DESIGNER PEPTIDOMIMETICS: SELF-ASSEMBLY AND PROTON SENSING PROPERTIES

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DESIGNER PEPTIDOMIMETICS: SELF-ASSEMBLY AND PROTON SENSING PROPERTIES

by

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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.

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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. Macrocycle **B3** resembles a calix[4]arene, while **B4** and **B5** adopt a sofa and lounger 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. Macrocycles 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 बिस्पपिडेन 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। डिज़ाइन 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 बिस्पपिडिन के आधार पर संश्लेषित किया जाता है। आंकड़ा-आठ टोपोलॉजी बनाने के लिए आवश्यक ढांचे में घुमाव बिस्पपिडिन और 1,3-बेंजीनडीकार्बोनिल लिंकर्स 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 पाड़ के रूप में बिस्पपिडीन 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**•$2H^+$) 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 (एएफएम) 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।

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SCHEMES

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. ^{1}H NMR spectra were recorded on Brucker-DPX-300 (^{1}H, 300 MHz; ^{13}C, 75 MHz) spectrometer using tetramethylsilane (^{1}H) as an internal standard. Coupling constants are in Hz and the ^{1}H 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
^{o}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
J	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