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Nucleic Acid Ligands Based on Carbohydrates

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Abstract. Sequence-specific nucleic acid ligands are important tools in chemistry and molecular biology and are thought to possess a considerable pharmaceutical potential. An overview of the structurally and mechanistically diverse approaches in the field with an emphasis on carbohydrates will be presented.
Carbohydrates are just beginning to emerge as a novel class of nucleic acid binding compounds. The detailed study of the factors affecting the site-selectivity of some recently discovered antitumor antibiotics, *e.g.* calicheamicin, has shed a new light on the role that oligosaccharides may play in nucleic acid recognition. Understanding these factors may aid in the rational design of novel nucleic acid ligands and their therapeutic application.

1. Introduction

Nucleic acids are attractive targets for the development of novel therapeutics. The sequence specific, high affinity complexation of single-stranded messenger-RNA or the corresponding double-stranded genomic DNA can, in principle, prevent the production of illness related gene products at the stage of translation or transcription, respectively (*Fig. 1*). The inherent advantage of this approach lies in the low abundance of a certain gene or the resulting messenger-RNA within an affected cell. This theoretically requires a much lower dosage of a potential therapeutic agent. However, it also necessitates very high affinities and selectivities. Nucleic acid ligands based on this concept would act as direct antagonists to the cellular proteins that control translation and transcription.

1.1. *Antisense* Agents

Proteins themselves are poor candidates for these purposes. There is no general code for the recognition of nucleic acids by proteins, a one to one correspondance of the protein sequence and individual nucleotides. In addition, their bioavailability is low due to the relative ease with which they are degraded and the slow transport across cell membranes. And they are difficult and rather expensive to produce. Oligonucleotides or rather synthetically derived analogs, on the other hand, are obvious candidates for this task [1]. In the *antisense* strategy, single-stranded oligonucleotides are designed to pair to an RNA target (sense strand) by forming a local duplex structure according to the *Watson-Crick* pairing rules. Translation is arrested by this complexation and the RNA strand is then degraded by RNase H, an enzyme which recognizes DNA-RNA heteroduplexes, leading to catalytic turnover of the *antisense* agent. In a different approach, single-stranded oligonucleotides are designed such as to form a local triple-helical structure with the third strand associated in the major groove of double-stranded DNA. In this case, the sequence specificity is derived from selective base triplet formation on the *Hoogsteen* face of purine bases (*Fig. 2*). Since all such oligonucleotide analogs are linear polymers their affinity and specificity can be altered at will by changing chain length and base sequence.

Several clinical trials are currently under way and although there have been some promising results, difficulties have been encountered in getting *antisense* oligonucleotides to work the way they were intended to [1e][2]. Selective delivery to the target tissue and potentially toxic side effects such as decreased blood clotting and inreased blood pressure are the main obstacles. An improved understanding of the fate of *antisense* oligonucleotides *in vivo* in conjunction with the development of novel, more specific analogs will be necessary to successfully generate therapeutics based on this concept.

1.2. *Small DNA-Binding Molecules*

In view of these difficulties, numerous academic and industrial research groups

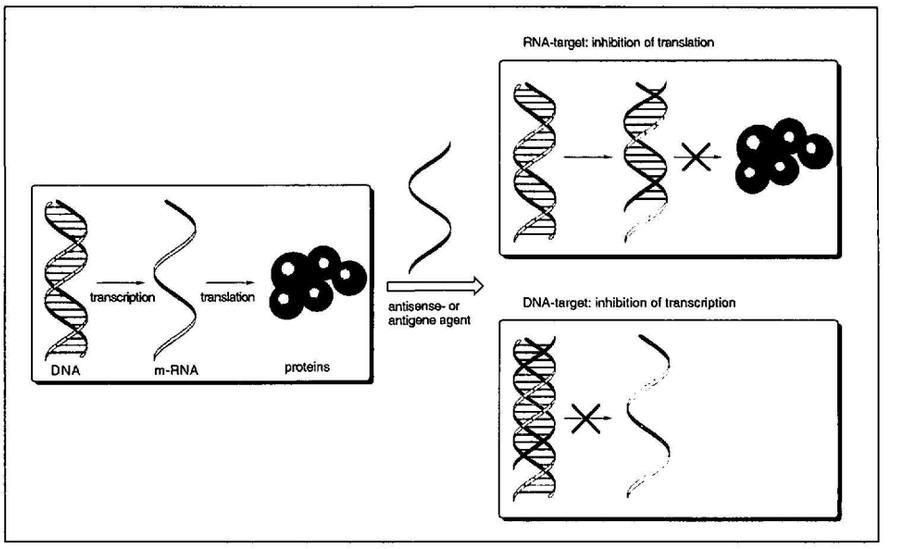

Fig. 1. *Schematic representation of a generalized antigene-concept.* By complex formation with messenger-RNA or the corresponding DNA antigene agents can shut down production of illness-related proteins at the stage of translation or transcription, respectively.

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are joined in an effort to evaluate alternatives to oligonucleotides, compounds of different structural types, with respect to their antigene properties. Studies of the binding modes of such molecules might lead to new strategies for the sequence-specific binding of nucleic acids.

The vast pool of natural products provides many small molecules that bind to nucleic acids, most of them of fungal or microbial origin. Unlike oligonucleotides, the majority of these small molecules interact with double-stranded nucleic acids *via* contacts within the minor groove. Two examples of such compounds are netropsin and distamycin (*Fig. 3*). These crescent-shaped polyamides composed of *N*-methylpyrrole amino acids bind to stretches of double-stranded DNA consisting of adenine/thymine base pairs. The sequence selectivity derives from H-bonding between the amide protons and the nitrogen and carbonyl oxygen of adenine and thymine on the floor of the minor groove. It was shown in NMR studies by *Wemmer* and coworkers that in the complex two distamycin molecules are aligned side by side in an antiparallel manner [3a]. Based on these findings, *Dervan* and coworkers successfully altered the sequence specificity of these molecules to include guanine/cytosine base pairs by substituting *N*-methylimidazole for *N*-methylpyrrole carboxamides at selected positions [3]. The affinity could be improved to subnanomolar levels by covalently linking the two individual halfs of these dimers [3e] (*Fig. 3*).

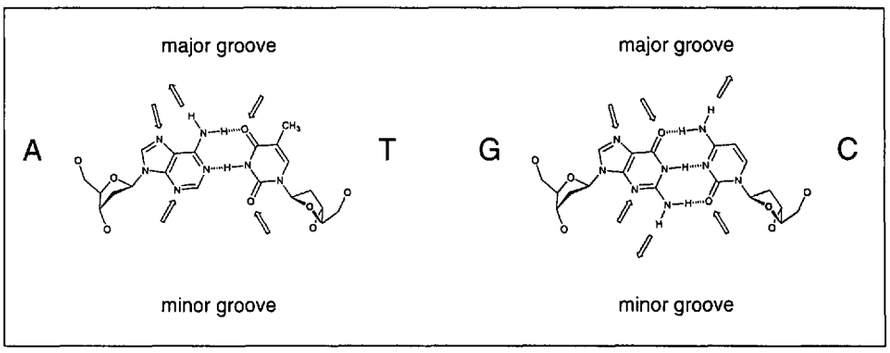

Fig. 2. *Substitution pattern of H-bonding donor and acceptor groups for adenine-thymine and guanine-cytosine base pairs in the major and minor groove*

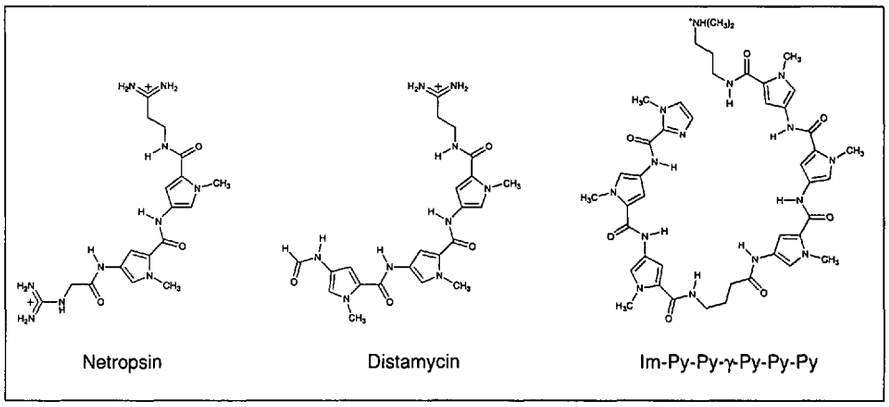

Fig. 3. *Structures of the minor groove binding drugs netropsin and distamycin.* By substituting *N*-methylimidazole for *N*-methylpyrrole carboxamides at selected positions of and covalently linking two individual molecules to a dimer *Dervan* and coworkers developed distamycin analogs, *e.g.*, Im-Py-Py-γ-Py-Py-Py, with altered sequence specificity and higher affinity [3].

2. DNA Ligands

Quite a few natural products have the capability not just to form complexes with DNA but to chemically modify it. Many compounds with antitumor activity belong into this category. *Figs. 4* and *5* show some examples of such naturally occurring compounds. Their mechanism of complexation with nucleic acids can be distinguished into four main categories, intercalation, binding *via* metal complexes, and binding mediated by a protein component or carbohydrate side chains. Intercalation consists of aligning an extended aromatic chromophore in between two base pairs of the double helix (*Fig. 6*). Driving force of this kind of interaction is π-stacking that extends over both strands and leads to an overall stabilization of the double helix. Many naturally occurring DNA ligands contain metal binding subunits. By complexing a metal, a net positive charge results which provides an attractive electrostatic force towards the negatively charged DNA and thus helps aligning the other parts of the molecule. Alternatively, certain DNA-ligands form dimeric (or higher order) complexes upon binding of a metal and only these can then interact with their target. However, these mechanisms alone cannot account for the sequence specificity displayed by the respective DNA-binding drugs. These as well as compounds without an aromatic moiety or an apparent metal binding site often contain additional carbohydrate side chains which have to be responsible for site selectivity. Carbohydrates might therefore be an interesting class of potential DNA ligands [5]. In recent years, several research groups have tried to develop synthetic DNA ligands based on the saccharide units of known DNA binders.

Certainly the most extensively studied group of DNA binding small molecules in recent years are the enediyne antibiotics, among them calicheamicin, esperamicin, and neocarcinostatin. Neocarcinostatin, a 1:1 complex of a protein and a chromophore compound has first been isolated 1965 [6a]. The protein is thought to mediate the sequence-specific recognition of double-stranded DNA, activation of the chromophore part then leads to double-strand cleavage. In 1985, *Nishida* and coworkers reported the structure of the neocarcinostatin chromophore (*Fig. 4*) which is responsible for the observed biological activity [6b]. This chromophore consisted of a highly unusual bicyclo[7.3.0]dodecadienediyne scaffold. Just two years later, the discovery of two compounds with potent cytostatic activity and a similar core structure, calicheamicin γ_1^I and esperamicin A_1 (*Fig. 4*), has been reported simultaneously by researchers at *Lederle Laboratories* [7] and *Bristol-Myers* [8]. Their unusual structure containing a bicyclic enediyne and their impressive anticancer activity intrigued the chemical and pharmaceutical community. Immediately, a race aimed at the total synthesis of calicheamicin set on, culminating in two reported synthesis by the groups of *Nicolaou* [9] and *Danishefsky* [10]. In 1989, a fourth structure of this type, dynemicin A, has been reported, again by researchers at *Bristol-Myers* [11], followed by the discovery of the kedarcidin [12] and C-1027 [13] chromophores (*Fig. 4*) adding to the arsenal of enediyne antibiotics. Of these latter compounds, the synthesis of dyne-

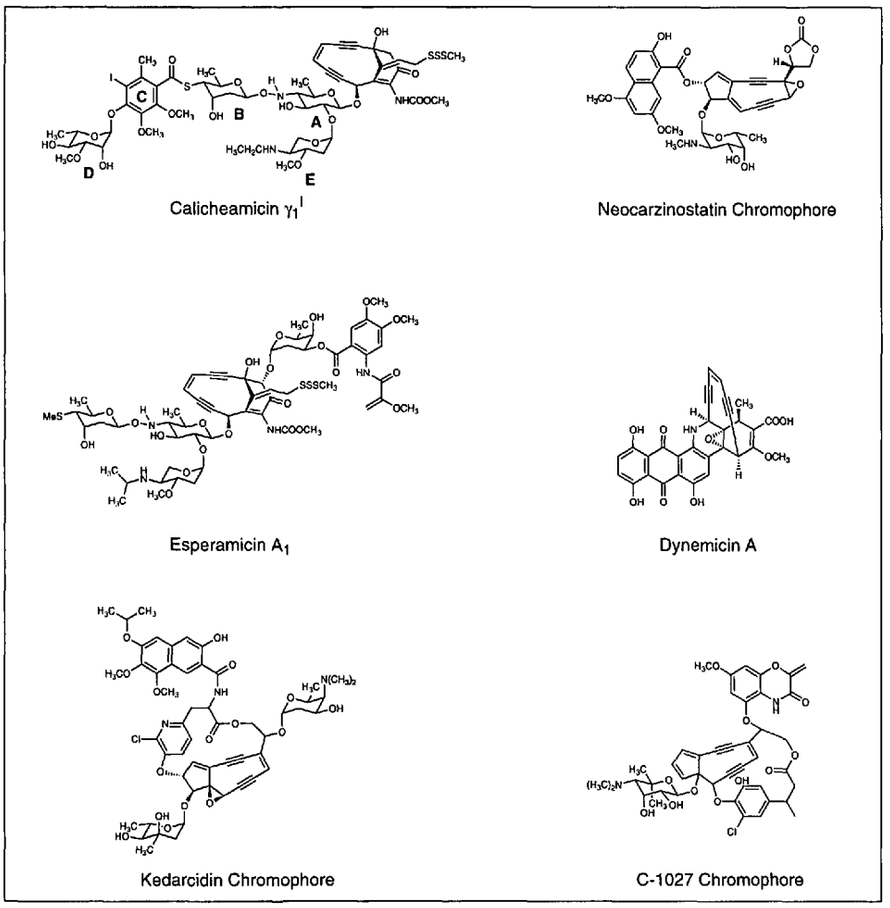

Fig. 4. *Structures of the recently discovered enediyne class of antitumor compounds.* These compounds are specific cutters of double-stranded DNA.

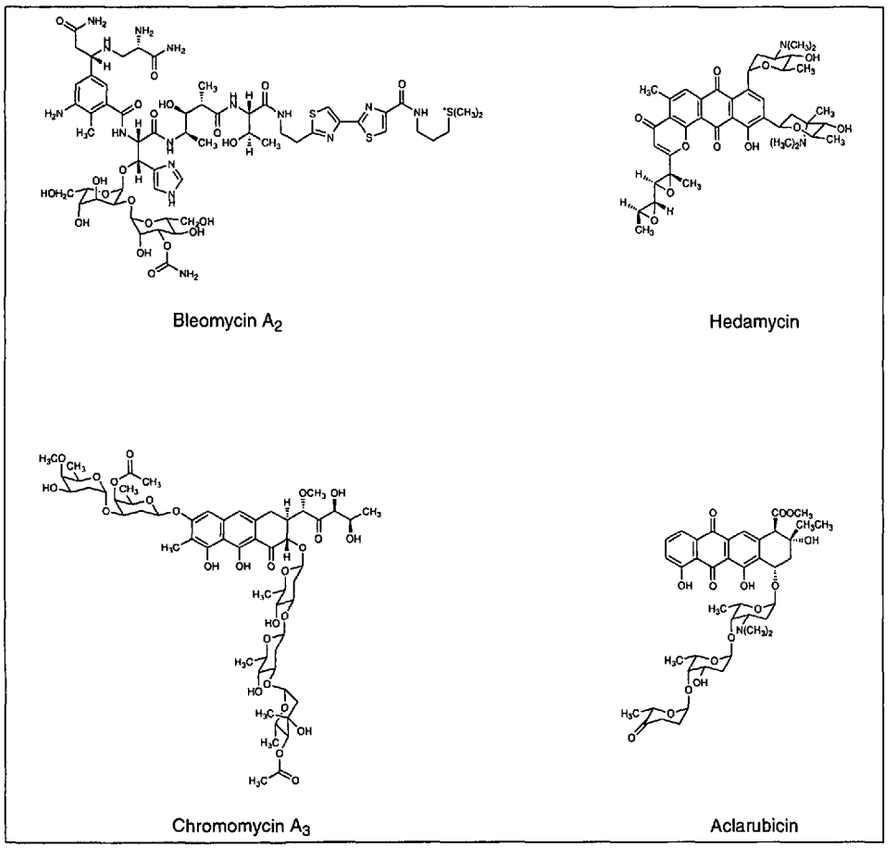

Fig. 5. *Further examples of DNA-binding antibiotics with carbohydrate side chains*

micin A has been reported by *Myers* and coworkers [14].

The mode of action of these enediyne antibiotics follows a similar pathway as shown in *Scheme 1* for calicheamycin. An external nucleophile attacks the trisulfide moiety remote of the enediyne core. The thiolate generated in this way undergoes an internal *Michael* addition leading to a conformational change of the chromophore. This brings the two acetylene units in close proximity and, in a spontaneous pericyclic process termed *Bergman* cyclization [15], a 1,4-aryl diradical is formed. This diradical then induces oxidative DNA cleavage by H-abstraction from 2-deoxyribose residues [16].

For most of the enediyne antibiotics mentioned above the recognition of double-stranded DNA relies on intercalation by a heterocyclic aromatic moiety, except for calicheamycin. Interestingly, it is calicheamicin which displays the highest sequence specificity in the recognition of double-stranded DNA.

2.1. *Calicheamicin*

Calicheamicin γ_1^I targets oligopyrimidine-oligopurine tracts of DNA with a preference for the sequence d(TCCT) [17a]; others include d(TCTC) and d(TTTT) [17b]. Additionally, it could be shown in competition experiments that calicheamicin binds in the minor groove [17a]. The presence of known minor groove binders, such as netropsin which binds to similar target sequences, prevents or greatly reduces the cleavage of double-stranded DNA by calicheamicin. Later, it became clear that most of the DNA recognition was due to the aryl-tetrasaccharide domain. *Kahne* and coworkers showed in NMR studies that the carbohydrate portion adopts a highly organized, extended conformation in solution [18]. They could further show that the oligosaccharide is organized into a shape that complements the shape of the minor groove by the presence of the unusual hydroxylamine glycosidic linkage connecting rings A and B (*Fig. 4*).

In a series of experiments *Danishefsky*, *Crothers* and coworkers could demonstrate that the oligosaccharide domain was indeed crucial for selective and tight binding to DNA [19]. In these experiments the cleavage properties of calicheamicinone, the aglycone of calicheamicin, were investigated. Calicheamicinone which cannot be obtained by degradation of the natural product, had been obtained in racemic or either enantiomeric form by total synthesis in the groups of *Danishefsky* [19b][20] and *Nicolaou* [9c]. *Dan-*

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ishefsky and *Crothers* found that calicheamicinone cleaves DNA with no apparent sequence selectivity and it primarily cleaves only one of the two target strands. These results suggested that the aryltetrasaccharide portion was indeed responsible for sequence selectivity.

The hypothesis of *Danishefsky* is further supported by the fact that esperamicin, which contains a similar carbohydrate fragment consisting of rings A, B, and E, does not display the same degree of sequence selectivity. Additionally, *Kahne* and coworkers investigated the cleavage selectivity of a calicheamicin analog termed calicheamicin T containing only the sugar residues A and E [17b]. This derivative exhibits only minimal sequence selectivity implying that the full terasaccharide is required for specific DNA recognition.

Several models have been put forth to rationalize the binding of calicheamicin to DNA [21]. It has been shown that a conformational change of DNA is induced upon binding of calicheamicin. Specifically, the oligopyrimidine regions of DNA display a certain flexibility and thus allow for a distortion of the sugar-phosphate backbone to accomodate the relatively rigid drug in the minor groove. The iodine of the aryl moiety is of particular importance to the binding event [21b]. It is thought to interact with the exocyclic amino group of a guanine residue. On substituting the iodine the binding affinity is reduced in the order I > Br > Cl > F > CH_3 > H. More structural information on the calicheamicin-DNA complex was obtained by several NMR investigations [22]. Recently, *Patel* and coworkers published a detailed solution structure of calicheamicin γ_1^I bound to a hairpin duplex of the sequence d(CACTCCTGGTTTTTCCAGGAGTG) by a combined NMR/molecular dynamics approach [22d]. The complementary fit of the carbohydrate portion and the DNA minor groove binding site d(TCCT) · d(AGGA) creates numerous *van der Waals* contacts as well as H-bonding interactions. The iodine and the sulfur atoms are engaged in H-bonds with the exposed proton of the amino groups of the 5'- and 3'- guanines, respectively. The sequence-specific carbohydrate binding orients the enediyne aglycon such that it is tilted relative to the helix axis and spans the minor groove placing the proradical centers adjacent to the cleavage sites. Specific localized conformational perturbations in the DNA have been identified from imino proton chemical shift differences and changes in sugar pucker patterns on complex formation. The helical parameters for the carbohydrate binding site are reminiscent of a B-form duplex while a widening of the minor groove is observed at the aglycon site.

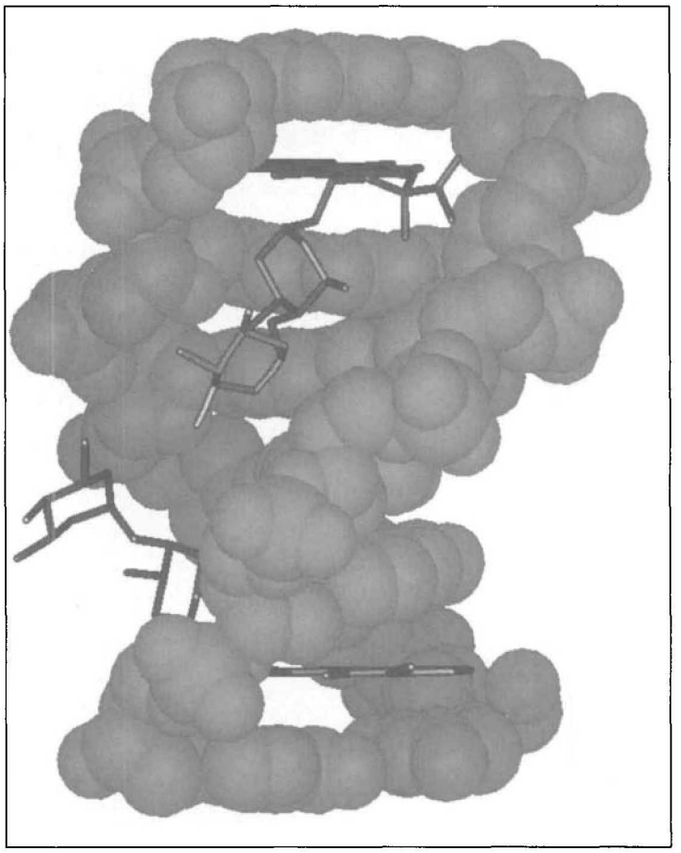
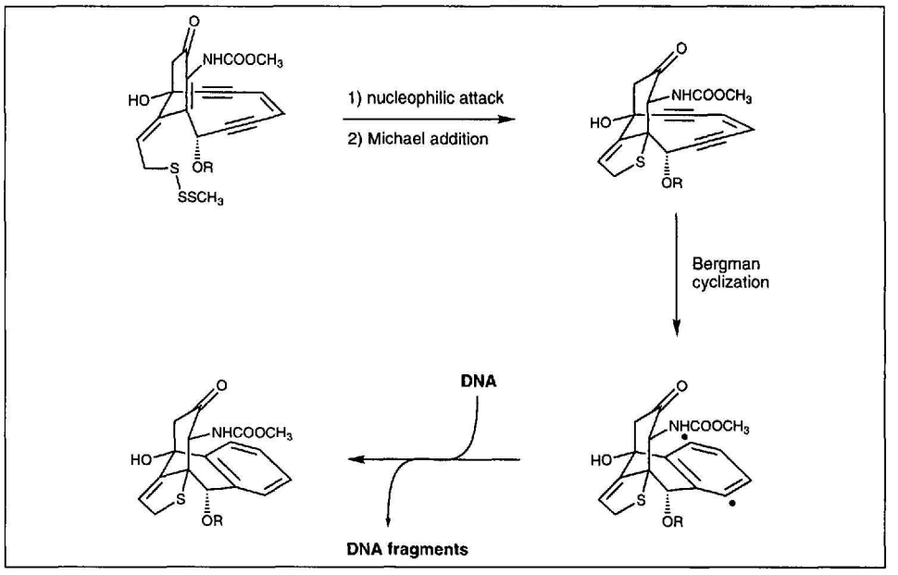


Fig. 6. *Structure of a synthetic anthracyclin antibiotic complexed with double-stranded DNA* [4]. The aromatic chromophore intercalates between two consecutive base pairs.

Scheme 1. *Mechanism of the Activation and DNA-Damage Displayed by Calicheamicin* γ_1^I



Based on the results of these structural studies the *Nicolaou* and *Danishefsky* groups began to evaluate the binding capabilities of the saccharide moiety of calicheamicin. This was possible only after the chemical synthesis of the corresponding methyl glycoside **9** (*Scheme 2*) had been accomplished since degradation studies of the natural product indicated that the carbohydrate could not be obtained intact in this manner [23]. *Nicolaou et al.* were the first to accomplish this synthesis [24a] [9b]; to date, two other syntheses have been reported [24b, c]. *Scheme 2* shows as an example the synthesis devised by *Kahne* and coworkers. It was then demonstrated that the methyl glycoside **9** binds to the same sites in a similar way as the parent natural product [21b, h][25] (*Scheme 3*).

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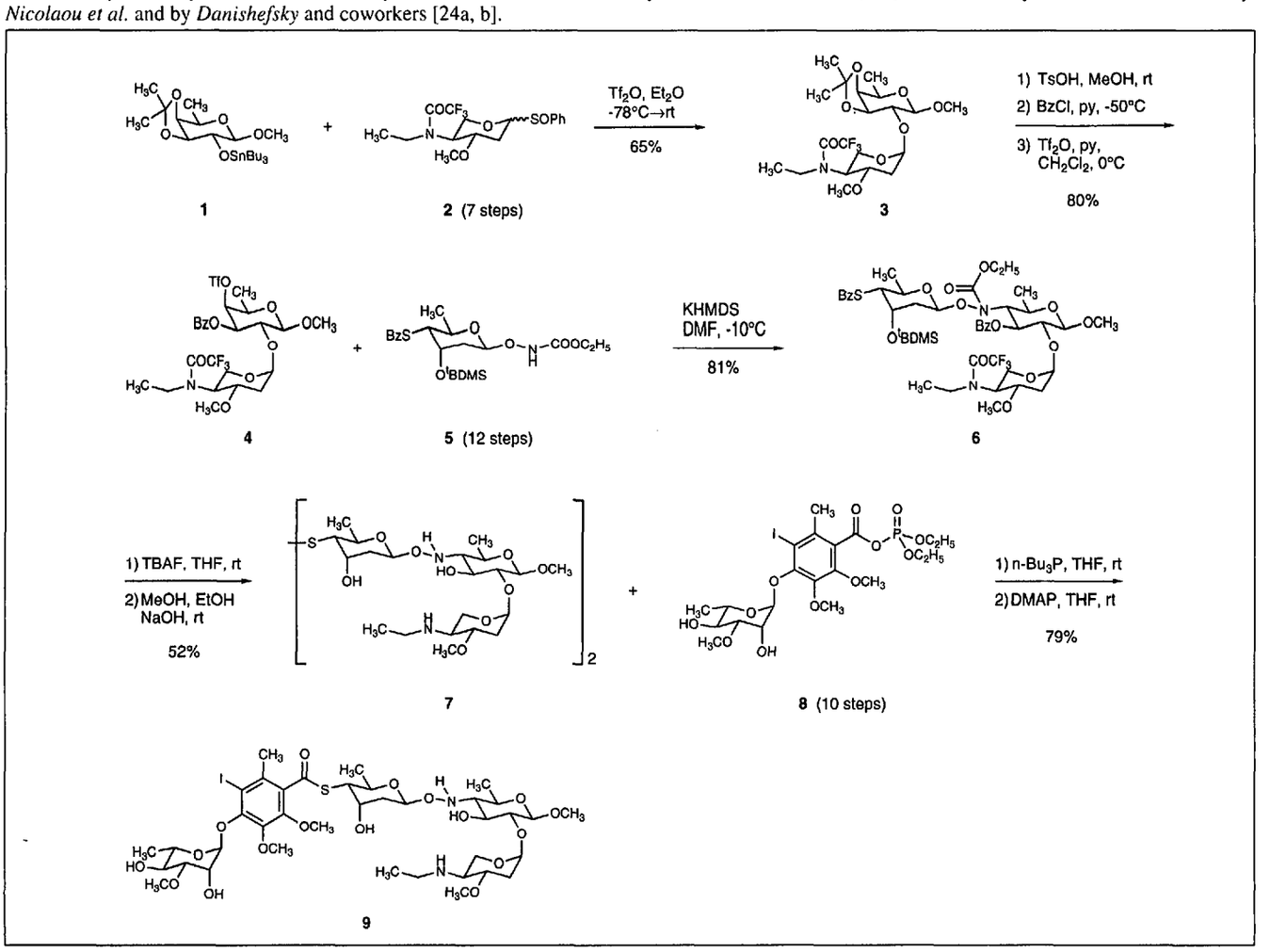
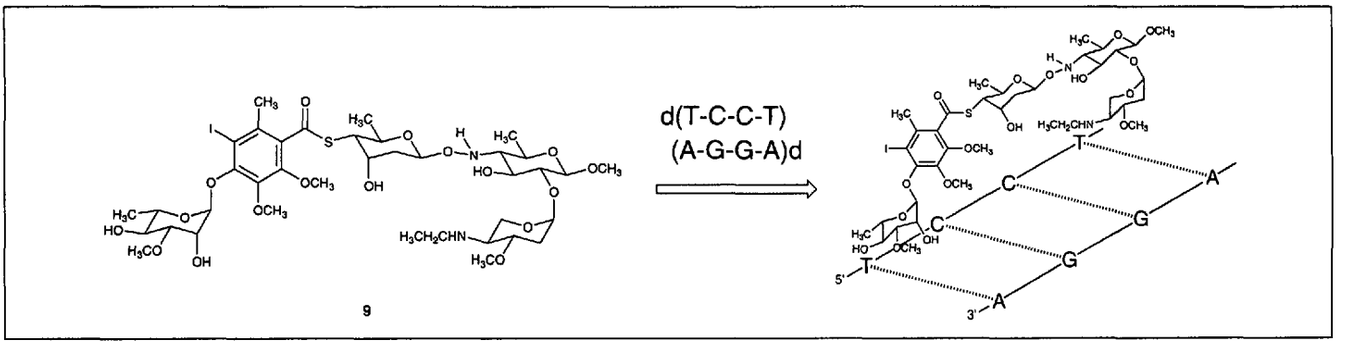
Schreiber, *Danishefsky*, and *Crabtree* subsequently went on to demonstrate the utility of the calicheamycin aryl-tetrasaccharide **9** as DNA ligand [26]. Calicheamicin methyl glycoside **9** inhibits the formation of DNA-protein complexes at micromolar concentrations in a sequence-specific manner and rapidly dissociates preformed complexes. Similar results were obtained by the same authors in an *in vivo* study. Calicheamicin methyl glycoside **9** effectively blocked the expression of a reporter gene under the control of the transcription factor NFAT. Thus, the proliferation of T cells was inhibited in a dose dependent manner. These observations mark the beginning of a novel strategy for the development of antigene agents based on carbohydrate-mediated recognition of DNA.

Nicolaou et al. later reported on the synthesis and the DNA-binding properties of a covalently linked dimer of the calicheamicin carbohydrate [27]. The synthesis starts with the known trichloroacetimidate intermediate **10** of the total synthesis of calicheamycin γ_1^I. A diethyleneglycol unit is introduced at the anomeric position of the A ring and then coupled with a second tetrasaccharide unit **10** leading to the dimer **11** shown in *Scheme 4*. This head-to-head dimer was shown to bind to its target sequence d(TCCT-XX-AGGA) with a 10-fold higher affinity as compared to the monomer. The authors could further demonstrate that dimer **11** is

Scheme 2. *Synthesis of the Calicheamicin Aryl-Tetrasaccharide* **9** *Devised by* Kahne *and Coworkers* [24c]. Two earlier syntheses were described by *Nicolaou et al.* and by *Danishefsky* and coworkers [24a, b].



Scheme 3. *Binding Site and Orientation of the Calicheamicin* γ_1^I *Aryl-Tetrasaccharide* **9** *in the Minor Groove of the Target DNA*



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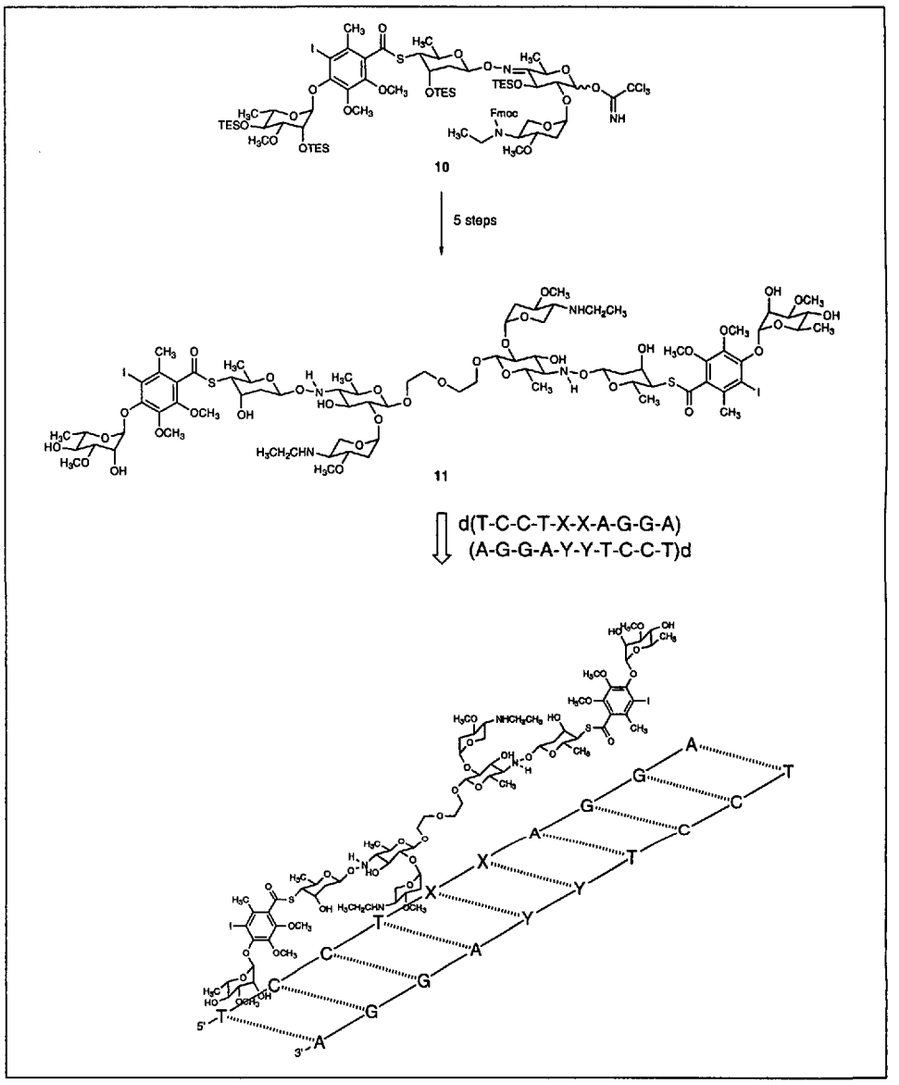
a more effective inhibitor of polymerase II in an *in vitro* transcription system. The effective concentrations of the oligosaccharide for inhibition of transcription factor binding and transcriptional inhibition are in the low micromolar range. It is noteworthy to realize, though, that at higher concentrations additional sequences such as d(TCTC), d(TTTT), and (TGGT) are recognized as well. The oligosaccharides derived from calicheamicin target certain preferred DNA sequences but they lack the kind of specificity that can be observed with proteins or oligonucleotide analogs.

2.2. *Chromomycin*

A binding mode completely different from the one displayed by calicheamicin was unveiled for the antitumor compound chromomycin A_3. Chromomycin A_3 (*Fig. 5*) is a member of the aureolic acid family of antibiotics and was first isolated in 1960 [28]. However, the structure was fully established only in 1982 [29]. It was shown that chromomycin binds to DNA preferably at GC-rich sites and that, upon binding, replication and transcription are inhibited [30]. In 1989, *Patel* and coworkers showed that chromomycin binds in the minor groove of DNA as an octahedral 2:1 drug-Mg^{2+} complex (*Fig. 7*) [31]. In the complex, the drug acts as a bidentate ligand. The metal is located in the middle of the minor groove at the central d(GC) step and is coordinated by the carbonyl group and the adjacent phenolate anion of the chromophore. The planes of the chromophores span an angle of 75° and are slightly tilted relative to each other. The extensively alkylated and acylated trisaccharide side chains adopt an extended conformation and are located along the minor groove. The sequence specificity of the chromomycin dimer for the d(GGCC) · d(GGCC) binding site is associated with the formation of H-bonds between the exocyclic amino groups of the guanine bases and oxygens within the aromatic and carbohydrate moieties of the drug. Additional intermolecular interactions consist of *van der Waals* contacts between aliphatic protons of the trisaccharide residues and the surface of the minor groove. The minor groove at the binding site is widened and shallow and the individual nucleosides within this central tetranucleotide segment share A-type conformational parameters.

The sugar residues in chromomycin A_3 do not just play a role in the sequence specificity towards DNA they also influence the stoichiometry of the complex with Mg^{2+}. *Kahne* and coworkers could show that the aglycone, chromomycinone, itself preferably forms a 1:1 complex in the presence of magnesium and not a 2:1 complex [32]. With a series of degradation products of chromomycine A_3 they showed that the trisaccharide side chain helps stabilizing the 2:1 complex with magnesium.

Scheme 4. *Synthesis and Binding Site of the Calicheamicin Saccharide Dimer* **11** *Described by* Nicolaou *and Coworkers* [27]



2.3. *Anthracycline Carbohydrate Side Chains*

The anthracyclines are another important class of antitumor antibiotics. Some of these compounds, *e.g.*, daunorubicin and doxorubicin are currently used in chemotherapy. They exert their biological activity by intercalation of a tetracyclic aromatic core between base pairs of double-helical DNA (*Fig. 6*), thereby inhibiting access of transcriptional factors and topoisomerases [4][33]. Many of these compounds contain carbohydrate side chains consisting of between one and three 2,6-dideoxy-L-pyranose residues which are connected *via* α-1,4-linkages. The saccharide side chains are in most cases required for optimal biological activity.

Single crystal X-ray and NMR studies have shown that the saccharide side chains are located in an extended conformation within the minor groove [34]. Upon drug binding, preferably at GC-rich sequences, the minor groove is widened and becomes shallow. In some cases an additional kinking of the double helix at the intercalation site is observed which leads the saccharide tails protruding partly into the solvent region.

Based on the assumption that the anthracyclines have evolved with carbohydrate side chains as a minor groove binding motif, *Harding* and coworkers synthesized the methyl glycoside **18** related to the aclarubicin (aclacinomycin A) carbohydrate moiety (*Scheme 4*) [35]. Preliminary binding studies were carried out by temperature-dependent UV melting curves

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with calf thymus DNA. Addition of the aclacinomycin trisaccharide **18** to the DNA solution resulted in only minor variations of the melting temperature of the DNA (< 1°). This indicates that the binding affinity of the carbohydrate is weak.

3. RNA Ligands

While quite a few classes of low-molecular weight compounds that bind to double-stranded DNA have been characterized, rather little is known about corresponding RNA ligands. One notable exception, though, are the aminoglycoside antibiotics.

Aminoglycoside antibiotics are basic pseudo-oligosaccharides (*Fig. 8*) which are produced by microorganisms and have found wide-spread use in therapy and as tools in molecular biology [36]. It has been known for quite some time that their potency stems from inhibition of protein synthesis. Streptomycin, for instance, interferes with translation by inducing misreading of the genetic code and by inhibiting translational initiation. The miscoding is believed to be the result of interference with a proof-reading step. *Moazed* and *Noler* showed in footprinting experiments that streptomycin binds to a single site within 16S ribosomal RNA in the vicinity of a pseudoknot structure [37a]. Neomycin B and related aminoglycosides bind to the A site of the 16S ribosomal RNA which comprises the decoding function of the ribosomes [37]. This affects the binding of aminoacylated transfer-RNA and leads to inhibition of translocation of the messenger-RNA and consequently to miscoding. Aminoglycosides also inhibit the splicing of several group I introns [38]. Group I introns are a class of functional RNA sequences that catalyze their own excision from a longer RNA strand as well as the religation of the remaining parent strand (exon) in a well characterized two-step process [39]. They share a common structural core with a binding site for the cofactor guanosine. *Von Ahsen et al.* have shown that aminoglycosides are competitive inhibitors of cofactor binding and further disrupt structural contacts in the catalytically active core.

The steady spread of illnesses caused by retroviruses, most prominently HIV, has lead to an intensified search for antiviral compounds that selectively bind to RNA. Recently, two groups independently reported on the sequence-selective recognition of certain substructures within the HIV RNA-genome by aminoglycoside antibiotics [40]. These substructures, Rev-response element (RRE) and transactivation responsive region (TAR), are binding sites for the viral regulatory proteins Rev and Tat, rich in stem-loop structures. RRE and TAR are of major importance in the viral replication cycle [41] and therefore attractive targets for therapeutic intervention [42]. The aminoglycoside binding sites are identical to the protein binding sites as has been shown by chemical footprinting and competition experiments and share a set of unusual structural features. RRE-RNA contains at this site consecutive G–A and G–G base pairs, TAR-RNA a bulge consisting of three pyrimidine nucleotides.

From the studies on the binding properties of aminoglycosides it seems evident that there is not a particular base sequence which is recognized by the structurally diverse class of aminoglycosides but rather a common structural motif. Experiments with combinatorial RNA-libraries that were selected for the aminoglycosides neomycin, tobramycin, lividomycin, or kanamycin additionally support this notion [43]. However, no high-resolution structural data is available yet for such a binding site or a complex between an aminoglycoside and its RNA-target.

It should further be noted that not only the structurally complex aminoglycoside antibiotics mentioned above display high affinity binding to RNA. Even chitosans – β-1,4-linked glucosamine oligomers – act as ligands for double-stranded RNA and effect a dramatic thermal stabilization of these complexes. Double-stranded DNA is complexed as well but undergoes a conformational change from B-form to A-form DNA [44].

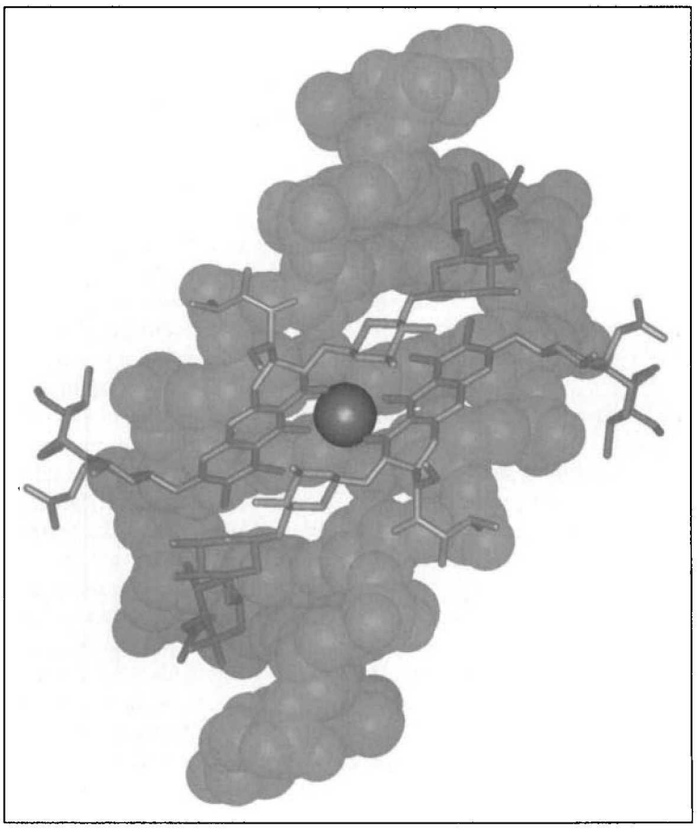

Fig. 7. *Structure of the 2:1:1 chromomycin A_3-magnesium-DNA complex described by* Patel *and coworkers* [31]

4. Conclusions

There exist a large number of nucleic acid-binding natural products which contain carbohydrate components. Although the role of these sugars, for the most part, is still unclear, it was shown in a few cases that the saccharide portion itself mediates the sequence-specific recognition. A comparison of the examples mentioned above, reveals similarities in the structure of the respective DNA- and RNA-binding glycoconjugates that may prove useful in the design of novel *antisense* agents.

DNA-binders, like for instance calicheamicin and chromomycin, have oligosaccharide side chains that are rather hydrophobic. There is rarely more than a single positively charged ammonium group present in the carbohydrate and many of the sugars are of the 2,6-deoxyhexopyranose type. Often, the remaining hydroxy groups are further alkylated or acylated. Thus, the recognition process relies on just a few H-bonding interactions

and is mostly driven by the hydrophobicity of the DNA-binding saccharides [45]. This suggests that rather the complementary shapes, and hence specific *van der Waals* contacts between the carbohydrates and the minor groove, may determine the site-selectivity displayed by many carbohydrate-containing minor groove binders. Additionally, the relatively rigid carbohydrates may take advantage of the sequence-dependent flexibility of double-stranded DNA to bind their target sites [5]. Furthermore, the hydrophobicity of DNA-binding carbohydrates may differentiate these from the hydrophilic and often negatively charged cell-surface saccharides which are involved in protein recognition and may enhance the cellular uptake of the respective drugs.

The known RNA-binding carbohydrates, on the other hand, display a completely different chemical and physical behavior. They are extremely hydrophilic due to extensive substitution of hydroxy function by ammonium groups. Deoxygenation or O-alkylation is not common for these aminoglycosides. They share, though, a similar rigidity as the DNA-binding saccharides since they mainly consist of pyranose subunits and a cyclohexitol part. To date, there is no detailed structural information available on the complexes of aminoglycosides with RNA. One can assume that enthalpy due to charge neutralization is a major factor in the interaction with RNA, in contrast to the recognition process of the DNA minor groove binders where entropic factors play a key role. These attractive forces can induce dramatic conformational changes as is seen for the transistion of B- to A-form DNA upon binding of aminoglycosides. However, the mechanisms by which aminoglycosides achieve sequence specificity in the recognition of RNA remains unclear and awaites further investigation.

Ligands that sequence-selectively bind to nucleic acids have a number of important uses as tools in chemistry and molecular biology and as therapeutics in medicine. Carbohydrates are a recent addition to the arsenal of such compounds. Their capabilities in the recognition of nucleic acids and the underlying mechanisms are just now beginning to be explored. Understanding these factors is a prerequisite for the development of new strategies in the design of nucleic acid binding agents and their pharamaceutical application.

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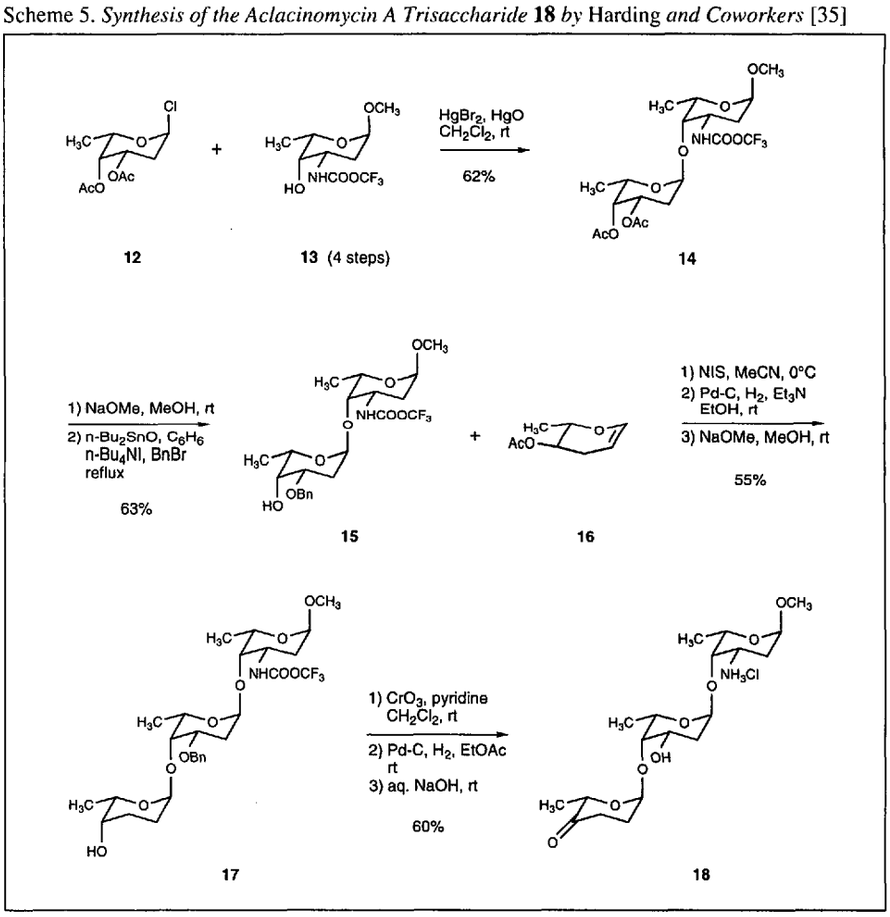
Scheme 5. *Synthesis of the Aclacinomycin A Trisaccharide* **18** *by* Harding *and Coworkers* [35]



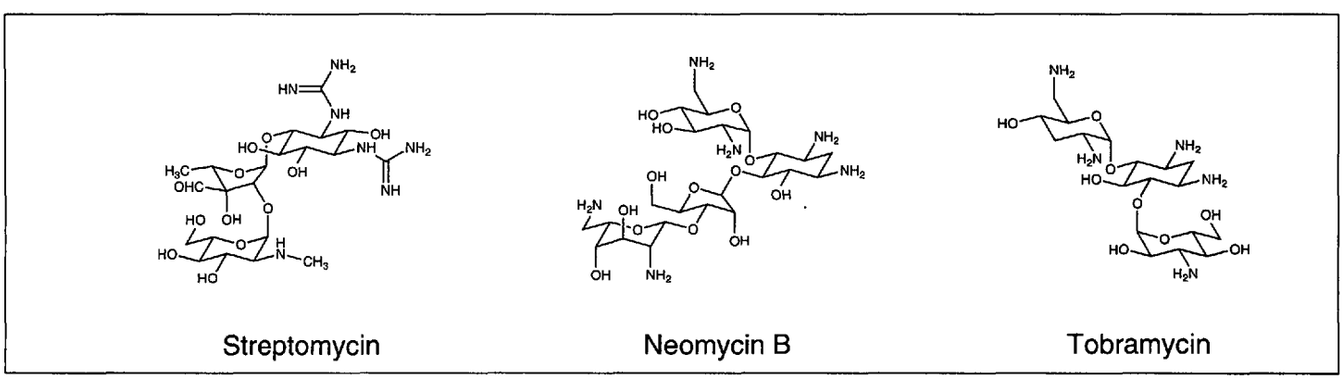


Fig. 8. *Structures of some representative examples of aminoglycoside antibiotics*

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