CHIMIA 2001, 55, No. 12

Chimia 55 (2001) 1041–1044

ISSN 0009–4293

Conformationally Restricted Oligonucleotide Analogues 'Made in Bern': A Mini Review

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Abstract: In our search for novel oligonucleotide analogues for use in therapy and DNA-based diagnostics we explored the concept of conformational preorganization as a means to improve duplex stability with complementary DNA and RNA. This research effort has led to a number of novel DNA analogues and has contributed to a deeper understanding of the interrelation between the structure of nucleosides and the properties of corresponding oligonucleotides. Some of these analogues show interesting biological properties. This article is a short review summarizing the properties of bicyclo-DNA and tricyclo-DNA, developed in our laboratory.

Keywords: Conformational restriction · DNA · Nucleosides · Oligonucleotides · RNA

1. Introduction

The discovery of the oligonucleotide-directed inhibition of gene expression on the level of translation by Zamecnik and Stephenson [1][2] more than two decades ago set the basis of what is called the antisense approach in human therapy. Conceptually, the approach is based on an oligonucleotide analogue that is complementary (antisense) in sequence to a mRNA of interest, leading to Watson-Crick duplex formation with its target, thus inhibiting its translation into a protein (Fig. 1).

One of the most appealing advantages of this concept over traditional small molecule protein targeting in drug design lies in the fact that only minimal structural knowledge about the target RNA (Fig. 2), namely its base sequence, is required. Its specificity to one unique target sequence can be triggered by the number of nucleotide residues in the chain, ideally leading to a statistically unique base sequence within the complete sequence space of the genome. The universal nature of antisense technology not only makes it thus a promising key instrument for tomorrow's therapies, but also a powerful tool in molecular biology for the manipulation of gene expression and the determination of protein function.

Our group is involved in a research effort towards the search for oligonucleotide analogues that bind with higher affinity to complementary DNA and RNA and that are more resistant to degradation by cellular enzymes than DNA and RNA. These key features are necessary requirements for diagnostic and therapeutic applications. In this context we started to apply the concept of conformational restriction to oligonucleotide analogues. The rationale was to preorganize the structure of an oligonucleotide single strand as to match the structure of the duplexed form. From this an entropic benefit upon duplex formation is expected, eventually leading to increased duplex stability. Over the years we have prepared a series of different conformationally constrained DNA analogues (Fig. 3). Here, a short summary of the properties of bicyclo-DNA and tricyclo-DNA, the most prominent representatives of this group is given.

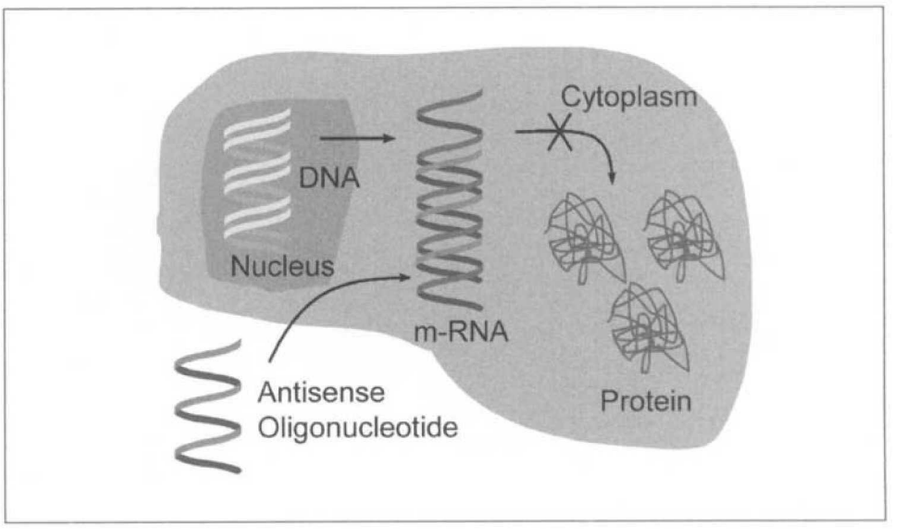

Fig. 1. Schematic representation of the antisense principle

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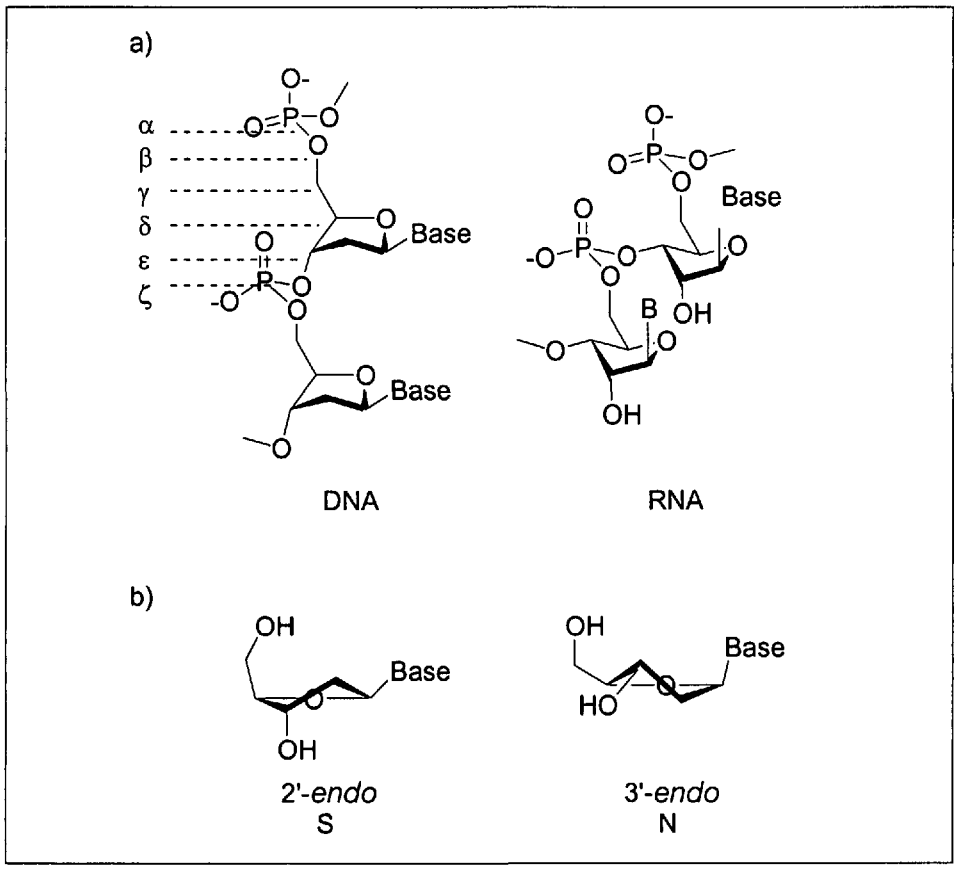

Fig. 2. a) The chemical structures of DNA and RNA, represented in their intrinsically preferred conformation, with indication of the backbone torsion angles describing the structure of the repetitive backbone unit; b) representation of the two major nucleoside conformations occurring in A- and B-DNA.

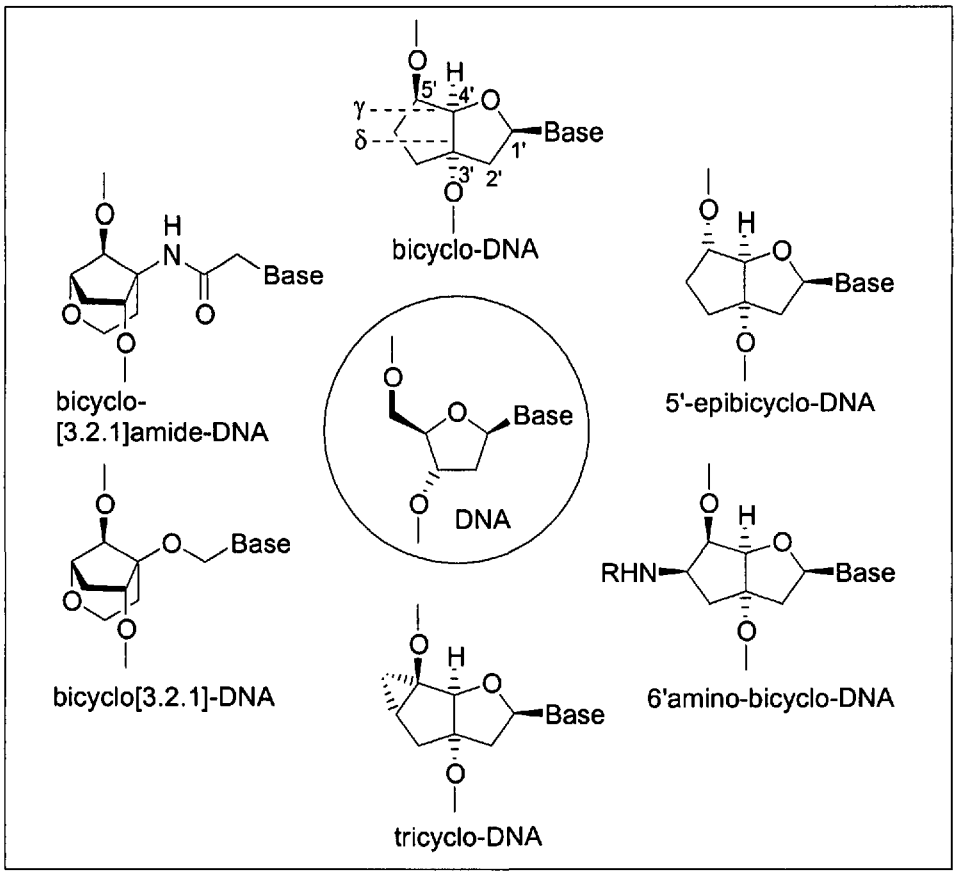

Fig. 3. Chemical structures and acronyms of a series of oligonucleotide analogues, displaying different aspects of conformational restriction, that were recently synthesized and evaluated in our laboratory.

2. Bicyclo-DNA

In our design of bicyclo-DNA (Fig. 3) we intended to include both backbone bonds (*cf.* torsion angle γ and δ) that lie within the carbon framework of a natural nucleoside in a ring structure, reasoning that the effect of restricting the C(4')–C(5') bond, which has minimal conformational restriction in the natural nucleosides, can contribute substantially to the preorganization of a corresponding oligonucleotide. Early molecular modeling of the nucleosides suggested that introduction of the carbocyclic ring into the [3.3.0]-scaffold would drive the furanose ring into a 2'-*endo*/1'-*exo* conformation (S-type conformation) as observed in B-DNA (Fig. 2) [3]. This prediction was fully supported by structural analysis of the mononucleosides in the solid state and in solution by X-ray crystallography and variable temperature NMR, resp. The configuration at C(5') (*R*) was chosen to match the geometry of torsion angle γ in DNA-duplexes of the A- and B-type (synclinal, +*sc*) as closely as possible. It was, however, clear from modeling and structural analysis of nucleoside monomers that the antiperiplanar (*ap*) arrangement of γ, orienting the hydroxy group in a pseudoequatorial position, is energetically preferred in bicyclo-nucleosides [3].

2.1. *Homopurine/Homopyrimidine Sequences of Bicyclo-DNA*

The first bicyclo-oligonucleotides to be investigated were homodecamers of the bases adenine and thymine. These were prepared in high yield using the well-established protocols of automated DNA synthesis from phosphoramidite building blocks [4][5]. Typically adenine homodecamers with the bicyclo-DNA backbone show equal to increased thermal and thermodynamic stability when complexed to natural DNA or RNA, resp. The opposite is the case for homo-thymidine sequences with the bicyclo-DNA backbone. A thermochemical analysis by means of differential scanning calorimetry (DSC) and isothermal titration calorimetry (ITC) revealed the higher thermodynamic stability to be entropic in origin. A structural investigation based on CD-spectroscopy, however, suggested that bicyclo-DNA purine strands prefer an association mode that is different from the Watson-Crick base-pairing mode (Fig. 4) [4]. Final proof for this came by introduction of a 1-deaza adenine base (Fig. 4) into the center of a bicyclic homo-A decamer [6]. Such a modified duplex displays almost fully restored pairing behav-

ior with regard to the non-modified bicyclo-DNA duplex, which ruled out Watson-Crick pairing in this system. The same results were also obtained with non-symmetric adenine- and guanine-containing homopurine sequences [6]. All these data taken together firmly allow the conclusion that within the homopurine sequence context bicyclo-DNA prefers the Hoogsteen and reverse-Hoogsteen over the Watson-Crick base-pairing mode. Structurally this change in association mode is solely triggered by the change of torsion angle γ from a *+sc* to an *ap* arrangement.

2.2. *Watson-Crick Base-pairing and Biological Stability*

DNA oligonucleotides with mixed-base sequences, for structural reasons, cannot form Hoogsteen duplexes unless changes in the preferred orientation of the glycosidic bond are taken into account. We had shown previously on the monomers by NMR-NOE spectroscopy that this is unlikely to occur in bicyclo-DNA. An investigation of a series of mixed-base bicyclo-DNA sequences revealed that Watson-Crick duplex formation in its own series as well as with complementary DNA and RNA readily occurs [7]. The thermal stability of heteroduplexes with DNA and RNA are in the same order as that of natural DNA. Base-pairing is sequence and orientation specific as is the case for DNA. Bicyclo-DNA is more stable against phosphodiesterases. A relative enhancement in stability of a factor of 10 against the enzyme snake venom phosphodiesterase, and a factor of 8 when incubated in fetal calf serum was measured.

2.3. *5'-Epi-bicyclo-DNA: Profiling Torsion Angle* γ

In order to complete the structure/affinity analysis around the C(4')–C(5') bond the bicyclonucleosides with inverted configuration at C(5') were prepared and incorporated into DNA (Fig. 3) [8][9]. This inversion brings about a shift of torsion angle γ to the *–sc* range as determined by NMR on the mononucleosides containing the bases adenine and thymine. The strong decrease in affinity towards complementary DNA of partially modified oligonucleotides, and the failure of a fully modified homothymidine decamer to form a duplex with its DNA complement shows the inability of this local structural arrangement for right-handed, helical Watson-Crick duplex formation. Taken all together, the general structure/activity profile for tor-

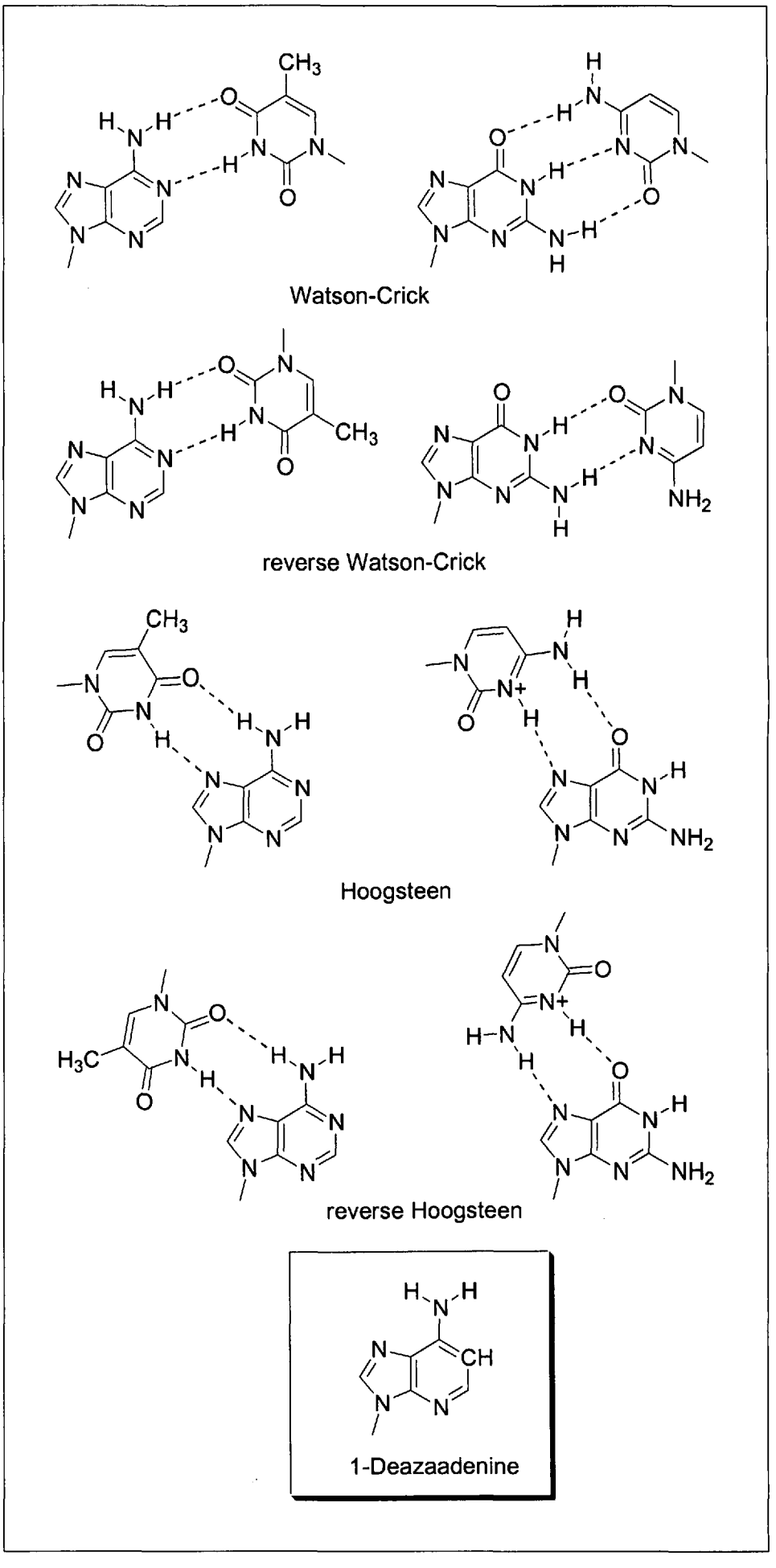

Fig. 4. Structures of different A-T (left) and G-C (right) base-pairs of relevance, and structure of 1-deazaadenine.

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sion angle γ, outlined in Fig. 5, emerges in which the *+sc* alignment in oligonucleotides leads to preferential Watson-Crick duplex formation, the *ap* alignment to preferential Hoogsteen- or reverse-Hoogsteen duplex formation, and the *–sc* alignment is not compatible with a pairing conformation.

3. Tricyclo-DNA

A second generation representative of the family of conformationally constrained DNA-analogues is tricyclo-DNA (Fig. 3). Originally the aim of the introduction of the cyclopropane ring in tricyclo-DNA was to further restrict conformational flexibility of the backbone and to correct, at least in part, for the geometry of backbone torsion angle γ. Taking advantage of intermediates along the synthetic pathway of bicyclo-DNA, tricyclo-nucleosides of the bases adenine and thymine became readily available [10]; they were converted into oligonucleotides and their complementary duplex formation investigated [11][12]. Tricyclo-DNA sequences resist enzymatic hydrolysis by the 3'-exonuclease snake venom phosphodiesterase. Homobasic adenine- and thymine-containing tricyclo-DNA octa- and nonamers are extraordinarily stable A-T base-pairing systems, not only in their own series but also with complementary DNA and RNA. Base mismatch formation is strongly destabilized. As in bicyclo-DNA, the tricyclo-DNA purine sequences preferentially accept a complementary strand on the Hoogsteen face of the base. A thermodynamic analysis reveals entropic benefits in the case of hetero-backbone duplex formation (tricyclo-DNA/DNA duplexes) and both an enthalpic and entropic benefit for duplex formation in the pure tricyclo-DNA series compared to natural DNA.

Meanwhile tricyclo-DNA oligonucleotides containing all four bases in a mixed-sequence context are available. Fully modified tricyclo-oligonucleotides efficiently form Watson-Crick duplexes with complementary DNA and RNA that show markedly increased thermal stabilities. Tricyclo-DNA turns out to be completely stable in heat-deactivated fetal calf serum and does not elicit RNaseH activity in duplexes with RNA (RNaseH is a ubiquitous cellular enzyme that catalyzes the degradation of an RNA strand in a RNA/DNA duplex) [13]. Especially the latter property does not particularly qualify tricyclo-DNA as an antisense probe against coding sequences of mature RNA, but it is a prerequisite for antisense oligonucleotides targeted to non-coding regions as *e.g.* intron sequences of pre-mRNA. We could recently show in a collaborative effort with the group of Prof. D. Schümperli at the University of Bern that aberrant pre-mRNA splicing *in vivo* in HeLa cells which stably express β-globin pre-mRNA carrying point mutations at the intron positions 654 and 705 [14] can be efficiently restored when targeting the aberrant splice site by tricyclo-DNA [13]. A direct comparison with a 2'-OMe phosphorothioate oligoribonucleotide of the same sequence and length showed enhanced activity of tricyclo-DNA by a factor of up to 100. Thus tricyclo-DNA is definitely an interesting antisense candidate for further exploration.

Acknowledgments

I am very much indebted to the enthusiastic efforts of all my former and present Ph.D. students and post-docs that have contributed to the success of our projects. Particularly involved in what has been reviewed here were Martin Bolli, Christopher Litten, Ralph Steffens, Markus Tarköy, and Dorte Renneberg. We all thank the Swiss National Science foundation for continuous financial support.

Received: October 17, 2001

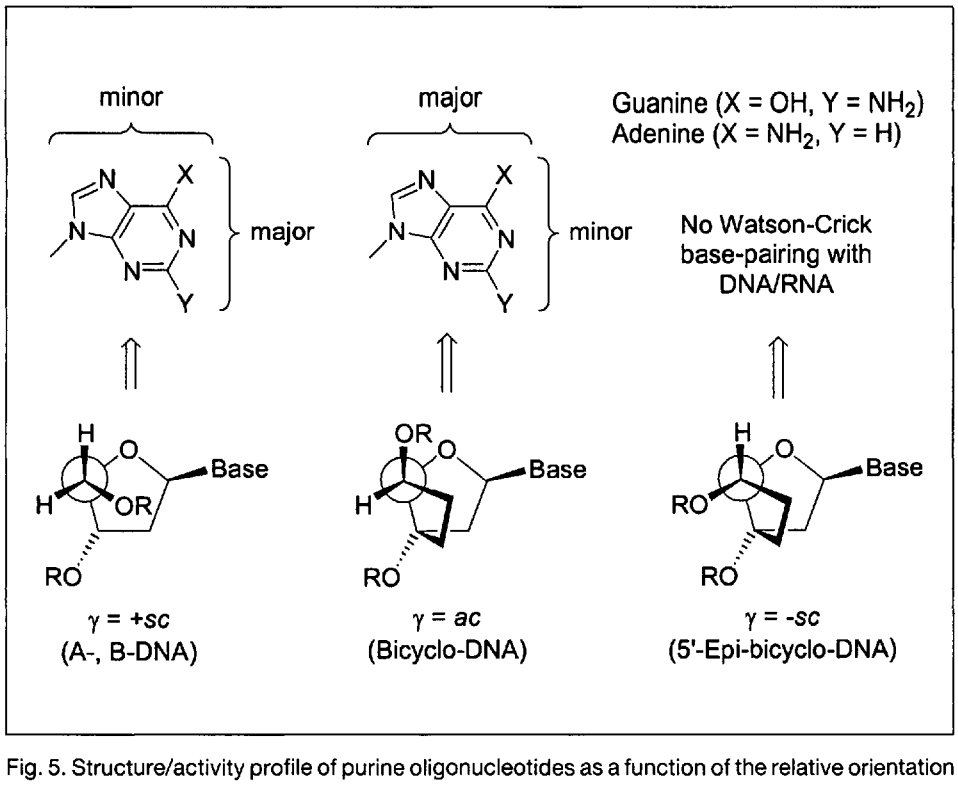

Fig. 5. Structure/activity profile of purine oligonucleotides as a function of the relative orientation of torsion angle γ.

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