

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

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SENSORS, SECONDARY STRUCTURE MIMETICS AND
SELF-ASSEMBLED MATERIALS**

by

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Department of Chemistry

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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-assembled 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-assembled 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.

TABLE OF CONTENTS

	Page No.
Certificate	i
Acknowledgements	ii
Abstract	v
Table of contents	vii
List of figures	ix
List of tables	xxv
List of schemes	xxvi
Notes	xxvii
List of abbreviations	xxx
CHAPTER 1: Introduction	1
1.1 Peptide-based anion receptors	3
1.2 Peptide-based self-assembled materials	10
References	35
CHAPTER 2: Design and synthesis of 1,3-aryl linked bis-urea based amino acids as anion receptors	
2.1 Introduction	47
2.2 Results and Discussion	48
2.3 Conclusion	69
2.4 Experimental Section	70
References	83

CHAPTER 3: Design and synthesis of 1,3-aryl linked bis-urea based peptides

And their anion induced conformational inversion

3.1 Introduction	87
3.2 Results and Discussion	87
3.3 Conclusion	121
3.4 Experimental Section	122
References	142

CHAPTER 4: Design and synthesis of bispidine based peptides as secondary structure mimetics

4.1 Introduction	145
4.2 Results and Discussion	146
4.3 Conclusion	171
4.4 Experimental Section	172
References	208

CHAPTER 5: Design and synthesis of polydiacetylene based peptides as self-assembling scaffolds for tissue engineering

5.1 Introduction	213
5.2 Results and Discussion	215
5.3 Conclusion	230
5.4 Experimental Section	231
References	247
Resume	253

LIST OF FIGURES

Figure No.	Description	Page No.
Figure 1.1	Various applications of peptide-based materials	2
Figure 1.2	Chemical structures of peptide-based chloride ion transporters developed by Schlesinger et al	4
Figure 1.3	Chemical structures of pseudopeptide-based anion receptors	5
Figure 1.4	Chemical structures of peptide-based anion receptors containing calixarene unit	6
Figure 1.5	Chemical structures of peptide-based anion receptors	7
Figure 1.6	Chemical structures of peptide-based anion receptors for carboxylate anions	8
Figure 1.7	Chemical structures of peptide-based anion receptors containing cryptands	10
Figure 1.8	Self-assembly of peptides into different types of structures	11
Figure 1.9	(a) Chemical structures of the cyclic D, L- α - peptides. (b) Schematic representation of the mode of interaction of the tube caps in forming heteromeric transmembrane channels	13
Figure 1.10	The self-assembling of peptide nanotubes composed of <i>cyclo</i> -[(1 <i>R</i> ,3 <i>S</i>)- ζ -Acc-D-Aa] ₃ units	14
Figure 1.11	Examples of cyclic peptides which forms nanotubes	15
Figure 1.12	Examples of acyclic dipeptides which forms nanotubes	16
Figure 1.13	Self-assembly of Phe-Phe and Phg-Phg into nanotubes and nanovesicles (Phg = phenylglycine)	18
Figure 1.14	Self-assembly of Phe-Phe into nanotubes and the	19

	transition of the CDPNTs into vesicles for oligonucleotide delivery	
Figure 1.15	Synthesis of a C ₃ -symmetric β -sheet peptide conjugate and schematic illustration of the self-assembly	20
Figure 1.16	Cyclic to linear peptide conformational switch using a reductive trigger	20
Figure 1.17	Structures of peptide rod-coil building blocks and their self-assembly into nanocapsule structures	21
Figure 1.18	Chemical structures of cyclic peptides self-assembled into vesicle-like nanostructures	22
Figure 1.19	Examples of peptide amphiphiles which self-assemble to form supramolecular β -sheet structures synthesized by Stupp et al.	23
Figure 1.20	Aggeli's peptides which forms β -sheet structure	24
Figure 1.21	Chemical structures of peptides which assemble to fibrillar structures	25
Figure 1.22	Bioconjugation of peptide stabilized gold nanoparticles using the standard sulfo-NHS-biotin labeling agent	26
Figure 1.23	Mimicking of collagen using peptide-dendrimer conjugate (a) The Z-protected TRIS scaffold (Z-TRIS[OH] ₃). (b) Chemical structure of collagen mimetic dendrimer TMA [TRIS [(Gly-Pro-Nleu) ₆ -OMe] ₃] ₃ (Nleu= <i>N</i> -isobutylglycine) and the cartoon representation of clustering of triple helices about the trimesic acid core.	27
Figure 1.24	Schematic description of the strategy for the build-up of biomimetic surfaces consisting of a supported polymerized peptide-amphiphile monolayer and schematic description of the topochemically controlled polymerization of diacetylenic amphiphiles in an organized monolayer architecture.	28

Figure 1.25	(a) Chemical structure of cRGD-PEG-PAsp carrying ethylenediamine (DET) units. (b) Schematic illustration of charge induced (electrostatic) polyplex micelle formation and its complexation with pDNA for the <i>in vivo</i> cellular uptake	30
Figure 1.26	Schematic drawing of self-assembly of amphiphilic block copolypeptides, its change in conformation with pH and the release of entrapped Fura-2 dye on pH change	31
Figure 1.27	Schematic representation of pH induced self-assembly of the diblock copolymer PGlu ₁₅ - <i>b</i> -PLys ₁₅ into vesicles	32
Figure 1.28	Temperature triggered self-assembly of an elastin-like polypeptide block copolymer (ELP _{BC}) to form multivalent spherical micelles	33
Figure 2.1	Changes in the ¹ H NMR spectrum (CDCl ₃ , 300 MHz) of B3 as a result of addition of TBAF	49
Figure 2.2	Changes in the chemical shift ($\Delta\delta$) for Hb proton of B3 versus number of equivalents of TBAF	50
Figure 2.3	Changes in the chemical shift ($\Delta\delta$) for Hd proton of B3 versus number of equivalents of TBAF	50
Figure 2.4	Changes in the chemical shift ($\Delta\delta$) for Hc proton of B3 versus number of equivalents of TBAF	51
Figure 2.5	Changes in the ¹ H NMR spectrum (CDCl ₃ , 300 MHz) of B3 in CDCl ₃ with the addition of varying amounts of TBACl	52
Figure 2.6	Changes in the ¹ H NMR spectrum (CDCl ₃ , 300 MHz) of B3 in CDCl ₃ with the addition of varying amounts of TBABr	53
Figure 2.7	Changes in the ¹ H NMR spectrum (CDCl ₃ , 300 MHz) of B3 in CDCl ₃ with the addition of varying amounts of TBAI	54

Figure 2.8	Job's plot for Hb of B3 with TBACl	55
Figure 2.9	Changes in ^1H NMR (CDCl_3 , 300 MHz) of B3 with the addition of varying amount of TBAHPO_4	56
Figure 2.10	Changes in the chemical shift ($\Delta\delta$) for Hd proton of B3 versus number of equivalents of TBAH_2PO_4	57
Figure 2.11	Changes in the chemical shift ($\Delta\delta$) for Hb proton of B3 versus number of equivalents of TBAH_2PO_4	57
Figure 2.12	Changes in ^1H NMR (CDCl_3 , 300 MHz) of B3 as a result of addition of TBAHSO_4	59
Figure 2.13	Chemical shift changes of the Hb and Hd protons (CDCl_3 , 300 MHz) of B3 as a result of addition of TBAHSO_4	60
Figure 2.14	Job's plot for B3 with TBAH_2PO_4	61
Figure 2.15	Job's plot for Hb of B3 with TBAHSO_4	61
Figure 2.16	Structure of B3 optimized at PBEPBE/6-311G(d,p) level of DFT	62
Figure 2.17	Structure of B3 - TBAH_2PO_4 complex optimized at PBEPBE/6-311G(d,p) level of DFT	63
Figure 2.18	X-ray crystal structure of B5	67
Figure 2.19	The self-assembly of B5 . Packing diagram showing the ribbon-like linear chain	68
Figure 2.20	The self-assembly of B5 . Structural representation of the interactions present in the solid state	68
Figure 2.21	^1H NMR ($\text{DMSO}-d_6$, 300 MHz) spectrum of B1	76
Figure 2.22	^{13}C NMR ($\text{DMSO}-d_6$, 75 MHz) spectrum of B1	76
Figure 2.23	^1H NMR (CDCl_3 , 300 MHz) spectrum of B2	77
Figure 2.24	^{13}C NMR ($\text{DMSO}-d_6$, 75 MHz) spectrum of B2	77
Figure 2.25	^1H NMR (CDCl_3 , 300 MHz) spectrum of B3	78

Figure 2.26	^{13}C NMR (CDCl_3 , 75 MHz) spectrum of B3	78
Figure 2.27	^1H NMR ($\text{DMSO}-d_6$, 300 MHz) spectrum of B4	79
Figure 2.28	^{13}C NMR ($\text{DMSO}-d_6$, 75 MHz) spectrum of B4	79
Figure 2.29	^1H NMR ($\text{DMSO}-d_6$, 300 MHz) spectrum of B5	80
Figure 2.30	^{13}C NMR ($\text{DMSO}-d_6$, 75 MHz) spectrum of B5	80
Figure 2.31	^1H NMR ($\text{DMSO}-d_6$, 300 MHz) spectrum of B6	81
Figure 2.32	^{13}C NMR ($\text{DMSO}-d_6$, 75 MHz) spectrum of B6	81
Figure 2.33	COSY (CDCl_3 , 300 MHz) analysis of B3 with TBAH_2PO_4	82
Figure 3.1	Chemical structures of the compounds C1-C6	88
Figure 3.2	^1H NMR (CD_3CN , 300 MHz) spectrum of C1	89
Figure 3.3	COSY (CD_3CN , 300 MHz) spectrum of C1	89
Figure 3.4	Selected region of COSY (CD_3CN , 300 MHz) spectrum of C1	90
Figure 3.5	NOESY (CD_3CN , 300 MHz) spectrum of C1	90
Figure 3.6	Selected region of NOESY (CD_3CN , 300 MHz) spectrum of C1	91
Figure 3.7	Selected region of NOESY (CD_3CN , 300 MHz) spectrum of C1	92
Figure 3.8	(a) CD spectrum of C1 in acetonitrile (100 μM). (b) Thermal denaturation curve for C1 at 233 nm. Titration experiment of C1 with varying amounts of TBAH_2PO_4 . (c) Changes in CD spectra and (d) changes in specific rotation with varying equivalents of TBAH_2PO_4	93
Figure 3.9	CD measurements to establish the intermolecular association in C1 (a) at different concentrations (b) in different solvent systems (c) at different proportions of	94

	ACN (acetonitrile): water solvent mixture	
Figure 3.10	Chemical shift of NHs versus % of DMSO added (v/v) to a solution of C1 in CD ₃ CN	96
Figure 3.11	The NH stretch region in the solution FT-IR spectrum of C1 . Spectra recorded are in acetonitrile and presented after subtraction of the spectrum of pure acetonitrile.	97
Figure 3.12	CD titration of C1 in acetonitrile with (a) TBAHSO ₄ , (b) TBACl, (c) TBABr, (d) TBAI	99
Figure 3.13	Demonstration of chiroptical property of C1 by CD. Addition of TBAH ₂ PO ₄ reverses the chirality and the water addition further revert it back to the original state.	100
Figure 3.14	Cartoon representation of conformational inversion of C1 and its chiroptical switching property with addition of water	101
Figure 3.15	Partial ¹ H NMR titration spectra of C1 with TBAH ₂ PO ₄ in CD ₃ CN. Peaks were assigned by COSY experiments. NOEs are represented by double headed arrows.	102
Figure 3.16	¹ H NMR (CD ₃ CN, 300 MHz) overlay spectra of C1 upon the addition of varying amounts of TBAH ₂ PO ₄	103
Figure 3.17	COSY (CD ₃ CN, 300 MHz) spectrum of C1 - TBAH ₂ PO ₄ complex	104
Figure 3.18	NOESY (CD ₃ CN, 300 MHz) spectrum of C1 -TBAH ₂ PO ₄ complex	104
Figure 3.19	Selected region of NOESY (CD ₃ CN, 300 MHz) spectrum of C1 - TBAH ₂ PO ₄ complex	105
Figure 3.20	Structure of the complex between C1 and phosphate optimized at PBEPBE/6-311G(d,p) level of DFT	105
Figure 3.21	Expected intermolecular H-bonding pattern in C1 and C1 - TBAH ₂ PO ₄ complex	106
Figure 3.22	Job's plot of C1 binding with TBAH ₂ PO ₄ by UV spectroscopy	107

Figure 3.23	Job's plot of C1 binding with TBAHSO ₄ by UV spectroscopy	107
Figure 3.24	Changes in (a) fluorescence spectra (b) UV spectra of C1 upon the addition of TBAH ₂ PO ₄	108
Figure 3.25	Temperature dependent fluorescence spectra of C1 (a) upon heating from 10 °C to 70 °C (b) upon cooling from 70 °C to 10 °C	108
Figure 3.26	COSY (CD ₃ CN, 300 MHz) spectrum of C3	109
Figure 3.27	Selected region of COSY (CD ₃ CN, 300 MHz) spectrum of C3	110
Figure 3.28	NOESY (CD ₃ CN, 300 MHz) spectrum of C3	111
Figure 3.29	Selected region of NOESY (CD ₃ CN, 300 MHz) spectrum of C3	112
Figure 3.30	Temperature dependent fluorescence spectra of C2 (a) upon heating from 10 °C to 70 °C (b) upon cooling from 70 °C to 10 °C	113
Figure 3.31	Temperature dependent fluorescence spectra of C3 (a) upon heating from 10 °C to 70 °C (b) upon cooling from 70 °C to 10 °C	113
Figure 3.32	(a) CD Titration experiment of C3 with varying amounts of TBAH ₂ PO ₄ , (b) changes in specific rotation of C3 with varying equivalents of TBAH ₂ PO ₄ , (c) CD melting experiment of C3 in acetonitrile, (d) CD of C2 in acetonitrile with TBAH ₂ PO ₄	114
Figure 3.33	CD titration of C3 in acetonitrile with (a) TBAH ₂ PO ₄ , (b) TBAHSO ₄ , (c) TBACl, (d) TBABr, (e) TBAI	115
Figure 3.34	Job's plot for C3 binding with TBAH ₂ PO ₄ by UV spectroscopy	115
Figure 3.35	Job's plot for C3 binding with TBAHSO ₄ by UV spectroscopy	116

Figure 3.36	Job's plot for C3 binding with TBACl by UV spectroscopy	116
Figure 3.37	(a) schematic representation of helical self-assembly into vesicles (b) SEM and (c) AFM images of C1 (d) cross-sectional analysis of C1 along the line shown in (c)	117
Figure 3.38	(a) SEM (b) AFM and (c) AFM cross-sectional analysis of C3 along the line shown in (b)	118
Figure 3.39	CD Titration of C4 with TBAH ₂ PO ₄	119
Figure 3.40	CD Titration of C5 with TBAH ₂ PO ₄	119
Figure 3.41	CD Titration of C6 with TBAH ₂ PO ₄	120
Figure 3.42	¹³ C NMR (DMSO- <i>d</i> ₆ , 75 MHz) spectrum of C1	132
Figure 3.43	HRMS of C1	132
Figure 3.44	¹ H NMR (CDCl ₃ , 300 MHz) spectrum of Z-Trp-Leu-OMe	133
Figure 3.45	¹³ C NMR (CDCl ₃ , 75 MHz) spectrum of Z-Trp-Leu-OMe	133
Figure 3.46	HRMS of Z-Trp-Leu-OMe	134
Figure 3.47	¹ H NMR (CD ₃ CN, 300 MHz) spectrum of C2	134
Figure 3.48	HRMS of C2	135
Figure 3.49	¹ H NMR (CDCl ₃ , 300 MHz) spectrum of Z-Leu-Trp-OMe	135
Figure 3.50	¹³ C NMR (CDCl ₃ , 75 MHz) spectrum of Z-Leu-Trp-OMe	136
Figure 3.51	HRMS of Z-Leu-Trp-OMe	136
Figure 3.52	¹ H NMR (CD ₃ CN, 300 MHz) spectrum of C3	137
Figure 3.53	¹³ C NMR (DMSO- <i>d</i> ₆ , 75 MHz) spectrum of C3	137
Figure 3.54	HRMS of C3	138

Figure 3.55	^1H NMR (CDCl_3 , 300 MHz) spectrum of C4	138
Figure 3.56	HRMS of C4	139
Figure 3.57	^1H NMR ($\text{DMSO}-d_6$, 300 MHz) spectrum of C5	139
Figure 3.58	^{13}C NMR ($\text{DMSO}-d_6$, 75 MHz) spectrum of C5	140
Figure 3.59	HRMS of C5	140
Figure 3.60	^1H NMR (CD_3CN , 300 MHz) spectrum of C6	141
Figure 3.61	^{13}C NMR (CDCl_3 , 75 MHz) spectrum of C6	141
Figure 4.1	Proline-inspired design of template for secondary structure nucleation	146
Figure 4.2	Synthesis and chemical structures of bispidine anchored peptides	147
Figure 4.3	The expanded amide NH region of the TOCSY (CDCl_3 , 300 MHz) spectrum of D3 in CDCl_3	150
Figure 4.4	Selected regions of ROESY (CDCl_3 , 300 MHz) spectrum of D3 . NOEs between Phe NH/Leu NH, Val NH/Ala NH, and Ala NH/Leu NH are marked in the spectrum	151
Figure 4.5	Selected regions of ROESY (CDCl_3 , 300 MHz) spectrum of D3 , ROEs between α CH/NH are marked in the spectrum	151
Figure 4.6	HSQC (CDCl_3 , 300 MHz) of D3	152
Figure 4.7	Chemical shift of NH protons versus % of DMSO added (v/v) to a solution of D3 in CDCl_3 for (a) Leu, Ala, Phe NHs, (b) Val NH	153
Figure 4.8	Observed NOEs in D3 are represented in double headed arrows, and H-bonds are represented by the dotted lines. Hydrogen bonding donor acceptors are identified from combined NMR and FTIR studies	153
Figure 4.9	The NH stratching region in the solution FT-IR	154

	spectrum of (a) D1 , (b) D2 , (c) D3 , (d) D6 and (e) D7 . Spectra presented are of 1mM solution of the compounds in chloroform after subtraction of the spectrum of pure chloroform	
Figure 4.10	Chemical shift of Leu NH versus % of DMSO added (v/v) to a solution of D1 in CDCl ₃	155
Figure 4.11	Chemical shift of NHs versus % of DMSO added (v/v) to a solution of D2 in CDCl ₃	155
Figure 4.12	Overlap of ten minimum energy structures of D3 obtained after performing MD simulation in (a) methanol (b) chloroform	157
Figure 4.13	CD spectra of (a) D1 , D2 (b) D3 (c) D5 (d) D6 (e) D7 (250-500 μM) in methanol. The molar ellipticity remained constant between 250-500 μM concentration range suggesting reduced aggregation effects	158
Figure 4.14	CD spectra showing little change in helicity of D3 ◇ in methanol, □ with 20% TFE and Δ with 40% TFE addition.	159
Figure 4.15	CD spectra of D4 ◇ in methanol and Δ after addition of 50% trifluoroethanol	160
Figure 4.16	CD spectra showing little change in helicity of D4 with temperature	160
Figure 4.17	(a) Changes in CD spectrum of D3 with addition of ◇ (0.0), □ (0.5), Δ (1.0), x (1.5), * (2) equivalents trifluoromethanesulfonic acid. (b) Changes in CD spectrum of D3 with addition of □ (0.0), x (1.0), * (2) equivalents methanesulfonic acid (MSA)	161
Figure 4.18	(a) HRMS of D3 recorded after the addition of 2 equivalents of trifluoromethanesulfonic acid for 2 h. (b) Protonation equilibrium of D3 and ¹ H NMR showing the	162

	protonated species after the addition of 1.5, 2 equivalents of MSA	
Figure 4.19	X-ray crystal structure of D3 (a) capped stick representation (b) backbone of D3 showing a right handed helical conformation. Peptide backbone is coloured in yellow. (c) intermolecularly H-bonded assembly in the solid state showing H-bonds between Val NHs and Phe COs	163
Figure 4.20	X-ray crystal structure of D3 (a) ribbon diagram based on X-ray structure and the chemical structure of D3 indicating hydrogen bonds (b) view along b axis (c) helical orientation of the peptide backbone, the hydrogen atoms and side chains were removed for clarity bispidine structure is colored in red and white (d) peptide backbone of D3 keeping the 1, 3 diaminopropane unit (-NH-CH ₂ -CH ₂ -CH ₂ -NH-), other part of bispidine structure is removed for clarity. (e) Peptide backbone of D3 without bispidine unit, the two peptide fragments adopt right handed helical conformation	165
Figure 4.21	HPLC chromatograms of D6 (a) Preparative HPLC after silica gel column chromatography (b) Analytical HPLC after purification by preparatory HPLC	167
Figure 4.22	¹ H NMR spectra of D6 in DMSO- <i>d</i> ₆ was taken before and after adding D ₂ O. Spectra were recorded at different intervals (a) D6 in DMSO- <i>d</i> ₆ , after addition of D ₂ O (b) after 10 min. (c) 40 min. (d) 80 min. (e) 133 min. (f) 156 min. (g) 193 min. (h) 343 min. (i) 403 min. (j) 17 h	168
Figure 4.23	TOCSY (DMSO- <i>d</i> ₆ , 300 MHz) spectrum of D6	168
Figure 4.24	The expanded amide NH region of the TOCSY spectrum of D6 in DMSO- <i>d</i> ₆	169
Figure 4.25	The expanded region of TOCSY spectrum of D6 in DMSO- <i>d</i> ₆	169

Figure 4.26	ROESY (DMSO- <i>d</i> ₆ , 300 MHz) spectrum of D6	170
Figure 4.27	ROESY (DMSO- <i>d</i> ₆ , 300 MHz) spectrum of D6 . NOEs between Phe NH/Val NH and NH/C ^α H are marked in the spectrum	170
Figure 4.28	¹ H NMR (CDCl ₃ , 300 MHz) of compound 2	191
Figure 4.29	¹³ C NMR (CDCl ₃ , 75 MHz) of compound 2	191
Figure 4.30	¹ H NMR (CDCl ₃ , 300 MHz) of compound D1	192
Figure 4.31	¹³ C NMR (CDCl ₃ , 75 MHz) of compound D1	192
Figure 4.32	¹ H NMR (DMSO- <i>d</i> ₆ , 300 MHz) of compound D2	193
Figure 4.33	¹³ C NMR (CDCl ₃ , 75 MHz) of compound D2	193
Figure 4.34	¹ H NMR (CDCl ₃ , 300 MHz) of compound Br-Gly-Ala-Val OMe	194
Figure 4.35	¹³ C NMR (CDCl ₃ , 75 MHz) of compound Br-Gly-Ala-Val OMe	194
Figure 4.36	¹ H NMR (CDCl ₃ , 300 MHz) of compound 3a	195
Figure 4.37	¹³ C NMR (CDCl ₃ , 75 MHz) of compound 3a	195
Figure 4.38	¹ H NMR (CDCl ₃ , 300 MHz) of compound D3	196
Figure 4.39	¹³ C NMR (CDCl ₃ , 75 MHz) of compound D3	196
Figure 4.40	HRMS of compound D3	197
Figure 4.41	¹ H NMR (CDCl ₃ , 300 MHz) of Br-CH₂-CO-NH-Val-Phe-OCH₃	197
Figure 4.42	¹ H NMR (CDCl ₃ , 300 MHz) of compound 3b	198
Figure 4.43	¹³ C NMR (CDCl ₃ , 75 MHz) of compound 3b	198
Figure 4.44	¹ H NMR (CDCl ₃ , 300 MHz) of compound D4	199
Figure 4.45	¹³ C NMR (CDCl ₃ , 75 MHz) of compound D4	199

Figure 4.46	HRMS spectrum of D4	200
Figure 4.47	¹ H NMR (CDCl ₃ , 300 MHz) of compound D5	200
Figure 4.48	HRMS of the compound D5	201
Figure 4.49	¹ H NMR (CDCl ₃ , 300 MHz) of Br-CH₂-CO-Leu-Val-OMe	201
Figure 4.50	HRMS of Br-CH₂-CO-Leu-Val-OMe	202
Figure 4.51	¹ H NMR (CDCl ₃ , 300 MHz) of compound 3c	202
Figure 4.52	¹³ C NMR (CDCl ₃ , 75 MHz) of compound 3c	203
Figure 4.53	HRMS of compound 3c	203
Figure 4.54	¹ H NMR (DMSO- <i>d</i> ₆ , 300 MHz) of compound D6	204
Figure 4.55	HRMS of compound D6	204
Figure 4.56	¹ H NMR (CDCl ₃ , 300 MHz) of compound 3d	205
Figure 4.57	¹³ C NMR (CDCl ₃ , 75 MHz) of compound 3d	205
Figure 4.58	HRMS of compound 3d	206
Figure 4.59	¹ H NMR (CDCl ₃ , 300 MHz) of compound D7	206
Figure 4.60	¹³ C NMR (CDCl ₃ , 75 MHz) of compound D7	207
Figure 4.61	HRMS of Compound D7	207
Figure 5.1	A general representation of self-assembly and topochemical polymerization of diacetylenes	214
Figure 5.2	(a) Design rationale for generating hydrophobic and hydrophilic surfaces (b) Structures of Lys-appended diacetylene monomers E1-E4 with different amino protecting groups and chemical structures of polydiacetylenes E5-E9	218
Figure 5.3	Raman spectra of (a) E3 and (b) E5	219

Figure 5.4	Raman spectra of (a) E4 and (b) E6	220
Figure 5.5	GPC analysis of E6 (a) showing the complete chromatogram (b) Expanded region of polymer peak	221
Figure 5.6	(a) AFM (b) SEM images of E3 SEM image of E4	222
Figure 5.7	(a) SEM image (b) TEM image and (c) AFM image for E5	222
Figure 5.8	(a) SEM image and (b) AFM image of E6	223
Figure 5.9	(a) SEM image (b) TEM image of E7 and (c) SEM image of E8	223
Figure 5.10	Photographs of water droplets on the polymers (a) E5 , (b) E6 , (c) E7	224
Figure 5.11	DPSC culture on polymers E5 , E6 , E7 , E8 and E9 . Fluorescence microscopy (a-g) after 4 h in culture followed by staining with FDA (green, vital staining) and PIES (red, dead cells). (a) polymer E5 , (b) polymer E6 , (c) polymer E7 , (d) polymer E8 , (e) polymer E9 , (f) glass cover-slip (negative control), (g) toxic control (DPSC rinsed with 4% formaldehyde). Scale bar = 100 μ m. (h) Graph showing the cell counts of DPSC attached on polymers E5 , E6 , E7 and E8 , after 4 h, 24 h, 48 h and 72 h of incubation (right side)	225
Figure 5.12	Cytoskeletal organization of DPSC on polymers E5 , E6 , E7 and E8 , after 24 h and 72 h of incubation (left and right pictures, respectively). Cells were stained with Hoechst (blue, nuclei staining) and phalloidin (red, actin cytoskeleton staining). (a) polymer E5 , (b) polymer E6 , (c) polymer E7 , (d) polymer E8 , (e) glass cover-slip control	227
Figure 5.13	Osteogenic differentiation assay: DPSC cultured on the	229

various polymers in osteogenic medium for 3 and 6 days (left and right pictures, respectively). A glass cover-slip was used as control. Cells were stained with Hoechst (blue, nuclei staining) and alkaline phosphatase antibodies (red, ALP staining). Background colors (green-yellow) are due to autofluorescence of polymers. (a) polymer **E5**, (b) polymer **E6**, (c) polymer **E7**, (d) polymer **E8**, (e) glass cover-slip. Scale bar = 30 μm

Figure 5.14	^1H NMR (CDCl_3 , 300 MHz) of compound E1	239
Figure 5.15	^{13}C NMR (CDCl_3 , 75 MHz) of compound E1	239
Figure 5.16	HRMS of compound E1	240
Figure 5.17	^1H NMR (CDCl_3 , 300 MHz) of compound E3	240
Figure 5.18	^{13}C NMR (CDCl_3 , 75 MHz) of compound E3	241
Figure 5.19	HRMS of compound E3	241
Figure 5.20	^1H NMR (CDCl_3 , 300 MHz) of compound E2	242
Figure 5.21	^{13}C NMR (CDCl_3 , 75 MHz) of compound E2	242
Figure 5.22	HRMS of compound E2	243
Figure 5.23	^1H NMR ($\text{DMSO}-d_6$, 300 MHz) of compound E4	243
Figure 5.24	^{13}C NMR ($\text{DMSO}-d_6$, 75 MHz) of compound E4	244
Figure 5.25	HRMS of E4	244
Figure 5.26	^1H NMR (CDCl_3 , 300 MHz) of compound E5	245
Figure 5.27	^1H NMR ($\text{DMSO}-d_6$, 300 MHz) of compound E6	245
Figure 5.28	^1H NMR ($\text{DMSO}-d_6$, 300 MHz) of compound E7	246
Figure 5.29	^1H NMR (CDCl_3 , 300 MHz) of compound E8	246

LIST OF TABLES

Table No.	Description	Page No.
Table 2.1	Changes in $\Delta\delta$ of the different protons of B3 with the addition of different anions	58
Table 2.2	Association constants for B3 with anions in CDCl_3	60
Table 2.3	Crystal data and structure refinement for B5	65
Table 2.4	Showing important H-bonds in the crystal structure of B5	66
Table 3.1	Different conformations and energy of C1 optimized at PBE/PBE/6-311G (d,p) level of DFT	98
Table 4.1	Table showing the ratio of major to minor conformers of D1-D5 in CDCl_3 based on ^1H NMR (CDCl_3 , 300 MHz) studies	149
Table 4.2	Possible H-bonding pattern and H-bond distances calculated from MD for D2	156
Table 4.3	Crystal data and structure refinement for D3	164
Table 4.4	Hydrogen bond parameters of D3 as observed in the X-ray structure	166
Table 4.5	Dihedral angles derived from X-ray crystal structure of D3	166
Table 5.1	Table describing the molecular weight distribution of the polymer	221
Table 5.2	Sessile drop water contact angles of films of polymers E5, E6, E7, E8 and E9	224

LIST OF SCHEMES

Scheme No.	Description	Page No.
Scheme 2.1	Synthesis of bis-urea molecules B1-B3	48
Scheme 2.2	Cyclization of bis-urea molecules B1-B3 to give imidazolidinediones B4-B6	64
Scheme 5.1	General scheme for the synthesis of protected polymers	217

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. ^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. 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 μ L aliquot of the sample solution was drop-casted on a glass coverslip, dried and coated with $\sim$ 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 μ L 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 $K\alpha$ = 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
$^{\circ}\text{C}$	Degree centigrade
AFM	Atomic force microscopy
ALP	Alkaline phosphatase
aq.	Aqueous
Ar	Aryl
ArH	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	Electron spray 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