SYNTHESIS, CHARACTERIZATION AND PHOTOCHEMICAL STUDY OF POTENTIALLY EMITTING TETRAZINE DERIVATIVES

Sachin D. Vahile

A Thesis

Submitted to Graduate College of Bowling Green State University
in Partial Fulfillment of the Requirements for the Degree of

AUGUST 2009

MASTER OF SCIENCE

Committee:

Dr. Pavel Anzenbacher Jr., Adviser

Dr. Thomas H. Kinstle

Dr. Douglas C. Neckers
ABSTRACT

Dr. Pavel Anzenbacher Jr.

Tetrazines are aromatic compounds that consist of a six membered ring containing four nitrogens. They are not as much explored as light emitting materials as compared to other common organic compounds. Still they are likely to be important for as substrates for HEDM (High Energy Density Materials), sensors (for anions), important chromophoric materials (absorption and emission wavelengths in distinct regions), fluorescent probes (gives color to an organic compound) and possible day to day standard and useful organic molecular reference in photo physical studies. Much research still needs to be done on tetrazines in order to understand comparisons with the benzene ring with regard to reactivity, electron affinity, and photooptical properties as well as reaction mechanisms, as well as different types of reactions.

A series of tetrazine derivatives with varying substitution patterns were successfully synthesized, characterized and preliminary photochemical studies were performed using them. Special quantitative $^{13}$C NMR techniques were employed in characterizing these compounds. Their emission wavelengths were found to be independent of the excitation wavelength. Longer singlet excited state life times of monosubstituted tetrazines were obtained which may be due to the formation of an intramolecular charge transfer state. A study of solvent effect based on polarity on these life times would be interesting to characterize the nature and stability of the formed charge transfer state.
DEDICATION

I would like to dedicate this dissertation to my parents, Dattatraya B. Vahile and Lakshmi D. Vahile, my brothers Ganesh and Santosh, my sisters-in-law and all my nephews and a niece for their inspiration, motivation, faith, and unlimited support throughout my studies, and for the future.

Special dedication to Anna, Appa, Mamachi Aai and Aajji…….
ACKNOWLEDGEMENT

I would like to take this opportunity to express my deep sense of indebtedness and gratitude to my adviser Dr. Pavel Anzenbacher Jr. for his leadership, professionalism and continued optimism, which make him an exceptional advisor. I am grateful for his time, effort and editing skills. His constant support, the trust he placed in my abilities, encouragement and timely interventions made this thesis possible and a reality.

I highly appreciate my committee member Dr. Thomas H. Kinstle who helped me in various ways: as an organic teacher, as a synthetic problem solver, as a landlord and so many other ways. I also extend my gratefulness to Dr. Douglas C. Neckers for supporting me in my class work.

I highly appreciate Dr. Grygori Zyryanov who encouraged and motivated me in the field of synthetic organic chemistry. He introduced me to new synthetic and characterization techniques.

I would like to say thanks to my lab mates Dr. Shin-ya Takizawa, Dr. Pavel Savechenkov, and Cesar Perez for their useful guidance, helpful discussions and continuous support.

I would also like to say thanks to all the members of the OLED team and the Sensor team for their generous help and kind support which made the research work very enjoyable.

I wish to thank Nora Cassidy, Alita Frater, Folake Hannan, Lisa Rood, Jackie Otiso, Mary Toth, Larry Ahl, Craig Bedra, D.Y Chen, and Jedrzej Romanowicz for all the administrative and instrumental help in the department.
Last but not the least, I wish to extend my deepest gratefulness to my parents and family members for years of love and support without whom this work would have never be accomplished.
# TABLE OF CONTENTS

## PART 1

1.1 Background  
1.2 Introduction  
1.3 Fluorescence and Phosphorescence  
1.4 History of Electroluminescent Devices  
1.5 Structure and Working Principle of OLED Devices  
1.6 References

## PART 2

2.1 Background  
2.2 Introduction  
2.3 Materials and Methods  
2.4 Results and Discussion  
2.4.1 Synthesis Pathway for Precursor  
2.4.2 Synthetically Targeted Substituted Tetrazines  
2.4.3 Experimental  
2.5 Photo Physical Studies  
2.6 Quantitative NMR Studies  
2.7 References

## PART 3

3.1 Conclusion  
3.2 Future Directions of the Research
PART 1:

1.1 Background:

The discovery of electroluminescence from organic compounds (small molecules or polymers) has led to the development of an intense interest in the field of organic optoelectronics. Small organic materials that can be used in Organic Light Emitting Diodes (OLED), sensors, and emissive semiconductors with characteristic photo physical and electronic properties. Recently OLEDs have attracted significant attention because of the advantages they can offer in the fabrication of lighting devices and imaging devices such as television screens, flat computer displays, and flexible displays. The main advantage in comparison to traditional technologies is the high luminescence efficiency that can be achieved, the lower driving voltage required, and the lack of a back light source, which allows for the production of thinner devices and lower battery power consumption.

The potential importance of this technology is also obvious from the growth of the OLED market. In 2007 the world’s OLED market was estimated to be approximately $1.7 billion, and predictions are that it would hit $10.9 billion by 2012 and 15.5 billion by 2014. As of today OLED technology has found commercial applications mainly for displays in consumer electronics such as in TV, digital cameras, and displays in cars.
1.2 Introduction:

In the OLED device, light is generated in the process of electroluminescence. This phenomenon arises from the electron-hole recombination that occurs when a voltage is applied to semiconductor materials sandwiched between two electrodes. The semiconductor materials are deposited in OLEDs as layers. These layers are hole transport layer, emissive layer, and electron transport layer. Indium tin oxide (ITO) deposited on a glass substrate is usually used as an anode, while the cathode is usually aluminum.

When a voltage is applied to this sandwich device, electrons are injected from the cathode into the electron-transport layer, while holes are injected into the hole-transport layer. These charges travel through the respective transport layers until a recombination process occurs. When the electrons and holes combine they create excited states in the emissive material that subsequently decay with emission of light.\textsuperscript{1-2}

OLED devices are fabricated by vapor-deposition process especially in small molecules while in polymers they are deposited by wet techniques like spin coating, inkjet printing and screen printing. The small molecules, in order to be considered useful
OLED materials should be thermally stable, highly fluorescent in the solid state, should be deposited uniformly as films by vacuum evaporation, and preferably also be capable of transporting electrons.

In general, there are two main types of emissive materials used in OLED applications: conjugated polymers and metallocomplexes (low molecular weight) which include organic chromophores or metallocomplexes.¹⁻²

1.3 Fluorescence and Phosphorescence:

![Jablonski Diagram](image)

**CHARACTERISTIC TIMES**

<table>
<thead>
<tr>
<th>Process</th>
<th>Time Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorption</td>
<td>$10^{-15}$ s</td>
</tr>
<tr>
<td>Vibrational relaxation</td>
<td>$10^{-12}$ - $10^{-10}$ s</td>
</tr>
<tr>
<td>Lifetime of the exited state $S_1$</td>
<td>$10^{-10}$ - $10^{-7}$ s - fluorescence</td>
</tr>
<tr>
<td>Intersystem crossing</td>
<td>$10^{-10}$ - $10^{-8}$ s</td>
</tr>
<tr>
<td>Internal conversion</td>
<td>$10^{-11}$ - $10^{-9}$ s</td>
</tr>
<tr>
<td>Lifetime of the excited state $T_1$</td>
<td>$10^{-6}$ - 1 s - phosphorescence</td>
</tr>
</tbody>
</table>
1.4 History of Electroluminescent Devices:

The first electroluminescent (EL) experiment was reported in 1965 by Helrich et al. The luminescence was quite inefficient requiring a drive voltage of more than 100 V to achieve significant light output. Later Vincent et. al. attempted to reduce the EL drive voltage below 30 V by using a thin organic film, but the quantum efficiency of the EL diodes was only about 0.05%. Significant progress on the performance of EL in devices was made by Tang and VanSlyke in 1987. They used aluminum (III) tris (8-quinolinolate) (Alq3) as an emissive and electron transport layer to obtain an external quantum efficiency of 19%. Three years later Holmes et. al. reported that the conjugated polymer, poly(p-phenylenevinylene) could be used as a polymer emitting layer in an EL device. Though the Holmes group obtained a low efficiency, they opened the door for the use of polymers in EL devices.

![Fig.3 Tang and VanSlyke's configuration of EL cell and molecular structure](image)

Recently, EL based devices have begun to obtain a small market share. OLED based displays include those in cell phones, i-pods, computers, televisions and other lighting applications.

1.5 Structure and Working Principle of OLED Devices:

The basic OLED consists of a stack of thin organic layers sandwiched between a transparent anode and a metallic cathode. The organic layers comprise a hole injection layer, a hole transport layer, an emissive layer, and an electron transport layer. When an
appropriate voltage (typically between 2 and 10 volts) is applied to the cell, the injected positive and negative charges recombine in the emissive layer to produce light (electroluminescence). The structure of the organic layers and the choice of anode and cathode are designed to maximize the recombination process in the emissive layer, thus maximizing the light output from the OLED device. Holes are injected from the transparent anode, typically transparent indium/tin oxide. Electrons are injected from a low work function cathode, typically aluminum or calcium.

**Fig. 4** General OLED Configuration adapted from reference 5.

Organic materials have been considered for the fabrication of practical EL devices because they have high fluorescence/phosphorescence quantum efficiency in red, green and blue regions and hence they are well suited for multi color display applications.
On applying voltage across the OLEDs electrical current flows from the cathode to the anode through the organic layers. The cathode gives electrons to the emissive layer of organic molecule. The anode removes electrons from the conductive layer deposited with organic molecules which is equivalent to giving electron holes to the conductive layer. At the boundary between the emissive layer and the conductive layers, electrons finds electron holes. When an electron finds an electron hole, the electron fills the hole and the electron gives up energy which excites molecules in the emissive layer and when these molecules return to the ground state they emit a photon of light. The color of the light depends on the HOMO-LUMO gap of organic compound used in the emissive layer.

A major challenge in OLEDs development is tuning the devices such that holes and electrons meet in equal quantities in the emissive layer. The mobility of the holes (i.e. carbocationic charges) is much lower than that of electrons (carboanionic charges) in the organic compounds. Light emission can occur from either singlet or triplet excitons. The
singlet to triplet formation is one to three. The overall efficiency of fluorescent OLEDs is less than that of phosphorescent OLEDs.

Improvement in the efficiency and the stability (lifetime) of EL devices and the tuning of color using different emitting materials still remains a challenge. Considerable advances must also be made for full color displays which require three primary colors, i.e. blue, green, and red emitting materials. Due to a high band gap energy, blue emitting materials have a low affinity for the electrons from the cathode in OLEDs. The design and synthesis of blue emitting materials suitable for the fabrication of stable OLEDs remain major obstacles. The working lifetime of the blue emitters is very low (7,000 hours) as compared to green (40,000 hours) and red (80,000 hours) emitters.

1.6 Reference:


PART 2:

2.1 Background:

Tetrazines are heteroaromatic compounds in which four nitrogens are present in a benzene like six membered ring. Depending on the arrangement of nitrogens in the ring there are three major isomers, 1,2,3,4-tetrazine, 1,2,3,5-tetrazine and 1,2,4,5-tetrazine, of which 1,2,4,5-tetrazine is the most common. The three isomers along with numbering of the atoms are shown in Fig. 6.

![Fig.6 Structure of Tetrazines](image)

Two substituents can be located on the two carbon atoms of the ring and can be identical (symmetrical or s-tetrazines) or different (unsymmetrical or u-tetrazines). The substituent’s include alkyl, alkenyl, alkynyl, aryl, heteroaryl, alkoxy, alkylmercapto- and other heteroatom. If s-tetrazine is considered as a benzene derivative with four CH groups replaced by nitrogen atoms, the symmetry is lowered from $D_{6h}$ to $D_{2h}$ or $D_{3h}$.

Tetrazines are bright, colorful compounds and their stability depends on the attached substituents. Simple aliphatic and even some aromatic substituted examples are not sufficiently stable thermally and/or photochemically. Tetrazines are fluorescent both in solid and solution phase. So if we are able to increase their thermal and photochemical stability, by increase their melting point, and increase their quantum yields, both in solution and solid state, then they can become prominent emissive materials for organic light emissive devices.
2.2 Introduction:

1,2,4,5-Tetrazine compounds were first synthesized more than 100 years ago and have gained much attention from chemists during the last decades. Several synthetic methods have been developed and many applications have been found in various fields such as organic synthesis, crop protection, pyrotechnics (high nitrogen content energetic materials), etc. Very recently, research to obtain new compounds for their optical and electrochemical properties has become increasingly active because these compounds have a huge potential especially in sensor applications.

This search for new compounds with particular and unusual optical and electrochemical properties is still very active, given their huge potential as emissive layers and building sensors. The fluorescence and reversible electroactivity are particularly interesting properties in this respect, because fluorescent and/or electroactive molecules may be quenched within a given time scale and therefore lead to sensing components dependent on the quenching agent. The tetrazine family appears a very promising and fascinating class of compound for this purpose. They are highly colored and reversibly electro active heterocycles. They display the following special properties:

They have a very high electron affinity, which make them reducible at high to very high potentials (actually they are among the electron poorest heterocycles),

They have a low lying π* orbital resulting in an n- π* transition in the visible light range. In addition to this all the tetrazine family compounds are fluorescent in solution and are also fluorescent in the crystalline state. This behavior places them among the smallest crystalline organic fluorophores in the visible range ever prepared, and therefore makes them especially attractive for potential applications as sensors.
In detail the most distinct character of tetrazines is their high electron affinity, which results from the replacement of four CH group by four more electronegative nitrogen atoms on the prototypical aromatic ring. In fact they are most electron poor C-N heterocycles\textsuperscript{12-15} and consequently are reduced at high to very high potentials (-0.8 ~ -0.4 V vs Ag\textsuperscript{+}/Ag). The other remarkable character of tetrazine is their low lying $\pi^*$ orbital resulting in n- $\pi^*$ transition in the visible range.

Two important requirements for an organic compound to be emissive material in OLED devices mainly are,

1. The organic compound must be fluorescent in solid and solution state.
2. It also should have high quantum yield of fluorescence along with good stability.

Substituted tetrazines are fluorescent in solid and solution state, and they also have high quantum yields, especially mono substituted one. But they are not sufficiently stable at relatively high temperatures because they tend to decompose easily. So in order to make them stable it is important to use a substituent which will make them stable and raise the melting point while at the same time enhance or at least not badly affect the fluorescent properties and quantum yields.

2.3. Materials and Methods:

Mass spectra were recorded on a Shimadzu GCMS-QP5050A instrument with a direct probe (ionization 70 ev). Matrix assisted laser desorption ionization (MALDI) spectra were obtained on a Bruker Daltonic Omniflex instrument (N₂ laser, 337 nm). Melting points were uncorrected. Bruker NMR spectrometers (working frequency 300.0 or 500.0 MHz for 1H) were used to record the NMR spectra. CDCl₃, DCM-d₂, DMSO-d₆, and Pyridine-d₅ were the solvents used for NMR, and chemical shifts relative to tetramethylsiane at 0.00 ppm are reported in parts per million (ppm) on the δ scale. Absorption and fluorescence spectra were recorded on a Shimadzu UV-2401 spectrophotometer and a Fluoroog-3 spectrometer, respectively. All measurements were carried out at room temperature unless otherwise specified.
2.4. Results and Discussion:

2.4.1. Synthesis Pathway for Precursor (3,6-Dichloro-1,2,4,5-tetrazine):

![Synthetic scheme](image)

- **a)** $\text{H}_2\text{N}-\text{NH}_2\text{H}_2\text{O}, 1,4$-dioxane, 100°C
- **b)** Pentane-2,4-dione, H$_2$O, R.T. for 30 min then 70°C for 4 hrs.
- **c)** NO$_2$, NMP, R.T., 30 min.
- **d)** 2$\text{H}_2\text{N}-\text{NH}_2\text{H}_2\text{O}, \text{CH}_3\text{CN}, 85^\circ\text{C}, 20$ min.
- **e)** 1,3,5-Trichloro-[1,3,5]triazinane-2,4,6-trione, Acetonitrile, 0°C

Fig. 8 Synthetic scheme, reagents and conditions.

**Triaminoguanidine Monohydrochloride:**

![Synthetic scheme](image)

Hydrazine monohydrate (34.1 g, 0.68 mole) was added to a slurry of guanidine hydrochloride (19.1 g, 0.20 mole) in 1,4-dioxane (100 ml) while stirring. The mixture was heated under reflux for 2 hours. After that time the mixture was cooled to ambient temperature, the product was collected by filtration, washed with 1,4-dioxane, and dried to give pure triaminoguanidine monohydrochloride, (98%)$^{17}$, m.p. 240°C (lit. m.p. 238-240°C); $^{13}$C NMR (D$_2$O): $\delta$ 159.61.
3,6-Bis(3,5-dimethylpyrazol-1-yl)-1,2-dihydro-1,2,4,5-tetrazine:

\[
N\text{H}_2N\text{-NH}_2 + O\text{C}-\text{O} \rightarrow \text{H}_2\text{O}
\]

R.T. for 30 min. then 70°C for 4 hrs.

Fig. 10 Synthesis of 3,6-bis(3,5-dimethylpyrazol-1-yl)-1,2-dihydro-1,2,4,5-tetrazine.

To a solution of triaminoguanidine monohydrochloride (7.03 g, 0.05 mole) in water (50 ml) was added 2,4-pentanedione drop wise while stirring at 25°C. After the mixture had been stirred for 30 min., it was heated at 70°C for 4 hours. During that time a solid precipitated from the solution. The product was filtered from the cooled mixture, washed with water, and dried to yield of crude which was purified by recrystallization using hot ethanol to give pure 3,6-bis(3,5-dimethylpyrazol-1-yl)-1,2-dihydro-1,2,4,5-tetrazine, (85%), m.p. 151°C (lit. m.p. 149-150°C)\(^{17}\); \(^1\)H nmr (CDCl\(_3\)): \(\delta\) 2.25 (s, 6H), 2.51 (s, 6H), 5.95 (s, 2H), 8.09 (bs, 2H); \(^13\)C nmr (CDCl\(_3\)): \(\delta\) 13.47, 13.79, 109.86, 142.30, 145.78, 149.95.

3,6-Bis(3,5-dimethylpyrazol-1-yl)-1,2,4,5-tetrazine:

\[
N\text{H}_2N\text{-NH}_2 + \text{NO}_2 \rightarrow \text{NMP, R.T., 30 min.}
\]

Fig. 11 Synthesis of 3,6-bis(3,5-dimethylpyrazol-1-yl)-1,2,4,5-tetrazine.

A slurry mixture of 3,6-bis(3,5-dimethylpyrazol-1-yl)-1,2-dihydro-1,2,4,5-tetrazine (13.06 g, 0.05 mole) in 50 ml 1-methyl-2-pyrrolidinone was stirred for 15 min. \(\text{NO}_2\) gas was generated by dissolving 10 g. of Copper wire in 10 ml of concentrated HNO\(_3\). The gas was passed through it for 30 min. A color change was observed from pale yellow-orange to red. Ice cold water was added to the solution which was then filtered to obtain of 3,6-bis(3,5-dimethylpyrazol-1-yl)-1,2,4,5-tetrazine, (99%), m.p. 226°C (lit. m.p. 222-
224°C$^{17}$; $^1$H nmr (CDCl$_3$): $\delta$ 2.40 (s, 6H), 2.75 (s, 6H), 6.20 (s, 2H), $^{13}$C nmr (CDCl$_3$): $\delta$ 13.86, 14.65, 111.89, 143.78, 154.47, 159.33.

3,6-Dihydrazino-1,2,4,5-tetrazine:

![Fig. 12](image)

To a slurry of 3,6-bis(3,5-dimethylpyrazol-1-yl)-1,2,4,5-tetrazine (13.5 g, 0.05 mole) in acetonitrile (150 ml) was added hydrazine hydrate (5.5 g, 0.11 mole) drop wise at room temperature. After the addition was completed, the mixture was refluxed for 20 minutes. The mixture was then cooled to room temperature, filtered, and washed with acetonitrile. It was then recrystallized using DMSO and methanol to give 3,6-dihydrazino-1,2,4,5-tetrazine as brown powder$^{17}$ (25%), m.p. 160°C (lit. m.p. 158-159°C$^{17}$); $^{13}$C nmr (DMSO$_d_6$): $\delta$ 163.84.

3,6-Dichloro-1,2,4,5-tetrazine:

![Fig. 13](image)

To a slurry of 3,6-di(hydrazine)-1,2,4,5-tetrazine (12.5 g, 0.088 mol) in acetonitrile (350 ml) at 0°C was added a solution of trichloroisocyanuric acid (40.8 g, 0.18 mol) in acetonitrile (250 ml) drop wise over 30 minutes. After the addition was finished the reaction vessel was allowed to warm to room temperature and stirred for 20 minutes. The
white insoluble precipitate was removed by filtration and the volatiles removed from the resulting orange solution under vacuum to give crude 3,6-dichloro-1,2,4,5-tetrazine as an orange solid. Sublimation under nitrogen at 70°C using a cold finger at -78°C gave 3,6-dichloro-1,2,4,5-tetrazine as orange powder, (51%), m.p. 150°C; (lit. m.p. 151-152°C); $^{13}\text{C nmr (CDCl}_3\text{): } \delta 168.10$. 
2.4.2 Synthetically Targeted Substituted Tetrazines:

Fig. 14 Synthesized Target Compounds.
2.4.3 Experimental:

3-Chloro-6-methoxy-1,2,4,5-tetrazine (I):

![Chemical structure of 3-Chloro-6-methoxy-1,2,4,5-tetrazine](image)

Fig. 15 Synthesis of 3-chloro-6-methoxy-1,2,4,5-tetrazine.

To 40 ml of anhydrous methanol was added activated MgSO₄. The mixture was stirred for 15 minutes. 3,6-Dichloro-1,2,4,5-tetrazine (0.4 g, 2.6 mmol) was added and this reaction mixture was stirred for 1 hour. The MgSO₄ was removed by filtering and the solvent was evaporated. Purification was done by a chromatographic column with petroleum ether: ethyl acetate (8:2) as eluents. The column yielded pure 3,6-dichloro-1,2,4,5-tetrazine (67%) as an orange powder, (67%), m.p. 63°C; ¹H nmr (CDCl₃): δ 4.35 (s, 3H), ¹³C nmr (CDCl₃): δ 57.35, 164.53, 166.97.

3,6-dimethoxy-1,2,4,5-tetrazine (II):

![Chemical structure of 3,6-dimethoxy-1,2,4,5-tetrazine](image)

Fig. 16 Synthesis of 3,6-dimethoxy-1,2,4,5-tetrazine.

3,6-Dichlorotetrazine (0.305g, 0.002 mol) was dissolved in 20 ml acetonitrile and to this solution was added a solution of sodium methoxide (0.227g, 4.2 mmol) in 4 ml anhydrous methanol were added drop wise. After 3 hour stirring the solvent was evaporated and the crude product was purified by silica gel column chromatography using dichloromethane as an eluent to afford pink crystals. (85%), m.p. 62-63°C. ¹H NMR (CDCl₃): δ 4.26 (s, 6H), ¹³C NMR (CDCl₃): δ 56.35, 166.01.
**3,6-Di-tert-butoxy-1,2,4,5-tetrazine (3):**

![Synthesis of 3,6-di-tert-butoxy-1,2,4,5-tetrazine.](image)

3,6-Dichlorotetrazine (0.2g, 0.00132 mol) was dissolved in 20 ml dry THF. To this solution was added a solution of potassium t-butoxide (0.296g, 2.64 mmol). After 30 min. stirring the solvent was evaporated and the crude product was purified by silica gel column chromatography using dichloromethane as eluent affording pinkish violet oil (85%), $^1$H NMR (CDCl$_3$): $\delta$ 1.68 (s, 18H), $^{13}$C NMR (CDCl$_3$): $\delta$ 27.62, 84.18, 164.88.

**3-Chloro-6-benzoxy-1,2,4,5-tetrazine (4):**

![Synthesis of 3-benzoxy-6-chloro-1,2,4,5-tetrazine.](image)

Sodium bicarbonate (0.036g) and magnesium sulfate (0.07g) along with a stir bar were added to an overnight dried pressure resistant reaction tube. Benzyl alcohol (3.41 ml 33 mmol) and dry DCM (5 ml) were added and the solution was stirred for 15 min. 3,6-dichlorotetrazine (0.5g, 3.3 mmol) was then added into it and the reaction mixture were heated to 100°C for 2 hr. then cooled to room temperature. The solvent was evaporated and the crude product was purified by silica gel column chromatography using
dichloromethane. Recrystallization from cyclohexane afforded in 57% yield, as a pink solid, m.p. 90-92°C. $^1$H NMR (CDCl$_3$): $\delta$ 5.72 (s, 2H), 7.37-7.48 (m, 3H), 7.52-7.62 (dd, 2H) $^{13}$C NMR (CDCl$_3$): $\delta$ 72.06, 128.84, 128.88, 133.69, 164.45, 166.5.

**3,6-Dibenzoxy-1,2,4,5-tetrazine (5):**

![Synthesis of 3,6-dibenzoxy-1,2,4,5-tetrazine.](image)

3,6-Dichlorotetrazine (0.1g, 0.66 mmol), benzyl alcohol (0.068 ml 0.66 mmol) and sodium hydride (60% in oil) (0.026g, 0.66 mmol) in THF (25 ml) were mixed together and the solution was refluxed for 4 days. It was then cooled to room temperature. The solvent was evaporated and the crude product was purified by silica gel column chromatography using dichloromethane affording pink solid. (45%), m.p. 118-120°C. $^1$H NMR (CDCl$_3$): $\delta$ 5.63 (s, 4H), 7.33-7.49 (m, 6H), 7.50-7.60 (dd, 4H) $^{13}$C NMR (CDCl$_3$): $\delta$ 71.15, 128.55, 128.72, 128.82, 134.65, 165.95.

**3-Chloro-6-ethoxy-1,2,4,5-tetrazine (6):**

![Synthesis of 3-chloro-6-ethoxy-1,2,4,5-tetrazine.](image)
A mixture of 20 ml anhydrous ethanol and activated MgSO₄ was stirred for 15 minutes, then 3,6-dichloro-1,2,4,5-tetrazine (0.2g, 1.3 mmol) was added and the reaction mixture was stirred for 1 hour. The MgSO₄ was removed by filtration and the solvent was evaporated. Purification by chromatography using dichloromethane as eluent yielded pure 3-chloro-6-ethoxy-1,2,4,5-tetrazine as an orange powder, (75%), m.p. 65°C; ¹H NMR (CDCl₃): δ 1.58-1.62 (t, 3H), 4.73-4.78 (q, 2H) C NMR (CDCl₃): δ 14.2, 67.0, 164.2, 166.6.

3-Chloro-6-(8-quinolinoxy)-1,2,4,5-tetrazine (7):

![Fig. 21 Synthesis of 3-chloro-6-(8-quinolinoxy)-1,2,4,5-tetrazine.]

To a previously dried pressure resistant tube along with 0.1g of MgSO₄ was added 0.025g of NaHCO₃ and (0.24g, 1.65 mmol) of 8-hydroxyquinoline. After adding 20 ml of dry DCM and stirring for 15 min. under nitrogen atmosphere, 3,6-dichloro-1,2,4,5-tetrazine (0.22g, 1.46 mmol) was added and this reaction mixture was stirred overnight. The MgSO₄ was removed by filtration and the solvent was evaporated. Recrystallization from cyclohexane afforded a pink solid. (64%), m.p. 153-154°C; ¹H NMR (CDCl₃): δ 7.39-7.47 (dd, 1H), 7.60-7.70 (dd, 1H), 7.73-7.80 (dd, 1H), 7.81-7.89 (dd, 1H), 8.20-8.27 (dd, 1H), 8.62-8.71 (dd, 1H) C NMR (CDCl₃): δ 120.4, 122.1, 126.6, 126.4-127.3, 130.0, 136.4, 139.5, 148.3, 150.3, 164.9, 168.5.

3-Chloro-6-isopropoxy-1,2,4,5-tetrazine (8):
To 20 ml of anhydrous isopropyl alcohol was added activated MgSO₄. After stirring for 15 minutes, 3,6-dichloro-1,2,4,5-tetrazine (0.2g, 1.3 mmol) was added and the reaction mixture was stirred for 1 hour. The MgSO₄ was removed by filtration and the solvent was evaporated. Purification by chromatography (DCM eluent) yielded pure 3-chloro-6-isopropoxy-1,2,4,5-tetrazine as an orange liquid, (60%), ¹H NMR (CDCl₃): δ 1.55 (d, 6H), 5.54 (m, 1H) ¹³C NMR (CDCl₃): 21.5, 75.0-75.5, 164.0, 166.5.

3-Chloro-6-sec-butoxy-1,2,4,5-tetrazine (9):

To 20 ml of anhydrous 2-butanol was added activated MgSO₄. After stirring for 15 minutes, 3,6-dichloro-1,2,4,5-tetrazine (0.2g, 1.3 mmol) was added and the reaction mixture was stirred overnight. The MgSO₄ was removed by filtration and the solvent was evaporated. Purification by chromatography with dichloromethane as eluent yielded pure 3-chloro-6-isobutoxy-1,2,4,5-tetrazine as an orange liquid, (45%), ¹H NMR (CDCl₃): δ 1.00-1.07 (t, 3H), 1.46-1.51 (d, 3H), 1.76-2.1 (m, 2H), 5.29-5.41 (m, 1H) ¹³C NMR (CDCl₃): 18.3, 18.9, 26.8, 72.5, 164.3, 166.7.

3-Chloro-6-isobutoxy-1,2,4,5-tetrazine (10):
To 20 ml of anhydrous isobutyl alcohol was added activated MgSO₄, after stirring for 15 minutes, 3,6-dichloro-1,2,4,5-tetrazine (0.2g, 1.3 mmol) was added and the reaction mixture was stirred overnight. The MgSO₄ was removed by filtration and the solvent was evaporated. Purification by chromatography with dichloromethane as eluent yielded pure 3-chloro-6-isobutoxy-1,2,4,5-tetrazine as an orange liquid, (55%), ¹H NMR (CDCl₃): δ 1.06-1.18 (d, 6H), 2.15-2.35 (m, 1H), 4.28-4.6 (d, 2H) ¹³C NMR (CDCl₃): 18.9, 27.8, 73.9, 164.2, 166.8.

3,6-Diisobutoxy-1,2,4,5-tetrazine (11):

To 20 ml of anhydrous isobutyl alcohol was added activated MgSO₄ and after stirring for 15 minutes, 3,6-dichloro-1,2,4,5-tetrazine (0.2g, 1.3 mmol) was added and stirring at reflux temperature was continued overnight. The MgSO₄ was removed by filtration and the solvent was evaporated. Purification by chromatography with dichloromethane as eluent yielded pure 3,6-diisobutoxy-1,2,4,5-tetrazine as an orange liquid, (80%), ¹H NMR
(CDCl₃): δ 1.06-1.15 (d, 12H), 2.20-2.33 (m, 2H), 4.24-4.46 (d, 4H) ¹³C NMR (CDCl₃): 19.0, 27.9, 75.8, 166.1.

1,1′:4′,1″-Terphenyl-4-ol:

![Synthesis of 1,1′:4′,1″-Terphenyl-4-ol.](image)

4-Bromophenol (0.2g, 1.2 mmol) and 4-biphenylboronic acid (0.336g, 1.7 mmol) were dissolved in 5 ml of dry DMF. Nitrogen gas was purged through it for 10 min. while stirring, then 1.75 ml of 2N potassium carbonate solution was added to it and again the reaction mixture was purged through nitrogen gas for 10 min. Then Pd₂(dba)₃ (0.06g, 0.065 mmol) was added to the reaction mixture. It was then heated to 70°C for 36 hours, the suspension was cooled, poured into 50 ml of 1 N NH₄Cl solution, and extracted with 50 ml of EtOAc twice. The combined organic layers were washed with brine, dried with sodium sulphate and filtered. Impurities were removed by recrystallization by using Ethanol. The residue was washed again with ethanol and the solvent was evaporated to yield white solid. (64%), m.p. >260°C ¹H NMR (DMSO-d₆): δ 6.73-7.00 (d, 2H), 7.35-7.43 (m, 1H), 7.44-7.53 (m, 2H), 7.53-7.62 (d, 2H), 7.62-7.8 (m, 6H), 9.63 (s, 1H) ¹³C NMR (DMSO-d₆): 115.8, 126.4, 126.5, 127.1, 127.4, 127.7, 129.0, 130.3, 138.05, 139.25, 139.85, 157.43.

3-Chloro-6-(4[p-biphenyl]phenoxy-1,2,4,5-tetrazine (12):
Fig. 27 Synthesis of 3-chloro-6-terphenyloxy-1,2,4,5-tetrazine.

3,6-Dichlorotetrazine (0.1g, 0.66 mmol), terphenylalcohol (0.16g, 0.66 mmol) and sodium bicarbonate (0.052g) in THF (25 ml) were added together and the solution was refluxed for 72 hours. It was then cooled to room temperature, the solvent was evaporated and the crude product was purified by silica gel column chromatography using dichloromethane to afford an orange solid. (67%), m.p. > 260°C. $^1$H NMR (DMSO$_d_6$): $\delta$

7.37-7.43 (t, 1H), 7.47-7.57 (dd, 4H), 7.72-7.77 (d, 2H), 7.78-7.86 (dd, 4H), 7.87-7.94 (d, 2H) $^{13}$C NMR (CDCl$_3$): Couldn’t get it because of poor solubility.

**Di-9,9’-Carbazolyl-3,6-tetrazine (13):**

Fig. 28 Synthesis of di-9,9’-carbazolyl-3,6-tetrazine.

3,6-Dichlorotetrazine (0.1g, 0.66 mmol), carbazole (0.22g, 1.32 mmol) and sodium hydride (60% in oil) (0.052g, 1.32 mmol) in THF (25 ml) were added together and the solution was refluxed overnight. After cooling to room temperature, the solvent was evaporated and the crude product was purified by silica gel column chromatography using dichloromethane to afford a red solid. (89%), m.p. > 260°C. $^1$H NMR (CDCl$_3$): $\delta$
7.41-7.745 (dd, 4H), 7.60-7.64 (dd, 4H), 8.19-8.22 (d, 4H), 8.86-8.99 (d, 4H) $^{13}$C NMR (CDCl$_3$): Couldn’t get it because of poor solubility.
2.5 Photo Physical Studies:

Absorption Spectra:

Fig. 29 UV-Absorption spectra of 3-chloro-6-methoxy-1,2,4,5-tetrazine.

Emission Spectra:

Fig. 30 Emission spectra of 3-chloro-6-methoxy-1,2,4,5-tetrazine.
The UV/visible spectra of 3-Chloro-6-methoxy-1,2,4,5-tetrazine were recorded in dichloromethane solution. The absorption maxima obtained at 327 nm corresponds to a $\pi$- $\pi^*$ transition, and the less intense band in the visible region at 520 nm, corresponds to an n- $\pi^*$ transition, which is responsible for the orange color of the compound. The florescence spectra of 3-Chloro-6-methoxy-1,2,4,5-tetrazine was recorded in solution, and the maximum emission wavelength was found to be 567 nm. It will be interesting to study fluorescence in the solid state.

Study of absorption and emission spectra along with measurements of quantum yield and lifetimes were performed for all of the mono and di-substituted tetrazine derivatives. For quantum yield measurements Rhodamine-B was used as an internal standards.

<table>
<thead>
<tr>
<th>Compound (in DCM)</th>
<th>$\lambda_{abs.}$</th>
<th>$\lambda_{emi.}$</th>
<th>Quantum Yield ($\Phi$) %</th>
<th>Lifetime ($\tau_F$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,6-dichlorotetrazine</td>
<td>229-307-514</td>
<td>560</td>
<td>11</td>
<td>58.42</td>
</tr>
<tr>
<td>Methoxychlorotetrazine</td>
<td>269-327-520</td>
<td>567</td>
<td>30</td>
<td>159</td>
</tr>
<tr>
<td>Dimethoxytetrazine</td>
<td>275-345-524</td>
<td>570</td>
<td>10</td>
<td>45.72</td>
</tr>
<tr>
<td>Ethoxychlorotetrazine</td>
<td>227-329-521</td>
<td>565</td>
<td>31</td>
<td>157.63</td>
</tr>
<tr>
<td>Isoproxychlorotetrazine</td>
<td>229-331-524</td>
<td>562</td>
<td>33</td>
<td>153.77</td>
</tr>
<tr>
<td>Isobutoxychlorotetrazine</td>
<td>229-329-521</td>
<td>567</td>
<td>30</td>
<td>150</td>
</tr>
<tr>
<td>Sec.butoxychlorotetrazine</td>
<td>229-332-523</td>
<td>560</td>
<td>43</td>
<td>152</td>
</tr>
<tr>
<td>Quinolinoxychlorotetrazine</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Benzyloxychlorotetrazine</td>
<td>229-328-521</td>
<td>565</td>
<td>16</td>
<td>67.14</td>
</tr>
<tr>
<td>Dibenzyloxytetrazine</td>
<td>229-345-527</td>
<td>571</td>
<td>11</td>
<td>51.21</td>
</tr>
<tr>
<td>Cz-Tetrazine-Cz</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>----------------------</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Terphenyloxychlorotetrazine</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Diterterbutoxychlorotetrazine</td>
<td>229-347-532</td>
<td>590</td>
<td>11</td>
<td>70.77</td>
</tr>
</tbody>
</table>

*Compound is not emissive in solution state.

2.6 Quantitative NMR Spectral Studies:

$^1$H decoupled $^{13}$C experiment without NOE (Inverse Gated) for integration (C13IG):

Sometimes, the integrals of the carbon signals becomes of interest e.g. to determine the number of equivalent carbon atoms in the molecule. In this case, the NOE (Nuclear Overhauser Effect) enhancement- which may be quite different for the C atoms in one molecule- must be totally relaxed before acquisition. The NOE transfer takes place during decoupling and thus a very long recycle delay d1 is needed between scans, where no decoupling is applied. Decoupling is applied however during acquisition to collapse the multiplets into singlets and thus gain signal to noise. The parameter set for this “inverse gated” experiment is called C13IG and the pulse program used is zgig. $^1$H decoupling is executed during acquisition only, using a composite pulse decoupling (CPD) scheme defined by parameter cpd2. The CPD sequence which is used for 1H decoupling is the WALTZ-16 sequence. The decoupling power is defined by the parameter pl12. The value for pl12 should be entered in the edprosol table by the NMR user and are called up in the experiment as usual by typing getprosol. The recycle delay d1 has to be about 30-60 seconds long in order to get rid of partial NOE enhancement and to obtain reliable integration information.$^{21,22}$
The integrals of a $^{13}$C spectrum with inverse gated $^1$H decoupling (C13IG) yield valuable information on the number of equivalent C atoms per peak. It has to be stated though, that the spectrum has to be acquired with a long enough, recycle delay $d_1$ in order to accumulate the signals of fully relaxed $^{13}$C atoms. The other important reason for long $d_1$ times is to make sure that the NOE enhancement- which is built up during the decoupling during the acquisition- is fully decayed. Only then, the integration values will make sense. Using the zgig pulse program with $90^\circ$ pulses, $aq+d_1$ should be about $5*T_1(^{13}$C), where $T_1$ is the spin-lattice relaxation time of $^{13}$C. For typical small organic molecules $T_1$ values vary between a few hundred milliseconds and several seconds (especially quaternary carbon atoms have long $T_1$ times). The zgig30 pulse program which uses $30^\circ$ pulse can be used with shorter $d_1$ values. Nevertheless, a good estimation is to use $d_1$ values of 30-60 seconds.$^{21,22}$
Fig. 32 Quantitative NMR spectra of Benzoxychlorotetrazine and Dibezoxytetrazine C13IG (inverse gating).
The NMR result obtained for compound 5 and 6 are shown above. The recycle
delay time $d_1$ used was 20 sec and the flip angle $p_1$ was 7$\mu$s i.e. 21° for 5 and 10$\mu$s (30°)

2.7 References:
1. Sauer, J. 1,2,4,5-Tetrazines. In Comprehensive Heterocyclic Chemistry; Boulton, A. J.
   2001, 137, 265190.
12. A. Weissberger (Consulting Editor), The Chemistry of Heterocyclic Compounds, A
    Series of Monographs: The 1,2,3- and 1,2,4- triazines, tetrazines and pentazines,
tel-00160587, version 1 - 6 Jul 2007


PART 3:

3.1 Conclusion:

A series of novel substituted tetrazines were successfully synthesized and characterized. Different types of reactions like aromatic nucleophilic substitution and Suzuki couplings were successfully performed. Also reaction conditions were optimized within the parameters of time, temperature, and reagent ratio in order to obtain the desired product in good yield.

Characterization was performed by using standard characterization techniques like Melting Point (MP), $^1$H NMR, $^{13}$NMR. Inverse gated $^{13}$C NMR experiment was for the first time successfully done to distinguish the mono and disubstituted tetrazines. Absorption and emission spectra were recorded in DCM. Emission spectra was found to be independent of excitation wavelength. Quantum yields ($\Phi$) and fluorescence lifetimes ($\tau_F$) were also successfully measured. Longer life times and relatively higher quantum yields were observed for mono substituted tetrazines. This may be due to their unsymmetrical nature which induces a dipole moment which is slightly stabilizing the singlet excited state via intramolecular charge transfer.
3.2 Future directions of the research:

The effect of solvent polarity on the lifetime and quantum yield will be interesting to study, since this can give an insight to the nature of intra molecular charge transfer. Femto second transient absorption studies will help in understanding the kinetics of the decay process.
Triaminoquainidine Monohydrochloride

Bruker

Acquisition parameters:

- Instrument: Bruker
- Frequency: 200 MHz
- Sample concentration: 100 mg/mL in DMSO-d6
- Temperature: 300 K

Spectroscopic data:

- Chemical shifts (ppm):
  - H1: 2.25
  - H2: 2.93
  - N: 1.67

- Coupling constants (Hz):
  - J1: 9.8 Hz
  - J2: 12.3 Hz

- Integration:
  - H1: 30
  - H2: 30
  - N: 30

Other parameters:

- Resolution: 0.05 ppm
- Signal to noise ratio: 800:1
- Peak width at half height: 0.3 Hz

Analysis:

- The spectrum shows the expected resonances for the triaminoquainidine moiety.
- The chemical shifts and coupling constants are consistent with the reported data for triaminoquainidine monohydrochloride.

Conclusion:

The spectrum is in good agreement with the known characteristics of triaminoquainidine monohydrochloride, confirming the chemical identity of the sample.
SV-27-H1-3,6-Bis[3,5-dimethylpyrazol-1-yl]-1,2-dihydro-1,2,4,5-tetrazine
3,6-Bis(3,5-dimethylpyrazol-1-yl)-1,2,4,5-tetrazine
C13-3,6-Bis[3,5-dimethylpyrazol-1-yl]-1,2,4,5-tetrazine
Ethoxycarbostyrilazine
### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>OLED</td>
<td>Organic Light Emitting Diode</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
</tr>
<tr>
<td>NLO</td>
<td>Nonlinear optical</td>
</tr>
<tr>
<td>HOMO</td>
<td>Highest Occupied Molecular Orbital</td>
</tr>
<tr>
<td>LUMO</td>
<td>Lowest Unoccupied Molecular Orbital</td>
</tr>
<tr>
<td>EtOAc</td>
<td>Ethyl Acetate</td>
</tr>
<tr>
<td>tert</td>
<td>Tertiary</td>
</tr>
<tr>
<td>sec</td>
<td>Secondary</td>
</tr>
<tr>
<td>ISC</td>
<td>Intersystem Crossing</td>
</tr>
<tr>
<td>ns</td>
<td>Nanosecond</td>
</tr>
<tr>
<td>s or sec</td>
<td>Second</td>
</tr>
<tr>
<td>min</td>
<td>Minute</td>
</tr>
<tr>
<td>h or hr</td>
<td>Hour</td>
</tr>
<tr>
<td>nm</td>
<td>Nanometer</td>
</tr>
<tr>
<td>M</td>
<td>Moles per Liter</td>
</tr>
<tr>
<td>ml</td>
<td>Milliliter</td>
</tr>
<tr>
<td>L</td>
<td>Liter</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>MHz</td>
<td>MegaHertz</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>-------------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>Kcal</td>
<td>Kilo Calorie</td>
</tr>
<tr>
<td>Conc.</td>
<td>Concentration</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin Layer Chromatography</td>
</tr>
<tr>
<td>GC</td>
<td>Gas Chromatography</td>
</tr>
<tr>
<td>MS</td>
<td>Mass Spectrometry</td>
</tr>
<tr>
<td>MP</td>
<td>Melting Point</td>
</tr>
<tr>
<td>°C</td>
<td>Degree Celsius</td>
</tr>
<tr>
<td>mol</td>
<td>Mole</td>
</tr>
<tr>
<td>mmol</td>
<td>Millimole</td>
</tr>
<tr>
<td>v or V</td>
<td>Volume</td>
</tr>
<tr>
<td>s</td>
<td>(for NMR) Singlet</td>
</tr>
<tr>
<td>d</td>
<td>(for NMR) Doublet</td>
</tr>
<tr>
<td>t</td>
<td>(for NMR) Triplet</td>
</tr>
<tr>
<td>q</td>
<td>(for NMR) Quartet</td>
</tr>
<tr>
<td>m</td>
<td>(for NMR) multiplet</td>
</tr>
<tr>
<td>UV</td>
<td>Ultra Violet</td>
</tr>
<tr>
<td>IR</td>
<td>Infra Red</td>
</tr>
<tr>
<td>S</td>
<td>Singlet Excited State</td>
</tr>
<tr>
<td>T</td>
<td>Triplet Excited State</td>
</tr>
<tr>
<td>hv</td>
<td>Photon</td>
</tr>
</tbody>
</table>