PREPARATION AND STUDIES OF RATIOMETRIC FLUORESCENT CHEMOSENORS BASED ON THE ATTENUATION OF EXCITATION ENERGY TRANSFER

A Dissertation

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DEDICATION

This dissertation is dedicated to my late parents

Mr. Dadhi Ram Sharma

&

Mrs Maheshori Devi Sharma

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Sincere acknowledgments are given to my parents Mr. Dadhi Ram Sharma and Maheshori Sharma, to whom this dissertation is dedicated.

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LIST OF ABBREVIATION AND SYMBOLS

$(dppp)NiCl_2$	1,3-bis (diphenylphosphino)propane dichloro nickel
^1H NMR	proton nuclear magnetic resonance spectroscopy
Ac_2O	acetic anhydride
$CHCl_3$	chloroform
CPs	conjugated polymers
d	doublet, deuterated
DCM	dichloromethane
DI	deionized water
DMF	dimethylformamide
DMSO	dimethylsulfoxide
DNT	dinitrotoluene
DPA	di-2-picolylamine
dvtms	Pt(0)-divinyl-tetramethylsiloxane complex
Et_2O	diethyl ether
FTIR	Fourier Transform Infrared Spectroscopy
FRET	Fluorescence Resonance Energy Transfer
g	gram
HOMO	Highest Occupied Molecular Orbital
HPTS	8-hydroxy-pyrene-1,3,6-trisulphonic acid
Hr	hour
Hz	hertz
ICT	Intermolecular Charge Transfer

i-Pr_2NH	diisopropylamine
ITO	indium tin oxide
LUMO	Lowest Occupied Molecular Orbital
m	multiplet
MCM	Mobil Composition of Matter
MeOH	methanol
mp	melting point
n-BuLi	n-butyllithium
PA	polyacetylene
PAn	polyaniline
PEDOT	poly(3,4-ethylenedioxy)thiophene
PET	Photoinduced Electron Transfer
ppm	parts per million
PVCZ	poly(N-vinylcarbazole)
r.f.	retention factor
rt	room temperature
s	singlet
SAM	self-assembled monolayer
SEM	scanning electron microscopy
SNAFI	seminaphthofluorescein
SANRF	seminaphthorhodafluor
t	triplet
TBAF	tetrabutylammonium fluoride
TEA	triethylamine

THF	tetrahydrofuran
TIPSA	triisopropylsilyl acetylene
TMS	trimethylsilyl
TNT	trinitrotoluene
UV/Vis	ultraviolet visible
XPhos	2-dichlorohexylphosphino-2',4',6'-triisopropylbiphenyl ($ArPCy_2$)
δ	delta (chemical shift)
λ	lamda(wavelength)
μm	micrometer
XPS-	X-ray Photoelectron Spectroscopy

ABSTRACT

Molecular devices possessing π-electron conjugated scaffold demonstrate unique capabilities as fluorescent chemosensors for the trace detection of analytes in a variety of environments. Important properties of these compounds such as charge transport and exciton migration, emission intensity etc. can be simply attenuated by external stimuli or analyte binding, leading to considerable changes in observable signals. The main goal of this Ph.D. dissertation was to study the possibility to control excitation energy migration in conjugated systems. Towards this goal, we designed surface-immobilized monodispersed oligo(*p*-phenylene ethynylene)s (OPEs) as a general basis for thin-film ratiometric fluorescent chemosensors. The sensor molecules have been functionalized with a specific analyte receptor at one end and a linkage for the covalent attachment to a glass surface at the opposite end. Upon surface immobilization these sensor molecules form a highly ordered monolayer with the receptors positioned at the monolayer surface. Analyte binding to the receptor causes attenuation of the HOMO–LUMO gap at the receptor terminus leading to the ratiometric change in fluorescent emission. The thin-film sensors for L-cysteine, pH and Zn^{2+} were prepared and studied in detail to uncover a number of unusual properties, thus demonstrating the potential of this platform as a universal foundation for designing a broad range of fluorescent ratiometric thin-film chemosensors.

CHAPTER 1 FLUORESCENT CHEMOSENSORS – A GENERAL OVERVIEW

1.1. Introduction

Chemical sensing generally refers to the continuous monitoring of the analytes present in the various samples of interest.[1] Chemistry, biology, environmental sciences and medical sector constantly require more accurate, highly sensitive, very selective sensors with ease of operation. For these applications, there are always countless numbers of practically important analytes which are always in need of qualitative and quantitative detection.

It is very useful to develop sensors for analytes, which are involved in the biological processes. Such analytes could be metal cations such as sodium, potassium, calcium, which are actively involved in the transmission of nerve impulses, muscle contractions, and regulation of cellular activities. The detection of toxic analytes involved in creating various abnormal conditions such as Alzheimer's disease is also equally important. Likewise, mercury, lead, cadmium ions, which are toxic for plants and animals, are in need of detection more precisely in order to maintain safer and more benign environment. Various industrial explosives and hazardous chemicals can be released into the environment due to explosions and buried landmines [2] in the process of storage and decontamination of warfare agents. The importance of more reliable as well as real time sensing systems is even more pronounced due to the war on terrorism.[3,4] In addition, simple and robust chemical sensing could play an important role from small laboratories to larger industrial settings in order for the control of food quality and safety.

There is always an immense need for reliable and less expensive sensing methods for the wide range of clinical bioprocesses, and in the area of chemical as well as environmental monitoring. Therefore, it is necessary to extend the range of detectable and quantifiable analytes. In sensor design, we can exploit the large number of interactions that nature provides.

For those analytes that cannot be detected with available receptors, new receptors can be synthesized and novel sensors for various applications can be designed.[5] In addition to a selectively binding receptor, a suitable signal transduction should be implemented into a practical sensor. Fluorescent technique has become one of the most powerful transduction methods[6] in order to report an event of chemical recognition. Based on fluorescence lifetime, anisotropy as well as the intensity or the wavelength change of fluorescent bands, numerous microscopy and spectroscopic techniques have been developed and applied for sensing applications.[6] These techniques are very sensitive and bear the capability to detect even single molecules. Another advantage of the fluorescence technique is that it does not consume analytes and a reference is typically not required. Due to the ability of light to travel without any wave-guide, it is possible to carry out remote monitoring in combination with fluorescence.[6] The possibility of forming different designs based on various aspects of the fluorescence wavelength, lifetime, intensity, anisotropy, and energy transfer is one of the advantages of the fluorescence detection.[6,7]

A milestone in fluorescent detection was established by Tsien and coworkers in 1980 by the successful demonstration of fluorescent reporters to signal a molecular recognition event based on calcium ion chelate receptors covalently linked to simple aromatic π-electron conjugated dyes as chromophores.[8-10] Major efforts have been given since then towards the rational design of fluorescent indicators,[11-13] but the challenges to build sensors still remain because a general universal strategy to the implementation of sensing probes in functional devices without the loss of sensitivity has not been developed yet.

1.2. Sensor vs Indicator

In the past, researchers commonly referred to any molecular indicators as “sensors”. This

notion has been criticized and it was recommended that only integration of fluorescent indicators into a device could form a sensor.[10] Later, in 1993, Czarnik overcame this confusion by suggesting a concept of "Chemosensor". According to that concept, *"a fluorescent sensor is a compound of abiotic origin that complexes to an analyte reversibly with a concomitant fluorescent signal transduction"*.[14-15] All other systems should be called "indicators".

1.3. Fluorescent Indicators – Traditional Design

The usual design of fluorescent indicators involves two fragments: a moiety responsible for molecular recognition of the analyte and a fluorophore to signal the recognition event. Traditionally, three strategies are employed in designing fluorescent molecular indicators for chemical sensing in solution.[1,16]

i) **Intrinsic Fluorescent Probes:** These are molecules with mechanism for signal transduction through the interaction of analyte with a ligand that is a part of the π-system of the fluorophore. [17,18]

ii) **Extrinsic Fluorescent probes:** These are also known as conjugates[23] in which, the receptor fragment and the fluorophore are covalently connected but separated electronically by a spacer. [14-15,17,25-26]

iii) **Chemosensing ensemble:** This is based on competitive assay in which the fluorophore-receptor ensemble selectively dissociates upon the addition of appropriate competing analyte, which interacts with the receptor affording a detectable response of the fluorophore.[22-24]

1.4. Fluorescent Materials for Chemosensing

Deposition of indicators on a solid support in order to get a sensor was the next milestone in sensor development. The best and most used approach in this direction is the

immobilization method. Of the several methods that have been employed along this direction, the most common one is entrapment of sensitive probes into a polymer matrix (Figure **1.1**).[25] However such simple entrapment of the sensor dyes into the matrix affords inhomogeneity leading to various problems such as low photostability caused by bleaching of the probe. This results in loss in reproducibility, and reduced life-time.[16] These shortcomings could be minimized by covalently linking the probes into a matrix, and this approach towards practical robust chemosensors has attracted significant attention.[1] In other useful methods, fluorophores can be confined or covalently immobilized on a surface in such a way that the probes align in a specific direction. Silica microspheres,[26-27] glass and gold surfaces,[28] quantum dots,[29-30] Langmuir-Blodgett films,[31] etc. are some typical examples of materials used in combination with fluorescent probes to fabricate covalently immobilized fluorescent materials for sensory applications.

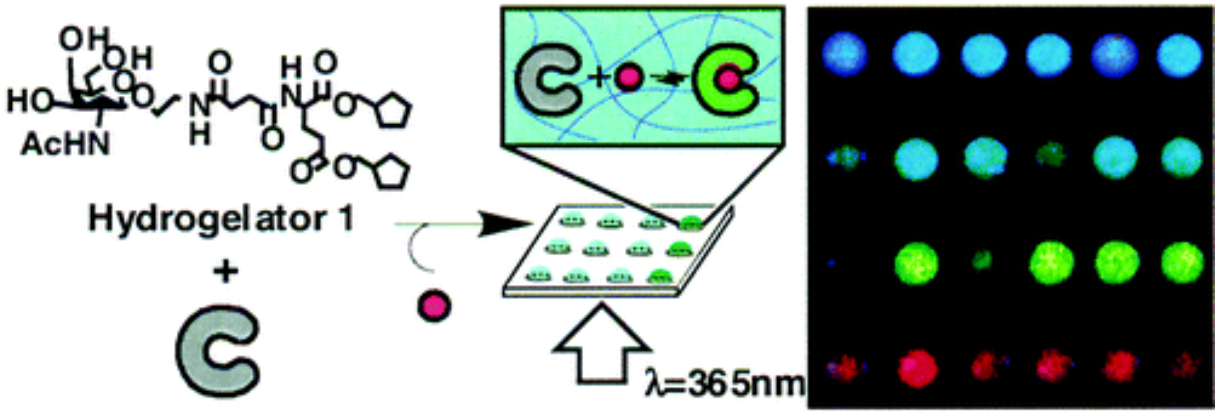

Figure 1.1 Immobilization by entrapment of sensitive probe in a polymer matrix *

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In the following section, application of conjugated polymers, molecularly imprinted polymers, mesoporous materials, and glass and gold surfaces will be discussed in view of their relevance to chemosensor design.

1.4.1. Sensors Based on Conjugated Polymers and Oligomers

Conjugated polymers (CPs) are the polyunsaturated compounds consisting only of either sp- or sp^2 hybridized atoms in their backbone **(Figure 1.2)**. Extended π-electronic conjugation along the backbone creates an analog of a molecular wire with an interaction between the molecular orbitals resulting in valence band (filled with electrons) and unfilled conduction band – an electronic structure typical for inorganic semiconductors. This structural feature of organic CPs make them highly useful in the design of optical and optoelectronic devices.

Conjugated polymers form a foundation of the important fluorescent methods for the trace detection of analytes in a variety of environments. Their delocalized electronic structure enables electronic coupling between repeating units acting as opto-electronic segments and efficient intra- and inter-chain energy transfer. While the inter-chain energy transfer occurs by a through-space (Förster) mechanism,[32] the intra-chain energy transfer involves both through space and through bond (Dexter) pathways. [33]

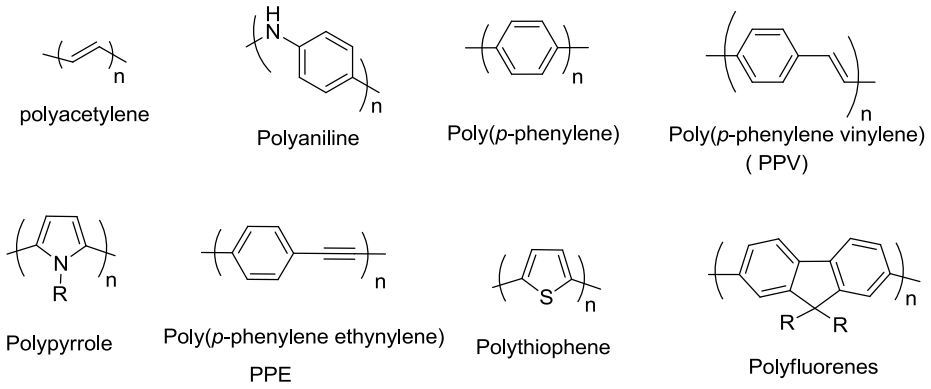

Figure 1.2 Some representative conjugated polymers

A significant improvement in energy transfer efficiency in conjugated polymers has been realized by maximizing electronic conjugation along the polymer backbone which increases the contribution of the through-bond Dexter energy transfer mechanism.[34] The exciton migration (along with other important properties such as charge transport (conductivity), fluorescent emission etc.) can be simply perturbed by external stimuli, leading to considerable changes in quantifiable output signals (Figure **1.3**).[35]

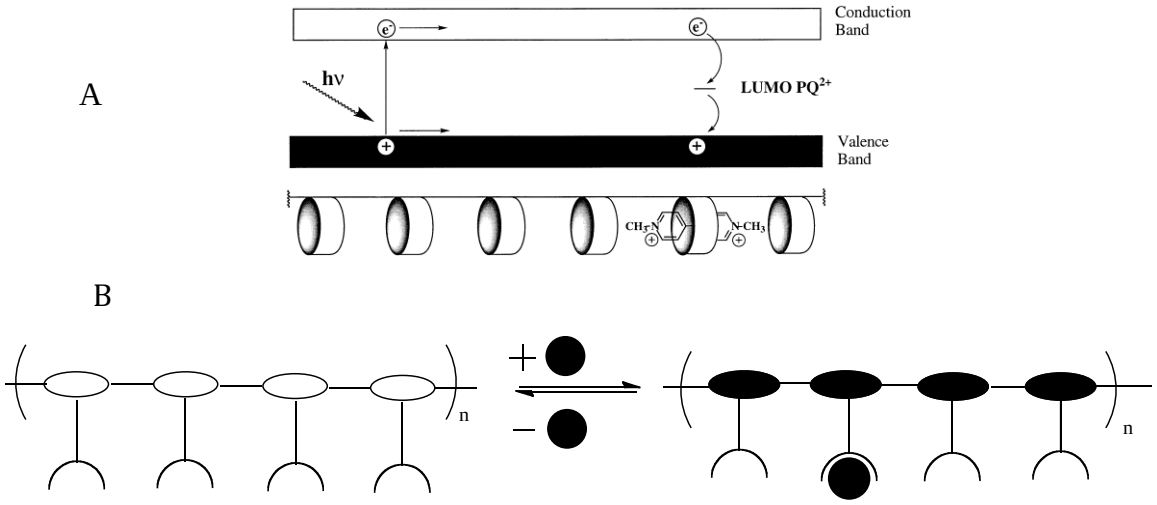

Figure 1.3: (A) Mechanism of exciton migration and electron transfer fluorescence quenching of a conjugated polymer upon interaction with a quenching analyte (B) Interaction of a small concentration of analyte with the receptor produces amplification in fluorescence quenching*

Conjugated polymers can produce strong luminescence with the efficiency being dependent on the delocalization and polarization of the electronic structure. **Figure 1.3** shows the amplification of molecular recognition signal via random migration of excited states (excitons) along the conjugated polymer chain. This figure also explains the mechanism of fluorescence quenching upon analyte binding known as photoinduced electron transfer fluorescent quenching.

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Absorption of light photon by CP causes an electron to be promoted to the conduction band, which thus creates an exciton - a tightly bound electron-hole couple. Further exciton migration along the polymer backbone occurs as a random walk.[36-37] A trapping site is produced upon analyte binding, whereby the excitation is effectively deactivated by electron transfer quenching. In this event, the low energy LUMO of the quencher participates in directing excitons to the ground state in a non-radiative process, resulting in quenching the polymer's fluorescence.[38-39]

Bazan and coworkers have extensively used water-soluble conjugated polymers for biosensor applications.[40-43] Conjugated fluorescent poly(2,2'-bipyridyl-phenylenes-vinylene)s,[44] poly(*p*-phenylene ethynylene)s,[45] and others[46] have been used for the detection of metal ions. The "Turn on" fluorescent sensors for metal ions have been designed by Jones and coworkers (Figure **1.4**).[47-48]

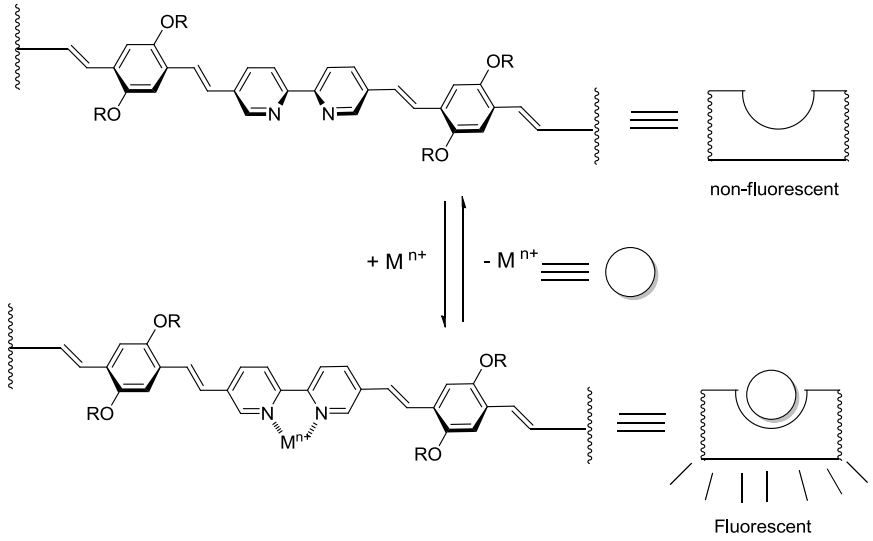

Figure 1.4: Scheme and mechanism of metal ion detection ("turn on") using pyridine-based conjugated polymers.[44]

The N-atom present in these compounds is responsible for quenching the fluorescence by Photoinduced Electron Transfer (PET) mechanism. The coordination of a metal ion with the N-

lone pair of electrons decreases the availability of free electrons for PET quenching, thus resulting in "Turning on" the fluorescent emission.

Poly(*p*-phenylene ethynylene) (PPE) is one of the best known examples of conjugated fluorescent polymers which exhibit a lower band gap at the terminus when linked with an anthracene based receptor group, and shows dominant emission from electronic states localized at the terminus groups.[34,49] Three different groups, Wang,[50] Fujiki,[51] and Swager,[52-53] designed conjugated polymers for fluoride ion sensing. The sensor shown in Figure **1.5** operates based on the reactivity of fluoride ion towards the phenols protected with bulky silyl protecting groups. In order to achieve amplification, the receptor was electronically coupled with a poly(*p*-phenylene ethynylene) through a thiophene ring. The formation of a small number of local band gap traps upon reaction with fluoride resulted in facile electron-exchange (Dexter) energy transfer from the polymer to the dye to provide an amplified fluorescence "turn-on" sensory methodology. The polymer sensor showed approximately was 100-fold higher sensitivity than a corresponding small indicator.

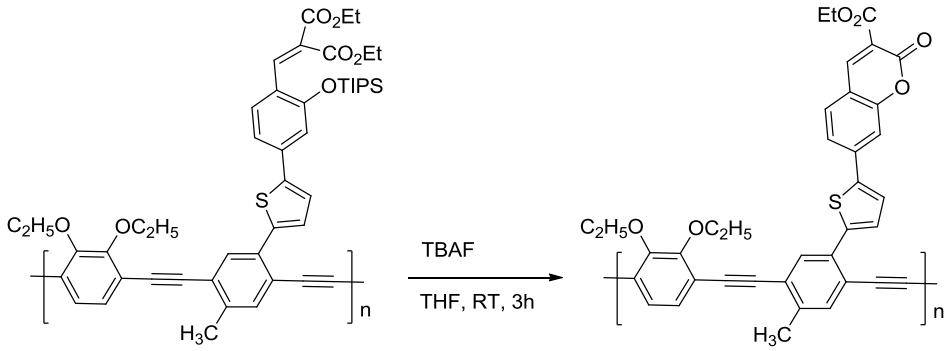

Figure 1.5: Principle of fluoride ion sensing by a PPE polymer. [53]

Along the same line, conjugated polymer sensors have been used often for the detection of nitroaromatic explosives, such as TNT and DNT, (Figure **1.6**).[4, 54] Vapors of TNT and DNT

quench the fluorescence of the polymer due to the strongly electron-deficient nature of the nitroaromatic analytes.

In recent years, a number of fluorescent imprinted polymers which rely upon incorporation of a small fluorescent reporting molecule have been exploited for sensing various biomedically important targets such as histamine,[57] L-Triptophan,[58] cyclicGMP,[59] D-fructose,[60] creatinine[61] and many more.[59-60,62] In these systems, the analyte-polymer interaction results in fluorescence quenching ("turn off" sensor), on the other hand the enhancement of fluorescence ("turn on" sensor) is more convenient practically and is desirable in the area of sensor design. Takeuchi and co-workers recently have developed a new fluorescent imprinted polymer which incorporates a small fluorescent molecule receptor and responds to the analyte-polymer interaction with a high enhancement in fluorescent intensity.[64]

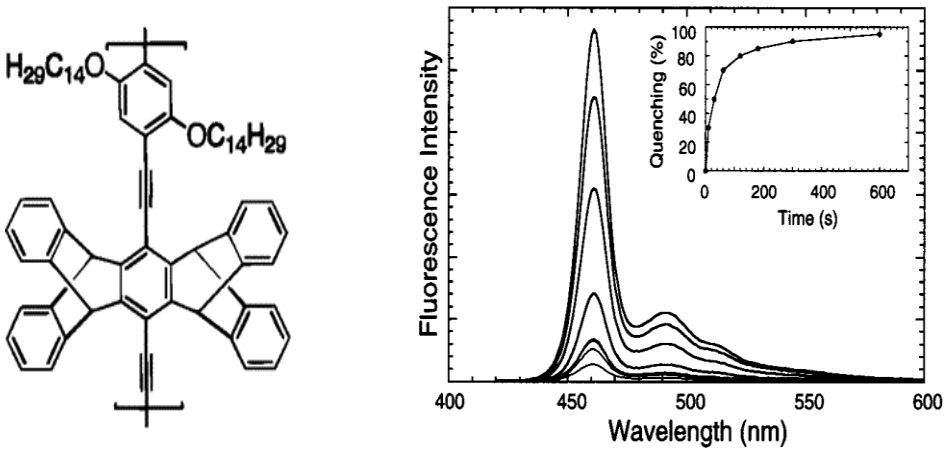

Figure 1.6: TNT sensing polymer and the time-dependent fluorescence intensity of a 25 Å film of this polymer upon exposure to TNT vapor (room temperature) at 0, 10, 30, 60,120, 180, 300, and 600 s (top to bottom), and the fluorescence quenching (%) as a function of time (inset)*

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For the first time, a systematic study using chemically synthesized intrinsically fluorescent molecularly imprinted conjugated polymers was carried out in 2007 in the Nesterov group by Li and Kendig [64] where fluorescent detection of analyte binding was employed for TNT detection. Upon binding to the imprinted binding sites, the analyte interacts with the fluorescent conjugated polymer thus causing quenching of its fluorescence. Relatively poor overall selectivity offsets the advantages of ease of synthesis of fluorescent imprinted polymers[65] however these are considered as suitable materials to be applied as multisensor arrays.[66]

1.4.3. Mesoporous Materials

Materials with pores in the range of 20-500Å in diameter are generally called mesoporous materials. Largely increased surface area of such materials provides a large number of sites for sorption of various compounds of interest. These materials have numerous applications in catalysis, separation and many other fields.

The active surfaces of mesoporous materials have been used for various applications including the design of optoelectronic devices. A new class of silica-based materials called MCM (Mobil Composition of Matter)[67] was described in 1992. These materials are prepared from surfactant/block copolymer as a template using sol-gel chemistry. Several methods of functionalizing the inner and outer surface of such materials have been developed in order to achieve composite materials showing more desirable adsorption, ion exchange, extraction, or catalytic properties.[68] Monofunctionalization of the inner surface, or successive inclusion of different organic functionalities, can be performed by post synthesis through covalent incorporation of organic compounds giving rise to higher ordered hybrid materials, which are considered a first step towards "biomimetic" or sensitive nanomaterials.[69] Dye molecules, as receptors, can be covalently attached to these materials to enhance the stability against extraction

or other work-up processes. Anchoring fluorescent dyes to the mesosporous materials inside the pores made these materials suitable for optical sensing. To prepare such materials, fluorescent dyes (e.g. fluorescein) were first modified with triethoxysilane followed by co-condensation with tetraethoxysiliane (TEOS) successfully for the first time in 1992 (**Figure 1.8**).[70]

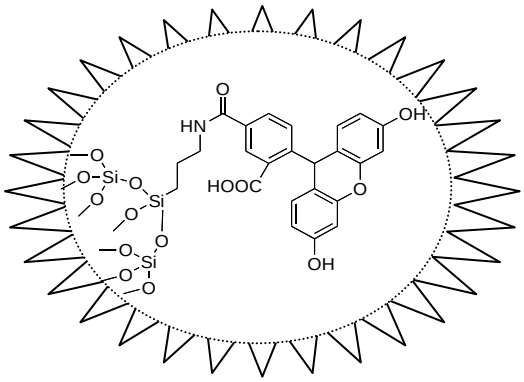


Figure 1.7: Schematic structure of the carboxyfluorescein-modified mesoporous materials[75]

Several techniques have been reported for immobilization of dyes in the mesoporous materials.[71] Mesoporous thin-films covalently modified with fluorescein dye revealed a very fast response to pH variations.[71] This was the first report of using the mesoporous materials for optical sensing. A pH sensor with extended working range designed by Brinker and co-workers[72] was involved in the patterning of surfaces with mesoporous materials. Selective de-wetting of SAM modified substrates resulted in mesostructures, which were then modified covalently with fluorescent probes forming a microfluidic system as a pH sensor. In a similar way, optical sensing of various gas mixtures using mesoporous molecular sieves anchored with rhodamine dyes was reported in the literature. For example, a trace amount of SO_2 gas was detected by fluorescence quenching.[73] Similar systems have been designed for the detection of metal ions[74]and anions[75] as well.

1.4.4. Sensors Based on Covalent Immobilization on Glass and Gold Surfaces

There are several ways to enhance the sensitivity of chemodetecting systems by minimizing problems such as leaking and instability. These are the common drawbacks of the physical entrapping or sol-gels methods. By immobilizing the sensing molecules on the glass or gold surface, the interaction between the sensing moiety and the analyte can be maximized, hence minimizing the leaking. Surface confinement of the sensor molecules enhances the physical stability of the sensor device. Immobilized monolayers (self-assembled monolayers (SAMs)), adsorbed on the gold surface or covalently bound to the silica surface (glass or quartz) are useful platforms for chemical sensing[76] because of the ability to produce a quick response due to the uniform alignment of the molecules with their receptors facing the analyte-sensor interface. Such immobilized monolayers are easy to make, synthetically flexible, and can be tailored readily as chemically active surfaces,[76-77] such systems (e.g. surface-immobilized monolayers on gold) were particularly useful for electrochemically based chemo- and biosensing. However their use for fluorescent sensing is very limited due to fluorescence quenching by the metal surface; having a spacer of specific length between the fluorophore and the support can minimize this effect. [78-79]

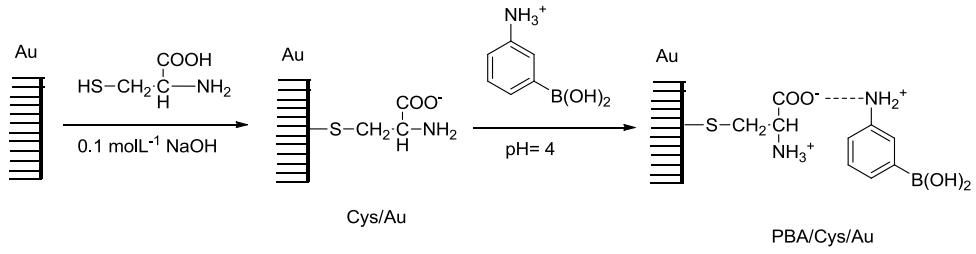
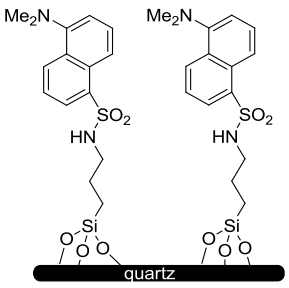


Figure 1.8: Schematic representation of Gold surface based glucose sensor.[78]

Among the very few publications found in literature which involve fluorescent sensing using gold surface attached monolayers, are fluorescent isopthalic acid adsorbate;[80] sensors for

glucose and other saccharides[81] are the typical ones (**Figure 1.9**) Unlike gold surfaces, glass is transparent to light and does not quench fluorescence, therefore it has been used extensively to develop surface-immobilized fluorescent chemosensors and assays for biological studies.[82, 83]

This was also used for analyzing the assembly of donor and acceptor chromophores in the mixed monolayers.[84] The successful covalent immobilization of dyes on the glass surface for efficient pH sensing was achieved by Saari and co-workers in 1982.[85] In this sensing system, a fluorogenic agent aminofluorescein was covalently immobilized on a glass surface. Fluorescent intensity of the monolayer changed upon exposure to the various pHs. The fluorescent intensity increases sharply between pH 3 to 6. The soluble fluorescein also demonstrated qualitatively similar responses on changing pHs. This showed only small change at pH values above 8. This was an indication of immobilization of aminofluorescin on the glass surface. The fluorescent sensors for halides and pseudohalides based on the covalent immobilization of fluorescent acridinium and quinolinium indicators on glass surface were also reported.[86-87] In these sensors, the sensing event was based on fluorescent quenching by the halides. In addition to versatility, covalent immobilization of fluorescent indicators was found to increase the long-term stability of the sensing device. Porous glass materials also provide robust solid supports that could be easily modified by various chemical reactions.[88]



Dansyl-modified-monolayer for sensing β-cyclodextrin

The progress in the development of the chemistry of surface immobilized monolayers has created a new paradigm in design of fluorescent chemosensors. Currently, there are few methods being developed for the chemical modification of self-assembled silane-based monolayers via surface reactions.[89] The components of fluorescent sensors can be covalently attached to glass, silica and quartz, which may involve single or multiple step synthesis. This process can be

conveniently and successfully achieved due to the affinity of trialkoxysilanes or halosilanes towards the hydroxylated surface of the substrates to afford self-assembled monolayers.[28,90] In this field, the work by Reinhoudt[91] and coworkers is particularly noteworthy. They prepared the first fluorescent sensor immobilized on a glass surface for monitoring the concentration of β-cyclodextrin solution in the millimolar range. Their sensor consisted of a dansyl-modified self-assembled monolayer. The attachment of the sensor molecule on glass surface was facilitated by 3-aminopropyltriethoxysilane (APTES) moeity. The detection of β-cyclodextrin by this sensor was measured by the enhancement of fluorescent intensity and hypsochromic shift of the emission maxima from 510 nm to 480 nm. In a different publication from the same group, first example of the detection of metal ions by a surface-immobilized fluorescent sensor was reported, which provided an alternative to the physical immobilization of fluoroionophores in membranes.[92] After these two groundbreaking papers, there was considerable development in the area of surface-immobilized fluorescent sensors. In addition to plane surfaces, preparing sensors using nanoparticles, microspheres, and polymer beads also became popular.

1.5. Goal of This Research Project

Several approaches towards the design of new materials for chemodetection have been proposed. However, there is still a need for reliable sensors for important targets. Especially critical is the availability of real-time reporting fluorescent chemosensors. Based on the available literature, covalent immobilization of fluorescent probes was established to be an advantageous method in terms of device implementation because it can produce more reusable sensors with high stability. The combination of multiple sensors on a surface can lead to the development of microsensor arrays, which can detect multiple analytes simultaneously.

In this dissertation project, I have designed end-capped small π-electron conjugated systems where the excitation energy migration efficiency is controlled by chemical change of the terminal group. The initial goal was to build novel thin-film molecular sensors for various target analytes, such as cysteine and homocysteine, which are abundant in blood plasma. The motivation for building thin-film molecular sensors came from the advantages of thin-film over solution based sensors. A major advantage is an easy implementation into simple practical devices. In order to achieve a simple easy-to-handle device, we designed a sensor molecule in a way which would allow self-assembly of the sensor molecules on the solid surface that would result in sensing units facing the thin-film surface (SAM approach), therefore reducing the fraction of receptor units in the bulk of the film and ensuring that almost all analyte-receptor interactions take place on the surface.

In the course of this Ph.D. project, we have designed and prepared surface-immobilized ratiometric fluorescent chemosensors for a number of biomedically important analytes such as L-cysteine, pH, Zn^{2+} ion and nitric oxide (NO). In the following chapters, the design, synthesis and detailed studies on these sensors will be discussed. One of our first choices was to develop a ratiometric florescent sensor for L-cysteine because of its importance in the field of biomedical application. Another target was sensing pH, which is ideal for various biomedical as well as industrial applications. Detection and sensing various metal ions by the use of reliable methods is critical and we chose Zn^{2+} target as an example.

CHAPTER 2 CHEMICALLY CONTROLLED AMPLIFIED RATIOMETRIC FLUORESCENCE IN SURFACE-IMMOBILIZED END-CAPPED OLIGO (*p*-PHENYLENE ETHYNYLENE)S*

2.1. Introduction

Efficient transport of excited states in extended π-electron conjugated molecular systems makes them especially valuable as a basis for designing chemosensing and optoelectronic devices.[52] Chemosensory devices greatly benefit from signal amplification resulting from the facility of this process.[39,93] In the case of isolated molecules (e.g. in dilute solutions) the intramolecular energy migration dominates via both through-space Förster and through-bond Dexter mechanisms,[94,95] although this process is often considered not to be very efficient.[96-98] If a higher energy fluorophore (donor) is chemically linked to a lower energy fluorophore (acceptor), and the electronic interaction between these two groups is relatively weak, the resulting dyad may exhibit dual fluorescent emission upon excitation of the higher energy fluorophore. Furthermore, the relative ratio of the two emission bands can be controlled by attenuation of the energy transfer within the dyad (Energy transfer cassette).[99-100] This principle has been previously explored in the design of ratiometric fluorescent sensors, where energy transfer between the donor and acceptor units was controlled either by changing the effective spatial separation between the fluorophores,[101] or by chemical modification of one of the two units.[102-104]

Compared to intramolecular energy transfer in dilute solutions, much higher efficiency can be achieved in molecular aggregates and solid films where the exciton migration occurs as a

*Most of the contents of this chapter has been published in the Journal of the American Chemical Society, and reproduced by the permission[124].

three-dimensional process both by inter- and intramolecular pathways.[105] In such systems, the energy migration becomes particularly sensitive to small electronic perturbations of the donor and/or acceptor units.

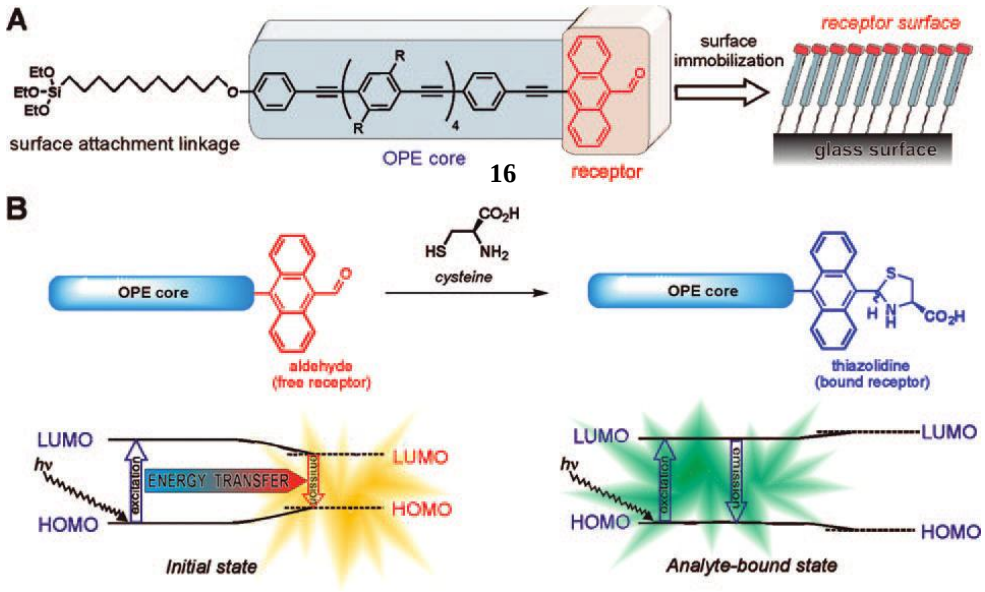


Figure 2.1. (A) General structure of end-functionalized OPE and its immobilization on glass surface; (B) Reaction of **16** with cysteine and a schematic diagram to show origin of the ratiometric fluorescent response.

We were particularly interested in studies of energy transfer in monodisperse oligo(*p*-phenylene ethynylene)s (OPEs) terminated with a lower energy gap group and uniformly immobilized on a surface to yield thin films. Upon achieving the uniform molecular arrangement, these systems display a very efficient energy transfer. Remarkably, this energy transfer process is extremely sensitive to chemical modifications of the film, producing a dramatically enhanced ratiometric fluorescent response on such events. We were the first to discover this unprecedented phenomenon.

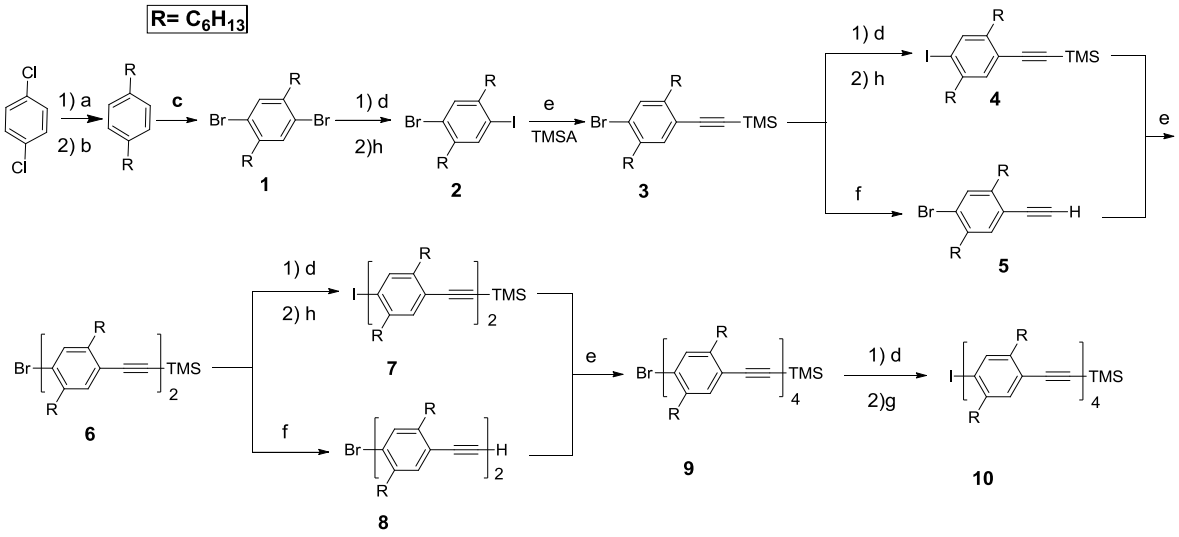
2.3. Synthesis of Oligo(*p*-phenylene ethynylene)

The required OPE core was prepared by iterative convergent synthesis. The synthesis began with a nickel-catalyzed Kumada cross-coupling[106] reaction. 1,4-Dichlorobenzene reacted

with n-hexylmagnesiumbromide to afford 1,4-dihexylbenzene in high yield. It was further brominated to yield 1,4-dibromo-2,5-dihexylbenzene.[107] One bromo substituent in this compound was then converted into an iodo group to afford 1-bromo-2, 5-dihexyl-4-iodobenzene (compound **2**).[108] (**Scheme 2.1**)

The process of iodination involved lithiation by n-BuLi at -78 ^{0} C followed by the treatment with iodine or 1,2-diiodoethane in THF. The compound **2** was subjected to Sonogashira coupling[109] with trimethylsilylacetylene, resulting in a high yield of 1-bromo-4-[(trimethylsilyl)ethynyl]-2,5-dihexylbenzene (compound **3).**

Scheme 2.1.Synthesis of the Tetramer Core **10**

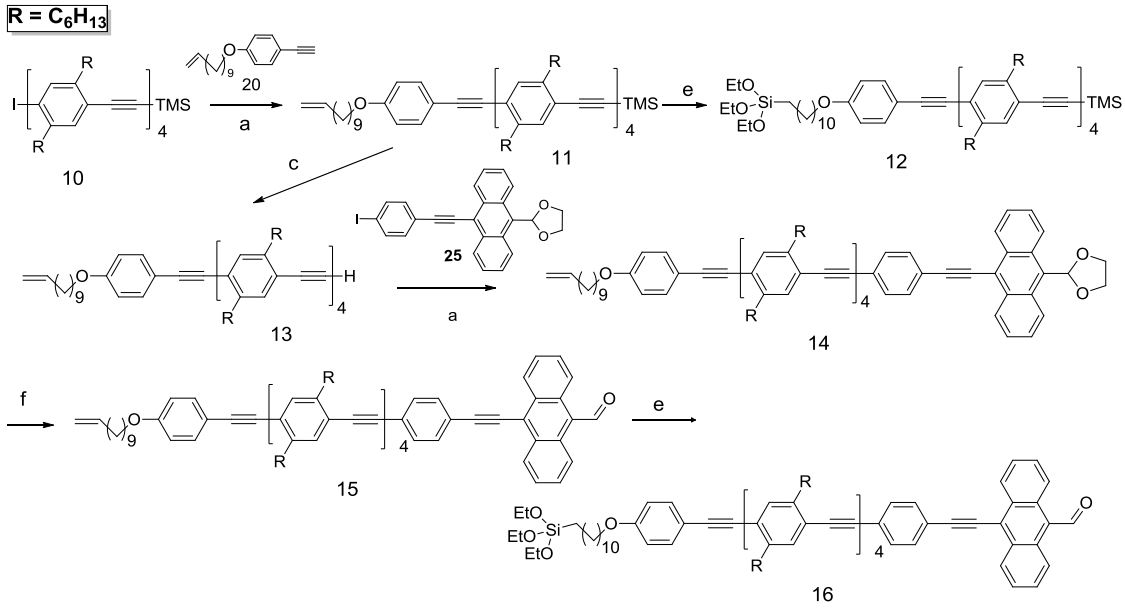


Reaction conditions: a) Mg,$C_6H_{13}Br$; b) $Ni(dppp)_2Cl_2$, ether, 45 ^oC; c) Br_2, I_2, CH_2Cl_2; d) n-BuLi,3 h , THF, 1 h, -78 ^oC ; e) $Pd(PPh_3)_4$,CuI, Toluene:iPr$_2$NH(7:3), 24 h, 55 ^{o} C; f) KOH/ MeOH, THF, 1 h; g)$C_2H_4I_2$, THF, 1 h, -78 ^{o} C; h) I_2

One half of the compound **3** was converted into the iodo compound **4** following the method mentioned above. The other half was desilylated by using KOH/MeOH to afford the acetylene **5**. The iodo compound **4** and acetylene **5** were coupled with each other to afford the

dimer **6**. The same sequence was repeated one more time to yield the tetramer compound **9.** The obtained tetramer was converted into the iodide **10** (**Scheme 2.1**), which was further functionalized with ω-undecenyl chain via a similar cross-coupling reaction with the acetylene **20** (**Scheme 2.2**) to yield the compound **11**.

Scheme 2.2. Preparation of chemosensor **16** and related compounds



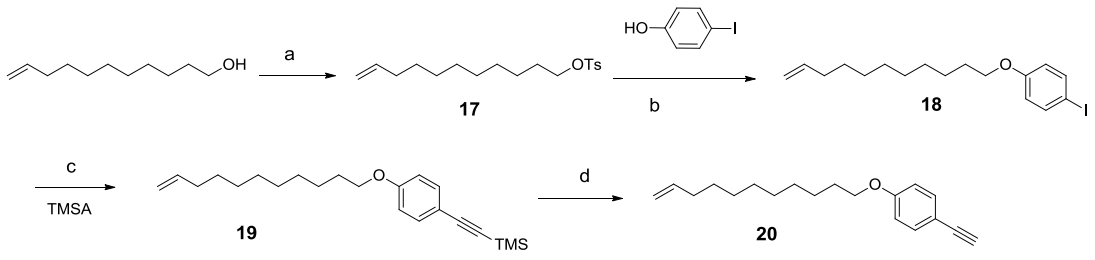
Reaction conditions: a) $Pd(PPh_3)_4$,CuI, Toluene:*i*Pr_2NH(7:3), 24 h, 55 ^oC; b) n-BuLi, 3 h, THF, 1 h, -78 ^{o} C; c) KOH in MeOH, THF, 1 h; e) Karstedt's catalyst, $(EtO)_3SiH$, toluene, 1 h; f) PTSA, Acetone/Chloroform, 4 h.

The presence of the ω-undecenyl functionality was required for further attachment of the complete sensor molecules to a glass surface (*vide infra*). The introduction of this substituent at this stage was also desirable in order to enhance the solubility of the compounds to be synthesized in the steps ahead. A few synthetic details require further discussion. The iodination of the bromides **1**,**3**, **6**, and **9** was carried out at -78 ^{0}C to avoid by-products arising from ortho-metallation. Some impurities were still produced as was determined by the presence of extra signals in the aromatic region of ^{1}H NMR spectra. Although their formation could not be

avoided, they could be easily removed by column chromatography on silica gel. In the case of the monomer **3** and dimer **6**, the iodination reaction was efficient (75-85% conversions) when 1.1 equivalent of n-BuLi and 1.5 equivalents of iodine were used. However, in the case of the tetramer **9**, iodination required using 1,2-diiodoethane instead of iodine. The TMS-acetylene desilylation reaction appeared to be very efficient using KOH/MeOH (3.0 equivalent of KOH) for smaller oligomers **3** and **6** but the efficiency of this reaction was found to diminish with the increase in size of the molecule most likely due to solubility problems, resulting in many hard-to-remove impurities that lowered the total yield. The replacement of KOH with TBAF (tetrabutylammonium fluoride) in THF as the deprotecting reagent for compound **11** substantially improved the yield of the desilylated compound **13**.

For the synthesis of the surface-attachment linker precursor **20**, ω-undecenyl alcohol was tosylated to give the compound **17** and reacted with 4-iodophenol to afford the compound **18**, which upon Sonogashira coupling with TMS-acetylene afforded compound **19**. Desilylation of **19** using KOH in methanol afforded the acetylene compound **20**.

Scheme 2.3. Synthesis of ω-undecenyl linkage **20**



Reaction conditions: a)TsCl, Pyridine, 0 ^{o} C; b) K_2CO_3, KI, Methyl ethyl ketone, reflux;
c) $Pd(PPh_3)_4$, CuI, toluene:iPr$_2$NH; d) KOH in Methanol/THF

In all synthetic steps involving coupling between aryl iodides and acetylenes, reactions were found to go to completion 24 to 72 hours at 45 to 65 ^{0} C, depending on the precursors, with

the product yields in the range of 50-94%. The yield was strongly affected by the presence of trace oxygen, dilution and the amount of copper catalyst used. Despite all our attempts, homo-coupling (Glaser coupling)[110] of acetylene compounds to yield bis-acetylenes could not be completely avoided. Nevertheless, we were able to minimize its effect and increase the yields of the desired products. Since acetylene homocoupling is caused by the presence of even a small amount of copper catalyst and a trace of oxygen, carrying out the reaction in oxygen-free conditions allowed us to obtain a relatively high yield of the desired Sonogashira cross-coupling products. Nevertheless, in the case of preparation of the compound **11**, the fraction of the oxidative homo-coupling by-product was especially significant, despite our attempts to ensure air-free conditions.

^{1}H NMR spectroscopy and high-resolution mass spectrometry were used as important tools for characterization and structure confirmation of the compounds synthesized. In the case of 1,4-dibromo-2,5-dihexylbenzene, a distinct ^{1}H NMR signal (singlet) at 7.49 ppm was observed for protons in the *ortho* position relative to bromine atom of the benzene ring. The success of iodination reaction was confirmed by a noticeable shift of this singlet from 7.49 to 7.74 ppm due to the replacement of bromine atom by iodine.

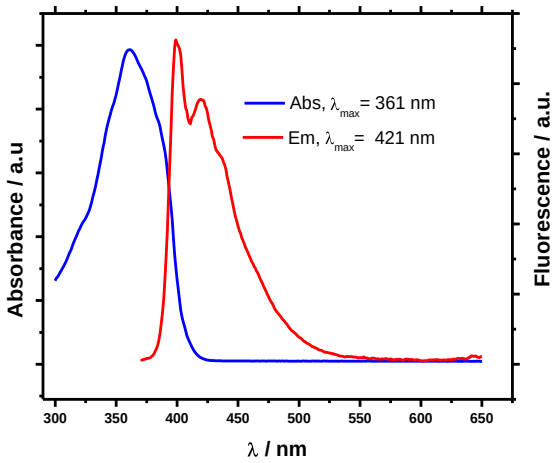


Figure 2.2. Absorption and emission spectra of tetramer **11** in $CHCl_3$

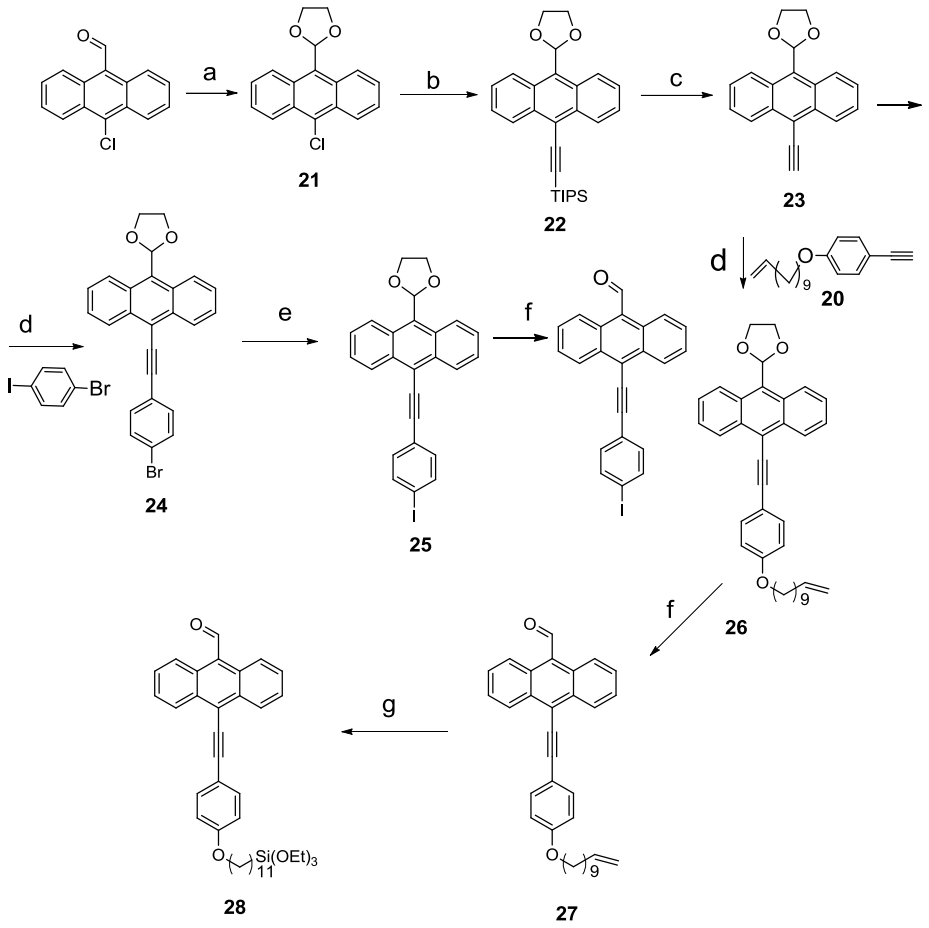
The absorption maximum for the tetramer **11** was found to be at 361 nm, with the emission maximum at 421 nm (**Figure 2.2**). Both were the typical values for oligo(*p*-phenylene ethynylene) compounds.

2.4. Synthesis of Receptor 25

Compound **25**, the precursor to cysteine receptor for the proposed molecular sensor, was synthesized starting from commercially available 10-chloroanthraldehyde. The aldehyde group of 10-chloroanthraldehyde was first protected by conversion into the acetal compound **21 (**Scheme **2.4)**. The Sonogashira coupling of **21** with TIPSA (tri-isopropylsilylacetylene) afforded compound **22**. Because of the low reactivity of aryl chlorides under typical Sonogashira coupling conditions, the cross-coupling between chloride **21** and TIPS-acetylene was carried out using a more reactive catalytic system $PdCl_2(CH_3CN)_2$ with 2-dichlohexylphosphino-2'4'6'-tri-isopropylbiphenyl (XPhos) ligand in the presence of excess Cs_2CO_3 as was recently described by Buchwald.[111] The silyl-protected acetylene **22** was deprotected with TBAF/THF to yield the acetylene **23**. It was then cross-coupled with 1-bromo-4-iodobenzene, which afforded bromide **24**. The need to increase reactivity for the next step coupling forced us to find a way to replace Br with I. We found that Buchwald's "aromatic Finkelstein reaction"[13] worked relatively well. It was carried out by using NaI, in the presence of CuI and trans-N^1,N^2-dimethyl-1,2-cyclohexanediamine ligand. The yield was 80% after recrystallization of **25** from hexane-dichloromethane**.**

The ^{1}H NMR data for the compounds **21-24** were consistent with the proposed structures as before. Conversion of the bromide **24** to the iodide **25** was confirmed by the shift of the doublet corresponding to hydrogens ortho to the halogen from 7.57 to 7.80 ppm.

Scheme 2.4. Synthesis of the Cysteine Receptor



Reaction conditions: a) Ethylene glycol, PTSA, Benzene; b) $Pd(CH_3CN)_3Cl_2$, Ar.PCy_2, Cs_2CO_3, 24h; c) TBAF, THF, 5 min; d) $Pd(PPh_3)_4$,CuI, Toluene:*i*Pr_2NH(7:3), 24 h, 55 ^oC ; e)NaI, CuI, trans-N'N''-dimethyl-1,2-cyclohexadiamine; f) PTSA, Acetone/chloroform, 4 h; g) Karstedt's catalyst, $(OEt)_3SiH$, toluene, 1 h.

The cross-coupling reaction between compound **13** and the receptor unit **25** was carried out under regular Sonogashira coupling conditions using $Pd(PPh_3)_4$ and CuI catalysts in toluene and diisopropylamine and afforded 54% of the cross-coupled product **14 (Scheme 2.2)**. The acetal protecting group in the compound **14** was removed by the treatment with *p*-toluenesulfonic acid (PTSA) in acetone and chloroform, which afforded 60% of the aldehyde (compound **15**). The same approach was successfully employed to convert **26** to **27** (**Scheme 2.4**). In both cases, the deprotection of precursors **14** and **26** was accompanied by the appearance

of an aldehyde peak (singlet) with the chemical shift about 11.5 ppm and disappearance of four –OCH_2CH_2O- protons signals in ^1H NMR spectrum. The high downfield chemical shift of the aldehyde signal was likely to be due to the extended π-electron conjugation in **15** and **27**.

The Uv/vis spectra of the acetal protected receptor unit **25** showed a band with maxima at 407 and 432 nm, and the corresponding emission spectrum showed a band with maxima at 439 and 466 nm (**Figure 2.3A**). Both spectra showed mirror symmetry and possessed fine vibronic structure typical of the anthracenyl group. The acetal-protected sensor **14** showed a broad, featureless absorption band at about 390 nm which corresponds to absorption of the OPE core (**Figure 2.3B**). The significant bathochromic shift of this band relative to the same band in the receptorless tetramer compound **11**(361 nm, **Figure 2.2**) was likely due to the more extended π-electron delocalization in **14**.

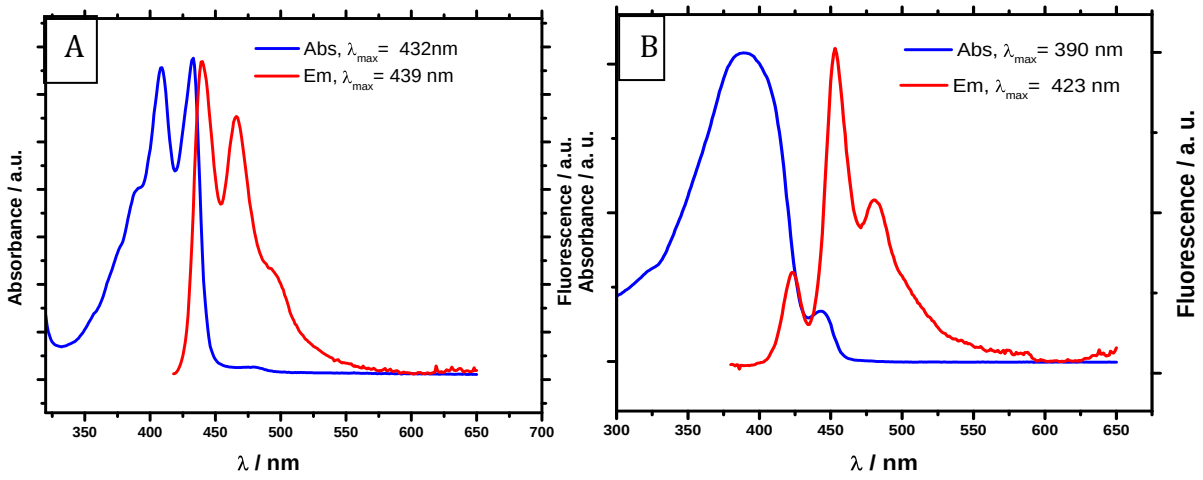


Figure 2.3. Absorption and emission spectra of (A) receptor unit **25**, (B) compound **14**

A small additional band at 442 nm was due to the absorption of the anthracenyl receptor. The major band in the fluorescence spectrum of **14,** with a maximum at 460 nm, was likely due to the emission of the terminal anthacenyl unit, therefore indicating a significant degree of intramolecular energy transfer from the OPE core to the receptor. The residual emission

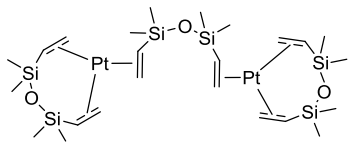
maximum at 423 nm coincided with the emission maxmum of the pure tetramer core **11**, and was likely due to the incomplete energy transfer to the terminal anthracenyl receptor in **14**.

2.5. Hydrosilylation Reaction

Covalent attachment of the prepared sensor molecules to a solid support required finding optimal conditions to carry out hydrosilylation of the terminal alkene group in the ω-undecenyl linkage. The most commonly used hydrosilylation reaction conditions require $H_2PtCl_6.6H_2O$ as the catalyst.[112] However, our attempts to carry out this reaction under such conditions were unsuccessful, most likely due to the high reactivity of the acetylene (C≡C) bonds in the OPE core as well as complete degradation of the aldehyde group. Attempts to hydrosilylate similar acetal-protected precursor **14** were also unsuccessful.

Even compounds without an aldehyde group (TMS-protected precursor) (**10**, **11** and **13)** could not be hydrosilylated under these conditions. In further attempts, we prepared TIPS-protected acetylenes since TIPS is a more robust protecting group. However, we were not successful in hydrosilylating this compound.

To our satisfaction, we found that a Pt(0) catalytic system, the Karstedt's catalyst,[113] allowed us to carry out the hydrosilylation reaction successfully. With a very small amount of this catalyst, we successfully hydrosilylated the alkene compounds **15** and **27** with triethoxysilane (**Schemes 2.2** and **2.4**).



Pt(0)-divinyl-tetramethylsiloxane complex(dvtms) (Karstedt's catalyst)

The extent of hydrosilylation could be easily monitored by ^1H NMR spectroscopy, which showed disappearance of the alkene proton signals at about 5.8 ppm and appearance of new signals from the triethoxy group at about 1.2 and 3.8 ppm. Due to the difficulties in purification, the crude products were used for surface immobilization.

2.6.Results and Discussions

The 10-formylanthracenyl-terminated OPE **16** (**Figures 2.1A** and **2.1 B** and Scheme **2.2**) was chosen since anthracene derivatives are very convenient low energy gap units for energy transfer studies in (*p*-phenylene ethynylene) systems.[114,115] Immobilization of **16** on glass slides occurred smoothly from a solution in toluene, and afforded the monolayer film with a relatively uniform surface coverage as observed by AFM microscopy (*vide infra*).

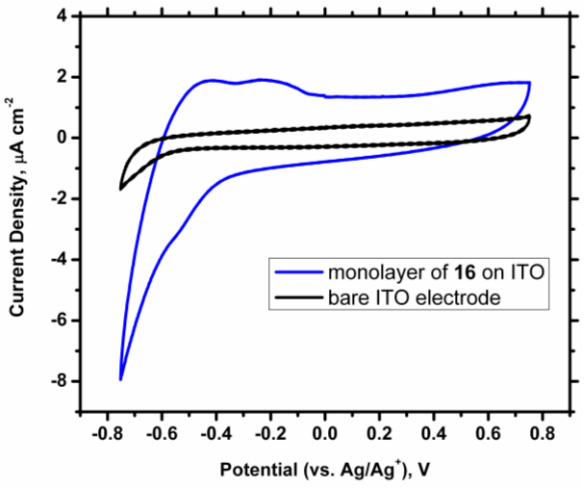


Figure 2.4. Cyclic voltammogram of a monolayer of **16** immobilized on ITO/glass surface. Experimental conditions: 0.1 M Bu_4NPF_6 in CH_2Cl_2, sweep rate 0.1 V s^{-1}

The surface density (estimated from electrochemical studies on the monolayer film immobilized on ITO/glass surface) was 2.5×10^{-11} Mol cm^{-2} (**Figure 2.4**), which corresponded to the occupied area of 7 nm^2 per molecule. With the theoretical estimate ranging from 2 to 4 nm^2 per molecule, this indicated a relatively densely packed film. Absorption spectra of **15** in $CHCl_3$ solution and the immobilized monolayer of **16** possess similar features, with the major band at ~380 nm from the OPE core, and a much less intense band at ~450 nm corresponding to the absorption of the anthraldehyde receptor. In the fluorescence spectrum in dilute $CHCl_3$ solution, the most intense band with a maximum at 416 nm corresponds to emission from the OPE core.

Relatively inefficient intramolecular energy transfer from the OPE core to the anthracenyl acceptor leads to appearance of the less intense broad band at 520 nm (**Figure 2.5**).

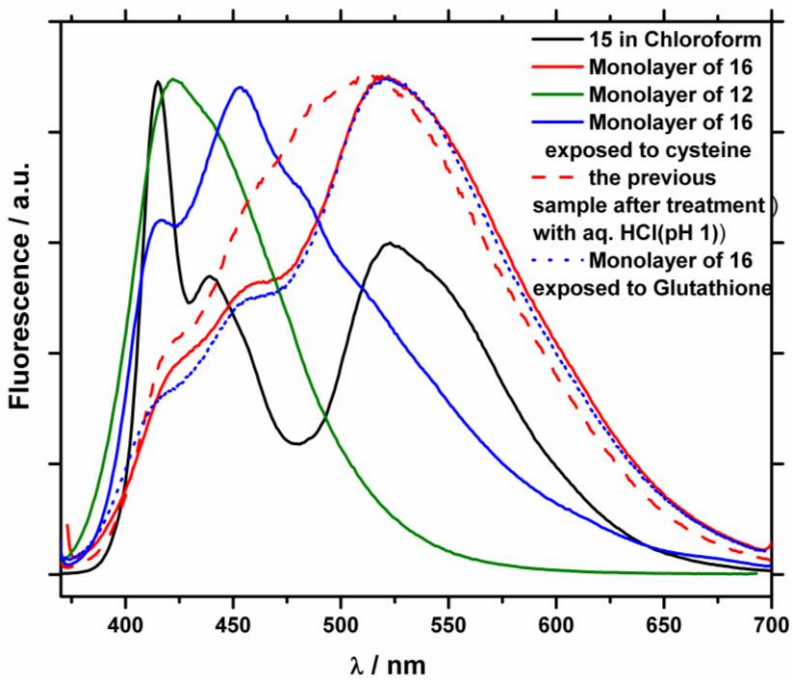


Figure 2.5. Normalized fluorescence spectra of **15** in $CHCl_3$ (fluorescence quantum yield 0.08), monolayer of **12**, and monolayer of **16** before and after exposure to aq. L-cysteine (10 mM) and glutathione (20 mM), as well as recovery of the initial fluorescence pattern after treatment of the monolayer pre-exposed to L-cysteine with aq. HCl (pH 1)

While not efficient in solution, the energy transfer process becomes highly efficient in the densely packed organized monolayer film, where it results in the dominating anthracenyl emission, with only a small residual band of the OPE core (**Figure 2.5**). That the 520 nm main emission band in the film indeed originated from the anthracenyl group, and not produced from intermolecular interactions of the closely packed OPE moieties (or aggregation-related excimer formation)[115-117] was confirmed by the presence of only a single OPE band at 430 nm in the fluorescence spectrum of the immobilized monolayer of the reference compound **12** lacking the anthracenyl group (**Figure 2.5**).

Exposure of a glass slide modified with a monolayer of **16** to a 10 mM aqueous buffered solution of L-cysteine led to significant diminution of the 520 nm anthracenyl emission band, and simultaneous growth of the OPE band with a maximum at 450 nm (**Figure 2.5**). This ratiometric behavior follows the principle schematically outlined in **Figure 2.1B**. Reaction with cysteine results in aldehyde to thiazolidine conversion, which perturbs the electronic structure of the anthracenyl receptor terminus resulting in the diminished energy transfer from the OPE core to the end group, with concomitant intensity increase of the OPE emission band. Thus, the system clearly exhibited the expected ratiometric behavior.

A more systematic study revealed a gradual intensity increase of the 450 nm emission band upon exposure of the monolayer to increasing concentrations of L-cysteine (**Figure 2.6**). The observed intensity increase was significant for cysteine concentrations ranging from 0.1 to 10 mM, and resulted in a 3-fold total enhancement of the fluorescence quantum yield, with the 450 nm band being the major contributor. This increase was in agreement with much higher fluorescence quantum yield of the OPE vs. the anthracenyl fluorophore (0.41 for **11** vs. 0.02 for **27**). Whereas the OPE emission band showed monotonic intensity increase, the behavior of the anthracenyl band at 520 nm was more complex. Exposure of the monolayer to low concentrations of L-cysteine resulted in the expected, albeit small, decrease of the anthracenyl band intensity. However, with increasing concentration of L-cysteine, the intensity started increasing again, eventually surpassing its initial value in the pristine monolayer (**Figure 2.6**). This can be understood by considering the significant enhancement of the OPE emission upon blocking intramolecular energy transfer to the acceptor fluorophore. This enabled delivering (via an intermolecular pathway) more excitation energy to the unreacted molecules of **16** surrounding cysteine-bound **16** in the monolayer film, therefore enhancing their anthracenyl emission.

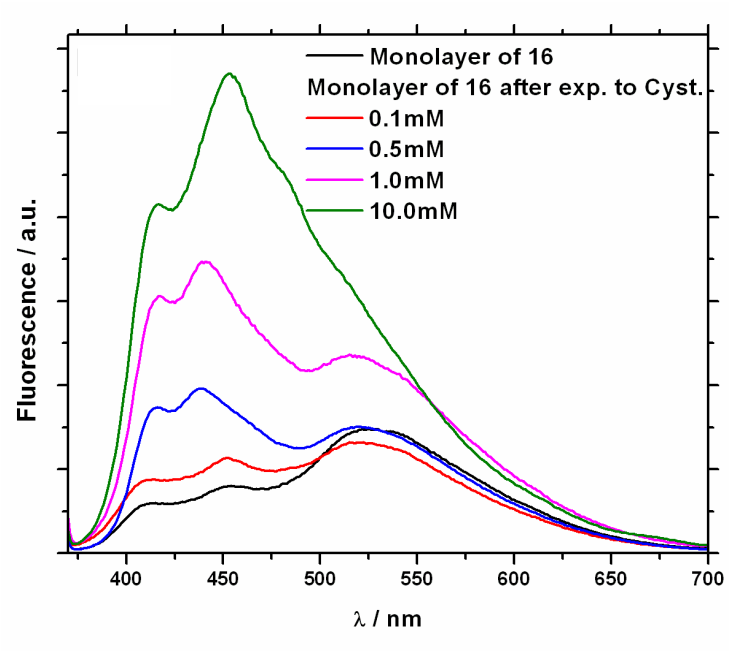


Figure 2.6. Change in fluorescence of the monolayer of **16** upon exposure to increasing concentrations of L-cysteine

This observation necessarily implies that even upon exposure to high cysteine concentrations the majority of aldehyde groups in the monolayer remained intact, and only a small fraction of them reacted on the film surface. Indeed, in a control experiment, a surface-immobilized monolayer of the anthracenyl receptor **27** showed only a subtle fluorescent response on a prolonged exposure to aqueous cysteine solution (**Figure 2.7**).

The lack of response was likely due to the unfavorable reaction equilibrium of the anthraldehyde receptor toward thiazolidine formation. Indeed, to test the general reactivity of anthraldehyde towards cysteine, we studied the reaction in a bulk solution. Anthraldehydes **30(a,b)** proved completely unreactive in a variety of different experimental conditions (refluxing ethanol, or aqueous DMF at pH 8 and above 140 ^{o} C), and quantitative recovery of the starting material was observed in all cases (**Scheme 2.5**). In contrast, *p*-bromobenzaldehyde resulted in almost quantitative formation of the corresponding thiazolidine **29**.

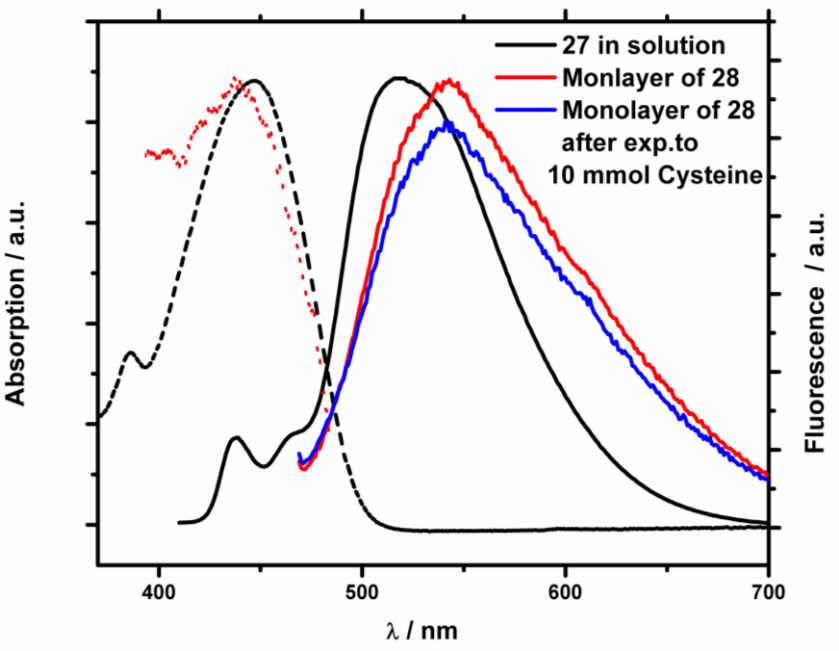
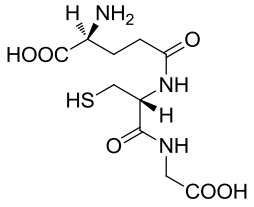


Figure 2.7. Absorption (dash line) and fluorescence (solid line) spectra of **27** in $CHCl_3$ (extinction coefficient (λ=432 nm) 1.13×10^4, fluorescence quantum yield 0.02), and excitation (dash line) and fluorescence (solid lines) spectra of monolayer of **28** before and after exposure to aq. Cysteine (10 Mm)

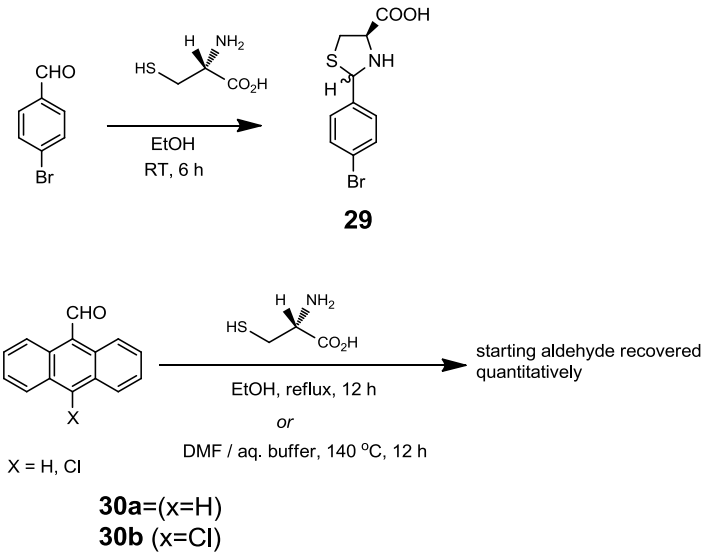
The lack of reactivity of the anthraldehydes with cysteine could be explained by the higher stabilization of the aldehyde functionality due to the more extended π-electron delocalization (which is also consistent with the abnormally high chemical shift for the CHO protons in ^{1}H NMR spectra, *vide supra*). That the formation of the "elusive" thiazolidine was the actual reason for the observed ratiometric response was evidenced by the finding that exposure of the monolayer of **16** to a cysteine-free buffer or to an aqueous solution of the closely related (but not capable of forming a thiazolidine) glutathione did not produce any fluorescence change (**Figure 2.5**).



L-Glutathione

In order to further prove that thiazolidine formation was the primary reason for the observed fluorescence response, we performed the hydrolysis of the monolayer previously exposed to L-cysteine in acidic conditions.

Scheme 2.5. Conversion of anthracenyl aldehydes into thiazolidines in solution



Recovery of the initial fluorescence pattern after treatment of the cysteine-exposed monolayer of **16** with dilute HCl (**Figure 2.5**) was observed which likely stemmed from hydrolysis of the surface thiazolidine groups. Also, trace thiazolidine formation in the monolayer was found in the XPS experiments (**Figure 2.7**).

It is to be noted that the N1s and S2p peaks were not detected in the monolayer before exposure to L-cysteine, and therefore indicated the presence of chemically bonded nitrogen and sulfur most likely due to formation of the thiazolidine compound in the L-cysteine treated films.

Surface morphology of the surface-immobilized monolayer was studied by AFM. The monolayer formed on the glass surface was found to be uniform and well-ordered and slightly reorganized after its exposure to cysteine. (**Figure 2.8**

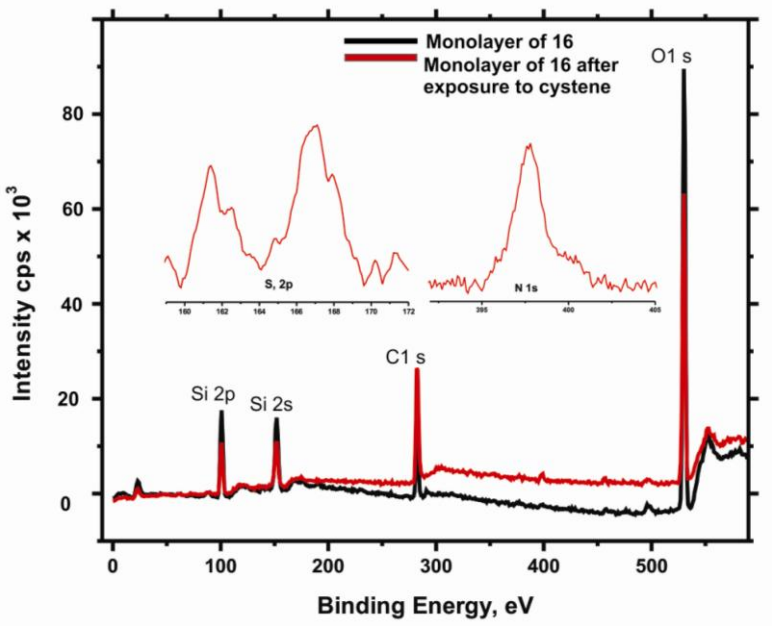


Figure 2.7. Survey XPS spectra of the monolayer of **16** immobilized on a glass surface before and after exposure to aqueous L-cysteine solution (10 mM). Insets show separately acquired spectra of N1s and S2p regions in a sample after exposure to L-cysteine

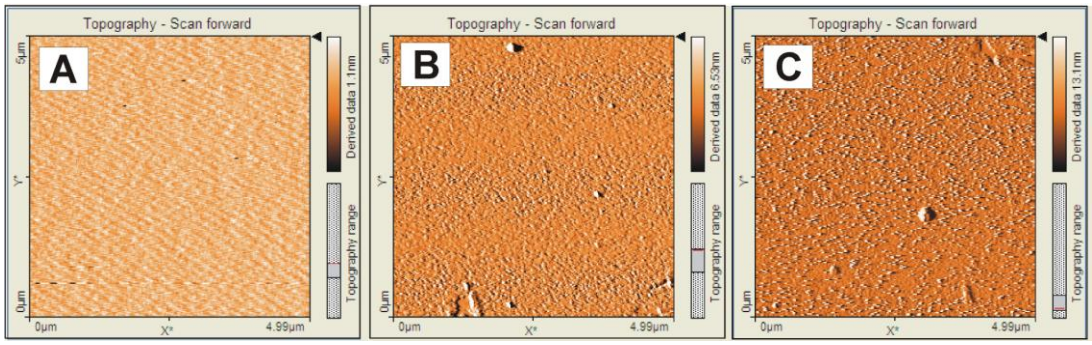


Figure 2.8. AFM images illustrating evolution of surface topography of a glass substrate: (A) Activated glass slide; (B) Glass slide modified with a covalently immobilized monolayer of **16** after annealing; (C) A monolayer of **16** after exposure to L-cysteine solution.

2.7. Conclusion

The observation that the small extent of the chemical conversion in the surface-immobilized monolayer of OPE **15** could trigger a significant ratiometric fluorescent response is without precedent. Although more additional studies are required to completely understand this

unusual “turn-on” amplification, the improved molecular organization in the monolayer likely plays a role in it. Indeed, a spin-cast film of the sensor **16** showed completely irreproducible and inconsistent in fluorescence responses. On the practical side, this “turn on” amplification phenomenon can provide a useful platform to increase optical gain in fluorescent chemodetection.

CHAPTER 3 COVALENTLY IMMOBILIZED FLUORESCEIN-CAPPED OLIGO (*p*-PHENYLENE ETHYNYLENE) S AS RATIOMETRIC FLUORESCENT pH SENSORS

3.1. Introduction

Development of approaches for the continuous monitoring of pH in environmental, biomedical, and bioprocess applications has attracted considerable attention in the recent years.[6,11,52,118-120] A substantial amount of work has been done in the field of design of fluorescent probes based on single fluorophore,[120] known as fluorescent indicators.[121] Typical pH indicators display response only in fluorescent intensity.[120] In addition, some compounds such as 8-hydroxy-pyrene-1,3,6-trisulfonic acid (HPTS), seminaphthofluoresceins (SNAFl), and seminaphthorhodafluores (SNARF) compounds have been researched as dual-band fluorescent sensors.[119] However, signal variation due to photobleaching, differences in source intensity as well as sensitivity to position and orientation of the sensor molecules with respect to analyte are the well-known drawbacks of the intensity-based measurements.[120,122] In order to circumvent the drawbacks of intensity-based sensors, ratiometric detection has been proposed as an alternative approach.

One important parameter to consider while designing ratiometric chemosensors is the magnitude of the range of signal ratios of emission intensities at two different wavelengths for a chemosensor in analyte-bound and analyte-unbound forms upon excitatin at the same wavelength.[123] This factor is the measure of the dynamic range and the sensitivity of the chemosensor with respect to the analyte concentration.[10] Availability of sensor compounds with well-resolved peaks for the analyte-bound and analyte-free forms with reasonable emission intensities makes fluorescent chemosensing more plausible.[123]

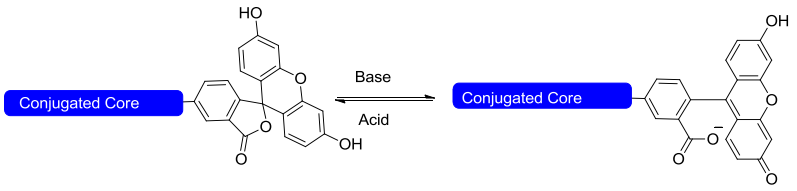


Figure 3.1: Structural changes in fluorescein receptor upon increasing and decreasing pH.

In the previous chapter, we presented a general concept for the design of ratiometric fluorescent chemosensors.[124] It is based on the energy transfer modulation by the HOMO-LUMO gap alteration originating from analyte binding to the receptor (**Figure 2.1**). The initial proof-of-concept study involved a cysteine chemosensor.[124] As a continuation of the project, in order to broaden the spectrum of chemosensors based on this principle, we developed a ratiometric fluorescent pH sensor based on the fluorescein-terminated surface-immobilized OPE core. Fluorescein is known to exhibit fluorescence change at various pHs due to reversible opening and closing of its lactone ring upon switching between basic and acidic conditions **(Figure 3.1**). Our idea here was to combine a pH-responsive fluorescein receptor with an OPE core to construct a ratiometric fluorescent sensing system. In order to develop a thin-film sensor, the system was then immobilized on a glass surface resulting in a film where all the sensing receptors stayed on the surface, therefore facilitating the maximum analyte-receptor interaction and providing enhanced sensitivity. In such a system, energy transfer occurs from OPE as a higher energy gap fluorophore to the lower energy gap fluorophore fluorescein, thus altering the relative intensities of the two separate bands corresponding to these two fluorophores. This lowers the emission intensity of the OPE band due to energy migration towards the terminal receptor. The initial intensity ratio changes when a chemical transformation happens during a chemosensing event at the receptor terminus.

The energy transfer in such systems takes place either by a through-bond process called Dexter exchange mechanism[94] or by a through-space pathways called Förster dipole-induced dipole mechanism[94] (often called Fluorescence Resonance Energy Transfer, FRET). Typically the energy transfer involves a combination of both processes.[125] The pH sensor described in this chapter shows how efficient utilization of these two mechanisms can be used to achieve remarkable sensing performance.

The donor-acceptor distance, relative orientation of the transition dipoles, and the extent of spectral overlap of the emission of the donor and the absorption of the acceptor (overlap integral)[125] are the main factors controlling the energy transfer efficiency. Of these two mechanisms, Förster energy transfer is better understood. In the recent years, significant development in the design and application of FRET based fluorescent probes has resulted in efficient sensors for various applications.[126]

3.2. Design and Synthesis

The donor OPE component of the sensor compound **34** was synthesized as shown in Scheme **3.1.** The Compound **10** was prepared as described in the previous chapter.[124] One of the most problematic issues with the preparation of the cysteine sensor described therein was hydrosilylation of the terminal alkene group in the sensor molecule. Although we were able to find suitable experimental conditions for this reaction in the case of sensor **16**, application of the same conditions to the similarly built pH sensor did not result in the required product. Considering these difficulties, we developed an alternative and more general way of installing the terminal triethoxysilane linkage, which required amine functionality in the conjugated OPE core. Thus, the iodide compound **10** was coupled with 4-aminophenyl acetylene **40** (**Scheme 3.2**) to afford the compound **30**, which was then converted into the triethoxysilyl-terminated

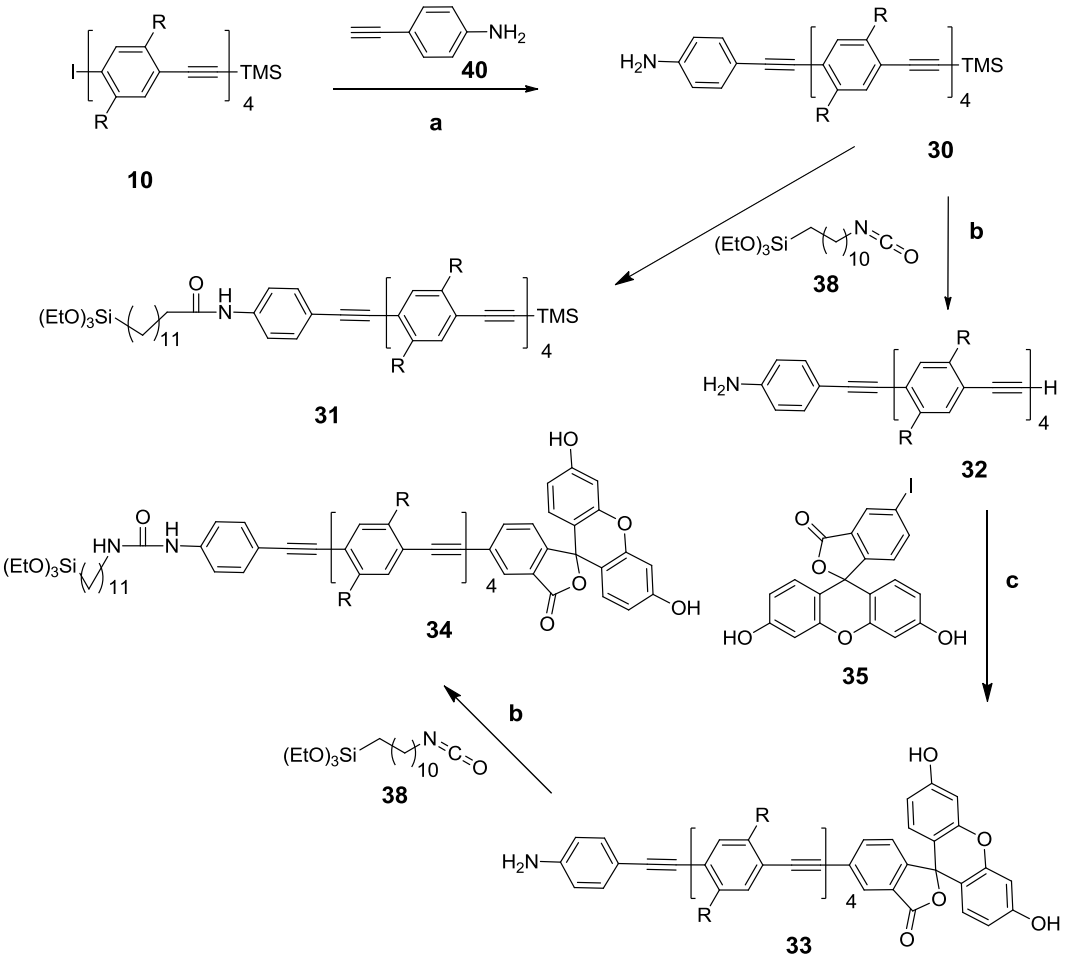
reference compound **31** using amine condensation reaction with isocyanide **38** (**Scheme 3.1**). The compound **30** was also desilylated to afford acetylene compound **32.**

The Sonogashira coupling of the acetylene compound **32** with 5-iodofluorescein **35**, (synthesized as shown in the **scheme 3.2)**, afforded the complete pH sensor compound **33** (Scheme **3.1**).

Transformation of the sensor **33** into the triethoxysilyl precursor for surface immobilization was achieved by reacting with the isocyanate **38**. This reaction was very efficient at room temperature and afforded a quantitative yield of the product **34** (**Scheme 3.1**). The isocyanate linker **38** was synthesized as shown in scheme **3.2**, starting from commercially available ω-undecenoic acid. In addition to the "immobilization ready" complete sensor compound **34**, we also prepared a receptor-only compound **36** (**Scheme 3.2**) required for comparison studies.

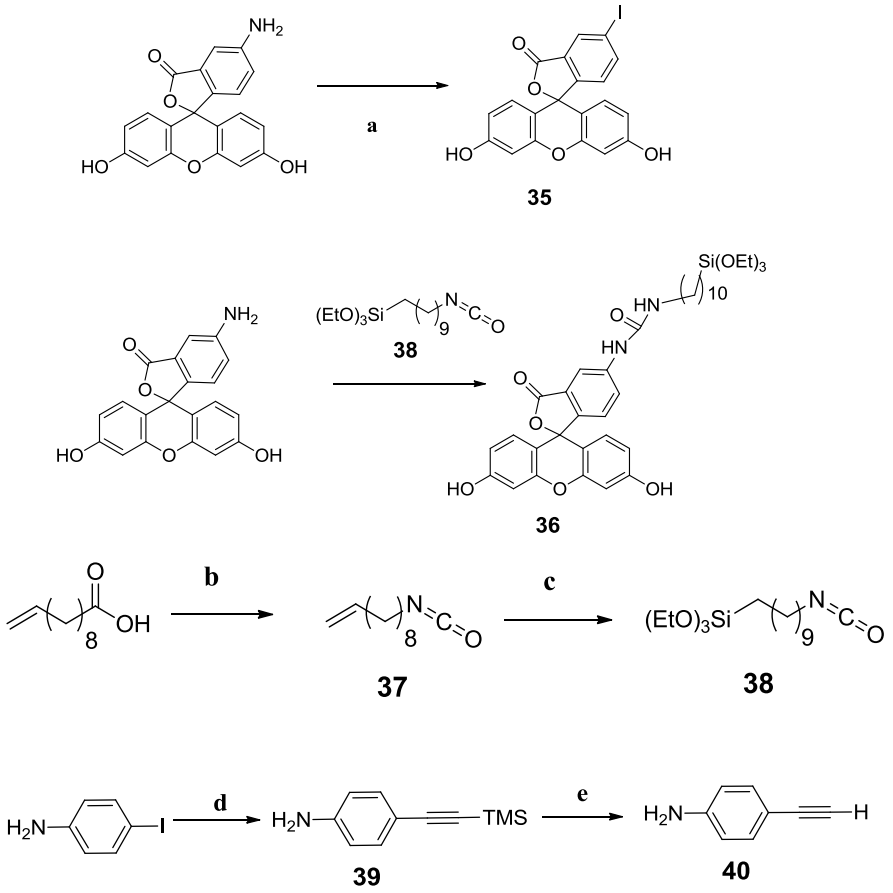
All the triethoxysilyl compounds (**31**, **34** and **36**) were immobilized on a glass surface from solutions in toluene (**31**, **34**) or DMF(**36**), to form covalently immobilized monolayer films. In this process, the glass slides were successively washed with chloroform, methanol, acetone, and DI water and dried under a stream of nitrogen. The slides were activated by treating with Piranha solution[127] upon sonication for 30 minutes. The 0.2 mM solution of the compounds **34** and **31** in dry toluene and **36** in DMF were used for immersion of activated glass slides under air free conditions at 80 ^{o} C for 2 hours. After completing immobilization, the slides were washed with chloroform, sonicated to remove unbound materials and then dried to afford surface immobilized monolayers. In the case of monolayer of **31**, in order to overcome the intermolecular aggregation in the monolayer, a mixed monolayer was prepared by using a mixture of the **31** and dodecyltriethoxysilane in the ratio of 1:3.

Scheme 3.1: Synthesis of the OPE core **30**, reference compound **31,** complete pH sensor **33** and "immobilization-ready" precursor **34**.



Reaction conditions: a) $Pd(PPh_3)_4$, CuI, Toluene:*i*Pr_2NH(7:3), 24 h, 55 ^oC ; b) KOH/MeOH, THF; c)$Pd(PPh_3)_4$, CuI, DMF:*i*Pr_2NH, 36 h, 60 ^{o} C.

Scheme 3.2. Synthesis of 5-iodofluorescein **35**, reference **36** and *p*-aminoacetylene **40**



Reaction conditions: a) 1) $NaNO_2$, HCl; 2)KI; b) DPPA,Et_3N, MeCN, 50 ^{o} C, 2h; c) $(EtO)_3SiH$, Karstedt's catalyst; d) 1) TMSA,$Pd(PPh_3)_3$, CuI,THF,iPr_2NH, 40 ^{o} C,12 h; e) K_2CO_3, MeOH, rt 12 h.

3.3. Spectral Studies of Fluorescein at Various pHs

Photophysical studies of the pH receptor **35** in aqueous solutions at various pH values revealed that it acted as a pH sensor (**Figure 3.2**). The threshold shift in absorption spectra happened between pH 3 and 4. A similar trend was observed in the emission behavior, where the emission intensity change was observed between pH 2 to 3 and it remained unchanged above pH 3. It was clearly noticed that the spectral response of the receptor itself upon exposure to various pH values was limited to a narrow pH range and therefore this compound could not be used to measure a broad range of pH values.

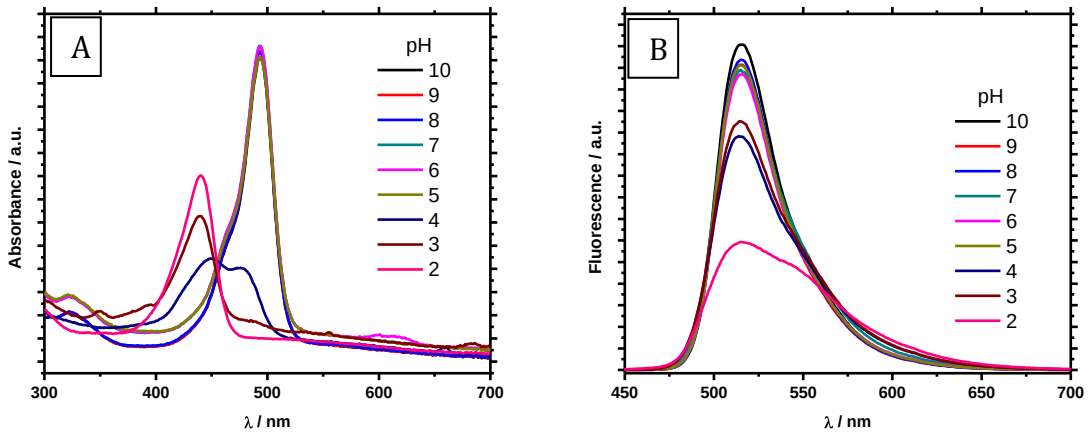


Figure 3.2. Absorption (A) and emission (B) spectra of 5-iodofluorescein 35 in aqueous solutions at various pHs (concentration of **35,** 40.0 μM)

3.4. Studies of pH Response of the Surface-Immobilized Sensor

The fluorescent response of the surface-immobilized monolayer of **34** showed a clear ratiometric behavior (**Figure 3.3A**). At low pH (2.0) the emission spectrum was dominated by the OPE band (at 420 nm). A gradual increase in pH to about 10 resulted in a gradual decrease in the 420 nm OPE band with a simultaneous increase in the fluorescent intensity of the 530 nm fluorescein band (**Figure 3.3 A**). The reversible ratiometric response was occurring in an unexpectedly broad pH range (2-10), which was in striking contrast to the response of

fluorescein itself in an aqueous solution (threshold switching in the much narrower pH range, *vide supra*). In order to determine the origin of the ratiometric response, we acquired the pH dependent emission spectra of the surface-immobilized sensor upon direct excitation of the fluorescein receptor (**Figure 3.3 B**). In this case, the similar enhancement of fluorescence intensity was observed upon increasing pH in the range 2-10. This increase was consistent with the intensity enhancement of the 540 nm emission band obtained upon excitation of the OPE core. Therefore, one could conclude that the fluorescein response indeed came from the chemical conversion of the receptor, not due to pH dependent variation of the energy transfer efficiency. On the other hand, simultaneous decrease of the intensity of the OPE band at 420 nm could be caused by the increased energy transfer efficiency from the OPE to fluorescein chromophore. Indeed, in the control experiments with only OPE conjugated core **31,** the monolayer of **31** showed practically no response on changing pH (**Figure 3.5A**). In initial experiments, the core monolayer exhibited a much broader fluorescence band possibly due to intermolecular aggregation. In contrast, such aggregation was likely suppressed in the monolayer of sensor compound **34** due to the steric restrictions of the bulky fluorescein terminus. The aggregation was overcome by using mixed monolayer prepared from the OPE core compound **31** mixed with dodecyltriethoxysilane(1:3).

In sharp contrast with the fluorescein incorporated into the complete sensor compound **34,** the surface-immobilized fluorescein **36** showed threshold response on pH changes (Figure **3.4 B**). This behavior was similar to that of fluorescein in aqueous solution (**Figure 3.2 B**). This indicated that surface immobilization alone did not significantly alter the photophysical behavior of fluorescein. Only when it was incorporated into a conjugated π-electron system, it started showing the gradual response on changing pH over a broad range.

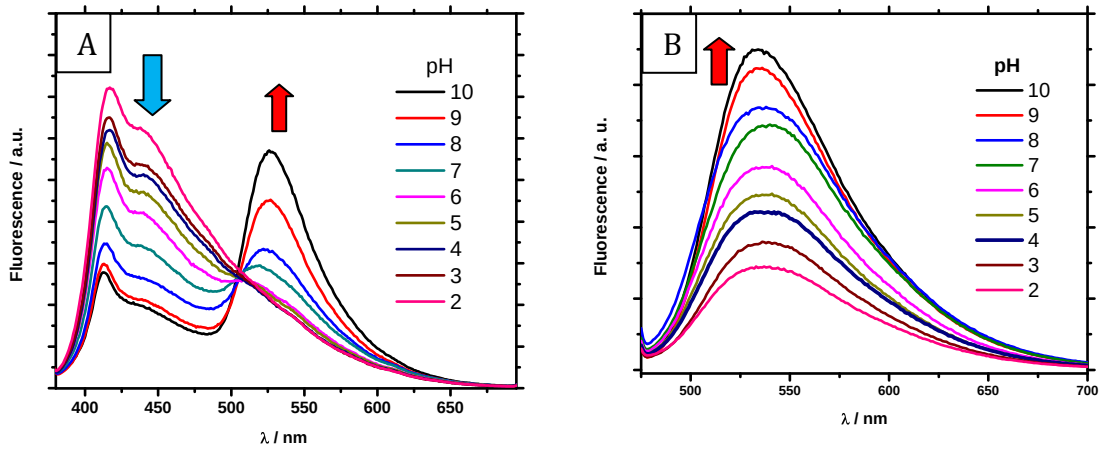


Figure 3.3. (A) Ratiometric response of the surface-immobilized pH sensor thin-film (compound 34) at various pHs. B) The same spectra acquired upon excitation at 440 nm.

The reasons for this phenomenon are not clear at this point. Our explanation could simply involve alteration of reactivity of fluorescein in the thin-film monolayer due to the non-polar environment provided by the OPE core. If this is the case, this could be a very useful feature for designing various ratiometric sensors with the extended detection range. It is also likely that the ordered and organized monolayer could provide certain enhancement of the energy transfer-related effects (although it might not play a substantial role here since direct excitation of fluorescein unit also gave similar gradual fluorescent response on changing pH).

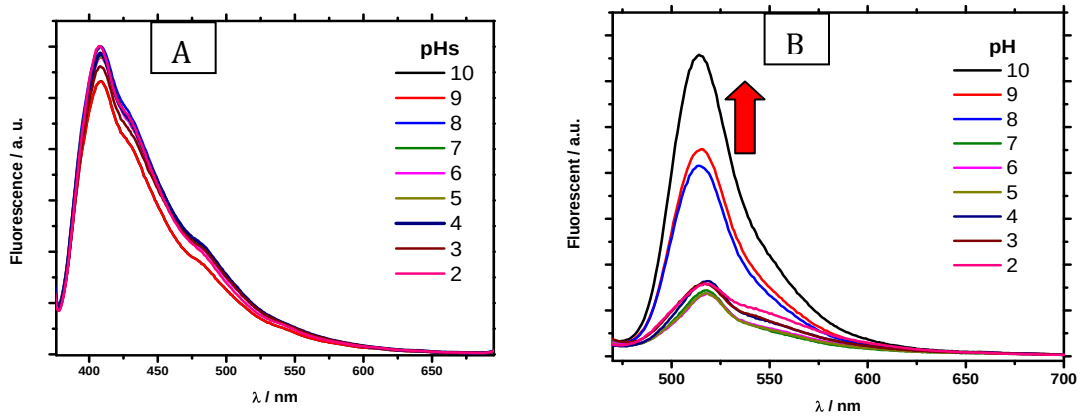


Figure 3.4. A) Fluorescence properties of surface immobilized OPE backbone (mixture of OPE and dodecyl silyl compound) and B) the surface-immobilized receptor **36** at various pH values

In any case, this very interesting phenomenon requires further studies to deepen our understanding of the fundamental reasons behind it.

3.6. Conclusions

We demonstrated that surface-immobilized Fluorescein-terminated OPE serves as an excellent broad-range pH probe which generates a ratiometric fluorescent response upon changes in pH. In contrast, the fluorescent receptor unit itself exhibited only a threshold fluorescence response over a narrow pH range. The perfect reversibility of the sensing makes this thin-film sensor even more promising from the practical perspectives. The successful application of the energy transfer modulation as a basis for design of thin-film ratiometric chemosensors for the case of pH sensor also illustrates generality of this approach.

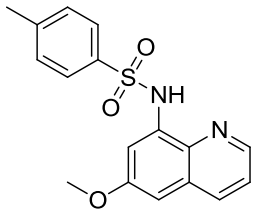
CHAPTER 4. THIN-FILM SENSORS FOR FLUORESCENT RATIOMETRIC DETECTION OF METAL IONS

4.1. Introduction

Fluorescent chemosensing has been a very popular area of research in the recent years because it provides a very useful and simple technique to quantify *in vitro* and *in vivo* biologically important analytes, of which metal ions are among the important ones.[128] In a typical fluorescent sensor, a receptor is linked with a fluorophore, which converts the analyte recognition by the receptor into a fluorescence signal.[17] There is always a strong demand for new design of fluorescent chemosensors with higher selectivity, efficiency and easy in handling. Metal ions such as Ca^{2+}, Na^{+}, Zn^{2+}, and Cu^{2+} are important biological analytes. There is always a need of more accurate, sensitive and selective sensing methods for them. In addition, metal cations such as Hg^{2+}, Pb^{2+}, and Cd^{2+} are common toxic industrial pollutants, which need to be continuously monitored. A number of fluorescent sensing methods have been developed for metal ion detection in the recent years.[52] In the course of our studies on design of thin-film fluorescent ratiometric chemosensors, we have designed, synthesized and characterized fluorescent ratiometric chemosensors for L-cysteine[124] and pH, which were described in the previous two chapters. In our approach, a fluorophore linked with a receptor is covalently connected to another fluorophore, oligo(*p*-phenylene ethynylene)(OPE) with a HOMO-LUMO energy gap different than that of the receptor terminus. In principle, when the receptor interacts with an analyte, the HOMO-LUMO energy gap of the fluorophore associated with it alters consequently leading to the change in the energy transfer process within the dyad which produces a ratiometric fluorescent response. Depending on the fluorophore, receptor, and analyte of interest, the possible mechanism of modulation of such energy transfer can vary.

Zinc ion (Zn^{2+}) has become an important target because of its biological significance. Zinc is the second most abundant transition metal ion in the human body, after iron. Zn^{2+} is believed to be an essential factor in many biological processes including brain functioning, immune function and mammalian reproduction.[129,130] Besides, zinc is also responsible for some pathological processes like Alzheimer's disease, epilepsy, ischemic stroke, infantile diarrhea etc. in living organisms.[131] Most zinc ions are tightly bound to enzymes and proteins, however free zinc metal pools are found in brain, intestine, pancreases, and retina.[129] Although, Zn^{2+} is spectroscopically silent due to its d^{10} electronic configuration, a large number of fluorescent chemosensors for Zn^{2+} have been designed and extensively studied.[132]

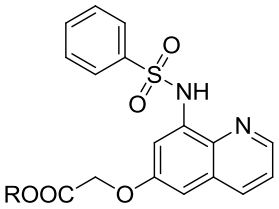
Previously, atomic absorption spectroscopy and radioactive tracing experiments have been used to show release and uptake of Zn^{2+} upon neuronal stimulation[133] and to diagnose brain tumors,[134] but there were serious adverse effects of exposing samples to ionizing and potentially toxic γ-radiation and they are fruitless for applications that require subcellular resolution. In addition to this, both atomic absorption spectroscopy and radioactive tracing techniques were incompetent of distinguishing between labile and tightly bound zinc pools. [134] These reasons forced to rely on the traditionally used invasive histochemical methods. These issues were solved after the development of fluorescent probes that provided a "turn-on" increase or ratiometric response to Zn^{2+}. These are preferred because these types of readouts provide superior spatial and temporal resolution compared with their corresponding "turn-off" counterparts. Ratiometric sensors are particularly valued for their potential to quantify Zn^{2+} concentrations by minimizing correction factors associated with variations in excitation source, emission collection efficiency, and sample thickness. The first and most widely used fluorescent sensor for imaging Zn^{2+} in biological samples is 6-methoxy-8-*p*-toluenesulfonamidoquinoline[135] an aryl sulphonamide derivative of 8-aminoquinoline (TSQ). Since then many other derivatives of TSQ have been



6-methoxy-8-*p*-toluenesulfonamidoquinoline

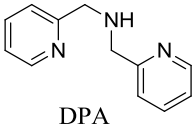
suggested because of the usefulness in identifying labile Zinc stores in the brain and other parts of the body. These derivatives include Zinquin A [136] and Zinquin E.[136]

Fluorescent chemosensing generally utilizes such observables as change of fluorescent intensity, fluorescent lifetime, shift of fluorescent wavelength or ratio of fluorescent intensities change if used with dual fluorophores. These changes are observed because of the energy transfer process that takes place beween the fluorophores. The energy transfer in these processes occurs either by photoinduced electron transfer (PET) or intermolecular charge transfer (ICT).[2]



R= H, Zinquin A
R=Et, Zinquin E

A typical Zn^{2+} sensor relies upon nitrogen-lone pair coordinated with metal ions which results in the increased redox potential of the receptor and the HOMO of the receptor sinks lower than that of the fluorophore blocking the PET process (**Figure 4.1**). Hence, PET mechanism always leads to the sensing fluorescent enhancement based on "TURN ON" behavior. On the other hand, ratiometric sensing is more signal-selective and practically convenient. For ratiometric measurements, an intermolecular charge transfer (ICT) is more preferred and has been widely used in designing ratiometric sensors. Such systems typically lack spacer between receptor and fluorophore and form an electronically conjugated system with electron rich and electron poor termini so that intermolecular charge transfer from donor to the receptor would be enhanced upon excitation.[149] In contrast, in PET based sensor, a fluorophore is connected to a receptor through a spacer with a relatively high energy due to non-bonded electron pair, which can be transferred to the excited



DPA

fluorophore resulting in fluorescence quenching. The coordination with non-bonding electron pair blocks fluorescence quenching leading to the "turn on" fluorescent enhancement. Popular di-2-picolylamine (DPA) based sensors functioning is based on the PET mechanism.

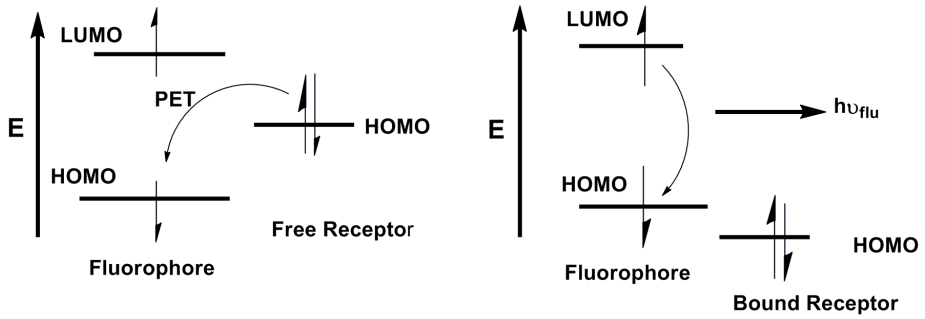


Figure 4.1. Principle of "turn on" fluorescent sensing based on blocking the photoinduced electron transfer (PET) by a metal coordinating to a receptor

When a fluorophore is directly connected without a spacer to form a π-elecron conjugated system with electron deficient termini, ICT from the electron donor to the receptor would be enhanced upon an excitation by light. When a receptor acting as a donor within the fluorophore interacts with a cation, it reduces the electron donating ability of the receptor resulting in the blue shift of the emission spectrum. In the similar manner, if a cation plays the role of an electron acceptor, the interaction between the receptor and the cation would result in red shifted emission. The coordination of Zn^{2+} with quinoline derivatives (*vide supra*) is the typical example of the ICT mechanism.

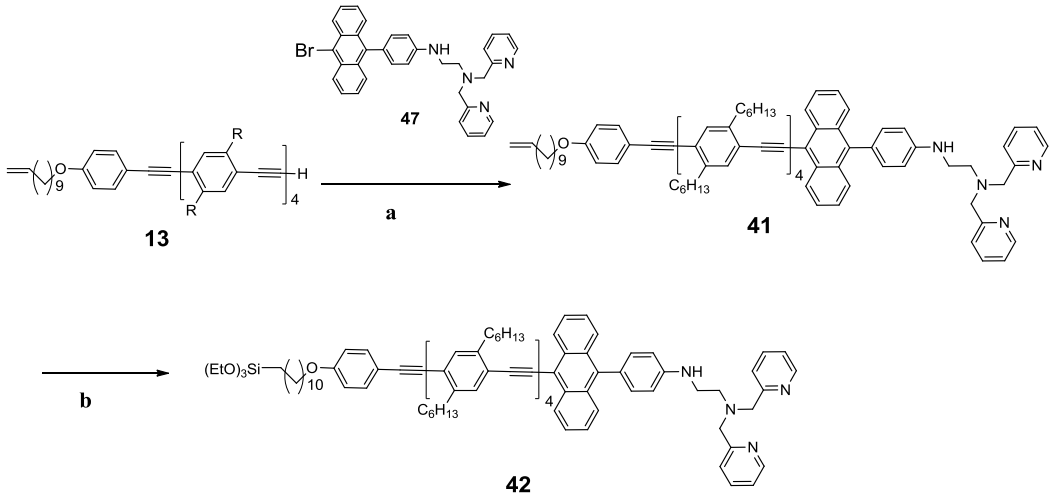
To extend our general concept of energy transfer controlled ratiometric sensors, we designed a ratiometric fluorescent Zn^{2+} chemosensor **42 (Scheme 4.1)**. The receptor unit incorporates a Zn^{2+}-specific DPA (di-2-picolylamine) linked with an anthracene fluorophore which is attached to oligo(*p*-phenylene ethynylene) (OPE) core. To build a surface-immobilized thin-film sensor, another end of the conjugated OPE core was terminated with a silyl anchor and covalently immobilized on the glass surface. We hypothesized that binding of Zn^{2+} cation by

receptor would alter the electronic structure of the receptor fluorophore therefore lowering its HOMO and leading to the ratiometric fluorescence response.

4.2. Synthesis

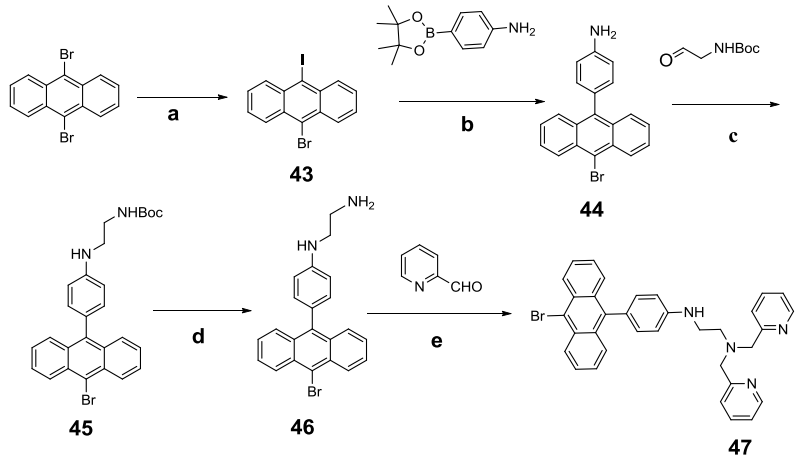
Zn^{2+} ion sensor **41** was synthesized by coupling of the conjugated OPE core **13** with Zn^{2+} receptor **47**. The resulting sensor compound **41** was then subjected to hydrosilylation to afford the immobilization precursor **42**, which was further immobilized on the glass surface to form a monolayer film of the fluorescent Zn^{2+} sensor. The OPE core **13** was synthesized as described in the Chapter 2. The terminal fluorophore with Zn^{2+} receptor was synthesized as shown in **Scheme 4.2**. Iodination of 9,10-dibromoanthracene afforded 9-bromo-10-iodoanthracene **43** in high yield. Subsequent coupling with commercially available *p*-(pinacolboronato)aniline gave the aniline compound **44**. The aniline **44** was subjected to reductive amination with N-Boc protected 2-aminoacetaldehyde to afford compound **45**, which upon deprotection produced diamine **46**. Finally, reductive amination of 2-pyridinecarboxaldehyde with **46** afforded Zn^{2+} receptor fluorophore **47**.

Scheme 4.1. Synthesis of Zn^{2+} sensor **41** and immobilization precursor **42**



Reaction conditions: a) $Pd(PPh_3)_4$, CuI, toluene:*i*Pr_2NH(7:3), 65 ^{0} C, 36 h; b) Karstedt's catalyst, $(OEt)_3SiH$,toluene, 2 h.

Scheme 4.2. Synthesis of Zn^{2+} receptor



Reaction conditions : a)1) n-BuLi; 2) I_2,THF, - 78 ^{o} C; b) $Pd(PPh_3)_4$, K_2CO_3, toluene, $EtOH/H_2O$, 95 ^{o} C, 24h, c) $NaCNBH_3$, CH_3COOH, CH_2Cl_2,RT, 4h, d) TFA/CH_2Cl_2; e)$NaBH(OAc)_3$,CH_2Cl_2

4.3. Results and Discussions

Spectroscopic studies of the compounds **47** and **41** in CH_2Cl_2 solutions showed some positive response on adding Zn^{2+} (**Figure 4.2**). However, the response with the receptor compound **47** was more pronounced than with the complete sensor molecule **41**. Although the exposure of the sensor compound **41** to various concentration of Zn^{2+} did display a ratiometric behavior, it was not very consistent. The change in absorption of the receptor **47** upon adding various concentration of Zn^{2+} was practically not detectable; however there was a sharp change in the emission spectrum. A substantial blue shift of 45 nm was observed upon addition of 0.5 equivalents of Zn^{2+} ions. Upon further addition of Zn^{2+} a noticeable fluorescent intensity enhancement of the new band was observed.

Similar experiments with the sensor compound **41** revealed ratiometric responses at much lower concentration of Zn^{2+} ions. There was a remarkable response even upon addition of 0.1 equivalents of Zn^{2+} ions (**Figure 4.2**).

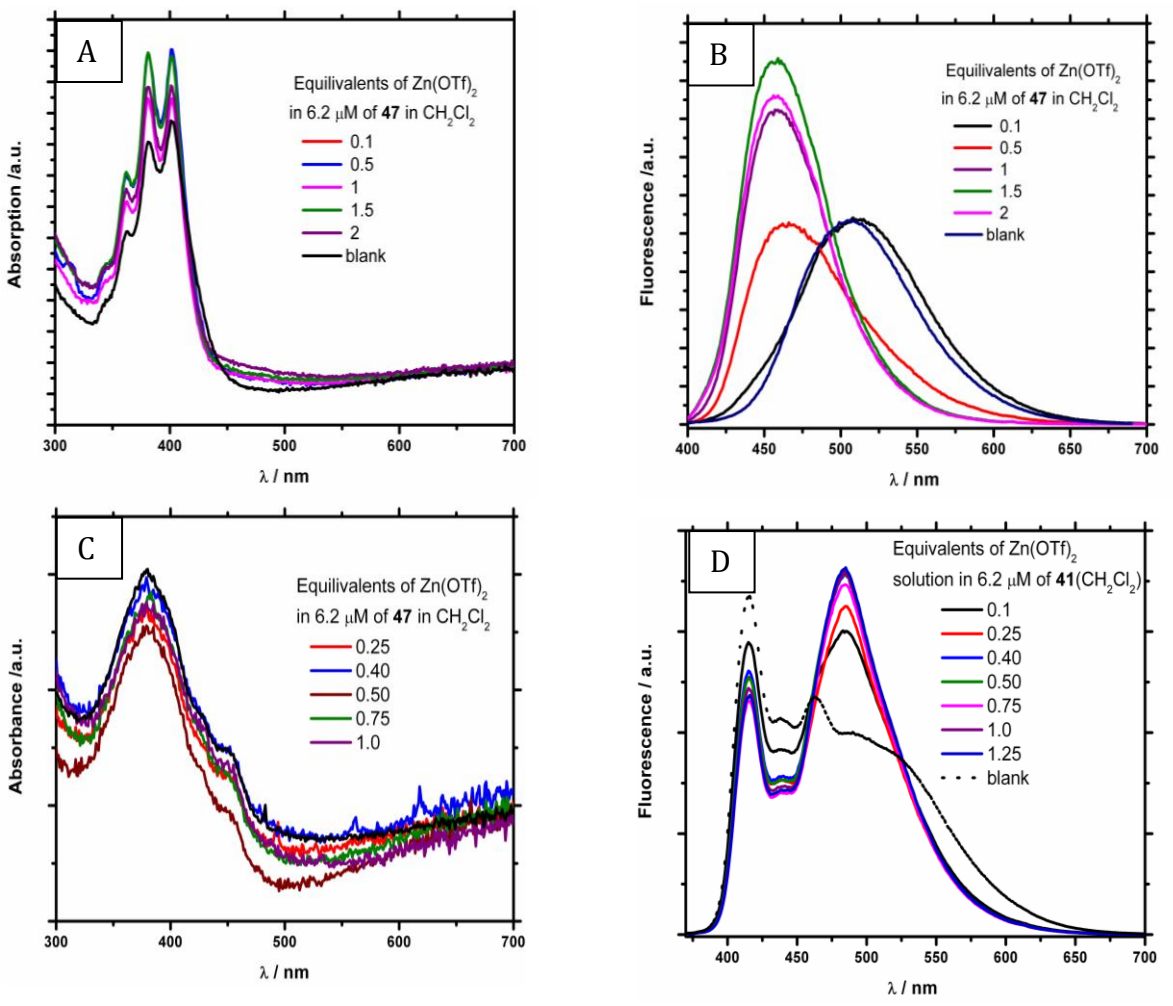


Figure 4.2. Change in absorption (A) and emission (B) spectra of the receptor **47**, and absorption (C) and emission (D) spectra of the sensor **41** upon adding various concentrations of $Zn(OTf)_2$ in CH_2Cl_2

Unfortunately, this behavior was not very consistent upon further increase of Zn^{2+} concentration. With the data available, we could not offer any reasonable rationale for these observed changes. Furthermore, when the sensor **41** was covalently immobilized on the glass surface, the resulted thin-film sensor did not show any consistent response upon exposure to various Zn^{2+} concentrations.

4.4. Conclusions

The new ratiometric fluorescent Zn^{2+} sensor was designed and synthesized. The sensor compound displayed the ratiometric responses when exposed to Zn^{2+} solutions. The observed behavior was not systematic and could not be rationalized with the data available. Further studies will be required to successfully complete this project.

CHAPTER 5 EXPERIMENTAL

5.1. Experimental Details

All reactions were performed under an atmosphere of dry nitrogen. Melting points were determined in open capillaries and are uncorrected. Column chromatography was performed using silica gel (Sorbent Technologies, 60 Å, 40-63 μm) slurry packed into glass columns. Tetrahydrofuran (THF), dichloromethane, ether, and toluene were dried by passing through activated alumina, and *N*,*N*-dimethylformamide (DMF) – by passing through activated molecular sieves, using a PS-400 Solvent Purification System from Innovative Technology, Inc. The water content of the solvents was periodically controlled by Karl Fischer titration (using a DL32 coulometric titrator from Mettler Toledo). All other solvents were additionally purified and dried by standard techniques. High purity $Pd(PPh_3)_4$ was obtained from Strem Chemicals, Inc. All other reagents were obtained from Aldrich and Alfa Aesar and used without further purification. Indium tin oxide (ITO) coated glass slides with 8-12 Ohm/sq. surface resistivity were purchased from Delta Technologies, Ltd. ^{1}H NMR spectra were recorded at 250, 300 and 400 MHz and are reported in ppm downfield from tetramethylsilane. UV-visible spectra were recorded on Varian Cary 50 UV-Vis spectrophotometer. Fluorescence studies were carried out with a PTI QuantaMaster4/2006SE spectrofluorimeter. Fluorescence quantum yields were determined using ethanol solution of Coumarin 6 ($\Phi = 0.78$[138]) or 0.1 M H_2SO_4 solution of quinine sulphate ($\Phi = 0.55$[139]) as standards. Atomic Force Microscopy (AFM) images were acquired in a tapping mode with easyScan 2 AFM (Nanosurf AG). High-resolution mass spectra were obtained at the LSU Department of Chemistry Mass Spectrometry Facility using an ESI or MALDI-TOF method, and a peak matching protocol to determine the mass and error range of the molecular ion.

5.2. Synthetic Details

5.1.1. General Procedure for Iodination

To a stirred solution of an aryl bromide in THF at -78 °C, a solution of *n*BuLi was added dropwise and the reaction mixture was stirred for 3 h. A solution of iodine (or 1,2-diiodoethane) in THF was added dropwise and stirred for an additional 1 h at -70 °C, and was allowed to warm to room temperature overnight. The resulting solution was concentrated to approximately 30% of the initial volume and treated with a 20% $Na_2S_2O_3$ aq. solution. The product was extracted with CH_2Cl_2, washed with water and saturated NaCl solution, and dried over anhydrous Na_2SO_4. After concentration in vacuo, a crude product was purified by recrystallization or column chromatography.

5.1.2. General Procedure for Cross-Coupling Reaction (Sonogashira Coupling)

A mixture of acetylene, aryl iodide, $Pd(PPh_3)_4$ and CuI in toluene – diisopropylamine (7:3) was stirred in a sealed flask in argon atmosphere for 24-72 hours at 45-65 °C. The mixture was cooled to room temperature and passed through a short column with silica gel eluted with chloroform to separate inorganic impurities. Concentration in vacuo afforded a crude product that was further purified by column chromatography on silica gel.

5.1.3. General Procedure for Desilylation

To a stirred solution of KOH in MeOH, a solution of a TMS-protected acetylene in THF was added dropwise and stirred for 40 minutes at room temperature. The resulting solution was concentrated to 30% of the initial volume and then extracted with diethyl ether, washed with water, NaCl and dried over anhydrous Na_2SO_4. The crude product was purified by column chromatography.

5.3. Preparation of Immobilized Monolayer Modified Slides

5.3.1. Cleaning and Activation of Glass Slides

Microscope glass cover slides (22×22 mm, Slip-Rite No. 1) were sonicated in chloroform for 10 min and then washed with copious amounts of methanol, acetone and DI water, and dried under the flow of nitrogen. The dried slides were sonicated with 25 ml of piranha solution (prepared by mixing conc. H_2SO_4 and 30% H_2O_2 7:3) for 30 min, washed with DI water and dried under the flow of nitrogen.

5.3.2 Preparation of ITO-Covered Glass Slides

Rectangular ITO-covered glass slides (approx. 1.1×2.5 cm) were ultrasonicated in CH_2Cl_2 for 20 min., followed by rinsing with acetone and deionized water. The pre-cleaned slides were subjected to an RCA-type cleaning procedure by keeping in a water – 30% H_2O_2 – 30% aqueous NH_3 (5:1:1) mixture at 70 °C for 1 hour. The substrates were then rinsed with copious amount of deionized water and dried in N_2 flow at room temperature for 2 h.

5.3.3. Surface-Immobilization Procedure

The freshly activated glass (or ITO/glass) slides were immersed into 0.2 mmol solutions of triethoxy silyl terminated compounds in toluene (or DMF) and kept at 80 °C for 2 h. After cooling to room temperature, the slides were rinsed with copious amount of chloroform upon ultrasonication. After drying, the monolayer-modified slides were annealed by heating to 80 °C in aqueous buffered solution (pH 9.5) for 2 h. The annealed slides were washed with water and dried under the flow of nitrogen.

5.4. Exposure to Cysteine and Glutathione

The compound **16**-modified glass slides were immersed into buffered (pH 9.5) aqueous

solutions of L-cysteine with varying concentrations, or glutathione (20 mM), and were kept at 80 °C for 20 minutes. Then the slides were thoroughly rinsed with DI water, dried, and subjected to absorption and fluorescence measurements.

5.5. Hydrolysis of Surface Thiazolidine Groups

The compound **16**-modified glass slides exposed to cysteine as described above were immersed into an aqueous HCl solution (pH 1) and kept there at 80 °C for 1 h, then it was thoroughly rinsed with DI water, dried, and subjected to fluorescence measurements.

5.6. pH Dependent Fluorescent Studies on the pH Sensor

Aqueous annealed surface-immobilized thin film pH sensors and the corresponding films were carefully placed in a rectangular open neck cuvette in a way that the position of slide was at 45^{0} to the incident light. The solutions of different pH values were successively added to the cuvette, one by one and the spectra were acquired at each pH values.

5.7. XPS Measurements for OPE for Cysteine Sensor Monolayer

XPS data were acquired with a Kratos AXIS 165 system with a monochromatic Al Ka source and a hemispherical electron energy analyzer. The pressure in the analyzing chamber was less than 2×10^{-9} torr. Survey spectra were recorded with 160 eV pass energy, and 150 W X-ray beam powers. High-resolution elemental spectra were recorded with 40 eV pass energy and 150 W X-ray beam powers. The normal emission angle-integrated, high-resolution scans with 15-20 eV windows were acquired for C1s, O1s, N1s, S2p, Si2s, and Si2p regions. These spectra were averaged over 40 scans each to obtain a good signal-to-noise ratio.

5.8. Evaluation of Surface Density of the Monolayer of 16

Cyclic voltammetry measurements were performed using an Autolab PGSTAT 302 potentiostat. The measurements were carried out using a three-electrode system with monolayer-modified ITO/glass working electrode (electrode area ~ 1.9 cm^2), $Ag/AgNO_3$ non-aqueous reference electrode, and a Pt gauze counter electrode in 0.1 M Bu_4NPF_6 solution in CH_2Cl_2 as supporting electrolyte. The reference electrode was checked against ferrocene standard every time before and after the experiments was performed, and the measured potentials were corrected based on the Fc/Fc^+ redox potential value.

The surface coverage density was determined based on the measured area of the redox peak corresponding to reduction of anthraldehyde groups. The value was corrected by the corresponding data for the bare ITO electrode. Assuming the reduction being a one-electron process, the surface density G was estimated using the formula:

$$\Gamma = \frac{Q}{F}$$

where Q is the redox peak area (C cm^{-2}), and F is Faraday constant (96500 C mol^{-1}). For the current case (Q = 2.45×10^{-16} C cm^{-2}, after correcting for the ITO surface roughness[140]), the surface density G was estimated as 2.54×10^{-11} mol cm^{-2}.

1-Bromo-2,5-dihexyl-4-iodobenzene (**2**) was prepared following the literature procedure.[141,142]

((4-Bromo-2,5-dihexylphenyl)ethynyl)trimethylsilane (**3**) was prepared following the general procedure **5.1.2**. A reaction of a mixture of 28.0 g (0.062 mol) of **2**, 9.56 ml (6.69 g, 0.0683 mol) of TMS-acetylene, 0.36 g (0.31 mmol) of $Pd(PPh_3)_4$, and 0.06 g (0.31 mmol) of CuI in 250 ml of iPr$_2$NH – toluene (3:7) afforded, after column chromatography on silica gel (eluent

hexane), 17.2 g (89%) of **3** as a colorless oil, R_f 0.78. ^{1}H NMR (250 MHz, acetone-D_6) δ 7.46 (s, 1H), 7.33 (s, 1H), 2.78 - 2.63 (m, 4H), 1.68 - 1.50 (m, 4H), 1.45 - 1.25 (m, 12H), 0.95 - 0.85 (m, 6H), 0.25 (s, 9H).

((2,5-Dihexyl-4-iodophenyl)ethynyl)trimethylsilane (**4**) was prepared following the general procedure **5.1.1**. A reaction of 17.2 g (0.041 mol) of **3,** 28.1 ml (0.044 mol) of 1.6 M *n*BuLi in hexanes, 13.54 g (0.053 mol) of iodine in 250 ml of THF afforded, after column chromatography on silica gel (eluent hexane), 19.5 g (80%) of **4** as a colorless oil, R_f 0.71. ^{1}H NMR (250 MHz, acetone-D_6), δ 7.75 (s, 1H), 7.31(s, 1H), 2.75 - 2.64 (m, 4H), 1.72 - 1.50 (m, 4H), 1.45 - 1.20 (m, 12H), 0.95 - 0.82 (m, 6H), 0.25 (s, 9H).

1-Bromo-4-ethynyl-2,5-dihexylbenzene (**5**) was prepared following the general procedure **5.1.3**. A reaction of 17.5 g (0.042 mol) of **4** in 150 ml of THF and 7.0 g (0.125 mol) of KOH in 225 ml of methanol afforded, after column chromatography on silica gel (eluent hexane) 14.2 g (98%) of **5** as a colorless oil, R_f 0.76. ^{1}H NMR (250 MHz, acetone-D_6) δ 7.46 (s, 1H), 7.37 (s, 1H), 3.87 (s, 1H), 2.85 - 2.60 (m, 4H), 1.75 - 1.55 (m, 4H), 1.50 - 1.20 (m, 12H), 0.95 - 0.80 (m, 6H).

TMS-protected dimer 6 was prepared following the general procedure **5.1.2**. A reaction of 14.87 g (0.032 mol) of **4**, 12.3 g (0.035 mol) of **5**, 0.18 g (0.16 mmol) of $Pd(PPh_3)_4$, and 0.03 g (0.16 mmol) of CuI in 300 ml of *i*Pr_2NH – toluene (3:7) afforded, after column chromatography on silica gel (eluent hexane), 20.69 g (93%) of **2** as a yellowish oil, R_f 0.67. ^{1}H NMR (250 MHz, acetone-D_6) δ 7.52 (s, 1H), 7.45 (s, 1H), 7.41 (s, 1H), 7.34 (s, 1H), 2.90 - 2.63 (m, 8H), 1.80 - 1.55 (m, 8H), 1.50 - 1.25 (m, 24H), 0.95 - 0.80 (m, 12H), 0.26 (s, 9H).

Aryl iodide 7 was prepared following the general procedure **5.1.1**. A reaction of 10.5 g (0.015 mol) of **6**, 10.5 ml (0.017 mol) of 1.6 M solution of *n*BuLi in hexanes, 5.8 g (0.023 mol)

of iodine in 200 ml of THF afforded, after column chromatography on silica gel (eluent hexane), 9.61 g (86%) of **7** as a yellowish oil, R_f 0.65. ^{1}H NMR (250 MHz, acetone-D_6) δ 7.80 (s, 1H), 7.42 (s, 1H), 7.41 (s, 1H), 7.34 (s, 1H), 2.92 - 2.70 (m, 8H), 1.80 - 1.55 (m, 8H), 1.50 - 1.25 (m, 24H), 0.98 - 0.82 (m, 12H), 0.26 (s, 9H).

Arylacetylene 8 was prepared following the general procedure **5.1.3**. A reaction of 8.53 g (12.4 mmol) of **6** in 100 ml of THF, 2.1 g (3.78 mmol) of KOH in 200 ml of methanol afforded, after column chromatography on silica gel (eluent hexane), 7.31 g (97%) of **8** as a slightly pink solid, R_f 0.64, mp 39-40 °C (lit.[142] m.p. 39°C). ^{1}H NMR (250 MHz, acetone-D_6), δ 7.52 (s, 1H), 7.45 (s, 1H), 7.41 (s, 1H), 7.38 (s, 1H), 3.93 (s, 1H), 2.95 - 2.63 (m, 8H)), 1.82 - 1.55 (m, 8H), 1.50 - 1.15 (m, 24H), 1.00 - 0.85 (m, 12H).

TMS-protected tetramer 9 was prepared following the general procedure **5.1.2**. A reaction of 8.72 g (11.8 mmol) of **7**, 7.31 g (11.8 mol) of **8**, 0.10 g (0.089 mmol) of $Pd(PPh_3)_4$, and 0.017 g (0.089 mmol) of CuI in 300 ml of *i*Pr_2NH – toluene (3:7) afforded, after column chromatography on silica gel (eluent hexane), 11.65 g (80%) of **9** as a greenish-yellow solid, R_f 0.67, mp 77 °C (lit.[140] mp 77 °C). ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.38 (s, 1H), 7.36 - 7.26 (m, 7H), 2.82 - 2.57 (m, 16H), 1.80 - 1.55 (m, 32H), 1.50 - 1.20 (m, 32H), 0.88 - 0.75 (m, 24H), 0.25 (s, 9H). UV ($CHCl_3$) λ_{max} 359 nm ($\varepsilon = 4.45\times10^4$), fluorescence ($CHCl_3$) λ_{max} 421 nm.

Iodide 10 was prepared following the general procedure **5.1.1**. A reaction of 9.35 g (7.62 mmol) of **9**, 5.7 ml (9.14mmol) of 1.6 M solution of *n*BuLi in hexanes, and 2.58 g (9.1 mmol) of 1,2-diiodoethane in 300 ml of THF afforded, after column chromatography on silica gel (eluent hexane), 6.69 g (69%) of **10** as a yellow solid, R_f 0.65, mp 85-87 °C (lit.[142] mp 85 °C). ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.68 (s, 1H), 7.43 - 7.30 (m, 7H), 2.92 - 2.57 (m, 16H), 1.85 - 1.50 (m, 16H), 1.50 - 1.20 (m, 48H), 1.00 - 0.75 (m, 24H), 0.27 (s, 9H).

Pentamer 11 was prepared following the general procedure **5.1.2**. A reaction of 2.0 g (1.6 mmol) of **9,** 0.75 g (2.8 mmol) of **10**, 0.036 g (0.03 mmol) of $Pd(PPh_3)_4$, and 0.006 g (0.03 mmol) of CuI in 100 ml of *i*Pr_2NH – toluene (3:7) afforded, after column chromatography on silica gel (eluent hexane : chloroform 10:1), 1.24 g (56%) of **11** as a greenish-yellow sticky solid, R_f 0.52, mp 101-103 °C. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.43 (d, *J* = 8.6 Hz, 2H), 7.38-7.26 (m, 8H), 6.86 (d, *J* = 8.6 Hz, 2H), 5.88 - 5.72 (m, 1H), 5.05 - 4.86 (m, 2H), 3.96 (t, *J* = 6.5 Hz, 2H), 2.90 - 2.65 (m, 16H), 2.04 - 1.95 (m, 2H), 1.85 - 1.55 (m, 24H), 1.50 - 1.20 (m, 54H), 0.95 - 0.80 (m, 24H), 0.25 (s, 9H). UV ($CHCl_3$) λ_{max} 374 nm, fluorescence ($CHCl_3$) λ_{max} 412 nm (Φ = 0.41).

Compound 12. One drop of platinum(0)-1,3-divinyl-1,1,3,3-tetramethyl-disiloxane complex (Karstedt's catalyst, 0.1 M solution in poly(dimethylsiloxane)) was added at room temperature to a solution of compound **11** (23.8 mg, 0.017 mmol) in 6 ml of toluene, and stirred for 10 min. Triethoxysilane (26.5 ml, 0.168 mmol) was added to the resulting solution and stirred for 1 h at room temperature. The reaction mixture was concentrated in vacuo, dissolved in toluene, filtered through a 0.22 mm PTFE filter, and concentrated in vacuo to afford **12** in quantitative yield as a greenish-yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (d, *J* = 8.9 Hz, 2H), 7.45 - 7.28 (m, 8H), 6.86 (d, *J* = 8.9 Hz, 2H), 3.96 (t, *J* = 6.5 Hz, 2H), 3.95 - 3.72 (m, 8H), 2.95 - 2.65 (m, 16H), 1.90 - 1.55 (m, 24H), 1.50 - 1.15 (m, 67H), 0.95 - 0.85 (m, 24H), 0.25 (s, 9H).

Compound 13 was prepared following the general procedure **5.1.3**. A reaction of 1.14g (0.81 mmol) of **11** in 100 ml of THF and 0.14g (2.5 mmol) of KOH in 20 mL of MeOH afforded, after column chromatography on silica gel (eluent hexane-chloroform 8:1), 0.96 g (87%) of acetylene **13** as a greenish-yellow solid, R_f 0.55, mp 66-68 °C. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.47 (d, *J* = 8.9 Hz, 2H), 7.45 - 7.25 (m, 8H), 6.86 (d, *J* = 8.9 Hz, 2H), 5.93 - 5.70 (m, 1H), 5.06 -

4.85 (m, 2H), 3.96 (t, *J* = 6.5 Hz, 2H), 3.28 (s, 1H), 2.88 - 2.65 (m, 16H), 2.12 - 1.99 (m, 2H), 1.84 - 1.55 (m, 16H), 1.50 - 1.15 (m, 62H), 0.95 - 0.75 (m, 24H).

Acetal-protected aldehyde 14 was prepared following the general procedure **5.1.2**. A reaction of 0.34 g (0.253 mmol) of **13**, 0.24 g (0.504 mmol) of **21**, 30 mg (0.0253 mmol) of $Pd(PPh_3)_4$, and 4.9 mg (0.0253 mmol) of CuI in 35 ml of $i Pr_2NH$ – toluene (3:7) afforded, after column chromatography on silica gel (eluent chloroform – hexane 1:2), 0.31 g (73%) of **14** as a yellow solid, R_f 0.42. The product contained some unidentified impurities, and was used for the next step without further purification. ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.72 (d, *J* = 8.6 Hz, 2H), 8.56 (d, *J* = 8.6 Hz, 2H), 7.75 (d, *J* = 8.6 Hz, 2H), 7.63 - 7.25 (m, 16H), 7.08 (s, 1H), 6.86 (d, *J* = 8.6 Hz, 2H), 5.92 - 5.71 (m, 1H), 5.08 - 4.86 (m, 2H), 4.58 - 4.43 (m, 2H), 4.32 - 4.21 (m, 2H), 3.95 (t, *J* = 6.4 Hz, 2H), 2.90 - 2.72 (m, 16H), 2.10 - 1.95 (m, 2H), 1.85 - 1.55 (m, 16H), 1.50 - 1.15 (m, 62H), 0.95 - 0.75 (m, 24H).

Aldehyde 15. A solution of 200 mg (0.118 mmol) of **14** and 5 mg (0.026 mmol) of *p*-toluenesulfonic acid monohydrate in 15 ml of acetone and 5 ml of chloroform was stirred at room temperature for 5 h. The reaction mixture was poured into water, extracted with CH_2Cl_2 (3×100 ml), washed with saturated aq. $NaHCO_3$ (3×50 ml), water, and dried over Na_2SO_4. Concentration in vacuo afforded a crude product which was purified by column chromatography on silica gel (eluent hexane – chloroform 1:1) to yield 150 mg (77%) of **15** as a yellow solid, R_f 0.55, mp 143-145 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.51 (s, 1H), 8.95 (d, *J* = 8.8 Hz, 2H), 8.76 (d, *J* = 8.8 Hz, 2H), 7.77 (d, *J* = 8.2 Hz, 2H), 7.74 - 7.64 (m, 4H), 7.61 (d, *J* = 8.6 Hz, 2H), 7.46 - 7.25 (m, 10H), 6.86 (d, *J* = 8.6 Hz, 2H), 5.90 - 5.72 (m, 1H), 5.05 - 4.85 (m, 2H), 3.96 (t, *J* = 6.4 Hz, 2H), 2.92 - 2.73 (m, 16H), 2.10 - 1.98 (m, 2H), 1.83 - 1.60 (m, 16H), 1.50 - 1.15 (m, 62H), 0.98 - 0.76 (m, 24H). MS (MALDI-TOF) *m/e* 1648.11 (calcd for $C_{122}H_{150}O_2$ 1648.17). UV ($CHCl_3$) λ_{max} 379 nm ($\varepsilon = 1.26\times10^6$), fluorescence ($CHCl_3$) λ_{max} 421 nm (Φ = 0.08).

Compound 16. One drop of platinum(0)-1,3-divinyl-1,1,3,3-tetramethyl-disiloxane complex (Karstedt's catalyst, 0.1 M solution in poly(dimethylsiloxane)) was added to a solution of **15** (98.3 mg, 0.062 mmol) in 15 ml of toluene and stirred at room temperature for 10 min followed by addition of triethoxysilane (0.11 ml, 0.62 mmol). The resulting mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated in vacuo, dissolved in toluene, filtered through a 0.22 mm PTFE filter, and concentrated in vacuo to afford **16** in quantitative yield as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.52 (s, 1H), 8.95 (d, *J* = 8.8 Hz, 2H), 8.76 (d, *J* = 8.8 Hz, 2H), 7.77 (d, *J* = 8.2 Hz, 2H), 7.74 - 7.65 (m, 4H), 7.61 (d, *J* = 8.2 Hz, 2H), 7.54 - 7.48 (m, 2H), 7.46 - 7.31 (m, 8H), 6.86 (d, *J* = 8.2 Hz, 2H), 3.96 (t, *J* = 6.4 Hz, 2H), 3.84 - 3.77 (m, 6H), 2.82 - 2.75 (m, 16H), 2.08 – 1.95 (m, 2H), 1.79 - 1.05 (m, 91H), 0.94 - 0.82 (s, 24H).

1-(4-Tolylsulfonyl)-10-undecene (**17**) was prepared following the literature procedure.[143]

4-Iodo-1-(10-undecenyloxy)benzene (**18**). A mixture of 2.93 g (0.013 mol) of 4-iodophenol, 8.63 g (0.027 mol) of **17**, 3.68 g (0.266 mol) of K_2CO_3, and 0.22 g (1.33 mmol) of KI in 200 ml of ethyl methyl ketone was refluxed for 60 h. The mixture was allowed to cool down to room temperature, and the solid precipitate was removed by filtration. The filtrate was concentrated in vacuo, and the crude product purified by column chromatography on silica gel (eluent ethyl acetate – hexane 1:3) to afford 4.7 g (96%) of **18** as a colorless oil, R_f 0.80. ^{1}H NMR spectrum was in agreement with the literature data.[8]

1-(Trimethylsilyl)-2-(4-(10-undecenyloxy)phenyl)acetylene (**19**). A mixture of 4.12 g (10.1 mmol) of **18**, 1.98 g (2.85 ml, 20.2 mmol) of trimethylsilylacetylene, 0.12 g (0.1 mmol) of $Pd(PPh_3)_4$, 20 mg (0.1 mmol) of CuI in 100 ml of *i*Pr_2NH – toluene (3:7) was stirred in a sealed flask at 55 °C for 30 h. After allowing to cool to room temperature, the reaction mixture was passed through a short column with silica gel eluted with $CHCl_3$. Concentration in vacuo

afforded 3.78 g (100%) of **19** as a yellowish oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (d, *J* = 8.6 Hz, 2H), 6.77 (d, *J* = 8.6 Hz, 2H), 5.90 - 5.72 (m, 1H), 5.05 - 4.85 (m, 2H), 3.91 (t, *J* = 6.5 Hz, 2H), 2.06 - 1.96 (m, 2H), 1.80 - 1.68 (m, 2H), 1.47 - 1.20 (m, 12H), 0.21 (s, 9H).

4-(10-Undecenyloxy)phenylacetylene (**20**). A solution of 3.70 g of **19** in 170 ml of THF was added dropwise to a stirred solution of 3.1 g (0.055 mol) of KOH in 150 ml of methanol and stirred at room temperature for 40 min. The reaction mixture was concentrated in vacuo to approximately 30% of its initial volume; poured into water, extracted with CH_2Cl_2 (3×100 ml), the organic fraction was washed with water, and dried over anhydrous Na_2SO_4 overnight. After concentration in vacuo, the crude product was purified by column chromatography on silica gel (eluent hexane) to afford 2.7 g (92%) of **20** as a colorless oil, R_f 0.34. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.36 (d, *J* = 8.6 Hz, 2H), 6.77 (d, *J* = 8.6 Hz, 2H), 5.91 - 5.72 (m, 1H), 5.05 - 4.85 (m, 2H), 3.93 (t, *J* = 6.5 Hz, 2H), 2.96 (s, 1H), 2.12 - 1.94 (m, 2H), 1.84 - 1.66 (m, 2H), 1.55 - 1.20 (m, 12H). HRMS *m/e* 271.2070 $[M+H]^+$ (calcd for $C_{19}H_{27}O$ 271.2062).

2-(10-Chloro-9-anthracenyl)-1,3-dioxolane (**21**). A mixture of 5.0 g (20.8 mmol) of 10-chloroanthraldehyde, 5.16 g (4.64 ml, 83.2 mmol) of ethylene glycol, and 34 mg (0.18 mmol) of *p*-toluenesulfonic acid monohydrate was refluxed with Dean-Stark adaptor for 21 h. After allowing to cool down to room temperature, the reaction mixture was washed with aq. $NaHCO_3$ (three times), water, and saturated NaCl solution, and dried over anhydrous Na_2SO_4. Concentration in vacuo afforded crude product which was recrystallized from hexane – dichloromethane mixture to yield 5.26 g (89%) of **21** as yellow needle-like crystals, mp 149-150 °C. ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.64 - 8.52 (m, 4H), 7.63 - 7.48 (m, 4H), 7.07 (s, 1H), 4.54 - 4.46 (m, 2H), 4.30 - 4.22 (m, 2H).

2-(10-Triisopropylsilylethynyl-9-anthracenyl)-1,3-dioxolane (**22**). The reaction was performed utilizing the conditions developed by Gelman and Buchwald.[145] A mixture of 0.5 g

(1.76 mmol) of **21**, 0.48 g (0.59 ml, 2.64 mmol) of TIPS-acetylene, 4.7 mg (0.018 mmol) of $PdCl_2(CH_3CN)_2$, 25.3 mg (0.053 mmol) of 2-(dicyclohexylphosphino)-2',4',6'-triisopropyl-1,1'-biphenyl (X-Phos), 1.47 g (4.5 mmol) of Cs_2CO_3 in 20 ml of acetonitrile was stirred for 24 h at 90 °C. After allowing to cool to room temperature, the reaction mixture was poured into water, extracted with diethyl ether, washed with water and conc. NaCl, and dried over anhydrous Na_2SO_4. After concentration in vacuo, the crude product was purified by column chromatography on silica gel (eluent hexane – ethyl acetate 3:1), and a fraction with R_f 0.45 afforded 0.45 g (60%) of **22** as dark-red oil crystallizing on standing. Although the product contained some unidentified impurities, it was used for the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.69 (d, *J* 7.0 Hz, 2H), 8.53 (d, *J* = 7.0 Hz, 2H), 7.60 - 7.45 (m, 4H), 7.06 (s, 1H), 4.55 - 4.43 (m, 2H), 4.32 - 4.21 (m, 2H), 1.38 - 1.12 (m, 21H).

2-(10-ethynyl-9-anthracenyl)-1,3-dioxolane (**23**). A solution of TBAF (10.8 ml of 1 M solution in THF, 0.01 mmol) was added dropwise to a solution of 1.55 g (3.6 mmol) of **22** in 30 ml of THF, and the resulting mixture was stirred at room temperature for 5 min, poured into a saturated aq. NH_4Cl solution, extracted with hexane – ethyl acetate (3:1), washed with water, saturated NH_4Cl and NaCl solutions, and dried over anhydrous Na_2SO_4. After concentration in vacuo, the crude product was purified by column chromatography on silica gel (eluent hexane – ethyl acetate 3:1), and the fraction with R_f 0.32 afforded 0.63 g (51%) of **23** as a brown-red solid. Although the product contained some unidentified impurities, it was used for the next step without further purification. ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.65 (d, *J* = 7.5 Hz, 2H), 8.55 (d, *J* = 7.5 Hz, 2H), 7.62 - 7.45 (m, 4H), 7.07 (s, 1H), 4.56 -4.44 (m, 2H), 4.31 - 4.20 (m, 2H), 4.03 (s, 1H).

2-(10-(4-Bromophenyl)ethynyl-9-anthracenyl)-1,3-dioxolane (**24**). A mixture of 0.63 g (2.3 mmol) of **23**, 0.65 g (2.3 mmol) of 1-bromo-4-iodobenzene, 80 mg (0.06 mmol) of

$Pd(PPh_3)_4$, 13 mg (0.06 mmol) of CuI in 30 ml of *i*Pr_2NH – toluene (3:7) was stirred at 45 °C for 24 h. After allowing to cool to room temperature, the reaction mixture was poured into water, extracted with CH_2Cl_2, washed with water, conc. NaCl, and dried over anhydrous Na_2SO_4. After concentration in vacuo, the crude product was purified by recrystallization from hexane – CH_2Cl_2 mixture to afford 0.66 g (67%) of **24** as a yellow crystalline solid, mp 210-212 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.68 (d, *J* = 7.5 Hz, 2H), 8.56 (d, *J* = 7.5 Hz, 2H), 7.70 - 7.40 (m, 8H), 7.07 (s, 1H), 4.61 - 4.41 (m, 2H), 4.33 - 4.18 (m, 2H).

2-(10-(4-Iodophenyl)ethynyl-9-anthracenyl)-1,3-dioxolane (**25**). The reaction was performed utilizing the conditions developed by Klapars and Buchwald.[156,157] A mixture of 0.62 g (1.45 mmol) of **24**, 0.48 g (3.18 mmol) of NaI, 0.08 g (60 ml, 0.58 mmol) of *trans-N,N'*-dimethyl-1,2-cyclohexanediamine, 0.01 g (0.007 mmol) of CuI in 10.0 ml of dioxane was stirred at 110 °C for 24 h. The mixture was cooled down to room temperature, diluted with conc. aq. NH_3 solution, poured into water, extracted with CH_2Cl_2, washed with water, conc. NaCl, and dried over Na_2SO_4. After concentration in vacuo, the residue was dissolved in ethyl acetate and passed through a short column with silica gel eluted with ethyl acetate. The crude product was recrystallized from hexane – dichloromethane to afford 0.55 g (80%) of **25** as a yellow crystalline solid, mp 216-218 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (d, *J* = 8.8 Hz, 2H), 8.56 (d, *J* = 8.9 Hz, 2H), 7.77 (d, *J* = 8.4 Hz, 2H), 7.65 - 7.50 (m, 4H), 7.47 (d, *J* = 8.4 Hz, 2H), 7.07 (s, 1H), 4.58 - 4.45 (m, 2H), 4.35 - 4.20 (m, 2H). HRMS *m/e* 477.0377 $[M+H]^+$ (calcd for $C_{25}H_{18}IO_2$ 477.0351). UV ($CHCl_3$) λ_{max} 407 (0-1), 432 (0-0) nm, fluorescence ($CHCl_3$) λ_{max} 439 (0-0), 466 (0-1) nm.

10-(4-Iodophenyl)ethynyl-9-anthraldehyde. A mixture of 100 mg (0.21 mmol) of **25** and 5 mg (0.026 mmol) of *p*-toluenesulfonic acid monohydrate in 15 ml of acetone was stirred for 4 h at room temperature. The reaction mixture was poured into water, extracted with CH_2Cl_2

(3×30 ml), the organic fraction was washed with water (3×100 ml), saturated $NaHCO_3$ (3×30 ml), and dried over anhydrous Na_2SO_4 overnight. After concentration in vacuo, the crude product was purified by column chromatography on silica gel (eluent chloroform – hexane 1:1) to afford 78 mg (86%) as a yellow solid, R_f 0.6, mp 211-215 °C. ^{1}NMR (400 MHz, $CDCl_3$) δ 11.48 (s, 1H), 8.91 (d, *J* = 8.9 Hz, 2H), 8.68 (d, *J* = 8.3 Hz, 2H), 7.79 (d, *J* = 8.4 Hz, 2H), 7.73 - 7.56 (m, 4H), 7.47 (d, *J* = 8.4 Hz, 2H). HRMS *m/e* 433.0101 $[M+H]^+$ (calcd for $C_{23}H_{14}IO$ 433.0089).

2-(10-(4-(10-Undecenyloxy)phenyl)ethynyl-9-anthracenyl)-1,3-dioxolane (**26**). A mixture of 54 mg (0.20 mmol) of **23**, 81 mg (0.22 mmol) of **20**, 11.5 mg (0.01 mmol) of $Pd(PPh_3)_4$, and 1.9 mg (0.01 mmol) of CuI in 10 ml of *i*Pr_2NH – toluene (3:7) was stirred in inert atmosphere in a sealed flask at 55 °C for 36 h. After allowing cooling down to room temperature, the reaction mixture was passed through a short column with silica gel eluted with $CHCl_3$, and concentrated in *vacuo*. The crude product was further purified by column chromatography on silica gel (eluent chloroform – hexane 1:1), to afford 72 mg (70%) of **26** as a yellow solid, R_f 0.67. The product contained some unidentified impurities, and was used for the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) d 8.72 (d, *J* = 9.1 Hz, 2H), 8.54 (d, *J* = 9.1 Hz, 2H), 7.67 (d, *J* = 8.8 Hz, 2H), 7.58 - 7.46 (m, 4H), 7.07 (s, 1H), 6.94 (d, *J* = 8.8 Hz, 2H), 5.88 - 5.72 (m, 1H), 5.05 - 4.86 (m, 2H), 4.54 - 4.46 (m, 2H), 4.32 - 4.22 (m, 2H), 4.00 (t, *J* = 6.5 Hz, 2H), 2.08 - 1.98 (m, 2H), 1.85 - 1.72 (m, 2H), 1.54 - 1.20 (m, 14H).

10-(4-(10-Undecenyloxy)phenyl)ethynyl-9-anthraldehyde (**27**). A solution of 72 mg (0.14 mmol) of **26** and 5 mg (0.026 mmol) of *p*-toluenesulfonic acid monohydrate in 20 ml of acetone was stirred for 4 h at room temperature. The reaction mixture was poured into water, extracted with CH_2Cl_2 (3×50 ml), the organic fraction was washed with water (3×100 ml), saturated $NaHCO_3$ (3×30 ml), and dried over anhydrous Na_2SO_4. After concentration in vacuo, the crude product was purified by column chromatography on silica gel (eluent chloroform –

hexane 1:1) to yield 55 mg (60%) of **27** as a yellow solid, R_f 0.64, mp 112-115 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.51 (s, 1H), 8.97 (d, *J* = 8.8 Hz, 2H), 8.77 (d, *J* = 8.5 Hz, 2H), 7.79 - 7.60 (m, 6H), 6.98 (d, *J* = 8.6 Hz, 2H), 5.87 - 5.73 (m, 1H), 5.03 - 4.86 (m, 2H), 4.04 (t, *J* = 6.5 Hz, 2H), 2.10 - 2.00 (m, 2H), 1.88 - 1.77 (m, 2H),1.58 - 1.18 (m, 12H). HRMS *m/e* 475.2648 $[M+H]^+$ (calcd for $C_{34}H_{35}O_2$ 475.2637). UV ($CHCl_3$) λ_{max} 432 nm ($\varepsilon = 1.13\times10^4$), fluorescence ($CHCl_3$) λ_{max} 518 nm ($\Phi = 0.02$).

10-(4-(11-(Triethoxysilyl)undecyloxy)phenyl)ethynyl-9-anthraldehyde (2**8**). One drop of platinum(0)-1,3-divinyl-1,1,3,3-tetramethyl-disiloxane complex (Karstedt's catalyst, 0.1 M solution in poly(dimethylsiloxane)) was added to a solution of **27** (55 mg, 0.011 mmol) in 6 ml of toluene and stirred at room temperature for 10 min followed by addition of triethoxysilane (0.20 ml, 1.15 mmol). The resulting mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated in vacuo, dissolved in toluene, filtered through a 0.22 mm PTFE filter, and concentrated in vacuo to afford **28** in quantitative yield as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$), δ 11.52 (s, 1H), 8.98 (d, *J* = 8.6 Hz, 2H), 8.77 (d, *J* = 8.3 Hz, 2H), 7.80 - 7.60 (m, 6H), 6.98 (d, *J* = 7.8 Hz, 2H), 4.03 (t, *J* = 6.6 Hz, 2H), 3.90 - 3.85 (m, 6H), 2.10 - 0.75 (m, 29H).

4-Aminophenylacetylene 40 was prepared from the reaction of 4-iodoaniline (8.70 g, 39.73 mmol) with TMS-acetylene (3.19 g, 43.70 mmol) in the presence of $Pd(PPh_3)_2Cl_2$ (545 mg, 0.78 mmol) and CuI (275 mg, 1.43 mmol) in 75.0 mL of THF:*i*Pr_2NH(2:1) upon stirring under air-free conditions at 40 ^{0}C for 12 h. After allowing to cool down to the room temperature, the reaction mixture was poured into water and extracted with ether three times. Organic fractions were combined and washed with brine and dried over anhydrous Na_2SO_4. After concentration *in vacu*, it afforded brown oil, which was redissolved in dichloromethane, filtered through a short plug of silica gel and concentrated to afford a yellow solid **39**, which was used

without further purification. It was dissolved in 60.0 mL of methanol and vigorously stirred with potassium carbonate (13.52 g, 97.83 mmol) for 12 h at room temperature. The reaction mixture was poured into a saturated solution of ammonium chloride and extracted with ethyl acetate. The organic fractions were combined, washed with brine, and dried over anhydrous Na_2SO_4. It was then concentrated in vacuo and filtered through silica gel using EtOAc as eluent to afford 3.35 g (72%) of **40** as a brown solid compound, (mp. 99-100^{o}C, lit. mp 104-105 ^{0} c[144]). ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.29 (d, *J*=8.6 Hz, 2H), 6.65 (d, *J*=8.7 Hz, 2H), 3. 85 (s, br, 2H), 2.95 (s, 1H).

11-Isocyanoatoundec-1-ene (37). Diphenylphosphoryl azide (15.0 g, 11.75 mL, 54.44 mmol) was added dropwise to the mixture of ω-undecenoic acid (10.03 g, 54.44 mmol), triethylamine (5.44 g, 7.5 mL, 53.81 mmol) in 50.0 mL of acetonitrile and the reaction mixture was stirred at 50^{o}C for 2 h. After concentration in *vacuo,* the crude product was dissolved in 50.0 mL of CH_2Cl_2, diluted to 2.0 L volume with hexanes under nitrogen, and allowed to settle until thick oil formed at the bottom of the solution. The hexane solution was decanted off and concentrated and the residue subjected to Kugelrohr distillation (95^{o}C, 10 mmHg), resulting in 7.32 g (74 %) of the compound **37** as a colorless oil. ^{1}H NMR (250 MHz, $CDCl_3$), δ 5.88-5.72 (m, 1H) 5.03-4.90 (m, 2H), 3.28 (t, *J* = 6.9 Hz, 2H), 2.08-2.0(m, 2H), 1.66-1.55 (m, 2H), 1.31 (s, 10H).

Triethoxy-11-Isocyanatoundecenylsilane (38). A reaction mixture of 3.93 g (21.69 mmol) of **37** was stirred at r.t with 1.0 mL (0.1M) Karstedt's catalyst solution for 10 min. 5.74g (6.45 mL, 43.37 mmol) of triethoxysilane was added dropwise to the resulting solution and stirred for 12 h under air-free conditions. After concentration in *vacuo*, the oily residue was subjected to Kugelrohr distillation (80^{o}C, 0.10 mmHg) to yield 4.42 g (59%) of the compound **38** as a colorless oil. ^{1}H NMR (250 MHz $CDCl_3$,) δ 3.75 (q, *J* = 6.9 Hz, 6H), 3.02 (t, *J* = 6.6 Hz, 2H), 1.55-1.51 (m, 2H), 1.44-1.22 (m, 21H), 0.53-0.57 (m, 2H).

5-Iodofluorescein (35). 5-aminofluorescein (1.0 g, 2.88mmol) at was stirred with 10 mL of 12N HCl at 0 ^{0}C for 2 min. followed by dropwise addition of a solution of 0.25 g (3.6 mmol) of $NaNO_2$ in 5.0 mL of water. In 2 minutes, after the addition was complete, 4.78 g (0.29 mol) of KI in 8.0 mL of water was added to drop wise at 0 ^{0}C. Ice bath was removed and stirred for 1.0 h at room temperature. After extraction with a mixture of 2-propanol and chloroform (1:3) three times, the organic fractions was washed with $Na_2S_2O_3$ solution and dried over anhydrous Na_2SO_4. After concentration in *vacuo*, the crude product was purified by column chromatography on silica gel (eluent 10% MeOH in CH_2Cl_2) A fraction with R_f 0.34 afforded 1.2 g (92%) of **35** as reddish solid**,** mp. 334-336^0 C (lit. mp. 323-325 ^{0} C [147]). ^{1}H NMR (400 MHz, DMSO-D_6), δ 8. 10.12 (s, br, 2H), 8.29 (s, 1H), 8..08 (m, 1H,) 7.08-7.04 (m,1H,), 6.66-6.54 (m, 6H)**.**

TMS protected amine compound 30 was prepared following the general procedure **5.1.2** The mixture of 293 mg (0.23 mmol) of the iodide compound **10**, 32 mg (0.28 mmol) of 4-ethynylaniline, 23 mg (0.023 mmol) of $Pd(PPh_3)_4$, 1 grain of CuI in 8.00 mL of toluene:*i*Pr_2NH (7:3) was stirred under argon at 55 ^{0}C for 36 h. After cooling down to room temperature, concentration in *vacuo*, the crude product was purified by column chromatography in silica gel (eluent: hexane-chloroform (1:1). A fraction with R_f 0.6, afforded 130 mg (45%) of the pure product as greenish sticky solid **30**, mp. 130-134 ^{o} C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (m, 10H), 6.65 (d, *J*=8.52 Hz, 2H) 3.82 (s, 2H), 2.85-2.72 (m, 16H), 1.71-1.69 (m, 16H), 1.41-1.32 (m, 48H), 0.92-0.87 (m, 24H), 0.26 (s, 9H).

Acetylene compound 32 was prepared following the general procedure **5.1.3.** To a stirred solution of 13 mg (0.24 mmol) of KOH in 10 mL of MeOH, a solution of 100 mg (0.08 mmol) of compound **30** in 20 mL of THF was added dropwise at room temperature. After stirring for 1 h at room temperature the reaction mixture was poured into water, extracted with

CH_2Cl_2, washed with water and brine, dried over anhydrous Na_2SO_4. After concentration in *vacuo*, the crude product was purified by column chromatography on silica gel, eluent: hexane-chlorofoem (1:1). A fraction with R*f* 0.54 afforded 80 mg (85%) of the compound **32** as a green solid, mp. 118-121 ^{0} C. ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.35-7.24 (m, 10H), 6.63 (d, 2H, *J*=8.5Hz,) 3.38 (s, 2H), 3.75-3.70 (m, 1H) 2.82-2.70 (m, 16H), 1.68-1.55 (m, 16H), 1.30-1.28 (m, 48H), 0.92-0.87 (m, 24H).

Amine Compound 33 was prepared following the general procedure **5.1.2.** A mixture of 110 mg (0.92 mmol) of the amino compound **32**, 63.0 mg (0.14 mmol) of 5-iodofluorescein **35**, 11 mg (9.2 mmol) of $Pd(PPh_3)_4$, and 1 small grain of CuI in 6.0 mL of anhydrous DMF and 4.0 mL of *i*Pr_2NH was stirred for 36 h under argon at 60 ^{0}C. The reaction mixture was cooled down to room temperature and passed through a short plug of silica gel using chloroform as an eluent. The solution was concentrated in *vacuo* and further purified by chromatography in silica gel (eluent 10% Methanol in CH_2Cl_2. A fraction with R_f 0.42 afforded 86 mg (61%) of the pure compound **33** as a reddish brown solid, mp. 248-254 ^{0}C. ^{1}H NMR (400 MHz, $CDCl_3$), δ 8.23 (s, 2H), 7.74-7.37 (m, 12H), 7.02-6.98 (m, 2H) 6.66-6.60 (m, 4H), 2.84-2.82 (m, 16H), 1.71-1.69 (m, 16H), 1.41-1.32 (m, 48H), 0.92-0.87 (m, 24H).

Triethoxy Compound 34 A mixture of 46.2 mg (0.03 mmol) of compound **33** and 10.5 mg (0.03 mmol) of **38** of triethoxy(10-isocyanatoundecyl)silane, in DMF was stirred for 4 h at 80 ^{0}C. The reaction was cooled down to room temperature. After concentration in *vacuo*, the crude product was further purified by chromatography using silica gel (eluent: ethyl acetate). It was concentrated in vacuum, afforded 28.2 mg (26%) of the compound **34** as a reddish brown solid. ^{1}H NMR (400 MHz, $CDCl_3$), δ 8.13 (s, 1H), 7.74--7.34 (m, 18H), 7.19-7.13 (m, 6H), 6.86-6.49 (m, 4H), 5.09-5.05 (m, 1H), 4.86-4.77 (m, 1H), 3.84-3.79 (m, 6H), 3.30-3.21(m, 4H), 2.85-2.82 (m, 16H), 2.84-2.70 (m, 16H), 1.72-1.69 (m, 16H), 1.42-1.25 (m, 57H), 0.90-0.82 (m, 24H).

Triethoxy Compound 36. A mixture of 40 mg (0.115 mmol) of 5-aminofluorescein **35** and 40 mg (0.115 mmol) of triethoxy(11-isocyanatoundecyl) silane was stirred at 80 ^{0}C in DMF for 4 h. A small portion of the mixture was concentrated and dried and NMR was taken. With the exception of few peaks corresponding to triethoxy(10-isocyanatoundecyl)silane ^{1}H NMR was consistent with the compound **36**, as brown solid. ^{1}H NMR (400 MHz DMSO-D_6), δ 10.07 (s, 2H), 7.95 (s, 1H), 7.0-6.9 (m, 1H), 6.97-6.52 (m, 6H), 6.51 (s, 1H), 5.79-5.72 (m, 2H), 3.84-3.33 (m, 6H), 3.54-3.51 (m, 1H), 1.5-0.99 (m, 23H), 0.52-0.49 (m, 2H).

4-(10-bromoanthracen-9-yl)aniline (44). A mixture of 3.0 g (7.86 mmol) of 9-bromo-10-iodoanthracene, 1.89 g (8.65 mmol) of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline, 8.67 g, (62,88 mmol) of K_2CO_3, 0.09 g (0.078 mmol) of $Pd(PPh_3)_4$ was stirred in the mixture of 60.0mL toluene 12.0 mL EtOH, 24.0 mL water for 15 h at 85 ^{0}C in argon. The reaction mixture was cooled down to room temperature and extracted with CH_2Cl_2. The organic fraction was washed successively with water, brine and dried over anhydrous Na_2SO_4. After concentration in *vacuo*, the crude product was purified by column chromatography using CH_2Cl_2 as eluent. A fraction with R_f 0.5 afforded 2.2 g (81%) of the product **44** as a yellow crystalline solid, mp. 152-154 ^{0} C. ^{1}H NMR ($CDCl_3$), 400 MHz, δ 8.58 (d, 2H, *J*= 8.8 Hz), 7.75 (dd, 2H, J_1 = 8.8Hz, J_2 = 8.7Hz), 7.57-7.54 (m, 2H), 7.38-7.34 (m, 2H), 7.16 (dd, 2H, J_1 = 7.2 Hz, J_2 =7.1 Hz), 6.87 (d, 2H, *J* = 8.4 Hz), 3.84 (s, br, 2H).

N-(N-Boc-2-aminoethyl)-4-(10-bromoanthracene-9-yl)aniline (45). A mixture of 1.0 g (2.84 mmol) of the compound **44** and 452 mg (2.84 mmol) of tert-butyl-2-oxoethylcarbamate in 10 mL of CH_2Cl_2 was stirred at room temperature under nitrogen for 20 min. This was followed by the addition of 178 mg (4.3 mmol) of $NaBH_3CN$ and 65 mL of glacial AcOH was stirred for 6 hours at room temperature. Then it was poured into water, extracted with CH_2Cl_2, the organic fraction was washed three times with large volume of water and then washed with 10% NaOH

and brine, dried over anhydrous Na_2SO_4. After concentrated in *vacuo*, the crude product was purified chromatography in silica gel. A fraction with R_f 0.52 afforded 1.2 g (86%) of **45** as a yellow crystalline solid**,** mp. 163-166 ^{0} C. ^{1}H NMR (400 MHz, $CDCl_3$), δ 8.58 (d, 2H, *J*= 8.8 Hz), 7.75 (dd, 2H, J_1 = 8.8Hz, J_2= 8.9 Hz), 7.57-7.54 (m, 2H), 7.38-7.34 (m, 2H), 7.16 (dd, 2H, J_1 = 7.2 Hz, J_2 = 6.9Hz), 6.87 (d, 2H, *J* = 8.36 Hz), 5.3 (s, 1H), 3.59 (s, 1H), 3.48-3.40 (m, 2H), 3.1-2.9 (m, 2H), 0.88 (s, 9H).

Amine Compound (46). To a stirred solution of 0.773 g, (1.15 mmol) of **45** in 20.0 mL of CH_2Cl_2, TFA (2.0 mL) was added dropwise and the resulting mixture was stirred for 2 h at room temperature. After concentration in *vacuo* to remove TFA, the crude product was dissolved in CH_2Cl_2, successively washed with water, 20% aq. NaOH and brine, dried over anhydrous Na_2SO_4. After concentration in *vacuo*, the crude product was purified by chromatography in silica gel (eluent: CH_2Cl_2). A fraction with R_f 0.54 afforded 506 mg (58%) **46** as a yellow crystalline solid, mp. 171-173 ^{0} C. ^{1}H NMR (400 MHz, $CDCl_3$), δ 8.58 (d, 2H, *J* = 8.8 Hz), 7.81 (dd, 2H, J_1 = 8.8Hz, J_2 = 8.3 Hz), 7.59-7.54 (m, 2H), 7.38-7.34 (m, 2H), 7.20 (d, 2H, *J* = 6.9 Hz,) 6.84 (d, 2H, *J* = 8.4 Hz), 3.49 (s, 2 H), 3.33 (t, 2H, *J* = 5.5 Hz), 3.07 (t, 2H, *J* = 6.0 Hz).

Zn Receptor 47. A mixture of 441 mg (1.13 mmol) of **46**, 483 mg (4.52 mmol) of 2-pyridinecarboxaldehyde, 7.18 g (3.4 mmol) of $NaBH(OAc)_3$ in 30 mL of CH_2Cl_2 was stirred under nitrogen at room temperature for 12 h. After extraction with CH_2Cl_2 it was washed successively with of water, 20% aq. NaOH, and brine, dried over anhydrous Na_2SO_4. After concentration in *vacuo*, the crude product was purified by recrystallization from hexane -CH_2Cl_2 mixture to afford 420 mg (36%) of **47** as a yellow solid, mp. 177-180 ^{0}C. ^{1}H NMR (400 MHz, $CDCl_3$), δ 8.62-8.55 (m, 4H), 7.81-7.12 (m, 14H), 6.70 (d, 2H, *J* = 8.4 Hz), 5.31(s, 1 H), 3.81 (t, 1H, *J*=5.5Hz), 3.33 (t, 2H, *J* = 5.5Hz), 3.07 (t, 2H, *J* = 6.0Hz).

Zn Sensor Compound 41. A mixture of 61 mg (0.045 mmol) of **13**, 64 mg (0.11 mmol) of **47**, 20 mg (0.02 mmol) of $Pd(PPh_3)_4$, 1 grain of CuI, and 10 mL of toluene: *i*Pr_2NH (7:3) was stirred for 36 hr under air-free conditions at 70 ^{0} C. The reaction mixture was cooled down to room temperature, concentrated under *vacuo*, the crude mixture was purified by chromatography in silica gel (eluent, Hexane-Chloroform 1:1). A fraction with R_f 0.42 afforded 40 mg (45%) of **41** as a dark brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.91-8.85 (m, 1H), 8.71 (d, *J* = 8.8 Hz, 2H), 8.64 (d, *J* = 4.3 Hz, 2H), 8.55-8.52 (m, 1 H), 7.79-7.55 (m, 9H), 7.47-7.34 (m, 14H), 7.20-7.16 (m, 4H), 7.07-6.91 (m, 2H), 6.91-6.86 (m, 2H), 6.72 (d, *J* = 8.52 Hz), 6.59-6.67 (m, 1H), 5.83-5.79 (m, 1H), 5.02-4.92 (m, 2H), 4.01-3.90 (m, 2H), 3.82-3.81 (m, br, 2H), 3.45-3.34 (m, 2H), 3.10-3.06 (m, 2H), 2.85-2.80 (m, 16H), 2.06-2.04 (m, 2H), 1.92-1.55 (m, 16 H), 1.57-1.219 (m, 76 H), 0.99-0.04 (m, 24H).

Zn Sensor Compound 42. A drop of Karstedt's catalyst was added to a solution of 35 mg (0.02 mmol) of the compound **41** in 10 mL of dry toluene and stirred for 10 min. for catalytic induction in inert atmosphere. To the stirring mixture solution, triethoxysilane 32.8 mg (0.2 mmol, 40 μL) was added and stirred for 6 h at room temperature. The reaction mixture was concentrated in *vacuo*, dissolved in 10 mL of dry toluene under nitrogen and filtered through 0.2 μm TFT filter. The solution was again concentrated to afford compound **42** quantitatively as a reddish brown solid. ^{1}H NMR data shows all peaks of the compound 41 with additional peaks of triethoxy compound. The presence of double bond in the compound indicates that hydroslylation occurred in the alkyne area.

CHAPTER 6. SUMMARY AND FUTURE WORK

6.1. Summary

During the course of these Ph.D. studies, we have designed and developed a novel general platform towards thin-film fluorescent ratiometric chemosensors. The sensor design is centered on a π-electron conjugated oligo(*p*-phenylene ethynylene) (OPE) core with covalently attached receptor fluorophore for specific interaction with a target analyte. The other end of the OPE core is fitted with a triethoxysilyl-functionalized linkage for covalent immobilization on glass surface to facilitate formation of a stable and molecularly ordered monolayer film. The operational principle of the sensor is based on attenuation of the photoexcitation energy transfer between OPE core and terminal receptor caused by electronic perturbations at the receptor site due to the analyte binding. The attenuation of energy transfer results in alteration of intensities ratio of the OPE core and the receptor fluorophore emission bands, i.e. produces the desired ratiometric response. Our initial proof-of-concept studies involved developing a ratiometric sensor for cysteine. Successful preparation of this sensor not only allowed to establish validity of the general strategy, but revealed a number of very unusual (and not clearly understood yet) phenomena such as "turn-on" fluorescent amplification. With this success, we continued further development of this strategy and designed and prepared a pH-responsive sensor. This thin-film sensor showed reliable and reversible ratiometric response in a broad pH range. Detailed studies on this sensor allowed gaining some insight into the fundamental aspects of its functioning, and to obtain additional information to guide further development of this strategy. Based on this information, we attempted to incorporate some additional sensing response triggering mechanisms (such as photoinduced electron transfer) into the sensor design. As an example of

such a sensor, a Zn(II) ratiometric sensor was developed and synthesized. Although, as of now, the Zn(II) sensor did not show expected behavior, it did provide a rational basis for further development. Overall, the outcomes of this dissertation serve as an ultimate proof of the validity of our strategy as a general universal platform, which allows converting practically any small-molecule single-wavelength fluorescent sensor into a thin-film ratiometric fluorescent sensor.

6.2. Future Outlook

We will expand this novel strategy for building a broad variety of practically useful fluorescent sensors. The choice of receptor is only limited by the requirement to have a substantial electronic perturbation upon interacting with analyte, and many suitable receptor/practically important analyte combinations can be envisioned. With a variety of sensors available, we will build microsensor arrays for facile and simultaneous multiple analyte sensing (Figure 6.1). The spatially addressable ensembles of different receptors can be fabricated through direct self-assembly or using one of the SAM nanopatterning techniques. Incorporation of such arrays into practical analytical devices ("lab-on-the-chip") will result in the very powerful, reliable, robust and simple devices for in-field detection.

We will also attempt to self-assemble the molecular sensors on the surface of silica microspheres (or even nanospheres) to create stable and inert microsensors for *in vivo* optical monitoring of bioanalytes (Figure 6.1). A stabilized colloidal suspension of these microspheres may be injected into a living organism's blood stream and will allow for direct monitoring of target analytes in the blood by fluorescence through a fiber optics probe. Furthermore, the molecular sensor can be chemically modified to have absorption and emission in the wavelength range of 600-800 nm. The longer wavelength light penetrates easier through living tissues, with the transparency maximum in the 600-800 nm windows. This will raise the possibility of the

direct monitoring inside living organisms by using laser-based confocal microscopy imaging from outside of the living body.

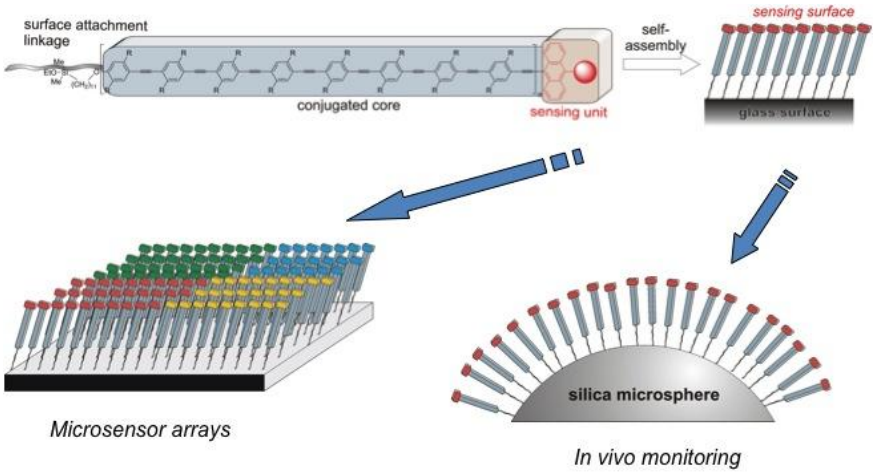


Figure 6.1. Fluorescent microsensor arrays and SAM-functionalized particles – two possible future strategic developments based on the outcomes of this dissertation

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Zinc in Viable Pancreatic Islet Cells Using a Specific Fluorescent Probe for Zinc.
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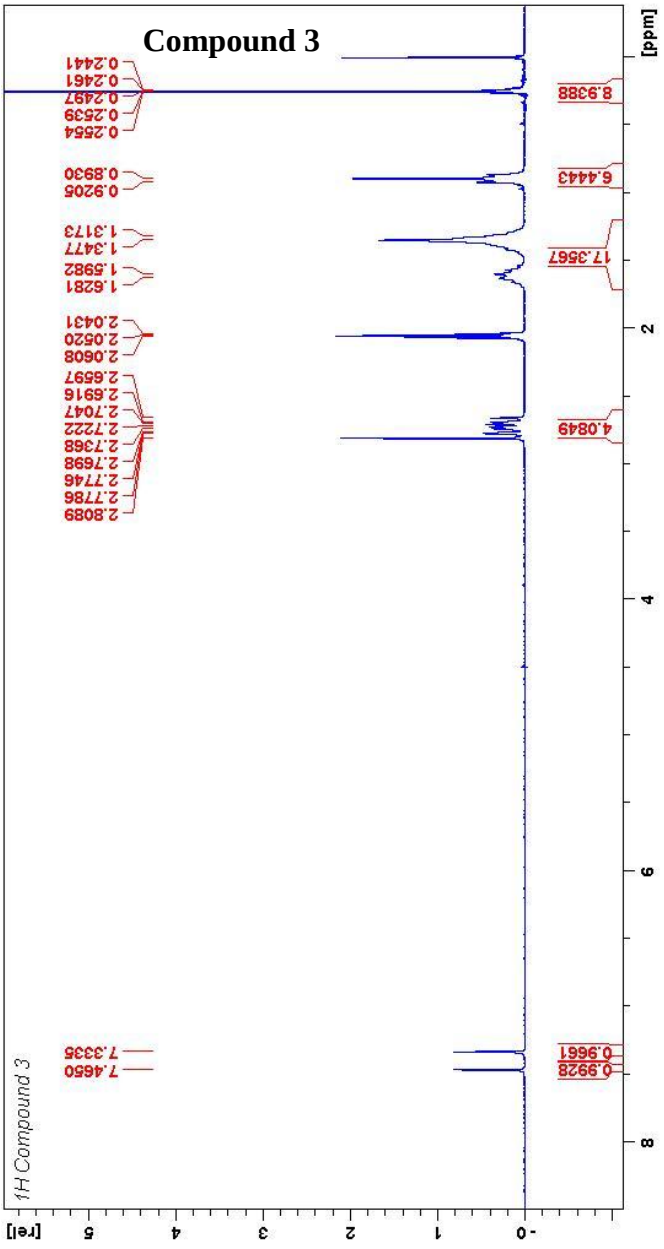
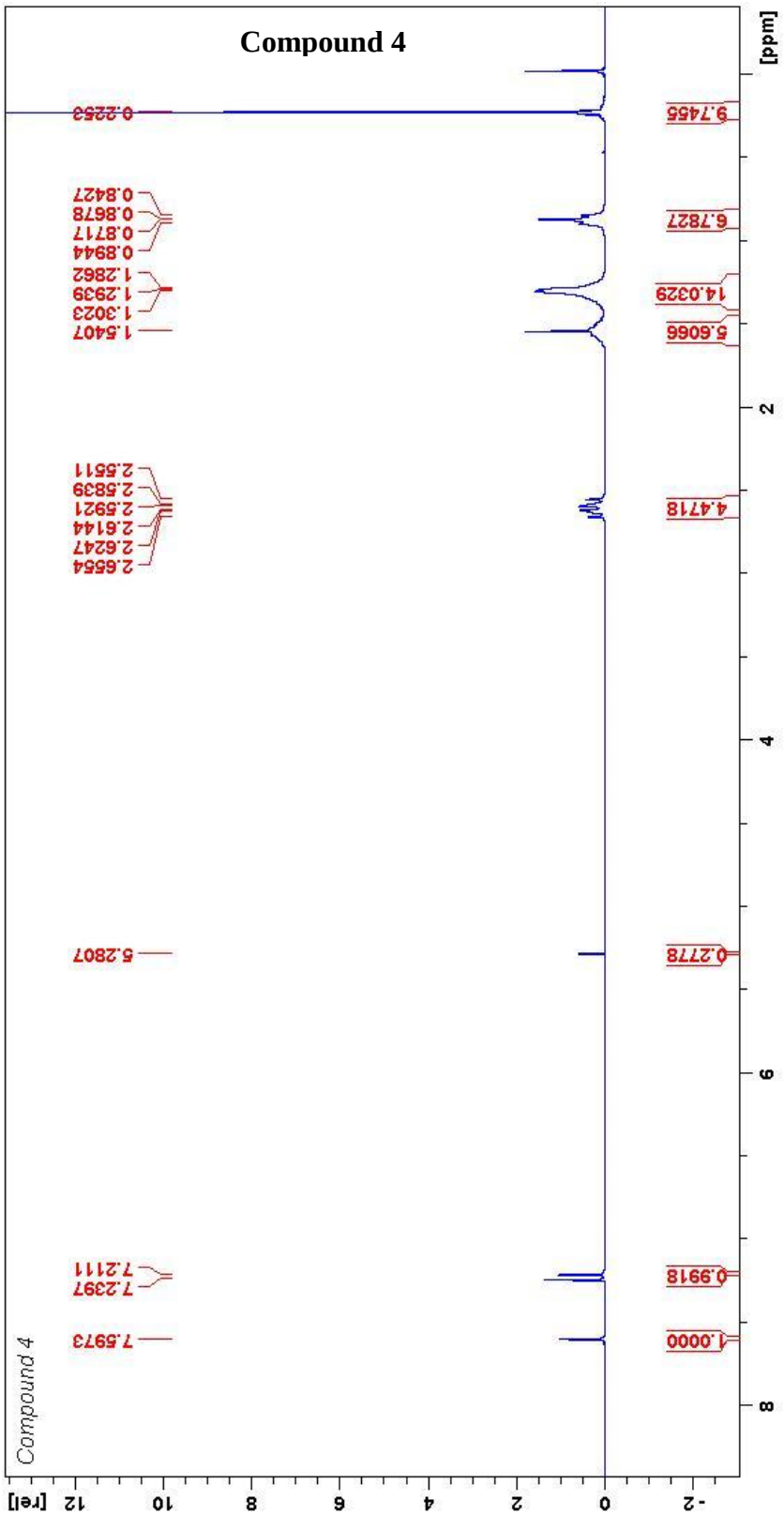
APPENDIX A. ^{1}H NMR SPECTRA

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Compound 4
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5.6066
4.4718
0.2778
1.0000
0.9918
0.2253
0.8427
0.8678
0.8717
0.8944
1.2862
1.2939
1.3023
1.5407
2.5511
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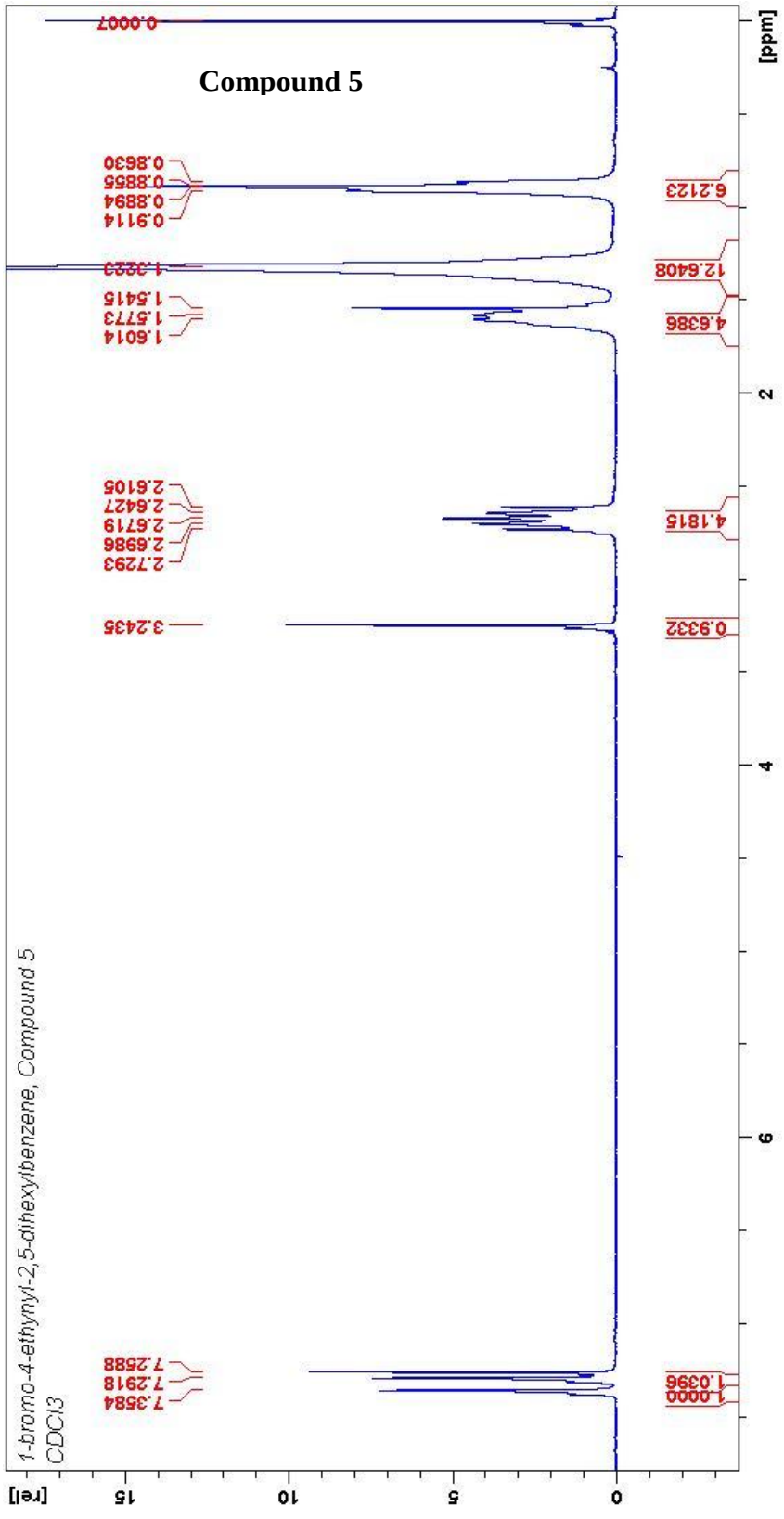
2.5921
2.6144
2.6247
2.6554
5.2807
7.2111
7.2397
7.5973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7.2588
3.2435
2.8105
2.6427
2.6719
2.6986
2.7293
1.6014
1.5773
1.5415
1.3223
0.9114
0.8894
0.8855
0.8630
0.0007
1.0000
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0.9332
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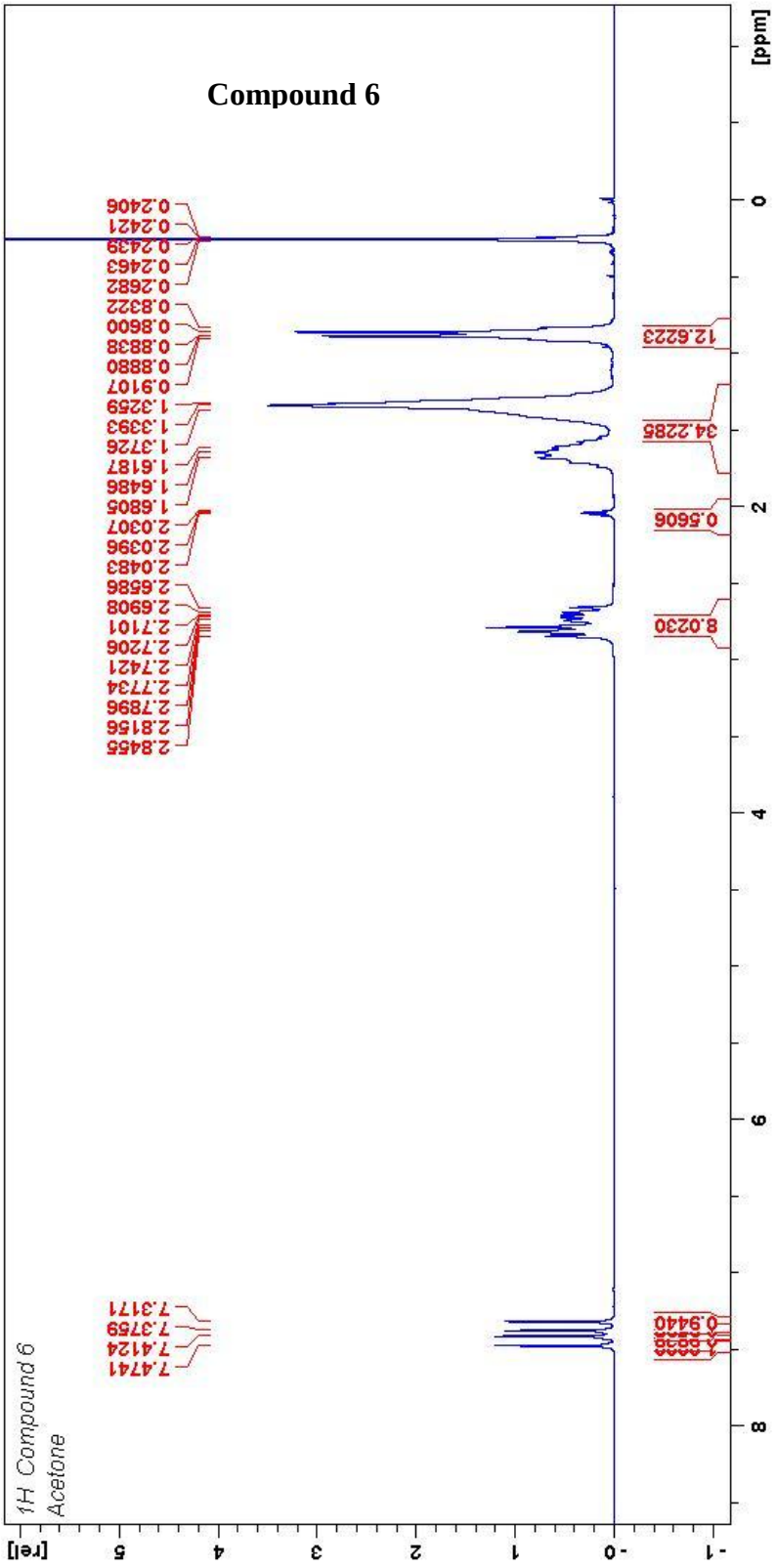
12.6408
4.6386
[rel]
[ppm]

Compound 6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Acetone
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[ppm]
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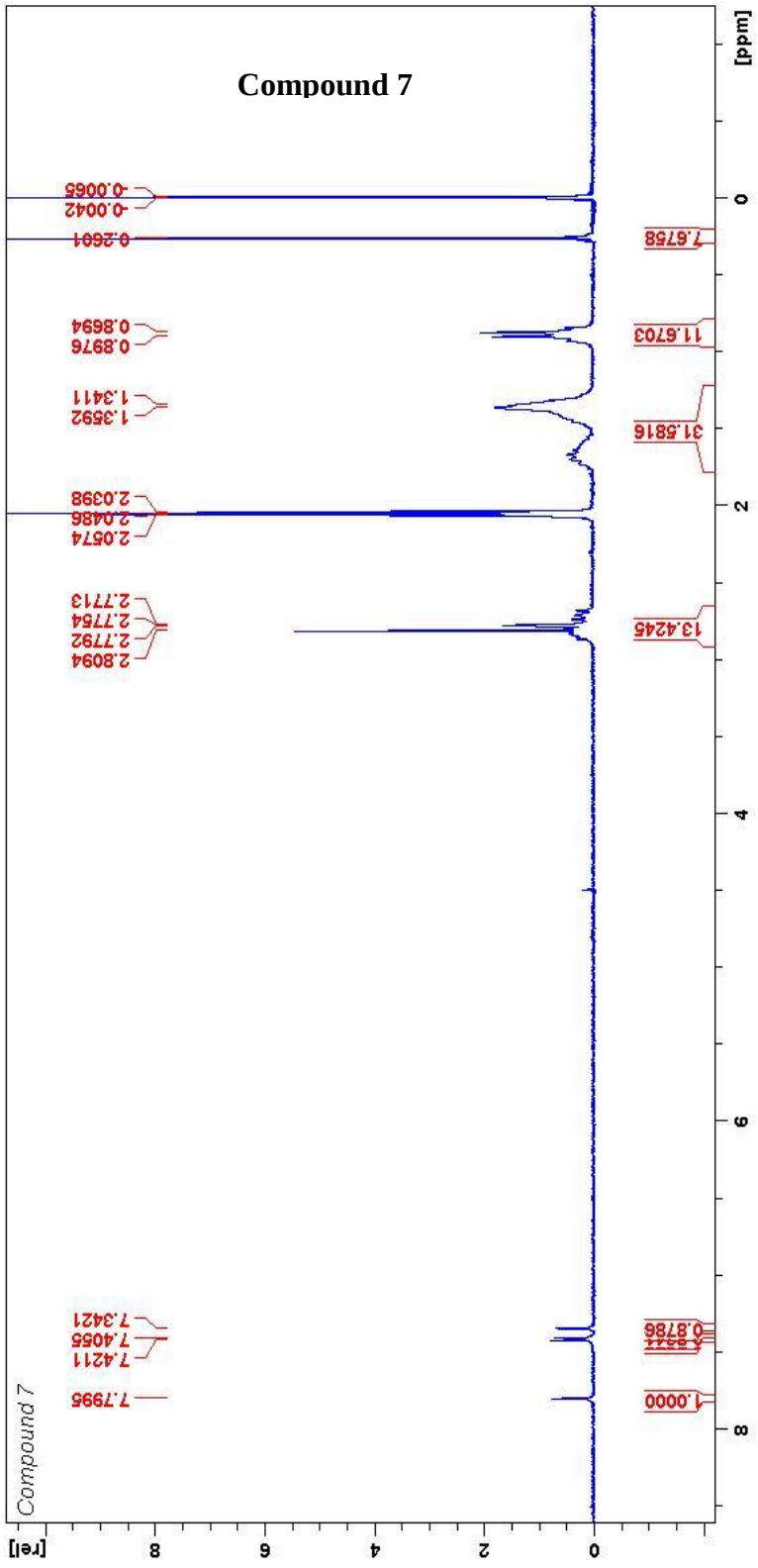
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[ppm]
[rel]
Compound 7
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2.7792
2.7754
2.7713
2.0574
2.0486
2.0398
1.3592
1.3411
0.8976
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1.0000
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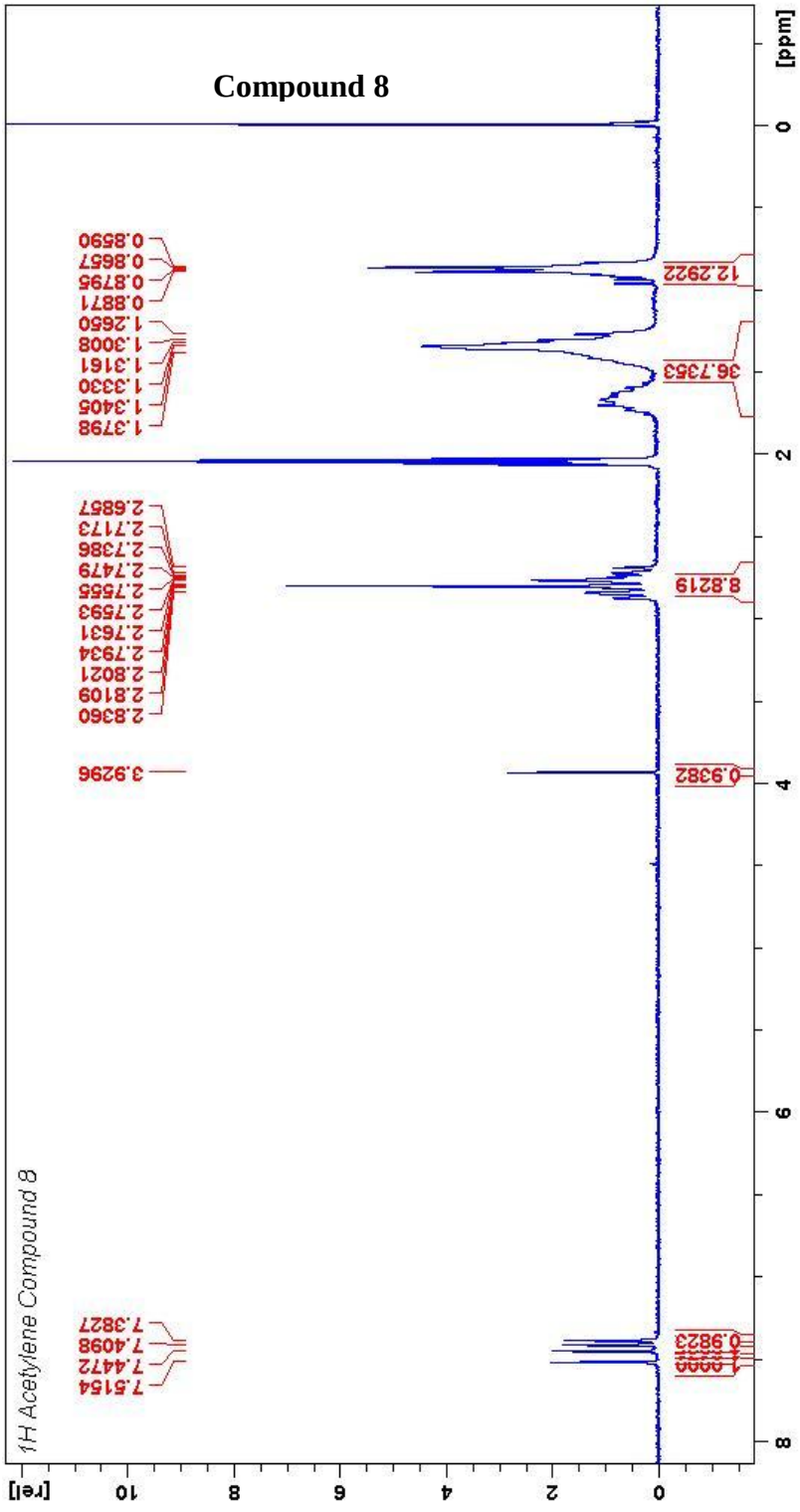
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1H Acetylene Compound 8
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1.0000
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7.4472
7.4098
7.3827
3.9296
2.8360
2.8109
2.8021
2.7934
2.7631
2.7593
2.7555
2.7479
2.7386
2.7173
2.6857
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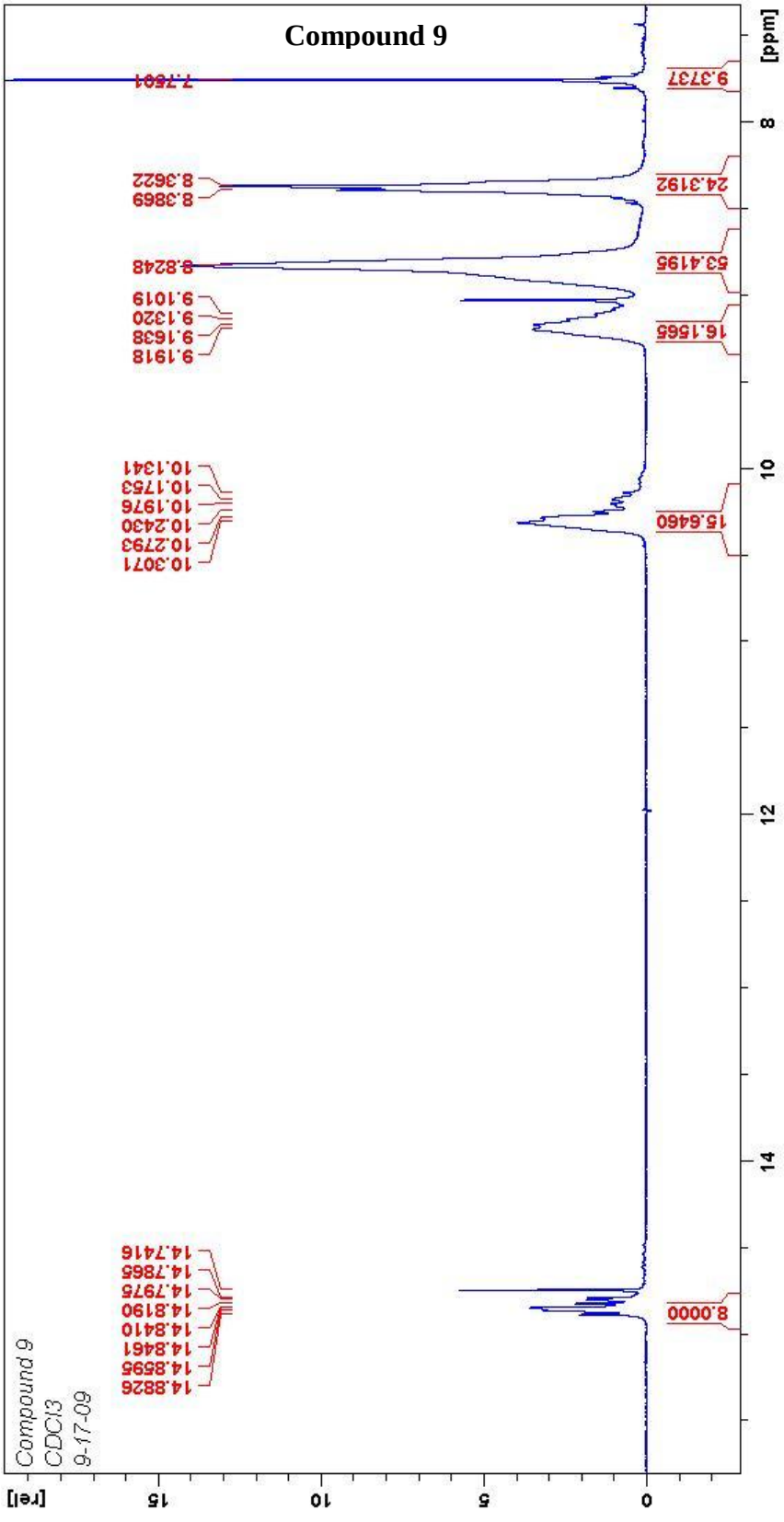
0.8657
0.8590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Compound 9
CDCl3
9-17-09
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9.1918
10.1341
10.1753
10.1976
10.2430
10.2793
10.3071
14.7416
14.7865
14.7975
14.8190
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14.8595
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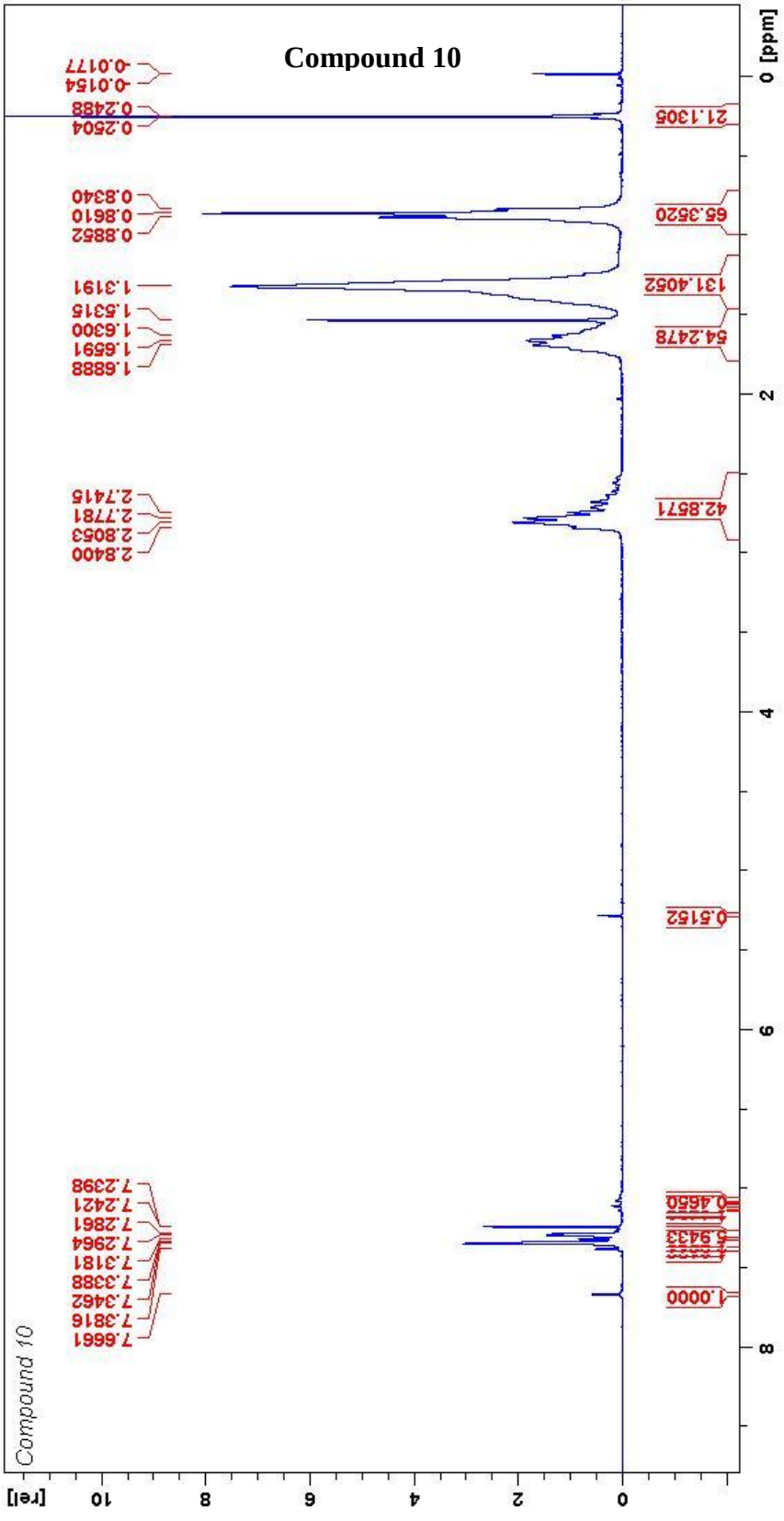
8.0000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Compound 10
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0.8852
1.3191
1.5315
1.6300
1.6591
1.6888
2.7415
2.7781
2.8053
2.8400
7.2398
7.2421
7.2861
7.2964
7.3181
7.3388
7.3462
7.3816
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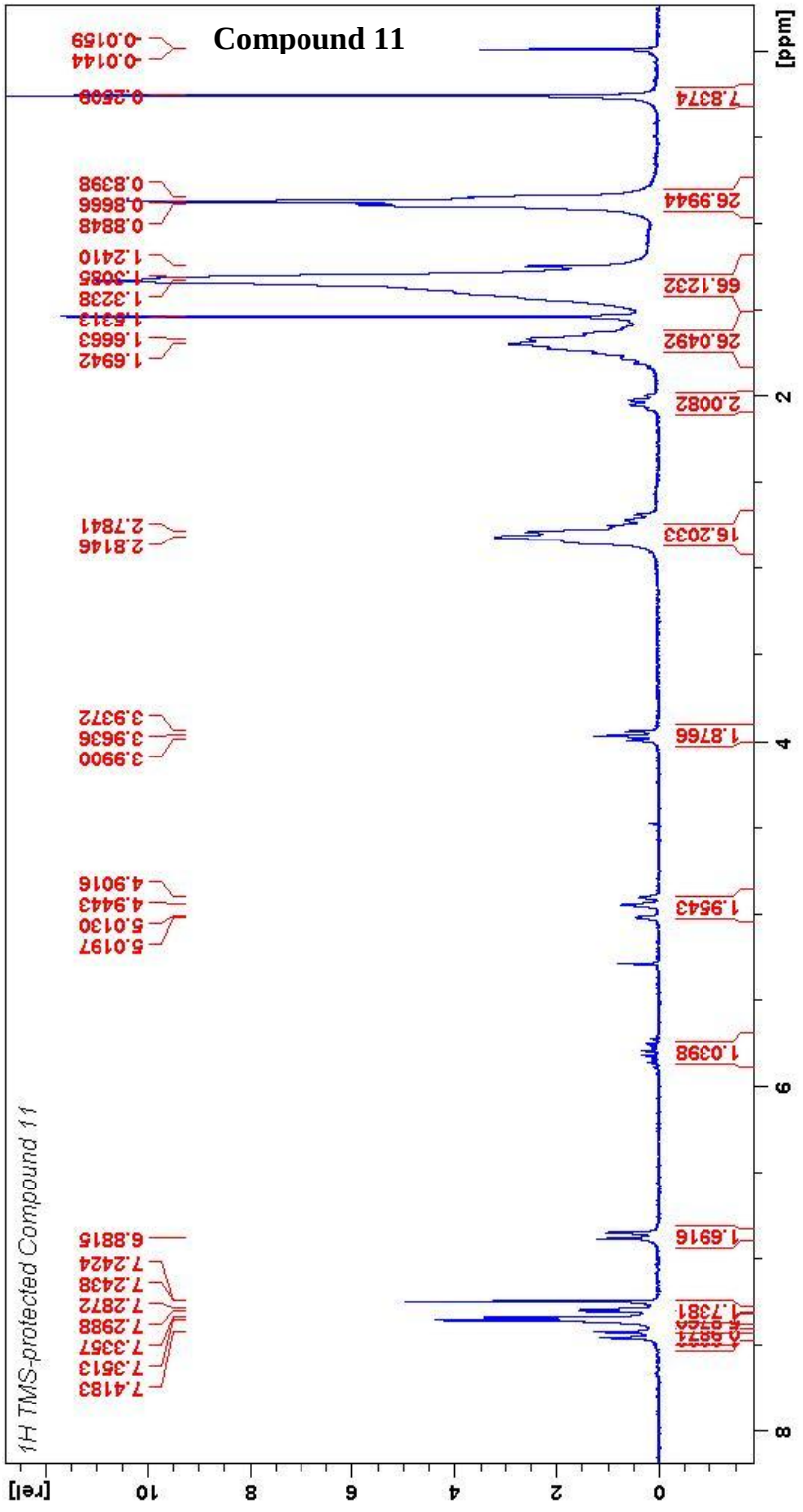
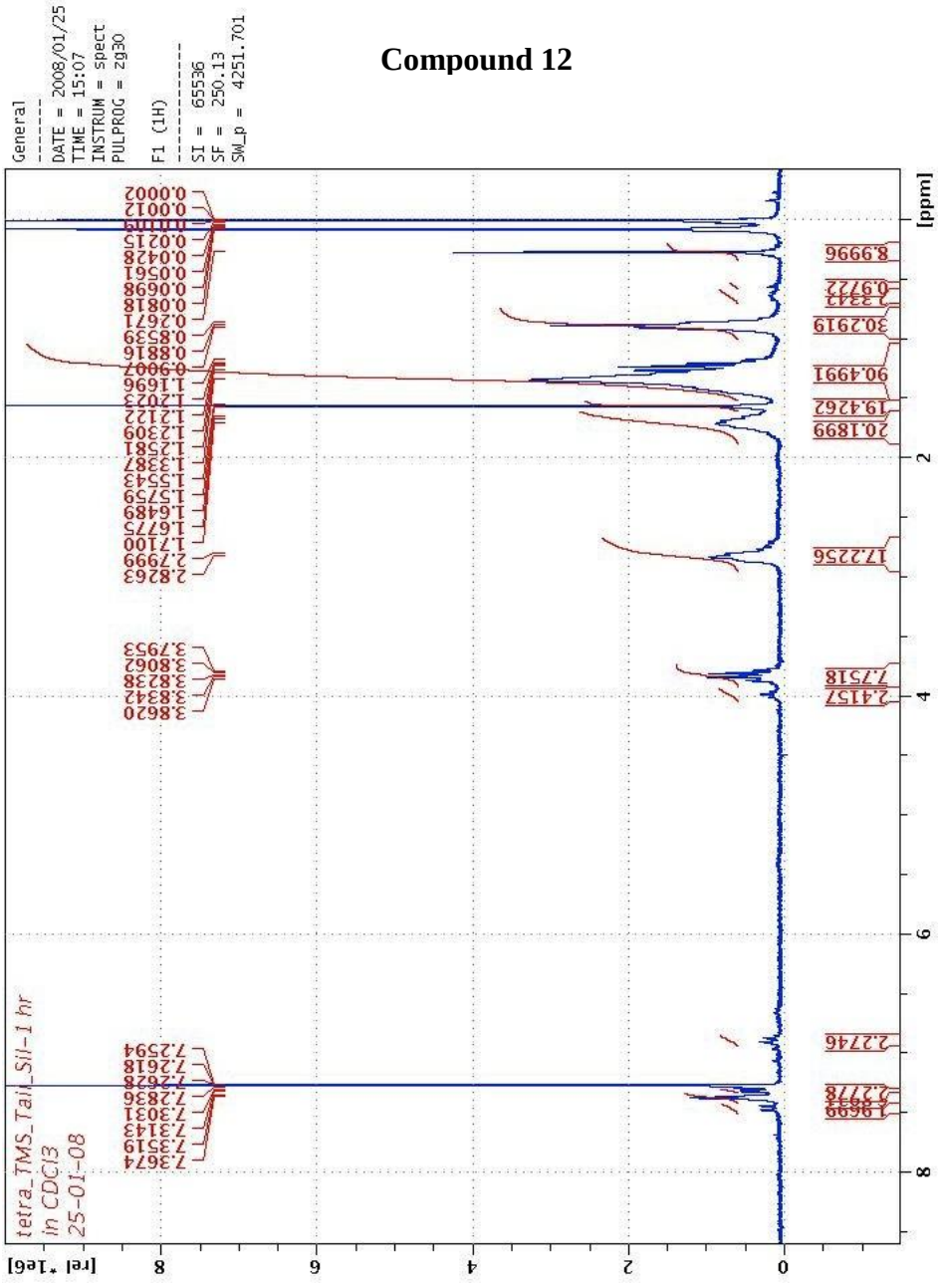
1.0000


Compound 11
1H TMS-protected Compound 11
[rel]
[ppm]

Compound 12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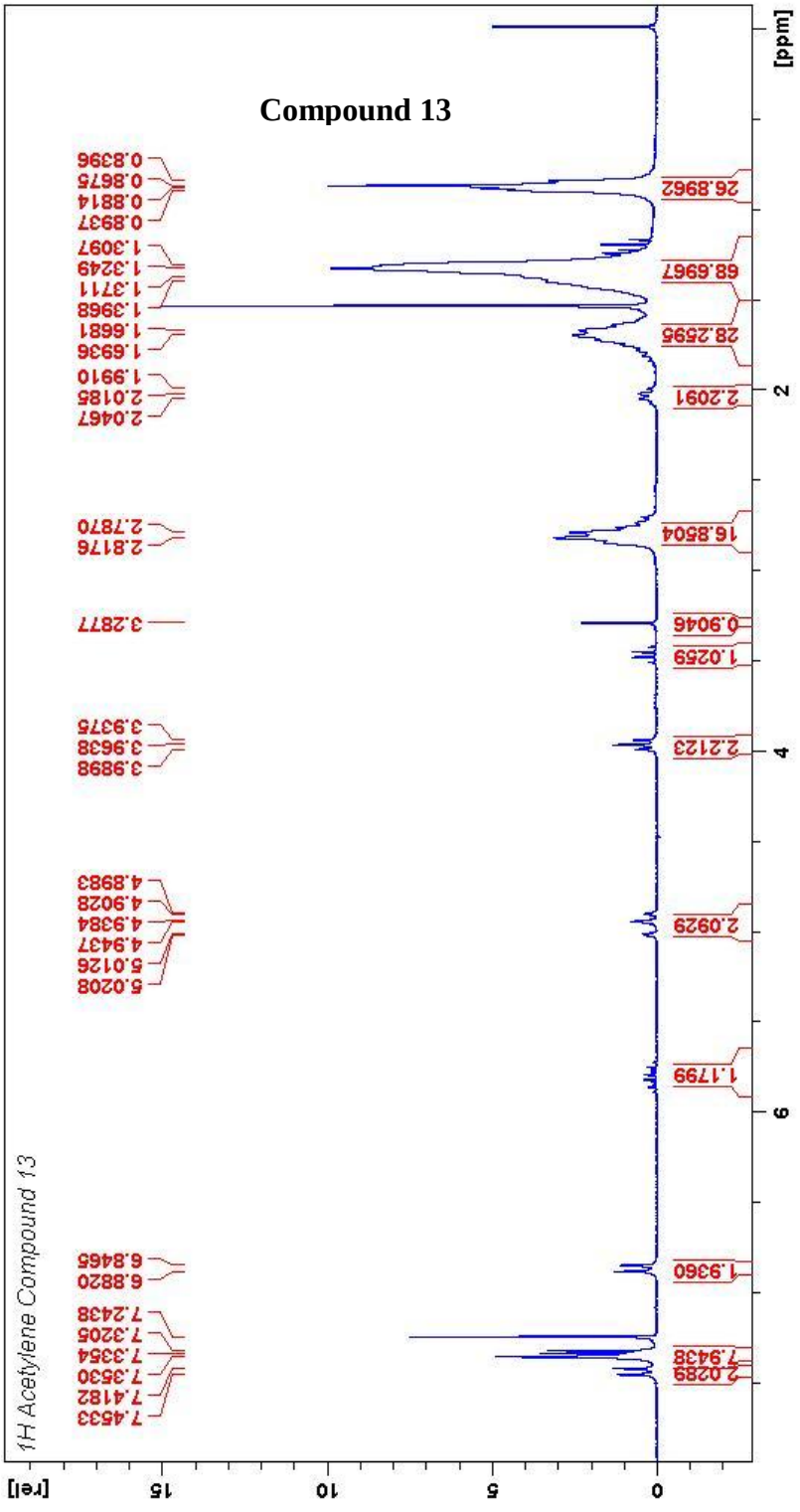
[rel * 1e6]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1H Acetylene Compound 13
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7.9438
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1.1799
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4.9437
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4.9028
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5.0208
5.0126
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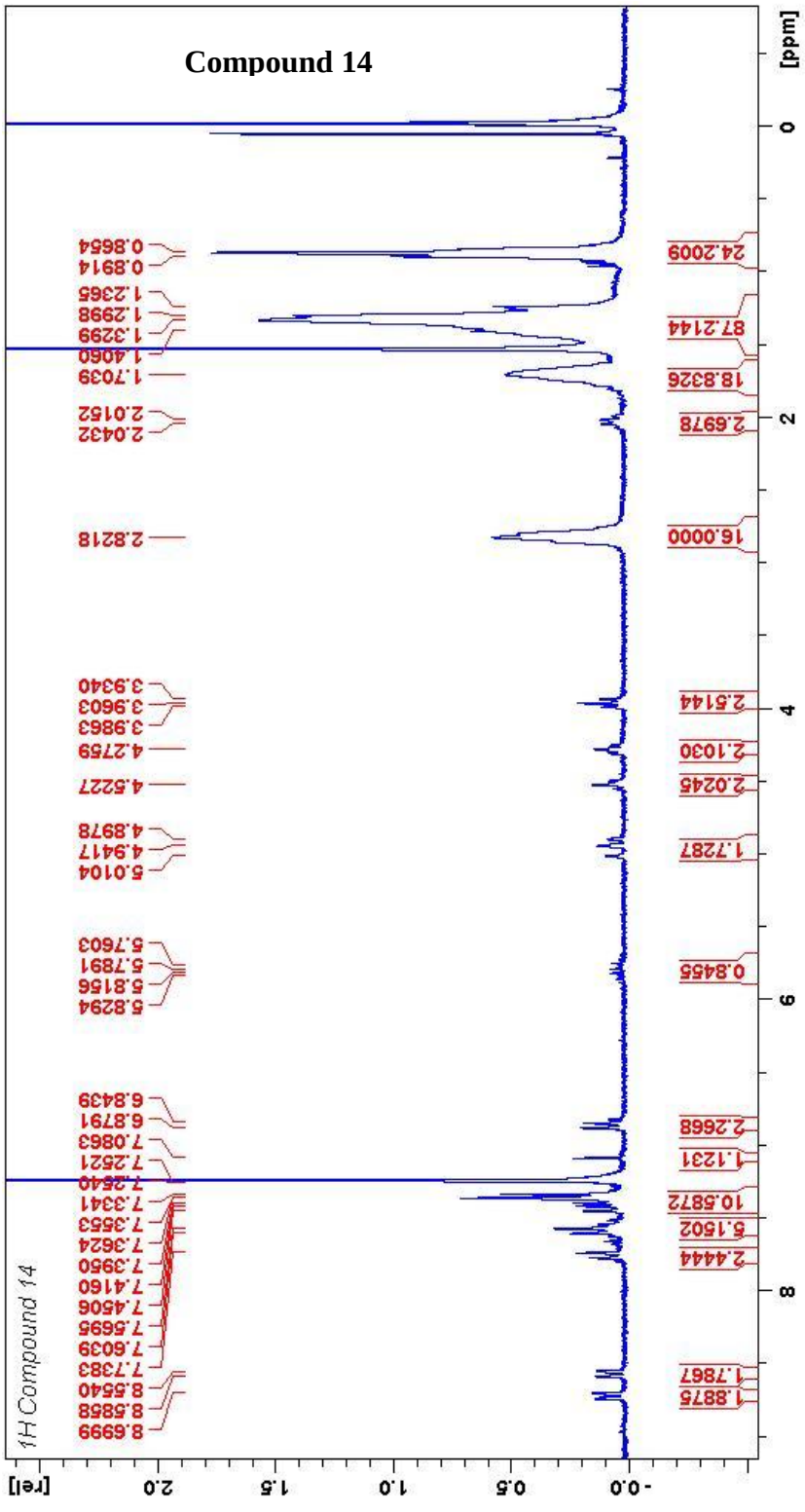
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1H Compound 14
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5.7603
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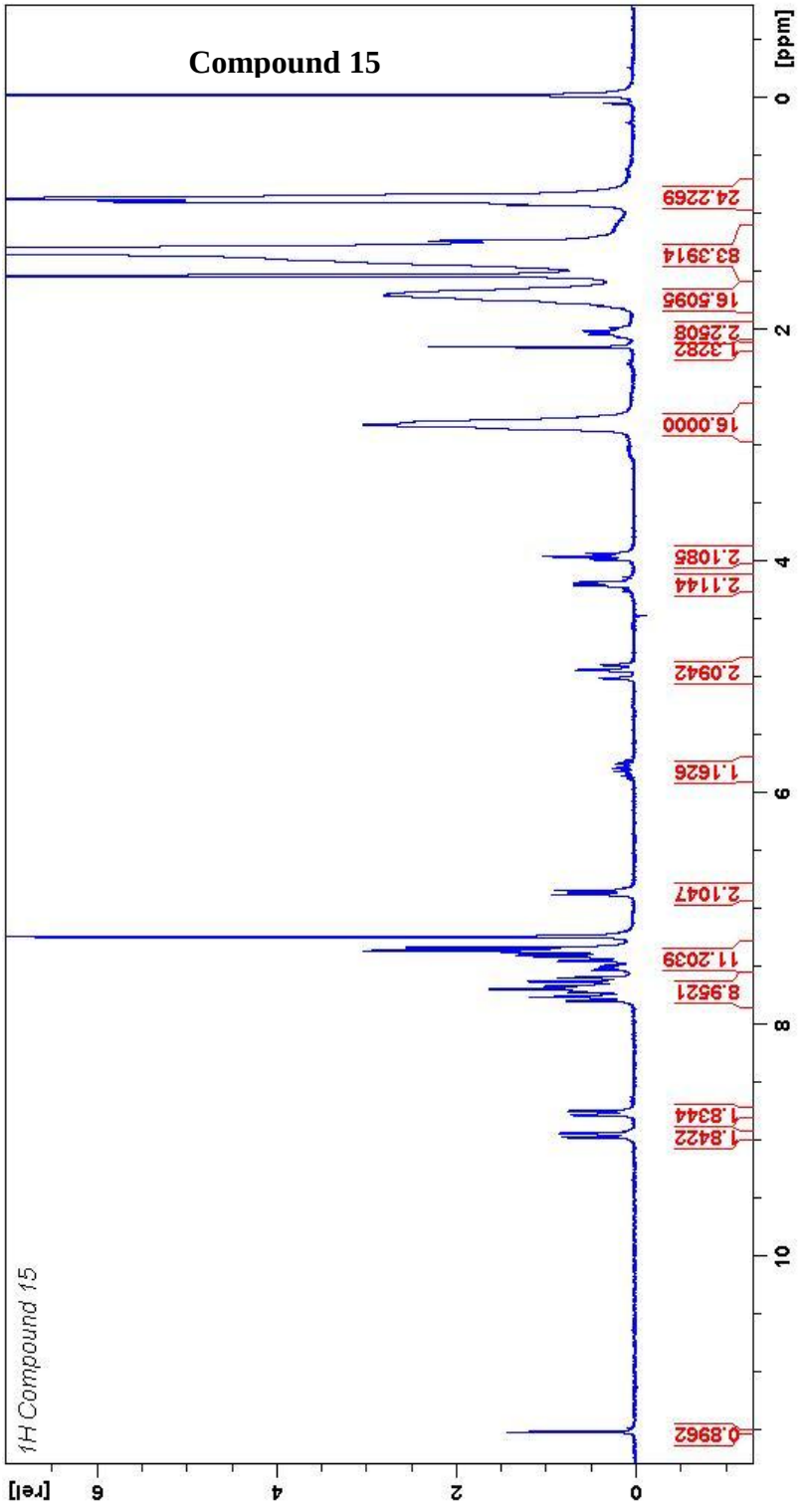
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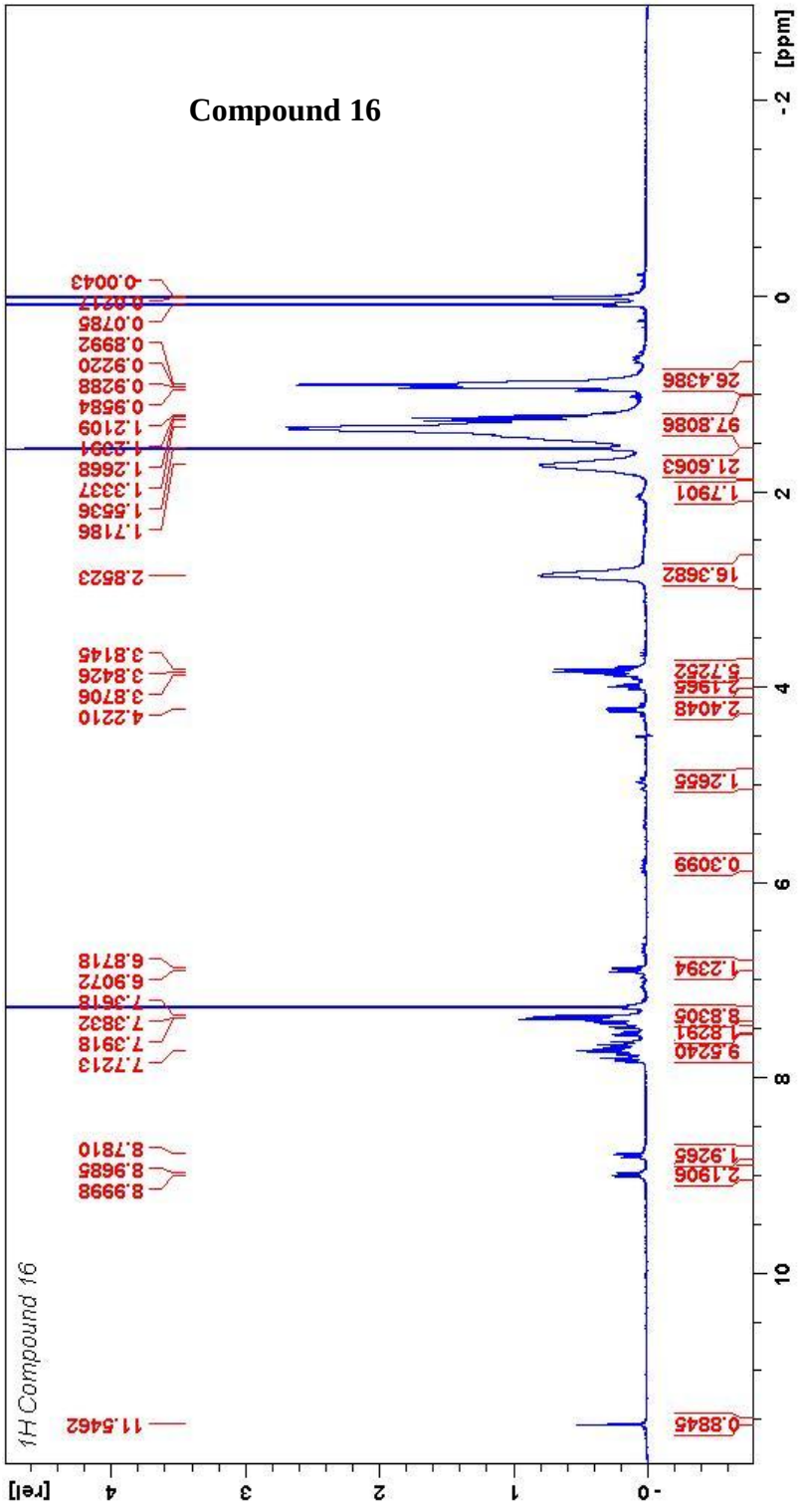
Compound 16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6.8718
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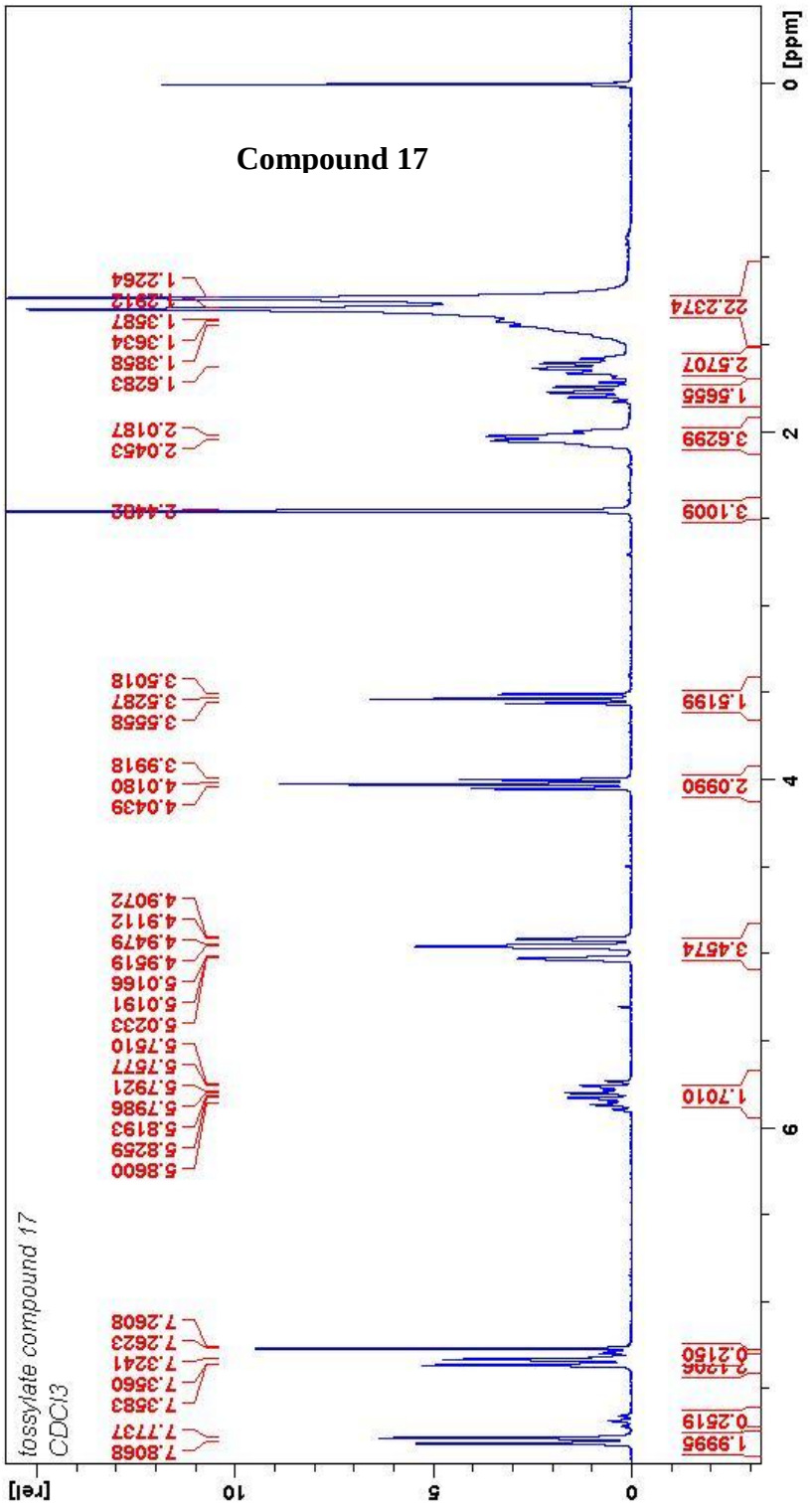
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tossylate compound 17
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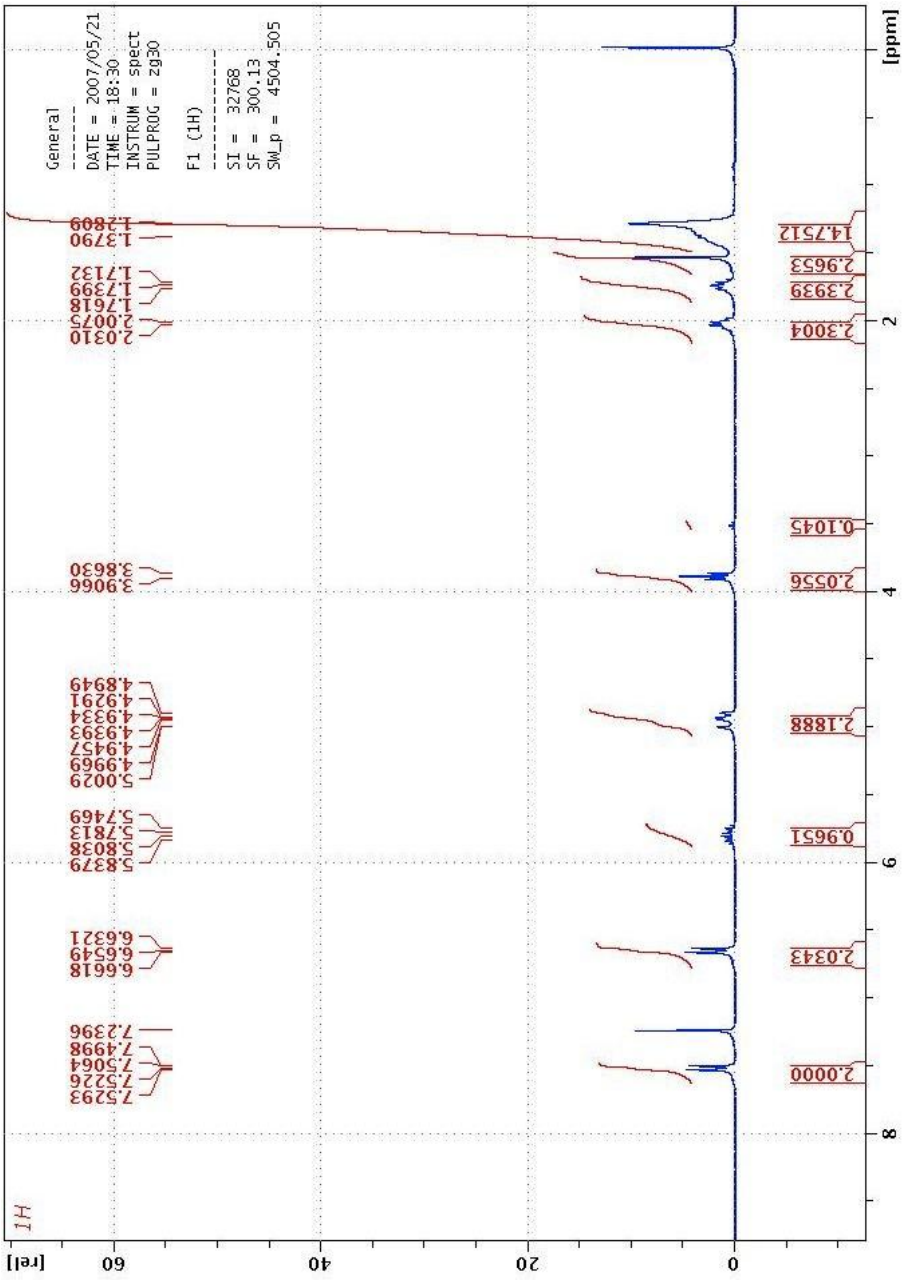
Compound 18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2.0075
2.0310
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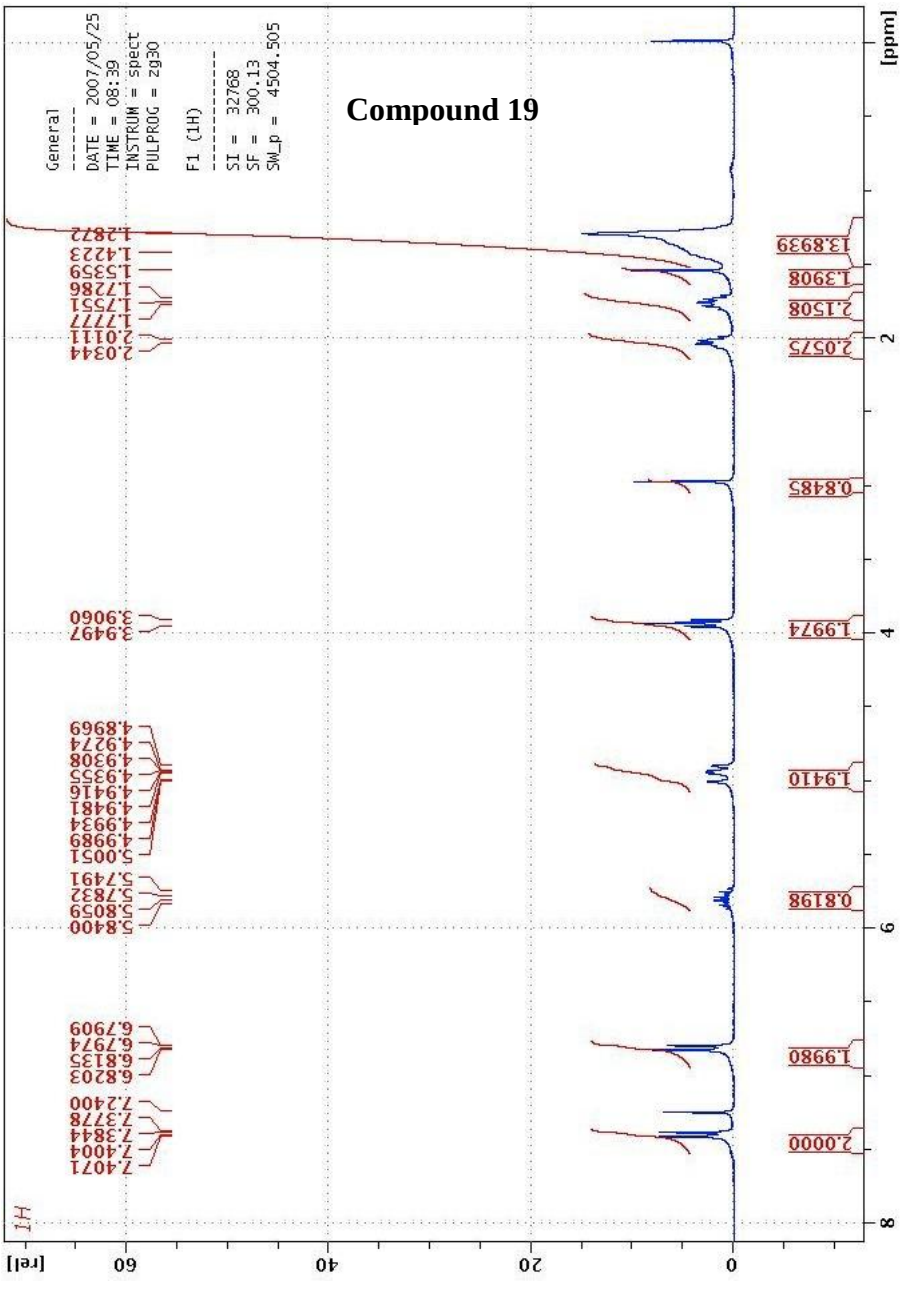
[ppm]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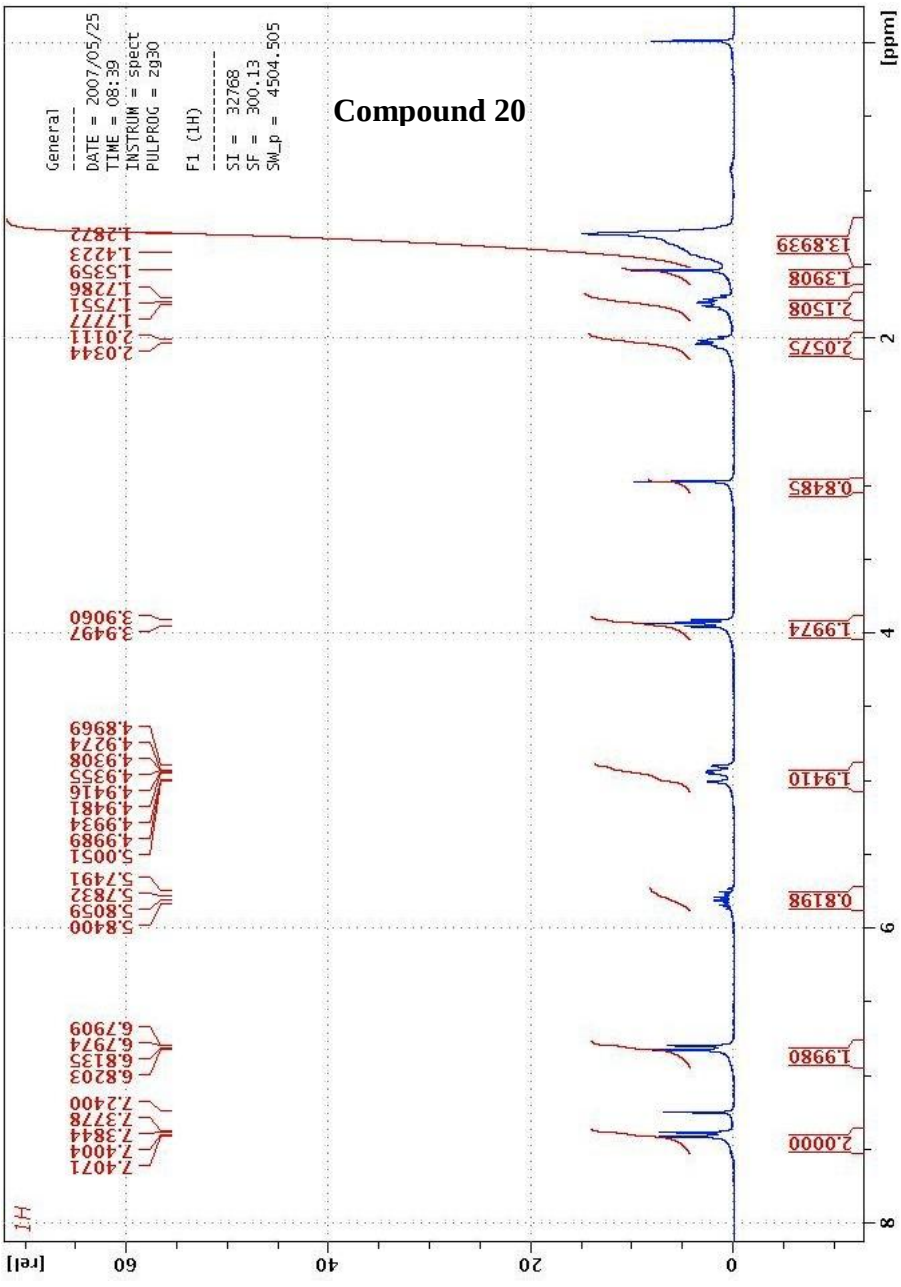
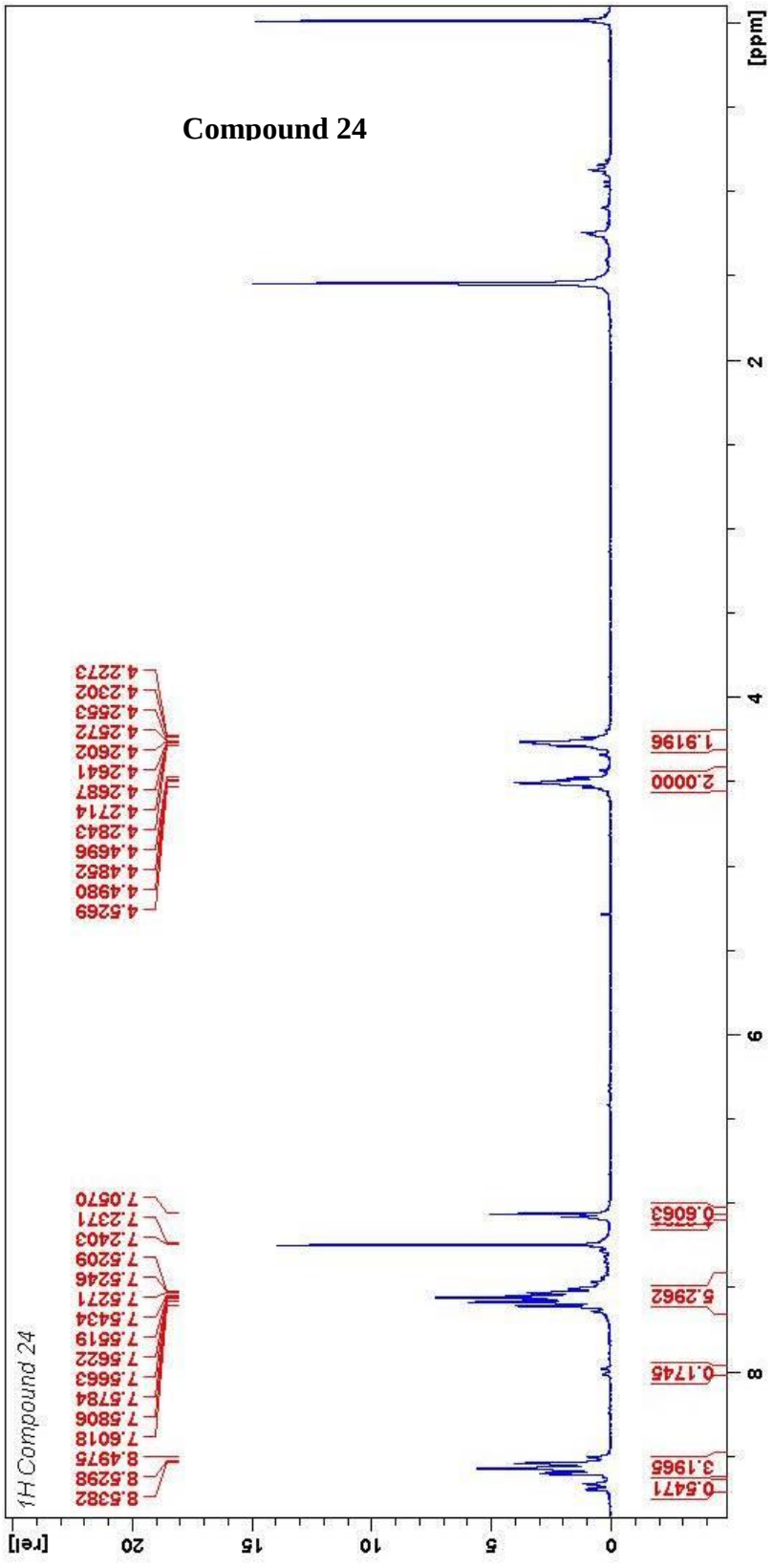
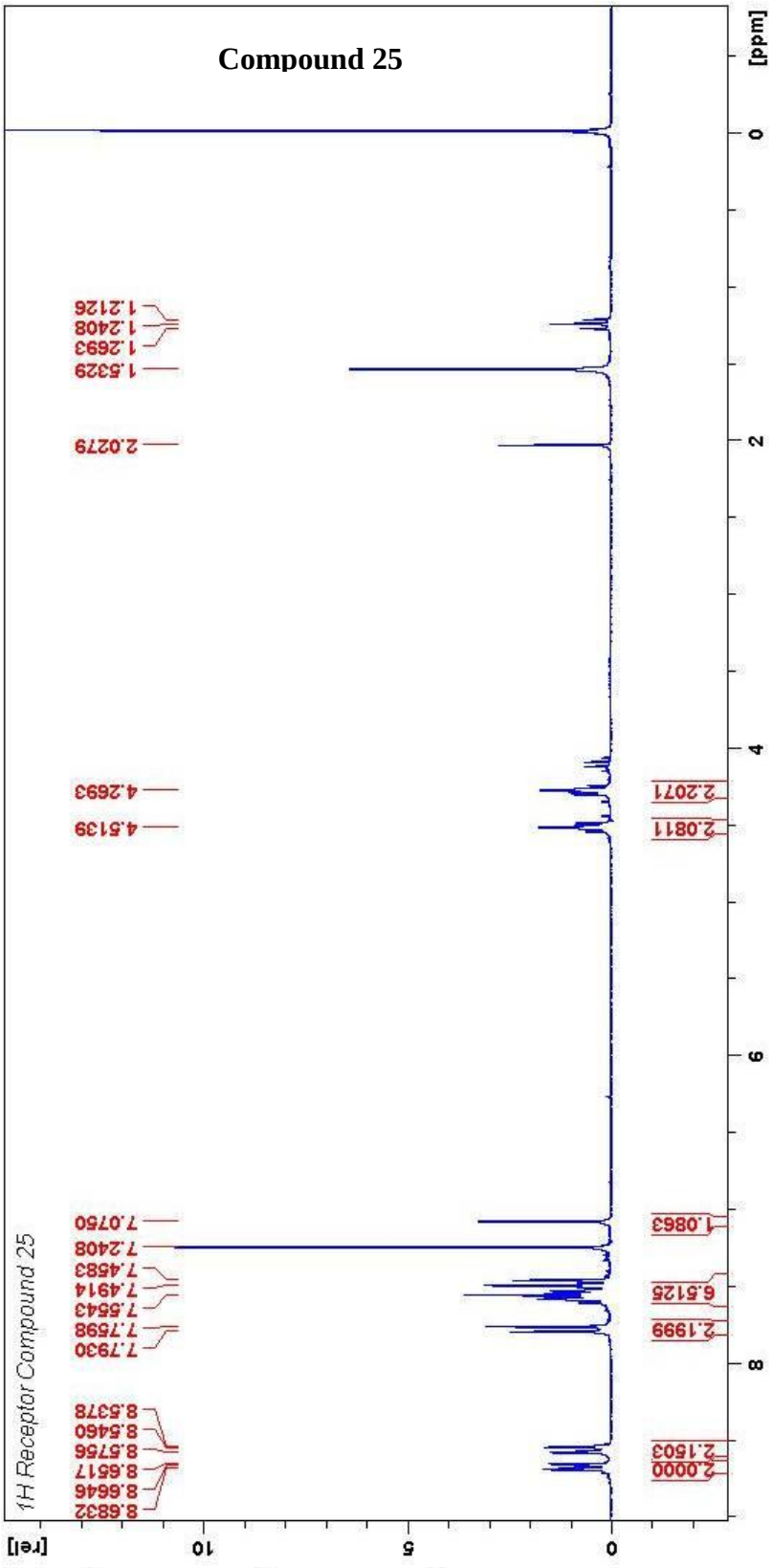
0.8198
1.9980
2.0000

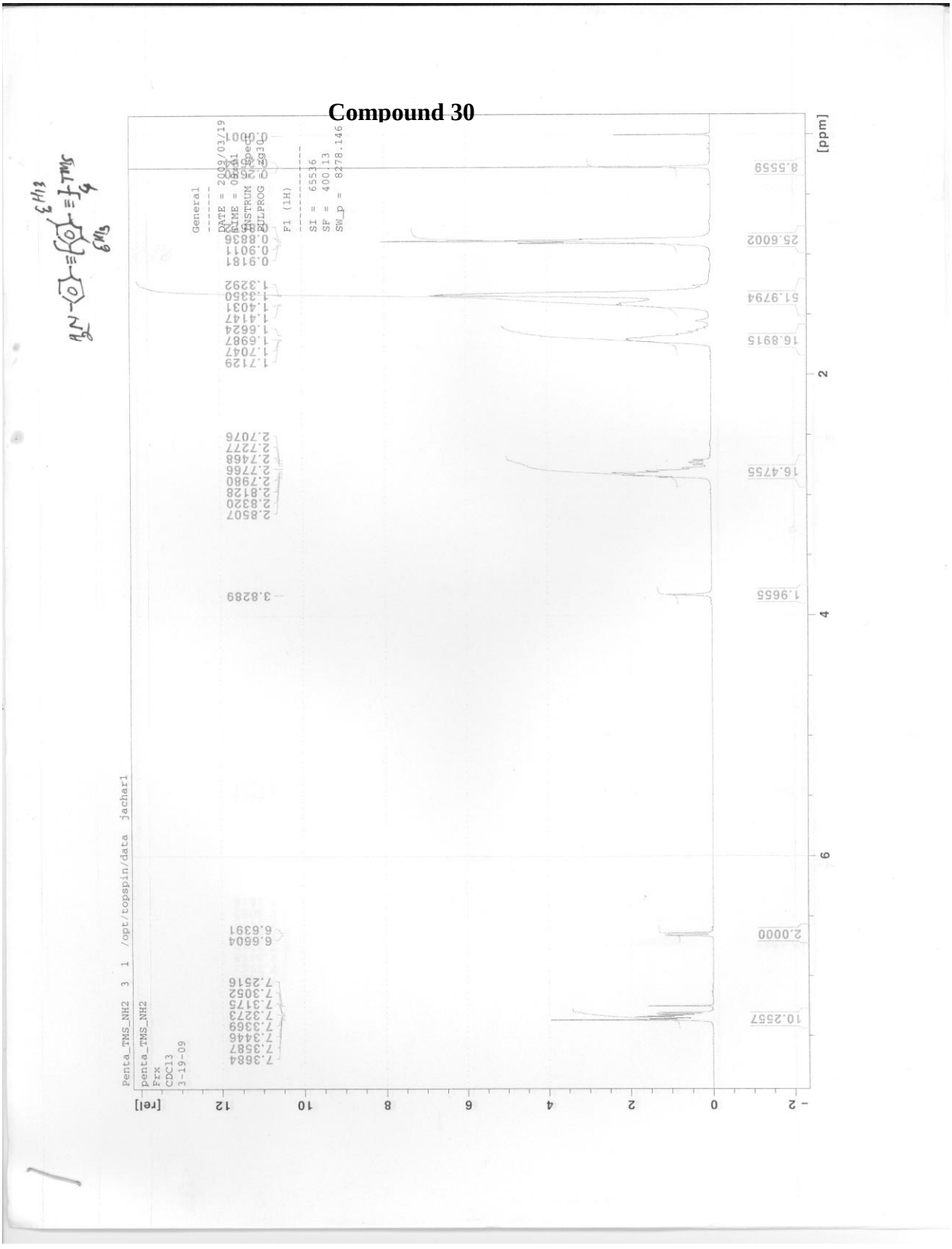

Compound 20
General
DATE = 2007/05/25
TIME = 08:39
INSTRUM = spect
PULPROG = zg30
F1 (1H)
SI = 32768
SF = 300.13
SW_p = 4504.505
[ppm]
[rel]
H1

Compound 24

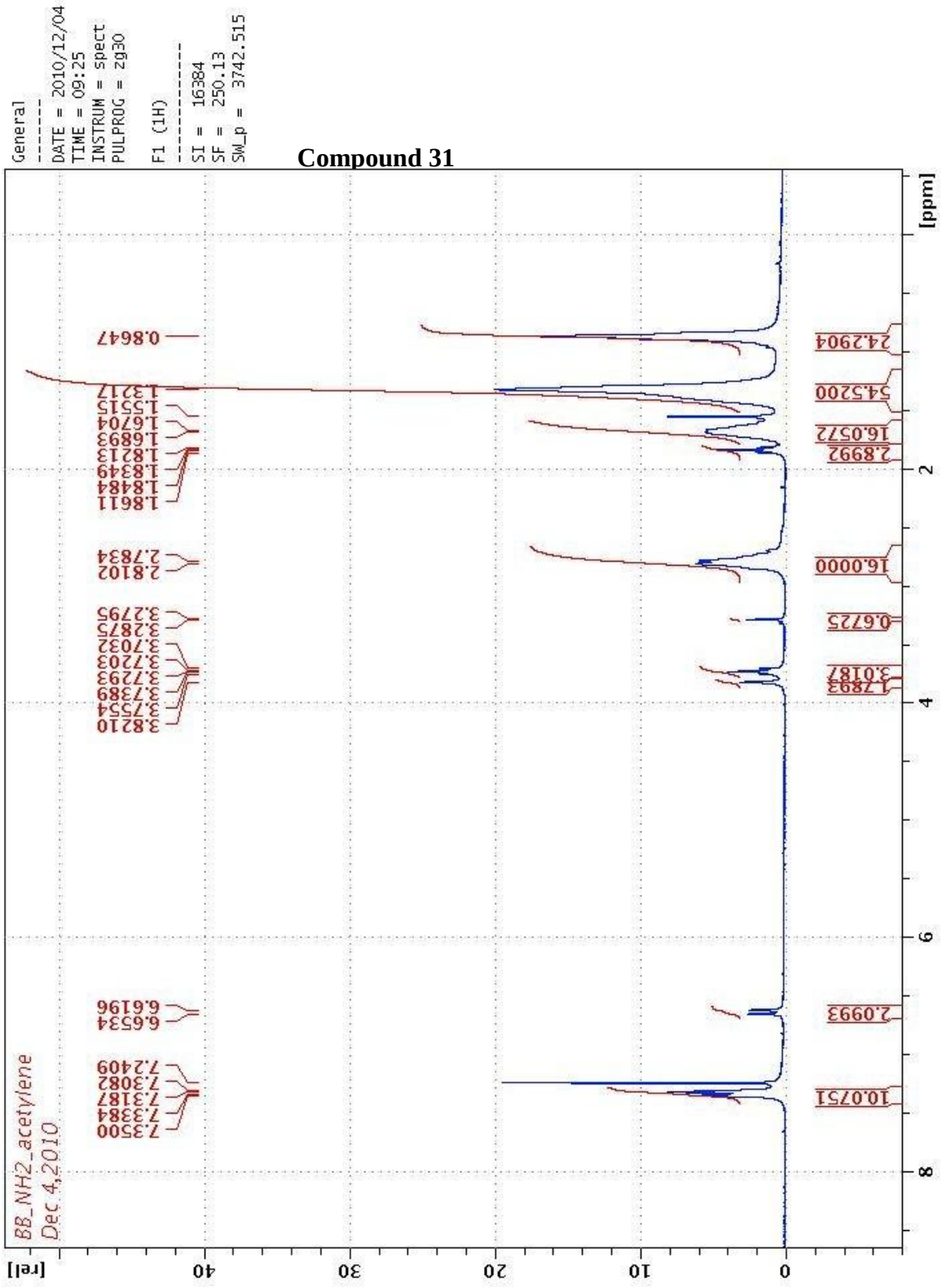

1H Compound 24
[rel]
[ppm]
8.5382
8.5298
8.4975
7.6018
7.5806
7.5784
7.5663
7.5622
7.5519
7.5434
7.5271
7.5246
7.5209
7.2403
7.2371
7.0570
4.5269
4.4980
4.4852
4.4696
4.2843
4.2714
4.2687
4.2641
4.2602
4.2572
4.2553
4.2302
4.2273
0.5471
3.1965
0.1745
5.2962
0.6063
2.0000
1.9196


Compound 25
1H Receptor Compound 25
[rel]
[ppm]
10
5
0
2
4
6
8
8.6832
8.6646
8.6517
8.5756
8.5460
8.5378
7.7930
7.7598
7.5543
7.4914
7.4583
7.2408
7.0750
4.5139
4.2693
2.0279
1.5329
1.2693
1.2408
1.2126
2.0000
2.1503
2.1999
6.5125
1.0863
2.0811
2.2071

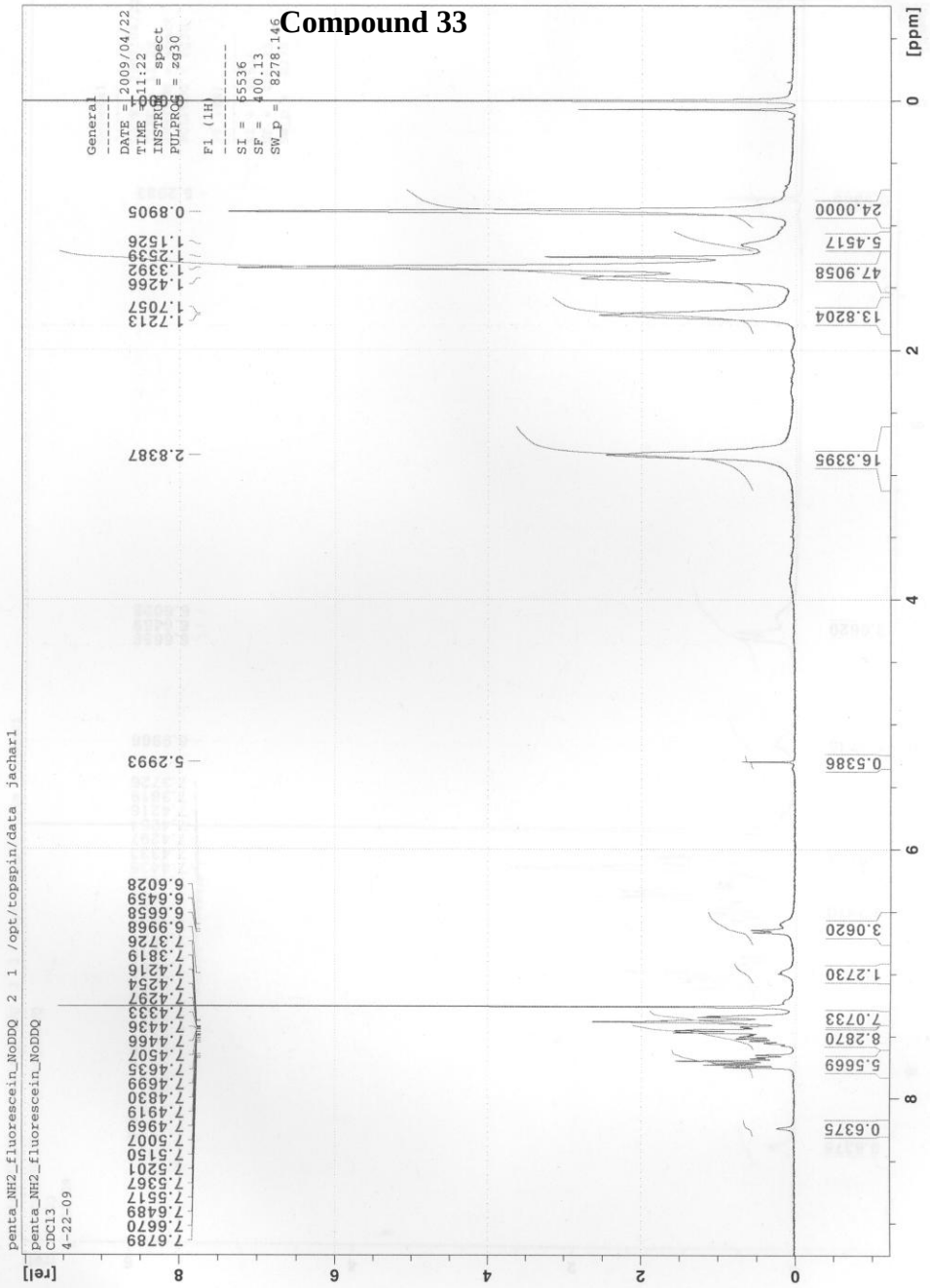
Compound 30


Penta_TMS_NH2 3 1 /opt/topspin/data jachar1
penta_TMS_NH2
Prx
CDCl3
3-19-09
General
DATE = 2009/03/19
PULPROG = zg30
F1 (1H)
SI = 65536
SF = 400.13
SW_p = 8278.146
[rel]
[ppm]
8.5559
25.6002
51.9794
16.8915
16.4755
1.9655
2.0000
10.2557
0.0001
0.8836
0.9011
0.9181
1.3292
1.3350
1.4031
1.4147
1.6624
1.6987
1.7047
1.7129
2.7076
2.7277
2.7468
2.7766
2.7980
2.8128
2.8320
2.8507
3.8289
6.6391
6.6604
7.2516
7.3052
7.3175
7.3273
7.3369
7.3446
7.3587
7.3684

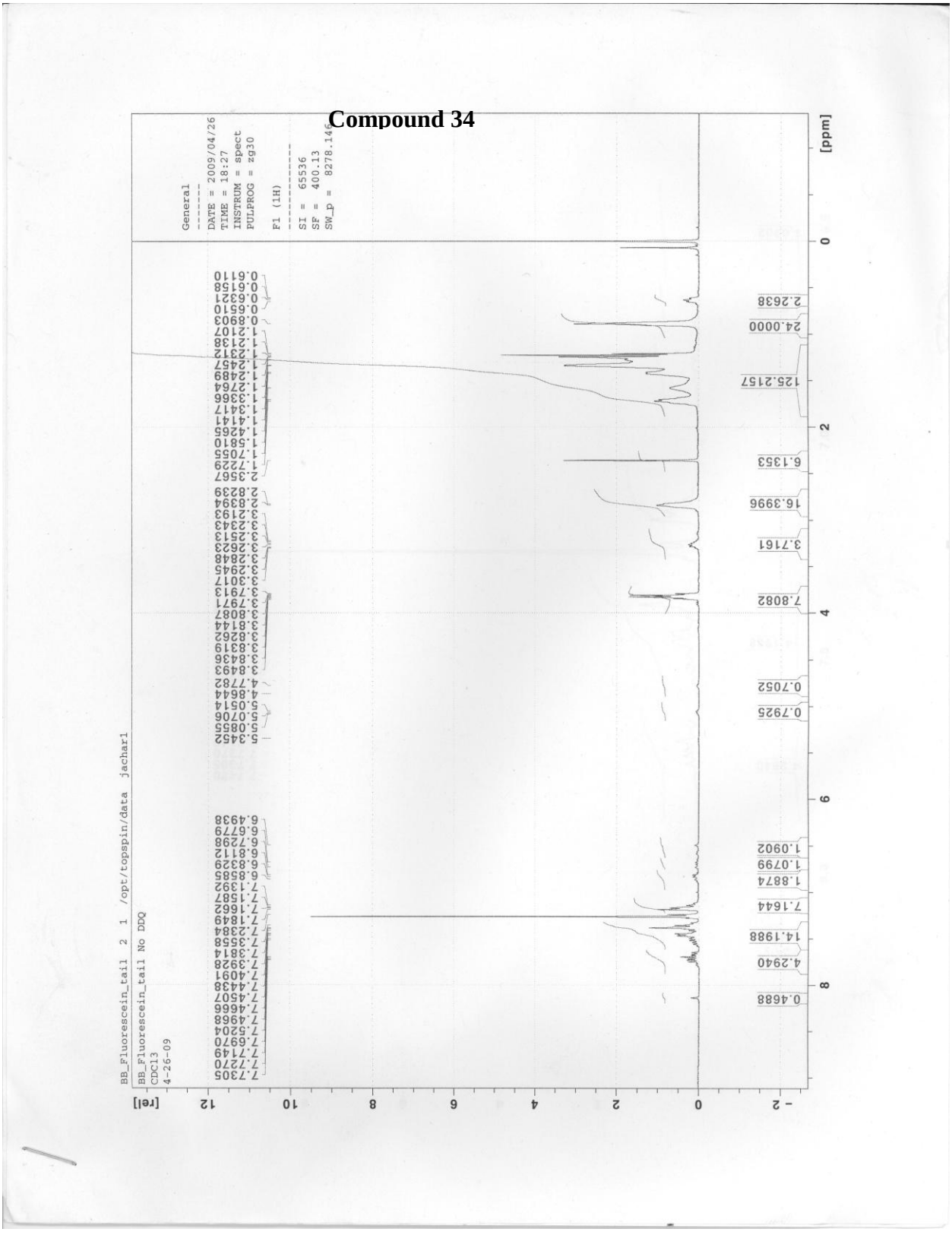
Compound 31


General
DATE = 2010/12/04
TIME = 09:25
INSTRUM = spect
PULPROG = zg30
F1 (1H)
SI = 16384
SF = 250.13
SW_p = 3742.515
BB_NH2_acetylene
Dec 4,2010
[rel]
[ppm]
0.8647
1.3217
1.5515
1.6704
1.6893
1.8213
1.8349
1.8484
1.8611
2.7834
2.8102
3.2795
3.2875
3.7032
3.7203
3.7293
3.7389
3.7554
3.8210
6.6196
6.6534
7.2409
7.3082
7.3187
7.3384
7.3500
24.2904
54.5200
16.0572
2.8992
16.0000
0.6725
1.7893
3.0187
2.0993
10.0751

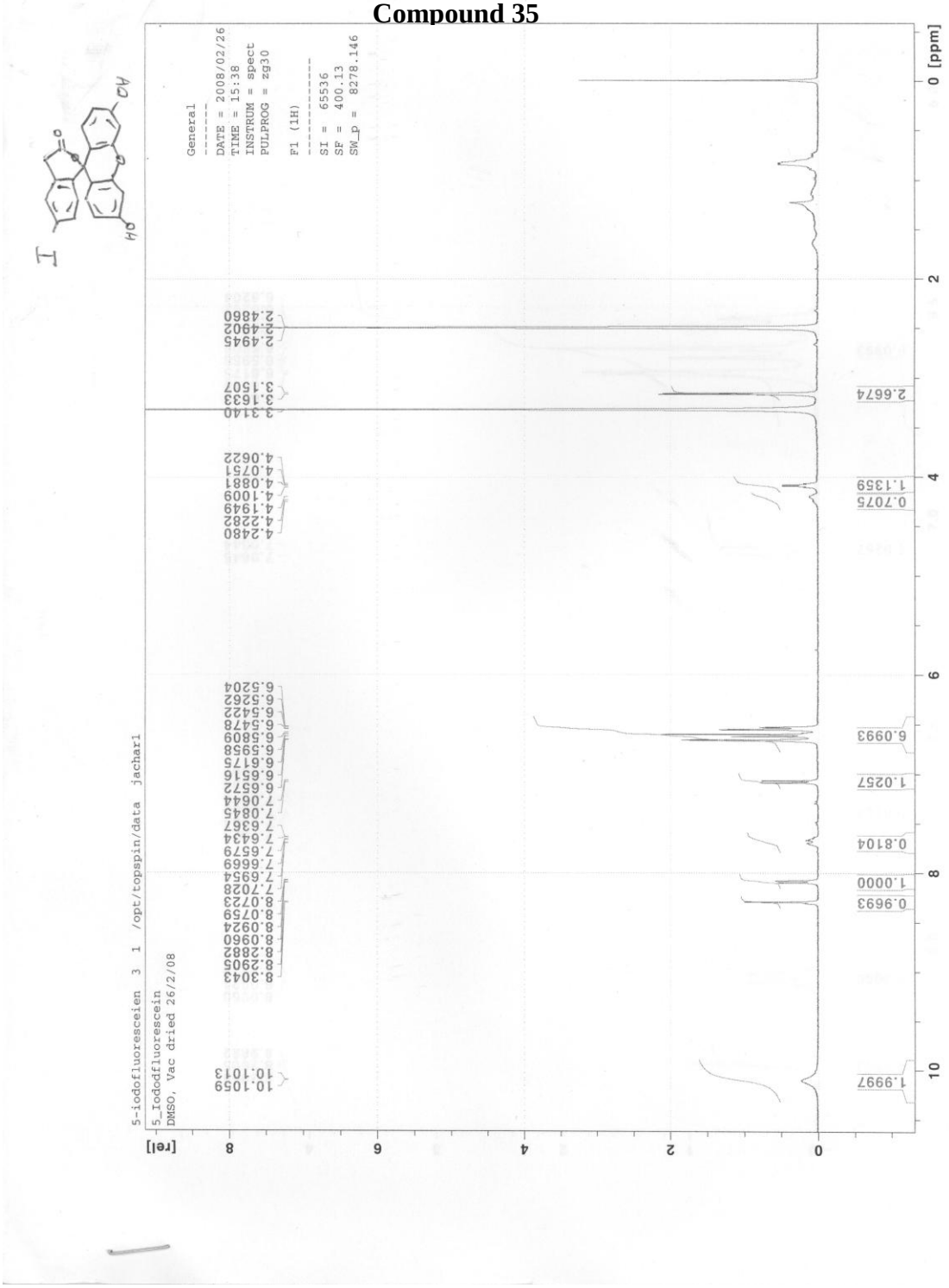
Compound 33


penta_NH2_fluorescein_NoDDQ 2 1 /opt/topspin/data jachar1
penta_NH2_fluorescein_NoDDQ
CDCl3
4-22-09
General
DATE = 2009/04/22
TIME = 11:22
INSTRUM = spect
PULPROG = zg30
F1 (1H)
SI = 65536
SF = 400.13
SW_p = 8278.146
[rel]
[ppm]
0.8905
1.1526
1.2539
1.3392
1.4266
1.7057
1.7213
2.8387
5.2993
6.6028
6.6459
6.6658
6.9968
7.3726
7.3819
7.4216
7.4254
7.4297
7.4333
7.4436
7.4466
7.4507
7.4635
7.4699
7.4830
7.4919
7.4969
7.5007
7.5150
7.5201
7.5367
7.5517
7.6489
7.6670
7.6789
24.0000
5.4517
47.9058
13.8204
16.3395
0.5386
3.0620
1.2730
7.0733
8.2870
5.5669
0.6375

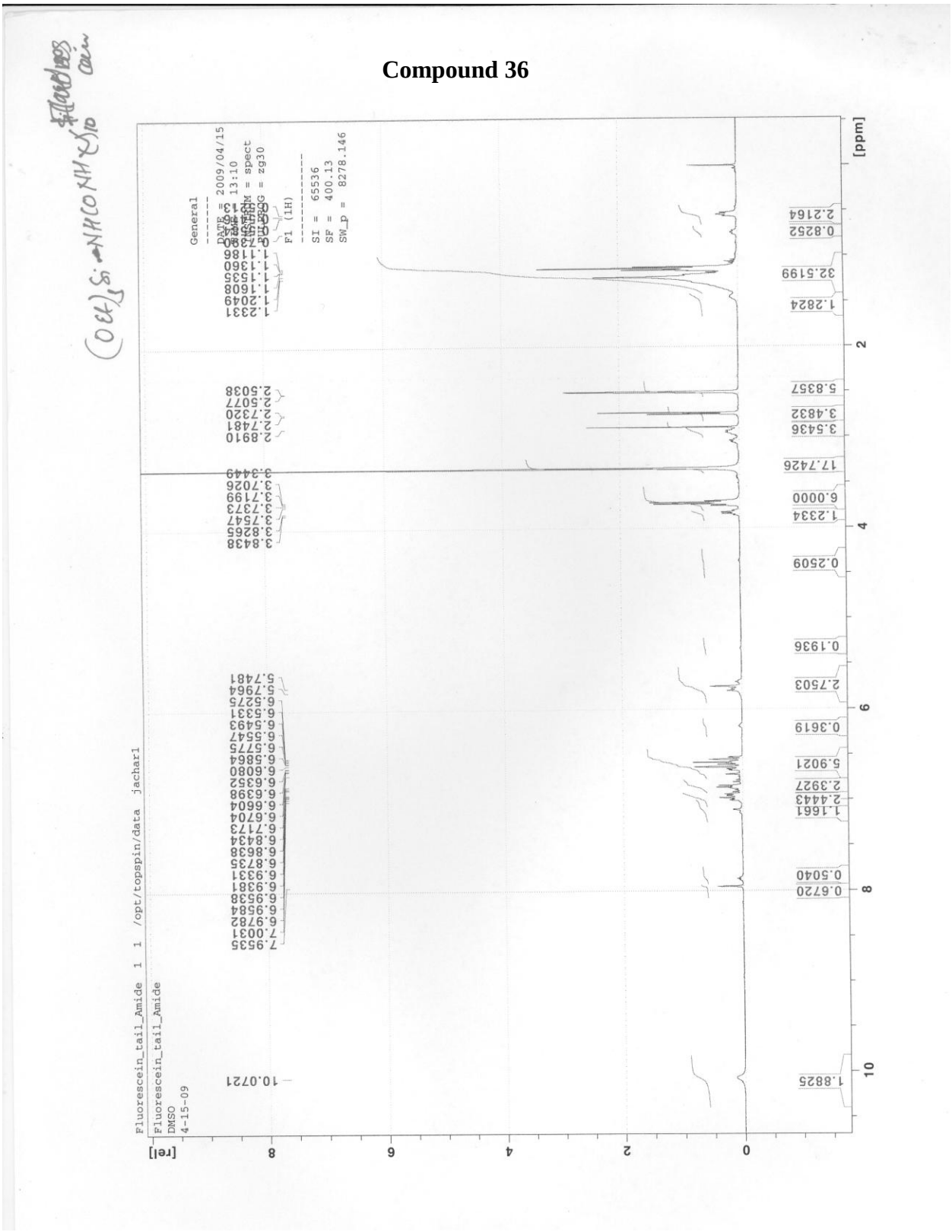
Compound 34


BB_Fluorescein_tail 2 1 /opt/topspin/data jachar1
BB_Fluorescein_tail No DDQ
CDCl3
4-26-09
General
DATE = 2009/04/26
TIME = 18:27
INSTRUM = spect
PULPROG = zg30
F1 (1H)
SI = 65536
SF = 400.13
[rel]
[ppm]

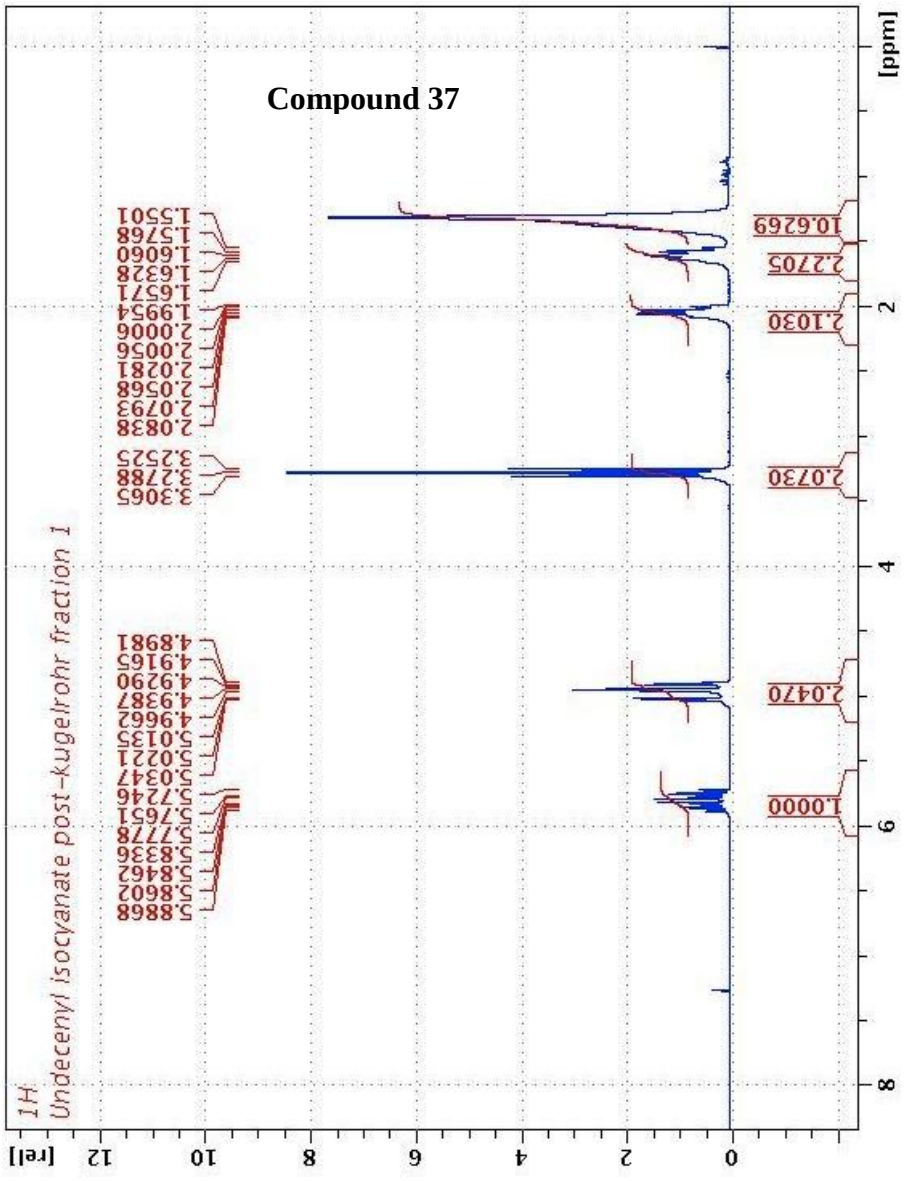
Compound 35


5-iodofluoresceien 3 1 /opt/topspin/data jachar1
5_Iododfluorescein
DMSO, Vac dried 26/2/08
General
DATE = 2008/02/26
TIME = 15:38
INSTRUM = spect
PULPROG = zg30
F1 (1H)
SI = 65536
SF = 400.13
SW_p = 8278.146
[rel]
[ppm]
I
HO
OH
O
10.1059
10.1013
8.3043
8.2905
8.2882
8.0960
8.0924
8.0759
8.0723
7.7028
7.6954
7.6669
7.6579
7.6434
7.6367
7.0845
7.0644
6.6572
6.6516
6.6175
6.5958
6.5809
6.5478
6.5422
6.5262
6.5204
4.2480
4.2282
4.1949
4.1009
4.0881
4.0751
4.0622
3.3140
3.1633
3.1507
2.4945
2.4902
2.4860
1.9997
0.8104
0.7075
0.6993
0.6993
1.0257
1.1359
2.6674

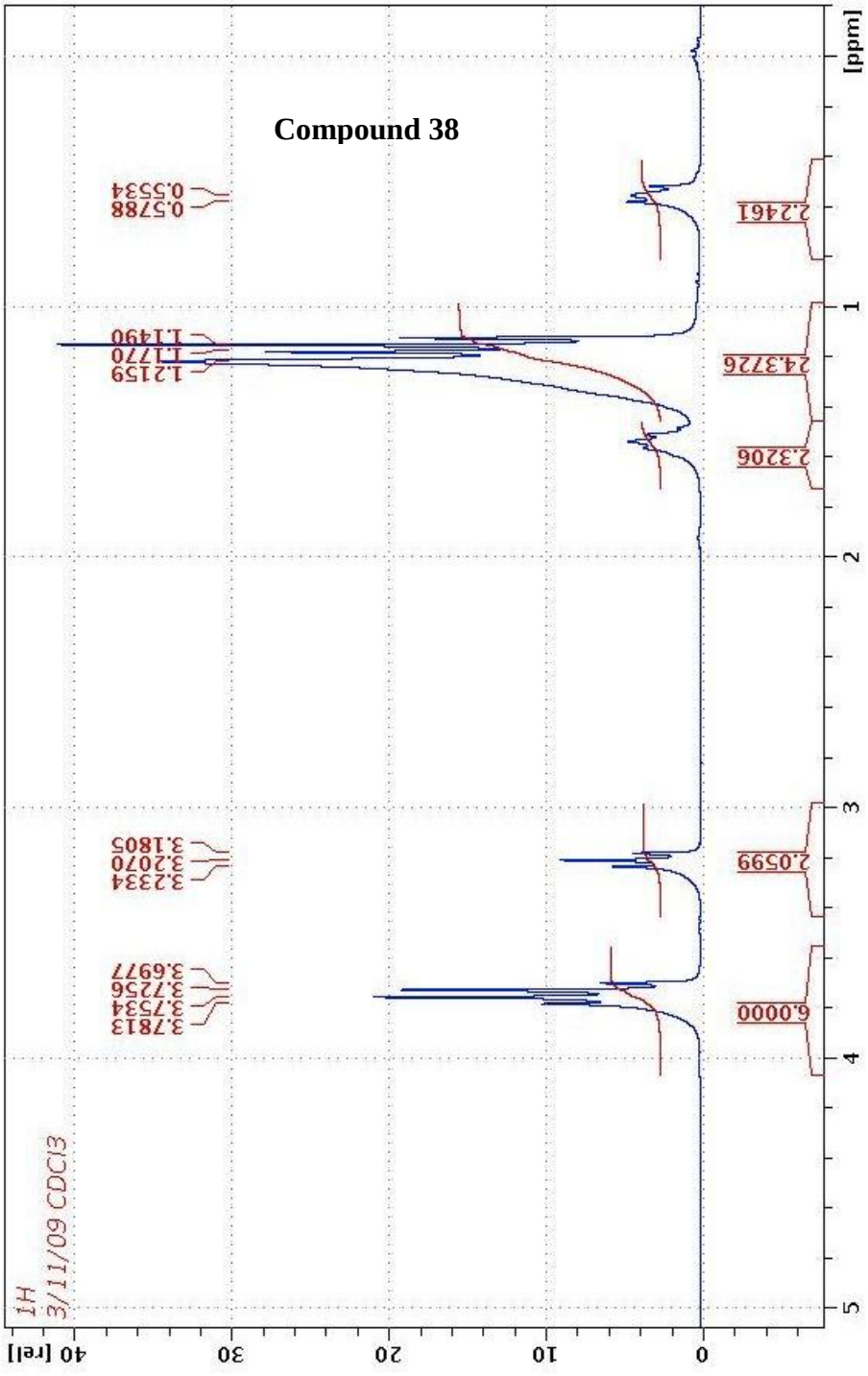
Compound 36

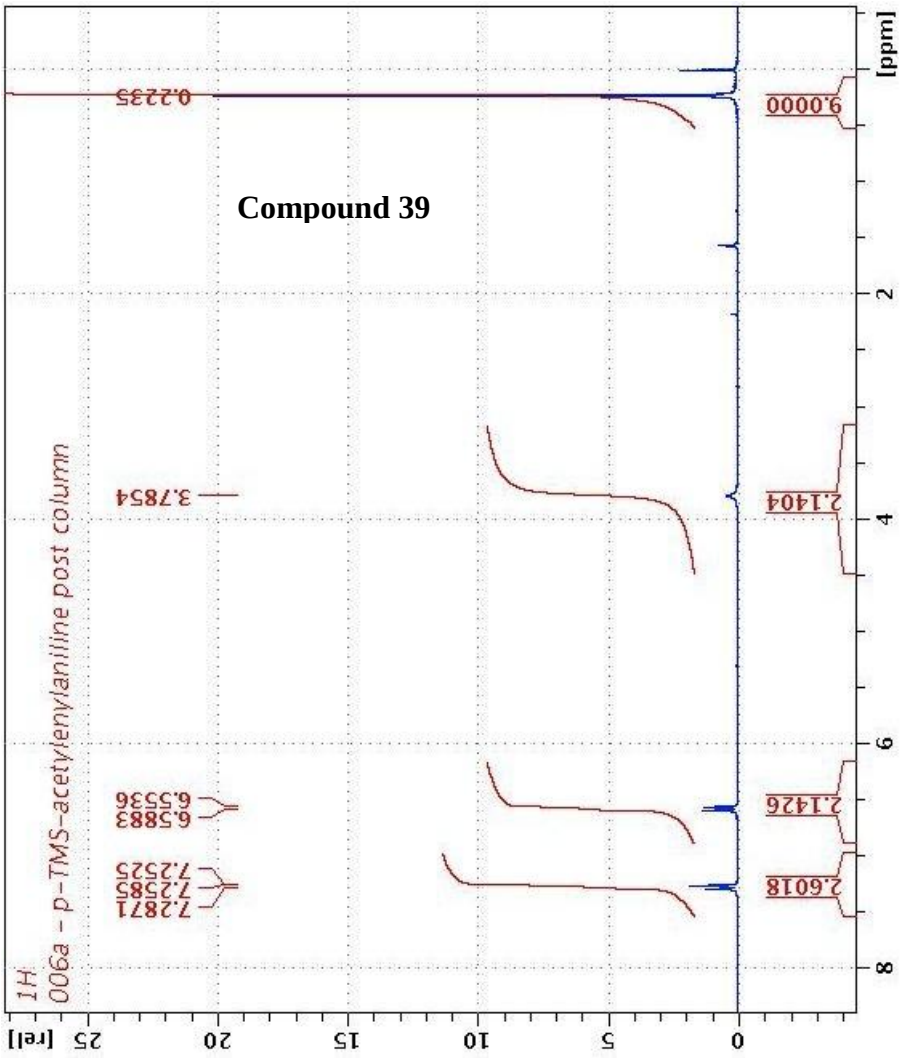

Fluorescein_tail_Amide 1 1 /opt/topspin/data jachar1
Fluorescein_tail_Amide
DMSO
4-15-09
[rel]
[ppm]
10.0721
7.9535
7.0031
6.9782
6.9584
6.9538
6.9381
6.9331
6.8735
6.8638
6.8434
6.7173
6.6704
6.6604
6.6398
6.6352
6.6080
6.5864
6.5775
6.5547
6.5493
6.5331
6.5275
5.7964
5.7481
3.8438
3.8265
3.7547
3.7373
3.7199
3.7026
3.3449
2.8910
2.7481
2.7320
2.5077
2.5038
1.2331
1.2049
1.1608
1.1535
1.1360
1.1186
1.8825
0.6720
0.5040
1.6611
2.4443
2.3927
5.9021
0.3619
2.7503
0.1936
6.0000
1.2334
0.2509
17.7426
3.5436
3.4832
5.8357
32.5199
1.2824
0.8252
2.2164

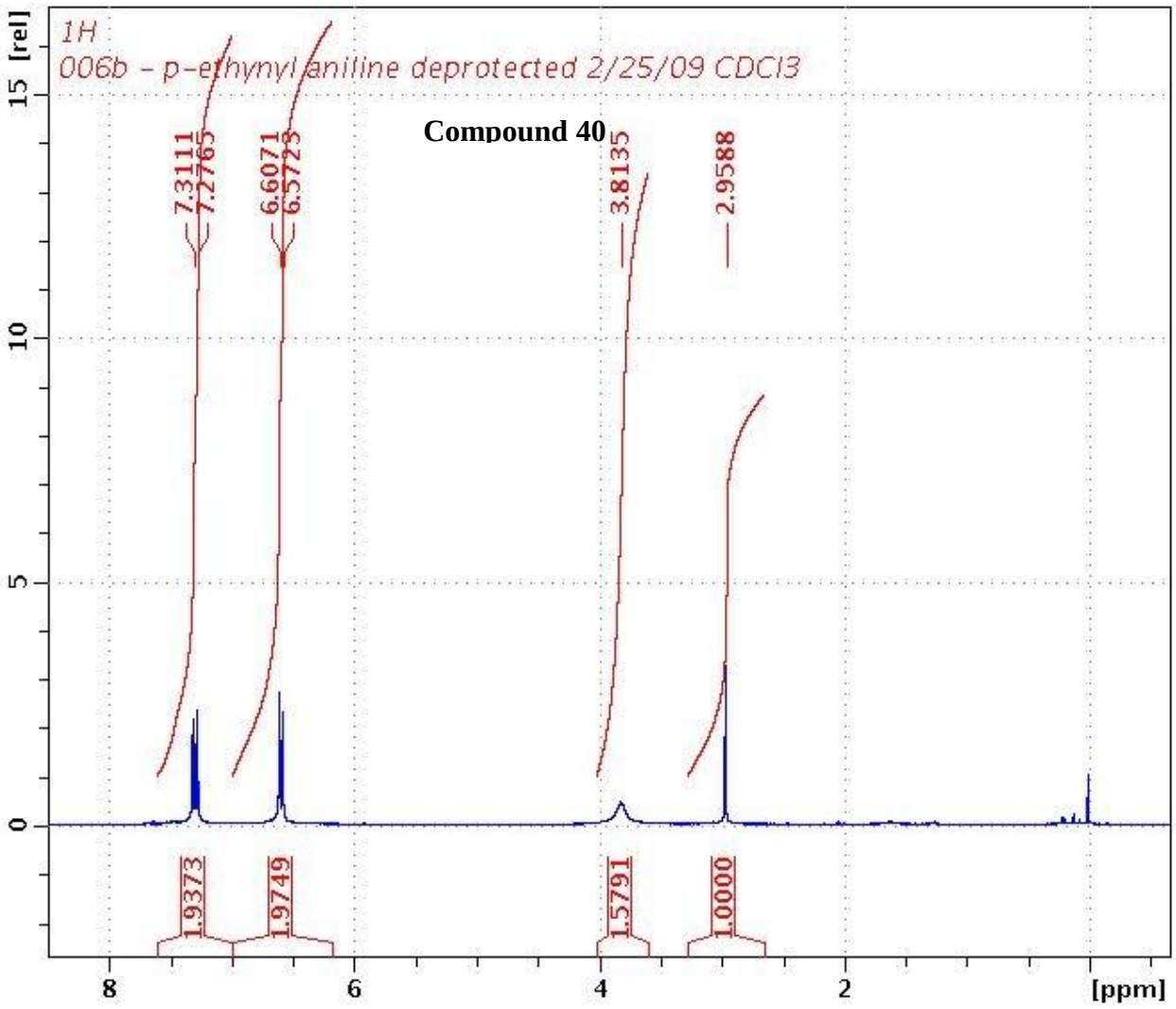
Compound 37


1H
Undecenyl isocyanate post-kugelrohr fraction 1
[rel]
[ppm]
10.6269
2.2705
2.1030
2.0730
2.0470
1.0000
3.3065
3.2788
3.2525
2.0838
2.0793
2.0568
2.0281
2.0056
2.0006
1.9954
1.6571
1.6328
1.6060
1.5768
1.5501
4.8981
4.9165
4.9290
4.9387
4.9662
5.0135
5.0221
5.0347
5.7246
5.7651
5.7778
5.8362
5.8462
5.8602
5.8868

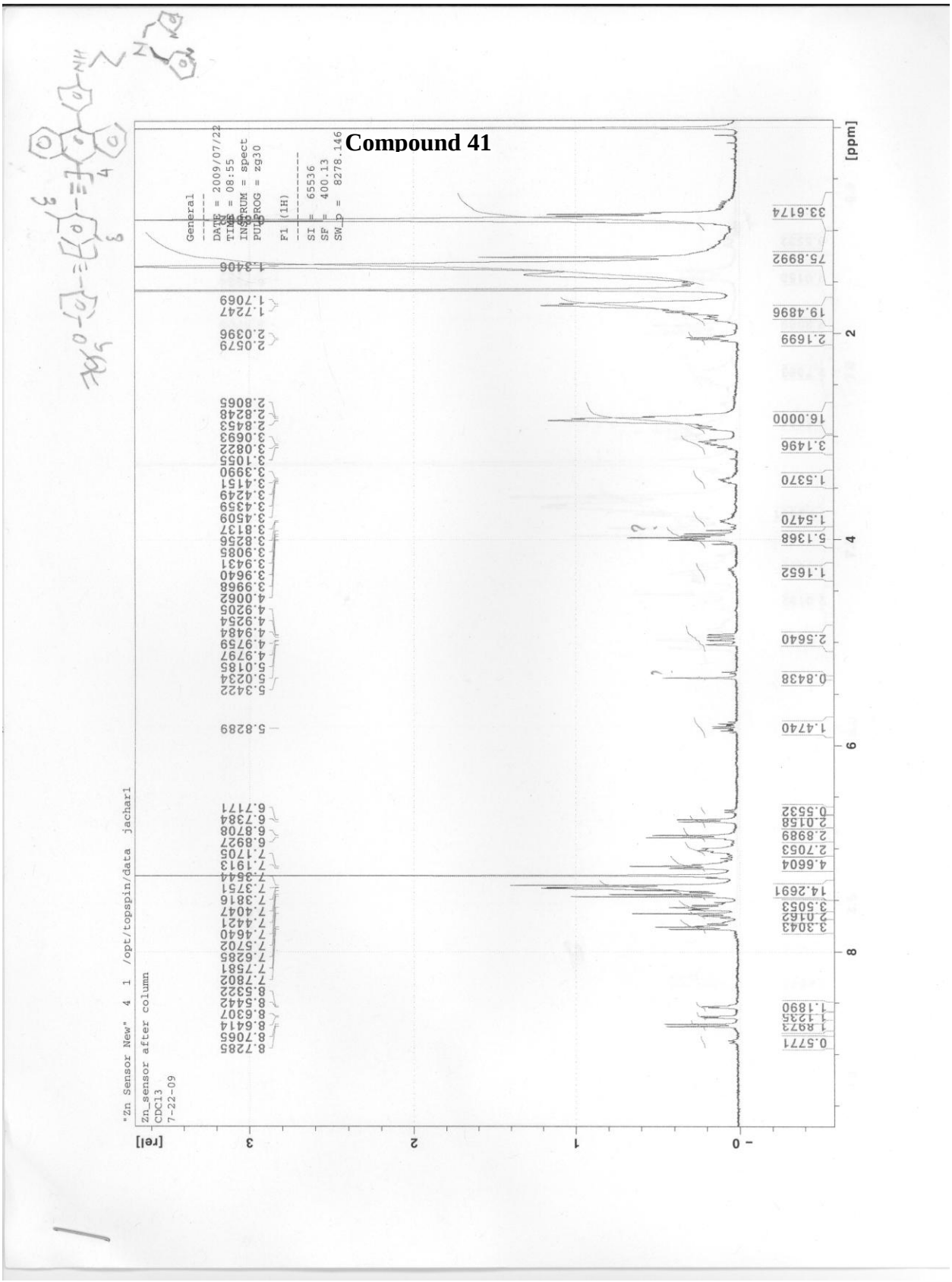
Compound 38


0.5534
0.5788
1.1490
1.1770
1.2159
3.1805
3.2070
3.2334
3.6977
3.7256
3.7534
3.7813
2.2461
24.3726
2.3206
2.0599
6.0000
[ppm]
1H
3/11/09 CDCl3
[rel]

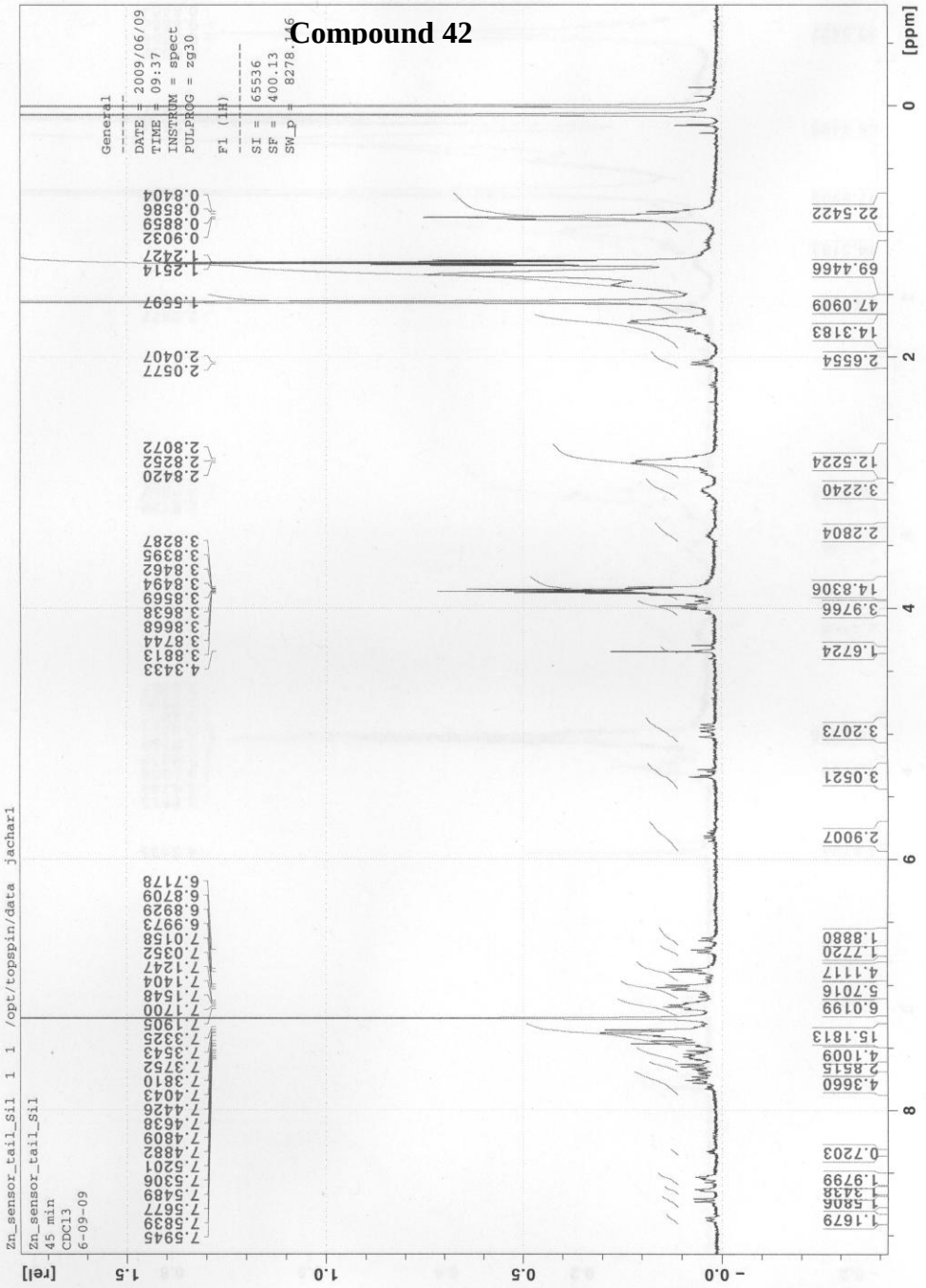

Compound 39
0.2235
3.7854
6.5883
6.5536
7.2871
7.2585
7.2525
9.0000
2.1404
2.1426
2.6018
1H
006a - p-TMS-acetylenylaniline post column
[rel]
[ppm]


1H
006b - p-ethynyl aniline deprotected 2/25/09 CDCl3
15 [rel]
10
5
0
7.3111
7.2765
6.6071
6.5723
3.8135
2.9588
1.9373
1.9749
1.5791
1.0000
8
6
4
2
[ppm]

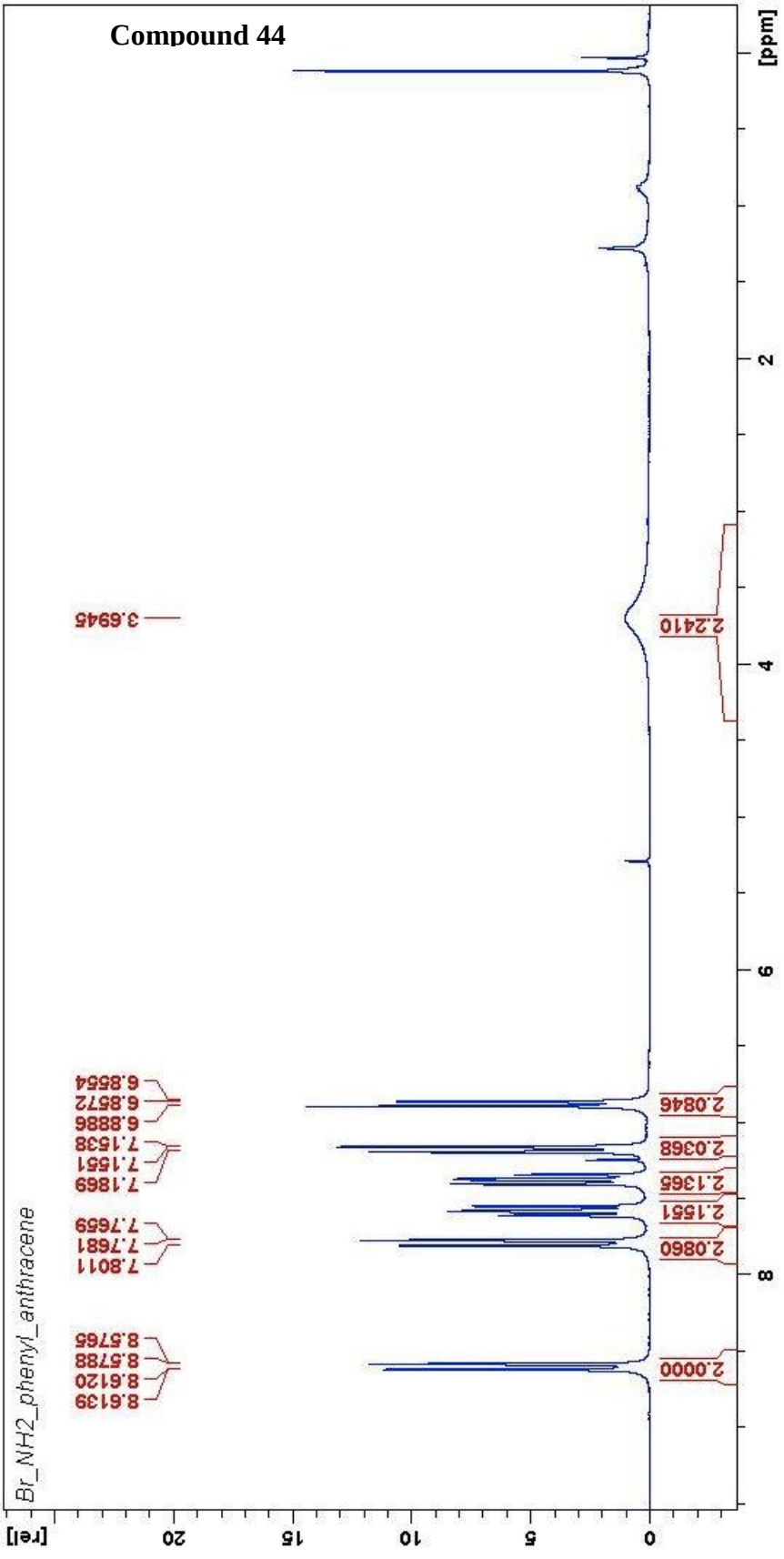
Compound 40


Compound 41
"Zn Sensor New" 4 1 /opt/topspin/data jachar1
Zn_sensor after column
CDCl3
7-22-09
General
DATE = 2009/07/22
TIME = 08:55
INSTRUM = spect
PULPROG = zg30
F1 (1H)
SI = 65536
SP = 400.13
SW_p = 8278.146
[rel]
[ppm]

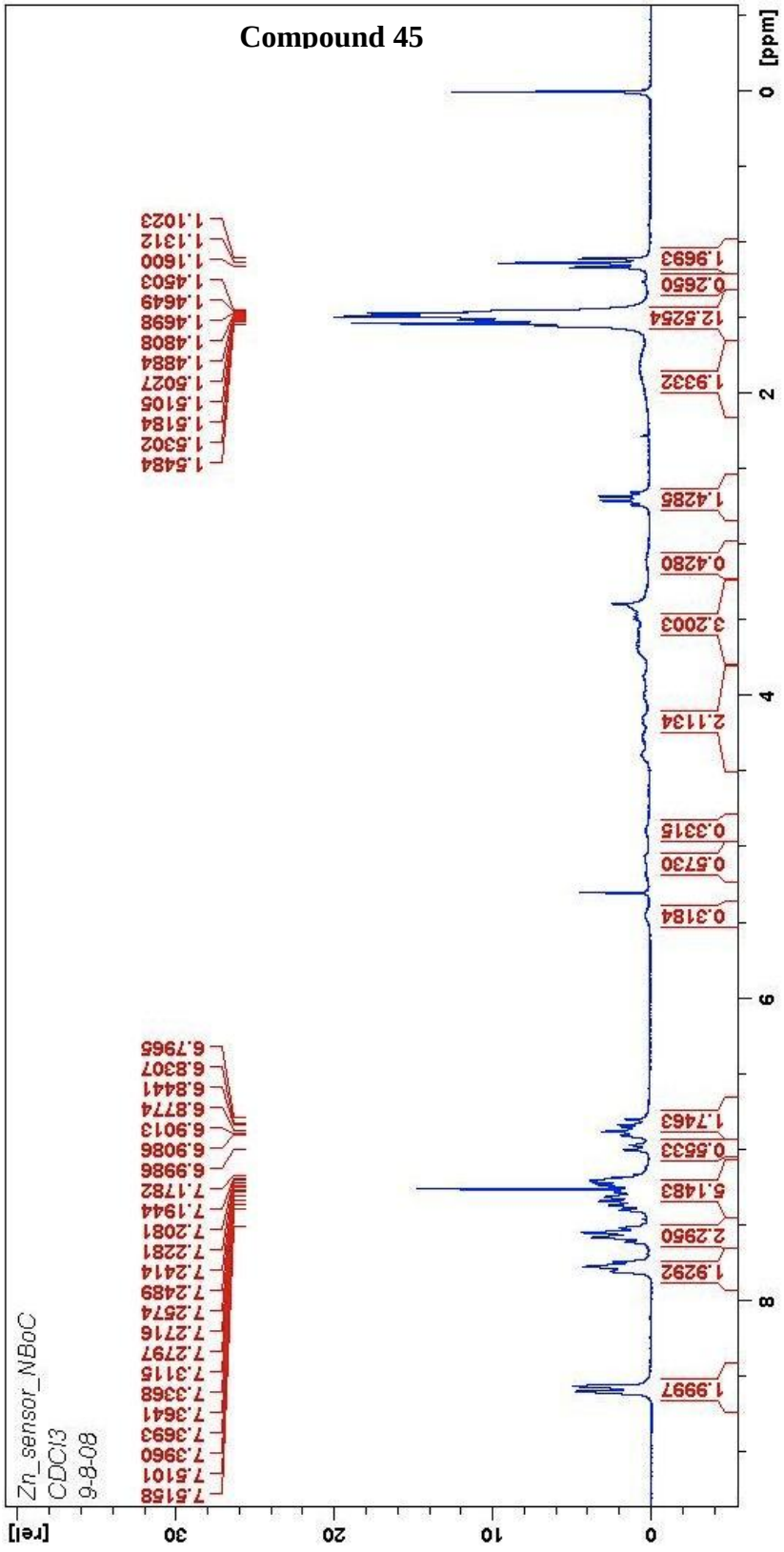
Compound 42


Zn_sensor_tail_Si1 1 1 /opt/topspin/data jachar1
Zn_sensor_tail_Si1
45 min
CDCl3
6-09-09
General
DATE = 2009/06/09
TIME = 09:37
INSTRUM = spect
PULPROG = zg30
F1 (1H)
SI = 65536
SF = 400.13
SW_p = 8278.146
[rel]
[ppm]

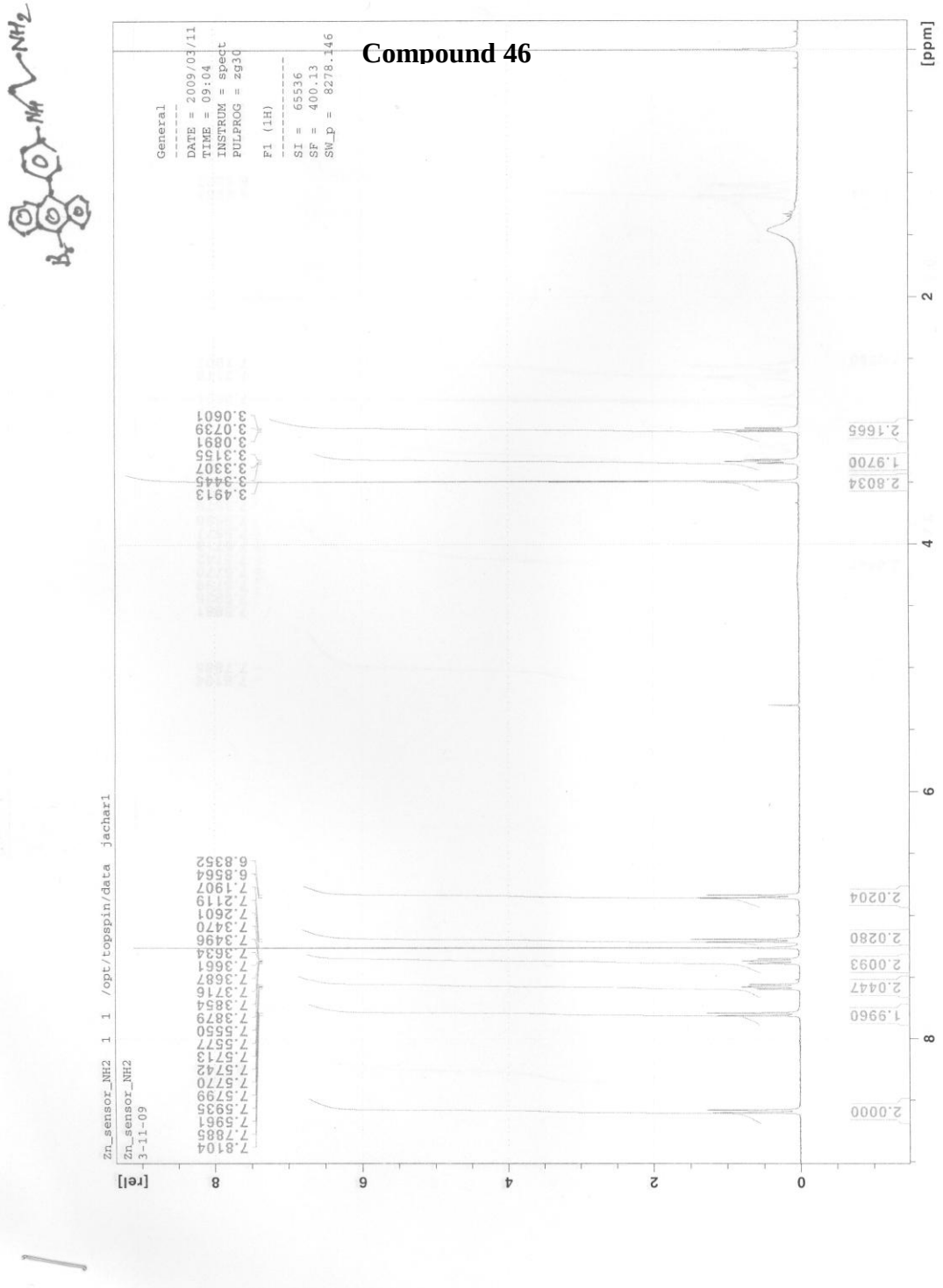
Compound 44


Br_NH2_phenyl_anthracene
[rel]
[ppm]
8.6139
8.6120
8.5788
8.5765
7.8011
7.7681
7.7659
7.1869
7.1551
7.1538
6.8886
6.8572
6.8554
3.6945
2.0000
2.0860
2.1551
2.1365
2.0368
2.0846
2.2410

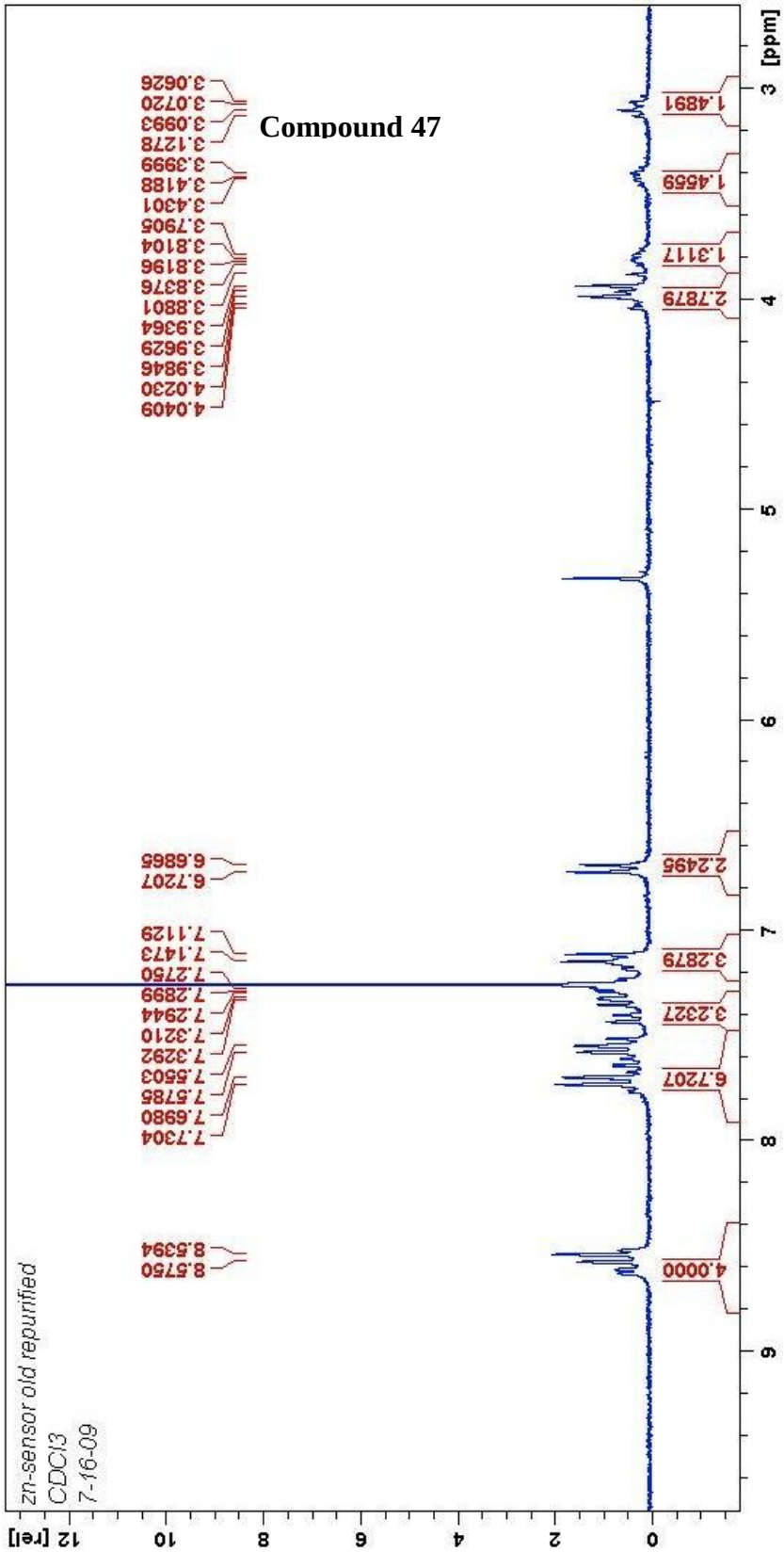
Compound 45


Zn_sensor_NBoC
CDCl3
9-8-08
[rel]
[ppm]
1.9997
1.9292
2.2950
5.1483
0.5533
1.7463
0.3184
0.5730
0.3315
2.1134
3.2003
0.4280
1.4285
1.9332
12.5254
0.2650
1.9893

Compound 46


Zn_sensor_NH2 1 1 /opt/topspin/data jachar1
Zn_sensor_NH2
3-11-09
General
DATE = 2009/03/11
TIME = 09:04
INSTRUM = spect
PULPROG = zg30
F1 (1H)
SI = 65536
SF = 400.13
SW_p = 8278.146
[rel]
[ppm]
2.0000
1.9960
2.0447
2.0093
2.0280
2.0204
1.9700
2.8034
2.1665

Compound 47


zn-sensor old repurified
CDCl3
7-16-09
8.5750
8.5394
7.7304
7.6980
7.5785
7.5503
7.3292
7.3210
7.2944
7.2899
7.2750
7.1473
7.1129
6.7207
6.6865
4.0409
4.0230
3.9846
3.9629
3.9364
3.8801
3.8376
3.8196
3.8104
3.7905
3.4301
3.4188
3.3999
3.1278
3.0993
3.0720
3.0626
4.0000
6.7207
3.2327
3.2879
2.2495
2.7879
1.3117
1.4559
1.4891
[rel]
[ppm]

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Jiba Raj Acharya received his master's degree in chemistry from Tribhuvan University, Kathmandu, Nepal, in 1996. He started his career as a teacher in Gyanodaya Higher Secondary School, Lalitpur, Nepal. He spent five years as a higher secondary school teacher and joined Birendra Military College, Bhaktapur, Nepal, in 2000 as a Post Graduate Teacher (PGT) equivalent to an assistant professor of chemistry. He also worked in Trichandra Multple College, Kathmandu, Nepal, for two years as an Assistant Professor of Chemistry. He joined Department of Chemistry at Louisiana State University in August 2004 as a PhD student.

He first joined Dr. Gudrun Schmidt's polymer research group in 2005 and worked until she resigned from LSU and moved to another university. During this time, He prepared biopolymer nanocomposite materials from guar gum derivatives and laponite RD nanoparticles and studied their viscoelastic properties. He then joined Dr. Evgueni Nesterov's research group in June 2006 and continued his research in the area of design, synthesis and studies on the ratiometric fluorescent surface-immobilized thin-film chemosensors. He will graduate in August 2011 with the degree of Doctor of Philosophy in chemistry.