Synthesis and DNA binding of 6-(alkylamino)indolo[1,2-b][2,7]naphthyridine-5,12-quinones

Deborah A. Davis, a Michael Cory, b Terri A. Fairley, b and Gordon W. Gribble*a

a Department of Chemistry, Dartmouth College, Hanover, NH 03755, USA
b Division of Organic Chemistry, Burroughs Wellcome Company, Research Triangle Park, North Carolina 27709

Email: ggribble@dartmouth.edu

This paper is to celebrate George A. Kraus for his many outstanding contributions to organic chemistry

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Abstract

We describe the synthesis of eight novel putative mono– and bis–DNA intercalators from a common precursor, 6-bromoindolo[1,2-b][2,7]naphthyridine-5,12-dione. Of these new indoloquinones, our data indicate that two are most likely DNA mono–intercalators, but weaker than ethidium bromide, and two others are DNA bis–intercalators. Our indoloquinones are inactive against mammalian topoisomerase II.

Keywords: Indole, DNA intercalation, amination, 6-bromoindolo[1,2-b][2,7]naphthyridine-5,12-dione, indoloquinone
Introduction

The antitumor activity and DNA affinity of amino-substituted quinones is well established, and several drugs in this category have seen utility in the cancer clinic; for example, mitomycin (1), ametantrone (2), mitoxantrone (3), pixantrone (4), and WEHI-150 (5) (Figure 1).

![Molecules](attachment:image)

**Figure 1**

We have previously synthesized and utilized the indolo[1,2-b][2,7]naphthyridine-5,12-dione (6) ring system to forge several members of the 6H-pyrido[4,3-b]carbazole family of antitumor alkaloids, including ellipticine (7), 9-methoxyellipticine (8), olivacine (9), 13-oxoellipticine (10), and ellipticine quinone (12) (Figure 2). A variation of our method allowed for the synthesis of 10H-pyrido[2,3-b]carbazoles (13) and 6,11-disubstituted-benzo[b]carbazoles (14). The shape similarity of quinone (6) with that of ellipticine quinone (12) and calothrixin B (15), both of which display antitumor activity, suggested that it would be fruitful to examine indoloquinone 6 and its amino-substituted derivatives for DNA binding, given the known enhancement of DNA binding and resulting biological activity imparted by the alkylamino side chains in antitumor quinones 2–5.
Figure 2

Fatefully, during attempts to isomerize indoloquinone 6 to ellipticine quinone (12) with various nucleophiles, we discovered the propensity for 6 to undergo oxidative dimerization at C-6 to give 16\textsuperscript{26} (Scheme 1).

Scheme 1

Nuc:\textsuperscript{−} = NaCN, KF, NaCl, Mg(OMe)\textsubscript{2}, NaSPh
Solvent = THF, DMF, DMSO, HMPA
This observation fortuitously provided a convenient synthesis of the C-6 alkylamino derivatives 17a-d and the bis-compounds 18a-d, which we describe herein. Furthermore, it was conceivable that compounds of type 17 (or 18) might irreversibly acylate DNA, given the tendency of N-acylindoles to undergo facile nucleophilic cleavage of the nitrogen-carbonyl bond\(^{27,28}\) (i.e., 17 $\rightarrow$ 19) (Figure 3).

![Figure 3](image_url)

**Results and Discussion**

The targeted 6-(alkylamino)indolo[1,2-b][2,7]naphthyridine-5,12-diones (17a-d) and bis[6-aminoidolo[1,2-b][2,7]naphthyridine-5,12-diones (18a-d) were synthesized from 1-(phenylsulfonyl)indole (20) as shown in Scheme 2. The synthesis of bromo indoloquinone (24) parallels our earlier synthesis of indoloquinone 6.\(^{15}\) The known 3-bromo-1-(phenylsulfonyl)indole (21), prepared quantitatively from 20 and bromine in methylene chloride, was treated with lithium di-isopropyl amide (LDA) at $-78^\circ C$, stirred at that temperature for one hour, and quenched with 3,4-pyridinedicarboxylic anhydride at $-100^\circ C$ to give the protected keto-acid (22) in 70% yield, after recrystallization from acetone to remove the minor regioisomer (< 2%). The phenylsulfonyl group was removed with K2CO3 in aqueous MeOH-water (84%), and the resulting deprotected keto acid (23) was cyclized to indoloquinone 24 in hot acetic anhydride (79%).

As noted earlier, our motivation to explore the reaction of 24 with various amines, and examine their DNA affinity, was that the aminoanthraquinones ametantrone (2) and mitoxantrone (3) show strong DNA affinity and good antineoplastic activity in clinical trials.\(^7\) In actuality, treatment of 24 with an excess of dimethylamine (25% aqueous solution) in THF at room temperature provided 5-dimethylaminoidolo[1,2-b][2,7]naphthyridine-6,11-dione (17a) cleanly in high yield. Similarly, compounds 17b and 17c were synthesized by allowing solutions of 24 in THF to react with excess N,N,N’-trimethylethylenediamine and 3-diethylaminopropylamine, respectively. These reactions doubtlessly proceed by a typical nucleophilic addition-elimination mechanism.\(^{29}\)
Interestingly, the reaction of 24 with hot dimethylformamide (DMF) produced 5-(methylamino)indolo[1,2-b][2,7]naphthyridine-6,11-dione (17d) in 86% yield (Scheme 2). This peculiar reaction was discovered during attempts to displace bromide from 24 with 2-amino-2-methyl-1,3-propanediol in hot DMF, since milder conditions (THF, rt) were unproductive. To confirm that the methylamino group had come from DMF rather than via the decomposition of 2-amino-2-methyl-1,3-propanediol, a solution of 24 was heated in DMF for 24 hours; once, again, there was a clean conversion to 17d. This phenomenon had been observed twenty years earlier by Lord and Peters, who observed that heating 1-chloroanthraquinone for 32 hours in DMF at reflux afforded a mixture of 1-dimethylaminoanthraquinone (25) and 1-methylaminoanthraquinone (26). Moreover, 25 was demethylated to 26 by additional heating in DMF for 73 hours. In contrast, 2-chloroanthraquinone gave only 2-dimethylaminoanthraquinone in refluxing DMF. These observations are wholly consistent with a demethylation mechanism involving assistance by the neighboring carbonyl group as shown for the formation of 17d from 24 (Scheme 3).
Our motivation for preparing the bis-indoloquinones \textbf{18a-d} was to attain novel DNA bis-intercalators, and we were encouraged by the results from our previous studies with bis-acridines.\textsuperscript{32} Indeed, as we found with bis-acridines, adjustment of the length and flexibility of the diamino-linkage (tether) can dramatically alter the DNA-binding of these compounds. Therefore, we prepared bis-indoloquinones \textbf{18a-d} using a method similar to the method used to synthesize compounds \textbf{17a} (Scheme 4). The reactions were run in the presence of excess \textbf{24} so as to encourage the formation of the bis-indoloquinones. Nonetheless, some mono-indoloquinones were still present in the reaction mixture after 24 hours of stirring at room temperature.

\textbf{Scheme 3}
The bromine in indoloquinone 24 is also susceptible to displacement by other nucleophiles (Scheme 5). Thus, treatment of 24 with (n-Bu)_4NCl in DMF gave an orange compound that was identical to an authentic sample of 6-chloroindolo[1,2-b][2,7]naphthyridine-5,12-dione (27). Similarly, 24 was treated with sodium methoxide in methanol to give a product identical to an authentic sample of 6-methoxyindolo[1,2-b][2,7]naphthyridine-5,12-dione (28). Such behavior is not unexpected as ß-haloenones are known to act as Michael acceptors for halogens, amines, alkoxides, and thiolates as well as carbon-based nucleophiles.

Scheme 5
Our results of DNA affinity studies for 17a-d and 18a-d are summarized in Table 1. The change in DNA melting, $\Delta T_m$, is the change in the thermal denaturation of DNA by a drug as a result of intercalation. From these results, it can be seen that compounds 17b and 17c are intercalators, although less effective when compared to a powerful intercalator such as the aminoacridine, quinacrine, which has a $\Delta T_m$ value of 24.9. The other experiment was to determine the slope of the calf thymus viscometric titration. When a drug intercalates into DNA, the DNA helix lengthens. Thus, the line of concern here is the ratio of the increase in DNA contour length versus the drug/nucleotide ratio. Unfortunately, in further tests, neither of these compounds exhibited any activity against mammalian topoisomerase II, which are enzymes that cause double-strand breaks in DNA, cause the DNA to unwind, and reseal the breaks. They are believed to be necessary for replication, since the helical winding of the DNA must be released to allow strand splitting in replication. Thus, it is currently believed that topoisomerase activity is crucial for useful anticancer activity. We find that 17a-d have no activity against mammalian topoisomerase II at a drug concentration of 100 µM.

Table 1. DNA affinity results for 17a–d and 18 b,c

<table>
<thead>
<tr>
<th>Compound</th>
<th>CT DNA$^a$</th>
<th>$\Delta T_m$ °C</th>
<th>Poly AT$^b$</th>
<th>Poly GC$^c$</th>
<th>Slope of Calf Thymus viscosity titration</th>
</tr>
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<tbody>
<tr>
<td>17a</td>
<td>0.3 ± 0.2</td>
<td>7.6 ± 0.03</td>
<td>3.7 ± 0.65</td>
<td>1.7 ± 0.2</td>
<td></td>
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<tr>
<td>17b</td>
<td>8.7 ± 0.05</td>
<td>7.2 ± 1.0</td>
<td>4.5 ± 1.8</td>
<td>0.5 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>17c</td>
<td>8.5 ± 0.02</td>
<td>7.0 ± 0.1</td>
<td></td>
<td>0.5 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>17d</td>
<td>0.56 ± 0.41</td>
<td>7.4 ± 1.4</td>
<td></td>
<td>0.5 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>18b</td>
<td>21.7 ± 7.4</td>
<td>9.9 ± 0.3</td>
<td></td>
<td>0.5 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>18c</td>
<td>7.8 ± 6.8</td>
<td>6.0 ± 0.8</td>
<td></td>
<td>0.5 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>–0.4 ± 0.2</td>
<td>9.9 ± 0.4</td>
<td></td>
<td>0.5 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Ethidium bromide</td>
<td>11.9 ± 1.1</td>
<td>9.9 ± 0.3</td>
<td>5.3 ± 0.7</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

$^a$CT is sonicated calf thymus DNA.
$^b$Poly AT is sonicated poly(dA)•poly(dT) homopolymer.
$^c$Poly GC is sonicated poly(dG-dC) with alternating GCGCGC on both strands.

Conclusions

We have synthesized eight novel indoloquinones, 17a-d and 18a-d for DNA binding studies. Of these, 17a and 17d do not bind to DNA as evidenced by the calf thymus DNA assay, suggesting that the pyridine ring system alone is insufficiently basic to become protonated in the presence of the polyphosphates of DNA. Moreover, as might be expected, the exocyclic amino group is rendered less basic by the C-5 carbonyl group (vinyllogous amide). In contrast, our data indicate that 17b and 17c are probably mono-intercalators. These two quinones show a binding profile similar to other DNA intercalators, and they exhibit a GC preference. By comparison to ethidium bromide, these two compounds are somewhat weaker intercalators. In contrast, indoloquinones 18b and 18c are likely bis-intercalators, since the high CT for 18b and its biphasic nature suggests a double intercalator. The lesser DNA affinity of 18c is probably imparted by the less electronegative oxygen atoms in the tether. Our eight new compounds, 17a-d and 18a-d, will serve in future biological studies.
Experimental Section

General. Melting points were determined on a Büchi 510 apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Perkin-Elmer 599 spectrometer and are referenced to the 1601 cm\(^{-1}\) band of polystyrene. \(^1\)H NMR (300 MHz) and \(^{13}\)C NMR (75 MHz) were recorded on a Varian XL-300 multinuclear Fourier transform spectrometer. Unitary resolution mass spectra (MS) were obtained on a Finnigan 4023 GC/MS system. High resolution mass spectra (HRMS) were recorded at the National Institutes of Health regional facility at the Massachusetts Institute of Technology. Ultraviolet (UV) spectra were recorded on a Hewlett Packard 8451A diode array spectrophotometer. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA. Analytical thin-layer chromatography (TLC) was performed on precoated Silica Gel 60 F\(_{254}\) plates from EM Reagents. Visualization was accomplished with 254 and 365 nm UV light, iodine vapor, ceric ammonium sulfate spray (3% in 10% sulfuric acid) or “van Urk’s reagent” spray (p-dimethylaminobenzaldehyde in ethanolic sulfuric acid). Flash chromatography was performed with EM Reagents Silica Gel 60 (230-400 mesh). All reactions were performed under a static head of predried (CaSO\(_4\) tower) nitrogen or argon in glassware that had been dried for at least 1 h at 135 °C. Benzenesulfonyl chloride and 3,4-pyridinedicarboxylic anhydride were distilled prior to use.

3-Bromo-1-(phenylsulfonyl)indole (21). To a stirred solution of 1-(phenylsulfonyl)indole\(^{36}\) (20) (20.0 g, 77.8 mmol) in CH\(_2\)Cl\(_2\) (125 mL) was added bromine (4.5 mL, 86 mmol) in CH\(_2\)Cl\(_2\) (100 mL) dropwise with stirring over 1 h at rt, during which time HBr gas was evolved. After stirring for an additional 2 h, saturated aqueous sodium thiosulfate solution (250 mL) was added and the biphasic mixture was stirred for 15 min. The layers were separated and the aqueous phase was extracted with CH\(_2\)Cl\(_2\) (3 x 50 mL). The combined organic phase was washed with saturated aqueous NaHCO\(_3\) (2 x 200 mL), H\(_2\)O (2 x 200 mL), brine (200 mL), dried (Na\(_2\)SO\(_4\)), and concentrated in vacuo to yield 26.1 g (100%) of 21 as a white solid: mp 234–236 °C (lit., mp 119–120 °C); IR (CHCl\(_3\)) 1605, 1585, 1445, 1370, 1265 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 7.15–8.15 (m, 9H), 7.63 (s, 1H); \(^{13}\)C NMR (CDCl\(_3\)) \(\delta\) 137.7, 134.2, 134.1, 129.4, 129.3, 126.8, 125.8, 124.7, 123.9, 120.0, 113.5, 99.8.

3-Bromo-1-(phenylsulfonyl)indol-2-yl 3-carboxy-4-pyridyl ketone (22). To a solution of lithium diisopropylamide (7.14 mmol) prepared from diisopropylamine (1.00 mL, 7.14 mmol) and n-butyllithium (1.22 M in hexane; 6.00 mL, 7.32 mmol) in dry THF (40 mL) under nitrogen at −78 °C was added dropwise with stirring over 20 min a solution of 21 (2.00 g, 5.95 mmol) in dry THF (40 mL). The mixture was stirred at −78 °C for 1 h then cooled to −100 °C and treated as rapidly as possible with a solution of 3,4-pyridinedicarboxylic anhydride (1.21 g, 8.11 mmol) in dry THF (40 mL) while maintaining efficient cooling and stirring. The mixture was allowed to warm slowly to rt with stirring over 18 h and then concentrated in vacuo. The resulting brown viscous oil was dissolved in H\(_2\)O (250 mL), cooled to 0 °C, and slowly acidified with dilute HCl. The resulting white precipitate was collected and dried in vacuo to give 2.53 g (88%) of crude product. Recrystallization from acetone yielded 2.01 g (70%) of 22 as a white powder: mp 236–238 °C (dec); IR (KBr) 1715, 1675, 1450, 1365, 1260, 1175, 1070, 950, 860, 755, 735, 675 cm\(^{-1}\); \(^1\)H NMR (DMSO-\(d_6\)) \(\delta\) 9.07 (s, 1H), 8.97 (d, 1H, J 4.8 Hz), 8.14 (d, 1H, J 8.0 Hz), 7.98 (d, 1H, J 8.0 Hz), 7.79–7.56 (m, 7H), 7.50 (m, 1H); \(^{13}\)C NMR (DMSO-\(d_6\)) \(\delta\) 184.7, 166.5, 153.0, 150.1, 144.9, 136.6, 136.4, 135.1, 133.5, 129.72, 129.67, 128.6, 127.1, 126.7, 125.7, 123.0, 121.8, 115.7, 110.9; MS \(m/e\) 486 (M\(^+\)+2), 484 (M\(^+\)), 328, 326, 264, 220, 164, 141, 77 (100%). Anal. Calcd for C\(_{23}\)H\(_{13}\)BrN\(_2\)O\(_5\)S + C\(_3\)H\(_2\)O: C, 53.05; H, 3.52; Br, 14.71; N, 5.15; S, 5.90. Found: C, 52.86; H, 3.30; Br, 14.95; N, 5.25; S, 6.05.

3-Bromoindol-2-yl 3-Carboxy-4-pyridyl ketone (23). A magnetically stirred solution of keto acid 22 (1.00 g, 2.06 mmol), K\(_2\)CO\(_3\) (1.2 g, 8.7 mmol), H\(_2\)O (8 mL), and MeOH (25 mL) was heated under reflux for 45 min. The mixture was cooled and the MeOH was removed in vacuo. The dark, oily residue was dissolved in H\(_2\)O (100
mL), cooled to 0 °C, and slowly acidified with dilute HCl with stirring. The yellow precipitate was collected by filtration and dried in vacuo to yield 0.49 g of 23. An additional 0.11 g of product was obtained by continuous extraction of the filtrate with CH₂Cl₂ to give a total of 0.60 g (84%) of 23: mp 194–195 °C; IR (KBr) 1710, 1625, 1505, 1335, 1260, 1230, 740 cm⁻¹; ¹H NMR (DMSO-d₆) δ 12.38 (s, 1H), 9.22 (s, 1H), 8.94 (d, J 4.3 Hz, 1H), 7.61 (d, J 4.3 Hz, 1H), 7.50 (m, 2H), 7.41 (m, 1H), 7.20 (m, 1H); ¹³C NMR (DMSO-d₆) δ 183.0, 165.6, 153.6, 151.8, 150.9, 148.6, 136.5, 131.0, 127.1, 124.6, 123.0, 121.6, 121.5, 120.6, 113.2. A satisfactory analysis could not be obtained for this product so it was used directly in the next step.

6-Bromoindolo[1,2-b][2,7]naphthyridine-5,12-dione (24). A solution of 23 (0.60 g, 1.7 mmol) in Ac₂O (25 mL) was heated at 80 °C for 4 h. After cooling, most of the solvent was removed in vacuo, then H₂O (50 mL) and CH₂Cl₂ (50 mL) were added. The layers were separated and the aqueous phase was further extracted with CH₂Cl₂ (2 x 50 mL). The combined organic phase was washed with H₂O (2 x 100 mL) and brine (100 mL), dried (Na₂SO₄), and adsorbed onto silica gel. Flash chromatography with EtOAc gave 0.45 g (79%) of 24 as a yellow-green powder. Recrystallization from CH₂Cl₂/hexane gave the analytical sample as fine yellow needles: mp 230–232 °C (dec); IR (CHCl₃) 2980, 2920, 1705, 1680, 1600, 1535, 1450, 1365, 1335, 1255, 1220 cm⁻¹; ¹H NMR (DMSO-d₆) δ 9.35 (s, 1H), 9.16 (d, J 4.7 Hz, 1H), 8.54 (d, J 7.9 Hz, 1H), 8.18 (d, J 4.7 Hz, 1H), 7.75 (m, 2H), 7.56 (m, 1H); ¹³C NMR (DMSO-d₆) δ 174.1, 155.4, 150.0, 147.9, 134.7, 131.4, 131.2, 126.2, 126.0, 122.0, 120.8, 119.0, 118.3, 116.6, 108.7; MS m/e 328 (M⁺, 100%), 326 (M⁺, 100%), 300, 298, 272, 270, 247, 219, 191, 164, 114. Anal. Calcd for C₁₅H₇BrN₂O₂: C, 55.07; H, 2.16; Br, 24.43; N, 8.56; O, 9.78. Found: C, 54.82; H, 2.18; Br, 24.53; N, 8.49; O, 9.88.

6-Dimethylaminindo[1,2-b][2,7]naphthyridine-5,12-dione (17a). To a solution of 24 (100 mg, 0.31 mmol) in THF (25 mL) was added excess Me₂NH (25% aqueous solution, 20 mL). The mixture was stirred at rt for 6 h, during which time the solution became bright orange. The solution was poured into EtOAc (100 mL) and washed with saturated aqueous NaHCO₃ (2 x 50 mL), H₂O (2 x 50 mL) and brine (50 mL), then dried (Na₂SO₄) and concentrated to give an orange powder. Recrystallization from THF-hexane gave 73 mg (82%) of 17a as small orange needles: mp 240–242 °C; IR (CHCl₃) 3050, 1680, 1575, 1545, 1495, 1410, 1370 cm⁻¹; ¹H NMR (CDCl₃) δ 9.62 (s, 1H), 8.98 (d, 1H, J 4.0 Hz), 8.86 (d, 1H, J 9.6 Hz), 8.06 (d, 1H, J 4.0 Hz), 8.00 (d, 1H, J 9.6 Hz), 7.62 (m, 1H), 7.33 (m, 1H), 3.49 (s, 6H); MS m/e 291 (M⁺), 276 (100%), 262, 249, 233, 220, 192, 164, 102, 77, 44; Anal. Calcd for C₁₇H₁₃N₂O₂: C, 70.08; H, 4.50; N, 14.42. Found: C, 69.87; H, 4.39; N, 14.41.

6-[(N-[2-(Dimethylamino)ethyl]methylamino)indolo[1,2-b][2,7]naphthyridine-5,12-dione (17b). To a solution of 24 (65 mg, 0.20 mmol) in THF (5 mL) was added excess N,N'-trimethylethlenediamine (0.10 mL, 0.78 mmol). The mixture was refluxed for 1 h, during which time the solution became dark orange. The solution was cooled and poured into EtOAc (50 mL) and washed with saturated aqueous NaHCO₃ (2 x 25 mL), H₂O (2 x 25 mL) and brine (25 mL), then dried (Na₂SO₄) and concentrated to give a red powder. Recrystallization from CH₂Cl₂-hexane gave 57 mg (83%) of 17b as short red needles: mp 129–131 °C; IR (CHCl₃) 2950, 2800, 1675, 1630, 1570, 1530, 1495, 1460, 1410, 1370, 1330 cm⁻¹; ¹H NMR (CDCl₃) δ 9.62 (s, 1H), 8.99 (d, 1H, J 5.1 Hz), 8.87 (d, 1H, J 8.1 Hz), 8.08 (d, 1H, J 5.1 Hz), 8.01 (d, 1H, J 8.1 Hz), 7.64 (m, 1H), 7.35 (m, 1H), 3.95 (t, 2H), 3.50 (s, 3H), 2.68 (t, 2H), 2.22 (s, 6H); ¹³C NMR (CDCl₃) δ 174.7, 168.0, 157.9, 153.9, 151.0, 150.9, 141.4, 137.5, 131.6, 124.9, 124.5, 124.3, 124.0, 118.5, 118.3, 57.6, 54.8, 45.7, 43.1; MS m/e 348 (M⁺), 303, 290, 277, 262, 248, 233, 80, 58 (100%); Anal. Calcd for C₂₀H₂₀N₂O₄ + 0.5 H₂O: C, 67.21; H, 5.92; N, 15.67. Found: C, 67.78; H, 5.76; N, 15.23.

6-[3-(Diethylamino)propylamino]indolo[1,2-b][2,7]naphthyridine-5,12-dione (17c). To a solution of 24 (56 mg, 0.17 mmol) in THF (5 mL) was added excess 3-diethylaminopropylamine (0.10 mL, 0.63 mmol). The mixture was refluxed for 1 h, during which time the solution became dark orange. The solution was cooled and poured into EtOAc (50 mL) and washed with saturated aqueous NaHCO₃ (2 x 25 mL), H₂O (2 x 25 mL) and brine...
(25 mL), and then dried (Na₂SO₄) and concentrated to give an orange semi-solid. Recrystallization from CH₂Cl₂-hexane gave 42 mg (66%) of 17c as an orange powder: mp 102–103 °C; IR (CHCl₃) 3450, 2980, 2940, 2820, 1675, 1635, 1575, 1550, 1450, 1350 cm⁻¹; ¹H NMR (CDCl₃) δ 9.58 (s, 1H), 8.95 (d, 1H, J 4.3 Hz), 8.68 (d, 1H, J 8.6 Hz), 8.02 (d, 1H, J 4.3 Hz), 7.96 (d, 1H, J 8.6 Hz), 7.62 (m, 1H), 7.29 (m, 1H), 3.96 (m, 1H), 3.90 (m, 2H), 2.63–2.46 (m, 6H), 1.34–0.87 (m, 8H); ¹³C NMR (CDCl₃) δ 172.6, 168.4, 156.6, 153.5, 153.0, 151.2, 141.2, 138.4, 132.7, 124.9, 124.7, 124.6, 120.5, 118.0, 117.6, 50.0, 46.9, 44.8, 28.0, 11.6; MS m/e 376 (M⁺), 290, 263, 248, 100, 86 (100%), 72, 58. Anal. Calcd for C_{22}H_{24}N₄O₂: O.31 mmol) in DMF (20 mL) was heated at 130 °C for 24 h. The dark orange red solution was cooled and poured into saturated aqueous NaHCO₃ (50 mL) and extracted with CH₂Cl₂ (3 x 25 mL). The combined organic phase was washed with H₂O (2 x 50 mL) and brine, dried (Na₂SO₄), concentrated in vacuo, and dried for 12 h at 60 °C in vacuo to give a brick-red solid. Recrystallization from CH₂Cl₂-hexane gave 0.073 g (86%) of 17d as a dark red powder: mp 278–280 °C; IR (CHCl₃) 3400, 2930, 2860, 1720, 1675, 1635, 1535, 1450, 1360, 1330 cm⁻¹; ¹H NMR (CDCl₃) δ 9.52 (s, 1H), 8.99 (d, 1H, J 4.5 Hz), 8.74 (d, 1H, J 8.7 Hz), 8.07 (d, 1H, J 4.5 Hz), 8.01 (d, 1H, J 8.7 Hz), 7.68 (m, 1H), 7.35 (m, 1H), 3.70–3.45 (bs, 1H), 3.56 (s, 3H); MS m/e 277 (M⁺, 100%), 248, 220, 192, 164, 104, 91, 77. Anal. Calcd for C_{16}H_{12}N₂O₂: O.25 H₂O: C, 68.20; H, 4.11; N, 14.91. Found: C, 68.47; H, 3.97; N, 14.60.

6-Methylaminindolo[1,2-b][2,7]naphthyridine-5,12-dione (17d). A solution of 24 (0.102 g, 0.31 mmol) in DMF (20 mL) was heated at 130 °C for 24 h. The dark orange red solution was cooled and poured into saturated aqueous NaHCO₃ (50 mL) and extracted with CH₂Cl₂ (3 x 25 mL). The combined organic phase was washed with H₂O (2 x 50 mL) and brine, dried (Na₂SO₄), concentrated in vacuo, and dried for 12 h at 60 °C in vacuo to give a brick-red solid. Recrystallization from CH₂Cl₂-hexane gave 0.073 g (86%) of 17d as a dark red powder: mp 278–280 °C; IR (CHCl₃) 3400, 2930, 2860, 1720, 1675, 1635, 1535, 1450, 1360, 1330 cm⁻¹; ¹H NMR (CDCl₃) δ 9.52 (s, 1H), 8.99 (d, 1H, J 4.5 Hz), 8.74 (d, 1H, J 8.7 Hz), 8.07 (d, 1H, J 4.5 Hz), 8.01 (d, 1H, J 8.7 Hz), 7.68 (m, 1H), 7.35 (m, 1H), 3.70–3.45 (bs, 1H), 3.56 (s, 3H); MS m/e 277 (M⁺, 100%), 248, 220, 192, 164, 104, 91, 77. Anal. Calcd for C_{16}H_{12}N₂O₂: O.25 H₂O: C, 68.20; H, 4.11; N, 14.91. Found: C, 68.47; H, 3.97; N, 14.60.

6-Chlorindolo[1,2-b][2,7]naphthyridine-5,12-dione (27). To a solution of 24 (50 mg, 0.15 mmol) in DMF (5 mL) was added (n-Bu)₄NCl (50 mg, 0.18 mmol). The mixture was stirred at rt for 12 h and then poured into H₂O (25 mL). The precipitate was collected by filtration, dried and recrystallized from CH₂Cl₂-hexane to yield 29 mg (71%) of 27 that was identical with an authentic sample by TLC, mp, and MS: mp 220–221 °C (lit. mp 219–220 °C); MS m/e 282 (M⁺, 100%), 254, 247, 226, 219, 191, 164, 114.

6-Methoxyindolo[1,2-b][2,7]naphthyridine-5,12-dione (28). A solution of 24 (0.0579 g, 0.177 mmol) in 2:1 THF-MeOH (10 mL) was added dropwise to a solution of NaOMe (from 0.012 g, 0.52 mmol, of Na) in MeOH (10 mL) at 0 °C. The mixture was allowed to warm to rt over 2 h and then poured in H₂O (50 mL) and extracted with EtOAc (3 x 25 mL). The combined organic phase was washed with H₂O (2 x 50 mL) and brine (50 mL), then dried (Na₂SO₄) and concentrated in vacuo to give an orange solid. Recrystallization from CH₂Cl₂-hexane gave 0.0321 g (65%) of 28 that was identical with an authentic sample by TLC and mixed melting point: mp 212–215 °C (lit. mp 214–219 °C); MS m/e 278 (M⁺, 100%), 199, 183, 152, 129, 77, 57.

N,N'-Octylenebis[6-aminindolo[1,2-b][2,7]naphthyridine-5,12-dione] (18a). To a solution of 24 (0.50 g, 1.5 mmol) in THF (25 mL) was added 1,8-diamino-octane (0.10 g, 0.70 mmol). The mixture was refluxed for 6 h, then cooled, poured into saturated aqueous sodium bicarbonate solution (100 mL) and extracted with CH₂Cl₂ (3 x 50 mL). The combined organic phase was washed with saturated aqueous sodium bicarbonate solution (50 mL), water (50 mL), and saturated aqueous sodium chloride solution (50 mL), then dried (Na₂SO₄) and adsorbed onto silica gel. Elution first with EtOAc, then acetone, then 16:3:1 acetonene-MeOH-TEA yielded 0.27 g (48%) of the di-HBr salt of 18a as an orange powder: mp 140–145 °C (dec); IR (CHCl₃) 3460, 3020, 2940, 2880, 1700, 1680, 1630, 1575, 1545, 1455, 1360 cm⁻¹; ¹H NMR (DMSO-d₆) δ 11.93 (bs), 9.38–8.52 (m, 6H), 8.03–7.04 (m, 8H), 3.02 (m, 4H), 1.64–0.80 (m, 12H): Anal. Calcd for C_{38}H_{22}N_{3}O_{4} + 2HBr: C, 57.16; H, 4.29; N, 10.52. Found: C, 58.69; H, 4.67; N, 10.57.

N,N'-[N-Methyl-4-aza-heptylene]bis[6-aminindolo[1,2-b][2,7]naphthyridine-5,12-dione] (18b). To a solution of 24 (0.50 g, 1.5 mmol) in THF (25 mL) was added 3,3'-diamino-N-methylidipropylamine (0.10 g, 0.70 mmol). The mixture was refluxed for 6 h, then cooled, poured into saturated aqueous sodium bicarbonate solution (100 mL) and extracted with CH₂Cl₂ (3 x 50 mL). The combined organic phase was washed with saturated aqueous sodium bicarbonate solution (50 mL), water (50 mL), and saturated aqueous sodium...
chloride solution (50 mL), then dried (Na₂SO₄) and adsorbed onto silica gel. Elution first with EtOAc then acetone then 16:3:1 acetone-MeOH-TEA yielded 0.19 g (43%) of 18b as an orange-red powder: mp 170–175 °C (dec); ¹H NMR (CDCl₃) δ 9.41 (s, 2H), 8.85 (m, 2H), 8.44 (d, 2H, J 8.4 Hz), 7.85 (d, 2H, J 5.5 Hz), 7.52 (d, 2H, J 8.2 Hz), 7.01 (m, 2H), 6.69 (m, 2H), 3.95 (m, 4H), 2.80 (m, 4H), 2.48 (s, 3H), 2.07 (m, 4H); MS m/e 638 (M⁺). Anal. Calcd for C₃₇H₃₃N₂O₄ + 2H₂O: C, 65.97; H, 5.13; N, 11.55. Found: C, 66.16; H, 5.13; N, 11.83.

N,N’-(4,9-Dioxo-dodecylene)bis[6-aminindo[1,2-b][2,7]naphthyridine-5,12-dione] (18c). To a solution of 24 (0.50 g, 1.5 mmol) in THF (25 mL) was added 4,9-dioxo-1,12-dodecanediamine (0.15 mL, 0.70 mmol). The mixture was refluxed for 6 h, then cooled, poured into saturated aqueous sodium bicarbonate solution (100 mL) and extracted with CH₂Cl₂ (3 x 50 mL). The combined organic phase was washed with saturated aqueous sodium bicarbonate solution (50 mL), water (50 mL), and saturated aqueous sodium chloride solution (50 mL), then dried (Na₂SO₄) and adsorbed onto silica gel. Elution first with EtOAc, then acetone, then 16:3:1 acetone-MeOH-TEA yielded 0.22 g (45%) of 18c as an orange powder: mp 165–170 °C (dec); ¹H NMR (CDCl₃) δ 9.23 (s, 2H), 8.98 (m, 2H, 8.62 (d, 2H, J 8.0 Hz), 8.02 (m, 2H), 7.94 (d, 2H, J 8.1 Hz), 7.60 (m, 2H), 7.28 (m, 2H), 3.92 (m, 4H), 3.63 (m, 4H), 3.54 (m, 4H), 2.07 (m, 4H), 1.78 (m, 4H); MS m/e 697 (M⁺). Anal. Calcd for C₄₀H₃₆N₆O₆ + 1.75 H₂O: C, 66.04; H, 5.13; N, 11.55. Found: C, 66.16; H, 5.13; N, 11.83.

N,N’-[Methylene-4,1-phenylenebis[6-aminindo[1,2-b][2,7]naphthyridine-5,12-dione] (18d). To a solution of 24 (0.73 g, 2.22 mmol) in THF (25 mL) was added 4,4’-methylenedianiline (0.20 g, 1.0 mmol). The mixture was refluxed for 6 h, then cooled, poured into saturated aqueous sodium bicarbonate solution (100 mL) and extracted with CH₂Cl₂ (3 x 50 mL). The combined organic phase was washed with saturated aqueous sodium bicarbonate solution (50 mL), water (50 mL), and saturated aqueous sodium chloride solution (50 mL), then dried (Na₂SO₄) and adsorbed onto silica gel. Elution first with EtOAc, then acetone, then 16:3:1 acetone-MeOH-TEA yielded 0.16 g (23%) of 18d as a red powder: mp 239 °C (dec); IR (CHCl₃) 3420, 3020, 2965, 1685, 1630, 1615, 1595, 1570, 1550, 1365, 1210 cm⁻¹; ¹H NMR (CDCl₃) δ 9.70 (s, 2H), 9.04 (d, 2H, J 5.1 Hz), 8.72 (d, 2H, J 8.5 Hz), 8.11 (d, 2H, J 5.1 Hz), 7.62 (m, 2H), 7.29 (m, 4H), 7.03 (d, 4H, J 8.4 Hz), 6.68 (d, 4H, J 8.4 Hz), 3.96 (s, 2H), 3.63 (bs, 2H); Anal. Calcd for C₄₃H₂₆N₆O₄: C, 74.78; H, 3.79; N, 12.17. Found: C, 75.02; H, 4.81; N, 12.04.

Procedure for determination of ΔTₘ values. The thermal denaturation studies were done by the method of Cory³⁴ on a Varian 2290 UV-visible spectrophotometer with a heating rate of 18 °C/h. The five cuvettes were on a 2 min cycle time with a 5 sec dwell time. ΔTₘ values were calculated from the printout using the difference between the compound Tₘ and the DNA. The calf thymus DNA had a Tₘ of 56.8 °C.

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