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Reaching into the Major Groove of B-DNA: Synthesis and Nucleic Acid Binding of a Neomycin−Hoechst 33258 Conjugate

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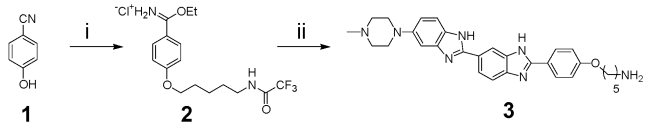
Recognition of duplex DNA by small molecules (polyamides, minor groove binders) and triplex forming oligonucleotides (TFOs, major groove binders) is a promising approach to a chemical solution for DNA recognition.[1] Several intercalators as well as various DNA minor groove ligands have been shown to bind to DNA double and triple helices.[2] Among the DNA groove binders, Hoechst 33258, its derivatives, and conjugates have been extensively studied for DNA duplex binding with a preference for A·T rich sequences.[3] We have recently shown neomycin to be a triplex-specific groove binder.[2] Neomycin, an aminoglycoside, had largely been known to bind different RNA structures.[4−8] Recent studies from our lab have, however, revealed that aminoglycosides (in particular, neomycin) can bind to other A-form structures.[9] The stabilization of poly(dA)·2poly(dT) triplex,[10] small oligomeric triplexes,[11] DNA·RNA hybrid duplexes,[12] RNA triplex,[2] and hybrid triplexes by neomycin has recently been reported by us. Aminoglycosides most likely bind in the major groove of these structures (much like RNA, as the A-form nucleic acids have a narrower major groove).[9] The B-form duplex has a much larger major groove and does not provide a good shape complementarity to aminoglycoside binding. Among all the aminoglycosides, neomycin was shown to be the most effective triplex groove binder that remarkably stabilized DNA and RNA triple helices with little effect on the double helices.[2,11] This work has complemented the success in development of DNA duplex specific groove binders in the past few decades, among which netropsin, distamycin, and Hoechst 33258 have been the lead compounds. We wished to investigate if a molecule such as neomycin can be forced into the B-form DNA major groove. Another intriguing question in this regard was whether the binding would be driven by Hoechst 33258 (duplex selective groove binder) or neomycin (triplex selective groove binder). Such ligands with minor/major groove recognition are also promising for development of inhibitors of transcription factors.[13] Herein, we report the synthesis and nucleic acid binding of a novel neomycin−Hoechst 33258 conjugate. The conjugate shows remarkable stabilization of DNA duplexes and destabilization of the DNA triplex.

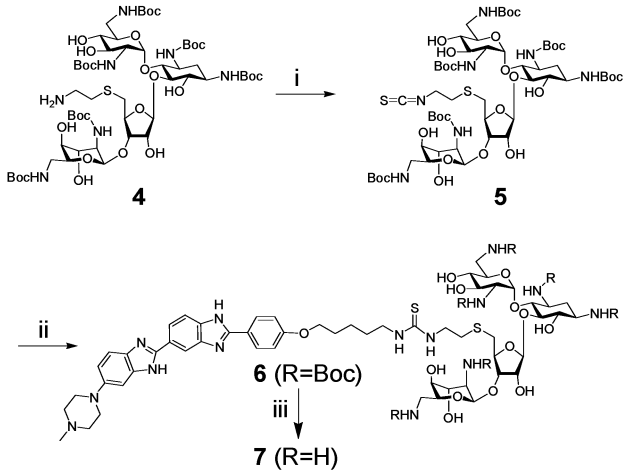
Starting with commercially available *p*-cyanophenol **1**, a Mitsunobu-type reaction with protected amino alcohol (*N*-trifluoroacetamido protection of 5-aminopentan-1-ol) allowed for successful conversion to imidate ester **2**, which could be subsequently coupled with 2-(3,4-diaminophenyl)-6-(1-methyl-4-piperazinyl) benzimidazole (prepared in five steps utilizing reported procedures)[14−16] to give the desired Hoechst 33258 linker component **3** necessary for ultimate conjugate synthesis (Scheme 1) (a similar approach has been reported by McLaughlin).[17,18] Using procedures developed by Tor[19] and our laboratory,[20,21] successful preparation of an electrophilic derivative of neomycin was then accomplished (Scheme 2). Starting from the natural product neomycin B, which is commercially available as the trisulfate salt, Boc (*tert*-butoxycarbonyl) protection of the six amino groups followed by conversion to 2,4,6-

Scheme 1 [a]



[a] Reagents and conditions: (i) (a) 5-trifluoroacetamido-1-pentanol, PPh_3, DIAD, dioxane, rt, 2 h, 84%; (b) HCl(g), EtOH, 0 °C, quant.; (ii) (a) 2-(3,4-diaminophenyl)-6-(1-methyl-4-piperazinyl) benzimidazole, HOAc, reflux, 4 h, 38%; (b) K_2CO_3 in 5:2 MeOH:H_2O, rt, overnight, 94%.

Scheme 2 [a]



[a] Reagents and conditions: (i) 1,1′-thiocarbonyldi-2(1*H*)-pyridone, cat. DMAP, CH_2Cl_2, rt, 20 h, 95%; (ii) **3**, pyridine, rt, overnight, 72%; (iii) 1:1 CH_2Cl_2, TFA, rt, 3 h, quant.

triisopropylbenzenesulfonyl derivative and subsequent substitution by aminoethanethiol gave rise to the protected neomycin amine,[19] compound **4**. Treatment of **2** with 1,1′-thiocarbonyldi-2(1*H*)-pyridone using a catalytic amount of DMAP gave isothiocyanate[21,22] derivative **5**, which was coupled with bis(benzimidazole) **3** and deprotected to give conjugate **7**.

The thermal stability of DNA triple and double helices in the presence of neomycin, Hoechst 33258, and neomycin−Hoechst 33258 conjugate **7** was investigated using thermal denaturation monitored by UV absorbance. It was found that **7** displays a marked effect on the stability of poly(dA)·poly(dT) duplex when compared to both neomycin (which is known to have no effect on the thermal stability of duplex DNA) and Hoechst 33258, which displayed some degree of stabilization of duplex DNA (Figure 1, left).

In the absence of ligand, the melting profile of poly(dA)·2poly(dT) is biphasic with $T_{m3\rightarrow2}$ = 34 °C and $T_{m2\rightarrow1}$ = 72 °C. As depicted in Figure 1 (left), the dissociation of duplex DNA in the presence of **7** occurs at a higher temperature (>95 °C) than that of DNA in the presence of Hoechst 33258 (86 °C) and neomycin (72 °C, unchanged when compared to native duplex melting), suggesting that **7** stabilizes the duplex better than the individual parent

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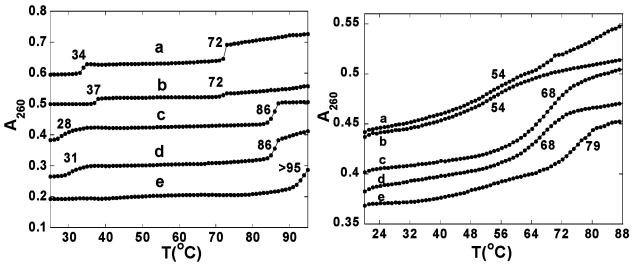

Figure 1. (Left) UV melting profile of poly(dA)·2poly(dT) in the presence of (a) no ligand, (b) 2 μM neomycin, (c) 2 μM Hoechst 33258, (d) 2 μM neomycin + 2 μM Hoechst 33258, and (e) 2 μM **7**. Samples of DNA (15 μM/base triplet) in buffer (10 mM sodium cacodylate, 0.5 mM EDTA, 150 mM KCl, pH 7.2) containing ligand were analyzed for UV absorbance at 260 nm from 20 to 95 °C using a temperature gradient of 0.2 °C/min. (Right) UV melting profile of d(CGCAAATTTGCG)$_2$ in the presence of (a) no ligand, (b) 1 μM neomycin, (c) 1 μM Hoechst 33258, (d) 1 μM neomycin + 1 μM Hoechst 33258, and (e) 1 μM **7**. Samples of DNA (1 μM/duplex) in BPES buffer (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM EDTA, 185 mM NaCl, pH 7.0) containing ligand (1 μM) were analyzed for UV absorbance at 260 nm from 20 to 95 °C using a temperature gradient of 0.2 °C/min.

compounds. Samples containing both neomycin and Hoechst 33258 displayed no difference in T_m from that observed with the individual molecules. It is important to note that triplex melting was not observed for poly(dA)·2poly(dT) in the presence of **7**, suggesting that drug binding prevents the third strand polypyrimidine from binding in the major groove.

A comparison was then made with a self-complementary DNA duplex d(CGCAAATTTGCG)$_2$ well-known for Hoechst 33258 affinity.[3] UV melting showed increased stability of the duplex in the presence of **7**, with a $\Delta T_m = 25$ °C (Figure 1 (right)), compared to $\Delta T_m = 14$ °C for Hoechst 33258.[3]

Further studies of numerous duplex DNA 22-mers of varying G/C content (breaking up stretches of A/T base pairs) were carried out (please see Supporting Information for the sequences). In all cases where stretches of at least four A/T base pairs were present, ΔT_m for **7** was at least 10 °C higher than that for Hoechst 33258. Duplex stabilization by **7** follows the selectivity shown by Hoechst 33258 (Figure 2a), whereas neomycin has no effect on the stabilization of any duplex. Hoechst 33258 is well-known to have a primary preference for A/T stretches as low as four base pairs, suggesting that the binding-induced thermal stabilization by **7** is largely controlled by the Hoechst 33258 moiety's ability to bind to its required stretch of A/T base pairs.

A model depicting the possible binding of **7** to a 12-mer duplex is shown in Figure 2b. Computer modeling suggests that electrostatic and H-bonding contacts between neomycin and sites within the major groove compete somewhat with the otherwise deep minor groove binding of Hoechst 33258 (Figure 2b). As Hoechst 33258 binds in the minor groove, neomycin is unable to be completely buried in the major groove (due to the linker size). Despite this constraint, conjugate **7** prefers the duplex, suggesting that neomycin can be forced into the major groove of a B-form DNA duplex. In retrospect, this could be due primarily to the larger binding constants observed between Hoechst 33258 and duplex DNA[3] ($\sim 10^8$ M^{-1}) as opposed to neomycin binding to triplex (10^5-10^6 M^{-1}).[11] Conjugates of different linker sizes can then perhaps be designed

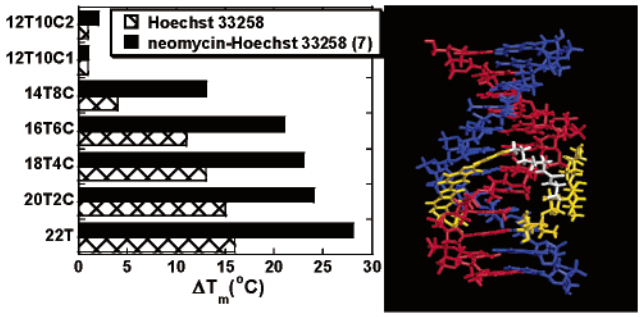

Figure 2. (a) (Left) Bar graph of ΔT_m for 22-mer duplexes in the presence of 4 μM Hoechst 33258 and 4 μM neomycin−Hoechst 33258 **7** obtained from UV melting profiles (solution conditions were identical to those in Figure 1b). (b) (Right) Computer model of neomycin−Hoechst 33258 (**7**) (yellow, linker atoms shown in white) docked in the DNA major−minor grooves.

to target a structure of preference and should aid in the development of even more selective and potent conjugates. Development of such dual-recognition ligands is being further explored in our laboratories and will be reported in due course.

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Supporting Information Available: UV scans/melts, synthesis/characterization of conjugate **7** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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