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SYNTHESIS AND CHARACTERIZATION OF PERFLUORINATED AMPHIPHILIC 1,2,3-TRIAZOLES COMPOUNDS

by

Suat TÜYSÜZ

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APPROVAL PAGE

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Suat TÜYSÜZ

M. S. Thesis – Chemistry
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Supervisor: Assist. Prof. Dr. Sedat COŞGUN

ABSTRACT

A series of analogues of perfluorinated amphiphilic 1,2,3-triazole compounds have been synthesized. 2-perfluoroalkyl-1-iodoethanes were used as starting materials Because of the destabilising influence of the perfluoro group on the 2-methylene protons, These products are substrates for which the nucleophilic substitution of the iodides becomes delicate. However, using the azide ion in the phase transfer method and well-adapted experimental conditions allows its substitution with good yields and Perfuoroalkylated triazole obtained in this way are subsequently used as hydrophobic moieties for the synthesis of amphiphilic peptides derivatives. To examine the physicochemical properties such as surface tension and critical micelle concentration, several tests have been performed. Measurements on a very large scale analogues of the perfluorinated hydrophilic segment (amino acids) have shown how the surface tension of aqueous solutions of the compounds synthesized reveal their surfactant properties. Antimicrobial activity tests have also showed how these compounds are efficient on several bacteria and fungi as biocides.

Keywords: Amphiphile, 1,2,3-Triazole, Peptide, Aminoacid Based, Surfactant Behavior, Surface Tension, Critical Micelle Concentration, Antioxidant.

PERFLORLU AMFİFİLİK 1,2,3-TRİAZOL BİLEŞİKLERİNİN SENTEZİ VE KARAKTERİZASYONU

Suat TÜYSÜZ

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ÖZ

Perflorlanmış amfifilik 1,2,3-triazol bileşiklerinin bir dizi analogları sentezlenmiştir. 2-perfloralkil-1-iodoetan başlangıç malzemeleri olarak kullanılmıştır. Çünkü metile protonlara perfloro grup denge bozucu etkisi nedeniyle, bu ürünlerin iyodürler arasında nükleofilik yer değiştirme kolayca gerçekleştirmektedir. Bununla birlikte, deney koşullarında azid iyonu faz transfer yöntemi iyi adapte olup iyi verim sağlamaktedır. Bu şekilde elde edilen Perfuoroalkylated amfifilik triazol sonradan peptidler türevlerinin sentezi hidrofobik kısımların olarak kullanılır. Fizikokimyasal özelliklerinin belirlenmesi için yüzey gerilimi ve kritik misel konsantrasyonu gibi bir çok test ile karakterize edildi. Perflorlanmış hidrofilik kısımlı (amino asit) arasında çok büyük bir ölçekte analogları ile ilgili ölçümler sentezlenen bileşiklerin sulu çözeltileri arasında yüzey gerilimi kendi yüzey aktif madde özelliklerini nasıl olduğu göstermiştir. Anti-mikrobiyal etkinlik testleri de bu bileşiklerin biyosit gibi çeşitli bakteri ve mantarlar üzerinde nasıl etkilediğini göstermiştir.

Anahtar Kelimeler: Amfifil, 1,2,3-Triazole, Peptit, Aminoasit,Baz, Yüzey Davranış, Yüzey Gerilimi, Kritik misel Konsantrasyon, Antioksidan.

This thesis is dedicated to my wife

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LIST OF SYSMBOLS AND ABBREVIATIONS

SYMBOL/ABBREVIATION

ATR	:	Attenuated Total Reflectance
BOP	:	Benzotriazolyloxytrisdimethylammonium
CMC	:	Critical Micelle Concentration
DCM	:	Dichloromethane
DMF	:	Dimethylformamide
DMSO	:	Dimethylsulfoxide
Et_3N	:	Triethylamine
EBSD	:	Electron Backscatter Diffraction
EPMA	:	Electron Probe Micro-Analyzer
EtOAc	:	Ethylacetate
EtOH	:	Ethanol
FT – IR	:	Fourier Transform Infrared Spectroscopy
MeOH	:	Methanol
MIC	:	Minimum Inhibitory Concentration
NMR	:	Nuclear Magnetic Resonance
POM	:	Polarized Optical Microscopy
SEM	:	Scanning Electron Microscopy
THF	:	Tetrahydrofuran
TLC	:	Thin Layer Chromatograph

CHAPTER 1

INTRODUCTION

1.1 GENERAL INFORMATION ABOUT THE SURFACTANTS

Surfactants are wetting agents that lower the surface tension of a liquid, allowing easier spreading, and lower the interfacial tension between two liquids. Surfactants reduce the surface tension of water by absorbing at the liquid-gas interface. They also reduce the interfacial tension between oil and water by absorbing at the liquid-liquid interface. Many surfactants can also assemble in the bulk solution into aggregates. Examples of such aggregates are vesicles and micelles.

A vesicle is a relatively small, intracellular, membrane-enclosed structure that stores or transports substances within a cell. Vesicles form naturally because of the properties of lipid membranes; and a micelle is an aggregate of surfactant molecules dispersed in a liquid colloid. A typical micelle in aqueous solution forms an aggregate with the hydrophilic "head" regions in contact with surrounding solvent, sequestering the hydrophobic tail regions in the micelle centre. The most important term about the micelle formation is the critical micelle concentration which will be discussed in the following pages.

1.2 AMPHIPHILIC MOLECULES

Amphiphile is a term describing a chemical compound possessing both hydrophilic and hydrophobic properties. Such a compound is called amphiphilic or amphipathic. Conventional amphiphiles contain a polar head group and one or two hydrophobic tails. The polar head can be a range of functional groups, including a carboxylic acid, an amino acid, a sugar, a phosphate or a quaternary amine. Similarly, there is much variation in the number, length, and flexibility (saturation, cyclic etc.) of the hydrophobic tails (Estrof, L.A.; Hamilton, A. D.; 2004).

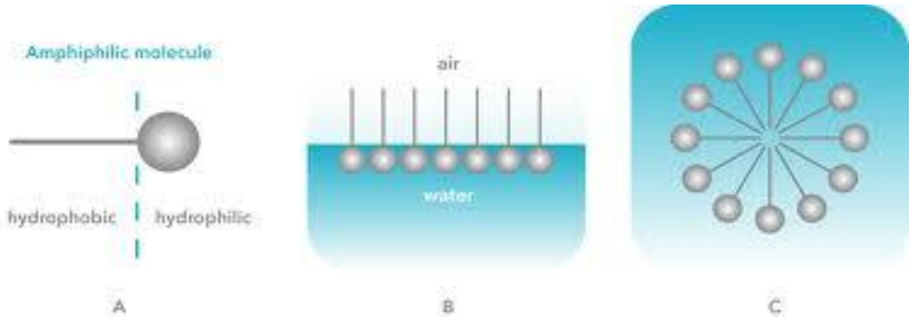


Figure 1.1 Amphiphilic molecule design imaging.

Amphiphilic molecules such as surfactants and lipids are made up of a hydrophobic tail and a hydrophilic head group. Depending on the chemical structure of the molecule, as well as solution properties such as ionic strength, temperature and composition in mixed systems, they may self-assemble to form a variety of different structures with re-spect to size, shape, flexibility, polydispersity etc. It is possible to rationalize and predict the structural behaviour of self-assembled structures (micelles, vesicles, lyotropic liquid crystalline phases, perforated bilayers, microemulsions etc) formed by amphiphilic molecules (Kunitake et al.; 1981).

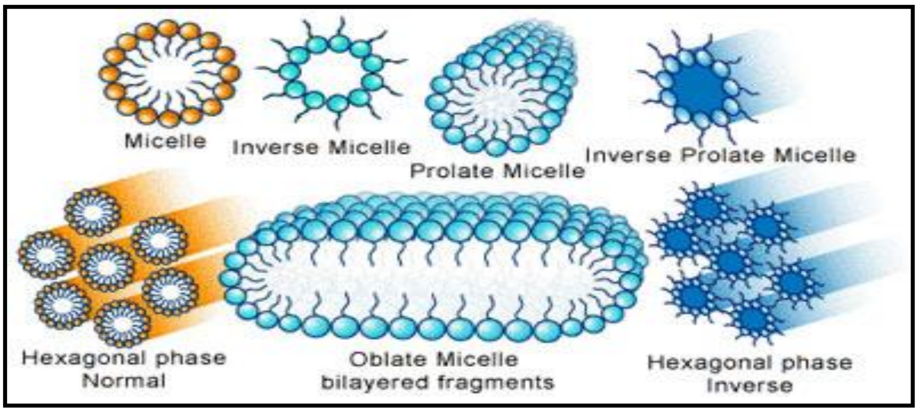


Figure 1.2 Amphiphilic molecule behaviour of self-assembled structures.

In a survey of over 60 amphiphiles, they identified a number of possible aggregate morphologies, including globules, vesicles, lamella, roads, tubes and disks. They identified the flexible tail, rigid segment and hydrophilic head groups as essential elements for stable self- assemblies (Kunitake et. al.; 1981).

Amphiphilic molecules namely surfactants, appear as suitable candidates, as they can form well- characterized supramolecular structures (Gérardin et. al.; 2010). When appropriately grafted with amino acid moieties they may conduct to stimuli-sensitive hydrogels through weak noncovalent interactions between gelator molecules and entrapped solvent molecules (Fu, X. et. al.; 2007 & Suzuki, M. et. al.; 2005).

Amino acid-based amphiphilic gelators have been paid much attention in recent past because of their biocompatibility (Khatua, D.; 2006), biodegradability in nature (Wang, C. et al.; 2011), and eco-friendly nature (Kaneko, D. et al.; 2002). A low molecular weight compound in organic solvent due to obtain gelator molecules depends on strongly on the structural fragments of the molecule. Therefore amphiphilic molecules are ideal for this reason because of their characteristic structure (Mohmeyer, N. and Schmitt, H.W.; 2005).

1.3 FLUORINATED SURFACTANTS

Perfluorocarbons, surfactants where all the hydrogens in the hydrophobic moiety have been replaced by fluorine, have been much less studied than the corresponding hydrogenated surfactants, despite their technical interest due to their potential usefulness (Ogita, M.; 1998). Fluorine is the most electronegative of all elements, and it's dense electron cloud has very low polarizability. On the other hand, the reduced conformational freedom allowed to perfluorinated tails produces bulky and stiff chains; hence the aggregates of perfluorinated amphiphiles have a tendency to form a structure with less surface curvature (Sengers, J.V.; 2000). These properties confer special characteristics on these amphiphiles compared with the corresponding hydrogenated ones such as great compressibilities and viscosities but lower internal pressures and refractive index (Krafft, M.P.; 1998).

However, the best known relationship between these molecules is the one that refers to their cmcs: the cmc of a perfluorinated surfactant is approximately equal to that of a hydrocarbon surfactant with a hydrocarbon chain 1.5 times longer, at a fixed temperature, than the fluorocarbon chain (Matsuoka, K.; 2003). A comparative study of perfluorinated and nonfluorinated surfactant systems is interesting in many ways for example, from a theoretical point of view hese two systems can be used to study the hydrophobic effect or to try to predict a priori the physicochemical properties of the perfluorinated compounds on the basis of the properties of their corresponding hydrogenated counterparts.

1.4 CLASSIFICATION OF FLUORINATED SURFACTANTS

Most commonly, surfactants are classified according to polar head group. A non-ionic surfactant has no charge groups in its head. The head of an ionic surfactant carries a net charge. If the charge is negative, the surfactant is more specifically called anionic; if the charge is positive, it is called cationic. If a surfactant contains a head with two oppositely charged groups, it is termed zwitterionic.

1.4.1 Anionic Fluorinated Surfactants

Ionic surfactants dissociate in water and form a surface active ion with an oppositely charged counter ion. The surface active ions of anionic surfactants bear a negative charge. Anionic fluorinated surfactants bear a negative charge. Anionic fluorinated surfactants can form water insoluble ion pairs with cationic species and are usually not compatible with most cationic surfactants. Anionic surfactants are the most important class of fluorinated surfactants. Based on the hdrophile structure, anionic fluorinated surfactants can be divided into four main categories:

Carboxylates	$R_fCOO^-M^+$
Sulfonates	$R_fSO_3^-M^+$
Phosphates	$R_fOP(O)O_2^{2-}M_2^+$

Where R_f is a fluorine containing hydrophobe and M^+ an inorganic or an organic cation.

1.4.2 Cationic Fluorınated Surfactants

In cationic fluorinated surfactants the fluorinated hydrophobe is attached directly or indirectly to a quaternary ammonium group, a protonated amino group, or a heterocyclic base. Cationic surfactants dissociate in water, forming a surface active positively charged ion and a negatively charged counter ion. Like anionic surfactants, cationic surfactants are sensitive to electrolytes and pH of the medium. Cationic surfactants are usually incompatible with anionic surfactants. Cationic fluorinated surfactants adsorb on negatively charged surfaces. Because most surfaces and particles are negatively charged, the adsorption can be advantageous or disadvantageous, depending on the intended use of the surfactant. For example, adsorption on clay and sludge in wastewater cleaning systems simplifies the removal o cationic fluorinated surfactants from effluents (Kissa, E.; 1994).

Perfloroalkylamides: $C_7F_{15}CONH(CH_2)_3N^+CH_3I^-$

Ester linkages: $C_6F_{13}SO_2O(CH_2)_3N^+(CH_3)_3I^-$

1.4.3 Zwitterionic Fluorınated Surfactants

Zwitterionic (amphoteric) surfactants have both cationic and anionic centers attached to the same molecule. The cationic part is based on primary, secondary, or tertiary amines or quaternary ammonium cations.

Zwitterionic surfactants (amphoteric surfactants) are characterized by having two distinct and opposite charges on the molecule at either adjacent or non-adjacent sites. The presence of both a positive and negative charge renders the molecule overall neutrally-charged at neutral pH. Some types of zwitterions are susceptible to pH changes in a solution and may become completely cationic or anionic in acidic or basic environments. The positively-charged site is typically a quaternized ammonium ion, but can also be a phosphonium ion, while the negatively-charged site can be one of a variety of anionic groups, such as sulfate, carboxylate, or sulfonate. There are several common categories of zwitterions used in hair care formulations, such as the betaines and amphoacetates (Banat, I. M.; 1995).

Dodesil betain $C_{12}H_{25}N^+(CH_3)_2CH_2COO^-$

1.5 DISTINCT PROPERTIES OF FLUORINATED SURFACTANTS

Due to effective overlapping of orbitals, the C–F bond is the most stable single bond found in organic chemistry (485 kJ/mol as compared to 425 kJ/mol for a standard

C–H bond). The bond strength further increases with increasing fluorine substitution, and was reported to reach 531 kJ/mol in terminal C-F groups (Kissa, E.; 2001). As a consequence, fluorocarbons are thermally and chemically very stable. They are also inert biologically. Furthermore, the dense electron cloud of the fluorine atoms results in a repellent sheath that protects the perfluorinated chain against the approach of reagents (Figure 1.3).

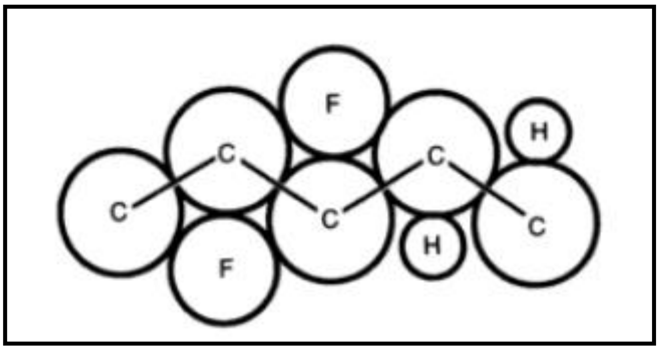


Figure 1.3 Steric effects of fluorocarbons.

1.5.1 Thermal Stability

The stability of fluorinated surfactants results from a strong C-F bond and effective shielding of carbon by fluorine atoms. The atomic radius covalently bonded fluorine is only 0.72 angstrom. Because of their small size, fluorine atoms can shield a perfluorinated carbon atom without steric stres (Fletcher, P. D. I.; 1997). Although the unique chemistry of fluorinated organic compounds is not completely understood, the stability of the C-F bond is very important in industrial applications.

The thermal stability of fluorinated surfactants is based on the outstanding stability of the fluorocarbon hydrophobe. Fluorocarbons are usually more stable than the corresponding surfactants (Riess, J.G.; 1994). However, hydrophilic functional groups exist on the structure of the fluorinated surfactants are usually less stable than the fluorocarbon group and may lower the thermal stability of the fluorinated surfactant. Hence, not all fluorinated surfactants are thermally stable. Perfluoroalkanecarboxylic acids and perfluoroalkanesulfonic acids are the most stable fluorinated surfactants.

1.5.2 Critical Micelle Concentrations

The critical micelle concentration of a surfactant solution can be determined by measuring surface tension, electrical conductivity, osmotic pressure, light scattering, viscosity, dye solubilization and other physical properties (Dekker, M.; 1997). Also new techniques are developing to determine the cmc in aqueous solutions such as using optical fibers, plastic cladding silica fibers (PCS) and plastic optical fiber (POF) (Muller, N.; 1967).

In analogy to surfactants with a hydrocarbon chain, the critical micelle concentrations for fluorinated surfactants in aqueous solution depend on the fluorocarbon chain length and the counter ion and decrease with increasing hydrophobe chain length.

The greater hydrophobicity of the fluorocarbon chain enhances the amphibilic character of the surfactant and the increases surface activity, reflected in surface tensions below 20 dyn / cm (mN/m) and lower cmc values. Perfluorination reduces cmc values about 3-4 times per $–CF_2$- group. The A value does not change considerably with the type of the surfactant. Because the relation expressed by above equation is exponential, the cmc value of fluorinated surfactants is approximately equal to that of a hydrocarbon surfactant with a hydrocarbon chain 1,5 – 1,7 times longer than the fluorocarbon chain (Miyazawa, H.; 2003).

1.5.3 Surface Tension

Fluorinated surfactants cause the largest reduction in the surface tension of water due to the very weak cohesive energy of the fluorocarbon chain. The low polarizability of fluorine results in low van der Waals interactions between fluorinated chains and low cohesive energy densities in liquid fluorocarbons. These low interactions are responsible for many of the most valuable properties of fluorocarbons, such as very low surface tensions excellent spreading properties, high fluidity, low dielectric constant, high vapor pressure, high compressibilities and high gas solubilities. Fluorinated surfactants also show surface activity in organic solvents (Igawa, K.; 2003).

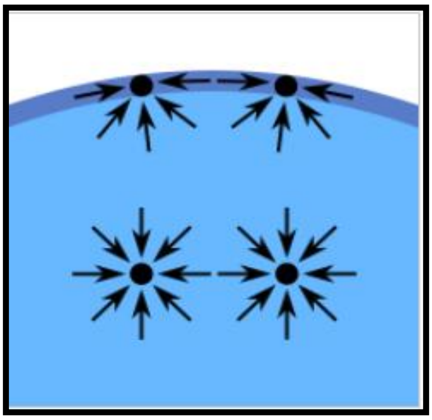


Figure 1.4 Surface tension.

1.6 1,2,3-TRIAZOLE RING SYSTEM

The 1,2,3-triazole ring system has been the subject of considerable research mainly due to its usefulness in synthetic organic chemistry and also because of the pharmacological properties shown by some of its derivatives (Jagerovic, N.; 2006).

Triazoles have found wide use in pharmaceuticals, agrochemicals, dyes, photographic materials and corrosion inhibition etc. (Fan, W. Q.; 1996). For example, there are numerous examples in the literature including anti-HIV activity, anti-microbial activity against Gram positive bacteria, selective b3 adrenergic receptor agonism by means of triazole compounds (Genin, M.J.; 2000). Several methods have been described for the synthesis of [1,2,3]-triazoles. Among them, the most important and useful one is the cycloaddition of azide with alkyne (Katritzky, A. R.; 2003). However, this reaction usually needs elevated temperature and also forms a mixture of 1,4 and 1,5 regioisomers for unsymmetrical alkynes. Recently, studies on 1,4 versus 1,5 regioselectivity were reported. Sharpless (Bertrand, F.; 2001) used Cu(I) salt as a catalyst to promote the reaction of azide with terminal alkynes to give in high regioselectivity 1,4-substituted products. Meldal (Song, J.J.; 2005) also regios-electively synthesized 1,4-substituted [1,2,3]-triazoles by 1,3-dipolar reaction of azides with polymer-supported terminal alkynes. The most classical approach to the synthesis of 1,2,3-triazoles involves therma l 1,3-dipolar cycloaddition of azides with alkynes, as initially proposed by Huisgen (Mobius R.; 1965).

1.7 HISTIDINE

Histidine (abbreviated as His or H) is an α-amino acid with an imidazole functional group. It is one of the 22 proteinogenic amino acids. Histidine was first isolated by German physician Albrecht Kossel in 1896. Histidine is an essential amino acid in humans and other mammals. It was initially thought that it was only essential for infants, but longer-term studies established that it is also essential for adult humans (Kopple, J. D.; Swendseid, M. E.; 1975).

The imidazole sidechain of histidine is a common coordinating ligand in metalloproteins and is a part of catalytic sites in certain enzymes. In catalytic triads, the basic nitrogen of histidine is used to abstract a proton from serine, threonine, or cysteine to activate it as a nucleophile. In a histidine proton shuttle, histidine is used to quickly shuttle protons. It can do this by abstracting a proton with its basic nitrogen to make a positively charged intermediate and then use another molecule, a buffer, to extract the proton from its acidic nitrogen. In carbonic anhydrases, a histidine proton shuttle is utilized to rapidly shuttle protons away from a zinc-bound water molecule to quickly regenerate the active form of the enzyme. Histidine is also important in haemoglobin in helices E and F. Histidine assists in stabilising oxyhaemoglobin and destabilising CO-bound haemoglobin (Swanson, K.; 2009).

1.8 HISTAMINE

Histamine is an organic nitrogen compound involved in local immune responses as well as regulating physiological function in the gut and acting as aneurotransmitter (Marieb, E.; 2001). Histamine triggers the inflammatory response. As part of an immune response to foreign pathogens, histamine is produced by basophils and by mast cells found in nearby connective tissues. Histamine increases the permeability of the capillaries to white blood cells and some proteins, to allow them to engage pathogens in the infected tissues (Di Giuseppe, M. et al.; 2003).

Histamine has two basic centres, namely the aliphatic amino group and whichever nitrogen atom of the imidazole ring does not already have a proton. Under physiological conditions, the aliphatic amino group (having a pK_a around 9.4) will be protonated, whereas the second nitrogen of the imidazole ring ($pK_a \approx 5.8$) will not be protonated (Paiva, T. B.; 1970). Thus, histamine is normally protonated to a singly charged cation.

1.9 TAURINE

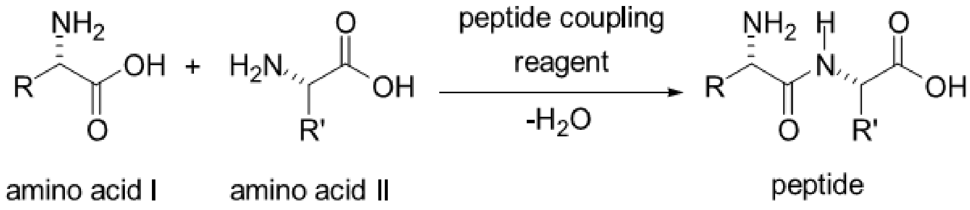
Taurine, or 2-aminoethanesulfonic acid, is an organic acid widely distributed in animal tissues. It is a major constituent of bile and can be found in the large intestine and accounts for approximately 0.1% of total human body weight (Huxtable, R.J.; 1992). Taurine has many fundamental biological roles such as conjugation of bile acids, antioxidation, osmoregulation, membrane stabilization and modulation of calcium signaling. It is essential for cardiovascular function, and development and function of skeletal muscle, the retina and the central nervous system. Taurine is unusual among biological molecules in being a sulfonic acid, while the vast majority of biologically occurring acids contain the more weakly acidic carboxyl group. While taurine is sometimes called an amino acid, and indeed is an acid containing an amino group, it is not an amino acid in the usual biochemical meaning of the term, which refers to compounds containing both an amino and a carboxyl group.

Taurine is essential for cardiovascular function, and development and function of skeletal muscle, the retina and the central nervous system (Huxtable, R.J.; 1992).

Taurine is conjugated via its amino terminal group with chenodeoxycholic acid and cholic acid to form the bile salts sodium taurochenodeoxycholate and sodium taurocholate. The low pKa of taurine's sulfonic acid group ensures this moiety is negatively charged in the pH ranges normally found in the intestinal tract and, thus, improves the surfactant properties of the cholic acid conjugate.

1.10 PEPTIDE COUPLING

Peptides are synthesized by coupling the carboxyl group or C-terminus of one amino acid to the amino group or N-terminus of another. Due to the possibility of unintended reactions, protecting groups are usually necessary. Chemical peptide synthesis starts at the C-terminal end of the peptide and ends at the N-terminus. This is the opposite of protein biosynthesis, which starts at the N terminal end.



Scheme 1.1 Peptide synthesis.

1.11 ASSESSMENT OF FLUORINATED SURFACTANTS IN TERMS OF THEIR BIOLOGICAL ACTIVITIES

Fluorinated chains confer unique properties to molecules. These properties are the foundations of new therapies based, for example, on fluorocarbons for oxygen delivery (blood substitutes) or liquid ventilation procedures that are currently in advanced human clinical trials. Therefore, the in vivo behavior of fluorocarbons has been intensively investigated (Shinoda, K.; 1972). The situation is quite different where fluorinated amphiphiles are concerned.

The dominant characteristics of fluorinated surfactants are their high surface activity and their strong tendency to self-aggregate into stable, well-organized supramolecular assemblies such as vesicles and tubules. Because of their high tendency to collect at interfaces, fluorinated surfactants can be used for the formulation of a range of multi-phase colloidal systems including direct and reverse fluorocarbon emulsions, microemulsions, gels, dispersions, aerosols, etc. Many of these colloidal systems have potential as drug delivery systems (Krafft, M.P.; 2001).

CHAPTER 2

EXPERIMENTAL PART

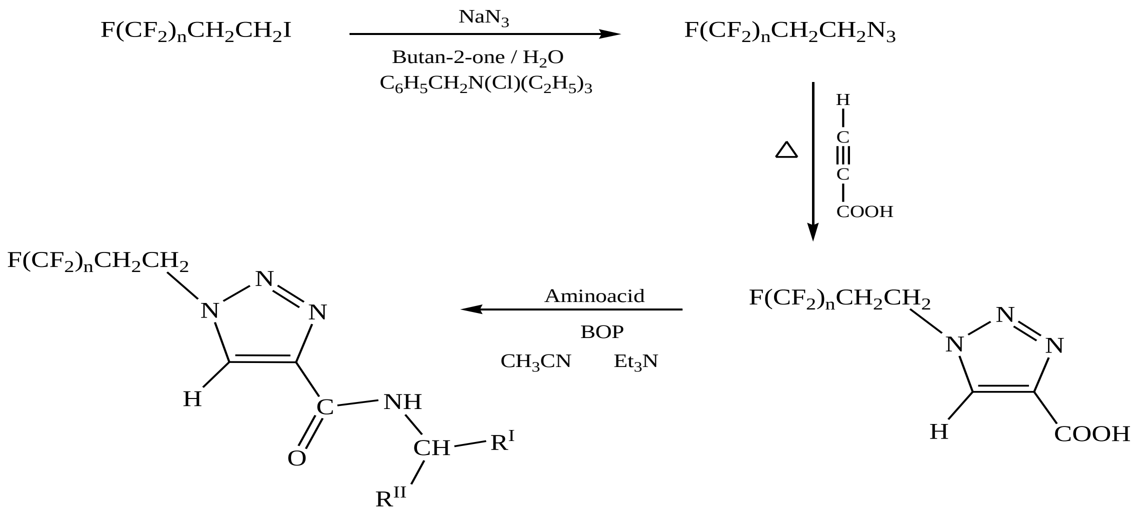
2.1 MATERIAL AND METHODS

2.1.1 Instruments and Materials

The ^1H NMR spectra were recorded on a Bruker Ultra Shield Plus, Ultra long hold time 400 MHz NMR spectrometer. The chemical displacements are reported (in parts per million (ppm) compared to the reference with trimethylsilane (TMS). Following solvent peaks were used as international standards: DMSO-d_5:2.50 ppm (^1H NMR), acetone-d_6: 2.05 ppm (^1H NMR), chloroform-d_3 ($CDCl_3$): 7.26 ppm (^1H NMR), MeOH-d_4: 3.31 ppm (^1H NMR), H2O-d_2: 4.80 ppm (^1H NMR). Multiplicities are described using the following abbreviations: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet and br=broad. FT-IR spectra were recorded on Bruker spectrophotometer in the range of 500-4000cm^{-1}. Melting points were measured on chassis KOFFLER Bench equipment. Some of reactions were controlled by thin layer chromatography (TLC) on silica gel and spots were detected either by UV-visible light or by charging with I_2 vapor or ninhydrine solution or in some cases $KMnO_4$ solution in H_2SO_4 used.

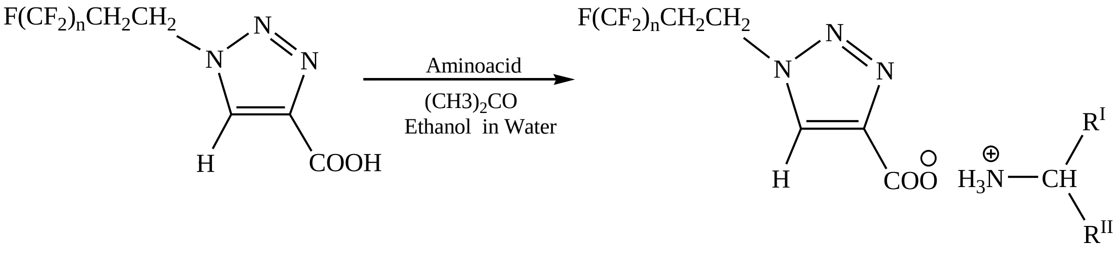
Reagent quality solvents were used without purification. Dichloromethane was distilled over molecular sives, triethylamine was distilled and kept over sodium hydroxide before use. Amino acids and saturated fatty acids were from Sigma, Fluka, Acros or Alfa Aesar. Other chemicals were purchased from Fluka, Merck or Sigma companies.

2.2 SYNTHESIS METHODOLOGY



Scheme 2.1 Cycloaddition of 2-perfluoroalkyl-ethyl-azides with alkynes and Methodology of synthesis of perfluorinated aminoacid analogues.

The synthesis starts with commercial 2-perfluoroalkyl-1-iodoethanes (Scheme 2.1). Because of the destabilising influence of the perfluoro group on the 2-methylene protons, these products are substrates for which the nucleophilic substitution of the iodides becomes delicate (Selve, C.; 1991). After obtaining the perfluorinated 1,2,3-triazole analogues, the following step consists of grafting an amino acid. We chose a classical method using benzo-triazolyl-oxy-tris(dimethylamino)-phosphonium hexafluorophosphate (BOP) as peptide coupling reagent.



Scheme 2.2 2-perfluoroalkyl-ethyl-1,2,3-Triazole and Methodology of synthesis of perfluorinated aminoacid salt form.

2.3 SYNTHESIS

2.3.1 Synthesis of 2-perfluoroalkyl-ethyl-azides

12,5 mmol (1 equiv) 2-perfluoroalkyl-ethyl-1-iodo was dissolved in 70 ml 2-butanone. 3,75 mmol (3 equiv) sodium azide, 12, 5 mmol (1 equiv) benzyltriethyl-ammonium chloride and 50 ml dionized water was added reaction ballon. The medium was stirred under reflux at 110 ^oC for 16 hours. After the reaction was complete which extraction was done three times with 50 ml diethyl ether, following washing with 75 ml of water. Then, the organic phase was dried with $MgSO_4$ and vacuum-filtered. The solvent evaporated by rotary evaporator.

$$\underset{n=4,6,8}{F(CF_2)_nCH_2CH_2I} \xrightarrow[\substack{\text{Butan-2-one / } H_2O \\ C_6H_5CH_2N(Cl)(C_2H_5)_3}]{NaN_3} F(CF_2)_nCH_2CH_2N_3$$

Scheme 2.3 Synthesis of 2-perfluoroalkyl-ethyl-azides.

Table 2.1 Some physical properties of the product derivatives.

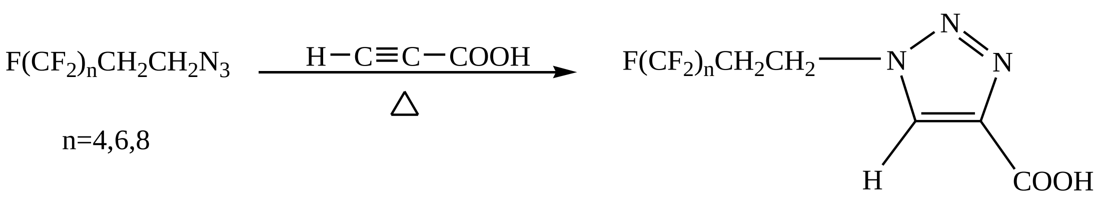
Compound	n	$M_{w\ theoretical}$ (g/mol)	Yield (%)	$Rf_{(EtOAc)}$	Appearence
1a	4	289,10	82	0,54	Yellow Liquid
1b	6	389,12	80	0,52	Yellow Liquid
1c	8	489,13	81	0,47	Yellow Liquid

NMR ^{1}H ($CDCl_3$**) :** (δ) : **a**:1,56 ppm (s, 2H) ;**b:**2,37 ppm (t, 2H)

IR :ν[C=O] : 2106 cm^{-1} ;ν[C-F] : 1100-1300 cm^{-1}

2.3.2 Synthesis of 2 -perfluoroalkyl -ethyl -1,2,3 -Triazole

9,26 mmol (1 equiv) 2-perfluoroalkyl-ethyl-azides was dissolved in 15 ml acetone. 9,26 mmol (1 equiv) propiolic acid is added. The medium was stirred under reflux at 65 ^{o}C for 6 hours. After the reaction was complete extraction was done three times with 50 ml diethyl ether, following washing with 75 ml of hexane. Then, the organic phase dried with $MgSO_4$ and vacuum-filtered and the solvent evaporated by rotary evaporator.



Scheme 2.4 Cycloaddition with termal alkynes.

Table 2.2 Some physical properties of products.

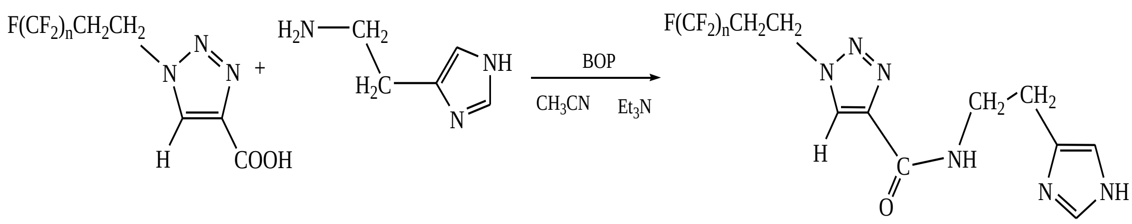
Compound	n	$M_{w\ theoretical}$ (g/mol)	Yield (%)	Melting Point (^{o}C)	Appearence
2a	4	359,15	95	145	White Solid
2b	6	459,16	96	189	White Solid
2c	8	559,18	96	195	White Solid

NMR ^{1}H (DMSO) **:** (δ) : **a**: 12,2 ppm (s, 1H); **b:** 7,6 ppm (s, 1H)**; c:** 4,5 ppm (t,2H) ; **d:** 2,1ppm (m, 2H).

IR :ν[C=O] : 1690 cm^{-1} ;ν[C-F] : 1100-1300 cm^{-1}

2.3.3 Synthesis of 2 -perfluoroalkyl –ethyl -1,2,3 –Triazole -Histamine

2,6 mmol (1 equiv) 2-perfluoroalkyl-ethyl-1,2,3-Triazole was dissolved in 25 ml of acetonitrile (CH_3CN). In a sequence 2,6 mmol (1 equiv) BOP coupling reagent, and 5,2 mmol (2 equiv) Histamine was added. The mixture was stirred at room temperature for 30 minutes. And then triethylamine (Et_3N) 7,8 mmol (3 equiv) was added drop by drop until 30 minutes. The reaction was left to take place for overnight at room temperature under the constant stirring. After the reaction was complete the white precipitate was filtered and washed with acetonitrile and water and then the solid part was dried in etuv.



Scheme 2.5 Synthesis of 2 -perfluoroalkyl–ethyl-1,2,3–Triazole–Histamine.

Table 2.3 Some physical properties of products.

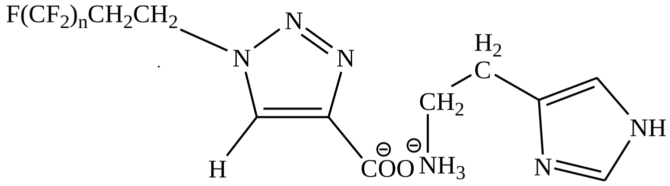
Compound	n	$M_{w\ theoretical}$ (g/mol)	Yield (%)	Melting Point (^oC)	Appearence
3c	8	652,31	72	230	White Solid

NMR ^{1}H (DMSO) **:** (δ) **: a**: 11,2 ppm (d, 1H); **b:** 8,52 ppm (s, 1H); **c:** 8,02 ppm (t, 1H); **d:** 7,6 ppm (s, 1H); **e:** 7 ppm (s, 1H); **f:** 3,5 ppm (t, 2H); **g**:3,3 ppm (m,2H); **h:** 2,9 ppm (t, 2H); **i**: 2,09 ppm (m,2H).

IR : ν[C=O]: 1706 cm^{-1} ; ν[C(O)] amide: 1645 cm^{-1}; ν[C-F] : 1100-1300 cm^{-1}

2.3.3.1 Synthesis of 2-perfluoroalkyl-ethyl-1,2,3-Triazole-Histamine Salt

In a beaker, 1,78 mmol (1 equiv) 2-perfluoroalkyl-ethyl-1,2,3-Triazole was dissolved in 10 ml asetone. In another beaker 1,78 mmol (1 equiv) histamine dihydrochloride was dissolved in 1 ml ethanol and 9 ml water of mixture. After that triazol solution was added dropwise over histidinate solution. The reaction was left to one hour at room temperature under the constant stirring. Finally the white precipitate was filtered and dried in etuv.



Scheme 2.6 The structure of 2-perfluoroalkyl-ethyl-1,2,3-Triazole-Histamine Salt.

Table 2.3.1 Some physical properties of products.

Compound	n	$M_{w\ theoretical}$ (g/mol)	Yield (%)	Melting Point (^oC)	Appearence
4c	8	652,31	95	210	White Solid

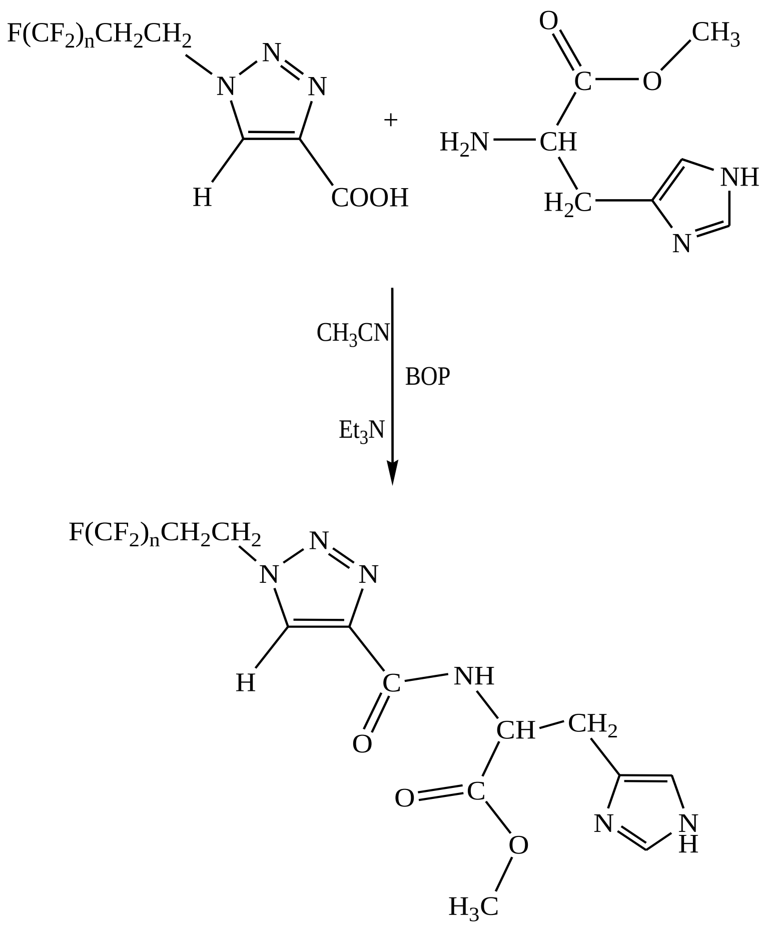
NMR ^{1}H (DMSO) **:** (δ) **: a**: 9,24 ppm (d, 1H) ; **b:** 8,18 ppm (s, 1H) ; **c:** 7,5 ppm (d, 1H); **d:** 7,24 ppm (t, 3H); **e:** 7 ppm (d, 1H); **f:** 3,9 ppm (m, 2H); **g**:3,8 ppm (t,2H) **h:** 2,85 ppm (t, 2H); **i**: 2,09 ppm (m,2H).

IR :ν[C=O]: 1689 cm^{-1} ; ν[C(H)] amine: 3099 cm^{-1}; ν[C-F] : 1100-1300 cm^{-1}

2.3.4 Synthesis of 2-perfluoroalkyl-ethyl-1,2,3-Triazole-Histidine

2,6 mmol (1 equiv) 2-perfluoroalkyl-ethyl-1,2,3-Triazole was dissolved in 25 ml of acetonitrile (CH_3CN). In a sequence 2,6 mmol (1 equiv) BOP coupling reagent, and

5,2 mmol (2 equiv) L-methyl histidine dihydrochloride was added. The mixture was stirred at room temperature for 30 minutes. And then triethylamine (Et_3N) 7,8 mmol (3 equiv) was added drop by drop until 30 minutes. The reaction was left to take place for overnight at room temperature under the constant stirring. After the reaction was complete the white precipitate was filtered and washed with acetonitrile and water and then the solid part was dried in etuv.



Scheme 2.7 Synthesis of 2-perfluoroalkyl-ethyl-1,2,3-Triazole-Histidine.

Table 2.4 Some physical properties of products.

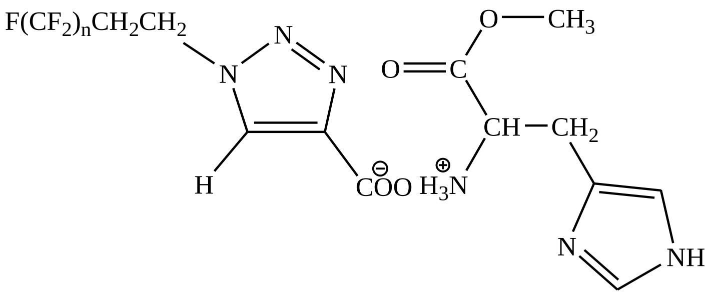
Compound	n	$M_{w\ theoretical}$ (g/mol)	Yield (%)	Melting Point (^oC)	Appearence
5c	8	710,34	86	190	White Solid

NMR ^{1}H (DMSO) **:** (δ) **: a**: 8,5 ppm (s, 1H); **b:** 8,01 ppm (t, 1H); **c:** 3,98 ppm (t, 2H); **d:** 3,8 ppm (m, 2H); **e:** 3,75 ppm (m, 2H); **f:** 2,2 ppm (m, 2H); **g**:2,06 ppm (s,1H).

IR : ν[C=O]: 1711 cm^{-1} ; ν[C(O)] amide: 1690 cm^{-1}; ν[C-F] : 1100-1300 cm^{-1}

2.3.4.1 Synthesis of 2-perfluoroalkyl-ethyl-1,2,3-Triazole-Histidine Salt

In a beaker, 1,78 mmol (1 equiv) 2-perfluoroalkyl-ethyl-1,2,3-Triazole was dissolved in 10 ml asetone. In another beaker 1,78 mmol (1 equiv) L-methyl histidinate dihydrochloride was dissolved in 1 ml ethanol and 9 ml water of mixture. After that triazol solution was added dropwise over histidinate solution. The reaction was left to one hour at room temperature under the constant stirring. Finally the white precipitate was filtered and dried in etuv.



Scheme 2.8 The structure of 2-perfluoroalkyl-ethyl-1,2,3-Triazole-Histidine Salt.

Table 2.4.1 Some physical properties of products.

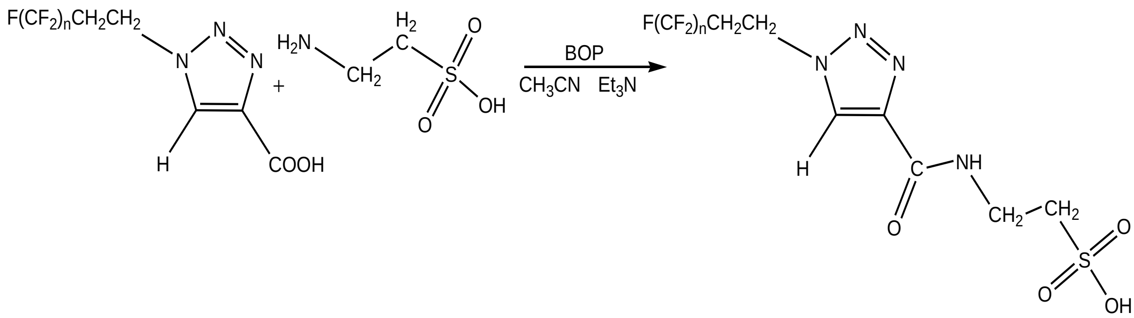
Compound	n	$M_{w\ theoretical}$ (g/mol)	Yield (%)	Melting Point (^{o}C)	Appearence
6c	8	652,31	95	165	White Solid

NMR ^{1}H (DMSO) **:** (δ) **: a**: 8,02 ppm (s, 1H); **b:** 7,53ppm (t, 3H); **c:** 3,9 ppm (t, 2H); **d:** 3,8 ppm (m, 2H); **e:** 3,75 ppm (t, 2H); **f:** 3,2 ppm (m, 2H); **g**:3,1 ppm (s,1H).

IR :ν[C=O]: 1689 cm^{-1} ; ν[C(H)] amine: 3097 cm^{-1}; ν[C-F] : 1100-1300 cm^{-1}

2.3.5 Synthesis of 2-perfluoroalkyl-ethyl-1,2,3-Triazole-Taurine

2,6 mmol (1 equiv) 2-perfluoroalkyl-ethyl-1,2,3-Triazole was dissolved in 25 ml of acetonitrile (CH_3CN). In a sequence 2,6 mmol (1 equiv) BOP coupling reagent, and 5,2 mmol (2 equiv) Taurine was added. The mixture was stirred at room temperature for 30 minutes. And then triethylamine (Et_3N) 7,8 mmol (3 equiv) was added drop by drop until 30 minutes. The reaction was left to take place for overnight at room temperature under the constant stirring. After the reaction was complete the white precipitate was filtered and washed with acetonitrile and water and then the solid part was dried in etuv.



Scheme 2.9 Synthesis of 2-perfluoroalkyl-ethyl-1,2,3-Triazole-Taurine.

Table 2.5 Some phsical properties of compound.

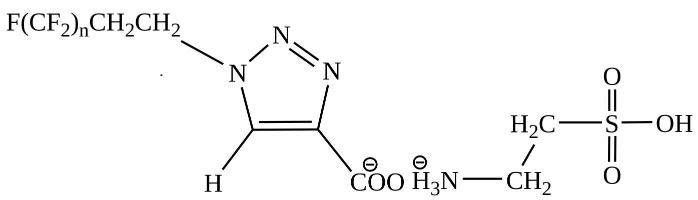
Compound	n	$M_{w\ theoretical}$ (g/mol)	Yield (%)	Melting Point (^oC)	Appearence
7c	8	666,31	59	175	White Solid

NMR ^{1}H ($CDCl_3$) **:** (δ) : **a** : 8,52 ppm (s, 1H) ; **b:** 8,01 ppm (s, 16H) **; c:** 3,9 ppm (d, 2H); **d:** 3,7 ppm (d, 2H); **e:** 3,5 ppm (s, 2H); **f:** 2,09 ppm (m, 12H), **g:** 1,9 ppm (t, 1H)

IR :ν[C=O] : amide 1711 cm^{-1} ; ν[C-F] : 1100-1300 cm^{-1}

2.3.5.1 Synthesis of 2-perfluoroalkyl-ethyl-1,2,3-Triazole-Taurine Salt

In a beaker, 1,78 mmol (1 equiv) 2-perfluoroalkyl-ethyl-1,2,3-Triazole was dissolved in 10 ml asetone. In another beaker (1 equiv) Taurine was dissolved in 1 ml ethanol and 9 ml water of mixture. After that triazol solution was added dropwise over Taurine solution. The reaction was left to one hour at room temperature under the constant stirring. Finally the white precipitate was filtered and dried in etuv.



Scheme 2.10 The structure of 2-perfluoroalkyl-ethyl-1,2,3-Triazole-Taurine Salt.

Table 2.5.1 Some physical properties of products.

Compound	n	$M_{w\ theoretical}$ (g/mol)	Yield (%)	Melting Point (^{o}C)	Appearence
8c	8	652,31	95	205	White Solid

NMR ^{1}H (DMSO) **:** (δ) **: a**: 8,5 ppm (s, 1H); **b:** 8,01 ppm (t, 1H); **c:** 3,98 ppm (t, 2H); **d:** 3,8 ppm (m, 2H); **e:** 3,75 ppm (m, 2H); **f:** 2,2 ppm (m, 2H); **g**:2,06 ppm (s,1H).

IR : ν[C=O]: 1711 cm^{-1} ; ν[C(O)] amide: 1690 cm^{-1}; ν[C-F] : 1100-1300 cm^{-1}

CHAPTER 3

RESULTS AND DISCUSSION

A series of analogues of perfluorinated amphiphilic 1,2,3-triazole compounds have been synthesized. 2-perfluoroalkyl-1-iodoethanes were used as starting materials Because of the destabilising influence of the perfluoro group on the 2-methylene protons, These products are substrates for which the nucleophilic substitution of the iodides becomes delicate. However, using the azide ion in the phase transfer method and well-adapted experimental conditions allows its substitution with good yieldsand Perfuoroalkylated triazole obtained in this way are subsequently used as hydrophobic moieties for the synthesis of amphiphilic peptides derivatives. Either amino acid based amphiphilic molecules were synthesized by well known coupling reacting with BOP reagent or DCC/ DMAP. Especially BOP reagent was used more than other coupling reagents because of in the presence of the reagent the yield is higher. (See chapter 2 methodology process)

3.1 2-perfluoroalkyl-ethyl-azide

$$F(CF_2)_nCH_2CH_2N_3$$

$n = 4, 6, 8$

Scheme 3.1 The structure of 2-perfluoroalkyl-ethyl-azide.

2-perfluoroalkyl-ethyl-azide was confirmed by NMR spectroscopy and ATR. The proton NMR of as shown in Figure 3.1. Aliphatic chain group protons appear on the right of the spectrum respectively.

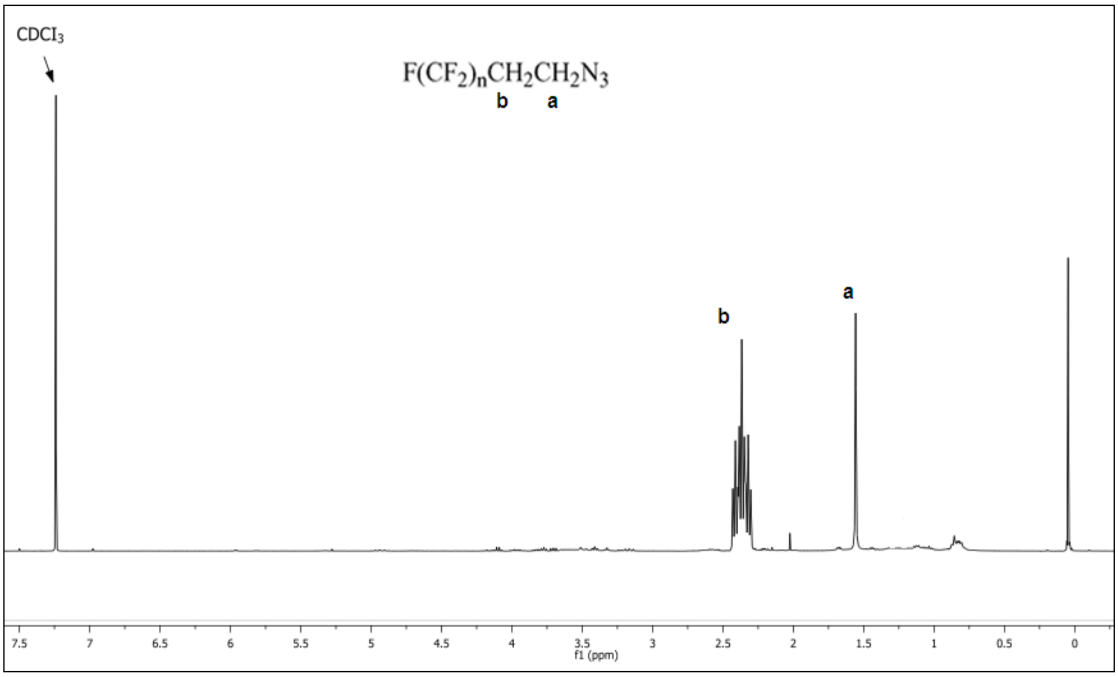


Figure 3.1 ^{1}H NMR spectrum of 2-perfluoroalkyl-ethyl-azides.

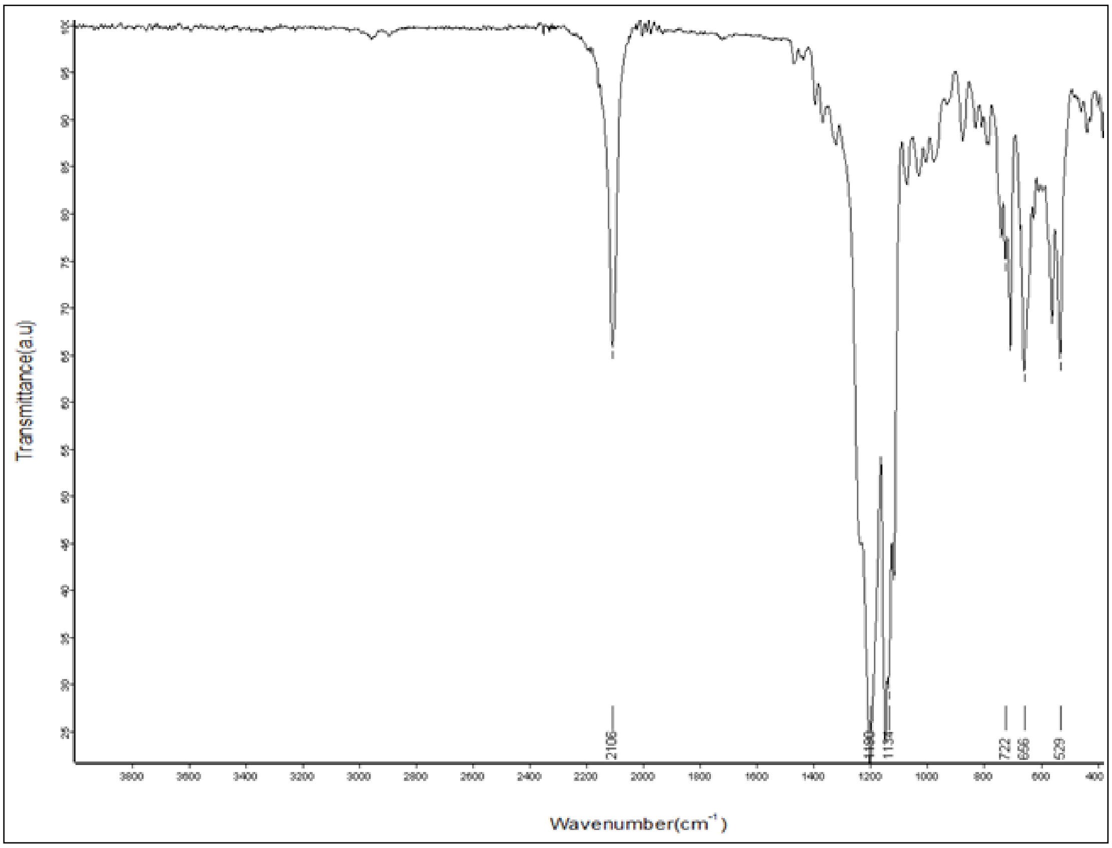
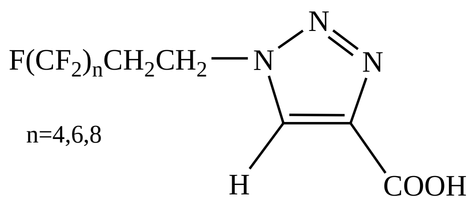


Figure 3.2 ATR spectrum of 2-perfluoroalkyl-ethyl-azides.

In ^1H-NMR spectrum of compound, the triplet at 1,56 ppm belongs to the methyl chains $-CH_2$ protons assigned to protons of **a**. The multiplet at 2,37 ppm belongs to the methyl chains $–CF_2–CH_2$-protons assigned to protons of **b**.

In ATR spectrum of compound, the band at 2106 cm^{-1} belongs to the $–N_3$ groups and the band at 1100-1300 cm^{-1} belongs to the C-F group.

3.2 2-perfluoroalkyl-ethyl-1,2,3-Triazole



Scheme 3.2 The structure of 2-perfluoroalkyl-ethyl-Triazol.

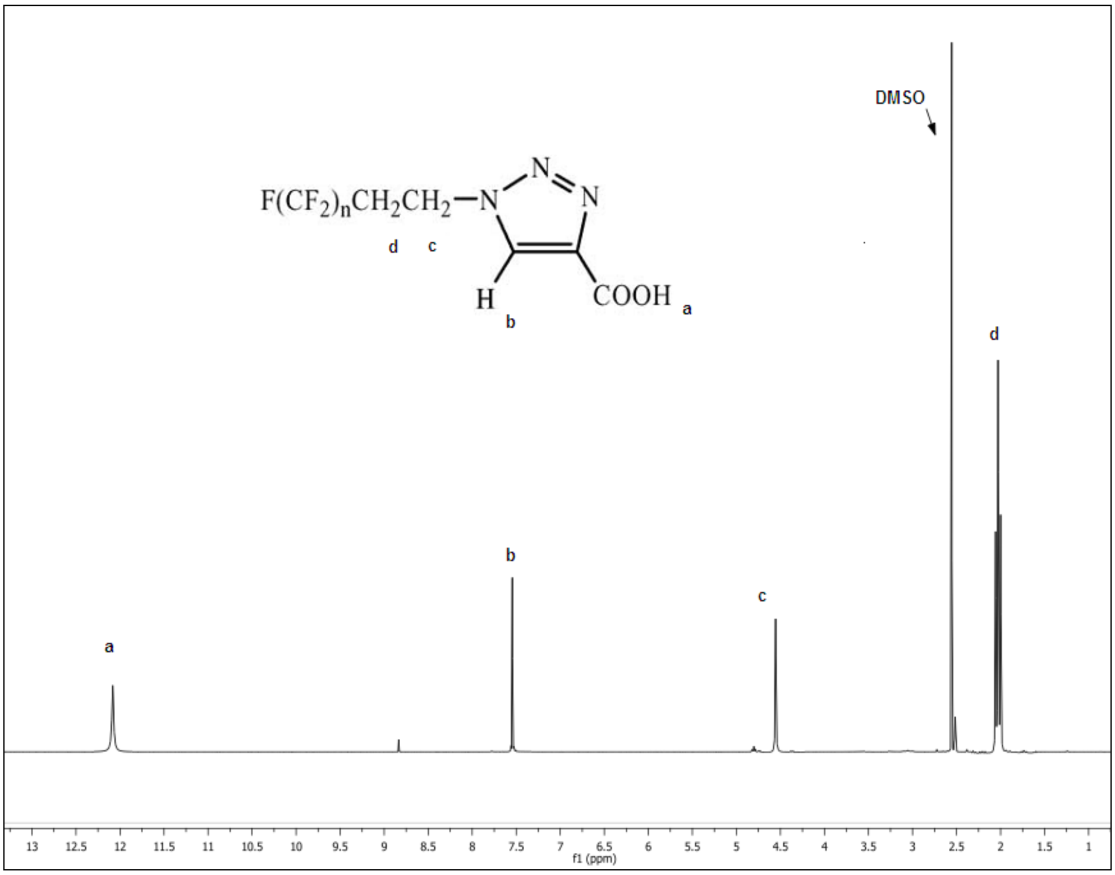


Figure 3.3 ^{1}H NMR spectrum of 2-perfluoroalkyl-ethyl-Triazol.

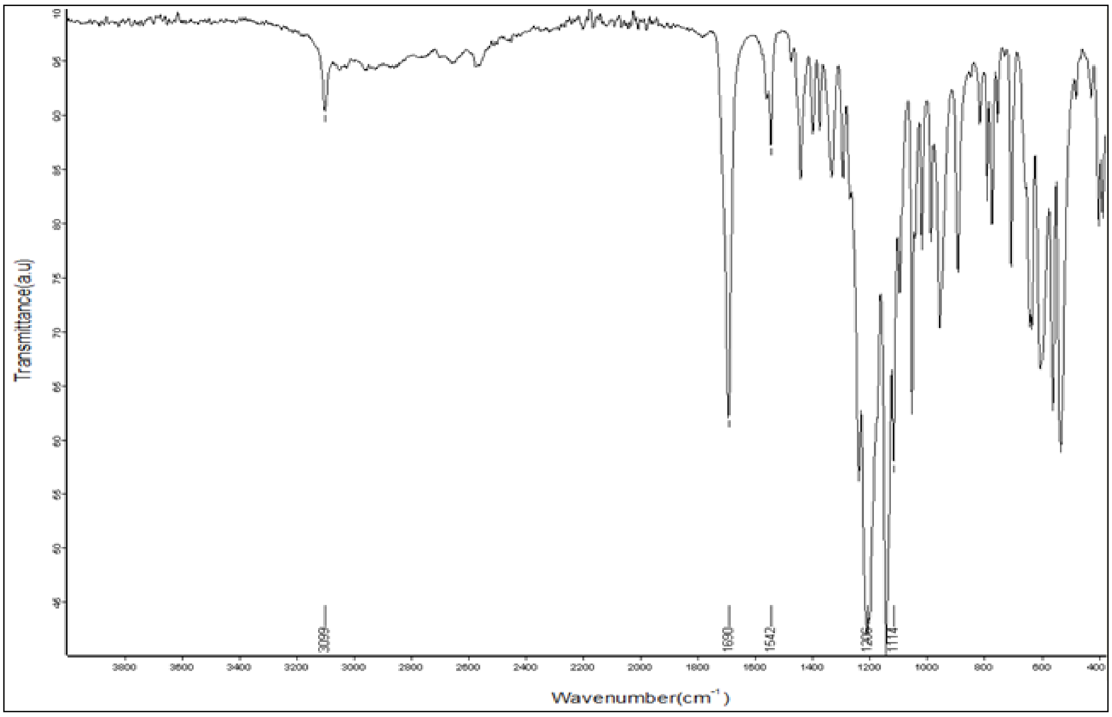
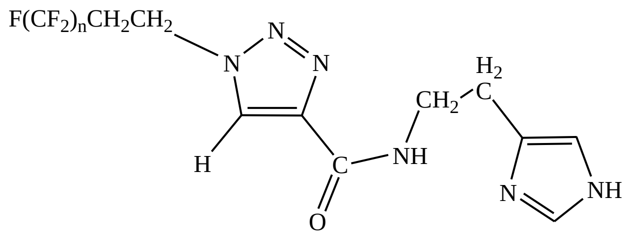


Figure 3.4 ATR spectrum of 2-perfluoroalkyl-ethyl-Triazol.

In ^{1}H-NMR spectrum of compound, the singlet at 12,2 ppm belongs to carboxylic acid proton assigned to protons of **a**. The singlet at 7,6 ppm belongs to the triazole ring and is assigned to the proton of **b**. The triplet at 4,5 ppm belongs to the methyl chains -CH_2 protons assigned to protons of **c**. The multiplet at 2,1 ppm belongs to the methyl chains –CF_2–CH_2- protons assigned to protons of **d**.

In ATR spectrum of compound, the band at 1690 cm^{-1} belongs to carboxylic acid C=O groups and the band at 1100-1300 cm^{-1} belongs to the C-F group .

3.3 2-perfluoroalkyl-ethyl-1,2,3-Triazole-Histamine



Scheme 3.3 The structure of 2-perfluoroalkyl-ethyl-1,2,3-Triazole-Histamine.

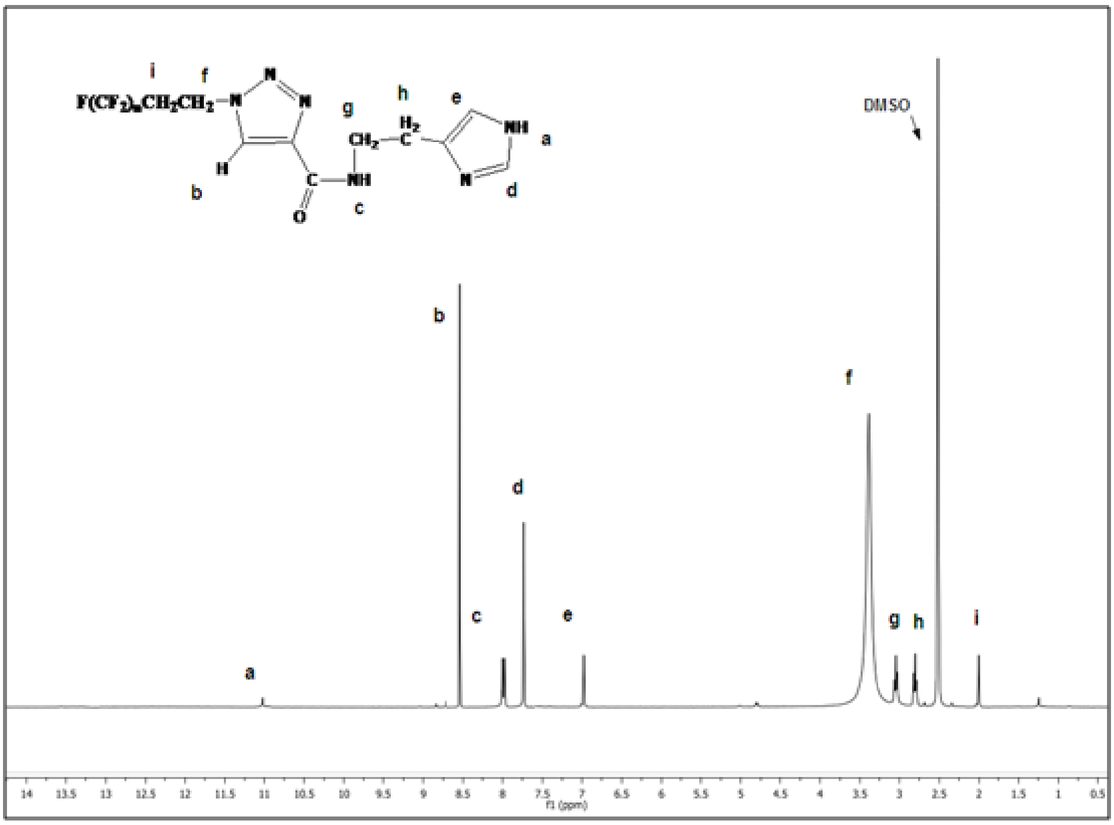


Figure 3.5 ^{1}H NMR spectrum of 2-perfluoroalkyl-ethyl-1,2,3-Triazole-Histamine.

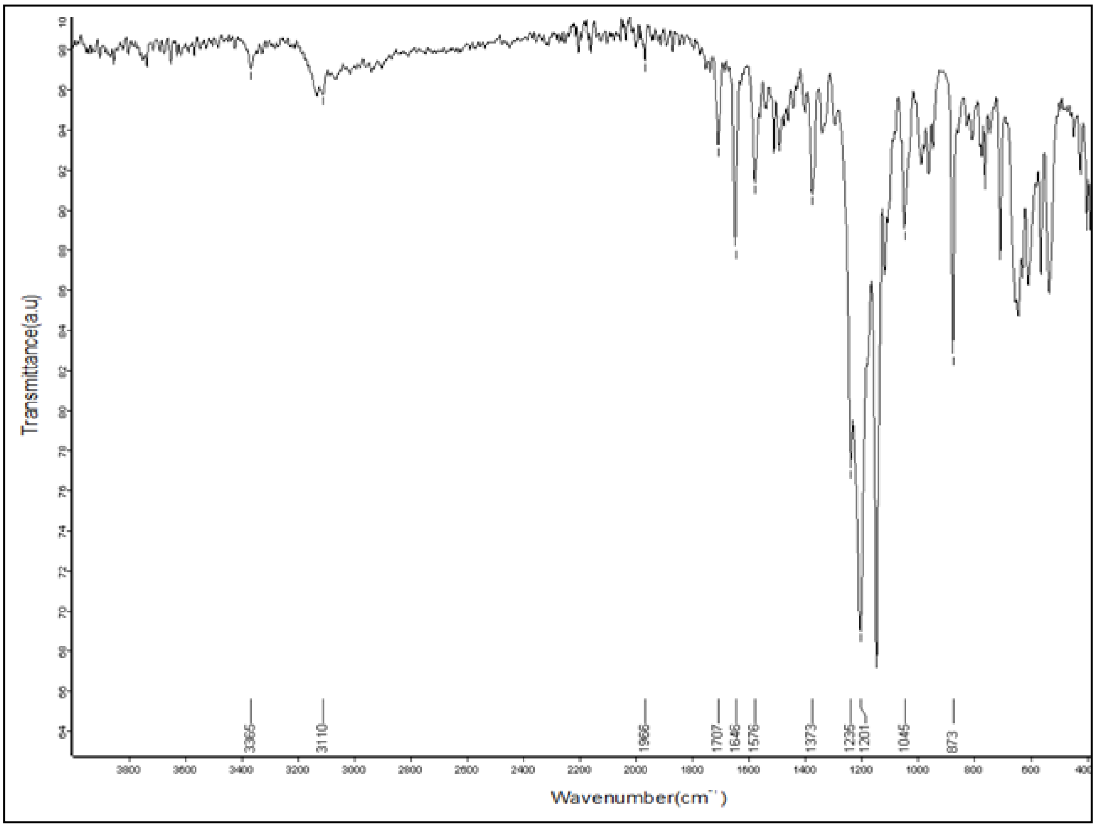
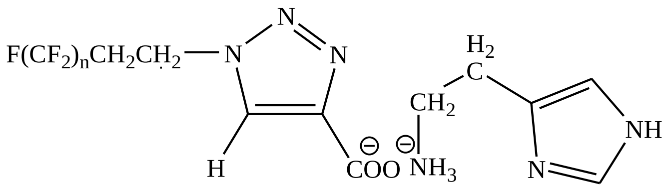


Figure 3.6 ATR spectrum of 2-perfluoroalkyl-ethyl-1,2,3-Triazole-Histamine.

In ^{1}H-NMR spectrum of compound, the duplet at 11,2 ppm belongs to imidazole NH protons assigned to protons of **a.** The singlet 8,52 ppm belongs to triazole -CH protons is assigned to protons of **b**. The triplet at 8,02 ppm is assigned to the seconder amide protons **c**. The singlet at 7,6 ppm is assigned to the imidazol –CH protons is assigned to protons of **d**.

In ATR spectrum of compound, the band at 1706 cm^{-1} belongs to the carbonyl group and the band at 1645 cm^{-1} belongs to the amide bond. The band at 1100-1300 cm^{-1} belongs to the C-F group.

3.3.1 2-perfluoroalkyl-ethyl-1,2,3-Triazole-Histamine Salt



Scheme 3.4 The structure of 2-perfluoroalkyl-ethyl-1,2,3-Triazole-Histamine Salt.

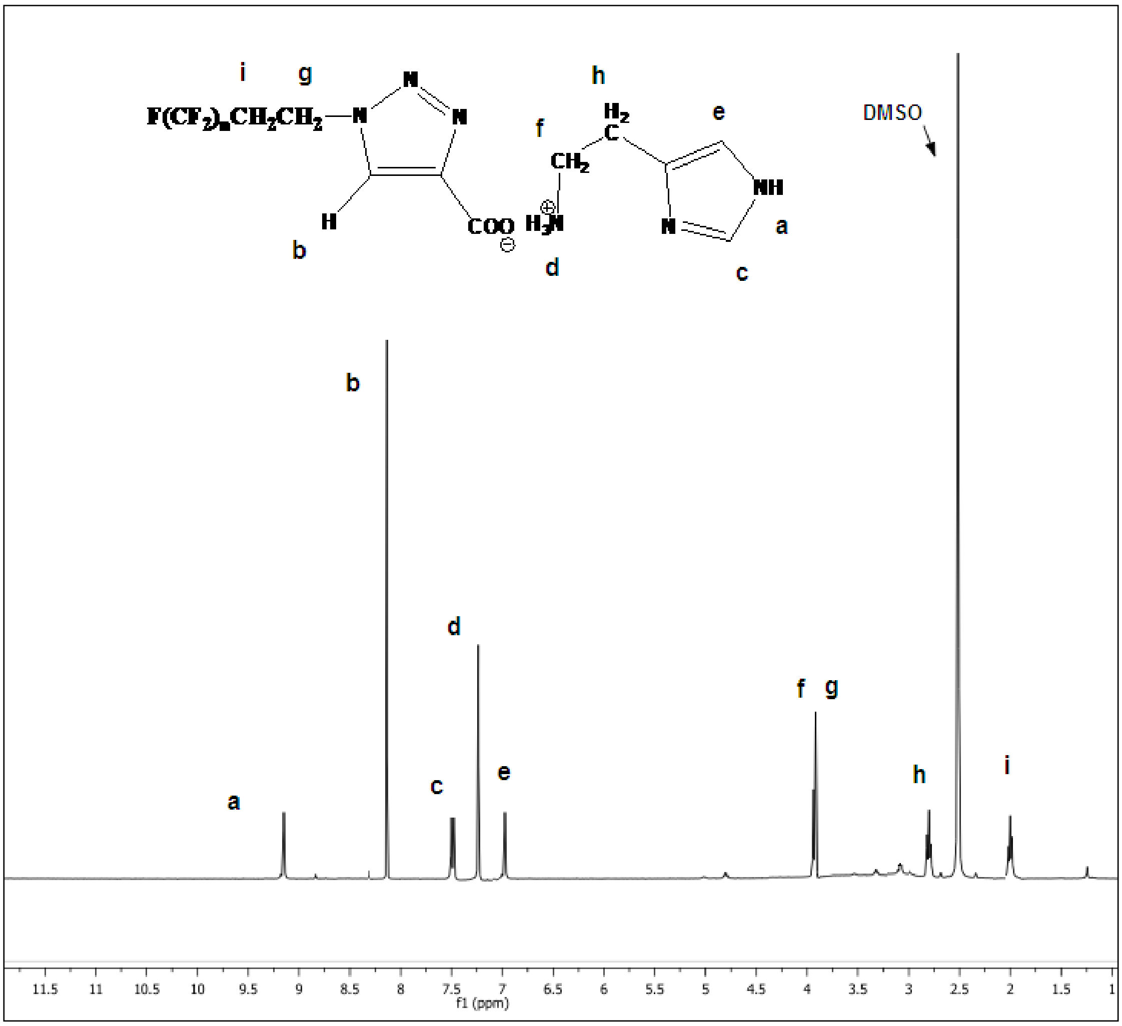


Figure 3.7 ^{1}H NMR spectrum of 2-perfluoroalkyl-ethyl-1,2,3-Triazole-Histamine Salt.

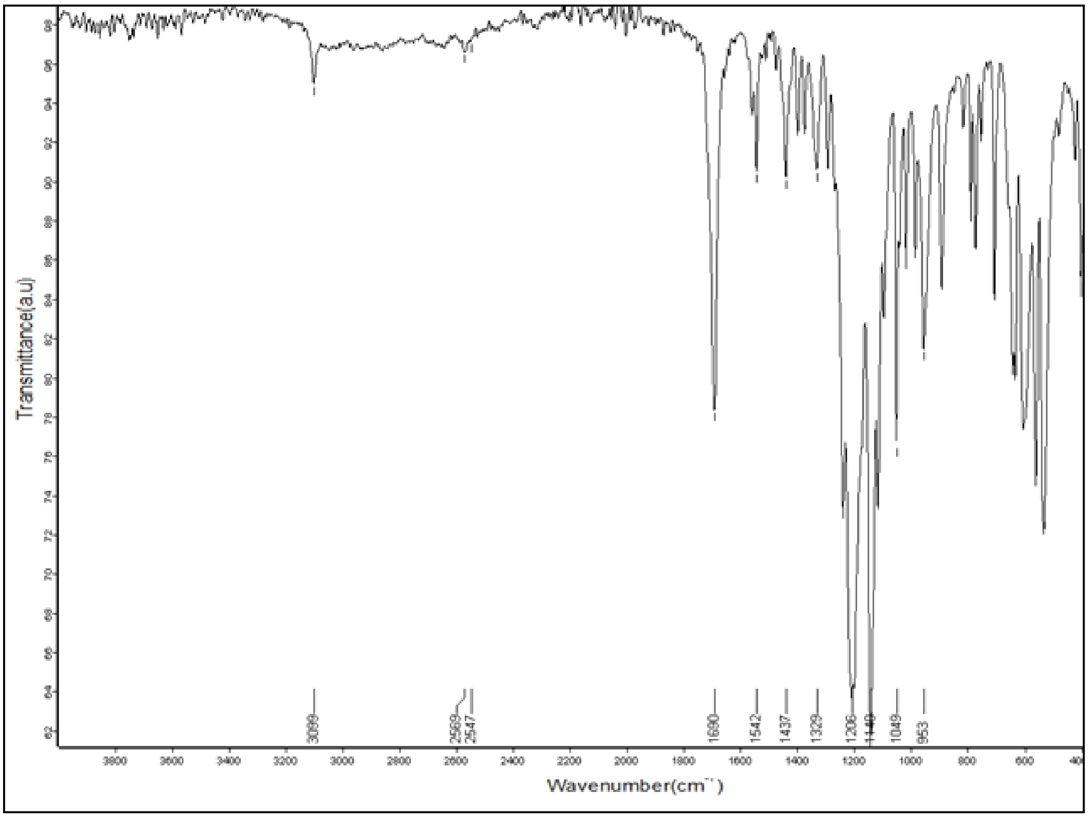
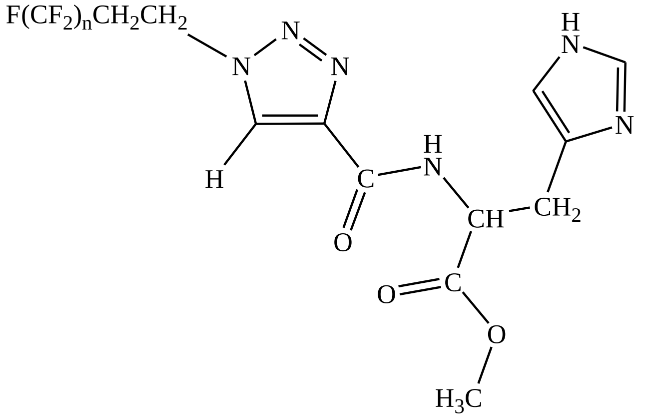


Figure 3.8 ATR spectrum of 2-perfluoroalkyl-ethyl-1,2,3-Triazole-Histamine Salt.

In ^{1}H-NMR spectrum of compound, the duplet at 9,24 ppm belongs to imidazole NH protons assigned to protons of **a.** The singlet 8,18 ppm belongs to triazole -CH protons is assigned to protons of **b**. The duplet at 7,5-7 ppm is assigned to the imidazol –CH protons is assigned to protons of **c** + **e**. The triplet at 7,24 ppm is assigned to the amine protons **d**.

In ATR spectrum of compound, the band at: 1686 cm^{-1} belongs to the carbonyl, and the bands at 3099 cm^{-1} amine bond and 1100-1300 cm^{-1} belongs to the C-F group.

3.4 2-perfluoroalkyl-ethyl-1,2,3-Triazole-Histidine



Scheme 3.5 Synthesis of 2-perfluoroalkyl-ethyl-1,2,3-Triazole-Histidine.

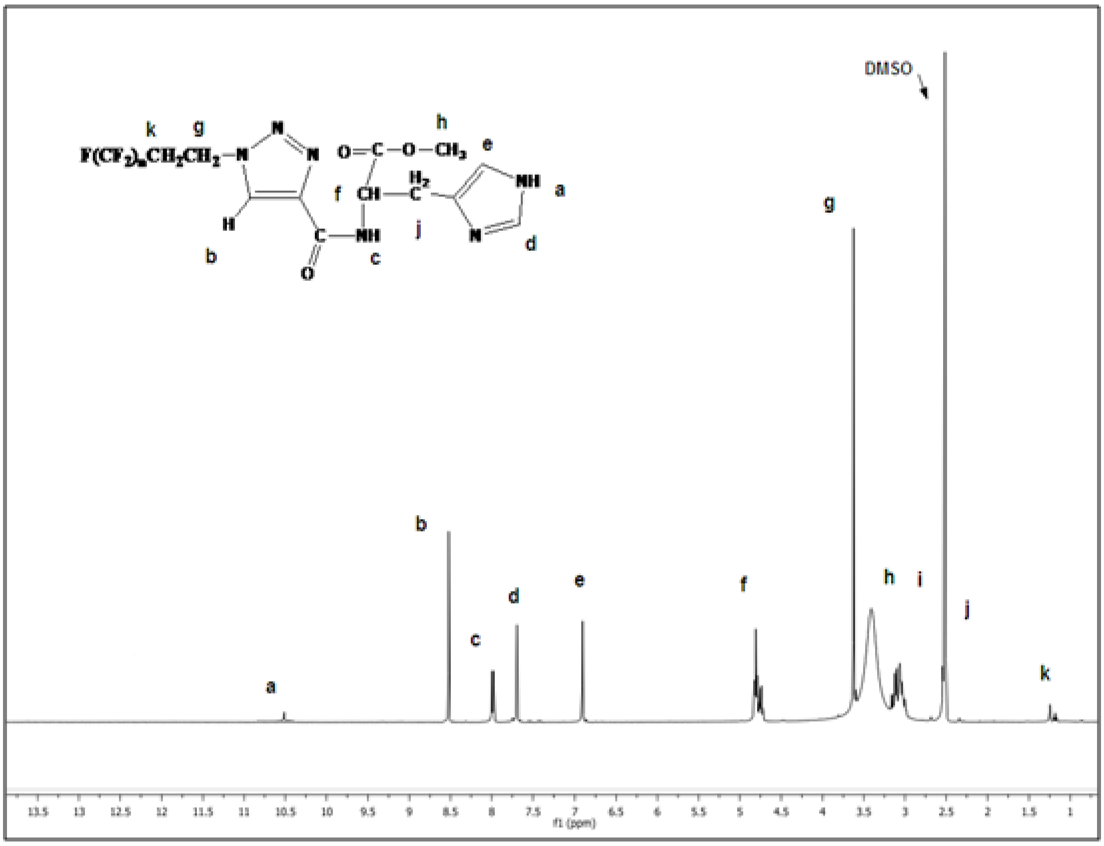


Figure 3.9 ^{1}H NMR spectrum of 2-perfluoroalkyl-ethyl-1,2,3-Triazole-Histidine.

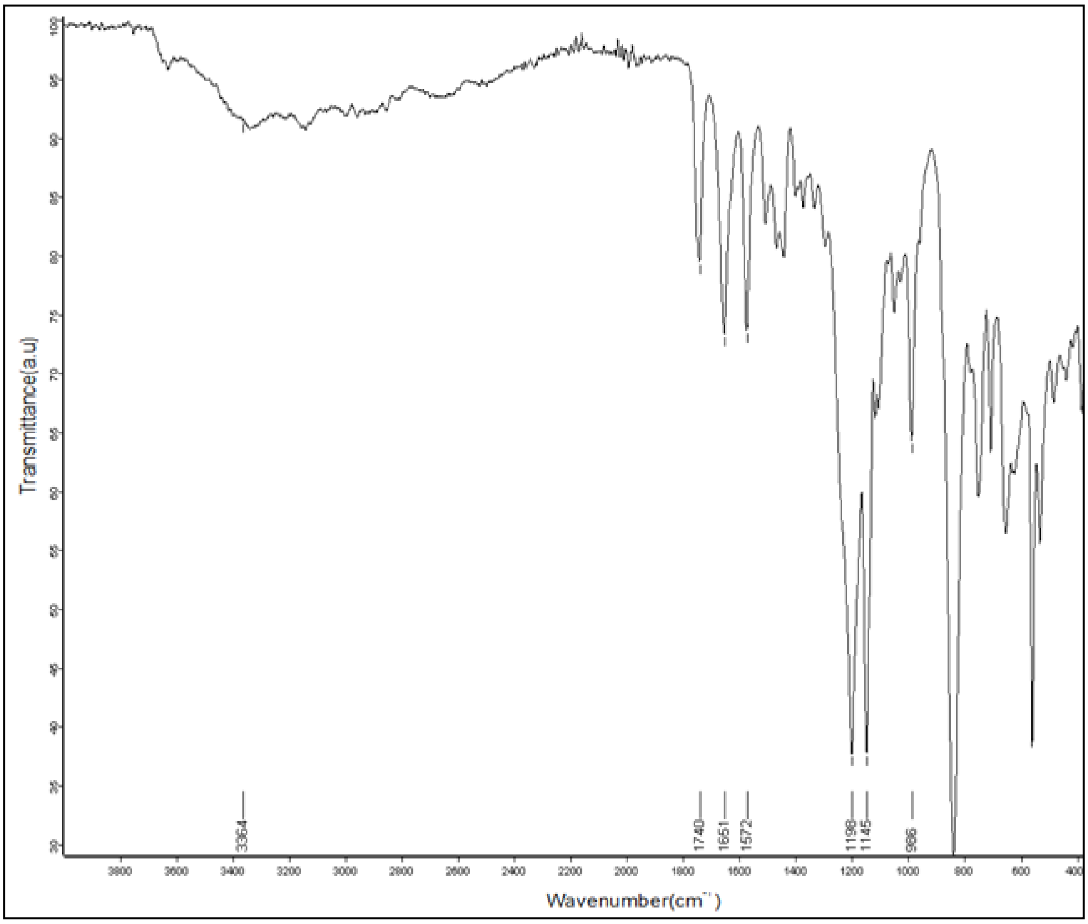
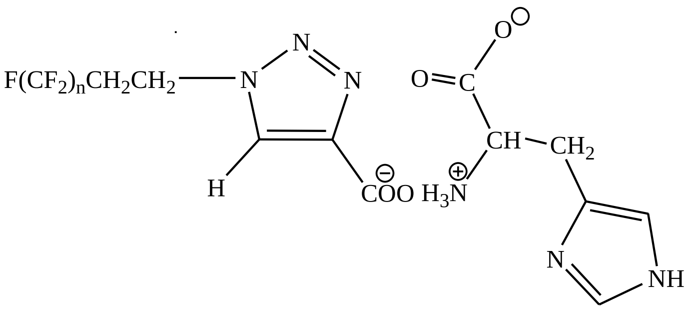


Figure 3.10 ATR spectrum of 2-perfluoroalkyl-ethyl-1,2,3-Triazole-Histidine.

In ^{1}H-NMR spectrum of compound, the duplet at 10,5 ppm belongs to imidazole NH protons assigned to protons of **a.** The singlet 8,54 ppm belongs to triazole -CH protons is assigned to protons of **b**. The dublet at 8,02 ppm is assigned to the amide protons **c**. The singlet at 7,68 ppm is assigned to the imidazol –CH protons is assigned to protons of **d.**

In ATR spectrum of compound, the band at 1748 cm^{-1} belongs to the carbonyl group and the band at 1651 cm^{-1} belongs to the amide bond. The band at 1100-1300 cm^{-1} belongs to the C-F group.

3.4.1 2-perfluoroalkyl-ethyl-1,2,3-Triazole-Histidine Salt



Scheme 3.6 Synthesis of 2-perfluoroalkyl-ethyl-1,2,3-Triazole-Histidine Salt.

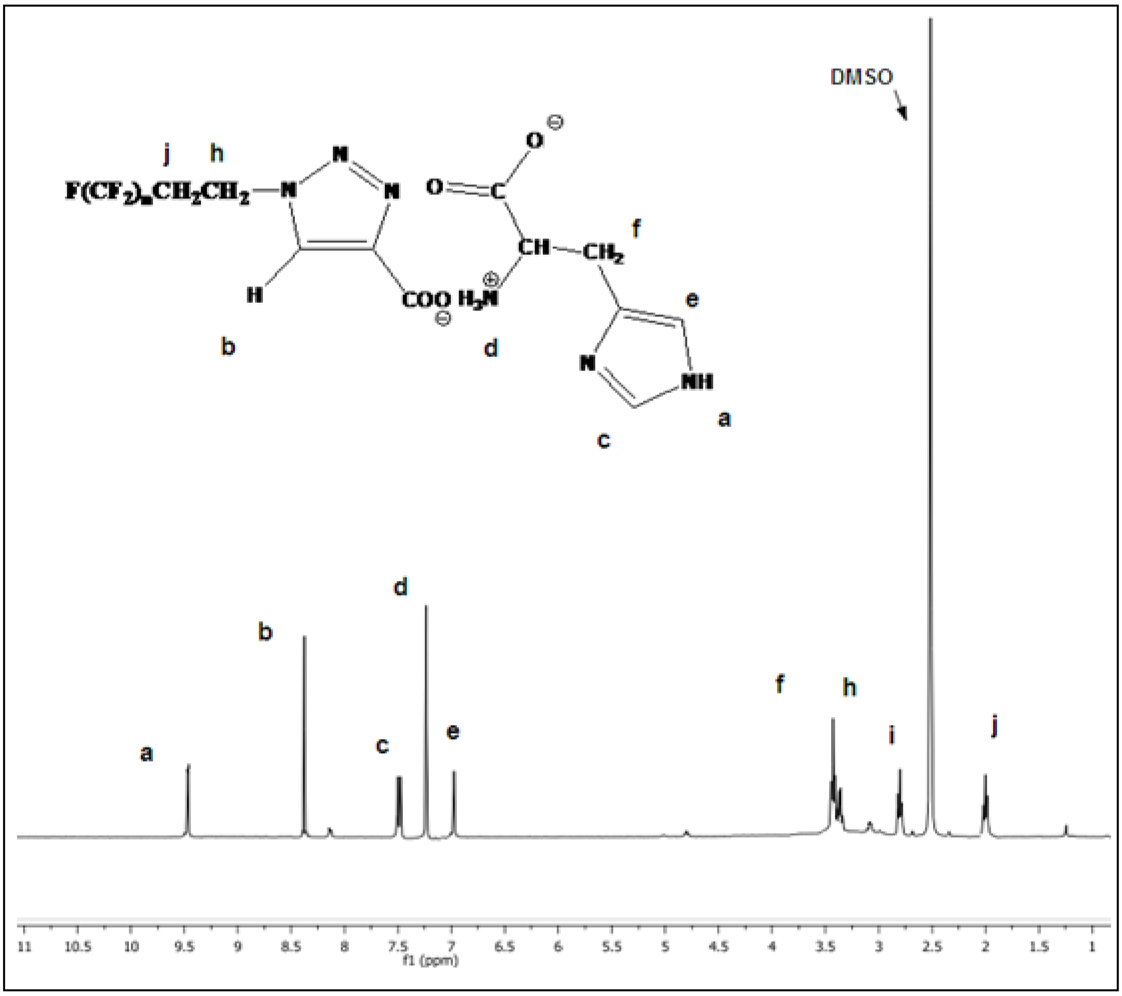


Figure 3.11 ^{1}H NMR of 2-perfluoroalkyl-ethyl-1,2,3-Triazole-Histidine Salt.

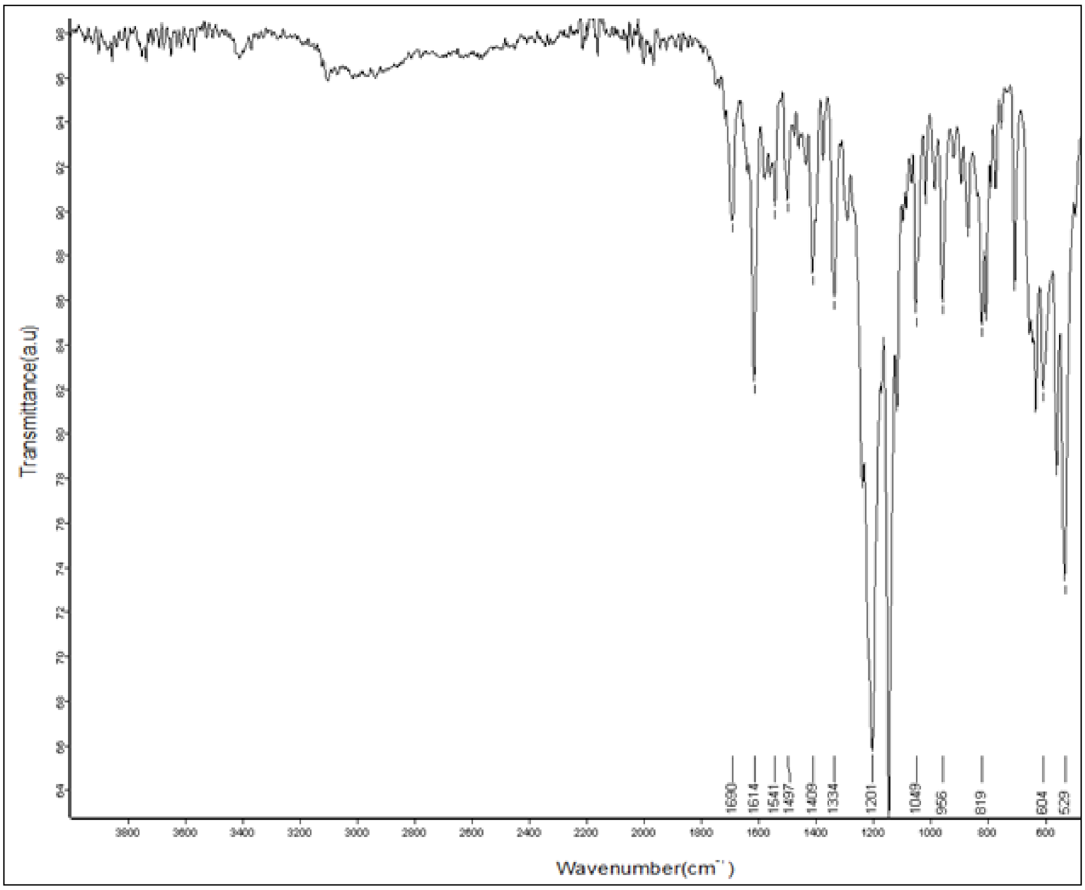
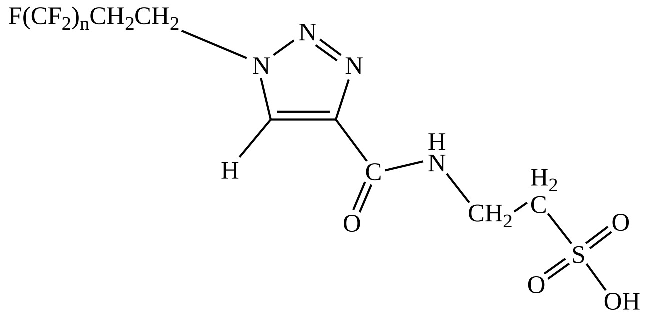


Figure 3.12 ATR spectrum of 2-perfluoroalkyl-ethyl-1,2,3-Triazole-Histidine Salt.

In ^{1}H-NMR spectrum of compound, the duplet at 9,5 ppm belongs to imidazole NH protons assigned to protons of **a.** The singlet 8,5 ppm belongs to triazole -CH protons is assigned to protons of **b**. The duplet at 7,5-7 ppm is assigned to the imidazol –CH protons is assigned to protons of **c** + **e**. The triplet at 7,25 ppm is assigned to the amine protons **d**.

In ATR spectrum of compound, the band at: 1690 cm^{-1} belongs to the carbonyl, and the bands at 3100 cm^{-1} amine bond and 1100-1300 cm^{-1} belongs to the C-F group.

3.5 2-perfluoroalkyl-ethyl-1,2,3-Triazole-Taurine



Scheme 3.7 Synthesis of 2-perfluoroalkyl-ethyl-1,2,3-Triazole-Taurine.

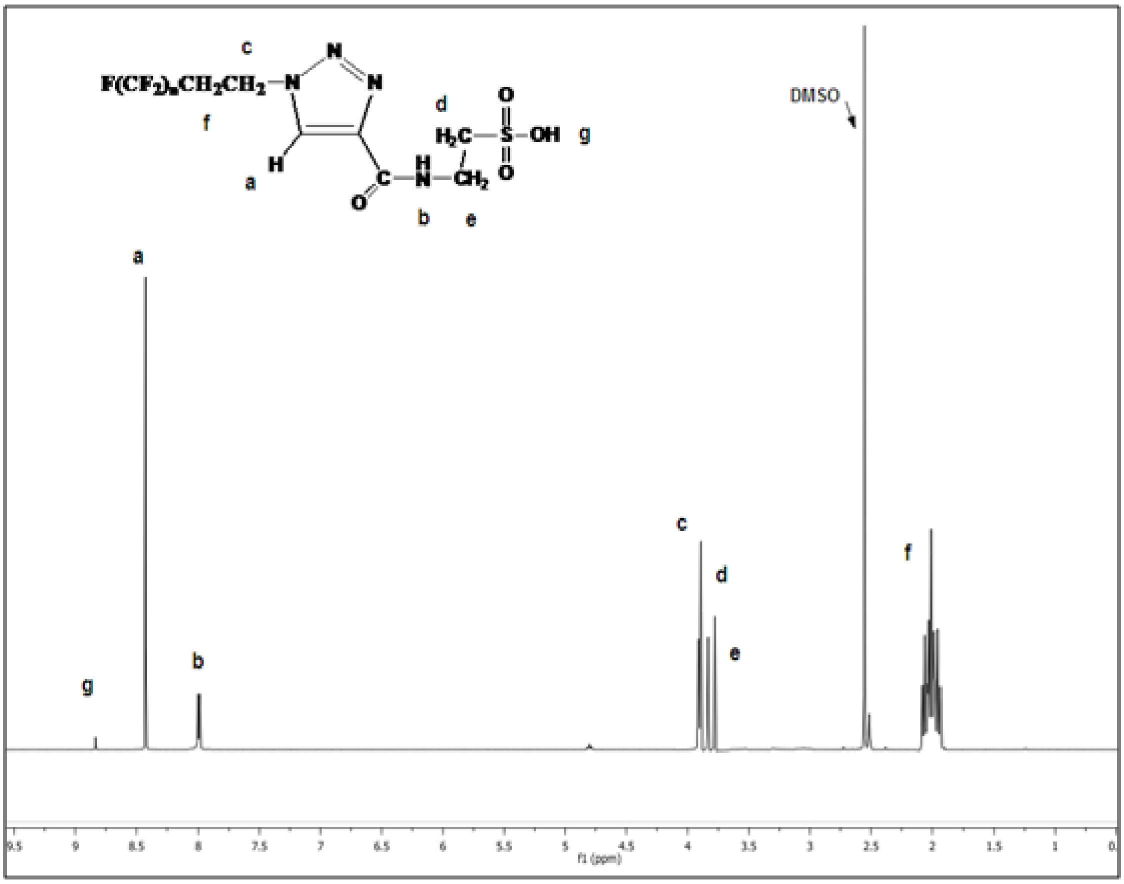


Figure 3.13 ¹H NMR of 2-perfluoroalkyl-ethyl-1,2,3-Triazole-Taurine.

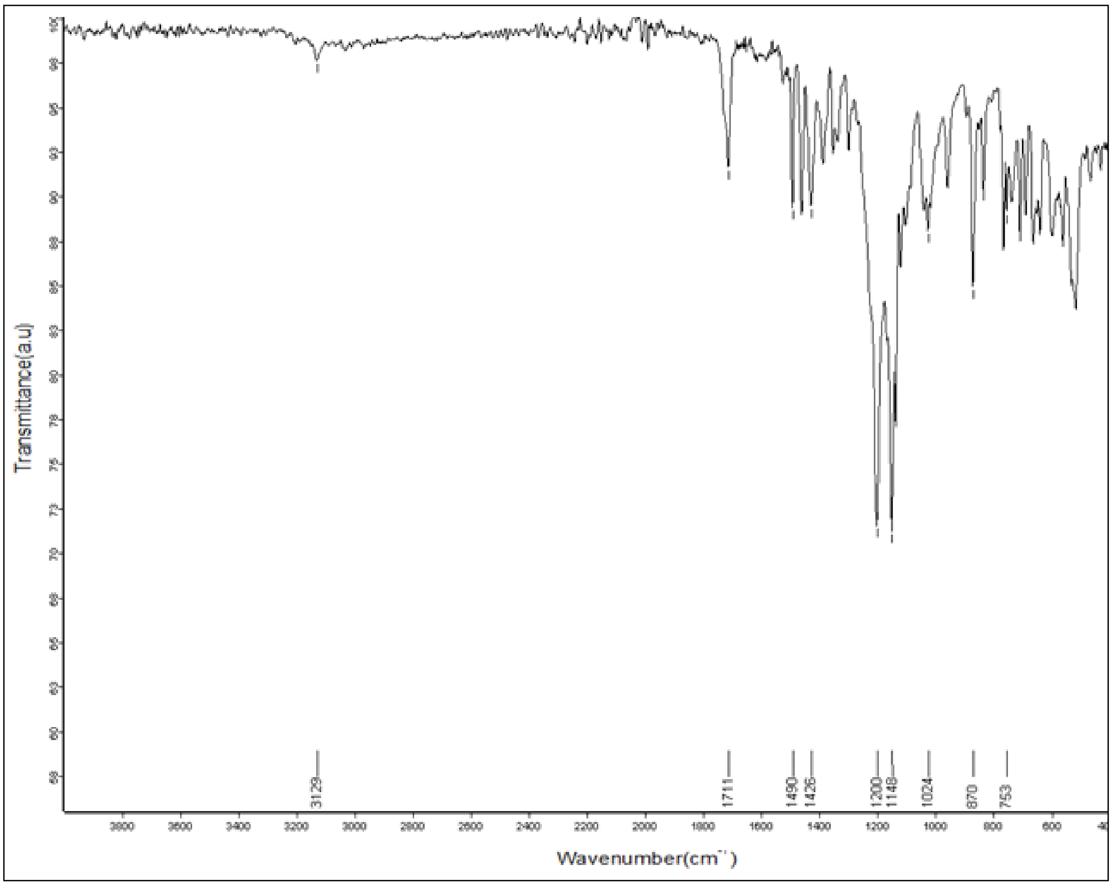
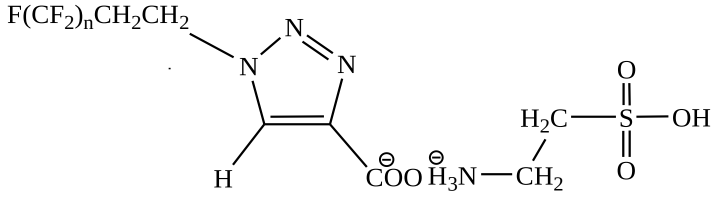


Figure 3.14 ATR spectrum of 2-perfluoroalkyl-ethyl-1,2,3-Triazole-Taurine.

In ^{1}H-NMR spectrum of compound, the singlet at 8,52 ppm belongs to triazole -CH protons assigned to protons of **a**. The triplet 8,01 ppm belongs to amide -NH protons is assigned to protons of **b**. The triplet at 8,8 ppm belongs to -OH protons is assigned to protons of **g**.

In ATR spectrum of compound, the band at 1711 cm^{-1} belongs to the carbonyl group and the band at 1690 cm^{-1} belongs to the amide bond. The band at 1100-1300 cm^{-1} belongs to the C-F group.

3.5.1 2-perfluoroalkyl-ethyl-1,2,3-Triazole-Taurine Salt



Scheme 3.8 Synthesis of 2-perfluoroalkyl-ethyl-1,2,3-Triazole-Taurine Salt.

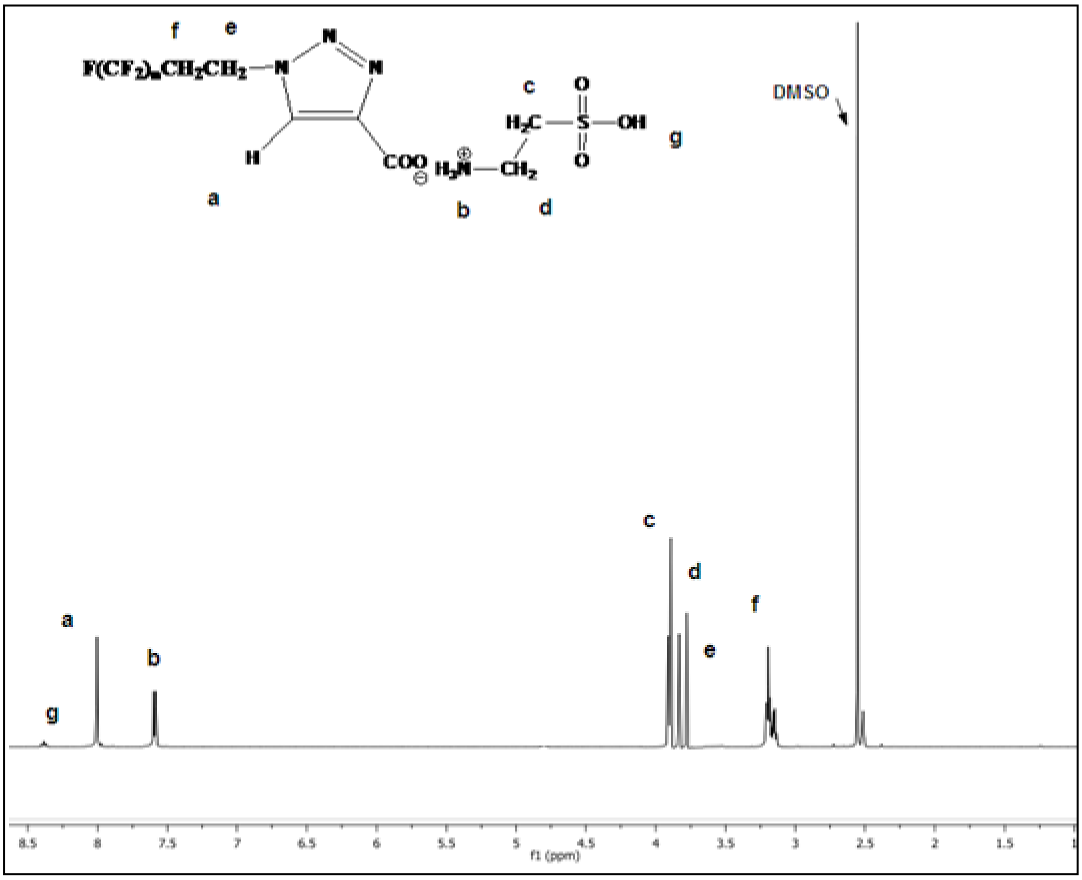


Figure 3.15 ^{1}H NMR of 2-perfluoroalkyl-ethyl-1,2,3-Triazole-Taurine Salt.

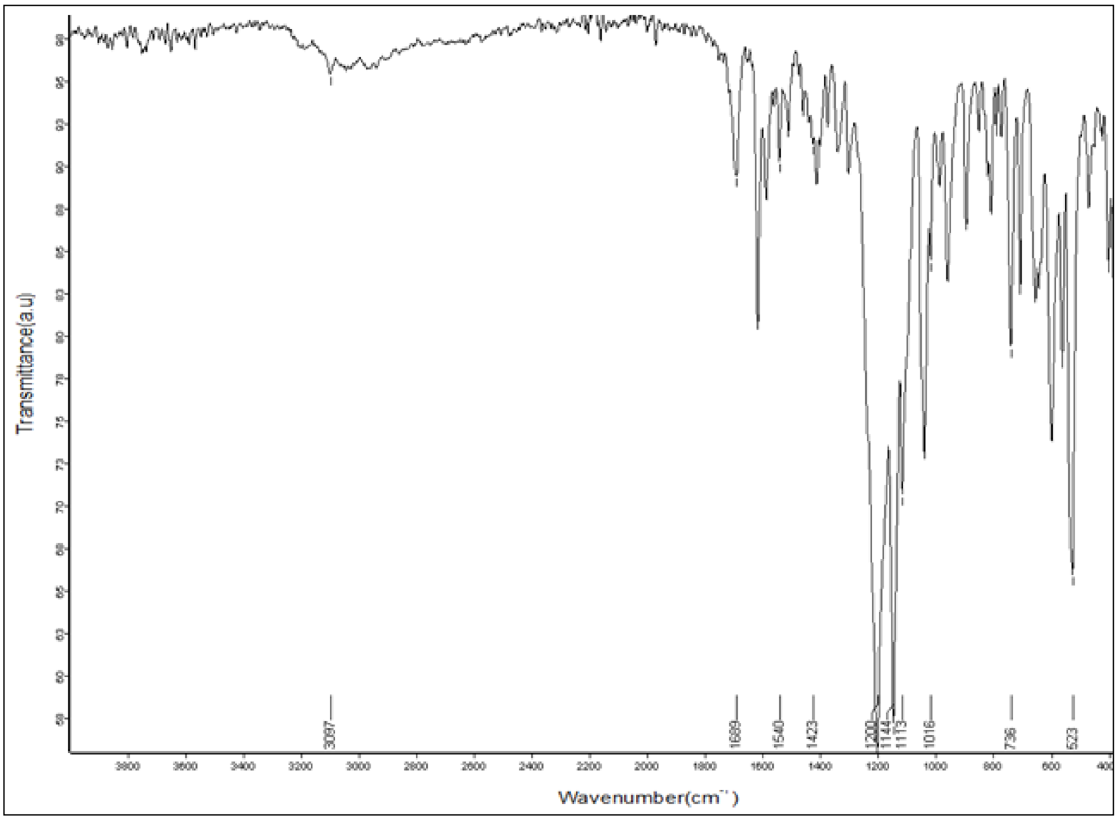


Figure 3.16 ATR spectrum of 2-perfluoroalkyl-ethyl-1,2,3-Triazole-Taurine Salt.

In ^{1}H-NMR spectrum of compound, the singlet at 8,02 ppm belongs to imidazole NH protons assigned to protons of **a.** The triplet 7,53 ppm belongs to amine-NH protons is assigned to protons of **b**. The multuplet at 3,9-3,8-3,75 ppm is assigned to the methylene –CH protons is assigned to protons of **c**+d+**e**. The singlet at 8,4 ppm is assigned to the alcohol protons **g**.

In ATR spectrum of compound, the band at 1689 cm^{-1} belongs to the carbonyl group and the band at 3097 cm^{-1} belongs to the amine bond. The band at 1100-1300 cm^{-1} belongs to the C-F group.

CHAPTER 4

APPLICATIONS

4.1 INTRODUCTION

Investigating physicochemical characteristics, antimicrobial activities and the synthesized compounds constitutes the application part of our work.

As it is stated before fluorinated chains confer unique properties to molecules. These properties are the foundations of new therapies based, for example, on fluorocarbons for oxygen delivery (blood substitutes) or liquid ventilation procedures that are currently in advanced human clinical trials (Mayot. E. et al 2005). Therefore, the in vivo behavior of fluorocarbons and information on the biological effects and toxicity of such compound is very important.

The dominant characteristics of fluorinated surfactants are their high surface activity and their strong tendency to self-aggregate into stable, well-organized assemblies such as micelles, vesicles and tubules. Because of their high propensity to collect at interfaces, fluorinated surfactants can be used for the formulation of a range of multi-phase colloidal systems including direct and reverse fluorocarbon emulsions, microemulsions, gels, dispersions, aerosols, etc. Many of these colloidal systems have potential as drug delivery systems. So investigations on the surface activity and micelle formation properties of synthesized molecules have particular importance.

4.2 PHYSICOCHEMICAL CHARACTERIZATION

Physicochemical characterization of compounds has included measurements of surface tensions and critical micelle concentrations and scanning electron microscopy (SEM) and obtaining polarized optical microscopy (POM) images of the surfactants.

4.2.1 Determination of Surface Tensions and Critical Micelle Concentrations

Surface tension and critical micelle concentration measurements are performed using Wilhelmy plate technique. Wilhelmy plate is a thin plate that is used to measure equilibrium surface or interfacial tension at an air-liquid or liquid-liquid interface.

In this method, the plate is oriented perpendicular to the interface, and the force exerted on it is measured (Figure 4.1).

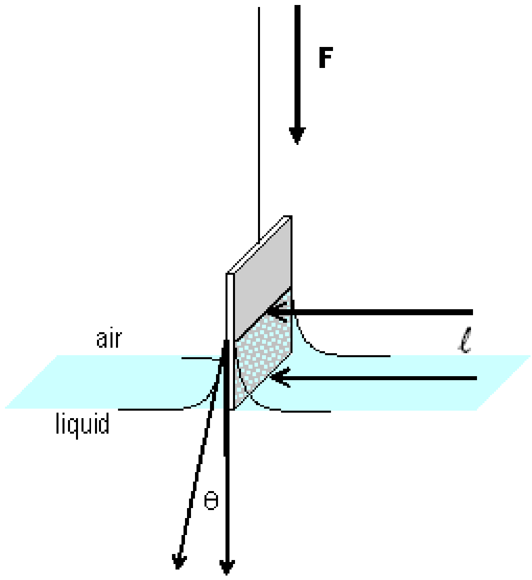


Figure 4.1 Representation of Wilhelmy plate technique.

The Wilhelmy plate consists of a thin plate usually on the order of a few centimeters square. The plate is often made from glass or platinum which may be roughened to ensure complete wetting. The plate is cleaned thoroughly and attached to a scale or balance via a thin metal wire. The force on the plate due to wetting is measured via a tensiometer or microbalance and used to calculate the surface tension (σ) using the Wilhelmy equation:

$$\sigma = \frac{F}{l \cdot \cos\theta} \tag{4.1}$$

Where l is the wetted length of the Wilhelmy plate and θ is the contact angle between the liquid phase and the plate. In practice the contact angle is rarely measured, instead either literature values are used, or complete wetting ($\theta = 0$) is assumed.

4.2.1.1 Results

We have measured the surface tension γ of aqueous solutions of the compounds at different concentrations C. The curves $\gamma = f(\log C)$ are shown in Fig. 4.3. The intersection of the linearly decreasing and the horizontal branch of each curve yields immediately the value of the critical micelle concentration (cmc) and the minimal surface tension γcmc. The intersecting solid lines shown in Fig. 4.3 are the result of a linear regression calculations based on the corresponding experimental values.

In Figure 4.2, collection of surfactant monomers on the surface and micelle formation is illustrated. Firstly monomers cover all the surface of liquid by aligning the hydrophobic chains outside of the surface. Then micelles start to form after critical micelle concentration.

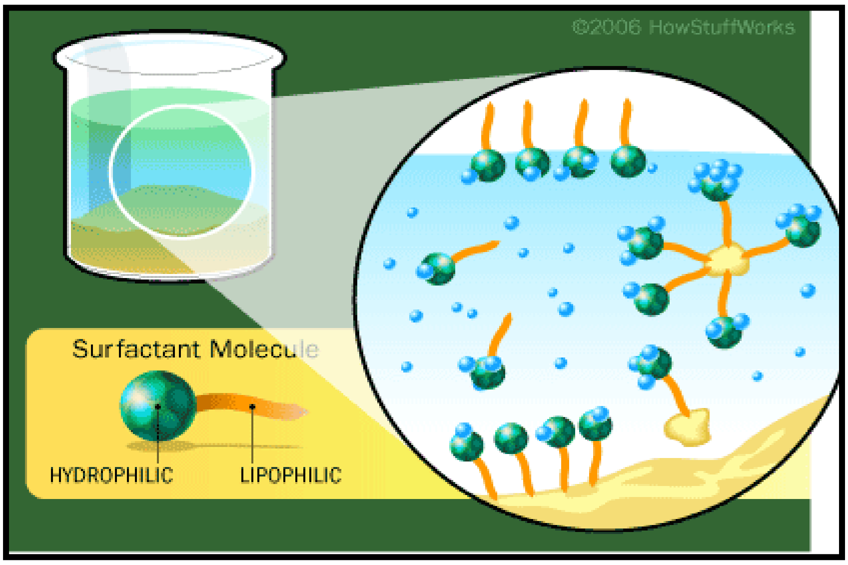


Figure 4.2 Behaviours of amphiphilies in water.
(http://static.howstuffworks.com/gif/play-doh-surfactant.gif)

The change of surface tension by dissolution of some compounds originates from the adsorption of the solute on to the surface tension of the solutions. The relationship is given by the Gibbs' adsorption isotherm equation, expressed as:

$$\Gamma = \frac{-1}{2.303RT}\frac{\partial\gamma}{\partial \log C} \tag{4.2}$$

Where γ(mN / m^{-2}) is the surface tension of the solute. Γ (gamma) is the adsorption amount at the surface or the surface excess concentration (mol / cm^2). It simply describes the number of moles of surfactant monomers per cm^2 of surface area (Drew, M. 1992 and Rosen, MJ. 1978). T is the absolute temperature, and R = 8.314 J mol^{-1} K^{-1}. From the surface excess concentration, the area per molecule at the interface *A* expressed in square angstroms is calculated from the relationship:

$$A = \frac{10^{16}}{\Gamma N} \tag{4.3}$$

Where N is the Avogadro's constant and Γ is in mol/cm^2.

Table 4.1 summarizes the results of these measurements and calculations. Very low surface tension values were obtained for histamine, histidine and taurine derivatives (surface tension of water is 72,8 mN /m). It was also observed that increasing number of -CF_2 groups in perfluoro chain increases the surface activity by lowering the surface tension values. Lowest surface tension values are obtained for taurine derivatives if hydrophobic chains are chosen same. In Table 4.2, for comparison, we have included the values obtained by other authors (Krafft, M.P. 2001) for compounds with structures similar to the synthesized surfactants. It may be noticed that the γcmc-values of the perfuorinated amphiphilic products synthesized in this work are very close to those usually found for this type of compounds.

Table 4.1 Minimal surface tension (γcmc) and critical micelle concentration (cmc) for aqueous solutions of compounds.

$F(CF_2)_n$-Triazol-Histamine				
	cmc (mol/l)	γ (mN/m)	Γ (mol/cm^2) x 10^{-10}	A (Wilhelmy) (A°2)
F8-TA-Hi	2,15 10^{-4}	17,59	7.23	83.89

$F(CF_2)_n$-Triazol-Histidine				
	cmc (mol/l)	γ (mN/m)	Γ (mol/cm^2) x 10^{-10}	A (Wilhelmy) (A°2)
F8-TA-His	1.64 10^{-4}	16,47	5.08	97.75

$F(CF_2)_n$-Triazol-Taurine				
	cmc (mol/l)	γ (mN/m)	Γ (mol/cm^2) x 10^{-10}	A (Wilhelmy) (A°2)
F8-TA-Tau	3,33 10^{-4}	15,392	0.84	25,4

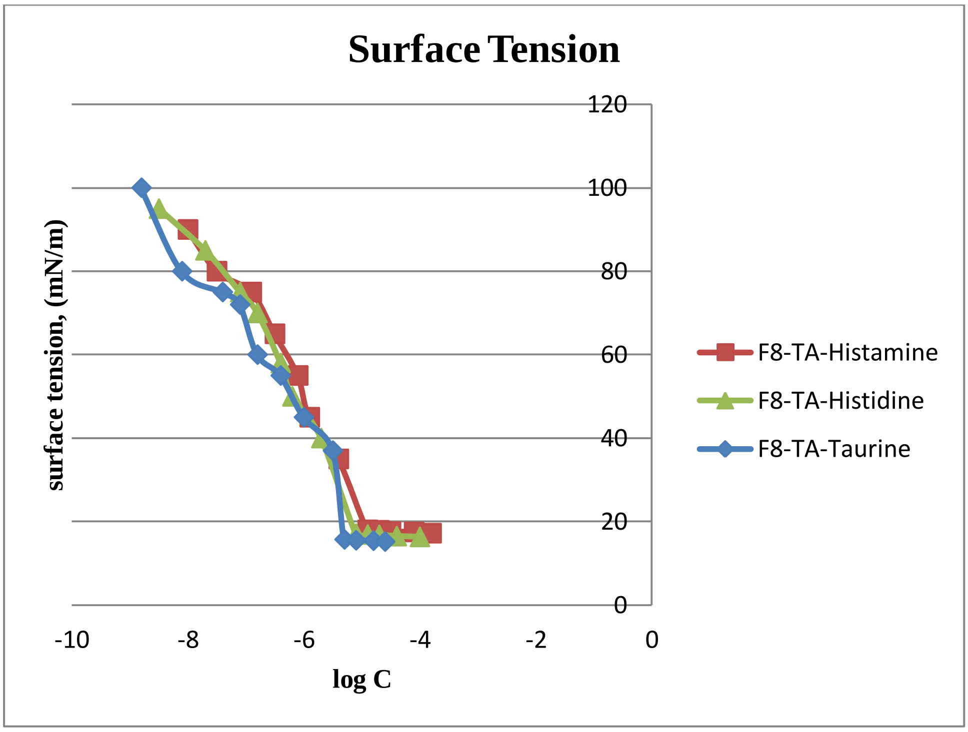


Figure 4.3 Surface tension measurements of surfactant.

Table 4.2 Minimal surface tension (γcmc) and critical micelle concentration (cmc) values for analogous compounds reported in the literature.

Compound	*γcmc (± 1 N/m)*	*cmc x 10^{-4} (mol/l)*
RF5-carnosine, HCl	19	29.5
$F(CF_2)_6$-CH_2-$(OC_2H_4)_4$-OH	18	1,17
$F(CF_2)_7$-CH_2-$(OC_2H_4)_4$-OH	17	0,41
$F(CF_2)_6$-CH_2-$(OC_2H_4)_4$-OCH_3	18	1,15
$F(CF_2)_7$-CH_2-$(OC_2H_4)_4$-OCH_3	17	0,39
$F(CF_2)_6$-CH_2-C(O)-Gly-Sar-$GlyNH_2$	17	15
$F(CF_2)_8$-CH_2-C(O)-Gly-Sar-$GlyNH_2$	16	1,3
$F(CF_2)_6N_3PC$	23	7,6
$F(CF_2)_6NH_3PC$	25	38

The surfactants prepared by Hamdoune and coworkers (Hamdoune, F. 1992) represent a series of non-ionic lipo-oligopeptide amphiphiles bearing a perfuorinated alkyl chain. These products are rather water soluble and their γcmc values range from 16 to 19 mN/m. For these non-ionic perfuoro-lipo-peptides the addition of two $–CF_2$ groups leads to a decrease of the γcmc by a factor of about 10. In a similar way, the increase in chain length by one $–CF_2$ group in the non-ionic surfactants synthesized by Achilefu and coworkers (Papadopoulos, D. 1999) lowers the γcmc by a factor of 3. The properties of our compounds compare favorably with these observations. Perfluorination reduces the γcmc by a factor 9-10 for $–CF_2$ groups. When we apply same analysis for perhydrogenation, we see that perhydrogenation decreases the γcmc value by a factor 1-2 per $–CH_2$ group.

Although the hydrophobic tails of the two compounds are identical, the hydrophilic parts of the molecules differ significantly. Critical micelle concentration is mostly related with the hydrophobic group and it might be concluded that perfluoro group is more dominant to appoint the cmc values.

When increase the length of hydrophobic chains an increment in the Γ values, which describes the amount in number of surfactant monomers per unit area, has observed. This might be because; increasing hydrophobic tail's efficiency causes the monomers behave more water hating by allowing them oriented on the surface. If we choose same series of hydrophobic groups, such as F4 series, we see that a lowering in the Γ values is observed. For example F8 systems have lower values than F6 and F4 systems. Again this is because increasing hydrophilic group's efficiency causes lower collection of monomers on the surface; since water-hating is lowered.

A change in the *A* values (describes the area of a hydrophilic head on surface of the water) depending on the length of perfluoro and perhydrogenated chains has also observed. An increment in the length of either of these chains causes a lowering in *A* values.

In summary, the above results show clearly that the products synthesized in this work are indeed, as expected, typical surfactants. The comparisons with analogous molecules reveal a classical behavior both for their surface activity and for their critical micelle concentrations.

4.2.2. Polarized Optical Microscopy

Polarized optical microscopy is a good technique to obtain the information on morphology and surface characterization of both crystalline materials and surfactants. Amphiphilic molecules have rich phase diagrams, with much novel morphology. In dilute solution, the surfactants do not form any particular structure. However, as the concentration is increased, the amphiphiles condense into well defined structures such as micelles, vesicles, hexagonal arrays or lamellas (Figure 1.2).

Polarized optical microscopy images of Histamine, Histidine and Taurine with Perfluorinated Triazoles systems are shown in Figure 4.4. In both images fan-like structures (structures having ordered domains in the texture) are observed (Achilefu, S. 1994).

a) b)

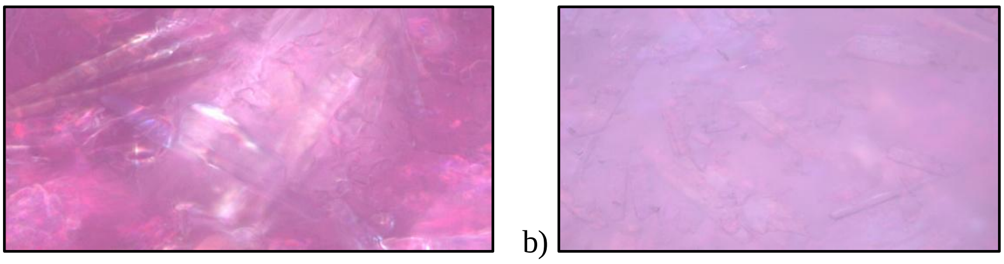


Figure 4.3 Polarized optical microscopy images of a) 2-perfluoroalkyl–ethyl-1,2,3–Triazole-Histamine b) 2-perfluoroalkyl-ethyl-1,2,3-Triazole-Histamine Salt. (Scale bar is 200 µm. T=25 ^{o}C).

In Figure 4.4 POM images of Triazole-histidine and Triazole-histidine-Salt systems after 55 ^{o}C heating are observed. As it can be seen in the images, any considerable morphological changes depending on the temperature increase have not been resolved.

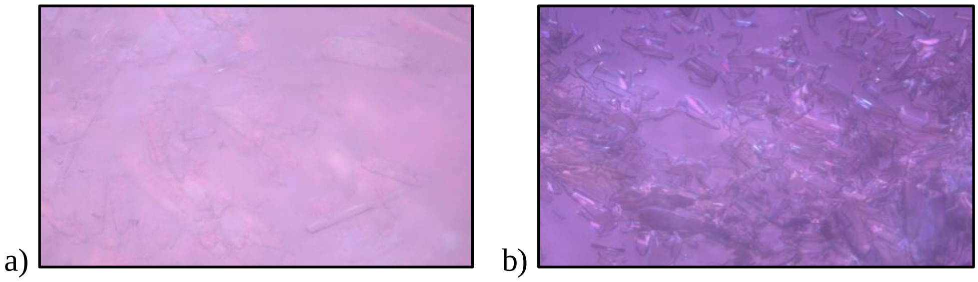


Figure 4.4 Polarized optical microscopy images of a) 2-perfluoroalkyl–ethyl-1,2,3–Triazole-Histidine b) 2-perfluoroalkyl-ethyl-1,2,3-Triazole-Histidine Salt. (Scale bar is 200 μm T=25 ^{o}C).

POM images of 2-perfluoroalkyl–ethyl-1,2,3–Triazole-Taurine and Salt systems are shown in Figure 4.5. Vesicle-like structures are seen in the images.

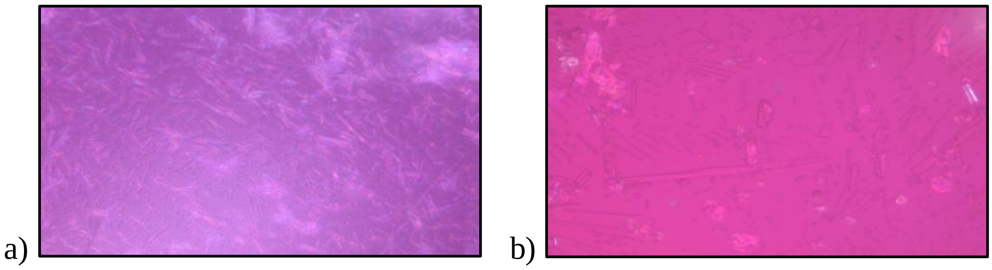


Figure 4.5 Polarized optical microscopy images of a) 2-perfluoroalkyl–ethyl-1,2,3–Triazole-Taurine b) 2-perfluoroalkyl-ethyl-1,2,3-Triazole-Taurine Salt. (Scale bar is 200 μm T=25 ^{o}C)

4.2.3 Scanning Electron Microscopy

The scanning electron microscope (SEM) uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. The signals that derive from electron-sample interactions reveal information about the sample including external morphology (texture), chemical composition, and crystalline structure and orientation of materials making up the sample. In most applications, data are collected over a selected area of the surface of the sample, and a 2-dimensional image is generated that displays spatial variations in these properties. Areas ranging from approximately 1 cm to 5 microns in width can be imaged in a scanning mode using conventional SEM techniques (magnification ranging from 20X to approximately 30,000X, spatial resolution of 50 to 100 nm). The SEM is also capable of performing analyses of selected point locations on the sample; this approach is especially useful in qualitatively or semi-quantitatively determining chemical compositions (using EDS), crystalline structure, and crystal orientations (using EBSD). The design and function of the SEM is very similar to the EPMA and considerable overlap in capabilities exists between the two instruments.

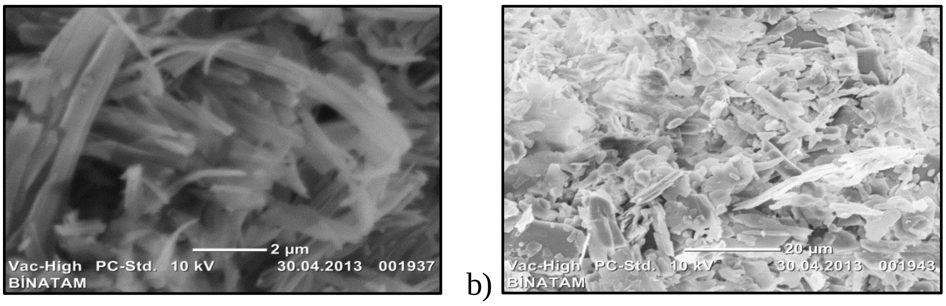


Figure 4.6 Scanning electron microscopy images of a) 2-perfluoroalkyl–ethyl-1,2,3–Triazole-Histamine b) 2-perfluoroalkyl-ethyl-1,2,3-Triazole-Histamine Salt.

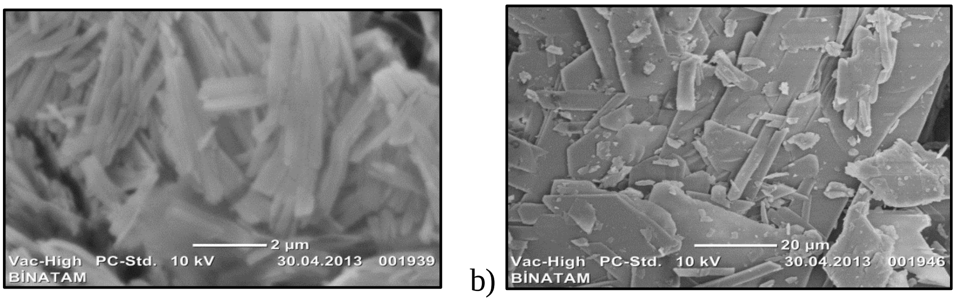
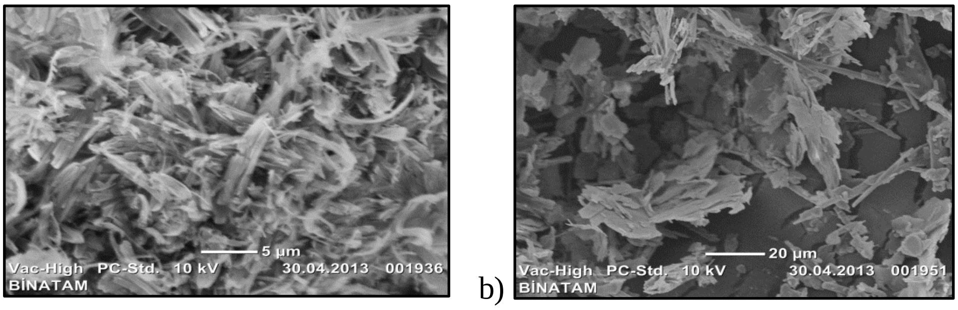


Figure 4.7 Scanning electron microscopy images of a) 2-perfluoroalkyl–ethyl-1,2,3–Triazole-Histidine b) 2-perfluoroalkyl-ethyl-1,2,3-Triazole-Histidine Salt.



a) b)

Figure 4.8 Scanning electron microscopy images of a) 2-perfluoroalkyl–ethyl-1,2,3–Triazole-Taurine b) 2-perfluoroalkyl-ethyl-1,2,3-Triazole-Taurine Salt.

4.3 ANTIMICROBIAL ACTIVITY DETERMINATION

Antibacterial and antifungal evaluations of our molecules were run using MIC (minimum inhibitory concentration) measurements on five bacteria i.e. *Gr (+): Basillus subtilis, Staphylococcus aureus, Gr (-): E. coli, Pseudomonas aeroginosa, Pseudomonas mirabilis, Citrobacter freundii, Klepsiella pnemonia* and three fungi; *Candida utilis, Candida albicans* and *Cryptococcus neoformans.* The MIC is defined as the lowest concentration of an antimicrobial agent that will inhibit the growth of a microorganism after incubation. Three independent experiments were undertaken for each compound. The choice of these strains allows us to study a broad spectrum of micro-organisms including Gram-positive Gram-negative bacteria and fungi. The MICs were taken as the minimal concentration showing no growth (turbidity) after 24 h of incubation at 37 ^{o}C for bacteria and after 48 h of incubation at 30 ^{o}C for fungi. MICs were determined using a Broth serial dilution method (a procedure outlined by National Committee for Clinical Laboratory Standards (NCCLS)) (Hamdoune, F.; 1992). The final inoculation (inoculums) approximately was 10^5cfu /ml. Adjustment was made by optical density measurement for bacteria and fungi (at a wavelength of 600 nm). The antimicrobial behavior were evaluated in comparison with six efficient commercial references i.e. Ampicillin, Kanamicin, Tetracylin, Eritromicin, Nalidic acid, Streptomicin for bacteria and two references Amphotricin-B, Nystatin for fungi. Since the stock solutions of compounds were prepared by using DMSO as solvent, the inhibitory effect of solvent on microorganisms were also undertaken.

4.3.1 Results

MIC ($\mu g\ ml^{-1}$) values for antibiotics on bacteria:

Bacteria/ Antibiotic	*Ampicillin*	*Kanamicin*	*Tetracylin*	*Eritromicin*
E. coli	> 256	16	2	128
P. aeroginosa	128	2	1	< 0,125
P. mirabilis	16	128	128	> 256
C. freundii	8	16	16	256
K. pnemonia	0,125	0,125	>256	2
S. aureus	128	>256	256	>256
B. subtilis	> 256	> 256	8	< 0,125

Bacteria/ Antibiotic	*Nalidic acid*	*Streptomicin*	*DMSO*
E. coli	16	> 256	256
P. aeroginosa	2	64	128
P. mirabilis	> 256	>128	256
C. freundii	64	8	256
K. pnemonia	> 256	8	128
S. aureus	> 256	>256	256
B. subtilis	8	16	256

MIC ($\mu g\ ml^{-1}$) values for compounds on bacteria

Bacteria/ Antibiotic	*F8-TA-His*	*F8-TA-Hist*	*F8-TA-Tau*
E. coli	> 256	> 256	> 256
P. aeroginosa	> 256	128	128
P. mirabilis	> 256	> 256	> 256
C. freundii	> 256	> 128	> 128
K. pnemonia	> 256	> 256	> 256
S. aureus	> 256	> 256	> 256
B. subtilis	16	> 256	> 256

MIC ($\mu g\ ml^{-1}$) values for antibiotics on fungi:

Fungus/ Antibiotic	*Amphotricin-B*	*Nystatin*
C. albicans	0,250	0,50
C.utilis	8	4
C. neoformans	16	0,5

MIC ($\mu g\ ml^{-1}$) values for compounds on fungi:

Fungi/ Antibiotic	*F8-TA-His*	*F8-TA-Hist*	*F8-TA-Tau*

C. albicans	128	256	>256
C.utilis	8	16	>256
C. neoformans	128	128	>256

4.3.2 Discussion of the Results

Since the perfluorinated surfactants have benefits in the applications of biologically important procedures such as oxygen delivery in emergency or drug delivery applications, in vivo characterization of compounds is very important. Antimicrobial activities of our compounds have been investigated based on the criteria:

How strong is the F_n-Triazole- aminoacid structure to kill the microorganisms?

Does attachment of histamine, histidine and taurine influence the activity?

How important is the length of perfluoro chain for activity?

Although the proposed mechanisms of action of different amphibilic antibiotics are numerous and not completely clarified, the first common step in all the presented mechanisms is related to the amphiphilic character of these compounds which allows them to strongly interact with the cell membrane (Krafft, M.P. 2001). Surfactant-like interaction with the cytoplasmic membrane of bacteria results in the loss of permeability properties of the membrane. Thus, a large part of their antimicrobial activity is conditioned by their surface active properties and consequently by the length of the hydrophobic chains (Pernak, J.; 2001). Since in our compounds we both have perfluorinated tail and perhydrogenated tail, the hydrophobic character of the molecules are quite strong and they have good surface active properties. Moreover, bicatenary surfactants (surfactants having hydrophobic tails and hydrophilic head group as it is seen our molecules) lead to much lower critical micelle concentration values and much greater efficiency in reducing surface tension than expected. So these surfactants should be considered as good candidates for biocide purposes.

However from the comparisons of results with activities of commercially available antibiotics we can say that the antimicrobial activity results of our compounds are quite unexpected from the point of view of antimicrobial activity is directly related with surface activity. In general the antimicrobial activities of the compounds are low and MIC values are greater than 256 $\mu g\ ml^{-1}$.

For compounds F8-TA-His and F8-TA-Hist not different results were determined but for F8-TA-Tau the MIC values halves. So, it implies that increasing the perfluorinated chain may cause an increasing in the antimicrobial activity.

In general, it is noteworthy that no direct relationships can be established between the MIC values and cmc, surface tension values or hydrophilic / hydrophobic character of the molecules. This can be explained by the complexity and the great number of components of the antibacterial effect of antibiotics in which the surfactant effect at the surface of the bacterial cell represents only a small part of the whole effect of the antibacterial agent. In deed, what was purposed from this work is not designing strong antibiotics for several antimicrobisms. This study includes in vivo characterization of our compounds.

CHAPTER 5

CONCLUSION AND PERSPECTIVES

In this thesis project a series of analogues of perfuorinated triazole were synthesized. Commercially available 2-perfuoroalkylethyl iodide was used as starting materials and they were substituted with an azide group and than triazole ring. Perfluoroalkylated analogues obtained in this way were subsequently used as hydrophobic moieties for the synthesis of amphiphilic aminoacid derivatives. To examine the physicochemical properties, surface tension and critical micelle concentration measurements were done and polarized optical microscopy images and scanning electron microscope were taken. Measurements on very large scale analogues of the perfluorinated hydrophilic segment (amino acids,) have revealed their surfactant properties. Antimicrobial activity test showed how these compounds are strong to kill several bacteria and fungus.

Synthesizing new surfactants by choosing the hydrophilic heads from different amino acids (histamine, histidine, and taurine) and investigation of antioxidant properties, biocompatibilities and toxicities of compounds are our next purposes to accomplish.

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