Heteroaromatic Oligoamides with dDNA Affinity

Hans-Christoph Gallmeier[a] and Burkhard König*[a]

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Heteroaromatic oligoamides are selective ligands for sequence-specific dDNA binding, many artificial oligoamides modeled on naturally occurring compounds and their dDNA binding motifs having been prepared over the last ten years. The large number of reported individual compounds does not allow each structure to be covered within the scope of this review, so we concentrate on examples of the most successful linear and tethered oligoamide structures, such as hairpin, H-pin, or cyclic structures, and the ensemble of heteroaromatic amino acids used as their building blocks. This provides an overview of which chemical structures are currently in use for sequence-specific dDNA recognition by artificial ligands and what they can achieve.

1. Introduction

The genetic information of all known organisms, with the exception of some viruses, is stored as double-stranded deoxyribonucleic acid (dDNA).[1−4] The stored information is accessed (expressed) biochemically by specific protein−dDNA interactions, which are either reversible or irreversible. Reversible interactions are classified into three different types:[5−7] electrostatic interactions, intercalation, and binding into the minor or major groove of dDNA. Classic intercalators, such as ethidium bromide, which insert with their arene group between two base pairs of dDNA,[8] generally show only low sequence specificity of insertion. Most investigated low molecular weight (< 1500 Dalton) organic molecules[9] interact with dDNA in the minor groove. This binding process can be described by two steps.[10] In the first, electrostatic and hydrophobic interactions transfer the ligand from solution into the dDNA minor groove. In the case of positively charged compounds, such as distamycin, this results in a dDNA counter-ion exchange. In the second step, various specific interactions are established between the bound ligand and the functional groups of the base pairs of the dDNA. The interactions usually include a combination of hydrogen bonds, hydrophobic and van der Waals contacts, and electrostatic interactions.[5]

The large number of DNA-binding heteroaromatic oligoamides can be classified and ordered in many different ways: by their binding motifs, by the heterocycles included, by their binding strengths, or by several other properties. We have used two classification schemes in this review: 1) the origins of the compounds, either as natural or as artificial DNA-binding oligoamides, and 2) the principles by which single oligoamide strands, naturally occurring or synthetic, are tethered to dimers and oligomers to enhance binding strength and selectivity. This allows us to cover examples of all known structural types of DNA-binding heteroaromatic oligoamides in this review.

[a] Institut für Organische Chemie, Universität Regensburg, 93040 Regensburg, Germany

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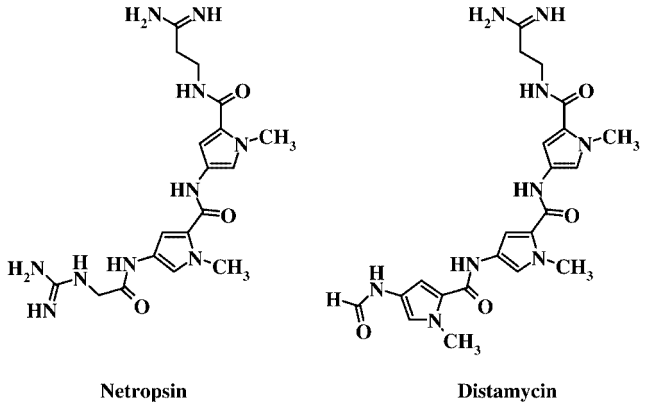
Hans-Christoph Gallmeier (left) was born in 1972 in Regensburg, Germany. He studied chemistry at the University of Regensburg and completed his Ph.D. project on the topic of the review in the group of Prof. König in December 2002. He joined OSRAM Opto Semiconductors in early 2003.

Burkhard König (right) received his doctorate in 1991 from the University of Hamburg under the direction of Prof. de Meijere. He continued his scientific education as a postdoctoral fellow with Prof. M. A. Bennett, Canberra, Australia, and Prof. B. M. Trost, Stanford, U. S. A. In 1996 he obtained his "Habilitation" at the Technical University of Braunschweig and since 1999 he has been Professor at the University of Regensburg, Germany. His current research interests focus on intermolecular interactions and their use in molecular recognition and self assembly.

MICROREVIEWS: This feature introduces the readers to the authors' research through a concise overview of the selected topic. Reference to important work from others in the field is included.

2.1 Natural dDNA-Binding Heteroaromatic Oligoamides

The peptides netropsin[11] and distamycin[12] (Scheme 1), consisting of two and three *N*-methylpyrrole-carboxamides, respectively, were the first natural dDNA-binding oligoamides identified. They bind preferentially in the minor groove of AT-rich sequences[13] of dDNA and show activity against some tumors, bacteria, and viruses.[14,15]



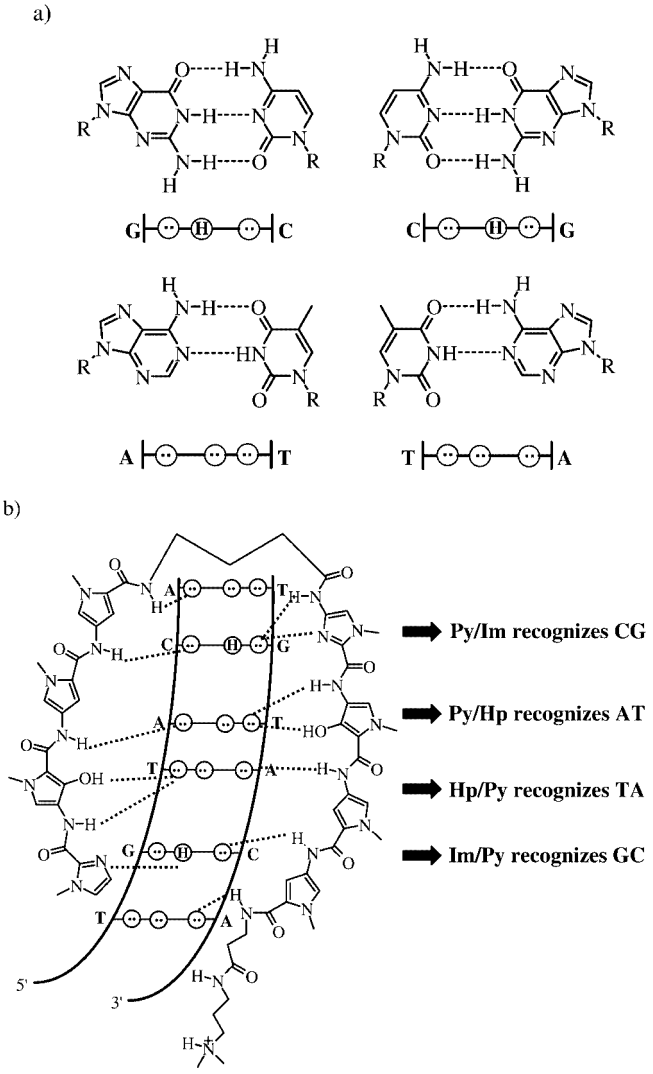
Scheme 1. The natural dDNA-binding oligoamides netropsin and distamycin

While netropsin forms complexes with suitable dDNA of 1:1 stoichiometry,[16−18] both 1:1 and 2:1 dDNA-substrate complexes are observed for distamycin.[19−21] Wemmer et al. were able to show by NMR studies that the binding motif attained varies with the concentration of distamycin and the base pair sequence of the dDNA.[22,23] In 2:1 complexes, two distamycin molecules bind into the minor groove of dDNA in an antiparallel fashion. Two opposite *N*-methylpyrrole-carboxamides of the two distamycin molecules bind specifically to one AT base pair. The *N*-methylpyrrole ring of one distamycin is superimposed by the peptide bond of the other.[22]

2.2 Artificial dDNA-Binding Heteroaromatic Oligoamides

Dervan et al. developed artificial oligoamides for selective recognition of all four Watson−Crick base pairs by variation of the heteroaromatic amino acids, based on the observed binding motifs of the natural oligoamides (Scheme 2).[24] A pyrrole (Py)/imidazole (Im) pair in opposite oligoamide strands distinguishes between the CG and the GC base pairs (reverse orientation Im/Py) and separates them from AT and TA.[25−28] With Py/Py, a preference for AT over GC or CG is achieved, but discrimination between AT and the reverse orientation TA is not possible.[22,23,25−29]

The problem was solved by the introduction of an additional heterocycle, 3-hydroxy-*N*-methylpyrrole (Hp).[30] The Hp/Py combination binds selectively to the TA base pair, while Py/Hp recognizes the AT base pair.[31−33] The dDNA-binding ligands form hydrogen bonds to the electron pairs of nitrogen atom N3 of the purine bases, oxygen atom O2 of the pyrimidine bases, and the guanine 2-amino group.[22,34] The selective recognition of all four

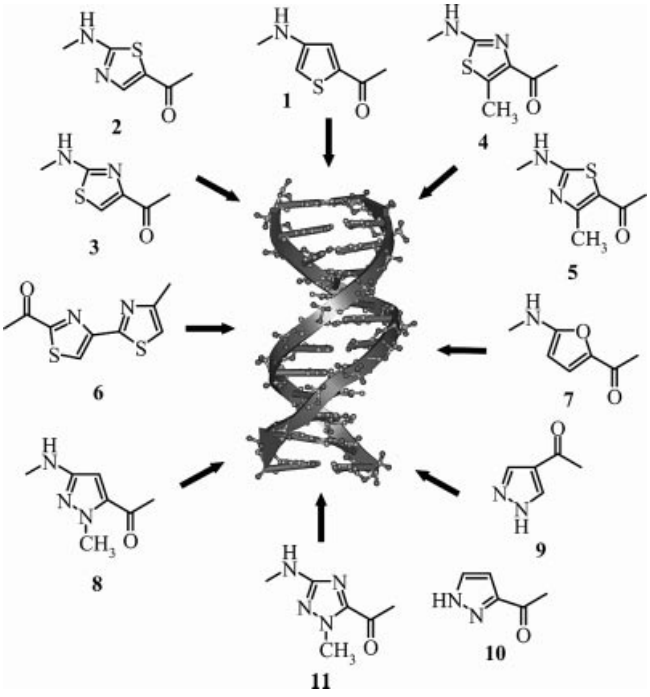


Scheme 2. Molecular recognition in the minor groove of dDNA; (a) hydrogen bonds between complementary Watson−Crick base pairs; circled points symbolize electron pairs of nitrogen N3 of purines and oxygen O2 of pyrimidines; circled H atoms symbolize the 2-amino group of guanine; R = sugar-phosphate backbone of DNA; (b) binding motif of a hairpin oligoamide with a 5′-TGTACA-3′ dDNA sequence; dashed lines symbolize hydrogen bonds

Watson−Crick base pairs in the minor groove of dDNA allows predictable and specific binding to base pair sequences. With heteroaromatic oligoamides that are able to cross cell membranes, even the regulation of gene expression with small molecules becomes possible.[34−40]

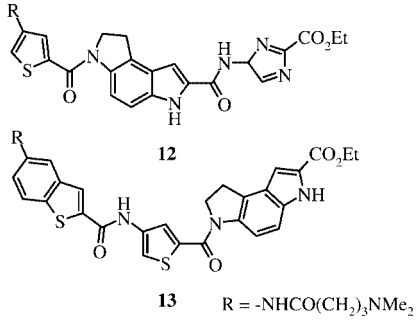
Many other five-membered ring heterocycles besides *N*-methylpyrrole, *N*-methylimidazole, and *N*-hydroxypyrrole have been used in the synthesis of oligoamides (see Scheme 3). Such synthetic analogues of the natural occurring compounds netropsin and distamycin have been named lexitropsins, as suggested by Lown et al.[41]

Some examples of compounds in which different heterocycles of these types have been incorporated are discussed in the following chapter, while more information about derivatives not mentioned is available in the cited references.



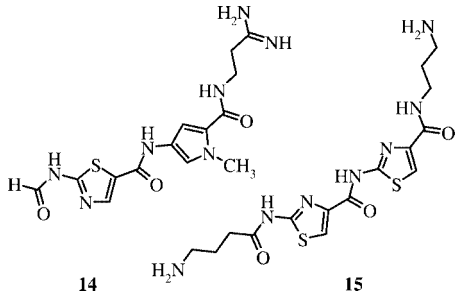
Scheme 3. Five-membered ring heterocycles found in lexitropsins

Boger et al.[42,43] synthesized a library of 2640 analogues of distamycin, from which two oligoamides with thiophene amino acid **1** have particular interesting properties. Compound **12** has a very high affinity for poly[dA]-poly[dT] DNA, of 2.5×10^6 M^{-1}, the second highest of all 2640 tested compounds.[44] An assay on cytotoxic activity against L1210 cells identified compound **13** as having 100 times higher activity than the natural product distamycin A.[45]



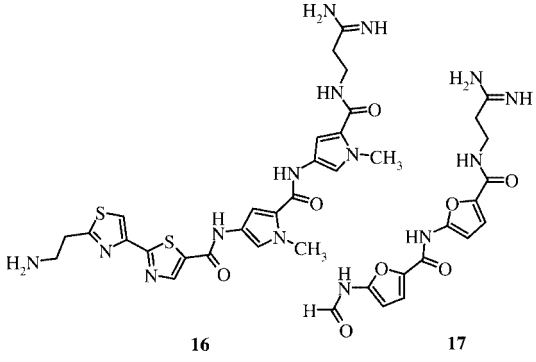
Scheme 4. Compounds **12** and **13** from Boger's distamycin analogues library

Oligoamides incorporating thiazole building blocks **2**, **3**, **4**, or **5** show two opposite trends in their dDNA binding selectivity. Depending on the orientation of the sulfur atom in the thiazole group, they either bind a GC base pair or show a strict AT binding preference.[46–49] Compound **14** (Scheme 5), in which the sulfur atom points into the minor groove of a DNA double helix, does not bind to GC base pairs. The related compound **15** (Scheme 5), in which the sulfur atom points away from the minor groove, shows its highest affinity for alternating purine-pyrimidine sequences, such as 5′-TATGAC-3′ and 5′-TATGAC-3′.[50,51]



Scheme 5. Related thiazole derivatives **14** and **15** with opposite binding selectivities

The bis(thiazole) unit **6** (Scheme 3), found in antitumor-active compounds of the bleomycin type, has binding affinity to GC base pairs.[52] The netropsin part of compound **16** (Scheme 6) directs the drug to AT-rich areas of a dDNA strand, while the bis(thiazole) unit **6** allows its binding to a pyrimidine-guanine-pyrimidine motif.[53,54]

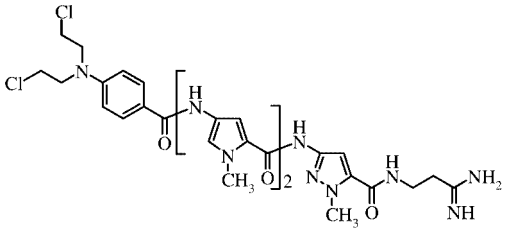


Scheme 6. Structures of compounds **16** and **17**

The bis(2,5-disubstituted) furan **17** (Scheme 6) binds to a sequence of four base pairs, binding to a GC pair in positions 1, 2 or 3 of the binding site being possible, while position 4 must be an AT base pair to produce affinity.[55,56]

Tallimustin (FCE24517)[57] is an analogue of distamycin, in which the formyl group is replaced by an alkylating unit. The compound is strongly cytotoxic, with a broad antitumor activity.[58] Similarly to distamycin, the compound shows a preference for AT base pair binding. The replacement of one or several *N*-methylpyrroles by pyrazole **8** (Scheme 3) gives compounds with the expected GC binding preference. Compound **18**, with one pyrazole, shows the same activity as tallimustin against L1210 cell lines, but with a decreased in vivo toxicity.[52d,59]

Only one reference for the use of a triazole **11** (Scheme 3) in the synthesis of carboxamides is to be found in the literature.[60] The prepared netropsin analogues, in which one or both pyrroles were replaced by a 1,2,4-triazole, and distamycin analogues in which one pyrrole was replaced by 1,2,4-triazole, showed only a low binding affinity to poly(dA)-poly(dT) DNA relative to the natural products. Ne-



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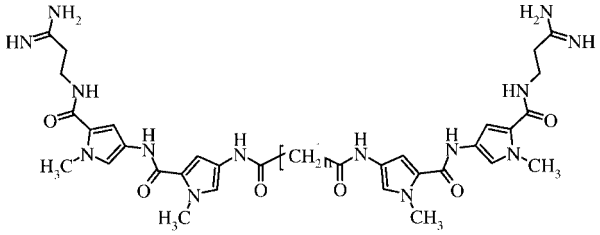
Scheme 7. Molecular structure of tallimustin analogue **18**



19 - 28 : n = 1 - 10

Scheme 8. Bis(netropsin) derivatives **19**−**28**, connected head-to-head by a flexible linker

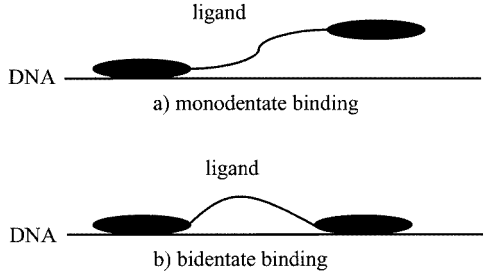
ither could any increased affinity for poly(dG)-poly(dC) DNA relative to the imidazole derivatives be found.

3. Principles for Connection of Heteroaromatic Oligoamides

For selective recognition of a single binding site in the human genome, which consists of approximately three billion base pairs, a ligand capable of recognizing at least 15 to 16 sequential base pairs is necessary.[61,62] First attempts focussed on the design of oligoamides with four, five, and six *N*-methylpyrrole-carboxamide moieties and aimed at the selective binding of six, seven, or eight consecutive AT base pairs. In theory, an oligoamide with $n-1$ five-membered heterocyclic carboxamides, or n amide bonds, should be able to bind $n+1$ subsequent base pairs.[62,63] However, binding studies revealed that maximum binding affinity was already reached with the pentaamide.[64] This observation is explained by the rigid structure of the growing oligoamide chain, which does not follow the curvature of the dDNA[65,65] and by the fact that an *N*-methylpyrrole-carboxamide moiety is 20% longer than the base pair it binds.[66] This results in a mismatch situation in the growing oligoamide chain.

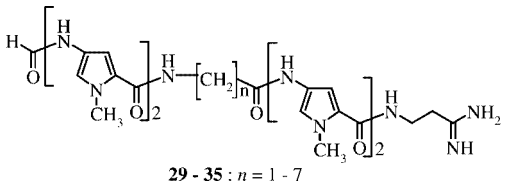
To solve the problem of non-complementary geometry and to increase specificity and affinity, dimeric, trimeric, and tetrameric oligoamides have been tethered to more extended assemblies. In the following section we give a summary of the structures and principles used to connect such short oligoamides.

A simple covalent linkage of two netropsin or distamycin molecules or their derivatives can be achieved by use of a polymethylene chain. Lown et al. reported the synthesis of bis(netropsins) **19**−**28**[67−69] (Scheme 8), connected head-to-head by CH_2 spacers.[70] The affinity constants of the compounds were determined by an ethidium bromide displacement assay[45] on calf thymus dDNA, but the affinities measured ($0.9-7.2 \times 10^7$ M^{-1}) were in the same range as for the monomeric netropsin.[68] DNase I footprinting studies[71] revealed that bis(netropsin) derivatives with a minimum spacer length of three CH_2 groups are able to undergo bidentate binding (Scheme 9) to dDNA. Because of the flexibility of the linker group, however, monodentate binding is also observed.[69,70]



Scheme 9. Schematic representation of (a) monodentate and (b) bidentate binding of a ligand in the minor groove of dDNA

Analogously to the described head-to-head linkage, a head-to-tail connection of two netropsins through a polymethylene chain is also possible. Scheme 10 shows such bis(netropsin) derivatives **29**−**35**, reported by Guo et al.[72,73] The relative affinity constants of the compounds were determined by ethidium bromide displacement assay with poly(dA)-poly(dT) DNA to be in the order of $0.55-1.67 \times 10^9$ M^{-1}.[45,73]

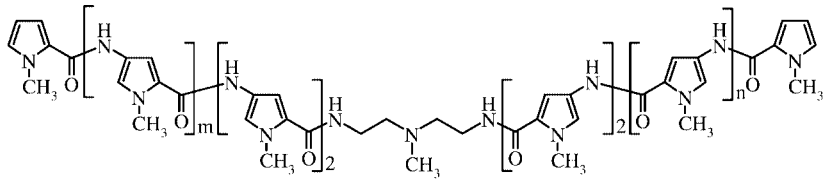


29 - 35 : n = 1 - 7

Scheme 10. Bis(netropsins) **29**−**35** with head-to-tail polymethylene linkages

The expected preference for AT binding was confirmed by much lower affinity constants for calf thymus (ca. 10^6 M^{-1}) and poly(dG)-poly(dC) DNA (ca. 10^5 M^{-1}). MPE·Fe^{II} footprinting studies[74] resulted in the conclusion that the bis(netropsin) analogues with n = 2, 4, and 6 bind in a bidentate fashion to dDNA, while the compounds with n = 1, 3, 5, and 7 prefer monodentate binding. For the bis(lexitropsins) with n = 2, 4, and 6 an increased cytotoxic activity against some human cancer cell lines was found. This is explained by effective binding of these compounds to dDNA, because the derivatives with an uneven number of methylene groups do not show comparable activity.[73]

Bis(distamycin) derivatives with tertiary amines within the covalent linker structure were first reported by Thomas et al.[75] in 2001 (Scheme 11).



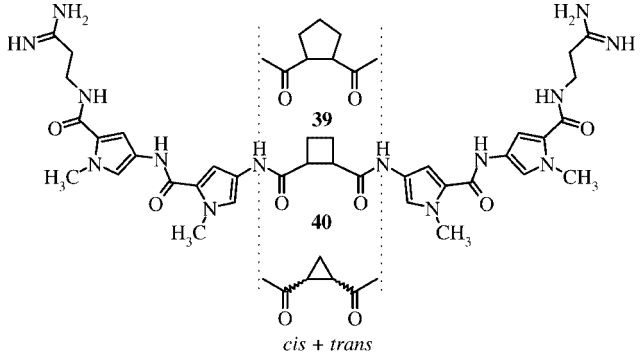
36 : $m = 0, n = 0$
37 : $m = 0, n = 1$
38 : $m = 1, n = 1$

Scheme 11. Tail-to-tail-linked bis(lexitropsine) **36**−**38**

Unlike the compounds discussed above, the distamycins are connected in a tail-to-tail fashion. The positively charged ammonium group, crucial for dDNA binding and sufficient water solubility of the compound, is now located within the linker unit. The tertiary amine is protonated under physiological conditions.

Compounds **36**, **37**, and **38** show a significantly increased affinity for poly[(dAT)]-poly[(dAT)] DNA in relation to the monomeric oligoamides. The largest increase in affinity, 33-fold if compared to the monomeric compound, was observed for bis(lexitropsin) **36**.[76] Upon binding to dDNA, two bis(lexitropsins) become arranged in an antiparallel orientation in the minor groove and interact with 8−10 AT base pairs.

To overcome fully the problem of monodentate versus bidentate binding modes, more rigid linkers were employed to connect netropsins (Scheme 12).[77] Such compounds interact with eight to ten subsequent base pairs and do not bind to shorter sequences.[70,78]



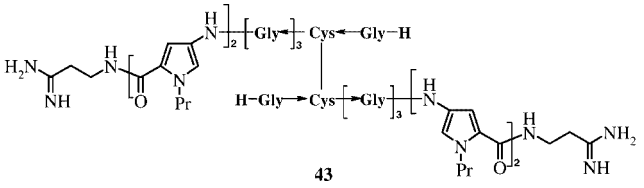
39
40
cis + *trans*
41, 42

Scheme 12. Examples of bis(netropsins) **39**−**42** with rigid linker groups

Differences in binding affinities were found for *cis*- and *trans*-cyclopropyl-connected bis(netropsins) **41** and **42**.[79,79] These bis(netropsins) interact with suitable dDNA in a two-step mechanism of monodentate and subsequent bidentate binding, which depends on salt concentration.

In the case of the *trans* compound, stable complexes with DNA are observed at high salt concentrations, while the *cis* compound does not show such a binding mode. When tested for their ability to inhibit dDNA topoisomerase enzyme activity, the *trans*-bis(netropsin) was more active, which again can be explained by its stronger bidentate binding.

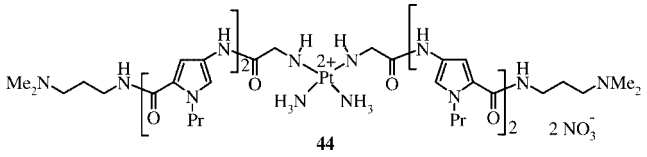
Another linker motif is achieved with peptide-bridged bis(oligoamides).[80−82] Of the molecules reported, the oligoamide **43** (Scheme 13) shows the most interesting properties.[82] The compound has an increased affinity for base pair sequences that possess a central GC unit, such as $(AT)_3(GC)_2(AT)_3$, relative to the homologous $(AT)_n$ sequence. The observation is explained by a binding motif in which both netropsin parts bind three AT base pairs each while the central peptide unit shows a preference for GC binding. Unlike in the case of the previously discussed methylene-linked bis(lexitropsins), the linker unit here contributes to the overall binding specificity.



43

Scheme 13. Example of a peptide-linked lexitropsins **43**; the arrows indicate the orientation (N−C$^{\alpha}$−C′) of the peptide; Pr = propyl

An example of a dimeric oligoamide in which the two components are linked by the metal complex *cis*-diamineplatinum(II) is shown in Scheme 14.[83−88]



44

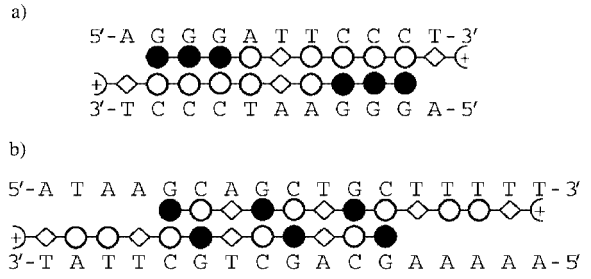
Scheme 14. A *cis*-diamineplatinum(II)-linked bis(netropsin); Pr = propyl

Bis(netropsin) **44** forms two types of aggregates with poly[(dAT)]-poly[(dAT)] DNA and DNA oligomers of the sequence 5′-CC(TA)$_n$CC-3′ with n = 4, 5, 6.[86,89] Either the ligand binds in its extended conformation to eight to nine subsequent AT base pairs {e.g., in poly[(dAT)]-poly[(dAT)] DNA}, or the complex adopts a hairpin structure with both netropsin units binding in the minor groove in parallel orientation. According to molecular modeling studies, the two complexes convert into one another by rotation of the N−C$^{\alpha}$ and C$^{\alpha}$−C′ single bonds of the glycines next to the

platinum(II) linker. In the hairpin structure the pyrrole heterocycles are superimposed and interact with nonequivalent positions in the minor groove. One of these interactions is stronger than the other.

Furthermore, compound **44** can be used to cleave dDNA. Upon X-ray radiation of a platinum(II)−bis-(netropsin)−dDNA complex, selective dDNA cleavage at the binding sites of **44** was observed. Each of the DNA strands is cleaved only once for each binding site.

The discovery of a 2:1 ligand/dDNA binding motif in 1989, in which two distamycins bind antiparallel in the minor groove of dDNA in a side-by-side arrangement, paved the way for the development of new high-affinity dDNA binding ligands. Head-to-tail covalent linkage of the oligoamide units by aliphatic amino acids solved the problem of the noncomplementary geometry of extended oligoamides and the dDNA and gave rise to increased affinity and specificity. β-Alanine (β) proved to be an ideally suited linker for this purpose.[89,90] Assemblies in which two such amino acids occupy opposite positions of the double strand show an affinity for AT and TA base pairs 20 times better than for GC or CG.[90,91] The linker unit therefore contributes to the overall binding selectivity of the extended oligoamide ligands. This strategy makes selective recognition of a sequence of 11 base pairs within a dDNA possible (Scheme 15, a).

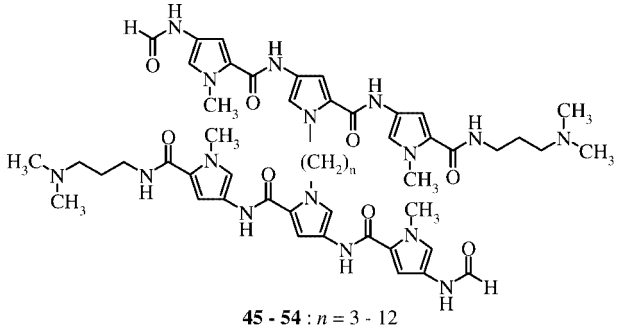


Scheme 15. Schematic representation of linear oligoamides binding to dDNA; black circles: imidazole; white circles: pyrrole; diamonds: β-alanine; +: under physiological conditions protonated (dimethylamino)propylamine; (a) fully overlapping homodimer binding to a sequence of 11 base pairs; (b) partly overlapping homodimer recognizing 16 base pairs

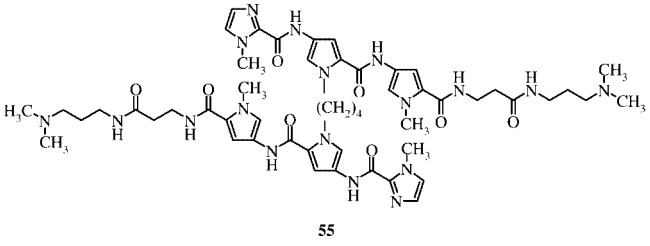
As confirmed by NMR investigations, the two eight-membered ring oligoamides bind into the minor groove of dDNA in antiparallel orientation with full overlap of both oligoamides.[90] Sequence-specific binding to 16 subsequent base pairs in the minor groove of dDNA was first reported in 1998, by Dervan et al.[91] A partly overlapping homodimer consisting of *N*-methylpyrrole, *N*-methylimidazole, and β-alanine was used for recognition, and an association constant of $K_a \geq 3.5 \times 10^{10}$ M^{-1} was reported (Scheme 15, b).

Although the formation of 2:1 ligand/dDNA complexes occurs in a cooperative way, their formation is unfavorable, relative to the binding of only one ligand molecule, in entropic terms. A suitable strategy to enhance affinity and specificity further is through covalent connection of the two antiparallel oligoamide strands. This transforms the 2:1 association binding motif of two oligoamides into a bidentate binding with a 1:1 stoichiometry. A simple way to do so is by covalent linkage of two of the heterocyclic nitrogen atoms.[92]

Several such compounds, commonly known as H-pin structures,[93] have been reported by Dervan et al.[94−96] and Lown et al.[94,97−103] Circular dichroism titration experiments with compounds **45** to **54** (Scheme 16) revealed an increase in binding strength to poly[(dAT)]-poly[(dAT)] DNA with 1:1 stoichiometry with increasing *n* (*n* = 4 to 10[94,100]). Affinity constants of H-pins **45**−**54** for poly-[(dAT)]-poly[(dAT)] and poly(dA)-poly(dT) DNA were determined by ethidium bromide displacement assay.[45] Relative to the monomeric oligoamides, the compound with *n* = 10 shows a 14000-fold increase in affinity for its bidentate binding. Hydrophobicity of the ligands is also of importance. Compounds analogous to lexitropsins **45**−**54**, but possessing a 3,6-dioxooctanediyl linker, show a further increase in affinity by a factor of 10. Oligoamides with H-pin structure are accessible by a solid-phase synthesis procedure.[95] Oligoamide **55** (Scheme 17), which was prepared by this method, binds to dDNA with 180-fold greater affinity than the unlinked oligoamides.



Scheme 16. Lexitropsins covalently tethered by a methylene linker

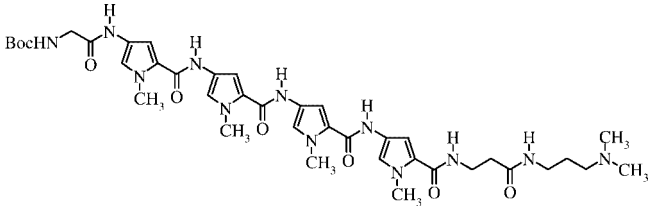


Scheme 17. Example of an *N*-methylpyrrole-*N*-methylimidazole H-pin oligoamide

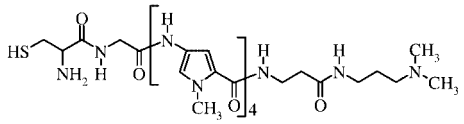
Immobilization on surfaces may be viewed as a different approach by which to couple and organize DNA-binding oligoamides.[104] Compounds **56** and **57**,[105,106] bearing terminal amino or thiol groups for anchoring, were prepared by modified solid-phase procedures[107] for heteroaromatic oligoamide synthesis. Immobilization of **56** on Fractogel® EMD Epoxy in basic aqueous solution yielded a polymeric material showing affinity for double-stranded plasmid pTri-Ex™-1 DNA. The binding capacity and kinetics are poorer

than those obtained by use of commercial ion exchange resins, however.[108]

The thiol group of **57** allows immobilization on gold surfaces. Although a binding constant to calf-thymus dDNA of $K = 10^4$ L/mol was determined for **57** in its cysteine-protected (PMB, Boc) form by ethidium bromide displacement assay, the dDNA affinity dropped below 10^3 L/mol after immobilization on a gold surface.



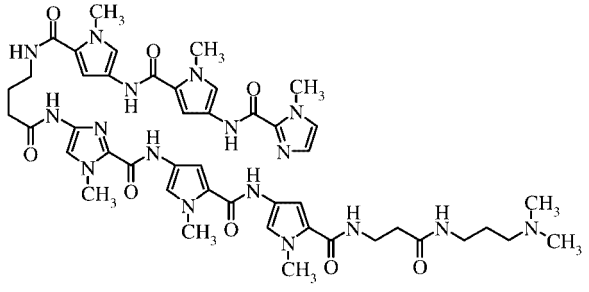
56



57

Scheme 18. Oligoamides with amino or thiol groups for anchoring to surfaces

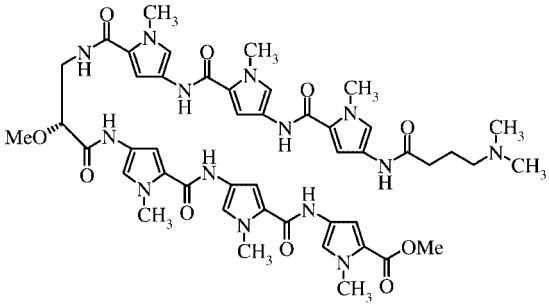
A major breakthrough in the rational design of dDNA binding ligands was the synthesis of oligoamides with hairpin structures.[109] Such compounds bind to dDNA with affinities and specificities of the same order as those of naturally occurring dDNA-binding proteins.[32,33,110−113] The optimal turn structure for the connection of the two oligoamide strands has been found to be γ-aminobutyric acid (γ-turn) (Scheme 19).[112]



58

Scheme 19. ImPyPy-γ-ImPyPy-β-Dp (**58**) − an example of an oligoamide with a hairpin structure

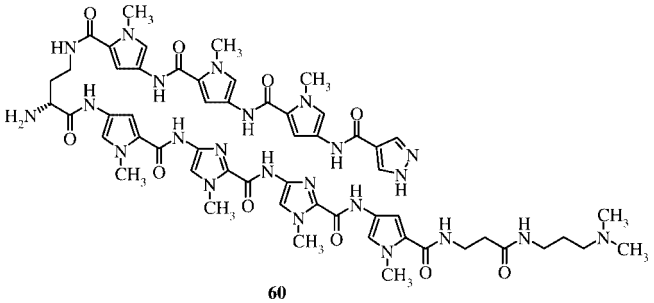
The use of glycine or β-alanine to connect the two oligoamides yielded compounds with no or very little affinity for the target dDNA. Even a linker consisting of several methylene groups gave a reduced affinity of the oligoamide relative to γ-aminobutyric acid, with three methylene groups. The longer linker induces a turn structure, but the resulting geometry is not as well suited for binding into the minor groove as with γ-aminobutyric acid.[114] Boger et al. devised an alternative structure to the γ-turn linkers,[115] using an (*R*)-α-methoxy-β-alanine spacer to link two Py-PyPy oligoamides (Scheme 20) and observing, contrary to the case of the use of unsubstituted β-alanine, a hairpin structure.



59

Scheme 20. (*R*)-α-Methoxy-β-alanine-bridged oligoamide **59**

Replacement of an *N*-methylimidazole of an Im/Py pair of a hairpin oligoamide with 3-pyrazole **60** (Scheme 21) increases the affinity and specificity for a GC base pair. This can be explained by the formation of a specific hydrogen bond between the exocyclic guanine amino group and nitrogen atom N2 of the 3-pyrazole. A general increase in affinity and specificity of hairpin derivatives is observed upon addition of a β-alanine to the C-terminus.[116] The terminal amino acid and the γ-turn moiety show affinities for AT/TA base pairs 200−400 times stronger than for GC.[117]



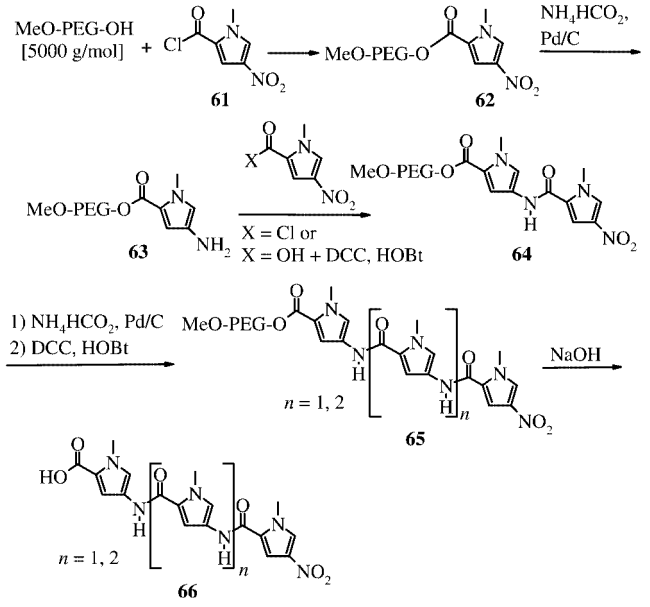
60

Scheme 21. Pyrazole-carboxamide hairpin **60**

The affinities and specificities of the hairpin structures are in general stronger than those of the H-pin compounds. This is illustrated by compound **58** (Scheme 19), which binds to the sequence 5′-TGTCA-3′ ($K_a = 2.0 \times 10^8$ M^{-1}) with twenty times the affinity and three times the specificity of the comparable H-pin lexitropsin **55** (Scheme 17, $K_a = 9.3 \times 10^6$ M^{-1}).[95]

A facile synthetic route to hairpin oligoamides is provided by solid-support procedures[118] reported by Dervan et al., which use *tert*-butoxycarbonyl (Boc)[110,119] or fluorenyl-9-methoxycarbonyl (Fmoc) protecting groups.[120]

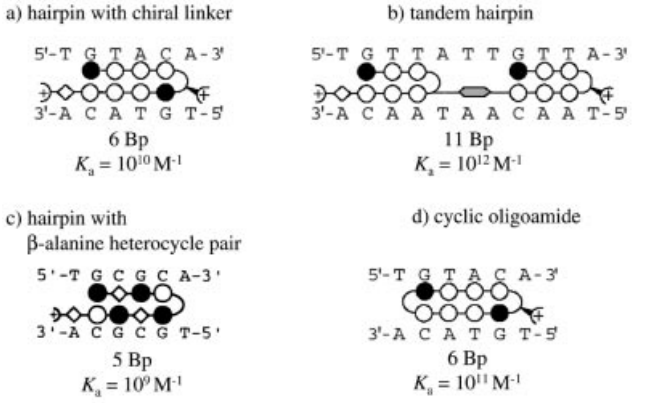
An alternative synthetic route for the preparation of short Py/Im oligoamides is synthesis on methoxypoly(ethylene glycol) as a solubilizing polymer.[121,122] The procedures cannot be automated and are restricted to short oligoamides, but they overcome potential solubility problems and



Scheme 22. Synthesis of short pyrrole oligoamides on methoxy-poly(ethylene glycol) as solubilizing polymer support

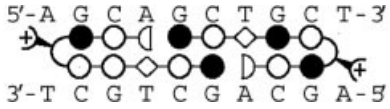
allow a facile and cheap synthesis of building blocks, which may be subsequently combined to produce larger ligands.

Other binding motifs based on the successful hairpin structures have also been developed (Scheme 23). The introduction of an amino group into the prochiral position of the γ-amino acid linker generates a second positive charge within the hairpin molecule under physiological conditions (Scheme 23, a).[123,124] Such a modification increases the affinity of the ligand for the target dDNA by a factor of 10, whereas no loss in binding specificity is observed.[126]



Scheme 23. New polyamide dDNA binding motifs; K_a: equilibrium-association constant; Bp: base pairs; black circles: imidazole; white circles: pyrrole; diamonds: β-alanine; +: (dimethylamino)-propylamine moiety, protonated under physiological conditions; curved line in (a), (b) and (d): (*R*)-2,4-diaminobutyric acid; curved line in (c): γ-aminobutyric acid

An extension of the binding site to ten base pairs without increase in the molecular mass of the ligand was achieved by cooperative association of two chiral hairpin structures (Scheme 24).[127] The affinity and specificity of binding are similar to those of conventional hairpin structures, which would bind to five base pairs.[110,125]



Scheme 24. Schematic representation of chiral hairpin oligoamides showing cooperative binding; half-circles represent $(CH_2)_2$−OH moieties; see caption of Scheme 23 for all other symbols

Another option is the coupling of the turn and end amino groups of two chiral hairpin structures with a dicarboxylic acid such as valeric acid, which yields so-called tandem hairpin structures (Scheme 23, b).[126] Dervan et al. were able to show that this produces an extension of the binding site and increased affinity of the dDNA binding ligand. The oligoamide shown in Scheme 23 (b), with six heterocyclic rings in each sub-hairpin, binds with picomolar affinity to the sequence 5′-TGTTATTGTTA-3′ and with a 4500-fold specificity relative to 5′-TGT*C*ATTGT*C*A-3′ (mismatch base pairs are underlined).[130]

Tethering of the linkers of two hairpins yields linker−linker-bridged tandem hairpins.[127] However, the synthesized tandem oligoamides, with six heterocyclic rings each, show slightly lower affinity constants, though still of a maximum of $7.5 \times 10^{10}\ M^{-1}$, on binding to 10 subsequent base pairs.

The microstructure of dDNA depends on its base pair sequence,[128−131] so some of these sequences are more difficult to bind than others. An example illustrating this is the oligoamide ImPyImPy-γ-ImPyImPy-β-Dp, which was prepared to bind 5′-TGCGCA-3′, but shows only moderate affinity of $K_a = 3.7 \times 10^7\ M^{-1}$ with this sequence.[132] It was speculated that the selective exchange of an *N*-methylimidazole or an *N*-methylpyrrole for the more flexible amino acid β-alanine might help to overcome the structural hindrance of the target dDNA sequence. The prepared analogous derivative Im-β-ImPy-γ-Im-β-ImPy-β-Dp (Scheme 23, c), in which an Im/β pair had been introduced, shows subnanomolar affinity ($K_a = 3.7 \times 10^9\ M^{-1}$) towards the above dDNA sequence.[136]

Cyclic polyamides bind to dDNA with even higher affinity than their hairpin analogues.[133−135] An example is shown in Scheme 23 (d): The cyclic oligoamide binds to the target dDNA sequence 5′-AGTACT-3′ with $K_a = 7.6 \times 10^{10}\ M^{-1}$, eight times higher than that of the comparable hairpin structure. The sequence specificity is similar to those of hairpin oligoamides.[138]

4. Conclusion

Results in this field over the last ten years have shown that specific recognition of dDNA sequences by minor

groove binding with synthetic heteroaromatic oligoamides is possible. Although not all factors influencing binding affinity and specificity are fully understood, it has been possible to rationally design and prepare ligands with binding properties similar to those of natural dDNA-binding proteins. Such artificial pyrrole-imidazole oligoamides seem to be less toxic than the natural products netropsin and distamycin,[24d] and so one focus of ongoing research is their use in molecular medicine to regulate gene expression in living cells.[36−41]

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