Synthesis, characterization and application of thermo-responsive [1] pseudorotaxane prepared by slippage approach

Chi Hin Wong

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DATE: July 13, 2017

STUDENT'S NAME: WONG Chi Hin

THESIS TITLE: Synthesis, Characterization and Application of Thermo-responsive \[1\] Pseudorotaxane Prepared by Slippage Approach

This is to certify that the above student's thesis has been examined by the following panel members and has received full approval for acceptance in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Chairman: Dr. Qiu Jianwen
Associate Professor, Department of Biology, HKBU
(Designated by Dean of Faculty of Science)

Internal Members: Prof. Cai Zongwei
Chair Professor in Chemistry, Department of Chemistry, HKBU
(Designated by Head of Department of Chemistry)

Dr. Ren Kangning
Assistant Professor, Department of Chemistry, HKBU

External Members: Prof. Mendes Paula M.
Professor
School of Chemical Engineering
University of Birmingham

Prof. Ng Kee Pui Dennis
Professor
Department of Chemistry
The Chinese University of Hong Kong

Proxy: Dr. Cheng Yuen Kit
Associate Professor, Department of Chemistry, HKBU
(as proxy for Prof. Mendes Paula M.)

In-attendance: Dr. Leung Ken C F
Associate Professor, Department of Chemistry, HKBU

Issued by Graduate School, HKBU

WONG Chi Hin

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy

Principal Supervisor:
Dr. Leung Ken C F (Hong Kong Baptist University)

July 2017
DECLARATION

I hereby declare that this thesis represents my own work which has been done after registration for the degree of PhD at Hong Kong Baptist University, and has not been previously included in a thesis or dissertation submitted to this or any other institution for a degree, diploma or other qualifications.

I have read the University’s current research ethics guidelines, and accept responsibility for the conduct of the procedures in accordance with the University’s Committee on the Use of Human & Animal Subjects in Teaching and Research (HASC). I have attempted to identify all the risks related to this research that may arise in conducting this research, obtained the relevant ethical and/or safety approval (where applicable), and acknowledged my obligations and the rights of the participants.

Signature: [Signature]

Date: July 2017
Abstract

This thesis is divided into three sections. The first section of the thesis includes the synthesis and characterization of a catechol-containing [2]pseudorotaxane which established a model for pseudorotaxane formation prepared through slippage method. The pseudorotaxane formation is performed in different solvents at elevated temperature in a period of time and the progress of pseudorotaxane formations were monitored with $^1$H NMR spectroscopy. The [2]pseudorotaxane had been successfully synthesized in acetonitrile (MeCN) at 60 °C for 5 d with 29% yield.

The second section of the thesis demonstrates the potential of the [1]pseudorotaxane to work as a nanovalve. The opening of valve had been investigated and quantified in the presence of external stimuli such as heat, ultrasound, pH and alternating magnetic field (AMF). Furthermore, a novel core-satellite Fe$_3$O$_4$ nanocomposite had been prepared for AMF responsive controlled drug released system. The cytotoxicity of the core-satellite Fe$_3$O$_4$ nanocomposite had also been investigated and quantified in human gingival epithelial cells and
human epithelial cell line, FaDu, from a squamous cell carcinoma of the hypopharynx. The core-satellite Fe₃O₄ nanocomposite showed non-cytotoxicity at concentration lower than 200 μg/mL and 100 μg/mL towards HGEPs and FaDu respectively.

The third section of the thesis illustrates the synthesis of a novel [1]pseudorotaxane from a signal compound which consists of a macrocycle and a coordination site through a slippage approach. The formation of mechanically interlocked molecules restricted the twisted intramolecular charge transfer (TICT) quenching process and an enhancement of fluorescence intensity was observed. With a potential to act as a fluorescent probe, the fluorescence and fluorescence-quenching nature of the [1]pseudorotaxane had been investigated and quantified in the presence of external stimuli such as base, acid and salt. Furthermore, a series of cations and anions had been screened. The results suggested that the [1]pseudorotaxane was a highly selective phosphate ion sensor and working with a linear operating mode.
Acknowledgements

I would like to give my sincere thanks to my supervisor, Prof. Ken Cham-Fai Leung for his guidance and helpful suggestions for my research project. Prof. Leung is not only an enthusiastic researcher, but also an excellent mentor who demonstrates great patience and forgiveness and offers encouragement during my study.

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Lastly, I wish to thank my family, friends and Eileen Yu for their tolerance and kindly support, especially during preparation of this thesis.

July 2017,

Chi-Hin Wong

Department of Chemistry

Hong Kong Baptist University

Hong Kong, HKSAR
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<th>Description/Unit</th>
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<tbody>
<tr>
<td>Δ</td>
<td>Change; Heating</td>
</tr>
<tr>
<td>2D</td>
<td>Two-dimensional</td>
</tr>
<tr>
<td>°C</td>
<td>Degree Celsius</td>
</tr>
<tr>
<td>δ</td>
<td>Chemical shift in parts per millions</td>
</tr>
<tr>
<td>AMF</td>
<td>Alternating magnetic field</td>
</tr>
<tr>
<td>Ar</td>
<td>Aromatic</td>
</tr>
<tr>
<td>a.u.</td>
<td>Arbitrary unit(s)</td>
</tr>
<tr>
<td>Bn</td>
<td>Benzyl</td>
</tr>
<tr>
<td>Boc$_2$O</td>
<td>Di-tert-butyl dicarbonate</td>
</tr>
<tr>
<td>Calcd.</td>
<td>Calculated</td>
</tr>
<tr>
<td>c</td>
<td>complexed</td>
</tr>
<tr>
<td>d</td>
<td>Day(s); Doublet (spectral)</td>
</tr>
<tr>
<td>DB24C8</td>
<td>Dibenzo[24]crown-8</td>
</tr>
<tr>
<td>DB24C8-Osu</td>
<td>Dibenzo[24]crown-8-succinimide</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethylsulfoxide</td>
</tr>
<tr>
<td>equiv.</td>
<td>Equivalent</td>
</tr>
<tr>
<td>ESI</td>
<td>Electrospray ionization</td>
</tr>
<tr>
<td>ET</td>
<td>Energy transfer</td>
</tr>
<tr>
<td>Et</td>
<td>Ethyl</td>
</tr>
<tr>
<td>EtOAc</td>
<td>Ethyl acetate</td>
</tr>
<tr>
<td>g</td>
<td>Gram(s)</td>
</tr>
<tr>
<td>h</td>
<td>Hour(s)</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared</td>
</tr>
<tr>
<td>lbu/IBU</td>
<td>Ibuprofen</td>
</tr>
<tr>
<td>J</td>
<td>Coupling constant</td>
</tr>
<tr>
<td>kHz</td>
<td>Kilohertz</td>
</tr>
<tr>
<td>λ</td>
<td>Wavelength</td>
</tr>
<tr>
<td>L</td>
<td>Liter(s)</td>
</tr>
<tr>
<td>LAH</td>
<td>Lithium aluminum hydride</td>
</tr>
<tr>
<td>M</td>
<td>Molar (moles per liter)</td>
</tr>
<tr>
<td>m</td>
<td>Multiplet (spectral); Meter</td>
</tr>
<tr>
<td>M</td>
<td>Formula weight</td>
</tr>
<tr>
<td>MALDI-Tof</td>
<td>Matrix-assisted laser desorption/ionization time-of-flight</td>
</tr>
<tr>
<td>Me</td>
<td>Methyl</td>
</tr>
<tr>
<td>MeCN</td>
<td>Acetonitrile</td>
</tr>
<tr>
<td>MeOH</td>
<td>Methanol</td>
</tr>
<tr>
<td>meso</td>
<td>Mesoporous</td>
</tr>
<tr>
<td>MHz</td>
<td>Megahertz</td>
</tr>
<tr>
<td>mM</td>
<td>Millimole(s) per liter</td>
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<tr>
<td>mmol</td>
<td>Millimole(s)</td>
</tr>
<tr>
<td>mol</td>
<td>Mole(s)</td>
</tr>
<tr>
<td>m.p.</td>
<td>Melting point</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition/Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectrometry</td>
</tr>
<tr>
<td>m/z</td>
<td>Mass-to-charge ratio</td>
</tr>
<tr>
<td>NIR</td>
<td>Near Infrared</td>
</tr>
<tr>
<td>nm</td>
<td>Nanometer(s)</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic Resonance</td>
</tr>
<tr>
<td>NOSEY</td>
<td>Nuclear overhauser effect spectroscopy</td>
</tr>
<tr>
<td>NP(s)</td>
<td>Nanoparticle(s)</td>
</tr>
<tr>
<td>PAA</td>
<td>Poly(acrylic acid)</td>
</tr>
<tr>
<td>PEI</td>
<td>Polyethyleneimine</td>
</tr>
<tr>
<td>PhMe</td>
<td>Toluene</td>
</tr>
<tr>
<td>ppm</td>
<td>Part(s) per million</td>
</tr>
<tr>
<td>q</td>
<td>Quartet (spectral)</td>
</tr>
<tr>
<td>R²</td>
<td>correlation coefficient</td>
</tr>
<tr>
<td>Rf</td>
<td>Retention factor</td>
</tr>
<tr>
<td>r.t.</td>
<td>Room temperature</td>
</tr>
<tr>
<td>s</td>
<td>Second(s); Singlet (spectral)</td>
</tr>
<tr>
<td>SPION(s)</td>
<td>Superparamagnetic iron oxide nanoparticles(s)</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>TGA</td>
<td>Thermogravimetric analysis</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>TICT</td>
<td>twisted intramolecular charge transfer</td>
</tr>
<tr>
<td>uc</td>
<td>uncomplexed</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>USPION(s)</td>
<td>Ultra-small Superparamagnetic iron oxide nanoparticles(s)</td>
</tr>
<tr>
<td>Vis</td>
<td>Visible light</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission electron microscopy</td>
</tr>
<tr>
<td>TEOS</td>
<td>Tetraethyl orthosilicate</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
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Figure 4-7. a) Relative fluorescence intensity of titration between 25-H·PF$_6$ and Et$_3$N/TFA alternately (odd: 1 equiv. Et$_3$N; even: 1 equiv. TFA) and b) a plot of relative fluorescence intensity of 25-H·PF$_6$ (after treatment of Et$_3$N) retreated with TFA against time. (conc. = 0.01 mM in MeCN; slit width = 10, 10 nm)

Figure 4-8. a) Stacked fluorescence emission spectra of [1]pseudorotaxane 25-H·PF$_6$ with various amounts of KPF$_6$ and b) a plot of relative fluorescence intensity at 345 nm against the equiv. of KPF$_6$ used (conc. = 0.01 mM in MeCN; slit width = 10, 10 nm)

Figure 4-9. A plot of quenching fluorescence intensity (%) of [1]pseudorotaxane 25-H·PF$_6$ treated with different metal ions for 30 min where I = intensity of 25-H·PF$_6$ treated with metal ions; I$_o$ = intensity of 25-H·PF$_6$ (conc. = 0.01 mM in MeCN; metal ion = 1 equiv.)

Figure 4-10. A plot of relative quenched fluorescence intensity of [1]pseudorotaxane 25-H·PF$_6$ treated with different metal ions for 24 h

Figure 4-11. A plot of relative quenched fluorescence intensity of [1]pseudorotaxane 25-H·PF$_6$ treated with different anions for 30 min where I = intensity of 25-H·PF$_6$ treated with anions; I$_o$ = intensity of 25-H·PF$_6$ (conc. = 0.01 mM in MeCN; anion = 1 equiv.)

Figure 4-12. A plot of relative quenched fluorescence intensity of [1]pseudorotaxane 25-H·PF$_6$ treated with different anions for 24 h

Figure 4-13. a) Stacked fluorescence emission spectra of [1]pseudorotaxane 25-H·PF$_6$ with various amount of Na$_3$PO$_4$; b) a plot of relative fluorescence intensity at 345 nm against the equiv. of Na$_3$PO$_4$ used and c) a plot of relative fluorescence intensity at 345 nm against the first equiv. of Na$_3$PO$_4$ (conc. = 0.01 mM in MeCN; slit width = 10, 10 nm)
Chapter 1 – Introduction

1.1 Stimuli responsive materials

Stimuli responsive materials can adapt structural and property changes according to different environment such as temperature, pH, redox, light, etc.¹ These materials mimic nature processes occurred in living systems. Due to subtle changes in the system, these materials respond dramatically with respect to their intrinsic properties such as shape, chemical characteristics, formation of inter/intramolecular self-assembly or sol-to-gel transition.² Understanding the structural-property relationship, allow advancement in functional smart materials designs with properties such as drug delivery,³ chemosensor,⁴ molecular machine,⁵ etc.

Thermo-responsive materials are well-developed for biomedical application because of their unique properties.⁶ Some polymeric structures are thermo-responsive which undergo phase changes below or above particular temperatures (Figure 1-1) namely lower or upper critical solution temperature (LCST or UCST).⁷ ⁸ Owing to the development of co-polymer preparation, manipulation of LCST of tailor-made co-polymers is possible. Furthermore, most thermo-responsive polymer are non-cytotoxic towards human normal cells.⁹ Therefore, this class of materials has been widely employed in biomedical field.

In 2006, polymer vesicles for controlled drug encapsulation and release have
been reported.\textsuperscript{9} The thermoresponsive polymersomes were prepared by diblock copolymers of PEO-\textit{b}-PNIPAm with a narrow polydispersity. When temperature is exceeds LCST (37 °C), the block co-polymers become amphiphilic and self-assemble into vesicles for drug encapsulation. In contrast, the vesicles collapse when the temperature is lower than LCST thereby releasing the drug.

\textbf{Figure 1-1.} Schematic illustration of vesicles from PEO-\textit{b}-PNIPAm copolymer at 37 °C and 25 °C.

In 2016, a novel poly(vinyl alcohol) derivative with dual-responsive properties has been reported (Scheme 1-1).\textsuperscript{10} The hydroxy groups on PVA was activated by carbonyldiimidazole (CDI) to give compound 1 followed by reaction with \textit{N,N}-diethylethane-1,2-diamine (DEEDA) to obtained PVA derivative 2. The derivative 2 is stimuli-responsive towards temperature or pH for controlled drug release.
Scheme 1-1. Preparation of PVA derivative 2 with thermo-responsive and pH-responsive parts.

Apart from smart polymers, mechanically-interlocked molecules also play an important role in stimuli responsive materials. Rotaxane is a mechanically interlocked molecule that consists of two or more components held by intermolecular, non-covalent interactions including hydrogen bonding, electrostatic interaction, $\pi-\pi$ stacking, etc.\textsuperscript{11} In other words, rotaxane is one of the supramolecular architecture, which consist of a thread as an axle with two bulky stoppers at the end (dumbbell-shaped molecule) encircled with one or more macrocycle(s).

Rotaxane formation involves the assembly between a linear molecule thread and a macrocycle first to give a [2]pseudorotaxane then followed by end-capping of the linear molecule with two bulky molecules at both ends. The two bulky groups prevent the macrocycle from slipping out of the thread. This process referred to threading-followed-by-stoppering which yields a [2]rotaxane effectively. In 2013, our group reported a synthesis of type III-B rotaxane dendrimers by this approach.\textsuperscript{12} [2]Pseudorotaxane was formed by threading a succinimide activated
dibenzo[24]crown-8 (guest) into a dialkylammonium molecules (host) with two azide groups at the end. Then, [2]rotaxane was synthesized by stoppering the [2]pseudorotaxane with two bulky arylether and an acetylene unit by a high-yielding click reaction.

On the other hand, clipping is another method for constructing rotaxanes effectively by encircling acyclic precursors onto a dumbbell-shaped molecule. The acyclic precursors assembled and templated on the thread to form the ring of the rotaxane. In 2011, Beer’s group reported a synthesis of [2]rotaxane by clipping method with the aid of olefin cross metathesis by Grubb’s catalyst. The [2]rotaxane was formed by a ring closing metathesis of a dumbbell-shaped pyridinium thread with a vinyl-containing macrocycle precursor.

Besides the methods mentioned above, slippage is another approach to synthesize rotaxane. Pseudorotaxane can be formed by slipping the macrocycle through the stopper of the thread by heating the mixture of the precursors in an appropriate solvent system. The self-assembly between the macrocycle and the thread is driven by the formation of a thermodynamically favored pseudorotaxane.

In 1998, a detailed mechanistic study of pseudorotaxane formation through slippage approach had been reported (Figure 1-2). In this method, macrocycle and “dumbbell” rod are heated in an appropriate solvent so that the free energy of
activation ($\Delta G_{\text{on}}$) for the macrocycle to slip through the dumbbell’s stopper can be overcome. The supramolecular interactions stabilize the rotaxane-like structure and become more stable than its separate components by $\Delta G^\circ$. Therefore, the free energy of dissociation ($\Delta G_{\text{off}}^\circ$) becomes insurmountable when the mixture is cooled to ambient temperature (Figure 1-2).

**Figure 1-2.** Schematic illustration of pseudorotaxane formation through a slippage approach.

In this study, different dumbbell derivatives had been synthesized for investigation of the possibilities to prepare a kinetically stable pseudorotaxane (Scheme 1-2) with a macrocycle DB24C8. The derivatives were either (1) modifying the phenyl ring with bulky stopper groups such as $i$-Pr and $t$-Bu group the *para*-position, or (2) replacing the phenyl ring with sterically more encumbering
cycloalkyl rings such as cyclopentane, cyclohexane and cycloheptane.

\[
\begin{array}{cccc}
\text{R} & \text{H}_2 & \text{R} & \text{H}_2 \\
\text{4}^+ & \text{R} = \text{i-Pr} & \text{5}^+ & \text{R} = \text{t-Bu} \\
\end{array}
\]

**Scheme 1-2.** Modification of compound 3\(^+\) towards preparation of kinetically stable pseudorotaxanes.

For compound 5\(^+\) & 8\(^+\), the t-Bu and cycloheptane stoppers were too bulky to allow the slippage of macrocycle DB24C8 over them to form pseudorotaxane. These observations suggest that the system designed for slippage method is highly dependent on the steric effects. Compounds 4\(^+\) & 6\(^+\) have less bulky stoppers, i.e., i-Pr and cyclopentane, from which the macrocycle was allowed to slip over the stoppers at ambient temperature. However, the dissociation of the complexes was also observed at ambient temperature and therefore, the complexes formed between compounds 4\(^+\) or 6\(^+\) and DB24C8 are denoted as pseudorotaxane instead of a rotaxane. In contrast, the complexation between compound 5\(^+\) and DB24C8 did not proceed at ambient temperature implying that there was insufficient thermal energy to permit the slippage of macrocycle over cyclohexane stopper. Surprisingly, the \(\Delta G_{\text{on}}\) became surmountable at higher temperature (i.e. 40 °C) and the pseudorotaxane with strong rotaxane-like character was found after cooling the mixture to ambient temperature.

Based on the interesting findings, a novel dendrimer 9-H·PF\(_6\) with rotaxane-
like characteristics has been assembled under thermodynamic control through the slippage approach (Figure 1-3).\textsuperscript{16} The assembly of the rotaxane was proceeded in DCM at reflux for 90 d and successfully characterized by \textsuperscript{1}H NMR spectroscopy. Furthermore, the dissociation of rotaxane-like dendrimer has also been demonstrated in DMSO.

\textbf{Figure 1-3.} Molecular structure of two dendritic wedges assembled by slippage.

In addition, we reported a detailed study about the dissociation of [2]pseudorotaxane prepared by slippage approach under the influence of base/DMSO.\textsuperscript{17} The result showed that the [2]pseudorotaxane was relatively stable in the presence of base which required almost 60 days to dissociate completely at ambient temperature. In contrast, the dissociation of [2]pseudorotaxane only took 24 h for complete dissociation in DMSO.

In short, pseudorotaxane with a strong rotaxane-like character prepared by
slippage approach with cyclohexane as the stopper has been well studied. The stability of the pseudorotaxane and the condition of dissociation have also been investigated. These results suggest that the pseudorotaxane may be employed as a thermo-responsive molecular machine.

1.2 Rotaxane-based controlled drug release system

As aforementioned, rotaxane can be designed for different applications by shuttling motion of interlocked macrocycle on the dumbbell-shaped linear molecule by external stimuli. This property renders the rotaxane to act as a molecular machine to control the blocking of nanoparticles orifice. Recently, literature reports demonstrated applications of rotaxane in different areas such as rotaxane-based molecular muscle, electronic device, controlled release of drug and gene delivery.

Nanoparticles’ surface modification can be incorporated with supramolecular or molecular nanovalve machine as a mechanically movable component to control a substrate’s release. Nanoparticle can be a stopper of pseudorotaxane or rotaxane. Furthermore, two types of methods to trigger the opening and closing of the nanovalves were also introduced. A snap-top machine allows the release of drug molecules from the nanoparticles by removing the ring component from the binding
site through cleavage of covalent linkage of the stopper. Figure 1-4 shows a snap-top machine constructed by a [2]pseudorotaxane incorporating compound 10 and DB24C8. The snap-top nanovalve is stimuli-responsive towards pH change or competitive binding. When the system was incubated in a basic environment, the ammonium ion will be deprotonated and the macrocycle will be slipped off from the thread as a consequence the guest molecules will be released. On the other hand, there are metal cation such as K\(^+\) and Na\(^+\) which will compete with the ammonium thread, the valve will be opened through a competitive binding.

![Figure 1-4. Schematic illustration of a [2]pseudorotaxane acting as a snap-top machine.](image)

Another method is utilizing the concept of a bistable [2]rotaxane whereas the ring can be switched from one recognition site to another by external stimuli such as change in pH, light, redox reaction to open or close the valve to control the release of nanoparticles’ trapped substrate. Figure 1-5 shows a reversible molecular
valve which is a bistable [2]rotaxane 11 controlled by redox reaction. The [2]rotaxane nanovalve-modified nanoparticles can be closed (ring moving to orifice of nanoparticles) by oxidation and opened (ring moving away from the orifice) by reduction to achieve reversible open and close of the valve.

**Figure 1-5.** a) The structural formula of the [2]rotaxane molecular valve 11; b) procedures of operating the molecular valve.

In 2014, Zhao’s group reported a near infrared (NIR) controlled release drug delivery system by functionalizing the surface of mesoporous silica coating on gold nanorod with switchable [2]rotaxane 12⊂α-CD in which α-cyclodextrin was assembled on azobenzene binding site to act as capping agent (Figure 1-6). Encapsulation of doxorubicin was achieved by irradiating with ultraviolet(UV)
light. Its release is then triggered by irradiating the targeted site with non-invasive NIR. With such operation, the [2]rotaxane $12\subset\alpha$-CD acts as a nanovalve which can be turned on and off to control the drug release by irradiating with NIR and UV light respectively.

**Figure 1-6.** Photo-responsive rotaxane $12\subset\alpha$-CD modified gold nanorod for controlled release drug delivery system.

In 2007, pH-responsive polypseudorotaxane-modified mesoporous silica nanoparticles have been reported by Kim’s group for controlled drug release. A polypseudorotaxane was designed and synthesized as nanovalve by assembly of $\alpha$-cyclodextrin or $\gamma$-cyclodextrin which act as capping agents on polyethyleneimine (PEI). The nanovalve can be turned on with protonation of the PEI, which established an electrostatic repulsion force on the thread that extrude the
cycloextrin out of the thread and resulted in the release of guest molecules from nanoparticles.

### 1.3 Rotaxane-based chemosensors

Based on the unique properties of rotaxane, different molecular shuttles, switches and machines have been reported in the past decade. Reversible non-covalent interactions among components render manipulation of mechanically-interlocked architectures with co-conformational changes. The assembly/disassembly can be altered by applying external stimuli such as pH, metal ion, redox, etc.

Interestingly, a switchable rotaxane architecture modified with a fluorophore would achieve a chemsensor which would respond to external stimuli so that the fluorophore can be switched on/off because of the co-conformational change or assembly/disassembly switching processes.

In 2010, an anthracene-containing dynamic [2]rotaxane 13-H·PF₆ has been reported. The [2]rotaxane 13-H·PF₆ was prepared through a thermodynamic template-directed synthesis by imine formation between individual components 14-H·PF₆, 15 and 16. The supramolecular system has been well studied in the stability, exchange dynamic and response towards external stimuli such as water, salts, acids, and amines. The results show the dissociation rate of rotaxane in the
The presence of acid is much faster in the presence of water which suggests the [2]rotaxane 13-H·PF₆ can be potentially used as a fluorescent acid sensors (Scheme 1-3).

Scheme 1-3. Dissociation mechanism of 13-H·PF₆ in the presence of acid/water.

In the previous strategy, the dissociation of rotaxane allowed the anthracene to restore the fluorescence signal with a “turn-on” mechanism. In contrast, there are examples of rotaxane chemosensor with “turn-off” mechanism. In 2013, a novel diketopyrrolopyrrole-based [2]rotaxane for fluoride ion sensing has been reported. The [2]rotaxane consists of a robust signaling fluorophore 3,6-di(thiophen-2-yl)pyrrolo[3,4-c]pyrrole-1,4(2H,5H)-dione (DPP)-containing thread and a macrocycle cavity with unique topological constraints for fluoride ion binding. When fluoride ion was mixed with the rotaxane, a macrocycle shuttling was induced and a quenching of the fluorophore was observed. In this strategy, it possessed a “turn-off” mechanism for fluoride ion sensing.
1.4 Aim of project

In this project, we have focused on the design of molecular machine based on pseudorotaxane formation through a slippage approach by employing cyclohexane as the stopper. As aforementioned, the macrocycle slip through a cyclohexane stopper at elevated temperature such that a pseudoroataxane with strong rotaxane-like character can be obtained when the complex cooled down to ambient temperature. Based on this observation, a novel thermo-responsive material is designed and developed for different applications. There are three sections in this thesis. The first section of the thesis includes the mechanistic study of pseudorotaxane formation between a model compound and DB24C8 in different aprotic solvents. The study establishes a model for investigating the potential of the system for other application.

The second section of the thesis utilizes the pseudorotaxane as nanovalve for controlled drug release system. The pseudorotaxane nanovalve responds to external stimuli such as heat, ultrasound and alternating magnetic field for controlled drug release with magnetic iron oxide nanomaterials. The release profiles reveal the fact that pseudorotaxane can be used as thermo-responsive nanovalve for potential theranostic application.

The third section of the thesis includes the synthesis and characterization of
thermo-responsive [1]pseudorotaxane. The [1]pseudorotaxane is equipped with an aromatic amide moiety to act as a stimuli-responsive fluorescent probe. The fluorescence quenching nature of the [1]pseudorotaxane has been investigated in the presence of different stimuli such as base, acid and salt. The [1]pseudorotaxane has also been demonstrated as a good phosphate ion sensor with high selectivity.

1.5 Reference:


Disassembly of Nanoparticle Aggregates for Light-Up Colorimetric Sensing.


1-15. Ashton, P. R.; Baxter, I.; Fyfe, M. C. T.; Raymo, F. M.; Spencer, N.; Stoddart,


Chapter 2 – Synthesis and Characterization of a New
[2]Pseudorotaxane Through a Slippage Approach

2.1 Background

Pseudorotaxane resembles the general form of rotaxane, but without bulky stoppers on the thread. The macrocycle is temporally encircled on the thread through noncovalent interaction and therefore, the macrocycle can be readily dissociated.\textsuperscript{1,2}

Recently, the formation of pseudorotaxane based on the thermodynamically stable and template-directed slippage method has been reported. The use of cyclohexyl ring as a “pseudo-stopper” which hinders the dissociation of pseudorotaxane.\textsuperscript{3,4} Furthermore, the pseudorotaxane shows tunable dissociation rates by different external stimuli.\textsuperscript{4}

These properties give the potential of pseudorotaxane to act as a novel functional nanovalve that can respond to different external stimuli and therefore, a model compound is designed to investigate the formation of pseudorotaxane.

2.2 Synthesis of model compound 17-H·PF$_6$

Scheme 1 shows the synthetic procedure of compound 17-H·PF$_6$ which uses as a potential model compound for complexation study with effective blockage of DB24C8 from slipping away. Compound 18 was prepared according to the
literature\textsuperscript{5} with modification. Compound 18 was reacted with cyclohexanecarboxaldehyde with Dean-Stark apparatus and followed by imine reduction to obtain compound 17 with 69\% yield. Model compound 17-H·PF\textsubscript{6} was obtained by protonation with conc. HCl to pH 3 and followed by counterion exchange with sat. NH\textsubscript{4}PF\textsubscript{6} solution with 87\% yield.

Scheme 2-1. Synthetic scheme of compound 17-H·PF\textsubscript{6}.

2.3 Characterization of model compound 17-H·PF\textsubscript{6}

The \textsuperscript{1}H and \textsuperscript{13}C NMR spectra of model compound 17-H·PF\textsubscript{6} are shown in figure 2-1. The proton signals at $\delta$ 8.28 ppm can be attributed to the ammonium protons (NH\textsubscript{2}$^+$). The proton signals at $\delta$ 6.48 and 7.29–7.40 ppm can be attributed to the aryl protons (Ar–H). The proton signal at $\delta$ 4.95 ppm can be attributed to the benzyl protons (ArCH\textsubscript{2}O–). The proton signals at $\delta$ 2.82, 3.10 and 3.22 ppm can be
attributed to the aliphatic protons (H_a, H_b & H_c). Furthermore, the protons on cyclohexane (cyc–H) resonate as multiplets in region ranging from δ 0.88 to 1.82 ppm.

The carbon signals at δ 101.1, 107.8, 127.7, 128.1, 128.7, 136.8, 138.5, 160.4 ppm can be attributed to the aromatic carbons (ArC). The carbon signals at δ 70.1 ppm can be attributed to the benzyl carbons (ArCH_2O–). The carbon signals at δ 34.8, 49.9, 54.5 ppm can be attributed to the aliphatic carbons (ArCH_2 & –NH_2^+CH_2–). The cyclohexane carbons (cycC) resonate as four signals at δ 25.4, 25.9, 30.6, 32.5 ppm.

**Figure 2-1.** NMR (CDCl_3, 298 K) spectra of compound 17-H·PF_6 a) ^1^H NMR spectrum and b) ^1^C NMR spectrum. *: Solvent residue.

Also, compound 17-H·PF_6 has been analyzed by MALDI-ToF-MS (figure 2-
2). A singly charged molecular ion base peak (m/z) of 430.2768 was observed which are corresponded to the compound [17+H]^+ molecular ion with a theoretical value of 430.2741. All ¹H NMR, ¹³C NMR and mass spectra suggested the successful synthesis of model compound 17-H·PF₆.

![Mass spectrum of compound 17-H·PF₆.](image)

**Figure 2-2.** Mass spectrum of compound 17-H·PF₆.

### 2.4 Binding study of [2]pseudorotaxane formation between model compound 17-H·PF₆ and dibenzo[24]crown-8

Scheme 2-2 shows the proposed formation scheme of [2]pseudorotaxane 17-H·PF₆⊂DB24C8. Three solvents (MeCN, CHCl₃, THF) have been chosen for the study with relatively low donor number but with a relatively high boiling point
which fascinate the formation of [2]pseudorotaxane. Figure 2-3 shows the stacked $^1$H NMR spectra of the components of [2]pseudorotaxane while figure 2-3c shows the $^1$H NMR spectrum of compound 17-H-PF$_6$ and DB24C8 (1:1 mixture) by simple mixing shows similar chemical shift values and pattern when compared to the individuals.


Figure 2-3. Stacked $^1$H NMR (CDCl$_3$, 298K) spectra of a) 17-H-PF$_6$, b) DB24C8 and c) 1:1 mixture of 17-H-PF$_6$ and DB24C8. *: Solvent residue.
Figure 2-4 shows the stacked $^1$H NMR spectra of [2]pseudorotaxane formation after a reaction of 3 d in different solvents. In the cases of MeCN and CHCl$_3$, the $^1$H NMR spectra show difference when compared to that of the 1:1 mixture. The chemical shift protons signal $H_a$, $H_b$ and $H_c$ are shifted and a new proton signal corresponded to the benzyl proton of complexed [2]pseudorotaxane observed next to the original one which indicated the formation of [2]pseudorotaxane. In the case of THF, the protons signal $H_a$, $H_b$ and $H_c$ disappeared and spectra became messier. Furthermore, no complexed [2]pseudorotaxane was observed when study performed in THF.

**Figure 2-4.** Stacked $^1$H NMR (CDCl$_3$, 298K) spectra of a) 1:1 mixture of 17-H·PF$_6$ and DB24C8, b) after complexation in MeCN for 3 d, c) after complexation in CHCl$_3$ for 3 d and d) after complexation in THF for 3 d. *: Solvent residue. uc: uncomplexed. c: complexed.
Figure 2-5 shows the stacked $^1$H NMR spectra of [2]pseudorotaxane formation after a reaction of 8 d in different solvents. After incubation for 8 d in MeCN and CHCl$_3$, more complexed [2]pseudorotaxane was observed which was indicated by the increment of the proton signal. Furthermore, the ratio of integral between complexed [2]pseudorotaxane and uncomplexed components of [2]pseudorotaxane is 1:2 was shown in complexation study in MeCN after 8 d.

**Figure 2-5.** Stacked $^1$H NMR (CDCl$_3$, 298K) spectra of a) 1:1 mixture of 17-H·PF$_6$ and DB24C8, b) after complexation in MeCN for 8 d, c) after complexation in CHCl$_3$ for 8 d and d) after complexation in THF for 8 d. *: Solvent residue. uc: uncomplexed. c: complexed.

Figure 2-6 shows the $^1$H NMR spectra of [2]pseudorotaxane formation after a reaction of 15 d in MeCN. After a 15 d complexation, the ratio of integral between complexed [2]pseudorotaxane and uncomplexed components is 1:2 which has
similar result when compared to the study for 8 d. Therefore, the formation of [2]pseudorotaxane attained an equilibrium after the complexation study performed for 8 d.

Figure 2-6. $^1$H NMR (CDCl$_3$, 298K) spectra of a) 1:1 mixture of 17-H·PF$_6$ and DB24C8 and b) after complexation in MeCN for 15 d. *: Solvent residue. uc: uncomplexed. c: complexed.

2.5 Synthesis and characterization of [2]pseudorotaxane 17-H·PF$_6$⊂DB24C8

After the binding study, the results show that the [2]pseudorotaxane obtained in MeCN with the highest yield. Scheme 2-3 shows the synthetic scheme of [2]pseudorotaxane 17-H·PF$_6$⊂DB24C8. [2]Pseudorotaxane 17-H·PF$_6$⊂DB24C8 was synthesized by incubating compound 17-H·PF$_6$ and DB24C8 in MeCN at 60 °C for 5 d with 29% isolated yield.

Figure 2-7 shows the partial $^1$H NMR spectra of 17-H·PF$_6$⊂DB24C8 and its
free components. There are new pattern of sharp peaks observed after formation of [2]pseudorotaxane which suggest that the [2]pseudorotaxane was successfully synthesized. In particular, the aryl protons signal at \( \delta = 6.48 \) ppm of 17-H-PF₆ split into two new protons signals at \( \delta = 6.22 \) and 6.41 ppm. Also, benzylic methylene proton, Hₓ, are remarkably shifted from \( \delta = 3.22 \) ppm in 17-H-PF₆ to \( \delta = 3.58 \) ppm in 17-H-PF₆⊂DB24C8.


Figure 2-7. Partial \(^1\)H NMR (CDCl₃, 298 K) spectra of 17-H-PF₆⊂DB24C8 and its free components: DB24C8 and 17-H-PF₆. *: Solvent residue. uc: uncomplexed.
also, 17-H·PF<sub>6</sub>⊂DB24C8 has been analyzed by ESI-Tof-MS (figure 2-8). A singly charged molecular ion base peak (m/z) of 878.4822 was observed which are corresponded to [17-H⊂DB24C8-H]<sup>+</sup> molecular ion with a theoretical value of 878.4838. <sup>1</sup>H NMR and mass spectra suggested the successful synthesis of [2]pseudorotaxane 17-H·PF<sub>6</sub>⊂DB24C8.

![Figure 2-8. ESI Mass spectrum of 17-H·PF<sub>6</sub>⊂DB24C8.](image)

### 2.6 Conclusion

Thread 17-H·PF<sub>6</sub> has been successfully synthesized to act as a model compound for binding study in the formation of [2]pseudorotaxane 17-H·PF<sub>6</sub>⊂DB24C8 through slippage approach. The [2]pseudorotaxane obtained with the highest
isolated yield 29% when the formation was performed in MeCN at 60 °C for 5 d. The result establishes a good model for the design of nanovalve and chemosensor through a slippage approach.

2.7 General information

Reactions were performed under nitrogen unless otherwise stated. Chromatography purifications were performed on silica gel (SiO$_2$) with the indicated eluents. Deionized water was obtained from Milli-Q ICW3000 water system. All solvents and reagents were in reagent grade and used as received without further purification.

$^1$H and $^{13}$C NMR spectra for structural characterization were recorded with Bruker Avance 400 ($^1$H: 400 MHz; $^{13}$C: 101 MHz) spectrometer at 297 K. All NMR samples were prepared in CDCl$_3$ unless otherwise stated. Spectra were calibrated internally using the CHCl$_3$ residual peak in CDCl$_3$ ($^1$H: $\delta = 7.26$, $^{13}$C: $\delta = 77.2$ ppm). Chemical shifts were reported as parts per million (ppm) in $\delta$ scale and coupling constants ($J$) were reported in hertz. Matrix-assisted laser desorption/ionization time of flight (MALDI-Tof) mass spectra were measured on a Bruker SolariX 9.4T mass spectrometer. The reported molecular mass ($m/z$) values correspond to the most abundant monoisotopic masses.
2.8 Experimental section

**Compound 17**

Benzyl-protected compound 18 was prepared according to the literature.\(^5\) Compound 18 (2.12 g, 6.4 mmol) and cyclohexanecarboxaldehyde (0.71 g, 7.0 mmol) were dissolved in PhMe (150 mL). The reaction mixture was heated at reflux for 12 h with a Dean-Stark apparatus to remove the condensed water molecules. The mixture was concentrated under reduced pressure. The residue was then dissolved in mixture of THF (30 mL) and MeOH (6 mL) and followed by the addition of sodium borohydride (0.36 g, 9.5 mmol) in 0 °C. The reaction mixture was then stirred at room temperature for 12 h. Water was added to the reaction mixture and extracted with EtOAc three times. The organic fraction was combined and washed by brine for three times. The organic fraction was then dried over anhydrous MgSO\(_4\). The mixture was filtered and concentrated under reduced pressure. The residue was finally purified by column chromatography with EtOAc as the eluent. The purified product 17 was yellowish-brown liquid (1.88 g, 69 %).
$R_f$: 0.19 (EtOAc). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ = 7.53–7.31 (m, 10H, ArH), 6.55 (s, 3H, ArH), 5.05 (s, 4H, PhCH$_2$O–), 2.89 (t, $J$ = 6.8 Hz, 2H, PhCH$_2$–), 2.80 (t, $J$ = 6.9 Hz, 2H, –CH$_2$NH–), 2.50 (d, $J$ = 6.7 Hz, 2H, –CH$_2$NH–), 1.75 (t, $J$ = 13.8 Hz, 5H, cyc-$H$), 1.53–1.44 (m, 2H, cyc-$H$ & NH), 1.26–1.17 (m, 3H, cyc-$H$), 0.98–0.90(m, 2H, cyc-$H$). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ = 159.9, 142.5, 136.9, 128.5, 127.9, 127.4, 107.9, 99.8, 69.9, 56.6, 51.1, 37.9, 36.6, 31.5, 26.7, 26.1. HRMS (MALDI-TOF): C$_{29}$H$_{36}$NO$_2$ $^+ [M+H]^+$: calcd 430.2741; found 430.2757.

**Compound 17-H·PF$_6$**

![17-H·PF$_6$](image)

A solution of compound 17 (1.21 g, 2.8 mmol) in 30 mL MeOH was stirred at 0 °C. The solution was acidified with conc. HCl until the pH of the solution reached at pH 3 and the resulting solution was stirred at ambient temperature for 2 h. The solution was dried under reduced pressure and the residue was re-dissolved in acetone (30 mL). A solution of sat. NH$_4$PF$_6$ (3 mL) was added to the reaction and the resulting solution was stirred at ambient temperature for another 2 h. The reaction mixture was evaporated to dryness and the residue was partitioned between
water (30 mL) and CHCl₃ (15 mL). The aqueous layer was further extracted with CHCl₃ (3 × 15 mL). The combined organic layer was dried over anhydrous MgSO₄ and the filtrate was dried at reduced pressure to give compound **17-H·PF₆** (1.31 g, 81%) as a pale-yellow powder. M.P.: 154.7 – 156.1 °C. Rf: 0.45 (EtOAc). ¹H NMR (400 MHz, CDCl₃) δ = 8.28 (s, 2H, –NH₂), 7.41–7.28 (m, 10H, ArH), 6.48 (s, 3H, ArH), 4.95 (s, 4H, PhCH₂O–), 3.21 (dd, J = 10.3, 5.5 Hz, 2H, PhCH₂–), 3.10 (dd, J = 10.3, 5.5 Hz, 2H, –CH₂⁺NH₂–), 2.82 (d, J = 6.5 Hz, 2H, –CH₂⁺NH₂–), 1.81 (d, J = 11.8 Hz, 3H, cyc-H), 1.69 (d, J = 13.1 Hz, 2H, cyc-H), 1.61 (d, J = 12.2 Hz, 1H, cyc-H), 1.22–1.06 (m, 3H, cyc-H), 0.96 (dd, J = 22.7, 11.1 Hz, 2H, cyc-H). ¹³C NMR (101 MHz, CDCl₃) δ = 160.4, 138.5, 136.8, 128.7, 128.1, 127.7, 107.8, 101.1, 70.1, 54.5, 49.9, 34.8, 32.5, 30.5, 25.9, 25.4. HRMS (MALDI-TOF): C₂₉H₃₆NO₂⁺ [M+H]⁺: calcd 430.2741; found 430.2768.

**[2]Pseudorotaxane 17-H·PF₆⊂DB24C8**
A solution of compound 17-H-PF$_6$ (0.15 g, 0.26 mmol) and DB24C8 (0.12 g, 0.26 mmol) in MeCN (10.4 mL) was heated at 60 °C in Schlenk flask for 5 d. The resulting solution was evaporated to dryness and the residue was partitioned between water (10 mL) and DCM (5 mL). The aqueous layer was further extracted by DCM (3 × 5 mL). The combined organic layer was dried over anhydrous MgSO$_4$ and the filtrate was evaporated to dryness. Flash column chromatography with EtOAc and gradient to acetone on silica gel of the residue gave the [2]pseudorotaxane 17-H-PF$_6$⊂DB24C8 (0.066 g, 29%) as a yellow paste. $R_f$: 0.08 (EtOAc). $^1$H NMR (400 MHz, CDCl$_3$) δ = 7.46–7.28 (m, 10H, ArH), 6.91–6.85(m, 8H, ArH), 6.41 (t, $J$ = 2.1 Hz, 1H, ArH), 6.22 (d, $J$ = 2.1 Hz, 2H, ArH), 4.92 (s, 4H, PhCH$_2$O–), 4.23–4.06 (m, 8H, –CH$_2$O–), 3.94–3.78 (m, 8H, –CH$_2$O–), 3.78–3.62 (m, 8H, –CH$_2$O–), 3.59 (s, 2H, PhCH$_2$–), 3.11 (dd, $J$ = 13.1, 6.8 Hz, 2H, –CH$_2$+NH$_2$–), 2.87–2.71 (m, 2H, –CH$_2$+NH$_2$–), 1.52 (t, $J$ = 10.7 Hz, 5H, cyc-H), 0.90 (dd, $J$ = 15.4, 7.9 Hz, 4H, cyc-H), 0.65 (dd, $J$ = 21.5, 10.4 Hz, 2H, cyc-H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ = 160.2, 147.6, 138.8, 137.0, 128.7, 128.1, 127.5, 122.0, 112.8, 107.9, 100.5, 100.1, 71.2, 70.6, 70.0, 68.3, 54.7, 49.3, 35.4, 32.9, 30.3, 25.8, 25.5. HRMS (ESI-Tof-MS): C$_{53}$H$_{68}$NO$_{10}$$^+$ [M+H]$^+$: calcd 878.4838; found 878.4822.
2.9 Reference:


Chapter 3 – Thermo-Responsive Controlled Drug Delivery System Based on Pseudorotaxane Capped Mesoporous Iron Oxide Nanoparticles

3.1 Background

In this project, new components for [1]pseudorotaxane formation are coupled on the surface of mesoporous iron oxide nanoparticles (meso-Fe$_3$O$_4$ NPs) to act as a nanovalve that prevents a premature release of drug molecules from the core. The [1]pseudorotaxane contains a cyclohexane-ended ammonium thread and a dibenzo[24]crown-8 which can demonstrate reversibly the slippage and extrusion processes at elevated temperature, altering by the polarities of solvent (Figure 3-1).¹

Dopamine, which is a catechol-containing compound, can act as an efficient anchor to functionalize the surface of iron oxide NPs.² Therefore, novel [1]pseudorotaxane components can be synthesized with catechol-containing group for facile modification of iron oxide NPs.

Figure 3-2 shows the schematic diagram of controlled release drug delivery system. The meso-Fe$_3$O$_4$@19:20 NPs were protonated at low pH while ibuprofen was incubated at the same time. The closing of nanovalve by a [1]pseudorotaxane formation was performed in MeCN at 60 °C to facilitate a drug loading and
prevented the escape of drug molecules. Then, dissociation of [1]pseudorotaxane could be controlled by applying external stimuli such as heat, change of pH and ultrasound, therefore, the nanovalve would be opened to trigger the release of ibuprofen.

Figure 3-1. a) The chemical structure of novel [1]pseudorotaxane precursors 19 and 20 and b) schematic diagram of precursors 19 and 20 functionalized meso-Fe$_3$O$_4$ NPs. (not drawn in scale)

The [1]pseudorotaxane formed on the surface of the meso-Fe$_3$O$_4$ NPs and act as a closed nanovalve to prevent the escape of drug molecules from the iron oxide core to achieve a controlled release drug delivery system. The system could be triggered by an increased temperature followed by the release of drug. By increasing the temperature, it provides extra energy to overcome the energy barrier for the extrusion of macrocycle through the cyclohexane.$^1$ Furthermore, ultrasound
which is a non-invasive diagnosis technique commonly used for medical purpose, may act as another way to trigger release of drug molecules from the system by enhancing intermolecular mechanical collision as well as the release of drugs from the NPs.\textsuperscript{7,8}

\textbf{Figure 3-2.} Schematic diagram of [1]pseudorotaxane used as nanovalve for controlled release of drug. (not drawn in scale)
Furthermore, meso-Fe$_3$O$_4$ NPs, which are magnetically responsive materials, can be used for magnetic resonance imaging (MRI) contrasting agent.\textsuperscript{9,10} Also, they can generate heat by applying alternating magnetic field to induce a release of drug molecules from the system.\textsuperscript{11,12}

Owing to the partial hydrophobic properties of the surface and core of NPs, the system can be used as a hydrophobic drug carrier such as ibuprofen, which is an anti-inflammatory drug (NSAID) shows induction of apoptosis of prostate tumor cells.\textsuperscript{13,14} The controlled release system prevents premature release of drug which mean less drug lost in the transportation in body, such that the dose of drug can be reduced and the efficiency of drug delivery can be enhanced.

Furthermore, core-satellite nanocomposite becomes popular recently because of its multifunctionality by constructing with different materials.\textsuperscript{15,16} In this chapter, a novel core-satellite Fe$_3$O$_4$ nanocomposite was constructed by supramolecular interaction between multidomain mesoporous Fe$_3$O$_4$ core for drug encapsulation with single domain ultra-small Fe$_3$O$_4$ satellite NPs acting as a heat source.
3.2 Synthesis and characterization of precursors 19 & 20

3.2.1 Synthesis of precursors 19 & 20

Scheme 3-1 shows the synthetic procedure of dopamine-cyclohexane precursor 19. Compound 18 was reacted with cyclohexanecarboxaldehyde in toluene (PhMe) and heated under reflux with a Dean-Stark apparatus. The reaction mixture was reduced by sodium borohydride in tetrahydrofuran (THF) at 0 °C to give the amine compound 21 with 68% yield in two steps. Finally, compound 21 was deprotected by hydrogenolysis with 10% Pd/C as catalyst in methanol to give the target precursor 19 with 97% yield.

Scheme 3-1. Synthetic scheme of novel dopamine-cyclohexane precursor 19.

Scheme 3-2 shows the synthetic procedure of novel dopamine-dibenzo[24]crown-8 precursor 20. Compound 22 was prepared by reaction between compound 18 and DB24C8-OSu in DCM for 24 h to give compound 22 with 78%
Finally, compound 22 was deprotected by hydrogenolysis with 10 % Pd/C as catalyst in methanol to give target precursor 20 with 97% yield.

**Scheme 3-2.** Synthetic scheme of novel dopamine-dibenzo[24]crown-8 precursor 20

### 3.2.2 Characterization of precursors 19 & 20

#### 3.2.2.1 Nuclear magnetic resonance (NMR) spectroscopy

The $^1$H and $^{13}$C NMR spectra of dopamine-cyclohexane precursor 19 are shown in figure 3-3. The proton signals at $\delta$ 6.47, 6.63, 6.67 ppm can be attributed to the aryl protons (Ar–H). The proton signals at $\delta$ 2.74, 2.80 and 2.98 ppm can be attributed to the aliphatic protons (ArCH$_2$ & –NHCH$_2$–). Furthermore, the protons on cyclohexane (cyc–H) resonate as multiplets in region ranging from $\delta$ 0.97 to 1.78 ppm.

The carbon signals at $\delta$ 115, 116, 119, 127, 144, 145 ppm can be attributed to the aromatic carbons (ArC). The carbon signals at $\delta$ 34, 49, 52 ppm can be attributed
to aliphatic carbons (ArCH$_2$ & $-\text{NHCH}_2$—). The cyclohexane carbons (cycC) resonate as four signals at $\delta$ 25, 26, 30, 31 ppm.

Figure 3-3. NMR (CD$_3$OD, 297 K) spectra of precursor 19 a) $^1$H NMR spectrum and b) $^{13}$C NMR spectrum. *: solvent residue.

The $^1$H and $^{13}$C NMR spectra of dopamine-dibenzo[24]crown-8 precursor 20 are shown in Figure 3-4. The protons on aromatic ring (Ar–$H$) resonate as multiplets ranging from $\delta$ 6.68 to 7.19 ppm. The proton signal at $\delta$ 6.49 ppm can be attributed to the amide proton (–CONH–). In addition, the protons on ethylene glycol (–CH$_2$O–) resonate as multiplets ranging from $\delta$ 3.77 to 4.14 ppm. The proton signals at $\delta$ 3.49 and 2.7 ppm can be attributed to the aliphatic protons (ArCH$_2$ & $-\text{NHCH}_2$–).
The carbon signal at δ 167 ppm can be attributed to carbonyl carbon (–CONH–). The aromatic carbons (ArC) resonate as multiple signals in a region ranging from δ 112 to 151 ppm. In addition, the ethylene glycol carbons (–CH₂O–) resonate as multiple signals in the region ranging from δ 69 to 71 ppm. The carbon signals at δ 35 and 42 ppm can be attributed to the aliphatic carbons (ArCH₂ & –NHCH₂–). These results support the successful synthesis of precursors 19 and 20.

![Figure 3-4. NMR (CDCl₃, 297 K) spectra of precursor 20 a) ¹H NMR spectrum and b) ¹³C NMR spectrum. *: solvent residue.](image)

3.2.2.2 Mass spectrometry (MS)

Precursors 19 and 20 have also been characterized by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-Tof-MS). Singly
charged molecular ion base peaks (m/z) of 250.1806 and 650.2592 were observed which are corresponded to the precursor [19+H]^+ molecular ion with a theoretical value of 250.1801 and the precursor [20+Na]^+ molecular ion with a theoretical value of 650.2572, respectively (Figure 3-5). These results of MALDI-Tof-MS further confirmed the successful synthesis of the precursors 19 and 20.

**Figure 3-5.** Mass spectra of a) precursor 19 and b) precursor 20.

In addition, singly charged molecular ion base peak (m/z) of 268.1962 was also observed which is corresponded to the precursor [19+H_3O]^+ molecular ion with a theoretical value of 268.1907. The observation can be attributed to the strong intermolecular hydrogen bonding between hydroxyl groups of catechol and water. Furthermore, singly charged molecular ion base peaks (m/z) of 628.1627 and 666.2408 were also observed which is corresponded to the precursor [20+H]^+ and [20+K]^+ molecular ions with a theoretical value of 628.2752 and 666.2317 respectively.
3.2.2.3 Infrared (IR) spectroscopy

Precursors 19 and 20 were also characterized by IR spectroscopy that can further confirm the functional groups of the compounds. Figure 3-6 shows the IR spectrum of precursor 19. In principle, N–H stretching absorption peak of secondary amine ranges from 3300 to 3400 cm\(^{-1}\), C–N stretching ranges from 1000 to 1250 cm\(^{-1}\) and N–H wagging ranges from 660 to 900 cm\(^{-1}\). The observed absorption peaks 3390, 1179 and 748 cm\(^{-1}\) of precursor 19 suggest that it has an amine functional group.

![Figure 3-6. IR spectrum of precursor 19.](image)

Figure 3-7 shows the IR spectrum of precursor 20. The spectrum showed C=O stretching ranges from 1640 to 1690 cm\(^{-1}\) and N–H bending ranges from 1550 to 1640 cm\(^{-1}\), revealing an amide group. Also, C–O stretching ranges from 1000 to 1300 cm\(^{-1}\) that indicates the presence of an ether group. From the IR spectrum, the absorption peaks 1635, 1600 and 1120 cm\(^{-1}\) of precursor 20 suggest that it has an amide functional group as well as crown ether with C–O ether linkage.
Figure 3-7. IR spectrum of precursor 20.

3.3 Synthesis and characterization of meso-Fe₃O₄@19·20 NPs

3.3.1 Synthesis of meso-Fe₃O₄@19·20 NPs

Figure 3-8 shows the synthetic procedure of meso-Fe₃O₄ NPs. The Fe₃O₄(NAA) NPs were synthesized by dissolving FeCl₃·6H₂O, polyacrylic acid (PAA), sodium acetate in diethylene glycol and ethylene glycol co-solvent system at 200 °C in a Teflon-lined stainless steel autoclave according to literature with modifications. The Fe₃O₄(NAA) NPs with average size 100 nm was obtained after the solvothermal reaction and the size of the particles can be easily tuned by changing the ratios of diethylene glycol and ethylene glycol. Then, Fe₃O₄(NAA)@SiO₂ NPs were obtained by coating with an approximately 10 nm silica layer through sol-gel reaction to prevent ripening process between the Fe₃O₄(NAA) NPs and fusion to from large grains during calcination. The Fe₃O₄(NAA)@SiO₂ NPs were then thermally treated at a rate 10 °C/min at 550 °C under argon for 6 h. The meso-Fe₃O₄@SiO₂ NPs were obtained and re-dispersed in aqueous NaOH solution (1 M).
After stirring for 8 h, the meso-Fe$_3$O$_4$ NPs were washed with water and ethanol (EtOH) for several times. Finally, the synthesized meso-Fe$_3$O$_4$ NPs were dried in vacuum.

![Diagram of synthesis](image)

**Figure 3-8.** Schematic diagram of synthesis of meso-Fe$_3$O$_4$ NPs. (not drawn in scale)

Figure 3-9 shows the schematic diagram of preparation of meso-Fe$_3$O$_4$@19-20 NPs. Precursors 19 and 20 were dissolved in DMF first with equal molar concentration. Then, meso-Fe$_3$O$_4$ NPs were dispersed in the solution and followed by addition of TEA at 50 °C for 24 h. The NPs were collected by magnetic separation and washed with ethanol, water and acetone under ultrasonication for three times each. The meso-Fe$_3$O$_4$@19-20 NPs were dried in vacuum overnight and prepared for drug loading and drug release profile studies.
Figure 3-9. Schematic diagram of coupling between precursors 19 and 20 with meso-Fe₃O₄ NPs. (not drawn in scale)

3.3.2 Characterization of meso-Fe₃O₄@19-20 NPs

3.3.2.1 Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) is a useful microscopic technique to analyze the size and also morphology of NPs. By transmitting an electron beam through the specimen, the contrast between specimen and background would produce an image which can be used to investigate the interested NPs.

Fe₃O₄(PAA) NPs with an average diameter of 100 nm have been successfully synthesized with good monodispersity. Figure 3-10 shows the TEM images of Fe₃O₄(PAA) NPs that are agglomerative structures consist of many smaller primary grains. The as-synthesized Fe₃O₄(PAA) NPs were coated with silica layer with 10 nm to prevent further agglomeration during calcination and the TEM images of Fe₃O₄(PAA)@SiO₂ core/shell NPs are shown in figure 3-11.
Figure 3-10. TEM images of Fe$_3$O$_4$(PAA) NPs.

Figure 3-11. TEM images of Fe$_3$O$_4$(PAA)@SiO$_2$ NPs.

The Fe$_3$O$_4$(PAA)@SiO$_2$ NPs were calcinated in argon at 550 °C for 6 h to obtain meso-Fe$_3$O$_4$@SiO$_2$ NPs and their TEM images are shown in figure 3-12. The images show that there was some void space presence between silica layer and Fe$_3$O$_4$ core due to the removal of PAA in the Fe$_3$O$_4$ core. The TEM images of meso-Fe$_3$O$_4$ NPs are shown in figure 3-13. The NPs were obtained by dissolution of silica layer in NaOH solution (1 M) and the images showed that the average size of the meso-Fe$_3$O$_4$ NPs is quite similar to Fe$_3$O$_4$(PAA) NPs after calcination. Also, some void space can be observed between the primary grains.
Figure 3-12. TEM images of meso-Fe₃O₄@SiO₂ NPs.

Figure 3-13. TEM images of meso-Fe₃O₄@SiO₂ NPs.

After coupling of precursors 19 and 20 with meso-Fe₃O₄ NPs in DMF, the TEM images of the product meso-Fe₃O₄@19·20 NPs are shown in figure 3-14. The average diameter and morphology of meso-Fe₃O₄@19·20 NPs are almost the same as that of the meso-Fe₃O₄ NPs.
Figure 3-14. TEM images of meso-Fe$_3$O$_4$@19·20 NPs.

3.3.2.2 IR spectroscopy

IR spectroscopy is another important tool for investigate the functional group presence in the modified NPs. Although the signals of functional group on the NPs are generally weaker when compared to free molecules, some of the signals indicate the successful coupling of precursors 19 and 20 on the meso-Fe$_3$O$_4$ NPs. Figures 3-15 shows the IR spectra of meso-Fe$_3$O$_4$@19·20 NPs. IR absorptions of N–H stretching, C–H stretching, C=O stretching, C–O stretching, and C–N stretching are presence in the spectrum of meso-Fe$_3$O$_4$@19·20 NPs.
Figure 3-15. IR spectrum of meso-Fe₃O₄@19·20 NPs.

3.3.2.3 Thermogravimetric analysis (TGA)

Meso-Fe₃O₄@19·20 NPs were further investigated by thermogravimetric analysis (TGA). The weight loss indicates the decomposition of the organic fraction in the sample and usually in a temperature range from 150 to 500 °C.⁵

The TGA spectra of meso-Fe₃O₄@19·20 and meso-Fe₃O₄ NPs were shown in figures 3-16 and 3-17 respectively. A major weight losses were observed in the range from 150 to 500 °C. In figure 3-16, the weight loss of 12.48% from 150 to 500 °C is mainly attributed to the decomposition of precursor 19 and 20 on the surface of the NPs. In contrast, figure 3-17 shows a 2.23% weight loss from 150 to 500 °C indicating the decomposition of the residue PAA. From the TGA spectra, the percentage weight loss from 150 to 500 °C of meso-Fe₃O₄@19·20 is 12.48%
and that of meso-Fe₃O₄ is 2.23%, indicating there was 10.25% more organic fraction on the nanoparticle after coupling reaction. These results suggest a successful coupling of the precursors 19 and 20 with the meso-Fe₃O₄ NPs. Hence, meso-Fe₃O₄@19·20 NPs have been successfully synthesized.

Figure 3-16. TGA spectrum of meso-Fe₃O₄@19·20 NPs.

Figure 3-17. TGA spectrum of meso-Fe₃O₄ NPs.
3.3.2.4 X-ray diffraction (XRD) analysis

X-ray diffraction (XRD) analysis is a powerful tool to analyze the crystallinity of a compound. Since there may be a change in crystallinity of the Fe₃O₄ NPs after a reaction at elevated temperature, a XRD analysis was performed to investigate the crystallinity of meso-Fe₃O₄ NPs after calcination in argon. Figure 3-18 shows the XRD pattern of meso-Fe₃O₄ NPs. Diffraction peaks are mainly observed at 2θ = 30.2 °, 43.2 °, 57.1 ° and 62.7 ° which are corresponding to a face-centered cubic phase of Fe₃O₄ (JCPDS no. 01-071-6336). Therefore, meso-Fe₃O₄ NPs retain its crystallinity after calcination in argon.

Figure 3-18. XRD pattern of meso-Fe₃O₄ NPs
3.3.2.5 X-ray photoelectron spectroscopy (XPS)

X-ray photoelectron spectroscopy (XPS) is a powerful and quantitative spectroscopic tool to investigate the elemental composition and chemical state.

Figure 3-19 shows the XPS spectrum of meso-Fe₃O₄@19·20 NPs in N 1s region, which indicates three chemical environments of nitrogen such as amide, C–N linkage and amine. The binding energy of 400.5, 399.4 and 398.4 eV signals are corresponding to the chemical environment of amide, C–N linkage and amine respectively, indicating the successful coupling of precursors 19 and 20 on meso-Fe₃O₄ NPs. Furthermore, the ratio between precursors 19 and 20 can be found from XPS. The percentage concentration of amide and amine is 36.67 and 31.94 % respectively which suggested that the precursors 19 and 20 were coupled onto the surface of meso-Fe₃O₄ NPs in 1.15:1 ratio.

Figure 3-19. XPS spectrum of meso-Fe₃O₄@19·20 NPs in N 1s region.
3.3.3 Drug loading process

According to the previous binding study, the [1]pseudorotaxane formation showed the best result when performed in MeCN with 29% yield. Therefore, the loading process was conducted in MeCN to achieve the highest loading.

![Diagram of drug loading process](image)

**Figure 3-20.** Schematic illustration of drug loading process. (not drawn in scale)

Figure 3-20 shows a scheme of loading process. The solution of meso-$\text{Fe}_3\text{O}_4@\text{19·20}$ and ibuprofen in DCM which acidified with AcOH was sonicated for 1 h and continued stirring for another 24 h at ambient temperature. The NPs were collected by magnetic separation and re-dispersed in MeCN. After that, a valve closing process was conducted in MeCN at 60 °C for 5 d.

The loading percentage has been examined by HPLC. The ibuprofen-loaded NPs were heated in PBS at 65 °C for 3 d to ensure all drugs have been released from the NPs. The resulting solution was then investigated by HPLC to obtained the loading percentage.
Figure 3-21. A plot of peak area at $\lambda = 218$ nm against concentration of ibuprofen.

Figure 3-21 shows the calibration curve of ibuprofen and that the result shows a good linearity. The loading percentage of ibuprofen has been calculated as $1.66\pm0.11\%$.

3.3.4 Drug Release Profile

3.3.4.1 Release of drug triggered at 55 °C

Ibuprofen was encapsulated in meso-Fe$_2$O$_4@19\cdot20$ NPs and drug release profile was investigated with different stimuli. The released of ibuprofen was triggered by heating the NPs at 55 °C with or without sonication. A control experiment has also been performed. Besides, all analyses have been repeated for three times to obtain the standard deviation for investigate the reproducibility. From Figure 3-22a, the release of drug of NPs without any stimuli was performed in PBS (pH 7.38) at 37 °C and monitored by HPLC to trace the concentration of ibuprofen. The result
showed that there was approximately 20% of ibuprofen released from the NPs, indicating that there are some drug were not completely blocked by the nanovalve and therefore, some premature release of drug was observed. From Figure 3-22b, the NPs was heated at 55 °C at 30 min and 60 min for 2 min to trigger the release of drug. From the curve, there was drastic increased in concentration of ibuprofen which indicated the open of nanovalve and allowed the release of drug from the NPs. Furthermore, there was around 60% of drug released from the NPs which was 40% more than the NPs without any stimuli.

Besides heat, ultrasound was also employed as one of the stimuli. Ultrasound is a non-invasive way to provide extra energy to mechanically trigger the release of drug from NPs. From Figure 3-22c, the NPs were heated and sonicated for 2 min (55 °C; 45 kHz & 480 W power) to trigger the release of drug. The result shows that there is a drastic increase of drug concentration at 30 & 60 min and there is a total about 70% of drug released from NPs.

From Figure 3-22d, the NPs were sonicated for 2 min to study any release of drug from NPs. The result shows that there is a total about 30% of drug released from NP but without any drastic increase in drug concentration at 30 & 60 min, indicating that heat is the key stimulus to trigger the release of drug.
Figure 3-22. Release profile of IBU-Fe$_3$O$_4$@NPs performed in PBS (pH 7.38) at 37 °C and the release of drug was triggered with different stimuli: a) No Stimuli; b) heat at 55 °C for 2 min; c) heat & sonication for 2 min (45 kHz & 480 W power) and d) sonication for 2 min

3.3.4.2 Release of drug triggered at 43 °C

As aforementioned, heat is the key stimulus to trigger the release of drug from the NPs. However, 55 °C was too high and will cause damage to normal human tissue. To solve this problem, a lower temperature should be employed to trigger the drug release and therefore, 43 °C was chosen for this purpose because of two main reasons. First of all, 43 °C can induce apoptosis of cancer cell without affecting adjacent normal tissue. Secondly, the magnetic core can be heated up to 43 °C by applying alternating magnetic field which is a non-invasive way to heat up the magnetic NP core to trigger a drug release.
**Figure 3-23.** Release profile of IBU-Fe₃O₄@19-20 NPs performed in PBS (pH 7.38) at 37 °C and the release of drug was triggered with different stimuli: a) heat at 43 °C for 2 min; b) heat at 43 °C & sonication for 2 min (45 kHz & 480 W power)

From Figure 3-23a, the NPs were heated at 43 °C to trigger the release of drug encapsulated in NPs. However, the result shows that there is no triggered release of drug from the system. It is because the system lacks sufficient energy to allow the opening of valve. In order to achieve the release of drug at a lower temperature, ultrasound was employed to provide extra energy for the opening of valve. From Figure 3-23b, the NPs were heated and sonicated at 43 °C for 2 min which successfully triggered the release of drug from NPs. At 30 & 60 min, drastic increments of drug concentration have been observed and there is a total about 70% of drug released from the NPs suggesting that ultrasound provided extra energy to the system to mechanically trigger the release of drug.
3.3.4.3 Release of drug triggered at various pH

The controlled drug release system was proved to be triggered at lower temperature with ultrasound. To investigate the potential of application in cancer chemotherapy which has a more acidic physiological environment compared to normal cells, the release of drug was triggered at various pH values. From Figure 3-24a, the NPs were heated and sonicated at 43 °C & pH 3.01. In an acidic condition, the total drug release was approximately 20% which was comparative to the result of the release without stimuli. It is because the ammonium ion of the nanovalve could not be deprotonated completely and therefore, the drug could not be released from the system due to the steric hindrance of the nanovalve.

From Figure 3-24b, the NPs were heated and sonicated at 43 °C & at pH 5.03 which is similar to the acidic environment in cancer. The total drug release is around 60% and shows a drastic increase in drug concentration at 30 & 60 min, indicating the drug was successfully released from the NPs. In pH 5.03, the ammonium ion could be partially deprotonated which allowed the extrusion of crown-ether due to a weaker binding between the dialkyl amine and crown-ether. The extrusion of crown-ether implied the opening of nanovalve which allowed the release of drug from the system.
Figure 3-24. Release profile of IBU-Fe$_3$O$_4$@19c20 NPs performed in PBS at 37 °C and the release of drug was triggered with heat at 43 °C & sonication for 2 min (45 kHz & 480 W power): a) pH 3.01; b) pH 5.03 & c) pH 7.38

From Figure 3-23b (also shown in Figure 3-24c for better comparison), the NPs were heated and sonicated at 43 °C & pH 7.38 which is the normal physiological environment in human body. It shows the best drug release percentage among three trials and that the total drug release is approximately 70%. It is because of the slightly basic environment; the ammonium thread could be completely deprotonated and more valve could be opened in order to release more drugs from the NPs.

In short, the extent of drug release was pH dependent because of the deprotonation of ammonium thread. More drug can be released in a basic environment due to the higher extent of deprotonation of thread. At pH 5.03 which
is slightly acidic, the drug can also be triggered, implying that the system may also work fine in physiological environment in cancer.

3.4 Synthesis and characterization of USPION@23 NPs

3.4.1 Synthesis of compound 23

Compound 18 was reacted with 4-fluorobenzaldehyde in MeOH and heated at reflux. The reaction mixture was reduced by sodium borohydride in tetrahydrofuran THF/MeOH (v/v = 5:1) at 0 °C to give the amine compound 24 with 76% yield in two steps. Finally, compound 24 was deprotected by hydrogenolysis with 10% Pd/C as catalyst in methanol to give the target precursor 23 with 93% yield (Scheme 3-3).

Scheme 3-3. Synthetic scheme of precursor 23
3.4.2 Characterization of compound 23

3.4.2.1 Nuclear magnetic resonance (NMR) spectroscopy

The $^1$H and $^{13}$C NMR spectra of dopamine-cyclohexane precursor 23 are shown in Figure 3-25. The proton signals at δ 6.58, 6.72, 7.16 and 7.56 ppm can be attributed to the aryl protons (Ar–H). The proton signals at δ 2.88, 3.19 and 4.19 ppm can be attributed to the aliphatic protons (ArCH$_2$ & –NHCH$_2$–).

The carbon signals at δ 116.6, 116.7, 116.8, 117.0, 121.0, 128.5, 128.6, 129, 133.4, 133.5, 145, 147, 163 and 166 ppm can be attributed to the aromatic carbons (ArC). The carbon signals at δ 33, 50 and 51 ppm can be attributed to aliphatic carbons (ArCH$_2$ & –NHCH$_2$–).

![NMR spectra of precursor 23](image)

**Figure 3-25.** NMR (CD$_3$OD, 297 K) spectra of precursor 23 a) $^1$H NMR spectrum and b) $^{13}$C NMR spectrum. *: solvent residue.
3.4.2.2 Mass spectrometry

Precursor 23 has been characterized by MALDI-Tof-MS. A singly charged molecular ion base peak (m/z) of 262.1246 was observed which is corresponded to the precursor [23+H]^+ molecular ion with a theoretical value of 262.1237 (Figure 3-26). These results of MALDI-Tof-MS further confirms the successful synthesis of precursor 23.

Figure 3-26. Mass spectrum of precursor 23.

In addition, as the precursor with a relatively low molecular weight and therefore some noise has been observed because of the interference came from the matrix employed in the MALDI-Tof-MS during the analysis.
3.4.3 Synthesis of USPION@23 NPs

The synthesis of ultra-small superparamagnetic iron oxide nanoparticles (USPION) was performed according to literature with modification. The as-prepared oleic acid capped USPION dispersed in PhMe was mixed with precursor 23 in 0.01 M AcOH to perform ligand exchange. The ligand exchange was completed when all NPs dispersed in aqueous phase and therefore, USPION@23 was synthesized through ligand exchange (Figure 3-27).

![Synthetic scheme of USPION@23](image.png)

**Figure 3-27.** Synthetic scheme of USPION@23. (not drawn in scale)

3.4.4 Characterization of USPION@23 NPs

3.4.4.1 Transmission electron microscopy (TEM)

USPION@23 NPs with an average diameter of 13 nm have been successfully synthesized with good monodispersity. Figure 3-28 shows the TEM images of USPION@23 NPs that consist of single domain crystals with high crystallinity.
which can act as a good heat source under the influence of alternating magnetic field.

**Figure 3-28.** TEM images of USPION@23 NPs.

### 3.4.4.2 IR spectroscopy

Figure 3-29 shows the IR spectrum of USPION@23 NPs. IR absorptions of N–H stretching, C–H stretching, C=C stretching (aromatic), C–O stretching, and C–F stretching are presence in the spectrum of USPION@23 NPs. The result confirms the functional groups of USPION@23.

**Figure 3-29.** IR spectrum of USPION@23 NPs.
3.4.4.3 X-ray photoelectron spectroscopy

Figure 3-30 shows the XPS spectrum of USPION@23 NPs in F 1s region, indicating the chemical environments of fluorine C–F linkage. The binding energy of 685.0 eV signal is corresponding to the chemical environment of C–F linkage which indicate successful coupling of precursors 23 on USPION.

![XPS spectrum of USPION@23](image)

**Figure 3-30.** XPS spectrum of USPION@23.

3.5 Preparation of core-satellite Fe₃O₄ nanocomposite

To achieve a controlled drug release in biological system, another source of non-invasive external stimuli should be employed. However, meso-Fe₃O₄@19 20 NPs
are a secondary nanostructure consists of agglomerated primary magnetic domains which cannot respond to an alternating magnetic field for localized heating. In order to overcome the problem, a novel core-satellite Fe$_3$O$_4$ nanocomposite has been prepared through the pseudorotaxane formation between meso-Fe$_3$O$_4$@19-20 and USPION@23 (Figure 3-31). The components play with their own duties, meso-Fe$_3$O$_4$@19-20 is used for drug encapsulation and USPION@23 is used for the heat source and magnetic hyperthermia when respond to external alternating magnetic field.

![Image](image.png)

**Figure 3-31.** Preparation of core-satellite Fe$_3$O$_4$ nanocomposite. (not drawn in scale)

According to previous study, the [1]pseudorotaxane formation between the precursors 19 and 20 on NPs was achieved in 29% yield. Despite the [1]pseudorotaxane, there are some free precursor 20 on the surface of the NPs and thereby, different amount of USPION@23 was incubated with the meso-Fe$_3$O$_4$@19-20. The USPION@23 and meso-Fe$_3$O$_4$@19-20 were self-assembled to form a core-satellite nanocomposite through supramolecular interaction. The
core-satellite nanocomposites with different ratio have been investigated with TEM.

**Figure 3-32.** TEM images of meso-Fe$_3$O$_4$@19c20 and USPION@23 with ratio 1:1 (w/w).

Figure 3-32 shows the TEM images the core-satellite Fe$_3$O$_4$ nanocomposite formed between meso-Fe$_3$O$_4$@19c20 and USPION@23 with ratio 1:1 (w/w). The result shows that there is excess amount of USPION@23 and observed as many individual NPs.

**Figure 3-33.** TEM images of meso-Fe$_3$O$_4$@19c20 and USPION@23 with ratio 5:1 (w/w).
Figure 3-33 shows the TEM images of the core-satellite Fe₃O₄ nanocomposite formed between meso-Fe₃O₄@₁₉₋₂₀ and USPION@₂₃ with a ratio 5:1 (w/w). The result shows much better assembly when compared to that with ratio 1:1 (w/w). The TEM images show a core Fe₃O₄ modified with some smaller size Fe₃O₄ NPs resembling a core-satellite Fe₃O₄ nanocomposite. However, there are still some individual USPION@₂₃ were observed (marked with red circle).

Figure 3-34. TEM images of meso-Fe₃O₄@₁₉₋₂₀ and USPION@₂₃ with ratio 20:1 (w/w).

Figure 3-34 shows TEM images the core-satellite Fe₃O₄ nanocomposite formed between meso-Fe₃O₄@₁₉₋₂₀ and USPION@₂₃ with a ratio 20:1 (w/w). The TEM images show the best formation of core-satellite Fe₃O₄ nanocomposite
among other ratios. Every core meso-Fe₃O₄@₁₉₋₂₀ has surrounded with a layer of USPION@₂₃ which resembles the satellite around the core with an overall size 110 nm. Form the above result, the core-satellite Fe₃O₄ nanocomposite has been successfully prepared through supramolecular interaction.

3.6 Alternating magnetic field (AMF) as an external stimulus

3.6.1 Temperature increment under the influence of AMF

An AMF generator (100 kHz, 2900 W) has been employed in this experiment to investigate the potential of core-satellite Fe₃O₄ nanocomposite acting as a therapeutic agent for magnetic hyperthermia. Although the power of AMF generator used in this experiment is much lower than the commercial one (300 kHz, 15 kW), the core-satellite Fe₃O₄ nanocomposites with a ratio 20:1 (w/w) showed good response to the external AMF.

Figure 3-35. A plot of temperature rise against time of AMF applied to core-satellite Fe₃O₄ nanocomposite.
Figure 3-35 shows a plot of temperature rise against time of AMF applied to the system. In general, a higher concentration of nanocomposite would result in larger temperature rise. In the first 10 min, the temperature is gradually increased and reaches to a plateau after 10 min. The plateau is observed because of the heat loss to the surrounding compensated the heat generated from nanocomposite. As there is no net heat flow and therefore, the rise in temperature was retarded.

3.6.2 Release of drug triggered by AMF

Since the core is a thermo-responsive drug carrier, the release profile of core-satellite Fe₃O₄ nanocomposite with a ratio 20:1 (w/w) was investigated. The release of drug has been triggered by external AMF for 10 min at 30 & 60 min. Figure 3-36 shows the release profile of core-satellite Fe₃O₄ nanocomposite performed in PBS (pH 7.38) at 37 °C. There is an overall 90% of ibuprofen released from the nanocomposite after 90 min which is 70% more than that performed without stimuli (Figure 3-22a). The result reveals that the satellite USPION@23 act as a good heat source under the influence of AMF and the heat transfer to the core. The nanovalve on IBU-Fe₃O₄@1920 was opened due to the heat transfer from the satellite and thereby ibuprofen was released from the system.
3.7 Cytotoxicity of core-satellite Fe₃O₄ nanocomposites

To aim as drug delivery systems, biocompatibility of the nanocomposites was investigated. The nanocomposites should be non-cytotoxic towards the target cells and the cell viabilities (%) were determined by Cell Counting Kit-8 (CCK-8). The relative cell viabilities (%) of nanocomposites towards human gingival epithelial cells (HGEPs) and FaDu cells (human epithelial cell line) were determined and shown in Figure 3-37.

For ibuprofen-loaded core-satellite Fe₃O₄ nanocomposite (IBU-CSFe₃O₄) and core-satellite Fe₃O₄ nanocomposite (CSFe₃O₄), they showed non-cytotoxic towards both cells at various concentration 12.5–200 μg Fe/mL and 6.25–100 μg
Fe/mL corresponding to HGEPs and FaDu respectively. The results reveal the nanocomposites could be act as drug delivery system with good biocompatibility.

![Figure 3-37](image)

**Figure 3-37.** Viability of a) HGEPs and b) FaDu exposed to core-satellite Fe₃O₄ nanocomposite at various concentration.

### 3.8 Conclusion

The precursors 19 & 20 for [1]pseudorotaxane components and meso-Fe₃O₄ NPs have been successfully synthesized. Furthermore, the catechol-containing precursors 19 & 20 were successfully attached on the surface of meso-Fe₃O₄ NPs. Also, the meso-Fe₃O₄@19-20 NPs have been characterized by TEM, IR, TGA, XPS and XRD. The release profile of IBU-Fe₃O₄@19-20 NPs reveal that the NPs
can act as thermo-responsive drug carrier at higher temperature (55 °C) and dual responsive at lower temperature (43 °C) with ultrasound. On the other hand, USPION@23 has been successfully synthesized and characterized for self-assembling of a novel core-satellite Fe₃O₄ nanocomposite. The release profile of core-satellite Fe₃O₄ nanocomposite has been investigated with alternating magnetic field and that the nanocomposite is potentially used as therapeutic agent for magnetic hyperthermia and thermo-responsive drug carrier. Last but not least, the cytotoxicity analysis shows core-satellite Fe₃O₄ nanocomposite with good biocompatibility.

3.9 General information

Reactions were performed under nitrogen unless otherwise stated. Chromatography purifications were performed on silica gel (SiO₂) with the indicated eluents. Deionized water was obtained from Milli-Q ICW3000 water system. All solvents and reagents were in reagent grade and used as received without further purification. All particles were prepared with sonication by Elma Transsonic Tl-H-10 at 35 kHz with 70% power.

¹H and ¹³C NMR spectra for structural characterization were recorded with Bruker Avance 400 (¹H: 400 MHz; ¹³C: 101 MHz) spectrometer at 297 K. All NMR
samples were prepared in CDCl$_3$ unless otherwise stated. Spectra were calibrated internally using the CHCl$_3$ residual peak in CDCl$_3$ ($^1$H: $\delta = 7.26$; $^{13}$C: $\delta = 77.2$ ppm) and the CH$_3$OH residual peak in CD$_3$OD ($^1$H: $\delta = 3.31$; $^{13}$C: $\delta = 49.0$ ppm). Chemical shifts were reported as parts per million (ppm) in $\delta$ scale and coupling constants ($J$) were reported in hertz. Matrix-assisted laser desorption/ionization time of flight (MALDI-Tof) mass spectra were measured on a Bruker SolariX 9.4T mass spectrometer. The reported molecular mass ($m/z$) values correspond to the most abundant monoisotopic masses.

Thermogravimetric analysis (TGA) spectra were obtained by Perkin Elmer thermal gravimetric analyzer 6 with stated temperature range. Infrared (IR) spectra were obtained by Nicolet Magna 550 series II FTIR with potassium bromide (KBr) pellet. Surface analyses of NPs were performed by Tecnai G2 20 S-TWIN Transmission Electron Microscope (TEM). Analyses of NPs crystallinity were performed by Bruker AXS D8 Advance X-Ray Diffractometer (XRD). X-ray photoelectron spectroscopy (XPS) analysis were performed by Sengyang SKL-12 electron spectrometer equipped with a VG CLAM 4 MCD electron energy analyzer.
3.10 Experimental section

**Compound 19**

![Chemical structure of Compound 19](image)

Compound 21 (0.88 g, 2.05 mmol) was dissolved in MeOH and followed by the addition of 10 wt. % Pd/C in a catalytic amount. The reaction mixture was stirred for 24 h at 40 °C in hydrogen. The reaction mixture was filtered and the filtrate was concentrated under reduced pressure to yield compound 19 as a pale yellow solid (0.50 g, 97%). M.P.: 227.8 °C (Decomp.). *R*$_f$: 0.06 (EtOAc). $^1$H NMR (CD$_3$OD) δ = 6.54 (t, $J$ = 7.2 Hz, 2H, ArH), 6.41 (d, $J$ = 7.8 Hz, 1H, ArH), 2.96 (t, $J$ = 8.0 Hz, 2H, PhCH$_2$), 2.68 (t, $J$ = 7.5 Hz, 4H, –CH$_2$NHCH$_2$–), 1.52 – 1.61 (m, 6H, cycH), 1.03 – 1.20 (m, 3H, cycH), 0.81 – 0.89 (m, 2H, cycH). $^{13}$C NMR (CD$_3$OD) δ = 146.9, 145.7, 129.3, 121.1, 116.9, 116.8, 55.1, 51.1, 36.6, 32.8, 31.7, 27.2, 26.7. HRMS (MALDI-Tof): C$_{15}$H$_{24}$N$O_2$ $^+$/[M+H]$^+$ calcd: 250.1801, found: 250.1806.

**Compound 20**

![Chemical structure of Compound 20](image)
Compound 22 (1.15 g, 1.42 mmol) was dissolved in MeOH and followed by the addition of 10 wt. % Pd/C in catalytic amount. The reaction mixture was stirred for 24 h at 40 °C in hydrogen. The reaction mixture was filtered and the filtrate was concentrated under reduced pressure to yield a pale yellow glassy paste (0.87 g, 97%). $R_f$: 0.04 (EtOAc). $^1$H NMR (CDCl$_3$) $\delta = 7.17$ (t, $J = 6$ Hz, 2H, ArH), 6.86 – 6.49 (m, 8H, ArH), 6.48 (d, $J = 7.2$ Hz, 1H, –NHCO–), 4.11 – 4.09 (m, 4H, –OCH$_2$–), 4.03 (s, 2H, –OCH$_2$–), 3.92 – 3.77 (m, 18H, –OC$_2$H$_2$–), 3.49 (s, 2H, –CH$_2$NHCO–), 2.69 (s, 2H, PhCH$_2$–). $^{13}$C NMR (CDCl$_3$) $\delta = 167.7, 151.2, 148.5, 148.4, 148.0, 144.2, 143.0, 130.7, 126.6, 124.8, 121.3, 121.2, 120.2, 120.1, 115.7, 115.1, 113.7, 113.6, 112.0, 71.1, 71.02, 70.95, 69.7, 69.6, 69.4, 69.0, 68.9, 68.6, 41.4, 34.5. HRMS (MALDI-Tof): C$_{33}$H$_{41}$NO$_{11}$Na$^+$ [M+Na]$^+$ calcd: 650.2572, found: 650.2592.

**Compound 21**

![Compound 21](image)

Benzyl-protected dopamine 18 was prepared according to literature in two step with overall 82% yield.$^2$ Compound 18 (1.01 g, 3.0 mmol) and cyclohexanecarboxaldehyde (0.37 g, 3.3 mmol) were dissolved in toluene (200
mL). The reaction mixture was heated at reflux overnight with a Dean-Stark apparatus to remove the condensed water molecules. The mixture was concentrated under reduced pressure. The residue was then dissolved in mixture of THF (30 mL) and MeOH (3 mL) and followed by the addition of sodium borohydride (0.17 g, 4.5 mmol) in 0 °C. The reaction mixture was then stirred at room temperature for 12 h. Water was added to the reaction mixture and extracted with EtOAc three times. The organic fraction was combined and washed by brine for three times. The organic fraction was then dried with anhydrous magnesium sulfate (MgSO₄). The mixture was filtered and concentrated under reduced pressure. The residue was finally purified by column chromatography with EtOAc as the eluent. The purified product 21 was yellowish-brown liquid (0.88 g, 68%). $R_f$: 0.23 (EtOAc). $^1$H NMR (CDCl₃):

$\delta = 7.45 – 7.42$ (m, 4H, ArH), $7.36 – 7.28$ (m, 6H, ArH), $6.86$ (d, $J = 8.12$ Hz, 1H, ArH), $6.80$ (s, 1H, ArH), $6.72 – 6.70$ (m, 1H, ArH), $5.13$ (d, $J = 6.8$ Hz, 4H, PhCH$_2$O–), $2.76$ (t, $J = 6.7$ Hz, 2H, PhCH$_2$–), $2.68$ (t, $J = 6.7$ Hz, 2H, –CH$_2$NH–), $2.40$ (d, $J = 6.7$ Hz, 2H, –NHCH$_2$–), $1.67$ (d, $J = 3.6$ Hz, 5H, cycH & –CH$_2$NHCH$_2$–), $1.42 – 1.36$ (m, 2H, cycH), $1.26 – 1.14$ (m, 3H, cycH), $0.89 – 0.80$ (m, 2H, cycH). $^{13}$C NMR (CDCl₃): $\delta = 148.9, 147.4, 137.5, 137.4, 133.7, 128.48, 128.47, 127.8, 127.75, 127.38, 127.35, 121.6, 115.8, 115.4, 71.5, 71.3, 51.4, 56.7, 56.7,$
Compound 22

![Compound 22](image)

Compound 18 (0.59 g, 1.77 mmol) and DB24C8-OSu (1.04 g, 1.77 mmol) were dissolved in DCM. The reaction mixture was stirred at room temperature for 24 h. Then, the mixture was washed with water and 1 M HCl three times each. The organic fraction was dried over anhydrous MgSO₄. The mixture was then filtered and concentrated. The residue was purified by column chromatography with EtOAc: MeOH (10:1) then gradient to CHCl₃: MeOH (10:1) as eluent. The product compound 22 was a white solid (1.12 g, 78%). M.P.: 137.3 – 138.9 °C. Rf: 0.45 (EtOAc). ¹H NMR (CDCl₃) δ = 7.45 – 7.41 (m, 4H, ArH), 7.37 – 7.28 (m, 7H, ArH), 7.10 (d, J = 8 Hz, 1H, ArH), 6.88 (t, J = 7.2 Hz, 5H, ArH), 6.82 (s, 1H, ArH), 6.78 (d, J = 8.4 Hz, 1H, ArH), 6.73 (d, J = 8 Hz, 2H, ArH), 6.02 (s, 1H, –NHCO–), 5.12 (d, J = 11.6 Hz, 4H, PhCH₂O–), 4.15 (d, J = 7.6 Hz, 8H, –OCH₂–), 3.91 (s, 8H, –OCH₂–), 3.82 (d, J = 7.2 Hz, 8H, –OCH₂–), 3.64 – 3.59 (m, 2H, –
Compound 23

\[
\begin{align*}
\text{HO} & \quad \text{H} & \quad \text{N} & \quad \text{F} \\
\text{HO} & \quad \text{Ph} & \quad \text{Ph}
\end{align*}
\]

Compound 24 (1.12 g, 2.54 mmol) was dissolved in MeOH and followed by the addition of 10 wt. % Pd/C in a catalytic amount. The reaction mixture was stirred for 24 h at 40 °C in hydrogen. The reaction mixture was filtered and the filtrate was concentrated under reduced pressure to yield compound 23 as a pale yellow solid (0.71 g, 93%). M.P.: 192.7 °C (Decomp.). \( R_f \): 0.09 (EtOAc). \( ^1H \) NMR (400 MHz, MeOD) \( \delta = 7.57 \) (s, 2H), 7.16 (s, 2H), 6.72 (s, 2H), 6.59 (s, 1H), 4.20 (s, 2H), 3.19 (s, 2H), 2.89 (s, 2H). \( ^{13}C \) NMR (101 MHz, MeOD) \( \delta = 165.9, 163.4, 146.5, 145.4, 133.5, 133.4, 129.0, 128.58, 128.55, 121.0, 117.0, 116.81, 116.76, 116.7, 51.5, 50.0, 32.6. \) HRMS (MALDI-Tof): \( C_{15}H_{17}FNO_2^+ \) [M+H]⁺ calcd: 262.1238, found: 262.1246.
Compound 24

Compound 18 (1.51 g, 4.5 mmol) and 4-fluorobenzaldehyde (0.62 g, 5.0 mmol) were dissolved in MeOH (50 mL). The reaction mixture was heated at reflux for 12 h. The mixture was concentrated under reduced pressure. The residue was then dissolved in mixture of THF (30 mL) and MeOH (6 mL) and followed by the addition of sodium borohydride (0.26 g, 6.8 mmol) in 0 °C. The reaction mixture was then stirred at room temperature for 12 h. Water was added to the reaction mixture and extracted with EtOAc three times. The organic fraction was combined and washed by brine for three times. The organic fraction was then dried with anhydrous magnesium sulfate (MgSO₄). The mixture was filtered and concentrated under reduced pressure. The residue was finally purified by column chromatography with EtOAc as the eluent. The purified compound 24 was yellowish-brown liquid (1.36 g, 68%). \( R_f \): 0.19 (EtOAc). \(^1\)H NMR (400 MHz, CDCl₃) \( \delta = 7.49 – 7.42 \) (m, 4H, ArH), 7.40 – 7.27 (m, 6H, ArH), 7.24 – 7.18 (m, 2H, ArH), 6.99 (ddd, \( J = 8.7, 5.8, 2.5 \) Hz, 2H, ArH), 6.87 (d, \( J = 8.1 \) Hz, 1H, ArH), 6.80 (d, \( J = 2.0 \) Hz, 1H, ArH), 6.71 (dd, \( J = 8.1, 2.0 \) Hz, 1H, ArH), 5.14 (s, 4H, \( \cdots \)
PhCH₂O–), 3.72 (s, 2H, PhCH₂NH–), 2.80 (t, J = 7.1 Hz, 2H, PhCH₂–), 2.72 (t, J = 6.6 Hz, 2H, –CH₂NH–), 1.54 (s, 1H, –NH–). ¹³C NMR (101 MHz, CDCl₃) δ = 163.2, 160.8, 149.0, 147.5, 137.6, 137.4, 136.02, 135.99, 133.4, 129.7, 129.66, 128.6, 127.88, 127.86, 127.5, 127.4, 121.7, 115.9, 115.4, 115.3, 115.1, 71.6, 71.4, 53.2, 50.5, 35.8. HRMS (MALDI-Tof): C₂⁹H₂₉FNO₂⁺ [M+H]⁺ calcd: 442.2177, found: 442.2160.

**Preparation of superparamagnetic Fe₃O₄(PAA) NPs**

Superparamagnetic Fe₃O₄(PAA) NPs were synthesized by solvothermal reaction according to the literature procedure with modification.³ By dissolving FeCl₃·6H₂O (0.54 g, 2 mmol) in EG/DEG (4/16 mL v/v) co-solvent system, an orange solution was obtained. PAA (60 mg) and NaOAc (1.5 g) were dissolved in the reaction mixture. The resultant brownish yellow solution was transferred into a Teflon-lined stainless-steel autoclave and heat to 200 °C for 12 h. The autoclave was cooled to room temperature and the black precipitate was washed with water (50 mL) five times. The Fe₃O₄(PAA) NPs with an average diameter of 100 nm were successfully synthesized.

**Preparation of Fe₃O₄(PAA)@SiO₂ NPs**

The Fe₃O₄(PAA)@SiO₂ NPs were synthesized by sol-gel reaction according to the literature procedure with modification.³ As-synthesized Fe₃O₄ particles (300 mg)
were dispersed in a mixture of ethanol (500 mL), water (50 mL) and NH$_3$ solution
(16 mL) under ultrasonication for 4 h. A tetraethyl orthosilicate (TEOS) solution
(2.5 mL in 15 mL ethanol) was added to the resultant mixture in 40 min and then
further sonicated for 40 min. The solid product was collected by magnetic
separation and washed with ethanol and water successively for five times.

**Preparation of meso-Fe$_3$O$_4$ NPs**

The meso-Fe$_3$O$_4$ NPs were prepared by calcination in tube oven at 550 °C for 8 h
(10 °C/min) under argon. Then, particles were re-dispersed in 1 M NaOH for 8 h to
remove the silica coating. The NPs were collected by magnetic separation and
washed with water five times. The NPs were dried under vacuum and collected as
black powder.

**Preparation of meso-Fe$_3$O$_4$@19-20 NPs**

The meso-Fe$_3$O$_4$ NPs (100 mg) were dispersed in DMF (4 mL) under
ultrasonication for 2 h. Precursors 19 (62.7 mg, 0.1 mmol) and 20 (24.9 mg, 0.1
mmol) were dissolved in DMF (1 mL) and TEA (0.5 mL). Then, the mixture was
added to the NPs under ultrasonication. After an hour, the reaction was continued
under magnetic stirring for 24 h at 50 °C. The product was collected by magnetic
separation and washed with EtOH, water, acetone three times each. The product
was dried under vacuum and collected as a black powder.
**Preparation of USPION@23**

The oleic acid capped USPION was prepared according to literature. The as-prepared oleic acid capped USPION (50 mg) was dispersed in PhMe (10 mL) under sonication for 2 h. Precursor 23 (50 mg) was dissolved in 0.01 M AcOH. Two solution was mixed vigorously and sonicated for another 15 min. The completion of ligand exchange was indicated by the phase transfer of USPION from organic layer to aqueous layer. USPION@23 was collected with magnetic separation and washed with EtOH, acetone and water successively for three times each.

**Drug loading of IBU-Fe₃O₄@19⊂20 NPs**

Meso-Fe₃O₄@19·20 NPs (20 mg) and ibuprofen (40 mg) were dispersed in DCM (2 mL) at pH 3 and ambient temperature for 24 h. The mixture was collected with magnetic separation and re-dispersed in MeCN. The NPs was then heat at 60 °C for another 5 d. The NPs were collected by magnet and wash by CHCl₃ for three times under sonication. The NPs were dried under vacuum. The loading % was then investigated by HPLC.

**Preparation of core-satellite Fe₃O₄ nanocomposite**

The IBU-Fe₃O₄@19⊂20/meso-Fe₃O₄@19⊂20 (100 mg) was dispersed in MeCN and incubated with USPION@23 (5 mg) at ambient temperature for 24 h under
vigorouss stirring. The resultant nanocomposite was connected by magnetic separation and wash with water and EtOH successively for three times.

**Release profile of IBU-Fe$_3$O$_4@19\subset20$ NPs**

IBU-Fe$_3$O$_4@19\subset20$ NPs (3 mg) was dispersed in PBS (1 mL) and incubated at 37°C while measurements were taken each 10 min. During 30 & 60 min, different stimuli was applied to the system. The NPs were collected by magnetic separation and the supernatants were analyzed by HPLC.

**Temperature rise of core-satellite Fe$_3$O$_4$ nanocomposite under influence of AMF**

Core-satellite Fe$_3$O$_4$ nanocomposite (3 mg) was dispersed in PBS (1 mL) and put in the AMF generator (100 kHz, 2900 W). The temperature rise was recorded at different time.

**Release profile of ibuprofen-loaded core-satellite Fe$_3$O$_4$ nanocomposite**

Ibuprofen-loaded core-satellite Fe$_3$O$_4$ nanocomposite (3 mg) was dispersed in PBS (1 mL) and incubated at 37°C while measurements were taken each 10 min. During 30 & 60 min, AMF (100 kHz, 2900 W) was applied to the system for 10 min. The NPs were collected by magnetic separation and the supernatants were analyzed by HPLC.
Cytotoxicity of Ibu-SCFe\textsubscript{3}O\textsubscript{4} and SCFe\textsubscript{3}O\textsubscript{4} on human gingival epithelial cells and Fadu cells

The human gingival epithelial cells (HGEPs) (passage 4) were seeded in 96-well plate at a density of 1.5×10\textsuperscript{4} cells/well and incubated for 2 d until reaching the confluence of 80%. Then, the cells were treated with IBU-CSFe\textsubscript{3}O\textsubscript{4} and CSFe\textsubscript{3}O\textsubscript{4} containing various concentrations of Fe (200, 100, 50, 25 and 12.5 μg/mL) for 24 h. Then, the cell viabilities were determined using Cell Counting Kit-8 (CCK-8, Sigma-Aldrich, St Louis, USA). The media containing nanoparticles were replaced by the fresh medium with CCK-8 (100 μL medium and 10 μL CCK-8 reagent per well) and incubated with the cell for 2 h. The absorbance of the medium was read at 450 nm using SpectraMax M2 Microplate Reader (Molecular Devices, California, USA). Similar treatment was conducted on Fadu cells. Briefly, Fadu cells were seeded in the 96-well plate at a density of 1×10\textsuperscript{4} cells/well. After the overnight incubation, the cells were treated with IBU-CSFe\textsubscript{3}O\textsubscript{4} and SCFe\textsubscript{3}O\textsubscript{4} containing various concentrations of Fe (100, 50, 25, 12.5 and 6.25 μg/mL) for 24 h. Then, the cell viabilities were evaluated by CCK-8.
3.11 Reference:


Chapter 4 – Novel Chemosensor for Phosphate Ion based on [1]Pseudorotaxane Prepared by Slippage Approach

4.1 Background

Mechanically interlocked molecules have caught scientists’ interest because of their potential applications in being nanoscale artificial molecular machines such as rotating motors,1 shuttles,2,3 scissors,4 molecular muscle,5 etc. [1]Rotaxane is a unique mechanically interlocked structure because a single molecule threads through itself. Recently, various [1]-rotaxanes prepared by different synthetic routes such as self-threading/threading followed by capping/self-capping,6,7 covalent bond formation,8 and self-entanglement,9,10 have been reported.

On the other hand, fluorescent moieties based on aromatic amide have been reported recently.11,12 Because of the fluorescence “Off–On” behaviors can be well-controlled by introducing an effective obstacle to a twisted intramolecular charge transfer (TICT) quenching process, different chemosensors for alkaline earth metal based on this concept have been reported.13,14 However, most of the literatures are focused on the detection of cations.

Inspired by these innovative ideas, a novel thermodynamically stable [1]pseudorotaxane was prepared by a template-directed slippage approach for ion detection. The mechanically interlocked moiety acts as an effective obstacle to
TICT quenching process in order to enhance the emission intensity in the presence of ions.

4.2 Synthesis of [1]pseudorotaxane 25-H·PF₆

Scheme 4-1 shows the synthesis of [1]pseudorotaxane 25-H·PF₆. Amine compound 26 was prepared with high yield and purity according to literature. Amine compound 27 was prepared first by imine formation between amine 26 and cyclohexanecarboxaldehyde in methanol under reflux for 12 h, followed by the addition of NaBH₄ to reduce the imine into an amine. Compound 27 was obtained with an overall 64% yield in two steps. The secondary amine compound 27 was protected with a Boc group with di-tert-butyl dicarbonate with 96% yield. Boc-protected compound 28 was then tosylated with p-toluenesulfonyl chloride to give compound 29 in 76% yield. Subsequently, compound 29 was reacted through Williamson ether synthesis with 4-cyanophenol to prepare compound 30 in 72% yield. Cyano-compound 30 was then reduced by LiAlH₄ to obtain a primary amine 31 in 94% yield. Afterwards, Compound 31 was coupled with dibenzo[24]crown-8 succinimide (DB24C8-OSu) to obtain compound 32 with 74% yield. Compound 33 was prepared by deprotecting the Boc-protected compound 32 with TFA in DCM with 94% yield. Compound 33-H·PF₆ was prepared by protonating compound 33.
with conc. HCl to pH 3 and followed by counterion exchange with sat. NH₄PF₆ solution with 81% yield. Finally, [1]pseudorotaxane 25-H·PF₆ was prepared by incubating compound 33-H·PF₆ with KPF₆ in ACN with 32% yield through a slippage approach. [1]pseudorotaxane 25-H·PF₆ was synthesized in 9 steps with an overall 6% yield.

4.3 Characterization

4.3.1 $^1$H Nuclear magnetic resonance (NMR) spectroscopy

Stacked $^1$H NMR spectra of 33-H·PF$_6$ and 25-H·PF$_6$ are shown in Figure 4-1 with sharp signals. All the proton signals have been assigned and the proton signals with newly shifted patterns have also been observed which suggested that the [1]pseudorotaxane has been successfully synthesized. First of all, the aromatic proton $H_e$, $H_f$, and $H_g$ shifted downfield and $H_d$ shifted upfield which suggested a displaced edge-on $\pi-\pi$ interaction between the corresponding two phenyl rings. First of all, the aromatic proton $H_e$, $H_f$, and $H_g$ shifted downfield and $H_d$ shifted upfield which suggested a displaced edge-on $\pi-\pi$ interaction between the corresponding two phenyl rings.\textsuperscript{16} Also, the proton signals adjacent to the ammonium center, $H_a$ & $H_b$, were shifted from $\delta = 2.65$ and 4.09 ppm to $\delta = 2.75$ and 4.02 ppm, respectively. In particular, crown ether ethylene glycol proton, $H_m$ shifted downfield and split into a new pattern because of the hydrogen bond and electrostatic interaction between ammonium ion and crown ether.

To further analyze the chemical structure of the [1]pseudorotaxane, a two-dimensional NOSEY $^1$H NMR spectral analysis has been performed (Figure 4-2). Some cross peaks have been observed between DB24C8’s protons ($H_m$, $H_e$) and the triethylene glycol chain’s protons ($H_h$, $H_i$) and N–CH ($H_b$). For example, (i) DB24C8 proton $H_m$ and triethylene glycol proton $H_i$ and (ii) DB24C8 proton $H_l$ and protons $H_b$ & $H_h$. These results reveal that the triethylene glycol chain was folded to thread to the crown ether.
Figure 4-1. $^1$H NMR (CD$_2$Cl$_2$, 298 K) spectra of compound a) 33-H·PF$_6$ and b) 25-H·PF$_6$. *: solvent residue (f = free).

Figure 4-2. A partial 2D-NOSEY spectrum of [1]pseudorotaxane 25-H·PF$_6$. 
4.3.2 $^{13}$C Nuclear magnetic resonance (NMR) spectroscopy

Stacked $^{13}$C NMR spectra of $^{33}$-H·PF$_6$ and $^{25}$-H·PF$_6$ are shown in Figure 4-3 with sharp signals. The aromatic carbons and ethylene carbons of $^{25}$-H·PF$_6$ showed a new pattern because of the differences in chemical environment in $^{33}$-H·PF$_6$, revealing the successful synthesis of the $[1]$pseudorotaxane.

![NMR spectra](image)

**Figure 4-3.** $^{13}$C NMR (CD$_2$Cl$_2$, 298 K) spectra of compound a) $^{33}$-H·PF$_6$ and b) $^{25}$-H·PF$_6$. *: solvent residue.

4.3.3 Mass spectrometry (MS)

$[1]$Pseudorotaxane $^{25}$-H·PF$_6$ has also been characterized by Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-Tof-MS) by a deprotonation of $^{25}$-H·PF$_6$ with an excess amount of Et$_3$N. The deprotonated $[1]$pseudorotaxane was relatively stable at room temperature.$^{17}$ A singly charged
molecular ion base peak ($m/z$) of 953.4765 was observed which corresponded to the [1]pseudorotaxane [25+Na]$^+$ molecular ion with a theoretical value of 953.4770 (Figure 4-4). This result of MALDI-Tof-MS further confirmed the successful synthesis of [1]pseudorotaxane without other spices such as dimer, trimer, oligomer, etc.

**Figure 4-4.** Mass spectrum of [1]pseudorotaxane 25-H·PF$_6$.

### 4.3.4 UV/Visible absorption and fluorescence spectroscopies

The UV/Vis absorption spectra of [1]pseudorotaxane 25-H·PF$_6$ and 33-H·PF$_6$ are shown in Figure 4-5a. Both compound showed a similar absorption band at 240–300 nm which is a common absorption band of aromatic amide reported in literature.$^{18}$
Figure 4-5. a) UV/Vis absorption profile of [1]pseudorotaxane 25-H·PF₆ and 33-H·PF₆ and b) fluorescence emission spectra (excitation wavelength = 270 nm) of [1]pseudorotaxane 25-H·PF₆ and 33-H·PF₆. (conc. = 0.01 mM in MeCN; slit width = 10, 10 nm)

Fluorescence emission spectra with excitation at 270 nm, where most of the light absorbed by the aromatic amide moiety, of [1]pseudorotaxane 25-H·PF₆ and
compound 33-H·PF₆ are shown in Figure 4-5b. For both compounds, they give fluorescence at $\lambda_{\text{max}} = 345$ nm. Interestingly, the emission intensity of [1]pseudorotaxane 25-H·PF₆ was 7.5-fold higher than that of compound 33-H·PF₆ because of the well-controlled twisted intramolecular TICT quenching effect. For compound 33-H·PF₆, the phenyl amide bond can be relatively freely rotated and therefore the fluorescence signal was greatly quenched by the non-radioactive TICT process.¹⁹ In contrast, the rotation of the aromatic amide moiety in [1]pseudorotaxane 25-H·PF₆ was restricted by the formation of pseudorotaxane where the mechanically interlocked molecule plays an important role on controlling the TICT process. Furthermore, the formation of [1]pseudorotaxane brings the benzene rings adjacent to each other and act as an intramolecular energy transfer (IntraET) donor (Scheme 4-2).¹⁴

On the other hand, the second shoulder at $\lambda_{\text{max}} = 656$ nm corresponding to the phosphorescence of [1]pseudorotaxane 25-H·PF₆ and the lifetime of phosphorescence was measured as 4.41 $\mu$s.
4.4 Effect of external stimuli

4.4.1 Addition of base

Fluorescence emission spectra of [1]pseudorotaxane titrated with different amount of Et₃N have been obtained (Figure 4-6). Upon the addition of 0.1 equiv. of Et₃N, the fluorescence intensity of 25-H·PF₆ dropped to 40% and kept at the same level until 1 equiv. of Et₃N was added. With the increasing amount of Et₃N, the fluorescence signals have been decreased continuously. The results showed that the [1]pseudorotaxane is highly sensitive to base because of the deprotonation of the ammonium ion which allowed the rotation of the phenyl amide bond and therefore, the TICT quenching process is resumed. When the amount of Et₃N increased, the fluorescence intensity dropped to 150 a.u. which is similar to that of 33-H·PF₆, revealing that the [1]pseudorotaxane started to dissociate.
Figure 4-6. a) Stacked fluorescence emission spectra of [1]pseudorotaxane with various amount of Et₃N and b) a plot of relative fluorescence intensity at 345 nm against the equiv. of Et₃N used. (Relative intensity is defined as the ratio of the fluorescence intensity between each data point and 25-H-PF₆) (conc. = 0.01 mM in MeCN; slit width = 10, 10 nm).

To further confirm the hypothesis of quenching effect by base, titration of 25-H-PF₆ against Et₃N/TFA (trifluoroacetic acid) has been performed (Figure 4-7a). After the addition of 1 equiv. of Et₃N, the fluorescence emission was quenched because of TICT quenching process resumption. Afterwards, TFA was added to 25-H-PF₆ for re-protonation and the fluorescence emission was increased again. Since the secondary amine was protonated again, the DB24C8 reassembled on the ammonium ion which was mechanically interlocked as well as the phenyl amide bond again. As a result, the bond rotation was restricted again and acts as an obstacle to the relaxation process.

In addition, a plot of the relative fluorescence intensity of 25-H-PF₆ retreated with TFA against time is shown in Figure 4-7b. The intensity required approximately 6 min to attain the maximum intensity again, implying that the
reassemble process requires a relatively longer time for completion. The results reveal the fact that enhancement of fluorescence intensity is attributed to the mechanically interlocked pseudorotaxane acting as an effective obstacle to TICT quenching process.

**Figure 4-7.** a) Relative fluorescence intensity of titration between 25-H-PF₆ and Et₃N/TFA alternately (odd: 1 equiv. Et₃N; even: 1 equiv. TFA) and b) a plot of relative fluorescence intensity of 25-H-PF₆ (after treatment of Et₃N) retreated with TFA against time. (conc. = 0.01 mM in MeCN; slit width = 10, 10 nm).

Besides, the fluorescence intensity can only be restored to original after three times of acid/base addition. This is because of the successive addition of Et₃N/TFA resulted in a buffer in the mixture where 25-H-PF₆ cannot be completely deprotonated/protonated after several cycles of addition.

4.4.2 Addition of salt

The use of metal ion may be one of the possibilities for quenching the fluorescence emission by competitive binding towards the DB24C8. The fluorescence emission spectra of titration between 25-H-PF₆ and KPF₆ are shown in Figure 4-8. From the above result, the fluorescence emission intensity did not show a strong quenching effect when compared to the case of base addition. It is because the DB24C8 can
still encircle the ammonium ion and that the mechanically interlocked part acts as an effective obstacle to the TICT quenching process. Although the fluorescence emission intensity only drops to 70% of the original one, the decrement of the signal is due to the distortion of the well-assembled structure by the insertion of K$^+$ ion. The distortion brought the phenyl rings apart from the phenyl amide moiety and thereby, reducing the efficiency of intraET process as well as the fluorescence emission intensity.

**Figure 4-8.** a) Stacked fluorescence emission spectra of [1]pseudorotaxane 25-H$^\cdot$PF$_6$ with various amounts of KPF$_6$ and b) a plot of relative fluorescence intensity at 345 nm against the equiv. of KPF$_6$ used (conc. = 0.01 mM in MeCN; slit width = 10, 10 nm).

### 4.5 Ion sensing

#### 4.5.1 Metal ion sensing

The fluorescence quenching ability of the [1]pseudorotaxane with sixteen different metals ion have been studied. The fluorescence emission intensities were only
quenched insignificantly ($\sim 0 - 20\%$) with the addition of metal ion after an incubation for 30 min (Figure 4-9) or 24 h (Figure 4-10). It is believed that the size or the charge of the metal ion only play a minor role in distortion of the mechanically interlocked moiety as well as the restoration of the TICT quenching process.

**Figure 4-9.** A plot of quenching fluorescence intensity (\%) of [1]pseudorotaxane 25-H·PF$_6$ treated with different metal ions for 30 min where $I =$ intensity of 25-H·PF$_6$ treated with metal ions; $I_o =$ intensity of 25-H·PF$_6$ (conc. = 0.01 mM in MeCN; metal ion = 1 equiv.).
A plot of relative quenched fluorescence intensity of [1]pseudorotaxane 25-H·PF$_6$ treated with different metal ions for 24 h.

4.5.2 Inorganic anion sensing

A series of seven anions have been screened and that their quenching ability for the [1]pseudorotaxane have been verified by fluorescence emission spectroscopy. Among these anions, only phosphate ion shows significant quenching effect for 30 min (Figure 4-11) and 24 h (Figure 4-12). The use of either Na$^+$ or K$^+$ as the countercation shows no significant difference. This is because the phosphate ion interacts with the ammonium ion and amide proton by hydrogen bonding which distorted the mechanical bonding between ammonium ion and DB24C$_8$. As a result, the fluorescence emission was quenched by the phosphate ion due to the restoration of TICT quenching process. In addition, the system only requires 30 min to attain equilibrium which allows immediate detection of phosphate ion.
Figure 4-11. A plot of relative quenched fluorescence intensity of [1]pseudorotaxane 25-H·PF\(_6\) treated with different anions for 30 min where I = intensity of 25-H·PF\(_6\) treated with anions; I\(_0\) = intensity of 25-H·PF\(_6\) (conc. = 0.01 mM in MeCN; anion = 1 equiv.).

Figure 4-12. A plot of relative quenched fluorescence intensity of [1]pseudorotaxane 25-H·PF\(_6\) treated with different anions for 24 h.

The fluorescence emission spectra of [1]pseudorotaxane titrated against Na\(_3\)PO\(_4\) are shown in Figure 4-13a. Interestingly, the intensity of fluorescence emission varies with the amount of Na\(_3\)PO\(_4\) used. Furthermore, the intensity varies
with the first equiv. of $\text{PO}_4^{3-}$ ion and give a linear relationship with a good
correlation value (Figure 4-13c). Also, it reaches a plateau in the range of 2 – 5
equiv. and increases again when the amount of water content increased (Figure 4-
13b).

![Figure 4-13. a) Stacked fluorescence emission spectra of [1]pseudorotaxane 25-
H·PF$_6$ with various amount of Na$_3$PO$_4$; b) a plot of relative fluorescence intensity
at 345 nm against the equiv. of Na$_3$PO$_4$ used and c) a plot of relative fluorescence
intensity at 345 nm against the first equiv. of Na$_3$PO$_4$ (conc. = 0.01 mM in MeCN;
slit width = 10, 10 nm).](image)

The [1]pseudorotaxane 25-H·PF$_6$ showed a good selectivity towards
phosphate ion because of the strong hydrogen bonding formed between phosphate
ion, ammonium ion and amide group. Furthermore, the ionic size of the ion also
played another important role to the selectivity due to the torsion angle of amide
bonding. Because of the ionic size of phosphate ion was comparatively large and
therefore increased the torsion angle of amide bond. Based on the two factors
mentioned above, 25-H·PF$_6$ showed high selectivity towards phosphate ion.
4.6 Conclusion

A novel [1]pseudorotaxane 25-H·PF₆ has been successfully synthesized by a template-directed slippage approach. The fluorescence properties have been investigated by fluorescence spectrophotometry. The fluorescence emission intensity has been enhanced after the formation of [1]pseudorotaxane because the mechanically interlocked molecule act as an effective obstacle to TICT quenching process. Furthermore, a series of ions have been screened and only phosphate ion shows a significant quenching effect towards [1]pseudorotaxane among all other tested ions. The results suggest the novel [1]pseudorotaxane 25-H·PF₆ can act as a good phosphate ion sensor.

4.7 General information

Reactions were performed under nitrogen unless otherwise stated. Chromatography purifications were performed on silica gel (SiO₂) with the indicated eluents. Deionized water was obtained from Milli-Q ICW3000 water system. All solvents and reagents were in reagent grade with high purity and used as received without further purification. In addition, Sodium phosphate tribasic dodecahydrate (BioXtra grade) with purity ≥ 98.0% was used in the analysis.

¹H and ¹³C NMR spectra for structural characterization were recorded with
Bruker Avance 400 (\(^1\)H: 400 MHz; \(^{13}\)C: 101 MHz) spectrometer at 297 K. All NMR samples were prepared in CDCl\(_3\) unless otherwise stated. Spectra were calibrated internally using the CH\(_2\)Cl\(_2\) residual peak in CD\(_2\)Cl\(_2\) (\(^1\)H: \(\delta = 5.32\); \(^{13}\)C: \(\delta = 54.0\) ppm) and the CHCl\(_3\) residual peak in CDCl\(_3\) (\(^1\)H: \(\delta = 7.26\); \(^{13}\)C: \(\delta = 77.2\) ppm). Chemical shifts were reported as parts per million (ppm) in δ scale and coupling constants (\(J\)) were reported in hertz. Matrix-assisted laser desorption/ionization time of flight (MALDI-Tof) mass spectra were measured on a Bruker SolariX 9.4T mass spectrometer. The reported molecular mass (\(m/\text{z}\)) values correspond to the most abundant monoisotopic masses.

UV-Vis spectra were obtained by Agilent UV-Vis spectrometer Cary 300. Fluorescence spectra were obtained by Perkin Elmer LS 55 fluorescence spectrometer. All measurements have been repeated for three times to reduce error.

**4.8 Experimental section**

[1]Pseudorotaxane 25·HPF\(_6\)
A solution of compound 33-H-PF$_6$ (0.21 g, 0.20 mmol) and KPF$_6$(0.036 g, 0.20 mmol) in MeCN (1 mL) was heated at 65 °C in Schlenk flask for 24 h. The resulting solution was evaporated to dryness and the residue was partitioned between water (5 mL) and DCM (2.5 mL). The aqueous layer was further extracted by DCM (3 × 2.5 mL). The combined organic layer was dried over anhydrous MgSO$_4$ and the filtrate was evaporated to dryness. Flash column chromatography with DCM/MeCN (1:1) on silica gel of the residue gave the compound 25-H-PF$_6$ (0.065 g, 31%) as a white paste. $R_f$: 0.58. $^1$H NMR (400 MHz, CD$_2$Cl$_2$) $\delta =$ 7.57 (d, $J =$ 2.0 Hz, 1H, ArH), 7.49 (dd, $J =$ 8.5, 2.0 Hz, 1H, ArH), 7.38 (t, $J =$ 6.0 Hz, 1H, —CONH—), 7.24 (d, $J =$ 8.7 Hz, 2H, ArH), 7.21 (d, $J =$ 8.7 Hz, 2H, ArH), 7.15 (d, $J =$ 8.6 Hz, 1H, ArH), 7.12 – 7.05 (m, 4H, ArH), 6.82 (dd, $J =$ 11.7, 8.7 Hz, 4H, ArH), 4.42 (d, $J =$ 5.8 Hz, 2H, —CONHC$_2$H$_5$), 4.38 (dd, $J =$ 5.3, 3.3 Hz, 2H, CH$_2$O—), 4.35 (dd, $J =$ 5.3, 3.5 Hz, 2H, CH$_2$O—), 4.29 (dd, $J =$ 9.1, 6.5 Hz, 4H, CH$_2$O—), 4.09 – 4.01 (m, 6H, CH$_2$O– & CH$_2$NH), 3.79 (dd, $J =$ 5.4, 2.9 Hz, 8H, CH$_2$O–), 3.73 (dd, $J =$ 8.7, 4.9 Hz, 4H, CH$_2$O–), 3.67 (s, 4H, CH$_2$O–), 3.59 (s, 8H, CH$_2$O–), 2.75 (d, $J =$ 6.5 Hz, 2H, CH$_2$NH), 2.15 (s, 2H, –NH), 1.66 – 1.54 (m, 6H, cyc-H), 1.10 (t, $J =$ 9.7 Hz, 3H, cyc-H), 0.92 – 0.81 (m, 2H, cyc-H). $^{13}$C NMR (100 MHz, CD$_2$Cl$_2$) $\delta =$ 167.7, 160.1, 158.2, 151.5, 148.5, 148.3, 148.2, 132.1, 131.4, 129.3, 129.1, 123.9, 123.8, 122.9, 122.4, 117.4, 116.7, 116.4, 116.3, 115.5, 115.0, 70.9, 70.0, 69.9, 69.5,
Compound 27

Compound 27 was prepared by a reductive amination. A solution of compound 26 (12.67 g, 49.6 mmol) and cyclohexanecarboxaldehyde (5.84 g, 52.1 mmol) in MeOH (50 mL) was heated at reflux for 12 h. The resultant mixture was evaporated to dryness and the residue was re-dissolved in 30 mL anhydrous THF/MeOH (2:1). NaBH₄ (2.82 g, 74.4 mmol) was then added to the solution at 0 °C and stirred for 6 h. The resulting solution was dried in vacuum and re-dissolved in CHCl₃ (50 mL). The solution was then washed with water (3 × 25 mL). The organic extract was dried over anhydrous MgSO₄ and the resulting mixture was filtered through a pack of celite. The filtrate was evaporated to dryness. Flash column chromatography with EtOAc on silica gel of the residue gave the compound 27 (11.16 g, 64%) as a pale-yellow liquid. Rf: 0.21 (EtOAc). ¹H NMR (400 MHz, CDCl₃): δ = 7.25 – 7.18 (m, 2H, ArH), 6.92 – 6.83 (m, 2H, ArH), 4.15 – 4.09 (m, 2H, CH₂NH), 3.85 (dd, J = 69.2, 68.6, 68.5, 68.0, 67.7, 67.6, 67.53 (s), 67.45 (s), 67.3, 67.2, 53.8, 52.4, 43.7, 35.3, 30.4, 26.1, 25.6. HRMS (MALDI-TOF): C₅₂H₇₀N₂O₁₃Na[M+Na]+: calcd 953.4770; found 953.4765.
5.4, 4.2 Hz, 2H, CH$_2$O–), 3.75 – 3.67 (m, 8H, CH$_2$O–), 3.63 – 3.57 (m, 2H, CH$_2$O–),
2.43 (d, $J = 6.7$ Hz, 2H, CH$_2$NH), 2.13 (s, 2H, –NH & –OH), 1.77 – 1.61 (m, 5H, cyc-H),
1.47 (ddd, $J = 11.3$, 6.8, 3.4 Hz, 1H, cyc-H), 1.29 – 1.08 (m, 3H, cyc-H),
0.95 – 0.83 (m, 2H, cyc-H). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta = 157.8$, 133.0, 129.4,
114.7, 77.5, 77.2, 76.8, 72.7, 71.0, 70.5, 69.9, 67.6, 61.9, 56.2, 53.58, 37.99, 31.60,
26.82, 26.20. HRMS (MALDI-TOF): C$_{20}$H$_{34}$NO$_4$ [M+H]$^+$: calcd 352.2482; found
352.2463.

**Compound 28**

![ Compound 28 ]

A solution of compound 27 (11.16 g, 31.8 mmol) and di-tert-butyl dicarbonate (7.00
g, 32.1 mmol) in MeOH (40 mL) was stirred at 0 °C for 1 h and continued stirring
at ambient temperature for another 5 h. The resulting solution was dried in vacuum
and the residue was partitioned between dilute HCl (0.1 M, 50 mL) and EtOAc (25
mL). The aqueous layer was further extracted with EtOAc (3 × 25 mL). The
combined organic layer was dried over anhydrous MgSO$_4$ and the filtrate was
evaporated to dryness. Flash column chromatography with hexane/EtOAc (1:1) on
silica gel of the residue gave compound 28 (13.75 g, 96%) as a colorless liquid. $R_f$
0.61 (hexane/EtOAc = 1:1). $^1$H NMR (400 MHz, CDCl$_3$) $\delta = 7.11$ (t, $J = 10.0$ Hz,
2H, ArH), 6.84 (d, J = 8.6 Hz, 2H, ArH), 4.34 (d, J = 21.1 Hz, 2H, CH₂NH), 4.17 – 4.06 (m, 2H, CH₂O–), 3.89 – 3.79 (m, 2H, CH₂O–), 3.77 – 3.64 (m, 6H, CH₂O–), 3.62 – 3.53 (m, 2H, CH₂O–), 2.96 (dd, J = 38.2, 5.9 Hz, 2H, CH₂NH), 2.69 (s, 1H, –OH), 1.74 – 1.54 (m, 6H, cyc-H), 1.44 (d, J = 20.3 Hz, 9H, CH₃), 1.29 – 1.06 (m, 3H, cyc-H), 0.88 (s, 2H, cyc-H). ¹³C NMR (100 MHz, CDCl₃) δ = 157.8, 156.4, 156.0, 131.1, 130.9, 129.0, 128.4, 114.6, 79.5, 77.5, 77.2, 76.8, 72.6, 70.9, 70.4, 69.8, 67.4, 61.8, 52.5, 52.2, 50.5, 49.5, 36.9, 36.6, 31.0, 28.6, 26.6, 26.0. HRMS (MALDI-TOF): C₂₅H₄₁NO₆Na [M+Na]⁺: calcd 474.2826; found 474.2796.

**Compound 29**

![Compound 29](image)

A solution of compound 28 (13.75 g, 30.4 mmol), TEA (12.7 mL, 91.3 mmol) and DMAP (cat.) in DCM (100 mL) was stirred at 0 °C. A solution of TsCl (6.09 g, 31.9 mmol) in DCM (50 mL) was added dropwise to the above mixture at 0 °C. The mixture was stirred for another 12 h at ambient temperature. The resulting solution was evaporated to dryness and the residue was partitioned between dilute HCl (0.1 M, 100 mL) and DCM (200 mL). The organic layer was further washed with dilute HCl (3 × 50 mL), water (3 × 50 mL) and sat. NaCl solution (3 × 25 mL). The organic
layer was dried over anhydrous MgSO$_4$ and the mixture was filtered with a pack of celite. The filtrate was evaporated to dryness. Flash column chromatography with hexane/EtOAc (3:1) on silica gel of the residue gave the compound 29 (14.02 g, 76%) as a pale-yellow liquid. $R_f$: 0.67 (hexane/EtOAc = 3:1). $^1$H NMR (400 MHz, CDCl$_3$) $\delta = 7.77$ (dd, $J = 8.3$, 1.6 Hz, 2H, ArH), 7.31 (d, $J = 7.6$ Hz, 2H, ArH), 7.11 (s, 2H, ArH), 6.83 (d, $J = 8.0$ Hz, 2H, ArH), 4.34 (d, $J = 20.4$ Hz, 2H, CH$_2$NH), 4.18 – 4.11 (m, 2H, CH$_2$O–), 4.07 (s, 2H, CH$_2$O–), 3.79 (s, 2H, CH$_2$O–), 3.70 – 3.56 (m, 6H, CH$_2$O–), 2.96 (d, $J = 32.8$ Hz, 2H, CH$_2$NH), 2.41 (s, 3H, ArCH$_3$), 1.65 (dd, $J = 22.5$, 9.1 Hz, 6H, cyc-H), 1.44 (d, $J = 20.1$ Hz, 9H, CH$_3$), 1.14 (dd, $J = 29.6$, 8.0 Hz, 3H, cyc-H), 0.87 (s, 2H, cyc-H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta = 157.9$, 156.4, 156.0, 144.9, 133.0, 131.1, 129.9, 129.0, 128.4, 128.0, 114.6, 79.4, 70.8, 70.8, 69.8, 69.3, 68.8, 67.5, 52.5, 52.2, 50.5, 49.5, 36.9, 36.6, 31.0, 28.5, 26.6, 26.0, 21.7. HRMS (MALDI-TOF): C$_{32}$H$_{47}$NO$_8$SNa$[M+Na]^+$: calcd 628.2925; found 628.2956.

**Compound 30**

![Compound 30](image_url)

A solution of compound 29 (14.02 g, 23.1 mmol), 4-hydroxybenzonitrile (3.03 g, 25.5 mmol) and K$_2$CO$_3$ (10.55 g, 76.4 mmol) in acetone (150 mL) was heated at
reflux for 24 h. The resultant mixture was cooled down and filtered with a pack of celite. The filtrate was evaporated to dryness and the residue was partitioned between NaOH (0.1 M, 100 mL) and EtOAc (50 mL). The aqueous layer was further extracted with EtOAc (3 × 50 mL). The combined organic layer was dried over anhydrous MgSO\(_4\) and the filtrate was evaporated to dryness. Flash column chromatography with hexane/EtOAc (3:1) on silica gel of the residue gave the compound \(\mathbf{30}\) (9.21 g, 72%) as a colorless liquid. \(R_f\): 0.63 (hexane/EtOAc = 3:1).

\(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta = 7.52\) (d, \(J = 8.9\) Hz, 2H, Ar\(H\)), 7.10 (s, 2H, Ar\(H\)), 6.92 (d, \(J = 8.9\) Hz, 2H, Ar\(H\)), 6.82 (d, \(J = 8.6\) Hz, 2H, Ar\(H\)), 4.33 (d, \(J = 19.6\) Hz, 2H, \(CH_2NH\)), 4.17 – 4.11 (m, 2H, \(CH_2O\)), 4.11 – 4.04 (m, 2H, \(CH_2O\)), 3.88 – 3.79 (m, 4H, \(CH_2O\)), 3.71 (s, 4H, \(CH_2O\)), 2.96 (d, \(J = 30.7\) Hz, 2H, \(CH_2NH\)), 1.64 (dd, \(J = 23.4, 10.6\) Hz, 6H, cyc-\(H\)), 1.42 (d, \(J = 19.8\) Hz, 9H, \(CH_3\)), 1.22 – 1.06 (m, 3H, cyc-\(H\)), 0.86 (s, 2H, cyc-\(H\)). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta 162.1, 157.8, 156.3, 155.9, 133.9, 131.0, 130.8, 128.9, 128.3, 119.2, 115.3, 114.5, 104.0, 79.3, 70.9, 70.8, 69.8, 69.4, 67.8, 67.4, 52.4, 52.2, 50.4, 49.4, 36.8, 36.5, 30.9, 28.4, 26.5, 25.9. HRMS (MALDI-TOF): C\(_{32}\)H\(_{44}\)N\(_2\)O\(_6\)Na [M+Na]^+: calcd 575.3102; found 575.3100.
A solution of compound 30 (9.21 g, 16.7 mmol) in anhydrous THF (30 mL) was added dropwise to a suspension of LiAlH₄ (0.95 g, 25.0 mmol) in THF (50 mL) at 0 °C. The resulting mixture was stirred at ambient temperature for 4 h. The resulting mixture was added to an ice bath and the suspension was filtered through a pack of celite. The filtrate was evaporated to dryness and the residue was partitioned between water (100 mL) and CHCl₃ (50 mL). The aqueous layer was further extracted with CHCl₃ (3 × 50 mL). The combined organic layer was dried over anhydrous MgSO₄ and the filtrate was evaporated to dryness. Flash column chromatography with CHCl₃/EtOH (3:1) on silica gel of the residue gave the compound 31 (8.74 g, 94%) as a pale-yellow liquid. $R_f$: 0.31 (CHCl₃/EtOH = 3:1).

$^1$H NMR (400 MHz, CDCl₃) δ 7.14 (d, $J = 8.6$ Hz, 2H, ArH), 7.08 (s, 2H, ArH), 6.81 (dd, $J = 8.4$, 5.9 Hz, 4H, ArH), 4.31 (d, $J = 21.5$ Hz, 2H, CH₂NH), 4.09 – 4.00 (m, 4H, CH₂O–), 3.83 – 3.75 (m, 4H, CH₂O–), 3.72 (s, 2H, CH₂NH), 3.68 (s, 4H, CH₂O–), 2.94 (d, $J = 33.3$ Hz, 2H, CH₂NH), 1.90 (s, 2H, NH₂), 1.62 (dd, $J = 22.8$, 9.7 Hz, 6H, cyc-H), 1.41 (d, $J = 21.1$ Hz, 9H, CH₃), 1.22 – 1.03 (m, 3H, cyc-H),
0.92 – 0.77 (m, 2H, cyc-H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta = 157.8, 157.6, 156.2, \ldots, 52.3$, $52.0, 50.3, 49.3, 45.6, 36.7, 36.4, 30.8, 28.3, 26.4, 25.8$. HRMS (MALDI-TOF): C$_{32}$H$_{48}$N$_2$O$_6$Na $[M+Na]^+$: calcd 579.3404; found 579.3442.

**Compound 32**

![Compound 32](image)

A solution of compound 31 (3.17 g, 5.69 mmol) and dibenzo[24]crown-8-OSu (3.36 g, 5.69 mmol) in DCM (30 mL) was heated at reflux for 12 h. The resulting solution was evaporated to dryness and the residue was partitioned between water (50 mL) and CHCl$_3$ (25 mL). The aqueous layer was further extracted with CHCl$_3$ (3 × 25 mL). The combined organic layer was dried over anhydrous MgSO$_4$ and the filtrate was evaporated to dryness. Flash column chromatography with EtOAc on silica gel of the residue gave the compound 32 (4.35 g, 74%) as a white powder. M.P.: 98.7 – 99.4 °C. $R_f$: 0.43 (EtOAc). $^1$H NMR (400 MHz, CD$_2$Cl$_2$) $\delta = 7.42$ (d, $J = 1.8$ Hz, 1H, ArH), 7.36 (s, 1H, ArH), 7.25 (d, $J = 8.7$ Hz, 2H, ArH), 7.14 (d, $J = 8.6$ Hz, 2H, ArH), 6.94 – 6.81 (m, 10H, ArH & –CONH–), 4.49 (d, $J = 5.7$ Hz, 2H, –CONHCH$_2$),
4.35 (s, 2H, CH$_2$NH), 4.15 – 4.06 (m, 12H, CH$_2$O–), 3.88 – 3.79 (m, 12H, CH$_2$O–), 3.76 (d, $J = 4.9$ Hz, 8H, CH$_2$O–), 3.69 (s, 4H, CH$_2$O–), 2.98 (d, $J = 11.1$ Hz, 2H, CH$_2$NH), 1.67 (dd, $J = 27.4$, 12.1 Hz, 6H, cyc-$H$), 1.44 (d, $J = 20.3$ Hz, 9H, $CH_3$), 1.28 – 1.12 (m, 3H, cyc-$H$), 0.96 – 0.83 (m, 2H, cyc-$H$). $^{13}$C NMR (101 MHz, CD$_2$Cl$_2$) $\delta = 166.8$, 158.4, 158.2, 156.4, 156.0, 151.9, 149.30, 149.28, 148.8, 131.6, 131.3, 129.4, 129.1, 128.8, 127.8, 121.69, 121.67, 120.5, 114.80, 114.6, 114.48, 114.45, 113.3, 112.7, 79.4, 78.0, 71.5, 71.4, 71.1, 70.1, 70.02, 70.00, 69.9, 69.6, 69.52, 69.47, 69.45, 52.6, 50.5, 49.7, 43.6, 37.2, 36.9, 31.3, 28.5, 26.9, 26.3. HRMS (MALDI-TOF): C$_{57}$H$_{78}$N$_2$O$_{15}$Na [M+Na]$^+$: calcd 1053.5294; found 1053.5307.

**Compound 33**

A solution of compound 32 (4.35 g, 4.21 mmol) and TFA (5 mL) in DCM (30 mL) was stirred at ambient temperature for 6 h. The resulting solution was neutralized with sat. Na$_2$CO$_3$ solution and washed with water (3 × 15 mL). The organic layer was dried over anhydrous MgSO$_4$ and the filtrate was evaporated to give compound 33 (3.68 g, 94%) as a white powder. M.P.: 117.3 – 118.7 °C. $R_f$: 0.11 (EtOAc). $^1$H
NMR (400 MHz, CD$_2$Cl$_2$) δ = 7.38 (d, J = 2.0 Hz, 1H, ArH), 7.29 (dd, J = 8.4, 2.1 Hz, 1H, ArH), 7.25 (d, J = 8.6 Hz, 2H, ArH), 7.21 (d, J = 8.5 Hz, 2H, ArH), 6.91 – 6.81 (m, 9H, ArH), 6.50 (t, J = 5.6 Hz, 1H, –CONH–), 4.50 (d, J = 5.7 Hz, 2H, –CONHCH$_2$), 4.18 – 4.12 (m, 4H, CH$_2$O–), 4.12 – 4.06 (m, 8H, CH$_2$O–), 3.85 (dt, J = 8.9, 5.1 Hz, 8H, CH$_2$O–), 3.83 – 3.79 (m, 4H, CH$_2$O–), 3.76 (d, J = 3.1 Hz, 8H, CH$_2$O–), 3.69 (s, 4H, CH$_2$O–), 3.66 (s, 2H, CH$_2$NH), 2.41 (d, J = 6.7 Hz, 2H, CH$_2$NH), 1.76 – 1.62 (m, 6H, cyc-H & –NH), 1.43 (ddd, J = 11.2, 6.9, 3.4 Hz, 1H, cyc-H), 1.28 – 1.11 (m, 3H, cyc-H), 0.95 – 0.84 (m, 2H, cyc-H). $^{13}$C NMR (100 MHz, CD$_2$Cl$_2$) δ = 166.8, 158.5, 158.1, 152.0, 149.37, 149.35, 149.0, 131.4, 129.6, 129.4, 127.8, 121.70, 121.67, 120.3, 114.9, 114.6, 114.53, 114.48, 113.3, 112.8, 71.6, 71.5, 71.4, 71.13, 71.12, 70.20, 70.19, 70.11, 70.09, 70.05, 70.00, 69.8, 69.6, 69.5, 67.9, 67.8, 56.5, 53.7, 43.7, 38.4, 31.9, 27.1, 26.5. HRMS (MALDI-TOF): C$_{52}$H$_{70}$N$_2$O$_{13}$Na [M+Na]$^+$: calcd 953.4770; found 953.4757.

**Compound 33-H·PF$_6$**

A solution of compound 9 (3.68 g, 4.0 mmol) in 30 mL DCM/MeOH (5:1) was
stirred at 0 °C. The solution was acidified with conc. HCl until the pH of the solution reached at pH 3 and the resulting solution was stirred at ambient temperature for 2 h. The solution was dried under reduced pressure and the residue was re-dissolved in acetone (30 mL). A solution of sat. NH₄PF₆ (3 mL) was added to the reaction and the resulting solution was stirred at ambient temperature for another 2 h. The reaction mixture was evaporated to dryness and the residue was partitioned between water (30 mL) and CHCl₃ (15 mL). The aqueous layer was further extracted with CHCl₃ (3 × 15 mL). The combined organic layer was dried over anhydrous MgSO₄ and the filtrate was dried at reduced pressure to give compound 33-H·PF₆ (3.45 g, 81%) as a white glassy powder. M.P.: 74.2 – 75.7 °C. Rf: 0.61 (DCM/MeCN = 1:1).

1H NMR (400 MHz, CD₂Cl₂) δ = 7.37 (dd, J = 8.4, 1.5 Hz, 1H, ArH), 7.32 (s, 1H, ArH), 7.19 (d, J = 8.7 Hz, 2H, ArH), 7.15 (d, J = 5.8 Hz, 1H, ArH), 7.11 (d, J = 8.7 Hz, 2H, ArH), 7.00 – 6.91 (m, 5H, ArH & –CONH–), 6.88 (d, J = 8.7 Hz, 2H, ArH), 6.74 (d, J = 8.7 Hz, 2H, ArH), 4.42 (d, J = 5.9 Hz, 2H, –CONHCH₂), 4.24 – 4.12 (m, 8H, CH₂O–), 4.09 (dd, J = 5.4, 3.6 Hz, 2H, CH₂NH), 4.03 – 3.96 (m, 4H, CH₂O–), 3.90 – 3.81 (m, 8H, CH₂O–), 3.81 – 3.70 (m, 12H, CH₂O–), 3.65 (d, J = 2.2 Hz, 4H, CH₂O–), 2.65 (d, J = 6.8 Hz, 2H, CH₂NH), 1.63 (d, J = 10.1 Hz, 8H, cyc-H & –NH), 1.10 (t, J = 9.5 Hz, 3H, cyc-H), 0.91 – 0.80 (m, 2H, cyc-H). 13C NMR (100 MHz, CD₂Cl₂) δ = 167.8, 160.2, 158.4, 151.5, 148.7, 148.5, 148.3, 132.1,
HRMS (MALDI-TOF): C_{52}H_{70}N_{13}O_{13}Na[M+Na]^+: calcd 953.4770; found 953.4751.

4.9 Reference:


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Appendix

List of NMR spectra

1. \( ^1 \text{H} \) NMR spectrum of compound 17
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17-H·PF₆·DB24C8
CURRICULUM VITAE

Academic qualifications of the thesis author, Mr. WONG Chi Hin:

- Received the degree of Bachelor of Science (Honours) in Chemistry from the Chinese University of Hong Kong, November 2012.

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