Amphiphiles: Design, Syntheses, Studies on Their Biomolecular Interactions and Micellar Catalysis

THESIS

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by

VIKASH KUMAR

Under the Supervision of

Dr. Mainak Banerjee

and

Co-supervision of

Dr. Amrita Chatterjee

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CERTIFICATE

This is to certify that the thesis entitled **"Amphiphiles: Design, Syntheses, Studies on Their Biomolecular Interactions and Micellar Catalysis"** submitted by **Vikash Kumar,** ID No. 2011PHXF010G for award of Ph.D. Degree of the institute, embodies original work done by him under our supervision.

Mainak Banerjee

Signature in full of the Supervisor:

Place: BITS Goa

Name in capital block letters: **Dr. MAINAK BANERJEE**

Date: 27/6/17

Designation: Associate Professor

Signature in full of the Co-supervisor:

Place: BITS GOA

Name in capital block letters: **Dr. AMRITA CHATTERJEE**

Date: 27.06.2017

Designation: Associate Professor

Dedicated

To the memory of my father

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ABSTRACT

Title of the thesis: "**Tailor-Made Synthesis of Amphiphilic Molecules: Studies on their Morphoogical Features and Potential Applications**"

Amphiphiles having built-in hydrophilic and lipophilic properties and they can be classified in to different types e.g. conventional surfactants, gemini amphiphiles, bolaamaphiphiles etc. There are 7 chapters in this thesis, which are as follows.

Chapter I: *Introduction*

This chapter provides a general introduction on amphiphiles with an emphasis on their synthesis and applications.

Chapter II: *Synthesis, surface properties, DNA binding and cytotoxicity of* D*-Glucose based gemini surfactants*

This chapter describes synthesis of four new D-glucose derived *m-s-m* type quaternary ammonium gemini surfactants with variable spacer and tail length by a simple and efficient methodology utilizing the free C-3 hydroxy group of diisopropylidene glucose. Apart from showing good surface properties, these gemini surfactants showed low cytotoxicity by MTT assay on HeLa cell line. The DNA binding capabilities of these surfactants were determined by agarose gel electrophoresis, fluorescence titration, and DLS experiments. The preliminary studies by agarose gel electrophoresis indicated chain length dependent DNA binding

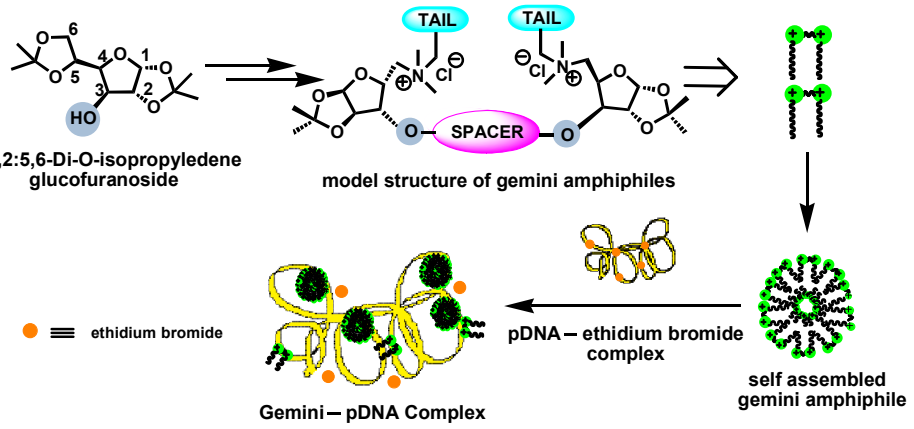
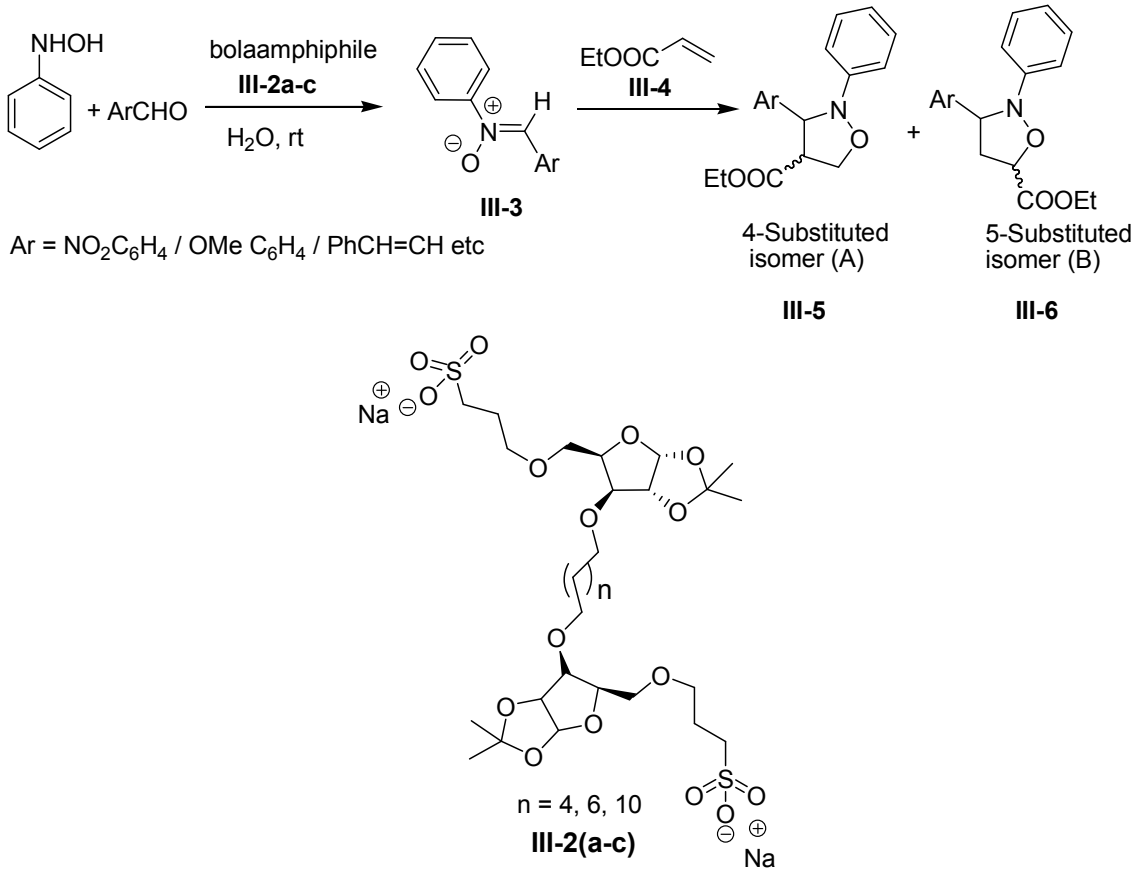


Fig. A. Graphical abstract for the synthesis of gemini surfactants and its applications

abilities, further supported by ethidium bromide exclusion experiments. Two of the D-glucose derived gemini surfactants showed effective binding with pET-28a plasmid DNA at relatively low N/P ratio.

Chapter III: *Synthesis and catalytic application of a novel class of D-glucose based bolaamphiphiles*

This chapter describes synthesis of three sugar based bolaamphiphiles from D-glucose. The resulting bolaamphiphiles showed unique aggregation behavior in aqueous solution. These sugar-based anionic bolaforms formed vesicles having diameter in nanometer range. After ageing the solution for 7 days tubular morphology has been seen as evidenced by TEM images. It was considered that the hydrophobic interior of the vesicles of bolaamphiphiles can form chiral nanoreactors in aqueous solution to carry out various reactions, which may show exceptional stereoselectivity. As a proof of concept, we have carried out dehydrative intramolecular [3+2] dipolar nitrone cycloaddition reactions in aqueous organized media of bolaamphiphiles to produce isoxazolidine diastereoisomers in highly stereoselective manner.



Scheme A. Bolaamphiphile catalyzed nitrone cycloaddition reaction

The bolaamphiphile **III-2c** with 12 carbon spacer, showed better diastereoselectivity for some cases and more interestingly with just 2 mol% loading. In addition, the reaction time is also less as compared with the reported SDS catalyzed reaction.

Chapter IV: *Synthesis and bio-medical applications of Tetraphenylethene (TPE)-based aggregation induced emission (AIE)-active fluorescent amphiphiles*

This chapter describes synthesis of three water soluble TPE derived cationic amphiphiles with variable glycol spacer. These amphiphiles have been prepared by a simple synthetic methodology from 4,4′-dihydroxybenzophenone. Their inherent AIE property was utilized to explore potential biotechnological applications. In this direction, apart from showing good BSA protein binding property, one of these amphiphiles with hexaethylene glycol spacer **(IV-6c)** showed good DNA binding efficiency, as evident by agarose gel electrophoresis and fluorescence titration. All TPE-amphiphiles were also showed comparatively low cytotoxicity while carrying out MTT assay on HeLa cell line.

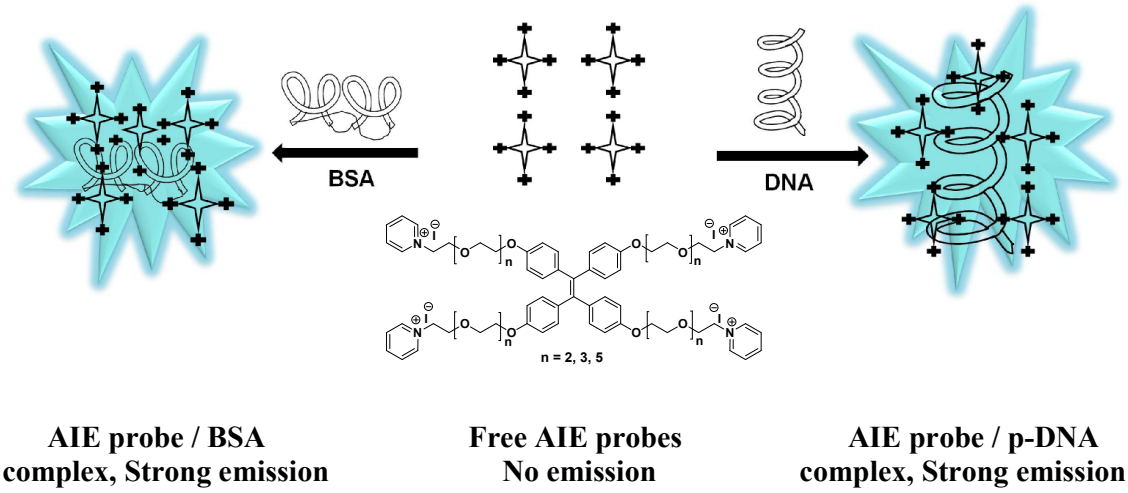


Fig. B. Graphical abstract for sensing mechanism for BSA and DNA

Chapter V: *Development of efficient and "green" synthetic methodology for 2-substituted benzimidazoles and benzothiazoles in aqueous micellar media*

This chapter describes development of an efficient synthetic method for chemoselective synthesis of 2-substituted benzimidazoles over 1,2-disubtituted benzimidazoles and benzothiazoles in organized aqueous media in the presence of a surfactant (viz. DBSA) as catalyst and I_2 as co-catalyst. The method described has the advantages of operational simplicity, excellent yields, high chemoselectivity, and clean and green reaction profile.

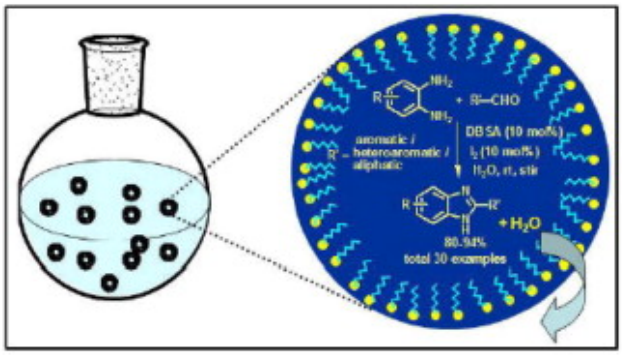


Fig. C. Graphical abstract for dehydrative synthesis of 2-substituted benzimidazole in aqueous micellar media.

Chapter VI: *Development of a mild and "green" synthetic methodology for 3-vinylchromones in aqueous micellar media*

This chapter describes development of a simple, mild, and eco-friendly method for the synthesis of 3-vinylchromones from 4-oxo-4H-1-benzopyran-3-carboxaldehyde (3-formylchromone) by simple Knoevenagel condensation with various active methylene compounds (AMC) in aqueous micellar media in the presence of catalytic amounts of cetyl trimethylammonium bromide (CTAB) and 1,4-diazabicyclo[2.2.2]octane (DABCO). In the case of malonic acid as AMC, the reaction resulted in formation of only Doebner decarboxylated products under the standard reaction condition. It has been also observed that 3-formylchromone derivatives primarily undergo tandem Knoevenagel and Michael reactions in the presence of >2 equiv. of ethyl acetoacetate to produce benzophenone derivatives, by opening of pyran ring, as the sole product in good yield.

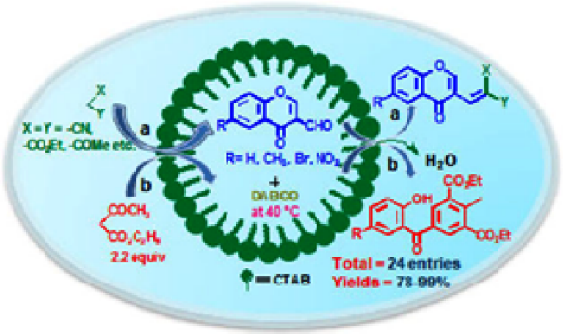


Fig. D. Graphical abstract for the vinylation of 3-formylchromone and effect of excess of ethylacetoacetate

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List of Abbreviations

Abbreviation	Description
AIE	Aggregation- induced emission
APCI-MS	Atmospheric-pressure chemical ionization mass spectrometry
δ	Chemical shift
%	Percentage
°	Degrees
λ	Wavelength
μ	micron
μM	micromolar
BSA	Bovine serum albumin
br	broad
Calcd.	Calculated
CMC	Critical Micelle Concentration
conc	concentrated
CTAB	Cetyl trimethylammonium bromide
^{13}C NMR	Carbon-13 nuclear magnetic resonance
d	Doublet
dil	Dilute
dd	Doublet of doublet
ddd	Doublet of doublets of doublets
DMF	N,N′-Dimethylformamide
DMSO	Dimethyl sulfoxide
dq	Doublet of quartets
dt	Doublet of triplets
equiv.	Equivalent
Et_2O	Diethyl ether
ESI-MS	Electrospray ionization mass spectrometry
ESI-Q-TOF MS	Electrospray ionization quadrupole time-of-flight mass spectrometry

g	Gram
^{1}H NMR	Proton nuclear magnetic resonance
HRMS	High Resolution Mass Spectrometry
IR	Infrared
J	Coupling constant
MALDI	Matrix-assisted laser desorption / ionization
m	Multiplet
mg	Milligram
mL	Millilitre
MLMs	Monolayer lipid membranes
mmol	Millimole
MHz	Megahertz
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
ng	Nanogram
NMR	Nuclear magnetic resonance
ppm	Parts per million
pDNA	Plasmid DNA
q	Quartet
R^2	Correlation coefficient
rt	Room temperature
s	Singlet
SDS	Sodium dodecyl sulphate
t	Triplet
TPE	Tetraphenylethylene
TEM	Transmission electron microscopy
THF	Tetrahydrofuran
TLC	Thin layer chromatography
UV visible	Ultraviolet visible
vs	Versus

CHAPTER I

Introduction

Molecular self-assembly is the association of molecules or ordered nanostructure/organised assembly without navigation from an external source.[1] It is prevalent in nature in the form of lipid bilayer, polymeric nucleic acids, quaternary structures of protein, colloids etc.[2] It offers unique directions for the fabrication of novel supramolecular structures and advanced materials. The noncovalent interactions that lead to the formation of molecular self-assembly include hydrogen bonds, van der Waals forces, hydrophobic interactions, electrostatic forces, dipole-dipole interactions, π-π-stacking etc. It has recently emerged as a new approach in chemical synthesis, nanotechnology, polymer science, materials and engineering. It creates a variety of materials at the molecular level. The nanomaterials formed using molecular self-assembly have been applied to study some complex and previously intractable biological phenomena. Molecular self-assembly is likely to play an increasingly important role to produce potentially useful functional materials for nano/bio-technology, nanomedicine etc. at present and in the future .[1-3]

Amphiphile is a term describing a chemical compound possessing both hydrophilic and lipophilic characteristics, and capable of self-assembling into various nanostructures. The cell membrane is an ideal example of molecular self-assembly of phospholipid based amphiphilic molecules. In cell membrane, the amphiphiles arrange themselves into bilayers, by positioning their polar groups towards the surrounding aqueous medium, and their lipophilic chains towards inside of the bilayer, defining a non-polar region between two polar ones. Mimicking this biological phenomenon a variety of artificial amphiphilic molecules have been synthesized which can self-assemble in solutions, at interfaces and in bulk, generating various nanoscale morphologies. The ability to generate desired nanoscale morphologies by synthesizing novel amphiphiles allows the amphiphilic systems to be tailored for specific applications.[4-6] For example, self-assembled amphiphiles can entrap various anticancer drugs in simple methods such as emulsion, nanoprecipitation and osmotic gradient method, and can perform specific delivery to the tumor cells.[7]

Amphiphiles (or surfactants) can be classified into various classes based on their structural features. Based on the charge on their polar head groups they can be classified as cationic,

anionic, non-ionic, catanionic surfactant (equal mixture of opposite charged surfactant), zwitterionic and amphoteric (where head group charge changes with the pH). On the other hand, surfactants can also be classified on the basis of number and type of linkage between polar heads and hydrophobic tails such as conventional surfactants, bolaamphiphiles, gemini amphiphiles, double chain or triple chain surfactants etc. The present document intend to follow this classification. Conventional amphiphiles comprise of a polar head and a hydrophobic tail. Whereas, bolaamphiphiles are made up of two polar heads joined by a hydrophobic spacer that may be a flexible alkyl chain or a rigid aromatic spacer and gemini amphiphile consist of two hydrophobic tails and two polar heads which are tethered by a spacer. Keeping in mind about the nature and its resources, during last decade, emphasis is given on the use of natural resources as starting materials for synthesizing these amphiphiles e.g. sugars, amino acids, fatty acids etc. The key advantages of using these resources are their bio-compatibility and ease of bio-degradation.[8-9]

In addition, our interest revolves around a special class of amphiphiles i.e. tetraphenylethylene (TPE) based amphiphiles which show fluorescence by aggregation induced emission (AIE). Because of this unique property such amphiphiles can emit light at solid state or in aggregated form, which make them attractive candidates in biotechnology, in particular, in image-guided delivery applications.

A huge literature is available in each of the various categories of amphiphiles discussed above, which is out of scope to cover in full details in this short review.

The following section will briefly discuss about the recent developments in (a) bolaamphiphiles, (b) gemini amphiphile, (c) TPE-based amphiphiles with an emphasis on their natural resources, aggregation behaviour and applications which are pertinent to this Ph.D. thesis. In addition, various dehydration reactions catalyzed by surfactants will be reviewed.

I. 1. Bolaamphiphiles

In recent years, bolaamphiphiles[10] have progressively gained importance because of their abilities to provide original supramolecular structures and advanced biomaterials.[11] Bolaamphiphiles (two-headed amphiphiles) are named after “bola”, which is a South American weapon made of two balls connected by a string. Bolaamphiphiles or bolaforms

consist of two polar headgroups connected to each other by one hydrophobic spacer.[12] A simple bolaamphiphile is represented in Fig. I-1.

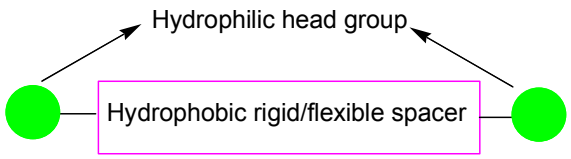


Fig. I-1. The common structure of a bolaamphiphile.

These amphiphiles can self-assemble into monolayer lipid membranes (MLMs), resembling the unusual architecture of natural archaeal macrocyclic bolaamphiphilic lipids.[13] As like conventional amphiphiles, the chemical functionality of the headgroups and spacer can be varied to change the aggregation properties. The structural differences, as compared to conventional amphiphiles, can result in different aggregate morphologies. For example, they can form a "bilayer" that is one molecule thick; i.e. bolaamphiphiles can form monolayer membranes instead of bilayer membranes. Kunitake *et al.* first reported the formation of aqueous monomolecular membranes from such bolaforms.[14] Since then, a number of research groups have reported the synthesis and self-assembly of bolaamphiphiles with different headgroups.[10,15]

As compared to the single-headed amphiphiles, the introduction of a second headgroup generally induces higher solubility in water.[16] Again depending on the length and flexibility of the linker, some bolaamphiphiles can fold in half and form micelles.[17] Such bipolar lipids offer several advantages for the construction of advanced liposomes that have characteristics of high mechanical and thermal stabilities due to the organization of the membrane.[18] The aggregation morphologies of bolaamphiphiles are as variable as their molecular structures.[19] Besides vesicles[11a,20] and lamellae,[21] disks,[22] rods, tubules,[23] ribbons, fibers[24] etc. in the nano- and micro-meter range are observed. Some chiral bolaamphiphiles derived from biomolecules even form chiral superstructures such as helices.[25] The structural diversity of the molecules, which are capable of self-assembly in solution, turns out to be manifold. Often compounds with structural elements of biological model are synthesized to take advantage of their intermolecular interactions. Specific examples of such residues are nucleosides,[26] sugars,[27] amino acids[28] or peptides[29] which can form strong hydrogen bonds leading to intermolecular aggregation.

The bipolar structure of the bolaforms have generated immense interest among researchers. A large variety of bolaamphiphiles (cationic, anionic, nonionic, zwitterionic) of different structures have been reported, so far. It was reported that bolaforms have weaker surface property but stronger self-aggregation property which is distinguised by high surface tension but low critical micellar concentration (CMC) than their traditional equivalents with the same hydrocarbon/head group ratio.[30] Since bolaamphiphiles are difficult to extract from natural membranes, scientists switched over to chemically synthesise bolaamphiphiles in order to mimic natural bolaforms. In the last two decades, scientists have focused on bolaamphiphiles from renewable resources to reduce their impacts on the environment.[31] Among various natural resources for the synthesis of bolaamphiphiles including carbohydrates (i.e. sugars), amino acids, fatty acids, sugar-based surfactants have gained increasing attention not only for their low toxicity but also for good biocompatibility and fast biodegradation. Their application as drug/gene delivery vehicles has also been explored quite extensively.[32]

After Kunitake's first report in early 1980's, the field of "bolaamphiphiles" has steadily progreesed mainly aided by Fuhrhop and his team.[10a,b] The early developments in this area is captured in an excellent review published by him and co-authors in 2004.[10b] However, the main intention of this review is to focus on the recent developments in the synthesis of bolaamphiphiles using natural resources and their applications. The following section is divided into two parts. The first part will discuss about the use of natural resources other than "sugars" for bolaamphiphiles with selective examples. Next to this, in a separate section, recent developments in "sugar-based" bolaamphiphiles, their aggregation morphologies and key applications are reviewed. All throughout emphasis will be given on the key developments in early of this century to till date.

I.1.1. Bolaamphiphiles from natural resources (other than sugars)

In recent years, natural resources like amino acids, fatty acids, nucleotides etc. have been used by number of research groups in the synthesis of bolaamphiphiles with a focus on their intriguing applications. In majority of the cases the bolaforms are having identical head groups joined by a linker. However, several of them are having two different head groups at the termini and they are categorised as unsymmetrical bolaamphiphiles.

Shimizu and co-workers have explored bolaamphiphiles with a variety of headgroups.[33] The amino acid derivatives (**I-1**, Fig. I-2) with terminal carboxylic acids show interesting

variation in aggregation with changing pH. Franceschi *et al*. reported another family of amino acid bolaform (**I-2,** Fig. I-2).[17] Study showed that the aggregation behaviour depends on the length of the linker, e.g. the molecules with shorter linkers showed a concentration-dependent transition from vesicles to fibers, whereas, linker with 20 methylene units forms gel. Frkanec *et al.* reported the aggregation behaviour of amino acid based bolaamphiphiles (**I-3** and **I-4**, Fig. I-2) by photo-isomerization of the double bond.[34] In another work Maity *et al.* described peptide bolaamphiphiles (**I-5**, Fig. I-2) with both tyrosine and tryptophan residues in the skeleton which forms hydrogels and further self-assemble into nanofibriller structure.[35] These nanofibers were used for stabilization of Pd nanoparticles which was subsequently used as an efficient catalyst for the deprotection of amino acids or peptides from *N*-terminal.

On the other hand, Sistach *et al.* synthesized anionic bolaaphiphile with L-alanine head group and varying linkers (**I-6**, Fig. I-2) and demonstrated its use as stabilizer of gold nanoparticles (NPs) at well below its CMC to avoid any micelle or vesicle formation in the solution.[36]

Apart from amino acids, fatty acids were also used as starting material for synthesis of bolaamphiphiles. Ewonkem *et al.* synthesized symmetric (**I-7**, Fig. I-2) and asymmetric cationic bolaamphiphiles from castor oil (a natural resource) that are having acetyl choline head groups which self-aggregate into nanovesicles and can act as potential drug delivery vehicle for water soluble drugs.[37] Tian *et al.* reported the thermodynamics of novel unilamellar vesicles from vitamin-derived zwitterionic 1,12-diaminododecanediorotate based bolaamphiphile (DDO) (**I-8**, Fig. I-2) in the presence of endogenous sodium cholate, a bile salt.[38]

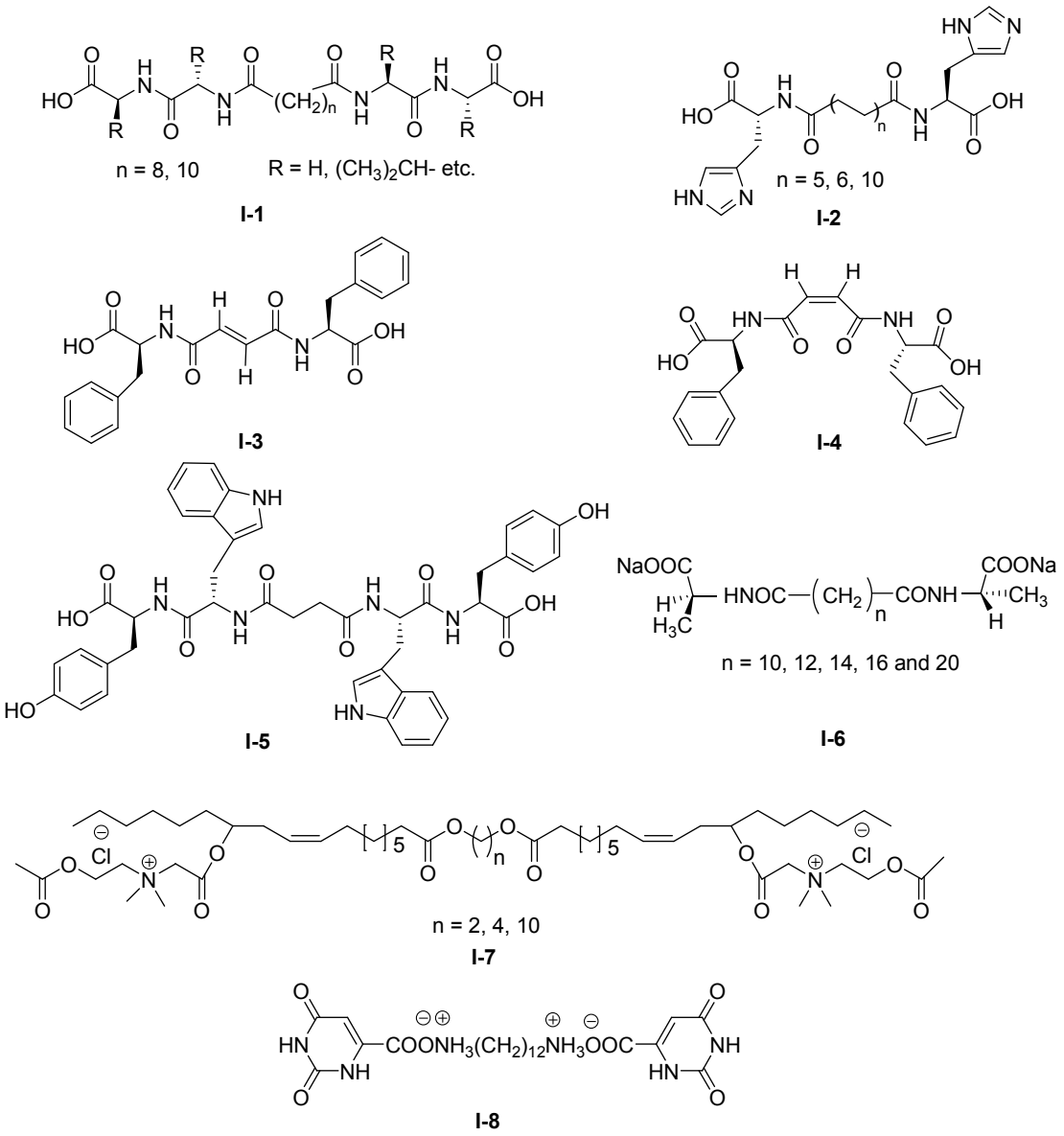


Fig. I-2. Representative examples of symmetrical bolaamphiphiles developed from various natural resources (other than sugars).

There are several examples of unsymmetrical bolaamphiphiles as well. Shimizu *et al.* reported that the hydrogen bonding of the end groups control the molecular organizations of the unsymmetrical nucleobase bolaamphiphiles (**I-9**, Fig. I-3).[24] These simple bolaamphiphiles can be precipitated as crystalline fibres. Maity *et al.* also described a fluorescent hydrogel formed by unsymmetrical peptide-based bolaamphiphiles (**I-10**, Fig. I-3), which self-assembles and undergoes a morphological transformation from nanovesicle to nanofibre.[39] The bolaform was formed in a single step using phenylalanine and tryptophan as

the starting materials. The hydrogel was formed by sonication and the transition from vesicle to fibre occurs because of stable ß-sheet type arrangement inside the nanofibres. It was studied that this peptide bolamaphiphile shows dose-dependent cytotoxicity and cell proliferation behaviour in MTT assay. Song *et al.* have studied the aggregation behaviour of unsymmetrical bolaamphiphiles (**I-11**, Fig. I-3) with a polymerizable linker depending on pH.[28] Increase in the pH of the solution caused fraying of helical ribbons into nanofibers accompanied by a sharp blue-to-red chromatic transition.

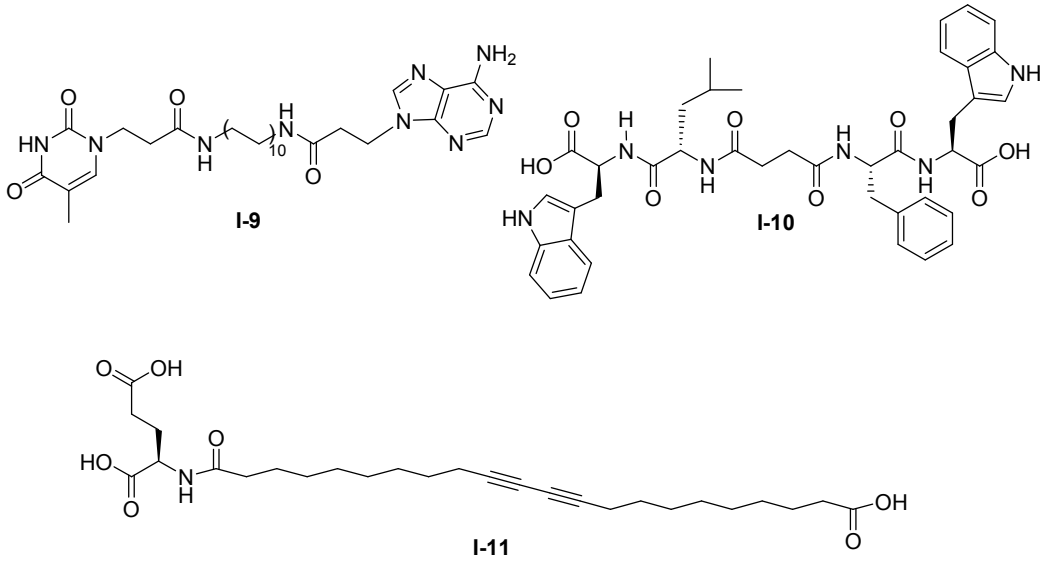


Fig. I-3. Representative examples of unsymmetrical bolaamphiphiles developed from various natural resources (other than sugars).

I.1.2. Sugar Based Bolaamphiphiles

Most of the sugar-based bolaamphiphiles available in literature are either nonionic or cationic but only one example of sugar-based anionic bolaamphiphile is reported. To the best of our knowledge sugar based zwitterionic bolaamphiphile has not been reported till date. In the following section these bolaamphiphiles are discussed as per their charge on the head groups.

I.1.2.1. Nonionic bolaamphiphiles

There are several examples of nonionic bolaamphiphiles which were synthesized and studied for their different applications using sugar as natural resource like D-glucose, D-xylose etc.

Shimizu and coworkers described the synthesis and self-aggregation property of 1-glucosamide **I-12** and 2-glucosamide bolaamphiphiles **I-13** (Table I-1, entry no. 1).[25] They reported that helical twisted fibres are formed when *n* (number of hydrocarbon linker) is even and platelets or amorphous solids are formed when *n* is odd, which exhibit the even-odd effect of connecting bridge.

Shimizu and co-workers also reported that the hydrogen bonding of the end groups control the molecular organizations of the nucleotide-appended bolaamphiphiles, **I-14** (Table I-1, entry no. 2).[40] They form gels because of the presence of solubilising deoxyribose and phosphodiester groups.

Jung *et al.* synthesized asymmetric sugar-based bolaamphiphile hydrogelators, **I-15**–**I-17** (Table I-1, entry no. 3).[41] Their gelation ability was examined using several analytical techniques including TEM. Their gelling ability is studied in organic solvents and water in the presence and absence of alkyl diammonium ions (guest molecules). Crown ether based hydrogel, **I-15** formed a helical fibre like structure in the presence and absence of guest molecules. Hydrogel stabilises due to the host-guest interaction and its synergistic effect by H-bonds.

Recently, Xu *et al.* described the synthesis of nonsymmetric bolaamphiphile composed of glucose head groups, alkyl side-chain and steroidal unit linked together by varying lengths of methylene spacer called as glycosteroidal bolaamphiphile, **I-18** (Table I-1, entry no. 4).[42] Side chain length dependent shape change was observed as a function of temperature with these kinds of bolaamphiphiles. Lamellar and columnar phases were observed by change in side chain length and temperature also.

In 2011, Jin *et al.* reported the synthesis of nonionic asymmetric bolaamphiphilic Zidovudine/Didanosine prodrug, zidovudine-phosphoryl-deoxycholyl didanosine (ZPDD), **I-19** using ribose sugar (Table I-1, entry no. 5).[43] **I-19** was useful as combination anti-HIV therapy for AIDS patient. It can deliver simultaneously two types of drug zidovudine (AZT) and didanosine (ddI) to targeted tissue. To synthesise this prodrug, deoxycholic acid was transformed into dehydrodeoxycholic acid before being used in coupling reaction. The prodrug was self-aggregated into vesicles and their stability was dependent on pH.

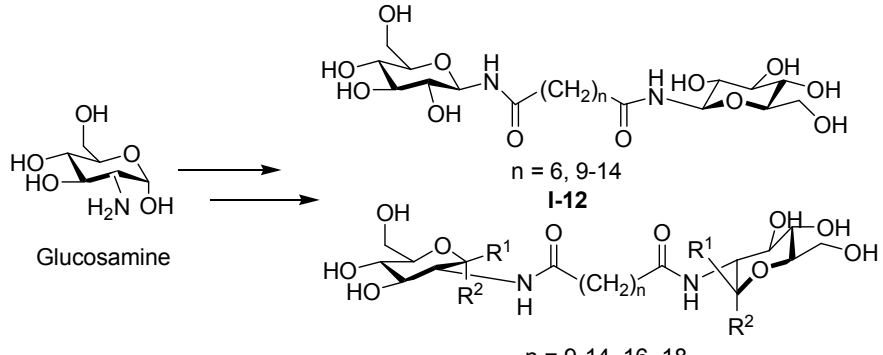
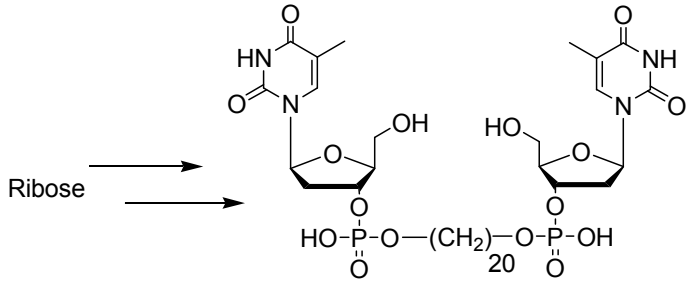
Lakhrissi *et al.* described an efficient synthesis of non-ionic, bis-galactobenzimidazolone based bolaamphiphiles, **I-20** using galactopyranose derivative (Table I-1, entry no. 6).[44] These bolaamphiphiles have fluorescence emission property that can be used for sensing of the cations like Cu^{+2}, which can form complex with the benzimidazolone moiety leading to quenching of fluorescence.

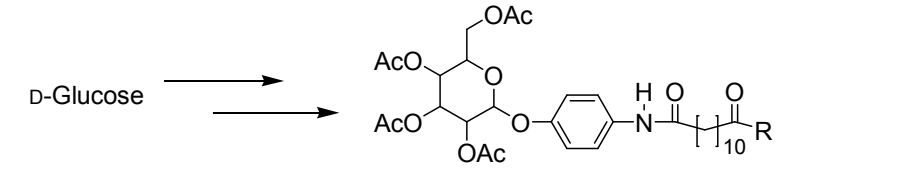
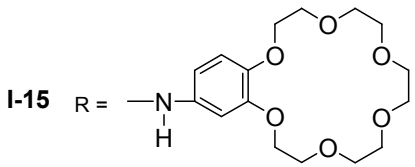
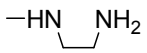
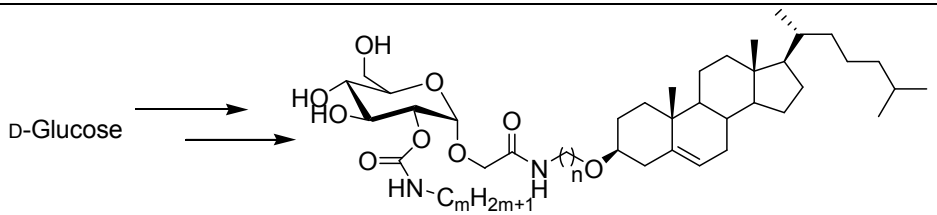
In 2012, Deleu *et al.* synthesized D-xylose based bolaamphiphile, **I-21** and studied its interfacial and self-assembling behaviour based on the presence or absence of unsaturation in its hydrophobic spacer (Table I-1, entry no. 7).[45] The presence of double bond does not affect the interfacial property at low compression but affects the monolayer stability at higher compression. They also pointed out that compound having saturated spacer could produce a better drug delivery formulation because of stable film formation apart from some side effect.

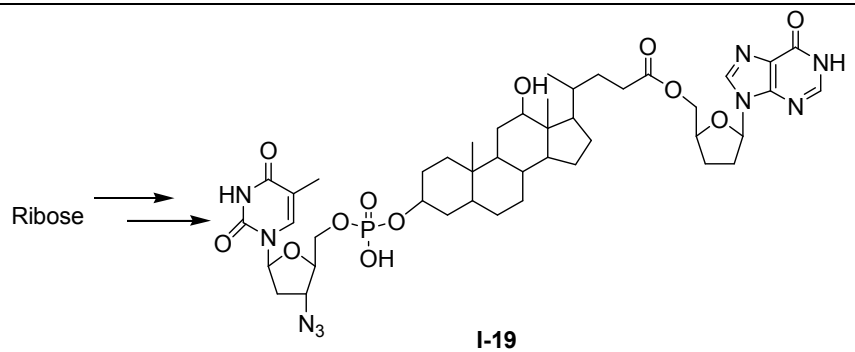
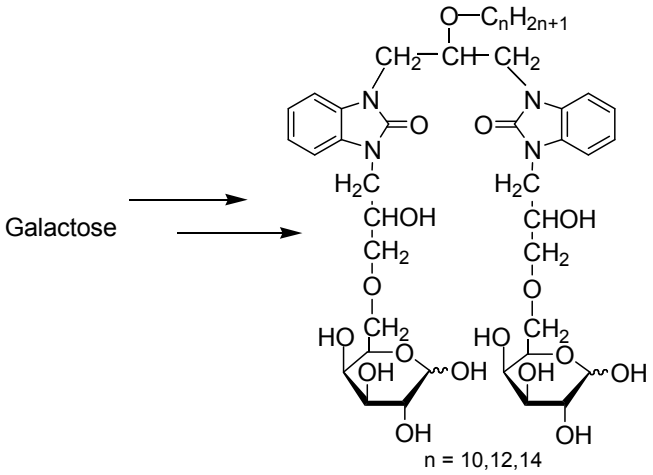
Recently, Zeng *et al.* synthesized few gluconolactone-modified polysiloxane based bolaamphiphiles, **I-22** and studied their micelle formation ability in solution and also studied that the polysiloxane moiety is responsible for their reduced surface tension (Table I-1, entry no. 8).[46]

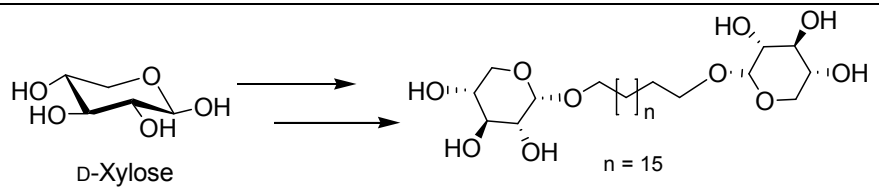
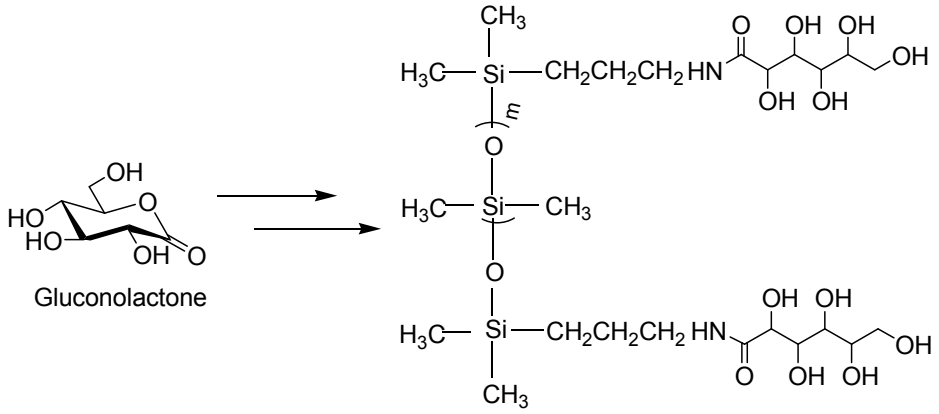
Very recently, Schmid *et al.* reported the synthesis of mannose functionalized oligothiophene (quarterthiophene) based symmetric enantiomeric bolaamphiphiles, **I-23**, **I-24** (Table I-1, entries no. 9 and 10).[47] The D-(+) and L-(-)-mannosidic enantiomers self-assemble into chiral aggregates that can be used for the loading of anti-cancer drug doxorubicin. The subsequent release of the drug from the complex is pH sensitive as observed in A 549 cancer cell line.

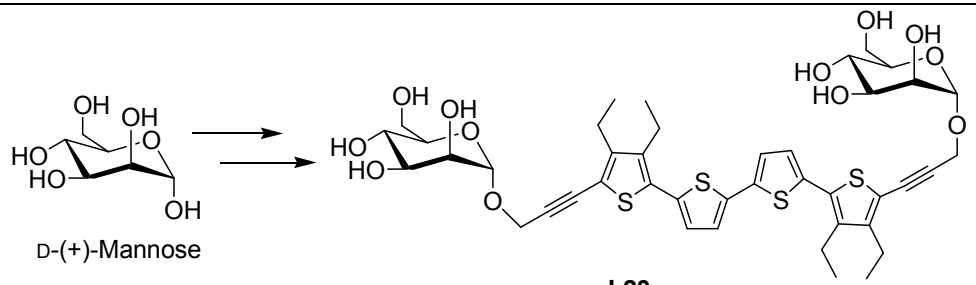
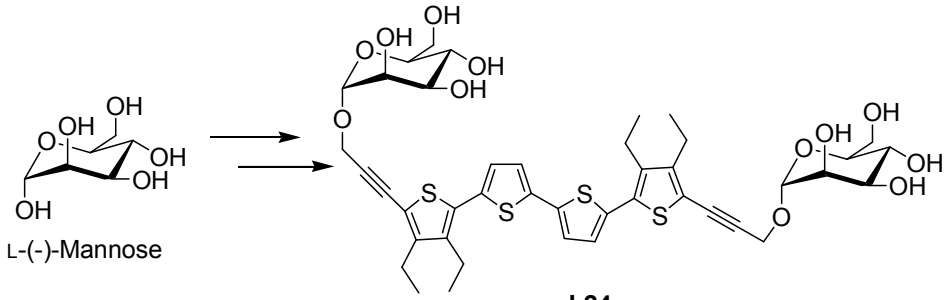
Table I-1: Nonionic bolaamphiphiles

Sl. No.	Bolaamphiphiles	Key study/Application	Ref.
1		self-aggregation behaviour (formation of helical twisted fibres, platelets etc.)	25
2		self-organizing behaviour (formation of DNA like nanofibres)	40

Sl. No.	Bolaamphiphiles	Key study/Application	Ref.
3	 **I-15, 16, 17**  **I-15** R = **I-16** R =  **I-17** R = —OH	self-aggregation behaviour (formation of hydrogel)	41
4	 n = 4, 6, 8, 10, 12 m = 4, 6, 8, 10, 12, 14, 16, 18 **I-18**	*self-organizing behaviour (formation of lamellar and columnar phases)*	42

Sl. No.	Bolaamphiphiles	Key study/Application	Ref.
5	 **I-19**	used in the formulation of anti-HIV combination therapy	43
6	 n = 10,12,14 **I-20**	Cu^{+2} ion sensing through its imidazole group	44

Sl. No.	Bolaamphiphiles	Key study/Application	Ref.
7	 **I-21**	Interfacial and membrane properties	45
8	 **I-22**	self-organizing behaviour (formation of spherical micelles)	46

Sl. No.	Bolaamphiphiles	Key study/Application	Ref.
9	 D-(+)-Mannose **I-23**	anti-cancer drug (doxorubicin) delivery	47
10	 L-(-)-Mannose **I-24**	anti-cancer drug (doxorubicin) delivery	47

I.1.2.2. Cationic Bolaamphiphiles

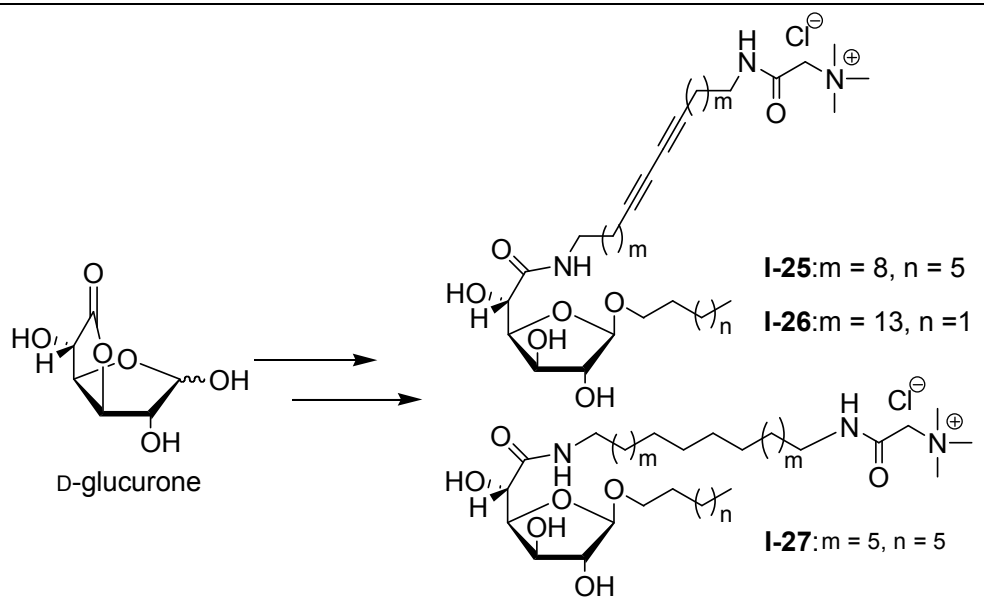
There are relatively less examples for catioinic bolaamphiphiles which were synthesized using sugar as natural resource and their potential applications have been explored.

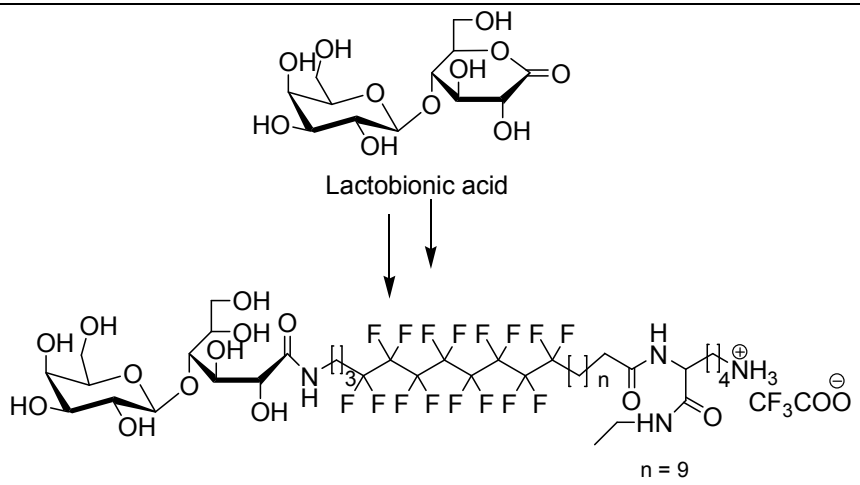
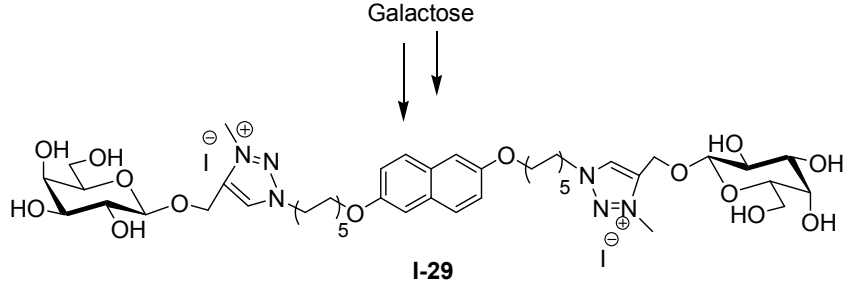
In 2008, Berchel *et al.* described the synthesis of unsymmetrical diacetylenic or saturated cationic bolaamphiphiles, **I-25**–**I-27** based on sugar and cationic glycine betaine moiety (Table I-2, entry no. 1).[48] These bolaamphiphiles were obtained through a sequential introduction of the D-glucurone headgroup and cationic glycine betaine moiety. It was reported that diacetylenic functionality and alkyl chain length affects its self-assembly behaviour in water causing polymorphism (multilamellar vesicles, tubule-like cylinder, dense network of filaments etc.).

Brunelle *et al*. synthesized and demonstrated the DNA complexation and non-viral gene transfection efficacy of a hemifluorinated disymmmetric sugar-based cationic bolaamphiphile **I-28** using lactobionic acid, a sugar acid composed of galactose and gluconic acid (Table I-2, entry no. 2).[49] Two different amino acids lysine and histidine were used as polar head groups and their effect on DNA complexation was studied. Bolaamphiphile having lysine head group and small fluorinated segment close to carbohydrate part showed good transfection efficiency.

In 2013, Yu *et al.* designed and synthesized substituted naphthalene based bolaamphiphile, **I-29** having galactose as hydrophilic part, which self-assembles into spherical structures due to hydrophobic and π–π interactions (Table I-2, entry no. 3).[50] Later on single walled carbon nanotubes (SWNTs) based biohybrid material was made using this bolaamphiphile and the agglutination property of the aggregates was compared against *E. Coli.*, which was more effective due to rich galactose surfaces of the SWNTs as compare to simple bolaamphiphile.

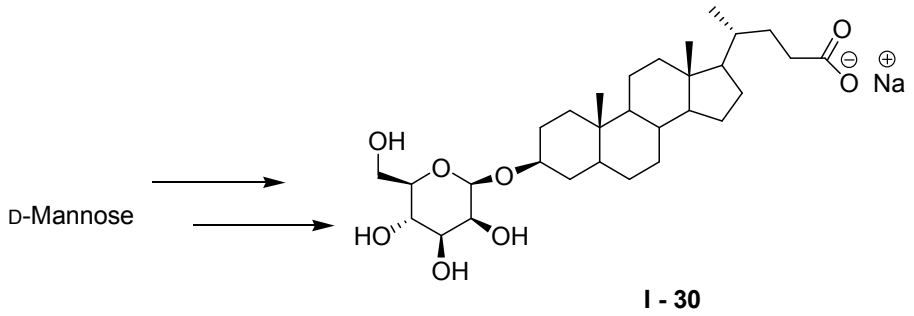
Table I-2: Cationic bolaamphiphiles

Sl. No.	Bolaamphiphiles	Key study/Application	Ref.
1		self-organizing behaviour (formation of multilamellar vesicles for **I-25**, **I-26** and tubule like structure for **I-27)**	48

Sl. No.	Bolaamphiphiles	Key study/Application	Ref.
2	 **I-28**	DNA complexation and gene transfection involving amino acid containing polar head groups	49
3	 **I-29**	*E. Coli* agglutination	50

I.1.2.3. Anionic Bolaamphiphile

To the best of our knowledge, only one sugar based anionic bolaamphiphile has been reported in literature till date. Gubitosi *et al.* have reported the sugar and bile acid based anionic asymmetric bolaamphiphile with rigid spacer, **I-30**. The bolaamphiphile (**I-30**) was synthesized by introduction of mannose residue at C-3 position of lithocholic acid (Scheme I-1).[51] 1,2,3,4,6-penta-*O*-acetyl-D-mannopyranoside was used as a sugar substrate as the starting material. **I-30** self-assembles into tubular scrolls in water which further transforms into single walled tubules in due course of time. They proposed that such system has potential application in supramolecular channel preparation.



I - 30

Scheme I-1

I.2. Gemini amphiphiles

The term gemini surfactant was coined by Menger and Littau in 1991 describing a novel type of surfactants that are formed by connecting two identical monomeric conventional surfactants through a spacer at the level of the head groups.[52] A simple gemini surfactant is represented in Fig. I-4.

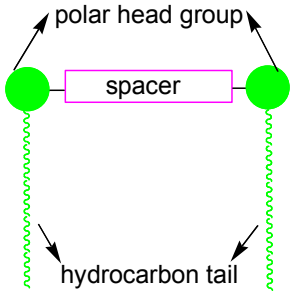


Fig. I-4. A schematic representation of a gemini surfactant.

Several other names have been given for this class of surfactants, such as dimeric surfactants[53] and siamese surfactant[54] depending on the type of their connections. The structural feature of these surfactants have opened a new field of research within surface and colloid chemistry because of their unique properties when dispersed in water. As far as the unique behaviour is concerned, geminis can be few orders of magnitude more surface active than conventional surfactants[55] and generally CMC is higher for longer chain than for shorter chain; just the opposite of the normal surfactants.[52b]

In 1991, Menger *et al.* assigned the word "gemini" to the bis-surfactant in which the spacer is rigid (e.g. a benzene or stilbene system). The idea was to examine how the separation of the two hydrocarbon chains within a surfactant molecule would change its properties. Later others extended this definition of gemini to include all such double-surfactants regardless of whether the spacers were rigid or not. Special mention should be given to Bunton *et al.*, who studied the catalysis of nucleophilic substitutions by "dicataionic detergents",[56] to Devinsky *et al.*, who reported the surface activity and micelle formation of some new "bisquaternary ammonium salts",[57] and to Okahara *et al.*, who synthesized and examined "amphipathic compounds with two sulfate groups and two lipophilic alkyl chains".[58] Since gemini surfactants have been shown to possess a variety of unique properties,[59] these compounds get worldwide attention. Geminis have shown promise in various commercial products including skin care,[60] antibacterial regiments,[61] construction of high porosity materials,[62] analytical

separations,[63] solubilization processes,[64] antipollution protocols,[65] in catalysis,[66] nanotemplating agents,[67] industrial detergency[68] etc.

As mentioned before all geminis possess at least two hydrophobic chains and two ionic or polar hydrophilic groups, and a variation exists in the nature of spacers.[69] Short or long methylene groups, rigid (stilbene), polar (polyether), and nonpolar (aliphatic, aromatic) groups are used as common spacers. The ionic group can be positive (ammonium) or negative (phosphate, sulphate, carboxylate), whereas, the polar nonionics may be polyether or sugar. The majority of geminis have symmetrical structures with two identical polar heads and two identical chains. A gemini with two C_m (*m* is the number of alkyl carbon atoms) tails and a C_s (*s* is the number of alkyl carbon atoms) spacer separating the quaternary nitrogen atoms can be represented as *m*-*s*-*m*. A sugar moiety can also be present as a spacer in the molecule.

They have unique properties as compared to their corresponding monomers such as high surface activity, unique aggregate morphology, low Krafft temperature, unusual rheological properties, better wetting ability etc.[70,71] The morphology of the gemini in polar solvent may vary depending on the structure of the molecule and its orientation. The long hydrocarbon chain tends to increase the surface activity. It is seen that increasing hydrophobicity makes the molecule insoluble, whereas, increasing hydrophilicity of the head groups may facilitate solubility in water. Hydrophilic groups in the spacer also increase the aqueous solubility. Increase in the number of carbons in the nonpolar chain increases both lipophilicity and surface activity with decrease in CMC.[72]

The field of "gemini amphiphiles" was started by Menger and his team. The early developments in this area is mentioned in an excellent review published by him & co-authors in 2000.[59] Moulik and co-workers also published a similar review on gemini amphiphiles in 2002 explaining its different properties.[73] However, the main intention of our review is to focus on the recent developments in the synthesis of gemini amphiphiles using natural resources and their applications. As like bolaamphiphils, the following section is divided into two parts. The first part will discuss about the use of natural resources other than "sugars" for gemini amphiphiles with selective examples. Next to this, in a separate section, recent developments in "sugar-based" gemini amphiphiles, their aggregation morphologies, and key applications are reviewed. The study on sugar based gemini amphiphiles has intensified in

21st century due to cheap, non-toxic, bio-degradable nature of sugar based starting material.[74,75] Once again, the following section will focus on the key developments in early of this century to till date.

I.2.1. Gemini amphiphiles from natural resources (other than sugars)

In recent years, natural resources like amino acids, fatty acids, nucleotides etc. have been used by number of groups in the synthesis of gemini amphiphiles with a focus on their important applications. In majority of the cases the geminis are having identical head groups joined by a spacer. However, several of them are having two different head groups and they are categorised as unsymmetrical geminis.

In 2008, Zhou *et al.* synthesized and studied the solution phase morphologies of few nonionic poly(ethylene oxide)-based gemini amphiphiles (**I-31**, Fig. I-5).[76] pH and the salt effect were also investigated on their surface properties. Zhu *et al.* synthesized and studied the aggregation behaviour of nonylphenol based anionic gemini amphiphile (**I-32**, Fig. I-5) having polyethylene chain as spacer.[77] These amphiphiles show lower CMC as compared to conventional ionic surfactants. In 2012, Ge *et al.* synthesized sulphonate based anionic gemini amphiphile (**I-33**, Fig. I-5) having different hydrophobic tail and their self-assembling behaviour was studied in bovine serum albumin (BSA) at physiological pH and room temperature.[78] Xie *et al.* reported the synthesis and surface property measurement of different zwitterionic gemini amphiphile, (**I-34**, Fig. I-5) based on alkylbetaine.[79] These amphiphiles are having lower CMC as compared to monomeric surfactant.

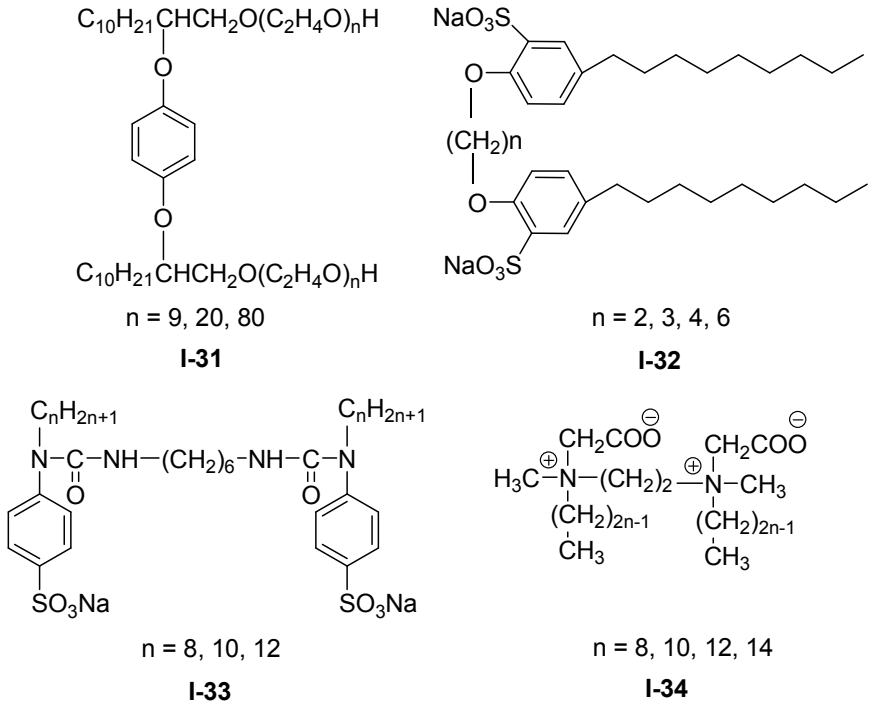


Fig. I-5. Representative examples of symmetrical gemini amphiphiles developed from various natural resources (other than sugars).

There are few examples of unsymmetrical geminis as well. Recently, Kawase *et al.* reported the monolayer behaviour of asymmetric gemini amphiphilic esters (**I-35**, Fig. 6) based on L-tartaric acid.[80] **I-35** (C_m-C_n) has two carboxyl groups and two alkanoyl groups, where m and n are the number of carbon atoms of hydrophobic alkanoyl group, m + n = 28. Pinazo *et al.* demonstrated the synthesis and application of amino acid based unsymmetrical gemini amphiphiles. They have synthesized gemini using various amino acids e.g. cystine (**I-36**, Fig. I-6), arginine, lysine and arginine containing glycerolipids.[81] These geminis are useful as foam and emulsion stabilizer in cosmetic and pharmaceutical industry.

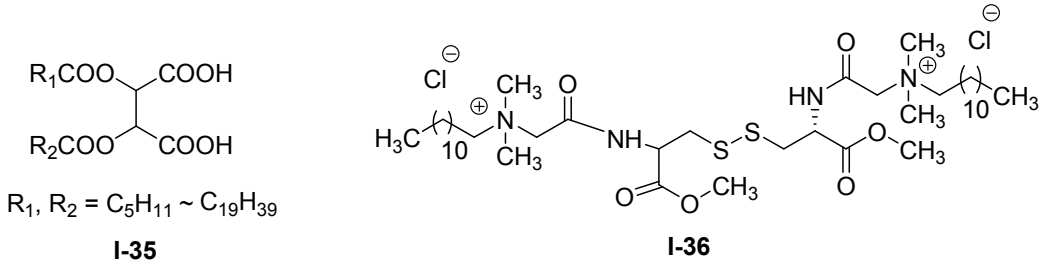


Fig. I-6. Some unsymmetrical gemini amphiphiles derived from different natural resources.

I.2.2. Sugar based gemini amphiphiles

This type of gemini amphiphiles can be subdivided into nonionic, cationic, anionic and zwitterionic. There are ample examples for sugar based nonionic and cationic bolaamphiphile. To the best of our knowledge synthesis of sugar-based anionic and zwitterionic gemini amphiphiles is not attempted so far.

I.2.2.1. Sugar-based nonionic gemini amphiphiles

The most common sugar residue in gemini amphiphile is D-glucose. In one such example, Pestman *et al.* synthesized D-glucose based nonionic bolaamphiphiles, with α,ω–diaminoalkanes, **I-37a** (Table I-3, entry no. 1) and their acetylated derivatives, **I-37b** (Table I-3, entry no. 1).[82] They have studied their aggregation behaviour in solution and reported that aggregation morphology is dependent on their spacer length (methylene units). When spacer length reduces to 6 methylene units from 10, vesicles become unstable and turn into thread like morphology upon cooling.

Fielden *et al.* synthesized and studied the transfection efficiency of nonionic reduced sugar based gemini amphiphiles, **I-38** (Table I-3, entry no. 2) which are connected through tertiary amino group with alkyl spacer (4-6 carbons) and a variable tail (saturated or unsaturated) of 12-18 carbons.[83] Their transfection efficiency was compared with commercial Lipofectamine Plus/2000 and showed that it was greater in case of unsaturated tail and comparable to the saturated alkyl tail (16 carbons). Their aggregate morphology was also studied at physiological and other pH.

Castro *et al.* studied the effect of linkage position, anomeric configuration, spacer functional group (ester, ether etc.) and nature of spacer on the alkyl glucoside based nonionic gemini amphiphiles with their monomeric part on interfacial properties. They have synthesized some short chain geminis, **I-39** using *n*-butyl-β-D-glucopyranoside linked through O-6 spacer (succinyl spacer) (Table I-3, entry no. 3) and several medium and long chain gemini surfactants.[84] They have mentioned that all the above factors affect interfacial property, even change in the configuration of anomeric carbon from α to β affects better packing of hydrophobic chains inside micelles which favours micellization over adsorption.

Johnsson *et al.* studied the aggregation behaviour of pH-sensitive reduced sugar (glucose) based nonionic amine functionalized gemini amphiphiles, **I-40** and **I-41** (Table I-3, entry no. 4) along with other amide-containing amphiphile.[85a] Their transfection activity was described by Wasungu *et al.*[85b] They mentioned that these compounds form bilayer vesicles at physiological pH but they change from lamellar to micellar morphology at endosomal pH (pH = 5-6.5) due to protonation. Wasungu *et al.* explained that these amphiphiles form lipoplex with DNA at physiological pH and then undergo transfection into cells of interest. This is because of lamellar morphology at physiological pH and nonlamellar at acidic pH. In case of amide-containing gemini, vesicles are anionic at pH<5 as compare to cationic in case of amine containing geminis (**I-40** and **I-41**). It was also mentioned that in case of gemini with amide head group only vesicles were obtained within experimental pH range.

Laska *et al.* described the synthesis, thermotropic and biological properties of D-glucose-based nonionic gemini amphiphile, **I-42** having 1,1′-ethylenebisurea spacer (Table I-3, entry no. 5).[86] They reported that upon heating liquid-crystalline mesophase was formed which is responsible for two phase transition point. After conducting various studies like biodegradation, antimicrobial assay they suggested that the amphiphiles are biodegradable and nontoxic to microbes like bacteria, molds etc.

Lakhrissi *et al.* investigated the surface and self-aggregation properties of several D-glucose based nonionic bis-benzimidazolone gemini amphiphiles, **I-43** derivative (Table I-3, entry no. 6).[87] They mentioned that these compounds are having very low CMC and high surface activity.

In 2012, Sharma *et al.* described the synthesis of D-glucose based nonionic gemini amphiphile, **I-44** and explored its application as reverse micellar system for encapsulation of D- and L-enantiomers of some UV absorbing aromatic α-amino acids like phenylalanine, tyrosine etc. in a nonpolar solvent like *n*- hexane (Table I-3, entry no. 7).[88] They found that the amphiphile in reverse micellar system encapsulates D-phenylalanine better than its L-form.

Liu *et al.* synthesized and studied the aggregation behaviour of a new nonionic alkyl *O*-glucoside based gemini amphiphile, **I-45** (Table I-3, entry no. 8).[89] **I-45** has been prepared by glycosylation of the gemini alkyl chains that are formed involving regioselective ring-

opening of ethylene glycol epoxides by the alkyl alcohols. The authors expressed its potential as nanocarrier for drug and gene delivery.

Sakai *et al.* synthesized the gluconamide type nonionic gemini amphiphiles Glu(*n*)-2-Glu(*n*), having varying hydrocarbon tails (n = 8, 10, 12) **I-46**, (Table I-3, entry no. 9) and described their surface properties and aggregation behaviour.[90] They mentioned that in case of n = 12, worm like micelle is formed above CMC.

In 2013, Guoyong *et al.* synthesized and studied the adsorption and aggregation behaviour of glucono *δ*-lactone based gemini amphiphile, **I-47** with Si-m-Si skeleton, where m is number of ethylene glycol units having tetrasiloxane tail (Table I-3, entry no. 10).[91] Short chain bulky siloxane moiety is responsible for reduction of surface tension. They reported the formation of spherical vesicles above CMC and also stated that their size was dependent on the value of m.

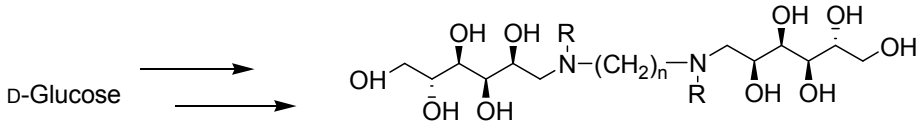
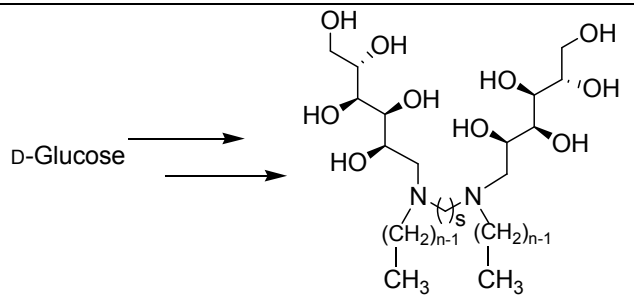
Recently, Xin *et al.* synthesized lactose based nonionic gemini amphiphile, **I-48** (Table I-3, entry no. 11) and it was immobilized on poly(styrene-*b*-(ethylene-*co*-butylene)-*b*-styrene, SEBS) elastomer through a pGMA (glycidyl methacrylate) spacer.[92] They mentioned that this complex polymer surfaces showed protein-resistant and anti-platelet adhesion property. Hemocompatibility of the modified surface was declined with increase in hydrophobic alkyl chain length.

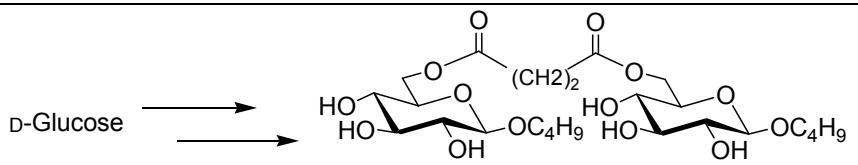
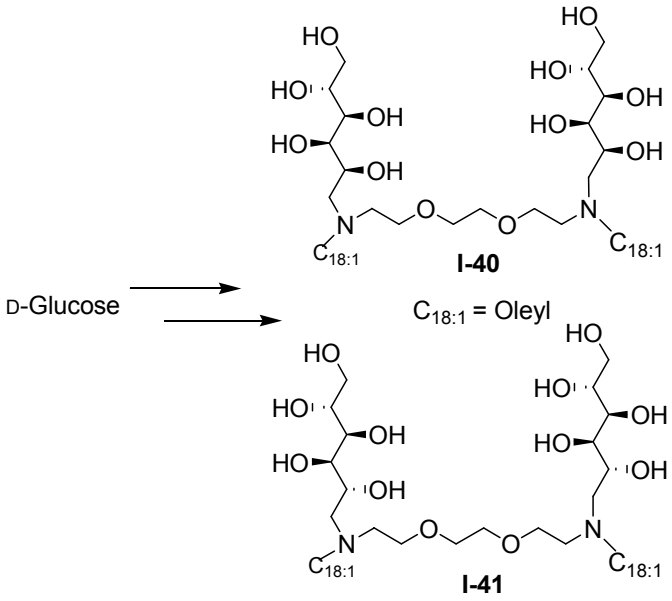
Menger *et al.* used trehalose (a disaccharide) as spacer in synthesizing nonionic gemini amphiphiles, **I-49** (Table I-3, entry no. 12).[93] **I-49** has an amide group with a long chain, which is in between spacer and alkyl tail and are water insoluble. **I-49** forms visible vesicular and tubular morphology in solution due to insolubility. Their CMCs are also lower than conventional surfactants.

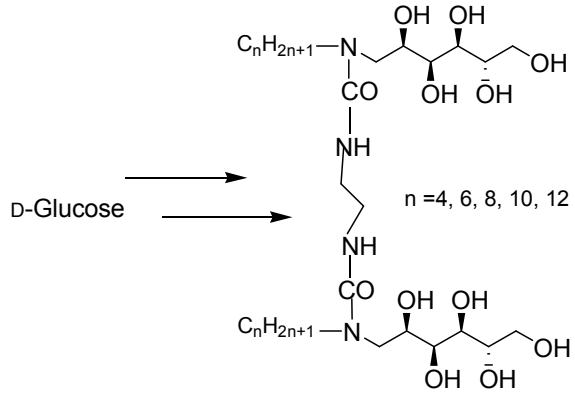
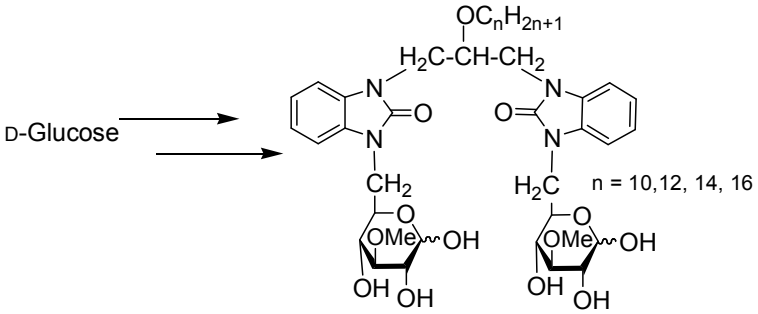
Yoshimura *et al.* synthesized and studied the self-aggregation property and biodegradability of lactobionic acid based gemini amphiphile, **I-50** containing peptide bond (Table I-3, entry no. 13).[94] They reported that **I-50** was synthesized by reacting adipoyl chloride with the respective monomeric surfactant *N*-alkyl-*N′*-lactobionylethylenediamine, which was obtained by reacting ethylenediamine with alkyl bromide and lactobionic acid. The gemini, **I-50** forms loosly bound micelles at low concentration above the CMC as compared to tightly bound

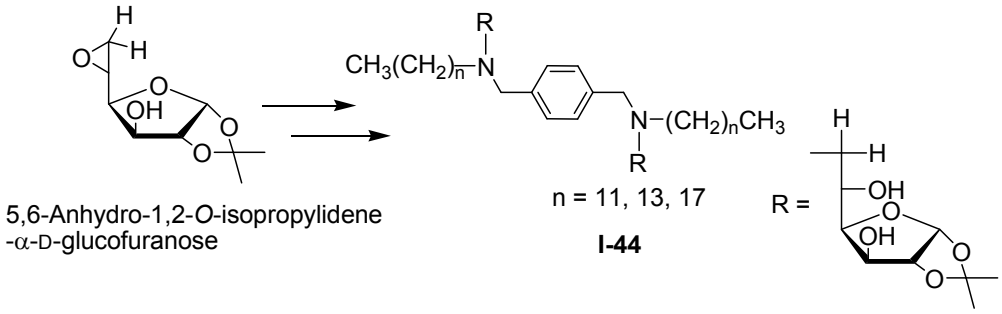
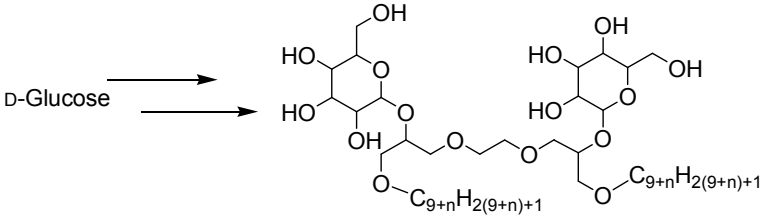
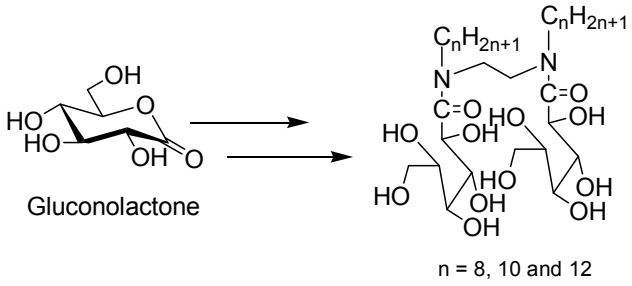
micelle at high concentration. They also mentioned that presence of tertiary amine in these molecules imparts slow biodegradation.

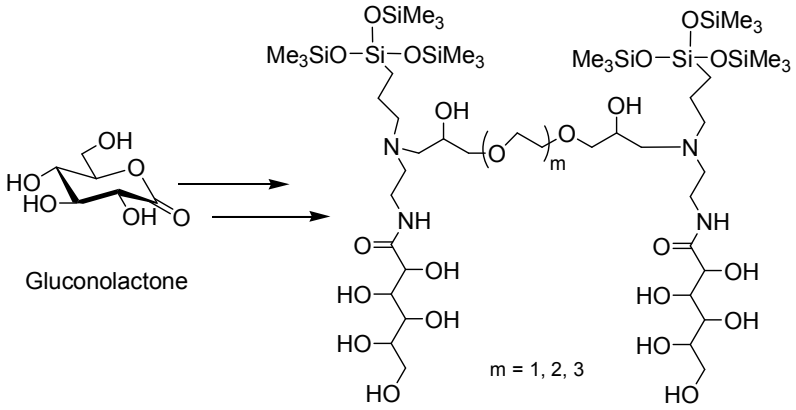
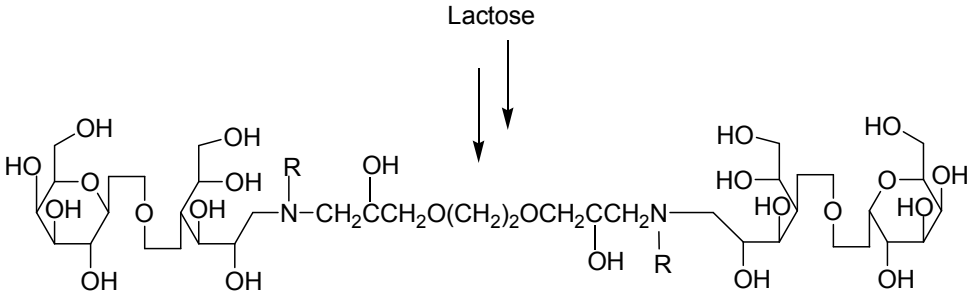
Table I-3: Nonionic gemini amphiphile

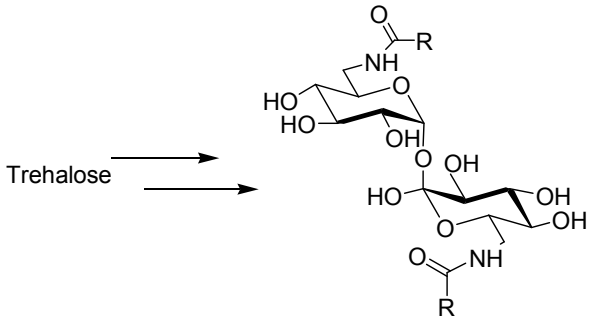
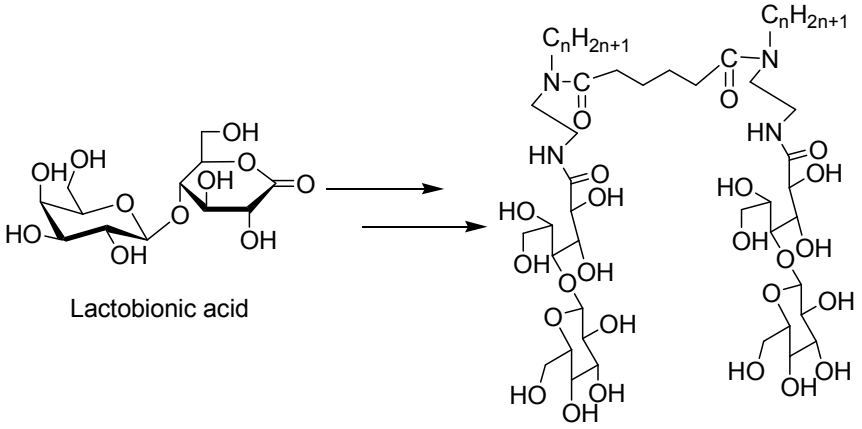
Sl. No.	Gemini amphiphile	Key study/Application	Ref.
1	 bis (*N*-tetradecanoyl-1-amino-1-deoxy-D-glucityl)alkanes n = 6, 8, 10 **I-37a :** R = H **I-37b :** R = $-\overset{O}{\overset{\|}{C}}-C_{13}H_{27}$	self-aggregation behaviour (formation of vesicles, thread like micelles)	82
2	 n = 12, 14, 16, 18, 18:1(oleyl) s = 4, 6 **I-38**	*in-vitro* transfection activity in an adherent Chinese hamster ovary cell line (CHO-K1)	83

Sl. No.	Gemini amphiphile	Key study/Application	Ref.
3	 **I-39**	effect of linkage position, anomeric configuration, spacer functionality, and the spacer type (rigid or flexible) on interfacial property	84
4	 **I-40** **I-41**	self-aggregation behaviour (formation of vesicles, micelles) and *in-vitro* transfection activity in CHO cells	85

Sl. No.	Gemini amphiphile	Key study/Application	Ref.
5	 **I-42**	thermotropic phase behaviour of pure surfactants	86
6	 **I-43**	surface property and self-aggregation behaviour (formation of submicellar aggregates)	87

Sl. No.	Gemini amphiphile	Key study/Application	Ref.
7	 **I-44**	encapsulation of aromatic α-amino acids in reverse micellar system	88
8	 **I-45**	surface property and self-aggregation behaviour (formation of vesicles, wormlike aggregates)	89
9	 **I-46**	adsorption and micellization behaviour	90

Sl. No.	Gemini amphiphile	Key study/Application	Ref.
10	 Gluconolactone m = 1, 2, 3 **I-47**	self-aggregation behaviour (formation of vesicles)	91
11	 Lactose $R = C_4H_9, C_8H_{17}, C_{12}H_{25}, C_{16}H_{33}$ **I-48**	surface modification of elastomer, preparation of protein resistant and anti-platelet adhesion surface	92

Sl. No.	Gemini amphiphile	Key study/Application	Ref.
12	 **I-49**	self-aggregation behaviour (formation of vesicles and tubules)	93
13	 **I-50**	self-aggregation behaviour (formation of micelles)	94

I.2.2.2 Sugar based cationic gemini amphiphiles

There are few examples for catioinic gemini amphiphiles which were synthesized from sugar as natural resource e.g. D-glucose and their different applications have been explored.

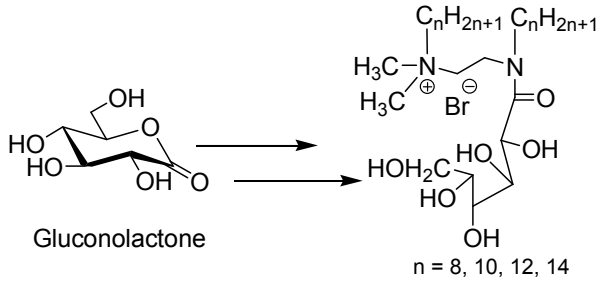
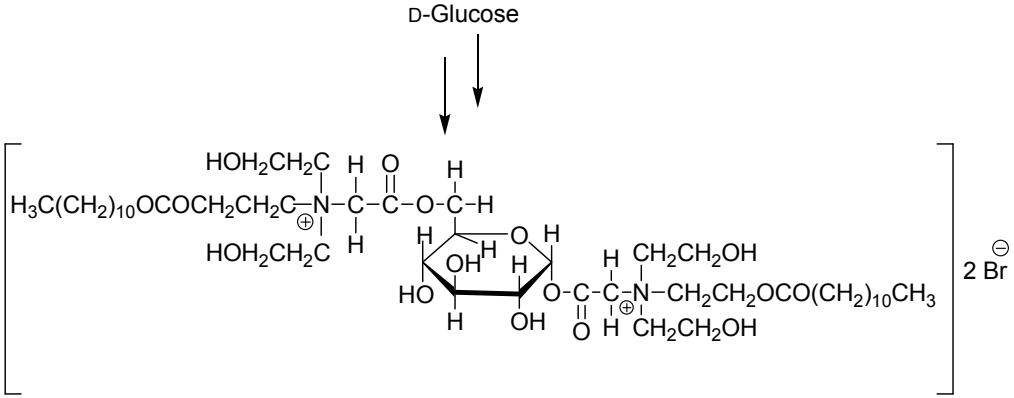
In 2006, Nyuta *et al.* synthesized and described the adsorption and self-aggregation property of D-(+)-gluconolactone based heterogemini amphiphile, **I-51** (Table I-4, entry no. 1) having a quaternary ammonium cation and other a non-ionic gluconamide as two different hydrophilic ends.[95] They mentioned that the aggregation morphology of **I-51** in solution depends on hydrocarbon chain length. Shorter chain length (n = 10, 12) leads to micelle formation, whereas, vesicles result at n = 14.

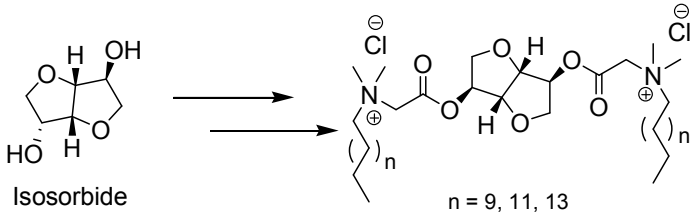
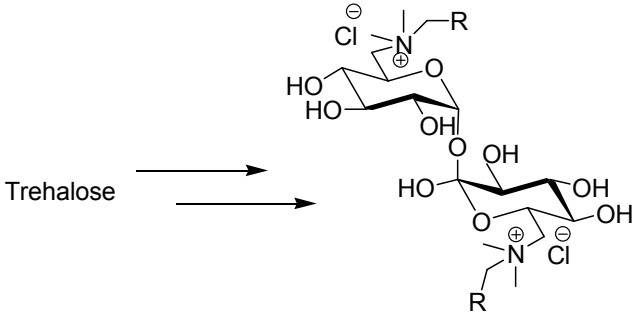
In 2008, Negm *et al.* synthesized D-glucose based **I-52** (Table I-4, entry no. 2) and fructose based new cationic gemini amphiphiles and studied their antimicrobial and antifungal properties.[96] The authors mentioned that antimicrobial and antifungal activities of **I-52** are comparable (or similar) to commercial surfactants (e.g. CTAB for antimicrobial and griseofulvin for antifungal activity).

Recently, Parikh *et al.* used isosorbide as spacer in building up cationic gemini apmphiphile, **I-53** and their aggregation bahaviour, DNA-binding and cytotoxicity properties were studied (Table I-4, entry no. 3).[97] They reported that their CMC, solubility and surface properties are better as compared to polymethylene spacer-based gemini amphiphile. These properties revealed that amphiphile with n = 9 tail length is a better candidate for gene therapy and amphiphile with n = 13 tail length is having the potential to treat cancerous (A549) human lung cells without affecting the normal cells.

Menger *et al.* extended their work with trehalose to develop cationic gemini amphiphile (**I-54**) as well (Table I-4, entry no. 4).[93] **I-54** is a quarternary ammonium gemini, which are tethered by a spacer and long chains, the ammonium groups ensure water solubility of this gemini amphiphile. The CMCs of these gemini amphiphiles are reported as lower than conventional surfactants.

Table I-4: Cationic gemini amphiphile

Sl. No.	Scheme	Application	Ref.
1	 **I-51**	adsorption property and self-aggregation behaviour (formation of micelles and vesicles)	95
2	 **I-52**	showed antimicrobial and antifungal activities	96

Sl. No.	Gemini amphiphile	Key study/Application	Ref.
3	 **I-53**	plasmid DNA (pEGFP-N1) binding ability	97
4	 **I-54**	self-aggregation behaviour (formation of micelles)	93

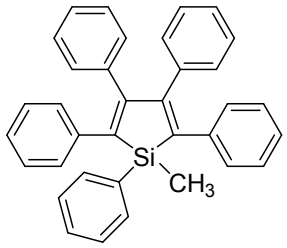
I.3. TPE based amphiphile

The detection and quantification of bioanalytes by fluorescent probes is emerging as an important area of research. This is due to the fact that fluorescence based techniques offer high selectivity, high sensitivity with low background noise.[98] In late 20th century, Friend *et al.* pointed out that many chromophoric dilute solutions are emissive but their thin films (solid state) were weakly emissive because of aggregate (excimer) formation.[99] Later on this phenomenon was called as aggregation quenching[100] or aggregation caused quenching (ACQ).

Tang *et al.* first proposed that aggregation property can be made benificial for enhancement of fluorescence emission intensity.[101] They reported that silole type of molecules (**I-55**, Fig. I-7) are typically non-fluorescent in solution phase (in organic solvents like ethanol, THF etc.) but become emissive upon addition of water (non-solvent) due to aggregation. Aggregation induced emission (AIE), a photophysical phenomenon, is opposite to the ACQ effect because it causes enhancement in fluorescence in the solid state or in aggregated form and is useful in several bio-technological and optical applications.[101,102] Tang *et al.* have proposed that fluorescence enhancement occurs in the aggregated state due to restriction in intramolecular rotation (RIR).[103]

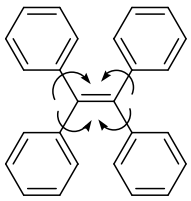
Over last ten years, a large number (and variety) of AIE luminogens (AIEgens) have been synthesized.[103,104] The AIE probes emit lights of various colours such as blue, green, yellow and red with high quantum yields (up to 85%).

Recently, Tang *et al.* have extensively used tetraphenylethene (TPE) (**I-56**, Fig. I-7) moiety in the construction of AIE based bioprobes.[98] TPE is actually olefin stator covered by phenyl rotors, which involves in RIR leading to AIE. These probes show AIE property upon binding with biomolecules such as DNA, BSA and give intense fluorescence in aqueous buffer solution. These interactions between probes and biomolecules are mostly non-covalent such as hydrophobic, electrostatic, π-π stacking etc. There are ample examples where TPE was successfully used as AIE active probe in various applications such as bioprobes, chemosensors, cell imaging etc.[98,102,105-110]



1-methyl-1,2,34,5-pentaphenylsilole

I-55



1,1,2,2-Tetraphenylethene (TPE)

I-56

Fig. I-7. A few common scaffolds of AIE luminogens.

Water solubility is an important factor, which determines the application of AIEgens in bio-systems.[111] Amphiphilic molecules are water soluble, if they are having sufficiently hydrophilic parts in them. Attachment of amphiphilic units to TPE core to make them water soluble is also a challenging area of research. The following section will discuss about the recent developments in TPE based amphiphiles and their applications.

Tang and co-workers have reported several water soluble TPE based probes **I-57**—**I-59** for different biological applications (Table I-5, entries no. 1-3).[98,106,107] Probe **I-57** was used for the detection of biomolecules DNA and BSA.[98] TPE core was synthesized by the reaction of 1,2-bis(4-hydroxyphenyl)-1,2-diphenylethene with α,ω–dibromoalkanes followed by amination. Quarternization of the terminal amine functionality afforded water soluble cationic probe, **I-57**. In aqueous buffer **I-57** binds with negatively charged DNA and protein through noncovalent interactions and shows fluorescence by AIE effect. On the other hand, **I-58** was used for DNA conformation study and also to study the folding process of G-Quadruplux (secondary structure of G_1- a guanine rich DNA strand).[106] McMurry coupling of 4,4′-bis(2-bromoethoxy)benzophenone formed the TPE core, which after quaternization afforded water soluble cationic TPE-amphiphile, **I-58**. Anionic TPE amphiphile, **I-59** was developed for the detection and quantitation of proteins, acetylcholinesterase (AChE) asaay and its inhibitor screening.[107] For the synthesis of **I-59**, dimethoxylated TPE derivative was synthesized in the first step, which was further converted to dihydroxylated TPE derivative and finally, attachment of sulphonate units was performed using 1,3-propane sultone to give water soluble anionic TPE derivative, **I-59**. They described that after binding with BSA, probe **I-59** gives fluorescence as a result of AIE. However, in presence of SDS, the quaternary folded structure of BSA chains get disturbed which results in quenching of

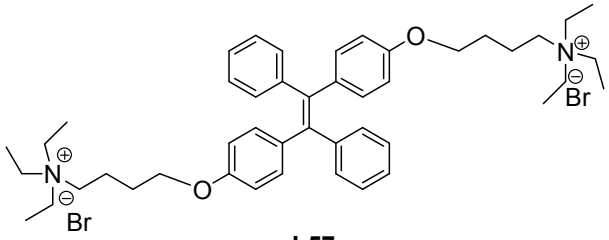
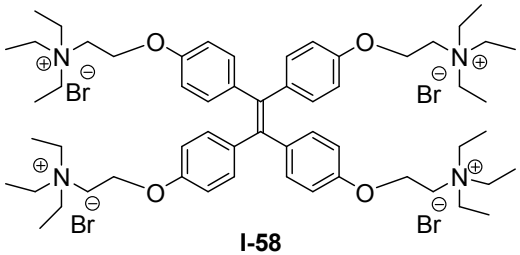
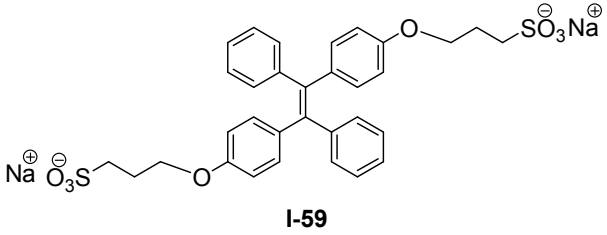
fluorescence because SDS occupy the hydrobhobic pockets of BSA which were earlier occupied by probe.[107a] Probe, **I-59** was also used to develop a fluorimetric method for AChE enzyme assay and also AChE inhibitor screening. Myristoylcholine, a susbtrate for AChE was used in this assay. Fluorescent aggregation complex between the probe and this substrate was de-aggregated in the presence of AChE which results in quenching of fluorescence.[107b]

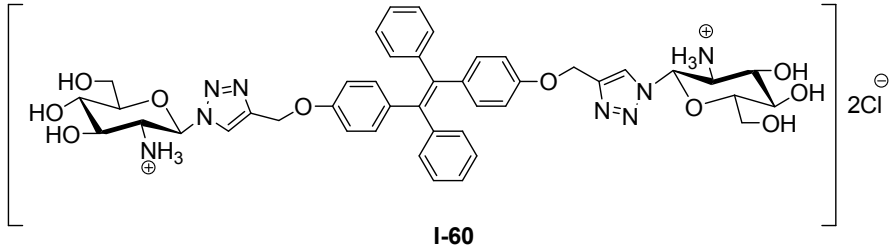
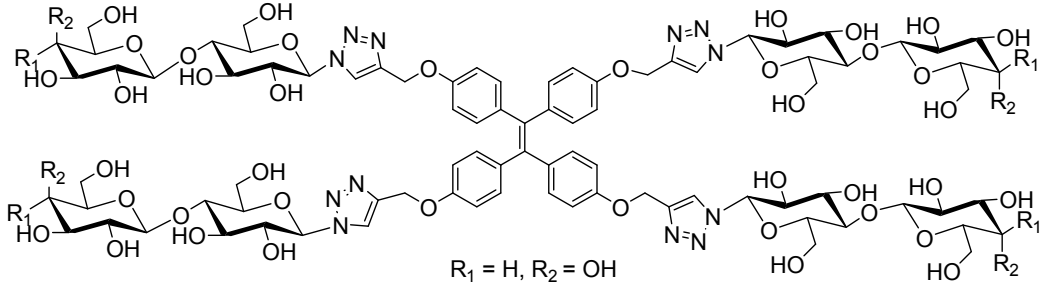
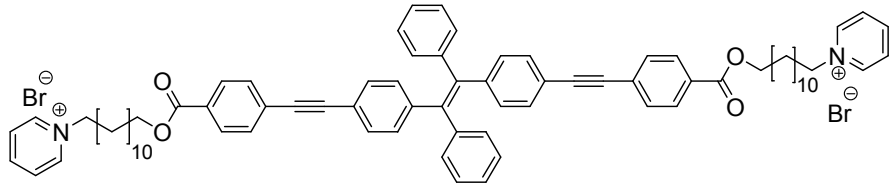
Apart from Tang and co-workers several other groups have also reported TPE based amphiphiles and their bio-technological applications. Chen *et al.* developed a water soluble cationic TPE amphiphile with glucosamine residue at the terminal (**I-60**) which was used for the detection of alkaline phosphatase enzyme (Table I-5, entry no. 4).[108] Notably, alkaline phosphatase is an important bio-marker of several diseases like diabebets, liver dysfunction etc. Glucosamine (in hydrochloride form) was joined with TPE by click chemistry to make water soluble probe. Aggregation induced fluorescence of heteroaggregation complex between **I-60** and monododecylphosphate (an amphiphile) was reduced in the presence of alkaline phosphatase enzyme because of de-aggregation.

In 2011, Hu *et al.* have synthesized a similar water soluble amphiphilic fluorescent probe, **I-61** for cholera toxin by modifying TPE core with lactosyl moiety. This probe was also synthesized by click reaction between propergyl-TPE and azido-functionalzed lactose sugar (Table I-5, entry no. 5).[109] Cholera toxin B sub unit interacts with the lactose unit of the probe and emits fluorescence signal by AIE phenomenon.

Recently, Xia *et al.* have developed a water soluble fluorescent probe, **I-62** for the labelling and mapping of HeLa cells using the self-aggregation property of this TPE based bolamphiphile and AIE property (Table I-5, entry no. 6).[110] In this case, the TPE core was extended by phenyl acetylinic unit by Sonogashira coupling. The compound self-assembles into monolayered nanostructures. The nanostructures shows good fluorescent emission property by AIE phenomenon, which was utilized in cell imaging.

Table I-5: TPE based amphiphiles and their applications

Sl. No.	TPE amphiphile	Key study/Application	Ref.
1	 **I-57**	detection of DNA, BSA	98
2	 **I-58**	DNA conformation study	106
3	 **I-59**	to study unfolding process of BSA and AChE assay	107

Sl. No.	TPE amphiphile	Key study/Application	Ref.
4	 **I-60**	probe for alkaline phosphatase	108
5	 R_1 = H, R_2 = OH **I-61**	cholera toxin B sensor	109
6	 **I-62**	for cell imaging	110

I.4. Surfactants as catalyst in "dehydration" reactions in water

Over the past few decades, growing environmental pollution pose huge threat on human health and ecology. Most of the industrial processes involve the use of toxic organic solvents and reagents that impact the environment. This warrants chemists to develop and use greener methodologies for organic transformations guided by green chemistry and its 12 principals.[111,112] It is pertinent to mention that nature uses water for most of its transformations, which is possible to mimic in synthetic protocols for organic transformations.[113] Water is cheap, non-toxic, non-flammable and readily available but may not be friendly solvent for many organic compounds. In addition, there is always a chance of hydrolytic decomposition which particularly makes dehydration reactions difficult to carry out in water. These problems were mostly solved by using surfactants, which form a kind of "nanoreactors" in aqueous media by forming colloidal, micellar or other organized phase. The hydrophobic interior of nanoreactors brings together the organic reactants in close proximity to facilitate the reaction between them. In last couple of decades, surfactants are extensively used as catalysts in aqueous media for various kinds of organic transformations, which have been comprehensively discussed in several review articles.[114a] In the present document, focus will be given on the various dehydration reactions in water in the presence of surfactants as catalyst. As mentioned in the earlier part, "dehydration reactions" are usually performed under anhydrous conditions and water was mostly avoided as the reaction media to carry out such reactions till the end of last century, however, use of surfactants made it possible to conduct "dehydration reactions" in water. The micellar nanoreactors formed by surfactants host the organic (hydrophobic) substrates inside it as the exterior is hydrophilic. It is often associated with the enhancement in the rate of reaction because the subtrates are in close proximity and are forced to interact with each other. In addition, once the water molecule is generated by the reaction it is ejected out to the hydrophilic exterior. The probable mechanism of "dehydration recation" in a micellar media is represented in Fig. I-8 with a typical example i.e. synthesis of *cis*-fused chromano[4,3-*c*]isoxazole.[114b]

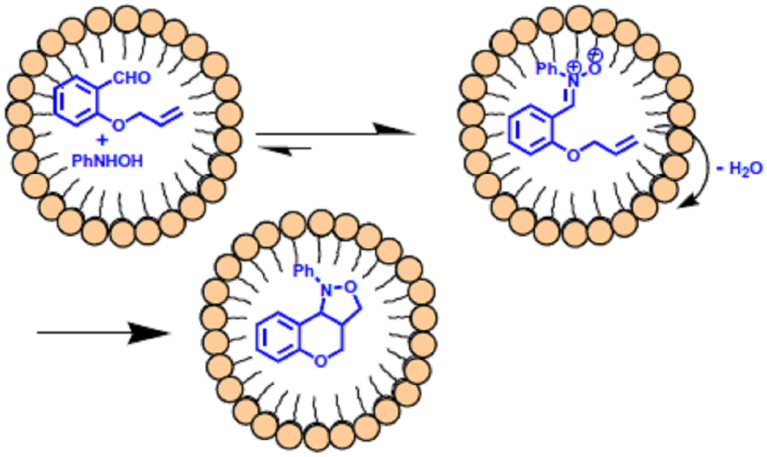


Fig. I-8. Dehydrative synthesis of *cis*-fused chromano[4,3-*c*]isoxazole.

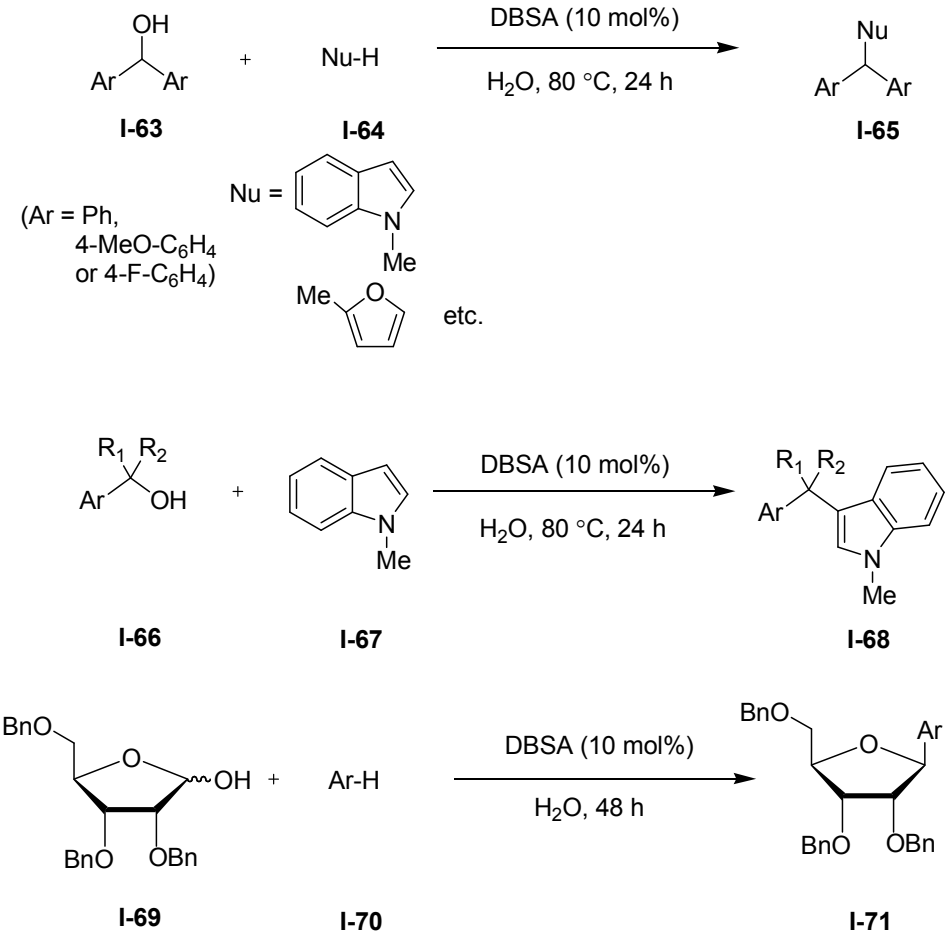
I.4.1. Dehydration reactions in micellar media

There are several types of dehydration reactions reported in literature, which are carried out in micellar media including C-C bond formation, C-N bond formation, C-O bond formation etc.

I.4.1.1. C-C bond forming reactions

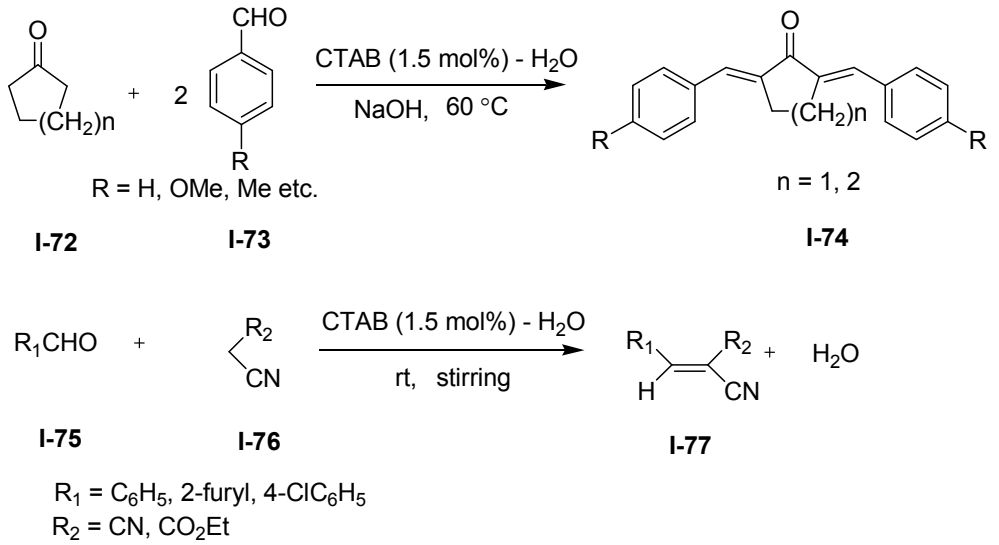
Surfactants were often used as catalysts to carry out dehydration reactions involving C-C bond formation to give several pharmaceutically important compounds, scaffolds of natural products through Knoevenagel condensation, crossed-aldol condensation etc.

Shirakawa *et al.* have developed a methodology for dehydrative nucleophilic substitution reactions between diaryl methanol and different nucleophiles in aqueous micellar media in the presence of DBSA as surfactant (Scheme I-2).[115] The same methodology was extended to the synthesis of 3-substituted indoles (**I-68)** by alkylation using different benzyl alcohols (**I-66)** in moderate to good yields. This methodology was also used for the stereoselective C-glycosylation of 1-hydroxy sugars (**I-69**) to afford biologically important product, **I-71.**



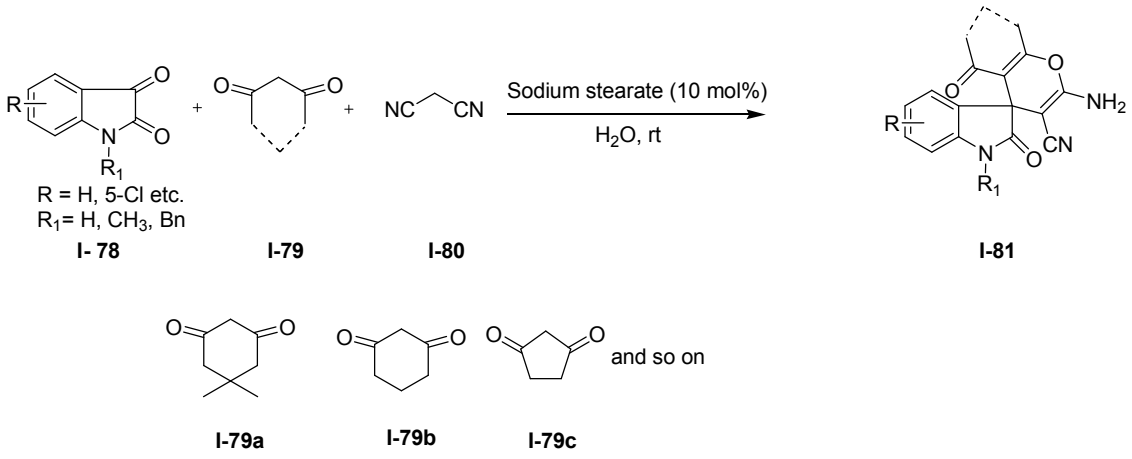
Scheme I-2

Shrikhande and co-workers developed a method for crossed-aldol condensation and Knoevenagel condensation in aqueous micellar media involving CTAB as catalyst (Scheme I-3).[116] They used different aromatic aldehydes (**I-73**) and cyclic ketones (**I-72**) in micellar media in order to get crossed-aldol products, α,α′-bis-benzylidene cycloalkanones (**I-74**) in excellent yields. Knoevenagel condensation was also tried using various aldehydes (**I-75**) and active methylene compounds (**I-76**) to afford the condensation products (**I-77**) at room temperature in high overall yields.



Scheme I-3

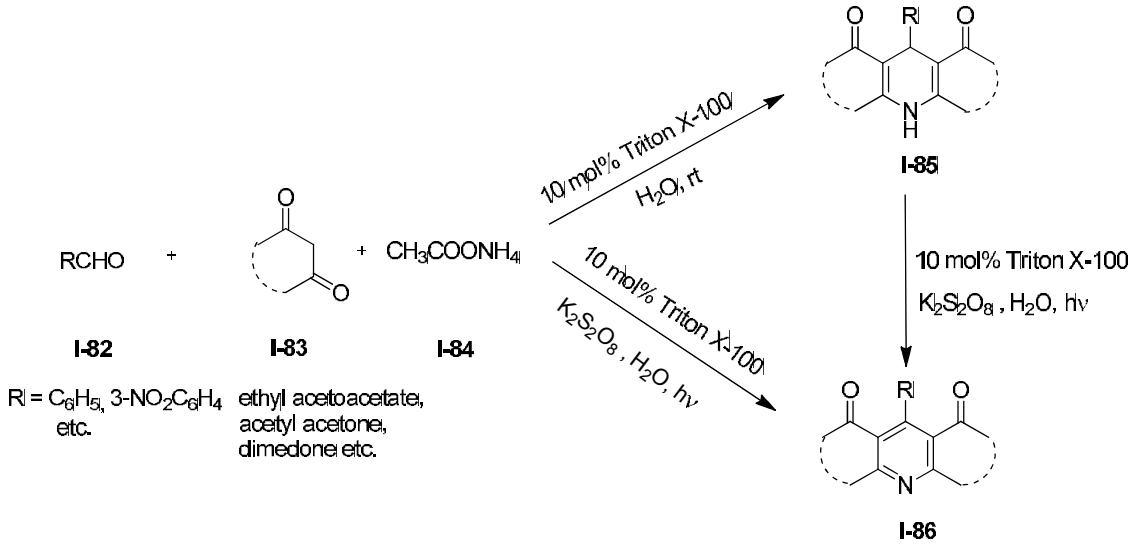
Multicomponent C-C bond formation reactions were also carried out in micellar media. Wang and co-workers described three component, one-pot reaction using fused chromenes for the synthesis of spirooxindoles derivatives (**I-81**) in good yields (>90%) using weakly basic sodium stearate as catalyst (Scheme I-4).[117] In the presence of surfactant malononitrile gets deprotonated and reacts sequentially with isatin (**I-78**) and 1,3-dicarbonyl compound (**I-79**) to afford the desired product (**I-81**). Similar methodology was also employed to synthesize different biologically important heterocycles taking 4-hydroxy-coumarin in place of 1,3-dicarbonyl compounds.



Scheme I-4

Ghosh *et al.* developed a one-pot method for the synthesis of 1,4-dihydropyridine derivative (**I-85**) in >80% yield in aqueous micellar media provided by a non-ionic surfactant, Triton X-

100. Aldehydes (**I-82**), 1,3-diketones (**I-83**) and ammonium acetate (**I-84**) were used as substrate in Hantzsch reaction (Scheme I-5).[118] They have also reported a method for direct synthesis of pyridine derivatives (**I-86**) by oxidation of 1,4-dihydropyridine derivative (**I-85**) in-situ in the presence of potassium persulphate as oxidant under visible light irradiation with almost 100% yield. Both dihydropyridines and pyridine moieties are of biologically significance. 1,4-Dihydropyridines are extensively used as calcium channel modulators and their oxidized counterpart targets several biological receptors.



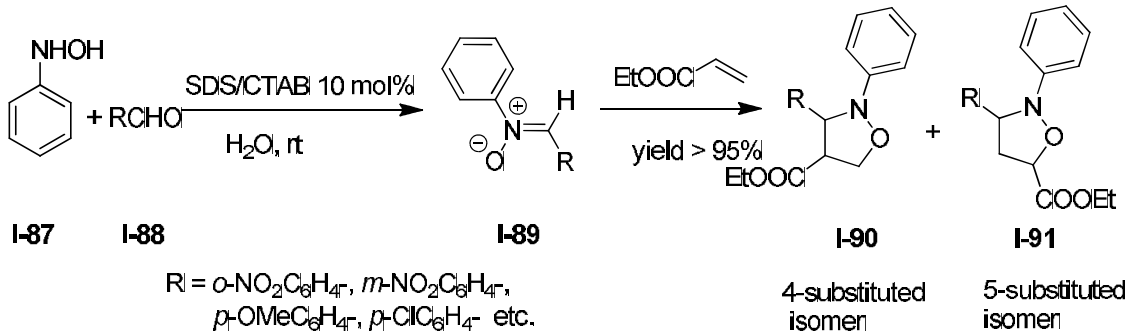
Scheme I-5

I.4.1.2. C-N bond forming reactions

There are several examples where surfactants were used as catalysts/co-catalysts to perform dehydration reactions involving C-N bond formation to afford different biologically active heterocycles including substituted indoles, qninoxalines, quinazolines etc. Several multi-component reactions are also reported using this type of greener methodology. Some examples are Mannich reaction, Kinugasha reaction, Kabacknik–Fields reaction etc.

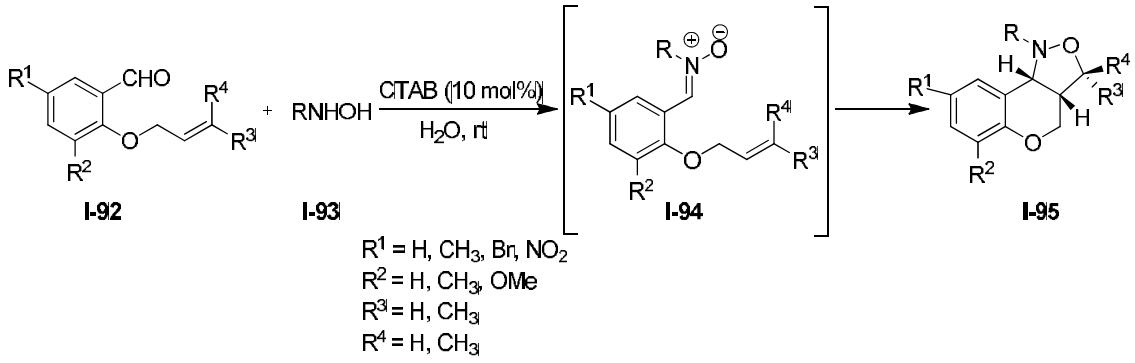
In this direction, Chatterjee *et al.* developed a greener methodology for nitrone formation followed by 1,3-dipolar cycloaddition in a single-pot in micellar media to synthesize isoxazoles derivatives (**I-90**, **I-91**) in a stereoselective manner (Scheme I-6).[119] To carry out this transformation two commercial surfactants SDS (anionic) and CTAB (cationic) were used and the results were compared. Both favoured *in-situ* nitrone formation (**I-89**) by the reaction of phenylhydroxylamine (**I-87**) with different aldehydes (**I-88**), which further

undergo 1,3-dipolar cycloaddition by reacting with ethyl acrylate to give the final products (**I-90**, **I-91**). Regioselectivity was controlled in the reaction which favours the formation of *trans*-5-substituted isoxazolidine (**I-91**) out of 4 regioisomers, *cis*/*trans* for 5-substituted (**I-91**) and *exo*/*endo* for 4-substituted product (**I-90**), as the major product. In few cases, the *endo*-4-substituted products predominate over others.



Scheme I-6

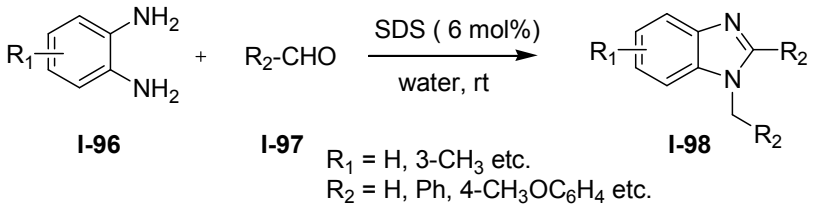
Chatterjee *et al.* also developed a green method for the synthesis of *cis*-fused chromano[4,3-c]isoxazoles (**I-95**) through intramolecular 1,3-dipolar cycloaddition of *O*-allylsalicaldehyde derivatives (**I-92**) and hydroxylamine (**I-93**) using CTAB as surfactant in water.[114b] Surfactant favours *in-situ* nitrone formation (**I-94**), which after cycloaddition gives chromanoisooxazoles (**I-95**) (Scheme I-7). Substituents on aromatic part of aldehyde (**I-92**) does not affect the yield. Isolation of products was also easy. The products were isolated by filtration after cooling.



Scheme I-7

Substituted benzimidazoles are biologically important *N*-containing heterocycles having antiulcer, antihypertensive, anticancer properties. Due to their biological importance several methods have been developed for these heterocyclic compounds including reactions in

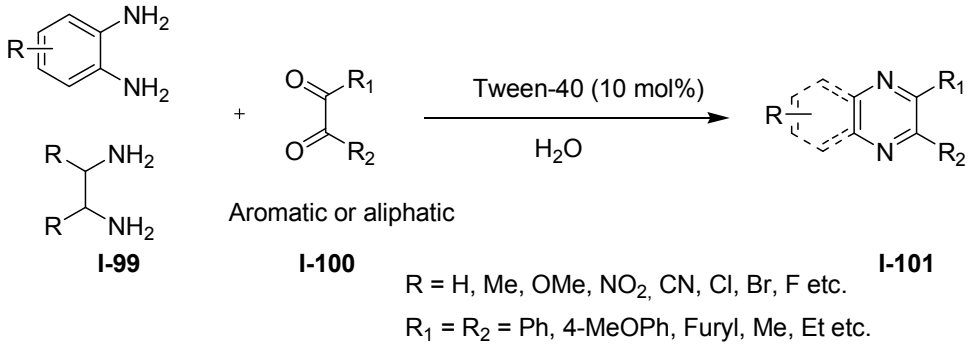
micellar media. In one such example, Ghosh *et al.* developed a one-pot method to synthesize 1,2-disubstituted benzimidazoles (**I-98**) selectively, from different aldehydes (aromatic, heteroaromatic and aliphatic) (**I-97**) and *o*-phenylenediamine (**I-96**) in water using SDS as catalyst (Scheme I-8).[120a] The reaction involves dehydrative imine formation followed by imtramolecular cyclization. They obtained 1,2-disubstituted benimidazoles as the major product with no 2-substituted benzimidazole. Overall yield of this reaction is excellent with an added advantage of reusability of surfactant catalyst.



Scheme I-8

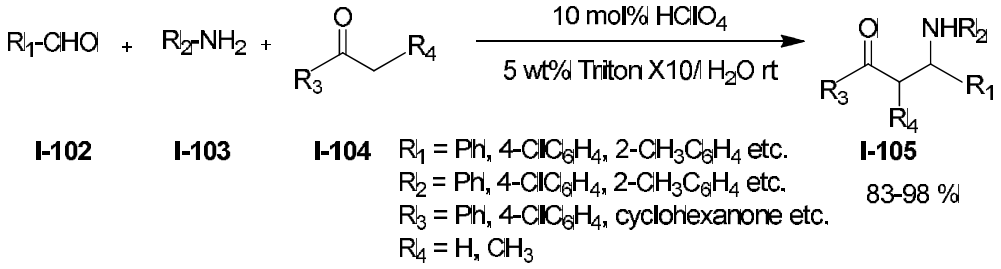
Similar work was reported by Bahrami *et al.* also for the synthesis of 1,2-disubstituted benimidazoles, 2-substituted benimidazoles and 2-substituted benzothiazoles using SDS as a surfactant catalyst.[120b]

Kumar *et al.* assessed the employability of approximately fifty surfactants at room temperature to synthesize biologically active quinoxalines (**I-101**) using 1,2-diamines (**I-99**) and 1,2-dicarbonyl compounds (**I-100**) (Scheme I-9).[121] Among all surfactants tested, neutral surfactant Tween 40 was found most suitable as catalyst in terms of yield of the final product (**I-101**) when compared with several Lewis/Brønsted acid combinations. This method is one of the many examples of better performances of aqueous micellar media over several organic solvents in terms of yield.



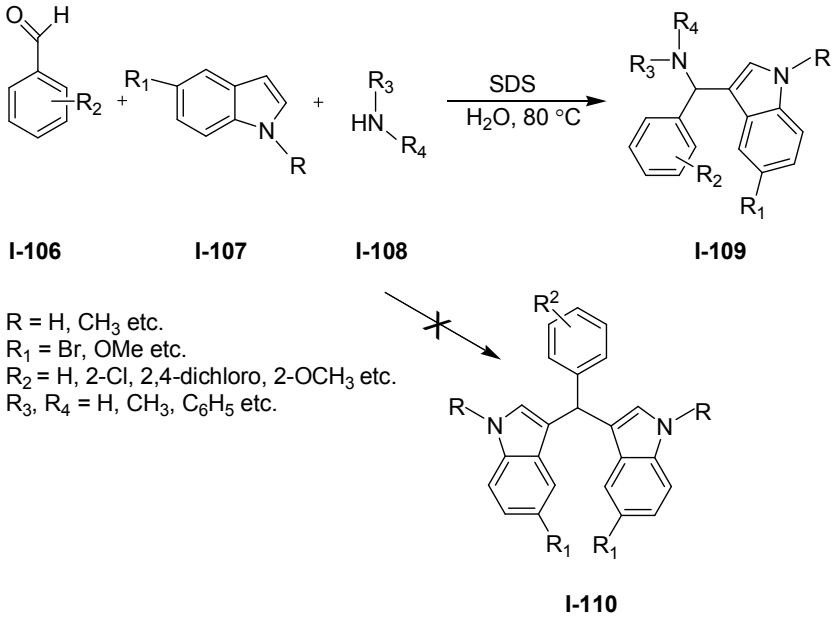
Scheme I-9

Lu *et al.* developed a method for Mannich reaction using perchloric acid as catalyst in the presence of Triton X10 as surfactant. Aromatic aldehydes (**I-102**) and aromatic amines (**I-103**) and various ketones (**I-104**) were used as substrates. The reaction requires shorter reaction time and affords good overall yields (83-98%) of the products (**I-105**) (Scheme I-10).[122] Moreover, products being insoluble in water can be purified by filtration itself. Dehydration step involves is the formation of aldemine.



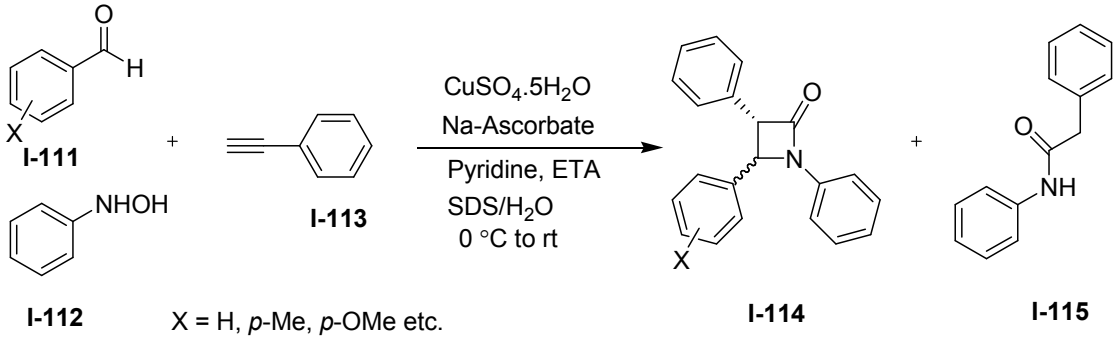
Scheme I-10

A multicomponent reaction is reported by Kumar *et al.*, who developed a green methodology to synthesize 3-amino alkylated indoles (**I-109**) using SDS as surfactant (Scheme I-11).[123] The three-component, one-pot Mannich-type reaction involving aldehyde (**I-106**), secondary amines (**I-108**) and indoles (**I-107**) affords 3-amino alkylated indoles (**I-109**) as the major products in good yields instead of *bis*-indole (**I-110**) derivative which is major product in case of other Brønsted and Lewis acids.



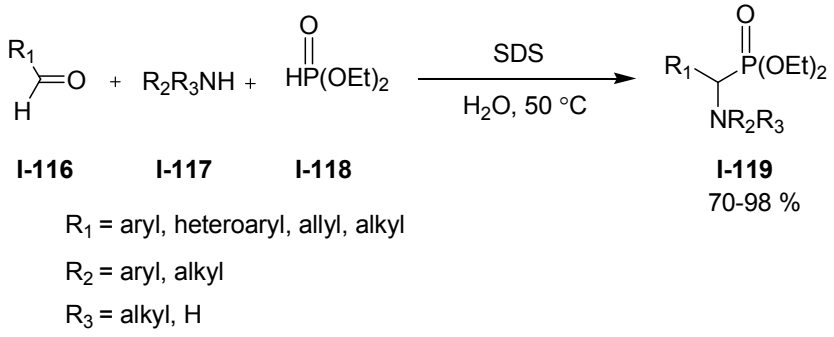
Scheme I-11

McCay *et al.* developed a green method for Kinugasha reaction to synthesize biologically active β-lactams (**I-114**) involving SDS as catalyst.[124] Aldehydes (**I-115**), phenyl hydroxyl amine (**I-112**) and phenyl acetylene (**I-113**) were used as substrates and reactions were carried out in aqueous micellar media in which *C*,*N*-diphenylnitrone formed as intermediate in-situ, which further reacts with Cu(I)-phenylacetylide to affords β-lactams (**I-114**) in moderate to good yields (45-85%) and an amide as co-product (**I-115**) (Scheme I-12). The substituents affect the reaction either by accelerating the cycloaddition process or by minimising the side product formation.



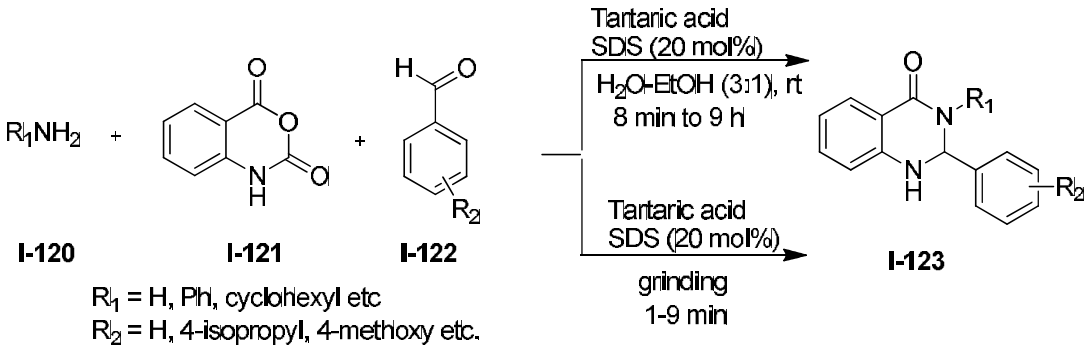
Scheme I-12

Sobhani *et al.* described a method to synthesize diethyl α-aminophosphonates (**I-119**) via Kabacknik–Fields reaction in one-pot using aldehydes (**I-116**), amines (**I-117**) and diethyl phosphite (**I-118**) in aqueous micellar media formed by SDS, in high overall yields (70-98%) with better chemoselectivity (Scheme I-13).[125] The development of this method is significant because α-aminophosphonates (**I-119**) are useful as enzyme inhibitors, peptide mimics, antibiotics etc.



Scheme I-13

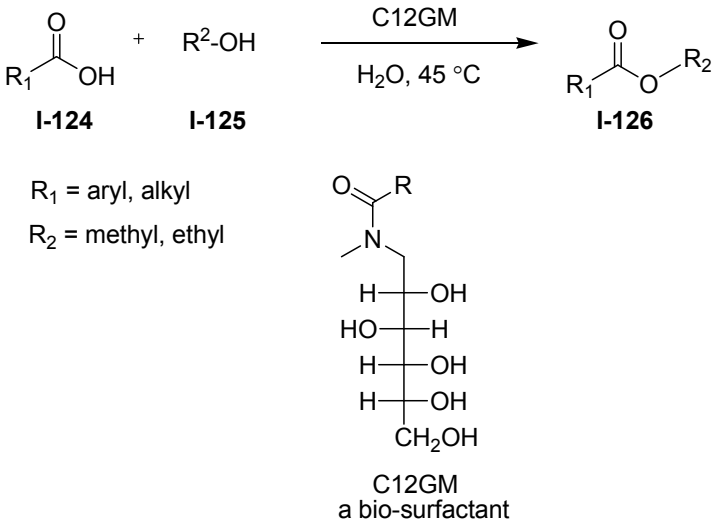
Sharma *et al.* developed a greener methodology to synthesize biologically important heterocycle 2,3-dihydro/spiroquinazolin-4(1*H*)-ones (**I-123**) which involves tartaric acid-SDS recyclable catalytic system and water-ethanol mixture (3:1) as solvent (Scheme I-14).[126] This multicomponnet reaction involves isatoic anhydride (**I-121**), amines (**I-120**) and various aldehyde/ketones (**I-122**). They have used two approaches (a) mechanical stirring at mild condition and (b) mechanochemical activation (grinding). Mechanical stirring in the solvent mixture affords desired product in good yield but the protocol can be further improved by mechanochemical activation because it is more effective under solvent free/solvent drop condition even in the presence of SDS without compromising the yield and moreover, reaction time was also less.



Scheme I-14

I.4.1.3. C-O bond forming reaction

There are only few examples where surfactant was used as a catalyst to carry out C-O bond forming dehydration reactions. One of the pioneer works in this area was carried out by Kobayashi *et al.* between carboxylic acids and alcohols.[127a] Recently, Rajabi *et al.* developed another green method for the esterification reaction between a range of aromatic and aliphatic acids (**I-124**) and methnol (or ethanol) in micellar media formed by a glucose based nonionic biosurfactant, C12GM (glucose-derived *N*-alkanoyl-*N*-methyl-1-25 glycamine) (Scheme I-15).[127b] Operational simplicity and high yield of esters (**I-126**) made this approach economically viable.



Scheme I-15

I.5. Gap in the existing research

In this Ph.D. work we targeted synthesis, characterization and studies on potential applications of three types of amphiphilic molecules namely a) sugar-based cationic gemini amphiphiles, b) sugar-based anionic bolaamphiphiles, c) Tetraphenylethene (TPE) based amphiphiles and studies on their potential applications. In addition, development of green and sustainable methods for various dehydration reactions catalyzed by commercial surfactants has been attempted.

The gap in the existing research which makes us interested to work in this direction is mentioned below.

- Gemini amphiphiles are having several bio-medical applications, particularly as gene delivery agents. It is documented in literature that cationic gemini amphiphiles can form positively charged lipoplexes with negatively charged DNA to act as a synthetic vector, which do not face electrostatic barrier while penetrating the biological cell surfaces. It is noteworthy to mention that the mechanism of gene transfer ability of conventional cationic surfactants is not fully understood and the major drawback of most of these non-viral/synthetic vectors is their poor transfection ability. So it is worthy to develop bio-compatible and effective gene delivery systems based on cationic gemini amphiphiles. After thorough literature survey it was found that glucose based cationic gemini surfactants are rare and their C-3 functionality was rarely used for the synthesis of gemini amphiphiles

and their DNA binding affinity. So, we were interested to design cationic gemini amphiphiles that join two monomeric units with quarternary ammonium group as polar head through a linker via C-3 functionality of diisoprypylidene glucose; study their surface property and DNA binding efficacy. We assumed that the natural angle and the partial conformational rigidity provided by the glucofuranoside ring to spacer-polar head-side chain would insist these molecules to stay in 'π' shape and thereby, they would form vesicles and/or micelles in solution with ease which could aid in enhancing binding abilities with plasmid DNA.

- Sugar-based amphiphiles are of special interest for pharmaceutical applications because of their natural origin and biocompatibility. So far, several sugar based neutral bolaamphiphiles have been reported and their morphologies in solution phase have been studied. Although a few sugar-based cationic bolaamphiphiles are reported with their physicochemical studies, reports on sugar-based anionic bolaamphiphiles are rare to the best of our knowledge. Being chiral these ionic bolaamphiphiles are expected to show unique aggregate morphologies. It is worthy to design and synthesize a series of sugar based anionic bolaamphiphiles to study their self-assembled nanostructures in solution.

- Although bolaamphiphiles have been used for a wide range of applications, another potential application of these molecules that remains practically unexplored is their ability to catalyze reactions in aqueous media. The bolaamphiphiles have all the properties of a normal surfactant and they are capable of forming vesicles in water. The in-built chirality of sugar-based bolaamphiphiles could be advantageous in order to get improved stereoselectivity in various organic transformations.

- Fluorescent probes are important in the field of bio-medical applications, particularly emission based fluorescent "light-up" probes. They can be applied in the detection and quantitation of proteins and nucleic acids. This is important because conventional dyes are carcinogenic in nature and their lipophilicity restricts their use. Tetraphenylethylene (TPE) is useful in making important building block for several luminescent materials because of its inherent AIE property in solid state. Water solubility and biocompatibility are two important factors for the use of AIE compounds in bio-technological applications. Few TPE based water soluble probes were reported for the detection and quantitation of DNA and proteins. However, they are mainly based on long alkyl chains with quaternary ammonium

ions at the terminal, which have profound toxicity. These facts indicate the requirement of the development of stable, water soluble, non-toxic fluorescent "light-up" bio-probes for the detection and quantitation of nucleic acids and proteins. These TPE based probes could become more water soluble and bio-compatible by incorporating glycol units in the chain and cationic pyridinium unit at the terminal respectively so that they would be more suitable for biotechnological applications.

- 1,3-Benzazoles (benzimidazole, benzothiazoles and benzoxazoles) are important heterocyclic scaffolds of several biologically active compounds and various reaction intermediates. There are several methods reported for their synthesis, which involves transition metal catalyzed reaction, solid phase supported synthesis etc. The reported procedures involves hazardous metal catalysts, reagents, organic solvents for reaction and extraction, which are not environmentally benign. Several eco-friendly solution phase methods were also developed for 1,2-disubstituted benzimidazoles but for 2-substituted benzimidazoles and benzothiazoles similar kinds of methods were rarely reported. So, it is worthy to develop a new and efficient "green" procedure in micellar medium provided by surfactants.

- Chromone is an important skeleton for different kinds of biologically important compounds. In this regard, 3-formyl chromone is an important starting material for these bio-active compounds because of the three different kinds of electron deficient centers specially aldehyde carbon. This scaffold is vulnerable to several nucleophiles, organic bases or acids, so, green and mild methods are required to functionalize C-3 position. There are few reports on Knoevenagel condensation to carry out the synthesis of 3-vinyl chromones. The drawbacks associated with these reported procedures are use of hazardous organic solvents and/or acids or bases and there are only few reports about green approaches for the vinylation of 3-formyl chromone. So, it is worthy to develop a new and efficient "green" protocol using micellar medium.

I.6. Objectives of the proposed research

1. Synthesis of sugar based gemini amphiphiles, characterization, study on their morphologies in solution phase and potential applications. (**Chapter 2**)

2. Synthesis of sugar based ionic bolaamphiphiles, characterization and an extensive study on

their morphologies in solution phase. (**Chapter 3**)

3. Synthesis, characterization and biological application of Tetraphenylethylene (TPE)-based amphiphilic molecules. (**Chapter 4**)

4. Use of amphiphilic molecules as catalysts for organic reactions in aqueous media. (**Chapter 3, 5 and 6**)

I.7. Outline of the thesis

Chapter I: Introduction

Chapter II: Synthesis, surface properties, DNA binding and cytotoxicity of D-Glucose based gemini surfactants

Chapter III: Synthesis and catalytic application of a novel class of D-glucose based bolaamphiphiles

Chapter IV: Synthesis and bio-medical applications of Tetraphenylethene (TPE)-based aggregation induced emission (AIE) -active fluorescent amphiphiles

Chapter V: Development of efficient and "green" synthetic methodology for 2-substituted benzimidazoles and benzothiazoles in aqueous micellar media

Chapter VI: Development of a mild and "green" synthetic methodology for 3-vinylchromones in aqueous micellar media

Chapter VII: Conclusions and future scope of the work

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CHAPTER II

Synthesis, surface properties, DNA binding and cytotoxicity of D-Glucose based gemini surfactants

II.1. Background of the present work

Over the past two decades, dimeric or gemini surfactants have generated considerable research interest with a view of developing 'next-generation' high-quality surfactants with various biological applications. Among them the cationic gemini surfactants draw major focus because of their simple synthetic strategies[1] and a broad range of bio-medical applications;[2-12] particularly, their use as gene delivery agents.[2-7]

Gene delivery is the introduction of a correct copy of gene/DNA across a biological cell membrane to the cell, to treat the acquired and inherited diseases e.g. cancer, neurogenerative diseases, hemophilia etc. It can be achieve by using synthetic, viral, or biological vectors.[3,13] As a synthetic vector it is well-documented that cationic gemini surfactants can form positively charged biocompatible complexes (lipoplexes) with negatively charged DNA,[13] which do not face much electrostatic barrier while penetrating the biological cell surfaces. In the last decade, this gene transfection ability of different cationic gemini surfactants has been reviewed.[13-15] However, the mechanism of gene transfer mediated by cationic surfactants is not fully understood[16,17] and thus, the strategy for the development of new delivery systems is merely empirical. In addition, the major disadvantage of most of these non-viral/synthetic vectors is their poor transfection efficacy. Therefore, the quest still remains to find out contemporary delivery systems that are more effective. Nonetheless, a large number of reports on the application of gemini surfactants as gene delivery vehicles have revealed that *m-s-m* type surfactants with small to medium spacer "*s*" length and medium length of side chains "*m*" and having a quaternary ammonium group as the polar head are among the leading candidates for this purpose.[13,14]

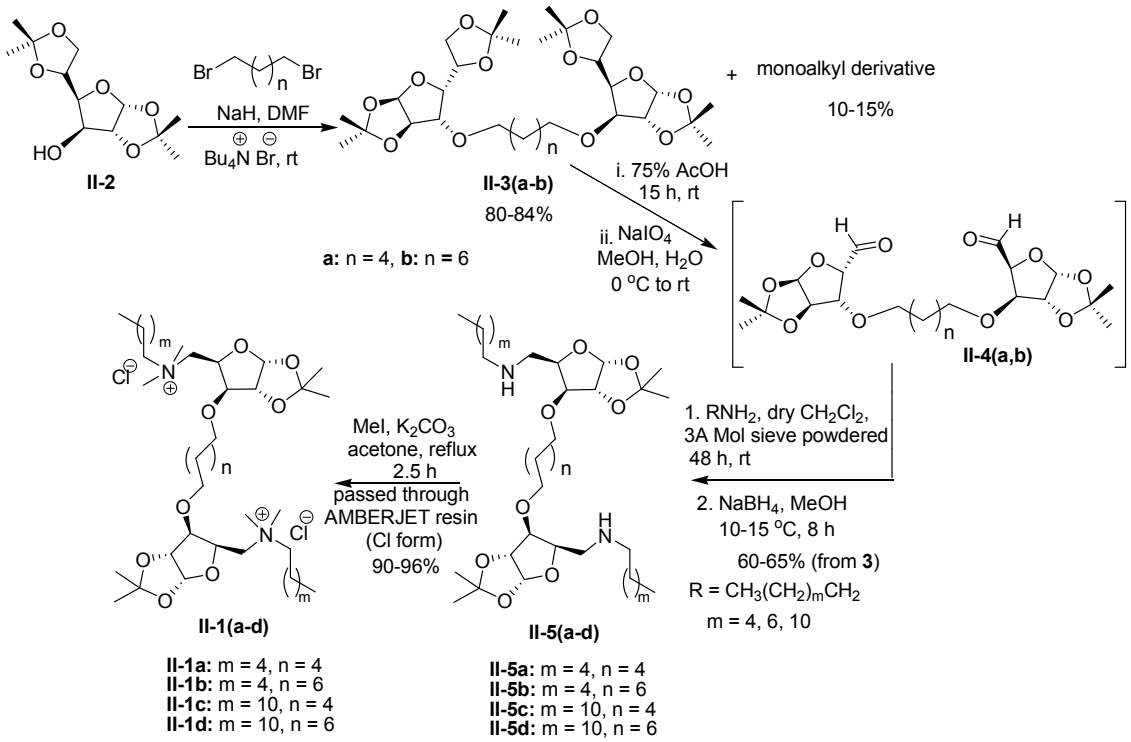
In most of the nonionic and ionic sugar based gemini surfactants, synthetic strategies utilize the functional group at anomeric carbon to link an aliphatic chain by imine or amide bond formation.[18] Some of them adopt longer routes involving several protection deprotection steps

for the syntheses of the desired surfactants.[19] However, C-3 functionality of glucose was rarely used for the construction of gemini surfactants.[19b] In this view, to further expand the available structures of sugar-based surfactants, we were interested in designing gemini surfactants that connect two monomeric units with quaternary ammonium group as the polar head by a linker via a sugar residue utilizing C-3 functionality of diisopropylidene glucose. We assumed, the natural angle and the partial conformational rigidity provided by the glucofuranoside ring to *spacer-polar head-side chain* would insist these molecules to stay in "π" shape and thereby, they would form vesicles and/or micelles in solution with ease which could aid in enhancing binding abilities with plasmid DNA. In this endeavor, we describe the development of an efficient synthetic strategy for a number of D-glucose derived *m-s-m* type cationic gemini surfactants having a quaternary ammonium group as the polar head (**II-1a-d**). In addition, their surface properties and preliminary studies on their DNA binding affinity have been studied.

II.2. Results and Discussion

II.2.1. Synthesis of gemini surfactants and their structural elucidation

D-Glucose was converted to 1,2:5,6-di-*O*-isopropyledene glucofuranoside (**II-2**) via a known procedure.[20] Compound **II-2** on alkylation with alkyl dibromide of variable chain length produced the initial building blocks (**II-3a,b**) in high yields (Scheme II-1). These building blocks (**II-3a,b**) on selective deprotection followed by periodate oxidation of the resulting diols produced relatively pure gemini aldehydes (**II-4a,b**). The aldehydes were found to be unstable in nature and were used up immediately for the next reaction without further characterization. The gemini amines (**II-5a-d**) were prepared from corresponding aldehydes (**II-4a,b**) by attachment of appropriate long chain amines via reductive amination. In the final step, the gemini amines were quaternized by refluxing with methyl iodide followed by passing through a bed of AMBERJET resin (in chloride form) to produce the cationic gemini surfactants (**II-1a-d**) with chloride as the counter anion in good overall yields. The compounds were found to be moderately soluble in water. However, the attempted acid catalyzed deprotection of acetonide group to promote solubility led to a complex mixture of inseparable products. Nonetheless, to our delight, the gemini surfactants, **II-1a-d** showed appreciable surface active properties and DNA binding affinity.



Scheme II-1. Synthetic route to the D-glucose derived gemini surfactants **II-1a-d**.

The structure of the new gemini surfactants (**II-1a-d**) have been established by ^{1}H NMR, ^{13}C NMR and ESI-MS. The formation of cationic gemini surfactants from the corresponding gemini amines was indicated by the appearance of two quaternary methyl singlets within the range δ 3.41-3.48 ppm for each part of -$N^{+}(C\underline{H}_3)_2$ for all the gemini surfactants and the downfield shift of one of the methylene protons next to quaternary nitrogen (-CH-C$\underline{H}_2$-N^{+}-) from the region of δ 2.90-3.00 ppm to ~ δ 4.70 ppm in ^{1}H NMR. In addition, the appearance of two methyl carbon signals at δ ~52.0 ppm for the two quaternary methyl carbons of each part of -$N^{+}(\underline{C}H_3)_2$ in ^{13}C NMR spectrum further supported the formation of desired products. The formation of these gemini surfactants (**II-1a-d**) were re-confirmed by ESI-MS (positive ion) mass spectroscopy. In each case, appearance of a base peak at *m/z* = (½ x MW-2Cl) (as *z* = 2) confirmed the formation of dicationic gemini surfactants. Therefore, peaks appeared at 343, 357, 427 and 441 for **II-1a**, **II-1b**, **II-1c** and **II-1d**, respectively. All the spectral studies clearly established the formation of the desired gemini surfactants.

II.2.2. Surface properties

II.2.2.1. Critical micellization concentration (CMC)

Geminis mostly possess unexpectedly low CMC values than the corresponding monomeric surfactants and CMC often reduces with the increase in hydrophobic character in the molecule.[21,22] The CMC values were determined from the conductivity versus concentration plots (Fig. II-1). The CMCs were further verified by steady-state fluorescence experiments using pyrene as the probe (Fig. II-2).[23] In the absence of micelles (below CMC) pyrene senses the polar environment of water molecules; the ratio of fluorescence emission intensities corresponding to the first and third vibrational peaks (I_I/I_{III}) is high. Above CMC, when micelles are present, and owing to the high hydrophobicity of pyrene molecules, these are solubilized in the interior micellar phase. This is a hydrocarbon-like solvent and therefore, the environment sensed by pyrene is less polar. As a result, the ratio I_I/I_{III} decreases.

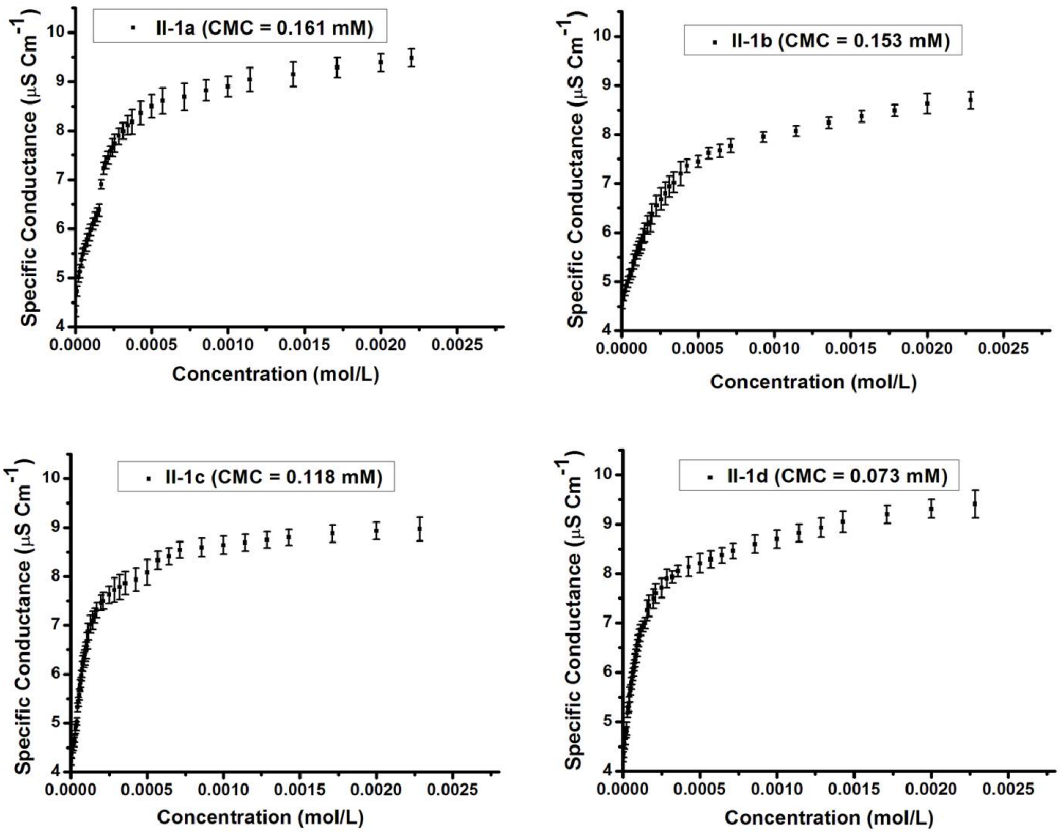


Fig. II-1. Specific conductivity vs. concentration plots of gemini surfactants, **II-1a-d**.

In Fig II-1, the point of deviation from sharp increase of specific conductance was considered as point of micellization and CMC values were obtained from the curves. The observed CMC of surfactants **II-1a, II-1b, II-1c** and **II-1d** are 0.161 mM, 0.153 mM, 0.118 mM and 0.073 mM, respectively. A general trend was observed: the CMC decreases with increasing chain length. The lower value of CMC for **II-1d** compared to others is a direct consequence of increased overall hydrophobicity of the former. The observed CMC values were of the same order of magnitude of previously reported sugar derived cationic gemini surfactants.[24,25] In addition, Menger et al.[24] and Negm et al.[25] also observed the tendency of lowering of CMC values with increase in chain length in a series of gemini surfactants, which matched with the results obtained with this new series of gemini surfactants. It is mention that the CMC values from conductometric measurements are obtained from the bulk solution which is unaffected by any interfacial events. To check the CMC values from conductometric measurements, steady-state fluorescence measurements were also performed to further verify them (Fig. II-2). From these experiments the CMCs of **II-1a**, **II-1b**, **II-1c** and **II-1d** were found as 0.155 mM, 0.150 mM, 0.122 mM, and 0.081 mM, respectively, which closely matched with the CMCs obtained by conductometric measurements.

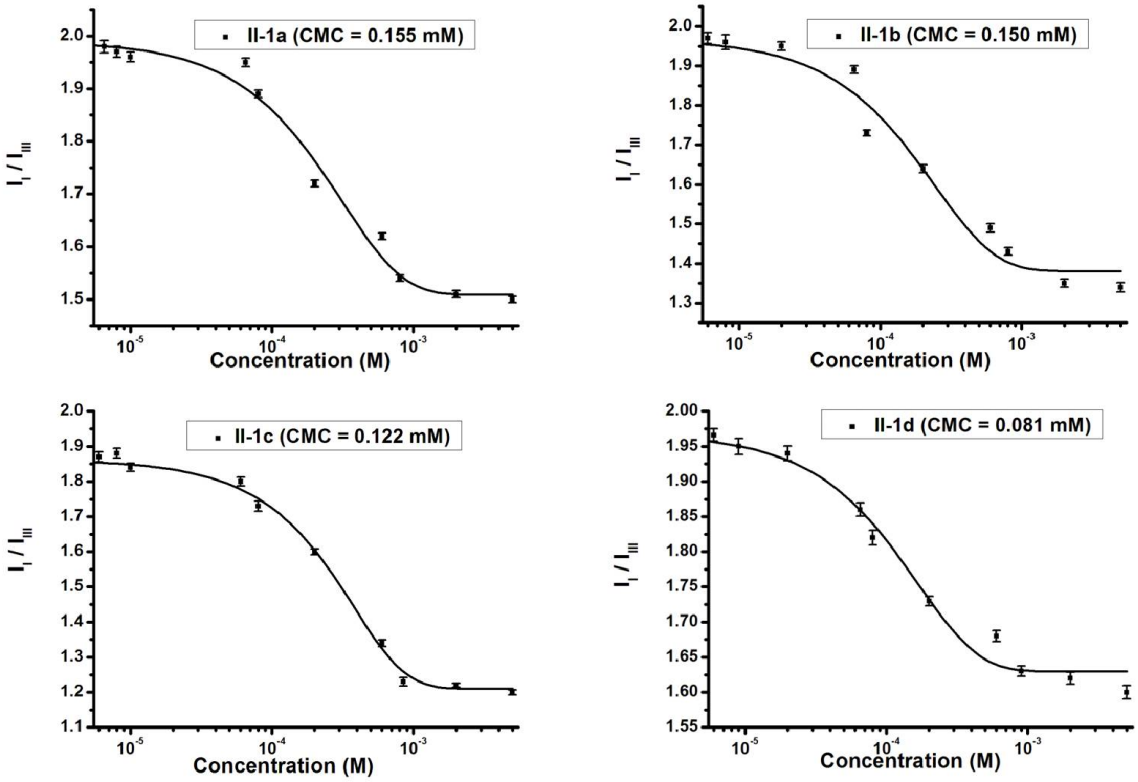
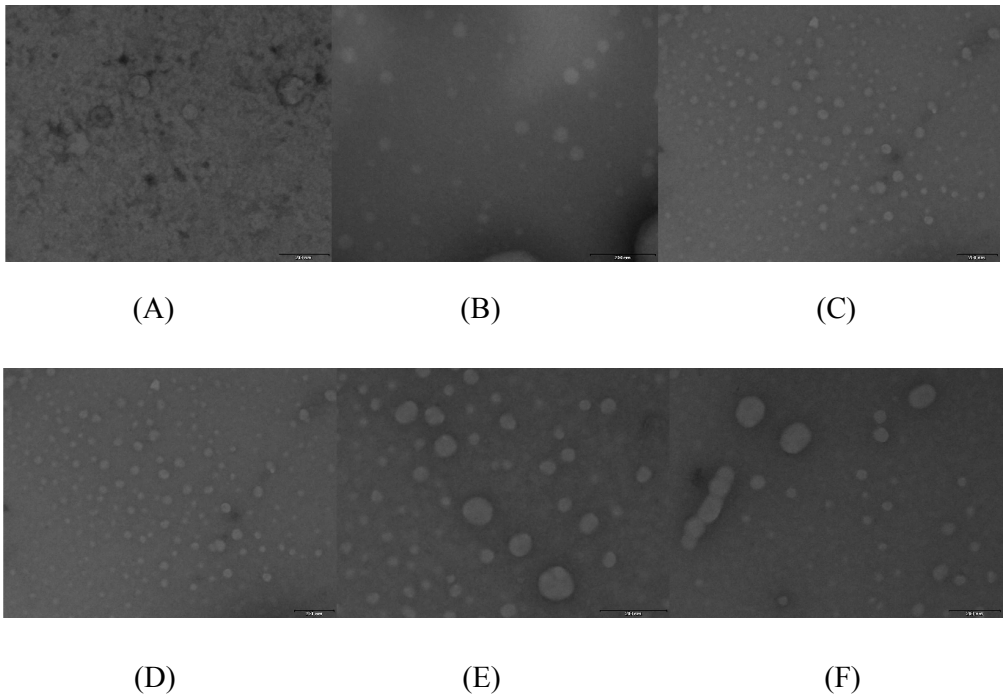


Fig. II-2. CMC curves of gemini surfactants obtained from steady-state fluorescence measurement.

II.2.2.2. Aggregation behavior of gemini surfactants in water

The aggregation morphologies of all the gemini surfactants in contact with water were investigated by TEM analysis (Fig. II-3).[26] It was found that they readily form micelle after 1 day aging of the solution except **II-1a,** where micelle formation occur rarely. In some cases, coexistence of other morphologies (e.g. vesicles) was also observed upon aging the surfactant solutions for longer time e.g., coexistence of both spherical micelle and vesicles was found in case of **II-1d** on standing the aqueous solution of surfactant for 7 days (Fig. II-3E). Furthermore, aggregation of spherical micelles to form a wormlike miceller arrangement was occasionally found (Fig. II-3F). It is presumed that the increased hydrophobic character of **II-1d** is advantageous for vesicle formation. Similar aggregation pattern was also observed in case of **II-1c**. However, vesicles were not observed both in the case of **II-1a** and **II-1b**.



(A) (B) (C)

(D) (E) (F)

Fig. II-3. TEM images of (A) occasional formation of micelle for (**II-1a**) after 1 day aging of the solution; (B), (C) & (D) spherical micelle for **II-1b-d** after 1 day aging of the solution; (E) coexistance of spherical micelles and vesicles for 12-8-12 (**II-1d**) after 7 days aging of the solution; (F) occasional aggregation of spherical micelles to form a wormlike miceller arrangement for 12-8-12 (**II-1d**).

II.2.3. DNA binding assay

II.2.3.1. Agarose gel electrophoresis

The physico–chemical properties of these molecules led us to assume a potential interaction with biological substrates such as DNA. Hence we decided to carry out preliminary studies on their DNA binding ability. The gemini surfactants (**II-1a-d**) were examined for their DNA binding capability by agarose gel electrophoresis (Fig. II-4). The commercially available monomeric surfactant, CTAB[27] was used as a reference material to determine the relative binding ability of these gemini surfactants. During gel electrophoresis it was observed that ethidium bromide (EB, 3,8-diamino-5-ethyl-6-phenylphenanthridiniumbromide) bands are present with equal intensity within the concentration range 12.5-100 μM for both CTAB and **II-1a** indicating no prominent binding with pDNA (pET-28a). In contrast, ethidium bromide band disappeared at 25 μM for **II-1d**, 37.5 μM for **II-1c** and 100 μM for **II-1b**. Disappearance of the bands may be explained considering the displacement of ethidium

bromide dyes by gemini surfactants in course of the formation of DNA-surfactant conjugate and thereby, the migration of DNA is retarded.[28,29] The number of cationic nitrogen of gemini surfactant required per phosphorous residue of pDNA (i.e. N/P ratio) for complete complexation was found to be close to 3 for **II-1d**, which is comparable with other cationic amphiphiles having excellent gene transfection ability.[28,30,31] Surfactant **II-1c** was found equally good to bind to DNA (N/P ratio: 4.2). **II-1b** binds to DNA but only at higher concentration.[32]

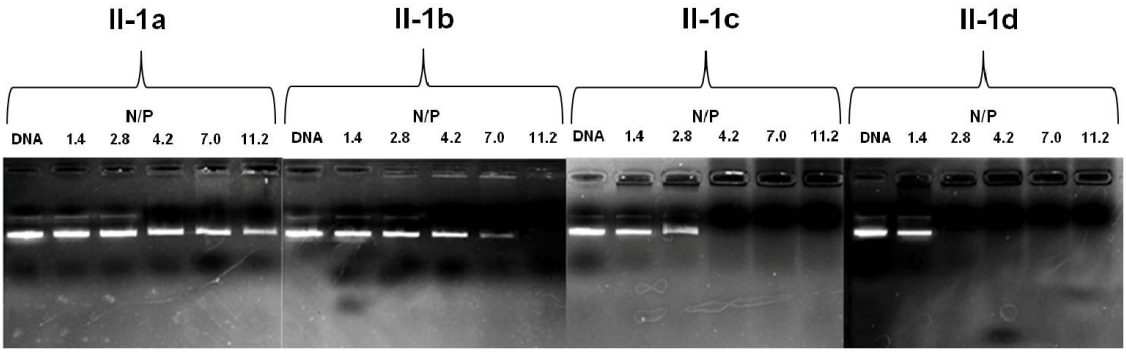


Fig. II-4. Agarose gel (1%) electrophoresis gel patterns of gemini-DNA complex of **II-1a-d** at concentrations 12.5, 25, 37.5, 62.5 and 100 μM (the corresponding N/P values are 1.4, 2.8, 4.2, 7.0 and 11.2, respectively).

II.2.3.2. Fluorometric titration

Fluorometric ethidium bromide exclusion assay was carried out to validate the results of agarose gel electrophoresis. It is well-documented that the fluorescence emission of ethidium bromide increases as a result of its intercalation between the DNA base pairs relative to that in water.[33] The extent of binding of a surfactant can be determined by its ability to displace EB from this intercalation complex leading to quenching of the fluorescence signal (up to 90%) due to formation of surfactant-DNA complex.[34] Thus, titration of EB-DNA complex with gemini surfactants were conducted by addition of surfactant solutions in several portions keeping concentration of each surfactant same to get a clear idea about the nature of binding of these surfactants with DNA. This experiment also revealed that binding is greatly influenced by alkyl chain length of the surfactants. It was observed that 50% fluorescence quenching occurs after addition of third portion of surfactant solution for **II-1d** and on the 4th addition for **II-1c** (Fig. II-5). Although **II-1b** could able to cause 50% quenching of fluorescence only after several additions of surfactant solutions, drop of fluorescence

intensity in case of **II-1a** was very nominal and 50% quenching was not reached within the concentration range that we have studied (Fig. II-5). Similar to the agarose gel electrophoresis, this experiment also explains better ability of long chain gemini to displace EB from DNA more efficiently and proves the chain length dependent binding behavior of this new series of gemini surfactants. Thus, it may be concluded that hydrophobic interactions cause enhancement in the rate of exclusion of EB from DNA. The exact concentration of the gemini surfactants (**II-1a-c**) required to displace ethidium bromide from

DNA, which brings about 50% decrease in fluorescence intensity, was determined by a plot of fluorescence intensity at 595 nm vs. concentration of surfactant (Fig. II-6). As expected, **II-1d** showed 50% of EB fluorescence decrease at lowest N/P ratio followed by **II-1c**. The exact concentration at which 50% decrease in fluorescence intensity occurs, as obtained from the graph, are 201.2 μM, 30.4 μM, 23.2 μM for **II-1b**, **II-1c** and **II-1d**, respectively. Presumably, a combination of the hydrophobic interactions[35] and restrictive conformational freedom imparted by the glucofuranoside ring may be playing an important role in strong binding with DNA for **II-1c** and **II-1d**. In a recent study, Dias et al.[36] described that increase in hydrophobicity of nonpolar part of surfactants influences association with DNA and hydrophilic ionic group opposes complexation. However, balance of both the forces is essential for the integrity of the double-helical structure of DNA. Thus, higher hydrophilicity of shortest chain gemini surfactant of this series, **II-1a** may be opposing complexation between surfactant and DNA. Whereas, as proper balance of hydrophilic and hydrophobic character in the molecule is arrived by increasing the tail length to 12 i.e. for **II-1c** and **II-1d**, they show better binding ability with DNA.

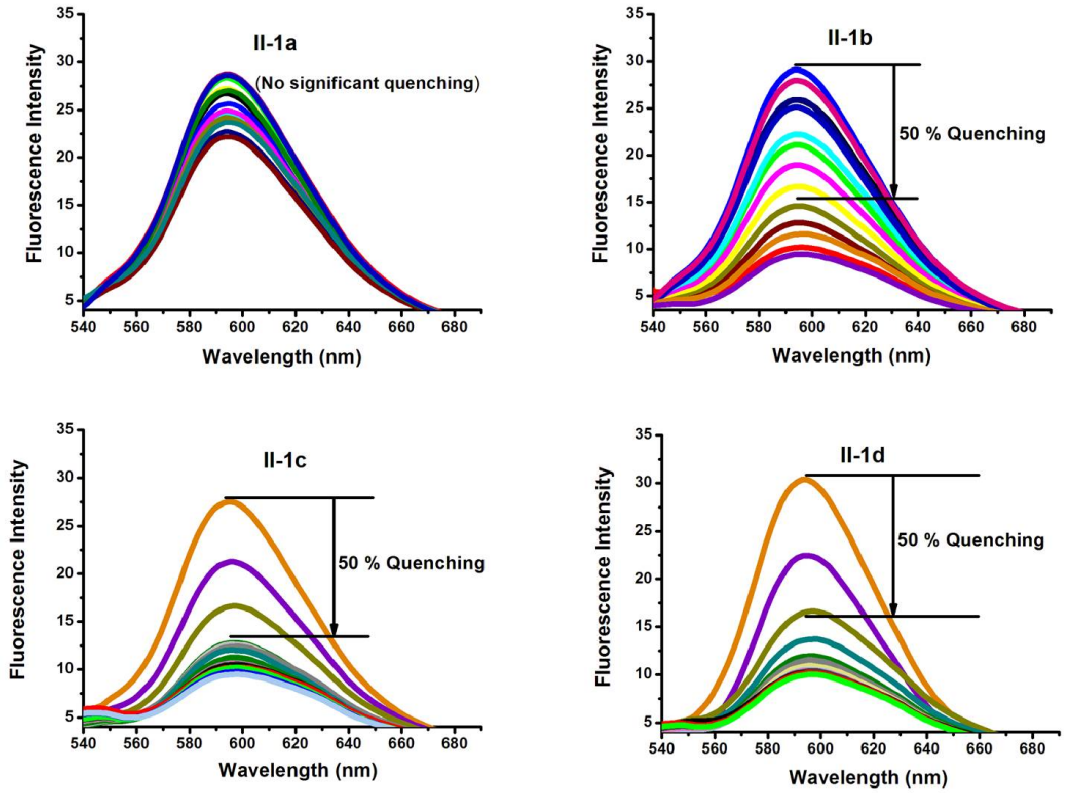


Fig. II-5. Fluorimetric titration of pDNA-etidium bromide complex with gemini surfactants 6-6-6 (**II-1a**), 6-8-6 (**II-1b**), 12-6-12 (**II-1c**) and 12-8-12 (**II-1d**).

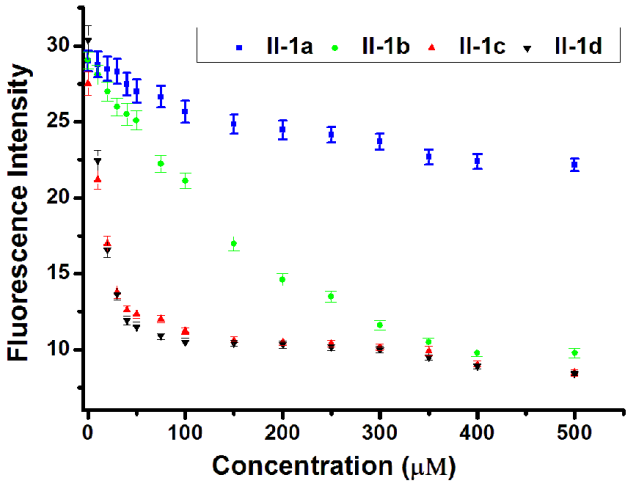


Fig. II-6. Fluorescence intensity observed at 595 nm vs. concentration (μM) plot of gemini surfactants **II-1a-d**.

II.2.3.3. Particle size determination of gemini-DNA conjugate

To estimate the size of the gemini-DNA conjugate, DLS experiment was carried out.[28] We have restricted this study to **II-1c** and **II-1d** only as they showed good binding affinity with DNA. Initially, the gemini-DNA conjugate at different N/P ratios were prepared by addition of increasing quantities of **II-1c** and **II-1d** from stock solutions to pDNA solution. It was observed that the particle size is relatively small at low N/P ratio (around 350 nm) for both **II-1c** and **II-1d**. In case of **II-1d**, the size of the particles in the solution increased rapidly above N/P 3.0 reaching a maximum at around N/P 4.0 (~ 1500 nm) and then sharply decreased till N/P 6.0, and continued to decrease slowly (Fig. II-7). A similar pattern was obtained for **II-1c**. In this case, the sharp increase in particle size was observed at around N/P 5.5. Thus, relatively higher gemini concentration probably favors aggregation of gemini-DNA complex into larger particles. However, further addition of gemini surfactants leads to decrease in particle size because of the strong positive charge on the complex.[37-39] Our observations are in line with recent reports on other cationic amphiphiles.[28,39] DLS experiments further established the fact that the geminis **II-1c** and **II-1d** strongly bind to pDNA at or above N/P ratio 4.2 and 2.8, respectively.

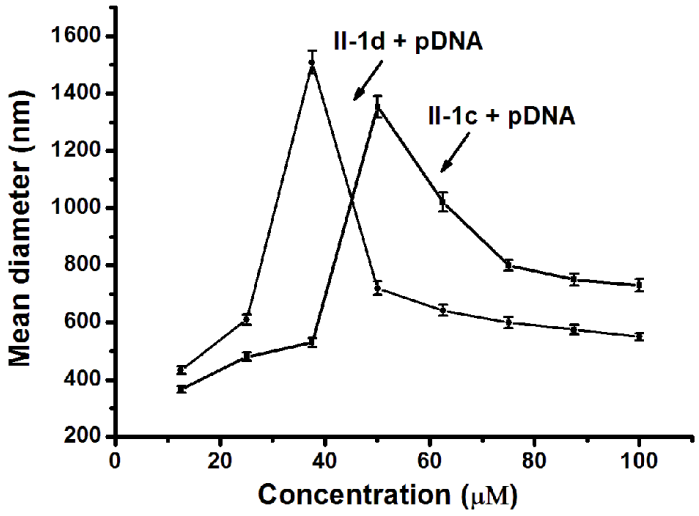


Fig. II-7. Mean diameters of gemini-pDNA complexes of **II-1c** and **II-1d** at different concentrations as measured by DLS.

II.2.4. Cytotoxicity

Low toxicity of nonviral delivery agents toward animal cell lines is crucial for gene delivery. Cytotoxicity of gemini surfactants **II-1a-d** were assessed on HeLa cell line using MTT assay. In the concentration range 10-100 μM of gemini surfactants with 24 h incubation, the observed cell viability was close to 80% for most of the cases (Fig. II-8). It was observed that the cytotoxicity gradually decreases with increase in alkyl chain length. The gemini surfactant **II-1d**, which was most efficient in binding to DNA, was found to be least toxic. Although not much is known about cytotoxicity of sugar derived *m-s-m* type cationic gemini surfactants, the decrease in cytotoxicity with increasing chain length of the surfactant molecules of similar skeleton has been observed by others.[40,41] In a recent study on cytotoxicity of simple *m-s-m* type gemini surfactants with ammonium as polar head group, Almeida et al.[40] observed that surfactants with longer chain length "m" are less toxic than shorter ones. Bhadani et al.[41] also reported decrease of toxicity with increase in alkyl chain length among gemini surfactants with pyridinium as polar head. The obtained results showed potential for this new series of gemini surfactants, especially **II-1d**, for further elaboration of studies for efficient gene delivery vectors.[32]

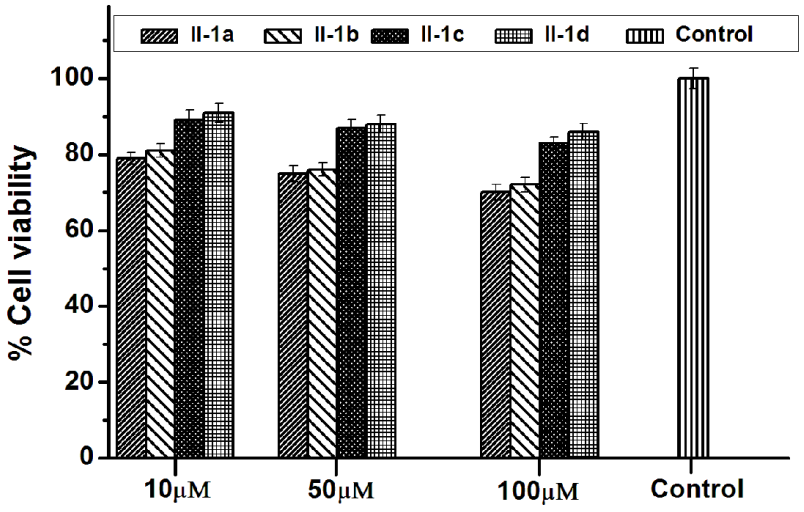


Fig. II-8. Cytotoxicity of gemini surfactants **II-1a-d** based on MTT assay.

II.3. Conclusion

In the present study, a simple and efficient synthetic methodology was developed by utilizing the free C-3 hydroxy group of diisopropylidene glucose for the synthesis of four new D-glucose derived gemini surfactants **II-1a-d**. They showed interesting surface properties and low CMCs. The microscopic studies revealed that they can spontaneously aggregate in aqueous solutions forming micelles, vesicles etc. DNA binding capabilities of these surfactants were determined by agarose gel electrophoresis, fluorescence titration, and DLS experiments. Preliminary studies revealed that two of the gemini surfactants, **II-1c** and **II-1d** possess good binding capability toward pDNA with relatively low N/P ratio. The MTT assay showed that the cytotoxicity of these new surfactants is low and it further decreases with increase in alkyl chain length. Finally, these new D-glucose derived gemini surfactants show excellent surface active properties, they have low cytotoxicity and two of them are potentially good candidates for gene delivery.

II.4. Experimental Section

II.4.1. Materials & methods

Dibromoalkanes, alkylamines and pyrene were purchased from Sigma-Aldrich and used without further purification.

Ethidium bromide, Dulbecco's Modified Eagle's Media (DMEM), Fetal Bovine Serum (FBS), Phosphate Buffer Saline (PBS) and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) were purchased from Himedia Laboratories, Mumbai. Plasmid DNA (pET-28a) was purchased from Bangalore GeNei, Bangalore, India. Other common reagents were procured either from SD Fine - Chem Limited, Mumbai, India or from Merck, India. Milli-Q (18MΩ) water was used in all experiments as per requirement. All the stock solutions of surfactants were prepared by using 10% DMF in Tris-EDTA buffer solution unless otherwise stated. Further dilutions were made by using Tris-EDTA buffer as per requirements.

NMR spectra were recorded on BRUKER NMR 300 MHz system using tetramethylsilane as an internal standard. The following abbreviations are used in reporting NMR data: s, singlet; brs, broad singlet; d, doublet; t, triplet; dd, doublet of doublets, dq, doublet of quartets; tdd, triplet of doublet of doublets; m, multiplet. Mass spectra were recorded on Waters Q-TOF

micro mass spectrometer or Brucker Esquire 3000 mass spectrometer using ESI as the ion source. Optical rotation were recorded on a JASCO B-2000 digital polarimeter. Conductivity was measured on a HANNA instruments auto temperature conductivity meter (model: HI 2300) fitted with a conductivity cell. Agarose gel was viewed under UV transilluminator (BIO-RAD, USA). Fluorescence studies were carried out on a JASCO FP6300 fluorescence spectrophotometer (JASCO Corp., Japan). Particle size of the DNA-surfactant conjugates was determined using a particle size analyzer (Delsa Nano S, Beckman Coulter, USA). Absorbance studies were carried out on a SHIMADZU UV–2450 UV-visible spectrophotometer (SHIMADZU Corp., Japan) for cytotoxicity assay.

II.4.1.1. Conductivity measurement

For conductivity measurement the solutions were kept in the cell at 25±0.1 °C with the help of a thermostat. The CMC of synthesized gemini surfactants were determined by adding adequate volumes of concentrated stock solutions of surfactants (0.1 M, 0.1 M, 0.05 M, 0.05 M stock solutions of gemini surfactants **II-1a**, **II-1b, II-1c** and **II-1d**, respectively) in measured amount of Milli-Q water (7 mL) so as to change the surfactant concentration well below and above CMC. The measured specific conductance was plotted against concentration (C). The point of deviation from sharp increase of specific conductance was considered as CMC.

II.4.1.2. Steady-state Fluorescence Measurement

CMC values were also determined by steady state fluorescence measurement in a quartz fluorescence cell by using a Shimadzu FP 6300 fluorescence spectrophotometer (Shimadzu corp., Japan) and pyrene. The CMC of gemini surfactants were determined by adding adequate volumes of concentrated stock solutions of gemini surfactants in pyrene solution. Pyrene was excited at 335 nm and the emission spectra were scanned from 350 to 450 nm. The plot of I_I/I_{III} against gemini surfactant concentration gave a sharp decrease in each case, which corresponds to the CMC value of the bolaamphiphile surfactants.

II.4.1.3. Transmission electron microscopy

Aqueous solutions of the cationic gemini surfactants (10^{-4} M) were prepared and aged for 1 to 7 days, and then applied to carbon coated copper grids. The samples were examined on a TECNAI G^2 FEI electron microscope operating at 60 kV.[26]

II.4.1.4. Agarose gel electrophoresis & Cytotoxicity assay

For agarose gel electrophoresis 120 ng of plasmid DNA (pET-28a) and 0.25, 0.5, 0.75, 1.25, and 2.0 μL of 1 mM solution of gemini surfactants were mixed with DNA loading dye and incubated. Then 20 μL of each reaction mixture was loaded on a 1% agarose gel made in Tris-acetate-EDTA buffer containing ethidium bromide. After completion of the assay, the gel was viewed under UV transilluminator and photographed.[32]

Cytotoxicity was performed using MTT assay. HeLa cell lines were grown in standard medium as per protocol and they were seeded at a density of 5 x 10^4 cells per well. After 24 h of incubation, the cells were supplemented with gemini surfactants at different concentrations. After incubation for 24 h, MTT was added per well and incubated for 4 h. Finally absorbance was measured after following standard protocol at 570 nm and cell viability was expressed as relative absorbance (%) of the sample vs control cells.[32]

II.4.1.5. Ethidium bromide exclusion experiment

Ethidium bromide exclusion experiments were carried out in a quartz fluorescence cell and fluorescence signal output was measured on a fluorescence spectrophotometer. The excitation wavelength was set at 520 nm and corresponding emission spectra were recorded. 1μg of pDNA (pET-28a) was used for each experiment. DNA solution was mixed with 1 μL of ethidium bromide (0.5 mg/mL) in the fluorescence cell. Autoclaved Tris-EDTA buffer (pH 8) was added to make a total volume of 2 mL. Titrations with gemini surfactants were done by sequential addition of surfactant solutions (2 μL of 10 mM surfactant stock solutions were added 5 times to obtain concentration range 10-50 μM, followed by addition of 1 μL of 50 mM stock solutions 2 times to achieve concentrations 75 and 100 μM, and then 8 additions of 2 μL of 50 mM stock solutions for concentration range 150-500 μM) and 10 min incubation at room temperature before recording the fluorescence spectrum. All observations were expressed by intensity vs. wavelength plots.

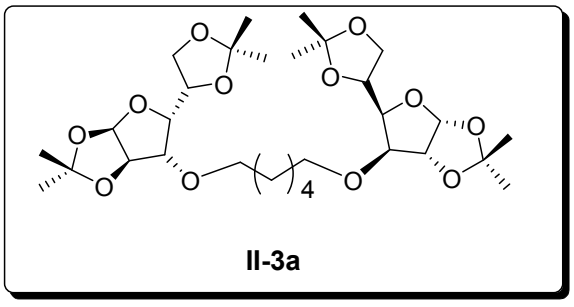
II.4.1.6. Particle size analysis

Particle size of the DNA-surfactant conjugates was determined with the following specifications: medium viscosity 0.8878 cP; refractive index (RI) medium 1.33; scattering

angle 90°; temperature 25 °C. The gemini surfactants and pDNA complexes were prepared according to desired concentrations (i.e. 12.5, 25.0, 37.5, 50.0, 62.5, 75.0, 87.5 and 100 μM) of Gemini surfactants using 1 μg of pDNA in Tris-EDTA buffer (pH 8.0) [the calculated N/P ratio are 1.4, 2.8, 4.2, 5.6, 7.0, 8.4, 9.8 and 11.2]. All the solutions were filtered through a 0.2 μm membrane filter of mixed cellulose acetate before preparation of the sample solutions. The sample solutions were incubated for 10 min at room temperature before measurements. The particle size measurements were repeated for 3 times for each sample and data were reported as the average.

II.4.2. General procedure for the preparation of initial building blocks of gemini surfactants (II-3a, b): The general procedure is illustrated by the preparation of **II-3a.**

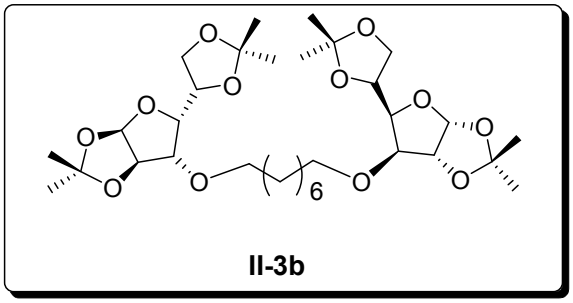
To a stirred suspension of sodium hydride (0.48 g, 12 mmol, 60% w/w in oil) in dry THF (5 mL) was added a solution of diisopropylidene glucose (**II-2**) (2.6 g, 10 mmol) in anhydrous THF (10 mL) at 0 ºC under nitrogen atmosphere. The mixture was stirred at room temperature for 1 h. It was re-cooled to 0 ºC and a solution of 1,6-dibromo hexane (0.77 mL, 5 mmol) in anhydrous THF (5 mL) and tetrabutylammonium iodide (370 mg, 1 mmol) were added one after another. The mixture was stirred at room temperature for 48 h and then quenched by addition of excess saturated NH_4Cl solution. After removal of THF the aqueous part was extracted with Et_2O (2 x 15 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated. Column chromatography of the crude product over silica gel (100-200 mesh) eluting with 8% EtOAc in petroleum ether (60-80 °C) afforded compound **II-3a**.



II-3a

1,6-Di-(5-(2,2-Dimethyl-[1,3]dioxolan-4-yl)-2,2-dimethyl-tetrahydro-furo[2,3-d][1,3]dioxol-6-oxy)-hexane **(II-3a):** Colourless liquid, yield: 84%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.32

(6H, s), 1.35 (6H, s), 1.42 (6H, s), 1.50 (6H, s), 1.34-1.61 (8H, m overlapped), 3.55 (4H, tdd, J = 6.3, 9.2, 28.0 Hz), 3.84 (2H, d, J = 3.0 Hz), 3.98 (2H, dd, J = 5.9, 8.5 Hz), 4.05-4.14 (4H, m), 4.26-4.33 (2H, m), 4.52 (2H, d, J = 3.7 Hz), 5.87 (2H, d, J = 3.7 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 25.4 (2C), 25.9 (2C), 26.2 (2C), 26.77 (2C), 26.83 (2C), 29.7 (2C), 67.2 (2C), 70.5 (2C), 72.5 (2C), 81.1 (2C), 82.1 (2C), 82.5 (2C), 105.2 (2C), 108.8 (2C), 111.7 (2C); IR (neat): 2986, 2935, 1456, 1375, 1215, 1164, 1078, 1022, 849, 756 cm^{-1}; ESI-MS: *m/z* 625 [M^+ +23]; $[\alpha]_D^{25}$: -28.57 (C 2.1, $CHCl_3$).



II-3b

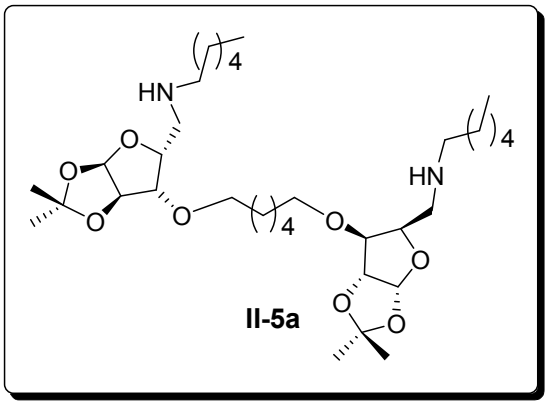
1,8-Di-(5-(2,2-Dimethyl-[1,3]dioxolan-4-yl)-2,2-dimethyl-tetrahydro-furo[2,3-d][1,3]dioxol-6-oxy)-octane **(II-3b):** Colourless liquid, yield: 80%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.32 (6H, s), 1.35 (6H, s), 1.30-1.35 (8H, m, overlapped), 1.42 (6H, s), 1.50 (6H, s), 1.50-1.63 (4H, m, overlapped), 3.55 (4H, tdd, J = 6.4, 9.2, 28.0 Hz), 3.84 (2H, d, J = 3.0 Hz), 3.98 (2H, dd, J = 6.0, 8.5 Hz), 4.02-4.18 (4H, m), 4.27-4.33 (2H, m), 4.52 (2H, d, J = 3.7 Hz), 5.87 (2H, d, J = 3.7 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 25.3 (2C), 25.9 (2C), 26.2 (2C), 26.7 (2C), 26.8 (2C), 29.3 (2C), 29.6 (2C), 67.1 (2C), 70.6 (2C), 72.4 (2C), 81.1 (2C), 82.0 (2C), 82.4 (2C), 105.2 (2C), 108.7 (2C), 111.6 (2C); IR (neat): 2985, 2929, 2857, 1457, 1375, 1252, 1215, 1077, 1021, 850 cm^{-1}; ESI-MS: *m/z* 653 [M^+ + 23]; $[\alpha]_D^{25}$: -24.48 (C 1.12, $CHCl_3$).

II.4.3. General procedure for the preparation of gemini amine (II-5a-d): The general procedure is illustrated by the preparation of **II-5a.**

A solution of **II-3a** (1.32 g, 2.2 mmol) in aqueous AcOH (75% v/v, 20 mL) was stirred for 15 h at 25 ºC. The reaction mixture was evaporated and the residue was co-evaporated with dry toluene. The syrupy liquid was dried under vacuum to get crude diols. The diols (1.09 g, 96%) was found sufficiently pure and used for the next step without further purification. To a solution of the diol (1 mmol) in CH_3OH (15 mL) was added dropwise a solution of $NaIO_4$ (2.4 mmol) in water (15 mL) at 0 ºC. The solution was allowed to warm up to 25 ºC and

stirring was continued for 3 h. The reaction mixture was filtered and the filtrate was concentrated. The residue was re-dissolved in $CHCl_3$ (20 mL), washed with 5% aqueous $Na_2S_2O_3$, brine, and then dried over anhydrous Na_2SO_4. Evaporation of the solvent afforded the aldehyde **II-4a**, which was found unstable in nature and used for the next step immediately without further purification.

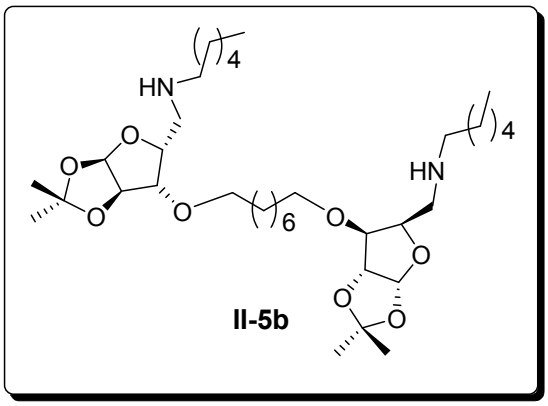
The crude aldehyde **II-4a** (1 mmol) was dissolved in dry CH_2Cl_2 (5 mL) and powdered 3Å molecular sieve (200 mg) was added into it. Then, hexyl amine (2.4 mmol) was added and the reaction mixture stirred at room temperature for 48 h. After complete imine formation MeOH (5 mL) was added and it was cooled to 0 ºC. Next, $NaBH_4$ (12 mmol) was added portion wise to the reaction mixture and it was stirred at room temperature for 8 h. The reaction mixture was filtered, the filtrate was evaporated under vacuum and the corresponding residue was chromatographed over silica gel (60-120) using 10% MeOH in CH_2Cl_2 to afford the desired amine **II-5a** in good overall yield from **II-3a**.



II-5a

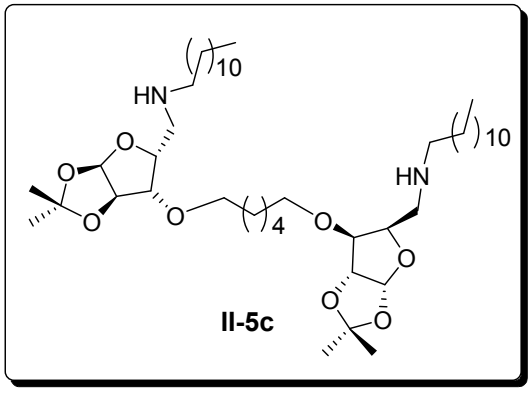
Hexyl-{6-[8-(5-hexylaminomethyl-2,2-dimethyl-tetrahydro-furo[2,3-d][1,3]dioxol-6-yloxy)-hexyloxy]-2,2-dimethyl-tetrahydro-furo[2,3-d][1,3]dioxol-5-ylmethyl}-amine **(II-5a):** Yellow liquid, yield: 60%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.87 (6H, t, J = 6.6 Hz), 1.31 (6H, s), 1.26-1.31 (12H, m), 1.49 (6H, s), 1.49-1.67 (6H, m, overlapped), 1.87 (6H, brs), 2.64 (4H, t, J = 7.2 Hz), 2.87 (4H, dq, J_1 = 4.8 Hz, J_2 = 12 Hz), 3.35-3.42 (2H, m), 3.57-3.64 (2H, m), 3.76 (2H, d, J = 3.3 Hz), 4.30-4.35 (2H, m), 4.54 (2H, d, J = 3.9), 5.90 (2H, d, J = 3.6 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 13.9 (2C), 22.5 (2C), 26.0 (2C), 26.2 (2C), 26.6 (2C), 29.2 (2C), 29.4 (2C), 29.5 (2C), 31.6 (2C), 47.9 (2C), 50.1 (2C), 70.2 (2C), 79.4 (2C), 82.1 (2C),

82.9 (2C), 104.7 (2C), 111.3 (2C); IR (neat): 2929, 2854, 1473, 1368, 1215, 1148, 1060, 1022, 868 cm^{-1}; ESI-MS: *m/z* 629 [M^+ + 1]; $[\alpha]_D^{25}$: -29.2 (C 2.1, $CHCl_3$).



II-5b

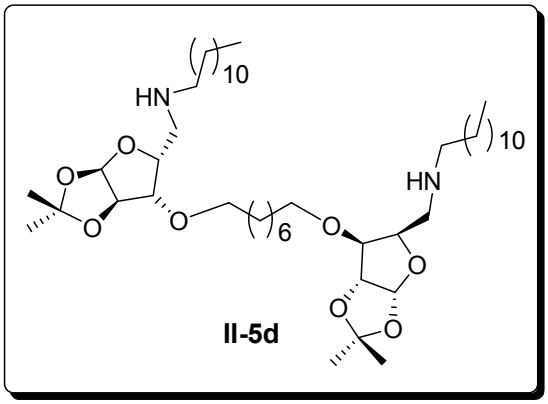
Hexyl-{6-[8-(5-hexylaminomethyl-2,2-dimethyl-tetrahydro-furo[2,3-d][1,3]dioxol-6-yloxy)-octyloxy]-2,2-dimethyl-tetrahydro-furo[2,3-d][1,3]dioxol-5-ylmethyl}-amine **(II-5b):** Yellow liquid, yield: 61%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.88 (6H, t, *J* = 6.7 Hz), 1.31 (26H, brs), 1.49 (6H, s), 1.49-1.67 (8H, m, overlapped), 2.93-3.02 (4H, m), 3.24 (4H, d, *J* = 5.1 Hz), 3.41-3.48 (2H, m), 3.59-3.66 (2H, m), 3.92 (2H, d, *J* = 3.2 Hz), 4.45-4.49 (2H, m), 4.56 (2H, d, *J* = 3.7 Hz), 4.80 (2H, brs, exchangeable), 5.92 (2H, d, *J* = 3.7 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 13.8 (2C), 22.4 (2C), 25.8 (2C), 26.2 (2C), 26.4 (2C), 26.6 (2C), 27.5 (2C), 29.1 (2C), 29.4 (2C), 31.3 (2C), 47.0 (2C), 49.1 (2C), 70.2 (2C), 77.4 (2C), 81.8 (2C), 83.1 (2C), 104.9 (2C), 111.8 (2C); IR (neat): 2927, 2855, 1461, 1375, 1215, 1164, 1080, 1019, 888, 860 cm^{-1}; ESI-MS: *m/z* 657 [M^+ + 1]; $[\alpha]_D^{25}$: -34.28 (C 0.35, $CHCl_3$).



II-5c

Dodecyl-{6-[6-(5-dodecylaminomethyl-2,2-dimethyl-tetrahydro-furo[2,3-d][1,3]dioxol-6-yloxy)-hexyloxy]-2,2-dimethyl-tetrahydro-furo[2,3-d][1,3]dioxol-5-ylmethyl}-amine **(II-5c):**

Yellow liquid, yield: 64%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.88 (6H, t, J = 6.1 Hz), 1.26-1.32 (40H, m), 1.32 (6H, s), 1.49 (6H, s), 1.49-1.55 (8H, m), 2.66-2.71 (4H, m), 2.87-3.00 (4H, m), 3.36-3.43 (2H, m), 3.56-3.64 (2H, m), 3.79 (2H, d, J = 2.4 Hz), 4.36 (2H, brs), 4.54 (2H, d, J = 3.6 Hz), 5.90 (2H, d, J = 3.6 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 13.9 (2C), 22.4 (2C), 25.7 (2C), 26.1 (2C), 26.5 (2C), 27.0 (2C), 29.1 (4C), 29.3 (4C), 29.4 (8C), 31.7 (2C), 47.6 (2C), 49.7 (2C), 70.0 (2C), 78.9 (2C), 82.0 (2C), 82.8 (2C), 104.7 (2C), 111.3 (2C); IR (neat): 2924, 2854, 1671, 1462, 1375, 1215, 1164, 1081, 1020 cm^{-1}; ESI-MS: *m/z* 797 [M^+ + 1]; $[\alpha]_D^{25}$: -22.66 (C 1.8, $CHCl_3$).

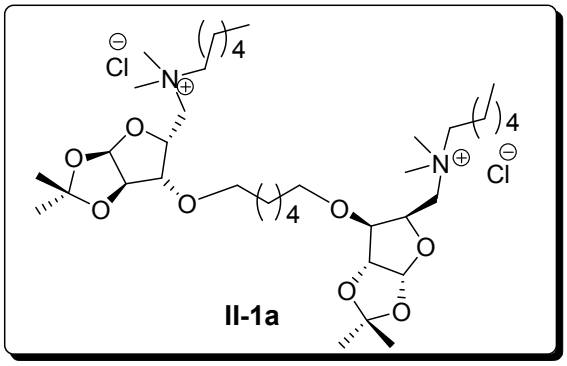


II-5d

Dodecyl-{6-[8-(5-dodecylaminomethyl-2,2-dimethyl-tetrahydro-furo[2,3-d][1,3]dioxol-6-yloxy)-octyloxy]-2,2-dimethyl-tetrahydro-furo[2,3-d][1,3]dioxol-5-ylmethyl}-amine **(II-5d):** Yellow liquid, yield: 63%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.88 (6H, t, J = 6.8 Hz), 1.26-1.32 (44H, m, overlapped), 1.32 (6H, s), 1.49 (6H, s), 1.49-1.60 (8H, m, overlapped), 2.64 (4H, t, J = 7.2 Hz), 2.82-2.98 (4H, m), 3.35-3.42 (2H, m), 3.56-3.64 (2H, m), 3.76 (2H, d, J = 3.1 Hz), 4.30-4.35 (2H, m), 4.54 (2H, d, J = 3.7 Hz), 5.90 (2H, d, J = 3.7 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 13.9 (2C), 22.5 (2C), 25.9 (2C), 26.1 (2C), 26.5 (2C), 27.1 (2C), 29.2 (2C), 29.36 (2C), 29.43 (8C), 29.46 (4C), 29.7 (2C), 31.7 (2C), 47.8 (2C), 50.0 (2C), 70.1 (2C), 79.3 (2 C), 82.1 (2C), 82.8 (2C), 104.7 (2C), 111.2 (2C); IR (neat): 2926, 2853, 1461, 1375, 1215, 1161, 1080, 1019, 888, 860 cm^{-1}; ESI-MS: *m/z* 826 [M^+ + 1], 825 [M^+]; $[\alpha]_D^{25}$: -33.12 (C 0.9, $CHCl_3$).

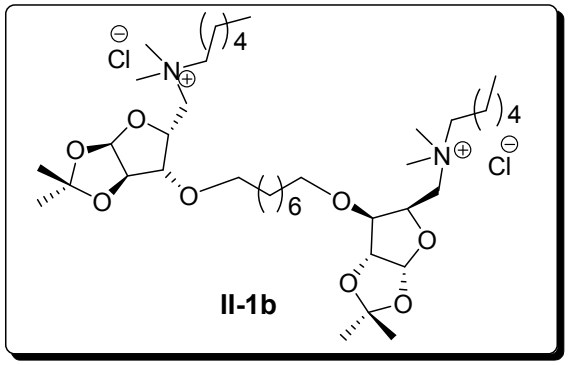
II.4.4. General procedure for the preparation of cationic gemini surfactant: quaternization of gemini amine (II-1a-d).

Gemini amines were dried under vacuum for several hours before use. The amine, **II-5a** (1 mmol) was dissolved in dry acetone (20 mL). To it K_2CO_3 (2.2 mmol) and methyl iodide (6 mmol) were added and it was refluxed for 2.5 h. Next, the reaction mixture was filtered through a bed of celite followed by anhydrous Na_2SO_4. The filtrate was passed through a column pre-packed with AMBERJET 4200 resin (in Chloride form). The eluent was concentrated to afford cationic gemini surfactant **II-1a**.

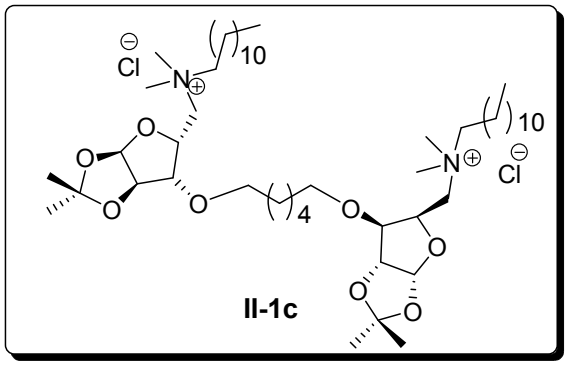


II-1a

Hexyl-{6-[8-(5-hexylaminomethyl-2,2-dimethyl-tetrahydro-furo[2,3-d][1,3]dioxol-6-yloxy)-hexyloxy]-2,2-dimethyl-tetrahydro-furo[2,3-d][1,3]dioxol-5-ylmethyltetramethylammonium dichloride **(II-1a):** Yellow viscous liquid, yield: 98%; ^{1}H NMR (CD_3OD, 300 MHz): δ 0.95 (6H, brs), 1.35 (6H, s), 1.41 (16H, s), 1.52 (6H, s), 1.63 (4H, brs), 1.85 (4H, *brs*), 3.25 (12H, s), 3.46-3.72 (10H, m), 3.97 (2H, d, J = 14.1 Hz), 4.09 (2H, d, J = 3 Hz), 4.59-4.62 (2H, m), 4.71 (2H, d, J = 3.9 Hz), 5.98 (2H, d, J = 3.6 Hz); ^{13}C NMR (CD_3OD, 75 MHz): δ 14.6 (2C), 23.7 (2C), 24.0 (2 C), 26.6 (2C), 27.2 (2C), 27.4 (4C), 31.0 (2C), 32.6 (2C), 52.5 (2 C), 53.1 (2C), 65.3 (2C), 67.5 (2C), 71.6 (2C), 75.4 (2C), 82.7 (2C), 85.4 (2C), 107.4 (2C), 113.6 (2C); IR (neat): 2926, 2885, 1636, 1445, 1364, 1122, 1064, 1020 cm^{-1}; ESI-MS: *m/z* 343 (z = 2); Anal. Calcd. for $C_{38}H_{74}Cl_2N_2O_8$: C, 60.22; H, 9.84; N, 3.70; Found: C, 59.98; H, 9.72; N, 3.81; $[\alpha]_D^{25}$: -25.46 (C 2.0, $CHCl_3$).

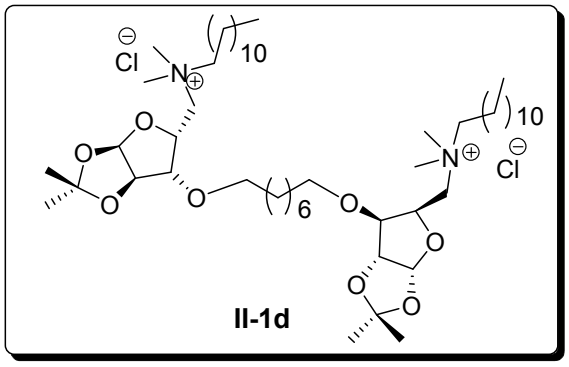


***Hexyl-{6-[8-(5-hexylaminomethyl-2,2-dimethyl-tetrahydro-furo[2,3-d][1,3]dioxol-6-yloxy)-octyloxy]-2,2-dimethyl-tetrahydro-furo[2,3-d][1,3]dioxol-5-ylmethyl}tetramethylammonium dichloride* (II-1b):** Yellow viscous liquid, yield: 96%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.89 (6H, brs), 1.25-1.40 (20H, brs), 1.32 (6H, s), 1.49 (6H, s), 1.49-1.67 (8H, m, overlapped), 3.43 (6H, s), 3.46 (6H, s), 3.39-3.69 (10H, m), 4.19 (2H, d, J = 3.3 Hz), 4.52-4.58 (4H, m), 4.72 (2H, d, J = 13.8 Hz), 5.92 (2H, d, J = 3.1 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 13.7 (2C), δ 22.2 (2C), 22.7 (2C), 25.5 (2C), 25.6 (2C), 25.9 (2C), 26.8 (2C), 29.0 (2C), 29.3 (2C), 31.1 (2C), 52.0 (2C), 52.2 (2C), 63.3 (2C), 66.2 (2C), 70.5 (2C), 73.6 (2C), 81.1 (2C), 83.0 (2C), 105.5 (2C), 112.0 (2C); IR (neat): 2927, 2892, 1654, 1452, 1361, 1122, 1064, 1020 cm^{-1}; ESI-MS: *m/z* 357 (z = 2); Anal. Calcd. for $C_{40}H_{78}Cl_2N_2O_8$: C, 61.13; H, 10.00; N, 3.56; Found: C, 60.80; H, 10.32; N, 3.69; $[\alpha]_D^{25}$: -23.8 (C 2.4, $CHCl_3$).



***Dodecyl-{6-[6-(5-dodecylaminomethyl-2,2-dimethyl-tetrahydro-furo[2,3-d][1,3]dioxol-6-yloxy)-hexyloxy]-2,2-dimethyl-tetrahydro-furo[2,3-d][1,3]dioxol-5-ylmethyl}-tetramethyl ammonium dichloride* (II-1c):** Yellow viscous liquid, yield: 91%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.88 (6H, t, J = 6.5 Hz), 1.26-1.36 (40H, m), 1.32 (6H, s), 1.49 (6H, s), 1.49-1.80

(8H, m, overlapped), 3.41 (6H, s), 3.43 (6H, s), 3.41-3.64 (10H, m), 4.21 (2H, brs), 4.53-4.58 (4H, m), 4.76 (2H, d, J = 13.7 Hz), 5.93 (2H, d, J = 3.0 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 13.8 (2C), 22.3 (2C), 22.6 (2C), 25.3 (2C), 25.7 (2C), 25.8 (2C), 26.6 (2C), 28.8 (2C), 28.9 (4C), 29.0 (2C), 29.1 (2C), 29.2 (4C), 31.5 (2C), 51.8 (2C), 52.0 (2C), 63.2 (2C), 65.7 (2C), 70.1 (2C), 73.4 (2C), 80.9 (2C), 82.8 (2C), 105.3 (2C), 111.8 (2C); IR (neat): 2936, 2843, 1633, 1487, 1236, 1142, 1061, 1001 cm^{-1}; ESI-MS: *m/z* 427 (z = 2); Anal. Calcd. for $C_{50}H_{98}Cl_2N_2O_8$: C, 64.84; H, 10.66; N, 3.02; Found: C, 64.58; H, 10.88; N, 2.86; $[\alpha]_D^{25}$: -19.5 (C 1.5, $CHCl_3$).



II-1d

Dodecyl-{6-[8-(5-dodecylaminomethyl-2,2-dimethyl-tetrahydro-furo[2,3-d][1,3]dioxol-6-yloxy)-octyloxy]-2,2-dimethyl-tetrahydro-furo[2,3-d][1,3]dioxol-5-ylmethyl}-tetramethyl ammonium dichloride **(II-1d):** Yellow viscous liquid, yield: 90%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.88 (6H, t, J = 6.7 Hz), 1.26-1.35 (44H, m), 1.32 (6H, s), 1.49 (6H, s), 1.49-1.59 (8H, m), 3.43-3.61 (22H, m), 4.19 (2H, brs), 4.52-4.70 (6H, m), 5.92 (2H, d, J = 3.00 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 13.7 (2C), 22.3 (2C), 22.6 (2C), 25.5 (2C), 25.7 (2C), 25.8 (2C), 26.6 (2C), 28.8 (2C), 28.9 (2C), 29.0 (2C), 29.1 (4C), 29.2 (6C), 31.5 (2C), 51.8 (2C), 52.0 (2C), 63.0 (2C), 65.9 (2C), 70.3 (2C), 73.4 (2C), 80.9 (2C), 82.8 (2C), 105.3 (2C), 111.8 (2C); IR (neat): 2967, 2845, 1621, 1484, 1113, 1024 cm^{-1}; ESI-MS: *m/z* 441 (z = 2); Anal. Calcd. for $C_{52}H_{102}Cl_2N_2O_8$: C, 65.45; H, 10.77; N, 2.94; Found: C, 64.98; H, 11.01; N, 2.86; $[\alpha]_D^{25}$: -28.9 (C 1.7, $CHCl_3$).

II.5. Notes & References

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[26] The TEM facility has been outsourced from SICART-CVM, Anand, Gujarat, India and method is obtained from Mr. Vikas A. Patel, Technical Assistant, SICART-CVM, Gujarat, India.

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[32] The experimental work has been performed in collaboration with Dr. Anasuya Ganguly and co-workers at Department of Biological Sciences, BITS Pilani-K. K. Birla Goa Campus and is not a part of any other thesis.

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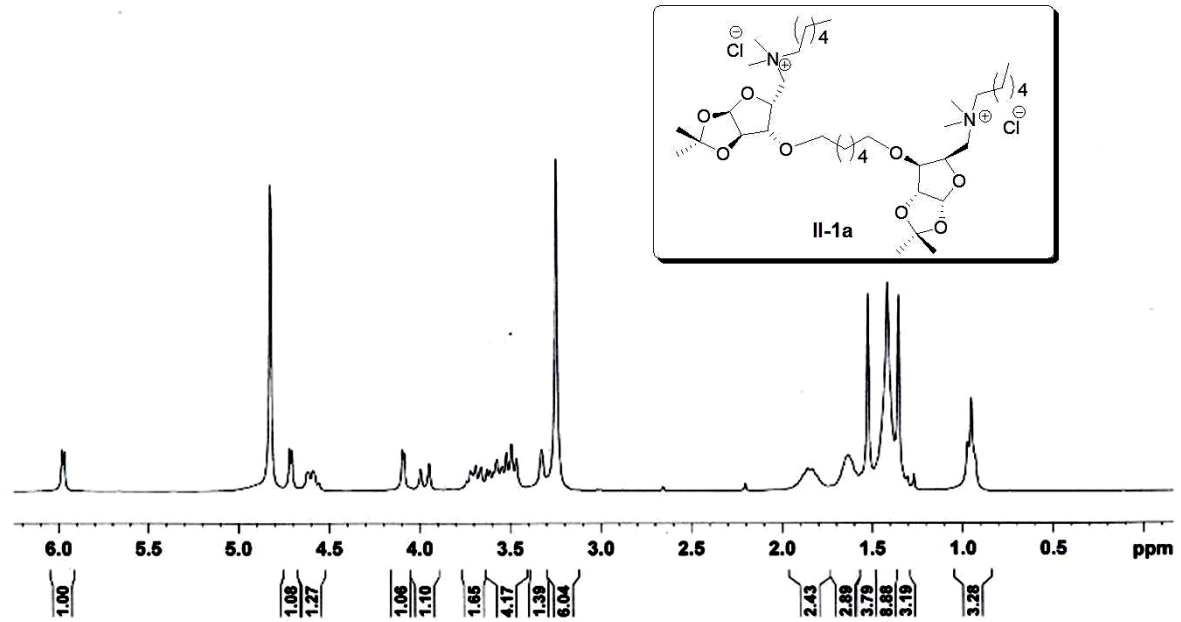
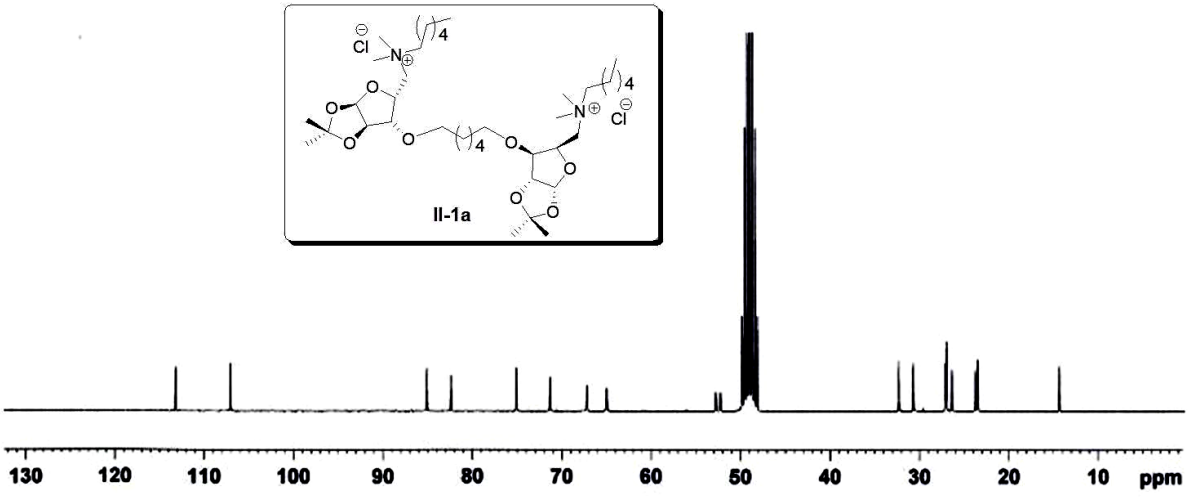
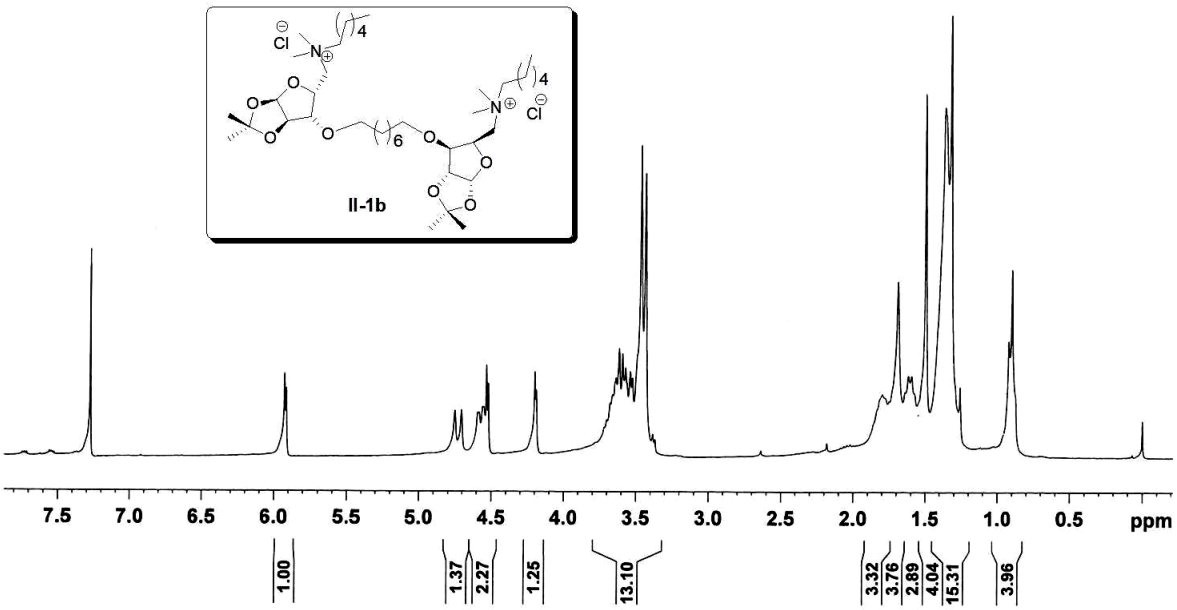
II.6 . Supporting Information (selected spectra)



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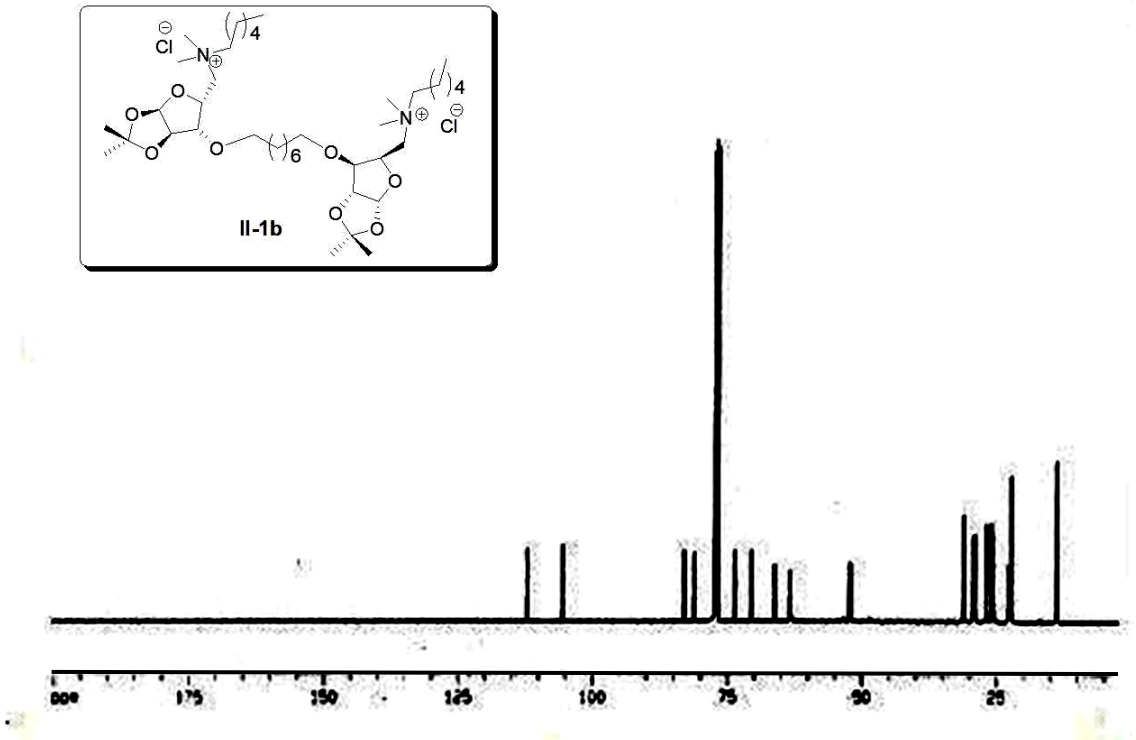
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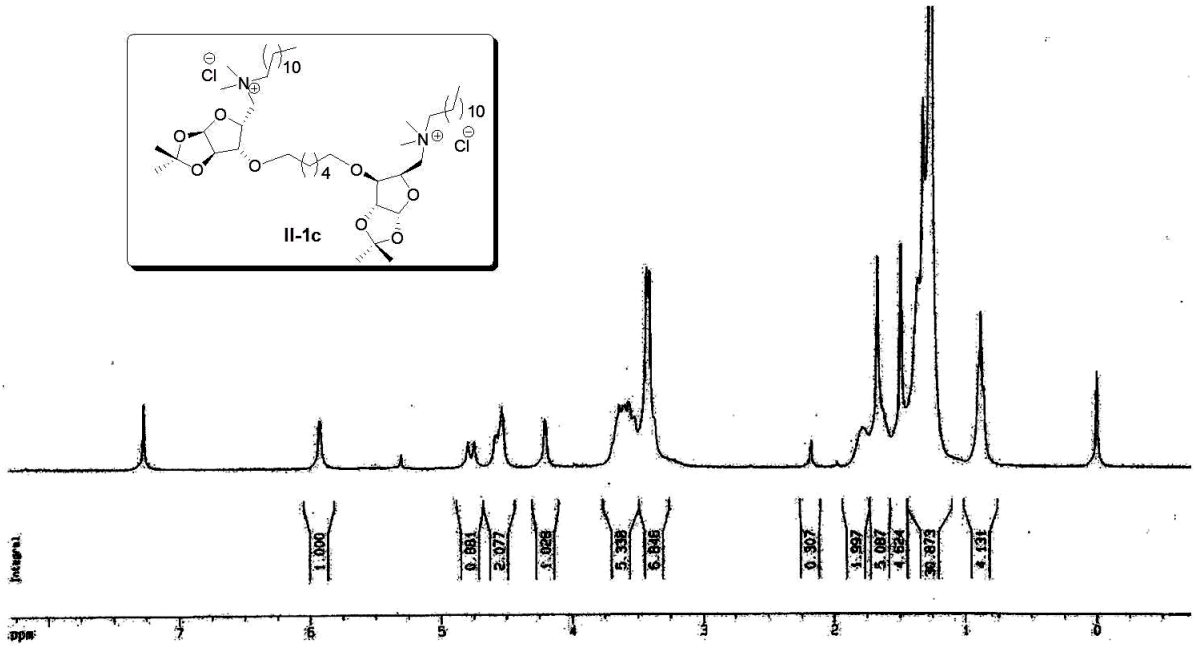
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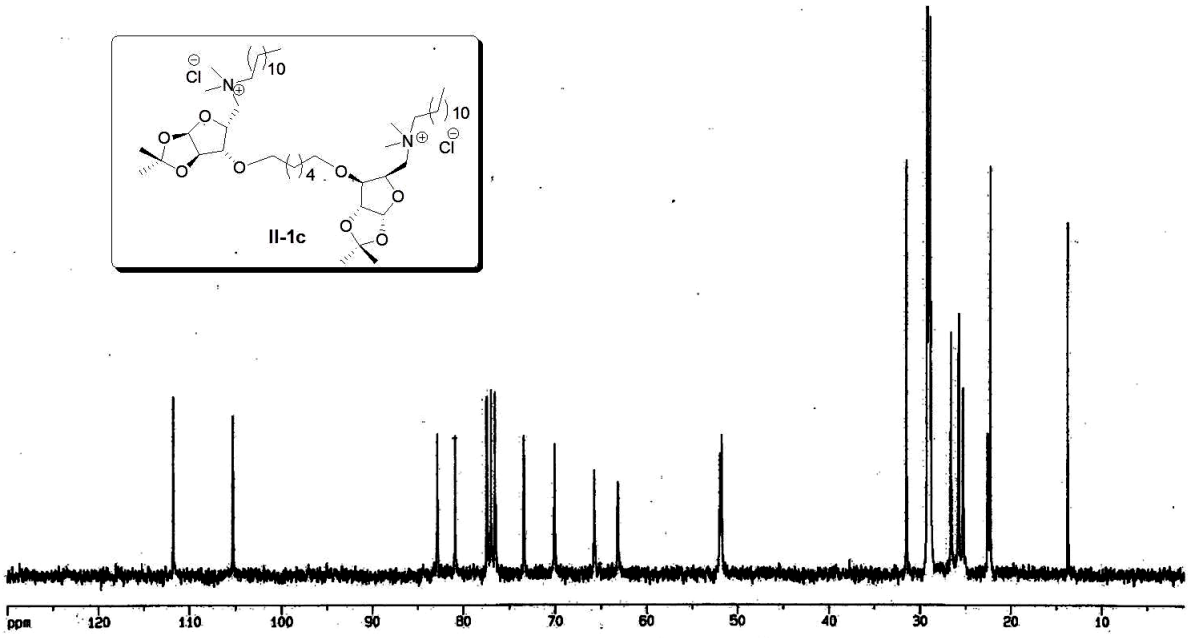
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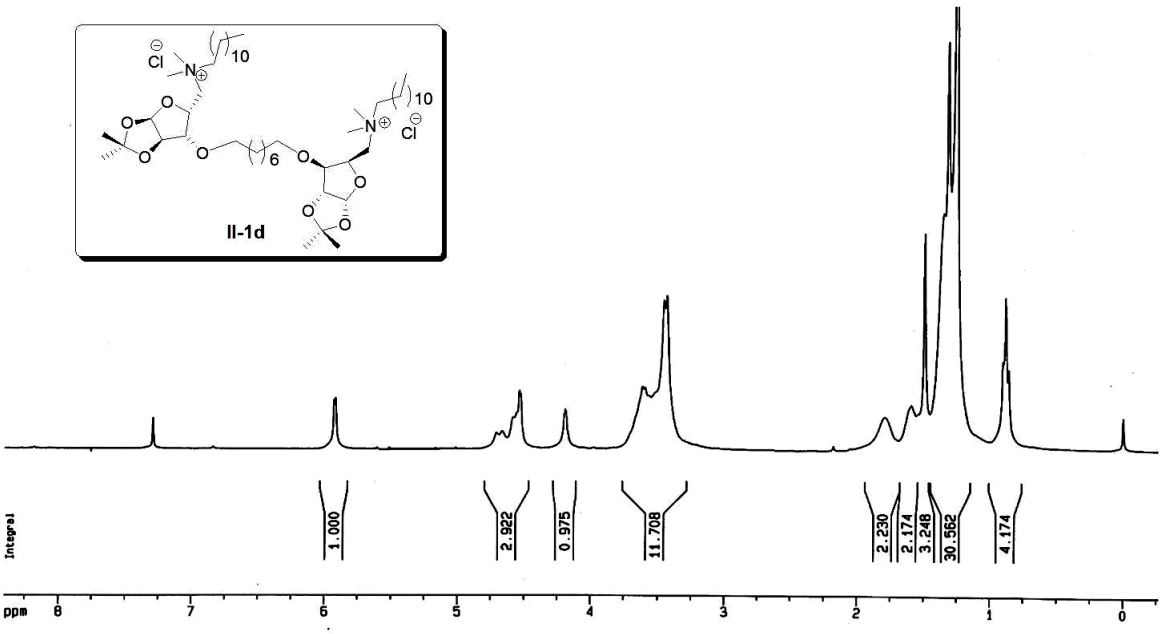
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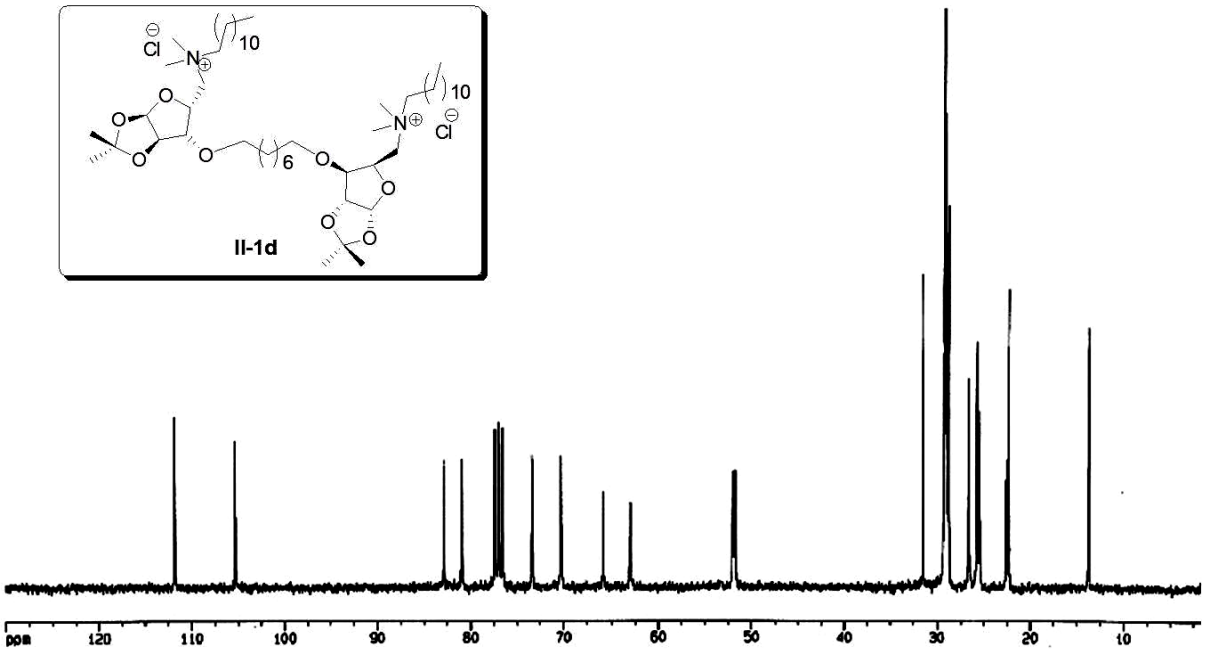
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ppm

CHAPTER III

Synthesis and catalytic application of a novel class of D-glucose based bolaamphiphiles

III.1. Background of the present work

Sugar-based bolaamphiphiles are of special interest for pharmaceutical and other applications because of their natural origin, biocompatibility and biodegradability. Among them a large variety of non-ionic and cationic sugar based bolaamphiphiles have been reported so far [refer to Chapter I, Section I.1.2]. However, there are very few reports available on sugar based anionic bolaamphiphiles. Most of the available bolaamphiphiles are made utilising the C-1 or C-2 functionality of glucose but C-3 functionality of glucose was rarely used for the synthesis of bolaamphiphiles.[1] We were interested to design bolamphiphiles that connects two monomeric units with anionic sulphonate as polar head by a linker via a sugar residue using C-3 of functionality of diisopropylidene glucose. Although bolaamphiphiles have been used for a wide range of applications, another potential application of these molecules that remains practically unexplored is their ability to catalyze reactions in aqueous media. The bolaamphiphiles have all the properties of a normal surfactant and they are also capable of forming vesicles in water. The in-built chirality of sugar-based bolaamphiphiles could be advantageous in order to get improved enantiomeric excess in asymmetric catalysis. Being chiral these ionic bolaamphiphiles are expected to show unique aggregate morphologies as well.

It is a well known fact that nitrones are important reaction intermediates, and are useful for the synthesis of several therapeutic agents e.g. antifungal,[2,3] antitubercular,[4] cytotoxic,[5-7] antiviral,[6,7] DNA intercalator[8], antimicrobial[9] etc. These therapeutic agents commonly have isoxazolidine backbone. Nitrones are mostly used as the dipole in 1,3-dipolar cycloaddition reaction to form isolated or fused isoxazole or isoxazolidine nucleus, which is mostly dependent on alkyne or alkene counterparts as dipolarophile like alkyl acrylates. These cycloaddition products are having specific regio- and stereo-selectivity, which depends on the manner by which fusion reaction happens.[10] Most of the conventional method to synthesize this pharmacologically important heterocyclic skeleton uses hazardous reagents, toxic

solvents, harsh reaction condition and are carried out in presence of a catalyst.[11] However, a few green methods are also available that includes the use of surfactant, microwave condition, mechanochemical methods etc.[12] In particular, Chatterjee and co-workers[12a] have developed a method using surfactant (SDS and CTAB) as catalyst in aqueous media. They mentioned that *in-situ* nitrone formation and 1,3-dipolar cycloaddition reaction occurs in a single pot that leads to isoxazole derivatives in stereoselective manner. They also mentioned that the reaction showed regioselectivity to *trans*-5-substituted isoxazolidine derivatives, however, in a few cases *endo*-4-substituted product predominates out of four regioisomers (*cis*/*trans* and *exo*/*endo*). We thought, the moderate regioselectivity can be improved by using a chiral bolaamphiphile as a catalyst. It is considered that the built-in chirality of the sugar-based bolaamphiphiles would play a crucial role in imparting higher regioselectivity to the final product mixture. In addition, lower CMC of bolaamphiphiles would ensure the reaction works at much lower catalyst loading. In this chapter, we describe the development of a synthetic route to a number of D-glucose derived anionic bolaamphiphiles having sulphonate as the polar head group (**III-2a-c**). Their surface properties and catalytic activity in 1,3-dipolar nitrone cycloaddition were studied.

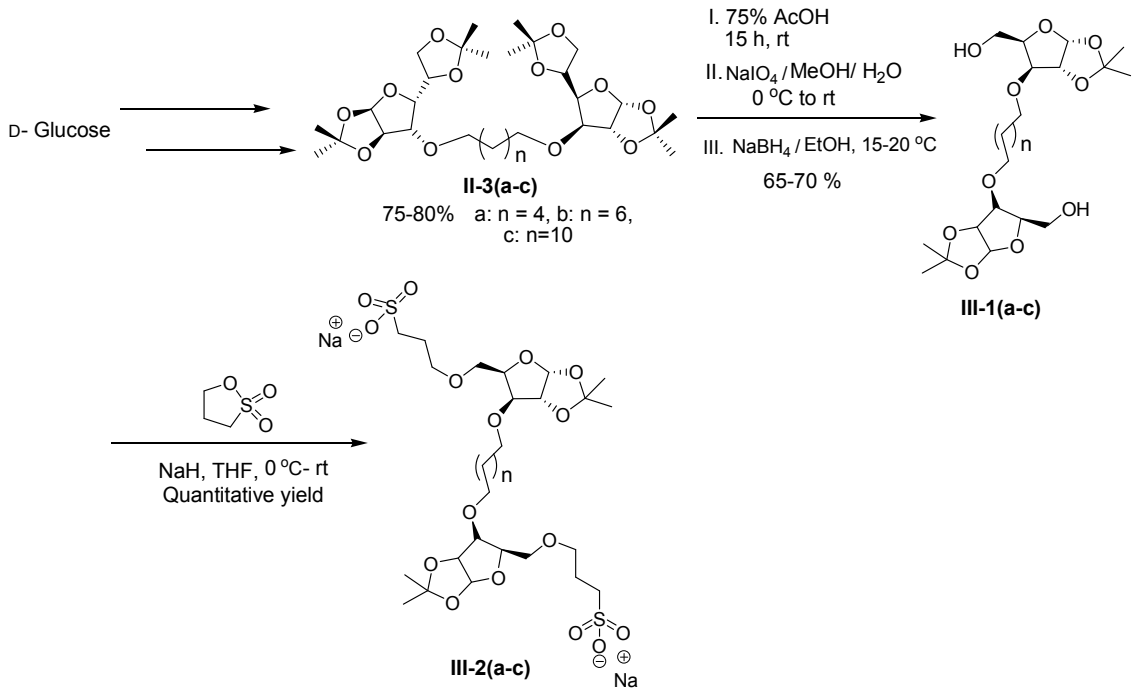
III.2. Results and Discussion

III.2.1. Synthesis of bolaamphiphiles and their structural elucidation

Compounds **II-3a-b** of Chapter II are common starting materials of gemini amphiphiles and bolaamphiphile except **II-3c**. They were converted to unstable aldehydes **II-4a-c** by deprotection followed by cleavage of corresponding diols by $NaIO_4$. The aldehydes were converted to alcohols (**III-1a-c)** by *in-situ* reduction using $NaBH_4$ (Scheme III-1). In the final step, the bolaamphiphiles were synthesized by treatment of alcohols (**III-1a-c**) with 1,3-propane sultone to produce the anionic bolaamphiphiles (**III-2a-c**) in good overall yields.

The structure of the new bolaamphiphiles (**III-2a-c**) have been confirmed by ^{1}H NMR, ^{13}C NMR, ESI-MS analysis. The formation of bolaamphiphile alcohols were confirmed by appearance of a 2H doublet at ~ 4.5-4.6 and a mass peak at *m/z* 485 $[M + 23]^+$ for **III-1a**, at *m/z* 513 $[M + 23]^+$ for **III-1b** and at *m/z* 569 $[M + 23]^+$ for **III-1c**, respectively. Appearance of a two proton multiplet at δ 1.81-1.94 for **III-2a**, 1.78-1.97 for **III-2b** and 1.78-1.97 for **III-2c** due to-$C\underline{H}_2$-SO_3^- and a two proton triplet at δ 2.87 for **III-2a**, 2.84 for **III-2b** and 2.85 for **III-2c** due to -O-$C\underline{H}_2CH_2CH_2$ SO_3^- in ^{1}H NMR and appearance of a peak at δ 48.0 (-

$\underline{C}H_2SO_3^-$) for all **III-2a**, **III-2b** and **III-2c** in ^{13}C NMR and their corresponding ESI-MS peaks at 352, 366 and 394 (*m/z*, z = 2; -ve mode) for **III-2a**, **III-2b** and **III-2c**, respectively confirm the product formation.



Scheme III-1. Synthetic route to D-glucose derived bolaamphiphiles **III-2(a-c)**.

III.2.2. Surface properties

III.2.2.1. Critical micellization concentration (CMC)

Bolaamphiphiles are generally having low CMC values as compared to their corresponding conventional surfactants.[13] They indeed showed lower CMC values as expected. The CMC values were calculated from the conductivity versus concentration plots (Fig. III-1). The CMCs were also confirmed by steady-state fluorescence experiments using pyrene as the probe (Fig. III-2). In the absence of micelles (below CMC) pyrene senses the polar environment of water molecules; the ratio of fluorescence emission intensities corresponding to the first and third vibrational peaks (I_I/I_{III}) is high. Above CMC, when micelles are present, and owing to the high hydrophobicity of pyrene molecules, these are solubilized in the interior micellar phase. This is a hydrocarbon-like solvent and therefore, the environment sensed by pyrene is less polar. As a result, the ratio I_I/I_{III} decreases.

In Fig. III-1, the point of deviation from sharp increase of specific conductance was considered as point of micellization and CMC values were obtained from the curves. The observed CMC of surfactants **III-2a**, **III-2b** and **III-2c** are 0.0076 mM, 0.0054 mM and 0.0038 mM, respectively. A general trend was observed: the CMC decreases with increasing chain length. The lower value of CMC for **III-2c** compared to others is a direct consequence of increased overall hydrophobicity of **III-2c**, which is having 12C spacer. The observed CMC values were of the same order of magnitude of previously reported sugar based bolaamphiphiles.[14] It is considered that the CMC values from conductometric measurements are obtained from the bulk solution which is unaffected by any interfacial events. CMCs were also determined by steady-state fluorescence measurements to verify the CMC values obtained from conductometric experiments. From these experiments the CMCs of **III-2a**, **III-2b**, and **III-2c** were found as 0.0052 mM, 0.0041 mM, and 0.0028 mM, respectively, which closely matched with the CMCs obtained by conductometric measurements.

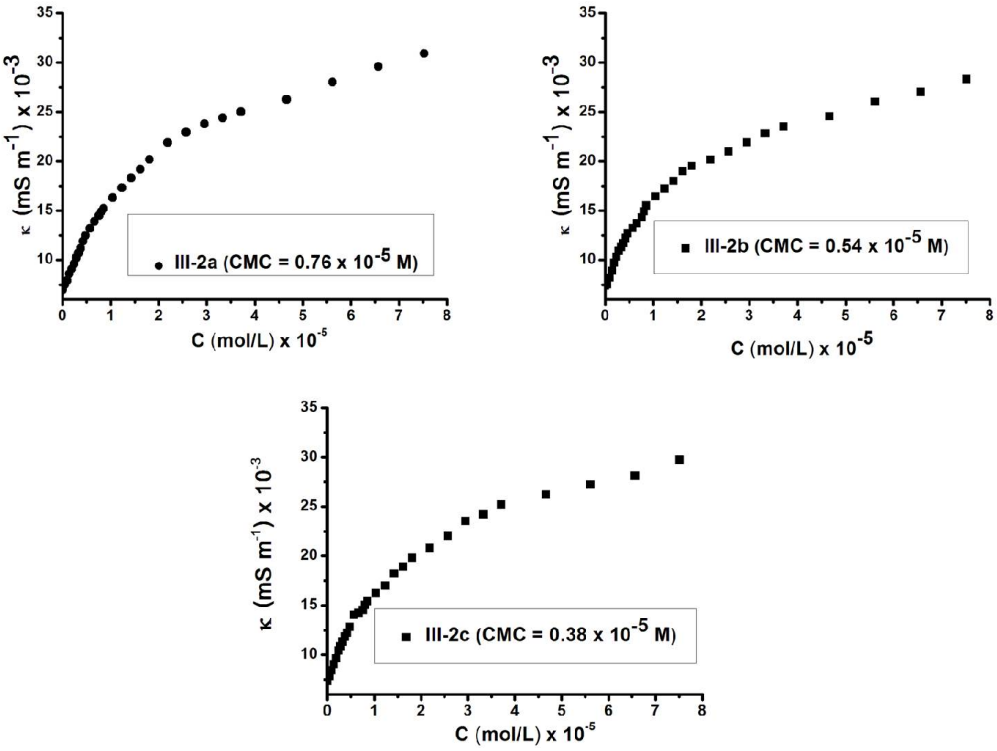


Fig. III-1. Specific conductivity vs. concentration plots of bolaamphiphiles, **III-2a-c**.

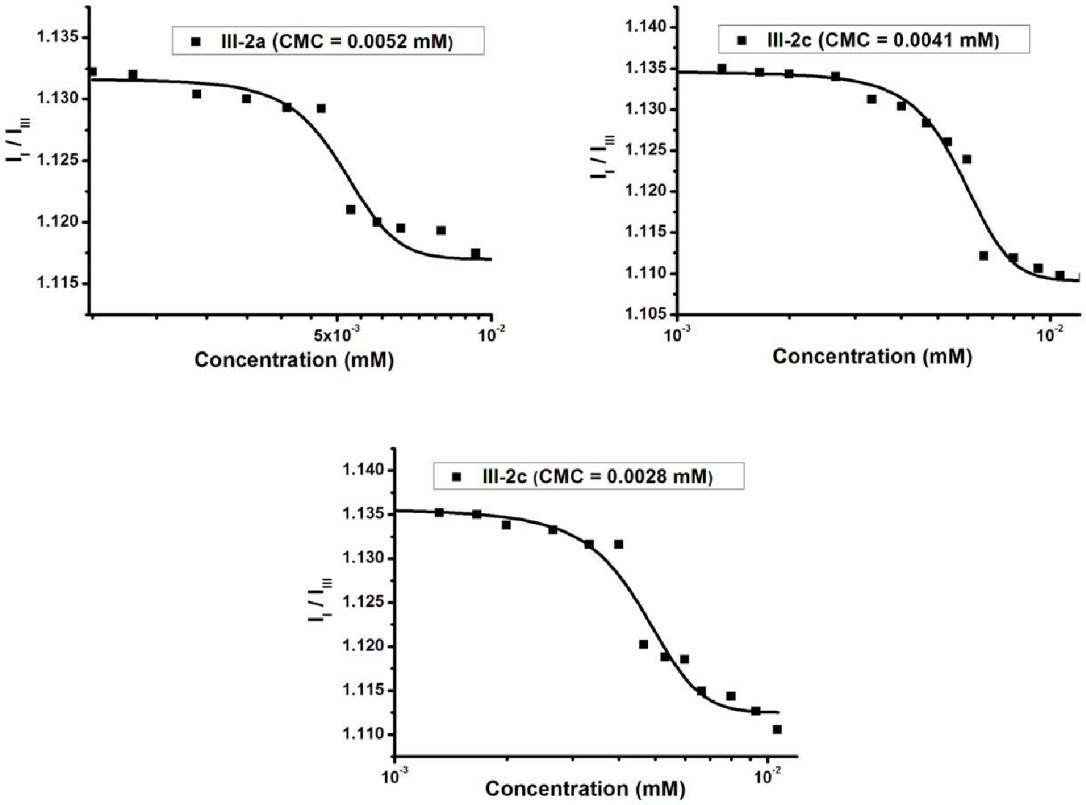


Fig. III-2. CMC curves of bolaamphiphiles obtained from steady-state fluorescence measurement.

III.2.2.2. Aggregation behaviour of bolaamphiphiles in water

The self-assembly of the bolaamphiphiles **III-2a-c** occurs rapidly under neutral condition at a concentration of 1 mg per mL of water. Room temperature sonication was sufficient to ensure the formation of stable supra-molecular assembly in aqueous solution. The structural morphologies of aggregation behaviour of bolaamphiphiles in aqueous solution at room temperature were determined by the aid of inverted microscope and transmission electron microscope.

TEM analysis revealed that micelle formation occurs in case of all bolaamphiphiles (Fig. III-3).[15] It was also found that initially they form micelle and vesicles that transform in to tubular morphologies upon standing the aqueous solution of surfactant for 7 days in case of **III-2b** and **III-2c**. Furthermore, in some cases twisted ribbon like morphology was also observed (Fig. III-3D-F). However, twisted ribbon like morphology was not observed in case of **III-2a**.

The molecular aggregation of anionic bolaamphiphiles to build up tubular and ribbon like morphology can be driven by hydrophobic interactions between the alkyl chains.

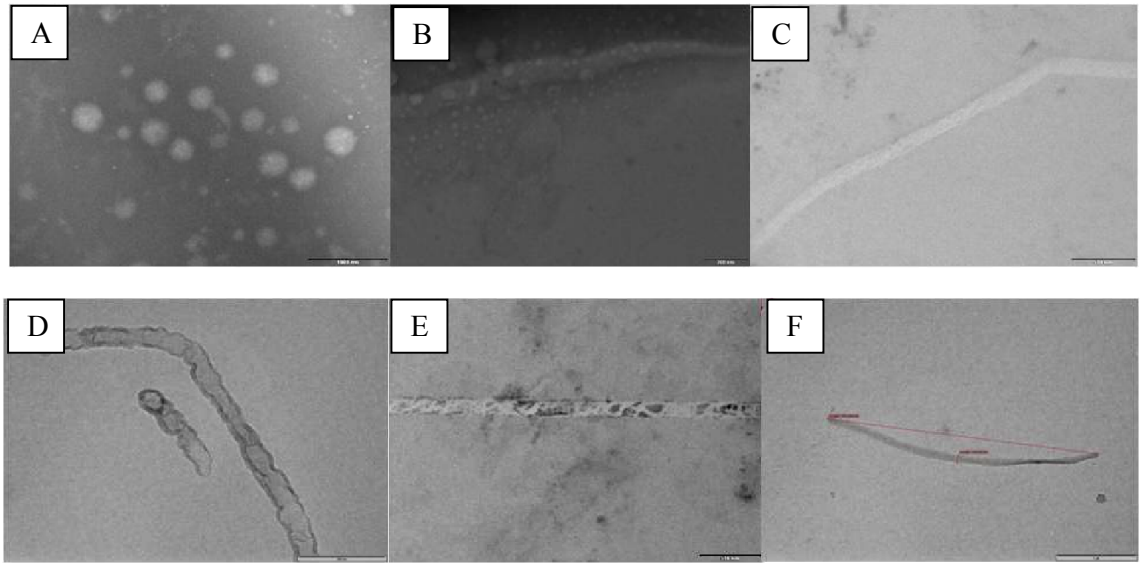


Fig. III-3. TEM images of (A) formation of micelle for (**III-2a-c**) after 1 day aging of the solution; (B-E) tubular morphology in case of **III-2b** and **III-2c** after 7 days aging of the solution; (F) twisted ribbon like morphology for **III-2c**.

III.2.3. Standardization of the reaction condition for catalytic properties of bolaamphiphiles

III.2.3.1. Characterization of reaction media

We started our work with a focus on optimizing the reaction conditions for catalytic reaction. In this direction, the formation of the emulsion droplets were confirmed by taking optical micrograph of different bolaamphiphiles (2 mol%) containing aqueous solutions of reactants before reaction would actually proceed (Fig. III-4). The experiment was carried out in IX-51 inverted microscope in which several emulsion droplets have been seen. This ensures that the proposed reaction can be carried out in this organized media.

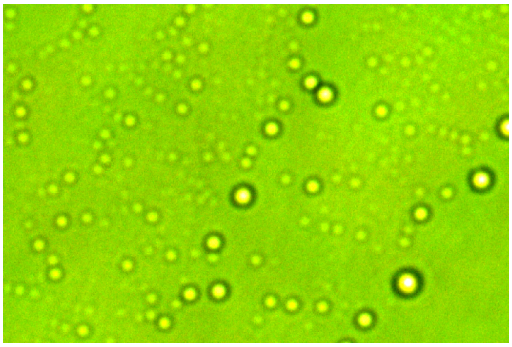


Fig. III-4. A typical optical micrograph of emulsion droplets formed in an aqueous solution of bolaamphiphile (**III-2c**), *m*-nitrobenzaldehyde and phenylhydroxyl amine.

Dynamic light scattering (DLS) experiments of the solution containing bolaamphiphile (**III-2c**), *m*-nitro benzaldehyde and phenylhydroxyl amine was also carried out in Delsa Nano S, Beckman Coulter particle size analyzer which confirmed that the size of emulsion droplets is mostly in the nanometer range (Fig. III-5). These droplets act as micellar nanoreactor to carry out the organic transformation inside their core.

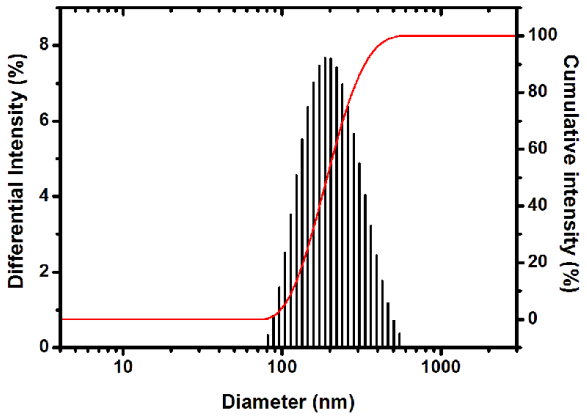
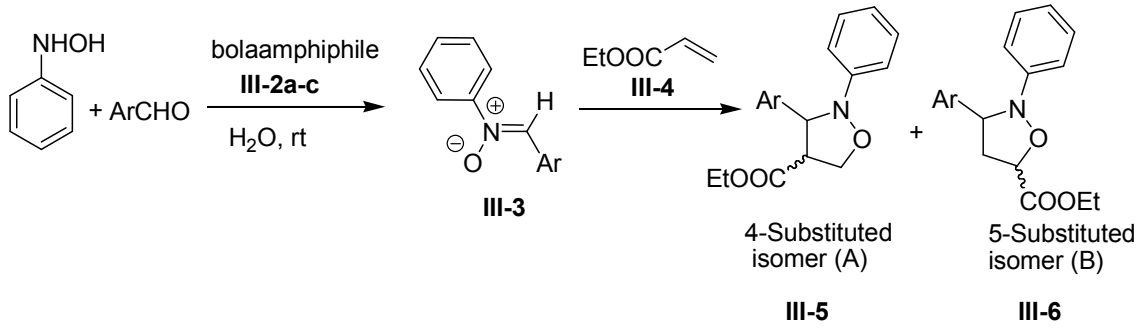


Fig. III-5. DLS data of bolaamphiphile (**III-2c**) containing solution showing formation of aggregates.

III.2.3.2. Selection of suitable bolaamphiphile and optimisation of reaction condition

To establish a suitable condition for water exclusion reaction, we screened catalytic activity of all three bolaamphiphiles (**III-2a-c**) for model reactions between one electron deficient aldehyde (viz. *m*-nitrobenzaldehyde) and one electron rich aldehyde (viz. *p*-

methoxybenzaldehyde) with phenylhydroxylamine and compared the results with the reported yields by Chatterjee et al. (Table III-1**)**.[12a] In this regard, the same model reactions were repeated by us using SDS as catalyst adopting the reported condition.[12a] The complete condensation of phenylhydroxylamine and aromatic aldehydes on an average took 1 h to afford intermediate nitrones (**III-3**). Once the nitrone completely formed, ethyl acrylate was added *in-situ* to the reaction mixture to carry out the cycloaddition reaction and it was allowed to stir at room temperature till the reaction got completed (Table III-1). As mentioned in table III-1, yields are generally very good (75-90 %). As expected, the reactions were successfully carried out with just 2 mol% of catalyst loading. It is noteworthy to mention that the amount of SDS used in the method reported by Chatterjee et al. was 5 fold.[12a] This is because of low CMC of synthesized bolamaphiphiles which allows formation of adequate numbers of micelles and vesicles in solution even at low concentrations. In addition, significant increase in the reaction rate was observed for bolaamphiles as compared to SDS (Table III-1). In general, the reactions produced 5-substituted products (**III-6**) preferably over 4-substituted products (**III-5**) for each of the bolaamphiphiles (**III-2a-c**). For 4-substituted products (**III-5**) the reaction is stereospecific in favor of *endo* isomer for all cases (Table III-1, entries 1-3, 7-9). For 5-substituted products (**III-6**), unpredictable diastereoselectivity was observed with bolaamphiphile **III-2c**, whereas, other bolaamphiphiles do not impose much diastereoselectivity. However, to our dismay, the synthesized bolaamphiphiles occasionally failed to achieve the regioselectivity and diastereoselectivity that are shown by SDS (Table III-1, entries 6, 10). Based on these observations bolaamphiphile **III-2c** was chosen for further study with an expectation of better diastereoselective implications. In a separate study, the concentration of bolaamphiphile **III-2c** was changed from 2 mol% to 5 mol% and 10 mol% on a model reaction with *m*-nitrobenzaldehyde and found that increased catalyst concentration does not make any significant improvement in terms of reaction time, yield and stereoselectivity (Table III-1, entries 4, 5).



Scheme III-2. Bolaamphiphile catalyzed nitrone cycloaddition reaction

Table (III-1): Selection of suitable bolaamphiphile catalyst:

entry	ArCHO	catalyst	mol% of catalyst	time (h)	%yield[a] (**III-5**:**III-6**)	**III-5** (exo: endo)	**III-6** (cis: trans)
1.	*m*-$NO_2C_6H_4$-	**III-2a**	2	12	80 (1:8)	endo only	1:2
2.	*m*-$NO_2C_6H_4$-	**III-2b**	2	10	76 (1:6)	endo only	1:2
3.	*m*-$NO_2C_6H_4$-	**III-2c**	2	12	84 (1:6)	endo only	2:5
4.	*m*-$NO_2C_6H_4$-	**III-2c**	5	10	85 (1:6)	endo only	2:5
5.	*m*-$NO_2C_6H_4$-	**III-2c**	10	10	82 (1:6)	endo only	2:5
6.	*m*-$NO_2C_6H_4$-	**SDS**	10	30	81	-	4:5
7.	*p*-$OCH_3C_6H_4$-	**III-2a**	2	36	78 (2:7)	endo only	4:5
8.	*p*-$OCH_3C_6H_4$-	**III-2b**	2	30	82 (2:3)	endo only	1:1
9.	*p*-$OCH_3C_6H_4$-	**III-2c**	2	30	85 (1:2)	endo only	2:3
10.	*p*-$OCH_3C_6H_4$-	**SDS**	10	76	84	-	*cis* only

[a]**III-5** and **III-6** are isolated separately and the yields presented in table **III-1** is the combined yield of them.

III.2.4. Scope & applicability on catalytic activity of bolaamphiphile III-2c for the synthesis of isoxazolidine derivative

After establishing the standard reaction condition, a series of aromatic aldehydes was treated with phenylhydroxylamine and then corresponding nitrone was treated with ethyl acrylate in the presence of 2 mol% of bolaamphiphile **III-2c** as catalyst in a single-pot (Scheme III-2). The methodology was found to be excellent in terms of yields and reaction time. Both aldehydes with electron withdrawing group (EWG) as well as electron donating groups (EDG) equally participated in the reaction to produce corresponding isoxazolidines in high yields (80-90%). As expected, reactions involving aromatic aldehydes with EWG completed at a faster rate (Table III-2, entries 1-3), whereas, aromatic aldehydes with EDG took longer time for complete conversion. The structures of the isoxazolidine derivatives (**III-5** and **III-6**) were established by ^{1}H NMR, ^{13}C NMR, CHN and ESI-MS, which matched reasonably well

with the reported data.[12a] In few selected cases HRMS data were also obtained. The 1,3-dipolar nitrone cycloaddition was found regioselective in the presence of bolaamphiphile **III-2c** as catalyst. Like previous cases, 5-substituted isoxazolidines (**III-6**) were favored over 4-substituted regiomers (**III-5**).

It was also observed that 4-substituted isomers (**III-5**) form as the minor product (only 10-12%) from aldehyde with EWG (Table III-2, entries 2, 3, 6, 7), whereas, the amount of **III-5** significantly increases (up to 30%) in the product mixture for the aldehydes with EDG (Table III-2, entries 4, 5). For compounds **III-5**(**b-g**) *endo* isomers were formed as the sole product and no trace of *exo* isomer was found in the reaction mixture (Table III-2, entries 2-7). Only in case of cinnamaldehyde negligible diastereoselectivity was observed (Table III-2, entry 8). The formation of *endo* isomer was established by a smaller coupling constant value between H_3-H_4 ($J_{3\text{-}4}$ = 5.5-6 Hz).[12a] For 5-substituted isomers (**III-6**) moderate distereoselectivity was observed except for 2,5-methoxybenzaldehyde.

Among the aldehydes with electron withdrawing groups (Table III-2, entry 1-3) only *m*-nitro benzaldehyde gives the *trans* isomer in maximum amount for 5-substituted product **III-6b** (Table III-2, entry 2) in comparison with SDS, which was confirmed by appearance of 1H doublet of triplet at 3.12 for *trans* isomer and 1H doublet of doublet of doublets at 3.07 for *cis* isomer due to C-4 protons of isoxazolidine skeleton and a mass peak at *m/*z 343 $[M + H]^+$ for **III-6b**.[12a,16] In case of *o*-nitrobenzaldehyde only 5-substituted isoxazolidine (**III-6a**) was obtained.

Among the aldehydes with electron donating groups also, *trans* isomer predominates over *cis* in comparison with SDS for **III-6** (Table III-2, entry 4). It has been further confirmed by appearance of 1H doublet of triplet at 3.10 for *trans* isomer and 1H doublet of doublet of doublets at 2.93 for *cis* isomer and a mass peak at *m/*z 328 $[M + H]^+$ for **III-6d**. For 2,5-dimethoxybenzaldehyde only *trans* product was formed (Table III-2, entry 5) which was confirmed by appearance of 1H doublet of doublet of doublets at 2.51 and 1H doublet of triplet at 3.07 and a mass peak at *m/*z 358 $[M + H]^+$ for *trans* isomer of **III-6e** but in addition to that *endo* product was also obtained as 4-substituted product **III-5e**. However, in case of aldehydes with *p*-chloro substitution, *cis* isomer predominates over *trans* (Table III-2, entry 6) for **III-6f**, which was confirmed by appearance of 1H doublet of doublet of doublets at 2.97 for *cis* isomer and 1H doublet of triplet at 3.06 for *trans* isomer and a mass peak at *m/*z

332 $[M + H]^+$ for Cl = 35 and 334 $[M + H]^+$ for Cl = 37 for **III-6d**.[12a,16] However, aldehydes like *p*-bromo benzaldehyde (Table III-2, entry 7), *trans* isomer predominates over *cis* for **III-6g,** which was confirmed by appearance of 1H doublet of triplet at 3.07 for *trans* isomer and 1H doublet of doublet of doublets at 2.97 for *cis* isomer and a mass peak at *m/*z 376 $[M + H]^+$ for Br = 79 and 378 $[M + H]^+$ for Br = 81.

The diastereomeric ratio in **III-6** was determined in a qualitative manner from the integration values of separate peaks of *cis* and *trans* isomers in ^{1}H NMR and ^{13}C NMR spectra of the inseparable mixture.[12a,16] It is noteworthy to mention that the yield and diastereoselectivity were poor in case of 2,5-methoxybenzaldehyde as substrate for SDS catalyzed reaction as reported by Chatterjee et al.[12a] However, it may be considered that the inherent chirality in the catalyst (**III-2c**) is not properly translated to the transition states of the reaction intermediates which is the consequence of inconsistent regio- and stereo-selectivity.

Table (III-2): Bolaamphiphile (III-2c) catalyzed water exclusion reaction

entry	ArCHO Ar = (a-h)	time (h)	%yield (**III-5:III-6**)	**III-5** (exo:endo)	**III-6** (cis:trans)
1.	a = *o*-$NO_2C_6H_4$-	12	82	-	2:3
2.	b = *m*-$NO_2C_6H_4$-	12	84 (1:6)	endo only	2:5
3.	c = *p*-$NO_2C_6H_4$-	12	92 (1:8)	endo only	3:4
4.	d = *p*-$OCH_3C_6H_4$ -	39	85 (1:2)	endo only	4:5
5.	e = 2,5-diOMe-C_6H_3-	15	81 (1:2)	endo only	trans only
6.	f = *p*-ClC_6H_4-	20	79 (1:6)	endo only	3:1
7.	g = *p*-BrC_6H_4 -	24	87 (1:6)	endo only	4:5
8.	h = C_6H_5CH=CH-	30	93 (2:3)	5:4	5:4

[a]**III-5** and **III-6** are isolated separately and the yields presented in table **III-2** is the combined yield of them.

III.2.5. Reusability of bolamaphiphile III-2c

One crucial aspect of a catalytic transformation is the reusability of the catalyst without much change in yield and stereoselectivity. This is a value addition to any process particularly from industrial perspective. As the products were easily taken up in the organic layer, water layer containing catalyst **III-2c** was reused for five consecutive cycles preserving diastereoselectivity and without observable change in the yields of the final products (Table III-3).

Table (III-3): Recovery and reuse of bolaamphiphile **III-2c**

Sl.No.	cycle[a]	time (h)	%yield (**III-5:III-6**)	**III-5** (exo:endo)	**III-6**[b] (cis:trans)
1.	1	12	84 (1:6)	endo only	2:5
2.	2	12	82 (1:6)	endo only	2:5
3.	3	12	83 (1:6)	endo only	2:5
4.	4	12	79 (1:6)	endo only	2:5
5.	5	15	78 (1:6)	endo only	2:5

[a]2 mol% of catalyst **III-2c** was added in the first cycle and the aqueous layer was reused in the subsequent cycles after extraction of products in the organic layer. [b]The ratio of isomers were obtained from the integration values of ^{1}H NMR/^{13}C NMR.

III.3. Conclusion

In conclusion, a simple and efficient route has been developed for the synthesis of a new series of a novel class of D-glucose based anionic bolaamphiphiles using the C-3 functionality of diisopropylidene glucose. They showed low CMC and interesting morphologies when dispersed in aqueous solution. These chiral anionic bolaamphiphiles were efficiently used to catalyze dehydrative 1,3-dipolar nitrone cycloaddition with just 2 mol% of catalyst loading. The corresponding isoxazolidine derivatives were formed with moderate to high regio- and stereo-selectivity. The bolaamphiphile with 12 carbon spacer **(III-2c)** was found to be a better candidate among the rest. The catalyst was successfully reused for five consecutive cycles without much change in activity.

III.4. Experimental Section

III.4.1. Materials & methods

Dibromoalkanes, 1,3-propane sultone, ethyl acrylate, nitrobenzene, different aromatic aldehydes and pyrene were purchased from Sigma-Aldrich and used without further purification. Other common reagents were procured either from SD Fine-Chem Limited, Mumbai, India or from Merck, India. Milli-Q (18MΩ) water was used in all experiments as per requirement.

^{1}H NMR and ^{13}C NMR spectra were recorded on BRUKER 300 MHz or BRUKER 400 MHz or JEOL 500 MHz NMR systems using tetramethylsilane as an internal standard. The chemical shifts are reported in parts per million (ppm) units relative to the solvent peak. The

following abbreviations are used in reporting NMR data: s, singlet; brs, broad singlet; d, doublet; t, triplet; dd, doublet of doublets, dq, doublet of quartets; dt, doublet of triplets; ddd, doublet of doublets of doublets; m, multiplet. Mass spectra were recorded on Waters Q-TOF micro mass spectrometer using ESI as the ion source. High-resolution mass spectra (HRMS) were obtained using an electrospray quadrupole time-of-flight (ESI-Q-TOF) mass spectrometer. Optical rotations were recorded on a JASCO B-2000 digital polarimeter. IR spectra were recorded with IR Affinity 1, Shimadzu. CHNS data were recorded using Vario elementar CHNS analyzer. Conductivity was measured on a HANNA instruments auto temperature conductivity meter (model: HI 2300) fitted with a conductivity cell. Fluorescence studies were carried out on a JASCO FP6300 fluorescence spectrophotometer (JASCO Corp., Japan). Particle size was determined using a particle size analyzer (Delsa Nano S, Beckman Coulter, USA). The reactions were monitored by thin layer chromatography (TLC) carried out on 0.25-mm silica gel on aluminium plates (60F-254) using UV light (254 or 365 nm) or naked eye for visualization. Column chromatography was performed on silica gel (60–120 mesh, Merck).

III.4.1.1. Conductivity measurement

For conductivity measurement the solutions were kept in the cell at 25±0.1 °C with the help of a thermostat. CMC of synthesized bolaamphiphiles were determined by adding adequate volumes of concentrated stock solutions of bolaamphiphiles in measured amount of Milli-Q water so as to change the surfactant concentration well below and above CMC. The measured specific conductance was plotted against concentration (C). The point of deviation from sharp increase of specific conductance was considered as CMC.

III.4.1.2. Steady-state Fluorescence Measurement

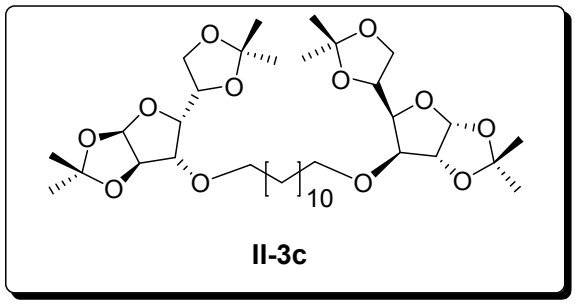
CMC values were also determined by steady state fluorescence measurement in a quartz fluorescence cell by using a Shimadzu FP 6300 fluorescence spectrophotometer (Shimadzu corp., Japan) and pyrene. CMC of bolaamphiphiles were determined by adding adequate volumes of concentrated stock solutions of bolaamphiphiles in pyrene solution. Pyrene was excited at 335 nm and the emission spectra were scanned from 350 to 450 nm. The plot of I_I/I_{III} against bolaamphiphile surfactant concentration gave a sharp decrease in each case, which corresponds to the CMC value of the bolaamphiphile.

III.4.1.3 Transmisison electron microscopy

Aqueous solutions of the anionic bolaamphiphiles (10^{-4} M) were prepared and aged for 1 to 7 days, and then applied to carbon coated copper grids. The samples were examined on a TECNAI G^2 FEI electron microscope operating at 60 kV.[15]

III.4.2. General procedure for the preparation of initial building blocks of gemini surfactants (II-3a, b, c):

The preparation and characterization of the initial building blocks (**II-3a** & **II-3b**) are discussed in chapter **II**, section **II.4.2**. Another building block **II-3c** was prepared by similar procedure for bolaamphiphile study.



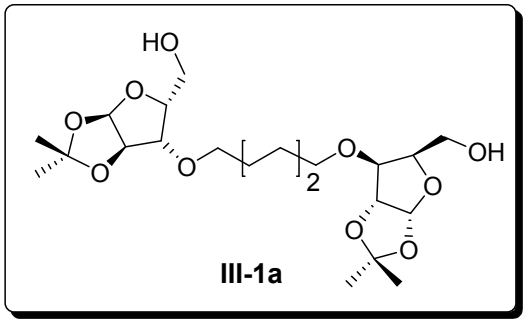
II-3c

1,12-Di-(5-(2,2-Dimethyl-[1,3]dioxolan-4-yl)-2,2-dimethyl-tetrahydro-furo[2,3-d][1,3]dioxol-6-oxy)-dodecane **(II-3c):** Colourless dense liquid, yield: 79%; ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.32 (s, 6H), 1.35 (s, 6H), 1.26-1.35 (m, 16H, overlapped peaks), 1.43 (s, 6H), 1.50 (s, 6H), 1.49-1.59 (m, 4H, overlapped peaks), 3.50-3.61 (m, 4H), 3.85 (d, J = 3.0 Hz, 2H), 3.98 (dd, J_1 = 6.0 Hz, J_2 = 8.7 Hz, 2H), 4.05-4.15 (m, 4H), 4.27-4.34 (m, 2H), 4.52 (d, J = 3.6 Hz, 2H), 5.87 (d, J = 3.9 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 25.4 (2C), 26.0 (2C), 26.2 (2C), 26.7 (2C), 26.8 (2C), 29.4 (2C), 29.6 (4C), 29.7 (2C), 67.2 (2C), 70.7 (2C), 72.5 (2C), 81.1 (2C), 82.0 (2C), 82.5 (2C), 105.2 (2C), 108.8 (2C), 111.7 (2C); IR (neat) ν: 2985, 2929, 2857, 1457, 1375, 1252, 1215, 1077, 1021, 850 cm^{-1}; ESI-MS: (*m/z*) 709 $[M + Na]^+$; $[\alpha]_D^{25}$: -28.26 (C 1.25, $CHCl_3$); *Anal.* Calcd. for $C_{36}H_{62}O_{12}$: C, 62.95; H, 9.10. Found: C, 63.16; H, 9.13.

III.4.3. General procedure for the preparation of bolaamphiphile alcohol (III-1a-c): The general procedure for bolaamphiphilic alcohols is illustrated by the preparation of **III-1a.**

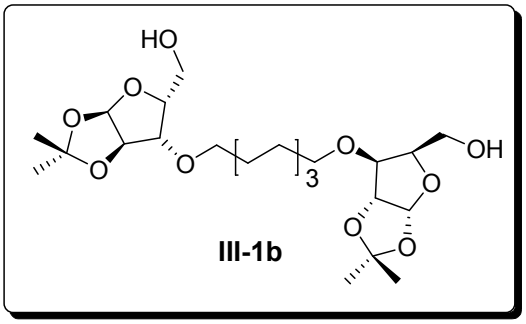
The initial building block **II-3a** was deprotected, followed by cleavage of diols to afford unstable aldehyde **II-4a** following the procedure described in chapter **II**, section **II.4.3**. Unstable aldehyde was reduced *in-situ* adopting the following procedure.

To a stirred solution of crude aldehyde, **II-4a** (2.5 mmol) in ethanol was added $NaBH_4$ (6 mmol) portionwise in ice-cold condition and the reaction mixture was stirred at room temperature for 3 h. Ethanol was removed and the residue was dissolved in minimum volume of water and then extracted with dichloromethane (2 x 15 mL); extracts were washed with brine, dried over anhydrous Na_2SO_4, and evaporation of the solvent under reduced pressure afforded the crude alcohol as colorless liquid. Column chromatography of the crude product over silica gel (100-200 mesh) eluting with 35% EtOAc in petroleum ether afforded compound **III-1a**.



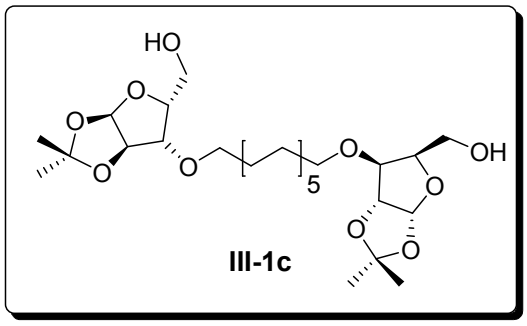
III-1a

({6-[6-(5-Hydroxymethyl-2,2-dimethyl-tetrahydro-furo[2,3-d][1,3]dioxol-6-yloxy)-hexyloxy]-2,2-dimethyl-tetrahydro-furo[2,3-d][1,3]dioxol-5-yl}-methanol **(III-1a):** Colourless oil, yield: 70%; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.33 (s, 6H), 1.35 (s, 4H), 1.50 (s, 6H), 1.57-1.59 (m, 4H), 2.47-2.48 (m, 2H, exchangeable), 3.43-3.45 (m, 2H), 3.61-3.65 (m, 2H), 3.89-3.94 (m, 6H), 4.27-4.29 (m, 2H), 4.56 (d, J = 3.7 Hz, 2H), 5.97 (d, J = 3.7 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 26.2 (2C), 26.6 (4C), 30.1 (2C), 62.2 (2C), 70.6 (2C), 80.0 (2C), 82.1 (2C), 83.6 (2C), 105.5 (2C), 111.3 (2C); ESI-MS: (*m/z*), 463 $[M + H]^+$; *Anal.* Calcd. for $C_{22}H_{38}O_{10}$: C, 57.13; H, 8.28. Found: C, 57.27; H, 8.30.



III-1b

{6-[8-(5-Hydroxymethyl-2,2-dimethyl-tetrahydro-furo[2,3-d][1,3]dioxol-6-yloxy)-octyloxy]-2,2-dimethyl-tetrahydro-furo[2,3-d][1,3]dioxol-5-yl}-methanol **(III-1b):** Colourless oil, yield: 65%; ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.26-1.40 (m, 8H, overlapped peaks), 1.33 (s, 6H), 1.49 (s, 6H), 1.54-1.56 (m, 4H, overlapped peaks), 2.45-2.65 (brs, 2H, exchangeable), 3.39-3.46 (m, 2H), 3.60-3.67 (m, 2H), 3.85-3.98 (m, 6H), 4.26-4.30 (dd, J_1 = 4.2 Hz, J_2 = 8.1 Hz, 2H), 4.56 (d, J = 3.9 Hz, 2H), 5.97 (d, J = 3.9 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 26.1 (2C), 26.7 (4C), 29.7 (2C), 29.9 (2C), 61.9 (2C), 70.8 (2C), 80.1 (2C), 82.2 (2C), 83.9 (2C), 105.4 (2C), 111.1 (2C); ESI-MS: (*m/z*) 513 $[M + Na]^+$; *Anal.* Calcd. for $C_{24}H_{42}O_{10}$: C, 58.76; H, 8.63. Found: C, 58.85; H, 8.66.



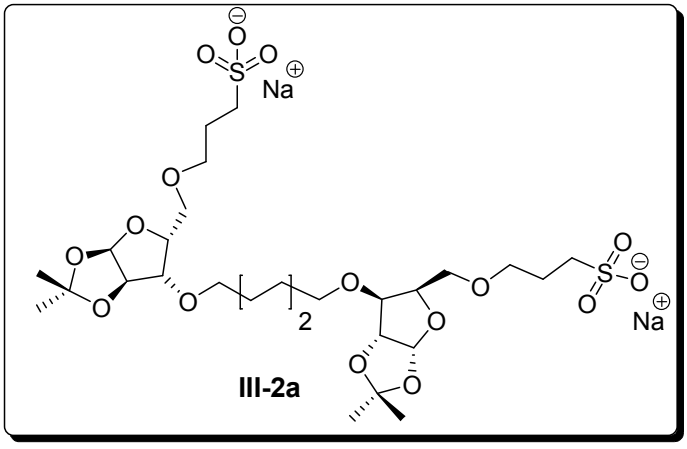
III-1c

{6-[8-(5-Hydroxymethyl-2,2-dimethyl-tetrahydro-furo[2,3-d][1,3]dioxol-6-yloxy)-dodecyloxy]-2,2-dimethyl-tetrahydro-furo[2,3-d][1,3]dioxol-5-yl}-methanol **(III-1c):** Colourless oil, yield: 68%; ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.26-1.40 (m, 16H, overlapped), 1.33 (s, 6H), 1.49 (s, 6H), 1.54-1.56 (m, 4H, overlapped), 2.45-2.46 (brs, 2H, exchangeable), 3.39-3.46 (m, 2H), 3.60-3.67 (m, 2H), 3.85-3.98 (m, 6H), 4.26-4.30 (dd, J_1 = 4.5 Hz, J_2 = 8.1 Hz, 2H), 4.57 (d, J = 3.6 Hz, 2H), 5.97 (d, J = 3.9 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 26.0 (2C), 26.3 (2C), 26.8 (4C), 29.3 (2C), 29.5 (2C), 29.6 (2C), 61.1 (2C), 70.5 (2C), 79.9 (2C), 82.4 (2C), 84.2 (2C),

105.1 (2C), 111.7 (2C); ESI-MS: (*m/z*) 569 $[M + Na]^+$; *Anal.* Calcd. for $C_{28}H_{50}O_{10}$: C, 61.52; H, 9.22. Found: C, 61.46; H, 9.27.

III.4.4. General procedure for the synthesis of bolaamphiphiles (III-2a-c): The general procedure illustrated by the preparation of **III-2a**.

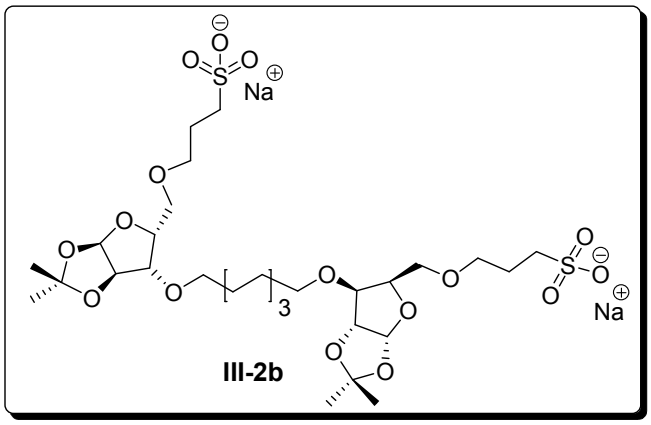
To a stirred suspension of sodium hydride (0.23 gm, 5.7 mmol, 60% wt in oil) in anhydrous THF (5 mL) under nitrogen was added a solution of alcohol **III-1a** (1.0 gm, 2.2 mmol) in THF (10 mL) at 0 ºC. The mixture was stirred at room temperature for 1 h then re-cooled to 0 ºC. A solution of 1,3-propane sultone (0.69 gm, 5.7 mmol) in anhydrous THF (5 mL) was added to the reaction mixture. After the mixture was stirred at room temperature for 15 h, it was cooled again by placing in an ice-salt bath and the reaction mixture was quenched by adding small pieces of ice. Then the solvent was evaporated under vacuum. The semisolid appeared was triturated with ether to remove excess sultone. The compound was again triturated with ethyl acetate to remove any left amount of starting material and non-polar impurity. Finally, compound was again dried in vacuum to furnish the water soluble pure bolaamphiphile **III-2a** as a off-white flake.



III-2a

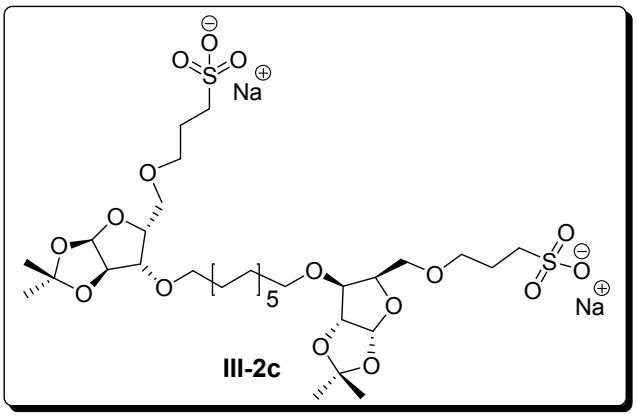
({6-[6-(5-Hydroxymethyl-2,2-dimethyl-tetrahydro-furo[2,3-d][1,3]dioxol-6-yloxy)-hexyloxy]-2,2-dimethyl-tetrahydro-furo[2,3-d][1,3]dioxol-5-yl}-methoxy propanesulphonate disodium **(III-2a):** Off-white solid, yield: 75%; mp. 120 °C (decomposed); ^{1}H NMR (300 MHz, D_2O): δ (ppm) 1.27 (s, 6H), 1.43 (s, 6H), 1.49 (brs, 4H), 1.81-1.94 (m, 8H), 2.87 (t, J = 7.5 Hz, 4H), 3.43-3.48 (m, 2H), 3.52-3.62 (m, 10H), 3.70-3.75 (m, 2H), 3.91 (d, J = 2.4 Hz, 2H), 4.30-4.32 (m, 2H), 5.91 (d, J = 3.6 Hz, 2H); ^{13}C NMR (75

MHz, D_2O): δ (ppm) 24.3 (2C), 25.0 (2C), 25.2 (2C), 27.0 (2C), 28.6 (2C), 48.0 (2C), 68.0 (2C), 70.0 (2C), 70.8 (2C), 79.1 (2C), 81.9 (2C), 82.1 (2C), 105.0 (2C), 113.0 (2C); ESI-MS: (*m/z*, -ve) 352 (z = 2); $[\alpha]_D^{25}$: –20.4 (C 1.94, $CHCl_3$); *Anal.* Calcd. for $C_{28}H_{48}Na_2O_{16}S_2$: C, 44.79; H, 6.44; S, 8.54. Found: C, 44.87; H, 6.51; S, 8.56.



III-2b

{6-[8-(5-Hydroxymethyl-2,2-dimethyl-tetrahydro-furo[2,3-d][1,3]dioxol-6-yloxy)-octyloxy]-2,2-dimethyl-tetrahydro-furo[2,3-d][1,3]dioxol-5-yl}-methoxy propanesulphonate disodium **(III-2b):** White solid, yield: 78%; mp. 110 °C (decomposed); ^{1}H NMR (300 MHz, D_2O): δ (ppm) 1.18 (s, 8H), 1.24 (s, 6H), 1.39 (s, 6H), 1.40-1.44 (m, 4H overlapped), 1.78-1.97 (m, 8H), 2.84 (t, *J* = 7.5 Hz, 4H), 3.40-3.51 (m, 2H), 3.53-3.59 (m, 8H), 3.67-3.70 (m, 2H) 3.87 (brs, 2H), 4.28 (brs, 2H), 5.88 (d, *J* = 2.7 Hz, 2H); ^{13}C NMR (75 MHz, D_2O): δ (ppm) 24.3 (2C), 24.7 (2C), 25.7 (2C), 26.0 (2C), 27.0 (2C), 29.5 (2C), 48.1 (2C), 68.6 (2C), 69.9 (2C), 70.5 (2C), 79.4 (2C), 82.2 (2C), 82.5 (2C), 104.8 (2C), 112.5 (2C); ESI-MS: (*m/z*, -ve) 366 (z = 2); $[\alpha]_D^{25}$**:** –19.1 (C 2.86, $CHCl_3$); *Anal.* Calcd. for $C_{30}H_{52}Na_2O_{16}S_2$: C, 46.26; H, 6.73; S, 8.23. Found: C, 46.15; H, 6.76; S, 8.24.



III-2c

{6-[8-(5-Hydroxymethyl-2,2-dimethyl-tetrahydro-furo[2,3-d][1,3]dioxol-6-yloxy) dodecyloxy]2,2-dimethyl-tetrahydro-furo[2,3-d][1,3]dioxol-5-yl}-methoxy propanesulphonate disodium (**III-2c**): White solid, yield: 80%; mp. 105 °C (decomposed); ^{1}H NMR (300 MHz, D_2O): δ (ppm) 1.20 (s, 8H), 1.24 (s, 6H), 1.43 (s, 6H), 1.40-1.45 (m, 8H, overlapped peaks), 1.78-1.97 (m, 12H), 2.85 (t, J = 7.5 Hz, 4H), 3.37-3.51 (m, 2H), 3.53-3.59 (m, 6H), 3.67-3.72 (m, 2H), 3.90 (d, J = 2.1 Hz, 2H) 4.28 (brs, 2H), 5.90 (d, J = 3.3 Hz, 2H); ^{13}C NMR (75 MHz, D_2O): δ (ppm) 22.8 (2C), 23.2 (2C), 24.4 (2C), 25.2 (2C), 25.6 (2C), 27.0 (2C), 28.4 (2C), 28.6 (2C), 48.0 (2C), 60.2 (2C), 67.9 (2C), 69.6 (2C), 70.8 (2C), 79.1 (2C), 82.0 (2C), 104.6 (2C), 112.6 (2C); ESI-MS: (*m/z*, -ve) 394 (z = 2); *Anal.* Calcd. for $C_{34}H_{60}Na_2O_{16}S_2$: C, 48.91; H, 7.24; S, 7.68. Found: C, 49.05; H, 7.28; S, 7.71.

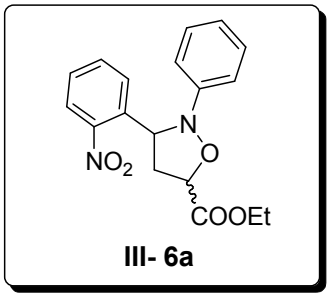
III.4.5. Catalytic reaction by bolaamphiphiles:

III.4.5.1. Preparation of Phenyl hydroxylamine[17]

A vigorously stirred mixture of nitrobenzene (13 g, 0.105 mol), NH_4Cl (6.5 g, 0.12 mol) and H_2O (200 mL) was maintained below 60 °C whilst zinc dust (90%, 15.4 g, 0.21 mol) was added in small portions during 15 min. The reaction mixture was stirred for 15 min after addition was complete, filtered while still warm, and the filter cake was washed with hot water (50 mL). The combined filtrates and washings were saturated with salt, and cooled to 0 °C, and the resulting solid was collected, dried. The crude phenyl hydroxylamine was recrystallized from petroleum ether.

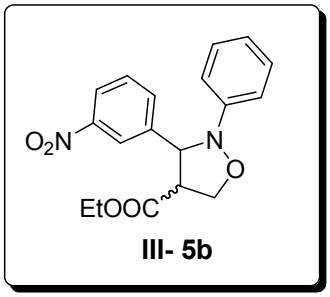
III.4.5.2. A general experimental procedure for bolaamphiphile catalyzed nitrone formation followed by cycloaddition reactions in water.

To a solution of bolaamphiphile (0.01 mmol) in H_2O (2 mL) were added an aldehyde (0.5 mmol), and phenyl hydroxylamine (0.6 mmol, 1.2 equiv) successively at room temperature in a 25 mL round bottom flask. The reaction was sonicated for 5 min and then stirred at room temperature. The reaction was monitored by TLC. After the disappearance of the aldehyde, ethyl acrylate (1 mmol, 0.1 mL) was added and the reaction mixture was stirred at room temperature. After stirring at the same temperature for the period of time listed in Table III-2, the product was extracted with ethyl acetate (2 x 10 mL), washed with brine, dried over anhydrous Na_2SO_4, concentrated and purified by silica gel column chromatography (60-120 mesh) using ethyl acetate-petroleum ether as eluent to afford the desired product(s).



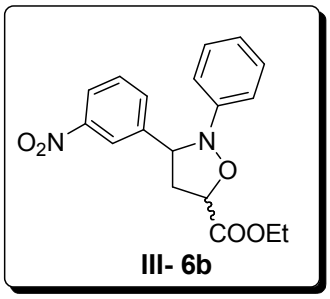
III- 6a

3-(2-Nitro-phenyl)-2-phenyl-isoxazolidine-5-carboxylic acid ethyl ester **(III-6a)**: Yellow oil, yield: 82%; *cis:trans* mixture of the diastereoisomers; ^{1}H NMR (400 MHz, $CDCl_3$): major isomer (*trans*) δ (ppm) 1.22-1.28 (m, 3H, overlapped peaks), 2.51-2.59 (m, 1H), 3.3-3.4 (m, 1H, overlapped peaks), 4.09-4.20 (m, 2H), 4.84 (t, J = 7.4 Hz, 1H), 5.57-5.63 (m, 1H), 6.97-7.11 (m, 3H), 7.25-7.32 (m, 2H), 7.47-7.53 (m, 1H), 7.67-7.71 (m, 1H), 8.05-8.22 (m, 2H); minor isomer (*cis*) δ (ppm) 1.22-1.28 (m, 3H, overlapped), 2.51-2.59 (m, 1H), 3.20-3.22 (1H, m, overlapped peaks), 4.09-4.20 (m, 2H), 4.67 (t, J = 7.2 Hz, 1H), 5.57-5.63 (m, 1H), 6.97-7.11 (m, 3H), 7.25-7.32 (m, 2H), 7.47-7.53 (m, 1H), 7.67-7.71 (m, 1H), 8.05-8.22 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): major isomer (*trans*) δ (ppm) 14.2, 41.9, 61.8, 66.7, 75.8, 114.9 (2C), 122.9, 125.1, 128.6, 128.9, 129.3 (2C), 134.4, 137.6, 147.4, 150.3, 169.8; minor isomer (*cis*) δ (ppm) 14.1, 41.0, 61.7, 66.3, 76.5, 115.5 (2C), 122.6, 125.3, 128.8, 129.2 (2C), 129.6, 134.3, 136.7, 147.5, 150.7, 170.3; ESI-MS: (*m/z*) 343 $[M+ H]^+$; *Anal.* Calcd. for $C_{18}H_{18}N_2O_5$: C, 63.15; H, 5.30; N, 8.18. Found: C, 63.02; H, 5.36; N, 8.24.



III- 5b

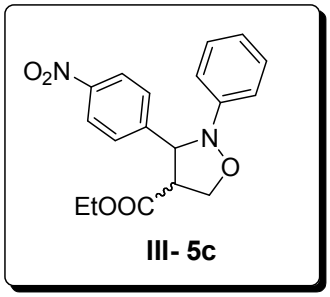
3-(3-Nitro-phenyl)-2-phenyl-isoxazolidine-4-carboxylic acid ethyl ester **(III-5b)**: Yellow oil, yield: 12%; *endo* isomer only; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.25 (t, J = 7.0 Hz, 3H), 3.55-3.61 (m, 1H), 4.18 (q, J = 7.1 Hz, 2H), 4.34 (t, J = 8.1 Hz, 1H), 4.43 (t, J = 8.4 Hz, 1H), 5.20 (d, J = 5.6 Hz, 1H), 7.0-7.10 (m, 3H), 7.27-7.31 (m, 2H), 7.60 (t, J = 8.0 Hz, 1H), 7.95 (d, J = 7.6 Hz, 1H), 8.19-8.22 (m, 1H), 8.46-8.47 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 14.1, 58.5, 61.9, 68.9, 71.2, 114.9 (2C), 121.8, 122.7, 122.9, 129.1 (2C), 130.0, 132.8, 143.8, 148.7, 150.0, 170.3; HRMS calculated for $C_{18}H_{18}N_2O_5$: 343.1294; found 343.1291 $[M + H]^+$.



III- 6b

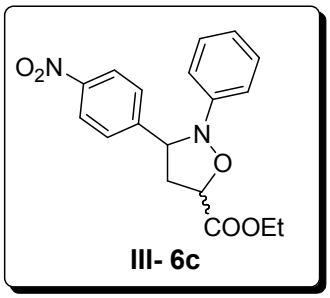
3-(3-Nitro-phenyl)-2-phenyl-isoxazolidine-5-carboxylic acid ethyl ester **(III-6b)**: Yellow oil, yield: 72%; mixture of the diastereoisomers; ^{1}H NMR (400 MHz, $CDCl_3$): major isomer (*trans*) δ (ppm) 1.26 (t, J = 7.3 Hz, 3H), 2.66-2.75 (m, 1H, overlapped peaks), 3.12 (dt, J = 4.4, 8.2 Hz, 1H), 4.21 (q, J = 7.2 Hz, 2H), 4.76-4.91 (m, 2H), 6.98-7.10 (m, 3H), 7.23-7.32 (m, 2H), 7.56-7.61 (m, 1H), 7.90 (q, J = 8.0 Hz, 1H), 8.16-8.20 (m, 1H), 8.37-8.39 (m, 1H); minor isomer (*cis*) δ (ppm) 1.25 (t, J = 7.3 Hz, 3H), 2.66-2.75 (m, 1H, overlapped peaks), 3.06 (ddd, J = 5.4, 7.4, 12.8 Hz, 1H), 4.16 (q, J = 7.2 Hz, 2H), 4.76-4.91 (m, 2H), 6.98-7.10 (m, 3H), 7.23-7.32 (m, 2H), 7.56-7.61 (m, 1H), 7.90 (q, J = 8.0 Hz, 1H), 8.16-8.20 (m, 1H), 8.37-8.39 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): major isomer (*trans*) δ (ppm) 14.1, 41.7, 61.8, 68.7, 75.2, 115.4 (2C), 121.9, 122.70, 123.2, 129.1 (2C), 129.8, 133.0, 143.4, 148.5, 149.9, 170.0; minor isomer (*cis*) δ (ppm) 14.0, 41.9, 61.7, 68.2, 76.3, 115.9 (2C), 121.8,

122.9, 123.2, 128.7, 130.0 (2C), 132.8, 142.9, 148.5, 150.4, 170.4; HRMS calculated for $C_{18}H_{18}N_2O_5$: 343.1294; found 343.1291 $[M + H]^+$.



III- 5c

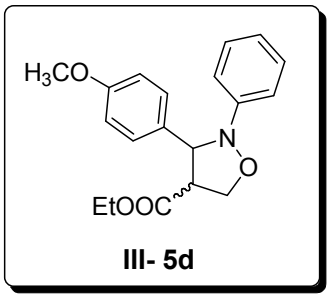
***3-(4-Nitro-phenyl)-2-phenyl-isoxazolidine-4-carboxylic acid ethyl ester* (III-5c):** Yellow oil, yield: 10%; *endo* isomer only; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.22 (t, J = 7.2 Hz, 3), 3.51-3.56 (m, 1H), 4.169 (q, J = 7.2 Hz, 2H), 4.31 (t, J = 7.9 Hz, 1H), 4.40 (t, J = 8.3 Hz, 1H), 5.17 (d, J = 5.8 Hz, 1H), 6.95-7.01 (m, 3H), 7.23-7.28 (m, 2H), 7.76 (d, J = 8.3 Hz, 2H), 8.25 (d, J = 8.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 14.1, 58.5, 61.9, 68.9, 71.3, 114.9 (2C), 122.6, 124.2 (2C), 127.5 (2C), 129.1 (2C), 147.5, 148.8, 150.0, 170.3; ESI-MS: (*m/z*) 343 $[M + H]^+$; *Anal.* Calcd. for $C_{18}H_{18}N_2O_5$: C, 63.15; H, 5.30; N, 8.18. Found: C, 63.16; H, 5.34; N, 8.14.



III- 6c

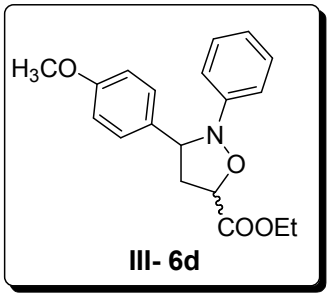
***3-(4-Nitro-phenyl)-2-phenyl-isoxazolidine-5-carboxylic acid ethyl ester* (III-6c):** Yellow oil, yield: 82%; *cis:trans* mixture; ^{1}H NMR (400 MHz, $CDCl_3$): major isomer (*trans*) δ (ppm) 1.26 (t, J = 7.2 Hz, 3H), 2.63-2.73 (m, 1H), 3.12 (dt, J = 8.6, 12.4 Hz, 1H), 4.16-4.24 (m, 2H), 4.77 (dd, J = 5.6, 7.7 Hz, 1H), 4.83-4.94 (m, 1H), 6.98-7.10 (m, 3H), 7.18-7.33 (m, 2H), 7.68-7.74 (m, 2H), 8.22-8.29 (m, 2H); minor isomer (*cis*) δ (ppm) 1.25 (t, J = 7.2 Hz, 3H), 2.63-2.73 (m, 1H), 3.07 (ddd, J = 5.5, 7.6, 12.4 Hz, 1H), 4.16-4.24 (m, 2H), 4.77 (dd, J = 5.6, 7.7 Hz, 1H), 4.83-4.94 (m, 1H), 6.98-7.10 (m, 3H), 7.18-7.33 (m, 2H), 7.68-7.74 (m, 2H), 8.22-8.29 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): major isomer (*trans*) δ (ppm) 14.1, 41.7, 61.7, 68.3, 76.3, 115.4 (2C), 123.2, 124.0 (2C), 127.7 (2C), 129.1 (2C), 147.5, 148.5, 149.9,

169.9; minor isomer (*cis*) δ (ppm) 14.0, 41.9, 63.3, 68.8, 75.6, 115.7 (2C), 122.8, 124.2 (2C), 127.6 (2C), 128.7 (2C), 147.4, 148.0, 149.9, 170.3; ESI-MS: (*m/z*) 343 $[M + H]^+$; *Anal.* Calcd. for $C_{18}H_{18}N_2O_5$: C, 63.15; H, 5.30; N, 8.18. Found: C, 63.07; H, 5.32; N, 8.22.



III- 5d

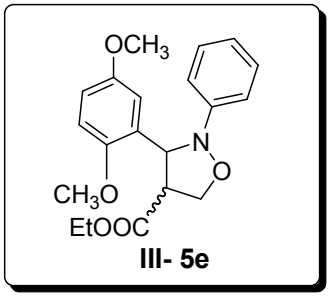
3-(4-Methoxy-phenyl)-2-phenyl-isoxazolidine-4-carboxylic acid ethyl ester **(III-5d):** Colourless oil, yield: 28%; *endo* isomer only; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.20 (t, J = 7.2 Hz, 3H), 3.52 (dt, J = 6.4, 7.7 Hz, 1H), 3.81 (s, 3H), 4.10-4.16 (m, 2H), 4.31 (dd, J = 6.7, 8.3 Hz, 1H), 4.38 (t, J = 8.3 Hz, 1H), 4.92 (d, J = 5.8 Hz, 1H), 6.89-7.02 (m, 5H), 7.19-7.26 (m, 2H), 7.45 (d, J = 8.5 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 14.1, 55.3, 58.6, 61.4, 68.9, 72.0, 114.2, 115.3 (2C), 122.2, 127.9 (2C), 128.8 (2C), 132.0, 133.2, 150.6, 159.2, 171.1; ESI-MS: (*m/z*) 328 $[M + H]^+$; *Anal.* Calcd. for $C_{19}H_{21}NO_4$: C, 69.71; H, 6.47; N, 4.28. Found: C, 69.60; H, 6.51; N, 4.22.



III- 6d

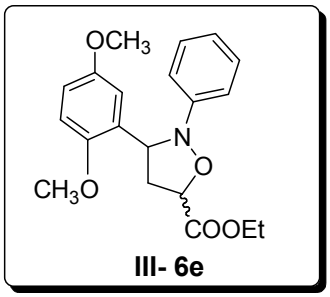
3-(4-methoxy-phenyl)-2-phenyl-isoxazolidine-5-carboxylic acid ethyl ester **(III-6d):** Colourless oil, yield: 57%; *cis*:*trans* mixture; ^{1}H NMR (400 MHz, $CDCl_3$): major isomer (*trans*), δ (ppm) 1.30 (t, J = 7.2 Hz, 3H), 2.66-2.74 (m, 1H), 3.10 (dt, J = 8.3, 12.6 Hz, 1H), 3.84 (s, 3H), 4.16-4.28 (m, 2H, overlapped peaks), 4.70 (t, J = 6.8 Hz, 1H), 4.78-4.82 (m, 1H), 6.88-7.11 (m, 5H), 7.20-7.30 (m, 2H), 7.40-7.44 (m, 2H); minor isomer (*cis*) δ (ppm) 1.26 (t, J = 7.2 Hz, 3H), 2.66-2.74 (m, 1H), 2.93 (ddd, J = 5.5, 7.2, 12.6 Hz, 1H), 3.84 (s, 3H), 4.16-4.28 (m, 2H, overlapped peaks), 4.35 (t, J = 8.6 Hz, 1H), 4.56-4.64 (m, 1H), 6.88-7.11 (m, 5H), 7.20-7.30 (m, 2H), 7.40-7.44 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): major

isomer (*trans*) δ (ppm) 14.2, 42.6, 55.3, 61.5, 68.7, 76.1, 114.1 (2C), 116.3 (2C), 122.5, 128.0 (2C), 128.8 (2C), 132.7, 150.9, 159.1, 171.0; minor isomer (*cis*) δ (ppm) 14.0, 42.6, 55.3, 61.5, 69.4, 75.4, 114.2 (2C), 115.9 (2C), 122.4, 127.9 (2C), 128.5 (2C), 132.1, 150.6, 159.3, 170.6; HRMS calculated for $C_{19}H_{21}NO_4$: 328.1549; found 328.1548 $[M + H]^+$.



III- 5e

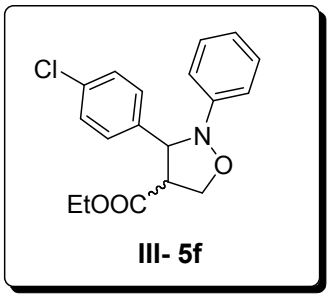
***3-(2,5-Dimethoxy-phenyl)-2-phenyl-isoxazolidine-4-carboxylic acid ethyl ester* (III-5e):** Colourless oil, yield: 27%; *endo* isomer only; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.23 (t, J = 7.2 Hz, 3H), 2.54 (ddd, J = 4.4, 7.4, 12.4 Hz, 1H), 2.89 (dt, J = 7.4, 12.4 Hz, 1H), 3.76 (s, 3H), 3.82 (s, 3H), 4.13-4.22 (m, 2H), 4.66 (t, J = 7.4 Hz, 1H), 5.13 (dd, J = 4.4, 7.4 Hz, 1H), 6.80-6.84 (m, 2H), 6.92-6.96 (m, 1H), 7.11-7.14 (m, 2H), 7.20-7.26 (m, 2H), 7.28 (d, J = 2.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 14.0, 39.9, 55.7, 55.9, 61.4, 64.3, 76.4, 111.2, 112.9, 113.4, 115.6 (2C), 121.9, 128.5 (2C), 129.8, 150.2, 151.5, 153.9, 171.0; ESI-MS: (*m/z*) 380 $[M + Na]^+$; *Anal.* Calcd. for $C_{20}H_{23}NO_5$: C, 67.21; H, 6.49; N, 3.92. Found: C, 67.35; H, 6.52; N, 3.94.



III- 6e

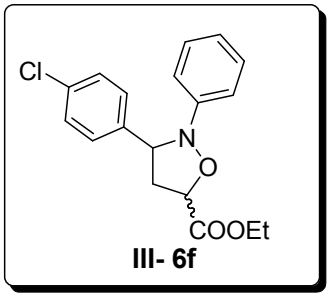
***3-(2,5-Dimethoxy-phenyl)-2-phenyl-isoxazolidine-5-carboxylic acid ethyl ester* (III-6e):** Colourless oil, yield: 54%; only *trans* isomer; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.23 (t, J = 7.2 Hz, 3H), 2.51 (ddd, J = 5.3, 7.0, 12.4 Hz, 1H), 3.07 (dt, J = 8.3, 12.6 Hz, 1H), 3.76 (s, 3H), 3.82 (s, 3H), 4.15-4.20 (m, 2H), 4.76 (t, J = 7.52 Hz, 1H), 5.08 (dd, J = 5.2, 8.2 Hz, 1H), 6.76-6.82 (m, 2H), 6.93-6.98 (m, 1H), 7.02-7.05 (m, 2H), 7.23-7.28 (m, 2H), 7.34 (d, J = 2.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 14.1, 41.0, 55.9, 55.8, 61.4, 64.5, 75.6,

111.2, 112.8, 113.5, 114.9 (2C), 122.1, 128.8 (2C), 130.5, 150.2, 151.2, 154.0, 170.2; ESI-MS: (*m/z*) 358 $[M + H]^+$; *Anal.* Calcd. for $C_{20}H_{23}NO_5$: C, 67.21; H, 6.49; N, 3.92. Found: C, 67.32; H, 6.53; N, 3.95.



III- 5f

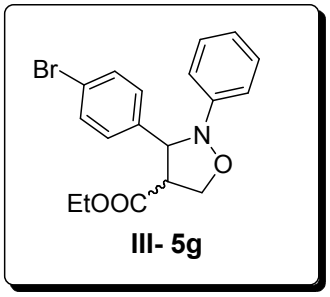
***3-(4-Chloro-phenyl)-2-phenyl-isoxazolidine-4-carboxylicacid ethyl ester* (III-5f):** Colourless oil, yield: 11%; *endo* isomer only; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.23 (t, J = 7.2 Hz, 3H), 3.50-3.56 (m, 1H), 4.16 (q, J = 7.2 Hz, 2H), 4.32 (dd, J = 7.2, 8.4 Hz, 1H), 4.40 (t, J = 8.2 Hz, 1H), 5.01 (d, J = 6.0 Hz, 1H), 6.97-7.00 (m, 3H), 7.24-7.28 (m, 2H), 7.38 (d, J = 8.4 Hz, 2H), 7.52 (d, J = 8.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 14.1, 58.5, 61.6, 68.9, 71.6, 115.1 (2C), 122.4, 128.1 (2C), 128.9 (2C), 129.1 (2C), 133.6, 139.8, 150.3, 170.7; HRMS calculated for $C_{18}H_{19}ClNO_3$ $[M + H]^+$: 332.1053; found 332.1050 For Cl = 35 and 334.1024; found 334.1069 $[M + H]^+$ For Cl = 37.



III- 6f

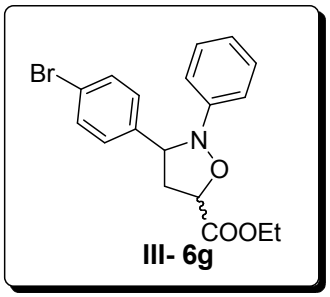
***3-(4-Chloro-phenyl)-2-phenyl-isoxazolidine-5-carboxylic acid ethyl ester* (III-6f):** Colourless oil, yield: 68%; *cis*:*trans* mixture; ^{1}H NMR (400 MHz, $CDCl_3$): major isomer (*cis*) δ (ppm) 1.25 (t, J = 7.2 Hz, 3H, overlapped peaks), 2.63-2.70 (m, 1H), 2.97 (ddd, J = 5.4, 7.3, 12.6 Hz, 1H), 4.17-4.24 (m, 2H), 4.73-4.79 (m, 2H), 6.97-7.09 (m, 3H), 7.22-7.31 (m, 2H), 7.33-7.38 (m, 2H), 7.44-7.47 (m, 2H); minor isomer (*trans*) δ (ppm) 1.25 (t, J = 7.2 Hz, 3H, overlapped peaks), 2.63-2.70 (m, 1H), 3.06 (dt, J = 8.3, 12.6 Hz, 1H), 4.17-4.24 (m, 2H), 4.73-4.79 (m, 2H), 6.97-7.09 (m, 3H), 7.22-7.31 (m, 2H), 7.33-7.38 (m, 2H), 7.44-7.47 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): major isomer (*cis*) δ (ppm) 14.0, 42.3, 61.6, 68.5,

76.1, 116.0, 122.7, 128.1 (2C), 128.6 (2C), 128.9, 129.0 (2C), 133.6, 138.8, 150.6, 170.7; minor isomer (*trans*) δ (ppm) 13.8, 53.1, 61.1, 67.6, 75.5, 115.2, 122.9, 128.2 (2C), 128.5 (2C), 129.0, 129.4 (2C), 133.9, 136.6, 150.0, 169.2; HRMS calculated for $C_{18}H_{19}ClNO_3$ $[M + H]^+$: 332.1053; found 332.1058 For Cl = 35 and 334.1024; found 334.1055 $[M + H]^+$ For Cl = 37.



III- 5g

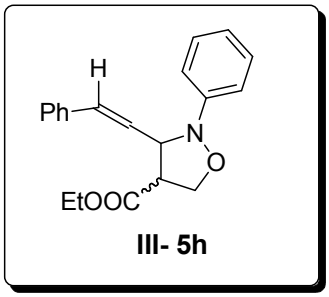
3-(4-Bromo-phenyl)-2-phenyl-isoxazolidine-4-carboxylic acid ethyl ester **(III-5g):** Colourless oil, yield: 12%; *endo* isomer only; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.23 (t, J = 7.2 Hz, 3H), 3.50-3.55 (m, 1H), 4.16 (q, J = 7.2 Hz, 2H), 4.32 (dd, J = 7.0, 8.5 Hz, 1H), 4.40 (t, J = 8.5 Hz, 1H), 5.0 (d, J = 5.8 Hz, 1H), 6.97-7.00 (m, 3H), 7.24-7.29 (m, 2H), 7.46 (d, J = 8.5 Hz, 2H), 7.54 (d, J = 8.5 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 14.1, 58.5, 61.6, 68.9, 71.6, 115.1 (2C), 121.7, 122.4, 128.4 (2C), 128.9 (2C), 132.0 (2C), 140.4, 150.3, 170.7; HRMS calculated for $C_{18}H_{19}BrNO_3$ $[M + Na]^+$: 398.0368; found 398.0365 For Br = 79 and 400.0347; found 400.0345 For Br = 81.



III- 6g

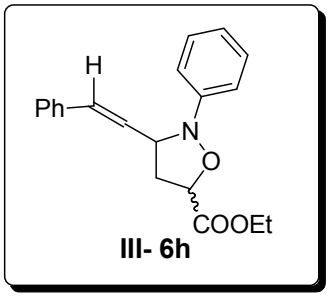
3-(4-Bromo-phenyl)-2-phenyl-isoxazolidine-5-carboxylic acid ethyl ester **(III-6g):** Colourless oil, yield: 75%; *cis*:*trans* mixture; ^{1}H NMR (400 MHz, $CDCl_3$): major isomer (*trans*) δ (ppm) 1.26 (t, J = 7.2 Hz, 3H), 2.62-2.71 (m, 1H), 3.07 (dt, J = 8.5, 12.8 Hz, 1H), f4.15-4.26 (m, 2H), 4.66-4.83 (m, 2H), 7.00-7.09 (m, 3H), 7.22-7.31 (m, 2H), 7.38-7.41 (m, 2H), 7.49-7.54 (m, 2H); minor isomer (*cis*) δ (ppm) 1.28 (t, J = 7.2 Hz, 3H), 2.62-2.71 (m, 1H), 2.97 (ddd, J = 5.6, 7.3, 12.6 Hz, 1H), 4.15-4.26 (m, 2H), 4.66-4.83 (m, 2H), 7.00-7.09

(m, 3H), 7.22-7.31 (m, 2H), 7.38-7.41 (m, 2H), 7.49-7.54 (m, 2H); ^{13} C NMR (100 MHz, $CDCl_3$): major isomer (*trans*) δ (ppm)14.1, 42.3, 61.6, 69.0, 76.1, 115.7 (2C), 121.5, 122.9, 128.4, 128.6 (2C), 129.7, 132.0 (2C), 139.5, 150.7, 170.6; minor isomer (*cis*) δ (ppm) 14.0, 42.2, 61.1, 68.5, 75.5, 116.0 (2C), 121.6, 122.6, 128.5, 128.9 (2C), 129.9, 131.9 (2C), 140.0, 150.3, 170.3; HRMS calculated for $C_{18}H_{19}BrNO_3$ $[M + H]^+$: 376.0548; found 376.0544 For Br = 79 and 378.0528; found 378.0533 For Br = 81.



III- 5h

2-Phenyl-3-styryl-isoxazolidine-4-carboxylic acid ethyl ester **(III-5h):** Light yellow oil, yield: 37%; *exo***:***endo* mixture; ^{1}H NMR (400 MHz, $CDCl_3$): major isomer (*exo*) δ (ppm) 1.18 (t, J = 7.0 Hz, 3H), 3.64 (two dt overlapped, dd, J = 7.8, 16.8 Hz, 1H), 4.10-4.19 (m, 2H, overlapped peaks), 4.30 (dd, J = 8.4, 11.6 Hz, 1H), 4.51 (t, J = 7.6 Hz, 1H), 4.75 (t, J = 8.0 Hz, 1H), 6.32 (dd, J = 8.0, 16.0 Hz, 1H), 6.73-6.81 (m,1H), 7.00-7.10 (m, 1H), 7.13-7.16 (m, 2H), 7.26-7.53 (m, 7H); minor isomer (*endo*) δ (ppm) 1.23 (t, J = 7.0 Hz, 3H), 3.50 (dd, J = 7.0, 13.8 Hz, 1H, overlapped peaks), 4.10-4.19 (m, 2H, overlapped peaks), 4.38 (t, J = 8.4 Hz, 1H), 4.63 (t, J = 6.4 Hz, 1H), 6.43 (dd, J = 7.2, 15.6 Hz, 1H), 6.73-6.81 (m, 1H), 7.00-7.10 (m, 1H), 7.13-7.16 (m, 2H), 7.26-7.53 (m, 7H); ^{13}C NMR (100 MHz, $CDCl_3$): major isomer (*exo*) δ (ppm) 14.3, 51.6, 61.2, 67.4, 70.3, 115.3 (2C), 122.7, 128.0, 128.6 (2C), 128.9, 129.1 (2C), 131.3, 133.3, 133.5, 136.3, 149.8, 169.5; minor isomer (*endo*) δ (ppm) 14.1, 55.8, 61.5, 68.7, 71.4, 115.6 (2C), 122.6, 128.1, 128.5, 128.7 (2C), 129.2 (2C), 132.1, 133.3, 133.5, 136.3, 150.36, 170.8; ESI-MS: (*m/z*) 346 $[M + Na]^+$; *Anal.* Calcd. for $C_{20}H_{21}NO_3$: C, 74.28; H, 6.55; N, 4.33. Found: C, 74.39; H, 6.58; N, 4.35.



III- 6h

2-Phenyl-3-styryl-isoxazolidine-5-carboxylic acid ethyl ester **(III-6h):** Light yellow oil, yield: 56%; *cis*:*trans* mixture; ^{1}H NMR (400 MHz, $CDCl_3$): major isomer (*cis*) δ (ppm) 1.28 (t, J = 7.1 Hz, 3H), 2.65 (ddd, J = 6.4, 8.1, 12.6 Hz, 1H), 2.80 (1H, ddd, J = 5.2, 7.2, 12.4 Hz), 4.22 (2H, dq, J = 2.0, 7.1 Hz), 4.37-4.41 (m, 1H), 4.77-4.82 (m, 1H), 6.30 (dd, J = 7.2, 16.0 Hz, 1H), 6.68 (d, J = 2.4 Hz, 1H), 6.99-7.05 (m, 1H), 7.17-7.20 (m, 1H), 7.23-7.30 (m, 4H), 7.32-7.35 (m, 2H), 7.37-7.42 (m, 2H); minor isomer (*trans*) δ (ppm) 1.32 (t, J = 7.1 Hz, 3H), 2.60 (dt, J = 5.6, 12.7 Hz, 1H), 2.87 (dt, J = 8.4, 12.6 Hz, 1H), 4.28 (q, J = 7.2 Hz, 2H), 4.31-4.36 (m, 1H), dd, 6.37 (dd, J = 7.2, 16.0 Hz, 1H), 6.72 (d, J = 2.4 Hz, 1H), 6.99-7.05 (m, 1H), 7.17-7.20 (m, 1H), 7.23-7.30 (m, 4H), 7.32-7.35 (m, 2H), 7.37-7.42 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): major isomer (*cis*) δ (ppm) 14.1, 40.0, 61.5, 68.6, 75.8, 116.0 (2C), 122.9, 126.5, 128.0, 128.5 (2C), 128.7 (2C), 128.9 (2C), 131.8, 136.3, 150.4, 171.0; minor isomer (*trans*) δ (ppm) 14.2, 39.8, 61.6, 67.8, 75.2, 116.8 (2C), 123.0, 127.6, 127.9, 128.6 (2C), 128.6 (2C), 129.0 (2C), 132.6, 136.4, 150.3, 170.7; ESI-MS: (*m/z*) 346 $[M + Na]^+$; *Anal.* Calcd. for $C_{20}H_{21}NO_3$: C, 74.28; H, 6.55; N, 4.33. Found: C, 74.43; H, 6.57; N, 4.34.

III.5. Notes & References

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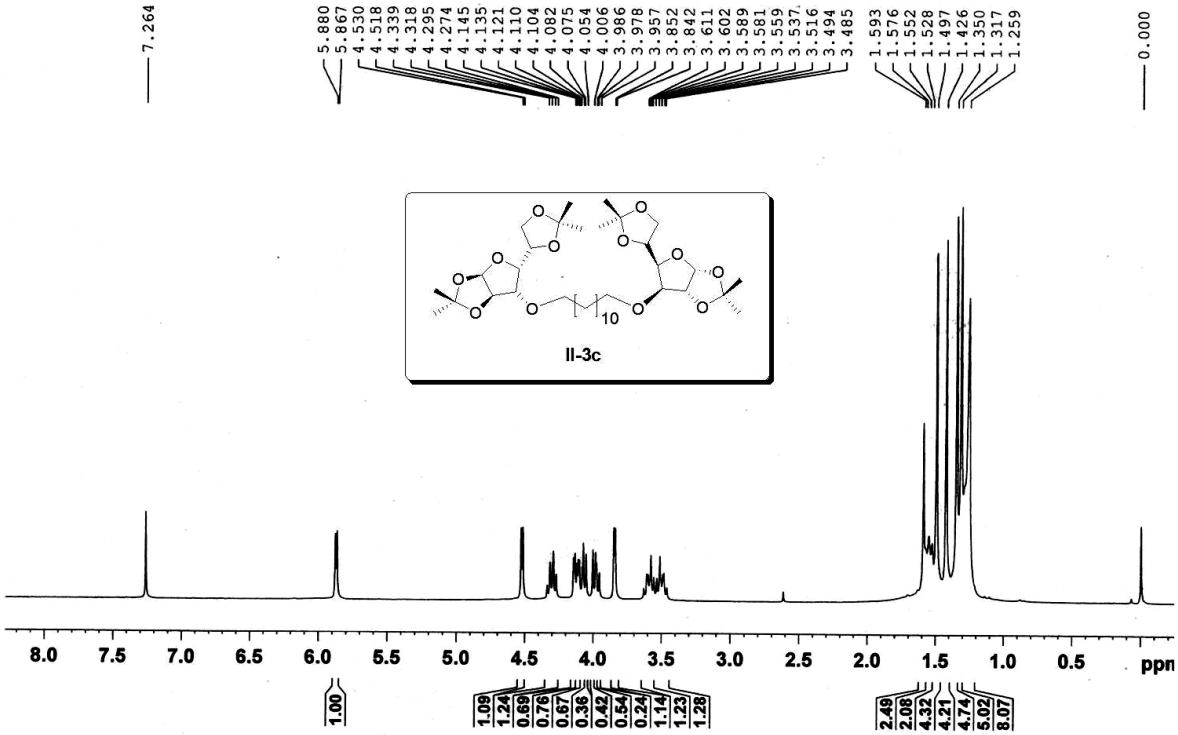
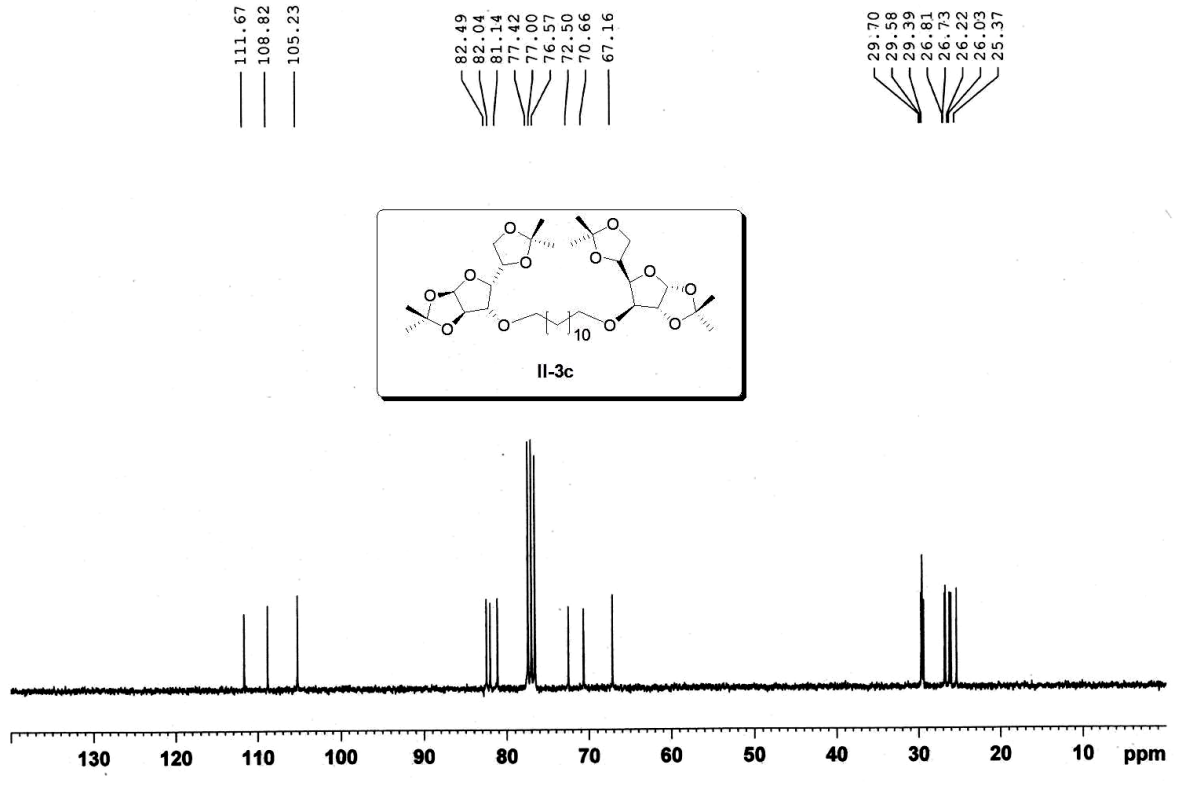
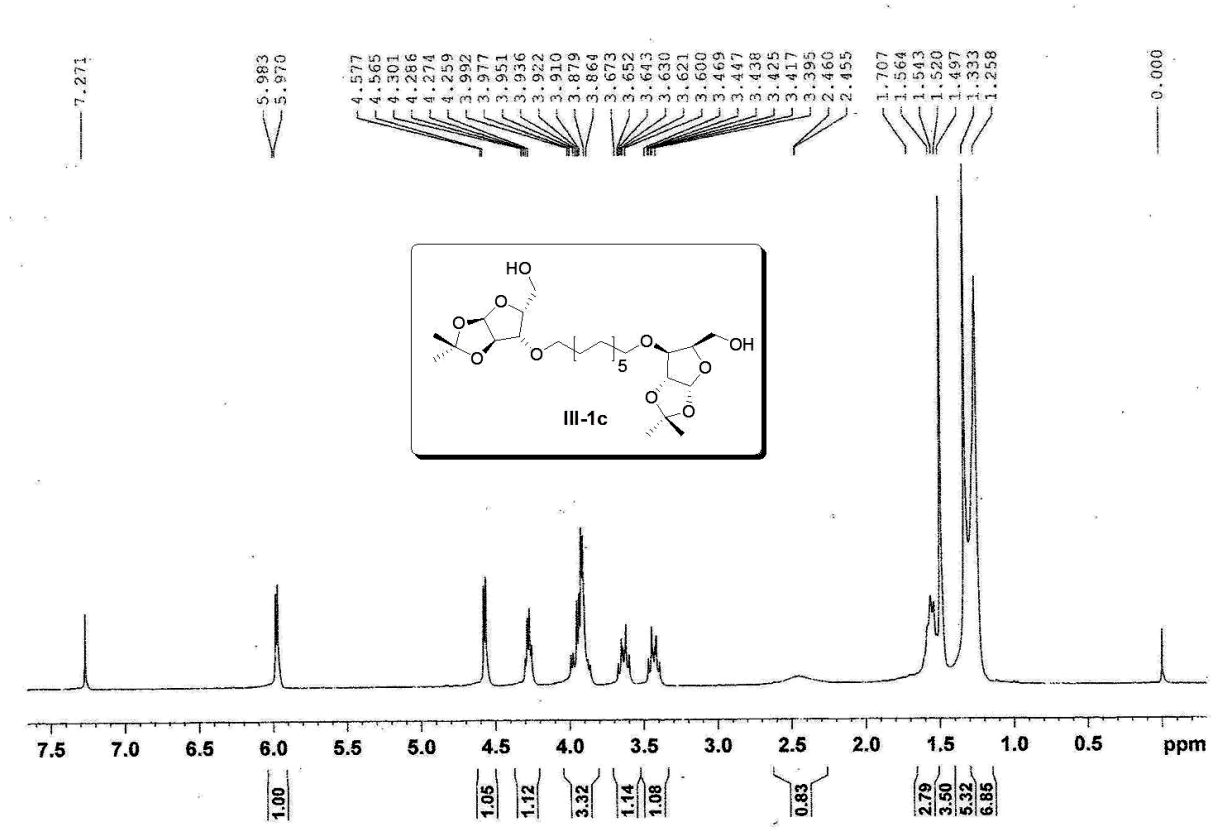
III.6. Supporting Information (selected spectra)



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3.922
3.910
3.879
3.864
3.673
3.652
3.643
3.630
3.621
3.600
3.469
3.447
3.438
3.425
3.417
3.395
2.460
2.455
1.707
1.564
1.543
1.520
1.497
1.333
1.258
0.000
HO
OH
5
III-1c
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
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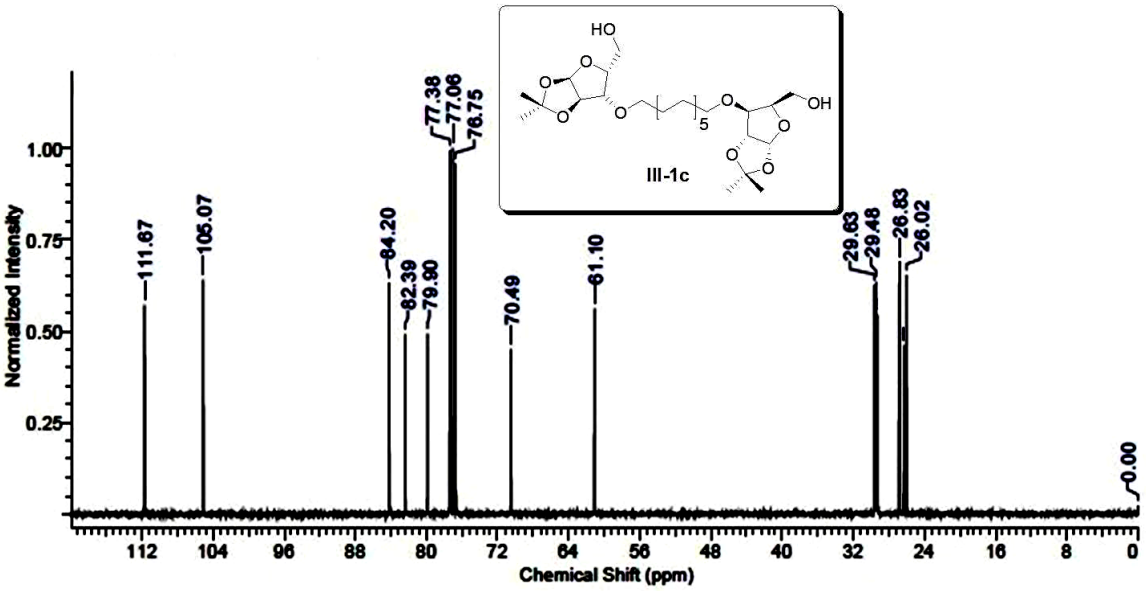
1.05
1.12
3.32
1.14
1.08
0.83
2.79
3.50
5.32
6.85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OH
5
III-1c
Normalized Intensity
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111.67
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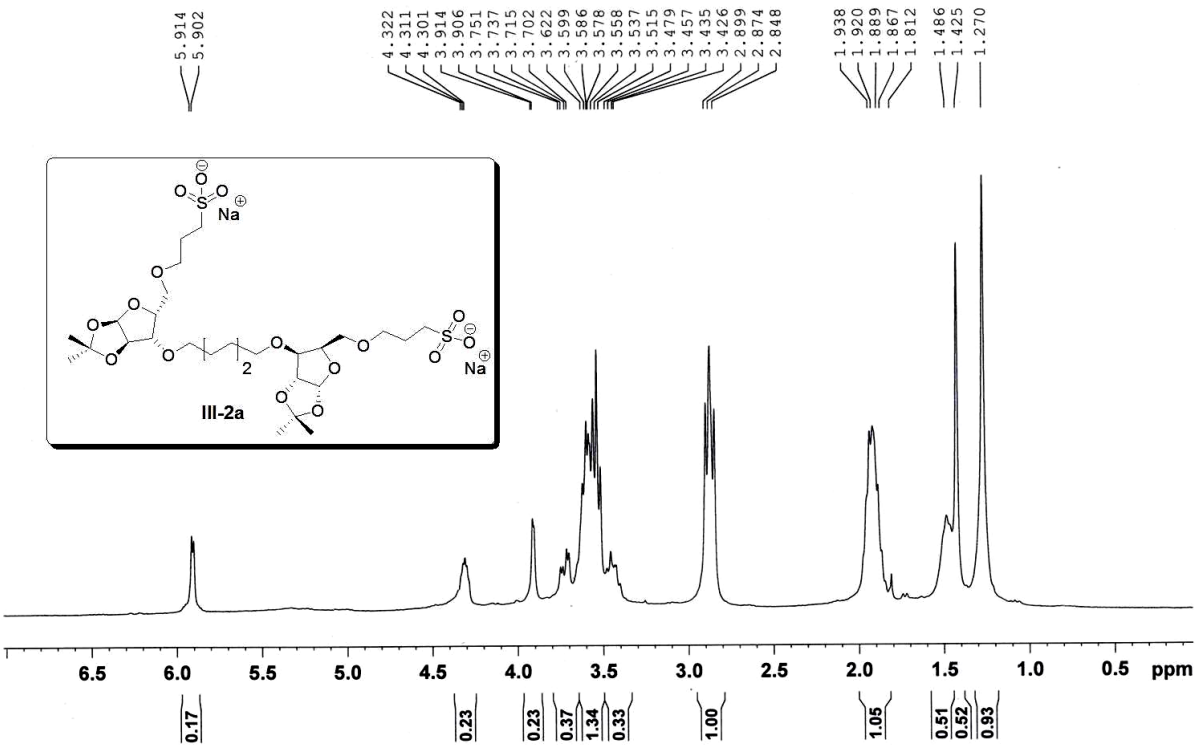
79.90
77.38
77.06
76.75
70.49
61.10
29.63
29.48
26.83
26.02
0.00
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0
Chemical Shift (ppm)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3.914
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3.715
3.702
3.622
3.599
3.586
3.578
3.558
3.537
3.515
3.479
3.457
3.435
3.426
2.899
2.874
2.848
1.938
1.920
1.889
1.867
1.812
1.486
1.425
1.270
III-2a
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
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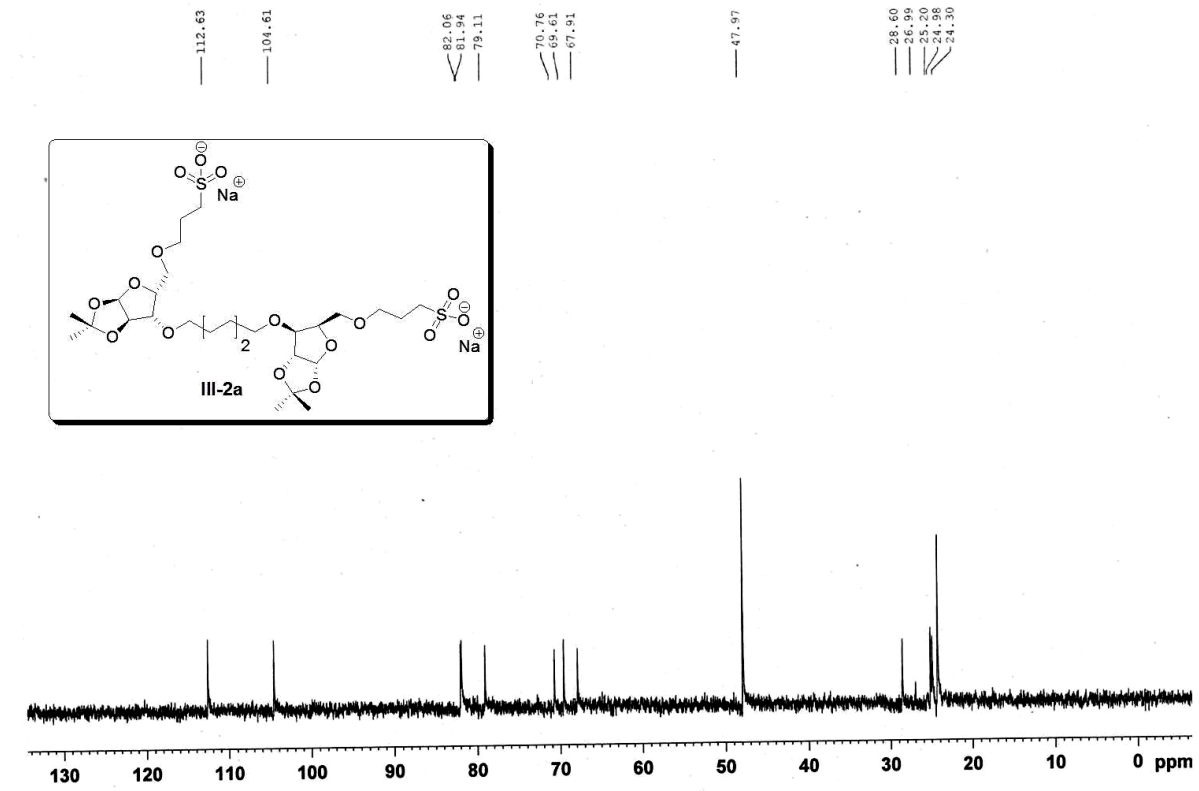
0.23
0.23
0.37
1.34
0.33
1.00
1.05
0.51
0.52
0.93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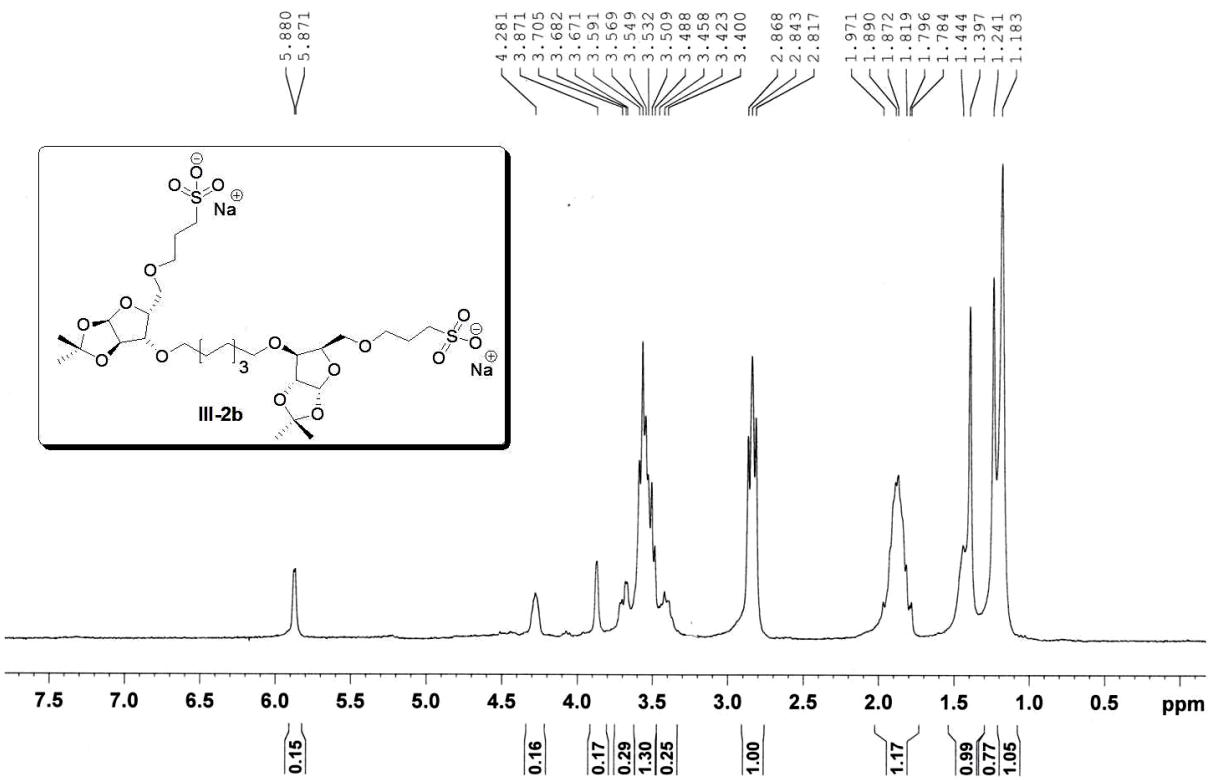
104.61
82.06
81.94
79.11
70.76
69.61
67.91
47.97
28.60
26.99
25.20
24.98
24.30
III-2a
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm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3.488
3.458
3.423
3.400
2.868
2.843
2.817
1.971
1.890
1.872
1.819
1.796
1.784
1.444
1.397
1.241
1.183
III-2b
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
0.15
0.16
0.17
0.29
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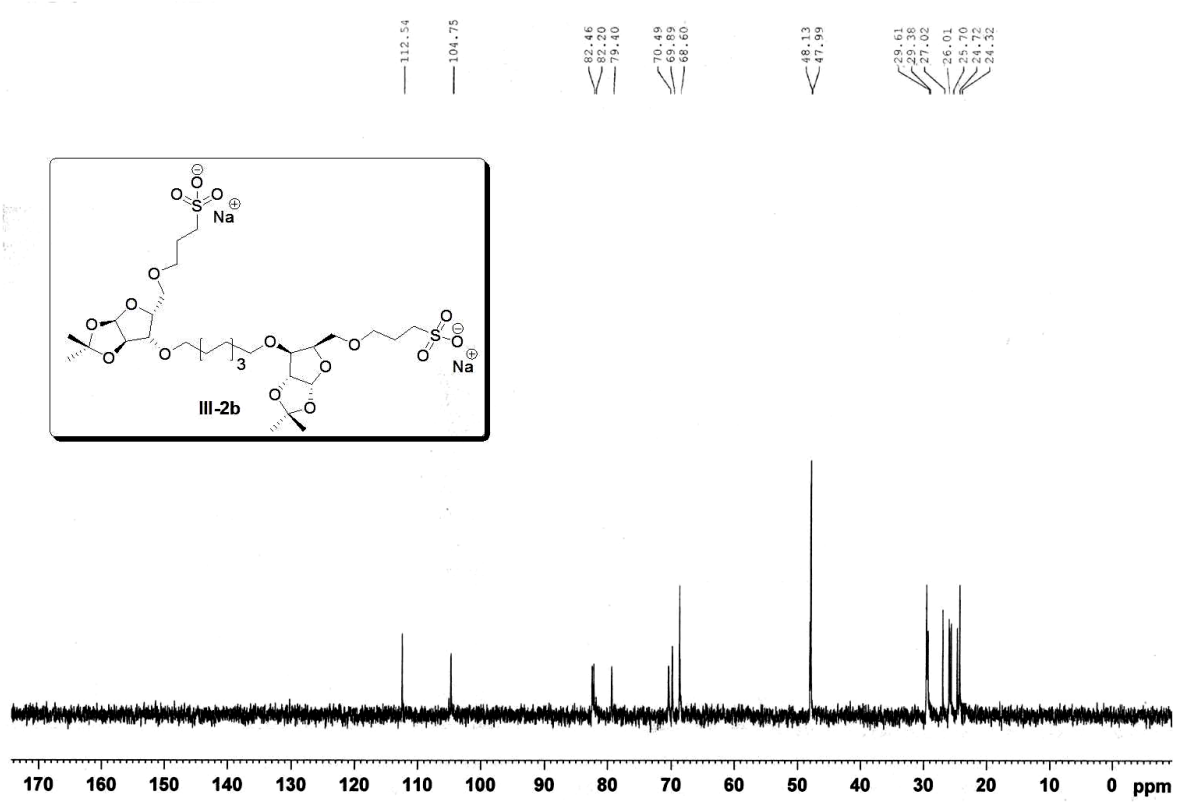
0.25
1.00
1.17
0.99
0.77
1.05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70.49
69.89
68.60
48.13
47.99
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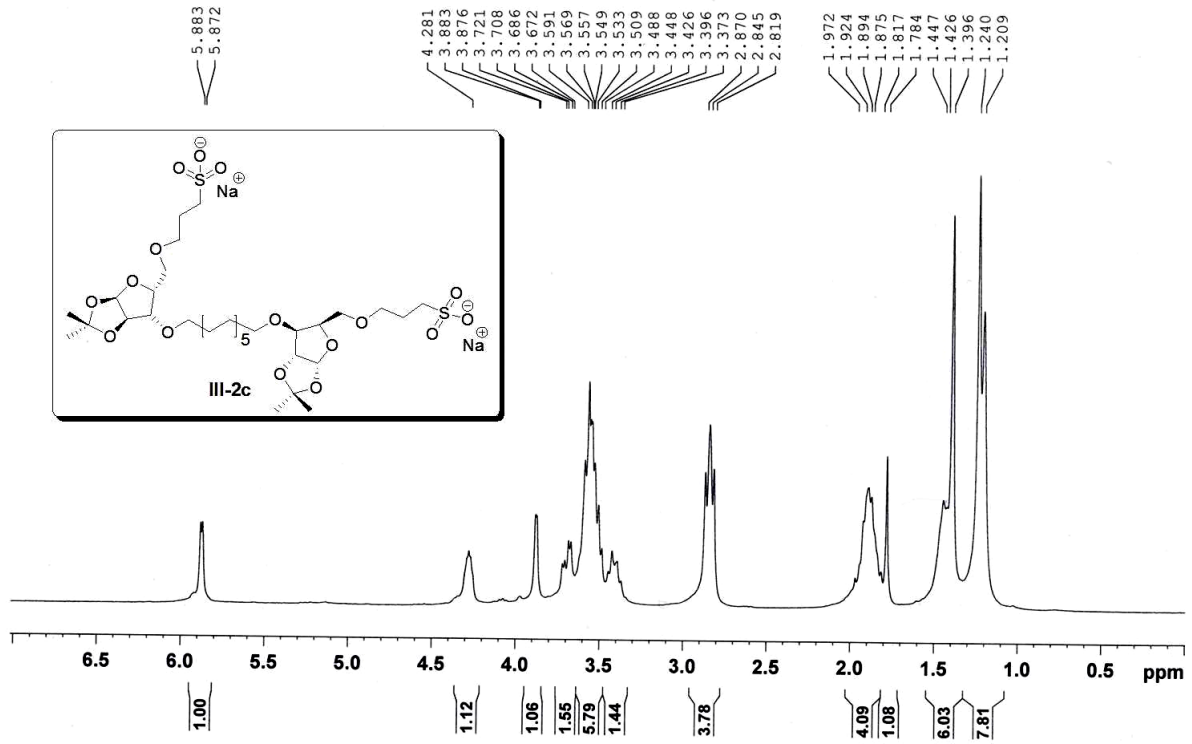
27.02
26.01
25.70
24.72
24.32
III-2b
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm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3.549
3.533
3.509
3.488
3.448
3.426
3.396
3.373
2.870
2.845
2.819
1.972
1.924
1.894
1.875
1.817
1.784
1.447
1.426
1.396
1.240
1.209
III-2c
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
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1.06
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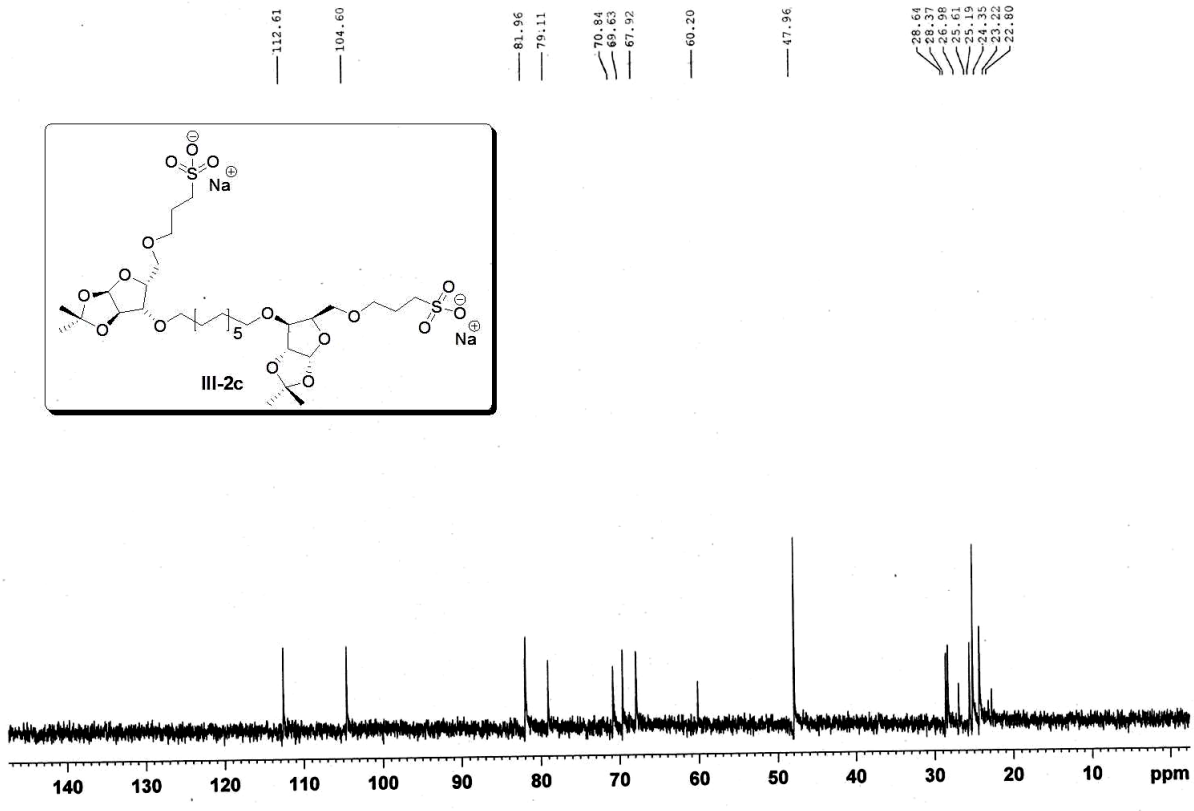
1.08
6.03
7.81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25.19
24.35
23.22
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III-2c
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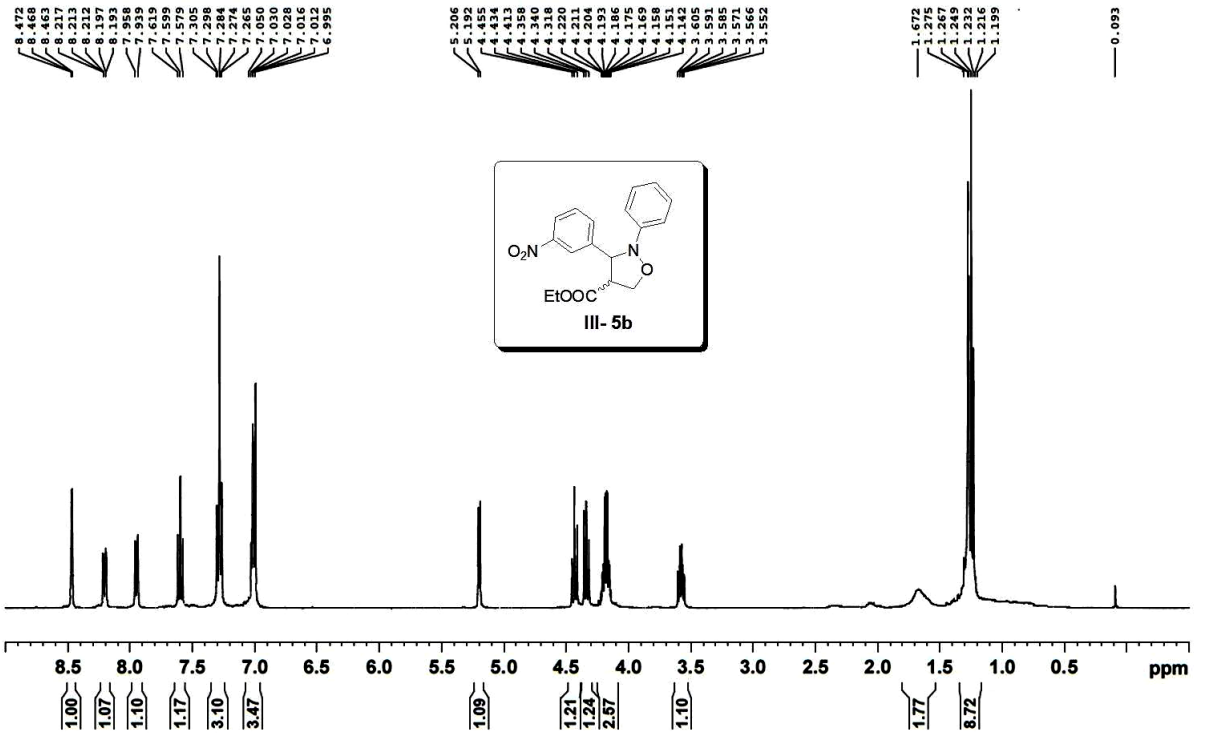
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm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7.274
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7.012
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5.192
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4.434
4.411
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4.220
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4.186
4.175
4.169
4.158
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4.142
3.605
3.591
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3.571
3.566
3.552
1.672
1.675
1.267
1.249
1.232
1.216
1.199
0.093
O2N
N
O
EtOOC
III- 5b
8.5
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7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
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1.17
3.10
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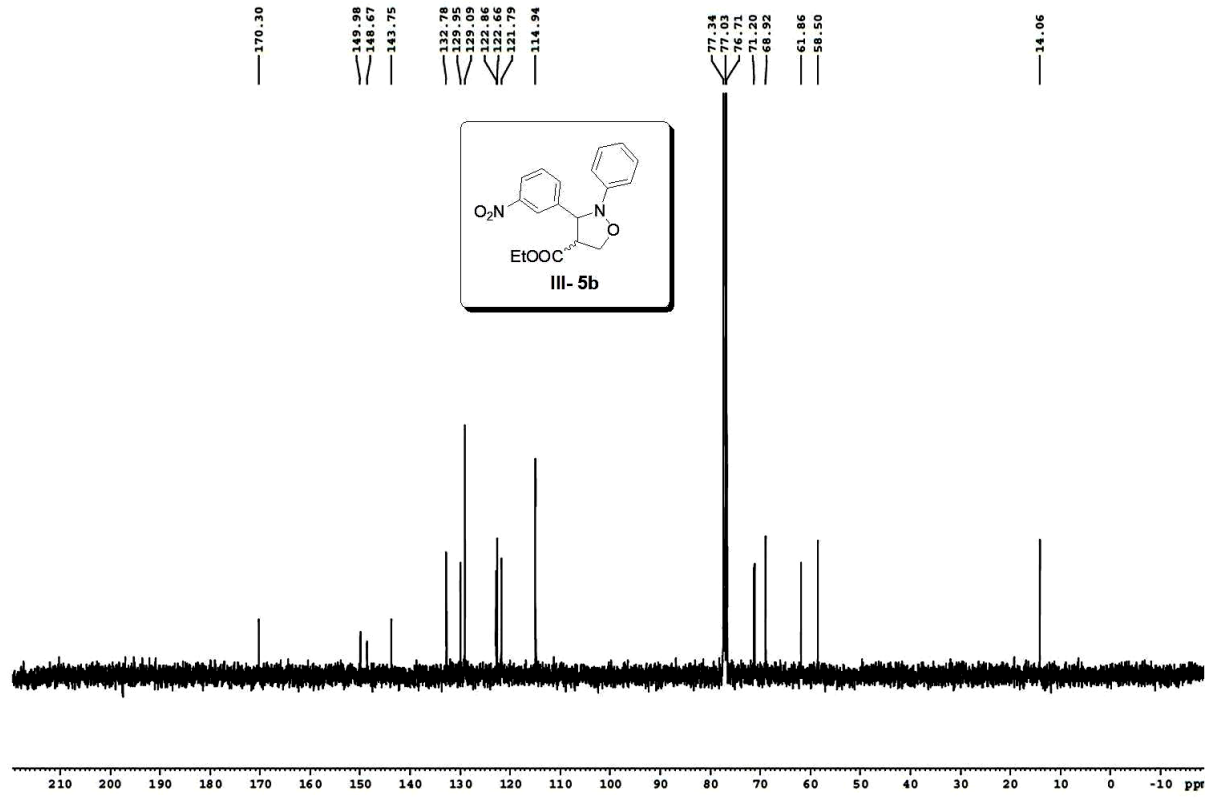
1.24
2.57
1.10
1.77
8.72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77.03
76.71
71.20
68.92
61.86
58.50
14.06
O2N
N
O
EtOOC
III- 5b
210
200
190
180
170
160
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130
120
110
100
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80
70
60
50
40
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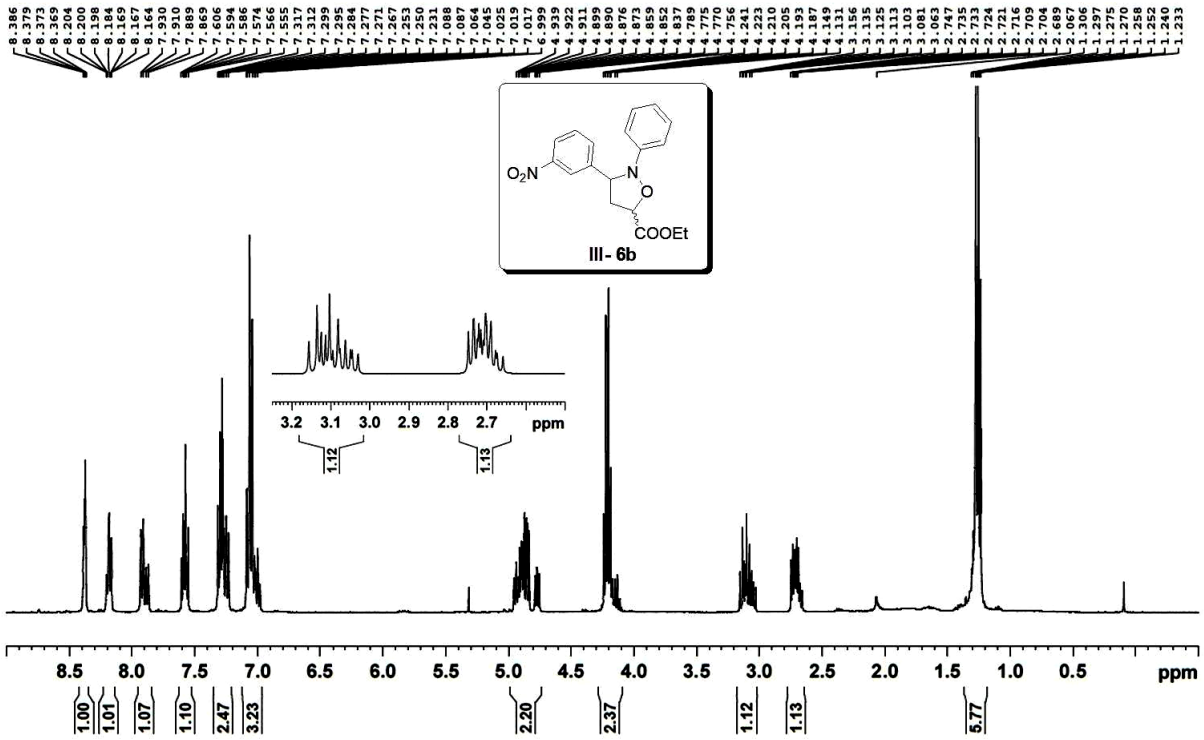
20
10
0
-10
ppm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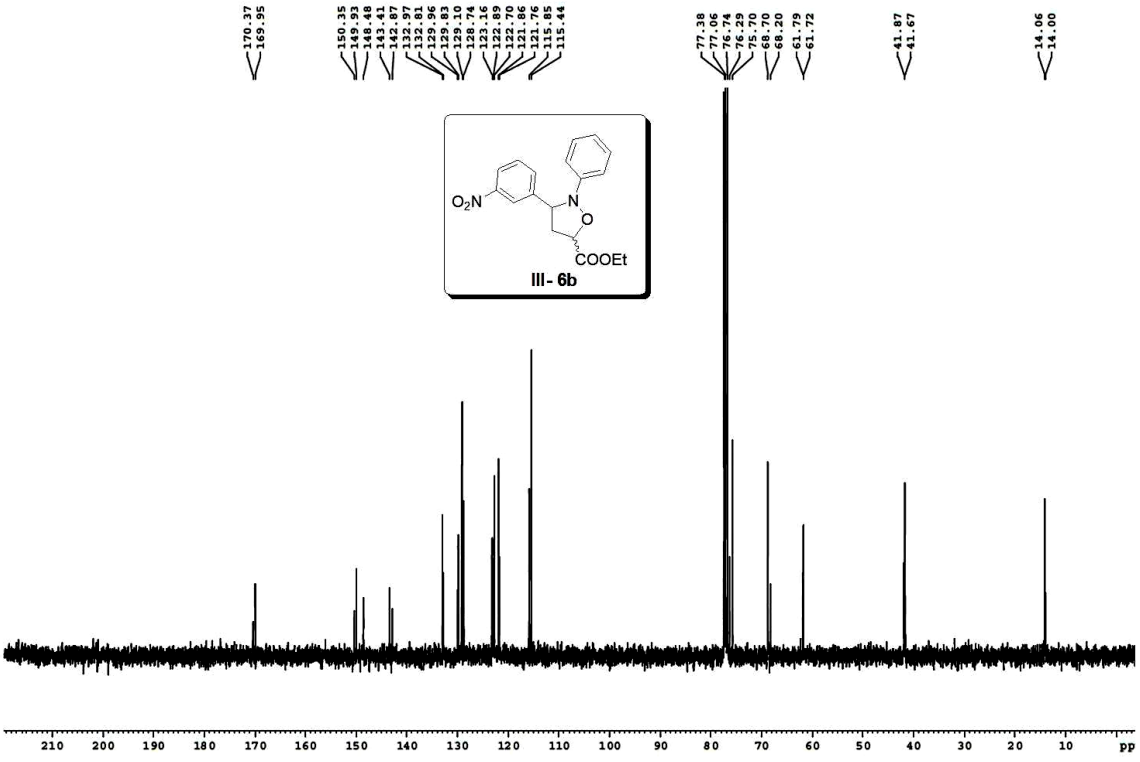
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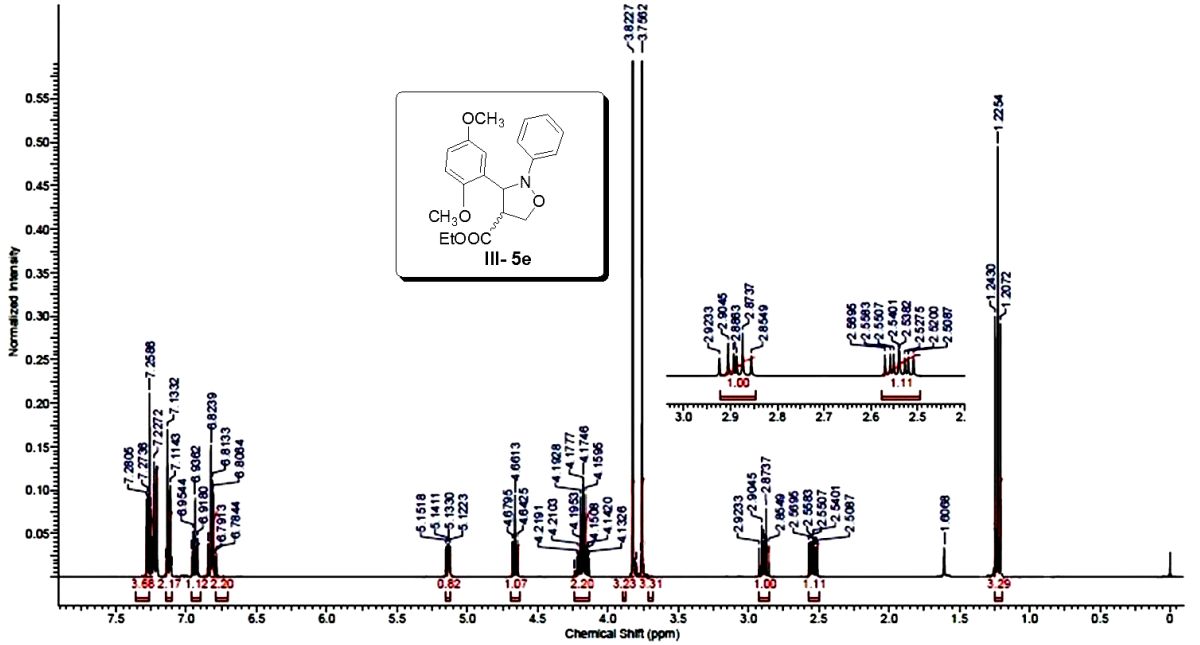
ppm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CH3O
EtOOC
III- 5e
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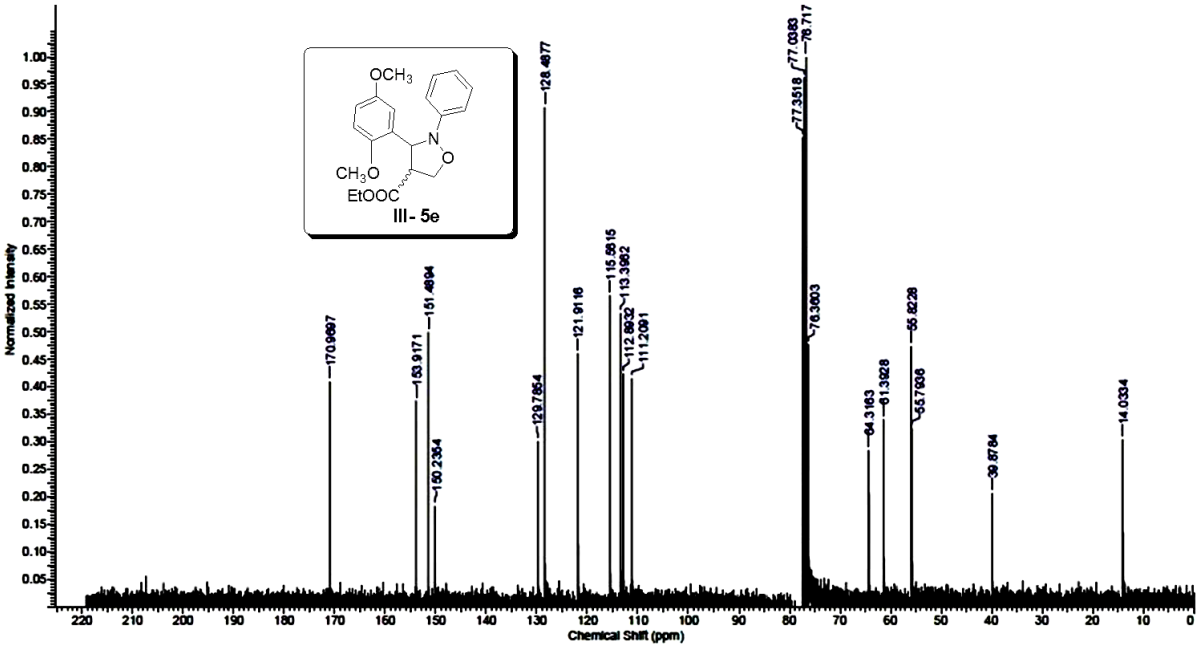
Chemical Shift (ppm)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CH3O
EtOOC
III- 5e
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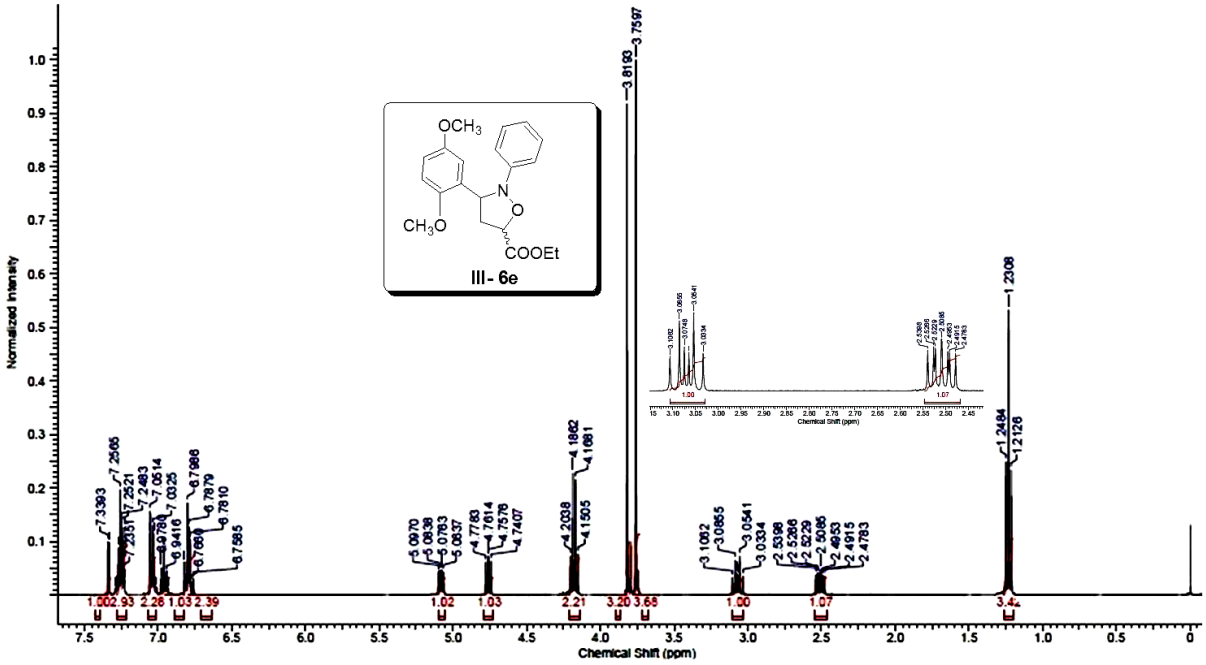
Chemical Shift (ppm)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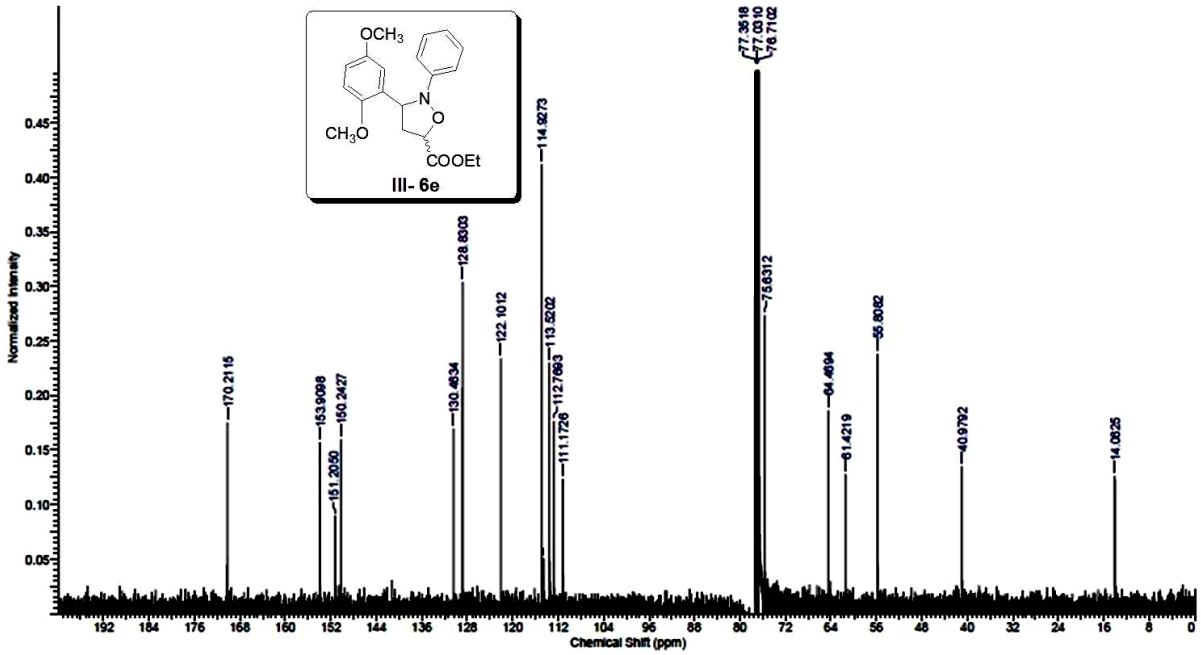
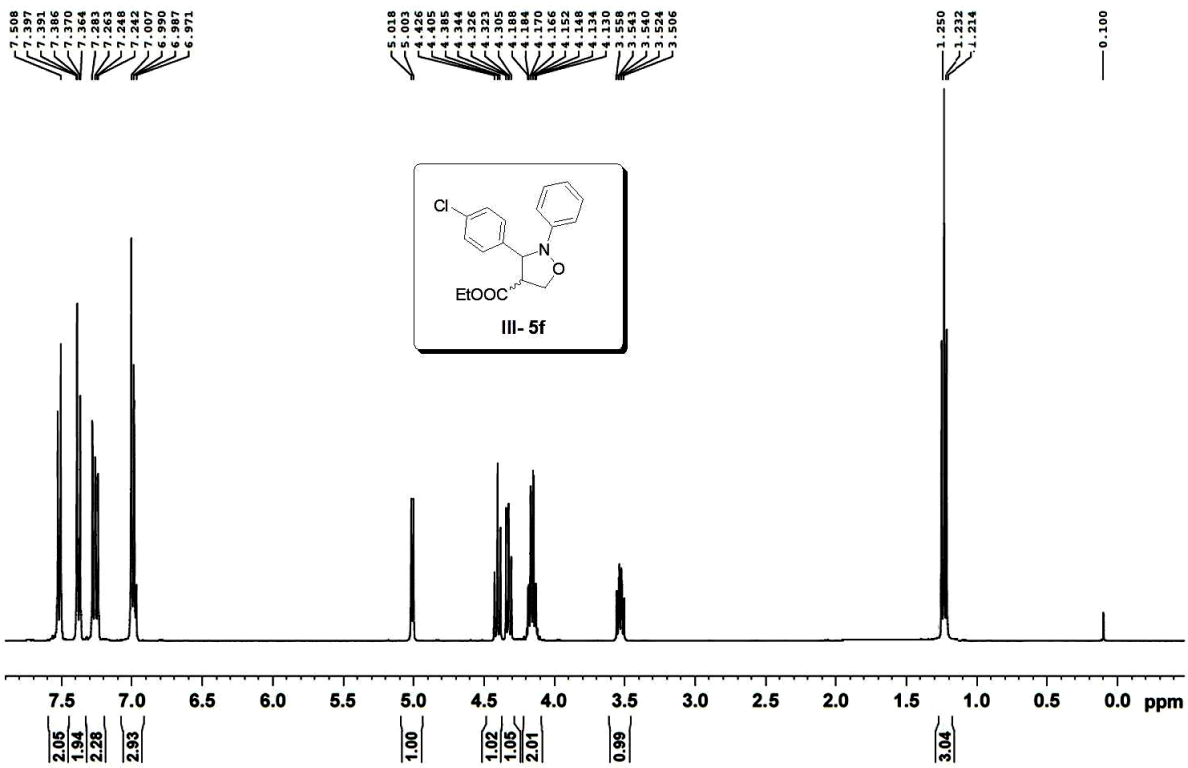

III- 6e
Normalized Intensity
Chemical Shift (ppm)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O
EtOOC
III- 5f
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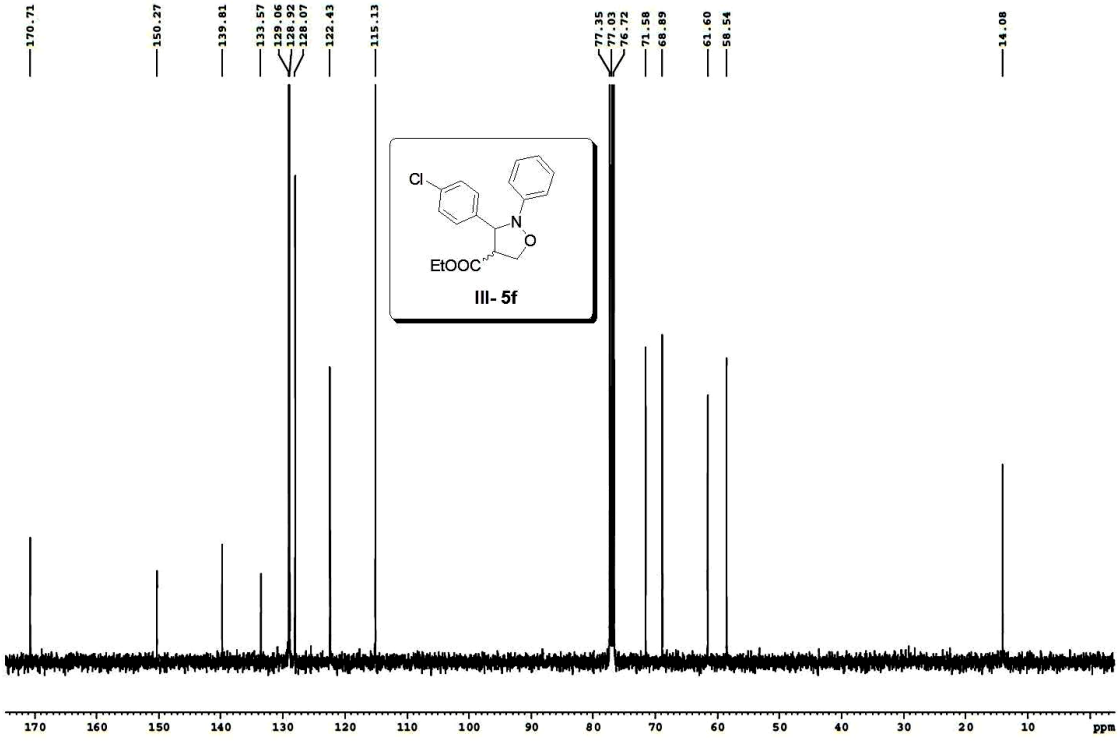
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71.58
68.89
61.60
58.54
14.08
Cl
N
O
EtOOC
III- 5f
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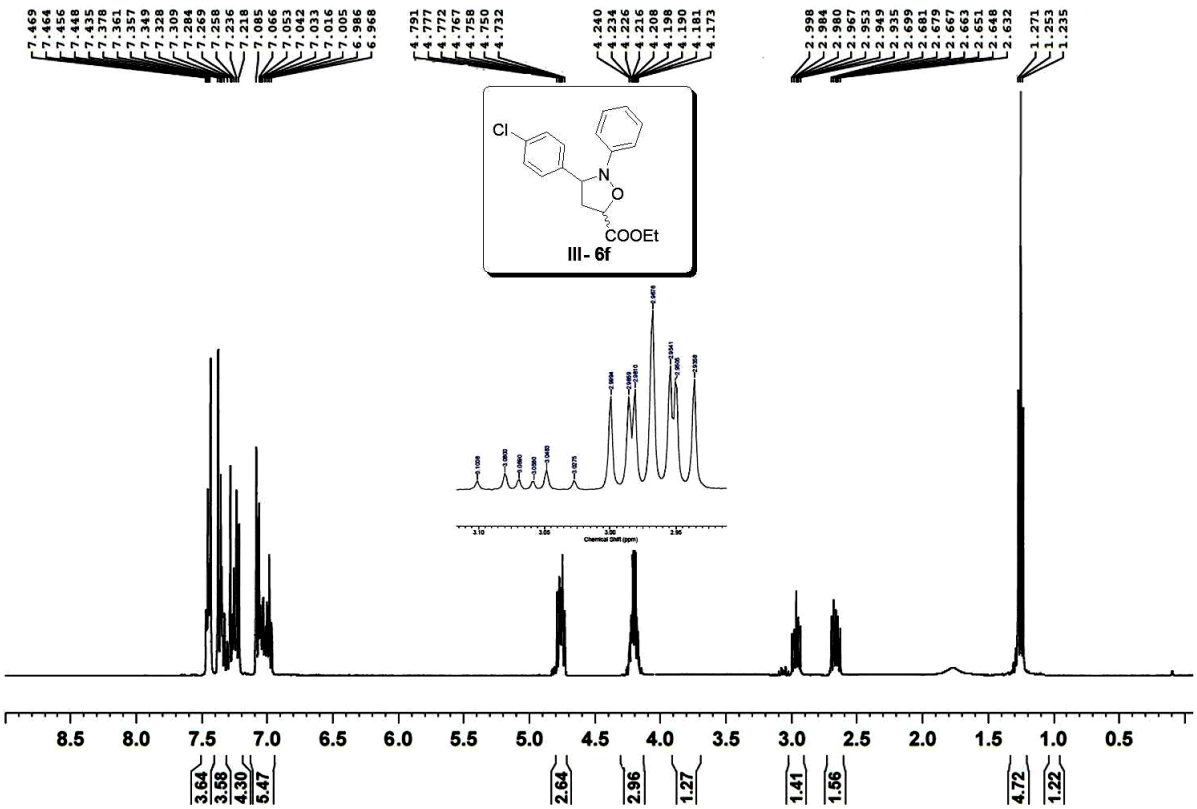
ppm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COOEt
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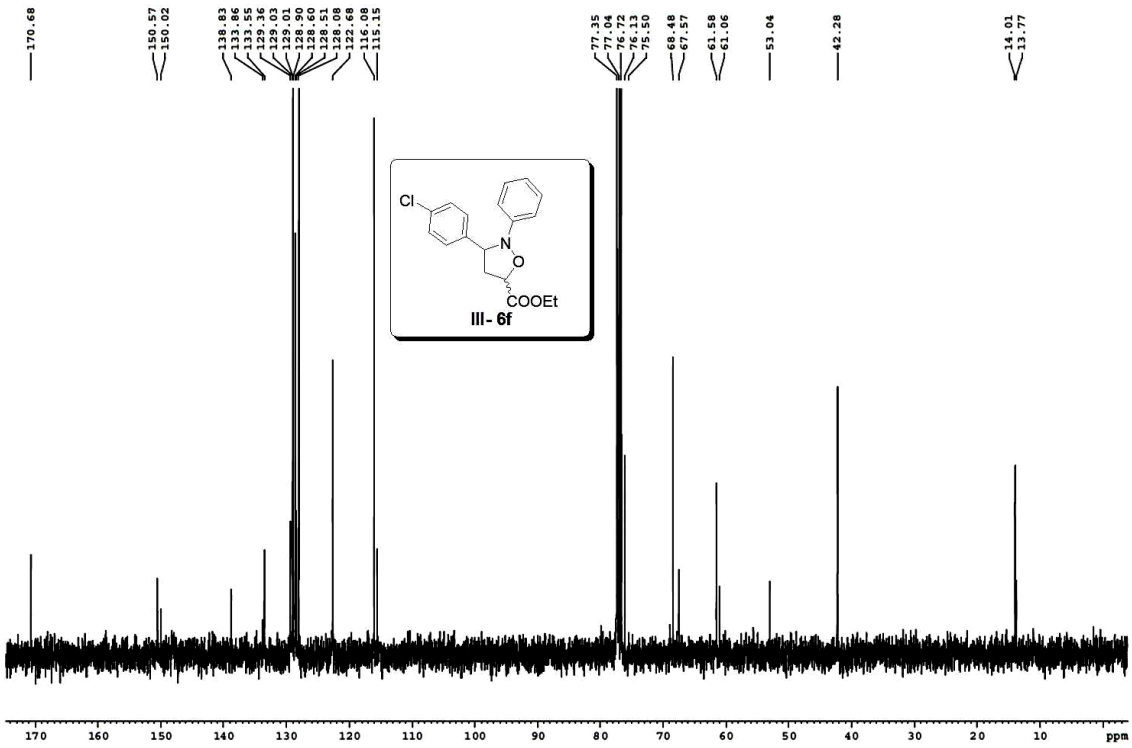
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75.50
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14.01
13.77
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O
COOEt
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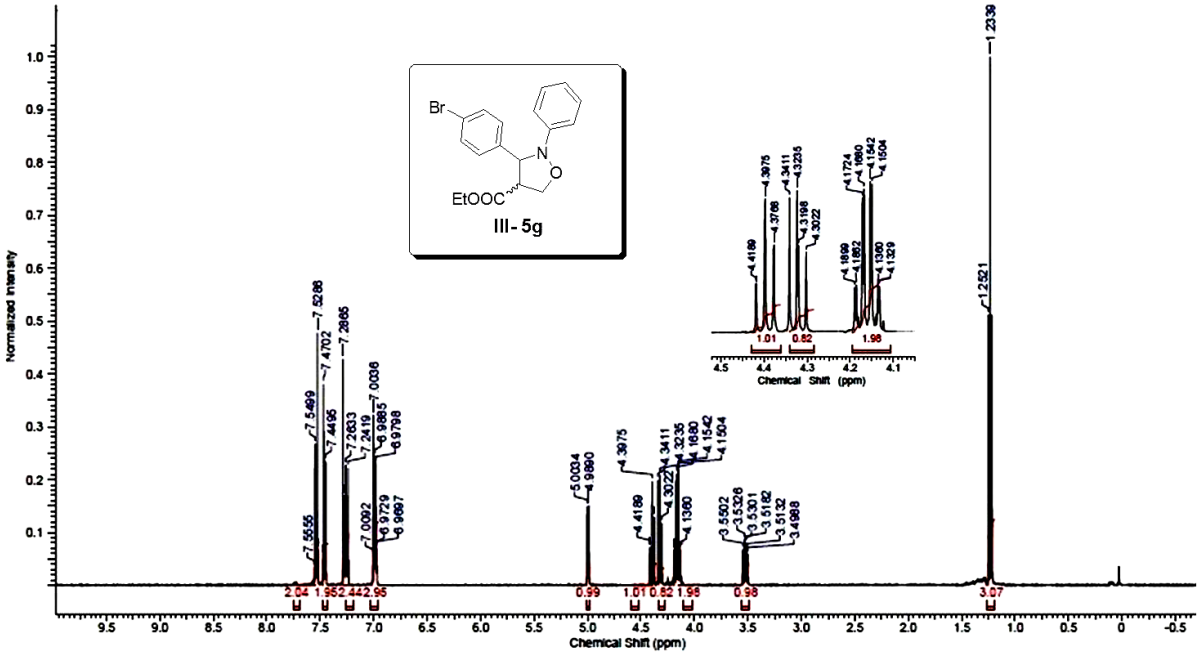
ppm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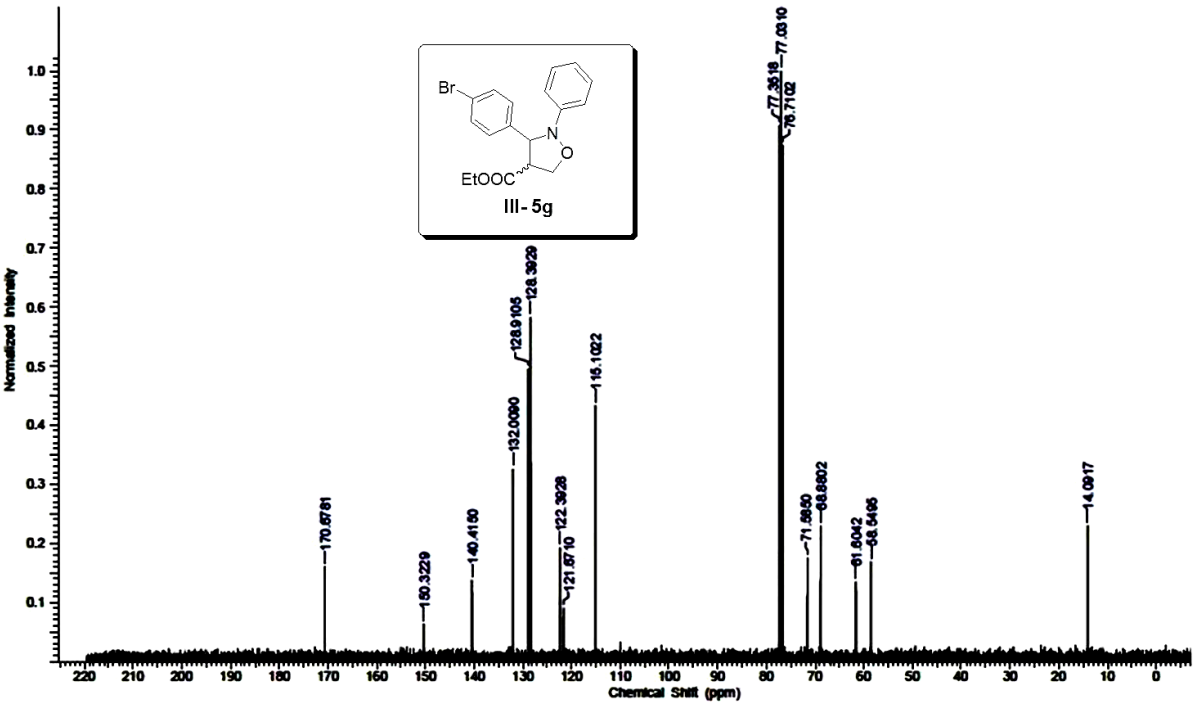
O
EtOOC
III-5g
Normalized Intensity
Chemical Shift (ppm)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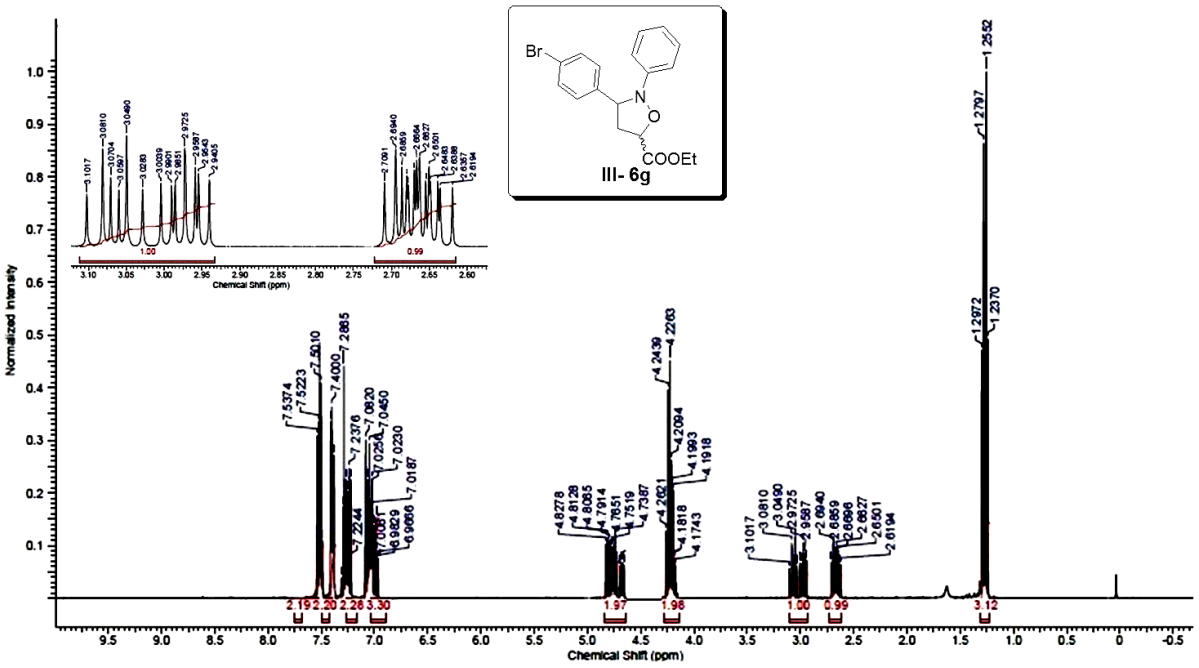
III-5g
Normalized Intensity
Chemical Shift (ppm)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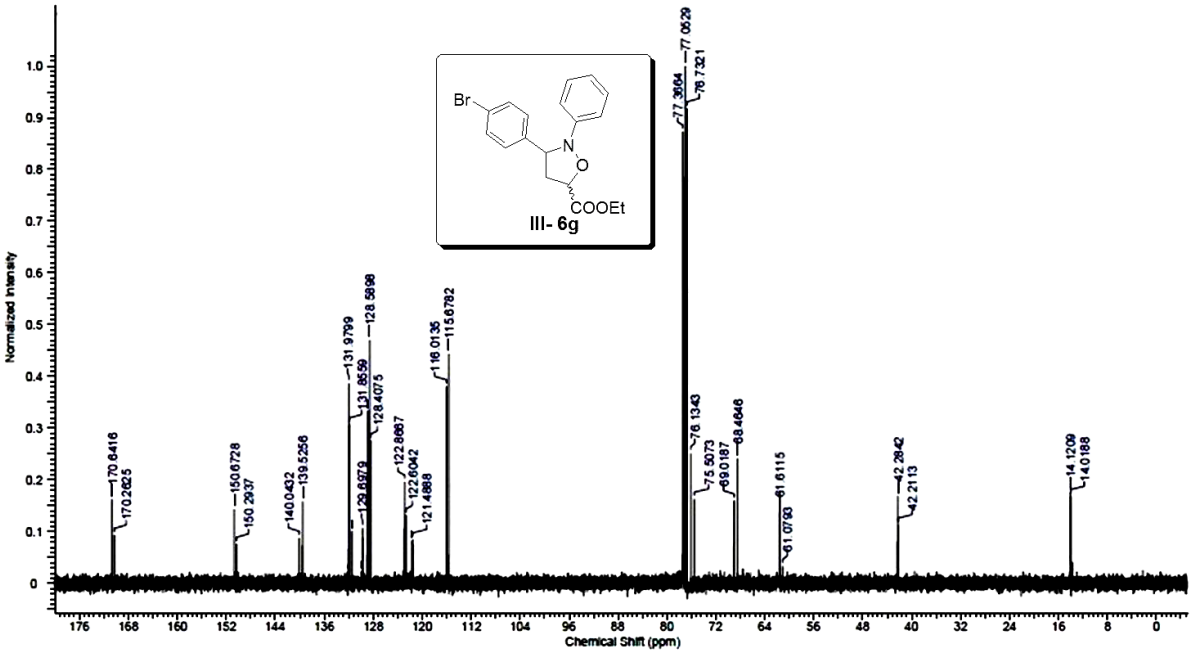
O
COOEt
III- 6g
Normalized Intensity
Chemical Shift (ppm)


Br
N
O
COOEt
III- 6g
Normalized Intensity
Chemical Shift (ppm)

CHAPTER IV

Synthesis and bio-medical applications of Tetraphenylethene (TPE)-based aggregation induced emission (AIE)-active fluorescent amphiphiles

IV.1. Background of the present work

Fluorescent probes are important analytical tools in the field of bio-technological research. Particularly, emission based fluorescent "light-up" probes are essential markers in genomics, proteomics and bioinformatics because they give the visual insight of the biological species. These probe emit fluorescence after interacting with biomacromolecules like nucleic acid and proteins.[1,2]

Several conventional methods are known for detection of proteins based on fluorescent enhancement, using conventional dyes like Nile Red, fluorescamine etc.[3], but they are not environmentally stable. Similarly ethidium bromide,[4a] Hoeschst dyes,[4b] cyanine derivatives[4c] etc. have been developed for nucleic acid detection. Particularly, ethidium bromide is a well established nucleic acid stain. However, its carcinogenicity restricts its use in molecular biology. As an alternative to this, SYBR-based dyes[5] are developed which were less carcinogenic but its lipophilicity restrict its use because of the requirement of harmful organic solvent to dissolve it. These facts warrant the development of stable, water soluble, non-toxic fluorescent "light-up" bio-probes for the detection and quantitation of nucleic acids and proteins.

As mentioned in Chapter I, Section I.3, tetraphenylethylene (TPE) is used as the building block in designing various luminescent functional materials because of its inherent AIE property in solid state. In this regard, biocompatibility and water-solubility are two vital factors which ensure AIE probes to be useful for different applications.[6] In recent years, Tang's group and other researchers have fabricated several TPE-based AIE active functional molecules for various biomedical applications such as detection and quantification of DNA,[7] proteins[8] and preparation of gene delivery vehicle.[9] There are also some reports about the water soluble cationic TPE molecules which are used for cell imaging.[10] These water soluble TPE probes are mainly having variable length alkyl chain spacer with quaternary ammonium

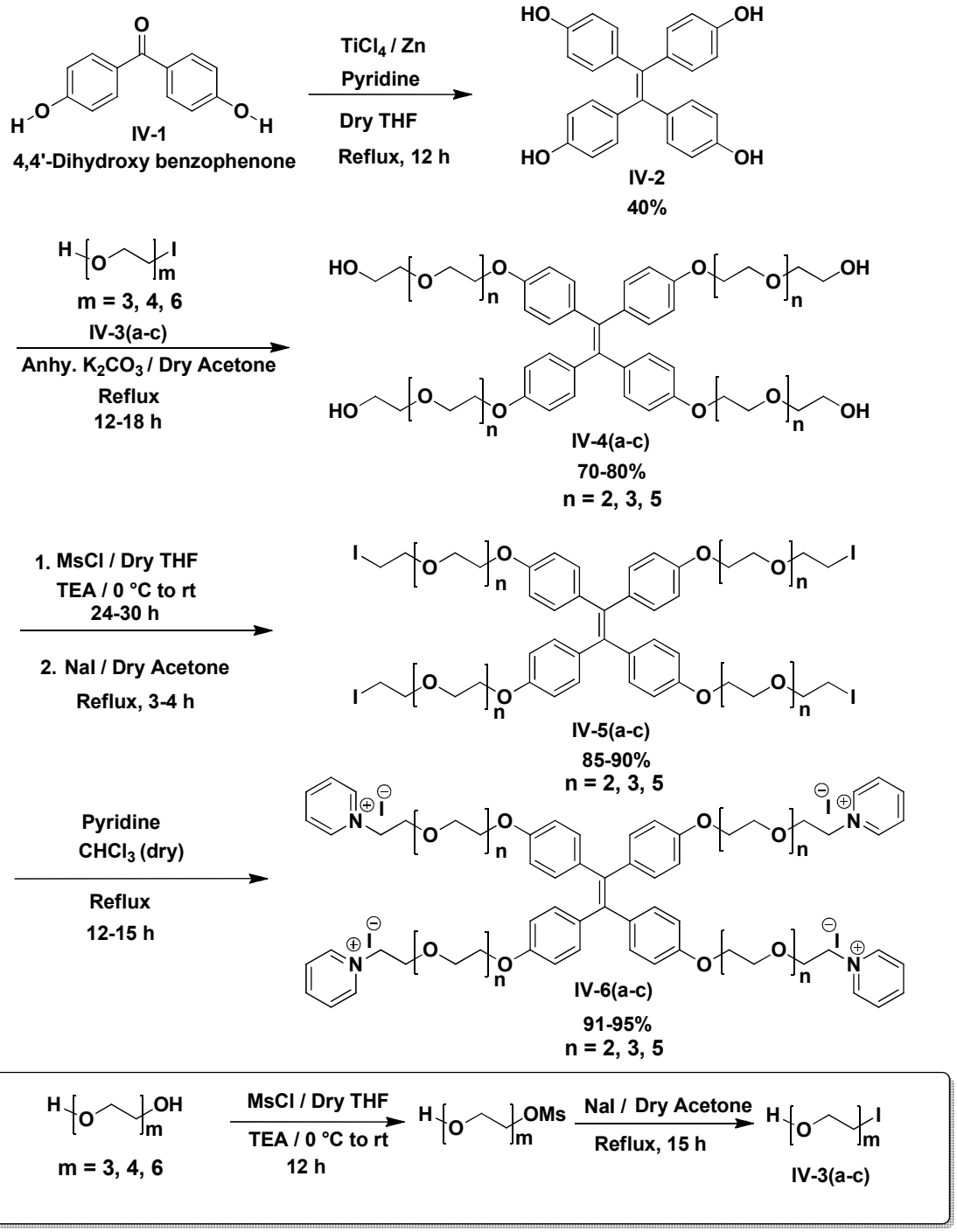
ions at the terminal as polar head, which some times cause toxicity.[11] These probes could be made more water soluble by incorporating glycol units in the chain. Moreover, cationic pyridinium unit is known to be more biocompatible[12] and use of this unit as polar head ensures the TPE-derived bioprobes would be more suitable for biotechnological applications.

Keeping these factors in mind, in this chapter, we describe the development of an efficient synthetic strategy for a number of water soluble TPE-based amphiphiles having a peripheral glycol chains with pyridinium group as the polar head (**IV-6a-c**). Their DNA and BSA binding affinities have been studied as a part of their potential applications as fluorescent bio-probes.

IV.2. Results and Discussion

IV.2.1. Synthesis of Tetraphenylethene (TPE)-based amphiphiles and their structural elucidation

Tetra(*p*-hydroxyphenyl)ethylene (**IV-2**) was synthesized using reported McMurry reaction in moderate yield from 4,4′-dihydroxybenzophenone.[13] Monoiodide derivatives of diffrenet ethylene glycols (**IV-3a-c)** have been prepared by mesylation of ethylene glycols of variable chain lengths then subsequent iodination of unstable mesyl derivative by refluxing with sodium iodide. Compound **IV-2** on reaction with monoiodide derivatives of different glycol units produced the TPE-glycols (**IV-4a-c**) in good yields. These compounds undergo mesylation to give the corresponding products in high yields, which were found to be relatively unstable and were immediately converted to iodide derivative (**IV-5a-c**) by refluxing with sodium iodide. In the final step, the iodide derivatives (**IV-5a-c**) were quarternized by refluxing with pyridine in chloroform to produce the desired cationic pyridinium TPE-amphiphiles (**IV-6a-c**) with iodide as the counter anion in very good overall yields (Scheme IV-1). The compounds are found to be freely soluble in water. It is noteworthy to mention that one of the pyridinium TPE-amphiphiles, **IV-6c** showed very good DNA and BSA binding affinity.



Scheme IV-1. Synthetic route to the Tetraphenylethene (TPE)-derived amphiphiles **IV-6a-c**.

The structure of the new TPE-amphiphiles (**IV-6a-c**) have been established by ^{1}H NMR, ^{13}C NMR, ESI-MS and CHN analysis. The formation of cationic pyridinium TPE-amphiphiles (**IV-6a-c**) from the corresponding iodides (**IV-5a-c**) was indicated by the appearance of desired number of heteroaromatic protons at downfield within the range δ 8.71-8.25 ppm in

^{1}H NMR, which ensures attachment of pyridine ring for all the TPE-amphiphiles. In addition, the appearance of two carbon (CH) signals at δ ~128.0-128.1 ppm, two carbon (CH) signals at δ ~144.7-144.8 ppm and one carbon (CH) signal at δ ~145.9-146.0 ppm for each pyridinium ring in ^{13}C NMR spectrum further supported the formation of desired products (**IV-6a-c**). The formation of these TPE-amphiphiles (**IV-6a-c**) were re-confirmed by ESI-MS (positive ion) mass spectroscopy. In each case, appearance of a base peak at *m/z* = (1/4 x MW-4I) (as *z* = 4) confirmed the formation of tetracationic TPE-amphiphiles. Therefore, peaks appeared at 293, 337, and 425 for **IV-6a**, **IV-6b** and **IV-6c**, respectively. All the spectral studies clearly established the formation of the desired TPE-amphiphiles (**IV-6a-c**).

IV.2.2. Protein binding assay

IV.2.2.1. Fluorimetric titration of Bovine serum albumin (BSA) with TPE based amphiphile (IV-6a-c):

Application of TPE-amphiphiles (**IV-6a-c**) as fluorescent bioprobes could be explored by analysing their interactions with biomacromolecules like proteins and DNA. Complexation of the water–soluble TPE based AIE compounds with Bovine serum albumin (BSA) (Fig.IV-1) was investigated by spectrofluorimetric titration in aqueous phosphate buffer (pH = 7.0) at 25 °C. The experiment was triplicated and similar results were found each time. It has been found that the pyridinium-TPE-amphiphiles (**IV-6a-c**) solutions in buffer (5 μM) in the absence of BSA are almost nonfluorescent, whereas, they show a fluorescence enhancement up to 5-6 fold by the addition of BSA. Fluorescent intensity recorded at 442 nm. The intensity of the probe solution increases up to 2 mg/mL of BSA. The linear range of I/I_0-1 vs. concentration of BSA plot for **IV-6c** is 0 - ~1.5 mg/mL. There is an unusual behavior in form of non-linear binding pattern of other two TPE derivatives (**IV-6a,b**). The fluorescence enhancement may be attributed to the fact that the native folded structure of BSA contains hydrophobic binding sites as pockets. The probes binds to these hydrophobic regions of BSA and move in to the hydrophobic pockets of their folded structures, where the rotation of the molecules are seized, hence inducing this complex to emit after aggregation.[14] So, AIE property makes the TPE-amphiphiles (**IV-6a-c**) as efficient probes for protein detection and quantitation.

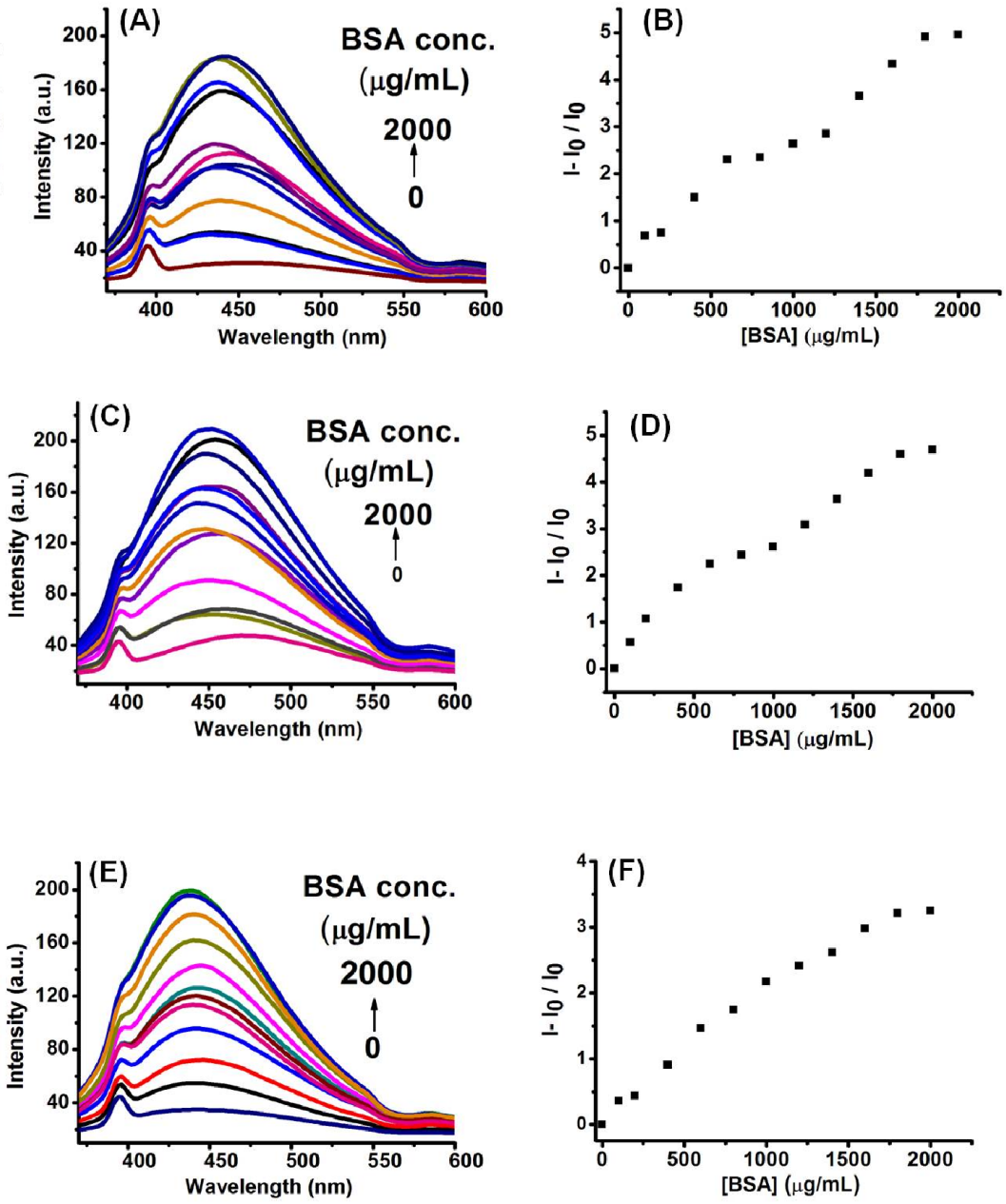


Fig. IV-1. (A,C,E) Change in fluorescence spectra of **IV-6(a-c)** (5 μM) upon addition of BSA in an aqueous phosphate buffer (pH = 7.0). (B,D,F) Plot of fluorescence intensity at 442 nm versus BSA concentration.

IV.2.3. DNA binding assay

IV.2.3.1. Fluorimetric titration of p-DNA with TPE-based amphiphile (IV-6a-c):

As a part of preliminary investigation on the efficacy of these systems as potential fluorescent probe for DNA and gene delivery agents, we studied the interaction of these cationic TPE-amphiphiles (**IV-6a-c**) with DNA (Fig.IV-2). We used pET-28a plasmid DNA as model for the spectrometric titration. A dilute solution of TPE-amphiphiles (**IV-6a-c**) in Tris-EDTA buffer was non-fluorescent. Addition of a small amount of plasmid DNA to the aqueous solution of TPE-amphiphiles (5 μM) turned on its emission. Increase of the DNA concentration further increased the fluorescent intensity. The fluorescence intensity at 484 nm increased rapidly at low DNA concentration and got gradually saturated (5.4 μg/mL) when the DNA concentration increases for amphiphile **IV-6c**. The linear range of the (I/I_0-1) vs. concentration plot in this case is 0-3.5 μg/mL. The change of I/I_0-1 versus plasmid DNA concentration can be easily fitted to the Boltzman function as shown in Fig IV-2. The plot displays a good linear range with an R^2 value of 0.9986 for **IV-6c**.[15] TPE-amphiphile **IV-6c** showed good binding efficiency with 6-7 fold fluorescence enhancement with plasmid DNA. In this case, binding is mainly electrostatic between positively charged TPE derivative and negatively charged DNA. However, two other pyridinium-TPE-amphiphiles (**IV-6a-b**) did not show reasonable binding with DNA. The shorter chain length of the glycol unit could be the possible reason for the unexpected results. Since only TPE-amphiphile, **IV-6c** has shown good binding behavior with plasmid DNA, this was used for further study in agarose gel electrophoresis experiment to check its efficacy as staining agent.

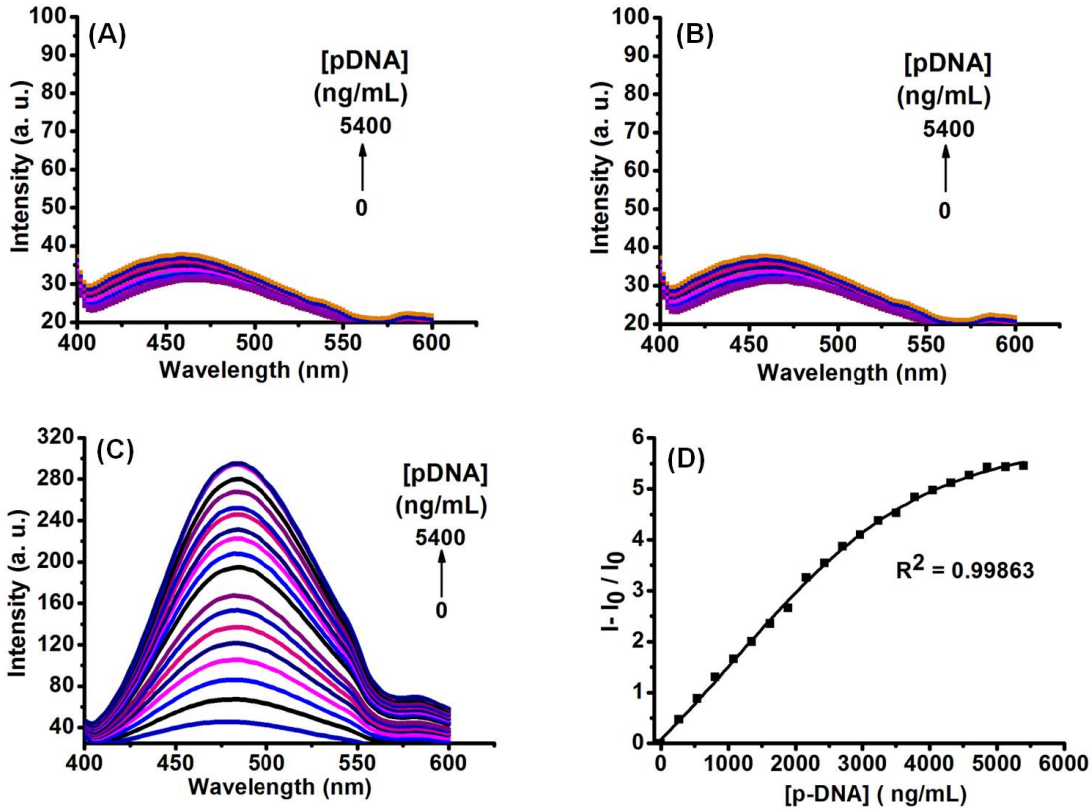


Fig. IV-2. (A-C) Flourimetric titration of pDNA with an aqueous solution of TPE-amphiphiles (**IV-6a-c**) (5 μM) in Tris-EDTA Buffer (D) Plot of $I-I_0/I_0$ at 484 nm versus the pDNA concentration for **IV-6c**. I_0 - emission intensity in the absence of pDNA. λ_{ex} = 350 nm [The other curves are not plotted as the binding affinity is very poor].

IV.2.3.2. Agarose gel electrophoresis

To examine whether the TPE-amphiphile **IV-6c** can be used as DNA staining and quantitation agent, gel electrophoresis experiment was carried out in agarose gel (1%) using pET-28a plasmid DNA (Fig. IV-3). It was found that TPE-amphiphile **IV-6c** effectively binds to pDNA and turns-on its fluorescence property by AIE phenomenon.[16] As shown in figure IV-3, after binding with the TPE-amphiphile (**IV-6c**), DNA bands become visible under UV illumination due to linear array of these molecules on the surface of DNA by electrostatic interactions to activate AIE effect which in turn causes rise in fluorescence. As expected, positively charged pyridinium unit and negatively charged phosphate of DNA strands are mainly involved in the interaction leading to aggregate formation. The binding pattern was favourably very good for **IV-6c** and it was found that as less as 0.25 μg of DNA can be detected in the presence of 10 μM of TPE-amphiphile **IV-6c**.[15]

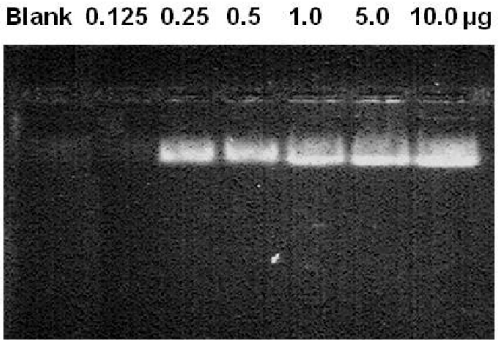


Fig. IV-3. Agarose gel (1%) electrophoresis gel pattern of TPE-amphiphile **IV-6c** (10 μM) – pDNA complex at different concentrations of pET-28a plasmid DNA (0, 0.125, 0.25, 0.5, 1, 5, 10 μg).

IV.2.4. Cytotoxicity

Low toxicity of fluorescent bio-probes is essential towards animal cell lines. Cytotoxicity of TPE-amphiphiles (**IV-6a-c**) was assessed on HeLa cell line using MTT assay.[16] Live HeLa cells were treated with these amphiphiles at a concentration range 10-100 μM. The percentage of viable HeLa cells were quantified and it was found that cell viability does not alter much. The cell viability was more than 90% even when the concentration of TPE-amphiphiles (**IV-6a-c**) was as high as 100 μM in culture medium (Fig. IV-4). These results also demonstrated that these new TPE-amphiphiles do not cause toxicity to living cells.[15]

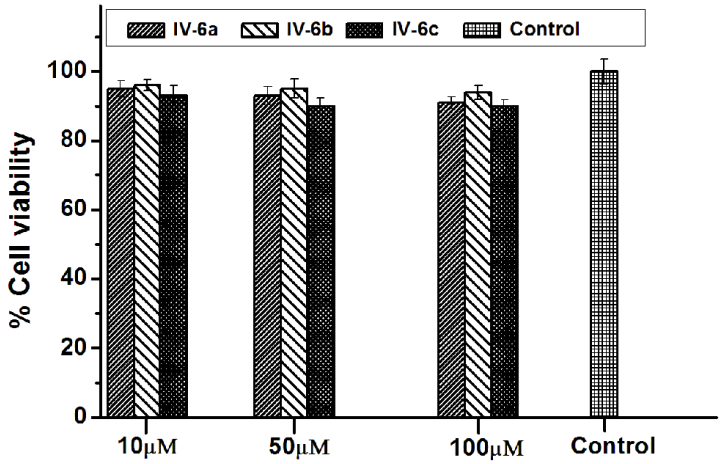


Fig. IV-4. Cytotoxicity of TPE-amphiphiles **IV-6a-c** based on MTT assay.

IV.3. Conclusion

In the present study, three water-soluble TPE-amphiphiles with pyridinium polar heads have been prepared using 4,4′-dihydroxybenzophenone as starting material in 91-95% yield and their DNA and protein binding abilities have been explored. It was observed that these TPE-amphiphiles (**IV-6a-c**) binds with BSA and negatively charged pDNA through hydrophobic and/or electrostatic interaction, which turns on fluorescence emission due to AIE. The AIE property enable them to be used as fluorescent "light-up" probes for the detection and quantitative analysis of biomacromolecules like pDNA and BSA. All TPE-amphiphiles (**IV-6a-c**) are found to be reasonably nontoxic. Therefore, **IV-6c** can also be used as staining agent for gel electrophoresis experiment in place of conventional dyes, which are mostly carcinogenic.

IV.4. Experimental Section

IV.4.1. Materials & methods

4,4′-dihydroxybenzopheneone, various ethylene glycols were purchased from Sigma-Aldrich and used without further purification. Dulbecco's Modified Eagle's Media (DMEM), Fetal Bovine Serum (FBS), Phosphate Buffer Saline (PBS) and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) were purchased from Himedia Laboratories, Mumbai. Plasmid DNA (pET-28a) was purchased from Bangalore GeNei, Bangalore, India. Other common reagents were procured either from SD Fine - Chem Limited, Mumbai, India or from Merck, India. Milli-Q (18MΩ) water was used in all experiments as per requirement. All the stock solutions of TPE-amphiphiles were prepared by using Tris-EDTA buffer solution or phosphate-buffered saline (PBS) (pH = 7.0) or Milli-Q (18Ω) water unless otherwise stated. NMR spectra were recorded on BRUKER NMR 300 MHz or 400 MHz systems using tetramethylsilane as an internal standard. The following abbreviations are used in reporting NMR data: s, singlet; brs, broad singlet; d, doublet; t, triplet; dd, doublet of doublets; m, multiplet. Mass spectra were recorded on Waters Q-TOF micro mass spectrometer or Brucker Esquire 3000 mass spectrometer using ESI as the ion source. CHN data were recorded using Vario elementar CHNS analyzer. Agarose gel was viewed under UV transilluminator (BIO-RAD, USA). Fluorescence studies were carried out on a JASCO FP6300 fluorescence spectrophotometer (JASCO Corp., Japan). Absorbance studies were

carried out on a SHIMADZU UV–2450 UV-visible spectrophotometer (SHIMADZU Corp., Japan) for cytotoxicity assay.

IV.4.1.1. Agarose gel electrophoresis and Cytotoxicity assay

For agarose gel electrophoresis, 0.125, 0.25, 0.5, 1.0, 5.0 and 10.0 μg of plasmid DNA (pET-28a) and 10 μM solution of TPE-amphiphiles were mixed so as to make 20 μL volume and incubated. Then each reaction mixture was loaded on a 1% agarose gel made in tris-acetate-EDTA buffer. After completion of the assay, the gel was viewed under UV transilluminator and photographed.[16]

Cytotoxicity was performed using MTT assay. HeLa cell lines were grown in standard medium as per protocol and they were seeded at a density of 5 x 10^4 cells per well. After 24 h of incubation, the cells were supplemented with TPE-amphiphiles at different concentrations. After incubation for 24 h, MTT was added per well and incubated for 4 h. Finally absorbance was measured after following standard protocol at 570 nm and cell viability was expressed as relative absorbance (%) of the sample vs control cells.[16]

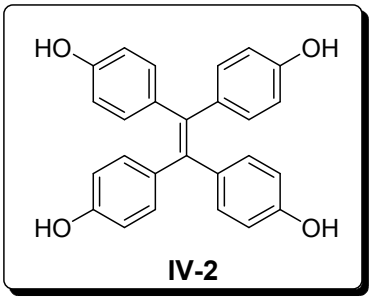
IV.4.1.2. Fluorimetric titration of BSA and p-DNA with TPE-based amphiphile:

Fluorimetric titration for BSA was carried out by adding aliquots of BSA solution in the aqueous phoshate buffer to 0.1 mL of a 0.1 mM stock solution of TPE-amphiphiles **IV-6a**, **IV-6b** and **IV-6c** followed by adding a proper amount of autoclaved aqueous phosphate buffer (pH 7) to acquire a 2 mL solution. The mixture was stirred for 60 min and then incubated for 10 min at room temperature prior to recording the fluorescence spectrum. All observations were expressed by intensity vs. wavelength plots.

Fluorimetric titration for plasmid DNA was carried out by adding aliquots of p-DNA solution in Tris-EDTA buffer to 0.1 mL of a 0.1 mM stock solution of TPE-amphiphiles **IV-6a**, **IV-6b** and **IV-6c** followed by adding adequate amount of autoclaved Tris-EDTA buffer (pH 8) to acquire a 2 mL solution. The mixture was subjected to vortex for few seconds and then incubated for 10 min at room temperature prior to recording the fluorescence spectrum. All observations were expressed by intensity vs. wavelength plots.

IV.4.2. Procedure for the preparation of Tetra(*p*-hydroxyphenyl)ethylene (IV-2):[13a]

A three-necked flask equipped with a magnetic stirrer was charged with zinc powder (3.1 g, 47 mmol) and 30 mL anhydrous THF under nitrogen atmosphere. The mixture was cooled to 0 to -5 °C and $TiCl_4$ (2.6 mL, 23.5 mmol) was slowly added by a syringe. The suspension was warmed to room temperature and stirred for 30 min, then heated at reflux for 2.5 h. The mixture was again cooled to 0 to -5 °C, charged with pyridine (0.9 mL, 11.3 mmol) and stirred for 10 min. The solution of 4,4′-dihydroxybezophenone (1 g, 4.7 mmol) in 10 mL of THF was added slowly. After addition, the reaction mixture was heated at reflux until the 4,4′-dihydroxybezophenone was consumed as revealed by TLC (~8 h). The reaction was quenched by addition of 10% aqueous K_2CO_3 solution and worked up with $CHCl_3$. The organic layer was collected and concentrated. The crude product was purified by column chromatography to give the desired product (**IV-2)** using 100-200 silica gel and 35% ethyl acetate in petroleum ether (60-80) as an eluent.

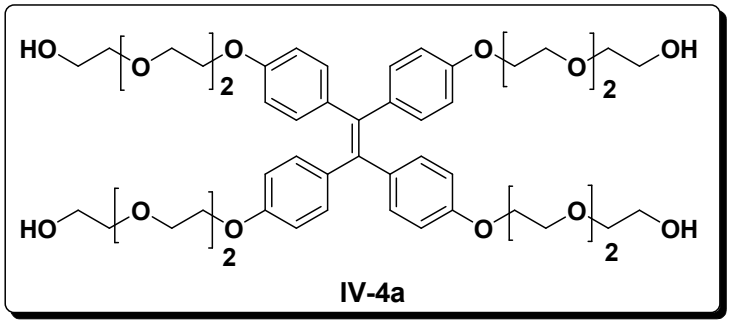


IV-2

Tetra(p-hydroxyphenyl)ethylene **(IV-2):**[13b,c] Off-white solid, yield: 40%; ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.48 (d, J = 8.4 Hz, 8H), 6.70 (d, J = 8.4 Hz, 8H), 9.22 (s, 4H, exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6): δ 114.9, 132.4, 135.5, 138.2, 155.8; ESI-MS: m/z 419 $[M + Na]^+$; Anal. Calcd. for $C_{26}H_{20}O_4$: C, 78.77; H, 5.09; Found: C, 78.92; H, 5.11.

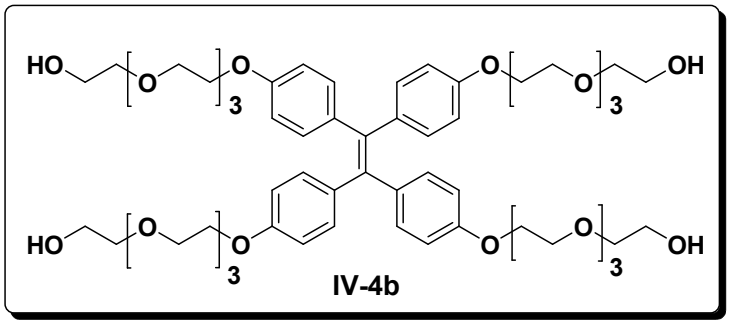
IV.4.3. General procedure for the preparation of amphiphilic TPE-glycols (IV-4a-c): The general procedure is illustrated by the preparation of **IV-4a.**

To a stirred solution of tetra(*p*-hydroxyphenyl)ethylene (**IV-2**) (0.7 g, 1.8 mmol) in dry acetone (7 mL) was added K_2CO_3 (9.76 g, 70.7 mmol) under nitrogen atmosphere. It was then refluxed for 2 h. Next, monoiodide derivative of triethylene glycol (**IV-3a**) (2.76 g, 10.6 mmol) in dry acetone (10 mL) was added drop by drop in the reaction mixture. The mixture was again refluxed for another 18 h. Then the reaction mixture was filtered and the filtrate was evaporated under vacuum and then the corresponding residue was chromatographed over silica gel (100-200) using 10% MeOH in chloroform to afford the desired compound (**IV-4a**).



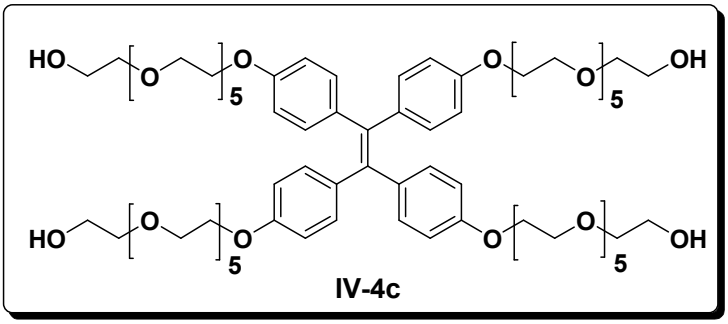
IV-4a

Compound IV-4a:[17] Colourless sticky thick liquid, yield: 74%; ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 3.48-3.68 (m, 40 H), 3.99 (brs, 8H), 6.69 (d, J = 8.4 Hz, 8H), 6.83 (d, J = 8.4 Hz, 8H); ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) 60.3, 67.0, 69.0, 69.8, 72.4, 113.7, 132.1, 136.4, 138.0, 156.8; ESI-MS: *m/z* 925 $[M + H]^+$; Anal. Calcd. for $C_{50}H_{68}O_{16}$: C, 64.92; H, 7.41; Found: C, 65.02; H, 7.44.



IV-4b

Compound IV-4b:[17] Colourless sticky thick liquid, yield: 70%; ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 3.41-3.54 (m, 48 H), 3.68 (brs, 8H), 4.00 (brs, 8H), 6.69 (d, J = 8.7 Hz, 8H), 6.83 (d, J = 8.7 Hz, 8H); ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 60.7, 67.2, 67.3, 69.4, 70.2, 72.8, 114.1, 132.5, 136.7, 138.4, 157.2; ESI-MS: *m/z* 1123 $[M + Na]^+$; Anal. Calcd. for $C_{58}H_{84}O_{20}$: C, 63.26; H, 7.69; Found: C, 63.38; H, 7.71.

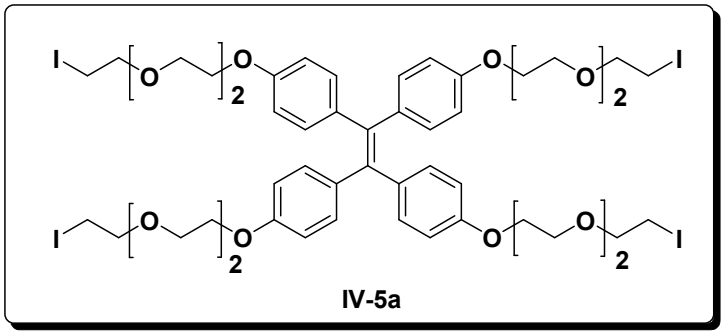


IV-4c

Cmpound IV-4c: Colourless sticky thick liquid, yield: 80%; ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 3.49-3.68 (m, 88 H), 3.99 (brs, 8H), 6.69 (d, J = 8.4 Hz, 8H), 6.83 (d, J = 8.4 Hz, 8H); ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) 60.3, 67.0, 69.0, 70.0, 72.4, 113.8, 132.1, 136.4, 138.0, 156.8; ESI-MS: *m/z* 1476 $[M + Na]^+$; Anal. Calcd. for $C_{74}H_{116}O_{28}$: C, 61.14; H, 8.04; Found: C, 61.30; H, 8.07.

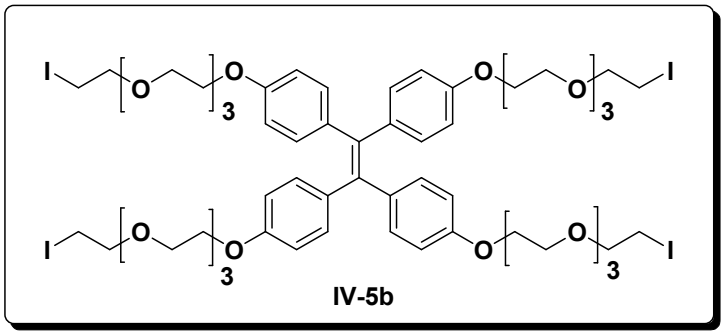
IV.4.4. General procedure for the preparation of iodide derivative (IV-5a-c) of amphiphilic TPE-glycols (IV-4a-c): The general procedure is illustrated by the preparation of **IV-5a.**

To a solution of **IV-4a** (0.7 g, 0.76 mmol) in dry THF (7 mL) was added triethylamine (0.46 g, 4.55 mmol) at 0 °C under nitrogen atmosphere. The mixture was stirred at room temperatre for 1 h. It was re-cooled to 0 °C and mesyl chloride (1.04 g, 9.10 mmol) was added into it. The mixture was stirred at room temperature for 24 h till the completion of the reaction. Then the THF was removed in vacuo and the obtained crude product was extracted with chloroform (2 x 15 mL). The combined organic extracts were washed with 5% HCl to remove excess triethyl amine. Then the extracts were washed with brine, dried over Na_2SO_4 and concentrated. Column chromatogtaphy of the crude product over silica gel (100-200 mesh) eluting with 2% MeOH in chloroform afforded the mesylated compound in high yield. The mesylated compound is relatively unstable and is immediately subjected to iodination. To a solution of mesylated compound (0.32 g, 0.26 mmol) in dry acetone (3 mL) was added sodium iodide (0.47g, 3.10 mmol) under nitrogen atmosphere. The mixture was then refluxed for 3 h. Then the reaction mixture was concentrated *in vacuo* to get crude residue, which was extracted with ethyl acetate (2 x 10 mL). The combined extracts were first washed with 10% $Na_2S_2O_3$ then with brine, dried over Na_2SO_4. Column chromatogtaphy of the crude product over silica gel (100-200 mesh) eluting with 1% MeOH in chloroform afforded the compound **IV-5a**.



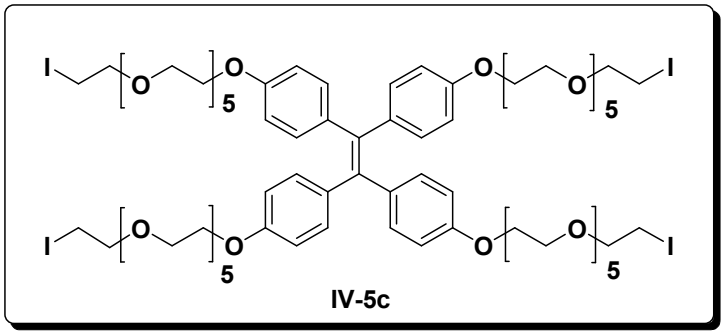
IV-5a

1,1,2,2-tetrakis(4-(2-(2-(2-iodoethoxy)ethoxy)ethoxy)phenyl)ethane **(IV-5a):** Colourless sticky thick liquid, yield: 88%; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 3.26-3.30 (m, 8H), 3.69-3.80 (m, 24H), 3.85-3.87 (m, 8H), 4.07-4.10 (m, 8H), 6.66 (d, J = 8.8 Hz, 8H), 6.92 (d, J = 8.8 Hz, 8H); ESI-MS: *m/z* 1387 $[M + Na]^+$; Anal. Calcd. for $C_{50}H_{64}I_4O_{12}$: C, 44.01; H, 4.73; Found: C, 43.89; H, 4.75.



IV-5b

1,1,2,2-tetrakis(4-(2-(2-(2-(2-iodoethoxy)ethoxy)ethoxy)ethoxy)phenyl)ethene **(IV-5b):** Colourless sticky thick liquid, yield: 85%; ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 3.25 (t, J = 6.9 Hz, 8H), 3.67-3.78 (m, 40 H), 3.83 (t, J = 4.5 Hz, 8H), 4.04-4.08 (m, 8H), 6.64 (d, J = 8.4 Hz, 8H), 6.90 (d, J = 8.4 Hz, 8H); ESI-MS: *m/z* 1563 $[M + Na]^+$; Anal. Calcd. for $C_{58}H_{80}I_4O_{16}$: C, 45.21; H, 5.23; Found: C, 45.07; H, 5.26.

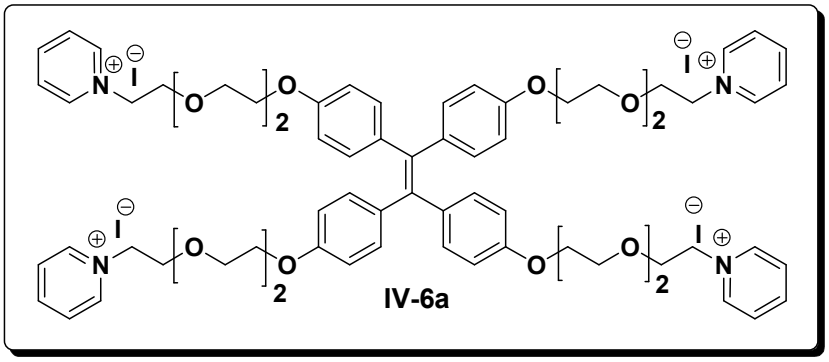


IV-5c

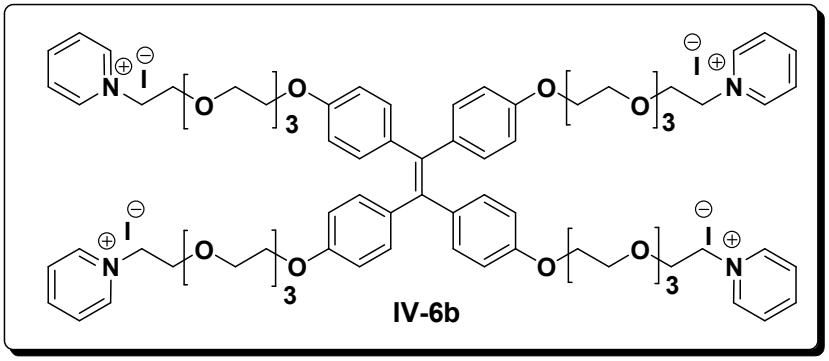
1,1,2,2-tetrakis(4-(2-(2-(2-(2-(2-(2-iodoethoxy)ethoxy)ethoxy)ethoxy)ethoxy)ethoxy) phenyl)ethene **(IV-5c):** Colourless sticky thick liquid, yield: 90%; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 3.26-3.29 (m, 8H), 3.66-3.79 (m, 72 H), 3.83-3.85 (m, 8H), 4.06-4.09 (m, 8H), 6.65 (d, J = 8.8 Hz, 8H), 6.91 (d, J = 8.4 Hz, 8H); ESI-MS: *m/z* 1893 $[M + H]^+$; Anal. Calcd. for $C_{74}H_{112}I_4O_{24}$: C, 46.94; H, 5.96; Found: C, 47.07; H, 5.98.

IV.4.5. General procedure for the preparation of cationic pyridinium amphiphile of TPE (IV-6a-c): The general procedure is illustrated by the preparation of **IV-6a.**

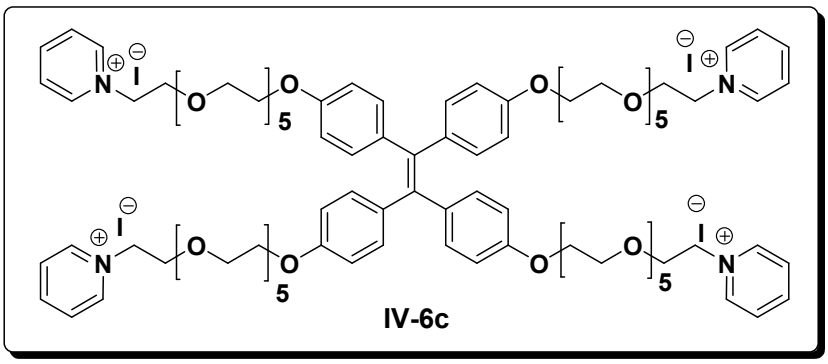
To a solution of **IV-5a** (0.2 g, 0.15 mmol) in dry pyridine (1.0 mL) was added dry chloroform (1.5 mL) under nitrogen atmosphere. It was then refluxed for 12 h till the completion of reaction. The reaction mixture was then dried in vacuo to remove chloroform and then cold diethyl ether (10 mL) was added into it to get a brown colour crude product after centrifugation. The crude product was then repeatedly washed with ethyl acetate (4 x 5 mL) followed by cold acetone (3 x 5 mL) to remove any impurity including trace amount of pyridine. The sufficiently pure final product was subjected to drying *in vacuo* to afford the pure compound **IV-6a**.



***1,1,2,2-tetrakis(4-(2-(2-(2-ethoxy)ethoxy)ethoxy)phenyl)ethene pyridinium tetraiodide* (IV-6a):** Light brown sticky liquid, yield: 91%; ^{1}H NMR (400 MHz, D_2O): δ (ppm) 3.49-3.58 (m, 28 H), 3.79-3.92 (m, 20 H), 6.52 (d, J = 8.4 Hz, 8H), 6.89 (d, J = 8.4 Hz, 8H), 7.83-7.86 (m, 8H), 8.27-8.31 (m, 4H), 8.71 (d, J = 6.0 Hz, 8H); ^{13}C NMR (100 MHz, D_2O): δ (ppm) 61.1, 67.2, 68.7, 68.9, 69.6, 69.9, 114.1, 128.1, 132.4, 137.0, 138.9, 144.7, 146.0, 156.5; ESI-MS: *m/z* 293 (z = 4); Anal. Calcd. for $C_{70}H_{84}I_4N_4O_{12}$: C, 50.01; H, 5.04; N, 3.33; Found: C, 50.15; H, 5.06; N, 3.34.



***1,1,2,2-tetrakis(4-(2-(2-(2-(2-ethoxy)ethoxy)ethoxy)ethoxy) phenyl)ethene pyridinium tetraiodide* (IV-6b):** Light brown sticky liquid, yield: 92%; ^{1}H NMR (400 MHz, D_2O): δ (ppm) 3.41-3.48 (m, 40H), 3.61 (brs, 8H), 3.82-3.85 (m, 16H), 6.47 (d, J = 8.6 Hz, 8H), 6.80 (d, J = 8.6 Hz, 8H), 7.82 (dd, J_1 = 6.9 Hz, J_2 = 7.8 Hz, 8H), 8.25-8.29 (m, 4H), 8.65 (d, J = 5.6 Hz, 8H); ^{13}C NMR (100 MHz, D_2O): δ (ppm) 61.0, 67.1, 68.7, 68.9, 68.6, 69.5, 69.8, 113.9, 128.0, 132.4, 136.9, 138.7, 144.7, 145.9, 156.4; ESI-MS: *m/z* 337 (z = 4); Anal. Calcd. for $C_{78}H_{100}I_4N_4O_{16}$: C, 50.44; H, 5.43; N, 3.02; Found: C, 50.61; H, 5.45; N, 3.04.



IV-6c

1,1,2,2-tetrakis(4-(2-(2-(2-(2-(2-(2-ethoxy)ethoxy)ethoxy)ethoxy)ethoxy)ethoxy) phenyl)ethene pyridinium tetraiodide **(IV-6c):** Light brown sticky liquid, yield: 95%; ^{1}H NMR (400 MHz, D_2O): δ (ppm) 3.47-3.57 (m, 68 H), 3.69 (brs, 12 H), 3.90-3.91 (m, 16H), 6.54 (d, J = 8.0 Hz, 8H), 6.81 (d, J = 8.0 Hz, 8H), 7.95-7.99 (m, 8H), 8.44-8.48 (m, 4H), 8.76 (d, J = 6.1 Hz, 8H); ^{13}C NMR (100 MHz, D_2O): δ (ppm) 61.1, 67.2, 68.7, 69.0, 69.5, 69.6, 69.8, 114.0, 128.1, 132.4, 136.9, 138.7, 144.8, 146.0, 156.5; ESI-MS: *m/z* 425 (z = 4); Anal. Calcd. for $C_{94}H_{132}I_4N_4O_{24}$: C, 51.09; H, 6.02; N, 2.54; Found: C, 51.23; H, 6.04; N, 2.55.

IV.5. Notes & References

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[16] The experimental work has been performed in collaboration with Dr. Anasuya Ganguly and co-workers at Department of Biological Sciences, BITS Pilani- K. K. Birla Goa Campus and is not a part of any other thesis.

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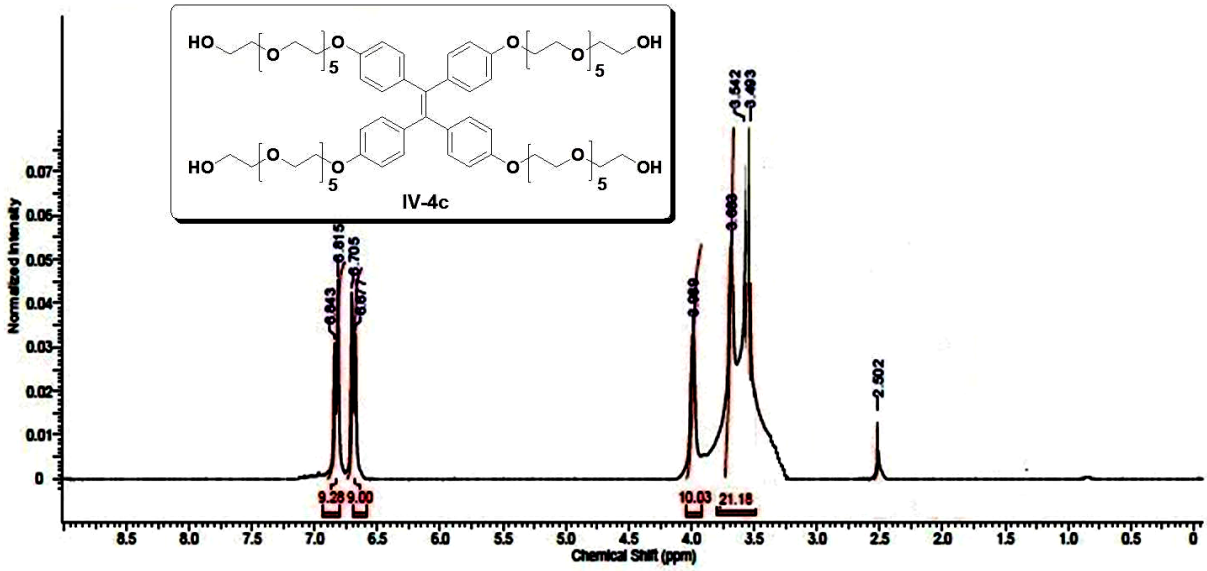
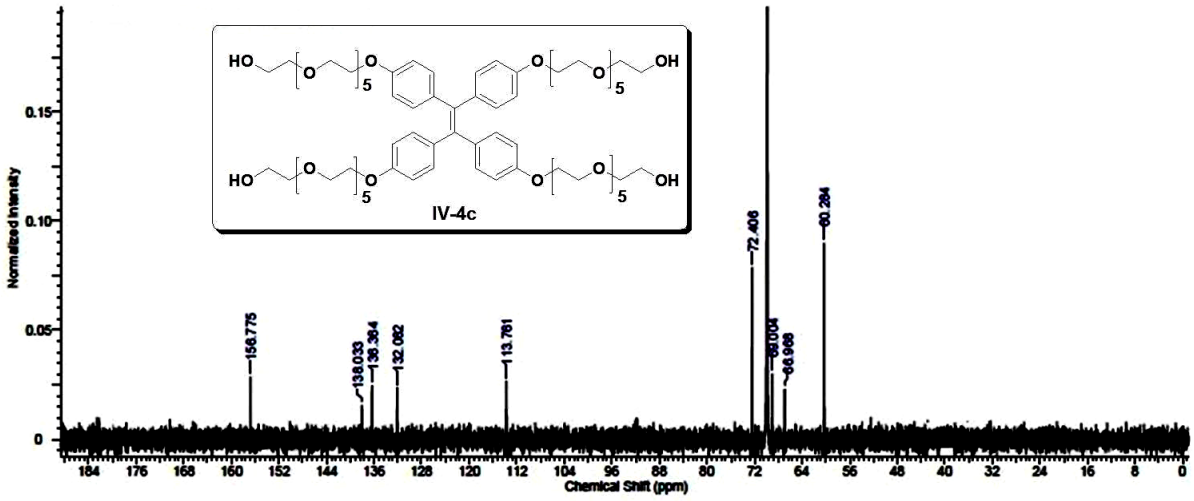
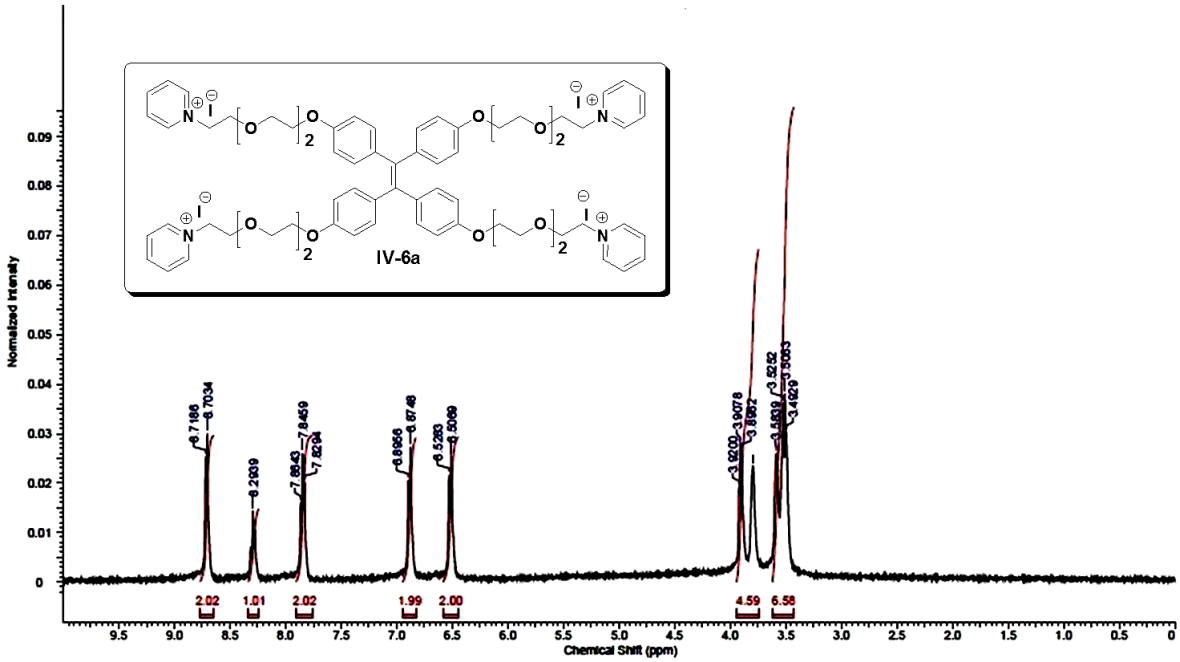
IV.6 . Supporting Information (selected spectra)






IV-6a
Normalized Intensity
Chemical Shift (ppm)
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8.7034
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8.8748
8.5283
8.5069
3.9209
3.9078
3.8862
3.5639
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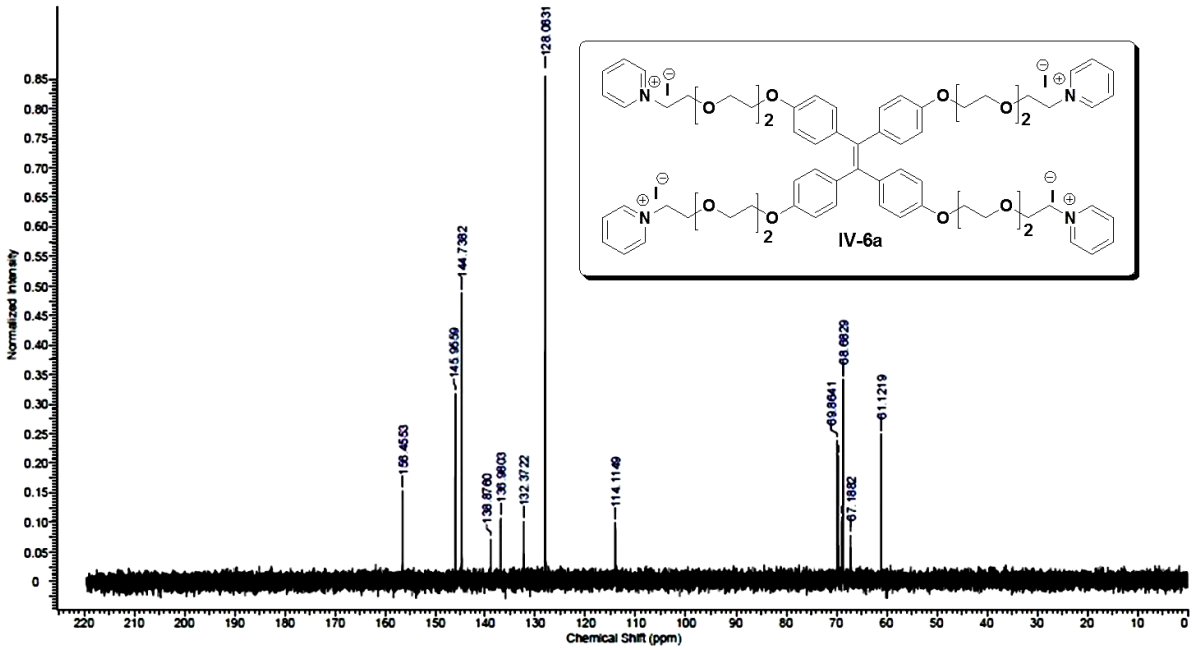
2.02
1.01
2.02
1.99
2.00
4.59
6.58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Normalized Intensity
Chemical Shift (ppm)
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69.8641
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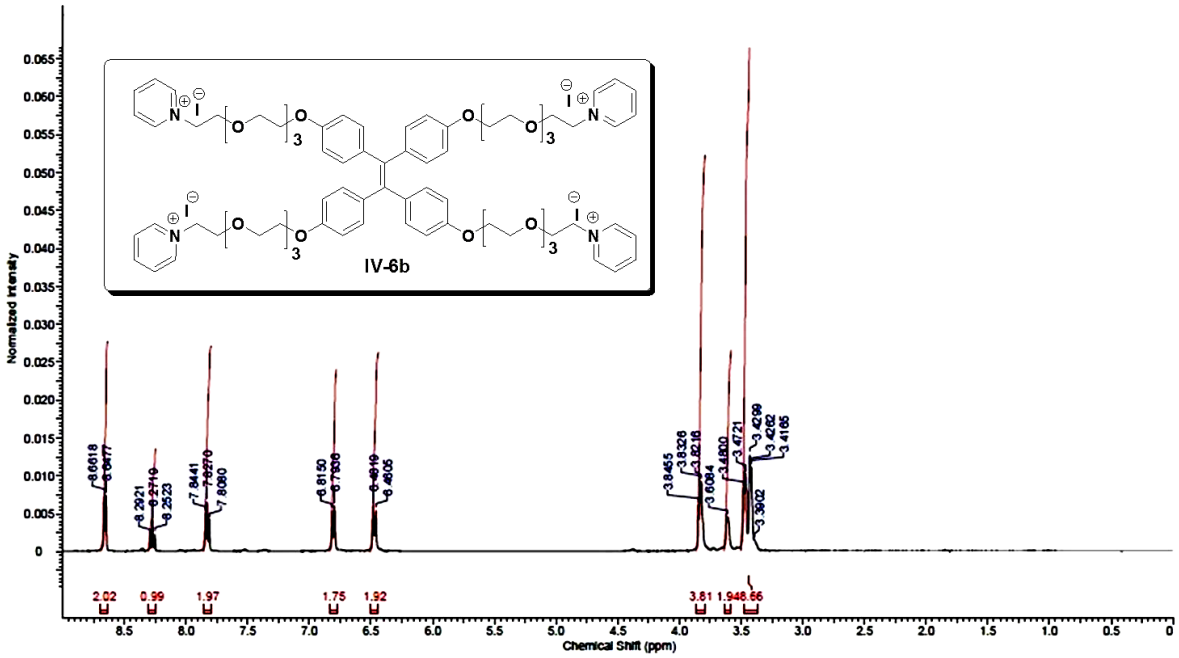
61.1219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Chemical Shift (ppm)
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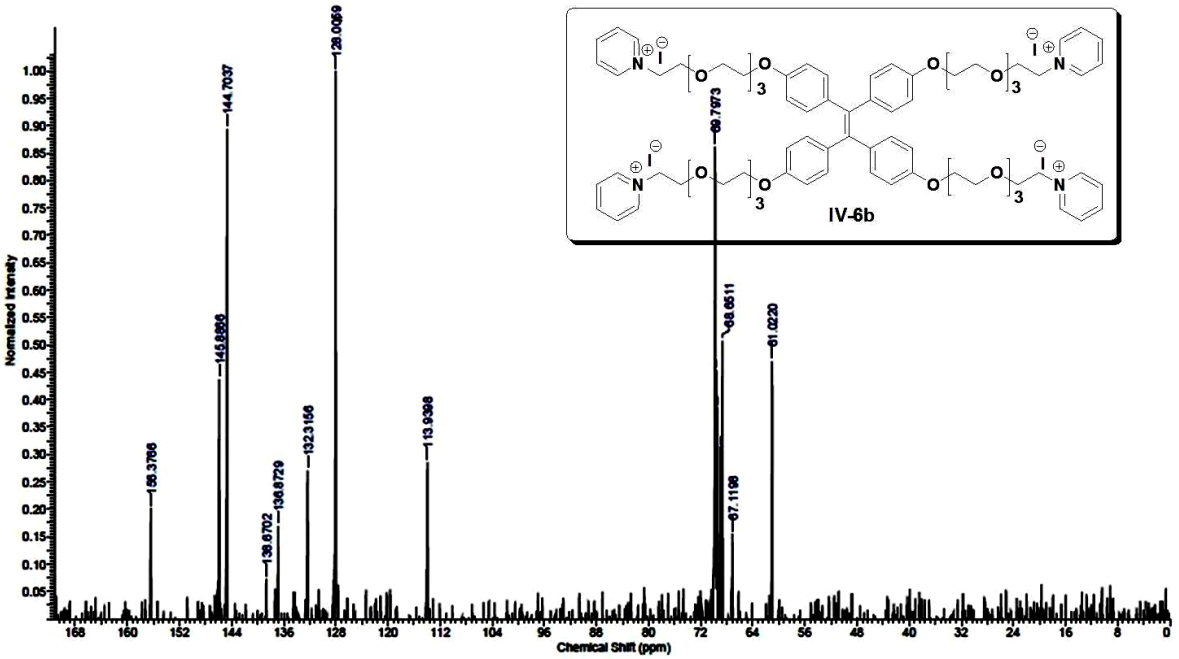
1.75
1.92
3.81
1.94
8.66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Normalized Intensity
Chemical Shift (ppm)
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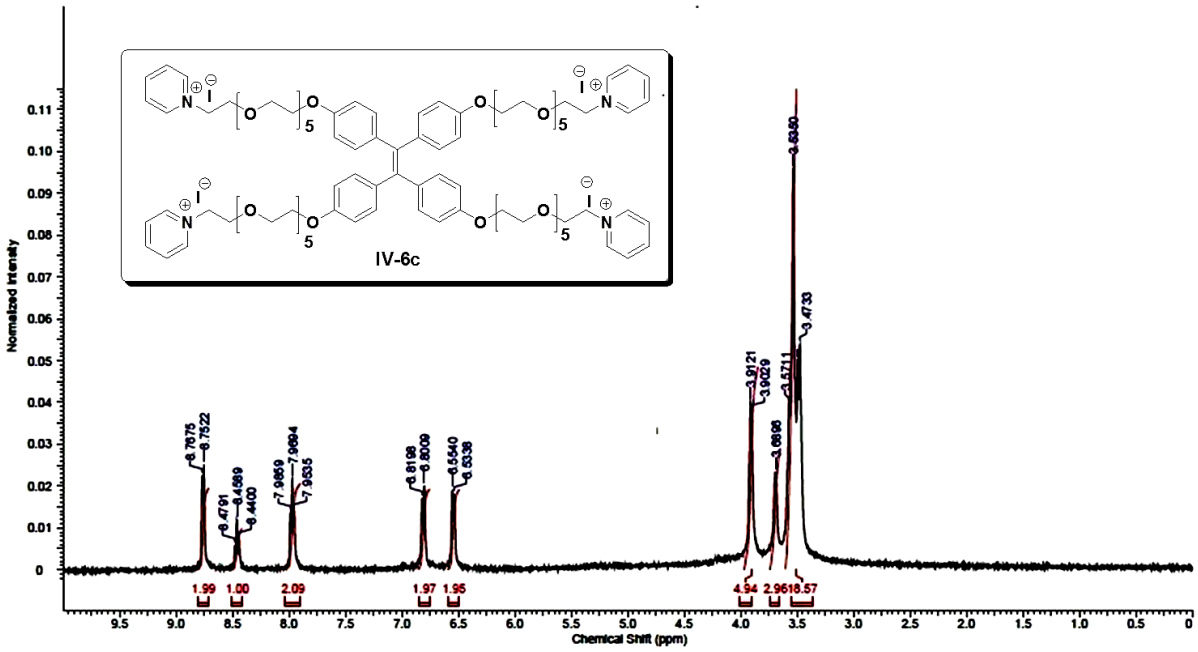
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6.8009
6.5540
6.5338
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3.9929
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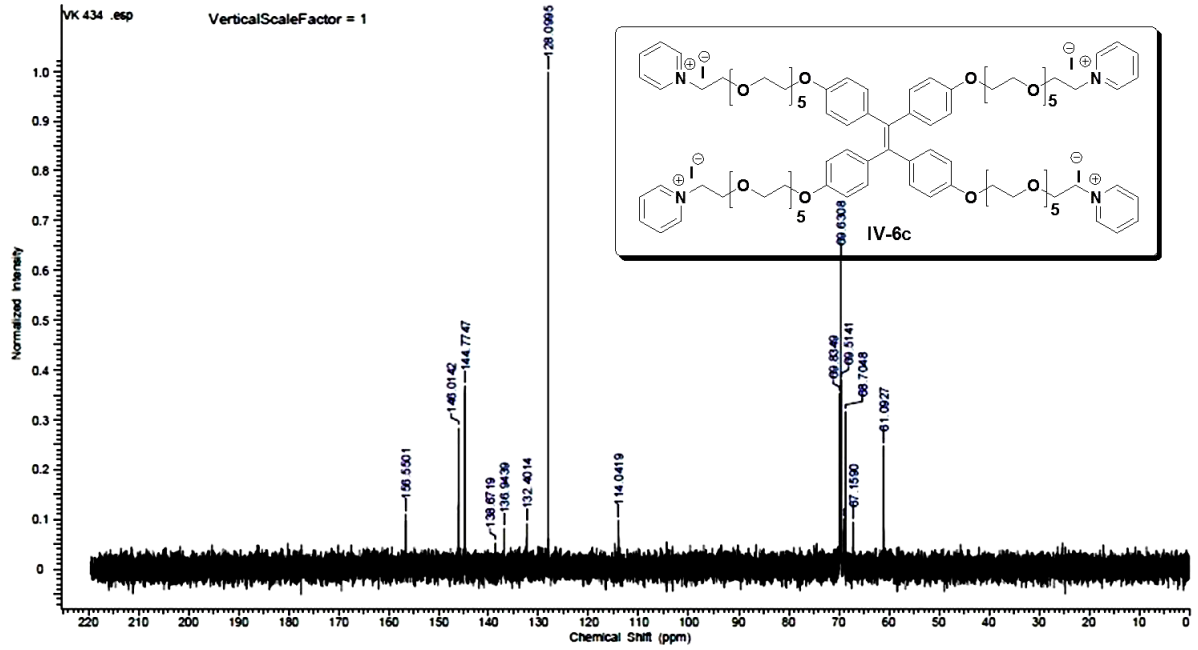
2.96
18.57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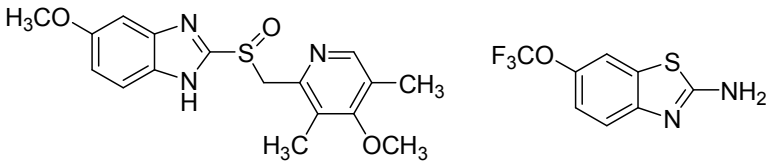
69.6308
69.5349
69.5141
68.7048
67.1590
61.0627

CHAPTER V

Development of efficient and "green" synthetic methodology for 2-substituted benzimidazoles and benzothiazoles in aqueous micellar media

V.1. Background of the present work

Benzimidazoles and benzothiazoles are ubiquitous heterocyclic scaffolds of biologically active compounds and natural products.[1] These heterocycles are of immense pharmaceutical interest as they show a range of pharmacological activities such as antibacterial, antiulcers, antihypertensives, antivirals, antifungals, anticancers, antihistaminics and antitubercular.[2] They are essential parts of various clinical medicines as well, for example, 2-substituted benzimidazole, Esomeprazole[3] is an anti-ulcerative drug, whereas, a benzothiazole derivative, Riluzole (Rilutek)[4] is used to treat motor neurone disease (Fig. V-1). In addition, they are important intermediates in various organic reactions[5] and key components of many functional materials.[6] This has led to the development of several methods for the synthesis of benzmidazole and benzothiazoles derivatives in recent times. The conventional methods for the synthesis of the benzimidazoles[7b,c] and benzothiazoles[7d] involve condensation of *o*-phenylenediamine / *o*-aminothiophenol with a carboxylic acid or its derivatives under severe dehydrating conditions.[7]



Esomeprazole **Riluzole**

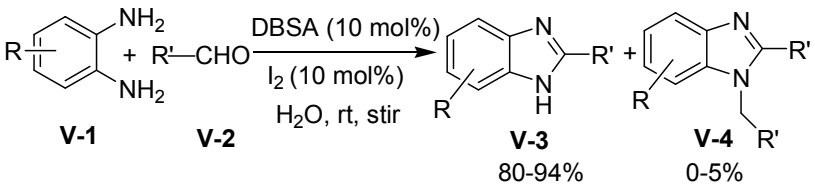
Figure V-1. Chemical structures of few clinically important 1,3-benzazoles.

Recently, various other routes have been developed for these heterocycles which include transition metal catalyzed cyclization of ortho-haloanilides,[8] metal catalyzed direct arylation via C–H bond activation,[9] solid-phase supported synthesis[10] and many others.[11] However, dehydrative Schiff's base formation followed by oxidative cyclization in one-pot between

ortho-functionalized anilines and aldehydes became the most popular method for these 1,3-benzazole derivatives[12-15] that comprises 1,3-benzimidazoles e.g. 2-substituted benzimidazoles, **V-3**[13] , 1,2-disubstituted benzimidazoles, **V-4**[14] and 1,3-benzothiazoles, **V-6**.[15] The reported procedures for this protocol involved a wide spectrum of reagents such as $In(OTf)_3$,[13a] $Sm(OTf)_3$,[13b] WO_x/ZrO_2,[13d] H_2O_2/CAN,[13e] *p*-TsOH/DMF[13j] for 2-substituted benzimidazoles and $HClO_4$-SiO_2,[14a] proline,[14c] Zn-proline,[14d] oxalic acid[14e] for 1,2-disubstituted benzimidazoles. Interestingly, acid catalyzed conditions favour 2-substituted benzimidazoles over 1,2-disubstituted benzimidazoles in most of the cases.[13a,13b,13j]

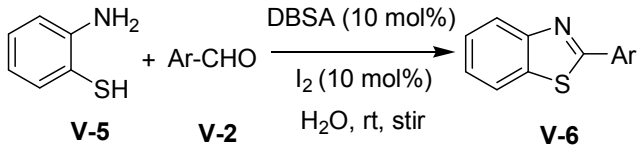
However, most of the methods used considerable amounts of metal catalyst, hazardous organic solvents for reaction and extraction processes, which are not environmentally benign. Moreover, several of these reactions were carried out at higher temperature and using costly reagents. Therefore, their utility is limited, especially in industrial applications. In last few years, several eco-friendly solution phase synthetic methods have been reported for 1,3-benzazoles[16-18] e.g. 1,2-disubstituted benzimidazoles[16] but similar methods for selective synthesis of 2-substituted benzimidazoles[16f,17] and for benzothiazoles[18] are rare. Therefore, the development of a new and efficient "green" protocol using micellar medium for this purpose is a worthy pursuit.

Synthesis of benzimidazoles involves a dehydration step and miceller condition has been successfully utilized, separately, by Bahrami et al.[16f] and Ghosh et al.[16g] for the construction of this heterocyclic system in the presence of mildly basic SDS as catalyst. It is interesting to note that both the methods predominantly yield 1,2-disubstituted benzimidazoles. As the literature survey reveals that acidic catalysts favour 2-substituted benzimidazoles over 1,2-disubstituted benzimidazoles,[13a,13b,13j] we assumed, acidic surfactant, DBSA (dodecylbenzenesulphonic acid) could be effective in achieving selectivity towards 2-substituted benzimidazole derivatives. The DBSA catalyzed chemoselective synthesis of 2-substituted benzimidazoles has been carried out in aqueous media in which iodine acts as co-catalyst to enhance selectivity of the desired product (Scheme V-1). The primary roles of DBSA are a) to assist in solubilizing the organic substrates in aqueous media by forming micelles or other organized phase and b) to act as a catalyst to promote condensation of *o*-diaminoarene with the aldehyde. Similarly, 2-substituted benzothiazoles has also been synthesized.



Scheme V-1. DBSA catalyzed synthesis of 2-substituted benzimidazoles.

The same methodology has also been utilized for the synthesis of 2-substituted benzothiazoles in high overall yields. The role of surfactant is same as in the case of 2-substituted benzimidazoles (Scheme V-2).



Scheme V-2. General scheme for the synthesis of 2-substituted benzothiazoles

V.2. Results and Discussion

V.2.1. Standardization of the reaction condition

V.2.1.1. Formation of micelles

We started our work with a focus on optimizing the reaction conditions. In this direction, the formation of the emulsion droplets were confirmed by taking optical micrograph of different surfactant containing aqueous solutions of reactants before reaction would actually proceed (Figure V-2). The experiment was carried out in IX-51 inverted microscope in which formation of several emulsion droplets of desired size have been seen. This ensures that the proposed reaction can be carried out in this media.

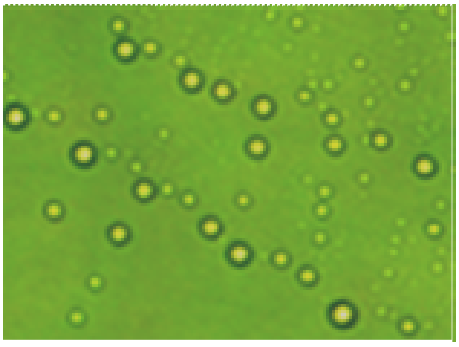


Figure V-2. A typical optical micrograph of emulsion droplets formed in an aqueous solution of DBSA, *o*-diaminoarene and benzaldehyde

Dynamic light scattering (DLS) experiments of those solutions were also carried out in Delsa Nano S, Beckman Coulter particle size analyzer which confirmed that the size of emulsion droplets is mostly in the nanometer range (Figure V-3). These droplets act as micellar nanoreactors to carry out the organic transformations inside their core.

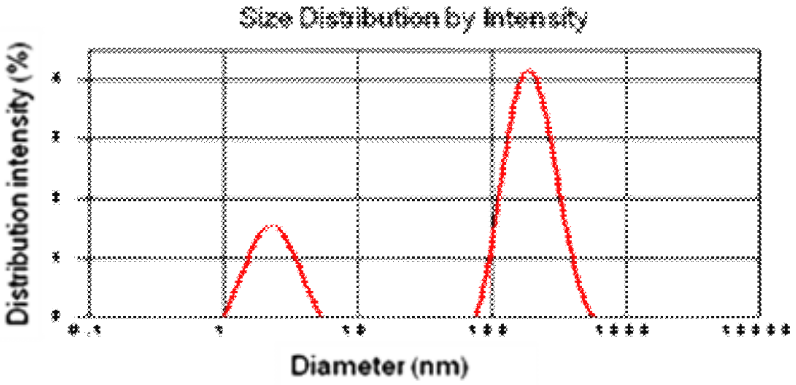


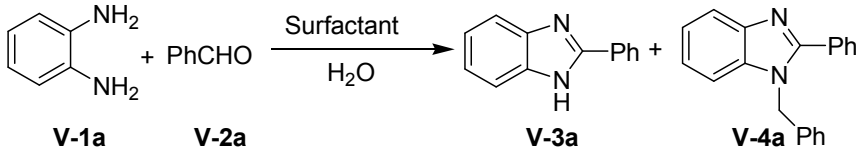
Figure V-3. DLS data of DBSA showing formation of aggregates.

V.2.1.2. Selection of suitable surfactant

To establish a suitable condition for the benzimidazole and benzothiazole synthesis, we screened catalytic activity of six surfactants on a model reaction between equimolar mixture of *o*-phenylenediamie (**V-1a**) and benzaldehyde (**V-2a**) to find out the best catalyst that induces higher selectivity to 2-phenylbenzimidazoles. To our delight, all six surfactants (DBSA, SDS, CTAB, Triton X100, Tween 20 and Tween 80) could catalyze the above reaction at variable rate to produce the desired products (**V-3a** and **V-4a)** in different proportions indicating a miceller condition is useful to carry out this condensation reaction (Table V-1). As expected, DBSA showed highest selectivity (Table V-1, entry 1) among all towards 2-phenylbenzimidazole (**V-3a**) with about 10% of undesired 1-benzyl-2-phenyl-1*H*-benzo[*d*]imidazole (**V-4a**). At elevated temperature, the rate of the reaction was increased with slight drop in the selectivity (Table V-1, entry 2). Whereas, SDS was found to be most suitable catalyst in terms of time required for the completion of the reaction with much reduced selectivity towards 2-phenylbenzimidazole (**V-3a**) (Table V-1, entry 4). The reaction was slower and selectivity was poor for other cases (Table V-1, entry 6−9). We also examined the chemoselectivity of SDS and DBSA on the same reaction upon addition of 2 equiv. of benzaldehyde at one portion. In case of SDS as catalyst, the result was close to what is reported by others[16f] showing pronounced selectivity towards 1,2-disubstituted benzimidazole (**V-4a**) but about 10% of 2-phenylbenzimidazole (**V-3a**) was also obtained

(Table V-1, entry 5). On the other hand, reaction was relatively slow in the presence of DBSA producing nearly equal proportion of both **V-3a** and **V-4a** even after 6 h (Table V-1, entry 3). From the above results we inferred that acidic nature of DBSA and slow reaction rate are helpful for the formation of 2-substituted benzimidazoles.

Table (V-1) Selection of suitable surfactant:



V-1a **V-2a** **V-3a** **V-4a**

Entry	Surfactant[a] (10 mol%)	No. of equiv of PhCHO	*T* (°C)	Time (h)	Yield of 3a (%)	Yield of 4a (%)
1	DBSA	1.0	Rt	6.0	72	10
2	DBSA	1.0	55	2.0	68	13
3	DBSA	2.0	Rt	6.0	52	44
4	SDS	1.0	Rt	1.0	44	26
5	SDS	2.0	Rt	0.5	10	78
6	CTAB	1.0	55	6.0	32	24[b]
7	Triton X-100	1.0	Rt	3.0	48	25
8	Tween 20	1.0	Rt	6.0	42	28
9	Tween 80	1.0	Rt	6.0	39	26[b]

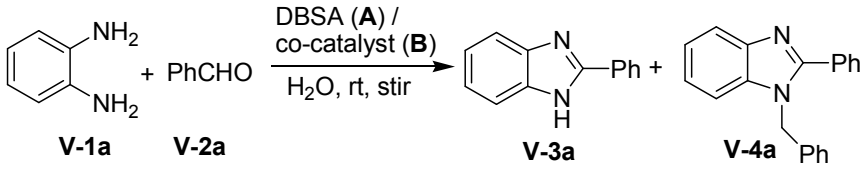
[a]The reaction was carried out between 0.5 mmol of *o*-phylenediamie (**V-1a**) and 0.5 mmol of benzaldehyde (**V-2a**) in the presence of 0.05 mmol of surfactants in 2 mL of water; [b]12-15% of *o*-phylenediamie was isolated along with the products.

V.2.1.3. Effect of co-catalyst and optimization of reaction condition

The excellent chemoselectivity induced by DBSA inspired us to investigate this transformation in details. Formation of some amount of undesired 1,2-disubstituted benzimidazole was still our concern. Literature survey revealed us that use of oxidizing agent under miceller condition[16f] increases selectivity towards 2-substituted benzimidazoles by quick conversion of monoimine into the aromatic system before diimine would form, which is the intermediate of 1,2-disubstituted benzimidazole.[14a] In this regard, various nonhazardous, easily available, cheap oxidizing agents are chosen to accelerate oxidative aromatization process from monoimine and thereby, minimize formation of **V-4a**. Initially, stoichiometric amounts of oxidizing agents I_2, H_2O_2, *p*-benzoquinone, ammonium persulfate and oxone were used separately for condensation of equimolar mixture of *o*-phenylenediamine (**V-1a**) and benzaldehyde (**V-2a**) in the presence of 10 mol% of DBSA in water to find out their influence in the final outcome on the product ratio (Table V-2).

Selectivity was improved at various extents towards the formation of **V-3a** in each case. However, both hydrogen peroxide and iodine were found equally suitable to bring about higher selectivity as well as to reduce reaction time (Table V-2, entry 1 and 5). Few more reactions were separately carried out with *o*-phenylenediamine and *p*-methoxybenzaldehyde / *p*-nitrobenzaldehyde in the presence of 10 mol% of DBSA and 1 equiv. of either H_2O_2 or I_2. The yields of corresponding 2-arylbenzimidazoles are as follows. For I_2, *p*-OMe: 86%, *p*-NO_2: 94%. For H_2O_2, *p*-OMe: 84%, *p*-NO_2: 88%. Based on the observed selectivity and considering the fact that iodine is milder and easier to handle, it was selected as an additive for further study. We presumed that the role of iodine could be two fold: a) to act as Lewis acid to increase electrophilicity of the imine bond and thus, facilitate cyclization process and b) to oxidize dihydroimidazole to corresponding aromatic system. In order to optimize the amount of iodine required for this condensation, we carried out reactions with various proportions of iodine at substoichiometric level keeping other conditions same. We were delighted to observe that use of 10 mol% of iodine is equally effective as like use of

Table (V-2) Standardization of reaction condition: effect of surfactant, effect of co-catalysts:



V-1a **V-2a** **V-3a** **V-4a**

Entry	mol % of A	Co-catalyst (B)	mol % of B	Time (h)	Yield of 3a (%)	Yield of 4a (%)
1	10	H_2O_2	100	2	90	3
2	10	*p*-Benzoquinone	100	5	82	5
3	10	Oxone	100	4	78	6
4	10	$(NH_4)_2S_2O_8$	100	4	75	8
5	10	I_2	100	1.5	89	0[a]
6	10	I_2	20	2	88	0[a]
7	10	I_2	10	2	92	0[a]
8	20	I_2	20	2	88	0[a]
9	5	I_2	05	6	74	6

[a]no trace of **V-4a** was found in the TLC.

stoichiometric amount to impose similar selectivity towards 2-phenylbenzimidazole (Table V-2, entry 7). This indicates that the primary role of iodine is to act as Lewis acid[19] to expedite cyclization process and presumably, oxidation is mostly done by the dissolved oxygen[13h,17a] in the system. We also examined that use of more than 10% of DBSA does not enhance chemoselectivity (or yield) (Table V-2, entry 8) neither use of less than 10% of DBSA is suitable for this transformation (Table V-2, entry 9). Therefore, we decided to use 10 mol% of iodine as co-catalyst along with DBSA (10 mol%) for this condensation reaction to achieve highest chemoselectivity towards 2-substituted benzimidazoles.

V.2.2. Chemoselective synthesis of 2-substituted benzimidazoles

A series of aromatic aldehydes was treated with various *o*-diaminoarenes under optimized reaction conditions. The developed process was found to be excellent in terms of yield and selectivity resulting in a variety of 2-substituted benzimidazoles in very high yields (Table V-3). The aldehydes with electron donating (Table V-3, entries 6, 7, 22, 28 etc.) as well as with electron withdrawing groups (Table V-3, entries 2, 14, 21, 27 etc.) participated in the reaction uniformly with no significant distinction in regards to the yields of the target products. Similarly, no distinct substituent effect was observed on the yields of 2-substituted benzimidazoles by varying substituents in *o*-diaminoarenes. Even sensitive substrates like furfuraldehyde (Table V-3, entry 9, 18 and 23) produced the desired product without any difficulty. The present method was fairly applicable to aliphatic aldehydes as well (Table V-3, entry 12, 26 etc.). However, reactions were sluggish for water soluble aldehydes viz. butyraldehyde (Table V-3, entry 29), which is quite expected as they will hardly stay inside the micelles. Apparently, the nature and position of the substitutions in the aryl rings did not have great influence on the reactivity. Most of the reactions were completed within 3 h. Only aldehyde with strong electron withdrawing group reacted relatively faster (Table V-3, entries 2, 14, 27 etc.) and reaction was little slow for an aldehyde with strong electron donating group (Table V-3, entries 6, 7, 22, 28 etc.), as expected.

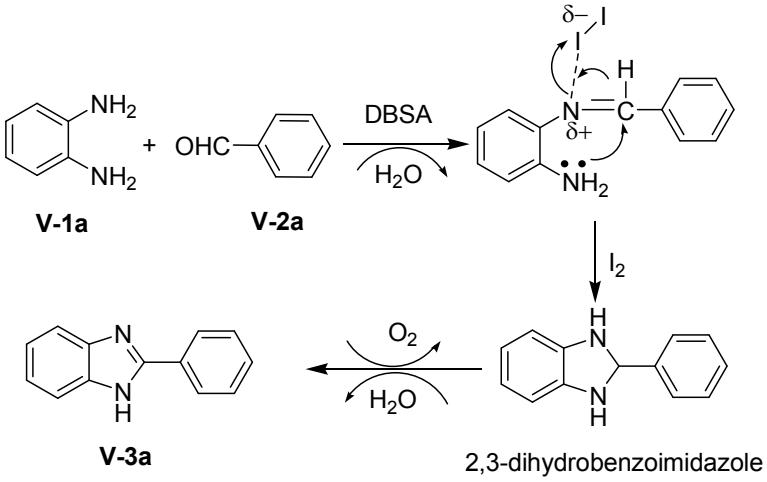
Table (V-3): DBSA–I_2 catalyzed synthesis of 2-substituted benzimidazoles

Entry	R	R′	Time (h)	Product	Yield of 3[a] (%)	Ref.
1	H	Ph	2.0	**V-3a**	92[b]	13d
2	H	4-$NO_2C_6H_4$	1.0	**V-3b**	94[b]	13d
3	H	3-ClC_6H_4	3.0	**V-3c**	80[c]	11k
4	H	4-ClC_6H_4	2.5	**V-3d**	89[c]	13d
5	H	4-BrC_6H_4	2.0	**V-3e**	87[c]	11k
6	H	2-OHC_6H_4	3.0	**V-3f**	84[c]	13d
7	H	4-$OCH_3C_6H_4$	5.0	**V-3g**	86[c]	13d
8	H	C_6H_5-CH=CH-	2.0	**V-3h**	84[d]	13a
9	H	Furan-2-yl	4.0	**V-3i**	80[d]	13d
10	H	Pyrrole-2-yl	2.0	**V-3j**	90[b]	
11	H	Thiophene-2-yl	2.5	**V-3k**	88[c]	13i
12	H	Cyclohexyl	6.0	**V-3l**	82[c,f]	17a
13	4-Methyl	Ph	2.0	**V-3m**	91[b]	16f
14	4-Methyl	4-$NO_2C_6H_4$	1.0	**V-3n**	93[b]	20
15	4-Methyl	3-ClC_6H_4	3.0	**V-3o**	79[c]	21a
16	4-Methyl	4-ClC_6H_4	2.0	**V-3p**	82[c]	21a
17	4-Methyl	2-OHC_6H_4	3.0	**V-3q**	84[c]	13h
18	4-Methyl	Furan-2-yl	4.0	**V-3r**	81[d]	
19	4-Methyl	Thiophene-2-yl	2.5	**V-3s**	86[c]	
20	4-Nitro	Ph	2.5	**V-3t**	94[b]	14f
21	4-Nitro	4-$NO_2C_6H_4$	2.0	**V-3u**	91[b]	
22	4-Nitro	4-$OCH_3C_6H_4$	6.0	**V-3v**	92[b]	21b
23	4-Nitro	Furan-2-yl	4.0	**V-3w**	83[d]	
24	4-Nitro	Pyrrole-2-yl	2.5	**V-3x**	88[d]	
25	4-Nitro	Thiophene-2-yl	3.0	**V-3y**	86[c]	
26	4-Nitro	Cyclohexyl	6.0	**V-3z**	81[c,f]	
27	4-Chloro	4-$NO_2C_6H_4$	1.5	**V-3aa**	90[b]	
28	4-Chloro	4-$OCH_3C_6H_4$	5.0	**V-3ab**	93[b]	
29	H	*n*-Bu	12.0	**V-3ac**	12[e,f]	16f

[a]all yields refer to isolated product; [b]isolated as sole product; [c]3-5% of 1,2-disubstituted benzimidazole was also isolated; [d]trace amount of other products were isolated, which could not be purified and characterized. [e]isolated yield of the product after 12 h; [f]the reactions are carried out at 40 °C.

Based on the above results a plausible mechanism has been proposed, which is believed to be the major pathway for this dehydrative condensation followed by cyclization reaction (Scheme V-3). The reaction is initiated by the formation of a Schiff's base when an aldehyde molecule comes in contact with an *o*-diaminoarenes inside the hydrophobic core of the micelle. This step is always inclined towards product as the water molecule is ejected out of the hydrophobic core as soon as it is formed. Next, iodine acts as a Lewis acid to make a partial bond with imine to increase its electrophilicity and facilitates attack of the other amino

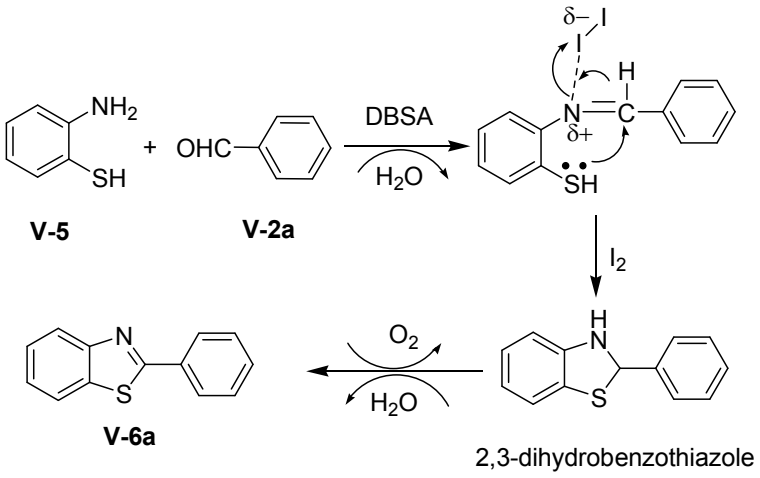
group to the imine carbon resulting in formation of dihydrobenzimidazole. In the final step the dihydrobenzimidazole gets oxidized to the product by the dissolved oxygen in water.



Scheme V-3. Mechanistic pathway of the formation of 2-phenylbenzimidazoles (**V-3a**) in micellar media.

V.2.3. Synthesis of 2-substituted benzothiazoles

To extend the application of this methodology, a series of aromatic and heteroaromatic aldehydes was treated with *o*-aminothiophenol under the optimized reaction condition for the synthesis of 2-substituted benzothiazoles (**V-6**) (Scheme V-2**)**. The reaction methodolgy was found to be excellent in terms of yields, reaction time resulting in a variety of 2-substituted benzothiazoles in very high yields (Table V-4). The products were characterized by ^{1}H NMR, ^{13}C NMR and mass and compared with the reported data as and when possible. A similar mechanistic pathway like 2-substituted benzimidazole may be operated in case of benzothiazole as well. Thus, the reaction is initiated by the formation of a Schiff's base when an aldehyde molecule comes in contact with an *o*-aminothiophenol inside the hydrophobic core of the micelle. The product foarmation was facilitated by the ejection of one molecule of water out of the hydrophobic core. Iodine acts as a Lewis acid to activate the imine bond to facilitate nucleophilic attack of sulphur atom to imine carbon resulting in formation of dihydrobenzothiazole. In the final step, the dihydrobenzothiazole gets oxidized to the product in-situ by the dissolved oxygen in water (Scheme V-4**)**.



Scheme V-4. Mechanistic pathway of the formation of 2-phenylbenzothiazoles (**V-6a**) in micellar media.

It was found that the aldehydes with electron donating (Table V-4, entries 7-9) as well as with electron withdrawing groups (Table V-4, entries 2-6) participated in the reaction uniformly with no significant distinction with regard to the yields of the final products. The method was found equally suitable for heteroaromatic aldehydes (Table V-4, entries 10-13). Fast reaction ensures the sensitive substrates like furfuraldehyde (Table V-4, entry 10) would also afford the desired product in high yield. However, the variation in the substituents in the aryl ring did have some influence on the rate of the reaction. Although most of the reactions were completed within 60 min, presences of strong electron donating groups in the aldehyde residue delayed complete conversion to some extent (Table V-4, entry 8, 9). On the other hand, aldehydes with strong electorn withdrawing groups reacted faster (Table V-4, entries 5, 6, etc.), as per expectation. However, to our dismay, reaction of aliphatic aldehydes with *o*-aminothiophenol failed to produce expected results (Table V-4, entries 14, 15). As a token of demonstration, we carried out reaction of *o*-aminothiophenol with cyclohexanecarboxaldehyde and butaraldehyde. Only 40% of desired product (**V-6o**) was isolated after carrying out the reaction up to 1.5 h, whereas, no product was isolated in pure form from the other reaction. Apparently, the imine was formed in due course of time but the cyclization step was retarded by the poor reactivity of the aliphatic imine group. Therefore, we restricted our study to aromatic aldehydes only.

Table (V-4): DBSA–I_2 catalyzed synthesis of 2-substituted benzothiazoles

Entry	Ar	Time (min)	Product	% Yield of 6[a]	Ref.
1	Ph	60	**V-6a**	83	22
2	3-$NO_2C_6H_4$	60	**V-6b**	82	22
3	4-ClC_6H_4	60	**V-6c**	79	22
4	3-BrC_6H_4	60	**V-6d**	82	11g
5	4-FC_6H_4	30	**V-6e**	84	22
6	4-CNC_6H_4	60	**V-6f**	81	23
7	2-OHC_6H_4	120	**V-6g**	80	12b
8	4-OHC_6H_4	90	**V-6h**	79	24
9	4-$MeOC_6H_4$	90	**V-6i**	78	22
10	Furan-2-yl	75	**V-6j**	82	11d
11	Thiophene-2-yl	75	**V-6k**	85	22
12	Indole-3-yl	75	**V-6l**	87	23
13	Pyridine-4-yl	60	**V-6m**	88	22
14	Cyclohexyl	90	**V-6n**	40[b]	25
15	Butaryl	60	**--**	n.d.[c]	

[a]All yields refer to isolated product; [b]Corresponding imine was isolated as the major product; [c]No desired product was isolated and imine got decomposed during column chromatography; n.d. is not determined

V.3. Conclusion

In conclusion, a practical and 'green' method has been developed for chemoselective synthesis of 2-substituted benzimidazoles in organized aqueous media in the presence of DBSA (10 mol%) as catalyst and iodine (10 mol%) as co-catalyst. The same method has been extended to the synthesis of 2-substituted benzothiazoles as well. A broad range of 2-substituted benzimidazoles and 2-substituted benzothiazoles have been synthesized from o-diaminoarenes and *o*-aminothiophenol, respectively with a variety of aldehydes using this method in high overall yields. The operational simplicity, excellent yields of the products, and high chemoselectivity in case of 2-substituted benzimidazoles over 1,2-disubstituted benzimidazoles are some of the merits of this method, and furthermore, this method is cheap, safe and environmentally benign.

V.4. Experimental Section

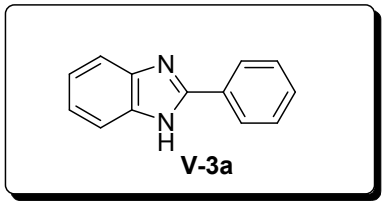
V.4.1. Materials & Methods

All solvents were obtained from local suppliers and were of research grade. All the aldehydes were purchased mainly from Sigma–Aldrich and used without further purification. Other common reagents were procured either from SD Fine-Chem. Limited, Mumbai, India or from Merck, India.

^{1}H NMR and ^{13}C NMR spectra were recorded on BRUKER 300 MHz or BRUKER 400 MHz or JEOL 500 MHz NMR systems using tetramethylsilane as an internal standard. The chemical shifts are reported in parts per million (ppm) units relative to the solvent peak. The following abbreviations are used in reporting NMR data: s, singlet; d, doublet; t, triplet; dd, doublet of doublets; m, multiplet; brs, broad singlet. Mass spectra were recorded on Waters Q-TOF micro mass spectrometer or Bruker Esquire 3000 mass spectrometer using ESI and APCI as the ion source. High-resolution mass spectra (HRMS) were obtained using an electrospray quadrupole time-of-flight (ESI-Q-TOF) mass spectrometer. Particle size was determined using a particle size analyzer (Delsa Nano S, Beckman Coulter, USA). The reactions were monitored by thin layer chromatography (TLC) carried out on 0.25-mm silica gel on aluminium plates (60F-254) using UV light (254 or 365 nm) or naked eye for visualization. Column chromatography was performed on silica gel (60–120 mesh, Merck).

V.4.2. General experimental procedure for the synthesis of 2-substituted benzimidazoles (V-3):

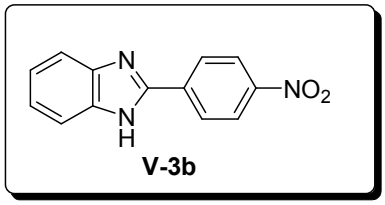
To a solution of DBSA (0.05 mmol) in H_2O (2 mL) were added an amine (0.5 mmol) and iodine (0.05 mmol). An aldehyde (0.5 mmol) was added portion wise at room temperature. The reaction was stirred at room temperature for several hrs (see Table V-3). The progress of the reaction was monitored by TLC. The completion of the reaction was indicated by separation of the organic phase from aqueous media. The aqueous layer was decanted. The organic part was taken in ethyl acetate, washed with saturated $NaHCO_3$, water, brine and then dried over anhydrous Na_2SO_4. The organic layer was concentrated and purified by silica gel chromatography.



V-3a

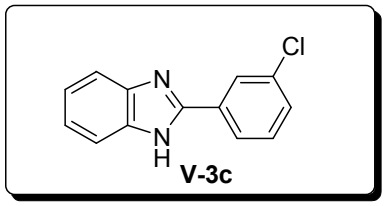
***2-Phenyl-1H-benzo[d]imidazole (V-3a)*:**[13d] Yellow color solid, yield: 92%; mp. 286-287 °C (Lit. 287-288 °C); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 7.22-7.23 (m, 2H), 7.47-7.71 (m, 5H), 8.22-8.24 (m, 2H), 12.97 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm 111.8, 119.4, 122.2, 123.0, 126.9 (2C), 129.4 (2C), 130.3, 130.7,

135.5, 144.3, 151.7; ESI-MS: (*m/z*) 195 $[M + H]^+$; *Anal.* Calcd. for $C_{13}H_{10}N_2$: C, 80.39; H, 5.19; N, 14.42. Found: C, 80.62; H, 5.18; N, 14.46.



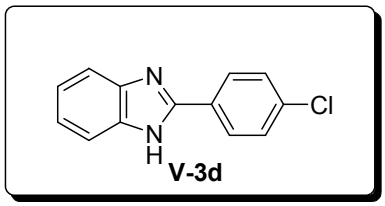
V-3b

***2-(4-Nitrophenyl)-1H-benzo[d]imidazole (V-3b)*:**[13d] Yellow color solid, yield: 94%; mp. 307-308 °C (Lit. 308-310 °C); ^{1}H NMR (300 MHz, CD_3OD): δ (ppm) 7.33-7.36 (m, 2H), 7.55-7.76 (m, 2H), 8.33 (d, J = 9.0 Hz, 2H), 8.43 (d, J = 9.0 Hz, 2H); ^{13}C NMR (75 MHz, CD_3OD): δ (ppm) 112.2, 119.8, 122.7 (2C), 124.0, 124.7, 127.7 (2C), 135.6, 136.4, 144.3, 148.1, 149.4; ESI-MS: (*m/z*) 240 $[M + H]^+$; *Anal.* Calcd. for $C_{13}H_9N_3O_2$: C, 65.27; H, 3.79; N, 17.56. Found: C, 65.17; H, 3.80; N, 17.58.



V-3c

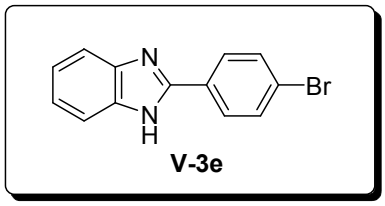
***2-(3-Chlorophenyl)-1H-benzo[d]imidazole (V-3c)*:**[11k] Yellowish white color solid, yield: 80%; mp. 230-231 °C (Lit. 232-233 °C); ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 7.18-7.19 (m, 2H), 7.40-7.58 (m, 4H), 8.12 (d, J = 7.5 Hz, 1H), 8.21 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ (ppm) 122.9, 125.5 (2C), 126.5 (2C), 130.0 (2C), 131.5 (2C), 132.8, 134.3 (2C), 150.3; ESI-MS: (*m/z*) 229 $[M + H]^+$ (major peak, for ^{35}Cl), 231 $[M + H]^+$ (minor peak, for ^{37}Cl); *Anal.* Calcd. for $C_{13}H_9ClN_2$: C, 68.28; H, 3.97; N, 12.25. Found: C, 68.49; H, 3.96; N, 12.27.



V-3d

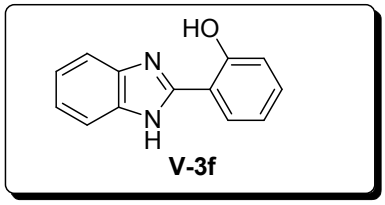
***2-(4-Chlorophenyl)-1H-benzo[d]imidazole (V-3d)*:**[13d] Light shiny brown color solid, yield: 89%; mp. 291-292 °C (Lit. 291-293 °C); ^{1}H NMR (400 MHz, CD_3OD): δ (ppm)

7.24-7.29 (m, 2H), 7.54 (d, J = 8.4 Hz, 2H), 7.57-7.64 (m, 2H), 8.05 (d, J = 8.4 Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ (ppm) 112.1, 119.5, 122.8, 123.9, 129.0 (2C), 129.8 (2C), 135.2, 135.9, 144.6 (2C), 151.0; ESI-MS: (*m/z*) 229 $[M + H]^+$ (major peak, for ^{35}Cl), 231 $[M + H]^+$ (minor peak, for ^{37}Cl); *Anal.* Calcd. for $C_{13}H_9ClN_2$: C, 68.28; H, 3.97; N, 12.25. Found: C, 68.46; H, 3.95; N, 12.26.



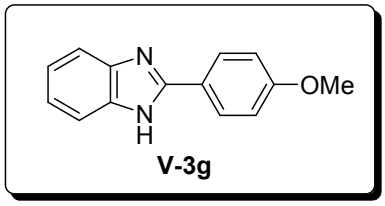
V-3e

***2-(4-Bromophenyl)-1H-benzo[d]imidazole (V-3e)*:**[11k] Light yellow color solid, yield: 87%; mp. 297-298 °C (Lit. 298-299 °C); ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 7.18-7.22 (m, 2H), 7.50 (s, 1H), 7.63 (d, J = 7.8 Hz, 1H), 7.74 (d, J = 7.2 Hz, 2H),), 8.10-8.14 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) 111.3, 118.6, 121.7, 123.0, 128.3, 129.0 (3C), 131.7, 134.6, 143.4 (2C), 149.9; ESI-MS: (*m/z*) 273 $[M + H]^+$ (for ^{79}Br), 275 $[M + H]^+$ (for ^{81}Br); *Anal.* Calcd. for $C_{13}H_9BrN_2$: C, 57.17; H, 3.32; N, 10.26. Found: C, 57.30; H, 3.31; N, 10.28.



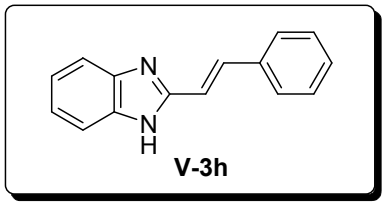
V-3f

***2-(2-Hydroxyphenyl)-1H-benzo[d]imidazole (V-3f)*:**[13d] White solid, yield: 84%; mp. 234-235 °C (Lit. 235-237 °C); ^{1}H NMR (300 MHz, CD_3OD): δ (ppm) 6.94-7.02 (m, 2H), 7.24-7.36 (m, 3H), 7.59-7.62 (m, 2H), 7.91 (d, J = 8.1 Hz, 1H); ^{13}C NMR (75 MHz, CD_3OD): δ (ppm) 113.0, 114.4, 116.8, 118.9 (2C), 122.5 (2C), 125.7 (2C), 131.2 (2C), 151.7, 158.1; ESI-MS: (*m/z*) 211 $[M + H]^+$; *Anal.* Calcd. for $C_{13}H_{10}N_2O$: C, 74.27; H, 4.79; N, 13.33. Found: C, 74.19; H, 4.80; N, 13.36.



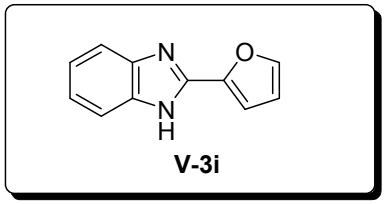
V-3g

***2-(4-Methoxyphenyl)-1H-benzo[d]imidazole (V-3g)*:**[13d] Light yellow color solid, yield: 86%; mp. 224-225 °C (Lit. 225-227 °C); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 3.84 (m, 3H), 6.64-6.76 (m, 3H), 6.83-6.88 (m, 1H), 6.92 (d, J = 8.4 Hz, 2H), 7.35 (d, J = 8.4 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 55.6, 112.3, 114.0 (2C), 116.6 (2C), 119.0, 120.7 (2C), 129.8, 131.4, 134.3, 137.6, 158.9; ESI-MS: (*m/z*) 225 $[M + H]^+$; *Anal.* Calcd. for $C_{14}H_{12}N_2O$: C, 74.98; H, 5.39; N, 12.49. Found: C, 75.16; H, 5.40; N, 12.51.



V-3h

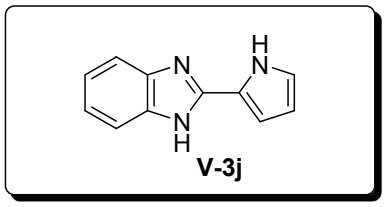
***E-2-Styryl-1H-benzo[d]imidazole (V-3h)*:**[15a] Pale yellow solid, yield: 84%; mp. 211-212 °C (Lit. 212-214 °C); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 7.10 (d, J = 15.8 Hz, 1H), 7.25-7.31 (m, 2H), 7.34 (m, 2H), 7.39 (m, 2H), 7.58 (m, 2H), 7.72 (m, 1H), 7.78 (d, J = 15.8 Hz, 1H), 12.93 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 111.1, 113.8 (2C), 119.7, 124.8 (2C), 126.3, 127.7, 128.8, 129.8, 135.2, 139.4, 142.3, 150.3, 162.8; ESI-MS: (*m/z*) 221 $[M + H]^+$; *Anal.* Calcd. for $C_{15}H_{12}N_2$: C, 81.79; H, 5.49; N, 12.72. Found: C, 81.95; H, 5.50; N, 12.74.



V-3i

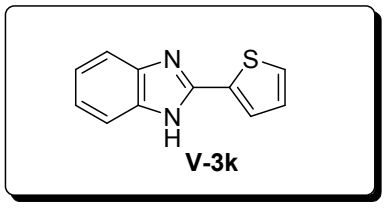
***2-(2-Furan-2-yl)-1H-benzo[d]imidazole (V-3i)*:**[13d] White solid, yield: 80%; mp. 284-285 °C (Lit. 284-286 °C); ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 6.69 (s, 1H), 7.15-7.18 (m, 3H), 7.52 (s, 2H), 7.90 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ (ppm)

111.0 (2C), 112.8 (2C), 122.7 (2C), 144.1, 145.2 (3C), 146.1; ESI-MS: (*m/z*) 185 $[M + H]^+$; *Anal.* Calcd. for $C_{11}H_8N_2O$: C, 71.73; H, 4.38; N, 15.21. Found: C, 71.57; H, 4.39; N, 15.23.



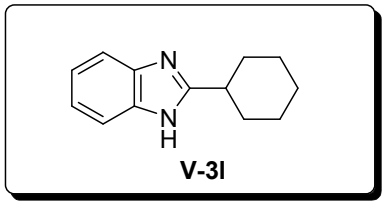
V-3j

***2-(1H-pyrrol-2-yl)-1H-benzo[d]imidazole (V-3j)*:** Yellow color solid, yield: 90%; mp. 268-270 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 6.19 (d, J = 1.5 Hz, 1H), 6.82 (d, J = 3.5 Hz, 1H), 6.90 (d, J = 1.5 Hz, 1H), 7.11 (m, 2H), 7.43 (d, J = 4.5 Hz, 1H), 7.55 (d, J = 4.7 Hz, 1H), 11.81 (s, 1H, NH), 12.49 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6): δ (ppm) 109.7 (2C), 110.8, 118.2, 121.4, 121.8 (2C), 123.2, 135.0, 144.3, 147.3; HRMS (ESI): *m/z* calcd for $C_{11}H_{10}N_3$ $[M + H]^+$ 184.0875, found 184.0872.



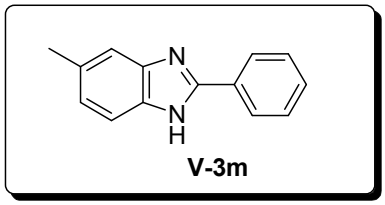
V-3k

***2-(2-Thienyl)-1H-benzo[d]imidazole (V-3k)*:**[13i] Brown red color solid, yield: 88%; mp. 330-331 °C (Lit. 330 °C); H NMR (500 MHz, DMSO-d_6): δ (ppm) 7.12-7.20 (m, 3H), 7.46 (d, J = 8.0 Hz, 1H), 7.57 (d, J = 7.2 Hz, 1H), 7.69 (d, J = 4.8 Hz, 1H), 7.79 (d, J = 3.2 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ (ppm) 111.6, 119.1, 122.3, 123.1, 127.2, 128.8, 129.3, 134.2, 135.2, 144.1, 147.6; ESI-MS: (*m/z*) 201 $[M + H]^+$; *Anal.* Calcd. for $C_{11}H_8N_2S$: C, 65.97; H, 4.03; N, 13.99. Found: C, 66.10; H, 4.02; N, 14.03.



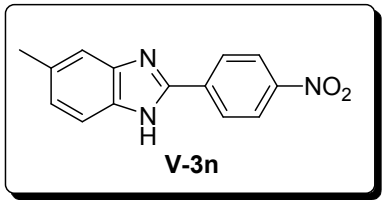
V-3l

***2-Cyclohexyl-1H-benzo[d]imidazole (V-3l)*:**[17a] White solid, yield: 82%; mp. 282-283 °C (Lit. 281-284 °C); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 1.31-1.52 (m, 2H), 1.67-1.79 (m, 2H), 1.84-1.88 (m, 2H), 2.08- 2.18 (m, 3H), 2.90-2.98 (m, 2H), 7.16-7.22 (m, 2H), 7.54-7.58 (m, 2H), 12.38 (br s, 1H, exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 25.5 (2C), 28.6, 33.5 (2C), 41.2, 116.2 (2C), 123.5 (2C), 139.8 (2C), 141.9; ESI-MS: (*m/z*) 201 $[M + H]^+$; *Anal.* Calcd. for $C_{13}H_{16}N_2$: C, 77.96; H, 8.05; N, 13.99. Found: C, 78.13; H, 8.07; N, 14.01.



V-3m

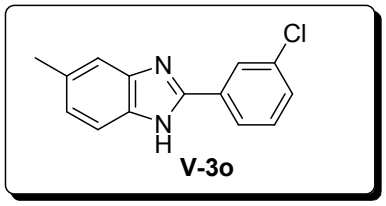
***5-Methyl-2-phenyl-1H-benzo[d]imidazole (V-3m)*:**[16f] Light brown color solid**,** yield: 91%; mp. 240-241 °C (Lit. 242-243 °C); ^{1}H NMR (300 MHz, CD_3OD): δ (ppm) 2.49 (s, 3H), 7.12 (d, J = 8.1 Hz, 1H), 7.41-7.61(m, 5H), 8.02 (d, J = 8.4 Hz, 1H), 8.07 (d, J = 6.8 Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ (ppm) 20.3, 113.8, 124.2, 126.3 (2C), 128.0, 128.7 (2C), 129.5 (2C), 130.9, 132.5, 151.5, 168.7; ESI-MS: (*m/z*) 209 $[M + H]^+$; *Anal.* Calcd. for $C_{14}H_{12}N_2$: C, 80.74; H, 5.81; N, 13.45. Found: C, 81.01; H, 5.83; N, 13.43.



V-3n

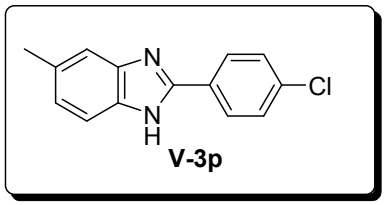
***5-Methyl-2-(4-nitrophenyl)-1H-benzo[d]imidazole (V-3n)*:**[20] Brownish yellow color solid, yield: 93%; mp. 142-144 °C (Lit. 143-145 °C); ^{1}H NMR (300 MHz, CD_3OD): δ (ppm) 2.50 (s, 3H), 7.16 (d, J = 8.1 Hz, 2H), 7.43-7.53 (m, 2H), 8.27 (d, J = 8.7 Hz,

2H), 8.39 (d, J = 8.7 Hz, 2H); ^{13} C NMR (100 MHz, CD_3OD): δ (ppm) 20.3, 123.7 (3C), 124.9, 126.8 (3C), 133.6, 135.3 (2C), 148.2 (2C), 148.9; ESI-MS: (*m/z*) 254 $[M + H]^+$; *Anal.* Calcd. for $C_{14}H_{11}N_3O_2$: C, 66.40; H, 4.38; N, 16.59. Found: C, 66.15; H, 4.39; N, 16.61.



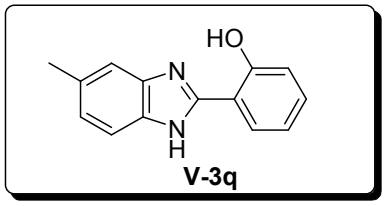
V-3o

***5-Methyl-2-(3-chlorophenyl)-1H-benzo[d]imidazole (V-3o)*:**[21a] Yellowish brown color solid, yield: 79%; mp. 137-138 °C (Lit. 139-140 °C); ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 2.39 (s, 3H), 7.00 (d, J = 7.9 Hz, 1H), 7.35 (s, 1H), 7.46-7.54 (m, 3H), 8.08 (d, J = 7.5 Hz, 1H), 8.17 (s, 1H); ^{13} C NMR (125 MHz, DMSO-d_6): δ (ppm) 21.9, 124.6, 125.4 (2C), 126.4 (2C), 129.9 (2C), 131.4 (3C), 132.9, 134.3, 149.9; ESI-MS: (*m/z*) 241 $[M - H]^+$; *Anal.* Calcd. for $C_{14}H_{11}ClN_2$: C, 69.28; H, 4.57; N, 11.54. Found: C, 69.18; H, 4.58; N, 11.55.



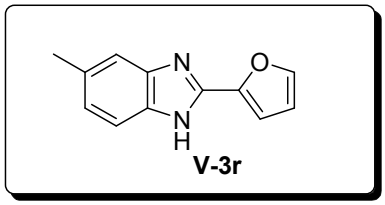
V-3p

***5-Methyl-2-(4-chlorophenyl)-1H-benzo[d]imidazole (V-3p)*:**[21a] Almond color solid, yield: 82%; mp. 116-117 °C (Lit. 118-119 °C); ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 2.46 (s, 3H), 6.97-7.02 (m, 1H), 7.28 (s, 1H), 7.37 (d, J = 8.0 Hz, 1H), 7.50 (d, J = 8.0 Hz, 1H), 8.12 (d, J = 8.6 Hz, 2H), 12.81 (d, J = 19.6 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ (ppm) 21.8, 111.5, 119.1, 124.0, 128.5 (2C), 129.7 (3C), 132.7, 133.6, 135.8, 142.4, 150.2; ESI-MS: (*m/z*) 243 $(M + H)^+$; *Anal.* Calcd. for $C_{14}H_{11}ClN_2$: C, 69.28; H, 4.57; N, 11.54. Found: C, 69.19; H, 4.58; N, 11.55.



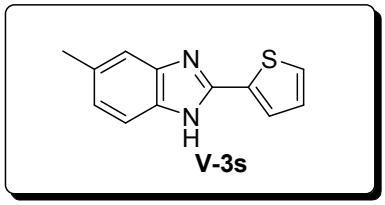
V-3q

***5-Methyl-2-(2-hydroxyphenyl)-1H-benzo[d]imidazole (V-3q)*:**[13h] Almond color solid, yield: 84%; mp. 198-199 °C (Lit. 199-200 °C); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 2.47(s, 3 H), 6.92-6.96 (m, 1H), 7.04-7.09 (m, 2H), 7.28-7.32 (m, 1H), 7.37 (d, J = 5.8 Hz, 1H), 7.48 (d, J = 7.6 Hz, 1H), 7.82(d, J = 7.6 Hz, 1H); ^{13}C NMR (100 MHz,$CDCl_3$): δ (ppm) 25.4, 117.1, 121.2 (2C), 123.1 (2C), 128.2, 129.3 (2C), 135.1 (2C), 136.6, 155.5, 162.0; APCI-MS: (*m/z*) 225 $(M + H)^+$; *Anal.* Calcd. for $C_{14}H_{12}N_2O$: C, 74.98; H, 5.39; N, 12.49. Found: C, 75.09; H, 5.42; N, 12.55.



V-3r

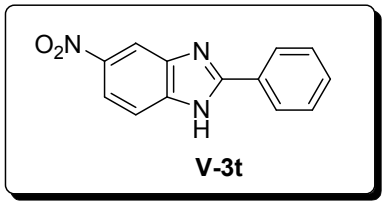
***2-(Furan-2-yl)-5-methyl-1H-benzo[d]imidazole (V-3r)*:** Brownish yellow color solid, yield: 81%; mp. 160-161 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 2.38 (s, 3H), 6.68 (dd, J_1 = 1.9 Hz, J_2 = 3.5 Hz, 1H), 6.98 (d, J = 7.9 Hz, 1H), 7.11 (d, J = 3.5 Hz, 1H), 7.29 (brs, 1H), 7.40 (brs, 1H), 7.88 (d, J = 1.9 Hz, 1H); ^{13}C (125 MHz, DMSO-d_6): δ (ppm) 21.8, 110.6, 112.8 (2C), 119.2, 124.0, 131.0, 143.9, 145.0 (2C), 146.2 (2C); HRMS (ESI): *m/z* calcd for $C_{12}H_{11}N_2O$ $[M + H]^+$ 199.0871, found 199.0874.



V-3s

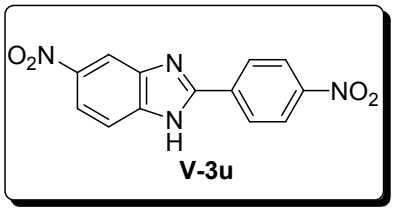
***5-Methyl-2-(thiophen-2-yl)-1H-benzo[d]imidazole (V-3s)*:** Yellow color solid, yield: 86%; mp. 226-227 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 2.38 (s, 3H), 6.97 (d, J = 7.6 Hz, 1H), 7.18 (d, J = 8.5 Hz, 1H), 7.19-7.52 (m, 2H), 7.66 (d, J = 4.9 Hz, 1H), 7.76 (d, J = 2.8 Hz, 1H), 12.74 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm)

21.8, 111.3, 118.6, 124.1, 126.8 (2C), 128.7 (2C), 128.9 (2C), 134.4, 147.2; HRMS (ESI): *m/z* calcd for $C_{12}H_{11}N_2S$ $[M + H]^+$ 215.0643, found 215.0642.



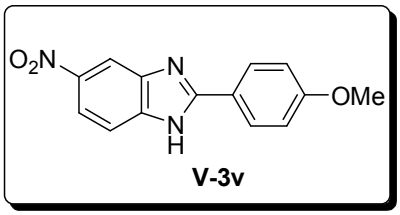
V-3t

***5-Nitro-2-phenyl-1H-benzo[d]imidazole (V-3t)*:**[14f] Brown color solid, yield: 94%; mp. 207-209 °C (Lit. 208-210 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.57 (d, J = 6.5 Hz, 3H), 7.74 (d, J = 8.9 Hz, 1H), 8.11-8.14 (m, 1H), 8.18-8.20 (m, 2H), 8.52 (s,1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 117.1, 123.0 (3C), 132.0, 133.8 (3C), 134.0 (2C), 135.9, 147.9, 161.0; APCI-MS: (*m/z*) 225 $[M + H]^+$; *Anal.* Calcd. for $C_{13}H_9N_3O_2$: C, 65.27; H, 3.79; N, 17.56. Found: C, 65.16; H, 3.75; N, 17.53.



V-3u

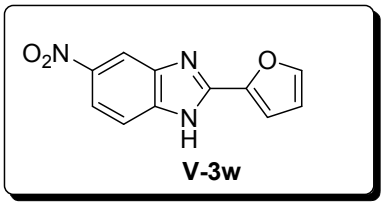
***5-Nitro-2-(4-nitrophenyl)-1H-benzo[d]imidazole (V-3u)*:** Brick color solid, yield: 91%; mp. 245-247 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 7.23 (d, J = 8.5 Hz, 1H), 7.62-7.68 (m, 2H), 7.80 (d, J = 8.5 Hz, 1H), 7.99 (d, J = 8.1 Hz, 1H), 8.10-8.16 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ (ppm) 118.4, 124.6, 125.0 (2C), 128.7 (2C), 129.2, 131.2, 132.1, 135.4, 143.7, 149.1, 167.7; HRMS (ESI): *m/z* calcd. for $C_{13}H_9N_4O_4$ $[M + H]^+$ 285.0624, found 285.0621.



V-3v

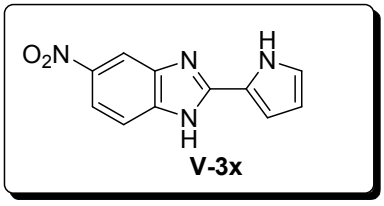
***5-Nitro-2-(4-methoxyphenyl)-1H-benzo[d]imidazole (V-3v)*:**[21b] Dirty yellow color solid, yield: 92%; mp. 236-238 °C (Lit. 237.2-239.5 °C); ^{1}H NMR (400 MHz, $CDCl_3$):

δ (ppm) 3.87 (s, 3 H), 7.03 (d, J = 8.1 Hz, 2H), 7.59 (brs, 1H), 8.02 (d, J = 8.1 Hz, 2H), 8.11-8.14 (m, 1H), 8.45 (brs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 59.1, 118.4 (4C), 122.2, 125.0 (2C), 132.6 (3C), 147.2, 166.0 (2C); ESI-MS: (*m/z*) 270 [M + H]$^+$; *Anal.* Calcd. for $C_{14}H_{11}N_3O_3$: C, 62.45; H, 4.12; N, 15.61. Found: C, 62.64; H, 4.11; N, 15.64.



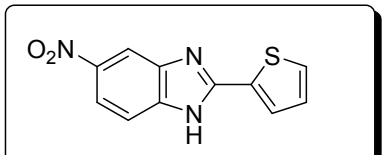
V-3w

***2-(Furan-2-yl)-5-nitro-1H-benzo[d]imidazole (V-3w)*:** Almond color solid, yield: 83%; mp. 222-223 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 6.75 (dd, J_1 = 1.5 Hz, J_2 = 3.4 Hz, 1H), 7.31 (d, J = 3.4 Hz, 1H), 7.67 (d, J = 8.9 Hz, 1H), 8.00 (s, 1H), 8.07 (dd, J_1 = 2.1 Hz, J_2 = 8.9 Hz, 1H), 8.38 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ (ppm) 113.3 (3C), 118.6, 143.3, 145.0 (2C), 146.4 (2C), 148.1, 148.3; HRMS (ESI): *m/z* calcd for $C_{11}H_8N_3O_3$ [M + H]$^+$ 230.0566, found 230.0564.



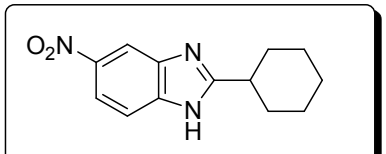
V-3x

***5-Nitro-2-(1H-pyrrol-2-yl)-1H-benzo[d]imidazole (V-3x)*:** Brown color solid, yield: 88%; mp. 256-258 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 6.22 (s, 1H), 6.95 (s, 1H), 7.00 (s, 1H), 7.60 (d, J = 8.8 Hz, 1H), 8.05 (dd, J_1 = 2.4 Hz, J_2 = 8.8 Hz, 1H), 8.29 (s, 1H), 11.99 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6): δ (ppm) 108.6, 110.3 (2C), 111.6, 112.0, 118.2 (2C), 122.0, 123.5, 142.7, 151.4; HRMS (ESI): *m/z* calcd for $C_{11}H_9N_4O_2$ [M + H]$^+$ 229.0726, found 229.0722.



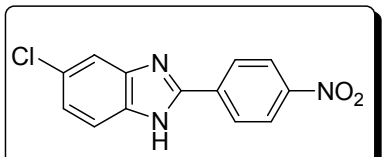
V-3y

***5-Nitro-2-(2-thienyl)-1H-benzo[d]imidazole (V-3y)*:** Light yellow color solid, yield: 86%; mp. 136-137 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 7.24 (d, J = 3.6 Hz, 1H), 7.67 (d, J = 8.2 Hz, 1H), 7.81 (d, J = 4.9 Hz, 1H), 7.90 (d, J = 3.0 Hz, 1H), 8.06 (d, J = 8.8 Hz, 1H), 8.38 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ (ppm) 118.6 (2C), 129.0 (2C), 129.2 (3C), 131.1, 132.8, 143.2, 151.9; HRMS (ESI): *m/z* calcd for $C_{11}H_8N_3O_2S$ $[M + H]^+$ 246.0337, found 246.0339.



V-3z

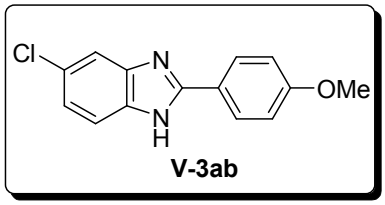
***2-Cyclohexyl-5-nitro-1H-benzo[d]imidazole (V-3z)*:** Yellow color solid, yield: 81%; mp. 142-144 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 1.27-1.44 (m, 3H), 1.51-1.68 (m, 3H), 1.79 (d, J = 11.9 Hz, 2H), 2.02 (d, J = 13.0, 2H), 2.78-2.82 (m, 1H), 7.78 (d, J = 7.8 Hz, 1H), 8.12 (dd, J_1 = 1.8 Hz, J_2 = 7.8 Hz, 1H), 8.41 (d, J = 1.8 Hz, 1H), 9.50 (brs, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6): δ (ppm) 25.4, 25.6 (2C), 31.2 (2C), 37.7, 129.0, 129.8 (2C), 131.1, 132.6, 142.7, 151.5; HRMS (ESI): *m/z* calcd for $C_{13}H_{16}N_3O_2$ $[M + H]^+$ 246.1243, found 246.1246.



V-3aa

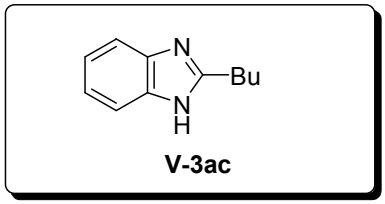
***5-Chloro-2-(4-nitrophenyl)-1H-benzo[d]imidazole (V-3aa)*:** Yellow color solid, yield: 90%; mp. 212-213 °C; ^{1}H NMR (400 MHz, CD_3OD in $CDCl_3$): δ (ppm) 5.61 (s, exchangeable, 1H), 7.22 (d, J = 8.8 Hz, 1H), 7.23-7.29 (m, 1H), 7.34 (d, J = 8.8 Hz,

1H), 7.57 (d, J = 4.8 Hz, 1H), 7.85-7.88 (m, 1H), 8.20 (d, J = 5.6 Hz, 1H), 8.24 (d, J = 5.6 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD in $CDCl_3$): δ (ppm) 115.3, 123.9, 128.1 (2C), 128.5, 130.7, 133.0, 138.8, 134.0 (2C), 147.2, 152.8, 157.1; HRMS (ESI): *m/z* calcd for $C_{13}H_9ClN_3O_2$ $[M + H]^+$ 274.0383, found 274.0381.



V-3ab

***5-Chloro-2-(4-methoxyphenyl)-1H-benzo[d]imidazole (V-3ab)*:** Buff color solid, yield: 93%; mp. 174-175 °C; ^{1}H NMR (400 MHz, CD_3OD in $CDCl_3$): δ (ppm) 3.88 (s, 3 H), 7.02 (s, 1H), 7.03 (dd, J_1 = 1.9 Hz, J_2 = 8.4 Hz, 1H), 7.20 (dd, J_1 = 1.9 Hz, J_2= 8.4 Hz, 1H), 7.50 (dd, J_1 = 2.8 Hz, J_2= 8.5 Hz, 1H), 7.56 (d, J = 1.9 Hz, 1H), 8.00 (dd, J_1 = 1.9 Hz, J_2= 7.8 Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD in $CDCl_3$): δ (ppm) 59.1, 117.7, 118.3 (3C), 119.1, 125.8, 126.7 (2C), 131.8, 132.2 (2C), 157.3, 165.3; HRMS (ESI): *m/z* calcd for $C_{14}H_{12}ClN_2O$ $[M + H]^+$ 259.0638, found 259.0639.



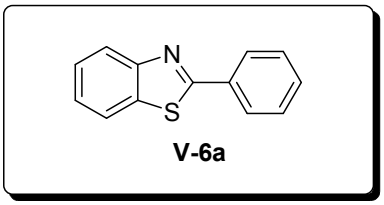
V-3ac

***2-Butyl-1H-benzo[d]imidazole (V-3ac)*:**[16f] Pale yellow solid, yield: 12%; mp. 149-150 °C (Lit. 150 °C); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.01 (t, J = 7.2 Hz, 3H), 1.40 (sext, J = 7.2 Hz, 2H), 1.84 (quin, J = 7.2 Hz, 2H), 2.94 (t, J = 7.2 Hz, 2H), 7.20-7.24 (m, 2H), 7.54-7.58 (m, 2H), 9.1 (s, 1H). [This particular compound was characterized by ^{1}H NMR & mp. only and matched with the reported values.]

V.4.3. General experimental procedure for the synthesis of 2-substituted benzothiazoles (V-6):

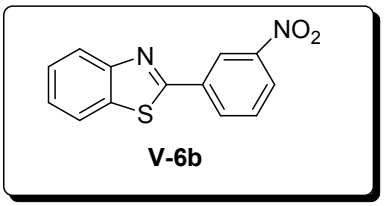
To a solution of DBSA (0.05 mmol) in H_2O (2 mL) were added *o*-aminothiophenol (0.5 mmol) and iodine (0.05 mmol). An aldehyde (0.5 mmol) was added in fractions at room temperature. The reaction was stirred at room temperature for required time (see Table V-4).

The progress of the reaction was monitored by TLC. The completion of the reaction was indicated by separation of the organic phase from aqueous media. The organic part was taken in ethyl acetate after separation of aqueous layer through decantation. Then ethyl acetate part is washed with saturated $NaHCO_3$, water, brine and then dried over anhydrous Na_2SO_4. The organic layer was concentrated *in-vacuo* and the product was purified by silica gel chromatography using ethyl acetate and petroleum ether mixture as an eluent.



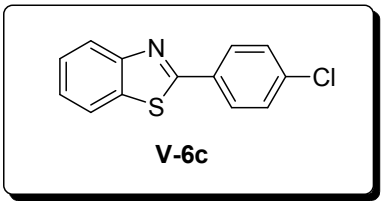
V-6a

***2-Phenylbenzo[d]thiazole (V-6a)*:**[22] Colorless solid, yield: 83%; mp. 113-114 °C (Lit. 111-115 °C); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.42 (t, J = 7.2 Hz, 1H), 7.52-7.57 (m, 4H), 7.94 (d, J = 7.8 Hz, 1H), 8.08-8.12 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 121.5, 123.1, 125.0, 126.3, 127.5 (2C), 129.0 (2C), 130.8, 133.5, 134.9, 154.1, 167.8; ESI-MS: (*m/z*) 212 $[M + H]^+$; *Anal.* Cald. for $C_{13}H_9NS$: C, 73.90; H, 4.29; N, 6.63. Found: C, 74.11; H, 4.28; N, 6.65.



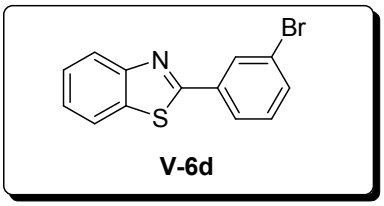
V-6b

***2-(3-Nitrophenyl)benzo[d]thiazole (V-6b)*:**[22] Colorless solid, yield: 82%; mp. 182-183 °C (Lit. 181-183 °C); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.44 (t, J = 7.5 Hz, 1H), 7.52 (t, J = 7.5 Hz, 1H), 7.65 (t, J = 7.5 Hz, 1H), 7.92 (d, J = 8.0 Hz, 1H), 8.10 (d, J = 8.0 Hz, 1H), 8.30 (d, J = 7.6 Hz, 1H), 8.39 (d, J = 7.6 Hz, 1H), 8.90 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 121.8, 122.3, 123.7, 125.1, 126.0, 126.8, 130.0, 132.9, 135.2, 135.3, 148.7, 153.9, 164.8; ESI-MS: (*m/z*) 257 $[M + H]^+$; *Anal.* Cald. for $C_{13}H_8N_2O_5S$: C, 60.93; H, 3.15; N, 10.93. Found: C, 60.83; H, 3.16; N, 10.94.



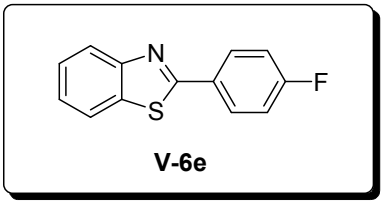
V-6c

***2(4-Chloropnenyl)benzo[d]thiazole (V-6c)*:**[22] Yellow solid, yield: 79%; mp. 114-115 °C (Lit. 114-116 °C); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.38-7.54 (m, 4H), 7.90 (d, J = 8.0 Hz, 1H), 8.01-8.10 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 121.7, 123.3, 125.4, 126.5, 128.7 (2C), 129.2 (2C), 132.1, 135.1, 137.0, 154.1, 166.6; ESI-MS: (*m/z*) 246 $[M + H]^+$ (major peak, for ^{35}Cl), 248 $[M + H]^+$ (minor peak, for ^{37}Cl); *Anal.* Cald. for $C_{13}H_8ClNS$: C, 63.54; H, 3.28; N, 5.70. Found: C, 63.73; H, 3.27; N, 5.71.



V-6d

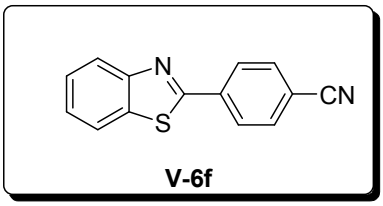
***2-(3-Bromophenyl) benzo[d]thiazole (V-6d)*:**[11g] Wine red solid, yield: 82%; mp. 84-85 °C (Lit. 83-84°C); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.33-7.54 (m, 3H), 7.60 (d, J = 8.0 Hz, 1H), 7.91 (d, J = 7.8, 1H), 7.98 (d, J = 7.8, 1H), 8.09 (d, J = 7.8 Hz, 1H), 8.29 (d, J = 1.5 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 121.7, 123.2, 123.5, 125.5, 126.1, 126.5, 130.3, 130.4, 133.7, 135.1, 135.5, 154.0, 166.1; ESI-MS: (*m/z*) 290 $[M + H]^+$ (for ^{79}Br), 292 $[M + H]^+$ (for ^{81}Br); *Anal.* Cald. for $C_{13}H_8BrNS$: C, 53.81; H, 2.78; N, 4.83. Found: C, 53.93; H, 2.77; N, 4.82.



V-6e

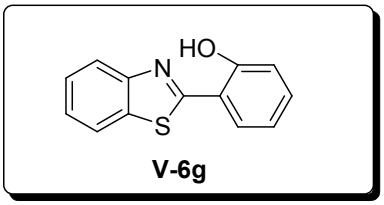
***2-(4-Fluorophenyl)benzo[d]thiazole (V-6e)*:**[22] White solid, yield: 84%; mp. 96-97 °C (Lit. 97-98 °C); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.19 (t, J = 7.6 Hz, 2H), 7.40 (t, J = 7.6 Hz, 1H), 7.51 (t, J = 7.6 Hz, 1H), 7.91 (d, J = 7.8 Hz, 1H), 8.07-8.11 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 116.1 (d, J = 22.0 Hz, 2C), 121.6, 123.2, 125.2, 126.4, 129.5 (d, J =

8.5 Hz, 2C)), 130.0 (d, *J* = 3.1 Hz), 135.1, 154.2, 164.5 (d, *J* = 250.3 Hz), 166.7; ESI-MS: (*m/z*) 230 $[M + H]^+$; *Anal.* Cald. for $C_{13}H_8FNS$: C, 68.10; H, 3.52; N, 6.11. Found: C, 68.02; H, 3.53; N, 6.12.



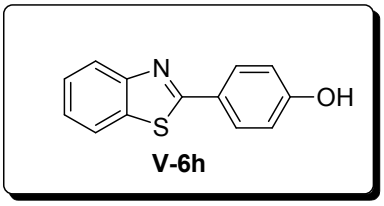
V-6f

***4-(Benzo[d]thiazol-2-yl)benzonitrile (V-6f)*:**[23] Greenish yellow solid, yield: 81%; mp. 172-173 °C (Lit. 171-173 °C); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.44 (t, *J* = 6.9 Hz, 1H), 7.54 (t, *J* = 6.9 Hz, 1H), 7.75 (d, *J* = 7.8 Hz, 2H), 7.93 (d, *J* = 7.6 Hz, 1H), 8.10 (d, *J* = 7.6 Hz, 1H), 8.17 (d, *J* = 7.5 Hz, 2H).); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 114.1, 118.2, 121.8, 123.8, 126.1, 126.8, 127.9 (2C), 132.7 (2C), 135.3, 137.4, 154.0, 165.3; ESI-MS: (*m/z*) 237 $[M + H]^+$; *Anal.* Cald. for $C_{14}H_8N_2S$: C, 71.16; H, 3.41; N, 11.86. Found: C, 71.33; H, 3.42; N, 11.88.



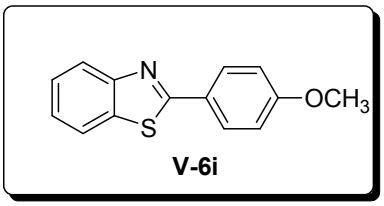
V-6g

***(2-Hydroxypnenyl)benzo[d]thiazole (V-6g)*:**[12b] Yellowish white color solid, yield: 80%; mp. 129-130 °C (Lit. 130-131 °C); ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 7.03-7.15 (m, 2H), 7.40-63 (m, 3H), 8.09-8.27 (m, 3H), 11.60 (s, 1H, exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) 116.3, 117.4, 119.0, 121.1, 121.8, 125.2, 126.2, 127.9, 132.1, 132.3, 151.3, 157.5, 168.9; ESI-MS: (*m/z*) 228 $[M + H]^+$; *Anal.* Cald. for $C_{13}H_9NOS$: C, 68.70; H, 3.99; N, 6.16. Found: C, 68.44; H, 3.99; N, 6.17.



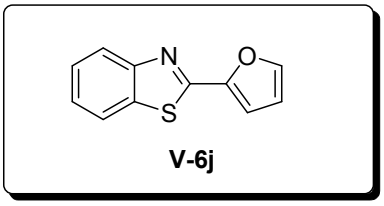
V-6h

***4-(Benzo[d]thiazol-2-yl)phenol (V-6h)*:**[24] White solid, yield: 79%; mp. 222-223 °C (Lit. 223-224 °C); ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 6.94 (d, J = 8.4 Hz, 2H), 7.37 (t, J = 7.5 Hz, 1H), 7.48 (t, J = 7.5 Hz, 1H), 7.91-8.05 (m, 4H), 10.23 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) 116.5 (2C), 122.5, 122.7, 124.5, 125.3, 126.8, 129.5 (2C), 134.6, 154.2, 161.0, 167.9; ESI-MS: (*m/z*) 228 $[M + H]^+$; *Anal.* Cald. for $C_{13}H_9NOS$: C, 68.70; H, 3.99; N, 6.16. Found: C, 68.91; H, 3.98; N, 6.17.



V-6i

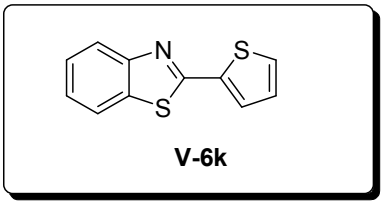
***2-(4-Methoxyphenyl)benzo[d]thiazole (V-6i)*:**[22] Yellow color solid, yield: 78%; mp. 122-123 °C (Lit. 122-124 °C); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 3.87 (s, 3H), 6.98 (d, J = 7.8 Hz, 2H), 7.36 (t, J = 7.6 Hz, 1H), 7.47 (t, J = 8.0 Hz, 1H), 7.88 (d, J = 7.8 Hz, 1H), 8.03 (d, J = 8.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 55.4, 114.3 (2C), 121.5, 122.8, 124.8, 126.2, 126.5, 129.1 (2C), 134.9, 154.3, 161.9, 167.8; ESI-MS: (*m/z*) 242 $[M + H]^+$; *Anal.* Cald. for $C_{14}H_{11}NOS$: C, 69.68; H, 4.59; N, 5.80. Found: C, 69.82; H, 4.58; N, 5.81.



V-6j

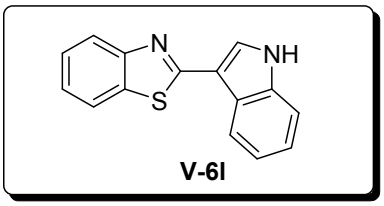
***2-(Furan-2-yl)benzo[d]thiazole (V-6j)*:**[11d] Yellow color solid, yield: 82%; mp. 101-102 °C (Lit. 100-102 °C); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 6.58 (s like, 1H), 7.19 (d, J = 3.3 Hz, 1H), 7.36 (t, J = 7.2 Hz, 1H), 7.48 (t, J = 7.2 Hz, 1H), 7.59 (s, 1H), 7.87 (d, J = 7.8 Hz, 1H), 8.05 (d, J = 7.8 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 111.4, 112.5, 121.5,

123.1, 125.2, 126.4, 134.3, 144.7, 148.8, 153.8, 157.5; ESI-MS: (*m/z*) 202 $[M + H]^+$; *Anal.* Cald. for $C_{11}H_7NOS$: C, 65.65; H, 3.51; N, 6.96. Found: C, 65.49; H, 3.52; N, 6.97.



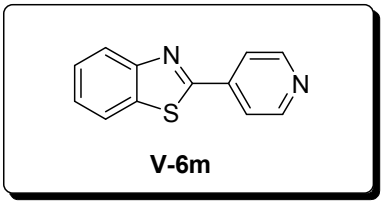
V-6k

2-(Thiophen-2-yl)benzo[d]thiazole (V-6k):[22] Colorless solid, yield: 85%; mp. 99-100 °C (Lit. 98-100 °C); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.14 (s like, 1H), 7.38 (t, J = 7.2 Hz, 1H), 7.46-7.51 (m, 2H), 7.66 (d, J = 3.2 Hz), 7.84 (d, J = 7.5 Hz, 1H), 8.04 (d, J = 8.1 Hz, 1H), ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 121.4, 123.0, 125.2, 126.4, 128.0, 128.6, 129.3, 134.7, 137.4, 153.7, 161.4; ESI-MS: (*m/z*) 218 $[M + H]^+$; *Anal.* Cald. for $C_{11}H_7NS_2$: C, 60.80; H, 3.25; N, 6.45. Found: C, 61.00; H, 3.26; N, 6.43.



V-6l

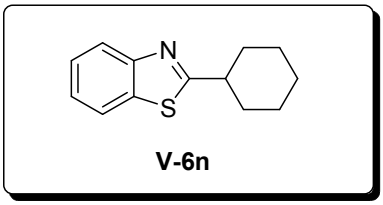
2-(1H-Indol-3-yl)benzo[d]thiazole (V-6l):[23] Dirty white solid, yield: 87%; mp. 170-171 °C (Lit. 171-172 °C); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.34-7.51 (m, 5H), 7.90-7.95 (m, 2H), 8.06 (d, J = 7.5 Hz, 1H), 8.47 (d, J = 7.2 Hz, 1H), 8.96 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 111.7, 112.5, 121.1, 121.3, 121.8, 122.2, 123.5, 124.2, 125.0, 126.1, 126.3, 133.9, 136.5, 153.8, 163.0; ESI-MS: (*m/z*) 251 $[M + H]^+$; *Anal.* Cald. for $C_{15}H_{10}N_2S$: C, 71.97; H, 4.03; N, 11.19. Found: C, 71.70; H, 4.04; N, 11.20.



V-6m

2-(Pyridine-4-yl)benzo[d]thiazole (V-6m):[22] Pale yellow solid, yield: 88%; mp. 133-134 °C (Lit. 133-135 °C); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.43 (t, J = 7.5 Hz, 1H), 7.52 (t, J = 7.5 Hz, 1H), 7.90-7.92 (m, 3H), 8.11 (d, J = 8.1 Hz, 1H), 8.74-8.76 (m, 2H); ^{13}C NMR (75

MHz, $CDCl_3$): δ (ppm) 121.1 (2C), 121.8, 123.9, 126.2, 126.8, 135.2, 140.4, 150.7 (2C), 154.0, 165.0; ESI-MS: (*m/z*) 213 $[M + H]^+$; *Anal.* Cald. for $C_{12}H_8N_2S$: C, 67.90; H, 3.80; N, 13.20. Found: C, 67.73; H, 3.80; N, 13.22.



V-6n

***2-Cyclohexylbenzo[d]thiazole (V-6n)*:**[25] Colorless liquid, yield: 40%; ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.30-1.50 (m, 3H), 1.61-1.89 (m, 4H), 1.92-1.94 (m, 2H), 2.20-2.25 (m, 2H), 3.12-3.16 (m, 1H), 7.34 (t, J = 7.5 Hz, 1H), 7.46 (t, J = 7.5 Hz, 1H), 7.86 (d, J = 7.8 Hz, 1H), 7.99 (d, J = 7.8 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 25.7, 26.1 (2C), 33.4 (2C), 43.5, 121.5, 122.6, 124.5, 125.8, 134.6, 153.1, 154.0; ESI-MS: (*m/z*) 218 $[M + H]^+$; *Anal.* Cald. for $C_{13}H_{15}NS$: C, 71.84; H, 6.96; N, 6.44. Found: C, 71.74; H, 6.95; N, 6.45.

V.4. References

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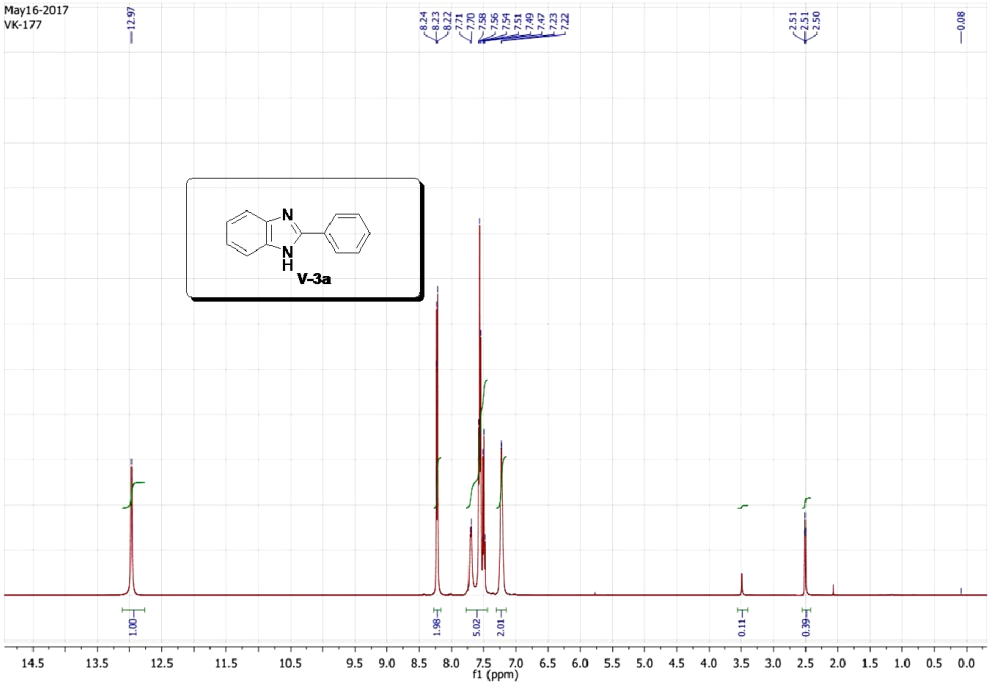
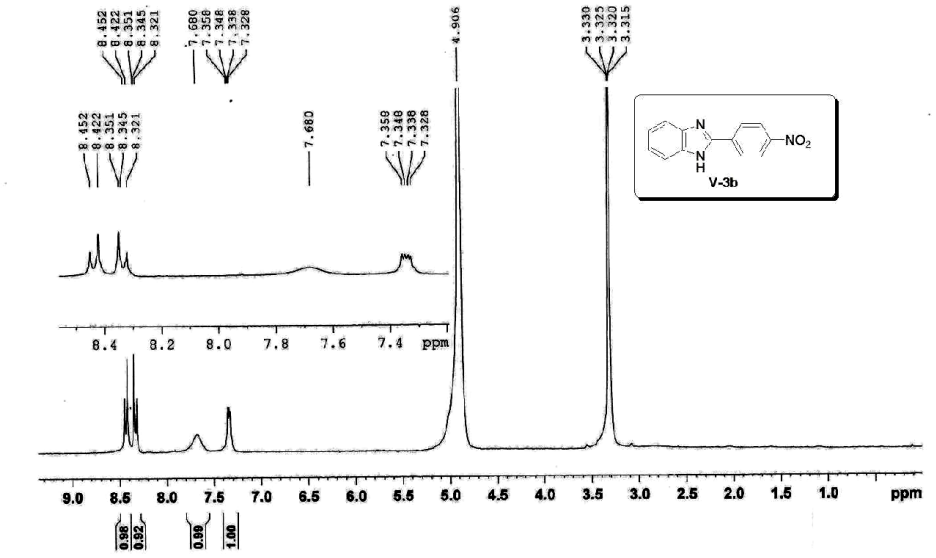
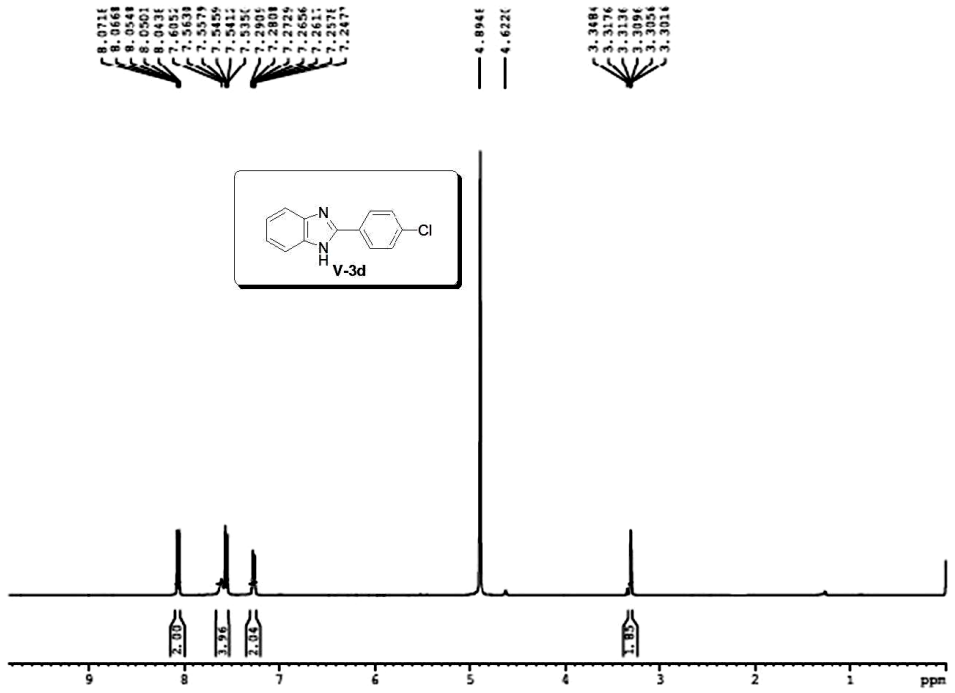
V.5 . Supporting Information (selected spectra)



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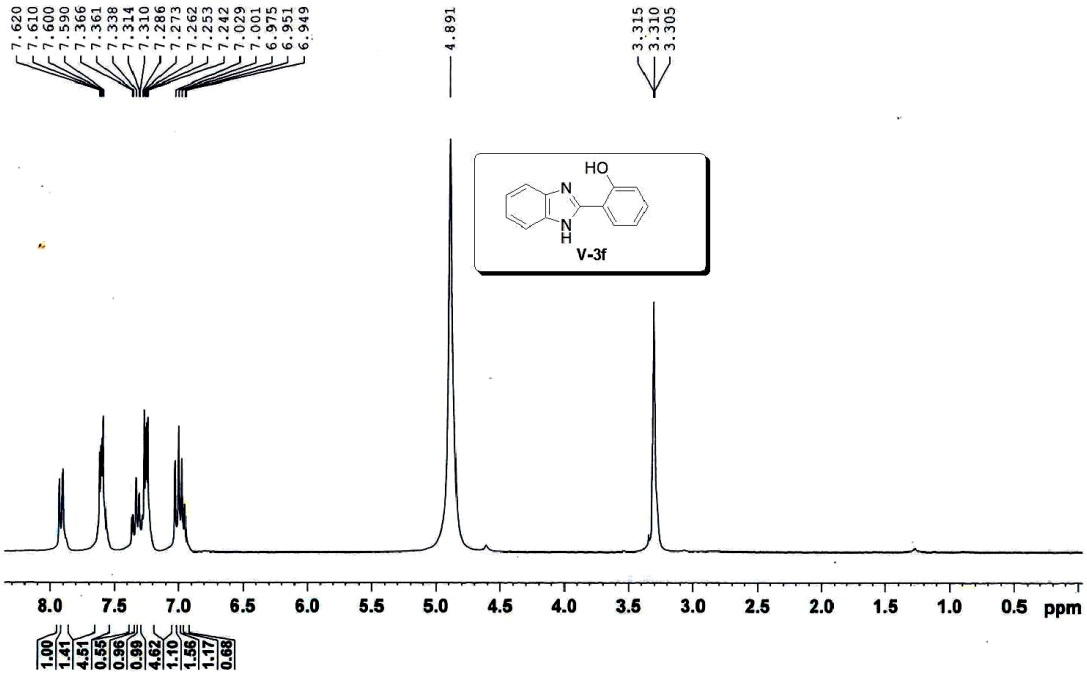
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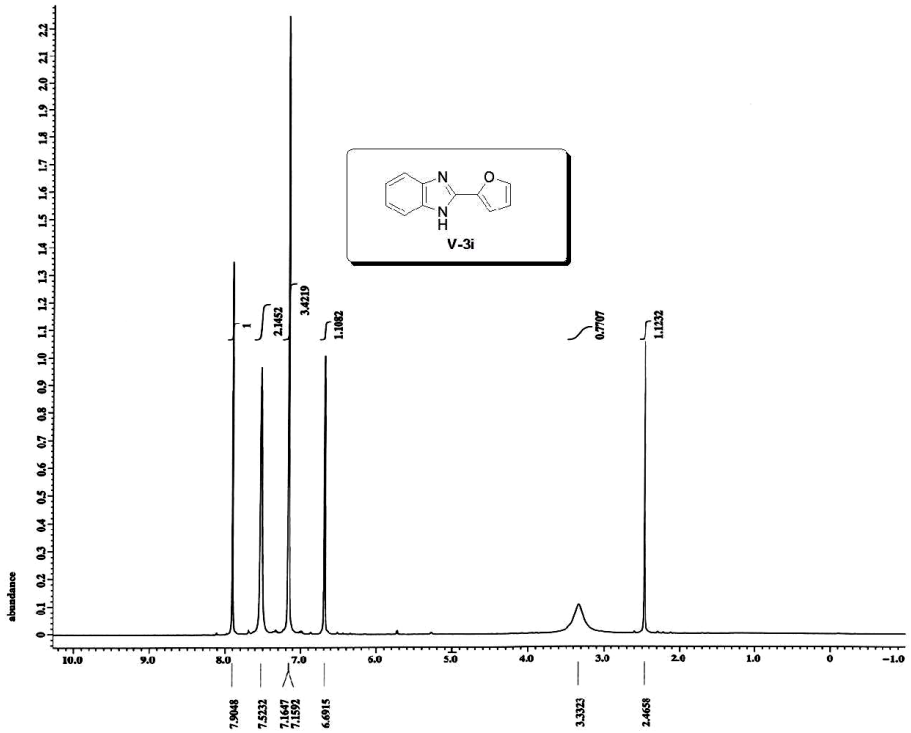
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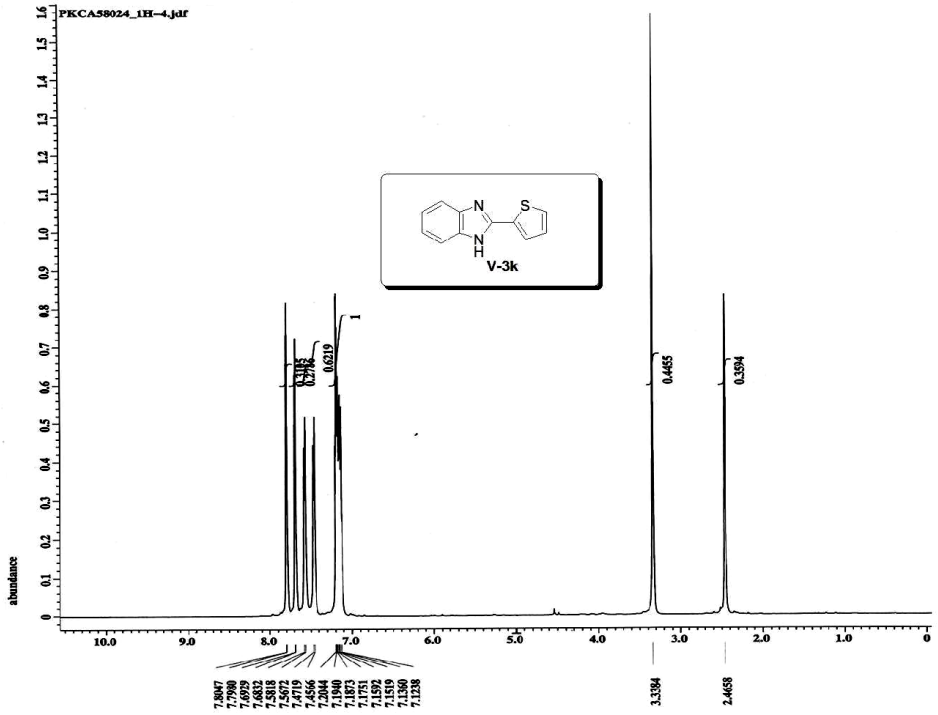
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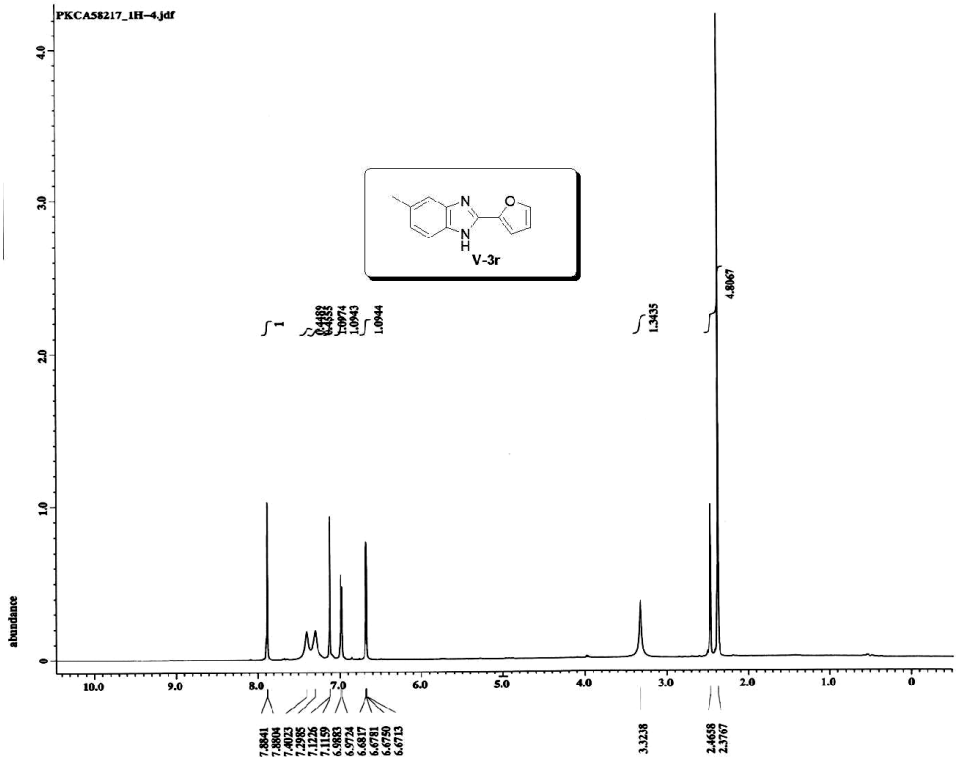
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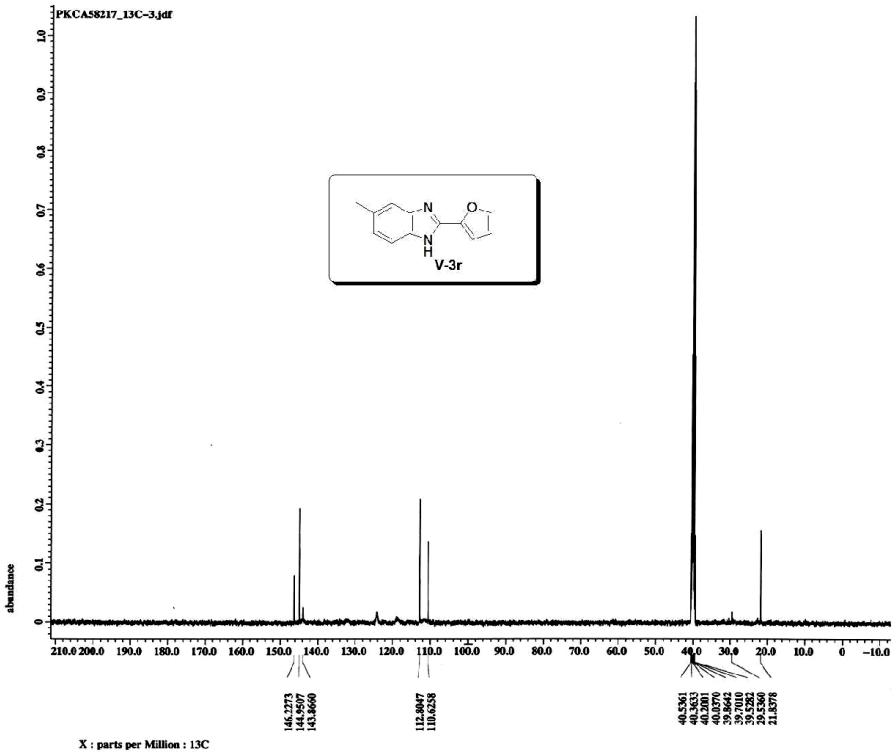
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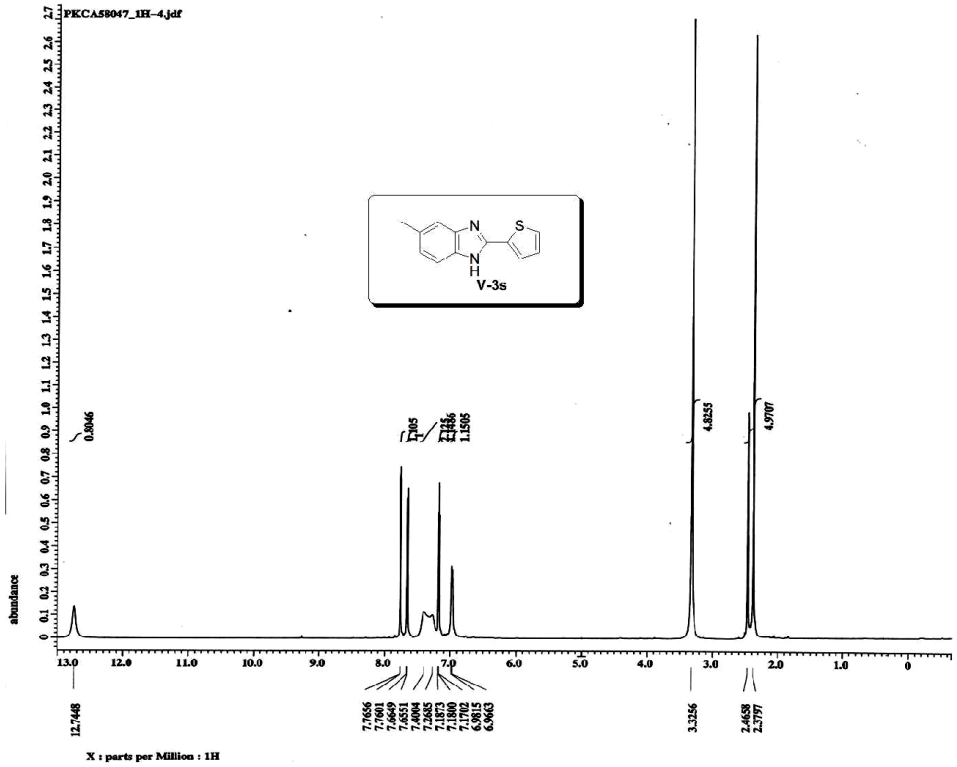
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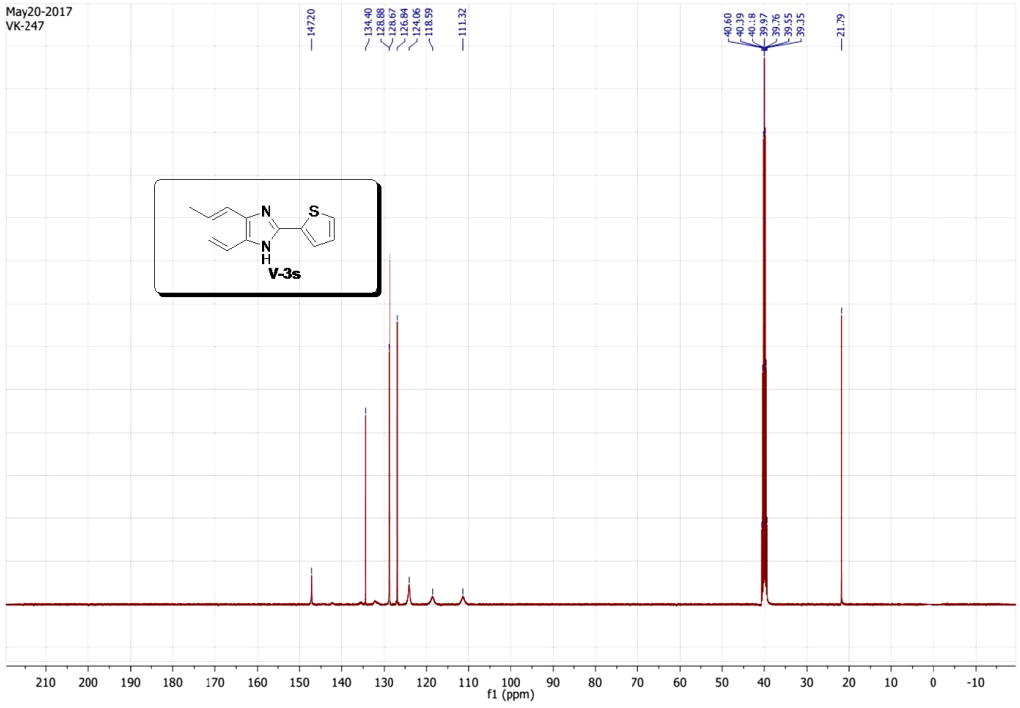
May20-2017
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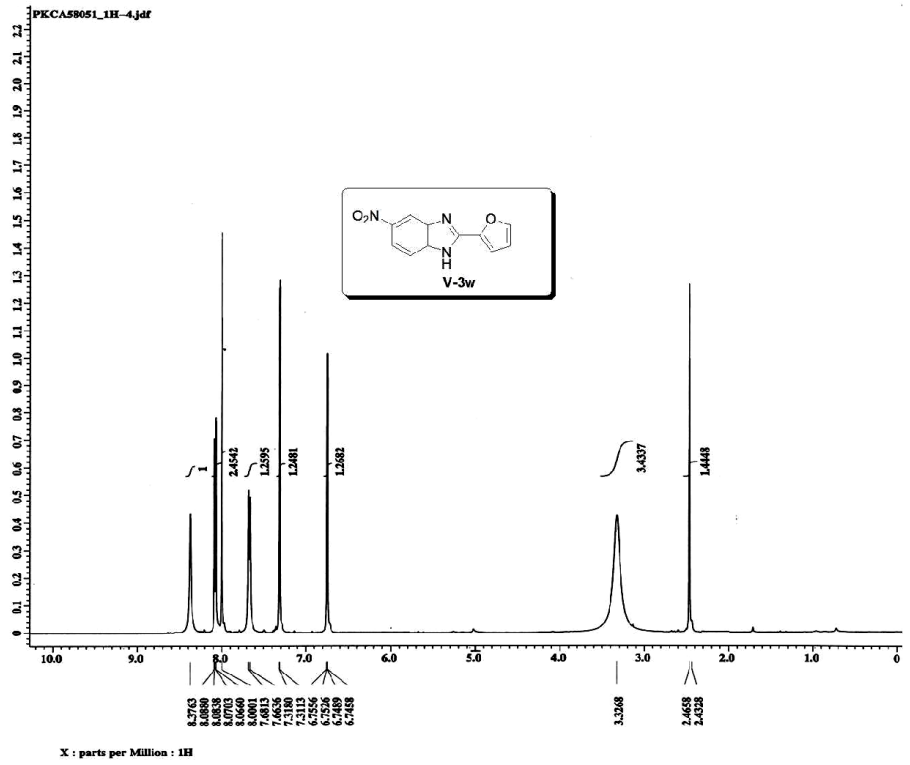
f1 (ppm)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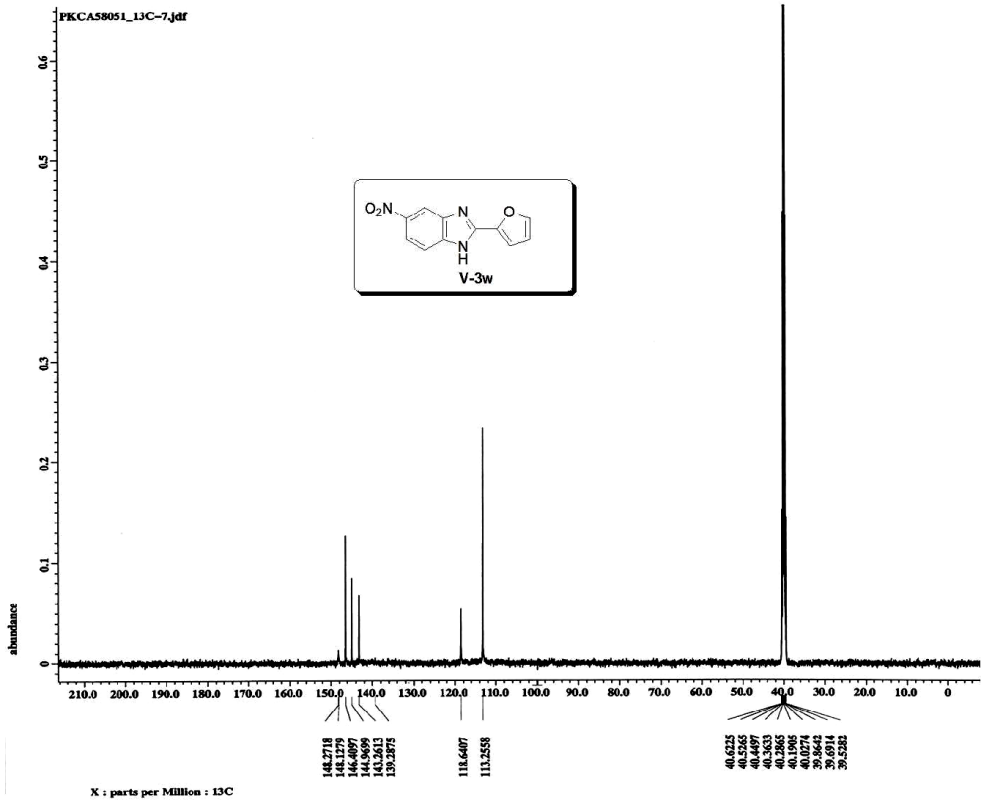
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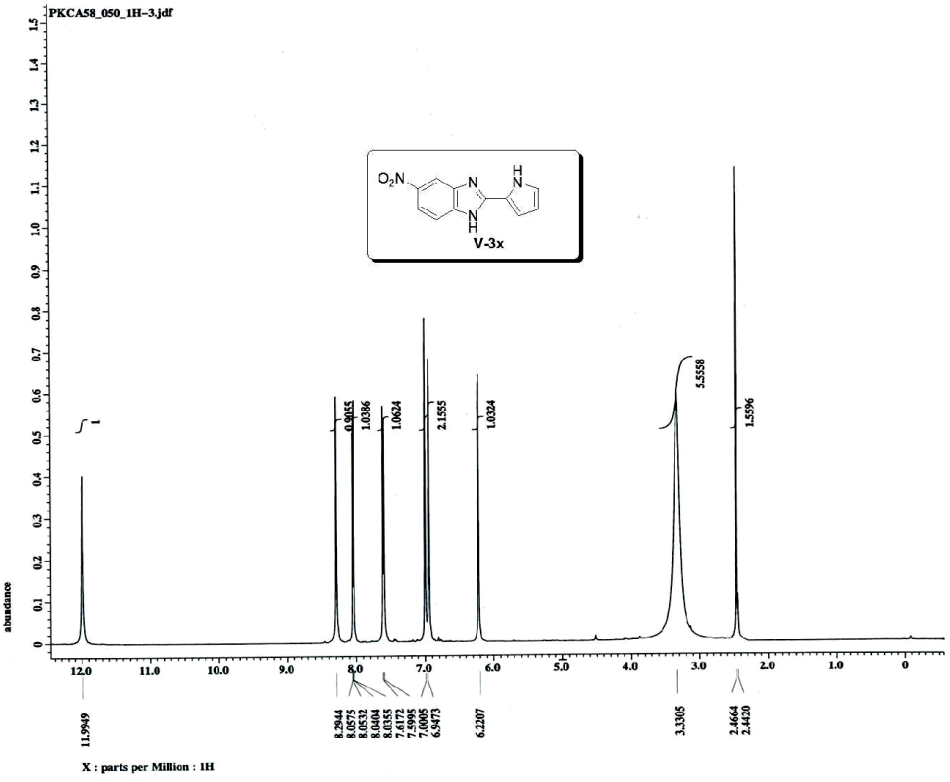
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V-3x
abundance
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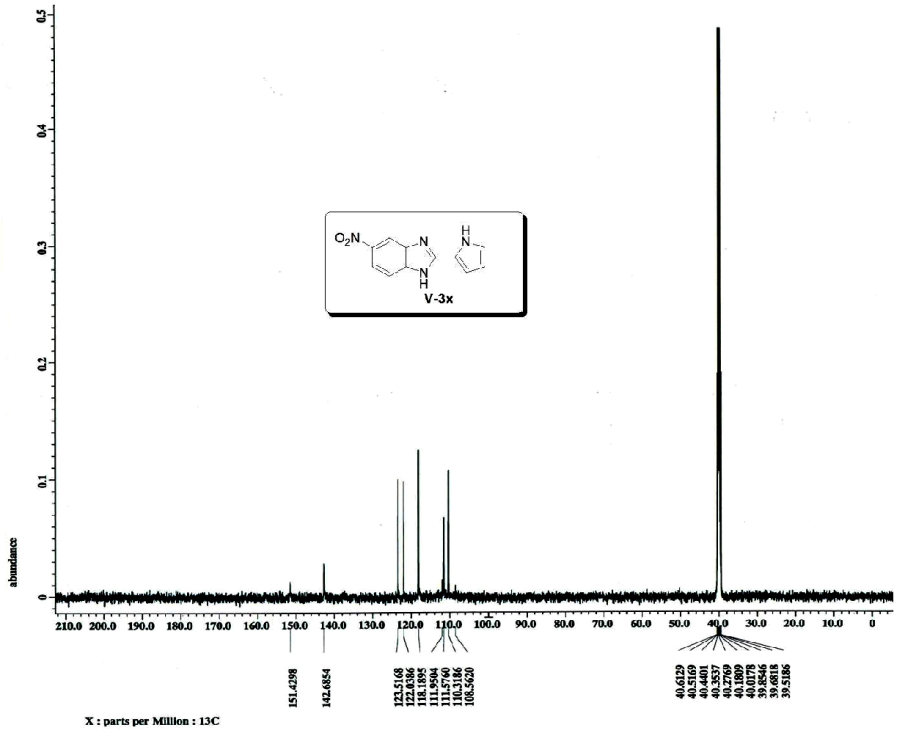
1.0624
2.1555
1.0324
5.5558
1.5596
11.9949
8.2044
8.0575
8.0532
8.0404
8.0355
7.6172
7.5995
7.0005
6.9473
6.2207
3.3305
2.4664
2.4420
X : parts per Million : 1H


V-3x
abundance
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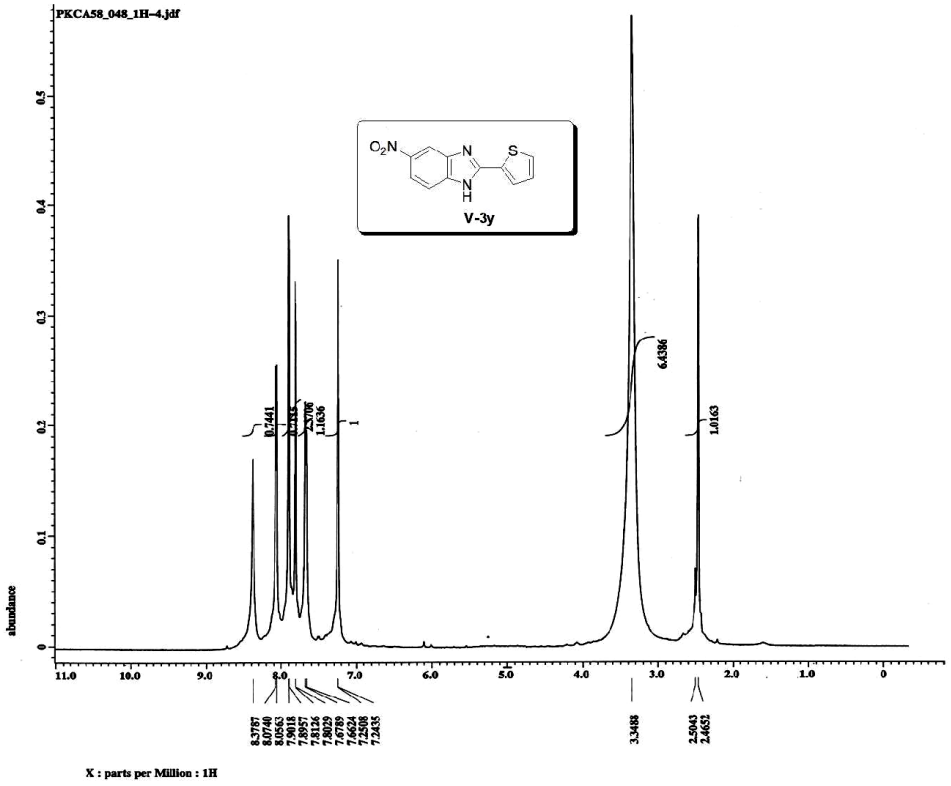
108.5620
40.6129
40.5169
40.4401
40.3537
40.2709
40.1809
40.0178
39.8546
39.6818
39.5186
X : parts per Million : 13C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V-3y
abundance
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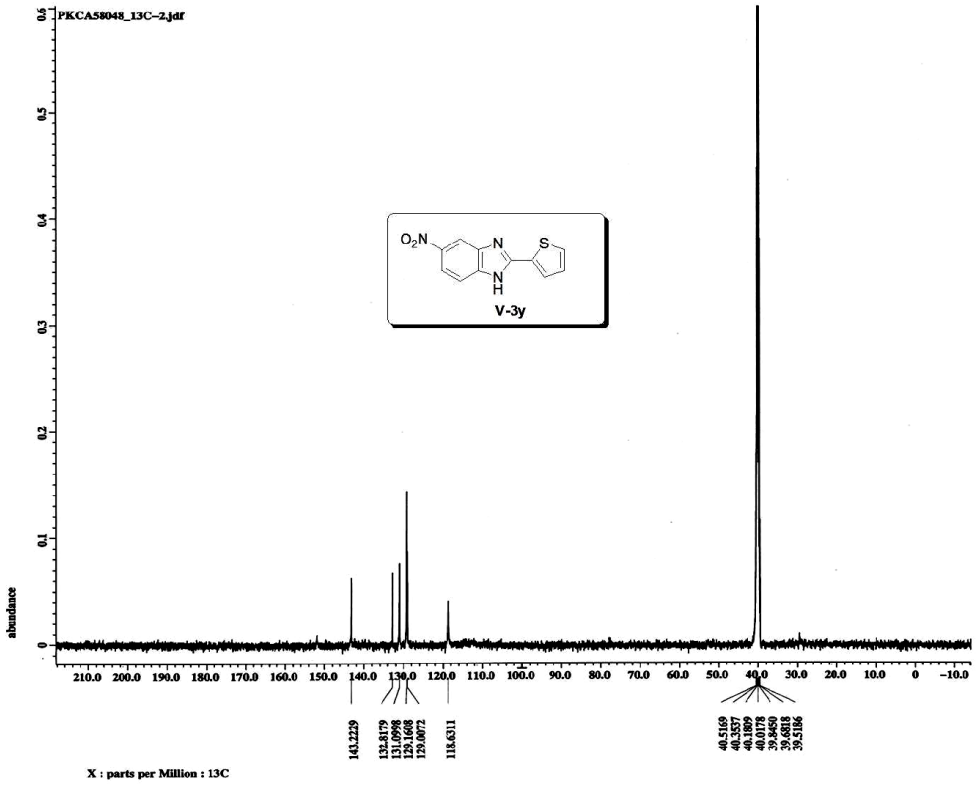
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V-3y
abundance
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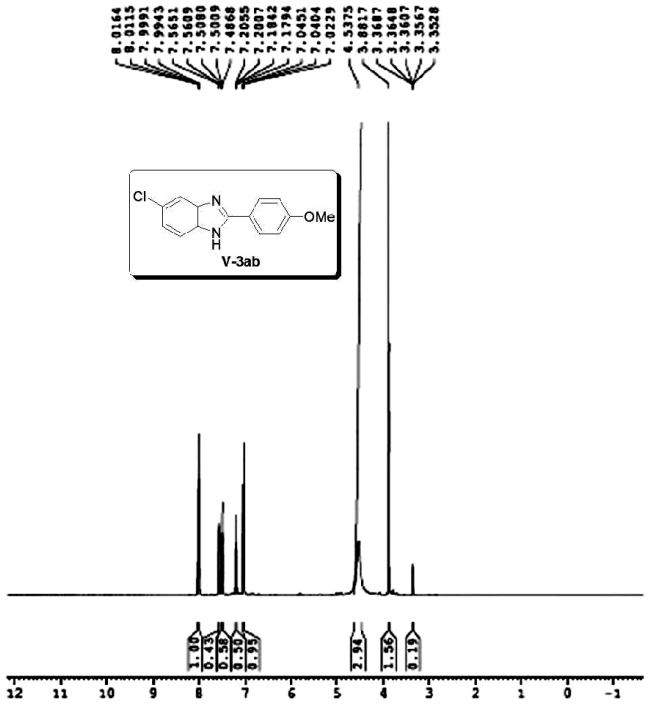
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7.9991
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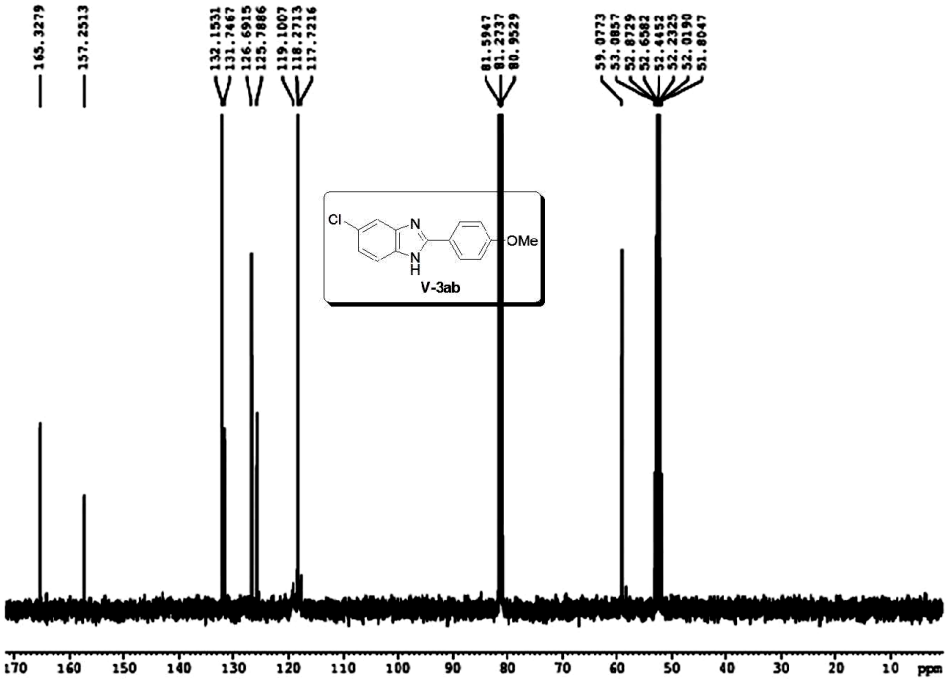
7.5651
7.5609
7.5080
7.5009
7.4868
7.2055
7.2007
7.1842
7.1794
7.0451
7.0404
7.0229
4.5375
3.8817
3.1647
3.1468
3.1457
3.1357
3.3528
Cl
N
N
H
OMe
V-3ab
1.00
0.45
0.58
0.50
0.53
2.94
1.56
0.19
12
11
10
9
8
7
6
5
4
3
2
1
0
-1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126.6915
125.7886
119.1007
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117.7216
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81.2737
80.9529
59.0773
53.0857
52.8729
52.6582
52.4152
52.2325
52.0190
51.8047
Cl
N
N
H
OMe
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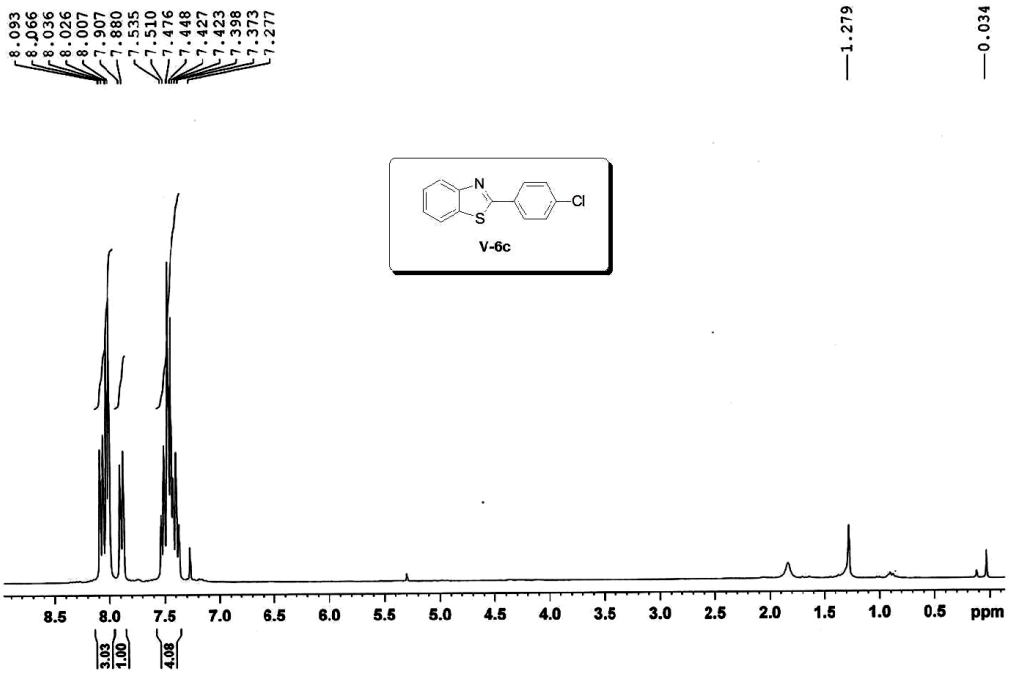
90
80
70
60
50
40
30
20
10
ppm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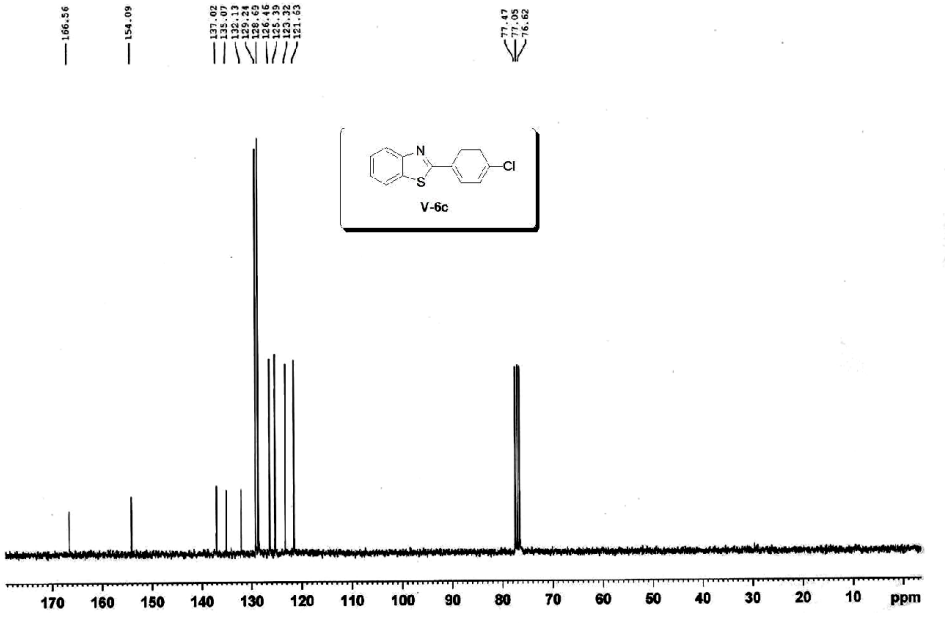
7.880
7.535
7.510
7.476
7.448
7.427
7.423
7.398
7.373
7.277
1.279
0.034
V-6c
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
3.03
1.00
4.08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129.24
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126.46
125.39
123.32
121.58
77.47
77.05
76.62
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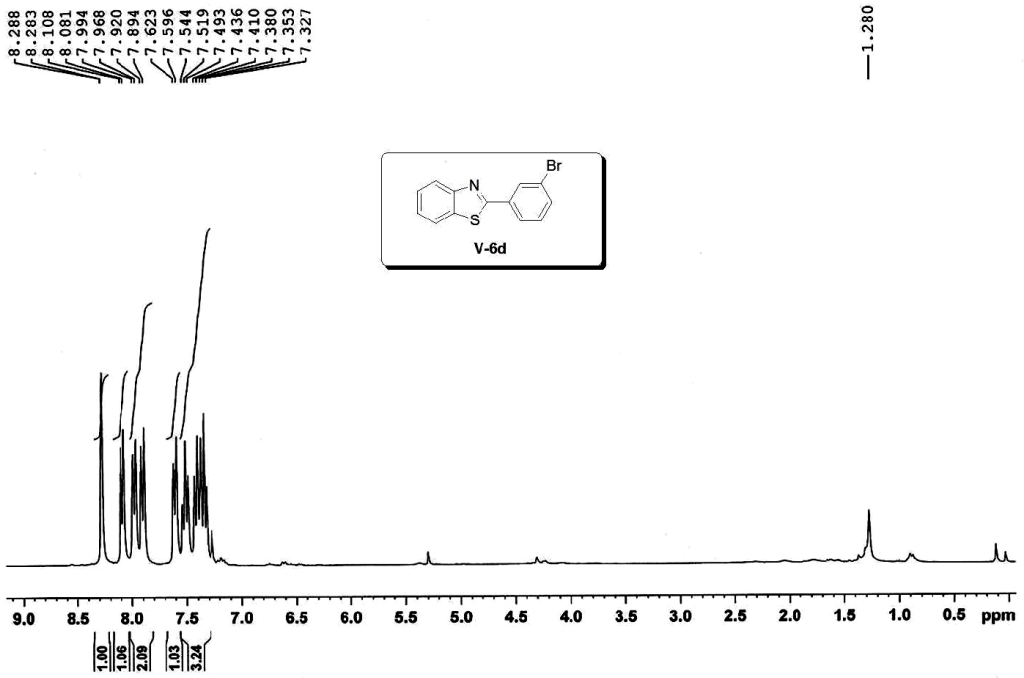
90
80
70
60
50
40
30
20
10
ppm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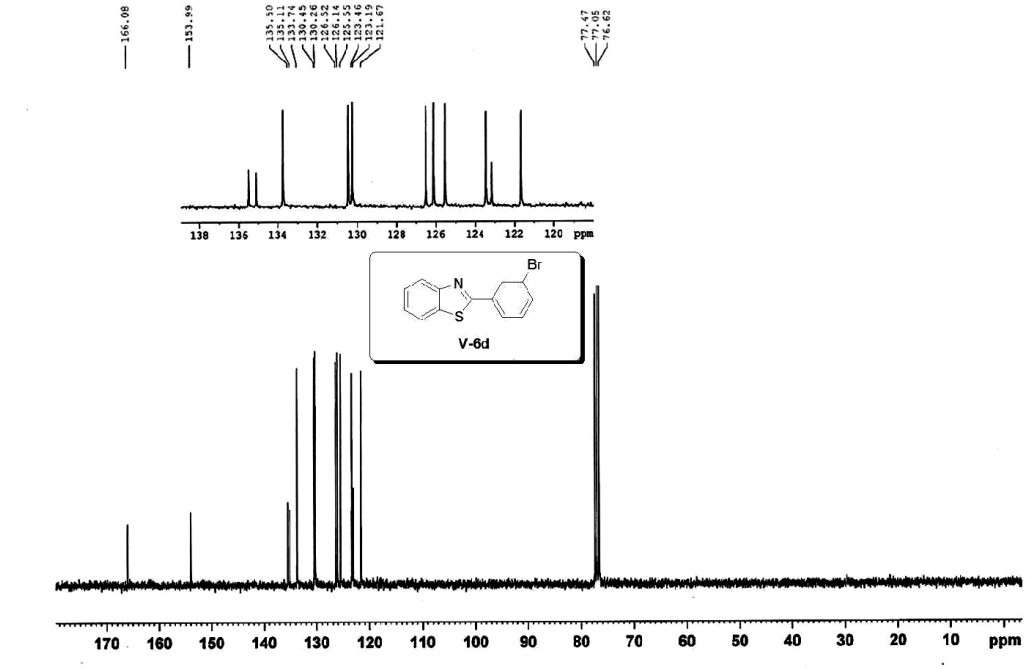
7.544
7.519
7.493
7.436
7.410
7.380
7.353
7.327
1.280
Br
N
S
V-6d
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
1.00
1.06
2.09
1.03
3.24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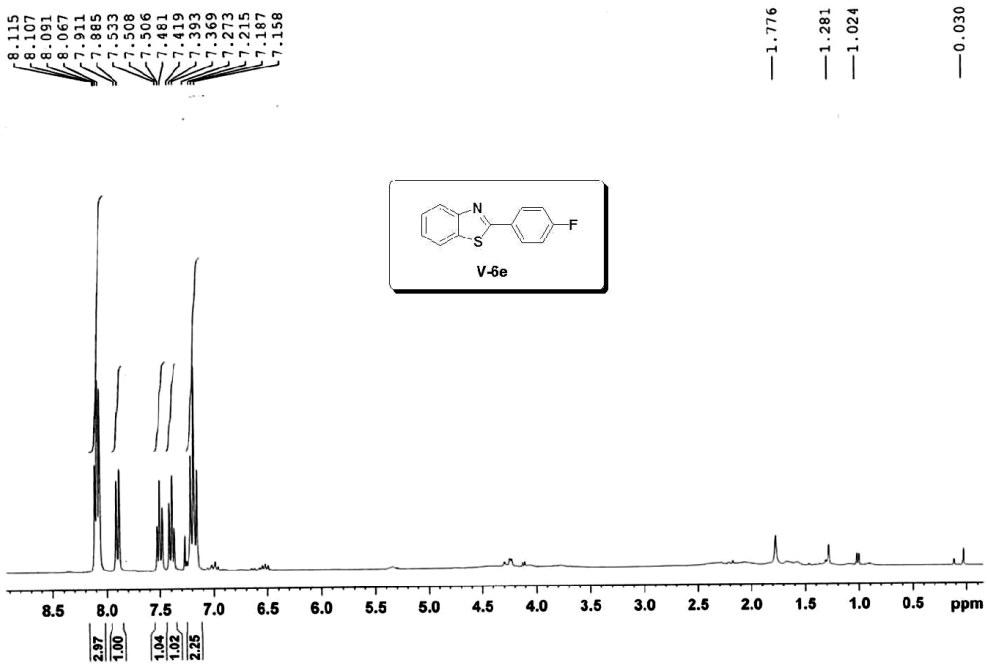
135.10
135.11
133.74
130.45
130.26
126.52
126.14
125.55
123.46
123.19
121.67
77.47
77.05
76.62
138 136 134 132 130 128 126 124 122 120 ppm
Br
N
S
V-6d
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm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7.508
7.506
7.481
7.419
7.393
7.369
7.273
7.215
7.187
7.158
1.776
1.281
1.024
0.030
V-6e
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
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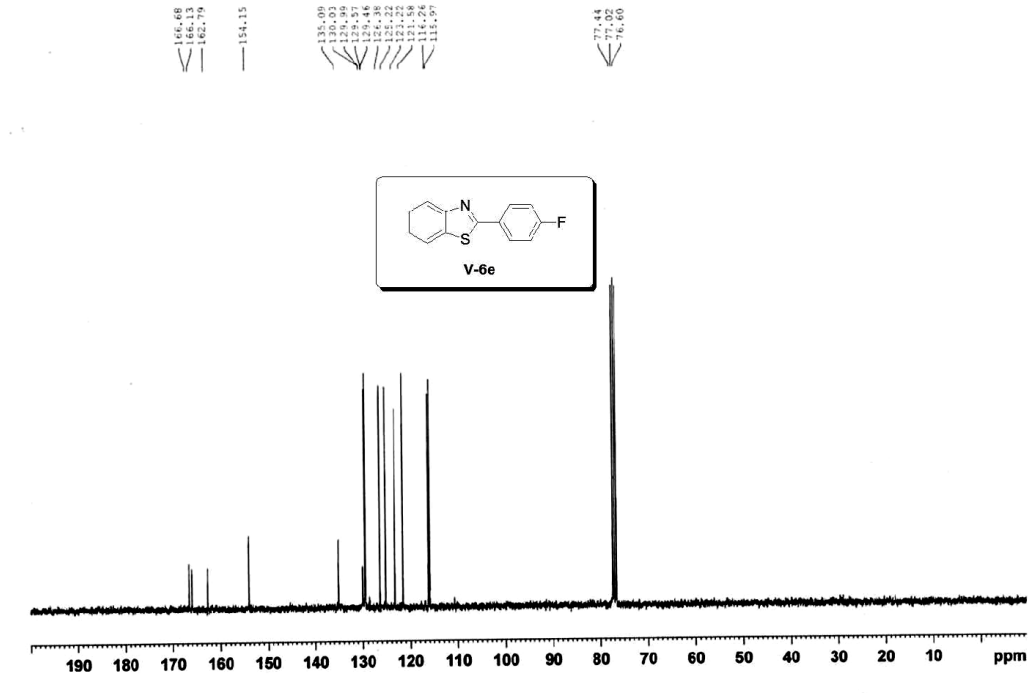
1.00
1.04
1.02
2.25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135.09
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129.57
129.46
129.36
125.22
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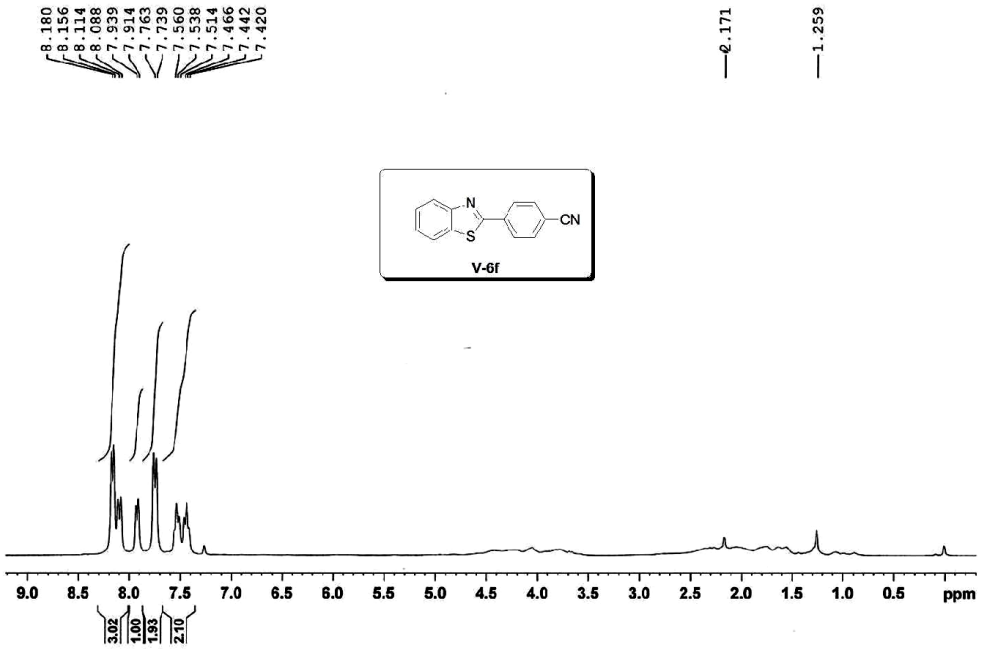
121.58
116.26
115.97
77.44
77.02
76.60
V-6e
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm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7.739
7.560
7.538
7.514
7.466
7.442
7.420
2.171
1.259
V-6f
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
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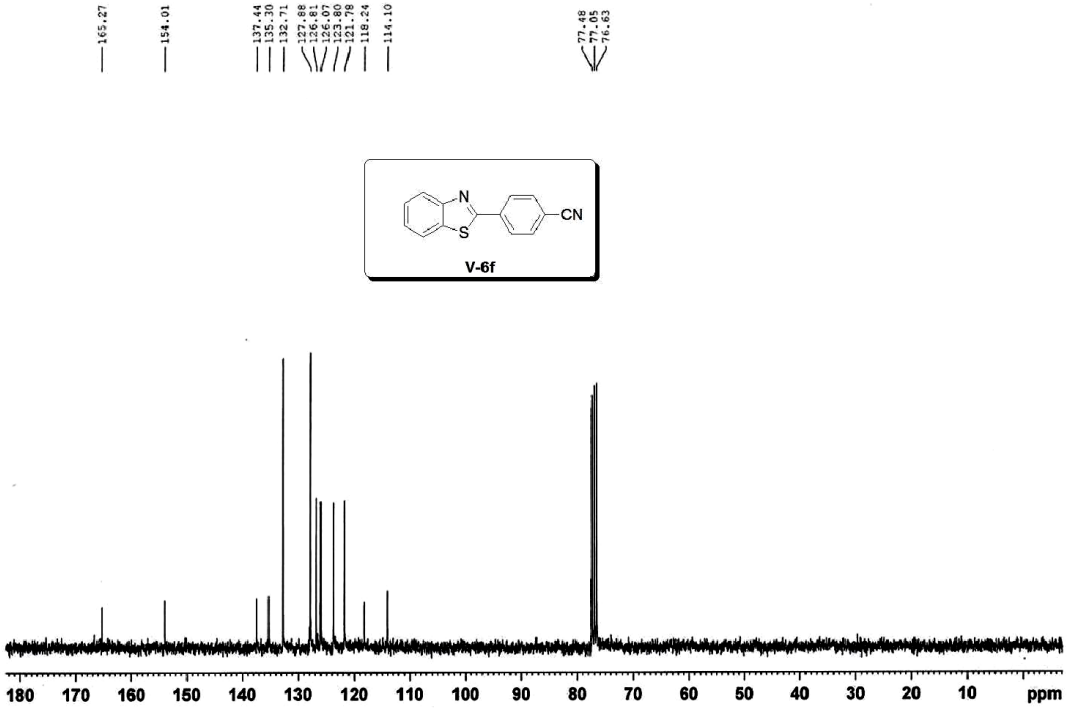
1.00
1.93
2.10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126.07
123.80
121.78
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114.10
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77.05
76.63
V-6f
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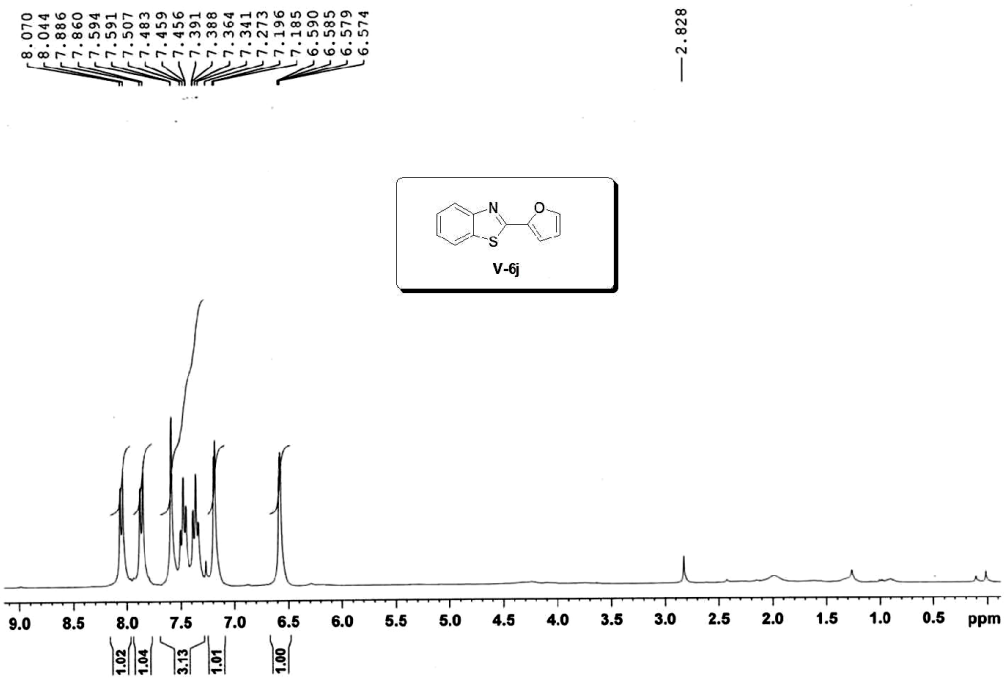
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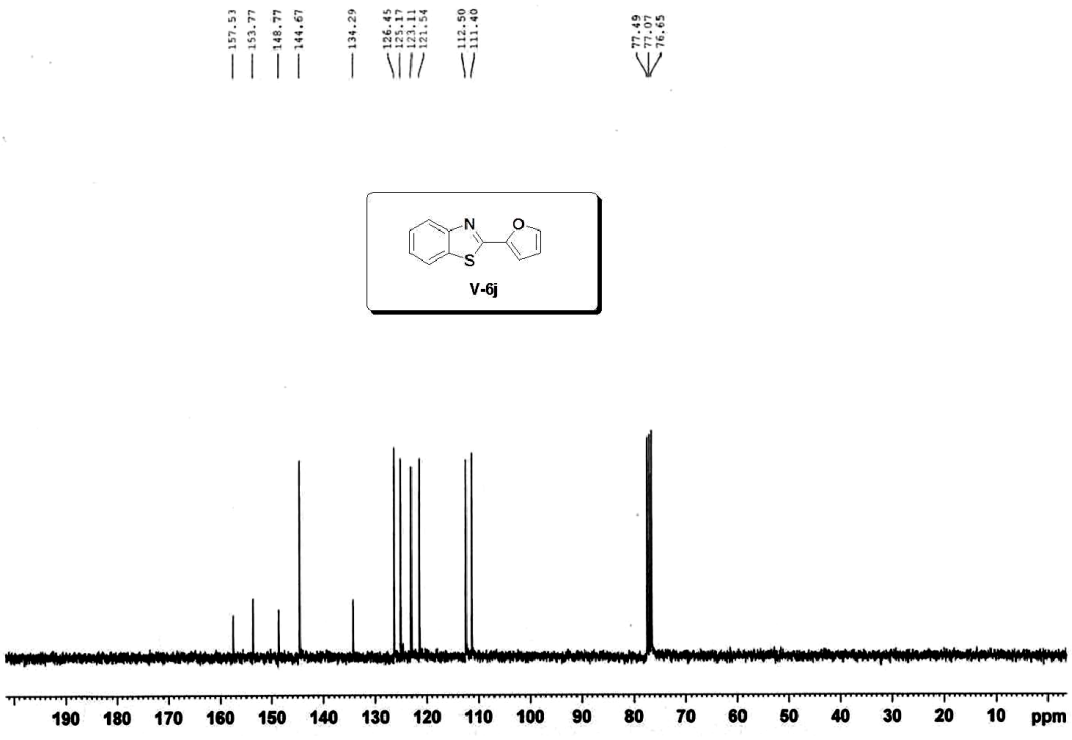
7.483
7.459
7.456
7.391
7.388
7.364
7.341
7.273
7.196
7.185
6.590
6.585
6.579
6.574
2.828
V-6j
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
1.02
1.04
3.13
1.01
1.00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125.17
123.11
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111.40
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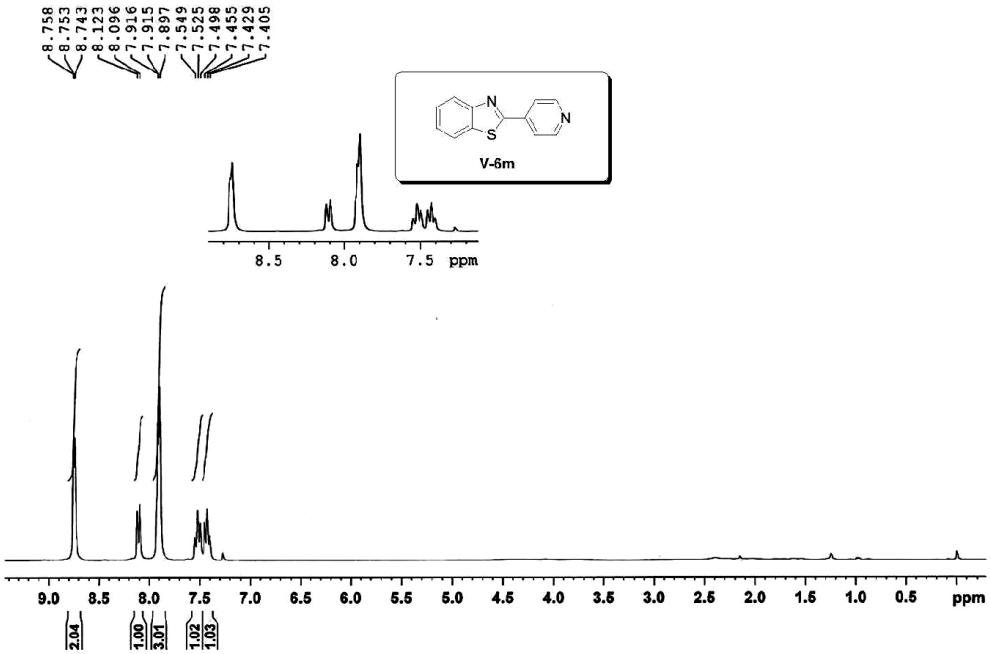
77.07
76.65
V-6j
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm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7.915
7.897
7.549
7.525
7.498
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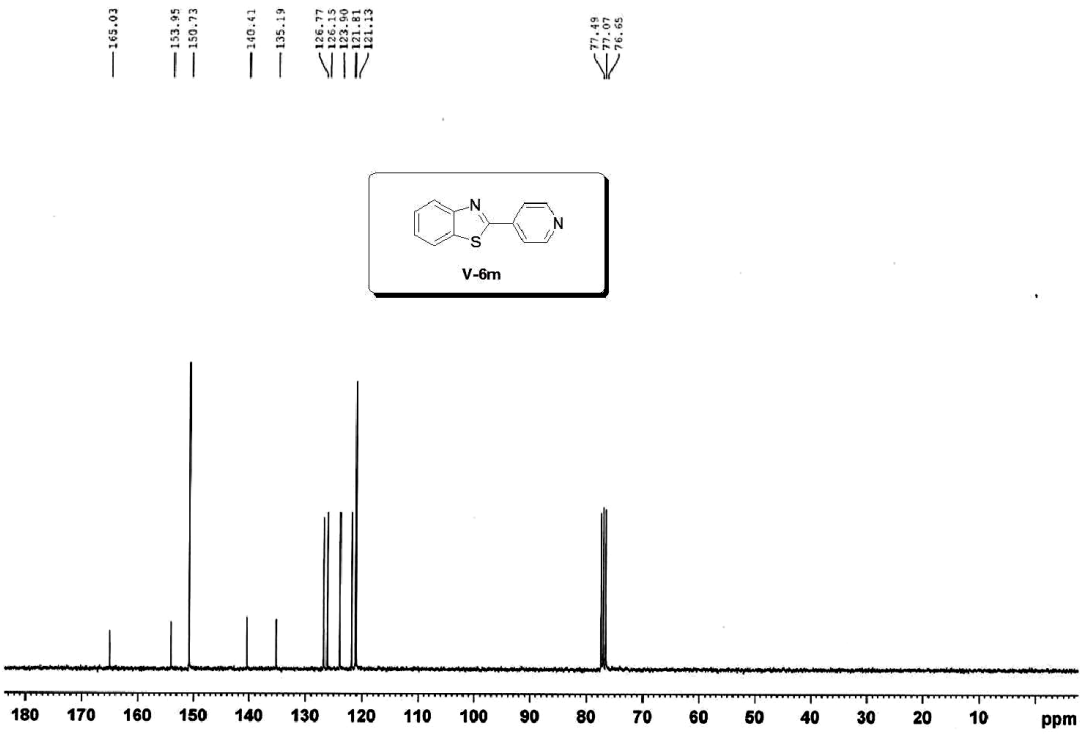
7.429
7.405
V-6m
8.5
8.0
7.5 ppm
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
2.04
1.00
3.01
1.02
1.03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153.95
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140.41
135.19
126.77
126.15
123.60
121.81
121.13
77.49
77.07
76.65
V-6m
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

CHAPTER VI

Development of a mild and "green" synthetic methodology for 3-vinylchromones in aqueous micellar media

VI.1. Background of the present work

Chromone is the structural scaffold of a variety of bioactive compounds of synthetic as well as of natural origin with great pharmaceutical importance.[1,2] From a synthetic viewpoint, 3-formylchromone remains as the key starting material for many of these important heterocyclic systems because of the availability of three electron deficient sites: the C-2 carbon, the aldehyde carbon and the C-4 carbon of the carbonyl group (Figure VI-1A). Therefore, 3-formylchromone is able to serve as a heterodiene as well as a dienophile or a Michael acceptor based on the available substrates and reaction condition leading to the construction of a great variety of heterocyclic systems.[3] At the same time, chemical modification of this scaffold is a delicate issue as valuable pharmacophores derived from this moiety are vulnerable to a number of nucleophiles, organic bases or strong acids due to its high reactivity.[4] Therefore, development of mild conditions for further functionalization of C-3 position of 3-formylchromane and pharmacological utilization draws significant research interests till date.[5] In particular, Knoevenagel condensation reactions of 3-formylchromones with compounds having active methylene group leading to formation of 3-vinylchromones were well studied.[6] Such types of condensations are mostly achieved by conventional methods in organic medium in the presence of acids or bases. However, present environmental concerns demand greener alternatives to construct these important heterocycles. So far, there are only limited numbers of green approaches for vinylation of 3-formylchromones by Knoevenagel condensation[7] including a catalyst free process in water at elevated temperature.[7b] Therefore, the development of a new and efficient "green" protocol for this purpose is worthy pursuit. To the best of our knowledge, Knoevenagel condensation of 3-formylchromone leading to 3-vinylchromones was not explored in a micellar medium. It is also mentioned that chromone derivatives bearing an acrylic acid residue at C-3 display antiallergic activity.[2d] These compounds were previously achieved only by refluxing the

reaction mixture in pyridine. We could also synthesise these biologically active compounds using this methodology.

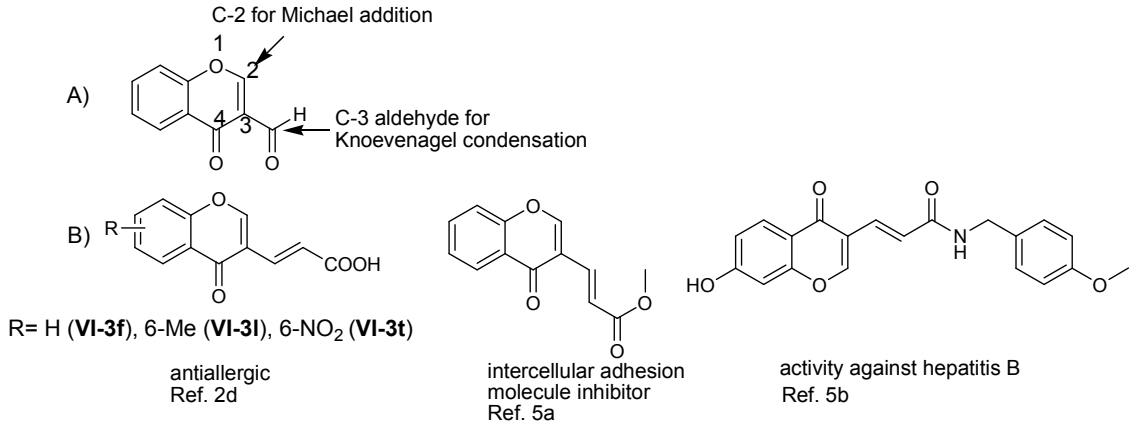
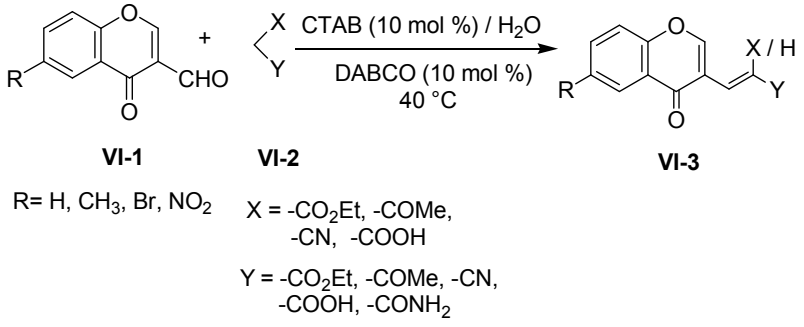


Figure VI-1. A) 3-Formylchromone and its various reactive centers, B) some pharmacologically active 3-vinylchromone derivatives.

VI.2. Results and Discussions

The CTAB catalyzed efficient synthesis of 3-vinylchromones by simple Knoevenagel condensation of 3-formylchromones with various active methylene compounds has been carried out in aqueous media in the presence of DABCO as co-catalyst (Scheme VI-1). We anticipated, a micellar medium will be helpful to carry out such reactions more efficiently at a much milder condition because both the reactants would preferably stay in close proximity inside the hydrophobic interior of the micelles allowing the reaction to proceed spontaneously. Indeed, the methodology worked out well for various 3-formylchromones and compounds containing active methylene group.



Scheme VI-1. Condensation of 3-formylchromones using various active methylene compounds (AMCs) in micellar media.

VI.2.1. Standardization of the reaction condition

VI.2.1.1. Formation of micelles

We started our investigation with a focus on standardizing the reaction conditions. First, the formation of the emulsion droplets were examined by taking optical micrograph of different surfactant containing aqueous solutions of reaction mixtures after 5 min of stirring at room temperature (Figure VI-2). The experiment was carried out in IX-51 inverted microscope in which several emulsion droplets of desired size have been seen. This ensures that the proposed reaction can be carried out in this media.

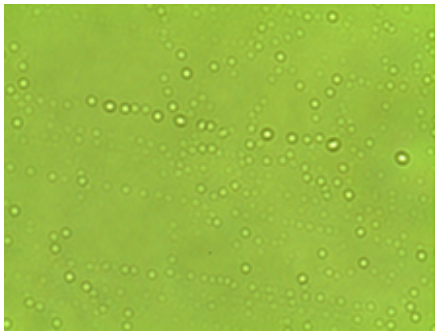


Figure VI-2. A typical optical micrograph of emulsion droplets formed in an aqueous solution of different 3-formylchromones and active methylene compounds in the presence of CTAB.

Dynamic light scattering (DLS) experiments of those solutions were also carried out in Delsa Nano S, Beckman Coulter particle size analyzer which confirmed that the size of emulsion droplets is mostly in the nanometer range (225-420 nm) (Figure VI-3). These droplets act as micellar nanoreactor to carry out the organic transformation inside their core.

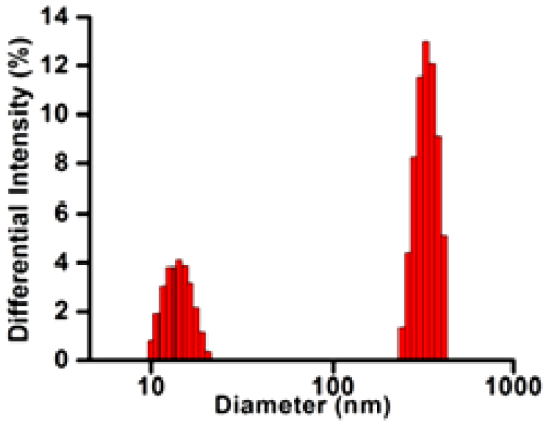
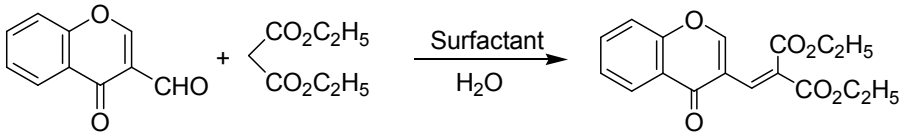


Figure VI-3. DLS data of CTAB showing formation of aggregates.

VI.2.1.2. Selection of suitable surfactant

To establish a suitable condition for the Knoevenagel condensation a thorough screening of various surfactants was carried out at the begining. We selected four different class of surfactants viz. SDS (anionic), DBSA (Brønsted acid), CTAB (cationic), and Triton X-100 (neutral) and conducted a model reaction between equimolar mixture of 3-formylchromone (**VI-1a**) and diethylmalonate (**VI-2a**) to find out the most suited surfactant for this reaction. It was found that three surfactants (SDS, CTAB, and Triton X-100) out of four could initiate the formation of the desired product (**VI-3a**) but the reaction was very sluggish at room temperature (Table VI-1, entry 1-3). Even gentle warming of the reaction mixture was not very effective in terms of the rate of formation of **VI-3a** (Table VI-1, entry 5-7). On the other hand, DBSA failed to initiate the reaction even after 12 h (Table VI-1, entry 4, 8). So, CTAB was found comparatively better in terms of % yield.

Table (VI-1) Selection of suitable surfactant:



VI-1a **VI-2a** **VI-3a**

Entry	Surfactant	mol% of surfactant	Time (h)	*T* (°C)	Yield of VI-3a (%)
1	SDS	10	12	rt	10[a]
2	CTAB	10	12	rt	16[a]
3	Triton X-100	10	12	rt	12[a]
4	DBSA	10	12	rt	nd
5	SDS	10	12	40	24[a]
6	CTAB	10	12	40	36[a]
7	Triton X-100	10	12	40	28[a]
8	DBSA	10	12	40	nd

[a] Incomplete conversion, starting material left out; nd: not determined

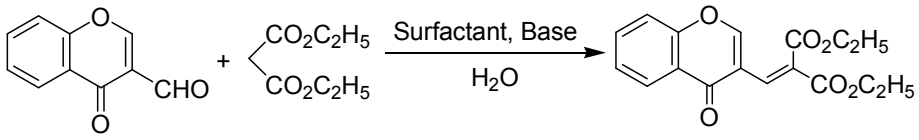
VI.2.1.3. Effect of co-catalyst and optimization of reaction condition

As only surfactant was not good enough to carry out the reaction, a suitable base was added as co-catalyst. Several bases were used to check their effect on % of yield of the Knoevenagel condensation product. In this regard, addition of 10 mol% of a suitable amine (such as DABCO) as co-catalyst boosted the rate of product formation by several fold (Table VI-2,

entry 1-5) in same model reaction between equimolar mixture of 3-formylchromone (**VI-1a**) and diethylmalonate (**VI-2a**). Furthermore, significant enhancement in rate was observed when the same reaction was carried out at 40 °C (Table VI-2, entry 2) instead of room temperature (Table VI-2, entry 1). In these cases, the model reaction was carried out using 10 mol% of CTAB as the surfactant. A variety of 2° and 3° amines were used as co-catalyst for the reaction. Among them DABCO was found to be most suitable in terms of yield and time required for completion of the reaction. Presumably, DABCO, being more hydrophobic in nature, prefers to stay inside the hydrophobic interior of micelles as compared to other bases used for this purpose and therefore, it could act as a better catalyst for this reaction. Once DABCO was identified as most suitable co-catalyst, the same model reaction was carried out with different surfactants and it was found that all the surfactants (10 mol%) could catalyze the condensation reaction in the presence of 10 mol% of DABCO at 40 °C to afford the desired product (**VI-3a**) in moderate to good yields (Table VI-2, entry 2, 6-8). Both DBSA and SDS were found less efficient as catalyst for this reaction, whereas, yields of the final product were high for both CTAB and Triton X-100. As expected, catalytic ability of DBSA-DABCO combination is poor as DBSA reduces the catalytic activity of DABCO by protonation (Table VI-2, entry 7). Among the rest, CTAB catalyzes the reaction better, presumably due to stronger binding of the CTAB with the substrates, which is expected as CTAB has higher hydrocarbon content in its core region than others.[8]

As a part of our study, we also varied mol% of both CTAB and DABCO, and changed temperature to arrive at the ideal reaction condition. As mentioned in table VI-2, dicrease in concentration of CTAB (Table VI-2, entry 12) or DABCO (Table VI-2, entry 9) in the reaction mixture slows down the reaction, whereas, use of excess CTAB (Table VI-2, entry 13) and DABCO (Table VI-2, entry 10, 11) hardly shows any effect on the reaction rate or the yield of final product (**VI-3a**). At the same time, the reaction temperature above 40 °C did not make a significant difference in the reaction rate and the yield (Table VI-2, entry 14, 15). Therefore, use of 10 mol% of CTAB as catalyst and 10 mol% of DABCO as co-catalyst and gentle heating (at 40 °C) was considered as optimum condition for further studies.

Table (VI-2) Standardization of the reaction condition: effect of surfactant, mol% of base and temperature



VI-1a **VI-2a** **VI-3a**

Entry	Surfactant	mol% of surfactant	Base	mol% of base	Time (h)	*T* (°C)	Yield of VI-3a (%)
1	CTAB	10	DABCO	10	4	rt	56[a]
2	CTAB	10	DABCO	10	1	40	78
3	CTAB	10	Piperidine	10	2.5	40	76
4	CTAB	10	Pyrrole	10	4	40	80
5	CTAB	10	L-Proline	10	6	40	72
6	SDS	10	DABCO	10	2	40	60
7	DBSA	10	DABCO	10	4	40	42[a]
8	Triton X-100	10	DABCO	10	2	40	71
9	CTAB	10	DABCO	05	2.5	40	68
10	CTAB	10	DABCO	20	1	40	75
11	CTAB	10	DABCO	100	0.75	40	82
12	CTAB	5	DABCO	10	3	40	48[a]
13	CTAB	20	DABCO	10	1	40	82
14	CTAB	10	DABCO	10	1	50	76
15	CTAB	10	DABCO	10	1	60	80

[a]Incomplete conversion, starting material left out.

VI.2.2. Condensation of 3-formylchromones with active methylene compounds

Various 3-formylchromones were treated with different compounds containing active methylene group in the presence of CTAB (10 mol%) and DABCO (10 mol%) at the optimized reaction condition. All the substrates were found to undergo smooth reaction to afford 3-vinylchromones in high yields within few hours (Table VI-3). The 3-vinylchromones was thoroughly characterized by ^{1}H NMR, ^{13}C NMR, ESI-MS, IR and CHN analysis, and compared with the reported data as and when possible. The condensation product was formed in stepwise manner, which includes removal of acidic proton from AMC by DABCO to give carbanion and then subsequent attack of carbanion to carbonyl carbon of 3-formylchromones to afford condensation product along by removal of water molecule (Figure VI-4). It is expected that the water molecule expels out of the inner core of the micelle due to opposite polarity.

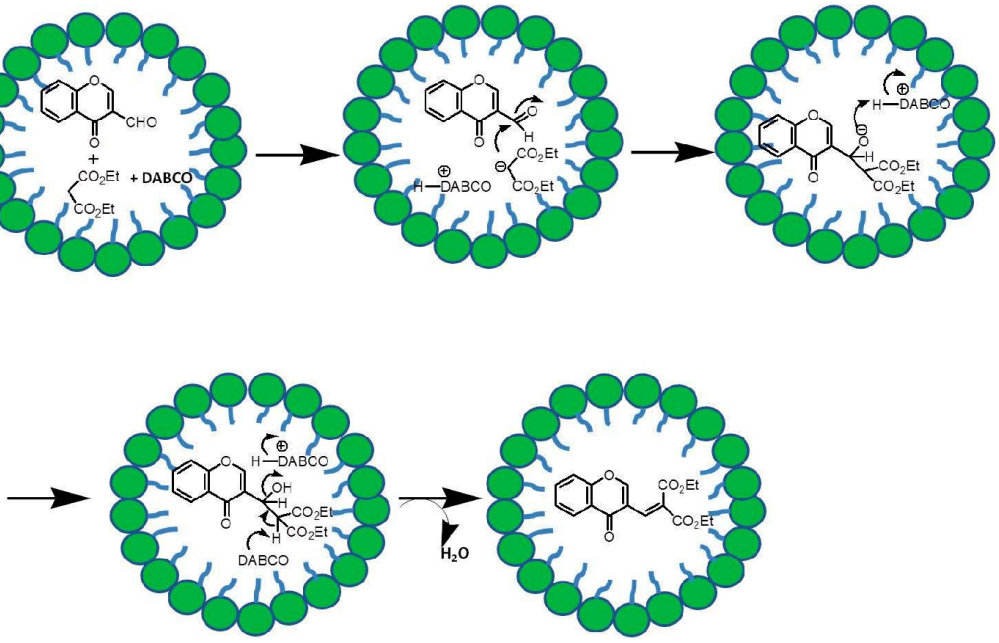
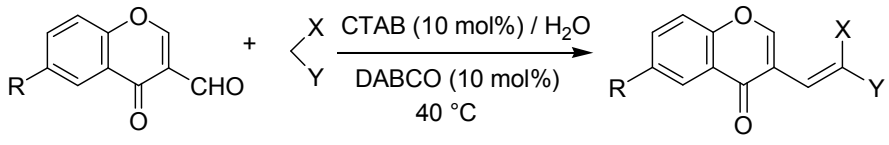


Figure VI-4. Mechanistic pathway of the formation of Knoevenagel product (**VI-3a**) in micellar media.

It was found that a substitution at the aromatic ring of 3-formylchromones does not have much influence in the final yield of the products. This is presumably because of the fact that the substituent is located far away from the reaction center. However, presence of electron withdrawing group does influence a little on the reactivity of the corresponding 3-formylchromone derivatives. Thus, the reactions involving 3-formyl-6-nitro-chromone (**VI-1d**) completed in shorter time (Table VI-3, entries 18, 19, and 20) than similar reactions with unsubstituted 3-formylchromones (Table VI-3, entries 1, 5, and 6). Among active methylene compounds malononitrile (**VI-2d**) reacted much faster than the rest to produce the desired condensation products in high yields (Table VI-3, entries 4 and 10). Interestingly, however, condensation of 3-formylchromones with malonic acid (**VI-2f**) afforded Doebner decarboxylated products (**VI-3f**, **VI-3l**, **VI-3q** and **VI-3t**; Table VI-3, entries 6, 12, 17 and 20, respectively) as the only isolated product as revealed by spectral studies and CHN analysis. The coupling constant (J = 15.6 Hz) of H-atoms across the olefinic double bond revealed that they bear *trans* geometry.[6d] We observed that the micellar medium is suitable to spontaneously carry out an *in situ* decarboxylation to afford these products in high yields. Moreover, the yields of these bioactive compounds using this method were

much higher than the previously reported procedure.[2d,6d] Although the yields were highly satisfactory for all the other entries, the reaction between ethyl acetoacetate (EAA) and 3-formylchromone did not afford the condensation product, **VI-3b** in high yield (Table VI-3, entry 2). It was observed that the major product of the reaction between equimolar mixture of **VI-1a** and **VI-2b** was a benzophenone derivative (**VI-4a**) (Scheme VI-2). The formation of the benzophenone derivative, **VI-4a** by opening of pyran ring by Knoevenagel condensation followed by Michael reaction was previously reported by W. D. Jones et al. in ethanolic medium in the presence of one equiv of organic base in poor yield.[9] This interesting result prompted us to carry out such reaction in the presence of excess EAA.

Table (VI-3): Condensation of 3-formylchromones with active methylene compounds (AMC):



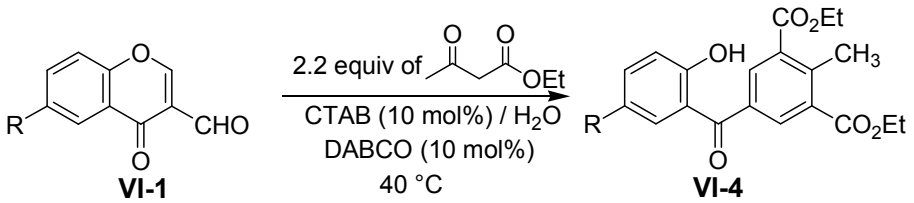
VI-1 **VI-2** **VI-3**

Entry	R	X	Y	AMC	Product	Time (min)	% Yield	Ref.
1	H	$-CO_2C_2H_5$	$-CO_2C_2H_5$	**VI-2a**	**VI-3a**	60	78	10
2	H	$-COCH_3$	$-CO_2C_2H_5$	**VI-2b**	**VI-3b**	60	22[a]	9
3	H	-CN	$-CO_2C_2H_5$	**VI-2c**	**VI-3c**	15	79	7c
4	H	-CN	-CN	**VI-2d**	**VI-3d**	10	81	7c
5	H	$-COCH_3$	$-COCH_3$	**VI-2e**	**VI-3e**	30	83	9
6	H	-COOH	-COOH	**VI-2f**	**VI-3f**	150	88	6d
7	H	-CN	$-CONH_2$	**VI-2g**	**VI-3g**	20	85	7c
8	6-Methyl	$-CO_2C_2H_5$	$-CO_2C_2H_5$	**VI-2a**	**VI-3h**	75	82	10
9	6-Methyl	-CN	$-CO_2C_2H_5$	**VI-2c**	**VI-3i**	15	96	7c
10	6-Methyl	-CN	-CN	**VI-2d**	**VI-3j**	10	83	7c
11	6-Methyl	$-COCH_3$	$-COCH_3$	**VI-2e**	**VI-3k**	40	79	--
12	6-Methyl	-COOH	-COOH	**VI-2f**	**VI-3l**	180	83	6d
13	6-Methyl	-CN	$-CONH_2$	**VI-2g**	**VI-3m**	30	85	7c
14	6-Bromo	$-CO_2C_2H_5$	$-CO_2C_2H_5$	**VI-2a**	**VI-3n**	40	92	--
15	6-Bromo	-CN	$-CO_2C_2H_5$	**VI-2c**	**VI-3o**	15	99	--
16	6-Bromo	$-COCH_3$	$-COCH_3$	**VI-2e**	**VI-3p**	30	84	--
17	6-Bromo	-COOH	-COOH	**VI-2f**	**VI-3q**	120	98	6d
18	6-Nitro	$-CO_2C_2H_5$	$-CO_2C_2H_5$	**VI-2a**	**VI-3r**	40	90	--
19	6-Nitro	$-COCH_3$	$-COCH_3$	**VI-2e**	**VI-3s**	15	90	--
20	6-Nitro	-COOH	-COOH	**VI-2f**	**VI-3t**	60	93	2d

[a]The reaction produced benzophenone derivative, **4a** in 32% yield.

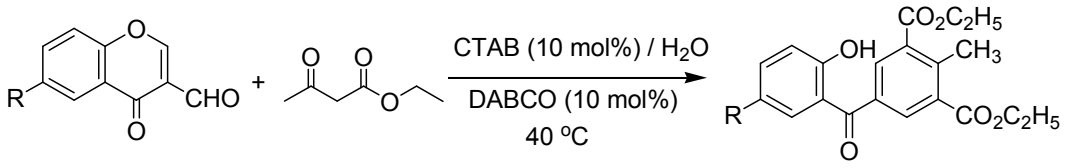
VI.2.3. Synthesis of benzophenone drivative

Encouraged by the fact that 3-formylchromone mainly undergoes tandem Knoevenagel and Michael reaction in the presence of EAA to produce benzophenone derivative, **VI-4a** by opening of pyran ring (Scheme VI-2) several reactions were carried out between various 3-formylchromones (1 equiv) and excess EAA (2.2 equiv) to ensure formation of benzophenone derivatives (**VI-4**) as the main product (Table VI-4). As expected, the reactions produced the benzophenone derivatives (**VI-4**) in high yields. None of simple Knoevenagel condensation product (**VI-3**) was observed in the final reaction mixture. Presumably, the benzophenone derivatives are formed by stepwise attack of two molecules of EAA to 3-formylchromones (Figure VI-5). At first, EAA undergoes Knoevenagel condensation to form expected 3-vinylchromones (**VI-3**). Next, another molecule of EAA goes for Michael addition followed by opening of the chromone ring and cyclization to afford the benzophenone derivatives, **VI-4**. Once again, it was found that the nature of the substitutions in the aryl ring of chromone moiety does not have great influence on the reactivity and the yield of the final product. All the reactions were completed within 2 h and the yields were in the range 82-90%. Although variation was made only in the chromone moiety, it is expected that a variety of acylacetate esters ($RCOCH_2COOR'$) would also undergo similar reactions to produce a benzophenone derivative.



Scheme VI-2. Synthesis of benzophenone derivative using various 3-formyl chromones and excess ethyl acetoacetate in micellar media.

Table (VI-4): Condensation of 3-formylchromones with ethyl acetoacetate:



VI-1 1.0 equiv + **VI-2b** 2.2 equiv → **VI-4**

Entry	R	Product	Time (h)	%Yield	Ref.
1	H	**VI-4a**	1.5	82	9
2	6-Methyl	**VI-4b**	2.0	88	--
3	6-Bromo	**VI-4c**	1.5	83	--
4	6-Nitro	**VI-4c**	1.0	90	--

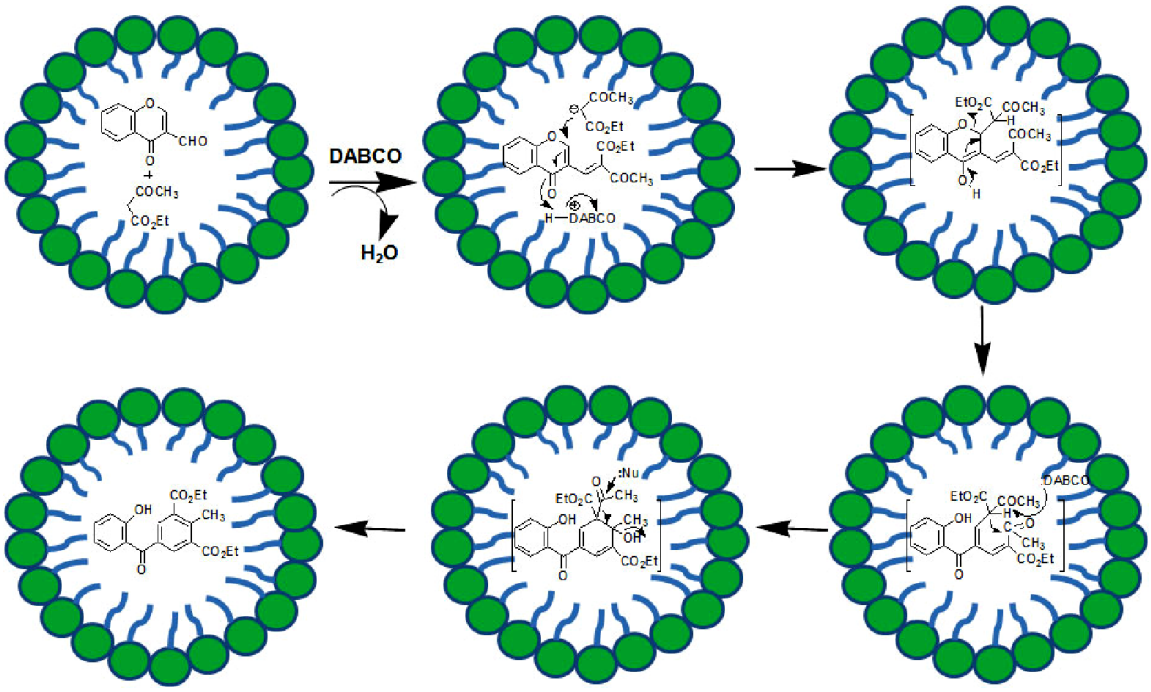


Figure VI-5. Mechanistic pathway of the formation of benzophenone derivative (**VI-4a**) in micellar media.

VI.3. Conclusion

A facile and 'green' method has been developed for the synthesis of 3-vinylchromones from 3-formylchromones using different active methylene compounds in organized aqueous media in the presence of 10 mol% of CTAB as cationic surfactant catalyst and 10 mol% of DABCO

as mild basic catalyst and mild heating at 40 °C. A broad range of 3-vinylchromone derivatives from various 3-formylchromones were obtained using this method in good overall yields. In case of malonic acid as active methylene compound, Doebner decarboxylated products were obtained. It has been also observed that 3-formylchromone derivatives primarily undergo tandem Knoevenagel and Michael reactions in the presence of more than 2 equiv. of ethyl acetoacetate to produce benzophenone derivatives, by opening of pyran ring, as the sole product in good yields. Overall, this method is environmentally benign, cheap, safe, high yielding, and a much improved method than other available methods for the synthesis of 3-vinylchromones from 3-formylchromones.

VI.4. Experimental Section

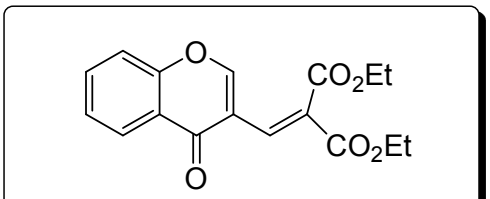
VI.4.1. Materials & Methods

The reagents were procured from commercial sources and were used without further purification. All solvents were obtained from local suppliers and were of research grade. All the chromones were purchased from Sigma–Aldrich and used without further purification. Other common reagents were procured either from SD Fine-Chem. Limited, Mumbai, India or from Merck, India.

^{1}H NMR and ^{13}C NMR spectra were recorded on BRUKER 300 MHz or BRUKER 400 MHz or JEOL 400 MHz NMR systems using tetramethylsilane as an internal standard. The chemical shifts are reported in parts per million (ppm) units relative to the solvent peak. The following abbreviations are used in reporting NMR data: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublets; dt, doublet of triplets; m, multiplet; brs, broad singlet. Mass spectra were recorded on Waters Q-TOF micro mass spectrometer or Bruker Esquire 3000 mass spectrometer using ESI as the ion source. The IR spectra were recorded in KBr pellets with IR Affinity 1, Shimadzu. CHN data were recorded using Vario elementar CHNS analyzer. Particle size was determined using a particle size analyzer (Delsa Nano S, Beckman Coulter, USA). Melting points of the compounds were determined using Melting Point Apparatus, Bio Techniques, India. The reactions were monitored by thin layer chromatography (TLC) carried out on 0.25-mm silica gel on aluminium plates (60F-254) using UV light (254 or 365 nm) or naked eye for visualization. Column chromatography was performed on silica gel (60–120 mesh, Merck).

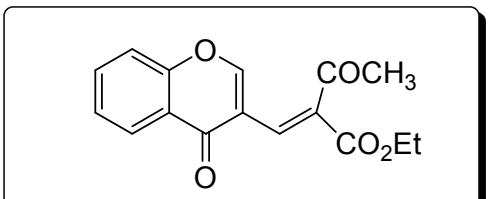
VI.4.2. General experimental procedure for the synthesis of Knoevenagel condensation products (VI-3):

To a solution of CTAB (0.05 mmol) in H_2O (2 mL) were added a 3-formylchromone (0.5 mmol), DABCO (0.05 mmol), and an active methylene compound (0.5 mmol) successively at room temperature in a 10 mL round-bottom flask. The reaction mixture was sonicated for few min and then stirred at 40 °C for required time as listed in table VI-2. The reaction was monitored by TLC. The crude product was extracted with ethyl acetate, washed with brine, the combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated in vacuum. The crude product was purified by column chromatography (silica gel, 60-120 mesh) using a mixture of ethyl acetate and hexane as the eluent.



VI-3a

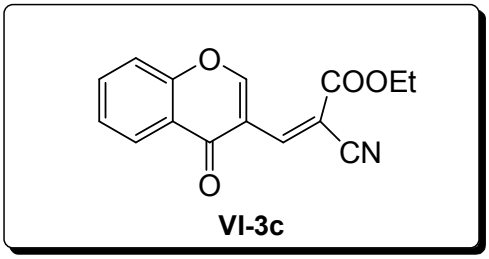
***Diethyl 2-((4-oxo-4H-chromen-3-yl)methylene)malonate (VI-3a)*:**[10] Light yellow solid, yield: 78%; mp. 109-110 °C (Lit. 111 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.29-1.37 (m, 6H), 4.28-4.38 (m, 4H), 7.43-7.49 (m, 2H), 7.68-7.74 (m, 1H), 7.78 (d, J = 0.6 Hz, 1H), 8.25 (dd, J_1 = 1.5 Hz, J_2 = 8.1 Hz, 1H), 8.32 (d, J = 0.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.0, 14.1, 61.7, 61.8, 118.2, 119.1, 123.8, 126.0, 126.4, 128.2, 133.1, 134.3, 155.9, 156.4, 163.9, 165.9, 175.1; IR (KBr): 3078, 1723, 1654 cm^{-1}; MS (ESI): *m/z* 339 [M + Na]$^+$; *Anal.* Calcd. for $C_{17}H_{16}O_6$: C, 64.55; H, 5.10. Found: C, 64.72; H, 5.11.



VI-3b

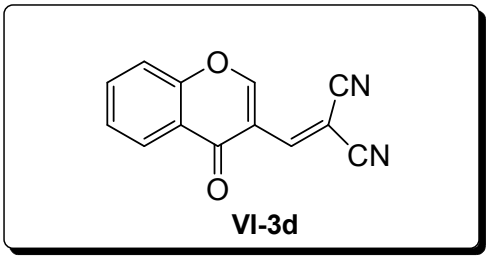
***(2E)-ethyl 3-oxo-2-((4-oxo-4H-chromen-3-yl)methylene)butanoate (VI-3b)*:**[9] Light yellow solid, yield: 22%; mp. 119-121 °C (Lit. 122 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.36

(t, J = 7.5 Hz, 3H), 2.5 (s, 3H), 4.32 (q, J = 7.4 Hz, 2H), 7.30-8.07 (m, 4H), 8.21 (dd, J_1 = 1.5 Hz, J_2 = 7.5 Hz, 1H), 8.37 (s, 1H); IR (KBr): 3038, 1703, 1684, 1652 cm^{-1}. [This particular compound was characterized based on ^1H NMR, mp. and IR only, and matching them with the reported data].



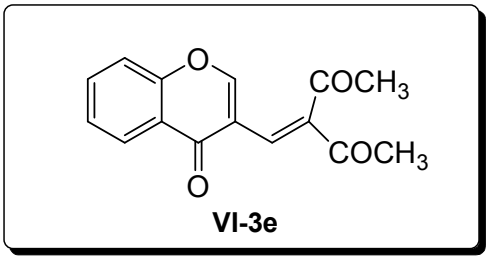
VI-3c

***(2Z)-Ethyl 2-cyano-3-(4-oxo-4H-chromen-3-yl)acrylate (VI-3c)*:**[7c] Light yellow solid, yield: 79%; mp. 125-126 °C; ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.40 (t, J = 7.3 Hz, 3H), 4.40 (q, J = 7.4 Hz, 2H), 7.49-7.57 (m, 2H), 7.74-7.81 (m, 1H), 8.26 (d, J = 7.5 Hz, 1H), 8.65 (s, 1H), 9.15 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 14.1, 62.9, 103.6, 115.4, 117.7, 118.5, 123.5, 126.5, 126.6, 134.9, 145.7, 155.8, 158.3, 161.5, 174.5; IR (KBr): 3039, 2222, 1729, 1662 cm^{-1}; MS (ESI): *m/z* 292 $[M + Na]^+$; *Anal.* Calcd. for $C_{15}H_{11}NO_4$: C, 66.91; H, 4.12; N, 5.20. Found: C, 67.07; H, 4.16; N, 5.24.



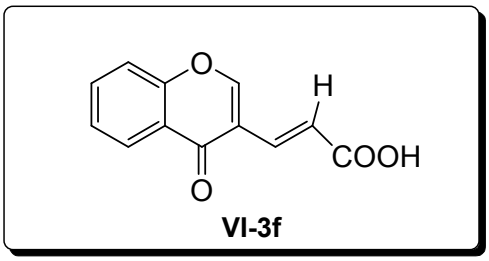
VI-3d

***2-((4-Oxo-4H-chromen-3-yl)methylene)malononitrile (VI-3d)*:**[7c] Light yellow solid, yield: 81%; mp. 184-185 °C (Lit. 186-187 °C); ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 6.96 (t, J = 7.8 Hz, 1H), 7.12 (d, J = 8.4 Hz, 1H), 7.50-7.61 (m, 2H), 8.27 (d, J = 2.4 Hz, 1H), 8.73 (d, J = 2.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 114.1 (2C), 118.6, 118.9, 119.3, 127.1, 132.3, 137.2, 144.0, 152.7, 163.1, 165.6, 196.1; IR (neat): 3156, 2233, 1722 cm^{-1}; MS (ESI): *m/z* 245 $[M + Na]^+$; *Anal.* Calcd. for $C_{13}H_6N_2O_2$: C, 70.27; H, 2.72; N, 12.61. Found: C, 70.10; H, 2.73; N, 12.58.



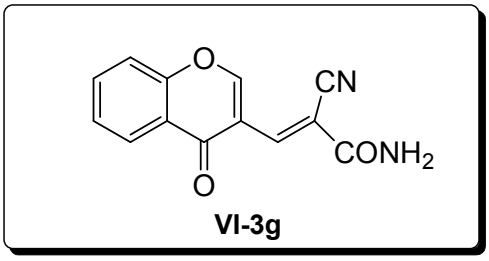
VI-3e

***3-[(4-Oxo-4H-1-benzopyran-3-yl) methylene pentan-2, 4-dione (VI-3e)*:**[9] Light yellow solid, yield: 83%; mp. 166-167°C (Lit. 168-170 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.37 (s, 3H), 2.50 (s, 3H), 7.45-7.53 (m, 3H), 7.73 (dt, J_1 = 1.5 Hz, J_2 = 8.8 Hz, 1H), 8.25 (dd, J_1 = 1.5 Hz, J_2 = 7.8 Hz, 1H), 8.32 (d, J = 1.2 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 26.3, 31.3, 118.3, 118.7, 123.5, 126.1, 126.2, 130.6, 134.5, 144.3, 155.9, 156.8, 175.4, 197.3, 204.0; IR (KBr): 3050, 1709, 1651 cm^{-1}; MS (ESI): *m/z* 279 $[M + Na]^+$; *Anal.* Calcd. for $C_{15}H_{12}O_4$: C, 70.31; H, 4.72. Found: C, 70.05; H, 4.73.



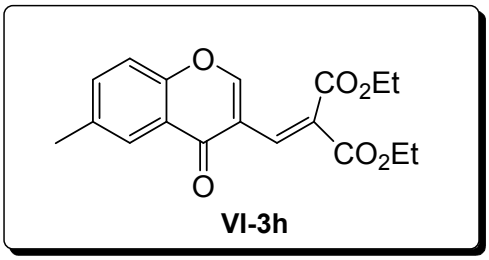
VI-3f

***(2E)-3-(4-oxo-4H-chromen-3-yl) acrylic acid (VI-3f)*:**[6d] Light yellow color solid, yield: 88%; mp. 252-253 °C (Lit. 253-254 °C); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.13 (d, J = 16.0 Hz, 1H), 7.43 (d, J = 16.0 Hz, 1H), 7.53-7.57 (m, 1H), 7.72 (d, J = 8.0 Hz, 1H), 7.83-7.87 (m, 1H), 8.13-8.15 (m, 1H), 8.89 (s, 1H), 12.46 (s, 1H, exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 118.2, 118.6, 121.5, 123.5, 125.5, 126.1, 134.6, 135.8, 155.1, 159.8, 167.9, 175.4; IR (KBr): 2971, 1715, 1665 cm^{-1}; MS (ESI): *m/z* 239 $[M + Na]^+$; *Anal.* Calcd. for $C_{12}H_8O_4$: C, 66.67; H, 3.73. Found: C, 66.58; H, 3.76.



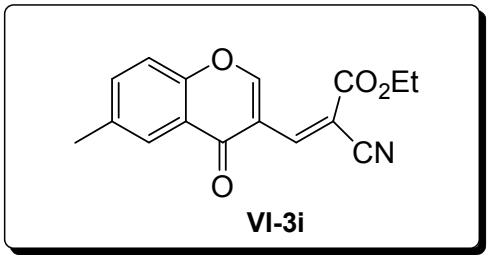
VI-3g

***(2E)-2-cyano-3-(4-oxo-4H-chromen-3-yl) acrylamide (VI-3g)*:**[7c] Light yellow color solid, yield: 85%; mp. 182-183°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.57-7.61 (m, 1H), 7.78 (dd, J_1 = 0.6 Hz, J_2 = 8.4 Hz, 1H), 7.85 (brs, 1H), 7.89-7.93 (m, 1H), 8.05 (brs, 1H), 8.15 (dd, J_1 = 1.5 Hz, J_2 = 8.4 Hz, 1H), 8.17 (d, J = 1.0 Hz, 1H), 9.11 (d, J = 0.8 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 110.2, 117.0, 119.0, 120.0, 124.2, 126.7, 127.8, 136.4, 143.3, 156.7, 159.7, 163.1, 175.4; IR (KBr): 3430, 3373, 3157, 2362, 2219, 1705, 1660 cm^{-1}; MS (ESI): *m/z* 263 $[M + Na]^+$; *Anal.* Calcd. for $C_{13}H_8N_2O_3$: C, 65.00; H, 3.36; N, 11.66. Found: C, 64.84; H, 3.37; N, 11.68.



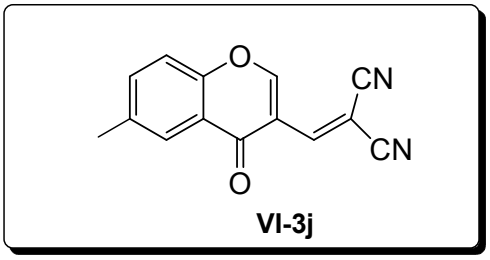
VI-3h

***Diethyl 2-((6-methyl-4-oxo-4H-chromen-3-yl)methylene)malonate (VI-3h)*:**[10] Pale yellow solid, yield: 82%; mp.119-120 °C (Lit. 120 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.24-1.36 (m, 6H), 2.47 (s, 3H), 4.27-4.38 (m, 4H), 7.37 (d, J = 8.0 Hz, 1H), 7.51 (dd, J_1 = 1.8 Hz, J_2 = 8.0 Hz, 1H), 7.78 (s, 1H), 8.03 (d, J = 1.2 Hz, 1H), 8.29 (d, J = 0.6 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 14.0, 14.1, 20.9, 61.7 (2C), 117.9, 118.8, 123.4, 125.6, 127.9, 133.2, 135.4, 136.1, 154.1, 156.3, 163.9, 165.9, 175.1; IR (KBr): 3070, 1727, 1658 cm^{-1}; MS (ESI): *m/z* 331 $[M + H]^+$; *Anal.* Calcd. for $C_{18}H_{18}O_6$: C, 65.45; H, 5.49. Found: C, 65.54; H, 5.52.



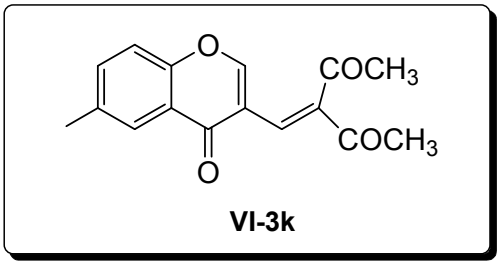
VI-3i

***(2Z)-Ethyl 2-cyano-3-(6-methyl-4-oxo-4H-chromen-3-yl)acrylate (VI-3i)*:**[7c] Light yellow solid, yield: 96%; mp. 118-119 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.40 (t, J_1 = 6.9 Hz, 3H), 2.49 (s, 3H), 4.39 (q, J_1 = 7.2 Hz, 2H), 7.44 (d, J = 8.7 Hz, 1H), 7.57 (dd, J_1 = 1.5 Hz, J_2 = 8.4 Hz, 1H), 8.06 (d, J = 1.4 Hz, 1H), 8.66 (d, J = 1.0 Hz, 1H), 9.13 (d, J = 0.8 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 14.2, 21.0, 62.8, 103.3, 115.5, 117.6, 118.3, 123.2, 125.9, 136.1, 136.9, 145.9, 154.1, 158.2, 161.6, 174.6; IR (KBr): 3055, 2234, 1710, 1662 cm^{-1}; MS (ESI): *m/z* 306 $[M + Na]^+$; *Anal.* Calcd. for $C_{16}H_{13}NO_4$: C, 67.84; H, 4.63; N, 4.94. Found: C, 67.75; H, 4.66; N, 4.91.



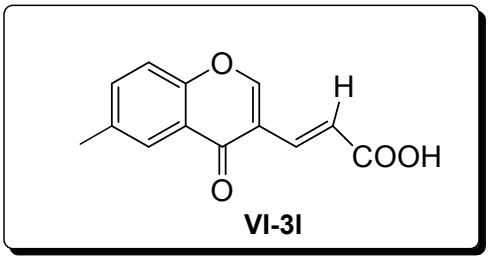
VI-3j

***2-((6-Methyl-4-oxo-4H-chromen-3-yl)methylene)malononitrile (VI-3j)*:**[7c] Light yellow solid, yield: 83%; mp. 216-217 °C (Lit. 217-218 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.50 (s, 3H), 7.46 (d, J = 8.4 Hz, 1H), 7.60 (dd, J_1 = 1.5 Hz, J_2 = 8.4 Hz, 1H), 8.05 (s, 1H), 8.30 (s, 1H), 9.11 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 21.1, 82.7, 112.6, 112.9, 117.5, 118.4, 123.0, 126.0, 136.6, 137.6, 151.4, 154.06, 158.6, 173.7; IR (KBr): 3031, 2234, 1698 cm^{-1}; MS (ESI): *m/z* 237 $[M + H]^+$; *Anal.* Calcd. for $C_{14}H_8N_2O_2$: C, 71.18; H, 3.41; N, 11.86. Found: C, 71.39; H, 3.40; N, 11.90.



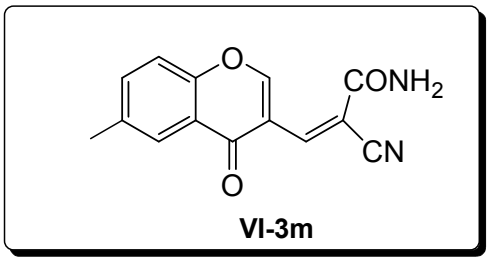
VI-3k

3-[(6-Methyl-4-oxo-4H-1-benzopyran-3-yl)-methylene]-pentan-2,4-dione **(*VI-3k*):** Light yellow solid, yield: 79%; mp. 138-139 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.34 (s, 3H), 2.45 (s, 3H), 2.46 (s, 3H), 7.36 (d, J = 8.5 Hz, 1H), 7.49-7.52 (m, 2H), 8.0 (s, 1H), 8.26 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 21.1, 26.4, 31.4, 118.1, 118.6, 123.3, 125.6, 130.9, 135.8, 136.4, 144.3, 154.3, 156.8, 175.6, 197.4, 204.2; IR (KBr): 3066, 1702, 1650 cm^{-1}; MS (ESI): *m/z* 271 $[M + H]^+$; *Anal.* Calcd. for $C_{16}H_{14}O_4$: C, 71.10; H, 5.22. Found: C, 71.24; H, 5.27.



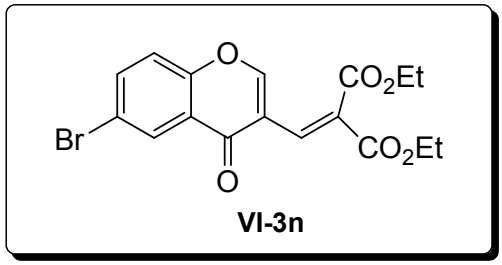
VI-3l

***(2E)-3-(6-methyl-4-oxo-4H-chromen-3-yl) acrylic acid (VI-3l)*:**[6d] Light yellow color solid, yield: 83%; mp. 259-261 °C (Lit. 260 - 261 °C); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.44 (s, 3H), 7.12 (d, J = 15.6 Hz), 7.42 (d, J = 15.7 Hz), 7.58-7.67 (m, 2H), 7.90 (d, J = 15.6 Hz, 1H), 8.85 (s, 1H), 12.50 (s, 1H, exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 20.5, 108.7, 117.5, 118.6, 122.7, 124.8, 136.2, 136.4, 142.2, 153.8, 158.3, 162.0, 174.1; IR (neat): 3064, 1724, 1661 cm^{-1}; MS (ESI): *m/z* 253 $[M + Na]^+$; *Anal.* Calcd. for $C_{13}H_{10}O_4$: C, 67.82; H, 4.38. Found: C, 67.63; H, 4.39.



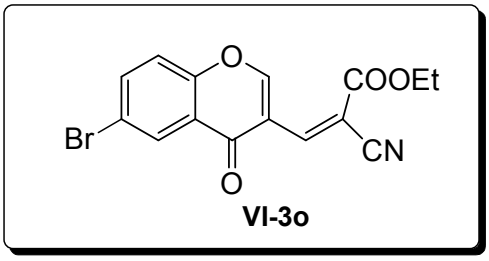
VI-3m

***(2Z)-2-cyano-3-(6-methyl-4-oxo-4H-chromen-3-yl)acrylamide (VI-3m)*:**[7c] Light yellow color solid, yield: 85%; mp. 198-199 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.45 (s, 3H), 7.65-7.73 (m, 2H), 7.84 (brs, 1H), 7.93 (d, J = 4.0 Hz, 1H), 8.04 (brs, 1H), 8.17 (d, J = 1.0 Hz, 1H), 9.10 (d, J = 0.8 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 20.5, 96.0, 114.5, 118.3 (2C), 121.2, 122.5, 124.6, 124.7, 132.9, 135.5, 136.0, 157.4, 159.6; IR (KBr): 3390, 3292, 2231, 2362, 1660, 1617 cm^{-1}; MS (ESI): *m/z* 277 [M + Na]$^{+}$; *Anal.* Calcd. for $C_{14}H_{10}N_2O_3$: C, 66.14; H, 3.96; N, 11.02. Found: C, 66.28; H, 3.95; N, 11.05.



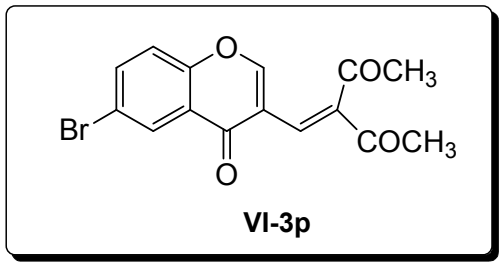
VI-3n

***Diethyl 2-((6-bromo-4-oxo-4H-chromen-3-yl)methylene)malonate* (*VI-3n*):** Light yellow solid, yield: 92%; mp. 67-68 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.25-1.34 (m, 6H), 4.26-4.34 (m, 4H), 7.35 (d, J = 8.8 Hz, 1H), 7.69 (s, 1H), 7.78 (dd, J_1 = 2.4 Hz, J_2 = 8.8 Hz, 1H), 8.30 (s, 1H), 8.34 (d, J = 2.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 14.1, 14.2, 61.9 (2C), 119.3, 119.6, 120.2, 125.1, 128.8, 129.0, 132.6, 137.3, 154.7, 156.5, 163.8, 165.8, 173.9; IR (KBr): 3055, 1717, 1659 cm^{-1}; MS (ESI): *m/z* 417 [M + Na]$^{+}$ for Br = 79, 419 [M + Na]$^{+}$ for Br = 81; *Anal.* Calcd. for $C_{17}H_{15}BrO_6$: C, 51.67; H, 3.83. Found: C, 51.82; H, 3.88.



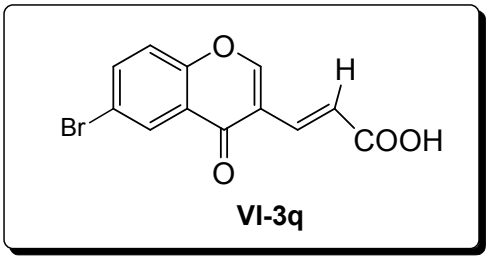
VI-3o

(*2Z*)-*Ethyl-3-(6-bromo-4-oxo-4H-chromen-3-yl)-2-cyanoacrylate* (*VI-3o*): Pale yellow solid, yield: 99%; mp. 185-186 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.38 (t, J = 7.1 Hz, 3H), 4.37 (q, J = 7.2 Hz, 2H), 7.44 (d, J = 8.7 Hz, 1H), 7.83 (dd, J_1 = 2.3 Hz, J_2 = 8.7 Hz, 1H), 8.37 (d, J = 2.3 Hz, 1H), 8.57 (d, J = 0.7 Hz, 1H), 9.10 (d, J = 0.7 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 14.2, 63.1, 104.3, 115.4, 118.0, 120.3, 120.6, 124.9, 129.2, 138.0, 145.2, 154.7, 158.2, 161.4, 173.4; IR (KBr): 3093, 2214, 1729, 1650 cm^{-1}; MS (ESI): *m/z* 370 $[M + Na]^+$ for Br = 79, 372 $[M + Na]^+$ for Br = 81; *Anal.* Calcd. for $C_{15}H_{10}BrNO_4$: C, 51.75; H, 2.90; N, 4.02. Found: C, 51.62; H, 2.94; N, 4.05.



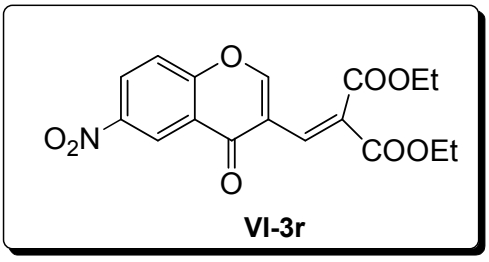
VI-3p

***3-[(6-Bromo-4-oxo-4H-1-benzopyran-3-yl)-methylene]-pentan-2,4-dione* (*VI-3p*):** Light almond color solid, yield: 84%; mp. 164-165 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.34 (s, 3H), 2.47 (s, 3H), 7.38 (d, J = 8.7 Hz, 1H), 7.44 (s, 1H), 7.78 (dd, J_1 = 2.5 Hz, J_2 = 8.9 Hz, 1H), 8.30 (s, 1H), 8.34 (d, J = 2.3 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 26.4, 31.4, 119.0, 119.7, 120.4, 124.8, 128.9, 130.2, 137.6, 144.9, 154.8, 156.9, 174.3, 197.3, 203.9; IR (KBr): 3067, 1697, 1657 cm^{-1}; MS (ESI): *m/z* 357 $[M + Na]^+$ for Br = 79, 359 $[M + Na]^+$ for Br = 81; *Anal.* Calcd. for $C_{15}H_{11}BrO_4$: C, 53.76; H, 3.31. Found: C, 53.63; H, 3.39.



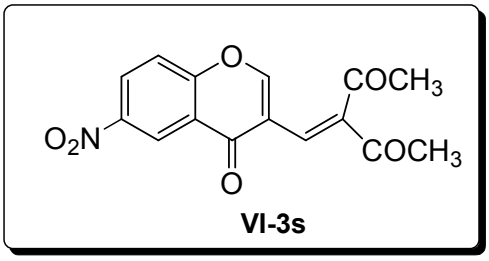
VI-3q

***(2E)-3-(6-bromo-4-oxo-4H-chromen-3-yl) acrylic acid (VI-3q)*:**[6d] Light almond color solid, yield: 98%; mp. 210-211 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.11 (d, J = 16.0 Hz, 1H), 7.41 (d, J = 16.0 Hz, 1H), 7.72 (d, J = 12.0 Hz, 1H), 7.99-8.02 (m, 1H), 8.19 (d, J = 3 Hz, 1H), 8.91 (s, 1H), 12.55 (brs, 1H, exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 118.6, 118.9, 121.4, 122.8, 124.2, 127.2, 136.0, 145.2, 156.7, 159.1, 164.5, 174.3; IR (KBr): 3071, 2928, 2851, 1738, 1672 cm^{-1}; MS (ESI): *m/z* 317 $[M + Na]^+$ for Br = 79, 319 $[M + Na]^+$ for Br = 81; *Anal.* Calcd. for $C_{12}H_7BrO_4$: C, 48.84; H, 2.39. Found: C, 49.03; H, 2.38.



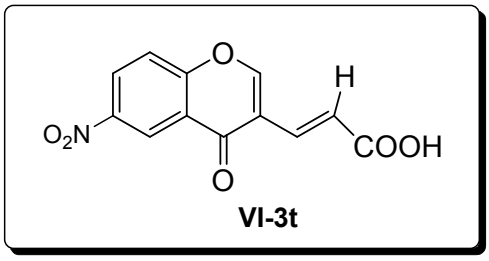
VI-3r

***Diethyl-2-((6-nitro-4-oxo-4H-chromen-3-yl)methylene)malonate (VI-3r)*:** Light yellow solid, yield: 90%; mp. 65-66 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.25-1.36 (m, 6H), 4.27-4.39 (m, 4H), 7.62 (d, J = 9.0 Hz, 1H), 7.68 (s, 1H), 8.37 (s, 1H), 8.54 (dd, J_1 = 2.8 Hz, J_2 = 8.9 Hz, 1H), 9.10 (d, J = 2.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 14.1, 14.2, 62.1 (2C), 119.9, 120.2, 123.2, 123.9, 128.6, 129.9, 131.7, 145.3, 156.5, 158.7, 163.6, 165.6, 173.8; IR (KBr): 3102, 1729, 1662 cm^{-1}; MS (ESI): *m/z* 384 $[M + Na]^+$. *Anal.* Calcd. for $C_{17}H_{15}NO_8$: C, 56.51; H, 4.18; N, 3.88. Found: C, 56.65; H, 4.17; N, 3.97.



VI-3s

***3-[(6-Nitro-4-oxo-4H-1-benzopyran-3-yl)-methylene]pentan-2,4-dione* (*VI-3s*):** Light brown solid, yield: 90%; mp. 107-108 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 2.24 (s, 3H), 2.37 (s, 3H), 7.39 (s, 1H), 7.96 (d, J = 9.3 Hz, 1H), 8.60 (dd, J_1 = 2.4 Hz, J_2 = 9.2 Hz, 1H), 8.70 (d, J = 2.4 Hz, 1H), 8.75 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 26.9, 31.2, 119.5, 121.4, 121.9, 123.5, 129.4, 131.1, 139.8, 145.3, 158.7, 160.1, 173.6, 198.3, 202.5; IR (KBr): 3068, 1677, 1630 cm^{-1}; MS (ESI): *m/z* 324 [M + Na]$^{+}$; *Anal.* Calcd. for $C_{15}H_{11}NO_6$: C, 59.80; H, 3.68; N, 4.65. Found: C, 59.68; H, 3.74; N, 4.69.

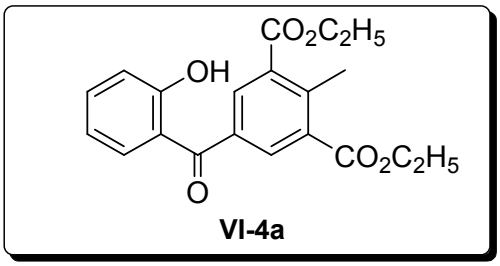


VI-3t

***(2E)-3-(6-nitro-4-oxo-4H-chromen-3-yl) acrylic acid (VI-3t)*:**[2d] Light almond color solid, yield: 93%; mp. 273-275 °C (Lit. 274-278 °C); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.12 (d, J = 15.8 Hz, 1H), 7.43 (d, J = 15.6 Hz, 1H), 8.00 (d, J = 8.0 Hz, 1H), 8.60 (dd, J_1 = 3.0 Hz, J_2 = 9.3 Hz, 1H), 8.80 (d, J = 2.8 Hz, 1H), 8.98 (s, 1H), 12.53 (s, 1H, exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 119.1, 121.5, 122.0, 122.9, 124.1, 129.1, 135.5, 145.2, 158.6, 160.7, 168.1, 175.0; IR (KBr): 2950, 1697, 1672 cm^{-1}; MS (ESI): *m/z* 284 [M + Na]$^{+}$. *Anal.* Calcd. for $C_{12}H_7NO_6$: C, 55.18; H, 2.70; N, 5.36. Found: C, 55.10; H, 2.71; N, 5.38.

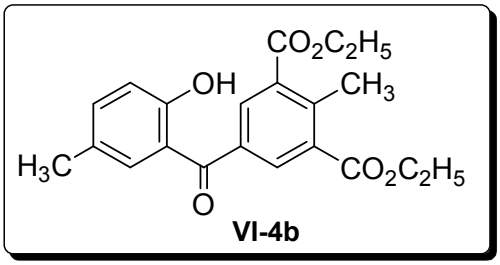
VI.4.3. General experimental procedure for the synthesis of benzophenone derivative (VI-4):

To a solution of CTAB (0.05 mmol) in H_2O (2 mL) were added a 3-formylchromone (0.5 mmol), DABCO (0.05 mmol), and an active methylene compound (1.1 mmol) successively at room temperature in a 10 mL round-bottom flask. The reaction mixture was sonicated for few min and then stirred at 40 °C for required time as listed in table 3. The crude product was extracted with ethyl acetate, washed with brine, dried over anhydrous Na_2SO_4 and the combined organic extracts were concentrated under vacuum. The crude product was purified by column chromatography (silica gel, 60-120 mesh) using 8-10% ethyl acetate in hexane as the eluent.



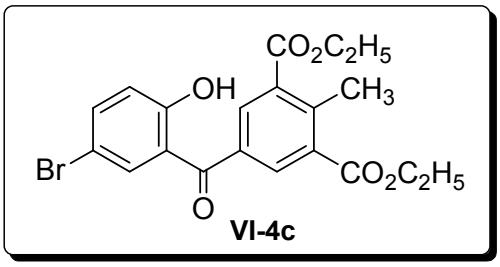
VI-4a

***Diethyl 5-(2-hydroxy benzoyl)-2-methyl-1,3-benzenedicarboxylate (VI-4a)*:**[9] Light yellow solid, yield: 82%; mp. 53-55 °C (Lit. 55-57 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.40 (t, J = 5.4 Hz, 6H), 2.80 (s, 3H), 4.40 (q, J_1 = 5.4 Hz, 4H), 6.90-6.93 (dt, J_1 = 0.8 Hz, J_2 = 8.8 Hz, 1H), 7.10 (dd, J_1 = 0.8 Hz, J_2 = 6.6 Hz, 1H), 7.53-7.56 (m, 2H), 8.20 (s, 2H), 11.85 (s, 1H, exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 14.2 (2C), 18.3, 61.7 (2C), 118.7, 118.8, 119.0, 132.9 (3C), 133.1, 133.4, 135.1, 136.8, 143.4, 163.3, 167.0 (2C), 199.4; IR (KBr): 2987, 1745, 1634 cm^{-1}; MS (ESI): *m/z* 379 $[M + Na]^+$; *Anal.* Calcd. for $C_{20}H_{20}O_6$: C, 67.41; H, 5.66. Found: C, 67.26; H, 5.68.



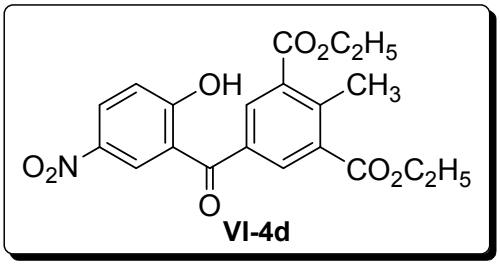
VI-4b

***Diethl-5-(2-hydroxy-5-methylbenzoyl)-2-methyl-1,3-benzenedicarboxylate* (*VI-4b*):** Light yellow solid, yield: 88%; mp. 75-77 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.42 (t, J = 8.0 Hz, 6H), 2.29 (s, 3H), 2.82 (s, 3H), 4.42 (q, J = 8.0 Hz, 4H), 7.02 (d, J = 8 Hz, 1H), 7.33 (d, J = 2.5 Hz, 1H), 7.38 (dd, J_1 = 2.5 Hz, J_2 = 8.0 Hz, 1H), 8.19 (s, 2H), 11.68 (s, 1H, exchangeable); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 14.2 (2C), 18.2, 20.5, 61.7 (2C), 118.4 (2C), 128.2, 132.7, 132.8 (2C), 133.3 (2C), 135.2, 137.9, 143.3, 161.2, 167.0 (2C), 199.3; IR (KBr): 2983, 1725, 1639 cm^{-1}; MS (ESI): *m/z* 371 $[M + H]^+$; *Anal.* Calcd. for $C_{21}H_{22}O_6$: C, 68.10; H, 5.99. Found: C, 67.89; H, 6.00.



VI-4c

***Diethl-5-(5-brmo-2-hydroxybenzoyl)-2-methyl-1,3-benzenedicarboxylate* (*VI-4c*):** Light yellow solid, yield: 83%; mp. 59-60 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.44 (t, J = 8.0 Hz, 6H), 2.86 (s, 3H), 4.44 (q, J = 8.0 Hz, 4H), 7.02 (d, J = 8.0 Hz, 1H), 7.65 (dd, J_1 = 1.5 Hz, J_2 = 8.0 Hz, 1H), 7.68 (d, J = 1.5 Hz, 1H), 8.19 (s, 2H), 11.76 (s, 1H, exchangeable); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 14.2 (2C), 18.3, 61.8 (2C), 110.6, 120.0, 120.7, 132.9 (3C), 133.6, 134.3, 135.0, 139.4, 144.1, 162.2, 166.7 (2C), 198.3; IR (KBr): 2979, 1725, 1634 cm^{-1}; MS (ESI) *m/z*: 457 $[M + Na]^+$ for Br = 79, 459 $[M + Na]^+$ for Br = 81; *Anal.* Calcd. for $C_{20}H_{19}BrO_6$: C, 55.19; H, 4.40. Found: C, 55.30; H, 4.44.



VI-4d

Diethl-5-(2-hydroxy-5-nitrobenzoyl)-2-methyl-1,3-benzenedicarboxylate **(*VI-4d*):** Light yellow solid, yield: 90%; mp. 75-76 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.42 (t, J = 8.0 Hz, 6H), 2.83 (s, 3H), 4.44 (q, J = 8.0 Hz, 4H), 7.24 (d, J = 8.0 Hz, 1H), 8.25 (s, 2H), 8.44 (dd, J_1 = 1.5 Hz, J_2 = 8.0 Hz, 1H), 8.59 (d, J = 1.5 Hz, 1H), 12.49 (s, 1H, exchangeable); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 14.2 (2C), 18.5, 61.9 (2C), 117.6, 119.8, 129.1, 131.3, 133.0 (3C), 133.5, 133.9, 139.6, 145.0, 166.5 (2C), 167.9, 198.4; IR (KBr): 2961, 1737, 1630 cm^{-1}; MS (ESI): *m/z* 424 $[M + Na]^+$; *Anal.* Calcd. for $C_{20}H_{19}NO_8$: C, 59.85; H, 4.77; N, 3.49. Found: C, 60.03; H, 4.79; N, 3.45.

VI.5. References

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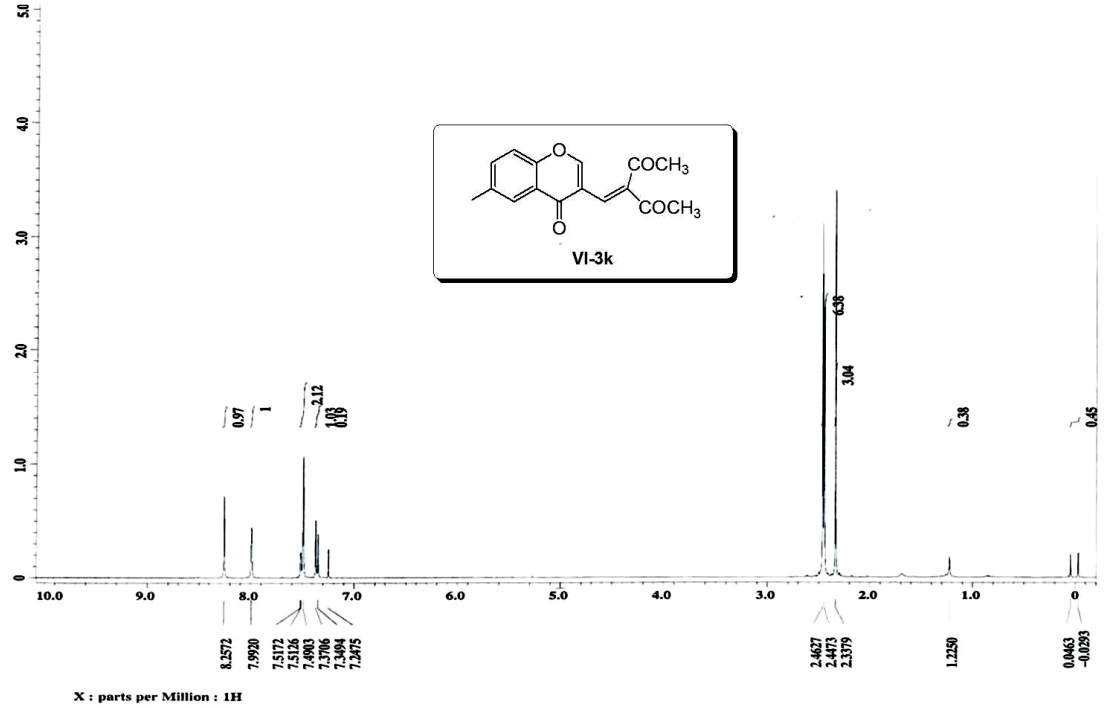
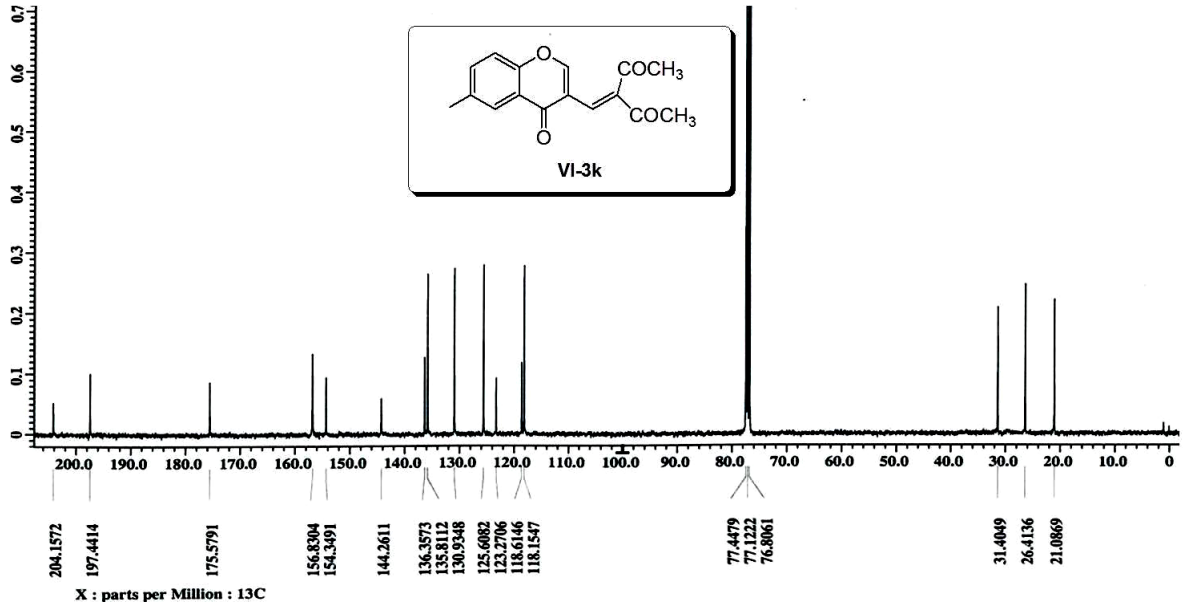
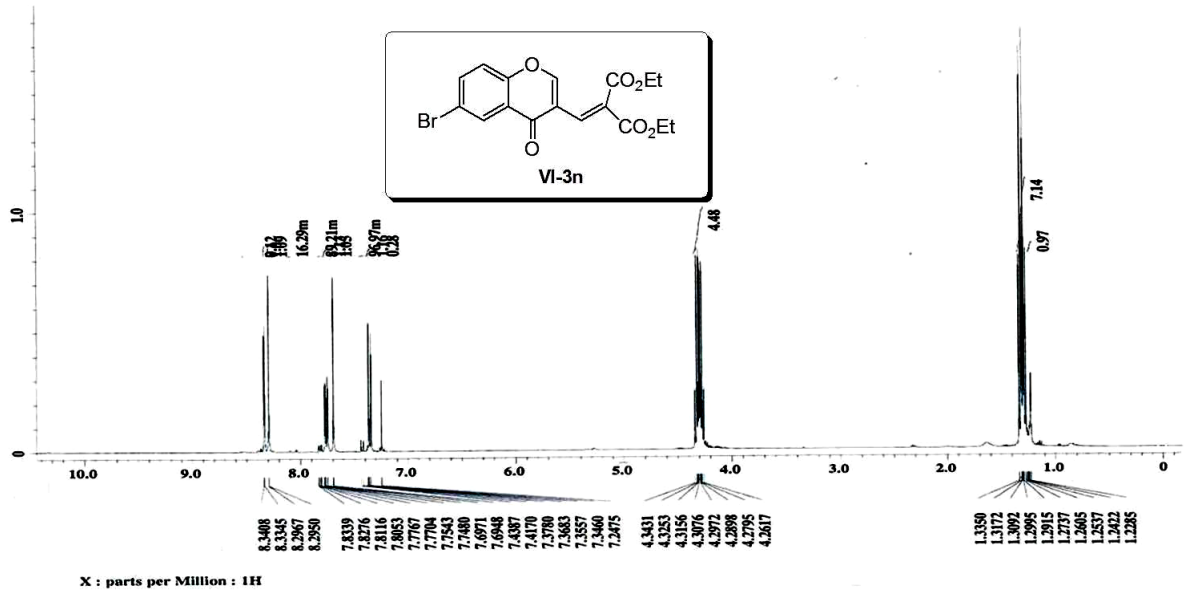
VI.6 . Supporting Information (selected spectra)



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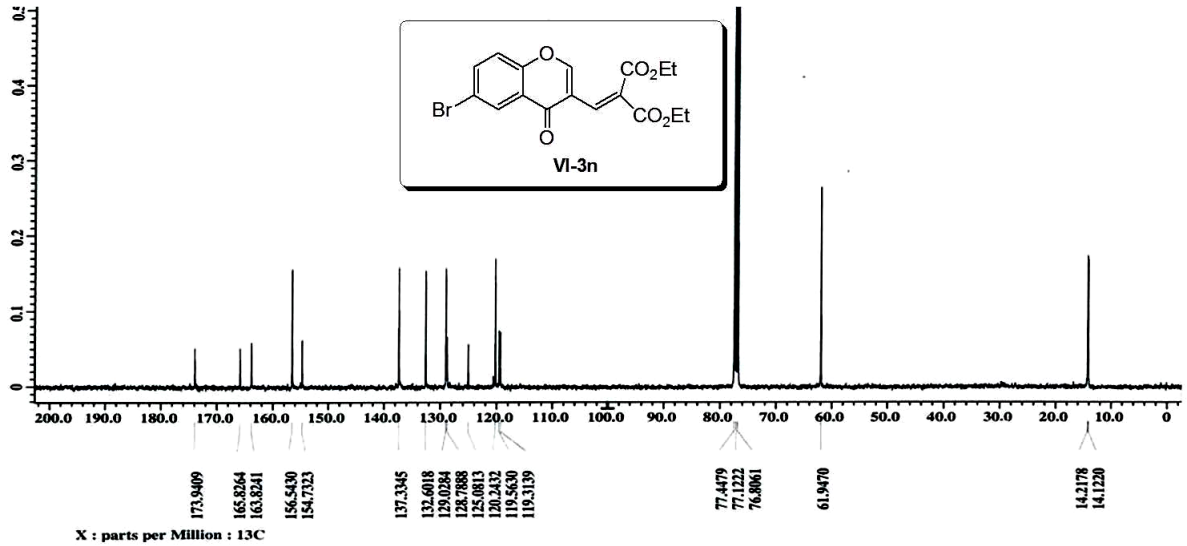
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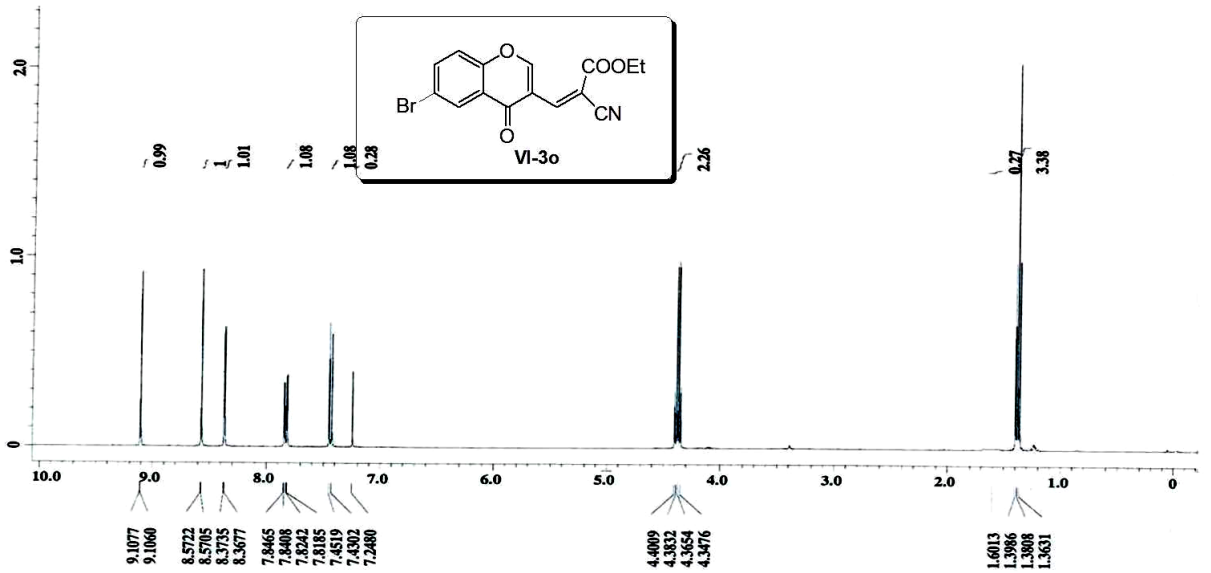
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O
COOEt
CN
O
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0.99
1.01
1.08
1.08
0.28
2.26
0.27
3.38
9.1077
9.1060
8.5722
8.5705
8.3735
8.3677
7.8465
7.8408
7.8242
7.8185
7.4519
7.4302
7.2480
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4.3654
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1.3631
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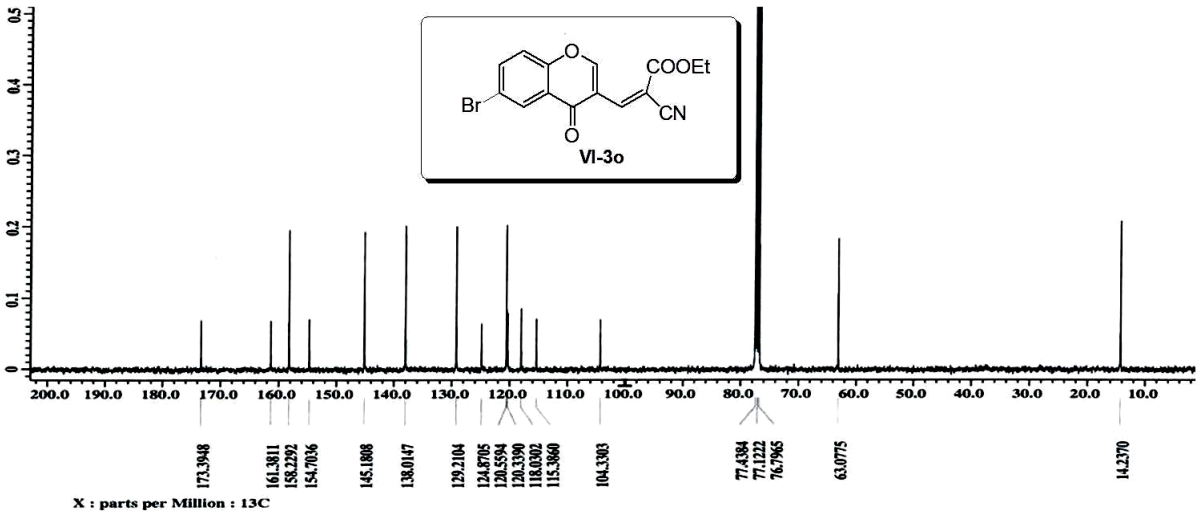
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COOEt
CN
O
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145.1808
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129.2104
124.8705
120.5594
120.3390
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104.3303
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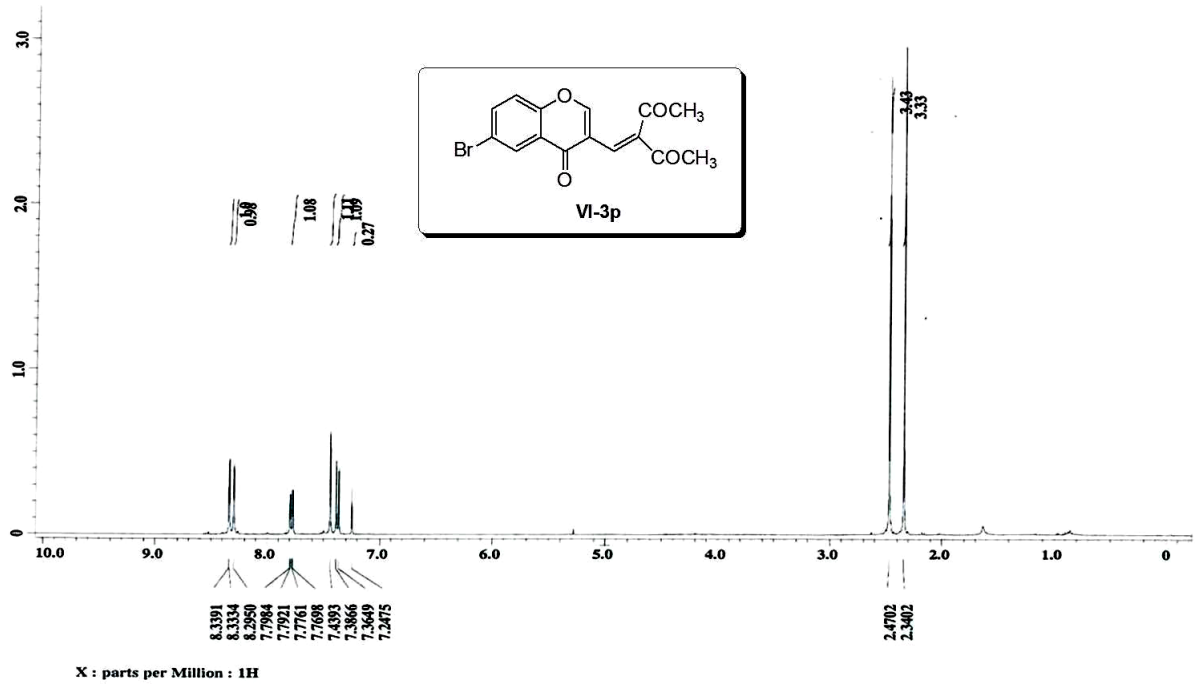
14.2370
X : parts per Million : 13C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O
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O
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1.08
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8.2950
7.7984
7.7921
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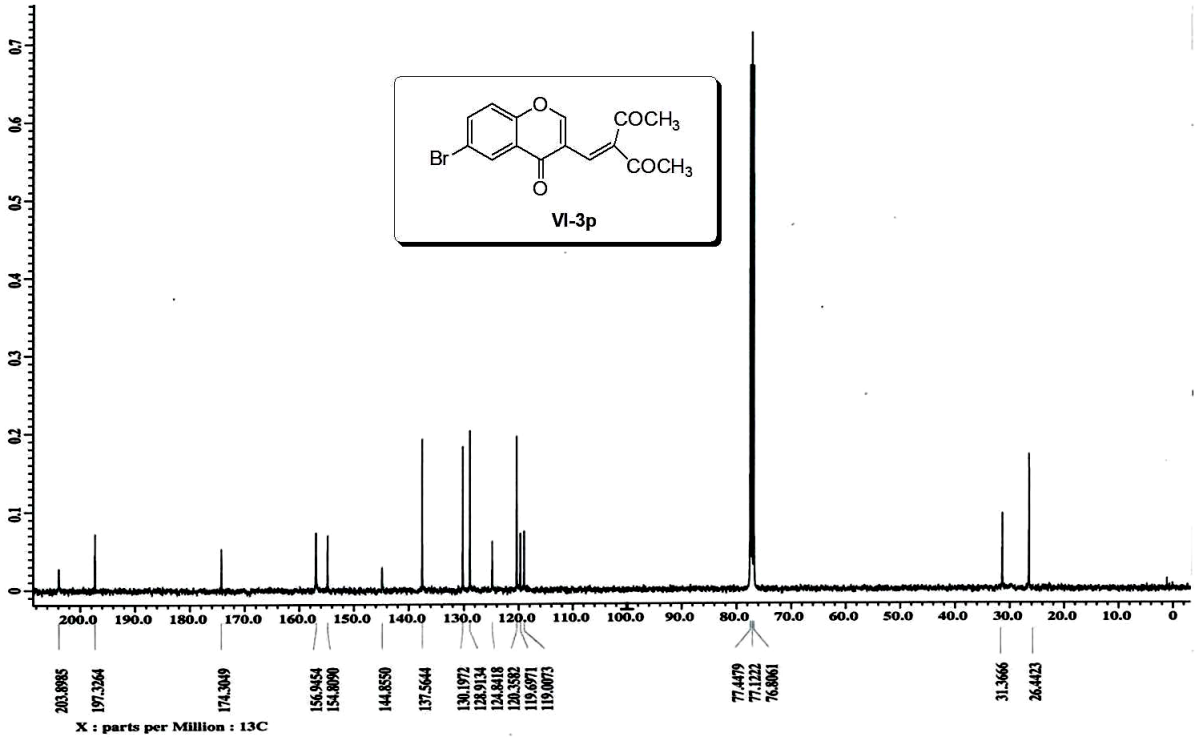
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O
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COCH3
O
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154.8090
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130.1972
128.9134
124.8418
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119.0073
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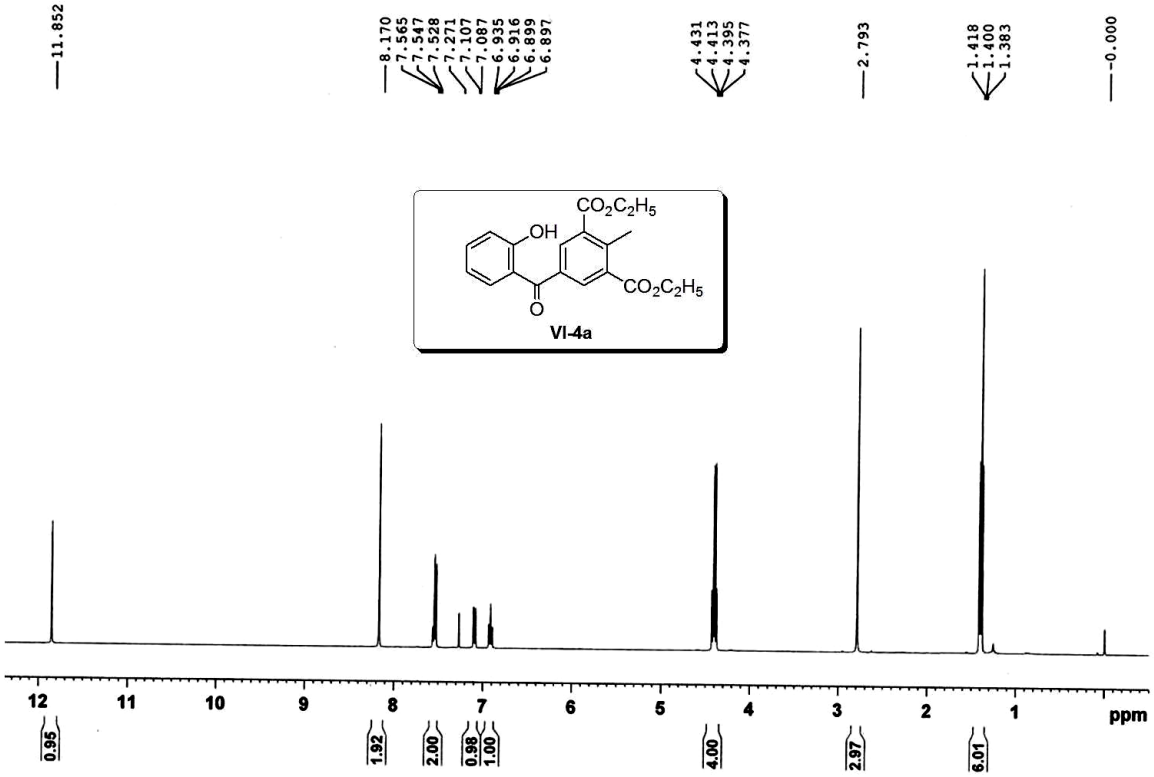
31.3666
26.4423
X : parts per Million : 13C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6.897
4.431
4.413
4.395
4.377
2.793
1.418
1.400
1.383
0.000
OH
CO2C2H5
CO2C2H5
O
VI-4a
12
11
10
9
8
7
6
5
4
3
2
1
ppm
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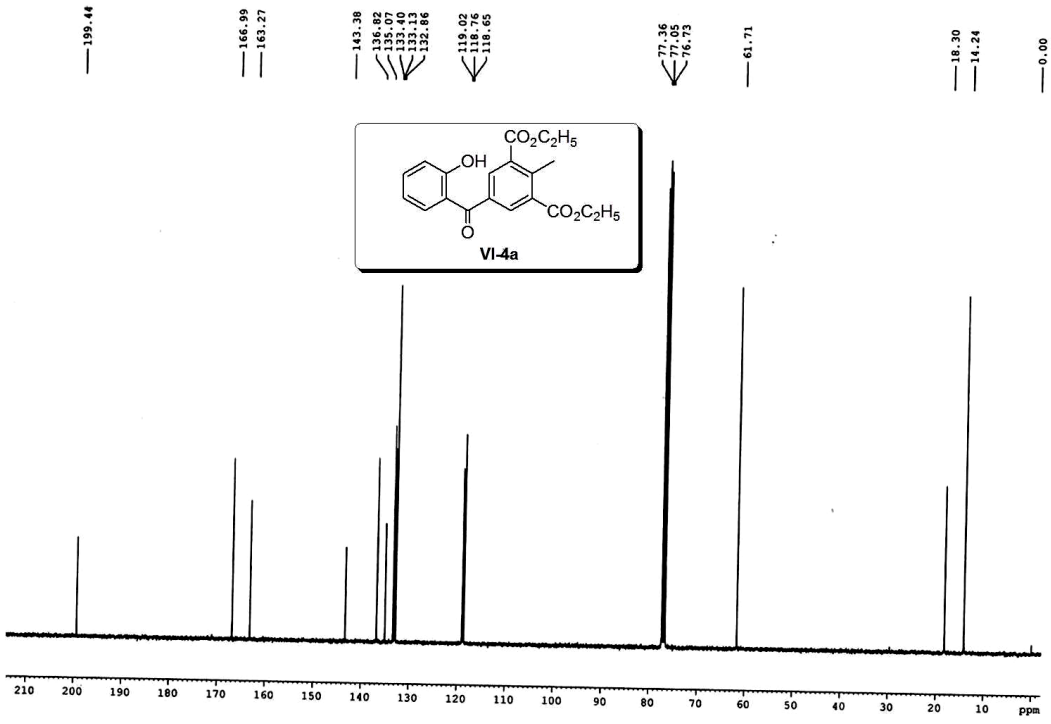
2.00
0.98
1.00
4.00
2.97
6.01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166.99
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143.38
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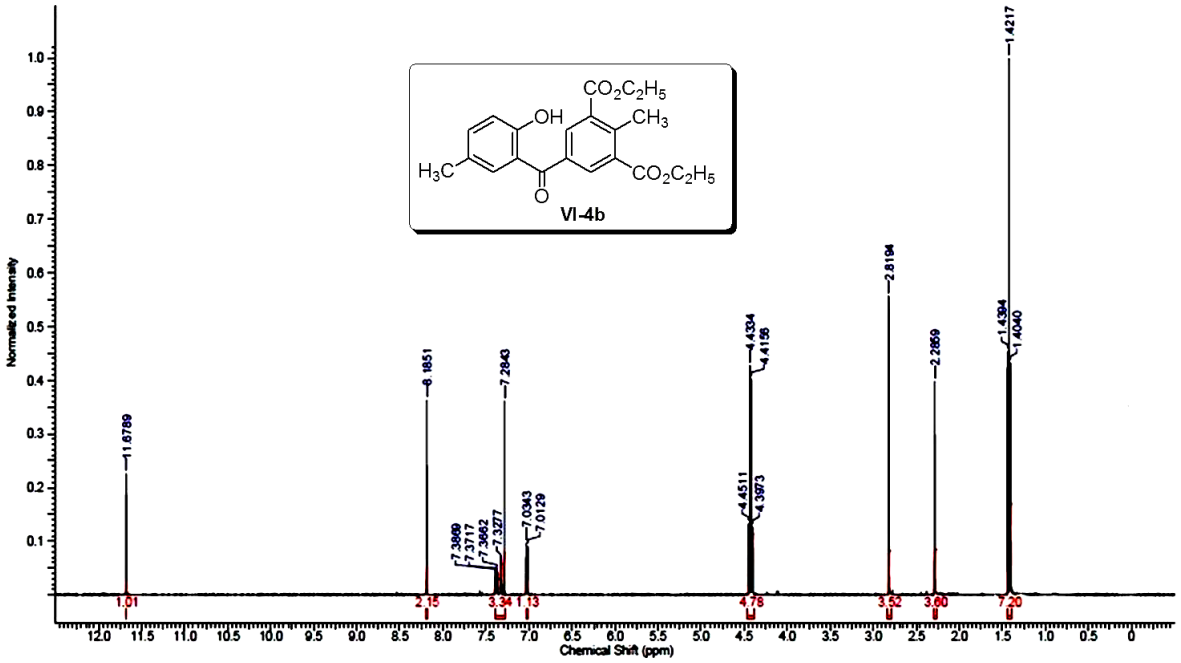
77.36
77.05
76.73
61.71
18.30
14.24
0.00
OH
CO2C2H5
CO2C2H5
O
VI-4a
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm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7.0343
7.0129
4.4511
4.4334
4.4156
4.3973
2.8194
2.2859
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1.4040
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1.13
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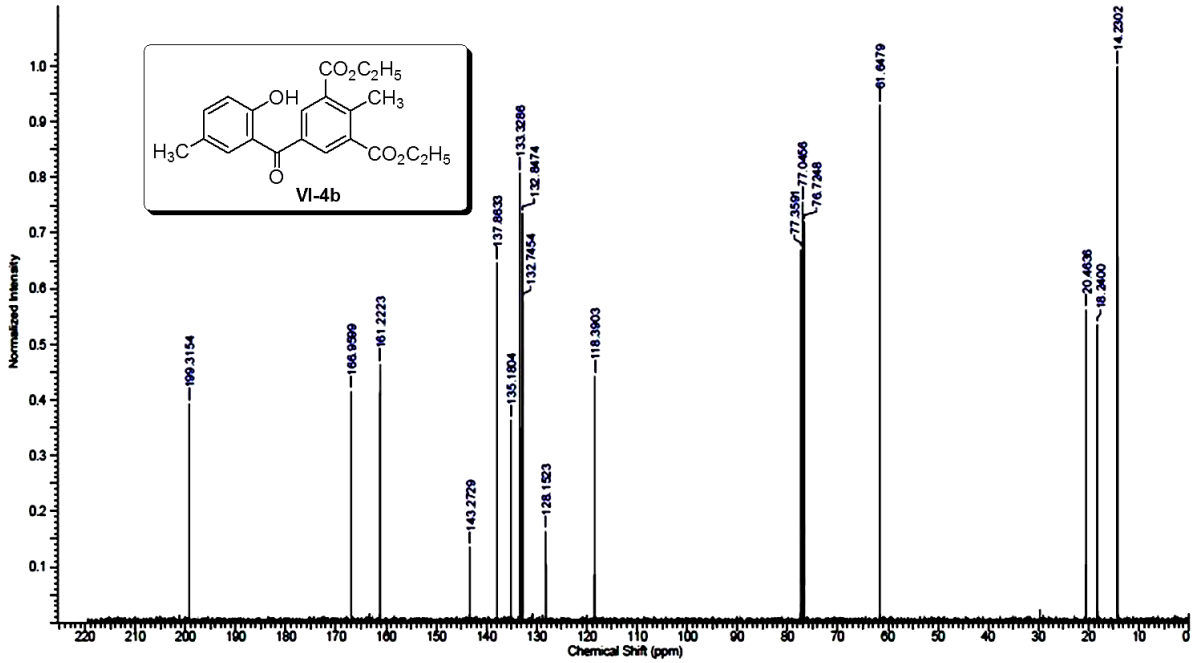
3.52
3.00
7.20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137.8633
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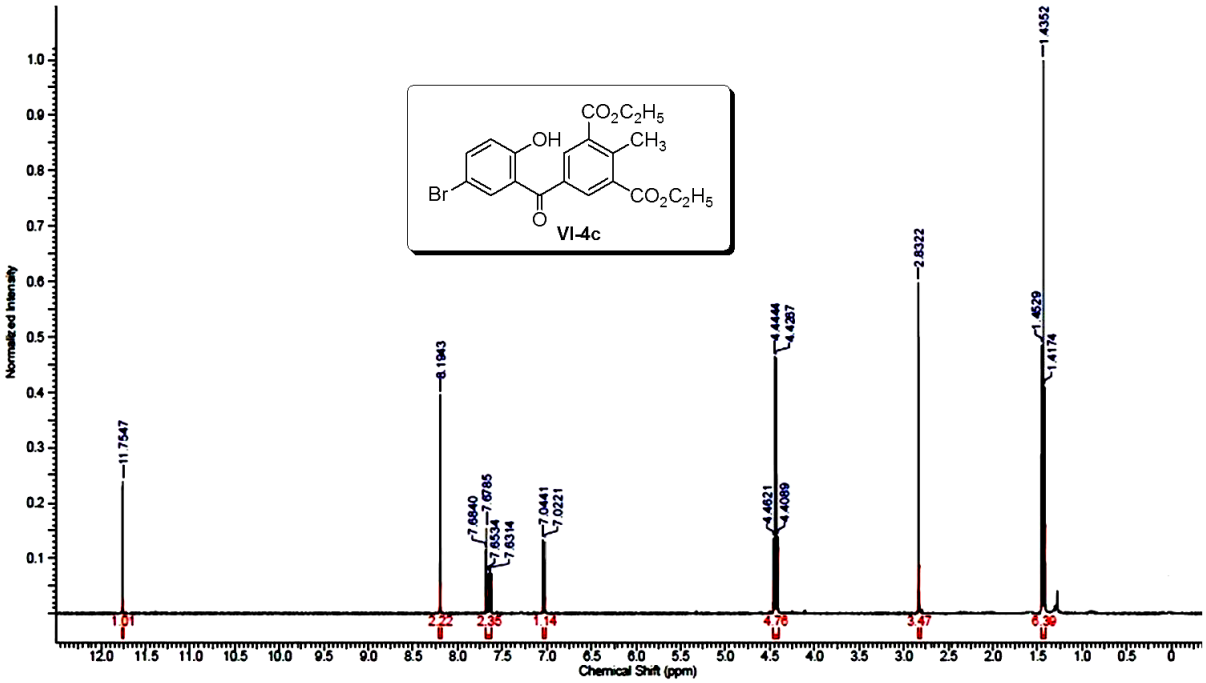
14.2302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7.0221
4.4444
4.4287
4.4521
4.4089
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1.01
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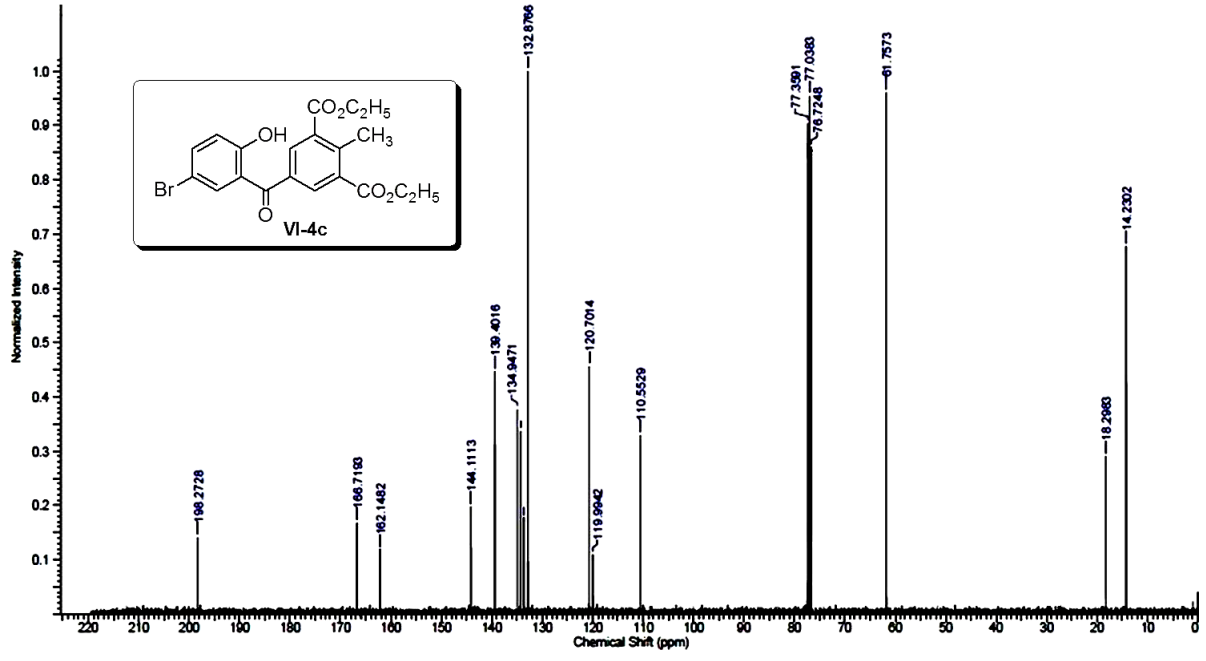
2.35
1.14
4.76
3.47
6.39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Normalized Intensity
Chemical Shift (ppm)
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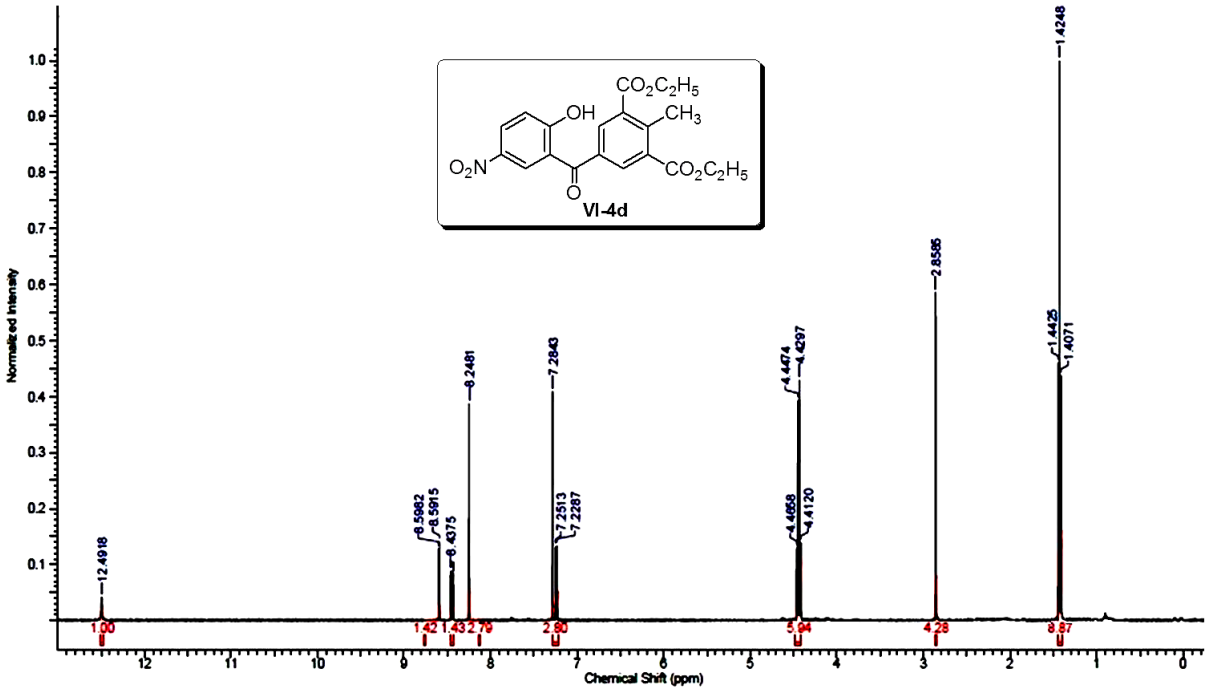
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Chemical Shift (ppm)
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4.4297
4.4058
4.4120
2.8585
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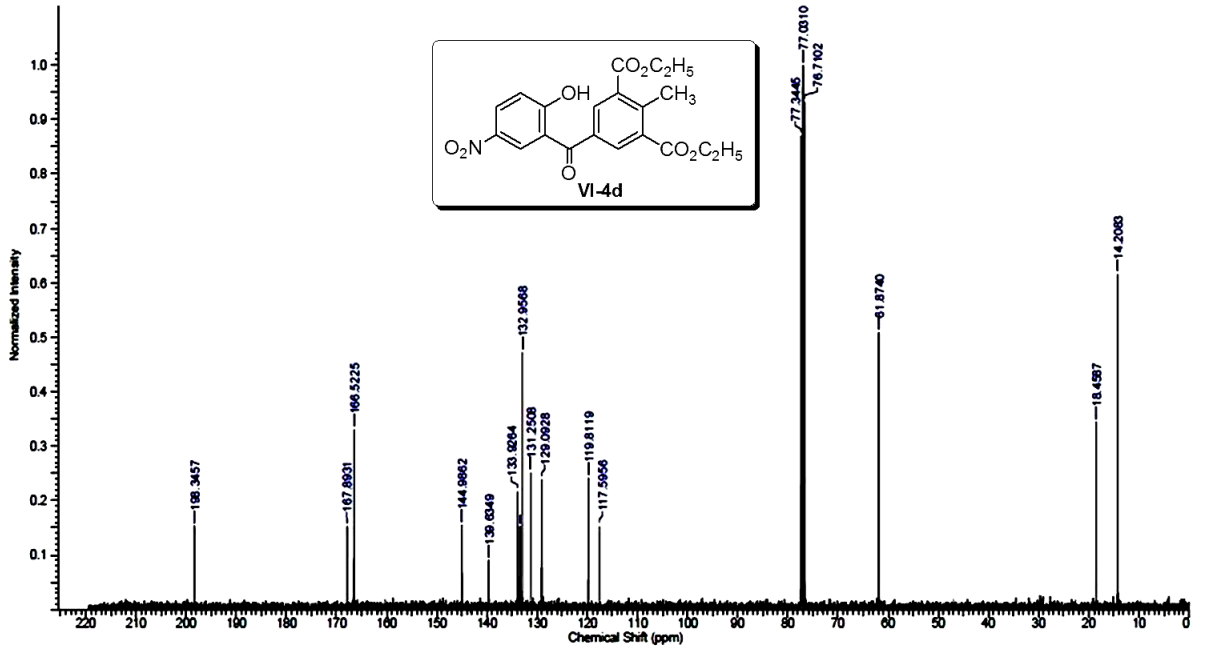
4.28
8.87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129.0928
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117.5956
77.3445
77.0310
76.7102
61.8740
18.4587
14.2068

CHAPTER VII

Conclusions and future scope of the work

VII.1. Conclusions

In the study of gemini amphiphiles, D-glucose based four compounds (**II-1a-d**) have been prepared. Apart from showing good surface and aggregation property, two of the gemini surfactants **II-1c** and **II-1d** showed good p-DNA binding capability at low N/P ratio. MTT assay also revealed that these gemini amphiphiles are relatively non-toxic (Chapter 2).

A simple methodology was also developed for the synthesis of three D-glucose based bolaamphiphiles and their surface property and catalytic activity in nitrone cycloaddition reaction have been explored, which lead to biologically active isoxazolidine derivatives. Out of three bolaamphiphile, **III-2c** has shown good aggregation morphology and catalytic activity because of the in-built chirality to get diastereoselective product, in comparison with commercial surfactant SDS (Chapter 3).

In another study, three water soluble cationic TPE-amphiphile with pyridinium polar head (**IV-6a-c**) have been synthesized using 4,4′-dihydroxybenzophenone as starting material and their protein and DNA binding efficiencies have been explored utilizing inherent AIE property of the TPE to act as fluorescent "light-up" bioprobes. One of the TPE amphiphiles viz. **IV-6c** showed good binding affinity with p-DNA, which has been confirmed by fluorimetric titration and gel electrophoresis experiment. MTT assay revealed that it can be used as staining agent for nucleic acid (Chapter 4).

To develop a sustainable methodology for chemoselective syntheses of 1,3-benzazoles, an environmentally benign method has been developed for the synthesis of 2-substituted benzimidazoles and benzothiazoles in organized aqueous media in the presence of DBSA as catalyst and iodine as co-catalyst (Chapter 5).

A green method has also been developed for the synthesis of different 3-vinylchromones in organized aqueous media in the presence of CTAB as surfactant and DABCO as catalyst using 3-formyl chromones. In the case of malonic acid and excess ethyl acetoacetate, Doebner decarboxylated products were obtained as major products (Chapter 6).

VII.2. Future scope of the work

- The synthetic methodology developed for gemini surfactants can be explored to synthesize different kinds of other gemini surfactants using C-3 functionality of glucose. Synthesized gemini surfactants can also be used to study their other applications.

- Bolaamphiphiles **III-2a-c** can also be used as surfactant catalyst in other nitrone cylcoaddition like Kinugasa reaction to get biologically active β-lactams with improved enantiomeric excess.

- TPE amphiphiles viz. **IV-6c** can also be used further for gene transfection study because of non-toxicity and good DNA binding affinity.

List of Publications:

Publications related to thesis:

1. V. Kumar, D. G. Khandare, A. Chatterjee and M. Banerjee, DBSA Mediated Chemoselective Synthesis of 2-Substituted Benzimidazoles in Aqueous Media, *Tetrahedron Lett.*, 2013, **54**, 5505.
2. V. Kumar, A. Chatterjee, N. Kumar, A. Ganguly, I. Chakraborty and M. Banerjee, D-Glucose Derived Novel Gemini Surfactants: Synthesis and Study of Their Surface Properties, Interaction with DNA and Cytotoxicity, *Carbohydr. Res.*, 2014, **397**, 37.
3. V. Kumar, A. Chatterjee and M. Banerjee, A Mild and Efficient Route to 3-Vinyl Chromones in Aqueous Micellar Media, *Synth. Commun.*, 2015, **45**, 2364.
4. V. Kumar, A. Chatterjee and M. Banerjee, "Synthesis of Novel D-Glucose based Anionic Bolaamphiphiles and Their Catalytic Application in 1,3-Dipolar Nitrone Cycloaddition Reactions" *Catal. Commun.*, 2017, **94**, 77.
5. V. Kumar, A. Chatterjee and M. Banerjee, "Synthesis of Tetraphenylethene-based AIE-Active Water Soluble Amphiphiles: Studies on Their Binding with Protein and DNA**"** (**Manuscript under preparation**).

Other publications:

1. V. Kumar, M. Banerjee and A. Chatterjee, A reaction based turn-on type fluorogenic and chromogenic probe for the detection of trace amount of nitrite in water, *Talanta*, 2012, **99**, 610.
2. D. G. Khandare, V. Kumar, A. Chattopadhyay, M. Banerjee and A. Chatterjee, An aggregation-induced emission based "turn-on" fluorescent chemodosimeter for the selective detection of ascorbate ions, *RSC Adv.*, 2013, **3**, 16981.
3. M. S. Majik, S. Tilvi, S. Mascarenhas, V. Kumar, A. Chatterjee and M. Banerjee, Construction and screening of 2-aryl benzimidazole library identifies a new antifouling and antifungal agent, *RSC Adv.*, 2014, **4**, 28259.
4. R. Pasumarthi, V. Kumar, S. Chandrasekharan, A. Ganguly, M. Banerjee and S. Mutnuri, Biodegradation of aliphatic hydrocarbons in the presence of hydroxycucurbit[6]uril, *Mar. Pollut. Bull.*, 2014, **88**, 148.

5. M. Banerjee, A. Chatterjee, V. Kumar, Z. T. Bhutia, D. G. Khandare, M. S. Majik and B.G. Roy, A simple and efficient mechanochemical route for the synthesis of 2-aryl benzothiazoles and substituted benzimidazoles, *RSC Adv.*, 2014, **4**, 39606.
6. Z. T. Bhutia, P. Geethika, A. Malik, V. Kumar, A. Chatterjee, B. G. Roy and M. Banerjee, *In-situ* mechanochemical synthesis of nitrones followed by 1, 3-dipolar cycloaddition: a catalyst-free, "green" route to *cis*-fused chromano[4,3-*c*]isoxazoles, *RSC Adv.*, 2015, **5**, 99566.

Conferences:

1. Vikash Kumar, Amrita Chatterjee and Mainak Banerjee "A reaction based fluorescent probe for the detection of nitrite in water" CARBO XXVI, Symposium on carbohydrate at the Interface of Chemistry and Biology, Indian Institute of Chemical Biology (CSIR), Jadavpur, Kolkata, 23-25 November,2011.

2. Vikash Kumar, Amrita Chatterjee and Mainak Banerjee "Sugar Based Bolaamphiphiles: From Morphological Studies to Catalytic Applications" International Conference on Global Trends in Pure and Applied Chemical Sciences (ICGTCS 2012), Asian Journal of Chemistry, Udaipur, Rajasthan, 3-4 March, 2012.

3. Vikash Kumar, Amrita Chatterjee and Mainak Banerjee. "D-Glucose Derived Novel Gemini Surfactants: Synthesis and Study of Their Surface Properties, DNA Binding, and Cytotoxicity", International Conference on Challenges in Chemistry and Biology of Carbohydrates (CARBO-XXVIII conference 2014), Forest Research Institute, Dehradun, 20-22 January, 2014.

4. Vikash Kumar, Amrita Chatterjee and Mainak Banerjee. "Design and synthesis of a novel class of D-glucose based bolaamphiphiles and their catalytic application in 1,3-dipolar nitrone cycloaddition reactions" Indo-UK International workshop on Advanced Materials and Their Applications in Nanotechnology (AMAN-2014), Department of Chemistry, BITS,Pilani-K. K. Birla Goa campus, 17-19 May, 2014.

5. Vikash Kumar, Amrita Chatterjee and Mainak Banerjee. "Tetraphenylethene - Based Water Soluble Fluorescent Probes having Aggregation Induced Emission (AIE) Characteristics for BSA, DNA Detection and their Quantitation" International Conference

on Nascent Developments in Chemical Sciences:Opportunities For Academia-Industry Collaboration (NDCS 2015), BITS,Pilani-Pilani campus, 16-18 October, 2015.

6. Vikash Kumar, Amrita Chatterjee and Mainak Banerjee. "Tetraphenylethene - Based Water Soluble Fluorescent Probes having Aggregation Induced Emission (AIE) Characteristics for BSA, DNA Detection and their Quantitation" National Conference on New Frontiers in Chemistry : From Fundamentals to Applications (NFCFA 2015), BITS,Pilani-K. K. Birla Goa campus, 18-19 December, 2015.

7. Vikash Kumar, Amrita Chatterjee and Mainak Banerjee. "Modular Synthesis of Tetraphenylethene-Based Aggregation Induced Emission (AIE) - Active Water Soluble Fluorescent Amphiphiles and Their Application on Binding and Quantitation of Protein and Plasmid DNA" Indo-UK International workshop on Advanced Materials and Their Applications in Nanotechnology (AMAN-2016), Department of Chemistry, BITS,Pilani-K. K. Birla Goa campus, 11-12 January, 2016.

BIODATA OF CANDIDATE

Personal details:

Name : Vikash Kumar

Date of Birth : 30-10-1978

Education : M. S. (Pharm.) (Medicinal Chemistry)
National Institute of Pharmaceutical Education and Research-Kolkata (NIPER-Kolkata), Jadavpur, Kolkata (2010)
B. Pharm., Dr. K.N.M.I.P.E.R., Modinagar, (UPTU, Lucknow) (2008)

Email : kumarniper@gmail.com

Scholarships and Awards:

1. Junior Research Fellowship, from Department of Science and Technology (DST), 27th January 2011-26th January 2013.
2. Senior Research Fellowship, from Department of Science and Technology (DST), 27th January 2013-31st March 2013.
3. Senior Research Fellowship, from Council of Scientific and Industrial Research (CSIR), 1st April 2013-31st March 2017.
4. Received fellowship from NIPER-Kolkata, Dept. of Pharmaceuticals, Govt. of India for M.S. (Pharm.): 2008-2010

Publications during Ph.D. Programme:

Listed in Appendix I.

BIODATA OF SUPERVISOR

Name	:	Dr. Mainak Banerjee
Current Position	:	Associate Professor Department of Chemistry Birla Institute of Technology and Science, Pilani - K. K. Birla Goa Campus. Zuarinagar, Goa - 403 726, India.
Date of Birth	:	03-04-1975
Education	:	Department of Chemistry, POSTECH, San 31, Hyoja Dong, Nam-Gu, Pohang-790784, Republic of Korea. Designation: **Postdoctoral Fellow,** Supervisor: Prof. Kimoon Kim (2007, Aug – 2009, Feb) Department of Chemistry Pusan National University, Busan- 609 735, Republic of Korea. Designation: **Postdoctoral Fellow** Supervisor: Prof. Ung Chan Yoon (2006, Aug – 2007, July) Ph.D. (Sc.) Jadavpur University (2006) (The experimental work was carried out in the Medicinal Chemistry division of IICB, CSIR, Kolkata-700032) M.Sc. (Chemsitry) University of Calcutta (1998)
Areas of Research	:	Heterogeneous catalysis Green chemistry Nanochemistry Method development
Email	:	mainak@goa.bits-pilani.ac.in mainak669@yahoo.co.in
Sponsored Research Projects	:	i) DST Fast Track Project for Young Scientists for the project "Redox-sensitive Vesicles of Cucurbit[7]urils as an Efficient Targeted Intracellular Drug Delivery Vehicle" (SR/FT/CS 023/2010, completed). Worth Rs. 24.15 lakhs as PI. ii) CSIR project "Synthesis of Metal-Organic-Porous-Material Based Efficient Chiral Heterogeneous Catalysts by Post-Synthetic Modification and Their Applications in Asymmetric Reactions" (project no. 02(0075)/12/EMR-II), worth 23 lakhs as PI (Co-PI Dr.

Rahul Banerjee of NCL Pune) (completed).

iii) CSIR project "Spectroscopic investigation of the properties of low-lying electronic states of some chemopreventive retinylnitrones and their comparison with retinyliminium ions" (project no. 01(2681)/12/EMR-II) worth 14 lakhs, as co-investigator (PI Dr. Anjan Chattopadhyay) (completed).

iv) CSIR project "Preparation of Magnetically Separable, Mesoporous TiO_2 Catalysts and Study of their Catalytic Properties" (project no. 02(147)/13/EMR-II), worth 16 lakhs as co-investigator (PI: Dr. Narendra Nath Ghosh).

v) Kurade Agro funded industrial project "Isolation of Natural Products from Kokum", as PI, Worth 2.5 lakhs, from 1/6/14-31/12/14 (completed).

Publications **:** 28 publications, Citations-653, h-index 12, i10-index 13

Key Publications:

1. First Total Synthesis of the 4a-Methyltetrahydrofluorene Diterpenoids (±) - Dichroanal B and (±) - Dichroanone, **Banerjee**, **M.**; Mukhopadhyay, R.; Achari, B.; Banerjee, A. K., *Org. Lett*., **2003**, *5*, 3931-3933.

2. A General Route to 4a-Methylhydrofluorene Diterpenoids: Total Syntheses of Taiwaniaquinones D and H, Taiwaniaquinol B, Dichroanal B and Dichroanone, **Banerjee**, **M.**; Mukhopadhyay, R.; Achari, B.; Banerjee, A. K. *J. Org. Chem.*, **2006**, *71*, 2787-2796.

3. Chiral Metal-Organic Porous Materials: Synthetic Strategies and Applications in Chiral Separation and Catalysis, Kim, K.; **Banerjee, M.**; Young, M.; Das, S., Invited review, *Top. Curr. Chem.*, **2010**, *293*, 115–153.

4. Postsynthetic Modification Switches an Achiral Framework to Catalytically Active Homochiral Metal-Organic Porous Materials, **Banerjee, M.**; Das, S., Young, M.; Choi, H. J., Huyn, M. H., Kim, K., *J. Am. Chem. Soc.,* **2009**, *131*, 7524–7525.

5. Supramolecular fishing for plasma membrane proteins using an ultrastable synthetic host-guest binding pair; Lee, D.-W.; Park, K. M.; **Banerjee, M**.; Ha, S. H.; Lee, T.; Suh, K.; Paul, S.; Jung, H.; Kim, J.; Selvapalam, N. Ryu, S. H.; Kim, K., *Nat. Chem.*, **2011**, *3,* 154–159.

6. DBSA Mediated Chemoselective Synthesis of 2-Sbustituted Benzimidazoles in Aqueous Media, Kumar, V.; Khandare, D. G.; Chatterjee, A.; **Banerjee**, **M.**, *Tetrahedron Lett.*, **2013**, *54*, 5505–5509.

7. D-Glucose Derived Novel Gemini surfactants: Synthesis and Study of Their Surface Properties, DNA Binding, and Cytotoxicity, Kumar, V.; Chatterjee, A.; Kumar, N.; Ganguly, A.; Chakraborty, I.; **Banerjee, M.**, *Carbhydr. Res.,* **2014**, *397*, 37–45.

8. Construction and screening of 2-aryl benzimidazole library identifies a new antifouling and antifungal agent, Majik, M. S.; Tilvi, S.; Mascarenhas, S.; Kumar, V.; Chatterjee, A.; **Banerjee, M.**, *RSC Adv*., **2014**, *4*, 28259–28264.

9. A simple and efficient mechanochemical route for the synthesis of 2-aryl benzothiazoles and substituted benzimidazoles, **Banerjee, M.**; Chatterjee, A.; Kumar, V.; Bhutia, Z. T.; Khandare, D. G.; Majik, M. S.; Roy, B. G., *RSC Adv*., **2014**, *4*, 39606-39611.

10. Amine functionalized tetraphenylethylene: A novel aggregation–induced emission based fluorescent chemodosimeter for nitrite and nitrate ions, Chatterjee, A.; Khandare, D. G.; Saini, P.; Chattopadhyay, A.; Majik, M. S.; **Banerjee, M.**, *RSC Adv.*, **2015**, *5*, 31479-31484.

11. A mild and efficient route to 3-vinylchromones in aqueous micellar media, Kumar, V.; Chatterjee, A.; **Banerjee, M**., *Synth. Commun.,* **2015**, *45*, 2364-2377.

12. Fluorescence Turn-on Chemosensor for the Detection of Dissolved CO_2 Based on Ion-Induced Aggregation of Tetraphenylethylene derivative, Khandare, D. G.; Joshi, H.; **Banerjee, M.**; Majik, M. S.; Chatterjee, A., *Anal. Chem.,* **2015**, *87*, 10871-10877.

BIODATA OF CO-SUPERVISOR

Name	:	Dr. Amrita Chatterjee
Current Position	:	Associate Professor Department of Chemistry Birla Institute of Technology and Science, Pilani - K. K. Birla Goa Campus. Zuarinagar, Goa - 403 726, India.
Date of Birth	:	09-12-1976
Education	:	Department of Chemistry and Center for *Electro-Photo Behaviors* in Advanced Molecular Systems, POSTECH, San 31, Hyoja Dong, Nam-Gu, Pohang-790784, Republic of Korea. Designation: **Postdoctoral Fellow,** Supervisor: Prof. Kyo Han ahn (2006, Jul–2009, March) Ph.D. (Sc.) Jadavpur University (2006) (The experimental work was carried out in the Medicinal Chemistry division of IICB, CSIR, Kolkata-700032) M.Sc. (Chemsitry) University of Calcutta (2000)
Areas of Research	:	Molecular Sensor Aggergation induced emission (AIE) based fluorescent sensor Nanoparticle based sensor Green Chemistry, Reactions in aqueous media.
Email	:	amrita@goa.bits-pilani.ac.in chatterjee.amrita@gmail.com
Sponsored Research Projects	:	DST Fast Track Project for Young Scientists for the project "Development of turn-on type Chemodosimer for Selective Recognition of Sulfhydryl-Containing Amino Acids and Peptides in aqueous media" (SR/FT/CS-092/2009, completed). Worth Rs. 19.86 lakhs as PI.
Publications	:	29 publications, Citations-741, h-index 13, i10-index 17

Key Publications:

1. Green synthesis of a benzothiazole based 'turn-on' type fluorimetric probe and its use for the selective detection of thiophenols in environmental samples and living cells, Khandare,

D. G.; Banerjee, M.; Gupta, R.; Kumar, N.; Ganguly, A.; Singh D.; **Chatterjee**, **A.**, *RSC Adv.*, **2016**, *6*, 52790-52797.

2. Fluorescence Turn-on Chemosensor for the Detection of Dissolved CO_2 Based on Ion-Induced Aggregation of Tetraphenylethylene derivative, Khandare, D. G.; Joshi, H.; Banerjee, M.; Majik, M. S.; **Chatterjee, A.**, *Anal. Chem.,* **2015**, *87*, 10871-10877.

3. Amine functionalized tetraphenylethylene: a novel aggregation-induced emission based fluorescent chemodosimeter for nitrite and nitrate ions, **Chatterjee**, **A.**; Khandare, D. G.; Saini, P.; Chattopadhyay, A.; Majik, M. S.; Banerjee, M., *RSC Adv*., **2015**, 5, 31479-31484.

4. An aggregation-induced emission based "turn-on" fluorescent chemodosimeter for the selective detection of Pb^{2+} ions, Khandare, D. G.; Joshi, H.; Banerjee, M.; Majik, M. S.; **Chatterjee, A.**, *RSC Adv*., **2014**, 4, 47076-47080.

5. A Reaction Based Turn-On Type Fluorogenic and Chromogenic Probe for the Detection of Trace Amount of Nitrite in Water, Kumar, V.; Banerjee, M.; **Chatterjee, A**., *Talanta*, **2012**, *99*, 610-615.

6. Selective Fluorogenic and Chromogenic Probe for Detection of Silver Ions and Silver Nanoparticles in Aqueous Media, **Chatterjee, A**.; Santra, M.; Won, N.; Kim, S.; Kim, J. K.; Kim, S. B.; Ahn, K. H., *J. Am. Chem. Soc.*, **2009**, *131*, 2040–2041.

7. A chemodosimeter approach to fluorescent sensing and imaging of inorganic and methylmercury species, Santra, M.; Ryu, D.; **Chatterjee, A**.; Ko, S.-K.; Shin, I.; Ahn, K. H., *Chem. Commun*., **2009**, 2115–2117.

8. Water Exclusion Reaction in Aqueous Media: Nitrone Formation and Cycloaddition in a Single Pot, **Chatterjee, A**.; Maiti, D. K.; Bhattacharya, P.K., *Org. Lett*., **2003**, *5*, 3967-3969.

9. A Green Chemical Approach for the *N*-alkylation of Aldoximes to form Nitrones in Organized Aqueous Media and their *in situ* Cycloaddition with Olefins. Hota, S. K.; **Chatterjee, A**.; Bhattacharya, P. K.; Chattopadhyay, P., *Green Chem.*, **2009**, *11*, 169–176.