route minimizes the potential steric complications but requires highly efficient and orthogonal chemistry that avoids incomplete couplings and undesired side reactions.⁵⁴

The convergent approach, pioneered by Fréchet and coworkers, involves the synthesis of the dendritic fragments separate from the core. The method is based on the growth from the periphery towards a central point, followed by coupling onto the core ultimately. In this strategy, the ultimate generation number is pre-determined, necessitating the synthesis of branched molecules of the various requisite sizes before in each generation. Generally, it affords dendrimers with higher structural homogeneity and monodispersity than the divergent approach. Convergent synthesis is versatile in the dendritic chains of different molecular composition that can be conjugated to a single core,
providing regional variations on the final dendrimer. Normally, this can yield dendrimers of higher purity, however it will be subjected to steric hindrance when the dendrons approach in higher generations and also frequently requires tiresome purification throughout the synthetic route.  

Compared with other carbohydrate-presenting scaffolds such as lipids, proteins, and polymers, the synthesis of well-defined glycodendrimers is in theory more straightforward and tunable in both the density and spacing of carbohydrate ligands. Similar to the synthetic approaches to dendrimers, glycodendrimers can also be synthesized following the two general strategies of the divergent and the convergent methods or a combination of these approaches.

Regarding metalloglycodendrimers, in the convergent synthesis, the ligand containing carbohydrates is first prepared, followed by metal complex formation. In the divergent method, a proper metalodendrimer is first formed, ultimately followed by conjugation of carbohydrate. The divergent approach is more commonly used for the construction of metalloglycodendrimers. The control over the clusterization and carbohydrate density are of extreme importance in carbohydrate-protein interactions.  

1.4.1.3. Self-assembly

Self-assembly of supramolecular dendritic molecules from their primary units into larger and more complex structures have lead to a wide range of potential applications, particularly in the fields of biomedicine and nanotechnology. However, dendrimers are mostly deficient of appropriate driving forces for self-assembly, therefore chemical or physical interactions are generally required and introduced. Amphiphilic dendrimers bearing hydrophobic and hydrophilic motifs have been used and made into various supramolecular assemblies as nanocarriers for the delivery applications. For metalloglycodendrimers, however, due to the strong coordination tendency of the resulting assemblies, the lack of sensitivity to external stimuli remains challenging.  

1.4.1.4. Properties and applications

In comparison with other nanoscale synthetic scaffolds (e.g., traditional polymers, Bucky balls, or carbon nanotubes), dendrimers have high structural diversity. The prototypical dendritic structures can be tuned using simple organic synthesis to change their chemical and physical properties to improve solubility, and to increase reactivity and stability. Particularly, it is possible to chemically modify the multiple functional groups that exist at the focal points, branching units, and mostly on the dendritic surface. This facilitates many potential applications for examples liquid crystals, catalysts, sensors, photovoltaics, bioactive dendrimer conjugates, targeted delivery, etc.
The main advantage of multivalent glycodendrimers compared to other glycomaterials is the very narrow dispersity, giving the ability to control the ligand density on the surface and well-defined molecular structure.\textsuperscript{51,65} Moreover, the metalloglycodendrimers can organize ligands in specific geometries, propagating carbohydrate clusters and the variations of clusters. Tuning of the multivalency and structural arrangements can be obtained by modifying the ligands or metal center.

A number of diverse dendrimers has been developed to study their properties, for example, pharmacokinetic properties (antibody-dendrimer, peptide-dendrimer conjugates).\textsuperscript{59} In particular, they have been extensively used in biological applications such as an efficient target for drug or gene delivery, diagnostics, imaging, vaccines, sensing and other therapeutics.\textsuperscript{1,51-53}

1.4.2. Liposomes

1.4.2.1. Introduction

Liposomes, defined as artificial microscopic spherical vesicles with particle sizes ranging from 30 nm to several micrometers were first discovered in 1960s by Bangham.\textsuperscript{66} Liposome properties vary considerably with differences in lipid compositions, surface charges, sizes and the method of preparations.\textsuperscript{51,67} Lipids consisting of hydrophilic head groups and lipophilic chains are generally able to self-assemble in aqueous medium \textit{via} available intermolecular forces as previously mentioned.\textsuperscript{66} The resulting bilayers can ultimately result in vesicular liposomes or micelles depending on the relative lengths, sizes and structures of the involved segment, as well as temperature, environmental and preparation conditions.

1.4.2.2. Preparation

Liposomes can be easily prepared from suitable natural or synthetic lipophilic entities such as fatty alcohols, cholesterol and non-toxic phospholipids. In particularly, cholesterol has been largely used to improve the bilayer characteristics of the liposomes. It improves the membrane fluidity, bilayer stability and reduces the permeability of water soluble molecules through the membrane.\textsuperscript{66} Many strategies to prepare vesicular liposomes \textit{via} self-assembly of amphiphilic structures have been developed. Principal synthetic approaches to multivalent aggregates include: i) direct self-assembly of appropriate amphiphilic molecules, for example based on poly(ethylene glycol), peptides and/or alkyl linkers with long hydrocarbon chains; and ii) incorporation of amphiphilic molecules with suitable lipid matrices at optimal molar ratios (usually ca. 1-10\%). The classical synthetic steps of liposome preparation involve the following four basic stages: i) drying down lipids from organic solvent; ii) dispersing the lipid in aqueous media; iii) purifying the resultant liposome; and iv) analyzing the final product.\textsuperscript{67} The diameter of liposomes can
be reduced by extrusion, homogenization or sonication. For some specific applications, liposomes have to be smaller than 100–150 nm, they can thus be achieved by sterile filtration (0.2 mm filters). For larger liposomes, the whole process must be performed aseptically although, in some cases, heat sterilization can be applied.68

1.4.2.3. Properties and applications
Following self-assembly, the liposomes possess compositional variability and interesting structural properties, for example, biocompatibility, biodegradability, low toxicity, and high capacity for drug encapsulation, leading to potentially enhance therapeutic activity. Liposomes have thus been extensively studied as drug delivery systems. Research on liposome systems has expanded considerably over the last 30 years. It has found increasing applications from carriers for numerous molecules as therapeutics to diagnostics, cosmetics, farming and chemical industry. Thereby, liposomes have definitely established their position in modern technology.51,67

1.4.3. Gold-surface functionalization

1.4.3.1. Introduction
Gold nanostructured materials are of great interest due to their favorable characteristics including facile preparation, high chemical and physical stabilities, unique optical properties and intrinsic biocompatibility.69 In addition, gold nanostructures can be readily surface functionalized with a variety of molecular moieties. For example, biomolecular targeting or fluorescent probes via the chemistry of the gold-thiol covalent bond formation have been developed and used in a wide range of applications.70-72 Moreover, the controllable optical properties of gold nanostructures are not restricted to single components but are also applied to composite nanostructures.70 This provides new directions in plasmon-enhanced applications, which is a growing research field.73,74

1.4.3.2. Preparation
In general, three major approaches have been developed to the surface functionalization of gold nanostructured materials. First, the one-pot approach involves the usage of thiol-modified ligands as capping ligands during the formation of gold nanoparticles. The second alternative is a post-synthetic ligand exchange protocol, in which gold nanoparticles-coated with a protective ligand is firstly prepared. Subsequently, the surface ligands are exchanged with the desired thiol-terminated ligands. The third protocol involves in situ coating during the formation of gold nanoparticles. For example, to functionalize gold nanoparticles with carbohydrates, the free hemiacetal-terminated carbohydrate ligands can act as mild reducing agents, which could form nanoparticles by reducing the gold salt. Recently, light-assisted gold surface functionalization
has been extensively explored. For example, a simple and versatile photo-initiated coupling protocol has been developed via light activation of perfluorophenyl azide moiety for surface functionalization.\textsuperscript{51,75-80}

1.4.3.3. Properties and applications

Gold nanostructures have unique optical properties.\textsuperscript{81} The so-called localized surface plasmon resonance (LSPR) absorption is based on the collective oscillation of conducting electrons in metal nanoparticle surfaces upon incident light excitation. The surface plasmon is highly sensitive to the dielectric environment close to nanoparticle surface.\textsuperscript{70,73} The plasmonic characteristics of metal nanostructures principally depend on their material composition, the medium, geometrical parameters, and interparticle distance of coupled particles.\textsuperscript{73} In particular, gold nanoparticles have intense and broad optical absorption, ranging from the visible to near infrared region. Although the principal mechanisms are relatively straightforward, the plasmonic research has only recently been developed to afford applications such as diagnosis, imaging, quantum optics, and particularly nanosensing, etc.\textsuperscript{72} They can overcome many challenges of involving the diffraction limit in conventional optical nanomaterials.\textsuperscript{81} Moreover, the manipulation of surface plasmon-enhanced two-photon luminescence or fluorescence is particularly attractive for biochemical sensing applications. It can provide high sensitivity by effectively repressing background noise, the 3D-imaging ability, and has minimal photodamage.\textsuperscript{82,83}

1.5. Aims of this thesis

The aim of this thesis is to:

- design, synthesis and evaluate glycodendrimers and metalloglycodendrimers for applications in self-assembly and controlled release
- design, synthesize and evaluate drug-conjugated lipids for applications in liposome formation and stimuli-responsive drug delivery
- design, synthesize and evaluate photoprobe for applications in plasmon-induced surface modification

In Chapter 2, a microwave-assisted regioselective deuteration reaction of 2,2'-bipyridine derivatives has been developed. The obtained products could be further used to study the dynamics of metal-ligand complexes. These dynamic systems were investigated based on coordination complexes of Fe\textsuperscript{II}, Co\textsuperscript{II}, Ni\textsuperscript{II}, Cu\textsuperscript{II}, or Zn\textsuperscript{II} with 2,2'-bipyridine-based ligands. This was then applied to the controlled assembly of supramolecular aggregates. The last part of this chapter deals with the design and synthesis of a new family of amphiphilic dendrimer
building blocks with versatile functional end groups. The dynamic self-assembly into supramolecular aggregates was studied.

In Chapter 3, two drug delivery systems based on supramolecular assemblies have been designed and developed. The first system was constructed from glycodendrimers/metalloglycodendrimers. The second system is based on ciprofloxacin-presenting liposomes. These liposomes contained an enzyme-degradable linker allowing controlled drug release. Lastly, a novel concept of plasmonic-induced surface functionalization has been developed using nanostructured gold. A photolabile-protecting group was cleaved upon IR radiation, followed by attachment of the thiolated fluorescent tag onto gold surfaces.
2. Synthesis and dynamics of bipyridine-based dendrimers and their supramolecular assemblies

(Paper I-III)

In this chapter, the microwave-assisted hydrogen-deuterium (H-D) exchange reaction of dimethyl-2,2′-bipyridine derivatives is first described. Second, the compositional self-adaptation of dynamic systems via selective coordination of 2,2′-bipyridine-based ligands to transition metals is discussed. Third, a new family of dynamic, amphiphilic dendrimers/metallo-dendrimers, based on bipyridine-core dendritic scaffolds were designed and synthesized via a convergent synthetic strategy. Their abilities to self-assemble into multifunctional supramolecular structures and controlled disassembly were investigated.

2.1. Microwave-assisted regioselective deuteration of dimethyl-2,2′-bipyridines

2.1.1. Introduction

H-D exchange reactions are important tools in NMR spectroscopy and mass spectrometry analysis. Consequently, methods for regioselective deuterium labeling are highly important for the production of deuterium-labeled reagents. Dimethyl-2,2′-bipyridine derivatives 1-3 (Figure 2.1) are well-known and broadly used as bidentate ligands for metal coordination chemistry, particularly in supramolecular chemistry. The regioselective introduction of deuterium atoms into the 2,2′-bipyridine core, however, remains challenging. Therefore, the development of general, low-cost, high-yielding, and chemo- and/or regioselective deuteration procedures for this class of structures are highly desired. In this context, microwave irradiation has been developed over the last decades to accelerate chemical reactions and enhance their selectivities.

2.1.2. Overview

We designed and developed an efficient regioselective method for the deuteration of 2,2′-bipyridine derivatives including 4,4′-dimethyl-2,2′-bipyridine (1), 5,5′-dimethyl-2,2′-bipyridine (2) and 6,6′-dimethyl-2,2′-bipyridine (3) under microwave irradiation conditions (Figure 2.1).
2.1.3. Deuteration under conventional heating

The H-D exchange reaction of bipyridine 1 was initially investigated for optimization of the reaction conditions under conventional heating conditions. All reactions were performed in D$_2$O for 24 h with variations of inorganic catalysts and loading amounts, as well as the reaction temperature. The results obtained were summarized in Table 2.1. Using Pd/C and Na$_2$CO$_3$ as the catalysts gave no desired H-D exchanged product of 1-d$_6$, even with increased catalytic amounts (Entries 1-6). Increasing the reaction temperature did not lead to any significant differences. The use of 1 M NaOD/D$_2$O catalyst provided slight H-D exchanged product 1-d$_6$ (Entry 7) with no byproducts observed. Although the H-D exchange reaction of compound 1 was ineffective under the conventional heating method, the results obtained suggested that deuteration in 1 M NaOD/D$_2$O is a possible condition (Table 2.1).

Table 2.1 Deuteration of compound 1 using conventional heating method

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst$^a$</th>
<th>Temperature (°C)</th>
<th>Recovery$^b$ (%)</th>
<th>% D$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pd/C</td>
<td>rt</td>
<td>quant</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Na$_2$CO$_3$</td>
<td>90</td>
<td>quant</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Na$_2$CO$_3$</td>
<td>reflux</td>
<td>quant</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Pd/C</td>
<td>reflux</td>
<td>quant</td>
<td>-</td>
</tr>
<tr>
<td>5$^d$</td>
<td>Pd/C</td>
<td>reflux</td>
<td>87</td>
<td>10 (1-d$_1$)</td>
</tr>
<tr>
<td>6$^d$</td>
<td>Na$_2$CO$_3$</td>
<td>reflux</td>
<td>quant</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>NaOD</td>
<td>reflux</td>
<td>quant</td>
<td>9</td>
</tr>
</tbody>
</table>

$^a$10% Pd/C (10 wt%) or Na$_2$CO$_3$ (10 wt%), and 1 M of NaOD/D$_2$O (>100 wt%) were used. $^b$Based on recovered yield. $^c$Deuterium incorporation was determined by $^1$H NMR spectroscopy and ESI-MS. $^d$10% Pd/C (30 wt%) or Na$_2$CO$_3$ (>100 wt%) were used.
2.1.4. Deuteration under microwave irradiation

The H-D exchange reaction of bipyridine 1 was then investigated using microwave irradiation in the presence of NaOD/D$_2$O with reaction temperature and time up to 170 °C and 3 h, respectively. The optimal microwave heating condition was obtained at 170 °C for 15 min, resulting in excellent H-D exchange yield for all six methyl protons with no deuterated bipyridine ring protons observed (Table 2.2, Entry 7). Decreasing the catalytic concentration showed reduced amount of product 1-$d_6$ along with the appearance of a small amount of 1-$d_4$ and 1-$d_5$, indicating a decrease in regioselectivity (Table 2.2, Entries 8-9). Increasing the catalyst concentration failed to improve the H-D exchange yield because of an unexpected explosion.

Table 2.2 Optimization of H-D exchange reaction conditions of compound 1 using microwave irradiation

<table>
<thead>
<tr>
<th>Entry</th>
<th>Time (min)</th>
<th>Temperature (°C)</th>
<th>Pressure (Bar)</th>
<th>% D$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>130</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
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<td>160</td>
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<td>7</td>
<td>15</td>
<td>170</td>
<td>15</td>
<td>97</td>
</tr>
<tr>
<td>8$^b$</td>
<td>15</td>
<td>170</td>
<td>14</td>
<td>78</td>
</tr>
<tr>
<td>9$^c$</td>
<td>15</td>
<td>170</td>
<td>14</td>
<td>82</td>
</tr>
</tbody>
</table>

$^a$Deuterium incorporation was determined by $^1$H NMR spectroscopy and ESI-MS. $^b$0.1 M of NaOD/D$_2$O was used. $^c$0.5 M of NaOD/D$_2$O was used.

2.1.5. Characterization and optimization of reaction conditions

Figure 2.2 shows the $^1$H and $^{13}$C NMR spectra of deuterated product 1-$d_6$ and starting material 1. After H-D exchange, the singlet peak of the methyl groups in compound 1 disappeared. In addition, the methyl carbons showed two extra carbon satellite signals in the $^{13}$C NMR spectrum, which is the characteristic C-D correlation pattern.
Furthermore, ESI-MS analysis was used to identify the desired deuterated product 1-d$_6$ in methanol (Figure 2.3a). The mass spectra showed a substantial product peak [1-d$_6$+Na]$^+$ at m/z 213.1267. The deuteration reaction of bipyridine 2 and 3 were further investigated using optimal microwave heating conditions at 190 °C for 3 h. ESI-MS analysis was used to monitor the exchange reaction in methanol to afford the resulting exchanged products, which are shown in Figure 2.3b and 2.3c.

The product from bipyridine 3 revealed the corresponding peak of [3-d$_6$+Na]$^+$ at m/z 213.1283. Additionally, the $^1$H and $^{13}$C NMR spectra demonstrated efficient deuteration of all six methyl protons in compound 3 with quantitative yield and 93% D incorporation (Scheme 2.1).
On the other hand, ESI-MS analysis of the H-D exchange products from bipyridine 2 showed that the reaction is less regioselective compared with either bipyridine 1 or 3. Various deuterated products were obtained, with compound 2-\textit{d}_4 as the major product (29%) and lower amounts of other deuterated compounds 2-\textit{d}_5 (23%), 2-\textit{d}_3 (19%), 2-\textit{d}_6 (14%), 2-\textit{d}_2 (10%), and 2-\textit{d}_1 (2%), respectively (Figure 2.3b, Scheme 2.2). Several conditions were studied including increasing temperature and/or time. However, no improvement in the regioselectivity was obtained.

Interestingly, the H-D exchange reactions of those three bipyridines were found to take place only at the methyl groups, with no deuteration of bipyridine ring protons. This could be explained by the higher acidity of the protons attached to the \(\alpha\)-carbon of the side-chains, which is one of the most important properties of alkylpyridines. This influence is the strongest for alkyl groups placed at position 2 and 4 of the pyridine ring, which correspond to the position 6 and 4 of 2,2'-bipyridine structures. The reactivity of the \(\alpha\)-protons at the side-chains was reported to decrease in the order of 4-methylpyridine > 2-methylpyridine > 3-methylpyridine (\(pK_a\) 30-37). Correspondingly, the methyl protons at the 6,6' and 4,4'-positions are more reactive in the H-D exchange reaction than the methyl protons at the 5,5'-positions.

2.1.6. Conclusions
A simple and convenient microwave-assisted method was developed, which provided a rapid and regioselective H-D exchange reaction at the methyl protons of the bipyridine structures in a deuterium oxide solution. In particular, isotopically pure \((\textit{d}_6)\)-4,4'-dimethyl-2,2'-bipyridine was obtained in quantitative yield within 15 min.

**Scheme 2.1 Optimal H-D exchange reaction conditions for bipyridine 3.**

**Scheme 2.2 The H-D exchange reaction of compound 2.**
2.2. Dynamic systems based on bipyridine-metal complexes

2.2.1. Introduction

Transition metal-ligand complexes are ubiquitous in chemistry and exhibit large structural diversity. Changing a ligand structure can influence its coordination behavior and hence the physical and chemical properties of the resulting transition metal complex. For example, different substitution patterns of 2,2'-bipyridine ligands are known to produce significant differences of the metal-ligand interactions. Therefore, metal-ligand recognition of dimethyl-2,2'-bipyridine complexes comprising of different methyl substitution patterns is of high interest to study.

2.2.2. Overview

5,5'-Dimethyl-2,2'-bipyridine L1 and deuterium-labeled 4,4'-dimethyl-2,2'-bipyridine L2 were readily prepared (Figure 2.4a), and metal coordination complexes for the first-row transition metals FeII, CoII, NiII, CuII and ZnII with 2,2'-bipyridine-based ligands were synthesized. Subsequently, the resulting dynamic systems for each metal homoleptic complexes were investigated using ESI-MS to allow swift verification of favored coordination for each metal, without the need to separate or isolate the optimal complexes. The kinetic and thermodynamic behaviors of these dynamical ligand-exchange systems were also determined (Figure 2.4b).

![Figure 2.4 a) Chemical structures of 2,2'-bipyridine derivatives and b) dynamic systems of metal-bipyridine complexes.](image-url)
2.2.3. Identification of ligand systems

In principle, to study a component mixture, it needs to be confirmed that the appropriate coordination complexes are analyzable by ESI-MS. A dynamic system of Zn(OTf)$_2$ and Fe(ClO$_4$)$_2$ coordinated with 5,5'-dimethyl-2,2'-bipyridine L1 and stable isotope-labeled ligand of $(d_6)$-4,4'-dimethyl-2,2'-bipyridine L2 were thus investigated. Deuterium labeled L2 was used to facilitate mass spectrometric analysis, where full qualitative and quantitative analysis were possible.

To prepare the homoleptic complexes of the dynamic system in this study, 3 eq. of both ligands were added into the solution of metal ions and the resulting system was subjected to ESI-MS analysis. The ESI-MS spectrum of Zn(L1)$_3$(OTf)$_2$ indicated significant peaks of [Zn(L1)$_3$]$^{2+}$ at m/z 308 and [Zn(L1)$_2$]$^{2+}$ at m/z 216, as well as [Zn(L1)$_2$OTf]$^+$ at m/z 581 as a minor signal (Figure 2.5a). Furthermore, the zinc complex of the isotopic ligand L2 was also studied with the same protocol. The data obtained demonstrated the divalent species of [Zn(L2)$_3$]$^{2+}$ at m/z 317 and [Zn(L2)$_2$]$^{2+}$ at m/z 222, as well as a small peak corresponding to [Zn(L2)$_2$OTf]$^+$ at m/z 593 (Figure 2.5b). Similarly, the spectra obtained from the Fe$^{II}$-bipyridine complexes identified intense peaks from each complex, i.e. [Fe(L1)$_3$]$^{2+}$ at m/z 304 and [Fe(L1)$_2$]$^{2+}$ at m/z 212 in the case of [Fe(L1)$_3$](ClO$_4$)$_2$ (Figure 2.5c), as well as [Fe(L2)$_3$]$^{2+}$ at m/z 313 and [Fe(L2)$_2$]$^{2+}$ at m/z 218 from [Fe(L2)$_3$](ClO$_4$)$_2$ (Figure 2.5d). The data obtained demonstrated that both bis- and tris-bipyridine complexes could be formed with Zn$^{II}$ and Fe$^{II}$ ions under the reaction conditions.
Figure 2.5 ESI-MS spectra of complexes a) Zn$^{II}$-L1; b) Zn$^{II}$-L2; c) Fe$^{II}$-L1; and d) Fe$^{II}$-L2; in methanol (concentration = 50 µM).

Furthermore, $^1$H NMR spectra of individual Zn$^{II}$ and Fe$^{II}$ complexes with L1 and L2 ligands were collected, together with those of free ligands L1 and L2 for comparison (Figure 2.6a-b and 2.6c-d, respectively). All signals from the bipyridine rings were clearly shifted for both the Zn$^{II}$ and Fe$^{II}$ complexes. Especially, the most deshielded signals at around 8.5 ppm shifted the most upfield upon complexation. Thus, the singlet H6/H6'-signal of ligand L1 (Figure 2.6a and 2.6c) and the doublet H6/H6' signal of ligand L2 (Figure 2.6b and 2.6d) showed large shifts to around 7.6, 7.3, 7.7, and 7.2 ppm, respectively. These results indicate that the obtained coordination complexes caused the additional shielding of bipyridine protons by the other neighboring ring systems in the metal-complex structures.
Figure 2.6 $^1$H NMR spectra of complexes a) Zn$^{II}$-L1; b) Zn$^{II}$-L2; c) Fe$^{II}$-L1; and d) Fe$^{II}$-L2 in CD$_2$Cl$_2$ solution.

2.2.4. Dynamics of mixed ligand systems

After the homoleptic Zn$^{II}$- and Fe$^{II}$-complexes were readily determined using ESI-MS and $^1$H NMR spectra, we next focused on studying the dynamic exchange of the different transition metal-bipyridine complexes. In addition to electronic and steric effects of the ligands around the metal center, another key feature, which can influence the equilibrium situation of dynamic ligand exchange, is the differences in solubility of the resulting compositions. However, if the differences in the solubility are sufficiently low, reversible coordination can lead to all available complex compositions. The reversible system between complexes of [Zn(L1)$_3$](OTf)$_2$ and [Zn(L2)$_3$](OTf)$_2$ is presented in Scheme 2.3 as an example.

\[
\begin{align*}
\text{[Zn(L1)$_3$](OTf)$_2$} & \quad \rightleftharpoons \quad \text{[Zn(L1)$_2$(L2)](OTf)$_2$} & \quad \rightleftharpoons \quad \text{[Zn(L1)L(L2)$_2$](OTf)$_2$} & \quad \rightleftharpoons \quad \text{[Zn(L2)$_3$](OTf)$_2$}
\end{align*}
\]

Scheme 2.3 Systemic equilibrium in a mixture of [Zn(L1)$_3$](OTf)$_2$ and [Zn(L2)$_3$](OTf)$_2$. 
Dynamic systems of the Zn$^{	ext{II}}$-bipyridine and Fe$^{	ext{II}}$-bipyridine complexes were first investigated by combining two solutions of the preformed heteroleptic complexes in a 1:1 ratio. The resulting solution was then analyzed using ESI-MS and $^1$H-NMR to determine the equilibrium time of the dynamic system in ambient settings. For the Zn(II)-bipyridine system, the mass spectra of the heteroleptic complex solution after 5 min (initial state) and after 2 h (at equilibrium) were recorded. The resulting coordination complexes of tris-bipyridine-Zn$^{	ext{II}}$ species could then be identified with distributed signals demonstrating the complex species (Figure 2.7a). Using the same preparative procedure, the heteroleptic complex solution from the Fe(II)-bipyridine system was also analyzed. This experiment implied that the time required to reach equilibrium was approximately 25 h (Figure 2.7c). The equilibrium time could be verified by comparative $^1$H NMR studies (Figure 2.7b and 2.7d).

![Figure 2.7 ESI-MS and $^1$H-NMR spectra of mixtures between a)-b) [Zn(L1)$_3$](OTf)$_2$ and [Zn(L2)$_3$](OTf)$_2$ after 2 h; and c)-d) [Fe(L1)$_3$](ClO$_4$)$_2$ and [Fe(L2)$_3$](ClO$_4$)$_2$ after 25 h in methanol solution.](image)

Similarly, in order to study the dynamics of the heteroleptic complex solution, pre-formed homoleptic complexes of bipyridine ligands (L1 and L2) with NiCl$_2$·6H$_2$O, CoCl$_2$·6H$_2$O and Cu(OTf)$_2$ were prepared. Subsequently, the ligand exchange reactions upon mixing the homoleptic complexes was monitored by ESI-MS. The reversible exchange times were observed, in comparison to the cases of Zn$^{	ext{II}}$- and Fe$^{	ext{II}}$-complexes, resulting in equilibrium...
times of 440, 320, and 150 min for those metals, respectively. Fe\textsuperscript{II}-based system was observed to take the longest time to reach equilibrium, and equilibrium times of the other metals followed the order of Zn (shortest) < Cu < Co < Ni < Fe (longest).

Moreover, the relative distribution of the resulting four complex components in each mixture of coordination complexes was also determined when the systems had reached equilibrium. Because the complexes containing ligand \textbf{L2} appeared more dominant in the ESI-MS spectra than those formed from ligand \textbf{L1}, a correction factor (\(\alpha\)) was thus used to compensate for the underrepresented intensities of the species composed of ligand \textbf{L1}. This investigation showed pronounced differences in the component distribution with variation of the metal core (Figure 2.8). The \([\text{Zn(L)}_3]\textsuperscript{2+}-complexes showed a distribution of approximately 1:4:4:1, with a relative preference for ligand \textbf{L1} compared to \textbf{L2}. A similar distribution was detected for the \([\text{Co(L)}_3]\textsuperscript{2+}-system, however in this case favoring ligand \textbf{L2}, whereas the homoleptic species were more pronounced in the \([\text{Fe(L)}_3]\textsuperscript{2+} and \([\text{Ni(L)}_3]\textsuperscript{2+} systems (2:3:3:2). The Fe\textsuperscript{II} showed a preference for ligand \textbf{L2}, on the other hand, Ni\textsuperscript{II} slightly preferred for \textbf{L1}. The Cu\textsuperscript{II}-systems displayed the largest differences between the ligands, which showed a significant preference for ligand \textbf{L2} over \textbf{L1} with an estimated complex distribution of 2:8:9:1. Consequently, the dynamics of the systems varied both with respect to overall equilibration time and complex distribution. These might be due to the differences in the preferred coordination geometry of each metal center and/or the steric and electronic effects in the different substitution patterns of both ligands.

![Figure 2.8 Normalized intensities for \(M\textsuperscript{II}/\textbf{L1} and \textbf{L2} (M; Zn, Fe, Cu, Co, and Ni), determined after equilibrium was reached for \([M(\text{L1}/\text{L2})_3]\textsuperscript{2+}; \quad [M(\text{L1})_3] \quad (turquoise), \quad [M(\text{L1})_2(\text{L2})] \quad (green), \quad [M(\text{L1})(\text{L2})_2] \quad (blue) and \quad [M(\text{L2})_3] \quad (red).}](image-url)
2.2.5. Kinetics of the dynamic systems

The exchange kinetics of each coordination system was further evaluated. Starting with the most labile species Zn(OTf)$_2$, the peaks for the ligand exchange products [Zn(L1/L2)$_3$]$^{2+}$ and [Zn(L1/L2)$_2$]$^{2+}$ were identified at $m/z$ 308-317 and 216-222, respectively. Over the course of the analysis, the relative peak intensities for the major peaks [Zn(L1/L2)$_3$]$^{2+}$ ($m/z$ 308, 311, 314, 317) reached plateaus as shown in Figure 2.9a. The rate constants for the four different species could be estimated to be $k_{Zn} = 0.6$-$2.5 \times 10^{-2} \text{M}^{-1}\text{min}^{-1}$ using CopasiUI (differential evolution method, second order reaction) with the system reaching a final equilibrium within 2 h (Table 2.3).

Subsequently, the exchange kinetics of the Fe$^{II}$ system was also investigated. The predicted mass peaks of [Fe(L1/L2)$_3$]$^{2+}$ and [Fe(L1/L2)$_2$]$^{2+}$ were found at $m/z$ 304-313 and 212-218, respectively. The relative peak intensities of the complexes ($m/z$ 304, 307, 310, 313) as functions of time is plotted in Figure 2.9b. The rate constant was calculated to be $k_{Fe} = 0.12$-$3.63 \times 10^{-3} \text{M}^{-1}\text{min}^{-1}$, which was considerably lower than the Zn$^{II}$ system (Table 2.3).

The investigation was additionally extended to other transition metals (Co$^{II}$-, Ni$^{II}$-, and Cu$^{II}$-bipyridine complexes) to explore the dynamic systems under the same conditions used for Zn$^{II}$ and Fe$^{II}$. The resulting time course graphs are showed in Figure 2.10, and the obtained kinetic data are summarized in Table 2.3. The Ni$^{II}$ system displayed the relatively similar behavior to Fe$^{II}$ with a higher exchange rate ($k_{Ni} = 0.27$-$1.94 \times 10^{-3} \text{M}^{-1}\text{min}^{-1}$), and the overall time to reach equilibrium was approximately 440 min. The Co$^{II}$ system showed a comparable behavior to what was observed for the Zn$^{II}$-complexes, although the rate constant was considerably lower ($k_{Co} = 0.38$-$3.45 \times 10^{-2} \text{M}^{-1}\text{min}^{-1}$). When Cu$^{II}$ was used as the metal center, the observed behavior was different from the other metals. The resulting analysis indicated partial irreversible
exchange between the complexes \((k_{Cu} = 0.16 \times 10^{-1} \text{ M}^{-1}\text{min}^{-1})\), although the time required to reach equilibrium was slightly longer than the Zn\textsuperscript{II} system. The obtained results demonstrate that all complex systems can reach equilibration under the selected conditions, and indicate the possibility of tuning the exchange behavior with different metal centers.

Figure 2.10 Time course graph of normalized intensity observed for \(M:\text{L1:~L2}, \) indicated for \([M(\text{L1/L2})_3]^{2+}, [M(\text{L1})_3]\) (turquoise), \([M(\text{L1})_2(\text{L2})]\) (green), \([M(\text{L1})(\text{L2})_2]\) (blue), and \([M(\text{L2})_3]\) (red).

The above studies indicate that ESI-MS could be used to determine the time taken to reach equilibrium and monitor the ligand exchange process, as well as to estimate the individual rate constant of the metal-complex system. The final complex distributions of the complexes could thus be obtained.

Table 2.3 Kinetic data for bipyridine-metal dynamic systems

<table>
<thead>
<tr>
<th>Metal</th>
<th>Equilibrium time (min)</th>
<th>Kinetic rate ( (k) ) ( (\text{M}^{-1}\text{min}^{-1})^{a} )</th>
<th>( \alpha^{b} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe\textsuperscript{II}</td>
<td>1500</td>
<td>(0.12-3.63 \times 10^{-3})</td>
<td>0.40</td>
</tr>
<tr>
<td>Zn\textsuperscript{II}</td>
<td>120</td>
<td>(0.60-2.54 \times 10^{-2})</td>
<td>0.81</td>
</tr>
<tr>
<td>Cu\textsuperscript{II}</td>
<td>150</td>
<td>(0-1.6 \times 10^{-1})</td>
<td>2.63</td>
</tr>
<tr>
<td>Co\textsuperscript{II}</td>
<td>320</td>
<td>(0.38-3.45 \times 10^{-2})</td>
<td>1.35</td>
</tr>
<tr>
<td>Ni\textsuperscript{II}</td>
<td>440</td>
<td>(0.27-1.94 \times 10^{-3})</td>
<td>0.90</td>
</tr>
</tbody>
</table>

\( ^{a}\)Estimated using CopasiUI with differential evolution method. \(^{b}\)Correction factor based on ESI-MS intensities. \(^{c}\)Data analysis resulting in partial irreversible exchange.
2.2.6. Conclusions

Dynamic coordination systems of the different transition metals and analogous bipyridine ligands were examined using ESI-MS and $^1$H NMR analysis. The equilibration rate of the systems were dependent on the metal species, in the order of Zn$^{II} >$ Cu$^{II} >$ Co$^{II} >$ Ni$^{II} >$ Fe$^{II}$, where Zn$^{II}$ undergoes the most rapid ligand exchange process. Some differences in the ligand preference were observed, with the more sterically hindered 5,5′-dimethyl-2,2′-bipyridine showing a slightly preference in complexing with Zn$^{II}$ and Ni$^{II}$. On the other hand, the other metals showed the preferred coordination with the 4,4′-dimethyl-2,2′-bipyridine ligand. The resulting dynamic systems were followed over time and the kinetic and thermodynamic behaviors were thus assessed. The results herein should have significance for studies of dynamic metal-enhanced supramolecular assemblies, as well as improved understanding of metal coordination chemistry in higher-order complex systems.

2.3. Design and synthesis of amphiphilic dendrimers toward multifunctional supramolecular assemblies

2.3.1. Introduction

The molecular structures of dendrons and dendrimers are typically prepared by a combination of convergent and/or divergent approaches. Their synthesis is highly tunable using straightforward organic synthesis$^{47-49}$ and thus dendrimers with different properties can be prepared.$^{56-58}$ Amphiphilic dendrimers have been seen as good candidates to create functional materials via self-assembly processes.$^6$ Their reversible binding is controlled by responsiveness to external stimuli.$^{100,101}$ This has resulted in a wide range of potential applications for dendrimers, especially in the fields of drug delivery, biomedicine and nanotechnology.$^6,102$ In spite of the tremendous achievements with traditional dendrimers,$^{103}$ the development of useful dendrimers remains a challenge.$^{15,18}$

Herein, we focused on the design and development of a new family of prototypical dendritic building blocks consisting of multiple functional head groups, which allow for further functionalization.$^{50,104}$ Furthermore, the driving forces such as hydrophobic effect, hydrogen bonding, and especially metal coordination were pursued for construction of supramolecular assemblies.

2.3.2. Overview

We designed a new family of amphiphilic structures consisting of polyamide branches bearing alkyne end groups as shown in Figure 2.11a. The self-assembly of these dumbbell-shaped amphiphilic molecules into supramolecular nanostructures was studied. These experiments were performed following two pathways: metal coordination and self-assembly (Figure 2.11b).
2.3.3. Synthesis of dendrons/dendrimers

Two types of amphiphilic dendrimers were synthesized using the convergent synthetic approach. Both types are based on polyamide-containing, alkyne-functionalized building blocks. The polyamide interior was made as follows: first ethylenediamine was monoprotected using Boc anhydride to give compound 4; Michael addition of 4 on acrylic ester yielded 5, which was converted by amidation with ethylenediamine to provide 6 as the first generation dendron. The second generation dendron 8 was synthesized by repeating the Michael addition and amidation steps on 6. Both dendrons were achieved in excellent yields as presented in Scheme 2.4.
Scheme 2.4 Synthesis of hydrophilic polyamide interiors (compounds 6 and 8).

The synthesis of the hydrophobic alkynyl exterior 12 started by esterification of benzoic acid 9, giving ester 10. This ester was subjected to reaction with propargyl bromide to give compound 11. Hydrolysis of ester 11 resulted in the desired exterior precursor 12 in good yield (Scheme 2.5).

Scheme 2.5 Synthesis of hydrophobic dialkyne exterior precursor 12.

Amide coupling of the interiors 6 or 8 with the exterior 12 afforded Boc-protected amphiphilic dendrons 13 or 14. Afterwards, the Boc was removed to give the DN1 and DN2 dendrons in good yields (Scheme 2.6).
Finally, the desired dendrimers DM1 and DM2 were obtained by amide coupling of dendrons (DN1 or DN2) to 2,2′-bipyridine-5,5′-dicarboxylic acid. Using this approach, both dendrimers could be obtained in moderate yields (Scheme 2.7).

2.3.4. Metal-dendritic ligand coordination

A new family of metalodendrimers was synthesized by metal coordination of the bipyridine-based dendritic ligands with iron (1:3, metal/ligand). The resulting Fe(II)-dendrimer complexes DM1-Fe and DM2-Fe were obtained in excellent yields (Scheme 2.8).
Scheme 2.8 Synthesis of metallo-dendrimers DM1-Fe and DM2-Fe.

The obtained metallo-dendrimers were investigated by UV-Vis spectroscopy. A comparison between the absorption spectra of both free dendritic ligands and the Fe(II)-dendrimer complexes are shown. The intense absorption band in the region of 500-600 nm, which represents the characteristic band of metal-ligand charge transfer, was observed.\textsuperscript{104,105} These effect of the metal-ligand coordination was also visible with the naked eye as the color changed from pale yellow to deep purple after the addition of Fe(II) (Figure 2.12).

Figure 2.12 (a) Schematic representation for the metallo-dendrimer synthesis for the supramolecular self-assembly; UV-Vis spectra in methanol for (b) DM1 and DM1-Fe; and (c) DM2 and DM2-Fe.
2.3.5. **Supramolecular self-assembly**

The self-assembly of the resulting dendrimers DM1 and DM2 and the metalloendrimers DM1-Fe and DM2-Fe were further investigated in aqueous solution using dynamic light scattering (DLS), transmission electron microscopy (TEM), and scanning electron microscopy (SEM) (Figure 2.13).

![Figure 2.13 Schematic representation the supramolecular self-assembly pathways of the dendritic ligands and their metalloendrimers.](image)

**Dynamic Light Scattering (DLS)**

DLS was employed to investigate the hydrodynamic size distribution of the amphiphilic dendrimer and their analogous metalloendrimer assemblies, and the results are shown in Figure 2.14. In summary, dendrimers DM1 and DM2 showed a relatively broad size distribution with the average hydrodynamic diameter of approximately 125 nm and 200 nm, respectively. The metalloendrimers DM1-Fe and DM2-Fe had an average diameter of approximately 155 nm and 190 nm, respectively. Overall, the DLS studies show an increase in the hydrodynamic radius when the dendritic generation was increased and/or the metal coordination was introduced. The metal-mediated self-assembly is probably due to the change in dendritic structural geometry or their structural orientation, leading to an increase in their hydrodynamic diameters.
Transmission Electron Microscopy (TEM)

Further characterization of the supramolecular assembly of the dendritic structures and metallo-dendrimers was provided by TEM as shown in Figure 2.15. TEM images revealed the formation of spherical assemblies with average diameters of approximately 115 ± 15 nm for DM1 (Figure 2.15a), 115 ± 35 nm for DM2 (Figure 2.15b), 150 ± 50 nm for DM1-Fe (Figure 2.15c) and 175 ± 25 nm for DM2-Fe (Figure 2.15d). A relative size difference from the DLS and TEM were observed (Figures 2.14 and 2.15). This could be explained by the fact that DLS measures the hydrodynamic radius while the samples were in the solvated state, whereas for TEM, the samples were under high vacuum.18,100,101,103,106-108
Figure 2.15 TEM images of (a) DM1; (b) DM2; (c) DM1-Fe; and (d) DM2-Fe.

Scanning Electron Microscopy (SEM)

The morphology and assembly behavior of the dendrimer assemblies were also confirmed by SEM images as shown in Figure 2.16. For DM1 and DM2, the micrographs revealed spherical assemblies with average diameters of 100 nm to 250 nm in diameter as shown in Figure 2.16a-b, respectively. In the case of metallo-dendrimer assemblies, the SEM images showed assembled particles, ranging from 100 nm to 800 nm in diameter (Figure 2.16c-d). Furthermore, the presence of the concave feature in particles of DM2-Fe was observed. This may indicate that the spherical assemblies could be hollow.

Figure 2.16 SEM images of (a) DM1; (b) DM2; (c) DM1-Fe; and (d) DM2-Fe.
In summary, the self-assembly of the dendrimers was demonstrated by the increase in sizes of metallodendrimer assemblies in comparison to the dendrimer analogues.

2.3.6. Biocompatible ligand competition for supramolecular disassembly

As these dendrimer assemblies are made through metal coordination, we envisioned that competitive binding might result in disassembly. To test this a competitive ligand, ethylenediaminetetraacetic acid (EDTA, 1.0 eq.) was added into the dendrimer solution to disturb the assembly. The UV-Vis results demonstrated a decreased absorbance of the MLCT band at 500-600 nm. This indicated the decomplexation of the bipyridine ligands (Figure 2.17a-b). The decomplexation for DM2-Fe is more efficient than DM1-Fe, probably due to the steric hindrance affected by the more branched ligand DM2, giving a less stable DM2-Fe. Moreover, it was clearly visible that the solution changed color after addition of the EDTA ligand, supporting the decomplexation of supramolecular assemblies (Figure 2.17c).

![Figure 2.17 Absorption spectra of (a) DM1-Fe; (b) DM2-Fe; and (c) photographs of (I) DM1-Fe, (II) DM1-Fe+EDTA, (III) DM2-Fe, and (IV) DM2-Fe+EDTA in methanol solution.](image)

2.3.7. Conclusions

A new class of dynamic dendritic structures based on a bipyridine core functionalized with alkynyl aryl ether groups was developed. These dendritic structures could be easily accessed using a convergent synthetic strategy and straightforward metal coordination. We have shown that these developed dendritic scaffolds could self-assemble into supramolecular nanostructures using DLS, TEM, and SEM. The supramolecular assemblies prepared from iron coordination could be successfully disassembled via a controlled ligand-
competitive decomplexation. These findings revealed that the metal coordination not only provides a flexible method for supramolecular construction, but also facilitates the controlled disassembly. Moreover, the presence of alkyne end groups could be of advantage in further surface coupling/functionalization for a wide variety of applications.
3.
Synthesis and applications of multivalent glycodendrimers, liposomes and gold nanoislands

(Paper IV-VI)

The development of nanoscience has progressed rapidly in recent years, with a diverse array of nanomaterials having been developed for a wide range of functional systems.\textsuperscript{38,73} For biomedical sciences in particular, the improved understanding of how nanosystems can be modified and how reactivity can be tuned by introduction of specific functionalities has provided a revolution for fields such as drug delivery. For example, a formulation system with built-in controlled-release nanocarriers capable of targeted drug delivery present many advantages over conventional drug carrier systems. Possible advantages include enhanced potency, minimized toxicity and improved convenience for patients. However, the formulation of drug delivery systems possessing controllable targeting and drug release functionality under physiological condition is still a great challenge.\textsuperscript{5} Furthermore, the research on plasmonic nanodevices and nanostructures has rapidly developed, affording potential applications for bionanosensing and imaging. Among them, gold is a well-investigated material with highly versatile and tunable properties. Gold nanostructures are thus of great interest for the fabrication of plasmonic nanostructures.\textsuperscript{70,71}

In this chapter, two drug delivery systems based on glycodendrimers and liposomes were developed. Firstly, we designed and synthesized a new family of surface-functionalized dendrimers with three analogous carbohydrate ligands that serve as bacterial targeting moieties. Secondly, cholesterol-conjugated ciprofloxacin with an enzyme-sensitive linker were prepared to form liposomes functioning as multivalent drug delivery vesicles. Lastly, plasmon-enhanced two-photon excitation for gold surface functionalization under laser irradiation was developed through covalent gold-thiol interaction with a fluorescent probe. The synthetic procedures, characterizations, and self-assembly conditions are described, and the assembled nanostructures have been studied with regard to specific biomedical applications.
3.1. Self-assembly of supramolecular glycodendrimers

3.1.1. Introduction

Inspired by the self-organization in nature, assembly of synthetic building blocks into hollow spherical nanostructures is a key strategy for the design of delivery vehicles.\textsuperscript{4,6} In this context, glycodendrimers/metalloglycodendrimers have been pursued for the controlled fabrication of supramolecular assemblies.\textsuperscript{50} Multivalent glycodendrimers capable of interaction with proteins can be used for targeting and controlled drug release.\textsuperscript{51,65} However, construction of coordination systems with sensitivity to external stimuli remains challenging.\textsuperscript{16} Here, Fe(II)-driven supramolecular self-assembly of bipyridine-based glycodendrimers has been proposed as a model system for constructing supramolecular nanocarriers to deliver bioactive entities into cells.\textsuperscript{50,54}

3.1.2. Overview

A novel family of bipyridine-based glycodendrimers with terminal carbohydrate moieties was synthesized as shown in Figure 3.1. The resulting dumbbell-shaped dendritic structures were self-assembled into functional supramolecular aggregates following two strategies: i) direct self-assembly by intermolecular interactions; and ii) metal coordination-triggered self-assembly (Figure 3.2). These assemblies were subsequently subjected to loading of pyrene, as well as pharmacologically important drugs such as ciprofloxacin (CIP) and camptothecin (CPT). Finally, stimuli-triggered and controlled release of guest molecules was investigated.
3.1.3. Synthesis of glycodendrimers

Synthesis of GDM1-Man, GDM1-Glc, and GDM1-Gal (generation I)

A convergent approach was applied for the syntheses of the desired glycodendrimers from commercially available starting materials (generation I and II). First, for glycodendrimers of generation I, the amide-branched interior 15 was readily prepared from ethylene diamine and benzylic acid 9 to provide the required branch 15 in high yield. Three acetylated carbohydrate analogues with D-mannose, D-glucose and D-galactose moieties were used to synthesize...
the carbohydrate derivatives 17-19, which contained azido-linker 16 for final dendritic exterior functionalization (Scheme 3.1).  

**Scheme 3.1 Synthesis of alkyne-functionalized benzoic amide derivative 15 and acetylated carbohydrate derivatives 17-19.**

Subsequently, alkyne-functionalized benzoic amide-branched 15 was coupled with carbohydrate-azide derivatives 17-19 via copper-catalyzed alkyne-azide cycloaddition in the presence of CuI in basic solution to afford the acetylated glycodendrons 20-22. The free primary amines of bipodal glycodendrons 23-25 were obtained after Boc-cleavage. Following amide coupling with acyl chloride core 26 in the presence of base, the acetylated glycodendrimers 27-29 were obtained in 47% to 78% yields. Finally, the protecting groups on the carbohydrate moieties were removed with NaOMe in methanol to provide access to the desired glycodendrimers (generation I) in moderate to high overall yields (Scheme 3.2).
Scheme 3.2 Synthesis of glycodendrimers generation I, GDM1-Man, GDM1-Glc, and GDM1-Gal.

Synthesis of glycodendrimer generation II, GDM2-Man

The branched layers could be synthesized as follows. Reduction of compound 11 with lithium aluminium hydride gave compound 30 in 80% yield. Subsequent chlorination of alcohol 30 with thionyl chloride yielded compound 31 in 97% yield. Nucleophilic substitution of 31 with dihydroxyl ester 10 under basic conditions gave compound 32. Finally, reduction of ester 32 with lithium aluminium hydride gave compound 33. The synthetic route toward the 2,2′-bipyridine core was conducted by bromination of compound 2 in the presence of N-bromosuccinimide and AIBN in CCl₄ to afford compound 34 (Scheme 3.3).
Scheme 3.3 Synthesis of branched interior 33 and dibromo-2,2′-bipyridine 34.

The acetylated glycodendron 35 could be accessed through copper-catalyzed alkyne-azide cycloaddition of branched interior 33 and acetylated α-mannose derivative 17 comprising an azido-linker in 97% yield. Ether bond formation of bipodal glycodendron 35 with a central core of 5,5′-dibromo-2,2′-bipyridine 34 under basic condition yielded acetylated glycodendrimer 36 in 97% yield. Finally, deacetylation of the mannoses with NaOMe in methanol provided glycodendrimer GDM2-Man in 85% yield (Scheme 3.4).
Scheme 3.4 Synthesis of glycodendrimer generation II, GDM2-Man.

Synthesis of metalloglycodendrimers, GDM1-Man-Fe and GDM2-Man-Fe

GDM1-Man, GDM1-Glc, GDM1-Gal and GDM2-Man were obtained with 19%, 31%, 13% and 46% overall yields, respectively. 1H and 13C NMRs, ESI and MALDI-TOF MS analysis were in full agreement with their structures. The resulting glycodendrimers GDM1-Man and GDM2-Man were then subjected to metal coordination with Fe(II) (1:3, metal/ligand). This resulted in Fe(II)-glycodendritic complexes GDM1-Man-Fe and GDM2-Man-Fe (Scheme 3.5). The maximal absorption of GDM2-Man ligand was at 286 nm, but red shifted to 305 nm in the case of GDM2-Man-Fe. The presence of a metal-ligand charge transfer band at 530 nm further implied successful complexation (Figure 3.3).
**Scheme 3.5** Formation of Fe(II)-glycodendrimer coordinative complexes, GDM1-Man and GDM2-Man. Reagents and conditions: (a) Fe(ClO$_4$)$_2$·xH$_2$O, DCM/MeOH (9:1), rt, 93% GDM1-Man-Fe, 95% GDM2-Man-Fe.

**Figure 3.3** UV-Vis absorption spectra of GDM2-Man and GDM2-Man-Fe in methanol ($10^{-4}$ M).

### 3.1.4. Supramolecular self-assembly

Mannose functionalized glycodendrimer generation II (GDM2-Man) and its Fe(II)-glycodendrimer analogue (GDM2-Man-Fe) were chosen for further investigation. The ability of dendrimers to self-assemble into supramolecular aggregates was studied (Figure 3.4). Subsequently, the aggregation behavior in aqueous solutions was evaluated using DLS, TEM and SEM, as described below.
Dynamic light scattering (DLS)

The DLS measurements provided important insights into the size distribution of the glycodendrimer assemblies. **GDM2-Man** revealed two clusters of particle size ranges. A sharp peak with relatively narrow size distribution and low polydispersity was observed, showing an average hydrodynamic diameter of approximately 260 nm. A broad peak with an average hydrodynamic diameter of 3,500 nm was also observed, indicating larger aggregates of higher polydispersity (Figure 3.5a). In comparison, **GDM2-Man-Fe** showed a single species with an average hydrodynamic diameter of approximately 250 nm with a relatively narrow size distribution (Figure 3.5b). This implies more homogeneous assemblies than with **GDM2-Man**. Moreover, zeta potential measurement can predict particle stability, where a large negative or positive zeta potential corresponds to high stability of systems. The zeta potential of **GDM2-Man** was -42 mV, indicating good stability of glycodendrimer assemblies.

![Figure 3.4](image1.png)

**Figure 3.4.** Two self-assembly pathways toward supramolecular aggregates.

![Figure 3.5](image2.png)

**Figure 3.5 Hydrodynamic size distributions of (a) GDM2-Man and (b) GDM2-Man-Fe.**
Transmission electron microscopy (TEM)

Further evidence for the morphology of the GDM2-Man and GDM2-Man-Fe assemblies was provided by TEM experiments. As shown in Figure 6, TEM images revealed the formation of spherical assemblies with diameters ranging from 150 to 200 nm for both glycodendrimer assemblies, which corresponded to the main peaks in the DLS. Furthermore, formation of small spherical aggregates for GDM2-Man was observed as shown in Figure 3.6a-b, and the dispersity was rather high according to the DLS and TEM results. Conversely, GDM2-Man-Fe was more uniform, well-formed aggregates of spherical morphology. Moreover, these assemblies showed a clear contrast between the interior and periphery, especially for GDM2-Man-Fe (Figure 3.6c-d), which indicates the possibility of a hollow structure for the spherical assemblies. As expected, the differences in size of resulting assemblies between DLS and TEM results were observed.

![TEM images](image)

Figure 3.6 TEM images of (a-b) GDM2-Man and (c-d) GDM2-Man-Fe.

Scanning electron microscopy (SEM)

The investigation of the glycodendrimers was further conducted using SEM (Figure 3.7). The SEM micrographs revealed spherical assemblies with diameters ranging from approximately a few hundred nanometers to a micrometer in GDM2-Man, and of about 200 nm in GDM2-Man-Fe, in accordance with previous results. In addition, the presence of visibly concave features (white arrow, Figure 3.7c) in GDM2-Man-Fe again indicated the possibility of hollow structures. The primary driving force for this unique self-assembly behavior is proposed to be the balance between solvent interactions with the hydrophilic carbohydrate exterior and dispersion interactions within the aryl ether segments, as well as the additional trigger by metal coordination in the case of GDM2-Man-Fe.
3.1.5. Host-guest encapsulation capability and release profiles

Pyrene loading and thermal-triggered release of GDM2-Man assemblies

Due to the possibility of hollow spherical assemblies of GDM2-Man glycodendrimer, the potential encapsulation ability of GDM2-Man host was assessed. Here, a non-polar molecule, pyrene was chosen as a fluorescent guest. This molecule fulfills the requirements regarding for encapsulation due to its hydrophobicity providing efficient binding to the interior hydrophobic parts of the host. In addition, the solubility difference enables a facile separation of host and unencapsulated guest. The initial studies of the guest encapsulation and release were performed in two-phase solution of chloroform/water with uptake and release measured by fluorescence spectroscopy. After the guest uptake (3.6 mM of pyrene, in 1 mL CHCl₃) to a pre-equilibrated aqueous solution of GDM2-Man (0.18 mM, 1 mL, 1 eq.) was carried out at room temperature, the encapsulation capacity was evaluated (Figure 3.8a). As shown in Figure 3.8b, the guest-load percentage was monitored over a period of time. The loading quickly increased over the first 3 days, and gradually reached 99% after a period of approximately 7 days. The amount of guest loaded was determined by comparison to the standard calibration curve of pyrene emission, resulting in a 6.1 μmol of pyrene per mg of GDM2-Man (99% pyrene loading). Furthermore, confocal laser scanning microscopy (CLSM) was used to image the encapsulating. The results are presented in Figure 3.8c, revealing the presence of fluorescent-labeled capsules (blue) that can be compared to non-fluorescent-labeled capsules (gray) as shown in Figure 3.8d. This supported the hypothesis of formation of spherical nanocapsules with guest encapsulation ability.

Figure 3.7 SEM images of (a) GDM2-Man and (b-c) GDM2-Man-Fe.
Figure 3.8 (a) UV-Vis absorption spectrum of GDM2-Man-pyrene encapsulation at room temperature; (b) pyrene loading profile of GDM2-Man ($\lambda_{ex}$ 339 nm, $\lambda_{em}$ 392) in chloroform solution; and CLSM images: (c) fluorescence and (d) overlay bright/fluorescence of pyrene-loaded particles.

We further proceeded to investigate if the assemblies could be used for drug delivery, where guest release would occur upon switching from the “self-assembly” to the “disassembly” forms in the same two-phase solution. Release of pyrene from the host interior into chloroform was accompanied by an increase in fluorescence intensity. Upon heating from 20 °C to 60 °C, the entrapped fluorescent probes were successfully released into the chloroform solution. Rapid release initially occurred at 30 °C, with further release occurring at 60 °C (Figure 3.9). A control experiment was performed in parallel, in which less than 5% of leaked GDM2-Man-pyrene was detected. These results demonstrate that the pyrene guest molecules can indeed be entrapped into glycodendritic hosts. The reversible disassembly to release the guest molecules can also occur in response to an increase in temperature.
Figure 3.9. Temperature-dependent release of pyrene-loaded GDM2-Man ($\lambda_{ex}$ 339 nm, $\lambda_{em}$ 385) in chloroform solution; (a) fluorescence emission spectrum and (b) released percentage of pyrene.

Drug loading and release profiles

After assessing the pyrene release from GDM2-Man, encapsulation of the antimicrobial drug ciprofloxacin (CIP, 0.2 mg/mL, 1 mL) as well as the anticancer drug (S)-(+-)camptothecin (CPT, 0.02 mg/mL, 1 mL) were individually carried out. Similarly, a chloroform solution of each drug was directly added into an aqueous solution of GDM2-Man or GDM2-Man-Fe (1 mg/mL, 1 mL). After three days of incubation, non-entrapped drugs were removed by washing with chloroform several times. UV-Vis and fluorescence analysis were performed on the chloroform-phase samples. According to the calibration curves, the loading capacity was determined to be 0.604 $\mu$mol of CIP and 0.057$\mu$mol of CPT per mg of the glycodendritic host (Figure 3.10).

Figure 3.10 (a) Chemical structures of CIP and CPT; (b) UV-Vis absorption spectra displaying the characteristic peaks of CIP and CPT compared with glycodendritic hosts, GDM2-Man and GDM2-Man-Fe in methanol ($10^{-4}$-$10^{-5}$ M; and (c) loading profiles of CIP and CPT into GDM2-Man and GDM2-Man-Fe glycodendrimer delivery systems in two-phase solution of CHCl$_3$/H$_2$O over 4 d.
The drug release profiles of CIP and CPT from glycodendrimer capsules over 48 h in two-phase solution at two different pH values are demonstrated in Figure 3.11. The results showed that the encapsulation stability is decent, with relatively low drug release in the absence of external stimuli for both glycodendrimer systems. Upon lowering pH to weakly acidic, the release from GDM2-Man increased from 55% to 97% for CIP and from 56% to 65% for CPT (Figure 3.11a). In the case of GDM2-Man-Fe, on the other hand, pH at neutral or acidic conditions showed an insignificant influence on drug release efficiencies with roughly 10% increase (Figure 3.11b). This is probably because of the protonation of the bipyridine moieties in the glycodendrimers, which can disrupt the assemblies, leading to reversible disassembly. In addition, the acidic solution could enhance the water solubility of the drugs, resulting in release to the water phase. CPT is more hydrophobic compared with CIP, providing stronger host-guest interaction.\textsuperscript{114,115} That could explain why CPT release was slower and lower amount in overall than CIP in both glycodendrimer systems.\textsuperscript{116} Moreover, the results obtained indicate that the glycodendrimer delivery system had a modest to excellent pH-responsive behavior. Since the extracellular tumor tissues and intracellular endosomes or lysosomes are acidic environments, the development of pH-triggered drug delivery systems could be of high utility in clinical cancer therapy.\textsuperscript{116,117}

![Figure 3.11](image)

**Figure 3.11** Controlled release profiles of CIP and CPT drugs-loaded glycodendrimer delivery systems in two-phase solution of CHCl\(_3\) and buffer (pH 5.0 and 7.4). (a) Drug-loaded GDM2-Man; and (b) drug-loaded GDM2-Man-Fe nanocarriers.

### 3.1.6. Conclusions

A new class of glycodendrimers and metalloglycodendrimers were developed following a convergent synthetic strategy. The self-assembly into functional supramolecular capsules was investigated using DLS, TEM and SEM, showing spherical morphology with sizes in the hundred-nanometer range. The supramolecular capsules were assessed with respect to guest encapsulation
using a pyrene probe, resulting in efficient uptake. The release of the probe could subsequently be triggered by thermal stimuli, leading to partial release of the guest. Furthermore, these glycodendrimer capsules were applied to well-known antibacterial drug CIP and anticancer drug CPT, and controlled drug release via pH stimuli was obtained. These studies indicated that the developed glycodendrimer nanocarriers could be of potential use as an encapsulation/release delivery system with carbohydrate targeting function for drug delivery and cancer therapy applications.

3.2. Ciprofloxacin-presenting liposomes

3.2.1. Introduction

Fluoroquinolone antibiotics such as ciprofloxacin are broad-spectrum antibiotics for the treatment of serious bacterial infection. They can act against both Gram-negative and Gram-positive pathogenic bacteria. Chemical functionalization and/or modification of the drug have been progressively developed to overcome problematic drug-resistant bacteria. Liposomes have shown potential in addressing the drug-resistance problem, where cholesterol has been principally used as anchor to improve the fluidity and stability of liposomal bilayer, preventing liposome aggregation and minimizing the permeability of hydrophilic molecules through the bilayer of liposomes. The ciprofloxacin structure can be introduced into the liposomal system via chemical conjugation with anchoring moieties. In particular, the use of cleavable linkages is interesting for feasible controlled release of active-drug species.

3.2.2. Overview

To address the challenge of drug-resistance, we aimed to develop a new strategy to accomplish the delivery of ciprofloxacin to the site of bacteria infection. Here, we focused on liposome systems, which give a multivalent drug-presenting structure as well as controllable drug release. The key molecule used for the liposomal construction is a cholesterol-conjugated ciprofloxacin (CIP-Chol), which acts as a prodrug. Dipalmitoylphosphatidylcholine (DPPC) was used as the lipid component for the liposome formation (Figure 3.12).
Figure 3.12 Chemical structures of cholesterol-conjugated ciprofloxacin (CIP-Chol) and dipalmitoylphosphatidylcholine (DPPC).

The multivalent drug-presenting liposomes were prepared in two steps. CIP-Chol was first synthesized, and the drug-functionalized liposomes through cholesterol anchoring of CIP-Chol were then constructed. Finally, ciprofloxacin-presenting liposomes were tested for bacterial-triggered drug release for killing bacteria (Figure 3.13).

Figure 3.13 Overview of multifunctional ciprofloxacin-based liposome system for drug delivery.

3.2.3. Synthesis of CIP-Chol

The cholesterol-conjugated ciprofloxacin (CIP-Chol) could be easily synthesized in one step. The commercially available ciprofloxacin CIP and cholesteryl chloroformate Chol-Cl were allowed to react through nucleophilic substitution under basic conditions. After a simple purification by precipitation
from DCM/methanol, a pale yellow powder of desired CIP-Chol was obtained in 64% yield (Scheme 3.6).

![Scheme 3.6. Synthesis of CIP-Chol prodrug.](image)

**Optical properties of CIP-Chol**

The absorption and emission properties of CIP-Chol in methanol, THF, chloroform, acetone, diethyl ether and water were studied using UV-Vis and fluorescence spectroscopy. The resulting absorption spectra showed absorption bands in the range of 275-280 nm. A slight red shift and significantly increased absorbance was observed in the order of water, diethyl ether methanol, chloroform and THF, respectively. This might be because of the solvent-stabilized transition state of CIP-Chol, leading to bathochromic shifts (Figure 3.14a). In parallel, the emission spectra were also obtained with maximum emissions at around 430 nm. The emission intensity decreased in the order of chloroform, THF, methanol, diethyl ether, acetone, and water solution, respectively. This is possibly because of decreased solubility of CIP-Chol. In addition, a small blue shift (~20 nm) was observed in the case of diethyl ether that might be because of the lack of solvent stabilization compared to other solvents (Figure 3.14b).\(^\text{132}\)
3.2.4. Liposome formation

The ciprofloxacin-presenting liposomes were prepared from saturated DPPC and CIP-Chol via a classical protocol by hydration of a thin lipid film (Bangham method), and membrane dialysis. After the CIP-Chol was synthesized, a different amount of CIP-Chol:DPPC (0.5-5.0 wt%) were mixed in chloroform and gradually evaporated to dryness under vacuum. The resulting thin-film was then resolved in PBS solution (pH 7.4) at room temperature, and left overnight to form a liposomal suspension.

Dynamic light scattering

DLS was used to study the average diameter, size distribution and stability of the liposomes. The resulting liposomes showed a significant difference in diameter over a 30-day period, ranging from several tens of nanometers to a few micrometers. High polydispersities were obtained in the cases of 0.5, 1.0 and 5.0 wt% of CIP-Chol, however a relatively low polydispersity was obtained in 1.5 and 2.0 wt% CIP-Chol systems. The highest liposomal stability was in the case of 2.0 wt% with an approximate diameter of 400-500 nm after storage at ambient temperature for a period of one month (Figure 3.15, Table 3.1).
Figure 3.15 DLS results for liposomes obtained from 10 mg DPPC and a) 0.5 wt%, b) 1.0 wt%, c) 1.5 wt%, d) 2.0 wt%, and e) 5.0 wt% CIP-Chol in 1 mL PBS.

Zeta potential

Another important property of the resulting liposomes is the surface charge. It is principally dependent upon the nature of the liposomal structure and the medium. Moreover, a liposomal suspension with a large negative or positive zeta potential will tend to repulse each other and not aggregate, leading to considerably increased stability. This ciprofloxacin-grafted liposome system was expected to have carboxyl groups on the liposome surface, providing a negative surface charge. The results of the zeta potential measurements are summarized in Table 3.1. According to the obtained zeta potential, the 2 wt% CIP-Chol:DPPC system had the highest stability (Figure 3.16).

Table 3.1 Liposome size and zeta potential as determined by DLS

<table>
<thead>
<tr>
<th>Ratio of CPFX-Chol:DPPC (wt%)</th>
<th>Mean diameter (nm) at 1 d</th>
<th>Mean diameter (nm) at 30 d</th>
<th>Zeta potential (ζ, mV) at 30 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>72±7, 297±18</td>
<td>535±45</td>
<td>-4.01±0.44</td>
</tr>
<tr>
<td>1.0</td>
<td>52±3, 202±7</td>
<td>282±13</td>
<td>-3.99±0.29</td>
</tr>
<tr>
<td>1.5</td>
<td>246±9</td>
<td>423±56</td>
<td>-6.49±1.15</td>
</tr>
<tr>
<td>2.0</td>
<td>400±27</td>
<td>507±24</td>
<td>-22.5±1.07</td>
</tr>
<tr>
<td>5.0</td>
<td>75±4, 675±80</td>
<td>87±4, 2109±195</td>
<td>-1.68±0.27</td>
</tr>
</tbody>
</table>

The 2 wt% CIP-Chol:DPPC demonstrated high stability of the liposomes (Figure 3.16C). Under the same conditions, the pure DPPC liposome system showed a similar behavior (ζ = -7.75±0.45) (Figure 3.16A), whereas the 2 wt% of Chol:DPPC (ζ = -4.54±0.43) showed much higher polydispersity, leading to considerably less stable liposomes (Figure 3.16B). These results suggest that
the presence of **CIP-Chol** in the liposomes affords a steric barrier that could avert liposome aggregations and improve the stability.

![DLS monitoring of liposomes in 1 mL PBS for their stability studies](image)

**Figure 3.16** DLS monitoring of liposomes in 1 mL PBS for their stability studies (A) **DPPC** (100%); (B) 2 wt% **Chol**:10 mg **DPPC**; and (C) 2 wt% **CIP-Chol**:10 mg **DPPC**.

### 3.2.5. Drug-grafted liposome quantitation

The amount of drug on liposomes was determined using $^{19}$F qNMR. After lyophilization, the liposomes were dissolved in CDCl$_3$ for $^{19}$F qNMR analysis. The **CIP-Chol** showed a fluorine signal at -115.3 ppm (Figure 3.17b), whereas the corresponding peak of **CIP-Chol** in the liposome shifted to higher field at -120.19 ppm (Figure 3.17a). This is possibly due to the intermolecular interaction with **DPPC** during the liposome formation. Using 4-fluoroaniline as the internal standard with a fluorine signal at -125.8 ppm, the relative amount of liposomal **CIP-Chol** was calculated to be 0.9 wt% by peak integration comparison (Figure 3.17). This obtained **CIP-Chol**:**DPPC** ratio is in the expected typical range for liposomal formation.
Figure 3.17 $^{19}$F NMR spectra of a) CIP-Chol-presenting liposomes; and b) free CIP-Chol with 4-fluoroaniline standard in CDCl$_3$. The F Peak from CIP-Chol is marked as “∗” and that from the standard is marked as “♦”.

3.2.6. Enzyme-triggered drug release

The drug release capacity and controlled release properties of liposomes are important factors in drug delivery applications. The carbamate linkage of the CIP-Chol prodrug is relatively stable under physiological conditions, however, it responds to esterase stimulus. Therefore, we envisaged that the presence of the extracellular enzymes in particular bacteria could catalyze the cleavage of the carbamate bond, leading to drug release. In particular, cholesterol esterase (CHE, EC 3.1.1.13), which can catalyze the hydrolysis of cholesteryl esters to cholesterol and fatty acid, was expected to enable cleavage the drug-conjugate. CHE is usually found in mammalian tissues, yeasts, fungi and microbial organisms such as Pseudomonas fluorescens, P. aeruginosa, P. mendocina, Streptomyces lavendula, Fusarium, Saccharomyces cerevisiae, Streptomyces sp. X9, Rhodococcus sp.$^{135-138}$ In the case of CHE-lacking bacteria, the enzyme could be loaded with the liposomes for co-delivery (Scheme 3.7).

Scheme 3.7 Lipase-catalyzed hydrolysis of ester linkage in CIP-Chol grafted liposomes.
The kinetic study for the CHE-catalyzed hydrolysis of CIP-Chol was performed using $^1\text{H}$ NMR spectroscopy and ESI-MS spectrometry within 24 h. $^1\text{H}$ NMR spectra obtained are shown in Figure 3.18, in comparison with the hydrolysis products. The kinetic hydrolysis of the prodrug was calculated as the relative amount between CIP-Chol reactant and hydrolyzed ciprofloxacin as a function of time based on the integration of marked peaks in spectra (Figure 3.18).

![Figure 3.18](image)

**Figure 3.18** $^1\text{H}$ NMR spectra for kinetic study of CHE-catalyzed hydrolysis of the carbamate bond of CIP-Chol-grafted liposomes in CDCl$_3$.

As shown in Figure 3.19, the hydrolysis was relatively fast, which was evident in a few hours. Almost complete hydrolysis of CIP-Chol was achieved within 15 h. The obtained results indicate that ciprofloxacin-presenting liposomes enables a triggered drug release by CHE in the bacterial cell efficiently.

![Figure 3.19](image)

**Figure 3.19** CHE-catalyzed hydrolysis of CIP-Chol-grafted liposomes based on $^1\text{H}$ NMR analysis.
3.2.7. Antibacterial activity

The antibacterial properties of the ciprofloxacin-presenting liposomes were evaluated against different bacterial strains. Drug resistant strains of Gram-positive and Gram-negative bacteria including *Escherichia coli* ORN 208, *Staphylococcus epidermidis* 35984, and *Mycobacterium smegmatis mc² 155* were used as model bacteria. The resulting MIC data for free ciprofloxacin, CIP-Chol prodrug and the CIP-Chol-presenting liposomes are shown in Table 3.2. In order to evaluate the liposome activity with regard to both intact CIP-Chol prodrug and CHE-catalyzed hydrolysis of prodrug, samples were also prepared in the absence and presence of added CHE.

The MIC results of the free CIP-Chol prodrug against all three bacterial strains showed decreased antibacterial activity compared to free ciprofloxacin (Table 3.2, Entry 2). The presence of added CHE in this case did not significantly improve the drug activity (Table 3.2, Entry 3). However, the incorporated CIP-Chol in the liposome system exhibited considerably enhanced antibacterial efficacy. 16-20 times improvement for *E. coli* and *S. epidermidis* were observed compared to the free drug. An enhancement effect towards *M. smegmatis* was also recorded with a slight improved activity of approximately 8 times higher than the free ciprofloxacin (Table 3.2, Entries 4-6). Additionally, the MIC data of the CIP-Chol-presenting liposomes against *E. coli* and *S. epidermidis* showed at least 1,024 times enhanced activity (Table 3.2, Entries 4-6), compared to the free prodrug (Table 3.2, Entries 2-3). Similarly, an enhancement for *M. smegmatis* was also observed at least two orders of magnitude (Table 3.2, Entries 4-5). These MIC results indicate that the ciprofloxacin-presenting liposome system was significantly more effective than either the free drug control or the individual CIP-Chol prodrug. The presence or absence of added CHE in the liposome preparations did not provide any significant differences (Table 3.2, Entries 4-6), especially for the *E. coli* and *S. epidermidis* strains. For *M. smegmatis*, a slight improvement showed upon 18 h CHE action, whereas longer incubation lead to lower efficiency. The obtained results imply the possibility of bacteria-promoted hydrolysis of the prodrug linkage together with enhanced drug delivery to the targeted bacteria, compared to the individual prodrug.
Table 3.2 MIC results of ciprofloxacin presenting liposomes with three different bacteria

<table>
<thead>
<tr>
<th>Entry</th>
<th>Sample</th>
<th>E.Coli 208</th>
<th>S. epidermidis 35984</th>
<th>Mycobacterium smegmatis mc² 155</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ciprofloxacin</td>
<td>0.5</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>2</td>
<td>CIP-Chol</td>
<td>N.A.</td>
<td>12.8</td>
<td>N.A. &gt;12.8</td>
</tr>
<tr>
<td>3</td>
<td>CIP-Chol+CHE (18 h)</td>
<td>N.A. &gt;12.8</td>
<td>N.A. 12.8</td>
<td>&gt;12.8</td>
</tr>
<tr>
<td>4</td>
<td>CIP-Chol-LP</td>
<td>0.025</td>
<td>0.0125</td>
<td>0.05</td>
</tr>
<tr>
<td>5</td>
<td>CIP-Chol-LP+CHE (18 h)</td>
<td>N.A. 0.0125</td>
<td>N.A. 0.0125</td>
<td>0.05</td>
</tr>
<tr>
<td>6</td>
<td>CIP-Chol-LP+CHE (7 d)</td>
<td>0.025</td>
<td>0.0125</td>
<td>0.05</td>
</tr>
</tbody>
</table>

3.2.8. Conclusions

We have developed a liposomal drug delivery system, which enhanced the antimicrobial efficacy of ciprofloxacin towards different bacterial strains. The antibiotic entities, covalently conjugated with cholesterol, resulted in structurally stable, multivalent, and ciprofloxacin-presenting liposomes. Antibiotic release was achieved upon cholesterol esterase-catalyzed hydrolysis of the prodrug carbamate linkage. Although the conjugated prodrug showed less effective than the parent drug, MIC evaluation of the drug-presenting liposome system revealed the improvement in efficacy with E. coli ORN208, S. epidermidis 35984 and M. smegmatis mc² 155. Two to three orders of magnitude enhancement and 8-20 times higher activity compared to free ciprofloxacin and the individual prodrug were achieved. These results indicate that antibiotic-cholesterol conjugated-presenting liposome system can be applied for achieving enhancement of drug activities. Furthermore, the liposomal delivery system also enables the introduction of targeting entities for site-specific delivery to resistant bacterial strains.

3.3. Plasmon-assisted surface functionalization of gold nanostructures

3.3.1. Introduction

The advantages of light assistance, and diverse physical and chemical properties of gold nanostructures, especially the light manipulation for their surface functionalization have recently increased attention. Applications such as photothermal polymerization, plasmon-enhanced catalysis, light-induced vapor generation, photothermal therapy, and plasmon-triggered drug release of gold plasmonic nanomaterials have been studied.
3.3.2. Overview

We synthesized a thiol-derivatized fluorescent probe protected with a photolabile group, and applied this to gold-nanoisland films showing plasmonic hot-spots. Subsequently, the laser light with energy corresponding to double the wavelength of the absorption region of probe was applied to the gold surface. This resulted in two-photon excitation triggering cleavage of the photolabile moiety. The free thiol linkage-labeled probe was liberated and spontaneously attached onto the gold nanostructure surfaces (Figure 3.20). Both spectroscopic and microscopic techniques were used for the evaluation.

![Figure 3.20 Schematic representation of laser-induced surface plasmon-assisted surface functionalization of gold nanostructure.](image)

3.3.3. Design and synthesis of fluorescent probe molecule

Photolabile protecting groups bring an interesting feature because they do not require any reagent for cleavage, just light. This category of protecting groups opens the possibility of dealing with extremely sensitive molecules, otherwise incompatible with acids or bases.142 Thereby, photolabile 6-nitroveratryl bromide was selected for fluorescent-labeled thiol chains. It displays a maximum absorption at around 350 nm and thus enables a safer degradation process with a relatively long-wavelength of UV light.143

The synthetic procedure for the desired fluorescent probe, 1,8-naphthalimide derivative (NV-SNaph) is shown in Scheme 3.8. It was prepared in a three-step sequence in relatively high yield. The commercially available 4,5-dimethoxy-2-nitrobenzyl bromide 37 was converted to compound 38 in excellent yield.144 Subsequently, compound 38 was then condensed with the 4-bromo-1,8-naphthalic anhydride 39 to afford compound 40. Finally, the bromine at the 6-position of 40 was directly displaced with piperidine, to give the probe molecule (NV-SNaph) in moderate yield.145
Scheme 3.8 Synthesis of protected 1,8-napthalimide derivative NV-SNaph as fluorescent probe molecule.

3.3.4. Optical properties of fluorescent probe molecule

Analysis of the spectroscopic properties, UV-Vis absorption and fluorescence, of NV-SNaph in chloroform solution was assessed. The thiolated NV 2 as the control devoid of the fluorescence exhibited a maximum absorption band at around 350 nm (Figure 3.21, red line), which indicated a characteristic optical property of the NV moiety. The NV-SNaph probe did not just demonstrate a distinct maximum absorption of the napthalimide moiety at 411 nm (\(\lambda_{\text{abs}}\)) but also a minor absorption band of the NV moiety in the same region compared to compound 2 (Figure 3.21, black line). In addition, the fluorescence spectrum of the probe (inset in Figure 3.21) showed a maximum absorption peak at 513 nm (\(\lambda_{\text{em}}\)). It showed relatively high emission intensity even at low analyzed concentration. These results indicate that NV-SNaph would be an eligible fluorescent probe for further studies.

Figure 3.21 UV-Vis spectra of NV-SNaph (black line) and NV protected thiol linkage 2 (red line) in chloroform solution (10^{-5} M). Inserted fluorescence spectrum of NV-SNaph (10^{-7} M, \(\lambda_{\text{ex}} = 411 \text{ nm}, \lambda_{\text{em}} = 513 \text{ nm}\)).
3.3.5. Photolysis of fluorescent probe molecule

Furthermore, prior to conjugating the NV-SNaph probe to the gold surfaces, the photolytic capacity of NV junction under the influence of UV irradiation with a specific wavelength was investigated. Analysis of the photocleavage in chloroform solution was performed using a photochemical reactor at 350 nm, using NMR and UV-Vis spectroscopy for monitoring. The $^1$H-NMR spectra obtained with the monitoring time from 0 to 60 min UV irradiation at 350 nm are shown in Figure 3.22. Comparatively, the signal around $\delta \approx 4.19$ ppm, arising from the benzylic protons decreased gradually whereas a new peak at 4.10 ppm appeared, indicating the successful UV-triggered cleavage of NV-linker.

![Figure 3.22 $^1$H-NMR spectra of NV-SNaph in CDCl$_3$ before and after irradiation using UV photoreactor (350 nm, $I = 1.8-2.0$ mW/cm$^2$) for 0-60 min.](image)

Similarly, the photocleavage of the NV-SNaph probe using UV-Vis analysis was performed to confirm the photolysis under exposure at 350 nm using a photochemical reactor for 1 h (Figure 3.23). The probe displayed a maximum absorption band of the napthalimide moiety at 411 nm and a minor peak at around 320-340 nm of NV moiety that correspond to the initial study. Upon UV irradiation, the absorption spectra obtained changed significantly. A slow decrease in the absorbance at 411 nm was observed (b), while the absorbance of NV (a) gradually increased, along with a noticeable color change of the solution from bright lime green to pale greenish yellow (insert Figure 3.23). The resulting absorption change could in part be due to oxidation-induced intramolecular aggregation. A slight increase in absorbance at around 325-350 nm was observed, indicating the release of the NV aldehyde moiety.$^{148-150}$ These promising results obtained from both $^1$H-NMR and UV-Vis spectroscopy experiments supported that the NV-protected thiol linkage, NV-
**SNaph** could be cleaved under exposure to UV stimulus, releasing the active **SNaph** for covalent attachment to gold surfaces.

![Absorption spectrum](image)

**Figure 3.23** UV-Vis absorption spectra in CHCl$_3$ (10$^{-5}$ M) under irradiation in UV photoreactor (350 nm, $I=2.8$-3.2 mW·cm$^{-2}$) for 0-60 min. Inserted fluorescence of NV-SNaph (5 mg/3 mL) color changes before (left) and after (right) 350 nm UV irradiation for 30 min.

According to the absorption changes observed after the solution of NV-SNaph was exposed to UV irradiation (Figure 3.23), the photolytic cleavage was evaluated at the absorption wavelength of 345 nm (NV cleaved). The rate constant and half-life were determined to be 5.0±0.6 x 10$^{-2}$ min$^{-1}$ and 14 min, respectively (Figure 3.24).

![Absorbance vs. Time](image)

**Figure 3.24** Cleavage of NV-SNaph at 345 nm (rate constant 5.0±0.6 x 10$^{-2}$ min$^{-1}$, half-life = 14 min).

### 3.3.6. Probe fluorescence evaluation

A qualitative evaluation of the NV-SNaph probe towards gold surface functionalization was initially performed on gold-plated quartz crystals. The gold and quartz surfaces of the substrate were clearly distinguished (Figure...
3.25a). Basically, the gold surface is light absorbing, leading to a dark appearance under UV light (Figure 3.25b). On the other hand, when the fluorescent probe was applied to the quartz crystals and exposed to long-wavelength UV light, lime-green emission appeared on the gold surface as clearly seen by the naked eye (Figure 3.25c).

![Figure 3.25 a) Reference quartz crystals under visible light; b) crystal without NV-SNaph; and c) with adsorbing of NV-SNaph as lime-green layer under UV light.](image)

3.3.7. Gold surface functionalization via light-induced surface plasmon-enhanced two-photon excitation

Quartz crystal gold surfaces

Having confirmed the photolysis of the NV moiety in the NV-SNaph probe molecule, the gold surface functionalization was investigated. Confocal laser scanning microscopy (CLSM) was applied for this evaluation. Substrates of each quartz crystal and the gold-nanoisland substrates capable of surface plasmon effects were coated with the NV-SNaph solution (10 mg·mL⁻¹). Regarding the quartz crystal substrates, the confocal images of substrates in the absence and presence of 650 nm laser light irradiation showed no fluorescence (Figure 3.26b-c), although residual fluorescence was observed before removal of unreacted compound (Figure 3.26a). However, after the 350 nm UV irradiation on the substrates, green emission of the fluorescent probe was observed on the gold surfaces, whereas, almost no fluorescence could be detected on the quartz areas (Figure 3.26d-e). The results imply that the chemical functionalization took place onto the quartz crystal gold surfaces via the thiol-gold ligation.
Figure 3.26 Confocal images of the grafted NV-SNaph on quartz crystal substrates ($\lambda_{ex} = 405 \text{ nm, } \lambda_{em} = 505 \text{ nm}$): a) no irradiation before being washed (10X); b) no irradiation after being washed (63X); c) 650 nm laser irradiation for 30 min and washed (40X); and d)-e) 350 nm UV irradiation for 30 min and washed with magnification 10X and 63X, respectively.

**Gold nanoisland surfaces**

To evaluate the plasmonic effect for gold surface functionalization, the NV-SNaph-coated gold-nanoisland substrates were investigated using CLSM. For the non-irradiated control substrates, a very low degree of physical adsorption was detected following the washing step (Figure 3.27a). In contrast, irradiation using different wavelengths (350 and 650 nm) of light, resulted in large degree of residual fluorescence. This indicates covalent ligation of the fluorescent probe on the gold surfaces. In addition, higher amount and more uniform attachment of probes was obtained in the case of 650 nm laser irradiation (Figure 3.27c-d) compared to the 350 nm UV irradiation (Figure 3.27b). This implies that the plasmon-enhanced two-photon excitation can induce photolysis of photolabile-conjugate fluorescent probe and subsequent gold surface functionalization (Figure 3.27).

The results obtained indicate that the NV moiety is not cleavable by illumination using laser light of 650 nm at featureless substrates due to the light energy being outside the excitation band of the NV moiety. However, irradiation at 650 nm on the gold-nanoisland films produced energy at half the wavelength ($\lambda/2$) of the source, thereby inducing photolysis of the photolabile-conjugated molecules for gold surface functionalization. A bright zone with numerous green spots on the gold surfaces, resulting from the high-energy
light from the gold-nanoislands, can be observed from the CLSM images as 2D averages of z-stacks (Figure 3.27b-c) and 3D visualization (Figure 3.27d).

**Figure 3.27** Confocal images of the grafted NV-SNaph on gold-nanoisland substrates in 2D (z-axis average projection views, $\lambda_{\text{ex}}=405$ nm, $\lambda_{\text{em}}=505$ nm): a) no irradiation and washed (63X); b) 350 nm UV irradiation ($I=2.8-3.2$ mW·cm$^{-2}$) for 30 min and washed (63X); c) 650 nm laser irradiation ($I=2.0-2.3$ mW·cm$^{-2}$) for 30 min and washed (40X); and d) 3D reconstruction from 2D CLSM image (processing using Zen confocal software).

### 3.3.8. Conclusions

A new surface functionalization approach for gold nanostructures *via* covalent thiol-gold interaction towards gold nanomaterials was developed. This study indicates the principle of gold nanostructured device being capable of the plasmonic effect under the harmless laser illumination. This promising strategy not only offers the possibility for potential uses in nanosensing and imaging applications.
Concluding Remarks

This thesis mainly focused on the development of dynamic multivalent nanostructures with unique properties for drug delivery and molecular sensing applications.

In Chapter 2, a simple and convenient synthetic method for the direct H-D exchange reaction of dimethyl-2,2’-bipyridine analogues under microwave irradiation is demonstrated. This rapid and regioselective process produced deuterium-labeled products with a high degree of isotopic purity in quantitative yield. The self-adaptation of dynamic systems via selective coordination of dimethyl-2,2’-bipyridine-based ligands to transition metals has been successfully assessed by ESI-MS. The equilibrium time required for the ligand-exchange complexes depend on the metal species, which are in the orders of FeII, CoII, NiII, CuII, and ZnII from slowest to fastest. Furthermore, a new series of dynamic dendritic structures based on 2,2’-bipyridine-functionalized building blocks could be synthesized via a convergent synthetic strategy. These developed dendritic building blocks straightforwardly coordinated with FeII toward metallo-dendrimers and multi-potent alkyne-presenting dendritic surfaces provides the opportunity for further functionalization with a variety of structures. The supramolecular dendrimer assemblies were obtained with the controlled disassembly via competitive ligand exchange with EDTA.

In Chapter 3, a new class of glycodendrimers with a carbohydrate periphery has been developed. The dynamic assembly of the supramolecular glycodendrimer/metalloglycodendrimer toward multivalent nanostructures has been achieved. They were effectively applied as nanocarriers for targeting drug delivery, resulting in efficiently drug loading. Disassembly of these dynamic delivery systems could be controlled by thermal or pH stimuli, resulting in guest release. Different scaffold of a ciprofloxacin-presenting liposome with a lipase-sensitive linker for controlled drug release were also developed. These ciprofloxacin-presenting liposomes showed significant improvement of antibacterial activity over the individual prodrug against various bacterial species, as well as increased solubility leading to improved biocompatibility of the drug. Finally, a novel gold surface functionalization approach via plasmon-enhanced two-photon excitation has been developed. This method provides a facile attachment of fluorescein labeled-thiolated structures onto the gold-nanoisland surfaces under low frequency laser light illumination.

In conclusion, a variety of dynamic multivalent nanostructures have been developed. They allow for application with specific functionalization. The
resulting nanostructures could be further used as potential nanocarriers for drug delivery applications. Furthermore, a new concept of plasmon-induced gold surface functionalization offers a great promise for biosensing or bioimaging applications.
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***************************************************************
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Appendix A

The following is a description of my contribution to Publications I to VI, as requested by KTH.

Paper I: I contributed to the formulation of the research problems, performed the experimental work and wrote the draft manuscript.

Paper II: I contributed to the formulation of the research problems, performed the experimental work and wrote the draft manuscript.

Paper III: I contributed to the formulation of the research problems, performed the experimental work and wrote the draft manuscript.

Paper IV: I contributed to the formulation of the research problems, performed the experimental work and wrote the draft manuscript.

Paper V: I contributed to the formulation of the research problems, performed the majority of the experimental work and wrote the draft manuscript.

Paper VI: I contributed to the formulation of the research problems, performed the majority of the experimental work and wrote the draft manuscript.


