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Zusammenfassung

Die kovalente Bindung zwischen zwei Atomen ist dafür verantwortlich, dass diskrete Strukturen sich aus vielen Atomen bilden können und genau in dieser Anordnung bleiben und dem somit aufgebauten Molekül eine Stabilität und Lebensdauer verleiht. Lange Zeit war der Fokus der Chemiker auf das selektive und vor allem kontrollierte *Brechen* bzw. *Ausbilden*, solcher kovalenten Bindungen gesetzt. Jedoch wurde auch schnell klar, dass die Natur, wie wir sie kennen, ein hoch komplexes Konstrukt aus ineinander greifenden Prozessen ist, welche weder starr noch ausschließlich aus chemischen Reaktionen bestehen, indessen Bindungen gebrochen und wieder zusammengefügt werden. Abgeleitet aus dieser Erkenntnis, musste es weitere Bindungsarten geben, die solche Dynamiken innerhalb einer Struktur oder auch zwischen verschiedenen Strukturen ermöglichen. Genau mit solchen schwachen und reversiblen Bindungsarten beschäftigt sich der Bereich der supramolekularen Chemie, um *die Welt hinter der kovalenten Bindung* zu entdecken und zu verstehen. Mit Hilfe dieser Erkenntnisse können neue, größere und komplexere Konstrukte erschaffen werden, die völlig neue Eigenschaften und Möglichkeiten bieten, aber gleichzeitig auch helfen, die bio-chemischen Abläufe in der Natur zu verstehen und für die Menschheit zugänglich zu machen.

In dieser Dissertation werden drei Themen behandelt, alle stehen im Zusammenhang mit "*Supramolekularen Koordinationskäfigen*". Der erste Abschnitt beschäftigt sich mit der Integration von Ligandensystemen in artifiziiell hergestellten Oligonukleotidstrukturen. Diese Ligandensysteme sind in der Lage in Anwesenheit von Metallkationen käfigartige Strukturen des Types (L_4M_2) auszubilden, wodurch ein Konstrukt gebildet wird, der einen inneren Hohlraum beinhaltet. Dieser Hohlraum, kann abhängig von der Struktur, kleinere

Moleküle aufnehmen und/oder Reaktionsabläufe begünstigen. Die Ausbildung dieser Käfigstruktur läuft zwar gerichtet, aber nicht kontrolliert ab. Das heißt, sobald mehrere unterschiedliche, aber strukturell ähnliche Liganden an der Assemblierung des Käfigs beteiligt sind, kommt es in den meisten Fällen zu einer unkontrollierten Zusammensetzung. Weder die Anzahl der einzelnen Liganden noch die Positionierung der Liganden im Käfig kann dabei gesteuert werden. Um ein kontrolliertes Zusammenfügen der Liganden im Käfig zu gewährleisten, wurden die Liganden in Oligonukleotidstränge eingebettet, welche sich selektiv und kontrolliert, abhängig ihrer Sequenzen zusammensetzen und ein stabiles, dennoch reversibles Gerüst aufbauen. So werden die Liganden im Zentrum dieser Gerüststruktur preorganisiert, um in einem abschließenden Schritt in Anwesenheit der Metallkationen einen Käfig auszubilden, welcher sowohl der Anzahl, als auch der Positionierung der Liganden im assemblierten Oligonukleotidgerüst entspricht. In diesem Projekt, sollte die allgemeine Machbarkeit eines solchen Systems gezeigt und gleichzeitig ein generelles Konzept geschaffen werden, um supramolekulare Käfige aus derivatisierten, aber strukturell-verwandten Liganden selektiv und kontrolliert aufzubauen.

Im zweiten Abschnitt dieser Arbeit, wurde ein neuer Ansatz ausprobiert, basierend auf zwitterionischen Strukturen, um niedrig geladene supramolekulare Käfige aus dem organischen Lösungsmittel in reines Wasser zu überführen. Dazu wurde sich der Struktur der Sulfobetaine bedient, welche häufig auch in natürlich vorkommenden Verbindungen gefunden werden können und eine wichtige Rolle im Bereich der Tenside spielen. Der große Vorteil der hier eingesetzten Sulfobetaine ist die Selbststabilisierung der zwitterionischen Struktur, d.h. es werden keine zusätzlichen Gegenionen benötigt, wodurch dieses Motiv erstens relativ unabhängig von möglich koexistierenden Ionen und deren Konzentration ist und zweitens, in einem weiten pH Bereich existieren kann, ohne seine zwitterionische Form einzubüßen. Supramolekulare Käfige lassen sich mit unterschiedlichsten Ligandensystemen einfach und variabel zusammensetzen. Der hier beschriebene Käfig Typus (L_4M_2), besitzt eine im inneren liegende Kavität, in der entweder kleinere Moleküle eingelagert oder sogar Reaktionen mit ungewöhnlicher Selektivität begünstigt werden, wodurch sie Interessant für ein breites Spektrum von Anwendungen im Bereich der medizinischen oder der katalytischen Chemie sind. Häufig jedoch sind diese Ligandensysteme sehr hydrophob, da ein rigides aromatisches Gerüst eingesetzt wird. Dadurch lassen sich die korrespondierenden supramolekularen Strukturen nur in organischen Lösungsmitteln herstellen. Auch die gebildete Käfigstruktur, zeigt anschließend keine besonders vorteilhafte Affinität zu hydrophilen Umgebungen, wodurch sie sich nicht *in vivo* oder anderen wässrigen Umgebungen verwenden lassen. Ziel der Chemie, besonders im Bereich der "Grünen Chemie" ist es jedoch, den Verbrauch von organischem Lösungsmittel zu reduzieren und stattdessen vermehrt Wasser als Medium zu verwenden. Durch die Einführung des hier beschriebenen Ansatzes, könnten viele dieser bis dato hydrophoben supramolekularen Systeme in Wasser überführt werden, ohne die Käfigstruktur und deren Funktion massiv zu beeinflussen.

Im dritten Abschnitt dieser Arbeit, wurde versucht ein System zu entwickeln, welches ermöglicht, dem System nach innen gerichtete funktionelle Gruppen hinzuzufügen. Ziel dieses Ansatzes ist es, die innen liegende Kavität der Käfigstruktur zu nutzen, um dort Moleküle aufzunehmen und anhand der zur Verfügung stehenden funktionellen Gruppen, eine selektive Bindung bestimmter Moleküle zu ermöglichen bzw. Reaktionen mit Hilfe der nach innen gerichteten funktionellen Gruppen zu erreichen. Dazu wurde das aus dem ersten Abschnitt bekannte acridon-basierende Ligandensystem modifiziert, sodass sich ein Käfig mit nach Innen gerichteten funktionellen Gruppen bilden konnte. Dabei wurde eine nicht erwartete Dynamik entdeckt, welche zunächst an einem Ligandenderivat mit reduzierter Komplexität intensiv untersucht und in einem zweiten Schritt, in eine Käfigstruktur transferiert wurde. Die untersuchte Dynamik wird durch ein intramolekulares elektronisches Push-Pull System verursacht, welches sich zwischen dem elektronenreichen Teil des Moleküls und dem elektronenarmen endohedralen Rotor einstellt und so zu einer temporären Ladungstrennung innerhalb der Struktur führt. Dadurch wird die Bindungsordnung der formalen Doppelbindung reduziert, sodass diese einen Einzelbindungscharakter erhält und eine temporäre Rotation um die verbrückende Bindung ermöglicht. Durch systematische Veränderung der äußeren Einflüsse und Derivatisierung des Moleküls, konnte gezeigt werden, dass die Rotationsrate stark abhängig von der Stabilisierung der zwitterionischen Form ist. Die Existenz konnte zusätzlich bewiesen werden, indem die negative Ladung in Anwesenheit von starken Säuren eine reversible Protonierung aufwies. Durch die Protonierung hat sich ein hoch-fluoreszierende positiv-geladene Struktur gebildet, welche durch seine markante Adsorptionsbanden, als Acridinium-Ion identifiziert werden konnte. Durch die Integration des Push-Pull Systems in die Ligandenstruktur, ging diese Eigenschaft verloren, jedoch blieb die Rotationsdynamik. Letztere konnte ebenfalls im gebildeten supramolekularen Käfig nachgewiesen werden, wo sich die nach innen-gerichteten Rotoren weiterhin drehten. Interessanterweise konnten trotz der nach innen gerichteten Gruppen, kleinere Moleküle reversible eingelagert werden. Die Einlagerung von Moleküle hatte einen signifikanten Einfluss auf die Rotationsraten, welcher durch geeignete VT-NMR Experimente nachgewiesen werden konnte.

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Summary

Enzymes are nature's workhorses, holding a vast variety of functional properties for essential processes in life, including catalyzing reactions, transporting molecules or being an auxiliary in protein/RNA folding. Especially, the catalytic properties of enzymes produced a lot of interest in all areas of science, reaching from biology, over physics and chemistry and is even found nowadays to be part of informatics. In the chemistry the catalytic functions of enzymes have long been in the focus of organic and bio-inorganic scientists, who accumulated their focus of interest rather on the very core mechanism and coordination between active center and reagents, but neglected the active sites shell. The contributions of the spatial arrangements and the involved inter- and intramolecular interactions are essential for the allover enzymes functionality. Although consisting of rather simple building units, connected by covalent bonds, the non-covalent inter- or intramolecular forces, make them become extremely versatile in shape and function. The field of supramolecular chemistry is investigating such interactions and scientist put all their effort in understanding this chemistry in all its facets, in order to be able to mimic and reach natures functionalities and beyond. Starting with pure organic compounds, soon after, the area of supramolecular chemistry was extended by using metal coordination motifs, what greatly expanded the design of possible structural topologies, especially with respect to three-dimensional arrangements. Besides, also the combination with natural occurring building blocks promoted the creation of new functional structures, giving new information and insights to understand why nature's workhorses have evolved in the way they are and how the enzymes' unique functions can be made accessible to mankind. This work covers three projects dealing with the functionalization of supramolecular metal-mediated coordination cages. In the first two parts, two approaches, based on exohedrally functionalized ligands, are discussed. The first one aims for a controlled assembly of heteroleptic coordination cages. Those cages are interesting to create endohedrally functionalized cages, acting a molecular reaction vessel and mimicking the active site of an enzyme. In order to be able to control the arrangement of structurally related ligands into a cage structure, the assembly process is assisted by an externally attached DNA scaffold. In the second project, the anisole-based ligand design of the first project was reused to synthesize exohedrally modified charged and neutral ligands. These ligands are investigated for their ability to form low-charged water-soluble coordination cages in the presence of palladium(II) salts. In the last chapter the acridone-based ligand, taken from the first project, is endohedrally functionalized with various cyano acetate ester derivatives. The ligands, as well as their corresponding palladium-mediated coordination cages, are showing an unexpected but interesting intramolecular rotational motion. This motion is induced by a charge-separated state within its intramolecular push-pull system and can be significantly effected by various external influences, including guest encapsulation within the cage's cavity.

PART 1 The idea of a DNA scaffold supported assembly for a supramolecular architecture, is an approach to overcome the problem of statistical mixtures during the assembly of supramolecular heteroleptic metal-mediated coordination cages, from structurally related ligands. The system was designed to preorganize the involved ligands in a square-planar fashion by the preceded formation of an external DNA scaffold. For that purpose acridone- and anisole-based ligands were designed, synthesized and subsequently incorporated into DNA single strands, capable of forming a *Holliday* junction (HJ). The *Holliday* junction is a cruciform-shaped canonical DNA architecture, where the ligands shall be located at the central crossing section. In order to prove the approach on its feasibility and functionality, the following steps had to be accomplished.

In a first step the ligand was synthesized, including linkers and protecting groups, crucial for DNA solid phase synthesis, accompanied by a full characterization via NMR and ESI-MS and other methods. Followed by the incorporation of ligands into the respective DNA sequences with subsequent chromatographic clean-up and characterization by ESI-MS and common spectroscopic methods. As a third step, the assembly of the intended DNA structure was proved with help of thermal analysis, electrophoreses, fluorescence and circular dichroism spectroscopy. In sum, two ligand-containing *Holliday* junction systems were proved to be formed under the given conditions and their thermal stability were compared to an "unmodified" *Holliday* junction (containing no ligands), as a reference. In a last step the assembly of the intended metal-mediated coordination cage was investigated, in presence of the respective metal salts and the established conditions, aiming to achieve the proof of principle for the actual system.

None of the used transition metals gave a sufficient evidence for the formation of the intended embedded supramolecular coordination structure. However, the acridone-containing HJ exhibit an increase in denaturation temperature (T_M) of 13 °C compared to the unmodified HJ. Moreover, an observable bathochromic shift in the UV-vis and fluorescence spectrum

indicates the formation of a stable ligand involved J-aggregate. The aggregation of ligands does not only explain the increase of T_M , but also the inertness towards the metal cations. Moreover, a chiral transfer from the exterior DNA scaffold on the ligand aggregates within the *Holliday* junction is indicated by signals in circular dichroism spectra, vanishing upon complete disassembly. Although the formation of the embedded coordination structure could not be proved, to the best of our knowledge the here presented system is the first example of a fully assembled HJ, with artificial organic compound incorporated at its central crossing section and moreover, also exhibit a stabilization due to these artificial modifications. Apart from that, the system bears a huge potential for the design of an easily accessible scaffold for the investigation of uncommon aggregation partners in solution.

PART 2 The anisole-based ligand, that was also used for the DNA scaffold topic in part I, was exohedrally modified with either a diethanol amine group (neutral ligand) or a sulfobetaine motif (zwitterionic ligand). By introducing the polar groups, the allover polarity of both ligands is significantly increased. Thus, both can be solved in polar organic solvent or even aqueous medium. Furthermore, both ligands still allow the formation of supramolecular metal-mediated coordination cages, even in pure water. In addition, it was tried to introduce an endohedral functionality to the system, in form of phenolic hydroxyl group by simple deprotection of the anisole's inward pointing methoxy ether. The deprotection resulted in an unwanted cyclization reaction, giving an asymmetric ligand, that was not able to form any metal-mediated coordination structure at all. Although the approach of bringing coordination cages into aqueous medium by attaching polar residues, has been shown in literature before, the usage of self-stabilizing zwitter-ionic motifs, in order to create low-charged water-soluble coordination cages, has not been shown yet to the best of our knowledge. The synthesized ligands and cages were characterized by common analytical methods, as NMR, ESI-MS and further spectroscopic techniques. Unluckily,

the here presented cages did not show any evidence of guest encapsulation, with a small selection of neutral guest molecules, probably due to the lack of space within the cavity. To overcome this problem, the ethynyl bridges could be exchanged i.e. with phenyl linkers, slightly increasing the size of the endohedral void. With this change, the unwanted described cyclization reaction would be probably obliterated as well. However, still the zwitter-ionic approach could be easily transferred on other supramolecular architectures, making it possible to investigate the host-guest chemistry or related functionalities in an aqueous environments,^[1] providing for sure a quantity of new systems for the field of green chemistry.

PART 3 In the last part an endohedral modification of the acridone-based ligand, related to the ligand used in part I, with various cyano acetate esters is discussed. All derivatives showed an unexpected intramolecular rotational motion, caused by a push-pull system with a certain degree of charge separation, between the acridone nitrogen and the opposed cyano acetate ester. This motion was thoroughly investigated on the modified acridone backbone, before it was transferred into a bis-dentate ligand-system and subsequently into a supramolecular palladium-mediated coordination cage. Interestingly, under certain conditions the charge-separated state within the acridone predominates, so that the negative charge could even function as a weak base. At this stage the system can be reversibly protonated in the presence of strong acids, forming a highly fluorescent cationic acridinium motif. The whole process of protonation was investigated in detail by NMR, UV-, fluorescence spectroscopy and mass spectrometry. Besides the interaction with strong acids, the influences of the various used ester derivatives, as well as other external influences on the intramolecular electronics, as for example temperature and solvent polarity, were studied as well. It became apparent that all these above mentioned influences have either more or less influence on the intermolecular electronics of the push-pull system, giving a tool for the

adjustable control of the rotational motion. Based on these findings a system was designed in order to control the rotational motion by external stimuli. For that reason, the π -system was extended, ending in an azacrown ether motif, capable of interacting with metal cations. This interaction is intended to form an opposed electron-withdrawing motif, being in competition with the actual electron-withdrawing cyano acetate ester. The influence of the various metal cations on the intramolecular push-pull system was investigated by variable temperature NMR methods, as well as UV-vis spectroscopy. Nearly all investigated metal cations showed a clearly observable interaction with the azacrown ether, tracked by NMR and UV-vis spectroscopy. Yet, the effect on the intramolecular electronics was moderate and even not detectable for the rotational motion. As an exception, the protonation of the azacrown ether with strong acids is to be mentioned. Nevertheless, latter is not applicable for the usage with metal-mediated coordination cages, since strong acid can result in a disassembly of those structures. However, since some results indicating a small influence on the rotational motion, it is worth to continue the research on this topic, in particular since this approach could be used as a good starting point for several new systems in the field of supramolecular functional units,^[2,3] molecular sensing^[4] or organic light-emitting diodes (OLED).^[5]

General Introduction

Chapter II.1

Supramolecular Chemistry

The main focus in the area of supramolecular chemistry is the design and creation of functional systems, assembled from smaller building units to a highly complex architecture with distinct functionalities. Compared to the classical synthetic chemistry, where it is the measure of all things to form a new covalent bond between two atoms, supramolecular chemistry utilizes the non-covalent interactions between two molecules, to create uncommon spatial arrangements. As so often before *nature* is one of the most important paragons for the scientists and gave inspiration for structural motifs in the field of supramolecular chemistry. On the other hand, supramolecular structures are used to create biomimetics in order to understand the by far advantageous and well-developed functional units in biological systems, as for instance enzymes. Moreover, by combining artificial structural motifs from the supramolecular chemistry with natural occurring building blocks, the field of nanotechnology was pushed on a new level in the last years.

II.1.1 History

The field of supramolecular chemistry has its roots go back to the end of the 19th century,^[6] but first became in 1987 officially an own field of research, after Jean-Marie Lehn, who later was awarded with the Nobel prize together with Cram and Pederson, introduces the term *supramolecular chemistry* in 1978. But also before some important findings for the field of supramolecular chemistry were made, which are still contemporary and frequently investigated in the modern supramolecular chemistry. In 1894, already Emil Fischer proposed the concept of lock and key, what is a commonly used model in biology and medicine as well as supramolecular host guest systems. 1953 the structure of DNA was solved by Rosalind Franklin as well as Crick and Watson, the latter both received the Nobel Prize for their work. Curtis, Busch and Jäger reported between 1961 and 1964 new

macrocycles based on Schiff's bases, coordinating to various transition metals. Some years later, in 1967, Pederson published the first crown-ether, able to bind alkali and earth-alkali metals, making it possible to transfer them into organic solvents.^[7] 1969 the first *cryptand* was synthesized by Lehn, a three-dimensional analogon to the crown ether, but published it several years later.^[8] Some years later, in 1973, Cram reported the synthesis of the first *spherand*, a more rigid version of a *cryptand* that provides a almost spherical preorganized coordination environment for enveloped cations.

Figure II.1.1.1: Three supramolecular structures, the noble prize winners Pederson, Cram and Lehn were honored for. From left to right: Crownether (Pederson, 1967),^[7] Cryptand (Lehn, 1978),^[9] Spherand (Cram, 1973).^[10,11]

These structures mark the birth of supramolecular chemistry. Since then, supramolecular chemistry did not only stick to the coordination of metal cations, but evolved to an area of research which bears the possibility to innovative architectures with high diversity and complexity for versatile applications. Starting from novel approaches for the directed formation of new topologically interesting complexes, modern supramolecular chemistry also provided the tools for potential systems as molecular devices and machines, catalytic molecular flasks and hosting units for molecular recognition, but also as containers for drug transport.

In 2016 another Noble prize for supramolecular chemistry was awarded to Sauvage, Stoddart and Feringa, the originators of the modern supramolecular chemistry. The foundation stone was laid by Sauvage *et al.* in 1983 by establishing a new strategy for the syntheses mechanically interlocked structures.^[12] The so called "*catenanes*" are mechanically interlocked rings,^[13,14] which can only be separated by breaking a covalent bond withing one of these rings, thus, the structure can move freely but is highly stable at the same time. Structures like that have been reported before, but Sauvages' approach in synthesis was pioneering. By preorganizing the rings with an templating metal ion, before the rings were covalently interlocked, Sauvage could synthesize significant amounts of those *catenane* structures, which hitherto were synthesized under ultra high-dilution conditions with extremely low yields. Based on this strategy, further new intertwined topologies like links and knots could be designed.

Later, another supramolecular structure was established by Stoddart *et al.* The "*rotaxanes*" consists of an linear rod-like motif threaded through an molecular ring. Both ends of

the rod are capped with bulky stopper motifs, giving the whole system a dumbbell shape. As for the *catenanes*, a templated preorganization of rod and ring by non-covalent interactions between both, before covalently adding the stoppers, is the key step for an high yielded synthesis. In 1991 Stoddart could prove, that a the ring could move along the rod-like thread. Furthermore, a controlled ring movement between to distinct states, triggered by external stimuli (pH, chemical trigger or light irradiation) led to the so called "*shuttlebus rotaxane*". Based on the same principle Stoddart designed even more complex systems, as the molecular muscle (figure II.1.1.2a) or the nano elevator .^[15–17]

Figure II.1.1.2: The two famous supramolecular architectures of Stoddart *et al.*, for which amongst others, he has received the noble prize in chemistry in 2016, together with Sauvage and Feringa. a) molecular muscle based on a "*shuttlebus rotaxane*" structure, where to topological states can be trigged by external stimuli. b) Based on the same principle, more complex structures, as the so called "*molecular elevator*", were designed and synthesized.

Already in 1999, Feringa *et al.* reported an unidirectional molecular rotor. Since it shows a controlled motion in one direction, it was one of the first artificial molecular motors. The principle of function is further discussed in chapter V.1. Although, this dynamic unit is not a supramolecular system, non-covalent interactions were crucial for the design of the world's smallest car. Feringa *et al.* created in 2011 a nanocar with four of their above

mentioned molecular motors as wheels and with a total size of approx. 1 nm. Due to the unidirectional motion of the four incorporated molecular motors, Feringa *et al.* could prove that the nano car structure was able to move forwards on a copper surface in a controlled unidirectional fashion, what was so far an unique breakthrough in the field of molecular machines.

Figure II.1.1.3: Supramolecular nanocar by Feringa *et al.*. The whole structure consists of two identical building blocks merged together in the middle by bridging alkyne units. It formed a consistently aromatic rigid system, with two molecular motor units at the front and two at the back. The location where the rotational motion takes place is marked with circular arrows.

II.1.2 Concepts of Supramolecular Chemistry

Supramolecular Chemistry investigates the "*chemistry of the non-covalent bond*", means every attractive inter- or intramolecular force, that do not lead to a sharing of electrons between two atoms. Hence, the term "non-covalent" includes interactions between ions, dipoles, induced dipoles or spontaneous transient dipoles. The diverse combinations can be subcategorized into ion-ion, ion-dipole, hydrogen bonds and so on (figure II.1.2.4).

Interaction	Scheme	Energy [kJ mol ⁻¹]
Ion-Ion	a)	200 - 250
Ion-Dipole	b)	50 - 200
Halogen Bond	h)	50 - 180
Hydrogen Bond	c)	4 - 100
Dipole-Dipole	g)	5 - 50
Ion- π	e)	5 - 80
π - π	f)	1 - 50
Van-der-Waals	d)	< 5

Figure II.1.2.4: Non-covalent interactions in supramolecular chemistry. a)-h) show the non-coordinative interactions in a schematic fashion. In the table below, the above shown interactions are named and sorted by their strength, regarding the energy to break these attractive interactions, from strong to weak in a descending order.

Compared to covalent bonds with an bond energy of 300-400 kJ mol⁻¹, the non-covalent interactions are rather weak with energies between <5 - 250 kJ mol⁻¹, but the sheer amount of weak interactions in sum, that can occur within a system, have a significant contribution to the overall system stabilization that should not to be underestimated. The strongest interactions with bond energies about 50 - 250 kJ mol⁻¹ are the electrostatic interactions, including ion-ion, ion-dipole and dipole-dipole interactions with permanent dipoles. Ion-ion interactions are known from salts or acid-base pairs, ions or molecules with permanent charges of opposite polarity. Although the electrostatic forces are pretty strong between those pairs, yet the presence of water often makes these interactions break very easily. One of the most occurring and also extremely important dipole-dipole interactions are the hydrogen bonds. With an energy of maximum 100 kJ mol⁻¹ hydrogen bonds are less than half as strong as ion-ion interactions. However, the formation of several hydrogen bonds within a system lead in sum to a significant stabilization, as it can be seen i.e. in DNA helix structures, but do also play an important role in the host-guest interaction of supramolecular systems. Back to the example of DNA, besides the above mentioned hydrogen bonds, the π - π stacking between neighboring nucleobases have a substantial contribution in stabilization. These attractive forces can cause a gain in energy about 50 kJ mol⁻¹ and play an important role in the guest encapsulation in supramolecular chemistry, especially with regard to highly aromatic guests, as for example fullerene.

In the last years the rather weak but frequently occurring interactions moved into the focus of interest, like the Van-der-Walls interactions, which do also include London dispersion forces, belong to the weakest interactions with just 5 kJ mol^{-1} at maximum. They are caused by spontaneous formation of transient dipoles, by other ions, dipoles or induced dipoles and occur in all matter. The best known example for the immense forces that can be caused by Van-der-Walls interactions is the gecko reptile. Thanks to its special surface under its feet, it can form an enormous amount of Van-der-Walls interactions between itself and the surface it is walking on. All forces in total generate such a big attraction, that the gecko can walk smoothly headlong without becoming an victim of gravity.

Besides the here presented forces, which mainly contributes to the enthalpic part of energy, the hydrophobic effect has occupied an important role in the area of supramolecular chemistry, since more and more water-soluble systems appeared that offer the possibility of hydrophobic guest encapsulation. Although not fully understood yet, the hydrophobic effect seems to mainly influence the entropy with respect to the thermodynamics, at least at room-temperature and below.^[18,19] However, the contribution of the hydrophobic effect in combination with possible interactions between host and guest molecules, makes the encapsulation of guests within supramolecular cavities energetically so preferable, thus those systems become extremely attractive for the usage as containers or molecular reactions flask in aqueous medium.

With supramolecular structures the term "molecular self-assembly" is often connected. Self-assembly describes the process of congregation of building blocks, which arrange themselves to a supramolecular architecture of higher complexity. The composition of the formed construct depends on the involved non-covalent interactions and lead to the most energetically favorable structure, since the system always tries to form the thermodynamically most stable product. Moreover, the reversibility of the non-covalent interactions plays an important role on the way during the process of self-assembly, because spontaneously occurring wrong connections can be broken again for the benefit of more stable non-covalent forces or coordination bonds. Nowadays industry is taking advantage of this reversible bond formation. Some materials are made of or coated with supramolecular polymers, where damages in the surface consolidate again, due to the reformation of the non-covalent bonds. These materials are called "self-healing". However, during the assembly of supramolecular structures non-covalent or dynamic-covalent bonds are broken and formed several times until the system reaches the final product. But even after the system is situated in an equilibrium, the building blocks are detached from and reattached to the supramolecular structure. This situation is important to be considered in order to understand why the formation of heteroleptic supramolecular cages is not an easy task (chapter III.1).

The process of self-assembly can be subcategorized into two types,^[20,21] the strict self-

assembly and the directed self-assembly. In the case of the strict self-assembly, the final product is formed entirely spontaneously when all components are mixed in the right ratio under certain conditions. In contrast, during the directed self-assembly process a templating compound is involved, i.e. ions, solvent molecule or organic compounds of a certain shape. Those auxiliaries can later be removed or are integrated parts of the end product. Moreover, the template can be essential for the formation of a discrete structure, which could not be achievable in a simple strict self-assembly, as for the synthesis of catenanes reported by Sauvage^[22]

The thermodynamics of supramolecular structure formation is given by the *Legendre* transformation (equation (II.1.2)), which is received from the *Gibbs-Helmholtz* equation (equation (II.1.1)):^[23]

$$\left[\frac{\delta G/T}{\delta T} \right]_p = -\frac{H}{T^2} \quad (\text{II.1.1})$$

$$\Delta G = \Delta H - T\Delta S \quad (\text{II.1.2})$$

with (ΔG) as the free energy change in the system, (ΔH) as the enthalpy and (ΔS) as the change of entropy. The consideration of the contributions of (ΔH) and (ΔS) are crucial for a successful formation. The enthalpy includes the gain in energy due to the formation of non-covalent or dynamic covalent bonds within the supramolecular structure as well as from the enthalpy of solution. In contrary, the formation of an higher ordered architecture from several individual smaller molecules is less favorable with regard to the systems entropy. But since the assembly to an supramolecular structure mostly leads to a smaller overall solvation shell compared to those of the individual building blocks, the release of solvent molecules can push the entropic contribution in favor of the self-assembled structure. Nevertheless, due to the fact that both, enthalpy and entropy, have an effect on the thermodynamic favorability of the formation, it is not necessary that both values do favor the process, as long as one contribution has a predominant effect compared to the other. In general, the self-assembly of higher ordered structures from smaller building blocks can be distinguished in two assembly mechanism. The strict self-assembly (figure II.1.2.5a), where the building blocks spontaneously assemble to the desired supramolecular structure and the directed self-assembly, where an auxiliary templating molecule helps to direct the formation of the desired supramolecular structure. The auxiliary is interacting with the building units in a non-coordinative manner, in order to preorganize the building blocks, before the assembly takes place (figure II.1.2.5b).

Figure II.1.2.5: Strict vs. directed self-assembly. **a)** Schematic representation of a strict self-assembly. The involved building units assemble spontaneously into a discrete structure without any outer auxiliary influences. **b)** In contrast, an auxiliary molecule is needed, i.e. functioning as an templating agent, in order to form the desired structure and preventing the formation of other possible assemblies.

Some of the supramolecular architectures including any inside cavity, where smaller molecules of the right size and shape can be taken up. This feature is of particular interest due to the ability to use those structures as transport containers or molecular reaction vessels (chapters IV.1 and V.2). The investigation of such processes is called host-guest chemistry. The larger aggregate possessing the cavity to bind a molecule is referred as host (**H**), while the encapsulated smaller compound is called guest (**G**), which can be a single ion or larger charged or neutral molecule.^[20,24] The resulting structure is called a host-guest complex [**G@H**] (in literature also often shown as [**G**⊂**H**], Figure II.1.2.6).^[21,25]

Figure II.1.2.6: Supramolecular assemblies containing an inner cavity, are capable in most cases to incorporate and smaller molecule. Those structures are mostly referred as *HOSTs* (**H**), whereas the uptaken molecules are called *GUESTs* (**G**). The fitting between the host cavity and guest molecule as well as the number and strength of existing interactions between host and guest, affect the equilibrium between host-guest complex and unoccupied guest. Also outer given conditions, like solvent polarity, pH, etc. can have a tremendous effect on the host-guest equilibrium.

As for the assembly of the supramolecular structure, the formation of host-guest complex is in equilibrium, considering the uptake of the guest (**G**) into the host structure host (**H**) and the release of guest from the cavity. The situation in equilibrium depends as before on the thermodynamics. Therefore, the quality of interactions between host and guest predominately predetermines how strong the guest is bound. The thermodynamic stability of the host-guest complex is determined in practice by determining the binding- or association constant K_a (equation (II.1.3)).

$$K_a = \frac{k_1}{k_{-1}} = \frac{[G@H]}{[G][H]} \quad (\text{II.1.3})$$

In some systems, the ions function as guest and template, as it was shown in the modified suberone cage of Freye *et al.*, where the change of counterion in sterically hindered coordination cage, results in the formation of an interpenetrated double cage. (chapter V.2).^[26] Furthermore, in some artificial supramolecular coordination structures, containing more than one binding position, positive allosteric effects or cooperative binding could be observed,^[20,27] as it is known from natural occurring systems, i.e. oxygen binding in hemoglobin.^[28]

II.1.3 Supramolecular Coordination Cages

The topologies of pure organic supramolecular nano-architectures are already highly diverse,^[29] but can be even extended by combining them with the topologies of metal coordination environments. The introduction of these coordination geometries (figure II.1.3.7) expanded the possibly designable topologies in supramolecular assemblies by a multiple. Depending on the nature of the used metal ion, a various number of ligands can be connected in a certain angle over the metal ion. With this strategy the shape of the overall supramolecular structures is easier to design, since the outcoming spatial arrangement of the ligands is more predictable. For the connection of two ligands in a linear fashion, Ag^+ ions would be the first choice. Four ligands can be connected in a square planar fashion with palladium(II) or copper(II), for tetrahedral shapes Cu(I) or Zn(II) and octahedral coordination environment can be achieved with i.e. iron(II/III). Also, metal ions with higher coordination numbers, like lanthanides, have been used for the formation of supramolecular cages.^[30] The defined metal coordination environments give supramolecular chemists additionally an easy opportunity to go from two-dimensional to three-dimensional compounds, while keeping the overall structure rather small compared to pure organic structures.^[31] In addition, rare topologies could be obtained, which would not be obtainable by conventional organic synthesis, as i.e. the already mentioned catennanes (section II.1.1).

Figure II.1.3.7: The here shown geometric schemes represent various possible coordination geometries with different coordination numbers. The existing coordination geometry within a supramolecular structure, depends on the nature of the used metal, its state of oxidation, size and nature of the corresponding coordinating ligands and other factors.

Besides the precept of directionality by the metal coordination environment, the used organic ligands have a major impact on the spatial arrangement within the self-assembly, thus on the allover structure. The compound specific features of the ligands, like length, coordinating functional groups, angles, denticity, flexibility has to be considered as well as the metal preferable coordination environment. On top of this, the reaction conditions such as metal/ligand ratio, choice of counter anions, solvent, temperature, and reaction time will affect the cage formation.^[32] Typical geometries obtained from homoleptic assemblies are in general highly symmetric, often resembling the rather simple Platonic solids, e.g. tetrahedr,^[33] octahedra^[34] or cubes^[35] (figure II.1.3.8), but also more complex Archimedean solids are achievable.^[36,37] Moreover, other topologies of three-dimensional self-assembled coordination structures, like adamantoids, trigonal prism, helices, bowls, and many more were reported in the last years.^[38]

Figure II.1.3.8: The here presented topologies belong to the Platonic solids. These topologies can be frequently found in supramolecular metal-mediated architectures and provide in most cases an endohedral cavity, which can function either as a container or reaction vessel in presence of suitable guest molecules. It is worth to mention, that the above mentioned topologies are often formed by a combination of building blocks with a geometry deviating from the assembled structure.

Among all supramolecular coordination complexes, the three-dimensional structure of the supramolecular coordination cages have become a very promising architecture for a variety of applications, as containers or reaction environment on a nanoscale level, since the assembled coordination cages have a discrete cavity, able to interact with smaller encapsulated guest molecules. Due to the fact, that a large number of coordination cages are positively charged, negatively charged molecules can be encapsulated inside their void,^[39] but also the uptake of neutral guests are reported in some cases.^[40,41] Additionally, reactive species could be stabilized and prevented from further undesired reactions, by shielding them within the assemblies cavity.^[42,43] With regard to supramolecular structures as nanoscale reaction environment, examples of accelerated reactions were reported.^[44,45] In both cases, charged intermediates are stabilized while bound in the cavity of the highly charged supramolecular construct. A further example is unusual regioselectivity of a Diels-Alder cycloaddition within a highly charged coordination cage, due to the changed spatial situations within the supramolecular cavity.^[46] The highly charged supramolecular assemblies are furthermore soluble in water, compared to most other supramolecular structures, for this reason they gained interest in the field of green chemistry (chapter IV.1). Besides, further structures with unique functions, like catalytic reactivity,^[39,47,48] gas sequestration^[49] or segregation of mixtures^[50] could be realized in well-designed supramolecular metal-mediated coordination architectures.

Structures assembled from bis-mono dentate concave shaped ligands, have become one of the most prominent directions in the last years.^[38,51] Besides its mostly straight forward syntheses, the ligands can be combined with a variety of metal ions to form discrete coordination architectures with a stoichiometry of $[M_nL_{2n}]$. Examples in literature do

include metal ions as copper, nickel, palladium, platinum, cobalt and different lanthanides (La, Ce, Sm, Eu, Tb).^[51,52] For the latter, multi dentate ligands are used.^[53] Most reported ligands consisting of rigid, aromatic backbone, with the coordinating groups mainly connected via rigid alkyne or phenyl units, but also more flexible linker as piperazine, amides, ethers or simple alkane based linker have been reported.^[51] Compared to the coordination cages mentioned in previous paragraph, the total charge is rather low and located at the coordination sites, thus the cages are polar but not water-soluble and preferably form in aprotic polar solvents as acetonitrile or dimethylsulfoxide. Furthermore, due to their banana-shaped ligands, the cages contain an internal void, whose size depends on the ligands specific size, but also how the ligands are arranged around the coordination centers.

In figure II.1.3.9 a collection of possible cage structures derived from various banana-shaped ligand with the d^8 metals Pd^{II} or Pt^{II} , providing a square planer coordination environment for the ligands. All these structures were reported in the group of Prof. Clever, who has intensively researched $[M_nL_{2n}]$ coordination cages the last years. But also Fujita, Shionoya, Crowley, Yoshizawa and many many others, continuously expanding the the pool of delightful palladium-mediated supramolecular architectures.

Figure II.1.3.9: Schematic representation of self-assembled architectures based on metal(II) cations (e.g. Pd(II) or Pt(II)) and banana-shaped bis-pyridyl ligands L. Resulting architectures in clockwise order [M₃L₆] ring, c) [M₃L₆] double-trefoil knot, d) [M₄L₈] ring, e) [M₂L₃X₂] bowl (X = solvent), f) [M₂L₄] coordination cage, g) [M₄L₈] interpenetrated coordination cage, h) triple catenane trans-[(MBr₂)₂L₂]₃ and i) rhombicuboctahedral sphere [M₂₄L₄₈].

The shown hollow cage assemblies are all capable to encapsulate anionic^[54,55] or, in special case, neutral guests.^[56,57] The choice of solvent and counter anions can have a dramatic influence on the formation of structure. Under specific conditions, monomeric cages do form in presence of a specific counter anion and the matching amount an interpenetrated dimeric [M₄L₈] cage,^[58] while an additional change in solvent can prevent again the interpenetration process. Furthermore an excess of halide anion in general leads to the disassembly of the supramolecular structure, whereas in the structure of Zhu *et al.*, the excess of halide anions result in the formation of triple catennane.^[59] Substitution of the

pyridine donor group by a quinoline group yielded a bowl shaped $[M_2L_3(\text{Solv})_2]$ -structure, where the free coordination side of the missing ligand is occupied by two solvent molecules. Furthermore, a shorter version of the light switchable dithienylethene (DTE) based ligand, with an additional change in coordination angle forms in presence of Pd^{II} a mixture of three- or four membered rings ($[\text{Pd}_3\text{L}_6]$ or $[\text{Pd}_4\text{L}_8]$), when in open form.^[36] The light-switched closed isomer on the other hand forms a $[\text{Pd}_{24}\text{L}_{48}]$ rhombicuboctahedral sphere. Although the architectures of self-assemblies are usually quite predictable, sometimes still surprising spatial arrangement occur, such as formation of an entangled double-trefoil knot^[60] or the heteroleptic trans- $[\text{M}_2\text{L}^{\text{a}}_2\text{L}^{\text{b}}_2]$ cage with a "doubly bridged figure eight" topology.^[61]

Since the interest in using the cavities of supramolecular architectures as catalytic units^[62,63] or highly selective containers for chemical segregation^[64] or drug delivery^[65] has grown drastically, the question for suitable systems arose, where several independent functionalities can be united. In the field of supramolecular coordination cages the realization of such a system turned out not to be an easy endeavor, since scientist facing the problem of statistical mixtures for multi-component cages. The lability of metal ligand coordination, that ensures the correct assembly of the desired end structure, is for the formation of heteroleptic supramolecular structures a fundamental issue (figure II.1.3.10).^[66]

Figure II.1.3.10: Schematic representation of the assembly process for palladium-mediated heteroleptic cages. The assembly of heteroleptic highly symmetric architectures is still a challenging task in supramolecular chemistry, since a discrete assembly of those structures is hardly controllable without any geometric restriction or templating auxiliaries. Assuming an assembly of a supramolecular cage structure composed of two palladium ions and four different ligands $[\text{Pd}_2(\text{L}_\text{A}\text{L}_\text{B}\text{L}_\text{C}\text{L}_\text{D})]$, having all the same structure, only the backbones are slightly modified. This would not lead to a single discrete non-racemic structure, but rather in a statistical mixture, where approx. 55 assembled cages can be found.

Most of the above shown examples (figure II.1.3.9), are highly symmetric architectures that contain only one type of organic ligand and therefore only one functionality. Due to that fact, the chemistry of artificial supramolecular self-assemblies is still far apart from

the level of structural and functional complexity encountered in biological macromolecular hosts. Especially in terms of those systems containing highly accurately arranged functionalities within its cavity, for a specific binding or chemical conversion of encapsulated molecules. In order to overcome this disparity, rational approaches have been emerged the last years to realize heteroleptic assembly in a regulating integrative self-sorting fashion between metal ions and multiple ligand components. Considering the $[M_2L_4]$ coordination cage as an example, the possible resulting assemblies in a fully-dynamic two-ligand-system (L^a and L^b) can be categorized into three scenarios of self-sorting (figure II.1.3.11a):^[67]

1. $[Pd_2L_n^aL_{(4-n)}^b]$ - **Statistical mixtures** – forms cages with all imaginable ligand compositions, mostly encountered in the absence of complementary interactions between the two deployed ligands L^a and L^b .
2. $[Pd_2L^a_4] + [Pd_2L^b_4]$ - **Narcissistic self-sorting** – generates several discrete homoleptic species, occurs due to a significant energetic penalty arising from the combination of ligands L^a with L^b .
3. $[Pd_2L^a_2L^b_2]$ - **Integrative self-sorting** - giving a single preferred heteroleptic product, mostly caused by significant enthalpic factors originating from the combination of L_a and L_b in the assembly.

In the last years, several systems for the integrative self-sorting were reported, based on various interaction concepts. As an example for ligand interactions (figure II.1.3.11b) Hooley and co-workers examined in 2011 the formation of heteroleptic $[Pd_2L^a_2L^b_2]^{4+}$ cages by bis-monodentate ligands with identical geometries and donor types, but with endohedral modifications, owning different steric demands. Combination of the ligands containing an endohedral steric bulk with those that have a less steric demand, formed preferably $[M_2L^a_3L^b]$ cages, containing only one sterically demanding ligand.^[68]

In 2015, the group of Yoshizawa reported the guest templated self-sorting of anthracene-functionalized $[Pd_2L^a_2L^b_2]^{4+}$ cages using a fullerene as templating guest,^[69] with short and long versions of a bis-monodentate ligand from a previous study.^[70] In the absence of a guest template, an equimolar mixture of the two homoleptic cages was found to rearrange into a statistical mixture of cages, as in the reported system of Frank *et al.*^[66] In contrary, the presence of C_{60} resulted in guest encapsulation and simultaneous reorganization to a single type of heteroleptic cage $[C_{60}L^a_2L^b_2]^{4+}$.

Bloch *et al.* reported in 2016 an heteroleptic $[Pd_2L^a_2L^b_2]^{4+}$ assembly, based on the concept of shape complementarity. The combination of two ligands with complementary

coordination angles (L^a :60° and L^b : 120° differing from the usual 180°, led to a tilt of the square planar coordination axis. Therefore, only the *cis*-[Pd₂L^a₂L^b₂] species shows the right ligand arrangement to occupy all coordination sites of the square planar the Pd^{II} cation, which make this species enthalpically most favorable.^[71]

The utilization of steric control by introducing steric hindrance around the metal center has been widely investigated.^[72,73] However, this donor site engineering has been rarely used in the formation of assembling heteroleptic cages. Clever *et al.* reported in 2018 a system formed by acridone- and phenothiazine-based picolyl ligands with either in- or outward pointing methyl groups on the coordinating picolyl groups. The combination of acridone ligand with outward pointing picolyl and phenothiazine with inward pointing methyl group led to a clean formation of the *cis*-[Pd₂L^a₂L^b₂] cage.^[74,75] Apart from the steric control approach, secondary interactions can also initiate heteroleptic cage formation. Crowley and co-workers reported an ortho-amino-substituted pyridyl ligands capable to form an heteroleptic *cis*-[Pd₂L^a₂L^b₂]⁴⁺ coordination cages in combination with unmodified ligands through complementary hydrogen bonding.^[76]

Figure II.1.3.11: Concepts of self-sorting and designed sorting.^[67] **a)** Three possible self-sorting outcomes for an AB-system (two different ligands). In the integrative self-sorting case gives a single heteroleptic product, but not necessarily enantiopure. The assembly is often driven by significant enthalpic factors that originate from interactions of vicinal components within the assembly and can be achieved by secondary interactions or geometric principles through proper system design as shown in figure II.1.3.11b. Narcissistic self-sorting leads to coexisting homoleptic cage species. The outcome of statistical self-sorting is described by n^k species. In the here assumed AB-System, 6 species would be expected. **b)** Comparison of possible designs for a controlled assembly of heteroleptic structures: *ligand interaction* design is based on specific stabilizing interactions between opposing or neighbour ligands. *Guest templating* design aims on the same, but is achieved by an auxiliary templating guest molecule. The next is a geometric approach, with designed shape-complementarity ligands, allowing only opposite ligands to assemble in the most energetically stable structure. *Donor-site engineering* concentrates on the design of the coordinating ligand parts, either by the approach of additional stabilizing interactions or steric hindrance. The first three design aims for the ligands backbone, while last approach focuses on the donor site, more decoupled from ligands functionality. The disadvantage of the ligand interaction and guest templating approaches is the sacrifice of the cavity when using endohedral interactions.

Once, heteroleptic self-assembly strategies find a wider implementation in supramolecular design, the resultant interplay between tailored combinations of precisely positioned substituents promises enhanced functionality in nanoscale structures. However, the complexity of the assembled systems is even more increased by mixing more than two kinds of different ligands in presence of metal cations. Even now, there is no general approach for the formation of heteroleptic self-integrated coordination cages consisting two different ligands, not to mention assemblies with more than that. In part III a new possible approach for the formation of heteroleptic coordination cages is presented. The design is based on a highly programmable natural occurring polymer, also known as DNA, which could help to design an exterior scaffold for the preorganization of ligands within a self-assembly.

Furthermore, this DNA scaffold could prevent the uncontrolled and undesired detaching or exchange of ligands.

Chapter 11.2

Desoxyribonucleic acid

Since the discovery of its structure in 1953 by Franklin, Watson and Crick, the DNA was investigated intensively. Even after 65 years of research, still not all secrets of the DNA and its natural occurring structures could be revealed. Especially in medicine it becomes more and more important to understand the structure and behavior of DNA, in order to find the right therapies to treat diseases caused by defects or mismatches within the DNA. However, still there are unanswered question, that issue a challenge to scientist. In the last years DNA also appeared as a promising material in nanotechnology, due to its highly predictable nature. By observing the rules of the DNA assembly and well-designed sequences a portfolio of 2D-^[77] and 3D-^[78,79] functional nano-structures^[80–83] could be obtained, where especially the research of Seeman and Rothemund played a key role. In combination with the toolbox of artificially synthesized organic, inorganic or polymeric building blocks, a new field of research, termed "supramolecular DNA assembly" has been recently emerged.^[84]

11.2.1 Structural Motifs

Natural systems do provide both selectivity and functionality, but they also had a long time to evolve and develop effective solutions and to organize complex architectures that contain the desired sophisticated functions. Of the naturally evolved systems, DNA is a fascinating example of self-assembly and commonly known to be the carrier of our genetic code. Despite its rather simple building units and especially and with only four different nucleobases, it still can create a highly potent system which made it possible to stores the whole information of all existing living organisms. Having a closer look at the building units of DNA, the so called nucleotides, they can be subdivided into three parts (figure 11.2.1.1).

Figure II.2.1.1: General structure of nucleotides, DNA nucleobases and Watson-Crick base pairs. Single unit of DNA, the nucleotide, consists of two parts: the nucleoside, containing the nucleobase and the sugar backbone, for RNA this is the ribose, for DNA it is the deoxyribose and the phosphate. For the DNAs polymeric backbone, the deoxyribose and the phosphate are mainly responsible, by forming a covalent bond between the phosphate on the 3-C of the ribose, therefore formally called 3'-end and the alcohol group on the riboses' 5-C, formally called 5'-end. The naturally occurring nucleobases in DNA are subcategorized into purine bases, including the adenine (**A**) and guanine (**G**) nucleobases and the pyrimidine base, where thymine (**T**) and cytosine (**C**) belong to. Base pair formations between adenine and thymine and between guanine and thymine are the most stable nucleobase pairs existing in a DNA. Those pairs are referred to as Watson-Crick base pairs and can be predominately found in DNA double strands.

The repeating sugar moiety (deoxyribose) is connecting one of the four possible nucleobases (adenine (**A**), cytosine (**C**), guanine (**G**) or thymine (**T**)) to the negatively charged phosphate group. The latter forms the highly negatively charged shell of DNA. This unfavorable accumulation of charges is primarily compensated by surrounding mono- or divalent cations, like Na^+ , K^+ , Mg^+ , or Ca^+ , but makes the DNA constructs water-soluble.^[85] The inner core of the DNA structures is formed by the nucleobases. They are connected to the sugar and form the base pairs. Guanine and cytosine or adenine and thymine form the so called Watson-Crick base pairs, or canonical base pairs. They are the most occurring type of base pairing and can be found i.e. in mostly known DNA secondary structure, the B-DNA. The favored formation of three hydrogen bonds between **G** and **C** or two hydrogen bonds between **A** and **T**, makes DNA so predictable and is next to the π - π interaction between vicinal base-pairs, the predominant interactions responsible for the stabilization of the DNA secondary structures. Furthermore, nucleobases can show a *syn*- or *anti*-orientation in respect to the sugar, which plays an important role for the formation of secondary DNA structures.^[86]

Under physiological conditions, the most stable of the natural occurring canonical secondary structures is the B-DNA. Two complementary ssDNA stick together as right-handed double helix due to Watson-Crick base pairing and stabilized by the intramolecular hydrogen bonds and the above mentioned π - π stacking between vicinal nucleobases. Besides this DNA construct, several additional structures have been found in the last decades, as

i.e. the A-DNA, in which the B-DNA can be reversibly converted upon dehydration. At high concentrations of electrolytes, the left-handed Z-DNA can be formed, which has a completely different spatial arrangement to the other both structures and uses purine bases in syn configuration.

Figure II.2.1.2: Crystal structure representation of the most common natural occurring DNA double strands. From left to right: A-DNA,^[87] B-DNA,^[88] Z-DNA.^[89] The pale red dots represent counter cations detected during crystal analysis.

A further occurring structure is the so called DNA hairpin loop, which mainly formed in RNA, but can also be found in double stranded DNA structures. For this special phenomena the ssDNA has to contain palindromic or inverted repeating nucleobase sequences.^[90] More commonly for DNA is the cruciform structure, which contain a complementary palindromic sequences in both strands, by what two opposing hairpins are formed.^[91] As the last canonical DNA structure, the Holliday Junction or four-way junction, is to be mentioned. Playing an important role during homologous recombination and the DNA repair mechanism, it is transiently formed by two crossing double stranded DNA fragments. This structure is further discussed in section II.2.2.

Figure II.2.1.3: Schematic representation of various canonical secondary non B-DNA structures. From left to right: Hairpin, cruciform, Holliday Junction. The hairpin structure exhibits an separated palindromic sequence within one strand, which can form under certain conditions this intrastrand loop. The cruciform structure can be deduced from the hairpin, since it basically a double strand with two opposite hairpins, with one hairpin in each single strand, resulting in a cross-like topology. The Holliday Junction contains four single strands, where one partial sequence of each single is complementary to one of the other three strands, whilst the other partial sequence fits to another one of the remaining two strands. Applying this system to all of these strands result in two-dimensional four-stranded cruciform, which can transform under physiological conditions into other three-dimensional topologies (figure II.2.2.7).

Besides the Watson-Crick base pairs an alternative hydrogen bond pattern between adjacent nucleobases can be found in secondary DNA structures. In the non-canonical secondary structures,^[92] the so called Hoogsteen base pairings take place, by what the formation of new DNA architectures are facilitated. In figure II.2.1.4 the most prominent representatives of the non-canonical secondary DNA structures are shown.

Figure II.2.1.4: Crystal structures of non-canonical secondary DNA structures. The formation of those structures are promoted by forming the so-called *Hoogsteen* base pairs. **a)** The *G-Quadruplex* belongs to the most stable secondary DNA structures. It is formed mono- or poly-molecular guanine-rich sequences and develops square-planar layers, consisting of four guanines, one on each corner. Every guanine forms two hydrogen bindings to each of its adjacent guanines, four in total, by what an enormous stability is archived. Furthermore, the layers stick on each other and are further supported by ions, in general potassium cations, which are encapsulated between two layers, interacting with the inward directed guanine oxygens. **b)** The counterpart of the *G-Quadruplexes* are represented by the *i-Motif*, it can be formed by cytosine-rich sequences, but needs a lower pH, since the one of cytosine has to be protonated in order to form an $C \cdot C^+$ base pair. **c)** The triplet structure is a normal DNA double strand structure, where a third single strand nestles into the double strand along the major groove. Besides the hydrogen bindings between adenine and thymine, known from Watson-Crick base pairing, adenine is forming further hydrogen bindings to another thymine of the third single strand, in a Hoogsteen-like fashion.

In figure II.2.1.4a the crystal structure of a tetramolecular G-quadruplex is illustrated. It is mainly formed in guanine-rich sequences, and consists of stacked planar G-quartets, held together by Hoogsteen base pairing. In between those planar layers, small monovalent cations, like sodium or potassium, are enclosed, which contributes to the structures' stabilization.^[93] The Hoogsteen base pairing occurs in G-quadruplexes between the $H1$ and C_2-NH_2 with the $C6=O$ and $N7$ of the next guanine. Up next, the crystal structure of the i-motif is presented. The corresponding quadruple stranded secondary DNA structure to

the G-quadruplex. In contrast to the G-quadruplex there are no four bases in one layer, but alternating layers of $C \cdot C^+$ base pairs between opposite strand.^[94] Since an unusual hemiprotonated cytosine per base pair is crucial for its formation, the investigations the i-motif structure is mostly done under acidic conditions. Right below, the triplex structure is shown (figure II.2.1.4c). A helix consisting of three DNA single strand. The third ssDNA binds to the major groove of DNA double helix by Hoogsteen base pairing to an existing Watson-Crick base pair. Besides the shown base triplet $T \cdot A=T$, another possible triplet is $C^+ \cdot G \equiv C$, which also includes an hemiprotonated cytosine.^[95]

Apart from the natural occurring nucleotide building blocks, the exchange and modification of the individual nucleotide constituents, including phosphate backbone, sugar and nucleobase, was extensively investigated in order to create a toolbox, with which the features of DNA can be finely tuned. Especially during the design and creation of functional DNA architectures, as i.e. for drug delivery or cell biology applications,^[83] the natural occurring negatively charged form of DNA is not ideal, since it makes the penetration of membranes almost impossible. As a consequence, a portfolio of artificial DNA hybrid structures have been designed and examined in the last years. In figure II.2.1.5 some nucleotide modifications are illustrated.

Figure II.2.1.5: Modifications of the individual nucleotide constituents. In the green third a selection of phosphodiester modifications are shown. The subtle alteration of the phosphodiester (**P1**), uncharged triazole linker (**P2**)^[96] and a positively charged guanidinium linkage (**P3**)^[97]. In the red third a selection of sugar modifications and replacements are illustrated. An RNA based modification with open sugar for higher conformational freedom, named as unlocked nucleic acid UNA (**S4**) and its counterpart of the locked nucleic acid.^[98] **S5** is an exception, since both the phosphodiester and the sugar are replaced by an uncharged peptide sequence. The modification is called peptide nucleic acid (PNA). **S1** and **S2** are used linkers to incorporate supramolecular building block (SBB) in DNA and will be used for the systems described in the next chapter. In the blue third a selection of artificial nucleobases is presented. **MB1-3** show linkers for artificial nucleobases/ligands for metal base interaction. **B2** shows a pair of artificial nucleobases that undergoes hydrogen bonding. As a contrast, another class of artificial nucleobases is made for hydrophobic interactions (**B1**).

Among the very first modifications made to oligonucleotides were alterations of the phosphodiester backbone to stabilize the oligonucleotides towards enzymatic degradation within cells. Therefore, a number of studies have focused on modifications of the native phosphodiester linkage, reaching from subtle modification as the replacement the free acidic oxygen by an organic acid (phosphonoacetate, **P3**)^[96] over a complete exchange to an uncharged triazole linker (**P2**) to linkers with a positively charged guanidine linker,

reversing the complete overall charge of the natural occurring DNA. Besides the phosphate backbone, also the sugar linker connecting the backbone with the nucleobase, has been frequently modified. In order to achieve a higher or lower degree of flexibility for the nucleobases various sugar analogs were investigated. As a result, nucleic acid variations as the RNA based unlocked nucleic acid (UNA), with an opened ribose ring (**S4**) or its counterpart the locked nucleic acid (LNA), has been designed and examined.^[98] Meggers *et al.* have replaced the sugar completely with an glycol motif, connected with the phosphate backbone on 1- and 2-position. The nucleobase is attached to the 3-position (**S3**). Due to its containing stereocenter the effect of the incorporated enantiomers on stability and reactivity can be investigated. This motif has recently be used by our group to introduce pyridine or imidazole ligands in tetra- and telomeric G-quadruplex for various metal bindings.^[99–101] An exceptional derivative is the peptide nucleic acid (PNA) (figure II.2.1.5, **S5**). The complete phosphordiester-sugar framework is replaced by a peptide sequence. In contrast to natural occurring DNA its backbone is not charged, similar to the backbone modification **P2**, unless additional charged groups are introduced.^[102,103] **S4** is a frequently used linker for the incorporation of supramolecular building blocks in DNA, which gives a certain flexibility. Furthermore, the polyethylene glycol (PEG is easy to introduce to both the desired ligand and the oligonucleotide.^[84] **S5** is based on diethanolamine. The ethanol chains are connected to the phosphate backbone, the nucleobase or SBB is attached on the amine nitrogen. This structural motif is further discussed in chapter III.2

The third possibility to modify the nucleotide is a variation of the nucleobases nature, in terms of shape or functionality. This area of research makes probably the largest part of nucleotide modifications in the last years. There are mainly four concepts of modifications. Firstly, the hydrogen bonds are retained, but the shape of nucleobases and possible formed hydrogen bonds are varied, as seen for **B2**. Secondly, the hydrogen bonds are discarded, instead the interactions of base pairs are based on hydrophobicity and shape complementary, as it is the case for **B1**. In the third place, the base pairing is neglected and the large mainly polycyclic aromatic functional compounds are introduced to one of the strands, while the other strand has only a small or even no nucleobase at the opposite position. Due to its planarity the compound can stack with the adjacent nucleobase pairs.

Lastly, the field of metal-base paring has to be mentioned. The fact that metal ions, can and do interact with nucleic acid is already known for some time past. Under physiological conditions, canonical as well as non-canonical architectures are stabilized by the biologically ubiquitary ions Na^+ , K^+ , Mg^{2+} , Ca^{2+} mainly from the outside, since the highly negatively charged backbone is located there. The concentration and/or change of counter-cation has a direct influence on topology of secondary DNA structure, as i.e. can be observed for the Z-DNA, which is only formed at high electrolyte concentration or for the Holliday Junction,

which switches from the open form to the stacked form in presence of small concentration of magnesium.^[104] However these interactions are rather of unspecific nature, an example for more specific interactions with nucleic acid is the the G-quadruplex, whose structure is additionally stabilized by an cation ion sitting in between G-quartet layers and interact with the inward pointing guanine carbonyl groups, resembling to an ion sandwiched by two crownethers.^[105] Most prominent specific interaction of metal ions to natural occurring nucleic acid is probably the DNA cross-links caused by the anti-cancer therapeutic *cis-platin*, which are preferably formed between adenine and guanine nucleobases. Besides, in the last years further metal base pair interactions in unmodified canonical DNA has been found.^[106,107] On the one hand there is the insertion of Hg^{2+} between and T-T mismatch, on the other hand Ag^+ was found to bind in between a C-C mismatch; in both cases the involved nucleobases are deprotonated. Encouraged by this observation, a plethora of artificial nucleobases were developed, that can be introduced to canonical oligonucleotides, where one and more layers of base paring is replaced by an metal base pair.^[108] Due to the confined space between the base pair layers, flat coordination environment (linear, square-planar) are preferred but under certain conditions or secondary DNA topologies other coordination environments are possible. In figure II.2.1.5 some examples are shown (**MB1-3**). Inspired by the above mentioned metal interactions between natural occurring nucleobases, Müller *et al.* replaced the cytosine by imidazole and could prove the existence of imidazole- Ag^+ -imidazole artificial metal base pair within an intact DNA double helix (**MB1**).^[109] **MB3** shows one of the first artificial metal base pairs reported by Schultz *et al.*, a Cu^{2+} metal ion coordinated by pyridine and pyridine-2,6-dicarboxylate, or an nitrogen containing analogs, in a flat square planar fashion.^[110,111] As a last example, the DNA incorporated salen ligand reported by Clever *et al.* has to be mentioned. The combination of the metal base pair concept in combination with dynamic covalent chemist led to reversibly covalently bounded DNA double helix, showing an immense stabilization compared to the unmodified double strand.^[112,113]

The above described system are coined as *ligandosides*^[114] (figure II.2.1.6a), which includes all artificial ligands that are attached at the sugar backbone, where actually the normal nucleobases were located.

Figure II.2.1.6: Ligandoside vs. incorporated ligands. **a)** Schematic representation of published ligandoside containing secondary DNA structures. From left to right: double-stranded helix with opposite lying ligandosides coordinating to metal ion;^[109] three-way junction with ligandosides in the structures' central void capable of forming octahedral complexes with metal ions;^[115] triplex stabilized by three ligandosides, one per strand, coordinating to metal ion. **b)** Similar structures as threeway junctions or normal double helix structures have been designed with incorporated artificial ligands, capable of metal coordination.^[116] However, compared to the ligandoside approach, the sugar-phosphate backbone is suspended, what effects the stability of the secondary DNA architecture.

These ligandosides are rarely used in other secondary structures than the double helix, anyway, some examples have been reported by Shionoya *et al.* With the help of triple coordinated Ag^+ by pyridine ligandosides, the stabilization of a metal containing triplex structure could be obtained.^[117] Later another ligandoside based on 2,2'-bipyridine was used to stabilize a DNA three-way junction, with Ni^{2+} metal ion, coordinated in the middle of the DNA architecture by three ligandosides in an octahedral fashion.^[118] Some years later a fine tuned system was reported by Takezawa *et al.*, where, depending on the presence of Ni^{2+} metal ions, the system can reversibly switched between duplex and three way junctions.^[115] More commonly used in modified non-duplex DNA, especially in the field of "*supramolecular DNA assemblies*", are the here entitled "*DNA incorporated ligands*" (figure II.2.1.6b). For this kind of modification the whole nucleoside is replaced by the respective ligand, which is directly bound to the phosphate backbone. Representatives of those systems can be found in hairpin,^[119] duplex,^[116] g-quadruplexes,^[99,120] threeway junction structures^[121] and other higher order assemblies.^[84] These structures are further discussed in the next chapter (chapter III.1).

II.2.2 Holliday Junction

Already over 50 years ago Robin Holliday postulated a DNA construct consisting of two crosslinked DNA duplexes, which is formed during the process of intergenic homologous recombination,^[122] responsible for the combinations of chromosomal DNA, representing genetic variation, which in turn enables populations to adapt during the course of evolution.^[123] This cross-linked four-way junction was later named after R. Holliday,

therefore this DNA architecture is nowadays mainly known as Holliday Junction (HJ). From that day on the motif *Holliday junction* was intensively investigated from both, chemists and biologists, in terms of topological and functional appearance under physiological conditions,^[124–126] DNA-protein interactions,^[127,128] relevant mechanisms during the homologous recombination^[129] and of course structural elucidation.^[130–132] In general the HJ consist of, as the alternative name four-way junction let assume, four individual ssDNA, which contain two sequence segments, each complementary to a sequence segment of two other ssDNA. Carrying on this assembly motif, four connected DNA helix structures are formed with a junction in the middle. For this structural arrangement four possible isomeric main topologies were postulated the HJ can switch between (figure II.2.2.7). The other possible intermediate topologies the HJ can assume while transforming from one stable topology to another are neglected here.^[133] In contrast to natural occurring HJs, the artificial designed HJs are in most cases immobile, means that the complementary sequences are designed in a way that only one structure can be formed. In contrary, the huge genomic DNA contains several potential complementary sequence segments, meaning that the four individual arms may slide through the junction in a specific pattern till the largest possible complementary base pair overlap is obtained.

Figure II.2.2.7: Schematic representation of the four known possible isomers of a natural occurring *Holliday Junction*. The isomers can be categorized into open and stacked forms, whereby the stacked form can be subcategorized into parallel stack, where two neighbored oligonucleotide strands have the same orientation (3'→5', 3'→5'), whereas in the antiparallel form, the strand orientation is alternating (3'→5', 5'→3'). Most common form is the antiparallel stacked form, which can isomerize from the ISO-I to the ISO-II form, by passing the open stacked form as an intermediate structure.

The four major HJ species are the stacked X form, where a distinction is made between the parallel and anti-parallel assembly. In the parallel configuration two adjacent strands exhibit the same strand orientation, while the strand orientation in the anti-parallel isomer is alternating. Additionally, the anti-parallel orientated stacked X form of the HJ is further subcategorized into the Iso-I and Iso-II conformation, depending on its spatial arrangement. The open X form has a square planar topology (figure II.2.2.8a) and exists as the major species at low electrolyte concentrations,^[104,134] since in this form the repulsive forces between the negatively charged phosphate groups within the DNA backbone are minimized. It is worth to mention, that the open-X clearly shows a gap at the junction position, what should be kept in mind. From this topology the HJ can be directly converted into every other possible topology, which in turn mean that the open X is always an intermediate species, when the HJ transforms to any other topology. However, *in silico* investigation indicating that at higher salt concentrations the intermediate HJ conformer has already a rather tetrahedral character.^[104] Under physiological conditions, with electrolyte concentration above 100 mM or in presence of divalent cation ($> 2 \text{ mM Mg}^{2+}$),^[135] the preferred conformer is the anti-parallel stacked X structure, which is more densely packed compared to the open form (figure II.2.2.8, since the negatively charged DNA backbone is better shielded by the present cations. Having a closer look at the crystal structure, it becomes quite obvious that the two respectively HJ arms are arranged in line, forming almost one consistent duplex. Both of these pseudo duplex structures are twisted against each other. This twist is characterized with the so called inter duplex angle (IDA) and is ranged between 40° and 60° . Although often postulated, the parallel stacked-X isomer of the Holliday junction, was never really verified. Neither the results from the publication dealing with that topic have been clear enough to see the existence of this conformer as proofed, nor was this conformer ever crystallized.^[136,137] However, Lu *et al.* claim to create a parallel stacked-X HJ, by connecting two involved ssDNA of opposite arms.^[138]

Figure II.2.2.8: Crystal structures of open-X and stacked-X Holliday junctions. The open-X^[139] form was extracted from protein-DNA co-crystallisation. The stacked-X form of the HJ was solved by Cardin *et al.*^[140]

In the recent past, Mergney *et al.* reported a system including a parallel stacked-X like four-way junction to preset the orientation of a G-quadruplex motif, formed within a junction environment.^[141] This specific example will be further discussed in the next chapter (chapter III.1).

Concluding, the Holliday junction has been intensively investigated to the present day, however lots of structural and functional questions are still unanswered. That is probably one of the reasons, why the unmodified four-way junction motif is frequently used in quite well predictable DNA origami structures,^[79,130] but has never been used, to the best of our knowledge, in combination with metal base pairs or supramolecular ligand modifications, as it has been shown for almost all other known secondary DNA structures (section II.2.1).

Ligands for Metal-Mediated Coordination Cages Embedded in Oligonucleotide Scaffolds

Related Publications:

T. R. Schulte, M. Krick, C. I. Asche, S. Freye, G. H. Clever, *RSC Adv.* **2014**, *4*, 29724–29728.

Chapter III.1

Introduction

Historically, discrete assemblies derived from natural occurring building blocks (including DNA and proteins, but in this work only the DNA part is taken into account) were distinguished from those made of synthesized organic ligands, with or without the use of metal ions. Thus, although both, the field of supramolecular chemistry and the field of biotechnology, sharing certain approaches in the design, especially with regard to deployed symmetries, they evolved independently their own methods to create three-dimensional self-assembled structures.^[142,143] Even though most structural symmetries can be formed in both areas of research, they clearly differ in size. While the average cavity dimension for bio-derived systems ranges in the nanometer scale, the size of cavities for synthetic structures is rather in the region of several Å. This difference in size is most commonly accompanied with a difference in the cavities' hydrophobicity, what is not surprising since the smaller cavities of the synthetic structures are delimited in most cases by poly-aromatic ligand backbones. In contrast, the larger cavities are more framed than delimited by the bio-derived building blocks, which rather led in combination with its commonly polar shells to a hydrophilic interior of cavity. Due to these differences the choice of the most suitable system is highly dependent on a respective issue to be investigated, therefore the actual intention of desired system must be known at a very early stage and will be discussed in the next chapter. However, for most of the bio-derived architectures the water-solubility is inherent, whereas the formation of three-dimensional supramolecular structures of artificial organic ligand in water was quite a challenge. A more detailed introduction to this topic can be found in Chapter IV.1.

On the other hand, the architectures formed from naturally occurring building blocks are often lacking in rigidity especially in terms of the formation of discrete angles and vertices.^[144] The combination of both, bio-derived and synthetic building blocks gave completely new possibilities of designing three-dimensional structures, which showed even in large structures discrete shapes and a certain rigidity, compared to everything else that has been reported before in this area of research. In the following, some of those systems

are presented.

In 2004 Sleiman *et al.* and Han *et al.* independently reported the incorporation of metal coordination environments into oligonucleotide strands.^[145,146] In case of Han, a double strand triangle was designed, with iron(II) terpyridine complexes on both ends of the double strands forming the discrete vertices in the DNA structure. In a first step iron(II) is added to two non-complementary strands in order to form an octahedral iron complex between two single strands. Subsequently all strands are mixed together and the self-assembly of complementary oligonucleotides lead to formation of the triangle (figure III.1.0.1).

Figure III.1.0.1: Triangular DNA architecture by Han *et al.*. The assembled structure consists of six ssDNA, complementary to each other, having a terpyridine motif on one end. In presence of FeII cations, the single strands do form octahedral $\text{Fe}(\text{tpy-DNA})_2$ complex, which assembles in a subsequent step to three bridging double strand helices, giving the final triangular DNA architecture.^[145]

One year later, Krämer *et al.* reported another system for the incorporation of metal coordination environments into DNA.^[147] Also based on terpyridine, the ligand was attached on both ends of an oligonucleotide single strand. In absence of Fe^{2+} or Zn^{2+} the modified strand hybridized with a complementary strand. Upon addition of one equivalent $\text{Fe}(\text{II})$ or $\text{Zn}(\text{II})$, the double strand preferentially disassembles to gain the flexibility and form an intramolecular octahedral metal complex. In both cases this process is reversible, but interestingly, in contrast to $\text{Fe}(\text{II})$, a second equivalent of $\text{Zn}(\text{II})$ led to reformation of the double strand, where each terpyridine ligand is bound to one zinc cation (figure III.1.0.2) This example nicely illustrates how strongly the formation of metal complexes can affect the overall oligonucleotide structure depending on the choice of the metal ligand system.

Figure III.1.0.2: Terpyridine-DNA structure by Krämer *et al.*. In contrast to Sleiman's system, Krämer's structure consists of a DNA single strand with terpyridine attached to both ends and a complementary strand without any artificial attachments. In absence of transition metals, the most stable form is described by a double helix structure. In the presence of one equivalent Fe(II) or Zn(II), the double helix disassembles and an intramolecular [Zn(II)(terpy)₂] complex is formed. In contrast to Fe(II), a second equivalent of Zn(II) leads to reassembly of the starting double helix structure, but with both terpyridine motifs occupied by zinc cation.^[147]

However, Sleiman *et al.* reported a system where the coordination environment of the metal is predetermined by the hybridization of two single strands. These strands contained either a terpyridine or a phenanthroline moiety. By mixing two complementary strand together, double strands with different coordination environments were created, which show depending on the ligand pairs, a preferred coordination behavior with respect to the tested metal cations (figure III.1.0.3), accompanied with a significant increase of denaturation temperature. Fe(II) showed as in the examples before the best match with two terpyridine ligands and the highest stabilization in denaturation temperature, while two phenanthroline ligands form the most stable complex with Cu(I), that prefers an tetrahedral coordination environment. For the mixed ligand case Cu(II) binds strongest in a trigonal-bipyramidal fashion. Most interestingly, is the behavior of copper in the wrong coordination environment. Initially, Cu(I) binds weakly to the mixed ligand case, but after ca. 30 min it undergoes a spontaneous oxidation to Cu(II) to fit better into the trigonal-pyramidal coordination environment. The other way around with Cu(II) in the phenanthroline environment is also observable, but in this case the spontaneous reduction to Cu(I) took over 12 h.^[116]

Figure III.1.0.3: Mixed coordination DNA structure by Sleiman *et al.* The system consists of two coordination motifs, phenanthroline (phen) and terpyridine (terpy), attached to two complementary sequences. By assembling the complementary single strands to a stable double helix, three different coordination environments can be obtained. Each coordination environment, prefers a different metal cation to coordinate. From left to right: (terpy)₂ -> Fe(II), (phen)₂ -> Cu(I), (terpy)(phen) -> Cu(II).^[116]

In another example, Sleiman *et al.* used the the phenanthroline coordination environment within the oligonucleotidic strands. In the first one, a templated formation of a metal-DNA fourway single stranded junction with a chiral metal center is described. Two modified single strands with an internally integrated phenanthroline ligand are brought together by an with help of an templating strand. Then the metal ion is added to from the chiral phenanthroline metal complex. Subsequently the templating strand is removed and the chiral four-way single stranded junction with the metal complex in its branch remained (figure III.1.0.4).^[148] In a further example, Sleiman *et al.* designed a three-dimensional DNA prism with the phenanthroline coordination environments located at each of its vertices, where matching metals can coordinate in order to give further stabilization and rigidity to the DNA construct.^[149]

Figure III.1.0.4: Chiral single stranded Metal-DNA fourway junction by Sleiman *et al.*. The system consists of two DNA single strands with an central incorporated phenanthroline ligand. A partial sequence of each single strand is complementary to a third single strand. The assembly of all DNA strands give a structure, where both phenanthroline ligands are preorganized, before Cu(I) can coordinate in a subsequent step. In a last step, the non-modified single strand is removed from the structure with help of complementary single strand, obtaining a single stranded Metal-DNA fourway junction with a chiral metal coordination center.^[148]

Inspired by the Metal-DNA hybrid systems described above, we wanted to exploit the highly programmable folding patterns of DNA and the possibility to integrate metal coordinating ligand into oligonucleotides,^[150] to design a new system which shall solve one of the still existing problems in the field of supramolecular coordination cages. The formation of multi-functional heteroleptic coordination cages. Especially, the controlled

self-sorting between ligands with similar (or identical) coordinating groups is still a big challenge. Yet, it is highly desirable, as it would simplify the ligands synthesis and modular strategies to incorporate a combination of specific functionalities into the coordination cages.^[151] Although there are some examples for narcissistic self-sorting^[66,152] and social self-sorting^[153] for coordination cages, the systems lack a controlled formation, especially when more than two different ligands are present. Frank *et al.* showed this for some phenothiazine based $[\text{Pd}_4\text{L}_8]$ interpenetrated coordination cages. As long as the ligand had a similar size and coordination profile, the self-assembly of ligands ended in a mixture of cages with various ligand combinations, represented by the sum formula $[\text{Pd}_4\text{L}_x\text{L}_{8-x}]$. An statistical mixture of cages even occurs when assembling the pure homoleptic interpenetrated coordination cages before mixing them. Due to the reversible palladium-nitrogen coordination bond, the ligands can exchange. On the one hand it is an important feature on the way to the thermodynamically most stable structure, but on the other hand it promotes the formation of the statistical cage mixtures, due to the similar energies of the different cages, making it easy to interconvert. (figure III.1.0.5).

Figure III.1.0.5: Statistic mixtures of coordination cages occur during the assembly of $[\text{Pd}_2\text{L}^{\text{A}}_2\text{L}^{\text{B}}_2]$ with structurally related ligands. By mixing all building blocks immediately together at once, a statistical mixture is obtained. Assembly of the ligand pure coordination cages (green and red) in spatially separated environment was a possible approach, which allows the ligand pure cages to coexist after mixing them together. However, due to the reversible coordination bonds of the ligands to the palladium(II) cations, a thermodynamically more favorable statistical mixture of cages is obtained after a certain time.^[66]

In the last years several attempts were reported and a progress is clearly visible. Clever and Crowley reported palladium-mediated coordination cage systems that form quantitatively $[\text{Pd}_2\text{L}^{\text{A}}_2\text{L}^{\text{B}}_2]$ cages in a integrative self-assembled manner, either by steric hindrance^[75,76] or shape-complementary^[61,71] However, a general approach for the quantitative formation of heteroleptic cages from two different ligands or more was not found yet.

In order to find a possible DNA scaffold for the controlled formation of $[\text{Pd}_2\text{L}_4]$ coordination cages, Shionoyas' metal-stabilized three-way junction (figure II.2.1.6a, second structure) gave the inspiration to have a closer look at secondary canonical and non-canonical structures of DNA (section II.2.1). Therefore, the DNA architecture had to fulfill two crucial requirements. First, it had to be possible to integrate four ligands into its structure, in a certain proximity. And second, the ligands had to be preorganized in a square-planar manner, since the coordination of ligand to the metal occurs in the same geometry. Considering those two requirements, two structures were suitable for the desired purpose, the G-quadruplex and the HJ (4-way junction). But since the tetrameric G-quadruplex

does not give any control over the self-sorting of ligands, the structure would also lead to a statistical mixture and was therefore excluded, by what the HJ became our first choice (figure III.1.0.6).

Figure III.1.0.6: Approach of a controlled assembly of a metal-mediated coordination cage, supported by a preassembled Holliday junction scaffold. The system shall consist of four DNA single strands, containing an incorporated bis-monodentate ligand. In a first step, the DNA single strands are assembled into a Holliday junction, forming the intended scaffold, preorganizing the ligands at the central position of the DNA architecture. Subsequently, the metal cations are added, in order to form the desired coordination cage.

Chapter III.2

Results and Discussion

III.2.1 Idea and conception

For a non-statistical formation of an $[M_2L_4]$ cages derived from a ligand mixture with more than two different ligands, some basic requirements should to be fulfilled.

1. the formed cage structure must be significantly lower in energy compared to the other formed structures
2. the ligand exchange process must be inhibited
3. a preorganized positioning of ligands within the coordination structure.

As already mentioned in the introduction (section II.1.3), several successful attempts were reported for the creation of heteroleptic $[Pd_2L^a_2L^b_2]$ by Clever *et al.* based on bis-monodentate pyridine ligands with shape complementary or steric hindrance at the coordination site. Crowley *et al.* published an $[Pd_2L^a_2L^b_2]$ cage with free amine groups at the coordination site for hydrogen bonds with neighbored exohedrally modified ligands, to from preferentially the cis-isomere of the $[Pd_2L^a_2L^b_2]$ cage. Although all of these systems work for the specific ligands, they only fulfill the first requirement. Additional ligands would probably lead again to a statistical mixture, albeit with less possible species.

For the second requirement, the inhibition of the ligand exchange, no ideal concepts are known in the field of supramolecular coordination cages. Clever *et al.* have shown that the rate of ligand exchange is decreased in coordination structures, depending either on their complexity, means how strongly the ligands are interwoven with each other,^[60] or on

the metal-ligand interaction. When palladium(II) is replaced by platinum(II), the ligand exchange rate is drastically reduced, since the Pt-N coordination bond is kinetically more inert compared to the Pd-N coordination bond.^[24] On the other hand the correction of malpositioned ligands during the assembly process is also inhibited, what leads to extremely decelerated formation rates of the desired coordination cage. A further possibility, that has been already shown for supramolecular architectures and coordination cages, where covalent connections of ligands was achieved in a post-synthetic approach.^[154] Leigh *et al.* created a 'Star of David' catenane, where the ligands were preorganized by templating metals and subsequently covalently connected in a metathesis reaction.^[155] Thereafter, the ligands cannot exchange anymore. Transferred to the coordination cages, this method would require an initial formation of the heteroleptic cage that is stable for a certain time, until the post-synthetic reaction takes place. Furthermore the structure would be irreversibly connected, what's contrary the concept of supramolecular chemistry. However the connection of course could also be realized by dynamic covalent bonds.

The last requirement is the preorganized positioning of the ligands before forming the coordination cage, to avoid stereo isomers. For that reason it needs a system which assembles in a highly controllable and reproducible way. Since the integration of such function into the ligand system would probably be an unpredictable endeavor and nightmarish synthesis, outsourcing this function into an external scaffold would probably be the most auspicious approach.

The idea and concept of the here presented system actually was inspired by nature. Having a closer look to natural occurring enzymes, it becomes quite obvious that the actual active site, where the desired catalytic reaction takes place, is rather small, compared to the rest of the peptidic secondary structure. Although the residual structure does not necessarily contribute to the reactivity of the catalytic site, it is still the framework or scaffold, which places the functional groups within the active site at the right position, forming the cavity shape and shielding the active site from unwanted external influences. Additionally, it makes the active site soluble in water and contributes in many other ways. With other words, the peptidic scaffold around the active site is a crucial part of the enzyme structure, which offers additional features. However, creating such a peptidic scaffold of a specific shape is an highly complicated project. In nature enzymes are folded during the peptide synthesis in a certain way, often even with help of supporting enzymes, to achieve the desired functional topology. As soon as the structure is unfolded, e.g. due to high temperatures, the enzyme cannot simply be refolded into its native topology. This process, the so called '*denaturation*', is accompanied with a loss of function. Therefore, it is still a complicated and unsolved problem, to predict the structure and function of secondary peptidic structures formed from an synthesized polypeptide. Hence, another natural occurring polymer was chosen, which folding patterns are highly predictable, making

it suitable to create an external scaffold for the formation of heteroleptic coordination cages.

III.2.2 Acridone-based ligand embedded in *Holliday* junction

DNA is possibly the most predictable and programmable self-assembling material known, undergoing rapid, high fidelity association according to simple base-pairing rules. Though many self-assembling molecules are known, such predictable control over the resulting structure is scarce. DNA gives researchers a code to define which strands will pair, to form double helices that are structurally uniform and well-defined. For these reasons, DNA assembly has been exploited in the field of DNA nanotechnology to generate higher order functional architectures.

(a)

III.2.2.1 Design and syntheses acridone-based ligand

The first attempt to create a DNA incorporated ligand, suitable for an controlled cage formation, was based on the acridone backbone, which was previously used in various other supramolecular cages systems and is therefore already well characterized.^[20,41] For the incorporation in oligonucleotides, polyethylene glycol (PEG) linkers were chosen, which are known not to hinder the formation of self-assembled coordination cages from a previous publication.^[32] The exohedral modifications on the ligands, for the increase of solubility,

(b)

Figure III.2.2.1: MMFF model of first generation DNA-cage hybrid structure. Modelled in Sparten '10.^[156] The ligands are coordinated to palladium. The ONS sequences contain an extra T-T mismatch next to the integrated ligand.

were tested on its ability to form supramolecular cage structures. It could be shown that the attachment of PEG groups at the coordinating pyridine motifs did not hinder the ligands in the formation of supramolecular cages or even interpenetrated double cages. On this basis, ligand **9** was designed with an acridone backbone and pyridine attached polyethylene glycol linkers, suitable for the integration in ONS during DNA solid-phase synthesis. The ligand was synthesized in an eight step synthesis, followed by two additional steps to introduce the 4,4'-Dimethoxytrityl (DMT) and β -cyanoethyl phosphoramidite (CEP) groups, which are crucial for the subsequent DNA solid phase synthesis.

Scheme III.2.2.1: Synthetic path for ligand **11**. **a)** $\text{CH}_3\text{O}(\text{CH}_2)_2\text{O}(\text{CH}_2)_2\text{Br}$, K_2CO_3 , DMF; **b)** NIS, TFA, MeCN; **c)** DHP, TsOH, DCM; **d)** TsCl, NEt_3 , DCM; **e)** 3-Bromo-5-hydroxy pyridine, K_2CO_3 , NEt_3 ; **f)** Ethynyl-TMS, Pd-cat., CuI, NEt_3 , THF; **g)** K_2CO_3 , MeOH; **h)** Pd-cat., CuI, NEt_3 , THF; **i)** TsOH, DCM; **j)** DMT-Cl, 4-DMAP, NEt_3 , DCM; **k)** $\beta\text{-PA-Cl}$, $(i\text{Pr})_2\text{EtN}$, DCM.

The synthesis for **11** was achieved in two independent synthetic pathways, one for the acridone backbone and the other one for the pyridine arms with the attached protected PEG linkers. Subsequently both individual motifs (**7** and **2**) were merged in a Sonogashira cross-coupling reaction to obtain molecule **8**. In three further steps the DMT and CEP groups were asymmetrically introduced to the ligand.

At first the THP protecting group was introduced to one side of the diethylene glycol chain. After, the other hydroxy group was converted into a better leaving group with tosylchloride. Subsequently, the tosylate leaving group was substituted with of 3-bromo-5-hydroxy pyrdine. In a palladium catalyzed cross coupling reaction ethynyl trimethyl silane was introduced to the 3-postion of the pyridine compound and the TMS group was removed under mild basic condition in the next step, to obtain **7**. For the acridone, methoxy-ethoxy-ethyl bromide was attached at the acridone nitrogen in a first step, in order to increase the solubility of the later end product. Secondly, iodine was introduced regiospecificly to the 2- and 7-postion of the acridone in a TFA catalyzed electrophilic aromatic substitution reaction, by what the substituted acridone nitrogen exhibits a para-directing effect (+M effect), while the keto group contributes with an meta-directing effect (-M effect). The coupled product was deprotected under acidic condition with the organic acid p-toluenesulfonic acid (TsOH). In the subsequent step the DMT protecting group was introduced to one of the pyrdine attached PEG chains in a asymmetric substitution reaction. Since there was no regioselective control over the substitution reaction **10** was obtained with a max. yield of roughly 50 % with 25 % non-protected or double-protected compound, each, as side products. The non-protected ligand was immediately reused, while the the double-protected compound had to be deprotected in presence of 5% TFA in dichloromethane (DCM). As the last step the 2-cyanoethyl N,N-diisopropylchlorophosphoramidite was attached to the second unprotected PEG chain. The reaction was proceeded under inert conditions and also the product compound was stored in an inert atmosphere at low temperatures ($< -30^{\circ}\text{C}$), since the phosphorous(III) species is extremely sensitive towards oxygen or moisture. Therefore, preparation of **11** was performed on the same day, as it was used for the DNA solid phase synthesis (section III.2.2.2).

III.2.2.2 Synthesis of Ligand Embedded Oligonucleotides

The synthesized ligand **11** was incorporated into artificial oligonucleotide sequences known from literature to form a four-way junction. Seeman *et al.*^[157,158] published a sequence, designed with respect to sequence symmetry minimization rules and without longer repeating G sequences, in order to avoid the formation of non-Watson-Crick pairs (table III.2.2.1). Every single strand consists of two non-complementary sequences, whose complementary counterpart is located in the other single strands. In other words, the half of one single strand forms a double strand to another single strand, while the other half of the single strand is interacting with a third single strand. In this manner, four single strands can be wrapped up to the desired *Holliday* junction (HJ) structure (figure II.2.2.7). To give an easier overview, which sequences do match to each other, the complementary sequences, shown in table III.2.2.1, are highlighted in the same color. Besides, a second sequence was used from a recent paper by Mendoza *et al.*,^[141] also consisting of four single

strands. Here the complementary parts do not just vary in the nucleotide composition, as in the case of Seeman, but also in the length of each matching sequence. Latter are only mentioned for the sake of completeness, however, since no formation of the desired structure could be observed for the sequences of Mendoza *et al.*, neither by UV melting nor by PAGE electrophoresis experiments, the synthesized strands are not further discussed in the following course of this dissertation.

Table III.2.2.1: ONS sequences for MKDH01um and MKDH01. The sequences are known to form a Holliday Junction. In the case of the unmodified sequence, the position where the ligand is incorporated in MKDH01, is represented by an extra thymine, to create and T-T mismatches within the center of the structure. Additional T-T mismatches next to the ligand are not necessary due to the long PEG linkers, which provides plenty of space in the central position, according to the MMFF model (figure III.2.2.1).

	MKDH01um Sequences 5'→3'	MKDH01 Sequences 5'→3'
A	GCC ATA GTT TGA GCA CG	GCC ATA GTL TGA GCA CG
B	CGT GCT CAT GGA TTG CG	CGT GCT CAL GGA TTG CG
C	CGC AAT CCT CCG AAT GC	CGC AAT CCL CCG AAT GC
D	GCA TTC GGT ACT ATG GC	GCA TTC GGL ACT ATG GC

Besides the strands containing the incorporated artificial ligand, from now on referred as **MKDH01**, the same sequence was also synthesized without the ligand, but with a thymine as connecting link between the two non-complementary sequences within a single strand. The thymines shall form T-T mismatches in the middle of intended HJ structure, in order to imitate the void within the Holliday junction, caused by the sterical demanding ligands or cages. These strands, from now on referred as **MKDH01um**, or *MKDH01-unmodified*, shall function as an easy accessible reference system, to figure out, if the the intended Holliday junction, with the incorporated ligands, is able to be formed at all. And if so, what impact the ligands do have on the formation and stabilization of those ligand-containing modified *Holliday* junction, in comparison to the unmodified versions.

As already mentioned at the end of the previous section, the freshly synthesized phosphoramidite was instantly used for the oligonucleotide solid phase synthesis (SPS), since compound **11** was extremely labile and decomposed within hours, even under cooled inert conditions. Therefore the reaction mixture was filtered under inert conditions, to get rid of the formed solids and subsequently the solvent and residual *Hünig* base was evaporated *in vacuo*. Directly after, the weight of the residue was determined and a 50 mM solution in anhydrous MeCN was prepared, on the supposition that the reaction yielded at least 50% of the desired product. The syntheses for the above mentioned oligonucleotides (table III.2.2.1) were accomplished in DMT-ON mode, according to the procedure described in the appendix, section B.1.

Followed by a successful synthesis, the oligonucleotides were cleaved from the solid phase support over night in a 33% aqueous ammonia solution, while all base labile protecting groups on the used oligonucleotides were removed as well. Subsequently the purification of the synthesized ONSs was performed via *Reversed Phase HPLC* as described in the appendix in section B.2.a, to separate the desired sequences from all other fail-sequences, formed during the oligonucleotide SPS. For that purpose, the ONSs were synthesized in DMT-ON mode, where the last DMT protecting group is not cleaved, but remains on the last coupled nucleotide. The DMT-group is a hydrophobic residue, which leads to an increased interaction with the solid phase during RP-HPLC cleanup. Therefore, it virtually functions as an anchor, so that the ONSs still containing DMT have a longer retention time on column, compared to the other ONSs and are easier to separate. HPLC traces recorded at 260 nm for the clean strands are shown in the appendix, chapter A. Since the incorporated ligands have a specific absorbance at 375 nm, for MKDH01 strands **a-d**, HPLC traces at 375 nm were recorded as well. After HPLC clean up, the ONSs had to be desalted and the DMT protecting group was removed. For that reason each sample was transferred on solid phase support, as described in the appendix, section B.2.b. Under acid conditions the DMT moiety was cleaved from the ONSs and the ions were washed out, before the strands were released again from the solid phase support and dissolved in 1 mL of pure water, as a stock solution.

As a next step, the isolated ONSs were each characterized by ESI-MS analysis. The corresponding mass spectra for the specific ONS can be found as the HPLC traces before in chapter A of the appendix. As it can be clearly seen, there is no single peak for each charged state of the ONS, but rather accumulations of peaks. This phenomenon comes from statistical unspecific adducts of cations with the ONS. During the whole process of synthesis and clean up various counter cations have been dragged along and were exchanged on the phosphate backbone of the ONS, so that several peaks do appear with similar molecular weight and various amounts of sodium, potassium or ammonium ions. Nevertheless, all peaks belong to the same ONS and therefore, prove the integrity of the ONS. As a last step the concentration of the 1 mL ONS solutions for every single sequence was determined, as described in the appendix, section B.3.b. For that reason the extinction coefficient of the incorporated ligands at 260 nm was determined by dilution UV-vis experiments. Due to the bad solubility of ligand **9** in water, the extinction coefficient was determined in a 1:1 mixture of pure water and MeCN. Later a highly diluted solution of **9** in pure water was available, from which an extinction coefficient of $\epsilon_{260\text{nm}} = 26618 \pm 470 \text{ mol cm}^{-1}$ was determined (figure VI.2.3.1a). As a comparison, the same dilution series was also made for the 1:1 mixture of water and acetonitrile, by what an extinction coefficient of $\epsilon_{260\text{nm}} = 26299 \pm 1605 \text{ mol cm}^{-1}$ was obtained. Since both values are very similar, the MeCN seems not to have a significant influence on the absorption properties of the acridone ligand. Together with the revised extinction coefficients,^[159] the concentration of each

stock solution was calculated from the measured absorption at 260 nm of 1 μ l ONS stock solution, filled up with pure water to a total volume of 400 μ l. Knowing the concentration of each ONS solution, first samples could be prepared with all four necessary sequences, to form a *Holliday* junction structure.

III.2.2.3 Holliday Junction with Embedded Acridone-Based Ligand Ligand

III.2.2.3.1 Holliday Junction MKDH01um

First experiments were performed with the unmodified version of the desired Holliday Junction system. The samples were prepared with 2 μ M of each required single strand, in order to get a 2 μ M solution of the desired Holliday junction, 10 mM of cacodylate buffer adjusted to a pH of 7.3, 100 mM sodium perchloride and 5 mM of magnesium perchloride with a total volume of 800 μ l. The perchloride salts were used on purpose, since chloride ions can disturb the formation of coordination cages, especially when palladium salts are involved, while perchlorides belong to the class of non-coordinative anions, which only show an extremely weak interactions with metal cations. Under these conditions it is ensured, that excess amounts of anions within the sample solution does not compete with the coordination of ligand to the metal. The prepared sample was heated to 90 °C for a couple of minutes to disassemble any unspecific binding between the DNA single strands, ensued by the cooling down process, where the sample was cooled down with a rate of 0.5 °C min⁻¹ to end temperature of 4 °C. After, the sample solution was transferred into the cuvette, and topped with 200 μ l of silicon oil. The topped oil shall prevent evaporation, causing a higher concentration of the sample, making it difficult to compare the single melting and assembly rounds with each other. For every experiment two rounds of melting and assembly process were recorded. Additionally, for every UV-vis experiment the background was measured with the buffer and salt, but no DNA. The background was later subtracted from the actual experiments, getting only the effects caused by the DNA. Furthermore, for each melting experiment a second wavelength, where no absorption occurs (mostly 500 nm), was recorded as well to exclude artefacts caused by the instrument. In an ideal experiment both passes should look identical. Since this is not the case, an average curve was calculated from all the data of one experiment, if applicable. From this curve the denaturation temperature (T_M) could be determined in three different ways, as described in section B.4.b:^[160]

1. Manually, by determining the midrule between the upper and lower baseline of the melting curve. The crossing point equals the approximate temperature where 50 % of the the DNA structure is disassembled.

2. Forming the first derivative of the melting curve and determine the point of inflection, which equals the approximate denaturation temperature.
3. Calculating the fraction folded values and determine the T_M at $y = 0.5$ (equals $\alpha = 0.5$). In this work approx. T_M value was determined with help of the *DoseResp* sigmoidal fitting, which is also used to determine the LD_{50} value.

In order to get a better and more detailed insight into the chosen HJ system, not only the denaturation temperature for the full assembled MDHO1um and MKDH01 HJs was determined, but also for every single pair of complementary oligonucleotide sequences contained in the HJ (shown in chapter A). Interestingly the single pairs, contributing to the overall stability of the full assembled HJ, having in most cases a denaturation temperature very close to T_M value of the corresponding *Holliday* junction (table III.3.0.1). But it has to be taken into account, that partially complementary parts of the "non-complementary" sequence can also contribute to the determined denaturation temperature.

Figure III.2.2.2: Fraction folded plots for the unmodified HJ MKDH01um in presence (a) and absence (b) of magnesium cations. Both experiments were performed with 2 μM of each required single strand, corresponding to 2 μM Holliday junction in solution, 10 mM of cacodylate buffer pH of 7.3, 100 mM sodium perchloride and 5 mM of magnesium perchloride (a) or 0 mM of magnesium perchloride with a total volume of 800 μl . From the measured melting curve the fraction folded values were calculated and plotted. T_M was determined from an appropriate sigmoidal DoseResp fit.

Figure III.2.2.2a shows the fraction folded plot for the unmodified *Holliday* junction (red) and the corresponding sigmoidal fit (black dashed), from which a T_M of 41.0 °C was determined. The curve has a rather flat slope in the temperature range of 30 °C to 50 °C, indicating a rather low temperature dependency on the affinity constant, as it was described in section B.4.b.^[160] In literature the presence of Mg^{2+} has been described as a crucial element in the stabilization of the HJ, since the equilibrium of the predominantly existing isomer (open or stacked) seems to be sensitive to the magnesium ion concentration. This

sensitivity is attributed to the phosphates located at the branch on the crossing over strands, which are assumed to differ in proximity, geometry or both.^[138] However, the presence of magnesium does not only affect the choice of the prevailed isomer, but also has a significant effect on the stability of the *Holliday* junction. In figure III.2.2.2b the T_M was determined for the unmodified HJ under the same conditions as before, but without any $Mg(ClO_4)_2$. The drop of T_M to 31.0 °C shows that the absence of magnesium has a significant impact on the stability of the *Holliday* junction. It is worth to mention, that no significant change could be observed for the slope during transition, compared to the experiment with $Mg(ClO_4)_2$. The slope of transition is nearly unchanged, appearing also in a temperature range of approx. 30 °C. Suggesting, that there is no additional stability with respect to the temperature-dependency of the affinity constant.

Figure III.2.2.3: UV-vis (a) and TDS (b) spectra for the unmodified *Holliday* junction, all recorded in presence 5 mM $Mg(ClO_4)_2$. In the UV-vis spectrum (a) the absorbance patterns for the assembled (4 °C, black) and the disassembled (85 °C, red) are shown. The assembled DNA structure has a lower absorbance, compared to the free oligonucleotide strand (ONS). The TDS spectrum (b) shows the change in absorbance between the assembled and disassembled HJ. For that reason the absorbance of the assembled HJ (4 °C) is subtracted from the disassembled HJ (85 °C).

Comparison of the UV-vis absorption pattern show a significant change in absorption at 260 nm between assembled (black) and disassembled (red) double stranded DNA (dsDNA) (figure III.2.2.3a), which is used in all melting experiments. This change is known and characteristic for the assembly or disassembly of DNA double helices. Due to the formation dsDNA, the extinction coefficient is lowered, since the ssDNAs are forming a highly defined structure, with lots of newly formed interactions in between the nucleobases, as e.g. hydrogen bonding, stacking or electrostatic effects. These interactions cause a change in the absorbance behavior. The predominant effect the formation of π - π - stacking, giving a lowering in absorbance up to approx. one third of the initial one. This decrease is used in general as indicator of an assembled DNA structure and makes it possible to determine the denaturation temperature via various temperature UV-vis experiments.^[161,162] In figure III.2.2.3b the difference in absorbance between the denatured

DNA structure at high temperatures and the folded one at low temperature is plotted. These representation is called thermal differential spectrum (TDS), by what the change in absorbance between those two states can be shown in detail. Every TDS has a specific shape that is unique for each type of nucleic acid structure and can be seen as structural fingerprint.^[163]

Due to the fact that oligonucleotides have a chiral character, it is suitable for circular dichroism (CD). The nucleosides themselves rather have a small response in the CD, however, in a sequence of nucleic acids these small responses are summed up, giving a detectable signal in CD measurements. By assembling into the secondary or even tertiary architectures, the spacial arrangement of each structure produces a unique chiral profile that can be identified and investigated by circular dichroism measurement, from which structural information can be derived.^[164,165] Figure III.2.2.4a represents the recorded spectra for assembled (red) and the disassembled (green) unmodified HJ. The formation of the *Holliday* junction induces a significant change in the spectrum. The band at 250 nm decreases about 30 mdeg, while the band at 270 nm is not only increased by ca. 15 mdeg, but is also slightly shifted to approx. 280 nm. These changes in CD between assembled and disassembled HJ are more clearly illustrated in the TDS of the CD measurements (figure III.2.2.4b). Furthermore, the change in profile indicates the formation of a higher ordered structure, which can not unambiguously assigned to the HJ structure, since the CD curve also resembles the profile of a B-DNA structure, what is not very surprising, since the flanks of the HJ virtually consists of B-DNA double helices.^[166] However, most CD profiles for B-DNA do not show such an intense band at 250 nm and in fact, comparison with literature-given CD spectra of HJs confirms this intense band to be quite characteristic.^[167] Unfortunately it seems not to be possible to determine the actual isomer HJ from the measured CD, since the profile mainly depends on the sequences flanking the crossing section.

Figure III.2.2.4: CD spectra for the unmodified HJ MKDH01um. HJ MKDH01um sample contains 2 μM of each ssDNA, 100 mM NaClO_4 , 5 mM $\text{Mg}(\text{ClO}_4)_2$, 10 mM NaCaco pH = 7.2. (a) shows the CD spectra of MKDH01um (red) in comparison to its disassembled (green) form. (b) The TDS illustrates the difference in signal amplitude between the spectrum at 85 °C and the one at 4 °C, giving a unique shape for the respective DNA structure.

Although, the CD experiment indicates that a formation of the desired *Holliday* junction happens, the system was investigated with help of an additional method, in order to proof whether the intended HJ is formed from the four ssDNA. The polyacrylamide gel electrophoresis (PAGE) experiment is a very common method,^[168] either to identify DNA strands or to separate them. For that reason small amounts of DNA is run through a polyacrylamide gel in a saline buffer solution, while applying an electric field, which attracts the negatively charged DNA and forces it to traverse the polyacrylamide network. The method is comparable to the size exclusion chromatography (SEC). Since the secondary or tertiary DNA structure shall be kept intact during the experiment, the gel was run under non-denaturing conditions as described in section B.5. It is worth to mention, that all salts and buffers were already added to the unpolymerized gel solution, thus, the gel already contained the same salt concentration, as the running buffer. That shall avoid upcoming salt barriers during the sample run, which can have a tremendous effect on the running behavior of the samples, resulting in sophisticating observations. Furthermore, the experiments were performed under constant temperature control, in order to avoid unwanted disassembly of the samples or cracking of the used glass apparatuses, due to thermal stress. Additionally, in order to get the best possible resolution, provisions were made to obtain as sharp bands as possible for each single sample in the final gel. First of all, the gels were prepared with a stacking gel, by what the sample shall enter the separating gel as concentrated as possible and secondly, the density of each sample was increased before injecting them to the sample pocket. The function of the stacking gel is easily explained and a common method. By topping the more dense separation gel by the less dense stacking gel, the sample can run much faster at the beginning through the polyacrylamide network, thus, the sample is concentrated at the interface of the two gels, giving much sharper band during the PAGE experiment. To support the effect additionally, the samples

were mixed with a 40 % sucrose solution, increasing the density of the sample, by what the sample solution sinks to the very bottom of the pocket, avoiding a quick diffusion of the sample into pockets and without interfering the mobility of the DNA in the electric field. Figure III.2.2.5 shows the gel for the unmodified HJ. Besides each single strand (**a-d**) and of course the *Holliday* junction (**hj**) itself, all pairwise combinations of the ssDNAs were run as well (ab,ac,ad,bc,bd,cd). All samples were run side by side in one gel, making the differences in travel distance, depending on the size of the investigated structures, easier to compare. In addition, a DNA ladder, containing defined double stranded helices ranging from 10 base pairs (bp) up to 150 bp in 10 bp steps, was also run on the same gel as a reference (**M**). The lower gel illustrates the same experiment as above and only serves for a better overview. The combinations, where an higher ordered structure would be expected, are highlighted in green, while for the red marked lines only ssDNA should be observed.

Figure III.2.2.5: PAGE gel for the unmodified *Holliday* junction MKDH01um. The samples (125 ng per strand) were run in a 8% polyacrylamide gel with TBM running buffer, containing 2 mM $\text{Mg}(\text{ClO}_4)_2$ at 200V for about 3 h. Besides the samples, a DNA ladder (**M**) containing double helix DNA 10-150 glsbp in 10 bp steps, was run as well as a reference for travel distance and size orientation. The higher the band the bigger the size of the respective DNA structure. The single band in the **hj** indicates the existence of the supposed HJ.

The band for the supposed HJ has an obviously smaller travel distance compared to all other samples, indicating a clear difference in size. Although it is not one-to-one comparable with the here used DNA ladder, from the height of the observed band it can be deduced that the present structure consists of 30 to 40 base pairs, what fits to the HJ with its 32 bp. Taking into account that the more bulkier structure of the HJ, compared to double stranded helix, cause a concomitant shape-originated lower mobility within the polyacrylamide structure, the travel distance of the HJ is in full accordance to the

expected traverse and also fits to literature-given examples.^[169] Surprisingly, only for the combination **ab** a band for a higher ordered structure was observable, indicating a lack of robustness under the given conditions. With regard to the determined denaturation temperature (T_M), shown in chapter A, actually more band for the pairwise combinations were expected. Nevertheless, since the unmodified *Holliday* junction sequences seem to be capable of forming the intended DNA structure, in a next step the ligand-containing modified sequences were investigated in the same way.

III.2.2.3.2 Holliday Junction MKDH01

To our surprise, the denaturation temperature, received from the melting curve of the modified HJ, is 13 °C higher (figure III.2.2.2a) with respect to the unmodified one (figure III.2.2.2a) under the same conditions. In absence of $Mg(ClO_4)_2$ T_M is decreased to 47.5 °C, giving a difference of 6.5 °C or 14.5 °C with respect to the unmodified HJ under the same conditions. As already observed before, the range of transition is approx. 30 celsius, thus the presence of magnesium cations do not effect the range of transition, merely its positioning is affected.

Figure III.2.2.6: Fraction folded plots for the modified HJ MKDH01 in presence (a) and absence (b) of magnesium cations. Both experiments were performed with 2 μ M of each required single strand, corresponding to 2 μ M Holliday junction in solution, 10 mM of cacodylate buffer pH of 7.3, 100 mM sodium perchloride and 5 mM of magnesium perchloride (a) or 0 mM of magnesium perchloride (b) with a total volume of 800 μ l. From the measured melting curve the fraction folded values were calculated and plotted. T_M was determined from an appropriate sigmoidal DoseResp fit.

Having a look at the UV-vis spectra of the modified HJ, at high (black) and low temperatures, reveals the expected decrease in absorbance for the region between 220 nm and 300 nm, caused by the assembling process of the ssDNA. Next to this region, the characteristic absorption bands of the acridone ligand at 325 nm and 360 nm can be

found.^[20] In this context, it is noteworthy to mention, that those experience a bathochromic shift upon the assembly of HJ, suggesting an interaction of the ligands, either with the surrounding nucleic acids or among themselves. These interactions will be discussed in more detail later during this chapter. In the thermal differential spectrum (figure III.2.2.7b) the lucid red-shifting about 10-15 nm of the acridone absorption is more clear. In the region above 300 nm, the positive bands belong to the ligand of the disassembled DNA structure, while the subzero band represent the shifted acridone bands in the assembled HJ.

Figure III.2.2.7: UV-vis (a) and TDS (b) spectra for the modified *Holliday* junction MKDH01, all recorded in presence of 5 mM $\text{Mg}(\text{ClO}_4)_2$. In the UV-vis spectrum (a) the absorbance patterns for the assembled (4 °C, black) and the disassembled (85 °C, red) are shown. The assembled DNA structure has a lower absorbance, compared to the free oligonucleotide strand (ONS). Furthermore the ligand region above 300 nm experience a bathochromic shift upon assembly of the HJ. (b) A detailed illustration of the differences in absorption is displayed in the thermal differential spectrum (TDS).

Since the polyacrylamide gel electrophoresis (PAGE) gave so far the best visual evidences for an existing fully assembled *Holliday* junction, polyacrylamide gels were prepared for the ligand-containing modified ssDNA, as described before. In figure III.2.2.8 the PAGE is illustrated, again, the lines with an expected higher order structure are highlighted in green, while the others, where solely ssDNA should be observed, are marked in red. In this gel four separately prepared HJ samples we inserted into adjacent line, all of them showing sharp and intense short traveled bands, suggesting a successful and reproducible assembly of the desired HJ architecture. The HJ bands are located this time between 40 bp and 50 bp, thus even a little higher as the unmodified HJ (figure III.2.2.5). But taking in consideration that there are big aromatic ligands incorporated at the central position, this result is not very surprising. Although it is apparent that more bands for the dimeric combination (**ab**, **ad**, **bc** and **cd**) are visible, besides, also the pale shadow band do attract attention, especially those which were not expected to exist. As example, in the line **bd** no higher ordered dimeric structure would be expected, however, there is one. In line **ad** a very light but still visible band appears on the same height as the HJs. The

fact that all bands show a slight fluorescence when illuminated at 312 nm, portends that all bands contain the ligand-containing ssDNAs. This finding supports the theory, that the ligand either intercalates into the formed double helices or stack on each other, since these bands are not observable for the unmodified HJ (figure III.2.2.5). Yet, one would probably expect a more intense band for the intercalation of the ligands to the DNA, since acridone derivatives are known to intercalate quite strongly with a high affinity.^[170] Therefore the shaded bands rather speak for the less stable but still powerful ligand stacking.

Figure III.2.2.8: PAGE gel for the modified *Holliday* junction MKDH01. The samples (125 ng per strand) were run in a 8% polyacrylamide gel with TBM running buffer, containing 2 mM $\text{Mg}(\text{ClO}_4)_2$ at 200V for about 3 h. Besides the samples, a DNA ladder (M) containing double helix DNA 10-150 glsbp in 10 bp steps, was run as well as a reference for travel distance and size orientation. The higher the band the bigger the size of the respective DNA structure. The single band in the **hj** indicates the existence of the supposed HJ.

Since the ligands are embedded into a chiral environment, it was of interest to examine if the ssDNAs transfer its chiral character on the ligands and if this chirality can also be found in the assembled HJ structure, respectively. For that purpose, circular dichroism (CD) spectroscopic measurements were performed for the modified HJ system at high and low temperatures, as described in section B.4.c. In consense with the unmodified HJ, the disassembled structure (green) shows a postive band at 275 nm and a slightly weaker negative band at 250 nm, according to the postive *Cotton effect* already seen for the unmodified *Holliday* junction. In the region of ligand above 300 nm, there could be a small

broad band, but that cannot be confirmed with certainty, since the spectra seems to be a little offset, although the spectrum was background corrected. As for the unmodified HJ, the assembly of the modified HJ led to an increased band at 280 nm and a strongly decreased band at 250 nm, supporting the evidences for a fully assembled HJ. Interestingly, in the ligand region above 300 nm, two new bands appeared at approx. 330 nm and 375 nm, in total accordance to the bands appeared in the UV-vis spectra (figure III.2.2.7b). These bands can be seen more clearly in the thermal differential spectrum (TDS), figure III.2.2.9b and have the same sequence, negative band $\rightarrow$ positive band, as the pattern caused by the *Holliday* junction, indicating a chirality transfer from the DNA scaffold to the incorporated ligands within the assembled structure.

Figure III.2.2.9: CD spectra for the modified HJ MKDH01. HJ MKDH01 sample contains 2 μ M of each ssDNA, 100 mM NaClO_4 , 5 mM $\text{Mg}(\text{ClO}_4)_2$, 10 mM NaCaco pH = 7.2. (a) shows the CD spectra of MKDH01 (red) in comparison to its disassembled (green) form. (b) The TDS illustrates the difference in signal amplitude between the spectrum at 85 °C and the one at 4 °C, giving a unique shape for the respective DNA structure.

Furthermore, as mentioned before, due to the incorporated ligands, the ssDNAs are fluorescent in comparison to the unmodified strands. Therefore the differences in fluorescence for the assembled and disassembled HJ were also investigated by various temperature fluorescence spectroscopy (figure III.2.2.10). The disassembled structure at 85 °C (red) has maximum emission at 445 nm and smaller side band at 525 nm. In comparison, the maximum emission is shifted to 470 nm for the assembled HJ, while the side band is not only red-shifted about 10 nm to 535 nm, but also the intensity is more than doubled. The bathochromic shift observable in fluorescence spectroscopy between disassembled and assembled HJ, is probably caused by aggregation of the ligands, what is in good accordance to the results of the previous experiments. What kind of aggregation takes place is really hard to say from the here presented results, but will be discussed in detail at the end of this chapter.

Figure III.2.2.10: Fluorescence emission spectra for the modified HJ MKDH01 at 85 °C (red) and 4 °C (black). The HJ system shows an emission at 450 nm, when irradiated at 360 nm. Upon assembly of the HJ the emission band experience a bathochromic shift from 445 nm to 470 nm. Furthermore, the small band at 525 nm is shift about 10 nm and the intensity of emission is tripled.

The logical next step was the attempt of forming the intended embedded coordination cage. For that purpose the addition of the transition metal salts $\text{Pd}(\text{NO}_3)_2$, $\text{Cu}(\text{ClO}_4)_2$, $\text{Ni}(\text{ClO}_4)_2$ to the HJ and their influence on the melting behavior was investigated. In figure III.2.2.11a the fraction folded representation for the melting experiment of MKDH01 in absence and presence palladium(II) cations is shown. The green curve was recorded after the addition of 2 equivalence $\text{Pd}(\text{NO}_3)_2$, since two palladium(II) cations are needed for the formation of the coordination cage. It is obvious that there is no significant difference in the shape of the melting curve and also the denaturation temperature remained unchanged, what does not necessarily speak for an unsuccessful formation of a coordination cage within the HJ, but it is very unlikely that T_M does not change after the formation of a metal coordination complex, especially when compared to other DNA system where metal-coordination occurs.^[84,118,120] Likewise, there was also no significant difference in the UV-vis spectra between both experiments. However, the fact that the sample still showed fluorescence, what can be seen by the naked eye when irradiated at 365 nm with a UV lamp, rather speaks for no formation of a coordination cage. Particularly, because it is known that the fluorescence of acridone is quenched upon coordination to palladium(II) cations. Encouraged by the systems of Engelhard^[120] and Yoshizawa,^[171,172] the melting behavior in presence of nickel(II) or copper(II) cations was investigated as well (figure III.2.2.11b). In order to avoid a miscarriage of the experiment due a lack of metal cations, by i.e. unspecific interactions with the DNA scaffold, the experiments were performed with an excess in metal cations (10 equiv.), in particular since it is very unlikely that a slight excess of metal cations would prevent the formation of the embedded cage, as one might expect it for a supramolecular cage, consisting of isolated ligands. Under the same conditions the experiment was also performed with 10 equiv. of $\text{Pd}(\text{NO}_3)_2$, but unfortunately no usable data were obtained. It is assumed that this happened due to a technical issue, but cannot be said with certainty. However, since all measured samples

used in this experiment were derived from the same batch, it is assumed that it was not a problem of the sample composition, especially because all other samples showed an intact HJ system. However, there was no time to repeat, therefore the melting curve for palladium is missing. For the HJ, in absence of any transition metal, a denaturation temperature (T_M) of 56 °C was determined. In contrast, the same sample showed in presence of 10 equiv. $\text{Cu}(\text{ClO}_4)_2$ a lower T_M by about 2 °C, whereas the sample with 10 equiv. $\text{Ni}(\text{ClO}_4)_2$ has a 1.5 °C higher T_M . Although a change in melting temperature can be observed, the results rather speak for unspecific interactions of the metal cations with the DNA scaffold. The contrary effects on the denaturation temperature are maybe associated with the different preferred interaction of the here used metal cations with nucleic acids. While nickel preferably interact with the negatively charged phosphate backbone, leading to a stabilization of the double stranded helices, copper(II) exhibit a high affinity to the nucleobases, preventing an interaction with opposite nucleic acids, destabilizing the double helix.^[173] Since the metals are deployed in extremely low amounts, accordingly, the resulting effects on the HJ is weak as well. Moreover, as in the previous experiment the fluorescence is still observed after the experiment. If this can be used as an evidence for a not-existing coordination of ligand, cannot be said, since no suitable system could be found as a reference.

Figure III.2.2.11: UV-vis melting experiments for MKDH05 in presence of (a) $\text{Pd}(\text{NO}_3)_2$ and (b) $\text{Ni}(\text{ClO}_4)_2$ or $\text{Cu}(\text{ClO}_4)_2$. The samples contain 2 μM of each ssDNA, 100 mM NaClO_4 , 5 mM $\text{Mg}(\text{ClO}_4)_2$, 10 mM NaCaco pH = 7.3. **a)** MKDH01 in presence of 4 μM $\text{Pd}(\text{NO}_3)_2$. **b)** MKDH01 in presence of excess $\text{Ni}(\text{ClO}_4)_2$ or $\text{Cu}(\text{ClO}_4)_2$. From the same experiment with $\text{Pd}(\text{NO}_3)_2$ no usable data were received.

In the here presented chapter, it was proven, that an organic banana-shaped bis-monodentate ligand, suitable for the formation of Pd_2L_4 and Pd_4L_8 coordination cages, could be incorporated into ssDNA. These modified oligonucleotides were assembled into the intended *Holliday* junction (HJ), which was verified by UV-vis, CD and PAGE experiments, as well as by comparison to an appropriate reference system. However, the last step, the formation of a coordination cage within the HJ architecture could not be proven, neither

in presence of palladium(II), nor copper(II) or nickel(II) cations. The interaction between metal cations and ligands is probably prevented due to strong stacking between the highly aromatic ligands. The strength of the stacking effect is reflected by an stabilization of the HJ by 13 °C compared to the unmodified system. This stabilization could be caused either by aggregation of the ligands or by intercalation of the ligands into the close by double helices. Latter is mostly accompanied with quenching of fluorescence.^[174] Since this is not the case, a stacking between the ligands is more likely. Furthermore, a bathochromic shifting in the ligands' absorption and emission profile could be observed, indicating the formation of excimers or j-aggregates, what is confirmed by literature.^[175,176] Moreover, it is worth to mention that for the assembled *Holliday* junction, circular dichroism signals are appearing for the ligands' region of absorption. Since the signals show the same positive *Cotton effect* as the HJ itself, it is most likely that the DNA structure is transferring its chiral character on the ligands, resulting in chiral J-aggregates embedded in a HJ scaffold.

III.2.3 Anisol-based ligand embedded in *Holliday* junction

Based on the results presented in the previous chapter, the ligand system was completely redesigned in order to eliminate the problems arising from the the first generation ligand. The main focus in the ligand design was set to the reduction of the aromatic backbone to avoid the effect of stacking interactions between the ligand or the nucleobases. Furthermore the site for the linker attachment to the oligonucleotides was shifted from the pyridine arms to the ligand backbone in order to provide a higher degree of freedom for the ligand within the secondary DNA scaffold and preventing a disfavored arrangement of the ligand within the Holliday Junction that hinders the formation of the supramolecular cage. As before, a MMFF model of the desired embedded cage system was prepared, estimating the amount of oligonucleotide mismatches and the PEG linker length, needed to provide enough space for the supramolecular cage in the center of the DNA scaffold.

Figure III.2.3.12: MMFF model of 2nd generation DNA-cage hybrid structure. Modelled in Sparten '10.^[156] The ligands are coordinated to palladium. The ONS sequences contain and extra T-T mismatch next to the integrated ligand.

III.2.3.1 Design and syntheses anisol-based ligand

As mentioned above, the backbone and ONS coupling positions were redesigned for the new embedded ligand system. The aromatic tricyclic acridone backbone, was replaced by an simple aromatic benzene motif, which still provides the flat and rigid character of the acridone, but provides a clearly reduced aromatic plane for stacking effects. Furthermore, this motif was already used several times before in literature for the design of endo- or exohedral modified metal-mediated coordination cages.^[56,74,177] The pyridine arms must

Scheme III.2.3.2: Synthetic path for ligand **17**. **a)** KIO₃, KI, KCl, AcOH, H₂O; **b)** MeI, (CH₃)₂CO; **c)** NaBH₄, MeOH; **d)** SOCl₂, CH₂Cl₂; **e)** NH(CH₂CH₂OH)₂, (*i*-Pr)₂NEt, CH₃Cl; **f)** 3-Ethynyl-pyridine, Pd(PPh₃)₂Cl₂, CuI, NEt₃, DMF; **g)** DMT-Cl, 4-DMAP, NEt₃, DCM; **h)** β-PA-Cl, (*i*Pr)₂EtN, DCM.

be attached in 2- and 6-position to generate the typical banana-shape for the ligands. To achieve this objective, the phenol motif was used as starting compound, since its hydroxy group acts as a directing group during the synthesis. As the ligand shall be integrated into the ONS in way that it has more freedom in movement, the linkage had to be moved to a central position of the backbone, therefore it must be inevitably positioned in para position to the phenol hydroxy group. Furthermore the linkage motif has to be branched, ending in two terminal hydroxy groups, where the nucleotides can be attached during solid phase synthesis. Diethanolamine as an commercially available compound fulfilled all the requirement and seemed to be suitable for this purpose. However, first attempts to attach the diethanolamine directly to the aromatic backbone failed, what is why the phenolic backbone was replaced by 4-hydroxybenzaldehyde. The newly designed ligand **17** could be synthesized in an eight step synthesis, followed by two additional steps to introduce the DMT and CEP groups (scheme III.2.3.2).

Outgoing from the simple 4-hydroxybenzaldehyde, the compound was regioselective iodinated at the 3- and 5-position (2- and 6- position, considering the hydroxy group on position 1), by generating elementary iodine *in situ*. In a next step the hydroxy group was protected by etherification with MeOH. Subsequently the benzylic position was prepared for the introduction of the diethanolamine. First the benzyl aldehyde was reduced to the benzyl alcohol in presence of NaBH₄. In a second step, the newly formed benzyl alcohol was

immediately converted to benzyl chloride in the presence of thionylchloride. Subsequently the chloride was substituted with diethanolamine, followed by the introduction of the pyridine arms in palladium catalyzed Sonogashira cross-coupling to obtain the ligand **17**, whose chemistry is discussed in section IV.2.1. As in acridone-based ligand case (scheme III.2.2.1), again two equivalent hydroxy groups exist, by what the DMT group has to be introduced in a desymmetrization reaction, with a moderate yield. The double substituted side-product can be simply treated with acid to recover the starting material. In the last step the 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (CEP) was attached to the second unprotected free hydroxy group under inert conditions and used for the DNA solid phase synthesis on the same day, due to the same reasons mentioned before at the end of section III.2.2.1.

III.2.3.2 Synthesis of Ligand Embedded Oligonucleotides

For the next approach the sequence of the HJ system was kept, also the position of the ligand stayed the same. Synthesis of the oligonucleotide strand (ONS) via solid phase synthesis (SPS) worked straight forward, although it was not known whether the new link would couple at all during SPS. Due to the fact that the polyethylene glycol linkers are only half the size of those used before, the ligands were incorporated in a second sequence containing additional thymines on each side of the ligand, which should form T-T mismatches at the HJ crossing section, generating a little more space for the ligands at the central gap, as it is shown in the MMFF model (figure III.2.3.12). Also strands were synthesized with two T-T mismatches next to the ligands, but no usable melting curves could be obtained from this system, therefore they are neglected in this work.

Table III.2.3.2: ONS sequence table for MKDH05 and MKDH06. The sequences of MKDH05 are equal to those used for the first approach with the incorporated ligand **54** shown in the previous chapter (section III.2.2.1), but contains the more flexible second generation ligand **17**. In the sequences of MKDH06 extra T-T mismatches on both sides of the incorporated ligand are given, in order give more space for the assembly of the intended supramolecular cage structure at the central position of the Holliday junction.

	MKDH05 Sequences 5'→3'	MKDH06 Sequences 5'→3'
A	GCC ATA GTL TGA GCA CG	GCC ATA GTTL TTGA GCA CG
B	CGT GCT CAL GGA TTG CG	CGT GCT CATL TGGA TTG CG
C	CGC AAT CCL CCG AAT GC	CGC AAT CCTL TCCG AAT GC
D	GCA TTC GGL ACT ATG GC	GCA TTC GGTL TACT ATG GC

Moreover into the same sequences shown in table III.2.3.2 a simple imidazol ligand was incorporated instead of the bis-dentate ligands, in order to simplify the coordination

environment and check whether a coordination within the *Holliday* junction is possible anyway. Those sequences were completely characterized (chapter A, MKDH03 and MKDH04) and the the analytic give evidence for a successful formation of a HJ. It is worth to mention, that the sequence without additional T-T mismatches, shows a slightly smaller T_M (39 °C) compared to the unmodified *Holliday* junction (41 °C), indicating destabilization of the DNA structure, due to the artificial ligand. This effect becomes more apparent as additional T-T mismatches next to imidazol ligand were added. In that system the T_M was even decreased to (34 °C), giving a clear evidence for disturbance of HJ structure caused by the T-T mismatches, by what a destabilization occurs and actually was also expected for the other used ligands. However, since the interaction with transition metal cations was not investigated yet, the imidazol containing HJ systems are not further discussed in this thesis.

III.2.3.3 Assembly and characterization of Holliday junction with embedded anisol-based ligand

III.2.3.3.1 Holliday junction MKDH05

The preparation of the sample for MKDH05 was performed under the same condition as for the the previously discussed system MKDH01. In section III.2.3.3.1 the fraction folded plot, generated from the UV-vis melting experiments, is illustrated, from which a denaturation temperature (T_M) of 44 °C could be obtained. This T_M is only slightly higher compared to the unmodified HJ (41 °C) and significantly smaller compared to MKDH01 (54 °C), speaking for weaker interaction between the ligands or with the surrounding oligonucleotidic scaffold, as it was intended. Since it is known from literature,^[104,135] that under the used condition, high salt concentration and especially in presence of magnesium, the X-stacked HJ isomers preferably forms, the melting experiments were also performed in presence of small salt concentration (< 10 mM) and in absence of magnesium, in order to generate conditions where the formation of the open HJ isomer is prevailed. But no usable data was obtained from those melting experiments, giving rise to the assumption that the HJ is not formed at all under the given conditions, forcing us to stay with the high salt concentrations. But since the determined T_M speaks for a rather weak interaction of the ligands, further experiments were continued with the high salt concentrations.

Figure III.2.3.13: Fraction folded plots for the modified HJ MKDH05, performed with 2 μM of each required single strand, in order to get a 2 μM solution of the desired Holliday junction, 10 mM of cacodylate buffer adjusted at a pH of 7.3, 100 mM sodium perchloride and 5 mM of magnesium perchloride with a total volume of 800 μl . From the measured melting curve the fraction folded values were calculated and plotted. T_M was determined from an appropriate sigmoidal DoseResp fit.

The first hints for the aggregation of ligands within the HJ structure in the first generation system (MKDH01) were received from the UV-vis spectroscopy and its TDS. For the assembled structure as significant bathochromic shift in the ligand region was observed, known from the formation of J-aggregates. For the here discussed ligand that approach is a little more difficult, due to fact that the ligands' main absorption band (figure IV.2.1.2a) is overlapping with the absorption region of the oligonucleotides. Nonetheless, by comparing the UV-vis absorption of the unmodified HJ, it becomes apparent that the small band above 300 nm must belong to the ligand. On the first glance it looks like the ligand is experiencing a tiny bathochromic shift, as it can also be seen in the TDS (figure III.2.3.14b) at approx. 310 nm. What also can be seen, there is an jump at 350 nm, infrequently caused the UV-vis machine, as it changed the light sources and due to the sporadic occurring this jump was not obliterated by background corrections. Therefore, the observed bathochromic shift cannot be proofed with certainty, but since the shift is minimal compared to that one observed for the first generation ligand, it can be assumed that only weak interactions occur, if at all.

Figure III.2.3.14: UV-vis (a) and TDS (b) spectra for the modified *Holliday* junction MKDH05, all recorded in presence of 5 mM $\text{Mg}(\text{ClO}_4)_2$. In the UV-vis spectrum (a) the absorbance patterns for the assembled (4 °C, black) and the disassembled (85 °C, red) are shown. The assembled DNA structure has a lower absorbance, compared to the free oligonucleotide strand (ONS), indicating a successful assembly of the intended HJ.

CD measurements do support the previous findings. As for the previous investigated HJs, the same positive *Cotton effect* with the bands at 250 nm and 275 nm could be observed and they do exactly behave the same way upon the assembly process. Means, both bands become more intense and show nearly the same value for the negative as well as for positive amplitude. Likewise, the thermal differential spectrum (TDS) does not show any divergent results, not to speak of any evidence for a chirality transfer to the ligand, as it was seen for the MKDH01 system. Considering that only the linker moiety is incorporated into the DNA scaffold, but not the ligand itself, it would have been surprising if chirality had been transferred on the ligand. Especially, since link and ligand are connected over a flexible bridge, where rotation can occur, so that the ligand can adjust its positioning within the assembled structure and is not forced to follow the arrangement of the DNA scaffold. Therefore, it appears to be logic, that no signal was observed for the ligand region in the CD spectrum.

Figure III.2.3.15: CD spectra for the unmodified HJ MKDH05. HJ MKDH05 sample contains 2 μM of each ssDNA, 100 mM NaClO_4 , 5 mM $\text{Mg}(\text{ClO}_4)_2$, 10 mM NaCaco pH = 7.2. (a) shows the CD spectra of MKDH05 (red) in comparison to its disassembled (green) form. (b) The TDS illustrates the difference in signal amplitude between the spectrum at 85 °C and the one at 4 °C, giving a unique shape for the respective DNA structure.

As it can be seen in figure IV.2.1.2b the ligand is fluorescent and shows emission at 375 nm, when irradiated with 280 nm light. Even though it is not as intense as the acridone-based ligand, it can still be detected in the assembled HJ. Nevertheless, another mechanism must take place for the here presented system compared to the aggregation in the acridone-based system, since the intensity of fluorescence is almost doubled, while for MKDH01 (figure III.2.2.10) the emission is red-shifted and even a new band arose. It is really hard to say by what effect this increase in emission is initiated. Stacking effects can have multiple influences on the fluorescence of molecules, depending on the way of how the molecules are stacked.^[176,178] Hence, the current data is not sufficient to make a qualified statement about the reason. However, the fact that there is detectable fluorescence in the assembled structure, giving a good starting point for later investigation with respect to the formation of the intended coordination cage, as the fluorescence is totally quenched upon coordination to palladium(II) salts, as it is described in section IV.2.1.

Figure III.2.3.16: Fluorescence emission spectra for the modified HJ MKDH05 at 85 °C (red) and 4 °C (black). The HJ system shows an emission at 375 nm, when irradiated at 280 nm. Upon assembly of the HJ the amplitude of emission is increased about 50% of the starting intensity.

Although the formation of the fully assembled HJ is proved with a reasonable certainty from the results of the previous experiments, yet, the system was still examined in PAGE experiments. And in fact, again it was possible to observe a band, representing the supposed HJ (figure III.2.3.17, line **hj**) Not only the T_M is very similar to the unmodified Holliday junction, but also the appearance of the polyacrylamide gel. There are no visible bands for higher ordered structures, which would be expected for the pairwise combination of the strands **ab**, **ad**, **bc** and **cd**. But obviously the structures stability was not sufficient to survive in the electric field under the given conditions. This finding is showing again, that the thermal stability does not represent the allover stability of a system and can be totally different, depending on the forces acting on it. Further, it supports the assumption of a reduced or weaker stacking between the ligands in the actual system, otherwise one would expect the appearance of weak bands in the gel, similarly as it was observed for acridone-containing HJ (figure III.2.2.8).

Figure III.2.3.17: PAGE gel for the modified *Holliday* junction MKDH05. The samples (125 ng per strand) were run in a 8% polyacrylamide gel with TBM running buffer, containing 2 mM $\text{Mg}(\text{ClO}_4)_2$ at 200V for about 3 h. Besides the samples, a DNA ladder (M) containing double helix DNA 10-150 glsbp in 10 bp steps, was run as well as a reference for travel distance and size orientation. The higher the band the bigger the size of the respective DNA structure. The single band in the **hj** indicates the existence of the supposed HJ.

III.2.3.3.2 Holliday junction MKDH06

The obtained results for the HJ system MKDH06, which only differs from MKDH05 in two additional thymine, enclosing the incorporated ligands, show a pretty similar melting behavior. The T_M is 42 °C, a decrease of 2 °C compared to the previously presented HJ, what is consistent with the denaturation temperature of the unmodified *Holliday* junction MKDH01um. The additional thymines are forming T-T mismatches, leading on the one hand to a higher disorder in the central position of the HJ, accompanied with a decrease in stability. On the other hand the gap at the cross section is increased, giving the ligands more space to move and the formation of the intended coordination cage. Very similar to MKDH05, melting experiments were also performed at low salt concentrations (< 10mM) in order to shift the equilibrium to the open HJ isomer. However, the experiments resulted as before in unusable melting curve, giving no evidence for a formation of a higher ordered structure. Therefore, all subsequent analytics were also performed under the common conditions described in this thesis.

Figure III.2.3.18: Fraction folded plots for the modified HJ MKDH06, performed with 2 μM of each required single strand, in order to get a 2 μM solution of the desired Holliday junction, 10 mM of cacodylate buffer adjusted at a pH of 7.3, 100 mM sodium perchloride and 5 mM of magnesium perchloride with a total volume of 800 μl . From the measured melting curve the fraction folded values were calculated and plotted. T_M was determined from an appropriate sigmoidal DoseResp fit.

Analog to the UV-vis spectrum recorded for MKDH05, the spectrum of MKDH06 shows a small band above 300 nm which can be assigned to the ligand. Likewise, the TDS is indicating a slight bathochromic shift of the ligand band, suggesting weak interactions of the ligand with the surrounding DNA scaffold or with vicinal ligands. The jump at 350 nm is caused by the UV-vis instrument during the change of the light source, as explained before. Since this artefact was happening infrequently, it could not be compensated by background corrections.

Figure III.2.3.19: UV-vis (a) and TDS (b) spectra for the modified *Holliday* junction MKDH06, all recorded in presence of 5 mM $\text{Mg}(\text{ClO}_4)_2$. In the UV-vis spectrum (a) the absorbance patterns for the assembled (4 °C, black) and the disassembled (85 °C, red) are shown. The assembled DNA structure has a lower absorbance, compared to the free oligonucleotide strand (ONS), indicating a successful assembly of the intended HJ.

The CD spectrum shows the typical bands at 250 nm and 275 nm with a positive *Cotton effect*, as it was observed before. Likewise, the bands are increased upon assembly of the

HJ, so that the negative band at 250 nm and the positive band at 275 nm exhibit nearly the same height in amplitude, indicating a successful assembly of the supposed HJ. In contrast to the shorter MKDH05 system, a significant negative CD band could be observed, which can be better seen in the TDS. Interestingly, at 310 nm only one negative peak can be observed, therefore it is assumed that a second positive peak lies under the 275 nm peak, which result in a negative *Cotton effect*. If this is the case, the chirality is probably not transferred from the DNA scaffold to the ligand, since for that case the same orientation of the CD signals would be expected.^[6] However, although both do not share the same orientation, the formation of the HJ must have somehow induced an inverse chirality at the ligands.

Figure III.2.3.20: CD spectra for the unmodified HJ MKDH06. HJ MKDH06 sample contains 2 μM of each ssDNA, 100 mM NaClO_4 , 5 mM $\text{Mg}(\text{ClO}_4)_2$, 10 mM NaCaco pH = 7.3. (a) shows the CD spectra of MKDH06 (red) in comparison to its disassembled (green) form. (b) The TDS illustrates the difference in signal amplitude between the spectrum at 85 °C and the one at 4 °C, giving a unique shape for the respective DNA structure.

The fluorescence spectrum for the HJ system shows as before an emission at 375 nm, when it is irradiated with 280 nm light. The fluorescence is caused by the incorporated ligand as it was shown in figure IV.2.1.2b. Upon assembly of the HJ the fluorescence is significantly increased, even more than in the previous case for MKDH05. Assuming, this increase in fluorescence is caused by ligand involved stacking effects, the higher intensity of emission in MKDH06 correlates with the increased space at the HJ crossing section. This extra space seems to give the ligand the possibility of a better overlap with the vicinal ligands, causing an enhanced fluorescence not by means of higher or lower energy gaps, but a higher probability to get electrons into an excited state, thus, an increased yield.

Figure III.2.3.21: Fluorescence emission spectra for the modified HJ MKDH06 at 85 °C (red) and 4 °C (black). The HJ system shows an emission at 375 nm, when irradiated at 280 nm. Upon assembly of the HJ the amplitude of emission is three times higher, compared to the disassembled case.

The PAGE experiment for MKDH06 (figure III.2.3.22) does not show any surprising results and lines up with the prior shown polyacrylamide gels. In line **hj**, containing all four ssDNAs, a band for the supposed HJ is found, giving an additional evidence for an successful assembly of the intended *Holliday* junction. As mentioned before, it was expected to find also higher ordered structures for the lines **ab**, **ad**, **bc** and **cd**, since those contained two semi-complementary ssDNAs, capable to form a double stranded structure. However, under the given conditions, those seem not to be stable, thus, they could not be observed in the actual gel.

Figure III.2.3.22: PAGE gel for the unmodified *Holliday* junction MKDH06. The samples (125 ng per strand) were run in a 8% polyacrylamide gel with TBM running buffer, containing 2 mM $\text{Mg}(\text{ClO}_4)_2$ at 200V for about 3 h. Besides the samples, a DNA ladder (**M**) containing double helix DNA 10-150 gls in 10 bp steps, was run as well as a reference for travel distance and size orientation. The higher the band the bigger the size of the respective DNA structure. The single band in the **hj** indicates the existence of the supposed HJ.

III.2.3.4 Melting behavior in presence of transition metals

As it was already shown for the MKDH01 system, the melting behavior was also investigated in presence of the transition metals palladium(II), copper(II) and nickel(II), in order to find any evidence for the formation of an coordination structure. For that reason, three succeeding experiments were accomplished for the HJ systems MKDH05 and MKDH06. After each run a certain amount of the respective metal salt was added. The first run was performed in absence of any metal, followed by the second run, where two equivalents of the respective metal salt were added, the minimum amount to form the intended M_2L_4 structure. In the last run an excess of the metal salt was added, ending up with a total concentration of 20 μ M (5 equiv.) of the corresponding metal. Compared to MKDH01 with copper (figure III.2.3.23a) or nickel (figure III.2.3.23b), MKDH05 did not show any significant differences in T_M even in presence of 10 equiv. of the respective metal salt. One would have at least expected a similar behavior as seen before for MKDH01 (figure III.2.2.11), even though the metals hardly changed the denaturation temperature. In contrast, the addition of $Pd(NO_3)_2$ to the HJ gave a change in the melting profile and an increase in T_M about 3 °C compared to the starting situation. Nevertheless, it is believed that this increase in thermal stabilization is rather caused by an unspecific interaction of the palladium cations with the DNA scaffold, since after the experiment there was still fluorescence visible when irradiated with light of the wavelength 254 nm, what does not match with the quenched fluorescence observed for the ligand. In figure IV.2.1.2b, it was shown that the fluorescence is totally quenched due to the assembly of the coordination cages with palladium(II).

Figure III.2.3.23: UV-vis melting experiments for MKDH05 in presence of (a) Cu(ClO₄)₂, (b) Ni(ClO₄)₂ and (c) Pd(NO₃)₂. The samples contain 2 μ M of each ssDNA, 100 mM NaClO₄, 5 mM Mg(ClO₄)₂, 10 mM NaCaco pH = 7.3. For each metal three succeeding melting curves were recorded. 1. in absence of metal, 2. in presence of 4 μ M metal salt, 3. with an excess (20 μ M of the respective metal cation).

For MKDH06 the melting profiles look pretty divergent, although all samples were taken from the same batch. The sample with copper is only shown for the sake of completeness, however, since the melting profile looks already strange and the sample with 10 equiv. did not show any usable data, it is omitted in this discussion. In figure III.2.3.24b the addition of nickel gives a minimal stabilization about 2 °C, what correlates to the observation made for MKDH01 in presence of nickel(II) cations. Although the the starting T_M for the last experiment (figure III.2.3.24c) is too low for some reasons, it looked so far most promising, since in the presence of an excess palladium(II) salt, the melting profile seems to contain two denaturation processes. By fitting the curve with a *BiDoseResp* function two temperatures could be determined. The denaturation temperature for the first process is $T_M = 43.5^\circ\text{C}$ and $T_M = 64.0^\circ\text{C}$ for the second melting process. Unfortunately, comparison of the UV-vis spectra did not give any hint on second species or the formation of a cage. But having figure IV.2.1.2a in mind, one would not expect any big changes in the UV-vis

spectrum. Checking the sample with 254 nm light by hand, revealed again fluorescence which could be seen with the naked eye.

Figure III.2.3.24: UV-vis melting experiments for MKDH06 in presence of (a) Cu(ClO₄)₂, (b) Ni(ClO₄)₂ and (c) Pd(NO₃)₂. The samples contain 2 μ M of each ssDNA, 100 mM NaClO₄, 5 mM Mg(ClO₄)₂, 10 mM NaCaco pH = 7.3. For each metal three succeeding melting curves were recorded. 1. in absence of metal, 2. in presence of 4 μ M metal salt, 3. with an excess (20 μ M of the respective metal cation).

Anyway, in this case the fluorescence does not exclude the existence of coordination cage. On one hand, based on the melting profile two species seem to exist, therefore fluorescence can still be detected, if one of those is still fluorescence active. On the other hand it is not possible to say from a single measurement if this second species really exist or if it is just an artefact, therefore, the experiment has to be repeated in order to give a convincing answer to this question. Up to that point, it is a "*Schrödinger*" metal-mediated coordination cage embedded in a oligonucleotide scaffold.

Chapter III.3

Conclusion

In this chapter a new approach was investigated to form heteroleptic metal-mediated coordination cages in a controlled non-statistical fashion, by preorganizing the ligands in an exterior oligonucleotide scaffold. For that reason, four banana-shaped bis-monodentate ligands were incorporated into four different semi-complementary oligonucleotide sequences, capable to form a *Holliday* junction (HJ). In this cross-shaped secondary DNA structure, the ligands are gathered in the central position in order to form a cage in presence of suitable transition metal cations. The first acridone-based ligand was successfully synthesized and subsequently incorporated into the respective single stranded DNA (ssDNA). After cleanup and identification of the desired strands, the system was tested on its ability to form the intended *Holliday* junction. As a reference a second sequence was synthesized, where the ligand was replaced by a single thymine nucleobase, which should form T-T mismatches at the central position in the HJ, simulating a disorder within the structure, as it is caused by the incorporated ligands.

Table III.3.0.1: Comparison of denaturation temperature (T_M) for the investigated *Holliday* junction (HJ) systems. The T_M was determined for 2 μ M of the respective HJ with 100 mM NaClO₄, 5 mM Mg(ClO₄)₂ and 10 mM NaCaco at pH 7.3 in demineralized ultra-pure water. [1] Preliminary value, does not necessarily reflect the reality.

System	T_M [°C]	HJ	ΔT_M^{Cu} [°C]	ΔT_M^{Ni} [°C]	ΔT_M^{Pd} [°C]	Cage
MKDH01um	41.0	✓	-	-	-	-
MKDH01	54.0	✓	-2.0	2.0	-	✗
MKDH05	44.0	✓	0.5	0.5	3.0	✗
MKDH06	42.0	✓	-	1.0	6.0 & 26.5 ^[1]	✗

Both system were investigated with help of UV-vis melting experiments, various temperature fluorescence spectroscopy, various temperature CD spectroscopy and polyacrylamide gel electrophoresis (PAGE) experiments. The existence of both HJs could be proven by melting curves and especially, by a distinct band in the PAGE gel. Interestingly, the modified HJ has

a 13 °C higher denaturation temperature compared to the unmodified HJ (table III.3.0.1). This behavior can be explained by a stacking of the polyaromatic ligands within the HJ structure, comparable to the effect described in a three-way junction by Laing *et al.*^[179] Either the ligands intercalate into the nearby double helices or more likely, they do interact with each other, forming an aggregate. This aggregation is indicated by a significant bathochromic shift, observable for the ligand-specific UV-vis band, caused by the assembly of HJ and is known from molecules forming so called J-aggregates or excimers. What kind of aggregate is formed could not be elucidated with the here presented experiments. However, what is for sure, since all experiments are performed in a polar environment, the stacking process additionally benefits from the hydrophobic effect, making the aggregate so stable that no coordination cage could be formed in presence of any investigated transition metal salt.

Therefore the ligand system as well as how it was incorporated into the oligonucleotide was complete redesigned. The aromatic backbone was minimized to avoid the stacking efficiency and the linker motif was replaced by a diethanol amine motif and relocated to the ligands backbone, giving the whole system more flexibility. This new ligand motif was incorporated into the same sequence as it was used in the first approach. In addition, a second sequence with thymines, enclosing the ligand, were synthesized, in order to form T-T mismatches at the crossing section of the HJ. That approach gave more space for the formation of the intended coordination structure, since the shortened DNA linkers could provide too few space for this purpose. From the accomplished experiments the proof of an successful assembly of *Holliday* junction for both sequences was obtained, still, the results indicating an interaction of the ligands with each other or the surrounding oligonucleotide scaffold, even though in a mitigated form. Nevertheless, again no trustworthy evidences could be found for a formation of the intended coordination cage, neither in presence of palladium, nor nickel or copper.

Water-soluble Metal-Mediated Coordination Cages

Chapter IV.1

Introduction

In the context of the world wide chemical production, solvents are widely recognized to be one of greatest hazards for the environment. Apart from the high effort and energy consumption that is needed for production and processing of suitable amounts of organic solvents for industrial applications, they are highly toxic when exposed to organisms.^[180] Moreover, their inflammability or even explosion hazards led in the last years to several incidents in both academics and industry, some of them were even lethal.^[181] Hence, the reduction of the global organic solvent usage is one of the primary and probably most important aim in the new field of research "green chemistry".^[182] That presupposes of course the availability of alternative methods that are equal to existing solvent based procedures. Moreover, especially with regard to industrial applications, the economic sustainability of those methods is an important factor to be considered during the development, to make the changeover attractive to the companies.^[183]

In the last 20 years, since green chemistry became an own area of research, new solvent systems like ionic liquids,^[184] organic-aqueous tuneable system (OATS),^[185] switchable solvents^[180] or supercritical fluids,^[186] to name some, have been investigated and proofed to be adequate alternatives to many volatile organic compounds. However, they often suffer from a lack of flexible applicability and their costs.^[187] Concerning the economic factor and availability, water became more and more the focus of attention. Although at the first glance water seems to be furtherest from the classical organic solvents, what is the truth, new innovations combined with the physical and chemical knowledge, how organic compounds solvate in water, helped to transfer a range of classical organic syntheses into aqueous medium.^[188] Moreover, the usage of water as solvent can potentially improve reactivities and selectivities or simplify the workup procedures. In addition, in the context of organic chemistry in water, in some reaction uncommon reactivities and reverse selectivities could be observed compared to the classic reaction in organic solvents.^[189]

In biological systems almost all reaction proceed in water, where superior control of reactivity and selectivity can be obtained, even under highly complex reaction mixtures,

due to nature's well-developed functional units. To emulate this level of complexity, the interaction between solvent, reagent and functional units has to be understood, what also includes the physical properties of water and its uniqueness towards other solvents. For that reason, as so often before, scientist created model compounds based on artificial bio-derived biopolymers, oligonucleotides and -peptides,^[190,191] or metal-organic frameworks,^[192] that can mimic the functionalities in natural occurring structures. Especially, the field of supramolecular chemistry provided in the last years a versatile playground for the creation of complex three-dimensional architectures, from which several beautiful examples of water-soluble functional constructs derived.^[144]

An early example is reported by Raymond *et al.* consisting in a supramolecular polyanionic compound, where six bis-catechol based ligands self-assemble with four Ga(III) cations to form a tetrahedral water-soluble cage (Figure IV.1.0.1).

Figure IV.1.0.1: Supramolecular gallium-mediated coordination cage of Raymond *et al.*^[193] The cage has a tetrahedral topology, with gallium(III) cations sitting on the corners, connected by six ligands. Each gallium is coordinated by 3 ligands in octahedral fashion. The ligand is symmetric with catechol motif for coordination on both ends. Each ligand is bridging two vicinal gallium cations, giving a total charge of -12. Due this highly negative charge, this compact supramolecular architecture is soluble in aqueous medium.

Besides the fact, that Raymonds' cage is water-soluble, it additionally promotes an alternative reaction route of citronellal in water, when added to the reaction in catalytic amounts. While in absence of the gallium cage a dimerization of the substrate occurs, an intramolecular cyclization is preferred when the substrate is encapsulated in the polyanionic structure. This change in reaction selectivity is caused by several factors. First of all, due the hydrophobic effect the substrate goes preferentially inside the cages' cavity, further due to the highly charged environment the concentration of oxonium ions is spatially increased, as well as cationic transition states or intermediates are better stabilized.^[44,193] An inversed charged principle can be found in the cobalt cube of Ward *et al.*^[194] or the octahedral cage of Fujita *et al.*(Figure IV.1.0.2).^[45]

Figure IV.1.0.2: Highly charged cages by Ward and Fujita, both soluble in aqueous medium. **a)** The cage of Ward *et al.* shows a cubic topology with cobalt(II) ions sitting on each corner. Each cobalt is coordinated by three ligands in an octahedral fashion, therefore the cage consists of 8 cobalt(II) ions and 12 neutral ligands, lying on the cubes' edges. The supramolecular structure has an overall charge of 16+. **b)** Fujita's palladium coordination cage has a octahedral topology, with palladium(II) cations on each corner. The whole cage contains only four tridentate neutral ligands, which coordinate to three neighbored palladium ions, spanning a triangular surface. Since the palladium is coordinated in a square planar fashion and only two coordinating positions are occupied by the ligands, the residual coordination sites are capped by an ethylenediamine motif on each palladium cation. Each pyramidal substructure consists of two opposite lying ligands, turned 90 in respect to the second substructure. The overall charge is 12+.

Further water-soluble cages of other geometries and charges were also reported by Fujita with a pyramidal structure,^[46] Nitschke with a negatively charged tetrahedral complex or Yoshizawa with a positively charged $[\text{Pd}_2\text{L}_4]$ cage, containing neutral bis-pyridyl ligands with polyethylene glycol (PEG) chains. (Figure IV.1.0.3).^[195] Comparing all these water-soluble structures, regardless whether they have catalytic function or serve only as container, all of them are highly charged ($\geq 8+$), what seems to be one crucial requirement to get the accumulation of aromatic ligands into solution. As a second requirement, the charges have to be well distributed over the whole structure, as for example, Clever *et al.* showed several examples of highly charged $[\text{Pd}_4\text{L}_8]^{8+}$ interpenetrated cages that

are not soluble in water at all.^[58] However, both Yoshizawa and Crowley reported supramolecular structures based on bis-pyridyl ligands forming $[\text{Pd}_2\text{L}_4]^{4+}$ cages that are soluble in aqueous medium. Both achieved this extra solubility in the organic solvent water mixture by adding polyglycolic chains or free hydroxy groups to the ligands backbones.^[42,76]

Figure IV.1.0.3: Charged cages by Nitschke, Fujita and Yoshizawa. **a)** Tetrahedral cage by Nitschke *et al.*, consisting of iron(II) cations, sitting on each corner, bridged by six negatively charged ligand. In comparison to Raymond's gallium-mediated cage, the metal ions are coordinated by neutral nitrogen ligands, with negatively charged sulfonate groups located at the ligands backbone, which do not interact with the iron cations. Therefore, each charged motif within the supramolecular structure, needs its own counter ion, giving 16 counter ions in total and a total charge of 4- for the coordination cage itself. **b)** By changing the substitution pattern of the pyridines from *para* to *meta*, Fujita *et al.* synthesized a derivative of the previously described ligand (Figure IV.1.0.2b), which rather forms a bowl structure than an octahedral coordination cage, in presence of palladium(II). **c)** Yoshizawa *et al.* attached PEG chains to the backbone of the banana-shaped bi-dentate ligand, making its corresponding cage suitable to be solved in aqueous medium. A little later, introduction of a third PEG chain to the ligands backbone, led to a completely water-soluble capsule.^[196]

There are also some concepts of adding charged residues, like trimethylammonium or sulfonate groups, to the ligand backbones, which led also to an increase in solubility, but generates as well a highly charged species with lots of counter-anions, which can have a tremendous influence on the formation^[31] or solubility^[197] of the intended supramolecular architecture. In the previous chapter, it has already been shown that the addition of either glycol chains or hydroxy groups to the ligand backbone, does not necessarily drive the system to be water soluble. (Chapter III.2).

In this chapter, the anisole-based ligand (**55**), known from the previously discussed topic (Section III.2.3) is examined for its ability to form a $[\text{Pd}_2\text{L}_4]^{4+}$ cage in pure water. Furthermore, instead of the diethanolamine motif, a new concept of self-stabilizing zwitter-ionic moiety was attached to the ligands backbone, causing a significant increase in water-solubility for the ligand itself and its resulting supramolecular architecture without changing the overall charge. The reported strategy to introduce zwitter-ionic moieties,

successfully achieved to avoid organic solvents, and it shows an elegant general strategy that can be used to tune the cage solubility, without changing the net charge, resembling a similar approach found in natural compounds.

Chapter IV.2

Results and discussion

IV.2.1 Diethanolamin methoxy ligand

In chapter III.2, the formation of cage with ligand **L^{Acr}** (**9**) could not be verified due to the insufficient solubility of ligand and cage in water (Section III.2.2), otherwise the preferred stacking of the acridone ligands maybe could have been noticed earlier. However, also this time the ligand **17** was checked for the formation of $[\text{Pd}_2\text{L}_4]$ cage in water, to be sure that ligand can do form a supramolecular cage in water in the presence of palladium(II) cations, before it was build into the oligonucleotides. For that reason a 2.8 mM solution of ligand **L^{DEA}** (**17**) in D_2O was prepared. Since the ligand itself showed a rather low solubility in water at room temperature, the solution was heated to 60 °C, until a clear transparent homogeneous solution was obtained. After, 500 μl of the ligand solution were mixed together with an 50 μl solution of $\text{Pd}[(\text{MeCN})_4](\text{BF}_4)_2$ or $\text{Pd}(\text{NO}_3)_2$ (15 mM in D_2O) and heated for 1 h at 70 °C. The resulting mixture examined via ^1H -NMR and ESI-MS (Figure IV.2.1.1). At the beginning the samples were prepared with the palladium salts solved in MeCN or DMSO, since the solvation in pure D_2O quickly led to formation of the reduced palladium(0) species, precipitating as a black solid. The formation of palladium(0) species already occurs at room temperature and was even accelerated when solvent was heated in order to bring the palladium(II) salts into solution. To obtain a suitable palladium(II) solution, a preceded cooling of the deuterated water was necessary. Subsequently the palladium salt was added and solved by only applying ultra-sonification until a homogeneous solution emerged. The freshly prepared palladium(II) solution was immediately added to the ligand, leading to the formation of the cage species. As soon the metal ion is coordination by the pyridine motifs, the palladium(II) seems to be stable, since no further black precipitation was observable. In ^1H -NMR the typical significant down-field shift of the pyridine related proton signals (especially **a** and **b**) was observed for both samples, the one with $[\text{Pd}(\text{MeCN})_4](\text{BF}_4)_2$ and the one with $\text{Pd}(\text{NO}_3)_2$. Additionally, the methoxy signal is also significantly down-field

shifted, indicating a formation of the cage, since the methoxy group pointing inwards the cage, having the positively charged palladium(II) cations in direct vicinity. Although it was not in the focus of interest, it is worth to mention that the formation of cage was also observed by NMR in polar organic solvents, like MeCN and DMSO in the presence of the $\text{Pd}[(\text{MeCN})_4](\text{BF}_4)_2$ salt.

Figure IV.2.1.1: (a) ^1H NMR (500MHz). $[\text{Pd}_2\text{L}_4]$ cage can be formed in pure deuterated water with both $[\text{Pd}(\text{MeCN})_4](\text{BF}_4)_2$ as well as $\text{Pd}(\text{NO}_3)_2$. (b) ESI-MS of the cage formed from ligand **17** with $[\text{Pd}(\text{MeCN})_4](\text{BF}_4)_2$ in D_2O . Since ligand **17** is poorly soluble in water at room temperature, additional spectra were recorded with 10% of deuterated methanol to increase the amount of solved ligand in the NMR sample. The cage was formed upon addition of 0.5 equiv. $[\text{Pd}(\text{MeCN})_4](\text{BF}_4)_2$ in deuterated methanol. The sample was taken from NMR sample in pure D_2O . For the cage formed in presence of $\text{Pd}(\text{NO}_3)_2$ no cage peak was detectable in ESI-MS, but the formation was verified by NMR and fluorescence spectroscopy.

Due to the fact that the ligand showed a rather poor solubility in pure water and partially precipitated when the solution cooled down to room temperature, an additional NMR experiment was accomplished in an aqueous solvent mixture containing 10% deuterated methanol. The solubility of the ligand was significantly increased, thus a clear transparent solution was obtained at room temperature. The ligand compound showed the same chemical shifts for the aromatic region in NMR, merely the diethanol amine signals are

slightly upfield shifted in the aqueous solvent mixture. Nevertheless, upon addition of $[\text{Pd}(\text{MeCN})_4](\text{BF}_4)_2$, also solved in $d^4\text{-MeOH}$, almost exactly the same NMR spectrum could be obtained, compared to the cages measured in pure water.

Samples for ESI-MS measurements were directly taken from the pure water NMR samples and measured. The mass spectra clearly showed two main peaks, which could be assigned to the 3+ and 2+ species of $[\text{Pd}_2\text{L}^{\text{DEA}}_4]$ cage with tetrafluoroborate as counter anions. It is worth to mention, that the 4+ species was not found in the ESI-MS spectrum, thereof it may be inferred that one BF_4^- anion is encapsulated within the cage cavity, which in turn explains why only the 3+ and 2+ species can be seen. On the other hand it just could be a matter of the used condition during the ESI-MS experiment. For the cage formed with $\text{Pd}(\text{NO}_3)_2$ no satisfying ESI-MS spectra could be obtained, however, in order to verify its formation, further experimental measurements with help of UV-vis- and fluorescence spectroscopy were performed (figure IV.2.1.2).

Figure IV.2.1.2: UV-vis and fluorescence spectra for L^{DEA} and $[\text{Pd}_2(\text{L}^{\text{DEA}})_4]\text{X}_2$ ($\text{X}=\text{NO}_3$ or BF_4). **a)** All UV-vis spectra were recorded in H_2O with a total ligand concentration of 0.035 mM. **b)** All fluorescence spectra were recorded in H_2O with a total ligand concentration of 0.005 mM, irradiated with a wavelength of 280 nm. The ligand is fluorescent and emits light in the range of 350 nm and 500 nm, with a maximum of approx. 377 nm. In contrast, the assembled cage does not show any fluorescence.

In the UV-vis measurements a difference between the spectra of the ligand L^{DEA} and those of the cage solutions is visible. In addition the shape of absorption bands for both cage measurements are pretty similar, even though they differ in absorbance. Anyway, a clear proof for the cage formation with $\text{Pd}(\text{NO}_3)_2$ as metal salt could not be obtained from this method. As a consequence, the fluorescence of the same samples were measured, since it is known from previous works, that if the ligand shows any fluorescence, it is usually quenched upon coordination to the palladium cation. And in fact, for both cage sample no fluorescence was detectable. In contrast, the free non-coordinated ligand shows a significant emission band at 377 nm when irradiated with 280 nm.

Figure IV.2.1.3: PM6 model of cage $[\text{Pd}_2(\text{L}^{\text{DEA}})_4]$. The cage has a Pd-Pd distance of 11.5 Å and an average distance of 7.3 Å between two opposite methoxy groups. The values do match with those of the sulfobetaine cage (figure IV.2.3.7a), what is not really surprising, since both consists of the same backbone and only differ in the exohedrally attached motif.

Although plenty of crystallization attempts under various condition were tried, it was not possible to obtain a crystal suitable for single crystal X-ray diffraction experiments. Due to that reason, a computer assisted model of the cage structure was created *in silico* (figure IV.2.1.3).

IV.2.2 Diethanolamin hydroxy ligand

Due to the fact that the cage based on diethanol amine ligand L^{DEA} with the inward pointing methoxy group did not show any evidence for an encapsulation of small unpolar guest, we decided to synthesize an analog structure with a less steric endohedral group. Since the hydroxy group is needed in the first step of synthesis, it had to remain, therefore only the methyl group could be omitted. Anyway, from PM6 model the average distance to the opposite ligand was increased in average by 1 Å, which approximates the cavity size to the double cage inner cavity, described in figure IV.2.3.7. Concurrently, the cavity would of course become less hydrophobic, lacking the methyl motif, which makes it less favorable for unpolar guests. However, the new motif also can give the opportunity for a new range of possible applications, since the hydroxy groups are actually phenolic groups, means they are weak organic acids. Phenols are often used in the context of supramolecular structures or macrocycles for anion recognitions, but also small molecules with hydrogen bond acceptors.^[198] Moreover, a post-synthetic deprotonation could make the cavity suitable for metal cations or small positively charged molecules.

The synthesis of ligand **31** was accomplished analogously to the previous synthesis. The only change, was the introduction of a protection group for the phenol hydroxyl group. As several reactions has to be performed under basic conditions, the phenol would probably deprotonated, leading to side reaction or product. From pre-experiments with various protecting groups, including methoxymethyl acetal (MOM) and methyl ether, *tert*-butyldimethylsilyl (TBDMS) turns out to be the best choice. After introducing TBDMS in the second step, all further product could be obtained with high yields. As a last step the TBDMS had to be removed from compound **30**, but during the purification process, it was spontaneously converted to compound **32** (scheme IV.2.2.3).

Scheme IV.2.2.3: Synthesis of compound **30**. a) TBDMS-Cl, NEt₃, EtOAc; b) NaBH₄, MeOH; c) SOCl₂, NEt₃, DCM; d) DEA, (i-Pr)₂NEt, CHCl₃; e) 3-ethynyl-pyridine, Pd(PPh₃)₂Cl₂, CuI, NEt₃, DMF. In step f) a deprotection was planned with tetrabutylammonium fluoride to obtain **31**, but in presence of g) ΔT and moisture, **30** spontaneously reacted to **32** (scheme IV.2.2.4).

After column purification of the compound **30**, there were still visible signals from DMF, which was used during synthesis. Therefore, the residue was dried for several hours in high vacuum at 60 °C. Suddenly the compound showed a lot of new signals in the aromatic region, from which several looked doubled. Interestingly, the signals from the side chain were unchanged as well as signals of the TBDMS protecting groups were also still there, but a little up-field shifted. Mass analysis with ESI-MS showed peaks, fitting to the desired deprotected ligand **31** (shown in Figure IV.2.2.4), but there were no peaks for the substrate **30**, as an evidence for the thermally induced deprotection of the *tert*-butyldimethylsilyl group. However, ESI-MS analysis did not allow to identify the origin of the new NMR signals in the aromatic region. As a consequence, the mixture was purified via GPC and the isolated compounds were analysed. The resulting ¹H-NMR spectrum still showed the doubled aromatic signals, but the TBDMS signal were vanished. By further 2D-NMR experiments the resulted compound could be identified as the asymmetric compound **32**. The compound has undergone a intramolecular cyclization reaction between the

deprotected phenol hydroxyl group and one of the ortho-substituted 3-alkynyl pyridine arms, by what the whole system becomes asymmetric, thus the aromatic signals get doubled. Additionally, the formed hetero-aromatic five-membered ring contains a new proton, showing a typical chemical shift in the aromatic region, which explains the same mass as the desired unprotected compound **31**. This proton could be assigned to signal **j**. Since the additional proton was not provided anyway, it must come either from air moisture or the wet solvent.

Figure IV.2.2.4: ¹H NMR (500MHz). After synthesis of ligand **30** the compound was dried in vacuo to get rid of the residual solvent at 60 °C. The dried compound showed a doubling of the aromatic signals. Additionally, the signals of the *tert*-butyl-dimethyl silyl protecting group are slightly highfield shifting. After repurification the signals of the *tert*-butyl-dimethyl silyl protecting group vanished.

Since the complete ligand **30** was converted to the cyclized ligand **32**, the still available methoxy ligand **17** got again in the focus of interest, since the methoxy group is also protecting group for phenols. To avoid the undesired cyclization reaction, the deprotection reaction was accomplished with BBr₃ and dry solvent at low temperatures (scheme IV.2.2.4).

Scheme IV.2.2.4: Deprotection of **17** and subsequent intramolecular cyclisation. a) BBr_3 , DCM; b) ΔT , moisture.

Indeed, the deprotected ligand could be obtained. In $^1\text{H-NMR}$ all relevant signals could be observed, including the phenol hydroxy signal (i) (figure IV.2.2.5), since it was measured in DMSO. Attempts to measure the compound **31** in D_2O failed, the initially clear ligand solution turns within minutes into a suspension. Probably the cyclization reaction immediately occurs, when the unprotected ligand is exposed to water. Also wet organic solvents and time led to the intramolecular cyclization. The NMR sample measured in DMSO, was remeasured after two weeks, stored at room temperature. The resulting NMR spectrum, shows a almost complete conversion to the cyclized ligand **32**, this clearly indicates the instability of ligand **56** and the tendency to convert to ligand **32**.

Figure IV.2.2.5: $^1\text{H NMR}$ (500 MHz). Solving ligand **31** in organic solvent like DMSO does not lead to a direct intramolecular cyclization. Therefore, the existence of **31** is able to verify via NMR. But, remeasuring the same sample after two weeks (stored at room temperature) revealed that the intermolecular cyclisation still happens. All signals of compound **32** can be found, although signals (i,e and j) show a quite big downfield shift in the solvent DMSO compared to the solvent MeCN. Some non-converted compound **31** can also be found in the spectra, indicated with the red bars.

The attempt to preserve the deprotected ligand **31** by assembling it into a supramolecular coordination cage also failed. From NMR measurements only an nonassignable mess of

signals could be obtained. The standard self-assembly conditions used in this work requires heating of the cage solution at 60°C for at least 1 hour to obtain a completely assembled cage in solution for NMR. Since it was assumed, that heat expedites the cyclization reaction, another sample was prepared. The sample was stored at room temperature and measured every day for five days, giving the same result as described before, a intramolecular cyclisation of the product.

Additionally, the cyclized ligand was investigated for its ability to assemble in a supramolecular construct in the presence of $[\text{Pd}(\text{MeCN})_4](\text{BF}_4)_2$. The experiments were performed in acetonitrile, because ligand **32** was not soluble in water at all anymore. The resulting spectra showed absolutely no shifting of the pyridine signals, but surprisingly the signals for the diethanol amine (DEA) motif, plus the adjacent aromatic signals, exhibit a significant down-field shift, suggesting a favorable interaction between palladium and the DEA motif. In literature a few systems are reported, where triethanol amine or DEA-functionalized polymers acting as ligand for palladium(0) species in phosphine-free catalyst for Suzuki or Heck cross-coupling reactions..^[199–201] In contrast, the methoxy ligand **17** did not show any evidence of an interaction between palladium(II) and the DEA motif, thus the geometry of ligand **32** probably became so unfavorable for the coordination to the metal center, due to the intramolecular cyclization preventing the formation of a supramolecular metal-mediated coordination cage. In turn, the coordination to the diethanol amine came probably into favor, since clear shifts of the signals assigned to DEA could be observed, in presence of palladium(II). But to really proof this assumption, further experimental evidence is needed.

IV.2.3 Sulfobetaine methoxy ligand

In the last part of this chapter, the introduction of an new motif to supramolecular coordination cages is described, which make them water-soluble but does not change the total charge of the supramolecular architecture. As already mentioned in the introduction of this chapter all water-soluble supramolecular cages have a high total charge, which is additionally well distributed over the whole structure. The lower charged coordination cages contain methyl capped PEG groups or free hydroxyl groups, as shown for the ligand **17**.

Another coordination cage with an low total charge is reported by Nitschke *et al.*. The tetrahedral structure (figure IV.1.0.3a) consists of four iron(II) metal ions, each coordinated by three bis-bidentate nitrogen-based ligands. Each ligand contains two sulfonic acid moieties at the backbone, which are deprotonated in solution. Taken together, the total charge is consequently **-4**. However, since the architecture is very labile towards acidic conditions, 12eq. tetramethylammonium hydroxide had to be added, to neutralize the protons released by the sulfonic acid motifs and further providing non-coordinative counter-ions for the sulfonates. Additionally, each metal corner has a sulfonate co-counter-anion, makes in total 16 counter-ions for the whole structure. As a result a water-soluble supramolecular structure is obtained, with positive charges at metal vertices and negative charges at the edges.^[202]

Herein we introduce a new general approach for water-soluble coordination cages with zwitter-ionic sulfobetaine moieties, in order to create low-charged coordination cages, which have a lower dependency on the needed counter-ions and are more attractive for "*Green Chemistry*" with respect to bio-compatibility and atom-economics. The sulfobetaine moiety belongs to the amphoteric tensides and is an organic zwitter-ionic group, containing a positively charged tertiary ammonium motif and a negatively charged sulfonate group, hence the formal charge is neutral. They are often used in cosmetics, but also find applications as hydrotropes in chemical industry. Especially the self-stabilizing forms $\text{R-NMe}_2^+-(\text{CH}_2)_n\text{-SO}_3^-$ ($n=3,4$) retain their zwitter-ionic character over a wide range of pH, which makes them suitable to use under acidic and basic conditions.^[203,204] Additionally, they are highly bio-compatible and their water-solubility is largely independent from the surrounding ions.^[205,206]

Due to above listed points, the sulfobetaine motif could be very interesting and promising for water-soluble supramolecular coordination architectures, especially with regard to drug delivery containers and nanoscale reaction vessels.

Synthesis of the ligand (scheme IV.2.3.5) is based on the already presented ligand **L^{DEA}** (**17**). The first four steps of synthesis are identical. In contrast to the ligand **L^{DEA}**, dimethyl amine was introduced to the benzylic position. Subsequently, the sulfobetaine

motif was formed as precipitate in a ring opening reaction of the highly carcinogenic 1,3-propane sultone. The collected solid was further converted to the desired ligand by attaching the 3-ethynyl-pyridine arms with help of palladium-catalyzed Sonogashira cross-coupling reaction. Due to the fact that the ligand had to be purified by elaborate reversed phase liquid chromatography methods, it was also tried to couple the pyridine arms firstly and forming the sulfobetaine motif subsequently, but the the 1,3-propane sultone reacted with the pyridine as well, resulting in an even more complex product mixture, with lower yield and more side-products that had to be separated, hence, the first synthetic pathway is preferred.

Scheme IV.2.3.5: Synthetic path for ligand **24**. a) MeI, $(\text{CH}_3)_2\text{CO}$; b) NaBH_4 , MeOH; c) SOCl_2 , DCM; d) HNMe_2 , THF/ H_2O ; e) propane-1,3-sultone, 1,4-dioxane; 3-ethynylpyridine, $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$, CuI, NEt_3 , DMF.

After the full characterization of ligand, the system was investigated for its ability to form palladium-mediated coordination cages in water, as it was supposed to do. In fact, in the presence of $\text{Pd}(\text{NO}_3)_2$ the NMR spectra show a significant down-field shift of the pyridine related proton signals, indicating a coordination to the palladium(II) cations (figure IV.2.3.6). Additionally, as it was already described for the $[\text{Pd}_2\text{L}^{\text{DEA}}_4]$, a down-field shift for the inwards pointing methoxy group was observed as well. In contrary, the sulfobetaine signals (**f-j**) stayed virtually unchanged, indicating no interaction between the sulfobetaine and the palladium cations. Due to the fast reduction of Pd^{II} to the black Pd^0 species in the used aqueous $\text{Pd}(\text{NO}_3)_2$ solution, first cage formation experiments were accomplished with a $\text{Pd}(\text{NO}_3)_2$ solution in deuterated DMSO (figure IV.2.3.6a), lower cage spectrum), as it was already mentioned in section IV.2.1. However, it was expected, that the here described ligand (**24**) can also form a coordination cage in pure water without any additional organic solvent, since it was also possible for the less water-soluble ligand **L^{DEA}**. Especially, considering that the ligand on its own shows already a quite good solubility in water. Indeed, as it can be seen in figure IV.2.3.6a (upper cage spectrum) the cage is formed in presence of $\text{Pd}(\text{NO}_3)_2$ and deuterated water as solvent. Moreover, the

cage formation was further verified by ESI-MS experiments (figure IV.2.3.6b). Peaks for the $+3$ species with NO_3^- and Cl^- could be found as well as traces of the $+4$ species. It is worth to mention that also a $[\text{PdL}_2]_2^+$ species was found, superimposed by the $+4$ species of the cage. The fact that the main peaks contain free ligand, suggesting that the chosen ESI-MS settings for the experiment are not ideal and have to be improved, on the other hand, regarding the usage of the standard condition for ESI-MS measurements of cages, indicating a lower relative stability of the cage in comparison to other cages, as e.g. $[\text{Pd}_2(\text{L}^{\text{DEA}})_4]$ in figure IV.2.1.1b. To make a certain statement about this assumption, more evidences have to be supplied by further experiments.

Figure IV.2.3.6: (a) ^1H NMR (500MHz). The ligand **24** is soluble in pure deuterated water (500 μl , 2.8 mM). Addition of $\text{Pd}(\text{NO}_3)_2$ 50 μl , in deuterated H_2O (upper cage spectrum) or DMSO (lower cage spectrum) lead to formation of the Pd_2L_4 cage. The typical downfield shift of the signals **a** and **b** is observable due to coordination of the pyridine motif to the Pd_2^+ cation. Furthermore the methoxy signal is also shifting downfield, which is a hint that the group is pointing inwards to the cages cavity. (b) The measured ESI-MS shows besides the peaks for the ligand **24**, the peak of the $[\text{Pd}_2\text{L}_4]^{4+}$ as well as $[\text{Pd}_2\text{L}_4]^{3+}$ with either chloride or nitrate as counteranion.

Crystals could be obtained from slow evaporation of THF into an aqueous cage solution. The crystals were picked under low temperature conditions in cryo stream, however, unfortunately they were not suitable for X-ray diffraction analysis. Therefore the here presented structures (figure IV.2.3.7a) were obtained *in silico* from semi-empirical PM6 geometry optimization calculations. From the model a Pd(II)-Pd(II) distance of 12.06 Å was extracted, as well as an average distance of 7.5-8.6 Å between the methoxy group, what is in agreement with the values obtained from the PM6 calculation of structural related [Pd₂L^{DEA}₄] cage (figure IV.2.1.3a).

Having a low-charged palladium-mediated coordination cage in water, it was of interest to investigate the encapsulation of small apolar guest molecules. Especially with regard to take advantage of the hydrophobic effect. For that reason exemplary ¹H-NMR titration experiments (figure VI.1.7.1) were made with cyclohexane, naphthalene and 1,4-benzoquinone, which was used by Lusby *et al.* in a structural related cage of similar size.^[207] Unfortunately none of these guest molecules showed any evidence for encapsulation. In all cases, the signals of guest molecules could be seen in NMR, but there was no observable change in chemical shift of the inward pointing hydrogen signals, neither from the aromatic hydrogens nor from those of the methyl groups. This behavior rather suggest that no encapsulation occurs in presence of the guest molecules.

Two possible reasons, why the cage did not take up a guest molecules, are taken from topological deliberations. Either the cavity is too small, or the cavity is more polar than expected and rather prefers charged guest/anions over the uncharged ones. As a reference the crystal structure of the previously reported double cage is used, reported by Löffler *et al.*, since it was able to bind neutral guest molecules.^[20] From the PM6 model of the cage, the obtained distance between two opposite ligands is on average 0.5 Å smaller (ca. 6.8-7.7 Å) compared to the inner cavity of the double cage (ca. 7.7-8.6 Å), where the binding of the neutral guest molecules occurs. Since the methyl groups are rather directed to the sides of the cage, the oxygen of the inward pointing methoxy groups, consumes most of the cavities' space, so that small molecules, as the ones used for titration, should have enough space. Also the distance between the palladium cations (Pd-Pd → 11.5 Å) is comparable to the distance found in the interpenetrated double cage (Pd-Pd → 10.5 - 11.0 Å). In contrary, the charge of palladium cations is less shielded in the small cage, compared to the interpenetrated double cage, where the chloride anions show a close interaction with the palladium metal centers. Therefore the encapsulation of the counter-anion could be preferred towards the neutral guest molecule. Another difference to be mentioned are the open sites of the cage, which allows an easy uptake and release of the guest molecules, compared to the densely packed inner cavity of the interpenetrated double cage. However, to prove this theory, more titration experiments have to be made, especially with small anionic guest, in order to figure out whether the cage is just not

able to encapsulate neutral guests or whether the cage is not able to encapsulate guest molecules at all.

(a)

(b)

Figure IV.2.3.7: Comparison PM6 model of sulfobetaine cage with acridone interpenetrated double cage crystal structure $[\text{Cl}_2 + \text{C}_6\text{H}_6 @ \text{Pd}_2\text{L}_4]_8^+$.^[20] All counterions and guest molecules were omitted in order to have a better view on the cavities. The chloride containing acridone interpenetrated double cage was chosen on purpose, since it was the first cage in the Clever group capable of encapsulating neutral guest molecules. Compared to the sulfobetaine cage, its central cavity is slightly lower with 10.5 Å (11.3 Å for the sulfobetaine cage), but also a little broader with 8.2 Å (7.2 Å for the sulfobetaine cage). Furthermore, the sulfobetaine cage has rather open sites, which are occupied by the ligands in the acridone double cage. This situation makes it harder for the guest to enter the cavity, but also the leaving and there are more possibilities for interactions between host and guest, giving a higher sum of potential stabilizing forces.

Chapter IV.3

Conclusion

In this chapter two new motifs were presented to make low charged supramolecular $[\text{Pd}_2\text{L}_4]$ cages soluble in pure water. Although the diethanol amine motif was actually chosen as linker for the incorporation of ligand **L^{DEA}** (**17**) into oligonucleotides, it gives the small aromatic backbone the extra hydrophilicity to be solved in pure water at elevated temperatures. In presence of palladium(II) salts the ligand **L^{DEA}** can form a supramolecular coordination cage, which is soluble in water as well as in polar organic solvents, such as MeCN and DMSO. This result was important to continue the oligonucleotide project (section III.2.3.3), since it was not clear if the first generation oligonucleotide ligand (section III.2.2) can not form at all in aqueous medium or just in the oligonucleotide scaffold. Furthermore, the here presented results is the subsequent development of Yoshizawa's PEG chain approach and Crowley's hydroxyl group approach to make supramolecular $[\text{Pd}_2\text{L}_4]$ water-soluble and can be seen as a proof of principle, that a branched, dendron-like side chains ending in free hydroxyl groups is a viable motif for this purpose. At the same time, the cage has not lost its ability to be solved in polar organic solvents, resulting in interesting supramolecular containers for applications in phase transfer reactions, as it has been shown by Nitschke *et al.*^[208,209]

The synthesis of an analogous cage with an endohedral hydroxyl group instead of an inward pointing methoxy group failed, due to formation of an unexpected side product. In literature similar systems have been synthesized successfully without mentioning any synthetic problems.^[210,211] However, the side product could be identified as intramolecular cyclization product, resulting in the asymmetric benzofuran containing ligand (**32**). With an alternative synthesis route, the desired ligand **31** could be obtained, but it converted in presence of water directly to the intramolecular cyclized ligand, which made this system impractical for the intended application. It is worth to mention, that the assembly of the asymmetric ligands to a palladium-mediated coordination cage failed, probably due to the reduction of the angle between the coordination sites. Thus, the coordination to the metal center became less favored. In turn, the palladium cations seem to be coordinated by the

backbone-attached diethanol amine (DEA) moiety instead.

For the last system, the DEA motif was replaced by an zwitter-ionic sulfobetaine. The zwitter-ionic structure has a formal charge of zero, therefore it does not change the total charge in a supramolecular coordination structure, when introduced, compared to supramolecular cage consisting of neutral ligands. Furthermore it is self-stabilizing, means it does not need any additional counter ions, which is not only an advantage in terms of atom-economy, but also ensures that its water-solubility is not dependent on the provided counter ions. In addition, the here used sulfobetaine motif is known to keep its zwitter-ionic character over a large pH range,^[203,204] making the system suitable for reactions under basic or acidic conditions. Additionally, the sulfobetaine structure has shown an excellent biocompatibility, hence it was used for drug carrier systems in recent publications.^[212,213] The here presented approach bears a huge potential, not only giving low-charged water-soluble coordination cages, but also gives a chance to bring neutrally charged supramolecular architectures in to aqueous medium, concerning its suitability for applications in *Green Chemistry*.

Endohedrally Functionalized Metal-Mediated Coordination Cages

Related Publications:

M. Krick, J. Holstein, C. Würtele, G. H. Clever, *Chem. Commun.* **2016**, 52, 10411–10414.

M. Krick, J. J. Holstein, A. Wuttke, R. A. Mata, G. H. Clever, *Eur. J. Org. Chem.* **2017**, 2017, 5141–5146.

Chapter V.1

Push-Pull Rotor Systems based on Acridinylidene Cyanoacetic Esters

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V.1.1 Introduction

The study of dynamics in all size scales of molecular systems is an important area of research, especially in respect of understanding and controlling the dynamics in such important natural occurring systems, like enzymes. In order to delve into the operating principle and function of those highly specialized and complex systems, smaller systems, ranging from simple alkanes,^[214,215] over more complex organic^[216,217] or organometallic compounds,^[218,219] catalysts,^[220,221] to biopolymers such as DNA,^[222] frequently delved. The integration of such dynamic systems in higher order supramolecular scaffolds can help to create biomimetics systems, giving hints about nature's chemistry or even develop artificial analogues. A prominent example for the latter one is the rotaxane based supramolecular peptide synthesis machine reported by Leigh *et al.*^[223,224]

In the context of dynamic systems in artificial supramolecular constructs,^[225] functionalities such as gears,^[226] wheels,^[227] and rotaxanes^[228–230] have been used as components in molecular-scale machines.^[231] However, most of these dynamic systems are lacking of directional control.^[232] Feringa *et al.* hurdled this problem by designing a system, consisting of photo-interconvertible helical isomers, which are thermally stable.^[233,234] In 2010, Feringa *et al.* reported an ultra-fast rotating motor, operating in the manner as his previous systems, but based on a N-Boc protected acridinylidene stator, with an asymmetric substituted bulky five-membered ring as rotor.^[235]

Interestingly, Löffler *et al.* were also able to synthesize similar acridinylidene system with a sterically demanding adamantyl group instead of the five-membered ring.^[24] Moreover, since the system was based on the acridone ligand from previous works,^[20] four of those molecules were able to form an endohedrally functionalized supramolecular coordination cage. Compared to Feringa's acridinyldine compound, the adamantyl system didn't show any rotation but a dynamic flipping motion, by what the ligand is mirror-inverted. This motion could be observed for the free ligand, for the assembled cage and even with an encapsulated guest. It is noteworthy to mention, that the flipping rate is decreasing from free ligand to cage to the cage with encapsulated guest.

Motivated by this new way to modify the rather inert keton position in our banana-shaped ligands, we were interested in introducing functional groups to the inward pointing position, in order to create functional units for specific molecular recognition^[236,237] or catalyzed reactions within the cage cavities.^[238]

For the acridone-based ligand we were able to introduce malonitrile and 2-cyano acetate ethyl ester at the keto-position while maintaining the double bond. Although being aware of having a potential flipping motion, the found predominant rotational motion within the ligand was not expected, which is only observable in the asymmetric substituted derivatives. First evidence of an intramolecular dynamic was seen in the comparison of the NMR signals from ligands **L^{CN}** (**40**) and **L^{Et}** (**42**) in figure V.1.1.1.

Figure V.1.1.1: Comparison of ^1H NMR spectra (400 MHz, 298 K, $\text{d}^3\text{-MeCN}$) for ligands L^{CN} and L^{Et} . The signals of **a** and/or **a'**, highlighted in red, belong to the protons with the closest proximity to the malononitrile or 2-cyano-acetate ester substituent. The 2-cyano-acetate ester derivative shows a less downfield shifted broad signal in comparison to the symmetric ligand.

Contrary to the expectations the asymmetric ligand L^{Et} showed only one set of signals (figure V.1.1.1), what was at the first glance not a problem, but the absence of a proton signal was. The signal for the protons **a** could not be found. However, changing the solvent from chloroform to acetonitrile revealed the portion of a very broadened peak, we assumed to be **a**. It is noteworthy that similar derivatives of substituted acridinylidene were already synthesized in the mid 1950s by condensation of malononitrile or 2-cyanoacetate esters with acridone, by Dimroth *et al.*,^[239] but this report is lacking of any NMR spectroscopic data. But interestingly, they already proposed the existence of an intramolecular push-pull system and a zwitterionic structure, what gave a first hint towards the cause of the rotation. More recent publications examined only symmetrically substituted dicyano derivatives, which do not reveal this dynamic motion in NMR spectroscopic investigations.^[240,241]

During our studies, it became apparent that we could not investigate the rotational motion by dynamic NMR spectroscopy (DNMR) in the ligand structure, due to spin convolution in the ^1H NMR experiments between the **a** signals and some signals belonging to the pyridine arms. Therefore, we decided to investigate the dynamic motion in a simplified structure without the attached the pyridyl arms.

V.1.2 Results and discussion

V.1.2.1 Synthesis

In the synthesis of the endohedral substituted adamantyl ligand,^[24] acridone and adamanton were merged with help of the McMurry reaction, a reductive coupling reaction.^[242] This reaction is taking place in presence of TiCl_3 and a reducing agent. TiCl_3 plays the key figure in this reaction, since, due to its oxophilic character, it firstly activates the rather unreactive keto group, secondly brings the reactants close together by forming a titanium(III)-carbonyl complex, before the reduction occurs and lastly removes the oxygen from the system by forming TiO_2 . As a result the double bond between the two reactants is formed. A similar reaction has been used for a controllable molecular motor of Feringa et al.^[243] Starting from the commercially available acrid-9(10*H*)-one, all derivatives (**34**, **35** and **37**) were synthesized in a two step synthesis. To increase solubility, a hexyl chain was introduced to the acridone nitrogen via $\text{S}_{\text{N}}2$ substitution reaction using 1-bromohexane and K_2CO_3 . Subsequent, the cyano acetate esters were coupled to the *N*-hexyl acrid-9(10*H*)-one with help of a variation of the Knoevenagel condensation reaction.^[244] The Lehnert modification^[245,246] uses besides the base, titanium(IV) species to couple methylene compounds with aromatic ketone, where the titanium species is also responsible for the activation, followed by the elimination of the carbonyl oxygen.

Scheme V.1.2.6: Syntheses of aridiynlidne cyanoacetic esters B^{tBu} (**34**), B^{Et} (**35**) and B^{Ph} (**37**). a) K_2CO_3 , Hexylbromide, DMF. b) Cyano acetate derivatives ($\text{R}=\text{tBu}$, Et, Ph), NEt_3 , TiCl_4 , CH_2Cl_2

After full characterization of all derivatives at room temperature (section VI.3.1), there was still no evidence of a second set of proton signals, although the asymmetric substitution should cause this. Since, we still expected a dynamic motion within the molecule, a deep temperature ^1H NMR at $-80\text{ }^\circ\text{C}$ of B^{Et} (**35**) in d^8 -THF was measured. And indeed a second set of signals appeared and become distinguishable. Signals **a/a'** as well as **b/b'** show a quite big difference in chemical shift, while **c/c'** and **d/d'** show just a slight difference (figure V.1.2.6). Which is not surprising, concerning the different proximities of the protons toward the cyano acetate substituent. Anyway, from ^1H NMR it was not

possible to assign the signal **a'**, neither to the proton closest to the cyano nor to the ester substructure. And same for the signal **a**. Regarding the proximity of the substituent to the tricyclic backbone, we expected to find through-space contacts between the ethyl ester group and the proton signal **a'**, but not to proton **a**. First of all an ^1H - ^1H -COSY experiment was measured at $-80\text{ }^\circ\text{C}$ (creffig:bet-cosy), where all aromatic signals could be categorized to the left (**a** - **d**) or the right (**a'** - **d'**) substructure.

Figure V.1.2.2: ^1H - ^1H -COSY of **35**. 2.8 mM in d^8 -THF at $-80\text{ }^\circ\text{C}$. The enlarged NMR segment shows the coupling between the aromatic proton signals **a**-**d** and **a'**-**d'**.

The ^1H - ^1H -NOESY experiment at $-80\text{ }^\circ\text{C}$ allowed us to assign the signals **a** and **a'** to the right proton position in **B^{Et}** (**35**), as we found through-space contacts between the ethyl ester group (**e** and **f**) and the proton signal **a'**, but not to proton **a** (figure V.1.2.3). Furthermore, smaller cross peaks were observable between ethyl ester signal **e** and **b'**, as well as between **f** and **b'** and even **c'**. Therefore, it is quite clear that the less shifted signals (**a'**-**d'**) belonging to the site of the aromatic compound closest to the ester group, while (**a**-**d**) is on site of the cyano substituent.

Figure V.1.2.3: ^1H - ^1H -NOESY of **35**. 2.8 mM in d^8 -THF at -80°C . In the enlarged section A the contacts between the CH_3 group of the ethyl ester group (signal f) and the aromatic signals a', b' and c' are shown. The enlarged section B shows the contact of the aromatic proton signals a' and b' with the methylene group (signal e) of the ethyl substituent.

Although it was clear at this point, that the dynamic within the molecule seems to depend on the temperature and that the motion can drastically decelerated by cooling down the whole system, the question still remained what kind of dyanmic motion is responsible for this effect. In similar systems^[24,247] a flipping motion of the aromatic scaffold was reported .In our case a flipping would not explain the observed chemical exchange between a' and a in the NMR. Regarding the reported twist of the dicyano substituent in a dianionic form of an acirdinylidene derivate by Sutherland et al.^[248] and the proposed zwitterionic structure of Dimroth,^[239] a rotation of the cyano acetate ester is therefore the most likely explanation. The zwitter-ionic state occurs due to the intramolecular push-pull system between the rather electron-rich nitrogen and the electron-withdrawing substituent.

While having the charges separated, the former double bond that connect the stator and rotor, getting temporarily a single bond character, by what the substituent can rotate freely.

V.1.2.2 Influence of Temperature on Rotation

To get a better idea what parameters can have influence on the rotation motions, two further derivatives were synthesized. The *tert*-butyl derivative (**34**), should have similar influence on the system as the ethyl derivative, but is more bulky. In contrast, the phenyl derivative should show a different effect on the electronics compared to the aliphatic esters residues, while having a similar demand as the *tert*-butyl group. In figure V.1.2.4a all the aromatic region in ^1H NMR at room temperature for all three derivatives are shown.

Figure V.1.2.4: Comparison of **34**, **35** and **37**. All ^1H -NMR spectra were recorded in $\text{d}^3\text{-MeCN}$ at 298 K. Aromatic signals of phenyl group are asterisked. Reprinted with permission from reference.^[249] Copyright © 2017 Wiley-VCH.

Considering again the spectra in figure V.1.1.1, it becomes quite obvious that the slower rotation in **L^{Et}** is indicated by the broadening of signal **a/a'**, whereas the ester's less electron-withdrawing characteristic causes a fewer chemical shift of the signal **a/a'**. Keeping this in mind, the least shifted but most broadened signal **a/a'** in the spectrum of **B^{tBu}** (**34**), shows qualitatively as decrease in rotation, as well as a slight more electron-pushing effect of the *tert*-butyl group, as compared to **B^{Et}** (**35**). In contrary, **B^{Ph}** (**37**) has a higher chemical shift and sharper signal **a/a'** under the same measuring conditions, thus, it exhibits probably a higher spinning rate and has a higher electron-withdrawing character, as compared to the ethyl ester derivative. Therefore, it is obvious, that the size seems to have rather a minor influence on the rotation. The influence of the ester substituent on the intramolecular electronics seems to be the predominant effect, that changes the rate of rotation. Next, the influence on temperature on the three derivatives was investigated. For

that reason VT NMR measurement were made from -90°C to 60°C in 10°C steps. All VT NMR measurements were made in d^8 -THF, since it showed the best solubility, while giving the best range of measurable temperatures for the system. Figure V.1.2.5 shows the VT NMR measurement of **B^tBu** (**34**). First thing to be noticed is that a splitting into the signals **a** and **a'** already occurs a room temperature. The broadened signal at with chemical shift of 8.55 ppm belongs to the proton **a** and 7.55 ppm to the proton **a'**. In comparison, the same compound showed in d^3 -MeCN a single broadened signal at ca. 8.0 ppm. Hence, the solvent polarity seems to be another factor to influence the rotation in the system, which is described in section V.1.2.3.

Figure V.1.2.5: VT- ^1H NMR (400 MHz) from -90°C to 60°C of **B^tBu** 2.8 mM in d^8 -THF. Signals of protons **a/a'** are highlighted in red. Signals of protons **b/b'** are highlighted in green. The coalescence cannot be clearly determined, but lies approximately between 50°C and 60°C

As expected signals (**a/a'**) are separated at very low temperatures. The difference in chemical shift is ca. 1.00 ppm (400 Hz). Likewise, the nearest neighbored protons (**b/b'**) show as well a significant difference in chemical shift, ca. 0.13 ppm (50 Hz). Since we could not observe the merged signal above coalescence, the T_C is guessed and estimated to be between 50°C and 60°C . Compared to **B^tBu** (**34**), **B^{Et}** (**35**) has a bigger difference in chemical shift between the signals **a** and **a'** (ca. 1.13 ppm or 450 Hz), as well as for the signals of **b** and **b'** (0.18 ppm or 73 Hz). For this case the coalescence temperature is approx. 20°C . Furthermore, it is worth to mention that we observed a splitting of the ester's methylene signal **e** in **B^{Et}** (**35**) at very low temperatures (figure V.1.2.6, highlightet in dark green), which gave a first hint to a further dynamic process within the system, since the acutal rotation is already frozen-out. The signal splitting probably occurs due to inequivalence of the now diastereotopic protons **e_α** and **e_β**, caused by the molecule's deviation from a flat structure and emergence of axial chirality in the frozen-out

conformation (figure V.1.2.20. Rapid flipping between both degenerate conformations occurs at ambient temperatures but slows down at the lowest temperatures reached in our NMR investigation. Estimation of the flipping frequency ($k_{\text{flip}} = \frac{\pi \Delta \nu_0}{\sqrt{2}}$)^[250] gave a value of about 100 Hz at a T_C of -75°C with an maximal peak separation ($\Delta \nu_0$) approximated to 45 Hz.

Figure V.1.2.6: VT- ^1H NMR (400 MHz) from -90°C to 60°C of B^{Et} 2.8 mM in $\text{d}^8\text{-THF}$. Signals of protons **a/a'** are highlighted in red. Signals of protons **b/b'** are highlighted in green. Furthermore, the signal of the methylene group of the ethyl ester residue (CH_2CH_3) is additionally highlighted in dark green. Coalescence temperature is approx. 20°C .

This flipping motion was already described for a similar systems that contains a bent acridinylidene motif,^[24] mentioned in chapter V.1, carrying sterically demanding substituents at this position. As it already become apparent in figure V.1.2.4a, B^{Ph} (**37**) has a much faster rotation rate compared to the other both derivatives at the same temperature. In this case it was not possible to frozen-out the rotation, since even at -90°C the signals **a/a'** are already separated but still quite broad. And for the signals **b/b'** a splitting was not even observable.

Figure V.1.2.7: VT- ${}^1\text{H}$ NMR (400 MHz) from $-90\text{ }^\circ\text{C}$ to $60\text{ }^\circ\text{C}$ of **B^{Ph}** 2.8 mM in d^8 -THF. Signals of protons **a/a'** are highlighted in red. Signals of protons **b/b'** are highlighted in green. The coalescence temperature is approx. $-55\text{ }^\circ\text{C}$

The small change of the ester substituent causes already a difference in T_C of roughly $40\text{ }^\circ\text{C}$ between **B^{tBu}** and **B^{Et}**. Going to the phenyl derivative the difference to the next highest coalescence temperature becomes even $75\text{ }^\circ\text{C}$. Therefore the rotation motion predominantly dependent on the intramolecular push-pull system. Furthermore it is obvious that all dynamic motions in the here investigated derivatives are also dependent on temperature. By chance, a further flipping motion is revealed in VT NMR experiment due to the splitting of diastereotopic proton signals **e_α** and **e_β** in **B^{Et}**. In the next section the above mentioned dependency on the solvent polarity is further investigated.

V.1.2.3 Influence of Solvent Polarity on Rotation

As already implied in the previous section, there seems to be not only dependencies from temperature or electronic situation of the ester substituents on the rotation, but from the polarity of the used solvent as well. To get a better understanding of what effect the solvent polarity has on the speed of rotation, NMR and UV-vis experiments in different solvents were performed and compared. The system **B^{tBu}** is in *d*³-MeCN above coalescence temperature, indicated by a broadened but discrete signal for **a/a'**. Lowering the solvent polarity by using chloroform, pushes the coalescence temperature to higher temperatures, therefore the system is closer to coalescence temperature at 25 °C and the signal **a/a'** is more broadened. THF has the lowest solvent polarity, hence the system has the highest coalescence temperature *T_C* with 50-60 °C (figure V.1.2.5). Thus, the system must be definitively below *T_C* at 25 °C and indeed **a** and **a'** are split but still quite broadened. The signal **a'** is lying under the signals **c/c'** and **d/d'**.

Figure V.1.2.8: a) ¹H-NMR (400MHz) of **B^{tBu}** in various solvents of different polarity at 298 K. The polarity decreases from upper the spectrum to the bottom one. The proton signals **a/a'** and **b/b'** are highlighted in red. The sharpness of signals decrease with solvent polarity, qualitatively correlating with a decrease of the rotational frequency. b) UV-vis absorbance of **B^{tBu}** 0.005 mM in various solvents of different polarities. The system shows the highest bathochromic shift in water $\lambda_{\text{max}}(\text{H}_2\text{O}) = 454 \text{ nm}$ and highest hypsochromic shift in hexane $\lambda_{\text{max}}(\text{hexane}) = 436 \text{ nm}$. c) **B^{tBu}** 0.005 mM in various solvents of different polarities, see V.1.2.8b. Solvent polarity decreases from left (H_2O) to right (hexane).

In the UV-vis experiments various spectra of **B^{tBu}** in different solvent were recorded, reaching from very low solvent polarity (hexane) to a very high solvent polarity (H_2O).

Considering the comparison of all three systems in acetonitrile (figure V.1.2.4b), **B^{tBu}** (**34**) is expected to absorb at the lowest wavelength. From hexane ($\Delta_{\text{max}} = 436$ nm to water ($\Delta_{\text{max}} = 454$ nm the system undergo a bathochromic shift of 18 nm (figure V.1.2.8b). The color of the solution go from slightly greenish to yellow as seen in figure V.1.2.8c. **B^{Et}** (**35**) is settled in a similar range, it shows an absorbance of $\Delta_{\text{max}} = 439$ nm in hexane and $\Delta_{\text{max}} = 461$ nm in water, thus it has an bathochromic shift of 22 nm, what is only a slight change of the absorption behavior compared to **B^{tBu}** (figure V.1.2.9b).

Figure V.1.2.9: a) ^1H -NMR (400MHz) of **B^{Et}** in various solvents of different polarity at 298 K. The polarity decreases from the upper spectrum to the bottom one. The proton signals **a/a'** and **b/b'** are highlighted in red. The sharpness of signals decrease with solvent polarity, qualitatively correlating with a decrease of the rotational frequency. In d^8 -THF the a/a' signal is too broad to be distinguished from noise. b) UV-vis absorbance of **B^{Et}** 0.005 mM in various solvents of different polarities. The system shows the highest bathochromic shift in water $\lambda_{\text{max}}(\text{H}_2\text{O}) = 461$ nm and highest hypsochromic shift in hexane $\lambda_{\text{max}}(\text{Hexane}) = 439$ nm.

Likewise, the NMR shows, similar to the UV-vis, rather tiny changes (figure V.1.2.9a). In THF the coalescence temperature T_C is around 20 °C, therefore the signal **a/a'** is extremely broadened and not observable. The signals **a/a'** are merely sharpened in the other two NMR spectra. As expected, all signals of the **a/a'** protons are above coalescence in the recorded NMR spectra (figure V.1.2.10a) and the UV-vis spectra show a bathochromic shift, but double as high as these of **B^{tBu}** and **B^{Et}** with 42 nm (figure V.1.2.10b). Since the absorption in more polar solvent occurs at wavelength up to 493 nm), the colors of the used solution go from yellow, for low solvent polarity, to orange (figure V.1.2.10c).

Figure V.1.2.10: a) ^1H -NMR (400MHz) of B^{Ph} in various solvents of different polarity at 298 K. The polarity decreases from the upper spectrum to the bottom one. The proton signals **a/a'** and **b/b'** are highlighted in red. The sharpness of signals decrease with solvent polarity, qualitatively correlating with a decrease of the rotational frequency. b) UV-vis absorbance of B^{Ph} 0.005 mM in various solvents of different polarities. The system shows the highest bathochromic shift in water $\lambda_{\text{max}}(\text{H}_2\text{O}) = 493 \text{ nm}$ and highest hypsochromic shift in hexane $\lambda_{\text{max}}(\text{Hexane}) = 451 \text{ nm}$. c) B^{Ph} 0.005 mM in various solvents of different polarities, see V.1.2.10b. Solvent polarity decreases from left (H_2O) to right (hexane).

From the here presented results, it can be concluded so far that the dynamic rotation really takes place due to the proposed charge-separated transition state induced by the intramolecular push-pull system. All three systems show positive solvatochromism upon increase of solvent polarity from hexane to H_2O . B^{Ph} shows the largest bathochromic shift, B^{tBu} the smallest ($\text{B}^{\text{tBu}} < \text{B}^{\text{Et}} < \text{B}^{\text{Ph}}$). The effect of solvent polarity can also be seen in ^1H NMR measurements, since in unpolar solvents a clear broadening of the signals **a/a'** and **b/b'**, closest to the rotor, is observed. The low solvent polarity disfavors the charge separation state, hence the single bond character, accompanied by a decrease of rotation rate. In the case of B^{tBu} the rotation is decelerated to a spinning rate that is slow enough to observe a splitting of the signal **a/a'** into two distinguishable signals in ^1H NMR.

V.1.2.4 Determination of activation enthalpy and entropy

In order to obtain quantitative information about the thermodynamics of the intramolecular rotational motion, the principle of DNMR was used to obtain rate constants of rotation k_{rot} at various temperature from VT NMR experiemnts. With the help of the *Full Line Shape Analysis*,^[250] the rotation rate can be calculated from the peak widths of the exchanging proton signals. Since the rotation changes dependent on the measured temperature, the peak widths of every single peak of the observed exchanging signals $\mathbf{a}/\mathbf{a'}$ and $\mathbf{b}/\mathbf{b'}$ were extracted from the different temperatures in the NMR spectra (figures V.1.2.5 to V.1.2.7). With the equations (equations (V.1.1) to (V.1.3)) the rate constants could be calculated. For slow exchange, means signals below coalescence temperature T_C the equation (V.1.1) was used. For fast exchange or signals above T_C , equation (V.1.3) is applied. It should be noted that signals with small differences in chemical shift need different equations, both above and below T_C . But since the gap between the exchanging signals is quite big, the signals around T_C become very broadened and hardly distinguishable from noise, thus it is hard to determine the peak width. To avoid thereof resulting mistakes, these signals were not considered (figure V.1.2.11c).

$$k = \frac{\pi}{\sqrt{2}}(\Delta\nu_0^2 - \Delta\nu_e^2)^{\frac{1}{2}} \quad (\text{V.1.1})$$

$\Delta\nu_0$ = Distance between signals of exchange protons when no rotation occurs

$\Delta\nu_e$ = Distance between observed signals of exchange protons

$$k = \frac{\pi\Delta\nu_0}{\sqrt{2}} \quad (\text{V.1.2})$$

$$k = \frac{\pi\Delta_0^2}{2}((\Delta\nu_e)_{1/2} - (\Delta\nu_0)_{1/2})^{-1} \quad (\text{V.1.3})$$

$(\Delta\nu_0)_{1/2}$ = FWHM peak no rotation

$(\Delta\nu_e)_{1/2}$ = FWHM observed peak

Figure V.1.2.11: a) Determination of rotational constants from NMR data of **B^tBu**. The spinning rates were determined with full line shape analysis from experimental data at different temperatures. b) Additionally, rate constants were also obtained from DNMR simulations of the proton signals **a/a'** and **b/b'**, which are closest to the rotor group and show the biggest signal splitting.

Additionally, all measured spectra were simulated with SpinWorks^[251] and the rotation rates were also extracted from the simulations to compare them with the calculated values. Due to the restriction in the number of simulated exchanging nuclei to a total amount of five, the protons signals of **a/a'** and **(b/b')** were only simulated, since they show the biggest dynamic effect in the NMR spectra. To obtain the simulations as accurate as possible, chemical shift, coupling constants and peak-width of the simulated signals were fitted to signals of measured spectra at -90 °C, assuming that no rotation occurs anymore. For **B^{Ph} (37)** this was not possible, for this reason the chemical shifts for the non rotating situation were roughly estimated.

With help of the Eyring-Polanyi equation,^[252] equation (V.1.4), it is possible to obtain from kinetic value k_{rot} and the temperature the thermodynamic values: enthalpy of activation $\Delta^\ddagger H^\ominus$ and the entropy of activation $\Delta^\ddagger S^\ominus$ (equation (V.1.6)).

$$k = \frac{\kappa k_B T}{h} \exp\left(-\frac{\Delta^\ddagger G^\ominus}{RT}\right) \quad (\text{V.1.4})$$

$$\Delta^\ddagger G^\ominus = \Delta^\ddagger H^\ominus - T\Delta^\ddagger S^\ominus \quad (\text{V.1.5})$$

$$k = \frac{\kappa k_B T}{h} \exp\left(\frac{-\Delta^\ddagger H^\ominus}{RT} + \frac{\Delta^\ddagger S^\ominus}{R}\right) \quad (\text{V.1.6})$$

converted to equation (V.1.7), plotting $\ln(kT^{-1})$ versus T^{-1} gives a linear correlation, where $\Delta^\ddagger H^\ominus$ can be extracted from the slope of the plot and $\Delta^\ddagger S^\ominus$ from the y-intercept.

$$\ln \frac{k}{T^{-1}} = \frac{-\Delta^\ddagger H^\ominus}{R} \cdot \frac{1}{T} + \ln \frac{\kappa k_B}{h} + \frac{\Delta^\ddagger S^\ominus}{R} \quad (\text{V.1.7})$$

Figure V.1.2.12: Determination of rotational constants from NMR data of $\mathbf{B}^{\text{Et}}$. The spinning rates were determined with full line shape analysis from experimental data at different temperatures. **b)** Additionally, rate constants were also obtained from DNMR simulations of the proton signals $\mathbf{a/a'}$ and $\mathbf{b/b'}$, which are closest to the rotor group and show the biggest signal splitting.

Experimentally determined rotation constants k_{rotexp} , as well as from simulation k_{rotsim} were plotted (figure V.1.2.11b - V.1.2.13c) and the thermodynamic values $\Delta^\ddagger H^\ominus$ and $\Delta^\ddagger S^\ominus$ were calculated from linear fit of plotted data points. The activation entropies and enthalpies received from experimental determination and simulation (table V.1.2.1) are in good agreement to each other. As expect, the enthalpy for the rotation decreases from $\Delta H^\ddagger = 39.0 \text{ kJ mol}^{-1}$ for the tert-butyl derivative to $\Delta H^\ddagger = 34.3 \text{ kJ mol}^{-1}$ to the fastest rotor with an activation enthalpy of $\Delta H^\ddagger = 26.3 \text{ kJ mol}^{-1}$. Since the rotation rate k_{rot} increasing at higher temperature and decreases at lower temperatures it is obviously an endothermic process, therefore the positive value of $\Delta^\ddagger H^\ominus$ is in full accordance with the empirical evidences.

Figure V.1.2.13: Determination of rotational constants from NMR data of **B^{Ph}**. The spinning rates were determined with full line shape analysis from experimental data at different temperatures. **b)** Additionally, rate constants were also obtained from DNMR simulations of the proton signals **a/a'** and **b/b'**, which are closest to the rotor group and show the biggest signal splitting.

Interestingly, all derivatives show all nearly the same negative value for the entropy of activation ($-72 \text{ kJ mol}^{-1} \text{ K}^{-1}$), which indicates that the pathway for the rotation has a similar nature in all derivatives. The negative value could be explained by the rearranging of the surrounding solvent molecules to a higher ordered structure, due to the formation of the assumed charged-separated transition state.^[253] Comparing the entropies of activation for all derivatives the order is as expected as in all previous experiments ($\text{B}^{\text{Ph}} > \text{B}^{\text{Et}} > \text{B}^{\text{tBu}}$).

Table V.1.2.1: Activation enthalpies, entropies and Gibbs' free energies of $\mathbf{B}^{t\text{Bu}}$, $\mathbf{B}^{\text{Et}}$ and $\mathbf{B}^{\text{Ph}}$ determined from experimental and simulated rate constants.

	$\mathbf{B}^{t\text{Bu}}$ (34)	$\mathbf{B}^{\text{Et}}$ (35)	$\mathbf{B}^{\text{Ph}}$ (37)
$\Delta H_{\text{exp}}^{\ddagger}$ [kJ mol ⁻¹]	39.0 ± 0.7	34.3 ± 1.3	26.3 ± 0.8
$\Delta S_{\text{exp}}^{\ddagger}$ [kJ mol ⁻¹ K ⁻¹]	-70.8 ± 2.9	-72.5 ± 5.2	-71.4 ± 3.3
$\Delta G_{\text{exp}}^{\ddagger 298\text{K}}$ [kJ mol ⁻¹]	60.1	56.0	47.5
$\Delta H_{\text{sim}}^{\ddagger}$ [kJ mol ⁻¹]	37.8 ± 0.3	33.1 ± 0.5	24.4 ± 0.5
$\Delta S_{\text{sim}}^{\ddagger}$ [kJ mol ⁻¹ K ⁻¹]	-74.9 ± 1.2	-76.6 ± 1.9	-75.5 ± 1.9
$\Delta G_{\text{sim}}^{\ddagger 298\text{K}}$ [kJ mol ⁻¹]	60.1	56.0	46.9

V.1.2.5 Cyclic voltammetry

Since the dynamic motion is caused by an induced charge-separation within the intramolecular push-pull system, it was interesting to investigate how the electrochemical properties would change between the three derivatives. Cyclic voltammetry measurements were performed for that reason in dichloromethane with 1 molar tetra-*n*-butyl ammonium tetrafluoroborate. The cyclic voltammograms of **B^{tBu}**, **B^{Et}** and **B^{Ph}** (figures V.1.2.14b to V.1.2.14d) show all together a fully reversible reduction between -1.3 V and -1.6 V (vs. Ag/AgNO₃) with an increasing anodic shift from the *tert*-butyl to the phenyl derivate. The cyclic voltammograms (figures V.1.2.14b to V.1.2.14d) of all derivatives show a fully reversible reduction between -1.3 V and -1.6 V.

Figure V.1.2.14: a) Cyclic voltammogram of Ferrocene, 2.8 mM in DCM, 0.1 M nBu₄BF₄, recorded with a scan rate of 100 mV s⁻¹. b) Cyclic voltammogram of **B^{tBu}**, 2.8 mM in DCM, 0.1 M nBu₄BF₄, recorded with a scan rate of 100 mV s⁻¹. c) Cyclic voltammogram of **B^{Et}**, 2.8 mM in DCM, 0.1 M nBu₄BF₄, recorded with a scan rate of 100 mV s⁻¹. d) Cyclic voltammogram of **B^{Ph}**, 2.8 mM in DCM, 0.1 M nBu₄BF₄, recorded with a scan rate of 100 mV s⁻¹.

The measured voltages for reduction and oxidation were referenced versus Fc/Fc⁺, as internal standard to make them better comparable for the literature (table V.1.2.2.^[254])

Comparison of the cyclic voltammetric measurements is shown in figure V.1.2.15. It is obvious that an increasing anodic shift happen from -1.66 V for $\mathbf{B}^{tBu}$, over -1.60 V for $\mathbf{B}^{Et}$ to -1.45 V for $\mathbf{B}^{Ph}$ (all vs Fc/Fc^+). This observation is in full agreement with the absorption spectroscopic investigations (figure V.1.2.4b).

Figure V.1.2.15: Comparison of cyclic voltammetry measurements of all derivatives. Potentials corrected to Fc/Fc^+ . An anodic shift about 210 mV is observed from $\mathbf{B}^{tBu}$ to $\mathbf{B}^{Ph}$.

Table V.1.2.2: Reduction and oxidation voltages for $\mathbf{B}^{tBu}$, $\mathbf{B}^{Et}$, $\mathbf{B}^{Ph}$ extracted from cyclic voltammetry experiments. $E_{1/2}$ values were referenced to Fc/Fc^+ . Since the symmetrically substituted $\mathbf{B}^{CN}$ was not available for CV the values were taken from literature.^[241,255]

	E_{red} [V]	E_{red} [V]	$E_{1/2}$ [V]	$E_{1/2}$ vs. Fc/Fc^+ [V]
Fc/Fc^+	0.11	0.38	0.24	
$\mathbf{B}^{CN}$			-1.07 ^[241]	-1.41 ^[255]
$\mathbf{B}^{Ph}$	-1.32	-1.10	-1.21	-1.45
$\mathbf{B}^{Et}$	-1.49	-1.23	-1.36	-1.60
$\mathbf{B}^{tBu}$	-1.54	-1.28	-1.42	-1.66

To compare the experimental obtained energy values for the HOMO/LUMO gaps (E_g) from spectroscopic measurement (section V.1.2.2) and cyclic voltammetry with those obtained from DFT calculations (section V.1.2.7), additional CV measurements were made from -2.0 V to +2.0 V. From the CV measurements (figure V.1.2.16) the oxidation E_{onset}^{ox} and reduction E_{onset}^{red} onset for every derivative was graphically determined by taking the

potentials from the the intersection of the tangents between the baseline and the signal current (red dashed lines).^[256]

Figure V.1.2.16: Full cyclic voltometric measurements for all the derivatives **a)** B^{tBu} (34), **b)** B^{Et} (35) and **c)** B^{Ph} (37) (2.8 mM in DCM (nBu)₄BF₄) recorded with a scan rate of 100 mV s⁻¹ from -2.0 V to 2.0 V.

With these values the HOMO and LUMO energies were calculated with help of following equation:^[257] The calculated energies can be found in table V.1.2.3.

$$E_{HOMO/LUMO}(eV) = -(E_{onset}^{ox/red} - E_{1/2}(Fc/Fc+)) + 4.8eV \quad (V.1.8)$$

V.1.2.6 Crystal Structures Analysis

To get an better idea of the spatial arrangement of the here investigated derivates, crystalization attemps were made, to obtain crystals suitable for x-ray diffracton measurements. For that reason soltuions of $\mathbf{B}^{tBu}$, $\mathbf{B}^{Et}$ and $\mathbf{B}^{Ph}$ in various solvents (THF, CH_2Cl_2 , CHCl_3 , MeCN) were prepeared for either slow evaporation or liquid-liquid diffusion. Crystals of $\mathbf{B}^{tBu}$ and $\mathbf{B}^{Et}$ were obtained by liquid-liquid diffusion of MeOH into a solution of the respective compound in MeCN. For $\mathbf{B}^{tBu}$ only an derivate with iodine on the 2- and 7-postion ($\mathbf{I}_2\text{-}\mathbf{B}^{tBu}$) was suitable for x-ray crystal analysis. Both structures are shown in figure V.1.2.17. Additionally the already existing crystal structure of the dicyano derivate ($\mathbf{B}^{CN}$) is included,^[241,247] which resembles the hexyl chain derivate, although it merely has a butyl chain.

Figure V.1.2.17: Comparison crystal structures of acridinylidene derivatives, from left to right: *butyl*- $\mathbf{B}^{CN}$, $\mathbf{B}^{Et}$, $\mathbf{I}_2\text{-}\mathbf{B}^{tBu}$. The dicyano derivative crystal structure is already known and is shown here for a better comparison of the rotation group influence on the whole structure. The *tert*-butyl residue, a crystal with iodine on the 2- and 7-postion of the acridone was only suitable for X-ray crystal structure analysis. The dicyano acridinylidene derivative shows the fewest bending of tricyclic backbone, whereas the derivative with the *tert*-butyl ester shows the biggest bending of the backbone. Furthermore, compared to the symetric dicyano derivative, the others show a slight tilt of the rotating group in respect to the backbone.

It is easy to see that the molecules have a slightly bent butterfly structure, where the bending of the aromatic stator is increasing from the dicyano derivative to the *tert*-butyl one. $\mathbf{B}^{tBu}$ shows the most bent structure with an angle of $\Theta = 149^\circ$ (section V.1.2.6, whereas $\mathbf{B}^{Et}$ has an angle of $\Theta = 154^\circ$ and $\mathbf{B}^{CN}$ only of $\Theta = 164^\circ$. It is apparent that the derivatives show again the same order as in previous experiment. Regarding this order one would expect the structure of $\mathbf{B}^{Ph}$ to be in between $\mathbf{B}^{CN}$ and $\mathbf{B}^{Et}$. Furthermore, all structures show a twisted rotor with regard to the stator, also increasing from $\mathbf{B}^{CN}$ to

B^{tBu}. The dihedral angle for **B^{tBu}** is $\varphi = 19^\circ$, decrease for **B^{Et}** to $\varphi = 11^\circ$ and **B^{CN}** almost does not show any twist with $\varphi = 2^\circ$. The twist probably occurs as a reaction of the system to minimize the repulsion between the rotating substituent and the aromatic protons next to the bridging double bond.

Figure V.1.2.18: Angles of bended backbones extracted from the obtained crystal structures. The **I₂-B^{tBu}** shows the biggest bending with a angle of 149° . The other derivatives are as expected less bended with angles of 156° for **B^{Et}** and 164° for **B^{CN}**. **B^{Ph}** is expected to be between **B^{Et}** and **B^{CN}**.

Due to the asymmetric rotor group and the bent tricyclic aromatic stator, the structure should owe an axial chirality what give rise to the existence of enantiomeric conformations of all investigated derivatives. In the case of **B^{Et}** the asymmetric unit contained four molecules, and luckily two molecules of both enantiomers were found (figure V.1.2.19). In the crystal structure the enantiomers can exist side by side long enough to be distinguishable, since the dynamics are frozen-out. In solution, however, both enantiomers are quickly interconverting by either the rotation or the flipping motion.

Figure V.1.2.19: The asymmetric unit of the measured crystal of **B^{Et}** contains all in all four molecules, two of each enantiomer. The single enantiomers are shown in (figure V.1.2.20).

Moreover, the conformation shown in the crystal structure of **B^{Et}** (figure V.1.2.20) supports the assumption of an additional flipping motion in the molecules, since the methylene protons of the ester substituent also show a distinguishable chemical environment (e_α and e_β), exactly those protons which show a splitting in the VT NMR experiments at very low temperatures, when rotation was already frozen-out. Additional analysis of the bond lengths in the x-ray structure indicates a quinoidal character for **B^{Et}**, what indicates that the bridging connection between rotor and stator exist with a prevalent double bond character in solid state.

Figure V.1.2.20: The two existing enantiomers of **B^{Et}**, extracted from the asymmetric unit of the measured crystal. The diastereotopic protons, which are probably responsible for the splitting of the esters' methylene group in the VT-NMR of **B^{Et}** (figure V.1.2.6) are labelled with α and β . The hexyl chain is omitted for a better view.

V.1.2.7 Chemoinformatic Studies

Comparison of the UV-vis spectra of **B^{tBu}**, **B^{Et}** and **B^{Ph}** support this picture, since increasing the charge stabilization within the push-pull system goes along with a larger bathochromic shift (26 nm in MeCN from **B^{tBu}** to **B^{Ph}**) of the longest-wavelength maximum, corresponding to a decreasing magnitude of the HOMO and LUMO transition (figure V.1.2.4b).

To get a better understanding of the electronic situation and substituent effects in the system we performed density-function theory (DFT) geometry optimization for all derivatives (B3LYP/6-311+g(d,p) in PCM-MeCN) based on the crystal structure of **B^{Et}**. Furthermore, DFT transition state optimization (B3LYP/6-311+g(d,p)) were performed for all derivatives. Therefore, the structure of the transition state was assumed to be a flat tricyclic aromatic system with the rotor twisted 90 ° in respect to the stator, bridged by a single bond. Calculations gave for all three derivatives only a single imaginary frequency, which give a hint that the provided structure is similar to the real transition state, but do not necessarily represent reality.

Furthermore TD-DFT (CAMB3LYP-6311+g(d) in PCM-MeCN) calculations were employed to simulate UV-vis spectra (figure V.1.2.21) and assign the experimentally observed absorption bands to MO transitions.^[258,259] By default gaussian estimates a deviation of 0.4 eV from the calculated value.^[260] Therefore, the simulated spectra show pretty accurate predicted uv-vis band, although the the wavelength in 10 nm to short on average. Merely the phenyl derivative show higher deviation from the empirically observed wavelength, as well as the difference to **B^{Et}** is to small with only 7 nm instead of 22 nm. But this is probably attributed to the rather simple used level of TD-DFT calculation for here investigated system. However, it is worth to mention, that there are more accurate and complex methods that can better handle an intramolecular charge transfer.

Figure V.1.2.21: Simulated UV-vis spectra from TD-DFT calculations (CAM B3LYP-6311+g(d) in PCM-MeCN) for a) $\mathbf{B}^{tBu}$, b) $\mathbf{B}^{Et}$ and c) $\mathbf{B}^{Ph}$ made with Gaussian '09.^[261] d) Comparison of the simulated UV-vis spectra.

Table V.1.2.3: Comparison of HOMO-/ LUMO-energy levels for all three derivatives. All values are given in eV. [*] HOMO and LUMO energies were calculated from CV measurements (figure V.1.2.16). [**] Optical HOMO/LUMO gap were calculated from λ_{max} values observed in the UV-vis measurements.

	E_{HOMO} (DFT)	E_{LUMO} (DFT)	E_g (DFT)	E_{HOMO} (CV) ^[*]	E_{LUMO} (CV) ^[*]	E_g (CV)	E_g (opt) ^[**]
$\mathbf{B}^{tBu}$	-5.82	-2.58	3.24	-5.43	-3.28	2.15	2.76
$\mathbf{B}^{Et}$	-5.87	-2.66	3.21	-5.45	-3.33	2.12	2.73
$\mathbf{B}^{Ph}$	-5.94	-2.78	3.16	-5.47	-3.47	2.00	2.60

Anyway, as expected, modifications of the acceptor moiety mainly affects the LUMO energies with E_{LUMO} increasing from -2.78 eV for $\mathbf{B}^{Ph}$ over -2.66 eV for $\mathbf{B}^{Et}$ to -2.58 eV for $\mathbf{B}^{tBu}$. All values, empirically or *in silico* determined, are shown in table V.1.2.3. Although the values from CV measurements are underestimated, while the calculated ones are overestimated in respect to the energies received from UV-vis experiments, all together have the trend. Energy of HOMO-LUMO gap is decreased from $\mathbf{B}^{tBu}$ over $\mathbf{B}^{Et}$ to $\mathbf{B}^{Ph}$.

Figure V.1.2.22: Calculated frontier orbitals (B3LYP/6-311+g(d) in PCM-MeCN) of all examined derivatives in comparison. The energy level of the respective molecular orbitals is displayed next to the MO visualisation.

V.1.3 Protonation-Induced Fluorescence

Due to the idea of the charge-separated zwitterionic transition state, the question appeared if the negative charge, located at the rotating group, can be used to protonate the system. And if this is possible, what influence the different ester substituent have on the grade of basicity. Dimorth *et al.* described a possible protonation of a similar system with weak acids, associated with a change in color.^[239] First tries with weak acids like ammonium chloride and acetic acid didn't lead to the desired result. A slight change in color of the solution was observable, which is rather attributed to the change in the allover polarity of the solution due to the addition polar acids molecules, which leads to a better stabilization of the charge-separated state, as described in section V.1.2.3. Anyway, using hydrochloric acid, a change in color from dark red to dark green was observed. Unfortunately NMR delivered no usable information, since the spectrum showed a lot new signals which couldn't be assigned to any species. The usage of even stronger acids with non-coordinating conjugated bases, like trifluor acetic acid, showed a tremendous change in color. The addition of TFA led to change in color from dark red to slight greenish clear solution. Furthermore, upon irradiation with ultraviolet light at 365 nm the solution showed a strong fluorescence (figure V.1.3.23a). Interestingly, all derivatives (**34**, **35** and **37**) showed the same reaction upon the addition of an excess of TFA. Comparing the acidified solution in UV-vis, all three derivatives showed exactly the same spectra, with a absorption maxima at $\lambda_{\text{max}} = 260$ nm and a second one at $\lambda_{\text{max}} = 370$ nm (figure V.1.3.23c). Additional investigation of the solutions via NMR showed a very similar chemical shift of all aromatic protons, as well as for the methylene group attached to the aromatic nitrogen. These results leaves only the conclusion that in all three cases the similar structural motif must be formed. Comparing the measured UV-vis spectra with similar compounds from literature revealed that the observed absorption bands are highly characteristic for the acridinium motif, where a positive charge is located on the nitrogen of the tricyclic backbone.^[262,263]

Figure V.1.3.23: **a)** Protonation of B^{Et} with TFA leads to change in color (left). Upon irradiation with UV light ($\lambda = 365$ nm), the protonated species shows a strong fluorescence (right). **b)** Comparison 1H -NMR spectra of protonated derivatives. B^{tBu} (black), B^{Et} (green), B^{Ph} (orange). 2.8 mM in d^3 -MeCN with 0.2 M TFA. **c)** Comparison UV-vis spectra of protonated derivatives. B^{tBu} (black), B^{Et} (green), B^{Ph} (orange). 0.05 mM in MeCN with 0.2 M TFA.

V.1.3.1 Position of Protonation

In order to figure out where the protonation within the system occurs, the compounds **34**, **35** and **37** were investigated by NMR in acidified solutions. In scheme V.1.3.7 the two possible positions of protonation are proposed. In theory both positions are feasible, since in both cases the negative charge, generated in the charge-separated state, would be stabilized. Furthermore, in both cases the bridging double bond is reduced to single bond, by what the system is losing its chirality and the cyano ester group can rotate freely. However, compound **58** is a very unlikely system, since no comparable motif could be found in literature and was just here presented for the sake of completeness.

Scheme V.1.3.7: Protonation of **35**. The two possible species protonated species **35a** or **35b**, where the double bond is suspended.

Comparison of the NMR spectra of the non-protonated **B^{Et}** (**35**) and the protonated species, show a general downfield shift of all aromatic protons upon addition of acid. It is noticeable that signal for the hydrogen atoms of **d** and the methylene group **NCH₂**, both located closest to the aromatic nitrogen, experience the highest chemical downfield shift (figure V.1.3.24). What is in consensus with the proposed formation of an positively charged nitrogen within the acridinium motif. Additionally a new signal appeared at ca. 6.75 ppm (*). The new signal (*) corresponded to the protonated potions.

Figure V.1.3.24: Comparison of **35** and its protonated species **35a** or **35b**. 2.8 mM in *d*³-MeCN (top) and with additional 0.5 M TFA (bottom).

To find out the exact position, where the rotating groups are protonated, various ¹H and ¹³C NMR experiments were performed (figure V.1.3.25). After assigning all ¹H and ¹³C signals (figures V.1.3.25a and V.1.3.25b), the 2D NMR experiments led to the conclusion that **35a** was formed, as expected. Following findings speak for the formation of **35a**. First of all in the ¹H-¹H-COSY experiment (figure V.1.3.25c) only the diagonal peak is observable for signal *, what indicates that proton is isolated, since no coupling to other protons occurs. In the ¹H-¹³C-HSQC experiment, the signal (*) couples to signal 8, what is assumed to be the non-aromatic bridging carbon of the substituent (figure V.1.3.25d, therefore a protonation at the ester carboxy group can be excluded. The ¹H-¹³C-HMBC confirmed this assumption, since signal (*) couples to the carbon signals 6, 7, 9 and 10 what is only possible from assumed position.

Figure V.1.3.25: a) ¹H NMR (400 MHz) of protonated **B^{Et}** (**35a**) 2.8 mM in *d*³-MeCN with 140 mM trifluoroacetic acid (TFA). b) ¹³C-NMR (176 MHz) of protonated **B^{Et}** (**35a**) 2.8 mM in *d*³-MeCN with 140 mM TFA. The signals marked with x belong to the TFA molecules. Splitting of these signals occurs due to ¹³C-¹⁹F coupling. c) ¹H-¹H-COSY of protonated **B^{Et}** (**35a**) 2.8 mM in *d*³-MeCN with 140 mM TFA. The *-proton shows no coupling to other protons, what supports its isolated positioning at the rotor headgroup. d) ¹H-¹³C-HSQC of protonated **B^{Et}** (**35a**) 2.8 mM in *d*³-MeCN with 140 mM TFA. The *-proton couples to a C-atom, which is assumed to be at the position 8.

Figure V.1.3.26: ^1H - ^{13}C -HMBC of protonated **B^{Et}** (**35a**) 2.8 mM in d^3 -MeCN with 140 mM TFA. Since there are no protons that can couple to the C-atom at position 8, the coupling of the proton signal * to neighbored C-atoms were investigated. All 2J and 3J couplings of signal * are marked with green lines. Additionally, some other proton carbon couplings are highlighted to confirm the right assignment of the ^{13}C signals.

V.1.3.2 ^1H NMR titration experiments with TFA

In order to get more information about the process of protonation and especially the differences among the three derivatives **B^{tBu}**, **B^{Et}** and **B^{Ph}**, NMR titration experiments were performed (figures V.1.3.27, V.1.3.28 and V.1.3.30). In contrast to the VT NMR experiments from section V.1.2.2, all described experiments were in MeCN, due to solubility issues of the charged species in THF. Following the process of protonation it is noticeable, that **B^{Et}** seems to form the protonated species without any intermediates or byproduct, what is confirmed by UV-vis (section V.1.3.3). Furthermore, the reaction equilibrium is slowly shifted towards the protonated species with increasing TFA concentrations. At a concentration of approx. 13 mM first peaks of the protonated species appear for **B^{tBu}** (figure V.1.3.27) and **B^{Et}** (figure V.1.3.28). In contrast **B^{Ph}** first signal of the protonated species appear at a concentration of approx. 54 mM TFA (figure V.1.3.30), giving rise to the first evidence that the better stabilized negative charge is harder to be protonated. In the case **B^{tBu}** and **B^{Et}** the non-protonated species is not detectable anymore at a concentration above 130 mM, unluckily the steps in concentration are too far apart to make out a further difference between both derivatives. In contrary, **B^{Ph}** the signals of the non-protonated species disappearing first at higher concentration above 200 mM, as expected.

Figure V.1.3.27: ^1H NMR titration experiment of 2.8 mM **34** with TFA in deuterated MeCN. The titration was proceeded to an final TFA concentration of 0.5 M. It is clearly visible, that **34** (red) is converted into a new protonated species (green) by increasing the TFA concentration. All aromatic protons experience significant upfield shift, where signals for the protons **b**, **d** and NCH_2 show the highest upfield shift.

Figure V.1.3.28: ^1H NMR titration experiment of 2.8 mM **35** with TFA in deuterated MeCN. The titration was proceeded to an final TFA concentration of 0.5 M. It is clearly visible, that **35** (red) is converted into a new protonated species (green) by increasing the TFA concentration. All aromatic protons experience significant upfield shift, where signals for the protons **b**, **d** and NCH_2 show the highest upfield shift.

Additionally to the titration experiments, the reversibility of the protonation reaction of the system was also investigated. For this reason a further titration experiment was performed on B^{Et} , exemplaric for all derivatives (figure V.1.3.29). The compound solution was acidified with 125 equivalents of TFA till the derivative was completely protonated, subsequently 125 equivalents of the base triethylamine was added in several steps to

neutralize the excess of TFA and deprotonate the protonated species of **B^{Et}**. In fact, already after first addition of the base (25 equiv. NEt_3) the signals of **B^{Et}** reappeared. After the second addition (50 equiv. NEt_3) the deprotonated species was already completely formed back.

Figure V.1.3.29: ^1H NMR titration experiment of 2.8 mM **35** with TFA in deuterated MeCN. TFA was added until no unprotonated species (red) was detected in NMR anymore. After, NEt_3 was added until all TFA was completely neutralized and the unprotonated species was regained. This experiment proves the easy reversibility of the protonation process and the forth and back conversion between acridinyliene and acridinium species.

Figure V.1.3.30: ^1H NMR titration experiment of 2.8 mM **37** with TFA in deuterated MeCN. The titration was proceeded to an final TFA concentration of 1.05 M. It is clearly visible, that **37** (red) is converted into a new protonated species (green) by increasing the TFA concentration. All aromatic protons experience significant upfield shift, where signals for the protons **b**, **d** and NCH_2 show the highest upfield shift.

V.1.3.3 Determination of associations constants from UV-vis titration experiments with TFA

Additionally to the NMR experiments described in the previous section, the process of protonation for the derivatives ($\mathbf{B}^{t\text{Bu}}$, $\mathbf{B}^{\text{Et}}$, $\mathbf{B}^{\text{Ph}}$) was examined in UV-vis experiments. In all three cases TFA was added stepwise to a solution of ($\mathbf{B}^{t\text{Bu}}$, $\mathbf{B}^{\text{Et}}$ or $\mathbf{B}^{\text{Ph}}$ with an concentration of 0.035 mM, until no change in absorption was detectable. The absorption band at approx. $\lambda=450\text{--}470\text{ nm}$ show in all experiments a hypochromic shift as well as the absorption band $\lambda=290\text{ nm}$, whereas the acridinium specific absorption bands at $\lambda=370$ and $\lambda=260\text{ nm}$ arise. Moreover, isosbestic points can be found at the wavelength $\lambda_{\text{isosb}}=405\text{ nm}$, $\lambda_{\text{isosb}}=335\text{ nm}$ and $\lambda_{\text{isosb}}=270\text{ nm}$. This finding indicates a direct formation of the protonated species outgoing from starting material, in accordance with the ^1H NMR titration experiments described above (section V.1.3.2).

Figure V.1.3.31: **a)** UV-vis TFA titration of $\mathbf{B}^{t\text{Bu}}$ to protonated $\mathbf{B}^{t\text{Bu}}\text{-H}^+$ (**34a**), 0.035 mM in MeCN. Arrows indicate the hyper or hypochromic shift from low TFA – to high TFA concentration. **b)** Determination of association constant from UV-vis TFA titration experiment of $\mathbf{B}^{t\text{Bu}}$. Data was globally fitted with BindFit v0.5.^[264,265] Measured and fitted data points are shown for the wavelengths 259nm, 290nm, 369nm, 450nm. The determined constants are shown in table table V.1.3.4.

Figure V.1.3.32: **a)** UV-vis TFA titration of $\mathbf{B}^{\text{Et}}$ to protonated $\mathbf{B}^{\text{Et}}\text{-H}^+$ (**35a**), 0.035 mM in MeCN. Arrows indicate the hyper –or hypochromic shift from low TFA – to high TFA concentration. **b)** Determination of association constant from UV-vis TFA titration experiment of $\mathbf{B}^{\text{Et}}$. Data was globally fitted with BindFit v0.5.^[264,265] Measured and fitted data points are shown for the wavelengths 259nm, 290nm, 369nm, 450nm. The determined constants are shown in table V.1.3.4.

In accordance to the NMR titration experiments, increasing amounts of acid are needed to fully convert the different derivatives into their corresponding protonated species. While the protonated $\mathbf{B}^{\text{tBu}}$ was completely formed after 2300 equiv. of TFA (ca. 80 mM) (figure V.1.3.31). $\mathbf{B}^{\text{tBu}}$ already needed approx. 700 more equivalents (ca. 105 $\mathbf{B}^{\text{tBu}}$) to reach complete conversion (figure V.1.3.32). The highest concentration by far was used for $\mathbf{B}^{\text{Ph}}$, no change in absorption was reached after approx. 5000 equiv. TFA (ca. 190 mM) (figure V.1.3.33).

Figure V.1.3.33: **a)** UV-vis TFA titration of $\mathbf{B}^{\text{Ph}}$ to protonated $\mathbf{B}^{\text{Ph}}\text{-H}^+$ (**37a**), 0.035 mM in MeCN. Arrows indicate the hyper or hypochromic shift from low TFA – to high TFA concentration. **b)** Determination of association constant from UV-vis TFA titration experiment of $\mathbf{B}^{\text{Ph}}$. Data was globally fitted with BindFit v0.5.^[264,265] Measured and fitted data points are shown for the wavelengths 259nm, 290nm, 369nm, 450nm. The determined constants are shown in table V.1.3.4.

In order to determine the association constant of the protonation reaction for each derivative, the recorded data from UV-vis titration experiments was fitted with the help of BindFit.^[264,265] On that account the concentration was plotted versus the corresponding absorption for the wavelengths 259nm, 290nm, 369nm, 450nm. In figures V.1.3.31b, V.1.3.32b and V.1.3.33b the measured data is plotted together with the fitted data for corresponding derivate. All fits show a systematic divergence from the measured data, especially for the wavelength $\lambda=259$ nm. However, for the remaining wavelength the fit seems to match quite good. The association constants obtained from the fitted data are presented in table V.1.3.4. As expected the order association constant is $\mathbf{B}^{t\text{Bu}} > \mathbf{B}^{\text{Et}} > \mathbf{B}^{\text{Ph}}$. The easier the protonation can occur the higher the association constant. By implication, it is harder to protonate the better the negative charge is stabilized by the surrounding electron-withdrawing groups. Therefore, for the rotation, which is dependent on the stabilization of charge-separated structure, the order is reversed ($\mathbf{B}^{\text{Ph}} > \mathbf{B}^{\text{Et}} > \mathbf{B}^{t\text{Bu}}$).

Table V.1.3.4: Associations constants for protonation with TFA determined from UV-vis TFA titrations experiments for $\mathbf{B}^{t\text{Bu}}$, $\mathbf{B}^{\text{Et}}$ and $\mathbf{B}^{\text{Ph}}$.

	$\mathbf{B}^{t\text{Bu}}$	$\mathbf{B}^{\text{Et}}$	$\mathbf{B}^{\text{Ph}}$
$K_{\text{A}}^{\text{TFA}} [\text{mol}^{-1}]$	230.6 ± 19.6	118.7 ± 5.3	38.2 ± 1.7

V.1.3.4 Fluorescence titration experiments with TFA

Besides the change in color, the here investigated compounds became fluorophores upon protonation, with a strong green fluorescene emission with maximum at $\lambda^{\text{em}} = 490$ nm, caused by the formation of the acidinium motif.^[266] The dependency of the fluorescence properties on the concentration of acid was examined similar to the UV-vis experiments. To obtain the best excitation wavelength to get the maximal fluorescence, 3D fluorescence measurement were performed with 1 μM in presence of 72.5 mM TFA. In these measurement fluorescence spectra from 400 nm to 700 nm were recorded at various excitation wavelength ranging from 220 nm to 400 nm in 5 nm increments. The results of these experiments show for all derivatives a maximum emission wavelength of $\lambda^{\text{em}} = 490$ nm for the excitation wavelengths $\lambda^{\text{ex}} = 260$ nm and $\lambda^{\text{ex}} = 370$ nm (figures V.1.3.34a, V.1.3.35a and V.1.3.36a). It is noticeable that for both excitation wavelength the system is emitting at the same wavelengths ($\lambda^{\text{em}} = 490$ nm). Therefore it is pretty likely, that a non-radiative internal conversion takes place for the both excited states to the same lower energy level, before the radiative process occurs.

Figure V.1.3.34: a) 3D fluorescence measurement of 0.005 mM $\mathbf{B}^t\mathbf{Bu}$ in MeCN with 72.5 mM TFA. b) TFA fluorescence titration of 0.005 mM $\mathbf{B}^t\mathbf{Bu}$ in MeCN, λ^{ex} : 260 nm. c) TFA fluorescence titration 0.005 mM $\mathbf{B}^t\mathbf{Bu}$ in MeCN, λ^{ex} : 370 nm. d) Plot fluorescence intensity vs. TFA concentration of $\mathbf{B}^t\mathbf{Bu}$ for the excitation wavelengths $\lambda^{\text{ex}} = 260$ nm and $\lambda^{\text{ex}} = 370$ nm. Data was globally fitted with BindFit v0.5.^[264,265] Measured and fitted data points are shown for the emission measured with excitation wavelengths 260 nm and 370 nm.

Figure V.1.3.35: a) 3D fluorescence measurement of 0.005 mM B^{Et} in MeCN with 72.5 mM TFA. b) TFA fluorescence titration of 0.005 mM B^{Et} in MeCN, λ^{ex} : 260 nm. c) TFA fluorescence titration 0.005 mM B^{Et} in MeCN, λ^{ex} : 370 nm. d) Plot fluorescence intensity vs. TFA concentration of B^{Et} for the excitation wavelengths λ^{ex} = 260 nm and λ^{ex} = 370 nm. Data was globally fitted with BindFit v0.5.^[264,265] Measured and fitted data points are shown for the emission measured with excitation wavelengths 260 nm and 370 nm.

Figure V.1.3.36: a) 3D fluorescence measurement of 0.005 mM $\mathbf{B}^{Ph}$ in MeCN with 72.5 mM TFA. b) TFA fluorescence titration of 0.005 mM $\mathbf{B}^{Ph}$ in MeCN, λ^{ex} : 260 nm. c) TFA fluorescence titration 0.005 mM $\mathbf{B}^{Ph}$ in MeCN, λ^{ex} : 370 nm. d) Plot fluorescence intensity vs. TFA concentration of $\mathbf{B}^{Ph}$ for the excitation wavelengths $\lambda^{ex} = 260$ nm and $\lambda^{ex} = 370$ nm. Data was globally fitted with BindFit v0.5.^[264,265] Measured and fitted data points are shown for the emission measured with excitation wavelengths 260 nm and 370 nm.

Table V.1.3.5: Associations constants for protonation with TFA determined from fluorescence TFA titrations experiments for $\mathbf{B}^{tBu}$, $\mathbf{B}^{Et}$ and $\mathbf{B}^{Ph}$.

	$\mathbf{B}^{tBu}$	$\mathbf{B}^{Et}$	$\mathbf{B}^{Ph}$
K_A^{TFA} [mol ⁻¹]	365.6 ± 77.8	200.6 ± 44.1	0.07 ± 0.01

Based on the results from the 3D measurements, fluorescence titration experiments were performed at the above mentioned excitation wavelength. (figures V.1.3.34b, V.1.3.34c, V.1.3.35b, V.1.3.35c, V.1.3.36b and V.1.3.36c) Likewise to the UV-vis titration experiments the concentration of TFA was increased until no change in fluorescence intensity was observed, whereby, as before the amount of acid needed to reach this steady state increased from $\mathbf{B}^{tBu}$ to $\mathbf{B}^{Ph}$. The measured data plotted as fluorescence intensity vs. acid concentration and the curves are fitted with BindFit.^[264,265] Since BindFit is actually

not conceptualized for fluorescence data, the obtained association constants are not really expressive (table V.1.3.5), but explains the rather big deviation in comparison with the association constants determined by UV-vis. However, the values show the same trend as in the UV-vis experiments (figure V.1.3.37).

Figure V.1.3.37: a) Comparison of measured UV-vis data during TFA titration of all three derivatives **34**, **35** and **37** for the absorption wavelength 369 nm. b) Comparison of measured fluorescence data during TFA titration of all three derivatives **34**, **35** and **37** for the excitation wavelength 370 nm.

V.1.4 Conclusion

This chapter dealt with the syntheses of three asymmetrically substituted acridinylidene derivatives based on acridone, where the keton portion was substituted in a Knoevenagel-like reaction with various cyano acetate esters, without effecting the double bond situation. By introducing these electron-withdrawing substituents, an intramolecular push-pull system is formed, which induces a charge-separated state, by what the formal C=C double bond is significantly weakened and a spinning motion of the cyano acetate ester can occur. The rate of rotation is highly dependent on the electronic character of the ester substituent, temperature (section V.1.2.2) and solvent polarity (section V.1.2.3), what have been examined in various NMR and UV-vis experiments. Moreover, the choice of ester substituents revealed that the esters contribution to the electronic situation within the molecule has a predominant effect, towards its steric demand, on the rate of rotation. The rotation motion was observable by dynamic NMR spectroscopy, since a chemical exchange of the protons, closest to the asymmetric rotor occurs in terms of NMR time scale. As a result the NMR shows only one set of signals above coalescence temperature (high spinning rates) which is split into two signals below coalescence temperature (low spinning rate). Furthermore, kinetic data was extracted from VT NMR experiments with help of Full Line Shape Analysis, by what thermodynamic parameters $\Delta^\ddagger H^\ominus$ and $\Delta^\ddagger S^\ominus$ for each derivatives

could be determined. Thus, following order of derivatives can be made with regard to the rates of rotation: $\mathbf{B}^{t\text{Bu}} < \mathbf{B}^{\text{Et}} < \mathbf{B}^{\text{Ph}}$. Results from UV-vis (section V.1.2.3) and CV (section V.1.2.5) experiments as well as data from crystal structure analysis (section V.1.2.6) and DFT computational (section V.1.2.7) were found to be in full agreement with the NMR observations, showing a decrease of activation energy from electron donating to rather electron withdrawing ester substituents. Furthermore, treatment with trifluoro acetic acid induces strong fluorescence in all derivatives, due to reversible protonation (section V.1.3.2) of the bridging double bond between stator and rotor (section V.1.3.1), linked to the formation of an acridinium ion. Determination of the associations constants by UV-vis and fluorescence titration experiments (section V.1.3.3) revealed a reversed order ($\mathbf{B}^{t\text{Bu}} > \mathbf{B}^{\text{Et}} > \mathbf{B}^{\text{Ph}}$) for the ability to be protonated. What is in consensus with the previous results, since the better stabilized negative charge at carbon leads to a lower basicity. In the next chapter, the here described derivatives were integrated into banana-shaped ligands suitable for metal-mediated supramolecular cage formation. The supramolecular cages are investigated for their host-guest chemistry and what is the effect of guest uptake on the rotation motion.

Chapter V.2

Endohedral dynamics of push–pull rotor-functionalized cages

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V.2.1 Introduction

Dynamic processes can be observed in most functional structures in nature. Whole domains in proteins show dynamics which are triggered upon binding of substrates and can lead to a change in the overall structure and its reactivity.^[267] But also confined molecular environment can be found, which contain a combination of rigid and flexible elements, where the dynamics are often involved in the regulation of reactivity,^[268] adaption of structure for an specific binding^[269,270] or the shielding of catalytic sites.^[271] On account of these features, nature still can selectively perform reactions in highly complex biological system. Therefore, the investigation and understanding of those dynamics is crucial to develop and create new highly specialized systems for medicinal or industrial production purposes.

However, strategies for the development of adequate artificial environments are needed first, since the investigation within natural occurring systems is usually as complex as the acquisition. Especially the field of supramolecular chemistry transpired to be a potential supplier, as it provides a vast portfolio of organic structures with interesting non-covalently connected topologies, which can even be extended, by including metal-mediated self-assemblies. The latter turned out to be a particularly successful strategy for the generation of a large degree of structural and functional architectures, with helicates,^[272] entangled architectures^[273] and coordination cages,^[51] to be named as representatives. Yet, amongst those structures, the introduction of endohedral modifications remains a challenge and only few examples have been reported including inward-pointing hydrogen bond donors^[274] and acceptors,^[56] reactive residues inside helicates, cages and spheres,^[275,276] organocatalysts,^[277] and metal-coordination sites.^[278,279]

The term "*endohedral*" originally derives from the fullerene chemistry.^[280] Atoms,^[281] molecules,^[282] ions^[283] or clusters^[284,285] are encapsulated, rarely reversible, into fullerene, by what the inner volume is occupied, thus an structural modification of the fullerene is only possible on the outer shell. In covalent organic^[286] or non-covalent supramolecular constructs the situation is different. On account of the feasibility to create larger structures with more voluminous cavities, exohedral^[287,288] and endrohedral^[289–291] modifications are possible without losing the ability of guest encapsulation.^[24,292,293] In the last decades, countless self-assemblies with inlying cavities have been synthesized. These constructs were vigorously examined for their properties in selective recognition,^[294] host guest exchange,^[295,296] or catalytic conversions.^[297] Moreover, in recent years, the focus of research moved to the development of strategies for the incorporation of functionalities such as redox units,^[298] photo switches^[299,300] or specific binding sites.^[236] In addition complex supramolecular systems were equipped with advanced features, like stimuli-responsiveness, tuneable reactivity or allosteric regulation, functions mostly known from enzymes.

Clever et al. specialized in the structural motif of metal-mediated self-assembled coordination cages in form of $[\text{Pd}_n\text{L}_{2n}]$, which turns out to be a suitable for versatile structural and

functional application. Ranging from allosteric binding^[20] over light-responsive structural conversion^[300] and light induced charge separation^[301] to the formation of heteroleptic^[61,302] and also endohedral functionalized cages^[24,236,292,293]

First attempts to create endohedral functionalized $\text{Pd}_{2n}\text{L}_{4n}$ with banana-shaped ligands were shown by Freye *et al.*,^[26] Bulky aryl substituents were introduced at the keto position in the suberone-based ligands via Grignard addition. Although the endohedrally modified ligand does contain a hydroxy group, the mere presence of steric bulk led to the new behaviour towards its formation. Due to the change of available space, the ligand was not able to form a interpenetrated double cage in the presence of the initial BF_4^- anions. First the addition of one equivalent chloride anions was shifting the equilibrium towards a thermodynamically more stable double cage structure, where a single chloride anion occupied the inner cavity, due to the change in available space. In comparison to the unmodified suberone and acridone cage,^[20,27] the modified version showed a increased volume in the outer pockets and a smaller one for the inner pocket, where the chloride is located. The examination of the cages' spacial arrangement in crystal structure revealed that the endohedral substituents point rather to the sides of the cages than into central cavity. What is not surprising, since the carbonyl carbon exist in a sp^3 hybridized fashion after Grignard reaction took place (Figure V.2.1.1a). Therefore a new strategy to make the endohedral substituent pointing more inwards the cavity was needed.

Figure V.2.1.1: Model of the published structure of the endohedrally modified suberone based cage by Freye *et al.*^[26] **a)** Top view on the cage. The single hydrogen atom is almost perfectly pointing into the cages central cavity, whilst the aromatic motif, that was introduced in a Grignard reaction is turned to the outsides, occupying the cages open sites, what can be better seen in the cages' site view **b)**. Therefore the inner cavity remains free, allowing still the formation of an interpenetrated double-cage.

Also the change from the rigid aromatic ligand scaffold to a slightly more flexible ligand on the base of polynorbornene, similar to the system reported by Clever *et al.*,^[55] resulted in centrally functionalized ligands, that do self-assemble into $[\text{Pd}_2\text{L}_4]$ coordination cages

featuring endohedrally oriented strong H-bond donors. Due to the inwards pointing donor functionalities the cage was capable to selectively bind guest molecules with square-planar or octahedral orientated H-bond acceptors.^[236]

Figure V.2.1.2: Polynorbornene-Cage by *Johnstone et al.*^[236] **a)** Top view on the highly symmetric polynorbornene cage, with the four banana-shaped ligands coordinated to palladium(II) ions. **b)** Side view, with encapsulated $[\text{Fe}(\text{CN})_6]$ complex as guest. The guest complex is perfectly aligned with the ligands inward pointing hydrogen bond acceptors.

In 2016 Löffler *et al.*, published an endohedral functionalized cages, with four bulky adamantyl substituents. The functionalized cage units are based on the previously published acridone ligand, but with an adamantyl substituent at the former keton introduced by an McMurry reaction. It is worth to mention, that the double bond between the hetero-aromatic tricycle and the adamantyl is maintained, what was actually the crucial requirements of the ligands design, since this should make the substituent pointing inwards the cages cavity. But, crystal structure analysis of cage revealed, a bending of the acridone unit, by what the bulky substituent protruding sideways from its concave face. This spacial arrangement causes the adamantyl substituents occupying the entrances of the cage and only partially the inner cavity. Therefore, there was still free space within the cage available, thus an encapsulation of an guest molecules could still occur. However, compared to the modified suberone cage, the adamantyl ligand was only able to form a single cage, no matter what counter anion was used, due to the bulky substituents. Interestingly, the ligand showed a rapid flipping motion, by what the adamantyl ligand inverted itself. Interestingly, this motion even still happened for all ligands in the assembled cage, but due to the lack of space much slower and could even more decelerated upon the encapsulation of guest molecules.

Figure V.2.1.3: Crystal structure adamantyl cage by Löffler *et al.*^[24] **a)** Top view, the bulky endohedral adamantyl substituents are bended to the open sites of the cage, thus, rather pointing to a vicinal ligand than into the cages cavity. Due to that fact, the inner void provides enough space for guest encapsulation. However, in contrast to Freye's cage (Figure V.2.1.1, the adamantyl cage is not able to form an interpenetrated double-cage. **b)** Side view, adamantyl substituents occupying cage's open sites.

Encouraged by the previous results, we designed a next generation endohedrally functionalized ligand based on the acridone backbone with a inward pointing group of smaller size to create less repulsion between substituent and aromatic backbone and additionally introducing functional groups.

The previous chapter dealt with a detailed characterization of a rotational motion around a double bond within an acridone based compound, enabled by an charge separated state. This charge separation was induced by an intramolecular push-pull system, which has reduced the bond order between stator and rotor, allowing a rotation. This chapter deals with the integration of the dynamic motion into a ligand system, capable of forming a metal-mediated supramolecular cage structure. The ligand properties are compared to its model compound with regard to the rotation. Furthermore, the formation of the cage structure, the host-guest chemistry and especially the influence of guest uptake in the rotational motion are investigated.

V.2.2 Results and discussion

V.2.2.1 Synthesis

Similarity to the cyano acetate ester derivatives described in the previous chapter, the synthetic pathway started with the introduction of an hexyl chain to improve the solubility. Outgoing from the commercially available acrid-9/*10H*-one, hexylbromide was attached to the acridone via simple substitution reaction, with K_2CO_3 as base in DMF.

Scheme V.2.2.8: Synthesis ligand derivatives **39**, **42** and **43**. a) hexylbromide, K_2CO_3 , DMF; b) NIS, TFA, DMF; c) 3-ethynyl-pyridine, NEt_3 , $Pd(PPh_3)_2Cl_2$, CuI, THF; d) $(CN)CH_2COOR$, $TiCl_4$, NEt_3 , DCM.

In contrast to the published synthesis of the acridone ligand^[24] iodine was introduced as leaving group for the later performed palladium cross-coupling reaction. The reaction was realized under catalytic amounts of TFA, that forms in presence of *N*-iodosuccimide *in situ* an activated I^+ species, suitable for the nucleophilic aromatic substitution. Moreover, $+I$ -effect of hetero-aromatic nitrogen and $-I$ -effect of the carbonyl within acridone led to a selective substitution in 2- and 7-position. After, 3-ethynyl-pyridine arms were attached at 2- and 7-position with help of Sonogashira cross coupling reaction. At last malononitrile or cyano acetate esters were coupled to tricyclic aromatic backbone with the same procedure as described in section V.1.2.1 to obtain the symmetric ligand L^{CN} (**40**) and the three asymmetric ligands L^{tBu} (**41**), L^{Et} (**42**) and L^{Ph} (**43**).

Figure V.2.2.4: a) Comparison NMR of the ligands L^{tBu} , L^{Et} , L^{Ph} and L^{CN} in MeCN at 298 K. The signal **a/a'** is highlighted in red. b) UV-vis spectra of all ligands. The bathochromic shift from 466 nm for the ligand L^{tBu} (red) to 489 nm for the L^{Ph} (blue) correlates with the observations made in the NMR experiments. The more pronounced the electron withdrawing character of the headgroup, the stronger the bathochromic shift and the higher the single bond character of the exocyclic $\text{C}=\text{C}$ bond, leading to faster rotation. This is effect is in accordance with literature described systems.

As for the non-ligand compound (B^{tBu} (34), B^{Et} (35), B^{Ph} (37)) the ligands show all together only one set of signals for the aromatic protons, although two sets, one for each molecules site, are expected, since the introduction of the cyano acetate ester substituent decreased the compounds symmetry. Therefore it is obvious that the same intramolecular dynamic seems to take place.

Dynamic NMR spectroscopy revealed the same behavior in ^1H NMR measurements as the previously investigated systems (figure V.2.2.4a). While signal (**a/a'**) is extremely broadened for L^{tBu} , so that it is not distinguishable from noise, L^{Et} show already a small bump at ca. 8.2 ppm. In contrast L^{Ph} has a quite sharper signal at 8.4 ppm. It is noteworthy that again there is not only the broadening of signal, indicating qualitatively the difference in speed of rotation, but the average signal **a/a'** is downfield shifted the higher the electron-withdrawing character of the observed ligand derivative. Therefore, the symmetric dicyano ligand L^{CN} has the highest downfield shift in NMR and the sharpest peak, but latter is contributed to the unsuitability to observe the rotation by nmr in this symmetric system. However, the comparison of UV-vis measurements for all four ligands (figure V.2.2.4b), show again the distinctive bathochromic shift going along with an increased charge stabilization within the intramolecular push-pull system ($\text{L}^{\text{tBu}} > \text{L}^{\text{Et}} > \text{L}^{\text{Ph}} > \text{L}^{\text{CN}}$).

V.2.2.2 Dependence on temperature and solvent polarity

To get a better overview of how similar the ligands behave to their smaller model compounds from the previous chapter, their dependencies on temperature and solvent polarity, was also investigated. For that reason, for all derivatives **VT ^1H NMR** experiments were performed in THF as solvent, since it showed the best ratio in solubility to temperature range. In figure V.2.2.5 the VT NMR experiment for **L^{Et}** is shown in comparison with the **B^{Et}**. Qualitatively, both compounds seems to behave virtually identical in terms of change in rotation rate towards variation of temperature. Furthermore, although the signals **a** and **a'** have a slightly different chemical shift in both compounds (**a** = 7.50 ppm, **a'** = 8.62 ppm for **B^{Et}** and $\delta_{\mathbf{a}} = 7.70$ and $\delta_{\mathbf{a}'} = 8.75$) the gap in between both signals is nearly the same (420-450 Hz), another indication for similar rate of rotation.

Figure V.2.2.5: Comparison of VT ^1H NMR spectra (400 MHz, $d^8\text{-THF}$) of backbone B^{Et} and ligand L^{Et} . Both compounds show a similar behavior of rotation towards coalescence temperature. Furthermore, in both cases coalescence temperature is reached around 30 °C and the splitting of ethyl group protons is observable at low temperatures.

To examine the dependency on the solvent polarity further NMR experiments were recorded (Figure V.2.2.6). For that reason, exemplary NMR measurement were performed for the derivative **L^{Et}** (**42**) and its corresponding cage **C^{Et}** (**46**) in various solvent. Since the charged cages $[\text{Pd}_2\text{L}_4]^{4+}$ are only soluble in polar solvent, following solvents MeCN, MeNO₂, DMSO, MeOH were used. As expected, the peak width of the signal **a/a'** is decreased (increased rotation rate) with the increase of solvent polarity. However, the system has a higher rotation rate in DMSO compared to MeCN and MeNO₂, although DMSO has actually a lower relative polarity.^[303] A possible explanation for that is the amount of water within the solvents, since non-dry deuterated solvents were used.

Figure V.2.2.6: ¹H NMR spectra (400 MHz, 298 K) of ligand **L^{Et}** and cage **C^{Et}** $[\text{Pd}_2\text{L}^{\text{Et}}_4]$ in different solvents. The spinning rate of the rotor groups and the associated sharpness of the signal **a/a'** (red) depends on the polarity of the solvent with the rotational rate rising in the order MeCN>MeNO₂>DMSO>MeOH. In all cases, the sharpness of the **a/a'** signal increases upon cage formation.

V.2.2.3 Cage Formation

After the successful synthesis and characterization of the ligands **L^{tBu}**, **L^{Et}**, **L^{Ph}** and **L^{CN}**, the ability of the ligand derivatives to form supramolecular cages in the presence of the palladium cations was tested. Hence, a 2.8 mM solution of the ligands in deuterated acetonitrile were prepared and 0.5 equiv of $[\text{Pd}(\text{MeCN})_4](\text{BF}_4)_2$ were added. The mixture was heated to 70 °C for 1 h. Subsequently the formation was examined by NMR. Comparison of the NMR spectra of ligands and cage, shows that in both cases the amount of signal is the same, indicating the formation of a simple supramolecular cage in form of $[\text{Pd}_2\text{L}_4]$ likewise to the adamantyl cage.^[24] Furthermore, the characteristic general downfield shift of the pyridine signals, but especially for **g/g'** and **f/f'** sitting closest to the nitrogen, are observable. The shifting for those signals occurs due to the coordination of the ligand to

the palladium(II) cation and the consequent change in the electronic situation. Signal **a/a'** shows in systems a slight downfield shift as well, except of in the for **L^{Ph}**, there the chemical shift for **a/a'** stays almost constant.

Figure V.2.2.7: Comparison ^1H NMR ligand and assembled cage for **40**, **42**, **43** and **59**. Top spectra belongs to the free ligand (2.8 mM), bottom one is the assembled cage ((0.7 mM) with $[\text{Pd}(\text{MeCN})_4](\text{BF}_4)$). Everything was measured in deuterated MeCN. a) **40** and **44**; b) **60** and **45**; c) **42** and **46**; d) **43** and **47**. The signal of the protons (**a/a'**), closest to the rotating group, are highlighted in red.

It is worth to mention, that the rate of rotation is increased upon cage formation (Figure V.2.2.6). Besides the slight downfield shift of the signal **a/a'** compared to the ligand spectra, the signal seems also to be sharpened in the cage spectra. This finding can be easily explained, since the electron withdrawing part of the ligand and therefore the negative part of the charge separated state, is pointing inwards the cage's cavity, which is known from other examples in literature to host either the non-coordinating counteranions or negatively charged guest molecules.^[24,51,304] Thus, environment within the cage structure probably contributes to the stabilization of the charge-separated state, therefore the rotation rate is increased compared to the free ligand.

Moreover, the formation of cages **C^{tBu}**, **C^{Et}** and **C^{Ph}** was verified by ESI-MS. The samples for the mass were directly taken from the solution used in the NMR measurements. In all cases the $[\text{Pd}_2\text{L}_4]^{4+}$ peak was found to be the main species, besides, a species with five ligands was also observable. Although, the NMR spectra (figures V.2.2.7b to V.2.2.7d) showed a clean formation of the ligands, the ESI-MS measurement showed s

significant a amount of the free ligand with various additives, what could be a indication for a rather low stability, at least under the experimental conditions for the ESI-MS measurements.

Figure V.2.2.8: ESI-MS of cages (a) $[\text{Pd}_2\text{L}^{\text{Et}}_4]$ (b) $[\text{Pd}_2\text{L}^{\text{tBu}}_4]$ and (c) $[\text{Pd}_2\text{L}^{\text{Ph}}_4]$. Samples were directly taken from NMR samples shown above.

In figure V.2.2.9 the comparison of UV-vis spectra for L^{Et} and C^{Et} are depicted. There is a significant difference in the absorption between 200 nm and 400 nm, however, the absorption band distinctive for the stability push-pull system (or HOMO-LUMO gap) (Sections V.1.2.1 and V.1.2.7) stays almost unchanged, indicating that the intramolecular push-pull system stays intact, although a slight bathochromic shift from $\lambda_{\text{max}}^{\text{L}^{\text{Et}}} = 472 \text{ nm}$ to $\lambda_{\text{max}}^{\text{C}^{\text{Et}}} = 476 \text{ nm}$, what is not a huge difference, but correlates to the increased rotation rate upon

the cage formation, observed in the NMR experiments (Figure V.2.2.7) and the proposed stabilization of charge-separated state within the cage cavity.

Figure V.2.2.9: Comparison UV-vis spectra of L^{Et} (**35**) and C^{Et} (**46**). Both spectra were recorded in MeCN with the same ligand concentration (0.035 mM). The maximum at 472 nm for the ligand stayed intact, but is slightly shifted to 476 nm for the cage.

V.2.2.4 Crystal Structure Analysis

Crystals of L^{CN} , L^{Ph} and L^{Et} were suitable for X-ray diffraction measurements and from all of them a crystal structure could be obtained. Unfortunately, for L^{Bu} no crystal could be grown, what is the reason why the structure of L^{tBu} was optimized in DFT calculation (B3LYP/6-311+g(d,p) in PCM-MeCN) outgoing from the crystal structure of L^{Et} . It is not surprising that as in the model compounds (B^{Et} etc.) the tricyclic aromatic backbones are bent and the rotor group is slightly twisted depending on the substituent. Comparison of the angles shows immediately that the order from the most bent structure to the least bent structure is the same as for the model compounds ($L^{tBu} > L^{Et} > L^{Ph} > L^{CN}$), thus the angles are also very similar to their smaller analogs. L^{CN} has a 2 ° smaller bend compared to B^{CN} (164 ° (Figure V.1.2.18), whereas L^{Et} is more bent about 6 ° in comparison to B^{Et} . The calculated structure L^{tBu} has exact the same angle as the crystal structure of I_2-B^{tBu} , but since the calculation does not consider any packing effect and the model is calculated in solution, the angle in crystal structure could differ from the here present one. Furthermore, this time no coexisting enantiomers could be found in the asymmetric cell, anyway, from the previous VT NMR experiments (Figures VI.1.7.2a to VI.1.7.2c) it is obvious that the rotation motion takes place in all derivatives, hence,

for the three asymmetric derivatives both enantiomers should exist, in solid as well as in solution.

Figure V.2.2.10: Comparison crystal structures of ligand derivatives $\mathbf{L}^{\text{CN}}$, $\mathbf{L}^{\text{Et}}$ and $\mathbf{L}^{\text{Ph}}$. The here used structure of $\mathbf{L}^{\text{tBu}}$ was created *in silico* (B3LYP-6-311+g(d,p) in PCM-MeCN), since no crystal was suitable for X-ray diffraction analysis. The crystal structures represents the bending angles within the respective ligands, what is in total accordance to the general trend shown by all other experiments made so far ($\mathbf{L}^{\text{tBu}}$ (149°) < $\mathbf{L}^{\text{Et}}$ (150°) < $\mathbf{L}^{\text{Ph}}$ (162°) < $\mathbf{L}^{\text{CN}}$ (166°). $\mathbf{L}^{\text{tBu}}$ is calculated presence of solvent, thus, the here shown angle may differ from the real one.

V.2.2.5 Chemoinformatic Studies

Since no crystals suitable for X-ray diffraction analysis could be obtained for any of the cages $\mathbf{C}^{\text{tBu}}$, $\mathbf{C}^{\text{Et}}$ or $\mathbf{C}^{\text{Ph}}$, models for $\mathbf{C}^{\text{Et}}$ with the possible arrangement of the inward pointing rotors (Table V.2.2.1) were calculated and the thereof obtained relative energies were compared. Unexpectedly, the cage with two neighbored rotors of the same orientation (*cis*-2up/2down) has the lowest energy and not as expected the conformer, where the rotors of opposite ligands show the same orientation of the cyano acetates. (*trans*-2up/2down). However, finding is in accordance with the recent publication of Zhu *et al.*,^[75] where sterical demanding picolyl ligands, designed for the formation of heteroleptic coordination cages, rather form *cis* coordinated $[\text{Pd}_2\text{L}^{\text{in}}_2\text{L}^{\text{out}}_2]$ cages than *trans* isomer, with the supposed less crowded palladium coordination sphere. Nevertheless, since the difference in energy is only 2.6 kJ mol^{-1} , it is very likely that both isomers exist in solution, presumed that the energy barrier of interconversion between the *cis* and *trans* isomer is not too high. Furthermore, as expected, the most crowded isomer ("all on one side") has the highest relative energy, about 10 times higher with respect to the *trans* isomer 26.3 kJ mol^{-1} , indicating a lower probability to be formed as compared to the *cis* and *trans* isomers.

Table V.2.2.1: Comparison of relative energies of various cage stereoisomers, with respect to the positioning of vinical rotation group within the cage cavity. Values were determined *in silico* from DTF calculations (B3LYP-6-311+g(d,p) in PCM-MeCN). Lowest enegertic level was found for the *cis* isomer, with two opposite equi-directed adjoined cyano acetate ester groups.

	cis-2up/2down	trans-2up/2down	all on one side
Symmetry	C_i	S_4	C_4
Rel. E [kJ mol ⁻¹]	0	+2.6	+26.3

V.2.2.6 Protonation with TFA

Before the system was investigated for its host-guest chemistry, we were interested whether the ligand can form the same fluorescence active acridinium species as described for the model compounds (Section V.1.3), or if the pyridine motives interfere the protonation of the rotor group. Addition of an excess of TFA to a solution of **L^{Et}** in MeCN gave immediately the answer to that question. The solution showed only a slight change in color, as well as no fluorescence was observable upon irradiation with a 365 nm light. Neither further addition of TFA nor the direct solving of the ligands in pure TFA led to the desired formation of the fluorophore. To take a closer look what is happening to ligand in presence of the acid TFA, UV-vis titration experiments were performed. Interestingly, all of them showed at least a very similar behavior. While there is a hyperchromic shift for the absorption bands at approx. 300 nm and 395 nm, a hypochromic shift of the absorption band at approx. 280 nm and 350 nm are clear observable. However, the absorption band at 460-490 nm, which was used as indicator for the quality of the intramolecular push-pull system, stays almost unchanged, but experience a slight hypsochromic shift. At the first glance this behaviour is counter-intuitively, since one would rather expect a stabilization of the push-pull system due to the increased polarity, as it was observed before for the backbone system. Addition of TFA to the backbone derivatives led to a initial stabilization, thus a higher rotation rate due to the increase in solvent polarity, before concentration was high enough to protonate the rotor group (Figure V.1.3.27, 0-13 mM TFA). But obviously the protonation of the pyridine arms has a negative impact on the intramolecular electronic situation, which has a predominant effect on the push-pull system in comparison to change of solvent polarity. For **L^{Et}**, as an example, the titration experiment with TFA was also monitored in ¹H NMR measurement (Figure V.2.2.12).

Figure V.2.2.11: UV-vis titration experiments of all cyano acetate ligand derivatives **a) L^{tBu}**, **b) L^{Et}**, **c) L^{Ph}** (0.035 mM) with TFA in MeCN. The TFA was added in 13 steps to an all over concentration of 0.13 M. Hyper- and hypochromic shifts are indicated by arrows. In all spectra isosbestic points can be found, speaking for a concerted conversion into the protonated ligand species. Furthermore, a slight hypsochromic shift of 460 nm band, characteristic for the quality of the push-pull system, can be observed for all derivatives, indicating a less favored conditions for a charge separated state.

As expected, upon protonation of the pyridines the proton signals **g/g'** and **f/f'** are downfield shifted, but only half as much (0.15 ppm) with respect to the shift occurring upon coordination to palladium(II) cation during the cage formation (approx. 0.4 ppm). Whereas the signals **e/e'** and **d/d'** experience a rather large downfield shift (0.8 ppm and 0.65 ppm), while the shift in the cage is merely the half (Figure V.2.2.7c). However, the signals of the backbone are hardly effected by the protonation, although, against expectations the **a/a'** signals goes from a very broadened peak to a sharper and more defined peak with increasing TFA concentration, what indicates a increased rotation rate. That observation contradicts the hypsochromic shift in the UV-vis spectra, but shows definitely that rotating motion is maintained despite the presence of an excess TFA.

Figure V.2.2.12: ^1H NMR titration experiment of L^{Et} (**42**) with TFA. All pyridine signals (g/g' , f/f' , e/e' , d/d') are clearly downfield shifted, while the signals on the backbone experience only a slight downfield shift. In presence of high concentration of TFA the a/a' signal gets sharpen due to the increased solvent polarity.

Neither the formation of cage does effect the rotation motion significantly, nor does the coordination of the pyridines to the palladium. In contrast, in presence of TFA, the pyridines are protonated first and seem to prevent the protonation of the rotor group. Therefore, the titration of cage with TFA was investigated, since in metal-mediated supramolecular structure, the pyridines are blocked due the coordination to the the palladium(II) cations. Thus, it could be possible to protonate the rotor group again and generating the acridinium species, before the cage disassembles due to the high TFA concentration, resulting in a highly charged cage (8+) with a strong fluorescence.

Figure V.2.2.13: UV-vis comparison of L^{Et} and C^{Et} in presence and absence of TFA. All measurements were performed in MeCN with a total ligand concentration of 0.035 mM. **a)** Comparison between L^{Et} its corresponding protonated species. **b)** Comparison between the cage C^{Et} in presence and absence of TFA. **c)** Comparison of ligand and cage in presence of TFA. **d)** Comparison of ligand and cage in presence or absence of TFA.

The titration experiment (Figure V.2.2.13b) shows no real change in its absorption pattern upon addition of excess of acid, not even after addition of 4000 equiv. TFA (approx. 70 mM). The slight general hypochromic shift is caused by dilution. Comparison of figures V.2.2.13a and V.2.2.13b elucidates the huge difference in absorption changes for the ligand in the presence of acid, while there is virtually no change for the cages' case. Furthermore, figure V.2.2.9 compares the spectra for the cage in presence of acid and the protonated ligand. Although the spectra exhibit a certain resemblance, the difference was still sufficient to clearly distinguish both species. On the one hand, this finding suggest that the cage can withstand the presence of acid, but on the other hand, it shows that the backbone is neither protonated in the cage nor in the free ligand. Nevertheless, this apparent resistance of the cage towards acid was suspicious, hence, a second titration experiment was made, but monitored by 1H NMR spectroscopy (Figure V.2.2.14).

Figure V.2.2.14: ^1H NMR (400 MHz, $\text{d}^3\text{-MeCN}$) titration experiment of C^{Et} (2.8 mM) with TFA. Upon addition of TFA a new species is rising up, becoming more predominant with increased TFA concentrations (highlighted in green), while the signals of the starting material are slowly vanishing (highlighted in red).

And in fact, already at a concentration of 2 mM TFA, first small signals appear (green), which grow with increasing TFA concentration, while the cage signals slowly disappear. In figure V.2.2.15 the spectrum of the C^{Et} in presence of 80 mM TFA is compared with the spectra of the protonated ligand L^{Et} (green) and of the cage C^{Et} in absence of acid (red). And indeed, the appearing new signals belong to the protonated ligand. In summary, the cage disassembles in presence of acid, but it takes a abundant excess of acid to shift the equilibrium to the ligands side, what was probably the reason why the disassembling could not be seen in the UV-vis titration experiments.

Figure V.2.2.15: Comparison of ^1H NMR spectra of cage **46** and ligand **42** in presence of TFA. All spectra were recorded in $\text{d}^3\text{-MeCN}$. It can be clearly seen, that the generated species is the protonated ligand (highlighted in green) and coexists with the assembled cage (highlighted in red).

V.2.2.7 Guest encapsulation and its influence on the rotation

In previous $[\text{Pd}_2\text{L}_4]$ cage systems^[51,305,306] it became apparent that small anionic molecules are particularly suitable for the investigation of the host guest chemistry. In the structural related adamantyl system,^[24] Löffler *et al.* mainly used small aromatic bis-sulfonates. To get a better comparison of these two system, a selection of these bis-sulfonate guest molecules were used for the host-guest titration experiments (Scheme V.2.2.9). However, the focus of these studies was to find a combination, that represents the influence of the guest uptake on endohedral dynamic motion.

Scheme V.2.2.9: Guest molecules $\text{G}^1\text{-G}^5$ for ^1H -NMR host-guest titration experiments. All guests come with two tetra-*n*-butylammonium cations $(\text{nBu})_4\text{N}^+$ as counter-ions.

Löffler *et al.* described three cases for the encapsulation of guests in the host-guest titration experiments:

1. Slow exchange: The guest is encapsulated in the cage. Due to slow exchange new signals appear belonging to the $[G@Pd_2L_4]$ species that a growing with every addition of guest, while the signals of the free host disappear. Furthermore, the inward pointing protons experience a significant change in chemical shift, due to the changed chemical environment and/or interaction with the encapsulated guest molecule. Moreover the signals of the encapsulated guest can be observed as well. An excess of guest lead to a precipitation of the the cage.
2. Fast exchange: The guest is encapsulated in the cage. Due to fast exchange the signals of the chemical shift of the inward pointing protons is changing gradually with every addition of guest. An excess of guest leads to the precipitation of cage.
3. No encapsulation: The signals of the free cage do not change at all. The signals of the free guest appear. An excess of guest leads to the precipitation of cage.

In the beginning, the same conditions for the titration experiments were used as Löffler *et al.* have described for their adamantyl system. A solution of the respected guest was added to the preassembled adamantyl cage in acetonitrile. The procedure was repeated for the cage system C^{Et} with the guests G^1 - G^5 (Figures VI.1.7.3 to VI.1.7.7). Similar to the adamantyl cage G^2 and G^4 did show a slight shift for the the pyridine signals, indicating an encapsulation of guest but in fast exchange mode. The same behavior is seen for ferrocene guest (G^1) which showed an encapsulation with slow exchange in the adamantyl system. However, since we expected to have a better chance to observe the influence of the guest uptake on the rotation motion in a system where the encapsulation occurs in a slow exchange fashion, the titration experiments were performed with the more steric demanding guests G^3 and G^5 . Both experiments showed upon the first addition an extreme broadening of all signal in the NMR spectrum, though a broad significantly downfield shifted signal appeared, assumed to be inward pointing proton g/g' . This spectrum differs from the one, where fast exchange of the guest is assumed to occur. Probably in this case the encapsulation process is proceeded with a slow-exchange mechanism, what would explain the significant downfield shift of g/g' , however, the reason for the broadening of all signals upon guest encapsulation could be found.

Since the spectra showing broadening of the signals were not suitable for investigation of guest uptake influence on the dynamic motion, we concentrated on finding a good combination which shows fast exchange in the NMR titration experiments. Considering the previous findings (Section V.2.2.2), the titration experiments were repeated with the already used host guest combinations, but with various solvents and at different temperatures (Figures VI.1.7.8 to VI.1.7.10). The here shown examples are all from the

titration experiments with guest **G**⁵, as it emerged to be the most promising combination. Interestingly, in nitromethane the before described broadening occurs again, likewise to the experiments in acetonitrile. By performing the experiments at 60 °C the signals became again slightly sharper but were still too broad to gain any usable information (Figure VI.1.7.10). Under the same conditions but with methanol as solvent, the investigated system showed suddenly an encapsulation with a fast exchange rate. However, under both conditions precipitation of the cage was observable after adding more than 0.4 equivalents of guest to the cage solution. The encapsulation of the bis-sulfonate guest decreases the overall charge of the cage construct and therefore the solubility in methanol (Figure VI.1.7.9). In DMSO the combination **C**^{Et} and **G**⁵ showed already at room temperature very promising results. Signal **g/g'** is slightly downfield shifted, therefore the encapsulation seems to take place with a fast exchange rate. However, for the first time, a significant broadening of signal **a/a'** is visible with increasing concentration of guest (Figure VI.1.7.8), but since the spectrum suffered from ample signal broadening, the experiment was repeated at 60 °C (Figure V.2.2.17).

Figure V.2.2.16: Screening of proper conditions for host-guest experiments with $\mathbf{C}^{\text{Et}}$ and $\mathbf{G}^5$. **a)** NMR experiments with $\mathbf{C}^{\text{Et}}$ and guest $\mathbf{G}^5$ proceed in MeCN and DMSO at 298K or 333K. In MeCN host and guest undergo a slow exchange process, since a new singal appear, while the actual cage signal do disapper. Nevertheless, the whole spectra extremly boradend at 298K and 333K, making it difficult to assign the signals or determine any linewidth. In comparison, in DMSO host and guest undergo a fast exchange. The signals are much sharper, especially the $\mathbf{a/a'}$ signal, making it easier so observe any changes in linewidth upon guest addition. The signals of the respective guest molecules are marked by an asterisk. **b)** Comparison of signal $\mathbf{a/a'}$ for ligand $\mathbf{L}^{\text{Et}}$ ($\mathbf{42}$) and cage $\mathbf{C}^{\text{Et}}$ ($\mathbf{46}$) in DMSO at 298K and 333K.

To clarify again what influence solvent polarity and temperature do exert on the system, in figure V.2.2.16a the NMR spectra of same cage guest combination (0.7 mM $\mathbf{C}^{\text{Et}}$ with 1.0 equiv. $\mathbf{G}^5$) are illustrated, in acetonitrile at 25 °C and 60 °C and in DMSO at the same temperatures. First observation to be mentioned, the exchange rate of guest is changing from slow exchange to fast exchange in terms of the NMR timescale, when going from acetonitrile to DMSO. Furthermore, an increase in temperature accelerates the dynamics in the whole system, what makes the NMR signals which are liable to a dynamic sharper in terms of their relative peak width. Exactly this case is observable in figure V.2.2.16a for both experiments. By going from 25 °C to 60 °C all signals become sharper, what is in total accordance to VT NMR experiments (Section V.2.2.2), because all signals are liable to the intramolecular dynamic motion. The same effect occurs of course also in absence of the guest (Figure V.2.2.16b). A disadvantage of using DMSO as solvent is the overlapping of signals $\mathbf{d/d'}$ and $\mathbf{a/a'}$. It is noteworthy, that the measurement of the system in acetonitrile at 60 °C let most signals spawn from noise, what support our assumption that the extremely broadened spectra are caused by encapsulation on a slow exchange rate.

Despite the overlapping of signals, a careful analysis and line fitting including the resonance of the $\mathbf{a/a'}$ protons revealed that the encapsulation of the bis-sulfonate guest $\mathbf{G}^5$ slows down the spinning of the endohedral rotors. The line fitting of the signals $\mathbf{a/a'}$ and $\mathbf{d/d'}$ is shown on the right of figure V.2.2.17. The increase in peak width is accompanied by

the typical downfield shifts for the cage protons in contact with the guest (signals **g/g'**, **a/a'**, OCH_2CH_3). In figure V.2.2.18 the change of peak width for signals **a/a'** and **d/d'** are plotted vs. the concentration of guest.

Figure V.2.2.17: ^1H NMR titration (400 MHz, 333 K, d^6 -DMSO) of $[\text{Pd}_2\text{L}^{\text{Et}}_4]$ with $(\text{NBu}_4)_2\text{G}^5$. The chemical shifts of the inward pointing protons change gradually due to a fast exchange of the guest on the NMR time scale. Guest signals are marked with *. On the right line fittings analyses of the deconvoluted signals **d/d'** and **a/a'** are shown. Whereas the **d/d'** signals do not broaden upon guest addition, the signal assigned to **a/a'** clearly broadens with increasing amounts of the guest which indicates that the encapsulated guest slows down the spinning of the rotors inside the cavity.

Figure V.2.2.18: Correlation of peak width with the concentration of guest G^5 from line fitting analysis of the NMR titration data shown in figure V.2.2.17. The significant change in peak width of the **a/a'** signal indicates a decrease of rotation rate upon guest uptake, while the peak width of other signals stays nearly constant.

V.2.3 Conclusion

This chapter described the synthesis and characterization of four new endohedrally functionalized bis-monodentate banana-shaped ligands ($\mathbf{L}^{t\text{Bu}}$, $\mathbf{L}^{\text{Et}}$, $\mathbf{L}^{\text{Ph}}$ and $\mathbf{L}^{\text{CN}}$) based on the acridone backbone. These ligands possess electron-withdrawing substituents, either a symmetric malononitril group or an asymmetric cyano acetate ester group (*tert*-butyl, ethyl or phenyl) protruding sideways from its concave face. Although connected by a double bond, the substituents exhibit an rotational motion in respect to the polycyclic backbone, caused by an intermolecular backbone. This rotation motion is qualitatively observable in the asymmetric substituted derivatives by dynamic NMR spectroscopy. In contrast to the small model compound described in the previous chapter, the ligand does not form an acridinium moiety in presence of strong acid and therefore does not show any strong fluorescence. Instead, the pyridine arms are protonated, which does not seem to influence the intramolecular push-pull system considerably. The presence of palladium(II) cations lead to a clean formation of supramolecular homoleptic coordination cages, what could be verified by NMR and ESI-MS measurements. Moreover, the rotation is maintained within the cage and even slightly accelerated compared the free ligand. In presence of TFA the cage $\mathbf{C}^{\text{Et}}$ could not withstand, but relatively high concentrations of strong acid were needed for a complete disassembly. Furthermore, several host guest titration experiment were carried out, to find a system that clearly shows an effect on the endohedral rotational motion caused by the guest uptake. 2,7-naphtalene bis-sulfonate slowed the rotation significantly down, when encapsulated in the cage $\mathbf{C}^{\text{Et}}$. This guest-induced “breaking effect” complements the previous findings concerning the flipping motion in a related adamantyl cage.^[24] Based on the here present results, we were curious if the cages’ guest uptake affinity can be changed by an stimuli responsive tuning of the endohedral rotation.

Chapter V.3

Azacrown-Cyano-Acridinylidne-Ethyl Acetate

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V.3.1 Introduction

In the previous chapters we could show that the rotation of the electron-withdrawing substituent was induced by a reduced bond order, caused by the intramolecular push-pull system induced charge separation. The quality of the push-pull system could be significantly tuned by small variations in the electronic contribution of the ester substituent what led to a better stabilization of the charge separated state and therefore to a faster or slower rotation. Furthermore we were able to integrate this dynamic system in supramolecular coordination cage while maintaining the rotational motion in the inner cavity. Moreover, the uptake of an bis-anionic guest into the supramolecular construct resulted in a distinct deceleration of the rotation rate. Since the the guest uptake seems to influence the dynamic in the cage, the question was if the guest uptake/release behavior can be influenced by the speed of rotation.

Especially with regard to idea of using the cages' cavity as supramolecular container,^[307] reaction vessel^[45,308] or as drug delivery system,^[309] a stimuli responsive control over the host-guest chemistry could be of advantage. Concerning the reaction vessel, these system sometimes suffer from product inhibition,^[310] meaning, the formed product stays inside the cavity, since it has a higher affinity compared to the substrate. A stimuli-responsive change in binding affinity could resolve such problems. Far more interesting is this approach for the latter topic. In several publication already dozens of attempts for stimuli responsive drug delivery systems were presented and discussed,^[311] going from stimuli by pH,^[312] redox,^[313] heat,^[314] light^[315] to magnetic^[316] or even ultrasound.^[317]

In a previous work Han *et al.* could already show that the guest uptake and release behavior can be controlled by a light-triggered conversion from a flexible into rigid form of supramolecular coordination cage and *vice versa*.^[300] Motivated by these results we wanted to design a system based molecular rotor system where the electronics of the intramolecular push-pull system can be influenced on the electron-donating side. Hence it had to be a functionality that can be attached to the aromatic nitrogen instead of the hexyl chain.

Since, our cages are normally not soluble in water and the coordination cages do not tolerate higher concentration of acid, we decided for a crown ether motif to bind alkali or earth alkali metals, which seem not to have a significant effect on the metal-ligand coordination as long as their counter-anions are of non-coordinative nature.^[31] In literature some examples can be found, where azacrown ethers are integrated to the π system of an chromophore, whose intramolecular electronic situation is manipulated by binding of cations to the azacrown ether, accompanied by a change of color. This process is generally called as ionochroism.^[318] In the case of azacrown ether containing push-pull systems, the azacrown ether can be part of the electron-withdrawing motif, engendering a positive ionochroism^[319] within the system (indicated by a bathochromic shift in the UV-vis absorption), or on the other hand, becomes part of the electron-donating motif, causing a weakening of electron-pushing effect by binding a cation (indicated by a hypsochromic

shift in UV-vis absorption).^[320–322] Therefore, a system was designed fitting the latter approach, in order to get a weakening of the intramolecular push-pull system, causing a deceleration in rotation, intended to give a change in the host-guest affinity.

V.3.2 Results and discussion

V.3.2.1 Synthesis

As the synthesis turned out to be quite challenging, we did not immediately plan to synthesis the whole ligand, but had a recourse to our model compound **B^{Et}**, so that the design of the system could be tested first before it is transferred into a ligand or coordination cage.

Scheme V.3.2.10: Retrosynthetic analysis of the intended azacrown ether system. The final product is cut up in four parts. The cyano acetate ester, the acridone backbone and the opposing azacrown ether, splitted into the phenyl linker and the macrocycle itself.

From a retrosynthetic perspective, the azacrown ether was synthesized separately, before it was merged to the acridone backbone. In a last step the electron-withdrawing malonic acid derivative was introduced to complete the synthesis (Scheme V.3.2.10)

Scheme V.3.2.11: Synthesis path for compound **B_{Azacrown}^{Et} 52**. a) TsCl,NEt₃,DCM; b) N-phenyl diethanol amine,NaH,THF; c) KI,H₅IO₆,DCM/H₂O; d) acridone,dipivaloylmethane,N(nBu)₄OH,CuI,DMF; e) cyano ethyl acetate,TiCl₄,NEt₃,DCM.

Based on the retrosynthetic analysis the proposed synthetic steps (Scheme V.3.2.11) were processed by *Halil Calik*, in order to obtain **B_{Azacrown}^{Et} 52**. In a first step, tosylate was introduced to both hydroxy groups of the triethylene glycol to have a better leaving group for the next step in synthesis, where the azacrown ether is formed. The syntheses of the *N*-phenyl-aza-15-crown-5 ether was performed under high dilution conditions, as described in literature,^[323] to avoid the formation of byproducts. In a further reaction iodine was introduced to the phenyl ring in para position to the azacrown ether, what was crucial for the C-N coupling reaction in the next step. Since the reaction was only known for simple iod-phenyl compound but not for its anilin derivatives, a palladium catalyzed Buchwald-Hartwig amination reaction was performed, but without success. Despite variation of catalytic compounds, base and solvent the desired product could not be obtained. Also the change to a copper catalyzed Ullmann reaction initially did not lead to the desired product. Only after using bases with sterically demanding, non-coordinating conjugated acids, like tetrabutylammonium hydroxide, the coupled product could be isolated from reaction mixture. In the subsequent Lehnert modified Knoevenagel reaction the end product could be obtained as a red solid.

V.3.2.2 Comparison of $\mathbf{B}_{\text{Azacrown}}^{\text{Et}}$ to $\mathbf{B}^{\text{Et}}$ in terms of rotational motion

At first both systems $\mathbf{B}^{\text{Et}}$ and $\mathbf{B}_{\text{Azacrown}}^{\text{Et}}$ were investigated on their spinning behavior. Therefore VT NMR experiments were performed for $\mathbf{B}_{\text{Azacrown}}^{\text{Et}}$ and the spectra were compared to those of $\mathbf{B}^{\text{Et}}$ (Figure V.3.2.1). Qualitatively both spectra show a similar behavior on the variation of temperature, although the $\mathbf{B}_{\text{Azacrown}}^{\text{Et}}$ derivative seems to be slightly faster, what can be noticed by comparing the peak shape of the signal **a** at -40 °C. Furthermore, the signal **e** admittedly shows a -80 °C a broadening of the signal, but is not yet split into $\mathbf{e}_{\alpha}$ and $\mathbf{e}_{\beta}$ as it can be seen in the ^1H NMR spectrum of $\mathbf{B}^{\text{Et}}$. These findings indicating a slightly enhanced interaction of the electron-withdrawing and -donating function within $\mathbf{B}_{\text{Azacrown}}^{\text{Et}}$, what is not uprising, though, the azacrown nitrogen acts as an additional electron-donating part. This become quite apparent on the chemical shift of the signal **d/d'**. While all signals of the acridone exhibit only tiny almost negligible changes, **d/d'** experience an significant upfield shift (approx. 0.5 ppm), indicative for an increased electronic density.

Figure V.3.2.1: Comparison ^1H -NMR for **52** with **35** in d^8 -THF at temperatures between -80°C to +60°C. It is worth to mention that both compounds behave very similar at the same temperatures, indicating a very similar electronic situation in both systems.

To confirm the above mentioned differences, a qualitative analysis of the obtained data was accomplished. Therefore, the thermodynamic parameters $\Delta H^\ddagger$ and $\Delta S^\ddagger$ were determined from experimental and simulated data, as it was already done before for **B^{Et}** (Section V.1.2.4). The rate constants extracted from experimental and simulated VT NMR spectra are plotted in figure V.3.2.2. From the corresponding Eyring plots the enthalpy and entropy of activation were calculated, which are presented in table V.3.2.1 in comparison to those determined for hexyl chain analogon **B^{Et}**.

Figure V.3.2.2: Eyring plots of experimental determined (a) and simulated (b) rotation rates. The determined thermodynamic values from these plots are presented in table V.3.2.1.

Table V.3.2.1: Activation enthalpies, entropies and Gibbs' free energies of **B_{Azacrown}^{Et}** in comparison to **B^{Et}** calculated from experimentally determined rate constants and from simulations.

	B_{Azacrown}^{Et} (52)	B^{Et} (35)
$\Delta H^\ddagger_{\text{exp}}$ [kJ mol ⁻¹]	32.6 ± 1.3	34.3 ± 1.3
$\Delta S^\ddagger_{\text{exp}}$ [kJ mol ⁻¹ K ⁻¹]	-73.9 ± 5.3	-72.5 ± 5.2
$\Delta G^\ddagger_{\text{exp}}^{298\text{K}}$ [kJ mol ⁻¹]	54.6	56.0
$\Delta H^\ddagger_{\text{sim}}$ [kJ mol ⁻¹]	31.4 ± 1.3	33.1 ± 0.5
$\Delta S^\ddagger_{\text{sim}}$ [kJ mol ⁻¹ K ⁻¹]	-77.4 ± 5.0	-76.6 ± 1.9
$\Delta G^\ddagger_{\text{sim}}^{298\text{K}}$ [kJ mol ⁻¹]	54.5	56.0

Undoubtedly, there is a difference in the enthalpy of activation for **B_{Azacrown}^{Et}**, which is in total accordance to the qualitatively made assertion. It is noteworthy, for the value for the entropy of activation there is nearly no deviation, what suggest that the mechanism for the rotational motion is unchanged compared to the model compounds **B^{tBu}**, **B^{Et}** and **B^{Ph}**. Interestingly, in the UV-vis absorption spectrum there is virtually no difference, the maximum of absorbance is merely blue-shifted about 1 nm compared to **B^{Et}** (Figure V.3.2.3).

Figure V.3.2.3: Comparison UV-vis of $B_{\text{Azacrown}}^{\text{Et}}$ and B^{Et} . Both spectra were recorded with 2.8 mM of the respective compound in MeCN

V.3.2.3 Effect of Metal uptake in Azacrown on Acridinyline System

Based on literature^[321] sodium, magnesium, calcium, and barium perchlorate were chosen to investigate their impact on the push-pull system and the rotational motion when coordinating to the aza-15-crown-5 ether. For that reason UV-vis and NMR titration experiment were carried out with the respective metal and compound $B_{\text{Azacrown}}^{\text{Et}}$. Furthermore, control titration experiments were accomplished with B^{Et} to exclude unspecific metal interaction with the remaining system.

The UV-vis titration experiment with sodium perchlorate show a marginal hypsochromic shift in the region of interest (Figure VI.1.7.11b). Whereas in ^1H NMR a significant downfield shift is observed for protons signals **1** and **2** of the bridging phenyl motif (Figure VI.1.7.11a, red and green line indicate the approximate shifting routes), by what the the phenyl signal **2** even passes signal **1**. In contrary, the proton signal **3** experience a slight upfield shift, while the residual crown ether signal stay nearly unchanged. All that speaks for an weak interaction between sodium cation and crown ether, therefore it is not surprising that no observable change in rotation rate happened. Next, the higher charged earth alkali metal magnesium in form of $\text{Mg}(\text{ClO}_4)_2$ was investigated. In NMR a clearly more intense downfield shift of both phenyl signals occurs. Additionally, all protons in the crown ether next to an oxygen show a clear downfield shift as well, while signals **3** experience again a slight upfield shift. The more surprising it was to see absolutely no change in UV-vis (the general hypochromic shift is due to dilution) (Figure V.3.2.4a and section V.3.2.3). However, on the first glance also in this experiment did not show

any changes. Through the control experiment with $\text{Mg}(\text{ClO}_4)_2$ titrated to a solution of **B^{Et}** (**35**) an unspecific interaction could be excluded, since neither in NMR nor UV-vis a change could be noted.

Figure V.3.2.4: a) ^1H NMR (400 MHz, d^3 -MeCN) titration of **52** (2.8 mM) with $\text{Mg}(\text{ClO}_4)_2$. The approximate shifting routes for the signals **1** and **2** are indicated by a green or red line. Below, UV-vis titration experiment of **52** (0.035 mM) in MeCN. Upon addition of the magnesium salt, no significant hypsochromic shift could be observed. Only a slight hypochromic shift, what could also be caused by dilution. b) NMR and UV-vis control experiments under the same conditions with **B^{Et}** (**35**). Compound **35** does not show any evidence for an interaction with $\text{Mg}(\text{ClO}_4)_2$, neither in NMR nor in UV-vis experiments.

In literature it has been shown that calcium rather fits in the aza-15-crown-5 ether than potassium. The latter is more suitable for next bigger azacrown ether. Thus, the effect of calcium perchlorate was used in the next binding studies (Figure V.3.2.5). Similar to $\text{Mg}(\text{ClO}_4)_2$ the proton signals of the phenyl motif are downfield shifted, signal **1** about 0.2 ppm and signal **2** about 0.6 ppm. At a approximate concentration 8 mM of $\text{Ca}(\text{ClO}_4)_2$ signal **2** passes signal **1**. In addition crown ether signals **5-7** experience a stronger downfield shift than **4**. In contrast the methylene groups bound to the azacrown ether nitrogen are shifted upfield. UV-vis shows first time a significant blue-shift about 9 nm and a more intense hypochromia for the 270 nm absorption band compared to sodium. As in the examples before, the control experiment do not indicate any unwanted interaction between

the push-pull rotor compound and the used metal salt.

As a next salt, barium perchlorate was used for titration. Barium is compared to the previous used metals softer, thus it should better prefer the interaction with the nitrogen. On the other hand, it has a lower binding affinity towards the azacrown ether since the cation is too large to fit into the crown ethers cavity. Still, it is literature-known that the barium cation forms a 2:1 complex instead with the azacrown ethers.^[324,325] Nevertheless, titration experiments have shown a weaker interaction compared to calcium perchlorate (Figure VI.1.7.12). Although an interaction between azacrown and metal can be regarded as certain, the studies suffer from a lack of evidence for a clear decrease in the rotation rate upon metal binding. Hence, as a next step we tried to protonate the azacrown nitrogen, what should be the strongest reversible reaction for this system to gain an electron-withdrawing counterpart in the opposite direction in respect to the rotating cyano acetate ester.

Figure V.3.2.5: a) ^1H NMR (400 MHz, d^3 -MeCN) titration of **52** (2.8 mM) with $\text{Ca}(\text{ClO}_4)_2$. The approximate shifting routes for the signals **1** and **2** are indicated by a green or red line. Below, UV-vis titration experiment of **52** (0.035 mM) in MeCN. Upon addition of the calcium salt, a maximum blue-shift of 9 nm was observed. b) NMR and UV-vis control experiments under the same conditions with **B^{Et}** (**52**). In comparison to **52**, compound **35** does not show any chemical shift in NMR upon addition of $\text{Ca}(\text{ClO}_4)_2$, neither does it show any evidence of interaction with the calcium cation in UV-vis titration experiments.

Indeed, the protonation with TFA (Figure V.3.2.6) showed the strongest effect on comparison to the other experiments for both NMR and UV-vis. In UV-vis the addition of 100 equiv. TFA resulted in a hypsochromic shift of 16 nm. What equals almost the hypsochromic shift **B^{Et}** has experienced by changing the solvent from water to hexane (Figure V.1.2.9). Moreover, the phenyl signal **2** in NMR makes an tremendous downfield jump ending with a highest chemical shift in the recorded spectra. Besides, for the first time, signal **3** downfield shifted as well, while the remaining crown ether signals are slight shifted to a lower chemical shift. Both observation are a clear indication for the successful protonation of the azacrown nitrogen and for a significant change in the compounds intramolecular electronic situation. But the most interesting is the fact that the signal **a/a'** is getting considerably broader up to a concentration of 20 equiv. (approx. 60 mM), while the remaining aromatic signals on the acidinylidene motif stay unchanged. This proves that a electron-withdrawing motif in the reverse direction lead to a distinct deceleration of an dynamic motion. Anyhow, still the deceleration is only achieved by protonation, which is not desired in terms of making the system suitable for supramolecular coordination cages, hence the cages can disassemble under acidic conditions, as shown in figure V.2.2.14. Furthermore, the presence of an excess in ionic compounds or polar compounds induces a better stabilization of the charge separated state, responsible for the rotation, thus it accelerates the rotational motion again. This effect can be nicely seen in figure V.3.2.6a. The peak width of signal **a/a'** is decreased again with every addition of TFA above 20 equivalents.

Figure V.3.2.6: NMR and UV-vis titration experiments of compound **52** and TFA. **a)** ¹H NMR (400 MHz, *d*³-MeCN) titration with TFA. The approximate shifting routes for the signals **1** and **2** are indicated by a green or red line. The region where a change in the width of the **a/a'** signal can be observed is highlighted in blue. Until the addition of 10 equiv, a slight broadening of signal **a/a'** can be observed. Further addition leads to a sharpen of the signal again. It seems like two contrary effects are taking place. On the one hand the protonation of the azacrown ether nitrogen, accompanied by weakening the push-pull system and on the other hand the stabilization of the charge separated state by increasing the overall polarity due to addition of higher amounts of TFA. Furthermore, compared to the other titration experiments, signal **3** exhibit a significant downfield-shift. **b)** The UV-vis titration experiments of **52** (0.035 mM, MeCN) with TFA. The maximum hypsochromic shift of 16 nm is achieved upon a total addition of 100 equiv. of TFA. Also a significant hypochromic shift for the band at 270 nm could be observed.

In order to overcome the minor effects caused by weak interaction of alkali or earth alkali metals with the azacrown nitrogen, the focus was set on transition metals. The coordination of nickel, copper, zinc and cadmium to the azacrown ether were investigated by UV-vis. Additionally manganese was used, since it is the closest transition metal analogon to magnesium in biological systems.^[326] The experiments showed for manganese perchlorate and zinc tetrafluoroborate a blue-shift of 9 nm, thus comparable to calcium perchlorate (Figures VI.1.7.13a and VI.1.7.13b). Cadmium perchlorate led to a maximal hypsochromic shift of 11 nm, what is not an outstanding improvement, apart from that, cadmium belongs rather to the higher toxic transistion metal and was therefore discarded for further experiments (Figure VI.1.7.13c).

Nickel and copper showed a completely new behavior. While nickel looked very promising in the beginning (Figure V.3.2.7a), since addition of 10 equiv. $\text{Ni}(\text{ClO}_4)_2$ already gave a blue-shift of 12 nm (6 nm for 10 equiv. TFA), anyway, further addition ended in the generation of a well-known absorption pattern (Figure V.1.3.23c). The control experiment with B^{Et} led to the same result (Figure V.3.2.7b). A further NMR titration experiments with B^{Et} proved the suspicion about the protonation of the rotating group (Figure V.3.2.7c). Irradiation with UV light led to the characteristic fluorescence. Therefore we suspect the used $\text{Ni}(\text{ClO}_4)_2 \times 6 \text{H}_2\text{O}$ to function as strong acid and providing the H^+ for the formation of the protonated acridinium species. In the case of copper perchlorate, addition led to the same result for B^{Et} (Figure VI.1.7.14b), whereas for $\text{B}_{\text{Azacrown}}^{\text{Et}}$ the addition of the first equivalent brought off an broad absorption band going from 400 nm to 800 nm, whats typical for an radical species. Second equivalent led to another unknown absorption pattern, all further additions gave the already known acridinium specific absorption pattern (Figure VI.1.7.14a).

Figure V.3.2.7: UV and NMR titration experiments with $B_{Azacrown}^{Et}/B^{Et}$ and $Ni(ClO_4)_2$. **a)** UV-vis titration experiment with $B_{Azacrown}^{Et}$ (**52**, 0.035 mM, MeCN) with TFA. Upon addition of 10 equiv. nickel salt a hypsochromic shift of 12 nm was observable. Further addition led to a conversion of **52** to the corresponding protonated acridinium species, indicated by the characteristic absorption pattern (Figure V.1.3.23c). **b)** Control experiment with B^{Et} and the corresponding nickel salt. A formation of the protonated acridinium species is observable from the very first addition and is totally converted at total addition of 50 equiv. $Ni(ClO_4)_2$. **c)** 1H NMR (400 MHz, d^3 -MeCN) titration experiments with B^{Et} (**35**) and $Ni(ClO_4)_2$. The starting material is highlighted in red, the protonated acridinium species in green. For comparison purposes, a spectrum of the acridinium species, protonated with TFA, is shown on the very top.

V.3.2.4 Influence of alkali metal binding on the rotation rate in VT NMR experiments

Since the desired effect on the rotation rate was not clearly visible under standard conditions, we have compared the influence of temperature on the rotation rate in presence and absence of coordinating metals. Therefore VT NMR measurements in acetonitrile from -40 °C to 70 °C were accomplished, hence the metal salts had only a poor solubility in the preferable solvent THF. In figure V.3.2.8 the outcome for both measurements are compared. It is obvious, that a broadening of signals already occurs at 20 °C, while in the other case no visible change in the peak widths occurred. Aside from that, at -20 °C and below it looks like a splitting of the signals already occurs, while in the spectra without $Mg(ClO_4)_2$, the system is still above coalescence temperature, means still one set of signals. Unfortunately, we could not further follow the process, due to the fact, that acetonitrile freezes at -45 °C, thus as decrease in temperature was not possible. Furthermore, in virtue of the general broadening of the signals in the spectra, it was not possible to assign the newly upcoming peaks to any proton, therefore we can only assume that the system

remained intact. But, it is definitive indication that the presence of the earth alkali metal effects the system.

Figure V.3.2.8: Comparison of VT-NMR experiments with a) $\mathbf{B}_{\text{Azacrown}^{\text{Et}}}$ (2.8 mM) in MeCN and b) $\mathbf{B}_{\text{Azacrown}^{\text{Et}}}$ (2.8 mM) + 150 eq. $\text{Mg}(\text{ClO}_4)_2$ in MeCN. For both the spectra between $-40\text{ }^{\circ}\text{C}$ and $+70\text{ }^{\circ}\text{C}$ are shown. Due to the broadening and convolution of the signal it was not possible to assign the signals to the corresponding hydrogens in a reliable way.

V.3.3 Conclusion and Prospects

In conclusion, the here presented system was designed to decrease the rate of rotation in the already described push-pull rotor system. Since the rate of rotation and the rotational motion itself strongly depends on the quality of the intramolecular push-pull induced charge separation, in terms of stabilization, we tried to initiate a reverse directed electron-withdrawing motif that lowers the electron-donating ability of the heteroaromatic nitrogen towards the electron-withdrawing cyano acetate ester. Moreover, the system should satisfy the requirements of reversible formation of this electron-withdrawing moiety and a suitability for a later incorporation into supramolecular coordination cages. For that purpose the motif of the azacrown ether was chosen. Coupled to the π system, on the first glance it rather contributes as an electron donor, but can form in presence of suitable metals an reversible complex that exerts an electron-withdrawing effect on the intramolecular push-pull system.^[327–329] To examine the effect of metal-binding on the rotational rate, the here investigated model compound $\mathbf{B}_{\text{Azacrown}^{\text{Et}}}$ was synthesized on the basis of the already characterized compound $\mathbf{B}^{\text{Et}}$ (**35**), in order to have an easily comparable reference. The coordination of various metals, including alkali and earth alkali metals but also some transition metals, were investigated for their influence on the dynamic motion by UV-vis, NMR and VT NMR experiments. Additionally, as a reference for the assumed maximal electron-withdrawing effect, the protonation of the

azacrown nitrogen was examined as well. NMR provided evidence for the interaction of all investigated metals with the azacrown ether, although the observable effect ranged from almost non-existing to clearly visible. However, an explicit indication for a decrease in rotation upon metal coordination stayed off under experimental conditions. Whereas the presence of strong acids led to the protonation of the azacrown nitrogen, what showed a clear impact the systems' electronics and the first time a deceleration in rotation rate could be observed qualitatively. It is noteworthy that an excess of nickel and copper perchlorate led to protonation of the rotating cyano acetate ester group in both compounds (**B^{Et}** and **B_{Azacrown}^{Et}**), but could only be shown in NMR for the **B^{Et}** compound. A possible explanation for this unusual behavior could be the usage of the nickel and copper perchlorate hexa-hydrate ($M(\text{ClO}_4)_2 \times 6 \text{ H}_2\text{O}$), what possibly led to the *in situ* generation and simultaneously release of protons. But to be sure, further investigation are necessary. Since experiments under standard condition did not lead to the proof of concept. examination of the azacrown compound in presence of $\text{Mg}(\text{ClO}_4)_2$ with help of VT NMR measurements were carried out. The resulting NMR spectra revealed a notable change in the sensitivity of rotational rate towards the temperature, compared to the reference compound. Unfortunately, due to overall signal broadening and the lack of control experiments, these findings are not complete yet and can only be considered as promising indications for the successful control of rotational rate by an external stimuli. In further studies additional control experiments have to made to get a clear proof of principle. Furthermore, the change to another coordination site, e.g. terpyridine,^[330] with an stronger influence on the push-pull system electronics or better matching crown ether-metal pair^[320,331] as well as a fine tuning of the experimental conditions, could make the system being promising for either molecular sensor applications, dynamic stimuli responsive supramolecular systems, catalytic cavities or functional materials.

Experimental Section

VI

Chapter VI.1

Syntheses of Oligonucleotide Incorporable Ligands

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VI.1.1.1 2-((2-(Tetrahydro-2H-pyran-2-yl)oxy)ethoxy)ethan-1-ol (**3**) Scheme VI.1.1.13: Synthesis of compound **3**.

To a solution of diethylene glycol (25.37 mL, 265 mmol, 5.00 equiv.) and 4-methylbenzenesulfonic acid (0.91 g, 5.31 mmol, 0.10 equiv.) in CH_2Cl_2 (200 mL), 3,4-dihydro-2H-pyran (4.46 g, 53.1 mmol, 1.00 equiv.) was added dropwise at 0°C. The mixture was stirred for 1.5 h at room temperature. After, H_2O (200 mL) was added and the aq. phase was extracted with CH_2Cl_2 (3×50 mL). The combined organic phases were dried over MgSO_4 and the solvent was removed *in vacuo*. The residue was purified by means of column chromatography (SiO_2 , diethyl ether/ ethyl acetate 1:1). The product **37** (7.50 g, 39.4 mmol, 74 %) was obtained as a clear oil.

$^1\text{H-NMR}$ (300 M Hz, 298 K, CD_3CN): δ (ppm) = 4.67-4.55 (m, 2H, 5, 10), 3.82-3.44 (m, 10H, 1-4, 9), 1.83-1.42 (m, 6H, 6-8).

$^{13}\text{C-NMR}$ (176 MHz, 298 K, CD_3CN): δ (ppm) = 99.6, 72.8, 70.2, 66.6, 61.8, 60.7, 30.7, 25.5, 19.6.

VI.1.1.2 2-((2H-pyran-2-yl)ethoxy)ethyl 4-methylbenzenesulfonate (**4**)

Scheme VI.1.1.14: Synthesis of compound **4**.

To a solution of diethylene glycol (25.37 mL, 265 mmol, 5.00 equiv.) and 4-methylbenzenesulfonic acid (0.91 g, 5.31 mmol, 0.10 equiv.) in CH_2Cl_2 (200 mL), 3,4-dihydro-2*H*-pyran (4.46 g, 53.1 mmol, 1.00 equiv.) was added dropwise at 0°C. The mixture was stirred for 1.5 h at room temperature. After, H_2O (200 mL) was added and the aq. phase was extracted with CH_2Cl_2 (3×50 mL). The combined organic phases were dried over MgSO_4 and the solvent was removed *in vacuo*. The residue was purified by means of column chromatography (SiO_2 , diethyl ether/ ethyl acetate 1:1). The product **37** (7.50 g, 39.4 mmol, 74 %) was obtained as a clear oil.

^1H -NMR (300 M Hz, 298 K, CD_3CN): δ (ppm) = 7.80 (d, $^3J = 8.3$ Hz, 2H, 10), 7.33 (d, $^3J = 7.8$ Hz, 2H, 11), 4.58 (t, $^3J = 3.5$ Hz, 1H, 5), 4.22-4.10 (m, 2H, 9), 3.91-3.72 (m, 2H, 1), 3.91-3.72 (m, 2H, 3), 3.91-3.72 (m, 2H, 4), 3.91-3.72 (m, 2H, 2), 2.44 (s, 3H, 12), 1.88-1.41 (m, 6H, 6-8).

ESI-HRMS ($[\text{C}_9\text{H}_{18}\text{O}_4+\text{H}]^+$):	measured:	345.1359
	calculated:	345.1366

VI.1.1.3 3-Bromo-5-(2-(2-((tetrahydro-2H-pyran-2-yl)oxy)ethoxy)ethoxy)pyridine (5)

Scheme VI.1.1.15: Synthesis of compound 5.

$^1\text{H-NMR}$ (300 M Hz, 298 K, CD_3CN): δ (ppm) = 8.30 (d, $^4J = 2.6$ Hz, 1H, 12), 8.27 (d, $^4J = 1.9$ Hz, 1H, 10), 7.74-7.71 (m, 1H, 11), 4.56 (t, $^3J = 3.6$ Hz, 1H, 5), 4.26-4.17 (m, 2H, 1), 3.80-3.66 (m, 4H, 2, 3), 3.60 (t, $^3J = 4.8$ Hz, 9), 3.54 – 3.45 (m, 2H, 4), 1.72 – 1.35 (m, 6H, 6-8).

$^{13}\text{C-NMR}$ (176 MHz, 298 K, CD_3CN): δ (ppm) = 155.4, 142.2, 137.1, 123.8, 120.1, 92.1, 69.9, 68.8, 68.2, 66.1, 61.3, 30.2, 25.1, 19.1.

ESI-HRMS ($[\text{C}_{14}\text{H}_{20}\text{BrNO}_4+\text{H}]^+$):	measured:	346.0649
	calculated:	346.0648

VI.1.1.4 3-(2-(2-((Tetrahydro-2H-pyran-2-yl)oxy)ethoxy)ethoxy)-5-((trimethylsilyl)ethynyl)pyridine (6) **Scheme VI.1.1.16:** Synthesis of compound **6**.

$^1\text{H-NMR}$ (300 M Hz, 298 K, CD_3CN): δ (ppm) = 8.29 (s, 1H, 12), 8.26 (s, 1H, 10), 7.26 (s, 1H, 11), 4.63 (t, 1H, 5), 4.21-4.13 (m, 2H, 1), 3.96-3.82 (m, 4H, 2, 3), 3.79-6.69 (m, 2H, 4), 3.68-3.42 (m, 2H, 9), 1.93-1.44 (m, 6H, 6-8), 0.25 (s, 9H, 13)

$^{13}\text{C-NMR}$ (176 MHz, 298 K, CD_3CN): δ (ppm) = 154.0, 143.9, 138.3, 123.0, 118.9, 97.9, 83.7, 80.1, 69.6, 68.6, 67.6, 65.9, 30.0, 24.8, 18.8, 1.4.

VI.1.1.5 3-ethynyl-5-(2-(2-((tetrahydro-2H-pyran-2-yl)oxy)ethoxy)ethoxy)pyridine (**7**)

Scheme VI.1.1.17: Synthesis of compound **7**.

6 and K_2CO_3 in MeOH (90 mL) were stirred at room temperature for 3.5 h. The solvent was removed *in vacuo* and the residue was resolved in CH_2Cl_2 (150 mL). The mixture was washed with H_2O (2×75 mL) and brine (50 mL). The organic phase was dried over MgSO_4 and the solvent was removed *in vacuo*. The product **7** (2.57 g, 8.72 mmol, 99 %) was obtained as a brownish oil.

^1H -NMR (300 M Hz, 298 K, CD_3CN): δ (ppm) = 8.25 (d, $^4J = 2.8$ Hz, 12), 8.20 (d, $^4J = 1.6$ Hz, 10), 7.46 (dd, $^4J = 2.9$, 1.6 Hz, 11), 4.51 (t, $^3J = 3.6$ Hz, 1H), 4.36 (s, 1H, 13), 4.15 (dd, $^3J = 5.3$, 3.7 Hz, 4H, 2,3), 3.74-3.62 (m, 2H, 4), 3.57-3.53 (m, 2H, 1), 3.47-3.40 (m, 2H, 9), 1.67-1.30 (m, 6H, 6-8).

^{13}C -NMR (176 MHz, 298 K, CD_3CN): δ (ppm) = 154.7, 144.6, 138.9, 123.7, 119.5, 98.53, 84.4, 80.7, 70.3, 69.2, 68.3, 66.6, 61.7, 30.7, 25.5, 19.6.

VI.1.1.6 10-(2-(2-methoxyethoxy)ethyl)-2,7-bis((5-(2-(2-((tetrahydro-2H-pyran-2-yl)oxy)ethoxy)ethoxy)pyridin-3-yl)ethynyl)acridin-9(10H)-one (8)

Scheme VI.1.1.18: Synthesis of compound **8**.

2 (2.15 g, 3.92 mmol, 1.00 equiv.), **7** (2.40 g, 8.22 mmol, 2.10 equiv.), Pd(PPh₃)₂Cl₂ (275 mg, 0.39 mmol, 0.10 equiv.) and CuI (74.6 mg, 0.39 mmol, 0.10 equiv.) were solved in dry THF (15 mL). Triethylamine (2.00 mL, 15.7 mmol, 4.00 equiv.) was added and the reaction mixture was stirred at 70 °C over night. The cooled down reaction mixture was filtered through celite and the residue was washed with CH₂Cl₂ (3 × 10 mL). The solvent of the combined organic phases was removed *in vacuo* and the residue was purified by means of column chromatography (SiO₂, chloroform / methanol 50:1 → 10:1). The product **8** was obtained as a yellow-brownish resin.

¹H-NMR (300 MHz, 298 K, DMSO): δ (ppm) = 8.88-8.14 (br, 4H, d, f), 8.47 (d, ⁴*J* = 2.1 Hz, a), 7.99 (d, ³*J* = 9.2 Hz, 2H, c), 7.94 (dd, ³*J* = 9.2 Hz, ⁴*J* = 2.1 Hz, 2H, b), 7.66 (s, 2H, e), 4.76 (t, ³*J* = 6.0 Hz, 2H, NCH₂CH₂), 4.58 (d, ³*J* = 3.6 Hz, 2H, 5), 4.25 (t, ³*J* = 4.4 Hz, 4H, 1), 3.93 (t, ³*J* = 5.5 Hz, 2H, NCH₂CH₂), 3.80 (t, ³*J* = 4.5 Hz, 4H, 2), 3.79-3.70 (m, 4H, 9), 3.64 (dd, ³*J* = 4.8 Hz, 4H, CH₂CH₂OCH₃), 3.56-3.48 (m, 4H, 3), 3.35-3.28 (m, 4H, 4), 3.12 (s, 3H, OCH₃).

^{13}C -NMR (176 MHz, 298 K, DMSO): δ (ppm) = 179.5, 175.2, 142.9, 136.2, 131.5, 130.1, 128.8, 121.6, 118.0, 115.0, 92.1, 91.5, 71.3, 70.2, 69.9, 68.8, 68.1, 67.9, 66.1, 61.3, 58.1, 46.3, 30.2, 29.5, 25.1, 19.1.

ESI-HRMS ($[\text{C}_{50}\text{H}_{57}\text{N}_3\text{O}_{11} + \text{H}]^+$): measured: 876.4050
 calculated: 876.4066

VI.1.1.7 2,7-bis((5-(2-(2-hydroxyethoxy)ethoxy)pyridin-3-yl)ethynyl)-10-(2-(2-methoxyethoxy)ethyl)acrid-9-one (9)

Scheme VI.1.1.19: Synthesis of compound **9**.

8 (850 mg, 1.00 mmol, 1 equiv.) was solved in EtOH (100 mL) and HCl (1 M, 10 mL, 10.0 mmol, 10.0 equiv.) was added. The mixture was heated to under reflux for 1 h. After cooling down the solvent volume was reduced to ca. 10 mL, then CH₂Cl₂ (50 mL) was added. The mixture was washed with sat. NaHCO₃ solution (50 mL) and brine (30 mL) and the organic phase was dried over MgSO₄. The solvent was removed *in vacuo*. The product **9** (0.65 g, 0.92 mmol, 92 %) was obtained as a yellow-brownish solid.

¹H-NMR (300 M Hz, 298 K, CD₃Cl): δ (ppm) = 8.68 (d, ⁴*J* = 2.0 Hz, 2H, a), 8.49 (s, 2H, f), 8.31 (s, 2H, d), 7.83 (dd, ³*J* = 9.0 Hz, ⁴*J* = 2.0 Hz, 2H, b), 7.64 (d, ³*J* = 9.0 Hz, 2H, c), 7.36 (s, 2H, e), 4.64 (t, ³*J* = 6.3 Hz, 2H, NCH₂), 4.25-4.20 (m, 4H, 1), 4.01 (t, ³*J* = 6.3 Hz, 2H, NCH₂CH₂), 3.94-3.89 (m, 4H, 2), 3.82-3.77 (m, 4H, 3), 3.72-3.68 (m, 4H, 4), 3.68-3.64 (m, 2H, CH₂CH₂OCH₃), 3.54-3.50 (m, 2H, CH₂CH₂OCH₃), 3.36 (s, 3H, OCH₃).

¹³C-NMR (176 MHz, 298 K, CD₃CN): δ (ppm) = 176.4, 144.7, 141.8, 137.9, 136.5, 131.6, 122.9, 122.4, 116.1, 115.8, 91.6, 86.4, 77.2, 72.7, 72.0, 71.2, 69.4, 68.1, 67.9, 61.7, 59.2, 59.1, 46.4.

ESI-HRMS ($[\text{C}_{40}\text{H}_{11}\text{N}_3\text{O}_9+\text{H}]^+$):	measured:	708.2899
	calculated:	708.2916

VI.1.1.8 2-((5-(2-(2-(Bis(4-methoxyphenyl)(phenyl)methoxy)ethoxy)ethoxy)pyridin-3-yl)ethynyl)-7-((5-(2-(2-hydroxyethoxy)ethoxy)pyridin-3-yl)ethynyl)-10-(2-(2-methoxyethoxy)ethyl)acridin-9(10H)-one (10)

Scheme VI.1.1.20: Synthesis of compound **10**.

4,4'-Dimethoxytrityl chloride (522 mg, 1.54 mmol, 1.05 equiv.), 4-DMAP (9.00 mg, 0.07 mmol, 0.05 equiv.) und **9** (1.04 g, 1.47 mmol, 1.00 equiv.) was solved in CH_2Cl_2 (50 mL) and triethylamine (0.31 mL, 2.20 mmol, 1.50 equiv.) was added dropwise at 0 °C. The reaction mixture was stirred at room temperature over night, whereby the mixture warmed to room temperature. The mixture was diluted with CH_2Cl_2 and the organic phase was washed with 5 % NaHCO_3 solution (25 mL) and brine 25 mL). The organic phase was dried over MgSO_4 and the solvent was removed *in vacuo*. The residue was purified by means of column chromatography (SiO_2 , chloroform / methanol 50:1 $\rightarrow$ 10:1, with 1 % TEA). The product was obtained **10** was obtained as an orange foam.

^1H -NMR (300 M Hz, 298 K, CD_3Cl): δ (ppm) = 8.65 (dd, $^4J = 2.1$ Hz, 1H, a), 8.63 (dd, $^4J = 2.1$ Hz, 1H, a'), 8.38 (d, $^3J = 1.8$ Hz, 2H, f,f'), 8.29 (dd, $^4J = 2.8$ Hz, 2H, d), 8.27 (dd, $^4J = 2.8$ Hz, 2H, d'), 7.79 (dd, $^3J = 4.1$ Hz, $^4J = 2.2$ Hz, 1H, b), 7.76 (dd, $^3J = 4.1$ Hz, $^4J = 2.2$ Hz, 1H, b'), 7.61 (d, $^3J = 3.4$ Hz, 1H, c), 7.58 (d, $^3J = 3.4$ Hz, 1H, c), 7.48-7.44 (m, 2H, 8), 7.38-7.30 (m, 6H, 5, e, e'), 7.30-7.13 (m, 3H, 9, 10), 6.84-6.76 (m, 4H, 6), 4.60 (t, $^3J = 6.2$ Hz, 2H, NCH_2CH_2), 4.25-4.16 (m, 4H, 1, 1'), 3.99 (t, $^3J = 6.2$ Hz, 2H, NCH_2CH_2), 3.95-3.86 (m, 4H, 2, 2'), 3.83-3.62 (m, 14H, 3, 3', 4, 7, $\text{CH}_2\text{CH}_2\text{OCH}_3$), 3.53-3.47 (m, 2H, 4'), 3.34 (s, 3H, OCH_3), 3.27 (t, $^3J = 5.1$ Hz, 2H, $\text{CH}_2\text{CH}_2\text{OCH}_3$).

^{13}C -NMR (176 MHz, 298 K, CD_3Cl): δ (ppm) = 176.6, 158.5, 155.0, 154.4, 145.1, 144.9, 144.8, 142.0, 138.1, 136.7, 136.6, 136.4, 135.5, 131.9, 131.8, 130.2, 129.0, 128.5, 128.4, 128.3, 127.9, 126.8, 123.1, 123.0, 122.6, 120.6, 120.5, 116.4, 116.0, 115.9, 113.2, 91.7, 91.6, 86.7, 86.6, 72.8, 72.2, 71.3, 71.2, 69.7, 69.6, 68.3, 68.2, 68.1, 67.2, 63.3, 62.0, 59.3, 55.4, 55.0, 46.5.

ESI-HRMS ($[\text{C}_{61}\text{H}_{59}\text{N}_3\text{O}_{11}+\text{H}]^+$):	measured:	1010.4217
	calculated:	1010.4222

VI.1.1.9 2-((5-((7-((5-(2-(2-(Bis(4-methoxyphenyl)(phenyl)methoxy)ethoxy)ethoxy)pyridin-3-yl)ethynyl)-10-(2-(2-methoxyethoxy)ethyl)-9-oxo-9,10-dihydroacridin-2-yl)ethynyl)pyridin-3-yl)oxy)ethoxy)ethyl (2-cyanoethyl) diisopropylphosphoramidite (11**)**

Scheme VI.1.1.21: Synthesis of compound **11**.

10 (0.81 g, 0.80 mmol, 1.00 equiv.) and DIPEA (0.75 mL, 2.43 mmol, 3.00 equiv.) were solved in dry THF (5 mL) and stirred for 0.5 h at room temperature. 2-Cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (0.23 mL, 1.04 mmol, 1.30 equiv.) was added dropwise and the reaction was followed by thin layer chromatography. After completion, the solvent was removed *in vacuo* and the residue was resolved in dry acetonitrile. The mixture was filtered under inert conditions and the solvent was removed again *in vacuo*. The product **11** was obtained as an orange foam and was used immediately for DNA synthesis without further purification.

³¹P-NMR (300 MHz, 298 K, CDCl₃): δ (ppm) = 148.0. (peaks at 14 or 8 ppm are oxidized or hydrolyzed side products)

VI.1.2 Syntheses of the Methoxybenzylamine-based Ligand

VI.1.2.1 4-Hydroxy-3,5-diiodobenzaldehyde (**12**)

Scheme VI.1.2.22: Synthesis of compound **12**.

To a stirred solution of 4-hydroxybenzaldehyde (5.00 g, 40.9 mmol, 1.00 equiv.), potassium iodate (8.76 g, 40.9 mmol, 1.00 equiv) and potassium chloride (6.10 g, 81.9 mmol, 2.00 equiv.) in acetic acid/water (9:1, 150 mL), potassium iodide (13.6 g, 81.9 mmol, 2.00 equiv.) was added at room temperature. After 6 h, EtOAc (500 mL) was 10 % NaS_2O_3 solution (500 mL) were added. The organic layer was separated and the aq. phase was extracted with EtOAc (2×250 mL). The combined organic layers were washed with 10 % NaS_2O_3 solution (250 mL) and brine (250 mL) and dried over MgSO_4 . The solvent was removed *in vacuo*. The product **12** (14.8 g, 39.7 mmol, 97 %) was obtained as yellowish solid and could be taken for next synthesis without further purification.

$^1\text{H-NMR}$ (300 M Hz, 298 K, CD_3Cl_3): δ (ppm) = 9.74 (s, 1H, b), 8.20 (s, 2H, a), 6.28 (br, 1H, c).

ESI-HRMS ([C₇H₄I₂O₂+H]⁺):

measured: 374.8369

calculated: 374.8373

VI.1.2.2 3,5-Diiodo-4-methoxybenzaldehyde (**13**) Scheme VI.1.2.23: Synthesis of compound **13**.

To **12** (7.50 g, 20.1 mmol, 1.00 equiv.) solved in Actone (100 mL) K_2CO_3 (6.93 g, 50.2 mmol, 2.50 equiv.) was added and the suspension was stirred 15 min at room temperature. MeI (6.47 mL, 100 mmol, 5.00 equiv.) was added and the reaction mixture was heated over night under reflux. The cooled down mixture was washed with H_2O (100 mL) and brine (50 mL) and the organic layer was dried over $MgSO_4$. After, the solvent was removed *in vacuo*. The product **13** (7.40 g, 19.1 mmol, 95 %) was obtained as brownish white solid.

1H -NMR (300 M Hz, 298 K, CD_3Cl): δ (ppm) = 9.81 (s, 1H, b), 8.27 (s, 2H, a), 3.93 (s, 3H, c).

^{13}C -NMR (176 MHz, 298 K, CD_3Cl): δ (ppm) = 188.2, 163.9, 141.4, 135.5, 91.4, 61.0.

ESI-HRMS ($[\text{C}_8\text{H}_6\text{I}_2\text{O}_2+\text{H}]^+$):	measured:	388.8345
	calculated:	388.8350

VI.1.2.3 (3,5-Diiodo-4-methoxyphenyl)methanol (**14**) Scheme VI.1.2.24: Synthesis of compound **14**.

To a well stirring mixture containing **13** (4.00 g, 10.3 mmol, 1 equiv.) in CH₂Cl₂ (35 mL) and CH₃OH (35 mL) at 0 °C was added portionwise NaBH₄ (0.41 g, 10.8 mmol, 1.05 equiv.) and the mixture was stirred at rt for 3 h. Saturated NH₄Cl (10 mL) was then added. After removal of most of the solvents, the residue was taken into Et₂O (100 mL), washed with H₂O (2 × 50 mL) and the organic layer was dried over MgSO₄. The solvent was removed *in vacuo*. The product **14** (4.00 g, 10.2 mmol, 99 %) was obtained as white solid.

¹H-NMR (300 M Hz, 298 K, DMSO): δ (ppm) = 7.75 (s, 2, a), 5.31 (t, ³*J* = 5.9 Hz, 1H, d), 4.40 (d, ³*J* = 5.8 Hz, 2H, b), 3.73 (s, 3H, c).

¹³C-NMR (176 MHz, 298 K, DMSO): δ (ppm) = 157.0, 142.8, 137.4, 91.0, 60.7, 60.3.

VI.1.2.4 5-(chloromethyl)-1,3-diiodo-2-methoxybenzene (**15**) Scheme VI.1.2.25: Synthesis of compound **15**.

To a solution of **14** (3.90 g, 10.0 mmol, 1.00 equiv.) and triethylamine (1.39 mL, 10.0 mmol, 1.00 equiv.) in dichloromethane (20 mL) was slowly added thionyl chloride (0.87 mL, 12.0 mmol, 1.20 equiv.) at 0 °C. The solution was stirred at 0 °C for 15 min then at rt for 3 h. Dichloromethane (80 mL) was added and the solution was washed with water (50 mL), sat. NaHCO₃ solution (50 mL) then brine (50 mL). The organic layer was dried MgSO₄ and the solvent was removed *in vacuo*. The product **15** (3.95 g, 9.68 mmol, 97 %) was obtained as a brown oil, which was used in the next step without further purification.

¹H-NMR (300 MHz, 298 K, CD₃Cl): δ (ppm) = 7.78 (s, 2H, a), 4.44 (s, 2H, b), 3.86 (s, 3H, c).

¹³C-NMR (176 MHz, 298 K, CD₃CN): δ (ppm) = 159.1, 140.0, 137.1, 90.5, 60.9, 43.6.

VI.1.2.5 2,2'-((3,5-diiodo-4-methoxybenzyl)azanediyl)bis(ethan-1-ol) (**16**) Scheme VI.1.2.26: Synthesis of compound **16**.

A mixture of **15** (2.30 g, 5.63 mmol, 1.00 equiv.), diethanolamine (0.59 mL, 6.19 mmol, 1.10 equiv.) and DIPEA (1.25 mL, 7.05 mmol, 1.25 equiv.) in CHCl₃ (50 mL) was heated under reflux over night. After cooling down, CHCl₃ (50 mL) was added and the organic layer was washed with H₂O (50 mL) and brine (50 mL). The organic phase was dried over MgSO₄ and the solvent was removed. The product **16** (2.40 g, 5.01 mmol, 89 %) was obtained as a yellowish oil.

¹H-NMR (300 M Hz, 298 K, CD₃CN): δ (ppm) = 7.83 (s, 2H, a), 3.80 (s, 3H, c), 3.57 (s, 2H, b), 3.50 (t, ³*J* = 5.6 Hz, 3H, 2), 2.57 (t, ³*J* = 5.6 Hz, 1).

¹³C-NMR (176 MHz, 298 K, CD₃CN): δ (ppm) = 158.5, 141.3, 141.1, 90.6, 61.3, 60.2, 57.8, 56.7.

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VI.1.2.6 (2,2'-((4-Methoxy-3,5-bis(pyridin-3-ylethynyl)benzyl)azanediyl)bis(ethan-1-ol) (17)

Scheme VI.1.2.27: Synthesis of compound **17**.

To a mixture of **16** (1.00 g, 2.10 mmol, 1.00 equiv.), 3-ethynyl pyridine (0.64 g, 6.29 mmol, 3.00 equiv.), $\text{Pd(PPh}_3)_2\text{Cl}_2$ (0.15 g, 0.21 mmol, 0.10 equiv.) and CuI (0.40 g, 0.21 mmol, 0.10 equiv) in dry DMF (10 mL), triethylamine (0.65 mL, 4.61 mmol, 2.20 equiv.) was added. The mixture was stirred at 75 °C over night. After, the DMF was removed *in vacuo* and the residue was resolved in CH_2Cl_2 (50 mL) and filtered through celtie. The filtrate was washed with sat. aq. NaHCO_3 (30 mL) and brine (30 mL). The organic layer was dried over MgSO_4 and the solvent was removed *in vacuo*. The residue was purified by means of column chromatography (SiO_2 , CH_2Cl_2 / acetone 2:1). The product **17** (2.1 g, 4.91 mmol, 78 %) was obtained as yellowish brown resin.

$^1\text{H-NMR}$ (300 M Hz, 298 K, CD_3CN): δ (ppm) = 8.36 (dd, $^3J = 8.3$ Hz, $^4J = 1.4$ Hz, 2H, a/a'), 7.72 (ddd, $^3J = 8.5$, 6.8 Hz, $^4J = 1.5$ Hz, 2H, c/c'), 7.67 (dd, $^3J = 8.9$ Hz, $^4J = 1.2$ Hz, 2H, d/d'), 7.43 – 7.38 (m, 2H, f), 7.31 (ddd, $^3J = 8.2$, 6.9 Hz, $^4J = 1.2$ Hz, 2H, b/b'), 7.26 (tt, $^3J = 7.5$ Hz, $^4J = 1.3$ Hz, 1H, g), 7.16 – 7.10 (m, 2H, e), 4.43 – 4.35 (m, 2H, NCH_2), 1.95 – 1.86 (m, 2H, NCH_2CH_2), 1.54 – 1.44 (m, 2H, $\text{NCH}_2\text{CH}_2\text{CH}_2$), 1.42 – 1.28 (m, 4H, $\text{CH}_2\text{CH}_2\text{CH}_3$), 0.90 (t, $^3J = 7.1$ Hz, 3H, $\text{CH}_2\text{CH}_2\text{CH}_3$).

^{13}C -NMR (176 MHz, 298 K, CD_3CN): δ (ppm) = 165.5, 155.7, 151.7, 140.0, 134.0, 130.4, 129.3, 126.9, 122.7, 122.4, 121.1, 120.5, 116.0, 86.6, 48.6, 32.1, 27.5, 27.0, 23.3, 14.3.

Melting point: 169-170 $^\circ\text{C}$.

ESI-HRMS ($[\text{C}_{28}\text{H}_{26}\text{N}_2\text{O}_2]^+$):

measured:	423.2051
calculated:	423.2067

VI.1.2.7 (2-((2-(bis(4-methoxyphenyl)(phenyl)methoxy)ethyl)(4-methoxy-3,5-bis(pyridin-3-ylethynyl)benzyl)amino)ethan-1-ol (18)

Scheme VI.1.2.28: Synthesis of compound **18**.

TiCl₄ (255 mg, 1.34 mmol, 1.25 equiv.) was slowly added to *N*-hexyl-acridone (300 mg, 1.07 mmol, 1.00 equiv.) solved in dry CH₂Cl₂ (15 mL) and stirred for 15 min at room temperature. **36** (346 mg, 2.15 mmol, 2.00 equiv.) and triethylamine (2 mL) were added consecutively to the reaction mixture and heated under reflux for 24 h. The cooled down reaction mixture was filtered through celite and the residue was washed with CH₂Cl₂ (2 x 5mL). The solvent was removed in vacuo and the residue was purified by means of column chromatography (SiO₂, chloroform/methanol 40:1 → 20:1). The product **37** (271 mg, 0.64 mmol, 60%) was obtained as a dark purple solid.

¹H-NMR (300 M Hz, 298 K, CD₃CN): δ (ppm) = 8.74 (d, 1.2 Hz, 2H), 8.55 (dd, 4.9 Hz, 1.7 Hz, 2H), 7.79 (dt, 7.9 Hz, 2.0 Hz, 2H), 7.48 (s, 2H), 7.44 (m, 8.5 Hz, 1.4 Hz, 2H), 7.36-7.26 (m, 8H), 7.21-7.15 (m, 1H), 6.83 (d, 8.9 Hz, 4H), 4.14 (s, 3H), 3.73 (s, 6H), 3.61 (s, 2H), 3.56 (t, 5.3 Hz, 2H), 3.19 (t, 5.5 Hz, 2H), 2.77 (t, 5.5 Hz, 2H), 2.66 (t, 5.5 Hz, 2H). The proton of the hydroxy group was not detectable.

¹³C-NMR (176 MHz, 298 K, CD₃CN): δ (ppm) = 161.6, 159.1, 152.7, 149.41, 145.8, 138.9, 136.8, 135.9, 134.9, 130.5, 128.6, 128.4, 127.2, 123.6, 120.71, 117.3, 113.6, 113.5, 91.0, 88.8, 86.9, 62.3, 62.1, 59.4, 58.5, 56.6, 55.7.

ESI-HRMS ([C₂₈H₂₆N₂O₂]⁺):

measured:	423.2051
calculated:	423.2067

VI.1.2.8 2-((2-(Bis(4-methoxyphenyl)(phenyl)methoxy)ethyl)(4-methoxy-3,5-bis(pyridin-3-ylethynyl)benzyl)amino)ethyl (2-cyanoethyl) diisopropylphosphoramidite (19)

Scheme VI.1.2.29: Synthesis of compound 19.

¹H-NMR (300 M Hz, 298 K, CD₃CN): δ (ppm) = 8.74 (d, 1.2 Hz, 2H), 8.55 (dd, 4.9 Hz, 1.7 Hz, 2H), 7.79 (dt, 7.9 Hz, 2.0 Hz, 2H), 7.48 (s, 2H), 7.44 (m, 8.5 Hz, 1.4 Hz, 2H), 7.36-7.26 (m, 8H), 7.21-7.15 (m, 1H), 6.83 (d, 8.9 Hz, 4H), 4.14 (s, 3H), 3.73 (s, 6H), 3.61 (s, 2H), 3.56 (t, 5.3 Hz, 2H), 3.19 (t, 5.5 Hz, 2H), 2.77 (t, 5.5 Hz, 2H), 2.66 (t, 5.5 Hz, 2H). The proton of the hydroxy group was not detectable.

^{31}P -NMR (300 MHz, 298 K, CD_3CN): δ (ppm) = 146.4. (peak of unreacted phosphoamidite at 14 ppm.)

VI.1.2.9 5-(Benzylthio)-1*H*-tetrazole (**20**) Scheme VI.1.2.30: Synthesis of compound **20**.

To a solution of Benzyl thiocyanate (35.5 g, 0.24 mol, 1.00 equiv.) in 1,4-dioxane (200 mL) was added an aq. NH_4Cl solution (5 M, 50.0 mL, 0.25 mol, 1.05 equiv.). The mixture was degassed under reduced pressure with help of an ultra-sonic bath. To the resulting reaction mixture sodium azide (15.5 g, 0.24 mol, 1.00 equiv.) was added portionwise and the suspension was stirred at 90 °C for 6 h. After the reaction has been cooled down, the solvent volume was reduced *in vacuo* to approx. 50 mL. Ice was added to the remaining solution was acidified subsequently with hydrochloric acid until pH 2 was reached. The resulting precipitation was filtered off and resuspended in toluene (150 mL). The heavily stirring suspension was heated to 110 °C for 1 h. The aqueous layer was removed and acetonitrile (10 mL) was added to the remaining organic layer. The solution was kept heating for another 30 min at 110 °C. After, the solution was filtered off hot and the filtrate was allowed to cool down slowly, whereupon the product starts to crystallize from solution. The crystalline precipitation was collected and dried *in vacuo*. The product **20** (28.8 g, 0.15 mol, 63 %) was obtained as a white crystalline solid.^[332]

^1H -NMR (500 MHz, 298 K, DMSO): δ (ppm) = 7.40 (d, $^3J = 7.2$ Hz, 2H, c), 7.30 (t, $^3J = 7.4$ Hz, 2H, b), 7.24 ($^3J = 7.3$ Hz, 1H, a), 4.51 (s, 2H, d), (NH not observable).

^{13}C -NMR (125 MHz, 298 K, DMSO): δ (ppm) = 153.8, 136.7, 129.0, 128.6, 127.7, 36.1.

ESI-HRMS ($[\text{C}_8\text{H}_9\text{N}_4\text{S}+\text{H}]^+$):

measured: 193.0533

calculated: 193.0542

Chapter VI.2

Syntheses of Water-Soluble Ligands

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VI.2.1 Syntheses of Sulfobetaine Ligand

VI.2.1.1 1-(3-iodo-4-methoxy-5-methylphenyl)-N,N-dimethylmethanamine (**22**)

To a solution of **15** (1.00 g, 2.45 mmol, 1.00 equiv.) in THF (5 mL), an aq. solution of Me₂NH (40 % (w/v), 1.40 mL, 12.2 mmol, 5.00 equiv) was added. The mixture was stirred at room temperature until the completion of the reaction was observed by TLC. The solvent was removed and the residue was redissolved in EtOAc (50 mL). The organic layer was washed with sat. aq. NaHCO₃ solution (25 mL) and brine (25 mL). The organic phase was

Scheme VI.2.1.31: Synthesis of compound **22**.

dried over MgSO_4 and the solvent was removed *in vacuo*. The residue was purified by means of column chromatography (SiO_2 , pentane / ethyl acetate 10:1 $\rightarrow$ 2:1). The product **22** (0.95 g, 2.28 mmol, 93 %) was obtained as a brownish solid.

$^1\text{H-NMR}$ (300 M Hz, 298 K, DMSO): δ (ppm) = 7.74 (s, 2H, a), 3.74 (s, 3H, c), 3.29 (s, 2H, b), 2.13 (s, 6H, 1).

$^{13}\text{C-NMR}$ (176 MHz, 298 K, DMSO): δ (ppm) = 157.2, 139.5, 139.4, 91.0, 60.9, 60.2, 44.8.

ESI-HRMS ($[\text{CH}_{26}\text{N}_2\text{O}_2]^+$):	measured:	416.9079
	calculated:	416.9087

VI.2.1.2 3-((3,5-Diiodo-4-methoxybenzyl)dimethylammonio)propane-1-sulfonate (**23**)

Scheme VI.2.1.32: Synthesis of compound **23**.

To a solution of 1,3-propane sultone (0.15 mL, 1.71 mmol, 1.00 equiv.) in 1,4-dioxane (3 mL), a solution of **22** (750 mg, 1.80 mmol, 1.05 equiv.) in 1,4-dioxane (3 mL) was added at room temperature, over a period of 15 min. The reaction mixture was heated for 4 h under reflux. The milky suspension was cooled down with an ice-bath, subsequently the suspension was filtered and the collected solid was washed with acetone (2×10 mL), after, the solid was dried in a vacuum. The product **23** (700 mg, 1.30 mmol, 76 %) was obtained as a white solid.

$^1\text{H-NMR}$ (300 MHz, 298 K, MeOH): δ (ppm) = 8.08 (s, 2H, a), 4.45 (s, 2H, b), 3.90 (s, 3H, c), 3.60-3.49 (s, 2H, 2), 3.05 (s, 6H, 1), 2.90 (t, $^3J = 6.8$ Hz, 2H, 4), 2.36-2.31 (m, 2H, 3).

ESI-HRMS ($[\text{C}_{13}\text{H}_{19}\text{I}_2\text{NO}_4\text{S}+\text{H}]^+$):	measured:	539.9215
	calculated:	539.9197

VI.2.1.3 3-((4-Methoxy-3,5-bis(pyridin-3-ylethynyl)benzyl)dimethylammonio)propane-1-sulfonate (**24**)

Scheme VI.2.1.33: Synthesis of compound **24**.

23 (250 mg, 0.46 mmol, 1.00 equiv), 3-ethynyl pyridine (120 mg, 1.16 mmol, 2.50 equiv.), $\text{Pd(PPh}_3)_2\text{Cl}_2$ (20.4 mg, 0.05 mmol, 0.10 equiv) and CuI (13.3 mg, 0.07 mmol, 0.15 equiv.) were solved in dry DMF (5 mL). To the stirred reaction triethylamine (0.38 mL, 2.32 mmol, 5.00 equiv.) was added and the reaction mixture was stirred at 80 °C over night. The solvent was removed *in vacuo* and the residue was purified by means of column chromatography (*reversed phase* RP-C18 with Isolera, H_2O / MeCN 1:1). The product **24** (52 mg, 0.11 mmol, 23 %) was obtained as a greyish white solid.

$^1\text{H-NMR}$ (300 M Hz, 298 K, MeOH): δ (ppm) = 8.74 (d, $^4J = 1.9$ Hz, 2H, g), 8.57 (dd, $^3J = 5.1$ Hz, $^4J = 1.7$ Hz, 2H, f), 8.04 (dt, $^3J = 7.9$ Hz, $^4J = 1.9$ Hz, 2H, d), 7.84 (s, 2H, a), 7.51 (dd, $^3J = 8.0, 5.0$ Hz, 2H, e), 4.54 (s, 2H, b), 4.26 (s, 3H, c), 3.64-3.55 (m, 2H, 2), 3.10 (s, 6H, 1), 2.92 (t, $^3J = 6.8$ Hz, 2H, 4), 2.40-2.27 (m, 2H, 3)

ESI-HRMS ($[\text{C}_{27}\text{H}_{27}\text{N}_3\text{O}_4\text{S}+\text{H}]^+$):	measured:	490.1803
	calculated:	490.1795

VI.2.2 Syntheses of Hydroxybenzylamine-based Ligand

VI.2.2.1 (3,5-Diiodo-4-((trimethylsilyl)oxy)benzaldehyde) (**26**)

Scheme VI.2.2.34: Synthesis of compound **26**.

To a solution of **12** (9.90 g, 26.5 mmol, 1.00 equiv.) and *tert*-butyldimethylsilyl chloride (4.79 g, 31.8 mmol, 1.20 equiv.) in ethyl acetate (50 mL), triethylamine (7.34 mL, 53.0 mmol, 2.00 equiv.) was added. The reaction mixture was stirred over night at room temperature. The solids were filtered off and the solvent volume was reduced to the half. The solution was allowed to stand for 2 h. After, the solids were removed again by filtration and the solvent of the filtrate was removed *in vacuo*. The product **26** (11.9 g, 24.4 mmol, 92 %) was obtained as a white solid.

¹H-NMR (300 M Hz, 298 K, DMSO): δ (ppm) = 9.72 (s, 1H, b), 8.24 (s, 2H, a), 0.83 (s, 9H, Si(CH₂)₂C(CH₃)₃), -0.05 (s, 6H, Si(CH₂)₂C(CH₃)₃).

^{13}C -NMR (176 MHz, 298 K, DMSO): δ (ppm) = 189.3, 160.7, 140.7, 132.1, 86.6, 25.8, 17.8, -3.20.

ESI-HRMS ($[\text{C}_{13}\text{H}_{18}\text{I}_2\text{O}_2\text{Si}+\text{H}]^+$):	measured:	488.9235
	calculated:	488.9238

VI.2.2.2 ((3,5-Diiodo-4-((trimethylsilyl)oxy)phenyl)methanol) (**27**) Scheme VI.2.2.35: Synthesis of compound **27**.

To a well stirring mixture containing **26** (4.50 g, 9.21 mmol, 1.00 equiv.) CH_2Cl_2 (40 mL) and MeOH (40 mL) at 0 °C was added portionwise NaBH_4 (0.37 g, 9.68 mmol, 1.05 equiv.) and the mixture was stirred for further 3 h. After, sat. NH_4Cl solution (5 mL) was added and the solvent was reduced to the half. Et_2O (50 mL) was added and the organic layer was washed with H_2O (25 mL) and brine (25 mL). The organic phase was dried over MgSO_4 and the solvent was removed *in vacuo*. The product **27** (4.42 g, 9.02 mmol, 98 %) was obtained as a yellowish solid.

^1H -NMR (300 MHz, 298 K, DMSO): δ (ppm) = 7.78 (s, 2H, a), 5.29 (s, 1H, c), 4.41 (s, 2H, b), 1.07 (s, 9H, $\text{Si}(\text{CH}_3)_3$), 0.48 (s, 6H, $\text{Si}(\text{CH}_2)_2\text{C}(\text{CH}_3)_3$).

^{13}C -NMR (176 MHz, 298 K, DMSO): δ (ppm) = 153.2, 139.8, 138.0, 89.2, 60.7, 26.4, 18.6, -0.2.

VI.2.2.3 (4-(Chloromethyl)-2,6-diiodophenoxy)trimethylsilane (28)

Scheme VI.2.2.36: Synthesis of compound **28**.

To a solution of **27** (4.20 g, 8.57 mmol, 1.00 equiv.) and triethylamine (1.32 mL, 9.41 mmol, 1.10 equiv.) in CH_2Cl_2 (10 mL), thionyl chloride (0.75 mL, 10.3 mmol, 1.20 equiv.) was slowly added at 0 °C. The solution was stirred for 3 h, while slowly warming to room temperature. CH_2Cl_2 (90 mL) was added and the organic layer was washed subsequently with H_2O (30 mL), sat. aq. NaHCO_3 (30 mL) and brine (30 mL). The organic phase was dried over MgSO_4 and the solvent was removed *in vacuo*. The product **28** (3.82 g, 7.51 mmol, 88 %) was obtained as a brown oil and was used for the next step without further purification.

$^1\text{H-NMR}$ (300 MHz, 298 K, CD_3CN): δ (ppm) = 7.81 (s, 2H, a), 4.65 (s, 2H, b), 1.07 (s, 9H, $\text{Si}(\text{CH}_2)_2\text{C}(\text{CH}_3)_3$), 0.48 (s, 6H, $\text{Si}(\text{CH}_2)_2\text{C}(\text{CH}_3)_3$).

$^{13}\text{C-NMR}$ (176 MHz, 298 K, CD_3CN): δ (ppm) = 155.5, 139.6, 133.6, 86.7, 43.8, 25.8, 17.8, -3.2.

VI.2.2.4 2,2'-((3,5-Diiodo-4-((trimethylsilyl)oxy)benzyl)azanediy)bis(ethan-1-ol) (29)

Scheme VI.2.2.37: Synthesis of compound **29**.

A mixture of **28** (2.00 g, 3.93 mmol, 1.00 equiv.), diethanolamine (0.75 mL, 7.86 mmol, 2.00 equiv.) and DIPEA (1.34 mL, 7.86 mmol, 2.00 equiv.) in CHCl_3 (50 mL) was heated under reflux over night. After cooling down, CHCl_3 (50 mL) was added and the organic layer was washed with sat. aqueous NaHCO_3 (50 mL) and brine (50 mL). The organic phase was dried over MgSO_4 and the solvent was removed. The residue was purified by means of column chromatography (Biotage, SiO_2 , CH_2Cl_2 / Acetone 92:8 $\rightarrow$ 34:66) The product **29** (1.36 g, 2.35 mmol, 60 %) was obtained as a yellowish oil.

$^1\text{H-NMR}$ (300 M Hz, 298 K, CD_3CN): δ (ppm) = 7.77 (s, 2H, a), 4.41 (s, 2H, 3), 3.53 (s, 2H, b), 3.49-3.37 (s, 4H, 2), 2.56-2.47 (m, 4H, 1), 1.03 (s, 9H, $\text{Si}(\text{CH}_2)_2\text{C}(\text{CH}_3)_3$), 0.44 (s, 6H, $\text{Si}(\text{CH}_2)_2\text{C}(\text{CH}_3)_3$).

VI.2.2.5 2,2'-((3,5-Bis(pyridin-3-ylethynyl)-4-((trimethylsilyl)oxy)benzyl)azanediy)bis(ethan-1-ol)) (30)

Scheme VI.2.2.38: Synthesis of compound **61a**.

23 (1.40 g, 2.43 mmol, 1.00 equiv), 3-ethynyl pyridine (0.55 g, 5.34 mmol, 2.20 equiv.), $\text{Pd(PPh}_3)_2\text{Cl}_2$ (0.17 g, 0.25 mmol, 0.10 equiv) and CuI (0.05 g, 0.25 mmol, 0.10 equiv.) were solved in dry DMF (10 mL). To the stirred reaction triethylamine (1.34 mL, 9.70 mmol, 4.00 equiv.) was added and the reaction mixture was stirred at 80 °C over night. The solvent was removed *in vacuo* and the residue was purified by means of column chromatography (SiO_2 , dichloromethane 20:1 $\rightarrow$ 10:1). The product **24** (0.71 g, 1.35 mmol, 55 %) was obtained as a yellowish resin.

$^1\text{H-NMR}$ (300 M Hz, 298 K, DMSO): δ (ppm) = 8.73 (s, 2H, g), 8.59 (d, $^3J = 4.6$ Hz, 2H, f), 7.91 (dt, $^3J = 7.9$ Hz, $^4J = 2.1$ Hz, 2H, d), 7.58 (s, 2H,), 7.46 (dd, $^3J = 8.0$, 4.8 Hz, 2H, e), 4.46 (t, $^3J = 5.1$ Hz, 2H, 3), 3.60 (s, 2H, b), 3.49 (q, $^3J = 5.1$ Hz, 4H, 2), 2.57 (t, $^3J = 6.2$ Hz, 4H, 1), 0.98 (s, 9H, $\text{Si(CH}_2)_2\text{C(CH}_3)_3$), 0.27 (s, 6H, $\text{Si(CH}_2)_2\text{C(CH}_3)_3$).

VI.2.2.6 2,2'-((4-Hydroxy-3,5-bis(pyridin-3-ylethynyl)benzyl)azanediyl)bis(ethan-1-ol) (31)

Scheme VI.2.2.39: Synthesis of compound **31**.

To a solution of **17** (100 mg, 0.23 mmol, 1.00 equiv.) was solved in dry CH_2Cl_2 (5 mL) BBr_3 (1 M, 0.35 mL, 0.35 mmol, 1.50 equiv.) was added dropwise at 0 °C. The reaction mixture was allowed to warm to room temperature and was stirred over night. After, H_2O was added and the mixture was extracted with CH_2Cl_2 . The combined organic phases were dried over NaSO_4 and the solvent was removed *in vacuo*. The residue was purified by means of column chromatography (SiO_2 , dichloromethane / methanol 20:1 $\rightarrow$ 10:1). The product **31** (41.4 mg, 0.10 mmol, 43 %) was obtained as yellowish solid.

$^1\text{H-NMR}$ (300 M Hz, 298 K, $\text{d}^4\text{-MeOH}$): δ (ppm) = 8.74 (d, $^4J = 2.4$ Hz, 2H, g), 8.51 (dt, $^3J = 4.9$ Hz, $^4J = 2.2$ Hz, 2H, f), 8.02 (dt, $^3J = 7.8$ Hz, $^4J = 2.0$ Hz, 2H, d), 7.52 (s, 2H, a), 7.47 (dd, $^3J = 8.1$ Hz, $^4J = 4.9$ Hz, 2H, e), 4.58 (s, 2H, 3), 3.69 (s, 2H, b), 3.65 (t, $^3J = 5.9$ Hz, 4H, 2), 2.73 (t, $^3J = 5.8$ Hz, 4H, 1).

ESI-HRMS ($[\text{C}_{25}\text{H}_{23}\text{N}_3\text{O}_3 + \text{H}]^+$):	measured:	414.1825
	calculated:	414.1812

VI.2.2.7 2,2'-(((2-(Pyridin-3-yl)-7-(pyridin-3-ylethynyl)benzofuran-5-yl)methyl)azanediyl)bis(ethan-1-ol) (32)

Scheme VI.2.2.40: Synthesis of compound **32**.

30 or **31** are placed in an open flask, either as solid or solved in wet polar solvents (like DMSO, MeOH or MeCN) and heated for several ours at 60 řC. After, the solvent was removed and the residue was purified by means of column chromatography (SiO₂, dichloromethane / methanol 30:1 → 10:1) and subsequent purification with help of GPC. The product **32** was obtained as a yellow solid.

¹H-NMR (300 M Hz, 298 K, d³-MeCN): δ (ppm) = 9.16 (d, ³*J* = 2.4 Hz, 1H, i), 8.86 (d, ³*J* = 2.3 Hz, 1H, m), 8.60 (dt, ³*J* = 4.7 Hz, ⁴*J* = 2.1 Hz, 2H, h, l), 8.27 (dt, ³*J* = 7.9 Hz, ⁴*J* = 2.0 Hz, 1H, f), 8.01 (dt, ³*J* = 7.9 Hz, ⁴*J* = 2.0 Hz, 1H, j), 7.72 (d, ⁴*J* = 1.7 Hz, 1H, c), 7.57 (d, ⁴*J* = 1.7 Hz, 1H, a), 7.48 (dd, ³*J* = 8.0, 4.7 Hz, 1H, g), 7.43 (dd, ³*J* = 8.0, 4.7 Hz, 1H, k), 7.35 (s, 1H, e), 3.79 (s, 2H, b), 3.54 (s, 4H, 2, 5), 2.66 (t, ³*J* = 5.6 Hz, 4H, 1, 4).

ESI-HRMS ($[\text{C}_{25}\text{H}_{23}\text{N}_3\text{O}_3 + \text{H}]^+$):

measured: 414.1825

calculated: 414.1812

Chapter VI.3

Syntheses of Acridinylidene Derivatives

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Scheme VI.3.1.41: Synthesis of compound **34**.

VI.3.1 Syntheses of Backbone Derivatives

VI.3.1.1 2-Cyano-2-(10-hexylacrid-9-ynylidene)tert-butylacetate (**34**)

TiCl₄ (255 mg, 1.34 mmol, 1.25 equiv.) was slowly added to *N*-hexyl-acridone (300 mg, 1.07 mmol, 1.00 equiv.) solved in dry CH₂Cl₂ (15 mL) and stirred for 15 min at room temperature. 2-cyano-*tert*-butylacetate (304 mg, 2.15 mmol, 2.00 equiv.) and triethylamine (2 mL) were added consecutively to the reaction mixture and heated under reflux for 24 h. The cooled down reaction mixture was filtered through celite and the residue was washed with CH₂Cl₂ (2 x 5 mL). The solvent was removed in vacuo and the residue was purified by means of column SiO₂, chloroform/methanol 40:1 → 20:1). The product **34** (288 mg, 0.72 mmol, 67%) was obtained as a yellow brownish solid.

¹H-NMR (300 M Hz, 298 K, CD₃CN): δ (ppm) = 8.03 (s, 2H, a/a'), 7.62 (ddd, ³*J* = 8.6, 7.0 Hz, ⁴*J* = 1.6 Hz, 2H, c/c'), 7.53 (dd, ³*J* = 8.5 Hz, ⁴*J* = 0.9 Hz, 2H, d/d'), 7.20 (ddd, ³*J* = 8.1, 7.0 Hz, ⁴*J* = 1.1 Hz, 2H, b/b'), 4.36 – 4.19 (m, 2H, NCH₂), 1.86 (p, ³*J* = 7.7 Hz, 2H, NCH₂CH₂), 1.47 - 1.41 (m, 2H, NCH₂CH₂CH₂), 1.42 (s, 9H, e), 1.40 – 1.28 (m, 4H, CH₂CH₂CH₃), 0.90 (t, ³*J* = 7.1 Hz, 3H, CH₂CH₂CH₃).

^{13}C -NMR (176 MHz, 298 K, CD_3CN): δ (ppm) = 165.20, 151.63, 140.17, 132.85, 128.53, 121.79, 120.33, 120.13, 115.41, 94.60, 83.51, 47.87, 32.10, 28.01, 27.20, 27.03, 23.34, 14.27.

IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 2924, 2868, 2193, 1709, 1601, 1577, 1507, 1460, 1377, 1263, 1231, 1103, 751, 659.

Melting point: 94-95 $^{\circ}\text{C}$

ESI-HRMS ($[\text{C}_{24}\text{H}_{26}\text{N}_2\text{O}_2]^+$): measured: 375.2064

calculated: 375.2067

Scheme VI.3.1.42: Synthesis of compound **35**.

VI.3.1.2 2-Cyano-2-(10-hexyl-9-acridinylidene)ethylacetate (**35**)

TiCl₄ (255 mg, 1.34 mmol, 1.25 equiv.) was slowly added to *N*-hexyl-acridone (300 mg, 1.07 mmol, 1.00 equiv.) solved in dry CH₂Cl₂ (15 mL) and stirred for 15 min at room temperature. 2-cyanoethylacetate (243 mg, 2.15 mmol, 2.00 equiv.) and triethylamine (2 mL) were added consecutively to the reaction mixture and heated under reflux for 24 h. The cooled down reaction mixture was filtered through celite and the residue was washed with CH₂Cl₂ (2 x 5 mL). The solvent was removed in vacuo and the residue was purified by means of column chromatography (SiO₂, chloroform/methanol 40:1 → 20:1). The product **35** (316 mg, 0.84 mmol, 79%) was obtained as an orange solid.

¹H-NMR (300 MHz, 298 K, CD₃CN): δ (ppm) = 8.02 (d, ³*J* = 8.1 Hz, 2H, a/a'), 7.62 (ddd, ³*J* = 8.6, 7.0 Hz, ⁴*J* = 1.5 Hz, 2H, c/c'), 7.53 (dd, ³*J* = 8.7 Hz, ⁴*J* = 0.7 Hz, 2H, b/b'), 7.20 (ddd, ³*J* = 8.1, 7.0 Hz, ⁴*J* = 1.1 Hz, 2H, d/d'), 4.36 – 4.25 (m, 2H, NCH₂), 4.30 (q, ³*J* = 7.1 Hz, 2H, OCH₂), 1.92 – 1.81 (m, 2H, NCH₂CH₂), 1.49 – 1.41 (m, 2H, NCH₂CH₂CH₂), 1.40 – 1.28 (m, 4H, CH₂CH₂CH₃), 1.17 (t, ³*J* = 7.1 Hz, 3H, OCH₂CH₃), 0.91 (t, ³*J* = 7.1 Hz, 3H, CH₂CH₂CH₃).

^{13}C -NMR (176 MHz, 298 K, CD_3CN): δ (ppm) = 166.2, 152.9, 140.1, 133.5, 128.8, 121.8, 120.3, 119.9, 118.3, 115.4, 62.5, 48.0, 32.0, 27.2, 27.0, 23.3, 14.3, 14.2.

IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 2924, 2868, 2193, 1709, 1601, 1577, 1507, 1460, 1377, 1263, 1231, 1103, 751, 659.

Melting point: 94-95 $^{\circ}\text{C}$

ESI-HRMS ($[\text{C}_{24}\text{H}_{26}\text{N}_2\text{O}_2]^+$):	measured:	375.2064
	calculated:	375.2067

Scheme VI.3.1.43: Synthesis of compound 36.

VI.3.1.3 2-Cyanophenylacetate (36)

2-Cyanoacetic acid (1.00 g, 11.76 mmol, 1.00 equiv.), phenol (1.22 g, 12.93 mmol, 1.10 equiv.) 4,4'-dimethylaminopyridine (143 mg, 1.18 mmol, 0.10 equiv.) and *N,N'*-dicyclohexyl carbodiimide (2.67 g, 12.93 mmol, 1.10 equiv.) in CH_2Cl_2 (15 mL) were stirred at 0 °C. The reaction mixture was allowed to warm up to room temperature over 12 h. After that the reaction mixture was filtered through celite and the solvent was removed in vacuo. The residue was purified by means of column chromatography (SiO_2 , pentane/ethyl acetate 6:1 $\rightarrow$ 2:1). The product (1.53 g, 9.50 mmol, 81%) was obtained as red oil.

$^1\text{H-NMR}$ (300 MHz, 298 K, CD_3CN): δ (ppm) = 7.47 – 7.38 (m, 2H, c), 7.35 – 7.29 (m, 1H, d), 7.19 – 7.13 (m, 2H, b), 3.73 (s, 2H, a).

ESI-HRMS ($[\text{C}_{28}\text{H}_{26}\text{N}_2\text{O}_2]^+$):

measured:	161.0423
calculated:	161.0477

Scheme VI.3.1.44: Synthesis of compound **37**.

VI.3.1.4 2-Cyano-2-(10-hexyl-9-acridinylidene)phenylacetate (**37**)

TiCl₄ (255 mg, 1.34 mmol, 1.25 equiv.) was slowly added to *N*-hexyl-acridone (300 mg, 1.07 mmol, 1.00 equiv.) solved in dry CH₂Cl₂ (15 mL) and stirred for 15 min at room temperature. **36** (346 mg, 2.15 mmol, 2.00 equiv.) and triethylamine (2 mL) were added consecutively to the reaction mixture and heated under reflux for 24 h. The cooled down reaction mixture was filtered through celite and the residue was washed with CH₂Cl₂ (2 x 5 mL). The solvent was removed in vacuo and the residue was purified by means of column chromatography (SiO₂, chloroform/methanol 40:1 → 20:1). The product **37** (271 mg, 0.64 mmol, 60%) was obtained as a dark purple solid.

¹H-NMR (300 M Hz, 298 K, CD₃CN): δ (ppm) = 8.36 (dd, ³*J* = 8.3 Hz, ⁴*J* = 1.4 Hz, 2H, a/a'), 7.72 (ddd, ³*J* = 8.5, 6.8 Hz, ⁴*J* = 1.5 Hz, 2H, c/c'), 7.67 (dd, ³*J* = 8.9 Hz, ⁴*J* = 1.2 Hz, 2H, d/d'), 7.43 – 7.38 (m, 2H, f), 7.31 (ddd, ³*J* = 8.2, 6.9 Hz, ⁴*J* = 1.2 Hz, 2H, b/b'), 7.26 (tt, ³*J* = 7.5 Hz, ⁴*J* = 1.3 Hz, 1H, g), 7.16 – 7.10 (m, 2H, e), 4.43 – 4.35 (m, 2H, NCH₂), 1.95 – 1.86 (m, 2H, NCH₂CH₂), 1.54 – 1.44 (m, 2H, NCH₂CH₂CH₂), 1.42 – 1.28 (m, 4H, CH₂CH₂CH₃), 0.90 (t, ³*J* = 7.1 Hz, 3H, CH₂CH₂CH₃).

^{13}C -NMR (176 MHz, 298 K, CD_3CN): δ (ppm) = 165.5, 155.7, 151.7, 140.0, 134.0, 130.4, 129.3, 126.9, 122.7, 122.4, 121.1, 120.5, 116.0, 86.6, 48.6, 32.1, 27.5, 27.0, 23.3, 14.3.

IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 2172, 1747, 1669, 1570, 1487, 1407, 1364, 1266, 1178, 1044, 896, 742, 619, 532.

Melting point: 169-170 $^{\circ}\text{C}$.

ESI-HRMS ($[\text{C}_{28}\text{H}_{26}\text{N}_2\text{O}_2]^+$):	measured:	423.2051
	calculated:	423.2067

VI.3.2 Syntheses of Ligand Derivatives

VI.3.2.1 2-(2,7-Di[(pyridin-3-yl)ethynyl])10-hexyl-9-acridinylidene)malononitrile(**40**) Scheme VI.3.2.45: Synthesis of compound **40**.

TiCl₄ (255 mg, 1.34 mmol, 1.25 equiv.) was slowly added to **39** (300 mg, 1.07 mmol, 1.00 equiv.) dissolved in dry CH₂Cl₂ (15 mL) and stirred for 15 min at room temperature. Malononitrile (142 mg, 2.15 mmol, 2.00 equiv.) and triethylamine (2 mL) were added consecutively to the reaction mixture and heated to 70 °C under reflux for 24 h. The cooled down reaction mixture was filtered through celite and the residue was washed with CH₂Cl₂ (2 x 50 mL). The solvent was removed in vacuo and the residue was purified by column chromatography (SiO₂, chloroform / methanol 40:1 → 20:1) followed by purification via GPC. The product (482 mg, 0.91 mmol, 85 %) was obtained as an orange solid.

¹H-NMR (300 M Hz, 298 K, CD₃CN): δ (ppm) = 8.77 (dd, 4J = 2.2 Hz, 2H, g), 8.61 (d, 4J = 1.9 Hz, 2H, a), 8.57 (dd, 3J = 4.9, 4J = 1.7 Hz, 2H, f), 7.92 (dt, 3J = 7.9, 4J = 2.0 Hz, 2H, d), 7.90 (dd, 3J = 3.7, 4J = 1.9 Hz, 2H, c), 7.72 (d, 3J = 9.1 Hz, 2H, b), 7.40 (dd, 3J = 7.9, 4J = 4.9, 2H, e), 4.44 – 4.31 (m, 2H, NCH₂), 1.95 – 1.83 (m, 2H, CH₂) 1.61 – 1.44 (m, 2H, CH₂), 1.43 – 1.32 (m, 4H, CH₂CH₂CH₃), 0.94 (t, J = 7.0 Hz, 3H, CH₃).

^{13}C -NMR (176 MHz, 298 K, CD_3CN): δ (ppm) = 154.4, 151.8, 148.3, 139.2, 138.5, 136.5, 130.3, 123.5, 118.2, 116.8, 116.6, 114.9, 91.33, 87.49, 77.4, 70.7, 48.4, 31.5, 26.9, 26.7, 22.7, 14.1.

IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 2924, 2854, 2206, 1562, 1480, 1465, 1368, 1263, 1186, 1021, 895, 820, 806, 702, 627, 588.

UV/Vis (MeCN): λ_{max} (nm) [ϵ ($\text{dm mol}^{-3} \text{cm}^{-1}$)] = 287 [48500], 347 [62300], 505 [16000].

ESI-HRMS ($[\text{C}_{38}\text{H}_{32}\text{N}_4\text{O}_2]^+$): measured: 530.2326

calculated: 530.2339

EA: (C₃₈H₃₂N₄O₂ × H₂O)

calculated: %C 79.0 %H 5.4 %N 12.8

found: %C 79.8 %H 5.8 %N 12.2

VI.3.2.2 2-Cyano-2-(2,7-di[(pyridin-3-yl)ethynyl]10-hexyl-9-acridinylidene)tert-butylacetate (**41**)

Scheme VI.3.2.46: Synthesis of compound **41**.

TiCl₄ (255 mg, 1.34 mmol, 1.25 equiv.) was slowly added to **39** (300 mg, 1.07 mmol, 1.00 equiv.) dissolved in dry CH₂Cl₂ (15 mL) and stirred for 15 min at room temperature. 2-Cyano-*tert*-butylacetate (304 mg, 2.15 mmol, 2.00 equiv.) and triethylamine (2 mL) were added consecutively to the reaction mixture and heated to 70 °C under reflux for 24 h. The cooled down reaction mixture was filtered through celite and the residue was washed with CH₂Cl₂ (2 x 50 mL). The solvent was removed *in vacuo* and the residue was purified by means of column chromatography (SiO₂, chloroform / methanol 40:1 → 20:1) followed by purification via GPC. The product **41** (214 mg, 0.36 mmol, 33 %) was obtained as an orange solid.

¹H-NMR (300 M Hz, 298 K, CD₃CN): δ (ppm) = 8.74 (s, 2H, g/g'), 8.71 (s, 1H, a'), 8.54 (dd, ³*J* = 5.0 Hz, ⁴*J* = 1.7 Hz, 2H, f/f'), 7.80 (dt, ³*J* = 7.9 Hz, ⁴*J* = 2.0 Hz, 2H, d/d'), 7.76 (s, 1H, a), 7.69 (dd, ³*J* = 9.0 Hz, ⁴*J* = 2.0 Hz, 2H, c/c'), 7.29 (d, ³*J* = 8.9 Hz, 2H, b/b'), 7.28 (dt, ³*J* = 7.9 Hz, ⁴*J* = 4.8 Hz, 2H, e/e*), 4.23 – 4.09 (m, 2H, NCH₂), 1.90 – 1.81 (m, 2H, CH₂), 1.48 (s, 9H, h), 1.51 – 1.45 (m, 2H, CH₂), 1.43 – 1.32 (m, 4H, CH₂CH₂CH₃), 0.93 (t, ³*J* = 7.0 Hz, 3H, CH₃).

^{13}C -NMR (176 MHz, 298 K, CD_3CN): δ (ppm) = 163.7, 151.7, 148.2, 147.7, 139.1, 138.7, 134.7, 132.7, 129.9, 123.6, 118.4, 114.2, 97.3, 92.1, 86.6, 83.9, 47.8, 31.5, 29.8, 27.9, 26.7, 26.6, 22.8, 14.1.

IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 2932, 2198, 1719, 1575, 1481, 1460, 1370, 1250, 1163, 1144, 1105, 1022, 840, 801, 702.

VI.3.2.3 2-Cyano-2-(2,7-di[(pyridin-3-yl)ethynyl])10-hexyl-9-acridinylidene)ethylacetate (**42**)

Scheme VI.3.2.47: Synthesis of compound **42**.

TiCl₄ (255 mg, 1.34 mmol, 1.25 equiv.) was slowly added to **39** (300 mg, 1.07 mmol, 1.00 equiv.) dissolved in dry CH₂Cl₂ (15 mL) and stirred for 15 min at room temperature. 2-Cyanoethylacetate (244 mg, 2.15 mmol, 2.00 equiv.) and triethylamine (2 mL) were added consecutively to the reaction mixture and heated to 70 °C under reflux for 24 h. The cooled down reaction mixture was filtered through celite and the residue was washed with CH₂Cl₂ (2 x 50 mL). The solvent was removed *in vacuo* and the residue was purified by column chromatography (SiO₂, chloroform / methanol 40:1 → 20:1) followed by purification via GPC. The product **42** (481 mg, 0.83 mmol, 78 %) was obtained as a red solid.

¹H-NMR (300 M Hz, 298 K, CD₃CN): δ (ppm) = 8.78 (s, 2H, g/g'), 8.60 (dd, ³*J* = 5.0 Hz, ⁴*J* = 1.7 Hz, 2H, f/f'), 7.86 (dt, ³*J* = 7.9 Hz, ⁴*J* = 2.0 Hz, 2H, d/d'), 7.72 (dd, ³*J* = 9.0 Hz, ⁴*J* = 2.0 Hz, 2H, c/c'), 7.34 (d, ³*J* = 8.9 Hz, 2H, b/b'), 7.34 (dt, ³*J* = 7.9 Hz, ⁴*J* = 4.8 Hz, 2H, e/e*), 4.30 (q, ³*J* = 7.2 Hz, 2H, h), 4.24 - 4.18 (m, 2H, NCH₂), 2.01 - 1.88 (m, 2H, CH₂), 1.59 - 1.48 (m, 2H, CH₂), 1.47 - 1.33 (m, 4H, CH₂CH₂CH₃), 1.31 (t, ³*J* = 7.1 Hz, 3H, i), 0.94 (t, ³*J* = 7.0 Hz, 3H, CH₃).

^{13}C -NMR (176 MHz, 298 K, CD_3CN): δ (ppm) = 164.6, 151.6, 149.7, 147.9, 139.5, 138.9, 134.9, 123.5, 118.3, 115.6, 114.2, 94.7, 91.9, 86.6, 77.25, 62.3, 47.8, 31.4, 26.6, 26.5, 24.6, 22.6, 14.1, 14.0.

IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 2926, 2207, 1716, 1522, 1478, 1465, 1372, 1235, 1183, 1102, 1021, 823, 804, 702.

UV/Vis (MeCN): λ_{max} (nm) [(dm³ mol⁻¹ cm⁻¹)] = 272 [48000], 340 [44600], 472 [10600]

Melting point: 148-149 °C.

ESI-HRMS ($[\text{C}_{38}\text{H}_{32}\text{N}_4\text{O}_2]^+$):	measured:	577.2577
	calculated:	577.2598

EA: (C ₃₈ H ₃₂ N ₄ O ₂ × H ₂ O)	calculated:	%C 76.7	%H 5.8	%N 9.4
	found:	%C 76.3	%H 5.9	%N 9.2

VI.3.2.4 2-Cyano-2-(2,7-di[(pyridin-3-yl)ethynyl])10-hexyl-9-acridinylidene)phenylacetate(43)

Scheme VI.3.2.48: Synthesis of compound **43**.

TiCl₄ (255 mg, 1.34 mmol, 1.25 equiv.) was slowly added to **39** (300 mg, 1.07 mmol, 1.00 equiv.) solved in dry CH₂Cl₂ (15 mL) and stirred for 15 min at room temperature. **36** (346 mg, 2.15 mmol, 2.00 equiv.) and triethylamine (2 mL) were added consecutively to the reaction mixture and heated to 70 °C under reflux for 24 h. The cooled down reaction mixture was filtered through celite and the residue was washed with CH₂Cl₂ (2 x 50 mL). The solvent was removed in vacuo and the residue was purified by means of column chromatography (SiO₂, chloroform / methanol 40:1 → 20:1) followed by purification via GPC. The product (153 mg, 0.25 mmol, 23 %) was obtained as a dark red solid.

¹H-NMR (300 M Hz, 298 K, CD₃CN): δ (ppm) = 8.76 (s, 2H, g/g'), 8.57 (dd, ³J = 5.0 Hz, ⁴J = 1.7 Hz, 2H, f/f'), 8.42 (d, ⁴J = 2.0 Hz, 2H, a/a'), 7.90 (dt, ³J = 7.9 Hz, ⁴J = 2.0 Hz, 2H, d/d'), 7.82 (dd, ³J = 9.0 Hz, ⁴J = 2.0 Hz, 2H, c/c'), 7.65 (d, ³J = 8.9 Hz, 2H, b/b'), 7.41 – 7.35 (m, 4H, e/e*, i), 7.27 – 7.23 (m, 1H, j), 7.22 – 7.18 (m, 2H, h), 4.37 – 4.30 (m, 2H, NCH₂), 1.92 – 1.83 (m, 2H, CH₂), 1.52 – 1.45 (m, 2H, CH₂), 1.42 – 1.30 (m, 4H, CH₂CH₂CH₃), 0.90 (t, ³J = 7.0 Hz, 3H, CH₃).

^{13}C -NMR (176 MHz, 298 K, CD_3CN): δ (ppm) = 163.7, 151.7, 148.2, 147.7, 139.1, 138.7, 134.7, 132.7, 129.9, 123.6, 118.4, 114.2, 97.3, 92.1, 86.6, 83.9, 47.8, 31.5, 29.8, 27.9, 26.7, 26.6, 22.8, 14.1.

IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 2931, 2183, 1716, 1564, 1480, 1468, 1410, 1370, 1267, 1176, 1165, 1079, 810, 748, 703.

UV/Vis (MeCN): λ_{max} (nm) [($\text{dm}^3 \text{mol}^{-1} \text{cm}^{-1}$)] = 273 [38500], 345 [42900], 489 [9600].

ESI-HRMS ($[\text{C}_{42}\text{H}_{33}\text{N}_4\text{O}_2]^+$): measured: 625.2583

calculated: 625.2525

EA: (C₄₂H₃₂N₄O₂ × H₂O)

calculated:	%C 78.5	%H 5.4	%N 8.7
found:	%C 78.3	%H 5.8	%N 9.0

VI.3.3 Syntheses of Cage Derivatives

Scheme VI.3.3.49: Synthesis of palladium-mediated coordination cages **44**, **45**, **46** and **47** from ligands **40**, **41**, **42** and **43**.

To a solution of the respective ligand **40**, **41**, **42** and **43** (0.5 mL, 2.8 mM, 2.0 equiv.) in MeCN, a solution of $[\text{Pd}(\text{MeCN})_4](\text{BF}_4)_2$ (50 μL , 15 mM, 1.1 equiv.) in MeCN was added. Subsequently the mixed solutions were heated between 1-8 h. The completion of the cage assembly was monitored by ^1H NMR.

VI.3.3.1 $[\text{Pd}_2(\text{L}^{\text{CN}})_4](\text{BF}_4)_2$ (44)

Scheme VI.3.3.50: Synthesis of compound 44.

^1H -NMR (300 MHz, 298 K, CD_3CN): δ (ppm) = 9.32 (dd, $^4J = 2.2$ Hz, 2H, g), 9.02 (dd, $^3J = 4.9$ Hz, $^4J = 1.7$ Hz, 2H, f), 8.80 (d, $^4J = 1.9$ Hz, 2H, a), 8.09 (dt, $^3J = 7.9$ Hz, $^4J = 2.0$ Hz, 2H, d), 7.89 (dd, $^3J = 3.7$ Hz, $^4J = 1.9$ Hz, 2H, c), 7.71 (d, $^3J = 9.1$ Hz, 2H, b), 7.62 (dd, $^3J = 7.9$ Hz, $^4J = 4.9$ Hz, 2H, e), 4.47 – 4.24 (m, 2H, NCH_2), 1.92 – 1.78 (m, 2H, CH_2), 1.51 – 1.40 (m, 2H, CH_2), 1.37 – 1.24 (m, 4H, $\text{CH}_2\text{CH}_2\text{CH}_3$), 0.84 (t, $^3J = 7.0$ Hz, 3H, CH_3).

ESI-HRMS ($[\text{C}_{144}\text{H}_{108}\text{N}_{20}\text{Pd}_2]^{4+}$):	measured:	581.4256
	calculated:	581.4287

VI.3.3.2 $[\text{Pd}_2(\text{L}^{\text{tBu}})_4](\text{BF}_4)_2$ (45)

Scheme VI.3.3.51: Synthesis of compound 45.

$^1\text{H-NMR}$ (300 MHz, 298 K, CD_3CN): δ (ppm) = 9.17 (s, 2H, g/g'), 8.94 (dd, $^3J = 5.0$ Hz, $^4J = 1.7$ Hz, 2H, f/f'), 8.09 (dt, $^3J = 7.9$ Hz, $^4J = 2.0$ Hz, 2H, d/d'), 7.79 (dd, $^3J = 9.0$ Hz, $^4J = 2.0$ Hz, 2H, c/c'), 7.63 (dt, $^3J = 7.9$ Hz, $^4J = 4.8$ Hz, 2H, e/e*), 7.56 (d, $^3J = 8.9$ Hz, 2H, b/b'), 4.32 – 4.13 (m, 2H, NCH_2), 1.88 – 1.74 (m, 2H, CH_2), 1.42 – 1.28 (m, 2H, CH_2), 1.31 – 1.18 (m, 4H, $\text{CH}_2\text{CH}_2\text{CH}_3$), 1.01 (s, 9H, h), 0.92 (t, $^3J = 7.0$ Hz, 3H, CH_3).

ESI-HRMS ($[\text{C}_{160}\text{H}_{144}\text{N}_{16}\text{O}_8\text{Pd}_2]^{4+}$): measured: 656.2363
 calculated: 656.2359

VI.3.3.3 [Pd₂(L^{Et})₄](BF₄)₂(46)

Scheme VI.3.3.52: Synthesis of compound 46.

¹H-NMR (300 MHz, 298 K, CD₃CN): δ (ppm) = 9.19 (s, 2H, g/g'), 8.95 (dd, ³J = 5.0 Hz, ⁴J = 1.7 Hz, 2H, f/f'), 8.35 (s, 2H, a/a'), 8.10 (dt, ³J = 7.9 Hz, ⁴J = 2.0 Hz, 2H, d/d'), 7.78 (dd, ³J = 9.0 Hz, ⁴J = 2.0 Hz, 2H, c/c'), 7.63 (dt, ³J = 7.9 Hz, ⁴J = 4.8 Hz, 2H, e/e*), 7.57 (d, ³J = 8.9 Hz, 2H, b/b'), 4.30 – 4.21 (m, 2H, NCH₂), 4.00 (q, ³J = 7.2 Hz, 2H, h), 1.86 – 1.77 (m, 2H, CH₂), 1.45 – 1.37 (m, 2H, CH₂), 1.36 – 1.23 (m, 4H, CH₂CH₂CH₃), 0.87 (t, ³J = 7.1 Hz, 3H, i) 0.83 (t, ³J = 7.0 Hz, 3H, CH₃).

ESI-HRMS ($[\text{C}_{152}\text{H}_{128}\text{N}_{16}\text{O}_8\text{Pd}_2]^{4+}$):	measured:	628.2029
	calculated:	628.2046

VI.3.3.4 [Pd₂(L^{Ph})₄](BF₄)₂ (47)

Scheme VI.3.3.53: Synthesis of compound 47.

¹H-NMR (300 MHz, 298 K, CD₃CN): δ (ppm) = 9.18 (s, 2H, g/g'), 9.01 (dd, ³J = 5.0 Hz, ⁴J = 1.7 Hz, 2H, f/f'), 8.37 (d, ⁴J = 2.0 Hz, 2H, a/a'), 8.11 (dt, ³J = 7.9 Hz, ⁴J = 2.0 Hz, 2H, d/d'), 7.71 (dd, ³J = 9.0 Hz, ⁴J = 2.0 Hz, 2H, c/c'), 7.65 (dt, ³J = 7.9 Hz, ⁴J = 4.8 Hz, 2H, e/e*), 7.55 (d, ³J = 8.9 Hz, 2H, b/b'), 7.08 – 6.97 (m, 3H, h,j), 6.86 – 6.80 (m, 2H, i), 4.35 – 4.16 (m, 2H, NCH₂), 1.84 – 1.66 (m, 2H, CH₂), 1.45 – 1.31 (m, 2H, CH₂), 1.31 – 1.19 (m, 4H, CH₂CH₂CH₃), 0.83 (t, ³J = 7.0 Hz, 3H, CH₃).

ESI-HRMS ([C₁₆₈H₁₂₈N₁₆O₈Pd₂]⁴⁺): measured: 676.4550
calculated: 676.4547

VI.3.4 Syntheses of Azacrown Ethyl Acridinylidine

VI.3.4.1 (Ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl) bis(4-methylbenzenesulfonate)) (48)

Scheme VI.3.4.54: Synthesis of compound **48**.

To a solution of triethylene glycol (6.68 mL, 50.0 mmol, 1.00 equiv.) and triethylamine (27.4 mL, 200 mmol, 4.00 equiv.) in CH_2Cl_2 (100 mL), p-tosyl chloride (28.6 g, 150 mmol, 3.00 equiv.) was added. The reaction mixture was treated for 30 min at 50 °C in the ultrasonic bath. After cooling down, the white solid was filtered off and the solvent was removed *in vacuo*. After recrystallization (2x) from methanol the product **48** (16.9 g, 36.8 mmol, 74%) was obtained as a white solid.

^1H -NMR (300 MHz, 298 K, CD_3Cl): δ (ppm) = 7.78 (d, $^3J = 8.1$ Hz, 4H, 4), 7.34 (d, $^3J = 8.1$ Hz, 4H, 5), 4.13 (m, 4H, 3), 3.65 (t, $^3J = 4.8$ Hz, 4H, 2), 3.52 (s, 4H, 1), 2.44 (s, 6H, 6).

^{13}C -NMR (176 MHz, 298 K, CD_3Cl): δ (ppm) = 145.01, 132.97, 129.98, 128.08, 70.80, 69.33, 68.86, 21.76.

IR (ATR): $\tilde{\nu}$ (cm⁻¹) = 2898, 1597, 1439, 1348, 1170, 1132, 1015, 981, 909, 804, 777, 663, 582, 552, 488, 443.

ESI-HRMS ([C ₂₄ H ₂₆ N ₂ O ₂] ⁺):	measured:	459.1139
	calculated:	459.1147

VI.3.4.2 (*N*-Phenyl-1-aza-5-crown-15)) (**49**) Scheme VI.3.4.55: Synthesis of compound **49**.

Hexane (20 mL) was added to NaH (60% in mineral oil, 4.81 g, 120 mmol, 3.63 equiv.) and stirred at room temperature for 15 min. After the hexane was removed, dry THF (480 mL) was added. To this suspension phenyldiethanolamine (6.00 g, 33.1 mmol, 1.00 equiv.) and **48** (15.2 g, 33.1 mmol, 1.00 equiv.), both in THF (60 mL), were added dropwise within 80 min. The reaction mixture was heated under reflux over night. After cooling down, the mixture was filtered and the solvent was removed *in vacuo*. The residue was resolved in MeOH (50 mL) and $\text{NaClO}_4 \times \text{H}_2\text{O}$ (4.64 g, 37.8 mmol, 1.15 equiv.) in MeOH (50 mL) and the mixture was heated under reflux for 10 min. The solvent was removed *in vacuo* and EtOAc (30 mL) stirred for 5 min and removed again. The residue was recrystallized from ethyl acetate (350 mL). The sodium crown-ether complex was stirred in CH_2Cl_2 / H_2O (1:1, 60 mL) for 30 min. The aqueous phase was washed with CH_2Cl_2 (2×30 mL), the combined organic phases were dried over Na_2SO_4 and the solvent was removed *in vacuo*. The product **49** (3.55 g, 12.0 mmol, 36 %) was obtained as a transparent oil.

$^1\text{H-NMR}$ (300 MHz, 298 K, DMSO): δ (ppm) = 7.13 (dd, $^3J = 8.7, 7.3$ Hz, 2H, 1), 6.61 (d, $^3J = 8.2$ Hz, 2H, 2), 6.56 (t, $^3J = 7.2$ Hz, 1H, 8), 3.62 (t, $^3J = 6.2$ Hz, 4H, 3), 3.56-3.48 (m, 16H, 4-7).

^{13}C -NMR (176 MHz, 298 K, DMSO): δ (ppm) = 147.32, 129.13, 115.15, 111.09, 70.35, 69.46, 69.07, 67.91, 51.86.

IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 2862, 1597, 1505, 1345, 1117, 988, 938, 745, 693, 622, 511.

ESI-HRMS ($[\text{C}_{24}\text{H}_{26}\text{N}_2\text{O}_2]^+$): measured: 318.1700

calculated: 318.1681

VI.3.4.3 *p*-Iodo-*N*-phenyl-1-aza-5-crown-15(**50**) Scheme VI.3.4.56: Synthesis of compound **50**.

To a solution of **49** (1.00 g, 3.39 mmol, 1.00 equiv.) in CH_2Cl_2 (30 mL), KI (674 mg, 4.06 mmol, 1.20 equiv.) and H_5IO_6 , both solved in H_2O (30 mL), were added and the mixture was stirred for 3.5 h at room temperature. After, $\text{Na}_2\text{S}_2\text{O}_3$ solution (10 % (w/v), 50 mL) was added and the aq. phase was washed with CH_2Cl_2 (3×30 mL). The combined organic phases were dried over Na_2SO_4 and the solvent was removed *in vacuo*. The product **50** (945 mg, 2.24 mmol, 66 %) was obtained as brown oil.

$^1\text{H-NMR}$ (300 MHz, 298 K, DMSO): δ (ppm) = 7.39 (d, $^3J = 8.9$ Hz, 2H, 1), 6.48 (d, $^3J = 8.9$ Hz, 2H, 2), 3.61-3.43 (m, 20H, 3-7).

^{13}C -NMR (176 MHz, 298 K, DMSO): δ (ppm) = 147.01, 137.28, 114.02, 75.92, 70.34, 69.48, 69.07, 67.66, 54.92, 51.86.

IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 2863, 1586, 1493, 1352, 1115, 984, 937, 802, 745, 694, 506.

ESI-HRMS ($[\text{C}_{24}\text{H}_{26}\text{N}_2\text{O}_2]^+$):	measured:	422.1000
	calculated:	422.0828

VI.3.4.4 *N*-(*N*-phenyl-1-aza-5-crown-15)-acridin-9(10*H*)-one (51)

Scheme VI.3.4.57: Synthesis of compound 51.

50 (500 mg, 1.19 mmol, 1.00 equiv.), acrid-9(10*H*)-one (278 mg, 1.42 mmol, 1.20 equiv.) and CuI (11.3 mg, 0.06 mmol, 0.05 equiv.) were solved in dry DMF (40 ml). After addition of dipivaloylmethane (0.05 mL, 0.24 mmol, 0.20 equiv.) and tetra-*n*-butyl ammoniumhydroxide (40% in MeOH, 1.15 mL, 1.78 mmol, 1.50 Äq.), the reaction mixture was heated 48 h under reflux. HCl (50 mL, 3 M) and CH₂Cl₂ (100 mL) were added to the cooled-down reaction mixture. The organic phase was washed with aq. sat. NaHCO₃, aq. sat. NH₄Cl and brine. The organic phase was dried over Na₂SO₄ and the solvent was removed *in vacuo*. The residue was purified by means of column chromatography (SiO₂, pentane/ethyl acetate 1:1) and subsequent purification via GPC. The product **35** (106 mg, 0.22 mmol, 18%) was obtained as a yellowish solid.

¹H-NMR (300 MHz, 298 K, d⁶-DMSO): δ (ppm) = 8.35 (dd, ³*J* = 8.0 Hz, ⁴*J* = 1.4 Hz, 2H), 7.65 (ddd, ³*J* = 8.6, 6.9 Hz, ⁴*J* = 1.7 Hz, 2H), 7.31 (ddd, ³*J* = 7.9, 6.9 Hz, ⁴*J* = 0.9 Hz, 2H), 7.24 (d, ³*J* = 8.7 Hz 2H), 6.94 (d, ³*J* = 9.0 Hz, 2H), 6.90 (d, ³*J* = 8.6 Hz, 2H), 3.74 (t, ³*J* = 6.1 Hz, 4H), 3.64-3.57 (m, 16H).

^{13}C -NMR (176 MHz, 298 K, $\text{d}^6\text{-DMSO}$): δ (ppm) = 176.82, 148.11, 143.60, 133.82, 130.28, 126.37, 125.70, 121.52, 121.20, 117.48, 112.89, 70.46, 69.70, 69.05, 67.96, 52.48.

IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 1631, 1597, 1518, 1482, 1455, 1358, 1112, 760, 677, 621, 547.

ESI-HRMS ($[\text{C}_{24}\text{H}_{26}\text{N}_2\text{O}_2]^+$):	measured:	489.2378
	calculated:	489.2389

VI.3.4.5 Ethyl-2-(*N*-(*N*-phenyl-1-aza-5-crown-15)-acridin-9(10*H*)-ylidene-2-cyanoacetate (**52**)

Scheme VI.3.4.58: Synthesis of compound **52**.

1 M TiCl_4 (in CH_2Cl_2) (0.26 mL, 0.26 mmol, 1.25 equiv.) was slowly added to **51** (100 mg, 0.21 mmol, 1.00 equiv.) solved in dry CH_2Cl_2 (10 mL) and stirred for 15 min at room temperature. 2-cyanoethylacetate (243 mg, 2.15 mmol, 2.00 equiv.) and triethylamine (1.5 mL) were added consecutively to the reaction mixture and heated at 70 °C under reflux for 24 h. To the cooled down reaction mixture H_2O (40 mL) and CH_2Cl_2 (25 mL) were added. The separated organic phase was dried over Na_2SO_4 and the solvent was removed *in vacuo*. The residue was purified by means of column chromatography (SiO_2 , dichloromethane/methanol 20:1) and subsequent purification via GPC. The product **52** (33.0 mg, 0.06 mmol, 28 %) was obtained as a red solid.

^1H -NMR (300 MHz, 298 K, CD_3Cl): δ (ppm) = 8.22 (d, $^3J = 6.6$ Hz, 2H), 7.39 (ddd, $^3J = 8.5$, 7.0 Hz, $^4J = 1.4$ Hz, 2H), 7.17 (ddd, $^3J = 8.2$, 7.0 Hz, $^4J = 1.1$ Hz, 2H), 7.11 (d, $^3J = 8.9$ Hz, 2H), 6.88 (d, $^3J = 8.1$ Hz, 2H), 6.84 (d, $^3J = 9.0$ Hz, 2H), 4.26 (q, $^3J = 7.1$ Hz, 2H), 3.84 (t, $^3J = 6.2$ Hz, 4H), 3.71-3.65 (m, 16H), 1.28 (t, $^3J = 7.1$ Hz, 3H).

^{13}C -NMR (176 MHz, 298 K, CD_3Cl): δ (ppm) = 166.10, 153.02, 148.20, 141.11, 131.96, 130.65, 127.91, 126.31, 121.23, 120.68, 118.25, 116.28, 112.92, 89.34, 71.50, 70.47, 70.06, 68.53, 61.76, 53.09, 14.32.

IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 2863, 2180, 1703, 1602, 1505, 1456, 1354, 1227, 1093, 740, 622, 542.

ESI-HRMS ($[\text{C}_{24}\text{H}_{26}\text{N}_2\text{O}_2]^+$):	measured:	584.2749
	calculated:	584.2761

Appendix

Appendix A

Further Analytical Data

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Oligonucleotides Analytical Data

The here presented data represent the characterization of the synthesized modified and unmodified oligonucleotide strands used and described in this thesis. The measured data for the melting curves of the respective ONS system is shown as red solid line. The corresponding fit to determine the denaturation temperature is always represented as a black dashed line. Since there is no general method to determine the denaturation temperature (T_M), all established methods, described in the publication of Lacroix *et al.*,^[160] were used. At first, from the measured data, the denaturation temperature was determined with help of the baseline method. Secondly, T_M was determined from first derivative of the measured melting curve with help of a GaussAmp fit. Last denaturation temperature was received from the "*fraction folded*" method. From the newly generated curve the temperature was determined with a DoseResp fit, which is normally used for the determination of EH_{50} values.^[333]

A.1.a Oligonucleotide MKDH01aum

Sequence 5' → 3'

GCC ATA GTT TGA GCA CG

Sum Formula

Mol. Mass

5207.91 g mol⁻¹

HPLC Traces

HPLC Traces

A.1.b Oligonucleotide MKDH01bum

Sequence 5' → 3'

CGT GCT CAT GGA TTG CG

Sum Formula

Mol. Mass

 $5214.89 \text{ g mol}^{-1}$

HPLC Traces

HPLC Traces

A.1.c Oligonucleotide MKDH01cum

Sequence 5' → 3'

CGC AAT CCT CCG AAT GC

Sum Formula

Mol. Mass

5112.89 g mol⁻¹

HPLC Traces

HPLC Traces

A.1.d Oligonucleotide MKDH01dum

Sequence 5' → 3'

GCA TTC GGT ACT ATG GC

Sum Formula

Mol. Mass

5198.8952 g mol⁻¹

HPLC Traces

HPLC Traces

A.1.e Doublestrand Oligonucleotide MKDH01aum with MKDH01bum

Sequences

a: 5'-GCC ATA GTT **TGA GCA CG**-3'
 b: 3'-GCG TTA GGT **ACT CGT GC**-5'

Melting Curve

Melting Curve

Melting Curve

UV-vis

A.1.f Doublestrand Oligonucleotide MKDH01aum with MKDH01bum

Sequences

a: 5'-GCC ATA GTT TGA GCA CG-3'
d: 3'-CGG TAT CAT GGC TTA CG-5'

Melting Curve

Melting Curve

Melting Curve

UV-vis

A.1.g Doublestrand Oligonucleotide MKDH01bum with MKDH01cum

Sequences

b: 3'-GCG TTA GGT ACT CGT GC-5'
 c: 5'-CGC AAT CCT CCG AAT GC-3'

Melting Curve

Melting Curve

Melting Curve

UV-vis

A.1.h Doublestrand Oligonucleotide MKDH01cum with MKDH01dum

Sequences

c: 5'-CGC AAT CCT **CCG AAT GC**-3'
d: 3'-CGG TAT CAT **GGC TTA CG**-5'

Melting Curve

Melting Curve

Melting Curve

UV-vis

A.1.i Holliday Junction MKDH01um

Sequences

a: 5'-GCC ATA GTT TGA GCA CG-3'
 b: 3'-GCG TTA GGT ACT CGT GC-5'
 c: 5'-CGC AAT CCT CCG AAT GC-3'
 d: 3'-CGG TAT CAT GGC TTA CG-5'

Melting Curve

Melting Curve

Melting Curve

UV-vis

Circular Dichroism

A.2.a Oligonucleotide MKDH01a

Sequence 5' → 3'

GCA CGA GTL TGA TAC CG

Sum Formula

Mol. Mass

 $5673.10 \text{ g mol}^{-1}$

HPLC Traces

HPLC Traces

A.2.b Oligonucleotide MKDH01b

Sequence 5' → 3'

GCG TTA GGL ACT CGT GC

Sum Formula

Mol. Mass

5680.0841 g mol⁻¹

HPLC Traces

HPLC Traces

A.2.c Oligonucleotide MKDH01c

Sequence 5' → 3'

CGC AAT CCL CCG AAT GC

Sum Formula

 $C_{193}H_{235}N_{63}O_{103}P_{16}$

Mol. Mass

5578.09 g mol⁻¹

HPLC Traces

HPLC Traces

A.2.d Oligonucleotide MKDH01d

Sequence 5' → 3'

GCA TTC GGL ACT ATG GC

Sum Formula

Mol. Mass

5664.09 g mol⁻¹

HPLC Traces

HPLC Traces

A.2.e Doublestrand Oligonucleotide MKDH01a with MKDH01b

Sequences

a: 5'-GCC ATA GTL **TGA GCA CG**-3'

b: 3'-GCG TTA GGL **ACT CGT GC**-5'

Ligand

Melting Curve

Melting Curve

Melting Curve

UV-vis

A.2.f Doublestrand Oligonucleotide MKDH01a with MKDH01d

A.2.g Doublestrand Oligonucleotide MKDH01b with MKDH01c

Sequences

b: 3'-GCG TTA GGL ACT CGT GC-5'

c: 5'-CGC AAT CCL CCG AAT GC-3'

Ligand

Melting Curve

Melting Curve

Melting Curve

UV-vis

A.2.h Doublestrand Oligonucleotide MKDH01c with MKDH01d

A.2.i Holliday Junction MKDH01

Sequences

a: 5'-GCC ATA GTL TGA GCA CG-3'
b: 3'-GCG TTA GGL ACT CGT GC-5'
c: 5'-CGC AAT CCL CCG AAT GC-3'
d: 3'-CGG TAT CAL GGC TTA CG-5'

Ligand

Melting Curve

Melting Curve

Melting Curve

UV-vis

Circular Dichroism

Fluorescence

A.3.a Oligonucleotide MKDH03a

Sequence 5' → 3'

GCC ATA GTI TGA GCA CG

Sum Formula

 $C_{164}H_{209}N_{65}O_{98}P_{16}$

Mol. Mass

5152.03 g mol⁻¹

HPLC Traces

HPLC Traces

A.3.b Oligonucleotide MKDH03b

Sequence 5' → 3'

CGT GCT CAI GGA TTG CG

Sum Formula

$C_{164}H_{210}N_{62}O_{101}P_{16}$

Mol. Mass

5159.01 g mol⁻¹

HPLC Traces

HPLC Traces

A.3.c Oligonucleotide MKDH03c

Sequence 5' → 3'

CGC AAT CCI CCG AAT GC

Sum Formula

Mol. Mass

5057.02 g mol⁻¹

HPLC Traces

HPLC Traces

A.3.d Oligonucleotide MKDH03d

Sequence 5' → 3'

GCA TTC GGI ACT ATG GC

Sum Formula

Mol. Mass

5143.02 g mol⁻¹

HPLC Traces

HPLC Traces

A.3.e Holliday Junction MKDH03

Sequences

a: 5'-GCC ATA GTI TGA GCA CG-3'
 b: 3'-GCG TTA GGI ACT CGT GC-5'
 c: 5'-CGC AAT CCI CCG AAT GC-3'
 d: 3'-CGG TAT CAI GGC TTA CG-5'

Ligand

Melting Curve

Melting Curve

Melting Curve

UV-vis

Circular Dichroism

Fluorescence

A.4.a Oligonucleotide MKDH04a

Sequence 5' → 3'

GCC ATA GTT ITT GAG CAC G

Sum Formula

 $C_{184}H_{235}N_{69}O_{112}P_{18}$

Mol. Mass

 $5760.12 \text{ g mol}^{-1}$

HPLC Traces

HPLC Traces

A.4.b Oligonucleotide MKDH04b

Sequence 5' → 3'

CGT GCT CAT ITG GAT TGC G

Sum Formula

Mol. Mass

 $5767.10 \text{ g mol}^{-1}$

HPLC Traces

HPLC Traces

A.4.c Oligonucleotide MKDH04c

A.4.d Oligonucleotide MKDH04d

Sequence 5' → 3'

GCA TTC GGT ITA CTA TGG C

Sum Formula

Mol. Mass

5751.11 g mol⁻¹

HPLC Traces

HPLC Traces

A.4.e Holliday Junction MKDH04

Sequences

a: 5'-GCC ATA GTT **ITT** GAG CAC G-3'
b: 3'-GCG TTA GGT **ITA** CTC GTG C-5'
c: 5'-CGC AAT CCT **ITC** CGA ATG C-3'
d: 3'-CGG TAT CAT **ITG** GCT TAC G-5'

Ligand

Melting Curve

Melting Curve

Melting Curve

UV-vis

Circular Dichroism

A.5.a Oligonucleotide MKDH05a

Sequence 5' → 3'

GCC ATA GTX TGA GCA CG

Sum Formula

Mol. Mass

5393.32 g mol⁻¹

HPLC Traces

HPLC Traces

A.5.b Oligonucleotide MKDH05b

Sequence 5' → 3'

CGT GCT CAX GGA TTG CG

Sum Formula

 $C_{182}H_{221}N_{63}O_{101}P_{16}$

Mol. Mass

5400.30 g mol⁻¹

HPLC Traces

HPLC Traces

A.5.c Oligonucleotide MKDH05c

Sequence 5' → 3'

CGC AAT CCX CCG AAT GC

Sum Formula

 $C_{179}H_{219}N_{63}O_{97}P_{16}$

Mol. Mass

5298.31 g mol⁻¹

HPLC Traces

HPLC Traces

A.5.d Oligonucleotide MKDH05d

Sequence 5' → 3'

GCA TTC GGX ACT ATG GC

Sum Formula

Mol. Mass

 $5384.31 \text{ g mol}^{-1}$

HPLC Traces

HPLC Traces

A.5.e Holliday Junction MKDH05

Sequences

a: 5'-GCC ATA GTX TGA GCA CG-3'
 b: 3'-GCG TTA GGX ACT CGT GC-5'
 c: 5'-CGC AAT CCX CCG AAT GC-3'
 d: 3'-CGG TAT CAX GGC TTA CG-5'

Ligand

Melting Curve

Melting Curve

Melting Curve

UV-vis

Circular Dichroism

Fluorescence

A.6.a Oligonucleotide MKDH06a

Sequence 5' → 3'

GCC ATA GTT XTT GAG CAC G

Sum Formula

 $\text{C}_{202}\text{H}_{246}\text{N}_{70}\text{O}_{112}\text{P}_{18}$

Mol. Mass

 $6001.41 \text{ g mol}^{-1}$

HPLC Traces

HPLC Traces

A.6.b Oligonucleotide MKDH06b

Sequence 5' → 3'

CGT GCT CAT XTG GAT TGC G

Sum Formula

Mol. Mass

6008.40 g mol⁻¹

HPLC Traces

HPLC Traces

A.6.c Oligonucleotide MKDH06c

A.6.d Oligonucleotide MKDH06d

Sequence 5' → 3'

GCA TTC GGT XTA CTA TGG C

Sum Formula

Mol. Mass

5992.40 g mol⁻¹

HPLC Traces

HPLC Traces

A.6.e Holliday Junction MKDH06

Sequences

a: 5'-GCC ATA GTT **X**TT GAG CAC G-3'
b: 3'-GCG TTA GGT **X**TA CTC GTG C-5'
c: 5'-CGC AAT CCT **X**TC CGA ATG C-3'
d: 3'-CGG TAT CAT **X**TG GCT TAC G-5'

Ligand

Melting Curve

Melting Curve

Melting Curve

UV-vis

Circular Dichroism

Fluorescence

A.7 Further Data

A.7.a Host-Guest Titration Diethanolamin-Methoxy Ligand

Figure VI.1.7.1: ^1H NMR (400 MHz, D_2O) host guest titration experiments with **25** (0.7 mM and cyclohexane (a), 1,4-benzoquinone (b) and naphthalene (c). The signals of the corresponding guest molecules are highlighted in red.

A.7.b VT NMR experiments for the ligands L^{tBu} , L^{Et} , L^{Ph} and L^{CN}

(a)

(b)

Figure VI.1.7.2: a) VT- ^1H NMR spectra (400 MHz, d^8 -THF) of ligand L^{tBu} . The rotation rate and the associated signal sharpness of $\text{a/a}'$ (red) depend on the temperature. At low temperature two sets of signals are observable due to a low rotation rate. Higher temperatures increase the rotation rates and lead to one set of signals. The coalescence temperature is not reached in this experiment. b) VT ^1H NMR spectra (400 MHz, d^8 -THF) of ligand L^{Ph} . The rotation rate and the associated signal sharpness of $\text{a/a}'$ (red) depend on the temperature. At low temperatures, two sets of signals are observable due to a low rotation rate. Higher temperatures increase the rotation rate and lead to one signal. The coalescence temperature is approx. -10°C

A.7.c Cyano-acridinylidine ethyl acetate titration data

Scheme VI.1.7.59: Guest molecules G^1 - G^5 for ^1H -NMR host-guest titration experiments. All guests come with two tetra-*n*-butylammonium cations (nBu_4N^+) as counter-ions.

Figure VI.1.7.3: ^1H NMR titration (400 MHz, 298 K, CD_3CN) of C^{Et} ($[\text{Pd}_2\text{L}^{\text{Et}}_4]$) with $(\text{NBu}_4)_2\text{G}^1$. The chemical shifts of the inward pointing protons change gradually due to a fast exchange of the guest on the NMR time scale. Excess addition of G^1 leads to precipitation of the cage.

Figure VI.1.7.4: ^1H NMR titration (400 MHz, 298 K, CD_3CN) of C^{Et} ($[\text{Pd}_2\text{L}^{\text{Et}}_4]$) with $(\text{NBu}_4)_2\text{G}^2$. The chemical shifts of the inward pointing protons change gradually due to a fast exchange of the guest on the NMR time scale. Excess addition of G^2 leads to precipitation of the cage.

Figure VI.1.7.5: ^1H NMR titration (400 MHz, 298 K, CD_3CN) of C^{Et} ($[\text{Pd}_2\text{L}^{\text{Et}}_4]$) with $(\text{NBu}_4)_2\text{G}^3$. Upon addition of one equivalent of guest G^3 , the uprising of more downfield shifted signal g/g' from $[\text{G}_3@\text{Pd}_2\text{L}^{\text{Et}}_4]$ species is observable due to slow exchange of the guest.

Figure VI.1.7.6: ^1H NMR titration (400 MHz, 298 K, CD_3CN) of $[\text{Pd}_2\text{L}^{\text{Et}}_4]$ with $(\text{NBu}_4)_2\text{G}^4$. The chemical shifts of the inward pointing protons change gradually due to a fast exchange of the guest on the NMR time scale. Excess addition of G^4 leads to precipitation of the cage.

Figure VI.1.7.7: ^1H NMR titration (400 MHz, 298 K, CD_3CN) of C^{Et} ($[\text{Pd}_2\text{L}^{\text{Et}}_4]$) with $(\text{NBu}_4)_2\text{G}^5$. Upon addition of one equivalent of guest G^5 , the uprising of more downfield shifted signal g/g' from $[\text{G}^5 @ \text{Pd}_2\text{L}^{\text{Et}}_4]$ species is observable due to slow exchange of the guest. Excess addition of G^5 leads to precipitation of the cage.

Figure VI.1.7.8: ^1H NMR titration (400 MHz, 298 K, $d^6\text{-DMSO}$) of $[\text{Pd}_2\text{L}^{\text{Et}}_4]$ with $(\text{NBu}_4)_2\text{G}^5$. The chemical shifts of the inward pointing protons change gradually due to a fast exchange of the guest on the NMR time scale. In contrast to the situation in CD_3CN , in DMSO a fast exchange is observable for G^5 with $[\text{Pd}_2\text{L}^{\text{Et}}_4]$.

Figure VI.1.7.9: ^1H NMR titration (400 MHz, 333 K, CD_3OD) of $[\text{Pd}_2\text{L}^{\text{Et}}_4]$ with $(\text{NBu}_4)_2\text{G}^5$. The chemical shifts of the inward pointing protons change gradually due to a fast exchange of the guest on the NMR time scale. Addition of 0.4 eq of G^5 leads already to visible precipitation in the nmr tube.

Figure VI.1.7.10: ^1H NMR titration (400 MHz, 333 K, CD_3NO_2) of $[\text{Pd}_2\text{L}^{\text{Et}}_4]$ with $(\text{NBu}_4)_2\text{G}^5$. Upon addition of one equivalent of guest G^5 the $[\text{Pd}_2\text{L}^{\text{Et}}_4]$ cage transforms into $[\text{G}^5@ \text{Pd}_2\text{L}^{\text{Et}}_4]$. Excess addition of G^5 leads to precipitation of the cage.

A.7.d Aza-15-crown-cyano-acridinylidine ethyl acetate Titration Data

Figure VI.1.7.11: UV-vis and NMR titration experiments for **52** with NaClO_4 . **a)** ^1H NMR (400 MHz, $\text{d}^3\text{-MeCN}$) titration experiments of **52** and NaClO_4 ranging from 0 to 150 equiv. of added salt. As in previous titration experiments an downfield shifting of the bridging phenyl signals can be observed, which becomes more significant at higher salt concentrations. However, there is no significant change detectable for the a/a' signal, which turns out to be a characteristic indicator for a change in the push-pull system electronics. **b)** There is also just a minimal change in UV-vis titration experiment (0.005 mM **52** in MeCN). There maximum observable blue-shift of 3 nm, after adding 100 equiv of the corresponding sodium salt.

Figure VI.1.7.12: UV-vis and NMR titration experiments for **52** with $\text{Ba}(\text{ClO}_4)_2$. **a)** UV-vis experiment with 0.005 M **52** in MeCN. $\text{Ba}(\text{ClO}_4)_2$ was added until no shift of the 455 nm was detected. A maximum hypsochromic shift of 7 nm could be observed after addition of 100 equiv. of metal salt. **b)** Control experiment. Titration of $\text{Ba}(\text{ClO}_4)_2$ to B^{Et} (**35**). Except of a slight hypsochromic shift, due to the salt addition caused dilution, no shift of the push-pull system characteristic 455 nm band was observed. **c)** NMR titration experiment (400 MHz, $d^3\text{-MeCN}$) of $\text{B}^{\text{Et}}_{\text{Azacrown}}$ (**52**, 2.8 mM) with $\text{Ba}(\text{ClO}_4)_2$. A significant chemical shift for the phenyl hydrogens **1** and **2** is observable upon addition of 5 equiv. of the corresponding metal salt (shifting is highlighted by green and red line). Addition of further metal salt led to further slight shifting of those signals. It is worth to mention, that the signal **2** experience to highest downfield shift, what indicates an interaction between barium cation and azacrown, since that speaks for a shifting of electron-density towards the azacrown nitrogen. However, no significant change for the a/a' is observable. It seems that the signal becomes slightly sharper by increasing the amount of metal salt. This finding can be explained by an slight increase in stabilization of the charge-separated state, caused by the increased ion concentration within the sample solution.

Figure VI.1.7.13: UV-vis titration experiments of **52** with a) $\text{Mn}(\text{ClO}_4)_2 \cdot x\text{H}_2\text{O}$, b) $\text{Zn}(\text{BF}_4)_2 \cdot x\text{H}_2\text{O}$ and c) $\text{Cd}(\text{ClO}_4)_2 \cdot x\text{H}_2\text{O}$. All samples show a slight blue-shift upon addition of the corresponding metal salts. For all samples 100 equiv. of the corresponding metal salt was added in total, leading to a maximum blue shift of: 12 nm for $\text{Mn}(\text{ClO}_4)_2$, 10 nm for $\text{Zn}(\text{BF}_4)_2$ and 11 nm for $\text{Cd}(\text{ClO}_4)_2$.

Figure VI.1.7.14: UV-vis titration experiments of a) **52** and b) **35** with $\text{Cu}(\text{BF}_4)_2$. a) Addition of 1.0 equiv. copper salt to compound **52** (0.005 M in MeCN) led to a broad peak at ca. 675 nm, vanishing upon addition of second equivalent of the copper salt. The behavior can often be observed for the formation of radical species. Further addition led to the acridinium ion specific absorption pattern. b) The titration of $\text{Cu}(\text{BF}_4)_2$ to compound **35** (0.005 M) did not show this behavior upon addition of 1.0 or 2.0 equiv. copper salt, therefore these steps were neglected. However, the acridinium ion absorption pattern appeared as well for higher copper salt concentrations.

A.8 Crystal Data and Structure Refinement

Table VI.1.8.1: Data from X-ray diffraction analysis for compounds **B^{Et} (35)** and **I₂-B^{tBu}**. Both crystals were measured and refined by Dr. Julian Holstein.

Compound	B^{Et} (34)	I₂-B^{tBu}
CCDC number	1542074	-
Empirical formula	C96 H104 N8 O8	C26 H28 I2 N2 O2
Formula weight	1497.87	654.30
Temperature	100(2) K	100(2) K
Wavelength	1.54178 Å	0.71073 Å
Crystal system	Monoclinic	Monoclinic
Space group	P21	P2/n
Unit cell dimensions	a = 10.8878(6) Å b = 26.8038(15) Å c = 14.9978(9) Å α = 90 ° β = 110.114(3) ° γ = 90 °	a = 15.6998(12) Å b = 9.1687(7) Å c = 18.6041(14) Å α = 90 ° β = 105.955(3) ° γ = 90 °
Volume	4109.9(4) Å ³	2574.8(3) Å ³
Z	2	4
Density (calculated)	1.210 Mg m ⁻³	1.688 Mg m ⁻³
Absorption coefficient	0.609 mm	2.467 mm
F(000)	1600	1280
Crystal size	0.300 x 0.200 x 0.200 mm	0.100 x 0.100 x 0.100 mm
Theta range for data collection	3.138 to 68.245 °	2.983 to 35.019° °
Index ranges	-9<=h<=13, -31<=k<=31, -18<=l<=18	-25<=h<=25, -14<=k<=14, -30<=l<=30
Reflections collected	33136	229857
Independent reflections	14121 [R(int) = 0.0612]	11342 [R(int) = 0.0666]
Completeness to theta = 67.679 °	98.7 %	99.8 %
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7542 and 0.5472	
Refinement method	Full-matrix least-squares on F ²	Full-matrix least-squares on F ²
Data / restraints / parameters	14121 / 1 / 1017	11342 / 0 / 293
Goodness-of-fit on F2	1.031	1.118
Final R indices [I>2sigma(I)]	R1 = 0.0763, wR2 = 0.2256	R1 = 0.0264, wR2 = 0.0612
R indices (all data)	R1 = 0.0807, wR2 = 0.2303	R1 = 0.0352, wR2 = 0.0679
Absolute structure parameter	0.40(11)	
Extinction coefficient	n/a	n/a
Largest diff. peak and hole	0.542 and -0.406 e.Å ³	1.917 and -1.934 e.Å ³

Table VI.1.8.2: Data from X-ray diffraction analysis for compounds **40**, **42** and **43**. Compounds **40** and **42** were measured and refined by Dr. Julian Holstein. Compound **43** was measured by Dr. Christian Würtele and refined by Dr. Julian Holstein

Compound	L^{CN} (40)	L^{Et} (42)	L^{Ph} (43)
CCDC number		1478914	1478915
Empirical formula	C36 H27 N5	C38 H32 N4 O2	C46 H32 D8 N4 O3
Formula weight	529.62	576.67	704.87
Temperature	100(2) K	100(2) K	100(2) K
Wavelength	1.54178 Å	0.71073 Å	1.54178 Å
Crystal system	Monoclinic	Monoclinic	Triclinic
Space group	P2 ₁ /n	P2 ₁ /n	P-1
Unit cell dimensions	a = 10.9411(5) Å b = 7.5173(3) Å c = 31.9858(15) Å α = 90 ° β = 95.053(2) ° γ = 92.206(2) °	a = 11.4777(5) Å b = 15.9325(7) Å c = 17.0968(8) Å α = 90 ° β = 103.233(2) ° γ = 97.198(2) °	a = 9.7141(3) Å b = 13.7175(4) Å c = 13.8642(4) Å α = 90 ° β = 110.114(3) ° γ = 98.744(2) °
Volume	2620.5(2) Å ³	3043.4(2) Å ³	1808.46(9) Å ³
Z	4	4	2
Density (calculated)	1.342 Mg m ⁻³	1.259 Mg m ⁻³	1.294 Mg m ⁻³
Absorption coefficient	0.629 mm	0.079 mm	0.638 mm
F(000)	1112	1216	736
Crystal size	0.100 x 0.100 x 0.100 mm	0.844 x 0.744 x 0.542 mm	0.300 x 0.116 x 0.072 mm
Theta range for data collection	2.774 to 63.685 °	2.331 to 33.141 °	3.22 to 74.61 °
Index ranges	-12 ≤ h ≤ 12, -8 ≤ k ≤ 8, -37 ≤ l ≤ 37	-17 ≤ h ≤ 17, -23 ≤ k ≤ 24, -26 ≤ l ≤ 23	-12 ≤ h ≤ 12, -17 ≤ k ≤ 17, -17 ≤ l ≤ 17
Reflections collected	35316	114976	21094
Independent reflections	4275 [R(int) = 0.0529]	11572 [R(int) = 0.0375]	7238 [R(int) = 0.0382]
Completeness to theta =	63.685 ° / 99.0%	25.242 ° / 99.5%	74.61 ° / 97.7%
Absorption correction	Semi-empirical from equivalents	Semi-empirical from equivalents	Semi-empirical from equivalents
Max. and min. transmission	0.7542 and 0.5472	0.7485 and 0.6842	0.75 and 0.68
Refinement method	Full-matrix least-squares on F ²	Full-matrix least-squares on F ²	Full-matrix least-squares on F ²
Data / restraints / parameters	4275 / 0 / 371	11572 / 0 / 399	7238 / 1 / 499
Goodness-of-fit on F2	1.186	1.116	1.047
Final R indices [I > 2σ(I)]	R1 = 0.0584, wR2 = 0.1631	R1 = 0.0411, wR2 = 0.1192	R1 = 0.0477, wR2 = 0.1094
R indices (all data)	R1 = 0.0605, wR2 = 0.1639	R1 = 0.0473, wR2 = 0.1246	R1 = 0.0643, wR2 = 0.1183
Extinction coefficient	n/a	n/a	n/a
Largest diff. peak and hole	0.267 and -0.294 e.Å ³	0.829 and -0.284 e.Å ³	0.243 and -0.275 e.Å ³

Appendix **B**

Methods

Instruments for analytics

NMR (Nuclear magnetic resonance spectroscopy)

The NMR data was measured on the spectrometers Bruker AV 500 Advance NEO, Bruker AV 400 Advance III HD NanoBay, AV 500 Advance III HD, AV 600 Advance III HD, AV 700 Advance III HD and Agilent Technologies DD2. The chemical shifts were calibrated on tetramethylsilane (^1H , 0 ppm), CFCl_3 (^{19}F , 0 ppm), 85% phosphoric acid (^{31}P , 0 ppm). For the ^{13}C NMR spectra the signals of the solvents were used as the internally standard (CD_3CN : 1.32, 118.26 ppm, C_6D_6 : 128.06 ppm, CD_2Cl_2 : 54.00 ppm, CDCl_3 : 77.00 ppm, CD_3OD : 49.00 ppm, more). The chemical shift δ is given in ppm, the coupling constants J in Hz. All spectra were recorded at 25 °C, otherwise it is mentioned.^[334]

Electrospray ionization mass spectrometry

ESI-MS measurements were performed on a Bruker maXis ESI-TOF MS spectrometer on a Bruker timsTOF mass spectrometer including electron spray ionisation (ESI) (positive / negative mode). The timsTOF is a Hybrid Quadrupole / Atmospheric Pressure Ionization orthogonal accelerated Time-Of-Flight mass spectrometer using Trapped Ion Mobility Spectrometry (TIMS). For calibration of the TIMS and TOF devices an Agilent ESI-Low Concentration Tuning Mix was used. The quotients of mass to charge (m/z) are given and the relative intensity related to the basis peak ($I = 100$) are given in brackets.^[240,335]

Ultraviolet-visible spectroscopy

Standard UV-vis data was obtained from Agilent 8453 DAD UV-Visible Spectrophotometer, using standard quartz glass cuvettes suitable for the ultra-violet and visible light spectra. Thermal denaturation experiments and thermal difference spectra were measured with the JASCO V-650 and JASCO V-750 UV-Vis Spectrophotometer, both equipped with the PAC-743 Automatic 6/8-position Peltier Cell Changer.

Fluorescence spectroscopy

FS measurements were performed on the JASCO FP-8300 Spectrofluorometer with standard 10 mm quartz glass cuvettes suitable for fluorescence measurements in the UV-vis region. Additionally, the Unisoku Coolspek temperature-controlled cuvette holder was used for various temperature measurements above or below room temperature.^[336]

Cyclic voltammetry

CV was measured on a Metrohm Autolab PGStat101 CV setup. Measurements were performed in custom made glass cell, with air tight inlets for the platinum working electrode (WE), platinum wire counter electrode (CE) and the reference electrode (RE). Two additional Teflon valves were available for the degassing process.

Circular dichroism

CD measurements were performed on the qCD Chirascan CD-spectrometer from Applied Photophysics with temperature-controlled cuvette holder and an external nitrogen generator to ensure constant flushing of the whole machine, in order to avoid an accumulation of damaging ozone, generated during the active operation of the machine.

For all steps oligonucleotide strands (ONSs) are involved, ultra pure water (type 1, 18.2 M Ω cm) was used, received from Puranity TU-3 UV VWR *International GmbH*, if not mentioned otherwise. Furthermore in connection with ONS containing solution, solvent removing steps *in vacuo* are always performed in a vacuum centrifuge (S-Concentrator BA-VC-300H, *H. Saur*) at 60 °C.

B.1 DNA Solid State Synthesis

The synthesis of the oligonucleotide strands (ONSs) were performed on a K&A DNA Synthesizer H-8 by phosphoramidite solid phase synthesis on a 1 μ mol scale using standard methods for phosphoramidite synthesis on controlled pore glass (CPG). All used solution (table VI.2.1.1) were prepared with anhydrous solvent and additionally stored over molecular sieves prior to synthesis for more than 12 hours.

Table VI.2.1.1: Solution used during solid-phase synthesis of the oligonucleotides.

Purpose	Label	Composition
Detritylation	DCA	3% (<i>v/v</i>) dichloro acetic acid in anhydrous DCM
Activator	ACT	0.3 M BTT in anhydrous MeCN
Capping Reagent A	Cap A	6.5% (<i>w/v</i>) DMAP in anhydrous THF
Capping Reagent B	Cap B	2,6-lutidine / acetic anhydride / anhydrous THF 1:1:8 (<i>v/v/v</i>)
Oxidizer	OXI	0.02 M iodine in THF / pyridine / H ₂ O 7:2:1 (<i>v/v/v</i>)

The compound BTT (**20**) used as activation agent was synthesized as described in scheme VI.1.2.30. As unmodified nucleotides commercially available DMT and CEP protected DNA phosphoramidites (DMT-dT-CEP, DMT-dG(*i*Bu)-CEP, DMT-dA(Bz)-CEP, DMT-dC(Bz)-CEP). As the solid support commercially available CPG was used with the respective starting nucleoside already immobilized on the CPG and DMT protected. The cartridges for the synthesis were manually packed. The synthesis itself was performed with a slightly modified version of the built-in methods. Following steps were performed during coupling process, after each step the cartridge was washed with anhydrous MeCN, followed by drying step with argon.

1. The CPG was flushed three times with DCA solution.

2. The respective phosphoramidite and the activator were mixed with the ratio 1:1 (*v/v*). Coupling time for normal nucleoside was 0.5 min. For the ligands the coupling time was increased to 3.5 min.
3. For the capping process Cap A and Cap B were mixed 1:1 (*v/v*)
4. The CPG was treated with the oxidizer solution
5. An extra washing step with anhydrous MeCN was introduced to remove as much water as possible, which interfere the coupling process.

The cycle was repeated for every coupled nucleobase. The progress and success of coupling steps was monitored by internal trityl absorption measurements. All syntheses were performed in DMT-ON mode, means the last deprotection step is omitted. After synthesis the CPG was removed from the cartridge and suspended in concentrated aqueous ammonia solution (33%). The mixture was heated over night at 55°C to cleave the ONS from the solid support and in addition deprotected the nucleobases. The supernatant was transferred and the left over CPG was washed twice with H₂O (200 µl). A sample for ESI-MS was taken and subsequently the collected solutions were reduced to a volume of 300 µl *in vacuo*, followed by the purification process.

B.2 Oligonucleotide Purification

B.2.a Reversed Phase - HPLC

ONSs samples were purified DMT-ON on a Agilent 1260 Infinity I or II system. Analytic HPLC measurements were performed on a *Macherny Nagel* 250 Nucleodor 100-5 C18 ec analytic column (Ø 4.6 mm). The separation of ONSs were realized on a *Macherny Nagel* 250 Nucleodor 100-5 C18 ec semi-preparative column (Ø 10 mm) The used methods are presented in table VI.2.2.2. Initially, 0.1 molar triethylammonium acetate (TEAA) solution was used, but the high buffer concentration led to column problems and was therefore reduced to 50 mM, what gave very similar separation resolutions. All measurements and separation were at 60 °C column temperature, to avoid unwanted assemblies of the ONSs. The used methods for the HPLC purification and analysis can be found in table VI.2.2.2.

Table VI.2.2.2: HPLC methods used in for analytic investigation and ONS separation.

Method	Solvent	Gradient	Flowrate	Time
Analytic	A: 0.05 M TEAA, pH 7.0 B: 70:30 MeCN / A	B 0-80%	1 mL/min	35 min
Analytic	A: 0.05 M TEAA, pH 7.0 B: 70:30 MeCN / A	B 0-80%	3 mL/min	35 min

The HPLC traces were always recorded at 260 nm, additionally for the ONSs containing ligand **39** the specific absorption at 357 nm was also observed. The traces shown in chapter A were recorded after detritylation and desalting of the samples.

B.2.b Solid Phase Extraction

After HPLC purification the isolated ONS was desalted and the DMT-protecting group was removed via Solid Phase Extraction on *Waters* Sep-Pak C18 cartridges. After preconditioning the Sep-Pak material by washing with 2 x 2 mL MeCN, 2 mL 70:30 MeCN / 0.1 M TEAA, pH 7.0 and 2 x 2 mL 0.1 M TEAA, the ONS solution was 3 times slowly passed through the cartridge. The absorbed ONS were subsequently washed with 2 x 2 mL 0.1 M TEAA and treated with 3% (*v/v*) TFA for at least 1 min. A successful detritylation was indicated by temporary red colored solution. Afterwards, the bound ONS were washed with 2 x 2 mL H₂O. The deprotected ONSs were eluted from the C18 material with MeCN/H₂O 1:1 (*v/v*) and the solvent was removed *in vacuo* to a volume of roughly 500 µl. Less solvent has led to precipitation which was hardly resolvable. The ONS solution was filled up with H₂O to 1 ml. This solution was used for the characterization and all prepared samples used in all further described experiments.

B.3 Oligonucleotide Characterization

B.3.a Oligonucleotide Mass Analysis

The molecular mass of the ONSs were determined with help of ESI-MS. The samples were measured on *Bruker* maXis with TOF analyser or *Bruker* timsTOF, likewise equipped with a TOF analyser. The samples were prepared from 1 μ l ONS, directly taken from stock solution and diluted 9:1 with H₂O/MeOH or H₂O/MeCN.

The mass of the synthesized ONS was determined three times before purification and after purification and again after the desalting/deprotection step. Before purification the crude solution checked if the synthesis was successful and it contains the desired strand. After purification every collected fraction was analyzed for the containing ONS and once again after desalting and deprotection, to confirm an successful deprotection.

B.3.b Oligonucleotide Concentration Determination

The concentration of each ONSs stock solution was determined by UV-vis absorbance at 260 nm at 25 °C using the revised extinction coefficients for each nucleoside.^[159] Since the integrated ligands are aromatic systems, they also show an absorption at 260 nm, that has to be taken into account. Therefore the extinction coefficient of each used ligand was determined by simple UV-vis dilution experiments. For that reason a solution with a known concentration of ligand was gradually diluted and the absorbance of each dilution step was record at 260 nm. From the plot absorbance vs. concentration the determined absorbances were fitted by an linear regression, where the slope represents the extinction coefficient $\epsilon_{260\text{nm}}^{25^\circ\text{C}}$ of the respective ligand for 260 nm at 25°C.

Figure VI.2.3.1: Determination of the ligands extinction coefficient by UV-vis dilution experiments

The determination of concentration was initially performed by UV-vis measurement on the *Agilent* DAD HP-8453 UV-Vis spectrometer. For that reason 1 μl of each ONSs sample was diluted with water to a total volume of 400 μl and its absorbance was measured. With the help of the individual extinction coefficient for each ONS with simultaneous consideration of the dilution, the concentration of each stock solution could be obtained. Later the concentration was elucidated with a *Thermo Scientific* Nanodrop One, where the absorbance could be directly measured from a single drop (1 μl) of respective stock solution. For a better comparison, the previously determined ONS stock solution concentrations were checked again on the Nanodrop machine, but were matching with the old ones.

B.4 Spectroscopic Methods

B.4.a ONS sample preparation

DNA samples were prepared with 10 mM sodium cacodylate (pH = 7.3) or sodium phosphate (pH = 7.2) buffer, 100 mM NaCl or NaClO₄ and 2 mM MgCl₂ or Mg(ClO₄)₂. Each ONS belonging to a Holliday junction systems was added to obtain concentration of 2 μ M per strand, so 8 μ M in total, in order to end up with 2 μ M of the desired Holliday junction structure and filled up with water to end up with a total volume of 800 μ l. The samples were heated to 90 °C for 5 min and subsequently cooled down to 4 °C in 0.5 °C min⁻¹ steps. The samples were stored in the fridge at ca. 3 °C, when not used immediately. The respective metal salts were also prepared in ultra pure water with a concentration of 800 μ M, so that one equivalent of metal salt could be added in a 22 μ l portion.

B.4.b UV-vis Spectroscopy

Except of the determination of concentration all UV-vis measurements on ONSs were performed on the JASCO V-650 or Jasco V-750 UV-vis spectrometer, both equipped with a PAC-743 temperature-controlled 6-cell cuvette changer slide. The actual temperature was contentiously measured during experiments in water-filled cuvette, which in turn reduced the amount of max. simultaneously measured samples to 5. The UV-vis bandwidth was set to 2.0 nm, the data interval to 1 nm. For the measurement the 800 μ l of ONS samples were filled in Hellma Analytics 114-QS, quartz glass cuvettes with 10 mm path length, to ensure the that the beam complete transpasses the solution. To avoid condensation of moisture on the cuvettes outside during the cooling process, the cell holder were continuously flushed with dried air. Additionally, to avoid concentration change caused by unwanted evaporation during the heating process, each sample were topped with a thin layer of silicon oil before the cuvette was tightly closed with a PTFE lid. The volume of afterwards additions was kept as small as possible in comparison to the sample volume, in order to minimize the change in absorption due to dilution. Before and after melting curve measurements normal UV-vis spectra were recorded. Before the melting process a spectra at 4 °C was recorded and compared with the spectrum recorded at 4 °C after the melting curve measurements. Furthermore, after the melting curve experiment, spectra were recorded for 4 °C and 85 °C, from which the TDS was generated. All measured spectra were background corrected (cuvette with buffer and electrolyte).

In case of thermal denaturation experiments, absorption of the samples were record at a wavelength of 260 nm with an increase or decrease in temperature of 0.5 °C min⁻¹. For

initial thermal denaturation measurements two full cycles were measured. After addition of a metal salt, three complete cycles were measured. The denaturation temperature (T_M) was determined, if possible, in three ways:^[160]

1. Forming the midrule between the baselines of the disassembled and assembled strands. The crossing of the midrule with the recorded spectrum shows the melting temperature.
2. Calculating the first derivative of the recorded melting curve. The obtained plot was fitted with a Gaussian function. This method can become quite inaccurate, as soon as the denaturation process happened over a larger temperature range
3. Calculating the fraction folded values and determine the T_M at the y-value = 0.5 (or $\alpha = 0.5$, where α represent the fraction folded).

The most used method throughout the literature is to calculate the first derivative of the melting curve (method 2) and then to determine the its maximum either manually by estimating it in the graphic or by fitting the curve with a suitable regression function and extract the value from the fitting. It has the advantage that it is very easy to perform and no other data processing is necessary. The disadvantage of this method becomes clear, as soon as the signal-to-noise ratio is lowered, since this becomes even more dominant in the derivative curve, making it more difficult to locate the position of the maximum and results in a less accurate determination of T_M . Method 1 still comes from the time, when computer-assisted data analysis was not daily routine. However, this method is still valid and gives quite accurate values for T_M . In order to determine T_M the bisectrix between the lower (assembled DNA) and upper (disassembled DNA) baseline is graphically assigned and T_M is obtained from the intersection of bisectrix and melting curve. In the third method, T_M can be received from the fraction folded value $\alpha = 0.5$, where it is assumed that 50 % of the oligonucleotide is still assembled, or 50 % is disassembled. For that reason, the recorded melting curve based on absorption is converted to a fraction folded curve, ranging from completely unfolded ($\alpha = 0$) to completely folded ($\alpha = 1$) oligonucleotidic structure, so that $\alpha = 0.5$ can be easily assigned. This method can give more accurate results than the derivative method, however, the assignment of the corresponding baselines is rather a subjective approach. The needed fraction folded curve is plotted from the fraction folded values, which are calculated for the measured absorption values at the corresponding temperatures (A_{260}) and the linear regressions of the upper (A_{high}) and lower (A_{low}) baselines (section B.4.b).^[160]

$$\alpha = \frac{A_{260} - A_{high}}{A_{low} - A_{high}} \quad (B.1)$$

Besides the denaturation temperature, further thermodynamic characteristics could be obtained from melting curves. A relative thermodynamic stability can be deduced from the slope of the recorded melting curves. A broad transition indicates a rather weak temperature dependency on the affinity constants, giving rise to the assumption the structure has a lower stability, or a less favorable ΔG° , compared to a system with the same T_M but a steeper slope. By implication, a sharp transition (displayed by a steep slope, meaning a transition in a small temperature range) is indicating a strong dependency of temperature on the reaction free enthalpy, accompanied by a higher affinity constant. As an example, a system with a higher melting temperature but a broader transition can thus have the a lower relative thermodynamic stability, compared to another system with lower melting temperature but a sharper transition. Therefore, a simple correlation between T_M and ΔG° is improper and other factors as i.e. the shape of the melting curve have to be considered as well, in order to compare the relative thermodynamic stability of different systems.^[160]

B.4.c CD Spectroscopy

CD measurement of ONSs were performed on a *Applied Photophysics* qCD Chirascan machine with a temperature-controlled cuvette holder. For all measurements samples were taken from previous melting curve experiments, to ensure the existence of the Holliday junction species within the sample. All spectra were measured from 500 nm to 220 nm, with a bandwidth of 1 nm and a data interval of 1 nm with standard sensitivity. The cell chamber was continuously flushed with a nitrogen stream to avoid condensation on the outer cuvette during cooling process. Due to the low signal intensity the measurement was repeated three times and data was accumulated. The ONSs, were measured at 4 °C and 80 °C, each. All spectra were background corrected (cuvette + buffer + electrolyte) and smoothed (10pt, Savitzky-Golay).

B.4.d Fluorescence Spectroscopy

Fluorescence spectra were measured on a Jasco FP-8300 fluorescence spectrometer. The samples were directly taken from the CD measurement, heated to 90 °C and cooled down to 4 °C in 0.5 °C min⁻¹. For measurement including ONSs an Unisoku Coolspek temperature-controlled cuvette holder was used, to measure the samples at 4 °C and 80 °C. To determine the the the best excitation wavelength for the respective samples,

3D fluorescence measurement, as shown in section V.1.3.4 were made, with excitation wavelength range from 220 nm to 400 nm in 5 nm steps and an emission wavelength range from 400 nm to 700 nm with a scan rate of 500 nm min⁻¹. For the actual fluorescence measurements, the determined best excitation wavelength for the respective samples was used with a bandwidth of 2.5 nm and a bandwidth of 5 nm for the recorded emission wavelength. The spectra were recorded at a medium sensitivity and a scan rate of 200 nm min⁻¹.

B.5 PAGE Gel Electrophoresis

PAGE experiments were carried out under non-denaturing conditions on a *CBS Scientific* Dual-Vertical Mini-Gel system MGV-402. All PAGE gels were run in constant voltage mode at 200V for 3.5 h with running TBM buffer containing 89 mM Tris-borate, pH 8.3, with 2 mM Mg(ClO₄)₂. Gels were prepared in 15 × 20 (w × h) size and preconditioned with . Separating gel consists of 8% (w/v), 0.28% (w/v) bisacrylamide topped with a 4% (w/v), 0.14% (w/v) bisacrylamide stacking gel. For gel polymerization in both cases ammonium peroxodisulfate (APS) was used as radical starter, with N,N,N',N'-tetramethylethylenediamine (TEMED) as stabilizing agent. Furthermore running buffer was already added to gel solutions, conditioning of the gels with running buffer after polymerization led to bad separation and massively blurred signals. Samples were prepared as with 10 mM TB buffer 2 mM Mg(ClO₄)₂ and 12.5 ng DNA filled up to 10 µl with water. All samples were heated to 90 °C for 5 min and then cooled down to 4 °C in 0.5 °C/min steps. For loading the samples were mixed with 10 µl 40% (w/v) sucrose solution. For a better visualization of the running front some samples were prepared with a dye containing loading buffer, containing additionally 0.05% (w/v) bromphenol blue and 0.05% (w/v) xylene cyanol. For size comparison the O'RangeRuler 10 bp DNA Ladder of *Thermo Scientific* was used, containing DNA double strands differing in length by 10 base pairs from 10 to 150 base pairs. The gels were run at constant 40 °C, when not mentioned otherwise. After completion of the separation step, the gel was carefully removed and incubated in 250 mL TBM buffer containing 25 µl *Invitrogen* SYBR Gold Nucleic Acid Gel Stain for 20 min. After, the pictures were made with the Intas Gel IX 20 gel documentation system, illuminated with an extinction wavelength of 312 nm. All pictures were converted to black and white and the brightness and contrast was adjusted.

B.6 NMR Line-Shape Analysis

The line shape analysis of the measured ^1H -NMR spectra was made with help of the line fitting function in the program *MestreLab Research S.L* MestreNova NMR, with what the FWHM of the respective proton signals could be easily determined. All further values such as $\Delta\nu_0$ and $\Delta\nu_e$ could also be extracted from the respective NMR spectra. Subsequently, the values were used to calculate the experimental rotation constants with the suitable functions given in section V.1.2.4.

The simulated NMR spectra were made with the program *Spinworks 4.2.4*. Unfortunately the simulation of DNMR is limited to five nuclei, therefore only two exchanging proton pairs could be simulated at the same time. Since the signals (a/a') and (b/b') showed the most distinctive effect, these both signals were chosen for the simulation. First of all the chemical shift and coupling constants for all four signals (a, a', b and b') were determined from low temperature measured NMR spectra. These values were used with the assumption that virtually no exchange between the proton pairs a/a' and b/b' occurs. The four signals were then simulated with the given values. Since the shape of signals caused by coupling could not be obtained with only the four protons, one dummy signal for both protons c/c' was added in the simulation, since it was only possible to add one more nucleus to the DNMR simulation. However, due to the fact that the signals c/c' does not exhibit such a big shifting caused by the exchange in the VT NMR, the use of this dummy signals gives a nice peak pattern for the signals of interest (a/a' and b/b') without having a big effect on the simulation outcome. Next, the simulated NMR spectra were superimposed with the measured one and the values of the simulated signals were adjusted with an iterative fitting function (NUMMRIT).

After, the parameters for the DNMR3 simulation was set up. First of all T_2 , this parameter defines the linewidth in the absence of exchange. Since it is known from the experiments that the linewidth at very low temperatures equals almost 1 Hz, the default value for $T_2 = 1.0$ was used. Since the chemical exchange in the investigated system is mutual exchange, mutual exchange was activated and the number of species was set to 2, including both enantiomeric structures. Since in the mutual exchange only the rate constant $RC(1,2)$ is available, it was set to 0, since we assumed the no rotation, or chemical exchange, for initial simulated spectrum. The population factors can be omitted in the case of mutual exchange. As last the permutation vectors have to be set. For that reason the order of the simulated nuclei has to be known. In the here described case, signal a = nucleus 1, signal a' = nucleus 2, b = nucleus 3, b' = nucleus 4 and the dummy signal c = nucleus 5. Considering that the exchange occurs between a and a' or b and b' and c is mapping itself the values inserting for the Permutation Vector 2 are 2, 1, 4, 3, 5. These values do result in following permutation matrix:

$$\begin{pmatrix} 0 & 1 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 \end{pmatrix} \quad (\text{B.2})$$

With those settings, the rotation constant was varied till the simulated signals overlapped with the measured signals. The procedure was repeated for every recorded spectrum at the different temperatures and the determined rotations constants were afterwards plotted against the temperature likewise to those from experiments.

Appendix C

Overview

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C.4 Abbreviations

G	guest
H	host
K_a	association constant
^1H NMR	^1H NMR
bp	base pairs
CD	circular dichroism
CEP	β -cyanoethyl phosphoramidite
CPG	controlled pore glass
CV	cyclic voltammetry
DCM	dichloromethane
DEA	diethanol amine
DFT	density-function theory
DMAP	4-dimethylaminopyridine
DMT	4,4'-Dimethoxytrityl
DNMR	dynamic NMR spectroscopy
dsDNA	double stranded DNA
EA	elemental analysis
ESI-MS	electrospray ionization mass spectrometry
FS	fluorescence spectroscopy
FWHM	full width half maximum
GNA	glycol nucleic acid
GPC	gel permeation chromatography
HJ	<i>Holliday</i> junction
HPLC	high performance liquid chromatography
IDA	inter duplex angle
LNA	locked nucleic acid

MOM	methoxymethyl acetal
NMR	nuclear magnetic resonance spectroscopy
ONS	oligonucleotide strand
PAGE	polyacrylamide gel electrophoresis
PEG	polyethylene glycol
PNA	peptide nucleic acid
RNA	ribonucleic acid
SBB	supramolecular building block
SEC	size exclusion chromatography
SPS	solid phase synthesis
ssDNA	single stranded DNA
T _C	coalescence temperature
T _M	denaturation temperature
TBDMS	<i>tert</i> -butyldimethylsilyl
TBM	tris-borate magnesium
TDS	thermal differential spectrum
TFA	trifluoroacetic acid
THP	tetrahydropyranyl ether
TsOH	p-toluenesulfonic acid
UNA	unlocked nucleic acid
UV-vis	ultraviolet-visible spectroscopy
VT NMR	various temperature NMR

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