Towards better transfection systems – understanding the interaction between polyamines and DNA/RNA

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Aim

The aim of the project is to investigate the relationship between structure, stability and activity of complexes between polyamines and DNA or siRNA. The hypothesis is **that an improved understanding of the factors governing stability of complexes between polyamines and DNA or siRNA will lead to improved systems with better safety for transfection and gene therapy, which eventually can be used for in vivo purposes.**

Project description

Polyamines are the common name for a highly diverse class of compounds containing multiple amino groups. DNA or RNA has a structure with a backbone presenting phosphate groups to the surroundings. These phosphate anions form salts in the presence of cations, but when a molecule of DNA or RNA is mixed with a polyamine salt formation takes place, where some of the amino groups in the polyamine are converted into positively charged ammonium ions forming ionpairs with the phosphates on the backbone. We hypothesise that the exact nature of these complexes and their stability must depend on the length of the polyamine, the shape of the polyamine (linear or globular) and on the distance between the amino groups. While the influence of these factors has been addressed for polyethyleneimine-based systems[1], there is almost nothing known for other types of polyamines.

The naturally occurring linear polyamines spermine, spermidine and putresceine, which in nature form complexes with DNA and RNA have all a larger distance between the amino groups than polyethyleneimine. This must therefore affect the stability of the complexes, because it is known that the acid-base properties of polyelectrolytes such as polyamines depend on the distance between the amino groups. We will address these questions by investigating the complex formation and stability between linear oligomeric analogs of spermine and spermidine and DNA and siRNA.

Examples of projects:

It is possible to do synthetic projects, physicochemical projects, transfection studies *in vitro*, toxicity studies or combinations depending on research interests. The synthetic work will take place under supervision of Jørn B. Christensen, Department of Chemistry (NAT), while physicochemical, transfection and toxicity takes place under supervision of Hanne Mørck Nielsen and Camilla Foged both from the Department of Pharmaceutics and Analytical Chemistry (FARMA).

1: Synthesis of linear polyamines.

The polyamines needed for the project will be synthesized as shown in figure 1 based on the use of continous-flow synthesis in a microreactor to avoid overalkylations in the alkylation with the alkylsulfonylazetidine.

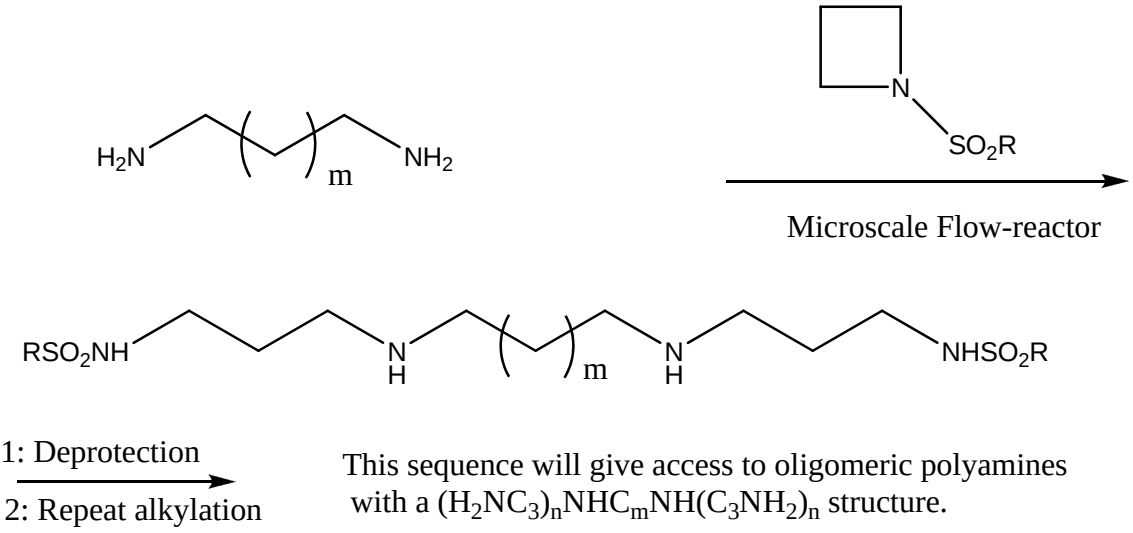


Figure 1: Synthesis of polyamines.

2: Synthesis of linear homopolyamines.

Oxazolines polymerises upon alkylation with an initiator on the ring nitrogen atom to give linear poly(ethylene)imine as shown in figure 2. The reaction is an example of a living cationic polymerisation, which means 1) that the average length of polymer can be controlled via the ratio between the initiator and the monomer and 2) the polymer is born with a reactive end, which can subsequently react with either another monomer to give a block copolymer or just be trapped with a suitable nucleofile.

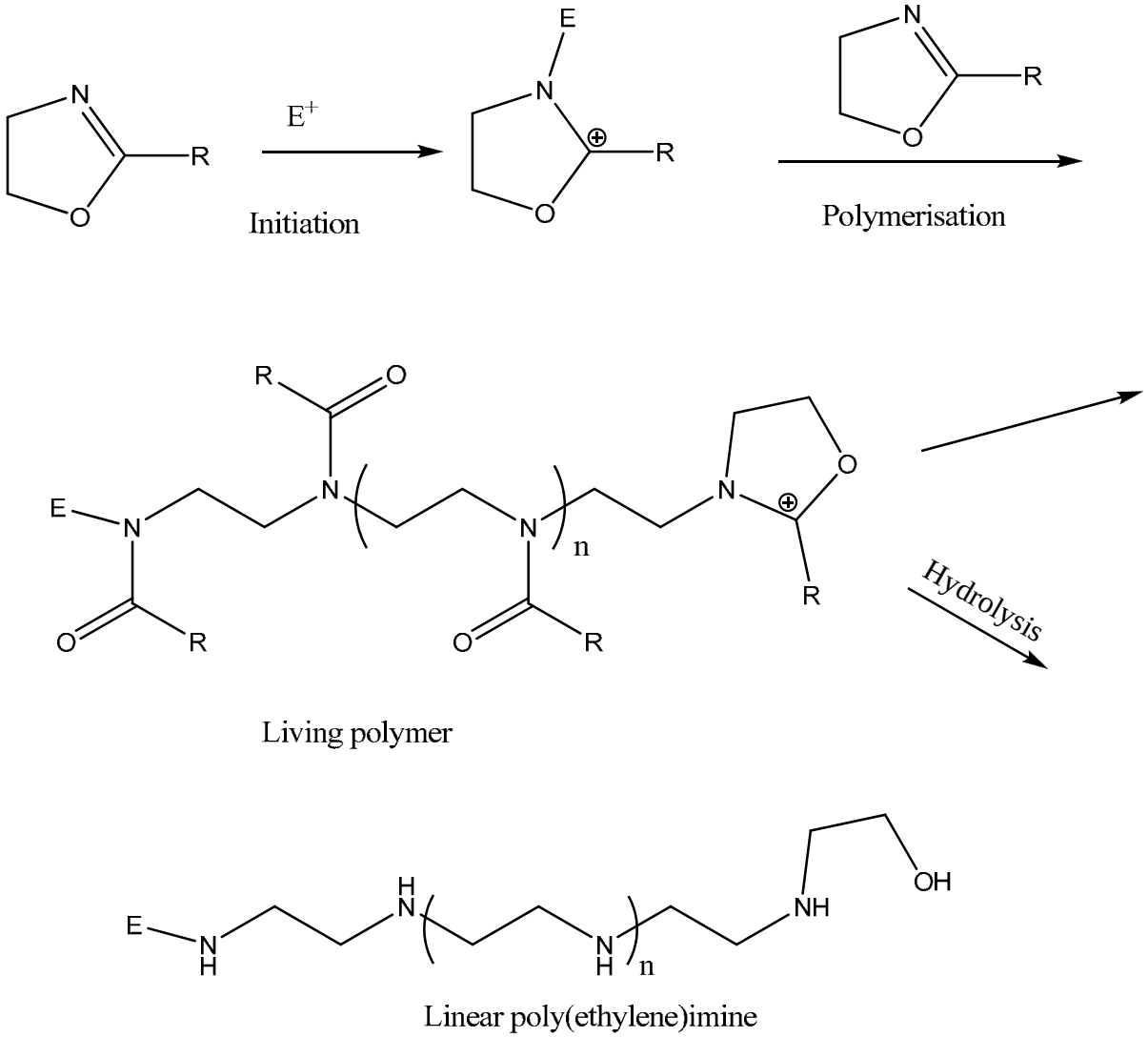
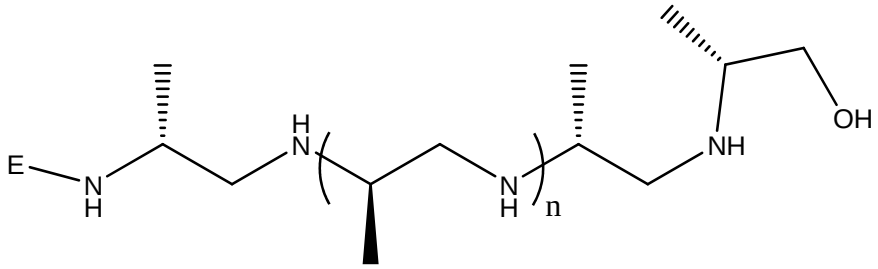
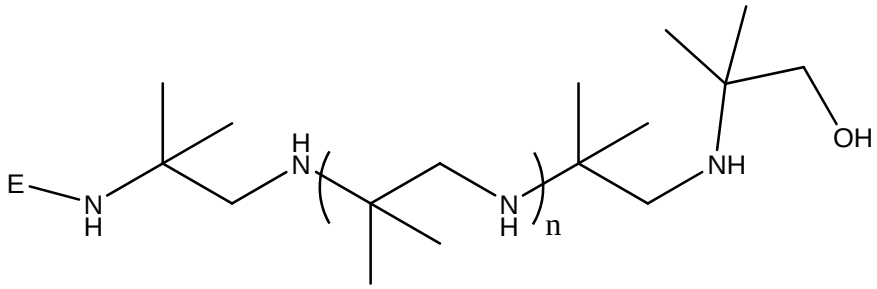


Figure 2: The cationic ring opening polymerisation of 2-alkyl-oxazolines.

There is only little known about other linear homopolyamines and a couple of interesting targets are shown in figure 3. The polymers will have to be characterized by standard techniques such as NMR and GPC, and in the case of the chiral polymer (a) it would be interesting to study the secondary structure in solution by CD-spectroscopy.



Linear chiral poly(propylene)imine (**a**)



Linear poly(methylpropylene)imine (**b**)

Figure 3: Substituted poly(ethylene)imines.

3: Polyamines and DNA/siRNA.

The formation of complexes between DNA/siRNA and a polyamine leads to polyplexes, which can be characterized by light scattering giving information on the size of the particles. The thermodynamic parameters of the complex formation can be determined by microcalorimetry and the morphology of the polyplexes can be studied by scanning probe microscopy (STM and AFM) The polymers by will be formulated with DNA/siRNA into polyplexes and characterized with respect to physicochemical properties, stability, in vitro transfection efficiency and toxicological profile.

Dendrimers and drugs.

Dendrimers are molecular trees built by repetitive branching from a core as shown on figure 4. Dendrons are the individual branches of the dendrimer. The physical and chemical behaviour of dendrimers and dendrons depends on the actual structure of the "dot" connected to form the structure: Amide-based dendrimers such as the PAMAM-dendrimers have properties similar to globular proteins and can host smaller molecules, polyphenylene-dendrimers are stiff molecules with highly interesting optical properties and silicon-dendrimers are highly unpolar molecular "blobs".

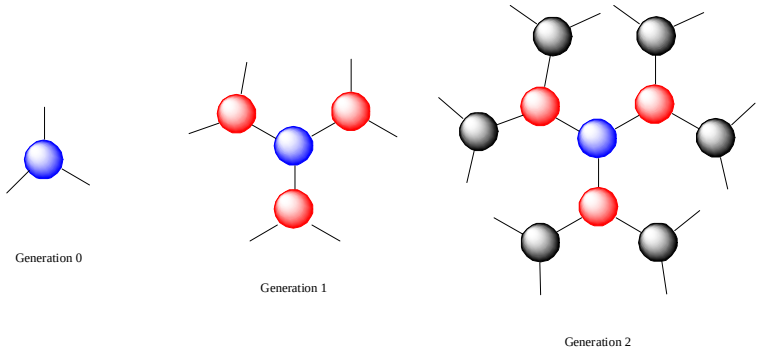


Figure 4: 3 Generations of a hypothetical dendrimer

4: Synthesis of dendrimers and dendrons.

Dendrimers with amino groups at the surface are known to form stable complexes with DNA and siRNA, which have been shown to be efficient for transfection, however very little is known about how the ideal dendrimer for this purpose should look like. We have been developing synthetic methodology for dendrimer synthesis for a number of years now, and we have now the tools for constructing dendrimers, that might help to provide the answer to that question.

5: Dendrimer-based antibiotics.

Multiresistant bacteria have become an increasing problem in recent years due to a combination of an increased (and non-critical) use of antibiotics combined with a lack of new antibiotics and leading microbiologists describes the present as "a return to the age without antibiotics"[16]. The most successful resistance mechanism found in bacteria is efflux-pumps, which are highly non-specific and allows the bacteria to dispose of unwanted compounds by transport through its outer membrane. We and others have previously shown, that multiresistant bacteria becomes susceptible to antibiotics, when exposed to a combination of efflux-pump inhibitors and antibiotics. We have also recently shown that small dendrimers are internalized in methicillin resistant staphylococcus aureus (MRSA, which is a major health concern in theses years). These observations open for the

use of conjugates between dendrimers and antibiotics as a new weapon against resistant bacteria. This will be achieved by synthezising conjugates between dendrimers and antibiotics and subsequently testing them in a checkerboard setup against relevant bacteria (for example MRSA). This project involves collaboration with microbiologists and it could be possible to spend part of the project at other universities.

6: Dendrimer-based drug-delivery.

PAMAM-dendrimers are capable of crossing biological barriers such as membranes and this opens for possibility of using dendrimers for drug-delivery.

7: Dendrimer-based drugs.

Many interactions in biological systems involve multivalent interactions between a ligand and a receptor. Dendrimers displays a large number of groups at the surface, which could be utilized for developing multivalent drugs.

Further reading:

"**Dendrimers, new molecular tools in medicine and biotechnology**"
Ulrik Boas, Peter Heegaard, Jørn B. Christensen
Royal Society of Chemistry 2006

"Poised to branch out"
Vivienne Marx
Nature Biotechnology 26, 729 – 732 (**2008**)

Heterocyclic Chemistry Projects

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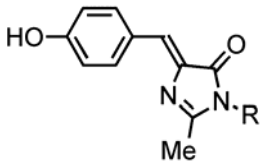
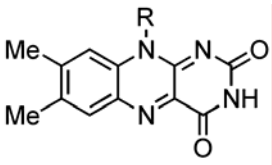
All projects are synthesis-driven and are based on heterocyclic chemistry and to some extent exploration of palladium-catalyzed reactions.

1) The Green Fluorescent Protein Chromophore
The Green Fluorescent Protein (GFP) is a light-sensitive protein that was first discovered in 1962 in the jellyfish *Aequorea Victoria*. It converts blue light into green light and has found wide applications as a marker protein for molecular and cell biology owing to these fluorescent properties. The covalently linked chromophore inside the protein is a *p*-hydroxybenzylidine-imidazolinone, formed autocatalytically from residues serine 65, tyrosine 66, and glycine 67. In this project, the aim is to develop new synthetic protocols for functionalizing the GFP chromophore and to investigate the optical properties of these compounds.



GFP chromophore

2) The Flavin Chromophore
Flavins constitute an important class of redox-active chromophores in Nature. They are co-factors in enzymes called flavoproteins that function as for example respiratory enzymes, dehydrogenases, oxygenases, photolyases, or blue-light sensitive plant photoreceptors, so-called BLUF proteins (Blue-Light Using FAD, FAD = flavin adenine dinucleotide). In this project, the aim is to synthesize derivatives that can be studied by gas phase spectroscopy in order to elucidate the intrinsic properties of this compound.



flavin (FAD)

Fluorescent DNA base analogues

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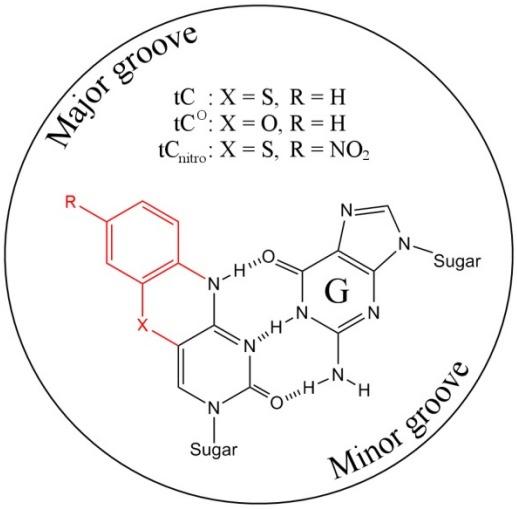
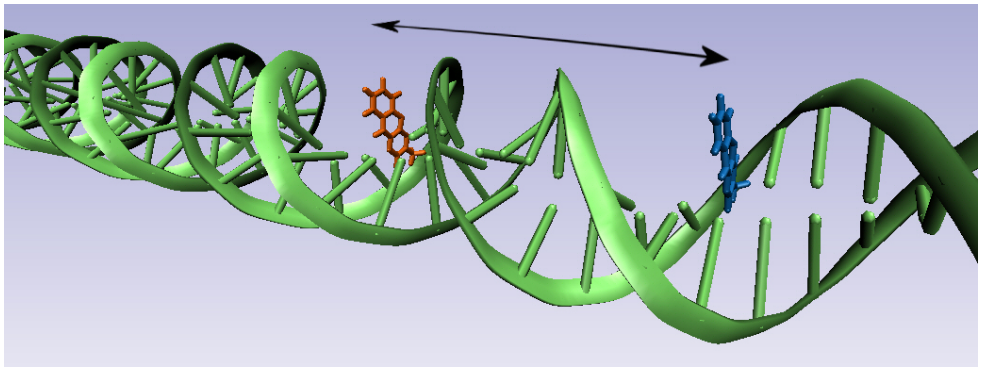
These projects concern the properties and utilization of DNA base analogues, mainly using spectroscopic methods.

Fluorescent DNA base analogues can be inserted in natural DNA and RNA without changing the structure, function or base-pairing pattern. Due to the fluorescent properties, the analogues can be used to reveal information about the dynamics both within the DNA double helix and about the overall macrostructure. However, the characteristics of the DNA base analogues have to be well designed and understood.



Projects within this area can for instance focus on the properties of DNA analogues both as monomers and inserted in DNA/RNA, and will involve absorption and emission spectroscopy, electrochemistry and quantum mechanical calculations. Other projects will focus on the use of these analogues in the study of DNA dynamics, and will involve use and development of fluorescent techniques such as anisotropy and energy tranfer.



Dynamic Combinatorial Chemistry in Water

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Tools: Synthetic chemistry, Supramolecular chemistry, Structure determination, LC/MS.

A typical project:

- A typical project has a substantial amount of synthetic organic chemistry combined with extensive use of modern analytical techniques such as LC/MS, NMR and X-ray crystallography.
- Another typical project has its focus on the analytical side, using LC/MS and NMR to elucidate the detailed structure of host-guest systems.

Objective: The purpose of the research program is to gain fundamental understanding of molecular recognition events in water – a key area in medicinal chemistry. We use dynamic combinatorial chemistry, an approach to discovery of new host-guest systems. This strategy enables the selection, synthesis and isolation of the best receptor in a mixture via a *survival of the fittest* approach. Ultimately the aim is to understand which interactions that govern the molecular binding events and to understand how to design artificial systems.

Introduction: Molecular-level understanding of the processes that govern complex biological systems and synthetic systems is important for the progress of many disciplines within the natural sciences such as bioorganic chemistry, chemical biology and in particular medicinal chemistry. A significant advancement for science would be if it was possible to recognise *any molecule* in a selective and strong manner using artificial systems.
In this research program an evolutionary synthetic chemistry approach will be pursued using dynamic combinatorial chemistry. In dynamic combinatorial chemistry a series of compounds are allowed to combine using reversible chemistry (covalent or non-covalent) to give a mixture of compounds: a dynamic combinatorial library (DCL). DCLs are responsive to external stimuli such as the addition of a 'template' that can be recognised and the library adapts according to the overall free energy of the reaction mixture. This is illustrated in figure 1.

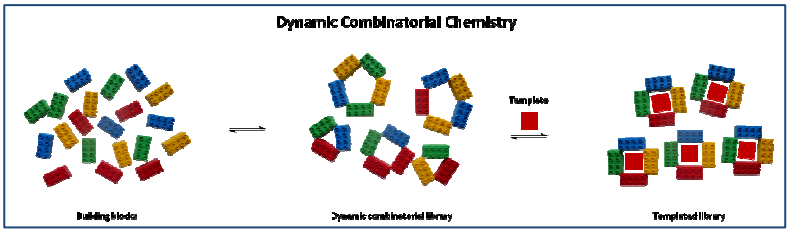

Fig. 1. (a) Schematic illustration of dynamic combinatorial chemistry. All chemical reactions are reversible and this provides the DCL. The template recognises, stabilises and amplifies the strongest binding component from the DCL – all in one step.

When a template is recognised, the library amplifies species that recognise the added template and thus shifts the equilibrium composition of the library. This approach has given rise to a number of highly complex and unexpected molecular receptors with very high binding affinities towards the templates. As reversible chemistry is being used, the process is under thermodynamic control, and will equilibrate to form the best receptor for the molecular targets. These targets can be small molecules or biomacromolecules such as proteins and DNA.

The chemistry: We develop new types of reversible chemical reactions and implement them in DCLs. Lately we have focused on disulfides, diselenides and thiosemicarbazides as new reactions for DCLs. We are also developing new receptors based on Biotin for small peptide fragments.

Synthesis of diazadioxa[8]circulenes and azatrioxa[8]circulenes for G-Quadruplex DNA binding

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Tools: Synthetic chemistry, Heterocyclic chemistry, Supramolecular chemistry.

A typical project:

- Synthesis, synthesis, synthesis! In a typical project the synthesis of diazadioxa[8]circulenes and azatrioxa[8]circulenes for binding G-quadruplex DNA will be targeted. A typical project has extensive use of modern analytical techniques such as LC/MS, NMR and X-ray crystallography.
- Another typical project has its focus on the analytical side. Once new [8]circulenes have been prepared, binding studies between these compounds and simple G-quadruplex structures will be carried out: the main tools will be LC/MS, UV/Vis, CD and NMR to elucidate the detailed structure of DNA binders.

Introduction: This project continues the exploration of the [8]circulene class of compounds. We will explore heteroaromatic [8]circulenes, namely the tetraoxa[8]circulenes, diazadioxa[8]circulenes and azatrioxa[8]circulenes (fig. 1). State-of-the-art in tetraoxa[8]circulene synthesis involves the acid promoted tetramerisation of 2,3-dialkyl-1,4-benzoquinoines or substituted naphthoquinones. This limits the scope of applications for tetraoxa[8]circulenes significantly, and in this project we seek synthesise functionalised [8]circulenes via direct functionalisation of tetra-*tert*-butyl-tetraoxa[8]circulene or through [8]circulene formation via substituted carbazoles to give azatrioxa[8]circulenes and/or diazadioxa[8]circulenes.

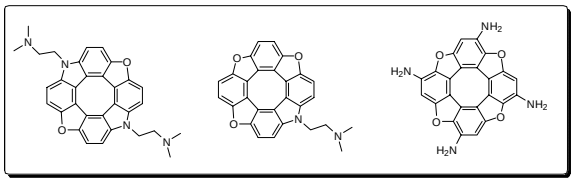

Fig. 1. Some heteroaromatic [8]circulenes targeted in this project.

We expect these materials to display unique optical and electrochemical properties and they are designed to display binding to G-quadruplex DNA. G-rich DNA and RNA sequences have a large tendency to form G-quadruplexes (fig. 2) and these types of structures are often present in nature. Especially in the telomer region of the genome these are important motifs. Selective recognition of these types of naturally occurring supramolecular motifs is highly desirable both from a fundamental research perspective, but also from a medicinal chemistry perspective.

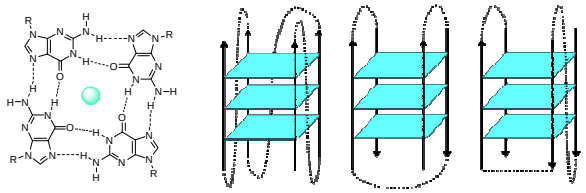

Fig. 2. Structure of G-quadruplex DNA.

The chemistry: New synthetic strategies for the synthesis of heteroaromatic [8]circulenes (for an example, see fig. 3). Exploration of the synthesis and properties of a new [8]circulenes for use in bioorganic-, medicinal- and materials chemistry. With new synthetic protocols for manipulation of [8]circulenes in place, their application in the molecular recognition processes and fluorescence marking of biomacromolecules will also be plausible.

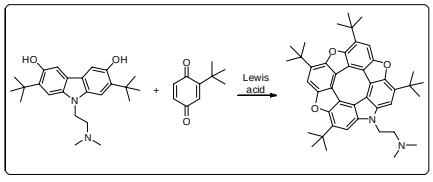

Fig. 3. Key step in azatrioxa[8]circulene.