SYNTHESIS AND CHARACTERIZATION OF BIOLOGICALLY ACTIVE PHENANTHROLINES, QUINOLINES AND RELATED MATERIALS

by

CHANDIMA ABEYWICKRAMA

A dissertation submitted to the Graduate Faculty in Chemistry in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

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This manuscript has been read and accepted for the Graduate Faculty in Chemistry in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

4/28/04
Date

Arthur D Baker
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4-29-04
Date

Gerald Koppel
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Dr. Arthur David Baker Arthur D Baker

Dr. Susan A. Rotenberg S. A. R. f. J

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Abstract

SYNTHESIS AND CHARACTERIZATION OF BIOLOGICALLY ACTIVE PHENANTHROLINES, QUINOLINES AND RELATED MATERIALS

by

Chandima Abeywickrama

Adviser: Professor Arthur David Baker

Derivatives of 1,4,5,12–tetraazatriphenylenes and analogues of dequalinium diiodide were synthesized and investigated.

In the first project, efficient preparations of 1,4,5,12-tetraazatriphenylene, also known as 4',7'-phenanthrolino-5,6:5'6'-pyrazine **1** (ppz) and a number of its substituted derivatives were developed. The condensation of 5,6-diamino-4,7-phenanthroline **6** with glyoxal provides a quantitative yield of ppz itself. This synthetic strategy avoids the disproportionation reaction and resulting minimum 50% loss of possible ppz product that occurs in the previously used preparation which involved the condensation of 4,7-phenanthroline-5,6-dione **2** with ethylenediamine. Use of dicarbonyl compounds other than glyoxal forms ppz derivatives including 2,3-diphenyl-1,4,5,12-tetraazatriphenylene

7, 2,3-dimethyl-1,4,5,12-tetraazatriphenylene **8**, 2,3-dipyridin-2-yl-1,4,5,12-tetraaza-triphenylene **9**, 1,8,9,10,17,18-hexaazaphenanthro[9,10-b]triphenylene **10**. Other ppz derivatives have been prepared *via* the condensation of diaminomaleonitrile with 4,7-phenanthroline-5,6-dione **2** and subsequent functional group transformations on the two cyano groups of the resulting 1,4,5,12-tetraazatriphenylene-2,3-dicarbonitrile **14**.

In the second project, protein kinase C (PKC) inhibitors related in structure to dequalinium salts were investigated. Structural changes were made with the ultimate goal of developing better therapeutic agents. The parent compound Dequalinium diiodide (C_{10}-DECA) **20c** gave the optimum potency in this series. However Dequalinium diiodide analogues based on 4-aminoquinoline and 4-*N,N*-dimethylaminoquinoline show similar potency to that of the parent compound. The methyl group at the second position does not contribute significantly to inhibitory activity. Substituted pyridine rings (rather than quinoline rings) reduce the potency. Unsubstituted quinoline rings are relatively inactive towards PKC. The quarternized exocyclic ring nitrogens of DECA analogues are less potent than that of quarternized ring nitrogens of DECA analogues. Other potent compounds were also discovered. e.g. the triphenylphosphine based DECA type analogues.

Gauss view 3.0/ Restricted Hartree-Fock, 6-31G Level calculations were performed on free bases and on quarternized model compounds with the goal of obtaining a structure-activity relationship.

Dedicated to my parents:

Anula Abeywickrama

and

Sugathadasa Abeywickrama

Who have been my inspiration and strength.

ACKNOWLEDGEMENTS

This has been a long journey, both emotionally and geographically. But I have been borne aloft, cushioned from the vicissitudes of fickle fortune, by family, friends, colleagues and students. Their invaluable contribution and support have made this endeavor less lonely and arduous.

The value of my family's support has been incalculable. My mother Anula Abeywickrama deserves my undying gratitude for blazing a trail in a part of the world where women were expected to take a subordinate role as adults. She not only excelled as a student, but as a teacher as well. She inspired me to become independent and to pursue my dreams no matter where they took me. She instilled in me the thirst for knowledge, which she said would be more enduring than any other pleasure. My father Sugathadasa Abeywickrama deserves special thanks, first for bucking tradition and marrying such an independent woman, but also for allowing his daughters to follow in her footsteps. He has made me feel safe all my life and continues to do so, though miles away.

My sister, Lakshmi Abeywickrama has eliminated the anxiety of leaving my family behind in Sri Lanka. With her marriage, she introduced two lovely people in my life, her husband Mohan Gunasinghe and my niece Ovini A. Gunasinghe.

I would like to thank my brother Anura Abeywickrama, who made me feel welcome when I came to the U.S. and made leaving home less painful. Thanks to my brother Dr. Anil Abeywickrama, who by his example and encouragement inspired me to pursue a scientific career. My late uncle Charles Vidana Patirana in Canada has been a source of comfort and a second father to me.

Assistance in finding my way through the complexities of this project, and the vast expanse of this field, requires a special expression of thanks to my Thesis adviser Dr. Arthur David Baker. He has been gracious and exceedingly patient with me, no matter how trivial the query, and never, once, gave any indication it was an imposition. He has been a great mentor and friend. Because of the freedom and the lack of pressure from Dr. Baker, I was able to be more productive. He also honed my skills in presenting a paper to an audience, increasing the impact of the presentation.

Dr. Susan A. Rotenberg deserves special thanks for giving me the opportunity to do some fascinating research. I got to see a first-class mind at work. My thanks to Dr. Klaus Grohmann, on my committee, for keeping a watchful eye over my project, making many helpful suggestions and also giving freely of his time and expertise.

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Dr Philip Barnett at City College Library, Ms. Everlyn Silverman and Ms. Marianne Conti at Queens College Library, were amazing in finding the important articles I needed when I was at my wits end.

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I would always count on, Dr. Gopal Subramaniam, Dr. Robert Engel, Dr. Cherice Evans, Dr. Jerome Schulman, Dr. Raymond Disch, Dr. Robert Bittman, Dr. George Axelrad, Dr. Bonnie Gersten, Dr. R. Bittman, Dr. Byun Hoesup and Dr. Wilma Saffran for the extra help on my project.

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Dr. Brian Schwartz deserves thanks for presenting me with a clear picture of the options I would have in the future. Dr. Hugh English, Dr. Beth Stickney and the late Ms. Adele Kapfur: Thank you for your excellent teaching! You added confidence to my writing.

For Jose Zambrana and Sharon Lall: I treasure your friendship and the important help at critical moments. Marie Thomas, Anh Tran, Joshua Mukhlall, Lu Xuequan, Liu Yidong, Sun Chaode, Charles Hicks, Oma Morgan, Baohe Chang, Delroy Coleman, John Pilsner and Namal Weeratunga brightened my life with their friendship.

I could not have proceeded with my experiments without the invaluable help of Queens College technicians and safety officers Robert Wurman, Joseph Badalamenti, Charles Ezeude, and Parmanand Panday. Thanks to Mr. Nimal Amaraweera who, like a superhero, came to my rescue in the lab when I wanted things fixed.

Special thanks to the Secretaries at Queens College and the Graduate Center Jeanne Deutsch, Molash Alemayehu, Alice Brickman, Diane Adebowale and Vivian Mason for always being so helpful whenever I needed them.

Thank you Dr. Sunil and family for your kindness and hospitality. I am fortunate to have Ms. Chang as a landlady whose proximity to the University made my life easier and more productive.

The staff at Public Safety deserves especial thanks: Ms. Georgina Medina, Mr. Ervin Maggette, Mr. Jose Sanchez, Ms. Calendar, Mr. Krishna Japsi, Mr. Roudy Jeanpierre and Mr. Lomax for being so accommodating and watching over me, especially when I left the campus at some ungodly hour.

Dr. K. Mahanama, Dr. A. Amarasekera, Dr. Nalin Goonasekera and Dr. Kirthi Premadasa: I feel very fortunate to have studied at Queens Collage. I owe you a great deal for your advice and suggestions when I decided to apply for admissions.

My Sri Lankan friends, Lenagala, Cryshanthi Suriapperuma, Dammika Herath, Athula Ranasinghe, Loy Abeywardana, and Thusitha Dharmaratne, are an important link that keeps me connected to my roots.

The venerable Kurunegoda Piyatissa, priest at the New York Buddhist temple and late venerable Mullewatte Chandrasiri in Sri Lanka provided spiritual comfort with the enlightening talks and religious services.

Thank you Mr. Walter Tavaris: words are inadequate to express my gratitude for all your help, while I was working on this project.

To all those mentioned, and to others not named, but who engaged me in and out of class on relevant issues my heartfelt thanks.

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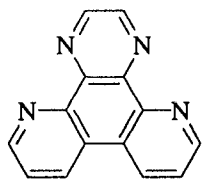
An Efficient Synthesis of 1,4,5,12–Tetraazatriphenylene and Derivatives

Introduction

The compound 1,4,5,12-tetraazatriphenylene, also known as 4',7'-phenanthrolino-5,6:5'6'-pyrazine **1** ("ppz") is a member of a relatively small family of planar heterocyclic molecules which contain three or more nitrogen atoms and four or more condensed rings. The molecular structures of several such substances are shown in Figure **1** and as noted there, many are biologically active. Compound **1** is also a member of an even more exclusive sub-set of such compounds in which the position of the nitrogen atoms allows the molecules to participate as bis-bidentate or bis-polydentate ligands toward a variety of metal ions. These ligands are noted in Figure **1.**[1-46]



1
ppz

Metal complexes containing such heterocycles often have a rich photochemistry and redox chemistry, and are used in studies of electron and energy transfer processes.[22,47,48] For example, early work from our laboratory described the use of ppz as a bridging ligand in the fabrication of the first luminescent complexes containing two ruthenium atoms (Figure **2**).[47] Luminescence in such complexes is a pivotal observation for the characterization of excited state properties. The amount of electronic communication between the metal centers connected by bridging ligands such as ppz is an area of intense

interest and both experimental and computational techniques have been applied.[24] Extensions of our early work with ppz have been abundant, as have related studies on other structurally similar heterocycles. Many reports detail the use of ppz and analogues as bridging ligands used to prepare bimetallic and polymetallic metal complexes, and in the fabrication of dendrimers and micro-porous network structures.[49-53,25] It has been pointed out that the bisbidentate bridging nature of ppz makes it a promising building block for constructing polynuclear systems where possibly lattice interpenetration is less likely due to the rigidity of the ligand.[23] Grove and coworkers have investigated the x-ray crystallographic structure of ppz, pointing out the occurrence of π stacking, and the effect on molecular dimensions of metal complexation and introduction of substituents.[6]

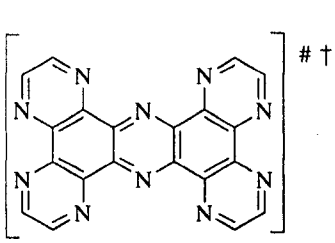
FIGURE 1. Planar Heterocycles Consisting of Four or More Condensed Rings and Three or More N Atoms[1-46]

Investigated / under investigation as DNA intercalators

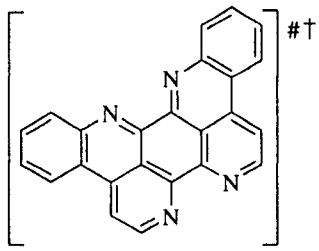
† documented as bis or polydentate ligands

* possible biomedical use

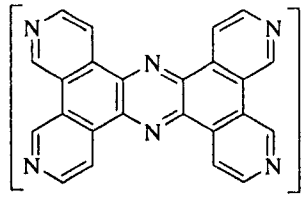
‡ other (see references)



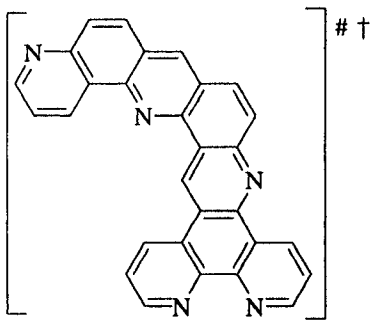
1,4,5,8,9,10,13,14,17,18-decaaza phenanthro[9,10-*b*]triphenylene/ 1,10-phenanthrolino [5,6-b]1,4,5,8,9, 12-hexaaxatriphenylene (phehat)[1]



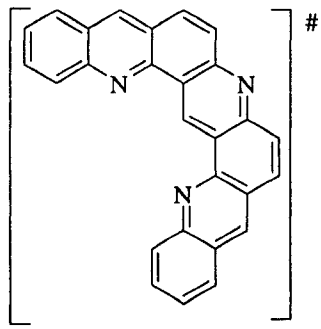
eilatin[2,3]



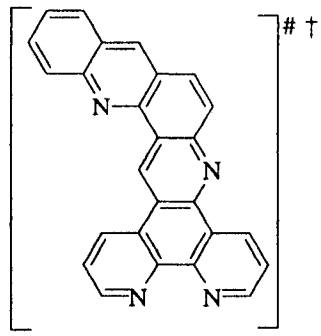
3,6,9,12,15,18-hexaazaphenanthro-[9,10-*b*]triphenylene/ tetrapyrido[3,2-*a*:2',3'-*c*:3",2"-h:2"', 3""-*j*]phenazine (tpphz)[4]



quinolino[5,6-*b*]phenanthrolino [1,10][5',6': *j*]phenanthroline[1,7][5]

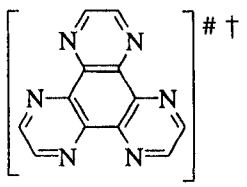


triazaheptacycle[8]

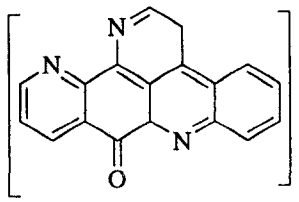


benzo[*b*]phenanthrolino[1,10] [5,6;*j*] phenanthroline-[1,7][5]

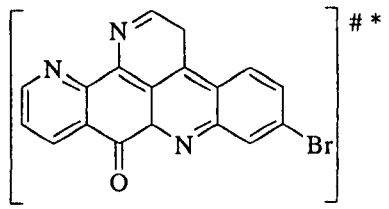
FIGURE 1 Continued



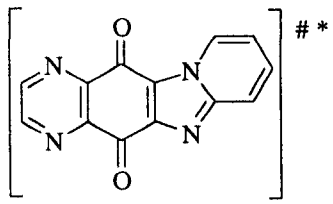
1,4,5,8,9,12-hexaaza triphenylene (hat)[1,6,7]



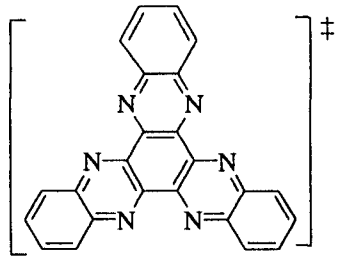
3,8a-dihydro-1,8,13-triazabenzo[*fg*]naphthacen-9-one /ascididemin[2,9]



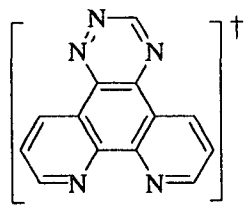
6-bromo-3,8a-dihydro-1,8,13-triazabenzo[*fg*]naphthacen-9-one[10]



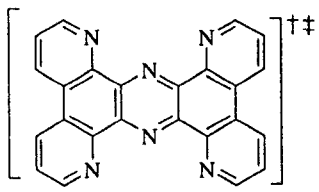
pyrido[1,2-*a*]imidazo[4,5-*g*] quinoxaline-6,11-dione[11]



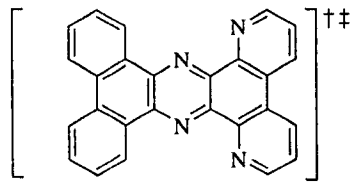
5,6,11,12,17,18-hexa azatrinaphthylene/ diquinoxalino [2,3-a:2',3'-c] phenazine[7,19,20]



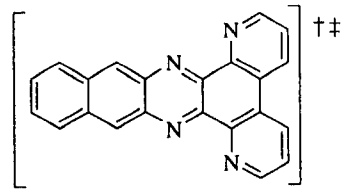
1,2,4,8,9-penta azatriphenylene[21]



1,8,9,10,17,18-hexaazaphenanthro [9,10-*b*]triphenylene /tetrapyrido [2,3-a:3',2'-c:2",3"-h:3"', 2"'-j]phenazine (tphz)[12-17]

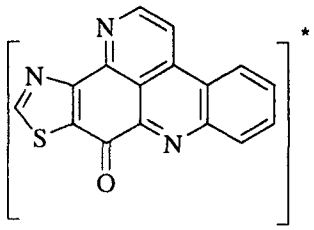


1,8,9,18-tetraazaphenanthro [9,10-*b*]triphenylene/ dibenzo[a:c] (dipyrido[2,3-h:2',3'-j]) phenazine ($dbdpzH_2$)[16,17]

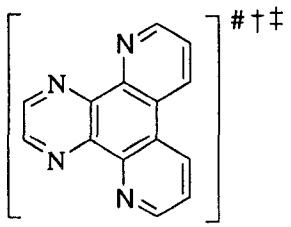


1,8,9,16-tetraazadibenzo[*a*,*c*] naphthacene / dipyrido(2,3-a;3',2'-c) benzophenazine (dpb')[18]

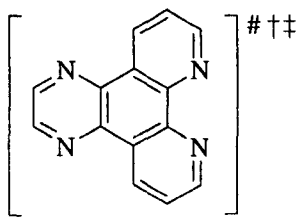
FIGURE 1 Continued



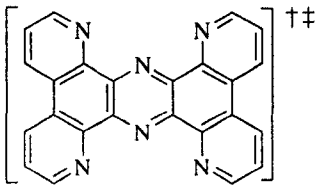
6-thia-3,4,8-triazabenzo[*de*] cyclopenta [*b*]anthracen-7-one/ kuanoniamine A[3]



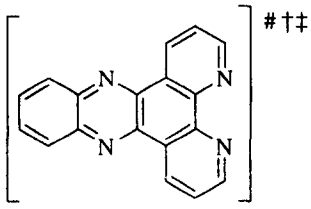
1,4,5,12-tetraaza-triphenylene/ ppz [1,22-32]



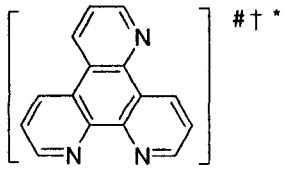
1,4,8,9-tetraazatriphenylene/ dipyrido[2,2-*d*:2',3'-*f*] quinoxaline (dpq)[1,33,34]



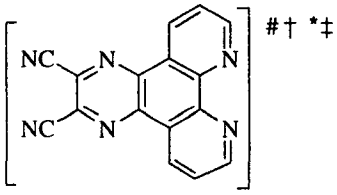
1,8,9,10,17,18-hexaaza-phenanthro[9,10-*b*]triphenylene /tetrapyrido[2,3-a:3',2'-c:2", 3"-h:3"',2"'-j]phenazine (tphz) [12-17]



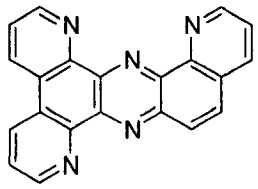
4,5,9,14-tetraazabenzo[*b*] triphenylene dipyrido[3,2-*a*:2',3'-*c*] phenazine(dppz)[1,4,33,35]



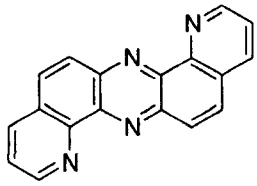
1,5,12-triazatriphenylene (pdphen) / pyrido(3,2-f) (1,7) phenanthroline[21,36-38]



1,4,8,9-tetraazatriphenylene-2,3-dicarbonitrile/ 6,7 - dicyano dipyrido[2,2-d:2',3'-f]-quinoxaline (dicnq)[33,34,39]

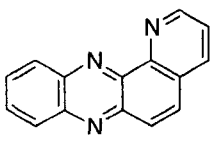


tripyrido(2,3-*a*:3',2'-*c*:3",2"-*j*) phenazine[12]

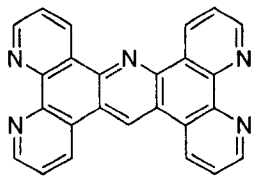


1,7,8,14-tetraaza-dibenzo[*a*,*h*] anthracene/ dipyrido(2,3-*a*:2',3'-*h*) phenazine[12]

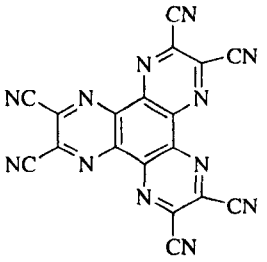
FIGURE 1 Continued



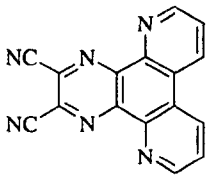
1,7,12-triazabenzo[*a*]anthracene / pyrido(2,3-*a*)phenazine[12]



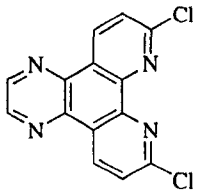
4,5,9,13,14-pentaazaphenanthro [9,10-*b*]triphenylene/ tetrapyrido[3,2-*a*:2',3'-*c*:3",2"-*h*:2",3"-*j*]acridine (tpac)[8]



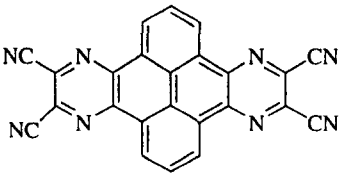
1,4,5,8,9,12-hexaazatriphenylene-2,3,6,7,10,11-hexacarbonitrile[40,41]



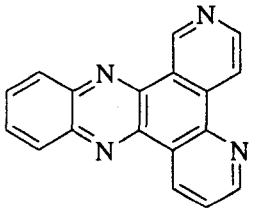
1,4,5,12-tetraazatriphenylene-2,3-dicarbonitrile[42,43]



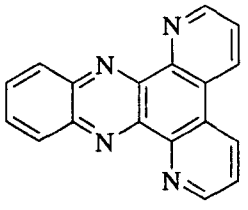
7,10-dichloro-1,4,8,9-tetraaza-triphenylene[33]



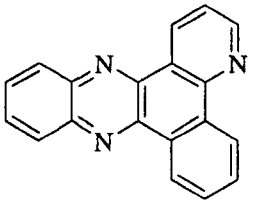
5,6,12,13-tetracyanpyreno[4,5-b:9,10-b']dipyrazine[42,43]



2,5,9,14-tetraaza-benzo[*b*] triphenylene[44]

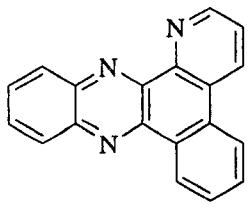


1,8,9,14-tetraaza-benzo[*b*] triphenylene[44,45]

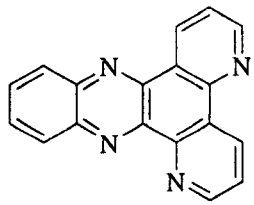


benzo[*a*]pyrido[2,3-*c*] phenazine[44]

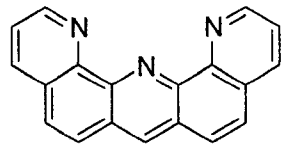
FIGURE 1 Continued



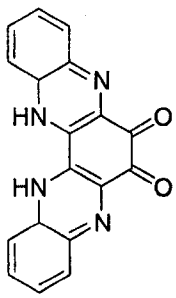
benzo[*a*]pyrido[3,2-*c*] phenazine[44]



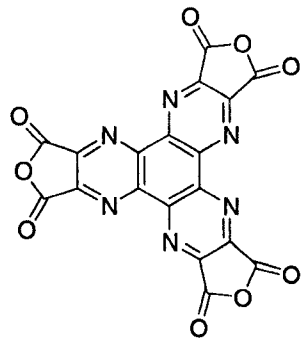
4,8,9,14-tetraaza-benzo[*b*] triphenylene[45]



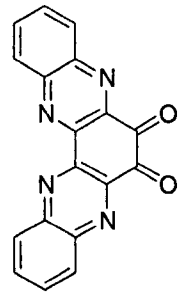
1,13,14-triaza-dibenzo[*a*,*j*] anthracene/ dipyrido(2,3-*a*:3'2'-*j*) phenazine[12,38]



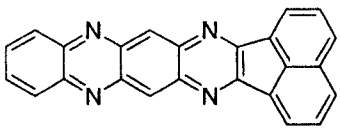
12a,13,14,14a-tetrahydro-5,8,13,14-tetraazapentaphene -6,7-dione[51]



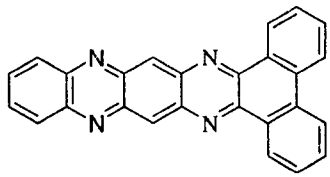
Hexaazatriphenylenehexa carboxylic acid trianhydride[30a]



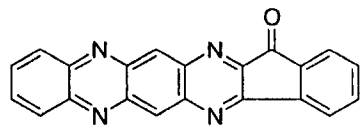
5,8,13,14-tetraaza-pentaphene-6,7-dione[51,52]



acenaphtho[1',2':5,6] pyrazino [2,3-b]phenazin e[46]

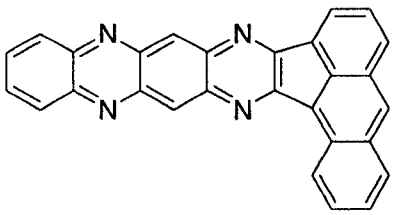


dibenzo[*a*,*c*]quinoxalino [2,3-*i*]phenazine[46]

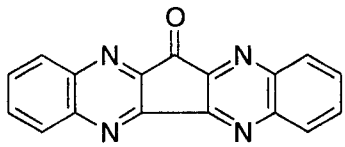


indeno[1,2-b]pyrazino [5,6-b]phenazine[46]

FIGURE 1 Continued

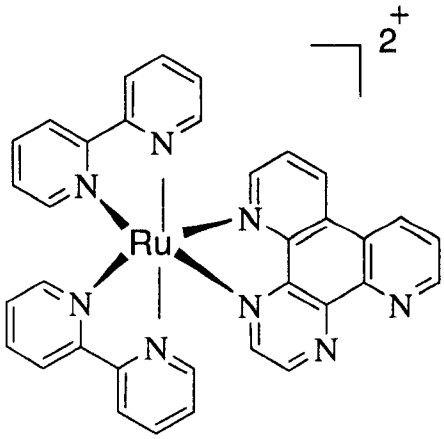


aceanthraceno[1',2':5,6]
pyrazino
[2,3-*b*]phenazin[46]

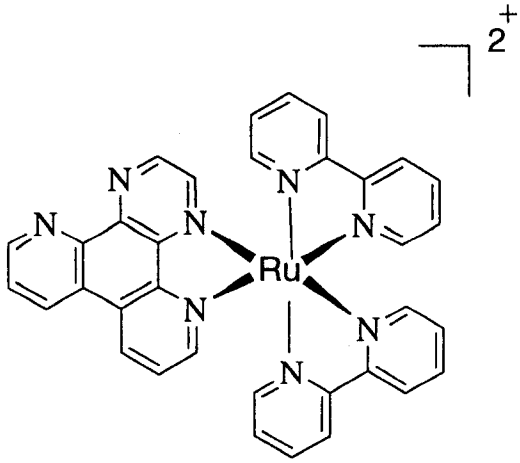


5,6,11,13-tetraazadibenzo[*b*,*h*]
fluoren-12-one[19,20]

FIGURE 2. Ruthenium(II) (bipyridine)$_2$ (ppz) Isomers



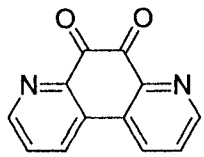
Λ-[Ru(ppz)(bpy)$_2$]$^{2+}$



Δ-[Ru(ppz)(bpy)$_2$]$^{2+}$

We have established that another most useful attribute of ppz, related to its planar structure, is its ability to act as a DNA intercalator. When ppz is part of a metal complex, significant changes in the photochemical properties of the complexes mentioned above

can occur, such as dramatic enhancement of luminescence intensity and shifts in absorption wavelengths (Figure **3** and **4**). These changes can be quantified and such measurements allow equilibrium binding constants to be calculated.[1,27-30,35] In its role as a DNA-intercalator ppz is one of a group of structurally related heterocycles that have also been investigated as DNA-intercalators. Many are noted in Figure **1,** but ppz is rare in this group of compounds in having multiple-metal binding sites, which we have utilized in developing DNA-cutting strategies.[28] In general, there is significant interest in such compounds as they have the potential to be sensitive diagnostic tools and novel chemotherapeutic agents.[9-11,37]

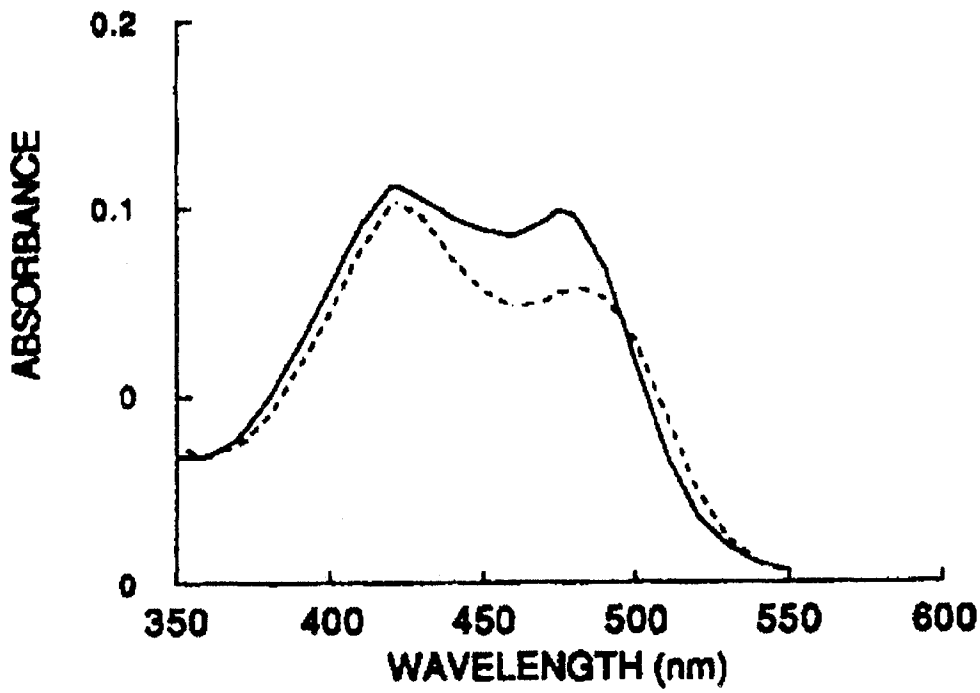


2
4,7-phenanthroline-5,6-dione

The immediate synthetic precursor to ppz in all published work has been the 4,7-phenanthroline-5,6-dione **2**. The structurally related 1,4,8,9-tetraazatriphenylene and 2,3-di-2-pyridylpyrazine (dpp) are similarly prepared from the corresponding 1,10-phenanthroline 5,6 dione and 1,2-dipyridin-2-ylethane-1,2-dione (2,2'- pyridil). In each case, a condensation with ethylenediamine is performed. However each condensation has its own idiosyncracies. In the case of **2**, the condensation produces a diimine which must be aromatized in a subsequent oxidative step,[31] but when 1,10-phenanthroline-5,6-dione is used, the intermediate diimine oxidizes under the reaction conditions to give 1,4,8,9-tetraazatriphenylene directly. Finally for ppz, the intermediate diimine **3** (2,3-dihydro-1,4,5,12-tetraazatriphenylene) undergoes a spontaneous disproportionation

reaction (Scheme **1**), producing ppz and the non-aromatic substance 1,4,4a,12b-tetrahydro-1,4,5,12-tetraazatriphenylene **4** in equal amounts. Thus the maximum theoretical yield of ppz in this final step is limited to about 50% and no methods have been found to-datefor oxidizing **4** to ppz.

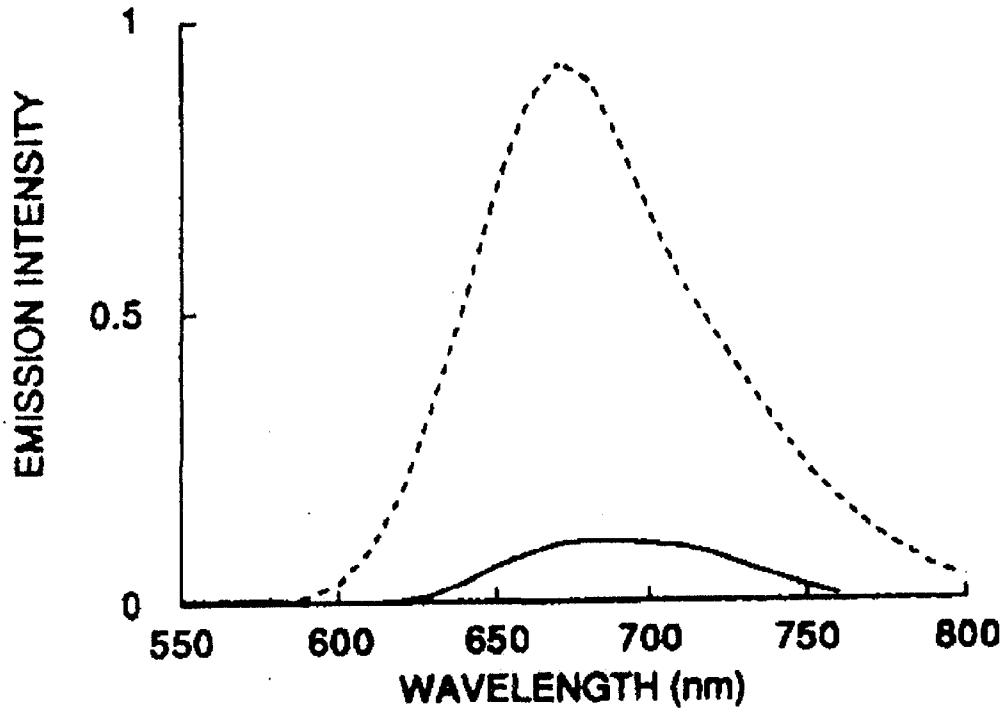
FIGURE 3. Visible Absorption Spectra of $Ru(ppz)(bpy)_2]^{2+}$



_____ = **complex alone**

---------- = **complex in the presence of calf thymus DNA**

FIGURE 4. Emission Spectra of $[Ru(ppz)(bpy)_2]^{2+}$

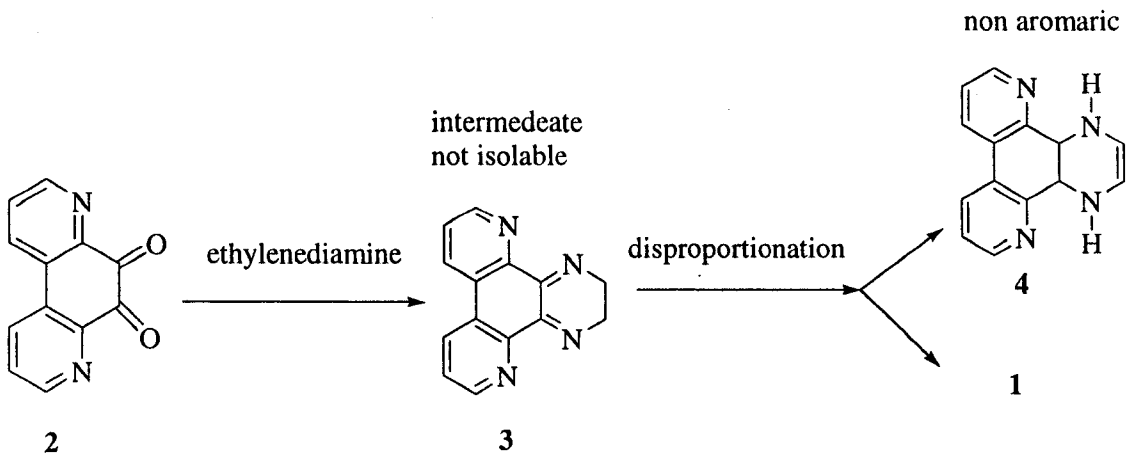


_____ = complex alone

---------- = complex in the presence of calf thymus DNA

Among our objectives has been the development of improved synthetic routes to ppz, and the preparation of substituted derivatives of ppz which could be potentially useful in fine-tuning the photochemical and DNA-intercalating properties of ppz and its metal complexes, and also for the construction of more elaborate structures in which the substituents can serve as a site for structural architecture. Only a few ppz derivatives have been reported in the past,[12-15,42,43] reflecting the observation that the known chemistry of 4,7-phenanthroline and its derivatives is sparse compared to that of 1,10-phenanthrolines. Here we report a more efficient synthesis of ppz, and the synthesis of several new ppz derivatives.

SCHEME 1. Synthesis of ppz via Disproportionation Reaction



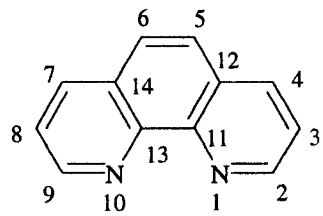
Results and Discussion

As noted in the Introduction, dione **2** is a logical precursor for the preparation of ppz, **1**. In principle it can be prepared via a number of strategies. Direct oxidation of 4,7-phenanthroline appears appealing, especially considering that the isomeric 1,10-pheanthroline is relatively easily oxidized to 1,10-pheanthroline-5,6-dione.[54] However, we investigated the action of several oxidizing agents on 4,7-phenanthroline, but did not find a suitable procedure for converting it to the desired dione. Failure to produce the dione *via* a number of other oxidative procedures have also been reported in earlier literature.[55] In an attempt to understand the difference between 1,10-phenanthroline and 4,7-phenanthroline, we performed molecular orbital calculations at the Gauss view 3.0/ 6-31G level. Although the Mulliken charges on the C_5 and C_6 carbon atoms are somewhat higher for 1,10-phenanthroline (C_5 -0.204 and C_6 - 0.204) than for 4,7-phenanthroline (C_5 -0.191 and C_6 - 0.191), the differences are small and unlikely to account for the observed differences in reactivities of the two isomers. However, more significantly, the calculations reveal that the HOMO of 1,10-phenanthroline has its highest electron density at the C_5 and C_6 positions, while that of the 4,7 isomer does not. The calculations show that the electron density in the HOMO is actually *lower* on the C_5 and C_6 carbon atoms than on any other carbon atoms in the 4,7-isomer (see Table **1** and Figure **5**). Thus attempts to oxidize 4,7-phenanthroline to the 5,6-dione might be doomed to failure.

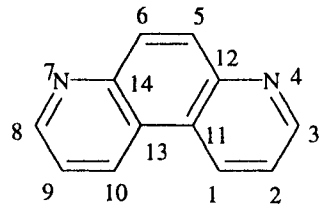
TABLE 1. Relative Electron Densities at the C Atoms in the HOMO's of 1,10 and 4,7-Phenanthroline, Calculated Using Gauss view 3.0/ 6-31G Level

Compound	Order of coefficients
1,10-phenanthroline	C_5 & C_6 >> C_2 & C_9 > C_{11} & C_{13} > C_4 & C_7 > C_{12} & C_{14} > > C_3 & C_8
4,7-phenanthroline	C_{12} & C_{14} ~ C_8 &C_3 ~ C_{11} & C_{13} >> C_1 & C_{10} > C_2 & C_9 > C_5 & C_6

FIGURE 5. Numbering of Atoms in 1,10-Phenanthroline and 4,7-Phenanthroline



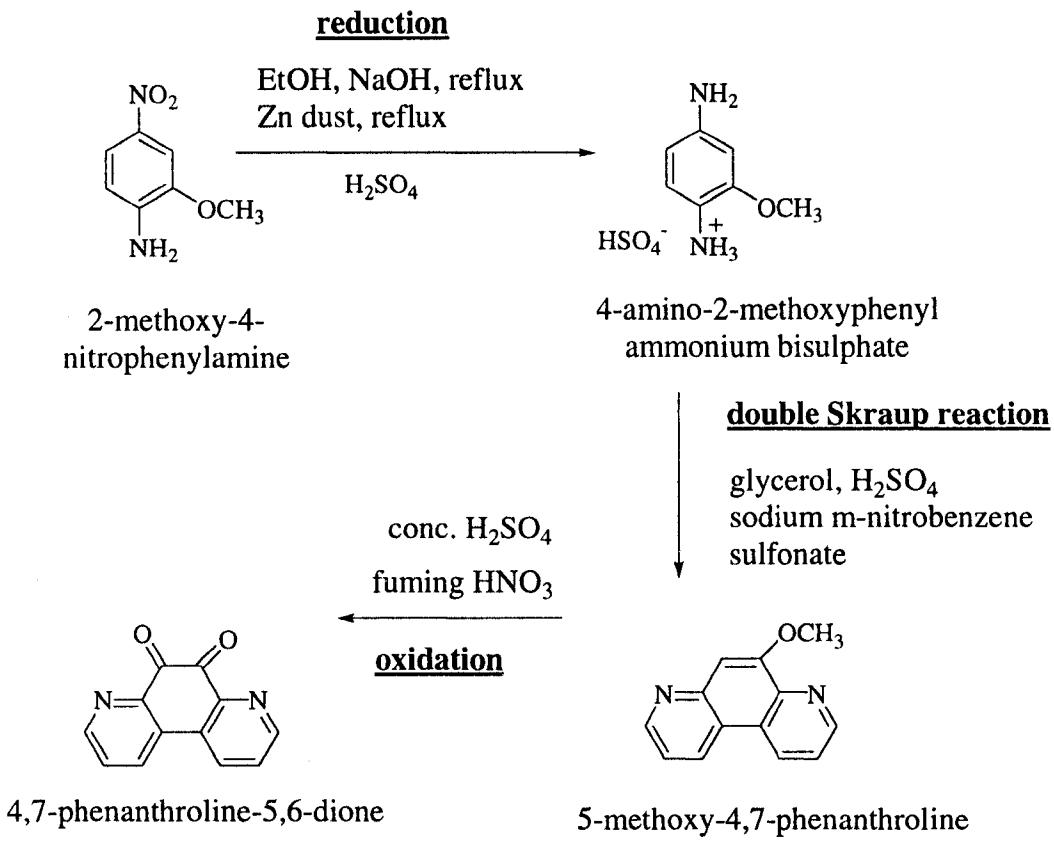
1,10-phenanthroline



4,7-phenanthroline

The dione **2** was once a product of the pharmaceutical industry.[58] Several preparative procedures are patented.[59-63] The most important of these are based on early reports[45] that 5-methoxy-4,7-phenanthroline is oxidized to the dione by heating with nitric and sulfuric acids. The starting 5-methoxy compound can be prepared via a double Skraup reaction on 2-methoxy-1,4-diaminobenzene,[45] which in turn can be prepared *via* reduction of 2-methoxy-4-nitroaniline. [45] We have fine-tuned the experimental details for the sequence of reactions needed to prepare ppz (Scheme **2**).[31]

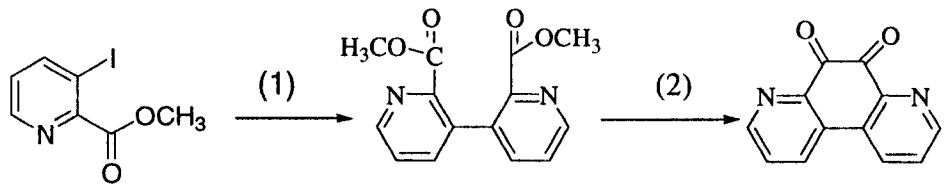
SCHEME 2. Synthesis of 4,7-Phenanthroline-5,6-dione: Use of Double Skraup Reaction



Although double Skraup reactions of diaminobenzene derivatives provide a viable route to 4,7-phenanthroline-5,6-diones, they are at best tedious to perform on a moderate to large scale, and at worst dangerous because runaway exothermic reactions sometimes occur. While we continue to rely on the Skraup reaction, and endeavor to find better and safe ways of performing this reaction, it will always prove tedious on a large scale as large amounts of concentrated sulfuric acids are used in the reaction and have to be neutralized. Furthermore, considerable tar formation occurs during the neutralization, making-workup difficult.

We decided to investigate a different strategy to prepare dione **2** - a C_8K mediated acyloin condensation[56a] of diethyl ester of 3,3´-bipyridine-2,2´-dicarboxylic acid. Acyloin condensations of aromatic esters are in general less common than those of aliphatic esters, but C_8K has been reported to be a reagent of choice in several cases. We planned to prepare the needed dipyridil diester **x** via a coupling reaction of a 3-halo precursor (Scheme **3**). Halopyridinecarboxylates in general would seem to be particularly useful key intermediates for this type of synthesis since the halogen substituent can be used in a variety of coupling reactions.

SCHEME 3. Modified Strategy for Preparation of 4,7-Phenanthroline-5,6-dione



3-iodo-pyridine-2-carboxylic acid methyl ester

3,3'-bipyridinyl-2,2'-dicarboxylic acid dimethyl ester
x

4,7-phenanthroline-5,6-dione

(1). $NiBr_2(PPh_3)_2$, Zn, Et_4NI in THF (2). C_8K

We investigated two approaches for the synthesis of the required halogenated pyridine ester (3-iodo-pyridine-2-carboxylic acid methyl ester).

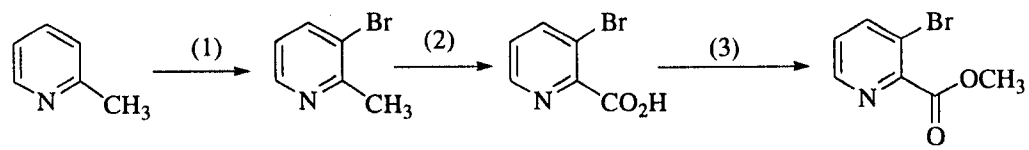
Method **1**: The use of 3-bromo-2-methylpyridine (3-bromo-2-picoline) (Scheme **4**)

Method **2**: The use of pyridine-2-carboxylic acid (picolinic acid) (Scheme **5**)

Method **2** was carried out by two routes designated below as "route **1**"and "route **2**".

In the first of these routes, our intention was to synthesize 3-bromo-2-picoline *via* bromination of 2-picoline, then perform a side chain oxidation followed by esterification to afford the desired halogenated ester. Unfortunately, as reported by Guthikonda *et al* [56a] the major product of the bromination of 2-methylpyridine is 5-bromo-2-methypyridine rather than the desired 3-bromo-2-methylpyridine (Scheme **4a**). In our hands, the ratio of 3-bromo : 5-bromo isomer was even less favorable than reported by Guthikonda *et al.* [56b] Therefore we decided to abandon this approach and to seek a better method (Method **2**) to synthesize the halogenated pyridine ester *via* a picolinic acid precursor (Scheme **5**).

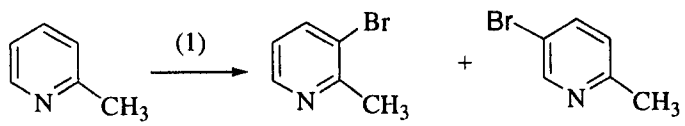
SCHEME 4. Schematic Representation Proposed for the Synthesis of Halogenated Pyridine Ester via 2-methylpyridine - Method 1



2-methylpyridine | 3-bromo-2-methyl pyridine | 3-bromopyridine-2-carboxylic acid | 3-iodopyridine-2-carboxylic acid methyl ester

(1). $N_2(g)$, $AlCl_3$, Br_2 (2). Oxidation (3)EtOH / H^+

SCHEME 4a. Synthesis of 3-bromo-2-methylpyridine



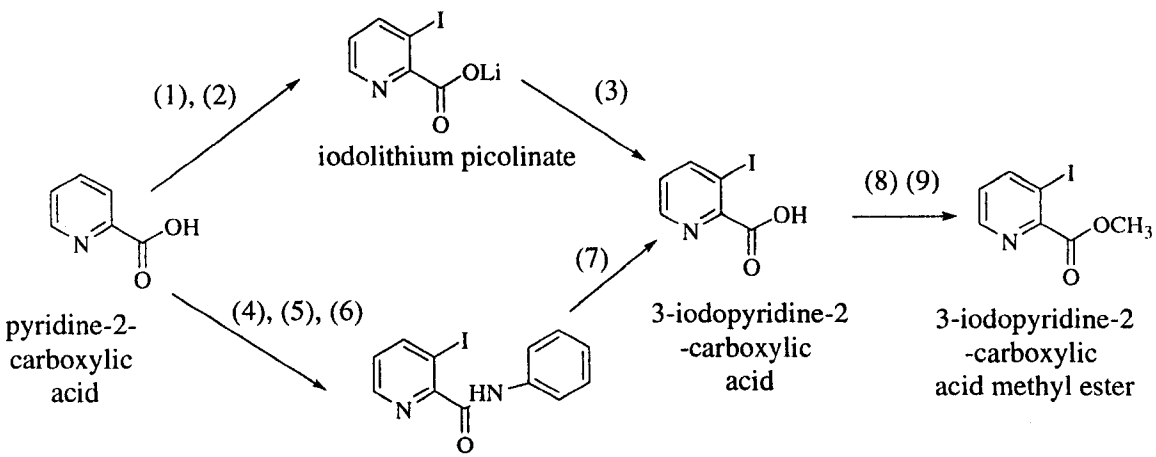
2-methylpyridine

3-bromo-2-methyl pyridine (minor product) 10%

5-bromo-2-methylpyridine (major product) 40%

(1). $N_2(g)$, $AlCl_3$, Br_2

SCHEME 5. Synthesis of Halogenated Pyridine Ester via Picolinic acid -Method 2



iodolithium picolinate

pyridine-2-carboxylic acid

3-iodopyridine-2-carboxylic acid

3-iodopyridine-2-carboxylic acid methyl ester

pyridine-2-carboxylic acid phenylamide

(1). BuLi, LTMP, THF (2). I_2, THF, H_2O (3). Amberlite® IR-120, MeOH, HCl (4). $SOCl_2$ (5). Ph NH_2/CH_2Cl_2, pyridine, DMAP (6). nBuLi, I_2,THF (7) HCl (8). $NaHCO_3$ (9). CH_3I, DMF

Route 1: consists of (4), (5), (6), (7), (8), (9) pathway

Route 2: consists of (1), (2), (3), (8), and (9) pathway

By method 2,[56b] halogenated pyridine-2-carboxylic acid was synthesized *via* two routes. In the first route, metallation of pyridine-2-carboxylic acid was achieved using 3 equivalents of lithium 2,2,6,6-tetramethylpiperidine (LTMP) in THF at 0 °C and after *in situ* formation of the lithium salt with 1 equivalent of BuLi at -75°C. The lithio derivative thus formed was treated with iodine to afford iodolithiumpicolinate. It had been reported [56b] that the attempts to form the corresponding halopicolinic acid *via* aqueous mineral acids (HCl, HF, H_2SO_4) were unsuccessful and therefore an ion exchange resin, Amberlite® IR-120 (hydrogen form) was used instead to afford the corresponding carboxylic acid which in turn was esterified to give the desired haloester. Even though this route looks simple and straightforward the yields of halogenated pyridine-2-carboxylic acid obtained were low.

It has been pointed out [56b] several experimental conditions are necessary to maximize the yield. Firstly 3 equivalents of LTMP are necessary to carry out a complete deprotonation of the pyridine-2-carboxylic acid lithium salt and hence optimize the yield, because there is a tendency that LTMP could react with the iodine electrophile.

Secondly, the temperature plays a vital role in the metallation step inorder to avoid the addition reactions to the carboxylate entity. [56c] Therefore the temperature should be kept below -25° C. In contrast in order to make the reaction go for a completion the literature reported [56b] temperature was 0° C, which implies the possibility of forming certain by-products during the metallation step.

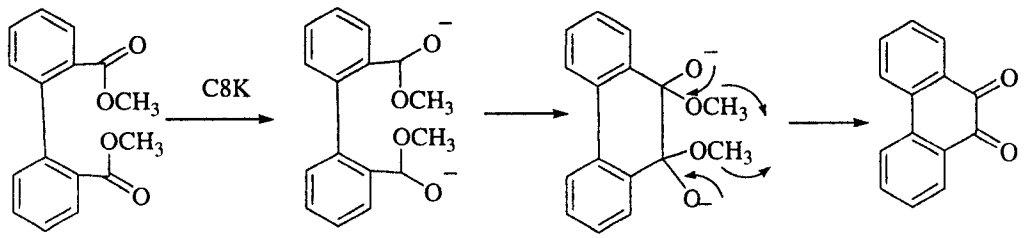
Therefore we searched for an alternative method to synthesize the halogenated pyridine-2-carboxylic acid *via* picolinic acid and a literature search led us to the approach designated as "route 2".[56d]

By employing this route[c] (Scheme **5**), pyridine-2-carboxylic acid was converted to an anilide (pyridine-2-carboxylic acid phenylamide). The subsequent hydrolysis of the halogenated anilide with boiling HCl afforded the corresponding halogenated carboxylic acid which was then esterified to give the desired 3-iodopyridine-2-carboxylic acid methyl ester. Even though the yields were reasonable with this route, the major drawback was the large number of steps involved and each intermediate needed a column chromatographic purification, such that the entire procedure was very time consuming.

Our intention was to couple the haloester with potassium-graphite intercalate, C_8K, (intramolecular acyloin condensation) to form the desired 4,7-phenanthroline-5,6-dione. However we have not done this as yet for two reasons.

The first is that attempts to cyclize the related 2,2´-dipyridil failed (see discussion), and secondly the yield of the halodiester were not sufficiently encouraging to make the overall methodology in method 2 attractive especially when taking into account the time consuming procedures needed for its preparation. Our idea for using C_8K came from earlier reports [56a] which describe ring-closure reactions with aromatic diketones and diesters (Scheme **6** and **7**). We hoped the dipyridil diester **x** would work in a similar manner to get the dione **2,** where the underlying idea was to convert compounds containing two pyridine rings to phenanthrolines using C_8K.

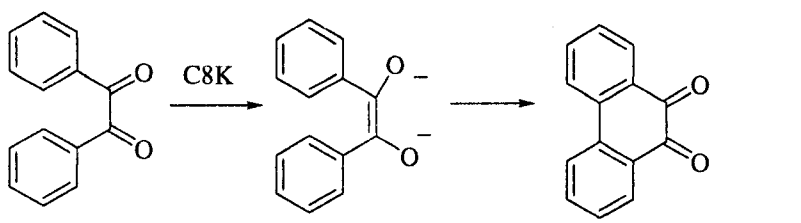
SCHEME 6. The Stepwise Reaction of C_8K with biphenyl-2,2'-dicarboxylic acid dimethyl ester [56a]



biphenyl-2,2'-dicarboxylic acid dimethyl ester

phenanthrenequinone

SCHEME 7. Reduction of Benzil with C_8K [56a]



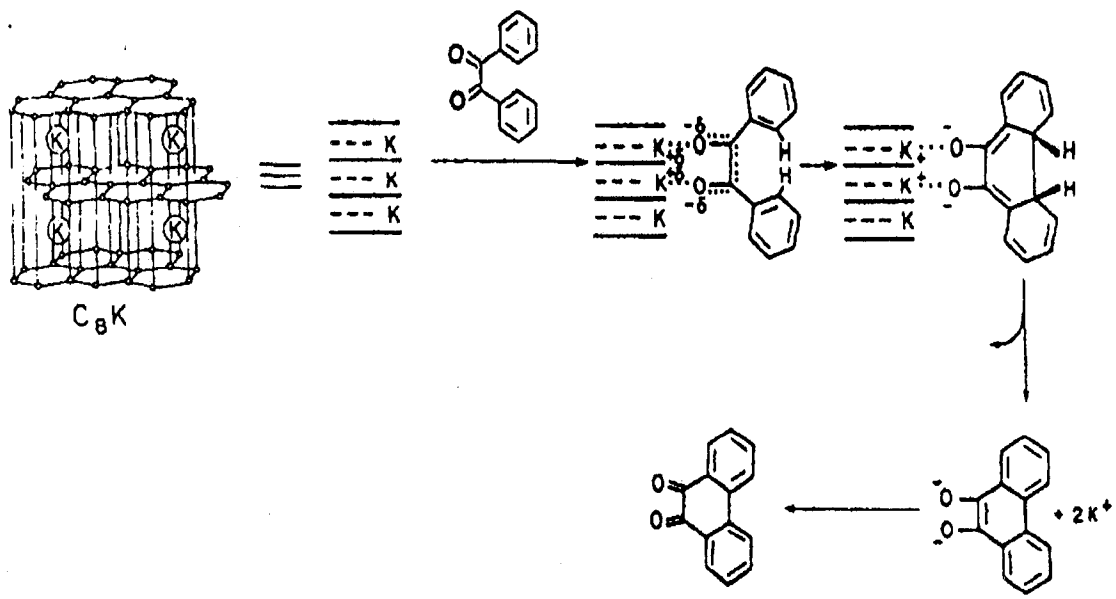
benzil

benzil dianion

phenanthrenequinone

Potassium-graphite intercalate, C_8K is in general an efficient and useful reducing agent. Potassium-graphite can be obtained by heating a mixture of graphite and potassium in the absence of air in a dry helium or argon atmosphere. In C_8K, potassium atoms are located in a highly ordered manner between the carbon layers of graphite. Because of its special structural feature, C_8K reacts much more selectively than nonintercalated dispersed potassium. A "layer edge mechanism" has been suggested to explain this specific behavior (Figure **6**). [56a]

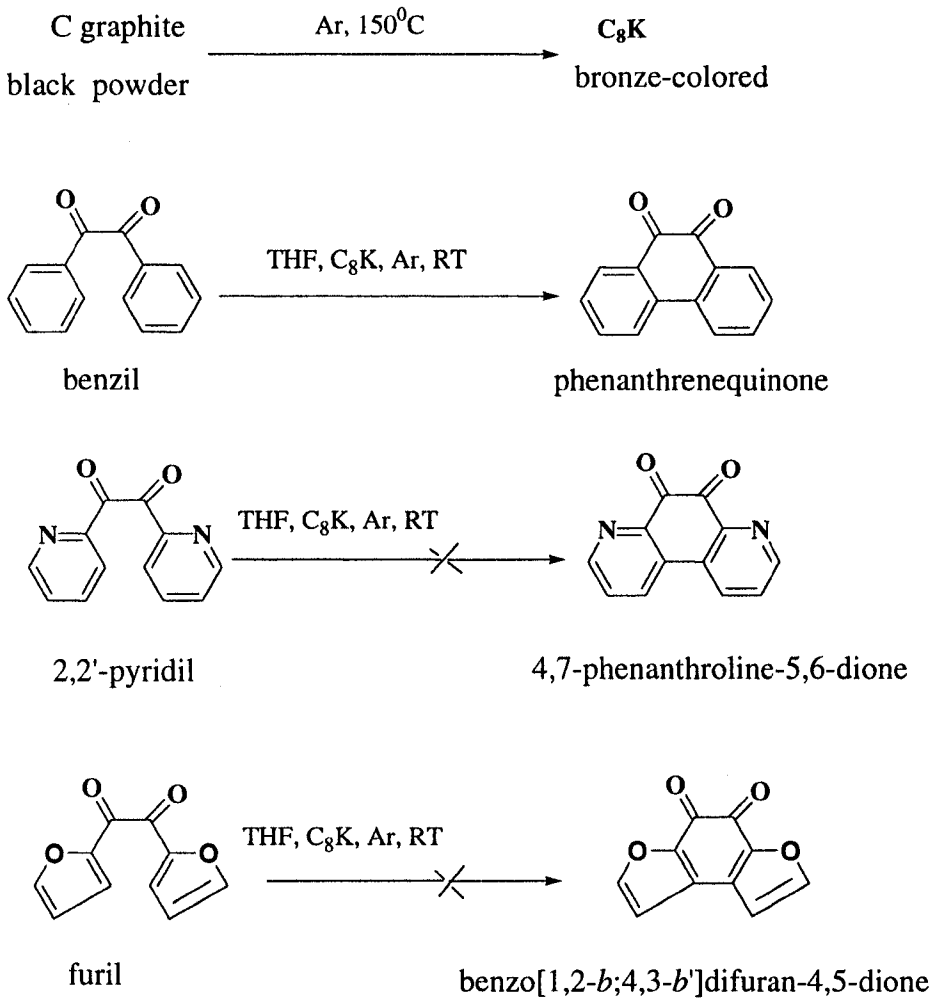
FIGURE 6. Layer Edge Mechanism of the Reaction of C_8K with Benzil [56a]



This strategy shown in Figure **8** appeared appealing for the preparation of 4,7-phenanthroline-5,6-dione from the readily available 2,2'-pyridil. As mentioned earlier, benzil is efficiently cyclized to phenanthrenequinone in good yield using C_8K (Scheme **7**).[56a] However, we found that 2,2'-pyridil is unreactive to C_8K (Scheme **8**). (Also we found that furil is not cyclized by this reagent). Perhaps the lack of reactivity of 2,2'-pyridil is because the needed conformation required for the desired cyclization is disfavored due to nitrogen-oxygen lone-pair/lone pair-repulsions. We are investigating whether metal complexation will force the required conformation and allow cyclization.

In a similar vein, we attempted to cyclize 2,3-dipyridin-2-yl-pyrazine to ppz directly using C_8K, and also photochemically using I_2 as this is a successful cyclization procedure in many other cases,[57] but these approaches have so far been unsuccessful.

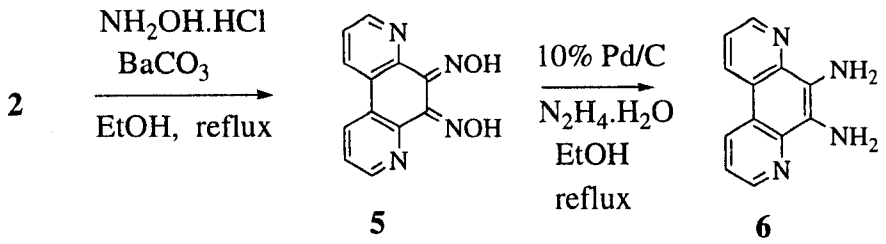
SCHEME 8. Reactions Employed with C_8K



However condensation of **2** with ethylenediamine in the final step of the ppz synthesis allows only 50% as the maximum attainable yield of ppz because the diimine intermediate **3** that is formed by the condensation is not isolable and undergoes a spontaneous disproportionation (Scheme **1**).

The isolated yield of ppz is in fact only about 20-30% in a typical experiment. Furthermore, this procedure does not allow an easy introduction of substituents onto the ppz molecule unless the ethylenediamine has substituents on it. With this in mind, we sought and developed modified routes to ppz and derivatives which avoid the disproportionation problem. One approach has centered on the formation of 4,7-phenanthroline-5,6-diamine **6** as a key intermediate, and its condensation with dicarbonyl compounds. Because the diamine has a double bond connecting the C_5 and C_6 positions, the condensation leads directly to a fully aromatic product, so there is no possibility of disproportionation. The needed diamine **6** had been previously reported by Case[13a] and by Meier and coworkers.[64] Only Meier *et al.* have given a preparative procedure with an overall yield of just 16% via tosylation of 5-amino-4,7-phenanthroline followed by nitration, hydrolysis, and reduction. The starting 5-amino-4,7-phenanthroline was obtained by amination of the corresponding bromo derivative, and the latter was made via a Skraup cyclization of nitrobenzene.[13b]

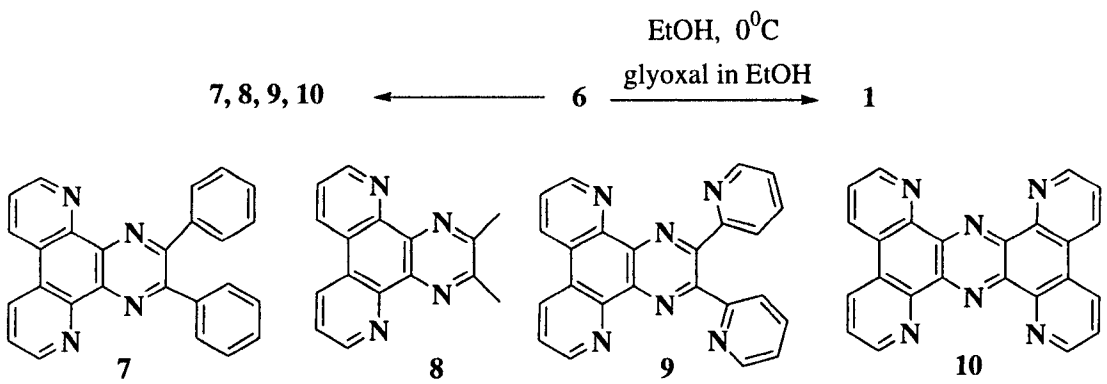
SCHEME 9. Synthesis of Diamine 6



In light of the difficulties of this multi-step sequence and the relatively poor overall yield, we explored an alternative route to diamine **6** from **2** (Scheme **9**).[65] The vacuum dried 4,7-phenanthroline-5,6-dioxime **5** (which TLC analysis showed to have the expected mixture of syn and anti isomers) was not purified, but was directly subjected to Pd/C catalytic reduction with hydrazine hydrate, giving tan colored **6** in 84% yield. The reaction was carried out under argon owing to the oxygen sensitivity of the free base **6**. With the desired substrate **6** in hand, we performed a condensation with glyoxal. The reaction mixture was kept under argon, and diluted glyoxal was added drop wise to **6** at 0 °C over 30 minutes. After filtration, beige colored ppz was isolated from the filtrate. This procedure is exceptionally clean and practicable. Furthermore, dicarbonyl compounds other than glyoxal can be used to prepare a family of ppz derivatives (Scheme **10**).

In this manner 2,3-diphenyl-1,4,5,12-tetraazatriphenylene **7**, 2,3-dimethyl-1,4,5,12-tetraazatriphenylene **8**, 2,3-dipyridin-2-yl-1,4,5,12-tetraazatriphenylene **9**,1,8,9,10,17,18-hexaazaphenanthro[9,10-b]triphenylene **10**, 1,4,5,12-tetraazatriphenylene-2,3-diol **11a**, 1,4-dihydro-1,4,5,12-tetraazatriphenylene-2,3-dione **11b**, 3-hydroxy-1H-1,4,5,12-tetra-azatriphenylene-2-one **11c** and N,N – bis-(6-amino-4,7-phenanthrolin-5-yl)-oxalamide **12** were easily prepared.

SCHEME 10. Synthesis of Ppz and Derivatives via Diamine 6



The compound **9** has been reported previously by two independent groups.[12,15] Only Case and coworkers[12] described the synthesis using 1,4-phenylenediamine as a precursor. The procedure involved Skraup cyclizations and resulted in a low overall yield of just 22%. Our preparation of **9** via the condensation of **6** with 2,2'-pyridil occurred smoothly with 70% yield, providing a clear advantage over the earlier procedures.

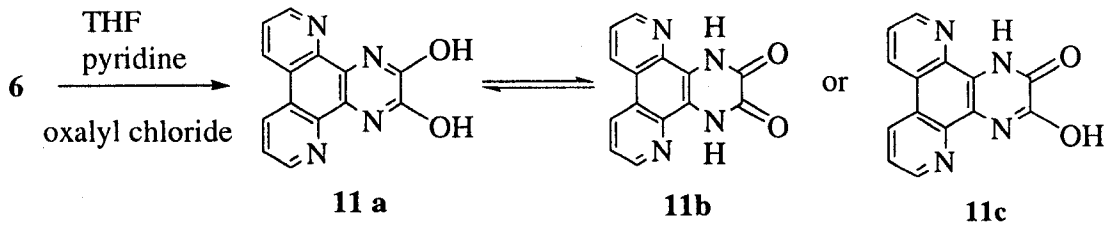
In 1966, Case and co-workers first described the synthesis[12] of **10** with overall yield of 58% by double Skraup reaction of 10,13-diacetamidodipyrido(2,3-*a*:3',2'-*c*)phenazine. The latter was obtained by condensation of **2** with N,N'-diacetyl-2,3-diamino-1,4-phenylenediamine. The catalytic reduction of N,N'-diacetyl-2,3-dinitro-1,4-phenylene-diamine afforded the diamine. Later in 1967, Case reported a similar procedure[13] to the one we describe here but, he used extra steps such as distillation of ethanol and recrystallization of the final product with DMSO to achieve **10** which we have found are not needed. Compound **10** was also synthesized by Bonhote *et al* via coupling of **2** with

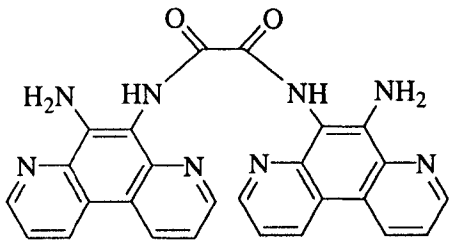
ammonia under reductive conditions involving many steps.[14] Here we report a condensation of **2** with **6** to directly afford the desired **10** through dehydration in 81% yield which provides improvements in terms of simplicity and yield relative to the earlier procedures. As noticed by Case, **10** is a brown solid which is soluble in methanol only in the presence of small amount of water.

The outcome of the condensation of **6** with oxalyl chloride is of interest because the product resulting from the reaction of one equivalent of each reactant may produce one or more of the tautomers 1,4,5,12-tetraazatriphenylene-2,3-diol **11a**, 1,4-dihydro-1,4,5,12-tetraazatriphenylene-2,3-dione **11b**, and 3-hydroxy-1H-1,4,5,12-tetraazatriphenylene-2-one **11c** (Scheme **11**). In fact, when the condensation was performed at -78°C in dilute solution, the major isolated product was N,N-bis-(6-amino-4,7-phenanthrolin-5-yl)-oxalamide **12** resulting from the intermolecular condensation of two equivalents of the diamine **6** and one equivalent of oxalyl chloride. It seems that the HCl produced after the reaction of one of the amine groups with oxalyl chloride protonated the second amine group and rendered it unavailable for further reaction. Therefore, in subsequent experiments, we used pyridine to capture the liberated HCl, thus leaving the second amine group unprotonated and available for the desired intramolecular reaction. This strategy was successful as indicated by mass spectrometry and ^{1}H NMR measurements. The presence of both carbonyl (1695 cm^{-1}) and N-H / OH peaks (br band at 3236 -3612 cm^{-1}) is consistent with the presence of tautomeric forms. A related system has also been described[19] in which tautomeric forms of quinoxalino(2,3-a)3,4-dihydroxyphenazine were discussed. The reaction of the structurally related phenazine-2,3-diamine with

oxalyl chloride is also described in the literature.[46,66] As in our work, the presence of carbonyl and NH/ hydroxyl groups was indicated by their IR data and one of the groups reported an ^{1}H NMR indication of N-H in their Experimental section.

SCHEME 11. Synthesis of 11a, 11b and 11c Tautomers





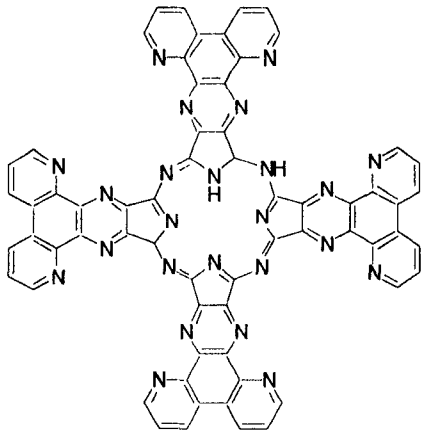
12

Another strategy for the preparation of substituted derivatives of ppz that we have investigated is the use of diaminomaleonitrile **13** instead of ethylenediamine in a condensation reaction with dione **2**. Because the product of this condensation reaction has a double bond between the two amino bearing carbon atoms, condensation leads directly to an aromatic dicyano derivative of ppz (disproportionation not possible). Then the cyano groups can be transformed into other functional groups.

The synthesis of 1,4,5,12-tetraazatriphenylene-2,3-dicarbonitrile **14** has previously been reported[43] with 66% yield via acetic acid catalysed condensation of **2** with excess **13**. In our study, we first attempted the synthesis by employing a simple condensation of **2** with **13** in ethanol. Although the related dicyano product was recovered from a corresponding condensation of 1,10-phenanthroline-5,6-dione by this method, it failed for **2**. In accordance with literature precedent[33] for other cyano derivatives, we found that in the case of **13**, because of the influence of cyano groups, a hemiaminal was formed instead of the Schiff base. Nevertheless, more forcing conditions did accomplish the formation of the desired dicyano product in a higher yield (80%) than that reported in the earlier synthesis mentioned above.

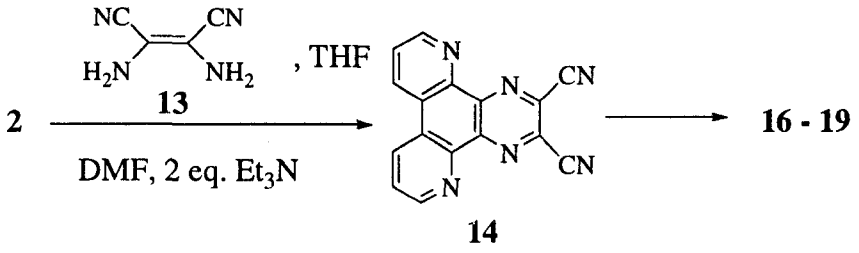
Thus, we employed the condensation of **2** and **13** in THF (Scheme **12**). TLC analysis indicated two major spots, for the formation of the Schiff base along with another intermediate, probably the hemiaminal. After removal of THF and treatment of the crude product with DMF and triethylamine completely converted the remaining hemiaminal to the Schiff base. At this point TLC indicated only one spot. The brown - black crude solid obtained was subjected to a silica gel column to yield brown colored **14** in 80% yield.

A dark brown -black material remained on top of the column. We believe it is likely to be the novel phthalocyanine analog **15**, resulting from the self-condensation of **14**, because dicyano copounds are often precursors for phthalocyanines.[40] This side product is highly insoluble, and thus its purification and characterization are as yet incomplete.

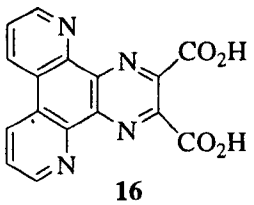


15

SCHEME 12. Synthesis of Dicyano Compound 14 and Other New ppz Derivatives



14



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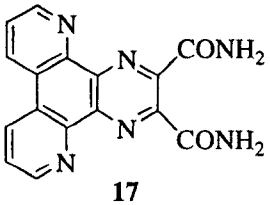

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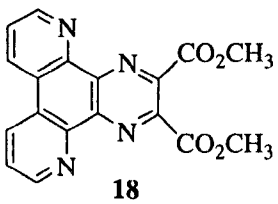

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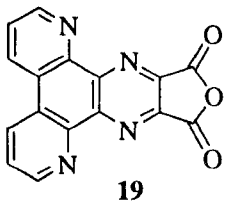


19

We converted the dicyano compound **14** to several other new ppz derivatives, specifically 1,4,5,12-tetraazatriphenylene-2,3-dicarboxalic acid **16,** 1,4,5,12-tetraaza-triphenylene-2,3-dicarboxylic acid diamide **17,** 1,4,5,12-tetraazatriphenylene-2,3-dicarboxylic acid dimethyl ester **18** and 11-oxa-1,8,9,13-tetraazacyclopenta-[*b*]tri-phenylene-10,12-dione **19**, all of which were prepared from **14** by functional group transformations. Traditional standard functional group transformation methods sometimes resulted in difficulties. Some modifications were necessary and ultimately proved successful (see Experimental section).

We were successful in converting **14** to dicarboxylic acid **16** by acidic hydrolysis. The dicarboxylate obtained was converted to the sodium salt and treatment with calculated amount of concentrated hydrochloric acid resulted in an ion exchange and precipitation of the less soluble dicarboxylic acid. Diamide **17** formation was accomplished by stirring **14** with concentrated sulfuric acid for 3 days followed by neutralization to get diamide **17** as a cream colored precipitate. Diester **18** was obtained by acid catalyzed alcoholysis conditions. Even though the diamide **17** was poorly soluble in hot methanol the transformation was complete within 15 hours with sufficient acid catalyst. Diester **18** was isolated in 69% yield after neutralization and extraction to methylene chloride. The formation of anhydride **19** was accomplished by heating the dicarboxylic acid **16** with a large excess of freshly distilled acetic anhydride. The shift of the 1658 cm^{-1} peak to 1736.9 cm^{-1} in the product, along with the disappearance of the dicarboxylic acid OH signal at 3470 cm^{-1}, confirmed complete conversion to the anhydride.

In summary, efficient procedures have been developed for the synthesis of ppz and of several of its substituted derivatives. Several new ppz derivatives have been prepared (4,7-phenanthroline-5,6-dioxime **5**, 2,3-diphenyl-1,4,5,12-tetraazatriphenylene **7,** 2,3-dimethyl-1,4,5,12- tetraazatriphenylene **8**, 1,4,5,12-tetraazatriphenylene-2,3-diol **11a,** and tautomeric forms **11b** and **11c**, N,N–bis-(6-amino-4,7- phenanthrolin-5-yl)-oxalamide **12**, 1,4,5,12-tetraazatriphenylene-2,3-dicarboxylic acid **16**, 1,4,5,12-tetraazatriphenylene-2,3-dicarboxylic acid diamide **17,** 1,4,5,12-tetraazatriphenylene- 2,3-dicarboxalic acid dimethyl ester **18** and 11-oxa-1,8,9,13-tetraazacyclopenta[*b*]triphenylene-10,12-dione **19**) and improved procedures have been described for several known ppz derivatives (4,7-phenanthroline-5,6-diamine **6,** 2,3-dipyridin-2-yl-1,4,5,12-tetraazatriphenylene **9,** 1,8,9,10,17,18-hexaazaphenanthro[9,10-*b*]triphenylene **10** and 1,4,5,12-tetraazatriphenylene -2,3-dicaronitrile **14**).

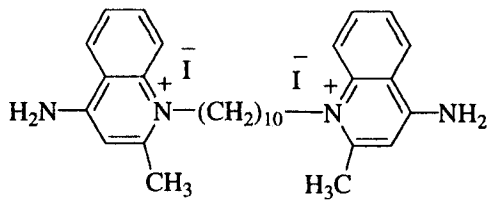
Several methods were employed to synthesize the needed precursor for ppz, 4,7-phenanthroline-5,6 dione **2**. Because of the tediousness of the double Skraup reaction, we explored a modified strategy (Scheme **3**). The idea was to subject a haloester of pyridine to nickel-catalyzed coupling to give the dipyridil diester **x.** Acyloin condensation of **x** would then afford the desired dione **2**. However, the low yield of haloesters and the failure of C_8K mediated cyclizations in pyridine rings, were not encouraging and we continue to investigate better routes to dione **2**.

CHAPTER 2

Synthesis of Dequalinium Analogues and Their Relative Activities Toward Protein Kinase C

Introduction

The term "kinase" is given to enzymes that transfer a phosphoryl group from adenosine triphosphate (ATP) to a protein substrate. Protein kinase C (PKC) is a particularly important member of the family of protein kinases. PKC actually exists as a family of isoforms which are designated by letters of the Greek alphabet (α, β, γ, δ, ε, η, θ, ζ, and λ). These isoforms are divided among three sub-categories designated as conventional (α, β, γ), novel (δ, ε, η, θ) and atypical (ζ and λ). In the present work PKCα was used exclusively. In addition to being Ca^{+2} dependent, PKCα is phospholipid-dependent and phosphorylates intracellular protein substrates on serine and threonine residues.[67] Protein kinase C's role in tumor formation and metastasis makes it an attractive target for chemotherapeutic agents that are able to inhibit its activity. Various quaternary salts in which a – $(CH_2)_n$ – linker bridges two quinoline rings via the ring nitrogen atoms have promise in this regard. Dequalinium diiodide (C_{10}-DECA) **20c,** also known as 1,1′-(1,10-decanediyl)bis(4-amino-2-methylquinolinium)diiodide is an important anti-tumor and anti-metastatic agent that recognizes and inhibits the activity of several isoforms of protein kinase C *in vitro* and *in vivo*.

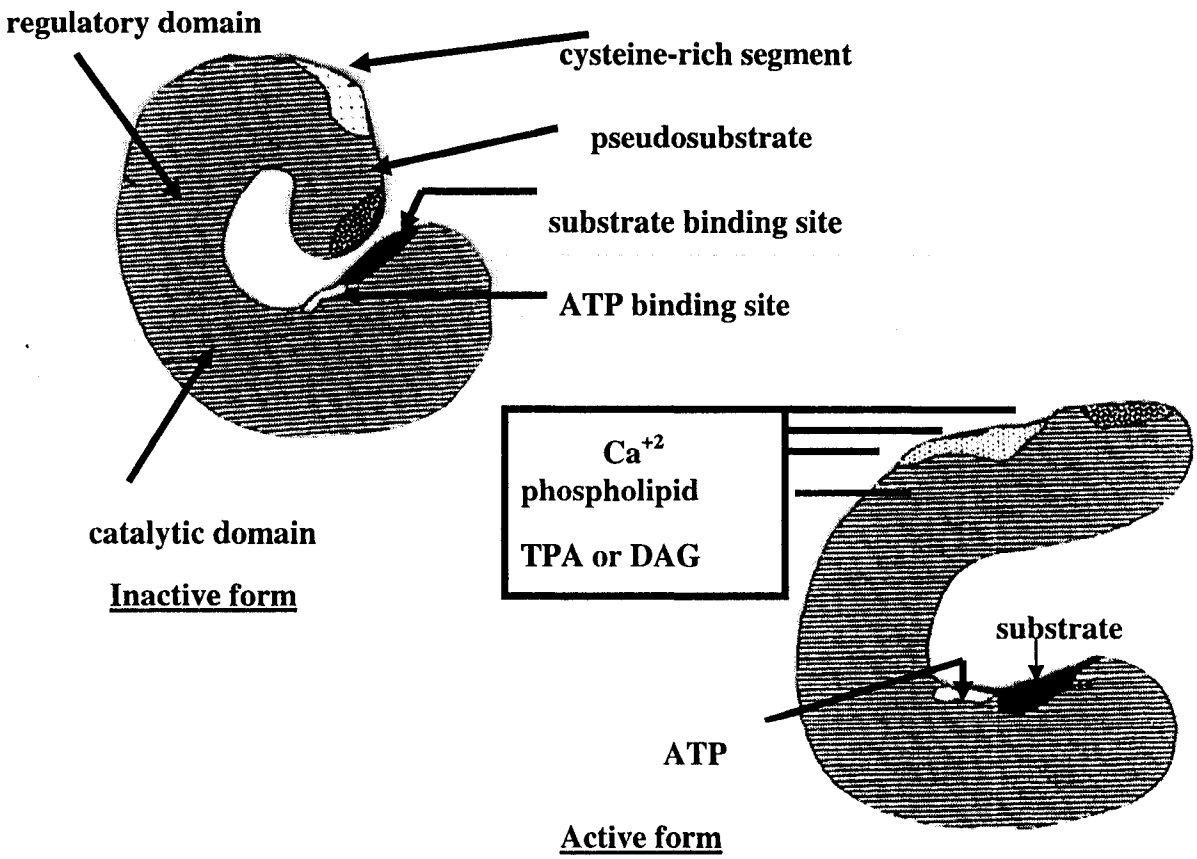


C_{10} - DECA
20c

PKC enzyme is a monomer consisting of a regulatory domain in the N-terminal region, notable for its two cysteine-rich segments and pseudosubstrate segment, and a catalytic domain in the C-terminal region that resembles catalytic domains of other protein kinases by the presence of a characteristic ATP-binding site.[67] PKC has significant hydrophobic character [68] and is believed to be primarily located in the cytosol and reversibly associated with cellular membranes during quiescence.[69] Current models of PKC structure (Figure **7**) depict inactive PKC as having its N-terminal pseudosubstrate segment tucked into the protein structure and in contact with the substrate binding site; this folded, inactive form undergoes a conformational change upon addition of PKC activators (Ca^{+2}, diacylglycerol (DAG) plus phospholipid, or phorbol ester tumor promoter plus phospholipid), resulting in activation of phospholipase C (PLC), and admitting the physiological substrates to the binding site.[70] The activated PLC hydrolyzes phosphatidylinositol-4,5-bisphosphate to diacylglycerol and inositol-1,4,5-trisphosphate (IP3). The IP3 causes the release of endogenous Ca^{+2} that binds to the cytosolic PKC and exposes the phospholipid binding site. The binding of Ca^{+2} promotes translocation of PKC to the membrane where it interacts with diacylglycerol, and becomes a fully active

enzyme. Tumor promoting phobol esters such as TPA, bind to the regulatory domain of the conventional and novel isoforms inducing translocation and activation of the enzyme. Tumor promoters have been used as a tool to selectively activate PKC and to study the participation of conventional and novel isoforms of PKC in a signaling pathway of interest.

FIGURE 7. Hypothetical Scheme Depicting the Inactive and Active Conformations of PKC [67]



PKC is of special importance because of its involvement in cellular signaling pathways that govern cell proliferation, differentiation, and movement. Substances that inhibit PKC activity have the potential to serve as chemotherapeutic agents because, by blocking PKC activity, they can limit or prevent tumor growth and metastasis.

Dequalinium diiodide had been used as an antimicrobial agent, e.g. as the active ingredient in mouthwash, and in other topical formulations, prior to its discovery as a PKC inhibitor.

C_{10} - DECA Timeline

1950 - Discovered as an anti-microbial agent (Allen & Hanburys Ltd., U.K)

1987 - Discovered as an anti-tumor agent (Dr. Lan Bo Chen, Dana Farber Cancer Institute)

1990 - Discovered as a PKC inhibitor (Dr. Susan Rotenberg, Columbia University)

IC_{50} is the concentration required to inhibit 50 % of PKC activity and this is used as the parameter to check the inhibitory potency of various DECA analogues on PKC. The lower the value of IC_{50} for a particular analogue of dequalinium, the more potent it is as an inhibitor.

Structure-activity analysis shows that certain structural attributes of DECA are critical for inhibition of PKC activity.

1. Effect of Monomeric and Dimeric Analogues of DECA on PKCα inhibition.

The parent DECA molecule, dequalinium diiodide (C_{10}-DECA) **20c**, consists of two aromatic moieties bridged by a 10-carbon alkyl linker. Based on previous observations with C_{10}-DECA, it has been suggested that the two aromatic moieties inhibit the activity of PKCα by coincident contact with two nonoverlapping target sites in the catalytic domain.[71]

The two point binding model indicates that the dimeric analogues which bind by both aromatic moieties are better inhibitors when compared to monomeric analogues (Figure **8**).[71] The use of linkers to form dimeric DECA analogues is found to increase the inhibitory activity compared to corresponding monomeric analogues. For example the experimental results given in Table **2** show that the $IC_{50} = 11 \pm 5$ μM for the C_{10}-dimer **20c** (C_{10}-DECA) is >300-fold lower than that observed for the C_1-monomer **22** for which

IC_{50} = 3590 ± 510 μM. The IC_{50} values of compound **21** and **22** imply that the nature of the linker contributes somewhat to the strength of binding of the individual aromatic moieties because potency of the C_{10}-monomer has improved many fold (IC_{50} = 117 ± 8 μM) when compared with that of the C_1-monomer (IC_{50} = 3590 ± 510 μM).

FIGURE 8. Scheme Depicting a Two Point Binding Model for C_{14} - DECA That Binds with *trans*-oid Geometry to the PKC Catalytic Domain.[71]

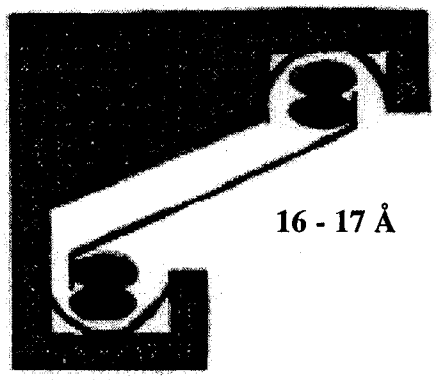
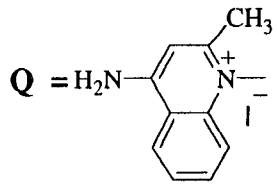


TABLE 2. Linker Distance and Geometry of DECA Analogues Determine Inhibitory Potency with PKCα [71]



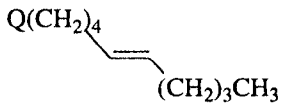
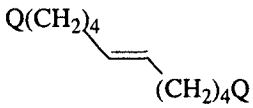
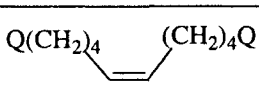
Compound number	Compound	IC_{50} (μM)	Spacer (Å)
20a	$Q(CH_2)_6Q$	54 ± 8	6.4
20b	$Q(CH_2)_8Q$	25 ± 9	8.8
20c	$Q(CH_2)_{10}Q$	11± 5	11.5 parent compound
20d	$Q(CH_2)_{12}Q$	5 ± 2	14.0
20e	$Q(CH_2)_{14}Q$	2.6 ±0.2	16.6
20f	$Q(CH_2)_{16}Q$	2.8 ± 0.2	19.1
21	$Q(CH_2)_9CH_3$	117 ± 8	_ monomer
22	QCH_3	3590 ± 510	_ monomer
23		137 ± 6	_ monomer

TABLE 2 Continued

24		12 ± 3	11.1
25		52 ± 12	10.5

2. Effect of Linker Geometry on PKCα Inhibition [71]

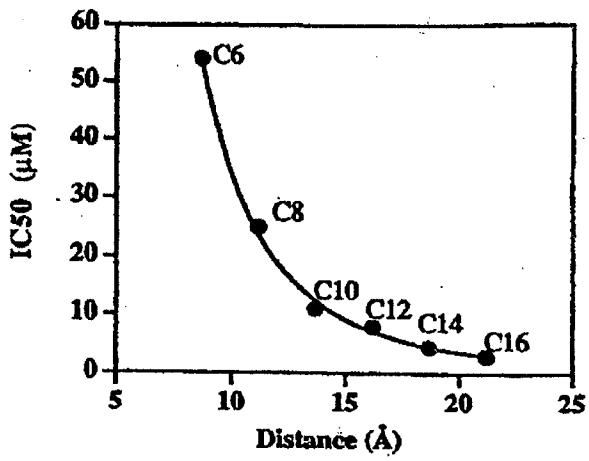
The DECA analogues **24** and **25** that bear a single site of unsaturation in the center of a C_{10}-linker (*cis* and *trans*) were used to explore the effect of spatial geometry of the linker on PKCα activity.[71] The *trans* isomer **24** was more potent and the IC_{50} of 12 μM was almost same as the saturated parent compound **20c** whereas the IC_{50} value of the *cis* isomer **25** was 52 μM. Since the *trans*-5-decene C_{10}-monomer **23** was as potent as the saturated C_{10}-monomer **21**, the *trans* geometry of the linker may not itself contribute to potency.[71] The differing IC_{50} value for compound **24** and **25** imply that the geometry of the linker may affect correct positioning of the aromatic moieties with the enzyme. The energy minimized structures obtained for the saturated C_{10}-DECA, *cis* and *trans* isomers indicate that the *cis* conformation is significantly different from the similar conformations obtained for the saturated and *trans* analogues. The lower inhibitory potency observed for the *cis* isomer may be the outcome of an altered spatial relationship of the

quinolinium ring moieties, because the *cis* isomer exhibits a pyridinium-pyridinium distance (12.7 Å) that is only 1 Å shorter than the analogous distance in the *trans* isomer (13.7 Å) and saturated C_{10}- DECA (14 Å). [71]

3. Effect of Linker Length on PKCα Inhibition [71]

Structure activity relationships obtained for C_{10}-DECA analogues having different linker lengths with 6,8,10,12,14,16 carbons indicate that as the length of the linker increases it acts as a better inhibitor (**Figure 9**).

FIGURE 9. Correlation of Linker Length with PKC_{α} Inhibition [71]



This work was directed at obtaining an improved understanding of the structural features of dequalinium that are important to its biological activity. Our approach was to synthesize analogues of dequalinium in which structural features of the quinolinium ring are systematically varied.

These structural features of DECA analogues to investigate were

(a).The length and nature of the linker

(b) The number of aromatic rings

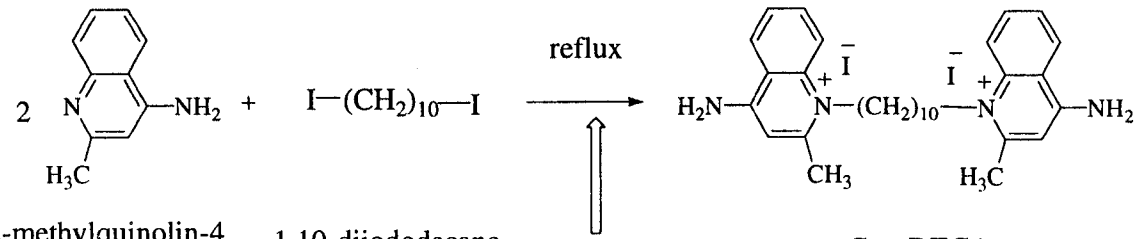
(c) The substitutent pattern around the rings

(d) The positions connected by the linker

(e) The nature of substituents

(f) The type of aromatic rings

SCHEME 13. Synthesis of Dequalinium Diiodide (C_{10}-DECA)



Various dequalinium diiodide (C_{10}-DECA) analogues were synthesized by refluxing 3-fold stoichiometric excess of the quinoline base with one equivalent of the 1,10 diiododecane linker using an appropriate solvent (2-butanone or 4-methyl-2-pentanol) to afford the C_{10}-DECA (Scheme **13**).

Two types of bases were used for the synthesis of DECA analogues.

Type 1 - consists of single aromatic moiety

Type 2 - consists of two aromatic moieties linked by exocyclic C_6 linker

Synthesis of the Type 1 - Quinoline Bases (Starting Material)

Starting materials 2-methylquinolin-4-ylamine, 2-methylquinoline, quinolin-4-ylamine, quinoline and pyridine-4-ylamine were purchased and dimethylquinolin-4-ylamine and dimethylpyridine-4-ylamine were synthesized by nucleophilic displacement of chloride ion from 4-chloroquinaldine and 4-chloropyridine respectively.

Synthesis of the Type 1 - Bases

Synthesis of 2-methylquinolin-4-ylamine (N,N-dimethylaminoquinoline) which consists of a single quinoline ring

The synthesis of the N,N-dimethylaminoquinoline was achieved by nucleophilic displacement of chloride ion from 4-chloroquinaldine. Phenol was used in the reaction to protonate the quinoline nitrogen atom thereby enhancing the susceptibility of the ring to nucleophilic attack.

Synthesis of the Type 2 - Bases

Synthesis of *N*,*N*-bis(2-methylquinolin-4-yl)decane-1,10-diamine which consists of two quinoline rings

The synthesis of *N*,*N*-diquinolin-4-ylhexane-1,6-diamine, **32** and *N*,*N*-bis(2-methyl-quinolin-4-yl)hexane-1,6-diamine **33** were achieved by an analogous procedure to that of the 2-methylquinolin-4-ylamine (N,N-dimethylaminoquinoline) except that the 1,6 diaminohexane was used as the nucleophile.

TABLE 3. Various Aromatic Moieties (Quinoline Bases) and the Corresponding DECA Analogues

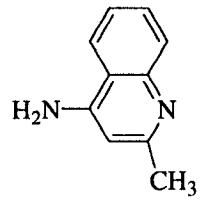
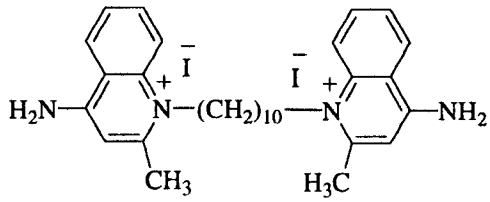
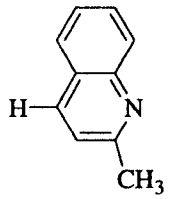
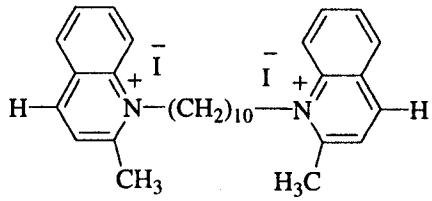
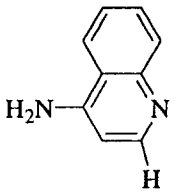
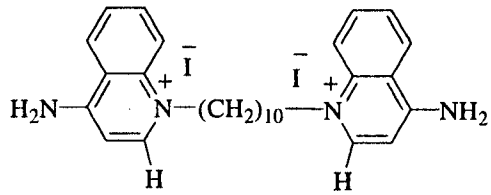
Aromatic moiety	C_{10}- Dequalinium Analogue
 2-methylquinolin-4-ylamine **20c-b**	 C_{10} - DECA **20c**
 2-methylquinoline **26-b**	 **26**
 quinolin-4-ylamine **27-b**	 **27**

TABLE 3 Continued

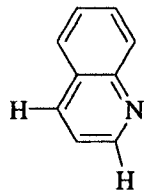
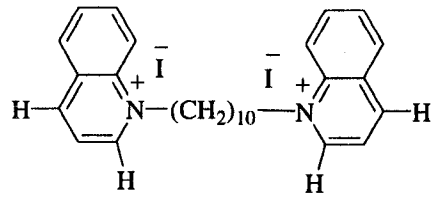
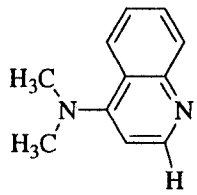
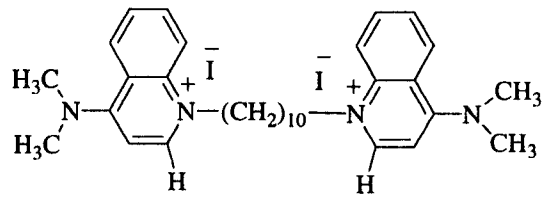
Aromatic moiety	C_{10}- Dequalinium Analogue
 quinoline **28-b**	 **28**
 dimethylquinolin-4-ylamine **29-b**	 **29**

TABLE 4. Various Aromatic Moieties (Quinoline Bases) and the Corresponding DECA Type Analogues

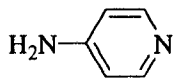
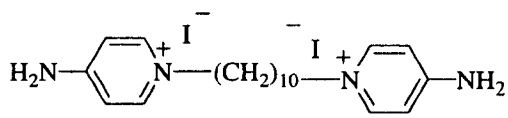
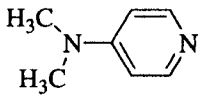
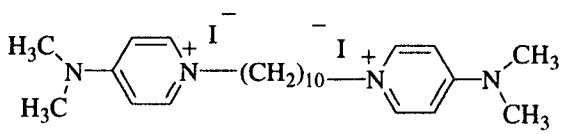
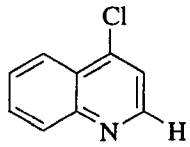
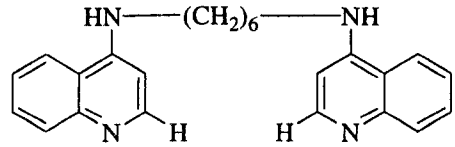
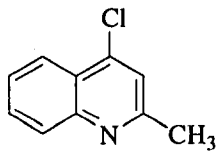
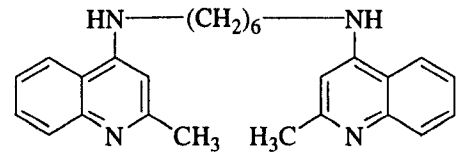
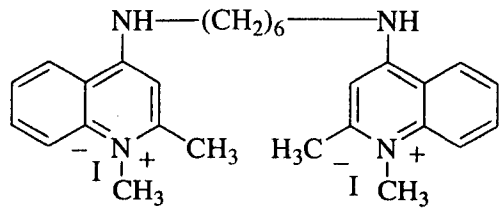
Aromatic moiety	C_{10}- DECA Type Analogue
 pyridin-4-ylamine **30-b**	 **30**
 dimethylpyridin-4-ylamine **31-b**	 **31**

TABLE 5. Various Aromatic Moieties and the Type 2 Bases (Consists of Two Aromatic Moieties Linked by Exocyclic C_{-6} Linker)

Aromatic moiety	Type 2 Base
 4-chloroquinoline	 *N*,*N*'-diquinolin-4-ylhexane-1,6-diamine **32**
 4-chloro-2-methylquinoline	 *N*,*N*'-bis-(2-methylquinolin-4-yl)hexane-1,6-diamine **33**

Synthesis of a DECA Type Analogue 34, with Dimeric Base 33 which Consists of Two Quinoline Rings Linked by Exocyclic C-$_6$ Linker

The type-2 quinoline base *N*,*N*-bis(2-methylquinolin-4-yl)hexane-1,6-diamine was refluxed with excess of methyl iodide for the methylation on ring nitrogen to give **34**.



34

Synthesis of DECA Type Analogues with Different Hetero Atoms, Different Groups and Different Sized Heterocyclic Rings

Various DECA type analogues (**35-40**) were synthesized by refluxing a 2.4-fold stoichiometric excess of the base with one equivalent of the 1,10 diiododecane linker.

TABLE 6. Different Bases (Different Hetero Atoms, Different Groups and Different Sized Heterocyclic Rings) and the Corresponding C_{10}-DECA Type Analogues

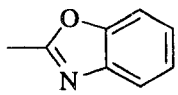
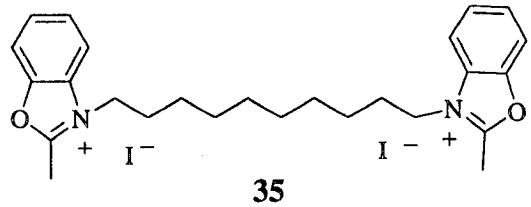
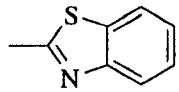
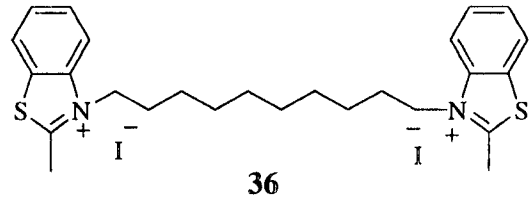
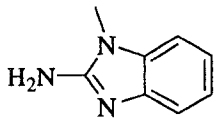
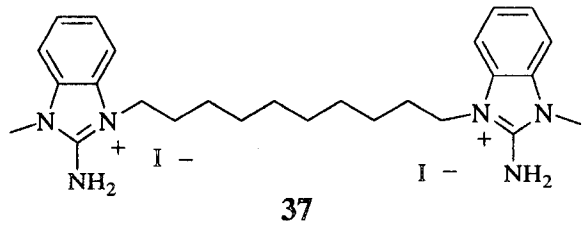
Base	C_{10}- DECA Type Analogue
 2-methylbenzoxazole **35-b**	 **35**
 2-methylbenzothiazole **36-b**	 **36**
 2-amino-1-methylbenzimidazole **37-b**	 **37**

TABLE 6 Continued

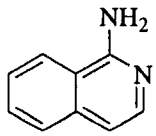
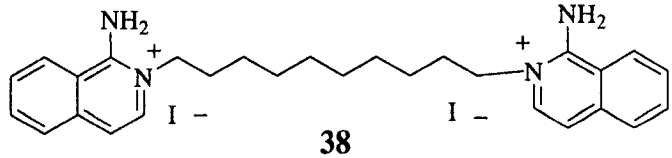
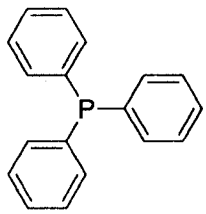
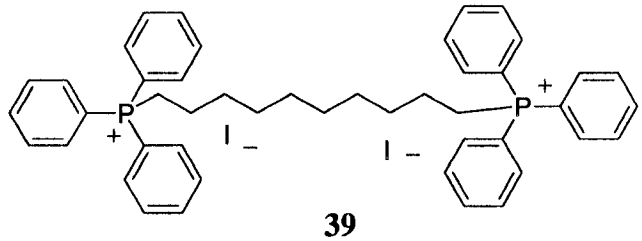
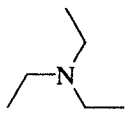
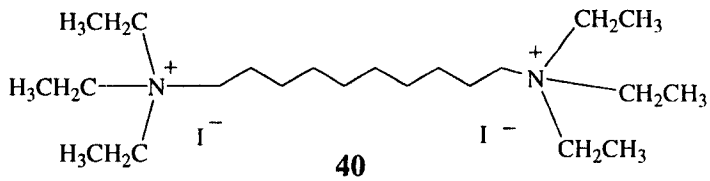
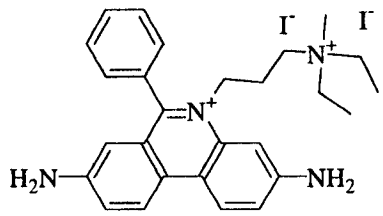
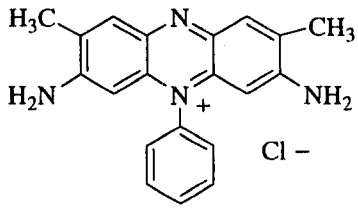
Base	C_{10}- DECA Type Analogue
 isoquinolin-1-ylamine **38-b**	 **38**
 triphenylphosphine **39-b**	 **39**
 triethylamine **40-b**	 **40**

TABLE 7. Other Quarternized Analogues



propidiumiodide
41



3,7-diamino-2,8-dimethyl-5-phenylphenazin
-5-iumchloride (Safranine O)
42

Assay of Dequalinium Analogues as PKC_{α} Inhibitors

(Assay of PKC_{α} catalytic activity in vitro)

Recombinant human PKC_{α} (95% pure) was used for testing DECA analogues *in vitro*.[71] To test the inhibitory potency of these compounds, the total catalytic activity of PKC_{α} (36 ng protein) was analyzed with increasing concentrations of a selected DECA analogue.

PKC Assay

Purified PKCα + Activators (Ca^{+2}, phospholipid)
[γ-^{32}P]-ATP
Different concentrations of DECA analogues
Peptide Substrate
↓
Phosphocellulose paper
↓
Phosphorylated peptide
↓
Analyze the radioactive content of peptide by ß Scintillation counting
↓
Determine IC_{50}

Assays were carried out on two different occasions for the DECA analogues reported here and hence in each case the parent C_{10}-DECA has its own IC_{50} value with respect to the experimental conditions employed at that time.

Table **8** and **9** - Assay series 1

Table **10** - Assay series 2

RESULTS

TABLE 8. IC_{50} Values for Various DECA Analogues and DECA Type Analogues

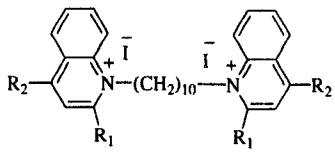
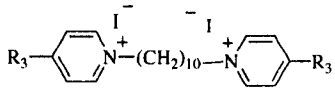
Number	Compound			IC_{50} (μM)
		R_1	**R_2**	
20C		CH_3	NH_3	11
26		CH_3	H	128
27		H	NH_2	15-20
28		H	H	inactive
29		H	$N(CH_3)_2$	17
		R_3		
30		NH_2		> 100
31		$N(CH_3)_2$		> 250

TABLE 9. IC_{50} Values for Bases 32 and 34 with Two Aromatic Moieties Linked by Exocyclic Ring Nitrogens and for DECA Type Analogue 34

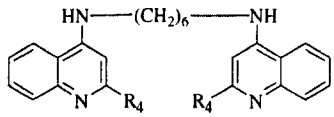
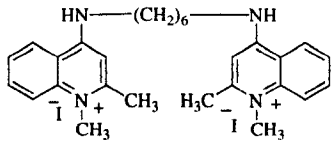
Number	Compound		IC_{50} (μM)
		R_4	
32		H	250
33		CH_3	250
34			> 250

Table 10. Molar Extinction Coefficients (ε), Wave lengths (λ_{max}, nm) and IC_{50} (μM) Values Obtained for DECA Type Analogues in the Assay Series 2

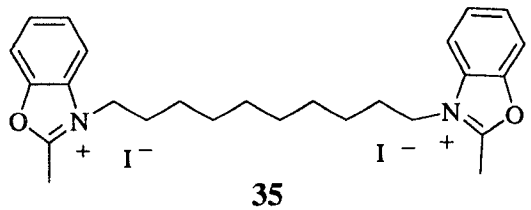
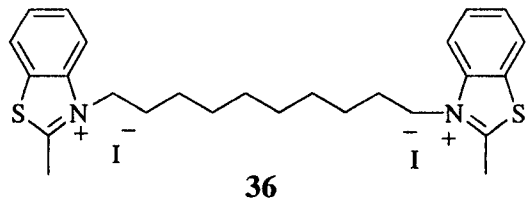
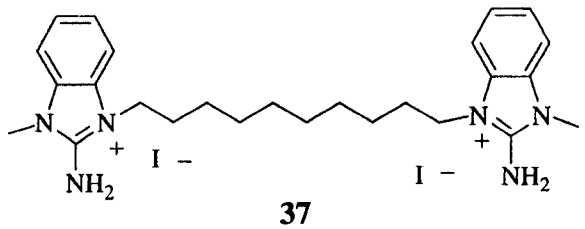
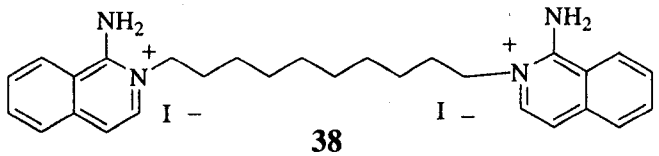
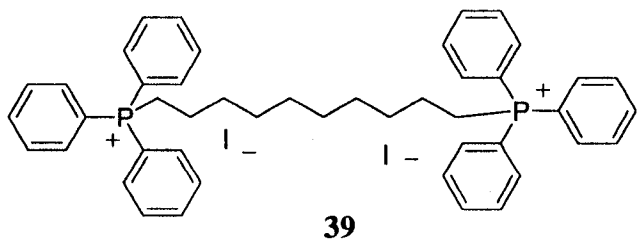
ε	λ_{max} (nm)	Compound	IC_{50} (μM)
21800	218.8	 **35**	105
71400	232.8	 **36**	>250
112800	231.6	 **37**	64.5
103600	233.6	 **38**	68.5
313500	230	 **39**	47.5

TABLE 10 Continued

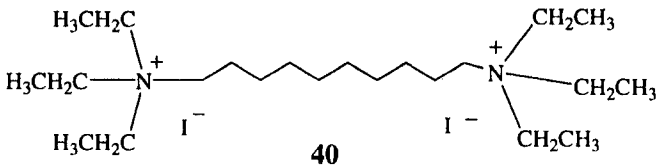
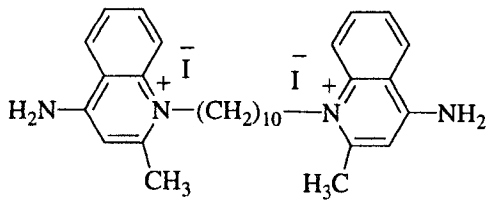
ε	λ_{max} (nm)	Compound	IC_{50} (μM)
35500	231.6	 **40**	>250
27000	333.0	 **20c**	45 (parent)

TABLE 10 Continued

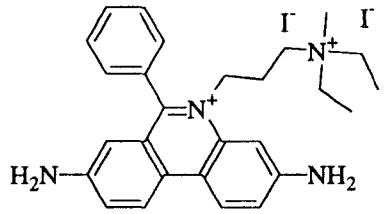
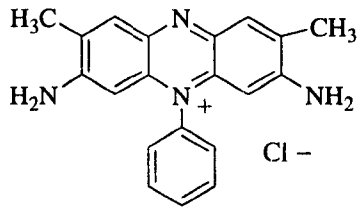
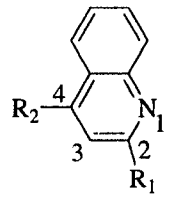
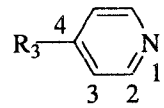
ε	λ_{max} (nm)	Compound	IC_{50} (μM)
67800	287.6	 **41**	65
52300	519.6	 **42**	89.5

TABLE 11. Partial Charges and Frontier Orbital Energies (*E* HOMO) for Quinoline Bases, Calculated using Gauss view 3.0 / 6-31G Level



Base	R_1	R_2	Charge		*E* HOMO (au)
			C_4	N_1	
20c-b	CH_3	NH_2	0.382	-0.662	-0.284
26-b	CH_3	H	-0.131	-0.626	-0.301
27-b	H	NH_2	0.378	-0.613	-0.287
28-b	H	H	-0.065	-0.579	-0.300
29-b	H	$N(CH_3)_2$	0.4830	-0.614	-0.273

TABLE 12. Partial Charges and Frontier Orbital Energies (*E* HOMO) for pyridine Bases, Calculated using Gauss view 3.0 / 6-31G Level



Pyridine base	R_3	Charge		*E* HOMO (au)
		C_4	N_1	
30-b	NH_2	0.363	0.558	- 0.322
31-b	$N(CH_3)_2$	0.4234	-0.555	- 0.310

TABLE 13. Partial Charges and Frontier Orbital Energies (*E* HOMO) for Bases 35-b to 40-b Calculated using Gauss view 3.0 / 6-31G Level

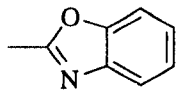
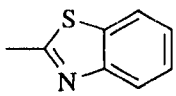
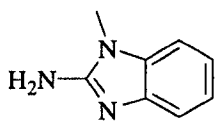
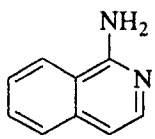
Base	Charge N	*E* HOMO (au)
 2-methylbenzoxazole **35-b**	-0.584	-0.306
 2-methylbenzothiazole **36-b**	-0.533	-0.308
 2-amino-1-methylbenzimidazole **37-b**	-0.655	-0.274
 isoquinolin-1-ylamine **38-b**	-0.634	-0.277

TABLE 13 Continued

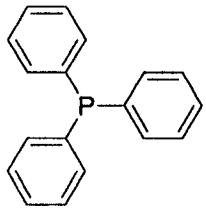
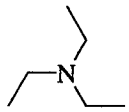
Base	charge	*E* HOMO (au)
 triphenylphosphine **39-b**	P 0.449	-0.262
 triethylamine **40-b**	N -0.552	-0.333

TABLE 14. Partial Charges and Frontier Orbital Energies (*E* HOMO) for Bases 41-b to 42-b, Calculated using Gauss view 3.0 / 6-31G Level

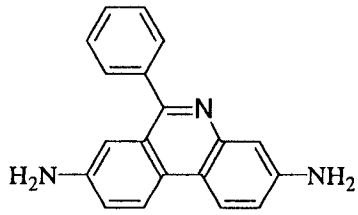
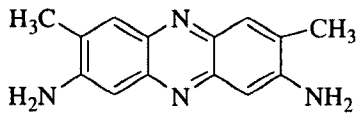
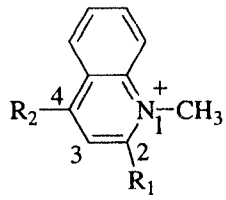
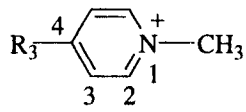
Base	Compound	Charge N	*E* HOMO (au)
41-b		-0.627	-0.241
42-b		-0.654	-0.261

TABLE 15. Partial Charges and Frontier Orbital Energies (*E* LUMO) for Quarternized Model Compounds of 20c-q to 29-q, Calculated using Gauss view 3.0 / 6-31G Level



Compound	R_1	R_2	Charge		*E LUMO* (au)
			C_4	N_1	
20c-q	CH_3	NH_2	0.506	-0.790	-0.079
26-q	CH_3	H	-0.056	-0.764	-0.106
27-q	H	NH_2	0.496	-0.743	-0.084
28-q	H	H	-0.056	-0.683	-0.115
29-q	H	$N(CH_3)_2$	0.531	-0.720	-0.079

TABLE 16. Partial Charges and Frontier Orbital Energies (*E* LUMO) for Quarternized Model Compounds of 30-q and 31-q, Calculated Using Gauss view 3.0 / 6-31G Level



Compound	R_3	Charge		*E LUMO* (au)
		C_4	N_1	
30-q	NH_2	0.459	0.624	-0.068
31-q	$N(CH_3)_2$	0.500	0.167	-0.065

TABLE 17. Partial Charges and Frontier Orbital Energies (*E* LUMO) for Quarternized Model compounds 35-q to 40-q, Calculated Using Gauss view 3.0 / 6-31G Level

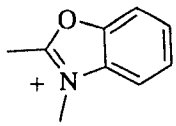
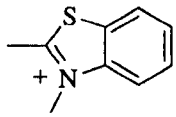
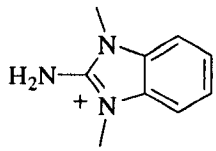
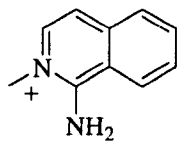
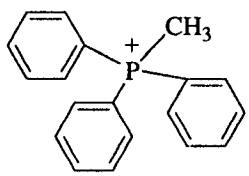
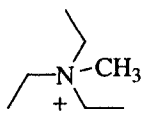
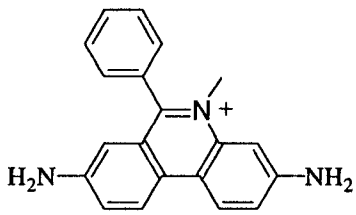
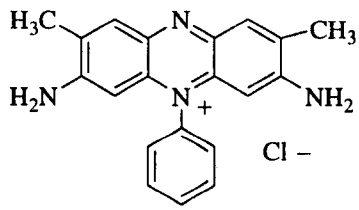
Compound	Base	Charge N	*E* LUMO (au)
35-q		-0.713	-0.077
36-q		-0.633	-0.087
37-q		-0.776	-0.023
38-q		-0.723	-0.077
39-q		Charge P 1.135	-0.022
40-q		-0.555	0.039

TABLE 18. Partial Charges and Frontier Orbital Energies (*E* LUMO) for Quarternized Model compounds of 41-q to 42-q, Calculated Using Gauss view 3.0 / 6-31G Level

Compound number	Compound	**Charge N**	*E HOMO*	*E LUMO*
41-q		-0.771	-0.362	-0.078
42-q		-0.837	-0.252	0.013

DISCUSSION

The dimeric nature of DECA is unique among the class of PKC inhibitors. The two point binding model of C_{10}-DECA (figure **8**) suggests that the interaction with DECA occurs within the catalytic domain of PKC, and is independent of its interaction with the regulatory domain of PKC.[71] This model also supports the idea that two aromatic moieties of a single DECA molecule (dimer) bind coincidentally to two target sites in the catalytic domain. It has been found that the dimeric analogues are in general better inhibitors than their corresponding monomeric counterparts.[71]

The series of DECA experiments we carried out suggested that certain structural features of dequalinium analogues contribute significantly to the efficiency of inhibitory action on PKC.

These include

(a) the length of the linker

(b) the role of the methyl and amino substituents

(c) the role of additional substituents and of substituents other than methyl and amino

(d) the number and nature of the aromatic rings.

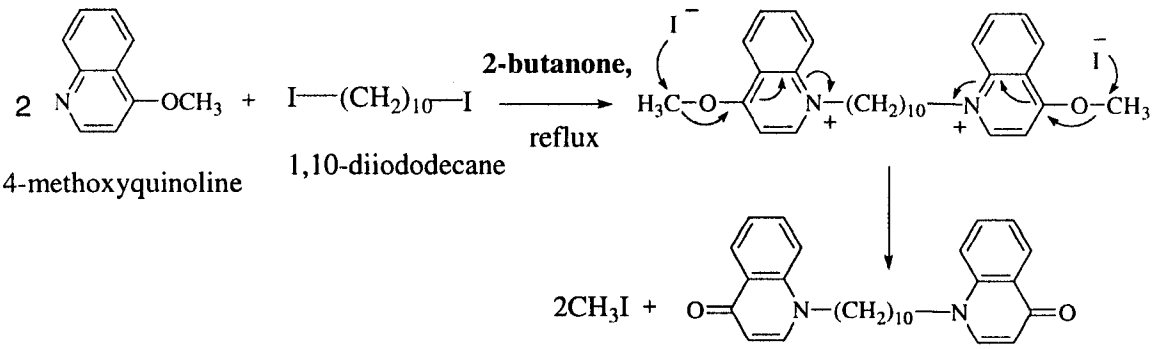
Earlier work[71] revealed that the length of the linker plays a significant role in determining inhibitory behavior. In general, there is a progressive increase in activity with

increasing linker length (Figure **9**). In the present work, we have explored the other aspects. In our first series of experiments we synthesized C_{10}-DECA analogues of different aromatic moieties by varying the nature of substituents, the substituent pattern around the ring, number of aromatic rings and positions connected by the linker. For comparison purposes, the linker length was kept constant to 10 carbons.

The similarity in the IC_{50} values of C_{10}-DECA (**20c**) and **27** suggests that the methyl group at the C-2 position is not highly significant for inhibitory potency. In contrast, the 4-NH_2 group does play an important role. Its removal results in a dramatic decrease in potency. The role of the $-NH_2$ group could in principle be related either to the hydrogen-bonding nature of the group, or to its ability to donate electron density into the quinoline ring *via* its resonance effect. To investigate this, we synthesized a DECA analogue in which a N,N-dimethylamino (-NMe_2) group replaces the $-NH_2$ group. This new analog proved to be about equally as active as the original amino compound. Thus, the electron donating ability of the amine group at the fourth position seems to be its critical role.

We also intended to examine another electron donating group (4-methoxy) regarding its effect on PKC potency. However the desired alkylation to form a DECA analogue could not be accomplished because a different reaction occurring instead (Scheme **14**). Microwave-assisted reactions were developed with appropriate solvent systems to synthesize some of the analogues.

SCHEME 14. Alkylation of 4-methoxyquinoline



4-methoxyquinoline

1,10-diiododecane

We also attempted to synthesize the DECA analogues that contained electron withdrawing groups. (4-carboethoxyquinoline, 4-chloroquinoline). However, presumably due to the electron deficiency of the ring nitrogen atom (N-1), none of the bases were good nucleophilles for alkylations and thus formation of dimeric salts were unsuccessful.

To investigate the importance of the number of aromatic rings, substituted pyridine bases rather than quinoline bases were used to synthesize some C_{10}-DECA analogues. We found that quinoline based analogues show much higher potency than the corresponding pyridine based derivatives.

The observation that the electron-donating ability of groups on the aromatic ring correlates with PKC inhibitory ability guided our further studies. We followed a two-pronged approach - synthesizing new compounds, and performing computational studies with Gauss view 3.0/ Restricted Hartree-Fock, 6-31G level software calculations. In this

computational work, we investigated the role of structural changes in three ways. First the precursors to the quarternized derivatives were investigated. Three properties were examined - the energy of the highest occupied molecular orbital (*E* HOMO), the Mulliken charge on the nitrogen atom (N-1) that was to be quarternized, and the Mulliken charge on C-4 atom of the heteroaromatic ring. Each provides an indicator of the electron density on the heteroaromatic ring. We also examined quarternized materials, but to simplify the calculations, performed them on model compounds in which a simple methyl group replaced the linker in the actual DECA analogues. Again we calculated Mulliken charges on the N-1 atom and also investigated the energy of the LUMO (*E* LUMO), of the materials. On the experimental side, we also investigated a number of analogues in which quarternized atoms other than N are present, and in which the pyridine component of the quinolinium system was replaced by more electron-rich five-membered rings such as oxazoles and thiazoles.

In the second phase of our work, we explored the activity of dequalinium analogues with different hetero atoms, different groups and different sized heterocyclic rings. Here we introduced other electron rich bases such as five and six membered rings of **35** and **36** analogues which contain oxygen and sulfur hetero atoms in addition to nitrogen atoms. For comparisons we used commercially available propidiumiodide **41,** and safranine O **42** and we also synthesized DECA-type analogues based on triethylamine and triphenyl-phosphine.

We were initially surprised that a simple C_{10} analogue based on triphenylphosphine as the parent base proved to be just about as active as the parent C_{10} - DECA itself. However, calculations show that triphenylphosphine has the highest *E* HOMO of all the free bases of C_{10} -DECA analogues or DECA type analogues that we examined. Thus in retrospect, its high potency is perhaps not so surprising with the addition of electron donating groups into the rings of this analogue. This finding provides an avenue for future study.

Propidium iodide **41** and 2-amino-1-methylbenzimidazole based dimeric salt **37** also show relatively high potencies which indicates the beneficial effect of amino groups. In **37**, the $-NH_2$ group is ortho to quarternized nitrogen and hence donates electron density into the quinoline ring *via* its resonance effect and the additional ring nitrogen also contributes towards its activity. In **41**, the positively charged nitrogen atom is resonance stabilized by the $-NH_2$ group. The same principle applies for **38**, where the $-NH_2$ is ortho to quarternized nitrogen. This salt shows a lower IC_{50} value of 68.5 μM. The effect of NH_2 can also be seen in Safranine O,**42** which shows an IC_{50} value of 89.5 μM.

To explore the effect of hetero atoms other than nitrogen we synthesized DECA type analogues using 2-methylbenzoxazole and 2-methylbenzothiazole bases. Both these are less potent when compared to that of **37** and **38** which implies that the electron donating groups play a more pivotal role in determining inhibitory potency than hetero atoms present in the ring.

The measured PKC inhibitory potency correlates well with three different numerical values obtained from the Gauss view calculations. These are the Mulliken charges on carbon 4, (C-4) *E* HOMO and *E* LUMO.

The structures of the free bases, quarternized model compounds and the results of the calculations are given in Tables **11-18**. There is a correlation between the HOMO energy (*E* HOMO) of the parent base and the activity of the DECA analogue made from it. The free bases of the active dequalinium analogues have *E* HOMO > -0.29 au. For example the free bases that are the precursors to C_{10}-DECA **20c**, *E* HOMO = -0.28 au, **27**, *E* HOMO = - 0.28 au and **29**, *E* HOMO = - 0.27 au. This correlation with HOMO energy applies even for the second series free bases of active analogues (2-amino-1-methyl-benzimidazole, isoquinoline-1-ylamine, and triphenylphosphine. Free bases of the other active analogs (propidiumiodide and safranine O) also show the similar trend mentioned above.

There is also a correlation between the C-4 charge of the C_{10} - DECA bases and the activity of their corresponding DECA analogues. All the free bases of active DECA analogues have positive charges at C-4 (Table **11**). The charge on the N-1 free bases did not show any correlation with their activities. In these free bases we looked at the *E* HOMO as a measure of electron richness in the ring systems.

Similarly the quarternized model compounds in Table **15** which are more active have a positive charge on C-4, when compared with that of the less active analogues whose C-4 charges are negative. The *E* LUMO of active quarternized model compounds have values greater than -0.1 au. This trend reflects even to the other active quarternized analogues **41-q** and **42-q**. The charges on N-1 of quarternized bases do not correlate in any obvious way with the PKC inhibitory activities.

TABLE 19. Correlation of the IC_{50} Values with *E* HOMO for Free Bases

E HOMO	IC_{50} μM
E HOMO > - 0.29	< 68.5 - 11
E HOMO < - 0.29	> 70 or inactive

TABLE 20. Correlation of the IC_{50} Values with C-4 for Free Bases

C-4 charge	IC_{50} μM
C-4 > 0.37	< 68.5 – 11
C-4 < 0.37	> 70 or inactive

In the case of pyridine based DECA analogues, the *E* HOMO of free bases are < -0.29 au and therefore irrespective of the charge on C-4 (C-4 > 0.37), *E* HOMO correlate with the inactivity of the corresponding DECA type analogues.

It seems that the IC_{50} values are not exactly reproducible for the same compound in different assays and therefore exact comparisons are somewhat problematic.

We also investigated how the positions connected by the linker affect the potency. The related analogues of unquarternized **32**, **33**, and methylated **34** where the two quinoline rings are linked through exocyclic nitrogen atoms were used for comparisons with DECA analogues that were quarternized through ring nitrogen atoms. The analogues of exocyclic linkers do not seem to be good inhibitors because the IC_{50} values obtained were extremely high (IC_{50} values were $\geq$ 250). There was no such correlation found between the inactivity of these analogues and the gauss calculations.

In summary we can say that potent new analogs of dequalinium diiodide were synthesized which show IC_{50} values approximately equal to that of the parent DECA compound.

EXPERIMENTAL SECTION

General Information.

Melting points were measured on a Electrothermal melting point apparatus and are uncorrected. Elemental analysis were performed by the Desert Analytics Laboratory, Arizona. ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 and 100 MHz on a Bruker spectrometer, respectively. Chemical shifts were expressed in ppm relative to TMS (0.00 ppm) or residual solvent signals; MeOD 3.30 ppm / 49.0 ppm and DMSO 2.50 ppm / 39.5). IR spectra were recorded on a Perkin-Elmer, 1600 series FT-IR spectrophotometer, and $CHCl_3$ was used as the solvent unless otherwise indicated (solids insoluble in $CHCl_3$ were ran as mulls using Nujol). Electrospray Ionization (ESI) mass spectra were recorded at the Hunter College of the City University of New York. Reaction solvents were distilled under N_2 as follows. Tetrahydrofuran (THF) and diethyl ether (Et_2O) from sodium and benzophenone immediately before use. Ethanol from magnesium ethoxide and Methanol from magnesium methoxide. methylene chloride (CH_2Cl_2), chloroform ($CHCl_3$), and acetonitrile (CH_3CN) from calcium hydride (CaH_2). Anhydrous N,N-dimethylformamide (DMF) and 2 M ammonia in methanol were obtained from Aldrich in SureSeal™ bottles. Flash chromatography and TLC were carried out with E. Merck silica gel 60 (230- 400 ASTM mesh) and Merck $60F_{254}$ (0.25 mm thick) sheets, respectively. All air and water sensitive reactions were performed in oven or flame - dried glassware.

4',7'-Phenanthrolino-5,6:5'6'-pyrazine (ppz) (1).

To a stirred solution of diamine **6** (200 mg, 0.952 mmol) in 10 mL anhyd EtOH under an argon atmosphere at 0 °C was added aqueous glyoxal (40%, 125.6 mg, 0.866 mmol) in absolute EtOH (6 mL) dropwise over 30 min. The solution was refluxed for 3.5 h and cooled in a refrigerator overnight. The red liquid mixture obtained was concentrated under vacuum, dissolved in few drops of MeOH and anhyd Et_2O (3 mL) was added and then the reaction mixture was filtered. The filtrate was concentrated to afford 221 mg (100%) of ppz **1** as a beige solid: mp 284 °C; ^{1}H NMR ($CDCl_3$) δ 7.78 (dd, 2H, *J* = 8.2, 4.4 Hz), 8.89 (dd, 2H, *J* = 1.6, 8.2 Hz), 9.21 (s, 2H), 9.23 (dd, 2H, *J* = 1.6, 4.4 Hz); ^{13}C NMR ($CDCl_3$) δ 124.3, 125.8, 131.1, 142.9, 145.2, 146.0, 151.3; LRMS (MH^+) calcd for *m/z*: $C_{14}H_8N_4$ 233.07, found 233.10.

4,7-Phenanthroline-5,6-dioxime (5).

An argon-flushed mixture of **2** (1.0 g, 4.76 mmol), $NH_2OH.HCl$ (1.16 g, 16.6 mmol) and (1.41 g, 7.14 mmol) of $BaCO_3$ in 70 mL anhyd EtOH was refluxed for 18 h. After the mixture was cooled to rt, the solvent was removed under reduced pressure, and the residue was treated with 120 mL of 0.2 M HCl. The solution was stirred for 45 min and filtered. The filtered solid was washed with water, EtOH, anhyd Et_2O and then dried under vacuum at 90 °C to afford 0.914 g (80%) of **5** as a yellow-brown solid: mp 240 °C (dec); IR 1698, 3447 cm^{-1}; LRMS (MH^+) calcd for *m/z*: $C_{12}H_8N_4O_2$ 241.06, found 241.10.

4,7-Phenanthroline-5,6-diamine (6).

A mixture of **5** (0.80 g, 3.33 mmol) and 10% Pd/C (0.80 g) in anhyd ethanol (200 mL) was flushed with argon and refluxed. A solution of 7.0 mL of $N_2H_4.H_2O$ in 30 mL of anhyd EtOH, were injected to the above mixture drop wise over an hour. After the solution was refluxed overnight, the hot mixture was passed through a pad of celite with suction, and the celite was thoroughly washed with boiling EtOH. The filtrate was concentrated. Then the residue was triturated with 60 mL of cold water and cooled in a refrigerator overnight. The tan solid obtained was filtered, washed with cold water, anhyd Et_2O and dried under vacuum to give 0.59 g (84%) of **6** as a tan solid: mp 218 °C; IR 1343,1630, 3425 cm^{-1}; ^1H NMR (DMSO) δ 5.52 (s, NH_2, 4H), δ 7.47 (dd, 2H, J = 8.3, 4.3 Hz), 8.85 (dd, 2H, J = 4.3, 1.4 Hz), 9.08 (dd, 2H, J = 8.3, 1.4 Hz); ^{13}C NMR (DMSO) δ 118.1, 118.2, 125.7, 131.2, 140.0, 148.7; Anal. calcd for $C_{12}H_{10}N_4$: C, 68.56; H, 4.79; N, 26.65, found C, 68.10; H, 4.65; N, 26.36; LRMS (MH^+) calcd for *m/z*: $C_{12}H_{10}N_4$ 211.09, found 211.10.

2,3-Diphenyl-1,4,5,12-tetraazatriphenylene (7).

Diamine **6** (250 mg, 1.19 mmol) was stirred in anhyd EtOH (15 mL) at rt and degassed with argon for 15 min. 1,2-Diphenylethane-1,2-dione (benzil) (270 mg, 1.31 mmol) was injected and refluxed for 3 h under argon. After cooling to rt, the product was filtered, washed with anhyd EtOH and Et_2O to afford 333 mg (73%) of **7** as a beige solid: mp 300 °C (dec); ^{1}H NMR ($CDCl_3$) δ 7.37 (m, 6H), 7.73 (dd, 4H, *J* = 6.6, 2.0 Hz), 7.79 (dd, 2H, *J* = 8.4, 4.4 Hz), 8.95 (dd, 2H, *J* = 8.4, 1.4 Hz), 9.27 (dd, 2H, *J* = 4.4, 1.4 Hz); ^{13}C NMR ($CDCl_3$) δ 123.8, 125.9, 128.3, 128.9, 130.3, 131.0, 138.8, 140.5, 145.4, 151.4, 154.4; Anal. calcd for $C_{26}H_{16}N_4$: C, 81.23; H, 4.20; N, 14.57, found C, 81.45; H, 3.98; N, 14.5; LRMS (MH^+) calcd for *m/z*: $C_{26}H_{16}N_4$ 385.14, found 385.10.

2,3-Dimethyl-1,4,5,12-tetraazatriphenylene (8).

Following the procedure described for **7**, diamine **6** (100 mg, 0.476 mmol) was condensed with butane-2,3-dione (45.1 mg, 0.524 mmol) in anhyd EtOH (8 mL) for 3 h under argon. The red brown reaction mixture was concentrated and purified by flash chromatography (2 M ammonia in MeOH / CH_2Cl_2 4.8: 0.4, *Rf* 0.45) to afford 102.8 mg (83%) of **8** as a white solid: mp 242 °C; ^{1}H NMR ($CDCl_3$) δ 2.99 (s, 6H); 7.76 (dd, 2H, *J* = 8.3, 4.4 Hz), 8.93 (dd, 2H, *J* = 8.3, 1.6 Hz), 9.26 (dd, 2H, *J* = 4.4, 1.6 Hz); ^{13}C NMR ($CDCl_3$) δ 123.5, 125.2, 131.0, 140.3, 145.4, 151.2, 155.2; Anal. calcd for $C_{16}H_{12}N_4$: C, 73.83; H, 4.65; N, 21.52, found C, 73.63; H, 4.50; N, 21.06; LRMS (MH^+) calcd for *m/z*: $C_{16}H_{12}N_4$ 261.11, found 261.10.

2,3-Dipyridin-2-yl-1,4,5,12-tetraaza-triphenylene (9).

Following the procedure described for **7**, diamine **6** (100 mg, 0.476 mmol) was condensed with 1,2-dipyridin-2-ylethane-1,2-dione (2,2'-pyridil) (100 mg, 0.476 mmol) in anhyd EtOH (8 mL) for 2 h under argon. The light brown residue was filtered, washed with anhyd EtOH and Et_2O. Purification by flash chromatography (2 M ammonia in methanol/ CH_2Cl_2 4.8: 0.4, *Rf* 0.67) to afford 128.7 mg (70%) of **9** as a white solid: mp >360 °C; ^{1}H NMR ($CDCl_3$) δ 7.26 (dd, 2H, *J* = 7.6, 4.8 Hz), 7.81 (dd, 2H, *J* = 8.4, 4.4 Hz), 7.88 (dd, 2H, *J* = 7.6, 7.6 Hz), 8.27 (d, 2H, *J* = 7.6 Hz), 8.38 (d, 2H, *J* = 4.8 Hz), 8.96 (dd, 2H, *J* = 8.4, 1.6 Hz), 9.29 (dd, 2H, *J* = 4.4, 1.6 Hz); ^{13}C NMR ($CDCl_3$) δ 123.1, 124.0, 125.2, 126.1, 131.1, 136.8, 140.9, 145.2, 148.4, 151.5, 153.6, 157.2; LRMS (MH^+) calcd for *m/z*: $C_{24}H_{14}N_6$ 387.13, found 387.10.

1,8,9,10,17,18-Hexaazaphenanthro[9,10-*b*]triphenylene (10).

Following the procedure described for **7**, diamine **6** (200 mg, 0.952 mmol) was condensed with dione **2** (220 mg, 1.048 mmol) in anhyd EtOH (8 mL) for 2 h under argon. The brown residue was filtered, washed with anhyd EtOH, Et_2O and dried to afford 296.3 mg (81%) **10** as a dark brown solid: mp > 360 °C; ^{1}H NMR (CD_3OD / D2O 10:1) δ 7.62 (dd, 4H, *J* = 8.0, 4.4 Hz), 8.32 (br, 4H), 8.77 (d, 4H, *J* = 8.0); (MH^+) calcd for *m/z*: $C_{24}H_{12}N_6$ 385.11, found 385.10.

1,4,5,12-Tetraazatriphenylene-2,3-diol (11a) and tautomeric forms (11b) and (11c).

To a vigorously stirred mixture of diamine **6** (100 mg, 0.476 mmol) in 100 mL THF and 5 equivalents of anhyd pyridine (0.2 mL, 2.3 mmol) under an argon atmosphere at -78 °C was added oxalyl chloride (66.48 mg, 0.524 mmol) dropwise. After the red colored mixture was stirred at -78 °C for 4 h under argon, the temperature was gradually raised to rt, stirred for 3 h and refluxed for 2 h. The orange colored mixture was filtered, filtrate was concentrated, 5 mL of water and 0.5 mL of saturated Na_2CO_3 was added, residue was filtered and recrystallized from CH_2Cl_2 to afford 69.1 mg (55%) of **11** as a light brown colored solid: mp 332 °C (dec); IR (nujol) 1695, 3236 - 3612 (br) cm^{-1}; ^1H NMR (DMSO) δ 7.83 (dd, 2H, J = 8.0, 4.0 Hz), 9.07 (br, 2H), 9.37 (dd, 2H, J = 8.0, 1.0 Hz), 11.86 (s, 2H); LRMS (MH^+) calcd for *m/z*: $C_{14}H_8N_4O_2$ 265.06, found 265.0.

N,N – Bis-(6-amino-[4,7] phenanthrolin-5-yl)-oxalamide (12).

To a stirred solution of diamine **6** (200 mg, 0.952 mmol) in 25 mL THF under an argon atmosphere at -75 °C was added oxalyl chloride (145.1 mg, 1.143 mmol) in THF (20 mL) dropwise over 1 h. The temperature of the brick red reaction mixture was gradually raised to rt, stirred for 3 h and refluxed for 3 h under argon. The residue was filtered and washed with THF and recrystallized from methanol to afford 171.5 mg (76%) of **12** as an orange colored solid: mp 283 °C (dec); IR (nujol) 1590, 1617, 1690, 3166, 3283.5, 3424.6 cm^{-1}; ^1H NMR (DMSO) δ 7.74 (br, 2H), 8.03 (dd, 2H, J = 8.4, 4.0 Hz), 8.88 (d, 2H, J = 4.0 Hz), 9.17 (d, 2H, J = 4.6 Hz), 9.40 (d, 2H, J = 8.4 Hz), 9.58 (br, 2H),

10.66 (s, 2H); LRMS (MH^+) calcd for *m/z*: $C_{26}H_{18}N_8O_2$ 475.16, found 475.0.

1,4,5,12-Tetraazatriphenylene-2,3-dicaronitrile (14).

Dione **2** (960 mg, 4.57 mmol) was dissolved in THF (117 mL) at room temperature under argon, and with stirring **13** (544 mg, 5.03 mmol) was added and refluxed for 2 h. The solvent was evaporated and the light brown residue was redissolved in anhyd DMF (56.4 mL) and 2 equivalents of Et_3N (1.26 mL) were added. The mixture was heated to 100 ^{0}C for 1 h, cooled to rt and the solvent was removed under reduced pressure. The black brown solid obtained was purified by flash chromatography. (2 M ammonia in MeOH / CH_2Cl_2 4.8: 0.4, *Rf* 0.47) to give 103.1 mg (80%) of **14** as a brown solid: mp > 360 ^{o}C ; ^{1}H NMR (DMSO) δ 8.10 (dd, 2H, *J* = 8.4, 4.4 Hz), 9.27 (dd, 2H, *J* = 4.4, 1.2 Hz), 9.42 (dd, 2H, *J* = 8.4, 1.2 Hz); ^{13}C NMR (DMSO) δ 114.6, 126.5, 128.0, 132.5, 132.7, 142.2, 142.5, 151.9; LRMS (MH^+) calcd for *m/z*: $C_{16}H_6N_6$ 283.07, found 283.10.

1, 4, 5, 12-Tetraazatriphenylene-2,3-dicarboxylic acid (16).

A solution of dicarbonitrile **14** (200 mg, 0.709 mmol) in 50% H_2SO_4 (14 mL) was heated under reflux for 3 h. After cooling the mixture to rt, diluted with cold water and neutralized with $NaHCO_3$. The mixture was then acidified by dropwise addition of concentrated HCl (ca. 4 mL) until initial precipitation. After stirring for 20 min an additional 10 mL of concd HCl was added until no more precipitation. The light yellow suspension was filtered, washed with cold water, cold acetone, Et_2O and dried in vacuum at 100 °C to afford 197.5 mg (87 %) of **16** as a light yellow solid: mp > 360 °C; IR 1658, 3470 cm^{-1}; ^1H NMR (DMSO) δ 8.01 (dd, 2H, J = 8.2, 4.4 Hz), 9.21 (dd, 2H, J = 4.4, 1.4 Hz), 9.38 (dd, 2H, J = 8.4, 1.4 Hz); ^{13}C NMR (DMSO) δ 125.3, 126.9, 132.4, 141.6, 143.6, 145.3, 151.2, 166.2; LRMS (MH^+) calcd for *m/z*: $C_{16}H_8N_4O_4$ 321.05, found 321.10.

1,4, 5,12-Tetraazatriphenylene-2,3-dicarboxylic acid diamide (17).

A solution of dicarbonitrile **14** (200 mg, 0.709 mmol) in concd H_2SO_4 (5 mL) was stirred at rt for 3 d and diluted by dropwise addition in to a vigorously stirred ice water (20 mL). The mixture was stirred and neutralized with solid $NaHCO_3$ until no more precipitation. The suspension was filtered, washed with cold water, acetone, Et_2O and dried in vacuum at 100 °C to afford 178.2 mg (79 %) of **17** as a beige colored solid: mp 260 °C (dec); IR (nujol) 1684, 3275 cm^{-1}; ^1H NMR (DMSO) δ 7.9 (s, 2H), 8.0 (dd, 2H, J = 8.1, 4.4 Hz), 8.22 (s, 2H), 9.22 (dd, 2H, J = 4.4, 1.4 Hz), 9.39 (dd, 2H, J = 8.1, 1.4

Hz); LRMS (MH^+) calcd for *m/z*: $C_{16}H_{10}N_6O_2$ 319.09, found 319.10.

1,4,5,12-Tetraazatriphenylene-2,3-dicarboxalic acid dimethyl ester (18).

To an argon-flushed solution of diamide **17** (200 mg, 0.629 mmol) in concd H_2SO_4 (4 mL) was injected anhyd MeOH (15 mL) at rt. The reaction mixture was refluxed for 12 h until the full consumption of the diamide **17** was observed (TLC, 2 M ammonia in MeOH / CH_2Cl_2 2.8 : 1.2, *Rf* 0.65). The brown mixture was cooled, water was added (25 mL), then the solution was filtered. The filtrate was neutralized with solid $NaHCO_3$. The product was extracted with CH_2Cl_2, the combined organic layers were washed with brine, dried ($MgSO_4$), and concentrated. The residue was dissolved in $CHCl_3$ (5 mL), filtered and the filtrate was concentrated to afford 151 mg (69%) of ester **18** as a pale yellow sticky substance: IR 1737 cm^{-1}; ^1H NMR (DMSO) δ 4.14 (s, 6H), 7.86 (dd, 2H, *J* = 8.4, 4.4 Hz), 8.96 (dd, 2H, *J* = 8.4, 1.2 Hz), 9.31 (dd, 2H, *J* = 4.4, 1.2 Hz); ^{13}C NMR (DMSO) δ 52.6, 124.1, 126.0, 130.2, 141.4, 143.0, 144.0, 150.9, 163.9; LRMS (MH^+) calcd for *m/z*: $C_{18}H_{12}N_4O_4$ 349.09, found 349.10.

11-Oxa-1,8,9,13-tetraazacyclopenta[*b*]triphenylene-10,12-dione (19).

A mixture of dicarboxylic acid **16** (250 mg, 0.781 mmol) and freshly distilled acetic anhydride (12 mL) was vigorously stirred and heated to 120 °C under argon, until all the acid had dissolved. The clear brown solution was cooled to rt, concentrated to about 2

mL, and precipitated with freshly distilled anhyd Et_2O. The brown solid obtained was washed with freshly distilled acetonitrile and the brown colored filtrate was concentrated and dried under vacuum to afford 153.3 mg (65%) of moisture - sensitive **19** as a brown solid: mp >360 °C; IR 1736.9, 1819.1 cm^{-1} ; ^1H NMR (DMSO) δ 8.06 (dd, 2H, J = 7.4, 3.5 Hz), 9.28 (br, 2H), 9.44 (br, 2H).

Skraup reaction was performed as described in the literature. [45]

3-Bromo-2-methylpyridine

2-picoline (4.65 g, 0.05 mol) was added under nitrogen to mechanically stirred solid aluminum chloride (20 g, 0.15 mol). The slurry was heated to 100 °C with stirring and bromine (4 g, 0.25 mol) was added over a period of 1 h. The heating was continued for another 0.5 h and the reaction mixture was poured into a mixture of 200 mL of ice water and 7.5 mL of conc. HCl. More HCl was added until the mixture was acidic. Excess $NaHSO_3$ solid was added and the mixture was left for 4 h at rt, decanted and washed with CH_2Cl_2. NaOH (50%) was used to make the aqueous phase alkaline and extracted with ether. Organic phase was washed with brine, dried and evaporated to give crude product which then separated by chromatographic column using ether and petroleum ether (1:9) to give the desired product (10%) as an oil. ^1H NMR ($CDCl_3$) δ 2.70 (s, 3H), 7.54 (d, H, J = 8.0), 8.30 (dd, H, J = 8.0, Hz), 8.69 (br, H).

3-Iodopyridine-2-carboxylic acid (via route 1)

To a solution of 2,2,6,6-tetramethylpiperidine (0.51 mL, 3 mmol) in THF (5 mL) at -78 °C under Ar, was added BuLi (4 mmol) in hexane (1.6 mL) dropwise. 15 min later, picolinic acid (0.12 g, 1 mmol) was added to this mixture. After 30 min at -78 °C, the temperature of the mixture was gradually increased to 0 °C over a period of 1 h and stirred for 30 min at 0 °C. The reaction mixture was then transferred dropwise to a cooled (0 °C) solution of iodine (3 mmol) in THF (5 mL), stirred for 15 min and allowed to reach to rt and continued the stirring for 1 h . Water (0.5 mL) was added to hydrolyze the excess base. (water saturated with sodium thiosulfate can also be used for hydrolysis step in order to remove the excess of iodine). The solvents were removed under vacuum, the residue was dissolved in water (2 mL) and the resulting solution washed with CH_2Cl_2 and Et_2O. The aqueous phase was evaporated to dryness and the residue was purified by flash chromatography (eluent: CH_2Cl_2: MeOH in NH_3 7:3) to give the corresponding lithium picolinate. It was then dissolved in MeOH (3 mL) and treated with Amberlite® IR-120 (2.5 g) and stirred for 30 min. The resin was removed by filtration, the filtrate was evaporated and the residue washed with acetone to afford 20% of halopicolinic acid as a beige powder. ^{1}H NMR (D_2O) δ 7.12 (dd, H, J = 8.0, 2.0 Hz), 8.27 (d, H, J = 8.0), 8.37 (d, H, J = 2 Hz). LRMS (M^+) calcd for *m/z*: C_6H_4NI 249.0, found 249.0.

Pyridine-2-carboxylic acid chloride·HCl (via route 2)

A mixture of pyridine-2-carboxylic acid (2.43 g, 0.019 mol) in $SOCl_2$ (1.5 mL) was stirred at room temperature for 2d under Ar. The reaction mixture obtained was poured into pentane (100 mL) and the precipitate was filtered off, washed with several portions of pentane (100 mL) and dried under vacuum to afford a 76 % yield of the product as a brick-red solid. mp 140 °C (dec).

Pyridine-2-carboxylic acid phenylamide

Pyridine-2-carboxylic acid chloride·HCl (2.79g, 0.019 mol) and a catalytic amount of DMAP was added to a stirred solution of aniline (1.98 mL, 0.019 mol) in CH_2Cl_2 (20 mL) and pyridine (10 mL). The resulting suspension was stirred under Ar for 12 h at rt. Saturated $NaHCO_3$ was added to quench the reaction mixture and the aqueous layer was extracted twice with CH_2Cl_2 and the combined organic phases were dried ($MgSO_4$), evaporated and purified by flash chromatography (hexane/ethyl acetate 4:1) to afford 57% of the pyridine-2-carboxylic acid phenylamide as white crystals. mp 109 °C. ^{1}H NMR ($CDCl_3$) δ 7.15 (m, 1H), 7.39 (dd, 2H), 7.46 (m, 1H), 7.77 (d, 2H), 7.93 (m, 1H), 8.31 (d, 1H), 8.64 (d, 1H), 10.06(s, br, 1H); ^{13}C NMR ($CDCl_3$) 119.7, 122.4, 124.3, 126.5, 129.1, 137.7, 137.8, 147.9, 149.9, 161.9.

3-Iodopyridine-2-carboxylic acid phenylamide

n-BuLi was added to a stirred mixture of anilide (0.04 mol) in THF at -78 °C and the stirring was continued for 30 min. The temperature of the reaction mixture was gradually increased to rt and stirred for 1 h. The reaction mixture was cooled again for -78 °C and the electrophile iodine (0.04 mol) in THF (100 mL) was added and stirred for 1 h. The temperature of the mixture was warmed up to rt and kept for 2 h. Then 25 mL of water was added and the organic layer was separated and aqueous layer was extracted with $CHCl_3$ (3 x 100 mL). The organic phase was washed with 10% aqueous sodium thiosulphate. The combined organic layers were dried ($MgSO_4$) and evaporated to afford a semisolid residue. It was purified by flash chromatography (CH_2Cl_2: MeOH / 6: 2) to give the haloanilide 60% as a beige solid. mp. 107 °C, ^{1}H NMR ($CDCl_3$) δ 7.13 (m, 2H), 7.38 (dd, 2H), 7.76 (d, 2H), 8.42 (dd, 1H), 8.59 (dd, 1H), 10.09 (s, br, 1H); ^{13}C NMR ($CDCl_3$) 89.4, 119.8, 124.4, 126.5, 129.0, 130.8, 137.7, 147.0, 147.6, 151.3, 161.2. LRMS (M^+) calcd for *m/z*: $C_{12}H_8N_2O_2I$ 324.0, found 324.1

3-Iodopyridine-2-carboxylic acid methyl ester

A solution of haloanilide (0.01 mol) in 15% HCl (60 mL) was heated to reflux for 15 h. The yellowish mixture was made alkaline with KOH (10%) and extracted with benzene. The organic layer was separated and the pH of the aqueous phase was adjusted approximately to 3.4 with HCl (10%). The white solid 3-iodo-pyridine-2-carboxylic acid which was separated as a white solid was filtered and immediately converted into the

methyl ester without further purification.

$NaHCO_3$ was added to a suspension of the 3-iodo-pyridine-2-carboxylic acid (0.004 mol) in water (30 mL) and the mixture was stirred until all solid had dissolved. The solvent was then removed under reduced pressure and the residue was dried under vacuum. The sodium salt of the residue was then subjected to methylation with methyl iodide (0.004 mol) in DMF (5 mL) at 0 °C for 4 h. The solvent was evaporated under reduced pressure and water was added (10 mL) to the residue and crude was extracted with $CHCl_3$ and purified by flash chromatography. ($CHCl_3$). ^1H NMR ($CDCl_3$) δ 3.98 (s, 3H), 7.35 (d, H), 8.37 (dd, H), 7.75 (d, H); LRMS (M^+) calcd for *m/z*: $C_7H_6INO_2$ 263.0, found 263.1

Preparation of C_8K

A three- neck flask was flushed with dry Ar and heated to about 300 °C, in order to dry the surface of the flask. After cooling to 150 °C under Ar, graphite powder (10.8 g) was placed and stirred. After 15 min, potassium metal (4.10 g) previously washed with pentane was added in small slices over a period of 40 min. The stirring at 150 °C was continued until the bronze-colored C_8K was formed. It was cooled to rt and kept under Ar.

Reduction with C_8K

Preparation of phenanthrolinequinone

To a freshly prepared C_8K (12.5 mmol) in freshly distilled anhyd THF (20 mL) was added benzil (0.525 g, 2.5 mmol) in THF (30 mL) and stirred at rt for 5 h under Ar. During this time hydrogen gas was evolved. The resulting reaction mixture was carefully quenched with water, filtered, extracted with CH_2Cl_2, evaporated and recrystallized with methanol to obtain phenanthrolinequinone (78%) as a red precipitate. m.p. 208 °C. ^1H NMR (DMSO) δ 7.52 (dd, 2H), 7.75 (dd, 2H), 8.01 (d, 2H), 8.27 (d, 2H).

Attempt to synthesize 4,7-phenanthroline-5,6 dione

To a freshly prepared C_8K (12.5 mmol) in freshly distilled anhyd THF (20 mL) was added 2,2′ pyridil (4.5 g, 2.5 mmol) in THF (30 mL), stirred at rt for 5 h under Ar and then refluxed for 2 h. The reaction mixture was carefully quenched with water, filtered, extracted with CH_2Cl_2, evaporated, and recrystallized with methanol. The desired product was not obtained. Starting material was recovered.

Attempt to reduce furil with C_8K

To a freshly prepared C_8K (12.5 mmol) in freshly distilled anhyd THF (20 mL) was added furil (4.27 g, 2.5 mmol) in THF (30 mL) and stirred at rt for 5 h under Ar. The reaction mixture was carefully quenched with water, filtered, extracted with CH_2Cl_2, evaporated and recrystallized with methanol. The desired product was not obtained. Starting material was recovered.

General Procedure for the Synthesis of C_{10}- DECA Analogues

Dequalinium diiodide analogue was synthesized by refluxing 3 - 2.2 fold stoichiometric excess of the quinoline base for 48 h or longer if necessary (monitor the reaction with TLC, CH_2Cl_2 : 2 M NH_3 in MeOH) with one equivalent of the 1,10 diiododecane linker using 2-10 mL of appropriate solvent (2-butanone at 90 ^{0}C for 48 - 72 h or 4-methyl-2-pentanol at 140 ^{0}C for 48-72 h). The precipitated salt was filtered, washed with the solvent used and subjected to a recrystallization or flash chromato-graphic separation to isolate the DECA analogue.

The reaction was monitored by thin layer chromatography (TLC) using 2 M ammonia in MeOH : CH_2Cl_2 system and the presence of 1,10 diiododecane was observed by TLC using hexane as the solvent.

C_{10}-DECA (20c)

2.4 equivalents of 4-aminoquinaldine (0.5 g, 3.16 mmol) was refluxed with 1,10-diiododecane (0.518 g, 1.32 mmol) in 2-butanone for 48 h. The white precipitate obtained was filtered, washed thoroughly with 2-butanone to yield 80% **20c** as a cream colored precipitate. ^{1}H NMR (DMSO) δ 1.31 (m, 10H), 1.72 (m, 6H), 2.74 (s, 6H), 4.46 (m, 4H), 6.65 (s, 2H), 7.71 (m, 2H), 8.02 (m, 2H), 8.17 (d, 2H), 8.46 (d, 2H), 8.84 (s, br, 4H); ^{13}C NMR (DMSO) δ 21.6, 25.8, 28.7, 29.7, 32.8, 47.9, 103.9, 116.6, 118.4, 124.3, 125.9, 134.4, 139.0, 155.0, 156.7.

Synthesis of Bases (Starting Materials)

Quinoline based starting materials 2-methylquinolin-4-ylamine, 2-methylquinoline, quinolin-4-ylamine, quinoline, dimethylpyridine-4-ylamine and pyridine-4-ylamine were purchased from Aldrich chemical company.

Synthesis of the Type 1 -Quinoline Bases (Starting Materials)

The starting materials dimethylquinolin-4-ylamine and dimethylpyridin-4-ylamine were synthesized by nucleophilic displacement of chloride ion from 4-chloroquinaldine and 4-chloropyridine respectively.

Synthesis of the Type 1 -Quinoline Bases - Method A

Synthesis of 2-methylquinolin-4-ylamine (*N,N*-dimethylaminoquinoline)

4-chloroquinaldine (3 g) was heated to 180 ^{0}C with approximately 10 g of phenol. *N,N*-dimethylamine, dried over quicklime, was passed through the solution for 2 h. The hydrochloride of the amine was separated and the excess of phenol was removed by steam-distillation. The clear solution was concentrated by evaporation on the water-bath. The resulting solution was cooled, made alkaline with NaOH and extracted to CH_2Cl_2 afford 2-methylquinolin -4-ylamine base (67%) as a brown liquid. ^{1}H NMR (DMSO) δ 2.89 (s, 12H), 6.79 (d, 2H), 7.45 (m, 2H), 7.65 (m, 2H), 8.01 (dd, 4H), 8.61 (d, 2H).

Synthesis of Type 2 Bases

Bases (**32** and **33**) with two aromatic moieties linked by exocyclic C_6 - linker were synthesized by following the general **Method A** described above (see later experimental section).

DECA Analogue 26

Following the procedure described for **C_{10}-DECA,** 2.4 equivalents of 2-methylquinoline (0.5 g, 3.49 mmol) was refluxed with 1,10-diiododecane (0.573 g, 1.45 mmol) in 2-butanone for 72 h. The cream colored precipitate obtained was filtered, washed thoroughly with 2-butanone to yield **26** (70%) as a cream precipitate. ^{1}H NMR (DMSO) δ 1.41 (m, 10H), 1.92 (m, 6H), 2.94 (s, 6H), 4.86 (m, 4H), 7.46 (d, 2H), 7.66 (d, 2H), 7.71 (m, 2H), 8.02 (m, 2H), 8.17 (d, 2H), 8.46 (d, 2H).

DECA Analogue 27

Following the procedure described for **C_{10}-DECA,** 2.4 equivalents of quinoline-4-ylamine (0.5 g, 3.47 mmol) was refluxed with 1,10-diiododecane (0.569 g, 1.44 mmol) in 2-butanone for 72 h. The cream colored precipitate obtained was filtered, washed thoroughly with 2-butanone to yield **27** (67%) as a cream precipitate. ^{1}H NMR (DMSO) δ 1.17 (m, 12H), 1.74 (m, 4H), 4.5 (t, 4H), 6.78 (d, 2H), 7.73 (m, 2H) 7.96 (m, 2H), 8.13 (d, 2H), 8.44 (d, 2H), 8.53 (d, 2H), 9.01 (d, br, 4H); ^{13}C NMR (DMSO) δ 25.5, 28.2, 28.4, 28.5, 53.5, 101.7, 116.8, 118.0, 124.2, 126.1, 134.2, 137.7, 145. 9, 157.5; Anal.

calcd for $C_{28}H_{36}I_2N_4$: C, 49.28; H, 5.28; I, 37.22; N, 8.21; found C, 49.15; H, 5.46; I, 37.55; N, 8.09.

DECA Analogue 28

Following the procedure described for **C_{10}-DECA,** 2.4 equivalents of quinoline-4-ylamine (0.5 g, 3.78 mmol) was refluxed with 1,10-diiododecane (0.636 g, 1.61 mmol) in 2-butanone for 72 h. The cream colored precipitate obtained was filtered, washed thoroughly with 2-butanone to yield **28** (73%) as a cream precipitate. ^{1}H NMR (DMSO) δ 1.19 (m, 12H), 1.84 (m, 4H), 4.65 (t, 4H), 7.05 (d, 2H), 7.48 (dd, 2H) 7.56 (m, 2H), 7.78 (m, 2H), 8.00 (d, 2H), 8.04 (dd, 2H), 8.69 (d, 2H).

DECA Analogue 29

Following the procedure described for **C_{10}-DECA,** 2.4 equivalents of dimethyl-quinolin-4-ylamine (0.5 g, 2.91 mmol) was refluxed with 1,10-diiododecane (0.477 g, 1.21 mmol) in 2-butanone for 72 h. The cream colored precipitate obtained was filtered, washed thoroughly with 2-butanone to yield **29** (73%) as a cream precipitate. ^{1}H NMR (DMSO) δ 1.24 (m, 12H), 1.79 (m, 4H), 3.46 (s, 12H), 4.56 (t, 4H), 7.01 (d, 2H), 7.70 (m, 2H), 8.01 (m, 2H), 8.14 (m, 2H), 8.16 (d, 2H), 8.62 (d, 2H); ^{13}C NMR ($CDCl_3$) δ 26.1, 28.4, 28.5, 28.9, 45.0, 55.0, 104.4, 117.9, 119.6, 125.6, 127.9, 134.0, 138.1, 146.3, 160.2; Anal. calcd for $C_{32}H_{44}I_2N_4$: H, 5.96; N, 7.59; found H, 5.53; N, 7.39; LRMS (M-I) calcd for $C_{32}H_{44}I_2N_4$ *m/z*: 611.1, found 611.2.

DECA Analogue 30

Following the procedure described for **C_{10}-DECA,** 3 equivalents of pyridine-4-ylamine (0.5 g, 5.32 mmol) was refluxed with 1,10-diiododecane (0.698 g, 1.77 mmol) in 2-butanone for 72 h. The cream colored precipitate obtained was filtered, washed thoroughly with 2-butanone to yield **30** (73%) as a cream precipitate. ^{1}H NMR (DMSO) δ 1.22 (m, 12H), 1.71 (m, 4H), 4.10 (t, 4H), 6.81 (s, 4H), 8.04 (d, 4H), 8.20 (d, 4H); ^{13}C NMR (DMSO) δ 26.2, 29.2, 29.6, 31.1, 57.8, 110.2, 143.7, 159.3; Anal. calcd for $C_{20}H_{32}I_2N_4$: C, 41.25; H, 5.5; I, 43.62; N, 9.6; found C, 41.19; H, 5.49; I, 43.23; N, 9.50.

DECA Analogue 31

Following the procedure described for **C_{10}-DECA,** 2.4 equivalents of 4-dimethyl-amino-pyridine (0.324 g, 2.65 mmol) was refluxed with 1,10-diiododecane (0.435 g, 1.10 mmol) in 2-butanone for 48 h. The white precipitate obtained was filtered, washed thoroughly with 2-butanone to yield **31** (78%) as a cream precipitate. ^{1}H NMR (MeOD) δ 1.34 (m, 12H), 1.88 (m, 4H), 3.27 (s, 12H), 4.21 (t, 4H), 7.01 (dd, 4H), 8.23 (dd, 4H); ^{13}C NMR (MeOD) δ 25.6, 28.6, 28.9, 30.5, 39.1, 57.5, 107.6, 141.7, 156.4; Anal. calcd for $C_{24}H_{40}I_2N_4$: C, 45.15; H, 6.32; I, 39.76; N, 8.78; found C, 44.09; H, 6.19; I, 39.55; N, 8.68; LRMS (M-2I)/2 calcd for $C_{24}H_{40}I_2N_4$ *m/z*: 191.1, found 191.2.

DECA Analogue 32

Following the **Method A** described above, 4-chloroquinoline (3 g, 18.4 mol) was heated to 180 ^{0}C with approximately 10 g of phenol. *N,N*-diaminohexane (1g, 5.8 mol) was added to the reaction mixture for 1 h. The crude product obtained was recrystallized with ethanol to afford **32** (67%) as pure crystals. ^{1}H NMR (MeOD) δ 1.53 (m, 4H), 1.83 (m, 4H), 3.45 (t, 4H), 6.55 (d, 2H), 7.51 (m, 2H), 7.76 (m, 2H), 7.81 (m, 2H), 8.21 (d, 2H), 8.35 (d, 2H). LRMS (MH^+) calcd for *m/z*: $C_{24}H_{26}N_4$ 371.1, found 371.0.

DECA Analogue 33

Following the **Method A** described above, 4-chloroquinaldine (2.06 g, 11.6 mmol) was heated to 180 ^{0}C with approximately 7.1 g of phenol. *N,N*-diaminohexane (1 g, 5.8 mmol) was added to the reaction mixture for 1 h. The crude product obtained was recrystallized with ethanol to afford **33** (60%) as pure crystals. ^{1}H NMR (MeOD) δ 1.48 (m, 4H), 1.73 (m, 4H), 2.65 (s, 6H), 3.52 (t, 4H), 6.83 (s, 2H), 7.68 (m, 2H), 7.84 (m, 2H), 7.92(m, 2H), 8.49 (d, 2H), 9.03 (t, 2H);. LRMS (MH^+) calcd for *m/z* : $C_{26}H_{30}N_4$ 399.0, found 399.2.

Synthesis of Type -2 dequalinium diiodide analogue - DECA Analog 34

(Two aromatic moieties linked by exocyclic ring nitrogens)

33 (0.246 g, 0.54 mmol) was refluxed with acetone and excess of methyl iodide(0.25 g) for 3 days inorder to methylate the ring nitrogen. The purple product obtained was dried and distilled with benzene using a Dean-Stark apparatus. The crude obtained was recrystallized with methanol to afford 80% of **34** as a pink precipitate. ^{1}H NMR (MeOD) δ 1.46 (m, 4H), 1.71 (m, 4H), 2.69 (s, 6H), 3.55 (t, 4H), 4.12 (s, 6H), 6.92 (s, 2H), 7.78 (m, 2H), 8.06 (m, 2H), 8.56 (d, 2H), 8.60 (d, 2H); LRMS (M-I) calcd for *m/z* : $C_{28}H_{36}N_4I_2$ 555.0 found 555.2.

DECA Analogue 35

2.4 equivalents of 2-methylbenzoxazole (2 g, 0.015 mol) was refluxed with 1,10-diiododecane (2.47 g, 0.006 mol) in 2-butanone for 24 h. The orange colored precipitate obtained was filtered, washed thoroughly with 2-butanone to yield 80 % **35** as an orange precipitate. ^{1}H NMR (MeOD) δ 1.41 (m, 12H), 2.00 (m, 4H), 3.14 (s, 6H), 4.61 (t, 4H), 7.81 (m, 4H), 8.00 (dd, 2H), 8.08 (dd, 2H); ^{13}C NMR (DMSO), δ 13.6, 27.4, 29.1, 29.9, 30.2, 48.8, 113.9, 115.4, 129.1, 130.1, 130.9, 149.4, 169.7; Anal. calcd for $C_{26}H_{34}I_2N_2O_2$: C, 47.29; H, 5.19; I, 38.43; N, 4.24; O, 4.85; found C, 47.05; H, 5.35; I, 38.65; N, 3.86; LRMS (M-2I)/2 calcd for $C_{26}H_{34}I_2N_2O_2$ *m/z*: 203.0, found 203.2.

DECA Analogue 36

Following the procedure described for **C_{10}-DECA,** 2.4 equivalents of 2-methyl benzothiazole (1 g, 6.7 mmol) was refluxed with 1,10-diiododecane (1.1 g, 2.79 mmol) in 2-butanone for 24 h. The purple colored precipitate obtained was filtered, washed thoroughly with 2-butanone to yield **36** (87 %) as a purple colored precipitate.

^{1}H NMR (DMSO) δ 1.28 (m, 8H), 1.31 (m, 4H), 1.84 (m, 4H), 3.22 (s, 6H), 4.71 (t, 4H), 7.81 (m, 2H), 7.89 (m, 2H), 8.34 (dd, 2H), 8.46 (dd, 2H); ^{13}C NMR (DMSO) δ 16.9, 25.9, 27.8, 28.6, 28.8, 49.2, 116.9, 124.6, 128.1, 129.1, 129.4, 140.8, 177.0; Anal. calcd for $C_{26}H_{34}I_2N_2S_2$: C, 45.09; H, 4.95; I, 36.65; N, 4.05; S, 9.26; found C, 45.09; H, 5.35; I, 36.34; N, 3.91; S, 8.88; LRMS (M-2I)/2 calcd for *m/z*: $C_{26}H_{34}I_2N_2S_2$ 219.01, found 219.1.

DECA Analogue 37

Following the procedure described for **C_{10}-DECA,** 2.4 equivalents of 2-amino-1-methylbenzimidazole (0.21 g, 1.41 mmol) was refluxed with 1,10-diiododecane (0.223 g, 0.588 mmol) in 2-butanone for 24 h. The light cream colored precipitate obtained was filtered, washed thoroughly with 2-butanone to yield **37** (77%) as a cream colored precipitate. ^{1}H NMR (MeOD) δ 1.33 (m, 12H), 1.78 (m, 4H), 3.71 (s, 6H), 4.17 (t, 4H), 7.38 (dd, 4H), 7.48 (dd, 4H); ^{13}C NMR (MeOD) δ 27.48, 29.0, 29.8, 30.3, 30.4, 44.2, 111.1, 111.3, 125.0, 131.1, 131.5, 151.3; Anal. calcd for $C_{26}H_{38}I_2N_6$: C, 45.36; H, 5.56; I, 36.87; N, 12.21; found C, 45.50; H, 5.59; I, 36.40; N, 11.86; LRMS (M-2I)/2 calcd for

m/z: $C_{26}H_{38}I_2N_6$ 217.1, found 217.2.

DECA Analogue 38

Following the procedure described for **C_{10}-DECA,** 2.3 equivalents of isoquinolin-1-ylamine (0.167 g, 1.16 mmol) was refluxed with 1,10-diiododecane (0.198 g, 0.504 mmol) in 2-butanone for 48 h. The light cream colored precipitate obtained was filtered, washed thoroughly with 2-butanone to yield **38** (79%) as a cream colored precipitate.

^{1}H NMR (MeOD) δ 1.41 (m, 12H), 1.88 (m, 4H), 4.27 (t, 4H), 7.25 (d, 2H), 7.70 (d, 2H), 7.79 (m, 2H), 7.81 (m, 4H), 8.48 (d, 2H); ^{13}C NMR (MeOD) δ 27.4, 28.4, 30.4, 30.5, 54.9, 113.9, 120.1, 126.1, 128.9, 130.5, 133.6, 135.7, 137.5,155.2; Anal. calcd for $C_{28}H_{36}I_2N_4$: C, 49.28; H, 5.32; found C, 49.07; H, 5.56; LRMS (M-2I)/2 calcd for *m/z* $C_{28}H_{36}I_2N_4$: 555.1, found 555.1.

DECA Analogue 39

Following the procedure described for **C_{10}-DECA,** 2.3 equivalents of triphenylphosphine (0.832 g, 3.17 mmol) was refluxed with 1,10-diiododecane (0.5 g, 1.27 mmol) in 2-butanone for 72 h. The white precipitate obtained was filtered, washed thoroughly with 2-butanone to yield **39** (83%) as a white precipitate. ^{1}H NMR ($CDCl_3$) δ 1.21 (m, 12H), 1.63 (m, 4H), 3.61 (m, 4H), 7.74 (m, 30H); ^{13}C NMR ($CDCl_3$) δ 22.5, 22.9, 23.4, 28.4 (J_{P-C} = 21 Hz), 30.2 (J_{P-C} = 16 Hz), 118.1 (J_{P-C} = 86 Hz), 130.6 (J_{P-C} = 12 Hz), 133.7 (J_{P-C} = 10 Hz), 135.1; Anal. calcd for $C_{46}H_{50}I_2P_2$: C,60.14; H, 5.49; I, 27.63; found C,

60.27; H, 5.43; I, 28.03; LRMS (M-2I)/2 calcd for *m/z* $C_{46}H_{50}I_2P_2$: 332.1, found 331.9, (M-I) calcd 791.15, found 791.3.

DECA Analogue 40

Following the procedure described for **C_{10}-DECA,** 2.4 equivalents of triethylamine (1.01 g, 9.9 mmol) was refluxed with 1,10-diiododecane (1.64 g, 4.15 mmol) in 2-butanone for 24 h. The cream precipitate obtained was filtered, washed thoroughly with 2-butanone to yield **40** (80%) as a cream colored precipitate. ^{1}H NMR (MeOD) δ 1.31 (t, 18H), 1.40 (m, 12H), 1.71 (m, 4H), 3.22 (m, 4H), 3.35 (q, 12H); ^{13}C NMR (MeOD) δ 8.10, 22.9, 27.5, 30.2, 30.4, 54.1, 58.3, Anal. calcd for $C_{22}H_{50}I_2N_2$: C, 44.3; H, 8.45; I, 42.55, N 4.70; found C, 44.4; H, 8.09; N 4.58, I, 42.44; LRMS (M-2I)/2 calcd for $C_{22}H_{50}I_2N_2$ *m/z*: 171.1, found 171.2, (M-I) calcd 469.2, found 469.3.

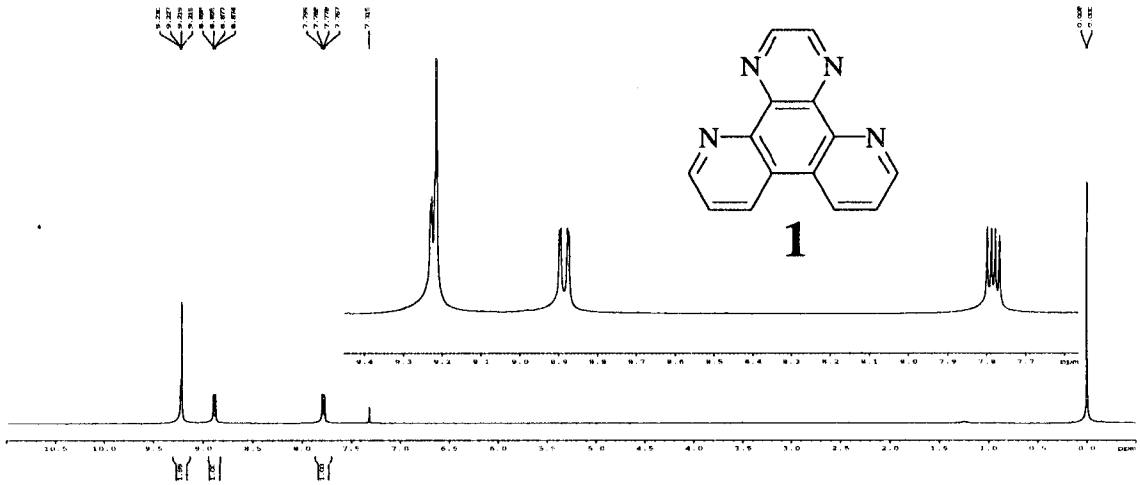
Assay of Dequalinium Analogues as PKC_α Inhibitors

Assay of PKC_α catalytic activity in vitro:

Recombinant human PKC_α (95% pure) (Pan Vera Corp., Madison, WI) was used for testing DECA analogues in vitro. To test the inhibitory potency of these compounds, the total catalytic activity of PKC_α (36 ng protein) was analyzed with increasing concentrations of a selected DECA analogue. PKC_α activity was measured in triplicate in the presence of activating cofactors (10 μg of phosphatidylserine, 0.5 mM Ca^{+2}) by the transfer of ^{32}P from [γ- ^{32}P] ATP to the modified pseudosubstrate peptide (RFARKGSLRQKNV). The phosphorylated product was isolated by applying the reaction medium to a square of phosphocellulose paper which retains only the peptide following several wash steps with water. The radioactive content of each square of phosphocellulose paper was analyzed by scintillation counting. The extent to which a given concentration of an analogue decreases the formation of phosphorylated product indicates the extent of PKC alpha inhibition. Stock solutions for DECA analogs were prepared in DMSO and standardized spectrophotometrically on a Perkin-Elmer Lambda II spectrophotometer

APPENDIX

^{1}H NMR and ^{13}C NMR Spectra



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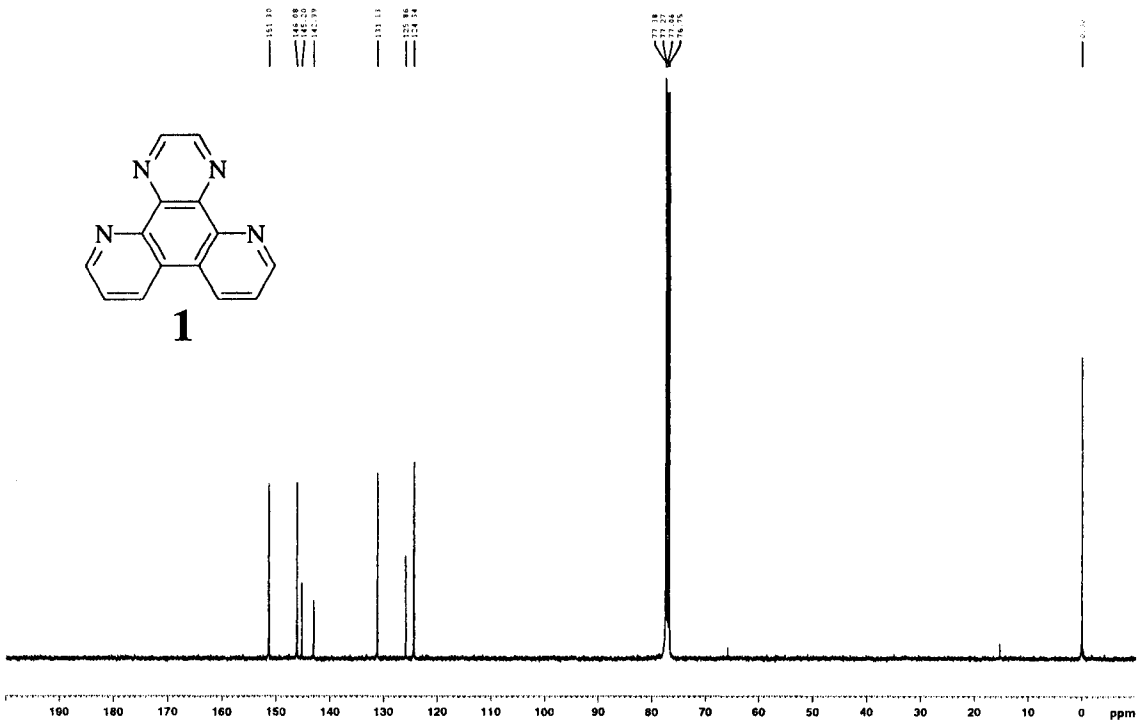

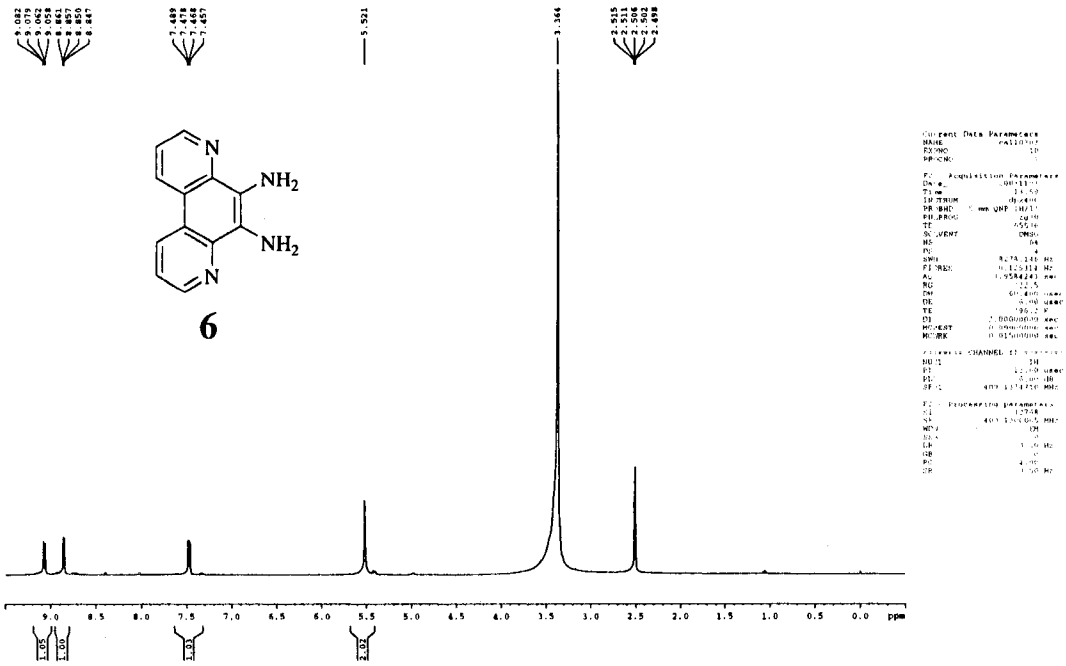

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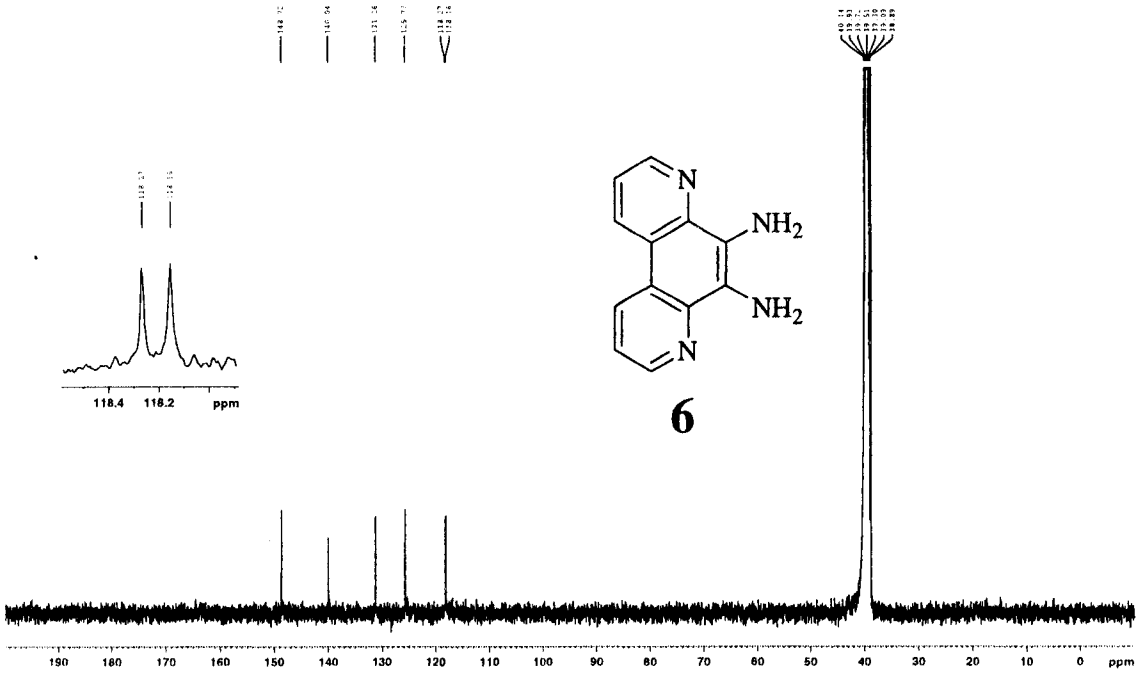

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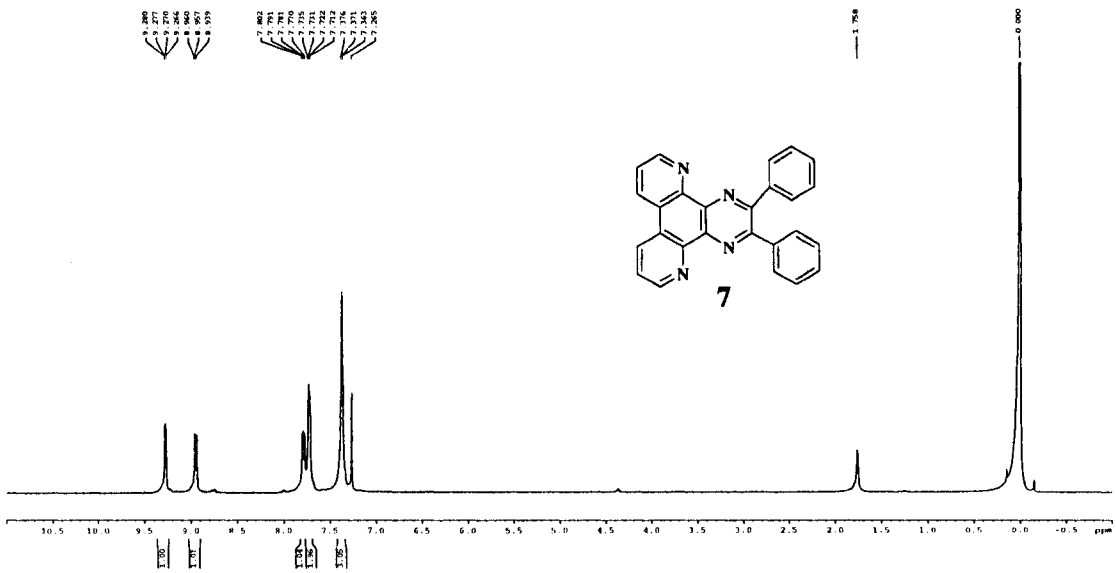

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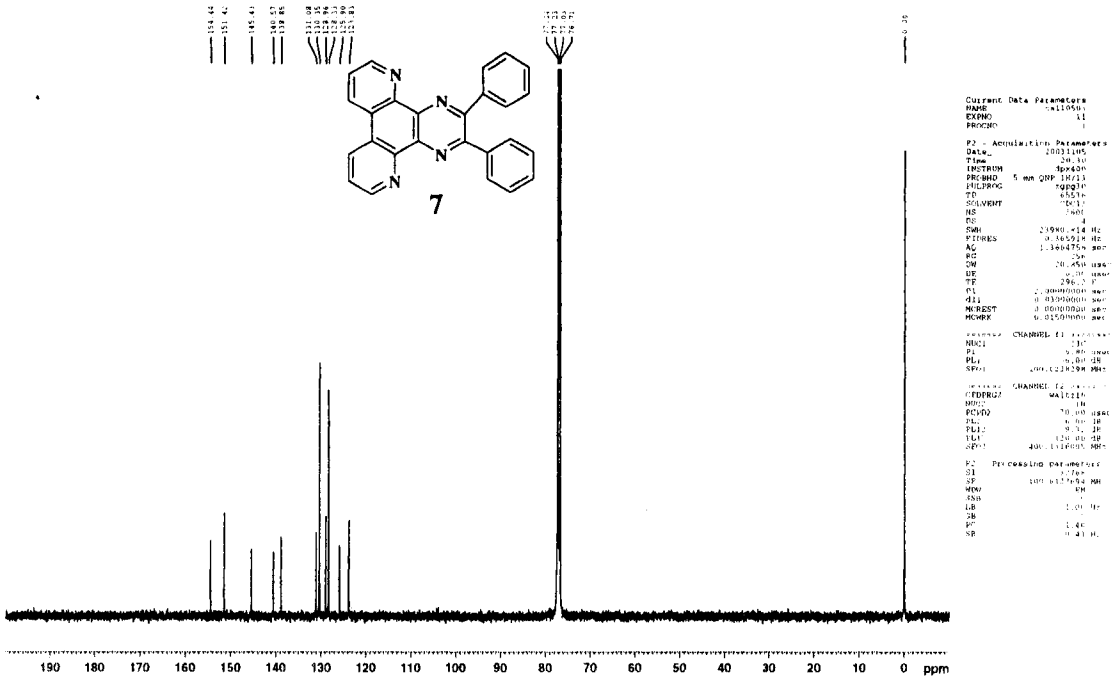

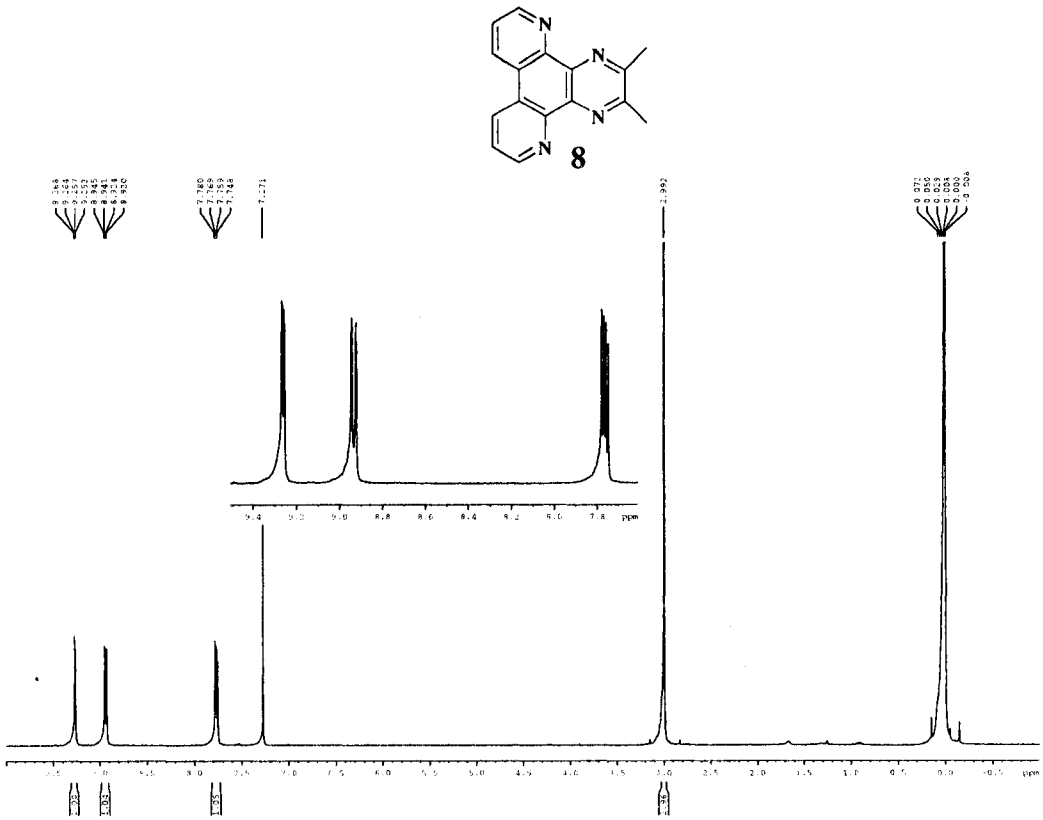

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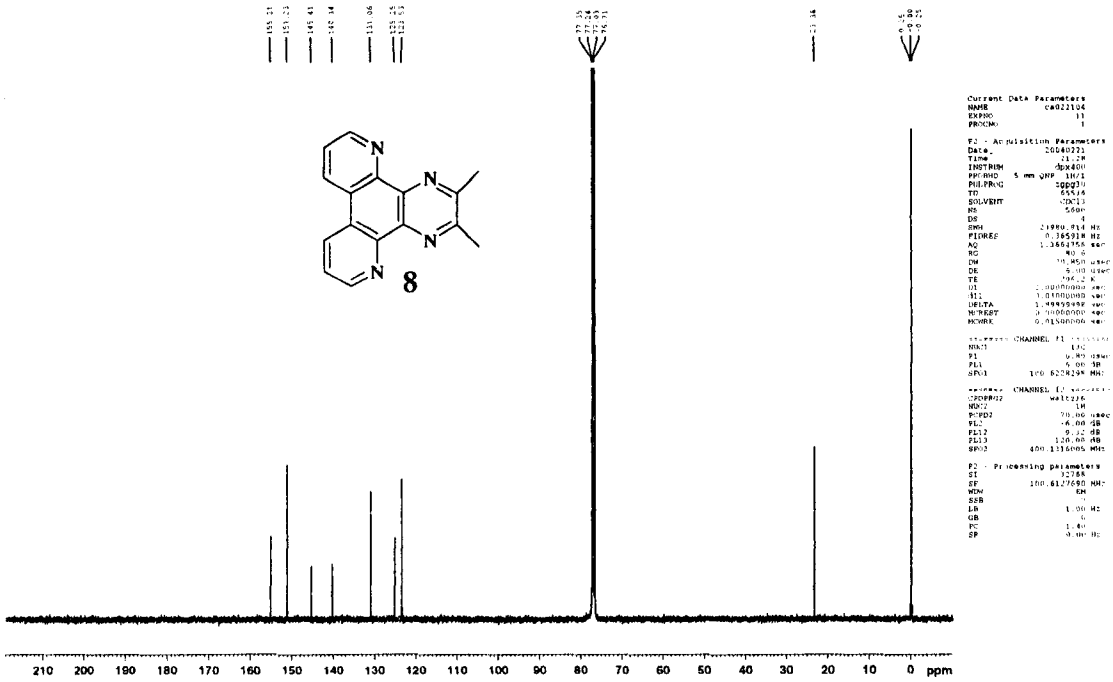
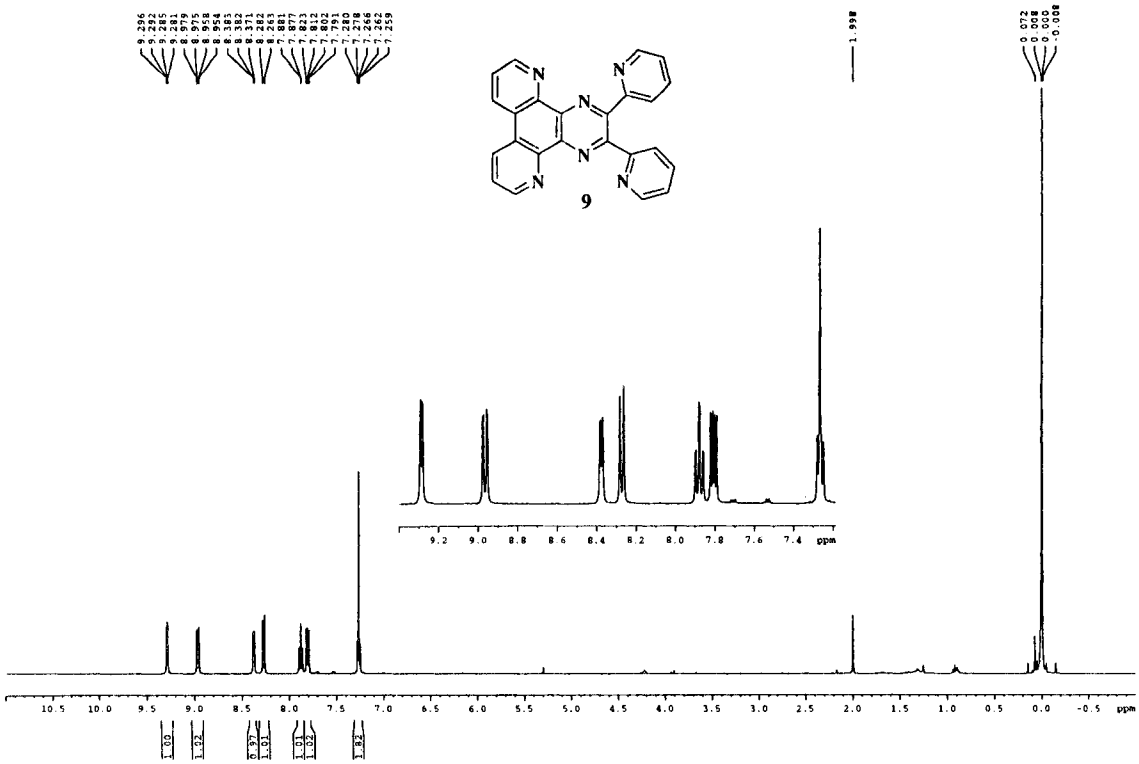
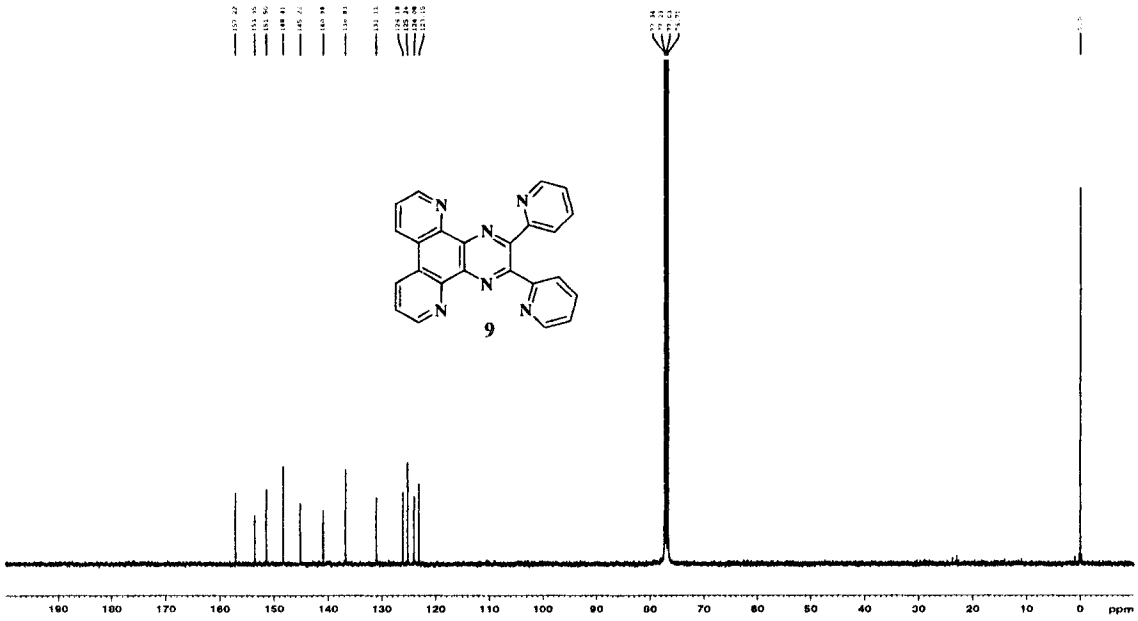
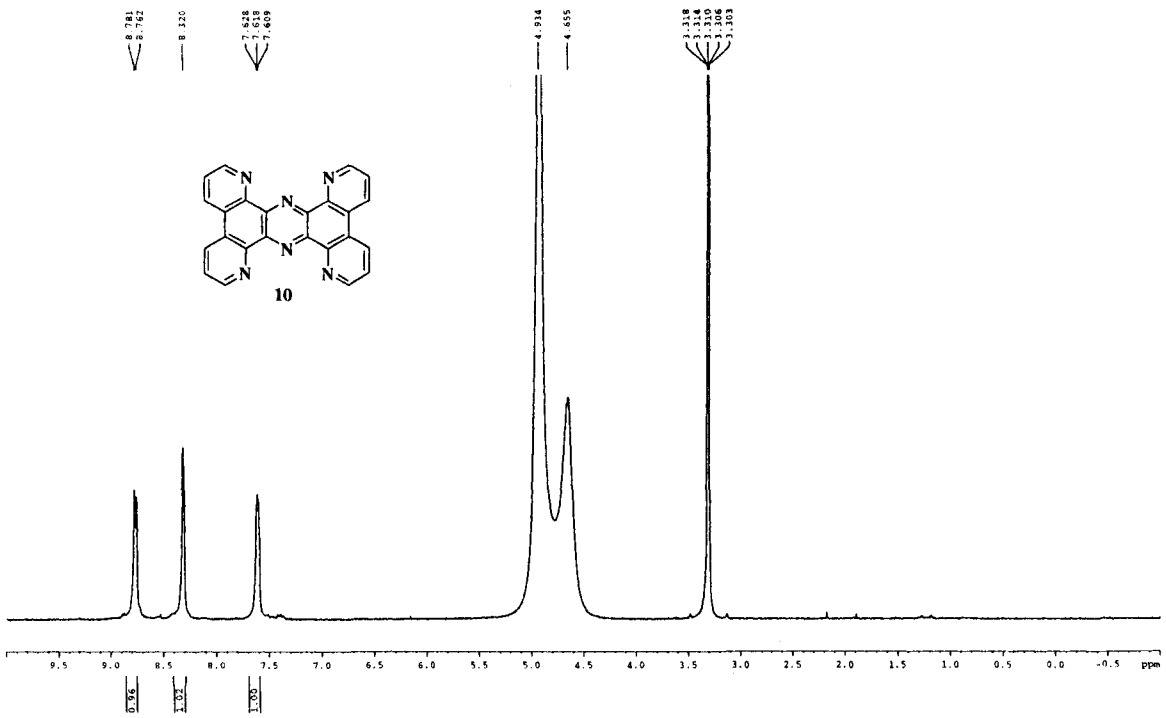
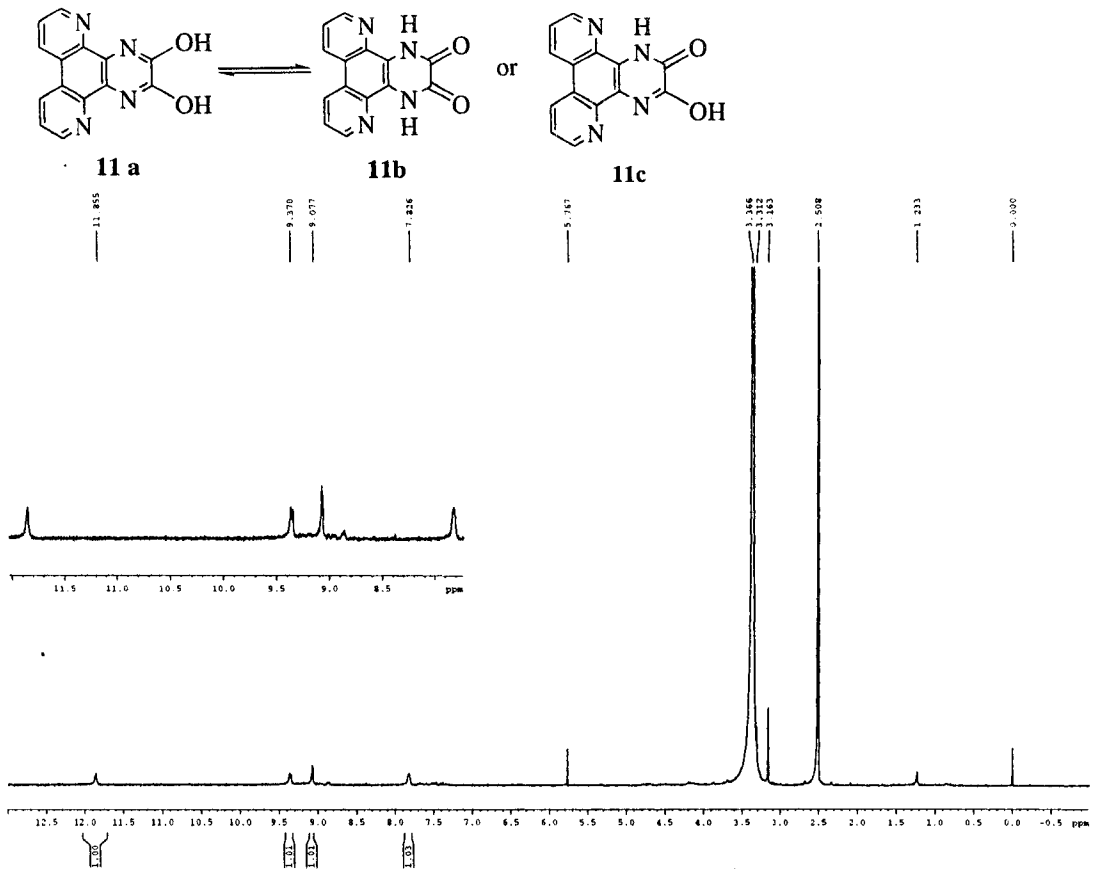


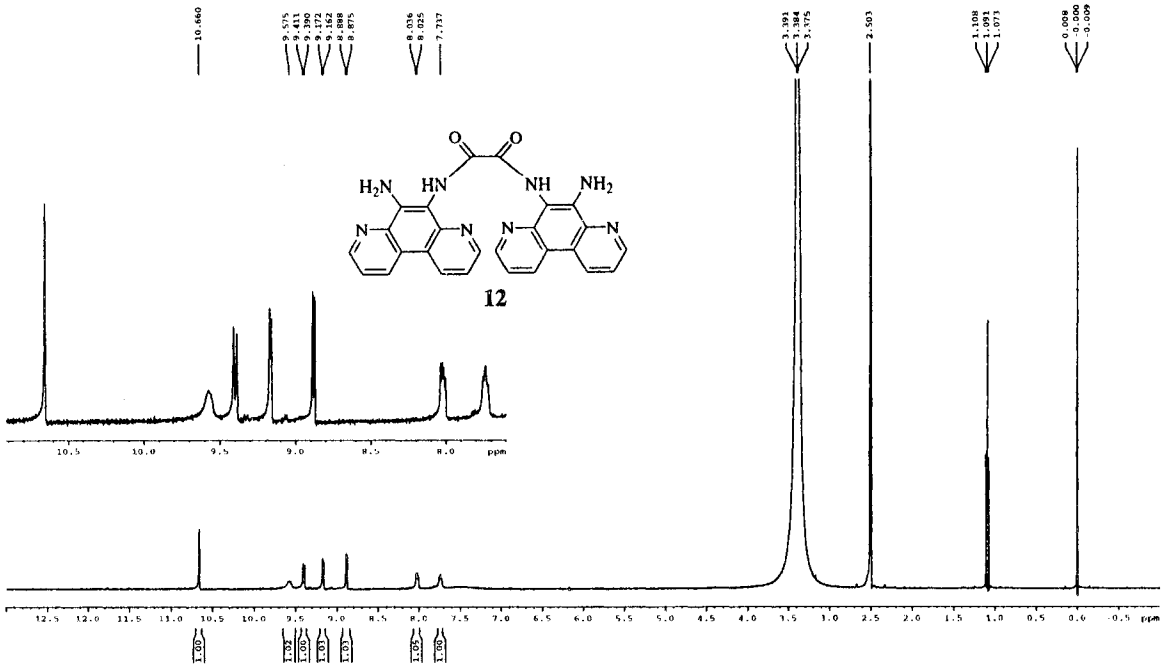


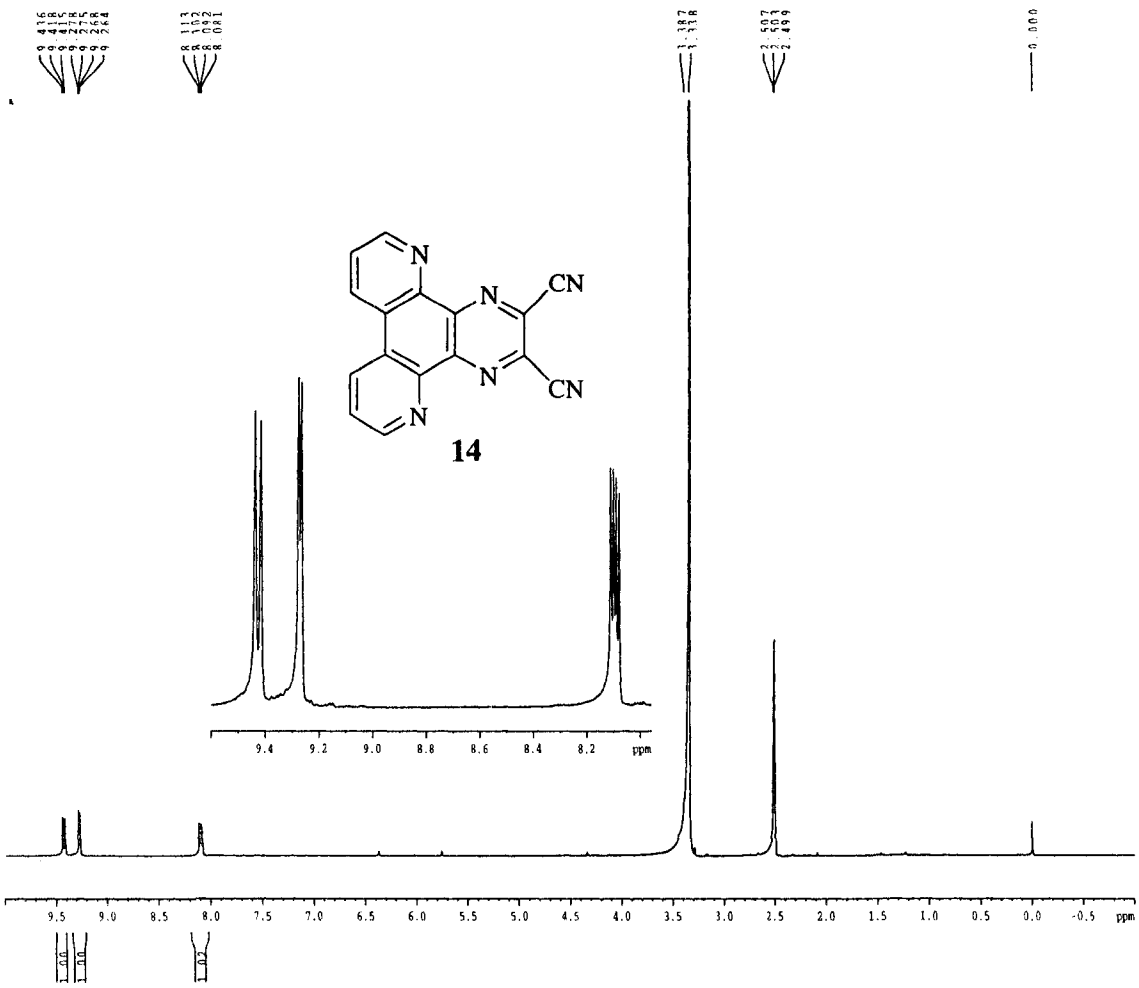


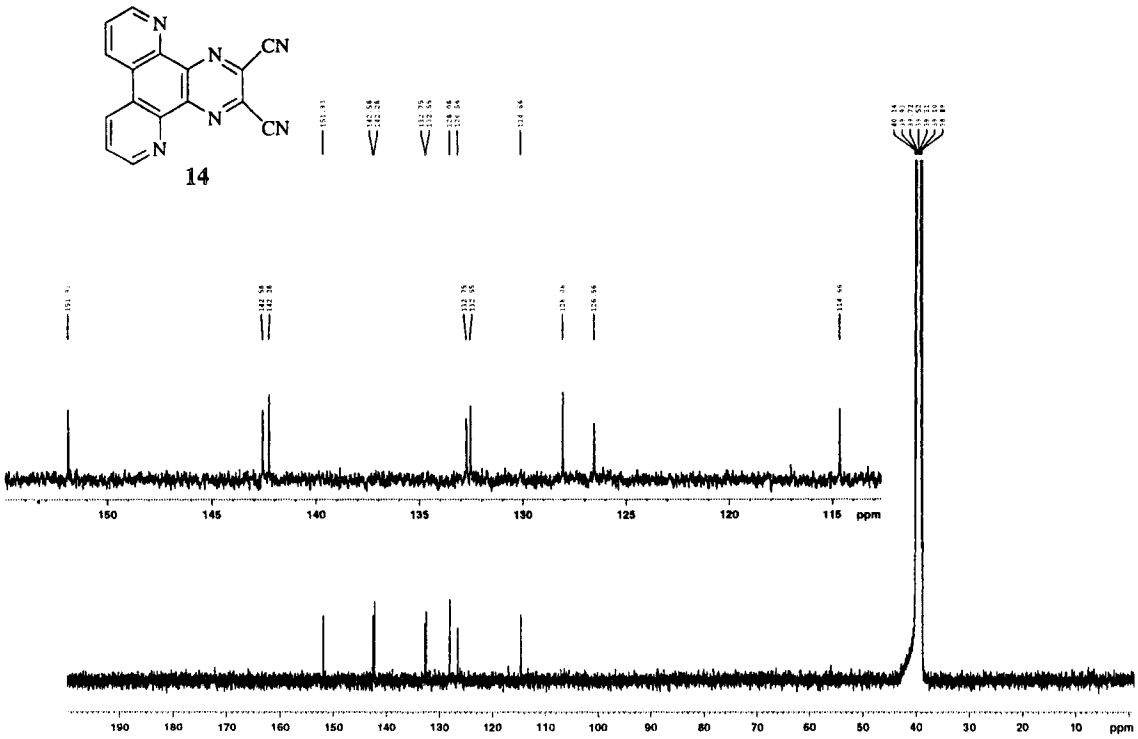


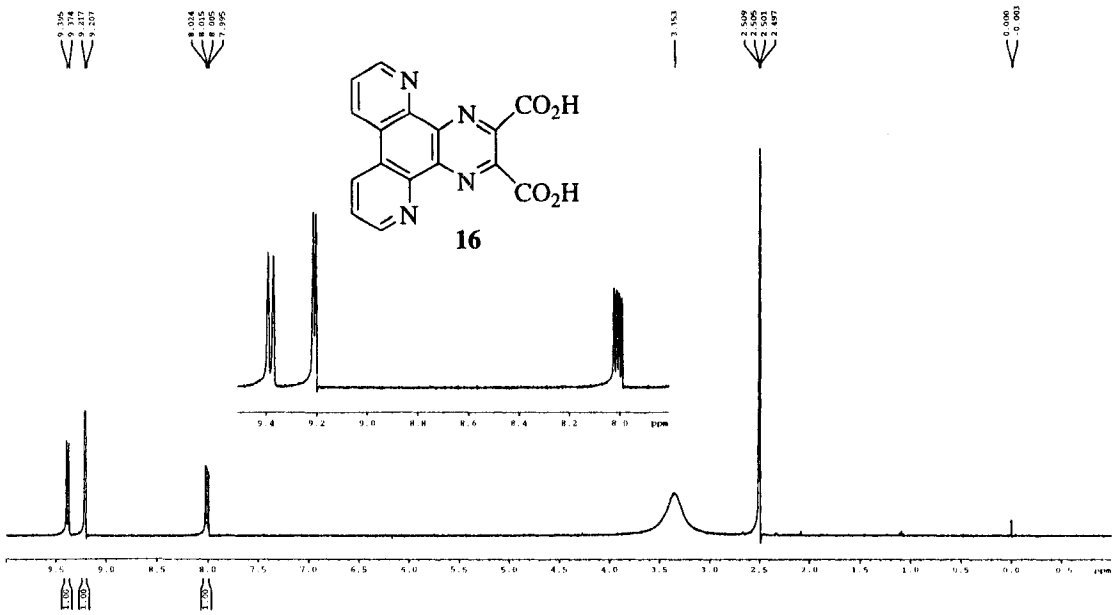


11 a **11b** **11c**









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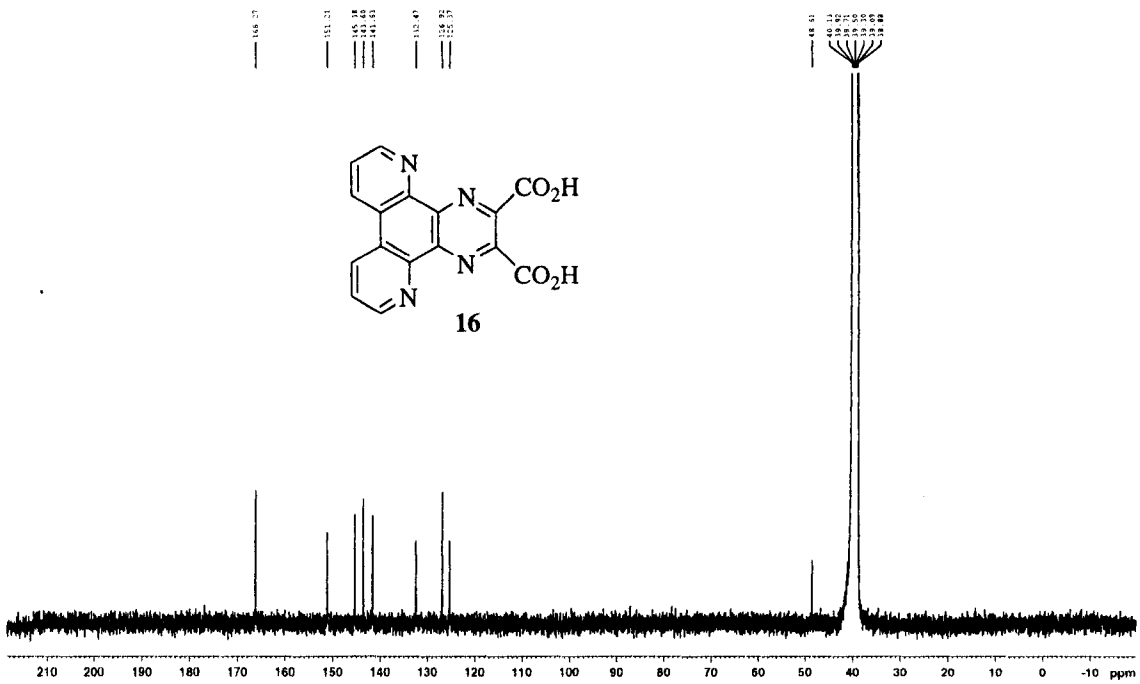

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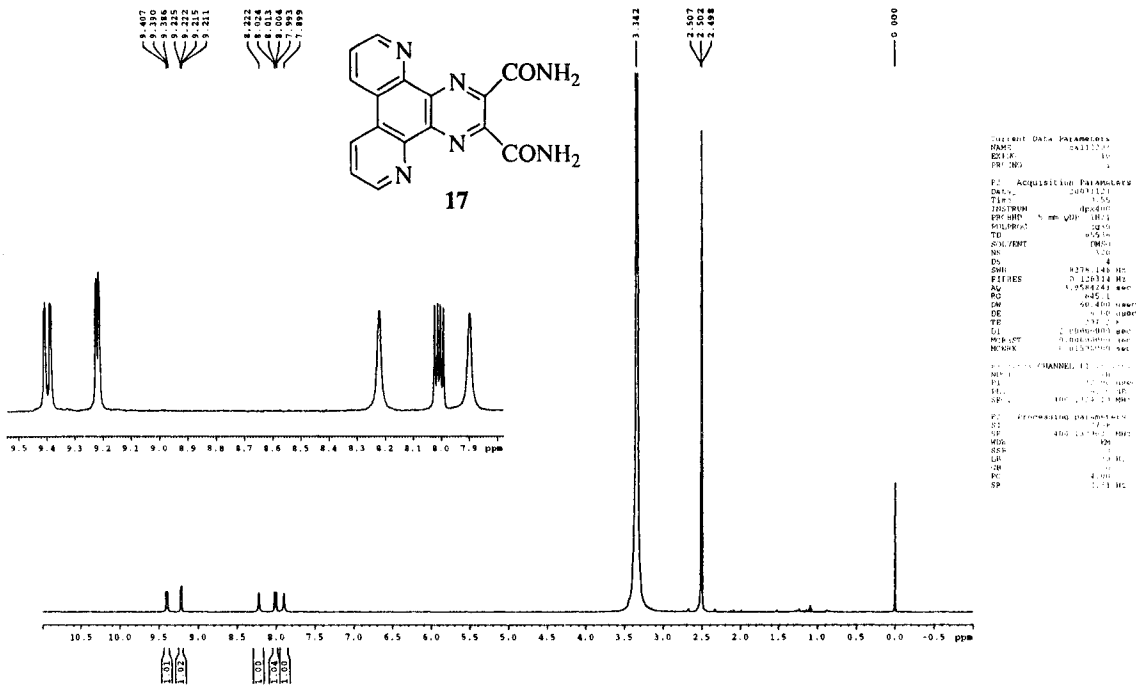

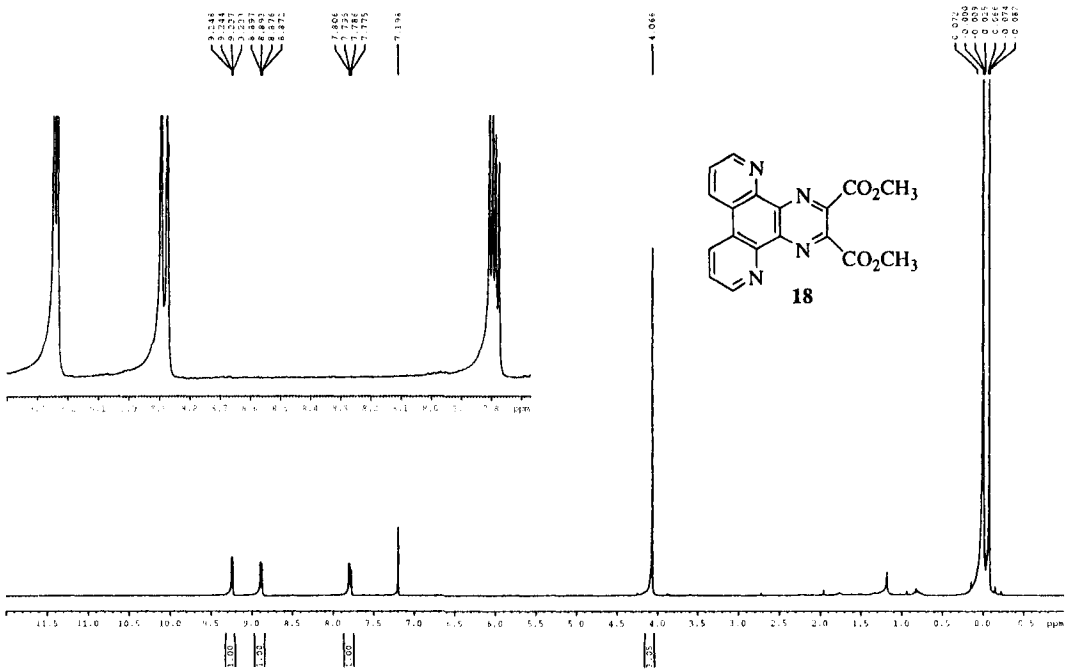

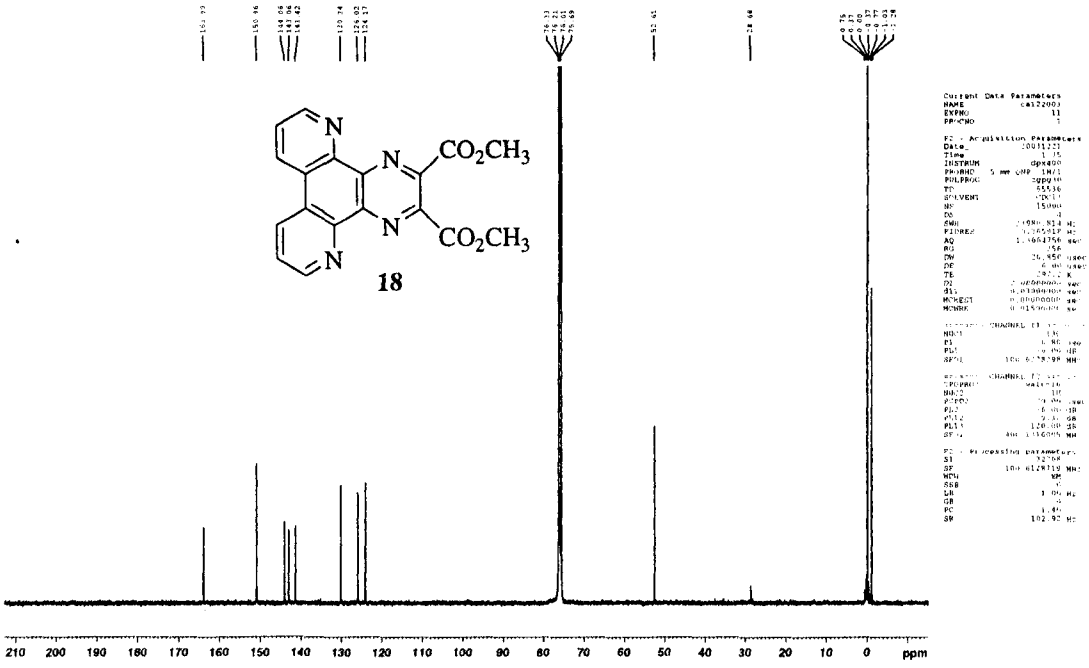

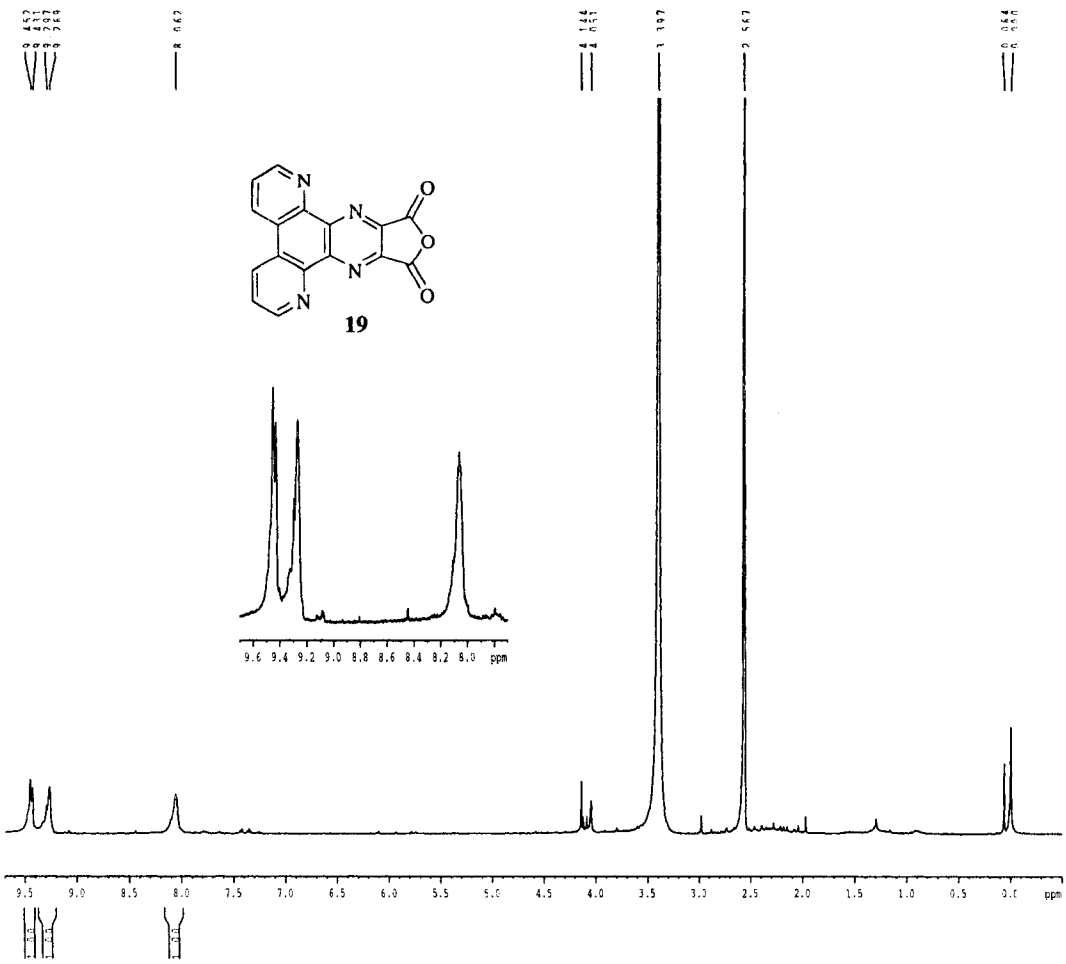

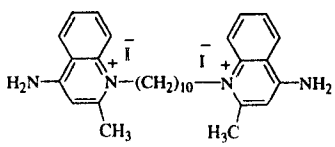

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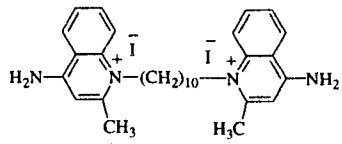

C_{10} - DECA
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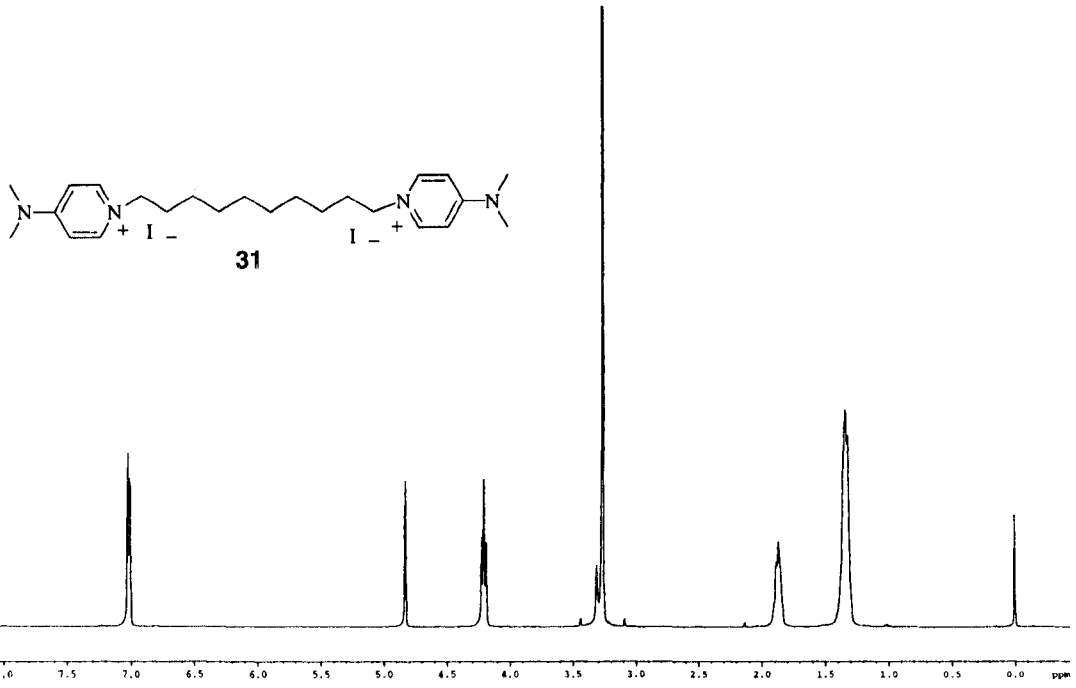

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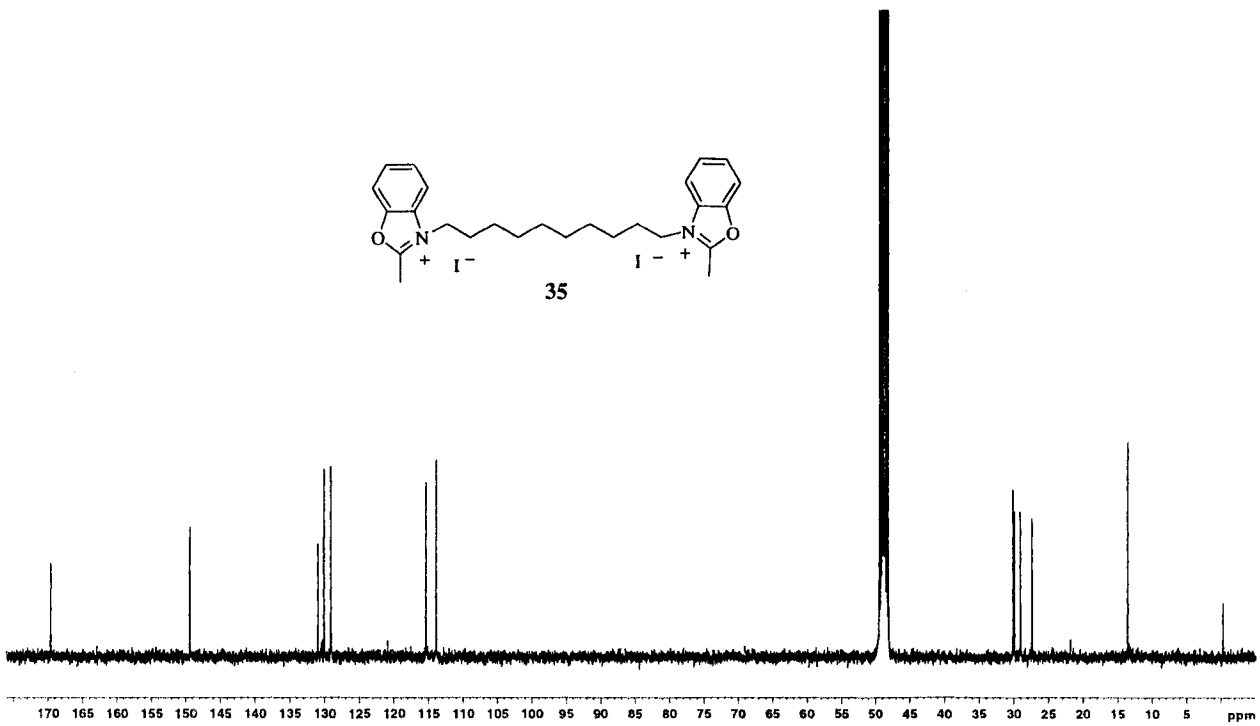

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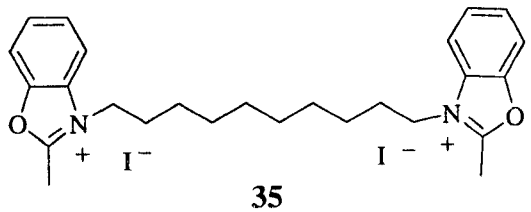

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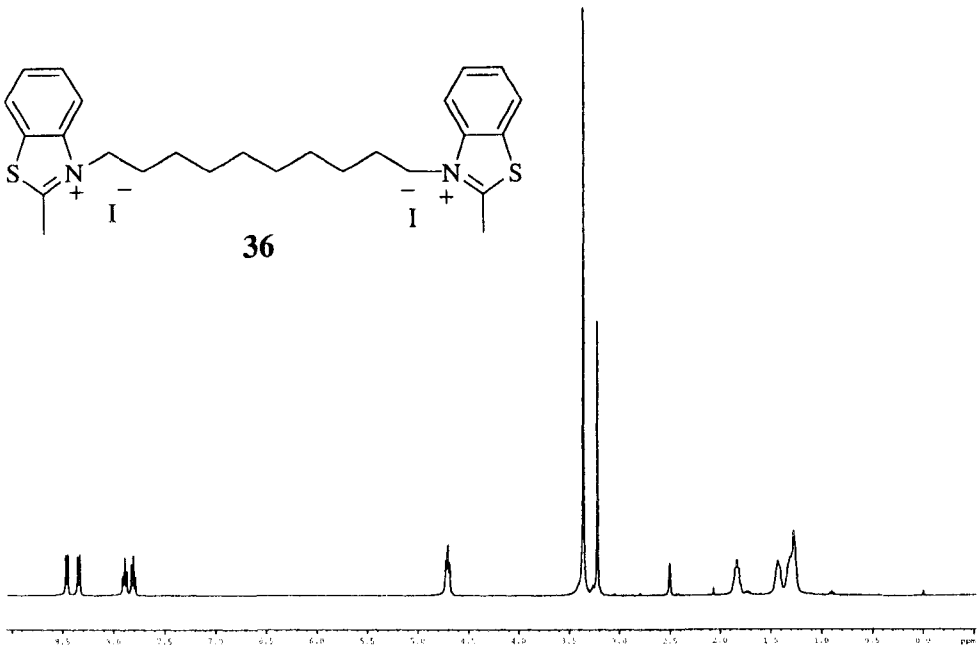

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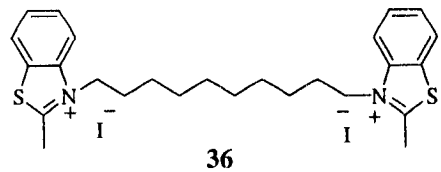

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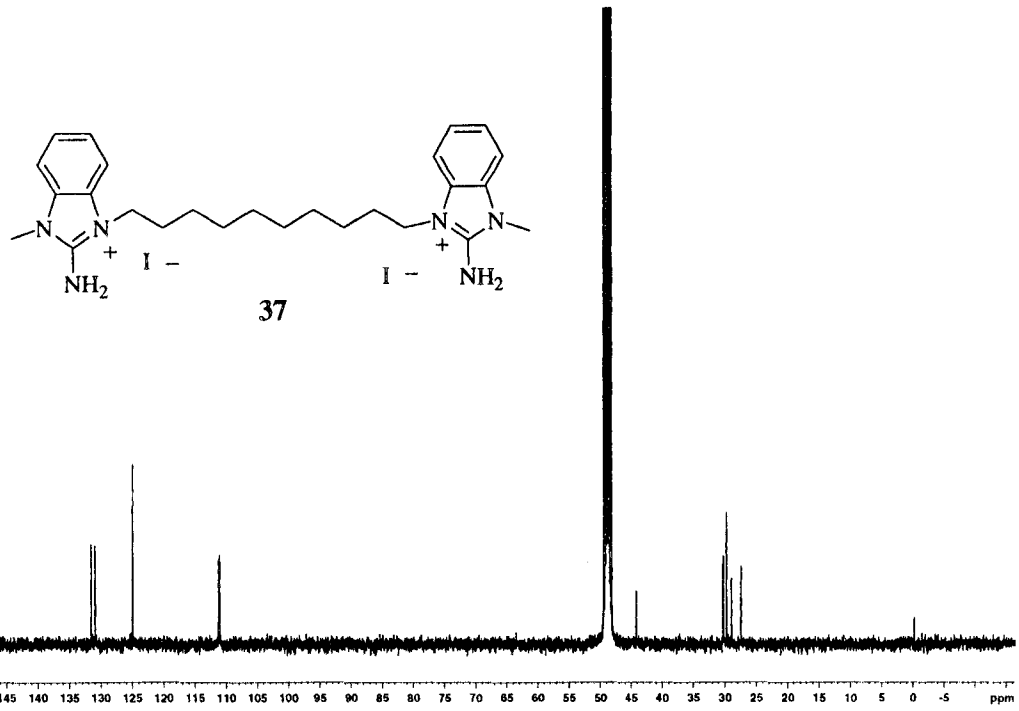

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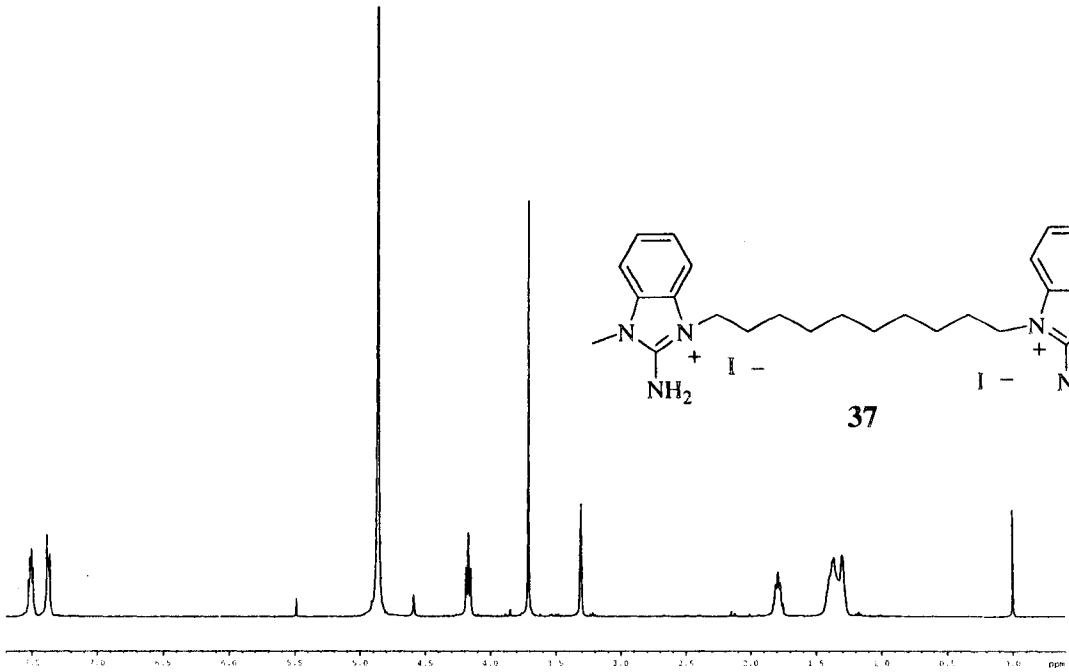

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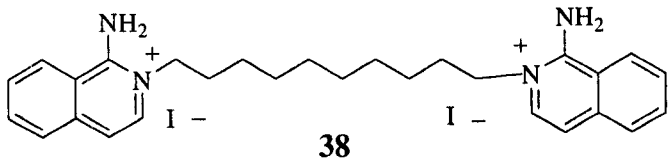

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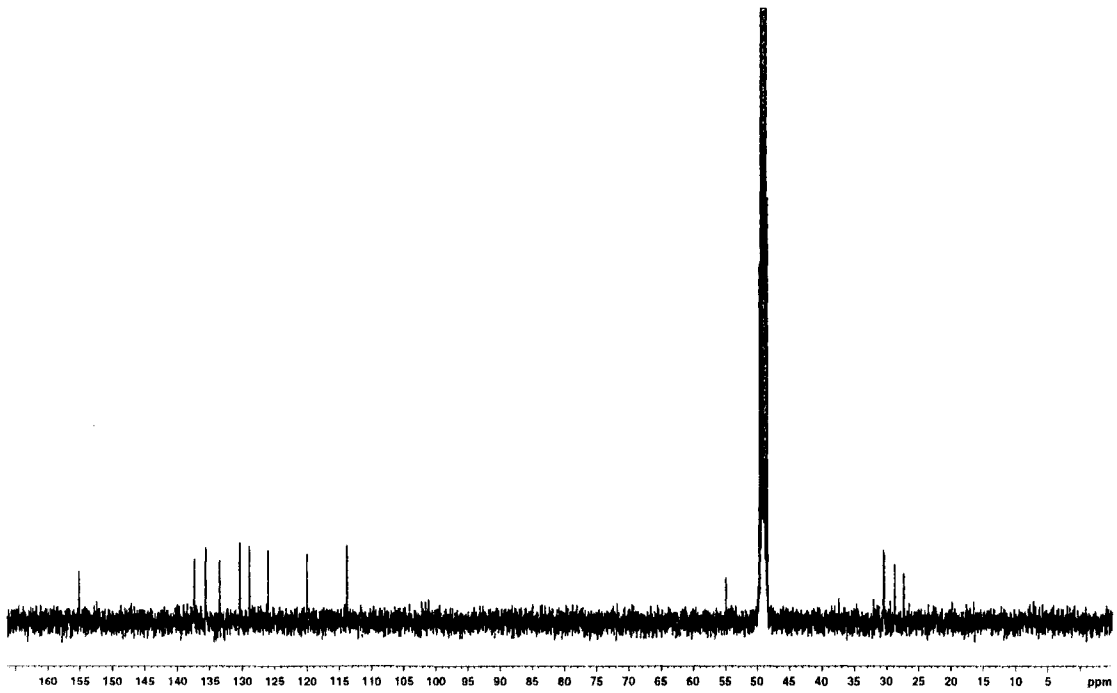

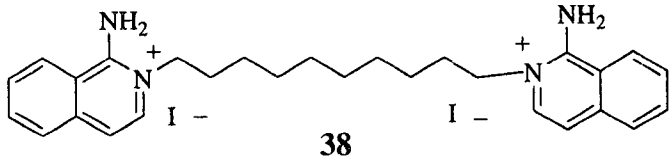

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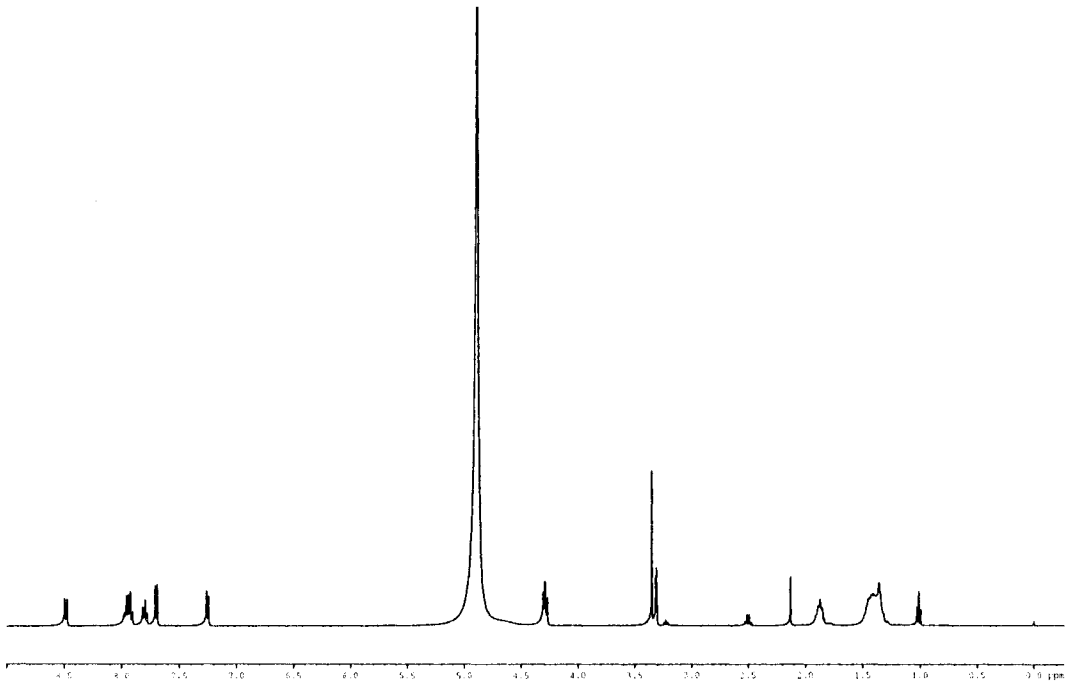

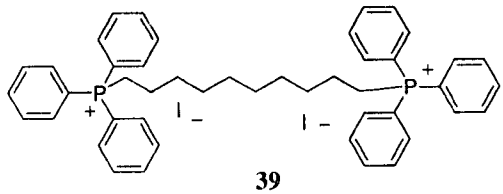

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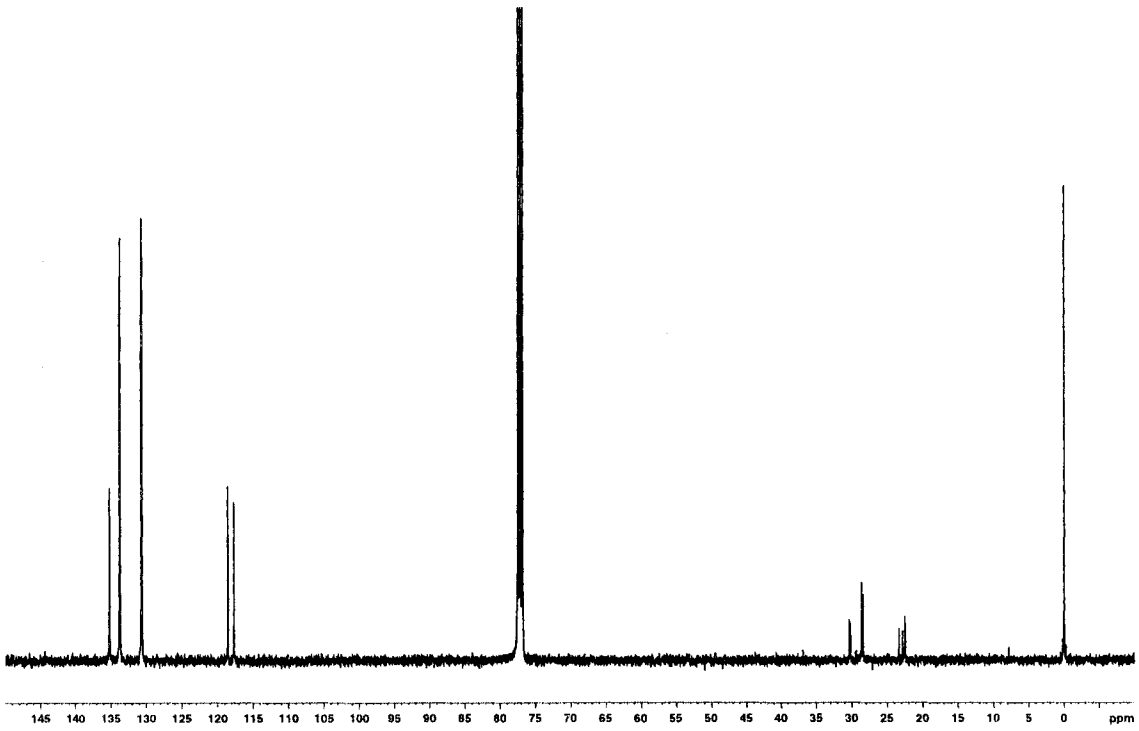

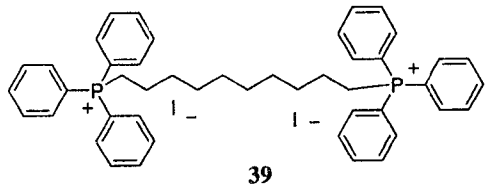

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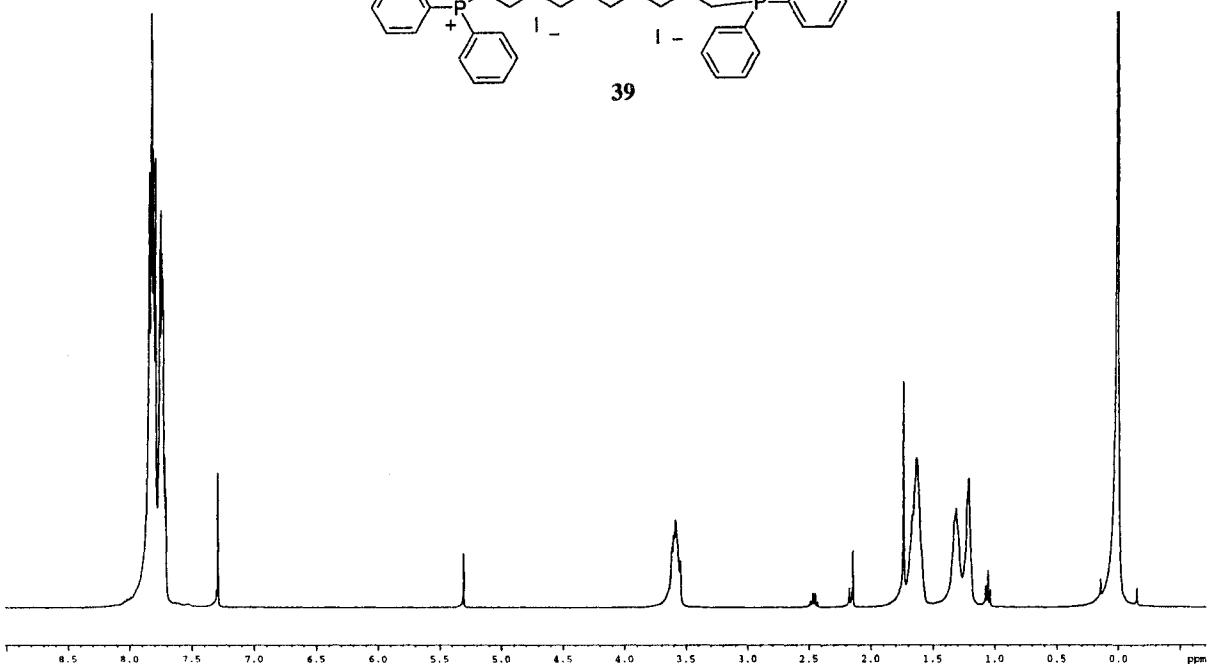

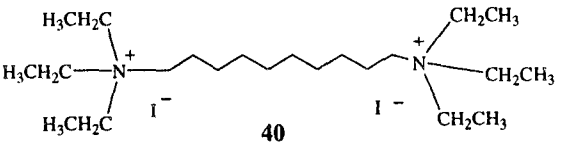

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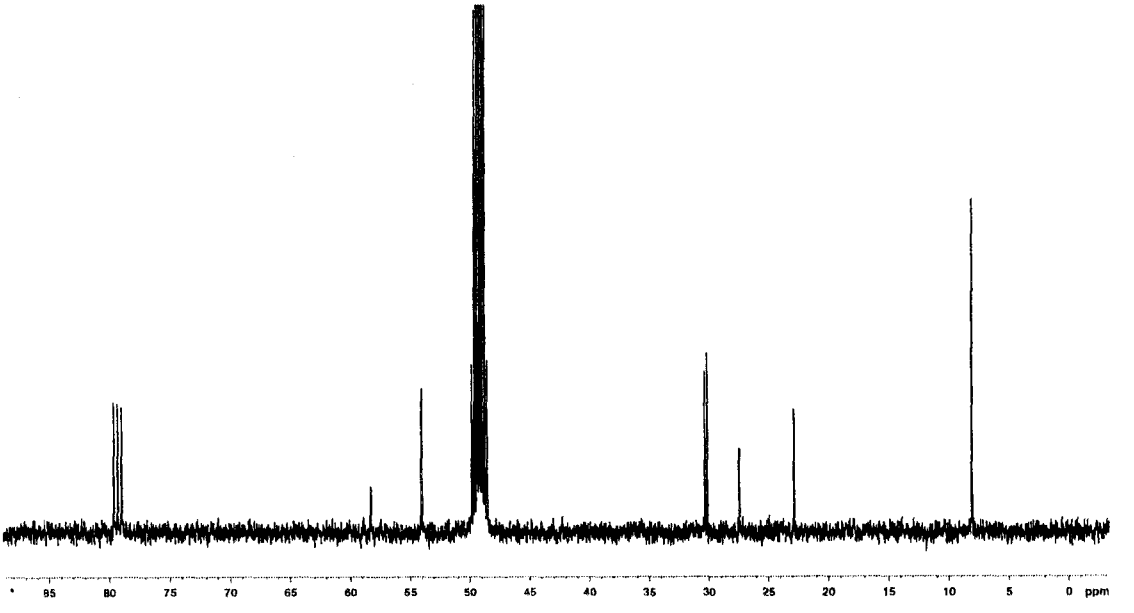

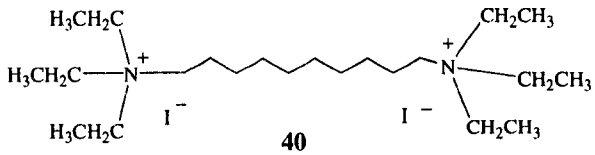

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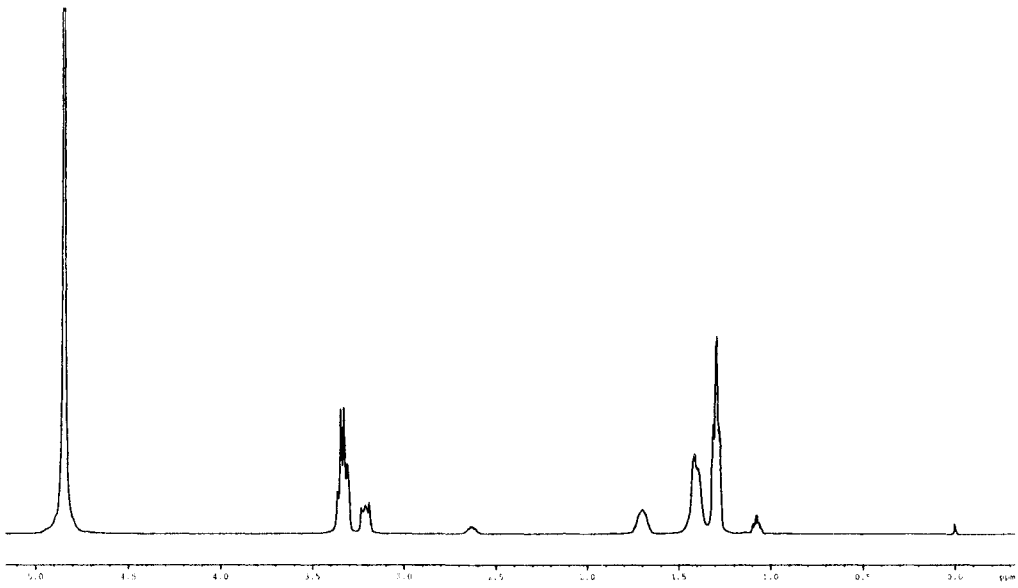

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