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Specific stabilization of DNA triple helices by indolo[2,1-*b*]-quinazolin-6,12-dione derivatives

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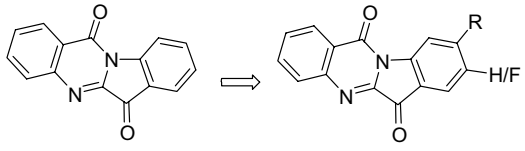
Abstract—Derivatives of indolo[2,1-*b*]quinazolinone containing aminoalkylamino side chains were synthesized as specific DNA triplex stabilizing agents. The aminoalkylamino side chains are essential for triplex stabilization. The position-8 fluorine atom or a methyl group to the nitrogen adjacent to the planar core can enhance triplex stability by 6 °C and the effect is additive. Conformational analysis reveals that the orientation of the side chain underlies the ability of this compound to stabilize a DNA triplex.

Antigene[1] and antisense[2] are two major strategies for selectively inhibiting gene expression. The antisense strategy utilizes oligonucleotides that specifically bind complementary mRNAs, resulting in arrested translation, and the antigene approach uses triplex-forming oligonucleotides (TFOs)[3] to interfere with transcription.[4] However, the use of TFOs has been hampered by the instability of triple helices under physiological conditions. Due to the tremendous therapeutic potential, stabilization of triple helix has attracted attention among researchers attempting to apply the antigene strategy.[5]

Large intercalators of polycyclic compounds were considered to bind to triplexes because of greater surface area for stacking interactions. Ethidium bromide was first demonstrated to stabilize the T·A*T triplex[6] while it destabilized the triplex containing 15 T·A*T and 7 C·G*C$^+$ due to repulsion between the two positive charges.[7] An ellipticine derivative was also able to stabilize T·A*T but not C·G*C$^+$ triplexes.[5a] The antitumor antibiotic coralyne chloride was then reported to stabilize both T·A*T and C·G*C$^+$ triplexes.[8] Hélène et al. further reported that benzopyridoindole derivatives bearing polyamino side chains preferentially stabilized the triplex over the duplex.[9] Bisamidoanthraquinones[10] and related sulfonamidoanthraquinones[11] were also reported to have selective stabilization on triplex.



tryptanthrin, **1**

2, R = $NH(CH_2)_3NH_2$

3, R = $NH(CH_2)_3N(CH_3)(CH_2)_3NH_2$

Tryptanthrin (**1**), indolo[2,1-*b*]quinazolin-6,12-dione, was originally reported to have antimicrobial activity[12] and to inhibit tuberculosis.[13] In recent years, tryptanthrin has attracted much attention as an aryl hydrocarbon receptor agonist,[14] anti-inflammatory agent,[15] inducer of caspase-3/Fas mediated apoptosis,[16] and anticancer agent.[17] In addition, polyamines are known to stabilize triplex DNA[18,19] due to the binding with the phosphate backbone, and they have been applied in gene therapy in many ways.[20] As part of our studies of quinazoline derivatives, we attempted to integrate polyamines onto tryptanthrin as triplex DNA stabilizing agents. The tetracyclic plane of tryptanthrin may interact with DNA through π–π stacking and hydrogen bonding, whereas the alkylamine offers binding with the third oligonucleotide via electrostatic interactions. A priori, derivatives **2**

Keywords: DNA triplex; Triplex stabilizing agents; Tryptanthrin; Indolo[2,1-*b*]quinazolin-6,12-dione; Thermal denaturation.
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and **3** would benefit from both of these interactions to stabilize triplex DNA.

Nucleophilic substitution of 5,6-difluoroisatin (**4a**) and 6-fluoroisatin (**4b**) with monoprotected 4-*tert*-butyloxycarbonylaminopropylamine (**5**) afforded 6-(*N*-Boc-aminopropylamino)isatins **6a** and **6b**, respectively, in low yields due to the side-reaction of the primary amine with the carbonyl group. Surprisingly, the same substitution of **4a** with monoprotected triamine *N*-(3-aminopropyl)-*N*-(3-Boc-aminopropyl)-*N*-methylamine (**7**) gave the diamine **8a** as opposed to the expected triamine product **9** (Scheme 1). As a consequence, **8b** was prepared by direct nucleophilic substitution of **4b** with *N*-(*tert*-butyloxycarbonylaminopropyl)-*N*-methylamine (**10**). Accordingly by using the reported method,[21] condensation of the resulting isatins **6a**,**b** and **8a**,**b** with isatoic anhydride (**11**) in the presence of *N*,*N*′-diisopropyl carbodiimide (CDI) followed by deprotection with TFA gave the TFA salts of corresponding tryptanthrin derivatives **2a**, **2b**, **12a**, and **12b**.

The triplex stabilizing ability of ligands **2a**, **2b**, **12a**, and **12b** in a 14C3 triplex (a 14-mer oligonucleotide binding to a 26-bp double helix) was evaluated by thermal denaturation using UV absorption spectroscopy, as shown in Figure 1. Both the ligand-free and ligand-bound triplexes demonstrated two well-resolved transitions, the transition of the triplex to the duplex followed by duplex melting to the single strands. The lower temperature $T_m^{3\to2}$ corresponds to the dissociation of the third strand from the duplex, and the higher temperature $T_m^{2\to1}$ corresponds to the melting of the duplex into two single strands. In the absence of ligand, the $T_m^{3\to2}$ and $T_m^{2\to1}$ of the 14C3 triplex were 20.5 °C and 59.0 °C, respectively (Fig. 1). A similar profile was observed in the presence of **1** (data not shown), suggesting that tryptanthrin per se has no stabilizing effect.

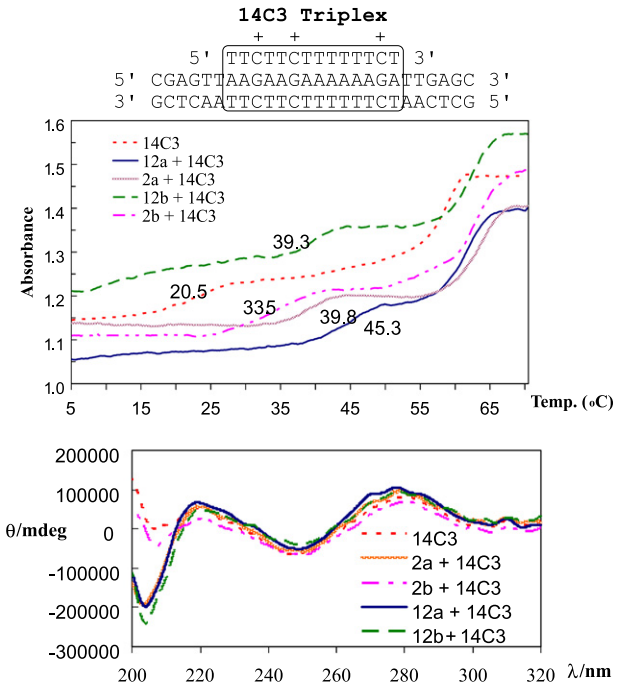
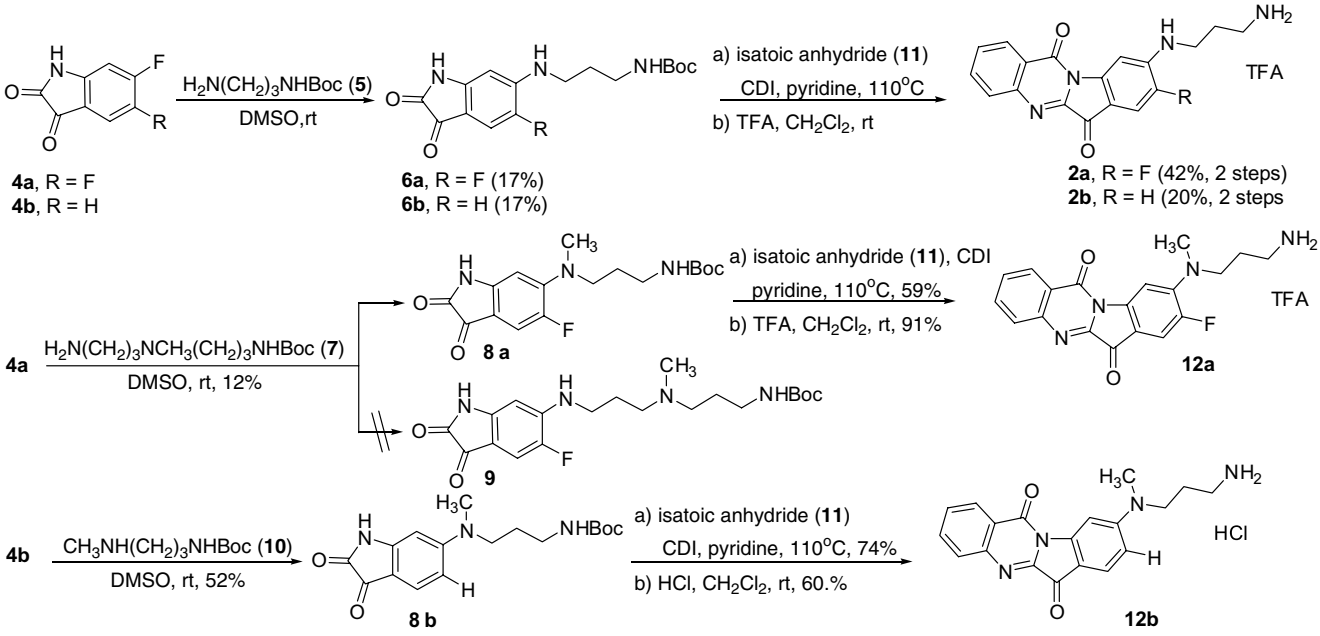


Figure 1. Sequence of the 14C3 triplex, UV denaturation and CD spectra of the 14C3 helices in the absence of ligand or in the presence of ligand. For UV profile, the temperature was increased at a rate of 0.5 °C/min. Numbers indicated are the $T_m^{3\to2}$. For CD measurement, the temperature was 38 °C. All ligands were measured at the concentration of 14.3 μM in 10 mM sodium cacodylate, pH 6.2, containing 0.1 M NaCl.

In the presence of 14.3 μM **2a** or **2b**,[22] the melting profile of the 14C3 triplex shifted to higher temperatures (Fig. 1). The stabilizing ability of the free base form of **2a** was also measured, and a comparable profile to that of the TFA salt **2a** was obtained, indicating that TFA did not interfere with the binding. This result lends



Scheme 1.

Table 1. Melting points (T_m) and changes in melting temperature (ΔT_m)[a] from the triplex to duplex (3 → 2) and from the duplex to single strands (2 → 1) for **2** and **12** binding to triplex 14C3

	Melting point (°C)		Stabilizing effect (°C)	
	$T_m^{3\to2}$	$T_m^{2\to1}$	$\Delta T_m^{3\to2}$	$\Delta T_m^{2\to1}$
14C3 (triplex)[b]	20.5	59.0		
2a + 14C3	39.8	63.8	19.3	4.8
2b + 14C3	33.5	64.7	13.0	5.7
12a + 14C3	45.3	61.8	24.8	2.8
12b + 14C3	39.3	62.3	18.8	3.3

[a] $\Delta T_m = T_{m(ligand+14C3)} - T_{m14C3}$.
[b] Values of $T_m^{3\to2}$ and $T_m^{2\to1}$ for the 14C3 triplex in the absence of any ligand were reported to be 18 °C and 58 °C, respectively, under the same conditions.[5a]

support to the idea that a polyamine chain may play an important role in binding. Notably, the triplex was greatly stabilized by **2a** and **2b**, with $T_m^{3\to2}$ values of 39.8 °C and 33.5 °C, respectively. Ligand **2a** had a $\Delta T_m^{3\to2}$ value of 19.3 °C, which was 6 °C higher than **2b** (13.5 °C) (Table 1). The only structural difference between **2a** and **2b** is the fluorine atom at the 8 position. Apparently, this fluorine plays some role in binding to the triplex. Ligands **2a** and **2b** had only minor effects on duplex melting, with $\Delta T_m^{2\to1}$'s of 4.8 °C and 5.7 °C, respectively.

The melting points of triplexes in the presence of **12a** and **12b** increased to 45.3 °C and 39.3 °C, respectively (Fig. 1). Therefore, **12a** and **12b** stabilized the triplex by 24.8 °C and 18.8 °C, respectively. They showed only minor effects on duplex DNA melting, with $\Delta T_m^{2\to1}$ values around 3 °C. As a result, **2a**, **2b**, **12a**, and **12b** are all triplex-specific binding agents that have little effect on the DNA duplex. Interestingly, the $\Delta T_m^{3\to2}$ value of **12a** was 6 °C higher than that of **12b**. These results demonstrate that the position-8 fluorine atom can enhance triplex stability by 6 °C. It is presumed that this fluorine might form hydrogen bonds[23] or van der Waals interactions[24] with the third strand.

In addition to the contribution of the F atom on triplex stability, a methyl group on the nitrogen adjacent to the planar core also has a significant effect on triplex stabilization. The $\Delta T_m^{3\to2}$ of **12a** (F/CH_3) was 6 °C higher than that of **2a** (F/H) and the $\Delta T_m^{3\to2}$ of **12b** (H/CH_3) was also

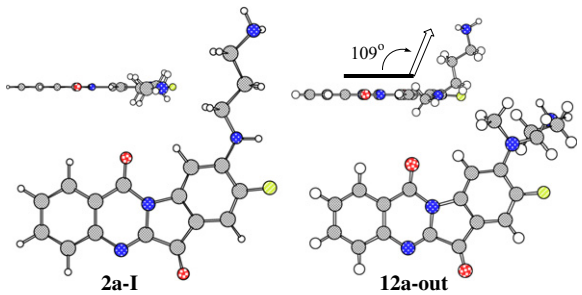
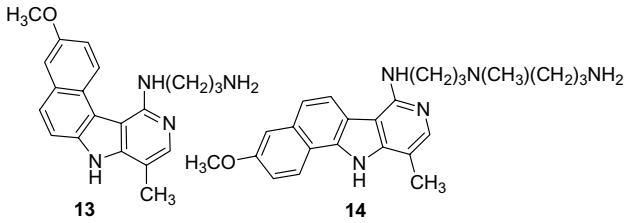


Figure 2. B3LYP/6-31G* conformational geometries of global minima **2a-I** and **12a-out**. The Newman projections are shown.

6 °C higher than that of **2b** (H/H). As a result, the $\Delta T_m^{3\to2}$ values of **2a** and **12b** are both 6 °C higher than that of **2b**. Ligand **12a**, containing both the F atom and a methyl group, had a 12 °C increase in $\Delta T_m^{3\to2}$ compared to **2b**. Thus, triplex stabilization by the F atom and the methyl group is additive. Triplex stabilization was further confirmed by CD spectroscopy at 38 °C (Fig. 1, and Scheme 1). A negative cotton effect around 210 nm is characteristic of a triplex. The characteristic negative bands around 210 nm[25] indicated triplex formation in the presence of **2a**, **12a**, and **12b**.

Benzopyridoindole derivatives **13** and **14** stabilized the same 14C3 triplex.[9] However, they also stabilized the



duplex. Due to the protonation on the aromatic nitrogens at pH 6.2 benzopyridoindoles without the side chain were able to stabilize the triplex.[9] Under the same condition, indoloquinazolinone was not protonated; the ligand lacking a side chain had no effect on the triplex stability. In addition, **2** and **12** exhibited triplex-specific stabilization with little effect on the duplex, suggesting that triplex stabilization arose from the interaction between the aminoalkylamino side chain and the triplex. We reasoned that the electrostatic interaction between the positively charged side chain[9] and the triplex was crucial for triplex DNA binding. Ligands **12a** and **12b**, specifically and favorably stabilizing the triplex, contain the same side-chain length as **2a** and **2b**. Apparently, the methyl group on the tertiary nitrogen plays a key role in enhancing stabilization.

Given that **2** and **12** specifically stabilize the triplex, we used DFT calculation[26] (B3LYP/6-31G*) to investigate whether the structural conformation of the side chains could explain the basis of the difference in triplex stabilization. The planar structure **2a-I** with the H atom on the secondary amine *syn* to the F atom is the most favored structure (Fig. 2). For **12a**, no planar structure could be located. In the preferred **12a-out**, the amino side chain and the tetracyclic plane are almost perpendicular. In general, the triplex stabilizing effect is enhanced by extension of the aromatic rings for π–π interaction or by lengthening side chains to polyaminoalkyl groups.[9] Nevertheless, **2** and **12** contain the same planar core and the same polyamine side-chain length but show distinct triplex stabilizing effects. Evidently, the methyl group of the tertiary amine in **12** affects the orientation of the side arm to allow suitable binding to the third strand. This distinct orientation may facilitate **12**-mediated triplex-specific stabilization.

Our results show that ligands **2** and **12** act as effective triplex DNA groove binders that remarkably stabilize

the triplex with little effect on the duplex DNA. The aminoalkylamino side chains in indolo[2,1-*b*]quinazolin-6,12-dione are essential for triplex stabilization. In addition, a fluorine atom at position 8 enhances the stabilization in this system. Our results may provide a new direction for the design of novel triplex DNA binders. It has been suggested that triplexes might play an important role in vivo.[27] Moreover, more studies have reported on triplex inhibiting promoter function[28] and preventing transcription.[29] Our preliminary results indicate that cancer cell growth is inhibited by exposure to derivatives **1**, **2**, and **12**. We are currently investigating the mechanism of anti-proliferation and the relevance of DNA triplex stabilization to biological functionality.

Acknowledgments

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