Top Curr Chem (2005) 253: 149–178
DOI 10.1007/b100446

Aminoglycoside–Nucleic Acid Interactions: The Case for Neomycin

Dev P. Arya (✉)

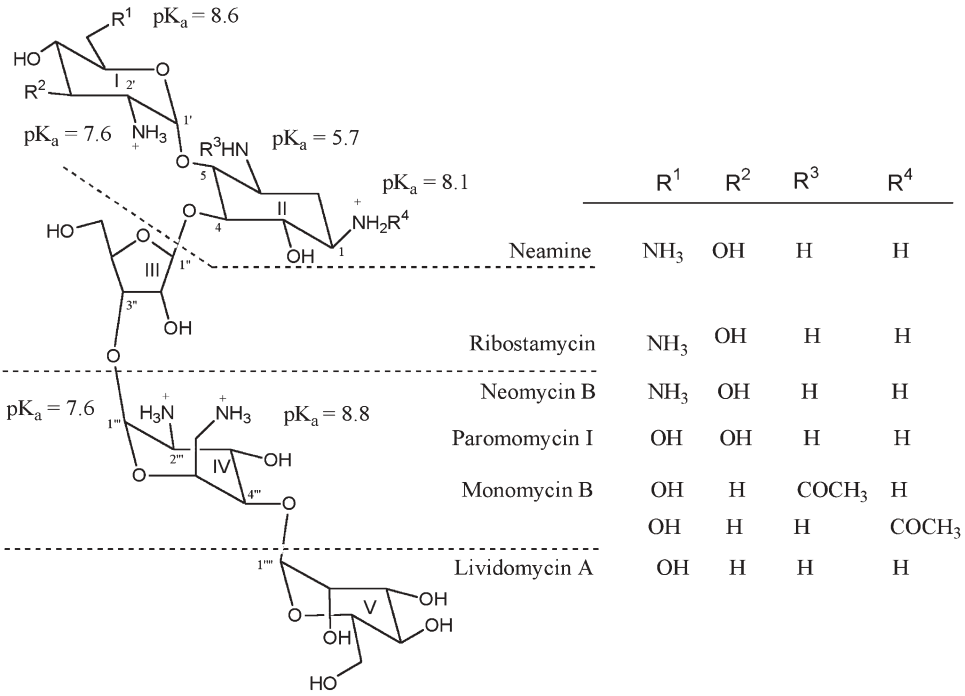
461 Hunter, Laboratory of Medicinal Chemistry, Department of Chemistry, Clemson University, Clemson, SC 29634, USA
dparya@clemson.edu

Abstract Aminoglycoside antibiotics are bactericidal drugs that have been at the forefront of antimicrobial therapy for almost five decades. The past decade (1990–2000) saw a resurgence in aminoglycoside-based drug development as their chemistry/mechanism of action became better understood. This work, however, had almost exclusively focused on targeting RNA. This review summarizes new developments (past 4–5 years) in aminoglycoside–nucleic acid interactions in the broader context of nucleic acid selectivity, not just RNA. Aminoglycoside binding to A-form nucleic acid structures is discussed, as is the development of novel conjugates for major-minor groove recognition of B-form DNA. Neomycin is chosen as the representative aminoglycoside and is revealed here to be an underutilized scaffold in nucleic acid recognition.

Keywords Aminoglycoside · Neomycin–Hoechst · A-form nucleic acids · B-form nucleic acids · PNA

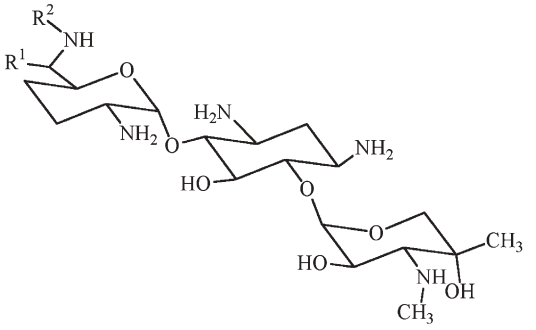
1 Aminoglycosides: An introduction

Aminoglycoside antibiotics (Scheme 1 and Scheme 2) are bactericidal agents that are comprised of two or more amino sugars joined in glycosidic linkage to

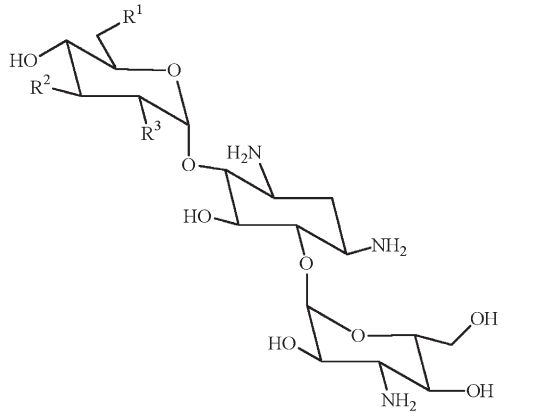


	R^1	R^2	R^3	R^4
Neamine	NH_3	OH	H	H
Ribostamycin	NH_3	OH	H	H
Neomycin B	NH_3	OH	H	H
Paromomycin I	OH	OH	H	H
Monomycin B	OH	H	$COCH_3$	H
	OH	H	H	$COCH_3$
Lividomycin A	OH	H	H	H

Scheme 1 Structures/pKas of aminoglycosides with a central ribose. Slightly different pKa values have been recently reported [6]



	R^1	R^2
Gentamicin C1	CH_3	CH_3
Gentamicin C2	CH_3	H
Gentamicin C1a	H	H



	R^1	R^2	R^3
Kanamycin A	NH_2	OH	OH
Kanamycin B	NH_2	OH	NH_2
Kanamycin C	OH	OH	NH_2
Tobramycin	NH_2	H	NH_2

Scheme 2 Structures of aminoglycosides (kanamycin and gentamicin families)

a hexose nucleus [1]. Though they exhibit a narrow toxic/therapeutic ratio, their broad antimicrobial spectrum, rapid bactericidal action, and ability to act synergistically with other drugs makes them highly effective in the treatment of nosocomial (hospital acquired) infections [2]. They are clinically useful in the treatment of urinary tract infections [3], lower respiratory infections, bacteremias, and other superinfections by resistant organisms [4]. Their greatest potential has been in combination drug regimens for the treatment of infections that are difficult to cure with single agents and for use in patients who are allergic to other classes of drugs [5]. Aminoglycosides (Schemes 1 and 2) contain a unique polyamine/carbohydrate structure, and have attracted considerable attention because of their specific interactions with RNA [6]. The bactericidal action of aminoglycosides is attributed to the irreversible inhibition of protein synthesis following their binding to the 30S subunit of the bacterial ribosome and thus interfering with the mRNA translation process. The miscoding causes membrane damage, which eventually disrupts the cell integrity, leading to bacterial cell death [7–10].

2
Aminoglycosides and Nucleic Acids: The Attraction for RNA?

After the discovery of streptomycin and other aminoglycosides by Selman Waksman in the early to mid-1940s as life-saving antibacterials against tuberculosis [11, 12], considerable efforts were focused on understanding their mechanism of action. In the decades that followed, and through seminal work by Davies and others [13, 14], ribosomal RNA came to be accepted as the target biopolymer responsible for drug action. In the early to late 1990s, as developments in nucleic acid synthesis, combinatorial biosynthesis, and the need for new drugs/targets for infectious diseases emerged, aminoglycosides were shown to bind to various RNA molecules. These include the 5′-untranslated region of thymidylate synthase mRNA [15], both Rev response element and transactivating response element RNA motifs [16–18] of HIV-1, a variety of catalytic RNA molecules such as group I introns [1, 19], ribonuclease P RNA [20], hairpin ribozyme [21, 22], hammerhead ribozyme [23–25], and hepatitis delta virus ribozyme [26, 27]. Aminoglycosides binding to HIV-1 RNA molecules have been shown to prevent binding of the cognate viral proteins Tat and Rev to TAR [28] and RRE [16], respectively. The glucose residues present in glycosylated DNA render the DNA inaccessible for enzymes, and thus help the pathogen escape degradation by host restriction enzymes [29, 30]. The literature of the past decade is rife with a large number of different RNA structures that aminoglycosides have been shown to bind. The reason for this RNA-centered development was understandable: aminoglycosides exhibit their antibacterial action through rRNA binding and show high affinity binding (K_d in the nanomolar range) to such RNAs. RNA rapidly became a target of drug development and discovery of such functional RNAs for drug development was a logical extension of an exploration of their extended activity. What was remarkable, however, was the almost complete absence of reports on the non-RNA structures targeted by aminoglycosides.

2.1
The Need for New Approaches: DNA vs RNA Recognition

RNA recognition has proven to be more challenging than DNA recognition by small molecules. Recognition of DNA•RNA hybrids by small molecules was virtually unexplored at the beginning of this century [31]. DNA-based intercalators and groove binders were the first to be examined for RNA recognition. These approaches met with limited success, due in large part to the different 3-D structures of functional RNA molecules. Sequence-specific RNA recognition has more similarities to recognition principles used in targeting proteins than to DNA duplexes. As with proteins, a distribution of charged pockets can provide a 3-D pattern that can be targeted specifically by compounds exhibiting structural electrostatic complementarity. Aminoglycosides have been shown

to provide complementary scaffolds where the positively charged ammonium groups displace several Mg^{2+} ions from their RNA binding sites [32–38]. An intriguing question in this regard was whether these scaffolds complementary to hairpin RNA structures could be extended for recognition of higher ordered RNA structures (triplexes, tetraplexes), DNA•RNA hybrids (duplex/triplex), and even purely DNA structures (duplex, triplex, tetraplex). Therefore, at the outset of our investigations, we wanted to investigate whether there were any other nucleic acids that aminoglycosides were capable of targeting and to try to understand their recognition principles in the larger context of nucleic acid selectivity. In this paper, using neomycin as the key example, I wish to show that our preliminary work of the past few years provides convincing evidence of the underutilized nature of aminoglycoside scaffolds in nucleic acid targeting.

3
The Nucleic Acid Triplex: Role of Aminoglycosides

The biochemical access to a living organism's genetic information (stored in DNA) is based on specific protein–DNA interactions. Predictive chemical principles for protein–DNA recognition are still considered complex, despite the recent progress using biological selection methods [39–43]. Recognition of duplex DNA by small molecules (minor groove binders-polyamides) [44–49] and oligonucleotides (major groove binders-DNA triple helices) [50–52] are promising alternate approaches to a chemical solution for DNA recognition. Triple strand formation has also been exploited to facilitate the delivery and enhance the sequence specificity of DNA-cutting reagents [51, 53, 54] and drugs [52, 55]. In addition, triple strand formation has been used to modify enzyme cutting patterns by selectively blocking enzyme binding sites in the major groove [56, 57]. In short, appropriately designed and constructed third strand oligonucleotides that hybridize to targeted duplex domains can be used to control gene-expression, serve as artificial endonucleases in gene mapping strategies, dictate or modulate the sequence specificity of DNA-binding drugs, and selectively alter the sites of enzyme activity. Ligands that increase the rates of association of a triplex forming oligonucleotide (TFO) to a target duplex thus have enormous potential in drug development and as tools for molecular biology.

Triple helix formation (see Fig. 1 for H-bonding in different types of triple helical structures) has been the focus of considerable interest because of possible applications in developing new molecular biology tools as well as therapeutic agents [58–64], and the possible relevance of H-DNA structures in biological systems [51, 65–67]. Intermolecular triplexes have aroused considerable interest as potential inhibitors of the expression of particular genes, since a sequence of either third-strand pyrimidines or purines, when 16–18 base pairs long, can be sufficient to be unique for recognition and binding to

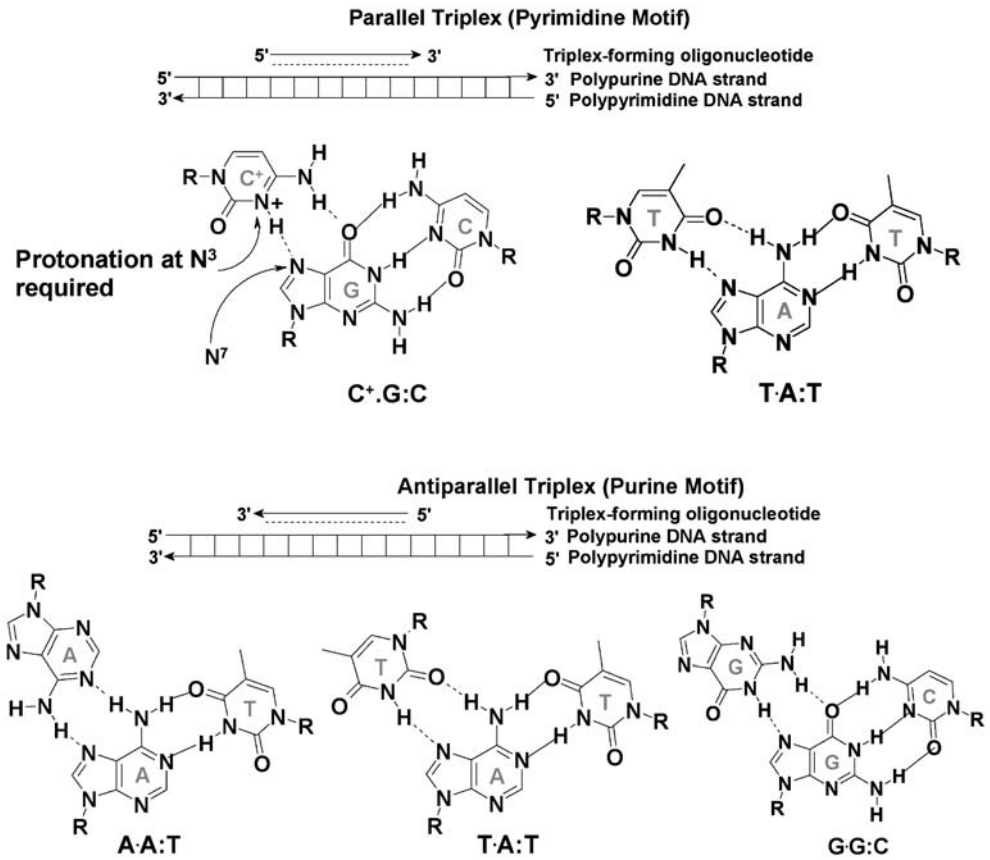


Fig. 1 Base interactions in parallel (pyrimidine motif *top*) and antiparallel (purine motif *bottom*) triple helices

defined single sites in a genome [52]. A number of experiments have now been reported that demonstrate the feasibility of the concept [68, 69].

$$\mathrm{dA{\bullet}dT} + \mathrm{dT} \rightleftharpoons \mathrm{dA{\bullet}2dT} \tag{1}$$

Association of a third strand with a duplex, however, is a thermodynamically weaker and a kinetically slower interaction than duplex formation itself (Eq. 1) [70, 71].

A number of intercalators, groove binders and polyamines have been used to stabilize triple helices [72–111]. The design of ligands that bind strongly to triple-helical structures and have a high discrimination between triplexes and duplexes opens new possibilities to control gene expression at the transcriptional level. There is a significant amount of high-resolution information on complexes of compounds that bind to both DNA and RNA by intercalation, and on compounds that bind in the DNA minor groove [48, 112]. Good models

exist for proteins and peptides that bind in the major groove of DNA and RNA [40–42]. There is, however, little information available for antibiotics that selectively bind DNA triplex grooves or RNA triplex grooves. We have recently reported on the importance of neomycin in narrowing the disparity between groove recognition of duplex versus triplex nucleic acids [113, 114]. Neomycin was shown as one of the first examples that bridge this gap and thus may lead to a novel understanding of the recognition principle(s) involved in selective targeting of triplex grooves. These results have shown that neomycin selectively stabilizes the DNA triplex without any effect on the DNA duplex.

3.1
Effect of Neomycin on a Polynucleotide Triplex

Neomycin selectively stabilizes DNA triplex without affecting the duplex [113–116]. Increasing the molar ratios of neomycin from 0–25 μM, r_{db} (ratio drug [neomycin]/base triplet)=1.67, increases the triplex melting point by nearly 25°C, whereas the duplex is virtually unaffected (Fig. 2).

3.2
Thermal Denaturation Studies with Poly(dA)•2poly(dT) in the Presence of Other Aminoglycosides and Diamines

Thermal analysis of poly(dA)•2poly(dT) in the presence of other aminoglycosides is shown as a bar graph in Fig. 3. At high concentrations (r_{db}=0.66–1.67, Fig. 3), most aminoglycosides with five or more amines are able to stabilize the triple helix (increasing $\Delta T_{m3\rightarrow2}$, without significantly affecting the $\Delta T_{m2\rightarrow1}$ values). The difference between the effectiveness of paromomycin and neomycin is quite remarkable. The structural difference between the two is a positively

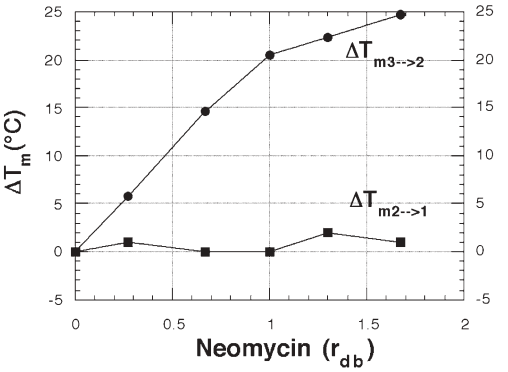


Fig. 2 Variation of triplex melting ($T_{m3\rightarrow2}$) and duplex melting ($T_{m2\rightarrow1}$) of poly(dA)•2poly(dT) as a function of increasing neomycin concentration; r_{db}=drug [neomycin]/base triplet ratio. Reprinted with permission from J Am Chem Soc (2001) 123(23):5385

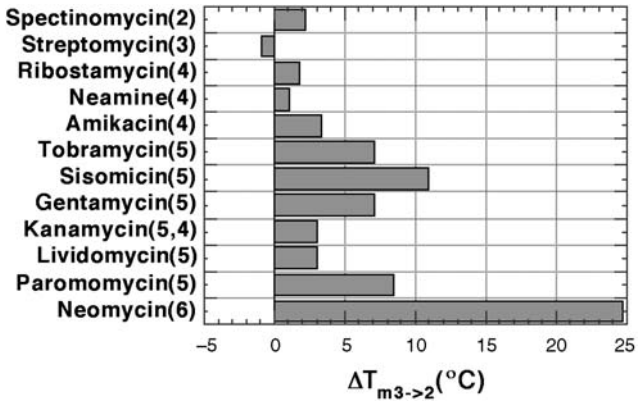
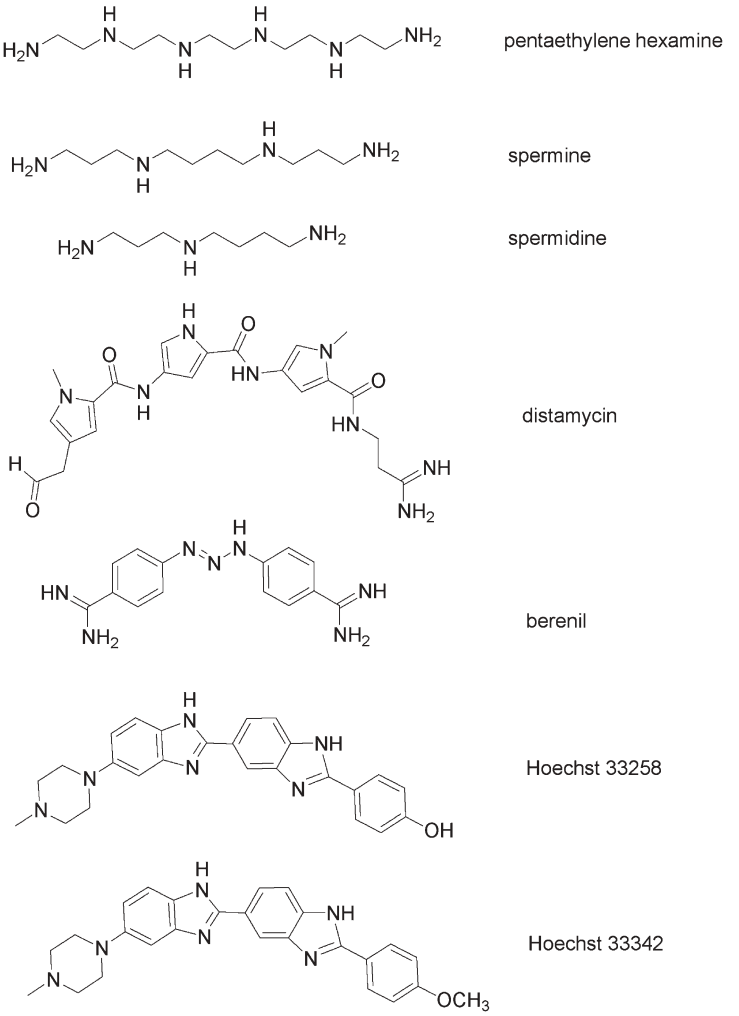


Fig. 3 Effect of aminoglycoside antibiotics on the melting of poly dA•2polydT triplex (r_{db}=1.67). Number of amines in each antibiotic is shown in parenthesis. Reprinted with permission from J Am Chem Soc (2001) 123(23):5385

charged amino group (present in neomycin), replacing a neutral hydroxyl (present in paromomycin). This leads to a difference of 10°C in $T_{m3\rightarrow2}$ values (r_{db}=0.66) and a difference of 16°C at r_{db}=1.67 [114]. At lower concentration of antibiotics (r_{db}=0.26), paromomycin has little effect on the stability of the triplex. Lividomycin, a paromomycin analog with a polyhydroxy hexose tether, is slightly less effective than paromomycin in increasing $T_{m3\rightarrow2}$ values under these conditions.

3.3 Stabilization of DNA Triple Helix Poly(dA)•2poly(dT) by Other DNA Groove Binders

In order to assess how neomycin compares to other ligands in stabilizing triplexes, thermal denaturation analyses of poly(dA)•2poly(dT) triplex in the presence of previously studied DNA minor groove binders (Scheme 3) has also been performed (Fig. 4). A comparison with groove binders, shown in Fig. 5, indicates that neomycin is much more active than the minor groove binders (berenil, DOC, DODC, DAPI, Hoechst 33258, Hoechst 33342). The minor groove binders previously studied have little preference for triple helix (berenil, distamycin and Hoechst dyes). Most groove binders stabilize the duplex as well as the triplex (Hoechst, berenil, distamycin) and some even destabilize the triplex (berenil, distamycin). The groove-binding ability of neomycin was extremely unique and presented a novel mode of triplex recognition. Neomycin, as opposed to other groove binders, differentiated the triplex grooves from those present in the duplex (Fig. 4).



Scheme 3 Structures of some groove binders known to bind duplex DNA

3.4 Thermodynamics of Drug Binding to the DNA Triplex (ITC)

An ITC-derived thermodynamic profile for neomycin binding to 12-mer intramolecular DNA triplex gave a binding constant of 2.0×10^5 M^{-1} (Fig. 5 and Table 1). The complexation is enthalpy-driven (81%), with little entropic contributions. The binding is salt-dependent, with higher salt leading to a decrease in the association equilibrium constant. A much higher binding constant of neomycin is observed with other nucleic acids-RNA triplex/DNA tetraplex (unpublished results).

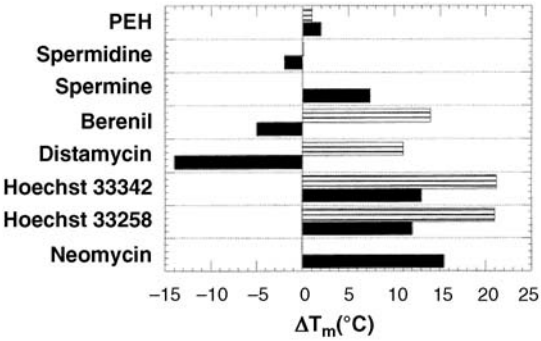


Fig. 4 Effect of 10 μM (r_{db}=0.66) groove binders on the DNA triplex melt, poly(dA)•2poly(dT) (*black bars*) and the duplex melt, poly(dA)•poly(dT) (*striped bars*). Distamycin does not show $T_{m3\rightarrow2}$ transition (20°C). *PEH* Pentaethylene hexamine. Reprinted with permission from J Am Chem Soc (2001) 123(23):5385

Table 1 ITC-derived thermodynamic profiles for the binding of neomycin to 5′–dA_{12}-x-dT_{12}-x-dT_{12}–3′ triple helix in 10 mM sodium cacodylate 0.5 mM EDTA, 150 mM KCl, pH 6.8 at 20°C

T (K)	ΔK ($\times10^5$ M^{-1})	ΔH (kcal•mol^{-1})	TΔS (kcal•mol^{-1})	ΔG (kcal•mol^{-1})	N (drug/triplex)
293	1.96±0.13	−6.9±0.3	0.18	−7.1±0.04	2.17±0.09

Reprinted with permission from J Am Chem Soc (2003) 125(13):3733.

3.5 CD/Molecular Modeling

Previous studies of neomycin have shown that it has a marked preference for binding to the larger Watson–Hoogsteen (W-H) groove of the triplex [116]. Ring I/II amino groups and Ring IV amines were proposed to be involved in the recognition process. CD/ITC studies indicate a five base triplet/drug binding site. The novel selectivity of neomycin was shown to be a function of its charge and shape complementarity to the triplex W-H groove (Fig. 6) [116].

A large number of molecules had previously been studied for triplex recognition. Before we began our investigations on aminoglycoside–triplex interactions, the goal of triplex-selective groove recognition had remained elusive. Neomycin has been shown to be the first molecule to selectively stabilize DNA triplex structures that include polynucleotides, small homopolymer, as well as mixed base triplexes [116]. This stabilization was shown to be based on neomycin's ability to bind triplexes in the groove with high affinity (based on viscometric and ITC titrations). Modeling/physicochemical results suggested a

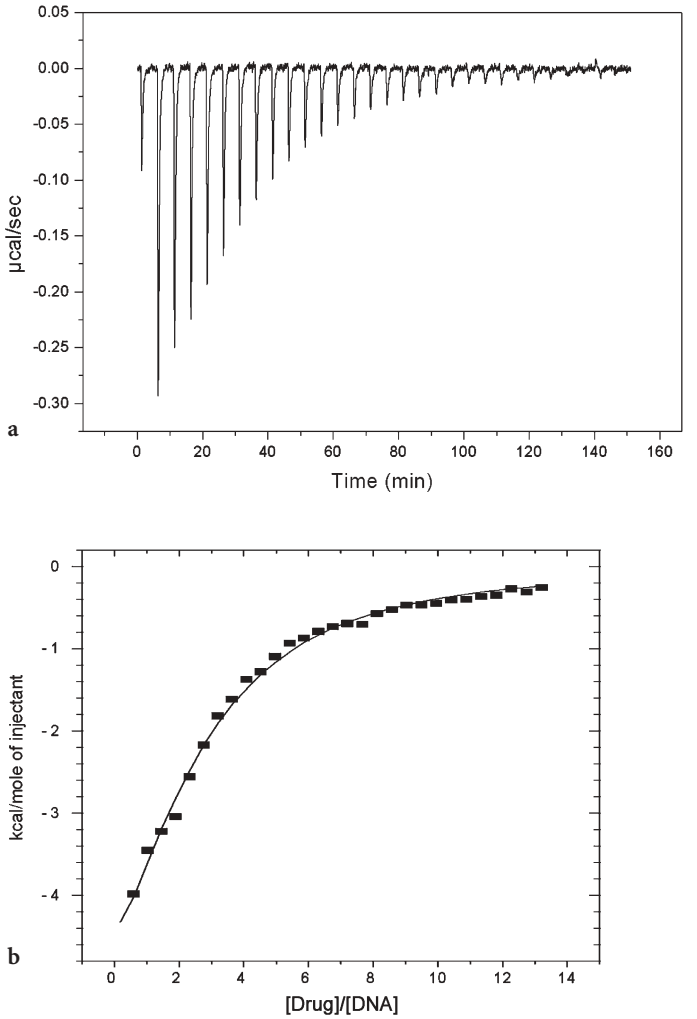


Fig. 5 **a** ITC profile of 5′-dA$_{12}$-x-dT$_{12}$-x-dT$_{12}$-3′ (4 µM/strand) titrated with neomycin (500 µM) in 10 mM sodium cacodylate, 0.5 mM EDTA, 150 mM KCl, pH 6.8 at 20°C. **b** Corrected injection heats plotted as a function of the [drug]/[DNA] ratio. The corrected injection heats were derived by integration of the ITC profile shown in Fig. 5a, followed by subtraction of the corresponding dilution heats derived from control titrations of drug into buffer alone. The *data points* reflect the experimental injection heats, while the *solid line* reflects calculated fit of the data. Reprinted with permission from J Am Chem Soc (2003) 125(13):3733

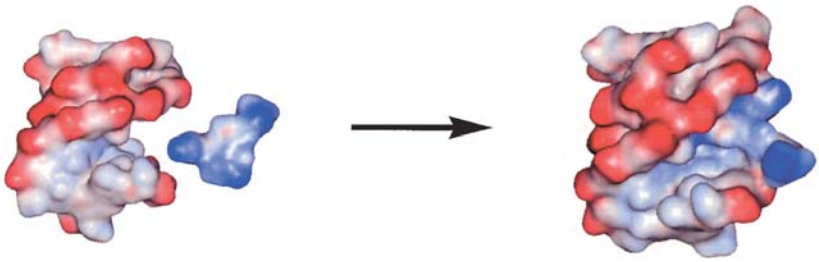


Fig. 6 Charge/shape complementarity of neomycin to the triplex W-H groove: Electrostatic surface potential maps of neomycin approaching the W-H groove of the triplex (*left*), and neomycin buried in the triplex groove (*right*). Reprinted with permission from J Am Chem Soc (2003) 125(13):3733

further preference of neomycin binding to the larger W-H groove. These findings will further contribute to the development of a new series of triplex-specific (DNA/RNA and hybrid) ligands, which may contribute to either antisense or antigene therapies.

4 DNA•RNA Hybrids

RNA•DNA hybrid duplexes are the primary targets for important enzymes that include ribonuclease-H and reverse transcriptase [117, 118]. Stable RNA•DNA triplexes normally adopt an A-type conformation and have been shown to inhibit RNA polymerase [119], DNAase-I, and RNAse [120]. Only six of the eight possible combinations of triplexes are stable under physiological conditions [121, 122]. Stabilization of poly(rA)•2poly(dT) and 2poly(rA)•poly(dT) triplexes can only be achieved under molar salt conditions [123]. Since these two triplexes could not be studied under the lower salt conditions used in competition dialysis assay, we investigated the effect of neomycin on these two triplexes using UV and CD thermal denaturation studies. Neomycin has been shown to stabilize the hybrid poly(rA)•poly(dT) duplex [113], and even induce poly(rA)•2poly(dT) triplex formation [113], much more effectively than previously reported ligands [100]. The effect of aminoglycosides on hybrid duplex and triplex structures showed that almost all aminoglycosides stabilized the hybrid poly(dA)•poly(dT) duplex (see Fig. 7). It is noteworthy that formation of these triple helices require molar salt in the absence of the drug, whereas micromolar neomycin concentration can induce the triplex formation. Recently, work from the Pilch lab [124] has also corroborated these findings and shown a high binding constant (10^7 M^{-1}) for aminoglycoside binding to small RNA•DNA hybrids.

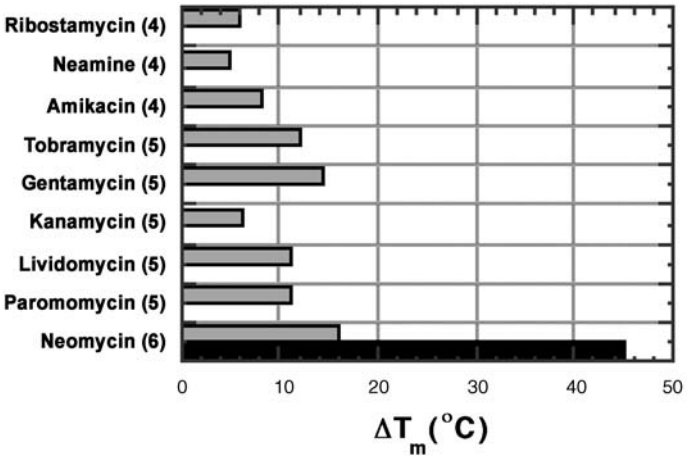
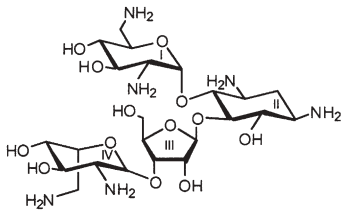


Fig. 7 Effect of added aminoglycoside (r_{db}=0.66) on the stabilization of rA•dT duplex (*gray*) and on inducing rA•2dT triplex (*black*). Number of amines in each aminoglycoside is shown in parenthesis. $\Delta T_{m3\rightarrow2}$ is calculated by assuming a $T_{m3\rightarrow2}$ of 10°C in the absence of neomycin (no transition seen). Reprinted with permission from J Am Chem Soc (2001) 123(44):11093

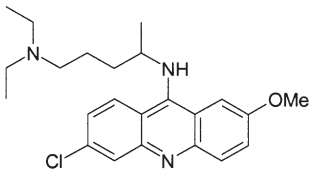
5
The A-Form Nucleic Acids

5.1
Competition Dialysis of Neomycin–Acridine Conjugate with Nucleic Acid Forms

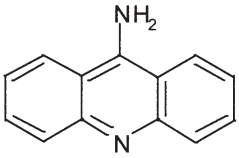
The remarkable ability of neomycin and other aminoglycosides to stabilize DNA, RNA, and hybrid triple helices has been reported and discussed above [113–116]. Neomycin has also been shown to induce the stabilization of hybrid duplexes as well as hybrid triple helices [113]. This significantly added to the number of nucleic acids (other than RNA) that aminoglycosides have been shown to target. A clear requirement then arose for a quantitative assay to determine the relative binding affinities for host triplex, duplex DNA, single-stranded (SS) DNA/RNA and other possible nucleic acid targets (tetraplex) for a given aminoglycoside ligand. Fortunately, a rapid technique has been established by Chaires for this exact purpose, using a thermodynamically rigorous competitive equilibrium dialysis method that exploits therapeutically useful drug concentrations [91, 125]. In the assay, solutions consisting of identical concentrations of different nucleic acid structures were dialysed simultaneously against a common ligand dissolved in appropriately buffered conditions. After equilibration, the amount of ligand bound to each DNA was measured by spectrophotometry. More ligand accumulated in the dialysis tube containing the structural form of highest binding affinity and, since all of the DNA samples were in equilibrium with the same free ligand concentration, the amount of ligand bound was directly proportional to the binding constant for each con-



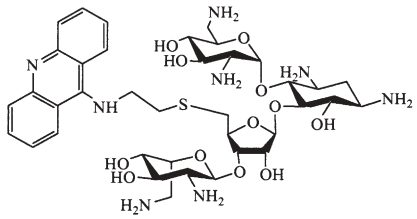
Neomycin



Quinacrine



9-aminoacridine



neomycin-acridine conjugate

Fig. 8 Structures of neomycin, aminoacridines, and the neomycin–acridine conjugate

formational form. Thus, comparison among the DNA samples gave a rapid and thermodynamically reliable indication of structural selectivity for any given ligand (an updated review on competition dialysis also appears in this volume).

Since aminoglycosides do not have a chromophore for spectrophotometric analysis, competition dialysis of three acridines with increasing positive charge was used to decipher aminoglycoside specificity (Fig. 8). Competition dialysis studies were carried out using 9-aminoacridine, quinacrine, and a neomycin–acridine (neo-acridine) conjugate [126] against 14 different nucleic acids. Going from acridine to neo-acridine, we were able to parse the effect of neomycin conjugated to the acridine chromophore. At first sight, dialysis of neo–acridine (Fig. 9) showed highly promiscuous binding with little preference for any specific nucleic acid structure, except for a clear preference for RNA triplex. Among comparable single strand, duplex, and triplex structures, maximum binding was always observed with the triplexes. This seemingly promiscuous binding yielded a different picture upon careful analysis of the dialysis data. All three drugs showed comparable binding to one nucleic acid: calf thymus DNA. Calf thymus DNA also represents a standard duplex DNA. This observation was used to replot the dialysis results to emphasize differences relative to that standard. These results, shown in Fig. 10, better illustrate the change in specificity of the different acridines toward different nucleic acids. While 9-aminoacridine and quinacrine showed a clear preference for DNA triplex, neo-acridine binding to RNA triplex is much greater than DNA triplex and even better than the natural aminoglycoside RNA target: eubacterial 16S A-site. Drug binding was

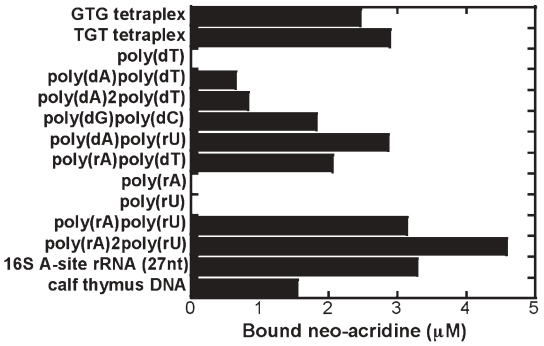


Fig. 9 Competition dialysis results of neo-acridine (1 μM) with various nucleic acids; 180 μL of different nucleic acids (75 μM per monomeric unit of each polymer) were dialyzed with 400 mL of 1 μM neo-acridine in BPES buffer (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM Na_2EDTA, 185 mM NaCl, pH 7.0) solution for 72 h. Reprinted with permission from J Am Chem Soc (2003) 125(34):10148

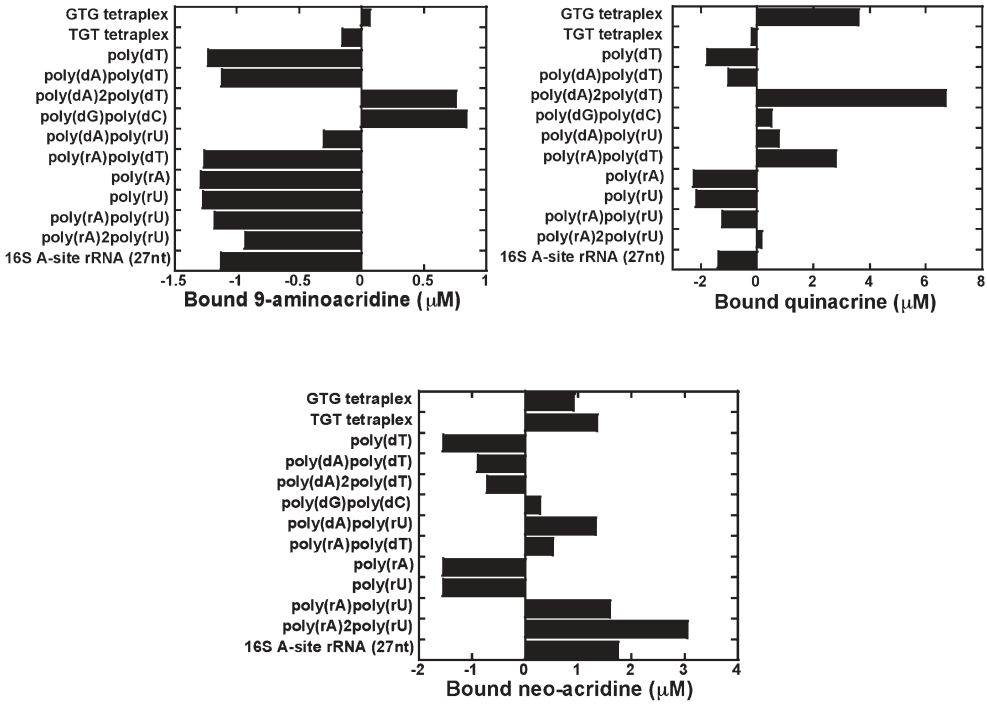


Fig. 10 Competition dialysis results (difference plots, with calf thymus DNA as reference) of 9-aminoacridine, quinacrine, and neo-acridine (1 μM) with various nucleic acids. Experimental conditions were identical to those for Fig. 6. Maximum binding of neo-acridine is observed with nucleic acids that can adopt the A-type conformation. Reprinted with permission from J Am Chem Soc (2003) 125(34):10148

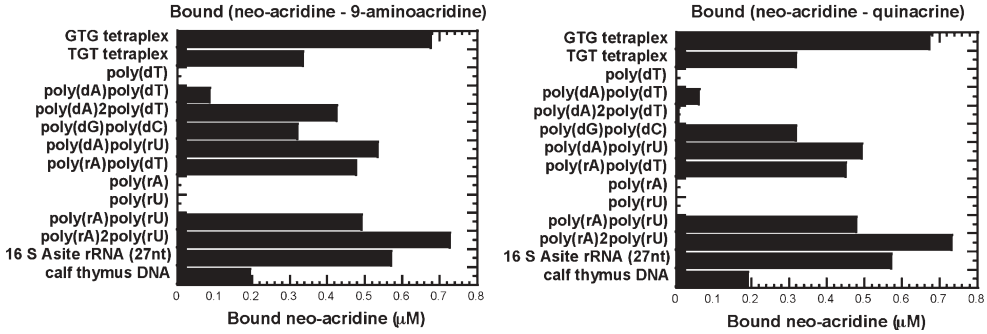


Fig. 11 Competition dialysis results of 100 nM drug: difference plots, neo-acridine minus 9-aminoacridine (*left*) and neo-acridine minus quinacrine (*right*). 180 μL of different nucleic acids (7.5 μM per monomeric unit of each polymer) were dialyzed with 400 mL of 100 nM ligand in BPES buffer (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM Na_2EDTA, 185 mM NaCl, pH 7.0) solution for at least 24 h

also observed with DNA as well as RNA duplex, and even with DNA tetraplex. The binding to DNA tetraplex was still lower than to the RNA triplex. RNA•DNA duplexes were better targets than DNA homoduplexes; poly(dA)•poly(rU) hybrid duplex being comparable in binding to the tetraplexes. Previous studies with aminoglycoside natural products have shown no effect on the stability of A•T-rich duplex DNA (in the presence of salt), suggesting weaker, nonproductive binding. Triplexes, then, are the targets of choice for neomycin. Neo-acridine shows a remarkable binding preference to RNA triplex that has not previously been observed. A big surprise, however, was the significant binding observed with the poly(dG)•poly(dC) duplex.

A competition dialysis assay using tenfold (100 nM) and 100-fold (10 nM) lower concentrations (nanomolar range) was also carried out. Results from dialysis under 100 nM drug concentration (Fig. 11) showed that neo-acridine favors nucleic acid forms that can adopt an A-type conformation. However, reliable results could not be obtained at 1 nM and 10 nM concentrations due to the low fluorescence intensity of the neo-acridine conjugate.

Neo-acridine binding to RNA triplex was also investigated by UV thermal melts, ITC, viscometric and CD titrations. Thermal denaturation in the presence of neo-acridine showed an increase in T_{m3-2} at low drug concentrations. At higher drug concentrations, the duplex was stabilized as well. We have previously shown neomycin to be one of the best stabilizers of an RNA triple helix [114]. Viscosity measurements showed a clear groove binding (as seen by shortening of RNA triplex length) upon titration of neomycin as well as neo-acridine into the triplex [127].

5.2
The Common Thread that Holds Together RNA Duplex/Triplex, DNA-RNA Hybrid Duplexes, DNA Tetraplexes, and the Poly(dG)•poly(dC) Duplex is the Propensity Towards an A-type Conformation

RNA duplex structures are known to adopt an A-type conformation, as are hybrid duplexes [128]. dG•dC-rich DNA duplex sequences [129] have also been shown to have a high propensity for A-form in the presence of cations, including neomycin [130], and CD studies have suggested the A-like solution conformation of G4 tetraplexes [131]. Further evidence of A-type preference was observed with the change in the CD spectrum of poly(dG)•poly(dC) upon inclusion of neo-acridine. A shift in λ_{max} from 257 nm to 267 nm, and increased signal in this range, in the presence of this drug, was strongly indicative of a B–A transformation, as observed by Wang [130] as well as Kypr [129, 131] in similar CD experiments. Additionally, the differences in binding to DNA•RNA hybrids can be attributed to the fact that poly(dA)•poly(rU) has been known to adopt an A-type conformation whereas poly(rA)•poly(dT) can exist in the B-form [128].

5.3
Importance of A-Form DNA and its Recognition

The polymorphism of DNA was noticed early after the discovery of its double helical structure [132]. The conformations of DNA have since been limited to two major distinctions: A-DNA and B-DNA (other less-well-known structures do exist). Both structures are of identical topology and hydrogen bonding patterns, but they differ largely in their overall shape (Fig. 12). B-DNA has long

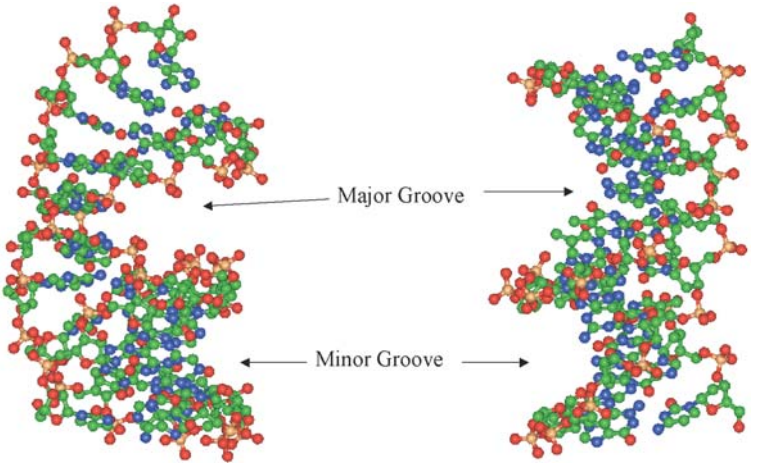


Fig. 12 Conformations of an A-type duplex (*left*) and a B-type duplex (*right*), generally seen for RNA•RNA and DNA•DNA duplexes, respectively. The B-form duplex has a much wider major groove

been believed to be the dominant biological conformation, implementing water molecules and biological cations appropriately within its structure. A-DNA, on the other hand, requires dehydrated conditions. The transition of B- to A-DNA is a reversible and cooperative process [133], in which the A-form is considered the higher-energy state. The underlying factors for this instability have been addressed, but with little success [134, 135].

Native DNA, which comprises the genetic information of all known free organisms, mostly adopts B-form under physiological conditions because it is associated with high humidity in fibers or with aqueous solutions of DNA. However, it is important to switch B-DNA into the A-form in a living organism since constitutive conformation of double-stranded RNA is predominantly A-type. RNA probably preceded DNA in evolution [136], so the basic mechanisms of genetic information copying are likely to have evolved on an A-form rather than B-form. In fact, the template DNA is induced by many polymerases into A-form at positions of genetic information copying in the microenvironment. Thus, DNA switching into A-form may influence replication and transcription of the genomes.

Understanding aminoglycoside A-form nucleic acid interactions then has underlying importance to the area of drug development as well as to the fundamental understanding of A-form recognition because:

1. Novel nucleic acid therapeutic targets can be identified with a better understanding of the thermodynamics and kinetics of molecular recognition involved in aminoglycoside specificity. We have already initiated such a program in the development of novel antimicrobial agents targeting novel RNA and DNA sequences [137, 138].
2. As opposed to B-form DNA recognition, very few small molecules (multivalent cations) [133, 139, 140] are known that select for A-form structural features. Aminoglycosides present a novel scaffold for groove recognition of A-form structures.
3. Aminoglycoside binding to such higher order structures (H-DNA triplex) has also been implicated in their toxic side effects [114]. A better understanding of aminoglycoside binding and selectivity can also help in a better understanding of toxic side-effects of these broad spectrum antibiotics.

6
From A- to B-Form Nucleic Acids: Using Organic Chemistry to Tune Aminoglycoside Selectivity

Aminoglycosides most likely bind in the major groove of A-form structures (much like RNA, as the A-form nucleic acids have a narrower major groove) [127]. The B-form duplex has a much larger major groove and does not provide a good shape complementarity for aminoglycoside binding (see Fig. 13). These findings have complemented the success in development of DNA duplex-spe-

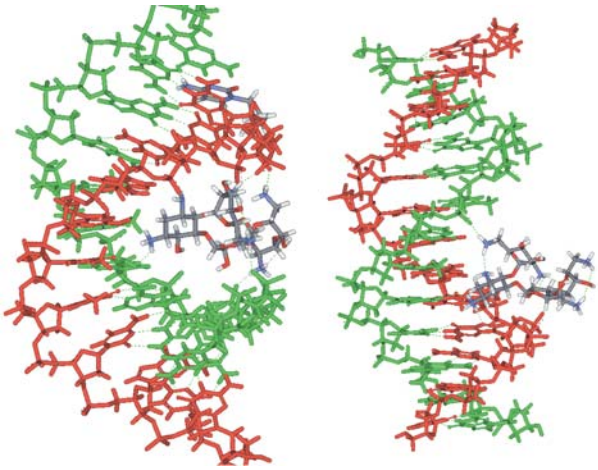
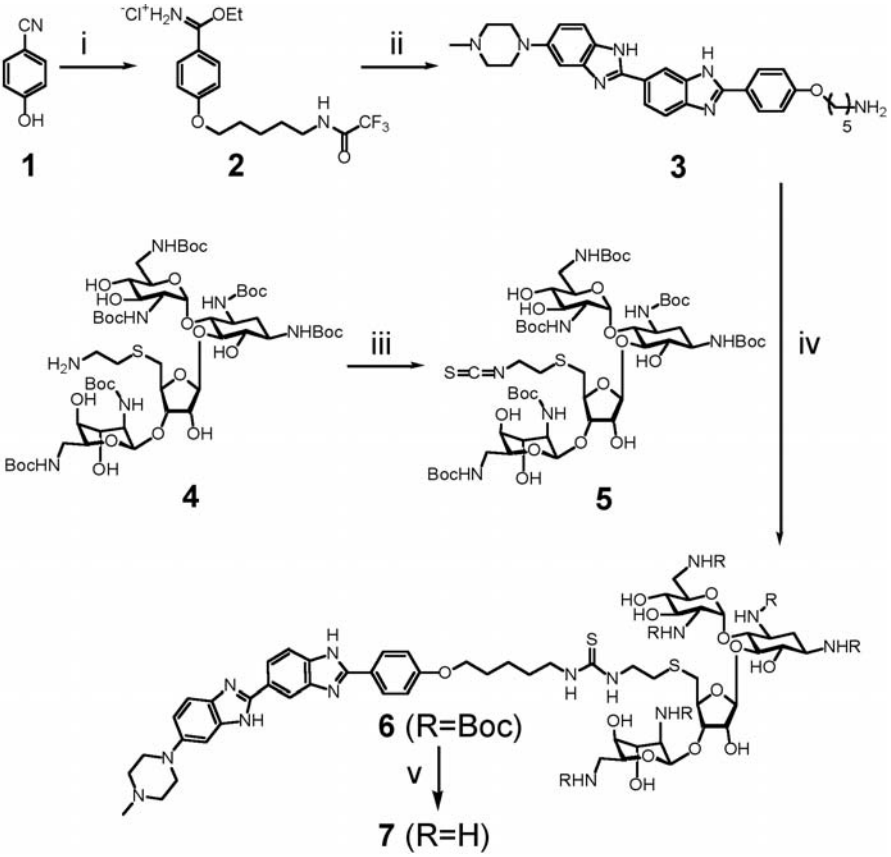


Fig. 13 Charge and shape complementarity of neomycin to the A-form major groove: Computer models of neomycin docked in the major groove of A-form DNA (*left*), and neomycin buried in the B-form major groove (*right*)

cific groove binders in the past few decades, among which netropsin, distamycin and Hoechst 33258 have been the lead compounds. We wished to investigate whether a molecule like neomycin could be forced into the B-form DNA major groove. Therefore, conjugation of neomycin to Hoechst 33258 was accomplished. 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 promising drug candidates for development of inhibitors of transcription factors [141]. To answer these questions, the synthesis and nucleic acid binding of a novel neomycin–Hoechst 33258 conjugate has been recently reported. The conjugate showed remarkable stabilization of DNA duplexes and destabilization of the DNA triplex.

Starting from the natural product neomycin B, which is commercially available as the tri-sulfate salt, Boc (*t*-butoxycarbonyl) protection of the six amino groups followed by conversion to 2,4,6-triisopropylbenzenesulfonyl derivative, and subsequent substitution by aminoethanethiol, gave rise to the protected neomycin amine [126] compound **4**. Treatment of **4** with 1,1′-thiocarbonyldi-2(1*H*)-pyridone using a catalytic amount of DMAP gave isothiocyanate derivative **5**, which was coupled with bis(benzimidazole) **3** and deprotected to give conjugate **7** (Scheme 4).

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** displayed 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



Scheme 4 Reagents and conditions: *i a* 5-trifluoroacetamido-1-pentanol, PPh_3, DIAD, dioxane, r.t., 2 h, 84%; *i b* HCl, EtOH, 0°C, quant.; *ii a* 2-(3,4-diaminophenyl)-6-(1-methyl-4-piperazinyl) benzimidazole, HOAc, reflux, 4 h, 38%; *ii b* K_2CO_3 in 5:2 MeOH:H_2O, r.t., overnight, 94%; *iii* 1,1′-thiocarbonyldi-2(1*H*)-pyridone, cat. DMAP, CH_2Cl_2, r.t. 20 h, 95%; *iv* **3**, pyridine, r.t., overnight, 72%; *v* 1:1 CH_2Cl_2, TFA, r.t., 3 h, quant. Reprinted with permission from J Am Chem Soc (2003) 125(41):12398

the thermal stability of duplex DNA) and Hoechst 33258, which displayed some degree of stabilization of duplex DNA (Fig. 14).

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 Fig. 14, 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). This suggests that **7** stabilizes the duplex better than the individual parent 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

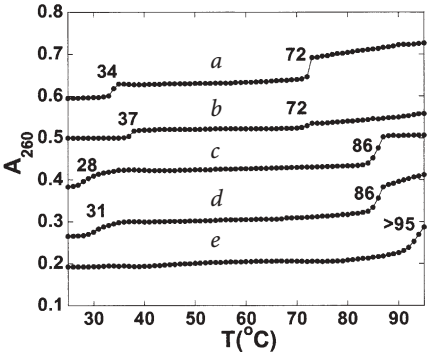


Fig. 14 UV melting profile of poly(dA)•2poly(dT) in the absence (*a*) and presence of 2 μM neomycin (*b*), 2 μM Hoechst 33258 (*c*), 2 μM Neomycin+2 μM Hoechst 33258 (*d*), and 2 μM Hoechst–neomycin conjugate (*e*). Samples of DNA (15 μM/base triplet) in buffer (10 mM Na cacodylate, 0.5 mM EDTA, 150 mM KCl, pH 7.20) containing ligand were analyzed for UV absorbance at 260 nm from 20–95°C using a temperature gradient of 0.2°C min^{-1}. Reprinted with permission from J Am Chem Soc (2003) 125(41):12398

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 [142]. UV melting showed increased stability of the duplex in the presence of **7**, with a ΔT_m=25°C, compared to ΔT_m=14°C for Hoechst 33258 [142].

Further studies of numerous duplex DNA 22-mers of varying G/C content (breaking up stretches of A/T base pairs) were carried out. In all cases where stretches of at least 4 base pairs were present, ΔT_m for **7** was at least 10°C higher than that for Hoechst 33258. Duplex stabilization by **7** followed the selectivity shown by Hoechst 33258 (Fig. 15a), whereas neomycin had 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 Fig. 15b. 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 (Fig. 15b). 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 [142] (~10^8 M^{-1}) as opposed to neomycin binding to triplex (10^5–10^6 M^{-1}) [116]. Conjugates of different linker sizes can then perhaps be designed to target

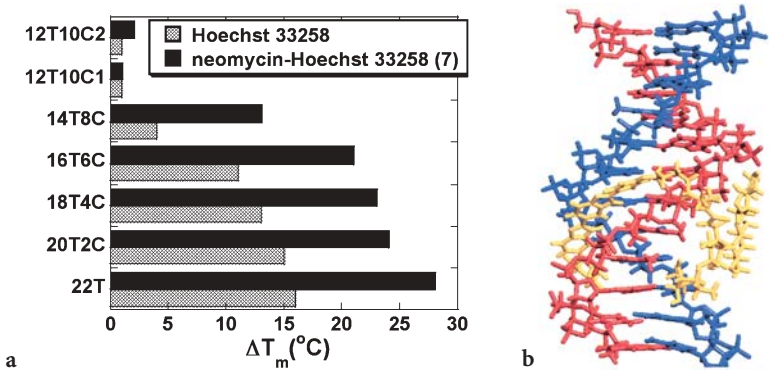


Fig. 15 **a** Bar graph of ΔT_m for 22-mer duplexes in the presence of 4 μM Hoechst 33258 and 4 μM neomycin–Hoechst 33258 7 obtained from UV melting profiles (solution conditions were identical to those for Fig. 14). Reprinted with permission from J Am Chem Soc (2003) 125(41):12398. **b** Computer model of neomycin–Hoechst 33258 (*yellow*, linker atoms shown in *white*) docked in the DNA major-minor grooves. Reprinted with permission from J Am Chem Soc (2003) 125(41):12398

a structure of preference and should aid in the development of even more selective and potent conjugates. Development of such dual recognition ligands opens up new avenues in targeting nucleic acids and is being further explored in our laboratories.

7
Targeting Nucleic Acids with Aminoglycoside–DNA and PNA Conjugates

7.1
RNA Sequence-Specific Aminoglycoside–ODN Conjugates

RNA has now become a well-established drug target [36, 143, 144]. Small molecules and antisense oligonucleotides are now being used to down-regulate gene expression. Vitravene, the first antisense drug, was approved by the FDA at the end of the 20th century [145]. RNA has distinct advantages in antibacterial and antiviral treatment. Primarily, appearance of drug resistance through point mutations in a conserved RNA motif among bacteria or viral strains is likely to be slow. Bacteria become resistant to ribosomal RNA-binding antibiotics through exchange of genetic material encoding RNA-modifying enzymes (typically methyltransferases and phosphotransferases), drug-modifying enzymes, or enzymes that affect drug transport [146, 147]. Therefore, if the structure of the nucleic acid-binding drug is novel, the emergence of resistance is likely to be slower than for protein targets (barring any novel efflux pump mechanisms). Antisense/antigene therapy can offer a viable alternative in tackling such resistance mechanisms.

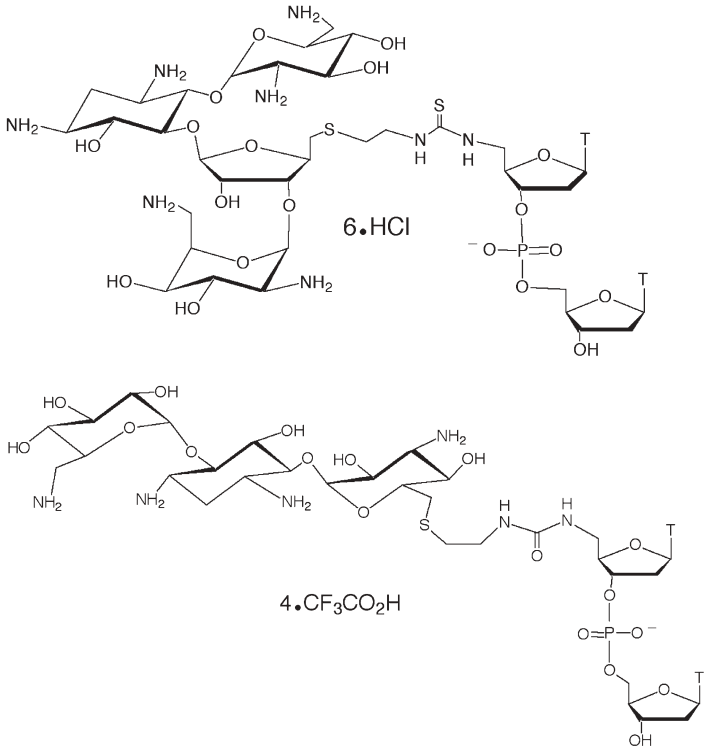
Recent findings that aminoglycosides can stabilize DNA/RNA triplexes [114, 115], hybrid duplexes [113], and that neomycin can even induce hybrid triplex formation [113] suggested that aminoglycoside–DNA conjugates could be effective models for targeting nucleic acids sequence specifically (via a hybrid duplex or triplex formation). Conjugation of an aminoglycoside to an ODN can assist in the following processes:

1. Delivery of aminoglycoside to a specific DNA/RNA site
2. Increasing the stabilization inferred by these hybrid duplex/triplex stabilizing agents
3. The unique structure of aminoglycosides can aid in cellular permeability/site-specific delivery of the ODN

To investigate the advantage of nucleic acid-based specificity coupled with aminoglycoside charge/shape complementarity, we have described a general strategy for the synthesis of covalently attaching aminoglycosides (neomycin) to nucleic acid analogs (DNA/PNA). Recently [148–150], ultrarapid functional genomics technologies have helped identify approximately 4,000 essential gene drug targets in 11 clinically relevant bacterial and fungal pathogens. In contrast, most antimicrobials prescribed today inhibit only a small fraction of this number of targets within bacterial and fungal pathogens. A comprehensive approach to identifying such essential drug targets in multiple pathogens can be combined with a complementary approach of developing antimicrobial agents that are sequence-specific to previously known, as well as rapidly identified, new RNA targets. Interestingly, a shotgun antisense technology was used as the key tool to identify these 4,000 essential genes, suggesting that oligos binding to these RNA targets will be able to selectively shut down protein synthesis. Additionally, the finding that aminoglycosides can stabilize DNA/RNA triplexes and DNA•RNA duplexes [113, 114, 116] suggests that neomycin–ODN conjugates could also be effective models for targeting nucleic acids sequence-specifically via triplex or hybrid duplex formation.

7.1.1
Synthesis of Aminoglycoside Isothiocyanates/ODN–Aminoglycoside Coniugate

The amino groups on rings I, II, and IV (neomycin) are necessary in recognizing and in stabilizing various nucleic acid forms (aminoglycosides without any of these amines do not stabilize DNA tniplexes as efficiently) [137]. The conjugates based on aminoglycosides must then retain these amines. The 5″-OH on ring III (neomycin) was chosen to provide the linkage to the nucleic acids (for ring numbering, please see Scheme 1). We recently reported the synthesis of neomycin isothiocyanate as a stable reagent that can be coupled to a variety of amines [137]. Scheme 4 shows the synthesis of neomycin isothiocyanate, starting from neomycin amine. The use of this isothiocyanate in the synthesis of a DNA 5′–aminothymidine dimer conjugated to neomycin and kanamycin also has been recently reported (Scheme 5) [137].

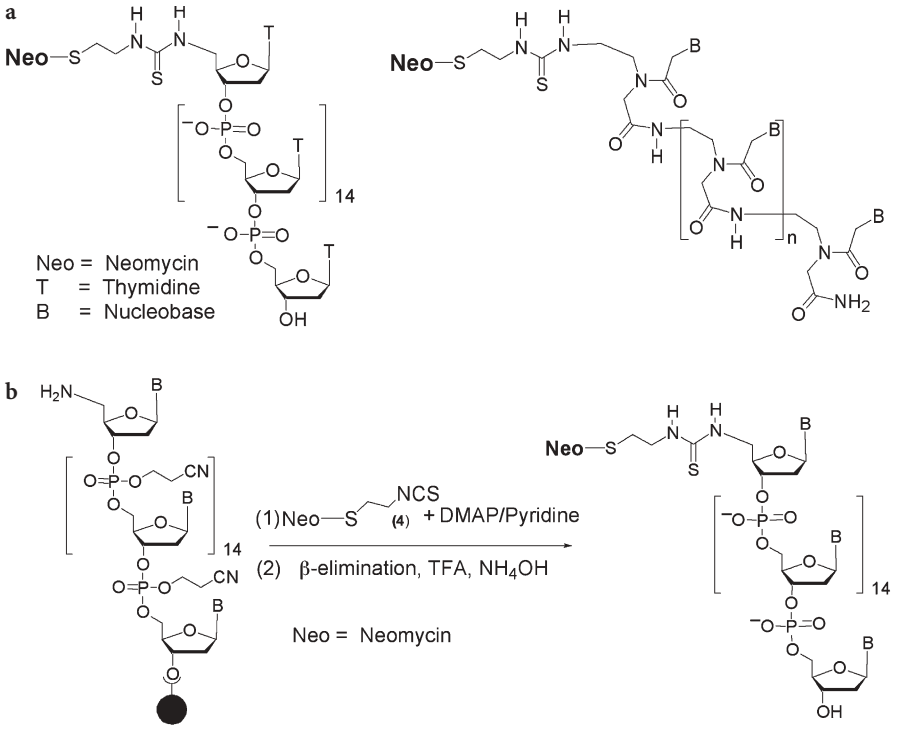


Scheme 5 Structures of neomycin–DNA and kanamycin–DNA dimers

7.1.2 Synthesis of Oligomeric Neomycin–ODN Coniugates

7.1.2.1 Neomycin–DNA/PNA Conjugate

The structures of generic neomycin DNA and PNA conjugates is shown in Scheme 6a and has been recently reported [138]. The synthesis of neomycin conjugated to 5′-end of a oligonucleotide $dT_{(16)}$ is shown in Scheme 6b. Neomycin is linked to the DNA via a thiourea linkage. Neomycin isothiocyanate (Scheme 6b) has been coupled to a 5′-amino-5′-deoxy ODN, which is easily prepared by incorporation of 5′-amino-5′ deoxythymidine (or cytidine) in a growing ODN chain. The synthesis of neomycin linked to a 16-mer DNA $dT_{(16)}$ has been reported [138]. The reactive amine at the 5′-end of $dT_{(16)}$ (Scheme 6b) was treated with a pyridine solution containing neomycin isothiocyanate and 4-dimethylaminopyridine (DMAP) for 12 h at room temperature, washed with trifluoroacetic acid (TFA), and deprotected from solid support with NH_4OH [138]. Having established that these conjugates can be synthesized on solid phase using conventional DNA synthesis, attention can now be devoted to syn-



Scheme 6 **a** Structure of a generic aminoglycoside–DNA/PNA conjugate. **b** Synthesis of neomycin–DNA conjugate on the solid phase

thesizing ODNs for targeting anticancer and antimicrobial DNA sequences of interest [138].

Although considerable advances have been made in antisense technology over the last few decades, there are still some issues that warrant active investigation [151]. These include:

1. Increasing the binding affinities (kinetic/thermodynamic) of ODNs to their target duplexes and single strands
2. Improving the delivery and uptake of oligonucleotides into any cell or tissue of interest
3. Enhancing the stabilities of the oligonucleotides inside the cells

Our preliminary work shows that aminoglycosides can considerably enhance the binding affinities of the ODNs to their duplex DNA target as well as to the single strand RNA targets. Further investigations of the molecular basis of such stabilizations and then using it to synthesize aminoglycoside–DNA/PNA conjugates with improved stability is being carried out in our laboratories. The approach could open up doors for developing sequence-specific anticancer and antimicrobial drugs.

8 Summary

Electrostatic complementarity has been successfully used to explain the structural basis of RNA binding to their aminoglycoside substrates [33–37]. Perhaps the best complementarity for aminoglycosides with a natural target is observed with eubacterial ribosomal 16S A-site. The structural basis of A-form specificity may be related to the closeness of the two negatively charged sugar-phosphate backbones along the major groove in A-DNA, which can be effectively neutralized by the multivalent positively charged amine functions of aniinoglycosides. Groove recognition of triplexes and tetraplexes has been an elusive feat, where such charged polyamine binding factors may be the key to opening this Pandora's box. While these findings do not question that aminoglycoside's mechanism of drug action involves binding to rRNA, they reevaluate, as a matter of biochemical principles, the common belief that aminoglycoside specificity is simply for RNAs, and subsequently unveil new targets for aminoglycoside-based drug development.

Acknowledgements I am greatly indebted to my students, postdoctoral researchers, and collaborators for their hard work and patience. Funding for this work was made available by Clemson University and the NSF (CHE/MCB-0134792).

References

1. Chow CS, Bogdan FM (1997) Chem Rev 97:1489
2. Kotra LP, Haddad J, Mobashery S (2000) Antimicrob Agents Chemother 44:3249
3. Santucci R, Krieger J (2000) J Urol 163:1076
4. Forge A, Schacht J (2000) Audio Neurootol 5:3
5. Gerding D (2000) Infect Control Hosp Epidemiol 21: S12
6. Kaul M, Barbieri CM, Kerrigan JE, Pilch DS (2003) J Mol Biol 326:1373
7. Moazed D, Noller HF (1987) Nature 327:389
8. Purohit P, Stern 5 (1994) Nature 370:659
9. Recht MI, Fourmy D, Blanchard SC, Dablquist KD, Puglisi JD (1996) J Mol Biol 262:421
10. Miyaguchi H, Narita H, Sakamoto K, Yokoyama 5 (1996) Nucleic Acids Res 24:3700
11. Waksman SA (1945) Science 102:40
12. Waksman SA, Lechevalier HA (1949) J Antibiot 109:305
13. Davies J (1965) Antimicrob Agents Chemother (1961–70): 1001
14. Davies J, Davis BD (1968) J Biol Chem 243:3312
15. Tok JBH, Cho J, Rando RR (1999) Biochemistry 38:199
16. Zapp ML, Stem 5, Green MR (1993) Cell 74:969
17. Hermann T, Westhof E (1998) Biopolymers 48:155
18. Mei H-Y, Galan AA, Halim NS, Mack DP, Moreland DW. Sanders KB, Hoa N. Truong, Czarnik AW (1995) Bioorg Med Chem Lett 5:2755
19. von Ahsen U, Noller HF (1993) Science 260:1500
20. Mikkelsen NE, Brannvall M, Virtanen A, Kirsebom LA (1999) Proc Natl Acad Sci USA 96:6155

21. Walter F, Murchie Al, Thomson JB, Lilley DM (1998) Biochemistry 37:14195
22. Eamshaw DJ, Gait MJ (1998) Nucleic Acids Res 26:5551
23. Tor Y, Hermann T, Westhof E (1998) Chem Biol 5: R277
24. Clouet-d'Orval B, Stage TK, Uhlenbeck OC (1995) Biochemistry 34:11186
25. Stage TK, Hertel KJ, Uhlenbeck OC (1995) RNA 1:95
26. Chia JS, Wu HL, Wang HW, Chen DS, Chen PJ (1997) J Biomed Sci 4:208
27. Rogers J, Chang AH, von Ahsen U, Schroeder R, Davies J (1996) J Mol Biol 259:916
28. Mei H-Y, Mack DP, Galan AA, Halim NS, Heldsinger A, Loo JA, Moreland DW, Sannes-Lowery KA, Sharmeen L, Truong HN, Czarnik AW (1997) Bioorg Med Chem 5:1173
29. van Leeuwen F, de Kort M, van der Marel GA, van Boom JH, Borst P (1998) Anal Biochem 258:223
30. Gao Y-g, Robinson H, Wijsman ER, Marel GAvd, Boom JHv, Wang AH-J (1997) J Amer Chem Soc 119:1496
31. Ren J, Qu X, Dattagupta N, Chaires J (2001)
32. Tor Y, Hermann T, Westhof E (1998) Chem Biol 5: R277
33. Hermann T (2000) Angew Chem Int Ed Engl 39:1890
34. Hermann T, Westhof E (1998) Biopolymers 48:155
35. Hermann T, Westhof E (1998) J Mol Biol 276:903
36. Hermann T, Westhof E (1998) Curr Opin Biotechnol 9:66
37. Hermann T, Westhof E (1999) J Med Chem 42:1250
38. Henry CM (2000) Chem Eng News 78:41
39. Greisman HA, Pabo CO (1997) Science 275:657–661
40. Wolfe SA, Nekludova L, Pabo CO (2000) Annu Rev Biophys Biomol Struct 29:183
41. Choo Y, Klug A (1997) Cur Opin Struct Biol 7:117–125
42. Nagai K (1996) Curr Opin Struct Biol 6:53
43. Ellenberger T (1994) Curr Opin Struct Biol 4:12
44. Dervan PB (1986) Science 232:464
45. Dervan PB, Burli RW (1999) Cur Opin Chem Biol 3:688
46. Gottesfeld JM, Turner JM, Dervan PB (2000) Gene Expr 9:77
47. Geierstanger BH, Wemmer DE (1995) Annu Rev Biophys Biomol Stnuct 24:463
48. Wemmer DE (2000) Annu Rev Biophys Biomol Struct 29:439
49. Kopka ML, Yoon C, Goodsell D, Pjura P, Dickerson RE (1985) Proc Natl Acad Sci USA 82:1376–1380
50. Felsenfeld U, Rich A (1957) Biochim Biophys Acta 26:457
51. Moser HE, Dervan PB (1987) Science 238:645
52. Praseuth D, Guieysse AL, Helene C (1999) Biochim Biophys Acta 1489:181
53. Francois J-C, Saison-Behmoaras T, Barbier C, Chassignol M, Thuong NT, Helene C (1989) Proc Natl Acad Sci USA 86:9702
54. Strobel SA, Moser HE, Dervan PB (1988) J Am Chem Soc 110:7927
55. Sun J-S, Francois J-C, Montenay-Garestier T, Saison-Behmoaras T, Roig V, Thuong NT, Helene C (1989) Proc Natl Acad Sci USA 86:9198
56. Maher III U, Wold B. Dervan PB (1989) Science 245:725
57. Hanvey JC, Shimizu M, Wells RD (1989) Nucleic Acids Res 18:157
58. Frank-Kamenetskii MD, Mirkin SM (1995) Annu Rev Biochem 64:65
59. Ganesh KN, Kumar VA, Barawkar DA (1996) In: Hamilton AD (ed) Supramolecular control of structure and reactivity. Wiley, p 263
60. Kool ET (1997) New J Chem 21:33
61. Radhakrishnan I, Patel DJ (1994) Biochemistry 33:11405
62. Shafer RH (1998) Prog Nucleic Acid Res Mol Biol 59:55
63. Thuong NT, Helene C (1993) Angew Chem Int Ed Engl 32:666

64. Kool ET (1998) Acc Chem Res 31:502
65. Wells RD, Collier DA, Hanvey JC, Shimizu M, Wohlrab F (1988) FASEB J 2:2939
66. Htun H, Dahlberg JE (1989) Science 243:1571
67. Htun H, Dahlberg JE (1988) Science 241:1791
68. Grigoriev M, Praseuth D, Robin P, Hemar A, Saison-Behmoaras T, Dautry-Varsat A, Thuong NT, Helene C, Harel-Bellan A (1992) J Biol Chem 267:3389
69. Grigoriev M, Praseuth D, Guieysse AL, Robin P, Thuong NT, Helene C, Harel-Bellan A (1993) Proc Natl Acad Sci USA 90:3501
70. Craig ME, Crother DM, Doty P (1971) J Mol Biol 62:383
71. Rougee M, Faucon B, Mergny JL, Barcelo F, Giovannageli C, Garestier T, Helene C (1992) Biochemistry 31:9269
72. Escude C, Sun JS, Nguyen CH, Bisagni E, Garestier T, Helene C (1996) Biochemistry 35:5735
73. Escude C, Nguyen CH, Mergny J-L, Sun J-S, Bisagni E, Garestier I, Helene C (1995) J Amer Chem Soc 117:10212
74. Escude C, Mohammadi S, Sun JS, Nguyen CH, Bisagni E, Liquier J, Taillandier E, Garestier T, Helene C (1996) Chem Biol 3:57
75. Escude C, Nguyen CH, Kukreti 5, Janin Y, Sun J-S, Bisagni E, Garestier T, Helene C (1998) Proc Natl Acad Sci USA 95:3591
76. Kim SK, Sun J-S, Garestier T, Helene C, Nguyen CH, Bisagni E, Rodger A, Norden B (1997) Biopolymers 42:101
77. Nguyen CH, Marchand C, Delage 5, Sun J-S, Garestier T, Helene C, Bisagni E (1998) J Am Chem Soc 120:2501
78. Tarui M, Doi M, Ishida T, lnoue M, Nakaike 5, Kitamura K (1994) Biochem J 304 (1): 271
79. Wilson WD, Mizan S, Tanious FA, Yao S, Zon U (1994) J Mol Recognit 7:89
80. Cassidy SA, Strekowski U, Wilson WD, Fox KR (1994) Biochemistry 33:15338
81. Wilson WD, Tanious FA, Mizan 5, Yao 5, Kiselyov AS, Zon U, Strekowski U (1993) Biochemistry 32:10614
82. Mergny JL, Duval-Valentin U, Nguyen CH, Perrouault U, Faucon B, Rougee M, Montenay-Garestier T, Bisagni E, Helene C (1992) Science 256:1681
83. Choi S-D, Kim M-S, Kim SK, Lincoln P, Tuite E, Norden B (1997) Biochemistry 36:214
84. Scaria PV, Shafer RH (1991) J Biol Chem 266:5417
85. Mergny J-U, Collier D, Rougee M, Montenay-Garestier T, Helene C (1991) Nucleic Acids Res 19:1521
86. Latimer LIP, Payton N, Forsyth U, Lee JS (1995) Biochem Cell Biol 73:11
87. Lee JS, Uatimer UP. Hampel KJ (1993) Biochemistry 32:5591
88. Denny WA (1989) Anticancer Drug Des 4:241
89. Pilch DS, Waring MJ, Sun J-S, Rougee M, Nguyen C-H, Bisagni E, Garestier T, Helene C (1993) J Mol Biol 232:926
90. Pilch DS, Shafer RH (1993) J Am Chem Soc 115:2565
91. Ren J, Chaires JB (2000) J Am Chem Soc 122:424
92. Strekowski U, Gulevich Y, Baranowski TC, Parker AN, Kiselyov AS, Lin S-Y, Tanious FA, Wilson WD (1996) J Med Chem 39:3980
93. Fox KR, Thurston DE, Jenkins TC, Varvaresou A, Tsotinis A, Siatra-Papastaikoudi T (1996) Biochem Biophys Res Cominun 224:717
94. Fox KR, Po]ucci P, Jenkins TC, Neidle 5 (1995) Proc Natl Acad Sci USA 92:7887
95. Haq I, Ladbury JE, Chowdhry BZ, Jenkins TC (1996) J Amer Chem Soc 118:10693
96. Kan Y, Armitage B, Schuster GB (1997) Biochemistry 36:1461
97. Durand M, Maurizot JC (1996) Biochemistry 35:9133
98. Durand M, Thuong NT, Maurizot JC (1994) J Biomol Struct Dyn 11:1191

99. Pilch DS, Kirolos MA, Breslauer KJ (1995) Biochemistry 34:16107
100. Pilch DS. Breslauer KJ (1994) Proc Natl Acad Sci USA 91:9332
101. Durand M, Thuong NT, Maurizot JC (1992) J Biol Chem 267:24394
102. Antony T, Thomas T, Shirahata A, Sigal LH, Thomas Ti (1999) Antisense Nucleic Acid Drug Dev 9:221
103. Basu HS, Marton U (1987) Biochem J 244:243
104. Musso M, Thomas T, Shirahata A, Sigal LH, Dyke MWV, Thomas TJ (1997) Biochemistry 36:1441
105. Thomas TJ, Kulkarni GD, Greenfield NJ, Shirahata A, Thomas T (1996) Biochem J 319:591
106. Thomas I, Thomas TJ (1993) Biochemistry 32:14068
107. Pallan PS, Ganesh KN (1996) Biochem Biophys Res Commun 222:416
108. Nagamani D, Ganesh KN (2001) Org Lett 3:103
109. Rajeev KG, Sanjayan GJ, Ganesh KN (1997) J Org Chem 62:5169
110. Potaman VN, Sinden RR (1995) Biochemistry 34:14885
111. Mamyama A, Katoh M, Ishihara T, Akaike T (1997) Bioconjug Chem 8:3
112. Dervan PB (2001) Bioorg Med Chem 9:2215
113. Arya DP, Coffee RL Jr, Charles I (2001) J Am Chem Soc 123:11093
114. Arya DP, Coffee RL Jr, Willis B, Abramovitch Al (2001) J Am Chem Soc 123:5385
115. Arya DP, Coffee RL Jr (2000) Bioorganic Med Chem Lett 10:1897
116. Arya DP, Micovic L, Charles I, Coffee RL Jr, Willis B, Xue U (2003) J Am Chem Soc 125:3733
117. Kohlstaedt LI, Wang J, Friedman J, Rice P, Steitz T (1992) Science 256:1783
118. Stein CA, Cohen JS (1988) Cancer Res 48:2659
119. Morgan AR, Wells RD (1968) J Mol Biol 37:63
120. Murray NL, Morgan AR (1973) Can J Biochem 51:436
121. Han H, Dervan PB (1993) Proc Natl Acad Sci USA 90:3806
122. Wang 5, Kool ET (1995) Nucleic Acids Res 23:1157
123. Riley M, Maling B, Chamberlin MJ (1966) J Mol Biol 20:359
124. Barbieri CM, Li T-K, Guo 5, Wang U, Shallop AJ, Pan W, Yang U, Gaffney BL, Jones RA, Pilch DS (2003) J Am Chem Soc 125:6469
125. Ren J, Chaires JB (2001) Methods Enzymol, vol 340. Academic, New York, p 99
126. Kirk SR, Luedtke NW, Tor Y (2000) J Am Chem Soc 122:980
127. Arya DP, Xue U. Willis B (2003) J Am Chem Soc 125:10148
128. Sanger W (1983) In: Cantor CR (ed) Principles of nucleic acid structure. Springer, Berlin Heidelberg New York, p 242
129. Stefl R, Trantirek U, Vorlickova M, Koca J, Sklenar V, Kypr J (2001) J Mol Biol 307:513
130. Robinson H, Wang AHJ (1996) Nucleic Acids Res 24:676
131. KyprJ, Fialova M, Chladkova J, Tumova M, Vorlickova M (2001) Eurbiophysi 30:555
132. Franklin RE, Goslin RU (1953) Acta Crystallography 6:673
133. Lvanov VI, Minchenkova LE (1995) Molekulyamaya Biologiya (Moscow) 28:780
134. Calladine CR, Drew HR (1984) J Mol Biol 178:773
135. Marky NU, Olson WK (1994) Biopolymers 34:121
136. Jeffares DC, Poole AM, Penny D (1998) J Mol Evol 46:18
137. Charles I, Xue U, Arya DP (2002) Bioorg Med Chem Lett 12:1259
138. Charles I, Arya DP (2004) Bioorg Med Chem Lett (in press)
139. Lu X-J, Shakked Z, Olson WK (2000) J Mol Biol 300:819
140. Robinson H, Gao Y, Sanishvili R, Joachimiak A, Wang AHJ (2000) Nucleic Acids Res 28:1760
141. White CM, Satz AL, Bruice TC, Beerman TA (2001) Proc Natl Acad Sci USA 98:10590
142. Haq I, Ladbury JE, Chowdhry BZ, Jenkins TC, Chaires JB (1997) J Mol Biol 271:244

143. Ecker DJ, Griffey RH (1999) Drug Discovery Today 4:420
144. Gallego JVU (2001) Acc Chem Res 34:836
145. Grillone LR (2001) In: Crooke ST (ed) Antisense drug technology: principles, strategies and applications. Dekker, New York, p 725
146. Neu HC (1992) Science 257:1064
147. Azucena E, Mobashery S (2001) Drug Resist Update 4:106
148. Elitra (2000) Bioworld Week 8:2
149. Elitra (1999) Bioworld Week 7:4
150. Haselbeck R, Wall D, Jiang B. Ketela T, Zyskind J, Bussey H, Foulkes JG, Roemer I (2002) Cur Pharm Des 8:1155
151. Vazquez ICM, Glazer PM (2002) Q Rev Biophys 35:89