

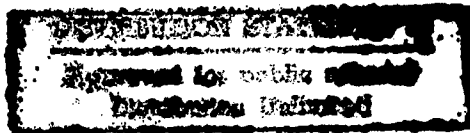
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3 CHELATORS EXHIBITING TRIPLE FLUORESCENCE
4

5 **Background of the Invention**

6 **1. Field of the Invention**

7 The present invention relates generally to chelators and more specifically to fluorescent
8 chelators.

9
10 **2. Background of the Invention**

11 1. Description of the Background Art

12 The use of fluorescence to detect metals provides a convenient way to measure low
13 concentrations of metals. Generally, a sample is exposed to a fluorescent chelating agent that
14 preferentially complexes with the metal of interest, either shifting or greatly enhancing the
15 fluorescence of the complexed ligand. However, these fluorescent chelates may be subject to
16 interference resulting in unreliable results and rarely exhibit the ability to preferentially distinguish
17 between various related metals.

18 The dual fluorescence of 4-(N,N-dimethylamino) benzonitrile, DMABN (Fig. 1a), in polar
19 solvent was discovered by Lippert more than 30 years ago. Lippert et al. *Angew. Chem.*, 1961, 73,
20 695. Several models have been proposed to address the cause for the unexpected, red-shifted
21 fluorescence band which is apparent in addition to the normal, fluorescent emission of the locally

1 excited state (LE). Grabowski et al proposed the twisted intramolecular charge transfer (TICT) state
2 to successfully account for the red-shifted fluorescence. Grabowski et al. Pure Appl. Chem., 1983,
3 55, 245. TICT refers to the state wherein an intramolecular charge transfer takes place between the
4 dimethyl amino donor group and the benzonitrile acceptor that is accompanied by a twisting motion
5 and orbital decoupling of the phenyl acceptor ring from the dimethyl amino donor group. The TICT
6 state has been observed in other molecular systems bearing donor and acceptor groups covalently
7 linked. When the N,N-dimethylamino group in DMABN is replaced by an amino propyl group, the
8 resulting compound, 3-(4-cyanophenyl)-1-N,N-dimethylamino-propane, forms an intramolecular
9 exciplex between the phenyl and amino end groups. The formation of an intramolecular exciplex
10 results from the flexibility of the alkyl chain, which links the donor and acceptor moieties close
11 enough spatially, that a charge transfer step is possible.

12 Research has been conducted toward the development of fluorescent sensor molecules
13 capable of sensitively and selectively monitoring heavy metal ion concentration. The covalent
14 attachment of benzonitrile to the aza group of a metal binding ionophore that uniquely combines the
15 properties of DMABN with the metal binding properties of metal binding ionophore has been
16 considered as one way to accomplish this goal. For example, Létard *et. al.*, *Recl. Trav. Chim. Pays-*
17 *Bas*, 1995, 114, 517-527, reported the dual fluorescence of 4-(1-aza-4,7,10-
18 trioxacyclododecyl)benzonitrile (DMABN-Crown4, Fig. 1c) and 4-(1-aza-4, 7,10,13-
19 tetraoxacyclopentadecyl)benzonitrile (DMABN-Crown5).

20 Benzonitriles bearing flexible, alkyl amino chains, e.g. 3-(4-cyanophenyl)-1-N,N-
21 dimethylaminopropane (CNP3NM, Fig. 1b), can form intramolecular exciplexes which arise due to

1 the conformational flexibility of the alkyl amino chain, and its ability to form a sandwich
2 configuration promoting excited state, charge transfer (Grabowski, Z.R. *Pure & Appl. Chem.*, **1992**,
3 64, 1249-1255). The propensity for intramolecular exciplex formation in CNP3NM is indicated by
4 the strong, red-shifted fluorescence observed, and the complete absence of LE emission. In contrast
5 to TICT state formation, the large distance and minimal mesomeric interaction apparent between D
6 and A causes a localized excited (LE) state precursor to be formed, wherein the excited state is
7 primarily localized on either D or A, depending upon which gives the lower S_1 energy. Van der
8 Auweraer *et. al.*, *J. Phys. Chem.*, **1991**, 95, 2083-2092, observed a correlation between
9 intramolecular exciplex formation and Hirayama's rule (Hirayama, F. J. *Chem. Phys.*, **1965**, 42,
10 3163-3171), which states that the most stable sandwich conformation will arise when $n=3$ for a
11 phenyl-(CH₂)_n-NMe₂ system.

13 Summary of the Invention

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15 Accordingly, it is an object of this invention to provide fluorescent molecules for the
16 detection of metals.

17 It is a further object of this invention to provide fluorescent molecules that alter their
18 fluorescent characteristics upon chelation or complexation with a metal.

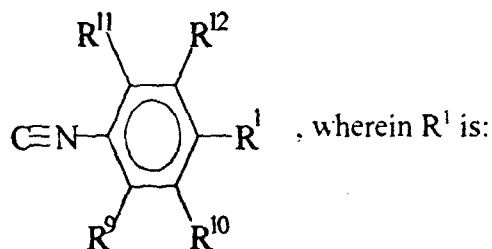
19 It is an additional object of the present invention to provide fluorescent molecules which
20 exhibit triple fluorescence, and which exhibit peak shifts when complexed or chelated with a metal.

1 It is a further object of the present invention to allow for the possibility of using fluorescence
2 to simultaneously determine the concentration of various metals within a mixture of metal ions

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4 These and additional objects of the invention are accomplished by complexation or chelation
5 of a metal with a novel fluorophore of the formula:

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an aliphatic group bearing at least one nitrogen substituted by the
benzonitrile group, at least one nitrogen 2 to 4 carbon atoms from the benzonitrile-
substituted nitrogen, and at least one additional heteroatom selected from the groups
consisting of N, O, and S, or

an azacyclo group having at least two ring nitrogens, at least one of the ring
nitrogens being substituted by the benzonitrile group and at least one of the ring
nitrogens being 2 to 4 carbon atoms from the benzonitrile-substituted ring nitrogen,
the azacyclo group having as ring members at least one additional heteroatom
selected from the group consisting of N, O, and S, said at least one additional
heteroatom having at least 2 carbons between it and the nearest of other ring member
heteroatoms; and

R⁹, R¹⁰, R¹¹, and R¹² are H or CH₃ and may be the same as or different from each other. In

1 this specification and the claims that follow, the terms "ring member", "ring nitrogen", or "ring
2 heteroatom" are used to distinguish those atoms, which constitute part of the azacyclo ring structure.
3 from atoms which are merely part of a pendant group attached to the azacyclo ring structure.
4

5 **Brief Description of the Drawings**

6
7 A more complete appreciation of the invention will be readily obtained by reference to the
8 following Description of the Preferred Embodiments and the accompanying drawings, wherein:

9 Fig. 1a through Fig. 1d show various fluorophores discussed in this specification.
10

11 Fig. 2 shows proposed structures and equilibria existing between the three excited-state
12 species generating the observed triple fluorescence of DMABN-Cyclam.
13

14 Fig. 3 is a graph showing relative fluorescent intensity vs wavelength for DMABN-Cyclam
15 in *n*-hexane (line a), toluene (line b), tetrahydrofuran (line c), 2-propanol (line d), ethanol (line e),
16 and water (line f). All concentrations were 25 μ M, with the exception of *n*-hexane, whose low
17 solubility made this concentration unattainable (the hexane emission has been normalized to that
18 seen in toluene.
19

20 Fig. 4 is a graph showing the effect of pH on the triple fluorescence of DMABN-Cyclam in
21 water: solid circles, LE; open squares, TICT/E; open triangles, E/LE; and solid triangles, TICT/LE.

1 thus perturbing the triple fluorescence peaks. Also, complexation of the fluorophore with a metal
2 red-shifts these peaks about 5 to about 10 nm. The type of peak permutation observed and amount
3 of red-shifting depends upon the complexed metal, the solvent system used, and the concentration
4 of metal in the solution.

5 The concentration of the fluorophore in solution is not critical, provided that the solution has
6 a sufficient concentration of fluorophore to generate detectable fluorescence, and provided that the
7 solution is sufficiently dilute to avoid the formation of intermolecular, rather than intramolecular,
8 exciplexes. These concentrations may be determined empirically for any given combination of
9 metal, fluorophore and solvent.

10 The fluorophore of the present invention may be used in both polar and non-polar, protic and
11 aprotic solvents. Typical solvents with which the compound of the present invention may be used
12 include ethanol, acetonitrile, H₂O, and mixtures (particularly solutions) thereof. In general, polar
13 solvents tend to red shift the TICT emission band and to increase the intensity of the TICT emission
14 band relative to the E and LE emission bands, since those solvents stabilize charge separation and
15 an open geometry. Conversely, non-polar solvents cannot stabilize charge separation, thus
16 increasing the intensity of the LE emission band relative to the TICT and E emission bands.

17 In aqueous solvents, the relative intensities of the peaks also varies with pH. Up to pH 3,
18 exciplex formation and emission band intensity generally increase with increasing pH, at which point
19 exciplex formation is at a maximum. Above pH 3, exciplex formation and emission band intensity
20 steadily decrease. Up to pH 3, TICT formation and emission band intensity generally increase. At
21 pH 3, TICT formation and emission band intensity is generally nearly level. Above pH 8, TICT

1 formation and emission band intensity steadily decline. Generally, LE formation and emission band
2 intensity steadily decrease with increasing pH, ultimately leveling off at pH 8 and above.

3 The fluorophore of the present invention selectively complexes transition metal ions, heavy
4 metal ions, and mixtures thereof. Typical metals complexed by the fluorophore of the present
5 invention include Zn(II), Cd(II), Cu(I), Cu(II), Ni(II), Pb(II), Hg(II), Fe(III), Al(III), and mixtures
6 thereof. Complexation of the fluorophore of the present invention perturbs the intensities and
7 positions of the three fluorescent peaks, by stabilizing or destabilizing the configurations that give
8 rise to those emissions. The nature and degree of this permutation depends upon both the solvent
9 system employed and the complexed metal ion. Generally, in highly protic solvents (e.g., aqueous
10 solvents) such as water at pH=7, complexation with metal ions causes a net decrease in TICT and
11 E state emission. Concurrently, in such solvents, complexation of the fluorophore of the invention
12 with metal ions increases LE emission, provided that the metal ions do not cause intracomplex
13 quenching of LE emission. Complexes of the fluorophore of the present invention with Zn^{2+} and
14 Cd^{2+} exemplify this behavior, causing an increase in LE emission. Complexation of the fluorophore
15 of the present invention with large, paramagnetic, or easily reducible cations, such as Hg^{2+} , Pb^{2+} ,
16 Cu^{2+} , and Ni^{2+} , however, decreases the observed LE emission, due to intracomplex quenching of LE
17 emission.

18 The effects of metal complexation of the fluorophore upon various emission bands differs
19 when an aprotic solvent, such as acetonitrile, or an organic solvent, such as ethanol, are employed.
20 Generally, in these solvents, complexation of the fluorophore of the present invention with metal
21 ions increases exciplex emission. Typically, TICT emission decreases, and LE emission increases.

1 upon complexation of the fluorophore with a metal ion in an aprotic or organic solvent. In some
2 cases, however, such as complexation of the fluorophore with Hg^{2+} , LE emission may be reduced
3 by the quenching behavior of a large, easily reducible ion. Additionally, the presence of
4 uncomplexed paramagnetic ions, such as Cu^{2+} , after complexation of the fluorophore in solutions
5 having greater than the stoichiometric concentration of the ion, may result in diffusional quenching
6 of the fluorophore:metal ion complex.

7 The effects of metal complexation of the fluorophore of the present invention upon
8 fluorescent emission at three separate emission bands, and in different solvents, or mixtures of
9 solvents, allows the fluorophore of the present invention to detect the presence of a large variety of
10 metals in a variety of different environments. For example, in environments where emission changes
11 in the TICT or LE emission bands, caused by complexation of the fluorophore with a metal ion, or
12 quenched by surrounding molecules, or otherwise difficult to detect, the presence of the metal ion
13 may be determined by monitoring the E emission band. Also, the unique effects of metals upon the
14 positions and intensities of the three fluorescence peaks allows for the possibility that the results may
15 be analyzed and evaluated heuristically to determine, either alone or with test results from other
16 analytical procedures, the metal ion compositions of solutions containing several metal ions.

17 The characteristics of the fluorophore of the present invention will also depend, to some
18 extent, upon its exact composition. For example, the benzonitrile component of the present
19 invention may be unsubstituted or substituted with methyl groups, if desired, in the meta and ortho
20 positions. The electron-donating character of the methyl groups should increase the stability of the
21 exciplex, but may also sterically hinder exciplex formation. Thus, typically, at least one of R^{10} and

1 R¹² will be H. The use of an aliphatic group for R¹, rather than an azacyclo group should enhance
2 exciplex formation because of its additional stability. However, aliphatic groups tend to form
3 weaker complexes with metals than do azacyclo groups.

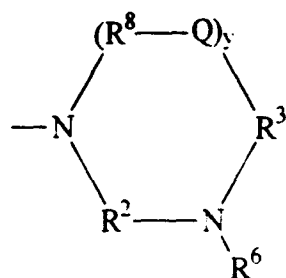
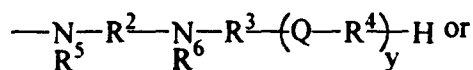
4 In R¹, at least one nitrogen (referred to herein as the neighboring nitrogen) should be located
5 within 2 to 4 carbons of the nitrogen bearing the pendant benzonitrile group (i.e., the benzonitrile-
6 substituted nitrogen). In the exciplex configuration, the neighboring nitrogen bears a δ^+ charge. If
7 the neighboring nitrogen is less than two carbons from the benzonitrile-substituted nitrogen, the
8 limited flexibility of the carbon chain hinders exciplex formation. If the neighboring nitrogen is
9 more than four carbons from the benzonitrile-substituted nitrogen, the large size of the carbon chain
10 sterically hinders exciplex formation. To allow for efficient metal binding, R¹ includes at least one
11 additional heteroatom, typically spaced 2 to 4 carbon atoms from the closest heteroatom. More
12 often, the heteroatoms of R¹ are spaced 2 or 3 carbon atoms from each other.

13 Generally, although additional heteroatoms improve metal complexation, the additional bulk
14 and structural limitations that arise when more than two additional heteroatoms are present may
15 hinder exciplex formation, depending upon the position of the additional heteroatoms relative to the
16 benzonitrile-substituted nitrogen and the neighboring nitrogen. Typically, R¹ having from 1 to 5
17 heteroatoms in addition to the benzonitrile-substituted nitrogen and the neighboring nitrogen strikes
18 the best balance between metal complexing ability and exciplex formation. Any additional nitrogen
19 heteroatoms in R¹ may be substituted with aliphatic groups that are substituted or unsubstituted.
20 Except for obvious steric hindrances (which depend upon the location of the aliphatic groups relative
21 to the benzonitrile-substituted and neighboring nitrogens and the heteroatoms which complex the

1 metal), and charge stability effects (which depend upon the distance of any electron-donating or
2 electron-withdrawing moiety in the aliphatic group from the benzonitrile-substituted and neighboring
3 nitrogens and the heteroatoms which complex the metal). the nature of the aliphatic group and
4 substitutions thereof should have at most a minor effect on the complexing and triple fluorescence
5 properties of the fluorophore of the present invention. Generally, atoms more than 4 carbons from
6 the benzonitrile-substituted and neighboring nitrogens and the heteroatoms which complex the metal
7 have a minimal impact on these properties.

8 In addition to the varying the number of heteroatoms, the nature of the additional heteroatoms
9 can also be varied to allow for greater differentiation between or preferential complexation among
10 various metals. In most cases, however, R¹ includes 3 or 4 nitrogens, and usually 4 nitrogens.

11 Typically, R¹ has one of the following structures:



15
16 wherein R² and R³ are unsubstituted C₂ to C₄ alkylene groups or C₂ to C₄ alkylene groups substituted
17 with an aliphatic group, and may be the same as or different from one another;

18 R¹ is -, an unsubstituted C₂ to C₄ alkylene group, or a C₂ to C₄ alkylene group substituted with
19 an aliphatic group, and may be the same or different from any other R¹ groups present and may be

1 the same or different from R² and R³;

2 Q is NH, NR⁷, O, or S, and may be the same as or different from any other Q group present;

3 R⁵, R⁶, and R⁷ are aliphatic groups and may be the same as or different from each other:

4 R⁸ is an unsubstituted C₂ to C₄ alkylene group or a C₂ to C₄ alkylene groups substituted with
5 an aliphatic group, and may be the same as or different from any other R⁸ group present; and

6 y is an integer having a value of from 1 to 5.

7 As can be seen from the above formula, in azacyclo structures, the ring size may be varied.

8 Typically, the ring includes 3 or 4 heteroatoms. Most often, the ring includes 4 heteroatoms.

9 Metal ion concentration in liquids can be quantitatively and qualitatively detected using the
10 fluorophore of the present invention. Quantitative detection can be accomplished when sufficient
11 fluorophore is available to complex with all metal ions present. The degree of fluorescent signal
12 perturbation will be proportional to the metal ion concentration. This detection can be accomplished
13 by determining the extent of emission frequency shifting and/or intensity change caused by
14 complexation for one, two, or all of the excited states responsible for fluorescent emission (TICT,
15 LE, and E). The extent of emission frequency shifting and/or intensity change may be determined
16 directly by comparison with a blank or concurrently analyzed standard solutions, or may be indirectly
17 determined by reference to a chart or other data based on previously analyzed standard solutions.

18 The triple fluorescence of the fluorophore of the present invention increases its versatility.
19 For example, if the fluorescent emission of one excited state is quenched by an interfering
20 compound, the metal may still be detected by examining the fluorescent emission of the other excited
21 states.

1 anions and were of the highest purity available (Aldrich or Alfa). Buffers (0.1 M) were prepared
2 using H₃PO₄ (pH 1-3), acetic acid (pH 4-5), NaH₂PO₄ (pH 6-8), NaHCO₃ (pH 9-11), Na₂HPO₄ (pH
3 12), and NaOH (pH 13), with the pH being adjusted using either HCl or NaOH while monitoring
4 with a pH meter. Intensity contributions from the three individual excited states were estimated
5 using the Voigt Amplitude function of Jandel's PeakFit 4.0.

6 Results and Discussion

7 **Solvent Dependence of Triple Fluorescence for DMABN-Cyclam.** The UV spectra for
8 DMABN-Cyclam in ethanol shows a single absorption peak at 295 nm. Excitation at this
9 wavelength leads to three emission peaks: 352 nm, 429 nm, and 473 nm (Fig. 3). The fluorescence
10 excitation spectra for these three emission wavelengths overlap at 295 nm, further indicating the
11 purity of this compound and that each of these emission bands arise from excitation of the same
12 ground state species. Intensity contributions and wavelength placement of the three excited state
13 species were determined using a peak fitting protocol discussed in the experimental section. Because
14 of the spectral overlap apparent between these three species, only qualitative information such as
15 relative intensity trends and approximate peak positions have been derived from this analysis. The
16 peak at 352 nm is attributed to the LE state emission of DMABN-Cyclam, and compares favorably
17 to that found for DMABN in THF ($\lambda_{\text{max}} = 354$ nm). *Recl. Trav. Chim. Pays-Bas*, **1995**, *114*, 517-527,
18 the entirety of which is incorporated herein by reference for all purposes. Identification of the two
19 additional, red-shifted emission peaks was accomplished by comparison to structural analogues of
20 DMABN-Cyclam, DMABN-Crown5 and CNP3NM. Létard *et. al.* reported that DMABN-Crown5
21 exhibits a TICT emission that red-shifts and increases in intensity as the solvent polarity is increased

1 from hexane (where the emission band was too weak to be measured accurately), to toluene (λ_{\max} =
2 406 nm), to acetonitrile (λ_{\max} =466 nm). *Id.* Similarly, DMABN-Cyclam, which differs from
3 DMABN-Crown5 primarily in the replacement of nitrogen binding sites for the oxygen atoms of the
4 crown ether, reports a similar trend in the emission spectra (Fig. 3): hexane (λ_{\max} = 390 nm \pm 10 nm),
5 toluene (λ_{\max} = 413 \pm 10 nm) and ethanol (λ_{\max} = 473 \pm 3 nm). We, therefore, attribute the third
6 emission band in ethanol at 473 nm to a TICT state. Unlike DMABN-Crown5, however, DMABN-
7 Cyclam has two additional amine groups linked by a short chain (n= 3 and n= 4) to the benzonitrile
8 acceptor. Petrov *et. al.*, *J. Phys. Chem.*, **1996**, *100*, 6368-6370, the entirety of which is incorporated
9 herein by reference for all purposes, have shown that azacrown ethers can readily form
10 intermolecular exciplexes with pyrene. for example. Despite the cyclic nature of the cyclam
11 functional group in DMABN-Cyclam, this molecule exhibits sufficient flexibility to form a sandwich
12 complex in the excited state that gives rise to an intramolecular exciplex peak (E) (Fig. 2). The
13 peak position identified for the exciplex peak of DMABN-Cyclam in hexane (λ_{\max} = 392 nm \pm 10
14 nm) compares favorably with that found for CNP3NM in isopentane (λ_{\max} = 390 nm). Van der
15 Auweraer, M.; Vannerem, A.; de Schryver, F.C. *J. Mol. Struct.*, **1982**, *84*, 343-351, the entirety of
16 which is incorporated herein by reference for all purposes.

17 Several trends are evident from Fig. 3 with regards to the effect of solvent polarity on the
18 triple fluorescence of DMABN-Cyclam. The TICT emission band red-shifts by more than 80 nm
19 and increases in intensity relative to the LE emission as solvent polarity is increased. The relative
20 intensity of the TICT band increases with solvent polarity due to a stabilization of the charge
21 separation. This type of behavior was also reported for DMABN-Crown4.⁷ The exciplex emission

1 does not red-shift quite as severely (43 nm), due to a smaller dipole moment compared with the
2 TICT configuration. The intensity of the TICT emission band increases relative to the exciplex
3 emission with increasing solvent polarity. This feature can be attributed to 1) increased stabilization
4 of the larger dipole moment evident in the TICT state; and 2) increased radiationless decay of the
5 exciplex by internal conversion in the more polar solvents. In fact, the intensity of the intramolecular
6 exciplex emission band is maximal in toluene, decreasing in intensity as the solvent polarity is
7 increased. This type of behavior has been observed for other amine intramolecular exciplexes,
8 including ((N,N-dimethylamino)-alkyl)phenanthrenes (Lewis, F.D.; Cohen, B.E. *J. Phys. Chem.*,
9 **1994**, *98*, 10591-10597, the entirety of which is incorporated herein by reference for all purposes)
10 and ω -(1-pyrenyl)- α -N,N-dimethylaminoalkanes. Swinnen et al. *J. Am. Chem. Soc.*, **1987**, *109*, 321-
11 330, the entirety of which is incorporated herein by reference for all purposes. Knibbe et al. were
12 the first to propose that the decrease in intermolecular exciplex fluorescence intensity with
13 increasing solvent polarity was due to ionic dissociation and the formation of nonfluorescent radical
14 ions in polar solvents. *J. Chem. Phys.*, **1967**, *47*, 1184-1185, the entirety of which is incorporated
15 herein by reference for all purposes. Because ionic dissociation is not possible in intramolecular
16 exciplexes. Mataga and co-workers suggested that a compact folded geometry is favored in nonpolar
17 solvents, and looser, more open geometries are apparent in polar solvents. Okada et al., *J. Phys.*
18 *Chem.*, **1981**, *85*, 3957-3960, the entirety of which is incorporated herein by reference for all
19 purposes. The loose geometry is akin to the solvent-separated radical ion pair which is formed in
20 intermolecular exciplexes, thereby explaining the quenching effect observed in more polar solvents.

1 In a similar fashion, it is expected that prior to excitation DMABN-Cyclam will conform to a unique
2 conformation in solvents of differing polarity. Presumably, DMABN-Cyclam optimally conforms
3 to a compact folded configuration in toluene, fostering enhanced intramolecular exciplex formation
4 and emission. As the polarity of the solvent is increased, solvation of the aza groups on the cyclam
5 moiety is enhanced, allowing DMABN-Cyclam to attain a more open configuration that decreases
6 intramolecular exciplex formation and emission. Similar behavior in other dye-linked aza crown
7 ethers has been reported. Collins et al., *J. Chem. Soc. Chem. Comm.*, 1997, 12, 1135-1136, the
8 entirety of which is incorporated herein by reference for all purposes.

9 The LE band exhibits an intensity minima in water because of the high polarity of this
10 solvent, and its capability for stabilizing the two charge transfer complexes. The small
11 hypsochromic shift evident in the LE band (5 nm) with decreasing solvent polarity indicates that
12 minimal charge transfer exists for this excited state species prior to formation of the charge transfer
13 states. It's possible that steric hindrance introduced by the cyclam moiety forces the diethyl amino
14 group to rotate out of the plane of the phenyl acceptor group, resulting in lower mesomeric
15 interaction between D and A. Support of this contention was found by Létard *et. al., supra.* who
16 reported a calculated twist angle for DMABN-Crown5 of 18° (compared to 1° for DMABN) which
17 was attributed to the repulsion of the large ring of the 5-azacrown ether by the *ortho*-hydrogens.

18
19 **pH Dependence of Triple Fluorescence for DMABN-Cyclam.** An improved understanding of
20 the interplay between the three excited state species can be achieved by examining the change in
21 fluorescence emission for DMABN-Cyclam with pH (Fig. 4). Examining first the effect of pH on

1 intramolecular exciplex emission (open triangles), this band is maximal at pH 3, dropping off rapidly
2 at more acidic pH, and decreasing in a nearly linear fashion at more basic pH values. The pK_a values
3 for cyclam have been measured by several workers, and are summarized by Bianchi et. al., *Coord.*
4 *Chem. Rev.*, 1991, 110, 17-113. At 25°C, there is reasonable agreement between the different
5 measurements for pK_1 (11.6 ± 0.16), pK_2 (10.44 ± 0.23) and pK_3 (1.55 ± 0.11). However, there is
6 considerable variability in the values determined for the pK_1 (0.94-2.41) of cyclam. The benzonitrile
7 aza group of DMABN-Cyclam will have the lowest pK_a value due to the strong, electron-
8 withdrawing nature of this group. By comparison to the pK_a 's recorded for aniline ($pK_a = 4.63$) and
9 diethylamine ($pK_a = 10.5$), the pK_a for this aza group are expected to be as much as four orders of
10 magnitude lower than that found in cyclam, particularly since the nitrile functionality makes this
11 group an even stronger acceptor. As the acidity drops below pH 2, DMABN-Cyclam will likely be
12 triply protonated, causing intramolecular exciplex formation to decrease dramatically due to the
13 inability of the protonated aza groups to donate charge to the phenyl acceptor group. As the pH
14 increases above pH 3, the intramolecular exciplex emission band steadily decreases. This appears
15 to be due to a shift in the equilibrium from the exciplex to the TICT state as the pH is increased.
16 Evidence for this shift exists in a plot of the change in TICT/E versus pH (open squares, Fig. 4). In
17 general, the TICT emission band slowly dominates the emission spectra as the pH increases, perhaps
18 due to the fact that unlike the exciplex state, the charge transfer step for the TICT state formation
19 requires no structural rearrangement.

20 The TICT emission band (closed triangles, Fig. 4), in similar fashion to the exciplex emission
21 band, passes through a maximum at pH 3. However, the emission band for TICT is nearly level

1 from pH 3-8, before steadily declining as the alkalinity is increased from pH 8-13. The decrease in
2 TICT emission seen above pH 8 is attributed to an increase in quenching by the exciplex aza groups.
3 The quenching of DMABN's TICT fluorescence has been reported by Wang et al. in the examination
4 of a series of tertiary amines. *Faraday Trans. 2*, 1988, 84(11), 1809-1823, the entirety of which is
5 incorporated herein by reference for all purposes. At pH values less than 2, DMABN-Cyclam will
6 be triply protonated, and TICT state formation will decrease due to the decrease in charge transfer
7 resulting from electrostatic repulsion with the three protonated nitrogens closely linked to the
8 benzonitrile aza group.

9 The LE emission intensity (solid circles, Fig. 4) reported a steady decrease in intensity with
10 increasing pH, ultimately leveling off at pH 8 and above. As DMABN-Cyclam is deprotonated and
11 charge transfer facilitated, the equilibrium will shift towards the formation of the two charge-transfer
12 states, E and TICT.

13
14 **Effect of Metal Complexation on Triple Fluorescence of DMABN-Cyclam.** Because the aza
15 groups responsible for metal cation binding are also directly involved with the formation of the two
16 charge-transfer excited state complexes (E and TICT), metal binding by DMABN-Cyclam should
17 have a dramatic influence upon the fluorescence intensity of the triple fluorescence. A series of
18 different metal cations were investigated in both protic (water, pH = 7, Table 1) and aprotic solvents
19 (acetonitrile, Fig. 5). In order to simplify the discussion, the metal ions have been classified as
20 follows: 1) diamagnetic metal cations- Zn^{2+} and Cd^{2+} ; 2) paramagnetic metal cations- Cu^{2+} and Ni^{2+} ;
21 and, 3) diamagnetic, heavy metal cations- Hg^{2+} and Pb^{2+} .

Table 1. Relative Change in LE, E, and TICT Emission Observed for the Addition of 12 μM of Each Metal Salt to a 25 μM Solution of DMABN-Cyclam in Buffered Water (pH=7)

| Metal ion | LE | E | TICT |
|-----------|------|------|------|
| none | 0.54 | 1.2 | 1.4 |
| Zn(II) | 2.2 | 1 | 1.2 |
| Cd(II) | 0.68 | 1.1 | 1.4 |
| Cu(II) | 0.42 | 0.9 | 1.2 |
| Ni(II) | 0.43 | 0.94 | 1.2 |
| Pb(II) | 0.51 | 1.1 | 1.4 |
| Hg(II) | 0.51 | 0.93 | 1.2 |

Consider first the situation evident for DMABN-Cyclam dissolved in water. As was mentioned earlier, DMABN-Cyclam likely conforms to an open geometry in this strongly polar solvent, a conformation lowering the extent of exciplex and LE emission, while favoring TICT state formation. Complexation of metal cations by DMABN-Cyclam in water (Table 1) causes a net decrease in the TICT and E state emission. Complexation of the cyclam aza groups by metal cations lowers the extent of charge transfer possible with the benzonitrile acceptor group, and hence, diminishes each of these charge transfer emission bands. In conjunction with the equilibrium shown in Fig. 2, as the TICT and E concentrations decrease, the LE emission increases. This was, in fact, observed for the diamagnetic metal cations. Zn^{2+} and Cd^{2+} , however, the paramagnetic and heavy

1 metal cations caused a decrease in the LE emission following complexation. Paramagnetic (Varnes
2 et al., *J. Am. Chem. Soc.*, **1972**, *94*, 946-950) and large or easily reducible cations (Akkaya et al., *J.*
3 *Am. Chem. Soc.*, **1990**, *112*, 3590-3593) are recognized for their intracomplex. quenching behavior,
4 and, apparently, are responsible for the decrease observed in LE emission.

5 A completely different set of results are obtained when the aprotic solvent, acetonitrile, is
6 used for the metal complexation studies. Summarized in Fig. 5 are the relative changes seen for LE
7 (solid), E (diagonal lines) and TICT (hatched lines) emission following the addition of 12 μM of
8 each metal salt to a 25 μM solution of DMABN-Cyclam in acetonitrile. Shown in Fig. 6 is an
9 example set of emission spectra indicating the influence of Zn^{2+} addition on the triple fluorescence
10 of DMABN-Cyclam in acetonitrile. The most dramatic difference realized from the situation
11 observed in water, is that in all cases, complexation of metal cations by DMABN-Cyclam caused
12 an increase in the exciplex emission. This result is surprising, considering that complexation of the
13 exciplex aza group would normally be expected to reduce the extent of charge transfer and, hence,
14 lower the exciplex emission intensity. Because the intramolecular exciplex emission does not
15 decrease, it can be concluded that in acetonitrile, the nitrogen involved in exciplex formation is no
16 longer complexed with the metal cation. This contention is also supported by the absence of any
17 blue-shifting seen in the exciplex emission. As was concluded previously from the study of
18 DMABN-Cyclam in solvents of varying polarity, DMABN-Cyclam forms a more compact, folded
19 geometry in acetonitrile wherein the exciplex aza group apparently does not participate in the
20 complexation of the metal cation. Apparently, complexation of a metal cation by the three remaining
21 nitrogens provides enough shielding to slow electron transfer from the benzonitrile radical anion

1 moiety. The dominating influence on the exciplex emission band arises from the elimination of
2 quenching amines due to the interaction of the remaining three aza groups with the complexed metal
3 cation. Additionally, binding of the metal cation may result in a new, metal/ligand, complex
4 geometry that further favors exciplex formation.

5 Reduction in the charge transfer character of the TICT aza group due to binding of a metal
6 cation in the ground state will result in at least three effects: 1) providing that the exciplex aza group
7 is not partaking in metal cation complexation, the exciplex emission will increase when quenching
8 from the TICT aza group is reduced; 2) TICT emission will decrease along with the reduced
9 capability for the complexed aza group to donate charge to the benzonitrile acceptor group; and, 3)
10 the LE emission band will increase with the shift in equilibrium from TICT to LE. The only
11 exception to the expected increase in LE emission was found for the metal cation, Hg^{2+} . This
12 reduction in emission intensity is attributed to the quenching behavior of this large and easily
13 reducible metal cation. For higher concentrations of paramagnetic metal ions, such as Cu^{2+} , there
14 is the additional impact of uncomplexed metal ions causing diffusional quenching of the [DMABN-
15 Cyclam: Metal ion] complex. This is shown in Fig. 7, which plots the change in triple fluorescence
16 seen for the sequential addition of Cu^{2+} to DMABN-Cyclam in acetonitrile. As the concentration
17 of copper cations becomes greater than the equimolar concentration of DMABN-Cyclam (25 μM),
18 the overall triple emission intensity can be observed to decrease in intensity due to quenching. The
19 paramagnetic effect is weak enough for this system that it is not observed until the addition of greater
20 than a stoichiometric amount of Cu^{2+} . This effect is perhaps related to a small coefficient of the
21 benzonitrile LUMO on the amino nitrogen complexed with the Cu^{2+} .

Docket No.: N.C. 78,806
Inventor's Name: Collins et al.

PATENT APPLICATION

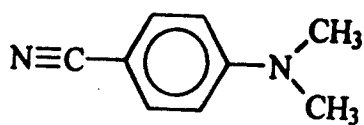
1 Obviously, many modifications and variations of the present invention are possible in light
2 of the above teachings. It is therefore to be understood that
3 the invention may be practiced otherwise than as specifically described.

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PATENT APPLICATION

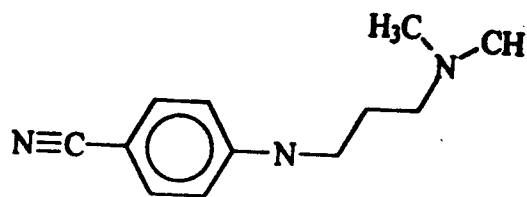
ABSTRACT

A polyfunctional amine, such as cyclam, substituted with 4-(N,N-dimethylamino) benzonitrile, exhibits triple fluorescence and complexes with metal ions. The complexation of metal ions with the fluorophore changes the triple fluorescence characteristics of the fluorophore. Thus, this substituted polyfunctional amine provides an effective indicator for the qualitative and quantitative detection of metals.



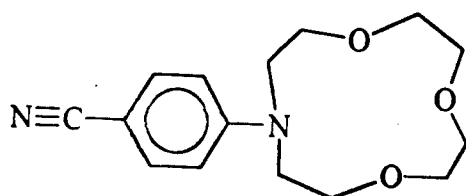
DMABN

Fig. 1a (Prior Art)



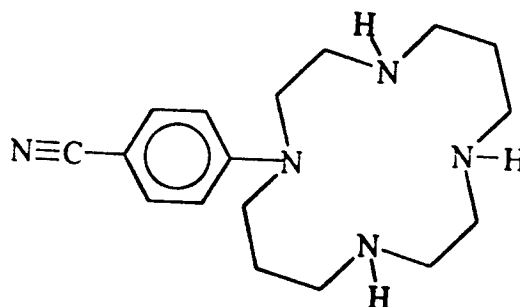
CNP3NM

Fig. 1b (Prior Art)



DMABN-Crown4

Fig. 1c (Prior Art)



DMABN-Cyclam

Fig. 1d

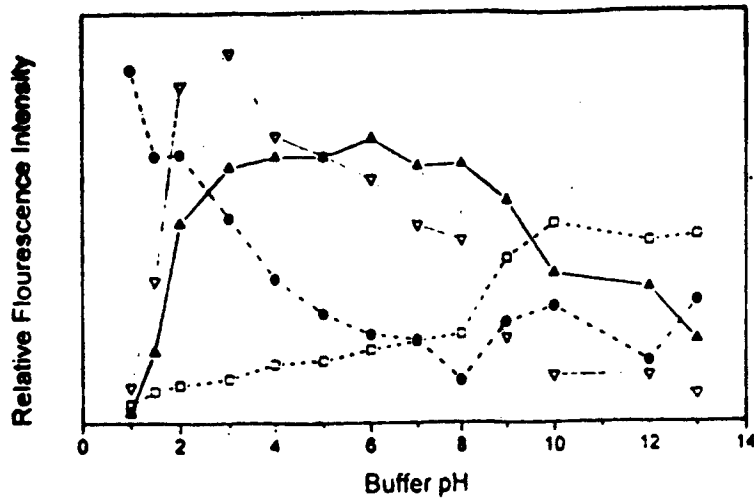


Fig. 4

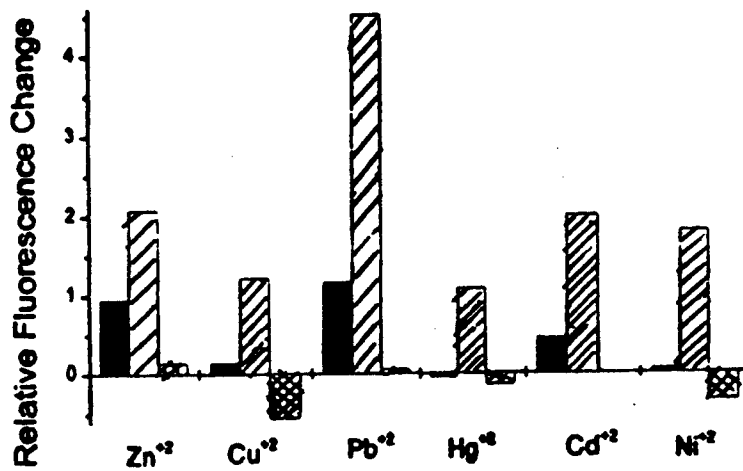


Fig. 5

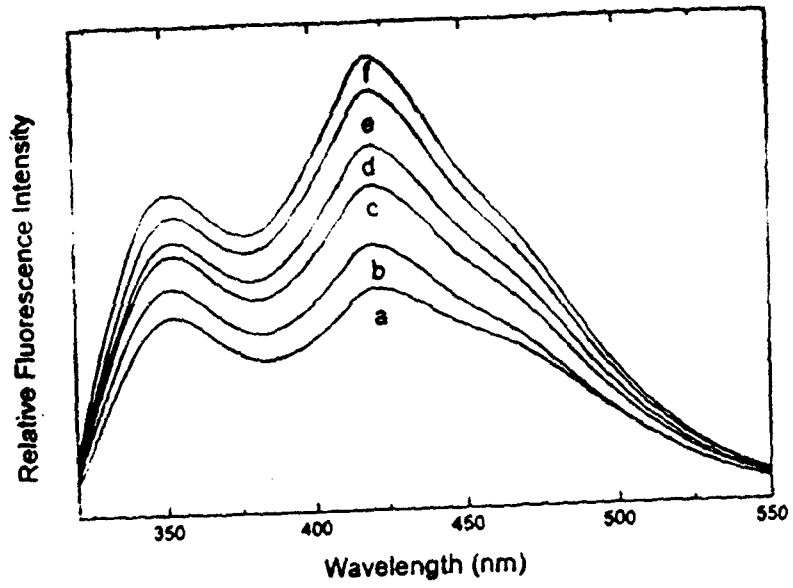


Fig. 6

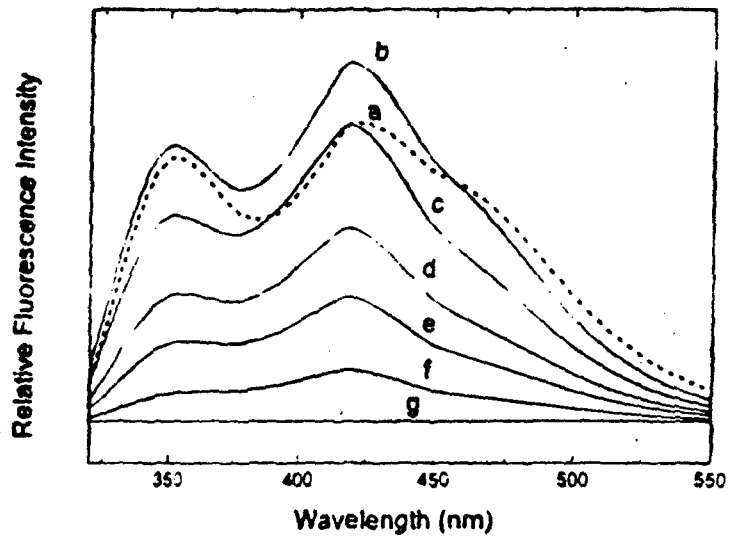


Fig. 7