

**DESIGNER PEPTIDOMIMETICS: SELF-
ASSEMBLY AND PROTON SENSING
PROPERTIES**

SAKSHI SHARMA



**DEPARTMENT OF CHEMISTRY
INDIAN INSTITUTE OF TECHNOLOGY DELHI
SEPTEMBER 2017**

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PROPERTIES**

by

SAKSHI SHARMA

Department of Chemistry

Submitted

In fulfillment of requirements of degree of Doctor of Philosophy

to the



Indian Institute of Technology Delhi

September 2017

Dedicated to my dear parents

(Madhu Bala Sharma and Mange Ram Sharma)

CERTIFICATE

This is to certify that the thesis entitled, “**Designer peptidomimetics: Self-assembly and proton sensing properties**”, being submitted by **Ms. Sakshi Sharma**, 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. Sakshi Sharma 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 the award of any degree or diploma.

Dr. V. Haridas

Associate Professor

Department of Chemistry

Indian Institute of Technology Delhi

New Delhi-110016

ACKNOWLEDGEMENTS

It is a great pleasure to me to pay my deepest gratitude to my supervisor, **Dr. V. Haridas**, Associate Professor, Department of Chemistry, I.I.T. Delhi, who has been of constant inspiration to me throughout the realization of this work not only through the groves of research but also through the jungles of reality. He always pushed me to bring my level best in research. I really enjoyed unpretentious criticisms of his during all of our research discussions.

My sincere thanks are due to the erstwhile and the present Head of the Department of Chemistry Prof. Ravi Shankar and Prof. Ramanan for providing the necessary facilities for carrying out my research. I am also greatly in debt to Prof. Nalin Pant, Prof. N. G. Ramesh, Prof. R. P. Singh, Prof. S. K. Pattanayek, and Prof. Sameer Sapra for their constant encouragement and useful discussions. It is indispensable to thank Prof. C. H. Suresh (Principal Scientist and Head, Inorganic and Theoretical, Chemistry Section CSTD, CSIR-NIIST, Thiruvananthapuram) for help in DFT calculations and Dr. Rajesh Gonnade (Principal Scientist & Associate Professor, AcSIR Center for Materials Characterization, CSIR-NCL, Pune) for help in single crystal diffraction. I gratefully acknowledge Mr. Keshav, Mr. Alok, Mr. J. P. Sharma, Mr. Bhupender, Mr. Narugopal and other technical staff of the Chemistry Department for their help during data acquisition. I would like to thank Department of Textile Technology-IIT Delhi for SEM images, Department of Physics and NRF IIT Delhi for AFM images and HR-TEM images.

I wish to thank everybody with whom I have shared experiences in life, from the people who played a significant role in my life, especially those who taught me to survive in difficult situation, to those who with the gift of their company made my days more enjoyable and worth living.

I would like to thank my chemistry teachers Mr. Kaushik, Mrs. Minakshi, Dr. Anju, Dr. Charu, Dr. H. R. Sharma, and Dr. Ram Sharan who first persuaded me and got me interested in the study of Chemistry. My lab mates Dr. Sarala Naik, Dr. Srikanta Sahu, Dr. Ram Prakash Verma, Dr. Ishanki Bhardwaj, Dr. Praveen Kumar P. P., Dr. Bijesh M. B., Appa Rao Sapala, Gopalakrishna, Dheepthi, Ajeet, Rohit, Prasun, Tanmay, Lakshay, Jyoti, Snigdha, Garima, Deepak, Anuj, Vipin, Govind, Akhilesh, Sameer, Dinesh, Rachit, and Sharan for their support shown towards me on and off the lab. I thank all my friends Pancham, Uma, Mona, Sushma, Swati, Shweta, Mayank, Rohit, Sajjan, Panchpuri, Labeesh, Mamta, Ashutosh, Mahendra, Jitender, Rituraj, Hemant, Mayukh, Ashmita, Nidhi, Vineet, Aditi, Ruchi, Pragati, Pushpanjali, Sandeep, Ved, Shridhar, Shiva, Poonam, Raunak and all those who made my research days more enjoyable. I thank IITD Scoop and Zerox facility, especially Dada for printing thesis. I must mention my all time favorite NESCAFE for boosting me.

Last but not the least, I thank my family: My Parents Mange Ram Sharma and Madhu Bala Sharma for their unconditional love, care, support and encouragement to pursue my interests, even when the interests went beyond boundaries of language, field, and geography. I express my sincere appreciation to my siblings Shikha Sharma, Kavita Sharma, Manish Sharma and Varun Sharma for affection and support. My cute niece Shravya Sharma for teaching me how to keep getting up every time you fall. My Grand Parents Late Sh. Om Prakash Sharma and Late Smt. Vidya Sharma for their love and blessings even though they are not physically present but they are always in my heart.

Finally, support from IIT Delhi and University Grant Commission, India in the form of research fellowship is gratefully acknowledged.

I would like to express my gratitude towards almighty for giving me strength, courage, and perseverance. Hare Krishna!

Sakshi Sharma

ABSTRACT

The thesis entitled “**Designer peptidomimetics: Self-assembly and proton sensing properties**” deals with the new design strategy for bispidine-based macrocyclic systems with a variety of topology having diverse properties. Their applications as a molecular scaffold for the sensing of proton and self-assembling properties have been studied. Topology has a dominant role in the macroscopic world as well as at the molecular level. Nature presents a great deal of complexity in terms of topology in macromolecules and small natural products. Topological features are thought to play a significant role in the functional properties of the biological systems. Therefore, molecules which adopt intriguing topology are increasingly becoming target of chemical synthesis.

Chapter 1 deals with a brief overview of the topology and importance of self-assembly. This chapter presents various molecular knots found in naturally occurring biological systems (DNA, RNA and proteins), and those designed and synthesized by chemists. Also, the role of distinctive molecular topology on self-assembly has been discussed briefly.

Chapter 2 describes the novel class of preorganized macrocyclic compounds with a variety of shapes having diverse stackabilization properties. Macrocyclic **B3** resembles a calix[4]arene, while **B4** and **B5** adopt a sofa and lounge type conformation respectively. X-ray crystallographic studies reveal that these macrocycles self-assemble solely utilizing non-conventional hydrogen bonding (C-H...O, CH... π) and π - π interactions. Theoretical studies support the observed topologies and the potential binding capability of **B3**.

Chapter 3 addresses the rational design strategy for the synthesis of hybrid macrocyclic peptides with unusual topological features. The design concept is based on the incorporation of turn units in the backbone of the macrocycle. Macrocyclic with twisted figure-eight (**C6**) and rigid cavity

(**C7**) are synthesized based on a bicyclic turn inducing bispidine scaffold. The kink in the structure required to form the figure-eight topology is provided by bispidine and the 1,3-benzenedicarbonyl linkers. The molecular structure and folding have been studied by X-ray crystallography, NMR, FT-IR, and CD.

Chapter 4 discusses about bispidine as a molecular scaffold for the sensing of H^+ . The modular structure ‘fluoro-spacer-amine’ containing anthracene unit has shown unique ability to sense proton according to the principle of photoinduced electron transfer (PET). The X-ray crystallography, mass spectroscopy and NMR data support **D2** with two bound protons (**D2·2H⁺**). Theoretical analysis has been done to calculate the gas phase proton affinity.

Chapter 5 deals with the design, synthesis and self-assembling properties of bispidine-based macrocycles **E5a-d** containing amino-acids. These compounds show self-assembly leading to spheres, which was confirmed by scanning electron microscopy (SEM), high-resolution transmission electron microscopy (HR-TEM) and atomic force microscopy (AFM). A detailed study on the self-assembly of these compounds, revealed that the replacement of bispidine (bicyclic unit) with monocyclic 1,5-diazocane changed the morphology from spheres to fibres. The spheres show encapsulation of fluorescent dye, rhodamine B. The spherical self-assembly opens up possibilities for the encapsulation of guest molecules and hence has potential use as carrier molecules for biological purposes.

सारांश

थीसिस "डिजाइनर पेप्टाइडोमेटिक्स: स्व-संयोजन और प्रोटॉन सेंसिंग गुण" विभिन्न गुणों वाले टोपोलॉजी के साथ बिस्पिडेन आधारित मैक्रोसायक्लिक सिस्टम के लिए नई डिजाइन रणनीति के साथ काम करता है। प्रोटॉन और स्व-संयोजन गुणों की संवेदन के लिए एक आणविक पाबंद के रूप में उनके आवेदनों का अध्ययन किया गया है। मैक्रोस्कोपिक दुनिया में और साथ ही आणविक स्तर पर टोपोलॉजी की प्रमुख भूमिका है। प्रकृति में अणुओं और छोटे प्राकृतिक उत्पादों में टोपोलॉजी के संदर्भ में जटिलता का एक बड़ा सौदा प्रस्तुत किया गया है। जैविक प्रणालियों के कार्यात्मक गुणों में मूलभूत सुविधाओं को महत्वपूर्ण भूमिका निभाने के लिए सोचा गया है। इसलिए, दिलचस्प टोपोलॉजी को अपनाने वाले अणु रासायनिक संश्लेषण का लक्ष्य बढ़ रहे हैं।

अध्याय 1 में टोपोलॉजी और स्व-संयोजन के महत्व का संक्षिप्त विवरण दिया गया है। यह अध्याय जैविक प्रणाली (डीएनए, आरएनए और प्रोटीन) और रसायनज्ञों द्वारा संश्लेषित अणु में पाए जाने वाले विभिन्न आणविक टोपोलॉजी को प्रस्तुत करता है। साथ ही, स्व-संयोजन पर विशिष्ट आणविक टोपोलॉजी की भूमिका पर संक्षेप में चर्चा की गई है।

अध्याय 2 विविध स्टैकबालाइजेशन गुण वाले विभिन्न आकृतियों के साथ अग्रगामी मैक्रोसायक्लिक यौगिकों का वर्णन करता है। मैक्रोसायकल B3 कैल्क्स[4]एरेन जैसा दिखता है, जबकि B4 और B5 क्रमशः एक सोफा और आरामकुर्सी प्रकार की रचना से मिलता है। एक्स-रे क्रिस्टलोग्राफिक अध्ययन से पता

चलता है कि मैक्रोसायकलस केवल गैर-पारंपरिक हाइड्रोजन बंधन (सी-एच...ओ, सी-एच... π) और π - π इंटरैक्शन का प्रयोग करते हुए स्व-संयोजन करते हैं। सैद्धांतिक अध्ययन ने कथित टोपोलॉजी और B3 की संभावित बाध्यकारी क्षमता का समर्थन किया।

अध्याय 3 असामान्य टोपोलॉजिकल विशेषताओं के साथ संकर मैक्रोसायक्लिक पेप्टाइड्स के संश्लेषण के लिए तर्कसंगत डिजाइन रणनीति को संबोधित करता है। डिजाइन की अवधारणा मैक्रोसायकल के मुख्य आधार में घुमाव वाले भागों के निगमन पर आधारित है। विकृत आंकड़ा-आठ (C6) और कठोर गुहा (C7) मैक्रोसायक्लिस को बाईसाइक्लिक बिस्पिडिन के आधार पर संश्लेषित किया जाता है। आंकड़ा-आठ टोपोलॉजी बनाने के लिए आवश्यक ढांचे में घुमाव बिस्पिडिन और 1,3-बेंजीनडीकार्बोनिल लिंक्स द्वारा प्रदान किया गया है। एक्स-रे क्रिस्टलोग्राफी, एनएमआर, एफटी-आईआर, और सीडी द्वारा आणविक संरचना और तह का अध्ययन किया गया है।

अध्याय 4 H^+ के संवेदन के लिए एक आणविक पाइ के रूप में बिस्पिडिन के बारे में चर्चा करता है। एन्थ्रेसिनेन यूनिट युक्त मॉड्यूलर संरचना 'फ्लुरो-स्पेसर-एमाइन' ने फोटो इंडेसड इलेक्ट्रॉन ट्रांसफर (पीईटी) के सिद्धांत के अनुसार प्रोटॉन को समझने की अद्वितीय क्षमता दिखायी है। एक्स-रे क्रिस्टलोग्राफी, मास स्पेक्ट्रोस्कोपी और एनएमआर डेटा D2 के साथ दो बाध्य प्रोटॉनों ($D2 \cdot 2H^+$) का समर्थन करता है। सैद्धांतिक विश्लेषण गैस चरण प्रोटॉन संबंध की गणना के लिए किया गया है।

अध्याय 5 बिस्पीडिन आधारित एमिनो एसिड युक्त मैक्रोसायक्लिस **E5a-d** के डिजाइन, संश्लेषण और स्व-संयोजन गुणों से संबंधित है। ये यौगिक गोलाकार स्व-संयोजन दिखाते हैं, जिसे स्कैनिंग इलेक्ट्रॉन माइक्रोस्कोपी (एसईएम), उच्च-रिज़ॉल्यूशन ट्रांसमिशन इलेक्ट्रॉन माइक्रोस्कोपी (एचआर- टीईएम) और परमाणु बल माइक्रोस्कोपी (एफएम) करके पुष्टि की गई थी। इन यौगिकों के स्व-संयोजन पर एक विस्तृत अध्ययन से पता चला कि मोनोसाइक्लिक 1,5-डायज़ोकैन के साथ बिस्पीडिन (बाईस्केलिस यूनिट) के प्रतिस्थापन ने गोलाकार आकृति को तंतुओं में बदल दिया। गोलाकार आकृति फ्लोरोसेंट डाई, रोडामाइन बी के इनकैप्सुलेशन दिखाते हैं। गोलाकार स्व-संयोजन अतिथि अणुओं के इनकैप्सुलेशन के लिए संभावनाएं खोलता है और इसलिए जैविक उद्देश्यों के लिए वाहक अणुओं के रूप में संभावित उपयोग हो सकते हैं।

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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.
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. ESI-MS were recorded with Bruker Micro-TOF- QII model and AB Sciex, 1011273/A 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. UV-Visible spectra were recorded in Shimadzu double beam spectrophotometer, UV-2400.
9. Emission spectra were recorded using HORIBA JOBIN YVON Scientific, fluoromax-4 spectrophotometer, with slit width of 5 nm.
10. SEM measurements were done using ZEISS EVO[®] and JEOL-JSM-5600 LV, instrument with an EHT of 20 kV.

11. AFM measurements were performed using Nanoscope Multimode AFM operating in tapping mode in air.
12. HR-TEM images were recorded using Philips CM 12 electron microscope.

LIST OF ABBREVIATIONS

%	percent
δ	chemical shift
$^{\circ}\text{C}$	degree centigrade
eV	electron volt
kV	Kilo volt
aq.	aqueous
AFM	Atomic force spectroscopy
Boc	t-butyloxycarbonyl
br	broad
Conc.	Concentrated
d	doublet
dd	double doublet
DCC	N,N'-Dicyclohexylcarbodiimide
DIEA	N,N-Diisopropylethylamine
DMF	N,N-Dimethylformamide
DMSO	Dimethyl sulfoxide
ESI-MS	Electrospray ionization mass spectroscopy
equiv.	Equivalents
F	Fluorescence intensity
g	gram
h	hour
Hz	Hertz

HR-TEM	High resolution transmission electron microscope
IR	Infrared
<i>J</i>	coupling constant
K	kelvin
Kcal/mol	Kilocalories per mol
KJ/mol	Kilojoules per mol
M	molar
μM	micromolar
mM	millimolar
m	multiplet
mg	milligram
mL	milliliter
min	minutes
mmol	millimoles
mol	mole
mp	melting point
m/z	ESI-MS/charge
NHS	N-hydroxysuccinimide
NMR	Nuclear magnetic resonance
ns	nanosecond
nm	nanometer
ppm	parts per million
q	quartet

RT	Room temperature
s	singlet
SEM	Scanning electron microscope
TEM	Transmission electron microscope
t	triplet
TLC	Thin layer chromatography
UV-vis.	Ultraviolet-visible