

Designing phosphate sensors based on supramolecular self-assembly strategies

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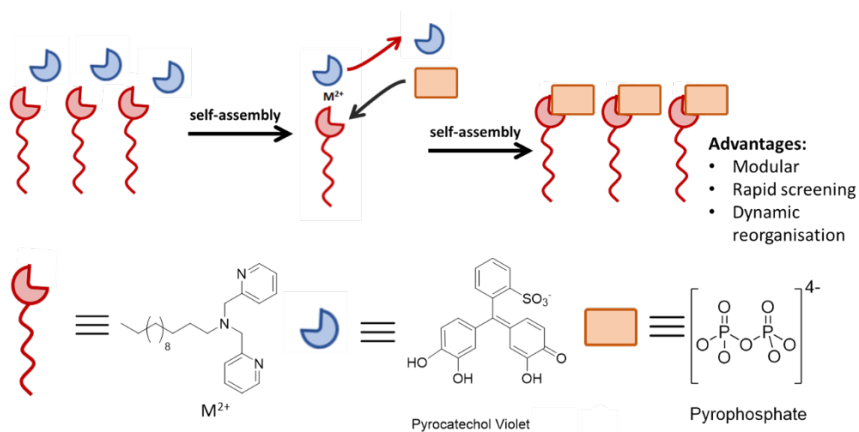
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Abstract

This thesis explores how molecular self-assembly can be harnessed to construct functional supramolecular receptors for the selective recognition of biologically relevant phosphate anions in water. By integrating principles of coordination chemistry, amphiphilic self-assembly, and indicator displacement assays (IDAs), a modular strategy was developed that eliminates the need for complex covalent scaffolds while enabling fine control over receptor geometry, selectivity, and responsiveness.

In the first part of this work, a family of dipicolylamine- Zn^{II} amphiphiles was designed and synthesised to investigate how hydrophobic chain length influences self-assembly and analyte recognition. Systematic studies revealed that mid-length amphiphiles, particularly C_8 -picolyl- Zn^{II} **37**, exhibited optimal behaviour: spontaneous vesicle formation, cooperative 2:1 host-indicator binding with pyrocatechol violet (**PV**), and selective displacement by pyrophosphate (PPi) under physiological buffer conditions. These results established clear structure-function relationships between molecular architecture, aggregation, and binding efficiency.



Building on these findings, the second part introduced bolaamphiphilic derivatives, in which two coordination sites were linked through hydrophobic spacers. The most effective of these, C_8 -di-picolyl- Zn^{II} **55**, displayed rapid and reversible PPi sensing driven by intramolecular coordination, confirming that molecular symmetry and flexibility govern the balance between stability and responsiveness.

In the final section, the design strategy was extended to thiol-based amphiphiles capable of forming silver-thiolate coordination polymers. The C_6 -picolyl-SH·Zn^{II} **72** system exhibited redox-switchable behaviour, assembling into disulfide-linked bolaamphiphiles that selectively recognised PPi with excellent selectivity over ATP, ADP, and citrate. Incorporation into a micellar matrix further enhanced signal clarity and reproducibility.

Collectively, these studies demonstrate that dynamic self-assembly provides a powerful and versatile route to functional receptor systems, capable of adaptive and selective molecular recognition. The principles developed here offer a foundation for future design of stimuli-responsive, metal-organic supramolecular sensors and catalytic materials operating in aqueous environments.

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Abbreviations

δ	chemical shift
$^{\circ}\text{C}$	degree Celsius
μ	micro
μL	microliter
μM	micromolar
$\gamma\text{-CD}$	γ -cyclodextrin
λ_{em}	emission wavelength
λ_{ex}	excitation wavelength
A	adenine
ATP	adenosine triphosphate
ADP	adenosine diphosphate
AIE	aggregation induced enhancement
ApA	adenylyl(3'→5')phosphoadenine
AMP	adenosine monophosphate
Asp	aspartic acid
ATP	adenosine triphosphate
a.u.	arbitrary unit
AuNPs	gold nanoparticles
BPR	Bromopyrogallol Red
Boc	<i>tert</i> -butyloxycarbonyl
(Boc) ₂ O	di- <i>tert</i> -butyl dicarbonate
br s	broad singlet
Brij O10	Polyoxyethylene (10) oleyl ether
C	cytosine
cAMP	cyclic adenosine monophosphate
CAC	critical aggregation concentration
Cbz	carbobenzyloxy group
CMC	critical micelle concentration
Coum	coumarin
cP	cyclic phosphate
C _{pp}	critical packing factor
cryo-TEM	cryo-transmission electron microscope
CTAB	hexadecyltrimethylammonium bromide
d	doublet
DBF	dibenzofulvene
DCC	<i>N,N'</i> -dicyclohexylcarbodiimide

DCL	dynamic combinatorial library
dd	doublet of doublets
DET	diethylenetriammonium
DFT	density function theory
DOPC	1,2-dioleoyl-sn-glycero-3-phosphocholine
DMP	dimethylphosphate
DPH	1,6-diphenyl-1,3,5-hexatriene
DPPC	dipalmitoylphosphatidylcholine
dt	doublet of triplets
DIAD	diisopropyl azodicarboxylate
DIPA	<i>N,N</i> -diisopropylaminoethanol
DIPEA	<i>N,N</i> -diisopropylethylamine
DLS	dynamic light scattering
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
DMP	dimethyl phosphate
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DNPP	2,4-dinitrophenyl phosphate
DPA	di-2-picolyamine
EDC·HCl	<i>N,N</i> -diisopropylethylamine hydrochloride
E _m	emission
EM	electron microscopy
ER	endoplasmic reticulum
et al.	and others
eq.	equivalent(s)
E _x	excitation
FAM	carboxylfluorescein
FI	fluorescence intensity
FRET	fluorescence resonance energy transfer
Fmoc	fluorenylmethyloxycarbonyl
g	gram
G	guanine
Gu	guanidine
GUVs	giant unilamellar vesicles
Glu	glutamic acid
h	hour(s)
HEPES	2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid
His	histidine

HoBt	hydroxybenzotriazole
HOMO	highest occupied molecular orbital
HPNPP	2-hydroxypropyl <i>p</i> -nitrophenyl phosphate
HRESIMS	high resolution electrospray ionisation mass spectrometry
Hz	Hertz
ICT	intramolecular charge transfer
IFE	inner filter effect
IR	infrared
<i>J</i>	coupling constant
LDA	linear discrimination analysis
LUVs	large unilamellar vesicles
m	multiplet
M	molar
Me	methyl group
MeCN	acetonitrile
MES	2-(<i>N</i> -morpholino)ethanesulfonic acid
mg	milligram(s)
min	minute(s)
mM	millimole(s)
mmol	millimole(s)
MOFs	metal-organic frameworks
mQ water	milli-Q water
m/z	mass to charge ratio
NI	naphthalimide fluorophore
nm	nanometer
NMR	nuclear magnetic resonance
OPPh ₃	triphenyl phosphine oxide
PAP	purple acid phosphatase
PCA	principal component analysis
Pd/C	palladium on carbon
PET	photoinduced energy transfer
Pic	bis(2-pyridylmethyl)amine
pKa	-log(acid association constant)
Pi	phosphate
PPi	pyrophosphate
PPPi	polypyrophosphate
PV	pyrocatechol violet
PR	pyrocatechol red
ppm	parts per million

q	quarte
R	unspecified alkyl group
R_0	Förster distance
R_f	retention factor
RNA	ribonucleic acid
RT	room temperature
s	singlet
SANS	small-angle neutron scattering
SDS	sodium dodecyl sulfate
Ser	serine
t	triplet
THF	tetrahydrofuran
TLC	thin-layer chromatography
TMR	tetramethylrhodamine
Trp	tryptophan
Tween 20	polyoxyethylene (20) sorbitan monolaureate
TSA	transition state analogue
UV-Vis	ultraviolet-visible
UpNP	uridine-3'- <i>p</i> -nitrophenylphosphate
wt. %	percentage weight

Attestation of Authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), not used artificial intelligence tools or generative intelligence tools (unless explicitly defined and referenced, along with the purpose of use), nor materials which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

Bhanumathi Bandi

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Chapter One

Introduction

1.1 Supramolecular Chemistry

1.1.1 Origins of Supramolecular Chemistry

Supramolecular chemistry is a relatively recent discipline concerned with molecular assemblies and the intermolecular forces that govern them. Often described as “chemistry beyond the molecule”, it focuses on non-covalent interactions such as hydrogen bonding, van der Waals forces, electrostatics, and π – π stacking. Although recognised as a distinct field only in the late 20th century, its foundations lie in several early seminal discoveries. In 1967, Charles J. Pedersen reported the synthesis of crown ethers, macrocyclic compounds that selectively complex metal ions.¹ This discovery demonstrated the principle of molecular recognition, showing that molecules can interact selectively with specific partners. Jean-Marie Lehn subsequently extended these ideas in the 1970s through the development of cryptands and introduced the term *supramolecular chemistry*.² Donald J. Cram further expanded the field by designing host molecules with cavities tailored to bind specific guests.³ Collectively, Pedersen, Lehn, and Cram were awarded the 1987 Nobel Prize in Chemistry for pioneering work on host-guest chemistry, which is widely regarded as the birth of supramolecular chemistry.

The discipline expanded further in the late 20th century with the development of mechanically interlocked molecules.⁴ Jean-Pierre Sauvage demonstrated the use of metal ions as templates for the synthesis of catenanes,⁵ while Fraser Stoddart pioneered the design of rotaxanes and molecular shuttles.^{6, 7} Ben Feringa’s work on light-driven molecular motors extended the field into dynamic systems capable of controlled motion.⁸ For these advances, Sauvage, Stoddart, and Feringa were awarded the 2016 Nobel Prize in Chemistry for the design and synthesis of molecular machines, highlighting the transition of supramolecular chemistry from static recognition to functional molecular devices.

Key concepts in supramolecular chemistry that will be examined in this thesis include *molecular recognition*, which refers to the ability of molecules to selectively interact with specific partners, much like a lock-and-key mechanism; *self-assembly*, which describes

the spontaneous organisation of molecules into ordered structures, typically driven by non-covalent interactions such as hydrogen bonding, van der Waals forces, and π - π stacking; and the concept of *host-guest chemistry*, which involves the formation of complexes between a host molecule (often possessing a cavity or pocket) and a guest molecule that fits within this binding site.⁹ These concepts not only form the foundation of synthetic supramolecular chemistry but are also crucial to biological systems. Proteins, DNA, and membranes all rely on non-covalent interactions for their structure and function. Inspired by this, supramolecular chemists often design artificial systems that replicate, and sometimes improve upon, natural processes. For example, synthetic receptors and hosts can be engineered to selectively bind ions or molecules through hydrogen bonding, electrostatic interactions, and other non-covalent forces. These artificial systems have found applications in sensing, catalysis, and drug delivery, demonstrating the real-world impact of supramolecular principles.¹⁰

1.1.2 From Host-Guest Chemistry to Molecular Machines

In 1967, Charles J. Pedersen discovered crown ethers, which, through their ability to selectively complex metal ions, made a foundational contribution to the emerging field of supramolecular chemistry.¹ His research demonstrated the importance of molecular recognition and self-assembly processes in chemical systems.¹¹ In the 1970s, Jean-Marie Lehn expanded the concept of molecular recognition by synthesising a variety of molecules capable of forming highly selective complexes with ions and small molecules.¹² For example, he developed cryptands, which consist of three polyether bridges that can bind to metal ions such as Na^+ , and K^+ . Lehn also introduced the term “supramolecular chemistry” and explored the concept of molecular complementarity and self-recognition.² He demonstrated that different cations, like Na^+ and K^+ , as well as larger cations, could be selectively bound depending on the size of the cavity and the substituents present (**Figure 1.1**). During the same period, Donald J. Cram further

expanded the field by designing host molecules with defined cavities to encapsulate guests selectively.¹³

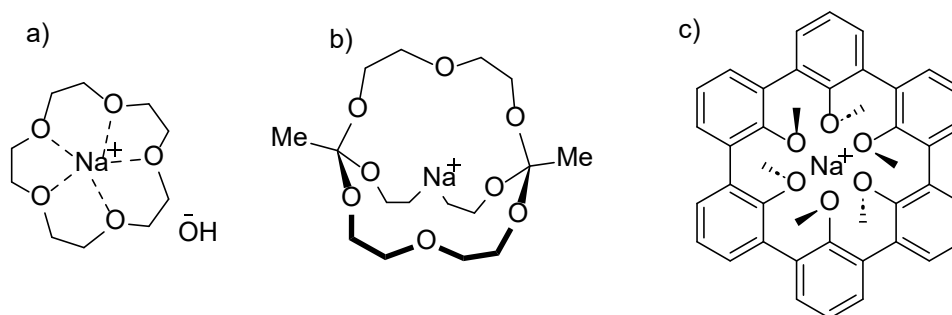


Figure 1.1. Examples of (a) a crown ether, (b) a cryptand and (c) a macrocyclic ring.

In the following decades, the scope of the field broadened to include mechanically interlocked molecular architectures. In the 1980s and 1990s, Jean-Pierre Sauvage, a French coordination chemist, made significant advances by using copper(I) complexes as templates for synthesising [2]catenanes (**Figure 1.2**) – mechanically interlocked structures composed of two interlinked macrocyclic rings.¹⁴ In these systems, the copper(I) ion is coordinated to two phenanthroline ligands, each embedded in a macrocycle, thus templating the interlocking of the rings and controlling their relative motion. By altering the oxidation state of the copper ion, Sauvage and co-workers demonstrated controlled, reversible motion of the catenane system, a landmark demonstration that paved the way for molecular switches and machines.⁵

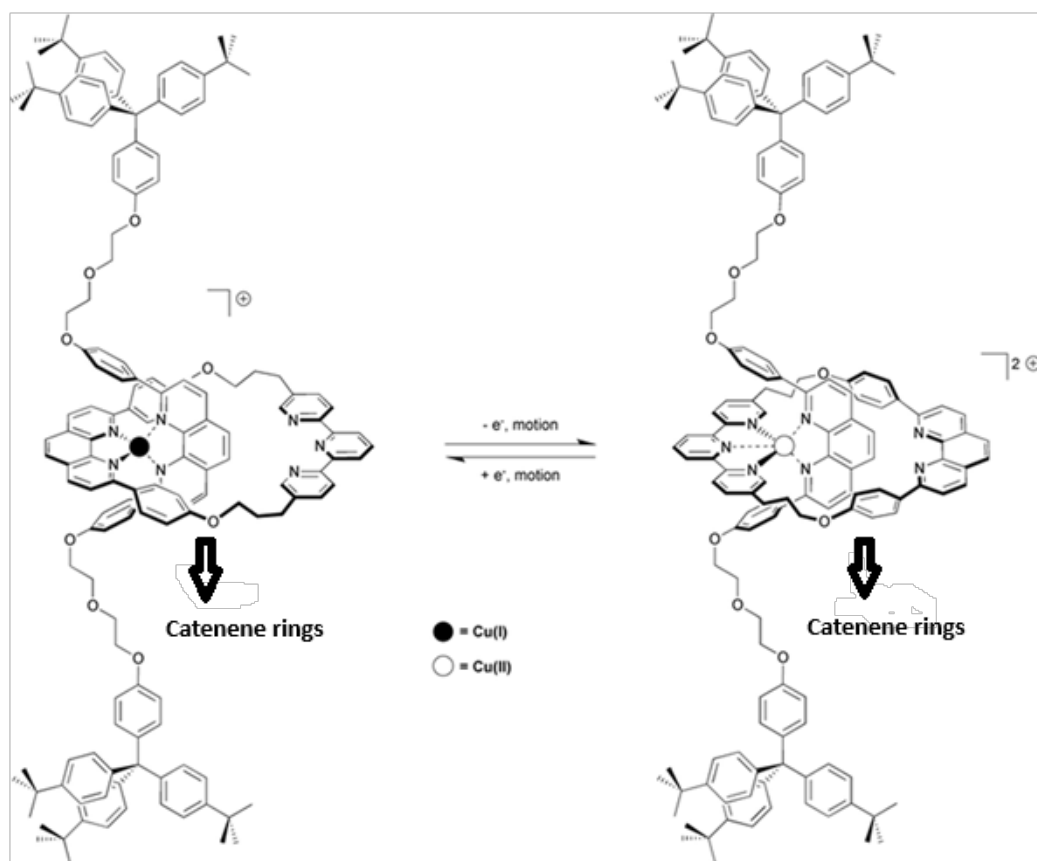


Figure 1.2. The synthesis and characterisation of copper (I) and copper (II) complexes with catenene macrocyclic rings. Adapted from Ref.[14] with permission ref[14]. Copyright (1998) American Chemical Society.

Similarly, Sir J. Fraser Stoddart was instrumental in the development of [2] rotaxanes, linear molecular shuttles in which a macrocyclic ring moves between distinct recognition sites (“stations”) on a threaded molecule (**Figure 1.3a**).¹⁵ The movement of the macrocycle could be triggered by chemical or electrochemical stimuli, enabling precise control of molecular motion. This work highlighted the concept of mechanical bonding, where topology rather than covalent bonding dictates the integrity of the system (**Figure 1.3b**).

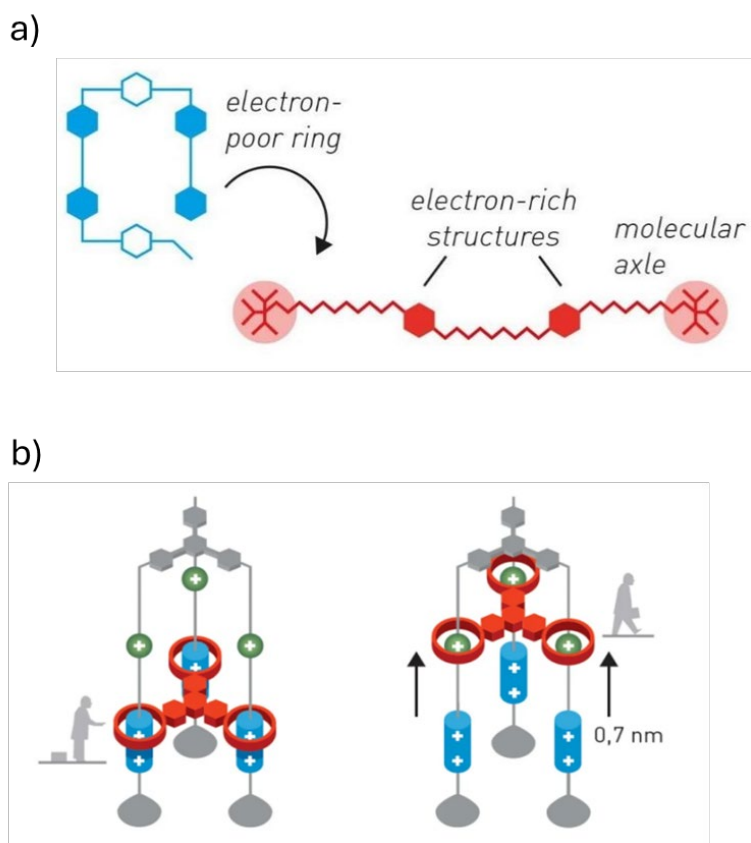


Figure 1.3. a) Alinear molecular shuttle b) molecular elevator. Source a and b: Ref[15]/Prof. J. Fraser Stoddart.

Building upon these advances, Ben L. Feringa pioneered the design of light-driven molecular motors.¹⁶ These systems comprise a rotor and stator connected by a double bond axis. The stator, with its chiral helical structure, imposes directionality, while the rotor undergoes photoinduced isomerisation to generate unidirectional rotation (**Figure 1.4**). This groundbreaking achievement provided the first example of a synthetic molecular motor, opening exciting new directions for nanoscale devices.¹⁷

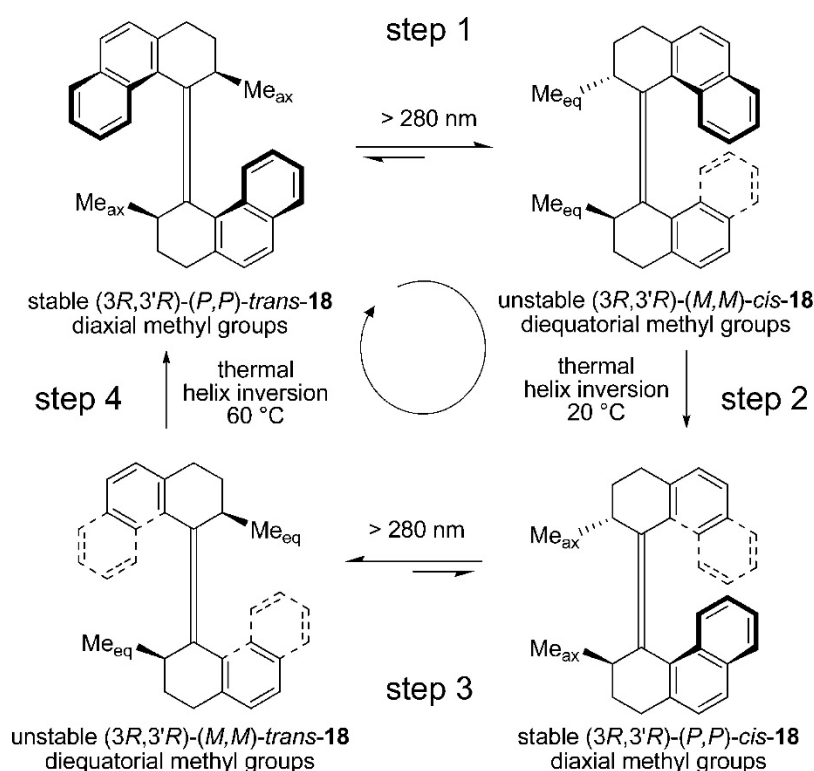


Figure 1.4. An example of Feringa's light-driven molecular motors. Adapted from Ref.[17] with permission ref[17]. Copyright (2001) American Chemical Society.

Together, the contributions of Sauvage, Stoddart, and Feringa demonstrated that supramolecular systems could be dynamic and functional. Collectively, the work of these scientists and others has established supramolecular chemistry as a thriving field, with applications extending across materials science, nanotechnology, drug delivery, sensing, and catalysis.¹⁰ The unique properties and functions of supramolecular assemblies continue to inspire chemists to create increasingly complex and sophisticated systems.

1.2 Anion Recognition

1.2.1 Introduction

Anions are negatively charged ions that play fundamental roles in biology, medicine, industry, and the environment. In living systems, they help maintain ionic balance,

regulate cellular signalling, and support essential biochemical processes.¹⁸ A particularly important class is the phosphate anions, which are central to energy metabolism and genetic information storage.¹⁹ For example, adenosine triphosphate (ATP) functions as the universal energy currency of the cell. Hydrolysis of one of its high-energy phosphate bonds releases free energies that powers cellular processes.²⁰ Pyrophosphate (PPi) is formed during biosynthetic reactions such as DNA and RNA polymerisation, where it serves as an important driving force for bond formation.²¹ Other phosphate-containing species, such as nucleotides and phosphorylated intermediates, are integral to signal transduction, enzyme regulation, and metabolism.²²

Beyond phosphates, other biologically relevant anions include chloride (Cl^-),²³ which regulates osmotic balance and neuronal signalling, and bicarbonate (HCO_3^-),²⁴ which buffers blood pH. In medicine, abnormal concentrations of anions are often linked to disease: for instance, impaired chloride transport causes cystic fibrosis, while phosphate mis-regulation is implicated in bone disorders.²⁵ Detecting and monitoring anions therefore has clinical importance.²⁶

In the environment, anions are equally critical. Nitrate (NO_3^-) and phosphate (PO_4^{3-}) are widely used in agriculture as fertilisers, yet their leaching into waterways contributes to eutrophication, algal blooms, and ecosystem damage.²⁷ Fluoride (F^-) is added to drinking water to protect against tooth decay, but excess fluoride can lead to fluorosis. Organophosphorus compounds, used as pesticides, illustrate the dual nature of anions and anionic species: they are highly effective in crop protection but pose risks to human health and environmental safety.²⁸ Selective and sensitive detection of such anions is therefore vital for both public health and environmental monitoring.

Supramolecular chemistry provides tools to address these challenges through anion recognition – the design of host molecules that can bind anionic guests with high affinity and selectivity.²⁹ Effective receptors can discriminate between anions based on size, geometry, charge distribution, and hydrogen bonding capacity. Strategies often exploit directional hydrogen bonding, for example, using functional groups such as ureas, thioureas, amides, or guanidinium units that act as hydrogen bond donors.³⁰⁻³²

Electrostatic attraction, hydrophobic effects, halogen bonding, and metal–ligand coordination are also used to enhance selectivity.

Applications of synthetic anion receptors are wide-ranging (**Figure 1.5**). These include²⁹

- Chemical sensing – detecting environmentally or biologically relevant anions such as phosphate, fluoride, or chloride.
- Medical diagnostics – designing receptors that report on anion concentrations in physiological fluids, aiding in disease detection.
- Environmental monitoring – tracking pollutants such as nitrate and phosphate in soil and water.
- Catalysis – stabilising anionic intermediates in organic transformations.
- Separation technologies – removing or concentrating anions from complex mixtures.

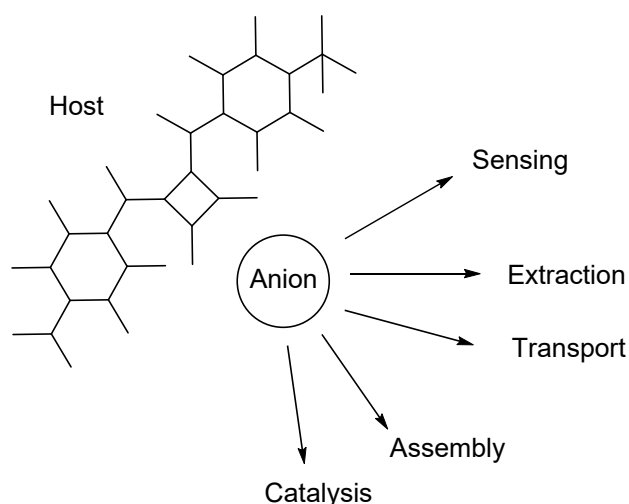


Figure 1.5. Different applications of anion binding.²⁹ Source: Adapted from Ref.[29] with permission ref[29]. Copyright (2015) American Chemical Society.

Despite this promise, significant challenges remain. Compared to cations, anions are generally larger, more polarisable, and more strongly solvated, especially in water.³³ Their geometries range from spherical halides to highly directional oxoanions, complicating receptor design. Overcoming the competition with solvation shells in aqueous media remains one of the major barriers to practical application.³⁴ Nonetheless,

continued advances in receptor design and a deeper understanding of non-covalent interactions are expanding the scope of anion recognition, with exciting opportunities for impact across biology, medicine, and environmental science.³⁵

1.2.2 Key challenges in anion recognition

Compared to cations, anions present unique challenges and opportunities in supramolecular recognition. Designing receptors that can selectively bind and detect specific anions requires careful consideration of their charge distribution, solvation, and geometry. A key difference arises from the concept of effective nuclear charge.

Size and Charge Considerations

Cations, having lost electrons, experience a greater effective nuclear charge because fewer electrons are available to shield the nucleus. This results in a smaller ionic radius that allows for stronger, directional electrostatic contacts with the receptor, allowing for the design of well-defined binding sites.³⁶ In contrast, anions contain additional electrons that increase electron-electron repulsion, expanding the electron cloud and producing a larger ionic radius relative to the neutral atom or its isoelectronic cation. For example, the fluoride anion (F^-) has a radius of 1.19 Å, compared with 1.16 Å for sodium (Na^+). Similarly, iodide (I^-) at 2.06 Å is significantly larger than caesium (Cs^+) at 1.81 Å. Anions therefore have charges that are more spread out and interact more weakly and non-directionally (**Table 1.1**).³⁷

Table 1.1. Radii of selected cations and their isoelectronic anions³⁷

Cation	Radii [$\text{\AA}$]	Anion	Radii [$\text{\AA}$]
Na^+	1.16	F^-	1.19
K^+	1.52	Cl^-	1.52
Rb^+	1.66	Br^-	1.67
Cs^+	1.81	I^-	2.06

Protonation States and pH Dependence

Many anions, particularly oxoanions, display pH-sensitive behaviour because their protonation states change depending on the surrounding environment. These species often derive from polyprotic acids, which can donate more than one proton in successive ionisation steps. A prime example is the phosphate system.³⁸ The tetrahedral phosphate ion (PO_4^{3-}) can exist in multiple protonated forms, from H_3PO_4 at low pH to PO_4^{3-} at high pH.³⁹ In aqueous solutions of intermediate pH, phosphate exists as a mixture of species (H_2PO_4^- , HPO_4^{2-} , and PO_4^{3-}), with distributions determined by the pKa values of the system (**Figure 1.6**). This complexity creates challenges for receptor design, as the target anion may exist in more than one form under physiological or environmental conditions.

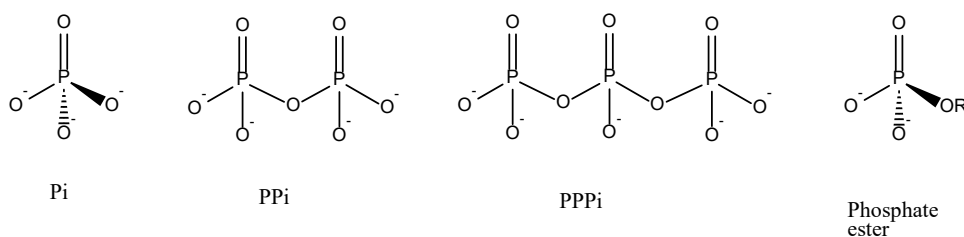


Figure 1.6. Different types of inorganic phosphates.

Solvation effects

In aqueous solution, many anions are strongly solvated due to their ability to form directional hydrogen bonds with water molecules. For example, fluoride (F^-) forms a particularly stable hydration shell through strong hydrogen bonding.⁴⁰ To bind such

anions, a receptor must first overcome the significant energetic cost of desolvation (removing water molecules from the solvation shell). Cations, by contrast, are typically smaller and more compact, and although some (e.g. Li^+ , Mg^{2+}) are also strongly hydrated, their solvation can often be more easily offset by coordination to electron-rich donor atoms in synthetic hosts. The relative ease or difficulty of displacing hydration shells is reflected in the Hofmeister series,⁴¹ which orders ions by their ability to stabilise or destabilise proteins and solutes in aqueous environments. Kosmotropes anions such as fluoride are strongly solvated, while larger, more polarisable anions such as iodide are less tightly solvated but more diffuse. Effective receptors for anions must provide multiple strong, directional interactions (e.g. hydrogen bonds, electrostatics) to outcompete water molecules.

Geometrical Diversity of Anions

Cations are often spherical (Na^+ , K^+ , Ca^{2+}) or adopt predictable coordination geometries (octahedral, tetrahedral). Anions, on the other hand, exhibit a wide variety of geometries, from simple spherical halides (Cl^- , Br^- , I^-) to planar carboxylates and nitrates (CO_3^{2-} , NO_3^-), tetrahedral phosphates and sulfates (PO_4^{3-} , SO_4^{2-}), and octahedral hexafluorophosphates (**Figure 1.7**).⁴² This geometrical diversity presents both opportunities and challenges: receptors can be tailored to match specific shapes, but the solvation shell and conformational flexibility of the anion must also be taken into account.

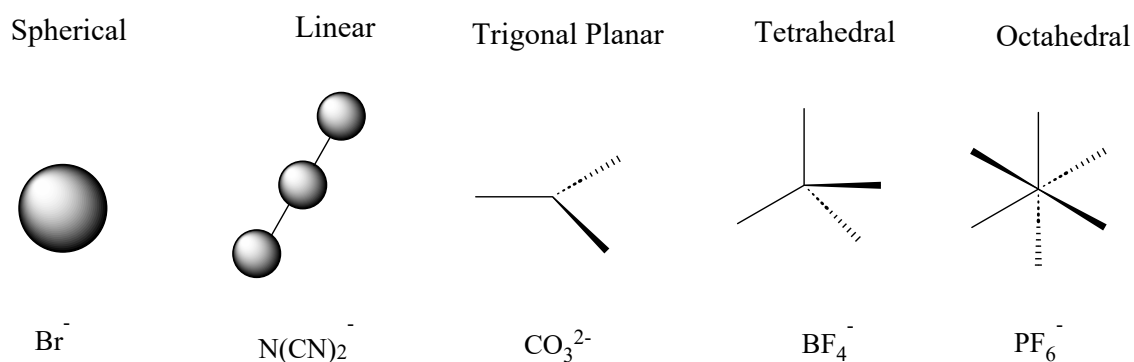


Figure 1.7. Standard geometries of anions and their shapes.

1.3 Non-Covalent Interactions in Anion Recognition

The design of receptors for cations is generally more straightforward than for anions, as cations bind strongly and directly through hard interactions like ion-dipole forces. Receptors can be designed to rely on robust donor-acceptor chemistry by featuring electron donors (O, N, S) for direct chelation, as seen in crown ethers and cryptands. Receptors to bind anions generally require a combination of carefully balanced, weaker forces, such as hydrogen bonding, halogen/chalcogen bonding, or hydrophobic effects, which are weaker, highly solvent-sensitive, and less reliable in water.⁴³

1.3.1 Hydrogen bonding

Hydrogen bonding is the most widely exploited interaction in anion recognition. The phenomenon was first discussed in 1912 by T. S. Moore and T. F. Winmill,⁴⁴ later elaborated by Latimer and Rodebush in the 1920s,⁴⁵ and given a full theoretical framework by Linus Pauling in the 1930s, who described its structural and energetic characteristics in detail.⁴⁶ A hydrogen bond arises when a hydrogen atom covalently attached to an electronegative atom (such as oxygen, nitrogen, or fluorine) interacts with a second electronegative atom bearing a lone pair of electrons. The resulting bond is strong and highly directional, typically 5–30 kJ/mol in strength – weaker than covalent bonds, but generally stronger than van der Waals interactions.⁴⁷

Two types are particularly relevant: **intermolecular hydrogen bonds**, which occur between different molecules (e.g. between water molecules in liquid water), and **intramolecular hydrogen bonds**,⁴⁸ which occur within the same molecule and can pre-organise the geometry of a receptor (**Figure 1.8**). In biology, hydrogen bonding is fundamental to the stability of DNA base pairs and plays a central role in enzyme catalysis by stabilising transition states. In supramolecular chemistry, hydrogen bonding remains one of the most important design principles for synthetic receptors, particularly those aimed at recognising highly solvated anions such as phosphates.⁴⁹

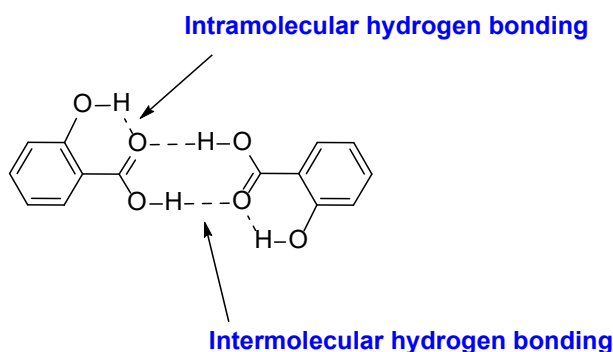


Figure 1.8. Inter- and intra-molecular hydrogen bonding.

Biological systems provide powerful examples of how multiple hydrogen-bonding interactions combine to achieve high selectivity in anion recognition. One of the best studied is the periplasmic phosphate-binding protein (PstS), a component of the high-affinity phosphate transport system in bacteria.⁵⁰ PstS contains a well-defined binding pocket lined with residues such as serine, threonine, and tyrosine, whose hydroxyl groups form hydrogen bonds with the oxygen atoms of the phosphate ion.⁵¹ These interactions are complemented by nearby positively charged residues, such as arginine or lysine, which provide additional electrostatic stabilisation. Together, hydrogen bonding and electrostatics give PstS both high affinity and high selectivity, ensuring efficient phosphate uptake even in environments where competing anions are present.⁵²

This example highlights the power of hydrogen bonding: its directionality allows the binding pocket to be pre-organised to suit the geometry of a specific guest, in this case phosphate. The study of proteins such as PstS **1** has inspired chemists to design artificial receptors that emulate these natural binding motifs (**Figure 1.9**). By mimicking the cooperative use of hydrogen bonding and electrostatics found in biological phosphate-binding proteins, it is possible to develop synthetic systems with enhanced affinity, selectivity, and sensitivity for phosphate detection.⁵³

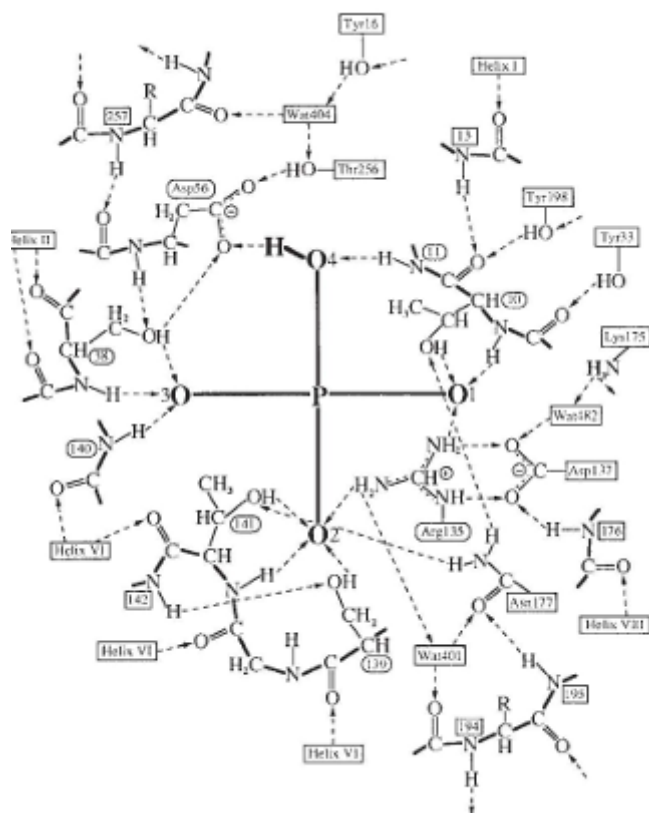


Figure 1.9. Structure of a phosphate-binding protein illustrating hydrogen bonding interactions with phosphate.⁵⁴ Source: Adapted from Ref. [54]. Copyright (1997) Nature Structural Biology.

1.3.2 Halogen and chalcogen bonds

Halogen and chalcogen bonds are non-covalent interactions that have attracted increasing attention in supramolecular chemistry for the design of anion receptors. Like hydrogen bonds, they are highly directional and tunable, but they rely on different electronic principles, providing opportunities where hydrogen bonding alone is insufficient.⁵⁵⁻⁵⁷

Halogen bonding arises when a covalently bound halogen atom (Cl, Br, I) engages in a non-covalent interaction with a Lewis base, such as the lone pair of an anion. This effect originates from the anisotropic distribution of electron density around the halogen: while the halogen is overall electron-rich, the extension of the covalent bond axis exhibits a region of electron deficiency known as the “ σ -hole.”⁵⁸

This region acts as a localized positive site, enabling halogen atoms to act as electrophilic hydrogen-bond analogues (**Figure 1.10**). The interaction is highly directional (often linear, close to 180°) and can be tuned by varying the halogen ($I > Br > Cl$) or by attaching electron-withdrawing substituents that deepen the σ -hole.⁵⁹

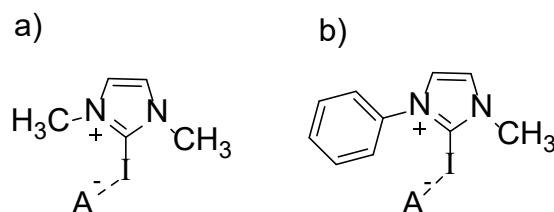


Figure 1.10. Examples of halogen bonding with functional groups bearing different σ -hole donor groups. Source: Adapted from Ref. [59].

Halogen bonding has proven particularly useful for anion recognition because of its strong, directional interactions and reduced sensitivity to solvation compared to hydrogen bonds.⁶⁰ For example, iodotriazole motifs incorporated into macrocyclic or acyclic frameworks have been developed that selectively bind dihydrogen phosphate ($H_2PO_4^-$) through convergent XB ($X = S, Se, \text{ and } Te$) donor sites.⁶¹ For instance, Beer and co-workers demonstrated that receptors bearing multiple iodotriazole **2** units engage phosphate via directional halogen bonds, giving measurable fluorescence changes that enable phosphate sensing in organic and mixed aqueous media.⁶² These systems demonstrate binding affinities comparable to, and sometimes stronger than, analogous hydrogen-bonding receptors, even under competitive conditions (**Figure 1.11**).

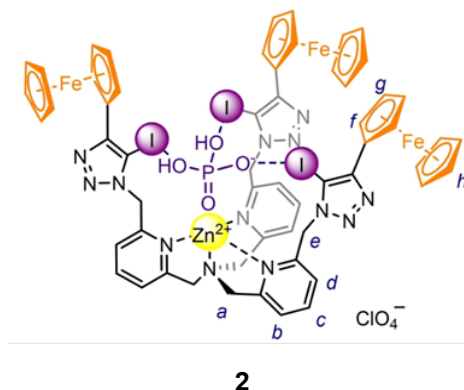


Figure 1.11. Example of a phosphate receptor featuring directional halogen-bonding, as reported by Beer and co-workers.⁶² Source: Adapted Ref.[62] from Chemistry-A European Journal and published by Wiley and Chemistry Europe.

Chalcogen bonding (ChB), involving group 16 elements (S, Se, Te), operates on a similar principle. A covalently bound chalcogen atom can also display a σ -hole, allowing it to interact with electron-rich species such as anions. ChB interactions tend to be weaker than XB ($X = S, Se, \text{ and } Te$) but can be highly effective when combined with preorganisation and cooperative effects in receptor design. A recent example includes a selenium-based ChB donors have been incorporated into receptor scaffolds to create highly preorganised binding pockets for phosphate species (**Figure 1.12**). Selenium based receptors are particularly promising for targeting oxyanions like phosphates and sulfates because the interaction can complement hydrogen bonding in multivalent recognition pockets.⁶³

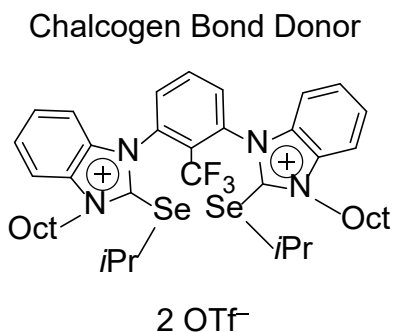


Figure 1.12. A selenium based chalcogen bond donor. Source: Reprinted from Ref.[63]/Paul D. Beer.

1.3.4 Charge-charge interactions

Electrostatic attraction between oppositely charged species is one of the most fundamental forces in supramolecular chemistry and plays a central role in anion recognition.⁶⁴ Receptors can be designed with positively charged functional groups, such as ammonium, guanidinium, or pyridinium moieties, which interact strongly with negatively charged anionic guests (**Figure 1.13**). These cationic groups provide both Coulombic attraction and, in some cases, directional hydrogen bonding (e.g. $\text{NH}\cdots\text{O}$ interactions in ammonium and guanidinium systems).

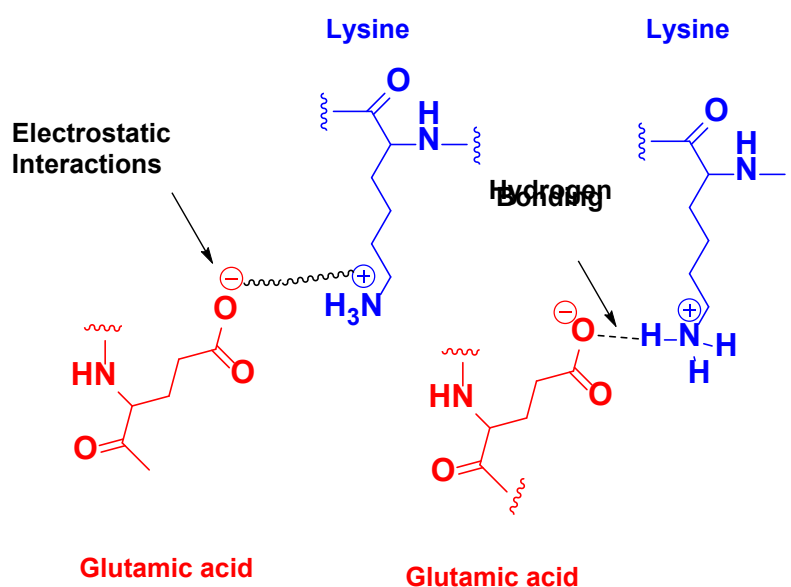


Figure 1.13. Hydrogen bonding and electrostatic interactions.

Although electrostatic interactions are substantially weakened in aqueous environments due to dielectric screening, they remain important contributors to anion recognition because they can help offset the high energetic cost of anion desolvation. For example, guanidinium groups bind oxyanions such as phosphates through a combination of electrostatic attraction and directional bidentate hydrogen bonding, providing enhanced affinity and selectivity in water.⁶⁵ Similarly, pyridinium-based receptors have been shown to bind phosphate and sulfate anions effectively, often with measurable optical responses when incorporated into sensor scaffolds.⁴³ The strength and selectivity of these

interactions depend on several factors, including the charge density of the receptor site, the geometry of the binding pocket, and the size and shape of the target anion. For instance, spherical halides interact primarily through Coulombic attraction, while polyatomic oxoanions like phosphate require receptors that can present multiple cationic sites arranged in a complementary geometry.⁶⁶

Electrostatic recognition is rarely used in isolation but instead works synergistically with hydrogen bonding, halogen bonding, and hydrophobic effects to enhance selectivity and affinity.⁶⁷ By integrating cationic motifs into supramolecular scaffolds, chemists have developed highly effective receptors for challenging targets such as phosphate, sulfate, and carboxylates, with applications in sensing, separation, and biomedical diagnostics.⁶⁸

1.3.5 Metal-ligand coordination

Metal-ligand coordination provides one of the most robust strategies for anion recognition, particularly in competitive aqueous environments. In these interactions, the anion functions as a Lewis base, donating a lone pair of electrons (commonly from oxygen atoms in oxoanions) to a vacant coordination site on a metal ion.⁶⁹ This results in a coordinate covalent bond, which is generally stronger and more directional than purely electrostatic interactions (**Figure 1.14**).

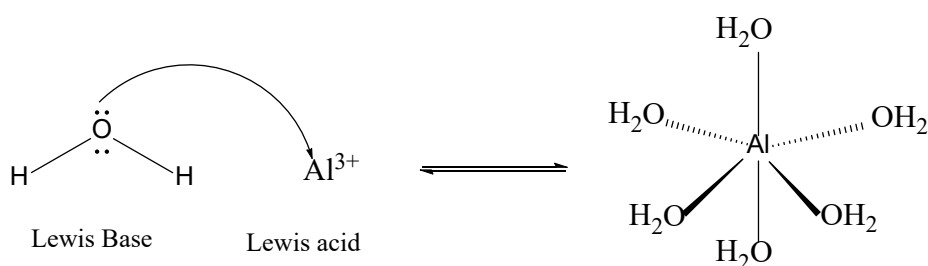


Figure 1.14. Aluminium ion acts as a Lewis acid and accepts the electrons from water and water acts as a Lewis base.

Transition-metal (d-block) ions are especially valuable in receptor design because they offer directionality through defined coordination geometries (e.g. octahedral, tetrahedral,

square planar). They also exhibit variable oxidation states, allowing control over binding strength and the potential for redox-responsive behaviour. The tunability of the selectivity will depend on the metal ion's hardness/softness and the donor properties of the ligand, in line with HSAB theory.

The stability of metal-anion complexes depends on factors such as the nature of the metal ion, the basicity and geometry of the anion, and the overall architecture of the receptor. For example, hard metal ions like Zn^{2+} or Al^{3+} strongly favour hard bases such as phosphate and sulfate, whereas softer metals (e.g. Hg^{2+} , Ag^+) preferentially bind softer anions like thiolates or iodide. These properties have been exploited in the design of phosphate sensors, especially with zinc (II)-based receptors **4** that readily coordinate to oxygen donors, as many Zn^{2+} complexes exhibit luminescent or colourimetric changes upon binding. Copper (II)-complex sensors have also been developed in which coordination of phosphate alters the electronic environment of the Cu^{2+} centre, producing measurable spectroscopic shifts (**Figure 1.15**).

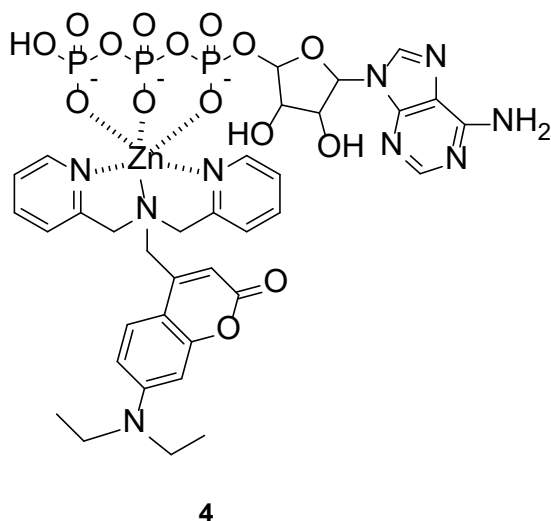


Figure 1.15. The design of the phosphate anions sensors with the Zn^{II} -based receptors.⁷⁰ Source: Reprinted from Ref.[70] with permission ref[70]. Copyright(2018) American Chemical Society.

1.3.6 Hydrophobic Effects

The hydrophobic effect is a fundamental principle of molecular recognition in aqueous solution. Although nonpolar species do not attract one another directly, they are effectively “pushed together” by water. This counterintuitive phenomenon was articulated by Walter Kauzmann in 1959 in his analysis of protein folding,⁷¹ and it has since become a central concept in biochemistry and supramolecular chemistry.⁷²

At the molecular level, water forms an extended hydrogen-bonding network. When a nonpolar group or molecule is introduced, water molecules reorganise into ordered “cages” around the solute, restricting their freedom of motion and decreasing the entropy of the system. The system minimises this unfavourable ordering by clustering nonpolar species together, thereby reducing the exposed hydrophobic surface area. The result is an apparent attraction between nonpolar groups – the hydrophobic effect.⁷²

In supramolecular chemistry, this effect is harnessed in the design of receptors that bind anions more effectively in water. For example, cationic calix [4] arenes **5** bearing ammonium groups can encapsulate phosphates or carboxylates within their hydrophobic cavity (**Figure 1.16**). The exclusion of water molecules from the binding pocket not only enhances electrostatic interactions between the receptor and the anion but also provides a favourable entropic contribution to binding. Similarly, cucurbiturils possess rigid hydrophobic cavities that can bind anions indirectly, often by stabilising ion pairs or by excluding water around the binding site, thereby amplifying recognition in aqueous environments.⁷³

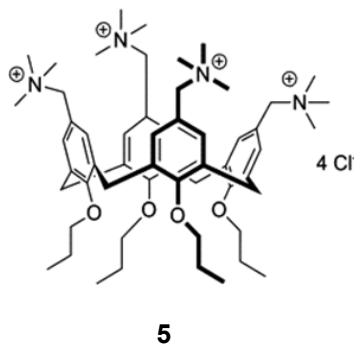


Figure 1.16. The structure of a cationic calix[4]arenes. Source: Adapted from Ref.[73] with permission ref[73]. Copyright (2010) International Journal of Pharmaceutics.

Hydrophobic effects also play a crucial role in self-assembled systems such as micelles and vesicles.⁷⁴ At the hydrophobic-hydrophilic interface, anions such as phosphate and pyrophosphate are concentrated and partially desolvated, making them more accessible to functionalised receptors located at the surface.⁷⁵ This principle has been exploited in indicator displacement assays and fluorescence sensors, where localisation at the hydrophobic interface enhances sensitivity and selectivity (**Figure 1.17**).²⁹

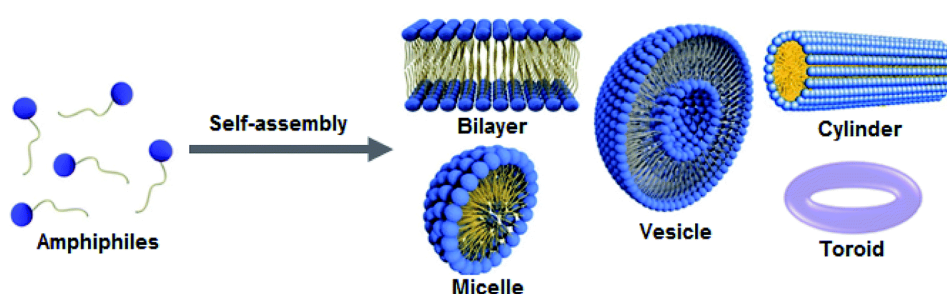


Figure 1.17. Amphiphiles, which possess hydrophobic tails and hydrophilic heads, self-assemble into various structures such as bilayers, micelles, vesicles, cylinders and toroids. Reprinted with permission from ref. ⁷⁴. Source: Adapted from Ref.[74] with permission ref[74]. Copyright (2020) RSC Advances.

Thus, by combining hydrophobic environments with other non-covalent interactions, chemists can enhance binding affinity and selectivity, even under competitive aqueous conditions where hydrogen bonding and electrostatics alone may be insufficient.

1.4 Synthetic Receptors for Assay Development

1.4.1 Construction of Synthetic Receptors

In nature, receptors are proteins finely tuned to recognise specific ligands. Their binding pockets are defined by the precise arrangement of functional groups, allowing multiple non-covalent interactions (hydrogen bonding, electrostatics, hydrophobic effects) to combine in a highly selective and potent manner. Inspired by these biological systems, chemists have sought to construct synthetic receptors that mimic this recognition

capability.

One common strategy is to attach functional binding groups onto a molecular scaffold such as a macrocycle (e.g. calixarenes, cyclodextrins, cucurbiturils) or an acyclic framework (e.g. urea-, thiourea-, or guanidinium-based receptors). By carefully arranging these groups, chemists can generate well-defined binding pockets that selectively accommodate particular guests, emulating the behaviour of natural enzymes (**Figure 1.18**).⁷⁶ This approach has yielded many highly selective cation receptors, including crown ethers, cryptands, and polyamine ligands. However, the design of selective anion receptors remains far more challenging due to the larger size, lower charge density, and high solvation energies of many anions.⁷⁷

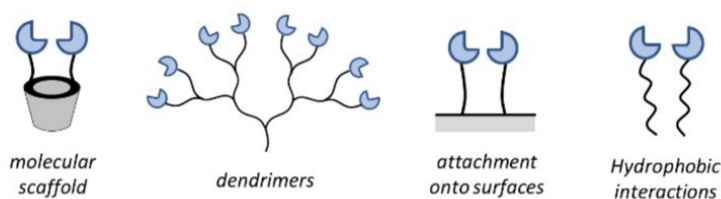


Figure 1.18. Comparison of different types of artificial receptors. Source: Adapted from Ref.[76] with permission ref[76]. Copyright (2021) Advanced Synthesis and Catalysts.

Synthetic receptors need not be restricted to small molecules. Binding sites can also be incorporated into larger constructs such as⁷⁸⁻⁸⁰

- Nanoparticles (e.g. gold nanoparticles or silica particles functionalised with cationic ligands) that present multiple binding sites on their surface. These benefit from multivalency and large surface areas, which enhance affinity and enable integration into sensing platforms.
- Polymers and dendrimers, which allow multiple functional groups to be positioned in flexible architectures, producing selective binding cavities within a macromolecular framework.

- Surface-immobilised systems, where receptors are tethered to electrodes, membranes, or solid supports to generate reusable sensing platforms for assays.

Unlike natural enzymes, which can undergo conformational changes upon ligand binding, most synthetic receptors are rigid and static. To address this limitation, supramolecular chemists have turned to self-assembly as a means of creating dynamic, adaptive recognition sites. Amphiphilic molecules, for instance, spontaneously assemble into structures such as micelles, vesicles, or bilayers in aqueous solution. Within these assemblies, hydrophobic pockets or polar head groups at the interface can create selective recognition sites for analytes.

An important advantage of self-assembled receptors is modularity: by mixing different amphiphiles in varying ratios, binding sites can be tailored and optimised without lengthy synthetic campaigns. This in situ adaptability makes self-assembly particularly attractive for assay development, where flexibility and rapid prototyping are essential. This will be further discussed in Section 1.6.

1.4.2 Indicator Displacement Assays

While many synthetic receptors are designed to bind analytes directly and generate an intrinsic signal, another powerful strategy separates the tasks of recognition and signal transduction. In this approach, the receptor provides selectivity for the analyte, while an external dye molecule acts as the signalling element. This bypasses the need to covalently attach a reporter group to the receptor, simplifying synthesis and greatly increasing flexibility. One of the most widely used techniques of this type is the indicator-displacement assay (IDA).⁸¹

In an IDA, a dye molecule (the indicator) binds reversibly to a receptor. When a target analyte is introduced, it competes with the dye for the binding site. If the analyte has a stronger affinity, it displaces the indicator, leading to a measurable change in the dye's optical properties. A schematic representation of this process is shown in Figure

1.19. The selectivity of the assay is dictated by the receptor, while the sensitivity and signal output are controlled by the choice of indicator (**Figure 1.19**).⁸²

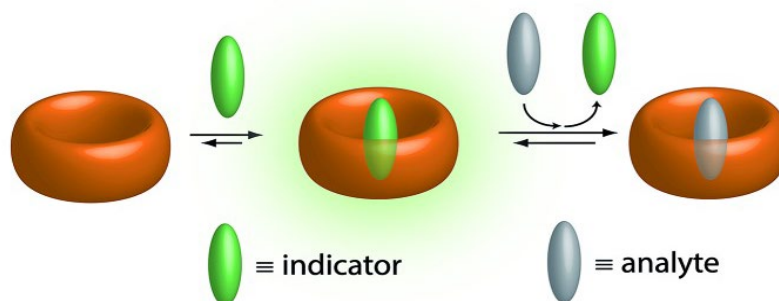


Figure 1.19. Schematic representation of Indicator Displacement Assays (IDAs).

Source: Reprinted with permission from Ref. 82. Copyright (2006) Elsevier B.V.

Both colorimetric and fluorescent IDAs are widely employed:^{81, 83}

- Colorimetric IDAs use dyes such as methyl orange or bromopyrogallol red, which exhibit distinct absorption spectra depending on whether they are bound or free. These changes can be quantified using UV-Vis spectroscopy, but they are often visible to the naked eye, making them attractive for simple, low-cost assays.
- Fluorescent IDAs employ dyes such as fluorescein, calcein, or pyrene, where displacement results in changes in fluorescence intensity or emission wavelength. Fluorescent IDAs are generally more sensitive than colorimetric ones, as fluorescence can be detected at much lower concentrations, often micromolar to nanomolar, using a fluorescence spectrometer.

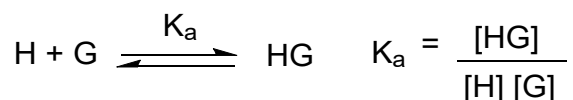
IDAs have proven particularly useful for anion detection, especially for challenging targets such as phosphate and pyrophosphate. These anions compete effectively with dye molecules for receptor binding, and their displacement can be readily monitored through changes in colour or fluorescence. Because of their modularity and adaptability, IDAs have found applications in biological monitoring, environmental analysis, and medical diagnostics, where reliable detection of anions is critically important.

1.4.3 Quantification of Host-Guest Interactions

Understanding the strength and selectivity of host-guest interactions is critical for evaluating receptor performance. One of the most widely used approaches for quantifying binding affinity is spectroscopic titration, in which incremental amounts of a guest are added to a solution of host, and the resulting physical changes are monitored. Several complementary spectroscopic techniques can be applied:⁸⁴

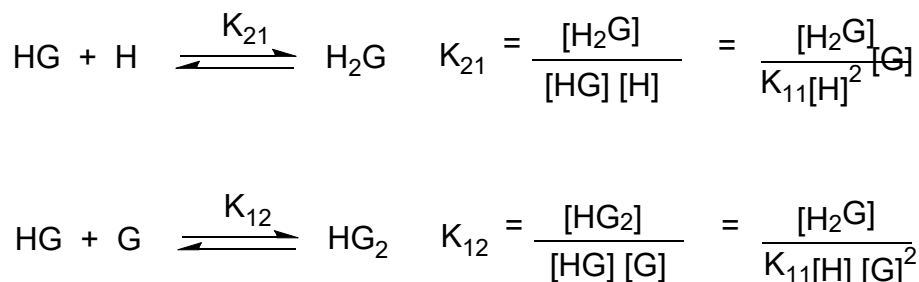
- UV-Vis absorption spectroscopy monitors changes in absorbance at specific wavelengths as binding perturbs the electronic environment of either host or indicator. This method is particularly useful in indicator-displacement assays.
- Fluorescence spectroscopy uses fluorogenic hosts or dyes whose emission intensity or wavelength shifts upon binding. Because fluorescence can be detected at very low concentrations, this technique is generally more sensitive than absorbance-based methods.
- NMR spectroscopy provides detailed information on the chemical environment of nuclei (often protons) in the host and/or guest. Binding is observed as chemical shift changes, signal broadening, or peak splitting. Depending on the exchange regime, binding constants can be extracted either directly (slow exchange) or through fitting of averaged signals (fast exchange).

From these measurements, binding isotherms are constructed by plotting the observed spectroscopic response against guest concentration. The data are then fitted to appropriate equilibrium models to extract association constants (K_a), which quantify the binding affinity. For simple 1:1 host-guest complexes, K_a describes the equilibrium:^{85, 86}



In more complex systems, alternative stoichiometries such as 2:1 (two hosts to one guest) or 1:2 (one host to two guests) may be observed. In these cases, additional equilibrium

constants are required, and the curve fitting becomes more computationally demanding. Fortunately, software packages such as BindFit (<http://supramolecular.org/>), have been developed to facilitate fitting of multi-site binding models, reducing the complexity of the analysis.



In practice, titrations are typically performed with host concentration held constant while varying the guest concentration. This ensures that the observed physical changes, e.g. shifts in absorbance, emission, or NMR signals, can be directly correlated to the progressive formation of host-guest complexes. By applying rigorous fitting procedures, accurate association constants can be determined, allowing quantitative comparisons of receptor performance across different systems.

1.5 Assays for Selective Detection of Phosphates

1.5.1 The Importance of Phosphates

Phosphorus, the 11th most abundant element in the Earth's crust, occurs almost exclusively in its oxidised forms as phosphates. The phosphate group, consisting of a central phosphorus atom tetrahedrally coordinated by four oxygen atoms, is one of the most fundamental motifs in chemistry and biology. Indeed, phosphates are essential across a remarkable range of contexts: they sustain life at the molecular level, underpin industrial and agricultural practices, and play a defining role in environmental cycles.⁸⁷

In biology, inorganic phosphate (Pi) and its condensed forms, pyrophosphate (PPi) and triphosphate (PPPi), are central to many energy-related processes. The hydrolysis

of adenosine triphosphate (ATP) releases P_i and PP_i , which drive countless biochemical reactions (**Figure 1.20**).⁸⁸ For example, DNA polymerisation requires the cleavage of ATP to release PP_i , and PP_i itself is involved in the biosynthesis of cyclic AMP, a key secondary messenger controlling hormone activity.⁸⁹ These processes highlight both the ubiquity and specificity of phosphate chemistry in living systems. However, the coexistence of related species such as ATP, ADP, and PP_i , creates a major challenge for molecular recognition: selective detection of one phosphate ion in the presence of others is critical for reliable bioanalytical measurements.⁹⁰ Without such selectivity, false positives may occur, undermining the utility of artificial receptors.

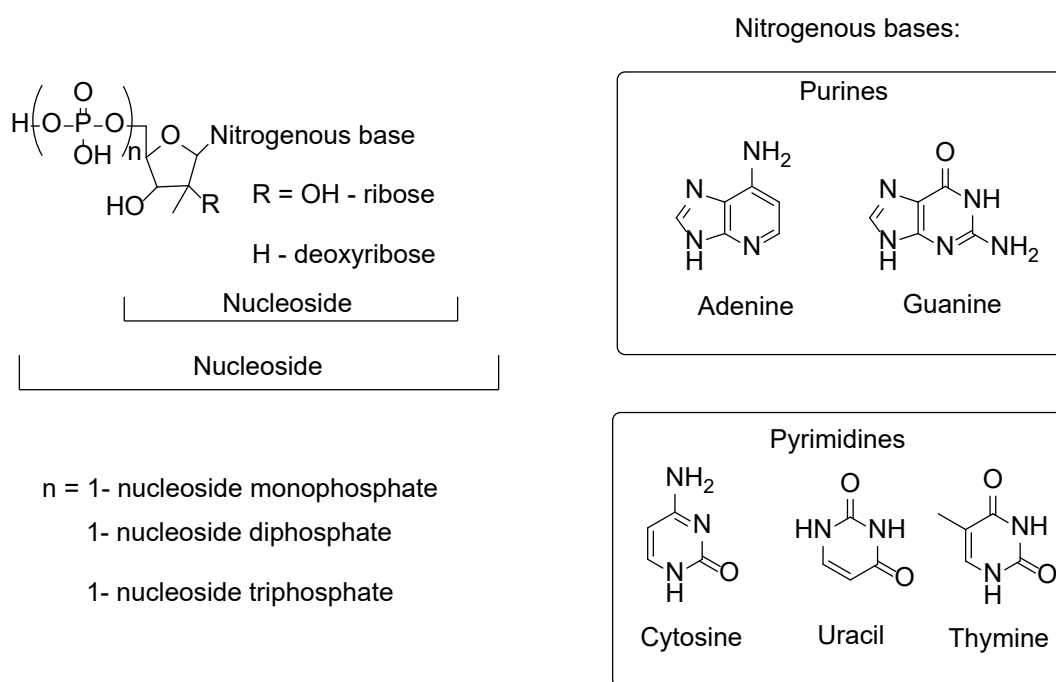


Figure 1.20. Different types of nucleoside phosphates.

Nucleoside phosphates also participate in intracellular signalling and metabolic regulation, ensuring that phosphate chemistry permeates nearly every aspect of cell biology.⁹¹ Proteins are similarly regulated by phosphorylation, a reversible modification in which phosphate groups are attached to amino acid side chains, particularly those of serine, threonine, and tyrosine (**Figure 1.21**). Such modifications can dramatically alter protein conformation, stability, and function, and they lie at the heart of cellular

communication and enzymatic control. Histidine phosphorylation, for instance, is a central feature of prokaryotic two-component signalling systems and illustrates how phosphate incorporation extends well beyond the canonical hydroxyl-amino acids.⁹²

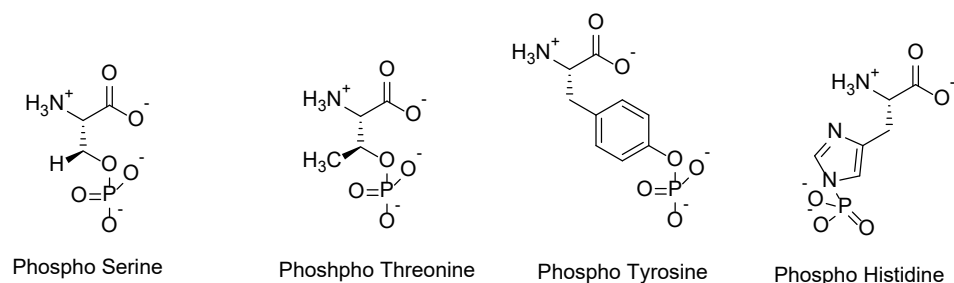


Figure 1.21. Different types of Phosphorylated amino acids.⁹²

The importance of phosphate extends beyond biological systems. In agriculture and industry, phosphates are indispensable as fertilisers, detergents, and food additives. Organophosphate derivatives, meanwhile, span a spectrum from pesticides to highly toxic chemical warfare agents such as sarin.⁹³ At the same time, excessive phosphate use has created pressing environmental challenges: agricultural runoff rich in phosphate fertilisers are a major driver of eutrophication, disrupting ecosystems and threatening water quality. These issues further emphasise the need for accurate and selective methods of phosphate detection.⁹⁴

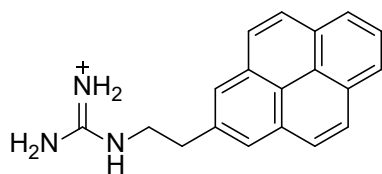
From this perspective, phosphate represents both an essential nutrient and a problematic pollutant, a molecular building block of life and a driver of environmental stress. Its complex chemistry, multivalency, strong hydration, and multiple protonation states make it one of the most challenging anions to recognise selectively in aqueous environments. The design of synthetic receptors and sensors that can meet this challenge remains a central goal in supramolecular chemistry and underpins the motivation for much of the work presented in this thesis.

1.5.2 Chromogenic Indicators for the Selective Detection of Phosphates

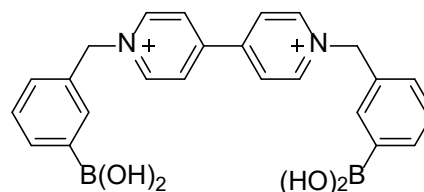
Given the central role of phosphate in biology, industry, and the environment, the development of synthetic sensors capable of selectively detecting phosphate-containing species has become an area of intense research.⁸⁸ A particular challenge lies in distinguishing between structurally similar species, for example, inorganic phosphate (Pi), pyrophosphate (PPi), and triphosphate derivatives such as ATP and ADP, which often coexist in complex biological or environmental samples.⁹⁵ Achieving such selectivity requires receptors that combine strong binding with precise discrimination, and equally importantly, transduction mechanisms that convert binding into a measurable optical or electronic signal. The following section surveys representative examples from the literature, highlighting how different receptor architectures and signalling strategies have been applied to develop chromogenic indicators for phosphate recognition and sensing.

Receptors utilising Charge-Charge Interactions

Incorporating positively charged functional groups such as guanidinium, imidazolium **6**, and polyammonium **7** moieties (**Figure 1.22**) into receptor frameworks is a common and effective strategy for phosphate sensing.⁹⁶ These groups exploit Coulombic attraction between the receptor and negatively charged phosphate species, helping to overcome the high hydration energies that normally prevent strong binding in aqueous media. Increasing the number of cationic sites within a receptor enhances overall affinity and improves selectivity by creating multiple interaction points, which leads to higher binding constants and improved sensitivity compared to neutral analogues.



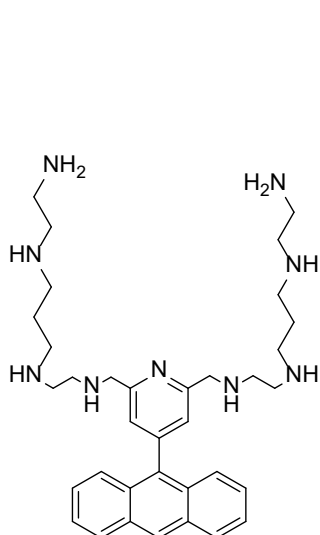
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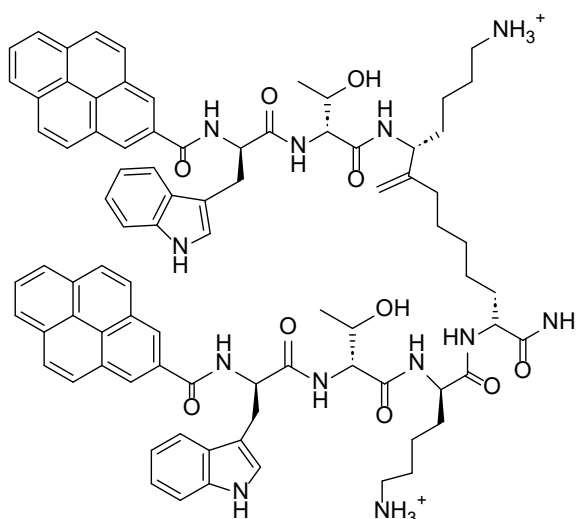
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Figure 1.22. Chemosensors featuring multiple charged groups (1. guanidinium-based, 2. viologen-based).⁹⁷ Chemosensors featuring multiple charged groups (1. guanidinium-based, 2. viologen-based).⁹⁷ Source: Adapted from Ref.[97]/ WileyVCH.

One of the earliest and most influential examples of this approach was reported by Czarnik and co-workers in 1994, who developed polyammonium chemosensors (**8** and **9**) capable of distinguishing between inorganic phosphate (Pi) and pyrophosphate (PPi) (**Figure 1.23**).⁹⁸ Their design featured two polyammonium arms positioned to interact with phosphate anions. While Pi could interact weakly, PPi was large enough to bridge both arms simultaneously, leading to enhanced binding. This size-selective interaction induced measurable changes in the fluorescence output, allowing the system to differentiate between Pi and PPi. This work highlighted the potential of size- and charge-based discrimination for phosphate sensing.



8



9

Figure 1.23. These chemosensors reported by Czarnik and coworkers, and Lehn and coworkers.^{99, 100} Source: Adapted from Ref.[100]/Journal of the Chemical Society.

Polyammonium-based chemosensors have been widely explored for phosphate recognition, with particular success in the detection of nucleoside phosphates. Although charged receptors often suffer from limited selectivity, carefully designed systems can overcome this limitation and achieve discrimination even between molecules with the same degree of phosphorylation.¹⁰¹ A notable example was reported by Yoon and co-workers, who developed a water-soluble imidazolinium-anthracene derivative **10** as a fluorescent chemosensor for nucleoside phosphates (**Figure 1.24**).¹⁰²

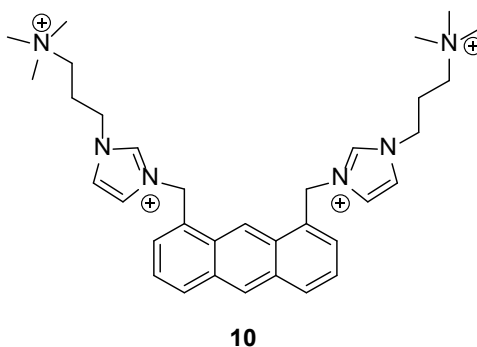


Figure 1.24. Water soluble chemosensor reported by Yoon and co-workers. **10**, and imidazolinium and anthracene derivative. Source: Adapted from Ref.[102] with permission ref[102]. Copyright (2020) Chemical Society Reviews.

This receptor displayed distinct fluorescence responses depending on the nucleobase: the emission of the anthracene unit **11** was quenched in the presence of guanosine triphosphate (GTP), whereas fluorescence was enhanced in the presence of adenine-based nucleotides such as ATP, ADP, and AMP in aqueous solution (**Figure 1.25**). Such contrasting behaviour allowed the system to differentiate between purine nucleotides despite their identical phosphorylation levels.¹⁰³

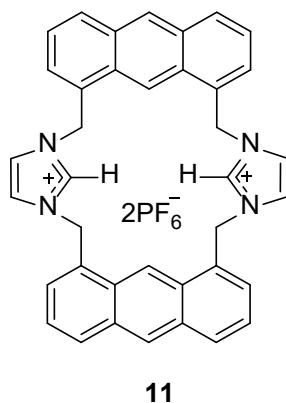


Figure 1.25. Water-soluble imidazolium-anthracene chemosensors designed to detect various nucleoside phosphates were reported by Yoon and co-workers.¹⁰⁴

Source: Adapted from Ref.[104] with permission ref[104]. Copyright (2011)

Royal Society of Chemistry.

The origin of this selectivity lies in the synergistic interplay of noncovalent forces. Electrostatic attraction between the cationic imidazolium groups and the phosphate anions drives binding, while π – π interactions between the aromatic anthracene unit and the nucleobase, combined with charge-assisted hydrogen bonding, further stabilise the complexes. Importantly, differences in the electronic structure and dipole moments of adenine versus guanine influence the binding geometry and photophysical response, leading to the observed variation in fluorescence.¹⁰⁴ This study demonstrated that by carefully combining multiple interaction modes, it is possible to design cationic chemosensors with remarkable selectivity, capable of distinguishing between structurally similar nucleoside phosphates under aqueous conditions.¹⁰⁵

Receptors utilising Hydrogen-bonding Interactions

Hydrogen bonding is another widely employed strategy for the recognition of phosphate species. In comparison to charged interactions such as metal coordination or electrostatics, hydrogen bonds are generally weaker in aqueous solution due to strong

competition from water molecules. This presents a critical challenge for chemosensor design: weaker binding can limit both sensitivity and selectivity.¹⁰⁶ Two main strategies have been developed to address this issue: the incorporation of multiple hydrogen-bonding interactions, which collectively provide enhanced affinity through cooperativity, and the construction of preorganised receptors, in which donor groups are held in precise spatial orientations to maximise binding efficiency.¹⁹

A wide range of hydrogen-bonding motifs have been incorporated into receptors, including ureas, thioureas, amides, squaramides, thiosquaramides, pyrroles, and imidazoles. These functional groups are particularly effective because they can donate and/or accept multiple hydrogen bonds. For example, ureas and thioureas can form bifurcated hydrogen bonds with phosphate oxygens, while squaramides and thiosquaramides are especially powerful donors due to their electron-deficient scaffolds, enabling high-affinity phosphate binding. Pyrrole and imidazole units, meanwhile, can serve as directional hydrogen bond donors or acceptors within heteroaromatic frameworks.¹⁰⁷

Several notable examples illustrate how hydrogen-bonding interactions can be exploited for selective phosphate detection. Zhu and co-workers reported a rhodamine spiro-lactam derivative **12** capable of selectively detecting ATP in buffered aqueous solution (20 mM HEPES, pH 7.2).¹⁰⁸ The receptor comprises a rhodamine fluorophore linked to a diethylenetriamine moiety that provides multiple hydrogen-bonding sites (**Figure 1.26**). In the absence of ATP, the rhodamine exists in its closed, non-fluorescent spirolactam form.¹⁰⁹ Upon binding ATP, hydrogen bonding between the diethylenetriamine unit and the phosphate groups induces ring opening, activating the rhodamine and producing a strong turn-on fluorescence signal. Additional π - π interactions between the rhodamine core and the adenine base of ATP are proposed to contribute to the remarkable selectivity of this chemosensor, which discriminates ATP over ADP, AMP, Pi, and PPi.

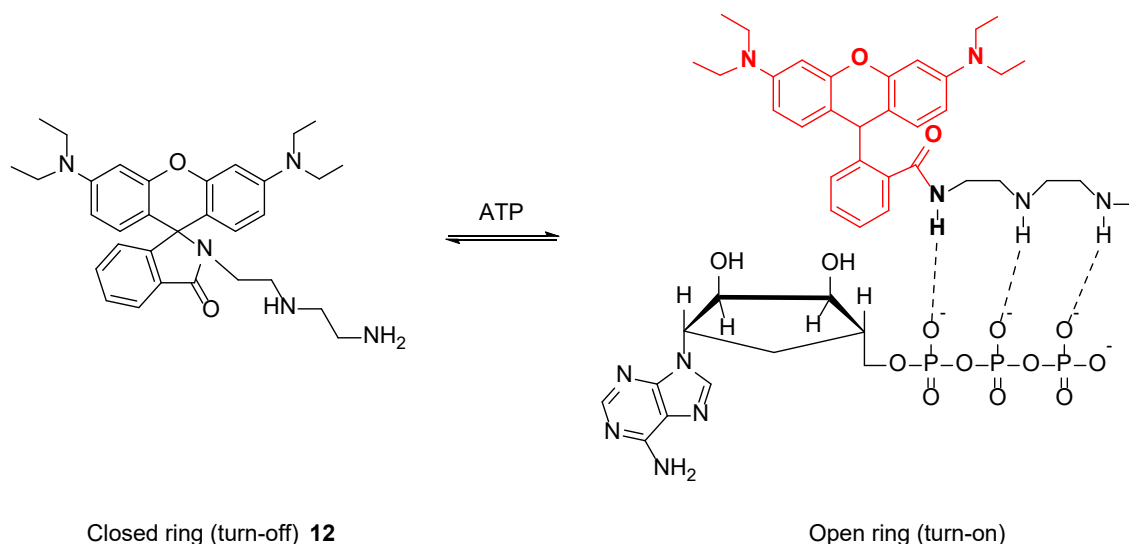


Figure 1.26. Colourimetric and fluorescent chemosensor rhodamine derivative reported by Zhu and co-workers.¹¹⁰ Source: Adapted from Ref.[110] with permission ref[110]. Copyright (2023) Journal of Coordination Chemistry.

Building on this scaffold, Zou and co-workers developed a ratio metric chemosensor by attaching a naphthalimide unit **13** to the rhodamine framework (**Figure 1.27**), enabling Förster Resonance Energy Transfer (FRET). This receptor displayed enhanced selectivity for ATP across a broad panel of competing analytes, including other nucleoside phosphates (ADP, AMP, CTP, GTP, UTP, TTP), inorganic phosphates (Pi, PPi, PPPi), and common anions such as sulfate, nitrate, and citrate.¹¹¹ ATP binding induced a marked colorimetric change and the strongest fluorescence response among the tested analytes. The authors attributed this to the cooperative effect of hydrogen bonding and π - π stacking, together with an optimal spatial fit between ATP and the receptor binding pocket.

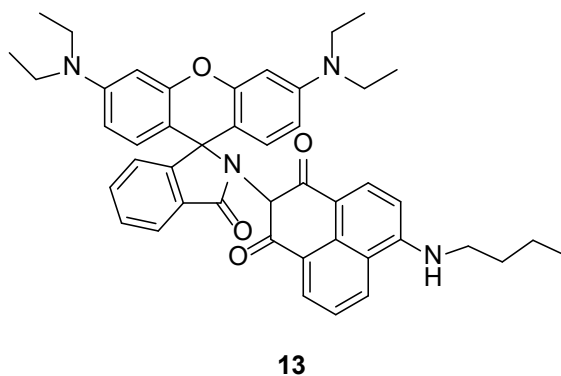
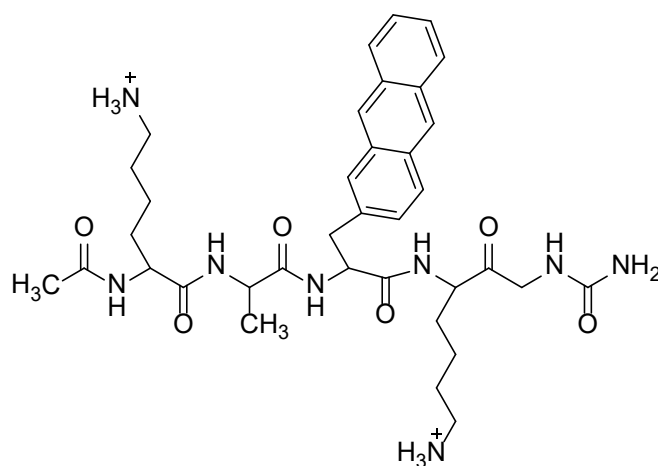


Figure 1.27. Ratio metric naphthalimide and rhodamine derivatives **13**. Source: Reprinted from Ref.[111] with permission ref[111]. Copyright (2014) Chemical Community.

A further example is provided by an amino-amide cage containing an anthracene fluorophore **14**, which is water soluble and exhibits unique fluorescence responses upon binding different anions (**Figure 1.28**). In buffered aqueous solution (50 mM Tris, pH 7.2), the receptor binds phosphate in a 1:2 host-guest stoichiometry, with an association constant significantly higher than that for oxalate or other dicarboxylates.¹¹² Interestingly, however, the fluorescence enhancement observed for phosphate was weaker than that for oxalate. This counterintuitive result was attributed to receptor deprotonation upon phosphate binding, which altered the electronic environment of the anthracene fluorophore and dampened the fluorescence response. This study highlights how subtle protonation effects can influence signal transduction, even when binding affinities are high.



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Figure 1.28. Anthracene based amino-amide cage fluorophore. ¹¹² Source: Adapted from Ref.[112] with permission ref[112]. Copyright (2020) Chemistry Open.

Receptors utilising Metal-ligand Interactions

Another powerful strategy for phosphate recognition is the incorporation of metal cations into receptor and chemosensor design. Phosphates are strong Lewis bases, and their oxygen atoms readily coordinate to metal centres. By carefully selecting the metal ion and ligand framework, it is possible to design receptors in which phosphate binding produces a measurable optical response.

A classic example is the fluorescein-Cu(II) system (**15** and **16**) (**Figure 1.29**). In this design, a Cu(II) centre is bound to fluorescein, resulting in quenching of the dye's emission due to the paramagnetic nature of the d⁹ Cu(II) ion. Upon addition of pyrophosphate (PPi), the strong affinity of PPi for Cu(II) leads to displacement of the fluorescein ligand. This restores fluorescence ("turn-on" response), providing a simple and highly selective method for PPi detection. The system exploits two key features of Cu(II): (i) its ability to quench fluorescence when directly coordinated to a chromophore, and (ii) its coordinative unsaturation, which allows binding of strongly chelating anions such as PPi. The chemosensor showed strong selectivity for PPi over a range of competing anions, including Pi, NCO⁻, Cl⁻, NO₃⁻, SO₄²⁻, N₃⁻, acetate, and benzoate, although discrimination among other polyphosphates was not extensively tested.

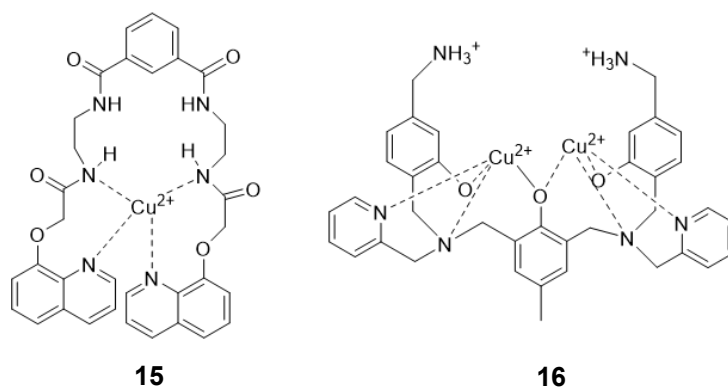


Figure 1.29. Cu (II) based receptors - macrobicyclic complex, **15**, reported by Taglietti and coworkers and ammonium appended bis copper (II) complex, **16**, reported by Chen and coworkers.¹¹³ Source: Adapted from Ref.[113] with permission ref[113]. Copyright (2014) Organic Letters.

A related approach was reported by Chen and co-workers, who developed a Cu(II)-based colorimetric chemosensor operating through an indicator displacement mechanism (**Figure 1.30**).¹¹³ In this system, PPI coordinates strongly to the Cu(II) centre, displacing a bound indicator dye and producing a measurable optical change. This intermolecular displacement process enabled selective detection of PPI in aqueous environments, again capitalising on the strong affinity between multivalent phosphate species and Cu(II).

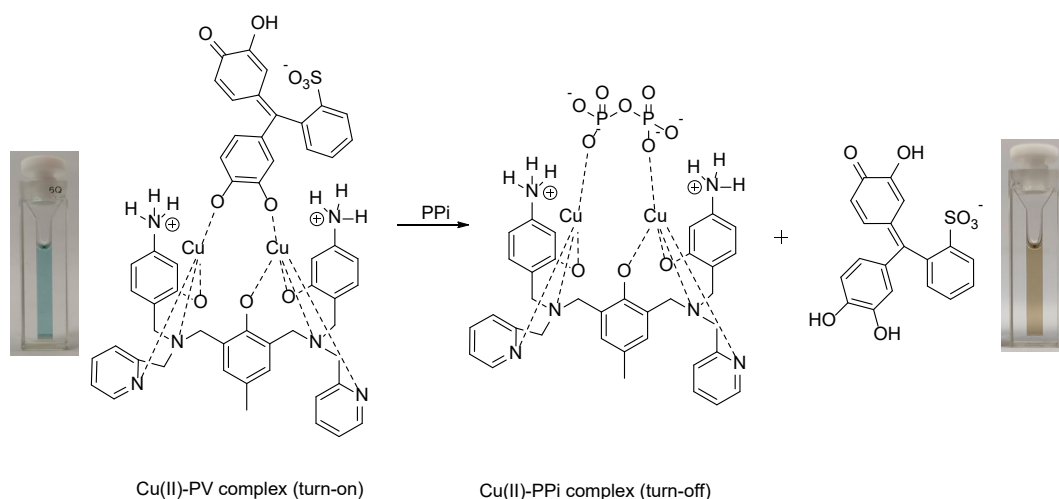


Figure 1.30. Cu(II) based receptors - macrobicyclic complex, **10** binding with **PV** and displacement of **PV** with PPI, reported by Chen.¹¹³ Source: Adapted from Ref.[113] with permission ref[113]. Copyright (2014) Organic Letters.

Lanthanide complexes (e.g., Eu^{3+} , Tb^{3+}) have also been explored for phosphate recognition, taking advantage of their unique photophysical properties such as long emission lifetimes and sharp f–f transitions (**Figure 1.31**).¹¹⁴ The ability to modulate the luminescence properties of lanthanide complexes by coordinating anions enables the detection of specific analytes in complex aqueous media. Recently, Butler and co-workers reported a cationic Eu(III) complex **17**, for the selective detection of ATP. This complex exhibits a luminescence increase upon the addition of ATP, which results from the displacement of a coordinated water molecule by the phosphate groups of ATPs. The interaction between the Eu(III) complex and ATP leads to a change in the coordination environment around the metal centre, affecting the luminescence properties of the complex. This example showcases the immense potential of lanthanide-based metal complexes as selective chemosensors for biologically relevant analytes in aqueous environments.¹¹⁵

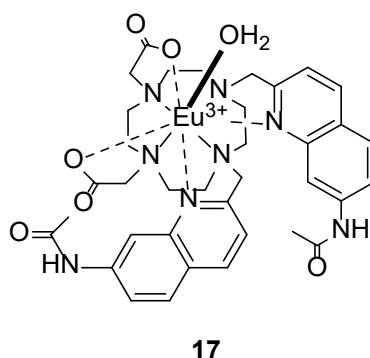


Figure 1.31. A lanthanide-based receptors, **17**, reported by Butler and co-workers.¹¹⁵ Source: Adapted from Ref.[115] with permission ref[115]. Copyright (2013) Chemical society Reviews.

Beyond Cu(II) , other transition metals have also been investigated. In particular, Zn(II) complexes have attracted significant attention due to their biological relevance, closed-shell d^{10} configuration (which avoids fluorescence quenching), and compatibility with aqueous environments. Zinc-based receptors often incorporate auxiliary binding motifs (e.g., amines, imidazoles, or pyridines) to pre-organise the binding site while leaving coordination positions accessible for phosphate ligation. These systems frequently display high selectivity for biologically important phosphates such as ATP and PPi and

will be discussed in more detail in the subsequent section.

Together, these examples demonstrate the effectiveness of metal-ligand coordination in phosphate chemosensor design. By combining the strong binding affinity of metal cations for phosphate oxygens with well-chosen signal transduction strategies (fluorescence quenching, displacement assays, or lanthanide luminescence), it is possible to achieve highly selective detection of phosphate species in aqueous environments.

1.5.3 Use of Dipicolylamine-Cation Coordination Complexes

Among metal-cation coordination complexes, those incorporating dipicolylamine (DPA) ligands have attracted particular attention for phosphate sensing. DPA acts as a tridentate ligand that binds Zn^{II} ions with high affinity and selectivity, leaving vacant coordination sites available for interaction with phosphate oxyanions (**Figure 1.32**).¹¹⁶ The resulting Zn-DPA complexes combine strong electrostatic interactions with adaptable coordination chemistry, enabling selective recognition of phosphate species in aqueous environments. DPA ligands are synthetically accessible and can be readily incorporated into a variety of scaffolds, making them highly versatile building blocks for chemosensor design.⁸⁸

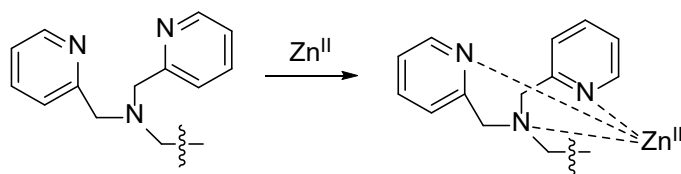


Figure 1.32. The dipicolylamino (DPA) ligand and corresponding Zn^{II} complex.¹¹⁶

Hamachi and co-workers reported a bis-Zn(DPA) chemosensor **18** in which two Zn(DPA) moieties were appended to an anthracene fluorophore (**Figure 1.33**).¹¹⁷ This system displayed strong binding to phosphate species in water, with fluorescence enhancement dependent on the presence of both Zn(DPA) arms (**Figure 1.33**). An analogous mono-Zn(DPA) derivative showed only weak responses, particularly toward phosphorylated peptides, highlighting the importance of cooperative binding by two zinc centres. This

work demonstrated that dual Zn(DPA) motifs are essential for achieving both sensitivity and selectivity in phosphate recognition.

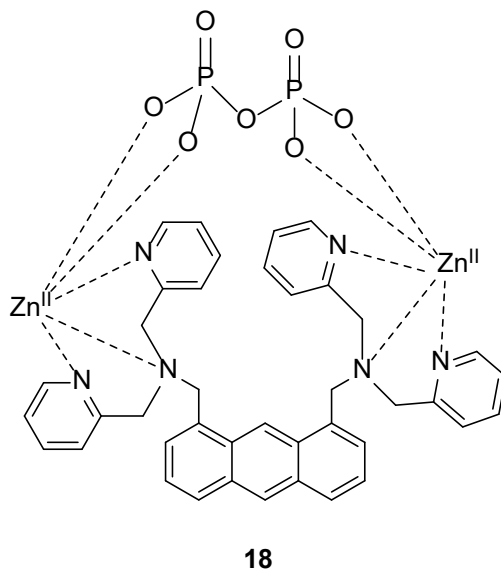
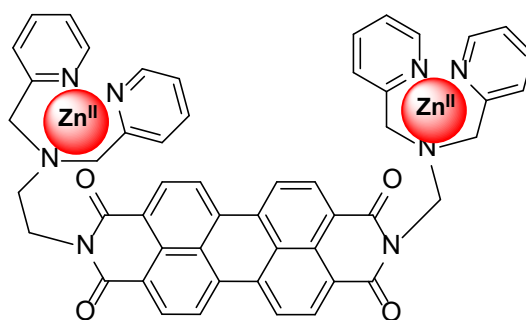


Figure 1.33. A Fluorescent probe bis-Zn (DPA) anthracene derivative **18** reported by Hamachi and co-workers, counterions omitted for the clarity. Source: Adapted from Ref.[117] with permission ref[117]. Copyright (2004) Journal of the Americal Chemical Society.

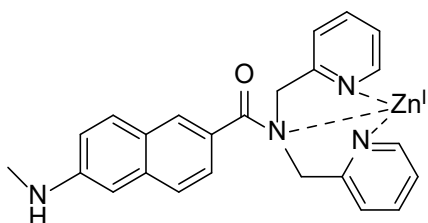
A complementary strategy was developed by Yoon and co-workers, who designed a bis-Zn(DPA) chemosensor **19** with a fluorescent perylene core (**Figure 1.34**).¹¹⁸ Unlike Hamachi's system, which targeted phosphorylated peptides, this receptor was optimised for nucleoside phosphates, showing pronounced selectivity for UDP and UTP over other nucleotides. The proposed binding mode involved a dual coordination mechanism: one Zn(DPA) arm binds the phosphate groups, while the second interacts with the uridine nucleobase. The rigid perylene spacer enforced an appropriate distance between the two Zn(DPA) centres, enabling simultaneous recognition of both components of the target analyte.¹¹⁹



19

Figure 1.34. Bis-Zn(DPA) chemosensor **19**, based around a fluorescent perylene core. Counterions omitted for clarity. Source: Adapted from Ref.[119] with permission ref[119]. Copyright (2007) Journal of the Americal Chemical Society.

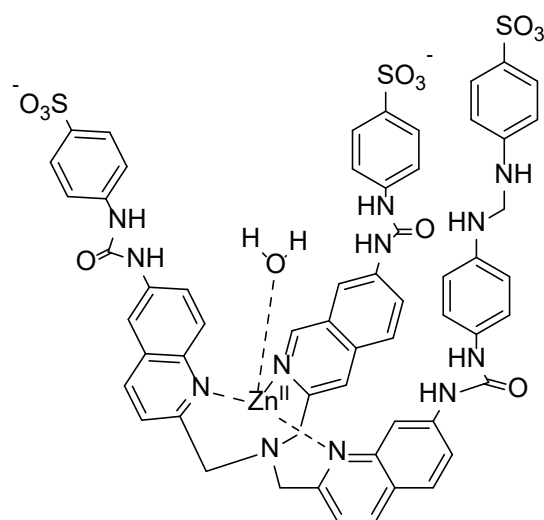
Another example is the acedan-based chemosensor **20**, which contains a single Zn(DPA) unit (**Figure 1.35**). This “turn-on” sensor was selective for ATP and ADP.¹¹⁷ The acedan fluorophore coordinates to Zn^{II}, quenching the intramolecular charge-transfer (ICT) fluorescence pathway. Upon addition of ATP or ADP, the phosphate groups bind preferentially to Zn^{II}, displacing the acetyl group and restoring ICT fluorescence. Additional π - π stacking between the adenine base and the naphthalene fluorophore further contributed to adenine nucleotide selectivity. Importantly, this system was successfully applied to the monitoring of ATP and ADP in live cells, highlighting the biological relevance of Zn(DPA)-based sensors.



20

Figure 1.35. Fluorescent acedan derivative **20** with a single Zn(DPA) moiety. Counterions omitted for clarity. Source: Adapted from Ref.[117] with permission ref[117]. Copyright (2004) Journal of the Americal Chemical Society.

More complex receptor architectures have also been reported. Butler and co-workers¹²⁰ developed a tripodal quinoline-based Zn(II) receptor **21** (Figure 1.36), which combines multiple interaction modes: direct Zn(II)-phosphate coordination, hydrogen bonding from appended urea groups, and π - π stacking with nucleobases. The receptor's binding cleft was tailored to favour the larger ATP molecule over ADP, resulting in association constants of $\log K_a = 5.21$ for ATP and $\log K_a = 3.86$ for ADP. Fluorescence enhancement was observed upon ATP binding, attributed to excimer formation between quinoline groups. This example illustrates how size complementarity, coupled with cooperative interactions, can impart both affinity and selectivity.¹²¹



21

Figure 1.36. Tripodal Zn^{II} complex **21** for the sensing of ADP, ATP and citrate.¹²¹ Source: Adapted from Ref.[121] with permission ref[121]. Copyright (2012) Chemical Communication.

The group of Kate Jolliffe has made extensive contributions to phosphate sensing through the development of indicator displacement assays (IDAs) based on Zn(DPA) receptors. In these systems, cyclic peptide scaffolds bearing Zn(DPA) motifs were coupled with chromogenic catecholate-based indicators such as pyrocatechol violet (**PV**), bromopyrogallol red (**BPR**), and pyrocatechol red (**PR**) (Figure 1.37).¹²² Binding of the indicator to Zn(DPA) produces a characteristic colour, which is displaced upon phosphate binding. For example, UV-Vis titrations of receptor **22** with **PV** showed clear spectral shifts at 640 and 444 nm corresponding to 1:1 complexation. Upon addition of PPi, the

indicator was displaced, reversing the spectral changes.¹²³

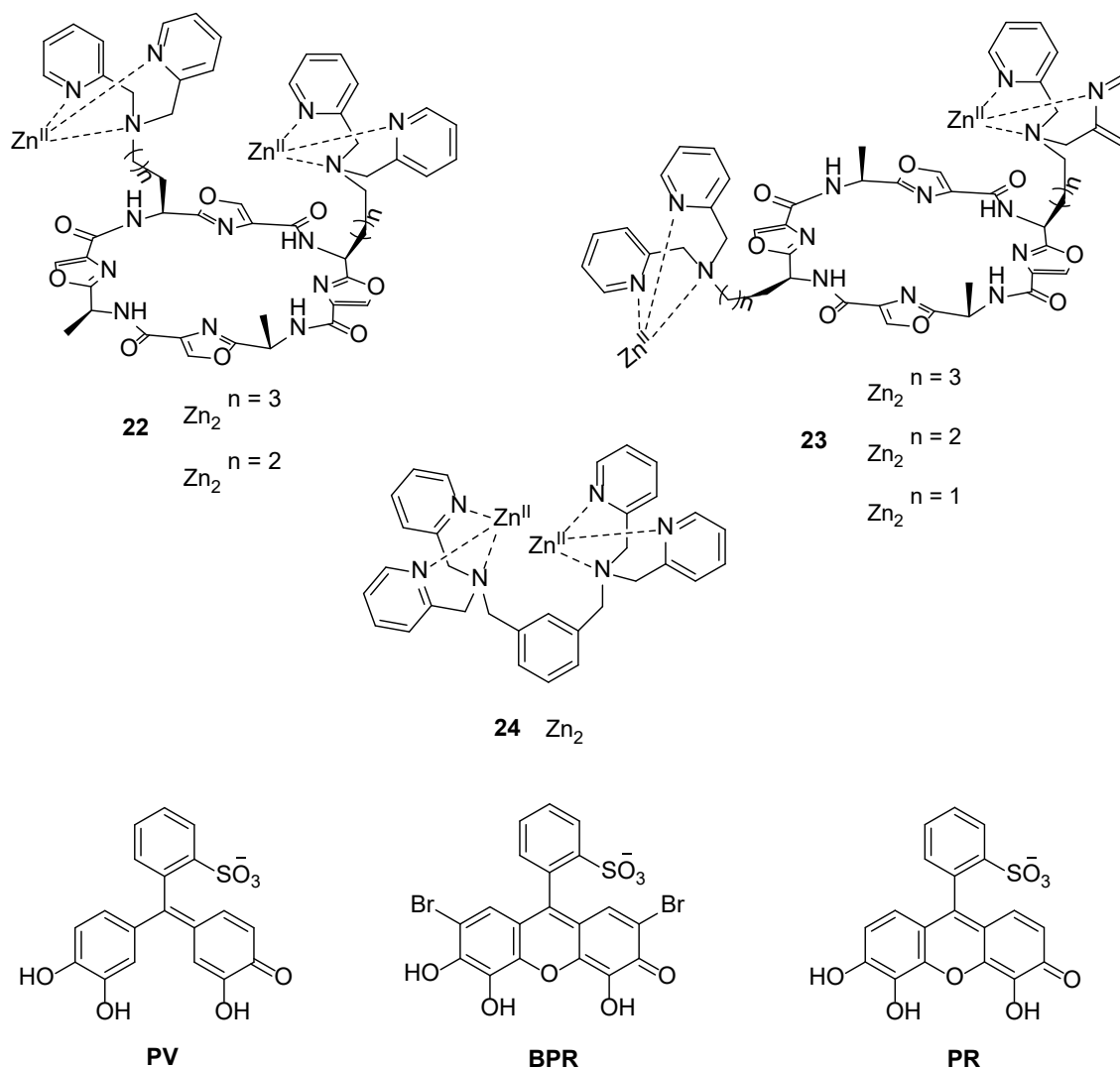


Figure 1.37. Structures of sensors consisting of dipicolylamine groups and chromophores containing catecholate groups, used for IDAs. Source: Reprinted with permission from ref. **123**. Copyright (2013) Chemical Science.¹²³

In these systems, the indicator first binds to the Zn^{II} ions chelated by the dipicolylamine ligands, and this complexation can be monitored spectroscopically. UV-Vis titration experiments were performed to determine the association constants between receptors and indicators in buffered solution. For example, binding of **PV** to receptor **22** was characterised by the appearance of a new absorption band at 640 nm accompanied by a decrease in the band at 444 nm, consistent with the formation of a 1:1 receptor-indicator

complex (**Figure 1.38a**, and **Figure 1.38b**). Upon subsequent titration with pyrophosphate (PPi), displacement of **PV** from the receptor was observed, with the 640 nm band diminishing and the 444 nm band reappearing. These spectral changes provided clear evidence of PPi competitively binding to the Zn (DPA) centres, displacing the **PV** indicator.

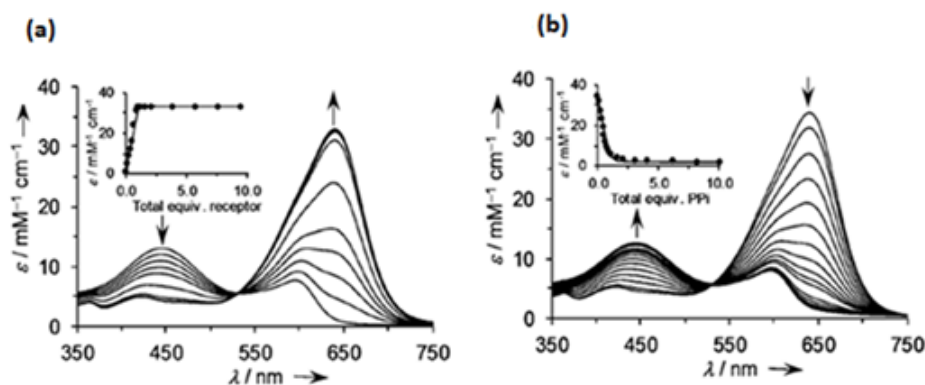


Figure 1.38. Changes in absorbance of **PV** and when (a) **PV** is added to varying concentrations of sensor **22**; and (b) when PPI is added in varying concentrations to the **PV**: sensor **22** complex. Source: Reprinted with permission from ref.123. copyright (2013) Chemical Science.¹²³

By systematically varying the peptide scaffold, Jolliffe and co-workers demonstrated that side-chain length between DPA groups strongly influences selectivity.¹²² Shorter linkers favoured displacement by PPI and ATP, while longer linkers allowed broader recognition, including ADP and citrate. Binding constants derived from UV-Vis titrations confirmed that selectivity could be tuned through scaffold modification and indicator choice.¹²⁴ This work provided an elegant demonstration of how IDAs can be adapted to discriminate among structurally similar phosphate species (**Figure 1.39**).

Fluorescent indicators have also been incorporated into Zn(DPA) IDAs, greatly enhancing sensitivity compared to colourimetric systems.¹²⁵ For example, a Zn(DPA) receptor **23** paired with a fluorescent catechol derivative **24** showed fluorescence quenching upon binding Zn^{II} (**Figure 1.40a**). Addition of PPI displaced the fluorophore, restoring fluorescence in a concentration-dependent manner (**Figure 1.40b**). This approach enabled quantitative detection of PPI in the presence of competing polyphosphates such as ATP.

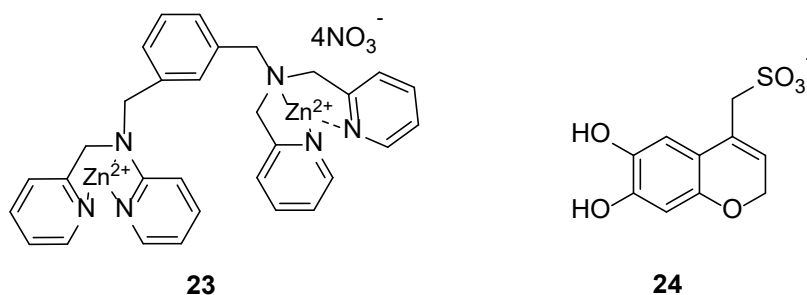


Figure 1.39. Structure of sensor **23** and fluorescent indicator **24** used for phosphate oxoanion sensing.¹²⁵ Source: Adapted from Ref.[125] with permission ref[125]. Copyright (2012) Chemistry-An Asian Journal.

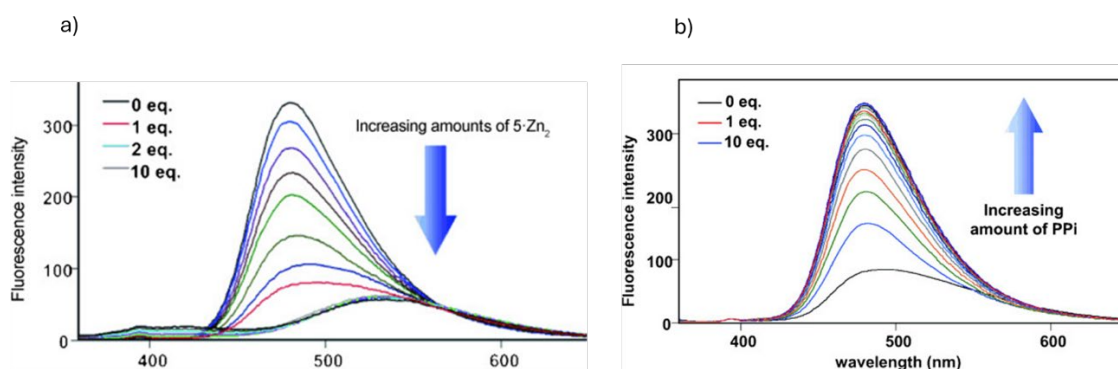
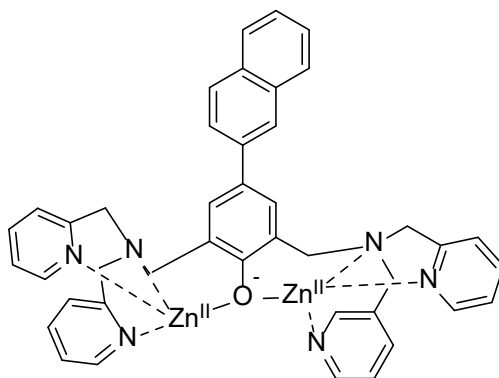


Figure 1.40. Fluorescence emission spectra of sensor **23** with (a) an increasing concentration of the host **23** in the presence of indicator **24**; and (b) with an increasing concentration of **PPI**. Source: Modified with permission from ref. 125. Copyright (2012) Chemistry-An Asian Journal.¹²⁵

Further advances include the development of fluorescent Zn(DPA) complexes capable of discriminating PPI from ATP and ADP under near-physiological conditions (pH 7.4, HEPES buffer, 145 mM NaCl). Notably, one naphthalene-based Zn(DPA) chemosensor **25** detected sub-stoichiometric concentrations of PPI even in the presence of 50 to 250-fold excesses of ATP or ADP, representing one of the first examples of a metal-complex sensor achieving reliable discrimination among closely related phosphate species (**Figure 1.41**).¹²⁶



25

Figure 1.41. Structure of a Zn^{II} -complex chemosensor **25** which can differentiate between PPI and ATP.¹²⁶ Source: Adapted from Ref.[126] with permission ref[126]. Copyright (2004) Journal of the Americal Chemical Society.

Overall, these studies underscore the versatility of $\text{Zn}(\text{DPA})$ complexes in phosphate recognition. By combining the strong binding affinity of Zn^{II} with adaptable ligand scaffolds, $\text{Zn}(\text{DPA})$ -based chemosensors can be tailored for specific phosphate species ranging from inorganic anions to nucleotides and phosphorylated peptides. The modularity of these systems, particularly when coupled with indicator displacement strategies, continues to make them one of the most productive approaches for phosphate sensing in supramolecular chemistry.

1.6 Aims of this Project

1.6.1 Designing a self-assembled host for PPI detection

In the literature described so far, most synthetic receptors have been constructed by covalently attaching the requisite functional groups for analyte binding (and, in the case of indicator displacement assays, indicator binding) onto a molecular scaffold (**Figure 1.42a**). The scaffold acts as a rigid framework that positions these groups in close proximity, allowing cooperative interactions and multivalency effects to drive selective recognition. While highly effective, this strategy often requires elaborate multistep

syntheses, and even small modifications to the receptor design can necessitate the preparation of new analogues from scratch.

In this research, we aim to explore an alternative approach: the use of self-assembly to bring together the functional groups required for analyte binding. Specifically, amphiphilic molecules will be employed, in which the recognition element is incorporated into the polar headgroup, while the hydrophobic tail drives spontaneous self-assembly into higher-order structures such as micelles or vesicles (**Figure 1.42 b**). At the interface of these assemblies, multiple recognition sites can be clustered, mimicking the cooperative binding observed in natural systems.¹²⁷

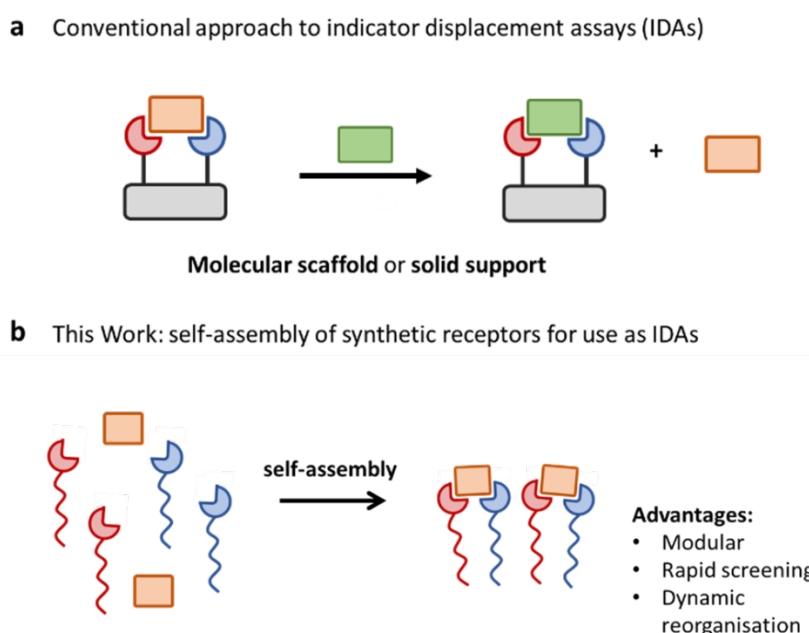


Figure 1.42. (a) Conventional approach to indicator displacement assays; (b) The focus of this thesis, the self-assembly of amphiphilic molecules for the formation of receptors capable of binding PPI.

The self-assembly approach offers several important advantages. First, it provides a modular platform: different amphiphiles bearing diverse recognition motifs can be mixed and matched, enabling rapid optimisation of binding properties without the need for complex covalent synthesis. Second, the dynamic and reversible nature of self-assembly allows receptors to adapt to different analytes and environments, offering flexibility not

easily achievable with rigid covalent scaffolds or dendrimeric architectures.¹²⁸ Finally, this modularity makes the strategy highly amenable to combinatorial design, where libraries of amphiphiles can be screened to identify the most effective combinations for a given analyte.

In principle, this approach could greatly streamline receptor development. Instead of laboriously synthesising entirely new molecules to test alternative binding arrangements, modifications can be introduced simply by changing the amphiphile composition or ratio. Such a system thus has the potential to accelerate the optimisation of supramolecular receptors, providing a versatile and synthetically straightforward route to selective anion recognition.

1.6.3 Previous work within the group

Our group has previously demonstrated that self-assembly can be harnessed to generate functional catalytic systems from amphiphilic molecules. Under appropriate conditions, amphiphiles bearing catalytic headgroups assemble into vesicular structures, in which multiple functional groups are clustered at the interface. This clustering enables cooperative substrate binding and catalysis, mimicking the multivalency observed in natural enzymes.¹²⁹ Notably, no catalytic activity was observed in the non-assembled form, highlighting the essential role of self-assembly. The driving force for assembly arises from the long hydrophobic tails of the amphiphiles, which are thermodynamically stabilised in water by forming micelles or vesicles (**Figure 1.43**).

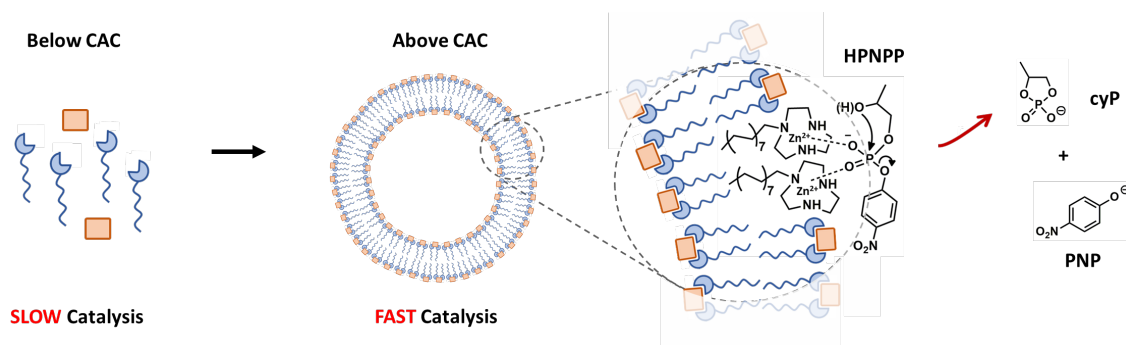


Figure 1.43. (a) The substrate-induced formation of amphiphilic molecules to form catalytically active sites on the surface of vesicular structures. Source: Adapted from Ref.[129] with permission ref[129]. Copyright (2018) Angewandte Chemie International Edition.

One example involved amphiphiles containing C_{16} -tethered 1,4,7-triazacyclononane (TACN) headgroups, which assembled into vesicles in the presence of the model phosphodiester substrate 2-hydroxypropyl-4-nitrophenyl phosphate (HPNPP) (**Figure 1.44**). The phosphate group of HPNPP interacts strongly with the TACN headgroups when coordinated to Zn^{II} , lowering the critical aggregation concentration and promoting vesicle formation.¹²⁹ Once aggregated, the close proximity of multiple catalytic sites enabled cooperative catalysis, leading to the spontaneous hydrolysis of HPNPP. This provided one of the first demonstrations of substrate-induced self-assembly of a supramolecular catalyst in our group.

Building on this work, we explored mixed self-assembly systems in which two different amphiphiles co-assemble to form cooperative catalysts. In particular, amphiphiles functionalised with TACN **26** and guanidinium (G) groups **27** (**Figure 1.44**) were combined in different proportions to generate a family of catalytic systems. TACN,¹³⁰ when coordinated to $Zn(II)$ or $Cu(II)$, promotes phosphodiester cleavage, while guanidinium groups stabilise transition states through hydrogen bonding and electrostatic interactions with phosphate substrates. Together, these complementary headgroups were expected to enhance catalytic efficiency. The mixed systems were tested with HPNPP, which upon cleavage produces a yellow p-nitrophenolate chromophore that can be

monitored by UV-Vis spectroscopy. Importantly, the TACN/G assemblies displayed synergistic catalysis: the rate of HPNPP hydrolysis was significantly higher for mixtures than for each amphiphile alone. The optimal activity was observed for a 1:3 TACN/G ratio, highlighting how catalytic performance can be tuned by simply varying amphiphile composition.

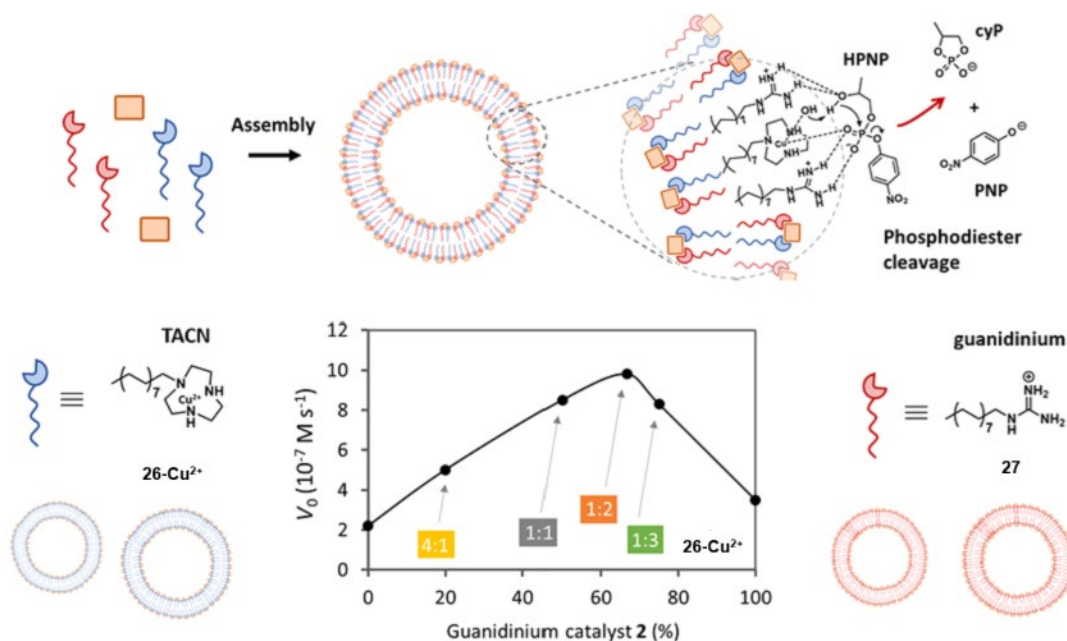


Figure 1.44. Representation of $C_{16}TACN \cdot Cu^{2+}$ ($26 \cdot Cu^{2+}$, blue) and C_{16} -guanidinium **27** (red) mixing to form heterogeneous vesicular assemblies that can catalyse the cleavage of HPNPP. Source: Adapted from Ref.[130] with permission ref[130]. Copyright (2018) Angewandte Chemie International Edition.

Taken together, these studies established that self-assembly provides a modular and adaptive strategy for catalysis, where functional headgroups can be mixed and matched to generate cooperative binding sites, and catalytic activity can be fine-tuned by adjusting amphiphile ratios. This work provides a strong foundation for extending the same principles to the design of self-assembled receptors for phosphate recognition and sensing.

1.6.4 Key Objectives of this Thesis

Building on the principles demonstrated in our group's catalytic systems, the present project seeks to apply self-assembly strategies to receptor design, with the ultimate goal of developing new indicator displacement assays (IDAs). Traditionally, IDAs rely on receptors constructed by attaching binding groups to rigid molecular scaffolds or surfaces, thereby creating defined binding pockets for the indicator and analyte. While this scaffold-based approach has produced many elegant systems, it often involves lengthy, multi-step syntheses, and any structural modification requires the preparation of new analogues, limiting the speed of optimisation.

In this work, we propose an alternative strategy: the use of amphiphilic molecules that spontaneously self-assemble into supramolecular architectures such as micelles or vesicles. By embedding recognition motifs into the polar headgroups of amphiphiles, the assembly process can cluster multiple binding sites at the hydrophilic interface. This arrangement provides a dynamic and modular means of generating selective binding pockets for both the indicator and the analyte, without the need for rigid scaffolds or complex synthetic routes. Such an approach offers the potential for rapid optimisation through combinatorial mixing of different amphiphiles, echoing the modularity demonstrated in our catalytic studies (**Figure 1.45**).

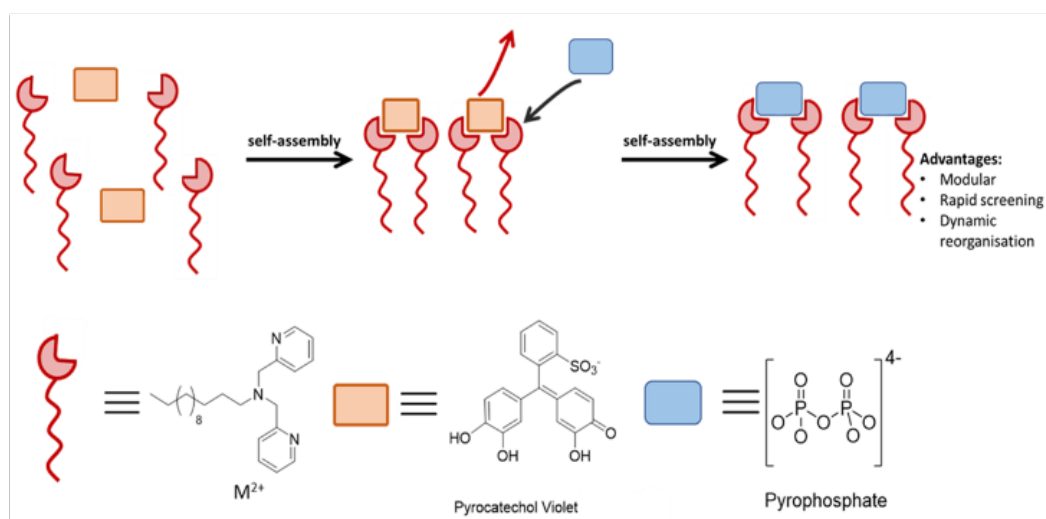


Figure 1.45. The substrate-induced formation of receptors self-assembly and modular, rapid screening, and dynamic reorganisation. Source: Adapted from Ref.[130] with permission ref[130]. Copyright (2020) Journal of the Americal Chemical Society.

For the binding motif, we will focus on dipicolylamine (DPA) headgroups, which are well-established for their strong affinity towards phosphate anions when complexed with divalent metal ions, particularly Zn^{II} . DPA- Zn^{II} complexes have been widely exploited in synthetic receptors for phosphate sensing due to their favourable coordination chemistry, water compatibility, and precedent in successful IDA systems. As a target analyte, we have chosen pyrophosphate (PPi), inspired by the work of Kate Jolliffe and co-workers. PPi is a biologically and environmentally significant anion, arising from processes such as DNA/RNA polymerisation and energy metabolism, and is structurally distinct from related phosphate oxoanions (e.g., Pi , ATP, ADP). Its high charge density and strong hydration make it a challenging but valuable analyte for selective sensing.

The overall aim of this project is to demonstrate that self-assembly can be employed as a viable and versatile strategy for receptor formation in IDAs. By designing amphiphiles with DPA headgroups and hydrophobic tails and testing their ability to self-assemble into receptor architectures capable of selectively binding PPi , we seek to establish proof of concept that dynamic, modular assemblies can rival traditional scaffold-based receptors in sensitivity, selectivity, and ease of development.

Chapter Two

**Self-Assembled Receptors for the
Detection of Pyrophosphate**

2.1 Overview

Chromogenic indicators that can distinguish between different phosphate-containing molecules are a subject of intense interest. In biological systems, anions play a multitude of vital roles. For example, pyrophosphate (PPi) and adenosine triphosphate (ATP) are crucial for numerous biochemical processes in the human body. DNA polymerisation requires the hydrolysis of ATP to release PPi, and PPi participates in the biosynthesis of cyclic adenosine monophosphate (cAMP), an important secondary messenger that controls the activity of many hormones. The ability to selectively detect one ion over another is a key factor for artificial receptors, as a target ion can be present together with structurally similar species. For example, PPi exists in the body alongside other phosphate oxoanions such as ATP and adenosine diphosphate (ADP).¹³¹ Sensors for anions are generally based around indicator-displacement assays (IDAs), where a synthetic receptor initially binds a chromogenic indicator such as a coloured molecule or a fluorescent probe. This selective detection of ions is a crucial aspect of the research, as it allows for the differentiation of target ions from other similar species.¹³²

Conventionally, IDAs are formed by placing the required binding groups onto molecular scaffolds or surfaces. This creates a binding pocket that allows for selective binding of the analyte.⁸² However, the design and optimisation of an IDA can be time-consuming due to the need for complex multi-step synthesis to produce the library of synthetic receptors required for screening. This work explores an alternative approach to accessing synthetic receptors by utilising the self-assembly of simple amphiphilic molecules and thiols to form the required receptors in situ. Self-assembly provides the flexibility to mix and match different components, enabling the rapid and efficient generation of numerous receptor variants with minimal synthetic effort. These systems offer greater flexibility, are easier to access, and enable the rapid optimisation of synthetic receptors.¹³³

We recently demonstrated that self-assembly driven by hydrophobic interactions is an effective strategy to form catalytically active pockets on the surface of vesicular

structures. The functional groups work cooperatively in the binding of the substrate, and the catalytic activity is the result of the simultaneous binding of multiple functional groups. These systems were exciting because they are highly modular, consisting of monomeric components. This can facilitate the introduction and mixing of different amphiphiles easily, without the need for lengthy chemical synthesis. Our idea was that, with minimal changes in design, we could use the same concept to construct vesicular structures that possess binding pockets on their surface for specific analytes. This strategy would allow for a variety of different amphiphiles to be quickly screened and allow rapid optimisation of an indicator displacement assay.¹³⁴

Inspiration for this work came from Jolliffe and co-workers, who have developed a range of colorimetric sensors based on peptides, both cyclic and linear, as scaffolds for positioning zinc(II) dipicolylamine Zn(DPA) binding sites that act as efficient Zn^{II} ions bind efficiently to the catecholate groups of the indicator, resulting in a color change that can be observed by visually.¹³⁵ This approach, which enables selective binding for pyrophosphate (PPi) compared to other polyphosphate species, including ATP, has the potential to revolutionise the field of biochemistry and molecular biology.¹³⁶ It allows for selective recognition of PPi even in complex media, such as cell growth buffers, opening new possibilities for research and applications. Using peptide scaffolds enables the creation of small receptor libraries, where the arrangement of the two Zn(DPA) binding sites can be subtly modified to evaluate factors affecting binding selectivity and affinity for PPi. This process involves systematically altering critical structural elements of the cyclic peptide ring size, including peptide length, amino acid composition, binding site positioning, and relative stereochemistry, to optimise PPi recognition meticulously.¹³⁵ Increased pyrophosphate selectivity was observed when the complexity of the media was raised from HEPES buffer to cell growth media (Krebs saline). Furthermore, the construction of pyrophosphate sensors with covalently attached and the colourimetric indicators they employed were catechol-type indicators such as pyrocatechol violet (**PV**), bromopyrogallol red (**BPR**), and pyrocatechol red (**PR**).

2.2 Aims of the Current Project

In the current chapter, we aim to utilise the above concepts for the self-assembly of receptors for phosphate-containing compounds. We plan to use amphiphiles for the formation of vesicle structures that have binding pockets on their surface that can selectively bind different phosphate-containing molecules. Self-assembly will allow us to screen several different amphiphiles to see which combinations work best (**Figure 2.1**). The ability of such systems to dynamically reorganise and the observation of this phenomenon is also a hot topic in systems chemistry.

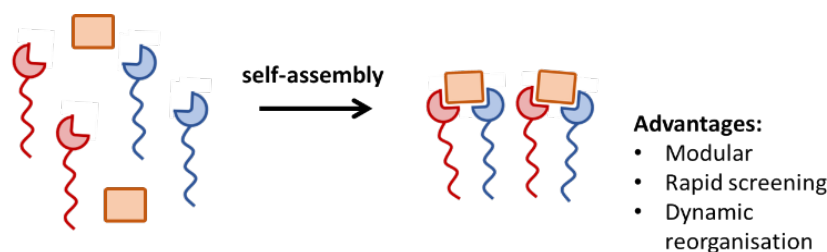


Figure 2.1. The substrate-induced formation of receptors self-assembly and modular, rapid screening, and dynamic reorganisation.

We aim to expand on the number of building blocks that can be used and combine them to test an array of different receptor combinations. To begin with, I synthesised amphiphiles containing the dipicolylamine (DPA) head group and a long hydrophobic tail (C₁₆) (**28**), as this system, including this ligand, had been previously shown by Jolliffe to be an effective phosphate sensor. The importance of the self-assembly of this ligand **28** in the formation of the supramolecular sensor can be assessed by comparing it to the control molecule **29**, which involves the same binding group but has a much shorter tail (C₄) (**29**). Molecule **29** is not expected to self-assemble under the conditions investigated. We expect to see a difference in the ability of **28** and **29** (**Figure 2.2**) to sense phosphates, as two dipicolylamine units must come together to bind phosphate oxoanions. Therefore, **28** should form a better sensor due to its capability to self-assemble and form a

supramolecular structure. **Scheme 2.1a** shows the simple one-step synthesis needed to make the above-mentioned ligands.

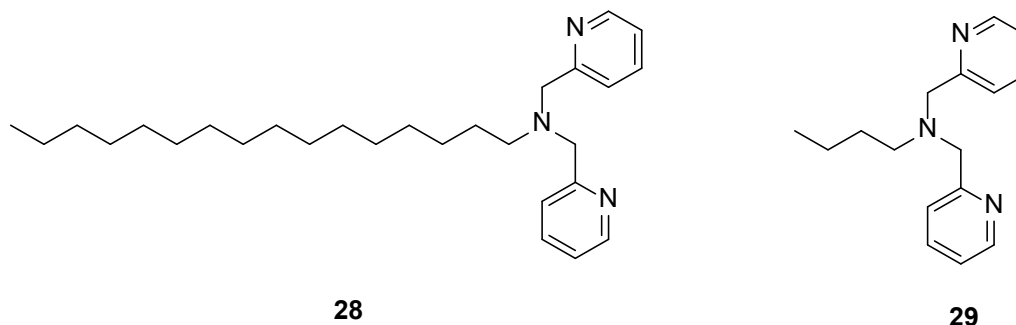
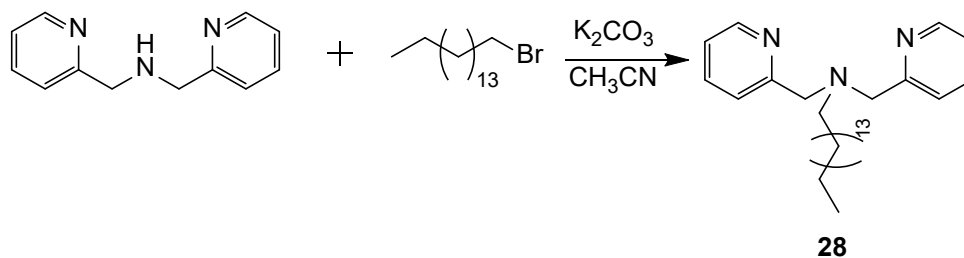


Figure 2.2. The structures of C₁₆-dipicolylamine (**28**) and C₄-dipicolylamine (**29**)

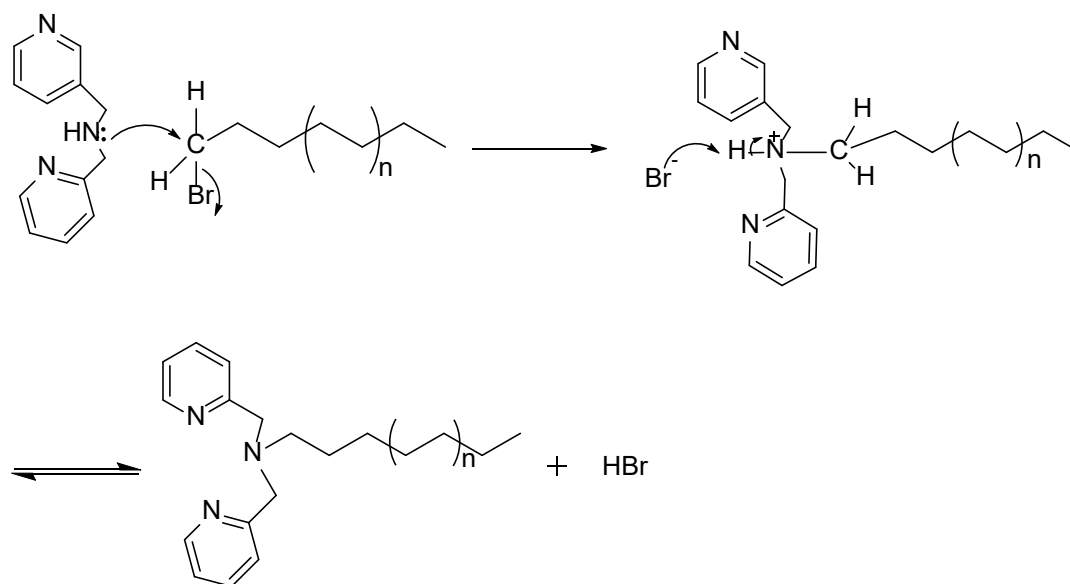
2.3 Initial Trials

2.3.1 Synthesis of the organic ligands for the self-assembled host

Our first target was to synthesise the picolyl-based ligands and investigate their self-assembly to form vesicle structures that have binding pockets on their surface that can selectively bind to different phosphate-containing molecules. We began by synthesising an amphiphile consisting of a dipicolylamine group and a 16-carbon hydrophobic tail, displayed in **Scheme 2.1a**. This was a simple synthesis consisting of just one step, the nucleophilic substitution of bromine for the dipicolylamine group via an S_N2 mechanism (**Scheme 2.1b**). We found the reaction itself to be successful and eventually purified the crude product by using flash column chromatography using alumina instead of silica.



Scheme 2.1a. Synthesis of amphiphilic ligand C₁₆-picolylamine (**28**).

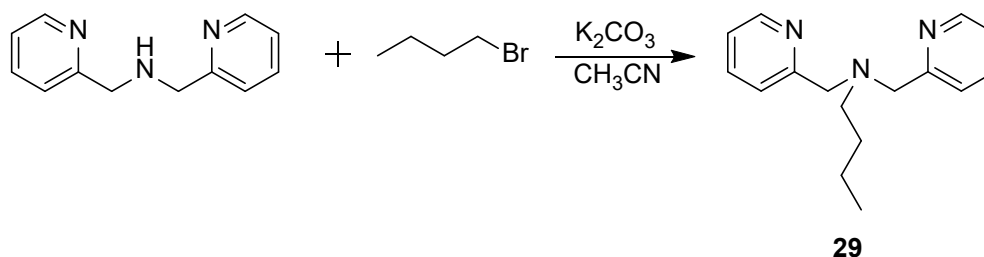


Scheme 2.1b. The S_N2 reaction mechanism for the formation of ligand C₁₆-picolylamine (**28**).

Originally, we used 10% MeOH in CH₂Cl₂ + 1% NH₄OH as the eluent system, however, this resulted in both spots on the TLC eluting together. We thought that this may be due to the polarity of the mobile phase being too high. Therefore, we reduced the polarity to 5% MeOH in CH₂Cl₂ + 1% NH₄OH. Once more, the two spots eluted together, and the NMR investigation showed that the product was present along with a high number of impurities. We, therefore, attempted the reaction again using 1-bromohexadecane in excess to assure that all the dipicolylamine reacted. Since 1-bromohexadecane is non-polar it should be easier to separate during flash chromatography than dipicolylamine. The reaction appeared to have reached completion after two days, though there was still dipicolylamine present in very small amounts (visible on TLC). Accordingly, we decided to change the stationary phase to alumina instead of silica to see if we could get a better separation of the starting materials to the product because silica is slightly acidic. In other words, it retains basic compounds whereas alumina is basic and is also less polar. We used 0.5% MeOH/CH₂Cl₂ as the eluent together with the alumina and this resulted in a clean separation of the product.

As a control molecule when examining the self-assembly of the amphiphilic

ligand, we synthesized the C₄-dipicolyl amine (**29**). This ligand was synthesized using the method optimised for compound **29** (Scheme 2.2). Flash chromatography using a gradient of solvents starting with CH₂Cl₂ and then increasing to 0.5% MeOH furnished the product with a yield of 84.7%



Scheme 2.2. Synthesis of control molecule C₄-dipicolylamine (**29**).

2.3.2 UV-Visible spectroscopy studies

Our current research aims to build on the previous investigations by Kate Jolliffe and coworkers, which showed that bis (Zn^{II}-DPA) with cyclic peptide rings displaced **PV** with 1:1 model response to various nucleoside phosphates, especially PPI. Similar Zn^{II}-DPA structures also have been reported to exhibit selectivity for pyrophosphate. To further investigate the titrations were conducted with C₁₆-picolyl-PV complex by using anion binding and repeating spectroscopic titrations with ATP, ADP, and AMP, as well as pyrophosphate (**Figure 2.3**).

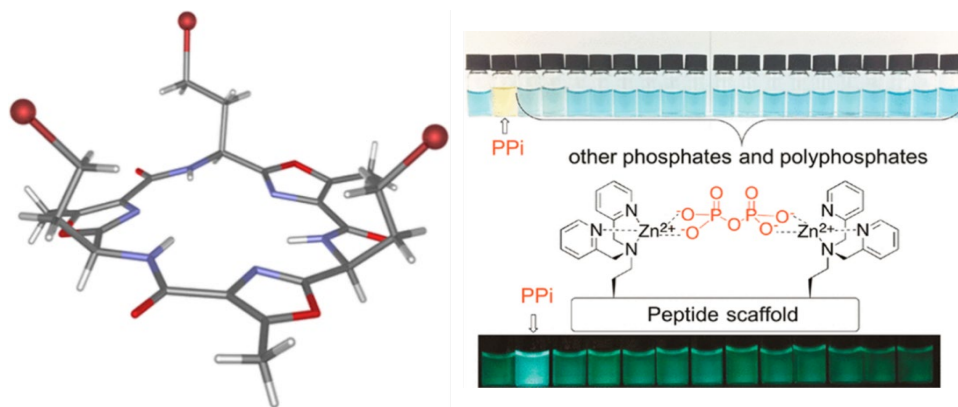


Figure 2.3. Different types of chemo sensors to selectively detect one type of anionic phosphate over other.¹²² Source: Adapted from Ref.[122] with permission ref[122]. Copyright (2012) Chemical Society Reviews.

This standard buffer setup provides a consistent environment for initial assessments of binding selectivity and affinity between chemosensors and target analytes. HEPES buffer is commonly used in biological studies due to its adequate pH buffering capacity in the physiological pH range (6.8 to 8.2). Additionally, including sodium chloride in the buffer helps maintain a physiological ionic strength, which can influence binding interactions through various mechanisms, such as electrostatic and osmotic effects. This approach enables the systematic study of molecular recognition systems with enhanced selectivity and sensitivity, as well as the investigation of relevant processes and analyte monitoring. These conditions involve a simple HEPES buffer at pH 7.4, with the addition of sodium chloride to simulate physiological ionic strength (5 mM HEPES buffer, 145 mM NaCl, pH 7.4).

2.3.3 Formation of the host-indicator (PV) complex

The UV studies we employed were based on previous work by Jolliffe *et al.* In their work they designed sensors that utilized the picolyl amine units to sense/bind phosphates. They explored the binding of their sensors with several dyes and used them to construct IDAs. Jolliffe *et al.* found pyrocatechol violet **PV** to be the most effective at binding to their

sensor, forming a 1:1 complex.¹²² Therefore, we decided to begin by assessing the binding of both our amphiphilic ligands **28** and **29** to **PV** to determine if this would be a suitable dye for our system. As the first step, we analysed the dye-sensor binding **28** with **PV**, and then the displacement of the dye from the sensor by PPI. **PV** typically has an absorbance peak between 350-500 nm and 550-640 nm, and the colour of the **PV** solution is yellow in the presence of HEPES buffer (5 mM), pH at 7.4 and NaCl (145 mM) in aqueous solution (**Figure 2.4**).

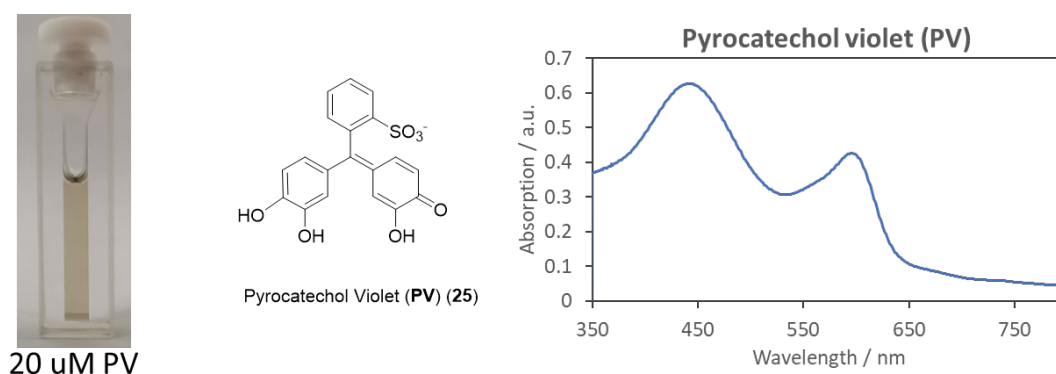


Figure 2.4. The absorbance spectra for pyrocatechol violet (**PV**).

Prior to assessing the dye-sensor binding, we took UV-Vis absorption spectra for both sensors **28** and **29** to see where our compounds absorb in the UV-Vis spectrum under our analysis conditions of HEPES buffer (5 mM), NaCl (145 mM) and pH at 7.4 in aqueous solution without Zn^{II} ion. C_{16} -picolyl (**28**) and C_4 -picolyl ((**Figure 2.5a**), (**29**) has a similar absorbance around 300 nm, but for sensor **28** (**Figure 2.5a**) a sharp, and higher intensity peak is observed. This could be due to sensor **28** self-assembling in solution.

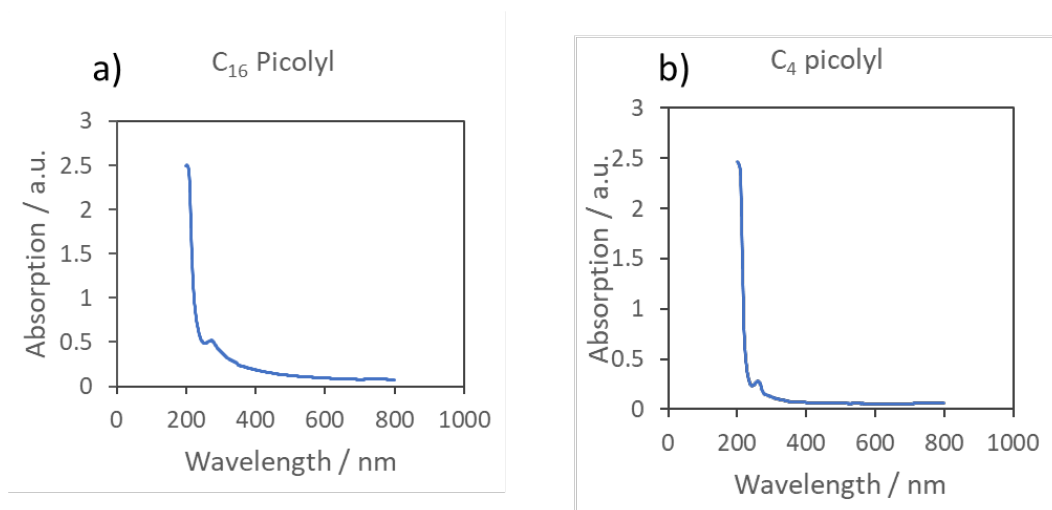


Figure 2.5. a) C₁₆-picolyl (**28**) without **PV** and Zn^{II} and b) C₄-picolyl (**29**) without **PV** and Zn^{II}

To begin with, amphiphile **28**, containing a di-picolylamine head group and a C₁₆ hydrophobic tail, was synthesised to investigate whether self-assembly could be used to effectively bind the colorimetric indicator **PV**. The addition of amphiphile **28**·Zn^{II} to a solution of 20 μM of **PV** in aqueous HEPES buffer (5 mM HEPES, 145 mM NaCl, pH 7.4) was found to induce a distinctive colour change from yellow to blue. This change is typically attributed to the binding of the catechol moieties in **PV** to two metal ions, generally fixed by ligands upon a molecular scaffold^{Butler, 2012 #366}. For our current system, this would require the dipicolyl headgroups to be brought together into proximity, either by dimerization or by their self-assembly to form supramolecular structures. We began by looking at the spectral changes resulting from the addition of ligand **28** at increasing concentrations, to a solution of **PV**. **Figure 2.7** shows the clear changes in the absorbance spectrum of the **PV** dye upon the addition of ligand **28** to the system. A small boost in absorbance is seen for the peak at 600 nm, with a new strong peak forming at 700 nm. Joliffe et al. described an increase in the peak along with a bathochromic shift (with 5 mM HEPES, 145 mM NaCl, pH 7.4), and we detected a minimal shift in wavelength with increasing concentrations. From the changes in the absorption spectrum of **PV** upon incremental additions of amphiphile **28**, we observed saturation of binding at approx. 2 equivalents (**Figure 2.6**), which suggests good binding affinity, though not as

strong as previous examples reported by Jolliffe and co-workers.

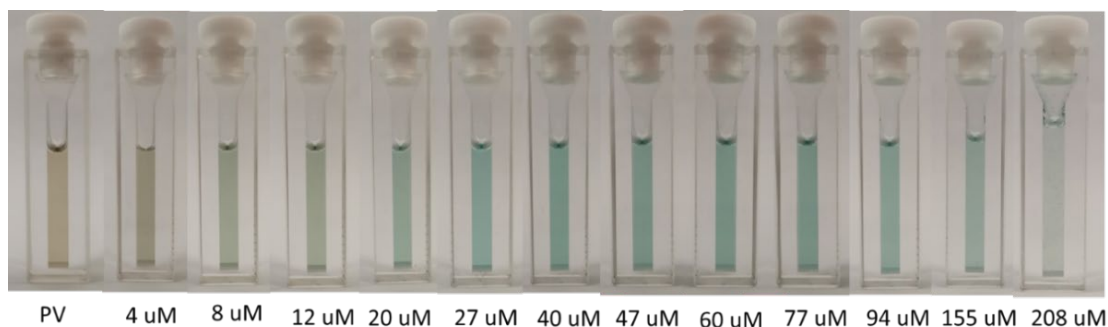


Figure 2.6. The colour of cuvette changes in absorbance of **PV** solution with increasing C_{16} -picoyl- Zn^{II} concentration until 208 μM .

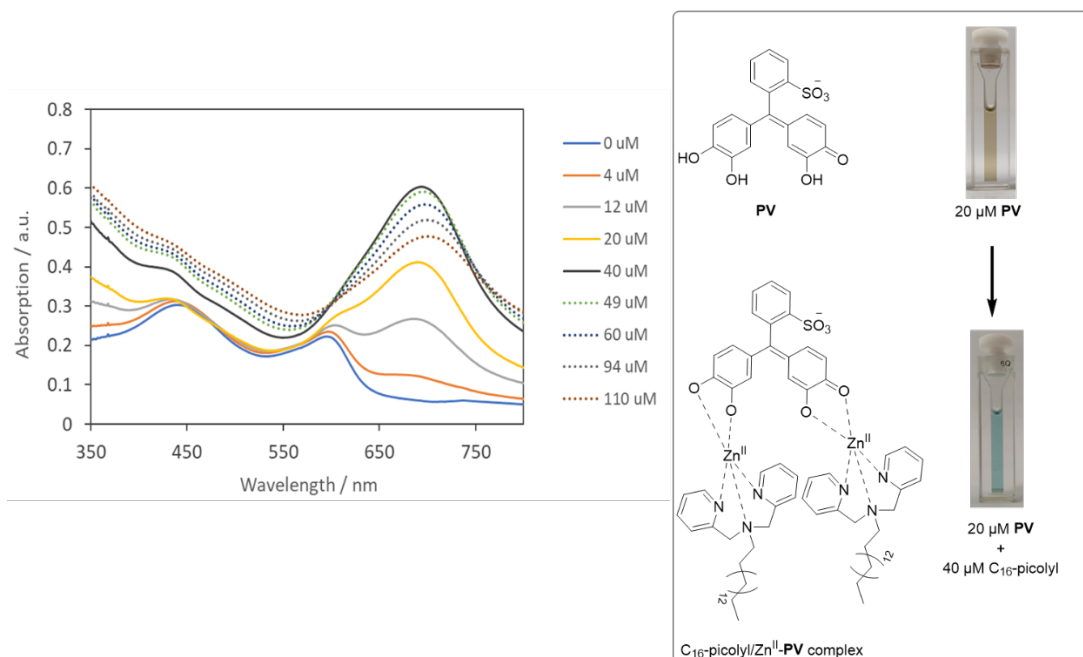


Figure 2.7. Changes in the absorbance of **PV** with increasing C_{16} -picoyl- Zn^{II} concentrations. Conditions: [HEPES buffer] = 5 mM, pH 7.4, [NaCl] = 145 mM, [**PV**] = 20 μM at 25 $^{\circ}C$. C_{16} -picoyl/ Zn^{II} concentrations = 0, 4, 12, 20, 40, 49, 60, 94 and 110 μM .

The rationale for this is that amphiphile C_{16} -picoyl- Zn^{II} (**28**· Zn^{II}) contains a long carbon chain, allowing this molecule to self-assemble in water. The dipicolylamine groups can bind Zn^{II} ions and when the sensor self-assembles, the zinc ions are brought close together

which allows for efficient binding to the catechol group of the dye. We observed that we needed two C₁₆-picolyl **28**·Zn^{II} ligands to bind one of the **PV** dyes. Jolliffe's system had a 1:1 binding of the sensor to **PV**, but they had two picolyl groups per sensor, so our results matched well. Control molecule **29** (C₄-picolyl·Zn^{II}) cannot self-assemble due to its much shorter hydrophobic chain. Therefore, we expect **28**·Zn^{II} to have stronger binding to **PV** than **29**·Zn^{II}, due to its ability to self-assemble.

NMR studies of **PV** and PPI were explored to aid our understanding of the interactions and binding mechanisms, conducted with **PV** and PPI in pH 7 buffer (5 mM HEPES, 145 mM NaCl, pH 7.4). These conditions were designed to induce a change in signal intensity when the sensor binds to the PV and the **PV** binds to the PPI. We prepared samples containing 1 eq. of **PV** with 2 eq. of **28**·Zn^{II} at pH 7.4 with HEPES buffer (5 mM) and NaCl (145 mM), all in an appropriate deuterated solvent, like D₂O. We studied **PV** (1 eq.) with PPIs (4 eq.) separately to observe any alternations in signal intensity or signal strengths and to detect the emergence of peaks that could suggest interactions between the substances and the creation of compounds. In this case, our observations indicated that both **PV** with PPI and **PV** itself gave the same signal intensity, indicating that **PV** does not bind with PPI (below **Figure 2.8**).

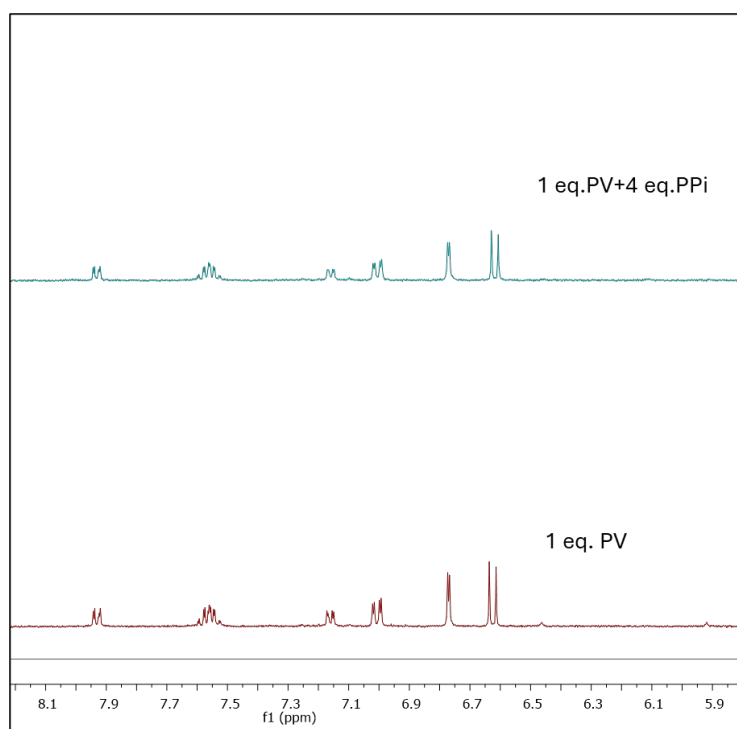


Figure 2.8. ^1H NMR spectra (400 MHz) with **PV** probe (below peak), and mixture of **PV** (1 eq.) and **PPI** (4 eq.) in HEPES buffer and pH = 7.4 in D_2O (above peak).

The **PV** (20 μM) chemical shifts were observed in the ^1H NMR spectrum from 6.5 to 8.5 ppm. With the addition of C_{16} -picolyl, the signal intensity reduces gradually, until at 40 μM of C_{16} -picolyl **28**, when we observe a completely flat line, indicating that **PV** (20 μM) has combined with the 40 μM of C_{16} -picolyl **28**. These two bind together when the C_{16} -picolyl **28** is added to the **PV** (20 μM) in the presence of HEPES 5 mM, a crucial component in the binding process. As a result, the chemical peak signal of **PV** disappeared at 40 μM of C_{16} -picolyl, indicating the formation of a **PV-C₁₆-picolyl complex** with a 1:2 ratio. A possible explanation for the observed NMR behaviour is that the addition of the C_{16} -picolyl ligand to the system induces a dynamic exchange process and/or the formation of larger supramolecular aggregates. As the amount of C_{16} -picolyl is increased, the ligand may interact with pyrocatecholate to form multiple species in equilibrium, causing the NMR signals to broaden due to chemical exchange on the NMR timescale. At approximately 2 equivalents of C_{16} -picolyl, this exchange may become most significant, resulting in severe peak broadening and the apparent disappearance of the resonances.

Therefore, the disappearance of the NMR peaks at 2 equivalents of C₁₆-picolyl is likely associated with the formation of dynamically exchanging or aggregated species in solution.

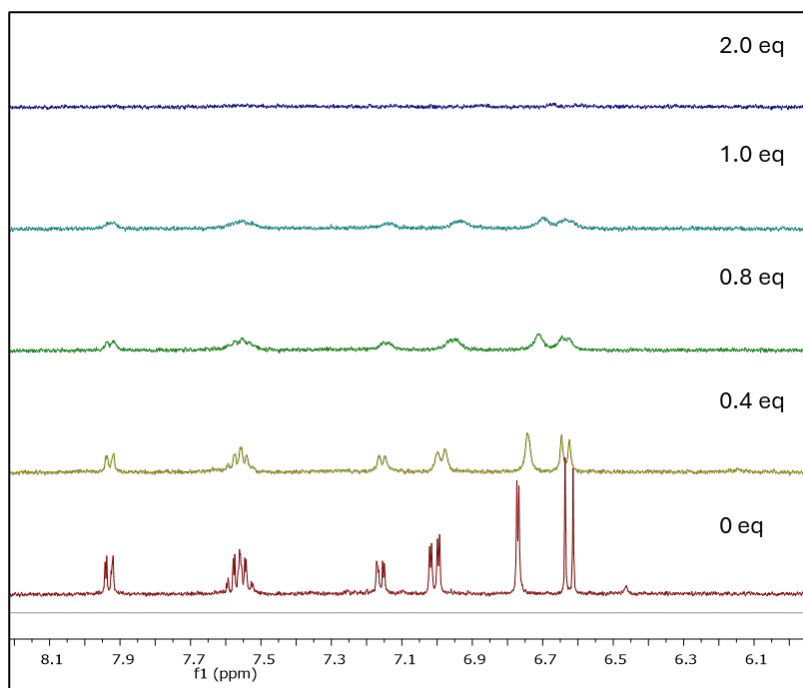


Figure 2.9. ¹H NMR spectra (400 MHz) from the probe **PV** and increasing the concentrations of C₁₆-picolyl·Zn^{II} = 0, 0.4, 0.8, 1.0, 2.0 equivalents in HEPES buffer and pH = 7.4 in D₂O.

2.3.4 Evidence for the formation of self-assembled structures

The next step is determining whether C₁₆-picolyl (**28**) or C₄-picolyl (**29**) are self-assembling or aggregating by monitoring with UV-visible spectroscopy. By increasing the concentrations of C₁₆-picolyl **28** (**Figure 2.10a**) and C₄-picolyl **29** (**Figure 2.10b**) individually from 0 to 204 μM, we observed that the baseline absorption of the solution for the C₁₆-picolyl sample increased with each addition, but not the solution containing C₄-picolyl. We concluded that C₁₆-picolyl aggregates or self-assembles, but the control molecule C₄-picolyl does not. It was also noticed that when C₁₆-picolyl was added in the absence of **PV**, turbidity was evident. For comparison, we also examined the corresponding control molecule, C₄-picolyl·Zn^{II}, there was no formation of the absorption

band at 700 nm, a result that underscores the crucial role of hydrophobic interactions in the formation of the self-assembled receptor.

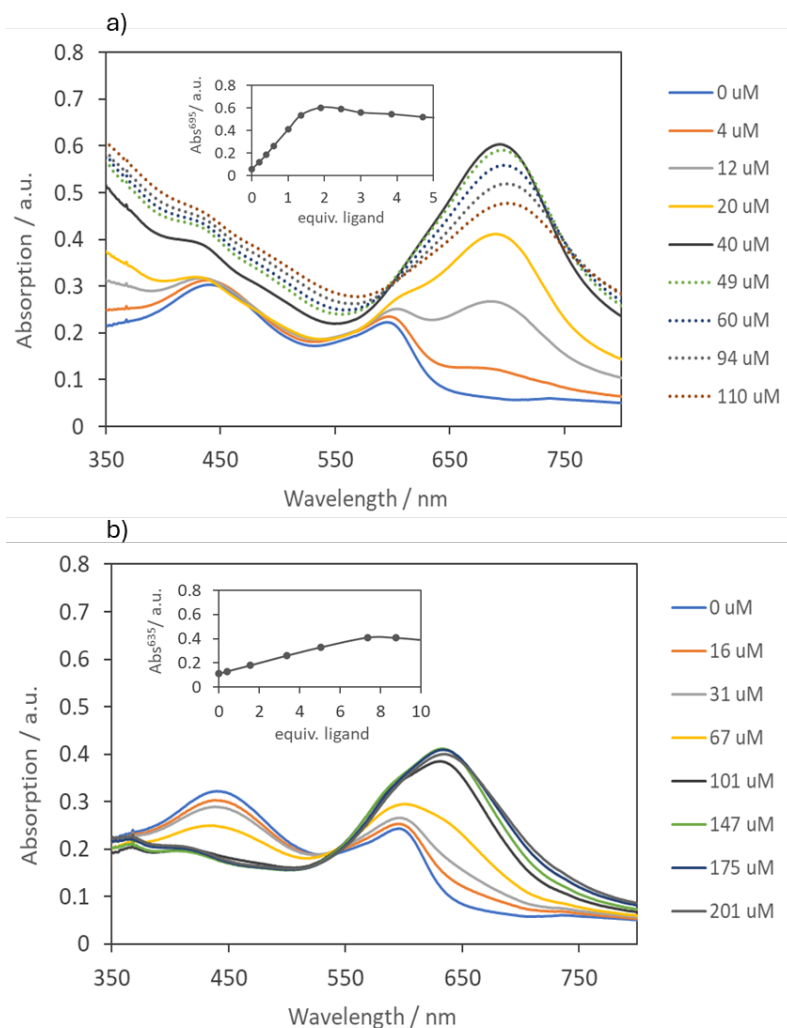


Figure 2.10. **a)** Changes in absorbance with increasing C₁₆-picolyl-Zn^{II} concentrations. Conditions: [HEPES buffer] = 5 mM, pH = 7.4, [NaCl] = 145 mM, [PV] = 20 μM at 25 °C. C₁₆-picolyl-Zn^{II} concentrations = 0, 4, 12, 20, 40, 49, 60, 94, and 110 μM. **b)** Changes in absorbance with increasing C₄-picolyl-Zn^{II} concentrations. Conditions: [HEPES buffer] = 5 mM, pH = 7.4, [NaCl] = 145 mM, [PV] = 20 μM at 25 °C. C₄-picolyl-Zn^{II} concentrations = 0, 16, 31, 67, 101, 147, and 201 μM.

We have previously shown that metal-chelating amphiphiles can form vesicular structures when they interact with strongly binding counterions, such as dialkyl phosphates, achieving critical aggregation concentrations as low as 10 μM. Further examination of

the $(\text{Zn}^{\text{II}})_2\text{PV}$ complexes formed under these conditions, using dynamic light scattering (DLS) and electron microscopy (EM), reveals that we are forming vesicular structures.

To confirm the formation of prominent vesicles of C_{16} -picolyl **28**, we employed dynamic light scattering (DLS), a modern particle characterization method used to determine size distributions.¹³⁷ With this technique, aggregates and particles dispersed in solution scatter light to varying extents. This scattering information enables us to determine the hydrodynamic diameter of the particles in the solution. These experiments were conducted in the presence of the **PV** indicator. We added 40 μM of C_{16} -picolyl **28** amphiphiles in the presence of 20 μM of **PV** and observed a monodisperse sample with an average hydrodynamic diameter of ~ 1000 nm (below **Figure 2.11a**). To confirm this observation, we examined these vesicles under a microscope to determine their size. The images of the C_{16} -picolyl $\cdot\text{Zn}^{\text{II}}$ vesicles in the microscope clearly show the presence of these large vesicles, of approximately 1 μm in diameter (**Figure 2.11b**)

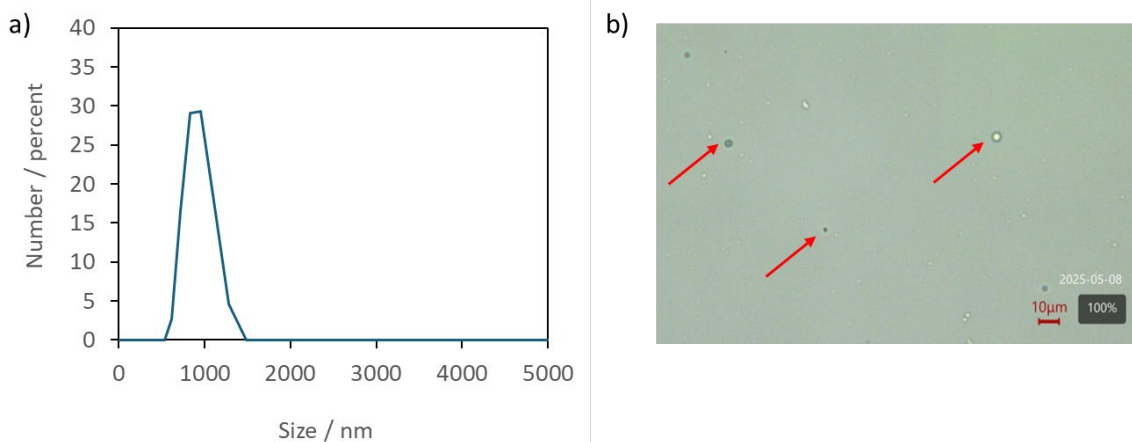


Figure 2.11. a) The hydrodynamic diameter of assemblies measured with dynamic light scattering (DLS) in the presence of **PV** (20 μM) for 40 μM C_{16} -picolyl $\cdot\text{Zn}^{\text{II}}$ system in aqueous HEPES. b) observed under a light microscopy. Conditions: [HEPES buffer] = 5 mM, pH = 7.4, [NaCl] = 145 mM, [**PV**] = 20 μM at 25 $^{\circ}\text{C}$.

To obtain more information on the nature of the aggregates formed and validate the DLS data for vesicle formation, we employed transmission electron microscopy (TEM). TEM also provided a significant finding in a solution of C_{16} -picolyl $\cdot\text{Zn}^{\text{II}}$ (40 μM) in the presence of **PV** (20 μM). The TEM results provided an explicit confirmation of the

presence of vesicular structures, not micelles, in the form of a big hollow circle, and these were observed to an average size of $\sim 300\text{-}700$ nm, which was in reasonably close agreement with the data obtained by DLS (**Figure 2.12**).

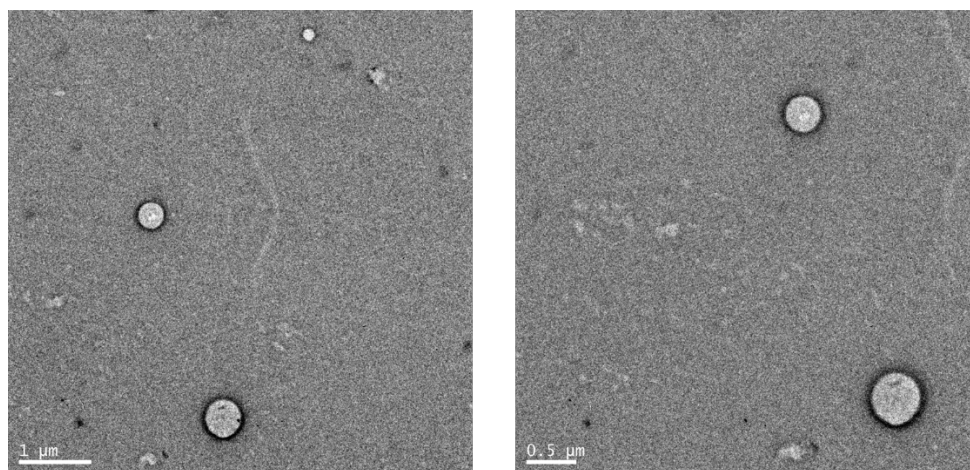


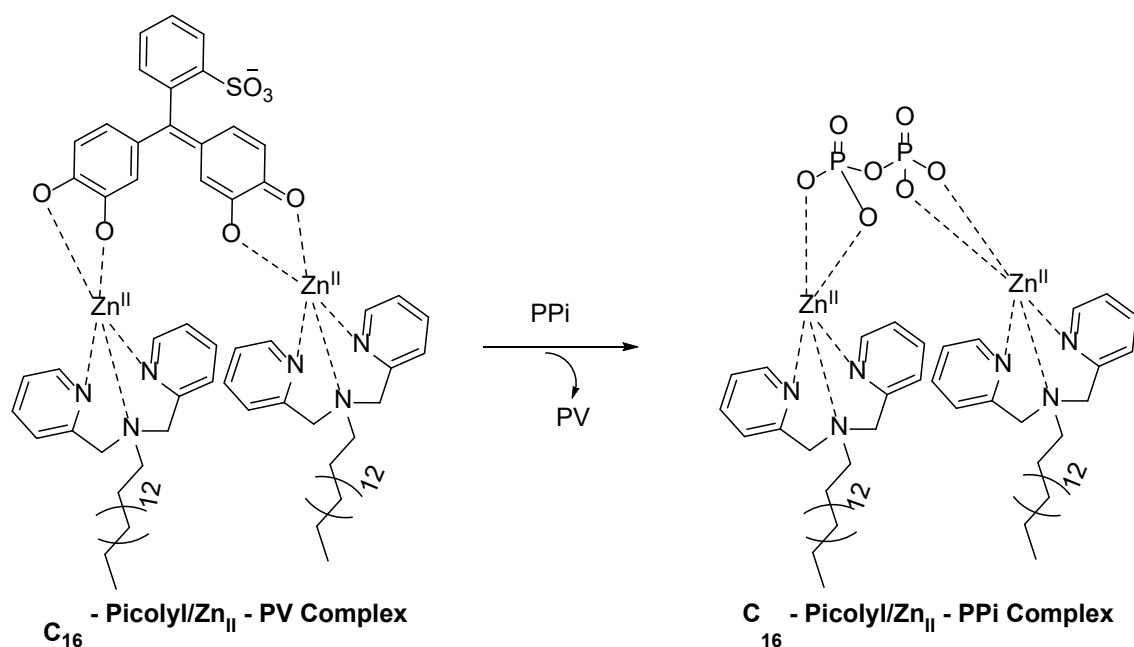
Figure 2.12. Representative TEM images of the vesicular structures of $\text{C}_{16}\text{-picolyl}\cdot\text{Zn}^{\text{II}}$ ($40\text{ }\mu\text{M}$) in HEPES solution. Conditions: [HEPES buffer] = 5 mM , $\text{pH} = 7.4$, $[\text{NaCl}] = 145\text{ mM}$, $[\text{PV}] = 20\text{ }\mu\text{M}$ at $25\text{ }^{\circ}\text{C}$.

2.3.5 Displacement of the indicator (PV) by PPi

From the above experiments, we established that our ligand had a strong binding affinity with the **PV** indicator. Next, we decided to investigate the effect of phosphate addition on the spectrum to determine if we could achieve a reversible change, similar to that observed in Jolliffe's system. For this, we examined the displacement of the indicator from the sensor in the presence of different phosphates, citrate, and inorganic ions. Our initial focus was on pyrophosphate and ATP, as similar indicators have been previously shown to respond most readily to these highly charged phosphates. Therefore, incremental additions of the phosphates were made to a 1:2 solution of the **PV** and $\text{C}_{16}\text{-picolyl}\cdot\text{Zn}^{\text{II}}$, and the absorbance was measured. With the addition of PPi and ATP, we detected minimal changes in the absorption spectrum, significant changes only at higher concentrations. Also, no colour change was observed until $500\text{ }\mu\text{M}$ of PPi and ATP were added individually. This indicated that there was weak binding of PPi and ATP and an

inability of these ions to displace the **PV** from the self-assembled complex.

Furthermore, we performed NMR studies at a 1:2 ratio of the C₁₆-picolyl-**PV** complex to determine whether PPI displaces it. We increased the concentrations of PPI in the C₁₆-picolyl-**PV** complex; however, we could not detect the **PV** peak until 10 equivalents, indicating that C₁₆-picolyl is not a suitable sensor for **PV**. These NMR experiments supported our UV experiments, providing crucial insights into the interaction of PPI and C₁₆-picolyl-**PV** complex (see **Scheme 2.3**).



Scheme 2.3. The complex formation of C₁₆-picoly-Zn^{II} - **PV** to complex formation of C₁₆-picoly-Zn^{II} - **PPI**.

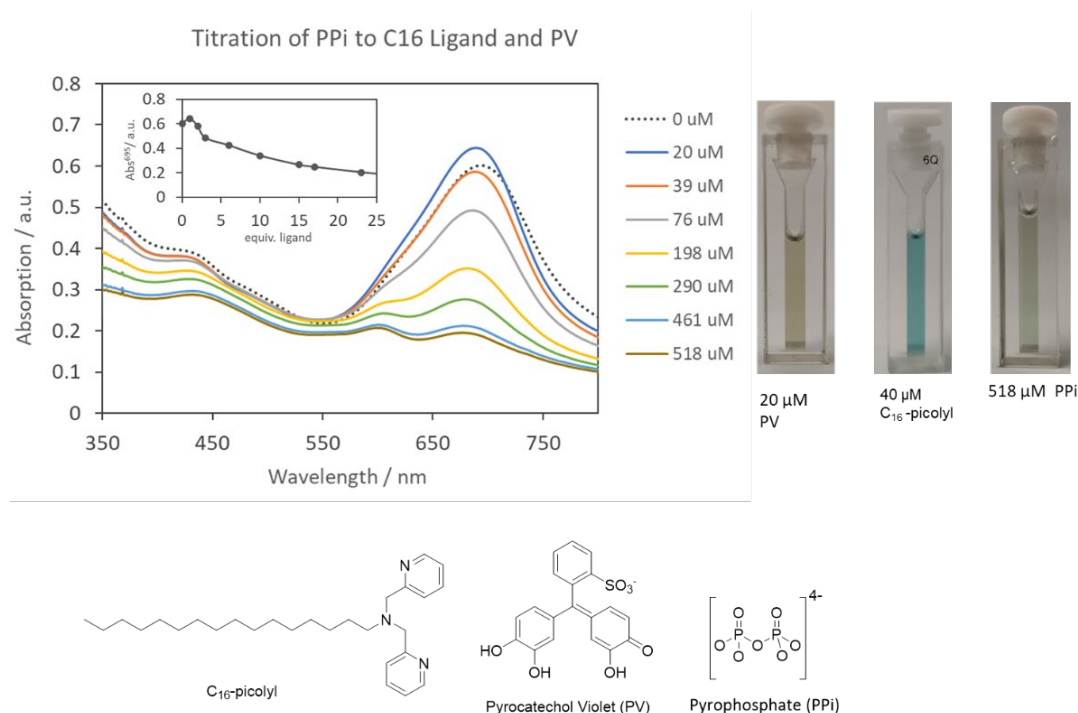
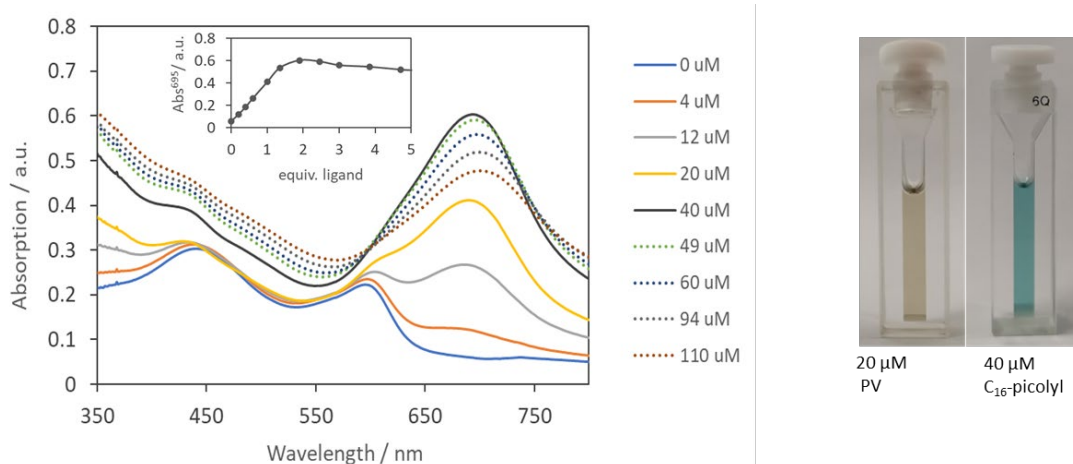


Figure 2.13. Absorbance spectra for the addition of PPI in the presence of C₁₆-picoly **28**. Conditions: [HEPES Buffer] = 5 mM, pH = 7.4, [NaCl] = 145mM, [PV] = 20 μ M, at 25 $^{\circ}$ C, [PV]: [C₁₆-picoly $\cdot$ Zn^{II}] = 1:2, [PPI] = 0, 20, 39, 76, 198, 290, 461 and 518 μ M.

Next, we investigated the behaviour of our control molecule, C₄-picoly **29**, to gain insight into the role of self-assembly. As in the previous example, we began by assessing the binding interaction between the indicator and ligand. **Figure 2.14** shows each ligand's absorbance spectra for PV titration. Notably, the absorption of PV changes dramatically upon the addition of C₁₆-picoly $\cdot$ Zn^{II} but with the addition of the control molecule C₄-picoly $\cdot$ Zn^{II} changes but requires slightly more PV. PV is a catechol-type indicator that requires two zinc ions to bind the catechol group. When binding occurs, a colour change is expected to be observed. In our system, a colour change is detected upon adding 40 μ M of the C₁₆-picoly $\cdot$ Zn^{II} sensor, from a dark yellow to blue (**Figure 2.14a**), at the expected ratio of 1:2 indicator/ligand. In this case, with the control C₄-picoly $\cdot$ Zn^{II} **29** (**Figure 2.14b**), we did not observe colour change until the addition of five equivalents of the ligand, which suggests much weaker binding. These results suggest that the presence of long hydrophobic chains has an important effect in inducing the self-assembly of the

supramolecular host, resulting in more effective binding with the **PV** indicator.

a)



b)

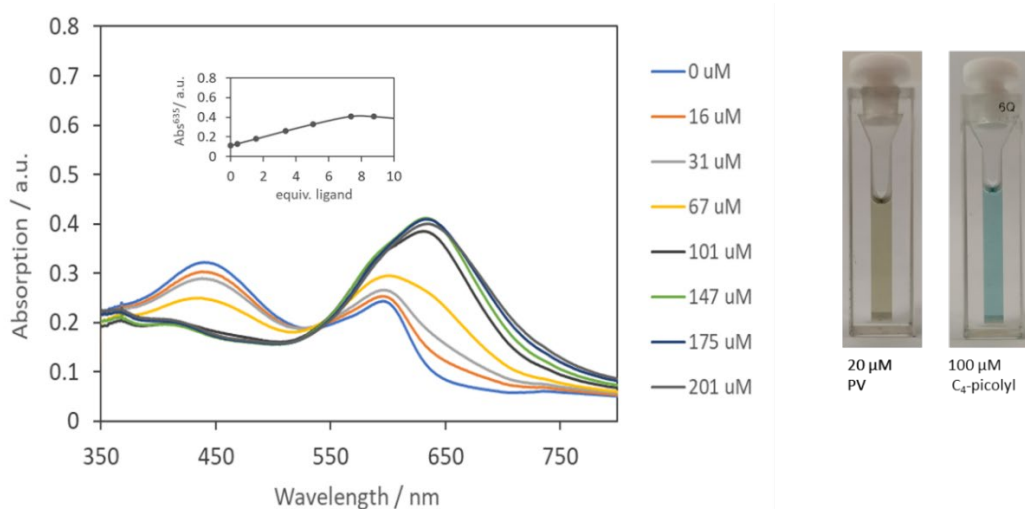


Figure 2.14. a) Changes in absorbance of **PV** solution with increasing C_{16} -picoyl· Zn^{II} concentrations until 110 μM. Conditions: [HEPES buffer] = 50 mM, pH 7.4, [NaCl] = 145 mM, [**PV**] = 20 μM at 25 °C. C_{16} -picoyl concentrations = 0, 4, 12, 20, 40, 49, 60, 94, and 110 μM. b) Changes in absorbance of **PV** solution with increasing C_4 -picoyl· Zn^{II} concentration until 201 μM. Conditions: [HEPES buffer] = 5 mM, pH 7.4, [NaCl] = 145 mM, [**PV**] = 20 μM at 25 °C. C_4 -picoyl concentrations = 0, 16, 31, 67, 101, 147, 175, and 201 μM.

Displacement of the indicator from the 1:5 **PV**/ C_4 -picoyl· Zn^{II} system was performed by titrating increasing amounts of pyrophosphate (**PPI**) and adenosine triphosphate (**ATP**) into the mixture. The objective was to determine whether these highly charged phosphates

could displace the **PV** dye from $C_4\text{-picolyl}\cdot\text{Zn}^{\text{II}}$ and cause a reversible change in the absorption spectrum, similar to that observed in Jolliffe's system. Upon adding PPI to the five equivalents of $C_4\text{-picolyl}\cdot\text{Zn}^{\text{II}}$ with **PV**, we required three equivalents to displace the **PV**, indicating a 1:3 ratio. The significant colour changes from blue to yellow upon displacement of PV by PPI underscores the importance of our findings. In contrast, for ATP, five equivalents were needed to displace the **PV**, resulting in a 1:5 ratio. Additionally, ADP did not alter the colour from blue to yellow, maintaining a light blue colour. The peak didn't return to its original level, even after the ratio of **PV** to ADP was 1:20. Citrate required seven equivalents to change the colour from blue to yellow. Still, it did not reach the original height of the peak, requiring a ratio of 1:7 to observe a colour change. PPI, ATP, ADP, and citrate were all not able to effectively displace **PV** from the $C_4\text{-picolyl}\cdot\text{Zn}^{\text{II}}$ complex.

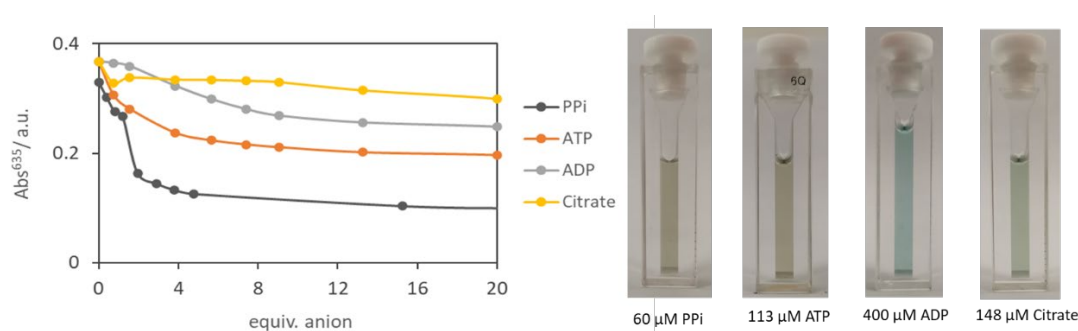


Figure 2.15. Absorbance spectra for the displacement of **PV** with other phosphate anions such as PPI, ATP, ADP, and citrate in the presence of $C_4\text{-picolyl}\cdot\text{Zn}^{\text{II}}$ at 100 μM . Conditions: [HEPES buffer] = 5 mM, pH 7.4, [NaCl] = 145 mM, [**PV**] = 20 μM at 25 °C. The ratios of [PV:PPI] = 1:3, [PV: ATP] = 1:5.5, [PV:ADP] = 1:20, and [PV:Citrte] = 1:7.

2.3.6 Investigation of an alternative indicator bromopyrogallol red (BPR)

The above studies show that self-assembly plays a role in the sensor's ability to bind **PV**. However, we were not able to efficiently displace the dyes from our sensor using PPI and

ATP, which suggests that the binding of our sensor to the **PV** is too strong. Therefore, we decided to examine the binding between our sensor and another dye, bromopyrogallol red (**BPR**). **BPR** exhibits a characteristic absorbance peak in the visible light range, typically between 500 and 600 nm which often occurs in the presence of specific analytes or under certain conditions containing HEPES buffer (5 mM), pH at 7.4 and NaCl (145 mM) in aqueous solution (**Figure 2.16** below) in chemo sensing systems. We observed that the colour of the cuvette is pink in the aqueous solution. This information can be valuable for designing chemosensing systems with improved sensitivity and selectivity for detecting specific analytes based on visual colour changes and spectral absorbance properties.

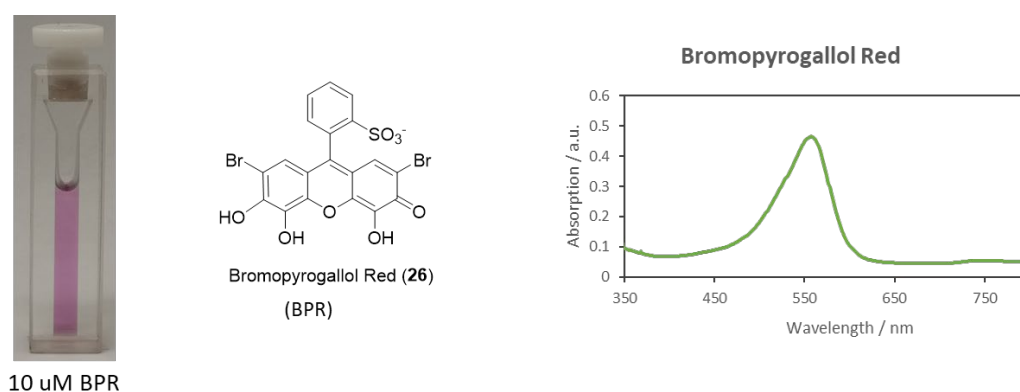


Figure 2.16. The absorbance spectra for **BPR** = 10 μ M and HEPES buffer (5 mM), pH at 7.4 and NaCl (145 mM) in aqueous solution at 25 $^{\circ}$ C.

Bromopyrogallol red (**BPR**) is another catechol-type dye due to its strong absorption properties and high sensitivity to environmental changes, which enables easy visual detection. **BPR** can also be used in various types of IDAs targeting different analytes, such as metal ions, anions, and biomolecules, making it a different and valuable tool in chemical sensing applications. **BPR** has been shown in the literature to bind to two zinc ions. {Beer, 2001 #56} Upon adding amphiphile C_{16} -picolyl- Zn^{II} to a solution of **BPR**, we observed significant changes to the UV spectrum and a colour change from pink to blue at 20 μ M (**Figure 2.17**). The peak at 558 nm underwent a bathochromic shift to 600 nm. This indicated efficient binding of our C_{16} -picolyl- Zn^{II} to **BPR**, which could be fitted to a 1:2 model of **BPR** and C_{16} -picolyl- Zn^{II} .

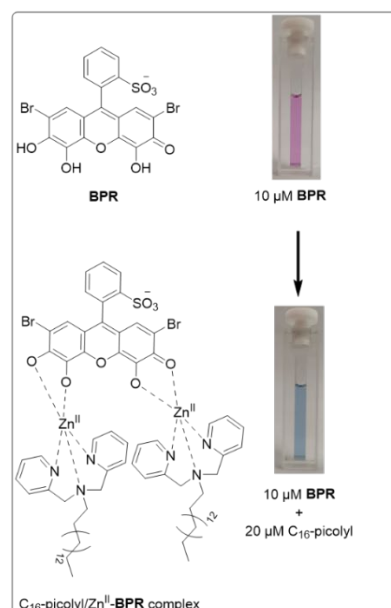
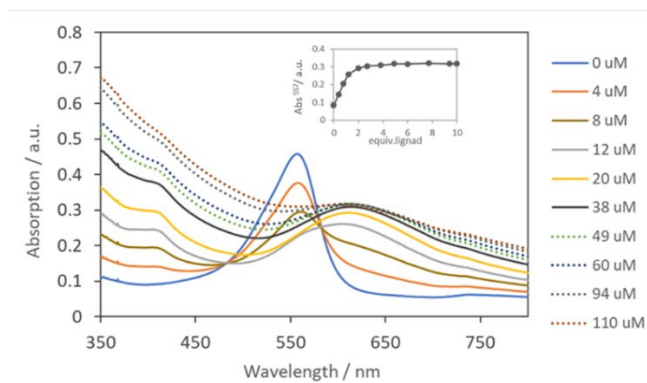


Figure 2.17. Absorbance spectra for the addition of C₁₆-picolyl. Conditions: [HEPES buffer] = 5 mM, pH = 7.4, [NaCl] = 145 mM, [BPR] = 10 μM, C₁₆-picolyl concentrations = 0, 4, 8, 12, 20, 38, 49, 60, 94, and 110 μM. at 25 °C.

There appeared to be a difference in how the compounds C₁₆-picolyl·Zn^{II} and C₄-picolyl·Zn^{II} scatter or absorb light when examined side by side. The absorbance of ligand C₁₆-picolyl·Zn^{II} was consistently higher, which could indicate its ability to assemble into supramolecular structures that scatter light and cause haziness, see **Figure 2.18** C₁₆-picolyl·Zn^{II} below. The change in **BPR** absorption was observed when compound C₁₆-picolyl·Zn^{II} was added and did not happen with reference compound C₄-picolyl·Zn^{II}. This underscores the importance of self-assembly in receptor formation.

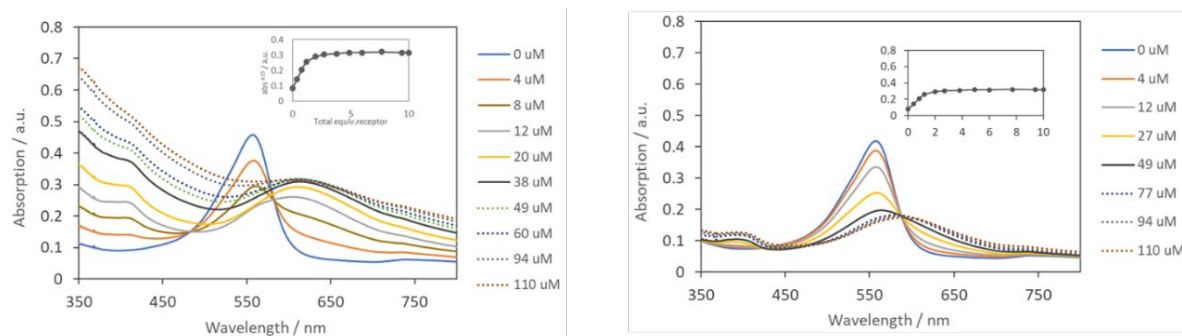


Figure 2.18. Absorbance spectra for the addition of increasing concentrations of C₁₆-picolyl·Zn^{II} (left) and C₄-picolyl·Zn^{II}. Conditions: [HEPES buffer] = 5 mM, pH = 7.4, [NaCl] = 145 mM, [BPR] = 10 μM, 1:2 ratio PV:[C₁₆-picolyl·Zn^{II}] and 1:5 ratio PV:[C₄-picolyl·Zn^{II}] at 25 °C.

Next, we looked into how the **BPR** dye moved from our receptor when PPis were used well as the initial solution had 20 μM of $\text{C}_{16}\text{-picolyl}\cdot\text{Zn}^{\text{II}}$ and 10 μM of **BPR**, which had a light blue colour and then added PPI in increasing amounts to try to replace the **BPR** probe to get back to its original pink colour, but similar to what was observed with the **PV** dye, we saw that displacing the **BPR** with PPI was sluggish (as shown in **Figure 2.19**). We also examined the displacement of **BPR** by the C4-picolyl ligand using phosphates such as PPI, which are known to have higher affinity. However, the dye was only minimally displaced from the sensor.

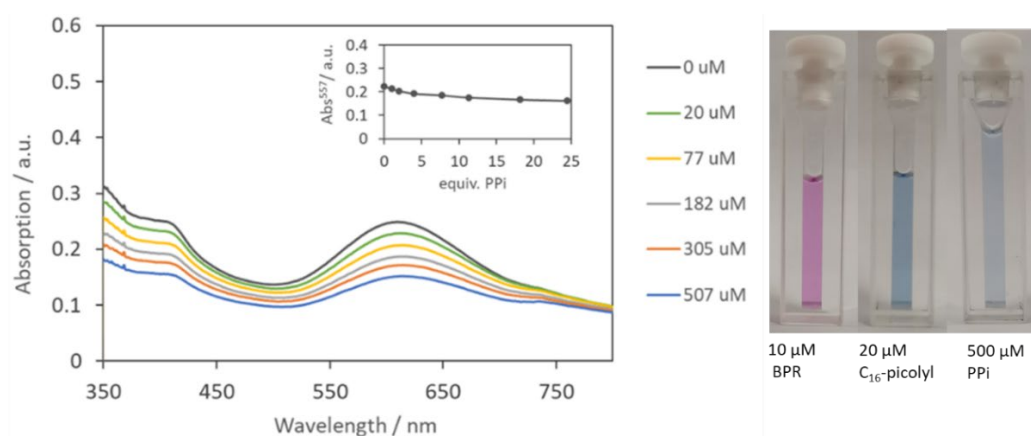


Figure 2.19: Absorbance spectra for the addition of PPI in the presence of $\text{C}_{16}\text{-picolyl}$ and Zn^{II} . Conditions: [HEPES Buffer] = 5 mM, pH = 7.4, [NaCl] = 145 mM, [BPR] = 10 μM , [BPR]: [$\text{C}_{16}\text{-picolyl}\cdot\text{Zn}^{\text{II}}$] = 1:2, [PPI] = 0, 20, 77, 182, 305, and 507 μM at 25 $^{\circ}\text{C}$.

Next, we examined the displacement of the indicator from the amphiphile $\text{C}_{16}\text{-picolyl}\cdot\text{Zn}^{\text{II}}$ in the presence of different phosphates, citrate and inorganic ions. Our initial focus was on pyrophosphate and ATP, as similar indicators have been previously shown to respond most readily to these highly charged phosphates. Therefore, incremental additions of the phosphates were made to a 1:2 solution of the **BPR**: $\text{C}_{16}\text{-picolyl}\cdot\text{Zn}^{\text{II}}$ and the absorbance was measured. For amphiphile $\text{C}_{16}\text{-picolyl}\cdot\text{Zn}^{\text{II}}$ with the addition of PPI, we detected changes in the absorption spectrum without significantly decreasing the peak at 557 nm to the ratio of 1:2 (**BPR** and amphiphile $\text{C}_{16}\text{-picolyl}\cdot\text{Zn}^{\text{II}}$). Also, we titrated with different phosphates, but none of them displaced **BPR** (**Figure 2.20**). This indicated there was

weak binding of PPI and an inability of these ions to displace the **BPR** from the self-assembled complex. In contrast, the addition of control molecule C_4 -picolyl·Zn^{II} to a solution of **BPR** resulted in a decreased signal at 550 nm, but without the appearance of the peak at 610 nm and a colour change. This result with control molecule C_4 -picolyl·Zn^{II} provided a clear contrast to the results with C_{16} -picolyl·Zn^{II}, highlighting the different responses of the indicators.

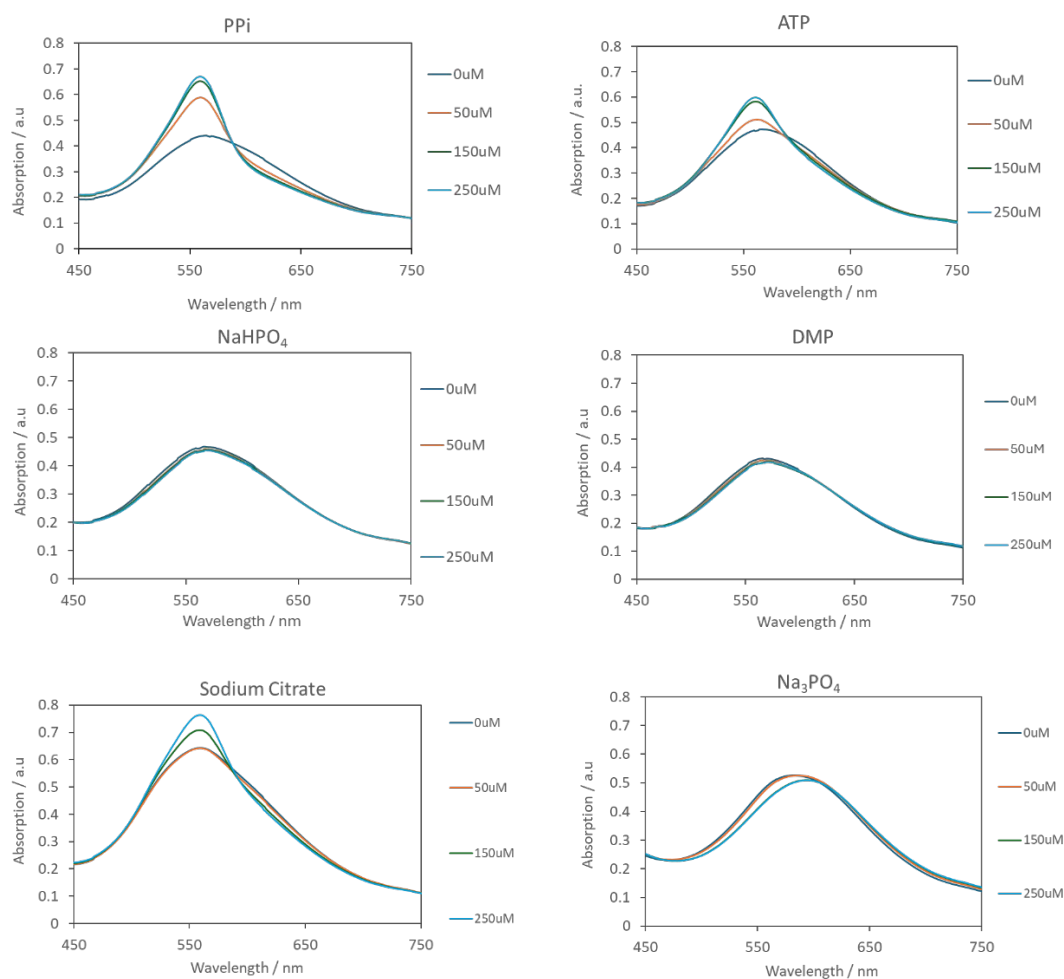


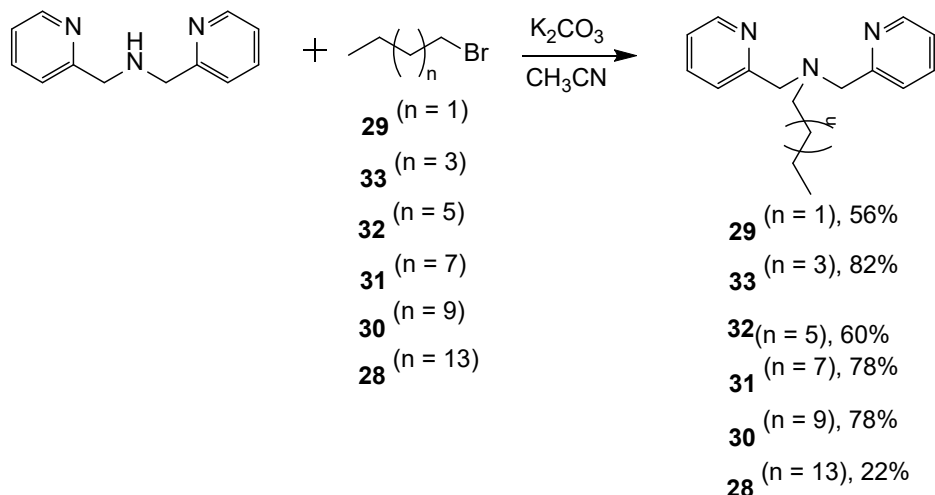
Figure 2.20. Absorbance spectra for the addition of different phosphates to amphiphile **44**. Conditions: [HEPES buffer] = 5 mM, pH 7.4, [NaCl] = 145 mM, [BPR] = 10 μ M, 1:2 ratio C_{16} -picolyl·Zn^{II} = 20 μ M, [phosphate] = 0, 50, 150 and 250 μ M at 25 $^{\circ}$ C.

2.4 Optimisation of the self-assembled receptor

2.4.1 Synthesis of the ligand analogues for the self-assembled host

Based on the results so far, it appears that modifying the length of the hydrophobic tail of ligand C₁₆-picolyl or adjusting the self-assembling conditions could increase its efficacy as a receptor for PPI or other ions of interest. We reasoned that by altering the length of the hydrophobic tail, we could tune the propensity for self-assembly and optimise the system in terms of both binding affinity to the indicator and therefore, the selectivity towards different phosphate analytes.

To investigate further how self-assembly can affect the effective formation of a supramolecular host, we synthesised the compounds C₁₂-picolyl **30**, C₁₀-picolyl **31**, C₈-picolyl **32**, and C₆-picolyl **33** by shortening the length of the hydrocarbon chain from C₁₆ to C₄ (**Scheme 2.4**). This series of picolyl compounds retains a head that functions as a chelating agent, allowing it to form complexes with metal ions or other desired substances. The amphiphilic nature of these molecules, with their hydrophobic tail and hydrophilic head group, is expected to facilitate their self-assembly into supramolecular structures such as micelles or vesicles in aqueous solutions. Such self-assembly would allow the formation of binding pockets involving two picolyl moieties, which have been shown to effectively complex indicators such as **PV**, and to bind target ions such as PPI.



Scheme 2.4. Synthesis of amphiphilic ligand C_n-picolyl amine.

2.4.2 Binding and displacement of pyrocatechol violet (PV)

First, we investigated the binding of C₁₂-picolyl **39** and **PV**. The binding isotherm was generated by titrating increasing concentrations of C₁₂-picolyl **39** into a buffered solution containing 20 μ M of **PV** (**Figure 2.21**). We observed that the binding isotherm was similar to that observed with amphiphile C₁₆-picolyl·Zn^{II}, with two equivalents of the ligand were required for the colour change of the indicator from yellow to blue-green. We were unable to obtain reliable fitting data to calculate binding constants due to the change in the baseline of the UV spectra upon the addition of increasing concentrations of C₁₂-picolyl **39**. This effect was more pronounced at higher concentrations of the ligand (see 100 μ M and above in **Figure 2.21**), and is attributed to the formation of supramolecular structures, likely vesicles, that are large enough to scatter light.

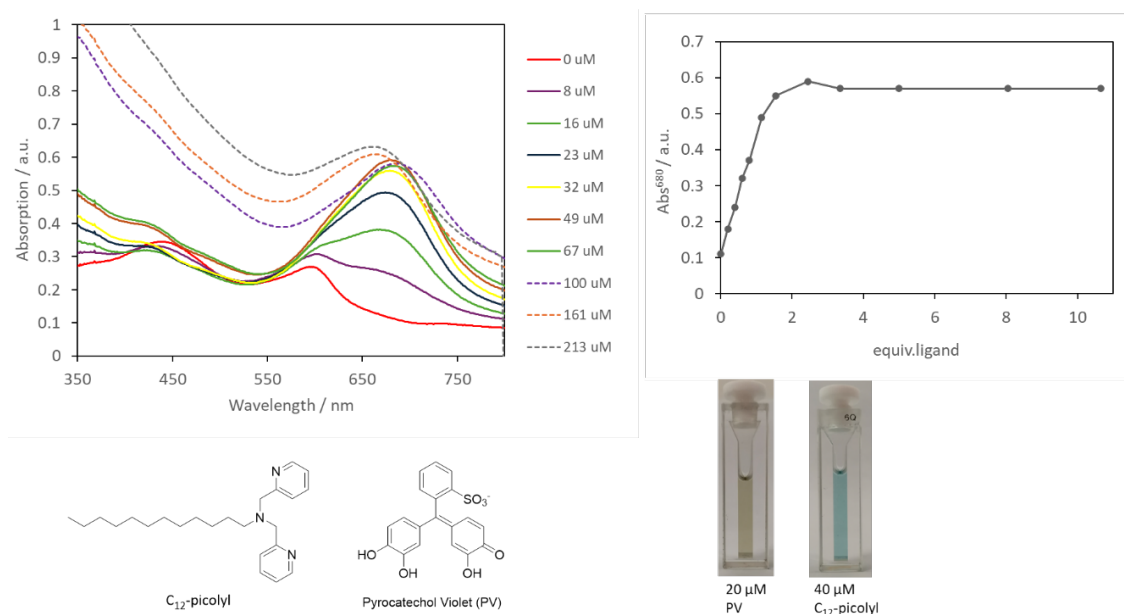


Figure 2.21. Changes in absorbance of **PV** solution with increasing C₁₂-picolyl·Zn^{II} concentration until 213 μ M. Conditions: [HEPES buffer] = 5 mM, pH 7.4, [NaCl] = 145 mM, [**PV**] = 20 μ M at 25 °C. [C₁₂-picolyl·Zn^{II}] concentrations = 0, 8, 16, 23, 32, 49, 67, 100, 161, and 213 μ M.

To determine whether or not there were differences in binding behaviour of this ligand compared to C₁₆-picolyl·Zn^{II}, we next explored the displacement of the C₁₂-picolyl

39·Zn^{II}-PV complex with phosphates such as PPI and ATP. The addition of PPI resulted in a significant colour change from green blue to yellow at 296 μM discernible by the naked eye. The amount of PPI required for this colour change was significantly lower than that required in the C₁₆-picolyl·Zn^{II} system, which required 500 μM to obtain a greenish colour without complete conversion to a yellow solution. This observation suggests that the shortening the chain length from C₁₆ to C₁₂ significantly changed the binding affinity of the self-assembled host to **PV**, such that it was much easier for PPI to displace **PV** from the C₁₂-picolyl **39**/Zn^{II} complex. However, the amount of PPI required to observe this colour change was still very high, at approximately 15 equivalents, indicating that the binding affinity of the indicator to the host was still too strong (**Figure 2.22**). Further displacement studies were performed using ATP, which required approximately 20 equivalents for a colour change. The small difference in the ability of PPI and ATP to displace **PV** from the self-assembled host indicates that this system would not be an effective indicator for discriminating between these two species.

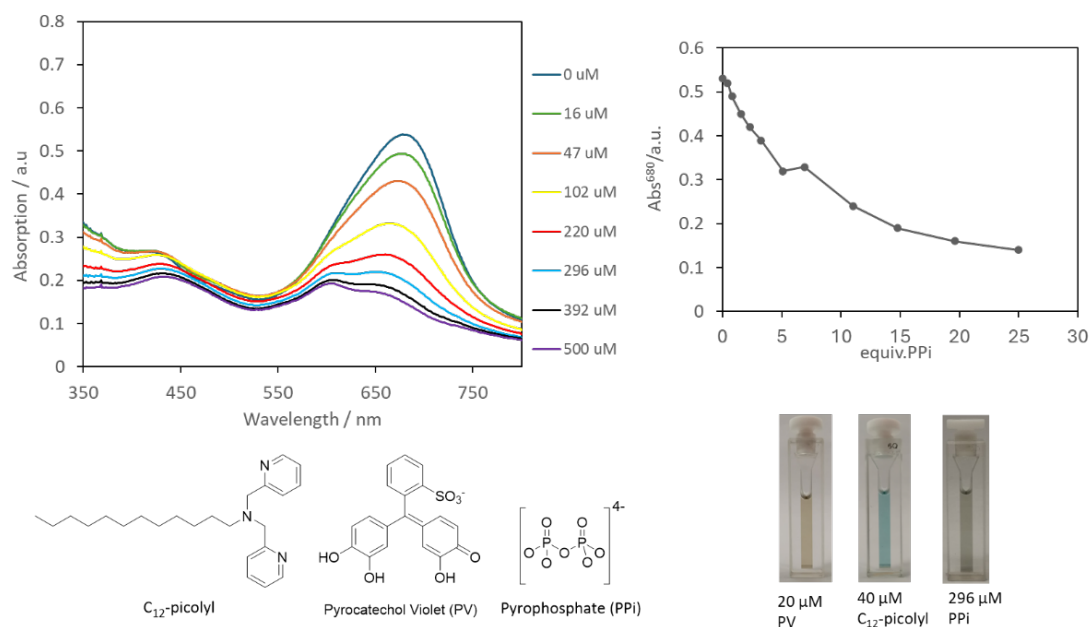


Figure 2.22. Absorbance spectra for the addition of PPI in the presence of C₁₂-picolyl **39**·Zn^{II}-PV. Conditions: [HEPES buffer] = 5 mM, pH = 7.4, [NaCl] = 145mM, [**PV**] = 20 μM , [C₁₂-picolyl **39**·Zn^{II}] = 40 μM , [PPI] = 0, 16, 47, 102, 220, 296, and 500 μM at 25 °C.

Next, we investigated the binding characteristics of a shorter analogue, C₁₀-picolyl ligand **38**. In the presence of indicator **PV** (20 μ M), we found that two equivalents of amphiphile C₁₀-picolyl·Zn^{II} were again required to induce a colour change in the solution, in line with what was observed with the longer analogues, and consistent with strong 1:2 binding between the **PV** and the picolyl moieties (**Figure 2.23**). Changes in the baseline of the absorption spectrum were again observed, which precluded the determination of binding constants using standard fitting models. In this case, we observe a decrease in absorption intensity (at 680 nm) at higher ligand concentrations, pointing to complex self-assembly phenomena that we could not explain. Importantly, strong binding with clear 1:2 stoichiometry was observed, which allowed us to conduct displacement studies using the analytes of interest.

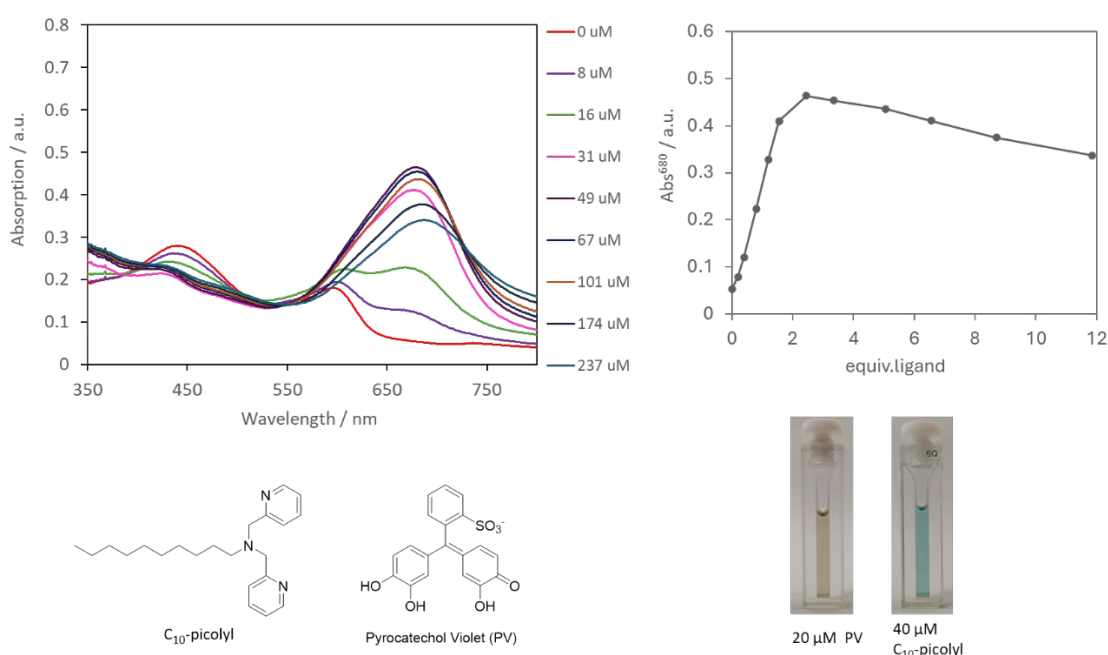


Figure 2.23. Changes in absorbance of **PV** solution with increasing C₁₀-picolylyl·Zn^{II} concentration until 213 μ M. Conditions: [HEPES buffer] = 5 mM, pH 7.4, [NaCl] = 145 mM, [**PV**] = 20 μ M at 25 $^{\circ}$ C. C₁₀-picolylyl·Zn^{II} concentrations = 0, 8, 16, 31, 49, 67, 101, 174, and 237 μ M.

The aim of the displacement studies was again to investigate whether these highly charged phosphates could displace the **PV** dye from the amphiphile C₁₀-picolylyl·Zn^{II} complex and bring about a reversible change in the absorption spectrum. It was observed that both the

addition of PPI and ATP were able to induce a reduction in the absorbance peak at 680 nm, and the reversal to a clear yellow solution, indicative of the presence of free **PV**. With this ligand, we found that 127 μM of PPI was required for this colour change, whereas for ATP, 227 μM was required (**Figure 2.25**). This is a significant reduction in the amount of analyte required for the displacement of **PV** when compared to C_{12} -picolyl- Zn^{II} and indicates that there is indeed an inversely proportional relationship between the length of the carbon chain in the amphiphile and the binding affinity of the amphiphile to **PV**. There is also now more difference between the concentration of PPI (6.4 eq.) and ATP (11.3 eq.) required to displace **PV**, indicating a greater degree of discrimination and positive steps towards a selective colorimetric indicator. Displacement experiments with ADP and citrate show that there was no evident effect on the 680 nm peak and no colour change after the addition of 20 equivalents of either analyte. These results further indicate that these self-assembled hosts have the potential to discriminate between different anions, with particular selectivity for PPI (**Figure 2.24**).

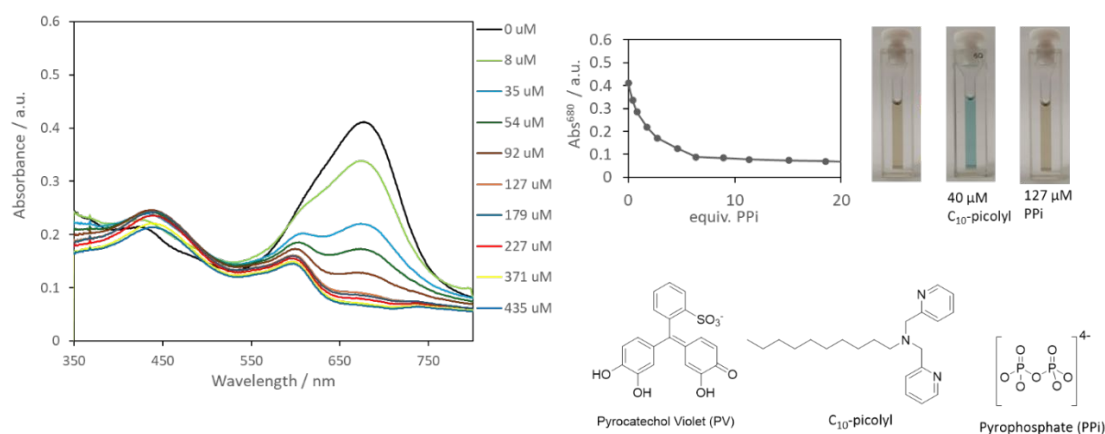


Figure 2.24. Absorbance spectra for the addition of PPI in the presence of C_{10} -picoly- Zn^{II} -**PV**. Conditions: [HEPES buffer] = 5 mM, pH = 7.4, [NaCl] = 145mM, [**PV**] = 20 μM , C_{10} -picoly **38**· Zn^{II}] = 40 μM , [PPI] = 0, 8, 35, 54, 92, 127, 179, 227, 371 and 435 μM at 25 $^{\circ}\text{C}$.

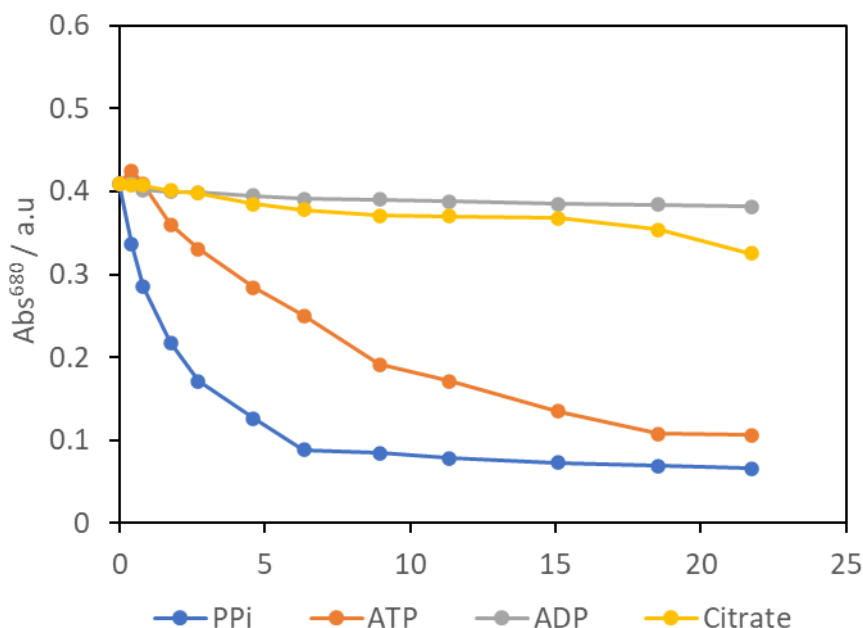


Figure 2.25. Absorbance spectra for the displacement of **PV** with the phosphate anions PPI, and ATP in the presence of C_{10} -picolyl·Zn^{II}. Conditions: [HEPES buffer] = 5 mM, pH 7.4, [NaCl] = 145 mM, [PV] = 20 μM, [C_{10} -picolyl·Zn^{II}] = 40 μM at 25 °C.

To further investigate the trend of decreasing binding affinity with shorter hydrophobic chains, we investigated the binding behaviour of C_8 -picolyl·Zn^{II} with **PV** (**Figure 2.26**). Upon titration of C_8 -picolyl·Zn^{II} to a solution of **PV**, it was noted that the colour change from yellow to blue green occurred at 40 μM of C_8 -picolyl·Zn^{II}, indicative of 1:2 binding **PV**/ C_8 -picolyl·Zn^{II} binding. However, from the binding isotherm, it can be seen that the absorption at 660 nm continues to increase at higher concentrations of C_8 -picolyl·Zn^{II}, plateauing at approximately 4-5 equivalents, suggesting that complete formation of C_8 -picolyl·Zn^{II}-**PV** complexes occur at higher concentrations. The binding isotherm also shows a much softer inflection point from 2-4 equivalents as the signal plateaus, as opposed to the much sharper turning points at 2 equivalents of ligand, seen for C_{12} -picolyl·Zn^{II} and C_{10} -picolyl·Zn^{II}. The much softer inflection point is an indication of the weaker binding of the C_8 -picolyl·Zn^{II} to **PV** and follows the trend of the binding affinity and displacement observed with this series of ligands.

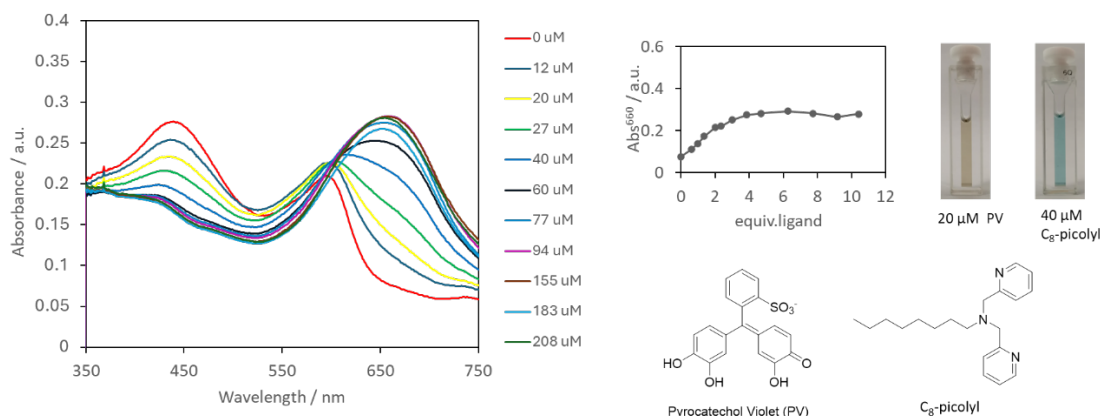


Figure 2.26. Changes in absorbance of **PV** solution with increasing C₈-picolyl·Zn^{II} concentration until 208 μM. Conditions: [HEPES buffer] = 5 mM, pH 7.4, [NaCl] = 145 mM, [**PV**] = 20 μM at 25 °C. C₈-picolyl·Zn^{II} concentrations = 0, 12, 20, 27, 40, 60, 77, 94, 155, 183, and 208 μM.

Fitting of the binding isotherm using BindFit v5.0, made available by Prof. Pall Thordarson and coworkers, allowed the determination of binding constants. We were able to perform fitting in this case, as we observed much less interference with the spectra upon the addition of increasing concentrations of the ligand. Fitting was performed using the UV 1:2 model (guest: host), using a constant concentration of the **PV** (guest) and increasing the concentration of the C₈-picolyl·Zn^{II} (host). While these binding isotherms are usually generated by a titration of the guest to a constant concentration of the host, this was not possible in our case as we were following changes in the UV absorption spectrum of the guest. It was therefore easier to vary the concentration of the host (C₈-picolyl·Zn^{II}), as this complex did not absorb in the spectral area of interest. Fitting of the binding isotherm obtained with 20 μM of **PV** into the 1:2 model revealed a low first association constant of 5.4 M⁻¹, and a much higher second association constant of 1.3 × 10⁷ M⁻¹. This is in line with expectations, showing very weak binding in the presence of a single ligand, and indicates that two equivalents of C₈-picolyl·Zn^{II} are required for effective binding to **PV**.

2.4.3 Confirmation of a 2:1 complex by NMR

The formation of the C₈-picolyl·Zn^{II}-**PV** complex was next examined using NMR experiments (**Figure 2.27**). C₈-picolyl·Zn^{II} was titrated into a solution of **PV** in D₂O buffered at pH = 7.4 using HEPES, and the complexation followed by examining the chemical shifts in the aromatic region (6.5 to 8.5 ppm). Following the addition of C₈-picolyl·Zn^{II}, the characteristic aromatic signals intensity slowly disappears, with complete disappearance at 2 equivalents of C₈-picolyl·Zn^{II} indicating a clear 1:2 binding ratio. Addition of excess C₈-picolyl·Zn^{II} then results in the appearance of the characteristic aromatic signals from the picolyl moiety, evident at 7.5, 8.0 and 8.6 ppm. The fact that these signals are not observed upon the addition of exactly two equivalents of C₈-picolyl·Zn^{II} indicates that this ligand was fully complexed with **PV** at that ratio, further supporting strong 2:1 binding.

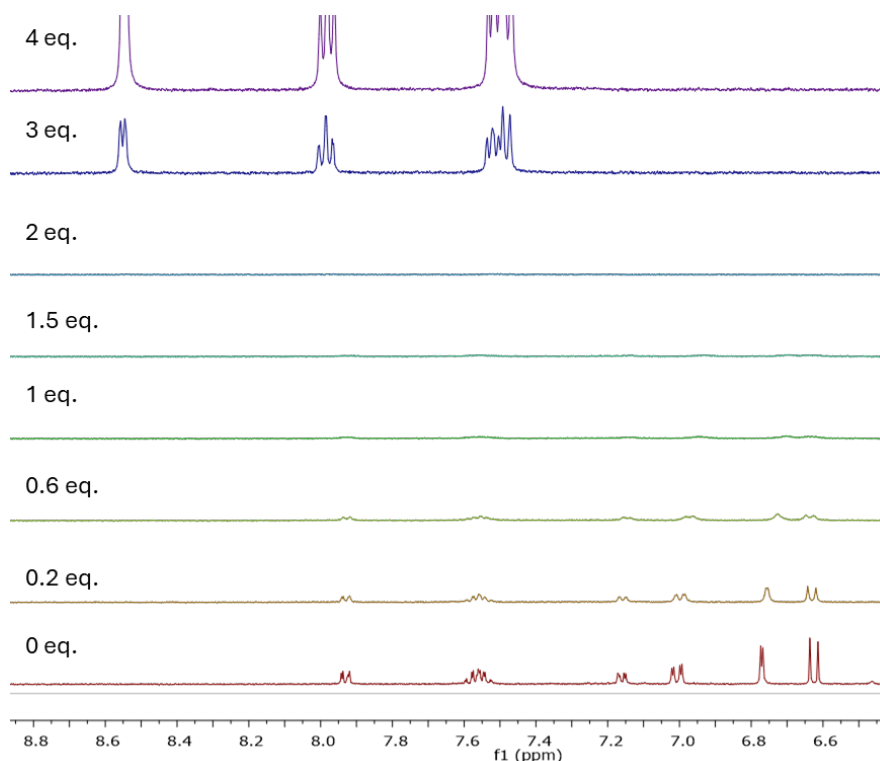


Figure 2.27. ¹H NMR spectra (400 MHz) of the indicator **PV** upon addition of increasing concentrations of C₈-picolyl **37**·Zn^{II}, at concentrations of 0, 0.2, 0.6, 1.0, 1.5, 2.0, 3.0, and 4.0 equivalents (bottom to top) in D₂O buffered with HEPES at pH = 7.4.

Displacement of the **PV** indicator from C₈-picolyl **37** was then attempted using PPI (**Figure 2.28**). The addition of increasing concentrations of PPI resulted in the reappearance of the aromatic signals, which are evident upon addition of 0.5 equivalents of PPI. Under these conditions, four equivalents of PPI are needed to fully displace the **PV** from the C₈-picolyl ligands. These NMR titrations confirm the 1:2 stoichiometry of C₈-picolyl·Zn^{II} to **PV** and show that this binding is reversible and thus has the potential to be used in an indicator displacement assay.

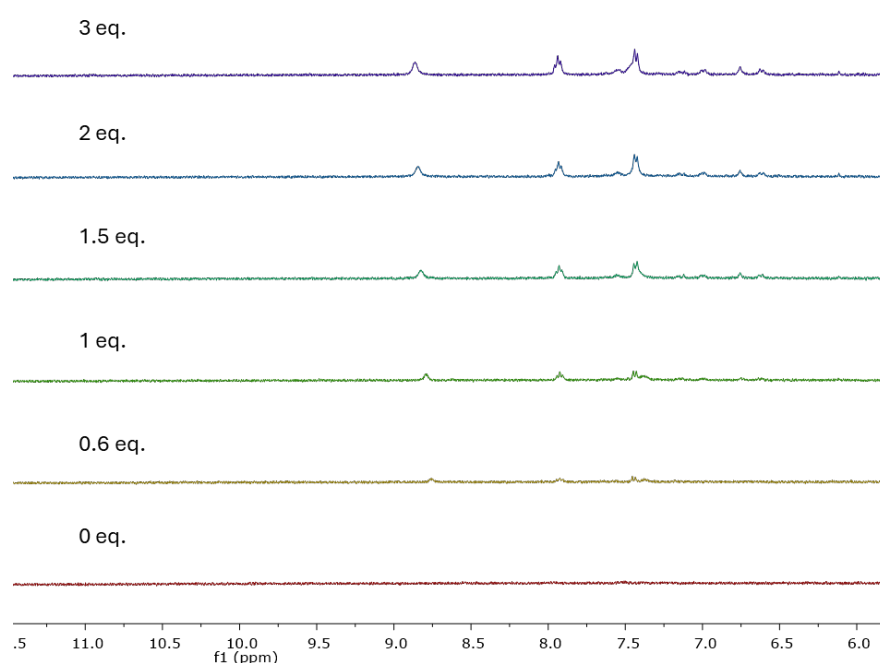


Figure 2.28. Increasing the concentration of PPI at 40 μ M C₈-picolyl·Zn^{II} **37** in the presence of 20 μ M **PV**, HEPES = 5 mM, and D₂O. Increasing the equivalents of PPI = 0, 0.5, 1.0, 1.5, 2.0, and 3.0 equivalents (bottom to top).

2.4.4 Characterisation of the self-assembled aggregates

To confirm the formation of aggregates of C₈-picolyl **37**, we measured the average size of the formed structures by dynamic light scattering (DLS), a technique commonly used for determining the size distribution of small particles. Aggregates and particles dispersed

in solution scatter light to different extents, and the scattering information can be used to determine the hydrodynamic diameter of the particles in solution. These experiments were performed in the presence of **PV** and C₈-picolyl·Zn^{II}, in a 1:2 ratio, in an aqueous solution containing HEPES buffer (5 mM) and NaCl (145 mM).

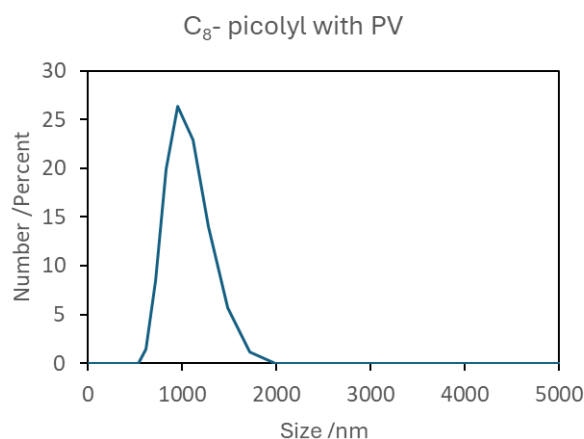


Figure 2.29. The hydrodynamic diameter of assemblies measured with dynamic light scattering (DLS) in the presence of **PV** (20 μ M) for the C₈-picolyl **37** system in aqueous HEPES buffer pH 7.4, NaCl = 145 mM, [C₈-picolyl·Zn^{II}] = 40 μ M at 25 $^{\circ}$ C.

Interestingly, we found that a solution of C₈-picolyl **37** (40 μ M) in the presence of **PV** (20 μ M) formed structures with a hydrodynamic diameter of 732 nm $\pm$ 164 nm (**Figure 2.29**). We then examined these samples using transmission electron microscopy (TEM; **Figure 2.30**). Staining with uranyl acetate was used to provide sufficient contrast for visualisation. The observed black hollow circles confirmed the presence of vesicular structures rather than micelles, and these were observed with an average size of ~50-100 nm (**37**).

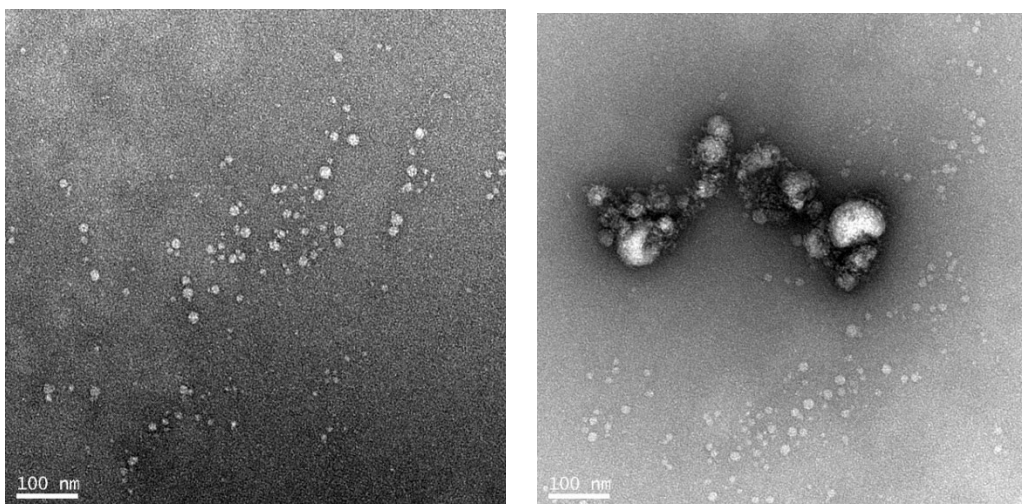


Figure 2.30. TEM data for the **PV** (20 μM), **C₈-picolyl 37** (40 μM) system in aqueous HEPES buffer pH 7.4, NaCl = 145 mM. Stained with uranyl acetate.

The CAC is the concentration of amphiphile at which aggregation occurs. Several methods for CAC determination have been published, including light scattering, electrical conductivity, surface tension, and spectrophotometric methods. While electrical conductivity and surface tension are straightforward for sample preparation, they may not be suitable for samples with low CAC values due to their low sensitivity. In contrast, spectrophotometric methods, known for their precision and high sensitivity, have emerged as a promising approach for CAC determination, even for values below 0.1 mM.

Fluorescence titration for the determination of the CAC was reported by Prazeres, who utilised **coumarin 40** as the fluorescence probe. This method plotted the shift in the emission intensity and its maximum wavelength against the addition of sodium dodecyl sulphate (**SDS**). The significant change in slope, a straightforward and clear indicator, indicated a change in the surroundings of **coumarin 40**, indicative of the aggregation of **SDS** amphiphiles in solution (**Figure 2.31**). Stuart and co-workers also reported a similar method using **Nile red** as the fluorescence probe.

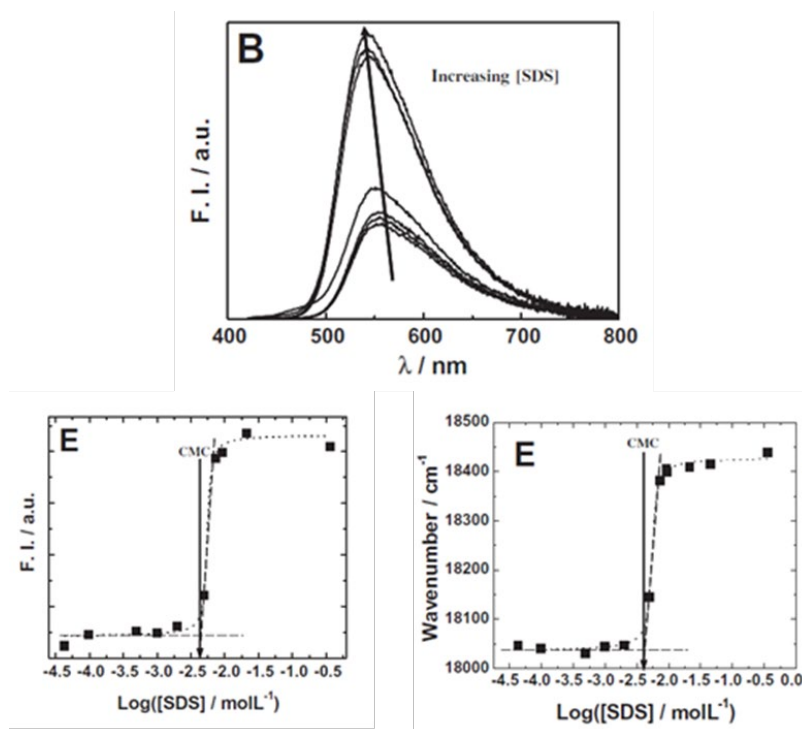


Figure 2.31. a) Fluorescence spectra of coumarin **40** in aqueous solutions containing SDS and concentration of SDS. Source: Adapted with permission from ref. 114. Copyright (2012) Elsevier Inc.

In this thesis, we determined the CAC of our amphiphiles of interest using fluorescence titrations. Our choice of Nile red as the fluorescent probe ($\lambda_{\text{ex}} = 550$ nm, $\lambda_{\text{em}} = 630$ nm in methanol) was due to its high sensitivity and the marked solvatochromic effect (see the structure of the Nile Red in **Figure 2.32**), which often results in marked changes in fluorescence intensity once aggregates are formed. Nile red, an apolar fluorescence probe, does not exhibit strong fluorescence in most polar solvents but becomes intensely fluorescent in hydrophobic environments. Upon formation of micelles or vesicles, Nile red is able to partition into the hydrophobic regions, where it is preferentially soluble, resulting in marked changes in its fluorescence characteristics. The CAC may be determined by measuring the change in fluorescence intensity at a fixed wavelength, or by following the changes in the emission maximum, which can shift significantly upon partitioning of Nile red into a hydrophobic environment. Furthermore, the low concentration of fluorophores required for CAC determination allows us to reasonably neglect the effect of the fluorescent probe on aggregation formation.

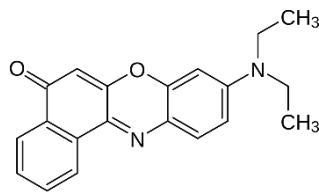


Figure 2.32. Chemical structure of Nile red

In practice, the CAC is determined by monitoring the changes in fluorescence intensity as amphiphiles are added to a solution containing Nile red. The CAC is usually the point where there is a change in the slope of a plot of the fluorescence vs amphiphile concentration. This is because the apolar Nile red probe becomes solubilised in the hydrophobic interior of the formed aggregates, causing significant and crucial changes in the fluorescence intensity.

To determine the CAC of C₈-picolyl **37**, increasing amounts of C₈-picolyl **37** was titrated into a HEPES buffer solution (pH 7.4) and NaCl (145 μ M), in the presence of **PV** (20 μ M) and Nile red (1 μ M) as a fluorescence probe. Adding a small amount of C₈-picolyl **37**·Zn^{II} to the solution increased fluorescence intensity at 600 nm (with an excitation at 550 nm), indicating the formation of micellar structures. Notably, the fluorescence intensity remained constant until the concentration reached 2.0 μ M, after which there was a sudden increase in intensity as the concentration of the amphiphile continued to rise. This change in slope, occurring between 4.5 and 5 μ M, estimates the amphiphile's CAC under these conditions. The CAC was estimated from the intercept of the trend lines by plotting the emission intensity against amphiphile concentration on a logarithmic scale (see **Figure 2.33** below). Using this method, we obtained a value of 7.15 μ M for the CAC.

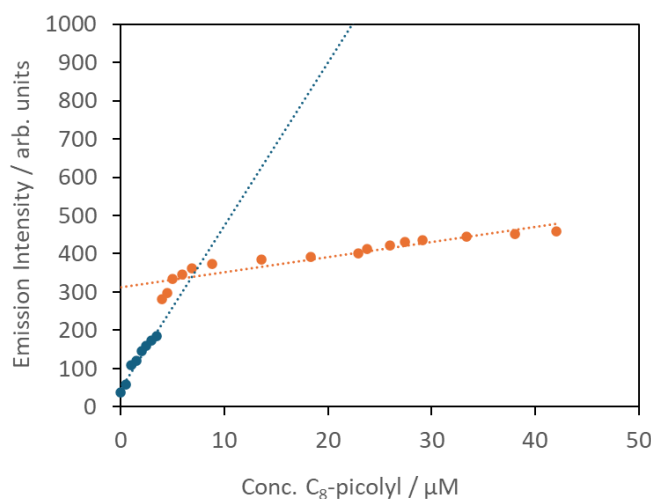


Figure 2.33. Emission intensity of Nile red (1 μM $\lambda_{\text{ex}} = 570 \text{ nm}$, $\lambda_{\text{em}} = 650 \text{ nm}$) at increasing concentrations of $\text{C}_8\text{-picolyl}\cdot\text{Zn}^{\text{II}}$ **37** in aqueous HEPES buffer solution (5 mM, pH 7.4).

2.4.5 Displacement of PV from the $\text{C}_8\text{-picolyl } \mathbf{37}\cdot\text{Zn}^{\text{II}}$ complex

Next, we examined the displacement of the indicator from the sensor $\text{C}_8\text{-picolyl } \mathbf{32}\cdot\text{Zn}^{\text{II}}$ in the presence of different phosphates. Our initial focus was on pyrophosphate and ATP, as similar indicators have been previously shown to respond most readily to these highly charged phosphates. Therefore, to a 1:2 solution of the **PV**: $\text{C}_8\text{-picolyl } \mathbf{37}/\text{Zn}^{\text{II}}$ complex, incremental additions of the phosphates were added, and the change in absorbance was measured. We detected a lot of changes in the absorption spectrum, with a significant decrease of the peak at 660 nm. This indicated that there was strong binding of PPi with 1:2 binding ratio of **PV** and PPi in **Figure 2.34**. We have performed some experiments with ATP, ADP and citrate. We observed that ATP (1:10) and other phosphates did not lead to the colour change from blue to yellow like PPi. This observation suggests an affinity towards PPi over other phosphates such as ATP, ADP and citrate indicating its potential to displace the **PV** from the self-assembled complex (below **Figure 2.35**).

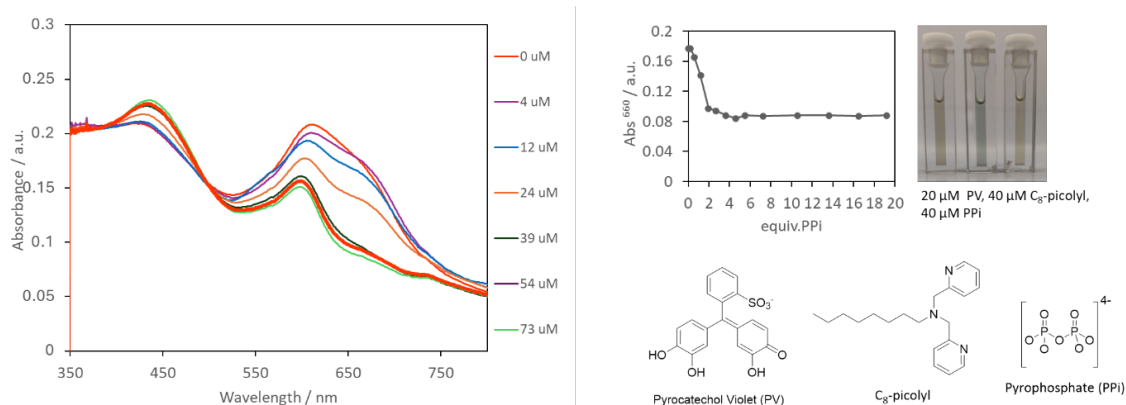


Figure 2.34. Absorbance spectra for the addition of PPI in the presence of C₈-picoly1 **37**/Zn^{II}-**PV**. Conditions: [HEPES buffer] = 5 mM, pH = 7.4, [NaCl] = 145 mM, [**PV**] = 20 μM, [**PV**]: [C₈-picoly1 **37**·Zn^{II}] = 40 μM, [PPI] = 0, 4, 12, 24, 39, 54, and 73 μM at 25 °C.

Figure 2.35 demonstrates that PPI displays superior selectivity compared to other phosphates, with a notable 1:2 ratio with C₈-picoly1·Zn^{II}, a distinction not shared by ATP and other phosphates. We observed that the displacement of ATP required 10 equivalents, and ADP required 15 equivalents. In contrast, citrate did not displace the **PV** until 20 equivalents, underscoring the exceptional selectivity of PPI among all other phosphates. The C₈-picoly1·Zn^{II} sensor is best suited for the displacement of **PV** by using PPI but no other phosphates. Our observation of C₈-picoly1·Zn^{II} UV absorbance is very low, so we cannot correctly determine whether these lower concentrations self-assemble or not. Therefore, we need to continue observing whether this sensor is a good sensor among the others.

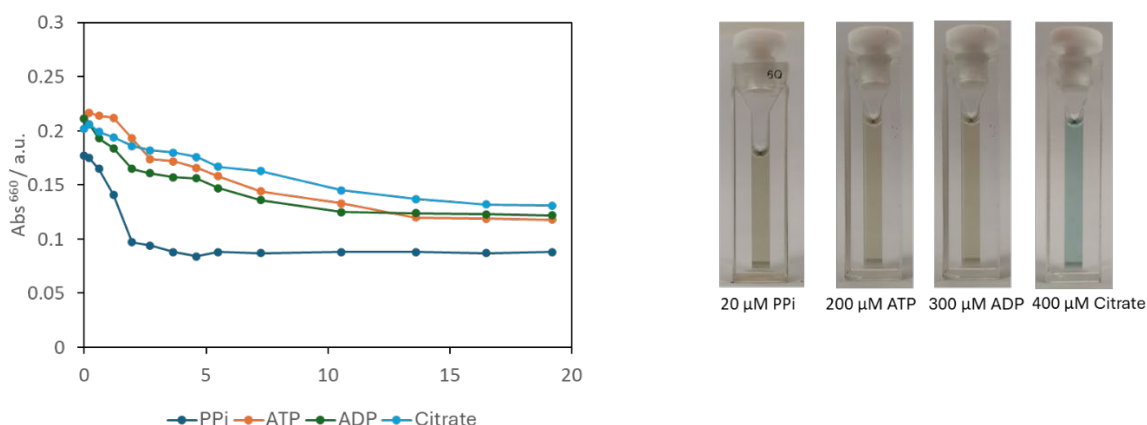


Figure 2.35. Absorbance spectra for the displacement of **PV** with other phosphate anions such as PPI, ATP, ADP and citrate in the presence of C_8 -picolyl- Zn^{II} [40 μ M]. Conditions: [HEPES buffer] = 5 mM, pH 7.4, [NaCl] = 145 mM, [**PV**] = 20 μ M at 25 $^{\circ}$ C.

2.4.6 Further optimisation of the **PV**- C_8 -picolyl 37/ Zn^{II} system

Due to the self-assembled nature of our system, we next investigated concentration-related effects that could influence the self-assembly of our host and therefore its binding to the indicator/analyte. While the NMR studies in **Section 2.4.3** indicated a clear 1:2 binding stoichiometry between C_8 -picolyl- Zn^{II} and **PV**, the curve from our UV titration of C_8 -picolyl- Zn^{II} to **PV** in **Section 2.4.2** did not indicate strong binding with 1:2 stoichiometry. We reasoned that this could be due to the large differences in concentrations of **PV** used in these two experiments, 100 μ M for the NMR titration, and 20 μ M for the UV titration. To investigate the effect of this concentration difference on binding, we first performed a titration with a fixed concentration of C_8 -picolyl- Zn^{II} (40 μ M) and added in increasing concentrations of **PV**, in HEPES = 5 mM and NaCl = 145 mM (**Figure 2.36**). The absorbance traces show graph below shows that at 20 μ M, the absorption trace has a maximum at 610 nm, due to the un-complexed **PV**, with a weaker peak at 672 nm, due to complexed **PV**. The peak at 672 nm becomes the maximum upon addition of 30 μ M of C_8 -picolyl- Zn^{II} and becomes stronger still at 40 μ M. These experiments suggests that at low concentrations of **PV**, more than 2 equivalents of C_8 -

picolyl·Zn^{II} are required to fully form the host-indicator complex.

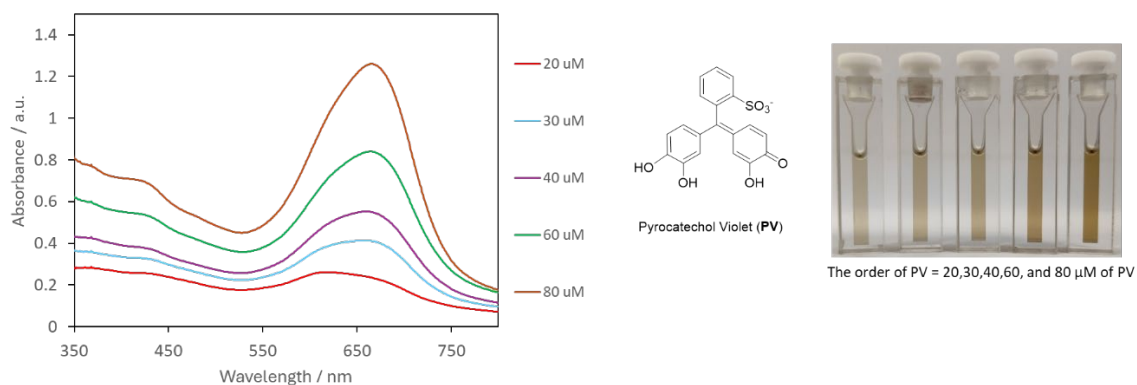


Figure 2.36. Increasing the concentration of **PV** with fixed 40 μM C₈-picolyl·Zn^{II} complex. The concentrations of **PV** = 0, 20, 30, 40, 60, and 80 μM at 25 °C.

We next performed further binding experiments beginning with 30 and 40 μM of **PV**, to compare with our initial titration which used 20 μM of **PV**. The below graphs show much tighter binding for the systems involving 30 and 40 μM of **PV** (**Figure 2.37**), indicating that the initial concentration of the ligands/indicator, have important consequences on the formation of the C₈-picolyl·Zn^{II}-**PV** complex.

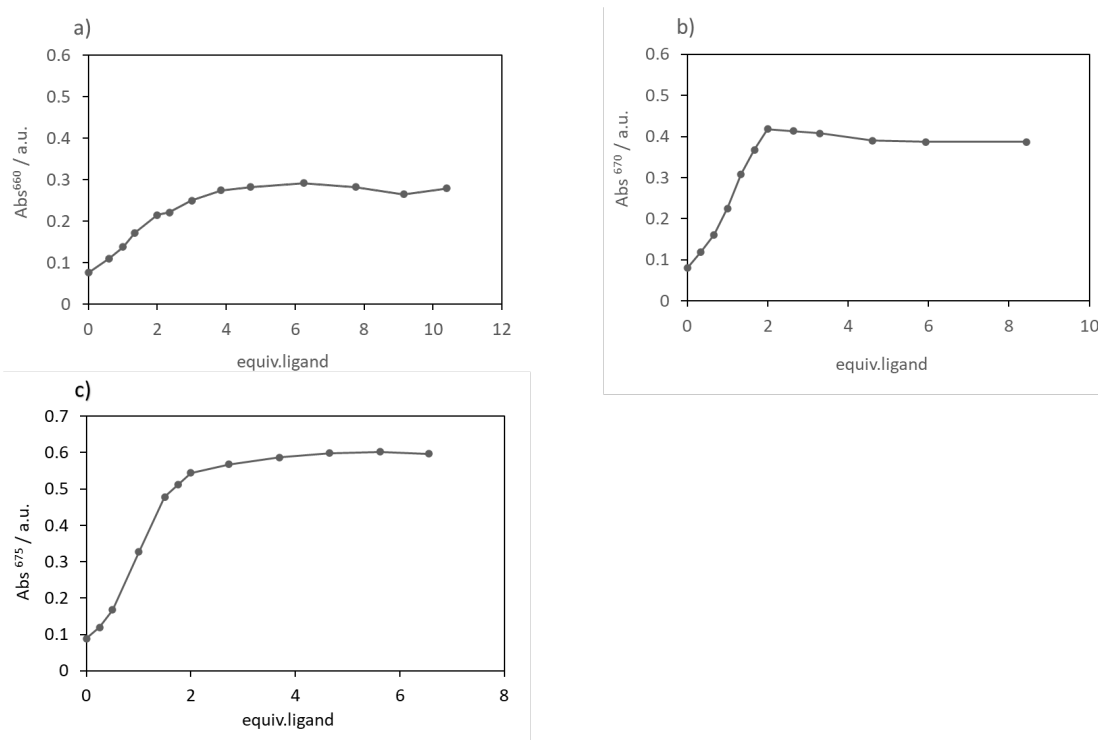


Figure 2.37. a) 20 μM of **PV** with increasing concentrations of C₈-picolyl·Zn^{II} b) 30 μM of **PV** with increasing concentrations of C₈-picolyl·Zn^{II} c) 40 μM of **PV** with increasing concentrations of C₈-picolyl·Zn^{II} at 25 °C.

Fitting of the binding isotherms using BindFit v5.0, allowed the determination of binding constants. As mentioned above, fitting of the binding isotherm obtained with 20 μM of **PV** into the 1:2 model revealed a low first association constant of 5.4 M^{-1} , and a much higher second association constant of $1.3 \times 10^7 \text{ M}^{-1}$ (**Table 2.1**). Interestingly, the binding isotherm obtained in the presence of 30 μM of **PV** revealed a first association constant of 15.7 M^{-1} , and a second association constant of 2.4×10^7 , both higher than with 20 μM of **PV**. These association constants were higher still in the presence of 40 μM **PV**, with 23.6 M^{-1} and $2.8 \times 10^7 \text{ M}^{-1}$ obtained for the first and second association constants, respectively. These results indicate that association constants increase at higher overall concentrations of indicator and host, which can have important implications for competitive binding with the analytes of interest.

Table 2.1. Binding constants at different concentrations of **PV**, and the equivalents of anion needed to observe a colour change.

PV conc. (μM)	K_{11} (M^{-1})	K_{12} (M^{-1})	PPi	ATP	ADP	Citrate
20	5.4	1.3×10^7	1:2	1:10	1:15	1:20
30	15.7	2.4×10^7	1:2	1:7	1:7	1:20
40	23.6	2.8×10^7	1:2	1:5	1:7	1:20

We next investigated the ease of displacement of the $\text{C}_8\text{-picolyl-Zn}^{\text{II}}\text{-PV}$ complex by different phosphates, to see how the concentration of the starting host-indicator complex can affect the selectivity of binding with different analytes. **Figure 2.38** shows the displacement studies beginning with 20, 30 and 40 μM of **PV**. As expected, at higher concentrations of **PV**, stronger beginning absorbance is seen, which would be advantageous for a colorimetric indicator, providing for enhanced sensitivity. However, it can be seen that the systems with a higher concentration of **PV** exhibits poorer selectivity when comparing the displacement between the different ions PPi, ATP, ADP and citrate. In the system with 20 μM of **PV**, 2 equivalents of PPi are required for the displacement of **PV** and for the solution to turn yellow, while 10 equivalents of ATP are

required to reach the same end point (this data is summarised in **Table 2.1**). In the system involving 30 μM **PV**, two equivalents of **PPi** are required for displacement of the **PV**, but this complex is also much more easily displaced by **ATP**, requiring only five equivalents. Moreover, in the system involving 40 μM of **PV**, displacement requires two equivalents of **PPi**, but only four equivalents of **ATP**, indicating even poorer selectivity (**Figure 2.38**). Thus, these experiments indicate that higher concentrations of the initial $\text{C}_8\text{-picolyl}\cdot\text{Zn}^{\text{II}}$ -**PV** complex have the potential to provide enhanced sensitivity, but poorer selectivity between different phosphate anions.

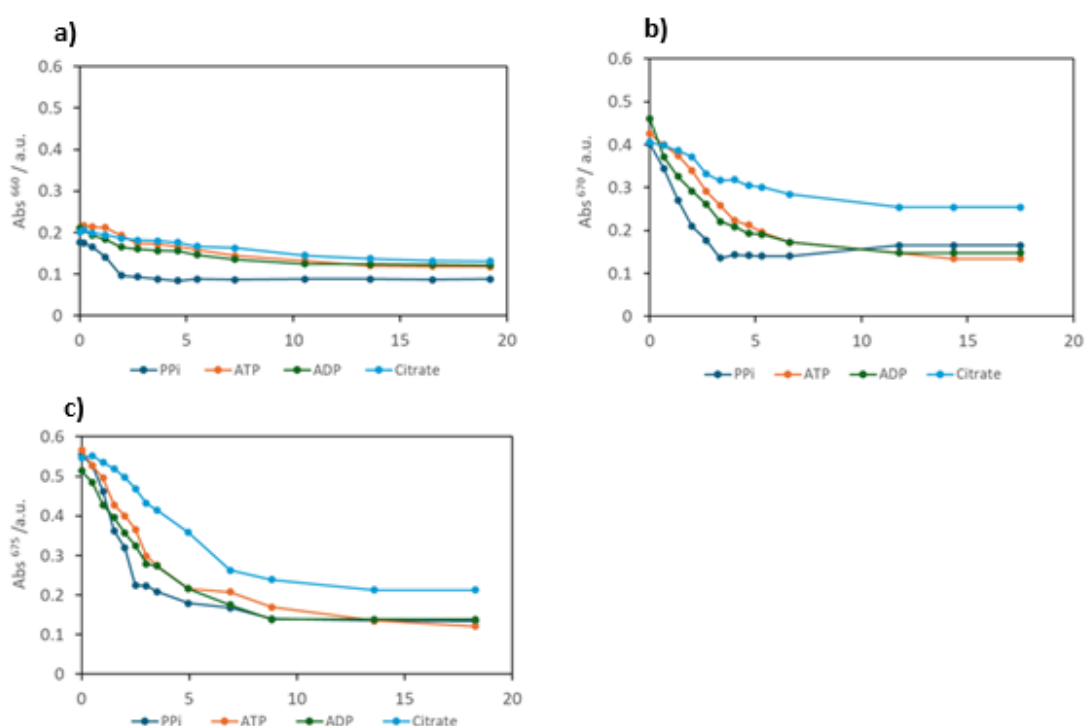


Figure 2.38. Different concentrations of **PV** with different phosphates in the presence of 1:2 ratio of **PV** and $\text{C}_8\text{-picolyl}\cdot\text{Zn}^{\text{II}}$ complex.

2.4.7 Studies with the shorter chain $\text{C}_6\text{-picolyl 36}/\text{Zn}^{\text{II}}$ ligand

To complete our study investigating the effects of changing the chain length of the $\text{C}_n\text{-picolyl}\cdot\text{Zn}^{\text{II}}$ amphiphiles, we next investigated the binding and displacement of $\text{C}_6\text{-picolyl 36}\cdot\text{Zn}^{\text{II}}$. We measured the absorption spectrum upon the addition of increasing concentrations of $\text{C}_6\text{-picolyl}\cdot\text{Zn}^{\text{II}}$ under the same conditions (HEPES = 5.0 mM, NaCl =

145 mM, pH = 7.4, **PV** = 20 at 25 °C) to observe its binding behaviour with **PV**. This experiment revealed that five equivalents of C₆-picolyl **36**·Zn^{II} were required to effectively bind with the **PV** and induce a colour change, demonstrating weaker binding than the C₈-picolyl **37**·Zn^{II} ligand, consistent with what we observed earlier (**Figure 2.39**).

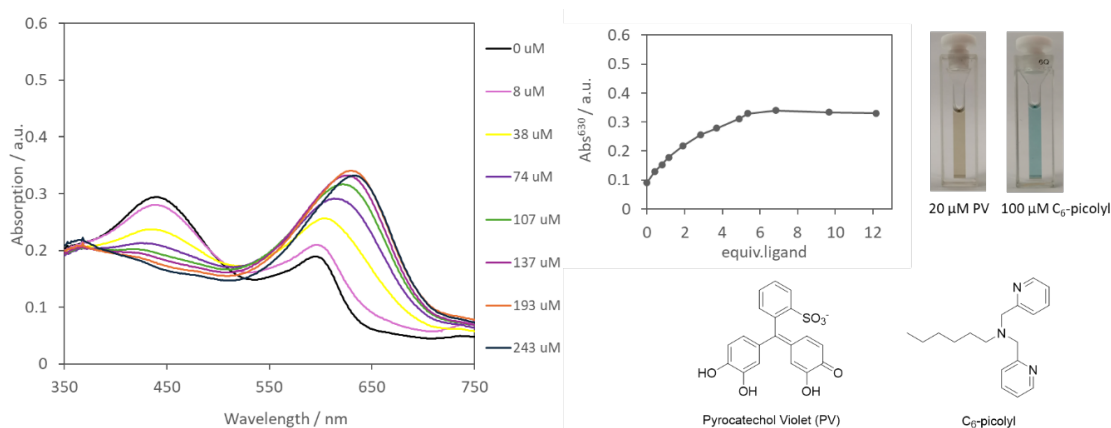


Figure 2.39. Changes in absorbance of **PV** with increasing C₆-picolylyl·Zn^{II} concentration until 208 μM. Conditions: [HEPES buffer] = 5 mM, pH 7.4, [NaCl] = 145 mM, [**PV**] = 20 μM at 25 °C. C₆-picolylyl·Zn^{II} concentrations = 0, 8, 38, 74, 107, 137, 193, and 243 μM.

Next, we investigated the displacement of the C₆-picolylyl·Zn^{II}-**PV** complex with PPI and ATP (**Figure 2.40**). We see that the complex undergoes a colour change following the addition of 5.5 equivalents of PPI (113 μM), whereas ATP needs 7.5 equivalents (150 μM) with respect to the **PV**. This demonstrates only minor differences in binding affinity between PPI and ATP, while the rest of the phosphates did not give a colour change even after the addition of 20 equivalents. These results suggest that the C₆ chain does not effectively form binding pockets that can efficiently bind to and discriminate between the phosphates ATP and PPI.

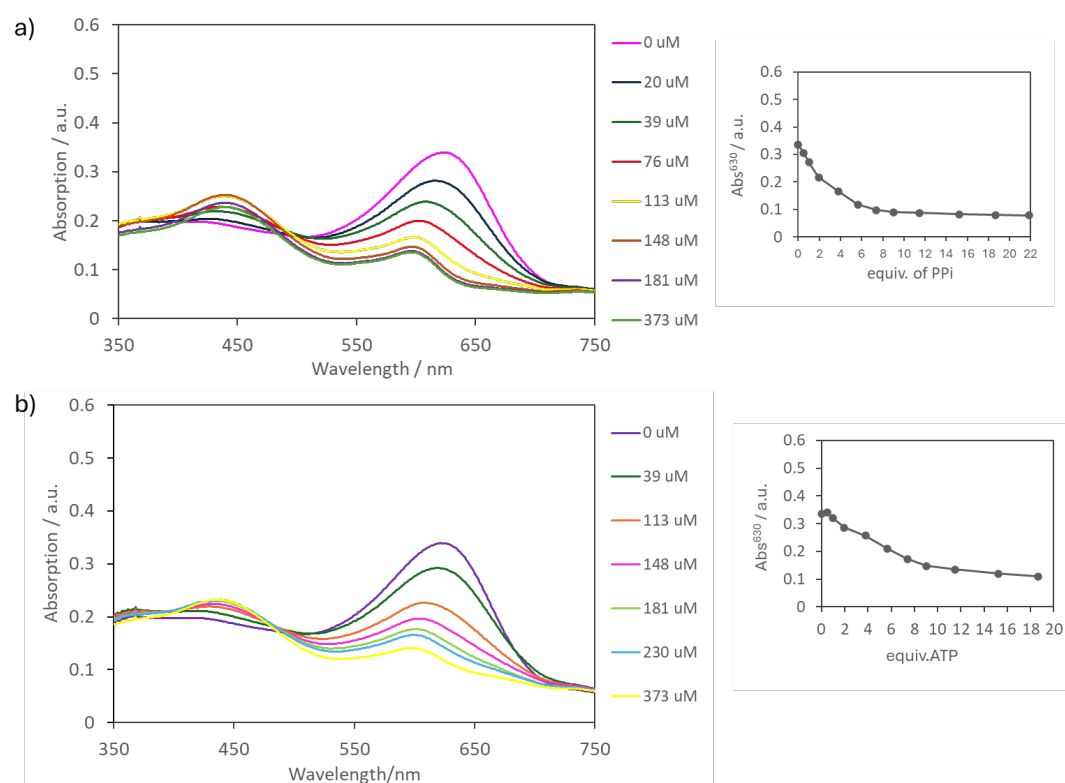


Figure 2.40. a) Absorbance spectra for the addition of PPI in the presence of C₆-picolyl·Zn^{II}-PV. Conditions: [HEPES buffer] = 5 mM, pH = 7.4, [NaCl] = 145 mM, [PV] = 20 μM, [C₆-picolyl·Zn^{II}] = 100 μM, [PPI] = 0, 20, 39, 76, 113, 148, 181, and 373 μM at 25 °C; b) Absorbance spectra for the addition of ATP in the presence of C₆-picolyl·Zn^{II}. Conditions: [HEPES Buffer] = 5 mM, pH = 7.4, [NaCl] = 145 mM, [PV] = 20 μM, [C₆-picolyl·Zn^{II}] = 100 μM, [ATP] = 0, 39, 113, 148, 181, 230 and 373 μM at 25 °C.

In this chapter so far, we have shown that our C₈-picolyl·Zn^{II} system offers the best balance of binding affinity with PV and the ability to discriminate between PPI and other phosphates, such as ATP and ADP. However, one potential weakness of this system is its highly dynamic nature, which is demonstrated in the concentration effects described in **Section 2.4.6**, where different concentrations of host result in different binding affinities with PV, and changes in the ability to discriminate between different phosphates. To induce greater stability to the system, we wondered if our self-assembled hosts could be embedded into micellar systems. The greater stability provided from a micellar matrix could remove concentration-dependent effects and make the system less susceptible to changes in the sample environment.

2.5 Incorporation within micellar matrices

2.5.1 Background

Surfactants are substances that contain a polar head and a non-polar tail (**Figure 2.41**). Due to their amphiphilicity, they tend to self-assemble in water to form micellar structures, thereby avoiding interactions between the nonpolar tail and the polar aqueous environment.¹³⁸ They have a variety of applications in industry, including as detergents in cleaning applications, emulsifiers in food and cosmetic formulations, or as dispersants in paints and coatings applications. Surfactants can be categorised as non-ionic, cationic, anionic and amphoteric surfactants, depending on the characteristics of the polar headgroup. In this section, we will examine the effect of incorporating our self-assembled receptors into different types of surfactants and investigate their effect on binding affinity and selective displacement by different phosphate analytes.¹³⁹

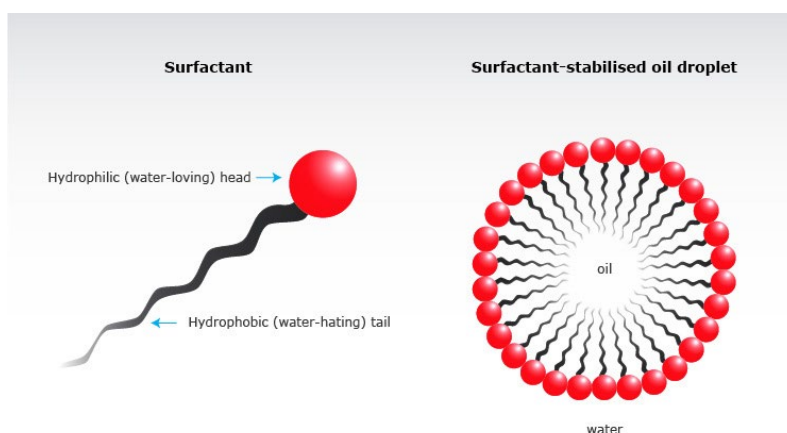


Figure 2.41. The general structure of a surfactant and a surfactant-stabilised oil droplet. Copy right 2012. University of Waikato, all rights reserved. Source: Adapted from Ref.[139] with permission ref[139]. Copyright (2025) Journal of the Americal Chemical Society.

2.5.2 Incorporation into cetyltrimethylammonium bromide (CTAB)

We began these experiments by investigating the incorporation of the self-assembled

receptors into a micellar matrix formed from cetyltrimethylammonium bromide (CTAB).¹⁴⁰ The addition of amphiphile C₁₆-picolyl·Zn^{II} to a solution of 20 μM of **PV** in aqueous HEPES buffer (5 mM, pH 7.4, 145 mM NaCl, at 25 °C) and 800 mM of CTAB led to the binding isotherm shown in **Figure 2.42**. One benefit of using a micellar matrix was observed immediately, with the absence of the significant changes in baseline at higher concentrations of C₁₆-picolyl·Zn^{II}, which was observed in the non-micellar system, due to the formation of C₁₆-picolyl·Zn^{II} aggregates. The more consistent baseline and smoother isotherm allowed a better fit into the BindFit models, and calculation of the association constants K_{11} and K_{12} (see **Figure 2.45**). Strong binding to **PV** was observed with a clear yellow to blue colour change observed upon the addition of two equivalents of C₁₆-picolyl·Zn^{II}.

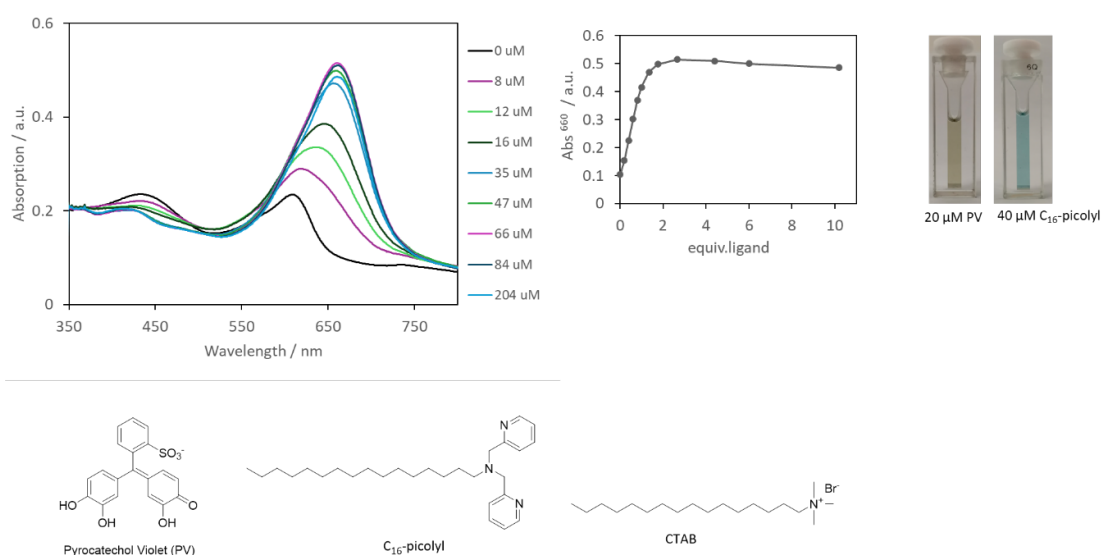


Figure 2.42. Changes in absorbance of **PV** solution with increasing C₁₆-picolyl·Zn^{II} concentration until 204 μM. Conditions: [HEPES buffer] = 5 mM, pH 7.4, [NaCl] = 145 mM, [**PV**] = 20 μM, [CTAB] = 800 μM at 25 °C. C₁₆-picolyl·Zn^{II} concentrations = 0, 8, 12, 16, 20, 35, 47, 66, 84, and 204 μM.

Our next step was to conduct the PPI experiment using 40 μM of C₁₆-picolyl·Zn^{II}, following the same procedure as in previous experiments (**Figure 2.43**). However, the spectra for this experiment were unexpectedly similar to those with C₁₆-picolyl ligand, even without the addition of micelles. At this concentration, we did not observe the anticipated yellow shift. Even when we increased the PPI concentration there is no

significant changes were observed.

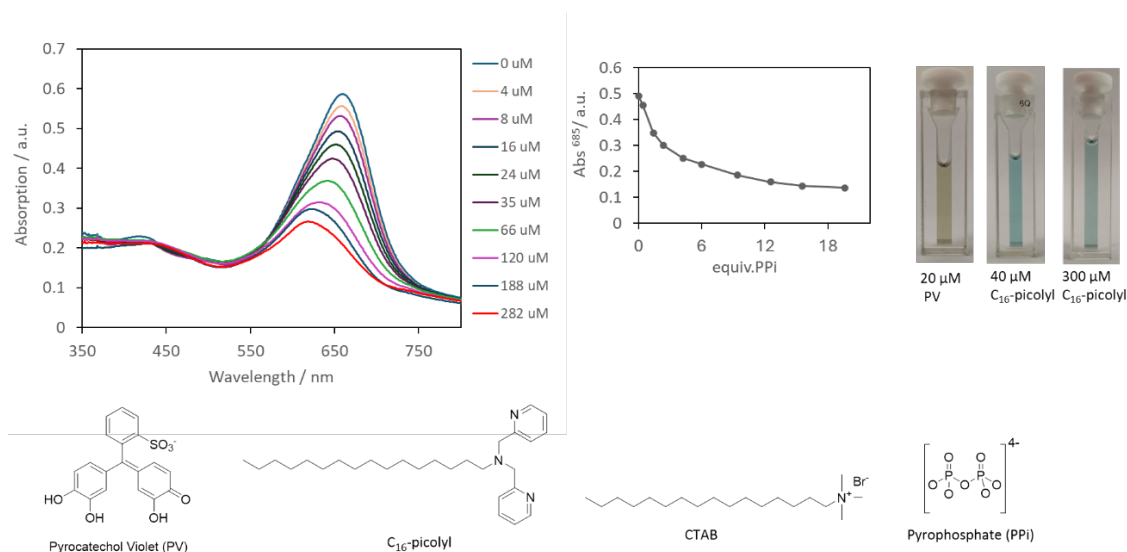


Figure 2.43. Absorbance spectra for the addition of PPI in the presence of C₁₆-picolyl·Zn^{II}. Conditions: [HEPES buffer] = 5 mM, pH = 7.4, [NaCl] = 145 mM, [PV] = 20 μM , [CTAB] = 800 μM , [C₁₆-picolyl·Zn^{II}] = 40 μM , [PPI] = 0, 4, 8, 16, 24, 35, 66, 120, 188, and 282 μM .

We next proceeded to investigate the binding isotherms of ligands containing different chain lengths, namely C₁₂-picolyl, C₁₀-picolyl, C₈-picolyl and C₆-picolyl, in the presence of 800 μM of CTAB (**Figure 2.44** and **Figure 2.45**), to see if we could observe a reduction in binding affinity at reduced chain lengths. Each ligand was tested for its ability to bind efficiently to **PV** under the same conditions, and each was shown to be able to effectively form a yellow complex upon addition of two equivalents of ligand (see **Table 2.2**).

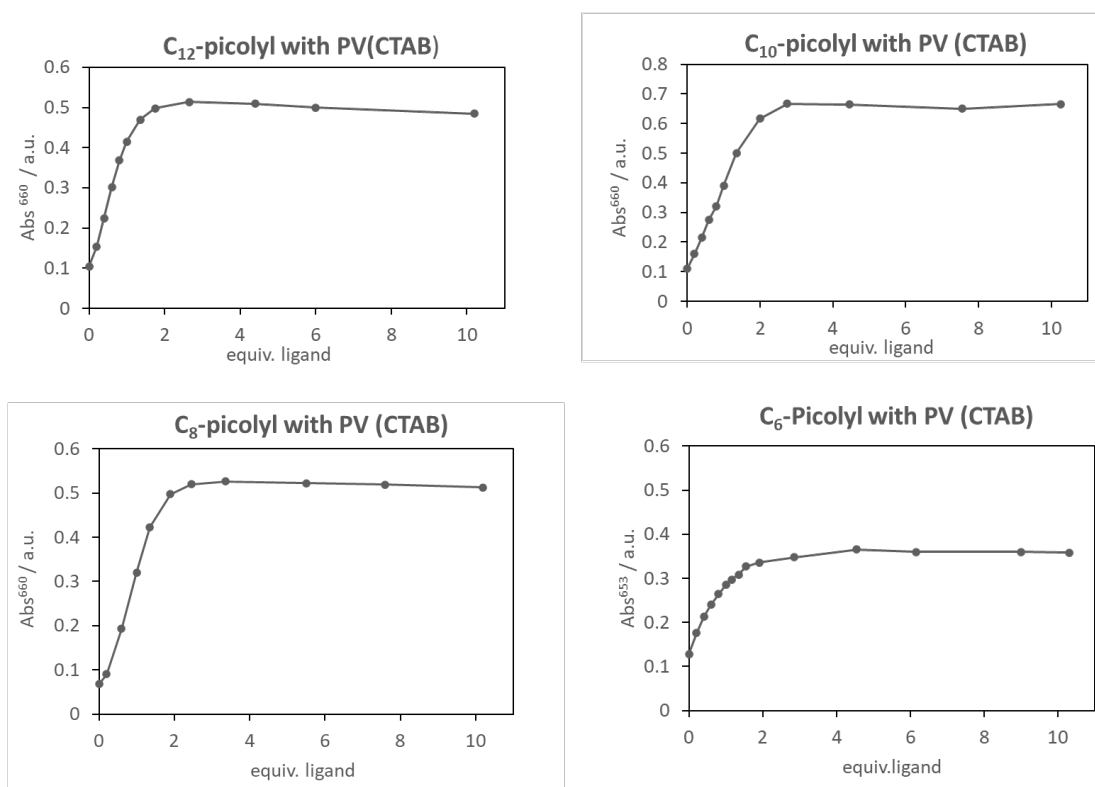


Figure 2.44. Addition of different ligands such as C₁₂-picolyl·Zn^{II}, C₁₀-picolyl·Zn^{II}, C₈-picolyl·Zn^{II}, and C₆-picolyl·Zn^{II} with the 20 μM of PV in the presence of [HEPES Buffer] = 5 mM, pH = 7.4, [NaCl] = 145 mM, [PV] = 20 μM, [CTAB] = 800 μM at 25 °C.

Table 2.2. Binding constants at different concentrations of PV, and the equivalents of anion needed to observe a colour change.

Ligand	K_{11} (M ⁻¹)	K_{12} (M ⁻¹)	PPI (eq.)
C ₁₆ -picolyl·Zn ^{II}			>20
C ₁₂ -picolyl·Zn ^{II}	5.6×10^3	2.5×10^8	12
C ₁₀ -picolyl·Zn ^{II}	1.8×10^{11}	4.0×10^6	10
C ₈ -picolyl·Zn ^{II}	7.4×10^7	1.1×10^6	8
C ₆ -picolyl·Zn ^{II}	1.5×10^5	4.3×10^3	8

Displacement studies could then be done using the same conditions (40 μM ligand/sensor

and 20 μM **PV** with CTAB) with the different amphiphiles with the addition of PPI. We observed that when reducing the length of the hydrophobic chain the ratio of PPI required to displace **PV** reduces; for example, if it is $\text{C}_{16}\text{-picolyl}\cdot\text{Zn}^{\text{II}}$, we are required to displace the **PV** with 15 eq. of PPI the ratio of PV with PPI is 1:15, but the blue colour is still the same; when we reduced the chain length at $\text{C}_{12}\text{-picolyl}\cdot\text{Zn}^{\text{II}}$, we required 12 eq. of PPI (1:12 (PV:PPI)) and observed a slight colour changed to yellow, at $\text{C}_{10}\text{-picolyl}\cdot\text{Zn}^{\text{II}}$ we required 10 eq. of PPI (1:10 (PV:PPI)). In contrast, at $\text{C}_8\text{-picolyl}\cdot\text{Zn}^{\text{II}}$, we required eight eq. of PPI (1:8 (PV:PPI)), and for $\text{C}_6\text{-picolyl}\cdot\text{Zn}^{\text{II}}$ required 8 eq of PPI(1:8 (PV:PPI)), Therefore, our sensor exhibits a good binding with **PV** in the presence of CTAB, but it is not suitable for the displacement of **PV**, as it requires a significant amount of PPI to displace **PV** (see **Figure 2.46** below).

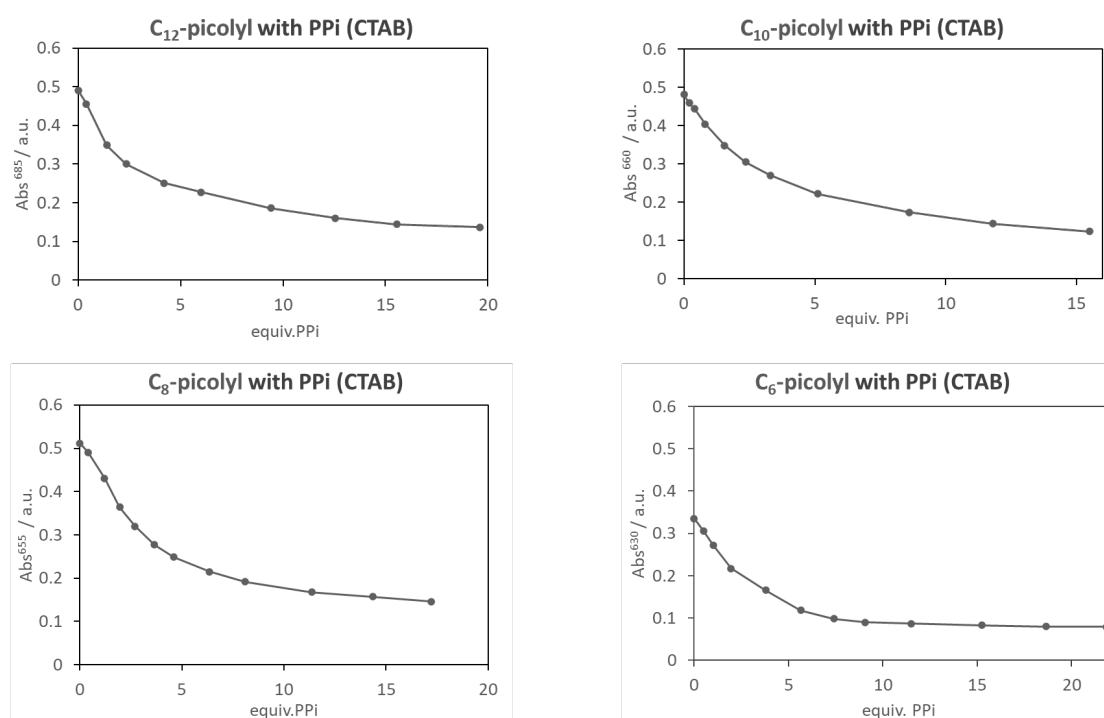


Figure 2.46. Titration of PPI with different amphiphiles such as $\text{C}_{12}\text{-picolyl}\cdot\text{Zn}^{\text{II}}$, $\text{C}_{10}\text{-picolyl}\cdot\text{Zn}^{\text{II}}$, $\text{C}_8\text{-picolyl}\cdot\text{Zn}^{\text{II}}$, and $\text{C}_6\text{-picolyl}\cdot\text{Zn}^{\text{II}}$ with 1:2 ratio of **PV** and amphiphiles.

When comparing our binding and selectivity results for $\text{C}_{16}\text{-picolyl}$ to $\text{C}_6\text{-picolyl}$ with CTAB, we observed that the self-assembled system exhibits superior binding with **PV** but does not displace **PV** with phosphates. In our quest to determine the best surfactant for

selectivity, we found that C₈-picolyl is the better test for the displacement of **PV** by using pyrophosphate (PPi). As a result, we have chosen to focus on the C₈-picolyl to ascertain whether it is the best option for selectivity when using different surfactants like Brij O10. Positively charged CTAB may give possibility for non-specific binding – shift to investigating with non-ionic surfactant Brij O10. Consequently, BrijO10 was selected to determine whether selective binding could be achieved while minimizing non- specific interactions.

2.5.3 Incorporation into the non-polar surfactant Brij O10

When we introduce Brij O10 to C₈-picolyl·Zn^{II} in the presence of **PV** (20 μM), we observe a binding pattern similar to CTAB, with a 1:2 ratio of **PV** and C₈-picolyl·Zn^{II} accompanied by a colour change from blue to yellow. Simultaneously, a new peak emerges from 550 to 750 nm, indicating that this system is favourable for **PV** binding. The self-assembly of the C₈-picolyl·Zn^{II} molecule leads to the dipicolylamine (DPA) binding groups and the zinc ions they bind being positioned adjacent to one another (see **Figure 2.47** below). We assume they are positioned adjacent to each other because it is known that two metal-binding units in close proximity is required for **PV** binding.

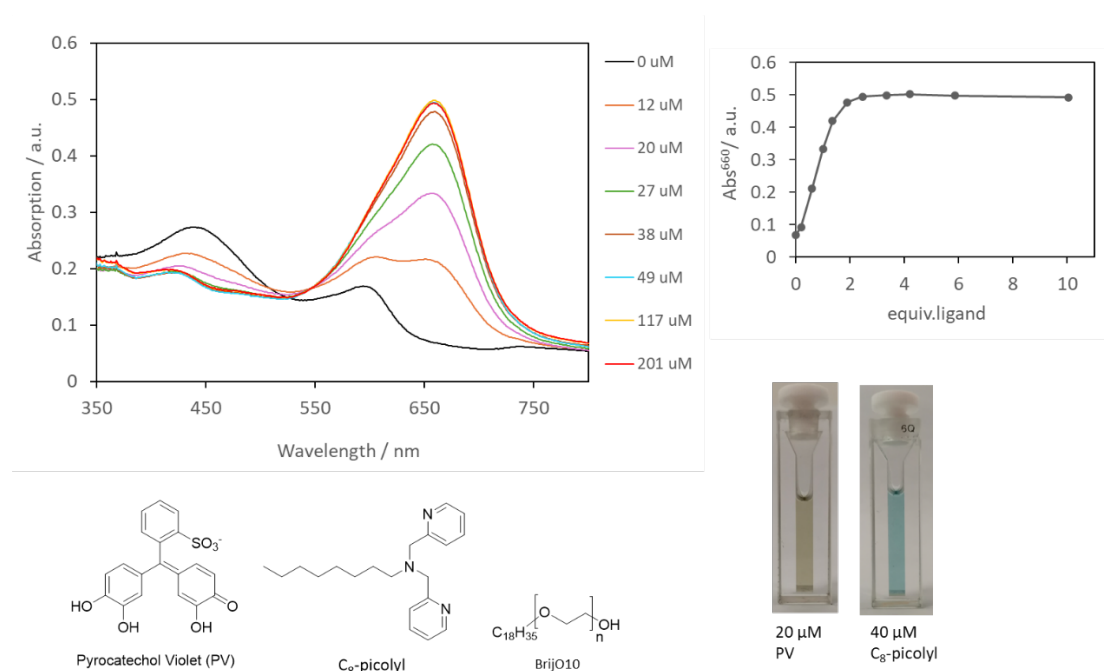


Figure 2.47. Changes in absorbance of **PV** solution with increasing C_8 -picolyl· Zn^{II} concentration until 201 μM . Conditions: [HEPES buffer] = 5 mM, pH 7.4, [NaCl] = 145 mM, [**PV**] = 20 μM , [Brij O10] = 800 μM at 25 °C. C_8 -picolyl **37** concentrations = 0, 12, 20, 27, 38, 49, 117 and 201 μM .

The detection of PPI by the C_8 -picolyl· Zn^{II} sensor was investigated under the conditions of 40 μM of C_8 -picolyl· Zn^{II} sensor and 20 μM **PV**, the absorbance results, as shown in **Figure 2.48**, reveal a significant change from blue to yellow at 80 μM of PPI. The absorbance spectrum for C_8 -picolyl· Zn^{II} indicates a substantial amount of PPI is required, with the **PV** (20 μM) necessitating 80 μM of PPI. To enhance these findings, we are working together to experiment with a combination of surfactants, aiming for superior results in the displacement of the **PV**.

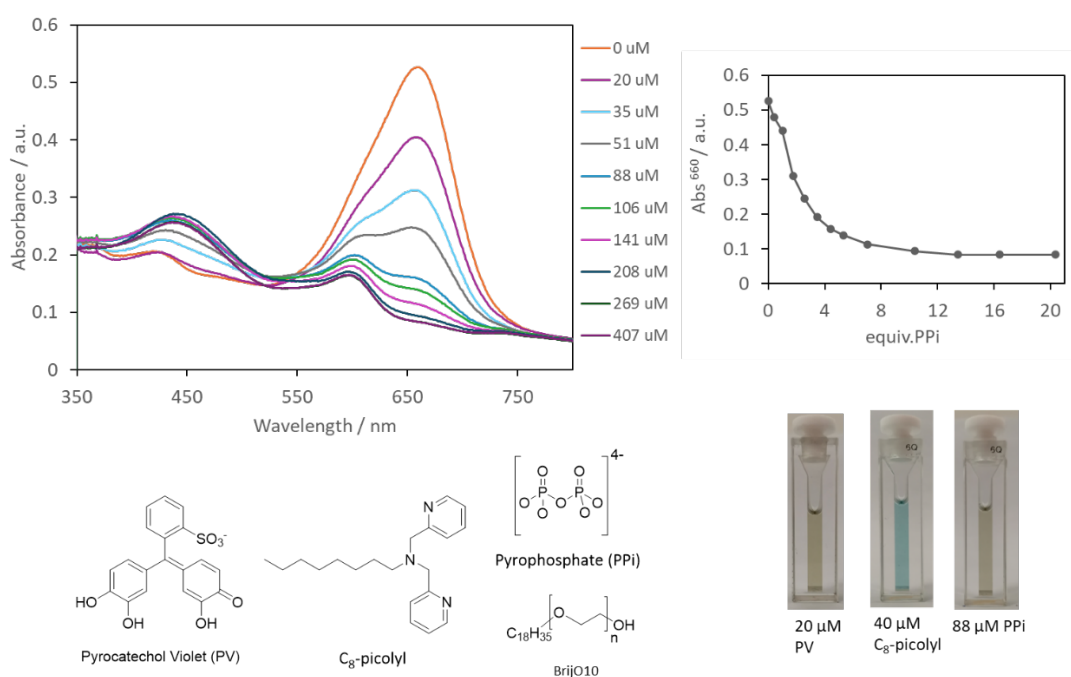


Figure 2.48. Absorbance spectra for the addition of PPI in the presence of amphiphilic ligands dipicolyl amine C_8 -picolyl· Zn^{II} . Conditions: [HEPES Buffer] = 5 mM, pH = 7.4, [NaCl] = 145 mM, [**PV**] = 20 μM , [Brij O10] = 800 μM , [C_8 -picolyl· Zn^{II}] = 40 μM , [PPI] = 0, 20, 35, 51, 88, 106, 141, 208, 269 and 407 μM at 25 °C.

We then tested the various phosphates, an important consideration in the development of

sensor systems. These studies carried out under the same conditions (40 μM of $\text{C}_8\text{-picolyl}\cdot\text{Zn}^{\text{II}}$ sensor and 20 μM of **PV**), have given encouraging results. We observed a colour change with 4.4 equivalents of PPi , whereas ATP required 11.5 equivalents, ADP required 10 equivalents, and citrate did not cause a colour change until 20 equivalents (see below graph) (**Figure 2.49**). This suggests that PPi is a better sensor than others. However, it also suggests that further selectivity in the displacement of **PV** will open the way for further development.

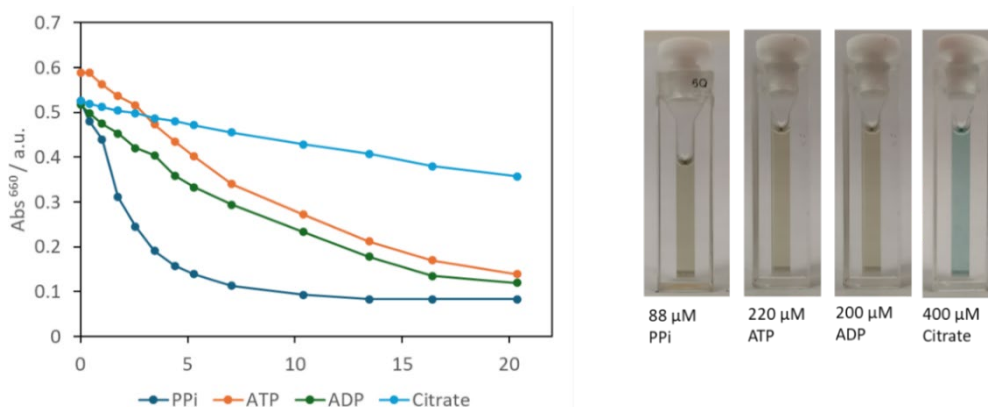


Figure 2.49. Absorbance spectra for the addition of different phosphates to the sensor **37**, conditions: [HEPES buffer] = 5 mM, pH = 7.4, [NaCl] = 145 mM, [**PV**] = 20 μM , 1:1 ratio $\text{C}_8\text{-picolyl}\cdot\text{Zn}^{\text{II}}$ = 40 μM , [Brij O10] = 800 μM .

2.5.4 Tuning of the micellar matrix

Our investigation into the relative binding affinity of our self-assembled receptor for the sensor **PV** versus PPi led us to test a mix of surfactants. We were particularly interested in SLS with CTAB and Brij O10, as SLS does not bind with the **PV**, allowing us to create a weak binding with our $\text{C}_8\text{-picolyl}\cdot\text{Zn}^{\text{II}}$ and easily displace the **PV** using PPi . However, the mixtures of SLS and CTAB, CTAB and Brij O10, and SLS and Brij O10 did not produce the desired results (**Figure 2.50**). This result may be due to the formation of mixed micelles that have different sizes, shapes, and surface properties than those made by each surfactant alone. Undeterred, we remain committed to the success of our project and decided to investigate another non-ionic surfactant Tween 20.

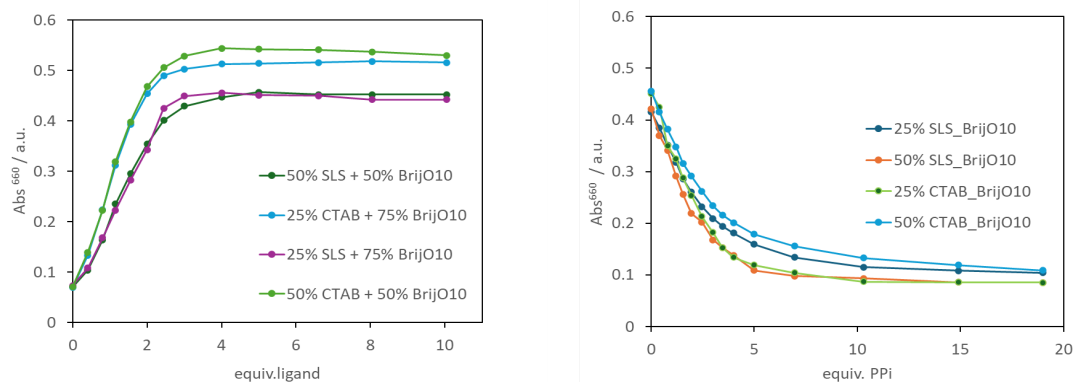


Figure 2.50. Absorbance spectra for the addition of different surfactants with C₈-picolyl·Zn^{II}. Conditions: [HEPES] = 5 mM, pH = 7.4, [NaCl] = 145 mM, [PV] = 20 μM, [Brij O10] = 800 μM, [CTAB] = 800 μM, [SLS] = 800 μM, and PPI at 2 eq. of C₈-picolyl·Zn^{II}.

Our investigation into the binding efficiency of the C₈-picolyl·Zn^{II} sensor with **PV** under the same conditions (20 μM **PV**, 5 mM HEPES, 145 mM NaCl) using Tween 20 has revealed a crucial need for optimization. The binding of **PV** required 3 eq of C₈-picolyl·Zn^{II} and thus, there is a need to optimize the combination of Brij O10 and Tween 20 to enhance sensitivity and selectivity. To achieve this, we tried different ratios of Tween 20 and Brij O10, and the most effective ratio for binding with the **PV** was identified. Specifically, we varied the ratio of 100% Tween 20 to 50% Tween, 25% Tween, 12.5% Tween, and 6.5% Tween and observed that 100% required 3 eq. of C₈-picolyl·Zn^{II}, 50% Tween required 2.5 eq. C₈-picolyl·Zn^{II}, 25% Tween required 2 eq. C₈-picolyl·Zn^{II}, 12.5% Tween required 2 eq. of C₈-picolyl·Zn^{II}, and 6.5 % Tween required 2 eq. of C₈-picolyl·Zn^{II} (see the graphs below **Figure 2.51**). Furthermore, we tried to take these different ratios of Tween with the different equivalents (eq) of C₈-picolyl·Zn^{II} to see which one is the best for the displacement of **PV**. We observed that 12.5% of Tween had good results because we required two equivalents of C₈-picolyl·Zn^{II}. The C₈-picolyl·Zn^{II} sensor is required to bind the **PV**, and the displacement of **PV** is also required with two equivalents of PPI, which is the best result. We show the graphs below to see the binding with the **PV** and displacement with the **PV** using PPI.

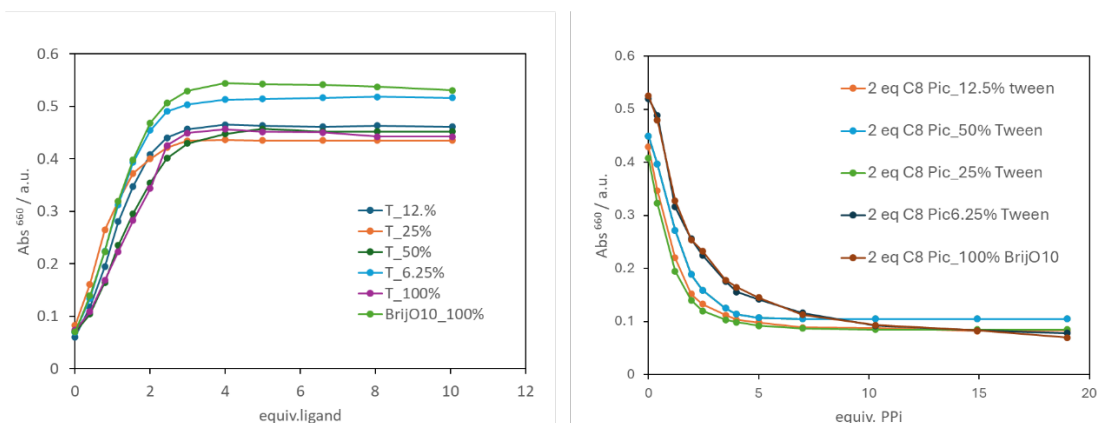


Figure 2.51. Absorbance spectra for the addition of Tween 20 and Brij O10 with different ratios in the presence of C_8 -picolyl- Zn^{II} sensor **37** and displacement with PPI, conditions: [HEPES buffer] = 5 mM, pH = 7.4, [NaCl] = 145 mM, [PV] = 20 μ M, [Brij O10] = 800 μ M, [Tween 20] = 800 μ M, and PPI at 2 eq. of C_8 -picolyl- Zn^{II} to PV.

From the changes in the absorption spectrum of **PV** upon incremental additions of C_8 -picolyl- Zn^{II} amphiphile, we observed efficient binding with the desired 1:2 PV: C_8 -picolyl- Zn^{II} stoichiometry (**Figure 2.52**). The colour change from yellow to blue also occurred at the 1:2 stoichiometry, which matched the results obtained in the presence of Brij O10.

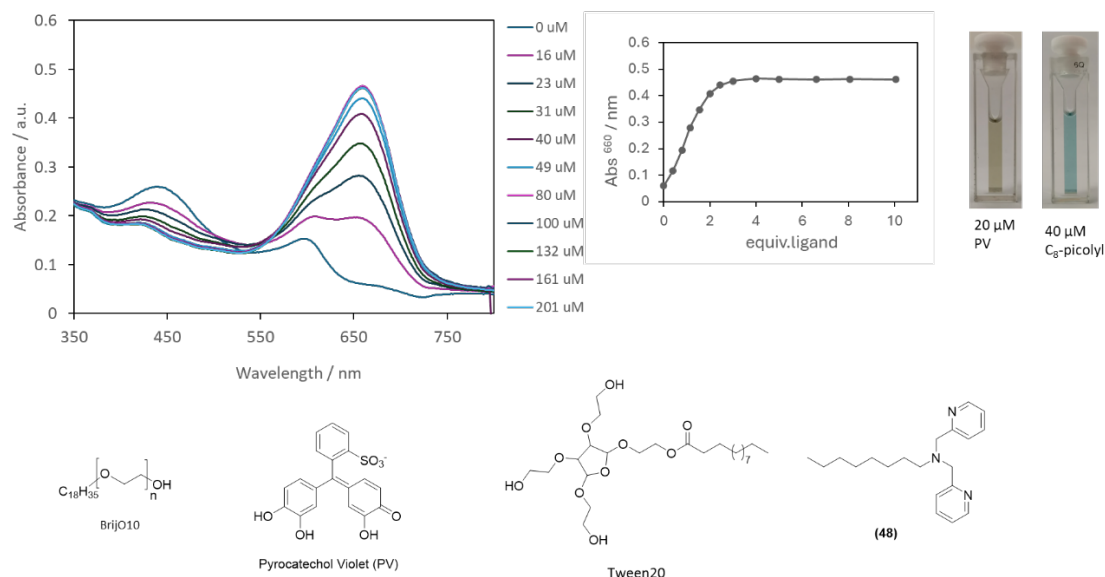


Figure 2.52. Changes in absorbance of **PV** solution with increasing C_8 -picolyl- Zn^{II} concentration until 201 μ M. Conditions: [HEPES buffer] = 5 mM, pH 7.4, [NaCl] = 145 mM, [PV] = 20 μ M, [Tween 20 and Brij O10 (12.5%:87.5%)] = 800 μ M at 25 $^{\circ}$ C. C_8 -picolyl- Zn^{II} concentrations = 0, 16, 23, 31, 40, 49, 80, 100, 132, 161 and 201 μ M.

Our study involved a detailed examination of the displacement of the indicator from the sensor upon the addition of PPI. We conducted a series of experiments, starting with a 1:2 solution of the **PV**: amphiphile C₈-picolyl·Zn^{II} complex. Incremental additions of phosphates were made, and the absorbance was measured. These results showed a decrease in the peak at 700 nm with high concentrations of PPI and a significant increase at 610 nm. A blue colour changes to yellow at 40 μM indicated a high binding affinity of PPI to the C₈-picolyl·Zn^{II} complex and an ability to displace **PV** from the self-assembled receptor. Interestingly, the **PV** complex with C₈-picolyl·Zn^{II} could be readily displaced with PPI, where most of the indicator was displaced upon adding two equivalents of PPI. A 2:1 complex was efficiently formed between amphiphile C₈-picolyl·Zn^{II} and **PV**, which could be displaced entirely upon adding two equivalents of PPI (**Figure 2.53**). These results suggest that the self-assembled micellar structures had higher **PV** binding affinity.

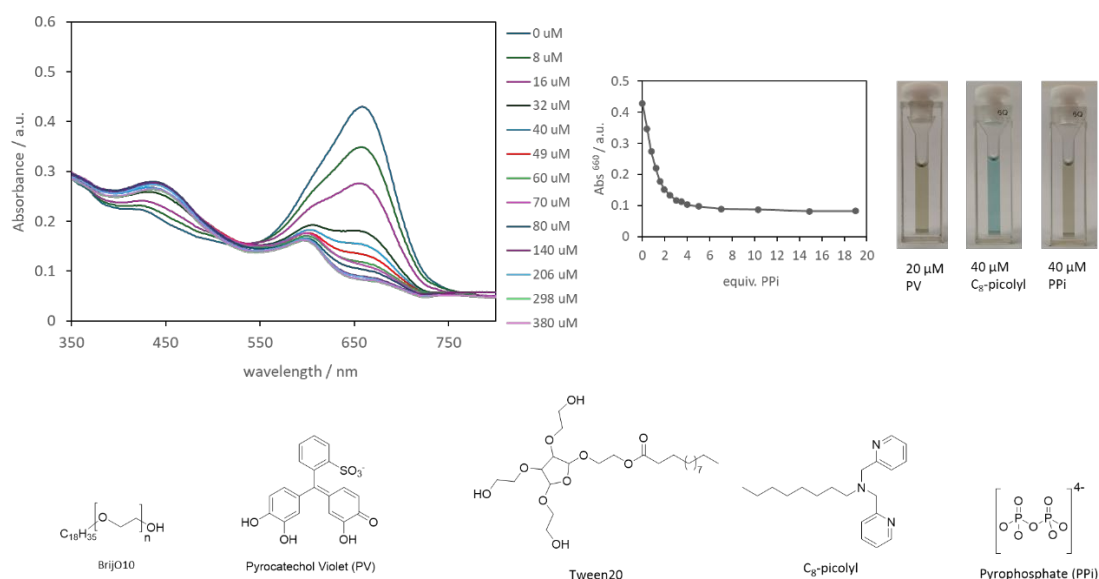


Figure 2.53. Absorbance spectra for the addition of PPI in the presence of amphiphilic ligands dipicolyl amine C₈-picolyl·Zn^{II}. Conditions: [HEPES Buffer] = 5 mM, pH = 7.4, [NaCl] = 145 mM, [**PV**] = 20 μM, [Brij O10] = 800 μM, [C₈-picolyl·Zn^{II}] = 40 μM, [PPI] = 0, 8, 16, 32, 40, 49, 60, 70, 80, 140, 206, 298, and 380 μM.

Additionally, the selectivity studies were conducted using the same conditions (40 μM ligand/sensor and 20 μM **PV** with 12.5% Tween and 87.5% Brij O10, HEPES = 5 mM,

NaCl = 145 mM) and several different phosphates (PPi, ATP, ADP, and Citrate). This involved adding phosphates in 2 eq. of C_8 -picolyl·Zn^{II} with **PV** to determine the selectivity for PPi as opposed to other competing anions under identical conditions. Interestingly, we achieved exceptional selectivity with PPi, surpassing other phosphates such as ATP, ADP, and citrate (see the **Figure 2.54** below). Furthermore, we introduced five equivalents of the selected phosphates, such as PPi, ATP, ADP, sodium phosphates, sodium biphosphates, and tyrosine, to the system and observed any colour changes (**Figure 2.54**). The C_8 -picolyl·Zn^{II} showed a distinct colour change when exposed to PPi with 2 eq but not to any other phosphate species. The spectra from each addition consistently show that the experimental process was thorough.

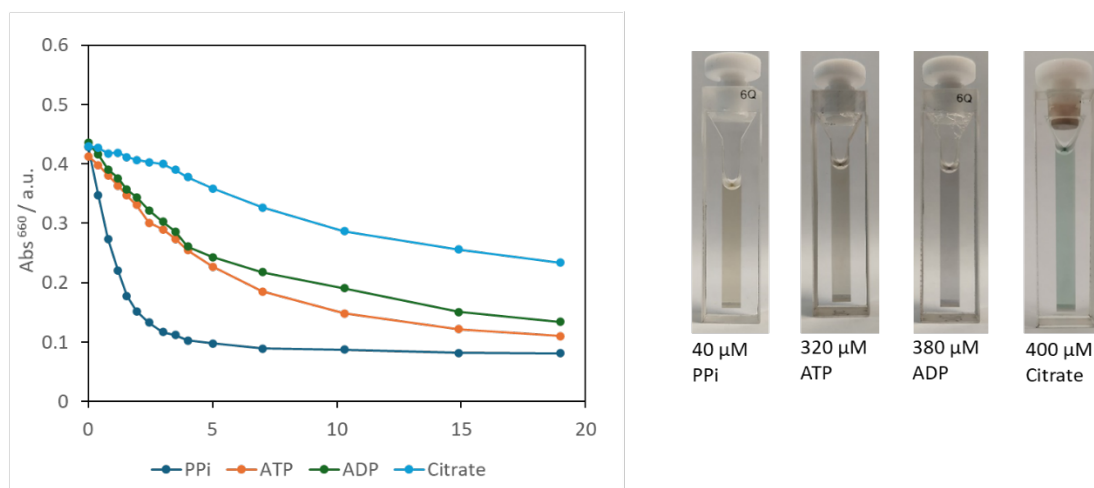


Figure 2.54. Absorbance spectra for the addition of different phosphates to the C_8 -picolyl·Zn^{II} sensor **37**, conditions: [HEPES buffer] = 5 mM, pH = 7.4, [NaCl] = 145 mM, [**PV**] = 20 μ M, C_8 -picolyl·Zn^{II} = 40 μ M, [Tween 20] and [Brij O10] (12.5%: 87.5 %) = 800 μ M [Phosphates] = PPi, ATP, ADP and citrate.

We were eager to investigate the selectivity of C_8 -picolyl·Zn^{II} for PPi as opposed to a wider range of phosphates, including PPi, ATP, ADP, AMP, DMP, phosphoserine, phosphotyrosine, sodium phosphate, sodium biphosphate, and citrate. Our experiments using 40 μ M of C_8 -picolyl·Zn^{II} in the presence of 20 μ M of **PV**, along with 100 μ M of different phosphates, maintaining a ratio of 5 equivalents under controlled conditions: HEPES buffer (5 mM), NaCl (145 mM), and a mixture of Brij O10 and Tween (87.5% to 12.5%) in aqueous solution. We found a colour change from blue to yellow only with PPi,

whereas no colour change occurred with the other phosphates (**Figure 2.55**). This suggests that PPI is highly selective for **PV** compared to the other phosphates tested.

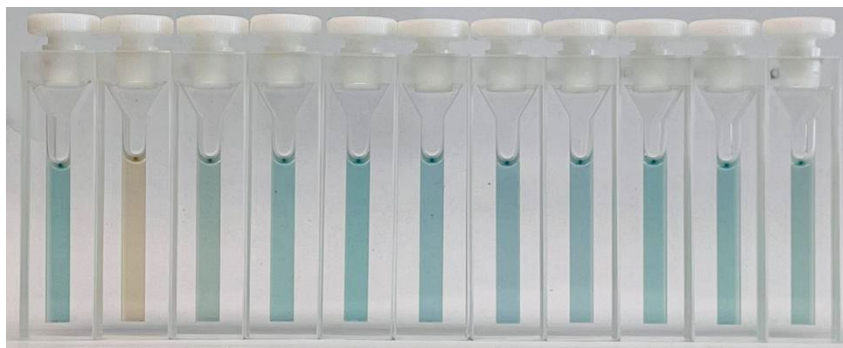


Figure 2.55. 1:2 mixtures of **PV** and C_8 -picolyl- Zn^{II} in 800 μ M Brij O10 (87.5%) and Tween (12.5%). with and without anions (sodium salts) at 5 eq. of different phosphates from left to right: No anion, PPI, ATP, ADP, AMP, DMP, phosphoserine, phosphotyrosine, sodium phosphate, sodium biphosphate, and citrate.

Table 2.3. Binding constants at different concentrations of **PV**, and the equivalents of anion needed to observe a colour change in the presence of different surfactants.

Surfactant	PV Binding	PPI	ATP
100 % Brij O10	1:2	1:4	1:11.5
93.75% Brij O10/ 6.25% Tween 20	1:2	1:2.5	1:10
87.5% Brij O10/ 12.5% Tween 20	1:2	1:2	1:16
75% Brij O10/ 25%Tween 20	1:2	1:2.5	1:15
50% Brij O10/ 50%Tween 20	1:2	1:3	1:15
100% Tween	1:3	1:3	1:16
100% CTAB	1:2	1:8	1:8
50% Brij O10/50% CTAB	1:2	1:6	1:6
75% Brij O10/25% CTAB	1:2	1:5	1:5
100% SLS	Not Binding	Not Binding	----
50% Brij O10/50 % SLS	1:3	1:3	1:7
75% Brij O10/ 25% SLS	1:2.5	1:2.5	1:5

2.6 Conclusions

In this chapter, we have demonstrated the development and evaluation of a self-assembled receptor system for the colorimetric detection of phosphate-containing analytes, using *in situ* formed amphiphilic dipicolylamine (DPA)-Zn^{II} complexes. Building on the principles of indicator displacement assays (IDAs) and inspired by the seminal work of Jolliffe and co-workers, we sought to replace traditional covalent receptor scaffolds with dynamically assembled amphiphilic architectures capable of forming their own binding pockets at the molecular interface.

Through systematic synthesis of amphiphiles bearing hydrophobic chains of varying length (C₄–C₁₆), we established that self-assembly plays a decisive role in enabling indicator binding and displacement. The long-chain amphiphiles (C₁₆, C₁₂) were found to readily self-assemble into vesicular aggregates, displaying strong binding to catechol-type indicators such as pyrocatechol violet (**PV**) and bromopyrogallol red (**BPR**), though with limited reversibility upon phosphate addition. Progressive shortening of the hydrophobic tail reduced aggregation propensity and indicator affinity, ultimately identifying **C₈-picolyl-Zn^{II}** as an optimal compromise between self-assembly, binding strength, and responsiveness. This system exhibited clear, reversible colour changes upon the addition of pyrophosphate (PPi), distinguishing it from ATP, ADP, and citrate, and confirming its selectivity under physiological buffer conditions.

Dynamic light scattering (DLS) and electron microscopy confirmed the formation of vesicular assemblies, while NMR experiments verified a 2:1 host-to-indicator stoichiometry and reversible binding behaviour – key hallmarks of an effective supramolecular IDA system. Incorporation of these receptors into micellar matrices such as CTAB, Brij O10, and mixed Brij O10/Tween 20 systems further improved control over aggregation and enhanced the reproducibility of binding and displacement events. Notably, the **12.5% Tween 20 / 87.5% Brij O10** mixed surfactant system produced the best overall performance, achieving full displacement of PV with just two equivalents of PPi while maintaining high selectivity.

Together, these findings demonstrate that self-assembly provides a viable and

modular strategy for constructing phosphate-selective receptors without the need for elaborate molecular synthesis. The tunability of the amphiphilic framework enables rapid optimisation of binding and selectivity through simple variation in molecular composition and environmental conditions.

The insights gained here form the foundation for the next stage of this research, where we extend the self-assembly concept to systems incorporating bolaamphiphilic versions of the molecules studied in this chapter.

Chapter Three

**Optimisation of a Picolyl-based
Bolaamphiphilic Receptor**

3.1 Introduction and Overview

3.1.1 Bolaamphiphiles

In 1986, Fuhrman and Fritsch introduced bolaamphiphiles (BAs), which are segmented amphiphilic molecules inspired by the structural properties of archaeal membrane lipids.¹⁴¹ Bolaamphiphiles are a special type of amphiphilic molecule composed of two polar hydrophilic head groups joined by a central hydrophobic, non-polar spacer. The “dumbbell-shaped” structure distinguishes them from conventional amphiphiles such as phospholipids, which typically comprise a single polar head and one or multiple hydrophobic tails. The structural uniqueness of BAs enables them to self-assemble into a vast range of supramolecular architectures, including monolayer membranes, nanofibers, nanotubes, vesicles, and ribbon-like aggregates. The self-assembly of BAs is extremely sensitive to the chemical nature of the head groups, the flexibility and chain length of the hydrophobic spacer, and various external factors, such as temperature, pH, and ionic strength.¹⁴²

Natural BAs are most notably found in the membrane lipids of *Archaea*, a group of extremophile microorganisms. These lipids, commonly referred to as tetraether lipids, span the entire membrane bilayer and confer exceptional thermal, chemical, and oxidative stability. Inspired by these natural systems, synthetic bolaamphiphiles have been developed with tunable molecular architectures for applications in nanotechnology, drug delivery, biomimetic membranes, and materials science (**Figure 3.1**). Their remarkable ability to form robust, monomolecular films that resist denaturation under extreme conditions makes them a versatile platform for designing functional nanomaterials and long-lasting nanomaterials, antimicrobial agents, hydrogels, delivery vehicles, and membrane-mimicking systems.^{143, 144}

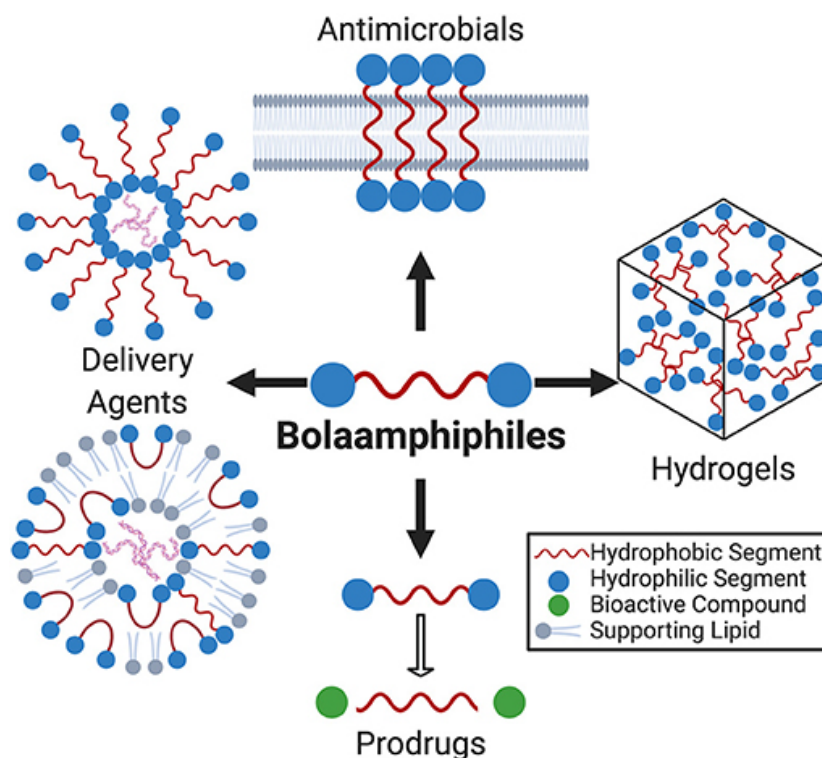


Figure 3.1. Bolaamphiphiles (BAs), due to their segmented amphiphilicity and rich self-assembly behaviour, have emerged as versatile building blocks for biomaterials relevant to therapy. Source: Adapted from Ref.[144] with permission ref[144]. Copyright (2025) Magnetic Resonance in Chemistry.

3.1.2 Previous Literature

In a significant study, Rothenbühler et al. (2018) reported the synthesis of a water-soluble, redox-active bolaamphiphile featuring a cyclopenta[hi]aceanthrylene core.¹⁴⁵ This compound self-assembles in aqueous solution to form supramolecular vesicles, driven by the balance between the hydrophobic aromatic core and the hydrophilic terminal groups (**Figure 3.2**). The resulting vesicles exhibit pronounced UV-Vis absorption across a broad spectral range, reflecting the extended conjugation of the aromatic core embedded within the bilayer-like shell. Notably, the vesicle morphology is responsive to redox changes, with oxidation and reduction modulating molecular packing and thereby altering aggregate stability and optical behaviour. These redox-active vesicles highlight the

potential of redox-switchable bolaamphiphiles to form stable, functional assemblies with tunable photophysical properties, making them suitable for applications in stimuli-responsive materials, sensor design, or drug delivery systems.

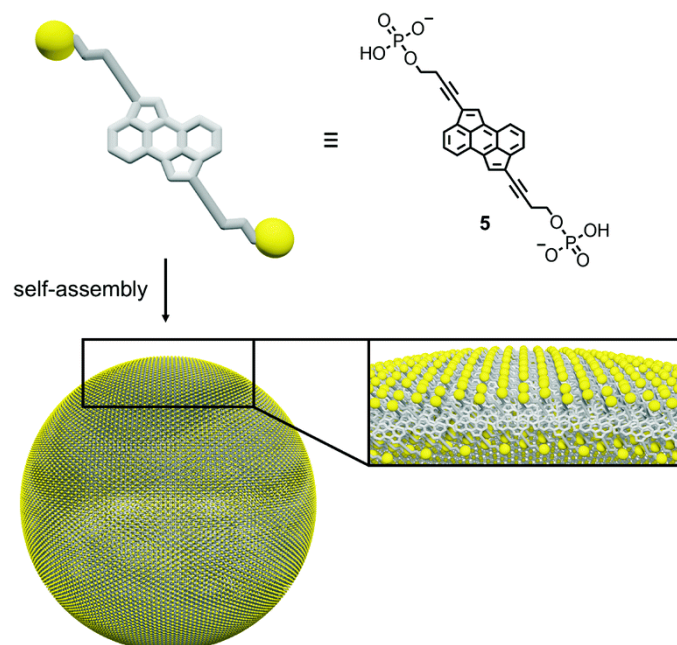


Figure 3.2. Formation of vesicular assemblies from bolaamphiphiles featuring a cyclopenta[hi]aceanthrylene core and terminal phosphates. Source: Adapted from Ref.[145] with permission ref[145]. Copyright (2004) Biochem.

Bolaamphiphilic molecules, typically represented as $X-(CH_2)_n-X$ where X denotes hydrophilic terminal groups such as $-COOH$ and NH_2 , possess the ability to form hydrogen bonds or dipole-dipole interactions with the aqueous phase.¹⁴⁶ Owing to this dual hydrophilic–hydrophobic character, bolaamphiphiles can self-assemble into ordered supramolecular architectures, including monolayer and multilayer crystalline films at the air-water interface, particularly upon coordination with transition metal ions (**Figure 3.3**). The molecular conformation adopted by bolaamphiphiles depends strongly on the flexibility of the hydrophobic spacer. Molecules containing flexible alkyl chains ($-(CH_2)_n-$) tend to adopt U-shaped conformations that allow both hydrophilic ends to interact with the aqueous phase, whereas those incorporating more rigid central units typically align in an extended, linear orientation. For example, the simple acid and amino

HOOC-(CH₂)_n-NH-COOH forms multilayer films on pure water with chain axes oriented parallel to the surface. Upon the addition of transition metal ions such as Cu²⁺ and Pb²⁺, these diacids undergo ion coordination to yield metal-carboxylate salts, leading to enhanced structural organisation and stability.¹⁴⁷ The U-shaped vs linear conformations of the bolaamphiphiles will be an important point of discussion later in this chapter.

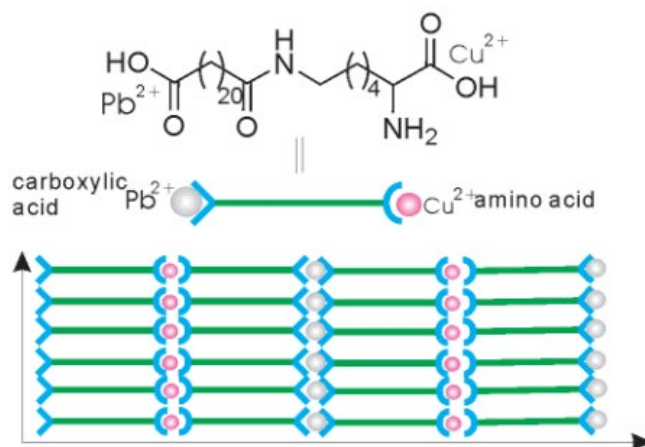


Figure 3.3. A bolaamphiphile with carboxyl and amino acid groups assembles in the presence of metal ions such as Pb²⁺ and Cu²⁺ on the water surface, leading to the largest molecular area and forming a linear shape. Source: Reprinted with permission from ref. 147. Copyright (2004) Chemical Reviews.

In another example, Furhop and Wang emphasised that the intramolecular geometry of bolaamphiphiles is primarily governed by the length and flexibility of the central hydrophobic spacer, and the symmetry and chemical nature of the terminal headgroups.¹⁴⁷ These molecular parameters play a crucial role in dictating how bolaamphiphiles self-assemble into higher-order structures. For instance, bolaamphiphiles with rigid, linear hydrophobic spacers and identical, symmetric headgroups generally adopt an extended rod-like conformation, favouring the formation of planar bilayers or vesicular membranes through close molecular packing. In contrast, when the central spacer is shorter, bent, or contains conformationally flexible segments, such as alkyl chains bearing polar functionalities, the molecules adopt a bent or wedge-like shape. This deviation from linearity induces molecular curvature, promoting the formation of non-lamellar morphologies such as micelles, helical fibres, or nanotubular assemblies.

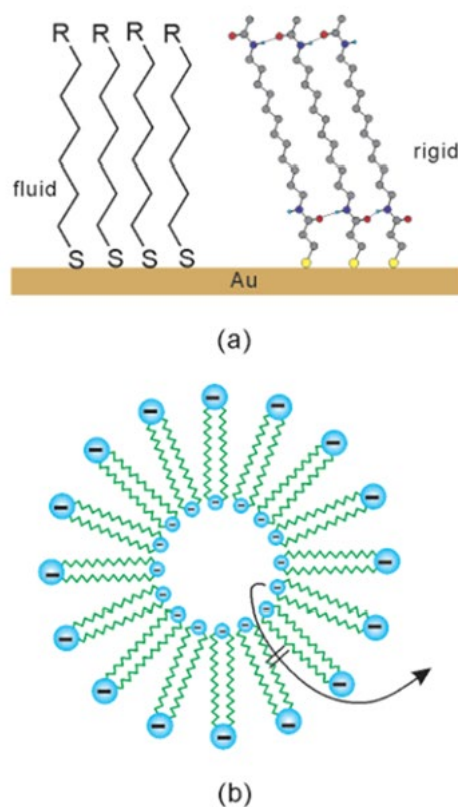


Figure 3.4. **a).** Bolaamphiphiles with linear and rigid hydrophobic spacers and identical symmetrical headgroups **b)** A monolayer lipid membrane of a vesicle with charged headgroups remains unchanged. Source: Reprinted with permission from ref. 147. Copyright (2004) Chemical Reviews 2004.

A representative example is a bolaamphiphile derived from glutamic acid, featuring glutamic acid headgroups at both termini and a flexible aliphatic chain in the centre. Due to the asymmetry of the headgroups and the conformational freedom of the spacer, this molecule preferentially forms helical nanofibres rather than vesicles. The resulting spontaneous curvature arises from asymmetric packing within the aggregates, exemplifying how subtle variations in molecular geometry and flexibility can dictate supramolecular morphology. This demonstrates the overarching principle that intramolecular architecture directly determines the curvature, packing, and ultimately the function of self-assembled bolaamphiphilic systems.¹⁴⁸

Min-Chul Kim and Sang-Yup Lee reported a biomimetic catalyst formed through the self-assembly of a bolaamphiphilic molecule containing histidine moieties for carbon dioxide sequestration.¹⁴⁹ The histidyl bolaamphiphile, bis(*N*- α -amidohistidine)-1,7-heptane

dicarboxylate (His-C₇), was synthesised and self-assembled to generate supramolecular structures analogous to the active site of the enzyme carbonic anhydrase (CA) upon coordination with Zn²⁺ ions. Spectroscopic analysis confirmed that the coordination between the imidazole groups of histidine and Zn²⁺ ions closely mimics the core geometry of the CA active site. The His-C₇ assemblies were first allowed to self-assemble for one hour, after which ZnCl₂ was introduced to promote the formation of the CA-mimetic catalyst via Zn²⁺-imidazole coordination within the aggregates. The resulting nanostructures were characterised using scanning electron microscopy and transmission electron microscopy, revealing well-defined self-assembled morphologies. Catalytic activity was assessed by monitoring the hydrolysis of *p*-nitrophenyl acetate (*p*-NPA) to *p*-nitrophenol (*p*-NP), with product formation quantified by UV-Vis spectroscopy at 400 nm using a pre-calibrated standard curve. Systematic evaluation of the catalytic performance as a function of zinc concentration, pH, and temperature demonstrated that the His-C₇/Zn²⁺ assemblies effectively replicate key features including zinc-mediated water activation, ester substrate hydrolysis, pH-dependent catalytic behaviour and the formation of a localised microenvironment that enhances substrate binding and reaction. These characteristics collectively reproduce essential functional aspects of carbonic anhydrase catalysis, providing a promising strategy for the design of self-assembled biomimetic catalysts (**Figure 3.5**). This example of a bolaamphiphile forming assemblies capable of selectively binding a specific substrate inspired us to explore bolaamphiphilic versions of the amphiphiles described in the previous chapter for the construction of an IDA for phosphate sensing.



Figure 3.5. a) Schematic representation of the hydration process of CO₂ through the self-assembly of the histidyl bolaamphiphilic molecule (His-C₇), which stimulates the active site of carbonic anhydrase, and b) chemical structure of His-C₇ (His = Histidine). Source: Reprinted with permission from ref. 148. Copyright (2013) Chemical Reviews.

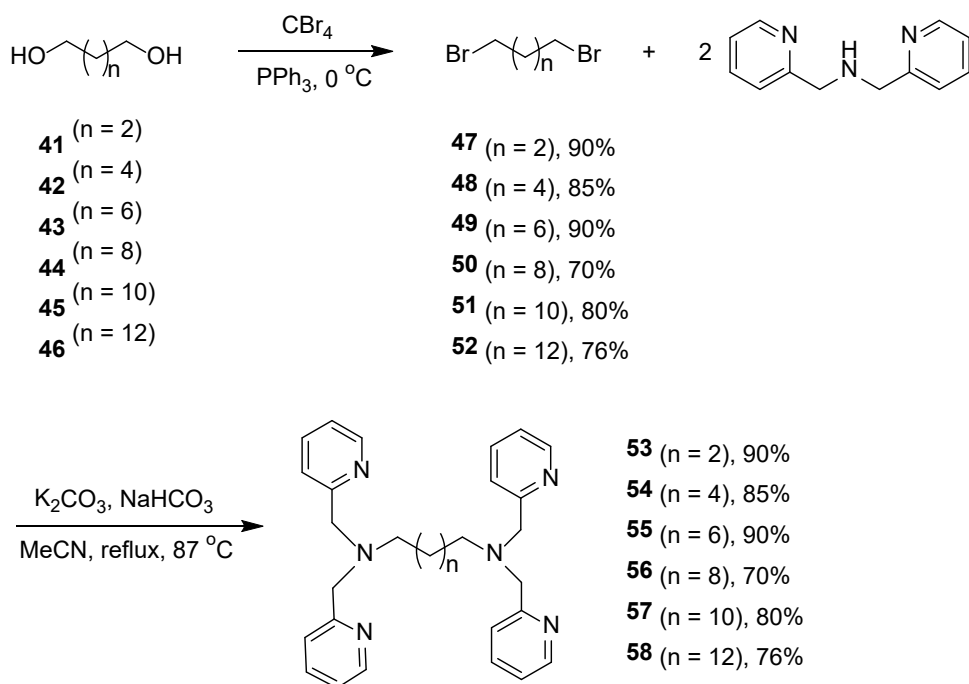
3.1.3 Aims of this Chapter

This aim of this chapter was to synthesise and analyse a series of di-picolyl bolaamphiphiles with varying alkyl chain lengths (C₁₄ to C₆) to evaluate their performance in zinc-based indicator displacement assays (IDAs). The study examined their binding to pyrocatechol violet (PV) and their ability to detect pyrophosphate (PPi) via indicator displacement, and the influence of molecular structure on sensing performance. Additionally, the research compared the effects of intramolecular versus intermolecular zinc coordination on indicator binding and PPi-induced displacement.

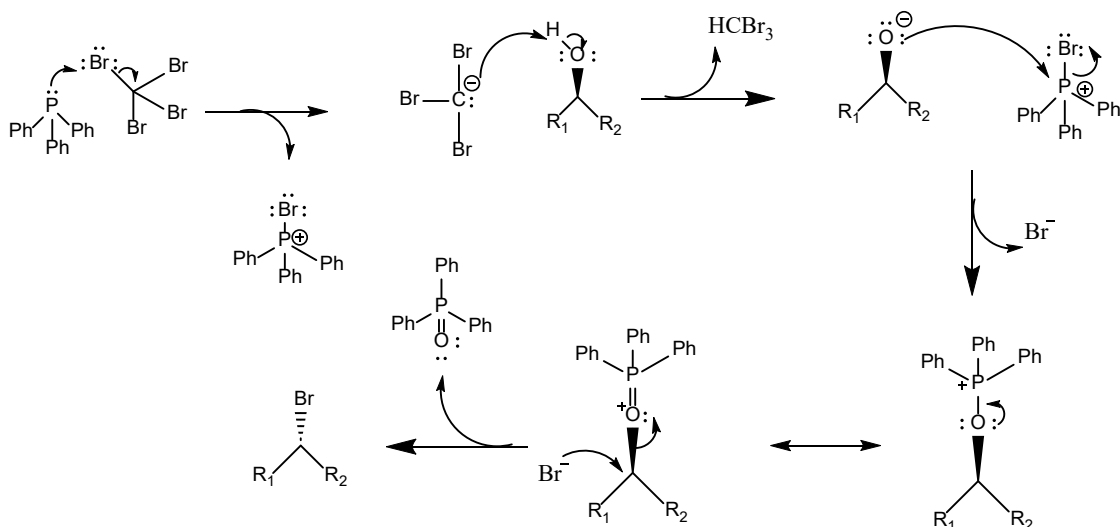
3.2 Initial Trials

3.2.1 Synthesis of the required ligands

Our next target was to synthesise the bis-picolyl-based ligands and investigate their self-assembly to form vesicle structures that have binding pockets on their surface that can selectively bind to different phosphate-containing molecules. So, we synthesised an amphiphile consisting of a di-picolylamine group and an n-carbon hydrophobic tail, displayed in **Scheme 3.1**. For this synthesis, we took 1,14-tetradecane diol in the presence of dry DCM, followed by an Appel reaction. The Appel reaction, an organic reaction mechanism that utilises carbon tetrabromide (CBr_4) to convert alcohols into alkyl bromides, owes its existence to the pioneering work of the German chemist Rolf Appel in the 1970s. The mechanism of the Appel reaction can be described in the following steps. The reaction begins with the interaction of triphenylphosphine (PPh_3) with carbon tetrabromide (CBr_4), resulting in the formation of a highly reactive phosphorus-containing intermediate, such as triphenylphosphonium bromide, which leads to the formation of an oxyphosphonium intermediate. The oxygen atom in the oxyphosphonium intermediate acquires a positive charge, making it a good leaving group. A bromide ion (Br^-) acts as a nucleophile and attacks the carbon atom bearing the leaving group in an $\text{S}_{\text{N}}2$ -type displacement followed by the formation of the alkyl bromide. The next step was the nucleophilic substitution of bromine for the di-picolylamine group via an $\text{S}_{\text{N}}2$ mechanism. We found the reaction itself to be successful. We eventually purified the crude product by using flash column chromatography using alumina instead of silica.



Scheme 3.1. Synthesis of the bis-picolyl-based ligands where n = 4, 6, 8, 10, and 12.



Scheme 3.2. Proposed mechanism of Appel conversion of diol to alkyl halide.

3.2.2 Initial Studies using C₁₄-di-picolyl-Zn^{II}

We began this study by investigating the ability of the bolaamphiphile C₁₄-di-picolyl **58** to bind to the indicator **PV**. UV-Vis titrations were conducted as in the previous chapter by adding increasing concentrations of the host C₁₄-di-picolyl **58** and Zn^{II} to a solution of

the indicator **PV** in HEPES buffer (HEPES = 5.0 mM, NaCl = 145 mM, pH = 7.4, **PV** = 20 μ M at 25 $^{\circ}$ C). The initial colour of the solution is yellow, indicative of unbound **PV**, and the solution turns blue upon effective complexation of **PV** with the picolyl-Zn^{II} moieties. **Figure 3.6** below shows the absorbance spectra for the titration of **PV** with C₁₄-di-picolyl-Zn^{II}. A bathochromic shift is seen in the absorption spectrum upon addition of increasing concentrations of C₁₄-di-picolyl **58**, with the formation of a new peak at 685 nm. The colour changes from yellow to blue following the addition of 60 μ M of C₁₄-di-picolyl-Zn^{II}, which is three times the concentration of the **PV** indicator. Given that each bolaamphiphilic receptor now contains two picolyl moieties, we would expect to see a 1:1 binding ratio between C₁₄-di-picolyl-Zn^{II} and **PV** if the binding affinity was high. The fact that we observe a 1:3 binding ratio between C₁₄-di-picolyl-Zn^{II} and **PV** suggests much weaker binding than what was generally observed with our self-assembled systems in the previous chapter.

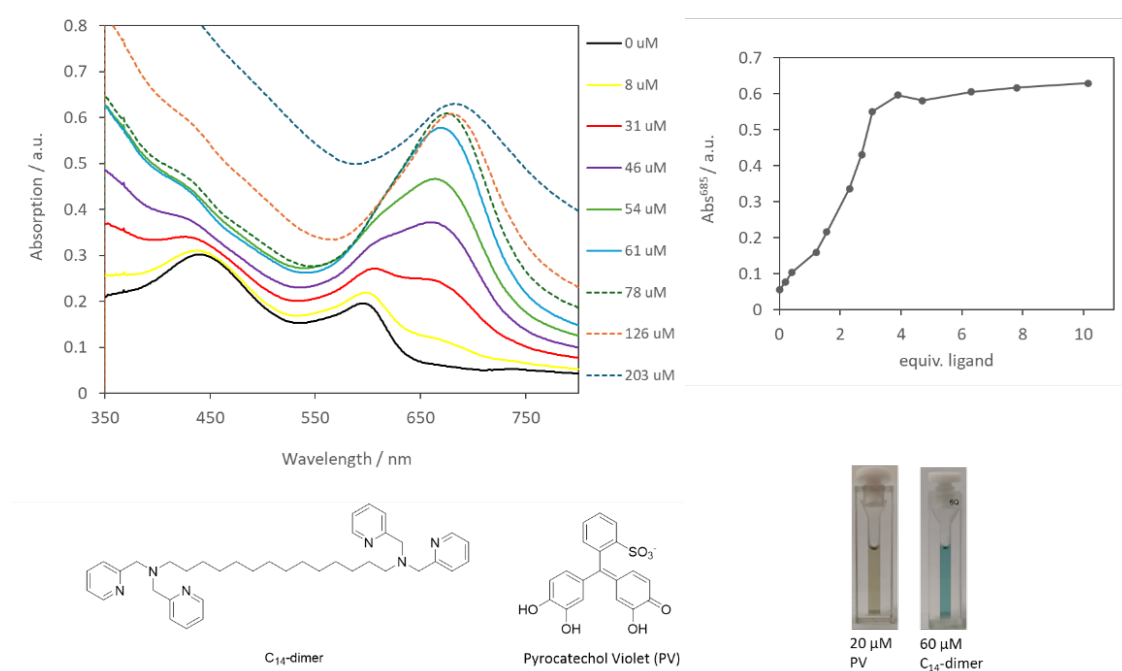
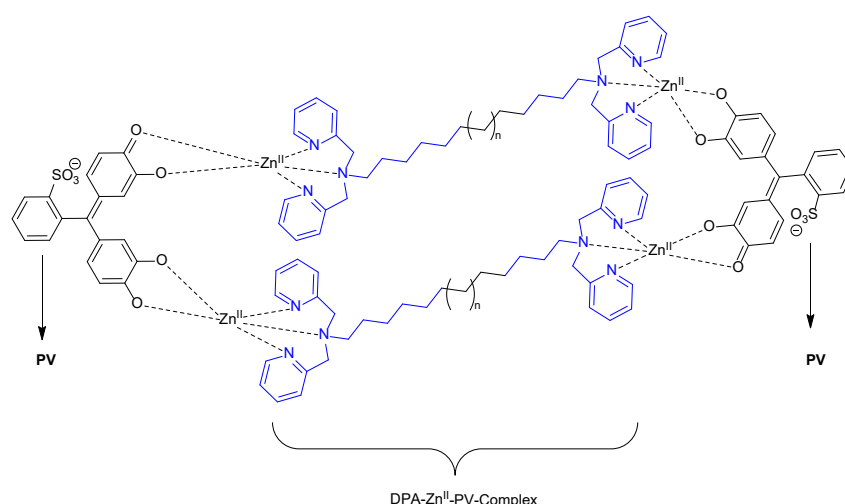


Figure 3.6. Changes in absorbance of **PV** solution with increasing C₁₄-di-picolyl-Zn^{II} concentration until 203 μ M. Conditions: [HEPES buffer] = 5 mM, pH 7.4, [NaCl] = 145 mM, [**PV**] = 20 μ M at 25 $^{\circ}$ C. C₁₄-di-picolyl-Zn^{II} concentrations = 0, 8, 31, 46, 54, 61, 78, 126, and 203 μ M.

A notable observation from our titration was a change in the baseline of the absorption spectrum, which became more prominent at higher concentrations. This behaviour was also observed in our amphiphilic systems investigated in the previous chapter, for amphiphiles possessing a hydrophobic chain with 12 or more carbon atoms. This was due to the formation of vesicular structures that were able to scatter light. We suspected that bolaamphiphile C₁₄-di-picolyl **58** was also forming vesicular structures in solution, as has been observed for similar bolaamphiphiles in the literature.¹⁵⁰ We therefore proposed that the **PV** indicator was in fact binding the ends of two different bolaamphiphilic ligands (**Scheme 3.3**) to form a tetrameric complex, which self-assembled to form vesicular structures.



Scheme 3.3. Formation of a di-picolylamine-Zn^{II}-**PV**-complex.

To investigate the presence of vesicles in the C₁₄-di-picolyl-Zn^{II}-**PV** mixture, we employed dynamic light scattering (DLS). We added 60 μM of C₁₄-di-picolyl-Zn^{II} bolaamphiphiles to 20 μM of the **PV** indicator under the same conditions as above (HEPES = 5.0 mM, NaCl = 145 mM, pH = 7.4, 25 °C). We observed a monodisperse sample with an average hydrodynamic diameter of ~1000 nm ± 344 (**Figure 3.7**), indicating the presence of large to giant vesicles. To confirm this observation, we examined these samples under a light microscope. The images of the C₁₄-di-picolyl-Zn^{II}

vesicles in the microscope confirm the presence of these large vesicles, with sizes in the vicinity of 1 μm . The sizes of these vesicles are large enough to significantly scatter light and is likely the culprit for the changes in the baseline of the absorption spectrum at increasing concentrations of $\text{C}_{14}\text{-di-picolyl}\cdot\text{Zn}^{\text{II}}$.

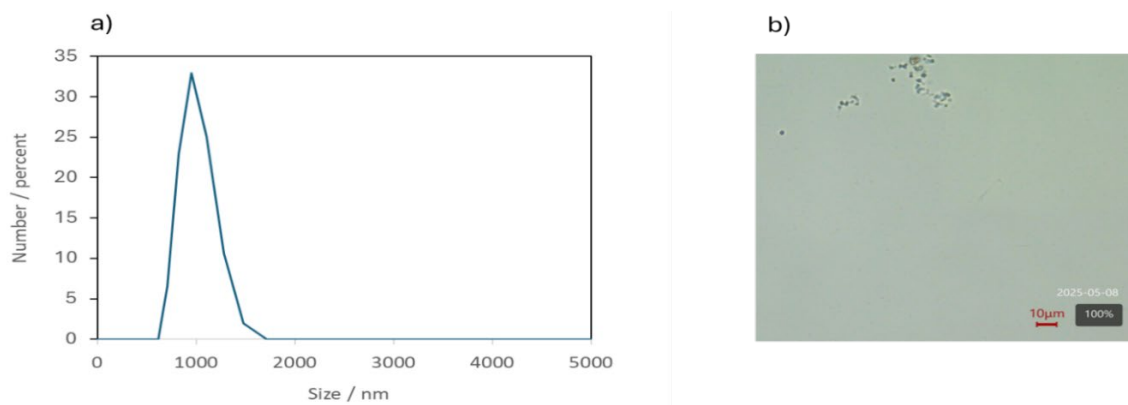


Figure 3.7. a) The hydrodynamic diameter of assemblies measured with dynamic light scattering (DLS) in the presence of **PV** (20 μM) for 40 μM $\text{C}_{14}\text{-di-picolyl}\cdot\text{Zn}^{\text{II}}$ system in aqueous HEPES. Conditions: [HEPES buffer] = 5 mM, pH = 7.4, [NaCl] = 145 mM, [**PV**] = 20 μM at 25 $^{\circ}\text{C}$. b) Under the same conditions, using an optical microscope.

We next used PPI to examine the displacement of **PV** from the self-assembled sensor. The host-indicator complex was first formed by the addition of 60 μM $\text{C}_{14}\text{-di-picolyl}\cdot\text{Zn}^{\text{II}}$ to 20 μM **PV** to form a blue solution (**Figure 3.8**). Upon the addition of increasing amounts of PPI, a decrease in the absorption peak at 685 nm was observed. The absorbance at 685 nm steadily decreases while the peak at 600 nm, due to unbound **PV**, becomes increasingly more prominent. However, colour change from blue to yellow occurs only after the addition of 400 μM of PPI, which is 20 times the amount of **PV** present. These results indicate that under these conditions, PPI is not able to efficiently displace **PV** from the host and therefore this system would not be a suitable IDA for the colourimetric detection of PPI.

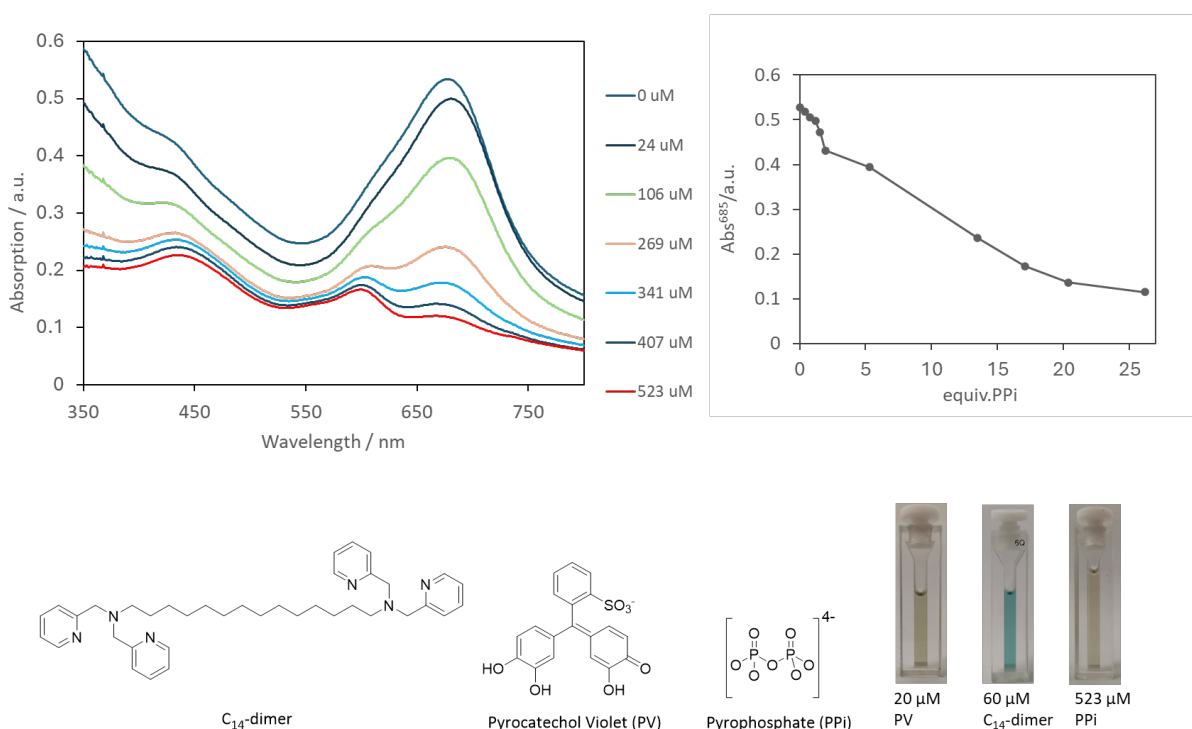


Figure 3.8. Absorbance spectra for the addition of PPI in the presence of amphiphilic ligand C_{14} -di-picolyl- Zn^{II} . Conditions: [HEPES buffer] = 5 mM, pH = 7.4, [NaCl] = 145 mM, [PV] = 20 μM , [C_{14} -di-picolyl- Zn^{II}] = 60 μM , [PPI] = 0, 24, 106, 269, 341, 407 and 523 μM .

We next investigated the displacement of the **PV** dye from our receptor C_{14} -di-picolyl- Zn^{II} using ATP. An initial solution containing 60 μM of C_{14} -di-picolyl- Zn^{II} and 20 μM **PV** was used, which possessed a blue colour. Next, ATP was added in increasing concentrations to displace the **PV** probe and return it to its original yellow colour. However, as in the case of PPI, we also observed a slow displacement of **PV** with ATP, requiring 523 μM to change the blue to yellow colour, which corresponds to a 1:26 ratio (**Figure 3.9**). The differences between PPI and ATP graphs are shown below, illustrating a small difference between the two, suggesting that the indicator is not selective for one analyte or the other. Furthermore, we attempted to displace **PV** using other anions, such as ATP, ADP and sodium citrate, but none of these were able to displace **PV**. Given the unsatisfactory results with this bolaamphiphile, we decided to investigate bolaamphiphiles of different chain lengths.

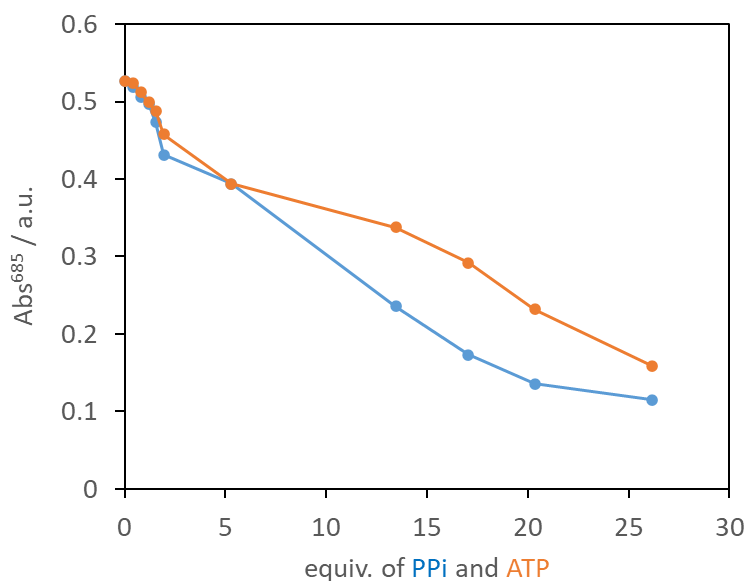


Figure 3.9. Comparing between PPI and ATP in the presence of 20 μM **PV** and 60 μM C_{14} -di-picolyl- Zn^{II}

3.2.3 Studies with C_{12} -di-picolyl- Zn^{II}

We next examined the potential of our shorter chain bolaamphiphile C_{12} -di-picolyl **57** to act as the host component of an IDA indicator. Absorbance spectra were taken as increasing amounts of C_{12} -di-picolyl **57**· Zn^{II} were added to a solution containing 20 μM **PV** (with HEPES = 5.0 mM, NaCl = 145 mM, pH = 7.4, at 25 °C). We observed a steady decrease in the peak at 600 nm, and the formation of a new peak at 670 nm, indicative of the complexation of **PV**. This was accompanied by a colour change from yellow to blue, which was observed upon addition of 31 μM of the host C_{12} -di-picolyl **57**· Zn^{II} (**Figure 3.10**). Compared to C_{14} -di-picolyl **57**· Zn^{II} , this represents a significant reduction in equivalents of host required to induce a colour change, which suggests more efficient binding between the host and the **PV** indicator. However, the determination of association constants by fitting the UV data into a model was not possible due to the significant changes in the baseline of the spectra.

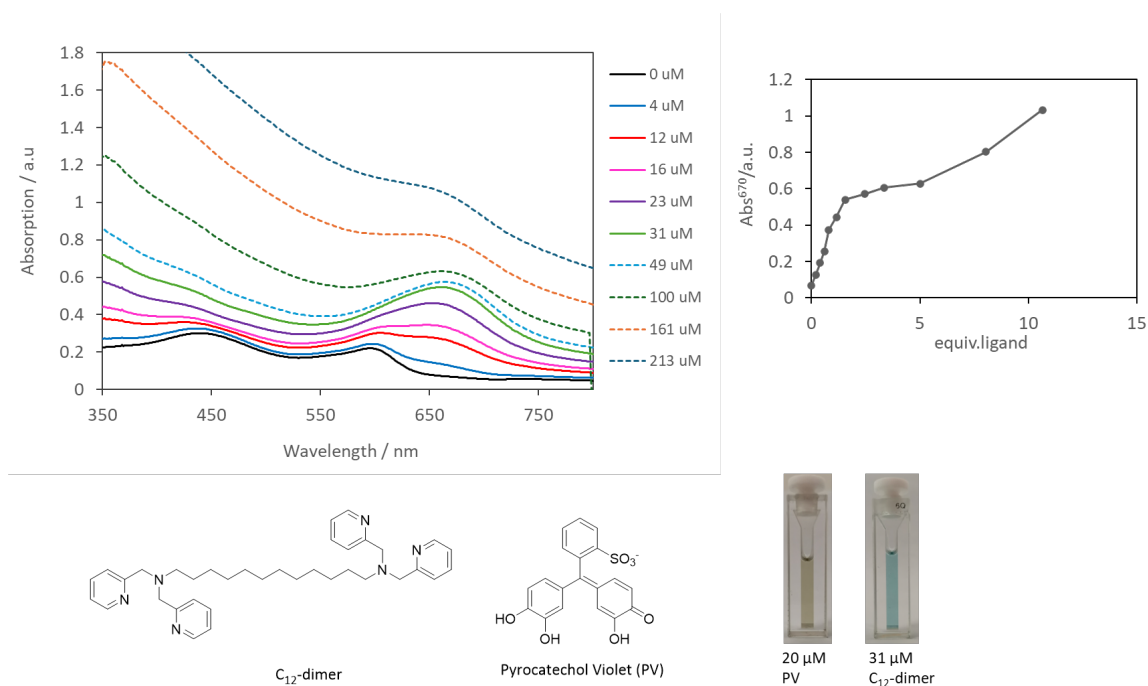


Figure 3.10. Changes in absorbance of **PV** solution with increasing C_{12} -di-picolyl- Zn^{II} concentration until 213 μM . Conditions: [HEPES buffer] = 5 mM, pH = 7.4, [NaCl] = 145 mM, [PV] = 20 μM at 25 $^{\circ}C$. C_{12} -di-picolyl- Zn^{II} concentrations = 0, 4, 12, 16, 23, 31, 49, 100, 161, and 213 μM .

Next, we investigated the displacement of the **PV** indicator from our receptor using PPI and ATP. An initial solution containing 31 μM of C_{12} -di-picolyl- Zn^{II} and 20 μM **PV** was used, which appeared blue. Next, increasing concentrations of PPI were added to displace the **PV** probe and return to the original yellow colour. With this system, 138 μM of PPI was required to observe this colour change, which is seven times the concentration of **PV**, and significantly less than the 20 equivalents needed with the C_{14} -di-picolyl- Zn^{II} host. These results suggest that C_{12} -di-picolyl- Zn^{II} is able to form binding pockets that are more effective for binding both the indicator **PV**, and the analyte PPI (**Figure 3.11**).

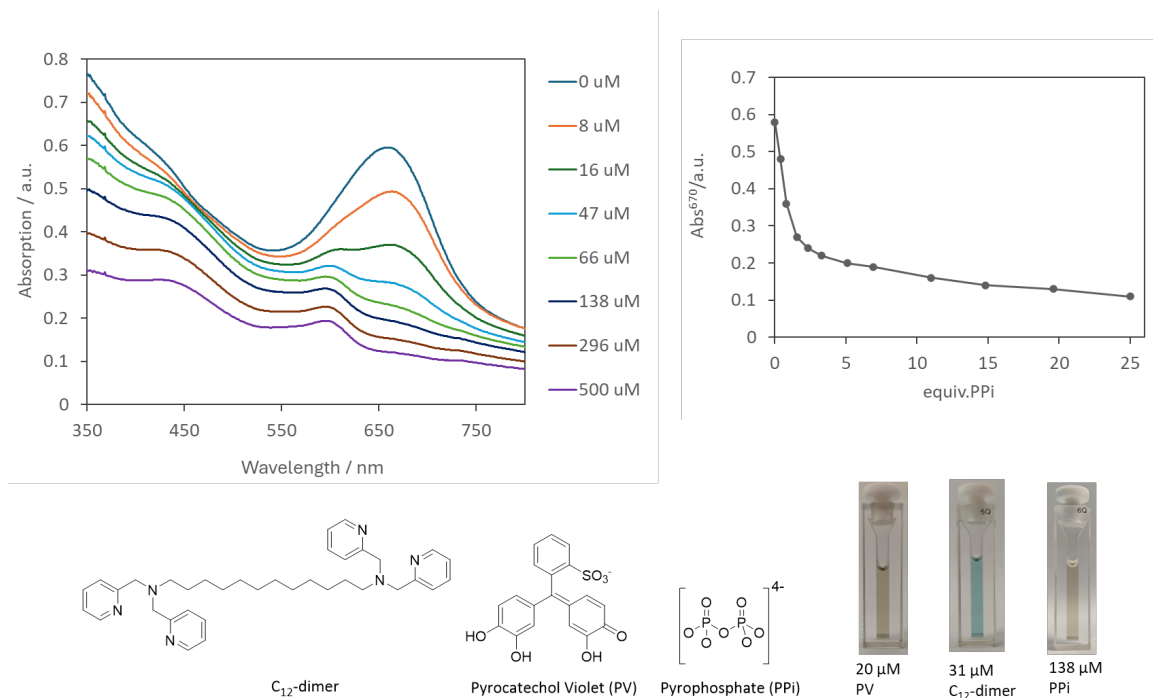


Figure 3.11. Absorbance spectra for the addition of PPI in the presence of amphiphilic ligands C_{12} -di-picolyl. Conditions: [HEPES buffer] = 5 mM, pH = 7.4, [NaCl] = 145 mM, [PV] = 20 μM , [PV]: [C_{12} -di-picolyl- Zn^{II}] = 1:7, [PPI] = 0, 8, 16, 47, 66, 138, 296, and 500 μM .

3.2.4 Studies with C_{10} -di-picolyl- Zn^{II}

Next, we explored the effectiveness of our next bolaamphiphile, C_{10} -di-picolyl **56**, to act as a host for the binding of PV. **Figure 3.12** shows that the changes in the absorbance spectra as increasing amounts of C_{10} -di-picolyl- Zn^{II} are gradually titrated into a solution containing PV (HEPES = 5.0 mM, NaCl = 145 mM, pH = 7.4, PV = 20 μM at 25 $^{\circ}C$). We observed the gradual decrease in the peak at 600 nm, coinciding with the emergence of a new peak at 635 nm. A distinct colour changes from yellow to blue was observed upon the addition of 20 μM of bolaamphiphile C_{10} -di-picolyl- Zn^{II} , suggesting strong 1:1 binding with the PV indicator. This suggests that C_{10} -di-picolyl- Zn^{II} **56** is much more effective at binding PV than the previously examined C_{12} -di-picolyl- Zn^{II} **57** and C_{14} -di-picolyl- Zn^{II} **58**. Interestingly, with this bolaamphiphile, we did not observe the very large changes in the baseline upon its addition, unlike the two analogues previously

investigated. The potential reasons for this difference are discussed later in this chapter. However, the slight changes in baseline (see how the absorbance starts to decrease at concentrations of the host greater than two equivalents) still prevented fitting of this data to obtain binding constants.

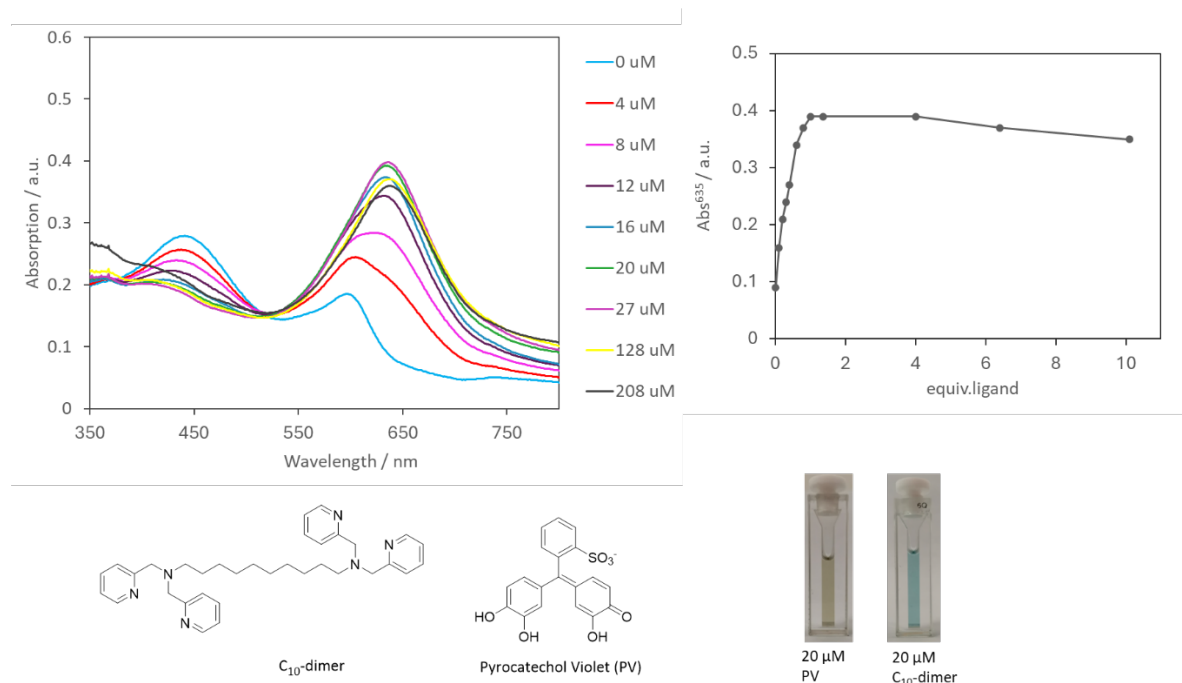


Figure 3.12. Changes in absorbance of **PV** solution with increasing C_{10} -di-picolyl- Zn^{II} concentration until 208 μM . Conditions: [HEPES buffer] = 5 mM, pH 7.4, [NaCl] = 145 mM, [**PV**] = 20 μM at 25 $^{\circ}C$. C_{10} -di-picolyl- Zn^{II} concentrations = 0, 4, 8, 12, 16, 20, 27, 128 and 208 μM .

The ability of this system to act as an IDA for detecting **PV** was next investigated. We began with an initial solution containing 20 μM of bolaamphiphile C_{10} -di-picolyl- Zn^{II} and 20 μM of **PV**, which had a distinct blue colour. We next gradually increased the concentration of PPI to displace the **PV** probe and return to the original yellow colour. Notably, we observed that lower concentrations of PPI, specifically 40 μM , were needed for the displacement of **PV** (**Figure 3.13**). This indicated that there was strong binding of PPI to the C_{10} -di-picolyl- Zn^{II} host, and that this binding was able to effectively displace **PV**.

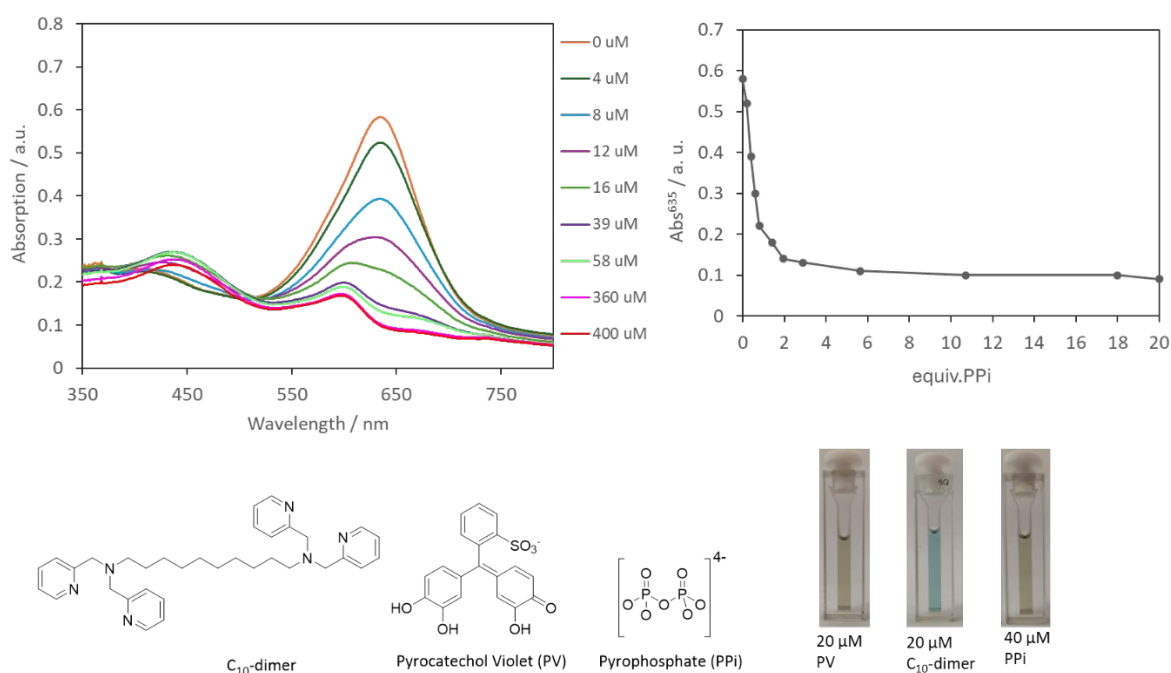


Figure 3.13. Absorbance spectra for the addition of PPI in the presence of amphiphilic ligand C₁₀-di-picolyl·Zn^{II}. Conditions: [HEPES buffer] = 5 mM, pH = 7.4, [NaCl] = 145 mM, [PV] = 20 μM, [C₁₀-di-picolyl·Zn^{II}] = 40 μM, [PPI] = 0, 4, 8, 12, 16, 39, 58, 360 and 400 μM.

To assess the selectivity of our system for PPI, ATP, ADP, and citrate, we prepared a solution containing 40 μM of bolaamphiphile C₁₀-di-picolyl·Zn^{II} and 20 μM of **PV**. Upon adding the analytes to the solution, we observed that 40 μM of PPI was required to displace the PV. In contrast, 200 μM of ATP and 360 μM of ADP were required to induce a colour change, and up to 400 μM of citrate did not change the colour from blue to yellow and did not allow the UV peak to return to the original **PV** peak (**Figure 3.14**). This indicates good selectivity of this IDA for PPI, with a 5-fold difference in analyte needed (compared to ATP) to reach the visibly discernible endpoint. However, when we compare these results to the peptide-based sensors from Jolliffe and co-workers, they were able to achieve more than a 5-fold difference in sensitivity. Therefore, we continued to investigate the performance of other analogues in our series of bolaamphiphiles.

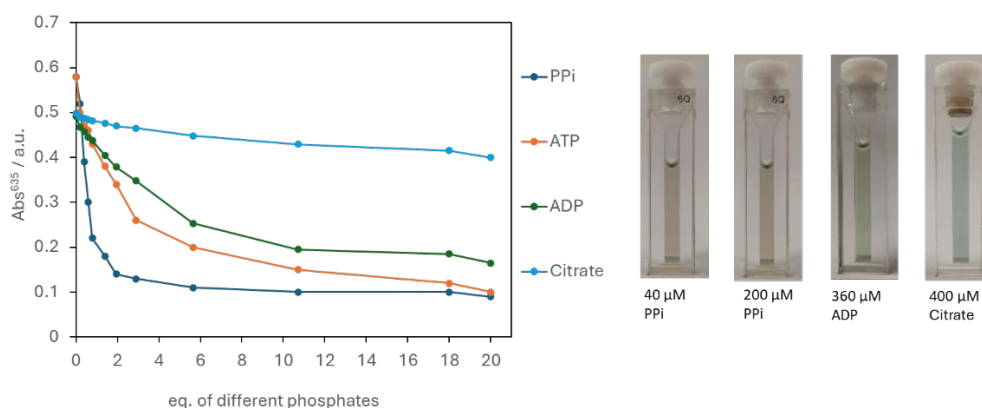


Figure 3.14. Comparing between PPI and ATP in the presence of 20 μM **PV** and 20 μM $\text{C}_{10}\text{-di-picolyl}\cdot\text{Zn}^{\text{II}}$

3.2.5 Studies with $\text{C}_8\text{-di-picolyl}\cdot\text{Zn}^{\text{II}}$ 55

We continued our UV studies by combining bolaamphiphile $\text{C}_8\text{-di-picolyl}\cdot\text{Zn}^{\text{II}}$ with **PV** to investigate binding affinity. We started by having a fixed amount of **PV** (20 μM) and adding increasing concentrations of our picolyl ligand $\text{C}_8\text{-di-picolyl}\cdot\text{Zn}^{\text{II}}$. **Figure 3.15** below shows how the spectrum changes with increasing concentrations of $\text{C}_8\text{-di-picolyl}\cdot\text{Zn}^{\text{II}}$, with the gradual decrease of the peak at 600 nm and the formation of a new peak at 640 nm. We observed a clear colour change from yellow to blue upon addition of 20 μM of $\text{C}_8\text{-di-picolyl}\cdot\text{Zn}^{\text{II}}$, again indicating strong 1:1 binding with **PV**. Fitting of the binding isotherm to a 1:1 binding model, using Bindfit gave a K of 8.5×10^6 , but with a very large error of 1200%. It was concluded that these systems are challenging to fit due to complex self-assembly phenomena that can affect the scattering of light and therefore the binding isotherm.

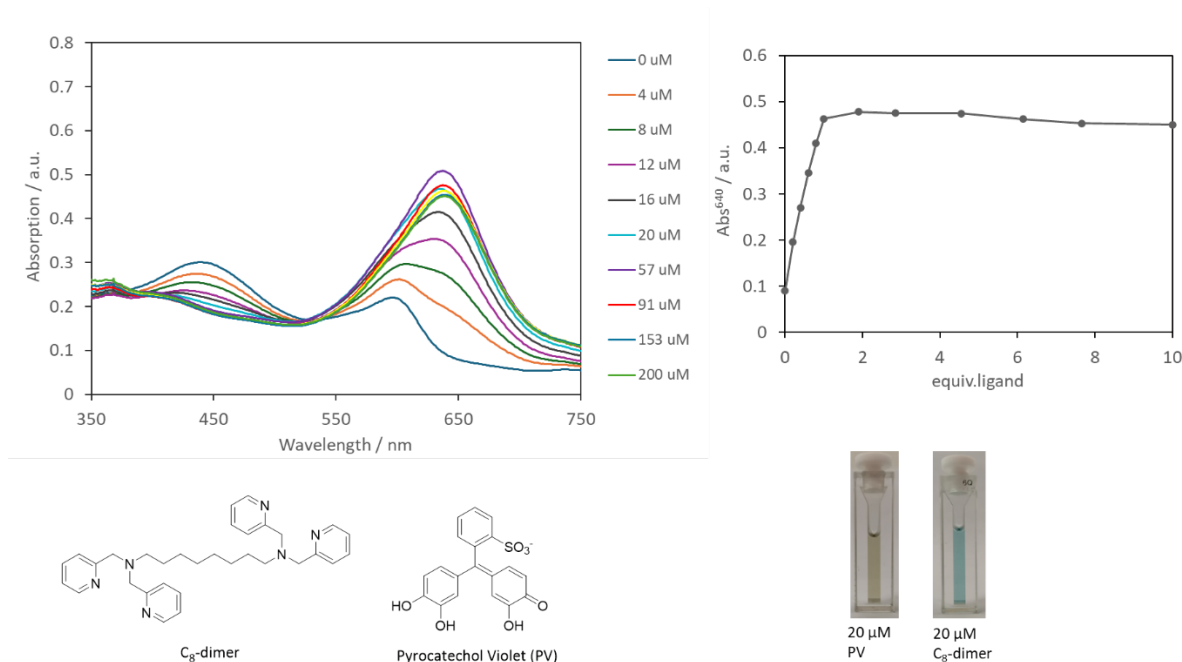


Figure 3.15. Changes in absorbance of **PV** solution with increasing C₈-di-picolyl·Zn^{II} concentration until 200 μM. Conditions: [HEPES buffer] = 5 mM, pH 7.4, [NaCl] = 145 mM, [**PV**] = 20 μM at 25 °C. C₈-di-picolyl·Zn^{II} concentrations = 0, 4, 8, 12, 16, 20, 57, 91, 153, and 200 μM.

Next, we tested whether this combination could be used as an IDA for PPI. We first combined 20 μM of the indicator **PV** and 20 μM of C₈-di-picolyl·Zn^{II} in solution and added increasing amounts of PPI to see if we could displace the C₈-di-picolyl·Zn^{II} from the indicator **PV**. Upon adding PPI, we observed a significant change in the spectrum upon addition of just the first aliquot (2 μM) of PPI, and a colour change from blue to yellow upon addition of 20 μM of PPI (**Figure 3.16**). The 1:1 binding ratio of C₈-di-picolyl·Zn^{II} to **PV** was as good as could have been hoped for and matched the performance of the peptide sensors developed by Jolliffe and co-workers. These results surpass those described earlier in this chapter, which all required more than one equivalent of PPI to displace **PV** from the host.

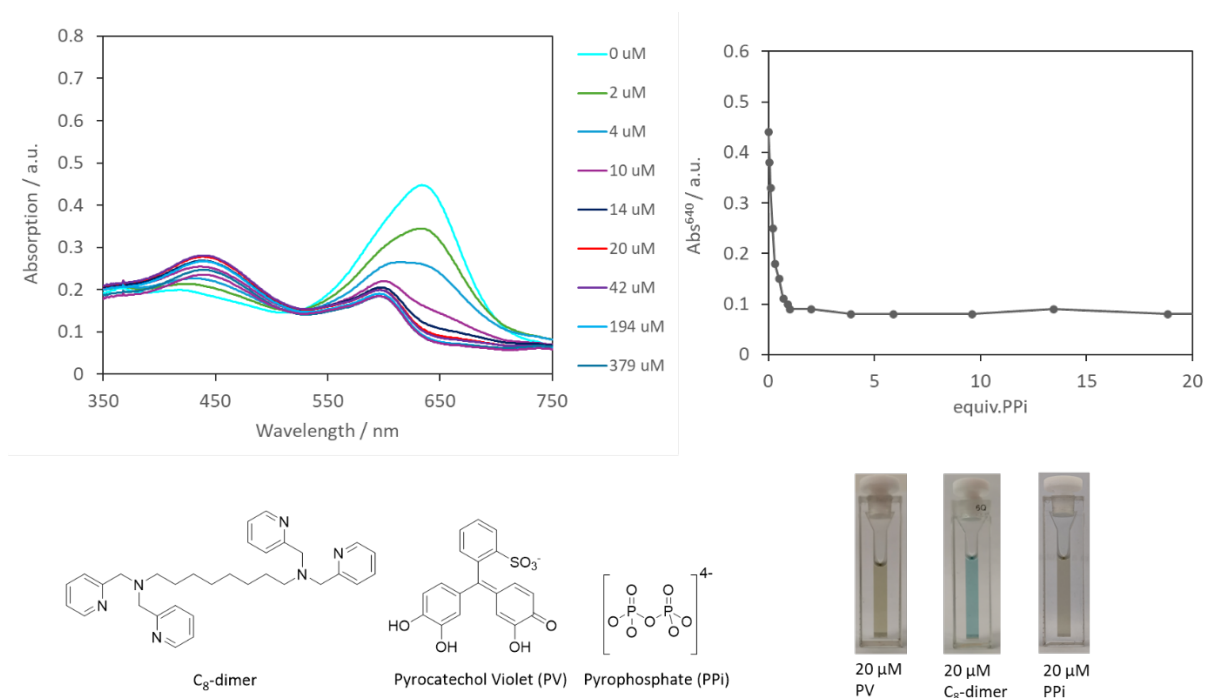


Figure 3.16. Absorbance spectra for the addition of PPI in the presence of amphiphilic ligands C₈-di-picolyl·Zn^{II}. Conditions: [HEPES buffer] = 5 mM, pH = 7.4, [NaCl] = 145 mM, [PV] = 20 μM, [C₈-di-picolyl·Zn^{II}] = 20 μM, [PPi] = 0, 2, 4, 10, 14, 20, 42, 194 and 379 μM.

To determine the selectivity of the IDA for PPI, the displacement of **PV** by ATP, ADP and sodium citrate was next investigated (**Figure 3.17**). Nine equivalents of ATP were required to induce a colour change back to yellow, whereas 17 equivalents of ADP were needed. Notably, the sample containing citrate did not change colour from blue to yellow even after the addition of 23 equivalents. Given that only one equivalent of PPI was required to induce a colour change, these results demonstrate excellent selectivity of the host for PPI and the promising potential of this system as a selective IDA.

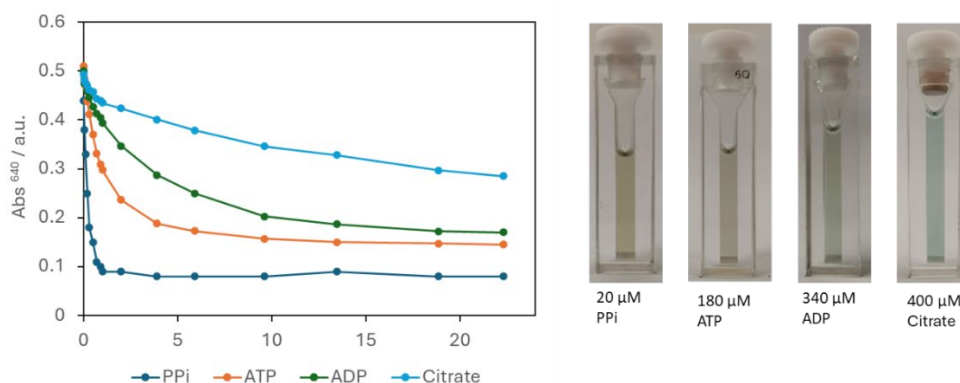


Figure 3.17. Absorbance spectra for the displacement of **PV** with other phosphate anions such as PPI, ATP, ADP and citrate in the presence of C_8 -di-picolyl- Zn^{II} [20 μ M]. Conditions: [HEPES buffer] = 5 mM, pH = 7.4, [NaCl] = 145 mM, [**PV**] = 20 μ M at 25 $^{\circ}$ C.

To demonstrate the general applicability of this system as a selective IDA sensor for PPI, we examined the colorimetric response of the mixture upon addition of a range of phosphates. The selectivity studies were performed using 20 μ M of C_8 -di-picolyl- Zn^{II} and 20 μ M of **PV**), to which was added 5 equivalents (100 μ M) of one of the following analytes: PPI, ATP, ADP, AMP, DMP, phosphoserine, phosphotyrosine, sodium phosphate, sodium biphosphate, and sodium citrate). **Figure 3.18** shows the result of this experiment, where only the sample containing PPI exhibited a colour change from blue to yellow, indicating displacement of the indicator and the presence of free **PV** in solution. All the other samples remained blue, indicating excellent selectivity of this system for PPI over similar anions.

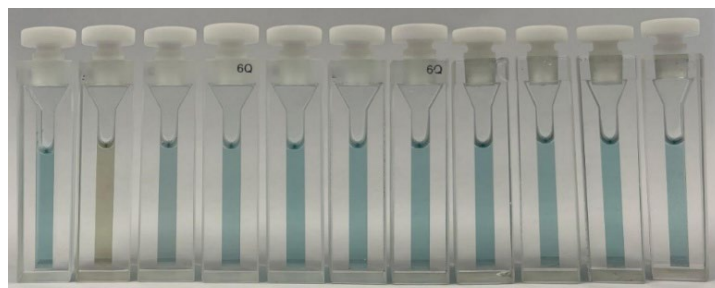


Figure 3.18. The colours of the 1:1 mixture of C_8 -di-picolyl- Zn^{II} -**PV** (20 μ M each) with and without anions (sodium salts) from left to right: No anion, PPI, ATP, ADP, AMP, cAMP, phosphoserine, phosphotyrosine, HPO_4^{2-} , PO_4^{3-} , and citrate (5 equiv. each).

3.2.6 Studies with C₆-di-picolyl·Zn^{II} and comparison with previous analogues

The binding and displacement experiments were also performed with our final bolaamphiphile C₆-di-picolyl·Zn^{II}, and these results are presented in **Figure 3.19a**, together with the other bolaamphiphiles in this series of hosts. Decreasing the hydrophobic chain by an additional two carbons resulted in weaker binding with **PV**, with two equivalents of the C₆-di-picolyl·Zn^{II} host now required to induce the colour change from yellow to blue. This indicates that the optimum chain length is C₈, which was the only host we found that was able to bind to **PV** in a 1:1 ratio. For each of the hosts, the equivalences required to obtain a colour change with 20 μM of **PV** are summarised in **Table 3.1**. It is interesting to note that the results with the C₁₄-di-picolyl·Zn^{II} and C₁₂-di-picolyl·Zn^{II} systems do not appear to follow the same trend as the C₁₀-di-picolyl·Zn^{II}, C₈-di-picolyl·Zn^{II} and C₆-di-picolyl·Zn^{II} systems, which means C₁₀-di-picolyl·Zn^{II}, C₈-di-picolyl·Zn^{II} and C₆-di-picolyl·Zn^{II} exhibited a consistent relationship between chain length and sensing performance. However, the C₁₄-di-picolyl·Zn^{II} and C₁₂-di-picolyl·Zn^{II} systems deviated from this pattern, suggesting that longer alkyl chains may alter the self-assembly behaviour or the zinc coordination environment, leading to different indicator-binding and displacement properties (**Figure 3.19b**). This suggests potentially different mechanisms may be in place, which will be discussed later in this chapter.

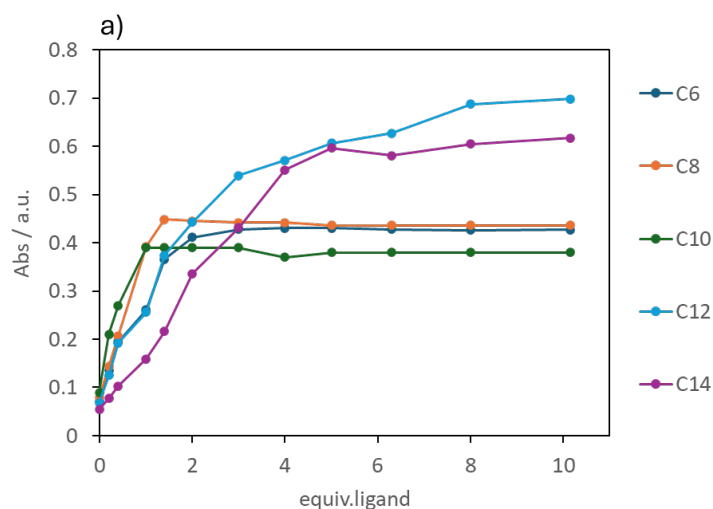


Figure 3.19a Binding isotherms for the different bolaamphiphiles examined in this chapter.

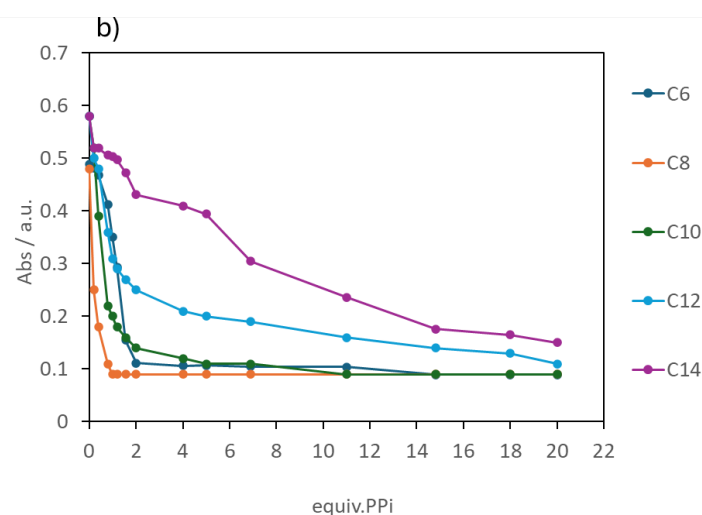


Figure 3.19b Displacement isotherms for the different bolaamphiphiles examined in this chapter.

Table 3.1. Summary of the binding affinity of the different bolaamphiphiles towards to **PV**, and the ease by which the resulting complex is displaced by **PPi** and **ATP**.

Host	Eq. required	PPi required	ATP required	Selectivity
di-picolyI·Zn ^{II}	yellow → blue	blue → yellow	blue → yellow	factor

C ₁₄	3.0	20	-	-
C ₁₂	1.5	6.9	-	-
C ₁₀	1.0	2.0	10	5
C ₈	1.0	1.0	9.0	9
C ₆	2.0	2.0	3.0	1.5

Through displacement experiments, it was found that **PV** could be displaced from the C₆-di-picolyl·Zn^{II} complex with two equivalents of PPI, accompanied by a colour change from blue to yellow (**Figure 3.20**). This suggests the formation of binding pockets that are much more efficient at binding PPI compared to **PV**. Further displacement experiments showed that three equivalents of ATP were required to displace **PV**, which demonstrates poor selectivity and an inability for the sensor to distinguish between PPI and ATP. This indicates that C₆-di-picolyl·Zn^{II} would not be a good bolaamphiphile for the further development of this IDA.

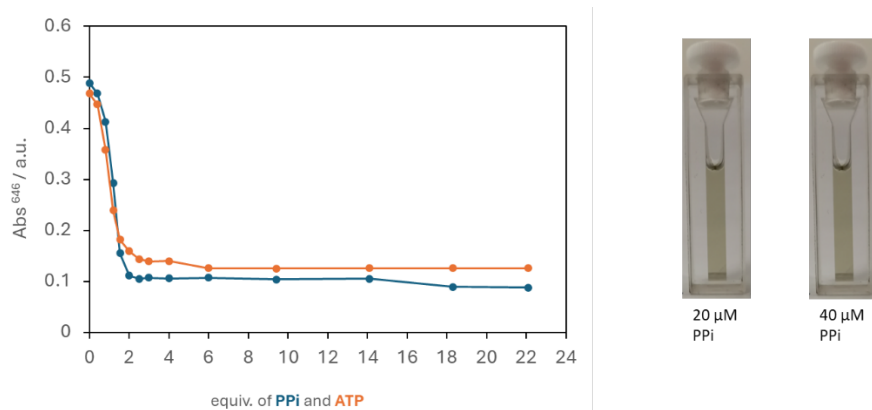


Figure 3.20. Absorbance spectra for the displacement of **PV** with other phosphate anions such as PPI, and ATP in the presence of C₆-di-picolyl·Zn^{II} (40 μM each). Conditions: [HEPES buffer] = 5 mM, pH 7.4, [NaCl] = 145 mM, [**PV**] = 20 μM at 25 °C.

3.3 Further characterisation of the C₈-di-picolyl·Zn^{II} system

3.3.1 NMR binding studies

Given that the C₈-di-picolyl·Zn^{II} bolaamphiphile was our most promising host for the development of a selective IDA for PPI, additional characterisation was performed to better understand this system. We first followed the binding and displacement of **PV** from C₈-di-picolyl·Zn^{II} using NMR spectroscopy, to confirm the 1:1 stoichiometry of binding. These experiments were performed in D₂O and required all the stock solutions, including the HEPES buffer, NaCl salt, **PV**, and host solutions to be prepared in D₂O. ¹H NMR spectra were taken as successive aliquots of C₈-di-picolyl·Zn^{II} was added to a solution containing **PV** (100 μM). We focused on the chemical shifts in the region of 6.5 to 8.5 ppm, as this is where the characteristic aromatic signals of **PV** and the picolyl groups appear (**Figure 3.21**). Prior to the addition of any C₈-di-picolyl·Zn^{II}, we can observe the characteristic peaks from the picolylamine groups, which consist of a doublet at 6.65 ppm, a singlet at 6.76 ppm, two doublets at 7.02 and 7.25 ppm, a multiplet at 7.55 ppm and a doublet at 7.90 ppm. Upon addition of 0.2 equivalents of C₈-di-picolyl·Zn^{II}, one can see the intensities of these signals decrease. These signals decrease even further in the presence of 0.4 equivalents of the bolaamphiphile and become small broad signals in the presence of 0.8 eq. of the bolaamphiphile. Upon the addition of a full equivalent of C₈-di-picolyl·Zn^{II}, these signals have completely disappeared, and a completely flat line is observed. This experiment demonstrates the clear 1:1 stoichiometry between **PV** and C₈-di-picolyl·Zn^{II}. As the concentration of the bolaamphiphile is increased further, new signals appear at 7.52 (doublet of doublets), 8.05 (triplet) and 8.55 (doublet), which can be attributed to the picolyl groups present in the host. These results are consistent with the UV titrations described above.

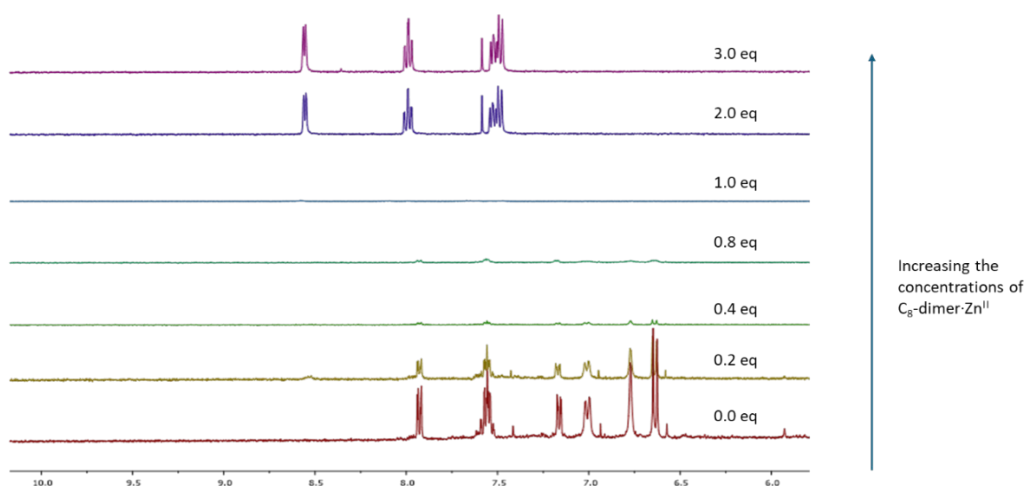


Figure 3.21. ^1H NMR spectra (400 MHz) from the probe **PV** (100 μM) and increasing the concentrations of $\text{C}_8\text{-di-picolyl}\cdot\text{Zn}^{\text{II}}$ = 0, 0.2, 0.4, 0.8, 1.0, 2.0, 3.0 equivalents in HEPES buffer at pH = 7.4 in D_2O .

Our next step was to conduct an experiment involving the $\text{C}_8\text{-di-picolyl}\cdot\text{Zn}^{\text{II}}$ -**PV** complex and PPI to investigate the displacement of **PV** with PPI. Our starting point has 100 μM of **PV** and 100 μM of $\text{C}_8\text{-di-picolyl}\cdot\text{Zn}^{\text{II}}$, which shows an absence of signals in the aromatic region (**Figure 3.22**). Upon the addition of 0.2 eq. of PPI, signals corresponding to the aromatic's protons of the picolyl group appear. Following the addition of 0.4 eq. of PPI, these signals become more prominent, and a singlet at 6.76 ppm and a doublet at 6.65 ppm also start to appear, which can be attributed to **PV**. The signals associated with **PV** continue to increase at higher concentrations of PPI, and further increases are seen between 2 and 3 equivalents of PPI, which suggests that more than 2 equivalents of PPI are required to fully displace **PV** from the host. This suggests that under these conditions, which possess a much higher concentration of the host and **PV**, the relative binding affinity of the system for **PV** vs PPI is different. We investigate concentration-related effects later on in this chapter.

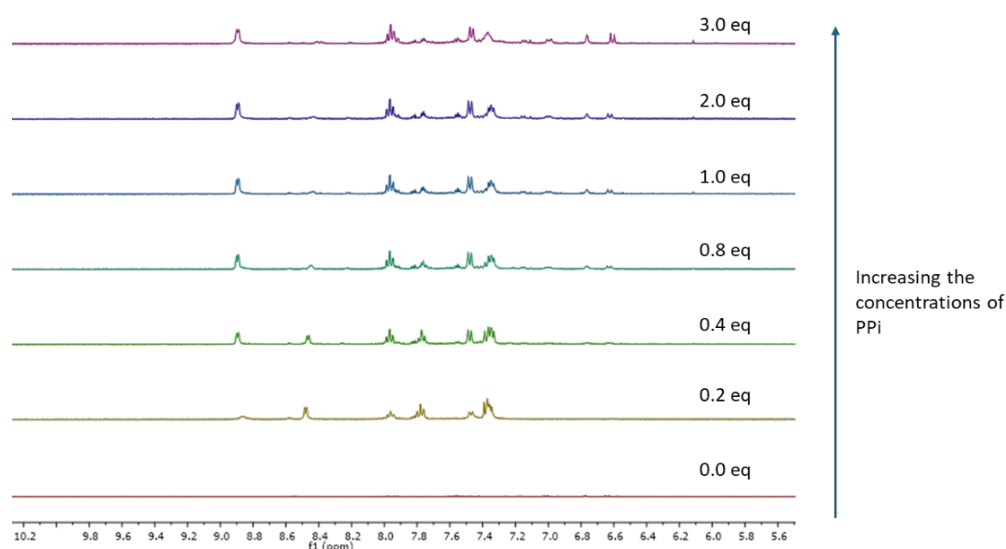


Figure 3.22. ^1H NMR spectra (400 MHz) from the probe $\text{C}_8\text{-di-picolyl-Zn}^{\text{II}}\text{-PV}$ (100 μM) complex with 1:1 ratio and increasing the concentrations of $\text{PPi} = 0, 0.2, 0.4, 0.6, 1.0, 2.0$ equivalents in HEPES buffer at $\text{pH} = 7.4$ in D_2O .

3.3.2 Investigation of the self-assembled structures formed

To determine whether the bolaamphiphile $\text{C}_8\text{-di-picolyl-Zn}^{\text{II}}$ formed individual monomeric receptors for **PV**, or assembled to form supramolecular structures in solution, we probed the solution using dynamic light scattering. The solution was first formed by adding 20 μM of $\text{C}_8\text{-di-picolyl-Zn}^{\text{II}}$ to 20 μM of **PV** in HEPES buffer (5 mM) and NaCl (145 mM). Dynamic light scattering revealed the presence of structures with a hydrodynamic diameter of $\sim 492 \text{ nm} \pm 268 \text{ nm}$, which suggests the formation of large vesicles, as this size is too large for micellar structures (**Figure 3.23**).

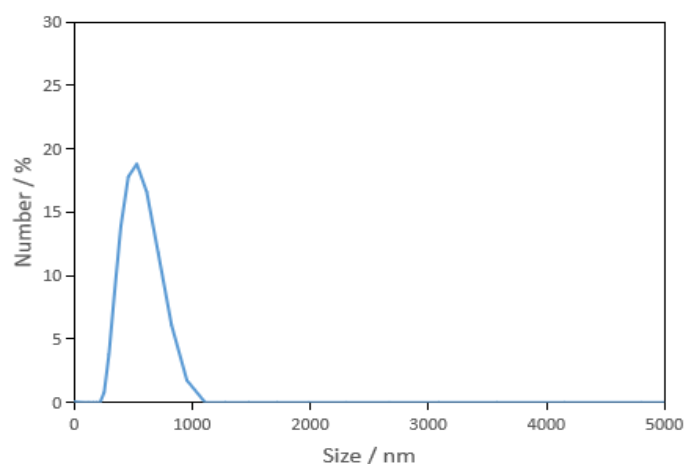


Figure 3.23. The hydrodynamic diameter of assemblies measured with dynamic light scattering (DLS) in the presence of **PV** (20 μM) for 20 μM $\text{C}_8\text{-di-picolyl}\cdot\text{Zn}^{\text{II}}$ system in aqueous HEPES. Conditions: [HEPES buffer] = 5 mM, pH = 7.4, [NaCl] = 145 mM, at 25 $^{\circ}\text{C}$.

Transmission electron microscopy (TEM) was employed to confirm the formation and morphology of vesicular structures in solution. A sample containing $\text{C}_8\text{-di-picolyl}\cdot\text{Zn}^{\text{II}}$ (20 μM) and **PV** (20 μM) was prepared in aqueous buffer. For grid preparation, a carbon-coated, formvar-supported copper grid was first glow-discharged under vacuum to render the surface hydrophilic and negatively charged, thereby promoting adsorption of aqueous samples. A droplet (40 μL) of the sample was pipetted onto a clean Parafilm surface, and the glow-discharged grid was gently floated on the droplet with the carbon-coated side facing down. After allowing adsorption for 45 sec, excess liquid was wicked off using filter paper. The grid was then stained with a 1% (w/v) aqueous solution of uranyl acetate for 45 seconds to enhance image contrast. Following blotting and complete drying, the grid was imaged using the transmission electron microscope. The microscope revealed images of hollow circles that confirmed the presence of vesicles. The majority of the structures seen ranged from 100 to 300 nm in diameter (**Figure 3.24**), which is smaller than the average obtained by DLS, but supports the presence of large vesicles. The difference in size visualised by the two methods could be due to either aggregation of smaller vesicles to form larger ones in the DLS samples, but it could also be that the larger vesicles were not as easily adsorbed onto the grids during the preparation of the TEM

samples.

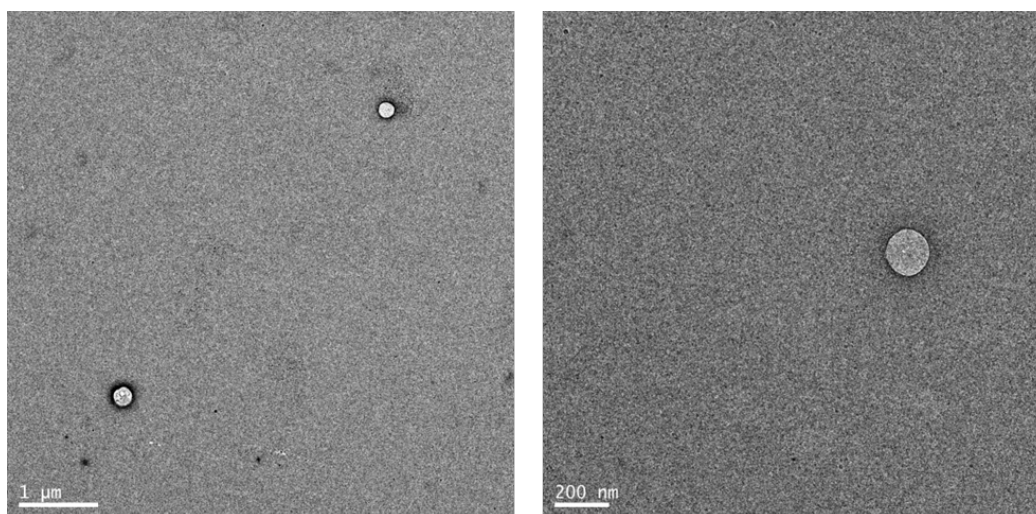


Figure 3.24. TEM images of sample of: **PV** (20 μM) and 20 μM $\text{C}_8\text{-di-picolyl-Zn}^{\text{II}}$ system in aqueous HEPES. Conditions: [HEPES buffer] = 5 mM, pH = 7.4, [NaCl] = 145 mM, at 25 $^{\circ}\text{C}$. Samples stained with uranyl acetate prior to imaging.

3.3.3 Determination of the critical aggregation concentration

A key parameter of vesicles is the concentration at which self-assembly occurs, known as the critical aggregation concentration (CAC). This parameter is analogous to the critical micellar concentration (CMC) for micelles, and can be determined using a variety of methods, typically using a tensiometer or fluorescence-based methods. We have chosen a fluorescence-based method for our studies due to the lower amounts of material required for this methodology, and its effectiveness in determining low CAC values, which we expected for our bolaamphiphiles. The CAC determination was performed by titrating an increasing amount of $\text{C}_8\text{-di-picolyl-Zn}^{\text{II}}$ into a HEPES buffer solution (pH 7.4), in the presence of **PV** (20 μM), and Nile red (1 μM), a hydrophobic fluorescent probe that preferentially partitions into hydrophobic environments. The sample was excited at 550 nm, and the intensity was measured at 600 nm as the bolaamphiphile was sequentially added. We were able to observe a change in the slope of the plot of the emission intensity vs bolaamphiphile concentration, which occurred at 6.7 μM . This inflection point indicates a change in the assembly state of the bolaamphiphiles, as Nile

red is solvatochromic and highly sensitive to the polarity of its surrounding environment. This CAC is very low, and interestingly, the CAC calculated in the absence of **PV** is also measured at a very low 5.0 μM (**Figure 3.25** and **Figure 3.26**). This suggests that $\text{C}_8\text{-di-picolyl}\cdot\text{Zn}^{\text{II}}$ forms self-assembled structures on its own, even in the absence of **PV**.

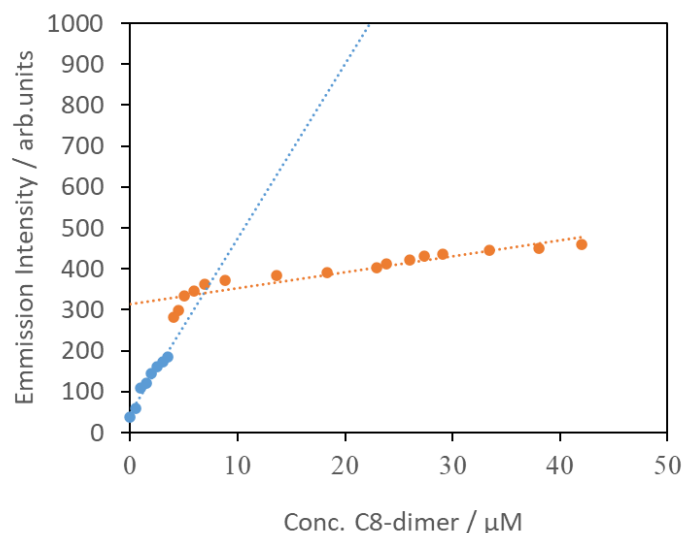


Figure 3.25. Emission intensity of Nile red (1 μM $\lambda_{\text{ex}} = 550$ nm, $\lambda_{\text{em}} = 660$ nm) at increasing concentrations of $\text{C}_8\text{-di-picolyl}\cdot\text{Zn}^{\text{II}}\cdot\text{PV}$ in aqueous HEPES buffer solution (5 mM, pH 7.4), [Nile red] = 1 μM .

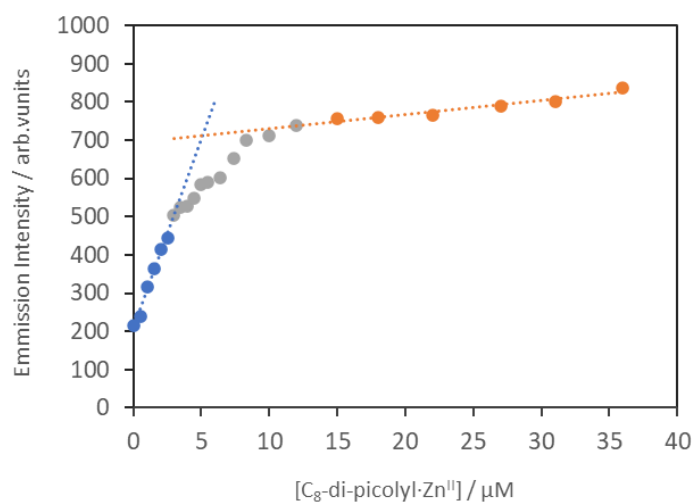


Figure 3.26. Emission intensity of Nile red (1 μM $\lambda_{\text{ex}} = 550$ nm, $\lambda_{\text{em}} = 660$ nm) at increasing concentrations of $\text{C}_8\text{-di-picolyl}\cdot\text{Zn}^{\text{II}}$ (in the absence of **PV**) in aqueous HEPES buffer solution (5 mM, pH 7.4), [Nile red] = 1 μM .

3.3.4 Intramolecular vs Intermolecular binding?

We reasoned that there were two ways by which these bolaamphiphiles could self-assemble to form vesicular structures, either with the bolaamphiphile extending the whole length of the vesicular bilayer, or with the bolaamphiphile folded into a U-shape and forming a double-headed amphiphile (**Figure 3.27**). In the first case, the **PV** indicator would be binding intermolecularly with two picolyl moieties from neighbouring bolaamphiphiles, while in the second example, **PV** would be binding intramolecularly with picolyl moieties within the same molecule. We hypothesised that we could distinguish between intermolecular and intramolecular binding by performing binding experiments at different concentrations of the bolaamphiphile. The idea is that intermolecular binding should be affected to a greater extent by the concentration of receptor than intramolecular binding, with the binding ratios not expected to change significantly for intramolecular binding.

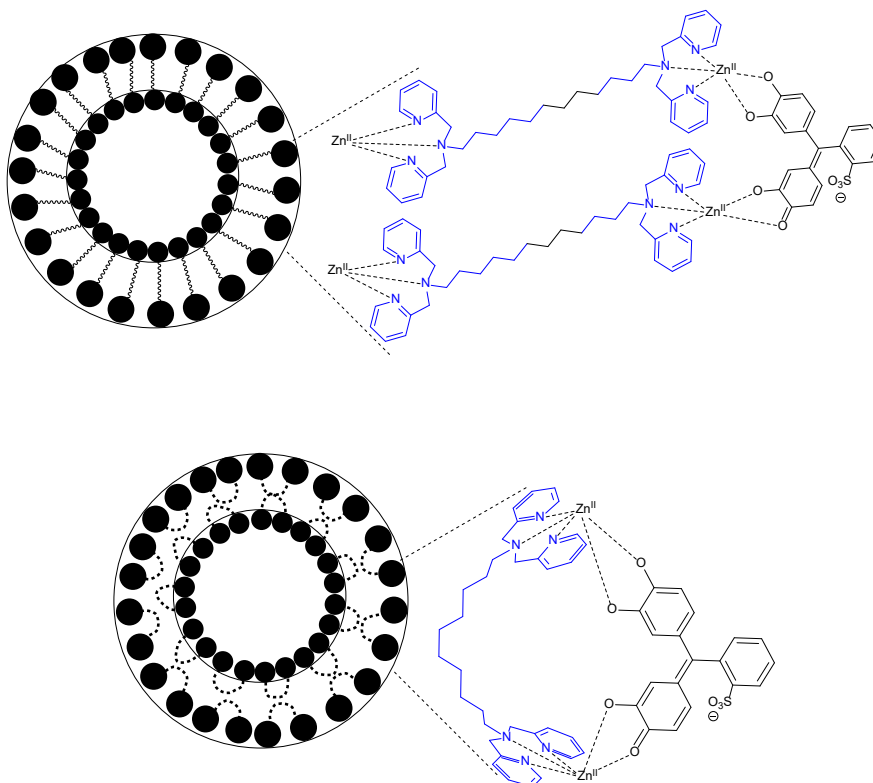


Figure 3.27. C₁₂-di-picolyll indicate intermolecular and intramolecular binding via the **PV** probe in the presence of Zn^{II}.

We performed experiments where we titrated increasing amounts of C₈-di-picoly^l·Zn^{II} to a buffered HEPES solution containing either 20 μM, 10 μM, or 5 μM of **PV** (**Figure 3.28**). Each of the 20 μM, 10 μM, and 5 μM samples needed about 1.0 equivalent of C₈-di-picoly^l·Zn^{II} to change colour from yellow to blue, showing that the binding stoichiometry was consistent at all tested concentrations (see **Table 3.2**). It is important to note that 5 μM is below the CAC of the bolaamphiphilic system, and the lack of a significant change in the binding ratio with this sample strongly indicates that the binding of the bolaamphiphile with **PV** is intramolecular.

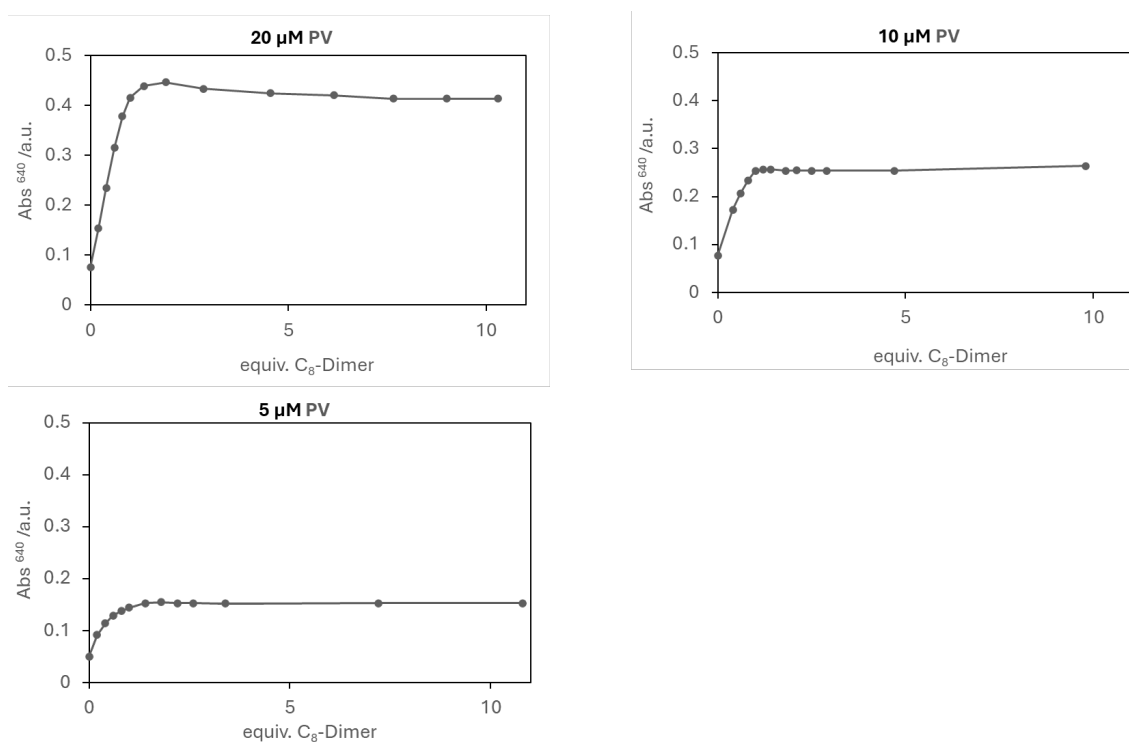


Figure 3.28. Changes in absorbance of **PV** solutions (20 μM, 10 μM, or 5 μM) with increasing concentrations of C₈-di-picoly^l·Zn^{II}. Conditions: [HEPES buffer] = 5 mM, pH 7.4, [NaCl] = 145 mM, [**PV**] = 20, 10 or 5 μM at 25 °C.

Table 3.2. Summary of the binding ratios required for colour change for C₈-di-picolyl·Zn^{II}, with different concentrations of **PV**.

[PV] (μM)	[C ₈ -di-picolyl·Zn ^{II}] (μM)	PV/C₈-di-picolyl·Zn^{II} ratio
20	20	1:1
10	10	1:1
5	5	1:1

For comparison, we performed the parallel titration experiments adding increasing amounts of C₁₄-di-picolyl·Zn^{II} to a buffered HEPES solution containing either 20 μM , 10 μM , or 5 μM of **PV** (**Figure 3.29a**). We had earlier predicted the picolylamine groups in C₁₄-di-picolyl·Zn^{II} were interacting in an intermolecular manner to bind to **PV**, as we assumed that this bolaamphiphile was self-assembling in a linear manner to form large vesicular structures. With 20 μM of **PV**, we had earlier observed that three equivalents of C₁₄-di-picolyl·Zn^{II} were required to induce the colour change from yellow to blue. Interestingly, when the **PV** concentration was reduced to 10 μM , which is just above the CAC of the system, the amount of C₁₄-di-picolyl·Zn^{II} required for the colour change reduced to 3.0 equivalents, but there was a significant decrease in the intensity of the absorption at 685 nm, significantly more than a 50% reduction compared to the system with 20 μM of **PV**. In the sample with 5 μM of **PV**, which is below the CAC of the system, we noticed that we were not able to obtain a colour change even after the addition of 10 equivalents of C₁₄-di-picolyl·Zn^{II}. These observations contrast the results obtained with C₈-di-picolyl·Zn^{II} and strongly suggest that C₁₄-di-picolyl·Zn^{II} binds to **PV** in an intermolecular manner, and at concentrations below the CAC, the self-assembly of C₁₄-di-picolyl·Zn^{II} does not occur and therefore binding with **PV** is negligible.

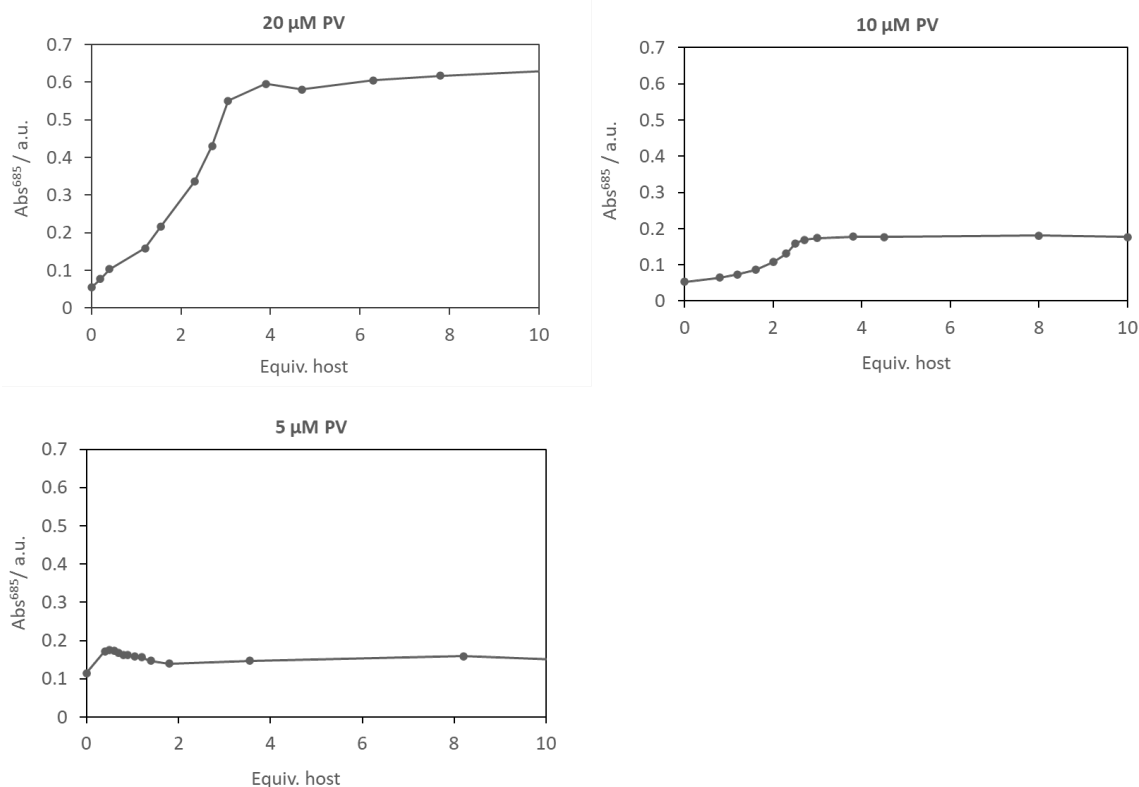


Figure 3.29. Changes in absorbance of **PV** solutions (20 μM , 10 μM , or 5 μM) with increasing concentrations of $\text{C}_{14}\text{-di-picolyl}\cdot\text{Zn}^{\text{II}}$. Conditions: [HEPES buffer] = 5 mM, pH 7.4, [NaCl] = 145 mM, [PV] = 20, 10 or 5 μM at 25 $^{\circ}\text{C}$.

3.4 Incorporation into Micellar Matrices

3.4.1 Surfactant and packing parameters

Given the dynamic nature of self-assembled systems and the commonly observed concentration-dependent effects, we decided to investigate the effect of incorporating our bolaamphiphiles within micellar matrices. Incorporation into a micellar matrix could also affect whether intramolecular or intermolecular binding to **PV** occurs, given the very different shapes of the bolaamphiphiles in each case, with a linear structure in the case of intermolecular binding, and a U-shape for intramolecular binding. A long linear bolaamphiphiles would be similar to a surfactant molecule that had zero or planar curvature (**Figure 3.30**) and generally favour the formation of lamellar or vesicular assemblies. On the other hand, a U-shaped structure would have a large polar head and a

smaller tail, possess a positive curvature, which generally favours the formation of micelles. We wanted to investigate whether the incorporation of the bolaamphiphiles into micelles could favour the formation of the U-shaped structure, and in addition, whether cleaner binding isotherms could be obtained that would allow the calculation of binding constants.

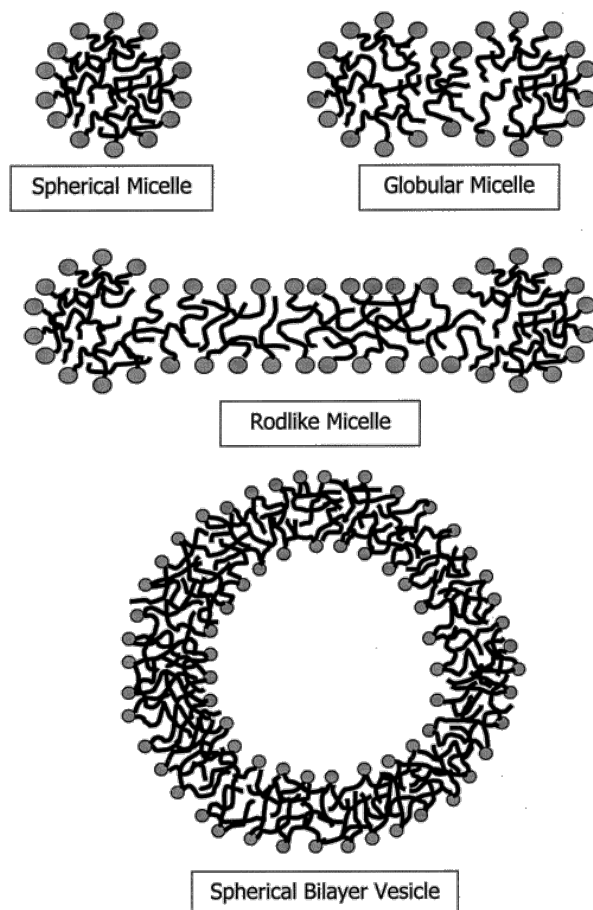


Figure 3.30. A schematic representation of surfactant aggregates in dilute aqueous solutions. Source: Reprinted with permission from ref. **150**. Copyright (1996) Surfactant Book.{Zahna, 1996 #91}

3.4.2 Incorporation into the non-polar surfactant Brij O10

We began by incorporating the bolaamphiphile C_{14} -di-picoly $1 \cdot Zn^{II}$ into a micellar matrix composed of the non-ionic surfactant Brij O10. The initial binding studies were

performed by the addition of increasing amounts of C_{14} -di-picolyl- Zn^{II} to a solution of 20 μM of **PV** in an aqueous HEPES buffer (5 mM, pH 7.4, 145 mM NaCl, at 25 $^{\circ}C$) containing 800 μM of Brij O10. Dramatically different UV spectra were obtained compared to the system in the absence of the micellar matrix (**Figure 3.31**). One key difference was that there was no longer a significant change in the baseline with successive UV spectra, which suggested we no longer had the formation of large vesicular aggregates that could scatter light. This can be attributed to the micellar matrix, with the assumption that all of the bolaamphiphiles in solution are now solubilised or incorporated within the matrix. Colour change from yellow to blue was induced upon the addition of 1 equivalent of C_{14} -di-picolyl- Zn^{II} , which suggests 1:1 binding. Incorporation of the bolaamphiphiles into the micellar matrix also resulted in a much cleaner binding isotherm and fitting with the BindFit tool using a 1:1 binding model estimated the binding constant to be approximately $1.3 \times 10^9 M^{-1}$.

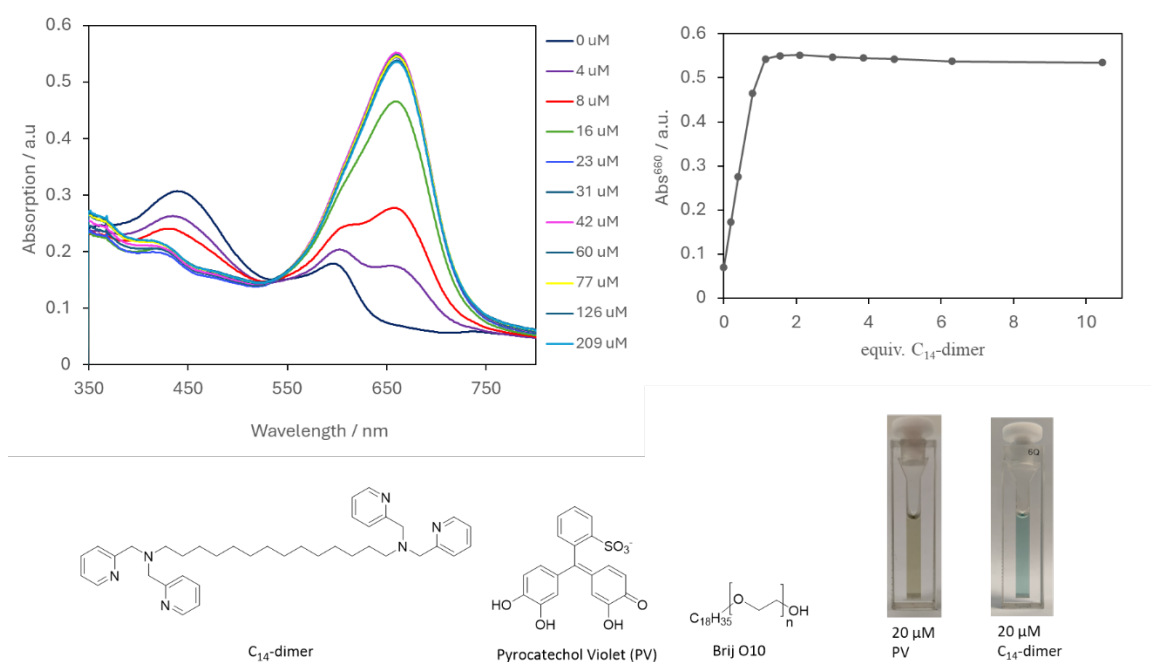


Figure 3.31. Changes in absorbance of **PV** solution with increasing C_{14} -di-picolyl- Zn^{II} concentration until 204 μM . Conditions: [HEPES buffer] = 5 mM, pH 7.4, [NaCl] = 145 mM, [**PV**] = 20 μM , [Brij O10] = 800 μM at 25 $^{\circ}C$. C_{14} -di-picolyl- Zn^{II} concentrations = 0, 4, 8, 16, 23, 31, 42, 60, 77, 126 and 209 μM .

Our next step was to conduct displacement experiments with PPI, following the same procedure as in previous studies. To a solution containing 20 μM of C_{14} -di-picoly $\text{l}\cdot\text{Zn}^{\text{II}}$ and 20 μM of **PV**, increasing amounts of PPI were sequentially added (**Figure 3.32**). The spectra obtained for this experiment were similar to those for the C_{14} -di-picoly l in the vesicular system, although much lower concentrations of PPI (7 equivalents) were required to displace the **PV**. While these concentrations are lower than the corresponding vesicular system, 7 equivalents is still high, and would not make for a sensitive receptor and effective IDA. We therefore continued to investigate the properties of our bolaamphiphiles of differing chain lengths.

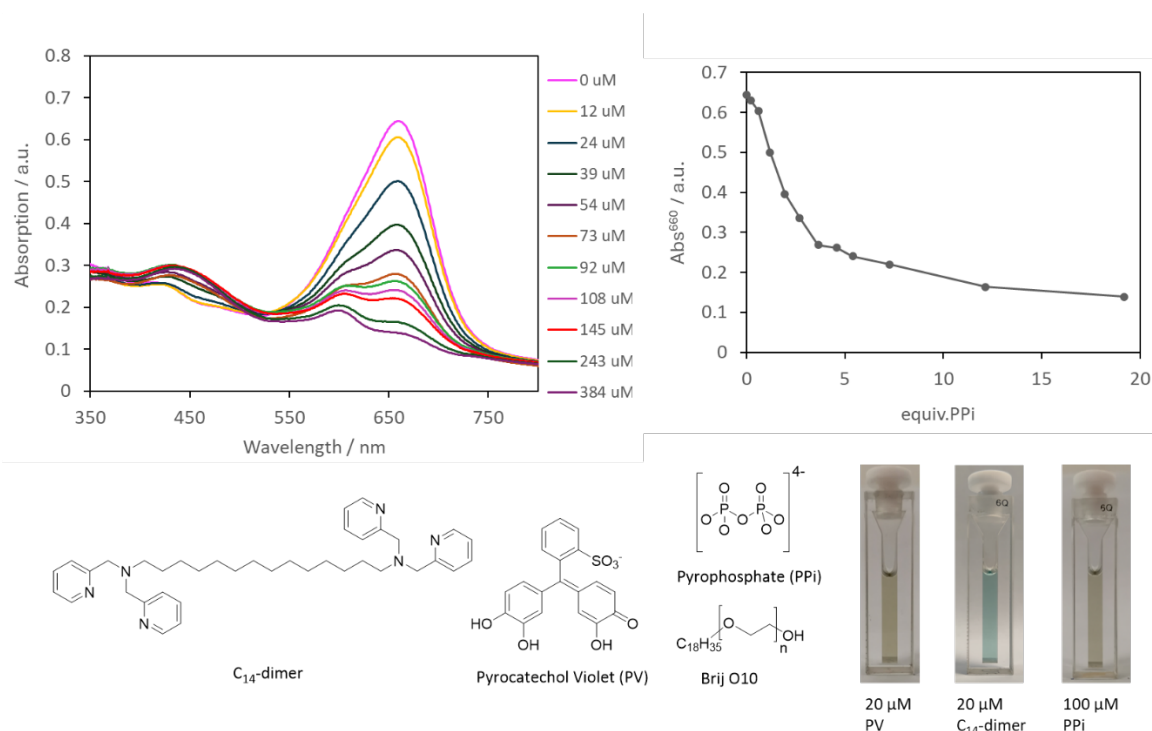


Figure 3.32. Absorbance spectra for the addition of PPI in the presence of amphiphilic ligands C_{14} -di-picoly l . Conditions: [HEPES buffer] = 5 mM, pH = 7.4, [NaCl] = 145 mM, [**PV**] = 20 μM , [Brij O10] = 800 μM , [**PV**]: [C_{14} -di-picoly $\text{l}\cdot\text{Zn}^{\text{II}}$] = 1:1, [PPI] = 0, 12, 24, 39, 54, 73, 92, 108, 145, 243, and 384 μM .

When we reduced the chain length from C_{14} to C_{12} , we observed a binding pattern similar

to that of C_{14} -di-picolyl· Zn^{II} , with a colour change occurring upon reaching a 1:1 ratio of **PV** to C_{12} -di-picolyl· Zn^{II} (**Figure 3.33a**). The use of a Brij O10 as a micellar matrix appears to have induced a change in the mode of binding, as it had with the C_{14} -di-picolyl· Zn^{II} bolaamphiphiles, with no significant change in the baseline of the UV-Vis spectra upon successive additions of the dipicolyl ligand. The binding isotherm was fitted with BindFit using a 1:1 model to estimate a binding constant of $1.5 \times 10^7 \text{ M}^{-1}$, which was a decrease with respect to the C_{14} -di-picolyl· Zn^{II} bolaamphiphile. The displacement study with PPI reflected this decrease in binding affinity, with a lower amount of PPI (4.4 equivalents) required to induce a colour change from blue back to yellow (**Figure 3.33b**).

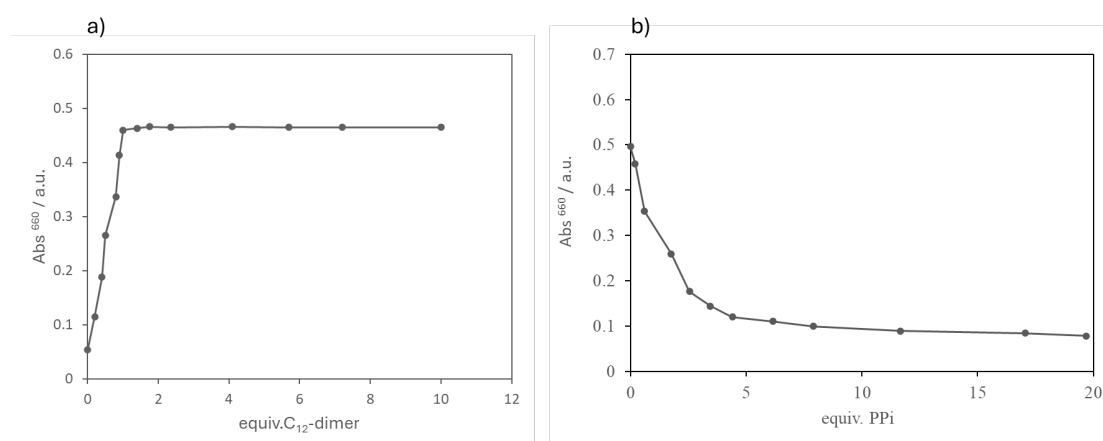


Figure 3.33. Changes in absorbance at 660 nm of a) a solution of **PV** with increasing C_{12} -di-picolyl· Zn^{II} concentration added; and b) the addition of PPI to a solution containing 20 μM of C_{12} -di-picolyl· Zn^{II} and 20 μM of **PV**. All solutions are with [Brij O10] = 800 μM , [HEPES buffer] = 5 mM, pH = 7.4, [NaCl] = 145 mM, at 25 $^{\circ}\text{C}$.

Next, we examined C_{10} -di-picolyl· Zn^{II} to see if the trend of the decrease in binding constant continued with decreasing chain lengths. To 20 μM of **PV** in aqueous HEPES buffer and 800 μM Brij O10 (5 mM HEPES, pH 7.4, 145 mM NaCl, and at 25 $^{\circ}\text{C}$) was added increasing concentrations of C_{10} -di-picolyl· Zn^{II} , and a 1:1 binding ratio was again observed to induce the colour change from yellow to blue (**Figure 3.34**). Fitting of the binding isotherm obtained a binding constant of $7.1 \times 10^7 \text{ M}^{-1}$, which is higher than that obtained for C_{10} -di-picolyl· Zn^{II} , but note that there are high errors associated with these calculations, of approximately 500%. Next, we performed experiments to displace **PV**

from the C_{10} -di-picolyl- Zn^{II} host, and we determined that only one equivalent of PPI was required to fully displace the **PV** and induce a colour change from blue to yellow. The ease by which the **PV** was displaced by PPI gives excellent potential for the development of a highly sensitive IDA.

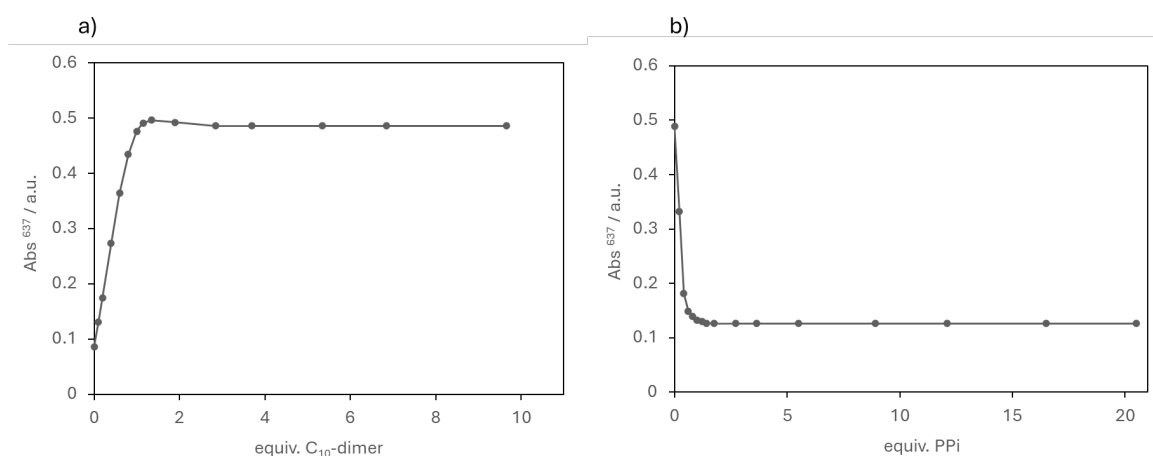


Figure 3.34. Changes in absorbance at 637 nm of a) a solution of **PV** with increasing C_{10} -di-picolyl- Zn^{II} concentration added; and b) the addition of PPI to a solution containing 20 μ M of C_{10} -di-picolyl- Zn^{II} and 20 μ M of **PV**. All solutions are with [Brij O10] = 800 μ M, [HEPES buffer] = 5 mM, pH = 7.4, [NaCl] = 145 mM, at 25 $^{\circ}$ C.

In addition to being highly sensitive for PPI, we needed to determine whether this system would respond *selectively* to PPI in the presence of similar phosphate anions. We next conducted displacement experiments with ATP, ADP and citrate, ions that have a high potential to be able to compete effectively with PPI. Our experiments revealed that 12 equivalents of ATP and 20 equivalents of ADP were required to induce a colour change from blue to yellow, while the addition of 20 equivalents of citrate did not induce a colour change. This shows that this system is highly sensitive to PPI, and also moderately selective (**Figure 3.35**).

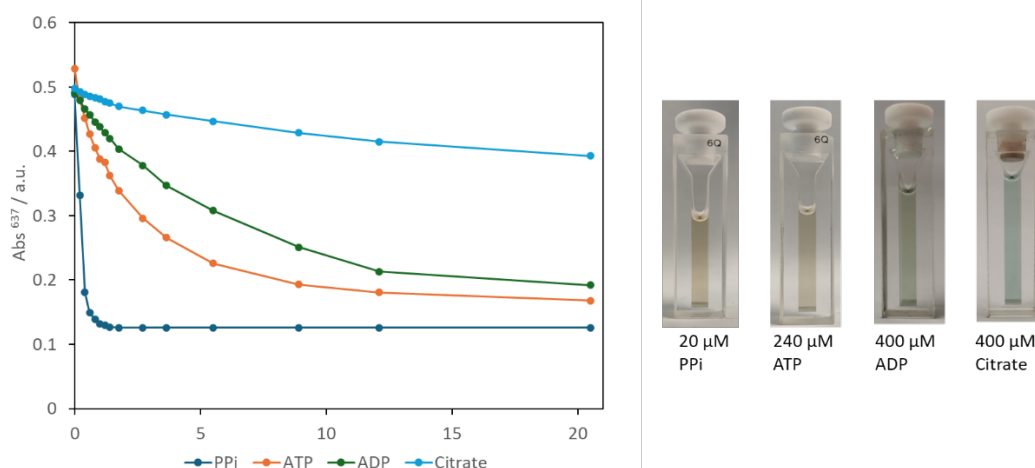


Figure 3.35. Absorbance changes at 637 nm during the displacement of **PV** with the anions PPI, ATP, ADP and citrate in the presence of C_{10} -di-picolyl- Zn^{II} [20 μ M]. Conditions: [HEPES buffer] = 5 mM, pH = 7.4, [NaCl] = 145 mM, [**PV**] = 20 μ M, Brij O10 = 800 μ M at 25 $^{\circ}$ C.

We subsequently investigated the binding behaviour of the next analogue in the series, the bolaamphiphile C_8 -di-picolyl- Zn^{II} . Addition of this host to a solution of **PV** in 800 μ M of Brij O10 (with HEPES = 5.0 mM, NaCl = 145 mM, pH = 7.4, **PV** = 20 μ M, Brij O10 = 800 μ M at 25 $^{\circ}$ C) again revealed a 1:1 binding ratio between the bolaamphiphile and **PV** (**Figure 3.36**). Note that these binding results for C_{10} -di-picolyl- Zn^{II} and C_8 -di-picolyl- Zn^{II} are similar to what was obtained earlier for the non-micellar system and support the assumption that intramolecular binding was also occurring in the earlier system. Fitting of the binding isotherm using BindFit obtained a binding constant of $8.2 \times 10^7 \text{ M}^{-1}$, which is similar to the binding constant obtained for C_{10} -di-picolyl- Zn^{II} . Note that the fit was quite poor this time, with a large degree of error (2900%), which indicates that the determination of binding constants may not be the best way to compare the utility of these different hosts.

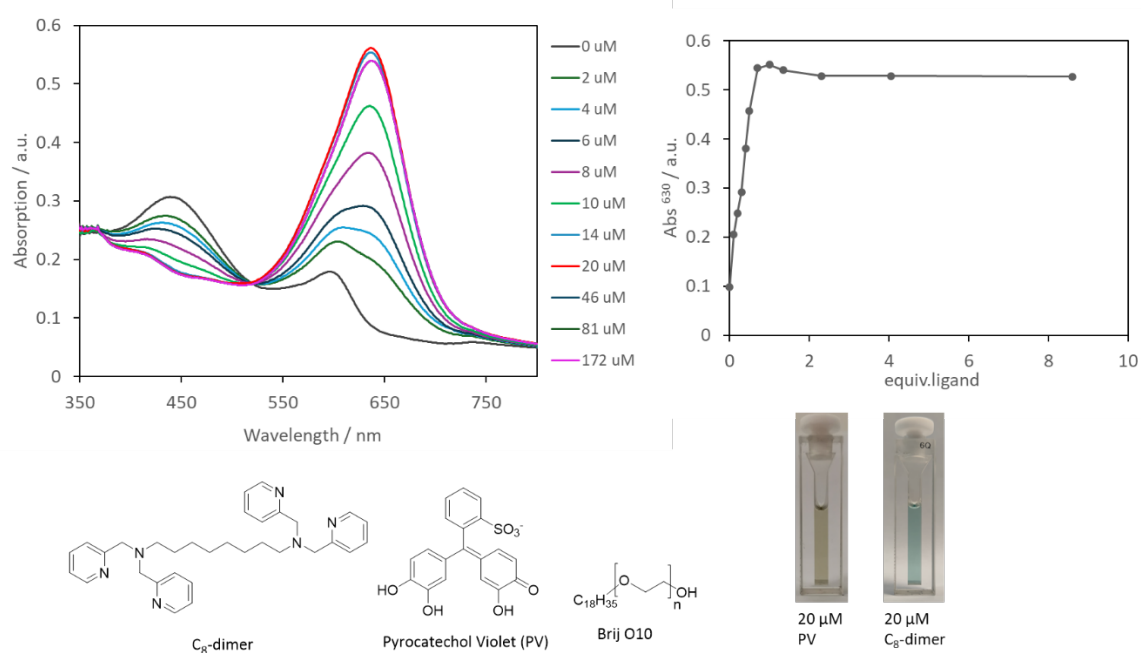


Figure 3.36. Changes in absorbance of **PV** solution with increasing C₈-di-picolyl·Zn^{II} concentration until 204 μM. Conditions: [HEPES buffer] = 5 mM, pH 7.4, [NaCl] = 145 mM, [**PV**] = 20 μM, [Brij O10] = 800 μM at 25 °C. C₈-di-picolyl·Zn^{II} concentrations = 0, 2, 4, 6, 8, 10, 14, 20, 46, 81, and 172 μM.

The C₈-di-picolyl·Zn^{II}-**PV** complex could again be efficiently displaced with PPI, with one equivalent required to induce a colour change from blue to yellow (**Figure 3.37**). The spectral changes appear much cleaner than for the non-micellar system. When compared to the other anions, 12 equivalents of ATP and 20 equivalents of ADP were required for the same colour change, whereas citrate was not effective even after the addition of 20 equivalents (**Figure 3.38**). As can be seen in the cuvette for citrate in **Figure 3.38**, the complex [C₈-di-picolyl·Zn^{II}-**PV**] remains a strong blue, unlike the cuvette in **Figure 3.36** for the [C₁₀-di-picolyl·Zn^{II}-**PV**] sample, which has turned greenish under the same conditions which was [HEPES buffer] = 5 mM, pH 7.4, [NaCl] = 145 mM, [**PV**] = 20 μM, [Brij O10] = 800 μM at 25 °C. This suggests that C₈-di-picolyl·Zn^{II} would be a more effective host than C₈-di-picolyl·Zn^{II}. Overall, these results indicate that C₈-di-picolyl·Zn^{II} is a highly sensitive and selective host for PPI, with a selectivity factor of 11 compared to ATP.

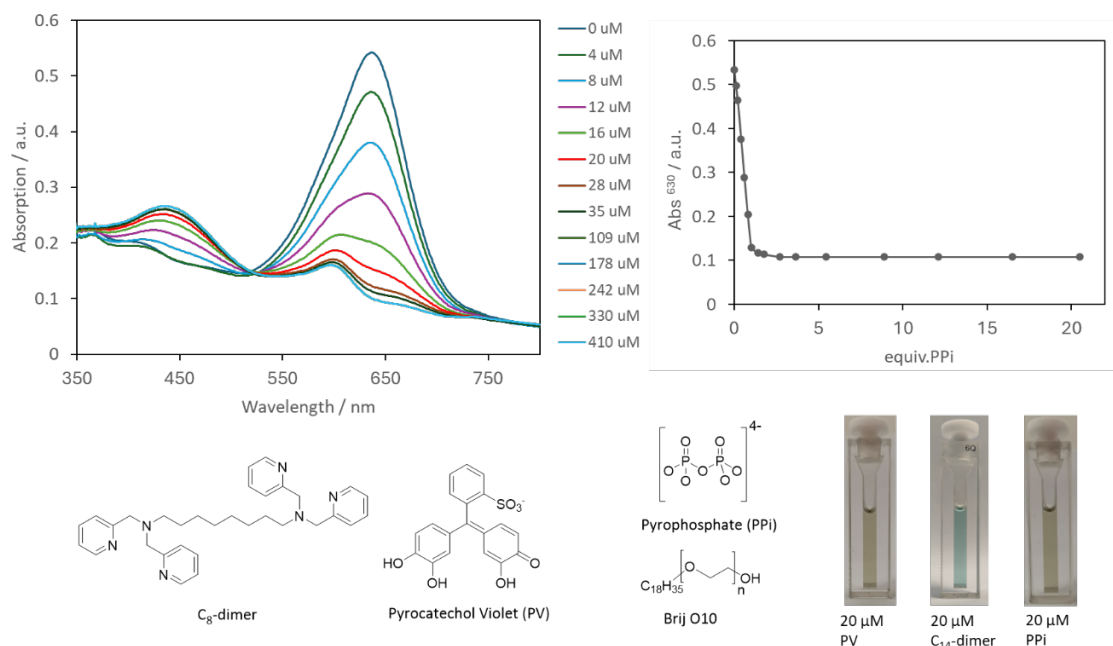


Figure 3.37. Absorbance spectra for the addition of PPI in the presence of amphiphilic ligands C_8 -di-picoly Zn^{II} . Conditions: [HEPES Buffer] = 5 mM, pH = 7.4, [NaCl] = 145 mM, [PV] = 20 μM , [PV]: [C_8 -di-picoly Zn^{II}] = 1:1, [PPI] = 0, 4, 8, 12, 16, 20, 28, 35, 109, 178, 242, 330, and 410 μM .

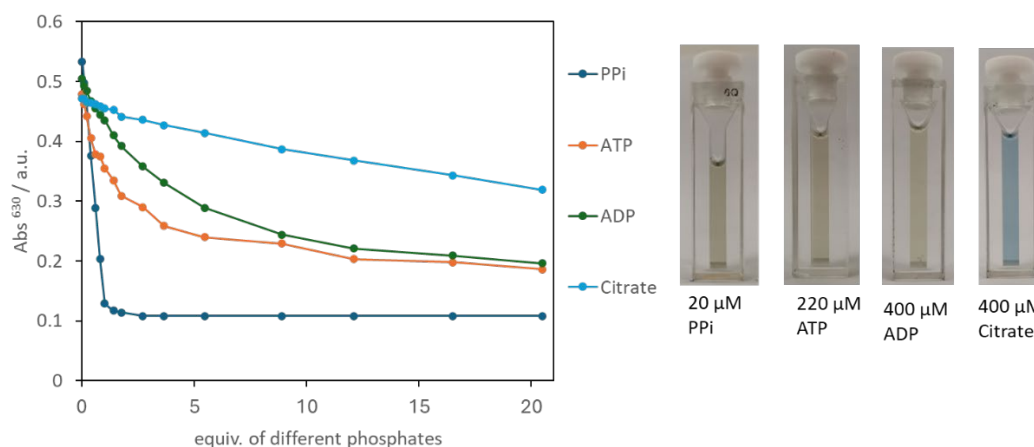


Figure 3.38. Absorbance changes at 630 nm during the displacement of PV with the anions PPI, ATP, ADP and citrate in the presence of C_8 -di-picoly Zn^{II} [20 μM]. Conditions: [HEPES buffer] = 5 mM, pH = 7.4, [NaCl] = 145 mM, [PV] = 20 μM , Brij O10 = 800 μM at 25 $^{\circ}C$.

To complete this series of experiments, we examined our final bolaamphiphile analogue C₆-di-picolyl·Zn^{II}. We added increasing concentrations of C₆-di-picolyl·Zn^{II} to a solution of **PV** under the same conditions (HEPES = 5.0 mM, NaCl = 145 mM, pH = 7.4, **PV** = 20 μM, Brij O10 = 800 μM, 25 °C) and observed a colour change from yellow to blue upon a 1:1 ratio between C₆-di-picolyl·Zn^{II} and **PV** (**Figure 3.39a**). The binding constant was calculated to be $1.3 \times 10^7 \text{ M}^{-1}$, which was similar to that observed for the other bolaamphiphiles in combination with the micellar matrix. This fit had significantly less error (116%) compared to the fit for C₈-di-picolyl·Zn^{II}. Subsequently, we tested for the displacement of **PV** with PPi to determine the sensitivity of the system to PPi. This experiment was performed with 20 μM of both C₆-di-picolyl·Zn^{II} and **PV**, and we observed that one eq of PPi is required to displace the **PV**, which demonstrated excellent sensitivity of the system for PPi (**Figure 3.39b**).

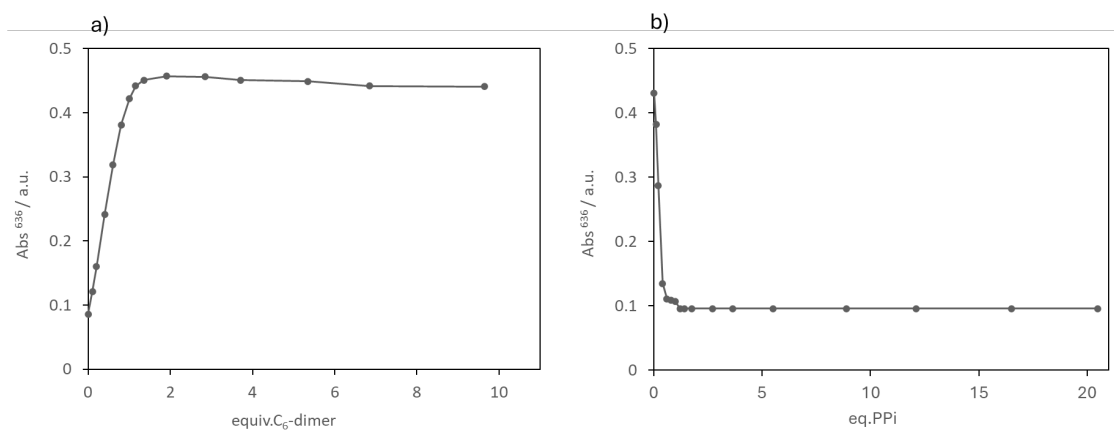


Figure 3.39. Changes in absorbance at 636 nm of a) a solution of **PV** with increasing C₆-di-picolyl·Zn^{II} concentrations; and b) the addition of PPi to a solution containing 20 μM of C₆-di-picolyl·Zn^{II} and 20 μM of **PV**. All solutions are with [Brij O10] = 800 μM, [HEPES buffer] = 5 mM, pH = 7.4, [NaCl] = 145 mM, at 25 °C.

To evaluate the selectivity of the C₆-di-picolyl·Zn^{II} for PPi, we tested its response to the closely related phosphate, ATP. Our results show that the C₆-di-picolyl·Zn^{II} showed no differential selectivity between PPi and ATP, as both required equimolar concentrations

(1 equivalent) to displace the **PV** complex and produce a colour change from blue to yellow (**Figure 3.40**). As shown in the figure below, the response curves for PPI and ATP are virtually identical, reaching maximum **PV** displacement at approx. 20 μM concentration. These results show that this combination would not be useful in the design of an IDA for PPI.

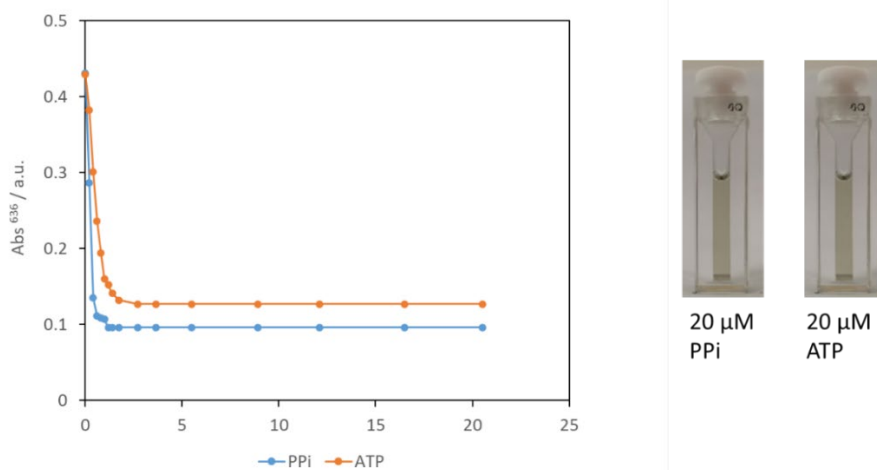


Figure 3.40. Absorbance changes at 630 nm during the displacement of **PV** with the anions PPI, and ATP in the presence of $\text{C}_6\text{-di-picolyl}\cdot\text{Zn}^{\text{II}}$ [20 μM]. Conditions: [HEPES buffer] = 5 mM, pH = 7.4, [NaCl] = 145 mM, [**PV**] = 20 μM , Brij O10 = 800 μM at 25 $^{\circ}\text{C}$.

3.4.3 Summary of results for the bolaamphiphiles in Brij O10

When we compare the different dimers in the presence of Brij O10 under the same conditions (HEPES = 5.0 mM, NaCl = 145 mM, pH = 7.4, **PV** = 20 μM , Brij O10 = 800 μM) at 25 $^{\circ}\text{C}$ to observe its binding behaviour and selectivity with **PV**, we observed that all of them bind with a 1:1 ratio of **PV**, and all dimers ($\text{C}_{14}\text{-di-picolyl}\cdot\text{Zn}^{\text{II}}$, $\text{C}_{12}\text{-di-picolyl}\cdot\text{Zn}^{\text{II}}$, $\text{C}_{10}\text{-di-picolyl}\cdot\text{Zn}^{\text{II}}$, $\text{C}_8\text{-di-picolyl}\cdot\text{Zn}^{\text{II}}$, and $\text{C}_6\text{-di-picolyl}\cdot\text{Zn}^{\text{II}}$), which is an excellent result. Still, we observed that when it is a long hydrophobic chain, the absorption band increases, and when we decrease the hydrophobic chain, the absorption band decreases (**Figure 3.41**).

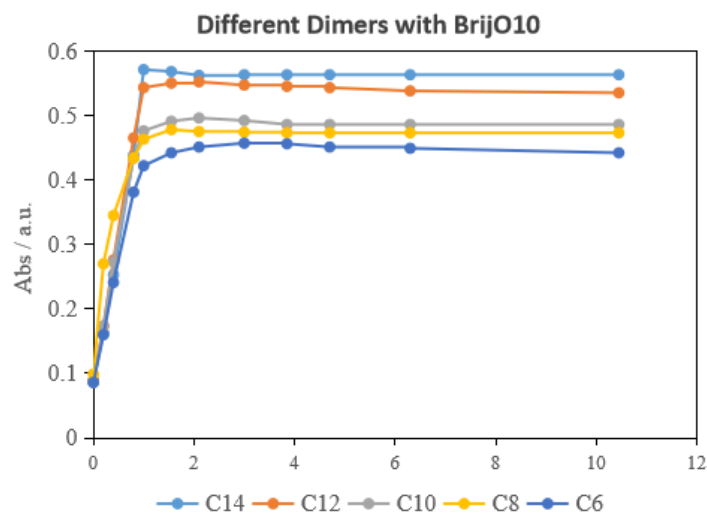


Figure 3.41. The differences between various dimers, including C₁₄-di-picolyl, C₁₂-di-picolyl, C₁₀-di-picolyl, and C₈-di-picolyl and C₆-di-picolyl, involve their binding to **PV** in the presence of Brij O10.

Table 3.3. Binding ratios observed at different concentrations of **PV**, and the equivalents of anion needed to observe a colour change in the presence of micelles

Dimers (Brij O10)	Binding with PV	<u>PPi</u>	ATP	ADP	Citrate
C ₁₄ -di-picolyl	1:1	1:5	1:20	---	---
C ₁₂ -di-picolyl	1:1	1:4	1:20	---	---
C ₁₀ -di-picolyl	1:1	1:1	1:12	1:20	---
C ₈ -di-picolyl	1:1	1:1	1:12	1:17	---
C ₆ -di-picolyl	1:1	1:1	1:1	1:5	---

Table 3.4. Binding ratios observed at different concentrations of **PV**, and the equivalents of anion needed to observe a colour change.

Dimers	Binding with PV	<u>PPi</u>	ATP	ADP	Citrate
C ₁₄ -di-picolyl	1:3	---	1:20	---	---
C ₁₂ -di-picolyl	1:2	1:4.4	1:20	---	---
C ₁₀ -di-picolyl	1:1	1:2	1:11	1:20	---
C ₈ -di-picolyl	1:1	1:1	1:9	1:17	---
C ₆ -di-picolyl	1:2	1:2	1:3	1:10	---

In the presence of micelles (see **Table 3.3** above), all dimers bind with the **PV** in a 1:1 ratio and the colour changes from yellow to blue. We observed that as we increased the dimer concentrations under the same conditions (HEPES = 5.0 mM, NaCl = 145 mM, pH = 7.4, **PV** = 20 μ M, Brij O10 = 800 μ M) at 25 °C, a new peak appeared and shifted from 550 to 700 nm. This indicates that the binding of dimers to **PV** is significantly more effective in micelles than vesicles (see **Table 3.4** above), where different ratios of dimers bind under the same conditions.

Furthermore, in micelles the displacement of **PV** with PPi occurs at lower concentrations than in vesicles. We observed in vesicles C₁₄-di-picolyl·Zn^{II}, PPi did not displace the **PV**, C₁₂-di-picolyl displaced by 4.4 equivalents whereas C₁₀-di-picolyl required two equivalents to displace the **PV** and C₈-di-picolyl is the best displacement with **PV** 1:1 ratio where in micelles C₁₄-di-picolyl required five equivalents of PPi, C₁₂-di-picolyl required four equivalents of PPi but C₁₀-di-picolyl and C₈-di-picolyl required only one equivalent of PPi. The selectivity is high for C₁₀-di-picolyl and C₈-di-picolyl in micelles, but poorer in the vesicular system.

3.5 Conclusions

This chapter has presented the design, synthesis, and evaluation of a family of picolyl-based bolaamphiphilic receptors for the colorimetric detection of phosphate-containing

analytes. Building on the self-assembly principles established in Chapter 2, we explored how intramolecular geometry, chain length, and the amphiphilic balance of bolaamphiphiles influence both their supramolecular morphology and their performance in indicator displacement assays (IDAs).

Systematic variation of the hydrophobic spacer length (C₆-C₁₄) revealed a clear relationship between molecular architecture, self-assembly, and sensing performance. Long-chain bolaamphiphiles such as **C₁₄-di-picolyl·Zn^{II}** and **C₁₂-di-picolyl·Zn^{II}** formed large vesicular aggregates in solution, as confirmed by DLS and microscopy, but exhibited inefficient and largely irreversible binding to the pyrocatechol violet (**PV**) indicator, requiring high receptor concentrations and showing poor displacement by pyrophosphate (PPi). Progressive reduction of the hydrophobic spacer length improved responsiveness, culminating in the optimal **C₈-di-picolyl·Zn^{II}** derivative, which displayed a clean 1:1 binding stoichiometry with **PV** and a highly selective, single-equivalent displacement by PPi. NMR titrations confirmed this 1:1 stoichiometry, while aggregation studies (DLS, TEM, fluorescence-based CAC analysis) demonstrated the spontaneous formation of vesicular assemblies at micromolar concentrations.

Further mechanistic analysis indicated that the optimal **C₈-di-picolyl·Zn^{II}** system operates primarily through **intramolecular binding**, facilitated by a U-shaped molecular conformation that allows both picolyl termini to coordinate the Zn^{II}-**PV** complex within a single molecule. In contrast, the longer C₁₄ analogue relied on intermolecular binding across adjacent molecules, consistent with its extended linear geometry and higher critical aggregation concentration. This distinction between intra- and intermolecular modes of coordination provides a mechanistic rationale for the observed differences in sensitivity and selectivity across the series.

Incorporation of these bolaamphiphiles into **micellar matrices** (particularly Brij O10) offered a powerful means of modulating aggregation and improving signal fidelity. In the micellar environment, all bolaamphiphiles exhibited 1:1 host-indicator binding behaviour with reduced light scattering and more consistent displacement responses. The **C₈-di-picolyl·Zn^{II}** and **C₁₀-di-picolyl·Zn^{II}** analogues again emerged as the most effective receptors, showing rapid and highly selective colorimetric responses

to PPi with negligible interference from ATP, ADP, or citrate.

Collectively, these findings establish **C₈-di-picoly¹·Zn^{II}** as the optimal structure within this series, balancing amphiphilicity, flexibility, and coordination geometry to produce a robust and selective self-assembled receptor for PPi. More broadly, the study demonstrates that fine-tuning molecular symmetry and chain length within bolaamphiphilic scaffolds enables control over self-assembly mode and analyte selectivity, offering a versatile design principle for supramolecular sensing systems.

Chapter Four

**Optimisation of a Thiol-based
Amphiphilic Receptor**

4.1 Introduction

4.1.1 Silver-thiolate coordination polymers¹⁵¹

Silver-thiolate coordination polymers are an intriguing class of supramolecular materials formed through the coordination of silver^I ions with thiolate ligands. The strong affinity of silver for sulfur atoms, combined with the versatile binding modes of thiolates (monodentate, bridging, or chelating), gives rise to a wide range of one-, two-, and three-dimensional polymeric architectures (**Figure 4.1**). These structures are stabilised not only by Ag-S coordination but also by secondary interactions such as Ag $\cdots$ Ag metallophilic interactions and π - π stacking between organic ligands.

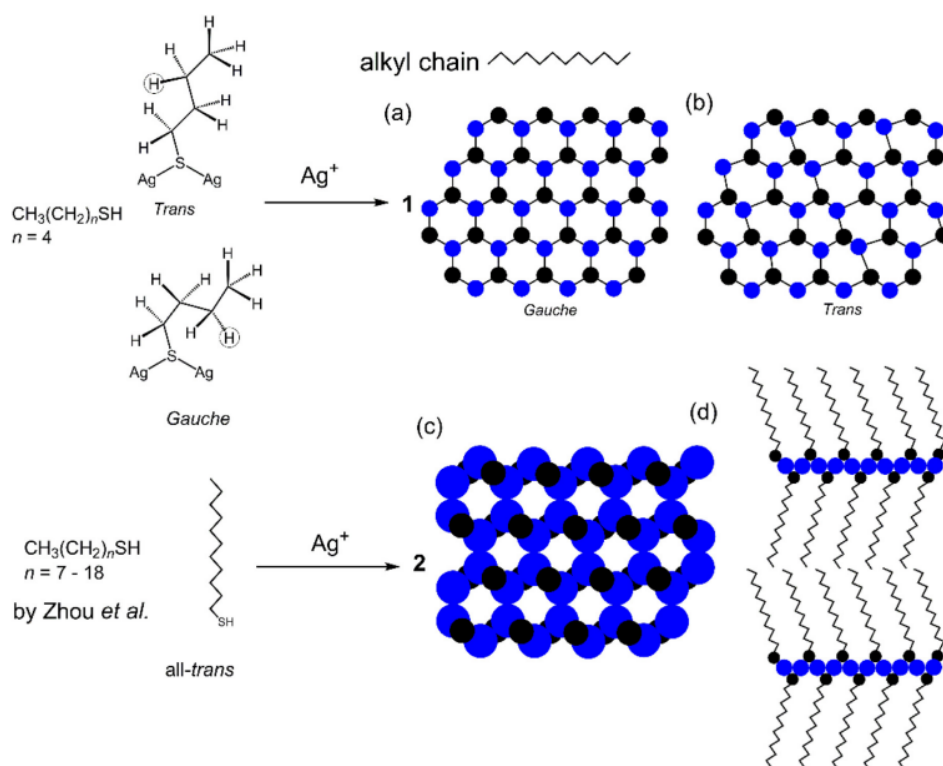


Figure 4.1. The combination of Ag^{I} and thiols is able to produce highly ordered polymeric structures that can be one-, two- or three-dimensional. Source: Reproduced with permission from Ref.[152]. Copyright (2021) Coordination Chemistry Reviews.¹⁵²

The potential applications of silver-thiolate coordination polymers are diverse. Literature

examples have shown polymers possessing luminescent properties that make them promising candidates for chemical sensors, light-emitting materials, and photocatalysts. Many silver-thiolate networks also exhibit antimicrobial activity, reflecting the well-documented biological activity of silver compounds,¹⁵³ which has attracted interest as biomedical coatings and antibacterial materials. Moreover, their structural versatility and dynamic coordination bonds make them suitable for ion exchange, gas storage, and separation technologies. Recent studies also point towards their utility in nanoelectronics and catalysis, where the combination of silver's electronic properties with robust polymeric frameworks offers unique opportunities.

A key property of silver-thiolate coordination polymers is their tunable optical behaviour, particularly luminescence, which is often sensitive to structural changes, metal-metal interactions, and the surrounding chemical environment.¹⁵⁴ Furthermore, their inherent modularity allows for the introduction of functional organic ligands, offering scope for fine-tuning porosity, surface chemistry, and photophysical properties. The modularity of these systems is a key reason why we want to explore these systems in this chapter.

4.1.2 Representative examples from the literature

Swanson and co-workers reported on Ag^I-alkanethiolate complexes, specifically Ag-S-(CH₂)_n-CH₃ for n = 5, 6, 9, 11, 15, and 17. Structural studies indicate that the Ag and S atoms in the thiol ligands are coordinated in a trigonal geometry.¹⁵² This arrangement results in a quasi-hexagonal inorganic core with a thickness of approximately 1 Å. The alkyl chains exhibit an all-*trans* conformation and self-assemble into a sandwich-shaped structure between the Ag-S backbones. The alkyl chains in each assembly are oriented perpendicular to the hexagonal Ag-S lattice, forming regular one-dimensional channels between adjacent layers. Building on previous models, a two-step, hierarchical self-assembly mechanism for [Ag(SR)]_n was proposed. In the first step, Ag atoms are linked by alkanethiolate ligands into an asymmetrically folded quasi-hexagonal two-dimensional lattice with alkyl chains extending on each side (see **Figure 4.2**). In the

second step, these two-dimensional building units form a sandwich bimolecular chain assembly in the third dimension through a secondary and complementary stacking of the alkyl chains.

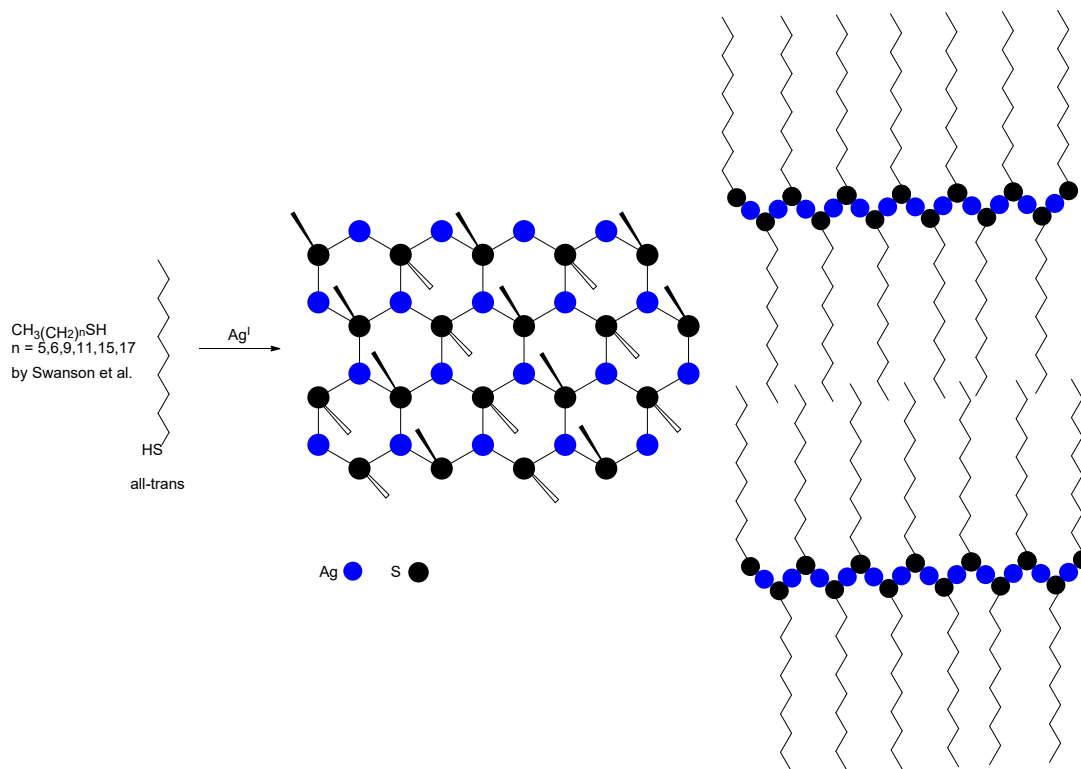


Figure 4.2. Proposed configuration and arrangements of S and Ag atoms of all-*trans* conformations depending on the orientation of alkyl chain reported Swanson et al. with $\text{CH}_3(\text{CH}_2)_n\text{SH}$ where $n = 5, 6, 9, 11, 15$ and 17 . The ligands are omitted for clarity. Reprinted with permission from Ref.[152]. Copyright (2021) Coordination Chemistry Reviews.

Jing-Jing Quan and co-workers¹⁵⁵ have reported the design of Ag^{I} -thiolate coordination polymers from a cysteine-derived ligand bearing a tetraphenylethylene (TPE) aggregation-induced emission (AIE) unit. In organic solvents such as THF and acetonitrile, the polymers form well-defined helical structures, with the chirality of the ligand (L- or D-) dictating the handedness, as shown by CD spectroscopy and microscopy. When the water fraction in $\text{H}_2\text{O}/\text{THF}$ mixtures exceeds $\sim 50\%$, the polymers aggregate, leading to enhanced AIE luminescence and inversion of supramolecular chirality. Importantly, this process is reversible: altering solvent composition allows disassembly

and reassembly, with partial recovery of the initial optical features. Subtle ligand modifications, such as the presence of a phenyl spacer, were found to strongly influence aggregation and emission sensitivity. The work demonstrates how Ag-S coordination combined with AIE-active ligands yields dynamic, chiral, and luminescent supramolecular materials, promising for sensing and adaptive photonic applications (**Figure 4.3**). The dynamic nature of such systems is a feature that we will explore later in this chapter.

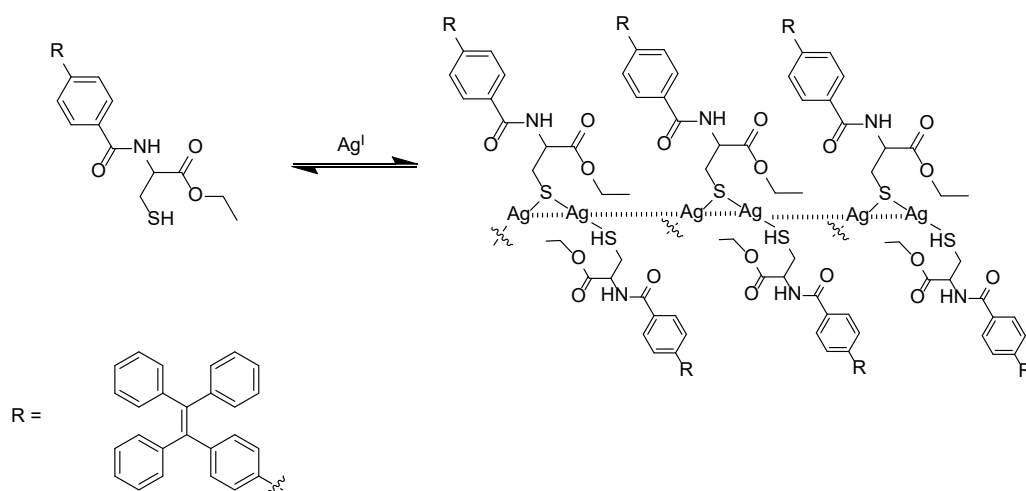


Figure 4.3. Formation of coordination polymers between Ag^{I} with a thiol ligand to form aggregates with enhanced AIE luminescence. Dashed lines through the backbone represent $\text{Ag}^{\text{I}}\text{-Ag}^{\text{I}}$ interactions. Reprinted with permission from Ref. [155]. Copyright (2023) Chemical Communications.

A key example from the literature, which has been an inspiration for the studies in this chapter, is a recent study by Shen et al. that describes the synthesis of coordination polymers where the thiol ligand is functionalised with a Zn^{II} -binding triazacyclononane (TACN) unit on the other end.¹⁵⁶ Upon addition of Ag^{I} ions, the thiols form one-dimensional Ag-SR coordination polymers through strong Ag-S bonds. Subsequent coordination of Zn^{II} to the TACN moieties generates multivalent catalytic sites in an array along the polymer backbone. The resulting assemblies exhibit phosphatase-like activity, efficiently catalysing the cleavage of the RNA model substrate 2-hydroxypropyl-4-nitrophenyl phosphate (HPNPP). The authors showed that the catalytic activity arises

from cooperative effects between adjacent Zn-TACN sites brought into close proximity by the Ag-SR framework (**Figure 4.4**).

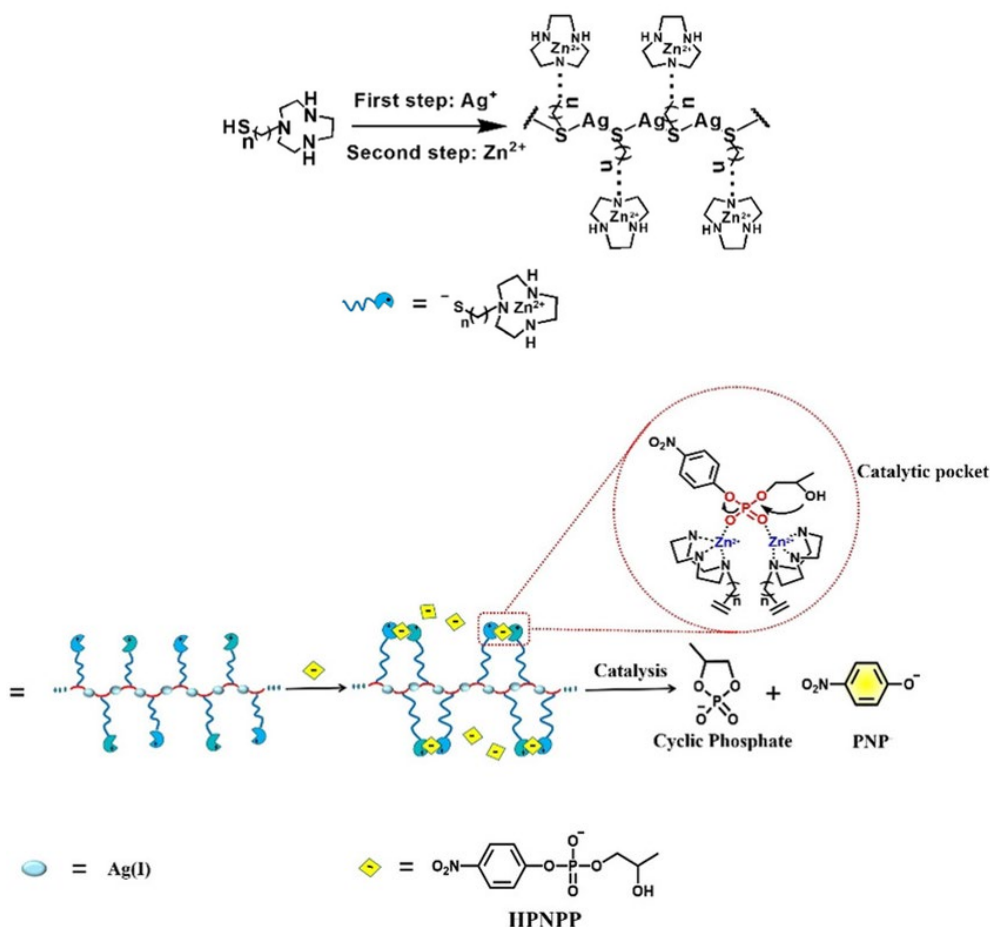


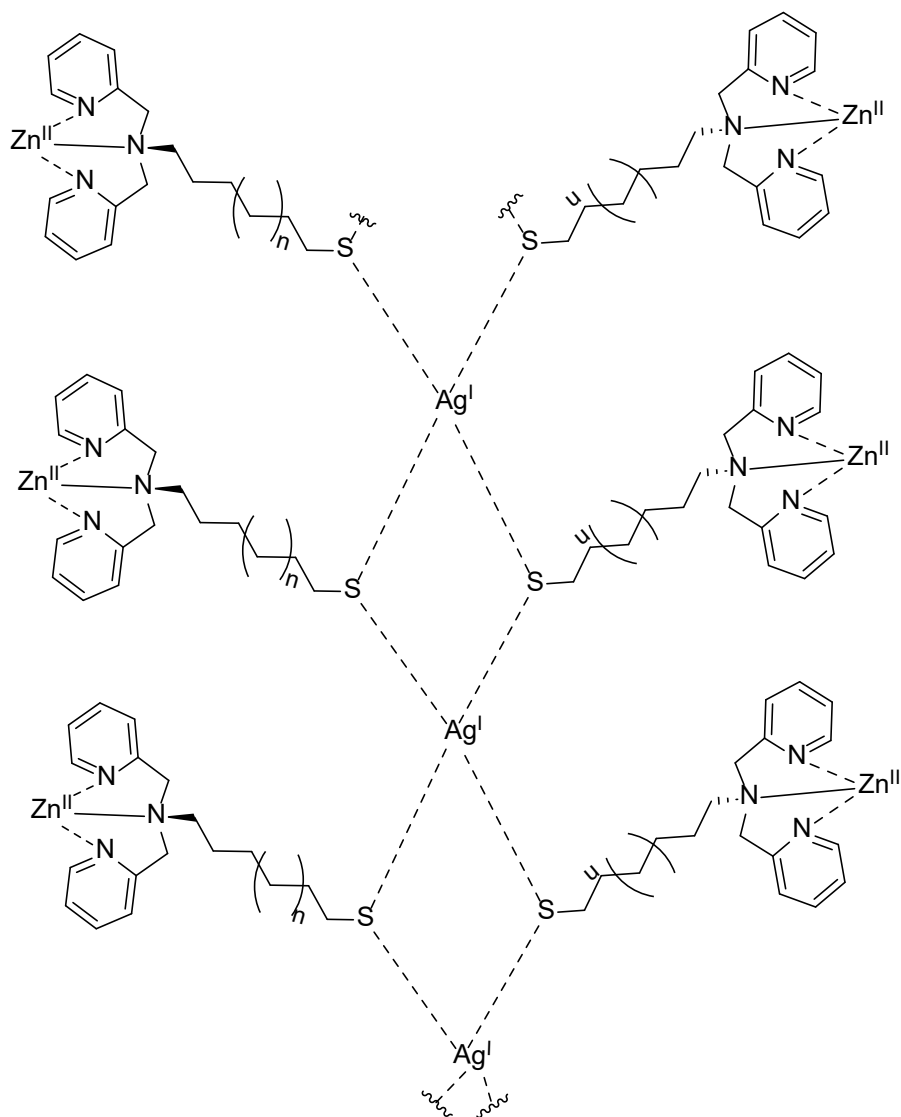
Figure 4.4. In situ formation of metal coordination polymers by using $\text{HS-C}_n\text{-TACN}$ and Ag^{I} , and the catalytic activity of this system in the presence of Zn^{II} in the hydrolytic cleavage of the RNA model substrate HPNPP. Reprinted with permission from Ref.[156]. Copyright (2021) Chemistry – A European Journal.

Importantly, the system demonstrated kinetic parameters ($k_2 \approx 109 \text{ M}^{-1}\text{s}^{-1}$) that rivalled or surpassed other artificial phosphatases, such as micelles,¹⁵⁷ vesicles,¹⁵⁸ and even gold nanoparticle-TACN assemblies. The activity was tunable through the hydrophobic chain length of the thiols, which controlled assembly stability and the polarity of the local catalytic microenvironment. Taken together, this work highlights how simple, modular self-assembly can be used to generate highly efficient artificial enzymes, providing a conceptual basis for developing self-assembled receptors and sensors in the present study.

4.1.3 Aims of this Chapter

The aim of this chapter is to extend the concepts introduced in the preceding sections – namely self-assembly, cooperative non-covalent interactions, and indicator displacement strategies – to a new class of systems based on silver-thiolate coordination polymers.¹⁵⁹ In the preceding chapters, we have shown that amphiphilic self-assembly can be harnessed to generate functional receptors suitable for the construction of indicator displacement assays for pyrophosphate. Building on this foundation, we now seek to explore how the strong affinity between silver^I and thiolate ligands can be exploited to construct dynamic coordination polymers that incorporate recognition elements within their frameworks. By combining Ag–S coordination with the modularity of ligand design, we aim to investigate whether these systems can mimic or even surpass traditional scaffold-based receptors in terms of adaptability, sensitivity, and selectivity. Ultimately, this chapter will evaluate the potential of silver-thiolate coordination polymers as versatile platforms for supramolecular recognition and sensing, using pyrophosphate sensing as a proof-of-concept.

Specifically, we aim to synthesise a series of thiols that terminate with a dipicolylamine Zn^{II}-binding headgroup, to investigate the potential to form hosts by coordination with Ag^I to form receptors that are able to reversibly bind colorimetric probes such as **PV**. Their hypothesis is that the addition of Ag^I will induce the formation of Ag^I-thiolate coordination polymers, which will then bring dipicolylamine groups into close proximity, allowing two neighbouring groups to bind cooperatively to the **PV** indicator. It has been shown that two equivalents of a Zn^{II} binding ligand are required for the binding of catechol-based indicators such as **PV**, and we aim to demonstrate that self-assembly is indeed crucial for the formation of an effective host (**Scheme 4.1**). We envision the need to synthesise a number of thiol analogues containing varying chain lengths, in order to tune the binding affinity, in a similar manner to what was performed in the preceding chapters. We aim also to investigate the reversibility of this process, to eventually design systems that are capable to responding to an external stimulus.



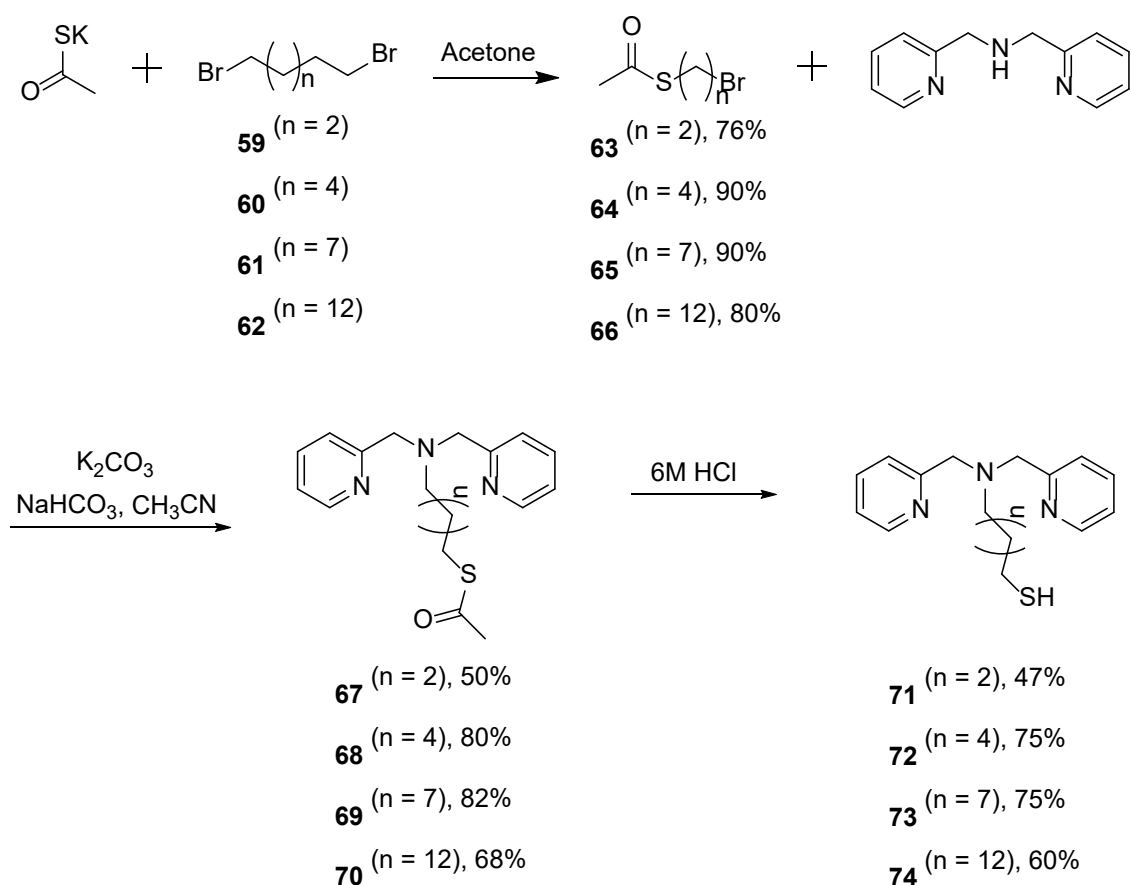
Scheme 4.1. The Ag^I-thiolate coordination polymers to be investigated in this chapter.

4.2 Initial Trials

4.2.1 Synthesis of the required thiols

The picolyl-containing thiols were synthesised via a three-step sequence involving two S_N2 substitutions. The synthesis began with 1,14-tetrabromodecane, and the substitution on one end of the molecule with potassium thioacetate with acetone as the solvent, providing the corresponding **C**₁₄ to **C**₄ thioesters in 76 to 90% yield. This was followed by substitution of the other terminal bromide by dipicolylamine, in the presence of

potassium carbonate to form thioesters **67-70** in 50 to 82% yield. Finally, hydrolysis of the thioester group formed the free thiols **71-74** in 47 to 75% yield. Earlier synthesis of molecules containing a dipicolylamine group (compounds C₁₄-picolyl SH **74**, C₉-picolyl SH **73**, C₆-picolyl SH **72**, C₄-picolyl SH **71**) in Chapter 2 encountered difficulties with purification via flash chromatography using silica due to the polarity of the dipicolylamine group. For this reason, we attempted the purification via alumina flash chromatography, which has often been shown to be more suitable for amines. However, purification still proved challenging, with slow elution and streaky spots observed for these final compounds, resulting in lower-than-expected yields.



Scheme 4.2. Synthesis of the thiol-based ligands, from C₁₄-picolyl-SH **74** to C₄-picolyl-SH **71**.

4.2.2 Initial Studies using C₁₄-picolyl-SH (74)

C₁₄-picolyl-SH **74** was first used in host-guest titration experiments to assess the ability to bind to the colourimetric indicator **PV**. Increasing concentrations of C₁₄-picolyl-SH **74**, were added into a buffered aqueous solution (HEPES = 5 mM, NaCl = 145 mM, pH = 7.4 and 25 °C) containing 20 μM of **PV**, Zn^{II} ions and Ag^I ions. **Figure 4.5** below shows the absorbance spectra for this titration. A bathochromic shift is observed in the spectrum as increasing amounts of C₁₄-picolyl-SH·Zn^{II}·Ag^I are added, with the formation of a new peak at 678 nm. A colour changes from yellow to blue is observed upon addition of 60 μM C₁₄-picolyl-SH·Zn^{II}·Ag^I which means **PV** and C₁₄-picolyl-SH·Zn^{II}·Ag^I in a 1:3 ratio. These results are in line with previous experiments featuring the amphiphile C₁₆-dipicolyl **28** and C₁₄-dipicolyl-dimer **74**, which both showed similar spectral changes.

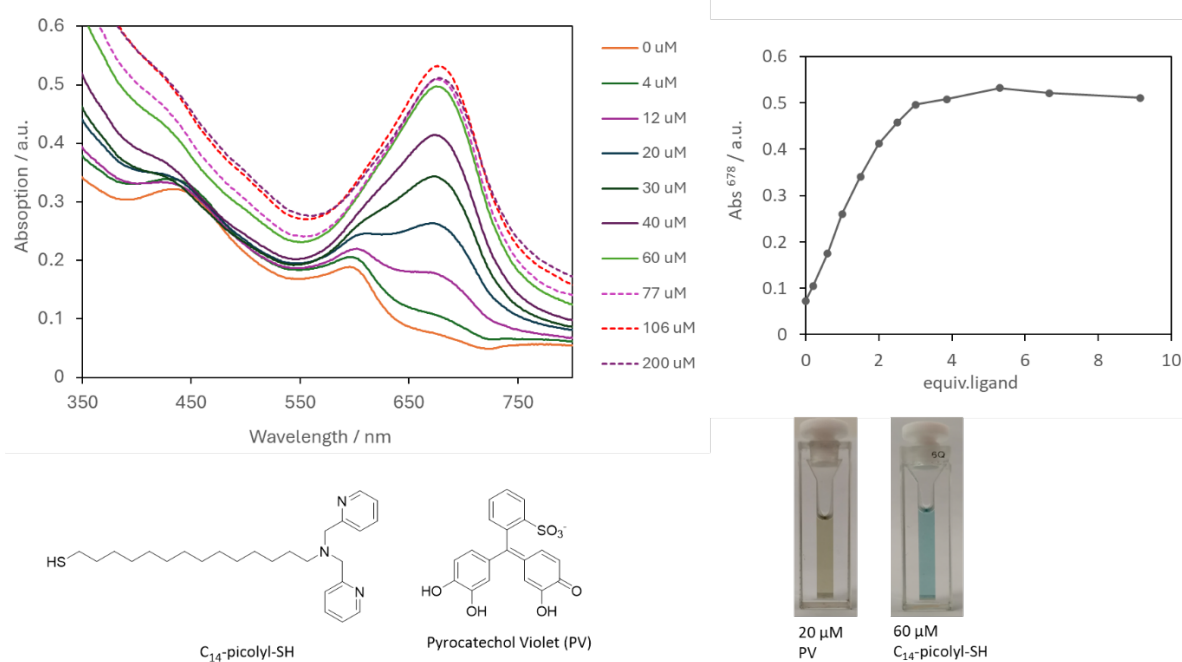
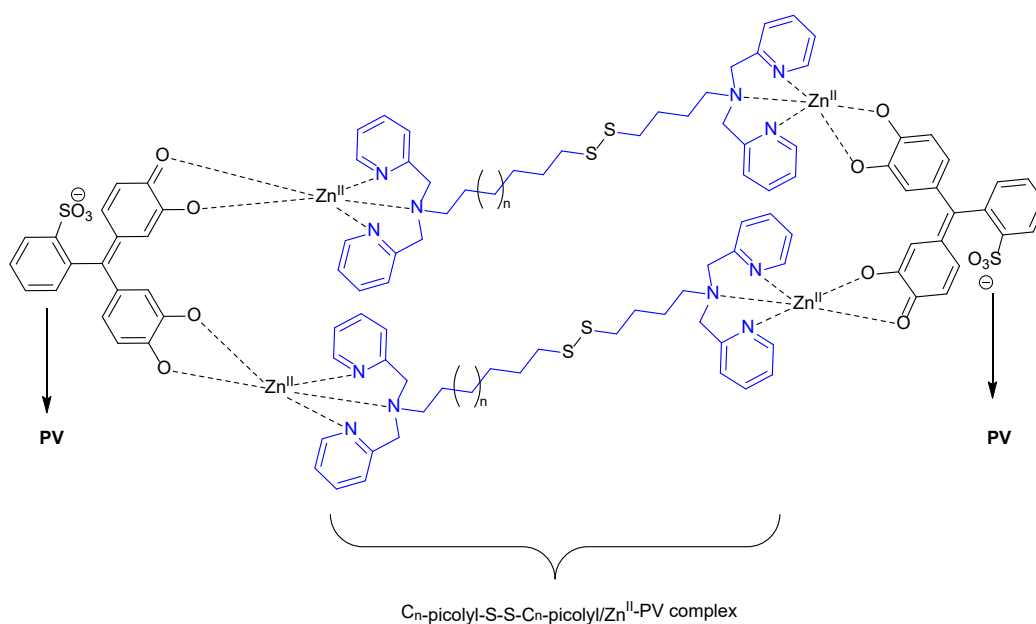


Figure 4.5. Changes in absorbance of **PV** solution with increasing C₁₄-picolyl-SH·Zn^{II}·Ag^I concentrations up to 192 μM. Conditions: [HEPES buffer] = 5 mM, pH 7.4, [NaCl] = 145 mM, [**PV**] = 20 μM at 25 °C. C₁₄-picolyl-SH·Zn^{II}·Ag^I concentrations = 0, 4, 12, 20, 30, 40, 60, 77, 106, and 192 μM.

Given the literature precedent for the formation of Ag^I-thiolate coordination complexes, we expected the formation of the complex shown in **Scheme 4.1** above, which contains a

central thread held together by Ag^{I} -thiolate coordination, with multiple picolyl ligands surrounding this central thread. It was thought that the coordination of Ag^{I} and thiol would result in the formation of structures that were more rigid than the vesicular and micellar assemblies examined in Chapters 2 and 3, which could potentially result in stronger binding to certain indicators/analytes, and/or greater selectivity due to the formation of more defined pockets. However, the initial UV results shown above indicate a 3:1 binding ratio between C_{14} -picolyl-SH and **PV**, suggesting weaker binding to **PV** compared to analogous systems examined in Chapter 2 (i.e., in comparison to C_{16} -picolyl **28** and C_{14} -picolyl **74**). Moreover, we also realised that C_{14} -picolyl-SH could potentially self-assemble to form vesicular assemblies like the systems examined in Chapter 2, or alternatively, dimerise via disulfide bonds to form bolaamphiphiles that then assemble as shown in Scheme 4.3 below.



Scheme 4.3. C_n -picolyl-SH· Zn^{II} binding dimerising to form bolaamphiphiles and then self-assembling and forming a complex with **PV**.

We therefore proceeded to investigate the ability of C_{14} -picolyl-SH **74** to bind **PV** in the absence of Ag^{I} ions, under the same aqueous buffer conditions (HEPES = 5 mM, NaCl = 145 mM, pH = 7.4 and 25 °C, with 20 μM **PV**). The rationale was that amphiphile C_{14} -

picolyl-SH contains a long carbon chain, which could potentially allow this molecule to self-assemble in water. The dipicolylamine groups can bind zinc ions, and when the sensor self-assembles, the zinc ions are brought close together, allowing for efficient binding to the catechol group of the dye. We observed that, under these conditions, in the absence of Ag^{I} , we still needed three equivalents of C_{14} -picolyl-SH· Zn^{II} to bind one equivalent of **PV** (**Figure 4.6**). However, we were unable to efficiently displace the **PV** dye from our sensor C_{14} -picolyl-SH· Zn^{II} using PPI, which suggests that the binding of our sensor to the **PV** is still too strong, despite the lower binding ratio.

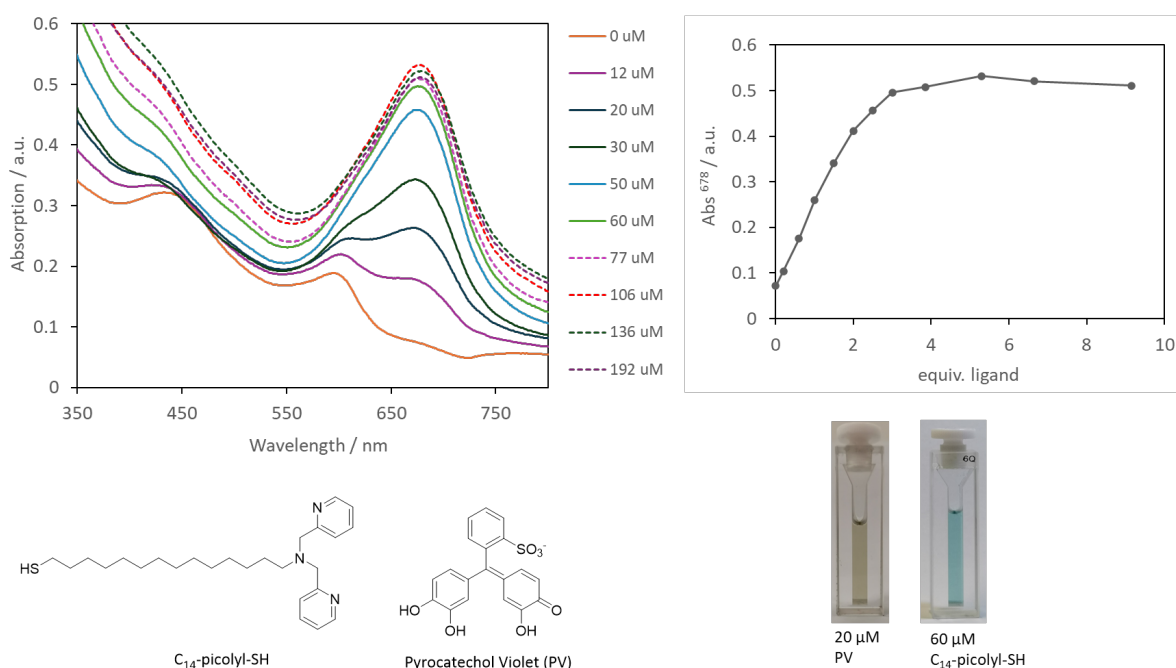


Figure 4.6. Changes in absorbance of **PV** solution with increasing C_{14} -picolyl-SH· Zn^{II} concentrations up to 192 μM . Conditions: [HEPES buffer] = 5 mM, pH 7.4, [NaCl] = 145 mM, [**PV**] = 20 μM at 25 °C. C_{14} -picolyl-SH· Zn^{II} concentrations = 0, 12, 20, 30, 50, 60, 77, 106, 136 and 192 μM .

As C_{14} -picolyl-SH· Zn^{II} was binding effectively to **PV** in the absence of Ag^{I} , we suspected that these amphiphiles were forming vesicles in solution. Examination of a solution containing 60 μM C_n -picolyl-SH· Zn^{II} and 20 μM **PV** (in HEPES buffer = 5 mM and NaCl = 145 mM) by DLS revealed the presence of large structures with a hydrodynamic radius of $974.6 \text{ nm} \pm 368.6 \text{ nm}$ (**Figure 4.7a**). Visualisation of the sample under a light

microscope (100 $\times$ magnification) revealed indeed circular and hollow structures, highly indicative of large vesicular structures (**Figure 4.7b**).

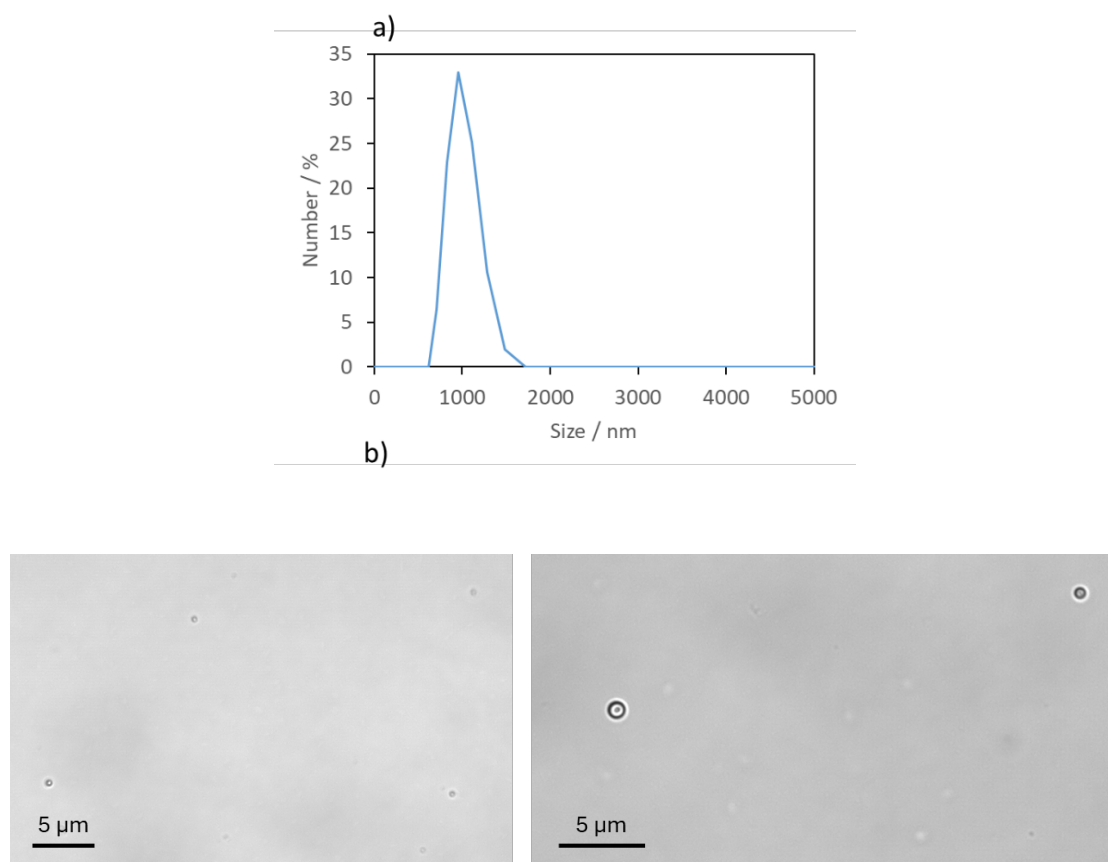


Figure 4.7. a) The hydrodynamic diameter of assemblies measured with dynamic light scattering (DLS) in the presence of **PV** (20 μ M) for C₁₄-picolyl-SH in aqueous HEPES buffer pH = 7.4, NaCl = 145 mM, [C₁₄-picolyl-SH·Zn^{II}] = 60 μ M at 25 $^{\circ}$ C, b) visualisation of the same sample using a light microscope (100 $\times$).

4.2.3 Further investigations using C₉-picolyl-SH (**73**)

We next examined the ability of C₉-picolyl-SH **73** to bind **PV** both in the presence and in the absence of Ag^I. Initial experiments with Ag^I (with [HEPES] = 5 mM, [NaCl] = 145 mM, pH = 7.4 and **PV** = 20 μ M at 25 $^{\circ}$ C). These results showed that 60 μ M of C₉-picolyl-SH·Zn^{II} and Ag^I were required to reach the colour change from yellow to blue. The change in the absorbance spectra of the **PV** was similar to what was observed previously, with the absorbance maximum shifting from 600 nm to 670 nm. However, three equivalents

of C_9 -picolyl-SH·Zn^{II}·Ag^I were required to induce a colour change, rather than the two equivalents with respect to **PV**, which we desired for a sensitive IDA (**Figure 4.8**). We therefore concluded that this system would not be a suitable host for a selective indicator for PPPi.

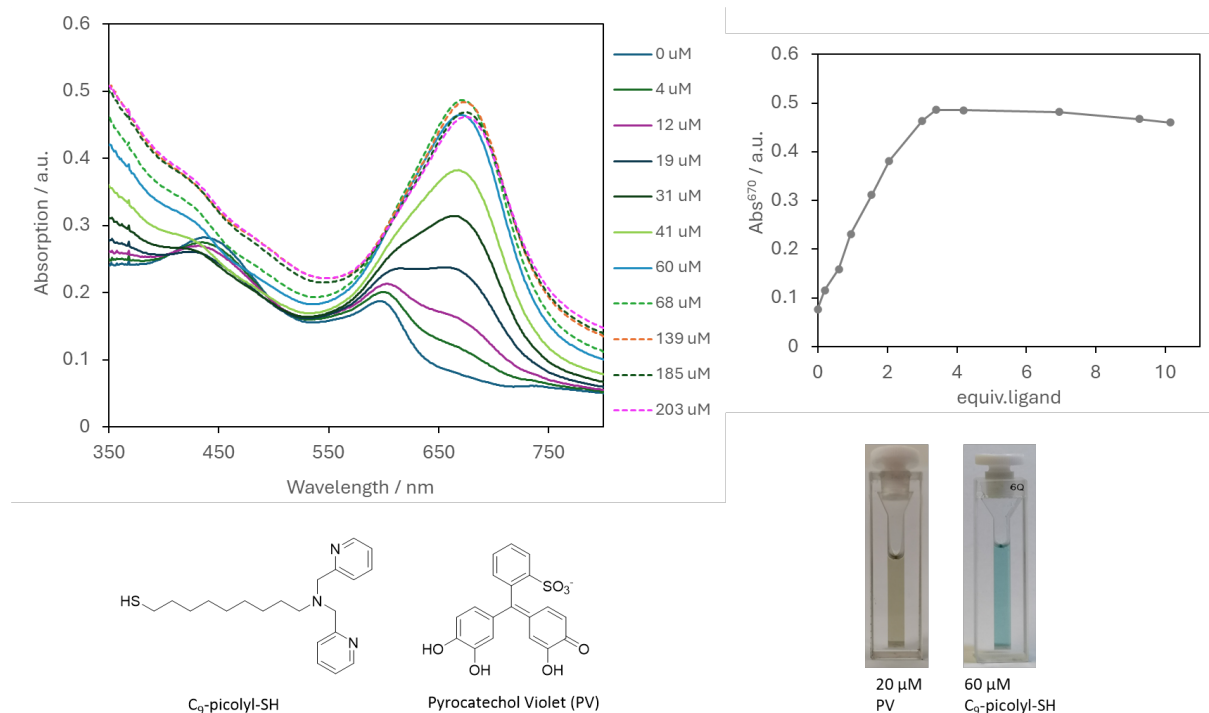


Figure 4.8. Changes in absorbance of **PV** solution with increasing C_9 -picolyl-SH·Zn^{II}·Ag^I concentrations up to 192 μ M. Conditions: [HEPES buffer] = 5 mM, pH 7.4, [NaCl] = 145 mM, [PV] = 20 μ M at 25 °C. C_9 -picolyl-SH·Zn^{II}·Ag^I concentrations = 0, 4, 12, 19, 31, 41, 60, 68, 139, 185 and 203 μ M.

Next, we examined the binding behaviour of C_9 -picolyl-SH **73** with 20 μ M **PV** in the absence of Ag^I. Upon adding C_9 -picolyl-SH to a **PV** solution, we noted significant changes in the UV spectrum and a dramatic colour shift from yellow to blue at 60 μ M (**Figure 4.9**). These findings, similar to those with C_{14} -picolyl-SH, suggest that **PV** necessitates three equivalents of C_9 -picolyl-SH·Zn^{II}, establishing a 1:3 ratio between **PV** and C_9 -picolyl-SH·Zn^{II}. As a result, we did not proceed further with this system and moved on to investigations with analogous thiols.

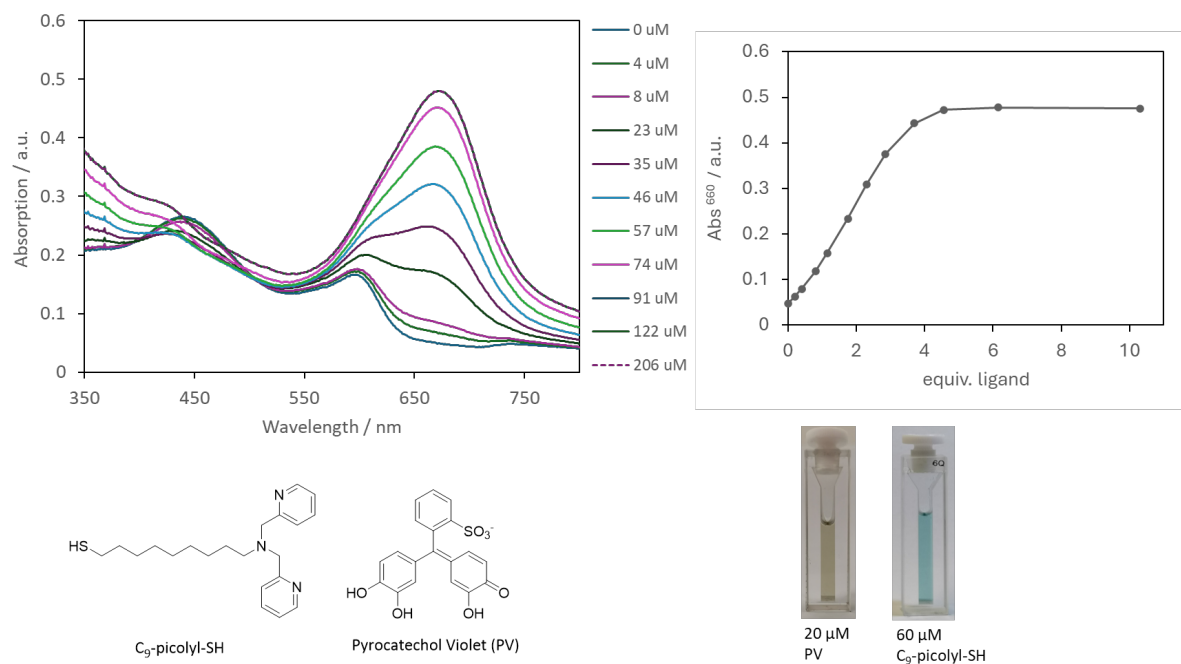


Figure 4.9. Changes in absorbance of **PV** solution with increasing C_9 -picolyl-SH·Zn^{II} concentrations up to 206 μ M. Conditions: [HEPES buffer] = 5 mM, pH 7.4, [NaCl] = 145 mM, [**PV**] = 20 μ M at 25 °C. C_9 -picolyl-SH·Zn^{II} concentrations = 0, 4, 8, 16, 23, 35, 46, 57, 74, 91, 122, and 206 μ M.

4.2.4 Further investigations using C_6 -picolyl-SH (72)

Next, we examined C_6 -picolyl-SH and explored its binding with **PV** in the presence of Ag^I. In an aqueous buffered solution containing 20 μ M **PV** (HEPES = 5 mM, NaCl = 145 mM, pH = 7.4 and 25 °C), we observed a colour change upon addition of 40 μ M of C_6 -picolyl-SH·Zn^{II} and Ag^I. This was a significant finding, given that the other analogues examined in this series all required three equivalents of the host to observe a colour change (**Figure 4.10**). There were significant changes in the absorbance spectra with increasing concentrations of C_6 -picolyl-SH·Zn^{II}·Ag^I, with a significant increase in the baseline seen from 400-550 nm, and a shift in the absorbance maximum at ~670 nm. This may suggest the formation of coordination polymers that interfere by light scattering, and that are also affecting the binding with **PV**.

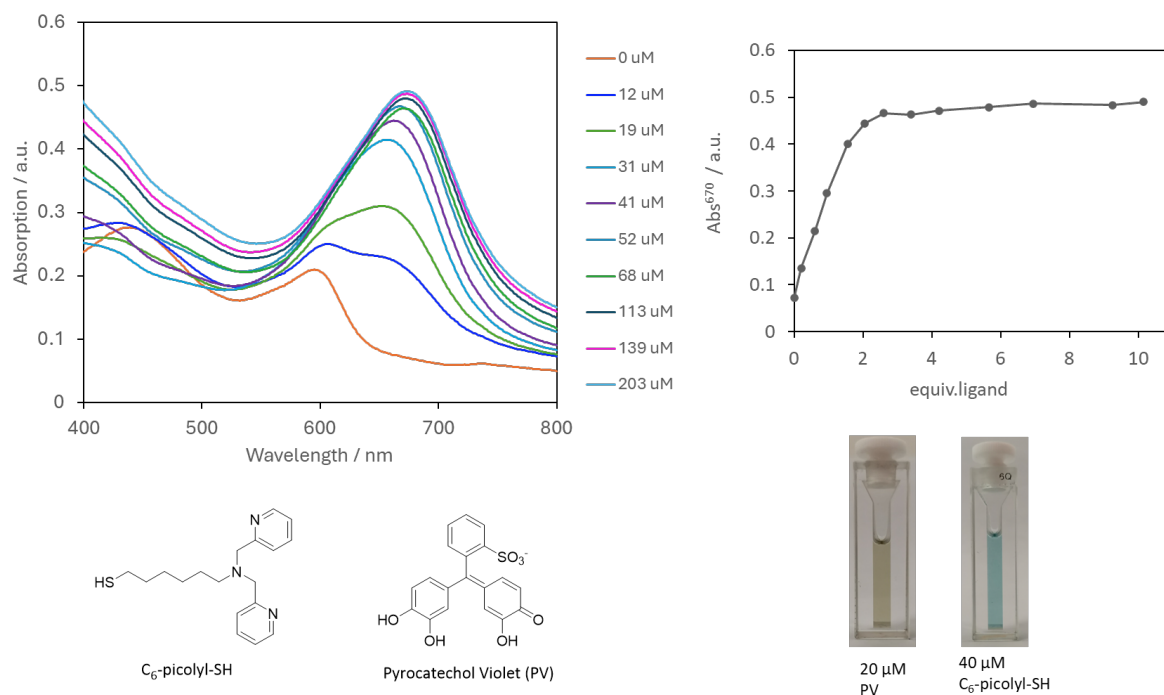


Figure 4.10. Changes in absorbance of **PV** solution with increasing C_6 -picolyl-SH·Zn^{II}·Ag^I concentrations up to 192 μ M. Conditions: [HEPES buffer] = 5 mM, pH 7.4, [NaCl] = 145 mM, [PV] = 20 μ M at 25 °C. C_6 -picolyl-SH·Zn^{II}·Ag^I concentrations = 0, 12, 19, 31, 41, 52, 68, 113, 139, and 203 μ M.

To probe the presence of supramolecular coordination polymers, we employed SEM to examine the C_6 -picolyl-SH·Zn^{II} system with and without Ag^I. In the presence of silver nitrate, we observed the presence of what appeared to be a network of globules (**Figure 4.11a, b**). We were not convinced that these images represented silver-thiolate coordination polymers, however, we were able to see a significant difference between these images and the images taken for the samples without Ag^I (**Figure 4.11c**). In the absence of Ag^I, discrete round shapes were observed, with diameters in the range of 1 μ M, which suggests the presence of large vesicles. In these images, we did not observe the extensive network of shapes observed in the presence of Ag^I. However, we were still not convinced that we were forming silver-thiolate coordination polymers, given that in the past, in unpublished work, we had been able to observe the formation of fibrous structures when triazacyclononane-based thiols and guanidinium-headed thiols were combined with Ag^I (see **Figure 4.11d**).

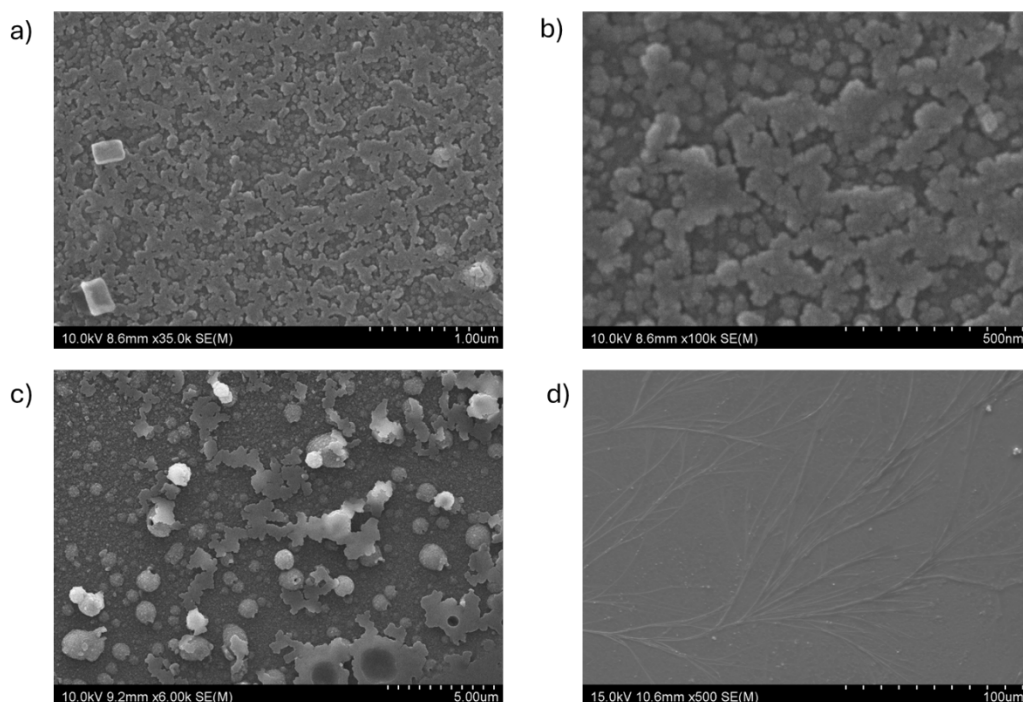


Figure 4.11. SEM images of C_6 -picolyl-SH·Zn^{II} a) and b) in the presence of Ag^I; c) in the absence of Ag^I; and d) from a previous experiment combining a picolyl-based thiol and a guanidinium-based thiol together with Zn^{II} and Ag^I ions.

In the literature, network structures similar to what we observed in **Figure 4.12a, b** has been attributed to the formation of Ag-thiolate coordination polymers. For example, Jing-Jing Quan and coworkers demonstrated that silver ions, when combined with a cystine thiol ligand in various solvents, including ethanol, methanol, and water, formed polymeric structures that are depicted in the SEM images shown in **Figure 4.12**. The images obtained for our C_6 -picolyl-SH·Zn^{II}·Ag^I system are in line with these images.

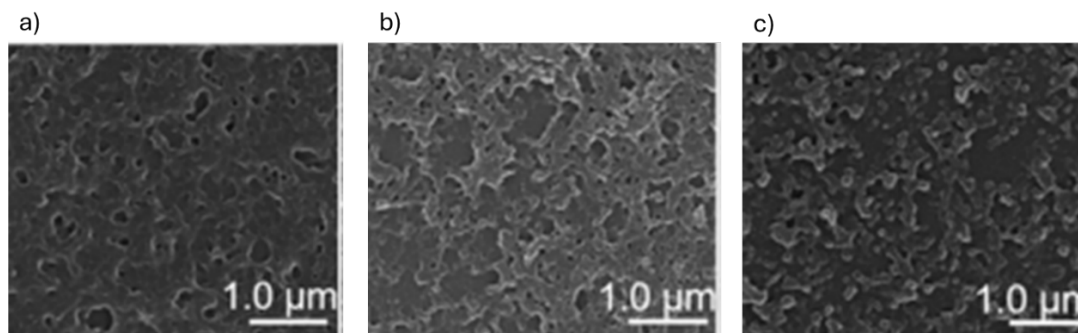


Figure 4.12. SEM images of supramolecular structures of Ag^I ion formed in ethanol (a), methanol (b), and water (c) in the presence of cystine thiol ligand.

Since we were able to establish that our C_6 -picolyl-SH·Zn^{II}·Ag^I complex exhibited 2:1 binding to the **PV** dye, a crucial next step was to establish if we could induce a reversible change upon the addition of PPI. To this end, we formed our host-guest complex by combining **PV** (20 μM) and 40 μM of C_6 -picolyl-SH·Zn^{II}·Ag^I and added increasing concentrations of PPI (**Figure 4.13**). Interestingly, we noticed that the displacement of **PV** from the PPI was inefficient, and eight equivalents of PPI were required to induce a colour change back to yellow. Further optimisation of this system was therefore required.

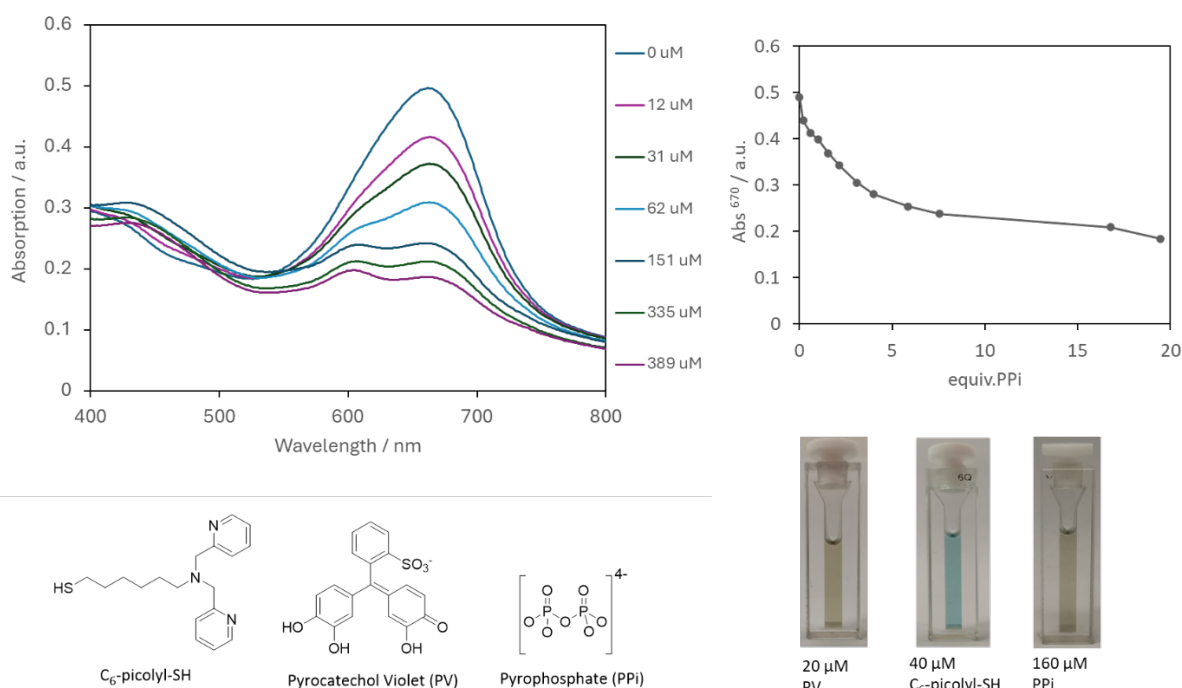


Figure 4.13. Absorbance spectra for the addition of PPI in the presence of amphiphilic ligand C_6 -picolyl-SH·Zn^{II}·Ag^I. Conditions: [HEPES buffer] = 5 mM, pH = 7.4, [NaCl] = 145 mM, [PV] = 20 μM, {[PV]: [C₆-picolyl-SH·Zn^{II}·Ag^I]} = 1:2, [PPI] = 0, 4, 12, 31, 43, 62, 80, 117, 151, 335 and 389 μM.

Next, we examined the behaviour of C_6 -picolyl-SH·Zn^{II} and 20 μM of **PV** in the absence of Ag^I (**Figure 4.14**). We were able to establish a 1:2 ratio between C_6 -picolyl-SH·Zn^{II} and **PV**, which was promising for the development of an IDA assay. To our delight, we were also able to show that the resulting **PV** complex could be efficiently displaced by 40 μM of PPI (**Figure 4.15**).

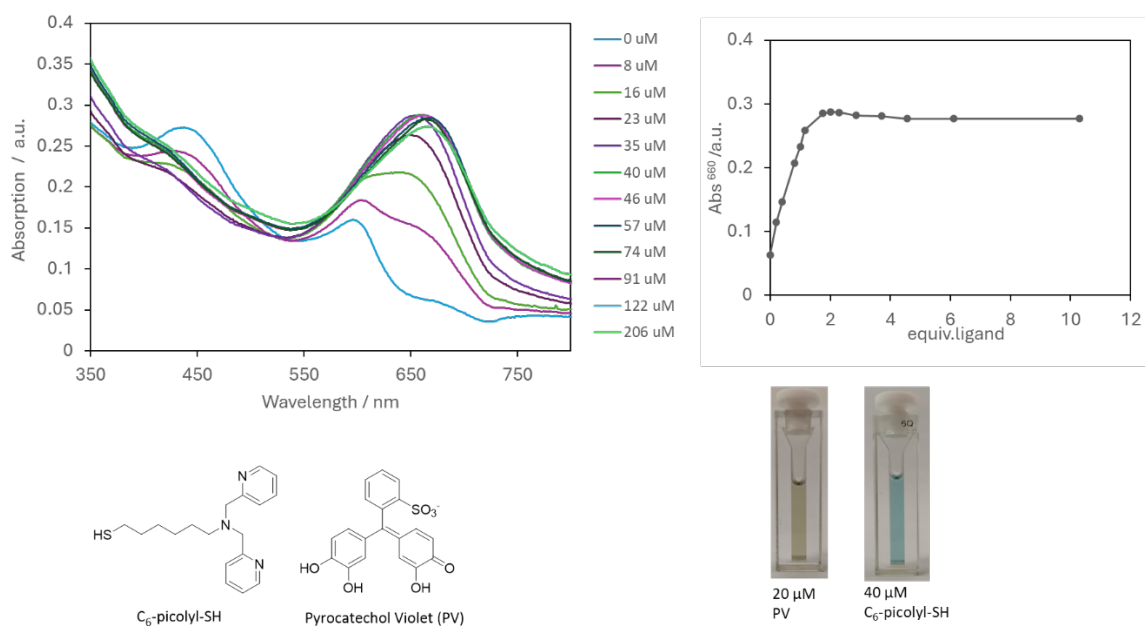


Figure 4.14. Changes in the absorbance of PV solution with increasing C_6 -picoly-SH·Zn^{II} concentrations up to 206 μ M. Conditions: [HEPES buffer] = 5 mM, pH 7.4, [NaCl] = 145 mM, [PV] = 20 μ M at 25 °C. C_6 -picoly-SH·Zn^{II} concentrations = 0, 8, 16, 23, 35, 40, 46, 57, 74, 91, 122, and 206 μ M.

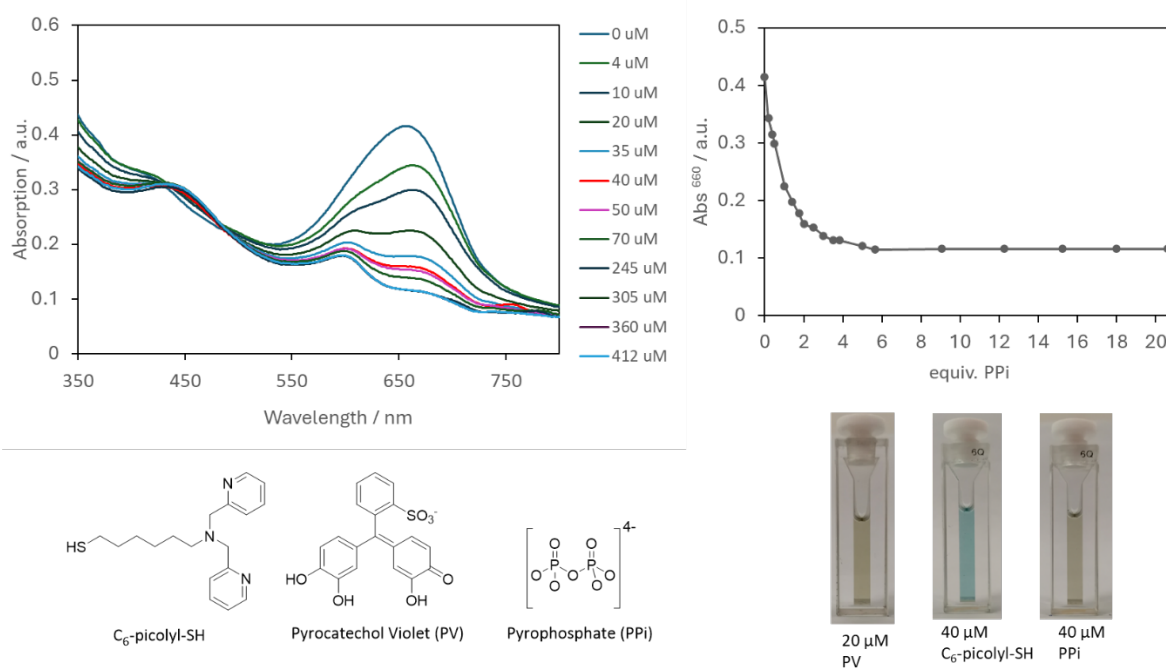


Figure 4.15. Absorbance spectra for the addition of PPI in the presence of amphiphilic ligand C_6 -picoly-SH·Zn^{II}. Conditions: [HEPES buffer] = 5 mM, pH = 7.4, [NaCl] = 145 mM, [PV] = 20 μ M, [PV]: [C_6 -picoly-SH·Zn^{II}] = 1:2, [PPI] = 0, 4, 10, 20, 35, 40, 50, 60, 70, 245, 305, 360 and 412 μ M at 25 °C.

The next step to determine whether this system could be used as an IDA for PPI, was to determine selectivity. We investigated the displacement of **PV** using the anions ATP, ADP, and citrate, and found that **PV** could be displaced with two equivalents of PPI whereas 20 equivalents of ATP were needed to obtain a greenish colour in the indicator, suggesting partial displacement. While ADP was able to induce a decrease in the absorbance at 660 nm, there was no colour change, even upon the addition of 25 equivalents. On the other hand, citrate was able to induce a colour change to green following the addition of 25 equivalents (Figure 4.16). These results show that this host-guest system shows over 10-fold selectivity for PPI over related phosphates such as ATP and ADP, giving it excellent potential for further development as an IDA.

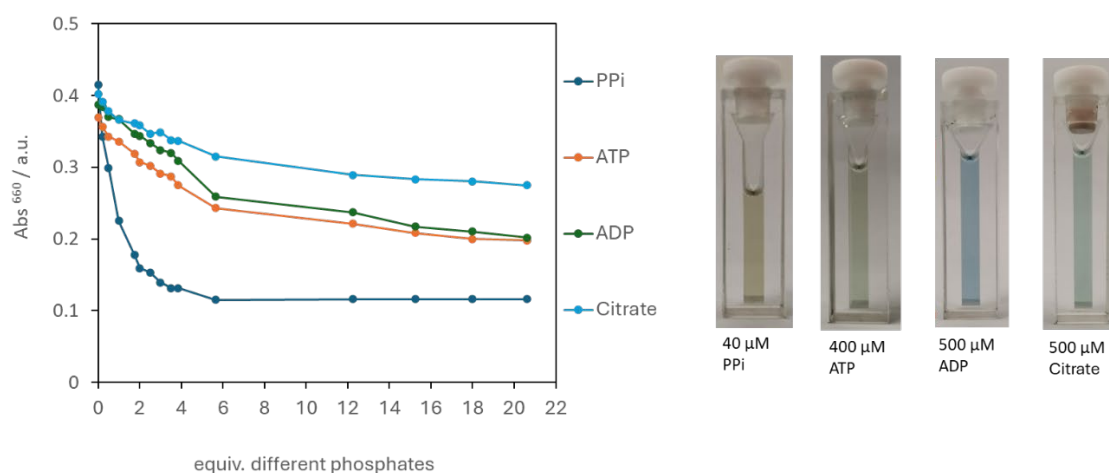


Figure 4.16. Absorbance spectra for the displacement of **PV** with anions such as PPI, ATP, ADP and citrate in the presence of C₆-picolyl-SH·Zn^{II} [40 μM]. Conditions: [HEPES buffer] = 5 mM, pH = 7.4, [NaCl] = 145 mM, [**PV**] = 20 μM at 25 °C.

Further selectivity studies were performed using 40 μM C₆-picolyl-SH·Zn^{II} and 20 μM **PV**, testing a variety of different phosphates (PPI, ATP, ADP, AMP, DMP, phosphoserine, phosphotyrosine, sodium phosphate, sodium biphosphate, and citrate (Figure 4.17). In each sample was added 100 μM (five equivalents) of the selected anion, and only the addition of PPI induced a colour change from blue to yellow, with the rest of the cuvettes remaining blue. Therefore, we were able to show that our supramolecular assembled host has good selectivity for PPI over other phosphates.

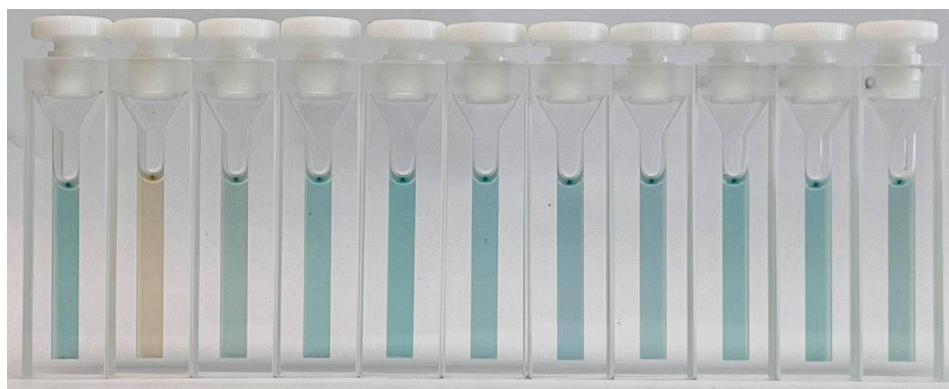


Figure 4.17. The colours of the mixtures of C_6 -picolyl-SH·Zn^{II} 40 μ M (each) and **PV** (20 μ M) with and without anions (sodium salts) from left to right: No anion, PPI, ATP, ADP, AMP, cAMP, phosphoserine, phosphotyrosine, HPO_4^{2-} , PO_4^{3-} , and citrate (5 equiv. each (100 μ M)).

4.2.5 Further investigations using C_4 -picolyl-SH (71)

Next, we investigated the behaviour of C_4 -picolyl-SH·Zn^{II} **71** to determine if there were any trends in binding behaviour associated with chain length. As in the previous examples, we began by assessing the binding of the indicator to the host in HEPES and 20 μ M **PV** ([HEPES] = 5 mM pH = 7.4, NaCl = 145 mM, 25 °C). Figure 4.18 shows the absorbance spectra for the titration of **PV** with each concentration of the C_4 -picolyl-SH·Zn^{II}·Ag^I. Crucially, no colour change was observed until the addition of 77 μ M of C_4 -picolyl-SH·Zn^{II}·Ag^I, which is less than the desired 2:1 binding. Interestingly, this shows that for this series of thiols, only the C_6 -picolyl-SH·Zn^{II}·Ag^I system was able to bind **PV** in the desired 2:1 ratio (**Table 4.1**).

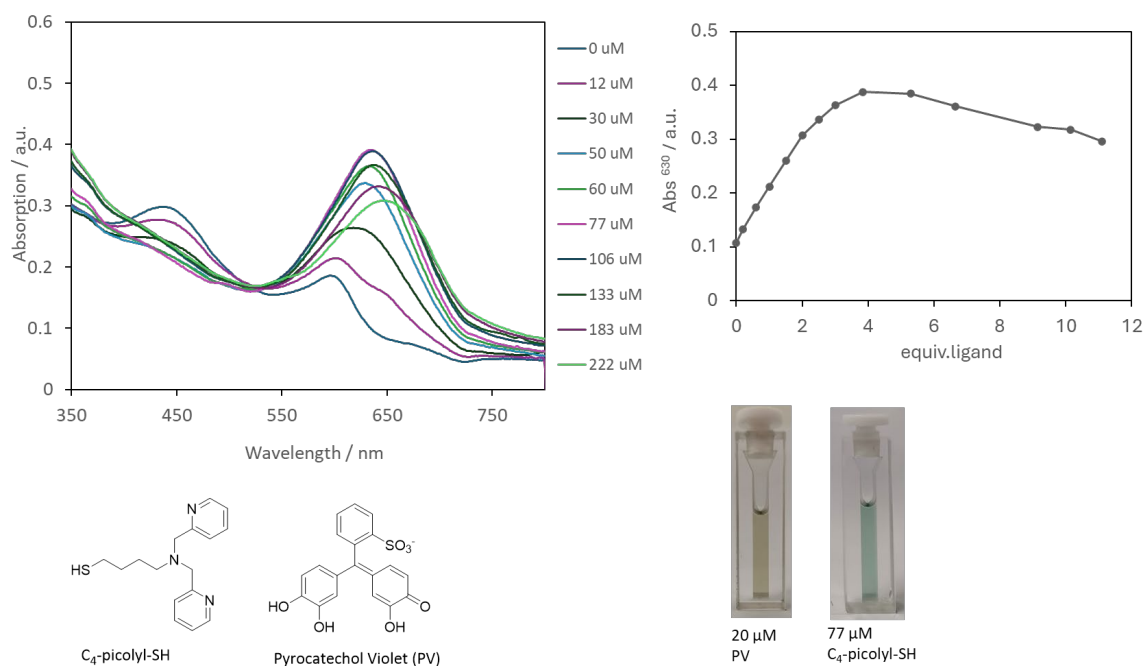


Figure 4.18. Changes in absorbance of **PV** solution with increasing C₄-picolyl-SH·Zn^{II}·Ag^I concentrations up to 192 μM. Conditions: [HEPES buffer] = 5 mM, pH 7.4, [NaCl] = 145 mM, [PV] = 20 μM at 25 °C. C₄-picolyl-SH·Zn^{II}·Ag^I concentrations = 0, 12, 30, 50, 60, 77, 106, 133, 183, and 222 μM.

Table 4.1. Summary of the experiments involving the binding of C_n-picolyl-SH·Zn^{II}·Ag^I with **PV**, and displacement with PPI.

Host	Equivalents for colour change with PV yellow → blue	Equivalents of PPI required to displace PV blue → yellow
C ₁₄ -picolyl-SH·Zn ^{II} ·Ag ^I	3	-
C ₉ -picolyl-SH·Zn ^{II} ·Ag ^I	3	-
C ₆ -picolyl-SH·Zn ^{II} ·Ag ^I	2	8
C ₄ -picolyl-SH·Zn ^{II} ·Ag ^I	4	-

Finally, we investigated the binding behaviour of C₄-picolyl-SH·Zn^{II} **71** with **PV** in the absence of Ag^I. Following the approach used in the previous examples, we first evaluated the binding of the indicator to the host in HEPES buffer containing 20 μM **PV** ([HEPES] = 5 mM, pH 7.4, NaCl = 145 mM, 25 °C). **Figure 4.19** presents the absorbance spectra

obtained from the titration of **PV** with varying concentrations of C_4 -picolyl-SH·Zn^{II}. In this case, no colour change was observed until the addition of 100 μ M C_4 -picolyl-SH·Zn^{II}·Ag^I, which is significantly more than the expected 2:1 binding ratio. Notably, within this series of thiols, only the C_6 -picolyl-SH·Zn^{II}·Ag^I complex demonstrated binding to **PV** in the desired 2:1 stoichiometry (**Table 4.2**).

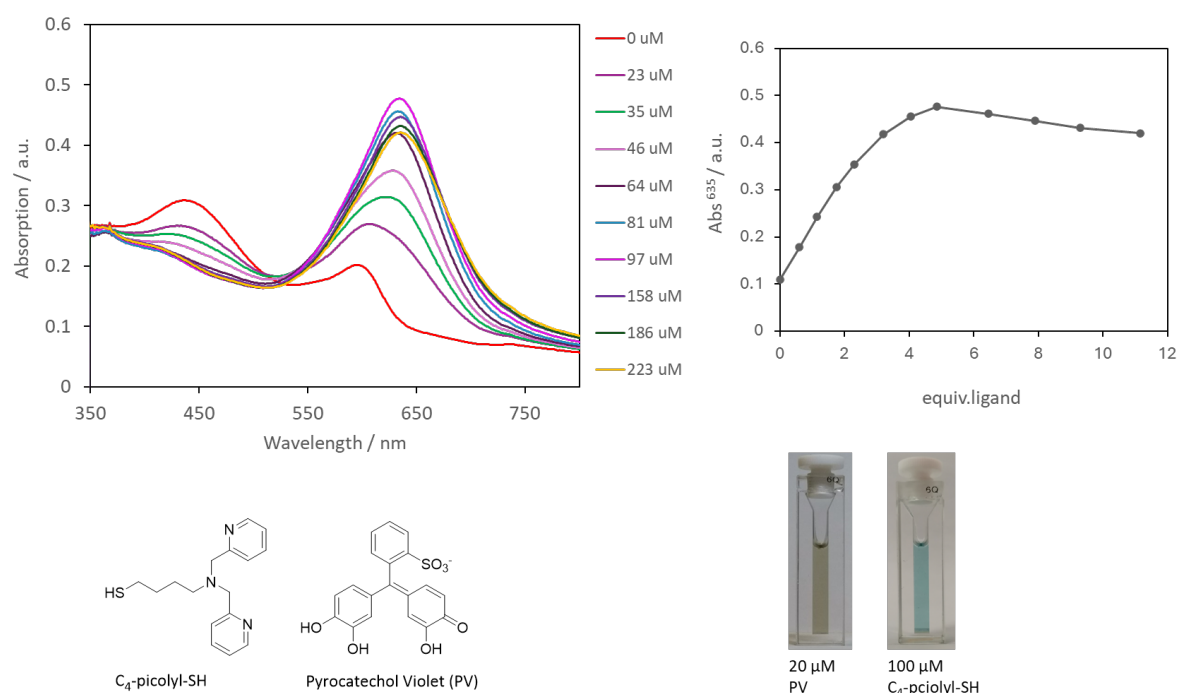


Figure 4.19. Changes in absorbance of **PV** solution with increasing C_6 -picolyl-SH·Zn^{II} concentrations up to 223 μ M. Conditions: [HEPES buffer] = 5 mM, pH 7.4, [NaCl] = 145 mM, [**PV**] = 20 μ M, at 25 °C. C_6 -picolyl-SH·Zn^{II} Ligand concentrations = 0, 23, 35, 46, 64, 81, 97, 158, 186 and 223 μ M.

Table 4.2. Summary of the experiments involving the binding of C_n -picolyl-SH·Zn^{II} with **PV** (in the absence of Ag^I), and displacement with PPI.

Host	Equivalents for colour change with PV yellow $\rightarrow$ blue	Equivalents of PPI required to displace PV blue $\rightarrow$ yellow
C_{14} -picolyl-SH·Zn ^{II}	3	-
C_9 -picolyl-SH·Zn ^{II}	3	-
C_6 -picolyl-SH·Zn ^{II}	2	2
C_4 -picolyl-SH·Zn ^{II}	5	-

4.3 Further characterisation and optimisation of the C₆-picolyl-SH·Zn^{II}-system

4.3.1 Determination of the supramolecular structure

Given that two Zn^{II}-binding units are required to be in proximity in order to effectively bind the indicator **PV**, it was likely that C₆-picolyl-SH·Zn^{II} was self-assembling into a supramolecular structure that allows the terminal dipicolylamine groups to be close together. One possibility is for C₆-picolyl-SH·Zn^{II} to self-assemble into micellar or vesicular structures, similar to the systems described in Chapter 2. While the thiol has a relatively short alkyl chain, it could be that **PV** is able to bind to two molecules of C₆-picolyl-SH·Zn^{II}, forming a two-tailed surfactant that has a higher propensity to self-assemble. Another possibility is for the amphiphiles to dimerise via a disulfide bond, forming bolaamphiphiles as shown in **Scheme 4.4**. Such structures would have a strong tendency to assemble, and the supramolecular structures formed could be stabilised by binding to **PV**. In both cases, two equivalents of C₆-picolyl-SH·Zn^{II} would be required to form an effective complex with **PV**, and in an ideal system, the resulting complex would be displaced by one equivalent of PPI.

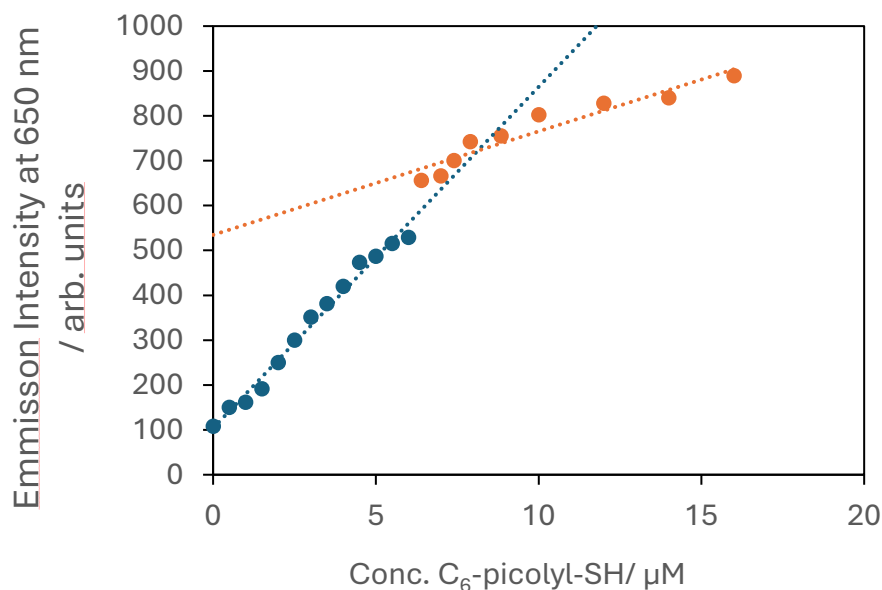


Figure 4.20. Emission intensity of Nile red (1 μM $\lambda_{\text{ex}} = 570 \text{ nm}$, $\lambda_{\text{em}} = 650 \text{ nm}$) at increasing concentrations of C₆-picolyl-SH·Zn^{II} in aqueous HEPES buffer solution (5 mM, pH 7.4).

The size of the supramolecular structures formed in solution was probed with DLS. We observed that the size of structures formed with C₆-picolyl-SH·Zn^{II} and **PV** was $1186 \text{ nm} \pm 459 \text{ nm}$, which suggests the formation of vesicular structures rather than micellar structures, given the large size (**Figure 4.21**). The presence of vesicles was supported by TEM images, which showed black hollow circles with sizes ranging from ~ 50 to $\sim 300 \text{ nm}$, roughly in line with the data obtained from DLS (**Figure 4.22**). These TEM images were prepared as described earlier, with staining using uranyl acetate.

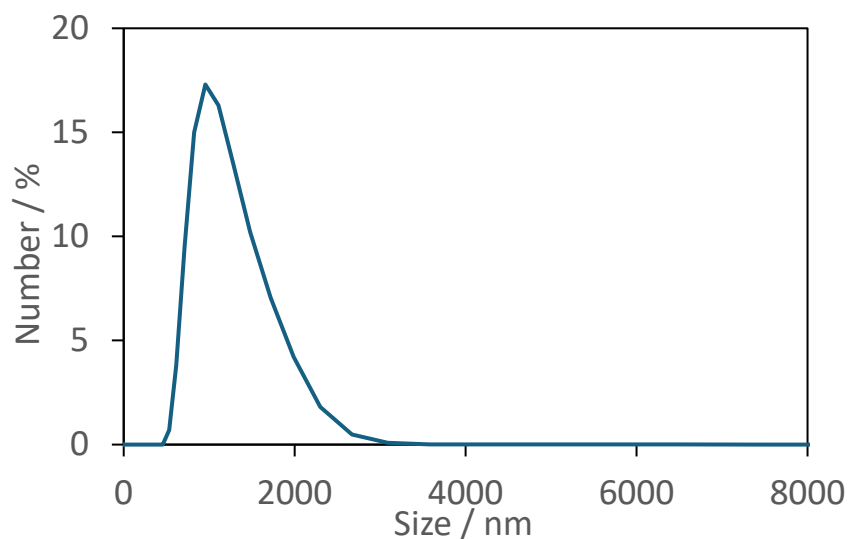


Figure 4.21. The hydrodynamic diameter of assemblies measured with dynamic light scattering (DLS) in the presence of **PV** (20 μM) for C_6 -picolyl-SH- Zn^{II} -**PV** system in aqueous HEPES buffer pH = 7.4, NaCl = 145 mM, $[\text{C}_6\text{-picolyl-SH}\cdot\text{Zn}^{\text{II}}] = 40 \mu\text{M}$ at 25 $^{\circ}\text{C}$.

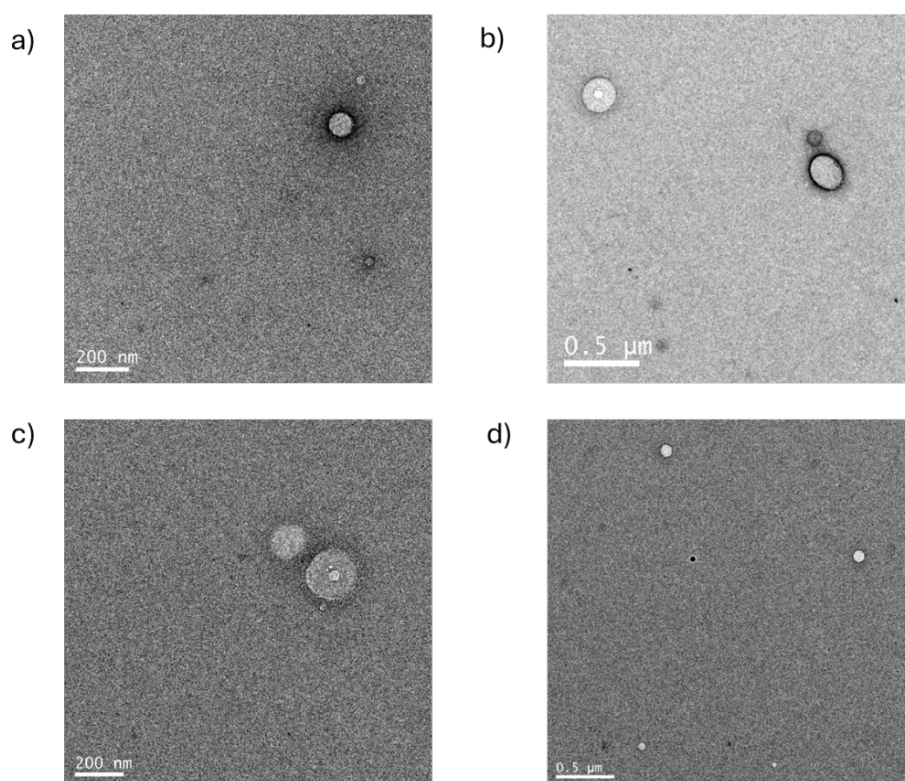


Figure 4.22. TEM images of C_6 -picolyl-SH- Zn^{II} (40 μM) in the presence of **PV** (20 μM), in aqueous HEPES buffer pH = 7.4, NaCl = 145 mM. Samples stained with uranyl acetate.

4.3.2 Switching experiments using redox reagents

To confirm whether dimerisation of thiols was occurring to form bolaamphiphiles, we performed a series of experiments under reductive and oxidative conditions. When C₆-picolyl-SH is in the presence of reducing agents like dithiothreitol (DTT), the formation of dimers can be prevented. The reducing agent keeps the thiol groups in their reduced state, which inhibits the formation of disulfide bonds between the thiol groups of proximal C₆-picolyl molecules. We conducted an experiment where we measured the absorbance spectrum of a mixture of 40 μ M C₆-picolyl-SH·Zn^{II} in combination with 20 μ M **PV**, in the presence of 25 μ M of DTT (in HEPES buffer (5 mM), pH 7.4 and NaCl (145 mM)). Under these conditions, the UV spectrum is similar to what is seen with **PV** on its own, with an absorbance maximum at 600 nm and no signal at 670 nm, with the solution remaining yellow in colour. Note that under standard conditions, in the absence of the reducing agent DTT, the solution would have already changed to a blue colour due to the complexation of C₆-picolyl-SH·Zn^{II} with **PV**. Crucially, the addition of 25 μ M hydrogen peroxide (H₂O₂) immediately induced the formation of a new peak at 670 nm, and a change in the colour of the solution to blue (see **Figure 4.23**, magenta line). These results strongly support the hypothesis that dimerisation of the thiols is occurring, to form bolaamphiphiles that are then able to effectively bind to **PV**.

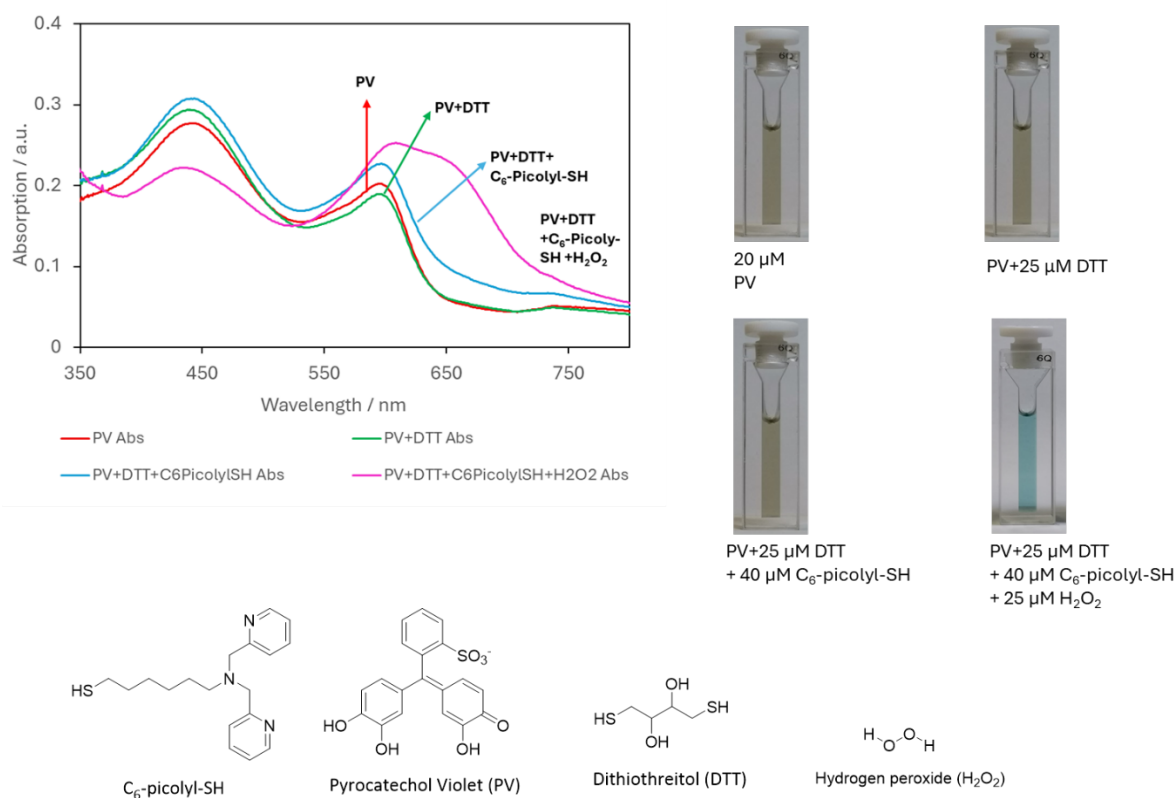


Figure 4.23. Switching experiments under different redox conditions, with 40 μM C₆-picoly-SH·Zn^{II}, in combination with 20 μM **PV**, and either 25 μM DTT or 25 μM H₂O₂.

To be certain that the addition of H₂O₂ did not induce the colour change from yellow to blue, we performed additional control experiments using the amphiphile C₆-picoly **36**. This compound does not possess a terminal thiol and would not be expected to respond to the reducing agent DTT or the oxidising agent H₂O₂. The addition of H₂O₂ to a solution of the control molecule C₆-picoly **36** and 20 μM **PV** in aqueous HEPES buffer (5 mM, pH 7.4, 145 mM NaCl) did not significantly change the spectrum of **PV**, with no formation of the absorption band at 670 nm (**Figure 4.24** below). The characteristic yellow-to-blue colour was not observed, which indicates that the addition of H₂O₂ does not cause the colour change on its own.

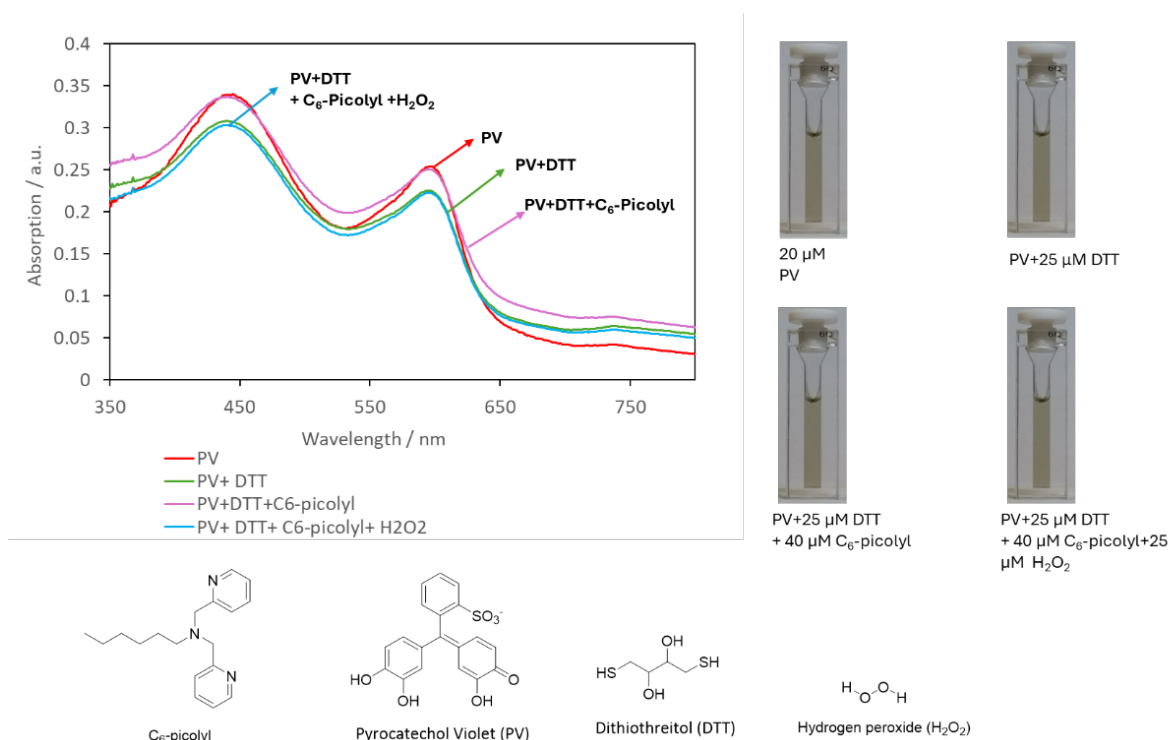


Figure 4.24. Switching experiments with the control molecule C₆-picolyl·Zn^{II} **36**, performed under different redox conditions, with 40 μM C₆-picolyl·Zn^{II}, in combination with 20 μM **PV**, and either 25 μM DTT or 25 μM H₂O₂.

4.3.3 Incorporation of C₆-picolyl-SH·Zn^{II} into a micellar matrix

In previous chapters, we had noticed benefits from incorporating the host into a micellar matrix, which provided cleaner binding isotherms that could then be modelled to determine binding constants. Thus, we conducted experiments in which increasing amounts of C₆-picolyl-SH·Zn^{II} were added to a solution containing the non-ionic surfactant Brij O10 (800 μM) and 20 μM **PV** in aqueous HEPES buffer (5 mM, pH 7.4, 145 mM NaCl, at 25 °C). **Figure 4.25** shows that two equivalents of C₆-picolyl-SH·Zn^{II} were required to induce a colour change from yellow to blue, which matches the results obtained in the absence of Brij O10.

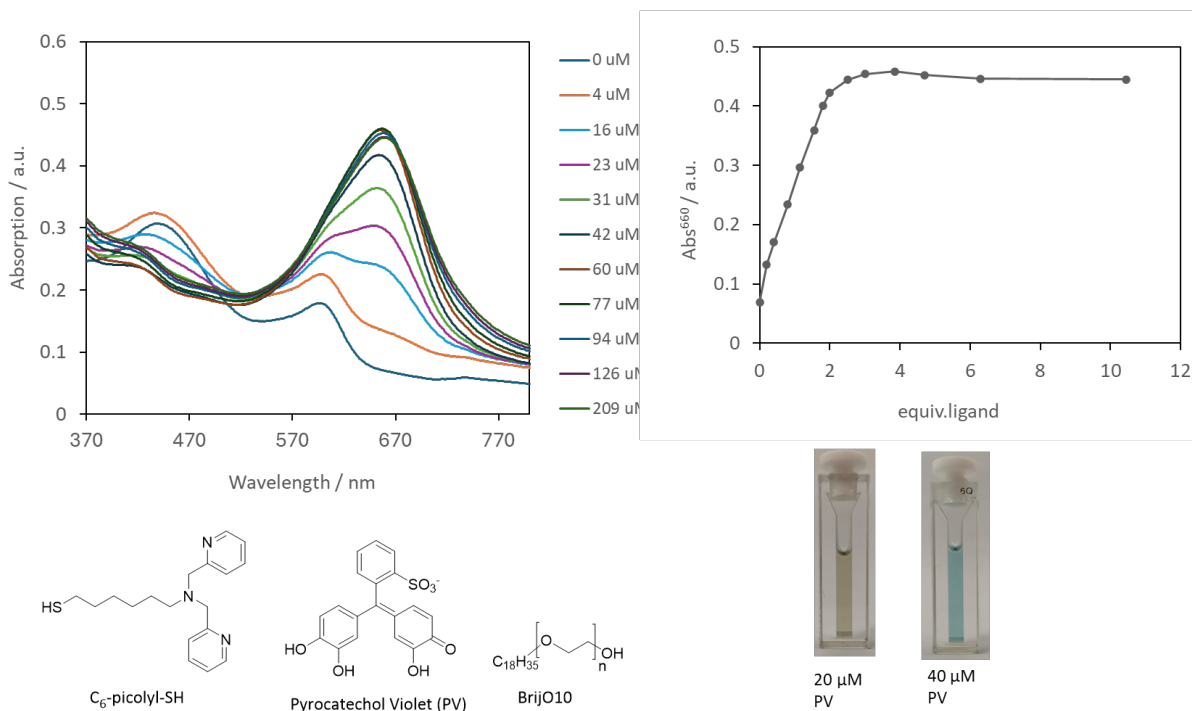


Figure 4.25. Changes in absorbance of PV solution with increasing C_6 -picolyl-SH·Zn^{II} concentrations up to 209 μ M. Conditions: [HEPES buffer] = 5 mM, pH 7.4, [NaCl] = 145 mM, [PV] = 20 μ M, [Brij O10] = 800 μ M at 25 °C. C_6 -picolyl-SH·Zn^{II} concentrations = 0, 4, 8, 16, 23, 31, 42, 60, 77, 94, 126 and 209 μ M.

Displacement of **PV** from the C_6 -picolyl-SH·Zn^{II} complex could be accomplished with 40 μ M PPI, with a clear colour change from blue back to yellow (**Figure 4.26**). Interestingly, this is the same concentration of PPI that is needed, when compared to the system in the absence of Brij O10. **Figure 4.27** below shows that this system is highly selective for PPI, with 20 equivalents of ATP required to induce a colour change, and at this concentration, the colour has not changed completely to yellow. With ADP, even upon addition of 20 equivalents, the colour of the solution remained blue. And with citrate, the addition of 20 equivalents results in the formation of a greenish colour. This micellar system therefore displays better selectivity for PPI than in the non-micellar system, with a selectivity greater than 20 over the other anions examined.

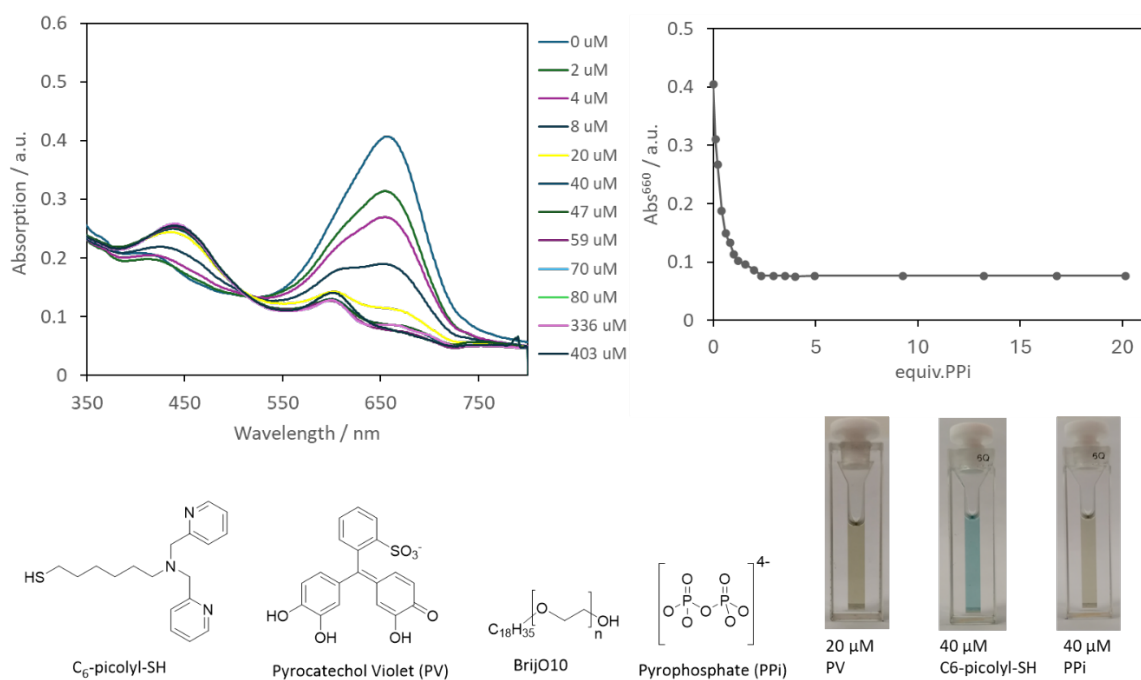


Figure 4.26. Absorbance spectra for the addition of PPI in the presence of amphiphilic ligand C₆-picolyl-SH·Zn^{II} within a Brij O10 (800 μM) micellar matrix. Conditions: [HEPES buffer] = 5 mM, pH = 7.4, [NaCl] = 145 mM, [PV] = 20 μM, [PV]: [C₆-picolyl-SH·Zn^{II}] = 1:2, [PPI] = 0, 2, 4, 8, 20, 40, 47, 59, 70, 80, 336, and 403 μM at 25 °C.

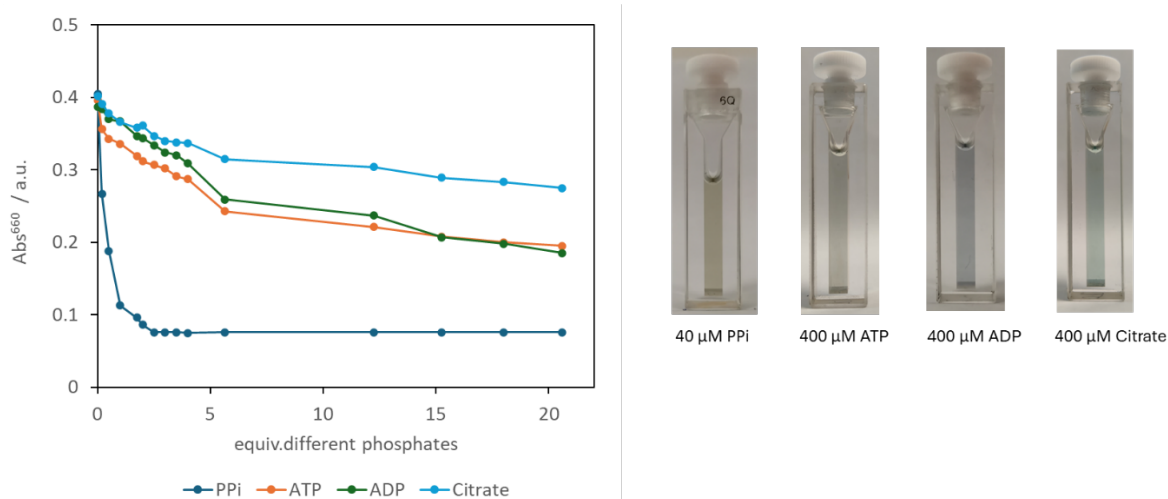


Figure 4.27. Absorbance spectra for the displacement of PV with anions such as PPI, ATP, ADP and citrate in the presence of C₆-picolyl-SH·Zn^{II} (40 μM). Conditions: [Brij O10] = 800 μM, [HEPES buffer] = 5 mM, pH = 7.4, [NaCl] = 145 mM, [PV] = 20 μM at 25 °C.

Table 4.3. Comparison of the selectivity of the vesicular and micellar systems involving C₆-picolyl-SH·Zn^{II} and **PV** (in the absence of Ag^I).

Analyte	Vesicular system	Micellar system (Brij O10 matrix)
PPi	2	1
ATP	>20	>20
ADP	>20	>20
citrate	>20	>20

Finally, to demonstrate selectivity for PPi, studies were conducted using 40 μM C₆-picolyl-SH·Zn^{II} and 20 μM **PV** in the presence of 800 μM of Brij O10. A variety of phosphates were tested, including PPi, ATP, ADP, AMP, DMP, phosphoserine, phosphotyrosine, sodium phosphate, sodium biphosphate, and citrate (as shown in **Figure 4.28** below). In each sample, 100 μM (five equivalents) of the selected anion was added. To our delight, only the addition of PPi caused a colour change from blue to yellow, while the other samples remained blue, similar to the vesicle system. This demonstrates that our supramolecular host exhibits strong selectivity for PPi over other phosphates also in the micellar system.

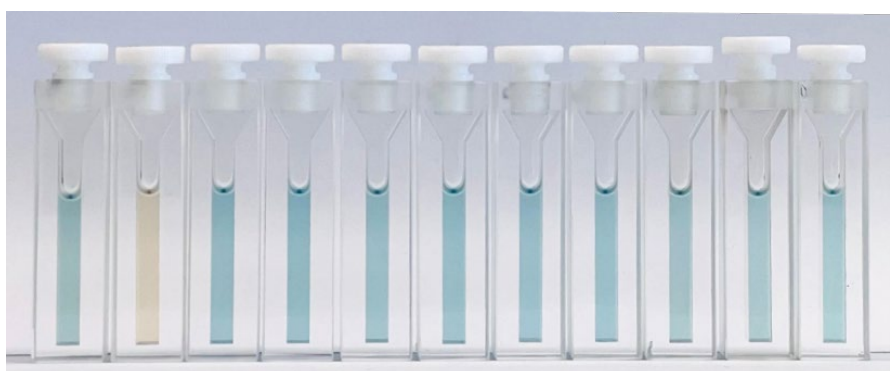


Figure 4.28. The colours of the mixtures of C₆-picolyl-SH·Zn^{II} 40 μM (each) and **PV** (20 μM) in the presence of Brij O10 (800 μM) with and without anions (sodium salts) from left to right : No anion, PPi, ATP, ADP, AMP, cAMP, phosphoserine, phosphotyrosine, HPO₄²⁻, PO₄³⁻, and citrate (5 equiv. each (100 μM)).

4.4 Conclusions

This chapter has explored the design, synthesis, and characterisation of a new class of thiol-based amphiphilic receptors capable of forming *in situ* silver-thiolate coordination polymers. Building on the supramolecular and self-assembly concepts developed in the previous chapters, this work aimed to investigate how **Ag-S coordination chemistry** could be exploited to generate dynamic receptor frameworks that integrate both metal-mediated and amphiphilic modes of assembly.

A series of picolyl-terminated thiols with varying alkyl chain lengths (C₄-C₁₄) were successfully synthesised and evaluated for their ability to form host-guest complexes with the colourimetric indicator pyrocatechol violet (**PV**) in the presence of Zn^{II} and Ag^I ions. Initial studies revealed that the longer-chain thiols (C₁₄, C₉) behaved similarly to conventional amphiphiles, forming vesicular aggregates with limited responsiveness and weak cooperativity. These systems required three or more equivalents of host to induce colour change and exhibited poor indicator displacement with pyrophosphate (PPi). In contrast, shorter-chain analogues displayed enhanced responsiveness, with **C₆-picolyl-SH·Zn^{II}** emerging as the optimal receptor within this series.

Spectroscopic, microscopic, and fluorescence studies demonstrated that the C₆ system self-assembles into **vesicular supramolecular structures** with a very low critical aggregation concentration (~8 μM). Under oxidative conditions, the formation of disulfide-linked dimers was confirmed through reversible redox switching experiments: treatment with the reducing agent DTT prevented complexation with **PV**, while oxidation with H₂O₂ restored binding and the characteristic yellow-to-blue colour change. These results provide strong evidence that **redox-controlled dimerisation** drives the formation of bolaamphiphilic species capable of cooperative **PV** binding through intramolecular Zn^{II}-picolyl coordination.

The C₆-picolyl-SH·Zn^{II} system exhibited efficient 2:1 host-indicator binding and selective 1:2 displacement by PPi, while maintaining excellent discrimination against ATP, ADP, and citrate. Integration of the receptor into a **Brij O10 micellar matrix** further improved the clarity of the spectral response, reproducibility of binding isotherms, and

overall selectivity. In this environment, complete indicator displacement was achieved with one equivalent of PPI and minimal response to competing anions, confirming the robustness of the system across different aggregation states.

Overall, this study demonstrates that dynamic Ag-thiolate and disulfide-driven self-assembly provide an effective strategy for constructing adaptive, redox-responsive supramolecular receptors. The results highlight the importance of fine-tuning molecular chain length, coordination environment, and redox state to control both self-assembly and analyte recognition. The emergence of the C₆-picolyl-SH·Zn^{II} system as a reversible, highly selective PPI sensor establishes a clear link between redox-controlled polymerisation and functional supramolecular sensing.

Chapter Five

Conclusions and Future Work

5.1 Conclusions

This thesis has demonstrated how molecular self-assembly can be harnessed to create functional supramolecular receptors for the selective sensing of biologically relevant phosphate anions. By combining concepts from coordination chemistry, amphiphilic self-assembly, and indicator displacement assays (IDAs), we have developed a modular approach to receptor design that circumvents the need for complex covalent synthesis. The central theme underpinning this work is that non-covalent self-assembly can generate, adapt, and optimise receptor structures dynamically, providing both structural flexibility and functional tunability.

In Chapter 2, amphiphilic ligands bearing dipicolylamine- Zn^{II} complexes were explored as self-assembled colorimetric receptors for pyrophosphate (PPi). We showed that aggregation in aqueous media is crucial to achieving cooperative binding between multiple metal-ligand units. Among a series of amphiphiles, the C_8 -picolyl- Zn^{II} system displayed optimal behaviour, forming vesicular aggregates with a 2:1 host-indicator binding ratio and a clean, reversible response to PPi. These findings demonstrated that hydrophobic chain length governs self-assembly, binding stoichiometry, and selectivity.

Chapter 3 extended these principles to bolaamphiphilic architectures, showing how structural symmetry and chain length affect intramolecular versus intermolecular coordination. Here, the C_8 -di-picolyl- Zn^{II} bolaamphiphile emerged as the most effective receptor, forming well-defined supramolecular assemblies and displaying a rapid, selective response to PPi. Importantly, this chapter established a clear structure–function correlation: shorter, more flexible spacers favour intramolecular binding and improved reversibility, while longer chains promote extended aggregates with diminished responsiveness.

In Chapter 4, the design principles were translated into thiol-based systems capable of Ag-S coordination polymerisation. This introduced an additional level of structural control through dynamic covalent (disulfide) and coordination (Ag-thiolate) bonding. Among the synthesised thiol amphiphiles, C_6 -picolyl-SH- Zn^{II} was found to form redox-responsive bolaamphiphilic assemblies with highly selective PPi sensing

capabilities. The system's reversible switching between reduced (thiol) and oxidised (disulfide) states was confirmed spectroscopically, establishing a proof-of-concept for redox-controlled assembly-disassembly of supramolecular sensors. Integration into micellar matrices such as Brij O10 further improved signal clarity and selectivity.

Collectively, these studies demonstrate that self-assembled amphiphilic and coordination systems can replicate and even surpass the selectivity of traditional covalent receptors. The modularity of the approach allows rapid tuning of receptor geometry and environment through simple chemical modification or environmental control, providing a versatile platform for future supramolecular sensor design.

5.2 Future Work

The findings presented in this thesis open multiple avenues for further investigation, both fundamental and applied. The development of amphiphilic and thiol-based receptors that can self-assemble and respond to analyte binding demonstrates the potential of self-assembly as a design strategy for creating functional supramolecular systems. However, a number of mechanistic and structural questions remain to be addressed in order to fully understand and exploit the behaviour of these assemblies.

A priority for future work will be to elucidate the detailed mechanisms of self-assembly and analyte recognition in these systems. While this thesis has provided spectroscopic and microscopic evidence for vesicle and bolaamphiphile formation, the precise molecular-level organisation, aggregation kinetics, and binding mechanisms remain to be quantified. Techniques such as isothermal titration calorimetry (ITC), NMR diffusion-ordered spectroscopy (DOSY), small-angle X-ray scattering (SAXS), and cryo-transmission electron microscopy (cryo-TEM) could provide direct insights into aggregate morphology and thermodynamic driving forces. Complementary computational approaches, including molecular dynamics (MD) simulations and density functional theory (DFT) calculations, would help visualise the spatial arrangement of metal-ligand units, the cooperativity of anion binding, and the energetic contributions of hydrophobic versus coordination interactions. A deeper mechanistic understanding would

allow rational fine-tuning of the system's assembly-disassembly equilibria and selectivity.

Another direction of considerable interest lies in broadening the chemical scope of the systems developed in this work. The dipicolylamine-Zn^{II} motif could be diversified to include other metal ions with complementary coordination geometries or redox properties. For example, replacing zinc with copper (II), cobalt (II), or nickel (II) may lead to distinct binding affinities or catalytic activity, while the introduction of lanthanide ions could confer luminescent or magnetic sensing capabilities. Extending the concept to recognise other biologically or environmentally relevant anions, such as nucleotides, phosphorylated peptides, carboxylates, or sulfates, would test the generality of the self-assembled receptor framework and expand its potential applications in biosensing and environmental monitoring. Similarly, modifying the amphiphilic ligands to include chiral centres or π -conjugated aromatic domains may enable selective enantio discrimination or optical readouts via circular dichroism and fluorescence.

The dynamic nature of the systems developed here also suggests promising opportunities for the creation of multi-stimuli responsive assemblies. The thiol-based systems demonstrated reversible control of self-assembly through redox chemistry. This principle could be expanded to include pH, light, temperature, or ion-exchange stimuli, introducing switchable or adaptive receptor architectures. For example, incorporating photochromic groups such as azobenzenes or spiropyrans could allow the reversible modulation of binding affinity via light-induced conformational changes. Similarly, redox-active metal centres or disulfide-thiol equilibria could be coupled to electron transfer processes, enabling electrochemical sensing or integration into smart materials that change colour or conductivity upon analyte recognition.

A further challenge and opportunity lie in translating these supramolecular systems into functional materials. Embedding the amphiphilic or coordination-based receptors within soft materials such as hydrogels, polymer membranes, or microemulsion droplets could lead to solid-state or semi-solid sensors capable of real-time signal transduction. These materials could be engineered to exhibit colourimetric or fluorescent responses to phosphate-containing species in biological fluids or environmental samples. Similarly, deposition of silver-thiolate coordination polymers as thin films or coatings

could generate luminescent or conductive sensor surfaces, potentially useful for integration into microfluidic or lab-on-chip devices. The high modularity and aqueous compatibility of these systems make them attractive candidates for biosensing, water quality monitoring, and environmental diagnostics.

In parallel, computational design and predictive modelling should be incorporated into future studies to accelerate discovery. By systematically varying ligand structures and metal combinations, and correlating experimental data with computational descriptors, it would be possible to establish quantitative structure-property relationships (QSPRs) that predict binding affinities, selectivity patterns, and aggregation behaviours. Such approaches could also inform the development of combinatorial libraries of amphiphiles, screened rapidly using high-throughput optical assays to identify optimal candidates for specific analytes. Integration with machine-learning frameworks could further refine design principles and lead to the discovery of unexpected self-assembled architectures.

Finally, an exciting avenue would be to explore catalytic and biomimetic applications of these self-assembled systems. The coordination polymer described in Chapter 4 already exhibited key structural motifs reminiscent of enzyme active sites, namely, multivalent metal-ligand arrays capable of cooperative binding and transition-state stabilisation. Future studies could leverage these architectures to catalyse biologically relevant reactions such as phosphoester hydrolysis, CO₂ hydration, or peptide cleavage, using the same dynamic self-assembly principles to regulate activity. By integrating recognition and catalysis, such systems would move toward artificial enzyme mimics that are both functional and adaptive, offering insights into the emergence of catalytic networks in prebiotic or synthetic cellular environments.

In summary, the research directions emerging from this thesis span a continuum from fundamental mechanistic studies to applied sensor and catalytic technologies. The central message, that self-assembly can be used as a modular design principle to build responsive supramolecular systems, provides a versatile foundation for future exploration. By combining synthetic innovation with advanced characterisation and modelling, the next generation of these systems could evolve into truly intelligent

molecular materials, capable of sensing, responding, and adapting to their chemical environment with precision and purpose.

Chapter Six
Experimental

6.1 General Information

All fine chemicals were sourced from commercial suppliers and were used directly without purification unless mentioned. Di-(2-picolyl)amine, 1-bromooctane, 1-bromodecane, 1-bromododecane, 1-bromohexadecane and 1,4-dibromobutane 1,6-dibromohexane, 1,8-dibromooctane, 1,10-dibromodecane, 1,12-dibromododecane, 1,14-dibromotetradecane, thioacetate, potassium carbonate, sodium carbonate, triphenyl phosphine, 1,14-dihydroxytetradecane, and hydrogen peroxide were purchased from Sigma Aldrich and NMR solvents, including deuterated methanol-*d*₄ and deuterated chloroform, were purchased from EURISO-TOP. Acetone and hexanes (analytical grade) were purchased from ECP chemicals, along with dichloromethane and ethyl acetate of laboratory quality. Hydrochloric acid 37% and methanol were purchased from Thermo Fisher Scientific. Celite 545 was purchased from VELP Scientific. Anhydrous magnesium sulphate was purchased from Scharlau. Palladium on carbon (10 wt.% loading, matrix activated carbon support) was purchased from Sigma Aldrich.

Organic compounds were synthesised and characterised by ¹H, ¹³C NMR, IR and HRMS and the data were compared to literature values. Flash column chromatography was performed using silica gel 60 (Aldrich) and a suitable eluent. Analytical TLC was performed on aluminium-backed plates pre-coated (0.25 mm) with Merck Silica Gel 60 F254 with a suitable solvent system and was visualised using UV fluorescence (254 nm) and/or developed with ninhydrin, phosphomolybdic acid (PMA) or potassium permanganate.

4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) was purchased from Sigma-Aldrich and used without further purification. The pH of buffer solutions was determined at room temperature using a Metrohm-632 pH-meter equipped with an Ag/AgCl/KCl reference electrode. In all cases, stock solutions were prepared using deionised water filtered with a Milli-Q water purifier (Millipore) and stored at 4 °C unless stated otherwise. Zn(NO₃)₂ was an analytical grade product, and the concentration of stock solutions was determined by atomic absorption spectroscopy. Stock solutions of the

PPi, ATP, ADP, DMP, and DMP substrates were prepared by weight, and their concentrations were confirmed by UV spectroscopy and adjusted if necessary.

^1H , ^{13}C and ^{31}P NMR spectra were recorded using a Bruker Ascend 400 NMR spectrometer operating at 400 MHz for ^1H nuclei and 101 MHz for ^{13}C nuclei. Chemical shifts (δ) are quoted in parts per million (ppm) and coupling constants (J) are in Hertz (Hz). Residual solvent peaks were used as the internal reference for ^1H and ^{13}C NMR. Abbreviations for multiplicity are as follows: s = singlet, br s = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublet, etc.).

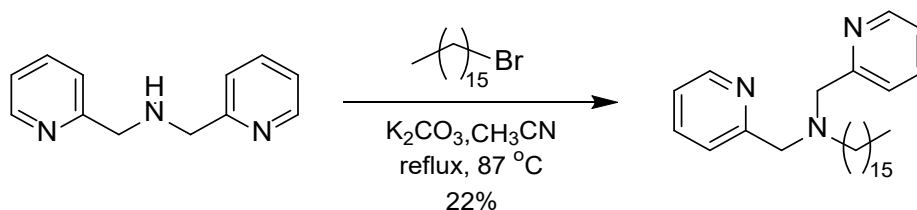
Infrared (IR) spectra were recorded using a Nicolet iS10 spectrometer (Thermo Fisher Scientific Inc.) with the absorption peaks expressed in wavenumbers (cm^{-1}) and recorded using a range of 450 to 4000 cm^{-1} . IR spectra were analysed using OMNIC 9.2.86. High resolution mass spectrometry (HRMS) measurements were obtained using a VG-70SE spectrometer at a nominal resolution of 5000 to 10000 as appropriate.

UV-visible spectroscopy was conducted using a Cary 100 UV-vis spectrophotometer (Agilent Technologies, model number: G9821A), equipped with a Cary temperature controller (Agilent Technologies, model number: G9844A). UV-vis spectra were recorded using Cary WinUV 4.20(468). Fluorescence measurements were recorded on a Varian Cary Eclipse Fluorescence spectrophotometer equipped with a thermos tatted cell holder. Nile red was added from a concentrated solution in EtOH (typically 2 μL of a 1 mM solution were present in a 1 mL solution).

Standard TEM images were recorded on a FEI Tecnai FEG20 or a FEI Tecnai 12 with Gatan cold stages and a Gatan Ultrascan 100 4 Mpixel digital camera. DLS analyses were performed on a Malvern Zetasizer Nano-S instrument using filtered milli-Q water and a filtered buffer solution.

6.2 Synthetic Procedures and Characterisation Data

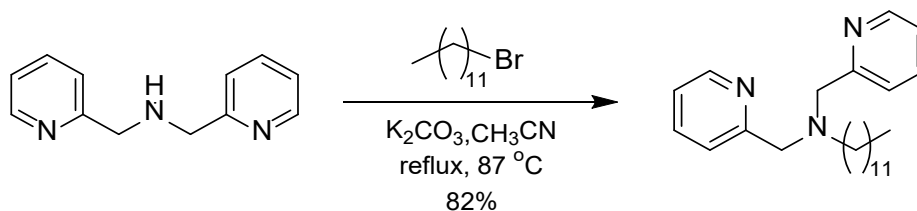
N-Hexadecyl-*N*-(2-pyridinylmethyl)-2-pyridinemethanamine (C₁₆-picolyl, 28)



To a solution of di-(2-picolyl) amine (0.50 g, 2.5 mmol, 1.0 eq.) in acetonitrile (20 mL) was added 1-bromohexadecane (0.63 mL, 2.0 mmol, 1.25 eq.) and potassium carbonate (1.04 g, 7.53 mmol, 3 eq.) followed by sodium carbonate (0.632 g, 7.53 mmol, 3.0 eq.). The reaction mixture was heated at reflux for 24 hours, followed by solvent evaporation under reduced pressure. The residue was added to distilled water (15 mL) and extracted with CH₂Cl₂ (3 x 8 mL). The organic layer was collected, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography on alumina gel (0.5:99.5 methanol/CH₂Cl₂) to yield the title compound (0.243 g, 0.57 mmol, 22%) as dark yellow oil.

¹H NMR (400 MHz, CD₃OD): δ (ppm) 8.45 (dd, J = 5.0, 1.7 Hz, 2H), 7.82 (td, J = 7.7, 1.8 Hz, 2H), 7.66 (t, J = 7.2 Hz, 2H), 7.36 – 7.20 (m, 2H), 3.80 (s, 3H), 3.33 (dt, J = 3.3, 1.6 Hz, 3H), 2.51 (dd, J = 7.2, 1.5 Hz, 4H), 1.54 (dt, J = 7.2, 1.3 Hz, 4H), 1.40 – 1.08 (m, 14H), 0.91 (m, J = 6.8 Hz, 9H). **¹³C NMR** (101 MHz, CD₃OD): δ (ppm) 147.9, 137.2, 123.5, 122.3, 59.8, 54.3, 31.6, 29.4, 26.9, 26.5, 22.3, 13.0. **IR** ν_{max} (film)/cm⁻¹: 3064, 3006, 2952, 2918, 2811, 1589, 1433, 1246, 1147, 1079, 995, 840, 718. **HRMS** found (ESI): MH⁺ 424.0922, C₂₈H₄₅N₃ requires 424.0923. The spectroscopic data were in agreement with literature values.¹⁶⁰

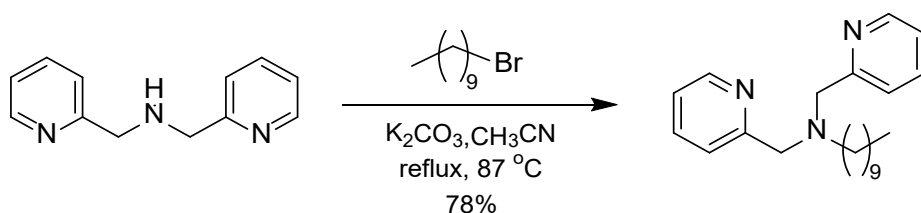
***N*-Dodecyl-*N*-(2-pyridinylmethyl)-2-pyridinemethanamine (C₁₂-picolyl, 39)**



To a solution of di-(2-picolyl)amine (0.399 g, 2.00 mmol, 1.0 eq.) in acetonitrile (8 mL) was added 1-bromododecane (0.50 g, 2.00 mmol, 1.0 eq.) and potassium carbonate (0.830 g, 6.01 mmol, 3.0 eq.) followed by sodium bicarbonate (0.505 g, 6.01 mmol, 3.0 eq.) flushed with nitrogen to keep inert atmosphere. The reaction mixture was heated at reflux for 24 hours, filtered with Celite, and removed under reduced pressure. The residue was added to distilled water (45 mL) and extracted with CH₂Cl₂ (3 x 15 mL) and dried with MgSO₄. The organic layer was collected, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography on alumina gel (30:70 EtOAc/hexane) to yield the title compound (0.600 g, 1.76 mmol, 78%) as orange-coloured semisolid.

¹H NMR (400 MHz, CD₃OD): δ (ppm) 8.45 (dd, *J* = 5.0, 4.2 Hz, 2H), 7.82 (td, *J* = 7.7, 5.1 Hz, 2H), 7.65 (d, *J* = 7.9 Hz, 2H), 7.30 (dd, *J* = 7.4, 1.3 Hz, 2H), 3.80 (s, 4H), 2.70 – 2.40 (m, 2H), 1.63 – 1.48 (m, 2H), 1.38 – 1.08 (m, 16H), 0.91 (t, *J* = 6.9 Hz, 3H).
¹³C NMR (101 MHz, CD₃OD): δ (ppm) 159.5, 147.9, 137.2, 123.4, 122.3, 59.8, 54.2, 31.6, 29.5, 29.1, 29.2, 29.0, 29.0, 26.9, 26.6, 22.3, and 13.0. IR ν_{max} (film)/cm⁻¹: 2898, 2789, 2345, 2098, 1795, 1609, 1537, 1435, 1395, 1184, 891, 676, 545, 456. HRMS found (ESI): MH⁺ 368.3060, C₂₄H₃₇N₃ requires 368.30602. The spectroscopic data were in agreement with literature values.¹⁶¹

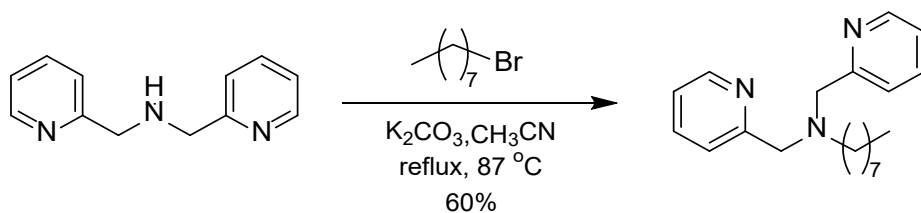
***N*-Decyl-*N*-(2-pyridinylmethyl)-2-pyridinemethanamine (C₁₀-picolyl, 38)**



To a solution of di-(2-picolyl)amine (0.452 g, 2.27 mmol, 1.0 eq.) in acetonitrile (7 mL) was added 1-bromodecane (0.50 g, 2.27 mmol, 1.0 eq.) and potassium carbonate (0.940 g, 6.81 mmol, 3.0 eq.) followed by sodium bicarbonate (0.572 g, 6.81 mmol, 3.0 eq.) flushed with nitrogen to keep inert atmosphere. The reaction mixture was heated at reflux for 24 hours, filtered with Celite, and the solvent removed under reduced pressure. The residue was added to distilled water (30 mL) and extracted with CH₂Cl₂ (3 x 12 mL) and dried with MgSO₄. The organic layer was collected, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography on alumina gel (40:60 EtOAc/hexane) to yield the title compound (0.600 g, 1.76 mmol, 78%) as dark orange semisolid.

¹H NMR (400 MHz, CD₃OD): δ (ppm) 8.45 (dd, *J* = 5.0, 1.4 Hz, 2H), 7.82 (td, *J* = 7.7, 1.8 Hz, 2H), 7.66 (d, *J* = 7.9 Hz, 2H), 7.30 (dd, *J* = 7.4, 1.1 Hz, 2H), 3.80 (s, 4H), 2.51 (dd, *J* = 7.8, 5.4 Hz, 2H), 1.53 (dt, *J* = 7.3, 4.2 Hz, 3H), 1.32 – 1.14 (m, 13H), 0.91 (t, *J* = 6.9 Hz, 3H). **¹³C NMR** (101 MHz, CD₃OD): δ (ppm) 159.5, 147.9, 137.2, 123.4, 122.3, 59.7, 54.2, 31.5, 29.2, 29.2, 29.0, 29.0, 26.9, 26.5, 22.3, 13.3. **IR** ν_{max}(film)/cm⁻¹: 2785, 2345, 1705, 1659, 1437, 1405, 1305, 1134, 801, 606, 545. **HRMS** found (ESI): MH⁺ 340.2748, C₂₂H₃₃N₃ requires 340.27472. The spectroscopic data were in agreement with literature values.¹⁶²

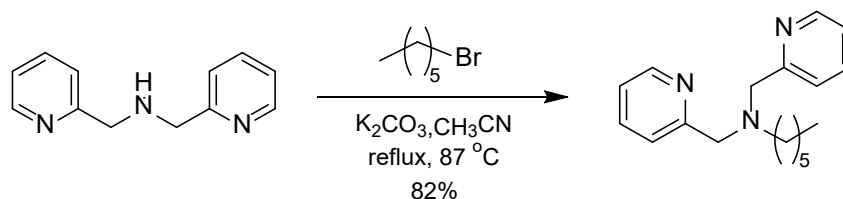
***N*-Octyl-*N*-(2-pyridinylmethyl)-2-pyridinemethanamine (C₈-picolyl, 37)**



To a solution of di-(2-picolyl)amine (0.509 g, 2.58 mmol, 1.0 eq.) in acetonitrile (10 mL) was added 1-bromooctane (0.50 g, 2.52 mmol, 1.0 eq.) and potassium carbonate (1.07 g, 7.76 mmol, 6.0 eq.) followed by sodium bicarbonate (0.652 g, 7.76 mmol, 6.0 eq.) flushed with nitrogen to keep inert atmosphere. The reaction mixture was heated at reflux for 24 hours, filtered with the Celite, and the solvent removed under reduced pressure. The residue was added to distilled water (40 mL) and extracted with CH_2Cl_2 (3 x 15 mL) and drying with $MgSO_4$. The organic layer was collected, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography on alumina gel (1:1 EtOAc/hexane) to yield the title compound (0.73 g, 0.70 mmol, 60%) as dark orange colour oil.

¹H NMR (400 MHz, CD_3OD): δ (ppm) 8.45 (dd, $J = 5.0, 1.5$ Hz, 2H), 7.81 (td, $J = 7.7, 2.1$ Hz, 2H), 7.65 (d, $J = 7.9$ Hz, 2H), 7.29 (dd, $J = 7.4, 4.3$ Hz, 2H), 3.79 (s, 4H), 2.61 – 2.42 (m, 2H), 1.65 – 1.46 (m, 2H), 1.38 – 1.11 (m, 13H). **¹³C NMR** (101 MHz, CD_3OD): δ (ppm) 159.5, 147.9, 137.2, 123.4, 122.3, 59.8, 54.2, 31.6, 29.0, 28.9, 26.9, 26.6, 22.3, 13.0. **IR** ν_{max} (film)/ cm^{-1} : 2957, 1668, 1607, 1559, 1497, 1366, 1205, 1050, 801, 676. **HRMS** found (ESI): MH^+ 312.2434, $C_{20}H_{29}N_3$ requires 312.24342. The spectroscopic data were in agreement with literature values.¹⁶³

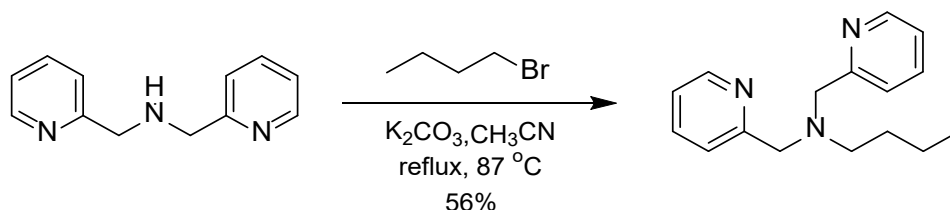
***N*-Hexyl-*N*-(2-pyridinylmethyl)-2-pyridinemethanamine (C₆-picolyl, 36)**



To a solution of di-(2-picolyl)amine (0.603 g, 3.02 mmol, 1.0 eq.) in acetonitrile (7 mL) was added 1-bromo hexane (0.50 g, 3.02 mmol, 1.0 eq.) and potassium carbonate (1.25 g, 9.08 mmol, 3.0 eq.) followed by sodium bicarbonate (0.763 g, 9.08 mmol, 3.0 eq.) flushed with nitrogen to keep inert atmosphere. The reaction mixture was heated at reflux for 24 hours, filtered with the Celite, and the solvent removed under reduced pressure. The residue was added to distilled water (40 mL) and extracted with CH₂Cl₂ (3 x 10 mL) and drying with MgSO₄. The organic layer was collected, and removing the solvent was under reduced pressure. The crude product was purified by flash column chromatography on alumina gel (1:1 EtOAc/hexane) to yield the title compound (0.700 g, 2.46 mmol, 82%) as dark orange colour semisolid.

¹H NMR (400 MHz, CD₃OD): δ (ppm) 8.45 (dd, J = 5.0, 1.7 Hz, 2H), 7.82 (td, J = 7.7 1.8 Hz, 2H), 7.63 (t, J = 8.0, 1.5 Hz, 2H), 7.30 (dd, J = 5.0, 1.0 Hz, 2H), 3.79 (s, 4H), 2.62 – 2.40 (m, 2H), 1.60 – 1.46 (m, 2H), 1.36 – 1.20 (m, 6H), 0.88 (dt, J = 7.1, 1.2 Hz, 3H). **¹³C NMR** (101 MHz, CD₃OD): δ (ppm) 159.5, 147.9, 137.2, 123.4, 122.3, 59.8, 54.2, 31.3, 26.6, 26.6, 22.2, 12.9. **IR** ν_{max} (film)/cm⁻¹: 2857, 1745, 1559, 1497, 1405, 1345, 1234, 801, 676, 545. **HRMS** found (ESI): MH⁺ 284.2121, C₁₈H₂₅N₃ requires 284.2121. The spectroscopic data were in agreement with literature values.¹⁶⁴

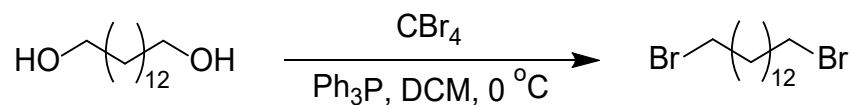
***N*-Butyl-*N*-(2-pyridinylmethyl)-2-pyridinemethanamine (C₄-picolyl, 29)**



To a solution of di-(2-picolyl) amine (0.500 g, 2.51 mmol, 1.0 eq.) in acetonitrile (20 mL) was added 1-bromobutane (0.34 mL, 3.3 mmol, 1.25 eq.) and potassium carbonate (1.03 g, 7.53 mmol, 3.0 eq.) followed by sodium carbonate (0.632 g, 7.53 mmol, 3.0 eq.) flushed with nitrogen to keep inert atmosphere. The reaction mixture was heated at reflux for 24 hours, filtered with the Celite, and the solvent removed under reduced pressure. The residue was added to distilled water (20 mL) and extracted with CH_2Cl_2 (3 x 10 mL). The organic layer was collected, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography on alumina gel (1:99 methanol/ CH_2Cl_2) to yield the title compound (0.362 g, 1.42 mmol, 56%) as a dark yellow oil.

1H NMR (400 MHz, CD_3OD): δ (ppm) 8.72 – 8.28 (m, 2H), 7.88 – 7.78 (m, 2H), 7.65 (d, $J = 7.9, 1.9$ Hz, 2H), 7.43 – 7.25 (m, 2H), 3.85 (s, 3H), 3.33 (dt, $J = 3.3, 1.6$ Hz, 2H), 2.57 (dd, $J = 1.8, 1.3$ Hz, 2H), 1.63 – 1.50 (m, 2H), 1.32 (dt, $J = 7.4, 4.3$ Hz, 2H), 0.86 (t, $J = 7.4$ Hz, 3H). **^{13}C NMR** (101 MHz, CD_3OD): δ (ppm) 159.0, 147.9, 137.3, 123.5, 122.4, 59.7, 54.1, 28.7, 20.01, 12.8. **IR** ν_{max} (film)/ cm^{-1} : 3064, 3006, 2848, 1469, 1147, 995, 840, 779. **HRMS** found (ESI): MH^+ 354.9823, $C_{16}H_{21}N_3$ requires 354.9824. The spectroscopic data were in agreement with literature values.

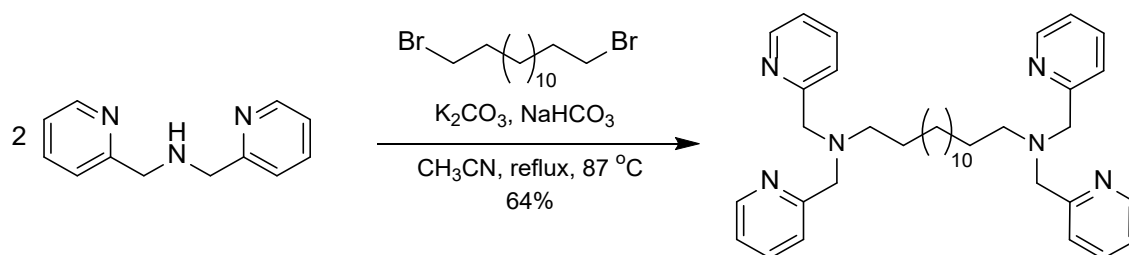
1,14-Dibromotetradecane (52)



To a solution of 1,14-tetradecanediol (0.2 g, 1.0 mmol, 1.0 eq.) in the presence of dry DCM (7.0 mL) was added tetrabromoethane (1.3 g, 4.1 mmol, 4 eq.) and triphenylphosphine (1.1 g, 4.1 mmol, 4.0 eq.) at $0\text{ }^\circ\text{C}$ under nitrogen gas. The solution was stirred at the same temperature for 2 h, and then hexane (10.0 mL) was added. The reaction mixture was filtered to remove triphenylphosphine oxide. The filtrate was concentrated, and the resulting crude was purified by column chromatography on silica gel (1:1 EtOAc/hexane) to yield the title compound (0.24 g, 76%) as a light-yellow colour liquid.

^1H NMR (400 MHz, CDCl_3) δ = 3.34 (t, J = 6.9 Hz, 4H), 1.83 – 1.73 (m, 4H), 1.48 (s, 2H), 1.35 (dt, J = 8.0, 5.1 Hz, 2H), 1.20 (d, J = 5.8 Hz, 16H). **^{13}C NMR** (101 MHz, CDCl_3) δ = 34.07, 32.85, 29.59, 29.53, 29.44, 28.78, 28.19. **IR** ν_{max} (film)/ cm^{-1} : 2956, 1668, 1559, 1446, 1366, 1234, 1146, 1050, 851, 676. **HRMS** found (ESI): MH^+ 355.5222, $\text{C}_{14}\text{H}_{28}\text{Br}_2$ requires 355.5223. The spectroscopic data were in agreement with literature values.¹⁷¹

N^1,N^1,N^4,N^4 -Tetrakis(2-pyridinylmethyl)-1,14-tetradecanediamine (58)

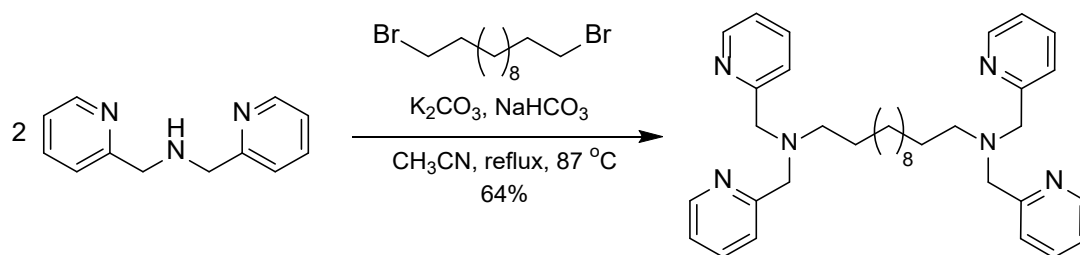


To a solution of di-(2-picolyl) amine (0.149 g, 0.754 mmol, 2 eq.) in acetonitrile (10.0 mL) was added 1,14-dibromotetradecane (0.104 g, 3.77 mmol, 1.0 eq.) and potassium

carbonate (0.311 g, 2.26 mmol, 3.0 eq.) followed by sodium bicarbonate (0.189 g, 2.26 mmol, 3.0 eq.) flushed with nitrogen to keep inert atmosphere. The reaction mixture was heated at reflux for 24 hours, filtered with the Celite, and the solvent removed under reduced pressure. The residue was added to distilled water (20 mL) and extracted with CH_2Cl_2 (3 x 10 mL). The organic layer was collected, and removing the solvent was under reduced pressure. The crude product was purified by flash column chromatography on alumina gel (1:1 EtOAc/hexane) to yield the *title compound* (0.072 g, 0.37 mmol, 64%) as orange semi solid.

^1H NMR (400 MHz, CD_3OD) δ (ppm) 10.48 (t, J = 8 Hz, 4H), 9.90 (dd, J = 4.7, 1.3 Hz, 4H), 9.69 (t, J = 7.9 Hz, 4H), 9.53 – 9.24 (m, 4H), 6.95 (d, J = 7.2 Hz, 2H), 5.81 (d, J = 7.7 Hz, 5H), 4.79 – 4.35 (m, 16H), 3.87 – 3.12 (m, 18H). **^{13}C NMR** (101 MHz, CD_3OD): δ (ppm) 160.9, 149.3, 138.6, 124.8, 123.7, 61.2, 55.6, 30.7, 30.6, 30.6, 30.4, 28.3, 27.9. **IR** ν_{max} (film)/ cm^{-1} : 3372, 3064, 2922, 2847, 1674, 1598, 1473, 1381, 1307, 1147, 1092, 839, 721. **HRMS** found (ESI): MH^+ 593.4325, $\text{C}_{20}\text{H}_{57}\text{N}_3\text{O}_5$ requires 593.4326.

N^1, N^1, N^4, N^4 -Tetrakis(2-pyridinylmethyl)-1,12-dodecanediamine (C_{12} -dimer, **57)**

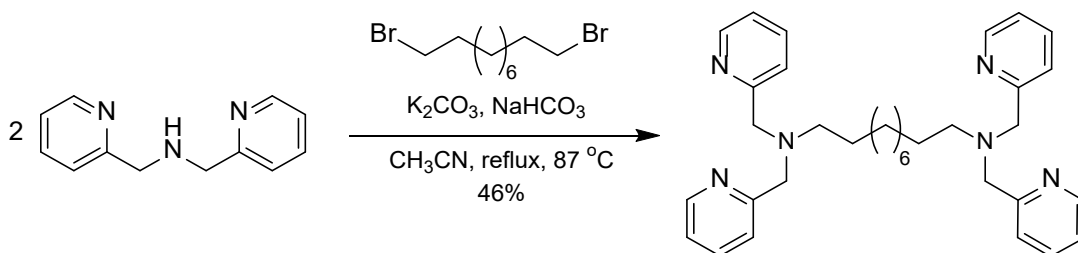


To a solution of di-(2-picolyl) amine (0.607 g, 3.05 mmol, 2.0 eq.) in acetonitrile (35 mL) was added 1,12-dibromododecane (0.50 g, 1.5 mmol, 1.0 eq.) and potassium carbonate (1.26 g, 9.14 mmol, 6.0 eq.) followed by sodium bicarbonate (1.05 g, 9.14 mmol, 6.0 eq.) flushed with nitrogen to keep inert atmosphere. The reaction mixture was heated at reflux for 24 hours, and the solution was filtered with Celite, and the solvent removed under reduced pressure. The residue was added to distilled water (40 mL) and extracted with

CH₂Cl₂ (3 x 15 mL). The organic layer was collected, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography on alumina gel (1:1 EtOAc/hexane) to yield the *title compound* (0.276 g, 1.52 mmol, 64%) as orange semi-solid.

¹H NMR (400 MHz, CD₃OD): δ (ppm) 8.44 (dd, J = 5.0 Hz, 4H), 7.78 (td, J = 7.7, 1.8 Hz, 4H), 7.63 (d, J = 7.9 Hz, 4H), 7.26 (dd, J = 7.4, 1.1 Hz, 4H), 3.78 (s, 2H), 2.72 – 2.39 (m, 8H), 1.76 – 1.28 (m, 4H), 1.34 – 0.98 (m, 18H). **¹³C NMR** (101 MHz, CD₃OD): δ (ppm) 159.6, 148.0, 137.1, 123.3, 122.3, 59.8, 54.2, 29.3, 29.3, 29.1, 26.9, 26.6. **IR** ν_{max} (film)/cm⁻¹: **HRMS** found (ESI): MH⁺ 563.9908, C₃₆H₄₈N₆ requires 563.9909.

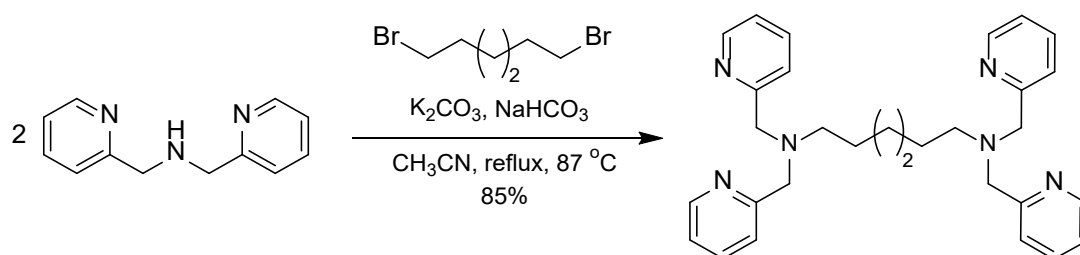
***N*¹,*N*¹,*N*⁴,*N*⁴-Tetrakis(2-pyridinylmethyl)-1,10-decanediamine (C10-dimer, 56)**



To a solution of di-(2-picolyl)amine (0.663 g, 3.33 mmol, 2.0 eq.) in acetonitrile (30.0 mL) was added 1,10-dibromodecane (0.5 g, 2.0 mmol, 1 eq.) and potassium carbonate (2.75 g, 19.9 mmol, 6.0 eq.) followed by sodium bicarbonate (1.68 g, 19.9 mmol, 6.0 eq.) flushed with nitrogen to keep inert atmosphere. The reaction mixture was heated at reflux for 24 hours, filtered with the Celite, and the solvent removed under reduced pressure. The residue was added to distilled water (60 mL) and extracted with CH₂Cl₂ (3 x 20 mL). The organic layer was collected, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography on alumina gel (1:1 EtOAc/hexane) to yield the *title compound* (0.208 g, 3.87 mmol, 46%) as orange semi solid.

¹H NMR (400 MHz, CD₃OD): δ (ppm) 8.33 (dd, J = 5.0 Hz, 4H), 7.69 (td, J = 7.7, 1.8 Hz, 4H), 7.53 (d, J = 7.9 Hz, 4H), 7.17 (dd, J = 7.4, 1.2 Hz, 4H), 3.68 (s, 8H), 2.45 – 2.31 (m, 4H), 1.48 – 1.32 (m, 4H), 1.24 – 0.99 (m, 12H). **¹³C NMR** (101 MHz, CD₃OD): δ (ppm) 159.5, 147.9, 137.1, 123.4, 122.3, 59.8, 54.2, 29.2, 29.0, 26.9, 26.6. **IR** ν_{max} (film)/cm⁻¹: **HRMS** found (ESI): MH⁺ 536.0134, C₃₄H₄₄N₆ requires 536.0135. The spectroscopic data were in agreement with literature values.¹⁶⁵

***N*¹,*N*¹,*N*⁴,*N*⁴-Tetrakis(2-pyridinylmethyl)-1,8-octanediamine (C₈-dimer, **55**)**

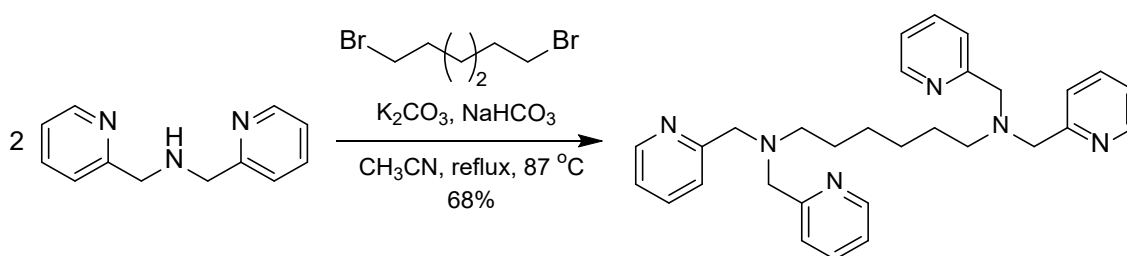


To a solution of di-(2-picolyl) amine (0.607 g, 3.05 mmol, 2.0 eq.) in acetonitrile (35 mL) was added 1,8-dibromooctane (0.50 g, 1.5 mmol, 1.0 eq.) and potassium carbonate (1.3 g, 9.1 mmol, 6 eq.) followed by sodium bicarbonate (1.05 g, 9.14 mmol, 6.0 eq.) flushed with nitrogen to keep inert atmosphere. The reaction mixture was heated at reflux for 24 hours, filtered with the Celite, and the solvent removed under reduced pressure. The residue was added to distilled water (40 mL) and extracted with CH₂Cl₂ (3 x 15 mL). The organic layer was collected, and removing the solvent was under reduced pressure. The crude product was purified by flash column chromatography on alumina gel (1:1 EtOAc/hexane) to yield the title compound (0.276 g, 4.88 mmol, 85%) as orange semi solid.

¹H NMR (400 MHz, CD₃OD): δ (ppm) 8.54 – 8.32 (m, 4H), 7.85 – 7.68 (m, 4H), 7.59 (t, J = 7.9 Hz, 4H), 7.35 – 7.11 (m, 4H), 2.47 (dd, J = 6.9, 1.3 Hz, 8H), 1.54 – 1.37 (m, 6H), 1.21 – 1.09 (m, 10H). **¹³C NMR** (101 MHz, CD₃OD): δ (ppm) 159.6, 148.1, 137.1, 123.3, 122.3, 59.9, 54.2, 29.0, 26.5, 26.6. **IR** ν_{max} (film)/cm⁻¹: 3388, 3049, 3012, 2935, 2853, 1588, 1463, 1357, 1152, 1073, 996, 746, 727. **HRMS** found (ESI): MH⁺ 509.3385,

$C_{32}H_{40}N_6$ requires 509.3387. The spectroscopic data were in agreement with literature values.¹⁶³

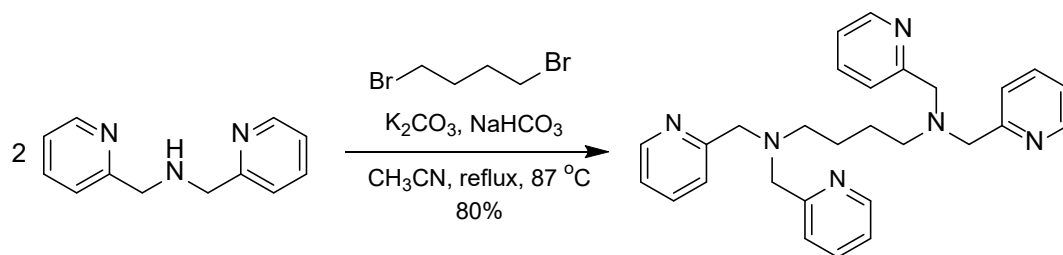
***N*¹,*N*¹,*N*⁴,*N*⁴-Tetrakis(2-pyridinylmethyl)-1,6-hexanediamine (C6-dimer, 54)**



To a solution of di-(2-picolyl) amine (0.816 g, 4.09 mmol, 2.0 eq.) in acetonitrile (35 mL) was added 1,6-dibromohexane (0.50 g, 1.5 mmol, 1.0 eq.) and potassium carbonate (3.39 g, 24.5 mmol, 6.0 eq.) followed by sodium bicarbonate (2.06 g, 24.5 mmol, 6.0 eq.) flushed with nitrogen to keep inert atmosphere. The reaction mixture was heated at reflux for 24 hours, filtered with the Celite, and the solvent removed under reduced pressure. The residue was added to distilled water (40 mL) and extracted with CH_2Cl_2 (3 x 15 mL). The organic layer was collected, and removing the solvent was under reduced pressure. The crude product was purified by flash column chromatography on alumina gel (1:1 EtOAc/hexane) to yield the title compound (0.337 g, 0.70 mmol, 68%) as orange semi solid.

¹H NMR (400 MHz, CD_3OD): δ (ppm) 12.44 – 12.27 (m, 4H), 11.83 – 11.61 (m, 4H), 11.52 (dd, J = 8.2, 2.3 Hz, 4H), 11.33 – 11.08 (m, 4H), 7.72 (d, J = 8 Hz, 8H), 7.27 (dt, J = 3.3, 1.6 Hz, 4H), 6.51 – 6.25 (m, 4H), 5.42 (s, 3H), 5.23 – 4.93 (m, 1H). **¹³C NMR** (101 MHz, CD_3OD): δ (ppm) 159.56, 147.92, 137.22, 123.42, 122.33, 59.83, 54.16, 26.61, 26.54. **IR** ν_{max} (film)/ cm^{-1} : 3063,, 3007, 2932, 2811, 1588, 1465, 1362, 1149, 1092, 893, 726. **HRMS** found (ESI): MH^+ 481.3073, $C_{30}H_{36}N_6$ requires 481.3074. The spectroscopic data were in agreement with literature values.¹⁶⁶

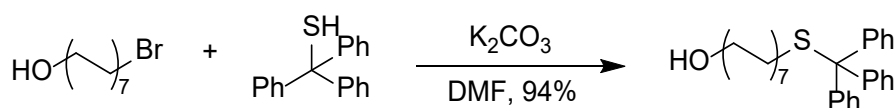
***N*¹,*N*¹,*N*⁴,*N*⁴-Tetrakis(2-pyridinylmethyl)-1,4-butanediamine (C4-dimer, 53)**



To a solution of di-(2-picolyl)amine (0.925 g, 4.63 mmol, 2.0 eq.) in acetonitrile (15 mL) was added 1,4-dibromobutane (0.50 g, 2.31 mmol, 1.0 eq.) and potassium carbonate (0.958 g, 13.88 mmol, 6.0 eq.) followed by sodium bicarbonate (0.583 g, 13.88 mmol, 6.0 eq.) flushed with nitrogen to keep inert atmosphere. The reaction mixture was heated at reflux for 24 hours, filtered with the Celite, and the solvent removed under reduced pressure. The residue was added to distilled water (40 mL) and extracted with CH_2Cl_2 (3 x 15 mL) and drying with MgSO_4 . The organic layer was collected, and removing the solvent was under reduced pressure. The crude product was purified by flash column chromatography on alumina gel (1:1 EtOAc/hexane) to yield the title compound (0.400 g, 8.83 mmol, 80%) as dark orange colour semisolid.

^1H NMR (400 MHz, CD_3OD): δ (ppm) 8.80 – 8.29 (m, 4H), 7.98 – 7.73 (m, 4H), 7.61 – 7.47 (m, 3H), 7.36 – 7.19 (m, 6H), 3.91 (d, 3H), 3.80 – 3.67 (m, 1H), 3.33 (dt, $J = 3.3$, 1.6 Hz, 8H), 2.50 (dd, $J = 7.9$, 1.3 Hz, 2H), 1.49 (dd, $J = 8$ Hz, 1H). **^{13}C NMR** (101 MHz, CD_3OD): δ 148.2, 137.2, 122.6, 122.3, 53.4. **IR** ν_{max} (film)/ cm^{-1} : 2857, 1698, 1559, 1497, 1050, 801, 676. **HRMS** found (ESI): MH^+ 451.8923, $\text{C}_{28}\text{H}_{32}\text{N}_6$ requires 451.8925. The spectroscopic data were in agreement with literature values.¹⁶⁷

14-(Tritylthio)tetradecane-1-ol (66)

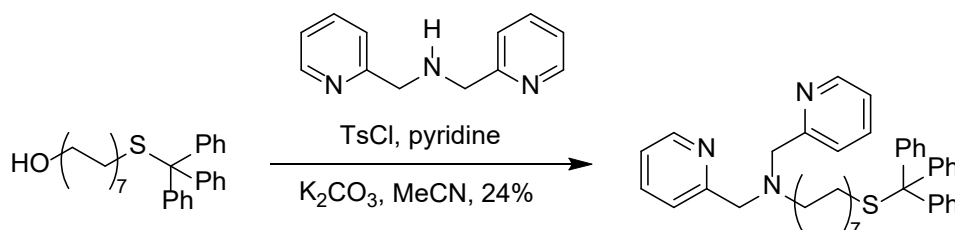


To a solution of 14-bromotetradecan-1-ol (0.500 g, 1.70 mmol) in anhydrous DMF (5 mL) was added triphenylmethanethiol (0.565 g, 2.05 mmol) and K_2CO_3 (0.352 g, 2.55

mmol). The reaction mixture was stirred at room temperature for 24 hours. 1 M HCl (20 mL) was added to the reaction. The product was extracted using CH₂Cl₂ (3 x 20 mL) and dried over MgSO₄. Flash chromatography on silica gel using 20% EtOAc/hexane gave the *title compound* (0.777 g, 1.59 mmol, 94%) as a white solid.

¹H NMR (400 MHz, CDCl₃): δ (ppm) 7.46 – 7.38 (m, 6H), 7.30 – 7.25 (m, 6H), 7.24 – 7.17 (m, 3H), 3.64 (t, J = 6.6 Hz, 2H), 2.13 (t, J = 7.3 Hz, 2H), 1.60 – 1.52 (m, 2H), 1.43 – 1.10 (m, 22H). **¹³C NMR** (101 MHz, CDCl₃): δ (ppm) 145.2, 130.3, 127.9, 126.6, 66.5, 63.3, 33.0, 32.2, 29.8, 29.7, 29.6, 29.3, 29.2, 28.7, 25.9. **IR** ν_{max} (film)/cm⁻¹: 3345, 3062, 2918, 2849, 1594, 1441, 1181, 1035, 740, 698. **HRMS** found (ESI): MH⁺, 511.3005, C₃₃H₄₄ONaS, 511.30051.

Bis((pyridine-2-yl)methyl)(14-(tritylthio)tetradecane)amine (70)



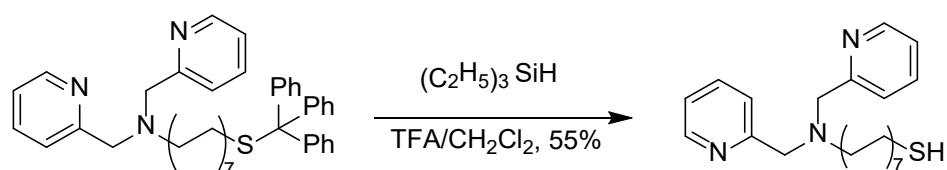
To a solution of 14-(tritylthio) tetradecane-1-ol **66** (1.15 g, 2.35 mmol) in anhydrous pyridine (10 mL) was added 4-toluenesulfonyl chloride (4.48 g, 23.5 mmol). The reaction mixture was stirred at room temperature for one hour. CH₂Cl₂ (20 mL) was added to the reaction and washed with 3% CuSO₄ (3 x 30 mL). This was dried over MgSO₄. The crude tosyl product was then used without further purification in the next step.

To a solution of crude tosyl product in anhydrous MeCN (10 mL) was added di-(2-picolyl) amine (0.313 g, 1.57 mmol) and K₂CO₃ (0.6496 g, 4.70 mmol). The reaction was refluxed for 20 hours. 1 M HCl (15 mL) was added, and the product was extracted into CH₂Cl₂ (3 x 20 mL) before being dried over MgSO₄. Flash chromatography on alumina initially using CH₂Cl₂, followed by 5% MeOH/CH₂Cl₂ to give the *title compound* (0.3818

g, 0.570 mmol, 24%) as a brown oil.

¹H NMR (400 MHz, CDCl₃): δ (ppm) 8.51 (d, J = 4.7 Hz, 2H), 7.63 (t, J = 7.6 Hz, 2H), 7.54 (d, J = 7.8 Hz, 2H), 7.42 – 7.39 (m, 6H), 7.29 – 7.25 (m, 6H), 7.22 – 7.17 (m, 3H), 7.15 – 7.10 (m, 2H), 3.80 (s, 4H), 2.52 (t, J = 7.3 Hz, 2H), 2.13 (t, J = 7.3 Hz, 2H), 1.57 – 1.48 (m, 2H), 1.43 – 1.33 (m, 3H), 1.25 – 1.13 (m, 20H). **¹³C NMR** (101 MHz, CDCl₃): δ (ppm) 160.4, 149.1, 145.2, 136.4, 129.8, 127.9, 126.6, 122.9, 121.9, 66.5, 60.7, 54.7, 32.2, 29.8, 29.7, 29.6, 29.6, 29.3, 29.2, 28.7, 27.5, 27.3. **IR** ν_{max} (film)/cm⁻¹: 3056, 2922, 2851, 1589, 1468, 1433, 1034, 742, 698. **HRMS** found (ESI): MH⁺, 670.4191, C₄₅H₅₆N₃S, 670.41895.

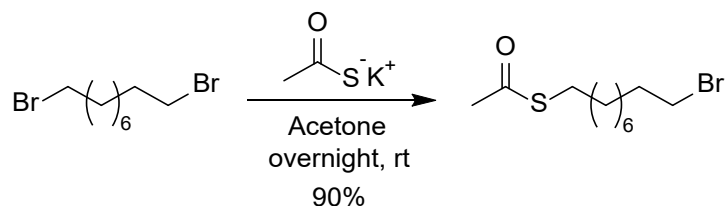
14-(Bis((pyridine-2-yl)methyl)amino)tetradecane-1-thiol (C₁₄-picolyl-SH, 74)



A solution of trityl-protected thiol **70** (0.060 g, 0.090 mmol) in anhydrous CH₂Cl₂ (1 mL) was cooled on ice. TFA (1 mL) was added, followed by triethylsilane (0.014 mL, 0.090 mmol). The reaction was stirred for 45 minutes. Flash chromatography was used to purify the compound on alumina, running initially in CH₂Cl₂ then increasing to 2% MeOH/CH₂Cl₂ to obtain the *title compound* (0.021 g, 0.049 mmol, 55%) as a yellow oil.

¹H NMR (400 MHz, CDCl₃): δ (ppm) 8.45 (d, J = 4.6 Hz, 2H), 7.62-7.49 (m, 4H), 7.34-7.30 (m, 6H), 7.22-7.16 (m, 6H), 7.14-7.07 (m, 5H), 3.93 (s, 4H), 2.59 (s, 2H), 2.02 (t, J = 7.3 Hz, 2H), 1.48 (s, 2H), 1.32-1.22 (m, 4H), 1.13-1.01 (m, 4H). **¹³C NMR** (101 MHz, CDCl₃): δ (ppm) 148.1, 144.0, 135.7, 128.6, 126.8, 125.5, 122.7, 121.6, 66.4, 58.7, 52.9, 30.8, 27.7, 27.4, 25.6. **IR** ν_{max} (film)/cm⁻¹: 2922, 2851, 1589, 1465, 1433, 1362, 1147, 1046, 995, 756. **HRMS** found (ESI): M⁺, 427.3009, C₂₆H₄₁N₃S, 427.30157.

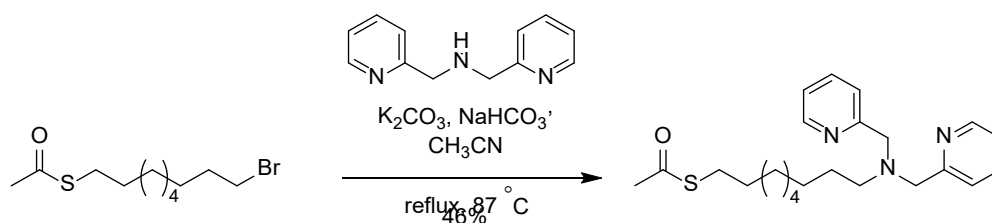
S-(9-Bromononyl) ethanethioate (**65**)



To a solution of corresponding 1,9-dibromononane **61** (0.30 mL, 1.52 mmol, 1.0 eq.) with potassium thioacetate in acetone (0.17 g, 1.52 mmol, 1.0 eq.), the reaction mixture was stirred at room temperature under nitrogen gas overnight. The reaction mixture was filtered, and the solvent evaporated under reduced pressure. The crude product was purified by flash column chromatography on silica gel (3:97 ethyl acetate/hexane) to yield of the title compound (0.25 g, 1.04 mmol, 90%) a yellow solid.

¹H NMR (400 MHz, CDCl₃): δ (ppm) 3.38 (t, *J* = 6.5 Hz, 2H), 2.85 (t, *J* = 5.5 Hz, 2H), 2.28 (s, 3H), 1.87 (m, 2H), 1.50 – 1.60 (m, 2H), 1.21 – 1.46 (m, 10H). **¹³C NMR** (101 MHz, CDCl₃): δ (ppm) 34.1, 32.9, 30.9, 29.6, 29.4, 29.3, 28.8, 28.4, 28.3. **IR** ν_{max} (film)/cm⁻¹: 2851, 2760, 2650, 1715, 1685, 1450, 1412, 1375, 740, 735, 723. **HRMS** found (ESI): MH⁺ 282.2785, C₁₁H₂₁BrOS requires 282.2786. The spectroscopic data were in agreement with literature values.¹⁶⁸

S-(9-(Bis(2-pyridinylmethyl)amino)nonyl) ethanethioate (**69**)

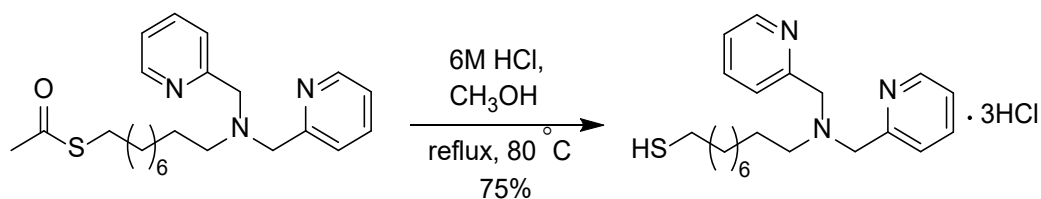


To a solution of *S*-(9-bromononyl) ethanethioate **65** (0.28 g, 0.99 mmol, 1.20 eq.) in acetonitrile was flushed with nitrogen to keep an inert atmosphere, followed by potassium carbonate (0.34 g, 2.48 mmol, 3.0 eq.), sodium bicarbonate (0.21 g, 2.48 mmol, 3.0 eq.)

and di-(2-picolyl) amine (0.17 g, 0.83 mmol, 1.0 eq.) was added. The reaction mixture was heated at reflux for overnight. The suspension was filtered by adding water and CH₂Cl₂, filtered through the separating funnel, and collected the organic layer. The solvent evaporated under reduced pressure. The crude product was purified by column chromatography on alumina gel (ethyl acetate/hexane 1:1) to yield the *title compound* (0.098 g, 0.31 mmol, 87%) as a yellow liquid.

¹H NMR (400 MHz, CD₃OD): δ (ppm) 8.21 (dd, $J = 7.5, 1.7$ Hz, 2H), 7.26 (td, $J = 7.7, 1.8$ Hz, 2H), 7.40 (d, $J = 7.9$ Hz, 2H), 7.03 (dd, $J = 7.4, 1.1$ Hz, 2H), 3.85 (s, 2H), 2.80 – 2.58 (m, 4H), 2.42 – 2.22 (m, 6H), 2.15 (s, 3H), 1.58 – 1.24 (m, 2H), 1.20 – 0.98 (m, 2H). **¹³C NMR** (101 MHz, CD₃OD): δ (ppm) 194.7, 165.5, 147.1, 145.7, 127.2, 123.4, 121.4, 60.9, 58.8, 56.1, 49.2, 48.9, 47.7, 47.4, 47.2, 29.3, 28.4, 28.1, 26.5, 26.2. **IR** ν_{max} (film)/cm⁻¹: 3210, 3090, 2856, 21768, 1659, 1446, 1396, 1166, 1090, 996, 871, 841, 676, 680. **HRMS** found (ESI): MH⁺ 357.0243, C₂₀H₂₇N₃OS requires 357.0244.

9-(Bis(2-pyridinylmethyl)amino)-1-nonanethiol (C₉-picolyl-SH, 73)

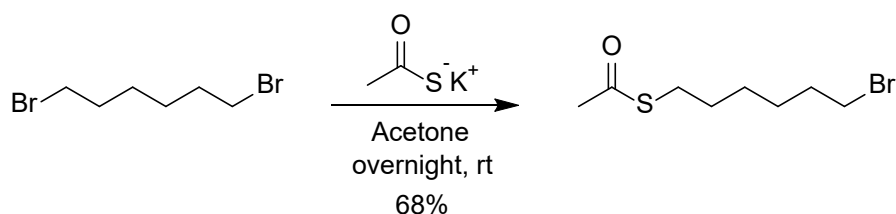


To a solution of *S*-(9-(bis(2-pyridinylmethyl)amino)nonyl) **69** ethanethioate (0.100 g, 3.02 mmol) in 1.5 mL methanol was flushed with nitrogen to keep an inert atmosphere, followed by adding 6 M HCl (5 mL). The reaction mixture was refluxed for 3 hours. The solvent was removed under reduced pressure to yield the *title compound* (0.045 g, 0.21 mmol, 75%) as a yellow semisolid, and nitrogen gas was passed to avoid oxidation.

¹H NMR (400 MHz, CD₃OD): δ (ppm) 8.92 (s, 2H), 8.44 (s, 2H), 8.25 (d, $J = 7.8$ Hz,

4H), 4.35 (s, 4H), 3.12 (s, 2H), 2.87 – 2.28 (m, 7H), 1.71 – 0.92 (m, 5H). **¹³C NMR** (101 MHz, CD₃OD): δ (ppm) 154.1, 148.2, 145.8, 137.5, 128.5, 96.0, 65.4, 34.3, 28.6, 27.8, 27.6, 26.5, 26.3, 25.7. **IR** ν_{max} (film)/cm⁻¹: 3210, 3015, 2941, 2876, 2351, 1724, 1712, 1614, 1541, 1435, 1370, 1216, 998, 766. **HRMS** found (ESI): MH⁺ 316.0201, C₁₈H₂₅N₃S requires 316.0204.

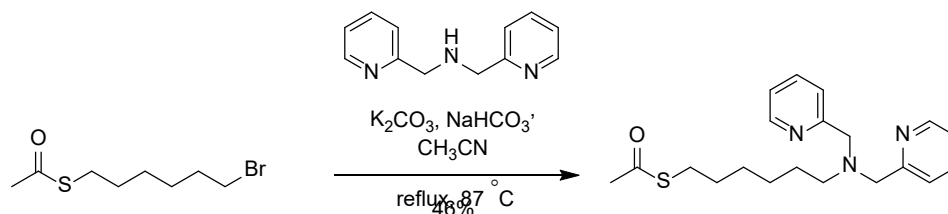
***S*-(6-Bromohexyl) ethanethioate (64)**



To a solution of corresponding 1,6-dibromohexane **60** (0.25 mL, 0.23 mmol, 1.0 eq.) with potassium thioacetate in acetone (4.0 mL), the reaction mixture was stirred at room temperature under nitrogen gas overnight. The reaction mixture was filtered, and the solvent evaporated under reduced pressure. The crude product was purified by flash column chromatography on silica gel (3:97 ethyl acetate/hexane) to yield of the title compound (0.250 g, 1.04 mmol, 68%) a yellow solid.

¹H NMR (400 MHz, CDCl₃): δ (ppm) 3.53 – 3.31 (m, 2H), 2.99 – 2.75 (m, 2H), 2.47 (d, J = 5.5 Hz, 2H), 2.39 (d, J = 4.8 Hz, 2H), 2.00 – 1.74 (s, 3H), 1.60 (dd, J = 7.4, 4.6 Hz, 2H), 1.46 – 1.34 (m, 2H). **¹³C NMR** (101 MHz, CDCl₃): δ (ppm) 195.8, 38.7, 33.7, 33.6, 32.7, 32.5, 30.6, 29.5, 28.9, 27.8, 27.6, 27.2. **IR** ν_{max} (film)/cm⁻¹: 2980, 2860, 2600, 1200, 1150, 780, 750. **HRMS** found (ESI): MH⁺ 239.0100; C₈H₁₅BrOS requires 239.0099. The spectroscopic data were in agreement with literature values. The spectroscopic data agrees with literature values.¹⁶⁹

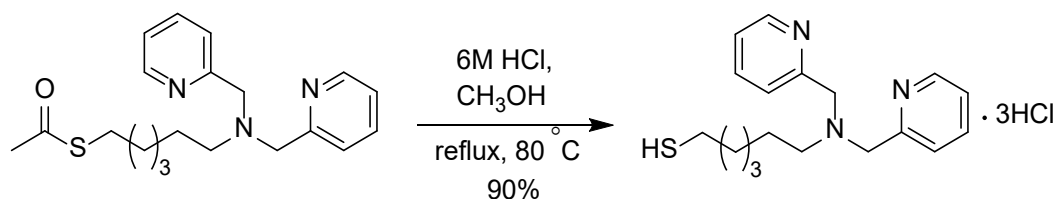
***S*-(6-(Bis(2-pyridinylmethyl)amino)hexyl)ethanethioate (68)**



To a solution of *S*-(6-bromohexyl) ethanethioate **64** (0.250 g, 1.04 mmol, 1.20 eq.) in acetonitrile was flushed with nitrogen to keep an inert atmosphere, followed by potassium carbonate (0.342 g, 2.48 mmol), sodium bicarbonate (0.208 g, 2.48 mmol) and di-(2-picolyl) amine (0.1734 g, 0.87 mmol, 1.0 eq.) was added. The reaction mixture was heated at reflux for overnight. The suspension was filtered by adding water and CH_2Cl_2 , filtered through the separating funnel, and the organic layer was collected. The solvent evaporated under reduced pressure. The crude product was purified by column chromatography on alumina gel (ethyl acetate/hexane 1:1) to yield the title compound (0.210 g, 0.59 mmol, 46%) as a yellow liquid.

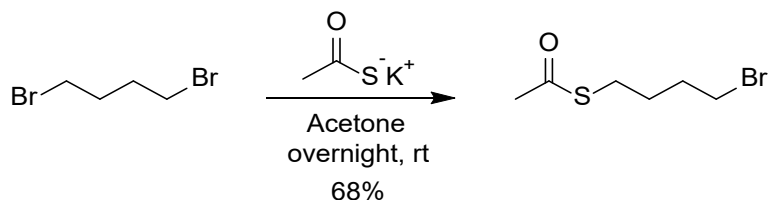
1H NMR (400 MHz, CD_3OD): δ (ppm) 8.31 (dd, $J = 7.0, 1.2$ Hz, 2H), 7.66 (td, $J = 7.7, 1.8$ Hz, 2H), 7.50 (d, $J = 7.9$ Hz, 2H), 7.13 (dd, $J = 7.4, 1.1$ Hz, 2H), 3.65 (s, 4H), 2.80 – 2.58 (m, 4H), 2.51 – 2.32 (m, 4H), 2.15 (s, 3H), 1.59 – 1.20 (m, 2H), 1.20 – 0.98 (m, 2H). **^{13}C NMR** (101 MHz, CD_3OD): δ (ppm) 195.7, 159.5, 148.1, 137.2, 123.4, 122.4, 59.9, 54.1, 48.2, 47.9, 47.7, 47.5, 47.3, 29.3, 28.4, 28.1, 26.5, 26.2. **IR** ν_{max} (film)/ cm^{-1} : 2956, 1668, 1559, 1446, 1366, 1146, 1050, 946, 851, 801, 676. **HRMS** found (ESI): MH^+ 357.02; $C_{20}H_{27}N_3OS$ requires 357.52. The spectroscopic data were in agreement with literature values.¹⁷⁰

6-(Bis(2-pyridinylmethyl)amino)-1-hexanethiol (C₆-picolyl-SH, 72)



To a solution of *S*-(6-(bis(2-pyridinylmethyl)amino)hexyl) ethanethioate **68** (0.100 g, 0.02 mmol, 1.0 eq) in methanol was flushed with nitrogen to keep an inert atmosphere, followed by adding 6 M HCl (5 mL). The reaction mixture was refluxed for 3 hours. The solvent was removed under reduced pressure to yield the title compound (0.090 g, 0.21 mmol, 90%) as yellow colour semi solid and passed the nitrogen gas to avoid oxidation. **¹H NMR** (400 MHz, CD₃OD): δ (ppm) 8.82 (s, 2H), 8.54 (s, 2H), 8.05 (d, J = 6.8 Hz, 4H), 4.35 (s, 4H), 2.87 – 2.28 (m, 7H), 1.71 – 0.92 (m, 6H). **¹³C NMR** (101 MHz, CD₃OD): δ (ppm) 153.1, 147.2, 141.8, 127.5, 126.5, 56.0, 55.4, 38.3, 28.6, 27.8, 27.6, 26.5, 26.3, 25.7. **IR** ν_{max} (film)/cm⁻¹: 3015, 2941, 2351, 1724, 1614, 1541, 1435, 1370, 1216, 998, 766. **HRMS** found (ESI): MH⁺ 316.0231; C₁₈H₂₅N₃S requires 316.0234. The spectroscopic data were in agreement with literature values.¹⁷⁰

S-(4-Bromobutyl) ethanethioate (63)

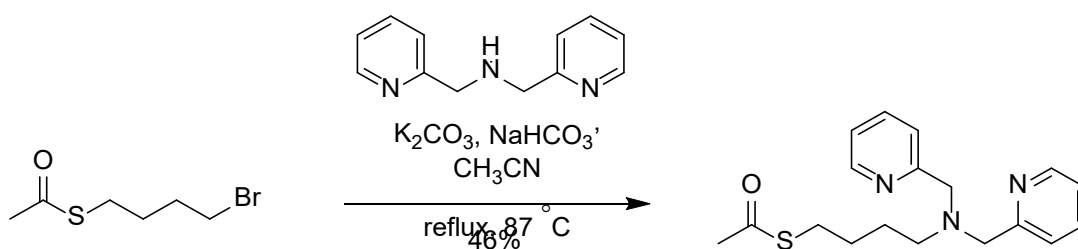


To a solution of corresponding 1,4-dibromobutane **59** (0.27 mL, 2.31 mmol, 1.0 eq.) with potassium thioacetate in acetone (0.29 g, 2.56 mmol, 1.1 eq.), the reaction mixture was stirred at room temperature under nitrogen gas overnight. The reaction mixture was filtered, and the solvent evaporated under reduced pressure. The crude product was

purified by flash column chromatography on silica gel (5:95 ethyl acetate/hexane) to yield of the title compound (0.37 g, 2.31 mmol, 76%) a yellow solid.

¹H NMR (400 MHz, CDCl₃): δ (ppm) = 3.44 (dt, J = 4.3, 1.7 Hz, 2H), 2.91 (q, J = 3.5 Hz, 2H), 2.35 (s, 2H), 2.11 – 1.89 (m, 1H), 1.85 – 1.72 (m, 1H), 1.62 (s, 3H). **¹³C NMR** (101 MHz, CD₃OD): δ (ppm) 56.5, 55.7, 38.3, 28.9, 27.9, 26.9. **IR** ν_{max} (film)/cm⁻¹: 3015, 2941, 2351, 1724, 1614, 1541, 1435, 1370, 1216, 998, 766. **HRMS** found (ESI): MH⁺ 209.9726; C₆H₁₁BrOS requires 209.9728. The spectroscopic data were in agreement with literature values.¹⁷²

***S*-(4-(Bis(2-pyridinylmethyl)amino)butyl) ethanethioate (67)**

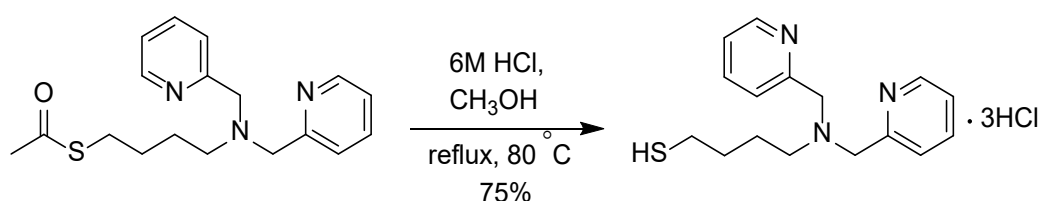


To a solution of *S*-(4-bromobutyl)ethanethioate (0.17 g, 1.04 mmol, 1.10 eq.) in acetonitrile was flushed with nitrogen to keep an inert atmosphere, followed by potassium carbonate (0.24 g, 3.13 mmol), sodium bicarbonate (0.14 g, 3.13 mmol) and di-(2-picolyl)amine (0.1734 g, 0.87 mmol, 1.0 eq.) was added. The reaction mixture was heated at reflux for overnight. The suspension was filtered by adding water and CH₂Cl₂, filtered through the separating funnel, and collected the organic layer. The solvent evaporated under reduced pressure. The crude product was purified by column chromatography on alumina gel (ethyl acetate/hexane 1:1) to yield the *title compound* (0.17 g, 1.04 mmol, 50%) as a yellow liquid.

¹H NMR (400 MHz, CD₃OD): δ (ppm) 8.31 (dd, J = 5.0, 1.7 Hz, 2H), 7.66 (td, J = 7.7, 1.8 Hz, 2H), 7.50 (d, J = 7.9 Hz, 2H), 7.13 (dd, J = 7.4, 1.1 Hz, 2H), 3.65 (s, 4H), 2.80 – 2.58 (m, 4H), 2.51 – 2.32 (m, 4H), 2.15 (s, 3H), 1.59 – 1.20 (m, 2H), 1.20 – 0.98 (m, 2H).

¹³C NMR (101 MHz, CD₃OD): δ (ppm) 195.7, 159.5, 148.1, 137.2, 123.4, 122.4, 59.9, 54.1, 48.2, 47.9, 47.7, 47.5, 47.3, 29.3, 28.4, 28.1, 26.5, 26.2. **IR** ν_{max} (film)/cm⁻¹: 2956, 1668, 1559, 1446, 1366, 1146, 1050, 946, 851, 801, 676. **HRMS** found (ESI): MH⁺ 357.0243; C₂₀H₂₇N₃OS requires 357.0244. The spectroscopic data were in agreement with literature values.

4-(Bis(2-pyridinylmethyl)amino)-1-butanethiol (C₄-picolyl-SH, 71)



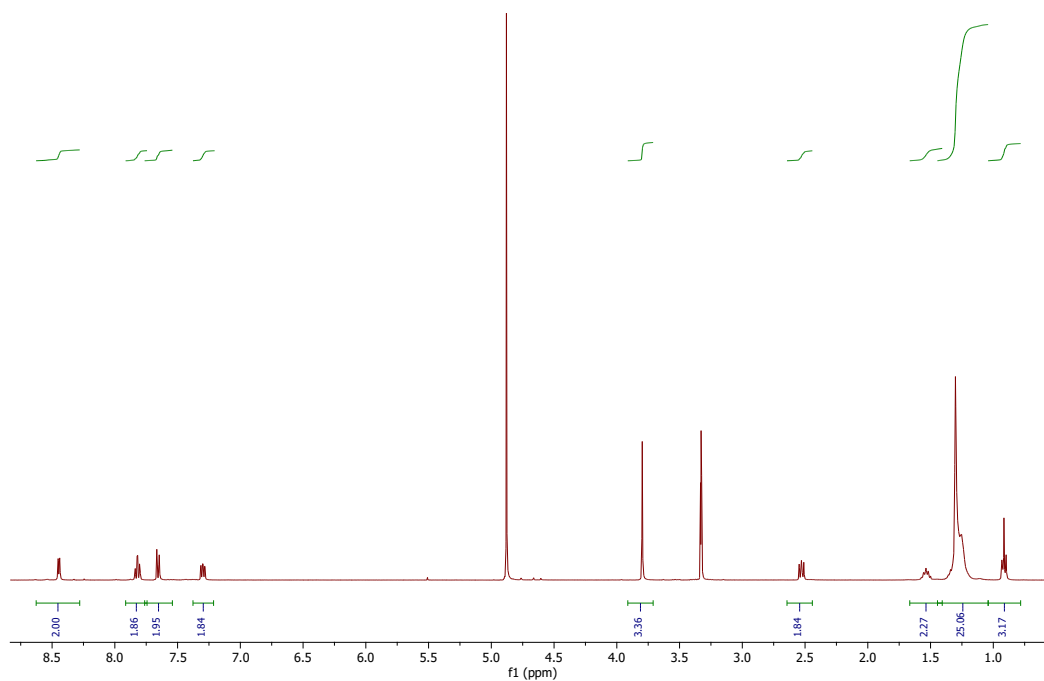
A solution of *S*-(4-(bis(2-pyridinylmethyl)amino)butyl)ethanethioate **67** (0.100g, 0.024) in methanol was flushed with nitrogen to maintain an inert atmosphere, followed by the addition of 6 M HCl (5 mL). The reaction mixture refluxed for 3 hours. The solvent was removed under reduced pressure to yield the *title compound* (0.090 g, 0.21 mmol, 90%) as yellow semi solid and passed the nitrogen gas to avoid oxidation.

¹H NMR (400 MHz, CD₃OD): δ (ppm) 8.82 (s, 2H), 8.54 (s, 2H), 8.05 (d, J = 6.8 Hz, 4H), 4.35 (s, 4H), 2.87 – 2.28 (m, 7H), 1.71 – 0.92 (m, 6H). **¹³C NMR** (101 MHz, CD₃OD): δ (ppm) 153.1, 147.2, 141.8, 127.5, 126.5, 56.0, 55.4, 38.3, 28.6, 27.8, 27.6, 26.5, 26.3, 25.7. **IR** ν_{max} (film)/cm⁻¹: 3015, 2941, 2351, 1724, 1614, 1541, 1435, 1370, 1216, 998, 766. **HRMS** found (ESI): MH⁺ 425.0954; C₁₈H₂₈N₃SCl₃ requires 425.0956. The spectroscopic data were in agreement with literature values.

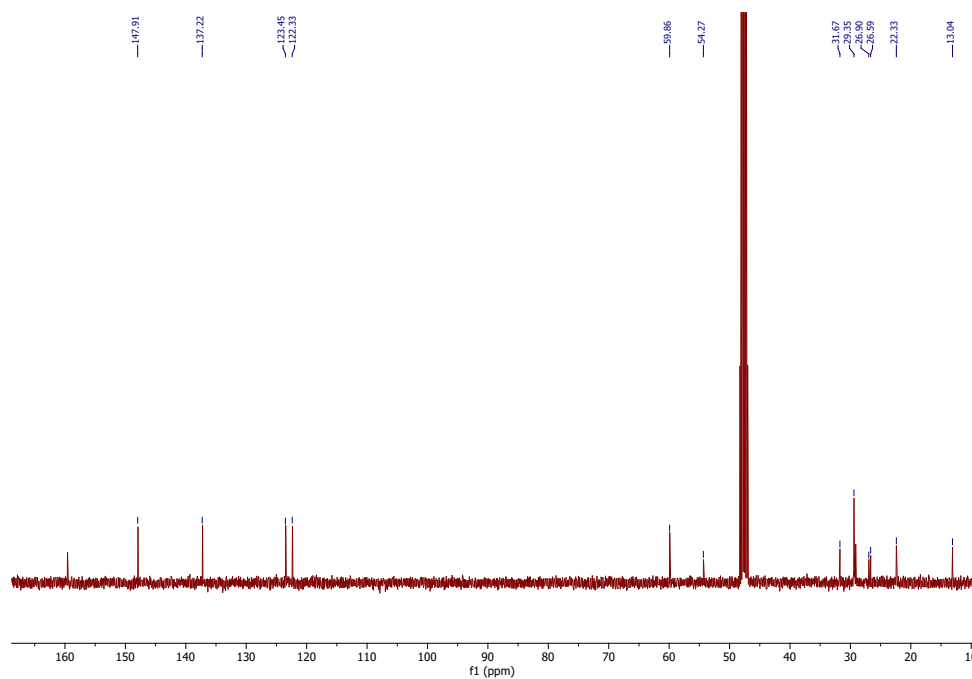
6.3 ^1H NMR and ^{13}C NMR Spectra for Synthesised Compounds

N-Hexadecyl-*N*-(2-pyridinylmethyl)-2-pyridinemethanamine (C_{16} -picolyl, 28)

^1H NMR (400 MHz, CD_3OD)

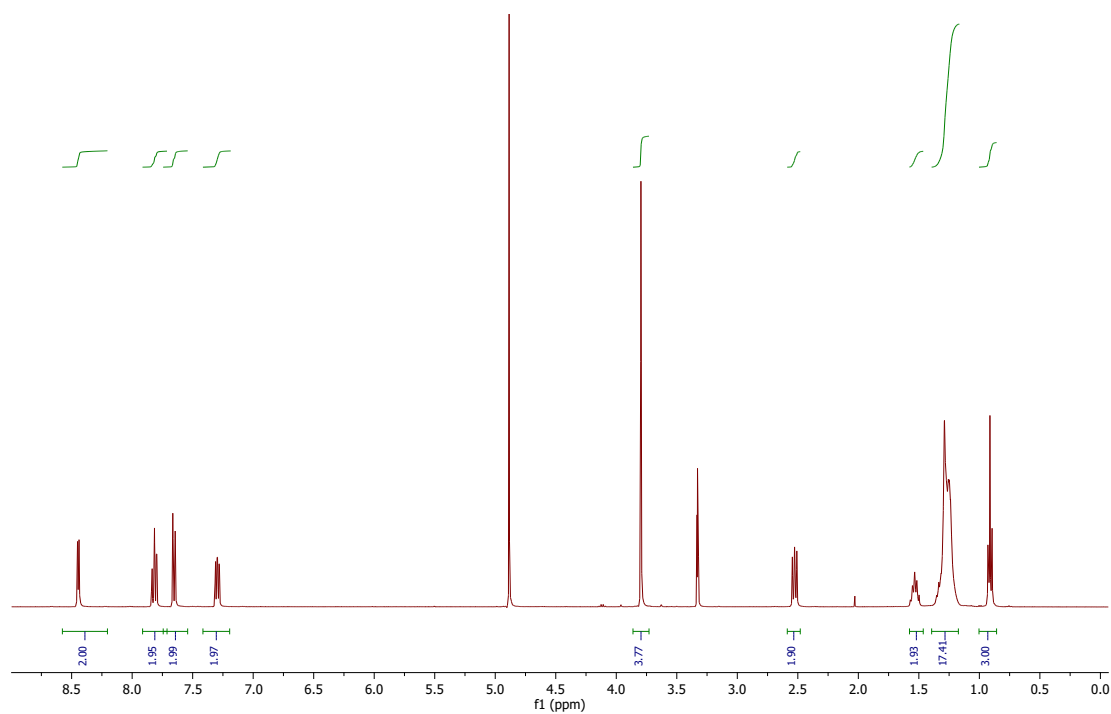


^{13}C NMR (100 MHz, CD_3OD)

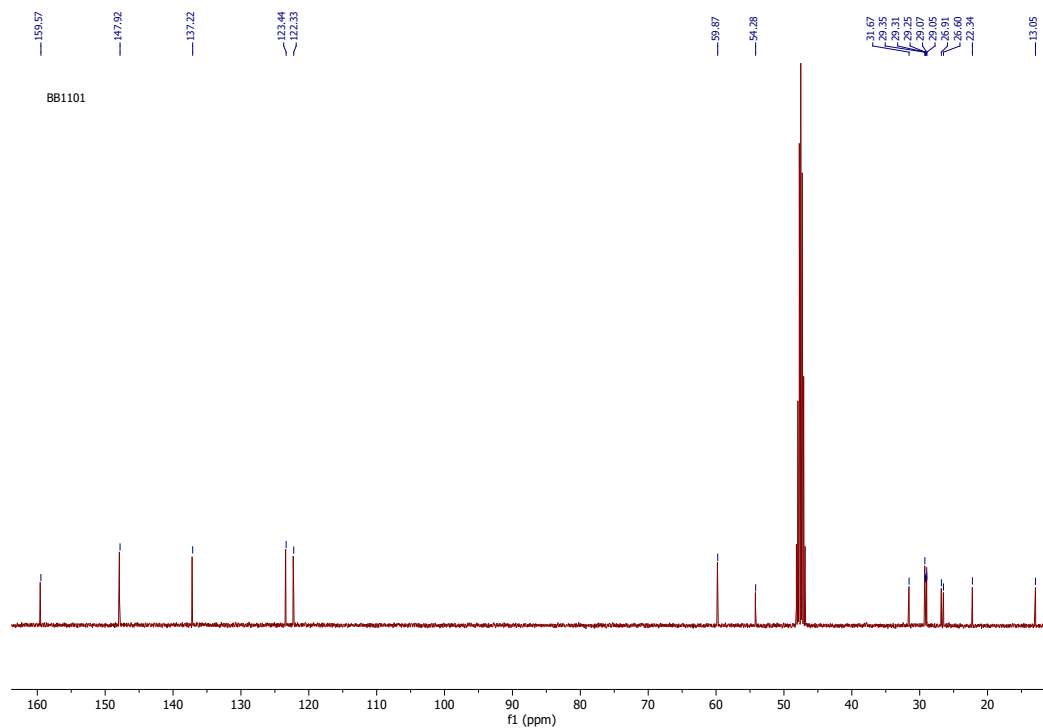


***N*-dodecane-*N*-(2-pyridinylmethyl)-2-pyridinemethanamine (C₁₂-picolyl, 39)**

¹H NMR (400 MHz, CD₃OD)

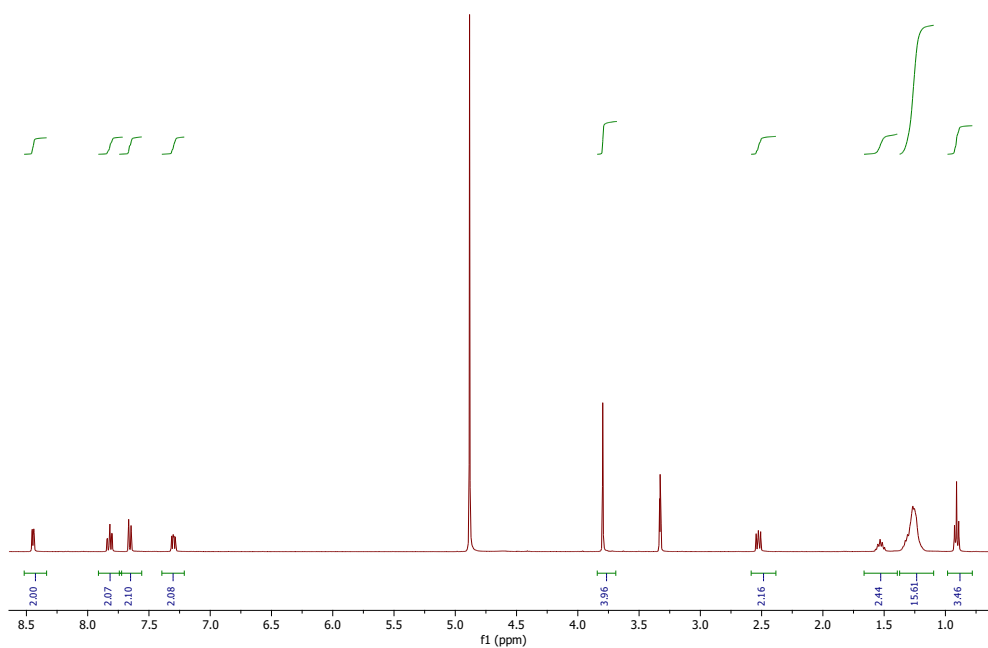


¹³C NMR (100 MHz, CD₃OD)

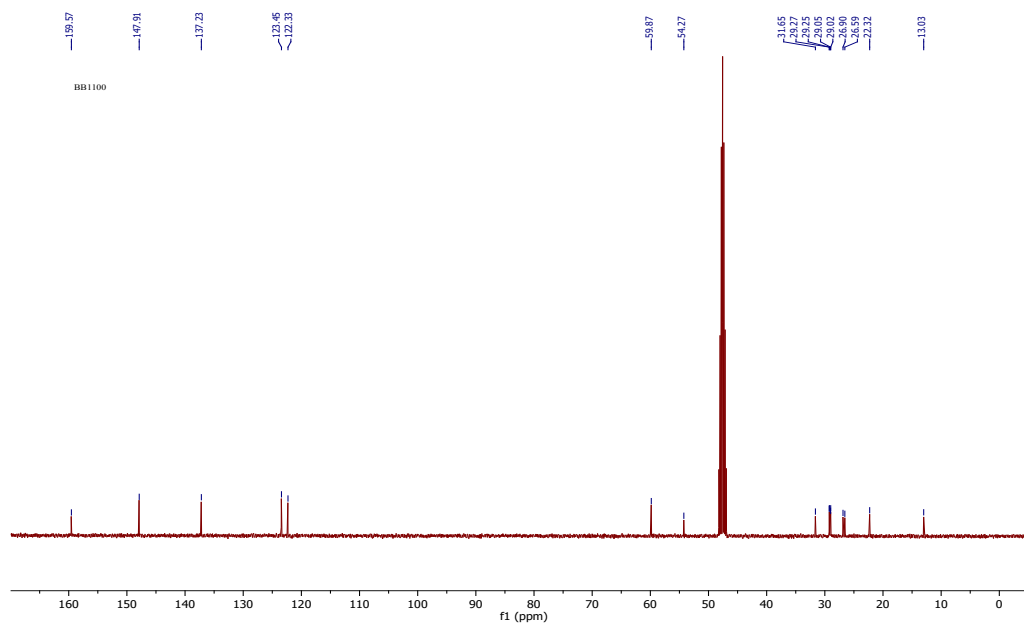


***N*-Decyl-*N*-(2-pyridinylmethyl)-2-pyridinemethanamine (C₁₀-picoyl, 38)**

¹H NMR (400 MHz, CD₃OD)

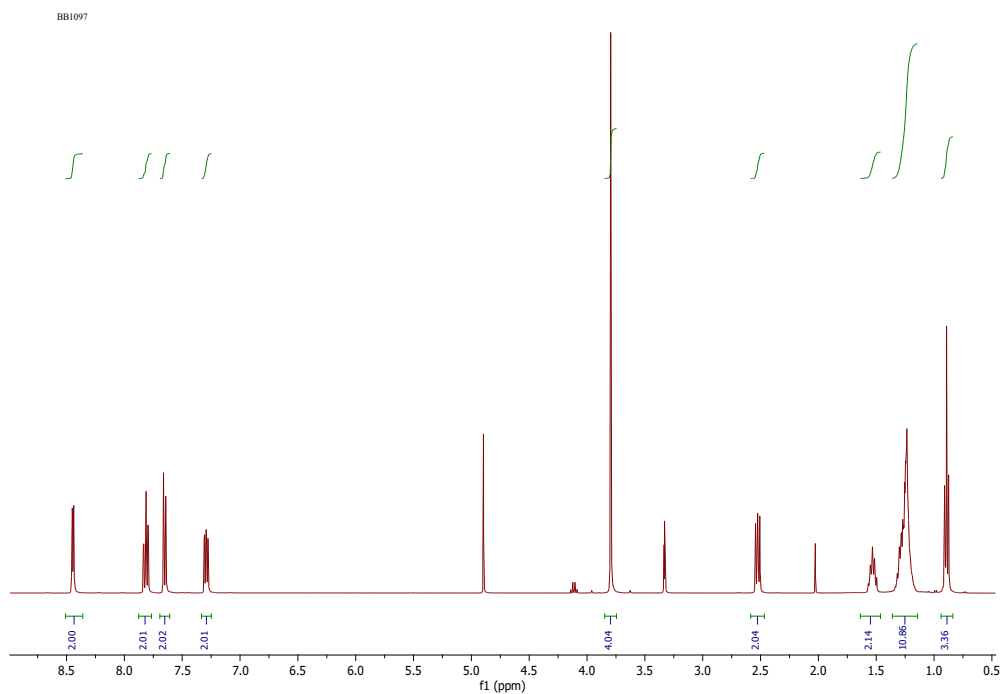


¹³C NMR (100 MHz, CD₃OD)

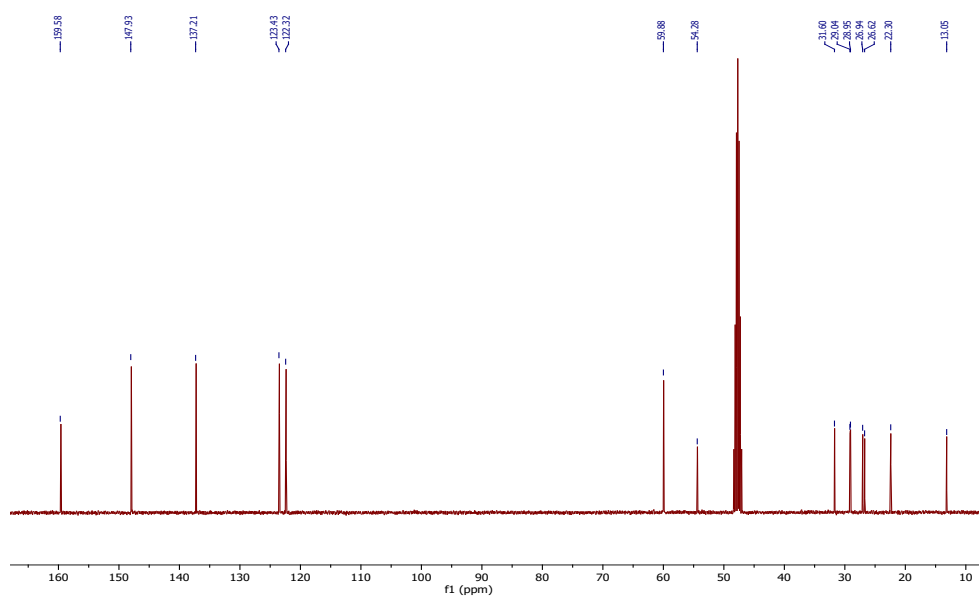


***N*-Octyl-*N*-(2-pyridinylmethyl)-2-pyridinemethanamine (C₈-picoyl, 37)**

¹H NMR (400 MHz, CD₃OD)



¹³C NMR (100 MHz, CD₃OD)

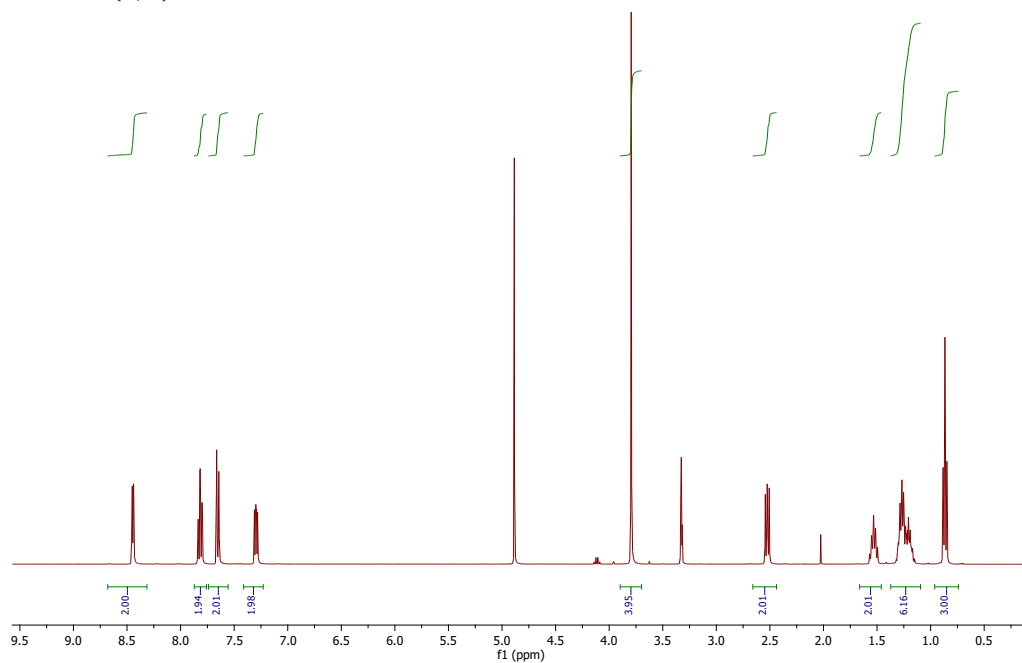


N-Hexyl-*N*-(2-pyridinylmethyl)-2-pyridinemethanamine (C₆-picoyl, 36)

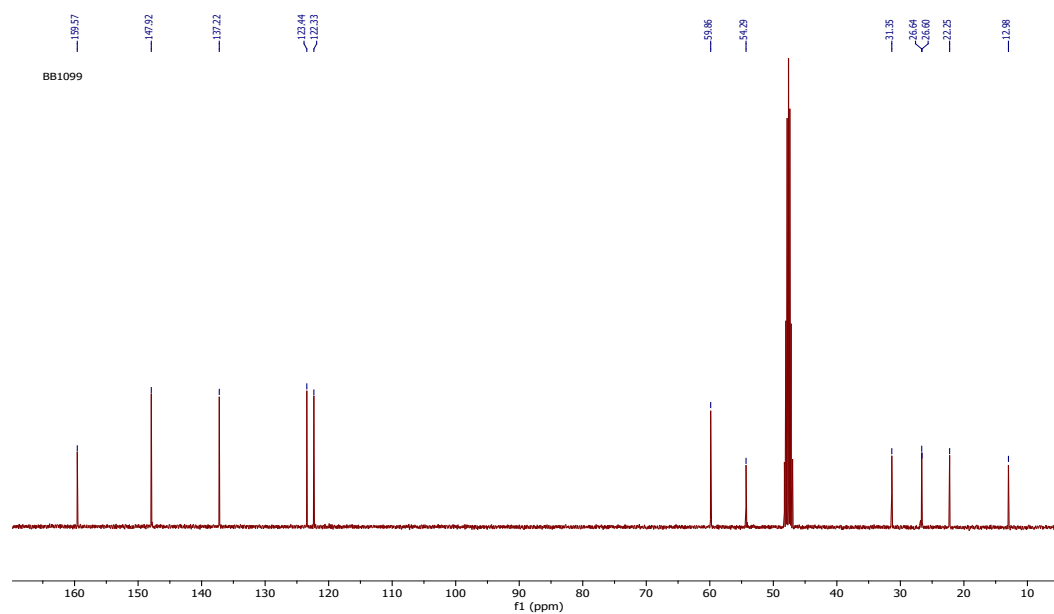
¹H NMR (400 MHz, CD₃OD)

BB1099 f2

PROTONRO MeOD {D:\data} BhanuB 19

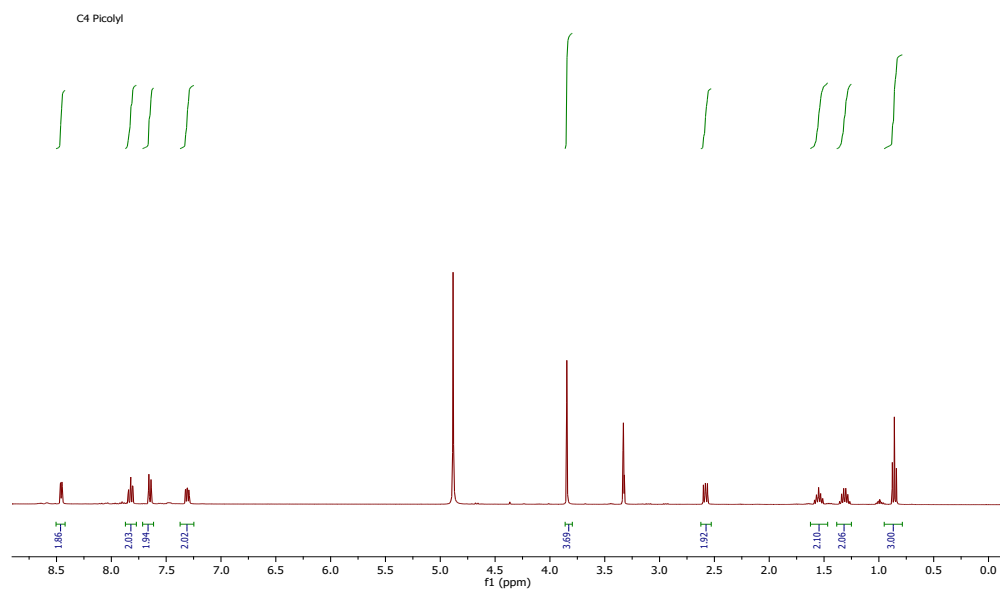


¹³C NMR (100 MHz, CD₃OD)

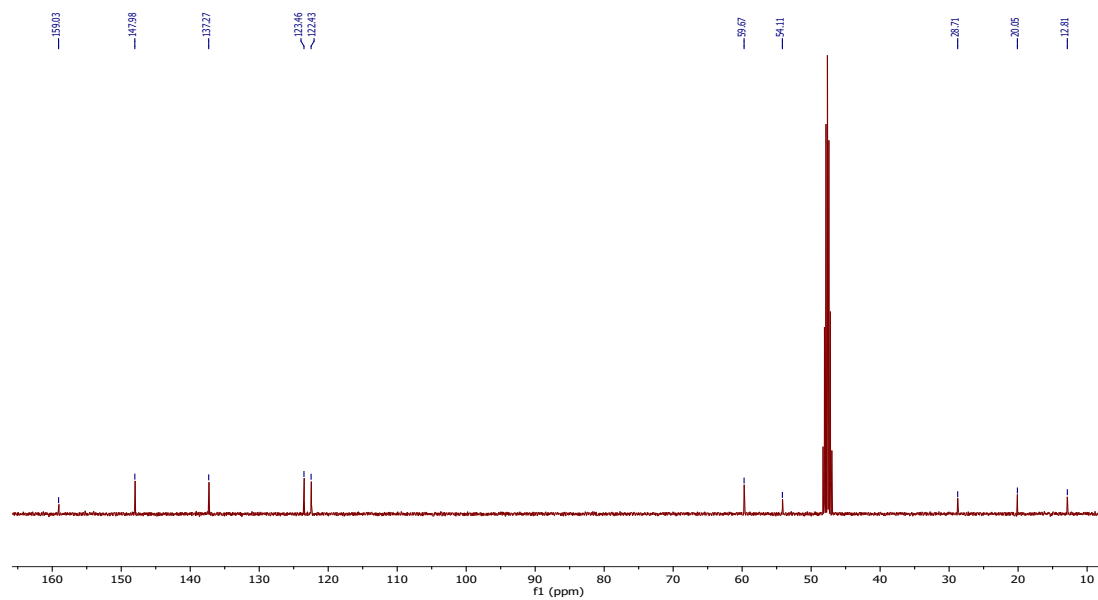


***N*-Butyl-*N*-(2-pyridinylmethyl)-2-pyridinemethanamine (C4-picoyl, 29)**

^1H NMR (400 MHz, CD_3OD)

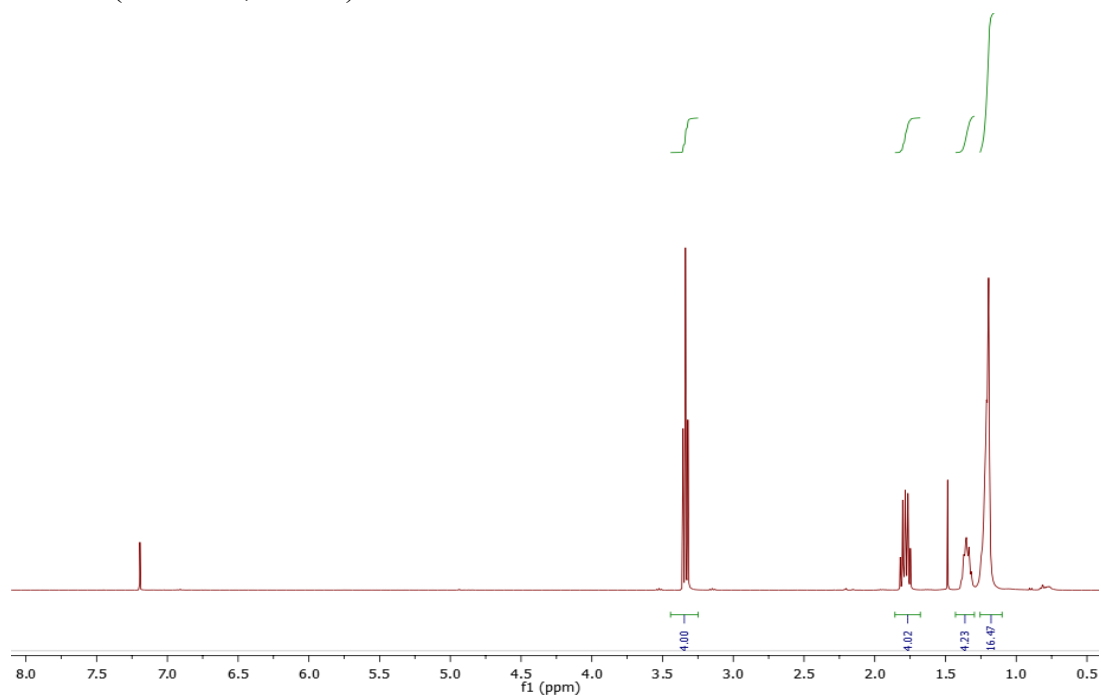


^{13}C NMR (100 MHz, CD_3OD)



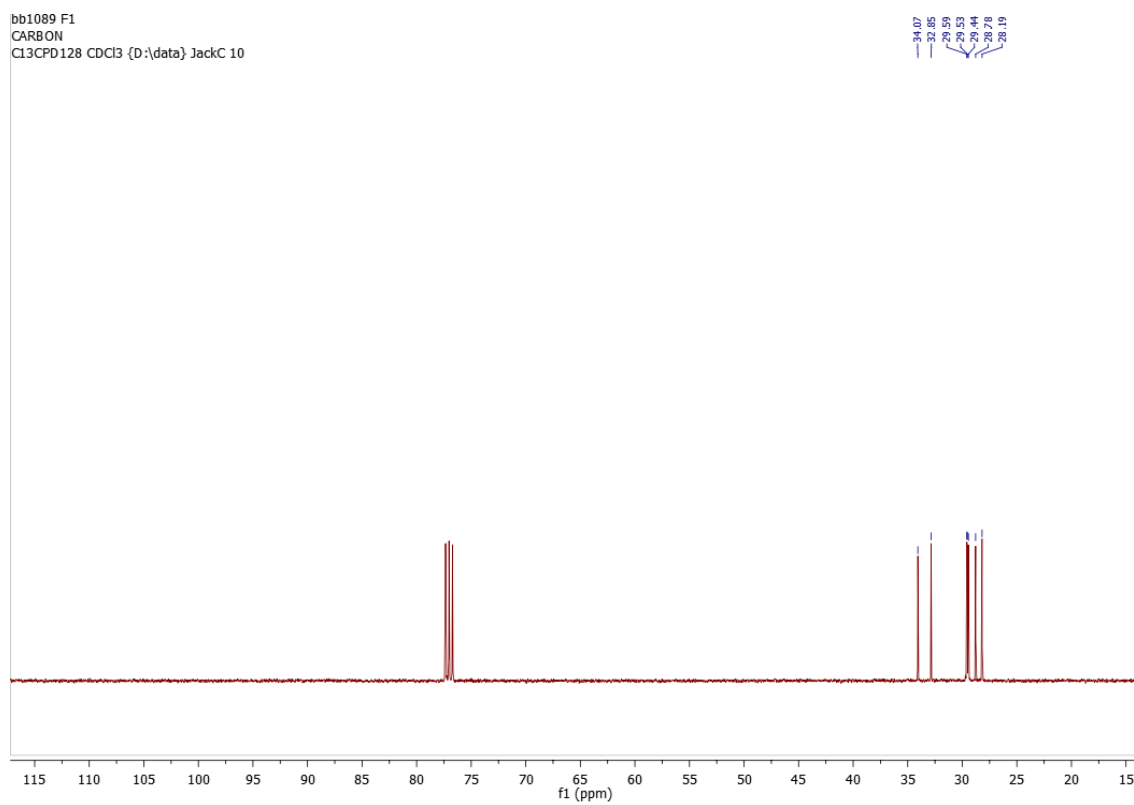
1,14-Dibromotetradecane (52)

^1H NMR (100 MHz, CDCl_3)



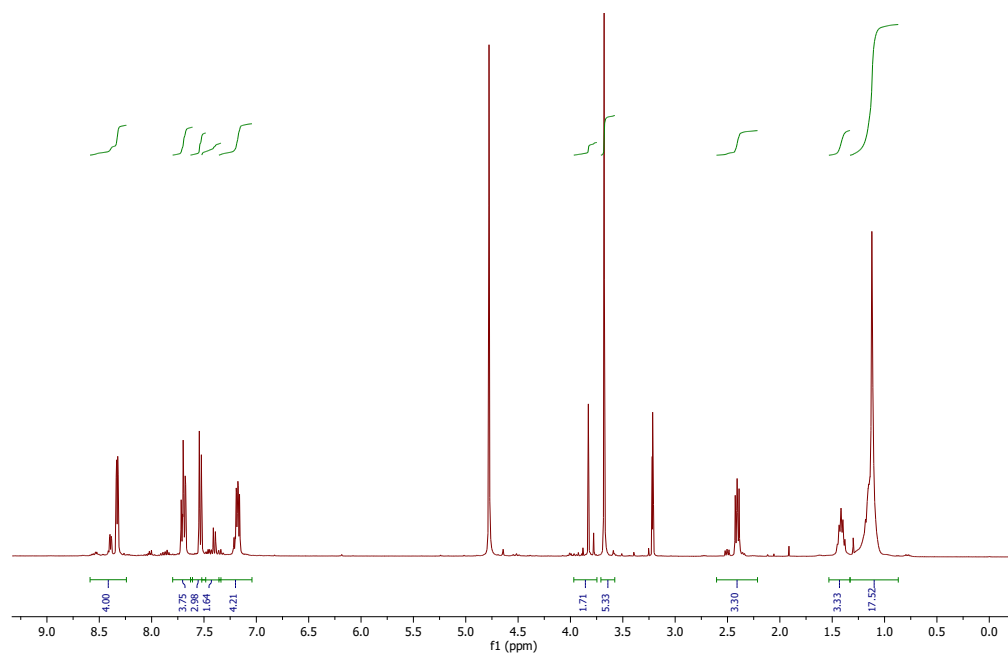
^{13}C NMR (100 MHz, CDCl_3)

bb1089 F1
CARBON
Cl3CPD128 CDCl_3 {D:\data} JackC 10

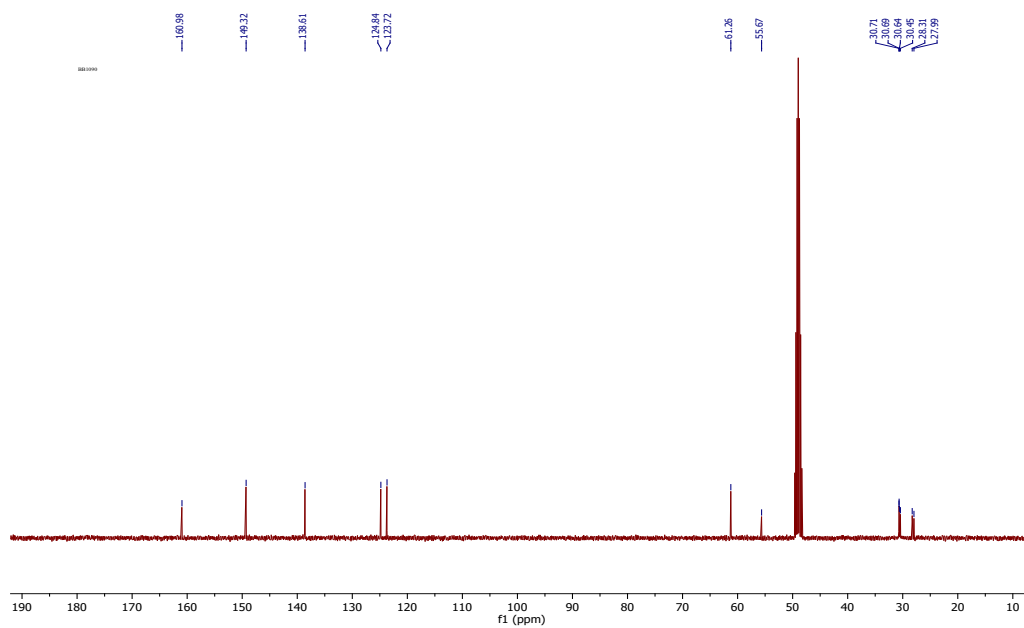


***N*¹,*N*¹,*N*⁴,*N*⁴-Tetrakis(2-pyridinylmethyl)-1,14-tetradecanediamine (C₁₄-dipicolyl, 58)**

¹H NMR (400 MHz, CD₃OD)

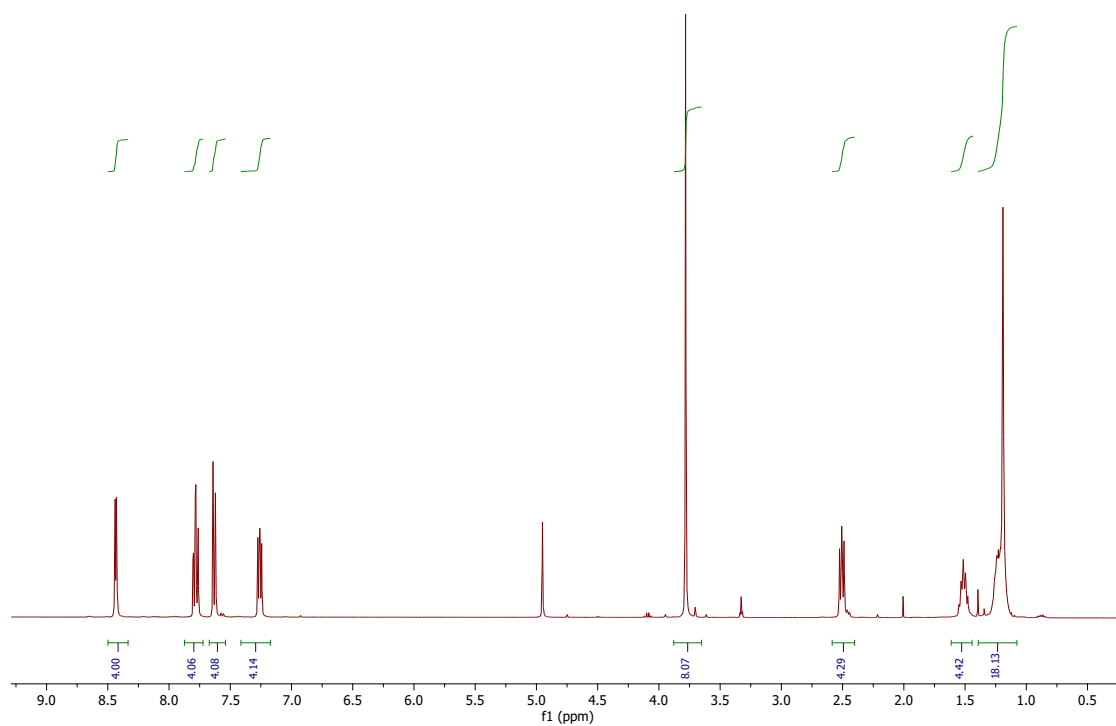


¹³C NMR (100 MHz, CD₃OD)

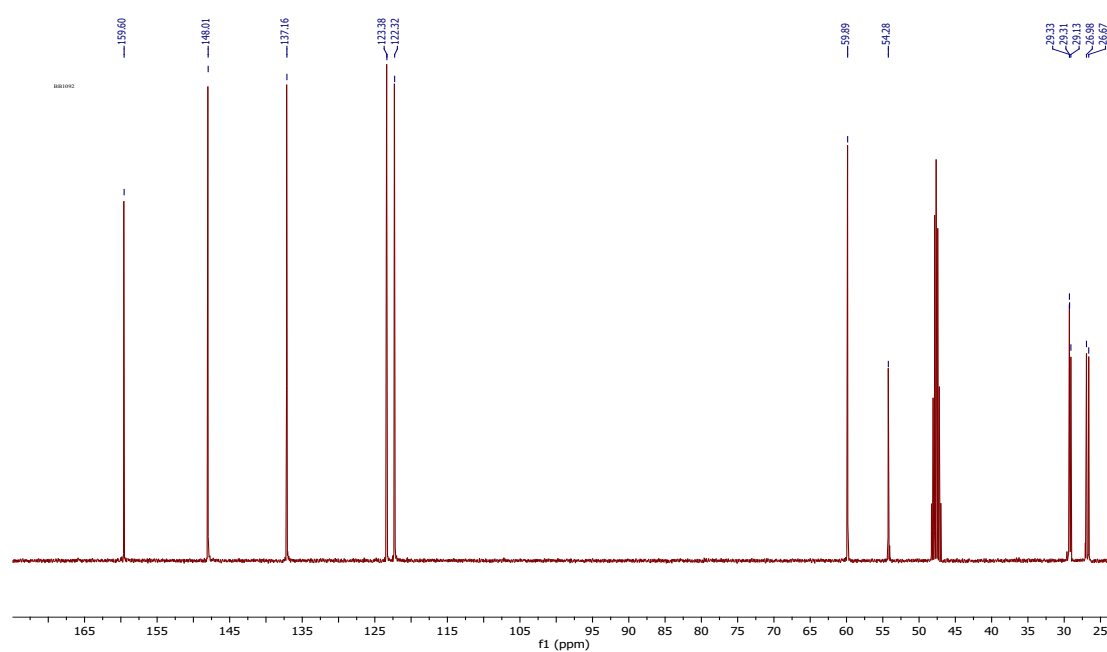


***N*¹,*N*¹,*N*⁴,*N*⁴-Tetrakis(2-pyridinylmethyl)-1,12-dodecanediamine (C₁₂-dipicolyl, 57)**

¹H NMR (400 MHz, CD₃OD)

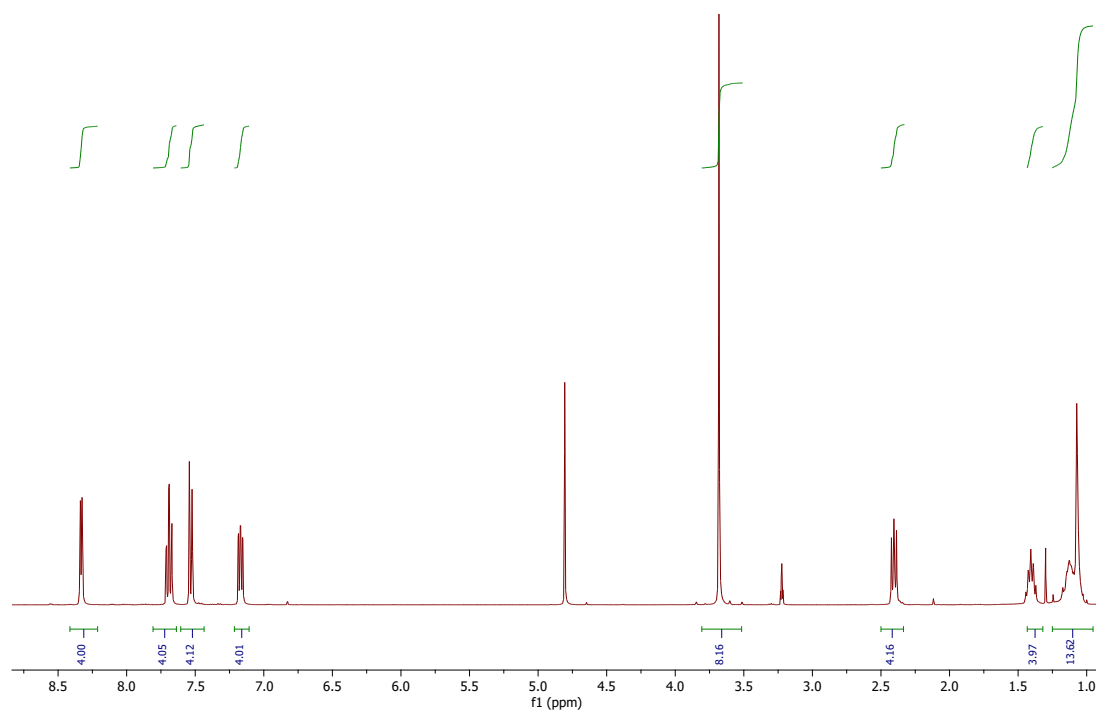


¹³C NMR (100 MHz, CD₃OD)

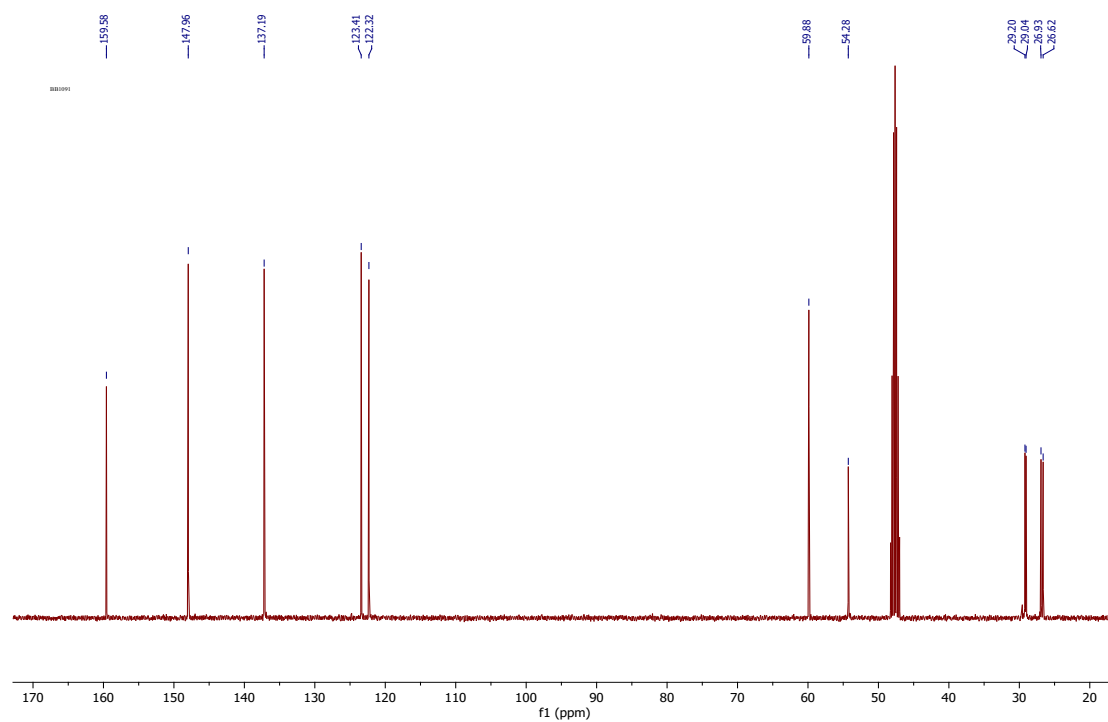


N¹,N¹,N⁴,N⁴-Tetrakis(2-pyridinylmethyl)-1,10-decanediamine (C₁₀-dimer, **56)**

¹H NMR (400 MHz, CD₃OD)

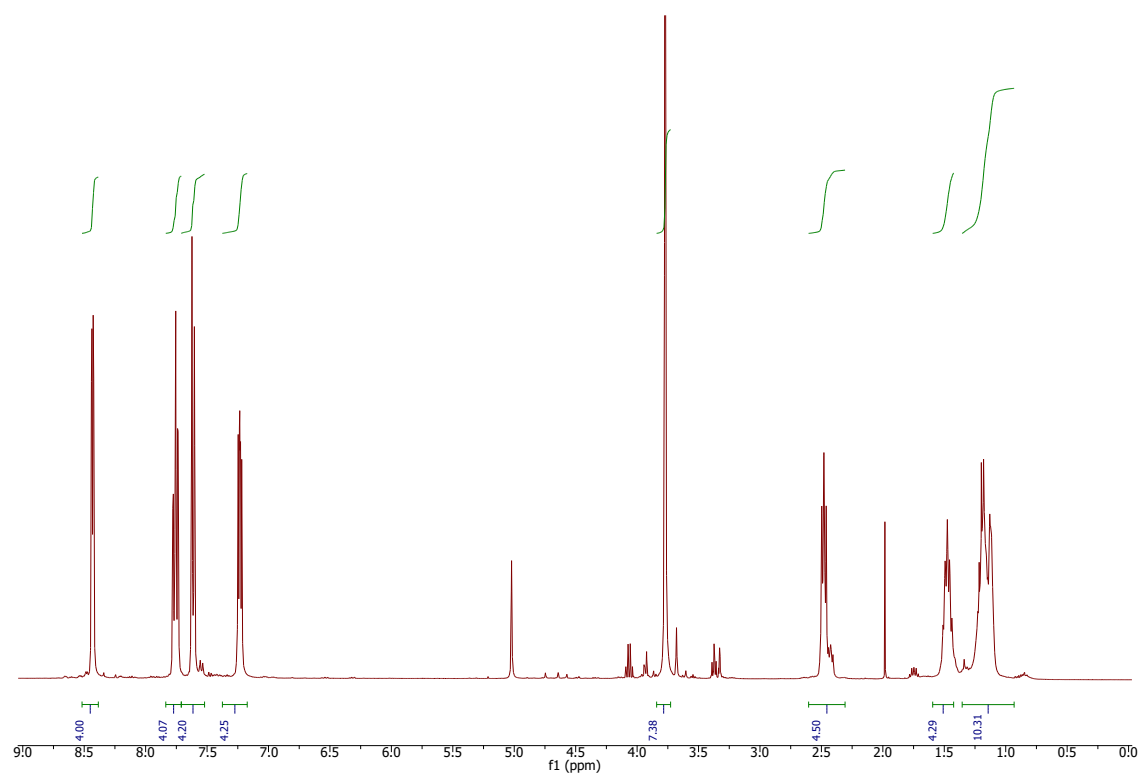


¹³C NMR (100 MHz, CD₃OD)

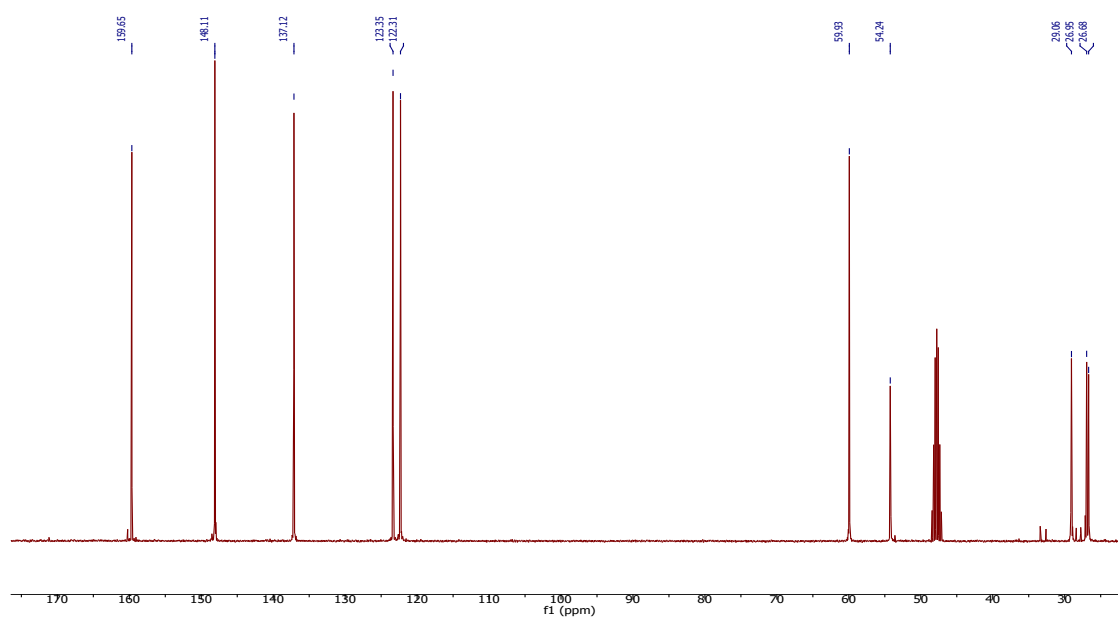


***N*¹,*N*¹,*N*⁴,*N*⁴-Tetrakis(2-pyridinylmethyl)-1,8-octanediamine (C₈-dipicolyl, 54)**

¹H NMR (400 MHz, CD₃OD)

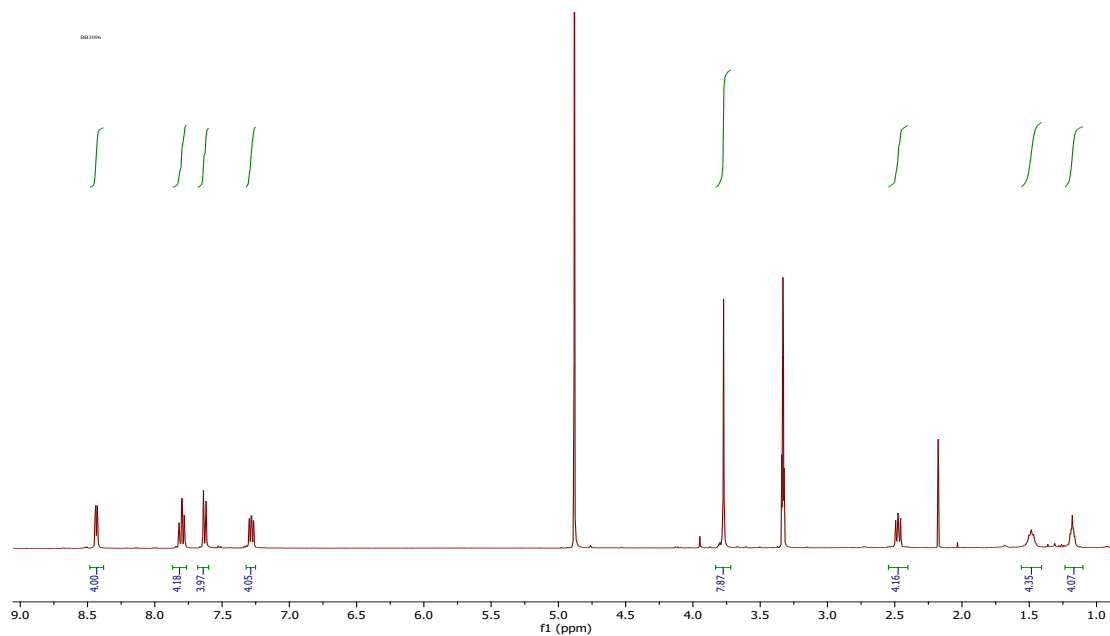


¹³C NMR (100 MHz, CD₃OD)

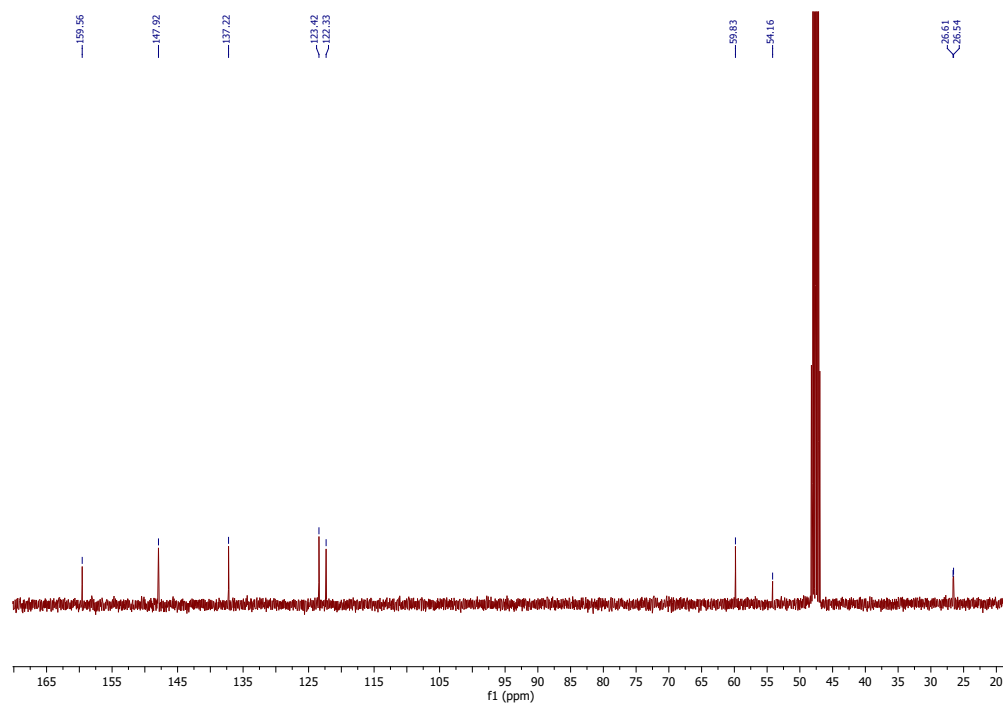


***N*¹,*N*¹,*N*⁴,*N*⁴-Tetrakis(2-pyridinylmethyl)-1,6-hexanediamine (C₆-dipicolyl, 54)**

¹H NMR (400 MHz, CD₃OD)

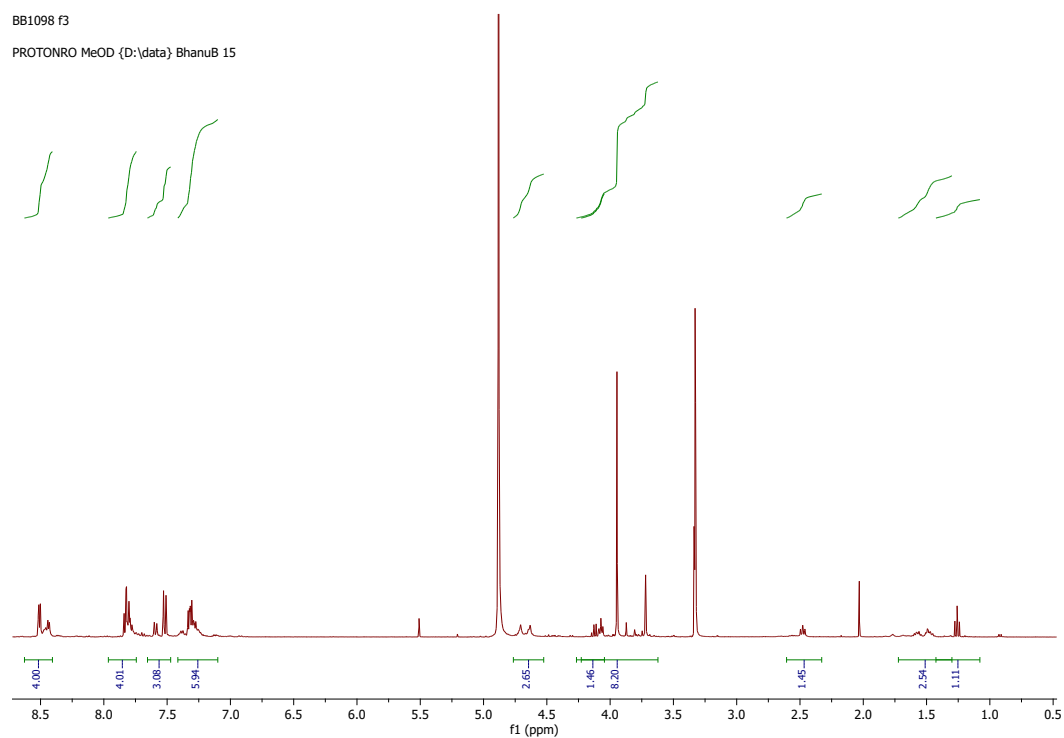


¹³C NMR (100 MHz, CD₃OD)

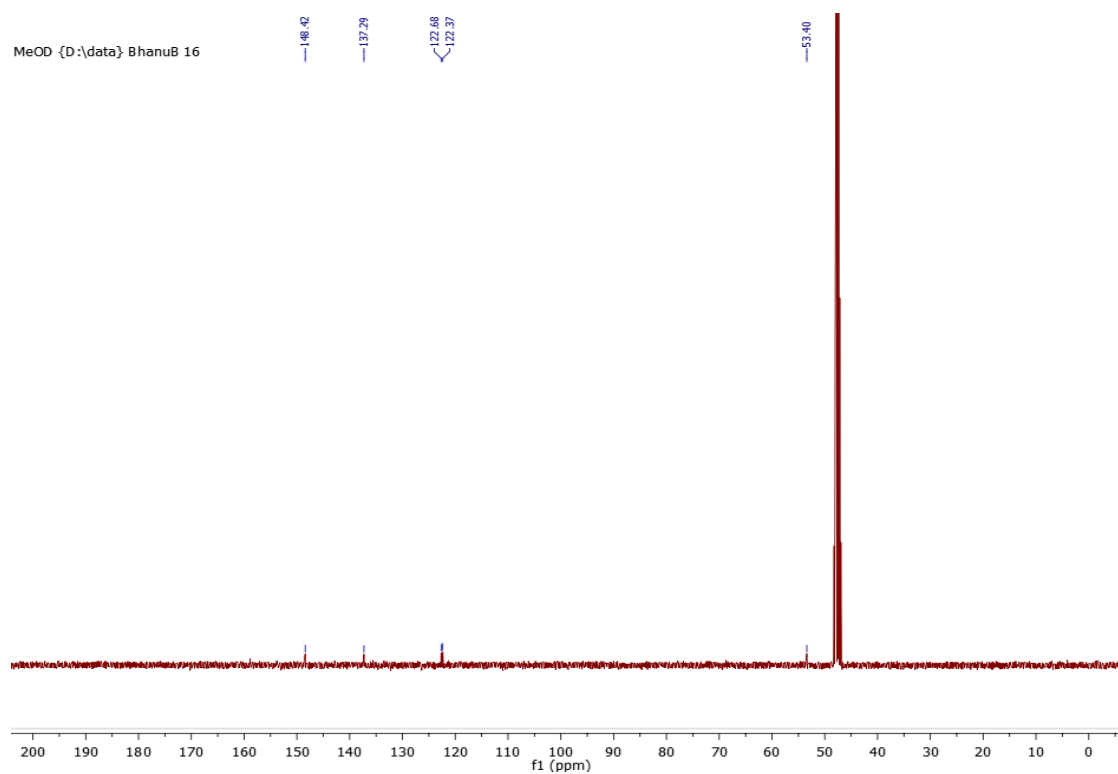


***N*¹,*N*¹,*N*⁴,*N*⁴-Tetrakis(2-pyridinylmethyl)-1,4-butanediamine(C₄-dipicolyl, 53)**

¹H NMR (400 MHz, CD₃OD)

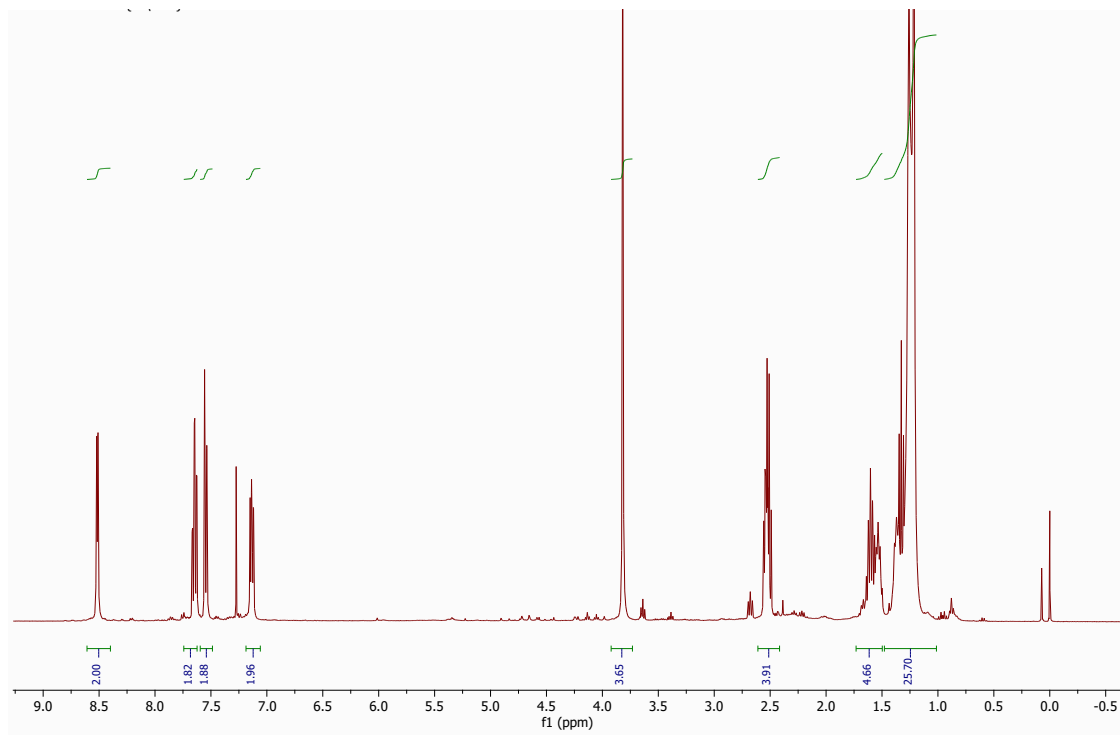


¹³C NMR (100 MHz, CD₃OD)

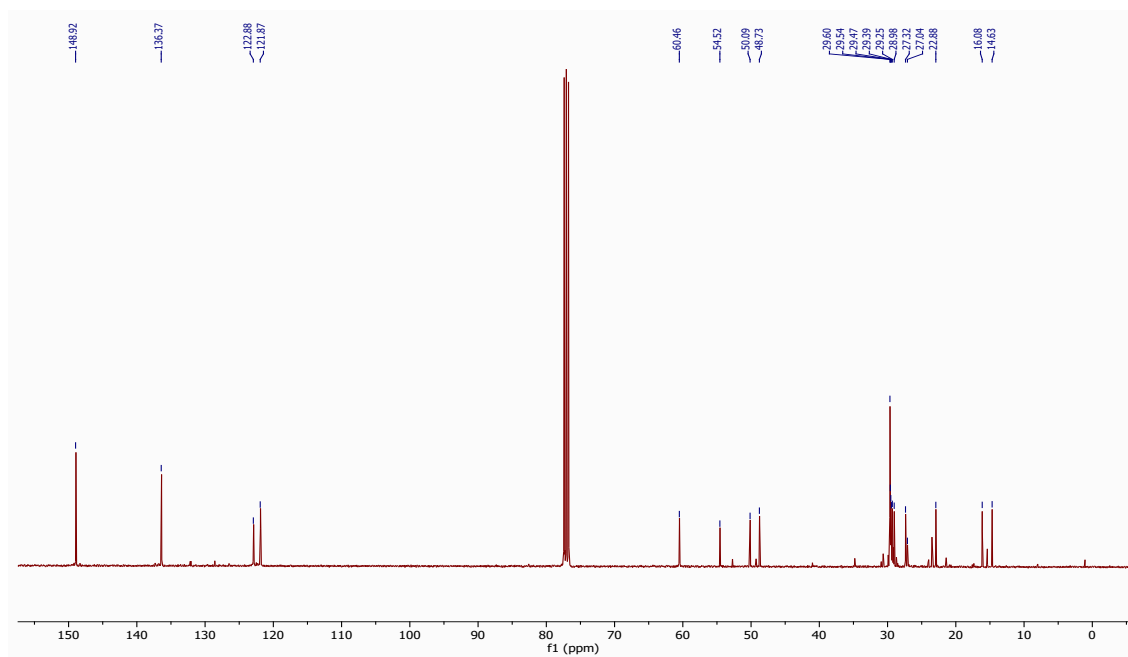


14-(Bis((pyridine-2-yl)methyl)amino)tetradecane-1-thiol (C₁₄-picolyl SH, 74)

¹H NMR (400 MHz, CDCl₃)

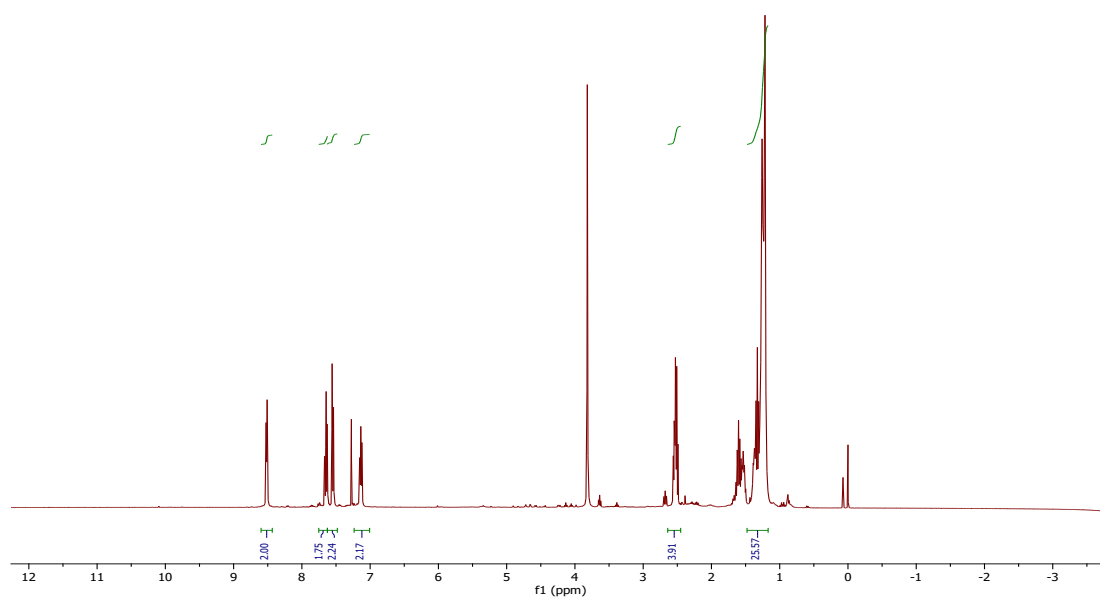


¹³C NMR (100 MHz, CDCl₃)

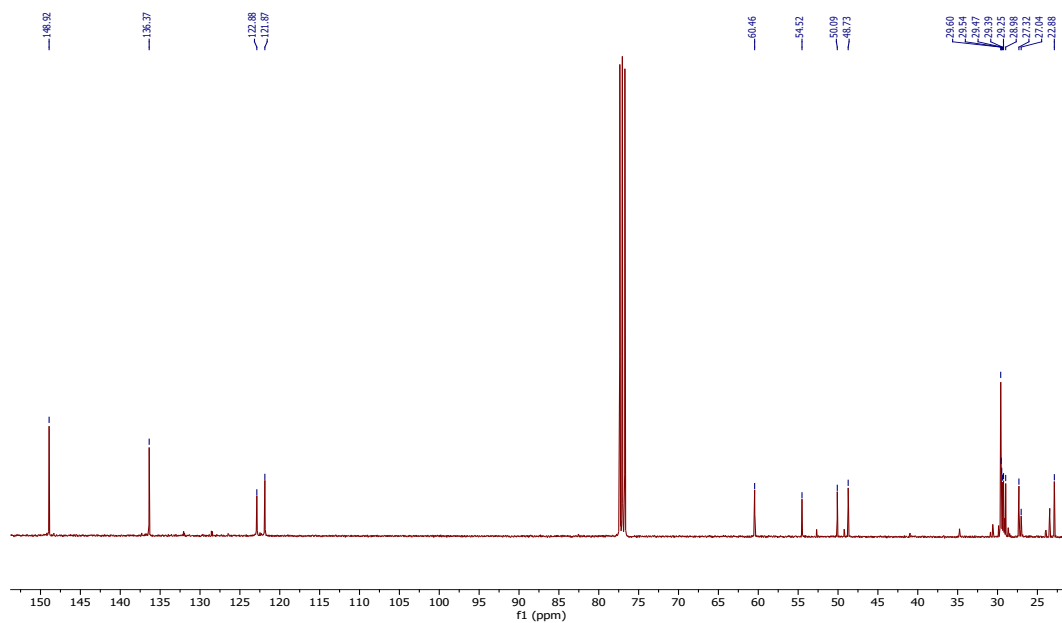


9-(Bis((pyridine-2-yl)methyl)amino)nonane-1-thiol (C₉-picolyl SH, 73)

¹H NMR (400 MHz, CDCl₃)

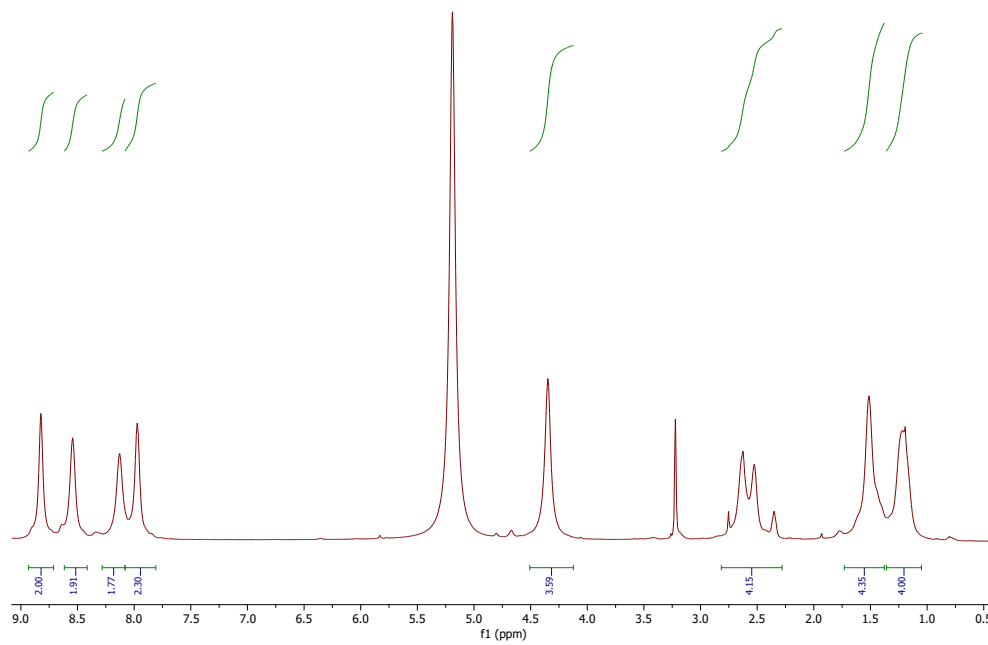


¹³C NMR (100 MHz, CDCl₃)

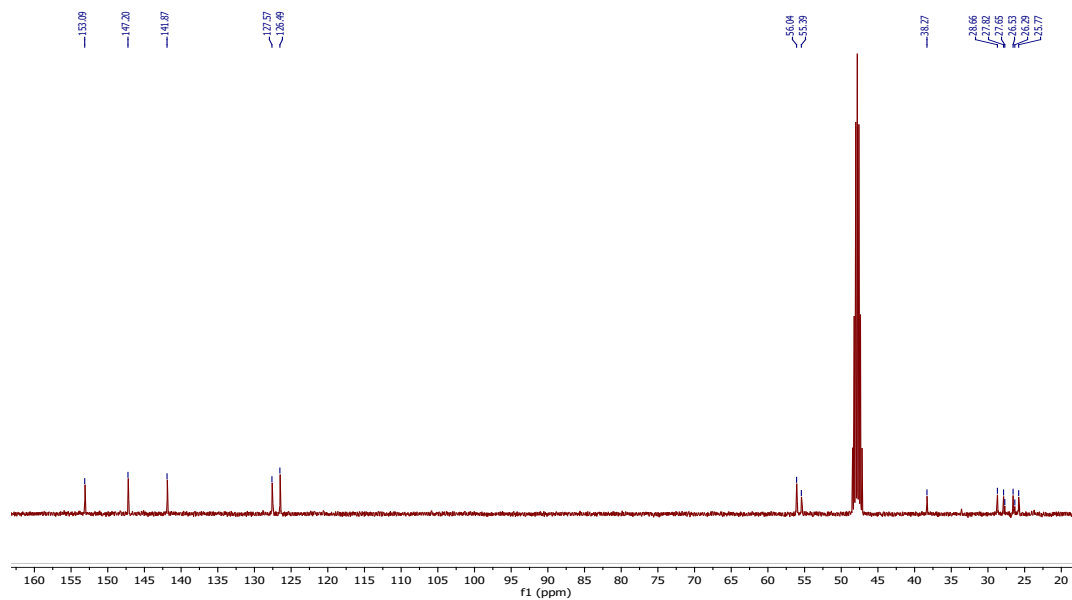


6-(Bis(2-pyridinylmethyl)amino)-1-hexanethiol (C₆-picolyl-SH, 72)

¹H NMR (100 MHz, CD₃OD)

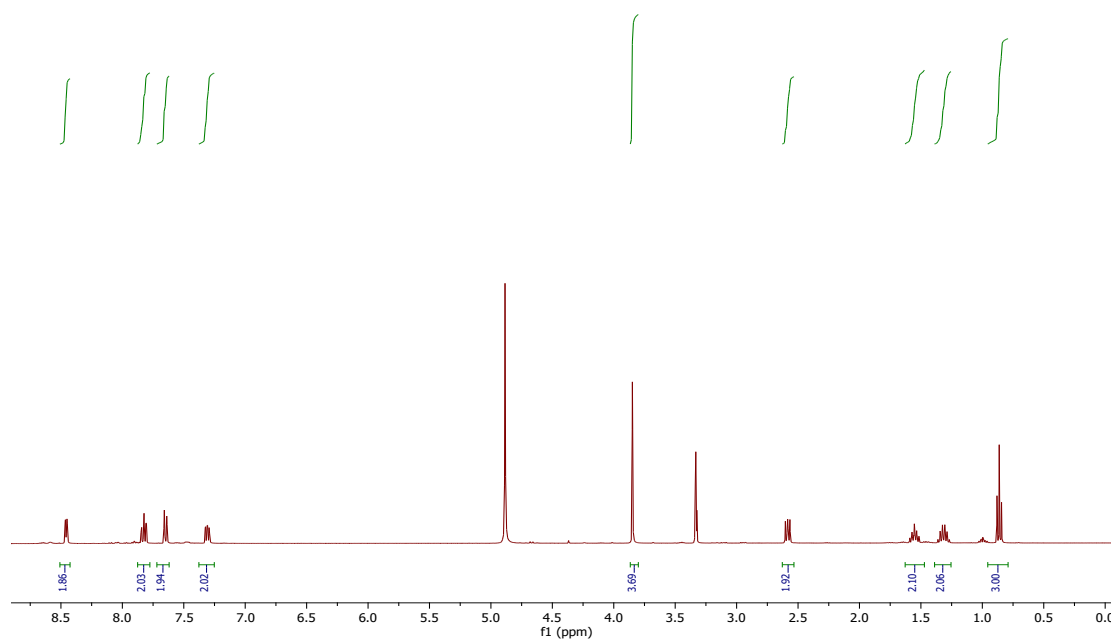


¹³C NMR (100 MHz, CD₃OD)

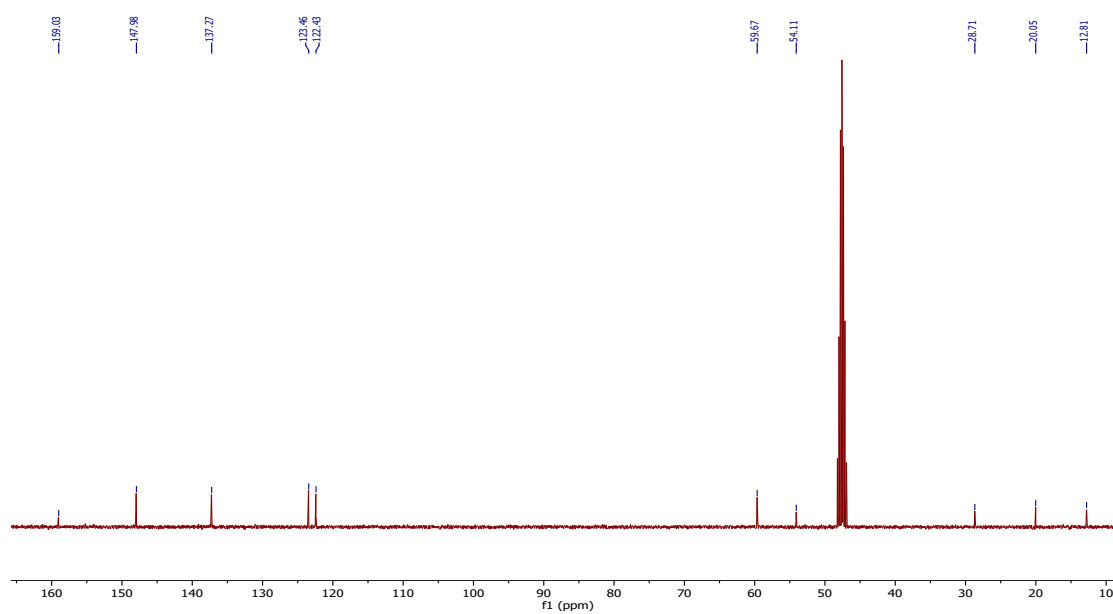


4-(Bis(2-pyridinylmethyl)amino)-1-butanethiol (C₄-picolyl-SH, 71)

¹H NMR (100 MHz, CD₃OD)



¹³C NMR (100 MHz, CD₃OD)



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