Helical Chromophoric Nanowires

een wetenschappelijke proeve op het gebied van de Natuurwetenschappen, Wiskunde en Informatica

Proefschrift

ter verkrijging van de graad van doctor aan de Radboud Universiteit Nijmegen, op gezag van de Rector Magnificus, prof. dr. C.W.P.M. Blom, volgens besluit van het College van Decanen in het openbaar te verdedigen op woensdag 13 oktober 2004 des namiddags om 1.30 uur precies

door

Pieter Antonius Johannes de Witte geboren op 25 augustus 1975 te Raalte
# Table of Contents

**Chapter 1: General Introduction**

1.1 Supramolecular chemistry: materials and nanotechnology ............................................... 1  
1.2 Chromophores .................................................................................................................. 3  
1.3 Naturally occurring chromophore assemblies .................................................................... 4  
1.4 Aim and outline of this thesis ............................................................................................. 6  
1.5 References .......................................................................................................................... 8  

**Chapter 2: Literature Survey**

2.1 Multi-chromophoric architectures; a literature survey ........................................................ 9  
2.2 Types of aggregates .......................................................................................................... 9  
2.3 Porphyrins ........................................................................................................................ 10  
2.4 Perylenes .......................................................................................................................... 20  
2.5 Polyisocyanides ............................................................................................................... 24  
2.6 References ........................................................................................................................ 32  

**Chapter 3: Porphyrin Pendant Polyisocyanides derived from Alanine.**

3.1 Introduction ....................................................................................................................... 35  
3.2 Results and discussion ..................................................................................................... 36  
3.3 Conclusions ..................................................................................................................... 55  
3.4 Experimental Section ....................................................................................................... 56  
3.5 References ....................................................................................................................... 63  

**Chapter 4: Synthesis and physical Characterisation of Porphyrin Nanorods.**

4.1 Introduction ....................................................................................................................... 65  
4.2 Results and discussion ..................................................................................................... 65  
4.3 Conclusions ..................................................................................................................... 97  
4.4 Experimental Section ..................................................................................................... 97  
4.5 References ....................................................................................................................... 102
Chapter 1

General Introduction

1.1 Supramolecular chemistry: materials and nanotechnology

In a recent review, it has been suggested\(^1\) that the chemical definition of a molecule should be modified and changed into “a collection of atoms held together by covalent and non-covalent bonds” This new definition clearly indicates how much supramolecular chemistry has become integrated into the field of chemistry as a whole. Mother nature, the highly cited, absolute number one supramolecular chemist, has indeed integrated both covalent and non-covalent interactions for the construction of functional, highly sophisticated systems during the long process of evolution.

Lehn, one of the 1987 Nobel prize winners for the development of the field of molecular recognition has divided the development of the emerging field of supramolecular chemistry into three overlapping stages:\(^2\), \(^3\) (i) Molecular recognition, which relies on design and preorganisation of molecules, enabling selective binding, information storage, processing, etc. It should be noted that selectivity is considered to be the Achilles heel of supramolecular chemistry, which is clear from the fact that the construction of selective supramolecular enzyme mimics, the biomimetic branch of supramolecular chemistry, remains a great challenge.\(^4\) (ii) Self-assembly which allows the ‘spontaneous’ but controlled assembly of molecular building blocks into thermodynamically stable supramolecular systems. This second stage is currently a great challenge for many supramolecular chemists. (iii) Self-organisation through selection in addition to design and assembly. This allows the construction of assembled architectures that are self-corrective and able to adapt to a change in the environment. Eventually adaptation leads to evolution when acquired features are conserved.
Chapter 1

and passed on. The ultimate goal of supramolecular chemistry is the development of self-assembling and self-evolving systems.

The current stage of (ii) comprises the manipulation of molecular building blocks that are programmed to express themselves at the supramolecular level. Supramolecular chemistry has advanced to a level that design of non-covalent structures is focused predominantly on the implementation of functionality. The self-organisation of functional supramolecular structures encompasses both discrete finite architectures and structures that are extended in one (e.g. polymolecular chains and fibres), two (e.g. layers), and three (e.g. solids) dimensions.

When functionality is introduced at the molecular level, a whole range of advanced materials becomes in principle available through supramolecular structuring of the monomeric building blocks. As a consequence of the ability of supramolecular chemistry to generate unique objects and materials, this discipline is envisaged to play a pivotal role in the development of the fields of nanoscience and nanotechnology. A literature search using the terms ‘supramolecular chemistry’ AND ‘materials’, shows a very strong increase in the number of publications in the last decade. Applying the terms ‘supramolecular’ AND ‘nanotechnology’, also reveals a sharp increase in papers in the last 4 years. From these results one may conclude that supramolecular chemists are strongly involved in new technological developments and even may take a leading role in it.

Figure 1 Results of a search in Scifinder using the keywords as stated, showing the increase in the number of papers dealing with supramolecular chemistry and (a) materials synthesis, and (b) nanotechnology.

Supramolecular materials prepared from molecular building blocks can be expected to display many of the target properties that are desired for application in electronic and photonic devices. They have already been constructed from a wide variety of molecular components and span wide length scales. An important class of these functional materials involves the use of organic dyes, especially in opto-electronic applications, where it is crucial to have the
chromophores ordered in well-defined arrangements to improve the efficiency of its function or to bring about new material properties.

1.2 Chromophores

Chromophores play an important role in our lives as dyes and pigments, but also because they are applied in a wide variety of technologies. Chromic phenomena, which are the origin of colour, occur through chemical and/or physical interactions with or between chromophores and can be grouped into five classes according to the technological application, see Figure 2.

![Figure 2 Classification of chromic phenomena (the causes of colour), grouped according to their technological application (drawing taken from reference [9], p3).](image)

In many of these applications, the performance of the material or device can be improved by controlling the organisation of the chromophores. Here, supramolecular chemistry can play a crucial role. One possibility for tailoring the properties of chromophores is to introduce molecular recognition elements within the chromophoric building blocks. Some chromophores already possess recognition elements naturally, e.g. the disk shaped porphyrins, phthalocyanines and perylenes, which have the proper geometry to form π-stacks. The supramolecular toolbox, however, contains many more assembly possibilities such as hydrogen bonding, electrostatic interactions, and Van der Waals interactions, many of which have been utilised to create multichromophoric assemblies.
1.3 Naturally occurring chromophore assemblies

Nature employs chromophores for energy and electron transport. To that end it mostly uses porphyrin-like systems that are organised in a manner that facilitates the transfer of excitation energy or electrons rapidly and with high efficiency. A particularly elegant example is the structure of the circular antenna complex of purple bacteria, LH2. With the determination of its crystal structure by Isaacs c.s.\textsuperscript{[12]} only one decade ago, the porphyrin community obtained an insight into how nature designs and constructs large porphyrin assemblies.

\textbf{Figure 3}  a) Side view of the circular LH2 complex with the B800 ring for light harvesting and perpendicular to that the B850 ring for excitation energy transfer. b) Idem, top view.

This antenna complex LH2 displays an incredible efficiency towards the harvest of sunlight. It consists of two rings of bacteriochlorophyll \textit{a} (Bchl \textit{a}) molecules. The outer, B800, ring (absorption maximum around 800 nm, hence the name) has 9 Bchl’s oriented with the planes parallel to the membrane surface in order to increase the efficiency of the light harvesting. This ring, together with the pendant carotenoids absorb the sunlight, spanning most of the solar spectrum. The excitation energy is transferred from the outer to the inner ring, consisting of 9 Bchl \textit{a} dimers, perpendicularly organised with respect to the membrane (B850). The Bchl’s within the outer ring have no interactions with one another, only with the Bchl’s from the inner ring, of which the chromophores are excitonically coupled. Whether the excitation energy is delocalised over the entire ring, or just over a few chromophores is still under investigation.\textsuperscript{[13, 14]} The Bchl \textit{a} (Figure 4a) is in fact a substituted magnesium chlorin (shown in grey), which is tightly embedded in a protein matrix and held in position by secondary interactions with the proteins, \textit{e.g.} coordination of histidine and formyl groups coordinating to the magnesium centre and hydrogen bonds between the acetyl carbonyl oxygen of Bchl \textit{a} and the proteins.

Several LH2 complexes are further grouped around a central LH1 ring, which consists of 16 self-assembled BChl dimers, resulting in 32 Bchl \textit{a} molecules arranged in a turbine geometry. The LH1 complex houses the photosynthetic reaction centre, where the collected energy is converted into a charge separated state. This in turn drives a proton pump, which initiates the
General introduction

synthesis of the energy rich molecule ATP and subsequently other processes important to life.\[15\]

![Diagram of ATP molecule](image)

**Figure 4** a) Structure of Bchl a with chlorin shown in grey. b) Structure of carotenoid.

The antennae are organised such that the excited state energy decreases when it is transferred from pigments at the periphery of the antenna towards the reaction centre, where the charge separation occurs.\[16\] In this way energy is funnelled efficiently to the reaction centre due to spectral overlap, and the wavelength range and cross-section for light absorption are greatly increased. The perfectly tuned energy gradient, the highly interacting chromophores, the ability to store the excess energy temporarily in the LH1 ring and the absorption of the solar light over a wide wavelength range makes this system one with a very high quantum efficiency (> 95 %) for absorbed photons.\[13, 17\]

In green bacteria, mainly the chlorosomes are responsible for light harvesting. The chlorosomes are constructed of Bchl c molecules that are self-assembled in rod-like tubular aggregates, containing up to several tens of thousands of Bchl’s. On the basis of various kinds of spectroscopic studies and theoretical models, an architecture has been proposed for these antennae, in which cylindrical BChl aggregates have diameters from 5 to 10 nm and lengths that can reach several hundreds of nanometers (Figure 5).\[18-20\]

![Diagram of tubular Bchl c aggregate](image)

**Figure 5** a) Schematic view of the tubular Bchl c aggregate as obtained from molecular modelling calculations. b) Schematic representation of the arrangement of Bchls in one cylindrical stack, the Z-axis is the axis connecting the Mg atoms in one stack.
Initially, the Bchl’s organise into stacks, which in turn form rod-like super-structures by forming a hydrogen-bonding network. The supramolecular structures can be envisaged as strands of interacting molecules that wind around the cylinder in a helical fashion, and are referred to as \(J\)-aggregates (see §2.1.3), since their absorption is red-shifted with respect to that of a single BChl molecule.

This is a beautiful example of natural supramolecular chemistry. Several of these rods form a hexagonal matrix that makes up the overall chlorosomal structure\[^{21, 22}\].

These rods display long-range molecular order of the BChl \(c\) molecules in a precise mutual position, ensuring highly efficient and directional energy transfer toward the reaction centres\[^{23}\].

These light harvesting systems have inspired numerous researchers to mimic the large number of chromophores involved and the well-defined architectural motifs in order to construct synthetic light harvesting systems.

### 1.4 Aim and outline of this thesis

In order to be able to use chromophores in molecular photonic and electronic devices, several key points need to be considered to obtain optimal performance:\[^{24}\] (i) ample electronic interactions between neighbouring chromophores is required for efficient energy transfer, (ii) long \(\pi\)-electron conjugation lengths which enhance charge mobility or optical non-linearity are needed, (iii) well-defined and rigid molecular structures should be constructed in order to avoid the formation of energy or charge sinks, which hamper the energy flow, (iv) high stability and solubility for processing is needed, and also (v) considerable length for use as wires in nanoelectronic devices.

A way to meet criteria (i)-(v) is to use a rigid scaffold, that precisely organises the chromophores. Polysiocyanides are such scaffolds (Figure 6, for a detailed description of these polymers, see § 2.4). Due to the special helical nature of these polymers, the side-groups are all placed at exact distances and precise positions with respect to each other. This ordering should allow for strong exciton interactions between the chromophores to occur over the entire polymer, at least in principle. By using amide functions in the side groups, hydrogen bonding arrays can be formed along the polymer backbone, which mimic the protein scaffolds of nature, even further rigidifying the polymer and stabilising the helical structure. In addition, and also of vital importance is the fact that the polymers can be synthesised with lengths of hundreds of nanometres.
This polymer based photonic system can also function as an interesting model system, since different parameters can be varied synthetically by tuning the chemical composition of the chromophore and the side arm spacer. A molecular modelling study on a short polyisocyanide fragment with porphyrin pendant groups, a 16-mer, showed that the overall helical structure is stable and that the polymer forces the porphyrins to stack on top of each other with a slight shift, which is also an intrinsic tendency of the porphyrins (Figure 7).

The general aim of the research presented in this thesis is the construction and characterisation of long, well-defined helical porphyrin and perylene arrays in order to obtain chromophoric nanowires capable of transferring energy over large distances, i.e. tens of nanometers. After the introductory Chapter 1 this thesis continues with an overview of the recent literature focussing on synthetic multi-porphyrin and multi-perylene arrays, constructed either using covalent or non-covalent synthesis (Chapter 2). The synthesis and characterisation of peptide-derived polyisocyanides with porphyrin pendants is described in Chapter 3. Although no long nanowires were obtained, a general synthesis protocol could be made for the construction of
long porphyrin pendant polyisocyanide wires, of which the synthesis and characterisation are described in Chapter 4. These polymers were found to be very long (87 nm) and capable of energy transfer over at least 10 nm. Reversible conformational switching of the porphyrins could be realised by changing the temperature. Zinc metal ions were incorporated into the porphyrins to obtain zinc porphyrin polyisocyanides (Chapter 5) which showed an optical behaviour that was different from the metal free polymers. Intramolecular coordination of alkoxy side chains to the zinc centres was found to make the polymers more stable to higher temperatures. In Chapter 6 the synthesis of perylene arrays is described. The same strategy as with the porphyrins was used, leading to long helical perylene fibres. The emission spectra of the perylene polymers were composed of two types of emissions, monomer-like and excimer emission. With confocal microscopy in combination with AFM it could be shown that the helical polymers display exclusively excimer emission. Single fibres of the polymers were observed to possess a left-handed helical structure by AFM. The last experimental chapter describes the attempts to form block copolymers based on the polymers mentioned above. It proved to be difficult to control the size of the polyisocyanide blocks to obtain well-defined copolymers.

1.5 References

Chapter 2

Literature Survey

2.1 Multi-chromophoric architectures; a literature survey
In this thesis two chromophores are used as key elements in the design and synthesis of multi-dye array structures: porphyrins and perylenes. This chapter deals with the properties of these dye molecules and recent examples of large defined chromophoric assemblies from the literature will be described. The emphasis of the review below is on the relationship between the structure and function of the designed assemblies.

2.2 Types of aggregates
Bringing chromophores in close proximity causes a reorganisation of the energy levels of the molecules due to electromagnetic interactions, resulting in an altered absorption spectrum.\cite{1, 2} Several types of aggregates of chromophores can be distinguished (Figure 1). When the transition dipole moments between the neighbouring molecules are aligned in a head-to-tail fashion, a so-called J-aggregate is formed, which displays a red-shifted and narrowed band in the absorption spectrum compared to its monomer. A cofacial aggregate, with parallel transition dipole moments, a H-aggregate, gives rise to a broadened, blue-shifted absorption spectrum. Aggregation leads to a splitting of the energy levels. For both J- and H-aggregates this results in one allowed and one forbidden transition due to the two possible vector sums of the transition dipole moments. The configurations depicted in Figure 1 give rise to an allowed transition due to their non-zero sum. Electrostatically, the transition dipole moments in the J-aggregate (Figure 1a) are attractive, hence leading to a lowering of the transition energy, while it is repulsive in the H-aggregate (Figure 1b). If the aggregate structure contains neighbouring molecules with non-parallel transition dipole moments (Herringbone structure), two transitions
are allowed, giving rise to a broadened or split spectrum. In reality, aggregates often show a combination of different interactions.

![Figure 1](image.png)

**Figure 1** a) Schematic representation of the structure of a J-aggregate. b) Idem of a H-aggregate. c) Herringbone structure.

### 2.3 Porphyrins

Porphyrins are a class of pigments that has been intensively studied for decades. The reason for this scientific interest is the many potential applications of these molecules, *viz.* in photodynamic therapy, as catalysts, and as mimics of natural pigments in artificial photosynthesis. Porphyrins and metalloporphyrins are extremely versatile building blocks for a variety of interesting materials.\(^{[3-5]}\) Initially, these compounds were only studied for their physical properties, and no effort was made to organise them into larger structures. They were merely deposited on surfaces, mixed with other materials, *etc.*

The structure of a porphyrin is shown in Figure 2a. The molecule consists of four pyrrole units that are linked by four methine bridges. The phenyl groups on the methine bridges (called the *meso* positions) are the usual substituents, which makes porphyrins synthetically accessible and functionalisable. The pyrrole protons are located at the so-called *β* positions. The core nitrogens of the porphyrin can either hold 2 protons (the free-base (FB) porphyrin), or a metal centre.

The porphyrin macrocycle is an aromatic system containing 22 \(\pi\) electrons, but only 18 of them are involved in a delocalised ring current. The aromatic character of porphyrins is evident from NMR spectroscopy. Due to the anisotropic effect of the porphyrin ring current, the NMR signals of the deshielded *meso* and *β* protons show up at low field (8 to 10 ppm), whereas the signals for the shielded protons on the inner nitrogen atoms are found at relatively high field (-2 to -4 ppm).

Metal porphyrins all display the same characteristic absorption spectra (Figure 2b), consisting of two so-called Q bands at 500-600 nm and a B- or Soret-band, around 400 nm.\(^{[6]}\) The Q bands originate from a transition to the lowest-energy first excited singlet state and have a molar extinction coefficient of \(\varepsilon \sim 1 \times 10^4\) M\(^{-1}\)cm\(^{-1}\). The B band originates from a transition to the second excited singlet state and has a molar extinction coefficient of \(\varepsilon \sim 10^5\) M\(^{-1}\)cm\(^{-1}\). In case of a free-base porphyrin, the visible absorption spectrum changes from a two-banded (D\(_{4h}\)-type)}
to a four-banded \( (D_{2h}\text{-type}) \) spectrum. This lowering of symmetry due to the presence of two protons splits the two Q-bands into four bands.\[^7\] When the core is fully protonated, a proton is attached to every nitrogen atom and the spectrum returns to the \( D_{4h}\text{-type} \), with the Q-band shifting to the red part of the spectrum. The absorption spectrum is only slightly affected by the substituents as the phenyl rings are not in resonance with the porphyrin plane.

\[ \text{Abs} \quad \lambda / \text{nm} \]

**Figure 2** a) Tetra- (or meso-)phenyl porphyrin, \( R \) can be varied. b) Uv-Vis absorption spectrum of a free base porphyrin (full line) and a zinc porphyrin (dashed line), inset shows an enlargement of the Q-band region.

In the following, a brief overview of porphyrin architectures will be given, restricted to systems with minimally 10 porphyrins that have recently appeared in the literature. For a review of smaller, oligomeric porphyrin arrays, several papers can be consulted.\[^8\text{-}11\]

2.3.1 Meso-meso linked porphyrin arrays

Beautiful examples of covalently linked large porphyrin arrays were published by Osuka and co-workers. Several giant porphyrin architectures could be synthesised in one, two and three dimensions using a Ag\(^+\)-salt-promoted meso-meso coupling reaction.\[^12\text{-}14\] Particularly, the meso-meso-linked zinc(II) porphyrin arrays are of fundamental interest, also in light of possible applications (Z in Figure 3a).\[^15\] The meso-meso-coupled porphyrin arrays possess split Soret bands as a result of exciton coupling. When the number of porphyrins in the array increases, the low-energy Soret band shifts to longer wavelength, while the high-energy Soret band remains at nearly the same wavelength (413 nm), resulting in a progressive increase in the splitting energy (Figure 3b).\[^16\] The red-shifting is a result of the coupling of the transition dipoles of all porphyrins that are parallel to the long axis of the polymer. The transition dipole moments perpendicular to the polymer long axis are orthogonal with respect to their neighbours and consequently are cancelled out. This orthogonality causes a relatively high conformational homogeneity.\[^17\] These arrays efficiently transfer singlet excitation energy over
large distances mediated by large electronic interactions between the porphyrins and as such offer promising light-harvesting capabilities. The lowest excited state can be considered as a Frenkel-type exciton, *i.e.* a strongly bound exciton, which has the excited electron in close proximity to the hole, due to the perpendicular geometry. The fluorescence spectra of the polymers also show a gradual red-shift with increasing porphyrin number. These polymers showed cooperative spontaneous emission (superradiance; an increase in rate of radiation due to in-phase emission[^18]), from which the radiative coherence length could be estimated[^17]. A plot of the natural fluorescence radiative lifetimes vs. the number of porphyrin units in Z showed a sharp linear decrease going from 1 to 6-8 porphyrins and becomes constant when more porphyrins are connected, indicating that the exciton delocalisation length for the S1 states is limited to ca. 6-8 porphyrin units.

![Figure 3](image)

*Figure 3* a) Chemical structure of the perpendicular porphyrin array (Z) with n up to 126, and the flat porphyrin array (T) with n up to 12 prepared by Osuka et al. b) Absorption spectra of polymers Z2-Z128 showing the increasing red shifts and extinction coefficients.

These monodisperse polymers were subsequently planarised with DDQ (2,3-dichloro-5,6-dicyano-1,4-benzoquinone) and Sc(OTf)₃ as oxidants which enabled the smooth transformation of meso-phenyl capped meso-meso-linked porphyrin arrays into meso-meso, β-β, β-β triply-linked porphyrin tapes[^19-21]. The unique character of the latter molecules is displayed in the absorption spectra. The lowest absorption band becomes increasingly red-shifted with increasing number of porphyrins, up to 12. This means that the effective conjugation length (ECL) is increasing constantly, and this is unique for electronically conjugated porphyrin arrays, which thus far had shown only conjugation saturation behaviour due to ECL[^22, 23]. Whereas the orthogonal arrays are considered to be photonic wires, the tapes should be
excellent electronic wires, since the band gap energy between the HOMO and LUMO levels has been reduced drastically.[24] This resembles a Wannier-type exciton, which has the excited electron loosely bound to the hole, giving it freedom to move around. Single molecules of Z and T (both hexamers) have been visualised by STM.[25]

![Image of UV-Vis-IR absorption spectra of oligomers T2 up to T6 and a porphyrin dimer in which the dihedral angle can be adjusted.](image)

**Figure 4** a) UV-Vis-IR absorption spectra of oligomers T2 up to T6. b) Porphyrin dimer in which the dihedral angle can be adjusted.

A compound intermediate between the two extreme forms of porphyrin arrays (planar and orthogonal), was synthesised in order to study the effect of the change in dihedral angle between the porphyrin planes on the delocalisation behaviour (Figure 4b).[26] This compound was prepared by selectively coupling two porphyrins on their meso-position, and by subsequently establishing a link between two neighbouring phenyl groups, to yield a so-called strapped bisporphyrin. By introducing a strap of variable length, it was shown that the electronic interaction between the two porphyrins increased with decreasing dihedral angle. Interestingly, the bisporphyrins are chiral and could be separated into their enantiomers on a chiral column. They displayed CD spectra that could not be accounted for by the exciton coupling theory due to additional charge transfer.

The dihedral angle could also be controlled by coordinating α,ω-diaminoalkanes to the zinc porphyrins in Z2.[27] It was found that 1,7-diaminoheptane (7DA) was capable of decreasing the dihedral angle from 90º to 50-70º. This caused red-shifted absorption and emission spectra and a four-fold splitting of the Soret band due to the change in interporphyrin electronic interactions. Addition of acetic acid restored the original dihedral angle by ‘capping’ of the amine groups. A reversible switch for excitation energy transfer could be developed by coupling a free-base porphyrin (FB) to Z2. Without coordination, energy transfer occurred from Z to FB. The direction of the excitation energy transfer could be inverted by adding 7DA. Upon excitation of FB excitation energy now became directed to the Z2. This process was reversible upon addition of acetic acid.
Energy transfer measurements have been performed on orthogonal porphyrin arrays (Z) in which one meso-meso ‘-bisphenylethynylporphyrin acceptor was linked to the end meso carbon of the orthogonal array. These compounds formed highly efficient molecular photonic wires because of the strong exciton interactions that are present in the arrays arising from the close proximity of the porphyrins and the lack of energy sinks owing to the well-defined orthogonal geometry.\[28]\]

The conductivity of single molecules of the porphyrin arrays Z48 and the tapes T8 was measured between nanoelectrodes. Z48 displayed diode-like behaviour which the authors ascribed to conformational heterogeneity, while T8 showed symmetric behaviour. The band gap of the tape structure was smaller than that of the perpendicular porphyrin array due to the stronger π-conjugation in the former structure, leading to a higher conductivity.\[29]\]

### 2.3.2 Dendritic multiporphyrin arrays

Several dendronised porphyrin architectures have appeared in the literature. Crossley et al. have used a polypropylene imine dendrimer as anchor to attach up to 64 porphyrins and have studied this dendrimer with time resolved fluorescence measurements.\[30]\] These measurements showed that in the system with 16 porphyrins energy transfer only occurred between the 4 porphyrins in one dendron, while interdendron transfer did not take place. The system with 64 dendrimers, on the other hand, displayed energy transfer due to strong coupling between the porphyrins over the entire dendritic surface, however, only 56 porphyrins were found to be involved. Two dendrons with 8 porphyrins resided either in or outside the dendrimer and effectively did not participate in the energy delocalisation on the surface.

Another porphyrin dendrimer system, with porphyrins inside the dendrimer arms, has been published by Aida and co-workers.\[31]\] The advantage of this system is the possibility to focus the excitation energy to the centre of the dendrimer. This was realised by placing a free-base porphyrin as an energy sink in the centre of the dendrimer and four dendritic wedges each with 7 zinc porphyrins in the periphery forming a star-shaped system. This compound clearly showed a very efficient energy transfer from the zinc to the free-base porphyrins. Interestingly, the four wedges were found to co-operate with each other, i.e. efficient energy transfer took place over the zinc porphyrins, before energy transfer occurred to the free base, like observed in the natural LH1 system that can retain excitation energy by delocalising it over the entire ring.

Hunter and his group have also used a dendrimer as scaffold for the synthesis of self-assembled porphyrin arrays.\[32]\] By end-capping an amino terminated third generation polypropylene imine
dendrimer with pyridine moieties, they attempted to synthesize a “zinc porphyrin ball”. However, due to the low solubility of the dendrimer and the low binding constants of the zinc porphyrin-pyridin complexes, no assembly could be detected. By creating a covalent porphyrin trimer, the binding constant was greatly increased, because the porphyrins in the trimer were found to bind in a co-operative fashion to the pyridine dendrimer. After binding of 3 trimers, most of the pyridine sites were blocked, and there was room for only 2 porphyrins of a 4th trimer, with the 3rd porphyrin dangling in space.

Another porphyrin array that was synthesised from dendronised porphyrins, in which a porphyrin is the centre of the dendrimer has been reported by Zimmerman c.s.\[33\] By incorporating tin in the porphyrins, these macromolecules could be connected using succinic acid as a bridging bidentate ligand (Figure 5). The exterior of the obtained porphyrin arrays was covered with homoallyl groups, which were polymerised to rigidify the dendritic shell. However, the porphyrins were removed again by a transesterification reaction to obtain a hollow nanocylinder, thus no studies were performed on the porphyrin array. This example shows the use of porphyrins as mere building blocks for the preparation of one dimensional objects.

Another non-covalent multi-porphyrin assembly was synthesised by Mihara and co-workers.\[34\] They developed amphiphilic polyamidoaminedendrimers terminated with α-helical peptides, which were capable of co-ordinating Fe(II) and Zn(II) porphyrins. Every porphyrin was bound by two α-helical peptides to form a multi metalloporphyrin array.

**Figure 5** a) Computer generated model of a hexameric tin-porphyrin dendrimer bridged by succinate ligands, side view. b) Idem, top view.

Another non-covalent multi-porphyrin assembly was synthesised by Mihara and co-workers.\[34\] They developed amphiphilic polyamidoaminedendrimers terminated with α-helical peptides, which were capable of co-ordinating Fe(II) and Zn(II) porphyrins. Every porphyrin was bound by two α-helical peptides to form a multi metalloporphyrin array.
2.3.3 Polymers with porphyrin side-groups

Daub and co-workers have recently reported on porphyrin appended cellulose polymers, in which the cellulose acts as the scaffold for the organisation of these dyes.\[35\] Cellulose has a helical structure and forms superhelices in the solid state. The degree of porphyrin substitution amounted to ca. 0.4. The absorption spectrum revealed a slight broadening of the Soret band and CD spectroscopy showed a helical arrangement of the porphyrins. Despite the elegant, natural helical scaffold, only poor interactions between the porphyrins could be realised.

A polyisocyanide was used as scaffold for the organisation of porphyrins already in 1985 by Nolte. It was attempted to produce a catalytically active polyisocyanide covered with porphyrins through grafting of these molecules onto the polyisocyanide backbone.\[36\] This resulted, however, in uncomplete coverage, hence no completely homogeneous porphyrin stack was obtained. Takahashi et al. developed a Pd-Pt $\mu$-ethynediyl dinuclear catalyst for the polymerisation of arylisocyanides.\[37, 38\] This catalyst induces a living polymerisation reaction, which results in precisely controlled molecular weights and the possibility to synthesise block-copolymers. Using this catalyst they polymerised porphyrin phenyl isocyanides and obtained a polymer with ca. 100 attached porphyrins (Figure 6a).\[39\] Variation of the monomer-catalyst ratio from 20:1 to 100:1 showed an increase in the absorption spectrum of a band at 400 nm (Figure 6b), showing that exciton interactions exist between the porphyrins. They did, however, not study the conformation of the porphyrins in the polymer.

![Chemical structure of a porphyrin pendant polyisocyanide.](image)

**Figure 6** a) Chemical structure of a porphyrin pendant polyisocyanide. b) UV-Vis absorption spectra of the porphyrin phenyl isocyanide monomer and the polymer with increasing number of monomeric units.

Using both zinc porphyrin (Zn) and free-base porphyrin (FB) substituted isocyanides, a triblock copolymer with composition Zn$_{10}$-FB$_2$-Zn$_{10}$ was synthesised.\[40\] This block copolymer system displayed energy transfer from Zn to FB with an efficiency of 35%.
The authors not only applied the polyisocyanide polymer as a scaffold for the porphyrins, but they also used the porphyrins to obtain information about the helical sense of the polyisocyanide. A triblock copolymer was synthesised with a central block of only two porphyrins and side blocks containing enantiopure menthylic side chains. The menthylic groups caused the formation of predominantly one helical form of the polymer, which was transferred to the central part. With the two porphyrins incorporated in the helix as spectator groups, the handedness of the polymer could be determined using the exciton chirality method.

2.3.4 Self-assembled porphyrin arrays

Kobuke c.s. have prepared imidazole-substituted zinc gable-porphyrins that form a mixture of oligomers containing linear and cyclic structures with a broad distribution of molecular weights. The diverse structures in the mixture could be reorganised to yield exclusively cyclic hexamers (Figure 7a) using the following ‘reorganisation principle’: a range of different structures is first formed in CHCl₃, subsequently, the coordination bonds between the imidazole and the zinc-porphyrins in the mixture are partly broken by addition of MeOH. After evaporation of the solvent at 25°C and redissolving in CHCl₃, the cyclic hexamers were obtained, as was concluded from GPC. The synthetic ring is reminiscent of the natural B850 ring with respect to imidazolyl-to-metal co-ordination, arrangement of the dimeric units in the macro ring, and the distance and orientation of the chromophores.

**Figure 7** a) Structural model of a self-assembled cyclic hexamer composed of 6 porphyrin dimers that is formed through axial co-ordination of imidazolyl groups to Zn, mimicking the natural B850 ring. b) Schematic structure of a self-assembled monolayer of Ga porphyrins, capable of electron transport upon photoillumination.
Based on the same imidazolyl porphyrin building blocks Kobuke et al. have also synthesised gadolinium porphyrins that self assembled in solution into a staircase arrangement. These porphyrins could be deposited on surfaces and the resulting structures had the same staircase structure (Figure 7b). When the porphyrins were processed as thin films, enhanced conductivity (ca. 5x) was measured when they were photo excited.

Drain et al. have reported on the self-assembly of a 21-component square planar porphyrin nonamer. The arrays are composed of four side porphyrins, four corner and one central porphyrin, allowing the use of three different (metal) porphyrins, each placed in the predetermined site of the supramolecule (Figure 8a). The aggregation process is dynamic, proceeding through several intermediate stages. The initially formed kinetic products are aggregates with dimensions of ca. 30 nm, which subsequently dissociate and reorganise to the thermodynamic product that is ca. 6x6x6 nm tall. By controlling a number of parameters, viz. the appended alkyl groups, solvent, temperature, porphyrin metal ion and surface chemistry when the squares are deposited on surfaces, the size and shape of the aggregates can be tuned. Once deposited on a surface, the aggregates showed to be stable for more than a year in ambient conditions. This stability is a key parameter in the development of nanoscaled devices.

\[ \text{Figure 8} \ a) \text{ Self-assembled 30 component metalloporphyrin nonamer which can further self-organise into a stack (not shown), of which the size can be tuned.} \ b) \text{ Schematic drawing of a porphyrin array that spans a lipid bilayer membrane and is capable of mediating electronic conduction between an electron donor D and an acceptor A after photoillumination.} \]
Drain has also used zinc porphyrins with hydrogen bonding groups to form porphyrin arrays that could be incorporated into lipid bilayer membranes and could function as photo-gated conducting channels (Figure 8b).

The porphyrins have either two diacetamidopyridyl groups or two uracil groups in opposite positions attached, which allows the formation of linear hydrogen bonding porphyrin arrays. These arrays will be preferentially formed inside the bilayer, the thickness of the latter also determines the size of the arrays. The membrane was spanned between a teflon partition, forming a two compartment cell separated by the membrane. One side contained a buffered solution with an electron donating species ($K_4Fe(CN)_6$) and the other one an acceptor species (anthraquinone sulphate), leading to an electrochemical potential. Upon photoillumination photocurrents that are solely due to the organised porphyrin assembly were measured. Control experiments with any of the individual porphyrins mixed into the bilayer-forming solution showed virtually no observable trans membrane photocurrent.

A well-known method to form large porphyrin aggregates is through (bio)polymer templation. A recent example was given by Koti et al. who described for the first time the formation of well defined porphyrin $J$-aggregates by the self-assembly of anionic porphyrins ($meso$-tetrakis-(4-sulfonatophenyl) porphyrin dianion, $H_4TPPS_4^{2-}$, pH=3), on a polylsine template. Only $\sim 1 \mu M$ of polylsine was needed for $J$-aggregate formation. Monomeric lysine residues were far less efficient in inducing aggregation (Figure 9a). Another type of nanorods, also constructed from ($H_4TPPS_4^{2-}$), was developed by De Paula and co-workers. They demonstrated that the diacid form of tetrakis(4-sulfonatophenyl)porphine self-assembles into rod-shaped $J$-aggregates in aqueous solutions containing an electrolyte, without the need of any assembly template or scaffold. These type of $J$-aggregates showed very similar absorption spectra. They even managed to construct aggregates of only 3.8 nm thick (Figure 9b,c).

![Figure 9](image)

**Figure 9** a) AFM image of the $J$-aggregate of TPPS formed after addition of poly-$L$-lysine. b) AFM image of a single TPPS rod obtained after immersing $H_4TPPS_4^{2-}$ in 0.3 M HCl (30 s). c) Idem, after immersing for 1h, resulting in the formation of bundles.
Balaban c.s. have developed porphyrins with different pyrimidine side-groups that self-organise into supramolecular porphyrin arrays. Introduction of one such group at the meso position of a zinc porphyrin resulted in the formation of a rigid tetramer. Attaching two pyrimidines in opposite meso positions led to the formation of extended aggregates, of which the structures were held together by hydrogen bonding, steric interactions and π-π interactions built into the monomeric species.\textsuperscript{[50]}

Shinkai\textsuperscript{[51]} et al. designed and synthesised porphyrins with ethyne bridged pyridyl groups. These compounds form co-ordination complexes with Pd, and upon addition of cis-Pd(II) capsules\textsuperscript{[52]} or extended linear compartmentalised aggregates (Figure 10a)\textsuperscript{[53]} were obtained, depending on the number of pyridines (4 and 8, respectively) present. When a chiral cis-Pd(II) complex (Figure 10b) bearing a BINAP ligand (\((R)-(+)\)-2,2′-bis(diphenylphosphino)-1,1′-binaphthyl) was used, the porphyrin assemblies displayed a chiral twist as was concluded from CD spectroscopy. Interestingly, the addition of the chiral cis-Pd(II) complex to these porphyrins induced a co-operative change in the CD signal, reminiscent of the Sergeants-and-Soldiers effect described by Green.\textsuperscript{[54]}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig10.png}
\caption{a) Structure of a linear compartmentalised porphyrin aggregate, which displays a chiral twist when the chiral cis-Pd(II) BINAP complex is used. b) Structure of the chiral cis-Pd(II) BINAP complex.}
\end{figure}

2.4 Perylenes

Perylenes are very stable, pentacyclic aromatic pigments that show strong fluorescence. The perylenes studied in this thesis are diimides of perylene-3,4,9,10-tetra(carboxylic acid. They form a group of environmentally stable and photo-stable, highly absorbing dyes with strong fluorescence that can reach unit quantum efficiency.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{fig8.png}
\caption{Chemical structure of perylene bisimide.}
\end{figure}
The high electron affinity of the molecules, together with the aforementioned properties makes them ideal candidates in photovoltaic devices. The absorption spectra of perylenes are composed of a \( \pi-\pi^* \) transition with large oscillator strength at 425 nm displaying several vibronic bands, and n-\( \pi^* \) transitions situated around 350 nm, which are not observed because they virtually have no oscillator strength.[55]

In light of applications in solar cells, it is desirable to have the perylenes organised in a well-defined manner over large distances (i.e. hundreds of nanometres). This facilitates charge migration and hence improves the efficiency of energy conversion. The tendency of the perylene molecule to self-assemble in solution can be used in this respect. Liquid crystalline perylene diimide derivatives so far have shown the highest charge carrier mobilities observed for discotic materials.[56] The liquid crystalline phase facilitates the processibility of the compounds.

Gregg and co-workers have studied self-assembly of perylene molecules in monolayers and thin films in light of their possible application in solar cells.[57-59] It turned out that, irrespective of the way the perylenes were deposited, in all instances they were found to align in self-assembled stacks parallel to the surface.

Antonietti et al. have used the concept of “ionic self-assembly” in which exceptionally high order of molecules can be reached by means of complexation of charged species with oppositely charged surfactants.[60] They synthesised perylenediimid-surfactant complexes (Figure 12) that assembled into highly ordered thermotropic liquid crystalline materials.

![Chemical structure of the complex of N,N'-bis(2-(trimethylammonium)ethylene)-perylene-3,4,9,10-tetracarboxyldiimide with dihexadecyl phosphate.](image)

Würthners group has synthesised thermotropic liquid crystals that formed fluorescent J-type aggregates by attaching 3,4,5-tridodecyloxybenzene groups to the nitrogen atoms of perylenes (Figure 13a).[61] The solubility of these perylene derivatives could be drastically increased when tetra(3-tert-butylphenoxo) substituents were attached to the bay positions of the perylene.[62] It was also possible to form supramolecular polymers via hydrogen bonding between perylene units and isophthalic acid (Figure 13b).[63] The assembly is hampered however, when bulky tert-butyl groups are present at the bay positions instead of phenyl groups.
Chapter 2

![Chemical structure of a perylene bisimide with 3,4,5-tridodecyloxyphenyl groups that forms a thermotropic liquid crystalline phase](image)

Figure 13 a) Chemical structure of a perylene bisimide with 3,4,5-tridodecyloxyphenyl groups that forms a thermotropic liquid crystalline phase. b) Ditopic diazadibenzoperylenes can form extended hydrogen bonded polymeric chains with isophthalic acid derivatives.

Perylenes can also be organised over large distances by using imide functionalities that form triple hydrogen bonds with melamine. This allows the construction of large chiral assemblies with chiroptical properties (Figure 14).[64]

![Intrinsically, perylene bisimides have hydrogen-bonding functionalities (grey), which can lead to extended assemblies when complementary melamine derivatives (b) are added, or (c) oligo(p-phenylene vinylenes) containing ureidotriazine hydrogen-bonding units.](image)

Figure 14 a) Intrinsically, perylene bisimides have hydrogen-bonding functionalities (grey), which can lead to extended assemblies when complementary melamine derivatives (b) are added, or (c) oligo(p-phenylene vinylenes) containing ureidotriazine hydrogen-bonding units.

Schenning et al. have reported the construction of large helical fibres (Figure 15) built up from a 1:2 complex of a perylene and oligoparavinyleneephendylene (OPV) moieties (Figure 14c), which organise into chiral J-stacks, as was concluded from CD spectroscopy.[65]

![AFM image of a spin-coated solution of the 1:2 perylene:OPV complex.](image)

Figure 15 AFM image of a spin-coated solution of the 1:2 perylene:OPV complex.
Since the OPV is a good electron donor, they could induce electron transfer from the OPV to the perylene after photoexcitation of the OPV, although no subsequent electron transport was reported.

In a similar system electron transfer was reported by Wasielewski and co-workers.\cite{66} They synthesised a large molecule in which four perylenes (Figure 16a), that both collect photons and accept electrons, are attached to a central zinc porphyrin, which acts as electron donor. This molecule was found to self-assemble into ordered nanoparticles (Figure 16b), primarily as the result of strong Van der Waals interactions. After photoexcitation of the nanoparticles, an ultrafast charge separation (3.2 ps) occurred and an electron was transferred from the zinc porphyrin core to the peripheral perylenes. The charge separation was much faster than in model compounds having only one perylene connected to the porphyrin. The electron migrates between several closely coupled electron acceptors over a distance of minimally 21 Å from the porphyrin. That means that at least six perylenes are involved in the site-to-site hopping of the electrons. This process resembles dye-sensitised charge injection into semiconductors.

![Chemical structure of perylene appended zinc porphyrin (a) that forms nanoparticles in solution (b).](image)

A new class of tetra-N substituted perylenes was introduced by Gade and co-workers.\cite{67,68} The tetra(amo)inyperelenes have highly negative oxidation potentials, which makes that they are not stable in air. By transforming them into carboxamides they could be stabilised (Figure 17a). These compounds aggregate in DMSO at high concentrations (10^{-3} M) as evidenced from the red shifted and broadened absorption spectra. AFM revealed that these aggregates were nano-sized crystalline rods, with lengths between 100 and 400 nm, widths of 40 nm, and a height of 2 nm (Figure 17b). While the length could be tuned by concentration, the thickness remained unchanged. The ability to tune the size of photo active perylene species is important for use
Chapter 2

and optimisation in devices. The only problem that needs to be tackled is increasing the fluorescence quantum yield.

\[ R = \text{fBu, 4-fBuPh} \]

**Figure 17** a) Chemical structure of tetra(carboxamido)perylene. b) AFM image of a solution of this perylene in DMSO (10^{-3} M) deposited on mica (dashed bar = 1 µm).

A thermophilic foldable polymer was synthesised composed of alternating DNA and perylene parts.\textsuperscript{[69]} This polymer displayed an improved ordering upon increasing the temperature, while normally, biopolymers lose their function at high temperature due to unfolding. This effect is the result of the aggregation of the perylenes, which is favoured by endothermic hydrophobic interactions between the perylenes, counteracting the exothermic hydrogen bonding interactions between the DNA bases at high temperatures. The folding of the perylene units could be followed by recording the concomitant colour change. After addition of complementary DNA strands, the perylene π-stacking was disrupted due to molecular recognition between the complementary strands. This process could be easily followed by optical spectroscopy and opens the way to DNA biosensors based on colour change.

### 2.5 Polyisocyanides

Polyisocyanides are obtained by polymerisation of isocyanide monomers. It was Millich who developed the first catalyst for the polymerisation of isocyanides: acid coated glass.\textsuperscript{[70]} Subsequently, Nolte *et al.* performed systematic studies on the use of transition metals as polymerisation catalysts of isocyanides and found that particularly Ni(II) was efficient to convert isocyanides into sterically crowded polymers (Figure 18a).\textsuperscript{[71]}

The backbone of a polyisocyanide is constructed solely from carbon atoms. Due to the fact that every carbon atom has a substituent, considerable steric hindrance is introduced. To minimise the steric crowding, the C-C bonds make a twist and adopt a helical structure. Millich proposed, on the basis of theoretical models, a 4\textsubscript{1} helical conformation (*i.e.* 4 repeat units per helical turn) for the main carbon chain in polyisocyanides.\textsuperscript{[70]}
2.5.1 Polyisocyanide conformation

The stability of the helix is dependent on the size of R. With R being t-butyl the helical conformation is completely locked because of the large steric interactions between the side groups. This polymer was separated by Van Beynen on a chiral column into the two optical antipodes, the P- and M-polymer. Although no chiral centres were present in this polymer, it displayed optical activity, proving the helical conformation.\textsuperscript{[72]} The helical structure is maintained even at elevated temperatures.\textsuperscript{[73]} When, however, the side groups are sterically less demanding, the helical conformation cannot be maintained, as was shown by theoretical models,\textsuperscript{[74, 75]} and by experiment.\textsuperscript{[76]} Theoretical studies have shown, that, when R = ethyl, several helical conformations exist, although the helical shape itself stays intact, while for R = H, depending on the calculation method, a disordered structure or a broad range of helical conformations are predicted.

In an experiment by Rosen \textit{et al.} poly (phenyl isocyanides) were shown to slowly loose their helical conformation in solution and to precipitate as random coil polymers. This shows that the most important factor governing the helical structure indeed is steric hindrance. Iyoda \textit{et al.} have performed similar studies on polyisocyanides derived from phenylalanine.\textsuperscript{[77]} Their study substantiated the idea that bulkiness is important in stabilising the helical conformation. By fine-tuning the molecular structure they found a polymer that showed reversible behaviour upon temperature. Yashima and co-workers have shown that poly (4-carboxyphenyl isocyanide) also shows these conformational changes and suggested that it exhibits a prochiral conformation, \textit{viz.} a zig-zag structure. This polymer was found to adopt a dynamic helical conformation when chiral amines were added.\textsuperscript{[78]}

An alternative conformation for polymers of isocyanides was suggested by Salvadori \textit{c.s.}\textsuperscript{[79]} This conformation predicts more accurately the CD spectral data observed for these polymers. This so-called \textit{syndio} conformation has the absolute minimum energy conformation, whereas the $4_1$ conformation was calculated to be a local minimum (Figure 19b).
2.5.2 Polymerisation mechanism

The first step in the polymerisation of isocyanides using a Ni(II) catalyst is the formation of a square-planar complex in which four isocyanides coordinate to the Ni(II) centre (Figure 20a). This complex can be isolated if bulky isocyanides are used (for example t-butyl isocyanide). A nucleophile, acting as initiator, coordinates to the Ni centre (b) and migrates to one of the isocyanides forming a carbene-like intermediate (c). Also this species can be isolated provided that the isocyanides are bulky. The carbene is now nucleophilic enough to attack its neighbour, which in turn becomes the nucleophile (d). The vacant position is occupied by an isocyanide from solution.

The mechanism presented in Figure 20 is called the merry-go-round mechanism.\textsuperscript{[71]} It plausibly explains some properties, like the helical shape and the ease of formation of a tightly coiled
helix (the Ni centre preorganises the isocyanides, which need hardly any further reorganisation to be incorporated into the polymer backbone). The actual mechanism may be more complicated but will not be discussed here.\textsuperscript{80}

Another efficient catalyst for the polymerisation of isocyanides is the Pd-Pt \(\mu\)-ethynediyl dinuclear complex, as was discussed in § 2.3.3. Iyoda \textit{et al.} have used two subsequent living polymerisation techniques to prepare polyisocyanides that are densely grafted with polystyrene and polystyrene-\textit{block}-polybutylacrylate.\textsuperscript{81} This was achieved by using their Pt-Pd complex catalyst to synthesise (co-) polyisocyanides with Br-functional side groups, from which an ATRP reaction (atom transfer radical polymerisation) could be performed (the ‘grafting from’ method), or by polymerising a polystyrene with an isocyanide end group (the ‘grafting through’ method). The molecular weights as calculated from GPC were much lower than expected, which was ascribed to the differences in hydrodynamic volumes between the rodlike polyisocyanide derivatives and the coil-like polystyrene standards.

Takahashi \textit{et al.} have prepared polyisocyanides with ferrocenyl groups.\textsuperscript{82} Using the Pd-Pt \(\mu\)-ethynediyl dinuclear complex, they succeeded in synthesising chiral ferrocene-containing polyisocyanides that exhibited a sharp response when an electrical stimulus was applied, generating a reversible conformational change, which is unique in responsive polymers. To prevent precipitation of the cationic ferrocenium species onto the electrode, a long aliphatic chain was introduced in the side chains. This modified polymer showed reversible changes in the absorption and CD spectra upon electrolytic redox and chemical oxidation, which caused repulsion between the ferrocenium cations in the side chains. This made the helix undergo a conformational change to a disordered polymer.

### 2.5.3 Stereochemistry of polyisocyanides

The stereochemical outcome of the polymerisation of isocyanides can be controlled with the group R in the monomer. Using enantiopure R*NC, one can produce the P (right handed) or the M (left handed) helix in excess, thus generating one of the possible diastereoisomeric products: (P)-(R), (M)-(S), (P)-(S), (M)-(R), of which the first two are enantiomers. The carbene like species reacts preferentially with either left or right neighbour, thereby fixing the handedness of the helical polymer. Stereoselectivity can also be obtained by using a chiral initiator or a sterically demanding chiral co-monomer, that slows down the formation of either the P- or the M-helix.
Chapter 2

2.5.4 Polyisocyanides derived from peptides

A new area in polyisocyanide chemistry was opened with the introduction of peptides as side groups of polyisocyanides by Cornelissen et al.,\cite{83,84} following earlier work by Van Beynen on aminoacid derived polyisocyanides. Cornelissen used dipeptides derived from alanine, glycine, and histidine, and synthesised different polyisocyanides (Figure 21). The introduction of peptides has interesting stereochemical consequences, and, in addition to this, it allows the formation of intramolecular hydrogen bonds between side chains \(n\) and \(n+4\) of the polymer (see below). Depending on the configuration of the constituting amino acids, in this way a highly defined conformation of the helical backbone could be obtained.

![Figure 21 Chemical structures of some dipeptide derived polyisocyanides synthesised by Cornelissen et al.](image)

These peptide polymers and their precursors were studied with the help of IR spectroscopy, which very clearly revealed the presence of the predicted hydrogen bonding arrays in the polymers.\cite{85} Some data are presented in Table 1. The isocyanide monomers IAA (isocyanoalanyl-alanine methyl ester) all showed a shift of the amide I and NH stretch vibrations to lower wavenumbers going from solution to the solid state, which is caused by the formation of hydrogen bonding arrays in the latter state, as was confirmed by X-ray analysis of crystals of \(L,L\)-IAA.\cite{86} The polymers on the other hand, showed amide I vibrations and N-H stretch vibrations at wavenumbers that are in agreement with the formation of hydrogen bonds between amide groups in side chains \(n\) and \(n+4\). For \(L\)-PIAG two N-H stretching vibrations were observed in CHCl\(_3\) at 3367 and 3298 cm\(^{-1}\), showing that in this case hydrogen bonding is only partly realised. NMR studies substantiated the formation of hydrogen bonds, except for PIAG, which showed a significantly smaller shift of the NH resonance when compared to other polymers. Optical rotations expressed per repeat unit, are one order of magnitude higher for PIAA’s and PIGA than for the corresponding monomers, which is suggestive of the formation of a helical secondary structure. No amplification of chirality was observed upon polymerisation of \(L\)-IAG, suggesting that the only partly formed hydrogen bonding arrays in \(L\)-PIAG are insufficient to secure the helical structure of this polymer. It is interesting to note that also PIGA showed amplification of chirality proving that this polymer has a secondary helical structure. This is remarkable, since the chiral centre is only present in the second amino acid.
Generally, polyisocyanides display an imine \( n-\pi^* \) absorption band at 250-350 nm.\(^{[87]}\) The CD spectra of these hydrogen bonded polymers all showed the same characteristic Cotton effect around 310 nm (Figure 22a).\(^{[88]}\) The highly oriented amide dipoles affect the \( n-\pi^* \) transitions, causing the observed particular shape of the CD spectrum, which made it difficult to establish the handedness of the helix. By attaching a diazochromophore to the polymer, which acts as a spectator group, the handedness could, however, be determined. The chromophores formed a right-handed helix, from which it was concluded that the polymer backbone was also right handed.\(^{[89]}\)

When PIAAs were heated to 55°C, a slight decrease in the CD intensity of the Cotton band at 309 nm was observed, which partially was restored after cooling. This effect was ascribed to a disruption of the hydrogen bonding arrays in the polymer chains. For entropic reasons the well-defined starting position was not regained,\(^{[88]}\) probably because of the sterically demanding substituents, which prevents a refolding. IR studies revealed that hydrogen bonds were only partially left after heating for longer periods.

The rigid character of polymers of isocyanopeptides is reflected in the formation of a nematic liquid-crystalline (LC) phase. For instance, a concentrated (10% w/w) solution of \( \text{L,L-PIAA} \) in CHCl\(_3\) displayed birefringence when visualised between crossed polarisers (Figure 22b).\(^{[88]}\)

The characteristic fingerprint texture points to a cholesteric arrangement of the macromolecules as observed before for concentrated solutions of stiff helical (bio)polymers.

It was found that in some cases, polymerisation of isocyanides can even be performed with \( \text{H}^+ \) as a catalyst.\(^{[86]}\) This turned out to be possible when the monomers possess the optimal configuration for hydrogen bonding and the polymers display only weak steric interactions between the side chains, leading to a swift polymerisation.

### Table 1
Overview of the different IR absorptions (cm\(^{-1}\)) of monomers and polymers of different dipeptide isocyanides ("split signal; average values are given, "broad band).  

<table>
<thead>
<tr>
<th></th>
<th>Monomer</th>
<th>Polymer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \nu_{\text{NH}} ) (CDCl(_3))</td>
<td>( \nu_{\text{NH}} ) (KBr)</td>
</tr>
<tr>
<td>( \text{L,L-IAA} )</td>
<td>3430(^{a})</td>
<td>3279</td>
</tr>
<tr>
<td>( \text{L,D-IAA} )</td>
<td>3410</td>
<td>3504</td>
</tr>
<tr>
<td>( \text{L-IGA} )</td>
<td>3425(^{a})</td>
<td>3256</td>
</tr>
<tr>
<td>( \text{L-IAG} )</td>
<td>3436(^{a})</td>
<td>3286</td>
</tr>
</tbody>
</table>
The hydrogen bonding arrays between the alanine residues n and n+4 in the side chains are shown in Figure 23a. Normally the optimal distance between the side-groups of a polyisocyanide is 4.2 Å. Strongest hydrogen bonding, however, occurs when the distance between the amide groups is 4.7 Å.[88] The ideal conformation of the helix, therefore, is not expected to be exactly ‘four-over-one’ as is observed for normal polyisocyanides, but slightly different, in order to allow for optimal hydrogen bonding.

The projection angle $\alpha$ between the n$^{th}$ and the n+4$^{th}$ side group (Figure 23b) can be calculated on the basis of the crystal structure of L,L-IAA. The distance between the middle of the backbone and the first amide is 5.4 Å.[90] From equation 1, $\alpha$ can be obtained, which amounts to $\alpha \sim 20^\circ$. This results in either 3.8 or 4.2 monomer units per turn.
2.5.5 AFM studies on polyisocyanopeptides

The size (distribution) of the polymers of isocyanopeptides could not be determined with conventional techniques like GPC or Maldi-TOF, while for other polyisocyanide polymers this had been possible. The supposed rigidity of the polyisocyanopeptides was confirmed by atomic force microscopy (AFM). This technique allows the observation of single polymer chains on a surface. The AFM study showed that the polymers are indeed very rigid. Normal (random coil) polymers are usually visible as large blobs on the surface, but the polyisocyanides can be distinguished as single molecules due to their rod-like appearance. Using this technique, the polymer contour lengths and dispersity could be determined. As an example, single polymer fibres of L,D-PIAA spin-coated from a chloroform solution onto mica are shown in Figure 24. Next to this, it was possible to determine the persistence length of the polymers by following the contours of the isolated polymer chains, as indicated by the bright white lines in Figure 24, drawn on polyisocyanide fibres in the AFM image. Previous solution measurements on non-hydrogen bonded polyisocyanides indicated a persistence length of 2-3 nm, while in this case a persistence length of 76 nm was measured.[91]

\[
\alpha = 2 \arcsin \left( \frac{\sqrt{(4.7)^2 - (4.2)^2}}{5.4} \right)
\]  

*eqn 1*

![Figure 24](image)

*Figure 24* AFM image of a spin-coated solution of L,D-PIAA in CHCl3. The bright contours (arrow) are due to the technique that was used to determine the contour length.

This number is in the same order of magnitude as the persistence length of ds-DNA (53 nm), which is considered to be a rigid rod-like macromolecule. Important for determining the persistence length of the polyisocyanopeptides is the fact that the molecules are equilibrated on
the mica surface to resemble the conformation in solution,[92, 93] which was proven to be the case.

2.6 References

Literature survey


The distance between the isocyanide carbon and the first amide was calculated from the co-ordinates of the atoms obtained from the crystal structure of the L-isocyanoalanyl-L-alanine methyl ester. The distance between the middle of the backbone and the isocyanide carbon was calculated to be 0.76 Å. The C=\(\text{N}\) distance in the helix was taken 1.268 Å.
Chapter 3

Porphyрин Pendant Polyisocyanides
Derived from Alanine

3.1 Introduction
Numerous synthetic multiporphyrin arrays have been developed as simple mimics of the natural antenna complexes. In general these arrays were designed to have a predefined organisation of the chromophores and as a consequence of the architecture they showed unique optical, electrical or electrochemical properties.\(^1,2\) In the majority of cases the construction of these arrays is achieved by stepwise covalent synthesis or via non-covalent, \textit{i.e.} supramolecular procedures in which secondary interactions are set in to form larger constructs. The latter approach usually involves a modular route which utilises covalent synthesis to construct the basic building blocks, and non-covalent synthesis to self-organise these building blocks to form the desired architectures. Both approaches have resulted in multi-chromophoric systems in which the porphyrins are organised into one, two or three dimensional arrays like fibres, circles, boxes, \textit{etc.}

Polymers (a covalent way of modular synthesis) have also been used to arrange porphyrin molecules, but these systems were either not designed to display energy transfer,\(^3-5\) or were invariably found to exhibit only limited order over small distances, \textit{i.e.} no more than a few nanometres.\(^6,7\)

It can be envisaged that, if the porphyrins are organised on a well-defined macromolecular scaffold, energy transfer is possible over larger distances. An intrinsically rigid polymer with porphyrin side groups would be ideal to achieve this goal. This concept was already tested by
our group, viz. by grafting the porphyrins onto a polyisocyanide backbone. This grafting, however, does not result in well-defined arrays and consequently, the resulting system lacked the long-range ordering due to defects.[8]

Recently, it was discovered that polyisocyanides prepared from isocyanodipeptides form exceptionally stable $\beta$-helical architectures due to the presence of hydrogen bonding arrays along the helical polymer backbone.[9] This secondary H-bonding network rigidifies the helix and prevents unwinding, a problem previously encountered with polyisocyanides.[10, 11] In addition, the chiral centres, present in the amino acid side chains, determine the handedness of the polymeric backbone. The similarity between these polyisocyanides and their natural counterparts ($\alpha$-helices, $\beta$-sheets and $\beta$-helices) suggests that they would be ideal scaffolds for the construction of mimics of the light harvesting antenna complexes.

The goal of the study presented in this chapter is to synthesise well-defined porphyrin arrays using polypeptide-like polyisocyanides as the macromolecular scaffold and to obtain efficient energy transfer over large distances (>100Å), as observed in the natural light harvesting systems.

In this chapter the synthesis and characterisation of a variety of different porphyrin isocyanide monomers are described and the study of their polymerisation with a nickel (II) catalyst.

3.2 Results and discussion

3.2.1 Synthetic strategy for porphyrin appended polyisocyanides

The initial synthetic strategy which was used is based on the incorporation of an amide bond in the spacer between the polyisocyanide backbone and the porphyrin in order to obtain a hydrogen bonding array along the polymer backbone, which will rigidify the polymer helix. Amino acids can be readily coupled using many simple coupling agents to form peptide bonds. In addition, the chirality present in these compounds may lead to chiral polymers, which may be homochiral if enantiopure amino acids are used.

Previous studies have shown that the choice of the amino acid is important for the conformational stability of the polymer.[12] Studying several alanine and glycine dipeptide derived polyisocyanides, Cornelissen showed that the ability to form hydrogen bonding arrays alone, is not enough to provide a well-defined structure: steric interactions also play a role in the architecture of the resulting helix.[13] It has been shown before that polyisocyanides lacking hydrogen bonds still can form stable helical structures if the side groups are bulky enough (see:
Chapter 2). It was, therefore, decided that the first amino acid (next to the isocyanide) should be L-alanine since it is chiral and has sufficient steric bulk.

\[
\begin{align*}
\text{Figure 1} & \quad \text{Target porphyrin pendant isocyanide in which the chiral centre provides the stereochemical control of the polymerisation, the amide bond the stabilising hydrogen bonding interactions, and the R group the solubility of the resulting polymer.}
\end{align*}
\]

The chemical structure of the porphyrin and the spacer between the alanine and the porphyrin can both be varied (Figure 1). To form the amide bond, either a second amino acid can be coupled to the first alanine, or an amino-functionalised porphyrin can be used. These approaches will be discussed in the next sections, with each paragraph describing the synthesis and characterisation of a particular polymer. The target polymers are shown in Chart 1.

\[
\begin{align*}
\text{Chart 1 Overview of the synthesised polymers.}
\end{align*}
\]
3.2.2 Polymer 23

Synthesis

The first approach to synthesise a polyisocyanide with pendant porphyrins is based on the existing poly (L-isocyanoalanyl-L-alanine methyl ester L,L-PIAA), which has been studied extensively in the Nijmegen group (see also Chapter 2). The structure of the target polymer 23 is shown in Chart 1. In contrast to the previously discussed PIAA’s the methyl group is replaced by a porphyrin. For the synthesis of the starting isocyanide 4 (Scheme 1), monohydroxyporphyrin 1 was prepared by the Adler method[14, 15] and coupled to dipeptide 2 using dicyclohexylcarbodiimide (DCC) and N,N-dimethyl aminopyridine (DMAP) as coupling reagents. During this reaction which leads to 3, two spots appeared on tlc (vide infra). The corresponding two products could be separated by column chromatography (silica gel) and the lower running product 3 (Rf = 0.17) was obtained pure.

Scheme 1 Synthesis of isocyanide monomer 4.(i) DCC/DMAP, CH₂Cl₂, 0°C; (ii) POCl₃/NEt₃, CH₂Cl₂, -5°C.

The conversion of 3 (the pure, lower running fraction on tlc) into the isocyanide 4 was achieved by dehydration with phosphorous oxychloride and triethylamine as base. The polymerisation of 4 was performed in dichloromethane using 1/125 equiv. of Ni(ClO₄)₂·6H₂O as catalyst. After 4 days, i.e. after ca. 80% of the isocyanide had been consumed and no additional reaction was visible on tlc (the product appears as a spot with a red flame at the baseline) the polymerisation was stopped. The polymer was purified by precipitation in methanol and washing with acetone. The infra-red spectrum of the product showed a shift of
the NH stretching vibration from 3326 to 3320 (with a shoulder at 3315) cm\(^{-1}\), which is indicative of the presence of amides involved in hydrogen bonding, in line with what is known for polymers of L,L-IAA, that also show similar shifts. The hydrogen bonding interaction is however not optimal, since in that case a large shift of the NH stretching frequency of 24 cm\(^{-1}\) is expected, as seen for the polymers derived from L,D-IAA. The \(^1\)H-NMR spectrum of 23 showed significant broadening of the porphyrin and alanine signals due to the restricted rotation imposed by the polyisocyanide backbone, next to sharp signals originating from the end-groups of the polymer.

**Racemisation**

For the synthesis of compound 3, the formylated dipeptide was activated with DCC. However, this procedure may lead to epimerisation (viz. racemisation of one of the chiral centres of a diastereoisomer, see Scheme 2). To quantify the amount of racemisation that occurred during the reaction, optical rotation studies were initially carried out. However, these studies were found not to be reliable, since the porphyrins strongly absorb at the used wavelength of 589 nm (Na lamp), which decreases the intensity of the transmitted light. It was evident, however, that the two spots observed on tlc showed different signs in rotations, the higher running spot having a positive rotation and the lower running spot a negative rotation. This experiment was repeated using a wavelength of 436 nm (Hg lamp) and comparable results were obtained. This result, together with the fact that the mass spectra were identical for both products (M = 843 g·mol\(^{-1}\)), is indicative of the formation of two diastereoisomers. During the synthesis of compound 3, it was repeatedly observed that the lower running spot on tlc was formed immediately after addition of DCC, while the higher spot appeared somewhat later. This could indicate that the lower spot is the desired product L,L-3 and the higher spot its epimer L,D-3, formed in a ratio L,L:L,D ~ 3:1.

![Scheme 2](image_url)

*Scheme 2* Mechanism for the epimerisation of dipeptides with DCC.
Absorption spectroscopy

The absorption spectrum of isocyanide 4 resembled the spectrum of a normal free-base porphyrin with the maximum of the Soret band at 420 nm (Figure 2a). This maximum is expected since substitution of porphyrins on the phenyl position does not significantly influence the π-electron system of the porphyrin chromophore. The spectrum of polymer 23 (Figure 2a) was found to be significantly broadened with respect to the monomer and split, exhibiting two bands with maxima at 404 and 419 nm. The splitting of the Soret band and the occurrence of a new, hypochromically shifted band (Δλ = −15 nm) suggests the presence of excitonic coupling between neighbouring porphyrins. Exciton theory generally predicts a blue-shift for porphyrins that are stacked face-to-face as in H-aggregates, a red-shift for porphyrins in a side-to-side geometry and two bands for a herringbone structure of the chromophores (see Chapter 2). This would suggest that, in polymer 23, the porphyrins are organised in a face-to-face arrangement. Figure 2 also shows the evolution of the Soret band during polymerisation. The fact that the 420 nm band does not disappear upon washing indicates that it is not due to the presence of monomeric isocyanide. The intensity of the Q-bands (Figure 2b) increased during polymerisation and shifted to the red by 1 nm. The exciton interactions are much weaker in the Q-bands than in the B-band, which is the result of the weak intensities of these bands. The ratio between the Q and the B-bands (B/abs Q) decreased, going from the monomeric isocyanide to the polymer indicating that the porphyrins are arranged differently after polymerisation.

**Figure 2** Absorption spectrum of isocyanide monomer 4 (dashed line) and its polymer 23 (full line) in CHCl₃. The Soret band in the polymer is split and has broadened, while the intensity of the Q-bands has increased. The right spectrum shows the Soret band change during the polymerisation reaction (time in days): t=0 (dashed), t=2 (dotted), t=4 (full line).
The observed features in the absorption spectrum of polymer 23 are ascribed to a well-defined packing of porphyrin molecules along the polyisocyanide backbone, imposed by the regularity of the helical structure and the hydrogen-bonding network. This well-defined arrangement results in distinct exciton interactions between the porphyrins. If the porphyrins would have been attached to a random-coil polymer, the absorption spectrum would be broadened due to the presence of multiple conformations.[4, 5]

Circular dichroism spectroscopy

While isocyanide 4 did not show any detectable CD signal, the circular dichroism spectrum of polymer 23 displayed several Cotton effects, see Figure 3, which originate from the split porphyrin Soret band at 405 and 419 nm. A positive couplet can be detected corresponding to the absorption band at 419 nm. This couplet is obscured by other CD bands in this area. In the wavelength region of the Q-bands, no CD signal was observed. When the concentration was increased (ca. 15x), another positive Cotton effect became apparent at 310 nm. This is the same Cotton effect as described for PIAA, and arises from the n-π* transition of the imine chromophores, which appear in the 250-350 nm region in the absorption spectrum. The CD effect at 310 nm is regarded as a proof for a well-defined arrangement of the side groups in the polymer, which is induced by the hydrogen-bonding arrays.[11]

![CD spectrum of 23](image-url)

**Figure 3** CD spectrum of 23 (CHCl₃, conc: 7.6·10⁻⁶M): upon polymerisation, Cotton effects appear in the Soret band of the porphyrin. When the concentration is increased 15x, a positive Cotton effect in the imine n-π* transition becomes visible at 310 nm, reminiscent of the Cotton effects present in polyisocyanopeptides PIAA.

This exciton coupled CD spectrum shows that the polymer backbone forces the porphyrins into a helical arrangement resulting in a CD effect. This CD effect arises from the fact that the
Chapter 3

Chirality present in the alanine groups is transferred in a stepwise fashion to the backbone of the polymer and then to the more distant porphyrins (see Figure 4).

**Figure 4** Schematic representation of the transfer of chirality in polymer 23. The chirality is transferred from the chiral centres of the alanine groups (*) to the polymer (1) and then to the porphyrins (2). There is no direct chirality transfer to the porphyrins.

There is no direct transfer of chirality from the alanine functions to the porphyrin, since the monomer, upon aggregation, does not exhibit a CD effect.

**Atomic force microscopy (AFM)**

AFM measurements on 23, spin-coated from solution (CH$_2$Cl$_2$ or CHCl$_3$) on mica did not result in the observation of long fibres. Instead, islands of aggregated polymers were observed at relatively high concentrations (~10$^{-6}$ M; Figure 5a). Upon dilution (~10$^{-7}$ M) the islands were observed to break up into the constituent single polymers as shown in Figure 5b.

**Figure 5** AFM images of polymer 23 spin coated on mica. The polymers tend to aggregate in monolayer islands (a). No rod shape structures pointing to single polymers can be detected, probably because they are too short (bar = 500 nm).
Subsequent dilution did not result in any further changes. Although the bright spots are thought to be single rigid-rod polyisocyanides with pendant porphyrins, AFM suggests that they are very short, too short for their rod-like conformation to be observed. The polymer contour lengths are estimated to be maximally 5-10 nm.

Conclusions
Polyisocyanides derived from alanine-alanine dipeptides can be used as a ‘chiral framework’ to which porphyrins can be attached. IR showed that the amides are partially involved in hydrogen bonding arrays, in a similar fashion as seen for polymers of L,L-IAA. In the absorption spectrum a broadening and splitting of the Soret band appeared after polymerisation indicating that exciton interactions exist between the porphyrin molecules within a polymer. These exciton interactions are also manifested in the CD spectrum, which indicates that the porphyrins are arranged in a chiral fashion. Polymer 23 has a major problem, in a sense that it is prepared from optically impure starting material. The coupling of the two amino acids via an N-formyl functional group using carbodiimide chemistry resulted in significant epimerisation. Generally for diastereoisomers, this problem can be overcome by including a crystallisation step, like for L,L-PIAA,[12] but this was found not to be possible for porphyrins 3 or 4. To avoid racemisation, Boc-protected amino acids should be used in the coupling reactions.

3.2.3 Polymer 24
Synthesis
The synthesis of polymer 24 started by coupling L-alanine to 6. Porphyrin 6 was obtained by the reduction of the corresponding cyano porphyrin precursor 5. This reduction can be performed with either LiAlH₄ or BH₃·THF as reducing agent. The coupling was carried out under standard conditions, viz. using dicyclohexyl carbodiimid and dimethyl aminopyridin (DCC/DMAP) as reagents to give 7, which was subsequently deprotected with TFA to yield 8. This compound was then formylated to give 9 using the p-nitrophenyl ester of formic acid (for-ONP). The resulting product was converted into the isocyanide 10 using either phosphorous oxychloride as dehydrating agent with triethyl amine as base or diphosgene and N-methyl morpholine as base. The latter procedure was found to give a cleaner reaction and a higher yield (74% compared to 51%). Both reactions are usually carried out at -30°C to -15°C, but in the case of porphyrin 9, no reaction was observed until the temperature was raised to 0°C. Attempts to polymerise 10 with Ni(ClO₄)₂·6H₂O as a catalyst were unsuccessful. Although tlc indicated the formation of some initial product, no further reaction took place and even after
ten days the composition of the reaction mixture had not changed. IR spectroscopy revealed the presence of significant quantities of unreacted isocyanide 10. This observation indicates that the monomer is very sterically hindered and, therefore, reluctant to undergo polymerisation.

![Chemical Structures](image)

**Scheme 4** Synthesis of an isocyanide monomer derived from a porphyrin with a methyl alanyl spacer. (i) LiAlH₄ (or BH₃), THF; (ii) Boc-L-ala, DCC/DMAP, CH₂Cl₂, 0°C; (iii) TFA, CH₂Cl₂; (iv) Formyl-ONP, CH₂Cl₂; (v) POCl₃/NEt₃ (or diphosgene/NMM), CH₂Cl₂, -12°C (or 0°C respectively).

To check if 10 could be polymerised at all, a copolymerisation reaction was carried out with isocyano-L-alanyl-L-alanine methyl ester (IAA) in a ratio of 1:10 and 1:1 (10:IAA). In the first test reaction, all the isocyanide 10 had disappeared after one day (as witnessed by tlc). In addition, the reaction mixture had become viscous, indicating the formation of long polymer fibres, similar to that seen for the homopolymerisation of IAA. Note that homopolymerisation of IAA with Ni(II) takes only a few minutes. The fact that monomer 10 can only be co-
polymerised with a smaller monomer and the fact this copolymerisation is much slower than the homopolymerisation of pure IAA, indicates that 10 is very bulky. The observed longer reaction time of the copolymerisation is indicative of the incorporation of a sterically demanding porphyrin isocyanide which slows down the overall polymerisation rate.

In the second copolymerisation experiment a 1:1 molar ratio of 10 and IAA was used. The reaction was stopped after 4 days, since it was observed that isocyanide 10 had not been fully consumed and did not react any further. No increased viscosity was observed for this reaction mixture. The fact that not all the porphyrin monomer 10 was consumed, together with the fact that the viscosity of the reaction mixture did not increase upon polymerisation, indicates that the polymerisation becomes blocked at some point during the reaction.

**Absorption spectroscopy**

The polymerisation and copolymerisation reactions of 10 were also followed by absorption spectroscopy. The UV-Vis absorption spectrum of the reaction mixture of 10 and the nickel catalyst showed no change, even not after 8 days (Figure 6). The incorporation of 10 in a random copolymer (initial ratio of monomers IAA:10 = 1:10) resulted in a slight broadening of the Soret band and a hypsochromic shift of 2 nm, which is ascribed to weak porphyrin-porphyrin interactions. Furthermore, a shoulder was observed around 400 nm, which is comparable to what is visible in the spectrum of 23.

![Absorption spectrum](image)

*Figure 6* Absorption spectrum of monomer 10 (CHCl₃, conc: ~ 3µM, dotted line) and of its copolymer 24 with IAA (conc: ~ 4µM, full line). The polymer spectrum is broadened and has a shoulder around 400 nm, indicating weak exciton interactions between the porphyrins.
The changes in the absorption spectrum indicate exciton interactions, but whether the porphyrins are organised as local dimers or are spread out over the polymer chain remains unclear.

Circular dichroism spectroscopy

The reaction mixture of the homopolymerisation of 10 did not show any measurable CD signal. The CD spectrum of the copolymer of 10 and IAA (ratio 1:10) however, revealed a small signal in the porphyrin absorption band (see arrows in Figure 7), indicating that the porphyrins are incorporated in a chiral polymer environment. In addition, also a positive Cotton effect in the region of the imine absorption band at 310 nm was observed. This is the signal previously recorded for the polymers of IAA, which have a well-defined hydrogen bonded arrangement of the side groups, showing that the structure of the co-polymer resembles that of the homopolymer of IAA.

The CD spectrum of the second copolymerisation reaction mixture (10:IAA = 1:1) revealed a very weak Cotton effect just above the level of the noise indicating that, although not all monomers 10 had been incorporated, the ones that were consumed still had large enough interactions to be detected. No CD signal was observed in the region of the imine n-π* absorption band, which is tentatively explained by the fact that in this case the polymers were so short that no well-defined hydrogen bonding arrays could be formed.

Figure 7 CD spectrum of copolymers of 24 and IAA (left: molar ratio 1:10, right: 1:1, solvent: CHCl₃). Weak Cotton effects can be seen in the Soret band of the porphyrin, indicating some coupling between helically arranged porphyrins. The left panel also shows a Cotton effect in the imine absorption band, as is seen for polymers of IAA with hydrogen bonding side groups.
Atomic force microscopy

Molecules of copolymer 10 and IAA spin coated from a CHCl₃ solution on mica, could be visualised by AFM. The results for the copolymer 10:IAA = 1:10 are depicted in Figure 8. The image (Figure 8a) shows a dense network of entangled fibres, which can be unraveled by diluting the solution (Figure 8b). Fibres of several hundreds of nanometres, identical to those measured for polyisocyanides of IAA, can be observed. Obviously, incorporation of another isocyanide than IAA in the polymer does not significantly alter the polymer architecture (this was already expected from the Cotton effect in the CD spectrum at 310 nm). The same experiment for the copolymer mixture 10:IAA = 1:1 showed a different picture (Figure 8c). No long fibres could be observed, but rather spherical objects that had a tendency to cluster. These spherical objects are assigned to the presence of fairly short polymer chains, as to be expected for a sterically hindered polymer.

Figure 8 AFM images of copolymers of 10 and IAA spin coated on mica. (a) A dense network of entangled fibres is observed for the copolymer (1:10). (b) Upon dilution, rigid fibres reminescent of polymers of IAA are seen. (c,d) Only spherical objects that have a tendency to aggregate are observed for copolymers (1:1) (bar = 500 nm).
Conclusions

The AFM results, together with the spectroscopic measurements, prove that isocyano monomer 10 cannot be readily polymerised to form a homopolymer, probably because it is too bulky, causing steric hindrance around the nickel centre during the catalysis. But by mixing it with a small monomer it can be polymerised, giving a polyisocyanide with porphyrins randomly attached to the polymer backbone. The fact that the polymers show a CD effect is due to weak exciton interactions and is additional proof of the incorporation of the porphyrins into a random block-copolymer. Hence, it is evident that porphyrins can be attached to polyisocyanides by co-polymerisation of 10 with a small co-monomer, but the copolymers do not show the well-defined ordering that was aimed for. This copolymer system can be compared with the published polyisocyanides that could only partly be grafted with porphyrins.[8]

3.2.4 Polymer 25

Synthesis

Since it was concluded from the previous experiments that homopolymer 24 cannot be readily formed due to steric crowding of monomers 10 around the nickel centre, it was decided to increase the spacer unit between the isocyano alanine and the porphyrin. Consequently, aminoporphyrin 11 was synthesised from porphyrin 1 using a Williamson reaction with aminopropyl bromide hydrobromide. The resulting compound was coupled to Boc-L-alanine using DCC/DMAP to give 12, which, after deprotection with TFA, gave porphyrin 13 in 72% yield after purification. Porphyrin 13 was formylated with formyl-ONP to give the required porphyrin 14 in 60% yield after purification. Finally, isocyanide 15 was prepared from 14 with phosphorous oxychloride and triethylamine. The isocyanide porphyrin 15 was polymerised with 1/300 equivalents of Ni(ClO₄)₂·6H₂O to give porphyrin polymer 25 after 1 day, in a yield of ca. 50%. IR spectroscopy (KBr) on porphyrin 15 showed the characteristic isocyanide stretching vibration at 2134 cm⁻¹ and an absorption band at 1686 cm⁻¹ which was ascribed to the amide carbonyl stretching vibration. Upon polymerisation a new band appeared at 1653 cm⁻¹ with a shoulder at 1618 cm⁻¹. The band at 1618 cm⁻¹ was assigned to the imine stretching vibration of polymer 25 that usually appears between 1600 and 1700 cm⁻¹. The absorption band at 1653 cm⁻¹ was assigned to the amide carbonyl stretching vibration in analogy to the assignment for the polymers of IAA.[11] The L,D-IAA monomer has been reported to display the following behaviour (see also §2.4.4): monitored as a solution in CHCl₃ the amide carbonyl stretch-band appears at 1688 cm⁻¹, whereas measured as a solid in KBr it is present at 1668 cm⁻¹. In the latter case hydrogen bonding between the amides occurs which is absent in solution.
After polymerisation of IAA, the amide band had even further shifted, viz. to 1659 cm\(^{-1}\) (in KBr). From this observation it is concluded that monomer 15 does not hydrogen bond in the solid state. The polymer, however, shows a large shift of the amide carbonyl to 1653 cm\(^{-1}\), which is indicative of hydrogen bonding, viz. between the amides of the \(n\)\(^{th}\) and the \((n+4)\)\(^{th}\) side-group.

\[
\text{Scheme 5 Synthesis of isocyanide monomer 15 and polymer 25. (i) Bromopropylamine hydrobromide, NaOH, DMF; (ii) Boc-L-ala, DCC/DMAP, CH\(_2\)Cl\(_2\), 0°C; (iii) TFA, CH\(_2\)Cl\(_2\); (iv) Formyl-ONP, CH\(_2\)Cl\(_2\); (v) POCl\(_3/\)NEt\(_3\), CH\(_2\)Cl\(_2\), -12°C.}
\]

NMR studies

The NMR-spectrum of porphyrin polymer 25 revealed significantly broadened peaks in the downfield region (porphyrin area) as well as in the peptide range (see Figure 9), which is characteristic for polymers. Besides the broadening, a clear upfield shift of the protons at the \(\beta\)-position of the porphyrins from 8.85 ppm to 8.28 ppm and an upfield shift from -2.77 ppm to -
2.92 ppm for the central porphyrin core protons was observed. These upfield shifts can be expected when a porphyrin experiences a ring current effect from another proximal porphyrin. Using these ring current shifts, the relative position of the porphyrins with respect to each other can be estimated (Figure 10).[^17] The expected changes in chemical shift of protons, positioned above a porphyrin as function of the height (z) with respect to the porphyrin plane and the distance (p) between the porphyrin cores, are shown. The distance between the planes of two facing sidearms (n and n+4) of the polyisocyanide backbone was assumed to be 4.2 Å (see Chapter 2). Assuming that the porphyrin planes are positioned perpendicularly to the polymer backbone, this value is taken as the distance between the planes of two facing porphyrins. In this case, the height of the protons of a porphyrin with respect to the planes of the top and bottom facing porphyrins is also z = 4.2 Å.

In Figure 10 a schematic representation of three porphyrins in the array is shown. The distance between the middle of the backbone and the middle of the porphyrin core was calculated to be 19.1 Å.[^18] Since the hydrogen bonding between the side arms is still intact, it is assumed that the projection angle between the side arms is 20° as was calculated for L,L-PIAA (see §2.4.4). This results in a distance between the porphyrin cores of p = 6.6 Å. When this value is taken as the distance p for the central core protons, the change in chemical shift for these protons can be derived from Figure 9.

![Figure 9 NMR spectra of monomer 15 (bottom spectrum) and of polymer 25 (top spectrum); arrows indicate upfield shifts of protons at the β-position (from 8.85 to 8.28 ppm) and at the core (from -2.77 to -2.92 ppm) of the porphyrin molecule.](image)

Since one porphyrin faces two other porphyrins the ring current shifts can be expected to be larger. The change in chemical shift using the calculated values for z and p is slightly more...
than zero, as can be seen in Figure 10, and is in good agreement with the observed upfield shift of \((-0.15)\) ppm in the NMR spectrum. The \(p\)-values correspond with an projection angle between the sidearms of 18°-21°. Therefore, the NMR data confirm the assumed angle of 20° between the sidearms. It must be stressed that this method is very approximate.

\[ \text{Figure 10} \quad \text{a) Schematic drawing of the porphyrin positions in the polymer. b) Isoshielding lines (numbers are shifts in ppm) as a function of height (z) and distance to the centre (p) of the porphyrin. In the present case } z = 4.2 \text{ Å and } p = 6.6 \text{ Å. c) Clarification of the distances } z \text{ and } p. \]

**Absorption spectroscopy**

The absorption spectrum of the polymer is shown in Figure 11a. After polymerisation the Soret band was found to be slightly shifted to 420 nm, with a shoulder on the high energy side of the absorption band when compared to the spectrum of the monomer, as was also observed for polymer 23 and the copolymer of 10 and IAA. In addition, the low energy side the Soret band was also clearly broadened, probably caused by a new band that can be ascribed to a \(J\)-aggregate (see CD spectroscopy). Relative to the Soret band the Q-bands had become more intense, a feature that has been observed before for polymer 23.

**Circular dichroism spectroscopy**

The possible helical arrangement of the porphyrin molecules in polymer 25 was investigated with the help of CD spectroscopy. For monomer 15 no CD-effect was observed. The polymer
Chapter 3

revealed a positive Cotton effect in the red side of the Soret band (see Figure 11b). The maximum of the CD curve was positioned at 433 nm and displayed a relatively large intensity compared to the other transitions. This feature will be discussed in more detail in Chapter 4. Compared to the CD spectrum of polymer 23 (Figure 7) the CD spectrum of 25 is significantly different, indicating a different ordering of the porphyrin molecules and hence the occurrence of different exciton interactions between the porphyrins in the two polymers. This effect can be ascribed to the fact that different spacers (differing in the number of amino acids) between the polymeric backbone and the porphyrins are present.

![Figure 11](image)

*Figure 11* a) Absorption spectra of monomer 15 (dashed line, conc: ~ 3µM) and polymer 25 (full line, conc ~ 4µM) in CHCl₃. b) CD spectrum of polymer 25 in CHCl₃ (absorption spectrum is depicted as a dotted line).

**Atomic force microscopy**

Polymer 25 was studied with the help of scanning probe techniques. To this end a drop of polymer 25 in chloroform was spin-coated on a freshly cleaved mica surface and observed with TM-AFM. The result is shown in Figure 12. Only spherical objects could be seen that were very homogeneous in size, with a height of 3 nm and a width of maximally 20 nm, which is mainly determined by the convolution of the AFM-tip. Rod-like objects were not observed. Upon dilution, the objects did not change in size but merely the density (number of spots per unit area) decreased. This observation, together with the fact that the objects were very homogeneous in size, suggests that these are single polymer molecules, and that these polymer molecules are not long enough to appear as rod-like objects. The small size could be the result of a premature precipitation of the polymer during the polymerisation reaction.
Conclusions

Polyisocyanide 25 with porphyrin pendants was successfully synthesised from their isocyanide precursors using 1/350 equiv. of a Ni(II) catalyst. IR measurements revealed the presence of intramolecular hydrogen bonds between the amide units in the side chains of the polymer. The absorption spectrum revealed excitonic interactions between the porphyrins, confirmed by CD spectroscopy, which also proved the helical ordering of the porphyrins. From the chemical shifts in the $^1$H NMR spectrum, the projection angle between porphyrins n and n+4 was estimated to be ca. 20º, in agreement with the predicted angle based on the hydrogen bonding arrangement in the polymer side arms. Although long polymers were expected, fibre-like structures were not observed with AFM, from which it was tentatively concluded that the polymers precipitate during the polymerisation reaction.

3.2.5 Attempts to prepare isocyanides from porphyrins with propyl-diaminoacid sidearms

Synthesis

To further study the effect of the spacer length and the composition of the porphyrin containing polyisocyanide, it was decided to add an extra glycine aminoacid as bridging unit between the polymer backbone and the porphyrin unit. For the synthesis of porphyrin isocyanide 17, a similar route as described for isocyanide 15 was followed. Aminoporphyrin 11 was coupled with the formylated dipeptide of alanine and glycine to give 16. The use of DCC was not expected to influence the optical purity of the dipeptide, since glycine is not an optically active aminoacid. To convert 16 into isocyanide 17 several attempts were undertaken using various dehydrating agents, also changing their ratios, and varying temperatures. Only an excess of the dehydrating agent was found to give conversion, but the obtained product was not the expected isocyanide. In another attempt it was tried to prepare isocyanide 21, which contains two L-
alanine units instead of an alanine and a glycine unit. Using standard DCC/DMAP coupling reagents, formamide 18 could be obtained, but the same problem arose as described for 17, viz. the final conversion to the isocyanide appeared to be unsuccessful. Regardless of all attempts, no isocyanide was obtained, but only a product that did not run on tlc.

![Scheme 6 Attempted synthesis of isocyanides 17 and 19.](image)

Strikingly, both isocyanide precursors 16 and 20 have the same length of their sidearms between the porphyrin and the formyl group. It was found that the reaction intermediate, phosphorylated 16 or 18 formed a spacer just long enough to fold back and fit into the porphyrin core (as was verified by a space filling CPK model, in which it was possible to fold the sidearm back into the porphyrin core), see Figure 13. This could prevent the final dehydration reaction to the isocyanide to occur.
To test if the porphyrin core has an influence on the formation of the isocyanide, 16 was converted into the corresponding nickel (II) analogue (16Ni) to block possible docking of the sidearm. Nickel was chosen, because nickel porphyrins have a low affinity for coordinating ligands. The UV-Vis absorption spectrum of 16Ni showed the characteristic two band structure of nickel porphyrins at 418 and 530 nm. The conversion to the isocyanide 17Ni was thought to occur (indicated by tlc), but the isocyanide was lost during the work-up procedure, probably because it formed an insoluble precipitate. Contrary to what is generally assumed, Ni-porphyrins apparently can be ligated when a large excess of (isocyanide) ligand is present. The precipitate probably consists of large three dimensional networks wherein isocyanide moieties are coordinated to nickel centres. Since these isocyanides can not be easily synthesised they were not studied further.

### 3.3 Conclusions

With the intention of synthesising long rod-like porphyrin polyisocyanides with hydrogen bonding arrays, different synthetic approaches have been investigated in which several structural parameters that can influence the polymerisation reaction have been varied. Polymer 23 contains two alanine units between the porphyrin moiety and the polymer backbone was found to possess hydrogen bonds between the amide groups and the porphyrins were found to be arranged in a helical fashion with exciton interactions between the chromophores. However, the coupling of a dipeptide via an N-formyl functional group using carbodiimide chemistry resulted in significant epimerisation. A Boc-protected alanine starting material should have been used instead of N-formylated alanine.

Experiments with isocyanide 10 revealed that steric hindrance around the nickel centre is an important issue during polymerisation. When the monomers are too bulky, polymerisation is hampered, which is the case for 10, where the porphyrin is relatively close to the isocyanide.
Chapter 3

function. The steric interactions could be minimised by increasing the distance between isocyanide group and the porphyrin. Alternatively, the steric bulk could be decreased during polymerisation by mixing 10 with IAA, leading to a random copolymer. When, however, a long spacer was applied between the isocyanide moiety and the porphyrin, viz. two alanines and a propyl unit, the synthesis of the isocyanide turned out to be impossible. This phenomenon was ascribed to the formation of an intermediate in which a phosphorous atom is inserted in the centre of the porphyrin.

With 25 it was found that the solubility of the polyisocyanide was high enough to prevent premature precipitation of the polymer. For the isolated short polymers 25, the projection angle between the 1st and 5th porphyrins could be estimated by 1H-NMR and was found to correspond to roughly 20°. This is in agreement with the value of 20° that follows from calculations based upon PIAA’s. The absorption spectra of the polymers all showed a broadening or splitting of the Soret band, indicative of exciton interactions between the porphyrins. CD studies revealed that the porphyrin functions of 25 are helically arranged along the polymer backbone.

3.4 Experimental Section

3.4.1 General methods and materials

All solvents were distilled prior to use under atmospheric pressure and a nitrogen atmosphere. Dichloromethane and chloroform were distilled from CaCl₂ and THF from Sodium. N-methyl morpholine and pyrrole were distilled under reduced pressure. All other chemicals were obtained commercially and used without further purification.

Thin layer chromatography analyses were performed on Merck precoated silica gel 60 F₂₅₄ plates. Flash column chromatography was performed using silicagel acquired from Acros. ¹H and ¹³C NMR spectra were recorded either on a Bruker WM-200, Bruker AC-300 or a Varian Inova 400 instrument at 297 K. Chemical shifts are reported in ppm relative to tetramethylsilane (δ = 0.00 ppm). FT-Infrared spectra were recorded on a Bio-Rad FTS 25 spectrometer. UV/vis spectra were measured on a Varian Cary 50 Conc spectrophotometer at ambient temperature. CD spectra were recorded on a Jasco 810 spectrophotometer equipped with a Peltier temperature control unit (Jasco PT-423 s/1) at 20°C unless stated otherwise. Melting points were measured on a Jeneval polarisation microscope THMS 600 equipped with a Linkham 92 hot stage temperature controller. Fluorescence spectra were recorded on a Perkin Elmer Luminescence Ls50B spectrometer at 25°C. FAB mass spectra were recorded on a VG-7070E mass spectrometer, with 3-nitrobenzyalcohol as matrix. Polyethylene glycol was used as calibrant for high accurate FAB measurements. MALDI-TOF spectra were measured on a Bruker Biflex III spectrometer, using dithranol as matrix. For highly accurate measurements, polyethylene glycol was used as internal reference.
3.4.2 Atomic force microscopy
AFM experiments were performed using a Nanoscope IIIa instrument from Digital Instruments. A solution of a polymer (~10⁻⁶M) in CHCl₃ was spincoated onto freshly cleaved Muscovite mica. All images were taken in tapping mode in air at room temperature. Commercial tapping-mode tips (Digital Instruments) with a typical resonance frequency around 300 kHz were used.

3.4.3 Synthesis

N-Formyl-L-alanine (FA)\(^{[23]}\)
In a 500 mL flask, L-alanine (6.32 g, 71 mmol) was dissolved in formic acid (200 mL) and the solution was cooled to 0 °C on an ice-bath. Then 70 mL of acetic anhydride was slowly added in 2 min. The mixture was allowed to warm to room temperature and stirred for 1 h. The solvent was evaporated in vacuum and the resulting white solid was recrystallised from acetone. \(^{1}\)H NMR (CD₃OD, 300.13 MHz) δ 7.91 (s, 1H, formyl), 4.29 (m, 1H, CH ala), 1.27 (d, 3H, CH₃) ppm.

N-Formyl-L-alanyl-L-alanine (2)
This dipeptide was synthesised using standard peptide coupling reactions, viz. by coupling Boc-L-alanine with benzyl protected L-alanine, followed by deprotection of the dipeptide, and formylation. After removal of the benzylic moiety by hydrogenation with Pd/C, the product was purified with the help of the counter current method. \(^{1}\)H NMR (CD₃OD, 300.13 MHz) δ 8.10 (s, 1H, HC(O)), 4.43 (q, 1H, CH ala next to acid), 4.31 (q, 1H, CH ala next to formamide), 1.41 (d, 3H, CH₃ ala next to acid), 1.39 (d, 3H, CH₃ ala next to formamide) ppm.

N-Formyl-L-alanyl-L-alanine methyl ester (FAA)
This compound was synthesised according to a literature procedure.\(^{[11]}\)

Isocyano-L-ala-L-ala (IAA)
This compound was synthesised according to a literature procedure.\(^{[11]}\)

5-(4-Hydroxyphenyl)-10,15,20-tris-(4-methylphenyl)porphyrin (1)
In a 1L flask, equipped with a reflux condenser, 4-hydroxybenzaldehyde (4.6 g, 0.038 mol) and p-tolualdehyde (13.5 g, 0.112 mol) were dissolved in propionic acid (500 mL) and the mixture was heated until near reflux. Then pyrrole (10.4 mL, 0.15 mol) was added and the solution was refluxed for 1 h. The reaction mixture was cooled to 4ºC and the resulting purple crystals were filtered off, washed with ethanol until the filtrate was colourless, and dried. The purple solid was purified by column chromatography (2x, silicagel, eluent: 0-3% MeOH in CHCl₃) to yield 1.13 gram (4.5%) of porphyrin 1 as a purple solid. \(^{1}\)H NMR (CDCl₃, 300.13 MHz) δ 8.85 (s, 8H, β-pyrrole), 8.09 (d, 6H, ArH meta to CH₃), 8.05 (d, 2H, ArH meta to OH), 7.54 (d, 8H, ArH meta to CH₃), 7.14 (d, 2H, ArH meta to OH), 2.70 (s, 9H, CH₃), -2.79 (s, 2H, NH). FAB-MS m/z 672 (M⁺).
N-For-L-ala-L-ala-porphyrin (3)

A solution of porphyrin 1 (300 mg, 0.45 mmol), dipeptide 2 (84 mg, 0.45 mmol), DCC (0.12 g, 0.58 mmol) and a catalytic amount of DMAP in 15 mL of CH₂Cl₂ was stirred for 14 h under a nitrogen atmosphere. The mixture was filtered and the residual DCU was washed with dichloromethane. The reaction resulted in the formation of 2 diastereomeric products, Rf = 0.17 and 0.21 (eluent 5% MeOH in CHCl₃). The filtrate was concentrated and subjected to column chromatography (2x, silicagel, eluent 5% MeOH in CHCl₃) resulting in the isolation of the product with Rf = 0.17 as a purple solid, yield: 59%.

1H NMR (CDCl₃, 300.13 MHz) δ 8.84 (q, 8H, β-pyrrole), 8.26 (s, 1H, formamide), 8.23 (d, 2H, ArH meta to OR), 8.09 (d, 6H, ArH meta to CH₃), 7.55 (d, 2H, ArH ortho to CH₃), 7.51 (d, 6H, ArH ortho to OR), 6.63 (d, 1H, NH amide), 6.29 (d, 1H, NH formamide), 4.95 (p, 1H, CH ala next to ester), 4.74 (p, 1H, CH ala next to formamide), 2.69 (s, 9H, CH₃ porphyrin), 1.76 (d, 3H, CH₃ ala), -2.80 (s, 2H, NH porphyrin) ppm. FAB-MS m/z 843 (M⁺).

Isocyano-L-ala-L-ala-porphyrin (4)

To a solution of porphyrin 3 (170 mg, 0.20 mmol) in 3 mL of CH₂Cl₂ at -10ºC under nitrogen was added triethylamine (NEt₃; 85 µl, 0.61 mmol). After 15 min, a solution of POCl₃ (38 µl, 0.39 mmol) in 0.5 mL of CH₂Cl₂ was added dropwise over a period of 30 minutes to the solution. The temperature was kept at -5ºC and the solution was stirred for another 30 min, after which an aqueous saturated solution of NaHCO₃ (2 mL) was added and the solution was allowed to stir for 5 min. The organic layer was separated, diluted to 20 mL, washed with water (2 x 10 mL), dried with MgSO₄ and evaporated. The obtained purple solid was subjected to column chromatography (silicagel, eluent 1% MeOH in CHCl₃). Yield: 56% of a purple solid. FT-IR (cm⁻¹, KBR): 3326 (NH), 2138 (NC), 1766 (ester), 1692 (amide). 1H NMR (CDCl₃, 300.13 MHz) δ 8.85 (q, 8H, β-pyrrole), 8.23 (d, 2H, ArH meta to OR), 8.09 (d, 6H, ArH meta to CH₃), 7.55 (d, 2H, ArH ortho to CH₃), 7.07 (d, 1H, NH amide), 4.98 (q, 1H, CH ala next to NC), 2.70 (s, 9H, CH₃ porphyrin), 1.81 (d, 3H, CH₃ ala next to ester), 1.76 (d, 3H, CH₃ ala next to NC), -2.80 (s, 2H, NH porphyrin) ppm.

Polymer 23

In a flask, protected from water with a CaCl₂ tube, was dissolved 4 (0.1 g, 0.12 mmol) in dichloromethane (0.5 mL). To this solution was added 0.008 equiv. of Ni(II), viz. 37 µL of a stock solution containing 0.50 g Ni(II)(ClO₄)₂·6H₂O in 46 mL CH₂Cl₂ and 4 mL EtOH. After stirring for 4 days, the reaction mixture was poured into a vigorously stirred mixture of H₂O/MeOH (80 mL, 3/7 v/v). The precipitate was filtered off and washed with water (2 x 10 mL), dried with MgSO₄ and evaporated. The residue was dissolved in CH₂Cl₂, precipitated in MeOH, filtered and washed again with acetone. Yield: 66 mg (66%) of a purple powder. FT-IR (cm⁻¹, KBR): 3320, shoulder at 3315 (NH), 1762 (ester), 1654 (amide), 1611 (C=N). 1H NMR (CDCl₃, 300.13 MHz) δ 9.7 (br, 16H, β-pyrrole, phenyl), 5.4-5.5 (br, 1H, CH ala), 3.0-3.5 (br, 15H, CH₃ toluyl, CH₃ ala), -3 (br, 2H, NH porphyrin) ppm. Beside these broad signals, sharp signals were observed from the end groups of the polymer: δ 8.86 (s, β-pyrrole), 8.09 (d, 6H, ArH meta to CH₃), 7.55 (d, 2H, ArH ortho to CH₃), 2.71 (s, CH₃ porphyrin), 1.76 (d, CH₃ ala), -2.79 (s, NH porphyrin) ppm.
5-(4-Cyanophenyl)-10,15,20-tris-(4-methylphenyl)porphyrin (5)

In a 1L flask, equipped with a reflux condenser, 4-cyanobenzaldehyde (4.98 g, 0.038 mol) and p-tolualdehyde (13.5 g, 0.112 mol) were dissolved in propionic acid (500 mL) and heated until near reflux. Then pyrrole was added (10.1 g, 0.15 mol) and the solution was refluxed for 1h. The reaction mixture was cooled to 0ºC and the purple crystals were filtered off, washed with ethanol until the filtrate was colourless and dried. The purple solid was purified by column chromatography (2x, silicagel, eluent 0-1% MeOH in CHCl₃) to yield 2.1 gram (8.4%) of porphyrin 1 as a purple solid. FT-IR (cm⁻¹, KBR): 2227 (CN).

1H NMR (CDCl₃, 300.13 MHz) δ 8.90 (d, 2H, β-pyrrole), 8.87 (s, 4H, β-pyrrole), 8.70 (d, 2H, β-pyrrole), 8.34 (d, 2H, ArH meta to CN), 8.09 (d, 6H, ArH meta to CH₃), 8.06 (d, 2H, ArH ortho to CN), 7.56 (d, 6H, ArH ortho to CH₃), 2.71 (s, 9H, CH₃), -2.79 (s, 2H, NH) ppm.

5-(4-Aminomethylphenyl)-10,15,20-tris-(4-methylphenyl)porphyrin (6)

To a solution of 5 (0.80 g, 1.18 mmol) in THF (200 mL) was added LiAlH₄ (180 mg). The mixture was stirred for 30 min under a nitrogen atmosphere and again LiAlH₄ (180 mg) was added. After stirring for 1h a reflux condensor was attached and the reaction mixture was refluxed for 20 min. After cooling to room temperature the flask was placed on an ice bath and to the reaction mixture was added 200 mL of aqueous 3N HCl. This mixture was extracted with CHCl₃ (2 x 200 mL). The combined organic layers were washed with aqueous saturated NaHCO₃ (2x) and water (2x), dried (MgSO₄) and evaporated to dryness. The product was purified by column chromatography (silicagel, eluent 5% MeOH in CHCl₃) to give 0.57 g of 6 as a purple solid (71%). 1H NMR (CDCl₃, 300.13 MHz) δ 8.85 (m, 8H, β-pyrrole), 8.17 (d, 2H, ArH meta to CH₂), 8.09 (d, 6H, ArH meta to CH₃), 7.69 (d, 2H, ArH ortho to CH₂), 7.55 (d, 6H, ArH ortho to CH₃), 4.21 (s, 2H, CH₂), 2.71 (s, 9H, CH₃), -2.78 (s, 2H, NH) ppm. FAB-MS m/z 687 (MH⁺)

5-(4-Aminomethylphenyl)-10,15,20-tris-(4-methylphenyl)porphyrin (6) alternative method

In a two-necked flask of 25 mL, placed on an ice bath and equipped with a rubber septum, porphyrin 5 (0.1 g, 0.15 mmol) was dissolved in 2.5 mL of THF under a dry nitrogen atmosphere. BH₃·THF (0.3 mmol, 0.3 mL of a 1M stock solution) was injected into the reaction mixture. After 2 h an aqueous concentrated HCl solution (ca. 0.3 mL) was added dropwise. The solvent was evaporated and the resulting slurry was dissolved in CHCl₃ and washed with aqueous 2N NaOH (2x) and water (2x). The organic layer was dried with MgSO₄ and evaporated. The product was purified by column chromatography (silicagel, eluent 5% MeOH in CHCl₃) to give 62 mg of 6 as a purple solid (62%).

Porphyrin 7

To a solution of 6 (190 mg, 0.28 mmol) and Boc-L-alanine (84 mg, 0.56 mmol) in 12 mL of CH₂Cl₂ under a nitrogen atmosphere were added DCC (91 mg, 0.56 mmol) and a catalytic amount of DMAP. After stirring for 14 h the mixture was concentrated and subjected to column chromatography (silicagel, eluent 0-1% MeOH in CHCl₃). The resulting product was purified by taking it up in dioxane to precipitate the DCU. The precipitate was filtered and washed with dioxane. The filtrate was concentrated to yield purple 7 (81%). 1H NMR (CDCl₃, 300.13
MHz) δ 8.86 (d, 2H, β-pyrrole), 8.85 (s, 4H, β-pyrrole), 8.80 (d, 2H, β-pyrrole), 8.16 (d, 2H, ArH meta to CH₂), 8.09 (d, 6H, ArH meta to CH₃), 7.64 (d, 2H, ArH ortho to CH₂), 7.55 (d, 6H, ArH ortho to CH₃), 6.81 (t, 1H, NHC₆H₅), 6.59 (d, 1H, NH), 4.21 (s, 2H, CH₂), 2.71 (s, 9H, CH₃), 1.54 (s, 9H, CH₃ boc), 1.52 (d, 3H, CH₃ ala), -2.78 (s, 2H, NH) ppm.

**Porphyrin 8**

In a 25 mL flask 7 (0.16 g, 0.2 mmol) was dissolved in dichloromethane (10 mL). Trifluoroacetic acid (1.0 mL, 12.8 mmol) was added and the reaction mixture was stirred for 1h at room temperature. After this period the solvent was evaporated under vacuum. The purple solid was taken up in dichloromethane and washed with an aqueous saturated solution of NaHCO₃ and subsequently with water (2x). The organic layer was dried over magnesium sulphate, filtered and evaporated to dryness. The purple solid was purified by column chromatography (silicagel, eluent 1-5 % MeOH in CHCl₃) to yield 90 mg (56%) of porphyrin 8 as a purple solid.

**Porphyrin 9**

FA (39 mg, 0.34 mmol) was suspended in a solution of 6 (230 mg, 0.34 mmol) in 15 mL of dichloromethane and this mixture was cooled on an ice bath. To this mixture were added DCC (77 mg, 0.37 mmol) and a catalytic amount of DMAP. The reaction mixture was stirred at 0ºC for 1 h under a nitrogen atmosphere and then 3 h at room temperature, after which the flask was placed on an ice bath and again DCC (40 mg, 0.19 mmol) was added. After 30 min the ice bath was removed and the reaction mixture was stirred for 2.5 h. The mixture was concentrated and subjected to column chromatography (2x, silicagel, eluent 0-2.5% MeOH in CHCl₃). The collected purple solid was dissolved in CHCl₃ and precipitated by addition of MeOH. The compound was filtered, washed with MeOH and dried to give 9 as a purple solid (160 mg, 60%). ¹H NMR (CDCl₃, 300.13 MHz) δ 8.86 (d, 2H, β-pyrrole), 8.85 (s, 4H, β-pyrrole), 8.79 (d, 2H, β-pyrrole), 8.24 (s, 1H, formyl H), 8.17 (d, 2H, ArH meta to CH₂), 8.08 (d, 6H, ArH meta to CH₃), 7.61 (d, 2H, ArH ortho to CH₂), 7.54 (d, 6H, ArH ortho to CH₃), 6.62 (t, 1H, CH₂NH), 6.34 (d, 1H, NH next to formyl), 4.81 (d, 2H, CH₂), 4.73 (p, 1H, CH ala), 2.70 (s, 9H, CH₃ porph), 1.55 (d, 3H, CH₃ ala), -2.79 (s, 2H, NH) ppm. FAB-MS m/z 785 (M⁺).

**Isocyanoporphyrin 10**

In a 10 mL flask, porphyrin 9 (75 mg, 0.089 mmol) was dissolved in dichloromethane (2.5 mL). The reaction mixture was cooled to -18 °C by placing it on an ice/salt bath and stirred under nitrogen. Triethylamine (0.04 mL, 0.30 mmol) was added and after stirring for 15 min, POCl₃ (15 µl, 0.190 mmol) was added in two portions over a period of 30 min. To the reaction solution was added ca. 5 mL of an aqueous saturated solution of NaHCO₃. After stirring for 5 min the mixture was poored into 20 mL of dichloromethane and extracted twice. The combined organic layers were washed with water (2x), dried over magnesium sulphate, filtered and evaporated to dryness. The product was purified by column chromatography (silicagel, eluent 0.75 % MeOH in CHCl₃). Yield: 35 mg (51%) of monomer 10 as a purple solid. ¹H NMR (CDCl₃, 300.13 MHz) δ 8.87 (d, 2H, β-pyrrole), 8.86 (s, 4H, β-pyrrole), 8.81 (d, 2H, β-pyrrole), 8.21 (d, 2H, ArH meta to CH₂), 8.09 (d, 6H, ArH meta to CH₃), 7.65 (d, 2H, ArH ortho to CH₂), 7.55 (d, 6H, ArH ortho to CH₃), 7.03 (t, 1H, NH ala), 4.85 (d, 2H, CH₂), 4.45 (q, 1H, CH ala), 2.70 (s, 9H, CH₃ porph), 1.81 (d, 3H, CH₃ ala), -2.78 (s, 2H, NH) ppm. FAB-MS m/z 767 (M⁺).
**Isocyanoporphyrin 10 (alternative route)**

To a solution of 9 (100 mg, 0.127 mmol) in 6 mL of CH$_2$Cl$_2$ was added 30 µL (0.270 mmol) of N-methylmorpholine. The mixture was cooled on an ice/salt bath at -18°C and diphosgene (8 µL, 0.064 mmol) was added. After 10 min the ice/salt bath was replaced by an ice/water bath and the mixture was stirred for 15 min, dropped into a stirred aqueous saturated NaHCO$_3$ solution (30 mL). The resulting mixture was extracted with CH$_2$Cl$_2$ (2x), the combined organic layers were washed with water (2x), dried (MgSO$_4$), and concentrated. The resulting solid was purified by column chromatography (silicagel, eluent 0.75% MeOH in CHCl$_3$). Yield 72 mg (74%) of purple 10.

**Homopolymer 24**

In a 10 mL flask, porphyrin monomer 10 (60 mg, 0.078 mmol) was dissolved in dichloromethane (1 mL) and a catalytic amount of Ni(II)(ClO$_4$)$_2$·6H$_2$O (25.9 µg in 0.1 mL of ethanol, 0.071 µmol) was added. Reaction times of up to 8 days did not result in conversion of the isocyanide monomer. Only in the first minutes a small amount of product was formed, as evidenced by tlc, but, according to tlc and IR nothing happened after this period.

**Block copolymer of 10 and IAA (molar ratio 1:10)**

Monomers 10 (10 mg, 0.013 mmol) and IAA (24 mg, 0.13 mmol) were dissolved in CH$_2$Cl$_2$ (6.5 mL). To this solution was added 0.002 equiv. of Ni(II) catalyst (0.033 mL of a stock solution of Ni(II)(ClO$_4$)$_2$·6H$_2$O (87.5 mg) in 100 mL CH$_2$Cl$_2$/EtOH, 97/3 v/v). After stirring for 40 h the solution was poured into a vigorously stirred mixture of 100 mL water/MeOH (30/70 v/v). The precipitate was collected and washed with acetone until the filtrate was colourless. The product was collected as a purple solid which, once dissolved had a strong tendency to turn green in solution.

**Block copolymer of 10 and IAA (molar ratio 1:1)**

Monomers 10 (10.1 mg, 0.013 mmol) and IAA (2.5 mg, 0.014 mmol) were dissolved in CH$_2$Cl$_2$ (4 mL). To this solution was added 0.002 equiv. of Ni(II) catalyst (0.033 mL of a stock solution of Ni(II)(ClO$_4$)$_2$·6H$_2$O (87.5 mg) in 100 mL CH$_2$Cl$_2$/EtOH, 97/3 v/v). After stirring for 4 days (the isocyanide was not completely consumed, but the polymerisation did not seem to proceed anymore) the solution was precipitated into a vigorously stirred mixture of 70 mL water/MeOH (20/50 v/v). The precipitate was filtered and the residue was washed with acetone until the filtrate was colourless.

**5-[4-(3-aminopropyloxy)phenyl]-10,15,20-tris-(4-methylphenyl)porphyrin (11)**

To a solution of porphyrin 1 (0.150 g, 0.22 mmol) in DMF (10 mL) in a 50 mL flask, was added crushed sodium hydroxide (0.180 g, 4.5 mmol). The mixture was stirred at room temperature for 20 min., and 3-bromopropylamine hydrobromide (0.053 g, 0.24 mmol) was added. After 1 h, again 3-bromopropylamine hydrobromide (0.053 g) was added. After 30 min. the solution was concentrated. The residue was dissolved in methanol/water (40/60 v/v) and extracted (2x) with dichloromethane. The organic layer was dried over magnesium sulphate, filtered and evaporated to yield 0.140 g (93%) of porphyrin 2 as a purple solid. The product was used without any further purification in the next reaction step, because column chromatography (silica gel as well as Merck Aluminumoxide 90 (activity III)) resulted in decomposition of the product. NMR and tlc showed the presence of some minor impurities. $^1$H NMR (CDCl$_3$, 300.13 MHz) δ 8.85 (s, 8H, β-pyrrole), 8.10 (d, 2H,
ArH meta to CH₂, 8.08 (d, 6H, ArH meta to CH₃), 7.55 (d, 6H, ArH ortho to CH₃), 7.28 (d, 2H, ArH ortho to CH₂), 4.35 (t, 2H, OCH₂), 3.13 (t, 2H, CH₂N), 2.70 (s, 9H, CH₃ toluyl), 2.14 (m, 2H, CH₂CH₂CH₂), -2.78 (s, 2H, NH porphyrin) ppm. FAB-MS m/z 730 (MH⁺). The mass and NMR spectra didn’t show any sign of doubly alkylated porphyrins.

5-[4-(3-tert-butyloxycarbonyl-L-alanyl-propyloxy)phenyl]-10,15,20-tris-(4-methylphenyl)porphyrin (12)
In a 100 mL flask porphyrin 11 (90 mg, 0.12 mmol) was dissolved in dichloromethane (10 mL) and the mixture was cooled to 0°C. Boc-L-alanine (1.1 equiv., 29.6 mg, 0.14 mmol) was added and the mixture was stirred under nitrogen at 0°C for 10 min. DCC (28.2 mg, 0.1356 mmol) and catalytic amount of DMAP were added. The mixture was allowed to warm to room temperature and stirred overnight. Again Boc-L-alanine (1.1 equiv.) and DCC were added and the mixture was stirred for another 3 h. The mixture was precipitated in hexane, the precipitate was filtered, dried and subjected to column chromatography (2x, silicagel, eluent: 0.5% MeOH in CHCl₃) after which the product 12 was obtained as a purple solid. Yield: 90 mg (83%).

1H NMR (CDCl₃, 200 MHz) δ 8.85 (s, 8H, β-pyrrole), 8.09 (d, 8H, ArH meta to CH₃), 7.52 (d, 6H, ArH ortho to CH₃), 7.22 (d, 2H, ArH ortho to OH), 6.72 (br, 1H, NH ala), 5.03 (br, 1H, NH ala), 4.25 (t, 2H, OCH₂), 4.00 (m, 1H, CH ala), 3.61 (m, 2H, CH₂N), 2.68 (s, 9H, CH₃ toluyl), 2.15 (m, 2H, CH₂CH₂CH₂), 1.47 (s, 9H, (CH₃)₃C), 1.41 (d, 3H, CH₃ ala), -2.77 (s, 2H, NH porphyrin).

Porphyrin 13
In a 25 mL flask porphyrin 12 (60 mg, 0.07 mmol) was dissolved in dichloromethane (6 mL). TFA (trifluoroacetic acid) (0.5 mL, 6 mmol) was added and the reaction mixture was stirred for 1h at room temperature. Hereafter, the solvent and the TFA were evaporated under vacuum. The resulting purple solid was taken up in dichloromethane and washed with an aqueous saturated solution of NaHCO₃ and subsequently with water (2x). The organic layer was dried over magnesium sulphate, filtered and evaporated to dryness. The purple solid was purified by column chromatography (silicagel, eluent 5 % MeOH in CHCl₃) to yield 27 mg (51 %) of porphyrin 13 as a purple solid.

Porphyrin 14
In a 50 mL flask porphyrin 13 (0.20 g, 0.27 mmol) was dissolved in dichloromethane (15 mL) and the mixture was cooled to 0°C. N-Formyl-L-alanine (0.032 g, 0.27 mmol) was added and the mixture was stirred under nitrogen at 0°C for 10 min. DCC (0.062 g, 0.30 mmol) and a catalytic amount of DMAP were added. The mixture was allowed to warm to room temperature and stirred overnight. Again N-formyl-L-alanine (0.008 g, 0.07 mmol) and DCC (0.016 g, 0.07 mmol) were added and the mixture was stirred for another 3 hrs. After precipitation in hexane, the solid was purified by column chromatography (silicagel, eluent 0-1.5% MeOH in CHCl₃) to yield 0.180 g (79%) of porphyrin 14 as a purple solid. 1H NMR (CDCl₃, 300.13 MHz) δ 8.85 (s, 8H, β-pyrrole), 8.23 (s, 1H, formyl), 8.12 (d, 2H, ArH meta to OCH₂), 8.10 (d, 6H, ArH meta to CH₃), 7.55 (d, 6H, ArH ortho to CH₃), 7.27 (d, 2H, ArH ortho to OCH₂), 6.46 (t, 1H, NHCH₃), 6.29 (d, 1H, NH next to formyl), 4.62 (m, 1H, CH), 4.34 (t, 2H, OCH₂) 3.67 (m, 2H, NCH₂), 2.71 (s, 9H, CH₃ toluyl), 2.20 (m, 2H, CH₂CH₂CH₂), 1.50 (d, 3H, CH₃ ala) -2.77 (s, 2H, NH porphyrin) ppm. ESI-MS (peak matched) m/z: 829.3863 (MH⁺) (calcd for C₅₄H₄₉N₆O₃: 829.3866).
Isocyanoporphyrin 15

In a 10 mL flask, porphyrin 14 (0.044 g, 0.053 mmol) was dissolved in dichloromethane (3.5 mL). The reaction mixture was cooled to -10°C and stirred under nitrogen. Triethyl amine (36.5 µl, 0.264 mmol) was added and after stirring for 20 min, POCl$_3$ (9.1 µl, 0.105 mmol) was added slowly in small portions over a period of 20 min. A side product was formed, running on tlc just above the product at the time when all the POCl$_3$ had been added. With addition of a little more NEt$_3$ this side product spot disappeared. The reaction mixture was taken up into an aqueous saturated solution of NaHCO$_3$ and the product was extracted (3x) with dichloromethane. The organic layer was dried over magnesium sulphate, filtered and evaporated to dryness. The product was purified by column chromatography (silicagel, eluent 0-1% MeOH in CHCl$_3$) to yield 20 mg (46%) of porphyrin 15 as a purple solid.

IR (cm$^{-1}$, KBr): 2134 (RNC).

$^1$H NMR (CDCl$_3$, 300.13 MHz) δ 8.85 (s, 8H, β-pyrrole), 8.13 (d, 2H, ArH meta to OCH$_2$), 8.09 (d, 6H, ArH ortho to CH$_3$), 7.35 (d, 2H, ArH ortho to OCH$_2$), 7.15 (br, 1H, NH ala), 4.39 (t, 2H, OCH$_2$), 4.35 (m, 1H, CH ala), 3.72 (m, 2H, CH$_2$N), 2.71 (s, 9H, CH$_3$ toluyl), 2.27 (m, 2H, CH$_2$CH$_2$CH$_2$), 1.74 (d, 3H, CH$_3$ ala), -2.77 (s, 2H, NH porphyrin) ppm.

$^{13}$C NMR δ 157.7, 165.5, 138.8, 136.8, 135.1, 134.0, 130.5, 126.9, 119.7, 119.0, 112.3, 67.0, 38.7, 29.6, 28.8, 22.6, 21.4, 19.9 ppm. ESI-MS (peak matched) m/z: 811.3755 (MH$^+$) (calcd for C$_{54}$H$_{47}$N$_6$O$_2$: 811.3760).

Isocyanoporphyrin 15 (alternative route)

To a solution of 14 (20 mg, 25 µmol) in 1.5 mL of CH$_2$Cl$_2$ was added 6 µL (54 µmol) of N-methyl morpholine. The mixture was cooled on an ice bath to 0°C and diphosgene (25 µL of a solution of 0.2 mL in 5 mL CH$_2$Cl$_2$) was added. Tlc showed complete conversion after 3 min. The mixture was dropped into a vigorously stirred aqueous saturated NaHCO$_3$ solution (30 mL). The resulting mixture was extracted with CH$_2$Cl$_2$ (2x), the combined organic layers were washed with water (2x), dried (MgSO$_4$), and concentrated. The resulting solid was purified by column chromatography (silicagel, eluent 1% MeOH in CHCl$_3$). Yield 15 mg (77%) of purple 15.

Polymer 25

In a 10 mL flask, porphyrin monomer 15 (20 mg, 0.025 mmol) was dissolved in dichloromethane (1 mL) and a catalytic amount of Ni(II)(ClO$_4$)$_2$.6H$_2$O (25.9 µg in 0.1 mL of ethanol, 0.071 µmol) was added. The polymerisation was followed by tlc (eluent: 5% MeOH in CHCl$_3$) which showed that after 1 day the reaction had ended. The reaction mixture was poured into 50 mL of methanol/water (7/3 v/v). The resulting precipitate was filtered and washed with methanol and acetone to yield ca. 10 mg (50%) of polymer 5 as a purple solid. IR (cm$^{-1}$, KBr): see text. $^1$H NMR (CDCl$_3$, 500 MHz) see Figure 9.

3.5 References

Chapter 3

[18] The distance between the centre of the porphyrin and the centre of the helix was calculated form the coordinates of a chem3D 4.0 (1997) image of isocyano-alaninyl-propyloxyphenyl porphyrin. The coordinate of the centre of the porphyrin was constructed in the direction of the O-C bond of the methoxy group. The distance from the core of the porphyrin to the 4-phenyl carbon was obtained from a labview image. The coordinate of the centre of the helix was constructed in the C=N direction.
Chapter 4

Synthesis and Physical Characterisation of Porphyrin Nanorods

4.1 Introduction
In chapter 3, the synthesis and characterisation of a series of porphyrin appended polyisocyanides were described, which were designed with the objective to construct rigid rod polymers that are covered with precisely defined arrays of porphyrins. From the results of the characterisation experiments it was concluded that well-defined porphyrin architectures indeed had been obtained, but that the systems were not long enough to be observed by AFM as rigid rod-like objects. In order to be able to synthesise really long (i.e. tens of nanometres), and well-defined arrays of porphyrins all stacked in a face-to-face geometry, a new approach toward the synthesis of long, porphyrin containing polyisocyanides was envisaged. In this chapter, the synthesis and physical characterisation of such polymers are described and discussed.

4.2 Results and discussion

4.2.1 Synthesis
From the experiments described in chapter 3 it became apparent that the spacer between the porphyrin group and the polymerisable isocyanide function should be long enough to overcome steric hindrance during the polymerisation reaction, but short enough to allow the synthesis of this isocyan function from the formamide precursor. Since the toluyl porphyrin polymer with the correct spacer (propyl alanine) was found to precipitate prematurely from the
polymerisation reaction mixture resulting in oligomers and small polymers, it was concluded that the porphyrin in the polymer side chains should be equipped with solubilising tails.

It was therefore decided to prepare porphyrin polyisocyanide 32, see Scheme 1. Its monomer is derived from the amino acid L-alanine in order to provide a peptide bond, which will lead to the β-helical structure of the polymer. The porphyrin is functionalised with aliphatic tails to increase the solubility of the resulting polymer. Monohydroxyporphyrin 25, which has three dodecyloxy tails was synthesised according to standard procedures and coupled to N-(3-bromopropyl)-phthalimide using K2CO3 as base to form 26. The phthalimide group was split off using H2NNH·H2O to the give 27. Alternatively, 27 could be directly obtained from 25 using a coupling with 3-bromopropylamine and NaOH as base. Mass spectrometry revealed that no doubly alkylated aminoporphyrin product (i.e. a propylaminopropyl spacer) was obtained. The overall yield was comparable for the two routes (72% and 74%, respectively). The resulting amino compound 27 was either reacted with Boc (t-butyl-oxycarbonyl) protected alanine to give 28 or immediately coupled to N-formyl-L-alanine using dicyclohexylcarbodiimide and N,N-dimethylaminopyridine to yield 30. In the former case, the Boc protecting group was split off with TFA and the product 29 was formylated using the p-nitrophenyl ester of formic acid to give 30. Product 30 was converted into the isocyanide 31 using diphosgene as dehydrating agent and N-methylmorpholine as base.

Polymerisation of 31 was carried out in CH2Cl2 under air in a reaction vessel protected from light using 3·103 equivalents of Ni(ClO4)2·6H2O as catalyst. Two different polymerisation reactions, which were dependent on the two synthetic routes used to obtain the formyl adduct 30, were found to take place. These two routes (the short route from 27 directly to 30 using N-formyl-alanine and the long route using Boc-alanine) had a strong effect on the polymerisation reaction time. The polymerisation of the isocyanide monomer that was synthesised using the short route (leading to 32a) took 4-5 days to finish (although the monomer was never completely consumed), whereas the polymerisation of the isocyanide obtained via the long route (leading to 32) took only a few minutes to be completed. Consequently, the latter polymerisation was found to be more efficient and cleaner (as deduced from tlc). The yields of polymerisation reactions amounted to 68% (32a) and 81% (32), respectively.

1H-NMR showed strong broadening of all the proton signals, suggesting that the polymers had a rigid structure. Both polymers displayed in the IR spectrum a C=N stretch vibration at 1605 cm⁻¹, which is indicative of the formation of a polyisocyanide. Furthermore, an N-H stretching vibration at 3265 cm⁻¹ next to one at 3313 cm⁻¹ was observed, which implies a structure in which the side chains form a hydrogen bonding network. The presence of the 3313 cm⁻¹
vibration, however, suggests that not all the amide functions are involved in hydrogen bonding, indicating that the hydrogen bonding arrays are not so well organised as in L,D-PIAA.\textsuperscript{[1, 2]} The hydrogen bonding network was further confirmed by the occurrence of an amide carbonyl vibration which was shifted to 1655 cm\textsuperscript{-1}.

\begin{scheme}
\textbf{Scheme 1} Synthesis and polymerisation of an isocyanide monomer derived from a porphyrin with a propyl-alanyl spacer. (i) Bromopropyl phthalimide, K\textsubscript{2}CO\textsubscript{3}, DMF; (ii) H\textsubscript{2}NNH\textsubscript{2}·H\textsubscript{2}O; (iii) Bromopropylamine hydrobromide, NaOH, DMF; (iv) Boc-L-ala, DCC/DMAP, CH\textsubscript{2}Cl\textsubscript{2}, 0°C; (iva) N-Formyl-L-alanine, DCC/DMAP, CH\textsubscript{2}Cl\textsubscript{2}, 0°C; (v) TFA, CH\textsubscript{2}Cl\textsubscript{2}; (vi) Formyl-ONP, CH\textsubscript{2}Cl\textsubscript{2}; (vii) diphosgene/NMM, CH\textsubscript{2}Cl\textsubscript{2}, O°C; (viii) Ni(II)/ethanol, CH\textsubscript{2}Cl\textsubscript{2}; (32a = polymer obtained via short route (iva), see text).
\end{scheme}
4.2.2 Absorption spectroscopy

The absorption spectra of monomer 31 and polymer 32 are depicted in Figure 1. The spectra of each of these compounds were identical when recorded in chloroform and in aromatic solvents such as toluene. Dramatic differences are observed when the monomer spectrum is compared to that of the polymer. The monomer displays a Soret band at 421 nm, whereas in the polymer this band has disappeared and two new Soret bands are present at 413 and 437 nm. The splitting of the bands and the very sharp absorption observed at 437 nm are indicative of the presence of well-defined and excitonically coupled stacks of porphyrin molecules. The absorption at 437 nm can be tentatively assigned to a $J$-type of aggregate (see Chapter 2). The absorption band of $J$-aggregates appears at higher wavelengths and is generally intense and narrow. The Soret band at 437 nm is attributed to an offset stacking of the 1$^{st}$ and 5$^{th}$ porphyrin and the blue shifted band at 413 nm is attributed to a combination of interactions between the 1$^{st}$ and 4$^{th}$ porphyrin and between the 1$^{st}$ and 2$^{nd}$ porphyrin in a side-on stacking geometry. The Q-bands exhibit very weak or no exciton coupling since they do not show clear shifts (the two lowest energy Q-bands have shifted 1 nm to the red).

In addition, a small absorption band at 307 nm is apparent in the UV-Vis spectrum at higher concentrations (shown in the inset spectrum in Figure 1). This absorption band is due to the n-$\pi^*$ transitions of the imine groups in the polymer backbone and appears in this wavelength region whenever polyisocyanides are present with dipeptide side groups that form well-defined arrays.

![Figure 1 Absorption spectra of monomer 31 (dotted line) and polymer 32 (full line) in CHCl₃. Inset shows the n-$\pi^*$ imine absorption at 307 nm (expanded 15x).](image-url)
The absorption spectrum of the polymer was found to be temperature dependent, indicating a slight conformational change in the porphyrin arrangement or partial aggregation between different polymers. These observations will be discussed later in §4.2.8.

### 4.2.3 Circular dichroism spectroscopy

The circular dichroism spectrum of a solution of 32 in CHCl$_3$ (Figure 2) showed a strong bisignate Cotton effect originating from the porphyrin Soret band at 437 nm and smaller bisignate signals with lower intensities corresponding to the blue shifted Soret band. The shape (sometimes completely inverted spectra were obtained) and the size of the CD signal were very sensitive to a variety of physical parameters, viz. temperature, ageing, etc. and the spectrum presented here is the final spectrum that remained constant, and all measurements have been performed on solutions of 32 displaying this spectrum (see § 4.2.12 for a discussion about this phenomenon). Interestingly, no CD signal was observed for the porphyrin monomer 31. The intense bisignate signal at 431 nm is nearly conservative and is blue shifted by 6 nm due to overlap of other transitions. This signal stems from coupling interactions between the 1$^{\text{st}}$ and the 5$^{\text{th}}$ porphyrin molecule in the polymer backbone, which are located on top of each other (vide infra). From the negative sign of this couplet a left-handed helical arrangement of the porphyrins can be inferred, according to the exciton chirality models developed for porphyrins.$^{[3-5]}$

![Figure 2 CD spectrum of polymer 32 in CHCl$_3$ (absorption spectrum depicted as dashed line).](image)

Since the CD signal is very strong, the porphyrin arrangement in this polymer may resemble those of large naturally occurring chromophore assemblies like the rod-shaped cylindrical aggregates involving thousands of BCl$_3$ molecules. In the case of large assemblies simple
theories like the exciton chirality theory may not be fully valid and conclusions about the orientation of the porphyrins are only tentative. The positive couplet at 400 nm is assigned to an exciton coupling between the 1st and 4th porphyrin which have a right-handed orientation with respect to each other in the porphyrin stack, as was observed by Takahashi et al. for related porphyrin polymers.[12]

Typical for amino acid derived polyisocyanides with hydrogen bonded side chains, like PIAA, is a Cotton signal corresponding to the n-\pi* transition at 310 nm, which always appears in this wavelength region. The other, non-hydrogen bonded polyisocyanides show signals that can range from 250 to ca. 360 nm, suggesting a different conformation of the side groups. The CD spectrum of 32 showed a negative Cotton effect with a minimum at 272 nm, (its intensity is low so it can only be detected at higher concentrations) which is assigned to this imine n-\pi* transition (Figure 3). This wavelength position has been observed before, viz. in the case of peptide-derived polyisocyanides that form hydrogen bonding arrays, but are not as well-defined as PIAA. In the applied concentration range, CD absorptions for the Q-bands are also visible (Figure 3).

![Figure 3 CD spectrum of a solution of polymer 32 (20x more concentrated) showing Cotton effects in the imine absorption and in the Q-band region.](image-url)

### 4.2.4 Fluorescence spectroscopy

The emission spectrum of monomer 31 showed a typical two-band profile of a free-base porphyrin, originating from the transitions Q(0-0) and its vibronic band Q(0-1). Polymer 32 displayed essentially the same two bands (Figure 4), but the Q(0-1) had gained in intensity with respect to Q(0-0). The maxima were hypsochromically shifted by 3 nm and the intensity had
decreased significantly to about 1/5 of the monomer emission, which can be ascribed to quenching effects of neighbouring porphyrins, further indicating that interactions exist between the stacked porphyrins.

![Emission spectrum of polymer 32 (CHCl₃).](image)

**Figure 4** Emission spectrum of polymer 32 (CHCl₃).

### 4.2.5 Atomic force microscopy

Single molecules of polymer 32 could be visualised by atomic force microscopy (AFM) in tapping-mode using spin-coated solutions of 32 on mica. The results are presented in Figure 5. At high concentration (∼10⁻⁵ M) networks of monolayer islands of aggregated polymers are visible (Figure 5a,b), as is often seen for rigid rod polymers. Diluting results in the formation of separated monolayer islands (Figure 5c-e) and at high dilution, isolated single polymer strands can be observed (∼10⁻⁷ M, Figure 5f,g). Further dilution did not result in any changes of the features. The images reveal that polymer 32 has a rod-like character as was expected for polyisocyanides with hydrogen bonding amino acid side chains.[¹, ⁶] The measured height of the single molecules was 4.2 (± 0.3) nm and that of the islands 4.7 (± 0.2) nm. CPK models indicate that the diameter of a polymer chain with stretched side groups is approximately 8.6 nm, and that of a chain without tails 5.6 nm. It has been often noted that due to the indentation of the tip the measured height is less than the real height.[⁷] The measured values, however are in good agreement with the calculated heights and confirm the presence of single molecules with lengths up to hundreds of nanometres. Polymers of phenyl isocyanides have been shown previously to exhibit fibre-like architectures.[⁸] Although the persistence lengths were not determined, those measurements indicated that the rigid character is not exclusively observed for hydrogen-bonded polyisocyanides.
Figure 5 AFM images of polymer 32 spincoated on mica (CHCl₃); (a,b) Structured network of connected monolayer islands (10⁴-10⁵ M); (c,d,e) Isolated islands formed after dilution clearly showing the constituent polymer fibres (10⁵-10⁶ M); (f,g) Single polymer molecules visible at very low concentrations (10⁻⁷ M). (Bar represents 100 nm.)
These polymers however transform to another, thermodynamically more favourable all-trans conformation (see Chapter 2). For the PIAA’s on the other hand, having amino acid side chains in a hydrogen bonding arrangement, it is suggested that the hydrogen bonds lock the helix into a rigid conformation. Without the hydrogen bonds, the helix is more flexible and can adopt different conformations.

4.2.6 Molecular weight determination

Since it was not possible to determine the molecular weight and polydispersity of 32 by MALDI-TOF or GPC due to the rod-like nature of the polymers, analysis of the AFM images was used to calculate these parameters. It has been shown by Möller et al. that the lengths of rigid, dendronised polymers can be reliably estimated by measuring the contours of these polymers on AFM images.\textsuperscript{[9, 10]} Cornelissen et al. have also used this procedure to estimate the molecular weights of a series of polyisocyanides derived from dipeptides.\textsuperscript{[1]} Several images of the type shown in Figure 5f,g were used to evaluate the polymer contour lengths. The results are shown in Figure 6.

![Figure 6](image)

**Figure 6** Histogram of the length distribution of 32, as calculated from the AFM images.

From the histogram of the AFM images the weight-average and number-average apparent length ($L_w$ and $L_n$) and the length polydispersity $PD$ were calculated using the following equations:

$$L_w = \frac{\sum_i N_i \cdot L_i^2}{\sum_i N_i \cdot L_i}, \quad L_n = \frac{\sum_i N_i \cdot L_i}{\sum_i N_i}, \quad PD = \frac{L_w}{L_n}$$

The former two values amounted to: $L_w = (87 \pm 7)$ nm and $L_n = (69 \pm 5)$ nm respectively, giving a $PD = 1.27 \pm 0.14$. This polydispersity is slightly lower than the one measured for L,L-PIAA.
(PD = 1.4), which was also polymerised using Ni(II) and considerably lower than the PD measured for L,D-PIAA (PD = 1.7). The high PD’s observed for L,L- and L,D-PIAA relate to a higher rate of propagation compared to the rate of initiation and consequently to less control over the degree of polymerisation.\textsuperscript{[11]} It is proposed that for the polymerisation of 32 an additional steric factor slows down the rate of propagation and hence lowers the PD. The length of 32 is considerably shorter than the length of the PIAA’s, while the monomer/catalyst ratio was much higher, again implying some steric hindrance.

Assuming that every monomer segment adds 1.05 Å to the polymer chain,\textsuperscript{[12]} a weight-average length of 87 nm corresponds to a degree of polymerisation of ca. 830 and a weight average molecular mass of 1.1·10\textsuperscript{6} Daltons. To date this is the largest porphyrin array reported in the literature, however, even larger arrays will be presented further on.

4.2.7 Control over the polymer length: varying the monomer/catalyst ratio

In total, three different catalyst/monomer ratios were used to prepare polymer 32; 1/50, 1/300, and 1/500 (32\textsubscript{50}, 32\textsubscript{300}, 32\textsubscript{500}, most of the physical studies have been performed on 32\textsubscript{300}). It should be noted that 32\textsubscript{50} was obtained using a different initiator (see Chapter 7), namely one used for the preparation of a block co-polymer. Figure 7 shows AFM images of spin-coated polymers 32\textsubscript{500} on mica. It is immediately evident from the AFM images that polymers 32\textsubscript{500} are longer than the 32\textsubscript{300} polymers. An often observed property of rigid rods is their tendency to aggregate and form bundles. This can already be seen in the height image (Figure 7a, arrow), where the brighter (higher) and broader fibres are the aggregated polymer molecules.

\textbf{Figure 7} AFM images of polymer 32\textsubscript{500} showing fibres longer than those seen for 32\textsubscript{300} (Left: height image. Right: phase image). Inset: the formation of bundles is apparent from the darker part of the fibre (scale bar = 1\,µm).
The phase image shows this phenomenon more clearly, viz. as dark parts in the fibre, which correspond to the brighter parts in the height image. This can be explained as a bundle of intertwined fibres, possibly a double helix to optimise their Van der Waals interactions. Therefore, the outer polymers have to wrap around a polymer fibre, possibly exposing a different surface compared to the single fibres, thus causing a different phase image. The height of the single polymer fibres amounted to $3.0 \pm 0.2$ nm and the height of the entwined fibres to $6.0 \pm 0.7$ nm. This suggests that the porphyrins of the neighbouring fibres are not intercalated, since in that case the height of the entwined fibre would be expected to be smaller, although further characterisation is needed to support this view. (For AFM images of polymer 32$_{50}$ see Chapter 7.)

Infrared spectroscopy revealed that for the short polymer 32$_{50}$, the vibrations at $3313 \text{ cm}^{-1}$ (amide not involved in hydrogen bonding) and $3265 \text{ cm}^{-1}$ (amide involved in hydrogen bonding network) were equally strong. Compared to 32$_{300}$ the absorption of the band at $3313 \text{ cm}^{-1}$ is more intense, which is explained by the fact that for 32$_{50}$, the ill-defined end-group sections become more important compared to the central well-defined body section of the polymer.

After polymerisation polymer 32$_{50}$ was purified by washing with acetone, which removes the monomer, and then by washing with a small amount of CH$_2$Cl$_2$, which removes the oligomers. As was observed from AFM microscopic images of the washing solutions, the polymers were very short (not shown). The absorption spectrum of this low molecular weight product showed a broadened monomer-like spectrum with a maximum at 422 nm. Since the abundant end-groups have no well-defined exciton interactions, these oligomers can be expected to show mainly a monomer-like spectrum with no CD signal. A small CD absorption band was, however, observed around 430 nm at the same position as the large bisignate signal of the long polymer, which can be attributed to an oligomeric well-defined $J$-type porphyrin stack in the central part of the polymer.

When the absorption spectra of the three polymers (32$_{50}$, 32$_{300}$, 32$_{500}$) are normalised and placed on top of each other, a trend is observed: the intensity of the blue bands at around 412 nm decreases with respect to the $J$-band at 437 nm when going to less catalyst in the polymerisation mixture, i.e. to longer polymers. In Figure 8 this is shown for a normalised $J$-band; the intensity of the blue band at 410 nm decreases with increasing monomer/catalyst ratio. Thus, it is found that the polymer length increases with increasing monomer/catalyst ratio. The increased intensity of the $J$-band reflects that a larger part of well-defined porphyrins is present in these polymers.
4.2.8 Variable temperature CD spectroscopy

In order to investigate the stability of the conformations of polymers 32, in particular of their helical structures, the temperature dependent behaviour of the CD absorptions was studied. The initial experiments were performed on the short polymer $32_{50}$ in CHCl$_3$. When the temperature was increased, the signals in the CD spectrum at 431 nm and 410 nm (Figure 9) were observed to decrease linearly, until no CD signal was observed at 42ºC. The signal went through an isodichroic point at the zero-point crossing of the bisignate signal, indicating a transition between two well-defined states.

![Figure 8 Normalised absorption spectra of the three different polymers $32_{50}$ (dashed line), $32_{100}$ (full line), $32_{200}$ (dotted line).](image)

Figure 8 Normalised absorption spectra of the three different polymers $32_{50}$ (dashed line), $32_{100}$ (full line), $32_{200}$ (dotted line).

![Figure 9 CD spectra of polymer $32_{50}$ showing a temperature dependent behaviour: the signals decrease to zero and do not reappear upon cooling. Right: graph of the CD signal at 427 nm as a function of the temperature revealing a linear decrease.](image)

Figure 9 CD spectra of polymer $32_{50}$ showing a temperature dependent behaviour: the signals decrease to zero and do not reappear upon cooling. Right: graph of the CD signal at 427 nm as a function of the temperature revealing a linear decrease.
The linear decrease monitored at 427 nm is depicted in Figure 9, right. After cooling to room-temperature the CD signal did not reappear. This behaviour can tentatively be ascribed to an unwinding of the polymer helix from a well-defined organisation of the porphyrins (state I) having a helical conformation, to a random coil polymer (state II) that has no CD signal and hence has no expression of chirality. After the temperature cycle a very small Cotton effect, just above the level of the noise, was still visible. This effect was measurable even after several temperature cycles and is believed to be caused by random intramolecular interactions of the porphyrins within the random coil polymer.

The same experiment was carried out on polymer 32\textsubscript{300} in CHCl\textsubscript{3}. The temperature dependent behaviour monitored for this molecule with CD spectroscopy was found to be completely different from that of 32\textsubscript{50}, see Figure 10 (the effect of heating on the n-\pi* transition will be discussed later, see Figure 12). Upon increasing the temperature the signal slightly dropped in intensity until 50°C after which a rapid decrease was observed. After this decrease the CD signal remained constant. The change in CD absorptions gave rise to several isodichroic points, viz. at 432, 424 and 402 nm, reflecting a transition between two distinct polymer conformations. Since there was still a significant CD signal left at 60°C, this result suggests that the porphyrins adopt a new conformation. It has been shown by Iyoda \textit{et al.} that polyisocyanides derived from phenyl alanine, having side groups with the appropriate bulkiness, can adopt different stable conformations depending on the temperature.\[13\]

\textbf{Figure 10} (a) CD spectra showing temperature dependent behaviour of polymer 32\textsubscript{300}. The CD signal decreases with increasing temperature, going through isodichroic points, reflecting two different conformations. (b) Graph of CD vs temperature monitored at 428 nm, highlighting the cooperative unfolding process.

When the sample of 32\textsubscript{300} was cooled down, the CD signal returned to almost its original level. This is in contrast to the earlier experiment with 32\textsubscript{50}, in which the helical porphyrin arrangement was found to disrupt irreversibly, presumably due to breaking of the hydrogen
bonds. In the case of the longer polymer the hydrogen bonding network is thought not to be irreversibly disrupted. The observed change in CD signal is probably the result of a change in the conformation of the porphyrins, which is a reversible process. This heating-cooling cycle could be repeated several times, during which the \( J \)-band gradually lost intensity. This indicates a slow unfolding of the porphyrin helix, analogously to what was observed for \( 32_{30} \). This process probably starts at the two polymer ends, and does not proceed far enough to completely unwind the helix.

When a complete temperature cycle was carried out, the CD signal of the polymer reappeared again. The curve was s-shaped and displayed some hysteresis. The amplitude of the CD signal was not fully regained with time. Five temperature cycles were carried out, and these showed a gradual decrease of the CD signal. The corresponding UV-Vis absorption spectrum revealed a concomitant decrease of the \( J \)-band and an increasing band at 420 nm. This suggests that the conformation of the polyisocyanide backbone converts from a well-defined helix conformation to a disordered coiled conformation as was observed for \( 32_{30} \). In line with this, the IR spectrum of \( 32_{300} \) showed that, after heating for prolonged periods, the band at 3313 cm\(^{-1}\) gained intensity at the expense of the band at 3265 cm\(^{-1}\).

![Figure 11](image)

**Figure 11** A heating(A)-cooling(B) cycle on polymer \( 32_{300} \) showing the hysteresis effect.

The effect of the temperature on the conformation of the polymer was also investigated by following the behaviour of the imine transition at 270 nm (Figure 12). At room temperature (20°C) the imine absorption exhibited a Cotton effect with a minimum at 270 nm. Upon heating up to 60°C, the intensity of this band did not change very much, only a slight blue-shift of ca. 1-2 nm was observed. After returning to 20°C the spectrum appeared to be nearly
identical to the starting spectrum. From this experiment, it can be concluded that, despite the clear impact heating has on the organisation of the porphyrin stacks, the central helical core of the polyisocyanide is hardly affected. The poly-imine structure is very sensitive to changes in hydrogen bonding and this experiment suggests that the hydrogen bonding network remains largely intact. It is known that polyisocyanides without hydrogen bonding arrays easily unwind upon heating. It should be noted that a small amount of unwinding can probably not be detected with this method.

![Graph](image)

**Figure 12** The effect of increasing the temperature on the Cotton effect at 270 nm.

The UV-Vis and CD experiments as a function of temperature enable one to draw a more or less complete picture of the changes that take place in the conformation of polymer $3_{2300}$. It is evident that the changes at around 50°C in the CD spectrum only occur in the Soret band and not in the imine band, indicating that the orientation of the porphyrin arrays alters whilst the conformation of the hydrogen bonded polymer backbone remains the same. A similar temperature dependent behaviour has been observed for self-assembled porphyrin stacks (composed of zinc porphyrins with amine side arms), which reversibly assemble at low temperatures and disassemble at high temperatures.$^{[14]}$ The porphyrins in polymer $3_{2300}$ can be envisaged as being a sort of a self-assembled array that behaves independently of the polymer. Although they are chemically linked to the polymer, the porphyrin molecules still have enough flexibility to exhibit a behaviour that is analogous to that of a supramolecular aggregate. Earlier studies with polymers of isocyanodipeptides without porphyrins have revealed that the hydrogen bond network can break up at higher temperature, while, somewhat surprisingly, this is not observed in the present case and the change in the porphyrin organisation is not caused by a disruption of the hydrogen bonds in the rigid polymer scaffold, but is merely caused by a

---

- 79 -
change in the $\pi-\pi$ stacking interactions of the porphyrins themselves. Since the hydrogen bonding network remains intact, the rigid polymer backbone is retained and the reorganisation is reversible. The only thing that happens after cooling, is that the porphyrins recover their energetically preferred arrangement.

In summary, it may be concluded that for the short polymers an increase of the temperature leads to a small, linear decay of the CD signal in the region of the porphyrin Soret band. This signal reaches zero and does not reappear which indicates that the helix unfolds irreversibly at higher temperature, probably starting at the ends of the polymer chain, reminiscent of the behaviour seen for other PIAA’s. It should be noted that this conclusion needs further proof by monitoring the n-$\pi^*$ transition during a temperature cycle. The unfolding of end group sections may also play a (minor) role in the case of the longer polymers (32300), the most important process, however, is the co-operative rearrangement of the porphyrin stacks from one conformation to another. The poly-imine structure appeared not to be affected by the temperature increase, and hence the observed changes can be attributed exclusively to a change in the porphyrin arrangement. Since the polymer backbone remains intact, the porphyrin assembly cannot break up completely like in a supramolecular aggregate, but is forced to adopt a new conformation, which upon cooling reverts to the original organisation of the porphyrins.

In order to study the effect of unfolding further, polymer 32300 was also dissolved in toluene and subjected to a temperature cycle up to 90°C. In a first set of experiments, the absorption spectra of an unheated solution and a solution that had been heated to 70°C for 2 mins and then cooled down, were recorded (Figure 13). Significant spectral changes were observed: the red-shifted $J$-band at 437 was found to disappear and two new blue shifted bands were detected with maxima at 416 and 407 nm. These changes indicate new exciton interactions between the porphyrins and are the result of a significant change in the orientation of the porphyrins. A blue shifted Soret band generally points to the formation of an $H$-aggregate, wherein the porphyrins are oriented face-to-face (see Chapter 2).
In a subsequent series of experiments the solution of 32300 in toluene was subjected to a temperature cycle up to 90°C and studied with CD spectroscopy. The CD spectrum in toluene (Figure 14a) at room temperature appeared to be different from that in chloroform. This is in contrast to the absorption spectrum that showed identical spectra in CHCl₃ and toluene (§4.2.2). In an aromatic solvent the porphyrins probably adopt a slightly different conformation, which is not apparent in the absorption spectrum, but results in an altered CD spectrum.

In an identical fashion to what was observed for 32300 in chloroform, the starting spectrum (dashed line, Figure 14a) changed upon increasing the temperature into another spectrum (full line). When the CD intensity at 409 nm was plotted vs the temperature (Figure 14b), a clear change was visible, starting at 33°C, and reaching a maximum at 55 °C, after which the intensity decreased again. However, upon lowering of the temperature, the original spectrum did not reappear, but merely the new conformation remained, even at low temperatures as is clear from a second temperature run (Figure 14b).

This result indicates that higher temperatures cause an irreversible conformational change, as was also evident from the absorption spectra. Apparently, the initially formed polymer is a kinetic product which transforms to a thermodynamically more stable helical conformation upon heating. The sharp transition at 50°C was also observed for 32300 in chloroform, and is ascribed to a conformational change of the porphyrins.

The different behaviour of 32300 in CHCl₃ and toluene can be explained by the fact that these solvents have different effects on the π-π stacking interactions of the porphyrins. In toluene the π-π stacking interactions between the porphyrins may be partly suppressed, because the
interactions between the porphyrins and toluene predominate.[15, 16] Keeping in mind that the polymerisation was performed in CHCl₃, in toluene the porphyrins probably tend to optimise the interactions with this aromatic solvent, hence showing an irreversible conformational change.

To summarise this part, it is concluded that the helical conformation of polymer 32₃₀₀ is almost completely maintained upon warming, although a small part (probably end groups) unwinds. Generally, when polyisocyanides have no bulky side groups, the polymer readily unfolds upon increasing the temperature to a more extended helical conformation, while in the case of bulky side groups, the helix remains intact.[13] In the case of polymer 32, the bulkiness of the side groups in combination with the hydrogen bonding arrays and the existing porphyrin-porphyrin interactions may lead to a stable helix. It seems that the porphyrins provide an extra stabilising interaction, since in the case of PIAA, the Cotton effect in the imine transition showed a clear decrease in intensity upon heating.[1] Contrary to the polymer backbone, the porphyrins can adopt different conformations with temperature, and as such could provide a unique temperature responsive switch, in which the one dimensionally ordered porphyrin array does not break up but merely changes conformation.

4.2.9 Resonance light scattering

In order to obtain more insight in the precise geometry of the porphyrins in these long rigid polymers the technique of resonance light scattering (RLS) was utilised. This technique has proven to be a sensitive and selective tool to investigate the electronic and geometrical properties of aggregated chromophores, such as synthetic porphyrins[17-19] and natural

---

Figure 14 a) CD spectra of 32₃₀₀ in toluene at room temperature before heating (dashed) and after heating (full line). b) CD intensity as a function of temperature, monitored at 409 nm, two consecutive runs are depicted.
chlorophyll $a$ arrays.$^{[20]}$ In particular, depolarised RLS measurements can give further information about the relative orientation of the stacked porphyrin molecules.$^{[21]}$

The RLS effect is an enhancement of the scattered light intensity in the red edge of an absorption band, which is related to a) an electronic coupling between adjacent chromophores, b) the size and the geometry of the aggregates in which the chromophores are present, and c) to the molar absorbance of the individual chromophores.$^{[17-19]}$ This phenomenon allows for the identification of the precise orientation of the chromophores in an aggregate and their interactions even when they are present in complex matrices. A simple quantum mechanical model for RLS, based on exciton-coupling theory, has been recently developed which addresses the relationship between the intensities of the observed RLS features and the electronic and geometrical properties of the aggregates.$^{[21]}$

The RLS spectrum of polymer $32_{300}$ (Figure 15) showed an intense peak (at least two orders of magnitude larger than the peak of the neat solvent) in the region of the Soret-band (444 nm), accompanied by a deep well in the range 408-438 nm, due to photon absorption. The enhancement observed for the scattered light intensity of the polymer is consistent with the presence of large domains of more than 25 ($n >> 25$) interacting chromophores.

These measurements were also performed on polymer $32a$ which was obtained using the alternative synthesis, namely via the direct coupling with formyl alanine (see § 4.2.1). The polymerisation time for this polymer was considerably longer and the nanorods as observed by AFM were shorter. The measured RLS spectrum however was identical to the one shown in Figure 15, which indicates that the polymer obtained via the alternative synthesis route, still
possesses a well-defined chromophoric array in which the porphyrin molecules are excitonically coupled.

In order to check if the previously synthesised polymers (Chapter 3) also exhibited extensive electronic coupling, the RLS spectrum of polymer 23 (see Chapter 3) was recorded. It showed profiles with minima in correspondence with the absorption features (due to photon loss by absorption; Figure 15). The intensity of the spectrum, however, was only slightly larger than that of the neat solvent. The absence of any detectable RLS effect points to very small polymers in which the number \( n \) of interacting porphyrins is less than 25 or to a high degree of disorder in the polymer structure. These results are in agreement with the earlier described idea that 23 is a short polymer, as was concluded from AFM images.

The theory developed for RLS predicts that, contrary to the scattering cross section, which is strongly dependent on the size of the aggregate, the value of the depolarisation ratio \( \rho_\lambda(90) \), is related only to the principal values of the polarisability tensor at the resonance wavelength. Assuming a parallel arrangement of the transition moments of the exciton-coupled porphyrin chromophores, and applying this simple model the slip angle \( \alpha \) between adjacent porphyrin planes can be calculated. The expected range for this ratio is \( 1/8 \leq \rho_\lambda(90) \leq 1/3 \), with the maximum value corresponding to \( \alpha = 90^\circ \) and a symmetric behaviour of this parameter around \( \alpha = 45^\circ \). To be sure that the depolarisation ratio was not affected by the very strong CD of the sample due to the chirality of the system, a standard sample was used that did not show any ellipticity. The polariser set-up was found not to be sensitive to the rotary power of the polymer. As a consequence it can be stated with confidence, that the calculated value is solely due to the depolarisation of the system, and that, in turn, this depolarisation is related to the excitonic interactions.

In addition to obtaining information concerning the number of interacting chromophores, depolarised RLS studies can also give details about the precise orientation of the chromophores in the polymeric stacks. Figure 16 shows the dispersion profile of the depolarisation ratio \( \rho_\lambda(90) \) at the absorption feature of polymer 32\textsubscript{300}. The value of \( \rho_\lambda(90) \) provides information about the geometry of the excited state of an aggregated species.\textsuperscript{[21]} The absorption feature at 437 nm has the appearance of a \( J \)-band and the corresponding \( \rho_\lambda(90) \) is 0.185. From this number an angle \( \alpha \simeq 30^\circ \) between porphyrin molecules 1 and 5 which are stacked 4.2 Å apart\textsuperscript{[12]} (\textit{vide infra}) can be calculated (see equations 17-19 in ref.\textsuperscript{[21]}) and a twist angle \( \beta \) (Figure 17) of 22º.\textsuperscript{[22]}

When the experiment on 32\textsubscript{300} was repeated in toluene (not shown), the value of \( \rho_\lambda(90) \) was found to correspond to 0.21, from which \( \alpha \simeq 28^\circ \) is derived. This slight difference in
organisation of the porphyrin side groups in chloroform and toluene is in agreement with the conclusion derived from the experiments described above, viz. that a very small difference in orientation exists between the porphyrins in toluene and CHCl₃.

![Figure 16](image)

**Figure 16** Dispersion profile (dashed line) of the depolarisation ratio in the absorption region of 32 in CHCl₃.

The measured angle $\beta$ is in complete agreement with the angle predicted from previous experimental results: crystal structures of L,L-IAA indicate that the hydrogen bonding network forces the dipeptide side chains to be 4.7 Å apart, while studies on polyisocyanides without hydrogen bonds predict an imine C₁-C₅ distance of 4.2 Å. In order to accommodate this difference, the helix would have to twist by a calculated angle $\beta$ of 20º (see §2.4.4). This value is in good agreement with the value of 22º that is derived from the RLS experiments. In addition, it is known that porphyrins have a preference for an off-set stacking,²³ and that an off-set stacking results in a red-shifted Soret band.

![Figure 17](image)

**Figure 17** Orientation of the porphyrins in polymer 32₁₀₀ shown are porphyrins 1 and 5 that display a slip angle of 30º (left) and hence a twist angle of 22º (right).
There are, however, two possible conformations that the porphyrins can adopt in order to form a $J$-aggregate: a conformation in which the porphyrin planes are perpendicular with respect to the polymer long axis (as shown in Figure 17), and a conformation in which they display a tilt with respect to the axis (vide infra). This problem will be treated in the next section.

4.2.10 Fluorescence anisotropy

In order to study more closely the orientation of the porphyrins in the polyisocyanide, fluorescence anisotropy measurements were performed on 32. Fluorescence anisotropy studies are based on the fact that when a chromophore is excited with polarised light, its emission will also be polarised. Due to rotational diffusion of the fluorophore and/or excitation energy transfer to neighbouring molecules, the polarised emission becomes depolarised. Fluorescence anisotropy measurements reveal the average angular change of the fluorophore in the time between excitation and emission. The steady-state fluorescence anisotropy $r$ is defined as

$$r = \frac{I_\parallel - I_\perp}{I_\parallel - 2I_\perp}$$

in which $I_\parallel$ and $I_\perp$ are the fluorescence intensities with the polarisation of the emission parallel and perpendicular to the polarisation of the excitation light, respectively. This value $r$ is to be corrected for polarisation dependent sensitivity of the detector and emission optics. The values for $r$ presented in this chapter are the corrected values.

When plane polarised light is incident on the sample, the absorption depends on the orientation of the absorbing transition dipole moments as the square of the cosine of the angle $\theta$ between the plane of the polarisation of the incoming light and the direction of the transition dipole.

The anisotropy expected for a simple chromophore with only one transition dipole moment that is co-linear with the emission dipole moment amounts to $r = 0.4$ in an isotropic medium. For porphyrins with two equally strong, degenerate perpendicular dipole moments, the maximum anisotropy is reduced to $r = 0.1$, since the polarisation is already lost in the plane of the porphyrin (referred to as a random absorber). From the measured anisotropy, the angle $\chi$ (the angle between the absorption, $\mu_{\text{abs}}$, and the emission, $\mu_{\text{em}}$, dipole moment) can be derived using the following expression:

$$r = \frac{1}{5} \left( 3 \cos^2 \chi - 1 \right)$$
The anisotropy of a monomeric tetra octyloxyphenyl porphyrin in toluene ($\lambda_{\text{exc}} = 420$ nm, $\lambda_{\text{det}} = 660$ nm) was measured and found to be $r = 0.0$. The theoretical value for porphyrins is 0.1 for in-plane transition dipole moments (viz. the Soret band), but due to rotation depolarisation, the orientation of the monomer molecules becomes fully randomised within the lifetime of the excited state. Slightly larger molecules, i.e. proteins have rotational times comparable to typical fluorescence lifetimes and as a result, fluorescence depolarisation measurements are a broadly used tool to estimate the hydrodynamic properties of molecules in solution and in biological membranes, since their rotational time is size (and viscosity) dependent.\cite{25}

On the other hand, if the molecule under study is large enough to be standing still on the timescale of the emission process, predictions can be made on the orientation of the chromophore(s) in the molecule. This assumption was made in order to be able to determine the orientation of the porphyrins in polymer 32 using fluorescence anisotropy measurements. Hence, conclusions about the orientation of the porphyrins are based on the assumption that the polymer 32 is immobile on the timescale of the emission. The correctness of this assumption was verified with a simple calculation to estimate the rotational correlation time $\phi$, which is given by\cite{24}

$$\phi = \frac{\eta V}{RT}$$

where $\eta$ is the viscosity, $V$ the molecular volume, $R$ the gas constant and $T$ the absolute temperature. In the case of polymer 32, these parameters amount to: $V \approx 5 \times 5 \times 80$ nm$^3$-molecule$^{-1}$ $\sim 1.3 \times 10^6$ cm$^3$-mole$^{-1}$; $T = 293$ K; $\eta = 0.6$ cP (toluene at 293 K); $R = 8.3 \times 10^7$ erg-mole$^{-1}$-K$^{-1}$. Substitution in equation 3 leads to $\phi \approx 0.3$ µs. This value applies to a globular shape, while in the case of rigid rods, the rotational correlation time is much higher.\cite{26} Fluorescence lifetimes for porphyrins are generally in the order of ns.\cite{27, 28} It can thus be safely assumed that the polymers are standing still during the fluorescence process. With the knowledge that rotational diffusion of polymer 32 is not contributing to the depolarisation, an expression is derived in the following section to relate the measured anisotropy to the orientation of the porphyrins in the polymer.

It was proven by the RLS studies (§4.2.9), that the Soret-band is extensively excitonically coupled, however, the emission arises from the Q-bands ($S_1-S_0$) in which the excitation is localised (the Q-bands do not exhibit excitonic coupling, see §4.2.2). As a consequence the emission polarisation is always parallel to the plane of the porphyrin, and circularly degenerate.
in the plane of the porphyrin. Hence it is possible to denote two fluorescence transition dipole moments: one that is oriented perpendicularly with respect to the helix axis ($\alpha_{\text{flu1}}$) and a second one that is tilted ($\alpha_{\text{flu2}}$) with respect to the polymer helix axis, each with a probability of 50%.

For ordered natural antenna complexes, with the BChl chromophores organised rotationally symmetric around the long aggregate axis (for a schematic drawing, see §1.3), such that all orientations of the transition dipoles are equally probable around this axis, an expression of $r$ was derived. It was assumed that the orientation of the absorption and emission dipoles are not correlated, and that the sample is isotropic. This expression reads:

$$r / 2 = 1 / 5 \left(0.5 \left(3 \cos^2 \beta_\mu - 1\right)\right) \left(0.5 \left(3 \cos^2 \beta_\nu - 1\right)\right)$$

Eqn. 4

where $\beta_\mu$ and $\beta_\nu$ are the angles between the symmetry axis of the complex and the absorption and emission dipole moments, respectively.

We can use equation 4 to determine the orientation of the porphyrins in polymer 32 since this polymer has rotational symmetry and the absorption and emission dipoles are not correlated because the porphyrins are excitonically coupled while the emission stems from the non-excitonically coupled Q-bands. Since polymer 32 has two emission dipoles with two different orientations with respect to the polymer axis, we have two different values for $\beta_\nu$. Using $\alpha_{\text{abs}}$ and $\alpha_{\text{flu}}$ instead of $\beta_\mu$ and $\beta_\nu$ we obtain

$$r / 2 = 0.2 \left(0.5 \left(3 \cos^2 \alpha_{\text{abs}} - 1\right)\right) \left(0.5 \left(3 \cos^2 \alpha_{\text{flu1}} - 1\right)\right) + 0.2 \left(0.5 \left(3 \cos^2 \alpha_{\text{abs}} - 1\right)\right) \left(0.5 \left(3 \cos^2 \alpha_{\text{flu2}} - 1\right)\right)$$

Eqn. 5

this rewrites to

$$r = 0.1 \times (3 \cos^2 \alpha_{\text{abs}} - 1) \times \left\{0.5 \times (3 \cos^2 \alpha_{\text{flu1}} - 1) + 0.5 \times (3 \cos^2 \alpha_{\text{flu2}} - 1)\right\}$$

Eqn. 6

wherein the $\alpha$'s represent the angles between the transition dipole moments and the helical axis of the polymer. Schematically, the conformation of the porphyrins is depicted in Figure 18. For polymer 32, with the organisation of the chromophore ensemble running along the helix axis, the absorption transition dipole moments can be divided into an overall perpendicular and parallel dipole moment with respect to the polymer helix axis. This assumption is valid if the porphyrins are strongly exciton coupled, which is the case for the bands at 405 and 437 nm in the absorption spectrum. The two angles of the transition dipole moments can now be substituted into equation 6, where $r_\parallel$ relates to $\alpha_{\text{abs}} = 0^\circ$ and $r_\perp$ to $\alpha_{\text{abs}} = 90^\circ$. 
\[ r_{\parallel} = 0.1 \times (3\cos^2 0 - 1) \times \{0.5 \times (3\cos^2 90 - 1) + 0.5 \times (3\cos^2 \beta - 1)\} \]
\[ = -0.2 + 0.3 \times \cos^2 \beta \]  
\textit{eqn. 7}

\[ r_{\perp} = 0.1 \times (3\cos^2 90 - 1) \times \{0.5 \times (3\cos^2 90 - 1) + 0.5 \times (3\cos^2 \beta - 1)\} \]
\[ = 0.1 - 0.15 \times \cos^2 \beta \]

The relation with the fluorescence transition dipole moments is as follows: \( \alpha_{\text{flu}1} = 90^\circ \) and \( \alpha_{\text{flu}2} = \text{tilt angle } \beta \). If the proposed exciton model is correct, the measured anisotropies (relating to the exciton bands at 405 and 437 nm) should relate as
\[ r_{\parallel} = -2r_{\perp} \]
\textit{eqn. 8}

\textit{Figure 18 a,b) Two possible tilted orientations of the porphyrins.}

The anisotropy values in equation 7 were calculated from measurements of which the results are presented in Figure 19. In this figure, the anisotropy is shown for different excitation wavelengths. It is clear that the fluorescence anisotropy depends on the wavelength of excitation. Excitation at 435 nm (the \( J \)-band, see §4.2.2) leads to an anisotropy value \( r = 0.05 \pm 0.005 \), while excitation at 405 nm leads to a much lower, negative anisotropy value \( r = -0.02 \pm 0.005 \). These anisotropy values demonstrate that the porphyrins indeed have a tilted geometry with respect to the long axis of the polymer, otherwise the value would be \( r = 0.1 \). If these values \( r = 0.05 \pm 0.005 \) and \( r = -0.02 \pm 0.005 \) are substituted in equation 7, \textit{viz.} in \( r_{\parallel} \) and \( r_{\perp} \) respectively (in conformance with equation 8), it follows that \( \beta = 24.1 \pm 1.3^\circ \) and \( 26.6 \pm 2.5^\circ \), which is in very good agreement, suggesting that the tilted conformation shown in Figure 18a is the correct one. The red-shifted \( J \)-band at 437 nm can thus be related to an orientation
parallel to the helix axis \( (\alpha_{437} = 0^\circ) \) and the blue-shifted exciton band at 405 nm to an orientation perpendicular to this axis \( (\alpha_{405} = 90^\circ) \).

Another possible conformation is depicted in Figure 18b. In this case the porphyrins are also tilted with respect to the helix axis. The two possibilities shown in Figure 18 can not be distinguished with the present technique. The conformation shown in Figure 18b, however, is less likely since it leads to increased steric hindrance between the porphyrin moieties and hence less favourable \( \pi-\pi \) interactions.

The effect of heating on the organisation of the porphyrins in toluene (see also §4.2.8), and consequently on the fluorescence anisotropy, was found to be very significant. Excitation of the porphyrins in polymer 32 at 405 and 420 nm (roughly the absorption maxima) resulted in both cases in an anisotropy value \( r = 0.055 \) (see Figure 20). Contrary to what was observed before heating, the anisotropy was now independent of the wavelength of excitation. This suggests that the porphyrin stacks have a uniform structure in which excitation mainly occurs in the plane of the porphyrins. The porphyrins probably are positioned in a parallel fashion with respect to each other and perpendicular with respect to the polymer axis. The measured anisotropy values indicate that the porphyrin molecules have a limited rotation during the lifetime of the excited state. Based on these results a tentative model can be derived in which the orientation of the porphyrins in the stacks changes from a tilted to a perpendicular geometry upon heating (Figure 21).
Figure 20 Fluorescence anisotropy displayed by polymer 32 heated to 70°C in toluene ($\lambda_{exc} = 405$ (light grey) and 420 nm (dark grey)).

Figure 21 Schematic drawing of the behaviour of a molecule of polymer 32$_{300}$ on heating in toluene. The porphyrins form a tilted stack with respect to the polymer long axis ($\beta$=25°; left) and change their conformation to a perpendicular arrangement after heating (right). One column of porphyrins with a J-geometry is shown in light grey for clarity.
4.2.11 Elastic light scattering

As mentioned in §4.2.3, the CD spectrum of polymer 32 was sometimes found to be completely inverted. In order to relate this phenomenon to possible aggregation of the polymer molecules in solution, elastic light scattering (ELS) experiments were performed. These measurements were carried out on a solution of \(32_{300}\) in dichloromethane and revealed the presence of nano-sized objects (schematically depicted in Figure 22b) with a correlation radius of \(~54\ \text{nm}\) (the correlation radius is that distance from the centre of the spherical aggregate at which the density of the aggregate amounts to 1/3 of the density in the centre). The optimal fitting of the measured data was performed using the Ornstein-Zernike equation for a statistical isotropic object (Figure 22a):\(^{[30]}\)

\[
S(k) \sim \frac{1}{1+(RK)^2} \quad \text{eqn. 9}
\]

where \(R\) is the correlation length for the density (size of aggregate) and \(K\) the exchange wavevector.

![Figure 22](image)

**Figure 22** (a) Elastic light scattering spectrum of 32 (dotted line), which was fitted (full line) using the model for a statistical isotropic object, schematically depicted in (b).

The ELS experiments revealed that a solution of polymer 32 with a CD spectrum exhibiting a positive couplet (the spectrum of Figure 23, *vide infra*) contained aggregates. On the other hand a solution with a negative (ultimate) couplet in the CD spectrum (as shown in Figure 2, §4.2.3), displayed no light scattering, indicating that the polymer molecules are not aggregated. The positive CD spectrum is thus ascribed to aggregated 32, whereas the non-aggregated polymer shows a negative bisignate signal in the CD spectrum.
4.2.12 Circular dichroism spectroscopy: a discussion

Circular dichroism spectroscopy is a powerful tool to investigate the conformations of helical species. However, for large helical aggregates containing many chromophores, there is still no good theory to relate the CD signals to the structure of the aggregate. The different features of the CD spectrum of 32, as presented in §4.2.3, will be further discussed in this paragraph and compared with analogous systems, reported in the literature.

The Soret band at 437 nm in the UV-Vis absorption spectrum (Figure 1) is ascribed to the formation of a $J$-structured aggregate of the porphyrins. Its intensity grows with increasing polymer length. The amplitude of the corresponding CD signal is disproportionately large and signals of this magnitude cannot be accounted for in terms of nearest-neighbour or next-to-nearest neighbour effects of the porphyrins alone. To account for these large magnitudes distant parts of the system must also be significantly coupled to each other.

When a CD spectrum of the reaction mixture in CHCl$_3$ is recorded, the intensities of the CD bands are even larger, viz. by an order of magnitude, than the one presented in Figure 2. The spectrum has a positive couplet and an asymmetric shape.

![Figure 23](image)

**Figure 23** a) CD spectrum of 32, immediately after polymerisation, inset shows magnification of Q-band region (CHCl$_3$; $\sim$10$^{-6}$ M). b) Idem, after heating.

After heating and subsequent cooling of the reaction mixture, the CD spectrum showed the reversed signal: a conservative negative couplet, similar to the spectrum described in §4.2.3, but with a much larger ellipticity (Figure 23b). Heating again, and scanning at 55°C made the signal decrease and reverts to the original level after cooling. This behaviour is identical to the behaviour described in §4.2.8, only the magnitude of the signal is higher. If the CD signals can be used as a measure of order it is apparent that an even better ordering exists of the porphyrins in the polymer immediately after polymerisation. Annealing, however, also leads to a slowly unwinding of the polymer molecules (as described in § 4.2.8). The CD spectrum in Figure 23b
of the reaction mixture of polymer 32 changed to the spectrum shown in Figure 2 after the workup and purification procedures.

Large CD signals have also been described for multiporphyrin/DNA complexes.\[^{31}\] As in these DNA complexes, the stacked porphyrins in 32 are electronically coupled, having periodic structural repeats that are responsible for the large circular dichroism signals. This “resonance effect” arises from excitonic interactions occurring over long distances. The porphyrin arrays in 32 can be considered to act as antennas, which is also the reason for the observed enhanced RLS. The fact that polymers of isocyanides have a high shape persistency facilitates the long-range interactions between the attached porphyrins. Similar phenomena have been reported for chiral anisotropic liquid crystalline phases, \(\text{e.g.} \) cholesteric phases\[^{32}\] and for thin films of helicenes.\[^{33}\] It is observed for these systems that the CD of the arrays is always orders of magnitude larger than the CD of the constituting building blocks.\[^{34}\]

Large CD effects and variation in CD intensities or sign have also been described for condensed phases of DNA, which are generally referred to as PSI (polymer and salt-induced) type CD. These large CD signals result from (\(i\)) the presence of a long-range chiral organisation in the aggregate, and (\(ii\)) delocalisation of the light-induced excitations in the chromophores throughout the entire system. Long-range coupling is possible when: (\(i\)) the aggregates or objects are large, \(i.e.\) of the same magnitude as the wavelength of the light (\(\sim 1/4 \lambda\)), (\(ii\)) the aggregate has a minimum density of chromophores (\(\sim 1 \text{ per } \text{nm}^3\)) and (\(iii\)) has a three-dimensional shape.\[^{35}\] The last requirement is essential since it favours the “collective response” of the chromophores in the aggregates. Uncondensed DNA (one-dimensional system) and biological membranes (two-dimensional) do not show these effects.\[^{36}\] The aggregation process affects the overall shape of the CD curves, which can vary widely because of the different sizes of the self-assembled species and the scattering contribution to the CD. PSI type CD spectra can be recognised by their large CD effects, the distorted (non-conservative) band shapes, the sensitivity to the preparation of the solutions, and, therefore, the difficulty in reproducing the spectra. Giant CD amplitudes observed for self-assembled porphyrin systems have been previously ascribed to this PSI-type CD effect.\[^{37}\] A very important difference between the above mentioned condensed DNA systems and polymer 32 is the fact that the latter polymer is a one-dimensional system. Since the CD spectrum of 32 is fully conservative and the length of the polymer is only tens of nanometres, it can be excluded that, unlike for the micrometre long DNA fibres, the single polyisocyanide fibres collapse in \(\text{CHCl}_3\) to form cylindrical aggregates, because they do not have the required \(\mu\text{m}\) dimensions.
Aggregates formed by 32, however, could display the PSI-type effect because they have a three dimensional shape and a size that corresponds approximately to 1/4λ.

On the other hand, one can argue that polymer 32 displays large CD magnitudes, even when it does not form aggregates in solution as shown by light scattering experiments. Hence, a three dimensional packing is not needed to show the delocalisation and collective response effect.

The BChl rod-like aggregates of the chlorosomes of green photosynthetic bacteria are also known to display variations in the CD signals. The CD spectra of these antenna systems can exhibit a strong variation with sample preparation.[38-41] Several explanations have been given for the sensitivity of the CD spectra.[42, 43] These include the following: (i) variation in sample preparation can cause variations in size of the aggregates and hence in exciton delocalisation length, which is reflected in the sign and amplitude of the CD signal; the sign of the CD signal may even invert. (ii) Calculations show that even very small changes in the orientation of the chromophores (i.e. helical conformation) can have a large effect on the signal.[42]

In contrast to these supramolecular BChl systems, the covalent polymer molecules 32 have a fixed size, they are defined physical entities in which the number of chromophores is constant, whilst in the described BChl system, a supramolecular aggregate is present.[44] In a test experiment monomer was added to the solution of the polymer in CHCl₃, but this addition did not change the structure of the bands in the UV-Vis absorption and CD spectra, rather a monomer-band appeared. So if there are any traces of monomer present in the polymer sample, it can be ruled out that they influence the spectra. The second reason (ii; see above), however, could hold for polymer 32. It could be that slight variations in the relative orientations of the chromophores lead to the observed variation of the CD signals. As was discussed in §4.2.8, conformational changes occur in time. The polymerisation reaction initially results in the formation of a kinetic product, which may undergo a transition to the thermodynamic product in solution (facilitated by heating). Hence, conformational changes may occur in the porphyrin stacks, leading to substantial changes in the CD spectrum.

4.2.13 Single molecule spectroscopy

Fluorescence spectroscopy was carried out on single molecules of polymer 32 using a confocal microscope with polarisation detection (measured in two perpendicular channels) and spectral sensitivity (for an introduction on confocal microscopy, see §6.2.7). To this end, a diluted solution of polymer 32 was spin-coated on a cleaned glass surface, to yield well-separated polymer fibres. The fluorescence spectra of approximately 30 single polymer molecules were measured (λexc = 514.5 nm, which is the Q-band), all of which showed the same behaviour. For
all the measurements, the intensity time trace (the intensity measured in time), the emission spectrum and the polarisation of the emission were recorded. The intensity of the fluorescence was found to decay continuously (Figure 24a), which is typical for an aggregate that contains many different emissive sites that are consecutively bleached. Both polarisation channels gave the same signal, which is in accordance with the presence of many emitters. From the absorption spectrum of 32 it was concluded that exciton interactions only exist in the Soret bands of the porphyrins, while the Q-bands showed no sign of interactions. Also the resonance light scattering measurements had shown that the energy delocalisation occurs in the B-bands and not in the Q-bands. Exciting the porphyrin in one of the Q-bands keeps the excitation energy on that site and the same porphyrin emits again. This satisfactorily explains the presence of the large number of emitters.

A future experiment would certainly be an experiment in which the Soret band is excited and the emission monitored. It is expected that the fluorescence emission will be polarised, since the energy can then be transferred over large distances.

The measured emission spectra matched the solution spectrum (Figure 24b, for the solution spectrum, see Figure 4), which proves that the emission comes from the porphyrin fibres. The emission profile shows two bands with different intensity and maxima that are slightly red-shifted with respect to the solution spectrum, viz. 660 vs 658 and 725 vs 724 nm respectively.

In further experiments on single polymers of 32 it would be of interest to address the question of exciton delocalisation by selective excitation in the Soret band (S₀→S₂). Moreover, it would be interesting to use a combined AFM-confocal setup to correlate any optical data with topographic information.

Figure 24 a) Time trace of a single molecule of porphyrin polymer 32. b) Emission spectrum of the same polymer.
4.3 Conclusions
The results presented in this chapter indicate that nanometre long, well-defined arrays of porphyrin molecules can be constructed by using isocyano alanine polymers as scaffolds. These polymers have a rigid chiral core to which the porphyrin chromophores are attached in a helical arrangement. The overall stack has an average length of 87 nm. The amino acid side chains form a hydrogen bonding array along the polymer, which causes the polymer to adopt a very rigid conformation.

The porphyrin molecules along the rigid polymer chain form intramolecular $J$-type of aggregates, and display a reversible conformational change upon heating in CHCl₃. However, the porphyrin organisation is lost when the polymer is heated for prolonged periods. In toluene, the porphyrins initially have a tilted geometry, but, upon heating, change their conformation to a perpendicular geometry.

The amplitudes of the CD bands are extremely large, which is ascribed to the formation of aggregates. However, after breaking up the aggregates by warming, the CD signals still show large amplitudes, which is caused by the exciton delocalisation over large distances along the porphyrin arrays. This phenomenon resembles the energy transfer in the natural antenna systems.

In the tilted geometry, as $J$-aggregate, a projection angle of 22° was calculated between porphyrins n and N+4 in the polymer chain in CHCl₃, whereas it was slightly less in toluene. The measured value of 22° is in agreement with the calculated angle.[45]

To the best of our knowledge the present polymer system is the first system displaying a unique and well-defined helical face-to-face porphyrin architecture involving hundreds of chromophores arranged over tens of nanometres.

4.4 Experimental Section

4.4.1 Atomic force microscopy
AFM experiments were performed using a Nanoscope IIIa instrument from Digital Instruments. A solution of 32 in CHCl₃ was spin-coated onto freshly cleaved Muscovite mica. All images were taken in tapping mode in air at room temperature. Commercial tapping-mode tips (Digital Instruments) were used with a typical resonance frequency ca. 300 kHz.

4.4.2 Molecular weight determination with AFM
For the analysis of the polymer molecular weight by AFM, several images, taken from different samples and different places on the mica substrate, were evaluated. Only molecules that were separated and had a consistent
height and phase appearance were measured. In total 1058 molecules were analysed. It was assumed that the overestimation of the length due to the shape of the AFM tip was equal to the overestimation of the width.

4.4.3 Fluorescence anisotropy

See §5.5.2.

4.4.4 Light scattering

Resonance light scattering experiments were performed on a Jasco mod. FP-750 spectrofluorimeter, using a synchronous scan protocol with a right angle geometry. Depolarised resonance light scattering measurements were carried out on the same spectrofluorimeter equipped with linear polarisers (Sterling Optics 105UV). The depolarisation ratio is defined as \( \rho_V(90) = \frac{I_{VH}}{I_{VV}} \), where \( I_{VH} \) and \( I_{VV} \) are the scattered light intensities with horizontal and vertical polarization, respectively. In order to correct the value of \( I_{VH} \) for the difference in transmission efficiency of polarized light from both excitation and emission monochromators, we used the equation: \( \rho_V(90) = G \times \frac{I_{VH}}{I_{VV}} \), where \( G = \frac{I_{HV}}{I_{HH}} \) is a correction factor. Depolarised dynamic light scattering measurements were made with a Malvern 4700 submicron particle analyser. Elastic light scattering experiments were performed with a home-built goniometer apparatus in the range 20º-150º (5.8-32.5 µm⁻¹ in the scattered wavevector range). The exciting light source was a 50 mW polarized Nd:YAG laser (532 nm). A Glan-Thompson polariser was placed before the photomultiplier.

4.4.5 Single molecule experiments

The experiments were performed with a confocal setup, of which the experimental details are outlined in the experimental part of Chapter 6. The polymer was excited at 514.5 nm and as a result a high laser power (15 kW cm⁻²) was used. Time traces of about 30 molecules were measured. Samples were prepared by drop casting CHCl₃ solutions of polymer 32 (∼10⁻⁸ M) on glass.

4.4.6 Synthesis

4-(Dodecyloxy)benzaldehyde (dba)

This compound was synthesised using a modified literature procedure, i.e. dodecyl bromide was used instead of hexadecyl bromide in cyclohexanone. \(^{[23]}\) \(^1\)H NMR (CDCl₃, 300.13 MHz)

\[ \delta 9.88 \text{ (s, 1H, HC(O)), 7.82 \text{ (d, 2H, ArH ortho to formyl), 6.98 \text{ (d, 2H, ArH ortho to formyl), 4.04 \text{ (t, 2H, OCH}_2), 1.81 \text{ (p, 2H, OCH(CH)CH), 1.46 \text{ (p, 2H, OCH(CH}_2OCH)}, 1.27 \text{ (m, 16H, aliphatic), 0.88 \text{ (t, 3H, CH}_3) ppm.} \]

Porphyrrin 25

A suspension of dba (15 g, 51.7 mmol) and 4-hydroxybenzaldehyde (2.09 g, 17.3 mmol) in propionic acid was heated until the compounds were dissolved. As soon as the temperature had reached 110°C freshly distilled pyrrole (4.66 g, 69.2 mmol) was added at once to the solution, whereupon the mixture was refluxed for 1h. After cooling, the obtained slurry was filtered and washed with ethanol until most of the polypyrrole side product was removed. After column chromatography (2x) (silicagel, eluent 0-2% MeOH in CHCl₃) compound 25 was isolated.
as a purple solid (0.95 g, 4.6%). $^1$H NMR (CDCl₃, 300.13 MHz) δ 8.86 (m, 8H, β-pyrrole), 8.10 (d, 6H, ArH meta to OR), 8.06 (d, 2H, ArH meta to O-aminopropyloxy), 7.28 (d, 6H, ArH, ortho to OR), 7.19 (d, 2H, ArH ortho to O-aminopropyloxy), 5.01 (br s, 1H, OH), 4.25 (t, 6H, OCH₂), 1.98 (p, 6H, OCH₂CH₂), 1.63 (p, 6H, OCH₂CH₂CH₂), 1.37 (m, 48H, aliphatic), 0.89 (t, 9H, CH₃), -2.77 (s, 2H, NH) ppm. $^{13}$C NMR (CDCl₃, 75.47 MHz) δ 158.9 (ArC ipso to OCH₂), 155.3 (ArC ipso to OH), 135.6, 134.7, 134.4 (all ArC), 131.1 (br, ArC next to ArN), 119.9, 119.5, 113.6, 112.7 (all ArC) 68.3 (ArOCH₂), 31.9 (ArOCH₂CH₂), 29.7-29.4 (m, CH₂), 26.2 (CH₃CH₂CH₂), 22.7 (CH₂CH₃), 14.1 (CH₃) ppm. HR-MALDI-TOF m/z: 1183.6860 (MH⁺) (calcd for C₈₈H₁₀₂N₅O₆: 1183.7901).

**Porphyrin 26**

To a solution of 25 (500 mg, 0.42 mmol) and N-(3-bromopropyl)-phthalimid (113 mg, 0.42 mmol) in DMF (25 mL) was added 500 mg of K₂CO₃. The flask was protected from light and the mixture was stirred under nitrogen at 110°C. After 2 hrs the DMF was evaporated in vacuo. The solid mixture was dissolved in CH₂Cl₂ and washed with water (3x). The organic layer was dried (MgSO₄) and evaporated. The purple solid was subjected to column chromatography (silicagel, eluent CHCl₃) to give the purple solid 3 (490 mg, 85%). $^1$H NMR (CDCl₃, 300.13 MHz) δ 8.86 (m, 8H, β-pyrrole), 8.10 (d, 6H, ArH meta to OR), 8.07 (d, 2H, ArH meta to OR), 7.91 (q, 2H, ArH phthalimid), 7.75 (q, 2H, ArH phthalimid), 7.27 (d, 6H, ArH ortho to OR), 7.16 (d, 2H, ArH ortho to OR), 4.34 (t, 2H, OCH₂), 4.25 (t, 6H, OCH₂), 4.09 (t, 2H, NHCH₂), 2.38 (p, 2H, NHCH₂CH₂), 1.98 (p, 6H, OCH₂CH₂), 1.65 (m, 6H, OCH₂CH₂CH₂), 1.31 (m, 48H, aliphatic), 0.90 (t, 9H, CH₃), -2.75 (s, 2H, NH) ppm. $^{13}$C NMR (CDCl₃, 75.47 MHz) δ 172.9 (C=O), 159.2, 158.6 (ArC ipso to OCH₂), 135.8, 135.2, 134.6 (all ArC), 131.1 (br, ArC next to ArN), 120.1, 119.7, 112.9 (all ArC) 68.6, 66.4 (ArOCH₂), 37.8 (CH₂NH₂), 32.2 (CH₂CH₂CH₂), 29.6 (m, CH₂), 26.2 (CH₃CH₂NH₂), 22.7 (CH₂CH₃), 14.2 (CH₃) ppm. HR-MALDI-TOF m/z: 1370.7770 (MH⁺) (calcd for C₉₁H₁₁₂N₅O₆: 1370.8534).

**Porphyrin 27**

To a solution of 26 (200 mg, 0.15 mmol) in THF (25 mL) at 60°C was added 3 mL of H₂NNH₂·H₂O. The reaction mixture was stirred for 2 hrs. After cooling to room temperature the hydrazine could be separated from the THF. The latter was evaporated in vacuo. The obtained purple solid was dissolved in CH₂Cl₂ and washed with aqueous 3N HCl, saturated aqueous NaHCO₃ solution and water (2x) consecutively, dried (MgSO₄) and concentrated. The solid was subjected to column chromatography (silicagel, eluent CHCl₃) to yield purple 27 (490 mg, 85%). $^1$H NMR (CDCl₃, 300.13 MHz) δ 8.86 (m, 8H, β-pyrrole), 8.09 (d, 8H, ArH meta to OR), 7.26 (d, 8H, ArH ortho to OR), 4.35 (t, 2H, OCH₂), 4.24 (t, 6H, OCH₂), 3.09 (t, 6H, NHCH₂), 2.13 (p, 2H, NHCH₂CH₂), 1.98 (p, 6H, OCH₂CH₂), 1.63 (m, 6H, OCH₂CH₂CH₂), 1.30 (m, 48H, aliphatic), 0.90 (t, 9H, CH₃), -2.74 (s, 2H, NH) ppm. $^{13}$C NMR (CDCl₃, 75.47 MHz) δ 158.9 (ArC ipso to OCH₂), 158.7, 135.6, 134.7, 134.4 (all ArC), 130.9 (br, ArC next to ArN), 119.8, 119.6, 113.8, 112.7 (all ArC) 68.3, 66.2 (ArOCH₂), 39.4 (CH₃NH₂), 31.9 (CH₂CH₂CH₂), 29.6 (m, CH₂), 26.2 (CH₂CH₂NH₂), 22.7 (CH₂CH₃), 14.2 (CH₃) ppm. FAB-MS m/z 1240 (M⁺).

**Porphyrin (27) alternative route**

For the synthesis of this compound a modified literature procedure was followed.[46] To a solution of 25 (350 mg, 0.30 mmol) in 10 mL DMF and 15 mL toluene was added crushed sodium hydroxide (0.245 g). After stirring for 20 min at room temperature 3-bromopropylamine hydrobromide (74 mg, 0.34 mmol) was added and the reaction
was continued for 3 h, upon which another 50 mg (0.23 mmol) of 3-bromopropylamine hydrobromide was added. The suspension was stirred for an additional 1 h and the reaction mixture was poured into water and extracted with dichloromethane (2x). The combined organic layers were washed with water, dried (MgSO₄) and evaporated to dryness. The resulting purple solid was subjected to column chromatography (silica gel, eluent 5% MeOH in CHCl₃) to yield 27 as a purple solid (276 mg, 74.2%). ¹H NMR (CDCl₃, 300.13 MHz) δ 8.86 (m, 8H, β-pyrrrole), 8.10 (d, 8H, ArH meta to OR), 7.27 (d, 8H, ArH ortho to OR), 4.35 (t, 2H, OCH₂), 4.25 (t, 2H, OCH₂), 1.48 (d, 3H, CH₃), 1.30 (m, 48H, aliphatic), 0.90 (t, 9H, CH₃), -2.75 (s, 2H, NH) ppm. FAB-MS m/z 1240 (M⁺).

**Porphyrin (28)**

Boc-L-alanine (27 mg, 0.140 mmol) was suspended in 7 mL CH₂Cl₂. To this suspension was added 27 (158 mg, 0.127 mmol). The flask was placed on an ice bath under nitrogen and to the mixture were added DCC (29 mg, 0.15 g, 0.11 mmol) was dissolved in dichloromethane (6 mL). TFA (0.5 mL, 6.4 mmol) was added and the reaction was stirred for 1 h at room temperature, after which the solvent was evaporated under vacuum. The purple solid was purified by column chromatography (silica gel, eluent: 1% MeOH in CHCl₃) to yield 143 mg (80%) of the purple product 28. ¹H NMR (CDCl₃, 300.13 MHz) δ 8.86 (m, 8H, β-pyrrrole), 8.10 (d, 8H, ArH meta to OR), 7.27 (d, 8H, ArH ortho to OR), 6.64 (t, 1H, NH), 4.35 (t, 2H, OCH₂), 3.68 (q, 2H, NHCH₂), 2.20 (p, 2H, NHCH₂CH₂), 1.96 (p, 6H, OCH₂CH₂), 1.67 (m, 6H, OCH₂CH₂CH₂), 1.48 (s, 9H, CH₃boc), 1.44 (d, 3H, CH₃ala), 1.30 (m, 48H, aliphatic), 0.90 (t, 9H, CH₃), -2.74 (s, 2H, NH) ppm. ¹³C NMR (CDCl₃, 75.47 MHz) δ 172.7 (C=O ala), 158.9, 158.4 (Ar C=O), 135.6, 135.0, 134.4 (all ArC), 130.9 (br, ArC next to ArN), 119.9, 119.5, 112.7 (all ArC) 68.3 (ArOCH₂ aliphatic), 66.5 (ArOCH₂ propyl), 50.3 (C(CH₃)₃), 49.0 (CH ala), 37.5 (CH₂NH), 33.9, 31.9, 29.5 (m), 25.5, 24.9 (CH₂ aliphatic), 28.3 (CH₃ boc), 26.0 (CH₂CH₂NH), 22.7 (CH₂CH₂), 18.4 (CH₃ ala), 14.1 (CH₃) ppm. HR-MALDI-TOF m/z: 1410.7886 (M⁺) (calcd for C₉₁H₁₂₂N₆O₇: 1410.9375).

**Amino porphyrin (29)**

In a 25 ml flask 28 (0.15 g, 0.11 mmol) was dissolved in dichloromethane (6 ml). TFA (0.5 ml, 6.4 mmol) was added and the reaction was stirred for 1 h at room temperature, after which the solvent was evaporated under vacuum. The purple solid was taken up in dichloromethane and washed with a saturated aqueous solution of sodium bicarbonate and subsequently with water (2x). The organic layer was dried over magnesium sulphate, filtered and evaporated to dryness. The purple solid was purified by column chromatography (silica gel, eluent 0-2.5 % MeOH in CHCl₃) to yield 90 mg (56%) of porphyrin 29 as a purple solid. ¹H NMR (CDCl₃, 300.13 MHz) δ 8.90 (s, 8H, β–pyrrrole), 8.14 (d, 2H, ArH meta to O-propyl), 8.11 (d, 2H, ArH meta to OC₁₂H₂₅), 7.75 (t, 2H, NH ala), 7.28 (d, 2H, ArH ortho to O-propyl), 7.27 (d, 6H, ArH ortho to OC₁₂H₂₅), 4.32 (t, 2H, OCH₂), 4.25 (t, 2H, OCH₂), 3.65 (q, 2H, NCH₂), 2.23 (p, 2H, NCH₂CH₂), 1.99 (p, 6H, OCH₂CH₂), 1.64 (p, 6H, OCH₂CH₂CH₂), 1.43 (d, 3H, alanine CH₃), 1.33 (m, 48H, aliphatic), 0.91 (t, 9H, CH₃), -2.74 (s, 2H, NH) ppm.

**Porphyrin (30)**

In a flask porphyrin 29 (142 mg, 0.108 mmol) was dissolved in dichloromethane (15 ml) and p-nitrophenyl ester of formic acid (formyl-ONP, 40 mg, 0.240 mmol) was added to the solution. The mixture was stirred for 4 h at room temperature and taken up in a saturated solution of sodium bicarbonate in water. The aqueous phase was extracted with dichloromethane (2x). The combine organic layers were washed with water (2x), dried over
magnesium sulphate, filtrated and evaporated. The product was purified by column chromatography (silicagel, eluent 0-1% MeOH in CHCl$_3$) to yield 121 mg (84%) of purple 30. $^1$H NMR (CDCl$_3$, 300.13 MHz) $\delta$ 8.86 (m, 8H, $\beta$-pyrrole), 8.22 (s, 1H, formyl), 8.12 (d, 2H, ArH meta to O-propyl), 8.10 (d, 2H, ArH meta to OC$_2$H$_2$), 7.26 (d, 8H, ArH ortho to OR), 6.50 (t, 1H, NHCH$_2$), 6.36 (d, 1H, HC(O)NH), 4.62 (p, 1H, CH), 4.33 (t, 2H, OCH$_2$), 4.24 (t, 6H, OCH$_2$), 3.65 (q, 2H, NHCH$_2$), 2.22 (p, 2H, NHCH$_2$CH$_2$), 1.97 (p, 6H, OCH$_2$CH$_2$), 1.63 (p, 6H, OCH$_2$CH$_2$CH$_2$), 1.50 (d, 3H, alanine CH$_3$), 1.30 (m, 48H, aliphatic), 0.89 (t, 9H, CH$_3$), -2.75 (s, 2H, NH) ppm. $^{13}$C NMR (CDCl$_3$, 75.47 MHz) $\delta$ 171.2 (CO, alanine), 160.3 (HCO, formyl), 158.5 (ArC ipso to OC$_2$H$_2$), 157.9 (idem), 135.2 (ArC meta to OC$_2$H$_2$ and O-propyl), 134.7 (CH, $\beta$-pyrrole), 134.0 (C, meso), 130.6 (br, ArC next to ArN), 119.5 (ArC ipso to meso C), 119.0 (idem), 112.3 (ArC ortho to OR), 76.9 (OCH$_2$), 66.1 (OCH$_2$), 47.3 (CH, alanine), 37.4 (HNCH$_2$), 31.5-22.3 (CH$_2$, aliphatic), 25.8 (CH$_2$, propyl), 18.1 (CH$_3$, alanine), 13.7 (CH$_3$) ppm. HR-MALDI-TOF m/z: 1338.882 (calcd for C$_{87}$H$_{112}$N$_6$O$_6$; 1338.880).

Porphyrrin (30) alternative route

FA (37.6 mg, 0.199 mmol, synthesis: see Chapter 3) was suspended in a solution of 27 (190 mg, 0.153 mmol) in 10 mL dichloromethane and the mixture was cooled on an ice bath. To this mixture were added dicyclohexylcarbodiimid (41.2 mg, 0.199 mmol) and a catalytic amount of dimethylaminopyridin. The reaction mixture was stirred for 1h at 0ºC under a nitrogen atmosphere and stirred overnight at room temperature. The dicyclohexylcarbodiimid (41.2 mg, 0.199 mmol, synthesis: see Chapter 3) was suspended in a solution of formamide (30) alternative route

Isocyanoporphyrin (31)

To a solution of the formamide 30 (30 mg, 22.4 µmol) in 6 mL CH$_2$Cl$_2$ was added 5.3 µL (47.4 µmol) of N-methylmorpholine. The mixture was cooled on an ice bath at 0ºC and over a period of 30 min diphosgene (1.4 mL, 11.3 mmol in 1 mL CH$_2$Cl$_2$) was added. After 10 min the ice bath was removed and the mixture was dropped into a vigorously stirred saturated aqueous NaHCO$_3$ solution (100 mL). The resulting mixture was extracted with CH$_2$Cl$_2$ (2x), the combined organic layers were washed with water (2x), dried (MgSO$_4$), and concentrated. The resulting solid was purified by preparative HPLC (reversed phase, eluent 1% MeOH in CHCl$_3$). Yield 20 mg (68%) of purple 31. FT-IR (cm$^{-1}$, KBR): 3313 (NH), 2134 (NC), 1685 (amide).

$^1$H NMR (CDCl$_3$, 300.13 MHz) $\delta$ 8.86 (m, 8H, $\beta$-pyrrole), 8.13 (d, 2H, meta to O-propyl), 8.10 (d, 6H, meta to OC$_2$H$_2$), 7.34 (d, 2H, ortho to O-propyl), 7.27 (d, 6H, ortho to OC$_2$H$_2$), 7.15 (t, 1H, NHCH$_2$), 4.39 (t, 2H, OCH$_2$), 4.25 (t, 6H, OCH$_2$), 4.34 (q, 1H, CH), 3.71 (q, 2H, NHCH$_2$), 2.26 (p, 2H, NHCH$_2$CH$_2$), 1.98 (p, 6H, OCH$_2$CH$_2$), 1.74 (d, 3H, alanine CH$_3$), 1.63 (p, 6H, OCH$_2$CH$_2$CH$_2$), 1.31 (m, 48H, aliphatic), 0.90 (t, 9H, CH$_3$), -2.75 (s, 2H, NH) ppm. $^{13}$C NMR (CDCl$_3$, 75.47 MHz) $\delta$ 166.2 (CO, alanine), 158.9 (ArC ipso to OC$_2$H$_2$), 158.3 (idem), 135.7 (ArC meta to OC$_2$H$_2$ and O-propyl), 135.1 (CH, $\beta$-pyrrole), 134.1 (C, meso), 131.0 (br, ArC next to ArN), 120.0 (ArC ipso to meso C), 119.5 (idem), 112.8 (ArC ortho to OR), 68.2 (OCH$_2$), 67.1 (OCH$_2$), 53.8 (CH, alanine), 38.7 (H$_2$NCH$_2$), 32.0-22.5 (CH$_2$, aliphatic), 26.1 (CH$_2$, propyl), 20.0 (CH$_3$, alanine), 14.2 (CH$_3$) ppm. HR-MALDI-TOF m/z: 1320.876 (calcd for C$_{87}$H$_{112}$N$_6$O$_6$; 1320.869).
Chapter 4

Polymer (32)

In a vessel protected from light monomer 31 (19 mg, 14 μmol) was dissolved in CH₂Cl₂ (1.5 mL). To this solution was added 1/300 eq. Ni²⁺ catalyst (0.35 mL of a solution of Ni(ClO₄)₂·6H₂O (2.5 mg) in 97 mL CH₂Cl₂ and 3 mL EtOH). The mixture was stirred for 1 hour, after which it was poured into 50 mL methanol/water (1/1 v/v). The precipitate was filtered and the residue washed with acetone until the filtrate was colourless, followed by washing with dichloromethane. The residue was dissolved in CHCl₃ and precipitated in EtOAc filtered and dried, resulting in 13 mg of a purple/red solid (68%). FT-IR (cm⁻¹, KBr): 3313 (NH), 3265 (NH) (ratio ca. 1:3), 1655 (amide), 1605 (C=N). ¹H NMR (CDCl₃, 400.15 MHz) δ from 9.0 to 6.7 (br with maxima at 8.7, 8.0, 7.2), 4.1 (br, OCH₃), from 2.5 to -1.0 (br with maxima at 1.6, 1.5, 1.3, 0.9, 0.7, 0.6, 0.3) ppm. ¹³C NMR (CDCl₃, 100.62 MHz) δ 32.2, 30.1, 23.1, 14.5 (aliphatic tails) ppm. For further characterisation see text. It was not possible to determine the molecular weight of polymer 2 by GPC due to severe tailing on the column. Maldi experiments turned out to be unsuccessful as well. Instead AFM was used: see text.

4.5 References

[22] The distance between the middle of the polyisocyanide backbone and the porphyrin centre was calculated to be 19 Å from CPK models and Chem3D.
Synthesis and physical characterisation of porphyrin nanorods


[44] Variations in size could occur in polymer 32 due to unwinding of the polymer at the end-groups sections. This would cause a variance in the length of helical porphyrin J-aggregate and hence the number of chromophores, consequently this could affect the relative position of the different bands. However, this explanation can be abandoned on the basis of the annealing experiments presented in §3.2.4

[45] The projection angle is calculated for polymer 25 (see Chapter 1), but is taken identical for polymer 32 since they are structurally the same, apart for the solubilising tails.

Chapter 5

Polyisocyanides containing Zinc Porphyrins

5.1 Introduction

Compounds containing multiporphyrin arrays are potentially interesting components of a new generation of performance materials\textsuperscript{[1]} (see also Chapter 2). The introduction of metals into the porphyrin cores of these materials is of particular interest since new properties may arise from this chemical modification. In addition, it opens the possibility to design and construct new architectures by using metal-ligand interactions. Metallo porphyrins have already been utilised as advanced materials in sensors, devices for data storage, \textit{etc.}\textsuperscript{[2]} The properties of these materials often are a consequence of the supramolecular organisation of the porphyrin molecules.

For the above reasons, it was thought to be of great interest to synthesise polyisocyanides with zinc porphyrin side chains and to study the effect of the addition of ligands to these polymers on their structural and physical properties. In principle, ligand interactions with zinc porphyrins may tune the optical properties of the polymers and change their conformation, \textit{e.g.} their stiffness.

In this chapter the synthesis and physical properties of two kinds of zinc porphyrin polymers (\ref{eq:33} and \ref{eq:34}) prepared from the free-base polymers discussed earlier in this thesis, are described. The first polymer (\ref{eq:33}) was used for preliminary binding studies with the ligand 1,4-diazabicyclo[2.2.2]octane (DABCO) using absorption, CD and fluorescence spectroscopy. The
second polymer (34) was studied more extensively, including both binding studies and more extensive studies of the optical properties of the polymer.

5.2 Results and discussion

5.2.1 Synthesis of polymer 33
Polymer 33 was prepared from the free-base porphyrin polymer 23 described in Chapter 3. To this end 23 was treated with zinc acetate in CHCl₃ as solvent to yield 33 in 97 % after washing with methanol and acetone.[3] The metal insertion reaction was followed by monitoring the disappearance of two of the Q-bands of the metal free derivative in the UV-Vis absorption spectrum (vide infra). The structure of 33 is shown in Figure 1a.

The size of the zinc(II) ion is too large to fit into the plane of the porphyrin. This stereochemical constraint results in a square-pyramidal co-ordination of the metal centre, leaving one co-ordination site available for external ligands. Solution studies have revealed no tendency of the monopyridine zinc(II) porphyrin to bind a second ligand molecule, e.g. pyridine, even at high concentrations of this ligand, due to the necessity of retaining the out-of-plane displacement of the zinc(II) ion.[4]

In order to learn more about the porphyrin organisation in polymer 33 and to be able to control the conformation of the porphyrins in this polymer, titration experiments were carried out with DABCO. DABCO is a bidentate ligand, known to coordinate very well to zinc porphyrins; it can be used to fix the porphyrins in a certain conformation.[5, 6] The centre-to-centre distance between two zinc porphyrins held together by DABCO is 7.6 Å,[7] resulting in a sandwich-type complex with the zinc ion pulled out of the plane by ~0.47 Å (Figure 1b).

![Figure 1a) Structure of polymer 33. b) Schematic drawing of the 2:1 zinc porphyrin-DABCO complex.](image)

The titration experiments were carried out in CHCl₃ and the UV-Vis absorption, CD and fluorescence spectra were monitored as a function of the DABCO concentration.
5.2.2 UV-Vis absorption spectroscopy

The absorption spectrum of 33 was found to change upon insertion of the zinc ion (see Figure 2). The Soret band at 421 nm became less intense and a new band appeared with a maximum at 407 nm. It is known that due to a stronger π-π stacking, zinc porphyrins are inclined to stack more co-facially than free base porphyrins, resulting in a blue shift of the Soret band. The insertion of zinc could be verified by following the disappearance of the Q-bands, since it is well established that the number of Q-bands changes from four to two as the result of the increase in symmetry upon metallation (see Chapter 2).

![Absorption spectra of free-base porphyrin polymer 23 (dashed line) and its zinc analogue 33 (full line); solvent CHCl₃, conc.: 4.1·10⁻⁶ M.](image)

The bidentate ligand DABCO was added in steps of 0.1 molar equivalents with respect to the number of monomeric units in the polymer. The latter number was calculated by dividing the weighted mass of the polymer by the molar mass of the monomer. The result of the addition of increasing amounts of DABCO on the absorption spectrum is shown in Figure 3. The Soret band was found to narrow and to display a bathochromic shift. At 409 nm an isosbestic point was observed, indicating that the reaction involved two distinct species: a polymeric DABCO-zinc porphyrin complex and a polymeric free zinc porphyrin.

Addition of DABCO caused the high energy component of the split Soret band to shift to longer wavelength. This red shift is characteristic for the binding of amine ligands to zinc porphyrins. At the high wavelength side of the spectrum a minor but significant shoulder could also be discerned. This shoulder around 440 nm is located at the same position as the sharp band that is visible in the absorption spectrum of polymer 32 (§4.2.2). In the latter polymer, this band became more intense and sharper as the polymer length increased. It was
ascribed to originate from strong Van der Waals interactions between the porphyrin side groups in the polymer.

Figure 3 a) Absorption spectra of polymer 33 in the presence of increasing amounts of DABCO; arrows indicate the observed changes. b) Titration curve as monitored at 420 nm (conc. of 33: 4.1·10^{-6}M, solvent: CHCl_3).

When the change in the absorption spectrum at one chosen wavelength ($\lambda = 420$ nm) is plotted vs. the number of equivalents of DABCO per porphyrin, a curve with an inflection point is obtained (Figure 3b). The inflection point was found to be slightly less than the expected value of DABCO/porphyrin = 0.5, which is fully in line with a sandwich-type complex and a non-cooperative binding event. From this observation and the fact that the spectra show an isosbestic point, it is concluded that a 1:2 DABCO:porphyrin complex within the polymer is formed. The changes in the absorption spectrum are relatively small, from which it can be deduced that the conformational changes are minor (vide infra). A proposed structure of the polymeric DABCO zinc porphyrin complex is schematically depicted in Figure 4.

Figure 4 Schematic drawing of the coordination of DABCO to polymer 33.

The observation of an isosbestic point is interesting, since not two, but three species can be imagined to be involved in the binding process (see Figure 5), viz. the non-ligated zinc porphyrin (a), the DABCO-porphyrin 1:1 complex (b), and the DABCO-porphyrin 1:2
complex (c). The complex of Figure 4c is relatively easily formed since the binding of the second porphyrin to DABCO in the supposedly stacked porphyrin architecture of polymer 33 is entropically free.\textsuperscript{[5]} We therefore tentatively propose that the only two species that participate in the binding, are the non-ligated porphyrin and the sandwich-type complex. The intermediate 1:1 complex is probably not observed, because it is immediately converted into the 1:2 complex. Another possibility is that the 1:1 complex has the same or nearly the same spectroscopic features as the 1:2 complex and as a result is not separately visible.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure5}
\caption{Different types of porphyrin complexes in polymer 33: a) non-complexed porphyrin, b) 1:1 complex, c) 1:2 complex.}
\end{figure}

5.2.3 Circular dichroism spectroscopy

The changes in the CD spectrum of polymer 33 as a function of the number of equivalents of DABCO added are shown in Figure 6. The initial CD spectrum showed small Cotton effects at 400-420 nm, \textit{i.e.} the region where the face-to-face interactions within the polymer occur and a stronger positive couplet for the edge-to-edge interactions with a zero-crossing at 431 nm.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure6}
\caption{a) CD spectra of polymer 33 in CHCl\textsubscript{3} as a function of the DABCO concentration; b) Change of CD intensity monitored at 440 nm as a function of the number of equivalents of DABCO per porphyrin.}
\end{figure}

Upon addition of DABCO, the intensity of the bisignate signal increased and shifted somewhat to the red (the zero-crossing of the couplet moved from 431 nm to 433 nm). The red shift is an indication of the binding of DABCO (see §4.2.2). The sign of the bands did not change, which
indicates that the conformation of the polymer does not change significantly, which is in line with the results of the UV-Vis study.

The increase in the intensity of the CD signal may be the result of a change in the distance between the chromophores. If DABCO does not fit perfectly, because it is too large or too small, it will influence the inter-porphyrin distance. Since the intensity (A) of the CD signal is inversely square dependent on the distance (r) between the porphyrins, i.e. $A \sim \frac{1}{r^2}$, this intensity increases when DABCO is added. The distance between the first and the fifth porphyrin is estimated to be ca. 7.2 Å, which is too small for optimal binding of DABCO. In order to bind, the DABCO has to push two porphyrins apart (porphyrin 1 and 5), which makes that the other porphyrins without DABCO come closer to each other in one stack (porphyrins 5 and 9). Due to the non-linear relationship between A and r, the overall signal increases. This is schematically represented in Figure 7.

In addition to this, the DABCO molecules may reduce the conformational freedom of the porphyrins, thereby fixing the porphyrin arrays, resulting in a more well defined polymer and perhaps a more intense CD signal.

\[1\ 5\ 9\ 13\ 1\ 5\ 9\ 13\]

Figure 7 Schematic drawing showing that DABCO changes the interporphyrin distances.

It is remarkable that the CD effect arises from the shoulder on the red side of the Soret band in the absorption spectrum, at the same position as observed for the CD spectrum of the well-defined long polymer 32 presented in chapter 4. In that sense, these polymers are comparable and it can be concluded that also in 33 the porphyrins are ordered in a J-type geometry like for polymer 32. For polymer 33, however, the absorption feature appears only as a broadening in the UV-Vis spectrum, because its polymer molecules are relatively short.

5.2.4 Fluorescence spectroscopy

The effect of the addition of DABCO to a solution of 33 in CHCl₃ on the emission properties of this polymer is shown in Figure 8 (only one emission band is shown because of the limited fluorescence window). The polymer was excited at its isosbestic point in the absorption spectrum (409 nm), in order to keep the absorbance constant during the experiment. The fluorescence signal was found to decrease and shift to the red upon addition of DABCO. The observed red-shift again is explained by the co-ordination of the amine ligand to the zinc centre. The decrease in fluorescence is tentatively explained by a stronger mutual quenching of
the porphyrins within the polymer, as a result of an increased ordering and a change in geometry induced by the DABCO. The emission monitored at 610 nm (see Figure 8b) showed an S-shaped profile, indicative of a cooperative process of DABCO binding. This phenomenon was not observed in the absorption or CD spectrum, hence it could be that with fluorescence spectroscopy specific porphyrins are selected, through which the cooperative process is uncovered.

![Figure 8](image_url)

Figure 8 a) Fluorescence spectra of polymer 33 in CHCl₃ at increasing DABCO concentrations. b) Change of the fluorescence at 610 nm as a function of the number of equivalents of added DABCO.

### 5.2.5 Synthesis of polymer 34

Polymer 34 was prepared from polymer 32₀₀₀ (§4.2.7) by reacting the latter with an excess of zinc acetate as described for polymer 33. The metallation reaction normally proceeds rapidly and quantitatively at room temperature, but in this case it was necessary to increase the temperature to 35°C to realise quantitative conversion. The need to increase the temperature for the reaction to occur indicates that 32₀₀₀ has a well-defined structure, as was already discussed in Chapter 4.

![Image of polymer 34](image_url)

The insertion of zinc could be verified by following the disappearance of two Q-bands in the absorption spectrum (Figure 10), from which it could be concluded that the conversion is at
least 99%. The $^1$H-NMR spectrum of 34 did not show a peak at -2.7 ppm, which is characteristic for a free-base porphyrin, confirming the high conversion.

5.2.6 Transmission electron microscopy

Polymer 32 does not contain a metal centre and, therefore, was not visible by transmission electron microscopy (TEM). Polymer 34, however, is completely decorated with zinc metal ions which makes it possible to visualise the polymers by TEM without any staining technique. Indeed, polymer 34 could be visualised by TEM (Figure 9). The electron micrograph showed a network of fibres having widths between 5-10 nm. Each fibre can, therefore, be ascribed to a single polymer chain.

Figure 9 Transmission electron micrograph of a solution of 34 (in CHCl$_3$) deposited on a carbon coated grid. Single polymer fibres with widths of 5-10 nm are visible (bar 200 nm).

5.2.7 UV-Vis Absorption spectroscopy

The absorption spectrum of polymer 34 in CHCl$_3$ was found to change dramatically upon insertion of the zinc metal ion (see Figure 10). As for polymer 33, the Soret bands seem to be inverted; the sharp band at 437 nm becomes smaller, whilst a new band appears at 406 nm. The Soret bands changed only slightly when the reaction was carried out at room temperature. Once the temperature was increased, the observed changes occurred. We ascribe this behaviour to a process in which zinc insertion in the porphyrins starts at the end groups of the polymer which are probably already accessible at room temperature. The absorption spectrum was found to be identical in toluene. The zinc containing polymer appeared to be very stable and heating to
70°C resulted in only minor effects on the absorption spectrum, unlike for the free-base derivative 32. This behaviour is ascribed to the occurrence of increased $\pi$-$\pi$ interactions\textsuperscript{[11]} between the zinc porphyrin molecules in the side chains of polymer 34 compared to the free-base porphyrins.

![Absorption spectra](image1.png)

**Figure 10** Absorption spectra of polymer 32 (dashed line) and its zinc analogue 34 (full line); inset highlights the $Q$-band regions of polymer 32 (dashed line) and 34 (full line), solvent CHCl$_3$.

When polymer 34 was dissolved in a coordinating solvent such as THF, significant spectral changes were observed (see Figure 11). The splitting of the Soret band became smaller and the whole band narrowed when compared to the spectrum in CHCl$_3$. The split Soret band has maxima at 417 and 424 nm and is asymmetric with two shoulders at 405 nm and at 435 nm. The Q-bands in THF had maxima at 557 and 598 nm, which is a red shift of 3 nm with respect to the spectrum in CHCl$_3$.

![Absorption spectra](image2.png)

**Figure 11** Absorption spectra of 34 in CHCl$_3$ (dashed line) and in THF (full line). The inset shows the Q-bands.
The coordination of THF probably increases the ground state energy of the porphyrin by increasing the electron density on the pyrrole ring nitrogens, which leads to a red-shift in the absorption spectrum.\cite{12} From the changes in the absorption spectra, it can be concluded that the coordination of THF alters the exciton coupling between the stacked porphyrins, which points to a change in the organisation of the porphyrins.

It is known that pyridine binds stronger to zinc porphyrins than THF.\cite{13} Addition of an excess of pyridine to a solution of polymer 34 in CHCl$_3$ had an even stronger effect on the absorption spectrum than THF (see Figure 12). The Soret band and the Q-bands showed a large bathochromic shift upon the addition of pyridine, the former having maxima at 421 and 429 nm with shoulders at 405 and 445 nm, while the latter appeared at 566 and 608 nm. In addition, the Q$_{0-0}$ transition had gained in intensity. The Soret-band still showed a split excitonic structure, which indicates that interactions exist between zinc porphyrins even when they are ligated with a pyridine molecule.

![Figure 12](image)

**Figure 12** Absorption spectra of 34 in different solvents: CHCl$_3$ or toluene (dashed), THF (dotted) and CHCl$_3$ with 1% pyridine (full); inset shows the Q-band region.

5.2.8 Fluorescence spectroscopy

The emission spectrum of polymer 34 (Figure 13) was recorded in both CHCl$_3$ and toluene, and was identical in these two solvents. Its intensity was reduced by 80% when compared to a reference compound (tetraoctyloxyphenylzinc porphyrin, Zn$_{TOOPP}$). The fluorescence spectrum of 34 shows more emission bands than normally observed for zinc porphyrins. The observed spectrum, however, might be composed of the emission spectra of zinc porphyrin (Zn) and...
free-base porphyrin (FB) species, as was also observed for systems that contain both Zn and FB and that are capable of energy transfer between the two species.\textsuperscript{[14]} Their combined emission spectra closely correspond to the spectrum of polymer 34 (Figure 13). This result might indicate that not all the porphyrins in the polymer had been metallated, and that still some free-base porphyrins were present in the polymer acting as an energy trap for the excitation energy. Even if the free-base porphyrins in polymer 34 are present in only very low quantities (as mentioned above, the metal insertion reaction resulted in a virtually complete conversion to the zinc porphyrin), the energy transfer can be expected to be very efficient due to the well-defined character of the polymer. Hence, emission can be detected of even very low quantities of free-base porphyrin.

Another prominent feature observed in the emission spectrum of 34 were two bands at the blue edge of the emission spectrum, \textit{viz.} at \(\sim608\) nm and \(\sim622\) nm, which is the Q(0,0) emission. This will be discussed later (\textit{vide infra}).

\begin{figure}[ht]
\centering
\includegraphics[width=\textwidth]{figure13.png}
\caption{Emission spectrum of polymer 34 in CHCl\textsubscript{3} (\(\lambda_{exc}=555\)nm); the dashed spectra are the emission spectra of Zn\textsubscript{OOPP} porphyrin (Zn) and a free-base porphyrin (FB). (Intensities of the latter two spectra are not correct.)}
\end{figure}

To test the hypothesis of energy transfer from Zn to FB in 34, an excitation spectrum was recorded with the emission fixed at 725 nm (the band assigned to purely free-base porphyrin emission). The resulting spectrum (Figure 14) revealed bands in the Q-region at 525 and 650 nm (indicated by the arrows in Figure 14), which unquestionably originate from a free-base porphyrin.
We may conclude that a small part of the porphyrin molecules in polymer 34 still exist in their free base form. From the absorption spectrum it is evident that this part indeed is very small since it cannot be detected. The observed emission, however, is considerable, and from this feature we may deduce that polymer 34 is capable of mediating energy transfer, allowing the excitation energy to be quenched by only a few free-base porphyrins. This result supports the conclusion drawn in Chapter 4 that coupling between the stacked porphyrins exists, resulting in energy transfer between the porphyrins.

These preliminary results indicate that the chromophoric arrays in 34 may act as simple antenna mimics. In order to obtain a better and more quantitative picture of the energy transfer process, a whole series of mixed FB/Zn copolymers with known FB/Zn ratios needs to be synthesised and studied. Due to the lack of time this was not possible.

As mentioned before, the emission spectrum of 34 contains two bands at the blue edge of the emission spectrum, viz. at ~608 nm and ~622 nm (Figure 13). The reference compound Zn_TOOPP was found to fluoresce at ~608 nm in toluene and at ~611 nm in THF, indicating a red-shift of the emission upon coordination of the THF to zinc. When pyridine was added to a solution of 34 in CHCl₃ or toluene, the emission clearly changed from the one in Figure 13, see Figure 15. The observed splitting of the Q(0,0) fluorescence band disappeared after pyridine had been added, and the band at 608 nm vanished, leaving solely the band at 622 nm. From these observations it can be concluded that two states exist for the zinc porphyrins in 34, a coordinated (622 nm) and a non-coordinated (608 nm) state. Remarkably, even in the case of the non-coordinating solvents CHCl₃ and toluene, still...
coordination to the zinc porphyrins occurs, as is evident from the emission at 622 nm. Since these solvents had been dried carefully, we tentatively assign the observed emission at 622 nm to an intramolecular coordination of the alkoxy oxygen atoms of the substituents on the porphyrins to the zinc centres (schematically shown in Figure 15b). The emission at 608 nm probably arises from zinc porphyrins that do not have such an intramolecular ligation. Apparently, ligation does not occur in an exactly 1:1 oxygen:Zn ratio over the entire polymer chain, most likely because this would cause too much strain.

In this context it is of interest to note that polymer 34 was found to be more stable than polymer 32 (see §5.3.3), which may now be interpreted in terms of the stabilising Zn-oxygen- ligations, which lead to a more rigid polymer conformation. The previous experiments reveal that pyridine is able to break up this intramolecular ligation between the zinc porphyrins, whereas THF is not strong enough to make the stacks dissociate completely.

![Fluorescence spectrum of 34 in CHCl₃ (exc. 405 nm) before (dashed line) and after (full line) the addition of pyridine, showing the disappearance of the band at 608 nm; b) Intramolecular ligation of an alkoxy oxygen atom to the zinc centre in the porphyrin array.](image)

Figure 15 a) Emission spectrum of 34 in CHCl₃ (exc. 405 nm) before (dashed line) and after (full line) the addition of pyridine, showing the disappearance of the band at 608 nm; b) Intramolecular ligation of an alkoxy oxygen atom to the zinc centre in the porphyrin array.

In the latter solvent, the two Q(0,0)-bands are still visible (Figure 16) confirming that THF, although it is a ligating solvent, is too weak a ligand to completely disrupt the stacks. In fact, the spectrum in THF is almost identical to the spectrum in CHCl₃ and toluene, apart from a blue shift of 4 nm (Figure 16). This is remarkable since THF did change the organisation of the zinc porphyrins in the stack as was evidenced from the absorption spectrum, which showed an altered exciton structure (see Figure 11). It should be kept in mind, however, that the Q-bands show no distinct excitonic interactions, and that the fluorescence originates from these bands. The shift of the second emission band from 622 nm to 618 nm may be caused by the change in the conformation of the porphyrins. These measurements suggest that energy transfer from Zn to FB has similar efficiencies in different solvents.
The excitation spectrum of 34 in the Soret band region (measured both in CHCl₃ and toluene) is identical to the absorption spectrum of 34 when the emission is detected at 725 nm, i.e. at the emission of the free-base porphyrins in the polymer. This result can be interpreted by assuming that the free-base porphyrins are predominantly located in the well-defined stacked region of the polymer chain, and not in the flexible end-groups. In this well-defined stack, the excitation energy can be transferred relatively easily along the array to the free-base traps. However, when the emission is set at 605 nm, a monomer-like band appears in the Soret region, showing that in this case the zinc porphyrins are not incorporated in the excitonically coupled rigid part of the polymer. This could be due to the presence of non-helical oligomers or end-groups. (The presence of monomeric zinc porphyrins that have not been incorporated in the polymer during the polymerisation reaction can be discarded in light of the results presented further on, see §5.3.5).

![Figure 16](image)

**Figure 16** Emission spectra of 34 in CHCl₃ (dotted line, \( \lambda_{\text{ex}} = 405 \text{ nm} \)) and in THF (full line, \( \lambda_{\text{ex}} = 425 \text{ nm} \)). The shifted \( \text{Q}_0 \text{-} \text{Q}_0 \) bands are indicated by the arrow.

### 5.2.9 Fluorescence anisotropy

Determination of the fluorescence anisotropy can give further information about the geometry of the porphyrins in polymer 34 (see also §4.2.10). The fluorescence anisotropy of the monomeric reference compound Zn_TOOPP in toluene was measured and amounted to \( r = 0.02 \). This value is lower than the theoretical value of 0.1 as a result of rotation depolarisation effects, but during its lifetime of 2 ns the polarisation is not completely randomised. The anisotropy of the reference free-base porphyrin monomer, tetraoctyloxyphenyl porphyrin...
Polyisocyanides containing zinc porphyrins

(\(\text{FB}_{\text{TOOPP}}\)) was measured to be \(r = 0\) (§4.2.10), which means that within the lifetime of 12 ns, the orientation of the molecule has fully randomised. When pyridine is added to \(\text{Zn}_{\text{TOOPP}}\), the fluorescence lifetime of the chromophore decreased to 1.5 ns\(^{[16]}\) leading to a higher anisotropy value of 0.025.

The results of fluorescence anisotropy measurements on polymer 34 in toluene are presented in Figure 17. The anisotropy value depends on the wavelength of excitation, and, like in the case of polymer 32 (§4.2.10), this indicates that the porphyrin stacks are tilted with respect to the polymer helix axis. Apart from this wavelength dependency, also different anisotropy values (for the same \(\lambda_{\text{ex}}\)) were found for the different bands at the blue side of the emission spectrum, \(\text{viz.}\) the bands at 608 and 622 nm, referred to as \(r_{\text{608}}\) and \(r_{\text{622}}\). When excited at 405 nm, these values corresponded to 0 and 0.02, respectively. Excitation at 435 nm likewise gave rise to different anisotropy values: \(r_{\text{608}} = 0.045\) and \(r_{\text{622}} = 0.035\). These results suggest that the ligated (622 nm) and non-ligated (608 nm) zinc porphyrins have different orientations with respect to the polymer axis.

When, however, excitation occurred in the ‘monomer’ region of the absorption spectrum of 34, \(\text{viz.}\) at 420 nm, a wavelength independent fluorescence anisotropy of \(r = 0.02\) was measured. This value is identical to the anisotropy found for \(\text{Zn}_{\text{TOOPP}}\), and is in line with the idea that the ill-defined end-groups can be selectively excited. These end-group porphyrins behave as monomers, showing identical rotation depolarisation behaviour.

\[ \begin{align*}
\lambda_{\text{ex}} & \quad \text{Int. / a.u.} \\
435 \text{ nm} & \quad 0.7 \\
420 \text{ nm} & \quad 0.6 \\
405 \text{ nm} & \quad 0.5 \\
\text{anisotropy} & \quad 0.10 \\
\text{anisotropy} & \quad 0.10 \\
\text{anisotropy} & \quad 0.09 \\
\text{anisotropy} & \quad 0.08 \\
\text{anisotropy} & \quad 0.07 \\
\text{anisotropy} & \quad 0.06 \\
\text{anisotropy} & \quad 0.05 \\
\text{anisotropy} & \quad 0.04 \\
\text{anisotropy} & \quad 0.03 \\
\text{anisotropy} & \quad 0.02 \\
\text{anisotropy} & \quad 0.01 \\
\text{anisotropy} & \quad 0.00 \\
\end{align*} \]

\(\text{Figure 17} \) Fluorescence anisotropy (right axis) in the emission spectrum (left axis) of polymer 34 as a function of the excitation wavelength (solvent: \(\text{CHCl}_3\)).

Since \(r_{\text{608}}\) is dependent on the wavelength of excitation, the species that emits at 608 nm cannot be a physical monomeric species, because it should then show a wavelength independent
anisotropy. The fact that the anisotropy differs with the wavelength of emission could point to a difference in orientation of the ligated and the non-ligated porphyrins. In §4.2.10, calculations, based on the observed anisotropy, were presented, which indicated that the porphyrins in polymer 32 had a tilted orientation. This may also be the case for polymer 34, however, this conclusion should be taken with some care, since there are different porphyrin species in the polymer, viz. FB and ligated and non-ligated Zn species.

Addition of pyridine (1 vol%) to a toluene solution of polymer 34 caused a change in the exciton structure. The fluorescence anisotropy (Figure 18) became be independent of the wavelength of excitation (λ_{ex} = 420 and 430 nm), which suggests that the porphyrins have an overall homogeneous arrangement, with their planes oriented perpendicularly to the helix axis, comparable to what was concluded for polymer 32 upon heating (see §4.2.10). The fact that the observed anisotropy was r = 0.045, suggests that the ligated zinc porphyrins are not perfectly organised (the anisotropy is lower than the theoretical value of r = 0.1) due to static or dynamic disorder.

![Figure 18](image)

**Figure 18** Fluorescence anisotropy in the emission spectrum of 34 in toluene after the addition of an excess of pyridine (λ_{ex} = 420 and 430 nm).

In analogy to polymer 32, the conformation of zinc polymer 34 can be altered. The driving force to overcome the energy barrier is either heat or coordination of a strong ligand. After heating 32, the porphyrin side chains were found to adopt the thermodynamically preferred orientation, which was maintained after cooling. The conformation of polymer 32 was kinetically trapped during polymerisation. Whether the change in conformation of 34 upon ligation is a kinetically or thermodynamically controlled process remains unclear, since the
pyridines were not removed afterwards (in analogy to cooling of 32) to verify if the porphyrins returned to their original conformation.

5.2.10 Circular dichroism spectroscopy
After incorporation of zinc in 32 to give 34, the CD spectrum changed, matching the changes observed in the absorption spectrum. The blue side of the Soret-band has a higher extinction coefficient and this also resulted in larger CD signals. The couplet at 431 nm was still present, although it had broadened. The same characteristics as described for polymer 32 were observed in the CD spectrum of 34, the bisignate signal at the lower energy side of the Soret-band formed a positive couplet, which after annealing inverted to give the final spectrum shown in Figure 19.

![Figure 19 CD spectra of polymer 32 (dashed line) and 34 (full line).](image)

5.2.11 Titration experiments with DABCO
Addition of DABCO to 34 induced the same shifts in the absorption spectrum as seen for pyridine. The excitonically split Soret-band narrowed and the Q-bands exhibited a bathochromic shift, indicating that the zinc is ligated by the DABCO (see Figure 20). The absorption and CD spectra of polymer 34 were monitored as a function of the DABCO concentration.
Addition of DABCO had a dramatic effect on the CD spectrum (Figure 21). Several experiments were carried out to precisely record the titration curves. An intersection point was already obtained at 0.1-0.2 equivalents of DABCO per porphyrin, whereas theoretically 0.5 equivalents are needed to accommodate all available zinc sites. This small number of DABCO equivalents can be explained by the fact that the porphyrins in the polymer are covalently coupled. In order to bind the DABCO, one of the porphyrins needs to rotate and this in turn forces the neighbouring porphyrins to twist as well. Thus, binding of DABCO probably causes a cooperative conformational change of the porphyrins in 34 and premature changes in the absorption and CD spectra.

After the theoretical saturation point of 0.5 equiv. of DABCO per porphyrin in 34, the absorption spectrum did not show any further detectable changes when more DABCO was added, even not after addition of a large (50-fold) excess, indicating that the association constant of the 1:2 DABCO:porphyrin dimer is much higher than that of the 1:1 DABCO:porphyrin coordination complex. Sanders et al. have previously shown that, in the case of physically linked porphyrin dimers, the transition from a 1:2 to a 1:1 complex was not observed even after addition of a 1000-fold excess of DABCO, the reason being the much higher binding constant of the dimer complex compared to the complex of the monomer. A similar porphyrin architecture is probably present in polymer 34.

An additional explanation may be the fact that our polymeric porphyrin system is even more rigid than the dimer of Sanders. The porphyrins in the polymer cannot alter their conformation such as to accommodate any more DABCO molecules.
Polysocyanides containing zinc porphyrins

Figure 21  a) Changes in the CD spectra of 34 on addition of increasing concentrations of DABCO (solv: CHCl₃). b) Change of the CD intensity, monitored at 425 nm, as a function of the number of equivalents of DABCO added per porphyrin in 34.

Strikingly, the negative couplet at 430 nm in the CD spectrum of 34 changed sign to become positive upon addition of DABCO. If the sign of the couplet can be related to the chirality of the porphyrin aggregate in the polymer, this result would mean that the chirality of the porphyrins inverts. This can be explained by considering Figure 22. In this figure, the porphyrins have a left-handed helical organisation, which becomes right-handed upon addition of DABCO, while the helicity of the polyisocyanide backbone remains the same.

Figure 22 Schematic drawing of the effect of coordination of DABCO to polymer 34, which leads to a reversal of the helicity of the porphyrin stacks.
5.2.12 Atomic force microscopy

AFM images of spin-coated solutions (CHCl₃) of polymer 34 on mica (Figure 23) showed the typical rod shape fibres observed previously for this type of polymers (see §4.2.5). No differences were observed compared to its free-base analogue 32. The figure showed brighter spots, which are due to the presence of entwined polymers having an increased height corresponding to $3.6 \pm 0.2$ nm, whereas the single fibres had heights of $1.6 \pm 0.2$ nm. Like for polymer 32, the AFM results show that the polymer fibres are entangled, but that the porphyrins probably do not intercalate, otherwise a lower value for the diameter would have been expected.

![AFM images of polymer 34](image)

*Figure 23* AFM images of a) polymer 34 in CHCl₃; b,c) idem after addition of DABCO showing clearly the tendency of the molecules to aggregate (conc. $\sim 10^{-7}$ M, bar = 1 µm).

Since spectroscopic studies had indicated that a structural change occurs in the porphyrin organisation when DABCO is added, it was of interest to study the effect of such an addition on the rigidity of the polymer by AFM. The change that occurred was the tendency of the polymer fibres to aggregate on the surface, even at a very low concentration of $\sim 10^{-7}$ M of polymer 34. The height of polymer 34 saturated with DABCO was found to be identical to the height of polymer 32. The entwined polymers showed heights of $5.5 \pm 0.4$ nm. Apparently, DABCO glues the polymers together, but its effect on the rigidity of the polymer chains is at yet unclear.

5.3 Conclusions

Two different zinc porphyrin polymers 33 and 34 have been synthesised and are discussed in this chapter. They show different behaviour upon the addition of DABCO. While the structure of 33 changed stepwise in a linear fashion on the addition of DABCO, forming a sandwich-type of complex, it changed in a co-operative way for 34. This difference is ascribed to the different conformations of the polymers; addition of DABCO causes a minor change in the
conformation of 33, whereas a relatively large rearrangement occurs for 34. This is due to the difference in the composition of porphyrins caused by the difference in spacer length.
While the zinc insertion was straightforward for 33, the reaction needed elevated temperatures to obtain 34, which is probably caused by the well-defined and rigid organisation of the porphyrins in 34. Nevertheless, the latter reaction resulted in incomplete insertion of zinc, as was established by fluorescence spectroscopy. The results of this study suggest that a few free-base porphyrins still remained in the polymer (maximally 1%), which made that they acted as an energy sink for the excitation energy. Fluorescence spectroscopy studies using several ligands indicated that the porphyrin molecules in 34 exhibit intramolecular ligation from alkoxy oxygen atoms of neighbouring porphyrins. This ligation stabilises the polymer structure to the extent that, upon heating to 70°C, no conformational changes occur, while for the free-base analogue 32 such changes do occur. Fluorescence anisotropy measurements revealed that the zinc porphyrins have a twisted arrangement with respect to the polymer helix axis. Addition of pyridine changes this arrangement to a perpendicular orientation with respect to this axis.
AFM measurements showed that the addition of DABCO to 34 causes clustering of the polymers, even at very low polymer concentration. Whether this addition changes the length of the polymer fibre by extending it like a spring could not be verified.

5.4 Experimental Section

5.4.1 General methods and materials
All solvents were distilled prior to use under atmospheric pressure and a nitrogen atmosphere. Dichloromethane and chloroform were distilled from CaCl$_2$, THF and toluene from Na. The solvents CHCL$_3$ and toluene used in §5.3.3-5.3.5 were dried over basic Al$_2$O$_3$. All other chemicals were obtained commercially and used without further purification. Thin layer chromatography analyses were performed on Merck silica gel 60 F$_{254}$ plates.

5.4.2 Spectroscopy
Absorption spectra were measured on a Varian Cary 50 Conc spectrophotometer at ambient temperature. The spectra presented in §5.2.7 were obtained on a Cary 5E Varian absorption spectrophotometer having monochromator slits with a band pass of 2 nm. CD spectra were recorded on a Jasco 810 spectrophotometer equipped with a Peltier temperature control unit (Jasco PT-423 s/1) at 25°C. Fluorescence spectra were recorded on a Perkin Elmer Luminescence Ls50B spectrometer at 25°C, except for §5.2.8-5.2.9 The experiments described in these paragraphs were performed A Fluorolog 3-22 (Jobin Yvon) fluorimeter. The fluorescence spectra are corrected for wavelength-dependent detection and the excitation spectra are corrected for wavelength-dependent
intensity of the lamp (via internal reflection cell). The monochromator slits were set at an excitation wavelength of 2 nm and emission wavelength of 1-2 nm (depending on the number of counts/photons per second).

5.4.3 Titration experiments
A stock solution, typically ca. 1 mg dissolved in 100 mL chloroform, was prepared from the porphyrin polymer. Another stock solution was prepared from the ligand, typically ca. 50 mg dissolved in 100 mL chloroform. This latter solution was diluted 10x. To the polymer stock solution (2 mL in a quartz 10 mm cuvette) were added portions of the amine solution using a 10-250 µl injection needle. The temperature was set at 25°C using a Peltier element (type Jasco PT-423 S/I) during CD measurements, while the UV-Vis measurements were performed at room temperature. For the fluorescence measurements the stock solutions were diluted 20x. The excitation wavelength was set at 409 nm, the isosbestic point in the absorption spectrum, with a slit of 10 nm. The emission spectrum was scanned in the range 550-700 nm, with a slit of 20 nm. The scan speed set on 120 nm/ min and the temperature at 25°C.

5.4.4 Microscopy
AFM experiments were performed using a Nanoscope IIIa instrument from Digital Instruments. A solution of a polymer (~10^{-6}M) in CHCl_3 was spin-coated onto freshly cleaved Muscovite mica. All images were taken in tapping mode in air at room temperature. Commercial tapping-mode tips (Digital Instruments) were used with a typical resonance frequency around 300 kHz. For electron microscopy experiments, samples were prepared by dripping a drop of a 10^{-6} M of polymer 34 in CHCl_3 on the electron microscope grid and subsequently turning these grids on their side on a filter paper. TEM images were obtained on a JEOL JEM-1010 microscope (60kV) equipped with a CCD camera.

5.4.5 Synthesis
Zinc porphyrin polymer 33
In a 25 ml flask, porphyrin polymer 23 (40 mg) was dissolved in CHCl_3 (2 ml). A 100-fold excess of zinc(II)acetate (ZnOAc_2·2H_2O) was added and the reaction mixture was stirred under nitrogen at room temperature. After one hour the chloroform was evaporated and the purple-red crystals were washed with methanol and acetone. For characterisation, see text. The insertion of zinc was accompanied by disappearance of 2 Q-bands in the absorption spectrum.

Zinc porphyrin polymer 34
Polymer 32 (30 mg) was dissolved in CHCl_3 and to this solution was added a 200-fold excess of zinc(II)acetate (ZnOAc_2·2H_2O). The mixture was heated to 40°C for 12 h, after which the product was washed with methanol and acetone. ^1H NMR (CDCl_3, 400.15 MHz) δ 9.1-8.8 (br, β-pyrrole), 8.2-7.9 (br, phenyl), 3.9-3.6 (br), 7.3-6.5 (br, phenyl, NH), 2.1-0.4 (br, aliphatic) ppm.

5.5 References
Polyisocyanides containing zinc porphyrins

[10] Data about the distance between porphyrins are not available, but it can be estimated that it is smaller than the 0.76 nm for polymer 32, since the spacer is shorter.
[17] One feature that stands out is the fact that these measurements were not exactly reproducible, i.e. the number of equivalents used to change the conformation varied between 0.1-0.2 (per porphyrin) and the end-state of CD signal varied, although the process always showed the same trend.
Chapter 6

Polyisocyanopeptides with pendant perylene functions

6.1 Introduction

The construction of well-defined perylene assemblies is an area of research which is attracting considerable interest since perylenes are promising components as n-type semiconductors used in organic photovoltaic cells. Their unique properties are the result of a strong absorptivity in the visible part of the spectrum, high thermal and photochemical stability and high electron affinity.\cite{1, 2}

The first organic solar cells were prepared by sandwiching an organic material between two different electrodes, which, after doping, resulted in power conversion energies ($\eta$) up to 0.3%.\cite{3} An improved design possessed two layers of $p$-type and $n$-type materials sandwiched between two electrodes, based on a copper phthalocyanine and a perylene compound respectively (see Figure 1a),\cite{4} resulting in an efficiency of 1%. The low conversion in these so-called excitonic solar cells\cite{5} is a result of the design, since charge separation only occurred at the $p$-$n$ interface. This is a problem because photo excited states cannot travel more than 20 nm, hence only excited states present in a very thin layer around the $p$-$n$ interface eventually result in charge separation. Outside this layer the excitations are lost in (non)-radiative decay processes. A pre-requrement for an efficient solar cell is that exciton diffusion should occur over a large enough area to create charge separation before it falls back to the ground state, or that a close contact exists between the donor and acceptor.
An improved design was offered with the introduction of the bulk-heterojunction solar cell (Figure 1b).\textsuperscript{[6-8]} In this improved concept the charge separation can take place everywhere between the electrodes, since the organic donor and acceptor material are mixed, providing a much larger interface with intimate contacts between the two materials (mostly semi conducting polymers), which results in efficiencies as high as 2.5\%.\textsuperscript{[9, 10]}

The bulk-heterojunction concept is the most promising one for all-organic solar cells, but thus far the energy conversion energy ($\eta$) has been rather low in all-organic devices compared to conventional inorganic cells. Improvement of $\eta$ requires both an increase in the exciton-diffusion length and in the transport of charge carriers. It has been shown that increased ordering of the perylene molecules can result in a higher exciton diffusion length\textsuperscript{[11-13]} and can stabilise the charge separated states, which slows down the charge recombination.\textsuperscript{[14]}

Additionally, increased order is expected to also favour charge transport. Ideally, the charge carriers should provide an obstacle free pathway for the migration of the charges to the electrodes. Conceptually, this might be achieved by using large organised assemblies of perylenes that can span the two electrodes (see Figure 2).

**Figure 1** (a) Double-layer p-n junction. Absorption of light creates an exciton (1) that diffuses to the p-n interface (2) to give charge transfer (3). The charges are transported to the electrodes (4). (b) Bulk-heterojunction concept: charge transfer can occur throughout the junction due to the nanoscopic mixing of the donor (dark) and acceptor (dashed) materials. (Images taken from Thesis P. van Hal, Eindhoven University, 2003.)

**Figure 2** Schematic drawing of a bulk-heterojunction solar cell in which the acceptor material is a well-organised perylene assembly (dashed) that possesses a large surface area contact with the donor material (dark) and bridges the electrodes for efficient charge transport.
In chapter 3 well-defined arrays of porphyrins, which are attached to a rigid polyisocyanide have been described. The resulting polymers form stiff rods with an average length of 87 nm, showing exciton diffusion over at least 10 nm. It is not unlikely that application of this type of polymers with perylenes instead of porphyrins could provide a way to construct perylene nanowires and possibly improve the efficiency of organic solar cells.

In this chapter, we describe the synthesis and characterisation of perylene pendant polyisocyanides, based on the concept of hydrogen-bonded side-groups that maintain the rigid helical structure of the polymer.

6.2 Results and discussion

6.2.1 Synthesis

For the synthesis of a perylene pendant polyisocyanide (8), the same strategy was followed as for the porphyrin polymer described in chapter 3. A mono-aminoperylene was coupled to L-alanine, which was subsequently transformed into the isocyanide monomer. The preparation of polymer 8 is shown in Scheme 1. The synthesis of \( N,N' \)-bis-(1-ethylpropyl)perylene-3,4:9,10-tetracarboxylic diimide 1 has been described previously. It was partially hydrolysed with potassium hydroxide in \( \text{tert-buthanol} \) to give the anhydride imide 2. Compound 2 was subsequently reacted with 1,3-diaminopropane in DMF at 100ºC to afford the asymmetric perylene diimide 3. The latter compound 3 was then reacted as a dispersion in CH\(_2\)Cl\(_2\) with \( \text{t-butyl-oxycarbonyl-(Boc)-L-alanine} \) using 2.2 equivalents of dicyclohexylcarbodiimide and \( N,N \)-dimethylaminopyridine as a catalyst. The Boc-protecting group was cleaved off with trifluoro acetic acid and the resulting amine was reacted without further purification with the formylating agent \( p \)-nitrophenylformate. The resulting product 6 was dehydrated with diphosgene with \( N \)-methyl morpholine as base to yield the isocyanide 7. Polymerisation was carried out using Ni(II) as a catalyst (0.002 equivalents), resulting in polymer 8. During the polymerisation reaction the colour of the reaction mixture changed within a few minutes from dark red to dark pink-red, indicating a change in the perylene organisation. After dilution the colour remained (light) pink even at concentrations as low as \( \sim 10^{-7} \) M, while in the case of monomeric perylenes, the colour changes from red (aggregated perylenes) to yellow (molecularly dissolved) upon dilution.

The \(^1\)H-NMR spectra showed that the CH\(_2\) protons of the ethyl propyl aliphatic tail of the perylenes are not equivalent. This inequivalence is ascribed to hybridisation of the nitrogen
into an umbrella-shape, that cannot flip due to steric hindrance, which forces one ethyl group aside into the shielding zone of the perylene group.

\[ \text{Scheme 1 Synthesis of perylene pendant polyisocyanide 8. (i) KOH, tert-butanol; (ii) 1,3-diaminopropane, DMF, 100°C, 2 h; (iii) Boc-L-ala, DCC/DMAP, CH}_2\text{Cl}_2, 0^\circ\text{C} ; (iv) TFA, CH}_2\text{Cl}_2; (v) Formyl-ONP, CH}_2\text{Cl}_2; (vi) diphosgene/NMM, CH}_2\text{Cl}_2, 0^\circ\text{C}; (vii) Ni(ClO}_4)_2/ethanol, CHCl}_3. \]

The infrared spectra of all precursors 1-7 clearly showed the presence of characteristic C-H out of plane wagging absorptions which were observed around 810 and 750 cm\(^{-1}\), the O=CN stretching vibrations around 1700 and 1660 cm\(^{-1}\), and the C=C aromatic stretching vibrations around 1590 and 1575 cm\(^{-1}\) as was reported for other perylenes.\(^{[17]}\) The infrared spectrum of isocyanide monomer 7 (Figure 3) additionally showed the characteristic isocyanide vibration at 2142 cm\(^{-1}\) and a N-H stretching vibration at 3355 cm\(^{-1}\). The amide stretching vibration normally occurs around 1690 cm\(^{-1}\), hence the observed vibration at 1689 in 7 is ascribed to an overlap of the amide and NC=O stretching vibrations. For polymer 8 (Figure 3) the IR spectrum showed the complete disappearance of the C≡N stretching vibration at 2139 cm\(^{-1}\), and the appearance of a N-H stretching vibration at 3286 cm\(^{-1}\) (with a shoulder at 3317 cm\(^{-1}\)). The significant shift of this band indicates the formation of a hydrogen bonding array, as was previously seen for polymer 32 (Chapter 3). The fact that, going from monomer 7 to polymer 8, a shift and a clear change in the relative intensities of the peaks between 1550 and 1700 cm\(^{-1}\)
occurred (see Figure 3), and that the NH vibration was shifted to a lower wavenumber, these facts in combination with the disappearance of the isocyanide peak at 2142 cm\(^{-1}\) all point to the formation of a polyisocyanide.

**Figure 3** Infrared spectrum of monomer 7 (dashed line) and polymer 8 (full line). Inset shows the region of the perylene carbonyl, imine and amide absorptions.

### 6.2.2 Absorption and circular dichroism spectroscopy

The absorption spectrum of the monomer 7 (Figure 4) showed the normal 4 bands in the region around 500 nm, viz. the \(\pi-\pi^*\) or HOMO-LUMO transitions in the long direction of the perylene (i.e. through the nitrogens), with maxima at 432, 459, 490 and 527 nm and another band at 373 nm with low \(\varepsilon\), which is the transition perpendicular to the first mentioned one. The spectrum of the polymer showed essentially the same bands, although they were red-shifted and their relative intensities had changed. In addition to this, the spectrum had broadened, which is clearly visible on the red side of the spectrum. These changes indicate that the perylenes are organised in a stacked arrangement.
Figure 4 Absorption spectra of monomer 7 (dashed line) and polymer 8 (full line) in CHCl₃.

The circular dichroism spectrum of the perylene polymer was nearly identical in CHCl₃ and in toluene and is shown in Figure 5. While monomer 7 (not shown) displayed no detectable CD effect, polymer 8 showed several positive Cotton effects in the absorption region of the perylene π-π* transitions. The Cotton effect centred around 310 nm stems from the imine chromophores of the polyisocyanide backbone. This indicates that the polymer has a similar structure as the PIAA’s (see Chapter 1) and that the incorporation of the perylene functions in the polymer has no significant influence on the structure of the helical backbone. Precise conclusions about the exact organisation of the perylenes and the helicity within the polymer cannot be easily made since the spectrum is non-conservative, meaning that the exciton chirality method can not be applied and that considerable theoretical calculations are required to obtain information about the structure of the polymer.
The temperature dependent behaviour of 8 as monitored by CD spectroscopy was evaluated both in CHCl₃ (up to 65°C) and in toluene (up to 90°C). The studies in both solvents revealed that the spectroscopic characteristics did not alter upon heating. In toluene, however, the signals decreased by ca. 25% upon heating to 90°C and returned to ca. 95% of the original value after a heating-cooling cycle. Interestingly, when compared to the porphyrin polymer 32 described in Chapter 3, the helical arrangement of the perylenes stayed intact upon heating, whereas in the case of polymer 32 the porphyrins changed conformation. The fact that the CD signal decreases is probably the result of a breaking up of the hydrogen bonds at the end of the polymer chain combined with an increased random thermal motion of the perylenes. The relative lack of sensitivity to a temperature increase indicates that the helical perylene architecture is very stable.

**6.2.3 Fluorescence spectroscopy**

The fluorescence spectrum of isocyanide 7, when photo excited in the absorption band at 492 nm, showed the characteristic profile of a monomeric perylene bisimide with a strong band at 535 nm and a weaker band at 578 nm. The emission spectrum of polymer 8 displayed the same profile, viz. bands at 535 and 578 nm, but had an additional broad band at 620 nm. This red shifted fluorescence band is indicative of the formation of excimers.
It is well known that an increase of the concentration of a solute often is accompanied by the appearance of a new structureless emission at longer wavelength, of which the intensity increases with concentration. For example, the violet fluorescence of a dilute pyrene solution changes to a blue fluorescence with increasing pyrene concentration. Förster has shown that this change can be explained by the formation of a pyrene excimer.\[18, 19\]

In general, the formation of intermolecular excimers is concentration dependent and is visible in the fluorescence spectrum as an upcoming excimer fluorescence band. Concentration dependent fluorescence studies on polymer 8, however, revealed that the relative intensity of the excimer band compared to the monomer bands did not change. This result clearly indicates that excimer formation in 8 is intramolecular in nature, i.e. it occurs within a single polymer stack. Although there is no concentration dependence of the excimer emission, some variation in the relative intensities of these bands is sometimes observed after sample preparation, possibly caused by some sort of aggregation phenomenon or precipitation.

### 6.2.4 Excimers

An excimer is a complex between identical molecules whose interaction is repulsive in the ground state but attractive in the excited state (in the case of non-identical molecules this is called an exciplex). The molecules in the complex are typically 0.3-0.4 nm apart. The fluorescence of excimers is usually red-shifted as compared to the monomer fluorescence, which is predicted by the exciton theory: after localised photo excitation of one of the molecules, the excitation is delocalised between the excimer molecules, resulting in a splitting of the energy level into two exciton states. Additionally, charge transfer interaction, leading to
ionic character, also plays a role in the stabilisation of the species. For perylene excimers the ratio between exciton interaction and charge transfer interaction has been determined to be 70:30.\cite{20} This is schematically depicted in Figure 7.

![Figure 7](image)

**Figure 7** Theoretical energy level diagram for interactions of excimers. The (M+M*) state is destabilised at short distances by the repulsion energy $E_R$. Due to exciton interactions, the excited state is delocalised and the energy level is split and lowered with a value of $E_S^{\text{EXC}}$. Additionally, charge transfer between exciton state and an ion pair occurs, leading to further stabilisation (energy $E_S^{\text{CT}}$). Excimers can be formed when the sum of $E_S^{\text{EXC}}$ and $E_S^{\text{CT}}$ is higher than $E_R$.

In order to learn more about the origin of the monomer and excimer-like emission spectra in polymer 8, a fractionation, in which the polymers are separated by size was carried out. Fractionation was achieved by repeated precipitation of the polymer samples in order to separate the longer polymer molecules. During the first two precipitation cycles, the supernatant solution was yellow, an indication of molecularly dissolved perylenes (as was substantiated by absorption spectroscopy, which showed a monomer-like spectrum). The emission spectra of the higher molecular weight fractions displayed a very strong excimer emission compared to the fractions containing the low molecular weight compounds (Figure 8). From this simple separation experiment the different architectures, which give rise to the different emission spectra could be resolved. The shorter polymers were observed to give monomer-like emission and the longer polymers predominantly excimer-like emission. For short polymers, end group effects become more pronounced compared to the longer polymers. It has been shown for polyisocyanides that the endgroups are in a random-coil conformation and that at least 10 monomeric units are needed to form a helix.\cite{21} Since the interactions between perylenes in random-coil polymers are expected to be ill-defined, these endgroups will exhibit monomer-like behaviour. The long polymers have almost all their perylenes in a well-
defined organisation and will display excimer-like emission, apart from their non-organised endgroups which emit like the monomers. The fluorescence quantum yield ($\Phi$) of the excimer species was determined to be $\Phi \approx 0.11$.\[^{[22]}\] Although the excimer species is the predominant species in the longer polymers, the quantum yield of the excimer fluorescence is so low, that there is still an observable contribution from the monomer-like emission of the endgroups at 535 nm.

![Emission spectra](image)

**Figure 8** Emission spectra of 8 before (dashed line) and after (full line) fractionation, which yields the longer polymers.

The occurrence of the two types of emissions becomes more clear when the excitation spectra are examined. The excitation spectrum of the emission band at 535 nm exhibits an absorption spectrum corresponding to that of a perylene monomer, whereas the excitation spectrum of the emission band at 620 nm is more similar to the absorption spectrum of the polymer (not shown). In the case of the fractionated polymer sample, which displays the intense excimer emission band at 620 nm, the excitation spectrum is almost identical to the absorption spectrum of the polymer (Figure 9).
Polyisocyanopeptides with pendant perylene functions

Figure 9 Absorption spectrum of 8 (dotted line; a) and the excitation spectrum of the fractionated polymer of 8 (full line, b). The emission spectrum of 8 is shown as a dashed line.

It was observed that the emission spectrum of a solution of the fractionated polymer shows a decrease of the excimer band in time (ca. 20% after 6 days at room temperature), while the monomer emission increased disproportionately higher at the same time. This phenomenon could be accelerated by sonication. This result may indicate that the polymer chains can unfold at their ends to give a more random coil conformation.

6.2.5 Fluorescence lifetime studies

The fluorescence lifetime of the singlet excited state (the time the molecule remains in the excited state, $\tau$) is a characteristic feature of excited molecules. The fluorescence lifetime of a monomeric perylene is typically around 4 ns.\textsuperscript{[23, 24]} The lifetime of the excited state can be determined with the help of time-resolved photoluminescence spectroscopy, in which the fluorescence intensity is measured as a function of time. The exponential decay of the fluorescence intensity as a function of time for $N$-formyl precursor 6 and polymer 8 were measured in CHCl$_3$ (Figure 10).
The decay of the precursor 6 could be fitted to a mono-exponential function with a lifetime of $\tau = 3.9$ ns, which is the fluorescence lifetime of a typical monomeric perylene molecule. The fluorescence decay of the polymer could be fitted to a double exponential function, which resulted in two lifetimes of $\tau = 3.9$ and $\tau = 19.9$ ns. The first can be assigned to the monomer-like fluorescence from the end groups or oligomers, whilst the second, much longer lifetime is typical for perylene excimers.\textsuperscript{[20, 25]} This lifetime is dependent on the conformation of the perylenes. The experiment was repeated using the non-fractionated polymer mixture (dashed spectrum Figure 8), which gave the same two lifetimes, but now with different ratios.

6.2.6 Atomic force microscopy

Spin-coated CHCl$_3$ solutions of 8 on mica were studied by AFM and revealed the presence of fibre-like structures (Figure 11a). These fibres had a height of 2.5 Å and a diameter of 5.5 Å \textit{(vide infra)} and are probably single polyisocyanide strands. Their length distribution is quite broad, with fibres ranging from a few nanometres up to a micrometer in length. In addition, AFM studies revealed that the polymers had a tendency to align, which is a common feature of rod-like objects. Upon dilution of the polymer solution no change in the observed characteristics, \textit{i.e.} the height appearance and length of the polymer strands, was observed. This, together with the fact that the height was consistent over the entire fibre length, whilst crossing points could clearly be distinguished (Figure 11c), indicates that single polymer molecules were imaged.
**Figure 11** AFM images of spin-coated CHCl₃ solutions of 8. a) Fibres with lengths up to 1 µm containing up to 10,000 perylene molecules (bar = 500 nm). b) Idem, high dilution. c,d) Assemblies of the fibres (arrow indicates crossing points, bar = 50 nm). e,f) Left-handed helical structures (highlighted) visible on the polymer at high magnification (pitch is 1.5 nm, bar = 10 nm).
Figure 11 shows that the measured width of the fibres is $5.5 \pm 0.2$ nm for samples in which these fibres are aligned side-to-side. This width matches well with that calculated by molecular modelling (5.0 nm). Although these dimensions are normally not reliable for isolated molecules, due to tip convolution, this is not a problem when a continuous monolayer is measured. The height of the fibres is measured to be 2.5 nm, which is only half the predicted diameter. We ascribe this difference to height anomalies as a result of tip-sample interactions. Especially the attractive capillary forces induced on the tip by the contamination layer, existing on surfaces measured in ambient conditions, are responsible for this phenomenon. The effective spring constant of the oscillating cantilever is changed by these forces and this causes a shift of the amplitude vs. frequency curve of the cantilever and hence a change in the acquired amplitude.[26]

Throughout the sample many fibres of hundreds of nanometres length, corresponding to several thousand perylene units incorporated into the polymer, can be distinguished. At higher magnification (Figure 11d) a fine structure can be observed on the single polymer fibres. Zooming in on one of the polymer fibres (Figures 11e,f) reveals the presence of a helical architecture. Measured on several samples, the helices were found to be exclusively left-handed and had a pitch of 1.5 nm. This is highlighted by the parallel bars in Figure 11f. To the best of our knowledge, this is the first observation of a helical structure in a single synthetic polymer fibre by AFM. Although it is obvious to relate the observed helicity to the helical backbone of the polycyanide polymers, the explanation of the measured pitch is not straightforward. Since the distance between the first and the second side-group in the polymer is $\sim 0.1$ nm, the measured pitch is tentatively interpreted as involving 15 perylene units, i.e. number 16 is on top of number 1 (Figure 12a). This would result in a projection angle between perylene 1 and 5 of $24^\circ$ (Figure 12b). This angle is remarkably similar to that measured for the porphyrin polysocyanides using depolarised RLS (Chapter 3) and to that calculated on basis of the hydrogen bonding arrays (Chapter 1). However, interactions between the polymer and the surface could have an effect on the observed pitch, so no direct comparison can be made.
Attempts to visualise the perylene polymers by scanning tunneling microscopy (STM) in order to obtain more information about the precise helical structure of the polymer failed, since the tip removed the fibres from the surface (graphite) upon scanning. No fibres were observed, even when the tunneling current was set as low as 0.1 pA, thus keeping the tip as high above the surface as possible. The sample, prepared by spin coating the same solution of \textit{8} as used for Figure 12a, on a freshly cleaved graphite plate, was first studied with AFM, which showed that the polymer fibres had a collapsed structure, possibly due to interactions with the graphite surface. Apparently, the polymer-surface forces are so strong that even these rigid rods collapse to form distorted polymer structures, which the STM tip cannot overcome.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure12.png}
\caption{a) Schematic side view of one pitch in an isolated fibre of \textit{8} observed with AFM involving 15 perylene units. b) Top view of helix with 5 perylene units. c) Schematic of 7 pitches observed with AFM.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure13.png}
\caption{AFM image of \textit{8} spin-coated on a graphite surface; the polymers molecules, although very stiff, collapse on the surface due to strong polymer-surface interactions (bar=500nm). Loops can sometimes be distinguished (see arrows).}
\end{figure}
6.2.7 Single molecule spectroscopy

The optical experiments described so far on precursors 6/7 and polymer 8 were all performed in solution and are ensemble measurements. Further insight into the optical properties of the compounds can be obtained from studies on single molecules, i.e. on single polymer fibres. In this way, features that would normally be lost in the ensemble of the bulk of molecules can be detected.\[27\] This is the case, for instance, if sudden changes occur in fluorescence intensities which are hidden in the bulk measurements due to the averaging over a multitude of chromophores.\[28\] Furthermore, single molecule experiments allow one to investigate how the optical properties vary in dependence of the size of the polymer molecule. Additionally, the intensity changes in time provide information about the number of independently emitting sites in a multichromophoric system.

To study polymer 8 at the single molecule level, the technique of fluorescence confocal microscopy (CFM) was used. CFM is a straightforward technique especially suitable to investigate thin samples, such as polymers deposited in submonolayer quantities onto a substrate. In order to study single molecule phenomena the molecules have to be well-separated on the surface, at least 200 to 300 nm apart, due to the diffraction limiting nature of the technique. The fluorescence count-rate arising from a single emitter is low and only detectable if the molecule has a high absorptivity and a high quantum yield, since background noise becomes an important factor when measuring at the single molecule level. Due to the high density of the applied excitation power, the single emitters are prone to photo-degradation and, therefore, photostable chromophores should be used. Perylenes are particularly suitable in this respect since they are stable, highly absorbing chromophores with a high fluorescence quantum yield. They have been used i) to retrieve conformational information\[29-31\] ii) as oligomeric species to study energy transfer\[32,33\] and iii) as optical probes to reveal information about the physical or chemical environment or, vice versa, the effect the environment has on the perylene molecule.\[34-38\]

Single molecule experiments on multi-perylene systems have been previously performed, viz. on dendrimers containing eight peryleneimides at their surface and a terylenediimide in the core.\[39-41\] Although the dendrimer was used as a scaffold to organise the perylene molecules, the arrangement of the latter molecules was not well-defined and showed conformational heterogeneity. A single molecule study on polymer 8 would offer a unique insight into the behaviour of perfectly organised multiperylene systems.
Initially, the perylene formamide 6 was studied as a reference compound with confocal microscopy ($\lambda_{\text{exc}} = 488$ nm). A $10 \times 10 \, \mu m$ confocal image of a drop casted solution of 6 in CHCl$_3$ (ca. $10^{-7}$-$10^{-8}$ M) on a glass surface is depicted in Figure 14a. The spots in the image represent emissions from single molecules of 6. The confocal image reveals that the emission has a well-defined polarisation, probably because the molecules do not alter conformation during the timescale of the measurement. In Figure 14b, the fluorescence intensity as a function of time (a timetrace) is shown for a single perylene molecule 6. The intensity is constant for a period of 6.5 s and then drops to zero due to irreversible photo bleaching of the molecule. This behaviour is typical for a single chromophore.

![Confocal image of perylene 6 and fluorescence time trace](image.png)

**Figure 14** a) Confocal image of perylene 6 ($\lambda_{\text{exc}}$=488nm, $\lambda_{\text{det}}$>510nm, $10 \times 10 \, \mu m$) showing emissive spots of single molecules. b) Fluorescence time trace of a single molecule of 6, i.e. from one isolated spot in a).

The same experiment was carried out on polymer 8 and a more complex behaviour was observed. A typical confocal image of a glass surface covered with polymers, spin-coated from a very diluted solution in CHCl$_3$ (ca. $10^{-7}$-$10^{-8}$ M), is presented in Figure 15. The image shows a scanned area of $10 \times 10 \, \mu m$ with spots of different shades of grey. Grey spots indicate the presence of a defined polarisation of the emitted light, whereas white spots correspond to unpolarised (or randomly polarised) emissions. In addition to the polarisation of the emission, two other properties were been investigated for the single perylene polymer molecules, viz. their emission spectra and their time traces.

Two different kinds of time traces could be distinguished when single emitters in the polymer sample were studied. The species yielding unpolarised emission, such as the white spot encircled by the full line in Figure 15a, showed a continuous decay of the fluorescence intensity in time, as depicted in Figure 15b. This result suggests that they originate from a large number of fluorescing sites, whose successive photo-bleaching leads to a continuous decrease
of the emission. The other type of emitter displaying a defined polarisation of emission behaved completely different. This was for instance the case for the grey spot encircled by the dashed line in Figure 15. Its intensity time trace (Figure 15b) showed sudden, stepwise fluctuations, indicating that only a few emissive sites are present, since a lot of sites would have resulted in an exponential decay. The steps are caused by the stepwise photo-bleaching of these few emissive sites. Strikingly, also collective dark states were observed, suggesting that energy transfer takes place between the perylene units, resulting in the observed collective behaviour. Such a behaviour has been previously described for perylene molecules attached to dendrimers.\cite{40} The absence of collective non-fluorescent states for the emitting species displaying the continuous decay suggests that these species emit independently.\cite{42}

![Figure 15](image)

**Figure 15** a) Confocal microscopic image of polymer 8 on a glass surface revealing emissive sites possessing either polarised or non-polarised fluorescence. b) Different time traces which arise from the polymer sample, x) exponential decay from the non-polarised emission sites (grey) and y) multi-step time trace from the polarised emission sites (black).

Interestingly, upon examination of the actual emission spectra of the two distinguished species, it appeared that the grey species, encircled by the dashed line, displayed a monomer-like emission spectrum (Figure 16b), whereas the white species with non-polarised emission displayed an excimer-like spectrum (Figure 16d), similar to that observed for polymer 8 in solution. The total fluorescence intensity of the grey species was composed of p- and s-polarised emission, as depicted in the time trace (Figure 16a). Clearly, the molecules have a preference for a certain orientation on the surface since the intensities of both polarisations are not equal. The time trace of the white spot on the other hand showed that both polarisations contribute equally to the total intensity (Figure 16c), indicating that the emission arises from a large number of chromophoric sites which are not perfectly aligned. Summarising, the correlation between the emission spectra and the time traces is as follows: the polymer
Polyisocyanopeptides with pendant perylene functions

molecules with the polarised, multi-step time-trace display monomer-like emission, while the polymer molecules with the unpolarised, continuous decay in time show excimer-like emission.

Figure 16 a) Fluorescence intensity trajectories for single fluorescent grey species (a) and white species (c), their emission spectra integrated over t=0-25 s time window are given in plots c) and d), respectively.

The observed different emission behaviour occurring in one sample, suggests that two different perylene ‘species’ are present in the polymer mixture, viz. perylenes that are well organised, probably located in the main part of the polymer, and perylenes that are dangling around, most likely in the ill-defined polymer end groups or in short oligomers. As mentioned before, the step-wise behaviour of the time traces of the ‘grey’ spots is reminiscent of the behaviour of the previously studied dendritic perylenes. The flexible polymer end-groups or the oligomers are related to a dendrimer in the sense that the polyisocyanide forces several perylenes together in a confined space and in a less ordered arrangement. Like in the dendrimers, after excitation, the energy is transferred to the perylene with the lowest lying state and from there emitted, hence explaining the polarised emission. The intramolecular energy transfer and the successive bleaching of the perylenes yields the multi-step behaviour in the intensity time trace.
The excimer emission stems from the well-defined central part of the polyisocyanides in which the perylenes are all located on top of each other in exactly the same geometry. From the observed continuous fluorescence decay of the single polymer fibres it may be tentatively concluded that many excimer sites are generated in the polymer chain all emitting in different directions, resulting in randomly polarised fluorescence. Figure 17 shows a drawing of a polyisocyanide fibre in which the different fluorescence behaviour of the central part of the polymer chain and the end-groups are schematically indicated.

![Figure 17](image-url)  

**Figure 17** Schematic representation of the suggested origins of the two types of emission (monomer-like and excimer-like) observed in one perylene-polyisocyanide sample.

The question whether the two types of behaviour are displayed by one single polymer molecule, or separately by short oligomers in the random coil conformation and by rigid-rod polymers cannot be answered solely by confocal microscopy measurements. An answer to this question may come from combined topographical and fluorescence measurements on single polymer molecules. Topographical information can be obtained from AFM, which enables rigid-rod polymers to be distinguished from oligomers and monomers. Experiments of this type are described in the next section.

### 6.2.8 Correlation of fluorescence and topography

Nearfield scanning optical microscopy (NSOM) is a technique that can measure topography and relate it to fluorescence.\[^{43, 44}\] Drawback, however, is the large size of the tip end, which makes it difficult to precisely measure small objects like our polymer molecules. It was decided therefore to connect the confocal setup to an atomic force microscope operating in
tapping mode (see Figure 18a). The tip of the AFM was aligned to the optical axis of the confocal microscope, which allowed the fluorescence and topography of the same area of the sample to be measured.

![AFM cantilever](image)

**Figure 18** a) Schematic drawing of the combined confocal microscope/AFM setup; the optical data are obtained from below the substrate and the topographical data from above. b) A dichroic mirror selectively blocks the monomer-like emission of 8, while the excimer emission is passed through, with a cut-off at 575 nm (dashed line).

In order to facilitate the optical measurements, a dichroic mirror was placed before the collection diodes. The dichroic mirror is a wavelength selection tool that reflects light below 575 nm and allows light above that wavelength to pass through. As a result it differentiates between monomer and excimer emission (see Figure 18b). In a confocal image this shows as red light for \( \lambda > 575\text{nm} \) and green light when \( \lambda < 575\text{nm} \). In this way, excimer emission can be differentiated from monomer-like emission.

A combined fluorescence and topographic image is presented in Figure 19. The confocal image is shown in (a) and the AFM image (tapping mode) is depicted in (b). Fibres can be seen with lengths ranging from 20-120 nm. The fact that they are shorter than the ones presented in Figure 11 could be caused by the fractionation experiment: next to removing the short oligomers, also the very long chains are lost. Another reason why no long polymers are seen could be that due to the polydispersity of the sample, the chance of finding a long polymer in extremely diluted solutions is small. The height of the fibres (2.5 nm) was found to be identical to the height measured for perylene polymers 8 on mica (see §6.2.6). Figure 19a shows that a clear correlation is present between topography and fluorescence, and, keeping the wavelength selection in mind, only those molecules emitting in the red (\( \lambda > 575\text{nm} \)) correlate with a topographic feature, while green species (\( \lambda < 575\text{nm} \)) do not possess any height.

Recording of the evolution of the emission intensities and the fluorescence spectra as a function of time revealed that the red species displayed a continuous decrease in fluorescence.
intensity, whereas the green species showed discrete intensity levels (Figure 19c). The fluorescence spectrum of the green species was similar to that of precursor molecule 6 (monomer-like emission), whereas the spectrum of the red species showed a broader, red-shifted fluorescence spectrum, indicating that the emission mainly arose from excimer sites.

**Figure 19** a) Confocal and b) AFM image from the same area of a diluted solution of polymer 8 spin-coated on glass (bar = 500 nm). c) Corresponding fluorescence intensity trajectory for an emitting species, with (black) and without (grey) topographic features in AFM. d) Idem for emission spectra.

Since the red emission originates from single polymer fibres that are visible by AFM, it is concluded that the helical perylene polyisocyanides emit via multiple, independent excimer sites which are created along the polymer chain during excitation. After photo-excitation, strong exciton coupling is expected to occur between the highly organised perylene side chains of the polymer. However, due to this well-defined and close-by organisation, the excitons are quenched via formation of excimer species between two or a few perylenes. The latter act as a sink for the exciton, limiting the extent of the delocalisation of the electronic excitation, and leading to the observed emission.
When the fluorescence intensity is plotted against the length of the single polymer fibres as measured by AFM, a clear linear correlation is observed (see Figure 20). This is to be expected, since the longer the polymer, the higher the chance of creating excimers.

![Graph showing linear correlation](image)

**Figure 20** a) Height profile for a line section of the AFM image showing features with a height of 2.5 nm, corresponding to individual polyisocyanide molecules. b) Plot of the fluorescence intensity (at t=0) vs. the polymer length for 18 selected single perylene polyisocyanide molecules.

The green emission in Figure 19 is assigned to short polyisocyanide oligomers in a random coil conformation, consisting of just a few perylene units. These species could not be detected with AFM due to the average noise level in the AFM images of ca. 1 nm (see Figure 20a). Two main factors are limiting the sensitivity: the roughness of the substrate (O2 plasma cleaned cover glass, which shows much better optical properties than mica), and the vibrations induced by the oil underneath the sample. This explains the fact that no topographic image could be obtained for the oligomers.

In conclusion, we have combined single molecule confocal fluorescence and tapping mode atomic force microscopy to unambiguously prove the occurrence of perylene containing polyisocyanides. With this technique it was possible to obtain detailed information about the optical properties of the polymer molecules.

The short oligomers turn out to be in a random coil conformation. They display monomer-like emission and show the behaviour of a single quantum system, i.e. several perylenes act as if they were one chromophore. The distance between the perylenes in these oligomers is too high for strong excitonic interactions to occur, but short enough for energy transfer to take place, which is demonstrated by the collective dark states observed in several of the time traces. Photo-bleaching was found to cause a stepwise decrease of the fluorescence intensity.

The longer polymers form a rigid helix with the perylenes organised in a well-defined assembly, resulting in excimer formation. Many excimers are formed along the single polymer fibre upon photo-illumination causing an exponential decay of the fluorescence intensity. The total fluorescence intensity was found to increase linearly with the length of the polymer fibre.
These experiments have also shown that, contrary to what was suggested in §6.2.7, the excimer emission and the monomer-like emission arise from two physically separated species (Figure 21). No polymers were found to exhibit both emissions, from which it could be concluded that, after excitation of the end-groups of a polymer, energy transfer occurs to the rigid part of the polymer, where the excitation energy is quenched by the excimer.

![Figure 21](image.png)

**Figure 21** Schematic representation of the origins of the two types of emission (monomer-like and excimer-like) found in one sample. From combined AFM/confocal measurements it was apparent that these species are physically separated.

### 6.3 Conclusions

Polyisocyanides have been prepared with perylene side groups, organised in a well-defined helical fashion along the polymer backbone. Analogously to the porphyrin-appended polyisocyanides described in Chapter 3, enantiopure L-alanine was used as a starting material to obtain helices of one particular handedness that have a hydrogen bonding array along the polymer backbone, which stabilises the helix. These hydrogen bonding arrays together with the strong π-π stacking interactions of the perylene side groups cause the rigid helical structure to be stable to at least 90°C. The fluorescence spectrum of these polymers displayed a broad structureless red-shifted emission band. Fluorescence decay studies revealed species with a decay time of 19.9 ns, which provides proof of the occurrence of intramolecular excimer formation upon photo-excitation of the perylene molecules in one polymer rod. This process is facilitated by the fact that the perylene moieties are organised in a close-by, face-to-face geometry, which is ideal for excimer formation. Single polymer fibres could be visualised by AFM, which revealed that the individual fibres had lengths of several hundred nm, incorporating several thousand perylenes, the longest being ca. 1 µm, which corresponds to a
Polyisocyanopeptides with pendant perylene functions

molecular weight of ca. $6 \times 10^6$ Daltons. AFM studies provided evidence for the helical nature of these polymers; a left-handed helical structure was observed on single fibres with a pitch of 1.5 nm. When this value is taken as a measure of the perylene organisation, it is calculated that the projection angle between the $1^{\text{st}}$ and the $5^{\text{th}}$ porphyrin is $24^\circ$, which is remarkably similar to the values found for the porphyrin appended polyisocyanides (Chapter 3).

Using a combination of confocal fluorescence microscopy and AFM it was possible to unambiguously show that excimers are only generated if the polymer chain is sufficiently long and has a helical, well-organised rigid structure. Furthermore, it was shown that coiled oligomers, although not well-organised, are still capable of energy transfer, as was obvious from the emergence of collective dark states.

6.4 Experimental section

6.4.1 Materials and methods

For an overview of the materials and methods employed see chapter 3.

6.4.2 Time-resolved fluorescence

The fluorescence measurements were performed on $\sim 10^{-6}$ M solutions of the particular perylene compound in CHCl$_3$. As a reference, compound 6 was used. Its decay could be fitted to a monoeponential decay function, from which a fluorescence lifetime of 3.9 ns was obtained ($\chi^2=1.513$). The fluorescence decay of the polymer was measured on the raw polymer mixture (with a small contribution to the total fluorescence of the excimer) and as the purified polymer, which shows mainly the excimer band. Fluorescence lifetimes were obtained after fitting of the fluorescence decay curves, which were recorded with the time-correlated single photon counting technique in the reversed mode using a micro channel plate photomultiplier (Hamatsu R3809u-51).

6.4.3 Fractionation experiment

Polymer 8 was dissolved in CHCl$_3$ (75 mL) and poured into MeOH (150 mL). The red precipitate was isolated after centrifugation (5000 rpm, 5 min). The colour of the supernatant solution was yellow, indicating the presence of monomeric perylenes. The precipitate was redissolved in CHCl$_3$ (30 mL) and to this solution THF (50 mL) was added. The mixture was centrifuged using the same conditions. The precipitate was isolated and dissolved in CHCl$_3$ (25 mL) and to this solution MeOH (30 mL) was added. The supernatant had changed from yellow to pink. The precipitate was obtained after centrifugation and the latter cycle was repeated.

6.4.4 Atomic force microscopy

AFM experiments were performed using a Nanoscope IIIa instrument from Digital Instruments. Solutions of 8 ($\sim 10^{-4}$-$10^{-5}$ M) in CHCl$_3$ were spin-coated onto freshly cleaved Muscovite mica. All images were taken in tapping...
mode in air at room temperature. Commercial tapping-mode tips (Digital Instruments) were used with a typical resonance frequency around 300 kHz.

6.4.5 Confocal microscopy and spectroscopy

The experimental setup for the confocal microscope experiments is depicted below. This setup allows for simultaneous measurement of the fluorescence spectrum, the time trace and the polarisation of emission. Circularly polarised excitation light at $\lambda=488$ nm with a power density at the sample of 1-2 kW cm$^{-2}$ was used. A polarising beam splitter allows for the discrimination of polarisation of the fluorescence, detected in two avalanche photodiodes (APD, SPCM-AQ-14; EG&G Electro Optics) and a CCD camera (Andor, DV437-BV). A dichroic beam splitter (Omega, 590DRLP) in front of the APDs split the fluorescence into two distinct spectral windows (APD1: $\lambda< 590$ nm; APD2: $\lambda>590$ nm). A direct vision prism in front of the CCD camera spread the emission light over its wavelength components and fluorescence spectra were collected using 1 s integration time. For the combined topography and fluorescence experiments (CFM/AFM) on single molecules, a home-built AFM head$^{[45]}$ was coupled to the confocal microscope, and connected to the xy-scanner stage, enabling the simultaneous collection of optical and topographical data.

![Schematic drawing of the confocal/AFM setup.](image)

**Figure 22** Schematic drawing of the confocal/AFM setup. Laser: Ar$^+$/Kr$^+$ (488 nm; continuous wave), ND = neutral density, NA = numerical aperture, CCD = charge coupled camera device, APD = avalanche photodiode.

6.4.6 Synthesis

$N$-(1-ethylpropyl)perylene-3,4:9,10-tetracarboxylic-3,4-anhydride-9,10-imide 2

The synthesis of this compound was performed according to a literature procedure.$^{[46]}$
Asymmetric perylene diamine 3

Compound 2 (0.197 g, 0.427 mmol) and 1,3-diaminopropane (1.0 mL, 11.95 mmol) were stirred in DMF (50 mL) at 100°C for 2 hrs. After cooling of the reaction mixture, the solvent and the 1,3-diaminopropane were removed by evaporation in vacuo. The residue was triturated with methanol (80 mL). The solid was filtered off and was subsequently dissolved in chloroform. This chloroform solution was filtered and after evaporation of the solvent in vacuo, 0.137 g (62%) of the product was obtained as a red solid. 1H NMR (CDCl3, 300.13 MHz) δ 8.60 (d, 2H, perylene), 8.51 (d, 2H, perylene), 8.46 (d, 2H, perylene), 8.41 (d, 2H, perylene), 5.07 (m, 1H, NCH), 4.27 (t, 2H, NCH), 6.47 (d, 1H, NH), 5.07 (m, 1H, NCH), 4.67 (p, 1H, CH ala), 4.28 (t, 2H, NCH), 0.99 (t, 6H, CHCH3) ppm. 13C NMR (CDCl3, 75.47 MHz) δ 163.5 (CO perylene), 134.6, 134.2, 131.4, 129.6, 129.3, 126.3, 126.2, 123.1, 123.0 (aromatic), 58.0 (NCH), 39.6 (NCH2), 38.1 (NCH2), 32.2 (NCH2CH2), 25.2 (NCHCH2), 11.5 (CH3) ppm. MS-MALDI-TOF m/z: 517.0 (M+; negative mode) and 517.9 (MH+; positive mode).

Perylene 4

Boc-L-alanine (58 mg, 0.31 mmol) was added to a suspension of compound 3 (145 mg, 0.28 mmol) in 50 mL of CHCl3 and the mixture was cooled on an ice bath under a nitrogen atmosphere. To this mixture were added dicyclohexylcarbodiimide (64 mg, 0.31 mmol) and a catalytic amount of dimethylaminopyridine. After 15 min, the reaction mixture was brought to room temperature. After 1 h again an amount of dicyclohexylcarbodiimide (64 mg, 0.31 mmol) was added and the mixture was stirred overnight at room temperature. The mixture was concentrated and subjected to column chromatography (silica gel, eluent 0-4% MeOH in CHCl3). The product was dissolved in CHCl3 and precipitated in di-isopropyl ether. The compound was filtered, washed with di-isopropyl ether and dried to give 4 as a red solid. 1H NMR (CDCl3, 300.13 MHz) δ 8.56 (d, 2H, perylene), 8.49 (d, 2H, perylene), 8.37 (d, 2H, perylene), 6.98 (t, 1H, NH), 5.24 (d, 1H, NH), 5.04 (m, 2H, CH propyl ethyl), 4.24 (t, 2H, NCH2), 3.34 (m, 2H, NCH2CH2), 2.26 (m, 2H, CHCH2CH2), 2.00 (m, 2H, CHCH2CH2), 1.97 (m, 2H, CHCH2CH2), 1.48 (s, 9H, Boc), 1.45 (d, 3H, CH ala), 0.97 (t, 6H, CHCH2CH2) ppm. 13C NMR (CDCl3, 75.47 MHz) δ 172.6 (CO, alanine), 163.7 (CO, perylene), 134.9, 134.1, 131.6, 129.5, 129.4, 126.4, 126.3, 123.2, 122.9, 122.8 (aromatic), 57.8 (NCH), 37.6 (NCH2), 36.1 (NCH2), 28.4 (Boc), 28.0 (NCH2CH2), 25.0 (NCHCH2), 18.9 (CH3 ala), 11.3 (CH3) ppm. FT-IR (cm−1, KBR): 1695, 1653 (O =CN), 1595, 1577 (C=C aromatic), 810, 746 (CH aromatic). HR-MS (FAB, NBA/PEG600) MH+: 689.2989 (calcld for C40H27N4O7: 689.2975).

Perylene 6

To a solution of compound 4 (90 mg, 0.13 mmol) in 15 mL of dichloromethane, 0.5 mL of trifluoro acetic acid was added and the mixture was stirred for 4.5 hrs. The solution was evaporated and the product was dissolved in CHCl3 (100 mL). The solution was washed with an aqueous saturated NaHCO3 solution (2 x 30 mL) and twice with water, dried (MgSO4) and filtrated. The resulting red filtrate was concentrated to a volume of ca. 20 mL. To this solution, containing 5, the p-nitrophenyl ester of formic acid (formyl-ONP, 40 mg, 0.240 mmol) was added and the mixture was stirred for 14 hrs. The reaction mixture was concentrated and subjected to column chromatography (0-4% MeOH in CHCl3), yielding 42 mg (53%) of 6 as a red solid. 1H NMR (CDCl3, 300.13 MHz) δ 8.67 (d, 4H, perylene), 8.61 (d, 2H, perylene), 8.58 (d, 2H, perylene), 8.26 (s, 1H, formyl), 6.93 (t, 1H, NH), 6.47 (d, 1H, NH), 5.07 (m, 1H, NCH), 4.67 (p, 1H, CH ala), 4.28 (t, 2H, NCH2), 3.33 (m, 2H, NHCH2), 2.26 (m, 2H, CHCH2CH2), 2.01 (m, 2H, CHCH2CH2), 1.96 (p, 2H, CH2CH2CH2), 1.51 (d, 3H, CH3 ala), 0.94 (t, 6H,
Chapter 6

CHCH₂CH₃ ppm. ¹³C NMR (CDCl₃, 75.47 MHz) δ 171.6 (CO, alanine), 163.8 (CO perylene), 160.6 (C formyl), 135.0, 134.2, 131.7, 129.4, 129.3, 126.4, 126.2, 123.3, 122.9, 122.6 (aromatic), 57.7 (NCH), 47.7 (CH ala), 37.5 (NCH₃), 36.2 (NCH₂), 27.7 (NCH₂CH₂), 24.9 (NCH₂CH₂), 18.9 (CH₃ ala), 11.3 (CH₃) ppm. FT-IR (cm⁻¹, KBR): 1695, (O=CN; shoulder 1655) 1646 (amide), 1595, 1577 (C=C aromatic), 810, 744 (CH aromatic). HR-MS (FAB, NBA/PEG600) MH⁺: 617.2383 (calcd for C₉₈H₆₃N₄O₃: 617.2400).

Isocyanide 7
To a solution of the formamide 6 (34 mg, 54.4 μmol) in 20 mL of CH₂Cl₂ was added 55 mg (0.55 mmol) of N-methylmorpholine. After 10 min the temperature was lowered to -30°C (acetone/CO₂) and over a period of 10 min diphosgene (0.14 mmol, 0.3 mL of a stock solution of 0.33 g diphosgene in 5 mL CHCl₃) was added. After vigorous stirring for 10 min, the resulting mixture was extracted with CH₂Cl₂. The mixture was precipitated in 150 mL of methanol/water (1/1 v/v). After filtration, the red product was dried. FT-IR (cm⁻¹, KBR): 3286 (and 3317), 1695, 1651 (asymmetric), 1594, 1577, (C=C aromatic), 810,744 (CH aromatic). HR-MS (FAB, NBA/PEG600) MH⁺: 599.2318 (calcd for C₉₈H₆₃N₄O₃: 599.2294).

Polymer 8
To a solution of monomer 7 (21 mg, 0.04 mmol) in CHCl₃ (4 mL) was added 1/500 equiv. of Ni²⁺ catalyst (0.21 mL of a solution of Ni(CIO₃)₂·6H₂O (6.0 mg) in 49 mL CHCl₃ and 1 mL EtOH). The polymerisation was finished after 5 min. After stirring for an additional hour the mixture was diluted with 50 mL of CHCl₃ and sonicated at 30°C to dissolve the polymer. The mixture was precipitated in 150 mL of methanol/water (1/1 v/v). After filtration, the red product was dried. FT-IR (cm⁻¹, KBR): 3286 (and 3317), 1695, 1651 (asymmetric), 1594, 1577, 810, 744. For further characterisation: see text.

6.5 References
Polyisocyanopeptides with pendant perylene functions

[21] Personal communication with G. Metselaar
[22] The quantum yield (QY) was estimated by dividing the fluorescence intensity by the intensity of the absorption band, assuming that the monomer has a QY of 1.
[28] Transitions to the triplet state, other dark states, bleaching.
Chapter 7

Towards amphiphilic block-copolymers derived from chromophoric polyisocyanides

7.1 Introduction

The ability to control the organisation of electroactive or optoactive molecules in three dimensions, opens the possibility to create advanced functional materials and devices (see Chapter 1). One approach to the construction of programmed 3D objects is the use of amphiphilic building blocks. Generally, amphiphiles have the possibility to organise themselves into three dimensional structures. The term amphiphile denotes the unification of two contradicting or ambivalent properties in one molecule. Most commonly it refers to hydrophilic-hydrophobic, viz. a water ‘loving’ part and a water ‘hating’ part. More generally, however, the term amphiphilic or amphiphatic covers properties like polar-apolar, hydrocarbon-fluorocarbon, or rigid-flexible. When the amphiphiles are dispersed in a specific solvent, they start to aggregate in order to minimise their surface energy. In most approaches hydrophobic interactions are the predominant driving force for this self-organisation. The size and shape of the aggregates (Figure 1) that are formed depend on the ratio of the headgroup size compared to that of the tail, as was described by Israelachvili.¹

The principles of self-assembly of amphiphiles are not only applicable to small molecules, but also to larger molecules such as diblock copolymers. It has been shown that a whole range of nanosized architectures can be constructed from such building blocks.²,³

If one is able to synthesise amphiphiles with functional groups and to organise these into structured assemblies, a range of functional nanoarchitectures would be accessible. Porphyrins
are particularly attractive components of such systems since they are multifunctional molecules and the properties of their assemblies can be tuned in different ways. One of our goals is to synthesise such porphyrin containing amphiphiles.

**Figure 1** Different aggregation morphologies: micellar rods (a), micelles (b), bilayers (c), vesicles (d) and inverted micelles (e).

In this chapter two different approaches towards the construction of amphiphilic polymers based on functional polyisocyanides are described, (i) the construction of block-copolymers consisting of a block of porphyrin functionalised polyisocyanide and a block of either polystyrene or PIAA, and (ii) the construction of a giant amphiphile based on the non-covalent interaction between a biotin functionalised polyisocyanide and the protein streptavidin (SAv).

### 7.1.1 Diblock copolymers: super amphiphiles

Amphiphilic diblock copolymers are a special class of amphiphiles since they are generally one order of magnitude larger in size than the most commonly known molecular amphiphiles, viz. the phospholipids. Amphiphilic block-copolymers are currently under intense investigation, because they can self-assemble into a wide variety of superstructures with unique properties.\(^1\) A very promising application of these architectures lies in the fabrication of nanomaterials, since the superstructure, and in turn the properties, can be varied by modifying the chemical composition of the constituent blocks or by altering physical parameters like temperature, pH, solvent *etc.*, under which self-assembly occurs. An interesting class of these macromolecular compounds are the rod-coil diblock copolymers, which consist of a rigid part and a flexible part.\(^2\) It was shown recently in our group that polyisocyanides derived from isocyno-L-
alanyl-L-alanine and isocyano-L-alanyl-L-histidine can be used as the rigid rod component in a diblock copolymer with polystyrene (PS$_{40}$-PIAA) to form a variety of superstructures, after saponification of the ester functions.$^{[11]}$ The resulting amphiphile was found to self-assemble into vesicles, fibres, bilayers and superhelical structures in water (Figure 2a,b). The same concept could be applied to block copolymers of thiophene functionalised isocyanides and styrene (PS-PIAT), in which case giant vesicles were formed after fusion of the initially formed smaller vesicles (Figure 2d-f).$^{[12]}$ The thiophene containing vesicles were extremely stable and the thiophene groups could be further polymerised to give electron conducting nanospheres. These two examples clearly demonstrate the power of the self-assembly of polyisocyanide-polystyrene block-copolymers to produce large functional architectures in basically two steps: monomer $\rightarrow$ block-copolymer $\rightarrow$ vesicle. The functionality is introduced in the monomer stage. In an overall hierarchical process the monomer is first polymerised to give the block-copolymer, which is then assembled to give the functional nano object.

![Figure 2](image)

**Figure 2** TEM images of (a) collapsed vesicles, and (b) left-handed superhelix (schematically represented in c), formed by PS-PIAA in a NaOAc buffer at pH = 5.6.$^{[11]}$ SEM images of vesicles obtained from PS-PIAT after 50 h (d), fusion process (e), and vesicles after polymerisation of the thiophene functions (f).$^{[12]}$

### 7.1.2 Giant amphiphiles

As an extension of the concept of super amphiphiles, a new type of macromolecular surfactant has been developed in our group, the so-called giant amphiphile. This type of compound consists of a protein or an enzyme and a polymer. The protein acts as the polar head group and the polymer as the apolar tail. These two components can be attached in three different ways:
(i) via cofactor reconstitution,[13] (ii) by direct attachment,[14] or (iii) by ligand-protein binding.[15] We intend to use the latter approach, which is based on the high affinity of the protein streptavidin (SAv) for biotin ligands. The giant amphiphiles obtained using the first two approaches were found to form spherical aggregates and micellar fibres, respectively, while maintaining at least part of their catalytic activity. Approach (iii) led to spherical structures when the giant amphiphiles were added to biotin-functionalised Ferritin proteins.

By changing the flexible polystyrene chains in the giant amphiphiles into more rigid (and chiral) polyisocyanide chains it should in principle be possible to generate a unique type of surfactant, which might self-assemble into a variety of alternative chiral objects. Similar conjugates have been prepared by Niemeyer et al. They functionalised rigid double-stranded (ds) DNA on both ends with a biotin moiety, which, after addition of SAv, formed oligomeric supramolecular networks.[16] Despite its tetravalent binding affinity for biotin, the SAv molecules were predominantly present as bi- or trivalent linkers between the ds DNA fragments. The initially formed cross-linked networks could be converted into circular structures by annealing. These circular architectures consisted of one ds DNA connected on both ends to one SAv, conceivable as a snake that bites its own tail.[17] Amphiphilic properties were, however, not discussed.

It was the objective of this research presented in this chapter to construct (i) novel types of super amphiphiles consisting of a polyisocyanide head group functionalised with chromophoric side chains, and a polystyrene tail, and (ii) new giant amphiphiles composed of a streptavidin head group and a functionalised polyisocyanide tail.

### 7.2 Polyisocyanide based amphiphiles

The first objective was the synthesis and characterisation of polyisocyanide-polystyrene rod-coil block-copolymers containing pendant porphyrin rings using the same approach as described previously for the thiophene functionalised block-copolymers. The overall aim was the development of a new route toward well-defined assemblies capable of directed energy transfer. This approach mimics in a more simple fashion the route used by nature to construct hierarchically self-assembled nano architectures such as photosynthetic antennae.

#### 7.2.1 Attempts to synthesise block copolymers based on chromophoric polyisocyanides

Previously, polystyrene-block-polyisocyanide copolymers have been synthesised and were found to form a variety of helical superstructures.[11] Isocyanides are usually polymerised with a Ni(II) catalyst (see Chapter 2), and the polymerisation is initiated by a nucleophile. In the
Towards amphiphilic block copolymers derived from chromophoric polyisocyanides

case of the copolymers of isocyanides and polystyrene, amino end-capped polystyrene 2 was used as the initiator. An initiator complex was formed upon addition of the polystyrene to the tetrakis t-butyl-isocyanide nickel (II) complex 1 and the resulting carbene complex 3 could be isolated as a yellow solid (Scheme 1). This complex was then used as the catalyst for the polymerisation of the isocyanide monomers.

\[
\begin{align*}
&\text{Scheme 1 Synthesis of different block-copolymers.}
\end{align*}
\]
Polymer 6a

The prepared carbene complex 3a was reacted with isocyanide monomer 4b to give block copolymer 6a. For this synthesis, 4b and 3 were mixed in a ratio of 50:1, which in principle should result in a diblock of 40 styrene units and 50 isocyanide units, corresponding to lengths of the blocks of ca. 10 nm (extended polystyrene chain) and 5 nm, respectively (see for schematic drawing Figure 3).

![Figure 3 Schematic representation of a di-block copolymer consisting of a flexible polystyrene block and a rigid polyisocyanide block with porphyrin pendant functions.](image)

After precipitation in acetone of a solution of the reaction mixture in chloroform, two fractions were obtained after filtration: a residue and a filtrate. $^1$H NMR spectra confirmed the presence of polystyrene and porphyrins in the filtrate. However, the NMR signals of the porphyrins were not broadened, suggesting that the porphyrins do not adopt a well-defined rigid arrangement, and the absorption spectrum of this filtrate indicated a monomer-like spectrum for the porphyrins with a (somewhat broadened) Soret band at 422 nm. This suggests that the polyisocyanide chain adopts a random coil-like conformation. The fact that this polymer is soluble in acetone suggests that a very short polyisocyanide block has been formed. In contrast to the filtrate, the $^1$H-NMR spectrum of the solid residue showed broadened signals of the protons of the alanyl-propyl porphyrin side-groups, while no signals in the polystyrene region (at 7.1, 6.4, 2.2, 1.6 ppm) could be detected. The UV-Vis absorption spectrum (not shown) of this residue in CHCl$_3$ was identical to the spectrum recorded for a polyisocyanide with a well-defined porphyrin arrangement (see §4.2.2). A sharp band at 437 nm and a broadened blue-shifted band at 414 nm were observed. In addition to this, the CD spectrum of this product displayed the same pattern as observed for the well-defined long porphyrin polyisocyanides (Figure 4a). These results indicate that the product contains well-defined rod-like molecules, however, it is unclear whether the polystyrene is attached to these polyisocyanide molecules. AFM measurements on the residue revealed rigid rod-like objects (Figure 4b), all having the same height (ca. 3.0 nm), while the shortest ones did not show a height of 3 nm, but appeared...
lower, probably because they are oligomers adopting a random coil conformation. The lengths of the rods were measured to be up to 100 nm, although the majority was too short to be detected as a fibre. The IR spectrum revealed absorptions at 3313 and 3265 cm$^{-1}$ in a ratio of ca. 1:2.

Figure 4 a) CD spectrum of residue of precipitated 6a in acetone, dissolved in CHCl$_3$, b) AFM image of 6a spin-coated on mica (bar = 500 nm).

**Polymer 6b**

The carbene complex 3a was reacted with isocyanide monomer 5 in a molar ratio of 3:5 = 1:50, in order to prepare block copolymer 6b, consisting of a polystyrene block and a perylene appended polyisocyanide block. Like in the case of the homopolymerisation of 5 (Chapter 6), the colour of the reaction mixture changed within a few minutes to deep pink-red and a precipitate was formed. Tlc indicated the formation of polymer as a red spot at the baseline. Due to the low solubility of the product, no $^1$H NMR spectrum could be recorded. The absorption spectrum was identical to that of the homopolymer, just as the CD spectrum was (Figure 5a). AFM studies of 6b spin-coated onto mica from a CHCl$_3$ solution revealed the presence of fibres, like observed for the homopolymer, ranging in size from tens to hundreds of nms (Figure 5b). These lengths suggest that the product copolymer does not possess the desired ratio of the two blocks, making that the amphiphilic character will be small and probably not useful.
Polymer 6c

Since it was observed that in the synthesis of block-copolymers of styrene and isocyanides with chromophore side groups, the length of the polyisocyanide block could not be controlled in a proper way it was decided to do experiments with 4a (Scheme 1) This monomer had previously been shown to yield the polymer 25 (see Chapter 3), and it was hoped that it would give block-copolymers in which the ratio of the two blocks would be more optimal for aggregation.

The polymerisation was carried out by adding the carbene complex 3a to a solution of isocyanide 4a in a ratio of 1:10 to eventually give block copolymer 6c. The product was extensively washed with acetone, after which $^1$H NMR showed the presence of both polystyrene and polyisocyanide fragments. CD spectroscopy of the product showed a small negative couplet at 425 nm (Figure 6a), which is different from the CD signal of the homopolymer of 4a (see Chapter 3). Although small, this couplet shows that the porphyrins are arranged in a helical fashion. AFM imaging revealed the presence of small spheres with heights of ca. 1 nm and some larger spheres with heigths of ca. 2.5 nm (Figure 6b). The latter objects are ascribed to polyisocyanide molecules in a helical conformation, in line with the conclusion from the CD experiments, while the former objects are probably polymers in a random coil conformation. Whether polystyrene is attached to the helical polyisocyanides or is not linked, giving rise to the 1 nm blobs, is not clear from these studies and requires more detailed investigations.
Towards amphiphilic block copolymers derived from chromophoric polyisocyanides

Figure 6 a) CD spectrum of 6c in CHCl₃. b) AFM image of 6c deposited on mica from a CHCl₃ solution.

The ability of 6c to form aggregates was tested by injecting a CHCl₃ solution of this compound in acetone, which selectively solubilises the polystyrene tail. AFM revealed the presence of aggregates of 200-350 nm diameter with heights of 50-70 nm, next to smaller structures with dimensions of 50-100 nm height and 5-6 nm thickness (Figure 7a). A magnified view of the phase image (Figure 7b) clearly showed a coarse structure on the large aggregates, an indication that they are constructed of smaller aggregates in a rather ill-defined manner, reminiscent of merely phase separated structures, and not the well-defined vesicular or micellar aggregates that were aimed for. It appears that the molecules are aligned, however, this may be due to a tip effect.

Figure 7 a) AFM image of 6c spin-coated on mica (CHCl₃, bar = 1µm). b) Idem, phase image.

A TEM study on this sample confirmed the view that phase segregation had occurred. Like in AFM, spherical objects were observed with diameters of 200-300 nm, while platinum shadowing confirmed the three dimensional shape of these objects (Figure 8). They appear to
be phase separated spheres, with typical dark impermeable centres and more transparent peripheries.

![Figure 8](image1.png)

**Figure 8** a) TEM image of a solution of 6c (CHCl₃) injected in acetone and deposited on a carbon coated grid. b,c) Idem, after Pt shadowing. e-g) Idem, SEM images.

**Polymer 9**

In a second approach, we investigated the possibility to construct a diblock copolymer of different isocyanides assuming that the polymerisation of isocyanides with nickel as a catalyst has a living nature. To this end a second isocyanide monomer has to be added to the polymerisation reaction mixture after the first monomer has been consumed. Although the living character of the polymerisation of isocyanides has been demonstrated in the case of block copolymerisation reactions using a Pt-Pd catalyst[18] (see also §2.3.3) and an electron deficient η₃-allylnickel trifluoroacetate catalyst,[19] the living nature of the polymerisation of IAA’s with nickel perchlorate has not yet been proven.

![Figure 9](image2.png)

**Figure 9** Schematic representation of a block copolymer consisting of two rigid polyisocyanide blocks, a PIAA block and a block containing porphyrin pendant groups.

**Ratio L₁-PIAA : 4b = 3:1**
Towards amphiphilic block copolymers derived from chromophoric polyisocyanides

We hoped that in this way a diblock co-polymer containing two rigid blocks, viz. a block of PIAA and a block of the polymer of porphyrin 4b could be synthesized. Schematically, this polymer is represented in Figure 9, showing the two polyisocyanide blocks in a ratio of 1:3. Once synthesised, the esters in the PIAA block can be (partly or completely) saponified to induce amphiphilic character.

The synthesis of this block copolymer was tried using the initiator complex 3b. This complex\(^{[20]}\) was chosen because it is known to produce relatively short polymers. To a solution of the complex in dichloromethane the monomer L,L-IAA was added to form the first block PIAA. After stirring for 10 minutes, monomer 4b was introduced. From tlc it was concluded that the reaction did not proceed further after two hours, although a considerable amount of 4b remained unreacted in the reaction mixture. Since during homopolymerisation of 4b more than 80% of the monomer is consumed, the lack of polymerisation is probably the result of the initially formed block of PIAA, which hinders the polymerisation of 4b. The absorption spectrum of 9 (Figure 10) revealed that the porphyrin was incorporated into the polymer (maximum at 422 nm), but not in a well-defined helical arrangement, since no J-band at 437 nm could be observed. The CD spectrum of 9 showed a small negative couplet at 435 nm, which arises from porphyrins in a helical arrangement. The magnitude of the CD signal, however, was small, indicating that the ordered helical part of the polymer was not the predominant architecture. A positive Cotton effect at 310 nm highlighted the presence of polymeric IAA.

AFM images of a spin-coated solution of 9 in CHCl\(_3\) showed small ill-defined spheres with heights of 1.4-2.0 nm and widths of 30-70 nm (Figure 11a), however, no rigid fibres were visible. This is in contrast to the long fibres observed for homopolymers of PIAA, and also in contrast to the fibres found for random co-polymers consisting of IAA and a porphyrin.
Chapter 7

isocyanide monomer (Figure 11b, see also §3.2.3). Some aggregation into micellar-like rods was observed in these samples of 9 (Figure 11c). Closer examination of these rods revealed that they had a length of several hundred nm and a diameter of tens of nms. Figure 7c shows a rod of 740 x 60 nm, the height of the rods varied from 5-10 nm.

![Figure 7](image)

**Figure 7** a) Fibres observed for random copolymers of IAA and a porphyrin isocyanide (bar = 500 nm). b) AFM image of 9 spin-coated on mica (CHCl$_3$, bar = 500 nm). c) Idem (bar = 250 nm).

It would be worthwhile to study these micellar rods further with optical techniques, such as the confocal-AFM technique presented in Chapter 6, in order to establish the possible presence of porphyrins within the aggregates. However, due to the lack of time this was not possible.

### 7.2.2 Conclusions

It proved difficult to construct super amphiphiles consisting of a chromophoric polyisocyanide block and a polystyrene or PIAA block. Either the polyisocyanides were too long compared to the polystyrene tail in order to obtain amphiphilic character, or the polyisocyanides were so short that they adopted a random coil conformation. Better control over the polymerisation length, therefore, is desired. Good control over the polymer lengths is obtained by using a μ-ethynediyl Pd-Pt complex as the catalyst (see Chapter 2). Unfortunately, the use of this catalyst is limited to certain monomers and does not allow the polystyrene block to be incorporated easily.

Further experiments are needed to confirm the presence of porphyrins in the aggregated structures that were observed by electron microscopy and AFM.
Towards amphiphilic block copolymers derived from chromophoric polyisocyanides

7.3 Synthesis of Biotinylated Polyisocyanides

The synthesis of giant amphiphiles from proteins and PIAA had not been tried before, hence it was decided to construct such amphiphiles, first from PIAA alone and at a later stage also from porphyrin appended polyisocyanides (Figure 12b). In order to synthesise polyisocyanide-based giant amphiphiles, the method of ligand-protein binding was applied using the very strong interaction between biotin and streptavidin. This research has also been described in the thesis of Dr. J. Hannink. A summary is presented below.

In order to obtain giant amphiphiles consisting of streptavidin and two rigid polyisocyanide chains, two different routes were investigated to incorporate a biotin moiety into a polyisocyanide polymer.

The first approach made use of a biotinylated initiator complex for the polymerisation of the isocyanides. This renders the biotin group immediately available for binding to streptavidin after polymerisation. Experiments were performed by first coupling an aminofunctional biotin to the initiator complex 1. Due to the difference in polarity of the biotin moiety and the initiator complex, the reaction could only be carried out in nitromethane with sonication at 60ºC. This complex was subsequently used as the initiator for the polymerisation of L,L-IAA to give 12 (Figure 12a). Unfortunately, no evidence could be obtained that the biotin moiety was incorporated into the polymer.

In a second approach, an initiator complex 3b containing a protected functional Fmoc group was used, as a precursor to other functionalities. After the polymerisation the functional group can be removed making it available for functionalisation with biotin. The initiator complex could be synthesized in dichloromethane and polymerisation with L,L-IAA was subsequently carried out to give 13 (Figure 12a). Proof of the incorporation of the Fmoc group into the polymer was obtained from UV-Vis absorption spectroscopy and ES mass spectrometry. Due to the lack of time further functionalisation of 13 could not be realised.

Interestingly, the contour lengths (as measured with AFM), using identical reaction conditions, were clearly lower for 12 and 13 than for the PIAA polymers obtained with ethanol as initiator and nickel as catalyst for the polymerisation reaction.\[21\]
Figure 12  
(a) Chemical structures of PIAA with a biotin (12) or Fmoc (13) functional group.  
(b) Schematic representation of the structure of a possible PIAA-SAvid conjugate,  
obtained after binding of the biotin containing polymers to SAvid.

7.3.1 Conclusions

From the experiments described in this section it is concluded that it is possible to link a Fmoc moiety to PIAA, which could open a route to form amphiphilic polymer-protein hybrids. In the future, it would be of interest to use this concept to create biotin functionalised systems that contain polyisocyanides with porphyrin pendant groups and to study the binding of these polymers to SAvid and subsequently study the self assembling properties of these bio-conjugates.

7.4 Experimental Section

Chemicals

All solvents were distilled prior to use. Thin layer chromatography analyses were performed on Merck precoated silica gel 60 F254 plates. NMR spectra were recorded on a Bruker AC-300 instrument at 297 K. Chemical shifts are reported in ppm relative to tetramethylsilane (δ = 0.00 ppm). FT-IR spectra were recorded on a Bio-Rad FTS 25 instrument. Absorption spectra were measured on a Varian Cary 50 Conc spectrometer. CD spectra were obtained on a Jasco J-810 spectropolarimeter.

Electron Microscopy

A solution of block-copolymer 6c in CHCl₃ (1mL) was injected into acetone (1mL). This mixture was drop casted onto an electron microscope grid and studied with TEM and SEM. TEM images were obtained with a JEOL JEM-1010 microscope (60kV) equipped with a CCD camera. SEM images were obtained with a JEOL JSM-6330F. Carbon coated electron microscope grids were commercially obtained from Electron Microscope Sciences.

AFM Microscopy

Imaging was performed with a Nanoscope III instrument from Digital Instruments operating in tapping mode at room temperature. Samples were prepared by spin coating (1800 rpm) a polymer solution in chloroform on freshly cleaved mica.
Towards amphiphilic block copolymers derived from chromophoric polyisocyanides

**Synthesis**

**Nickel complex 1**

This complex was synthesised according to a literature procedure.\(^\text{[22]}\)

**Initiator complex 3a**

Synthesis of this compound was performed as described in ref.\(^\text{[11]}\) The polystyrene was an amine end-capped polymer and had the following properties: \(M_n = 4.2 \times 10^3\), PD index = 1.05 (40 repeat units). Nickel complex 1 (7.28 mg, 0.012 mmol) was dissolved in 5 mL of \(\text{CH}_2\text{Cl}_2\) under a nitrogen atmosphere. It took ca. 15 min. to dissolve all compound to yield a yellow solution, after which the amine end-capped polystyrene 2 (55.2 mg, 0.013 mmol) was added as a solution in 4.4 mL \(\text{CH}_2\text{Cl}_2\). The mixture turned orange almost instantaneously.

**Di-block copolymer 6a**

An aliquot of 0.16 mL (1.09 mg, 0.23 \(\mu\)mol) of the mixture of 3a (see above) in \(\text{CH}_2\text{Cl}_2\) was added to a solution of 4b in \(\text{CH}_2\text{Cl}_2\) (15 mg, 0.011 mmol). After stirring for 1.5 h the reaction mixture was dropped into a stirred mixture of 40 mL of \(\text{MeOH}/\text{H}_2\text{O}\) (3/2 v/v). The precipitated product was filtered and washed with MeOH. The residue was dissolved in \(\text{CHCl}_3\) and precipitated in acetone. The resulting precipitate (a) was filtered and washed with acetone. The filtrate was evaporated to give a purple solid. The precipitated product (a) was also isolated to yield 10 mg of a purple solid. \(^1\)H NMR of filtrate (\(\text{CDCl}_3\), 300.13 MHz) \(\delta\) 8.86 (m, 8 H, \(\beta\)-pyrrole), 8.11 (d, 2H, meta to O-propyl), 8.10 (d, 6H, meta to OCH\(_2\)), 7.27 (d, 8H, ortho to OR), 7.3-6.8 (br, ArH Ph), 6.7-6.3 (br, ArH Ph), 4.37 (t, 2H, OCH\(_2\)), 4.32 (q, 1H, CH), 4.25 (t, 6H, OCH\(_2\)), 3.70 (q, 2H, NHCH\(_2\)), 2.25 (p, 6H, OCH\(_2\)), 1.97 (p, 6H, OCH\(_2\)), 1.7-1.2 (br, CH\(_2\)CHPh), 1.97 (p, 6H, OCH\(_2\)), 1.7-1.2 (br m, 54H, aliphatic, CH\(_2\)CHPh), -2.75 (s, 2H, NH) ppm. Product a: \(^1\)H NMR (\(\text{CDCl}_3\), 300.13 MHz) \(\delta\) 9.0 (br, \(\beta\)-pyrrole), 8.2 (br, ArH meta to O-propyl), 8.1 (d, 2H, meta to O-propyl), 7.3-6.8 (br, ArH Ph), 6.7-6.3 (br, ArH Ph), 4.3 (br, OCH\(_2\)), 4.2 (br, OCH\(_2\)), 3.8 (br, NCH\(_2\)), 2.2 (br, CH\(_2\)), from 2 to 0 (br with maxima at 1.6, 1.5, 1.3, 0.9, aliphatic), -2.8 (br, NH) ppm. IR: 3313, 3265 (ratio ca. 1:2), 1656, 1606, no sign of polystyrene (expected at 1493 and 1453).

**Diblock copolymer 6b**

To a solution of perylene isocyanide 5 (8 mg, 13 \(\mu\)mol) in 4 mL of \(\text{CH}_2\text{Cl}_2\) was added complex 3 (1.3 mg, 0.3 \(\mu\)mol). The mixture was stirred for 10 min. Aliquots of the reaction mixture were dissolved in \(\text{CHCl}_3\) for characterisation, see text.

**Di-block copolymer 6c**

Polystyrene initiator complex 3a (4.3 mg, 0.9 \(\mu\)mol) was added to a solution of porphyrin isocyanide 4a (7 mg, 8.6 \(\mu\)mol) in 2 mL of dichloromethane. The reaction mixture was stirred for 2 days, although tlc seemed to indicate that the reaction had already stopped after one day. After addition of another 2 mL of dichloromethane, the reaction mixture was precipitated in 30 mL of \(\text{H}_2\text{O}/\text{MeOH}\) (1/2 v/v). The mixture was filtered and the residue was washed with methanol and acetone until the filtrate was colourless. The precipitation procedure in a water/methanol mixture and washing with methanol and acetone was repeated again. \(^1\)H-NMR (300.13 MHz) \(\delta\) 9.0-8.5 (br, \(\beta\)-pyrrole), 8.2-7.6 (br, ArH meta to CH\(_3\)), 7.6-7.3 (br, ArH ortho to CH\(_3\)), 7.2-6.9 (br, ArH Ph), 6.8-6.4 (br, ArH Ph), 4.6-3.4 (br, CH ala, OCH\(_3\), NCH\(_2\)), 2.8-2.4 (br, CH\(_3\) porphyrin), 2.2-1.2 (br, CH\(_2\)CH\(_2\)CH\(_2\), CH\(_2\)CHPh, CH\(_3\) ala, CH\(_2\)CHPh), -2.7--3.0 (br, NH pyrrole).
Di-block copolymer 9

To a solution of 7 in 1.5 mL of CH₂Cl₂ (1.5 mg, 0.008 mmol) was added 0.05 equiv. of 3a (0.38 mg, 0.4 µmol). After stirring for 10 min, 1 mL of a solution of 4b in CH₂Cl₂ was added and the mixture was stirred for 15 h (although tlc indicated that the reaction did not proceed anymore after 2 hrs, while not all monomer was consumed). The reaction mixture was precipitated in 50 mL of H₂O/MeOH (1/4 v/v). After isolation, the purple precipitate was dissolved in CHCl₃ and precipitated in hexane to remove monomeric porphyrin. The precipitate was filtrated and the residue was dissolved in acetone. Characterisation, see text.

7.5 References

Summary

In this thesis a new method to arrange dye molecules (porphyrins and perylenes) in a well-organised fashion over extended lengths up to hundreds of nanometers is presented. This method was inspired by the naturally occurring light harvesting systems that are capable of efficient energy transfer over large distances (>100Å), involving tens or hundreds of bacteriochlorophyll pigments. It was envisioned that the arrangement of the dye molecules in a well-defined organisation would require a rigid polymeric scaffold. Polyisocyanides are helical, intrinsically rigid polymers that could serve this purpose (Figure 1a). It was recently discovered by our group that polyisocyanides prepared from isocyanodipeptides form stable $\beta$-helical architectures due to the presence of hydrogen bonding arrays along the helical polymer backbone. This hydrogen bonding network rigidifies the helix and prevents unwinding to a random-coil structure. Besides, the handedness of the polymeric backbone can be tuned by using the appropriate L- or D-amino acids.

Since it had previously been shown that grafting of polyisocyanides did not lead to a polymer that was completely substituted with porphyrins, it was decided to first prepare porphyrin and perylene isocyanides and to subsequently polymerise these monomers. In order to find the optimal conditions for the fabrication of well-defined porphyrin arrays, several polyisocyanides with porphyrin side groups were synthesized. From this initial exploration several parameters that are crucial for the successful synthesis of long porphyrin pendant polyisocyanides could be determined. Two factors were found to mainly determine the success of the polymerisation, viz. steric hindrance and enantiomeric purity of the monomers.

Subsequently, polymerisation reactions were carried out which led to polymers with an average length of ca. 100 nm and a dispersity of PD = 1.35. These numbers were determined by AFM, since it was impossible to use other techniques like GPC or Maldi-TOF to determine molecular weights. Spectroscopic studies revealed that in the side chains of the polymers hydrogen bonding arrays were present, but not as well-defined as in the case of other peptide derived polyisocyanides. The UV-Vis absorption spectra of the polymers showed a sharp band at 437 nm, indicative of porphyrin molecules arranged as J-aggregates. The intensity of this band was dependent on the length of the central, well-defined part of the polymers, which was verified by preparing polyisocyanides of different lengths. The J-band displayed a
reversible change upon heating in organic solvents. Studies in toluene revealed that the porphyrins initially had a tilted geometry, which, upon heating changed into a perpendicular geometry. Resonance light scattering (RLS) experiments showed that at least 25 porphyrins are involved in energy transfer along one stack. Depolarised RLS showed that the slip angle $\beta$ between 1$^{st}$ and the 5$^{th}$ porphyrin amounted to 22° (Figure 1b), in agreement with calculations. Fluorescence anisotropy was used to obtain additional information concerning the orientation of the porphyrins in the polymers. It was calculated from the anisotropy measurements that the porphyrins are tilted by ca. 25° with respect to the helix axis of the polymers (Figure 1c). The porphyrin containing polyisocyanides displayed intense CD bands, which is ascribed to exciton delocalisation over large distances in the polymers. This delocalisation phenomenon resembles the energy transfer processes occurring in the natural antenna systems.

![Figure 1](image-url)  

**Figure 1** a) Schematic representation of a helical polyisocyanide to which porphyrin chromophores are attached. b) Representation of the slip angle between the 1$^{st}$ and 5$^{th}$ porphyrin in a polyisocyanide chain. c) Illustration on how the porphyrins are twisted with respect to the central polyisocyanide helix.

The above described studies involved polymers with pendant free base porphyrins. Two of these polyisocyanides were converted into polymers with pendant zinc porphyrin groups. These two polymers differed in the orientation of the porphyrin units in their side chains. This resulted in different behaviour upon the complexation of the bivalent ligand 1,4-diazabicyclo[2.2.2]octane (DABCO), leading to sandwich-type of complexes. The formation of these complexes occurred in either a non-co-operative or a co-operative fashion, depending on the initial conformation of the porphyrin side chains. This was the result of a difference in the structure of the polymers caused by the presence of different spacer groups between the porphyrin and the polyisocyanide backbone. It was found that after metal insertion, a trace of free-base porphyrin was still present in the polymers, which made that these porphyrins acted
as energy sinks for the excitation energy. Fluorescence spectroscopy studies indicated that the zinc porphyrin molecules in the polymers were intramolecularly ligated by alkoxy oxygen atoms of neighbouring porphyrins. This ligation stabilised the polymer structure to the extent that, upon heating to 70°C, no conformational changes occurred, while for the analogous free-base porphyrin polymer such changes did occur. This co-ordination architecture is similar to the supramolecular arrangement found in the natural bacteriochlorophyll aggregates in the chlorosomes of green bacteria. Fluorescence anisotropy measurements revealed that the zinc porphyrins had a twisted arrangement with respect to the polymer helix axis. This arrangement could be switched upon the addition of pyridine, which changed it into an orientation that was perpendicular with respect to this axis.

These results show that it is possible to synthesise simple analogues of the rod-like bacteriochlorophyll aggregates found in the chlorosomes of green bacteria, which mimic not only the number of chromophores, but also the dimensions, architecture and chirality of the natural system.

To further explore the concept of organising pigments on rigid polymer scaffolds, polyisocyanides with perylene side groups were prepared. Perylenes are promising candidates as components in organic solar cells, *viz.* as n-type conductors. The same route was followed as developed for the synthesis of the porphyrin containing polymers, including the incorporation of an alanine moiety to provide for hydrogen bonding arrays along the polyisocyanide backbone. These hydrogen bonding arrays together with the strong π-π stacking interactions of the perylene side groups made that the helical conformation of the polymer was very stable. Upon photo-excitation, neighbouring perylenes were found to form intramolecular excimers within one polymer chain, which could be concluded from a broad structureless red-shifted band in the emission spectrum. Fluorescence decay studies revealed the presence of species with a lifetime of 19.9 ns, which was proof of excimer formation in the polymer rods. Single polymer fibres could be visualised by AFM, which revealed that the individual fibres had lengths of several hundreds of nanometers, incorporating several thousands of perylenes. AFM studies provided evidence of the helical nature of these polymers; a left-handed helical structure was observed to be present in single fibres displaying a pitch of 1.5 nm. From these AFM measurements the slip angle between the 1st and the 5th perylene was calculated to be 24°, which is in line with several other studies in this thesis on porphyrin appended polyisocyanides. Using a combination of confocal fluorescence microscopy and AFM it was possible to unambiguously show that excimers are only
generated if the polymer chain has sufficient length and a well-organised rigid structure. Furthermore, it was shown that coiled oligomers, although not well-organised, are still capable of energy transfer, as was obvious from the emergence of collective dark states.

The ability to control the organisation of porphyrins or perylenes in three dimensions, could be of interest to create advanced functional materials. Therefore, super-amphiphiles (amphiphilic block-copolymers) were designed containing at least one block of the functional polyisocyanide and another block of polystyrene. It was envisaged that these block-copolymers would self-assemble into a variety of 3D objects. It proved, however, to be difficult to construct these super-amphiphiles. Either the polyisocyanides were too long compared to the polystyrene tail in order to obtain amphiphilic character, or the polyisocyanides were so short that they adopted a random coil conformation. Sometimes, spherical or tubular aggregated structures were observed by electron microscopy and AFM, but further experiments are needed to establish their molecular structure.

As an alternative method to synthesise polyisocyanide-based amphiphiles, the method of ligand-protein binding was applied using the very strong interaction between biotin and streptavidin. To check the applicability of this method, first the coupling of streptavidin to more simple polyisocyanides, i.e. without porphyrins, was tested. Preliminary experiments confirmed that it was possible to link a 9-fluorenylmethoxycarbonyl (Fmoc) moiety to the polymer. This precursor compound can be used to prepare a biotinylated polyisocyanide, but this route was not studied further due to the lack of time. In the future, it would be of interest to use a biotin functionalised polyisocyanide with porphyrin pendant groups and study the binding of these polymers to streptavidin and subsequently study the self assembling properties of these bio-conjugates.

**Samenvatting**

In dit proefschrift wordt een nieuwe methode behandeld om organische pigmenten (porfyrines en perylenen) op een goed gedefinieerde manier in rijen te ordenen over lengtes van honderden nanometers. Deze methode is geïnspireerd op de licht invangende antennesystemen die deel uitmaken van de fotosynthesesystemen in planten en sommige
bacteriën. Deze antennes zijn opgebouwd uit honderden tot duizenden bacteriochlorofyl pigmenten, die in staat zijn om uiterst efficient en over grote afstanden (>100 Å) energie aan elkaar over te dragen. Een manier om pigmenten in rijen te ordenen tot een goed gedefinieerd geheel is, zo werd verwacht, het gebruik van een starre polymere drager, waaraan deze pigmenten kunnen worden vastgezet. Polysocyanides zijn schroefvormige, intrinsiek stijve polymeren, die als een staafrigheid, polymere drager zouden kunnen dienen (Figuur 1a). Recentelijk is in onze onderzoeksgroep ontdekt dat polysocyanides, afgeleid van isocyanodipeptides, stabiele, zogenaamde beta-helix structuren vormen als gevolg van de aanwezigheid van waterstofbruggen langs de ruggengraat van het polymer. Dit waterstofbrugnetwerk maakt dat het polymer zeer stijf is en voorkomt bovendien dat het polymer ontwindt en een spaghettivorm aanneemt. Daarnaast kan de schroef(helix)richting van het polysocyanide worden gekozen door de juiste L- en D-aminozuren te gebruiken.

Aangezien al eerder was aangetoond dat het vastzetten van porfyrines aan een separaat gesynthetiseerde polymere drager niet tot goede resultaten leidde, werd besloten om eerst de monomere isocyanides van porfyrines en perylenen te synthetiseren, en deze vervolgens te polymeriseren. Teneinde de optimale omstandigheden te vinden voor de fabricage van goed gedefinieerde rijen van porfyrines, werden verscheidene polysocyanides met porfyrinezijgroepen gesynthetiseerd. Uit dit exploratieve onderzoek konden verschillende parameters worden afgeleid die belangrijk zijn voor een succesvolle synthese van lange polysocyanides. Twee factoren bleken een hoofdrol te spelen: sterische hindering en enantiomere zuiverheid van de monomeren.

Vervolgens werden polymerisatiereacties uitgevoerd die polymeren opleverden met een gemiddelde lengte van ca. 100 nm en een dispersiteit van PD = 1.35. Deze waarden werden bepaald met AFM, aangezien dit onmogelijk was met de meer gangbare technieken zoals Maldi-ToF of GPC. Spectroscopische studies toonden het voorkomen van waterstofbruggen aan, hoewel deze waterstofbruggen niet zo goed gedefinieerd waren als in het geval van andere, van peptide afgeleide polysocyanides. UV-Vis absorptiespectra van de polymeren vertoonden een scherpe band bij 437 nm, hetwelk een indicatie was voor het voorkomen van een J-type aggregatie van de porfyrines in het polymer. De intensiteit van deze band was afhankelijk van het centrale, goed gedefinieerde deel van de polymeren, hetgeen werd bewezen door polymeren van verschillende lengtes te synthetiseren. Deze J-type absorptieband vertoonde een reversibele verandering bij verwarmen in organische
oplosmiddelen. Studies in tolueen brachten aan het licht dat de porfyrines aanvankelijk een gekantelde geometrie hadden, welke echter door verwarmen veranderde in een loodrechte ordening. Resonante lichtverstrooiingsexperimenten (RLS) toonden aan dat zeker 25 porfyrines in een rij betrokken zijn bij energieoverdracht langs de polymeerketen. Uit gedepolariseerde RLS metingen werd afgeleid dat de schuifhoek tussen de $1^\circ$ en $5^\circ$ porfyrine $22^\circ$ is (Figuur 1b), hetgeen in overeenstemming was met berekeningen. Fluorescentie-anisotropiemetingen werden gebruikt om additionele informatie te verkrijgen omtrent de precieze oriëntatie van de porfyrines. Aan de hand van deze anisotropiemetingen werd berekend dat de porfyrines $25^\circ$ gekanteld zijn ten opzichte van de centrale schroefas van het polymeer (Figuur 1c).

Het CD spectrum van de porfyrinepolymeren vertoonde intense banden, die werden toegeschreven aan excitondelocalisatie over grote afstanden in de polymeerketen. Dit fenomeen lijkt op de energie-overdrachtsprocessen die plaatsvinden in antennesystemen uit de natuur.

![Figuur 2](image1.png)

**Figuur 2** a) Schematische weergave van een schroefvormig polyisocyanide waaraan porfyrines zijn verankerd. b) Weergave van de schuifhoek tussen de $1^\circ$ en de $5^\circ$ porfyrine in een polyisocyanideketen. c) Weergave van de wijze waarop de porfyrines zijn gekanteld ten opzichte van de centrale polyisocyanide-schroefas.

De hierboven beschreven studies behelsten polymeren met vrije-base-porfyrines als zijgroepen. Twee van deze polyisocyanides werden omgezet in polymeren waarin zinkporfyrines als zijgroepen functioneren. Deze twee polymeren verschillen in de oriëntatie van de porfyrines in de zijketens. Dit resulteerde in verschillend gedrag na complexering van een bivalent ligand, 1,4-diazabicyclo[2.2.2]octaan (DABCO), waarbij sandwich-achtige complexen ontstaan. De vorming van deze complexen geschiedde ofwel op een manier die lineair afhankelijk is van de DABCO-concentratie, ofwel coöperatief, afhankelijk van de
initiële conformatie van de porfyrines. Dit verschil kon worden teruggevoerd op het verschil in ketens waarmee de porfyrines zijn verbonden met de ruggengraat van beide polymeren. Er werd gevonden dat na zinkinsertie nog steeds een kleine hoeveelheid vrije-base-porfyrines in de polymeren aanwezig was, die als energieputjes fungeerden voor de excitatie-energie. Fluorescentie-spectroscopische studies gaven aan dat de zinkporfyrine-moleculen in de polymeren intramoleculair werden gestabiliseerd door binding van de alkoxy-zuurstofatomen van naburige porfyrines. Deze ligandering stabiliseerde de polymeerstructuur zodanig dat bij verhitting tot 70° geen conformatieveranderingen optraden, terwijl voor het analoge vrije-base-porfyrinepolymeer zulke veranderingen zich wel voordeden. Deze supramoleculaire architectuur van de zinkporfyrines lijkt op de supramoleculaire schikking van bacteriochlorofyl moleculen in de chlorosomen van groene bacteriën. Fluorescentie-anisotropiemetingen brachten aan het licht dat de zinkporfyrines in de polymeren een gekantelde ordening aannamen ten opzichte van de polymere schroefas. Deze ordening kon worden veranderd door toevoeging van pyridine, hetgeen resulteerde in een oriëntatie loodrecht op de schroefas.

Bovengenoemde resultaten tonen aan dat het mogelijk is om simpele analoga te synthetiseren van de staafvormige bacteriochlorofylaggregaten uit groene bacteriën, die niet alleen het aantal pigmenten, maar ook de afmetingen, structuur en chiraliteit van het natuurlijke systeem nabootsen.

Om het concept van het organiseren van pigmenten aan rigide polymeren verder uit te werken werden polyisocyanides met peryleen-zijgroepen gesynthetiseerd. Perylenen zijn veelbelovende verbindingen voor toepassing als n-type geleiders in organische zonnecellen. Dezelfde route als beschreven voor de synthese van de porfyrinepolymeren werd gevolgd, inclusief de inbouw van alanine-eenheden om het polymeer van waterstofbruggen te kunnen voorzien. De waterstofbindingen, samen met de sterke π-π stapelinteracties tussen de perylenen, bleken voor een uitermate stabiele polymeerconformatie te zorgen. Foto-excitatie maakte dat intramoleculaire excimeren werden gevormd tussen nabijgelegen perylenen, hetgeen kon worden opgemaakt uit het voorkomen van een brede, roodverschoven, structuurloze band in het emissiespectrum. Met fluorescentieverval-metingen kon de aanwezigheid worden aangetoond van een deeltje met een levensduur van 19.9 ns, hetgeen een bewijs is voor de vorming van excimeren in de polymere staafjes. Afzonderlijke polymeervezels konden zichtbaar worden gemaakt met AFM. Dit bracht aan het licht dat de individuele polymeermoleculen lengtes konden bereiken van enkele honderden nanometers.
hetgeen overeenkomt met enkele duizenden peryleen moleculen. AFM-metingen leverden ook het bewijs dat de peryleen een schroefvormige structuur bezitten: een linkshandige schroef met een spoed van 1.5 nm werd waargenomen in afzonderlijke, moleculaire polymeerfibers. Hieruit werd een schuifhoek van 24° tussen het 1e en 5e peryleen berekend, hetgeen in overeenstemming is met verscheidene andere studies aan de porfyrienepolymeren in dit proefschrift. Door gebruik te maken van een combinatie van confocale fluorescentiemicroscopie en AFM was het mogelijk om ondubbelzinnig aan te tonen dat excimeren alleen gevormd worden als de polymeerketen voldoende lang is om een goed-gedefinieerde schroefstructuur aan te nemen. Bovendien werd aangetoond dat flexibele oligomeren, hoewel niet goed gedefinieerd, toch in staat zijn tot energie-overdracht. Dit bleek uit het voorkomen van collectieve ‘donkere toestanden’ van de fluorescentie in de tijd.

De mogelijkheid om de organisatie van porfyrines of peryleen in drie dimensies te sturen, kan van belang zijn voor het ontwerpen van nieuwe materialen. Als eerste aanzet hiertoe werden zogenaamde superamfifiele (amfifie blok-copolymeren) ontworpen, die tenminste één blok van het functionele polyisocyanide bevatten en een tweede blok van polystyreen. Er werd verwacht dat deze blok-copolymeren zich zouden organiseren tot een reeks van 3D-objecten. Het bleek echter lastig te zijn om deze superamfifiele in elkaar te sleutelen. De polyisocyanides waren ofwel te lang in vergelijking met het polystyreen-staartdeel om een goed amfifie karakter te induceren, ofwel ze waren zo kort dat ze niet netjes een rigide schroefvorm aannamen. Soms kondsten met electronenmicroscopie en AFM sferische of buisvormige aggregaten worden waargenomen, maar om de moleculaire structuur hiervan te ontrafelen zijn meer experimenten nodig. Een alternatief om amfifie copolymeren van polyisocyanides te synthetiseren, is gebruik te maken van een eiwit als hydrofiele component. Als eiwit werd streptavidine gekozen dat met behulp van biotine aan het polymer kan worden gekoppeld; deze moleculen hebben een bijzonder sterke interactie met elkaar. Om de toepasbaarheid van dit concept te testen werd eerst de koppeling tussen streptavidine en simpele polyisocyanides zonder porfyrines, uitgeprobeerd. Initiële experimenten bevestigden dat het mogelijk was om een zogenaamde Fmoc-groep aan het polymer te zetten, welke een voorloper is voor het biotinepolymer. Dit voorlopermolecuul kon door tijdgebrek niet verder worden bestudeerd. In de toekomst zou het interessant kunnen zijn om een biotine gefunctionaliseerd polyisocyanide met porfyrinezijgroepen te gebruiken en de binding met streptavidine te bestuderen en vervolgens na te gaan wat de zelfassemblerende eigenschappen zijn van deze biohybride amfifie verbindingen.

- 182 -


**Dankwoord**

Met een zeer goed gevoel kijk ik terug op mijn promotietijd. Dat het schrijven van het proefschrift een behoorlijke kluif was doet daar niets aan af. Het was een intense periode; ik heb genoten als promovendus op het Noltelab, maar ook buiten het lab heb ik een prachtige tijd gehad. Iedereen die hierbij betrokken was, wil ik daarvoor bedanken. Ik wil uit deze schare echter nog wat mensen in het bijzonder bedanken die nauw betrokken waren bij het onderzoek.

Roeland Nolte, promotor, jou wil ik bedanken voor de vrijheid die je me hebt gegeven om een eigen invulling te geven aan mijn onderzoek en ook voor het vertrouwen dat je in me stelde, ook al was dat wel eens met gefronste wenkbrowwen. Ik heb enorm veel geleerd, vooral doordat je veel verantwoordelijkheid op m’n bord legde. Daarnaast heb ik je persoonlijke invulling zeer op prijs gesteld.

Alan Rowan, copromotor, jou ben ik dankbaar voor je wilde ideeën en onstuurbare enthousiasme en dynamiek. Prettig dat dat gepaard ging met veel humor. Na een opbeurend gesprek met jou had ik het gevoel dat ik toch op het juiste spoor was. Ook op de congressen was je vaak aanstichter van een uitstekende sfeer.

Eén van de leuke kanten van het onderzoek was de kans om samen te werken en zo mijn onderzoek vanuit verschillende oogpunten te belichten.

I am grateful to Prof. Luigi Monsu Scolaru and especially to Dr. Mariangela Castriciano for the RLS experiments. Mariangela thank you for your help and kindness.

I thank Prof. Rabe from the Humboldt University for the opportunity to visit his lab in Berlin twice and allow me to become familiar with SPM.

In his lab I came to work with Dr. Paolo Samori, which was the beginning of a long and very pleasant collaboration. Paolo, I thank you, first of all for a wonderful time in Berlin. Furthermore, I am grateful that you had a great confidence in me and invited me to join you in Strasbourg to start up a new research group at the ISIS institute, which was a wonderful experience. I have enjoyed your easy going and honest attitude, thank you very much.

Prof. René Janssen en Dr. Edda Neuteboom, jullie bedank ik voor de hulp bij de synthese van de peryleenprecursors en de tijdsopgeloste fluorescencientemetingen. Daarnaast ben ik René erkentelijk voor de uitstekende feedback op mijn proefschrift.
Prof. Niek van Hulst en Dr. María García-Parajó bedank ik voor een plezierige en vruchtbare samenwerking. Maria I thank you also for the pleasant and pragmatic discussions. I am especially grateful to dr. Jordi Hernando: it was a truly pleasant experience to cooperate with you. Due to hard work and very good communication (we have visited each other regularly) we have been able to get some excellent results. I thank you very much for this and wish you a good start with your academic career in Barcelona. Enjoy the football!

Prof. Herbert van Amerongen en Rob Koehorst ben ik zeer erkentelijk voor hun hulp bij de fluorescentie(anisotropie)-metingen, en zeker ook voor alle hulp erna met het uitwerken van deze metingen.

Jan Gerritsen, jij hebt me de beginselen van AFM geleerd toen ik bij Vaste Stof Fysica stage liep en ook in de jaren daarop ben je nog betrokken geweest bij de metingen, onderhoud en discussies, waarvoor dank.

Studenten begeleiden, werkcolleges en voorlichtingsdagen geven, heb ik met veel plezier gedaan. Matthijs, al een half jaar na mijn aanstelling stond je op de stoep. Net begonnen, had ik nog geen vastomlijnd concept, waardoor jij je enthousiasme en leuke ideeën kon botvieren op een breed scala aan onderwerpen. Met een blikkeldoorzettingsvermogen en kritische blik werd de harde noot gekraakt en jij hebt er voor gezorgd dat we inzicht kregen in de factoren die belangrijk zijn voor een succesvolle polymerisatie, en dus heb je zo’n beetje de basis gelegd voor de rest van het onderzoek. Leuk dat je er als paranimf bij bent.

Pili, although I was not allowed to publish your results in my thesis since it was part of Jurry’s thesis work, there were still some important results that I could use. I have enjoyed it very much to work with you and I thank you for your dedication.

Rosana assisted me with lots of titration experiments, but, unfortunately, it was too short a period for you to fully enjoy the nice results. Thank you for your contribution.

Collega’s, daar moet je het van hebben! Geweldige sfeer op het lab, en niet in de laatste plaats door mijn directe collega’s van UL06. Allereerst waren Jeroen en Jurry daar al op het zojuist ingerichte lab. Jeroen, bedankt voor alle ideeën en discussies, en voor het beoordelen van het manuscript. Jurry, digitale onderhoudsmonteur tegen wil en dank, bedankt voor je computertips. Al snel kwam Dennis erbij, en dat betekende voor mij een perfecte zoorkastbuurman, veel gediscussieerd en gelachen vooral, ik bewonder je doorzettingsvermogen. Hartelijk dank voor de mooie tijd. Een aangenaam frisse wind gierde over het lab toen Aurélie kwam. Aurélie, jouw spontaniteit werkt aanstekelijk, ik vond het

Van mijn vrienden wil ik in het bijzonder Quinten ontzettend bedanken, want ik vond dat we een fantastische tijd hebben gehad in Nijmegen: veel uitgaan en sporten. Tjonge, wat een heerlijke, bloedfanatieke sparringpartner met fietsen in het schitterende Nijmeegse heuvellandschap, squashen en kroegsporten. Bedankt Q! Belangrijk zijn ook de vrienden van het Ardennenweekend, de mannen van de mannenvakantie, en de vrienden met wie ik in de loop der jaren mooie contacten heb opgebouwd, via middelbare school, sportvereniging, studentenhuis, bijbaantjes etc.

Mijn twee beste vrienden Paul (paranimf!) en San! Natuurlijk met resp. Marieke & kids en Hans. Paul ken ik al vanaf de eerste klas van de middelbare school en Sandy vanaf het 1e jaar scheikunde; er is in die tijd een ontzettend goede vriendschap onstaan, we hebben veel meegemaakt en gedeeld, daar ben ik jullie heel dankbaar voor.

Mijn ouders en zusje. Pap en mam, ik ben jullie erg dankbaar voor het feit dat jullie altijd voor me hebben klaar gestaan (heerlijk om thuis te komen) en vooral dat ik de vrijheid kreeg de keuzes te maken die me zinden. Lot, jij bedankt voor alles wat niets met mijn werk te maken heeft, fijne zus.

Tot slot richt ik me tot mijn lieve Maud. Nog geen drie jaar bij elkaar, en nu al het gevoel dat we alles hebben meegemaakt samen. Het was een bewogen tijd, zeker het jaar in Straatsburg. Ik ben je ontzettend dankbaar dat je zoveel moeite hebt gedaan (loftrompet), en zie uit naar een geweldige tijd die we voor de boeg hebben. Bedankt voor al je liefde!

Piet
Publications


Curriculum Vitae

Sponsors

The author acknowledges the following contributors: