



**SYNTHESIS OF MACROCYCLIC BISINTERCALATOR AND A TRIS-INTERCALATOR**

**BY**

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**THESIS**

**for the**

**DEGREE OF BACHELOR OF SCIENCE**

**IN**

**CHEMISTRY**

**College of Liberal Arts and Sciences**

**University of Illinois**

**Urbana, Illinois**

**1987**

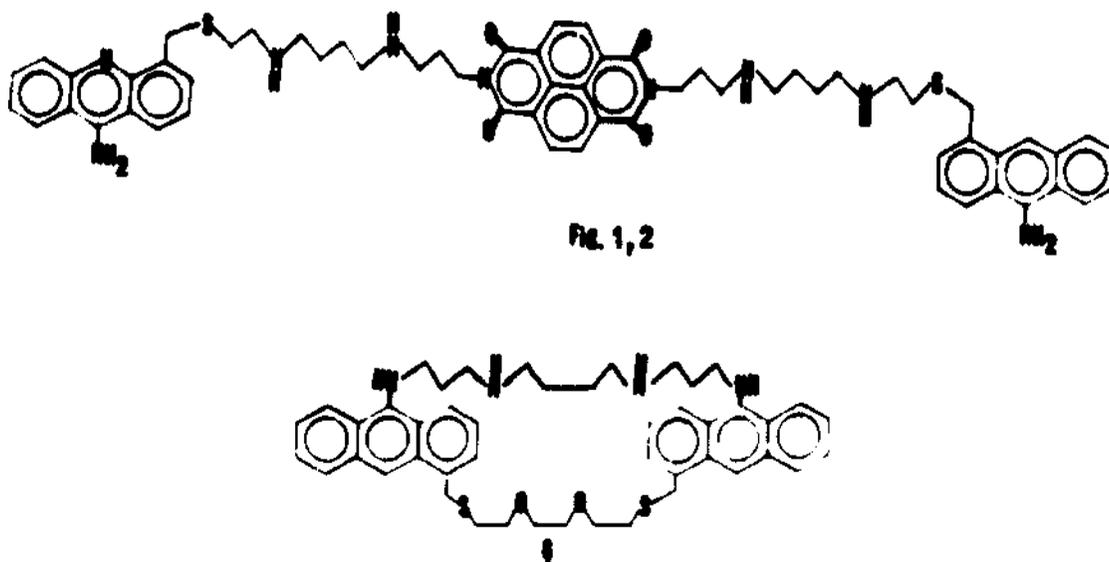
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## INTRODUCTION:

Within the wide range of cancer therapies and treatments, one particular strategy has focused on inhibiting the rapid replication of neoplastic cells. A logical cellular target for effecting this inhibition is RNA transcription. Studies by Lerman<sup>1</sup> demonstrated that aminoacridine, an early anti-tumor agent, binds to DNA and inserts or intercalates between adjacent base pairs. This intercalation and the conformational changes it induces is believed to block the action of RNA polymerase during transcription thereby promoting cytotoxicity.

The goal of our research has been the design and synthesis of a macrocyclic-bis-intercalator and tris-intercalator expected to intercalate with DNA through novel binding mechanisms. Our target compounds (Fig. 1 and 2) are designed to take advantage of the intercalating properties of acridine reported by Lerman.



In addition, there are four properties which the molecules should ideally possess: 1) A long residence time with a concurrently strong binding constant; 2) A sufficiently long linking chain in accordance with the neighbor exclusion principle; 3) A specificity for binding to the AT rich promoter region of RNA initiation; and 4) A moderate water solubility.

### Residence Time and Binding Affinity

Studies by Robertson<sup>2</sup> indicated that anti-tumor activity is closely correlated to binding affinity for DNA. Ideally, a DNA affinity of the same order of magnitude as RNA polymerase and its repressor is sought ( $10^{12} \text{ M}^{-1}$ ). Our target compound consists of two substituted acridine moieties connected by 9-9' and 4-4' linkages. Bis-acridine compounds are known to possess a high binding constant.<sup>3</sup> Therefore, it is reasonable to expect a compound incorporating these moieties to exhibit high DNA affinity.

However, attempts at increasing the DNA affinity led to the synthesis of anti-tumor agents which possessed strong binding constants and specificity for AT rich regions but without the attendant increase in anti-tumor effectiveness.<sup>4</sup> Kearn's<sup>5</sup> NMR studies provide a logical explanation for this discrepancy. His studies indicated that one of the acridines may dissociate and travel up the DNA helix through a "walking" mechanism. As a result of this "walking," the intercalator does not remain stationary at one DNA site long enough to block the progress of RNA polymerase as it proceeds up the helix, and thus exhibits no significant anti-tumor activity. Hence, a proposed anti-tumor agent should possess not only strong DNA affinity but also a long residence time at particular DNA sites.

Mandel<sup>6</sup> proposed that DNA "breathing" occurs when hydrogen exchange between base pairs in the DNA helix results in the transient opening of 10 or more base pairs. The AT base pairs are especially prone to this process and undergo exchange more frequently than GC pairs due to hydrogen bonding between only two hydrogens in the AT pair instead of three.

The utilization of a macrocycle design insures that our target compound will intercalate only when DNA breathes and is locally unwound. Subsequent closure of the double helix may trap the target compound at a particular DNA

site insuring a long residence time. Because of the anticipated high binding affinity of the macrocycle, it is also unlikely to relocate during future DNA breathing.

#### Neighbor Exclusion Principle:

According to the neighbor exclusion principle<sup>7</sup>, the continuity of the helix is disrupted at the intercalation site causing a conformational change of the deoxyribose rings making the adjacent base pairing site energetically unavailable. As such, bis-intercalation cannot occur unless the acridine rings are separated by two base pairs and a distance greater than 8.1 Å. The macrocycle design provides a large 'bite size' of approximately 16.1 Å to allow both acridine moieties to bind.

#### Affinity of Base Pairs

In studies by Canellakis<sup>8</sup>, it was determined that spermine diacridine inhibited initiation and elongation during transcription of RNA. The mechanism of inhibition was believed to involve blockage of the promoter region through binding of its AT rich base pairs. Since the macrocycle possesses a form of spermine diacridine, it is also expected to target the AT base pairs of the promoter region and intercalate when a transient opening of the helix occurs.

#### Water solubility:

Water solubility is an important consideration in the design of any intercalator since the DNA binding experiments are performed in an aqueous media simulating in vivo conditions. The primary role of the polyether side chain is to enhance the water solubility. In comparison, the spermine chain's cationic nature provides an affinity for polyanionic DNA and also enhance the

water solubility properties of the macrocycle.<sup>9</sup>.

#### TRIS-INTERCALATOR:

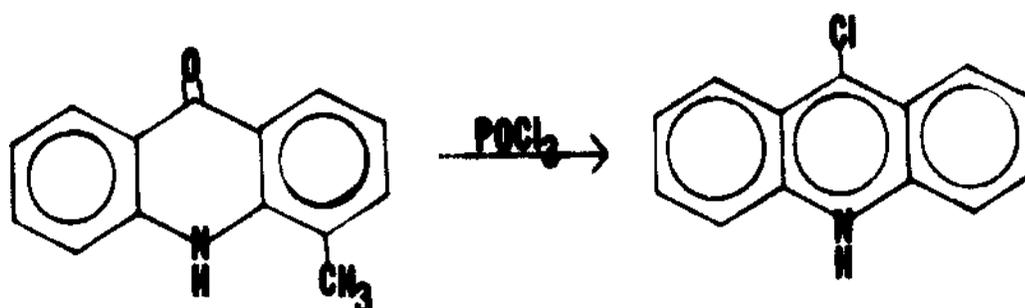
Similar to the macrocycle, the tris-intercalator also incorporates a novel binding mechanism. In studies by Yen<sup>10</sup>, an naphthalene diimide intercalator bound with an adamantyl in both major and minor groove was found to intercalate with its two linking chains lying in an alternate groove configuration. Similarly, the tris-intercalator can have chains in both grooves but additionally will insert two chromophores from both the major and minor groove. In order to assume this configuration, it is necessary for one of the acridine moieties to slide through the base pairs allowing the adamantyl substituent to bind to the recently vacated site. Once bound to the helix, two of the intercalation sites would have to dissociate simultaneously to travel to another binding site. This complex binding mechanism should increase the resident time by making dissociation from the DNA helix difficult (Figure 3).

#### RESULTS AND DISCUSSION:

##### **Macrocycle:**

The synthetic scheme depicted in Figure 4 summarizes the route chosen for the synthesis of the macrocycle. Initially, the scheme utilizes the synthesis of 4 outlined by Acheson.<sup>11</sup> The order of side chain addition was selected after consideration of the high reactivity of the polyamine and its potential for generating side products. Consequently, the synthesis of 6 begins with 9-chloro-4-methylacridine cyclized from the ullman coupling product, 1. Both of these reactions produce relatively clean products in high yields.<sup>12,13</sup>

These reactions are well preceded in the literature, and the only major concern in working with chloroacridines lies in their slow hydrolysis to acridone in the presence of air. Fortunately, acridone may be reconverted into its corresponding chloroacridine through refluxing with 2 equivalents of phosphorus oxychloride for 1 hour.



Bromination<sup>14</sup> of 2 with N-bromosuccinimide and benzoyl peroxide in carbon tetrachloride afforded 3 in 40% yield. While Acheson's previous bromination work had suggested that the route would produce a slightly higher yield, the starting material could not be reacted to completion without a substantial production of 4-dibromomethyl-9-chloroacridine. The spectroscopic data clearly distinguishes between the amount of bromination in each of the products. While the NMR shifts of the acridine hydrogens are very similar for all three of the chloroacridines, the methyl hydrogens shift dramatically from  $\delta$  2.91 to  $\delta$  5.38 upon monobromination and to  $\delta$  5.56 upon dibromination.

Although Acheson reported the synthesis of 4, the details of its preparation were not reported. Our procedure involves the nucleophilic displacement of bromine by the anionic thiol chain under low dilution conditions. Initial attempts at optimizing this route by varying the solvent

conditions were poor. In ethanol, no significant reaction with 4 occurred, and the starting material was subsequently recovered. Addition of methanol THF (1:1) generated 4 in a 24% yield, but the low yield and the production of numerous side products discouraged the use of this procedure. The final optimization conditions generated 4 in a 55% yield with the use of THF.

It is important to note that in the interpretation of the NMR data, several factors contributed to the positive identification of 4, 5, and 6. First, the extensive symmetry of these compounds provided relatively simple spectra to interpret. As such, unsymmetrical side products were readily detected by their complex splitting patterns. Secondly, the chemical shifts of the phenoxy, spermine, thiol, and acridine hydrogens did not extensively overlap which clearly verified the incorporation of these structural features. Finally, the ratio of the number of hydrogens for each structural feature determined the extent of its incorporation. For example, a comparison of hydrogens of the spermine chain with those of the thiol chain ensured that 2 spermine chains were not incorporated to the 9 and 9' positions of the acridine in the final step in the synthesis of the macrocycle.

In the preparation of 5, the procedure for the synthesis of spermine diacridine was utilized. Cannellakis<sup>15</sup> suggested that displacement of the chloride ions with phenoxy groups would facilitate the incorporation of the spermine chain. Consequently, 4 was added to a mixture of melted phenol and potassium hydroxide. The reaction was heated at 110 C for 1 1/2 h. to give 5 in a 31% yield. This precursor was then used to synthesize the macrocycle.

Addition of the spermine chain was accomplished under high dilution conditions to discourage the incorporation of 2 spermine chains to the 9 and 9' positions of the acridine. A solution of spermine and 5 was heated to 110-130C for 2 h. in melted phenol to yield 6 in 1.5% yield. Purification of 6

involved elution from a plug of silica with 30%  $\text{NH}_3$ -- $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$ . This inelegant procedure incorporated silica which was carefully removed upon filtration of a concentrated solution of 6. Had the macrocycle been soluble in water as previously expected, an ion exchange column could have been utilized. This lack of water solubility eventually disqualified 6 from being used in DNA binding studies, but it still maintains the distinction of being a new compound, and the first macrocycle synthesized to intercalate with DNA. At the present, the synthesis of a macrocycle with a higher solubility is underway in our lab.

#### Tris-intercalator:

The synthetic scheme depicted in figure 4 summarizes the route chosen for the synthesis of the tris-intercalator. Initially, we have been working on the synthesis of the spermidine protecting group, 8. Although Chantrapromma<sup>16</sup> reported the synthesis of 8 in 92% yield, he failed to publish the precise synthetic procedure, and until recently, we have only recovered inorganic barium salts. Fortunately, the full synthetic procedure was sent upon request, and the preliminary data on our synthesis of 8 appears promising.

#### EXPERIMENTAL SECTION

All solvents and reagents were of reagent grade quality unless otherwise noted. 2-Chloro-benzoic acid was purified according to Allen and McKee.<sup>12</sup> 0-toluidine was fractionally distilled over copper powder, and THF was distilled over sodium benzophenone prior to use. N-bromosuccinimide was recrystallized from water and dried in vacuo over  $\text{P}_2\text{O}_5$ . 1,2-Bis(2--mercaptoethoxy) ethane was furnished by Arduengo, but it may be alternatively prepared by Speziale<sup>17</sup>. All

column chromatography was performed according to Still<sup>18</sup>, using 14 cm of silica gel (32-63).

Nuclear magnetic Resonance spectra were recorded on a General Electric QE-300 (300 MHz) spectrometer using TMS as an internal reference. Mass spectra were recorded on a Finnegan-MAT CH-5 DF instrument, and melting points were obtained using a Thomas Hoover capillary melting point apparatus.

2'-Methyl-N-phenylanthranilic acid, 1:

2-Chlorobenzoic acid (2.43g, 15.52mmol), anhydrous potassium carbonate (2.47g, 1787mmol), and finely powdered copper (0.05g, 1.41mmol) were combined. O-toluidine (10.62g, 99.10mmol) was added and heated at reflux with an air-cooled condenser. After two hours, an additional equivalent of copper (0.05g, 1.41mmol) was added and reflux continued for 1 1/2 hr. The solution was allowed to cool; chloroform (100ml) was added; the reaction was filtered, and the copper catalyst was washed with 1N KOH (200ml). Following extraction of the basic layer with additional chloroform (300ml), it was acidified to pH 1 with 3M HCl. The precipitated white powder was filtered and dried in vacuo. Recrystallization from benzene yielded 2.57g (73%) of off-white crystals. Mp. 186°C. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 2.29(s, 3H), 6.71(t, J=7Hz, 1H), 6.84(d, J=9Hz, 1H), 7.12-7.22(m, 5H), 8.04(d, J=9Hz, 1H), 9.14(s, 1H). EI(M<sup>+</sup>227, 89.89%).

9-Chloro-4-methylacridine, 2:

A solution of 1 (1.47g, 6.47mmol) was heated at reflux with phosphorus oxychloride (4.96ml, 32.0mmol) for 1 1/2 h. The residue obtained after removal of excess phosphorus oxychloride in vacuo was diluted with chloroform (50ml), and extracted with additional chloroform (2 75ml). The organic layer was dried (MgSO<sub>4</sub>), filtered, and concentrated in vacuo. The crude solid was purified by

flash chromatography (dichloromethane) to afford 1.19g (81%) of a yellow solid, 2. Mp. 93-94°C. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 2.91 (s, 3H), 7.48 (t, J=6Hz, 1H), 7.60 (d, J=3Hz, 2H), 7.76 (t, J=6Hz, 1H), 8.24 (d, J=6Hz, 2H), 8.37 (d, J=6H, 2H). EI (M<sup>+</sup> 227, 100.00%).

4-Bromomethyl-9-chloroacridine, 3:

A solution of 2 (0.66g, 2.90mmol), N-bromosuccinimide (1.03g, 5.79mmol), benzoyl peroxide (0.03g, .124mmol) in carbon tetrachloride (5ml) was heated at reflux for 4h. The solid succinimide was filtered and washed with additional carbon tetrachloride (2 x 2ml) to obtain the filtrate. Removal of the solvent in vacuo afforded a crude light green solid. The solid was purified by flash chromatography (chloroform-hexane; 1:4) to afford 0.300g (34%) of pure yellow solid, 3. Mp. 172-173°C. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 5.38 (s, 2H), 7.56-7.68 (m, 2H), 7.82 (t, J=7Hz, 1H), 7.93 (d, J=6Hz, 1H), 8.27 (d, J=9Hz, 1H), 8.40 (d, J=9Hz, 2H).

1,2-Bis(2-(9-chloroacridinyl) methylthio) ethoxy) ethane, 4:

To a solution of 3 (1.83g, 5.98mmol) in THF (9ml), a separate solution of sodium hydride (0.287g, 12.02mmol) and 1,2-bis (2-mercaptoethoxy) ethane (0.545g, 2.99mmol) in THF (9ml) was combined. The reaction mixture was allowed to stir at room temperature for 4 1/2 h. The solution was added to methylene chloride (75ml) and extracted with water (75ml) followed by drying of the organic layer (MgSO<sub>4</sub>). Removal of the solvent in vacuo yielded a crude solid which was purified by flash chromatography (ether-dichloromethane; 2:98) afford .639g (34%) of pure dark yellow solid, 4. <sup>1</sup>H NMR (CDCl<sub>3</sub>): 2.75 (t, J=9Hz, 4H), 3.57 (s, 4H), 3.70 (t, J=6Hz, 4H), 4.55 (s, 4H), 7.49-7.62 (m, 4H), 8.20 (d, J=9Hz, 2H), 8.30 (d, J=9Hz, 2H), 8.37 (d, J=9Hz, 2H).

1,2-Bis(2-(4-(9-phenoxyacridinyl) methylthio) ethoxy) ethane, 5:

To a solution of 4 (1.15g, 1.82mmol), in melted phenol (31ml), finely powdered potassium hydroxide (.408g, 3.82mmol) was combined. The mixture was stirred at 110°C for 1 1/2 h. The hot phenol was transferred into 1 M NaOH (20ml), and the basic solution was extracted with chloroform (2 60ml) to obtain the organic layer, which was dried (MgSO<sub>4</sub>), filtered, and concentrated. Purification by flash chromatography (ether:dichloromethane;2:98) afforded .8886g (65%) of dark yellow, 5. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 2.80(t, J=9Hz, 4H), 3.61(s, 4H), 3.74(t, J=6Hz, 4H), 4.59(s, 4H), 6.82(d, J=6Hz, 4H), 7.02(t, J=6Hz, 4H), 7.24(t, J=6Hz, 4H), 7.32-7.44(m, 4H), 7.70-7.75(m, 4H), 7.98(d, J=9Hz, 2H), 8.03(d, J=9Hz, 2H), 8.26(d, J=9Hz, 2H).

2,6,11,15-tetraimino-25,28-dioxa-22,31-dithio-45,46-diaza-tricyclo[38,8,4<sup>16,20</sup>] tetrapentacontane-36,35,50,47,39,37,17,19,51,53,42,44-dodecaene, 6:

5 (55mg, .073mmol), phenol (13ml), and spermine (26mg, 0.074mmol) were added in consecutive order. The solution was maintained at 100°C for the first hour and 130°C for the second hour. The reaction was allowed to cool to room temperature, added to 4M KOH (120ml) and extracted with isopropanol:dichloromethane (1:9; 4 80ml). The organic layer was dried (MgSO<sub>4</sub>), filtered, and concentrated in vacuo. The crude solid was filtered through a pad of silica with acetone followed by NH<sub>3</sub>-CH<sub>3</sub>OH/CH<sub>2</sub>Cl<sub>2</sub> (1:9) to yield 1% of pure 6. <sup>1</sup>H NMR (CDCl<sub>3</sub>): 1.77(s, 4H), 2.08(s, 4H), 2.59(t, J=Hz, 4H), 2.75(s, 4H), 2.97(t, J=3Hz, 4H), 3.68(s, 4H), 3.72(t, J=6Hz, 4H), 3.98(t, J=4Hz, 4H), 4.31(s, 4H), 7.01(t, J=9Hz, 2H), 7.18(t, J=9Hz, 2H), 7.38(d, J=6Hz, 2H), 7.48(t, J=9Hz, 2H), 7.80(d, J=9Hz, 2H), 8.00(d, J=9Hz, 2H), 8.12(d, J=9Hz, 2H).

Trimethoxycarbonyl spermidine, 7:

To a biphasic solution of 1M NaOH (10ml) and methylchloroformate (1.18ml, 15.27mmol) in chloroform (10ml), spermidine (0.200g, 1.53mmol) was added. After 2 1/2 hr. of stirring, the organic layer was extracted with additional chloroform (15ml), dried ( $MgSO_4$ ), and concentrated in vacuo. Purification by flash chromatography (methanol:dichloromethane; 3:97) afforded .3721g (76%) of a clear oil, 7.  $^1H$  NMR ( $CDCl_3$ ): 1.51(m, 4H), 1.71(m, 2H), 3.15-3.31(m, 8H), 3.69(s, 9H).

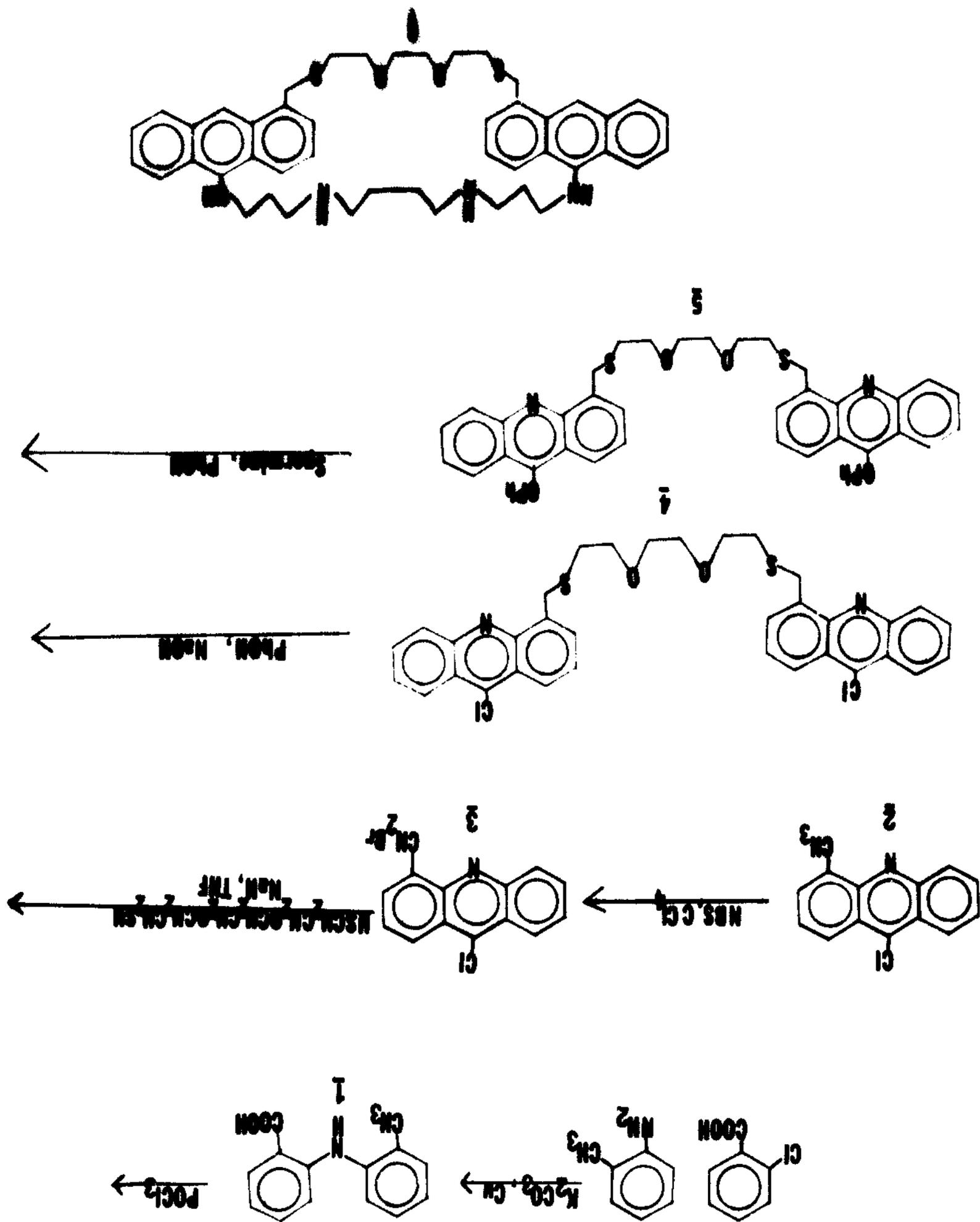
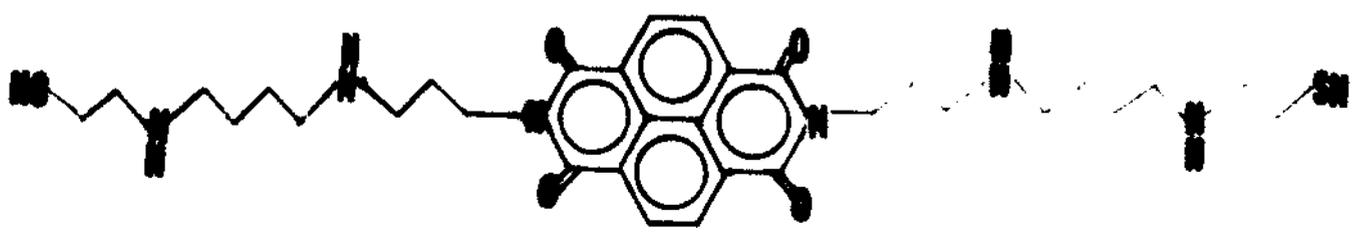
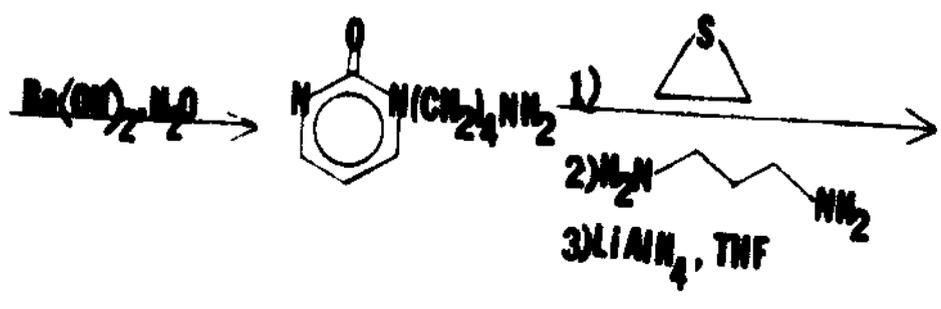
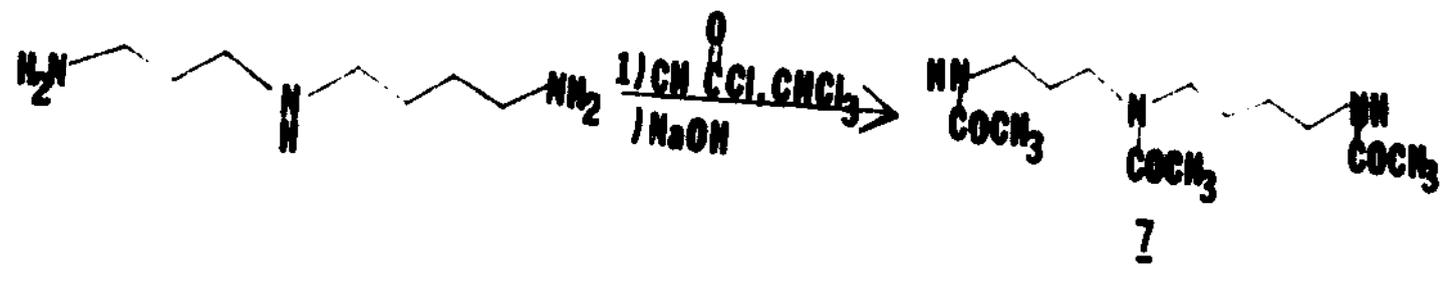


Fig 4



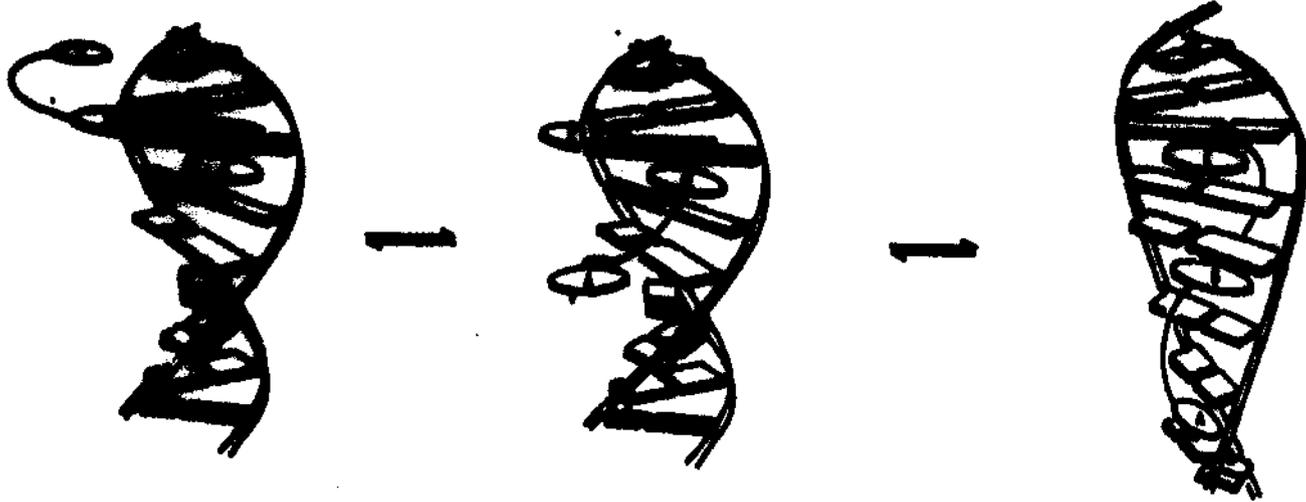
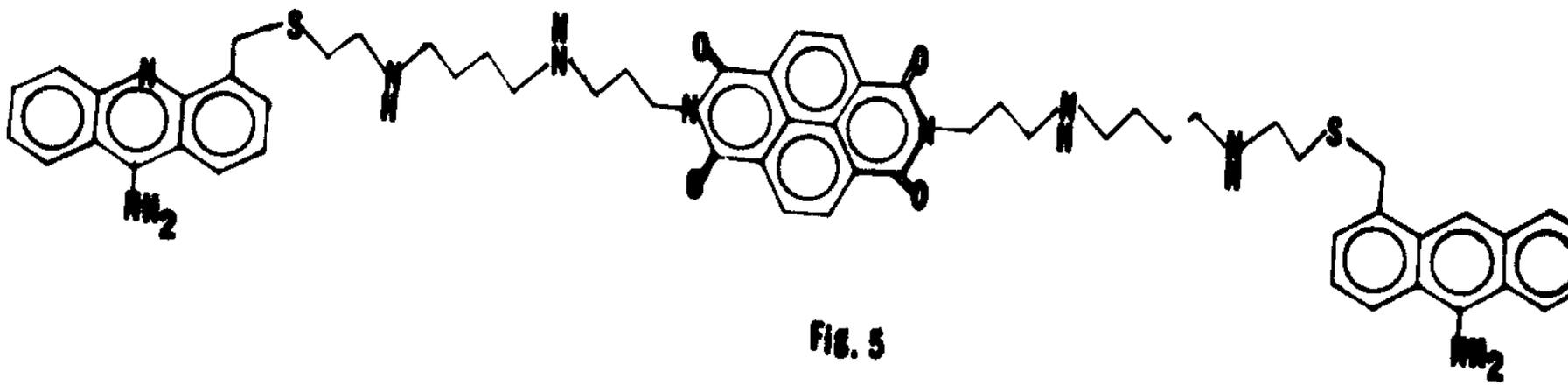
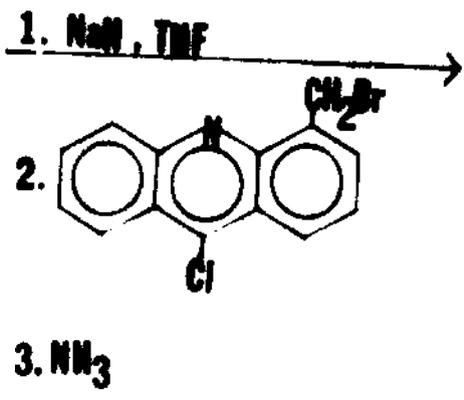


Fig 3

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