

DESIGN AND SYNTHESIS OF AMINO ACID-BASED SYSTEMS FOR ION RECOGNITION, SELF-ASSEMBLY AND CELLULAR UPTAKE STUDIES

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Design and synthesis of amino acid-based systems for ion recognition, self-assembly and cellular uptake studies

by

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Dedicated to my dear parents

(Dr. G.K. Sharma and Dr. Sarita Sharma)

CERTIFICATE

This is to certify that the thesis entitled, “**Design and synthesis of amino acid-based systems for ion recognition, self-assembly and cellular uptake studies**”, being submitted by **Ms. Ishanki Bhardwaj**, to the Indian Institute of Technology, Delhi, for the award of degree of ‘**Doctor of philosophy in Chemistry**’, is a record of bonafide research work carried out by her. **Ms. Ishanki Bhardwaj** has worked under my guidance and supervision and has fulfilled all the requirements for the submission of this thesis, which to my knowledge has reached the requisite standard. The results embodied in this thesis have not been submitted in part or in full, to any other University or Institute for award of any degree or diploma.

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ABSTRACT

This thesis, “**Design and synthesis of amino acid-based systems for ion recognition, self-assembly and cellular uptake studies**” deals with the design and synthesis of various peptide-based molecules. We have demonstrated the utility of these molecules for binding of ions and as new self-assembling systems. Molecular recognition of cations, anions and neutral molecules play a significant role in biology and chemistry. Cation transport is an important biological event that keeps the ionic balance in the body. Anions regulate the flux of key metabolites into and out of cells while maintaining osmotic balance. Incorporation of peptide in the molecules enhances molecular recognition ability and self-assembling properties of the molecules. Combination of self-assembling ability and biocompatibility of peptides can give an extra advantage in the design of peptide-based molecules. Self-assembled peptide systems are utilized for target oriented intracellular delivery purpose in the form of cell penetrating peptides (CPPs).

Chapter 1 deals with the discussion of history, current state and scope of the application of amino acid-based systems in molecular recognition, self-assembly and their application as intracellular delivery vehicles.

Chapter 2 describes the design and synthesis of self-assembling tryptophan-based peptides and their use as intracellular delivery vehicles. In this chapter, self-assembly is employed as a strategy for the design of vesicles that can load cargo into the interior of the self-assembled vesicles and traverse through the cell membrane with the cargo. Experimental results showed that **B3b** showed highest uptake efficiency while **B9b** has relatively less efficiency for cellular uptake as compared to other peptides.

Chapter 3 addresses the design, synthesis and self-assembly of disulphide-based macrocyclic systems and their use as heavy metal ion sensors. These macrocycles were demonstrated to show

distinct self-assembling features dependent upon the backbone moieties. **C4-C6** showed vesicular self-assembly, while **C8** showed flat bar-like morphology. A detailed study on the self-assembly of these macrocycles revealed that π - π interactions alone can play a pivotal role in the assembly process. Altering the positioning of aromatic units in the macrocycle can lead to a different organization. The binding studies of **C4-C8** towards metal ions revealed the affinity of **C4** towards Hg^{2+} ion while **C6** exhibited binding towards Cd^{2+} .

Chapter 4 discusses about design, synthesis, self-assembly and anion binding properties of glutamic and aspartic acid-based asymmetrical tripodal receptors. The binding property of these peptides towards tetrabutylammonium salts of different anions was analyzed in detail. They displayed varied self-assembling architectures, such as fibers and vesicles. The anion binding property of these molecules is used to fabricate wiffle-ball like assembly.

Chapter 5 deals with the design, synthesis, self-assembly and anion binding properties of urea functionalized peptides. The results from the binding studies showed that urea-cored compounds **E4** and **E5** have very weak affinity, while urea-triazole containing peptide **E10** turned out to be the most efficient receptor. **E10** acts as colorimetric sensor for fluoride and phosphate. Incorporation of triazole improved the anion binding ability of the urea-cored amino acid appended molecule. The self-assembling studies revealed that **E4-E6** and **E10** self-assembled into vesicles.

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NOTES

1. All amino acids used were of L-configuration. Unless otherwise stated, all reagents were used without further purification.
2. All solvents employed in the reaction were distilled or dried from appropriate drying agent prior to use.
3. Melting points were recorded in a Fisher-Johns melting point apparatus and were uncorrected.
4. IR spectra were recorded on a Nicolet, Protégé 460 spectrometer as KBr pellets.
5. ^1H NMR spectra were recorded on Bruker-DPX-300 (^1H , 300 MHz; ^{13}C , 75 MHz) spectrometer using tetramethylsilane (^1H) as an internal standard. Coupling constants are in Hz and the ^1H NMR data are reported as s (singlet), d (doublet), br (broad), br d (broad doublet), t (triplet), q (quartet), m (multiplet).
6. HRMS were recorded with Bruker MicrO-TOF- QII model using ESI-technique.
7. Reactions were monitored wherever possible by thin layer chromatography (TLC). Silica gel G (Merck) was used for TLC and column chromatography was done on silica gel (100-200 mesh) columns, which were generally made from slurry in hexane, hexane/ethyl acetate or chloroform.
8. Optical rotations were measured with a Rudolph Research Analytical Autopol® V Polarimeter. Concentrations are reported in g/100 mL.
9. UV-Visible spectra were recorded in Shimadzu double beam spectrophotometer, UV-2400.

LIST OF ABBREVIATIONS

%	percent
δ	chemical shift
$^{\circ}\text{C}$	degree centigrade
μM	micromolar
aq.	aqueous
AFM	Atomic force spectroscopy
Ar	Aryl
<i>ArH</i>	Aromatic proton
Ac	Acyl
Boc	t-butyloxycarbonyl
br	broad
Conc.	Concentrated
d	doublet
dd	double doublet
DCC	N,N'-dicyclohexylcarbodiimide
DIPEA	N,N'-Diisopropylethylamine
DMF	N,N-dimethylformamide
DMSO	dimethylsulfoxide
DLS	Dynamic light scattering
ESI-MS	Electrospray ionization mass spectroscopy
g	gram

h	hour
Hz	Hertz
HOSu	N-hydroxysuccinimide
HRMS	High resolution mass spectra
HR-TEM	High resolution transmission electron microscope
IR	infrared
J	coupling constant
m	multiplet
mg	milli gram
mL	milli liter
min	minutes
mmol	milli moles
mol	mole
Mp	melting point
m/z	mass/charge
NMR	Nuclear magnetic resonance
nm	nanomolar
ppm	parts per million
q	quartet
RT	Room temperature
s	singlet
SEM	Scanning electron microscope
TBAF	Tetrabutylammonium fluoride

TBACl	Tetrabutylammonium chloride
TBABr	Tetrabutylammonium bromide
TBAI	Tetrabutylammonium iodide
TBAH ₂ PO ₄	Tetrabutylammonium phosphate
TBAHSO ₄	Tetrabutylammonium sulfate
TEM	Transmission electron microscope
t	triplet
TLC	Thin layer chromatography
UV-vis.	Ultraviolet-visible
Z	Benzyloxycarbonyl