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# Bis-4-aminoquinolines: Novel Triple-Helix DNA Intercalators and Antagonists of Immunostimulatory CpG-Oligodeoxynucleotides

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**Abstract**—Six dimeric 2-(2-naphthyl)quinolin-4-amines with a linker between the amino groups and eight dimeric 2-(4-anilino)quinolin-4-amines linked between the anilino groups were synthesized and evaluated for their interaction with duplex/triplex DNA's and as antagonists of immunostimulatory oligodeoxynucleotides with a CpG-motif (CpG-ODN). The most powerful triple-helix DNA intercalator known to date, with high affinity toward T·A·T triplets and triplex/duplex selectivity, was found. The potent antagonism of immunostimulatory CpG-ODN by several bis-4-aminoquinolines is not related to their DNA interactions.


## Introduction

Despite their molecular simplicity, 4-aminoquinolines exhibit a variety of biological activities, and several simple derivatives are drugs currently in clinical use. 4-Aminoquinolines, depending on structure, are antimalarial, antiviral, antitumor, anti-inflammatory, analgesic, immunostimulatory, and hypotensive agents. Several derivatives are used to induce remission in systemic lupus erythematosus and rheumatoid arthritis, and others are reversible inhibitors of $(H^+/K^+)$-ATPase, $K^+$ channel blockers, and are useful in the treatment of cardiac arrhythmias. Interest in this class of compounds is not restricted to the field of medicinal chemistry as 4-aminoquinolines are also important agrochemicals due to their fungicidal, insecticidal, and pesticidal properties.[1]

We have reported previously that a 2-(2-naphthyl)quinolin-4-amine **2** (structure in Scheme 1) and analogues intercalate with poly(dT·dA·dT) and stabilize this triple-helix DNA structure with high selectivity in the presence of duplex DNA of any sequence.[2–6] When covalently attached to an oligo(dT) the 2-(2-naphthyl)quinolin-4-amine system strongly promotes binding of the oligomer to its duplex target.[7] An even stronger effect on the formation and stabilization of T·AT triplets has been observed for *intermolecular* interaction of bis[2-(2-naphthyl)quinolin-4-amine] **12** (structure in Scheme 1) which is a triple-helix DNA bis-intercalator.[8] These findings have important ramifications for the development of a therapeutic methodology known as the 'antigene' strategy.[9] This potential approach utilizes the triple-helix formed from the cellular duplex and an external third strand followed by stabilization of the triplex to directly regulate the expression of a selected gene. We have also shown that ligand **2** and its 2-aryl analogues intercalate, albeit weakly, with A-form duplex RNA,[10,11] and a strong correlation has been found between RNA interactions and anti-HIV-1 activity[11] of this class of antiviral agents.[11,12] More recently, we have reported that **2** and analogues are potent antagonists of immunostimulatory CpG-oligodeoxynucleotides (CpG-ODN) and bacterial DNA which contains the CpG motif.[13–15]

This report pertains to the synthesis and evaluation of bis-quinoline triplex-DNA intercalators that are analogues of **12**. Full synthetic details for **12**, undisclosed previously,[8] are also presented. We also report for the first time that several bis-quinolines synthesized as part of this work are potent antagonists of the CpG-ODN mediated immunostimulatory effect. With the results of DNA binding and the inhibition of the immunostimulatory effect for a large series of compounds, we confirm our previous suggestion[15] that the two phenomena are not related.

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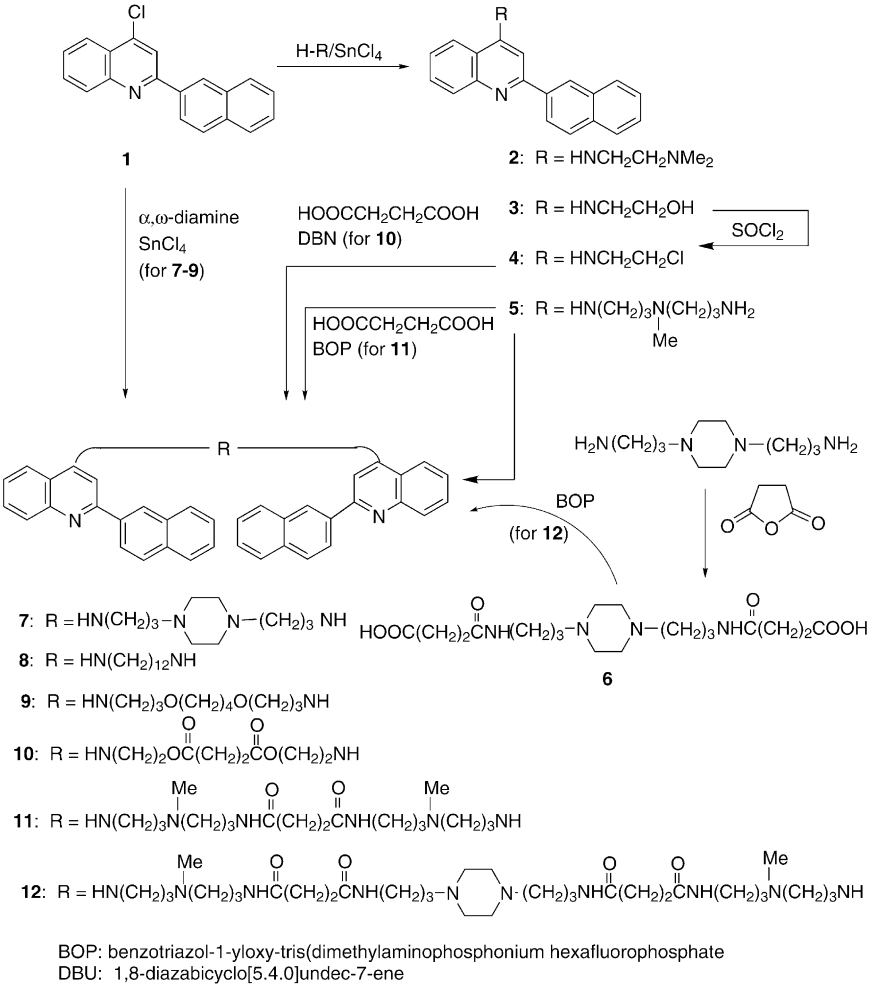



**Scheme 1.**

## Results and Discussion

### Synthesis

Two series of bis-quinolines were synthesized. Compounds **7**–**12** (Scheme 1) are derivatives of 2-(2-naphthyl)quinoline in which the two quinolines are linked at positions 4 and 4′ with an α,ω-diamine of various length, from 12 atoms in **7** and **10** to 38 atoms in **12**. Compounds **14**–**21** (Scheme 2) are bis(2-phenylquinolines) with an α,ω-diamine linker between positions 4 and 4′ of the phenyl groups. The length of the linker increases from 4 atoms in **14** to 14 atoms in **18** and **19**, and all compounds **14**–**21** are substituted at positions 4 and 4 of the quinolines with same side amino chain, as in **2**.

Bis-quinolines **7**–**9** were obtained by one-pot nucleophilic displacement of a chlorine atom in readily available 4-chloro-2-(2-naphthyl)quinoline (**1**) by the reaction with a α,ω-diamine in the presence of a catalytic amount of $SnCl_4$.[16] The remaining compounds **10**–**12** were synthesized by a stepwise construction of the linkage chain. In particular, a direct condensation of an intermediate aminoquinoline **5** with succinic acid or diacid **6** in the presence of BOP reagent gave the respective diamides **11** and **12**. On the other hand, a similar BOP-mediated reaction of an alcohol **3** with succinic acid furnished a diester **10** that was difficult to purify. This problem was overcome by synthesis of a chloro derivative **4** from **3** followed by coupling of **4** with succinic acid in the presence of DBU.[17]

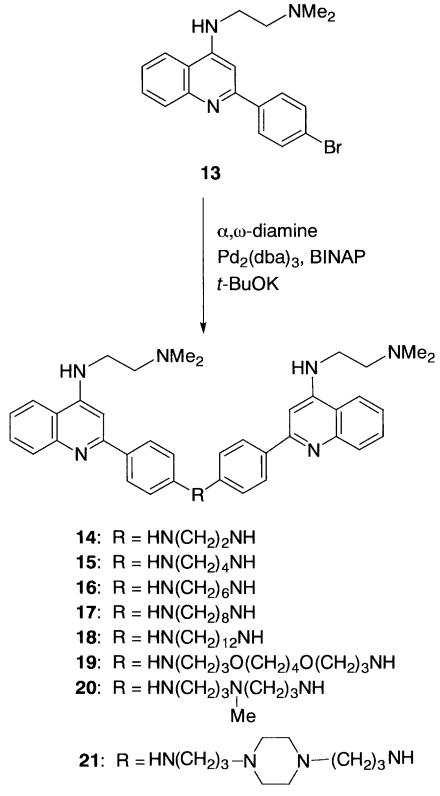
In an attempted synthesis of bis-quinolines **14**–**21** (Scheme 2) a bromophenyl derivative **13** or its fluorophenyl analogue (not shown) was treated with a dilithio reagent derived from the corresponding α,ω-diamine. In spite of the successful similar chemistry developed by us for 2-(4-aminophenyl)quinolines,[18] the desired bis-quinolines could not be obtained by this approach. Compounds **14**–**21** were synthesized by palladium(0) catalyzed amination of **13** with an α,ω-diamine.[19]

### DNA interactions: $T_m$ determinations

Interactions of ligands with duplex polydA·polydT and triplex polydA·2polydT were evaluated by thermal melting experiments under identical conditions with those reported previously for quinoline **2**,[2] for direct comparison. The results are given in Table 1. We have found a strong correlation between compound binding

affinity and the increase in DNA $T^m$.[20] Although the correlation is not completely linear, the agreement between binding affinity and *T*m increase is quite good within any series of compounds. In general, the *N*,*N*′-linked bis(quinolin-4-amines) **7**–**12** have the largest $T_m$ increases with triplex DNA and are superior to the phenyl-linked analogues **14**–**21** in their ability to selectively stabilize triple-helix DNA in the presence of duplex DNA. Of the latter series, only compound **18** shows stabilization of the triplex helix and triplex/duplex binding selectivity that are comparable to those of the reference quinoline **2**. By contrast, compounds **7** and **8** exhibit virtually no interaction with duplex DNA, and the increase in $T_m$ for triplex DNA in the presence of **7** is greater than the $\Delta T_m$ value for **2**. As with **2**, all bis-quinolines **7**–**12**, **14**–**21** interact weakly with $C^+ \cdot G \cdot C$ triple helix, as judged by small increases in *T*m, suggesting the T·A·T triplet specificity.

The differences in substituent effects among these compounds are expected to arise due to their different linking position on the intercalating ring system (note that the assumption of intercalation of these compounds is based on their structural similarity to previously characterized systems). We have previously shown[2] that the cationic substituent of **2** fits into the Watson–Crick groove of the TAT triplex. This groove is relatively wide in the TAT triplex and provides sufficient space for the substituent to optimize DNA interactions without unfavorable steric effects. This orientation of the substituent also allows optimum stacking of the quinoline




**Scheme 2.**

**Table 1.** $T_m$ increases[a] for polymer duplex and triplex structures with the quinoline derivatives and activities of the quinoline derivatives as antagonists of immunostimulatory CpG-ODN

| Compd | $\Delta T_m$ (°C) | | $EC_{50}$, nM |
|---|---|---|---|
| | Duplex DNA | Triplex DNA | |
| **2** | 5.5[b] | 35.6[b] | 9.1[c] |
| **5** | nd[d] | nd[d] | 18.0 |
| **7** | 0 | 38.7 | 72 |
| **8** | 0 | 24.9 | 590 |
| **9** | 3.6 | 32.2 | 14.0 |
| **10** | 5.7 | 35.7 | 160 |
| **11** | 21.7 | 41.0 | 16.0 |
| **12** | 14.1 | 39.7 | 7.0 |
| **14** | 14.3 | 21.7 | 95 |
| **15** | 14.3 | 16.7 | 15.8 |
| **16** | 12.7 | 20.3 | 1.8 |
| **17** | 12.7 | 21.9 | 13.5 |
| **18** | 6.0 | 34.0 | 19.5 |
| **19** | 9.1 | 17.4 | 2.6 |
| **20** | 19.6 | 19.1 | 118 |
| **21** | 23.7 | 10.5 | 513 |

[a]See Experimental for conditions.
[b]Determined previously.[2]
[c]Determined previously.[15]
[d]Not determined. The equilibrium dialysis results show a considerable interaction of **5** with duplex and triplex DNA with a stronger binding to the triplex (Professor Jonathan B. Chairs, private communication).

and naphthyl rings with the TAT base triplets in the intercalated complex. The linkers in **7**–**12** represent a direct extension of the cationic side chain of **2** and they can bind in the same orientation as with the linker in **2**. The cationic side chain of **14**–**21** can also fit into the same groove as with **2**. In this series, however, the para position of the phenyl ring is stacked over the third strand T. This orientation places the linking chain either into the AT Hoogsteen groove, which is relatively narrow in the TAT triplex, or into the TT groove that has all of the T-$CH_3$ groups and steric restrictions. The same linker substituent in the two compound series will, thus, be in different grooves and will interact with different groups on the DNA bases and backbone with different interaction and steric effects. These modeling based observations agree well with the experimental results indicating better binding of **7**–**12** than of **14**–**21** with the triplex. It is also of interest to consider the linker length required for bisintercalation into triplexes based on these new experimental results. With duplexes a hydrocarbon linker in the 6-8 methylene unit range is required for bisintercalation. Below that effective length one ring will intercalate and one will bind outside the base stack. In the series **14**–**21**, it is certain that **14** with two methylenes is too short for bisintercalation while **18** with 12 units has sufficient length for bisintercalation. Compounds **14**–**17** with 2, 4, 6 and 8 methylene groups have very similar $\Delta T_m$ values with the triplex suggesting that all of these compounds bind with only one ring intercalated. There is a dramatic jump in the $\Delta T_m$ of **18** and this suggests a switch for mono to bisintercalation. With the exception of **8**, **7**–**12** have $\Delta T_m$ values $>30\,^{\circ}C$ indicating that all of these compounds bind by bisintercalation.

A large number of different classes of bis-intercalators have been designed for interaction with double-helix DNA.[21,22] By contrast, bis-quinolines developed by us

are the only triple-helix bis-intercalators known to date. As judged by $\Delta T_m$ values for the triplex–ligand complex, bis-quinoline **7** is not only superior to the reference quinoline **2** but also to all monomeric triple-helix DNA intercalators reported by others, namely substituted anthraquinones[23,24] and a series of polyfused heteroaromatic compounds.[25] This conclusion was derived from analysis of the reported $\Delta T_m$ values obtained under low salt conditions and extrapolation of these values to the high salt conditions used in our $T_m$ measurements. These results provide important new information about recognition of DNA triplexes and they define many of the requirements for bisintercalation into triplexes. They will be useful for the design of new agents for triplex stabilization and development of antigene therapeutics.

### Antagonism of immunostimulatory CpG-ODN

Bacterial DNA, CpG-ODN, and phosphorothioate CpG-ODN are immunostimulatory by an unknown mechanism.[13,14] This effect is inhibited by substituted 2-arylquinolin-4-amines.[13–15] Evidence has been accumulating that such quinolines may be useful in the treatment of rheumatoid arthritis and systemic lupus erythematosus[26,27] and in the prevention of graft-versus-host disease in bone marrow transplant patients.[28]

In this paper, we report for the first time that bis-quinolin-4-amines are antagonists of immunostimulatory CpG-ODN. The most active compounds that inhibit the immunostimulatory effect in vitro at low nanomolar concentrations are phenyl-linked bisquinolines **16**,**19** followed by slightly less active quinoline-linked derivative **12**. With the DNA binding and biological activity data for a large set of compounds it was of interest to determine whether there is a correlation between the DNA interaction and activity results. As can be seen from Table 1, the $T_m$ increase for duplex DNA, triplex DNA, and the binding selectivities as measured by differences in $T_m$ increases do not parallel the biological activity values. Thus, neither duplex DNA nor triplex DNA is a likely target for bisquinoline antagonists of CpG-ODN. This conclusion is fully consistent with our previous suggestion derived from studies of a limited number of simple quinolines.[14,15]

## Conclusions

Novel bis-4-aminoquinolines that are powerful triple-helix DNA intercalators with high triplex/duplex selectivity and high affinity toward T·A·T triplets were described. The potent activity of several bis-quinolines as antagonists of immunostimulatory CpG-ODN is not related to their DNA interaction.

## Experimental

### General

All reactions were conducted under an atmosphere of nitrogen. Amorphous and oily products were transformed into hydrobromide or hydrochloride salts by using a general procedure. Mp's (Pyrex capillary) are not corrected. $^1$H NMR spectra (400 MHz) were obtained in DMSO-$d_6$ solutions with TMS as an internal standard.

**General procedure for bis-quinolines 7–9.** A mixture of 4-chloro-2-(2-naphthyl)quinoline (1, 0.29 g, 1 mmol), an α,ω-diamine H-R-H (0.05 mmol), and a catalytic amount of $SnCl_4$ (30 μL) was heated in a Parr bomb to 140 °C for 3.5 h. Compound **7** was crystallized from MeOH/$H_2O$ while purification of **8** and **9** involved silica gel chromatography eluting with AcOEt/MeOH (9:1) and crystallization from AcOEt or AcOEt/MeOH).

**1,4-Bis[3-[2-(2-naphthyl)quinolin-4-amino]propyl]piperazine (7).** Yield 60%; mp 192–198 °C; $^1$H NMR δ 1.90 (m, 4H), 2.48 (m, 4H), 3.35 (s, 8H), 3.52 (m, 4H), 7.15 (s, 2H), 7.44 (t, $J$ = 8 Hz, 2H), 7.50 (br, exchangeable with $D_2O$), 7.55 (m, 4H), 7.66 (t, $J$ = 8 Hz, 2H), 7.92 (d, $J$ = 8 Hz, 2H), 7.96 (m, 2H), 8.04 (d, $J$ = 8 Hz, 2H), 8.09 (m, 2H), 8.24 (d, $J$ = 8 Hz, 2H), 8.38 (d, $J$ = 8 Hz, 2H), 8.71 (s, 2H). Anal. calcd for $C_{48}H_{46}N_6 \cdot H_2O$: C, 79.52; H, 6.67; N, 11.60. Found: C, 79.39; H, 7.04; N, 11.70.

***N*,*N*′-Bis[2-(2-naphthyl)quinolin-4-yl]dodecane-1,12-diamine (8).** Yield 42%; mp 193–194 °C; $^1$H NMR δ 1.30 (m, 8H), 1.73 (m, 2H), 3.43 (m, 2H), 7.10 (m, exchangeable with $D_2O$, 2H), 7.11 (s, 2H), 7.40 (t, $J$ = 8 Hz, 2H), 7.54 (m, 4H), 7.63 (t, $J$ = 8 Hz, 2H), 7.90 (d, $J$ = 8 Hz, 2H), 7.94 (m, 2H), 8.00 (d, $J$ = 8 Hz, 2H), 8.07 (m, 2H), 8.25 (d, $J$ = 8 Hz, 2H), 8.39 (d, $J$ = 9 Hz, 2H), 8.71 (s, 2H). Anal. calcd for $C_{50}H_{50}N_4 \cdot 0.5H_2O$: C, 83.62; H, 7.19; N, 7.82. Found: C, 83.79; H, 7.46; N, 7.80.

***N*,*N*′-Bis[2-(2-naphthyl)quinolin-4-yl]4,9-dioxa-1,12-dodecanediamine (9).** Yield 40%; mp 115–117 °C; $^1$H NMR δ 1.44 (m, 4H), 1.93 (m, 4H), 3.31 (m, 4H), 3.42 (m, 4H), 3.58 (m, 4H), 7.10 (s, 2H), 7.53 (t, $J$ = 8 Hz, 2H), 7.57 (m, 4H), 7.78 (t, $J$ = 8 Hz, 2H), 7.96 (m, 2H), 8.06 (m, 6H), 8.22 (d, $J$ = 8 Hz, 2H), 8.26 (m, exchangeable with $D_2O$, 2H), 8.41 (d, $J$ = 8 Hz, 2H), 8.67 (s, 2H). Anal. calcd for $C_{48}H_{46}N_4O_2 \cdot H_2O$: C, 79.09; H, 6.64; N, 7.69. Found: C, 78.70; H, 6.69; N, 7.41.

**Bis[3-[2-(2-naphthyl)quinolin-4-amino]ethyl succinate (10).** A solution of **1** (420 m, 1.4 mmol) in 2-aminoethanol (1.0 mL) was heated to 130 °C for 1.5 h. Treatment with water (2 mL) was followed by extraction of the mixture with AcOEt, drying of the extract with $MgSO_4$, and concentration to give a residue of *N*-(2-hydroxyethyl)-2-(2-naphthyl)quinolin-4-amine (**3**). Compound **3** was purified by silica gel chromatography eluting with $CH_2Cl_2$/MeOH (5:1) followed by crystallization from MeOH; yield 68%; mp 146–148 °C. Anal. calcd for $C_{21}H_{18}N_2O$: C, 80.23; H, 5.77; N, 8.91. Found: C, 80.14; H, 5.62; N, 8.86.

A mixture of **3** (400 mg, 1.27 mmol), $SOCl_2$ (1.0 mL), and benzene (5 mL) was heated under reflux for 1.5 h. Following concentration on a rotary evaporator, the oily residue was treated with AcOEt (25 mL) and an aqueous solution of $NaHCO_3$ (10%, 10 mL), and the mixture was stirred briefly. The organic layer was sepa-

rated, dried ($MgSO_4$), and concentrated. The residue of *N*-(2-chloroethyl)-2-(2-naphthyl)quinolin-4-amine (**4**) was purified by silica gel chromatography eluting with hexanes/ether (1:2); yield 64%, an oil. HRMS calcd for $C_{21}H_{17}{}^{35}ClN_2$: *m*/*z* 332.1080; observed *m*/*z* 332.1073.

A solution of **4** (200 mg, 0.6 mmol) succinic acid (35 mg, 0.3 mmol), and DBU[3] (90 μL, 0.6 mmol) in anhydrous DMF (1.0 mL) was heated to 90 °C for 6 h. Following concentration on a rotary evaporator, the residue of **10** was subjected to silica gel chromatography eluting with $CH_2Cl_2$/*i*-PrOH (5:1). Then, compound **10** was transformed into a hydrobromide salt, and the salt was crystallized from AcOEt; yield 38% of **10**·2HBr·1.5$H_2O$; mp 260–268 °C; $^1$H NMR δ 2.61 (s, 4H), 3.71 (m, 4H), 4.34 (t, *J* = 6 Hz, 4H), 7.26 (s, 2H), 7.32 (bs, exchangeable with $D_2O$, 2H), 7.43 (t, *J* = 7 Hz, 2H), 7.54 (m, 4H), 7.64 (t, *J* = 7 Hz, 2H), 7.93 (m, 4H), 8.01 (d, *J* = 9 Hz, 2H), 8.01 (d, *J* = 9 Hz, 2H), 8.07 (d, *J* = 7 Hz, 2H), 8.21 (d, *J* = 9 Hz, 2H), 8.42 (d, *J* = 9 Hz, 2H), 8.76 (s, 2H). Anal. calcd for $C_{46}H_{38}N_4O_4$·2HBr·1.5$H_2O$: C, 61.40; H, 4.60; N, 6.23. Found: C, 61.17; H, 4.50; N, 6.59.

***N,N′*-Bis[3-[*N*-[3-[2-(2-naphthyl)quinolin-4-amino]propyl]methylamino]propyl]suc-cinamide (11).** A mixture of 1 (0.6 g, 2.1 mmol), bis(3-aminopropyl)methylamine (3 mL, 18 mmol) and $SnCl_4$ (0.05 mL) was stirred and heated to 135 °C for 4 h. Concentration at 120 °C/5 mmHg followed by treatment of the residue with EtOH (15 mL) and then with a mixture of EtOH and 47% HBr (1:1, 6 mL) gave a precipitate. The precipitate was crystallized from EtOH containing a few drops of 47% HBr to give *N*-[3-[*N*-(3-amino]propyl]quinolin-4-amine hexahydrobromide (**5**·6HBr), yield 64%, mp 232–235 °C. Anal. calcd for $C_{26}H_{30}N_4$·6HBr: C, 35.33; H, 4.11; N, 6.33. Found: C, 35.53; H, 3.97; N, 6.12.

A mixture of succinic acid (36 mg, 0.3 mmol), triethylamine (0.35 mL, 2.5 mmol), and BOP (225 mg, 0.6 mmol) in anhydrous DMF (4 mL) was stirred at 23 °C for 15 min before treatment with **5**·6HBr (530 mg, 0.6 mmol). The mixture was stirred at 23 °C for 24 h and then filtered. The solution was concentrated on a rotary evaporator and a residue of crude product **11** was transformed into a hydrobromide salt. The salt was crystallized from 95% EtOH containing a few drops of 48% HBr to give **11**·5HBr·5$H_2O$: yield 73%; mp 235–240 °C; $^1$H NMR δ 1.81 (m, 4H), 2.19 (m, 4H), 2.31 (s, 4H), 2.78 (s, 6H), 3.09 (m, 8H), 3.27 (m, 4H), 3.81 (m, 4H), 7.27 (s, 2H), 7.71 (m, 6H), 8.01 (m, 4H), 8.10 (m, 2H), 8.16 (m, 4H), 8.23 (d, *J* = 8 Hz, 2H), 8.62 (d, *J* = 8 Hz, 2H), 8.74 (s, 2H), 9.30 (b, exchangeable with $D_2O$, 2H), 9.55 (bs, exchangeable with $D_2O$, 2H). Anal. calcd for $C_{56}H_{68}N_8O_2$·5HBr·5$H_2O$: C, 49.02; H, 5.24; N, 8.19. Found: C, 48.89; H, 5.35; N, 8.55.

**1,4-Bis[3-[*N*-[3-[*N*-[3-[2-(2-naphthyl)quinolin-4-amino]-propyl]methyl-amino]propyl]succinamoyl]amino]propyl]-piperazine (12).** A solution of 1,4-bis(3-aminopropyl)piperazine (1.0 mL, 4.8 mmol) and succinic anhydride (1.0 g, 10.7 mmol) in anhydrous DMF (20 mL) was heated to 70 °C for 1 h. The resultant precipitate of 1,4-bis[3-[(3-carboxypropionamido)propyl]piperazine (**6**) was filtered, washed with EtOH, and crystallized from DMF: yield 57%, mp 179–180 °C. Anal. calcd for $C_{18}H_{32}N_4O_6$: C, 54.00; H, 8.05; N, 14.00. Found: C, 53.99; H, 8.20; N, 13.98.

Substitution of **6** for succinic acid in the procedure for **11** given above gave **12**·9HBr·2$H_2O$: yield 46%; mp 234–240 °C; $^1$H NMR δ 1.89 (m, 8H), 2.08 (m, 4H), 2.82 (m, 4H), 2.56 (m, 8H), 2.65 (s, 8H), 2.82 (s, 6H), 2.94 (m, 4H), 3.11 (m, 4H), 3.34 (m, 4H), 3.87 (m, 8H), 7.30 (s, 2H), 7.71 (m, 6H), 7.93 (bs, exchangeable with $D_2O$, 2H), 8.01 (t, *J* = 8 Hz, 2H), 8.08 (d, *J* = 8 Hz, 2H), 8.21 (m, 8H), 8.74 (d, *J* = 8 Hz, 2H), 8.81 (s, 2H), 9.38 (bs, exchangeable with $D_2O$, 2H), 9.93 (b, exchangeable with $D_2O$, 2H). Anal. calcd for $C_{70}H_{88}N_{12}O_4$·9HBr·2$H_2O$: C, 43.64; H, 5.28; N, 8.73. Found: C, 43.81; H, 5.43; N, 8.46.

**General procedure for bis-quinolines 14–21**

A solution of $Pd_2(dba)_3$ (5.4 mg, 0.005 mmol) and BINAP (15 mg, 0.025 mmol) in dioxane (25 mL) was stirred at 23 °C for 5 min before the addition of **13** (0.42 g, 1.1 mmol) and an α,ω-diamine H-R-H (0.5 mmol). The solution was treated with *t*-BuOK (0.32 g, 3.4 mmol) and the resultant red mixture was stirred and heated to 80 °C for 40 h. Treatment with AcOEt (60 mL) and filtration, then washing of the solution with brine, drying ($Na_2SO_4$), and concentration gave **14–21**. The product was purified by silica gel chromatography eluting with AcOEt/$Et_3N$/EtOH (35:3:1) and crystallization from EtOH/hexanes (**14–18**, **21**) or EtOH (hydrobromides of **19**, **20**).

***N,N′*-Bis[4-[4-[2-(dimethylamino)ethyl]amino]quinolin-2-yl]phenyl]ethane-1,2-diamine (14).** Yield 15%; mp 254–256 °C; $^1$H NMR δ 2.25 (s, 6H), 2.6 (t, *J* = 6 Hz, 2H), 3.35 (s, 2H), 3.42 (t, *J* = 6 Hz, 2H), 6.10 (br, 1H exchangeable with $D_2O$), 6.72 (d, *J* = 9 Hz, 2H), 6.82 (br, 1H exchangeable with $D_2O$), 6.83 (s, 1H), 7.32 (t, *J* = 8 Hz, 1H), 7.56 (t, *J* = 8 Hz, 1H), 7.80 (d, *J* = 8 Hz, 1H), 8.0 (d, *J* = 9 Hz, 2H), 8.08 (d, *J* = 8 Hz, 1H). $^{13}$C NMR δ 40.5, 41.5, 45.4, 57.2, 111.7, 117.8, 121.2, 122.9, 127.4, 128.1, 128.8, 128.9, 148.4, 149.6, 150.2, 156.8. Anal. calcd for $C_{40}H_{46}N_8 \cdot 0.5H_2O$: C, 74.16; H, 7.31; N, 17.29. Found: C, 73.90; H, 7.69; N, 17.26.

***N,N′*-Bis[4-[4-[2-(dimethylamino)ethyl]amino]quinolin-2-yl]phenyl]butane-1,4-diamine (15).** Yield 29%; mp 212–214 °C; $^1$H NMR δ 1.7 (s, 2H), 2.22 (s, 6H), 2.6 (t, *J* = 7 Hz, 2H), 3.15 (m, 2H), 3.48 (t, *J* = 7 Hz, 2H), 6.00 (br, 1H exchangeable with $D_2O$), 6.68 (d, *J* = 9 Hz, 2H), 6.80 (br, 1H exchangeable with $D_2O$), 6.85 (s, 1H), 7.32 (t, *J* = 8 Hz, 1H), 7.55 (t, *J* = 8 Hz, 1H), 7.76 (d, *J* = 8 Hz, 1H), 8.01 (d, *J* = 9 Hz, 2H), 8.08 (d, *J* = 8 Hz, 1H). $^{13}$C NMR 26.5, 40.4, 45.5, 57.2, 111.6, 117.8, 121.3, 123.0, 127.1, 128.2, 128.9, 148.5, 150.0, 150.1, 157.1. Anal. calcd for $C_{42}H_{50}N_8$·0.5$H_2O$: C, 74.64; H, 7.61; N, 16.57. Found: C, 74.92; H, 8.03; N, 16.95.

***N,N′*-Bis[4-[4-[2-(dimethylamino)ethyl]amino]quinolin-2-yl]phenyl]hexane-1,6-diamine (16).** Yield 35%; mp 172–174 °C; $^1$H NMR δ 1.6 (br m, 2H), 2.25 (s, 6H), 2.6

(t, $J=7$ Hz, 2H), 3.08 (t, $J=6$ Hz, 2H), 3.33 (t, $J=7$ Hz, 2H), 3.46 (t, $J=6$ Hz, 2H), 5.96 (br, 1H exchangeable with $D_2O$), 6.66 (d, $J=8$ Hz, 2H), 6.82 (br, 1H exchangeable with $D_2O$), 6.85 (s, 1H), 7.31 (t, $J=8$ Hz, 1H), 7.55 (t, $J=8$ Hz, 1H), 7.76 (d, $J=9$ Hz, 1H), 8.0 (d, $J=8$ Hz, 2H), 8.10 (d, $J=9$ Hz, 1H). $^{13}$C NMR δ 26.8, 28.9, 40.5, 42.7, 45.5, 57.3, 94.2, 111.7, 121.4, 123.2, 127.1, 128.3, 128.3, 129.2, 148.5, 150.1, 157.3. Anal. calcd for $C_{44}H_{54}N_8$: C, 76.05; H, 7.83; N, 16.11. Found: C, 76.21; H, 8.05; N, 15.93.

***N*,*N*′-Bis[4-[4-[2-(dimethylamino)ethyl]amino]quinolin-2-yl]phenyl]octane-1,8-diamine (17).** Yield 30%; mp 191–192 °C; $^1$H NMR δ 1.18 (m, 4H), 1.45 (m, 2H), 2.2 (s, 6H), 2.6 (m, 2H), 3.10 (t, $J=6$ Hz, 2H), 3.45 (t, $J=6$ Hz, 2H), 5.9 (br, 1H exchangeable with $D_2O$), 6.60 (d, $J=8$ Hz, 2H), 6.81 (br, 1H, exchangeable with $D_2O$), 6.85 (s, 1H), 7.31 (t, $J=8$ Hz, 1H), 7.55 (t, $J=8$ Hz, 1H), 7.75 (d, $J=8$ Hz, 1H), 7.97 (d, $J=8$ Hz, 2H), 8.06 (d, $J=8$ Hz, 1H). $^{13}$C NMR δ 26.9, 28.9, 29.1, 40.5, 42.8, 45.5, 57.2, 94.2, 111.7, 117.8, 121.4, 123.2, 128.3, 128.9, 129.2, 148.5, 150.1, 150.3, 157.3. Anal. calcd for $C_{46}H_{58}N_8$: C, 76.42; H, 8.08; N, 15.49. Found: C, 76.09; H, 8.57; N, 15.55.

***N*,*N*′-Bis[4-[4-[2-(dimethylamino)ethyl]amino]quinolin-2-yl]phenyl]dodecane-1,12-diamine (18).** Yield 30%; mp 174–176 °C; $^1$H NMR δ 1.3 (bm, 8H), 1.55 (t, $J=6$ Hz, 2H), 2.25 (s, 6H), 2.6 (t, $J=6$ Hz, 2H), 3.10 (t, $J=6$H), 3.48 (t, $J=6$ Hz, 2H), 5.90 (br, 1H exchangeable with $D_2O$), 6.60 (d, $J=8$ Hz, 2H), 6.80 (br, 1H exchangeable with $D_2O$), 6.85 (s, 1H), 7.30 (t, $J=7$ Hz, 1H), 7.55 (t, $J=7$ Hz, 1H), 7.75 (d, $J=8$ Hz, 1H), 7.98 (d, $J=8$ Hz, 2H), 8.08 (d, $J=8$ Hz, 1H); $^{13}$C NMR δ 26.8, 28.7, 29.0, 29.2, 29.2, 40.4, 42.7, 45.5, 57.2, 94.0, 111.6, 117.8, 121.3, 123.0, 127.0, 128.1, 128.9, 129.0, 148.5, 150.0, 150.1, 157.1. Anal. calcd for $C_{50}H_{66}N_8$: C, 77.08; H, 8.54; N, 14.38. Found: C, 76.86; H, 9.14; N, 14.56.

***N*,*N*′-Bis[4-[4-[2-(dimethylamino)ethyl]amino]quinolin-2-yl]phenyl]-4,9-dioxa-1,12-dodecanediamine (19).** Yield 30%; $^1$H NMR δ 1.58 (s, 2H), 1.81 (m, 2H), 2.93 (s, 6H), 3.21 (t, $J=6$ Hz, 2H), 3.5 (m, 4H), 4.05 (t, $J=6$ Hz, 2H), 6.8 (d, $J=8$ Hz, 2H), 7.03 (s, 1H), 7.66 (t, $J=8$ Hz, 1H), 7.90(t, $J=8$ Hz, 1H), 7.98 (d, $J=9$ Hz, 2H), 8.13 (d, $J=8$ Hz, 1H), 8.51 (d, $J=9$ Hz, 1H), 8.92 (br, 1H exchangeable with $D_2O$), 9.70 (br, 1H exchangeable with $D_2O$); $^{13}$C NMR δ, 26.2, 28.9, 37.7, 42.6, 54.2, 67.7, 70.1, 95.1, 111.8, 116.0, 117.2, 120.0, 123.3, 126.0, 130.3, 133.5, 138.2, 152.6, 152.9, 154.5. **19**·4HBr·4$H_2O$: mp 198–200 °C. Anal. calcd for $C_{48}H_{62}N_8O_2$·4HBr·4$H_2O$: C, 46.60; H, 6.51; N, 9.05. Found: C, 46.30; H, 6.26; N, 8.80.

***N*,*N*-Bis[3-[4-[4-[2-(dimethylamino)ethyl]amino]quinolin-2-yl]anilino]propyl]methylamine (20).** Yield 32%; $^1$H NMR δ 2.02 (m, 2H), 2.85 (s, 2H ), 2.95 (s, 6H), 3.3 (m, 4H), 3.51 (t, $J=6$ Hz, 2H), 4.2 (t, $J=6$ Hz, 2H), 6.86 (d, $J=9$ Hz, 2H), 7.06 (s, 1H), 7.68 (t, $J=8$ Hz, 1H), 7.93 (t, $J=8$ Hz, 1H), 8.03 (d, $J=9$ Hz, 2H), 8.2 (d, $J=8$ Hz, 1H), 8.56 (d, $J=8$ Hz, 1H), 9 (br, 1H exchangeable with $D_2O$), 9.8 (br, 2H exchangeable with $D_2O$); $^{13}$C NMR δ 23.2, 37.7, 42.6, 53.4, 54.2, 56.1, 95.2, 112.0, 116.1, 117.7, 120.0, 123.3, 126.1, 130.4, 133.5, 138.2, 152.2, 152.8, 154.6. **20**·5HBr·5$H_2O$: mp 219–222 °C. Anal. calcd for $C_{45}H_{57}N_9$·5HBr·5$H_2O$: C, 44.34; H, 5.94; N, 10.33. Found: C, 44.08; H, 5.96; N, 10.15.

**1,4-Bis[3-[4-[4-[2-(dimethylamino)ethyl]amino]quinolin-2-yl]anilino]propyl]piperazine (21).** Yield 35%; mp 128–130 °C; $^1$H NMR δ 1.73 (m, 2H), 2.25 (s, 6H), 2.4 (m, 4H), 2.6 (t, $J=6$ Hz, 2H), 3.12 (t, $J=6$ Hz, 2H), 3.45 (m, 4H), 5.98 (br, 1H exchangeable with $D_2O$), 6.65 (d, $J=8$ Hz, 2H), 6.79 (br, 1H, exchangeable with $D_2O$), 6.85 (s, 1H), 7.31 (t, $J=7$ Hz, 1H), 7.55 (t, $J=7$ Hz, 1H), 7.75 (d, $J=8$ Hz, 1H), 7.98 (d, $J=8$ Hz, 2H), 8.06 (d, $J=8$ Hz, 1H); $^{13}$C NMR δ 25.9, 40.1, 45.4, 53.0, 55.8, 56.0, 94.1, 111.6, 117.7, 121.2, 123.0, 127.2, 128.1, 128.9, 129.0, 148.4, 150.0, 150.2, 157.1. Anal. calcd for $C_{48}H_{62}N_{10}$ · 2.5$H_2O$: C, 69.96; H, 8.19; N, 16.98. Found: C, 69.96; H, 8.39; N, 16.40.

## Nucleic acid samples

PolydA·polydT, polydA and polydT from Phamacia were dissolved in PIPES 20 buffer (0.01 M piperazine-*N*,*N*′-bis(2ethanesulfonic acid), 0.001 M EDTA, 200 mM NaCl adjusted to pH 7.0. The concentrations of the polymer stock solutions were determined by using molar nucleotide extinction coefficients of 8600 $M^{-1}$ $cm^{-1}$ at 257 nm for poly(dA) and 8700 $M^{-1}$ $cm^{-1}$ at 260 nm for polydA·polydT. Triple helical complexes were prepared either by mixing a 1:1 ratio of polydA·polydT and polydT or a 1:2 ratio of polydA and polydT. The polymer stock solutions were added to 1 mL of PIPES 20 buffer. To anneal the polymers into a triple helix the mixed samples were heated to 90 °C and slowly cooled to room temperature.

## $T_m$ determination

Thermal melting curves for unbound DNA and compound complexes were determined on Varian Cary spectrophotometers in one cm pathlength cells as previously described.[2] Absorption changes at 260 nm were followed as a function of temperature and *T*m values were determined from first derivative plots. Compound stabilization of DNA samples was compared by the increase in $T_m$ ($\Delta T_m = T_m$ of the complex- *T*m of the free nucleic acid) they produce in PIPES 20 buffer at saturating amounts of the compound (ratio of 0.2 mol of compound to nucleic acid bases).

## Antagonism of immunostimulatory CpG-ODN

The ability of analogues to inhibit the action of CpG-ODN was assessed in vitro using WEHI 231 B-cell lymphoma cells as previously described.[13]

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