# PEPTIDE DESIGN: DESIGNER PEPTIDES AS ANION SENSORS, SECONDARY STRUCTURE MIMETICS AND SELF-ASSEMBLED MATERIALS

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*by*


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*Submitted*

*in fulfillment of the requirements*

*of the degree of*

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*to the*



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*Dedicated to my parents and my well -*

*wisher R. K. Menon*

# CERTIFICATE

This is to certify that the thesis entitled, "**Peptide design: Designer peptides as anion sensors**, **secondary structure mimetics and self-asssembled materials**", being submitted by **Miss**. **Sandhya Sadanandan**, to the Indian Institute of Technology Delhi, for the award of degree of '**Doctor of philosophy**', is a record of bonafide research work carried out by her. **Miss**. **Sandhya Sadanandan** 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

The thesis entitled "**Peptide design: Designer peptides as anion sensors**, **secondary structure mimetics and self-asssembled materials**", presents the results obtained from the research work carried out on the design, synthesis, characterization and applications of a variety of designer peptides as anion sensors, secondary structure mimetics and as a self-assembling scaffold for tissue engineering applications. The accompanied research work has been divided into the five chapters.

**Chapter 1** is a general introduction providing the backgrounds and literature required to follow up the research work carried out and presented in the subsequent chapters. As the work described in this thesis is mainly focused on peptide design and their applications, the design and synthesis of peptide-based materials, their spontaneous and stimuli responsive self-assembly, their biochemical applications along with recent developments have been discussed.

**Chapter 2** embodies the design and synthesis of amino acid-based molecules containing an aryl linked bis-urea moiety. The binding studies of these compounds with various anions are also demonstrated. The conversion of these 1, 3-aryl urea based compounds to imidazolidinediones and their self-assembly are also discussed.

**Chapter 3** provides a detailed discussion on the design, synthesis and supramolecular chiral assemblies of a series of aryl linked urea peptides. The chiral inversion of this supramolecular self-assembly upon anion binding is also demonstrated.

**Chapter 4** demonstrates the use of 3, 7-diazabicyclo [3.3.1] nonane as a scaffold for inducing helical structure to the attached peptides. Peptides containing four chiral α-amino acid residues are found to adopt a right handed helical conformation. The design, synthesis and conformational studies are discussed in detail.

**Chapter 5** provides the design and synthesis of a variety of self-assembling peptide systems containing a diacetylene moiety. These systems were used to prepare polydiacetylenes with different morphologies and studied their cell attachment, morphology and viability towards human mesenchymal stem cell adhesion and spreading.

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# NOTES

1. All amino acids used in the reactions were of L-configuration. Standard single/triple letter codes are used to represent the amino acids.
2. All solvents employed in the reaction were distilled or dried from appropriate drying agent prior to use. Unless otherwise stated, all reagents were used without further purification.
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. $^{1}$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. 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.
7. CD measurements were made using AVIV-420 spectropolarimeter. Quartz cell of 0.1 cm was used for the measurements.
8. UV-Vis spectroscopy - The absorption spectra were recorded on a Shimadzu UV-2450 spectrometer.
9. Fluorescence spectroscopy - The emission spectra were recorded in HORIBA JOBIN YVON Scientific, fluoromax - 4 spectrofluorometer with slit width of 5 nm

10. SEM images were recorded using ZEISS EVO Series Scanning Electron Microscope EVO 50 operating at an accelerating voltage of 0.2 − 30 kV. For SEM, a 10 μL aliquot of the sample solution was drop-casted on a glass coverslip, dried and coated with ~10 nm of gold.
11. HRTEM images were recorded on a Tecnai G2 20 electron microscope operated at an accelerating voltage of 200 kV. Samples were prepared by drop-casting the sample on 200 square mesh carbon-coated copper grids.
12. AFM imaging and measurements were performed using a Bioscope Catalyst AFM (Bruker Corporation, Billerica, MA) with silicon probes. 10 μL of the sample was dropcasted on a freshly peeled mica surface followed by drying under nitrogen flow. The standard tapping mode was used to image the morphology at room temperature in air. A spring constant of 20−80 N/m was used. A single third order flattening of height images with a low pass filter was done followed by section analysis to determine the dimensions of the images.
13. X-ray diffraction study was carried out on a BRUKER AXS SMART-APEX diffractometer with a CCD area detector (Mo Kα = 0.71073Å, monochromator: graphite). Frames were collected at T = 298 by ω,ϕ and 2θ-rotation at 10 s per frame with SMART. The measured intensities were reduced to $F^2$ and corrected for absorption with SADABS. Structure solution, refinement, and data output were carried out with the SHELXTL program. Non-hydrogen atoms were refined anisotropically. C-H hydrogen atoms were placed in geometrically calculated positions by using a riding model. Image was created with the Diamond program.

14. The water contact angles were measured using Kruss goniometer drop shape analysis system (DSA 100) at ambient temperature. Average water contact angles were obtained by measuring the same sample at five different positions.
15. GPC- Molecular weight distribution was analyzed by Waters HPLC 1525 (based on polystyrene calibration) equipped with a Styragel HR4 THF 7.8 × 300 mm column using refractive index detector.

# LIST OF ABBREVIATIONS

| | |
|---|---|
| % | Percent |
| δ | Chemical shift |
| $^{o}$C | Degree centigrade |
| AFM | Atomic force microscopy |
| ALP | Alkaline phosphatase |
| aq. | Aqueous |
| Ar | Aryl |
| Ar*H* | Aromatic proton |
| Ac | Acyl |
| Boc | t-butyloxycarbonyl |
| br | Broad |
| BSA | Bovine serum albumin |
| CA | Contact angle |
| CD | Circular dichroism |
| Conc. | Concentrated |
| COSY | Correlation spectroscopy |
| CuCl | Copper (1) chloride |
| d | Doublet |
| Da | Dalton |
| DA | Diacetylene |
| DCM | Dichloromethane |

| | |
|---|---|
| dd | Double doublet |
| DCC | N,N'-dicyclohexylcarbodiimide |
| DFT | Density functional theory |
| DIPEA | N,N'-Diisopropylethylamine |
| DMF | N,N-dimethylformamide |
| DMSO | Dimethylsulfoxide |
| DPSC | Dental pulp stem cells |
| ECM | Extra cellular matrix |
| ESI | Electronspray ionization |
| FDA | Fluorescein diacetate |
| Fmoc | Fluorenylmethyloxycarbonyl |
| g | Gram |
| GPC | Gel permeation chromatography |
| h | Hour |
| HPLC | High performance liquid chromatography |
| Hz | Hertz |
| HRMS | High resolution mass spectrum |
| HRTEM | High resolution transmission electron microscope |
| IR | Infrared |
| J | Coupling constant |
| m | Multiplet |
| MeOH | Methanol |

| | |
|---|---|
| μM | Micro molar |
| μm | Micro meter |
| mg | Milli gram |
| mL | Milli liter |
| min | Minutes |
| mmol | Milli moles |
| mol | Mole |
| Mp | Melting point |
| m/z | Mass/charge |
| NMR | Nuclear magnetic resonance |
| NOESY | Nuclear Overhauser effect spectroscopy |
| PA | Peptide amphiphile |
| PBS | Phosphate buffered saline |
| PDA | Polydiacetylene |
| PDI | Polydispersity index |
| PI | Propidium iodide |
| ppm | Parts per million |
| q | Quartet |
| RGD | Arginine - Glycine – Aspartic acid |
| ROESY | Rotating-frame Overhauser Spectroscopy |
| RT | Room temperature |
| s | Singlet |

| | |
|---|---|
| SEM | Scanning electron microscope |
| TBAF | Tetrabutylammonium fluoride |
| TBACl | Tetrabutylammonium chloride |
| TBABr | Tetrabutylammonium bromide |
| TBAI | Tetrabutylammonium iodide |
| TBAX | Tetrabutylammonium halide |
| TEM | Transmission electron microscope |
| TE | Tissue engineering |
| TFA | Trifluoroacetic acid |
| TOCSY | Total correlation spectroscopy |
| TMEDA | Tetramethylethylenediamine |
| t | Triplet |
| TLC | Thin layer chromatography |
| UV | Ultra violet |