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Synthesis and reactivity of new phenolic and/or heteroaromatic systems of potential practical interest

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Dottorato di Ricerca in Scienze Chimiche

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Sintesi e reattività di nuovi sistemi fenolici e/o eteroaromatici di potenziale interesse applicativo

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INTRODUCTION

The steady progress in industrial and biomedical field has stimulated in recent years a growing interest for the synthesis of organic molecules with specific chemical and structural feature to be used in the fields of nanotechnologies, biomedical chemistry or imaging. In many cases, the central structural elements of these compounds are made by phenolic and/or heteroaromatic systems that can generate complexes and highly conjugated structures with peculiar physicochemical properties useful in the optoelectronic or photovoltaic field for the realization of highly efficient molecular photonic devices such as molecular wires, switches, transistors and artificial light harvesting devices. This latter is the case of the complex structure shown in Figure 1, characterized by a star-shaped organization in which the benzene core is conjugated to twelve porphyrin rings through phenolic arms.

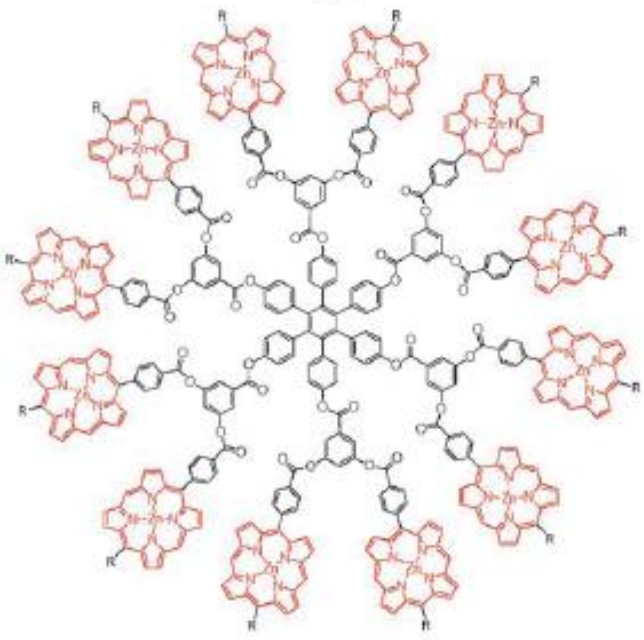


Figure 1. Structure of zinc(II)porphyrin dendrimers.

Due to this peculiar arrangement, this compound can mime the fast energy transfer of the natural photosynthetic system through two main energy-migration processes: interwheel (fast) and intrawheel (slow).[1]

Another recent application of this carbon-rich, highly conjugated organic molecules is as organic light-emitting diodes (OLED) for the realization of full-colour flat panel display.[2] During the past few years many examples of phenolic/heteroaromatic polymers have been synthesized with the aim of producing OLED characterized by a peculiar emission. Further studies have shown how it is possible to modulate the wavelength of the light emitted by choosing the appropriate substituent of the polymer as shown in Figure 2.[3]

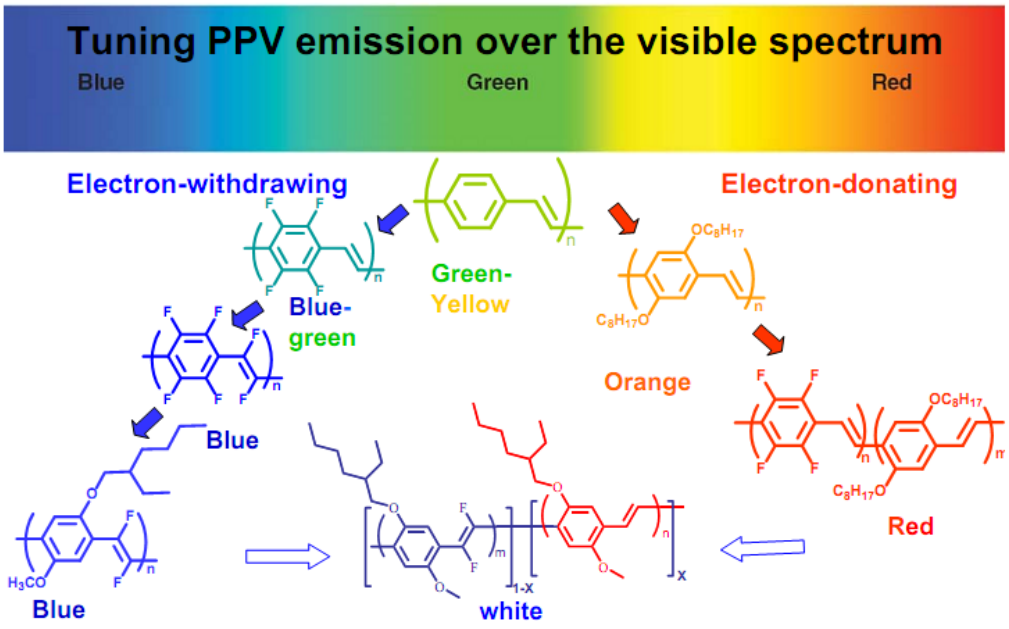


Figure 2. Modulation over the visible spectrum of the light emitted by OLED polymers.

Recently it has also been reported the use of star-shaped oligomers as OLED; this is the case, for example, of the compound shown in Figure 3, in which the triazatruxene core, linked to six carbazole units, exhibits different emitting maximum fluorescence

wavelengths ranging from 420 to 430 nm which may be served as good blue light-emitting material.[4]

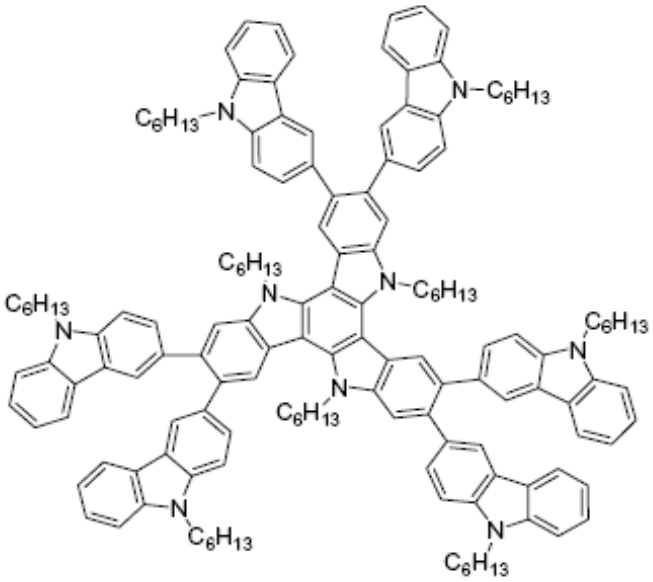


Figure 3. Triazatruxene-carbazole oligomer.

Due to their intrinsic photophysical and redox properties, i.e., they exhibit relatively intense luminescence and undergo reversible oxidation processes making them suitable as hole carriers.[5] Carbazole derivatives, including triazatruxenes, are also widely used as building blocks for potential organic semiconductors,[6] as well as for the realization of field-effect transistors and photovoltaic cells.[7]

Another important application of these phenolic/heteroaromatic compounds is as ion sensors, systems capable of converting the binding event into readable signal output through optical, electrochemical or magnetic resonance responses.[8] A very interesting class of compounds with ion-sensory properties is represented by 1,2,4,5-tetrasubstituted cruciforms. Cruciform fluorophores display two distinct molecular axes with either similar or dissimilar electronic properties; depending upon the

employed molecular design, cruciforms have found applications as electroactive organic glasses in thin-film transistors, organic single-layer device components, nonlinear optical materials, and most notably as responsive fluorophore cores. Responsive cruciform cores with 1,2,4,5-tetra-substituted benzenes, like those reported in Figure 4, display two transverse π-systems connected by a central benzene ring.[9] This specific structure leads to large metallochromic shifts in absorption and emission, like shown in Figure 4.

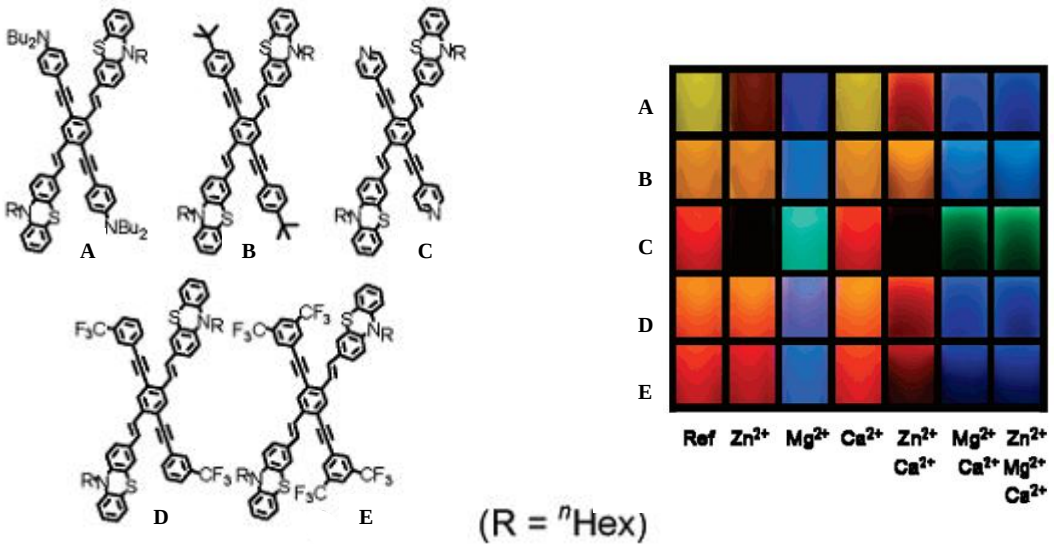


Figure 4. Phenothiazine-containing cruciforms and their shifts in absorption and emission when exposed to different metal ions.

Also indole-containing compounds have shown very interesting properties as sensor. This is the case of the dimer of pyrenopyrrole shown in Figure 5-A characterized by an excellent selectivity and sensitivity for the detection of fluoride ion.[8] The hydrogen bonding with fluoride ion, both in formation and in subsequent dissociation, provides remarkable colorimetric and fluorescent changes in the visible region that are advantageous for real-time and on-site application.

Compounds with a bisindol-3-yl-phenylmethane structure, such as that shown in Figure 5-B, are able to interact, in their oxidized form, with fluoride ions emitting an intense pink color (λ = 517 nm).[10] Other studies have also demonstrated that compounds with a bisindole skeleton (Figure 5-C) can behave as chemosensors, distinguishing different anions (i.e. F^-, AcO^-, I^-, Cl^-, NO_2^-) on the basis of the chemical shift values of the ^1H NMR spectrum of the complex.[11]

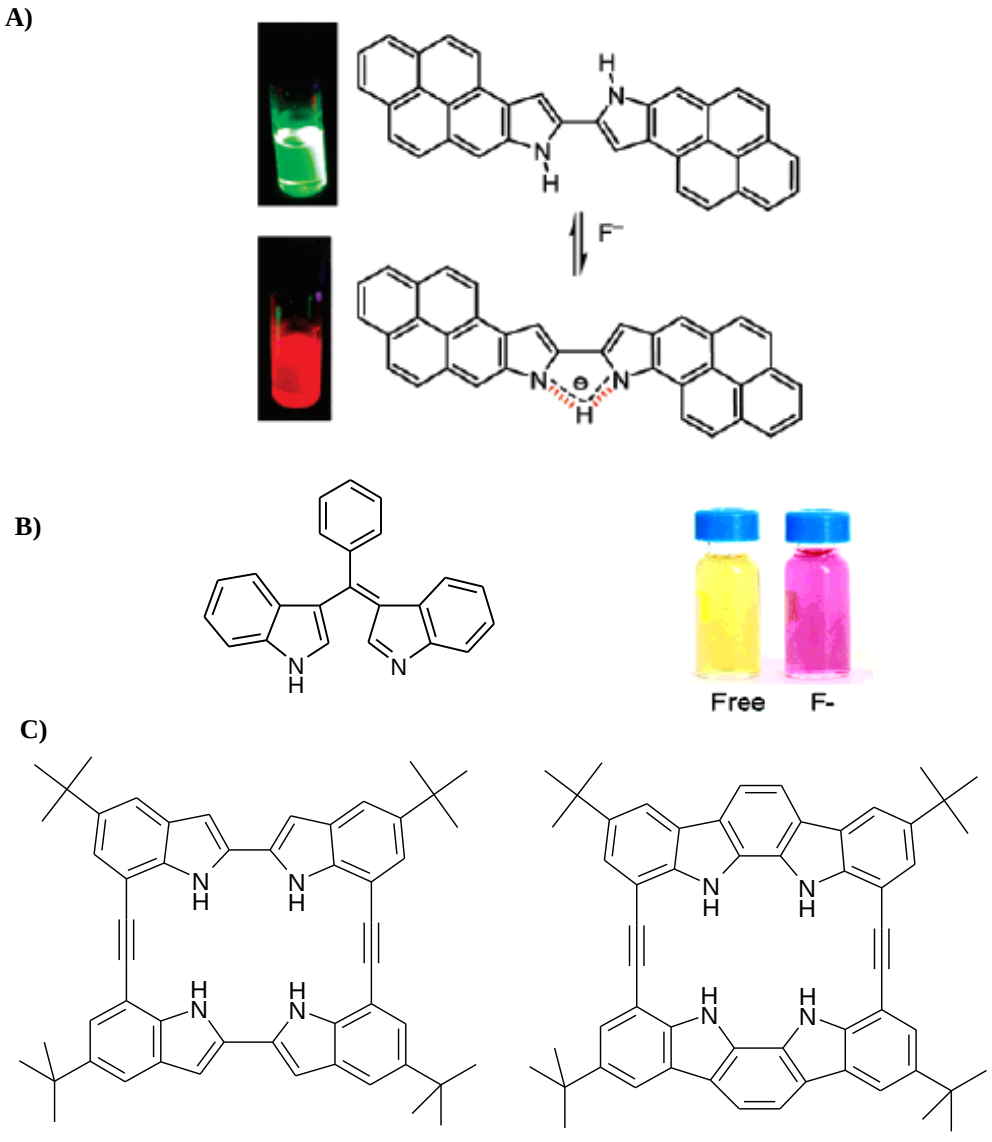


Figure 5. Color change induced upon addition of F^- to the dimer of pyrenopyrrole (A) and to the oxidized bis(indolyl)methane structure (B); (C) chemosensors with bisindole skeleton.

Taking into consideration that the Nobel Prize 2000 for chemistry was given to studies on polyacetylene,[12] it is not surprising that most of the research in the field of organoelectronics is focused on π-conjugated polymers. The study of these conducting and electroactive compounds started with the discovery of metallic electrical conductivity in oxidatively doped polyacetylene by Shirakawa, Heeger and MacDiarmid in 1977.[13] Extensive theoretical and experimental studies were initially adressed towards the properties of trans-polyacetylene **PA**[14] and then shifted towards environmentally more stable conjugated polymers such as poly-*p*-phenylene **PPP**,[15] poly-*p*-phenylenevinylene **PPV**,[16] polyanilin **PAni**,[17] polypyrrole **PPy**[18] and polythiophene **PT**[19] (Figure 6). These polymers overcame the limited stability of polyacetylene by stabilizing the polyene structure with heteroatoms or arene moieties.[20a]

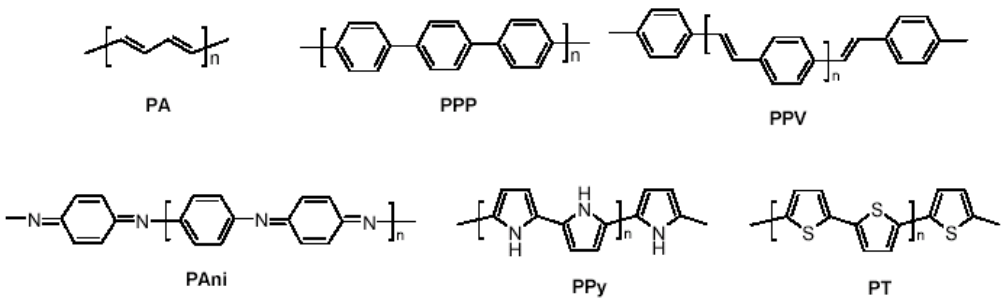


Figure 6. Representative examples of conjugated polymers: trans-polyacetylene **PA**, poly-*p*-phenylene **PPP**, poly-*p*-phenylenevinylene **PPV**, polyanilin **PAni**, polypirrole **PPy**, polythiophene **PT**.

As a result of enormous research efforts towards novel polymers with improved properties, new classes of materials which uniquely combine the electronic and optical properties of metals and semiconductors, with the processing advantages and properties of polymers, have been developed. In 1980s, a variety of bulk applications

such as antistatic coatings, electromagnetic shielding and energy storage devices were targeted. With the first report on electroluminescence of **PPV** in 1990,[20b] a new era of these conjugated polymers as active components for electro-optical devices was opened. New applications as laser dyes, photoconductors, organic light-emitting and non-linear optical materials became possible. In spite of outstanding achievements in the field of device fabrication, a fully understanding of the intrinsic electronic and optical properties of the conjugated polymers is still far from being complete. This is partly due to the fact that most synthetic polymerization reactions are statistical processes generating polydisperse materials. Therefore, the influence of the structures on macroscopic properties is hard to determine. This makes the improvement of such materials difficult.

Nonetheless, in recent years organic polymeric materials with extended π-conjugation continue to be the topic of widespread current interest due to their electrical, optical and structural properties. They can be designed and tailored to give materials with specialized properties for specific application towards the next generation of electronics and photonics.[21]

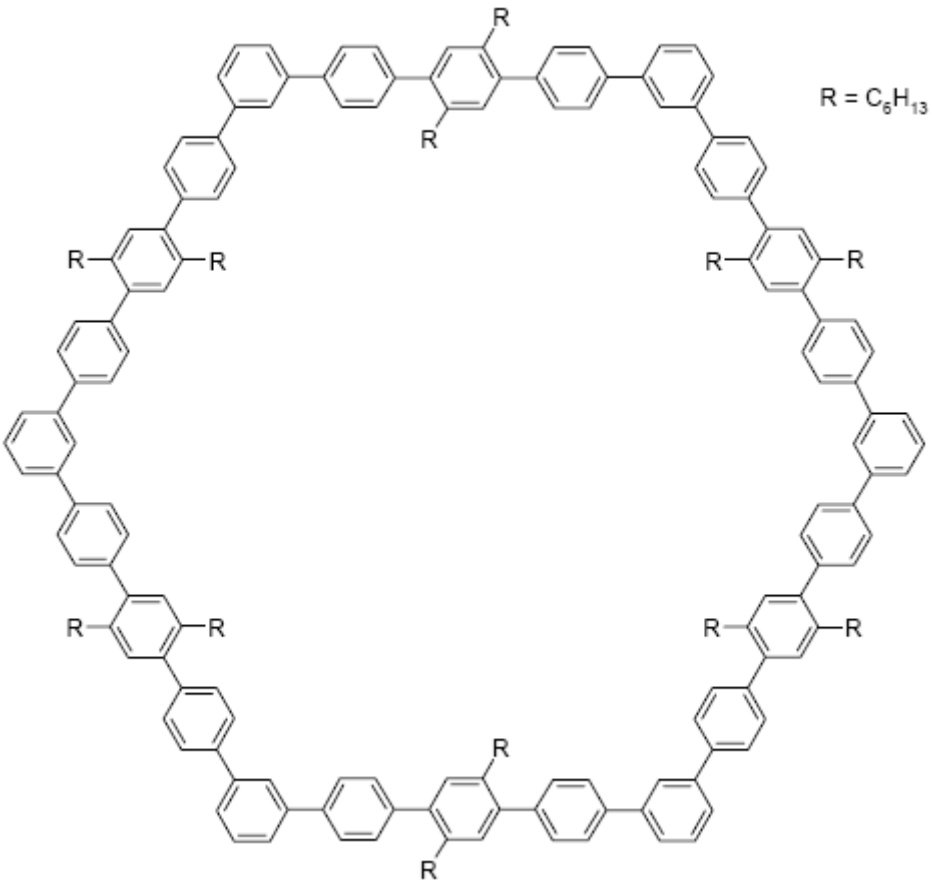
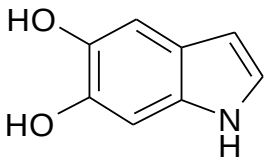
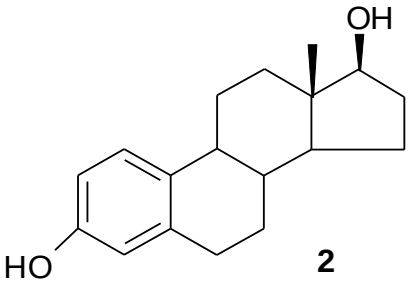


Figure 7. Cyclotetraicosaphenylene reported by Schlüter *et al.*[22]

Within this frame[23] my research project has been directed to the synthesis and characterization of a number of selected phenolic and/or heteroaromatic systems and their derivatives for preparation of new molecular scaffolds of potential practical interest. Most of the activity has therefore been directed to the preparation of new derivatives from 5,6-dihydroxyindole (**1**) and 17β-estradiol (**2**), selected as biologically relevant building blocks.



1



2

5,6-Dihydroxyindole (**1)** is a naturally occurring, catechol-containing heterocyclic compound[24] which provide the fundamental monomer precursors of eumelanins.[25] The unique physico-chemical properties of eumelanins, the characteristic black, insoluble, and heterogeneous biopolymers of human skin, hair, and eyes, have recently stimulated and inspired research efforts aimed at creating a new class of biologically inspired high-tech materials e.g. as light harvesting devices.[26] This is due to the peculiar set of optical and photochemical properties, including broadband monotonic absorption, intrinsic free radical character and switching threshold semiconductor behaviour, displayed by eumelanins.[26a]

The choice of **1** as key compound for the preparation of new molecular scaffolds was mainly due to its particular reactivity: in addition to the typical nucleophilicity in position 3, tipically shown by indoles in organic solvents and in acidic aqueous solutions, it shows a particular nucleophilic reactivity at the C-2 in neutral or alkaline aqueous solutions due to the partial dissociation of the hydroxyl group on the six position. This reactivity has been used for the preparation of 5,6-dihydroxyindole oligomers both to improve the current knowledge about the complex oxidative polymerization process leading to eumelanins, and to synthesize new prototypes based on the 5,6-dihydroxyindole skeleton for technologically-oriented functional materials. The results of these studies have been reported in Chapters 1 and 2, the first devoted to the synthesis of 5,6-dihydroxyindole oligomers and the second to the synthesis and reactivity of new alkynyl-derivatives of **1**.

17β-Estradiol (**2**) represents an important human and mammal steroidal hormone with estrogenic activity involved primarily in the control of sexual growth and behaviour.[27a]

The main body of studies on **2** have been traditionally focused on its biological activity, aimed also at the design and synthesis of pharmacologically active compounds. Only recently, some studies have shown how appropriate modifications of the steroidal skeleton can lead to versatile molecular scaffolds for the preparation of liquid crystals and self-assembling compounds. It has also been reported in the literature how the steroidal skeleton is used for the construction of photonic wires through a triplet-triplet excited-state energy transfer (TTET),[27b] or multielectron redox system with liquid crystalline behaviour.[27c-e] Starting from this observations, I have synthesized new alkynyl-derivatives of **2**. The results have been reported in Chapter 3.

The last chapter of this thesis, Chapter 4, concerns the results of experiments started during my Master thesis and regarding the synthesis and reactivity of 9- and 10-nitrolinoleic acids, two important nitrated derivatives of linoleic acid, under physiologically relevant conditions.

CHAPTER 1

SYNTHESIS OF 5,6-DIHYDROXYINDOLE OLIGOMERS

Introduction

Eumelanins: biosynthesis and properties

Among the broad variety of biopolymers found in nature, few have such profound and fascinating interdisciplinary implications at the crossroads of physics, chemistry, biology, and medicine as do the melanins. The reasons for this are rooted in the role of these pigments as the key components of the human pigmentary system[25,28,29] and their important socio-economic and clinical relevance, in relation to pigmentary disorders, such as malignant melanoma, the most aggressive of skin cancers.

Melanins can be classified into two distinct groups, pheomelanin (red) and eumelanin (black). Eumelanin and pheomelanin share a common biosynthetic origin from tyrosine via the common precursor dopaquinone, which is formed by the action of the enzyme tyrosinase in epidermal melanocytes (Figure 8).[30]

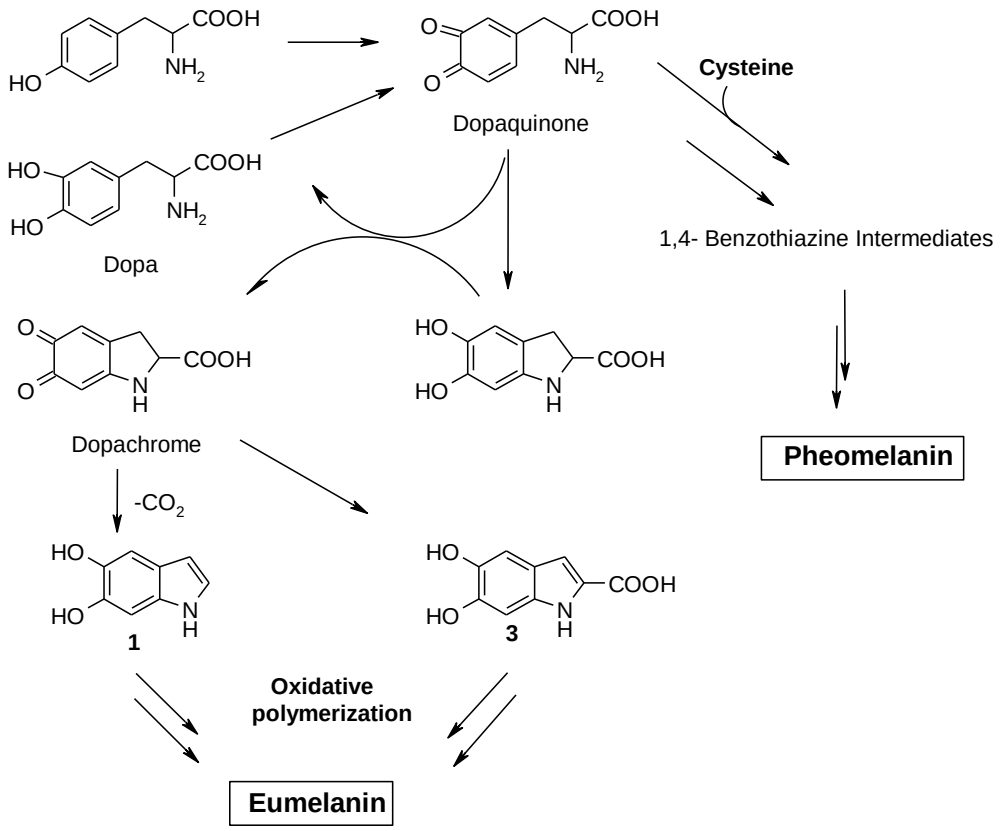


Figure 8. Biosynthetic pathways leading to eumelanin and pheomelanin production.

There remains a significant gap in knowledge between the structures of the initially formed molecules and those of the oligomers or polymers that make up the final pigments. What is now clear is that pheomelanin consists mainly of benzothiazine units, whereas eumelanins arise via 5,6-dihydroxyindole (DHI, **1**) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA, **3**), which are the final monomer precursors. Oxidative polymerization of **1** and **3**[24] then gives rise to the black-brown insoluble eumelanin.[31]

Working on eumelanins has traditionally been regarded as an intriguing, though sometimes frustrating, experience.[32] This is due to several challenging features of the system, including almost complete insolubility in all solvents, an amorphous

particulate character, and extreme molecular heterogeneity. This is the main reason why still to-date eumelanin's fundamental structure (if indeed the term "structure" can rightly be applied to such a highly heterogeneous material) is little understood.[32,33]

Eumelanins however do possess a number of physicochemical properties[34] that can be used to identify and quantify the system, such as a persistent electron paramagnetic resonance (EPR) signal, broadband monotonic optical absorption, peculiar excitation and emission properties,[35,36] and time dependent photodynamics.[37-39] Standard vibrational methods such as infrared absorption and Raman spectroscopy,[40,41] and more recently inelastic neutron scattering spectroscopy[42] have also been applied with varying degrees of success to study the vibrational finger-print of eumelanin precursors.

In the 1970s, McGinness and his associates showed that natural and synthetic eumelanin behave like amorphous semiconductors.[43,44] This result suggested that eumelanin consists of a very high molecular weight polymer made up of different units in various oxidation states and linked randomly[45] so to fit the band-gap semiconductor model. In the mid 1990s a different basic supramolecular architecture for eumelanin particles was proposed.[46-49] This model suggested protomolecular structures approximately 15 Å in size made up of four to five planar sheets of four-to-eight 5,6-dihydroxyindole units each stacked along the z direction with a graphite-like stacking spacing of 3.4 Å. In eumelanin from sepia ink, a sequence of aggregation steps has been suggested to account for the apparent three levels of structural organization (Figure 9).[50-53]

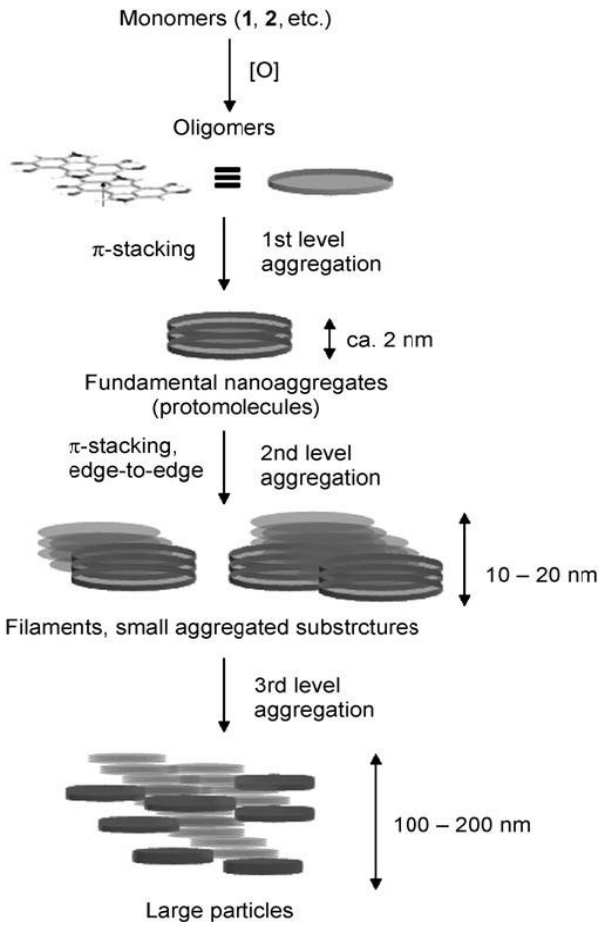


Figure 9. The hierarchical aggregate structure proposed for sepia eumelanin.

Numerous studies carried out using, for example, atomic force microscopy (AFM),[50,51,54] X-ray diffraction,[55] mass-spectrometry,[56] NMR spectroscopy,[57] and advanced quantum chemical calculations,[58-60] have addressed the eumelanin structure, and though most of them appear to support the stacked-aggregate picture, definitive proof for this model is missing.

With the structural debate still alive, research on eumelanins has seen a significant revival in interest in the past few years, far beyond the biomedical circles. This is due to recognition on the part of material science community of the unique

physicochemical properties of these black biopolymers and their potential applications in a variety of fields, from organoelectronics to gas sensing.

Physicochemical Properties and Applications

Optical and Photophysical Properties

The optical and photophysical properties of eumelanins are peculiar for these pigments and have been comprehensively reviewed by Meredith and Sarna.[34] As shown in Figure 10, the absorbance in the ultraviolet and visible spectrum is monotonic and broadband, it is featureless and fits a single exponential in wavelength space to a high degree of accuracy.

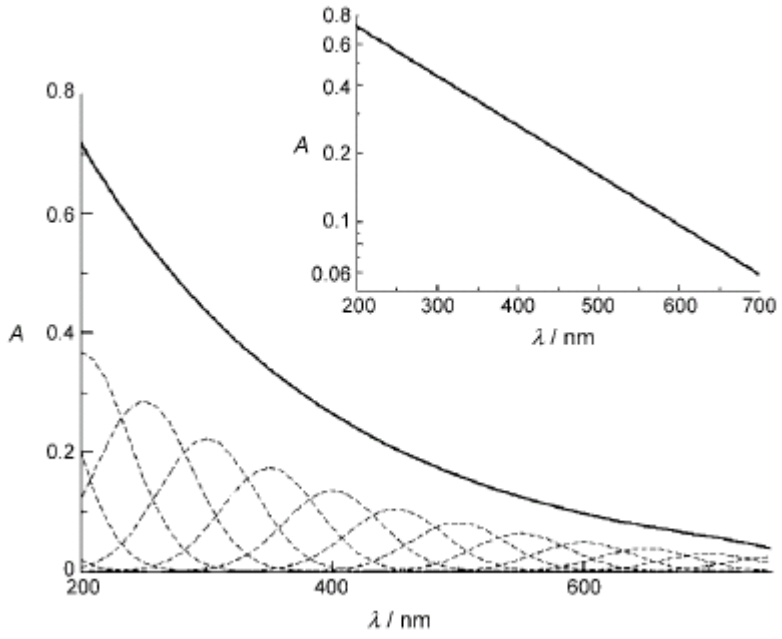


Figure 10. The broad-band absorption of eumelanin: the spectrum is monotonic and fits an exponential in wavelength space (insert shows the logarithmic–linear plot). The exponential shape can be fitted by a sum of Gaussians with full widths at half maxima characteristic of inhomogeneously broadened chromophores at room temperature.[61]

This kind of spectrum is atypical of organic chromophores which normally contain peaks corresponding to transitions between individual and distinct electronic states and/or vibronic states. As a matter of fact, eumelanin absorption looks more like that of an inorganic material than of an organic polymer.

Many studies have shown that synthetic and natural eumelanins do emit radiatively when appropriately stimulated by UV and visible radiation. However, eumelanins possess an extremely low radiative quantum yield. This means that more than 99.9% of all absorbed photons are subject to non-radiative dissipation, a very useful property for a photoprotectant. Other authors have also shown that the system displays slight hyperchromism: the polymerization process enhances the relative strength of the absorption versus the individual monomer units. Nighswander-Rempel et al.[35,36] have shown that the radiative emission is dependent upon the energy of the exciting radiation.

The collection of optical properties, has led to a reappraisal of the high molecular heterogeneity of eumelanins[45] in terms of the chemical disorder proposition.[61] In this simple model, the broad monotonic absorption of eumelanin is in fact envisaged as due to an ensemble average of all the individual chemically distinct species within the system. It has been calculated[62] that as few as eleven species are sufficient to create the smooth exponential profile across the UV and visible regions.

Electrical Properties

The electrical switching work of McGinness and coworkers[43] cemented the paradigm that eumelanins were amorphous organic semiconductors conferred by the indolic structure and the accompanying electronic delocalization.[62] Several studies have

shown that the electrical properties of solid-state eumelanin samples are also dependent upon the hydration state of the material.[63] This can be explained by the fact that water is capable of modifying the local dielectric constant of semiconductor systems. This does not prevent the system from being a semiconductor since a hygroscopic material such as eumelanin may well be expected to have an activation energy dependent upon hydration state, but caution needs to be exercised in measurements and interpretation.

Redox, Free Radical, and Ion-Binding Properties

One of the most remarkable features of eumelanins is their ability to undergo electron-transfer reactions. Although the quinone/hydroquinone nature of the eumelanin subunits is a reasonable basis for explaining the observed redox properties of this material, the chemical stability of the quinone groups is an issue that is not fully understood. It is believed that the quinone groups of eumelanin are mostly *o*-quinones related to 5,6-indolequinone.[64] However, free *o*-quinones, unlike *p*-quinones, are extremely unstable. It can be speculated that their stabilization in eumelanin arises from covalent linking of *o*-quinone subunits in the eumelanin oligomers and their subsequent aggregation. The modified redox properties of the bound monomers and their reduced accessibility as a result of steric hindrance may add to this stabilization.

An intriguing question is whether redox properties of eumelanin change with time. This is particularly relevant for eumelanin in the pigmented tissues of the human eye, such as retinal pigment epithelium, where melanin is formed early during fetal development and undergoes very little or no metabolic turnover.[65] Although no data

directly answering this question have yet been obtained, physicochemical analysis of retinal pigment epithelium (RPE) melanosomes from donors of different age suggest that their age-dependent changes in photoreactivity,[66] free-radical properties,[67] and antioxidant capacity[68] may be determined by modifications of the eumelanin oxidation state. Interestingly, Hong and Simon[69] using X-ray photoelectron spectrometry (XPS) have shown that in bovine choroidal melanosomes the content of C=O groups, compared to C-O, increases with the age of the animal. This observation may suggest that the choroidal melanosomes become more susceptible to oxidative stress with age. A distinct decrease in antioxidant efficiency of bovine and porcine RPE melanosomes was observed with experimental in vitro photobleaching.[70,71]

Melanin is the only biopolymer that both *in vivo* and *in vitro* contains relatively high concentration of persistent free radical centers that can easily be detected by EPR spectroscopy.[34] A growing body of experimental evidence suggests that more than one type of free radicals exists in eumelanins.[72,73] In particular, the EPR spectra appear to result from at least two different types of radicals: an *o*-benzosemiquinone anion radical that is strongly dependent on the pH value, is quite labile, and is associated with the well-hydrated portion of eumelanin, and another radical that is independent of pH value, but depends upon aggregation and, therefore, is probably associated with defects in the polymer backbone. The ability of melanin to bind metal ions is one of the basic physicochemical properties that affects the biological effects of this pigment. It has been estimated that the number of metal-ion binding sites in eumelanins is about 20% of the number of monomeric units in the polymer. This means that eumelanin is a good chelator of multivalent metal ions, such as Fe^{3+},

Mn^{3+}, Zn^{2+}, and Cu^{2+}.[34,74,75] The binding of metal ions may involve carboxy, amine, imine, phenol, and *o*-diphenol groups of eumelanin which have different association constants. Notably, the different binding sites of eumelanin can be activated at different pH values, which also determines the observable stability of the metal ion eumelanin complexes. A precise control of the sample pH is so required in the complexing process of metal ions with eumelanin.

Film Preparation

An absolute pre-requisite to the full realization of eumelanin-based materials within the organic electronic or optoelectronic arena is the production of device-quality thin films. Most solid-state optical and electrical measurements have been performed on compressed powders which are wholly unsuitable for devices because of their morphological variability. Control over the nanoscale morphology is at the heart of modern organic electronics research and technology development. Very recently, several groups have produced synthetic eumelanin thin films[76-78] and organically soluble eumelanin derivatives.[79,80] This was due to the fact that eumelanin thin films possess a number of physical properties that make them highly attractive for use in organic bioelectronic devices. As a biomaterial, eumelanin is inherently biocompatible. This is a very important property of any viable organic bioelectronic material.[81] Notably, Bothma et al.[26c] have reported the first device-quality synthetic eumelanin films showing enhanced optoelectronic functionality. The crucial step in the production of thin films was the solubilization of eumelanin in ammonium hydroxide so that when the ammonia and the melanin solution is spin-cast, the water evaporates along with the ammonia, leaving the synthetic melanin as uniform film.

The film appeared homogeneus, being free from voids or cracks. The films showed solid-state absorption coefficients between 10^7 and 10^6 m^{-1} (UV-to-IR) and showed a conductivity strongly depended on the hydratation state. Due to the ability of eumelanin to chelate metal ions, it was necessary to check that the films were not contaminated by metal ions, to avoid potential effects on conductivity. The key to the production of such films is an understanding of, and control over, the aggregation state of the system. It appears as though the insolubility of eumelanin is related to its supramolecular aggregation state. Breaking this aggregation without affecting the primary unit structure or properties is the secret which unlocks the potential of these materials. An overview of the physicochemical properties of eumelanin films and their possible application is reported in Figure 11.

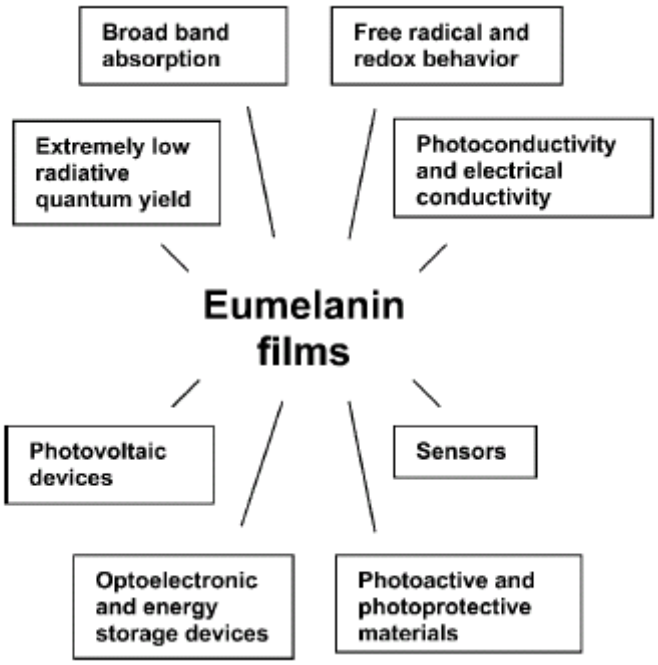


Figure 11. Physicochemical properties (top) and possible range of applications of eumelanin films (bottom).

Synthesis of 5,6-dihydroxyindole dimers

As explained in the introduction of this chapter, **1** is the key eumelanin building block that leads to eumelanin through an oxidative process. This polymerization reaction proceeds through an exceedingly complex mixture of oligomer intermediates that are still not well known. Recent studies[82] have demonstrated that the first stages of the polymerization can be populated at the dimer level by up to four main biindoles, **4**-**7**, sharing 2-linked indole units mainly via 2,4'- and 2,7'-coupling steps, as indicated by the isolation of biindoles **4** and **5** and triindoles **8** and **9**[82-84] as a common feature (Figure 12).[24,85]

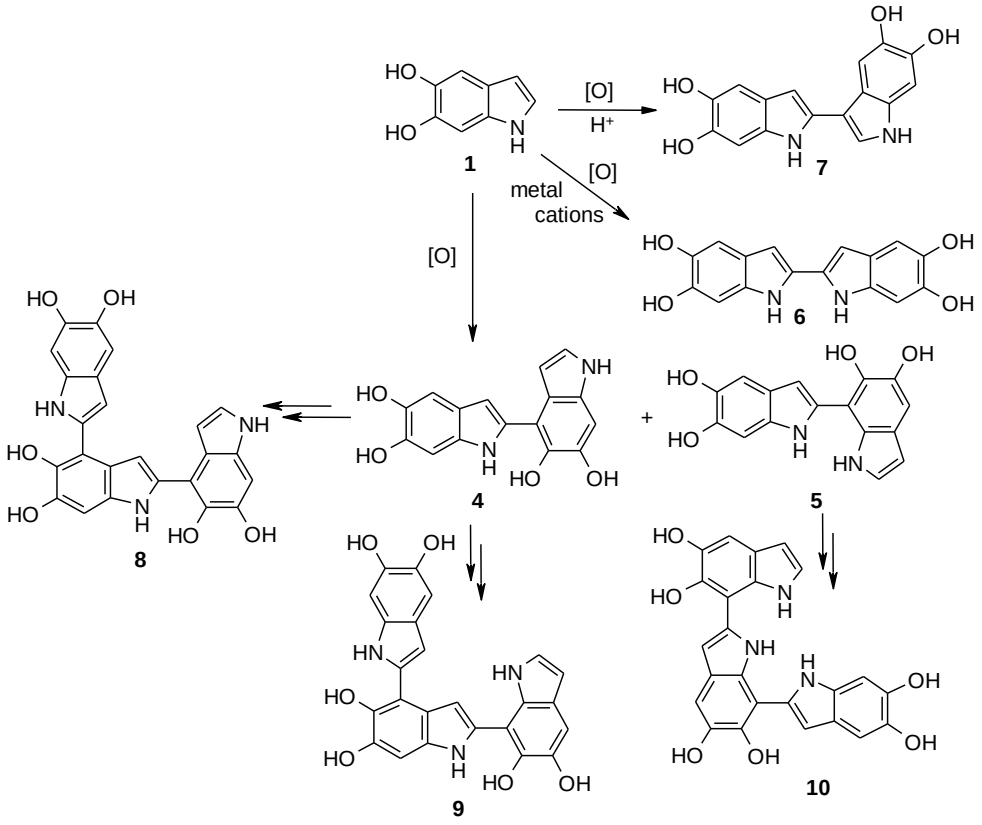


Figure 12. Structural diversity within the dimer group of **1** oligomers en route to eumelanin biopolymers.

Further insights into the structure of the oligomeric species generated during the oxidative polymerization of **1** have been hindered by the marked complexity of the reaction mixtures and the poor isolated yields.

The availability of a collection of 5,6-dihydroxyindole oligomers of variable molecular size is therefore pivotal for future advances in the structural characterization of eumelanin biopolymers[26a] as well as for the realization of bioinspired functional materials for technological applications.[26c,34] Interest in these synthetic targets is also spurred by the potential of indole-based scaffolds for the preparation of anion sensing architectures.[8,11,86-88]

Since the seminal paper by Khorana in 1948,[87] numerous synthetic efforts have been directed to the 5,6-dihydroxyindole system, as reviewed recently.[24,88] Reports describing preparations of 5,6-dihydroxyindoles include also a significant number of patents in which several practical applications of these advances are disclosed. Whereas literature on the synthesis of parent **1** is rich and varied, the current synthetic repertoire for the preparation of related dimers and other oligomers is surprisingly poor, the only exception being the synthesis of the 3,3'-biindole.[89] Considerable constraints to the possible synthetic plans are posed by the highly oxidizable *o*-dihydroxy functionality, which requires careful selection of protecting groups, reagents and reaction conditions. Accordingly experimental control over oxidation pathways of **1** represents at present the only means to gain access to small amounts of dimers for structural investigations. Thus biindoles **4** and **5** have been obtained in low yields by stopping the oxidation of **1** in the early stages, and under these conditions **4** is usually the main product. On the other hand, **6** can only be obtained in the presence of metal ions, e.g. Ni^{2+}, whereas **7** is formed by controlled

oxidation of **1** under acidic conditions. As a matter of fact, only **4** can be practically obtained in sufficient amounts for structural studies, whereas the other dimers, especially the 2,2'-biindole **6**, are difficult to prepare and are usually obtained in poor yields with significant impurities, requiring cumbersome chromatographic purification steps.

Based on these motivations, an important part of my research project has been devoted to the synthesis of the least accessible dimers of **1**, namely the 2,2'-, 2,3'- and 2,7'-biindoles **5-7.** The sequence relies on a judicious sequence of coupling and cyclization steps involving suitably protected *o*-ethynylaniline intermediates.

Initially the attention was focused on the least accessible 2,7'-linked biindole **5**. The strategic route to this target envisioned two key synthetic elements, namely the easily obtained *o*-ethynylaniline derivative ***I*** and its iodinated derivative ***II*** which could be suitably coupled to provide the desired 2,7'-linkage (Figure 13).

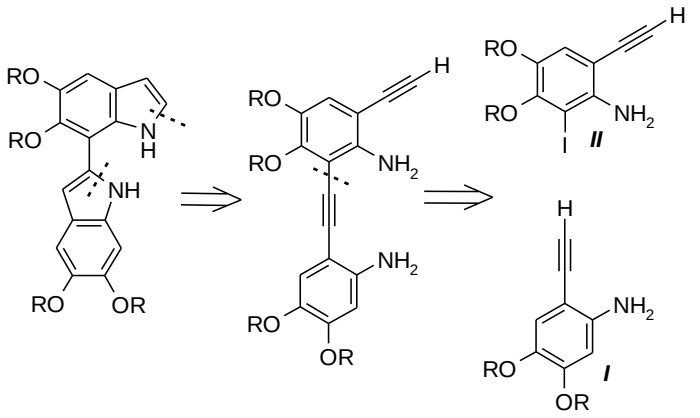
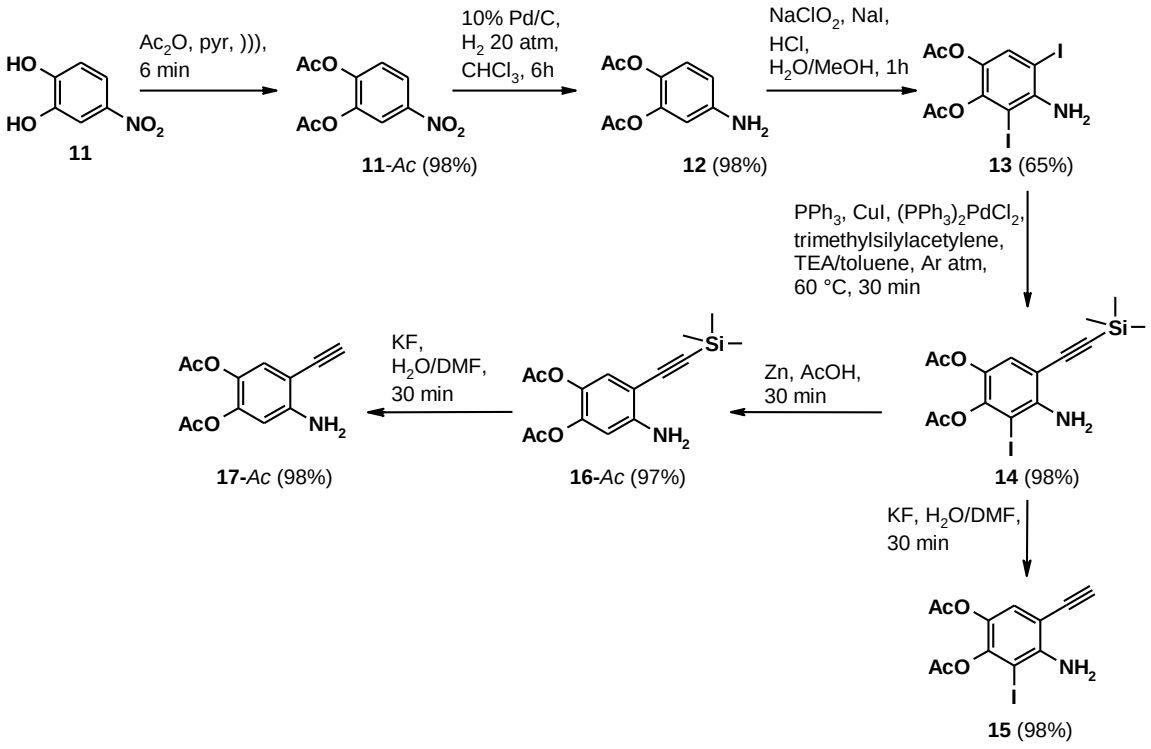


Figure 13. Retrosynthetic scheme showing possible assembly of the 2,7'-biindolyl scaffold from o-ethynyalinine derivatives.

Both ***I*** and ***II*** have been prepared from 4-nitrocatechol (**11**), chosen as the starting compound. Being aware of the high oxidizability of the cathecol functionality, in the

first step of the synthetic procedure we paid attention to suitable protection of the hydroxyl groups. This was achieved with acetic anhydride and pyridine in an ultrasound bath (320 W) allowing the derivatization to occur in a very short time (6 minutes) (Scheme 1).



Scheme 1. Synthesis of *o*-Ethynylaniline Building Blocks (**15**) and (**17**-*Ac*).

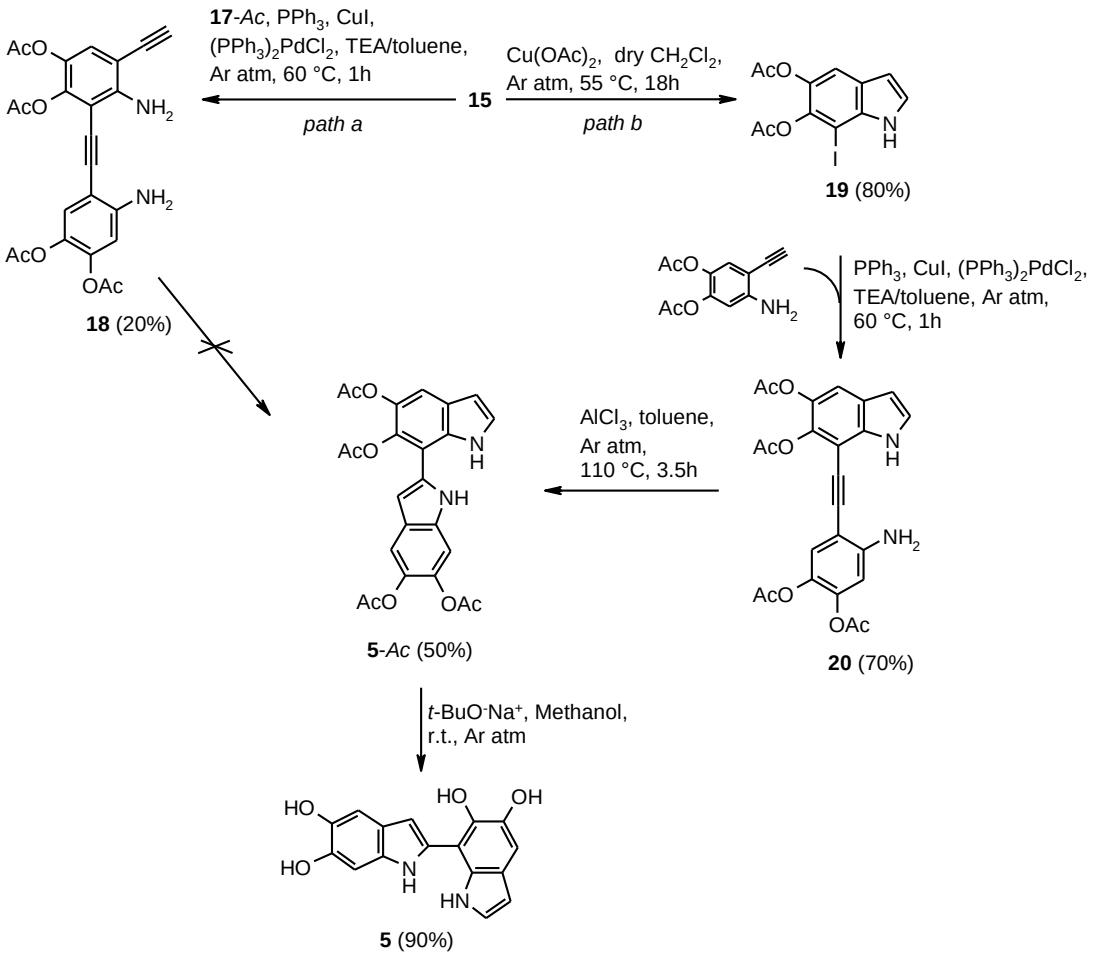
The acetyl-derivative of **11** was then subjected to catalytic hydrogenation to give the corresponding diacetoxyaniline **12**, that was iodinated with NaI/$NaClO_2$ according to a procedure developed in our laboratory to give the corresponding *o*,*o*-diiodoaniline **13**.[90]

A crucial role in this step was played by the protecting acetyl groups that blunt the electron-donating effect of the hydroxyl groups thus allowing a more controlled ring iodination.

The iodo-derivative **13** was converted into the corresponding 6-trimethylsilylethynyl-derivative **14** in good overall yield following a regioselective Sonogashira coupling[91] by using one molar equivalent of trimethylsilylacetylene, copper iodide, triphenylphosphine and palladium(II)dichlorobistriphenylphosphine as catalyst, in a 1:1 mixture of triethylamine-toluene. The efficient deiodination of **14** with zinc in acetic acid afforded, after desilylation with KF in DMF, the first key intermediate ***I*** (**17**-*Ac*). Unfortunately, all attempts made to stop the iodination reaction at the mono-insertion stage failed also under quite mild conditions.

The second key intermediate ***II*** (**15**) was obtained from **14** by removing the trimethylsilyl group with KF in DMF.

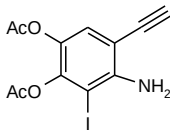
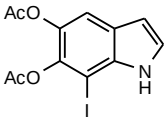
According to the retrosynthetic sequence of Figure 13, the 2,7' linkage was realized by reacting the two *o*-alkynylaniline intermediates **17**-*Ac* and **15** following the conditions of the Sonogashira coupling (Scheme 2-path a).



Scheme 2. Alternate routes to the 2,7'-biindolyl **5** via combination of the *o*-ethynylanilines **15** and **17**-*Ac*.

It was thus possible to isolate the intermediate **18** that was subjected to a concerted double intramolecular cyclization with CuI in dry DMF. Unfortunately, this latter step did not provided the desired biindole, due probably to the unsuitable reactivity of the *o*,*o*-disubstituted aniline ring, so an alternative synthetic path was pursued (Scheme 2-path b). The *o*-alkynylaniline **15** was initially subjected to intramolecular cyclization with $Cu(OAc)_2$ in dry CH_2Cl_2[92] leading to the formation, for the first time in high yields (80%), of 5,6-diacetoxy-7-iodoindole (**19**). The option of $Cu(OAc)_2$ as the final catalyst was the result of a systematic analysis carried out by using different conditions (Table 1).

Table 1. Cyclization of *ortho*-ethynylaniline **15** under different reaction conditions.

Substrate	Product	Reaction conditions	T (°C)	Time (h)	Yield (%)
		$FeCl_3/PdCl_2$/dry CH_2Cl_2	55	4	-
		CuI/DMF	110	5	30
 **15**	 **19**	CuI/TEA/toluene	130	1.5	50
		$AuCl_3$/dry EtOH/(ultrasound bath)	25	1.5	30
		$NaAuCl_4 \times 2H_2O$/ethanol	r.t.	4	70
		$Cu(OAc)_2/CH_2Cl_2$	55	18	80

The iodoindole **19** was then subjected to the Sonogashira coupling with the *o*-alkynylaniline **17**-*Ac* to afford the indolylethynylaniline **20**. This latter was treated with $AlCl_3$ to give the 2,7'-biindole **5**-*Ac*, and finally deacetylated by treatment with sodium *tert*-butoxide in methanol under an argon atmosphere to afford **5**, the real intermediate in the melanin biosynthesis. Pure **5** was completely characterized for the first time by NMR (Table 2).

Table 2. ^{1}H and ^{13}C resonances for pure **5** (Aceton-d_6)**.**

Carbon	δ_C (ppm)	δ_H (ppm) (mult., *J* (Hz))	Carbon	δ_C (ppm)	δ_H (ppm) (mult., *J* (Hz))
C-2	131.6[a]	-	C-2'	123.8	7.16 (t, 2.7)
C-3	100.8	6.74 (s)	C-3'	101.9	6.31 (t, 2.7)
C-4	104.5	6.99 (s)	C-4'	104.4	7.02 (s)
C-5	141.0[b]	-	C-5'	140.2[b]	-
C-6	143.3[b]	-	C-6'	140.4[b]	-
C-7	97.4	7.01 (s)	C-7'	104.3	-
C-8	131.8[a]	-	C-8'	128.8	-
C-9	122.3	-	C-9'	121.7	-
NH	-	9.86 (br s)	NH'	-	9.86 (br s)

[a] **Interchangeable**

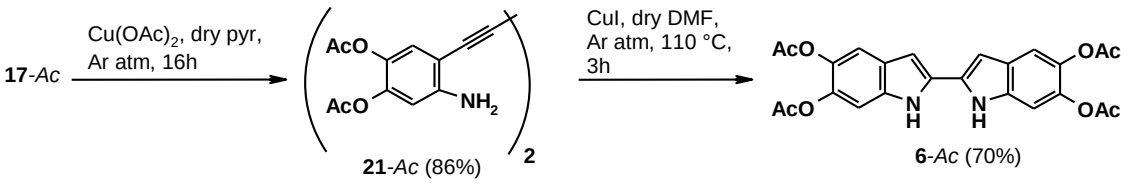
[b] **Interchangeable**

To the best of my knowledge, this is the first total synthesis of a 2,7'-biindole system that does not rely on heavily substituted preformed indole precursors.[93]

Once obtained the biindole **5**, the attention was focused on the synthesis of 2,2'-biindole **6**, a highly desirable target due both to its value as eumelanin precursor and as core structural motif for functional molecules.

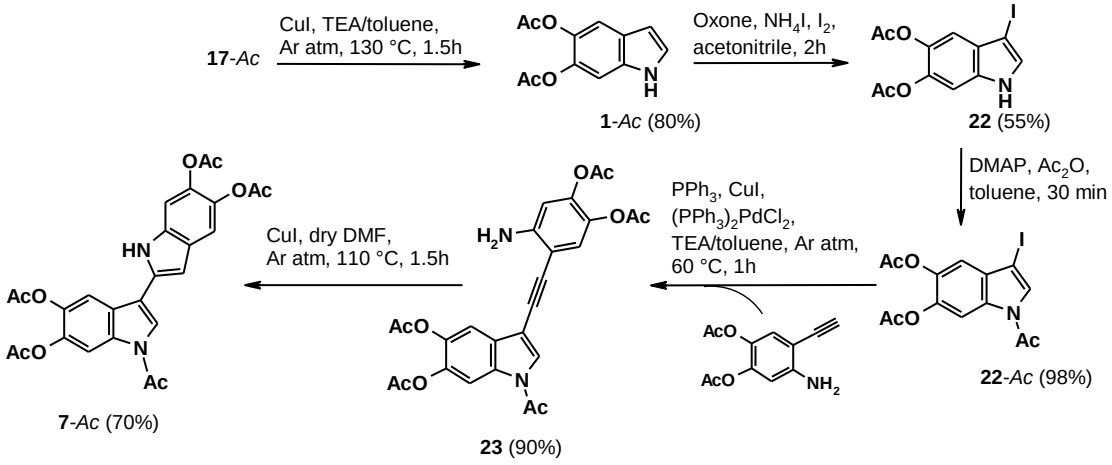
Even if the recent literature allowed identification of a number of possible strategies to prepare 2,2'-biindolyls,[11,94] a careful scrutiny of the synthetic plans led us eventually to opt for an extension of the above chemistry toward the preparation of **6** as its acetyl derivative. In brief, the *o*-ethynylaniline **17**-*Ac*, prepared as reported above, was subjected to oxidative dimerization with $Cu(OAc)_2$ in dry pyridine[95] to

give **21**-*Ac* (86%) which was treated with CuI[96] to give, after a double concerted intramolecular cyclization, the desired 2,2'-biindole derivative **6**-*Ac* (70%) (Scheme 3).



Scheme 3. Synthesis of 2,2'-Biindolyl **6**-*Ac*.

The successful implementation of the latter sequence was next applied to the synthesis of **7**-*Ac* (Scheme 4). In this case, **17**-*Ac* was converted into 5,6-diacetoxyindole (**1**-*Ac*) via CuI-promoted intramolecular cyclization and then regioselectively iodinated at the 3-position to give 5,6-diacetoxy-3-iodoindole (**22**) according to a recently developed preocedure.[90] The Sonogashira coupling of **22** with **17**-*Ac* under the typical conditions proved unsuccessful. However, the problem was circumvented by *N*-acetylating the indole substrate just prior to the Sonogashira coupling. The *N*-acetylation of **22** with acetic anhydride and *N*,*N*-dimethylaminopyridine makes the iodoindole derivative more prone to the Sonogashira coupling with **17**-*Ac*, giving the 3-alkynylsubstituted 5,6-diacetoxy-1-acetylindole **23** in good yields; this latter was subjected to the final cyclization step with CuI to afford **7**-*Ac* in good yields (Scheme 4).



Scheme 4. Synthesis of 2,3'-Biindolyl **7**-*Ac*.

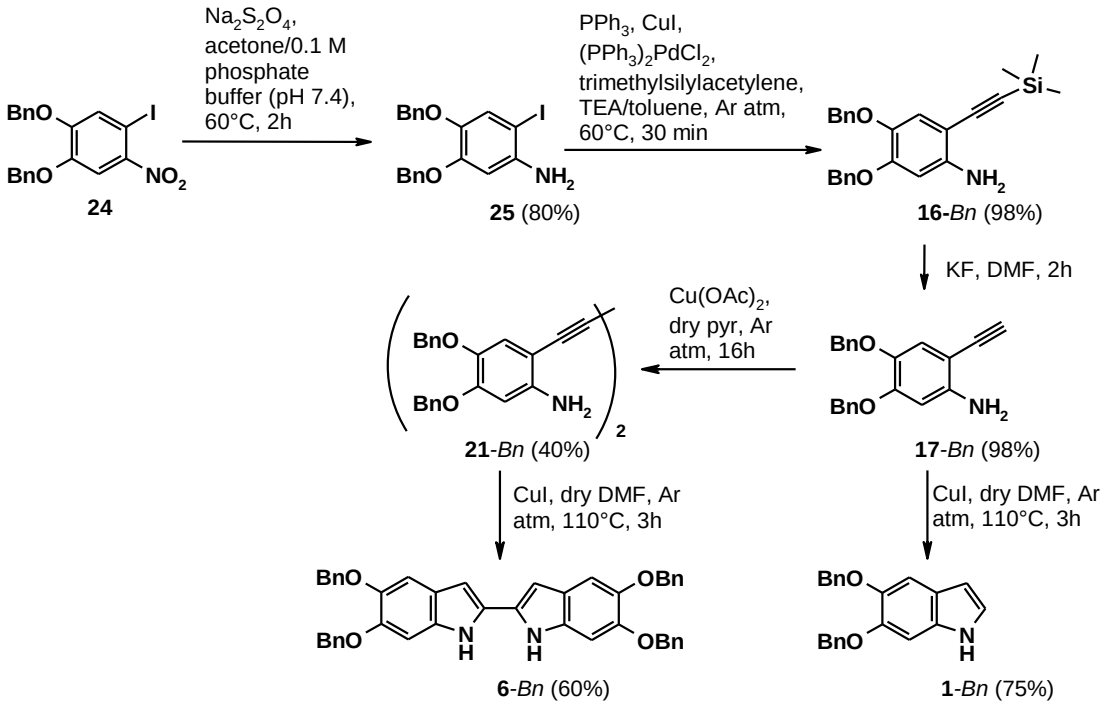
Attempts at synthesizing the biindole **4**-*Ac* by a proper variant of the unified strategy were twarted by difficulties encountered with the preparation of 4,5-diacetoxy-2-ethynyl-3-iodoaniline as the necessary intermediate. Since this dimer is easily available by oxidation of **1**, the research for alternate approaches was postponed to studies currently under development.

In separate experiments, an alternative synthetic strategy for the preparation of biindoles with a different protective group was developed. In particular, I have prepared the benzyl derivative of 2,2'-biindole (**6**-*Bn*). This group, in fact, could be easily removed by catalytic hydrogenation and was therefore judged useful for the purposes of this study.

As reported in Scheme 5, the 4,5-dibenzyloxy-2-nitroiodobenzene (**24**) was chosen as starting compound, and was readily obtained by the same sequence of steps reported in the previous synthesis of the 3,3'-biindolyl.[89]

A problem arose, however, when reduction to the corresponding iodoaniline **25** was attempted by hydrazine on activated carbon, as reported, because extensive deprotection of the catechol group occurred. The problem was circumvented by using sodium dithionite in a 1:1 mixture of acetone and 0.1 M phosphate buffer (pH 7.4) as reducing system, which allowed to obtain the iodoaniline derivative in good yields. The insertion of the alkynyl functionality was achieved via a classical Sonogashira coupling with trimethylsilylacetylene[91] followed by deprotection with potassium fluoride. The *o*-ethynylaniline **17**-*Bn* thus obtained was used to prepare 5,5',6,6'-tetrabenzyloxy-2,2'-biindolyl (**6**-*Bn*) in satisfactory overall yield using the same reaction conditions detailed in Scheme 3 for **6**-*Ac*. Interestingly, direct cyclization of **17**-*Bn* led to **1**-*Bn* in good yield, as described for **1**-*Ac*.

This procedure is probably one of the best so far developed for preparation of **1**-*Bn*.[97]



Scheme 5. Synthesis of 5,5',6,6'-Tetrabenzyloxy-2,2'-biindolyl (**6**-*Bn*).

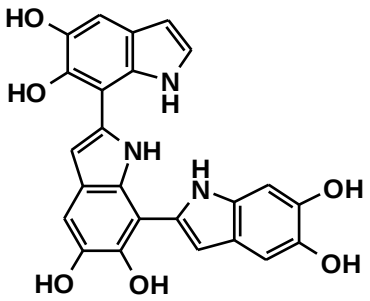
This route was at least as efficient as that based on the acetyl protecting group (Scheme 3) giving the dimer **6**-*Bn* with a similar number of steps and a good overall yield, so it provided a valuable alternative to the synthesis reported in Scheme 3.

Efforts to extend the above chemistry to the preparation of **5**-*Bn* were unsuccessful because it was difficult to prepare the crucial intermediate, 3,4-dibenzyloxy-6-ethynyl-2-iodoaniline, by mono-iodination of **17**-*Bn*. This was attributed to the electron-donating benzyloxy groups increasing the reactivity of the aromatic ring and preventing control over iodination.

Unfortunately, as for **5**, also 2,3'-biindole could not be developed using this synthetic path because of the unexpected difficulty to obtain 5,6-dibenzyloxy-3-iodoindole in satisfactory yields.

Synthesis of a 5,6-dihydroxyindole trimer

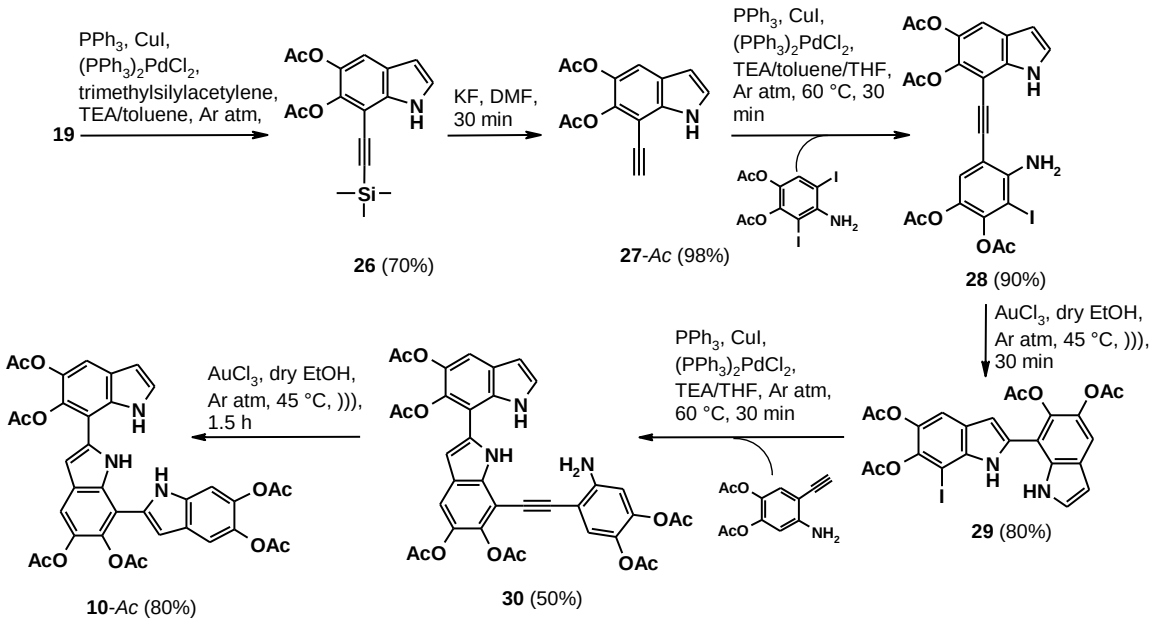
The versatile methodology developed before represent the first successful approach to a series of dimers of **1** and was envisaged to provide a convenient general strategy toward higher 5,6-dihydroxyindole oligomers. In extending that procedure, I have developed the first synthetic approach to a trimer of **1**, namely the 2,7':2',7''-triindole **10**.



10

The triindole **10** is considered an interesting target for several reasons: a) although its structure embodies the characteristic 2,7'-coupling mode of 5,6-dihydroxyindoles,[24] it has never been identified in the oxidation mixtures of **1**, and the availability of an authentic standard was expected to guide its detection during the polymerization process; b) the preparation of this missing triindole would integrate the current knowledge of the structural properties of 5,6-dihydroxyindole oligomers,[84b] and would provide a useful starting material for assembling high molecular polymers via the oligomer-oligomer coupling approach;[82,83] c) the triindole skeleton of **10**, featuring three nitrogen groups in a suitable disposition for ion coordination, also offers interesting opportunities for anion sensing.

The synthetic approach to **10** capitalizes on 5,6-diacetoxy-7-iodoindole (**19**) as the starting material, easily obtained from commercial **11** through the sequence of reactions reported in the previous section in Schemes 1 and 2.[98] The Sonogashira coupling on **19** with trimethylsilylacetylene led, after treatment with KF in DMF, to 5,6-diacetoxy-7-ethynylindole (**27**-*Ac*) which was reacted with the *o*,*o*-diiodoaniline **13**, previously prepared according to the procedure reported in the Scheme 1, to give the indolylethynylaniline **28** (Scheme 6).



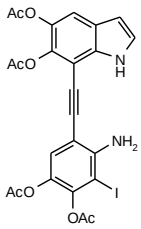
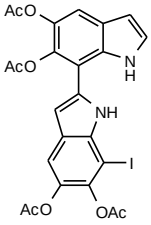
Scheme 6. Synthetic procedure for the preparation of trimer **10**-*Ac*.

Surprisingly, the cyclization of **28** to 7-iodo-2,7'-biindole **29** with $Cu(OAc)_2$ proved less efficient than in the case of **15**, possibly because of steric effects. However, a brief screening of some potential catalysts (Table 3)[99] showed that $AuCl_3$ or $NaAuCl_4 \cdot 2H_2O$, this latter with longer reaction times, could efficiently promote the reaction to give **29** in good yield (80%). A similar sequence of Sonogashira coupling applied on **29** with the *o*-ethynylaniline **17**-*Ac* followed by the intramolecular cyclization step mediated, also in this case, by $AuCl_3$, led to the desired **10**-*Ac*. The

structural assignment of the trimer was secured by extensive 2D NMR* and MS analysis also in comparison with trimers **8** and **9**.[85a]

The synthetic approach in Scheme 6 stems largely from the previously reported strategy to isomeric biindoles, however it features some aspects of general interest. In particular, as a result of a systematic screening of different catalysts under various reaction conditions (Table 3) has demonstrated that $AuCl_3$[100] and $NaAuCl_4 \cdot 2H_2O$[101] proved to be superior catalysts for the cyclization steps that led to the biindole **29** and **10**-*Ac*.

Table 3. Cyclization of *ortho*-ethynylaniline **28** under different reaction conditions.

Substrate	Product	Reaction conditions	T (°C)	Time (h)	Yield (%)
		$Cu(OAc)_2/CH_2Cl_2$	55	24	-
		CuI/TEA/toluene	130	4	-
		CuI (2 eq)/DMF	110	4	20
		CuI (5 eq)/DMF	110	3	5
		$Cu(OAc)_2$/dry methanol/(ultrasound bath)	70	5	10
28	**29**	LiCl/toluene	110	2	30
		$AlCl_3$/toluene	110	3.5	50
		$AuCl_3$/dry ethanol/(ultrasound bath)	45	1.5	80
		$NaAuCl_4 \times 2H_2O$/ethanol	r.t.	5	83

*Structural assignment was secured by comparing 1D and 2D NMR spectra registered in $(CD_3)_2CO$ with or without the addition of 5 μL of a 10% solution of NH_4Cl in H_2O (see Experimental Section).

There is no clear-cut explanation of why $Cu(OAc)_2$ works only well on the monomer precursor **15** and less efficiently on the bulkier substrates **28** and **30**, and why $AuCl_3$ and $NaAuCl_4 \cdot 2H_2O$ work better in the latter case. Although gold catalysts have been successfully utilized in the preparation of various indole systems from *o*-alkynylanilines,[92,101-104] the use of $AuCl_3$ to promote the cyclization of *o*-alkynylanilines has remained so far confined to few examples leading to 2-arylindoles.[100] Key changes introduced in the present methodology with respect to the previous $AuCl_3$ protocol[100] include higher *o*-ethynylaniline and catalyst concentration and a lower temperature (45 °C instead of 70 °C) with ultrasound activation, which resulted in shorter reaction times (30–90 min) without the need for extensive chromatographic purification. Comparable results in terms of yields, but with longer reaction time, have been obtained by using $NaAuCl_4 \cdot 2H_2O$ as reported.[101]

The development of this convenient access protocol to **10**, besides the intrinsic synthetic interest, allowed the probing of the generation of this trimer during 5,6-dihydroxyindole polymerization.

In Figure 14 are shown the LC-MS elutograms of the oxidation mixture of **1** with the system peroxidase/H_2O_2 in 0.05 M phosphate buffer, pH 7.4,[24,82,83] after work up and acetylation (UV_{254nm} trace; ESI(+)-MS trace) and of the coinjection of the same oxidation mixture with synthetic **10**-*Ac* (UV_{254nm} trace; ESI(+)-MS trace). The LC-MS profile of the reaction mixture appears very complex with a pattern of peaks that have been assigned only in part (**1**-*Ac*, *O*-acetylated dimers of **1**, *N*,*O*-acetylated trimers of **1**). Among these it has been possible to identify a group of peaks around 30 min (see expanded region) ascribable to *O*-acetylated trimers of **1**. Thanks to the

availability of synthetic standards it was possible to identify the main peaks at 28 and 35 min as the trimers **8**-*Ac* and **9**-*Ac*; only small amounts of the trimer **10**-*Ac* were formed in the mixture, the identification being possible thanks also to mass analysis showing for the peak at 32 min (peak **A**) the pseudomolecular ion peak at *m/z* 718 ($[M+Na]^+$) (Figure 15).

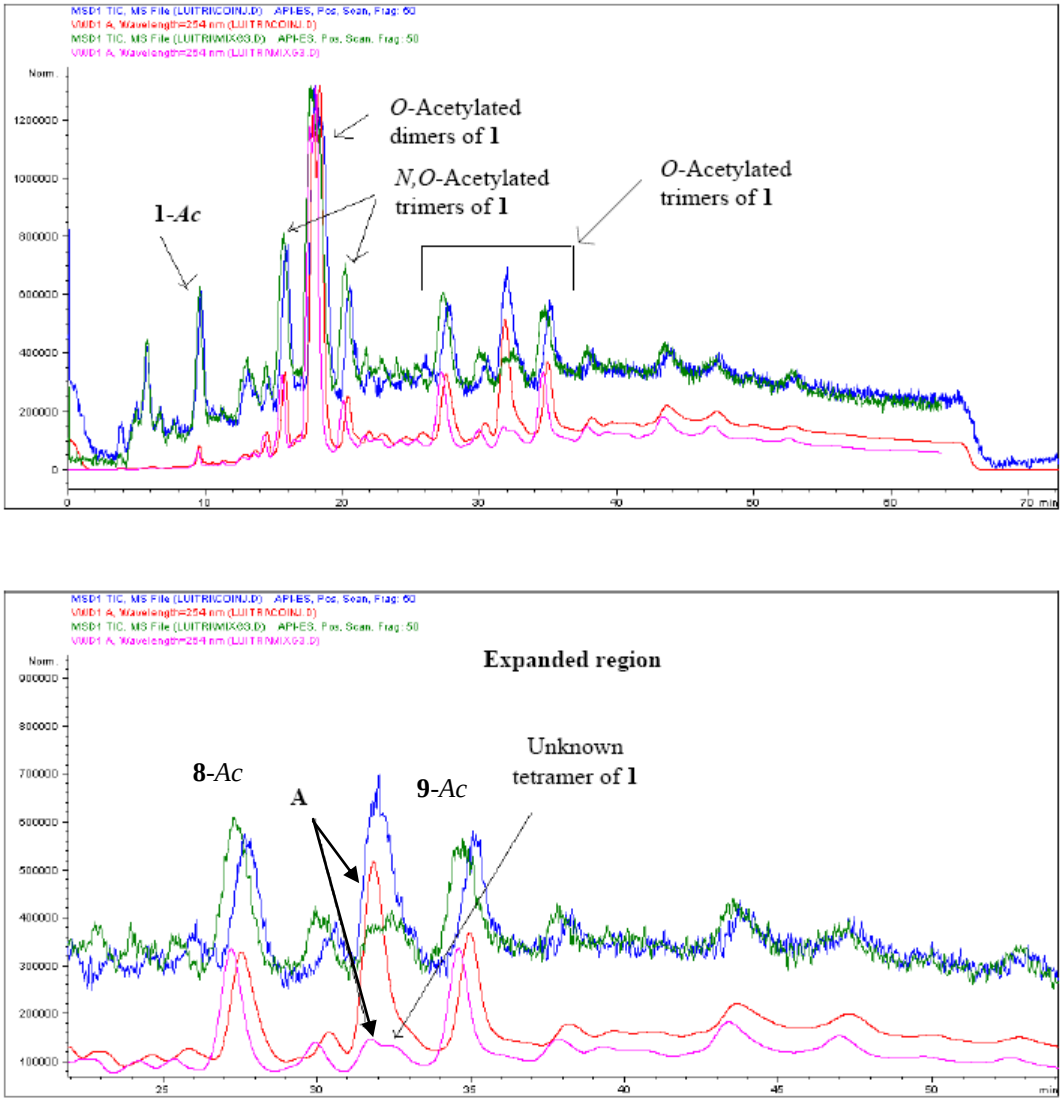


Figure 14. LC-MS elutograms of the oxidation mixture of **1**, after work up and acetylation, (UV_{254nm} trace; ESI(+)-MS trace) and of the coinjection of the same oxidation mixture with synthetic **10**-*Ac* (UV_{254nm} trace; ESI(+)-MS trace).

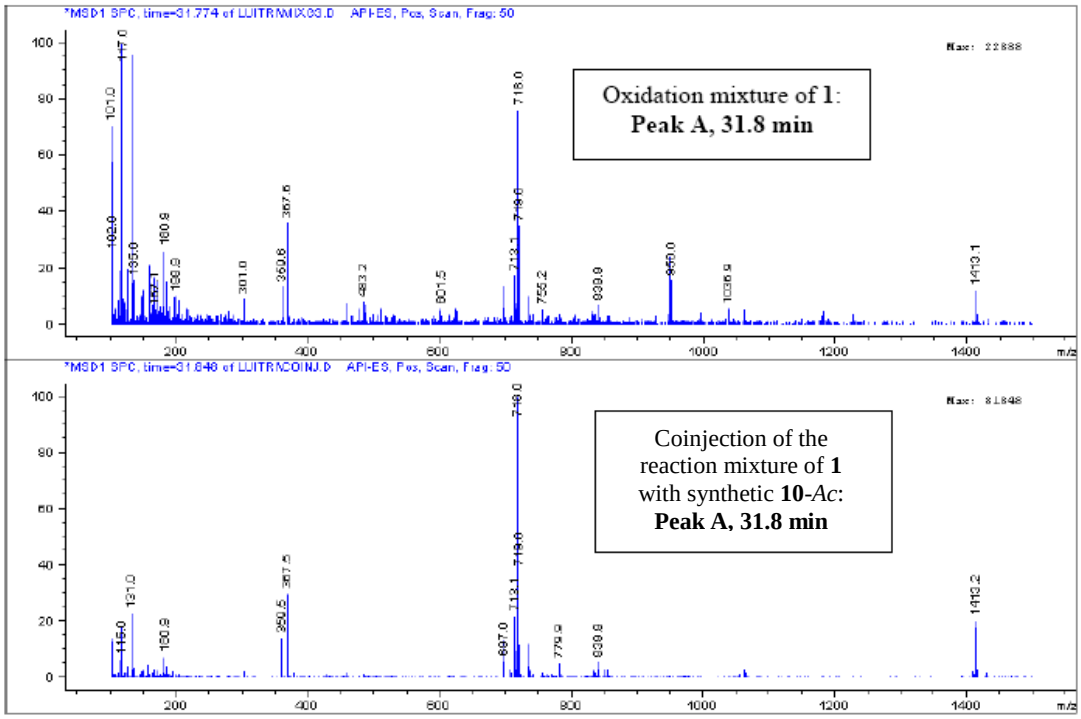
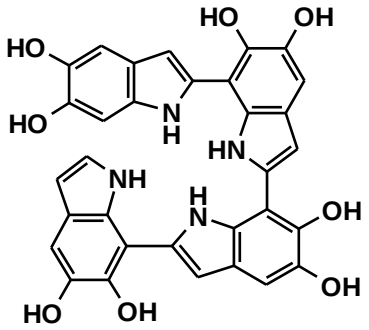


Figure 15. ESI(+)-MS spectra of the peak **A** (31.8 min) in the oxidation mixture of **1**, after work up and acetylation, and in the coinjection of the same oxidation mixture with synthetic **10**-*Ac*.

These data suggested that 2,4'- and 2,7'-bonds are of comparable importance in the oxidative dimerization of **1**, but the 2,4'-coupling mode prevails beyond the dimer stage, a finding which may have interesting mechanistic implications for eumelanin build-up.

Synthesis of a 5,6-dihydroxyindole tetramer

The excellent results obtained from the synthetic procedures developed for the preparation of dimers and trimers of **1** supported the possibility of extending this strategy toward the preparation of superior oligomers of **1**. On this basis, the synthesis of the new tetramer **31** was then investigated.



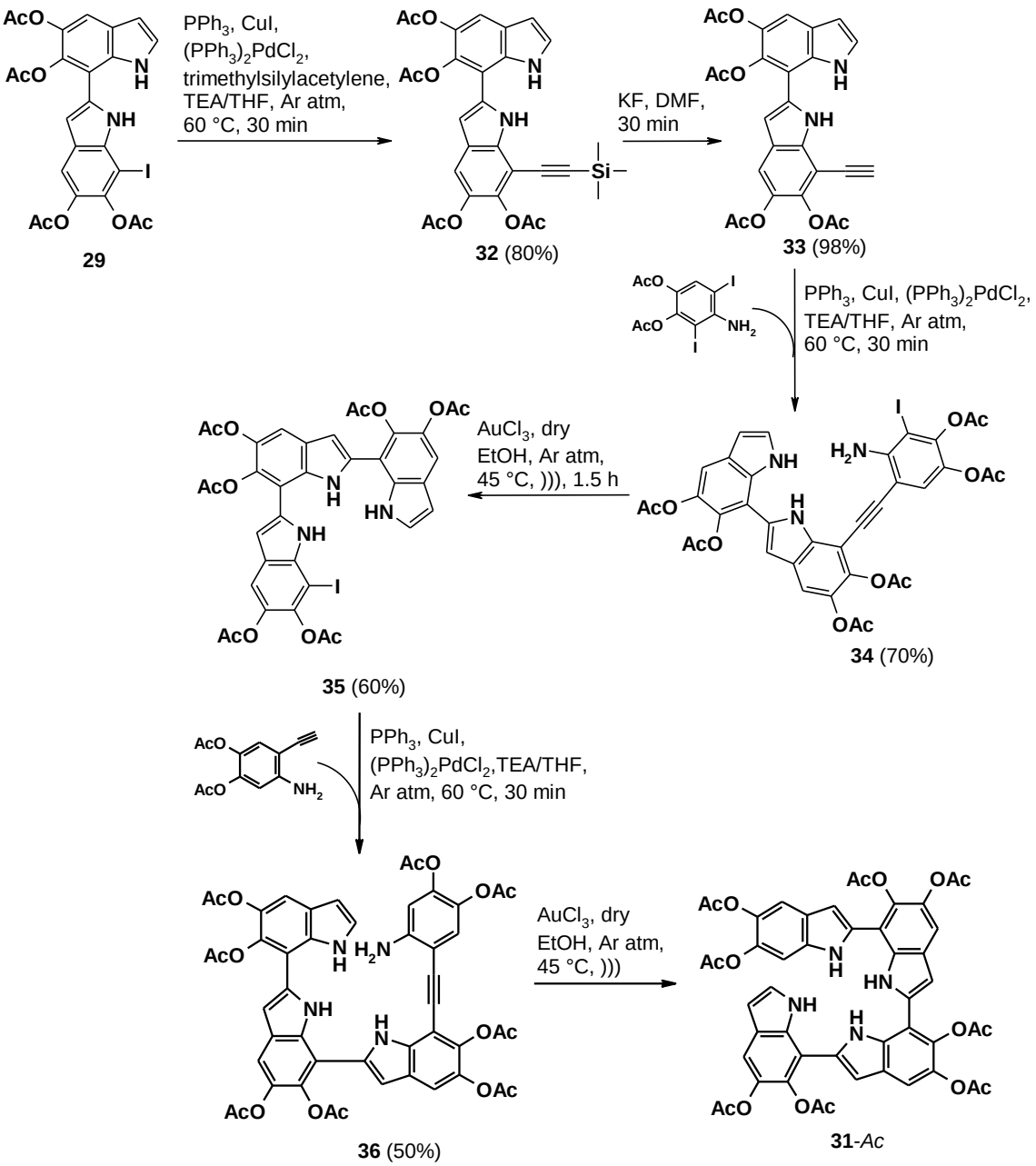
31

Also in this case the structure of **31** embodies the characteristic 2,7'-coupling mode of 5,6-dihydroxyindoles;[24] moreover, the addition of the fourth unit could confer to this compound particular properties useful for the preparation of complex molecular structures with possible applications for ion coordination or for the synthesis of highly ordered bioinspired polymers that could create efficient π-stacking interactions for supramolecular assembly.

Following the synthetic strategy previously described for the preparation of dimers and trimers of **1**, the tetrameric skeleton of **31** was obtained via a properly functionalized trimer that is able to accept the insertion of an o-alkynylaniline, precursor of the indole unit.

The first part of the synthetic procedure leading to the preparation of the tetraindole **31** was the same of that described for the synthesis of the triindole **10** up to the

iodinated biindole **29** (Scheme 7).[105] This latter was, then, treated with trimethylsylilacetylene following the reaction conditions of the Sonogashira coupling, and subsequently with KF in DMF to give the ethynyl-derivative **33** (Scheme 7). A second Sonogashira coupling carried out with the diiodoaniline **13** led to the formation of **34** that, after intramolecular cyclization with $AuCl_3$, afforded the desired iodinated triindole **35**. The triindole was treated with the *o*-ethynylaniline **17**-*Ac* under the conditions of the Sonogashira coupling to give the intermediate **36** that, after the last intramolecular cyclization step with $AuCl_3$, afforded the tetraindole **31**-*Ac*. This latter step is currently under investigation for yield optimization.



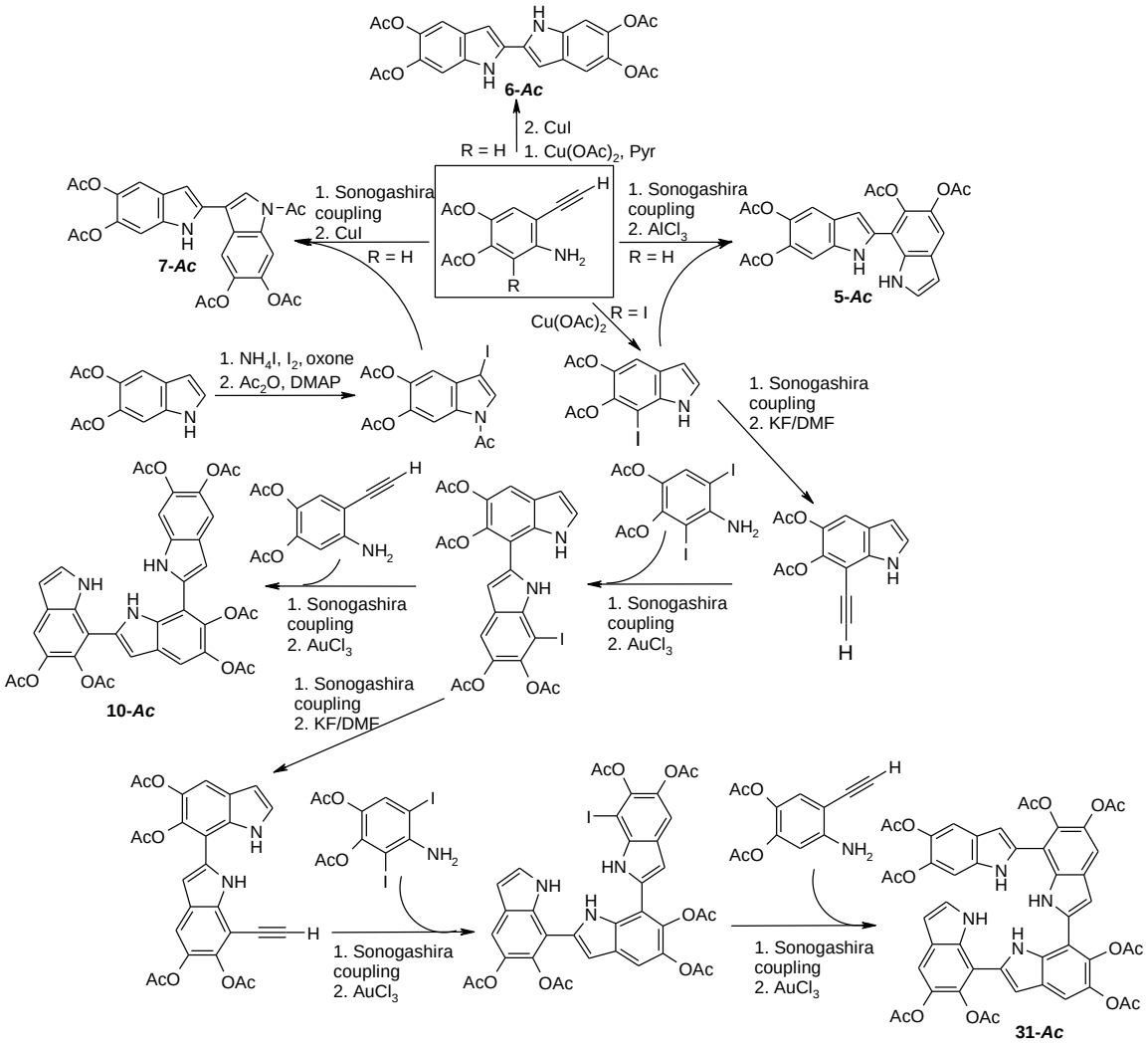
Scheme 7. Synthehtic procedure for the preparation of tetramer **31**-*Ac*.

Conclusions

In conclusion, in this chapter I have reported the first total synthesis of biindoles **5**-*Ac*, **6**-*Ac* and **7**-*Ac*, of the triindole **10**-*Ac* and the tetraindole **31**-*Ac*, based on the unified strategy summarized in Scheme 8. This allowed access to different oligomeric scaffolds and monomer derivatives by manipulation of only two key *o*-ethynylaniline building blocks, **17**-*Ac* and **15**, which, in turn, can be obtained from a common precursor, **14**.

Main outcomes include:

- expansion of the current repertoire of synthetic strategies for biindole, triindole and tetraindole derivatives;
- the first access to fundamental eumelanin building blocks in significant amounts for structural investigations and model chemical studies;
- an entry to indole derivatives of potential practical interest for the preparation of functional materials.



Scheme 8. Simplified scheme illustrating the unified strategy to 2,7'-, 2,2'- and 2,3'-biindoles (**5**-*Ac*, **6**-*Ac* and **7**-*Ac*), to the trimer **10**-*Ac* and to the tetramer **31**-*Ac* .

Further details about results shown in this chapter can be found in the following articles:

1. Capelli, L.; Manini, P.; Pezzella, A.; Napolitano, A.; d'Ischia, M. *J. Org. Chem.* **2009**, *74*, 7191–7194.

2. Capelli, L.; Manini, P.; Pezzella, A.; d'Ischia, M. *Org. Biomol. Chem.* **2010**, *8*, 4243-4245.

Experimental Section

Materials and methods. All commercially available reagents were used as received. Anhydrous solvents were purchased from commercial sources and withdrawn from the container by syringe, under a slight positive pressure of argon. All the solvents were of analytical grade quality. Compounds 1,2-dibenzyloxy-4-iodo-5-nitrobenzene (**24**) and 5,6-diacetoxy-3-iodoindole (**22**) were prepared according to reported procedures.[64,89]

FT-IR spectra were recorded on a FT-IR spectrophotometer. UV spectra were performed using a diode array spectrophotometer. NMR spectra were recorded with 200, 300, 400 and 500 MHz instruments. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$, CD_3OD and $(CD_3)_2CO$ using TMS as the internal standard; *J* values are given in Hz. Assignments with identical superscripts may be interchanged. $^{1}H,^{1}H$ COSY, $^{1}H,^{13}C$ HSQC, $^{1}H,^{13}C$ HMBC and ROESY experiments were run at 400.1 MHz using standard pulse programs. Mass spectra were registered in the electrospray ionization-positive ion (ESI+) mode and in the MALDI mode. ESI analysis were performed with the cone and the fragmentator voltages set at 4 kV and 80 V, respectively; nitrogen was used as carrier gas at a flow of 8 mL/min and the nebulizer pressure was set at 50 psi. MALDI analysis were performed on a spectrometer in the positive ion mode using 2,5-dihydroxybenzoic acid as the matrix. High resolution mass spectra were registered in the electrospray ionization-positive ion (ESI+) mode. Analytical and preparative TLC analyses were performed on F_{254} silica gel plates (0.25 and 0.5 mm, respectively). TLC plates were visualised using a

UV lamp (λ= 254 nm) and a fluorescence lamp (λ= 366 nm). Liquid chromatography was performed on silica gel (60-230 mesh). LC-MS analyses were carried out on an instrument equipped with an ESI ion source; an octadecylsilane-coated column (4.6 × 150 mm, 3.5 μ) at 0.4 mL/min was used. The eluant system was 0.1% formic acid (eluant A) and methanol (eluant B), starting with 40% solvent B for 1 minute, and then from 40% to 50% solvent B gradient, for 4 minutes, from 50% to 60% solvent B gradient, for 35 minutes, and finally with 60% solvent B for 20 minutes.

Catalytic hydrogenation was performed with a hydrogenator bomb at 20 atm. Ultrasound experiments were performed in a sonic bath at 320 W and at 25 °C and 45 °C.

Synthesis of 1,2-diacetoxy-4-nitrobenzene (11-*Ac*). A solution of **11** (1 g, 6.5 mmol) in acetic anhydride (5 mL) and pyridine (250 μL) was kept in an ultrasound bath at 320 W for 6 min and then the mixture was evaporated under reduced pressure to afford pure **11**-*Ac* (1.5 g, 98 % R_f = 0.47 eluant: petroleum ether/ethyl acetate 7:3 (v/v)).

11-*Ac*: ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 2.22 (6H, s, 2 × CH_3), 7.29 (1H, d *J* = 8.0 Hz, H-6), 8.01 (1H, d *J*= 2.6 Hz, H-3), 8.03 (1H, dd *J* = 8.0, 2.6 Hz, H-5); ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm) 20.3 (CH_3), 20.4 (CH_3), 119.5 (CH), 121.7 (CH), 124.0 (CH), 142.2 (C), 145.1 (C), 147.3 (C), 167.2 (C=O), 167.5 (C=O); MS (ESI+) *m/z* 240 ($[M+H]^+$), 262 ($[M+Na]^+$); HRMS (ESI) *m/z* $C_{10}H_{10}NO_6$ $[M+H]^+$ calcd 240.0508, found 240.0512.

Synthesis of 3,4-diacetoxyaniline (12). A solution of **11**-*Ac* (781 mg, 3.3 mmol) in chloroform (60 mL) was poured into a 100 mL hydrogenator bomb and treated with 10wt% Pd/C (209 mg). After 6 h the reaction mixture was filtered on celite and evaporated under reduced pressure to afford pure **12** (670 mg, 98% R_f = 0.55 eluant: petroleum ether/ethyl acetate 4:6 (v/v)).

12: ^{1}H NMR (200 MHz, CD_3OD) δ (ppm) 2.20 (3H, s, CH_3), 2.21 (3H, s, CH_3), 4.83 (2H, br s, NH_2), 6.50 (1H, d *J* = 2.6 Hz, H-2), 6.54 (1H, dd *J* = 8.6, 2.6 Hz, H-6), 6.88 (1H, d *J* = 8.6 Hz, H-5); ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm) 20.4 (CH_3), 20.5 (CH_3), 109.5 (CH), 112.7 (CH), 123.5 (CH), 133.6 (C-NH_2), 142.4 (C-O), 145.3 (C-O), 168.6 (C=O), 168.9 (C=O); MS (ESI+) *m/z* 210 ($[M+H]^+$), 232 ($[M+Na]^+$); HRMS (ESI) *m/z* $C_{10}H_{12}NO_4$ $[M+H]^+$ calcd 210.0766, found 210.0772.

Synthesis of 3,4-diacetoxy-2,6-diiodoaniline (13). The title compound was prepared according to a reported procedure[90] with slight modifications. In brief, a solution of **12** (600 mg, 2.9 mmol) in methanol (60 mL) was added to a solution of $NaClO_2$ (521 mg, 5.8 mmol) and NaI (1.7 g, 11.6 mmol) in water (75 mL). The final solution was treated with 12 M HCl (2.2 mL) under stirring and at room temperature. After about 1 h the reaction mixture was taken up in ethyl acetate (200 mL) and extracted with a saturated solution of sodium thiosulfate. The organic layers were dried over anhydrous sodium sulfate, evaporated under reduced pressure and the residue fractionated on silica gel (petroleum ether/ethyl acetate, gradient from 9:1 to 8:2) to afford pure **13** (860 mg, 65%, R_f = 0.78 eluant: petroleum ether/ethyl acetate 1:1 (v/v)).

13: ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm) 2.23 (3H, s, CH_3), 2.34 (3H, s, CH_3), 5.16 (2H, br s, NH_2), 7.65 (1H, s, H-5); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ (ppm) 20.1 (CH_3), 20.4 (CH_3), 75.2 (C-6), 78.9 (C-2), 133.7 (C-5), 134.2 (C-1), 145.1 (C-O), 147.1 (C-O), 167.3 (C=O), 168.7 (C=O); MS (ESI+) *m/z* 462 ($[M+H]^+$), 484 ($[M+Na]^+$), 500 ($[M+K]^+$); HRMS (ESI) *m/z* $C_{10}H_{10}I_2NO_4$ $[M+H]^+$ calcd 461.8699, found 461.8705.

Synthesis of 3,4-diacetoxy-2-iodo-6-(trimethylsilylethynyl)aniline (14). A solution of **13** (1.2 g, 2.6 mmol) in triethylamine (21.6 mL) and toluene (21.6 mL), was treated with PPh_3 (68.3 mg, 0.26 mmol), CuI (49.6 mg, 0.26 mmol), $(PPh_3)_2PdCl_2$ (91.3 mg, 0.13 mmol) and trimethylsilylacetylene (370 μL, 2.6 mmol) under an argon atmosphere at 60 °C. After 30 min the reaction mixture was extracted with a 10% water solution of NH_4Cl and toluene. The organic layers were dried over anhydrous sodium sulfate, evaporated under reduced pressure and the residue fractionated on silica gel (petroleum ether/ethyl acetate, gradient from 95:5 to 9:1) to afford pure **14** (1.1 g, 98%, R_f = 0.86 eluant: petroleum ether/ethyl acetate 1:1 (v/v)).
14: ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.25 (9H, s, $Si(CH_3)_3$), 2.22 (3H, s, CH_3), 2.34 (3H, s, CH_3), 4.79 (2H, br s, NH_2), 7.16 (1H, s, H-5); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 0.10 ($Si(CH_3)_3$), 20.4 (CH_3), 20.7 (CH_3), 80.2 (C-2), 100.0 (-C≡C-Si), 101.4 (-C≡C-Si), 105.0 (C-6), 126.4 (C-5), 133.0 (C-1), 144.3 (C-O), 147.8 (C-O), 167.0 (C=O), 168.4 (C=O); MS (ESI+) *m/z* 432 ($[M+H]^+$), 454 ($[M+Na]^+$), 470 ($[M+K]^+$); HRMS (ESI) *m/z* $C_{15}H_{19}INO_4Si$ $[M+H]^+$ calcd 432.0128, found 432.0133.

Synthesis of 3,4-diacetoxy-6-ethynyl-2-iodoaniline (15). A solution of **14** (566 mg, 1.3 mmol) in DMF (12 mL) was treated with a solution of KF (110 mg, 1.9 mmol) in water (2 mL) at room temperature and under vigorous stirring. After 30 min the reaction mixture was extracted with chloroform and a 10% water solution of NH_4Cl, dried over anhydrous sodium sulfate and evaporated under reduced pressure to afford pure **15** (457 mg, 98%, R_f = 0.53 eluant: petroleum ether/ethyl acetate 6:4 (v/v)).

15: ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 2.22 (3H, s, CH_3), 2.33 (3H, s, CH_3), 3.41 (1H, s, -C≡CH), 4.20 (2H, br s, NH_2), 7.16 (1H, s, H-5); ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm) 20.4 (CH_3), 20.7 (CH_3), 79.0 (-<u>C</u>≡CH), 80.3 (C-2), 83.6 (-C≡<u>C</u>H), 103.6 (C-6), 126.7 (C-5), 132.9 (C-1), 144.6 (C-O), 148.2 (C-O), 167.0 (C=O), 168.5 (C=O); MS (ESI+) *m/z* 360 ($[M+H]^+$), 382 ($[M+Na]^+$), 398 ($[M+K]^+$); HRMS (ESI) *m/z* $C_{12}H_{11}INO_4$ $[M+H]^+$ calcd 359.9733, found 359.9739.

Synthesis of 4,5-diacetoxy-2-(trimethylsilylethynyl)aniline (16-*Ac*). A solution of **14** (540 mg, 1.25 mmol) in acetic acid (18 mL) was treated with zinc (1.1 g, 16.8 mmol) at room temperature and under stirring. After 30 min the reaction mixture was taken up in ethyl acetate (50 mL), filtered and extracted with water. The organic layers were dried over anhydrous sodium sulfate and evaporated under reduced pressure to afford pure **16**-*Ac* (370 mg, 97%, R_f = 0.52 eluant: chloroform/ethyl acetate 9:1 (v/v)).

16-*Ac*: ^{1}H NMR (200 MHz, $(CD_3)_2CO$) δ (ppm) 0.20 (9H, s, $Si(CH_3)_3$), 2.16 (3H, s, CH_3), 2.18 (3H, s, CH_3), 5.09 (2H, br s, NH_2), 6.57 (1H, s, H-6), 7.00 (1H, s, H-3); ^{13}C NMR (50 MHz, $(CD_3)_2CO$) δ (ppm) 0.20 ($Si(CH_3)_3$), 20.1 (CH_3), 20.2 (CH_3), 99.8 (-<u>C</u>≡C-Si), 101.3 (-C≡<u>C</u>-Si), 104.5 (C-2), 108.8 (C-6), 126.6 (C-3), 133.1 (C-1),

144.6 (C-O), 148.9 (C-O), 168.1 (C=O), 168.8 (C=O); MS (ESI+) *m/z* 306 ($[M+H]^+$), 328 ($[M+Na]^+$), 344 ($[M+K]^+$); HRMS (ESI) *m/z* $C_{15}H_{20}NO_4Si$ $[M+H]^+$ calcd 306.1161, found 306.1157.

Synthesis of 4,5-diacetoxy-2-ethynylaniline (17-*Ac*). The title compound (277 mg, 98%, R_f = 0.41 eluant: chloroform/ethyl acetate 9:1 (v/v)) was prepared from **16**-*Ac* (370 mg, 1.21 mmol) following the same procedure reported for the synthesis of **15**. **17**-*Ac*: ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm) 2.21 (3H, s, CH_3), 2.24 (3H, s, CH_3), 3.91 (1H, s, -C≡CH), 5.14 (2H, br s, NH_2), 6.64 (1H, s, H-6), 7.09 (1H, s, H-3); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ (ppm) 20.0 (CH_3), 20.2 (CH_3), 79.8 (-C≡CH), 84.1 (-C≡CH), 103.7 (C-2), 108.9 (C-6), 126.8 (C-3), 133.2 (C-1), 144.6 (C-O), 149.1 (C-O), 168.1 (C=O), 168.8 (C=O); MS (ESI+) *m/z* 234 ($[M+H]^+$), 256 ($[M+Na]^+$), 272 ($[M+K]^+$); HRMS (ESI) *m/z* $C_{12}H_{12}NO_4$ $[M+H]^+$ calcd 234.0766, found 234.0760.

Synthesis of 5,6-diacetoxy-7-iodoindole (19).

A solution of **15** (900 mg, 2.5 mmol) in dry CH_2Cl_2 (63 mL) was treated with $Cu(OAc)_2$ (300 mg, 1.5 mmol) at 55 °C under an argon atmosphere. After 18 h the reaction mixture was brought to room temperature and extracted with ethyl acetate and water. The organic layers were collected, dried over anhydrous sodium sulfate and evaporated under reduced pressure to afford pure **19** (720 mg, 80%, R_f = 0.70 eluant: chloroform/ethyl acetate 9:1 (v/v)).

19: ^{1}H NMR (500 MHz, $(CD_3)_2CO$) δ (ppm) 2.28 (3H, s, CH_3), 2.36 (3H, s, CH_3), 6.69 (1H, br s, H-3), 7.43 (1H, s, H-4), 7.46 (1H, br s, H-2), 10.33 (1H, br s, NH); ^{13}C NMR (50 MHz, $(CD_3)_2CO$) δ (ppm) 20.3 (CH_3), 20.4 (CH_3), 73.4 (C-7), 104.0 (C-3), 114.8 (C-4), 125.6 (C-4a), 127.7 (C-2), 136.8 (C-7a), 137.4 (C-5), 140.1 (C-6), 168.2 (C=O), 169.1 (C=O); MS (ESI+) *m/z* 360 ($[M+H]^+$), 382 ($[M+Na]^+$), 398 ($[M+K]^+$); HRMS (ESI) *m/z* $C_{12}H_{11}INO_4$ $[M+H]^+$ calcd 359.9733, found 359.9741.

Synthesis of 4,5-diacetoxy-2-[2'-(5",6"-diacetoxyindol-7"-yl)-1'-ethynyl]aniline (20). A solution of **19** (140 mg, 0.39 mmol) in triethylamine (3.4 mL) and toluene (3.4 mL), was treated with **17**-*Ac* (182 mg, 0.78 mmol), PPh_3 (10.2 mg, 0.039 mmol), CuI (7.4 mg, 0.039 mmol) and $(PPh_3)_2PdCl_2$ (13.7 mg, 0.02 mmol) under argon atmosphere at 60 °C. After 1 h the reaction mixture was extracted with a 10% water solution of NH_4Cl and chloroform. The organic layers were dried over anhydrous sodium sulfate, evaporated under reduced pressure and the residue fractionated on silica gel (eluant chloroform) to afford pure **20** (127 mg, 70%, R_f = 0.46 eluant: chloroform/ethyl acetate 1:1 (v/v)).

20: ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm) 2.24 (3H, s, CH_3), 2.25 (3H, s, CH_3), 2.30 (3H, s, CH_3), 2.36 (3H, s, CH_3), 5.25 (2H, br s, NH_2), 6.57 (1H, t *J* = 2.4 Hz, H-3"), 6.69 (1H, s, H-6), 7.21 (1H, s, H-3), 7.43 (1H, t *J* = 2.4 Hz, H-2"), 7.47 (1H, s, H-4"), 10.85 (1H, br s, NH); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ (ppm) 19.6 (2 × CH_3), 19.7 (2 × CH_3), 85.8 (C-2'), 93.4 (C-1'), 102.7 (C-3"), 103.9 (C-2), 108.6 (C-6), 109.6 (C-7"), 114.8 (C-4"), 125.2 (C-4a"), 126.1 (C-3), 126.9 (C-2"), 132.9 (C-1), 133.9 (C-7a"), 136.8 (C-5"), 139.2 (C-6"), 144.3 (C-4), 148.1 (C-5), 167.6 (C=O), 168.0 (C=O), 168.4 (C=O), 168.6 (C=O); MS (ESI+) *m/z* 465 ($[M+H]^+$), 487

([M+Na]$^+$), 503 ([M+K]$^+$); HRMS (ESI) *m/z* $C_{24}H_{21}N_2O_8$ $[M+H]^+$ calcd 465.1298, found 465.1301.

Synthesis of 5,5',6,6'-tetraacetoxy-2,7'-biindolyl (5-*Ac*). A solution of **20** (50 mg, 0.11 mmol) in toluene (6 mL) was treated with $AlCl_3$ (14 mg, 0.11 mmol) under an argon atmosphere at 110 °C. After 3.5 h the reaction mixture was extracted with a 10% water solution of NH_4Cl and chloroform. The organic layers were dried over anhydrous sodium sulfate, evaporated under reduced pressure and the residue fractionated on silica gel (eluant: chloroform/ethyl acetate 65:35 (v/v)) to afford pure **5**-*Ac* (25 mg, 50%, R_f = 0.51 eluant: chloroform/ethyl acetate 65:35 (v/v)). ^{1}H and ^{13}C NMR resonances were in in good agreement with data reported in the literature.[85a]

Synthesis of 5,5',6,6'-tetrahydroxy-2,7'-biindolyl (5). A solution of **5**-*Ac* (50 mg, ca. 0.1 mmol) in MeOH (2 mL) was treated with sodium *t*-butoxide under an argon atmosphere for 1 min to obtain complete deacetylation. The crude mixture was then evaporated under reduced pressure and the residue characterized by MS and NMR. For ^{1}H NMR and ^{13}C NMR see Table 2. HRMS (ESI) *m/z* $C_{16}H_{12}N_2O_4$ $[M+H]^+$ calcd 296.0797, found 296.0792.

Table 2. ^{1}H and ^{13}C NMR resonances of compound **5** (Aceton-d_6)

Carbon	δ_C (ppm)	δ_H (ppm) (mult., *J* (Hz))	Carbon	δ_C (ppm)	δ_H (ppm) (mult., *J* (Hz))
C-2	131.6[a]	-	C-2’	123.8	7.16 (t, 2.7)
C-3	100.8	6.74 (s)	C-3’	101.9	6.31 (t, 2.7)
C-4	104.5	6.99 (s)	C-4’	104.4	7.02 (s)
C-5	141.0[b]	-	C-5’	140.2[b]	-
C-6	143.3[b]	-	C-6’	140.4[b]	-
C-7	97.4	7.01 (s)	C-7’	104.3	-
C-8	131.8[a]	-	C-8’	128.8	-
C-9	122.3	-	C-9’	121.7	-
NH	-	9.86 (br s)	NH’	-	9.86 (br s)

[a] Interchangeable

[b] Interchangeable

Synthesis of 2-[4’-(4”,5”-diacetoxy-2”-aminophenyl)-1’,3’-butadiinyl]-4,5-diacetoxyaniline (21-*Ac*). A solution of **17**-*Ac* (250 mg, 1.05 mmol) in dry pyridine (3.5 mL) was treated with $Cu(OAc)_2$ (228 mg, 1.15 mmol) at room temperature under an argon atmosphere. After 16 h the reaction mixture was filtered on celite, evaporated under reduced pressure and extracted with chloroform and a saturated solution of $NaHCO_3$. The organic layers were washed with brine, dried over anhydrous sodium sulfate, evaporated under reduced pressure and the residue fractionated on silica gel (cyclohexane/ethyl acetate, gradient from 7:3 to 6:4) to

afford pure **21**-*Ac* (205 mg, 86%, R_f = 0.16 eluant: chloroform/ethyl acetate 8:2 (v/v)).

21-*Ac*: ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 2.24 (3H × 4, s, CH_3), 4.35 (2H × 2, br s, NH_2), 6.47 (1H × 2, s, H-6, H-3"), 7.10 (1H × 2, s, H-3, H-6"); ^{13}C NMR (50 MHz, $(CD_3)_2CO$) δ (ppm) 20.1 (2 × CH_3), 20.2 (2 × CH_3), 79.3 (-C≡C-C≡C-, -C≡C-C≡C-), 102.5 (C-2, C-1"), 109.2 (C-6, C-3"), 127.2 (C-3, C-6"), 133.3 (C-1, C-2"), 145.5 (C-O), 150.6 (C-O), 168.1 (C=O), 168.8 (C=O); MS (ESI+) *m/z* 465 ($[M+H]^+$), 487 ($[M+Na]^+$), 503 ($[M+K]^+$); HRMS (ESI) *m/z* $C_{24}H_{21}N_2O_8$ $[M+H]^+$ calcd 465.1298, found 465.1304.

Synthesis of 5,5',6,6'-tetraacetoxy-2,2'-biindolyl (6-*Ac*). A solution of **21**-*Ac* (180 mg, 0.18 mmol) in dry DMF (2 mL) was treated with CuI (70 mg, 0.36 mmol) at 110 °C under stirring and under an argon atmosphere. After 3 h the reaction mixture was brought at room temperature and extracted with chloroform and a 10% water solution of NH_4Cl. The organic layers were dried over anhydrous sodium sulfate and evaporated under reduced pressure to afford pure **6**-*Ac* (124 mg, 70%, R_f = 0.51 eluant: chloroform/ethyl acetate 65:35 (v/v)). ^{1}H and ^{13}C NMR resonances were in good agreement with data reported in the literature.[85b]

Synthesis of 5,6-diacetoxyindole (1-*Ac*). A solution of **17**-*Ac* (100 mg, 0.4 mmol) in triethylamine (7 mL) and toluene (7 mL) was treated with CuI (82 mg, 0.4 mmol) at 130 °C and under an argon atmosphere. After 1.5 h the reaction mixture was extracted with chloroform and a 10% water solution of NH_4Cl. The organic layers were dried over anhydrous sodium sulfate and evaporated under reduced pressure to

afford pure **1**-*Ac* (80 mg, 80%). ^{1}H and ^{13}C NMR resonances were in good agreement with data reported in the literature.[24]

Synthesis of 5,6-diacetoxy-1-acetyl-3-iodoindole (22-*Ac*). A solution of **22** (500 mg, 0.140 mmol) in toluene (12 mL) was treated with acetic anhydride (600 μL) and DMAP (150 mg, 0.120 mmol) at room temperature. After 30 min the reaction mixture was extracted with ethyl acetate and water and the organic layers were dried over anhydrous sodium sulfate and evaporated under reduced pressure to afford pure **22**-*Ac* (550 mg, 98%, R_f = 0.73 eluant: chloroform/ethyl acetate 8:2 (v/v)).

22-*Ac*: ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 2.32 (3H × 2, s, CH_3), 2.60 (3H, s, $NCOCH_3$), 7.23 (1H, s, H-4), 7.57 (1H, s, H-2), 8.31 (1H, s, H-7); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 20.3 ($OCO\underline{C}H_3$), 20.4 ($OCO\underline{C}H_3$), 23.3 ($NCO\underline{C}H_3$), 66.4 (C-3), 111.5 (CH), 115.3 (CH), 130.2 (C), 130.6 (CH), 131.9 (C), 139.5 (C-O), 140.9 (C-O), 167.1 (C=O), 168.3 (2 ×□ C=O); MS (ESI+) *m/z* 402 ($[M+H]^+$), 424 ($[M+Na]^+$), 440 ($[M+K]^+$); HRMS (ESI) *m/z* $C_{14}H_{13}INO_5$ $[M+H]^+$ calcd 401.9838, found 401.9843.

Synthesis of 4,5-diacetoxy-2-[2'-(5",6"-diacetoxy-1"-acetylindol-3"-yl)-1'-ethynyl]aniline (23). The compound was prepared from **22**-*Ac* (112 mg, 0.28 mmol) following the same procedure reported for the synthesis of **20**. After 1 h the reaction mixture was worked-up as previously described and the residue fractionated on silica gel (eluant chloroform) to afford pure **23** (127 mg, 90%, R_f = 0.46 eluant: chloroform/ethyl acetate 65:35 (v/v)).

23: ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm) 2.24 (3H, s, $OCOCH_3$), 2.26 (3H, s, $OCOCH_3$), 2.33 (3H, s, $OCOCH_3$), 2.35 (3H, s, $OCOCH_3$), 2.72 (3H, s, $NCOCH_3$), 5.29 (2H, br s, NH_2), 6.69 (1H, s, H-6), 7.23 (1H, s, H-3), 7.61 (1H, s, H-4"), 8.12 (1H, s, H-2"), 8.30 (1H, s, H-7"); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ (ppm) 20.2 (2 × $OCOCH_3$), 20.3 (2 × $OCO\underline{C}H_3$), 23.5 ($NCO\underline{C}H_3$), 86.1 (C-2'), 89.5 (C-1'), 104.3 (C-2), 104.7 (C-3"), 109.1 (C-6), 112.2 (C-7"), 114.3 (C-4"), 126.6 (C-3), 128.6 (C-4a"), 131.3 (C-2"), 132.6 (C-7a"), 133.5 (C-1), 140.6 (C-5"), 141.7 (C-6"), 144.6 (C-4), 148.7 (C-5), 168.3 (2 × $O\underline{C}OCH_3$), 169.0 (2 × $O\underline{C}OCH_3$), 169.5 ($N\underline{C}OCH_3$); MS (ESI+) *m/z* 507 ($[M+H]^+$), 529 ($[M+Na]^+$), 545 ($[M+K]^+$); HRMS (ESI) *m/z* $C_{26}H_{23}N_2O_9$ $[M+H]^+$ calcd 507.1404, found 507.1409.

Synthesis of 5,5',6,6'-tetraacetoxy-1'-acetyl-2,3'-biindolyl (7-*Ac*). A solution of **23** (100 mg, 0.2 mmol) in dry DMF (2.1 mL) was treated under stirring with CuI (76 mg, 0.4 mmol) at 110 °C and an argon atmosphere. After 1.5 h the reaction mixture was brought at room temperature and extracted with chloroform and a 10% solution water of NH_4Cl. The organic layers were dried over anhydrous sodium sulfate and evaporated under reduced pressure to afford pure **7**-*Ac* (70 mg, 70%, R_f = 0.44 eluant: chloroform/ethyl acetate 4:6 (v/v)).

7-*Ac*: ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm) 2.28 (3H × 2, s, $OCOCH_3$), 2.33 (3H× 2, s, $OCOCH_3$), 2.75 (3H, s, $NCOCH_3$), 6. 96 (1H, s, H-3), 7.29 (1H, s, H-7), 7.39 (1H, s, H-4), 7.89 (1H, s, H-4'), 8.28 (1H, s, H-2'), 8.35 (1H, s, H-7'), 10.81 (1H, br s, NH); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ (ppm) 20.3 (4 × $OCO\underline{C}H_3$), 23.5 ($NCO\underline{C}H_3$), 101.3 (C-3), 105.8 (C-7), 112.1 (C-7'), 114.0 (C-4), 114.1 (C-3'), 114.8 (C-4'), 125.1 (C-2'), 126.3 (C-4a'), 127.2 (C-4a), 132.8 (C-2), 133.8 (C-7a'), 134.8

(C-7a), 137.5 (C-5), 139.3 (C-6), 140.4 (C-5'), 141.4 (C-6'), 168.9 (O<u>C</u>OCH$_3$), 169.0 (O<u>C</u>OCH$_3$), 169.1 (O<u>C</u>OCH$_3$), 169.2 (O<u>C</u>OCH$_3$), 169.7 (N<u>C</u>OCH$_3$); MS (ESI+) *m/z* 507 ([M+H]$^+$), 529 ([M+Na]$^+$), 545 ([M+K]$^+$); HRMS (ESI) *m/z* $C_{26}H_{23}N_2O_9$ [M+H]$^+$ calcd 507.1404, found 507.

Synthesis of 4,5-dibenzyloxy-2-iodoaniline (25). A solution of **24** (2 g, 4.3 mmol) in acetone (250 mL) was added to a saturated solution of $Na_2S_2O_4$ in 0.1 M phosphate buffer pH 7.4 (250 mL), at 60 °C and under vigorous stirring. After 2 h the reaction mixture was extracted with CH_2Cl_2 and the organic layers were dried over anhydrous sodium sulfate, evaporated under reduced pressure and the residue fractionated on silica gel (petroleum ether/ethyl acetate, gradient from 9:1 to 8:2) to afford pure **25** (1.5 g, 80%, R_f = 0.40 eluant: petroleum ether/ethyl acetate 8:2 (v/v)). ^{1}H and ^{13}C NMR resonances were in good agreement with data reported in the literature.[89]

Synthesis of 4,5-dibenzyloxy-2-(trimethylsilylethynyl)aniline (16-*Bn*). A solution of **25** (500 mg, 1.2 mmol) in triethylamine (9.5mL) and toluene (9.5mL) was treated with PPh_3 (30mg, 0.12mmol), CuI (22 mg, 0.12 mmol), $(PPh_3)_2PdCl_2$ (40 mg, 0.06 mmol), and trimethylsilylacetylene (900 μL, 6.3 mmol) under an argon atmosphere at 60 °C. After 30 min the reaction mixture was extracted with a 10% water solution of NH_4Cl and toluene. The organic layers were dried over anhydrous sodium sulfate and evaporated under reduced pressure to afford pure **16**-*Bn* (456mg, 98%, R_f = 0.53, eluant: petroleum ether/ ethyl acetate 8:2 (v/v)).

16-*Bn*: ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 0.27 (9H, s, $Si(CH_3)_3$), 3.80 (2H, br s, NH_2), 5.02 (2H, s,OCH_2Ph), 5.08 (2H, s, OCH_2Ph), 6.31 (1H, s, H-6), 6.96 (1H, s, H-3), 7.0-7.2 (10H, m, H-2, H-3, H-4, H-5, H-6/OBn); ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm) 0.47 ($Si(CH_3)_3$), 71.1 (OCH_2Ph), 73.1 (OCH_2Ph), 98.8 (-C≡C-Si), 100.1 (-C≡C-Si), 101.8 (C-6), 102.2 (C-2), 120.2 (C-3), 127-129 (10 × CH, C-2, C-3, C-4, C-5, C-6/OBn), 137.1 (C-1/OBn), 137.8 (C-1/OBn), 141.2 (C-1), 144.9 (C-4), 151.9 (C-5); HRMS (ESI) *m/z* $C_{25}H_{28}NO_2Si$ $[M+H]^+$calcd 402.1889, found 402.1893.

Synthesis of 4,5-dibenzyloxy-2-ethynylaniline (17-*Bn*). A solution of **16**-*Bn* (320 mg, 0.8 mmol) in DMF (7.2 mL) was treated with KF (70 mg, 1.2 mmol) at room temperature. After 2 h the reaction mixture was extracted with chloroform and a 10% water solution of NH_4Cl, and the organic layers were dried over anhydrous sodium sulfate and evaporated under reduced pressure to afford pure **17**-*Bn* (257 mg, 98%, R_f = 0.35, eluant: petroleum ether/ethyl acetate 7:3 (v/v)).

17-*Bn***:** FT-IR ($CHCl_3$) ν 2096 (-C≡C-), 3400-3500 (NH_2) cm^{-1}; ^{1}H NMR (300 MHz, $(CD_3)_2CO$) δ (ppm) 3.84 (1H, s, -C≡CH), 4.81 (2H, br s, NH_2), 5.02 (2H, s, OCH_2Ph), 5.08 (2H, s, OCH_2Ph), 6.60 (1H, s, H-6), 6.97 (1H, s, H-3), 7.3-7.5 (10H, m, H-2, H-3, H-4, H-5, H-6/OBn); ^{13}C NMR (50 MHz, $(CD_3)_2CO$) δ (ppm) 70.8 (OCH_2Ph), 73.1 (OCH_2Ph), 81.7 (-C≡CH), 82.7 (-C≡CH), 98.0 (C-2), 101.5 (C-6), 120.9 (C-3), 128-129 (10 × CH, C-2, C-3, C-4, C-5, C-6/OBn), 137.9 (C-1/OBn), 138.7 (C-1/OBn), 140.6 (C-1), 147.1 (C-4), 152.6 (C-5); HRMS (ESI) *m/z* $C_{22}H_{20}NO_2$ $[M+H]^+$calcd 330.1494, found 330.1489.

Synthesis of 2-[4'-(2"-Amino-4",5"-dibenzyloxyphenyl)-1',3'-butadiinyl]-4,5-dibenzyloxyaniline (21-*Bn*). A solution of **17**-*Bn* (682 mg, 2.1 mmol) in dry pyridine (7 mL) was treated with $Cu(OAc)_2$ (455 mg, 2.3 mmol) at room temperature under an argon atmosphere. After 16 h the reaction mixture was filtered on Celite, evaporated under reduced pressure and extracted with chloroform and a saturated solution of $NaHCO_3$. The organic layers were washed with brine, dried over anhydrous sodium sulfate, and evaporated under reduced pressure and the residue fractionated on silica gel (cyclohexane/ethyl acetate, gradient from 7:3 to 6:4) to afford pure **21**-*Bn* (272 mg, 40%, R_f = 0.44 eluant: cyclohexane/ethyl acetate 6:4 (v/v)).

21-*Bn***:** FT-IR ($CHCl_3$) ν 2200 (-C≡C-), 3400-3500 (NH_2) cm^{-1}; ^1H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm) 4.95 (2H × 2, br s, NH_2), 5.03 (2H × 2, s, OCH_2Ph), 5.12 (2H × 2, s, OCH_2Ph), 6.57 (1H × 2, s, H-6, H-3"), 6.93 (1H × 2, s, H-3, H-6"), 7.2-7.6 (10H × 2, m, H-2, H-3, H-4, H-5, H-6/OBn); ^{13}C NMR (50 MHz, $(CD_3)_2CO$) δ (ppm) 71.0 (2 × OCH_2Ph), 73.1 (2 × OCH_2Ph), 78.9 (-C≡C-C≡C-), 80.9 (-C≡C-C≡C-), 97.4 (C-2, C-1"), 101.5 (C-6, C-3"), 120.8 (C-3, C-6"), 128-129 (10 CH × 2, C-2, C-3, C-4, C-5, C-6/OBn), 138.1 (2 × C-1/OBn), 138.9 (2 × C-1/OBn), 141.0 (C-5, C-4"), 148.7 (C-1, C-2"), 153.5 (C-4, C-5"); MS (MALDI) *m/z* 657 ($[M+H]^+$); HRMS (ESI) *m/z* $C_{44}H_{37}N_2O_4$ $[M+H]^+$calcd 657.2753, found 657.2758.

Synthesis of 5,5',6,6'-tetrabenzyloxy-2,2'-biindolyl (6-*Bn*). A solution of **21**-*Bn* (170 mg, 0.25 mmol) in dry DMF (2.6 mL) was treated with CuI (99 mg, 0.50 mmol) at 110 °C under an argon atmosphere. After 3 h the reaction mixture was filtered on

Celite and extracted with chloroform and a 10% solution water of NH_4Cl. The organic layers were dried over anhydrous sodium sulphate and evaporated under reduced pressure and the residue was fractionated on preparative TLC (eluant: benzene/acetone 9:1 (v/v) plus 1% acetic acid) to afford pure **6**-*Bn* (102 mg, 60%, R_f = 0.62 eluant: benzene/acetone 9:1 (v/v) plus 1% acetic acid).

6-*Bn***:** UV-Vis (DMSO) λ 357, 376 nm; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm) 5.15 (2H × 2, s, OCH_2Ph), 5.17 (2H × 2, s, OCH_2Ph), 6.71 (1H × 2, s, H-3, H-3'), 7.06 (1H × 2, s, H-7, H-7'), 7.20 (1H × 2, s, H-4, H-4'), 7.3-7.6 (10H × 2, m, H-2, H-3, H-4, H-5, H-6/OBn), 10.40 (1H × 2, br s, NH); ^{13}C NMR (50 MHz, $(CD_3)_2CO$) δ (ppm) 71.8 (2 × OCH_2Ph), 72.2 (2 × OCH_2Ph), 98.0 (C-3, C-3'), 98.5 (C-7, C-7'), 106.8 (C-4, C-4'), 123.3 (C-4a, C-4a'), 127-129 (10 CH × 2, C-2, C-3, C-4, C-5, C-6/OBn), 131.3 (C-2, C-2'), 132.6 (C-7a, C-7a'), 138.3 (4 × C-1/OBn), 145.4 (C-5, C-5'), 147.3 (C-6, C-6'); MS (ESI+) *m/z* 657 ($[M+H]^+$), 679 ($[M+Na]^+$); HRMS (ESI) *m/z* $C_{44}H_{37}N_2O_4$ $[M+H]^+$calcd 657.2753, found 657.2762.

Synthesis of 5,6-dibenzyloxyindole (1-*Bn*). A solution of **17**-*Bn* (100 mg, 0.3 mmol) in dry DMF (3 mL) was treated with CuI (119 mg, 0.6 mmol) at 110 °C under an argon atmosphere. After 3 h the reaction mixture was filtered on celite and extracted with chloroform and a 10% solution water of NH_4Cl. The organic layers were dried over anhydrous sodium sulfate, evaporated under reduced pressure and the residue fractionated on preparative TLC (eluant: petroleum ether/ethyl acetate 6:4 (v/v)) to afford pure **1**-*Bn* (75 mg, 75%, R_f = 0.35 eluant: petroleum ether/ethyl

acetate 6:4 (v/v)). The identity of the compound was confirmed by comparison of NMR data with those of the commercially available product.

Synthesis of 5,6-diacetoxy-7-(trimethylsilylethynyl)indole (26). A solution of (**19**) (700 mg, 1.9 mmol) in a mixture of triethylamine (16.3 mL), toluene (11.1 mL) and tetrahydrofuran (5.2 mL) was treated with PPh_3 (51.1 mg, 0.19 mmol), CuI (37 mg, 0.19 mmol), $(PPh_3)_2PdCl_2$ (68.4 mg, 0.1 mmol) and trimethylsilylacetylene (278 μL, 1.9 mmol) at 60 °C under an argon atmosphere. After 30 min the reaction mixture was extracted with a 10% water solution of NH_4Cl and chloroform. The organic layers were collected, dried over anhydrous sodium sulfate, evaporated under reduced pressure and the residue fractionated on silica gel (petroleum ether/ethyl acetate, gradient from 90:10 to 85:15) to afford pure **26** (450 mg, 70%, R_f = 0.75 eluant: chloroform/ethyl acetate 95:5 (v/v)).

26: ^{1}H NMR (400 MHz; $(CD_3)_2CO$), δ (ppm) 0.28 (9H, s, $Si(CH_3)_3$), 2.28 (3H, s, CH_3), 2.33 (3H, s, CH_3), 6.55 (1H, t, *J* = 2.6, 3-H), 7.40 (1H, t, *J* = 2.6, 2-H), 7.45 (1H, s, 4-H), 10.53 (1H, br s, NH); ^{13}C NMR (50 MHz, $(CD_3)_2CO$), δ (ppm) 0.3 ($Si(CH_3)_3$), 20.1 (CH_3), 20.3 (CH_3), 96.6 (C≡C-Si), 102.3 (C≡C-Si), 103.3 (C-3), 103.9 (C-7), 115.7 (C-4), 126.8 (C-4a), 127.6 (C-2), 134.8 (C-7a), 137.5 (C-5), 140.1 (C-6), 168.2 (C=O), 169.1 (C=O); MS (ESI+) *m/z* 330 ($[M+H]^+$), 352 ($[M+Na]^+$), 368 ($[M+K]^+$); HRMS (ESI+) *m/z* $C_{17}H_{20}NO_4Si$ $[M+H]^+$ calcd 330.1161, found 330.1164.

Synthesis of 5,6-diacetoxy-7-ethynylindole (27-*Ac*). A solution of (**26**) (500 mg, 1.5 mmol) in DMF (15 mL) was treated with KF (61.5 mg, 1.1 mmol) under

vigorous stirring. After 30 min the reaction mixture was extracted with water and chloroform. The organic layers were collected, dried over anhydrous sodium sulfate and evaporated under reduced pressure to afford pure **27**-*Ac* (383 mg, 98% R_f = 0.50 eluant: chloroform/ethyl acetate 95:5 (v/v)).

27-*Ac*: ^{1}H NMR (400 MHz; $(CD_3)_2CO$), δ (ppm) 2.27 (3H, s, CH_3), 2.32 (3H, s, CH_3), 4.13 (1H, s, C≡C-H), 6.56 (1H, t, *J* = 2.6, 3-H), 7.44 (1H, t, *J* = 2.6, 2-H), 7.47 (1H, s, 4-H), 10.63 (1H, br s, NH); ^{13}C NMR (50 MHz, $(CD_3)_2CO$), δ (ppm) 20.1 (CH_3), 20.3 (CH_3), 74.5 (C≡C-H), 87.6 (C≡C-H), 103.3 (C-3), 104.1 (C-7), 115.9 (C-4), 125.9 (C-4a), 127.7 (C-2), 134.8 (C-7a), 137.5 (C-5), 140.1 (C-6), 168.8 (C=O), 169.1 (C=O); MS (ESI+) *m/z* 258 ($[M+H]^+$), 280 ($[M+Na]^+$), 296 ($[M+K]^+$); HRMS (ESI+) *m/z* $C_{14}H_{12}NO_4$ $[M+H]^+$ calcd 258.0766, found 258.0762.

Synthesis of 3,4-diacetoxy-6-[2'-(5",6"-diacetoxy-indol-7"-yl)-1'-ethynyl]-2-iodoaniline (28). A solution of **27**-*Ac* (500 mg, 1.9 mmol) in a mixture of triethylamine (17 mL), toluene (7 mL) and tetrahydrofuran (10 mL) was treated with **13** (897 mg, 1.9 mmol), PPh_3 (51 mg, 0.19 mmol), CuI (37 mg, 0.19 mmol) and $(PPh_3)_2PdCl_2$ (68.3 mg, 0.1 mmol) at 60 °C under an argon atmosphere. After 20 min the reaction mixture was extracted with a 10% water solution of NH_4Cl and dichloromethane. The organic layers were collected, dried over anhydrous sodium sulfate, evaporated under reduced pressure and the residue fractionated on silica gel (petroleum ether/ethyl acetate, gradient from 70:30 to 65:35) to afford pure **28** (1 g, 90%, R_f = 0.35 eluant: chloroform/ethyl acetate 98:2 (v/v)).

28: ^{1}H NMR (400 MHz; $(CD_3)_2CO$), δ (ppm) 2.25 (3H, s, CH_3), 2.30 (3H, s, CH_3), 2.36 (3H, s, CH_3), 2.37 (3H, s, CH_3), 5.43 (2H, br s, NH_2), 6.59 (1H, t, *J* = 2.9, 3"-

H), 7.33 (1H, s, 5-H), 7.46 (1H, t, *J* = 2.9, 2”-H), 7.49 (1H, s, 4”-H), 10.92 (1H, br s, NH); ^{13}C NMR (100 MHz, $(CD_3)_2CO$), δ (ppm) 20.3 (4 × CH_3), 80.5 (C-2), 87.2 (C-2’), 93.4 (C-1’), 103.4 (C-3”), 103.6 (C-7”), 104.3 (C-6), 115.9 (C-4”), 125.8 (C-4a”), 127.1 (C-5), 127.6 (C-2”), 133.8 (C-1), 134.7 (C-7a”), 137.5 (C-5”), 140.0 (C-6”), 146.0 (C-4), 149.0 (C-3), 167.5 (C=O), 168.6 (C=O), 169.0 (C=O), 169.2 (C=O); MS (ESI+) *m/z* 613 ($[M+Na]^+$); HRMS (ESI+) *m/z* $C_{24}H_{19}IN_2NaO_8$ $[M+Na]^+$ calcd 613.0084, found 613.0087.

Synthesis of 5,5’,6,6’-tetraacetoxy-7-iodo-2,7’-biindole (29). A solution of **28** (900 mg, 1.5 mmol) in dry ethanol (18 mL) was treated with $AuCl_3$ (60 mg, 0.15 mmol) under an argon atmosphere and kept in an ultrasound bath at 320 W and 45 °C. After 1 h and 30 min the mixture was extracted with a 10% water solution of NH_4Cl and chloroform. The organic layers were collected, dried over anhydrous sodium sulfate and evaporated under reduced pressure to afford pure **29** (720 mg, 80%, R_f = 0.70 eluant: chloroform/ethyl acetate 7:3 (v/v)).

29: ^{1}H NMR (400 MHz; $(CD_3)_2CO$), δ (ppm) 2.20 (3H, s, CH_3), 2.31 (2 × 3H, s, CH_3), 2.40 (3H, s, CH_3), 6.59 (1H, t, *J* = 2.5, 3’-H), 6.87 (1H, d, *J* = 1.7, 3-H), 7.43 (1H, t, *J* = 2.5, 2’-H), 7.48 (1H, s, 4-H), 7.50 (1H, s, 4’-H), 10.37 (1H, br s, NH), 10.67 (1H, br s, NH’); ^{13}C NMR (100 MHz, $(CD_3)_2CO$), δ (ppm) 20.0 (CH_3), 20.3 (2 × CH_3), 20.4 (CH_3), 73.4 (C-7), 102.6 (C-3’), 104.8 (C-3), 111.1 (C-7’), 114.6 (C-4, C-4’), 126.0 (C-4a, C-4a'), 127.5 (C-2’), 127.8 (C-2), 133.0 (C-7a, C-7a’), 137.4 (C-O), 137.5 (C-O), 137.6 (C-O), 140.4 (C-O), 168.2 (C=O), 169.0 (2 × C=O), 169.1 (C=O); MS (ESI+) *m/z* 591 ($[M+H]^+$), 613 ($[M+Na]^+$); HRMS (ESI+) *m/z* $C_{24}H_{20}IN_2O_8$ $[M+H]^+$ calcd 591.0264, found 591.0261.

Synthesis of 4,5-diacetoxy-2-[2'-(5",5''',6",6'''-tetraacetoxy-2",7'''-biindol-7"-yl)-1'-ethynyl]-aniline (30). A solution of **29** (200 mg, 0.34 mmol) in triethylamine (3 mL) and tetrahydrofuran (3 mL) was treated with **17**-*Ac* (130.4 mg, 0.56 mmol), PPh_3 (8.9 mg, 0.034 mmol), CuI (6.4 mg, 0.034 mmol) and $(PPh_3)_2PdCl_2$ (12 mg, 0.017 mmol) at 60 °C under an argon atmosphere. After 1 h the reaction mixture was extracted with a 10% water solution of NH_4Cl and chloroform. The organic layers were collected, dried over anhydrous sodium sulfate, evaporated under reduced pressure and the residue fractionated on silica gel (eluant chloroform/ethyl acetate 1:1 (v/v)) to afford pure **30** (118 mg, 50%, R_f = 0.50 eluant: chloroform/ethyl acetate 6:4 (v/v)).

30: ^{1}H NMR (400 MHz; $(CD_3)_2CO$), δ (ppm) 2.17 (3H, s, CH_3), 2.21 (3H, s, CH_3), 2.23 (3H, s, CH_3), 2.30 (3H, s, CH_3), 2.33 (3H, s, CH_3), 2.40 (3H, s, CH_3), 5.33 (2H, br s, NH_2), 6.58 (1H, t, *J* = 2.3, 3'''-H), 6.66 (1H, s, 6-H), 6.73 (1H, d, *J* = 1.7, 3"-H), 7.19 (1H, s, 3-H), 7.43 (1H, t, *J* = 2.3 Hz, 2'''-H), 7.48 (1H, s, 4'''-H), 7.51 (1H, s, 4"-H), 10.80 (1H, br s, NH"'), 11.05 (1H, br s, NH''); ^{13}C NMR (100 MHz, $(CD_3)_2CO$), δ (ppm) 20.1 (CH_3), 20.2 (2 × CH_3), 20.3 (2 × CH_3), 20.6 (CH_3), 86.3 (C-2'), 94.4 (C-1'), 102.4 (C-7"), 102.7 (C-3'''), 104.3 (C-3"), 104.5 (C-2), 109.1 (C-6), 111.5 (C-7'"), 114.7 (C-4'''), 115.2 (C-4"), 126.2 (C-4a"), 126.4 (C-4a'''), 126.8 (C-3), 127.6 (C-2'''), 133.1 (C-7a'', C-7a'''), 133.4 (C-1), 135.4 (C-2"), 136.6 (C-5'''), 137.7 (C-5", C-6"'), 140.1 (C-6''), 144.9 (C-4), 148.7 (C-5), 168.2 (C=O), 168.7 (C=O), 168.9 (C=O), 169.1 (C=O), 169.3 (2 × C=O); MS (ESI+) *m/z* 696 ($[M+H]^+$), 718 ($[M+Na]^+$), 734 ($[M+K]^+$); HRMS (ESI+) *m/z* $C_{36}H_{30}N_3O_{12}$ $[M+H]^+$ calcd 696.1829, found 696.1831.

Synthesis of 5,5',5",6,6',6"-hexaacetoxy-2,7':2',7"-triindole (10-*Ac*). A solution of **30** (200 mg, 0.29 mmol) in dry ethanol (4 mL) was treated with $AuCl_3$ (66.7 mg, 0.17 mmol) under an argon atmosphere and kept in an ultrasound bath at 320 W and 45 °C. After 30 min the mixture was extracted with a 10% water solution of NH_4Cl and chloroform. The organic layers were collected, dried over anhydrous sodium sulfate and evaporated under reduced pressure to afford pure **10**-*Ac* (160 mg, 80%, R_f = 0.54 eluant: chloroform/ethyl acetate 1:1 (v/v)).

10-*Ac*:* ^{1}H NMR (400 MHz; $(CD_3)_2CO$), δ (ppm) 2.17 (3H, s, CH_3), 2.22 (3H, s, CH_3), 2.26 (3H × 3, s, CH_3), 2.33 (3H, s, CH_3), 6.56 (1H, t, *J* = 2.8, 3"-H), 6.76 (1H, s, 3'-H), 6.87 (1H, s, 3-H), 7.32 (1H, s, 7-H), 7.39 (1H, s, 4-H), 7.41 (1H, t, *J* = 2.8, 2"-H), 7.45 (1H, s, 4"-H), 7.51 (1H, s, 4'-H), 10.66 (1H × 2, br s, NH, NH"), 10.80 (1H, br s, NH'); ^{1}H NMR (400 MHz; $(CD_3)_2CO$ plus 1% of NH_4Cl (10% in H_2O)), δ (ppm) 2.15 (3H, s, CH_3), 2.21 (3H, s, CH_3), 2.27 (3H × 3, s, CH_3), 2.33 (3H, s, CH_3), 6.56 (1H, t, *J* = 2.8, 3"-H), 6.76 (1H, s, 3'-H), 6.85 (1H, s, 3-H), 7.32 (1H, s, 7-H), 7.38 (1H, s, 4-H), 7.40 (1H, t, *J* = 2.8, 2"-H), 7.43 (1H, s, 4"-H), 7.50 (1H, s, 4'-H), 10.64 (1H, br s, NH"), 10.71 (1H, br s, NH), 10.74 (1H, br s, NH'); ^{13}C NMR (100 MHz, $(CD_3)_2CO$), δ (ppm) 20.3 (6 × CH_3), 102.7 (C-3"), 103.7 (C-3), 104.2 (C-3'), 106.2 (C-7), 111.7 (C-7"), 112.1 (C-7'), 114.1 (C-4), 114.4 (C-4'), 114.6 (C-4"), 126.1 (C-4a''), 126.5 (C-4a), 127.0 (C-4a'), 127.3 (C-2"), 129.5 (C-7a"), 132.8 (C-2), 133.1 (C-7a'), 133.5 (C-2'), 134.7 (C-7a), 136.5[a] (C-5'), 136.6[a] (C-5"), 137.4[b] (C-6'),

*Structural assignment was secured by comparing 1D and 2D NMR spectra registered in $(CD_3)_2CO$ with or without the addition of 5 μL of a 10% solution of NH_4Cl in H_2O.

137.6[b] (C-6”), 138.1 (C-6’), 139.3 (C-5), 169.0 (2 × C=O), 169.2 (2 × C=O), 169.3 (2 × C=O); MS (ESI+) *m/z* 696 ($[M+H]^+$), 718 ($[M+Na]^+$); HRMS (ESI+) *m/z* $C_{36}H_{30}N_3O_{12}$ $[M+H]^+$ calcd 696.1829, found 696.1826.

Intramolecular cyclization of 30 to 10-*Ac*.[101] .A solution of **30** (150 mg, 0.22 mmol) in ethanol (1.8 mL) was treated with $NaAuCl_4 \times 2H_2O$ (3.3 mg, 8.4 μmol) under an argon atmosphere. After 5 h the mixture was extracted with a 10% water solution of NH_4Cl and ethyl acetate. The organic layers were collected, dried over anhydrous sodium sulfate and evaporated under reduced pressure to afford pure **10-*Ac*** (125 mg, 83%, R_f = 0.54 eluant: chloroform/ethyl acetate 1:1 (v/v)).

LC-MS analysis of the oxidation mixture of 1. The oxidative polymerization of **1** under biomimetic conditions has been carried out as previously reported.[84a] In brief, a solution of **1** in 0.05 M phosphate buffer, pH 7.4, was treated with horseradish peroxidase (36 U/mL) and hydrogen peroxide (1.1 molar equivalents). After 25 s reaction time, the oxidation mixture was halted by the addition of a solution of sodium dithionite in water and worked up. After acetylation with acetic anhydride and pyridine overnight at r.t., the mixture was subjected to LC-MS analysis.

Synthesis of 5,5’,6,6’-tetraacetoxy-7-(trimethylsilylethynyl)-2,7’-biindole (32). A solution of **29** (352 mg, 0.6 mmol) in triethylamine (5 mL), tetrahydrofuran (3.8 mL) and toluene (1,2 mL) was treated with PPh_3 (16 mg, 0.06 mmol), CuI (11.3 mg, 0.06 mmol) and $(PPh_3)_2PdCl_2$ (25 mg, 0.03 mmol) and trimethylsilylacetylene (168 μL, 1.1 mmol) at 60 °C under an argon atmosphere. After 20 min the reaction mixture

was extracted with a 10% water solution of NH_4Cl and chloroform. The organic layers were collected, dried over anhydrous sodium sulfate, evaporated under reduced pressure and the residue fractionated on silica gel (eluant petroleum ether/ethyl acetate 7:3 (v/v)) to afford pure **32** (267 mg, 80%, R_f = 0.73 eluant: chloroform/ethyl acetate 9:1 (v/v)).

32: ^{1}H NMR (400 MHz; $(CD_3)_2CO$) 0.25 (9H, s, $Si(CH_3)_3$), 2.17 (3H, s, CH_3), 2.30 (2 × 3H, s, CH_3), 2.36 (3H, s, CH_3), 20.2 (2 x 3H, s, CH_3), 6.59 (1H, t *J* = 2.6, H-3'), 6.72 (1H, d *J* = 1.4, H-3), 7.44 (1H, t *J* = *2*.6, H-2'), 7.49 (2 × 1H, s, H-4, H-4'), 10.66 (1H, br s, NH), 10.71 (1H, br s, NH'); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) 0.7 ($Si(CH_3)_3$), 20.2 (2 x CH_3), 20.3 (2 x CH_3), 96.3 (-C≡C-Si), 102.3 (-C≡C-Si), 102.8 (C-3'), 104.4 (C-7), 104.5 (C-3), 111.5 (C-7'), 114.9[a] (C-4), 115.6[a] (C-4'), 126.3 (C-9, C-9'), 127.7 (C-2, C-2'), 133.1 (C-8, C-8'), 135.8 (C-O), 136.7 (C-O), 137.6 (C-O), 137.8 (C-O), 168.4 (C=O), 169.2 (C=O), 169.3 (C=O); 169.4 (C=O); MS (ESI+) *m/z* 561 ($[M+H]^+$), 583 ($[M+Na]^+$), 599 ($[M+K]^+$).

Synthesis of 5,5',6,6'-tetraacetoxy-7-ethynyl-2,7'-biindole (33). A solution of (**32**) (115.5 mg, 0.2 mmol) in DMF (2.1 mL) was treated with KF (8.5 mg, 0.1 mmol) under vigorous stirring. After 30 min the reaction mixture was extracted with water and chloroform. The organic layers were collected, dried over anhydrous sodium sulfate and evaporated under reduced pressure to afford pure **33** (98 mg, 98% R_f = 0.50 eluant: chloroform/ethyl acetate 95:5 (v/v)).

33: ^{1}H NMR (400 MHz; $(CD_3)_2CO$) 2.16 (3H, s, CH_3), 2.31 (2 × 3H, s, CH_3), 2.36 (3H, s, CH_3), 4.14 (-C≡CH), 6.59 (1H, t *J*=2.5, H-3'), 6.72 (1H, d *J*=1.7, H-3), 7.44 (1H, t *J*=2.5, H-2'), 7.50 (2 x 1H, s, H-4, H-4'), 10.73 (1H, bs, NH), 10.79 (1H, bs,

NH'); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) 19.8 (2 x CH_3), 19.9 (2 x CH_3), 74.8 (-C≡C-H), 87.5 (-C≡C-H), 101.0 (C-7), 102.3 (C-3'), 104.0 (C-3), 111.0 (C-7'), 114.4[a] (C-4), 115.3[a] (C-4'), 125.9 (C-9, C-9'), 127.2 (C-2, C-2'), 132.8 (C-8, C-8'), 135.8 (C-O), 136.2 (C-O), 137.2 (C-O), 137.3 (C-O), 168.1 (C=O), 168.6 (C=O), 168.8 (2 x C=O); MS (ESI+) *m/z* 489 ($[M+H]^+$), 511 ($[M+Na]^+$), 527 ($[M+K]^+$).

Synthesis of 4,5-diacetoxy-2-[2'-(5",5''',6",6'''-tetraacetoxy-2",7'''-biindol-7"-yl)-1'-ethynyl]-7-iodoaniline (34). A solution of **33** (75 mg, 0.15 mmol) in triethylamine (1.4 mL), toluene (400 μL) and tetrahydrofuran (1 mL) was treated with **13** (106 mg, 0.23 mmol), PPh_3 (4.2 mg, 0.016 mmol), CuI (3 mg, 0.016 mmol) and $(PPh_3)_2PdCl_2$ (6 mg, 0.008 mmol) at 60 °C under an argon atmosphere. After 20 min the reaction mixture was extracted with a 10% water solution of NH_4Cl and chloroform. The organic layers were collected, dried over anhydrous sodium sulfate, evaporated under reduced pressure and the residue fractionated on silica gel (eluant chloroform/ethyl acetate 8:2 (v/v)) to afford pure **34** (88 mg, 70%, R_f = 0.57 eluant: chloroform/ethyl acetate 8:2 (v/v)).

34: ^{1}H NMR (400 MHz; $(CD_3)_2CO$) 2.16 (3H, s, CH_3), 2.22 (3H, s, CH_3), 2.23 (3H, s, CH_3), 2.33 (3H, s, CH_3), 2.35 (3H, s, CH_3), 2.41 (3H, s, CH_3), 5.45 (2H, br s, NH_2), 6.58 (1H, t *J*=2.8, H-3'''), 6.74 (1H, d *J*=1.2, H-3"), 7.32 (1H, s, H-3), 7.43 (1H, t *J*=2.8 Hz, H-2'''), 7.47 (1H, s, H-4'''), 7.53 (1H, s, H-4"), 10.76 (1H, br s, NH"), 11.07 (1H, br s, NH'''); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) 20.2 (CH_3), 20.3 (2 × CH_3), 20.4 (2 × CH_3), 20.5 (CH_3), 79.9 (C-6), 87.1 (C-2'), 94.4 (C-1'), 102.1 (C-7"), 102.8 (C-3'''), 104.4 (C-2), 104.5 (C-3''), 111.5 (C-7'"), 114.9 (C-4'''), 115.8 (C-4"), 126.3 (C-9"), 126.6 (C-9'''), 127.2 (C-3), 127.8 (C-2'''), 133.2[a] (C-8''),

133.4[a] (C-8’’’), 133.8 (C-1), 135.6 (C-2”), 136.7 (C-O), 137.7 (C-O), 137.8 (C-O), 140.4 (C-O), 146.0 (C-4), 148.9 (C-5), 167.5 (C=O), 168.8 (C=O), 168.9 (C=O), 169.1 (C=O), 169.3 (2 × C=O); MS (ESI+) *m/z* 822 ($[M+H]^+$), 845 ($[M+Na]^+$), 734 ($[M+K]^+$).

Synthesis of 5,5’,5”,6,6’,6”-hexaacetoxy-7-iodo-2,7’:2’,7”-triindole (35). A solution of **34** (200 mg, 0.24 mmol) in dry ethanol (15 mL) was treated with $AuCl_3$ (40 mg, 0.1 mmol) under an argon atmosphere and kept in an ultrasound bath at 320 W and 45 °C. After 1 h and 30 min the mixture was extracted with a 10% water solution of NH_4Cl and chloroform. The organic layers were collected, dried over anhydrous sodium sulfate and evaporated under reduced pressure to afford pure **35** (120 mg, 60%, R_f = 0.62 eluant: chloroform/ethyl acetate 8:2 (v/v)).

35: ^{1}H NMR (400 MHz; $(CD_3)_2CO$) 2.19 (3H, s, CH_3), 2.21 (3H, s, CH_3), 2.26 (3H, s, CH_3), 2.27 (3H, s, CH_3), 2.32 (3H, s, CH_3), 2.38 (3H, s, CH_3), 6.56 (1H, t *J*=2.8, H-3”), 6.74 (1H, d *J* = 1.2, H-3’), 6.89 (1H, d *J* = 1.2, H-3), 7.43 (1H, s, H-4”), 7.44 (1H, s, H-4), 7.52 (1H, t *J*=2.8, H-2”), 7.53 (1H, s, H-4’), 10.61 (1H, br s, NH), 10.86 (1H, s, NH”), 11.13 (1H, br s, NH’); MS (ESI+) *m/z* 822 ($[M+H]^+$), 845 ($[M+Na]^+$).

Synthesis of 4,5-diacetoxy-2-[2’-(5”,5’’’,5’’’’,6”,6’’’,6’’’’-hexaacetoxy-2”,7’’’:2’’’,7’’’’-triindol-7”-yl)-1’-ethynyl]-aniline (36). A solution of **35** (150 mg, 0.2 mmol) in triethylamine (1.6 mL) and tetrahydrofuran (1.6 mL) was treated with **17**-*Ac* (64 mg, 0.3mmol), PPh_3 (5 mg, 0.02 mmol), CuI (3.5 mg, 0.02 mmol) and $(PPh_3)_2PdCl_2$ (6 mg, 0.01 mmol) at 60 °C under an argon atmosphere. After 20 min

the reaction mixture was extracted with a 10% water solution of NH_4Cl and chloroform. The organic layers were collected, dried over anhydrous sodium sulfate, evaporated under reduced pressure and the residue fractionated on silica gel (eluant chloroform/ethyl acetate 8:2 (v/v)) to afford pure **36** (93 mg, 50%, R_f = 0.29 eluant: chloroform/ethyl acetate 8:2 (v/v)).

36: ^{1}H NMR (400 MHz; $(CD_3)_2CO$) 2.14 (3H, s, CH_3), 2.17 (3H, s, CH_3), 2.20 (3H, s, CH_3), 2.22 (3H, s, CH_3), 2.25 (2 × 3H, s, CH_3), 2.28 (2 × 3H, s, CH_3), 5.15 (2H, br s, NH_2), 6.45-6.74 (3 × 1H, H-3", H-3‘‘‘, H-3‘‘‘‘), 6.68 (1H, s, H-6), 7.39[a] (1H, s, H-4"), 7.46[a] (1H, s, H-4‘‘‘), 7.50[a] (1H, s, H-4‘‘‘‘), 10.58[b] (1H, br s, NH), 11.03[b] (1H, br s, NH), 11.11[b] (1H, br s, NH); MS (ESI+) *m/z* 927 ($[M+H]^+$), 949 ($[M+Na]^+$), 965 ($[M+K]^+$).

Synthesis of 5,5’,5",5‘‘‘,6,6’,6",6‘‘‘-octaacetoxy-2,7’:2’,7":2",7‘‘‘-tetraindole (31-*Ac*). A solution of **36** (50 mg, 0.05 mmol) in dry ethanol (5 mL) was treated with $AuCl_3$ (15 mg, 0.04 mmol) under an argon atmosphere and kept in an ultrasound bath at 320 W and 45 °C. After 30 min the mixture was extracted with a 10% water solution of NH_4Cl and chloroform. The organic layers were collected, dried over anhydrous sodium sulfate and evaporated under reduced pressure to afford pure **31-*Ac*** (R_f = 0.27 eluant: chloroform/ethyl acetate 8:2 (v/v)).

CHAPTER 2

SYNTHESIS OF ALKYNYLDERIVATIVES OF 5,6-DIHYDROXYINDOLE

Introduction

Parallel to biomimetic polymerization studies,[24,82,83] increasing research work has been directed in recent years to exploring the potential of **1** as a versatile lead for the design and development of molecular scaffolds with tailored chemical and physical properties for potential applications.[26a,61] The expanding range of chemical opportunities that emerge from these studies is illustrated by the acid induced one-pot conversion to triazatruxene[85c] and benzylindolylquinoline derivatives[86] and the remarkable modular assembly of the first 5-membered indole macrocycle from 5,6-dihydroxy-1-methylindole.[106] Of particular relevance is also the behavior of the 3-iodo-derivative of DHI,[64] leading to a relatively stable quinone, the synthesis of the 3-thio-derivative of DHI[107] and the recent exploitation of the 3-thioglycosidation reaction of the 5,6-dihydroxyindole skeleton as an access route to the first water soluble DHI-based eumelanin-type polymers.[108]

A most attractive and so far unexplored strategy for the rational manipulation of 5,6-dihydroxyindoles was inspired by the current renaissance of the chemistry of aromatic alkynes which followed the development of the Sonogashira reaction.[91,109] The availability of efficient protocols for extending π-π conjugation has enabled the creation of linear molecular structures lacking *Z/E* isomerism and functioning as nanoscale "molecular wires", π-electron-based conducting organic structures, as well as self-assembling supramolecular systems.[9,110] The manifold theoretical issues and synthetic prospects offered by conjugating an ethynyl group to 5,6-dihydroxyindoles, have induced for the first time an integrated chemical investigation of the structural

properties and reaction behavior of 3- and 7-alkynyl-derivatives of **1** (**27**, **37**-**40**). Specific aims include:

1) to develop the synthetic chemistry of DHI derivatives possessing alkynyl substituents with special attention to the compatibility of the protocols with the notorious oxidizability of the *o*-diphenolic system;
2) to elucidate the acid-mediated reactivity, in view of the central importance in indole chemistry and related synthetic transformations.
3) to probe the impact of extended π-electron conjugation and nitrogen lone pair delocalization across the triple bond on the redox behaviour and protonation equilibria;
4) to elucidate the influence of the triple bond on the interunit electronic communication in conformationally mobile **1**-π-**1** system that can delocalize π electron density among the indole units in a flexible conformational mechanism or can establish π stacking interactions (Figure 16**)**, in the prospects of engineering novel eumelanin-alkyne hybrid polymers with tailored physical and electronic properties.

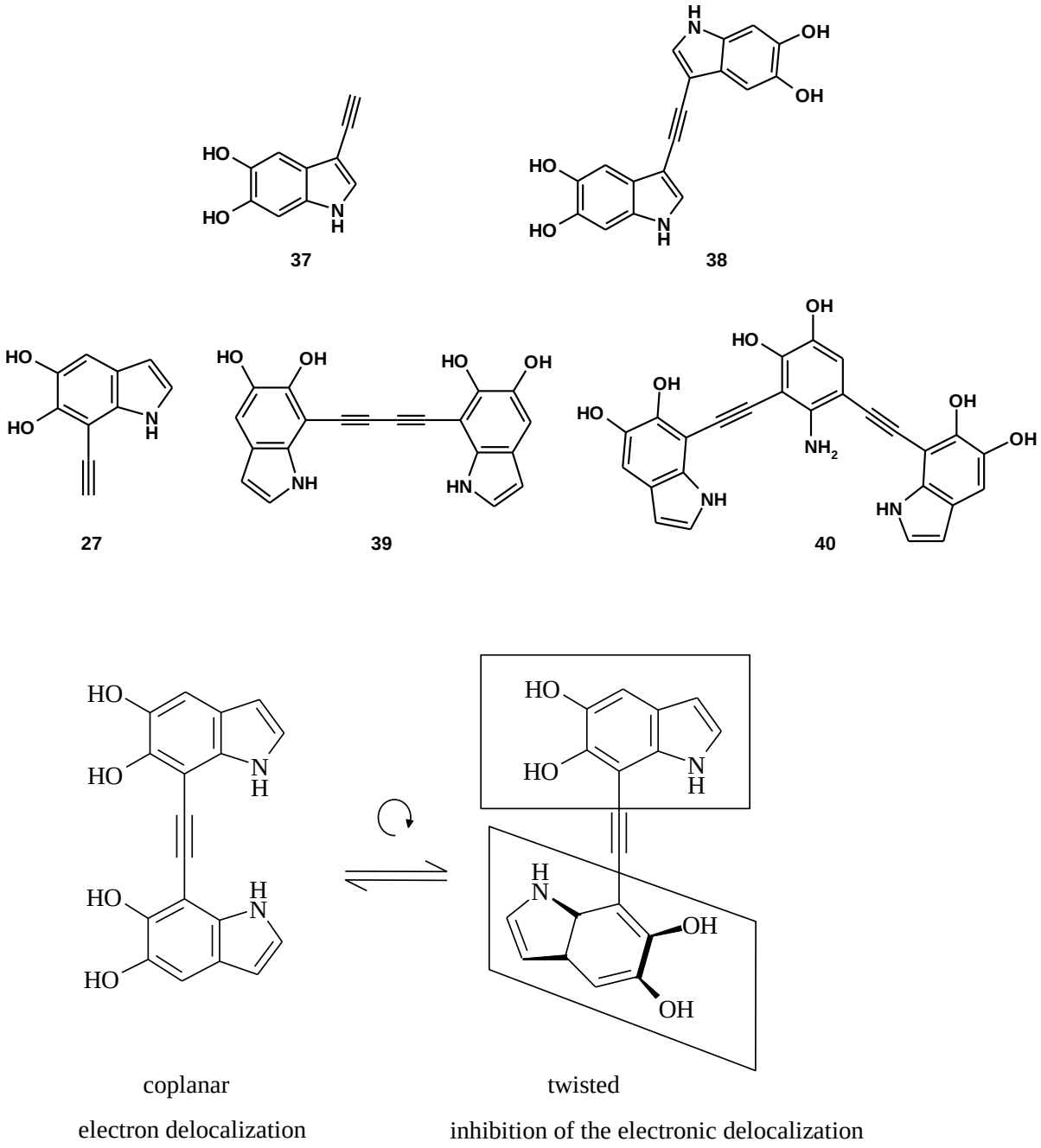
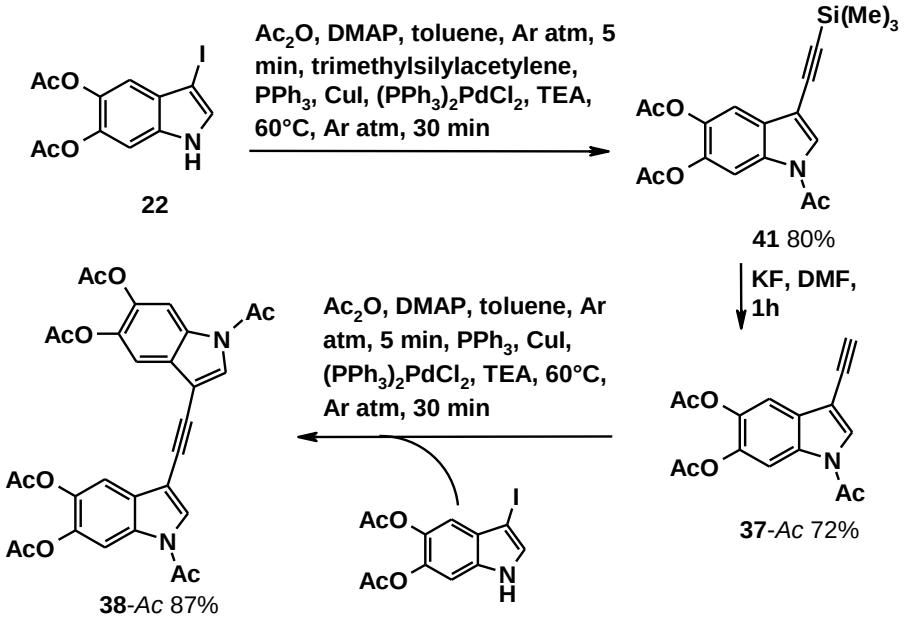


Figure 16. Delocalization of π electron density among the indole units.

Synthesis of 3-alkynyl-derivatives of 5,6-dihydroxyindole

The synthetic approaches toward the preparation of the 3-alkynyl-derivatives of **1**, **37** and **38**, require protection of the phenolic groups as acetyl derivatives, as previously discussed.[98] For both compounds, 5,6-diacetoxy-3-iodoindole (**22**)[64] was selected as the starting material (Scheme 9). Subsequent introduction of the ethynyl group by Sonogashira coupling[91,109] proved not to be straightforward, the main difficulty being related to the high electron density on the 3-position. This difficulty was circumvented by preliminary *N*-acetylation of **22** with acetic anhydride/*N*,*N*-dimethylaminopyridine (DMAP), followed by the in situ Sonogashira coupling with trimethylsilylacetylene. Desilylation of **41** then led to the desired **37**-*Ac*, the first alkynyl-derivative, in moderate overall yields.



Scheme 9. Synthesis of 3-ethynyl-5,6-dihydroxyindoles **37**-*Ac* and **38**-*Ac*.

Compound **37**-*Ac* then was subjected to further Sonogashira coupling with the iodoindole **22**; previously *N*-acetylated with Ac_2O/DMAP to afford the second alkynyl-derivative **38**-*Ac*.

Reactivity of 3-alkynyl-derivatives of 5,6-dihydroxyindole

In a first group of experiments I examined the reactivity of the alkynyl-derivatives of **1**, **37**-*Ac* and **38**-*Ac*, under different conditions. In particular, I studied the behaviour of these compounds under oxidative conditions of biological relevance and under acidic conditions.

A preliminary insight into the oxidation behavior of **37** and **38** in comparison with the parent DHI was obtained using two oxidizing systems typically used in melanin chemistry, namely Ni^{2+}/O_2 in 0.1 M HEPES buffer, pH 7.4, and horseradish peroxidase (HRP)/H_2O_2 in 0.1 M phosphate buffer, pH 7.4. Since the oxidative behaviour of these compounds is dictated by the reactivity of the catechol functionalty, compounds **37**-*Ac* and **38**-*Ac* were first deacetylated according to a previously established procedure involving careful treatment of the compounds with sodium *tert*-butoxide in methanol under an argon atmosphere.[98] After 10 minutes, the reaction mixtures were added to 0.1 M HEPES buffer, pH 7.4, and treated with Ni^{2+} under a vigorous flux of O_2. In the case of **37**, the oxidation proceeded with the rapid generation of a dark precipitate resembling DHI eumelanin. TLC analysis of the reaction mixture carried out at different intervals of time after dithionite reduction, ethyl acetate extraction and acetylation with Ac_2O/pyr, revealed the complete consumption of the starting material and the formation of a very complex mixture of products, all in trace amounts, eluding all attempts at isolation and characterization. A different course of the reaction was observed in the case of **38**; in fact, the

oxidation proceeded only slowly to give a brownish material with much unreacted starting material, as revealed by TLC analysis.

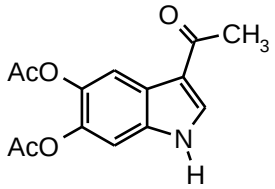
When HRP/H_2O_2 was used as the oxidizing system no significant improvement of the reaction outcome was observed. The failure to isolate oligomer intermediates under conditions in which DHI furnishes isolable dimers and trimers may be due to a more complex mode of polymerization of **37** and/or to the unfavorable physicochemical properties of the oligomer intermediates hindering their isolation. A detailed structural characterization of the dark eumelanin-like polymer from **37** was not pursued further at this stage but is worthy of further attention in relation to the physicochemical properties of the new alkynyl-functionalized eumelanin-like polymers.

In another series of experiments the stability and mode of decomposition of **37** and **38** under mildly acidic conditions was investigated. Considering that acid treatments induce cyclotrimerization of DHI initiated by protonation at C3,[85] it seemed of interest to determine whether this step is still important under such conditions or alternate pathways involving e.g. alkynyl group modification become prevailing.

Initially, **37** and **38** were allowed to react as the acetyl derivatives in 0.1 M phosphate buffer, pH 3. Under such conditions, no significant reactivity was observed.

Accordingly, in subsequent experiments both compounds were deacetylated with sodium *tert*-butoxide and then exposed to the acidic buffer. At different intervals of time aliquots of the solution were withdrawn, extracted with ethyl acetate and acetylated for TLC analysis. After 10 min, compound **37** was completely consumed and replaced by a main product which was isolated and characterized by spectral analysis. The ESI(+) mass spectrum indicated a pseudomolecular ion peak ($[M+H]^+$)

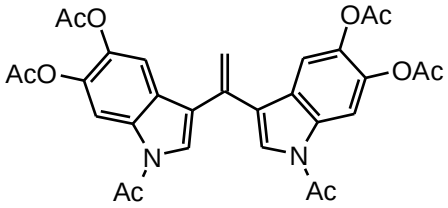
at *m/z* 276 whereas the ^{1}H NMR spectrum displayed a singlet in the aliphatic proton region at δ 2.39 (3H) correlating in the ^{1}H,^{13}C HMBC spectrum with a carbon signal at δ 195.1 indicating an acetyl group. On this basis, the compound was identified as 5,6-diacetoxy-3-acetylindole (**42**-*Ac*). This is one of the few examples of alkyne-to-ketone conversion reactions under mild conditions and in the absence of added catalysts.



42-*Ac*

In marked contrast to **37**, compound **38** did not exhibit significant reactivity in acidic buffer, remaining virtually unmodified after sufficiently prolonged periods of time. On this basis we returned to compound **37** to assess whether its enhanced reactivity could be exploited for synthetic purposes. It seemed of interest, in particular, to investigate the susceptibility of the triple bond to nucleophilic attack by DHI under acidic conditions, since the availability of cross-conjugated, ethylidene-spaced bisindoles was most desirable for preparing novel molecular scaffolds and to probe π-conjugation effects under polymerization conditions.

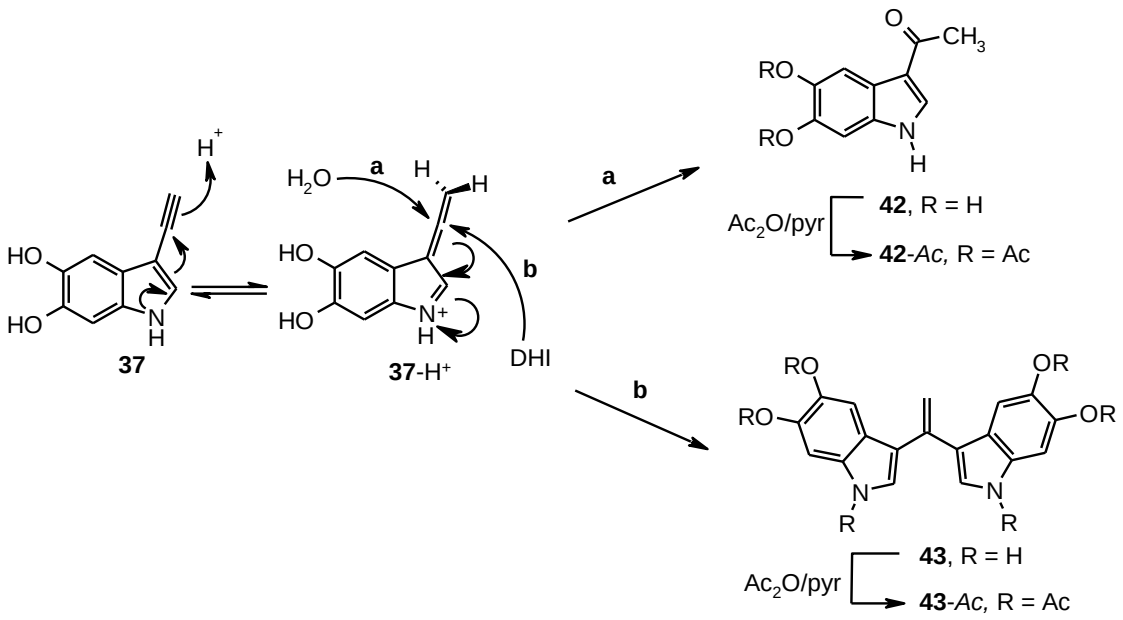
Accordingly, compound **37** was reacted in 0.1 M phosphate buffer, pH 3, in the presence of 1.5 molar equivalents of DHI. After few minutes, the starting material was consumed and a main product was detected by TLC. This was isolated after acetylation and identified as 3,3'-ethenylidenebis-5,6-diacetoxyindole (**43**-*Ac*).



43-*Ac*

The structural assignment was supported by complete spectral characterization, including: (a) the ESI(+) mass spectrum, showing a pseudomolecular ion peak ($[M+Na]^+$) at *m/z* 597; (b) the ^{1}H NMR spectrum, showing a 2H singlet at δ 5.74 and the resonances for three protons framed into a 3-substituted indole ring; (c) the presence of ten sp^2 carbon resonances (excluding the acetyl groups), consistent with the high molecular symmetry; (d) a distinct one-bond correlation in the ^{1}H,^{13}C HSQC spectrum of the singlet at δ 5.74 with a carbon resonance at δ 115.8, compatible with an olefinic methylene group.

The isolation of **43** disclosed a straightforward access route to a novel building block of potential synthetic and practical interest.



Scheme 10. Proposed mechanisms for the acid-induced conversion of **37** to products **42** and **43**.

Based on these results, it was concluded that the indole nitrogen exerts a potent activating effect on the triple bond greatly enhancing its reactivity with acids and directing protonation toward the β position. Symmetric operation of these effects on both sides of the triple bond, on the other hands, suppresses acid-induced reactivity in **38**. This behavior can be rationalized in terms of the inability of the protonated intermediate from **38** to give products due to the electron-donating effects of the second indole ring decreasing electrophilic reactivity toward nucleophiles.

Formation of the 3-acetyl derivative **42** conceivably proceeds via the classic alkyne hydration pathway. This would be triggered by initial protonation of triple bond followed by nucleophilic addition of water restoring the aromaticity of the pyrrole moiety (Scheme 10). Likewise, product **43** would result from nucleophilic attack of DHI to the protonated intermediate **37**-H^+, leading to the 3,3'-ethenylidenebis-5,6-dihydroxyindole derivative. Operation of an alternate mechanism involving

nucleophilic attack of DHI to **42** was ruled out by the observation that **42** was inert to acids in the presence of DHI and that no trace of **42** was present in the reaction mixture of **37** and DHI.

Based on these results it is possible to conclude that the effects of π-conjugating groups on the 5,6-dihydroxyindole system clearly depend on the site of substitution and that, in the case of compounds **37** and **38**, the interaction of the triple bond at C3 with the indole ring is restricted to the enamine portion of the pyrrole sector. Here, the role of the triple bond is to accept and delocalize the electrons donated by the NH lone pair. These conclusions were supported by a systematic Density Functional Theory (DFT) investigation of tautomerism, redox and protonation equilibria in **37** and **38** carried out by Professor Orlando Crescenzi (Department of Chemistry, University of Naples Federico II). The results, which will not be reported in detail, can be summarized as follows:

- Comparison of the free energies for the most stable species of **37** and DHI indicated a decreased tendency to oxidation of **37** with respect to the parent indole.
- Comparison of the free energies for the most stable species of **38** and DHI revealed a modest stabilizing effect of the triple bond toward oxidation for the 5,6-dihydroxyindole system.
- Rotation of the aromatic substituents around the connecting alkyne in **38** is characterized by very small barriers.

These data would point to the 2-electron oxidation product of **38** as a prototype of anomalous D-π-A systems of potential interest in organo-electronics and related applications.

Analysis of the protonation equilibria of **37** relative to DHI showed that in the case of reduced **37** the *N*-protonated allene is the most stable form and that protonation of DHI is favored at the 3-position, consistent with previous experimental observations (Figure 17).

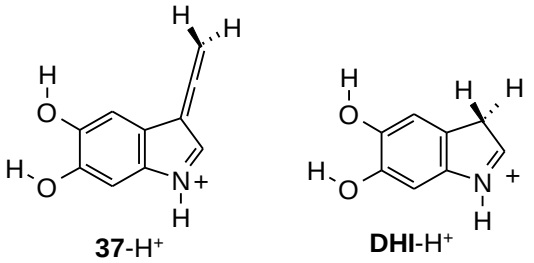
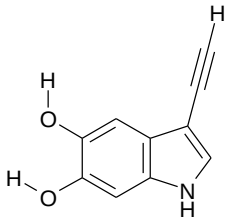
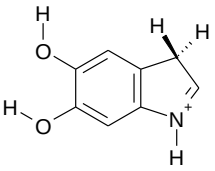
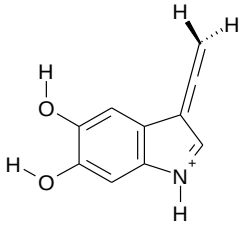
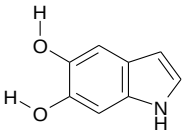


Figure 17. Structures of the most stable form of **37** and DHI, computed in water (PCM).

Comparison of the protonation free energies for the most stable species of **37** and DHI indicated a thermodynamically favored protonation equilibrium for **37**:

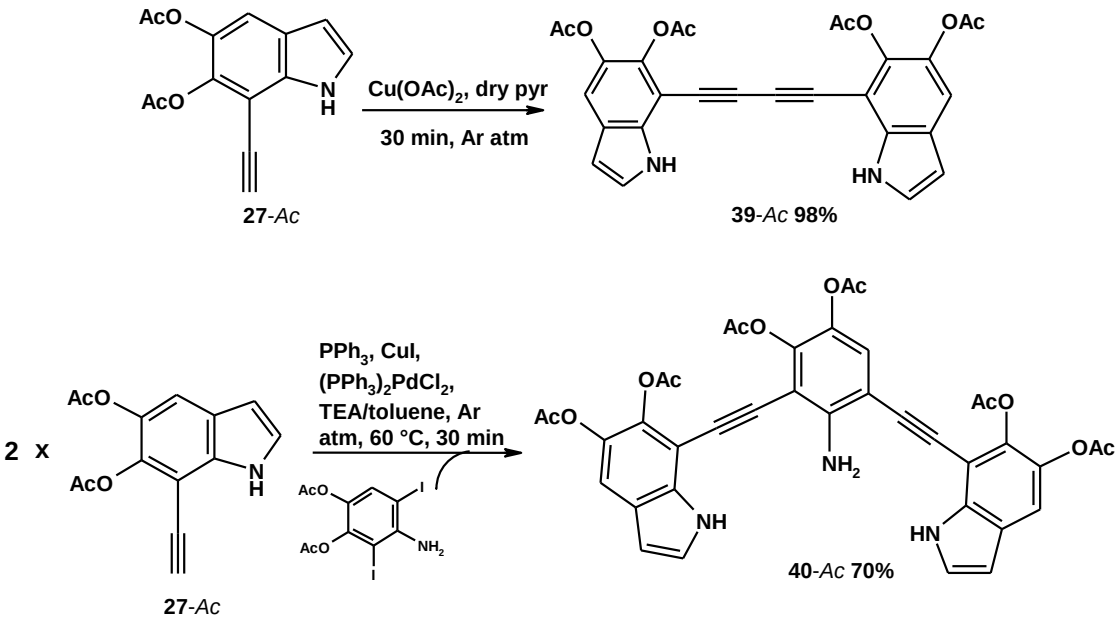
A	**B**	**C**	**D**	**A+B → C+D: ΔG (kcal/mol)**
				-5.7 (PCM)

This indicates that **37** is more basic than DHI.

These results were in good agreement with the experimentally observed reactivity of **37** in acids.

Synthesis of 7-alkynyl-derivatives of 5,6-dihydroxyindole

The synthetic access to the first 7-alkynyl-derivative of **1**, **27**, as the acetyl derivative, has been previously discussed in Chapter 1 (Scheme 6). Starting from this compound it has been possible to prepare the other two alkynyl-derivatives of **1**, that are **39** and **40**, as the acetyl derivatives. The former has been obtained via the oxidative coupling mediated by $Cu(OAc)_2$ (Scheme 11);[98] otherwise, **40**-*Ac* was prepared via the Sonogashira coupling with the diiodoaniline **13**, using a molar equivalent ratio between the alkyne and the diiodoaniline of 2:1



Scheme 11. Synthetic scheme for **39**-*Ac* and **40**-*Ac*.

At present, the reactivity and properties of compounds **27, 39** and **40** are currently under investigation. However, I report herein the preliminary data of a study on the spectrophotometric properties of **40**-*Ac*. Compound **40**-*Ac* exhibited in the UV-vis spectrum three maxima at 295, 318 and 371 nm; moreover, when excited at 266 nm,

it showed an intense blue fluorescence with emission centered at 420 nm. This data, coupled with the structural features of **40**-*Ac*, suggested possible applications as anion or cation sensor.

Preliminary experiments were carried out by treating compound **40**-*Ac* with 10 molar equivalents of different anions, such as CN^-, F^-, NO_2^-, Br^-, HSO_4^- and I^-. As shown in Figure 18, only F^- and CN^- induced a marked reduction of the maximum at 420 nm, quenching the blu fluorescence.

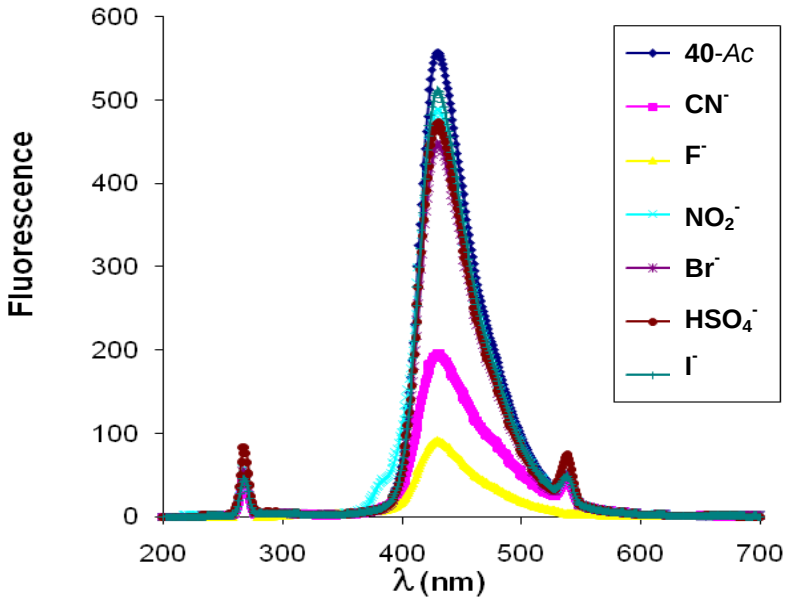


Figure 18. Fluorescence spectra of **40**-*Ac* in acetonitrile in the presence of different anions (10 eq).

To establish the concentration level for the maximum quenching effect, **40**-*Ac* was treated with varying concentrations of CN^-. The results shown in Figure 19 indicated a significant decrease in fluorescence only with 10 equivalents of the salt added to the solution (Figure 19).

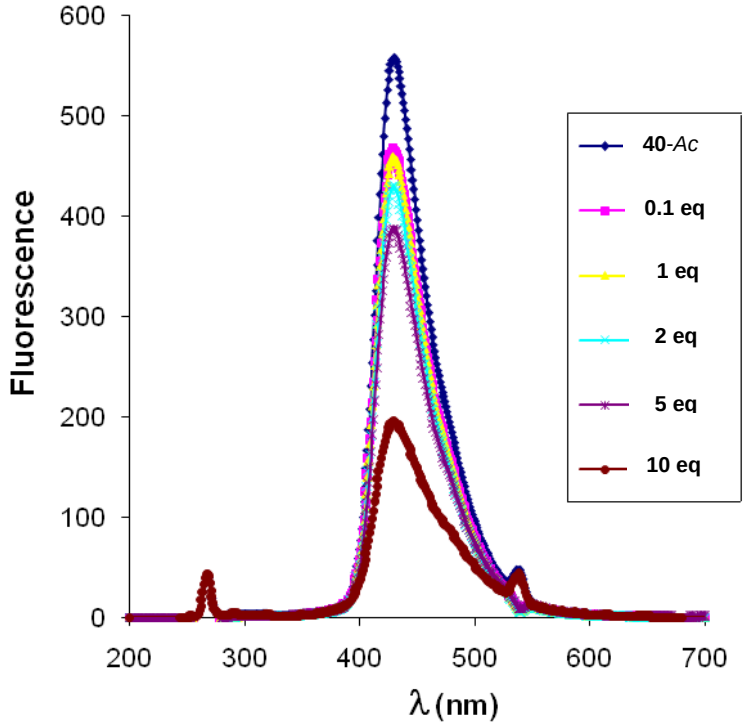


Figure 19. Fluorescence spectra of **40**-*Ac* in acetonitrile in the presence of increasing concentrations of cyanide ions.

Carrying out the same experiments by adding different cations to the solution of **40**-*Ac*, no significant change in the emission spectra were observed (Figure 20).

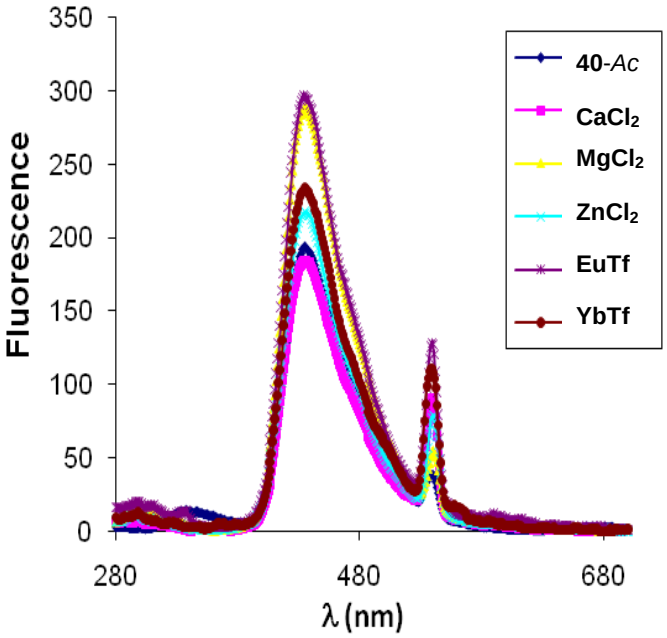


Figure 20. Fluorescence spectra of **40**-*Ac* in H_2O/acetonitrile 1:1 in the presence of different cations.

These data offer new interesting insights for the construction of on/off molecular structures that can monitor the presence of a specific anion through quenching of fluorescence.

Conclusions

This Chapter was devoted to the synthesis of compound **27**, **37**-**40**, interesting molecular systems for their structural and chemical profile and for the possible applications in the field of material sciences, e.g. for the design and synthesis of bioinspired functional materials and scaffolds through rational manipulation of the fundamental π-electron system and substitution patterns.

The synthetic strategy, also in this case, can be considered an extension of the procedures shown in Chapter 1 based on sequences of Sonogashira couplings and intramolecular cyclization followed, when appropriate, by an oxidative dimerization step.

In addition to the synthetic results, preliminary experiments carried out on **40**-*Ac* have shown the potentiality of this compound to chelate cations or anions with possible applications in the field of sensing agents. Of particular interest were also the results of the studies on the chemistry of 3-alkynyl-5,6-dihydroxyindoles, disclosing the influence of the 3-alkynyl group on the protonation equilibria and acid-mediated reactivity leading to unprecedented tranformations in ethynylindole chemistry;

In addition, the interunit electron delocalization in the 2-electron oxidation product of **38** would point to a novel "anomalous" prototype of D-π-A-like systems of potential interest for organoelectronics and related applications.

The scope of alkynylation as a means of rationally perturbing the π-electron system of 5,6-dihydroxyindoles to meet specific structural and synthetic requirements for

application purposes is currently under assessment but it is possible to hypothesize that positioning the substituent on the appropriate site, it could be possible to modulate the reactivity of the aromatic system for synthetic applications.

Further details about results shown in this chapter will be found in the following submitted article:

Capelli, L.; Crescenzi, O.; Manini, P.; Pezzella, A.; Barone, V.; d'Ischia, M. (**2010**), "Designing π-Electron-Extended Eumelanin Building Blocks: Synthesis, Quantum Chemical Investigation and Peculiar Acid-Mediated Chemistry of 3-Alkynyl-5,6-dihydroxyindoles."

Experimental Section

Materials and methods. All commercially available reagents were used as received. Anhydrous solvents were purchased from commercial sources and withdrawn from the container by a syringe under a slight positive pressure of argon. All the solvents were of analytical grade quality. Compound **22** was prepared according to a reported procedure.[64] For the synthesis of compound **27**-*Ac* see Experimental Section Chapter 1.

0.1 M HEPES Buffer, pH 7.4 and 0.1 M phosphate buffers, pH 7.4 and 3, were treated with Chelex-100 resin before use to remove transition metal contaminants. Fluorescence spectra were recorded on a Jasco FP-750 instrument, setting the excitation wavelenght at 266 nm.

NMR spectra were recorded with a 200 and 400 MHz instruments. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ using TMS as the internal standard. $^{1}H,^{13}C$ HSQC and $^{1}H,^{13}C$ HMBC experiments were run at 400.1 MHz using standard pulse programs.

Mass spectra were registered in the electrospray ionization-positive ion (ESI+) mode. ESI analysis were performed with the cone and the fragmentator voltages set at 4 kV and 80 V, respectively; nitrogen was used as carrier gas at a flow of 8 mL/min and the nebulizer pressure was set at 50 psi. High resolution mass spectra were registered in the electrospray ionization-positive ion (ESI+) mode.

Analytical and preparative TLC analyses were performed on F_{254} silica gel plates (0.25 and 0.5 mm, respectively). Liquid chromatography was performed on silica gel (60-230 mesh).

Spectrofluorimetric analysis

A solution of **40**-*Ac* (3.3 μM) in acetonitrile was treated with 10 molar equivalents of the appropriate anion (F^-, NO_2^-, Br^-, HSO_4^- and I^-) using sodium or potassium salts and was then subjected to spectrofluorimetric analysis. For concentration-dependence studies, a solution of **40**-*Ac* (3.3 μM) in acetonitrile was treated with 0.1, 1, 2, 5 and 10 molar equivalents of CN^-. The effects of cations were investigated by treating a solution of **40**-*Ac* (0.15 μM) in a 1:1 H_2O/acetonitrile mixture with 10 molar equivalents of the appropriate cation ($CaCl_2$, $MgCl_2$, $ZnCl_2$, EuTf, YbTf) prior to fluorescence determination.

Synthesis of 1-acetyl-5,6-diacetoxy-3-(trimethylsilylethynyl)indole (41). A solution of **22** (700 mg, 2.0 mmol) in toluene (16.2 mL) was treated under stirring and an argon atmosphere with acetic anhydride (840 μL) and *N*,*N*-dimethylaminopyridine (210 mg, 1.7 mmol). After 5 min triethylamine (16.2 mL), PPh_3 (51.3 mg, 0.2 mmol), CuI (37.8 mg, 0.2 mmol), $(PPh_3)_2PdCl_2$ (68.4 mg, 0.1 mmol) and trimethylsilylacetylene (1.4 mL, 10 mmol) were added to the reaction mixture and the temperature was raised to 60 °C. After 30 min the reaction mixture was extracted with a 10% water solution of NH_4Cl and toluene. The organic layers were collected, dried over anhydrous sodium sulfate, evaporated under reduced pressure and the residue fractionated on silica gel (eluant: cyclohexane/ethyl acetate

8:2) to afford pure **41** (593 mg, 80%, R_f = 0.56 eluant: cyclohexane/ethyl acetate 1:1 (v/v)).

41: ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.27 (3 × 3H, s, $Si(CH_3)_3$), 2.33 (2 × 3H, s, 2 × $OCOCH_3$), 2.60 (3H, s, $NCOCH_3$), 7.44 (1H, s, H-4), 7.63 (1H, s, H-2), 8.29 (1H, s, H-7); ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm) 0.05 (-$Si(CH_3)_3$), 20.6 (2 × OCO$\underline{C}H_3$), 23.5 (NCO$\underline{C}H_3$), 95.3 (-$\underline{C}$≡C-$Si(CH_3)_3$), 99.6 (-C≡$\underline{C}$-$Si(CH_3)_3$), 105.2 (C-3), 111.9 (C-7), 114.0 (C-4), 128.4 (C-9), 129.5 (C-2), 132.1 (C-8), 139.6 (C-5), 140.8 (C-6), 167.9 (N$\underline{C}OCH_3$), 168.6 (2 × O$\underline{C}OCH_3$); HRMS (ESI) *m/z* $C_{19}H_{22}NO_5Si$ $[M+H]^+$ calcd 372.1267, found 372.1270.

Synthesis of 1-acetyl-5,6-diacetoxy-3-ethynylindole (37-*Ac*). A solution of **41** (600 mg, 1.6 mmol) in DMF (14.8 mL) was treated with KF (139.2 mg, 2.20 mmol) under vigorous stirring. After 1 h the reaction mixture was extracted with water and toluene. The organic layers were collected, dried over anhydrous sodium sulfate and evaporated under reduced pressure to afford pure **37**-*Ac* (344 mg, 72% R_f = 0.68 eluant: chloroform/ethyl acetate 1:1 (v/v)).

37-*Ac***:** ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 2.32 (2 × 3H, s, 2 × $OCOCH_3$), 2.61 (3H, s, $NCOCH_3$), 3.25 (1H, s, -C≡CH), 7.49 (1H, s, H-4), 7.65 (1H, s, H-2), 8.29 (1H, s, H-7); ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm) 20.6 (2 × OCO$\underline{C}H_3$), 23.6 (NCO$\underline{C}H_3$), 74.5 (-$\underline{C}$≡CH), 81.8 (-C≡$\underline{C}$H), 104.0 (C-3), 111.9 (C-7), 113.9 (C-4), 128.3 (C-9), 129.9 (C-2), 132.0 (C-8), 139.6 (C-5), 140.8 (C-6), 167.8 (N$\underline{C}OCH_3$), 168.5 (2 × O$\underline{C}OCH_3$); HRMS (ESI) *m/z* $C_{16}H_{14}NO_5$ $[M+H]^+$ calcd 300.0872, found 300.0869.

Synthesis of 3,3'-(1,2-ethynediyl)bis-5,6-dihydroxyindole (38-*Ac*). A solution of **22** (400 mg, 1.12 mmol) in toluene (9.8 mL) was treated in an argon atmosphere under stirring with acetic anhydride (484 μL) and *N*,*N*-dimethylaminopyridine (122 mg, 1 mmol). After 5 min triethylamine (9.8 mL), PPh_3 (29.2 mg, 0.11 mmol), CuI (22 mg, 0.11 mmol), $(PPh_3)_2PdCl_2$ (40 mg, 0.056 mmol) and **37**-*Ac* (334 mg, 1.12 mmol) were added to the reaction mixture that was then heated to 60 °C.

After 30 min the reaction mixture was filtered and the solid washed with toluene and extracted with chloroform and a 10% water solution of NH_4Cl. The organic layers were collected, dried over anhydrous sodium sulfate and evaporated under reduced pressure to afford pure **38**-*Ac* (608 mg, 87%, R_f = 0.82 eluant: cyclohexane/ethyl acetate 2:8 (v/v)).

38-*Ac***:** ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 2.33 (4 × 3H, s, 4 × $OCOCH_3$), 2.65 (2 × 3H, s, 2 × $NCOCH_3$), 7.55 (2 × 1H, s, H-4, H-4"), 7.71 (2 × 1H, s, H-2, H-2"), 8.33 (2 × 1H, s, H-7, H-7"); ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm) 20.6 (4 × $OCO\underline{C}H_3$), 23.6 (2 × $NCO\underline{C}H_3$), 84.4 (-C≡C-), 104.7 (C-3, C-3"), 112.0 (C-7, C-7"), 114.0 (C-4, C-4"), 128.3 (C-9, C-9"), 129.2 (C-2, C-2"), 132.2 (C-8, C-8"), 139.7 (C-5, C-5"), 140.9 (C-6, C-6"), 167.9 (2 × $N\underline{C}OCH_3$), 168.6 (4 × $O\underline{C}OCH_3$); ESI(+)-MS *m/z* 573 ($[M+H]^+$); HRMS (ESI) *m/z* $C_{30}H_{25}N_2O_{10}$ $[M+H]^+$ calcd 573.1509, found 573.1512.

Oxidation reaction of alkynyl-derivatives: general procedure. A 0.01 M solution of **37**-*Ac* or **38**-*Ac* in methanol was degassed with an argon flux and then treated with sodium *tert*-butoxide (2 molar equivalents in the case of **37**-*Ac*, 4 molar equivalents

in the case of **38**-*Ac*). After 10 min, the reaction mixture was acidified to pH 3-4 with acetic acid and treated as follows:

- In a first group of experiments the reaction mixture was taken in 0.1 M HEPES buffer, pH 7.4, (3.5×10^{-3} M) and treated under vigorous stirring with 2 molar equivalents of $NiSO_4 \cdot 7H_2O$ under an oxygen flux.
- In a second group of experiments the reaction mixture was taken in 0.1 M phosphate buffer, pH 7.4, (2×10^{-3} M) and treated with horseradish peroxidase (20 U/mL) and H_2O_2 (2 molar equivalents in the case of **37**-*Ac*, and 4 molar equivalents in the case of **38**-*Ac*).

At regular intervals of time, aliquots of the reaction mixtures were reduced with sodium dithionite, acidified to pH 3-4 with 3 M HCl, and extracted with ethyl acetate. The organic layers were collected, dried over anhydrous sodium sulphate, evaporated under reduced pressure and acetylated with acetic anhydride and pyridine. The resulting mixtures were analyzed by TLC (eluant: chloroform/ethyl acetate 1:1 (v/v) for **37**-*Ac*; cyclohexane/ethyl acetate 2:8 (v/v) for **38**-*Ac*).

Reactivity of 37-*Ac* and 38-*Ac* under acidic conditions: general procedure. The appropriate compound was dissolved in methanol (0.022 M) and deacetylated with sodium *tert*-butoxide as reported above. After 10 min the reaction mixtures were acidified to pH 3-4 with acetic acid and taken up in 0.1 M phosphate buffer, pH 3, (0.01 M final concentration). At regular intervals of time, aliquots of the reaction mixtures were extracted with ethyl acetate and the organic layers collected, dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residues were treated with acetic anhydride (1 mL) and pyridine (50 μL), and the acetylated

mixtures analyzed by TLC (eluant: chloroform/ethyl acetate 1:1 (v/v), for **37**-*Ac*, cyclohexane/ethyl acetate 2:8 (v/v) for **38**-*Ac*).

Isolation of 3-acetyl-5,6-diacetoxyindole (42-*Ac*). A solution of **37**-*Ac* (100 mg, 0.34 mmol) in methanol (15 mL), previously degassed with an argon flux for 10 min, was treated under stirring with sodium *tert*-butoxide (68 mg, 0.68 mmol). After 10 min the reaction mixture was acidified to pH 3-4 with acetic acid and taken up in 0.1 M phosphate buffer, pH 3 (33 mL). After 10 min the reaction mixture was extracted with ethyl acetate and the organic layers were collected, dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue was then treated with acetic anhydride (2 mL) and pyridine (100 μL). The acetylated mixture was evaporated under reduced pressure and the residue fractionated on silica gel (eluant: chloroform/ethyl acetate 4:6 (v/v)) to afford pure **42**-*Ac* (20 mg, 21%, R_f = 0.33 eluant: chloroform/ethyl acetate 4:6 (v/v)).

42-*Ac***:** ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 2.35 (2 × 3H, s, $OCOCH_3$), 2.38 (3H, s, $COCH_3$), 7.04 (1H, s, H-7), 7.48 (1H, d, *J* = 2.8 Hz, H-2), 7.97 (1H, s, H-4), 8.84 (1H, br s, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 20.6 ($OCO\underline{C}H_3$), 20.8 ($OCO\underline{C}H_3$), 27.0 ($CO\underline{C}H_3$), 106.7 (C-7), 115.8 (C-4), 133.5 (C-3), 133.9 (C-2), 134.6 (C-9), 137.7 (C-8), 138.5 (C-5), 139.3 (C-6), 170.0 ($O\underline{C}OCH_3$), 170.2 ($O\underline{C}OCH_3$), 193.5 ($\underline{C}OCH_3$); ESI (+)-MS *m/z* 276 ($[M+H]^+$); HRMS (ESI) *m/z* $C_{14}H_{14}NO_5$ $[M+H]^+$ calcd 276.0872, found 276.0870.

Isolation of 3,3'-ethenylidenebis-5,6-diacetoxyindole (43-*Ac*). Compound **37**-*Ac* (100 mg, 0.34 mmol) was de-acetylated and taken up in 0.1 M phosphate buffer, pH

3, as described above, and then treated with DHI (74 mg, 0.5 mmol) under vigorous stirring. After 10 min the reaction mixture was extracted with ethyl acetate and the organic layers were collected, dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue was then treated with acetic anhydride (2 mL) and pyridine (100 μL). The acetylated mixture was evaporated under reduced pressure and the residue fractionated on silica gel (eluant: chloroform/ethyl acetate 1:1 (v/v)) to afford pure **43-***Ac* (36 mg, 18%, R_f = 0.66 eluant: chloroform/ethyl acetate 1:1 (v/v)).

43-*Ac*: ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 2.29 (2 × 3H, s, 2 × $OCOCH_3$), 2.34 (2 × 3H, s, 2 × $OCOCH_3$), 2.57 (2 × 3H, s, 2 × $NCOCH_3$), 5.74 (2H, s, -C=CH_2), 7.39 (2 × 2H, s, H-2, H-2”, H-4, H-4”), 8.40 (2 × 1H, s, H-7, H-7”); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 20.9 (4 × OCO$\underline{C}H_3$), 23.7 (2 × NCO$\underline{C}H_3$), 111.6 (C-7, C-7”), 114.3 (C-4, C-4”), 116.3 (=$\underline{C}H_2$), 122.4 (C-3, C-3”, $\underline{C}$=CH_2), 125.1 (C-2, C-2”), 126.3 (C-9, C-9”), 133.1 (C-8, C-8”), 139.4 (C-6, C-6”), 139.9 (C-5, C5”), 168.3 (N$\underline{C}OCH_3$), 168.4 (O$\underline{C}OCH_3$); ESI(+)-MS *m/z* 597 ($[M+Na]^+$); HRMS (ESI) *m/z* $C_{30}H_{26}NaN_2O_{10}$ $[M+Na]^+$ calcd 597.1485, found 597.1483.

Synthesis of 5,6-diacetoxy-7-[4’-(5’’,6’’-diacetoxyindol-7’’-yl)-1’-butadiynyl]-indole (39-*Ac*). A solution of **27**-*Ac* (50 mg, 0.19 mmol) in dry pyridine (650 μL) was treated with $Cu(OAc)_2$ (43 mg, 0.22 mmol) at room temperature under an argon atmosphere. After 30 min the reaction mixture was extracted with chloroform and 10% water solution of NH_4Cl. The organic layers were collected, dried over

anhydrous sodium sulfate and evaporated under reduced pressure to afford pure **39**-*Ac* (49 mg, 98%, R_f = 0.40 eluant: chloroform/ethyl acetate 6:4 (v/v)).

39-*Ac*: ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ (ppm) 2.30 (3H × 2, s, CH_3), 2.38 (3H × 2, s, CH_3), 6.62 (1H × 2, t *J*=2.7 Hz, H-3, H-3''), 7.49 (1H × 2, t *J* = 2.7 Hz, H-2, H-2''), 7.57 (1H × 2, t *J* = 2.7 Hz, H-4, H-4''), 11.02 (1H × 2, br s, NH, NH''); ^{13}C NMR (50 MHz, $(CD_3)_2CO$) δ (ppm): 19.2 (CH_3), 19.4 (CH_3), 74.8 (C-2', C-3'), 80.9 (C-1', C-4'), 102.7 (C-3, C-3''), 113.8 (C-7, C-7''), 116.4 (C-4, C-4''), 125.2 (C-4a, C-4a''), 127.1 (C-2, C-2''), 134.6 (C-7a, C-7a''), 136.5 (C-5, C-5''), 140.8 (C-6, C-6''), 167.3 (C=O), 168.2 (C=O); MS (ESI+) *m/z* 535 ($[M+Na]^+$).

Synthesis of 3,4-diacetoxy-2,6-di[2'-(5'',6''-diacetoxy-7''-indolyl)-1'-ethynyl]-aniline (40-*Ac*). A solution of **27**-*Ac* (183 mg, 0.71 mmol) in a mixture of triethylamine (3.2 mL) and toluene (3.2 mL) was treated with **13** (164 mg, 0.36 mmol), PPh_3 (9.4 mg, 0.04 mmol), CuI (6.8 mg, 0.04 mmol) and $(PPh_3)_2PdCl_2$ (12.6 mg, 0.02 mmol) at 60 °C under an argon atmosphere. After 30 min the reaction mixture was extracted with a 10% water solution of NH_4Cl and chloroform. The organic layers were collected, dried over anhydrous sodium sulfate, evaporated under reduced pressure and the residue fractionated on silica gel (petroleum ether/ethyl acetate, gradient from 70:30 to 65:35) to afford pure **40**-*Ac* (180 mg, 70%, R_f = 0.26 eluant: chloroform/ethyl acetate 9:1 (v/v)).

40-*Ac*: ^{1}H NMR (200 MHz, $(CD_3)_2CO$) δ (ppm): 2.28 (3H, s, CH_3), 2.29 (3H, s, CH_3), 2.30 (3H, s, CH_3), 2.31 (3H, s, CH_3), 2.35 (3H, s, CH_3), 2.37 (3H, s, CH_3), 5.71 (2H, br s, $-NH_2$), 6.5-6.6 (2H, m, H-3" × 2), 7.34 (1H, s, H-5), 7.4-7.5 (2H, m, H-2" × 2), 7.49 (1H, s, H-4"), 7.51 (1H, s, H-4"), 10.82 (1H, br s, -NH), 10.91 (1H,

br s, -NH); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ (ppm): 20.2 (4 × CH_3), 25.8 (CH_3), 27.2 (CH_3), 88.5 (2 × C-2'), 93.0 (2 × C-1'), 102.9 (2 × C-7"), 103.3 (2 × C-3"), 104.1 (C-6), 104.2 (C-2), 115.7 (C-4"), 116.0 (C-4"), 125.9 (2 × C-4a"), 127.7 (C-5), 127.8 (2 × C-2"), 133.7 (C-1), 134.5 (C-7a"), 134.6 (C-7a"), 137.4 (C-3, C-4), 137.4 (2 × C-5"), 149.6 (C-6"), 149.8 (C-6"), 168.0 (2 × C=O), 168.6 (C=O), 168.8 (C=O), 169.2 (2 × C=O); MS (ESI+) *m/z* 720 ($[M+H]^+$), 742 ($[M+Na]^+$), 758 ($[M+K]^+$).

CHAPTER 3

SYNTHESIS OF 17β-ESTRADIOL DERIVATIVES OF POTENTIAL PRACTICAL INTEREST

Introduction

17β-Estradiol (**2**) is an important steroidal hormone with estrogenic activity involved primarily in the control of sexual growth and behaviour. In addition to this, 17β-estradiol and structurally related estrogens possess both carcinogenic and neuroprotective properties that have been attributed to the inherent susceptibility of the phenolic A-ring to enzymatic or chemical oxidation. In particular, the involvement of **2** in breast and other human cancers would be the result of the metabolic conversion to the 2- and 4-hydroxy derivatives, termed the catecholestrogens, and the corresponding *o*-quinones, which can induce the critical initiation step of cancerogenesis via adduct formation with DNA and depurination processes. On the other hand, the neuroprotective action of estrogens would be related to, or at least complemented by, their potent antioxidant and free radical scavenging capacity.[111]

In connection with the latter role, recent studies have been aimed at developing new synthetic procedures for the preparation of oxidatively functionalized derivatives of **2** on the A-ring both for elucidation of the structural modifications suffered by **2** in oxidative settings and for the possible academic and industrial relevance e.g. in asymmetric synthesis,[112] in supramolecular chemistry,[113] in the quest for liquid crystal preparations or for the synthesis of anticancer compounds.[114]

Numerous articles reported in the literature described the synthesis of differently modified steroidal compounds; some of these are shown in Figure 21 and concern the preparation of: a) the catecholestrogens (**44** and **45**), obtained via a one-pot

procedure involving oxygenation of 17β-estradiol with the hypervalent iodine reagent *o*-iodoxybenzoic acid (IBX) under carefully controlled reaction and work-up conditions;[115] b) the *p*-quinols derivatives, an interesting class of estradiol-related compounds that have found application in estrogen replacement therapy, prevention and treatment of osteoporosis,[116] such as **46** that can be easily obtained via the oxidation of **2** with potassium permanganate;[117] c) a series of oligomers of **2** featuring the C2-C2' or C4-C4' linkage (i.e. **47** and **48**) obtained by the enzymatic oxidation of **2** with the system horseradish peroxidase/H_2O_2;[118] d) the nitroestradiols **49** and **50**, an important class of compounds for their anticancer activities that can be synthesized from **2** by treatment with nitric acid in acetic acid;[119] and the corresponding oxidation products **51** and **52** obtained with the system horseradish peroxidase/H_2O_2;[120] e) the halo-derivatives of **2**, such as the iodo-estradiols **53-55** and the bromo-estradiol **56**, that can be easily obtained via a simple procedure involving cheap and mild reagents $NaClO_2$/NaX (X = I or Br) in the presence of HCl.[90]

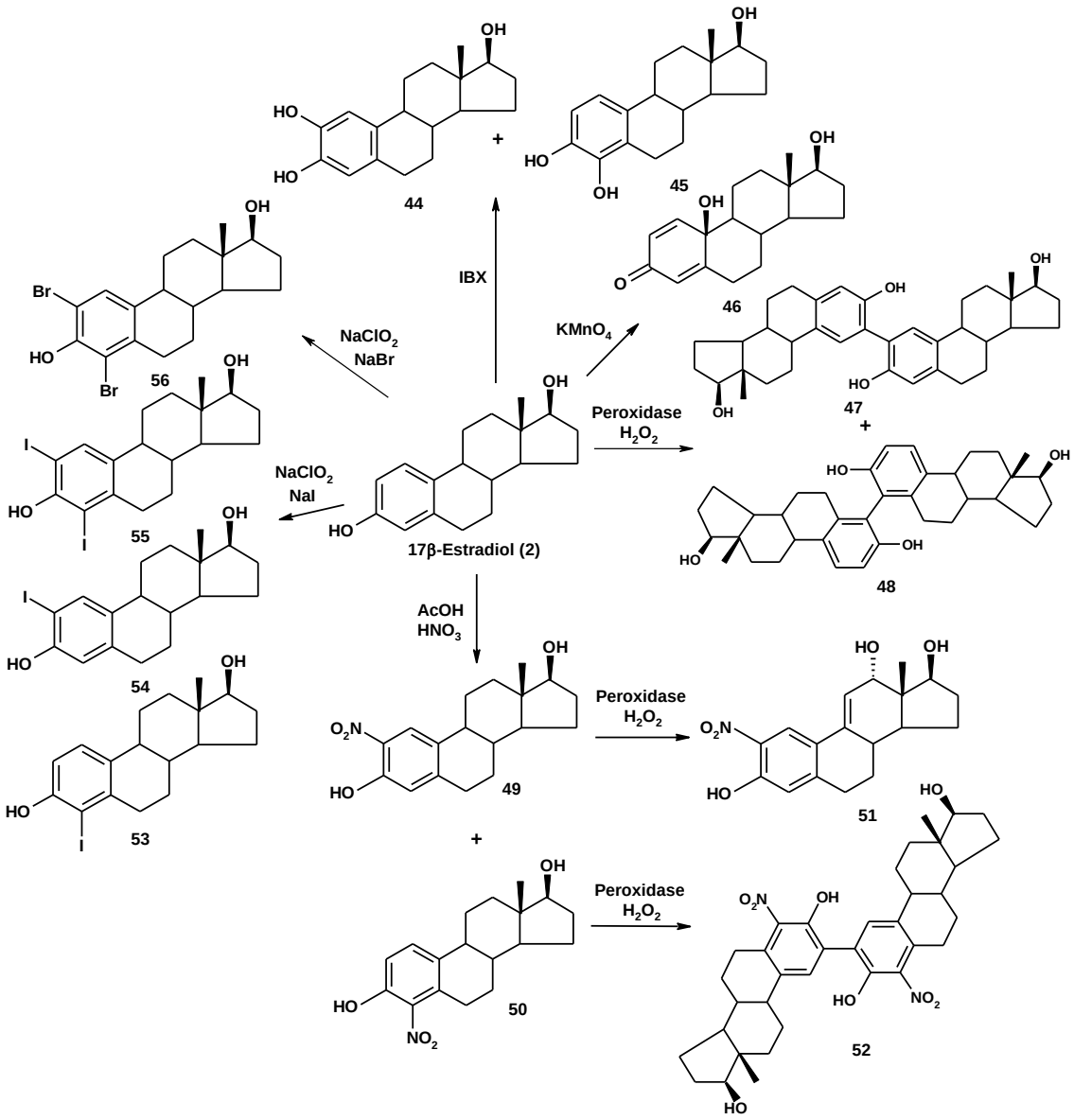


Figure 21. Main derivatives of 17β-estradiol.

In addition, a very innovative oxidation procedure have been explored in the research group in which I have worked during these years consisting in oxidation of **2** using solvent free conditions. These reaction conditions have several advantages: the total absence of the solvent reduces the environmental impact and inserts the process in the green chemistry field, that is aimed at the development of eco-compatible synthetic methodologies.

Compound **2** was so treated with 2 equivalents of $FeNH_4(SO_4)_2$ under solvent free conditions. The mixture was finely powdered in agate mortar, pestle and then put in a test tube at 150°C for 6 h. By this oxidation the main product isolated in 67% yield without the need for extensive purification, was identified as **57** (Figure 22).[121]

The hypothesized mechanism, outlined in Figure 22, involves dehydrogenation without opening of D ring, dehydration, methyl migration with tertiary carbocation formation and deprotonation to give a cyclopentaphenanthrene system.

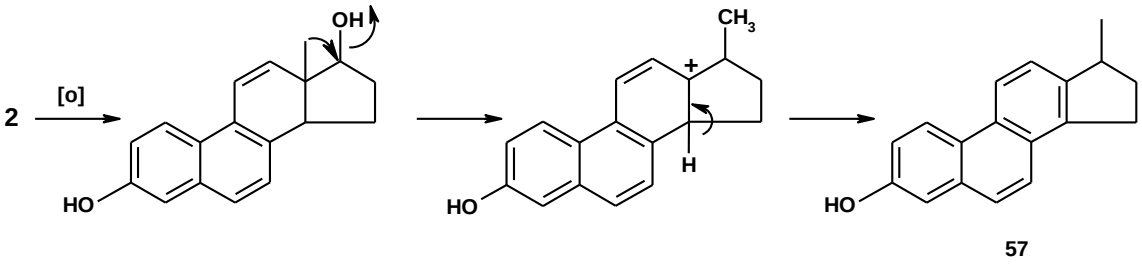


Figure 22. Schematic outline of the proposed mechanism of formation of **57.**

Compound **57** proved to be a good starting material for synthetic purposes: experiments carried out in our laboratories showed that **57** can be derivatized with the same systems used on 17β-estradiol (IBX, $NaClO_2$/NaI, $FeNH_4(SO_4)_2$, peroxidase/ H_2O_2) to give a set of novel structurally related compounds (Figure 23) of potential synthetic and practical interest.

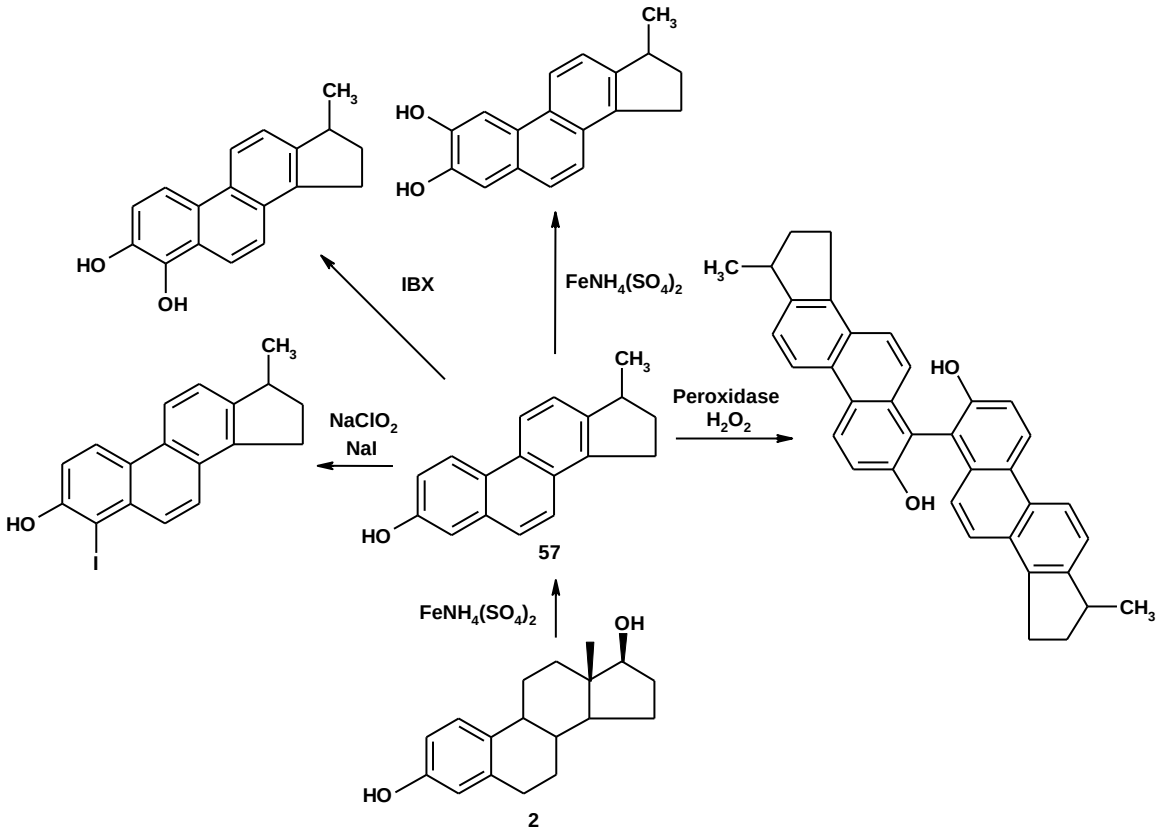
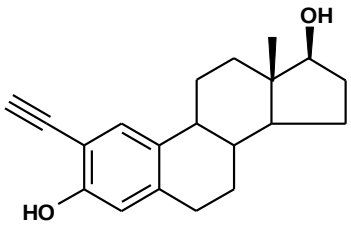
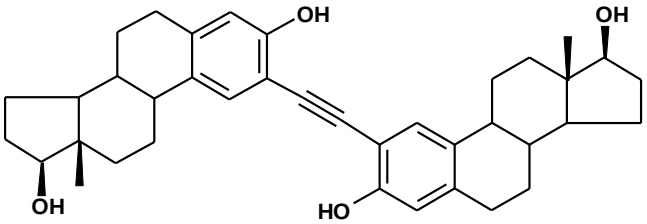


Figure 23. Main derivatives of **57** from 17β-estradiol.

With this background available, and with a view to synthesizing new molecular scaffolds, I have focused my attention on the preparation of alkynyl-derivatives of **2** with the same approach described in Chapter 2 in the case of the alkynyl-derivatives of 5,6-dihydroxyindole. In particular, I developed a new procedure for the preparation of 2-ethynyl-17β-estradiol **(58)** and 2-[(17β-estradi-2-yl)-1-ethynyl]-17β-estradiol **(59)** as new potential self-assembling supramolecular systems.[122]



58

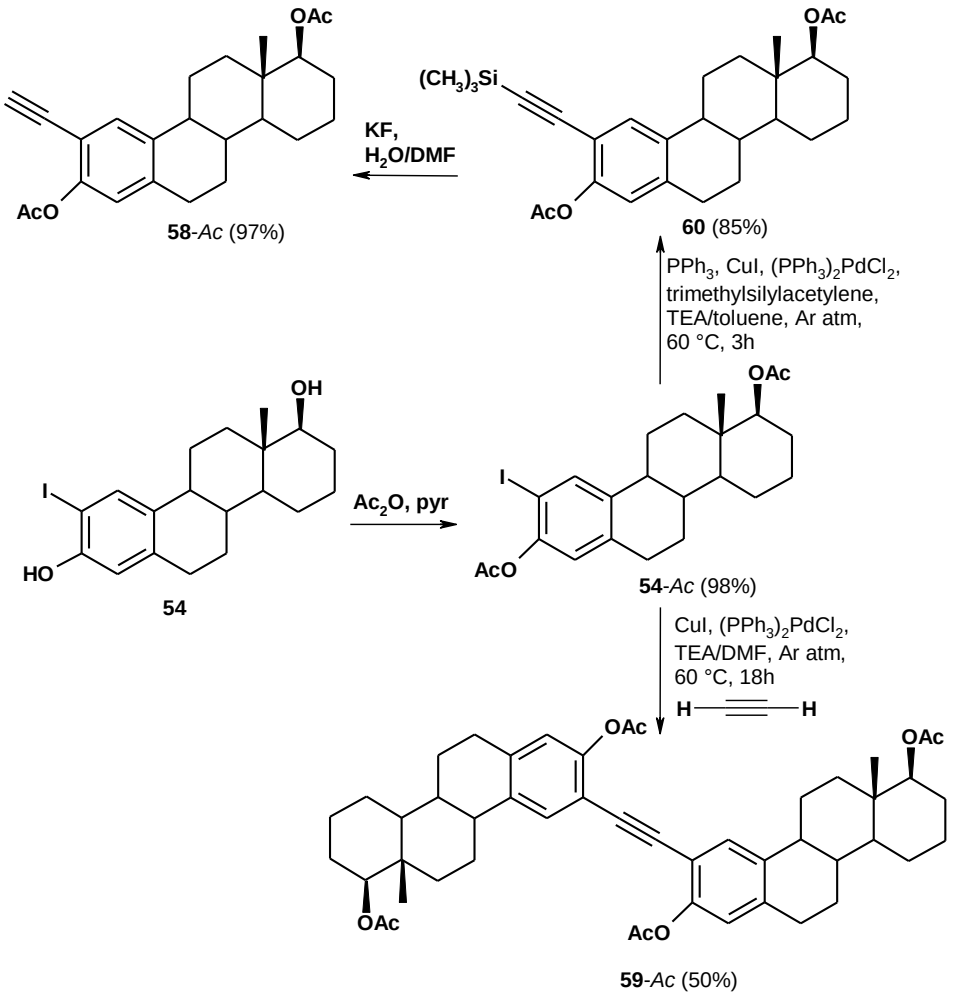


59

Synthesis of alkynylderivatives of 17β-estradiol

As previously described, the Sonogashira coupling is a key reaction for the generation of alkynyl derivatives via carbon-carbon bond formation. The synthetic approach utilized as the starting material 2-iodoestradiol (**54**), which was obtained under mild conditions by the previously mentioned procedure.[90] The iodinated sterol was initially protected at the phenolic hydroxyl group by acetylation (Scheme 12), which allowed insertion of the alkynyl functionality via the classical Sonogashira coupling conditions with trimethylsilylacetylene, CuI, PPh_3 and $(PPh_3)_2PdCl_2$ as catalysts, in a 1:1 mixture of triethylamine/toluene as solvent.[91] Treatment of the trimethylsilyl-derivative of **54**-*Ac* with potassium fluoride led to the desired 2-ethynyl-17β-estradiol as the acetyl derivative (**58**-*Ac*) in good yields.

In a variant of this procedure, the same compound **54**-*Ac* was subjected to Sonogashira coupling using acetylene instead of trimethylsilylacetylene and CuI and $(PPh_3)_2PdCl_2$ as catalysts, in triethylamine/DMF as the solvent, to furnish the new dimer **59**-*Ac* in satisfactory yields (50%) (Scheme 12).



Scheme 12. Synthesis of compound **58**-*Ac* and **59**-*Ac*.

Conclusions

The results shown in this chapter have corroborated the versatility of the steroidal skeleton as a valuable starting material for the preparation of innovative molecular systems. During my thesis I succeeded in preparing for the first time the alkynyl-derivatives of 17β-estradiol, **58**-*Ac* and **59**-*Ac*, using the Sonogashira coupling procedure on 2-iodo-17β-estradiol-bis-acetylated (**54**-*Ac*). These compounds may provide useful starting materials for the preparation of novel molecular scaffolds of potential interest as supramolecular systems, molecular wires or chiral compounds. Ongoing work in my laboratory is aimed at pursuing these goals.

Experimental Section

Materials and methods. All commercially available reagents were used as received. Anhydrous solvents were purchased from commercial sources and withdrawn from the container by syringe, under a slight positive pressure of argon. All the solvents were of analytical grade quality. Compound **54** was prepared according to a reported procedure.[90]

NMR spectra were recorded with 200, 300, and 400 MHz instruments. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$, using TMS as the internal standard; *J* values are given in Hz. $^{1}H,^{1}H$ COSY, $^{1}H,^{13}C$ HSQC, $^{1}H,^{13}C$ HMBC and ROESY experiments were run at 400.1 MHz using standard pulse programs. Mass spectra were registered in the electrospray ionization-positive ion (ESI+) mode. ESI analysis were performed with the cone and the fragmentator voltages set at 4 kV and 80 V, respectively; nitrogen was used as carrier gas at a flow of 8 mL/min and the nebulizer pressure was set at 50 psi. Analytical and preparative TLC analyses were performed on F_{254} silica gel plates (0.25 and 0.5 mm, respectively). TLC plates were visualised using a UV lamp (λ= 254 nm) and a fluorescence lamp (λ= 366 nm). Liquid chromatography was performed on silica gel (60-230 mesh).

Synthesis of 2-iodo-17β-estradiol-bis-acetylated (54-*Ac*). A solution of **54** (300 mg, 0.75 mmol) in acetic anhydride (3 mL) and pyridine (150 μL) was left at room temperature for 18 h. The reaction mixture was then extracted with chloroform and HCl 3 M. The organic layers were washed with water and then collected, dried over

anhydrous sodium sulphate and evaporated under reduced pressure to afford pure **54**-*Ac* (356 mg, 98 % R_f = 0.71 eluant: cyclohexane/ethyl acetate 6:4 (v/v)).

54-*Ac*: ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 0.76 (3H, s, CH_3), 1.1-1.9 (12H, m, H-7, H-8, H-11, H-12, H-14, H-15, H-16), 2.00 (3H, s, $OCOCH_3$), 2.28 (3H, s, $OCOCH_3$), 2.16 (1H, m, H-9), 2.74 (2H, m, H-6), 4.62 (1H, t, *J* = 8.0 Hz, H-17), 6.74 (1H, s), 7.61 (1H, s); ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm) 11.8 (CH_3), 21.1 ($OCO\underline{C}H_3$), 23.1 ($OCO\underline{C}H_3$), 26.1, 26.5, 27.4, 27.7, 36.5, 37.0 (CH_2), 42.6, 43.8, 49.5, 60.3, 82.3, 86.3 (CH), 125.0, 127.0, 134.0, 136.1, 141.6 (C), 169.2 ($O\underline{C}OCH_3$), 171.4 ($O\underline{C}OCH_3$). ESI (+)-MS *m/z* 483 ($[M+H]^+$).

Synthesis of 2-(trimethylsilylethynyl)-17β-estradiol-bis-acetylated (60). A solution of **54**-*Ac* (250 mg, 0.52 mmol) in triethylamine (4.25 mL) and toluene (4.25 mL) was treated with PPh_3 (13.6 mg, 0.05 mmol), CuI (9.9 mg, 0.05 mmol) and $(PPh_3)_2PdCl_2$ (18.2 mg, 0.03 mmol) and trimethylsilylacetylene (403 μL, 2.8 mmol) at 60 °C under an argon atmosphere. After 3h the reaction mixture was extracted with a 10% water solution of NH_4Cl and toluene. The organic layers were collected, dried over anhydrous sodium sulfate, evaporated under reduced pressure and the residue fractionated on silica gel (eluant petroleum ether/ethyl acetate 95:5 (v/v)) to afford pure **60** (200 mg, 85%, R_f = 0.75 eluant: cyclohexane/ethyl acetate 7:3 (v/v)).

60: ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 0.23 (9H, s, $Si(CH_3)_3$), 0.82 (3H, s, CH_3), 1.1-2.3 (13H, m, H-7, H-8, H-9, H-11, H-12, H-14, H-15, H-16), 2.06 (3H, s, $OCOCH_3$), 2.30 (3H, s, $OCOCH_3$), 2.83 (2H, m, H-6), 4.68 (1H, t, *J* = 8.0 Hz, H-17), 6.77 (1H, s), 7.40 (1H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 0.25 (3 $Si(CH_3)_3$),

11.8 (CH_3), 20.9 ($OCO\underline{C}H_3$), 23.0 ($OCO\underline{C}H_3$), 25.7, 26.6, 27.3, 29.2, 36.5, 37.8 (CH_2), 42.6, 43.5, 49.6, 82.4, 121.8, 130.0 (CH), 97.9, 100.2, 113.9, 123.2, 137.8, 139.0, 149.5 (C), 168.7 ($O\underline{C}OCH_3$), 170.9 ($O\underline{C}OCH_3$). ESI (+)-MS *m/z* 453 ($[M+H]^+$).

Synthesis of 2-ethynyl-17β-estradiol-bis-acetylated (58-*Ac*). A solution of **60** (200 mg, 0.44 mmol) in DMF (4.2 mL)) was treated with a solution of KF (51.4 mg, 0.88 mmol) in water (791 μL) at room temperature and under vigorous stirring. After 2 h the reaction mixture was extracted with toluene and water. The organic layers were collected, dried over anhydrous sodium sulfate and evaporated under reduced pressure to afford pure **58**-*Ac* (160 mg, 97%, R_f = 0.59 eluant: cyclohexane/ethyl acetate 6:4 (v/v)).

58-*Ac*: ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 0.83 (3H, s, CH_3), 1.1-2.4 (13H, m, H-7, H-8, H-9, H-11, H-12, H-14, H-15, H-16), 2.06 (3H, s, $OCOCH_3$), 2.35 (3H, s, $OCOCH_3$), 2.83 (2H, m, H-6), 2.95 (1H, s, C≡CH), 4.69 (1H, t, *J* = 8.0 Hz, H-17), 7.19 (1H, s), 7.23 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 11.8 (CH_3), 20.9 ($OCO\underline{C}H_3$), 23.0 ($OCO\underline{C}H_3$), 25.7, 26.6, 27.3, 29.2, 36.5, 37.8 (CH_2), 43.0, 43.9, 50.0, 82.8, 96.1, 122.4, 130.7 (CH), 113.0, 122.6, 129.3, 138.6, 140.5, 150.0 (C), 169.4 ($O\underline{C}OCH_3$), 171.4 ($O\underline{C}OCH_3$). ESI (+)-MS *m/z* 381 ($[M+H]^+$), 403 ($[M+Na]^+$), 419 ($[M+K]^+$).

Synthesis of 2-[(17β-estradi-2-yl)-1-ethynyl]-17β-estradiol-bis-acetylated (59-*Ac*). A solution of **54**-*Ac* (100 mg, 0.2 mmol) in triethylamine (1.4 mL) and DMF

(500 μL) was treated with CuI (4.4 mg, 0.02 mmol) and $(PPh_3)_2PdCl_2$ (5 mg, 0.007 mmol) under an argon atmosphere. The reaction mixture was then kept under a gaseous acetylene atmosphere at room temperature and under stirring. After 18 h the reaction mixture was extracted with a 10% water solution of NH_4Cl and chloroform. The organic layers were collected, dried over anhydrous sodium sulfate, evaporated under reduced pressure and the residue fractionated on silica gel (eluant cyclohexane/ethyl acetate 7:3 (v/v)) to afford pure **59**-*Ac* (38 mg, 50%, R_f = 0.55 eluant: cyclohexane/ethyl acetate 7:3 (v/v)).

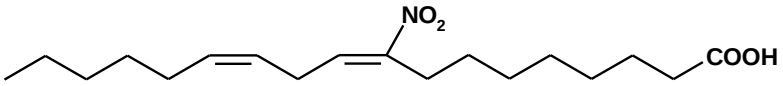
59-*Ac*: ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 0.83 (2 × 3H, s, CH_3), 1.1-2.4 (2 × 13H, m, H-7, H-7', H-8, H-8', H-9, H-9', H-11, H-11', H-12, H-12', H-14, H-14', H-15, H-15', H-16, H-16'), 2.17 (2 × 3H, s, $OCOCH_3$), 2.31 (2 × 3H, s, $OCOCH_3$), 2.86 (2 × 2H, m, H-6, H-6'), 4.68 (2 × 1H, t, *J* = 8.0 Hz, H-17, H-17'), 6.80/6.81 (2 × 1H, s), 7.43/7.46 (2 × 1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 11.8 (2 × CH_3), 20.9 (2 × $OCO\underline{C}H_3$), 23.0 (2 × $OCO\underline{C}H_3$), 2 × 25.7, 2 × 26.6, 2 × 27.3, 2 × 29.2, 30.1, 36.5, 2 × 37.8 (CH_2), 2 × 43.0, 43.9, 2 × 50.0, 82.8, 96.1, 122.2, 122.4, 130.1, 130.7, 131.5 (CH), 2 × 113.0, 2 × 122.6, 2 × 129.3, 2 × 138.6, 2 × 140.5, 2 × 150.0 (C), 169.4 (2 × $O\underline{C}OCH_3$), 171.4 (2 × $O\underline{C}OCH_3$). ESI (+)-MS *m/z* 734 ($[M+H]^+$), 757 ($[M+Na]^+$).

CHAPTER 4

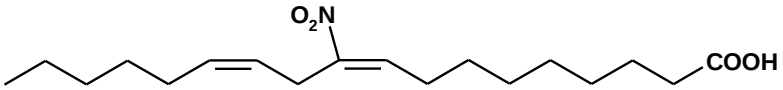
CHEMISTRY OF NITRATED LIPIDS

Introduction

A brief part of my doctorate studies has been aimed at completing the experiments of my master degree thesis project on the synthesis and reactivity of nitrolinoleic acids. Nitrated derivatives of unsaturated fatty acids have emerged during the past decade as important products of lipid modification under (patho)physiological conditions associated with oxidative stress and elevated production of nitric oxide (NO).[123,124] Although the detailed structural features of the nitrated fatty acids generated in vivo remain to be defined, a significant proportion of them appears to be present in the form of nitroalkene derivatives, such as the nitrooleic acids and the nitrolinoleic acids.[125,126] These latter comprise four isomeric derivatives of (*Z*,*Z*)-9,12-octadecadienoic acid exhibiting a conjugated nitro group on the terminal (9- and 13-) or inner (10- and 12-) positions of the (*Z*,*Z*)-1,4-pentadienyl system. So far, 10-nitrolinoleic acid ((9*E*,12*Z*)-10-nitrooctadecadienoic acid, **62**) and the 12-nitro isomer have been detected in human plasma and urine by LC-MS techniques,[127-129] whereas 9-nitrolinoleic acid ((*9E*,12*Z*)-9-nitrooctadecadienoic acid, **61**) and its 13-nitro isomer have been described only as products of chemical nitration of linoleic acid in an organic solvent.[130,131]



61



62

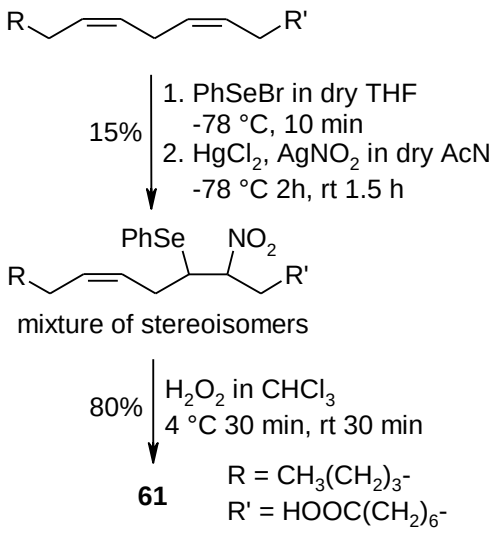
A substantial body of evidence indicates that nitrolinoleic acids behave as pluripotent signaling molecules that transducer the actions of NO via multiple mechanisms,[129,132,133] which in part are receptor-mediated and in part reflect the electrophilic and NO-releasing behavior of the nitropentadiene reactive moiety in a physiological milieu. The electrophilic nature of nitrolinoleates is deduced by their ability to cause post-translational modifications of protein residues[131,134,135] and by the occurrence in vivo of a significant proportion of vicinal hydroxynitro derivatives,[129,132] suggesting addition of water onto the double bond. The ability of nitrolinoleic acids to release NO is the focus of much interest. Experimental evidence derived from different experiments (EPR, chemiluminescence, deoxy- and oxymyoglobin assays)[132,136-139] suggests the release of NO as well as nitrite,[137,138] but the detailed pathways remain to be definitively assessed. In the presence of other lipids or amphiphiles at levels above the critical micellar concentration, nitrolinoleic acids are stabilized and become highly resistant to NO-release. This finding suggests that nitrolinoleic acids provide a hydrophobically stabilized NO reserve and, in view of their accumulation to detectable levels in lipophilic biological compartments, represent the single largest pool of bioactive oxides of nitrogen in the vasculature.[132] Apart from the above studies, current knowledge of the chemical behavior and fate of these nitroalkene fatty acid derivatives under physiologically relevant conditions is scanty and limited to the decomposition routes supposedly associated to their NO donor abilities. Surprising is also the lack of general information about the free radical oxidation pathways of the nitro-1,4-pentadienyl system, the reactive core of nitrated polyunsaturated fatty acids. A detailed elucidation of the chemical properties of nitrolinoleic acids under physiologically relevant conditions is therefore of

paramount importance for further progress toward an understanding of the signaling capabilities of these bioactive lipids. To this aim, access to the individual isomers is essential to advancing structure-activity knowledge and to rationally design new more active structural variants for therapeutic purposes. Starting from this viewpoint, I have developed the first convenient access route to pure **61** and **62** as free acids and, as a consequence, I have carried out a comparative investigation of their chemical behavior in aqueous phosphate buffer at pH 7.4. By integrating product analysis with mechanistic experiments, I have provided a comparative description of the basic chemistry of nitrolinoleic acids under physiologically relevant conditions. The remarkable position-dependent influence of the nitro group on the oxidation behavior of the 1,4-pentadienyl moiety has also been addressed with the aid of a density functional theory (DFT) investigation.

Preparation of Nitrolinoleic Acids

Unlike nitrooleic acids, for which simple access routes based on standard Henry condensation chemistry are available,[138,140] nitrolinoleic acids are difficult to synthesize. Their preparation currently relies on direct nitration of the parent fatty acid[126,128,130,131,141,142] because of the lack of synthetic routes assembling the C-18 nitrated fatty acid chain. Early methods based on acidic nitrite or NO_2^+-forming reagents led invariably to mixtures of regio- and stereoisomers in low yields, along with abundant side products, and only small amounts of **62** and the 12-nitro isomer as carboxylate esters could be obtained. More recently, a nitrophenylselenenylation/oxidation protocol has been reported to produce a statistical distribution of the four regioisomeric nitrolinoleic acids in good overall yields,[131] but their separation as free acids remained a difficult task. To provide access to individual nitrolinoleic acid isomers needed for probing the structural basis of their biological activities and fate, to determine if certain isomers are more reactive than others in competing autoxidation/NO-release and water addition pathways, and to understand why unsaturated lipids with the nitro group on terminal positions of the polyene systems (e.g., **61**) have so far eluded identification in vivo, it was necessary to devise expedient methods for preparation of representative isomeric nitrolinoleates as pure free acids. Nitrolinoleates **61** and **62** were thus selected for the purposes of the present study because of their appropriate positional isomerism. After an extensive search of reaction conditions with a variety of nitrating systems, it was found that the target nitroalkenes **61** and **62** could be conveniently obtained by a

modification of the reported nitrophenylselenenylation/oxidation procedure[131] through a judicious combination of experimental conditions and protection strategies. Compound **61** was prepared by a modified protocol in which linoleic acid was reacted with PhSeBr in THF at -78 °C and then with $HgCl_2$ and $AgNO_2$ in dry acetonitrile to produce regioisomeric nitrophenylselenenyl adducts (Scheme 13).[143] Preparative HPLC then afforded a main fraction containing a mixture of the appropriate stereoisomeric nitrophenylselenenyl adducts, which were treated with H_2O_2 to remove phenylseleninic acid and give the desired **61** in 12% overall yield.



Scheme 13. Preparation of **61**.

The proposed structural assignment was secured by TOCSY experiments showing cross peaks between the terminal methyl proton signal at δ 0.89 and resonances at δ 2.05, 5.35, and 5.52, due to H-14 allylic protons and H-12 and H-13 olefinic protons, in that order.

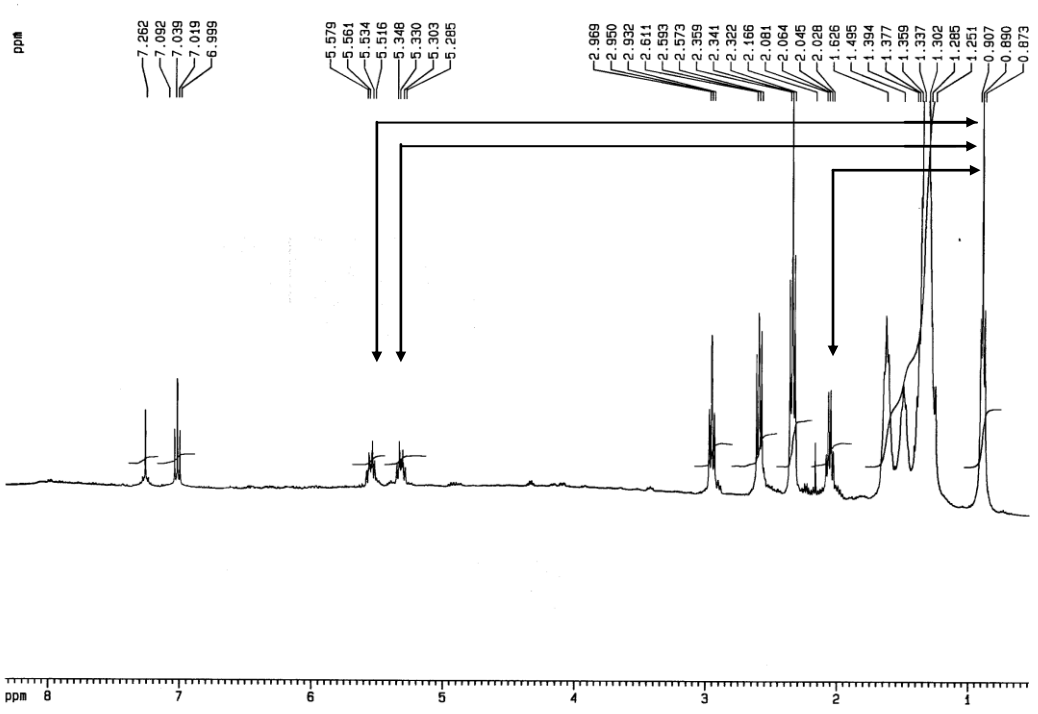
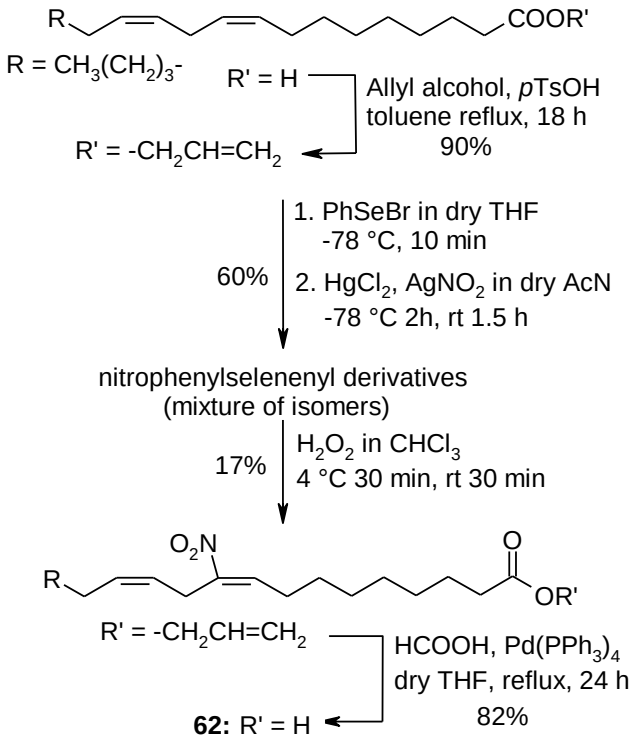


Figure 24. ^{1}H NMR spectrum of **61** ($CDCl_3$); selected TOCSY correlations are indicated by the arrows.

The same procedure was successfully extended to obtain ^{15}N-labeled **61** in 9% isolated yield using $Na^{15}NO_2$ in the place of $AgNO_2$. Preparation of **62** as free acid could not be achieved by the same protocol because of difficulties in the separation of the appropriate nitrophenylselenenyl intermediates or the final nitrolinoleates. Accordingly, an alternate strategy was devised which involved the use of the allylic ester of linoleic acid as the substrate. This protecting group was preferred over simple alkyl groups because of the facile removal by hydrogenolysis[140] and the presence of an additional double bond expected to improve chromatographic separation over silver nitrate-impregnated silica gel.[130] Indeed, nitrophenylselenenylation/oxidation of linoleic acid allylester as above followed by a chromatographic step gave pure **62**-allylester from which **62** was eventually obtained

in ca. 10% overall yield after a deprotection step with formic acid and $Pd(PPh_3)_4$ (Scheme 14).



Scheme 14. Preparation of **62**.

To summarize, the synthetic protocols described in Schemes 13 and 14 provide the first expedient access to single nitrolinoleic acids as free acids and in sufficient amounts for biological studies. They differ from previously published reports of nitrophenylselenenylation of fatty acids[131] in the modified experimental protocol, which relies on a low-temperature procedure favoring selective nitration of the Δ^9 double bond, and in the protection of the carboxyl group followed by advanced chromatographic separation strategies. In particular, access to **61** was made possible by preparative HPLC separation of the nitrophenylselenenylation mixture prior to H_2O_2 treatment, whereas preparation of **62** capitalized on the use of the linoleic acid

allylester as the substrate of the nitrophenylselenenylation protocol, allowing better separation of the final allyl nitrolinoleates and facile deprotection of the carboxyl group.

Reactivity of Nitrolinoleic Acids

The first step toward the definition of the reactivity of nitrolinoleic acids under physiologically relevant conditions was given by the comparative investigation of their relative stability. According to previous and related studies,[132,144] freshly prepared pure **61** and **62** at 20 μM concentration were dissolved in a mixture of 0.1 M phosphate buffer, pH 7.4, containing a 20% ethanol, and their rates of decomposition at 20 °C were monitored by HPLC with UV detection. Data reported in Figure 25 revealed a markedly faster decay of **61** relative to **62**: after 15 min nearly 80% of **61** is consumed whereas **62** was recovered unreacted. Under the same conditions, also linoleic acid remained virtually unchanged over more than 5 h (Figure 25).

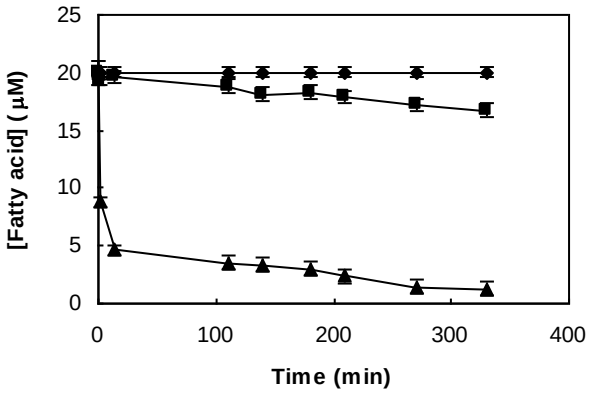


Figure 25. Consumption of linoleic acid (◆), **61** (▲) or **62** (■) with the reaction time during incubation in 0.1 M phosphate buffer, pH 7.4. All experiments have been carried out in triplicate, and data are expressed as average ± S.D.

The faster decay of **61** relative to **62** was also apparent from NMR analyses of the crude ethyl acetate extracts of the reaction mixtures. After 2 h incubation, complete conversion of **61** to a complex mixture of products was noted, whereas in the case of

62 a substantial proportion of unchanged starting material was present. Notably, even when stored dry in the cold, **61** was unstable compared to **62**: while pure samples of the former underwent extensive degradation after a month or so, the latter remained virtually unaffected during the same period.

Considering the high reactivity exhibited by **61** under physiologically relevant conditions, and on the basis of the well documented predisposition of nitrolipids to release NO in the aqueous medium, I have tested the NO-releasing abilities of **61** and **62** in phosphate buffer using the oxymyoglobin assay.[132,136,137] With this test the NO-release was monitored indirectly following the change of the spectrophotometric profile due to the conversion promoted by NO of oxymyoglobin ($MbFe^{II}O_2$) to metmyoglobin ($MbFe^{III}$).[132]

$$OxyMb + NO \rightarrow MetMb + NO_3^-$$

The results showed in Figure 26 indicated a faster decrease in the 580 and 543 nm maxima (characteristic of the visible α- and β-band absorbance of oxymyoglobin) and a more rapid increase in the 630 and 503 nm maxima (characteristic of metmyoglobin) in the case of **61**, suggesting faster NO-release from this fatty acid (Figure 26).

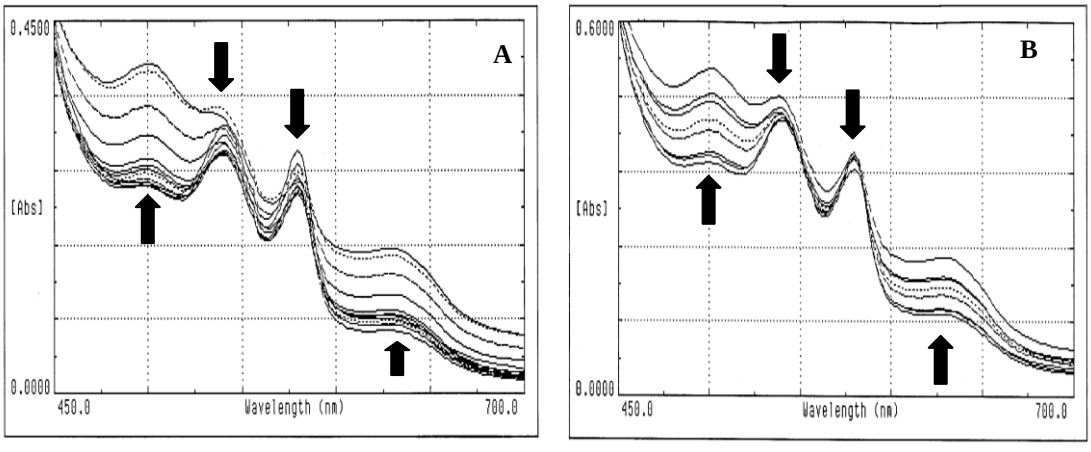


Figure 26. Spectrophotometric oxymyoglobin assay for **61** (A) and **62** (B).

These preliminary data show that both **61** and **62** are able to release NO in water and that rate of release is faster in the case of **61** as suggested by data reported in Figure 27 relative to the rate of oxidation of oxymyoglobin.

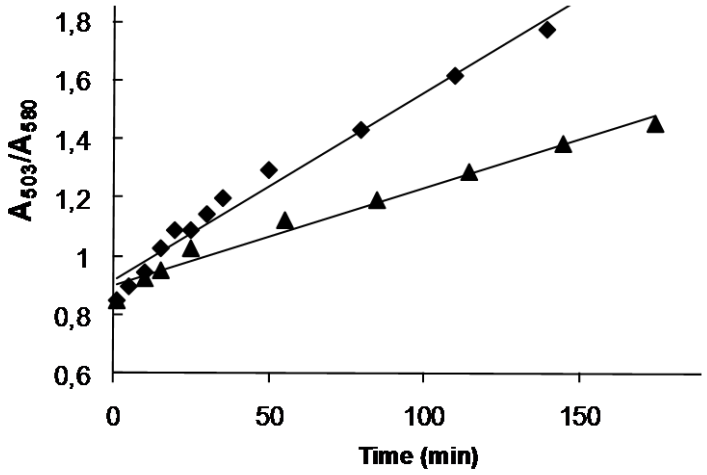


Figure 27. Change in the absorbance ratio A_{503}/A_{580} with the time during the oxidation of oxymyoglobin mediated by **61** (◆) or **62** (▲).

The second step toward the definition of the reactivity of nitrolinoleic acids was to isolate and characterize the products formed by the decomposition of the nitrolinoleic acids. The incubation of **61** in 0.1 M phosphate buffer, pH 7.4, resulted in the generation of a very complex mixture of products, as revealed by TLC analysis. After 2 h, the mixture was worked up and chromatographed on silica gel plates to give three main products, A (R_f = 0.20), B (R_f = 0.30), and C (R_f = 0.36), each detectable by a UV lamp (254 nm) and positive to the Griess reagent specific for nitrite-releasing substances. The same pattern was also obtained by reacting ^{15}N-labeled **61** prepared as previously described. All the isolated compounds were submitted to complete spectroscopic characterization.

Compound A exhibited in the ESI(+)-MS spectrum the pseudomolecular ion peaks at *m/z* 364 and 380 ($[M+Na]^+$ and $[M+K]^+$, respectively),[127] and in the ESI(-)/MS/MS spectrum the pseudomolecular ion peak $[M-H]^-$ at *m/z* 340, with its daughter ions at *m/z* 322, 294, 293 due to the loss of H_2O, NO_2 and HNO_2, respectively, and at *m/z* 46 due to $[NO_2]^-$. On the basis of extensive 1D and 2D NMR experiments the compound was thus formulated as (9*E*,11*E*)-13-hydroxy-9-nitro-9,11-octadecadienoic acid (**63**) (15% isolated yield).

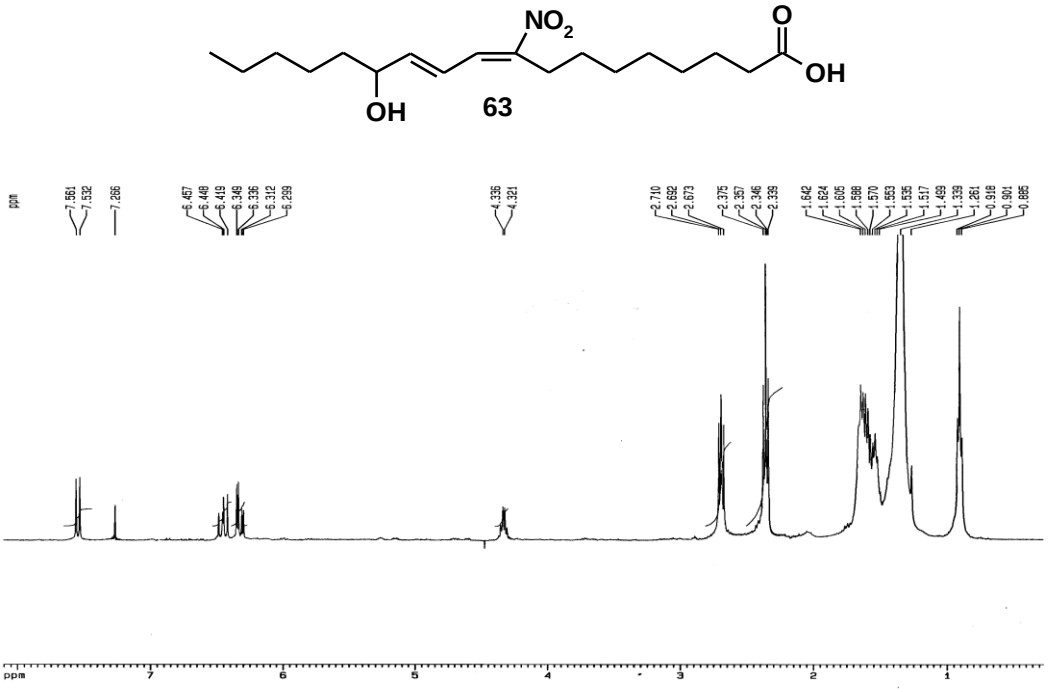
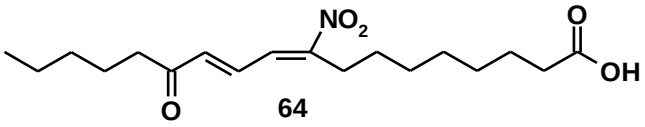


Figure 28. ^1H NMR spectrum ($CDCl_3$) of **63**.

The ^{15}N-labeled **63** consistently exhibited in the ESI(+)-MS spectrum the pseudomolecular ion peaks ($[M+Na]^+$ and $[M+K]^+$) at *m/z* 365 and 381, respectively, and in the $^1H,^{15}N$ HMBC spectrum two distinct cross peaks between the proton signals at δ 7.55 and 2.69 and the nitrogen resonance at δ 376.7. The structural assignment was also secured by the comparison of the spectroscopic data with those

reported in the literature for the corresponding methylester isolated from the reaction of 13-hydroxyoctadecadienoic acid with nitrite under acidic conditions.[145]

Compound B showed in the ESI(+) mass spectrum a pseudomolecular ion peak $[M+H]^+$ at *m/z* 340, whereas the ESI(-)/MS/MS analysis revealed the pseudomolecular ion peak $[M-H]^-$ at *m/z* 338 and its daughter ions at *m/z* 292 and 46 due to $[(M-NO_2)-H]^-$ and $[NO_2]^-$. The ^1H NMR spectrum (Figure 29) showed the same pattern of three olefinic proton signals displayed by **63**, with also a deshielded methylene proton signal at δ 2.79. A salient feature of the ^{13}C NMR spectrum was the signal at δ 199.2 suggestive of a conjugated keto group. The $^1H,^{15}N$ HMBC spectrum of the ^{15}N-labeled compound showed two distinct cross peaks between the proton signals at δ 7.51 and 2.79 and a nitrogen resonance at δ 374.9, while the MS spectrum indicated a pseudomolecular ion peak $[M+H]^+$ at *m/z* 341. Based on these data, the compound was identified as (9*E*,11*E*)-9-nitro-13-oxo-9,11-octadecadienoic acid (**64**) (9% isolated yield).



64

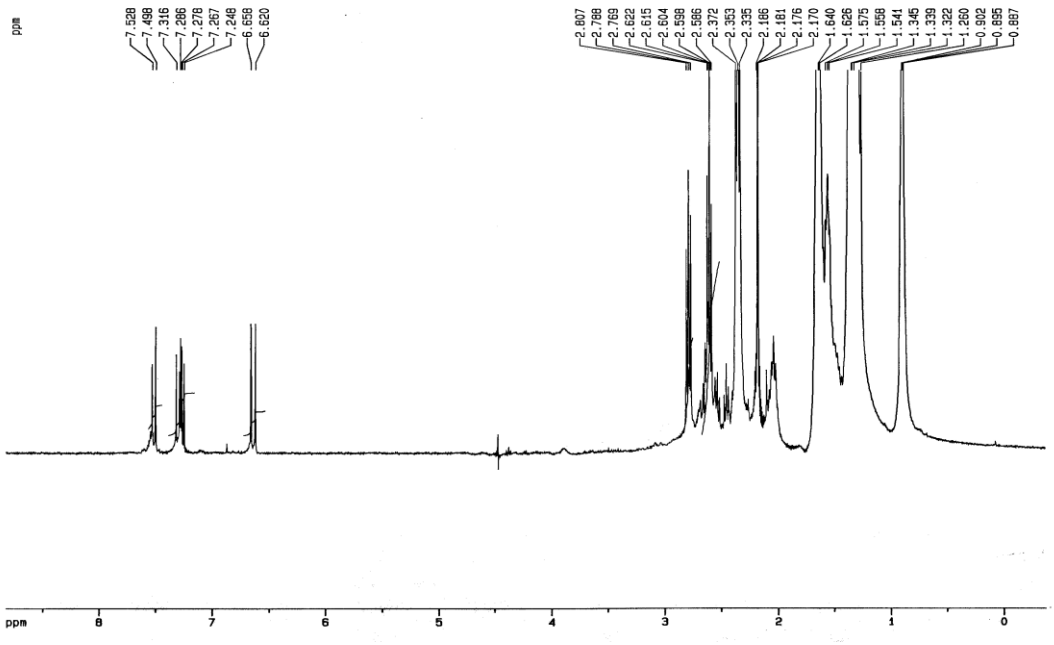
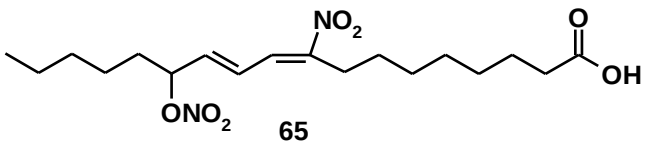


Figure 29. ^{1}H NMR spectrum ($CDCl_3$) of **64**.

The least polar compound C displayed in the ^{1}H NMR spectrum (Figure 30), besides the three typical olefinic proton signals, a double triplet at δ 5.41 giving a cross peak in the ^{1}H,^{13}C HMQC spectrum with a carbon signal at δ 83.0. Quite unexpectedly, the ^{1}H, ^{15}N HMBC spectrum of the labeled derivative revealed the presence of two nitrogen groups, one at δ 375.3 correlating with the proton signals at δ 7.48 and 2.70, and the other one at δ 338.4, suggestive of a nitrate ester,[146,147] correlating with the proton signal at δ 5.41. Accordingly, the compound was identified as the (9*E*,11*E*)-9-nitro-13-nitrate-9,11-octadecadienoic acid (**65**) (6% isolated yield).



65

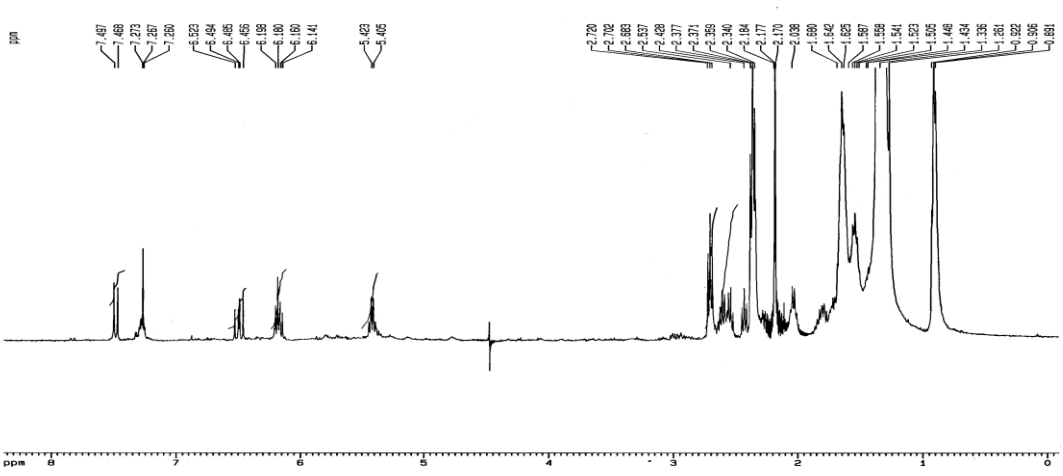


Figure 30. ^{1}H NMR spectrum ($CDCl_3$) of **65**.

The ESI(+) mass spectrum of **65** showed peaks for the $[M+Na]^+$ and $[M+K]^+$ ions at *m/z* 409 and 425, as well as peaks at *m/z* 324, 346, and 362, suggesting the loss of HNO_3 from the $[M+H]^+$, $[M+Na]^+$, and $[M+K]^+$ pseudomolecular ion peaks, respectively. The ESI(-)/MS/MS analysis showed the pseudomolecular ion peak $[M-H]^-$ at *m/z* 385 and its daughter ion at *m/z* 62 due to $[NO_3]^-$. The ^{15}N-labeled derivative gave the expected pseudomolecular ion peaks at *m/z* 411 and 427 ($[M+Na]^+$ and $[M+K]^+$, respectively) and peaks at *m/z* 325, 347, and 363 consistent with loss of $H^{15}NO_3$ from the pseudomolecular ion peaks. ESI(-)/ MS/MS experiments gave the pseudomolecular ion peak $[M-H]^-$ at *m/z* 387 and its daughter ion at *m/z* 63 assigned to $[^{15}NO_3]^-$.

Although products **63-65** account for a modest mass balance, data refer to isolated yields and therefore underestimate actual product yields. Scrutiny of the ^{1}H and ^{13}C NMR spectra of the crude reaction mixtures after **61** had completely disappeared revealed the expected patterns of resonances for **63-65** and traces of other unknown species. The same products **63-65** were also formed by decomposition of 20 μM **61**

(HPLC), suggesting that their formation is independent of substrate concentration. In a subsequent set of control experiments, it was found that: (a) in unbuffered water (pH around 5) the formation of products arising from the peroxidation of **61** was markedly slower with no detectable **65**; (b) under an argon atmosphere the formation of the products **63**-**65** was inhibited; (c) when **61** was allowed to decompose at room temperature as a dry film, the formation of **63** and **64** occurred but no detectable **65**.

To gain a deeper insight into the products resulting from aqueous decomposition of **61**, the crude reaction mixtures obtained from both the unlabeled and ^{15}N-labeled substrates were investigated by LC-MS. The analysis of the LC traces at 2 h reaction time indicated the presence, besides **63**, **64**, and **65**, of several additional species, one of which exhibited distinct $[M+Na]^+$ and $[M+K]^+$ pseudomolecular ion peaks at *m/z* 380 and 396, suggesting the 13-hydroperoxy derivative of **61**. Another species displayed a $[M+H]^+$ pseudomolecular ion peak at *m/z* 295, compatible with a dienone in which the nitro group was evidently lost. The lack of nitrogen was corroborated by the analysis of the mixture from ^{15}N-labeled **61**, revealing the same pseudomolecular ion for the same peak. Similar species generated by decomposition of nitrolinoleic acids have been reported previously.[132] Despite **61**, the decay of **62** in aqueous phosphate buffer at pH 7.4 gave, after 2 h, only one main product which was isolated by preparative TLC. The diagnostic features in the ^{1}H NMR spectrum (Figure 31) were two sets of multiplets at δ 4.44-4.46 and 4.06/3.90, showing one-bond correlations with carbon resonances at δ 91.7/92.5 and at δ 72.4/71.5, respectively. These and ^{1}H,^{1}H COSY, ^{1}H,^{13}C HMQC and ^{1}H,^{13}C HMBC data indicated for the product the structure of the (12*Z*)-9-hydroxy-10-nitrooctadec-12-enoic acid (**66**, 9% isolated yield), obtained as a 1:1 mixture of diastereoisomers (pairs of enantiomers).

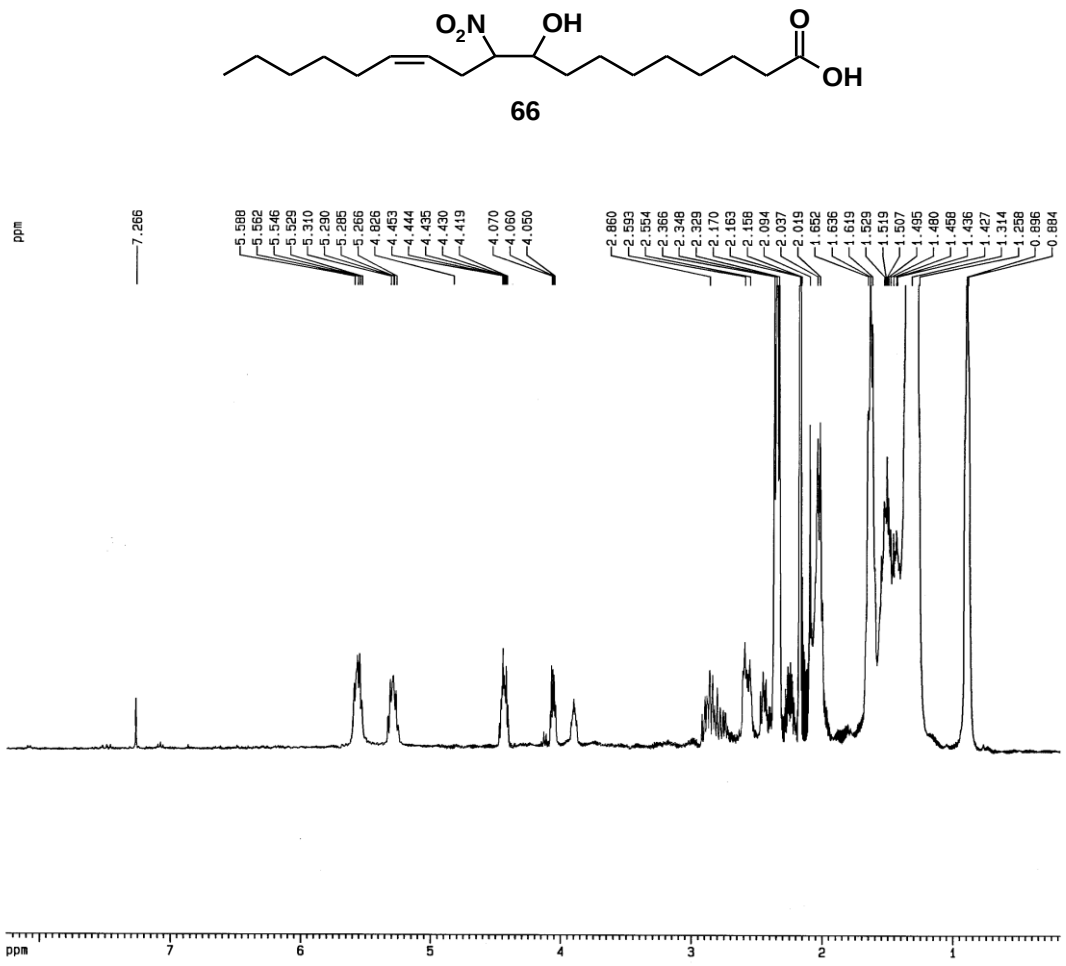
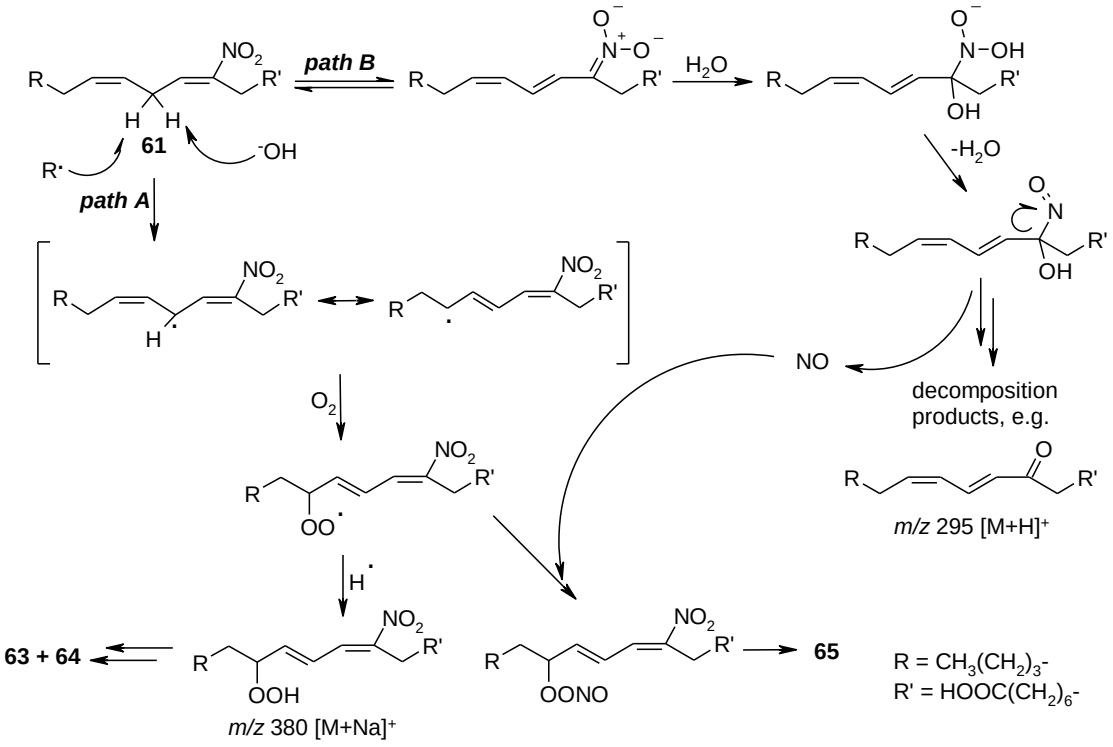


Figure 31. ^{1}H NMR spectrum ($CDCl_3$) of **66**.

This conclusion was supported by the results of the LC-MS analysis showing two species eluted under two very close peaks (16.8 and 17.2 min) with the same pseudomolecular ion $[M+H]^+$ at *m/z* 344.

Unreacted **62** accounted for about the 80% of the mixture after 2 h reaction time. Although no other single product could be isolated from the mixture, NMR and LC-MS investigations indicated the formation, besides **66**, of several species all in minute amounts.

Overall, the identification of the products deriving from the degradation of the nitrolinoleic acids suggested three main degradation channels of the nitropentadienyl system in neutral aqueous buffer, namely: (a) H-atom abstraction from the bis-allylic methylene group triggering the classic peroxidation chain reactions; (b) NO-release; (c) the nucleophilic addition of water onto the nitroalkene moiety. This latter path accounted for the formation of **66** by the decay of **62**,whereas the first two routes led to **63-65**, as outlined in Scheme 15.



Scheme 15. Mechanistic pathway proposed for the formation of compounds **63-65** from **61**.

The H-atom abstraction from the bis-allylic methylene group (path A) would result in a stabilized nitropentadienyl radical which would undergo coupling with oxygen to give **63** and **64** via the classic lipid peroxidation steps. This path is supported by the mass spectrometric identification of the hydroperoxy derivative and the

substantial inhibition of product formation in the absence of oxygen. NO-release is apparently a pH-dependent route triggered by a deprotonation equilibrium, and is involved in the formation of **65** at pH 7.4. A substantial deprotonation of the nitrolinoleic acids can be predicted at pH 7.4 based on a suggested pK_a value of 5 for the allylic methylene group[132] and would be favored in the case of **61**, from which a highly conjugated, resonance-stabilized nitronate structure can be generated. One viable mechanism for NO-release is based on the recently proposed modified Nef sequence[132] and is sketched in path B. In this route, the first formed nitronate anion would be converted to a hydroxynitroso intermediate which can undergo homolytic cleavage to form NO and an allylic radical. The latter might then react with oxygen to give eventually a dienone product, as suggested by the presence of a species in the LC-MS chromatogram with a pseudomolecular ion peak at *m/z* 295.

Alternative mechanisms, mutually non-exclusive, can account for NO-release: for example, the nitroalkene rearrangement to a nitrite ester followed by N-O bond homolysis to form NO and a carbon-centered radical,[136] but they cannot be distinguished based on the present results. Fast and effective coupling of NO with peroxyl radicals ($k > 10^{10}$ M^{-1} s^{-1})[148] produced by peroxidation of **61** followed by the rearrangement of the resulting peroxynitrite ester intermediate is likely to be involved in the formation of **65**. It may be relevant to notice, in this connection, that the efficient trapping of NO by the peroxyl radicals present in the mixture may bias estimation of NO release by chemiluminescence and other assays.

Product analysis suggested a higher oxidizability of **61** relative to **62**. Since H-atom abstraction from the 1,4-pentadiene system (C-H bond dissociation enthalpy (BDE) = 76.4 kcal mol^{-1})[149-151] dictates the oxidizability of polyunsaturated fatty acids, at

least a rough estimate of the relative abilities of nitrolinoleic acids to act as H-atom donors was desirable. Accordingly, the activity of **61** and **62** in the diphenylpicryl hydrazide (DPPH) radical quenching spectrophotometric test was determined against linoleic acid as a reference fatty acid.[152] This test, which is run in ethanol and is therefore independent of pH-related effects, enables measurement of the radical-scavenging properties of a given substance and is often used in the food industry to establish the rank order of antioxidants. The results in Figure 32 indicated more effective H-atom transfer to the DPPH radical from **61** than from **62** or linoleic acid. This trend can be taken to suggest a larger radical stabilizing effect of the nitro group on the terminal pentadienyl positions, making **61** more prone to H-atom loss and free radical formation.

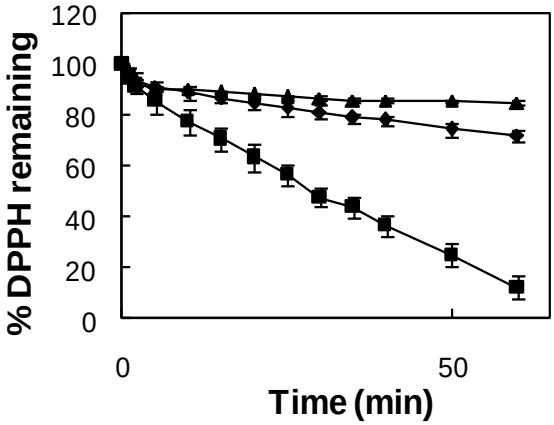


Figure 32.-Absorbance decay at 515 nm of a 100 μM solution of DPPH in ethanol after addition of: **61** (■), **62** (◆) and linoleic acid (▲). Final fatty acid concentration: 7.7 mM. All experiments have been carried out in triplicate and data are expressed as average ± S.D.

This conclusion was supported by a computational (DFT) investigation of the isomeric nitro-1,4-pentadienyl systems (Figure 33) carried out by Dr Marianna Arzillo and Professor Orlando Crescenzi (Department of Chemistry, University of

Naples Federico II), showing that compound **68R** is less stable than **67R** by 8.4 kcal mol^{-1}, with **67** and **68** almost isoenergetic.[153]

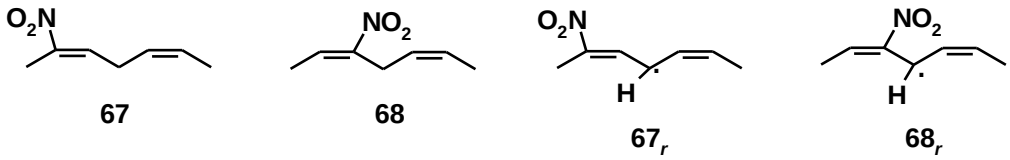


Figure 33. Simplified truncated structures **67** and **68** for the isomeric nitro-1,4-pentadienyl systems and the corresponding bis-allylic radicals **67R** and **68R**.

Conclusions

The preparation of two isomeric nitrolinoleic acids in pure form is reported for the first time (only the ester derivative of **62** was obtained previously),[130] and their markedly different chemical behavior is disclosed. Compound **61**, bearing a nitro group on the terminal position of the pentadiene moiety, is markedly unstable when exposed to phosphate buffer at pH 7.4, and partitions mainly between two concurrent degradation pathways, one resulting in NO-release presumably via a base dependent Nef-type reaction, and the other involving a typical peroxidation process. A remarkable outcome is the formation of the nitronitrate ester **65** involving probably trapping of NO by transient peroxyl radicals. Compound **62** decays at slower rate and gives mainly the stereoisomeric hydroxynitro derivatives **66** by nucleophilic addition of water. The differential behavior of **61** and **62** can be rationalized on the basis of DPPH radical quenching experiments and DFT calculations, indicating a higher H-atom donating ability and a larger radical stabilization when the nitro group is located on the terminal rather than inner positions of the 1,4-pentadienyl core. These results provide an underpinning for ongoing investigations of the physiological properties of nitrolinoleic acids and may orient the design of new pharmacologically active nitrated lipids. They also offer a plausible explanation as to why **61** and the isomeric 13-nitrolinoleic acid have so far eluded detection in biological systems.

Further details about results shown in this chapter can be found in the following article:

Manini, P.; Capelli, L.; Reale, S.; Arzillo, M.; Crescenzi, O.; Napolitano, A.; Barone, V.; d'Ischia, M. *J. Org. Chem.* **2008**, *73*, 7517-7525.

Experimental Section

Materials and methods. All commercially available reagents were used as received. Anhydrous solvents were purchased from commercial sources and withdrawn from the container by syringe, under a slight positive pressure of argon. All the solvents were of analytical grade quality.

Phosphate buffers, 0.1 M pH 7.4 and 50 mM + 100 μM EDTA pH 7.4, were treated with Chelex-100 resin before use to remove transition metal contaminants. Oxymyoglobin was prepared from horse heart myoglobin following a procedure described in the literature.[154] UV spectra were performed using a diode array spectrophotometer. NMR spectra were recorded with 200, 300, 400 and 500 MHz instruments. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ using TMS as the internal standard. ^{15}N spectra were recorded at 40.5 MHz using [^{15}N]urea as the internal standard (76.97 ppm in DMSO relative to NH_3 (liquid, 298 K) 0.0 ppm (^{15}N NMR)).[146] $^{1}H,^{1}H$ COSY, $^{1}H,^{13}C$ HSQC, $^{1}H,^{13}C$ HMBC, $^{1}H,^{15}N$ HMBC and TOCSY experiments were run at 400.1 MHz using standard pulse programs. For all isolated compounds ^{1}H and ^{13}C NMR resonances due to C-2 (CH_2), C-3/C-4/C-5/C-6/C-7/C-15/C-16/C-17 (CH_2) and C-18 (CH_3) groups appear in the $^{1}H/^{13}C$ NMR spectra at δ ($CDCl_3$) 2.34 (2H, t, *J* = 7.4 Hz)/33.6, 1.2-1.6 (16H, m)/22-32, 0.89 (3H, t, *J* = 7.2 Hz)/14.0, in the order. Purity of isolated compounds was determined by ^{1}H NMR analysis.

Analytical and preparative TLC analyses were performed on F_{254} silica gel plates (0.25 and 0.5 mm, respectively) using cyclohexane/ethyl acetate 70:30 (v/v)

containing 1% acetic acid (eluant a) or cyclohexane/ethyl acetate 90:10 (v/v) (eluant b). Silver-impregnated silica gel plates were prepared by elution with a 1 M solution of $AgNO_3$ in acetonitrile.[130a] Silver-impregnated silica gel was prepared by suspending in a 1 M solution of $AgNO_3$ in acetonitrile for 30 min and recovering the residue by filtration and drying under reduced pressure. TLC plates were visualised using a UV lamp (λ = 254 nm) and developed with the Griess reagent (1% sulphanilamide and 0.1% naphthylethylenediamine in 5% phosphoric acid),[155] $Ce(SO_4)_2$ (0.05 M in 10% H_2SO_4), or iodine as appropriate. Liquid chromatography was performed on silica gel (60-230 mesh). Preparative HPLC was carried out on an instrument coupled with a UV detector set at 254 nm. An octadecylsilane-coated column (22 × 250 mm, 10μm df) was used. The following isocratic conditions were used for elution: 1% acetic acid/acetonitrile 30:70 (v/v) (eluant I) at a flow rate of 40 mL/min. LC-MS analyses were carried out on a HPLC apparatus coupled with a quadrupole mass spectrometer. HPLC runs were performed on an octylsilane-coated column (4.6 × 150 mm, 5 μm df) using the following elution conditions: 0.5% formic acid (solvent A) and acetonitrile (solvent B), from 50% to 70% solvent B gradient, for 50 min, and then from 70% to 50% solvent B gradient, for 10 min (eluant II). Flow rate of 0.4 mL/min was used. (High resolution) mass spectra were registered in the electrospray ionization-positive ion (ESI+) mode with the cone and the fragmentator voltages set at 4 kV and 80V, respectively. ESI(-)/MS/MS analysis were carried out on a triple quadrupole mass spectrometer equipped with a Z-spray electrospray source. Negative ion mass spectra were recorded with the cone voltage set at 7 V. The source temperature was kept at 120 °C. Daughter ions were obtained with a collision energy of 10 V and a gas (Ar) pressure of 2.0×10^{-4} mbar.

Synthesis of (9*E*,12*Z*)-9-Nitroottadecadienoic Acid (61). The title compound was prepared according to a procedure reported in the literature with modifications.[126,128,131] In brief, a solution of linoleic acid (1 g, 3.6 mmol) in dry THF (6 mL) was added to a solution of PhSeBr (843 mg, 3.6 mmol) in dry THF (6 mL) at -78 °C under an argon atmosphere. After 10 min, $HgCl_2$ (1.26 g, 4.6 mmol) was added to the mixture followed by a solution of $AgNO_2$ (550 mg, 3.6 mmol) in dry acetonitrile (12 mL, aliquots of 1 mL over 30 min). After 2 h, the reaction mixture was taken at room temperature for 1.5 h, then filtered on Celite and washed extensively with diethyl ether. The surnatant was evaporated to dryness, taken up in $CHCl_3$, extracted with brine, and evaporated under reduced pressure after drying over anhydrous sodium sulfate. The residue was subjected to preparative HPLC (eluant I) to afford two fractions, **I** (t_R = 18-20 min) and **II** (t_R = 21-23 min). Fractions **I** and **II** were evaporated under reduced pressure and worked up as described above. The residues were taken up in $CHCl_3$ and treated separately with H_2O_2 (4 molar equiv) under vigorous stirring for 30 min at 4 °C and for additional 30 min at room temperature. After workup as above, residues were subjected to preparative TLC (eluant a) to afford from fraction **I** a 1:1 mixture of **61** and **62** (264 mg) and from fraction **II** pure **61** (135 mg, 12% yield, R_f = 0.50, eluant a).

61: ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 2.05 (2H, m, H-14), 2.59 (2H, t, *J* = 7.6 Hz, H-8), 2.95 (2H, t, *J* = 7.6 Hz, H-11), 5.34 (1H, m, H-12), 5.52 (1H, m, H-13), 7.02 (1H, t, *J* = 7.6 Hz, H-10); ^{13}C NMR (100 MHz $CDCl_3$) δ (ppm) 26.9 (CH_2), 27.1 (CH_2), 28.2 (CH_2), 123.3 (CH), 133.3 (CH), 134.4 (CH), 151.8 (C) 180.0 (C); LC-MS t_R = 43 min; ESI(+)-MS *m/z* 326 ($[M+H]^+$), 348 ($[M+Na]^+$), 364 ($[M+K]^+$); ESI(+)-HRMS for $C_{18}H_{31}NO_4$ calcd 325.2253 $[M+H]^+$, found 325.2249.

Synthesis of [^{15}N]-(9*E*,12*Z*)-9-Nitroottadecadienoic Acid ([^{15}N]61). The title compound was prepared as described for **61**, but using $Na^{15}NO_2$ (250 mg, 3.6 mmol) instead of $AgNO_2$. Two fractions were isolated, one consisting of pure [^{15}N]**61** (105 mg, 9% yields) and the other of a 1:1 mixture of [^{15}N]**61** and [^{15}N]**62** (198 mg).

[^{15}N]**61**: 1H NMR (300 MHz, $CDCl_3$) δ (ppm) 2.05 (2H, m, H-14), 2.59 (2H, dt, *J* = 7.6, 3.6 Hz, H-8), 2.95 (2H, t, *J* = 7.6 Hz, H-11), 5.34 (1H, dt, *J* = 7.2, 10.0 Hz, H-12), 5.52 (1H, dt, *J* = 7.2, 10.5 Hz, H-13), 7.02 (1H, dt, *J* = 7.6, 3.6 Hz, H-10); LC-MS t_R = 43 min; ESI(+)-MS *m/z* 327 ($[M+H]^+$), 349 ($[M+Na]^+$), 365 ($[M+K]^+$); ESI(+)-HRMS for $C_{18}H_{31}{}^{15}NO_4$ calcd 326.2223 $[M+H]^+$, found 326.2220.

Synthesis of allyl (9*Z*,12*Z*)-octadecadienoate. The title compound was prepared according to a reported procedure.[140] In brief, a solution of linoleic acid (1.0 g, 3.6 mmol) in toluene (2.4 mL) was treated with allyl alcohol (2.4 mL, 35.2 mmol) and *p*-toluensolfonic acid (2.0 mg, 0.011 mmol) under reflux and vigorous stirring. After 18 h the mixture was evaporated under reduced pressure, resolvated with diethyl ether and extracted first with NaOH 0.1 M and then with water. The organic layer was dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The residue was fractionated on silica gel (eluant b) to afford pure allyl (9*Z*,12*Z*)-octadecadienoate (1.0 g, 90% yield, R_f = 0.68, eluant b).

Allyl (9*Z*,12*Z*)-octadecadienoate: ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm)[156] 2.04 (4H, dt, *J* = 6.6, 6.3 Hz, H-14, H-8), 2.76 (2H, t, *J* = 5.7 Hz, H-11), 4.56 (2H, d, *J* = 6.0 Hz, -OCH_2-), 5.21 (1H, dd, *J* = 10.5, 0.9 Hz, -OCH_2CH=CH_2), 5.30 (1H, dd, *J* = 17.1, 0.9 Hz, -OCH_2CH=CH_2), 5.33 (4H, m, H-9, H-10, H-12, H-13), 5.91 (1H, ddt, *J* = 17.1, 10.5, 6.0 Hz -OCH_2CH=CH_2); ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 25.6

(CH_2), 29.0 (2 × CH_2), 64.8 (-OCH_2-), 118.0 (-OCH_2CHCH_2), 127.9 (CH), 128.0 (CH), 130.0 (CH), 130.1 (CH), 132.3 (-OCH_2CHCH_2), 173.4 (C).

Synthesis of allyl (9*E*,12*Z*)-10-Nitrooctadecadienoate (62-allylester). The title compound was prepared following the nitrophenylselenenylation protocol described for **61** with allyl (9*Z*,12Z)-octadecadienoate as the substrate. After workup, the residue was fractionated on silica gel using cyclohexane/ethyl acetate (gradient mixture from pure cyclohexane to cyclohexane/ethyl acetate 98:2) to afford the mixture of nitrophenylselenenyl-adducts of allyl (9*Z*,12*Z*)-octadecadienoate (1.0 g, 60% yield). This latter was taken up in $CHCl_3$ and treated with H_2O_2 (4 molar equiv) under vigorous stirring at 4 °C. After 30 min, the mixture was taken at room temperature for additional 30 min. The reaction mixture was then extracted with brine, and the organic layers were dried over anhydrous sodium sulfate, filtered, and evaporated. The residue was fractionated on silver-impregnated silica gel (eluant cyclohexane) to afford two fractions, one consisting of a mixture of **61**-allylester and **62**-allylester (98 mg) and the other of pure **62**-allylester (119 mg, 17% yield, R_f = 0.62, eluant b).

62-allylester: ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 2.12 (dt, *J* = 7.2 Hz, H-14), 2.23 (2H, dt, *J* = 7.6 Hz, H-8), 3.33 (2H, d, *J* = 7.2 Hz, H-11), 4.56 (2H, d, *J* = 6.0 Hz, -OCH-), 5.2-5.3 (1H, m, H-12), 5.23 (1H, dd, *J* = 10.5, 0.9 Hz, -OCH_2CH=CH_2), 5.31 (1H, dd, *J* = 17.1, 0.9 Hz, -OCH_2CH=CH_2), 5.48 (1H, m, H-13), 5.90 (1H, ddt, *J* = 17.1, 10.5, 6.0 Hz, -OCH_2CH=CH_2), 7.09 (1H, dt, *J* = 7.6, 3.9 Hz, H-9); ESI(+)-HRMS for $C_{21}H_{35}NO_4$ calcd. 365.2566 $[M+H]^+$, found 325.2563.

Deprotection of 62-allylester. A solution of **62**-allylester (119 mg, 0.3 mmol) in dry THF (5.84 mL) was treated with HCOOH (123 μL, 3.3 mmol) and $Pd(PPh_3)_4$ (18.8 mg, 16.3 μmol) under an argon atmosphere at 80 °C. After 24h, the reaction mixture was filtered on Celite, evaporated to dryness, and fractionated by preparative TLC (eluant a) to afford **62** (87 mg, 82% yield, R_f = 0.48, eluant a).

62: ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 2.12 (2H, dt, *J* = 7.2 Hz, H-14), 2.21 (2H, dt, *J* = 7.6 Hz, H-8), 3.33 (2H, d, *J* = 6.9 Hz, H-11), 5.26 (1H, m, H-12), 5.48 (1H, m, H-13), 7.08 (1H, dt, *J* = 7.6, 3.9 Hz, H-9); ^{13}C NMR (50 MHz $CDCl_3$) δ (ppm) 25.1 (CH_2), 27.9 (CH_2), 28.0 (CH_2), 123.8 (CH), 132.7 (CH), 136.2 (CH), 150.8 (C) 179.3 (C); LC-MS t_R = 42 min; ESI(+) *m/z* 326 ($[M+H]^+$), 348 ($[M+Na]^+$), 364 ($[M+K]^+$).

Autooxidation of nitrolinoleic acids: general procedure. The appropriate nitrolinoleic acid was dissolved under vigorous stirring in a solution of 0.1 M phosphate buffer, pH 7.4, containing a 20% of ethanol (final concentration: 1 mM). At regular intervals, aliquots of the reaction mixture were withdrawn and subjected to HPLC or LC-MS analysis. Similar experiments were carried out: a) starting with **61** at 200 μM; b) starting with either the nitrolinoleic acids at 20 μM; c) under an argon atmosphere; d) in dry film.

Spectrophotometric analysis

a) Oxymyoglobin assay. The assay was carried out according to a reported procedure.[132] A suspension of the appropriate nitrolinoleic acid (1.2 mM) in 50 mM phosphate buffer plus 100 μM EDTA pH 7.4 was treated under vigorous stirring with

a solution of oxymyoglobin (40 μM) in the same buffer. Absorption measurements were made at regular intervals monitoring oxymyoglobin (543 and 580 nm) and metmyoglobin (503 and 630 nm) absorption maxima.

b) 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH) assay. The assay was carried out according to a reported procedure.[152] **61** Or **62** or linoleic acid in ethanol (7.7 mM) was reacted under vigorous stirring with DPPH in ethanol (100 μM). Absorption measurements were made at regular intervals monitoring spectrophotometrically the decay of the nitroxyl radical at 515 nm.

Isolation of (9*E*,11*E*)-13-Hydroxy-9-nitro-9,11-octadecadienoic Acid (63), (9*E*,11*E*)-9-Nitro-13-oxo-9,11-octadecadienoic Acid (64), and (9*E*,11*E*)-9-Nitro-13-nitrate-9,11-octadecadienoic Acid (65). A solution of **61** (75 mg) in ethanol (44 mL) was added to 0.1 M phosphate buffer, pH 7.4 (178 mL) and kept at 37 °C and under vigorous stirring. After 2 h, the reaction mixture was acidified to pH 3 with HCl 3 M and extracted with chloroform. After drying over anhydrous sodium sulfate and evaporation of the solvent, the residue was fractionated by preparative TLC (eluant a) to afford **63** (11 mg, 15% yield, R_f = 0.20, eluant a), **64** (7 mg, 9% yield, R_f = 0.30, eluant a), and **65** (5 mg, 6% yield, R_f = 0.36, eluant a).

63: ^{1}H NMR (400 MHz,$CDCl_3$) δ (ppm) 1.2-1.6 (2H, m, H-14), 2.69 (2H, t, *J* = 7.6 Hz, H-8), 4.33 (1H, m, H-13), 6.32 (1H, dd, *J* = 14.8, 5.2 Hz, H-12), 6.45 (1H, dd, *J* = 14.8, 11.6 Hz, H-11), 7.55 (1H, d, *J* = 11.6 Hz, H-10); ^{13}C NMR (100 MHz $CDCl_3$) δ (ppm) 27.2 (CH_2), 37.8 (CH_2), 72.7 (CH), 122.8 (CH), 133.4 (CH), 150.0 (CH), 151.6 (C), 178.6 (C); LC-MS t_R = 20 min; ESI(+)- MS[127] *m/z* 324 ([M-

$H_2O+H]^+$), 364 ([M+Na]$^+$), 380 ([M+K]$^+$); ESI(-)/MS/MS *m/z* 340 ([M-H]$^-$), 322 ([(M-H_2O)-H]$^-$), 294 ([(M-NO_2)-H]$^-$), 293 ([(M-HNO_2)-H]$^-$), 46 ([NO_2]$^-$).

64: ^{1}H NMR (400 MHz,$CDCl_3$) δ (ppm) 2.62 (2H, t, *J* = 7.6 Hz, H-14), 2.79 (2H, t, *J* = 7.6 Hz, H-8), 6.64 (1H, d, *J* = 15.2 Hz, H-12), 7.28 (1H, dd, *J* = 15.2, 12.0 Hz, H-11), 7.51 (1H, d, *J* = 12.0 Hz, H-10); ^{13}C NMR (125 MHz $CDCl_3$) δ (ppm) 27.2 (CH_2), 42.3 (CH_2), 129.6 (CH), 137.3 (CH), 156.1 (CH), 158.2 (C), 177.9 (C), 199.2 (C); LC-MS t_R = 26 min; ESI(+)-MS *m/z* 340 ([M+H]$^+$), 362 ([M+Na]$^+$), 378 ([M+K]$^+$); ESI(-)/MS/MS: *m/z* 338 ([M-H]$^-$), 292 ([(M-NO_2)-H]), 46 ([NO_2]$^-$); ESI(+)-HRMS for $C_{18}H_{29}NO_5$ calcd. 339.2046 [M+H]$^+$, found 339.2049.

65: ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.80 (2H, m, H-14), 2.70 (2H, t, *J* = 7.6 Hz, H-8), 5.41 (1H, dt, *J* = 7.2, 6.8 Hz, H-13), 6.17 (1H, dd, *J* = 15.2, 7.2 Hz, H-12), 6.49 (1H, dd, *J* = 15.2, 11.6 Hz, H-11), 7.48 (1H, d, *J* = 11.6 Hz, H-10); ^{13}C NMR (100 MHz $CDCl_3$) δ (ppm) 27.2 (CH_2), 26.4 (CH_2), 83.0 (CH), 127.6 (CH), 129.3 (CH), 132.6 (CH), 153.8 (C), 179.0 (C); LC-MS t_R = 39 min; ESI(+)-MS *m/z* 324 ([M-HNO_3+H]$^+$), 409 ([M+Na]$^+$), 346 ([M-HNO_3+Na]$^+$), 425 ([M+K]$^+$), 362 ([M-HNO_3+K]$^+$); ESI(-)/MS/MS: *m/z* 385 ([M-H]$^-$), 62 ([NO_3]$^-$); ESI(+)-HRMS for $C_{18}H_{30}N_2O_7$ calcd 386.2053 [M+H]$^+$, found 386.2055.

Isolation of [^{15}N]-(9*E*,11*E*)-13-Hydroxy-9-nitro-9,11-octadecadienoic ([^{15}N]63), [^{15}N]-(9*E*,11*E*)-9-Nitro-13-oxo-9,11-octadecadienoic ([^{15}N]64) and [^{15}N]-(9*E*,11*E*)-9-Nitro-13-nitrate-9,11-octadecadienoic Acids ([^{15}N]65).

Compounds [^{15}N]**63**, [15N]**64**, and [^{15}N]**65** were prepared from [^{15}N]**61** following the same procedure described above for **61**.

[^{15}N]**63**: ^{1}H NMR (400 MHz,$CDCl_3$) δ (ppm) 1.2-1.6 (2H, m, H-14), 2.69 (2H, dt, *J* = 7.6, 3.6 Hz, H-8), 4.34 (1H, m, H-13), 6.32 (1H, dd, *J* = 14.2, 5.2 Hz, H-12), 6.45 (1H, dd, *J* = 14.8, 11.6 Hz, H-11), 7.55 (1H, dd, *J* = 11.6, 3.6 Hz, H-10); LC-MS t_R = 20 min; ESI(+)-MS^{127} *m/z* 325 ([M-H_2O+H]$^+$), 365 ([M+Na]$^+$), 381 ([M+K]$^+$).

[^{15}N]**64**: ^{1}H NMR (400 MHz,$CDCl_3$) δ (ppm) 2.61 (2H, t, *J* = 7.6 Hz, H-14), 2.79 (2H, dt, *J* = 7.6, 3.6 Hz, H-8), 6.64 (1H, d, *J* = 15.2 Hz, H-12), 7.28 (1H, dd, *J* = 15.2, 12.0 Hz, H-11), 7.51 (1H, dd, *J* = 12.0, 3.6 Hz, H-10); LC-MS t_R = 26 min; ESI(+)-MS *m/z* 341 ([M+H]+), 363 ([M+Na]+), 379 ([M+K]+); ESI(+)-HRMS for $C_{18}H_{29}{}^{15}NO_5$ calcd 340.2016 [M+H]$^+$, found 340.2020.

[^{15}N]**65**: ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.80 (2H, m, H-14), 2.70 (2H, dt, *J* = 7.6, 3.6 Hz, H-8), 5.41 (1H, ddt, *J* = 7.2, 6.8, 3.6 Hz, H-13), 6.17 (1H, dd, *J* = 15.2, 7.2 Hz, H-12), 6.49 (1H, dd, *J* = 15.2, 11.6 Hz, H-11), 7.48 (1H, dd, *J* = 11.6, 3.6 Hz, H-10); LC-MS t_R = 39 min; ESI(+)-MS *m/z* 325 ([M-H$^{15}NO_3$+H]$^+$), 411 ([M+Na]$^+$), 347 ([M-H$^{15}NO_3$+Na]$^+$), 427 ([M+K]$^+$), 363 ([M-H$^{15}NO_3$+K]$^+$); ESI(-)/MS/MS *m/z* 387 ([M-H]$^-$), 63 (^{15}[NO_3]$^-$); ESI(+)-HRMS for $C_{18}H_{30}{}^{15}N_2O_7$ calcd 388.1994 [M+H]$^+$, found 388.1996.

Isolation of (12*Z*)-9-hydroxy-10-nitro-12-ottadecenoic Acid (66) (Mixture of Stereoisomers). The reaction for compound **62** was carried out as described for **61**. After workup, preparative TLC afforded **66** (mixture of stereoisomers) (7 mg, 9% yield, R_f = 0.35, eluant a).

66: ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) (the superscripts a and b are referred to the two diastereoisomers) 1.2-1.6 (2H, m, H-8), 2.02 (2H, m, H-14), 2.51^b (1H, m, H-11), 2.60^a (1H, m, H-11), 2.78^b (1H, m, H-11), 2.91^a (1H, m, H-11),4.44-4.46^{a,b} (1H,

m, H-10), 3.90[b] (1H, m, H-9), 4.06[a] (1H, m, H-9), 5.29 (1H, m, H-12), 5.57 (1H, m, H-13); ^{13}C NMR (100 MHz CDCl3) δ (ppm) 25.0 (CH_2), 26.4[a] (CH_2), 28.2 (CH_2), 28.8[b] (CH_2), 71.5[b] (CH), 72.4[a] (CH), 91.7[a] (CH), 92.5[b] (CH), 121.9 (CH), 136.0 (CH), 179.1 (C); LC-MS t_R = 16.8 e 17.2 min; ESI(+)-MS *m/z* 344 ($[M+H]^+$), 366 ($[M+Na]^+$), 382 ($[M+K]^+$); ESI(+)-HRMS for $C_{18}H_{33}NO_5$ calcd 343.2359 $[M+H]^+$, found 343.2355.

LIST OF PUBLICATIONS

1) Capelli, L.; Crescenzi, O.; Manini, P.; Pezzella, A.; Barone, V.; d'Ischia, M. (**2010**). Designing π-Electron-Extended Eumelanin Building Blocks: Synthesis, Quantum Chemical Investigation and Peculiar Acid-Mediated Chemistry of 3-Alkynyl-5,6-dihydroxyindoles. *Submitted*.

2) Capelli, L.; Manini, P.; Pezzella, A.; d'Ischia, M. (**2010**). First synthetic entry to the trimer stage of 5,6-dihydroxyindole polymerization: *ortho*-alkynylaniline-based access route to the missing 2,7':2',7"-triindole. *Org. Biomol. Chem.* Vol. 8, pp.4243-4245.

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