# STUDIES DIRECTED TOWARDS DEVELOPMENT OF NEW MOLECULAR DIAGNOSTICS


By

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**Submitted**

*in fulfilment of the requirement of the degree of*

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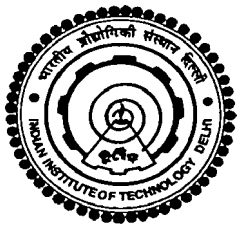
**to the**




**Indian Institute of Technology, Delhi**
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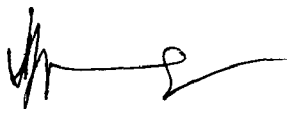
# CERTIFICATE

This is to certify that the thesis entitled "***STUDIES DIRECTED TOWARDS DEVELOPMENT OF NEW MOLECULAR DIAGNOSTICS***" being submitted by **Ms. Vandna Arora** to the Department of Chemistry, Indian Institute of Technology, Delhi, for the award of the degree of **Doctor of Philosophy** is a record of bonafide research work carried out by her.

Vandna Arora has worked under my guidance and supervision and has fulfilled the requirements for the submission of this thesis, which to my knowledge has reached the requisite standard.

The results contained in this thesis have not been submitted in part or in full to any other University or Institute for the award of any degree or diploma.



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# ACKNOWLEDGEMENTS

*The most popular of all Greek heroes, Hercules was the epitome of extraordinary strength and courage. Lesser mortals like me might possess a certain amount of the Herculean spirit, but definitely need support and guidance to achieve great heights. A fact that holds true for the thesis that you will read in the subsequent pages. It might seem to be an effort on my part to lay before the world my nature of work, but it would not have been possible had it not been for the constant support of my guide, my colleagues as well as my family.*

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# ABSTRACT

The thesis titled ***Studies Directed towards Development of New Molecular Diagnostics*** presents the research work carried out on the synthesis, characterization and evaluation of molecular receptor properties of some novel calix[4]arene derivatives. The research work embodied in the thesis has been divided into six chapters.

Calix[n]arenes constitute an important class of macrocyclic compounds that can serve as useful building blocks for designing molecular receptors for ionic and molecular recognition. **Chapter I** of the thesis presents an overview of the concepts of molecular recognition and the research work published on the synthesis, conformational analysis and application of calix[n]arenes during the past ten years with special emphasis on their applications in analytical chemistry and as sensor materials for various metal ions and neutral molecules.

**Chapter II** describes the synthesis and characterization of various regioselective formylated calix[4]arenes by using dichloromethylmethylether as the formylating agent and $TiCl_4$ and $SnCl_4$ as the catalysts. Optimization of various reaction parameters has been examined to achieve the synthesis of mono-, di-, tri- and tetraformylated derivatives of various alkoxycalix[4]arenes. Conformational analyses of these synthesized products have been achieved by $^1H$ NMR, NOESY, $^{13}C$ NMR and X-ray data.

**Chapter III** describes the synthesis of novel formazyl and tetrazolyl calix[4]arenes by two alternative routes. Formazyl calix[4]arenes have been synthesized by the condensation of formyl calixarenes with phenyl hydrazines followed by their coupling with diazonium salts. Alternatively, diazonium salts of selectively substituted

aminocalix[4]arenes have been coupled with phenyl hydrazones of aromatic aldehydes. Oxidation of formazyl calix[4]arenes, thus obtained, has been carried out by using N-bromosuccinimide, lead tetraacetate and sodium hypochlorite to yield the corresponding tetrazolyl calixarenes. The characterization of the compounds obtained has been achieved by IR, UV, NMR and FAB mass analysis.

Preliminary UV-visible spectral investigations conducted to evaluate the molecular recognition properties of the synthesized formazyl calixarenes towards transition metal ions have been described in **Chapter IV**. It has been observed that formazyl calix[4]arenes specifically interact with copper(II) ions and lead(IV) ions to produce intense colour changes. The nature of interaction of formazyl calix[4]arenes with Cu(II) and Pb(IV) has been evaluated by time dependent UV-visible studies.

The host-guest properties of inorganic and organic constrained systems represented by zeolites and calix[n]arenes, respectively, have been explored by carrying out some organic unit processes in the presence and absence of these constrained systems and the results have been presented in **Chapter V**. It has been observed that out of several reactions investigated, bromination of simple anilides in chloroform as the medium showed a remarkably high specificity and enhanced rates of bromination in the presence of zeolites and *p-tert*-butylcalix[4]arenes. A plausible rationale for the observed results has been suggested.

**Chapter VI** describes the standardization of the procedure for isolation of an important component of lac, i.e., aleuritic acid. Variation of saponification time, quantity of bleach added and effect of addition of different coagulants and polyelectrolytes on yield and

purity of aleuritic acid has been given in the Chapter VI. An HPLC assay system has been developed for testing the purity of aleuritic acid thus obtained.

# NOTES

1. All melting points reported in this thesis are uncorrected and were taken on an electric melting point apparatus(Toshniwal, India).
2. UV spectra were taken on Hitachi 330 and Perkin Elmer's(Lambda-3B) spectrophotometers.
3. IR spectra were recorded in KBr discs on a [5-DX]Nicolet FT-IR and Nicolet protégé 460ESP spectrophotometers.
4. $^{1}$H-NMR and $^{13}$C-NMR spectra were recorded in $CDCl_3$ and DMSO-$d_6$ on Jeol [JNM-FX-100] (IIT Delhi)and Bruker Spectrospin-300MHz FT-NMR spectrometers using TMS as internal standard and values reported are on δ scale. The abbreviation such as s, bs, d, t, q and m indicate singlet, broad singlet, doublet, triplet, quartet and multiplet respectively.
5. FAB-mass spectra of some of the synthesised compounds were recorded on a JEOLSX 103/DA-6000 Mass Spectrometer available at Central Drug Research Institute, Lucknow.m-Nitrobenzyl alcohol was used as the matrix.
6. Elemental Analysis was carried on Perkin Elmer's 240C-CHN Analyzer.
7. The completion of the reaction and the purity of the synthesised compounds were checked by TLC performed on silica gel coated glass plates using iodine for visualizing the spots.
8. Purification of several of the synthesised compounds was carried out by column chromatography over silica gel(60-120 mesh)obtained from Qualigens Fine Chemicals Limited, Mumbai.

9. The solvents used were purified and dried before use by procedures described in "Purification of Laboratory Chemicals" by W.L.F.Amarego and D.D.Perrin, Bath Press and Bath, Britain.

10. For the sake of convenience, the names of calixarenes used in this thesis have been shortened. For example, 5,11,17,23-tetra-tert-butyl-25,26,27,28-tetrahydroxycalix[4]arene has been usually referred as p-tert-butylcalix[4]arene or even parent calix[4]arene while 5,11,17,23,29,35-hexa-tert-butyl-37,38,39,40,41,42-hexahydroxycalix[6]arene and 5,11,17,23,29,35,41,47-octa-tert-butyl-49,50,51,52,53,54,55,56-octahydroxycalix[8]arene are referred as p-tertcalix[6]arene and p-tert-butylcalix[8]arene respectively. Similarly names of 25,26,27,28-tetrahydroxycalix[4]arene, 37,38,39,40,41,42-hexahydroxycalix[6]arene and 49,50,51,52,53,54,55,56-octahydroxycalix[8]arene have been shortened to tetrahydroxycalix[4]arene, hexahydroxycalix[6]arene and octahydroxycalix[8]arene and even further shortened to calix[4]arene, calix[6]arene and calix[8]arene.

11. Abbreviations used in this thesis are:

    THF – Tetrahydrofuran;

    DMF – N,N'-Dimethylformamide;

    $CDCl_3$- Deuterated chloroform;

    $CD_3OD$- Deuterated methanol;

    $D_2O$ – Deuterated water

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