Polyamidoamine dendritic fullerene derivatives for biological and material applications

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TESI DI DOTTORATO

Polyamidoamine dendritic fullerene derivatives for biological and material applications

(settore scientifico disciplinare CHIM /06)

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This thesis is dedicated to my father, whose love inspires me everyday.
“And now, I said, let me show in a figure how far our nature is enlightened or unenlightened: -- Behold! human beings living in a underground cave, which has a mouth open towards the light and reaching all along the cave; here they have been from their childhood, and have their legs and necks chained so that they cannot move, and can only see before them, being prevented by the chains from turning round their heads. Above and behind them a fire is blazing at a distance, and between the fire and the prisoners there is a raised way; and you will see, if you look, a low wall built along the way, like the screen which marionette players have in front of them, over which they show the puppets.”

The Republic, Book VII, Allegory of the Cave

Plato
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Abbreviations

([OMIM]BF_4): 1-methyl-3-n-octylimidazolium tetrafluoroborate
([BMIM]BF_4): 1-methyl-3-n-butyl imidazolium
Ab: antibody
AFM: atomic force microscopy
Boc: benzyloxy carbonyl
CBz: carboxy benzyl
CSS: charge separated state
DCTB: trans-2-[3-(4-tert-butylphenyl)-2-methyl-2-propenylidene]malononitrile
DBU: 1,8-diazabicyclo[5.4.0]undec-7-ene
DCC: dicyclohexyl carbodiimide
DCM: dichloromethane
DLS: dynamic light scattering
DMAD dimethyl acetylene dicarboxylate
DMAP 4-dimethyl aminopyridine
DMF: N,N-dimethylformamide
DMS: dimethyl sulfide
DMSO: dimethyl sulfoxide
EPO: erythropoietin
ESI: electro Spray Ionization
exTTF: extended-tetrathiafulvalene
Fc: ferrocene
FP: fulleropyrrolidine
HRTEM high resolution transmission electron microscopy
HOBt: hydroxybenzotriazole
Abbreviations

HOMO: high occupied molecular orbital
HPLC: high performance liquid chromatography
IL: ionic liquid
LUMO: low occupied molecular orbital
MALDI: matrix-assisted laser desorption/ionization
MW: microwave
NMR: nuclear magnetic resonance
o-DCB: orthodichlorobenzene
OPE: oligophenylethynyl
OPV: oligophenylenevinyl
P: porphyrin
P3HT: poly(3-hexylthiophene)
PAMAM: polyamidoamine
PCBM: [6,6]-Phenyl-C_{61}-butyric acid methyl ester
pDNA: plasmid DNA
PET: photoinduced electron transfer
SWCNTs: single walled carbon nanotubes
TFA: trifluoroacetic acid
TLC: thin layer chromatography
TTF: tetrathiafulvalene
TBADT: tetrabutylammonium decatungstate
TEG: triethylenglycol
TEM: Transmission Electron Microscopy
TTP: tetraphenyl porphyrin
VNPs: viral nanoparticles
ZnP: zinc porphyrin
Abstract

Fullerene C\textsubscript{60} science can be broadly divided into the study of three areas: (i) its reactivity that permits the obtention of modified fullerene derivatives, which can typically found (ii) biological and (iii) material applications.

The main goal of this thesis is the synthesis and characterization of a library of monoadducts, bisadducts and hexakisadducts of fullerene C\textsubscript{60} containing different generations of PAMAM dendron. On this purpose we have firstly studied the functionalization of fullerene C\textsubscript{60} by means of 1,3-dipolar cycloaddition. On this purpose, we moved from the classic conditions by employing MW irradiation as the heating source, combined with ionic liquids as the solvent phase in order to obtain remarkable differences in the reactivity and the polyaddition selectivity.

In a second stage we have been dealing with the separate synthesis of four different generations of PAMAM dendron and different Fulleropyrrolidine moieties including: monoadduct and five bisadducts isomers to finally attach them via amidation. We have complete this library with the employment of the Bingel-Hirsch reaction to attach twelve units of a PAMAM first generation dendron to the carbon cage to obtain a \(T_h\) symmetric hexakisadduct. As a result, a variety of fullerene derivatives with an enhanced water solubility was obtained, opening the door to their utilization for biological applications. Thus, we can distinguished between those containing terminal, positive-charged amines that can be used to efficiently complex oligonucleotides and those that contains one or more terminal-free carboxylic acid that can be used as anchor points for further functionalization.

Concerning to those potentially used for transfection, the broad range of examples described in this thesis will permit to examine the role of the dendron moiety, the fullerene, and the distribution of the positive charges around the fullerene sphere, as key points into the complexation and transfection processes. Furthermore, complexation studies of some of these derivatives has been performed, all of them exhibiting a high affinity towards DNA complexation, demonstrating the great potential of these derivatives for transfection.

In the last part of this thesis, we have focused on the synthesis of two porphyrin-dendrofullerene dyads with or without an amide linker. Since the water solubility usually goes hand in hand with a good number of charges we have incorporated an asymmetric tryspyridilporphyrin conferring three more positive charges to the final structure, that provides an additional solubility to the final molecule. This system is of the most interest since electron transfer processes could be studied in polar media. In addition, electrostatic interactions could be further exploited with negative charged systems to build up high complex systems.
Riassunto

Lo studio del Fullerene C\textsubscript{60} si concentra essenzialmente su tre aspetti principali: (i) la reattività, che porta all’ottenimento di potenziali derivati applicabili in (ii) campo biologico e nella (iii) ricerca di nuovi materiali.

Lo scopo principale di questo lavoro di tesi è la sintesi e la caratterizzazione di una libreria di monoaddotti, bisaddotti ed esa-addotti del fullerene C\textsubscript{60} modificati con diverse generazioni di dendron PAMAM.

A questo proposito è stata inizialmente studiata la funzionalizzazione del fullerene C\textsubscript{60} tramite la cicloaddizione 1,3-dipolare sfruttando l’irradiazione microonde combinata con liquidi ionici come mezzo solvente. L’ottimizzazione di questo approccio sintetico ha permesso di ottenere importanti risultati sul piano della resa e della selettività della poli-addizione.

In una seconda fase ci si è concentrati sulla sintesi separata di quattro diverse generazioni di dendron PAMAM e di varie unità fulleropirrolidiniche comprendenti: un monoaddotto e cinque isomeri di bisaddotto da legare ai dendroni attraverso reazioni di ammidazione. Questa libreria è stata completata con la sintesi di un esa-addotto simmetrico di tipo T\textsubscript{h}, ottenuto legando dodici unità di dendrone PAMAM di prima generazione alla sfera fullerenica tramite la reazione di Bingel-Hirsch.

Come risultato, è stata prodotta una batteria di derivati fullerenici dotati di notevole solubilità in acqua e di conseguenza di forte interesse per applicazioni biologiche. In particolare, all’interno della libreria si può distinguere una prima serie di derivati costituita da ammine terminali cariche positivamente, efficacemente impiegabili per la complessazione di oligonucleotidi, a cui se ne affianca una seconda contenente uno o più gruppi carbossilici terminali sfruttabili come punti di ancoraggio per successive funzionalizzazioni.

Nel corso della tesi si fa particolare riferimento all’applicabilità dei derivati come vettori per il trasferimento di geni attraverso la parete cellulare, sostenuta da studi di complessazione in gel elettroforesi da cui è emersa una forte affinità di alcuni campioni delle serie col DNA.

Nell’ultima parte di questa tesi verrà trattata la sintesi di due diadi porfirino-dendrofullereniche con e senza linker ammidico. Al fine di aumentare il numero di gruppi carichi dell’intera struttura e, con essi, la conseguente solubilità in acqua, è stata incorporata una tris-piridilporfirina recante tre azoti piridinici carichi positivamente.

Il sistema così progettato è di forte interesse in quanto consente lo studio di processi di trasferimento elettronico in solventi polari. Inoltre, le cariche positive possono essere ulteriormente sfruttate per interagire elettrostaticamente con gruppi carichi negativamente, formando strutture supramolecolari più complesse e introducendo nuovi risvolti applicativi.
Chapter 1

Introduction

“The roots of knowledge are bitter, but its fruit is sweet”
Aristotle
1.1 Fullerene \(\text{C}_{60}\), the most beautiful molecule in the world

Periodic Table of the Elements contains 118 chemical elements but a whole discipline is dedicated to the study of one of them, carbon, to its combinations with hydrogen and other carbon atoms and, to a lesser extent, with heteroatoms. Carbon’s prevalence can be briefly explained by means of a casual combination of a low/intermediate electronegativity (2.5) and the presence of 4 electrons in its valence shell. Thus, covalent bond is widely preferred with up to four atoms by means of single, double and triple bond, giving part to an amazing variety of molecules that represents more than 90% of all known chemical compounds. Thus, it is not difficult to understand why structures containing only carbon are so appealing for the scientific community.

Before 1985, mainly three carbon allotropes were known: amorphous carbon, graphite, and diamond. In 1985, a new era of carbon allotropes was opened thanks to the discovery of Harold Kroto, Robert Curl and Richard Smalley, later on recognized with the Nobel prize in 1996 (Figure 1.1).

The fullerene family was identified when traces of molecules containing only 60 and 70 carbon atoms were detected by mass spectroscopy during a simulation of the high-heat conditions of a red giant’s atmosphere, devoted to study the production of carbon clusters. Later on, the fullerene family increased continuously and the most recent recognition of a new fullerene structure is dated 2010, with the isolation of \(\text{C}_{90}\). However, \(\text{C}_{60}\) remains in a privileged position since (i) it is the most abundant, (ii) it can be produced in bulky quantities and with affordable coast, (iii) it is relatively inert in standard conditions (iv) it is high symmetric.

\(\text{C}_{60}\) possesses an unequalled beautiful structure that reminds the geodesic dome of the American architect Buckminster Fuller, built on the occasion of the Expo ’67 in Montreal (Figure 1.2, panel A).

The structure of fullerene \(\text{C}_{60}\) consists of 20 hexagons and 12 pentagons fused together into a hollow carbon sphere (Figure 1.2, panel B). Initially, \(\text{C}_{60}\) was considered as a “super aromatic molecule”, but X-ray diffraction revealed rather a polyenic structure.
As a consequence of this peculiar structure, fullerene $C_{60}$ presents a strong apolar character that renders it absolutely insoluble in all the polar solvents, and sparingly soluble in the other organic solvents. Commonly, toluene, carbon disulfide and orthodichlorobenzene are used to dissolve $C_{60}$, that gives solutions with a characteristic magenta color.

### 1.2 Fullerene $C_{60}$ Reactivity

$C_{60}$ presents two types of bonds: (i) those joining two hexagons (6,6 junctions, 1.38 Å); and (ii) those at the junction of a pentagon and a hexagon (5,6 junctions, 1.45 Å). The deviation from planarity of the double bonds, due to its geometrical constraints, results in an enhancement of the reactivity. The main driving force for the reactivity of fullerenes is therefore the relief of strain associated with the change in hybridization at the reacting carbons on the spherical surface from $sp^2$ to $sp^3$ (Figure 1.3). From the chemical point of view, $C_{60}$ can be mainly considered as an electrophilic reagent.

The demonstration that $C_{60}$ reacts readily with diazo compounds opened the access to many functional derivatives, which were familiarly called “fulleroids” (Figure 1.4). It was later discovered that “fulleroids” convert to methanofullerenes using different reaction conditions (Figure 1.4).
Several other approaches for the covalent functionalization of C\textsubscript{60} have been developed. Depending on the type of reaction, fullerene C\textsubscript{60} reactions have been divided in cycloadditions, nucleophilic additions and radical functionalization.

In the following paragraphs we will give a general overview on the variety of reactions that can be efficiently used to attached several functionalities to C\textsubscript{60}, focusing on the two most employed methodologies in fullerene chemistry: the Bingel-Hirsch reaction and the 1,3-dipolar cycloaddition.

### 1.2.1 Cycloadditions

[1+2] cycloadditions take place with divalent species such as carbene (Figure 1.5, route a), nitrenes\textsuperscript{7} and silylenes\textsuperscript{8} to obtain a huge variety of methanofullerene or heteroanalogues. In the addition of nitrenes, “azafulleroids” and azamethanofullerenes, precursors of azafullerenes (C\textsubscript{59}N), are obtained.\textsuperscript{9}

Alkynes and alkenes (Figure 1.5, route b) can undergo [2+2] cycloaddition reactions either photochemically or thermally.\textsuperscript{10} Moreover, C\textsubscript{60} oligomers have been prepared by solid state synthesis in the presence of KCN to give dimers,\textsuperscript{11} or under high pressure to obtain polymers.\textsuperscript{12}

Also [4+2] cycloadditions take place at 6,6 junctions. Diels-Alder reactions with 1,3 dienes have been described (Figure 1.5, route d).\textsuperscript{13} The main drawback is the stability of the cycloadduct \textit{versus} the retro-Diels-Alder reaction, that strongly depends on the electronic nature of the diene substituents.
1.2.1.1 1,3-dipolar cycloaddition

$C_{60}$ can react with dipoles to yield cycloadducts at the 6,6 junctions. The 1,3-dipolar cycloaddition of azomethine ylides is a powerful and widely used methodology since it was introduced by Prato and coworkers (Figure 1.5, route c),\textsuperscript{14} because of the versatility of the reaction and the stability of the resulting fulleropyrrolidines.\textsuperscript{15}

In this reaction, the ylide can be generated \textit{in situ} by thermal ring opening of aziridines or by decarboxylation of the iminium salts derived from the condensation of $\alpha$-aminoacids and aldehydes or ketones. In the first example sarcosine, formaldehyde
and C₆₀ were reacted together to give the corresponding N-methyl fulleropyrrolidine (Figure 1.6).

The retrocycloaddition of fulleropyrrolidines has been recently described by using high temperatures (180°C) while trapping the ylide intermediate with a metal and a dipolarophile in order to shift the equilibrium to the retrocycloaddition. The required harsh conditions demonstrate the high stability of the fulleropyrrolidines.

In an outstanding work, it has been possible to physically visualize fulleropyrrolidines attached in the vicinity of single walled carbon nanotube surface or in their internal cavity by using High Resolution Transmission Electron Microscopy (HRTEM) (Figure 1.7).

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Figure 1.6 The 1,3-dipolar cycloaddition leading to N-methyl fulleropyrrolidine.

Figure 1.7 HRTEM images of fulleropyrrolidines (C₆₀-C₃NH₂) attached to the surface of SWCNTs (a-c) Image simulations of C₆₀ derivatives (d-f) Corresponding atomic simulations (g-i).
1.2.2 Nucleophilic additions

1.2.2.1 Bingel reaction

The nucleophilic addition of bromomalonates to C\textsubscript{60}, the so-called Bingel or Bingel-Hirsch reaction\textsuperscript{18}, has been widely used in the design of new fullerene derivatives. The original conditions involved the treatment of diethyl bromomalonate with NaH in the presence of C\textsubscript{60} but many other modifications of the Bingel reaction have been developed (Figure 1.5, route e).\textsuperscript{19}

Thus, a strong base is used in order to abstract the acidic malonate proton, generating a carbanion, which reacts with the electron deficient fullerene double bond. This in turn generates a carbanion which displaces bromine in a nucleophilic aliphatic substitution to give an intramolecular cyclopropane ring closure (Figure 1.8).

Although methanofullerenes formed in the Bingel reaction are normally stable, the retro-Bingel reaction\textsuperscript{20} has been described both electrochemically\textsuperscript{21} and under mild chemical conditions.\textsuperscript{22}

1.2.2.2 Other nucleophilic additions

Organometallic compounds, such as organocopper\textsuperscript{23}, cyanide anions\textsuperscript{24}, organolithium or Grignard reagents\textsuperscript{25} can give nucleophilic reactions to C\textsubscript{60} with interesting results but strong drawbacks such as (i) low monoaddition selectivity (ii) low compatibility with other functional groups and (iii) high load requirement of organometallic reagents.

Variations of the well-known organolithium addition have permitted the synthesis of complex multifullerene derivatives\textsuperscript{26}. New aroylation and alkenylation reactions to C\textsubscript{60} have been performed by using Rh as catalyst and organoboron compounds (Figure 1.5, route f).\textsuperscript{27}
Other approaches have been also developed, involving zwitterions derived from dimethyl acetylene dicarboxylate (DMAD) and 4-dimethyl aminopyridine (DMAP) at room temperature (Figure 1.9). The proposed mechanism goes through (i) addition of the zwitterionic intermediate to the electrophilic fullerene, (ii) attack of the fullerene anion on the carbonyl carbon of the ester group and (iii) replacement of the DMAP group with a methoxy group.\(^{28}\)

\[
\text{DMAD} + \text{DMAP} \rightarrow \text{Fullerene} \rightarrow \text{Fullerene} + \text{DMAP}^+ + \text{OCH}_3^-
\]

**Figure 1.9** Fullerene zwitterions-mediated functionalization.

### 1.2.3 Radical functionalization

Radical functionalization has been less explored than the other derivatization methods previously described as a consequence of the high reactivity of radicals towards C\(_{60}\). As a matter of fact, a complex mixture of polyadducts is obtained, while the monoaddition has been only observed by mass spectroscopy and electron spin resonance (ESR).\(^{29}\)

Nonetheless in the last years, exciting results have been obtained. The well-known acyl radical reactions in organic chemistry have been successfully applied to fullerene. Thus, a single addition to the fullerene has been obtained, using a variety of aldehydes as acyl radicals precursors and tetrabutylammonium decatungstate [(n-Bu\(_4\)N)\(_4\)W\(_{10}\)O\(_{32}\), TBADT]. In a similar fashion, the direct addition of ethers to the fullerene has been recently achieved by a photochemically assisted free-radical approach, (Figure 10, top).\(^{30}\) Another example is given by the straightforward and general procedure to prepare dioxolanes on a 6,6 junction from aldehydes and ketenes. The reaction proceed via radical addition to C\(_{60}\) promoted by Fe\(^{3+}\) salts (Figure 1.10, bottom).\(^{31}\)
1.2.4 Multiple additions

The addition of an organic residue to a 6,6 junction of C\textsubscript{60} leads to monoadduct, but, depending on the reaction conditions, can also give a complex mixture of multiadducts (Figure 1.11). There are 8, 46 and 262 possible regioisomers for the bis, tris and tetrakis-additions of a symmetrical addend, respectively.

The second addition to a fullerene monoadduct is controlled by the frontier molecular orbitals\textsuperscript{32} while the control over the regiochemistry of multiple additions is only achieved in a few cases\textsuperscript{33} where the steric hindrance plays a main role.

1.2.4.1 Fulleropyrrolidine bisadducts

The double functionalization of the carbon cage can take place in eight different positions. If the second addition proceeds on the same hemisphere, cis-isomers are obtained while when the second
addend locates in the opposite hemisphere trans isomers are given. When the intermediate position is preferred, equatorial isomer is given (Figure 1.12).

![Figure 1.12](image_url). Relative positions of the addends in C_{60} bis-adducts.

This is clearly the reason why multiaddition has been generally disregarded since a hard and time-consuming purification is required in most of the cases to obtain pure bisadducts in low yields. However, from the chemical point of view multiadducts are of a great interest since the attachment of more than one unit dramatically decrease the aggregation observed on the analogue monoadducts.34

Focusing on the isolation of fulleropyrrolidines bisadducts, big efforts have been done to understand the behaviour of multiadditions in the last years. Thus, by controlling the stoichiometry of the reagents and the reaction conditions mono-, bis-, tris, etc., are formed with a moderate selectivity.

This methodology was followed in order to achieve the isolation and full analytical characterization of the whole series of the N-methyl pyrrolidine bisadducts (trans-1, trans-2, trans-3, trans-4, equatorial, cis-3, cis-2 and cis-1).35 Earlier work made possible the partial characterization of some of the patterns,36 while cis-3, cis-2 and cis-1 fulleropyrrolidine isomers were isolated for the first time. Moreover, the same strategy was applied in order to synthesize, isolate and characterize nine different trisadducts.37

1.2.4.2 Bingel-Hirsch multiadducts

A very ingenious approach to facilitate the isolation of a wide range of polyadducts was reported by Diederich et al.38 who introduced the so called tether-directed remote multifunctionalization, considered as one of the most powerful tools to control
multiaddition in the Bingel reaction. In this case, a combination of cyclopropanation and Diels-Alder reaction with a careful control of length, rigidity and geometry of the tethers allows the obtaining of bis, tris and hexakisadducts. By controlling the length and geometry of the tether, equatorial, cis-2,39 or even trans-240 and trans-141 can be almost exclusively obtained. Variations of this methodology and incorporation of macrocycles helps the formation of regioselective trisadducts and bisadducts.42

Concerning hexakisadducts, those with a T₃ symmetrical octahedral addition pattern are almost routinely synthesized by following the original procedure introduced by Hirsch43 and further improved by Sun.44

1.2.5 Non-covalent functionalization

Supramolecular chemistry allows the preparation of functional architectures by means of non-covalent interactions, including hydrogen bonding, π-π, and metal coordination. For example, C₆₀ derivatized with pyridyl groups can spontaneously coordinate to zinc and ruthenium tetraphenyl porphyrins.45 Other examples include the functionalization of fullerenes with multiple hydrogen bond motifs self-recognized.46 On the other hand, fullerene can participate directly in the self-assembly process through interactions with its curved π-system. C₆₀ can form inclusion complexes with bis-porphyrin hosts47 and with extended-tetrathiafulvalene tweezers (exTTF).48

Atoms, molecules, or ions can be trapped inside the cavity of a fullerene cage to form endohedral fullerenes. H₂, N₂, and a wide variety of noble gases and transition metal atoms have been successfully encapsulated.49 Physical methods for the preparation of endohedral fullerenes are tedious and with a clear lack of efficiency, reaching isolated yields of only 1%.50 Recently, a new creative concept has been developed, called “molecular surgery” to introduce guests inside the fullerene sphere (Figure 1.13). The procedure starts with a ring opening or a ring expansion generally induced by cycloadditions or radical-oxidating reactions in order to open a “hole” in the sphere and insert the desired species into the cage. After the encapsulation, the reconstitution of the cage has been achieved at high temperatures.51
As already discussed, pristine C_{60} is insoluble in water which is the main drawback that limits its compatibility with biological media and consequently its possible biological applications: pristine C_{60} could be potentially toxic to humans since, among others, it has been observed oxidative damage in the brain lipids of fishes, when exposed to fullerene’s aggregates in water.\(^\text{52}\) In order to overcome this problem, two main strategies have been proposed: (i) to partially mask the apolar fullerene surface, and (ii) to covalently modify the aromatic cage.

In the first approach, several methodologies have been successfully applied including: (a) fullerene complexation with cyclodextrins or calixarenes\(^\text{53}\) (b) co-solvation with polyvinylpyrrolidone in organic solvents;\(^\text{54}\) (c) inclusion into suspensions\(^\text{55}\) and (d) incorporation into artificial lipid membranes.\(^\text{56}\) Although promising results have been obtained following these approaches, it is also of great interest to maintain the intrinsic hydrophobicity of the fullerene sphere for some biological applications.

Regarding the covalent functionalization, several examples are available in the literature describing adducts with improved water-solubility thanks to the presence of sugars.\(^\text{57}\) For instance, the addition of 2-azidoethyl tetra-O-acetyl-\(\alpha\)-D-mannopyranoside to C_{60} resulted in two different water-soluble isomers that were separated and characterized.\(^\text{58}\)

Following this strategy, the same cycloaddition reaction was employed to prepare a bisadduct (Figure 1.14) that forms bilayer vesicles.\(^\text{59}\) These liposome-like structures could act as neuroprotective
agents owing to the fullerene intrinsic superoxide dismutation ability. Moreover, the vesicles are endowed with an internal cavity exploitable as a reservoir where a drug could be encapsulated and eventually slowly released into the cells.

A similar approach was used for the construction of the so-called fullerene sugar balls, in which the fullerene sphere is partially or totally surrounded by sugar units. Spherical micelles with a diameter of 5 nm were observed by TEM. Very recently, Nierengarten and coworkers reported the synthesis of a fullerene hexakisadduct, in which the whole sphere is covered by suitable sugar residues coupled to a polyfunctionalized fullerene by click chemistry, generating globular polytopic ligands.

The development of new methods to solubilize fullerene is a very active field that currently involves not only organic chemistry but also other disciplines, such as biochemistry. In fact in a recent work C_{60} derivatives were covalently attached to large biological structures, viral nanoparticles (VNPs), obtaining VNP-C_{60} hybrids with high solubility and biocompatibility. The cellular uptake of these constructs was demonstrated using labelled derivatives, prepared using click chemistry as the key step. Both VNPs and C_{60} retained their biological properties. It is most likely that the cell internalizing driving force is exerted by the viral portion while the fullerene acts as the cargo, exploitable, for example, in photodynamic therapy.

A valid route to obtain water-soluble C_{60} derivatives is also the conjugation of amino acids. Fullerene substituted phenylalanine derivatives, (Figure 1.15) dipeptides from a fullerene amino acid or multifullerene peptides are among the most representative examples.

Thus, the procedures described in sections 1.2.1-1.2.4 have been systematically used to attach different polar appendages in order to get biocompatible fullerene derivatives.

Dendritic architectures are among the most interesting addends that can be coupled to fullerene, since a variety of functional groups can be massively introduced in a controlled manner, from an initial growing core, and successively attached to the fullerene sphere by means of the methods previously discussed.

Bettreich and Hirsch developed the first pioneer work concerning dendrofullerenes, in which a second generation Newkome-type dendron containing 18 carboxylic groups was conjugated to fullerene C_{60} (Figure 1.16). As a consequence, this compound is characterized by one of the highest solubility for a fullerene derivative in water (34 mg/mL at pH 7.4).
After this encouraging results, the group of Hirsch has largely contributed to the development of the dendrofullerene science. Special attention has been focused on the attachment of chromophores mainly for the studies of donor-acceptor systems that possess a high solubility in polar solvents as methanol and water due to the presence of a dendron in the structure.°

1.3 Biological Applications of fullerene derivatives

The synthesis of water-soluble fullerene derivatives, though not straightforward, permitted to explore the use of fullerene based compounds in various biological and biomedical fields, including neuroprotection, antioxidation, DNA photocleavage, antibacterial and antiviral activity, and immunomodulation.° Herein we will mainly focus on: drug and oligonucleotide delivery.

1.3.1 Drug delivery systems based on $\text{C}_60$

$\text{C}_60$ can be considered as an interesting scaffold for drug delivery since it can be multifunctionalized, forms nanoparticles and/or acts as a drug absorbent,° and, moreover, its lipophilicity can be exploited to cross cell membranes.

Several groups have directed their efforts to the synthesis of appropriate fullerene derivatives able to bind and to deliver drugs intracellularly.

Few years ago, paclitaxel, one of the most promising drug against cancer,° was covalently attached to the fullerene sphere (Figure 1.17, top) via an ester bond, permitting the paclitaxel to retain its pharmacological activity. Indeed, the hydrolysis of the ester function resulted in the consequent release of the drug.
Moreover, it was demonstrated that this conjugate has a significant anticancer activity in vitro when administrated with a liposome formulation even though the IC$_{50}$ was 1.6 times higher than the analogous formulation containing the drug alone.

In a similar approach doxorubicin, another effective anticancer agent, was attached to the fullerene moiety (Figure 1.17, bottom). While the drug itself is sparingly soluble in water, the conjugation to fullerene increased its solubility, thus rendering doxorubicin more available for bioapplications. Following the incubation with cells, this construct was localized into the cytoplasm and, to a less extent, into the nucleus, where instead the free drug mainly accumulates. Despite this difference, the activity of doxorubicin was preserved.

Polyhydroxylated fullerenes, called fullerenols or fullerols, have also emerged as very promising candidates for delivering anticancer drugs. Indeed, these structures, although not perfectly defined in the number and the position of the hydroxyl groups, present the remarkable advantage of high solubility in polar solvents if compared to many other C$_{60}$ derivatives. Moreover, the functional groups can bind bioactive units, obtaining so far a high drug loading as the result of a multifunctionalized structure. A fullerenol model has been used to link doxorubicin and cis-platin. In the latter case the multifunctionalization implied

![Figure 1.17. Molecular structures of paclitaxel- (top) and doxorubicin-fullerene C$_{60}$ conjugates (bottom).](image-url)
also the cyclopropanation reaction thus introducing the carboxylic acids as anchor points for the ligation of the platin-based complex.\textsuperscript{73}

C\textsubscript{60} has been used to deliver not only anticancer drugs, but also other bioactive molecules like warfarin, a coumarin anticoagulant drug.\textsuperscript{74} The fluctuations of the concentration of warfarin in the blood, due to a rapid discontinuation, can provoke damages as thrombosis. It has been recently concluded that the biological profile of the warfarin can be modulated by its conjugation to fullerene.

In another example, erythropoietin (EPO), a hormone mainly produced by kidneys, was linked to fullerene and carbon nanotubes.\textsuperscript{75} The usual administration route of EPO is the intravenous injection, but its biological activity can be dramatically reduced since it is quite sensitive to the enzymatic degradation into the intestine. One interesting approach to optimize the EPO administration consisted in absorbing the molecule on porous materials containing fullerene. The pharmacokinetic parameters of these preparations were studied, and EPO bioavailability was strongly improved when fullerene was used, being almost three times higher than the bioavailability obtained by intraperitoneal administration.\textsuperscript{76}

Another methanofullerene with two terminal carboxylic acids was used as counterions to modulate the cellular uptake of polyarginines.\textsuperscript{77} This derivative behaves as an efficient activator of oligo/polyarginines in egg yolk phosphatidylcholine membrane, confirming its ability to cross phospholipidic barriers and easily penetrate into the cells. However, it was observed that the efficiency of this delivery strongly depends on the type of membrane.\textsuperscript{78}

Recently, it has been observed that fullerenes have the ability to penetrate through the skin, paving the way to new transdermal administration systems. The interaction with human epidermal keratinocytes was also analyzed to evaluate cytokine production and pro-inflammatory response in the presence of fullerene derivatives. Cytokine activity and cell viability were found to depend on the concentration of the fullerene derivatives.\textsuperscript{79} In another study, a fullerene-based peptide was synthesized and its ability to penetrate through flexed and un-flexed skin was analyzed.\textsuperscript{80} The experiments, carried out on porcine skin, revealed that mechanical flexion increased the penetration by compromising the permeability barrier of epidermis.

The first example of fullerene-immunoconjugates was reported in 2006, by using a novel multifunctionalized water-soluble C\textsubscript{60} derivative as fullerene scaffold (Figure 1.18). C\textsubscript{60} was modified by the Bingel-Hirsch
reaction affording the activated compound sketched on Figure 1.18, ready for the covalent attachment of the antibody (Ab) ZME-018 via the disulphide bridge exchange. This Ab specifically targets gp240 antigen, which is present in more than the 80% of the human melanoma cells. This protein is well-known as targeting agent toward melanoma and has already been used to deliver therapeutic agents into this type of cancer cells. Therefore, the strategy based on the use of fullerene scaffold is potentially advantageous since multiple attachment of different drugs on the polyfunctionalized fullerene cage could be performed, developing targeted, single-dose “drug cocktails”.81

1.3.2 Nucleic acid delivery

New advanced approaches in the treatment of genetic diseases are based on cellular delivery of foreign nucleic acids, including DNA, RNA, siRNA, and plasmid DNA (pDNA).82

In the last years, the attention has been focused on the development of efficient methods to deliver nucleic acids. A good methodology has to meet four basic criteria: (i) tight binding of the nucleic acid sequences, (ii) protection of the genetic material from the nucleases' degradation in the intercellular compartments, (iii) overcoming of different cell barriers, and (iv) absence of undesired side effects.83

After the first transfection strategies based on liposomes,84 several systems have been devised, classified in viral and non-viral vectors. Viral methods take advantage of the ability of viruses to inject their genetic material inside a host cell, but normally a high immune response is triggered. Alternatively, the non-viral approaches are based on the use of cationic systems like polymers or dendrimers,85 silica nanoparticles,86 gluco-nanoparticles87 or carbon nanotubes.88 Concerning the non-viral approach, it is still necessary to overcome the low efficiency and/or the acute immune response often observed in most of the tested systems.

In this context, fullerenes derivatives containing one or more positive charge on their cage have been explored as nucleic acid delivery carrier.89 This field was pioneered by Nakamura’s group, who synthesized a double-handed fullerene transfection derivative with four positive charges (Figure 1.19).90 The derivative resulted in binding and delivering double-stranded DNA into cells with efficiency comparable to commercial reagents.

Furthermore, after verifying the protein expression for a long period, it was observed that the pDNA-fullerene complex was stable into the cytoplasm.91
In order to understand the morphology of complexes between cationic fullerenes and pDNA, AFM analyses were performed. This derivative was able to fold a supercoiled DNA molecule into a single-molecule complex with the adhesion of DNA double strands displaying only a small increase of the DNA volume. This is certainly an advantage as other DNA-condensing agents based on lipids or dendrimers create larger and less structurally defined aggregates.

The cytotoxicity and in vitro transfection efficiency of a series of fullerene conjugates were tested, evidencing some structural requirements for the design of efficient transfection fullerene derivatives. Transfection efficacy is supposed to be strongly related not only to the applied protocols and/or cell types but also to the dimension of the DNA-vectors. The latter characteristic is intimately related to the structure of the carriers. In fact, different forces are implied in the interaction among nucleic acids and fullerene derivatives and the predominant interactions are due to the electrostatic charges between the phosphates and the ammonium groups, while hydrophobic interactions, hydrogen bonding, and van der Waals forces play a secondary role. When the complexation is based only on the charge interactions, the obtained constructs are bigger and generally less effective. Taking into consideration these important parameters, a library of aminofullerenes presenting slight variations on the same structural motif was synthesized. All cationic fullerenes were able to condense DNA into aggregates in the nano/micrometer scale, while only few of them were active in the delivery.

In addition, a different approach to obtain cationic fullerenes that can complex pDNA for gene delivery has been reported. Two types of multifunctionalized C\textsubscript{60} (Figure 1.20) were prepared by the 1,3-dipolar cycloaddition, thus obtaining a mixture of polyadducts which were methylated using an excess of iodomethane.
The cationic poly-N,N-dimethyl fulleryrrolidinium salts were used to evaluate their capacity to condense pDNA using surface plasmon resonance. A strong interaction between both units was measured with an association constant in the submicromolar range. These derivatives present the advantage of being totally water-soluble, compared to Nakamura’s compound, where potentially toxic DMF or DMSO was added as co-solvent, to completely solubilize it.94

Contrary to what hypothesized at the beginning, Remy et al. observed that systems with an isotropic distribution of positive charges could be also efficiently

Figure 1.20. Top: Molecular structures of polycationic fullerenes derivatives. Bottom right: Confocal microscopy images of Jurkat cells incubated with FITC-fullerene derivative (green fluorescence) showing the internalization into the cells.
employed as transfecting agents.\textsuperscript{95} Several generations of polycationic dendrimeric fullerene hexakisadducts were tested as potential vectors, finding them able to complex DNA into stable and positively charged polyplexes.

Finally, the first \textit{in vivo} gene delivery experiment with fullerene derivatives has been recently reported. Thus, a cationic tetraamino-fullerene (Figure 1.21), with a high water-solubility, was used to complex pDNA, and its efficiency and toxicity were compared to Lipofectin.\textsuperscript{96} No acute toxicity was reported for the fullerene complex in comparison to Lipofectin administration. The \textit{in vivo} delivery of insulin-2 gene corresponds to an increased plasma insulin level and a reduced blood glucose concentration, demonstrating the high potential of fullerene derivatives as gene delivery carriers.

\section*{1.4 Materials applications of fullerene derivative}

A large number of C\textsubscript{60} derivatives have been synthesized yielding new materials with exciting properties. In fact these derivatives can be organic electronic devices, superconductors, liquid crystals and hybrids or are really interesting for electron transfer and non-linear optic properties. Inhere the discussion will be focused on for donor-acceptor and photovoltaics examples.

\subsection*{1.4.1 Donor-acceptor systems}

The research on donor-acceptor systems involving fullerenes has attracted the attention trying to simulate the photosynthetic system and transforming light into chemical energy. In the natural photosynthetic reaction centre, several photoactive units are coupled together, so that several photoinduced electron transfer events take place after irradiation, giving a long distance and long-lived charge separated state in which the
energy is stored (Figure 1.22). The easiest approach to an artificial model consists of two different units (dyad), an electron-donor and an electron-acceptor, linked together by a covalent or non-covalent spacer.

C₆₀ have been widely used as an electron-acceptor because of its high electron affinity and small reorganization energy. A large number of example of dyads have been reported on the last years and the field has been extensively reviewed, and, since the overall lifetime of the radical pairs depends on the distance and orientation between the donor and the acceptor, a series of C₆₀ dyads with various electron donors including porphyrins, phthalocyanines and their metalated analogs tetraphiafulvalene (TTF), ruthenocene and ferrocene have been prepared, also using different donor-acceptor geometries.

Several dyads have been reported to give charge separated states with lifetimes over the μs. For instance, the ferrocene-fullerene dyad (Figure 1.23, left) gives radical pairs with lifetime of 2.5 μs, while for an exTTF-C₆₀ dyad (Figure 1.23, centre) 174 ns were measured. The longest radical-ion pair lifetime for a fullerene derivatives, a Zn chlorin-C₆₀ (Figure 1.23, right), has been reported by Fukuzumi, and resulted on 120 s at -150°C.

![Figure 1.22](image1.png)

**Figure 1.22** Schematic representation in an energy diagram of the processes of separation (CS), shift and recombination charge.

![Figure 1.23](image2.png)

**Figure 1.23** From left to right: Ferrocene, exTTF, and chlorin C₆₀ dyads.
Another example is given by the phtalocyanine-$C_{60}$ dyad (Figure 1.24) containing a polar triethyleneglycol (TEG) chain that self-aggregates into nanostructured nanotubules and, as consequence, an enhancement on the charge separation lifetimes up to 1 ms\textsuperscript{106}.

**Figure 1.24.** Structure of a phtalocyanine-$C_{60}$ dyad and TEM image showing nanotubular aggregates formed in water solution.

### 1.4.1.1 Porphyrin-$C_{60}$ dyads

Porphyrin-$C_{60}$ dyads are among the most investigated systems\textsuperscript{107}. Porphyrins (P) can be covalently introduced at position 2 of fulleropyrrolidines in the 1,3-dipolar cycloaddition reaction\textsuperscript{108}. These structures usually exhibit photoinduced electron transfer (PET) with charge separated state (CSS) with lifetime up to 230 $\mu$s at 25ºC. Multiple rigid spacers based on amides, diazo derivatives\textsuperscript{109} and rigid chiral binaphthyl compounds\textsuperscript{110} have been linked to $C_{60}$ not only by means of the 1,3-dipolar cycloaddition, but also using the Diels-Alder cycloaddition\textsuperscript{111} and the Bingel reaction,\textsuperscript{112} as in the case of a double-bridged P used to synthesize the P-$C_{60}$ dyad involving a trans-2 addition pattern (Figure 1.25).
This derivative is an hybrid between the covalent and the non-covalent interaction approach, in fact its photophysical studies showed a good electronic coupling and the ultra-fast formation of a CSS that can be considered as a reference point for \( \pi \)-stacked P-fullerene ensembles, even though there is covalent link.\(^{113}\) Also non-covalent interactions are used to build up the dyads, as Van der Waals forces, \( \pi \)-stacking interactions, axial coordination or hydrogen bonding. The affinity exhibited between fullerenes and porphyrins has been demonstrated by X-ray structures of co-crystals of different fullerenes and Ps,\(^{114}\) and by observing upfield shifts in \(^1\)H and \(^{13}\)C experiments in toluene when in solution. In other developed approaches P-based molecules host \( \text{C} \text{60} \) inside their cavities, allowing a supramolecular control of the electronic properties. For instance, P jaws,\(^{115}\) a kind of bis-P systems, can successfully interact with the curved \( \pi \)-system of \( \text{C} \text{60} \) and, in some cases, light-induced electron transfer was observed.\(^{116}\)

Fullerene derivatives containing a pyridyl substituent can bind metalloporphyrins by axial coordination, as the herein reported complex (Figure 1.26), that spontaneously assembles in solution when mixing a fulleropyrrolidine bearing a pyridine moiety with Zn(II) tetraphenyl porphyrin (ZnTPP). Intramolecular PET was observed in dichloromethane leading to a radical-pair with a lifetime of 8.6 \( \mu \)s.\(^{117}\) The formation of 1:1 complexes of \( \text{C} \text{60} \), functionalized with a pyridyl group, and a variety of metalloporphyrins have been observed both in solution and in the solid state.\(^{118}\)

Furthermore, when P and \( \text{C} \text{60} \) are functionalized with complementary hydrogen bond motifs, dyads can be also assembled in solution.\(^{119}\) A P-fullerene dyad (Figure 1.27) was assembled through a two-point amminidium-carboxylate binding motif with a high association constant of \( 10^7 \text{M}^{-1} \).

The photophysical studies showed a radical-ion pair states with a lifetime of 10 \( \mu \)s, comparable to those of the covalent dyads ones (\( \sim 1 \mu \)s).\(^{120}\)
Another example has been recently reported, in which a fullerene derivative containing a Hamilton receptor was synthesized and assembled with a ZnP resulting into the dyad (Figure 1.28). The association constant was in the order of $10^5 \text{M}^{-1}$, thanks to the 6-fold hydrogen-bonding motif. The photophysical measures revealed a radical-ion lifetime of 3.1 ns.\textsuperscript{121}

Another example has been recently reported, in which a fullerene derivative containing a Hamilton receptor was synthesized and assembled with a ZnP resulting into the dyad (Figure 1.28). The association constant was in the order of $10^5 \text{M}^{-1}$, thanks to the 6-fold hydrogen-bonding motif. The photophysical measures revealed a radical-ion lifetime of 3.1 ns.\textsuperscript{121}
1.4.1.2 Polyads

Donor-acceptor systems are not limited to one single electron transfer between two chromophores. In fact, as already mentioned, the photosynthetic reaction centre is comprised of a series of electron donors and acceptors that promote an energy transfer (ET) cascade. Following the same fashion, fullerene derivatives have been coupled to several photo- and electroactive units carefully selected and arranged, mimicking the multistep ET nature of the photosynthetic reaction centre. C₆₀ containing various units of porphyrins have been synthesized, principally triads and tetrads. Porphyrin (H₂P), Zinc Porphyrin (ZnP) and Ferrocene (Fc) with different structures have been linearly arranged and attached to C₆₀ to investigate their properties. A ZnP-H₂P-C₆₀ triad was reported in which energy transfer from the ZnP to the H₂P followed by an electron transfer from the excited H₂P to C₆₀ was observed. Fc-ZnP-C₆₀ and Fc-H₂P-C₆₀ triads with a terminal ferrocene unit were prepared, giving two consecutive electron transfer processes, in which an electron was transferred from the porphyrin to C₆₀ and then from Fc to the P. The combination of these two systems gives rise to a tetrad (Figure 1.29) in which a sequence of energy and multistep electron transfer events permitted to give a very high lifetime (380 µs).

Figure 1.29 A Fc-ZnP-H₂P-C₆₀ tetrad.

Also multicomponent polyads, based on linear and branched multi-P polyads, including a Fc-multi-P pentad, have been synthesized. In parallel, multinuclear Pc-C₆₀ polyads have been prepared by covalent or non-covalent interactions. One interesting example is the supramolecular triad of axially coordinated subphthalocyanine-triphenylamine-ZnP, which presents a long-lived charge-separated state of 6.6 µs (Figure 1.30).
Multiple donor–acceptor systems containing TTF have been also explored. These include P-C$_{60}$ triads bearing terminal TTF and exTTF moieties. Series of triads based on terminal exTTF or P linked to C$_{60}$ through oligophenylene vinylene (OPV) molecular wires of different lengths have been recently reported (Figure 1.31). After light irradiation, electron transfer occurred leading to up to a distance between charges of 40 Å.

Analogous systems have been prepared with Ps connected to C$_{60}$ through oligothiophene,$^{127}$ OPE$^{128}$ and combination of oligothiophene and OPV.

![Figure 1.30 A subphthalocyanine-ZnP-C$_{60}$ triad.](image)

![Figure 1.31 An exTTF- C$_{60}$ dyad containing an OPV linker.](image)
1.4.2 Photovoltaics

The excellent electron-accepting properties of fullerenes have been exploited in the preparation of organic solar cells, which are considered a possible alternative to silicon solar cells. [6,6]-Phenyl-C$_{61}$-butyric acid methyl ester (PCBM) (Figure 1.32) was first synthesized by Wudl group$^{129}$ and is the most widely used C$_{60}$ derivative in plastic solar cells, together with the C$_{70}$ analog PC$_{71}$BM.$^{130}$

Bulk-heterojunction photovoltaic cells are prepared from blends of soluble fullerene acceptors with polymers as electron donating materials, typically poly(3-hexylthiophene) (P3HT) or copolymers with thiophene units. This type of cells display a high power conversion efficiency, which ranges from 5 to 7.4%.$^{131}$ Many fullerene derivatives have been used in combination with different polymers, mainly methanofullerenes, [2+4] cycloadducts and fulleropyrrolidines. As methanofullerenes, PCBM is consider the most successful acceptor due to an excellent electron mobility. In addition, PCBM posses a good miscibility with conjugated polymers and a large number of structural variations in the PCBM structure have been performed, opening the door to a big number of fullerene-based solar cells systems with continuously improved efficiencies.$^{132}$ In a very recent work, a number of blends containing P3HT and various PCBM derivatives were tested as solar cells, founding a power conversion efficiencies in a range from 0.02% to 4.1% as a consequence of the different solubilities of the PCBM derivatives. This fact strongly affects the morphologies of its composite with P3HT and consequently the resulting efficiency.$^{133}$ Electron tomography technique was used in order to study the 3D morphology of blends of P3HT and PCBM and the influence on the power efficiency of the final system.$^{134}$

Concerning to [2+4] cycloadducts an impressive 6.5% efficiency has been recently achieved with a bulk-heterojunction polymer solar cell consisting on a blend of P3HT as an electron donor and an indene-C$_{60}$ bisadduct (Figure 1.33). In addition the results were found to be highly reproducible and independent to the active layer thickness in the range of 100-300 nm.$^{135}$

Fulleropyrrolidines have been used as well as electron acceptor for the construction of solar cells, presenting lower power conversion efficiencies but promising results.$^{136}$ For instance a novel cyanine-fullerene dyads have been used to build up a double-layer device obtaining an energetic conversion efficiency of 0.1% (Figure 1.34)$^{137}$ while a 0.02% was found for phthalocyanine-fulleropyrrolidine dyad thin films.$^{138}$
In a similar way, π-conjugated oligomer-tetrafullerene and P3HT were used to build a photovoltaic device exhibiting a quantum efficiency of 15%. Other functionalized fullerenes can be useful in order to design efficient solar cells. Endohedral fullerenes, as acceptor materials, have been used in combination with P3HT, observing 4% power conversion efficiency. Nonetheless, trannulenes (18π-annulenic fluorofullerenes) have been recently used as photoactive materials for solar cells in combination with polyfluorene polymers.

The large number of examples reported in literature and the stimulating results obtained so far confirm the photovoltaic field as one of the most realistic and developed applications for fullerene C$_{60}$.

Figure 1.34 A cyanine-C$_{60}$ dyad.
"It would be possible to describe everything scientifically, but it would make no sense; it would be without meaning, as if you described a Beethoven symphony as a variation of wave pressure”

Albert Einstein
2.1 Control over the 1,3-dipolar cycloaddition

The first part of this thesis has been focused on the deepened study of the main C_{60} derivatization method herein used, the 1,3-dipolar cycloaddition of azomethine ylides, in order to gather more useful information to be applied in the synthesis of dendrofullerenes, described in the next sections. The main goals of this part in fact were (i) the obtention of a protocol to increase the reaction yields and decrease the reaction time both in the mono and the polyaddition (ii) the control on the multiple addition in order to simplify the polyadduct mixture usually obtained when the 1,3-dipolar cycloaddition is performed following conventional protocols, with special attention on the bisadduct formation.

As already discussed in the introduction, the addition of azomethine ylides to fullerene C_{60} is a widely recognized and applied method for functionalizing C_{60}. The formidable success of this reaction can be explained by its selectivity in the attack on the [6,6] bonds and the easily introduction of many substituents/functional groups. In addition, the 1,3-dipolar cycloaddition can be conveniently accelerated when assisted by microwave (MW) irradiation as demonstrated in a large number of examples reported in literature. Another advantage of this functionalization methodology is the quasi-irreversibility of the 1,3-dipolar cycloaddition, as compared, for instance, with the electrochemical, photochemical and thermal instability of methanofullerene, Diels-Alder cycloadducts or isoxazolinofullerenes.

Nonetheless, Prof. Martin et al. have recently described how to obtain quantitative retrocycloaddition in fulleropyrrolidine derivatives. In this outstanding work, thermal and catalytic conditions were tested on five different fulleropyrrolidines. When the derivatives were heated to reflux in o-DCB for up to 24 hours, no changes were observed, while the addition of maleic anhydride resulted in the retrocycloaddition. The addition of a metal catalyst in combination with maleic anhydride was the key to obtain the quantitative retrocycloaddition in most of the cases. Noteworthy, prolonged heating of the fulleropyrrolidines, in the presence of either a dipolarophile excess and/or a Lewis acid catalyst, is required for quantitative conversion. Further studies on the mechanism of the retrocycloaddition revealed that the fulleropyrrolidine thermal treatment regenerates the 1,3-dipole, which is efficiently trapped by the dipolarophile. In addition, it was proved that the nature of the substituent on the pyrrolidine nitrogen atom has great impact on the retrocycloaddition process: the N-methyl or the unsubstituted pyrrolidinofullerenes efficiently undergo the retrocycloaddition reaction while the N-benzoxy derivatives are stable if treated in the same experimental conditions.

We have decided to deepen the study of the 1,3-dipolar cycloaddition and retrocycloaddition introducing the use of microwave irradiation, as heating source, and ionic liquids (ILs) as cosolvents to achieve a very efficient flash-thermal activation in conjunction with a strong activation of polar/ionic intermediates or reactants. Both features are associated with the electrolytic nature of ILs, and turn out to play a key role in the reactions under examination.
Ionic liquids are normally composed by bulky cations and anions, reason why the lattice energy is small than in a conventional ionic solid, so resulting into compounds with a low melting point (Figure 2.1). ILs have been successfully used as alternatives to molecular solvents, for a variety of organic transformations. Recognized advantages are their tunable properties, polar character, non volatility, thermal resistance, complementarity with water or other green media, and liquid electrolyte behaviour. Concerning their use with fullerenes, only few examples are reported in literature and solvophobic aggregation effects, dominating at low temperatures, discourage their use with $C_{60}$.

We started synthesizing seven different fulleropyrrolidine monoadducts 1-7 (Figure 2.2) containing different substituents. All of them were efficiently prepared by refluxing the correspondent aminoacid and aldehyde with fullerene $C_{60}$ in toluene, following the classic procedure described in literature.

Figure 2.1. Comparison between a normal ionic solid and an ionic liquid species.

Figure 2.2. Molecular structure of the seven fulleropyrrolidine monoadducts under investigation.
Thus, 2 mg of monoadduct 1-7 were suspended by sonication in 0.5 mL of 1-methyl-3-n-octylimidazolium tetrafluoroborate ([OMIM]BF₄) and later on heated by applying MW-irradiation (50 W) and controlling the temperature profile (T_{bulk}=180ºC) by simultaneous cooling with compressed air. The parallel cooling of the system allows a higher level of microwave power and the applied air flow is crucial in order to find a protocol applicable to a broad panorama of reactions.

As a result, in these conditions (Scheme 2.1), the retrocycloaddition is achieved quantitatively in 5-10 minutes (Table 2.1). The reaction can be conveniently monitorized by HPLC of the extracted mixture since pristine C₆₀ quickly precipitates from the reaction mixture that contains only IL media, avoiding in this way further reactions.
Table 2.1. MW assisted retrocycloaddition of fulleropyrrolidines 1-7 in 1-methyl-3-n-octylimidazolium tetrafluoroborate [OMIM]BF$_4$.

<table>
<thead>
<tr>
<th>entry</th>
<th>FP</th>
<th>Conv (%)</th>
<th>t(min)</th>
<th>R</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>1</td>
<td>&gt;99</td>
<td>10</td>
<td>21</td>
<td>180°C, 50W</td>
</tr>
<tr>
<td>b</td>
<td>2</td>
<td>&gt;99</td>
<td>5</td>
<td>19</td>
<td>180°C, 50W</td>
</tr>
<tr>
<td>c</td>
<td>3</td>
<td>&gt;99</td>
<td>5</td>
<td>8</td>
<td>180°C, 50W</td>
</tr>
<tr>
<td>d</td>
<td>4</td>
<td>45</td>
<td>10</td>
<td>1</td>
<td>180°C, 50W</td>
</tr>
<tr>
<td>e</td>
<td>5</td>
<td>&gt;99</td>
<td>5</td>
<td>70</td>
<td>180°C, 50W</td>
</tr>
<tr>
<td>f</td>
<td>6</td>
<td>&gt;99</td>
<td>5</td>
<td>87</td>
<td>180°C, 50W</td>
</tr>
<tr>
<td>g</td>
<td>7</td>
<td>&gt;99</td>
<td>5</td>
<td>119</td>
<td>180°C, 50W</td>
</tr>
</tbody>
</table>

As already discussed, in molecular solvents, the lack of substituents on the pyrrolidine ring as compound 1 has been shown to severely inhibit the retrocycloaddition. This calls for a stabilization of the incipient azomethine ylide in order for the reaction to proceed to an appreciable extent. Indeed, our conditions permit to conclude that the impact of the IL is remarkable as N-methyl fulleropyrrolidines bearing either aromatic or aliphatic substituent undergo >99% of cycloreversion. The only exception is the pyrene derivative 7, the solubility of which is low in IL media, rendering difficult the conversion to C$_{60}$.

Encouraged by these results, we decided to investigate the role of the ILs in the retrocycloaddition, demonstrating that in presence of IL the addition of either a further dipolarophile acceptor or a Lewis acid catalyst becomes unnecessary (Table 3.2). To confirm this conclusion, control experiments were carried out using FP 2, lowering the MW irradiation power to 12 W while introducing either maleic anhydride (Table 3.2, entry b) or AlCl$_3$ (Table 3.2, entry c) in the reaction mixtures.

Table 2.2. MW assisted retrocycloaddition of fulleropyrrolidine 2 in 1-methyl-3-n-octylimidazolium tetrafluoroborate [OMIM]BF$_4$.

<table>
<thead>
<tr>
<th>entry</th>
<th>FP</th>
<th>Conv (%)</th>
<th>t(min)</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>2</td>
<td>76</td>
<td>15</td>
<td>100°C, 12W</td>
</tr>
<tr>
<td>b</td>
<td>2</td>
<td>71</td>
<td>45</td>
<td>100°C, 12W, maleic acid</td>
</tr>
<tr>
<td>c</td>
<td>2</td>
<td>83</td>
<td>45</td>
<td>100°C, 12W, AlCl$_3$</td>
</tr>
</tbody>
</table>
The key role played by the ionic medium has been further addressed by introducing the use of \( \sigma \)-DCB as co-solvent and varying the IL nature for the retrocycloaddition of fulleropyrrolidine 2 which has been chosen as reference. Noteworthy, phases with ILs as dominating component (IL: \( \sigma \)-DCB=3:1) preserve the activating effect already discussed, although slowing down the overall reaction rate. The latter turns out to be almost suppressed when the organic solvent is the main component in the mixture (IL: \( \sigma \)-DCB=1:3) (Table 2.3, entries a-f). This observation speaks in favor of a specific effect exerted by the IL medium as a bulk which happens to be denatured when diluted in \( \sigma \)-DCB. In addition, as expected, IL with a different nature, like the 1-methyl-3-n-butyl imidazolium analogue ([BMIM]BF\(_4\)), affords sluggish reactions (Table 2.3, entries g and h) while quantitative cycloreversion is obtained only when in the presence of Al-containing counterpart ([BMIM]AlCl\(_4\)), (Table 2.3, entry i), featuring Lewis acid properties.

<table>
<thead>
<tr>
<th>entry</th>
<th>FP</th>
<th>IL</th>
<th>W (T(_\text{bulk}^\circ\text{C}))</th>
<th>Conv. (%)</th>
<th>t (min)</th>
<th>Solvent mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>2</td>
<td>[OMIM]BF(_4)</td>
<td>12(100)</td>
<td>68</td>
<td>45</td>
<td>IL: ( \sigma )-DCB=3:1</td>
</tr>
<tr>
<td>b</td>
<td>2</td>
<td>[OMIM]BF(_4)</td>
<td>50(180)</td>
<td>82</td>
<td>45</td>
<td>IL: ( \sigma )-DCB=3:1</td>
</tr>
<tr>
<td>c</td>
<td>2</td>
<td>[OMIM]BF(_4)</td>
<td>12(100)</td>
<td>25</td>
<td>45</td>
<td>IL: ( \sigma )-DCB=3:1</td>
</tr>
<tr>
<td>d</td>
<td>2</td>
<td>[OMIM]BF(_4)</td>
<td>50(180)</td>
<td>43</td>
<td>45</td>
<td>IL: ( \sigma )-DCB=3:1</td>
</tr>
<tr>
<td>e</td>
<td>2</td>
<td>[OMIM]BF(_4)</td>
<td>12(180)</td>
<td>0,02</td>
<td>45</td>
<td>IL: ( \sigma )-DCB=1:3</td>
</tr>
<tr>
<td>f</td>
<td>2</td>
<td>[OMIM]BF(_4)</td>
<td>50(180)</td>
<td>5</td>
<td>45</td>
<td>IL: ( \sigma )-DCB=1:3</td>
</tr>
<tr>
<td>g</td>
<td>2</td>
<td>[BMIM]BF(_4)</td>
<td>12(100)</td>
<td>0,9</td>
<td>45</td>
<td>IL: ( \sigma )-DCB=1:3</td>
</tr>
<tr>
<td>h</td>
<td>2</td>
<td>[BMIM]BF(_4)</td>
<td>50(180)</td>
<td>3,7</td>
<td>45</td>
<td>IL: ( \sigma )-DCB=1:3</td>
</tr>
<tr>
<td>i</td>
<td>2</td>
<td>[BMIM]AlCl(_4)</td>
<td>12(100)</td>
<td>&gt;99</td>
<td>45</td>
<td>IL: ( \sigma )-DCB=1:3</td>
</tr>
</tbody>
</table>

Table 2.3. MW assisted retrocycloaddition of fulleropyrrolidine 2 in IL: \( \sigma \)-DCB. In all reactions: 2 mg of fulleropyrrolidine in 0.5 mL IL phase, MW heating, magnetic stirring and cooling by compressed air at 40 psi. % of FP 2 conversion to \( \text{C}_{60}\) monitored by HPLC analysis.

According to the mechanism proposed by Martín,\(^\text{146}\) cycloreversion is highly influenced by the substituents of the fulleropyrrolidine. Thus, electron-acceptors on the \( \alpha \)-carbon, or electron-donors on the nitrogen, or both will boost up the progress of the retrocycloaddition. On this purpose, a 3:1 mixture of [OMIM]BF\(_4\):\( \sigma \)-DCB was selected, since the solubility of the reagents was satisfactory for all substrates, pinpointing the sole stereo-electronic impact of the FP on the process under examination.

Despite the strong reactivity enhancement brought about by the combined use of IL media and MW-irradiation, the cycloreversion rates remain significantly tuned by the
substituents on the pyrrolidine ring. This is clearly visualized from the reactivity ratios (R) calculated for FP 1-7, on the basis of estimated initial rates, and referenced to the slowest reacting pyrene derivatives 4. The reactivity trend results in the series 4<3<1<2<5<6<7 with R in the range 8-120 (Figure 2.3).

Figure 2.3. Time evolution of fulleropyrrolidines 1–7 retrocycloaddition in [OMIM]BF₄·DCB=3:1 (0.5 ml) under MW irradiation, 12 W, with cooling air at 40 psi, T_{bulk} = 100 °C. Estimated initial rates are as follow: \( R_0(4) = 2.3 \times 10^{-4} \) mmol L⁻¹s⁻¹; \( R_0(3) = 1.9 \times 10^{-3} \) mmol L⁻¹s⁻¹; \( R_0(2) = 4.7 \times 10^{-3} \) mmol L⁻¹s⁻¹; \( R_0(1) = 4.7 \times 10^{-3} \) mmol L⁻¹s⁻¹; \( R_0(5) = 1.6 \times 10^{-2} \) mmol L⁻¹s⁻¹; \( R_0(6) = 2.7 \times 10^{-2} \) mmol L⁻¹s⁻¹.

The electron-donating substituent on the nitrogen site turns out to strongly influence cycloreversion. On the other hand, the poor reactivity of the pyrene derivative 4 is confirmed and it is likely attributed to detrimental steric/aggregation effects.

2.1.1 Selective control over the multiaddition

We focused our attention also on the use of MW-activated IL phases to accelerate the thermal azomethine ylide cycloaddition, providing an additional tool for tuning the selectivity outcome on the polyadduct distribution (Scheme 2.2).
Prior to this, the best solvent composition was set for the model synthesis of FP 2, by changing the relative IL:o-DCB ratio and optimizing the overall C$_{60}$ conversion as exposed in Table 2.4.

<table>
<thead>
<tr>
<th>entry</th>
<th>[OMIM]BF$_4$:o-DCB</th>
<th>Conv. (%)</th>
<th>Mono-FP(%)</th>
<th>Selectivity</th>
<th>W</th>
<th>Solvent Vol (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>1:0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>12</td>
<td>0,5</td>
</tr>
<tr>
<td>b</td>
<td>4:1</td>
<td>18</td>
<td>6</td>
<td>0,5</td>
<td>12</td>
<td>0,5</td>
</tr>
<tr>
<td>c</td>
<td>2:1</td>
<td>21</td>
<td>9</td>
<td>0,8</td>
<td>12</td>
<td>0,5</td>
</tr>
<tr>
<td>d</td>
<td>1:1</td>
<td>64</td>
<td>22</td>
<td>0,5</td>
<td>12</td>
<td>0,5</td>
</tr>
<tr>
<td>e</td>
<td>1:2</td>
<td>58</td>
<td>21</td>
<td>0,6</td>
<td>12</td>
<td>0,5</td>
</tr>
<tr>
<td>f</td>
<td>1:3</td>
<td>60</td>
<td>30</td>
<td>1</td>
<td>12</td>
<td>0,5</td>
</tr>
<tr>
<td>g</td>
<td>1:3</td>
<td>64</td>
<td>44</td>
<td>2,2</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>h</td>
<td>1:9</td>
<td>52</td>
<td>38</td>
<td>3,0</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>i</td>
<td>0:1</td>
<td>15</td>
<td>15</td>
<td>-</td>
<td>20</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 2.4. Effect of [OMIM]BF$_4$:o-DCB ratio on the yield and selectivity of mono- and poly-FPs 2, obtained by MW-assisted reaction of C$_{60}$ with sarcosine and heptaldehyde. In all reactions: C$_{60}$ 0.14 mmol; sarcosine 0.28 mmol; heptaldehyde 0.56 mmol; T$_{bulk}$=100°C, under magnetic stirring and simultaneous cooling by compressed air. Fullerene conversion was monitored by HPLC analysis. % of mono-FP 2 monitored by HPLC analysis. Selectivity expressed as mono-FP/poly-FP ratio.

**Scheme 2.2.** Azomethine ylide cycloadditions to C$_{60}$ yielding mono 2 and polyadducts under MW irradiation, in IL:o-DCB mixtures.
For this purpose, in all the cases [OMIM]BF$_4$ was used as ionic liquid since gives finer fullerene dispersions than other imidazolinium-based ILs. Interestingly, under MW irradiation at 12 W, $T_{\text{bulk}}$=100ºC for 10 min, the change in solvent composition in favor of $\sigma$-DCB leads to a steady increase of the overall yield and of the mono-substitution selectivity (Table 2.4, entry f). This latter turned out to be remarkably affected by the relative solubility of reagents and products in the solvent system, which is mainly controlled by the IL content and by the C$_{60}$ concentration.

In addition, the selectivity reverts in favor of the monoadduct upon dilution (Table 2.4, entries g and h). For the IL-free reaction, which lacks the ionic absorbing component, the accelerating effect of MW irradiation, even when the MW power was increased up to 20 W, is very poor (Table 2.4, entry i). The results reported herein indicate that also in this case the phase IL:$\sigma$-DCB=1:3 gives the best results, guaranteeing both a fast reaction and a convenient selectivity control, which, possibly, depends on clustering/aggregation phenomena.

Yield optimization was also investigated by varying the relative ratio of the azomethine ylide precursors, sarcosine and heptaldehyde, and their additional recharge during the reaction that, in turn, was carried out at different C$_{60}$ concentrations (Table 2.5, entries a-f).

Under the optimal conditions for monoaddition, the yield of fulleropyrrolidine 2 levels off at 40% with no major effects of reagent recharging. Such limiting yield is probably attributable to equilibrium constraints, which dictate the observed product distribution, as a result of the thermally activated retrocycloaddition involving mono and polyfulleropyrrolidines.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Sarcosine/heptaldehyde</th>
<th>Conv (%)</th>
<th>Mono-FP (%)</th>
<th>Selectivity</th>
<th>V (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>1/1</td>
<td>34</td>
<td>16</td>
<td>0,9</td>
<td>0,5</td>
</tr>
<tr>
<td>b</td>
<td>2/4</td>
<td>60</td>
<td>30</td>
<td>1</td>
<td>0,5</td>
</tr>
<tr>
<td>c</td>
<td>10/20</td>
<td>95</td>
<td>4</td>
<td>0,04</td>
<td>0,5</td>
</tr>
<tr>
<td>d</td>
<td>2/4</td>
<td>58</td>
<td>42</td>
<td>2,9</td>
<td>1</td>
</tr>
<tr>
<td>e</td>
<td>4/8</td>
<td>69</td>
<td>44</td>
<td>1,8</td>
<td>1</td>
</tr>
<tr>
<td>f</td>
<td>2/4+2/4</td>
<td>72</td>
<td>41</td>
<td>1,3</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 2.5. In all reactions 0.14 mmol of C$_{60}$, [OMIM]BF$_4$:o-DCB=1:3; MW irradiation at 12 W, 10 min, $T_{\text{bulk}}$=100ºC, under magnetic stirring and simultaneous cooling by compressed air. Fullerene conversion monitored by HPLC analysis. Selectivity expressed as: Mono-FP/Poly-FP ratio.
Interestingly, a fast and quantitative conversion to polyadducts became accessible with an excess (10/20-fold) of sarcosine and heptaldehyde when exposed to reagents and to MW irradiation (Table 2.5, entry c).

### 2.1.2 Selective control over the bisaddition

Due to such efficient activation, we decide to study how the reaction selectivity is affected by these conditions. Thus, 1,3-dipolar cycloaddition of sarcosine and p-formaldehyde to fullerene C₆₀ was performed to examine the possible changes in FP 1 bisadduct distribution under equilibrium regime (Scheme 2.3).

![Scheme 2.3. Azomethine ylide cycloadditions to [60]fullerene, yielding monoadduct 1 and polyadducts under MW irradiation, in IL:o-DCB mixtures.](image)

We decided to use a higher concentration of C₆₀ compared to the precedent experiments in order to favor the formation of bisadducts. In a first approach the reaction mixture was irradiated for 10 minutes at 12 W using o-DCB as solvent, and analyzed by HPLC (Figure 2.4a). As a matter of fact a complex mixture of bisadducts and polyadducts can be distinguished in the HPLC chromatogram, similar to this that
can be found when heating in classic conditions. In contrast, the introduction of ionic liquid as co-solvent is key to obtain a simpler trace, with higher concentration of trans-1, trans-2, trans-3 and equatorial bis-FP 1 (Figure 2.4b).

Motivated by the possibility to get a better selectivity we decide to deal with the analysis of the time of irradiation and its effect on the bisadduct distribution. When the sample was irradiated for 20 minutes, a consequent increase on trans-1, trans-2, trans-3 and equatorial peaks was observed in the chromatogram, while the intensity of fullerene and the monoadduct peaks decreased. However the presence of an irregular baseline in the chromatogram indicates the formation of undesired higher polyadducts and other decomposed species which difficult the purification procedure.

Considering the previous restrictions, we have irradiated a mixture containing exclusively C_{60}, monoadduct 1 and its respective bisadducts (Figure 2.5a) at 12 W for 5 minutes using a mixture of IL:o-DCB=1:3 as solvent. Thus, we found a remarkable change in the HPLC profile where only monoadduct, trans-1, trans-2, trans-3 and a low peak of C_{60} were present. No traces of trans-4, cis 1-3 and equatorial were found (Figure 2.5b).
In order to quantify the variation on the bisadduct distribution by using only IL: o-DCB we have irradiated a mixture of C$_{60}$, p-formaldehyde and sarcosine solved in o-DCB for 5 minutes, avoiding in this way the formation of undesired higher polyadducts. The results obtained are collected in Table 2.6 in which it can be observed (i) an expected decreasing on the monoadduct peak area as a result of a more efficient heating provided by the presence of the IL, and (ii) a clear increasing on the formation of trans-1, trans-2 and trans-3 isomers.

Figure 2.5. HPLC chromatograms of a mixture containing C$_{60}$ monoadduct 1 and its bisadducts before MW irradiation in a mixture IL:o-DCB=3:1 (a) and after irradiation (b).
Table 2.6. Variation on the yield of bisadduct formation expressed as HPLC peak area percentage. Reactions: 0.014 mmol of fullerene, 0.055 of \( p \)-formaldehyde and 0.028 mmol of sarcosine at 12 W. Unreacted \( \text{C}_{60} \) area is not included. \(^{[a]}\)2 mL of dichlorobenzene, 2.5 minutes \(^{[b]}\)1.5 mL of \( o \)-dichlorobenzene and 0.5 mL of OMIM, 5 minutes.

These results are greatly advantageous when the isolation of some of the bisadducts is desired, but hard to explain exclusively by means of different aggregation behaviours.

In order to clarify these results, we have also performed DFT calculation on all the FP 1 bisadducts, reported in Figure 2.6.

The formation of such products results from the reaction between the LUMO of the m-FP 1 and the HOMO of the ylide, whose difference in energy (14.1 Kcal/mol, Figure 2.7) is much lower than that calculated between HOMO and LUMO (138.7 Kcal/mol). The FP LUMO displays an electron density which should favor equatorial, cis-2, trans-3 bisadducts.

**Figure 2.6.** Isomeric \( \text{C}_{60} \) bisadducts.
To assess the thermodynamic stability of the products under equilibrium regime, we have calculated the relative energy of the bisadduct derivatives, which have been reported in Figure 2.8.

The less stable isomers resulted to be the cis (1-3) and trans-4, followed by the trans-1 in agreement with the HPLC results, where the first four cited isomers have not been detected, while only a minor amount of trans-1 appeared.

These preliminary results partially explain the different changes of the HPLC profile commented above. It seems to be the collection of two different effects: the thermodynamic stability of the bisadducts and the different aggregation properties provided by each isomer as a result of the different ratio between fullerene covered surface/fullerene naked surface.

The optimization of this technique is of extreme interest since different factors, as stoichiometry, steric factors and power of irradiation can play an important role in order to obtain higher yields of conversion.

Figure 2.7. Energy level for HOMO e LUMO of m-FP and ylide.

Figure 2.8. Energy of bis-FP 1, with respect to equatorial, the most stable bisadduct.
2.2 Synthesis of dendrofullerene derivatives for gene and drug delivery

With the purpose of creating new efficient oligonucleotide and drug delivery efficient vectors based on fullerene cores we decided to focus our efforts on the synthesis of new monoadduct and bisadducts containing polyamidoamine (PAMAM) dendron functionalities. The possibility to attach a second unit of the dendron moiety to the fullerene sphere represents a chance to increase the biocompatibility of the final compounds while increasing the number of potential oligonucleotide or drugs unit to the system. In addition, a comparison on the transfection performance among the bisadduct isomers would be extremely advantageous to extract several considerations for the future design of more efficient systems.

PAMAM dendrimers were firstly studied by Tomalia in 1985, who described the synthesis of a new type of macromolecules defined as “starburst dendrimers”. One of the central core used by Tomalia et al is the ethylendiamine to which is possible to grow the dendrimer via Michael addition by using methacrylate and later on the correspondent product is reacted again with an amine (like ethylendiamine). The repetition of this sequence brings out the different generations of the PAMAM dendrimer (Figure 2.9).

Morphologic studies carried out on the first generation of the dendrimer show a non-defined structure while by increasing the dendrimer generation, a spheric structure is appreciated. One of the most valuable characteristics of these dendrimers is the high
solubility that they exhibit in polar solvents as MeOH and water, that renders them very interesting for biological applications, mainly as drug-delivery vectors. Indeed, several contributions have been done in this line proposing a mechanism called “click-in”. According to it, the drug will be placed on the inner cavity of the dendrimer, stabilized by ionic weak interactions and hydrogen bonding with the internal amide groups.

One of the most exciting examples concerns a third generation PAMAM dendrimer that has been used as a drug carrier of ibuprofene. Up to 78 molecules of ibuprofene can be loaded thanks to the interactions of the carboxylic groups of the drug with the terminal amine groups of the dendrimer. In addition, it has been demonstrated that 80% of the complexed ibuprofene can be internalized into the cell in one hour while the naked ibuprofene alone needs 3 hours.

PAMAM dendrimers are also very appealing as gene delivery carriers. Terminal amines of these dendrimers can be positively charged and bind by means of ionic bond the negative charged fractions of the oligonucleotides. The complex PAMAM-oligonucleotide could potentially penetrate the cellular membrane and release the oligonucleotide into the nucleus.

2.2.1 Synthesis of a fullerene moiety containing a terminal carboxylic acid

Prior to tackle the synthesis of the different generations of dendrons, we have focused on the preparation of mono and bisadducts of fulleropyrrolidine containing carboxylic acid functions that can be further used as anchor points for PAMAM dendrons.

On this purpose, the amino acid 9 was synthesized as reported in literature. Thus, commercial available ß-alanine tert-butyl ester was reacted with benzyl bromoacetate that selectively alkylates the amine giving a mixture of unreacted, mono alkylated and bis alkylated derivatives. This mixture was purified by chromatographic column in order to obtain the desired monoalkylated derivative 8. A further hydrogenation in presence of Pd/carbon mixture as catalyst permitted to obtain the unprotected amino acid 9.
To obtain the monoadduct 10, amino acid 9 was dissolved in toluene with p-formaldehyde and $C_{60}$ and refluxed for two hours, until no further progress on the reaction was detected by thin layer chromatography (TLC). As a result, a complex mixture of polyadducts, monoadduct and unreacted fullerene was obtained and further purified by column chromatography to achieve compound 10 with a 45% yield.

However, as exposed in the introduction, polyadducts are extremely appealing, for example to decrease the inherent aggregation of fullerene derivatives. With this aim, we decided to isolate some of the bisadducts of 10, but the polyadduct mixture obtained in the described conditions (reflux, 2h) contained only a small quantity of the desired bisadducts and amounts of tris and higher polyadducts, as observed by Electro Spray Ionization (ESI) analysis (Figure 2.10).

**Scheme 2.4.** Synthesis of β-aminoacid 9 and 1,3-dipolar cycloaddition on $C_{60}$ in classic conditions. (i) NEt₃, dioxane, r.t., 4 h (ii) H₂, Pd/C, EtOH, r.t., 12 h (iii) toluene, reflux, 2 h.

Figure 2.10 ESI spectra of the crude of the mixture of 10 in classic conditions.
Shorter times of reaction yielded a bigger quantity of unreacted C₆₀ but, at the same time, remarkably decreased the number of major polyadducts, facilitating the purification of five of the eight isomers: *trans-1, trans-2, trans-3, trans-4* and *equatorial* (Figure 2.11).

![Diagram of isomers](image)

**Figure 2.11.** Symmetry patterns of the eight possible bisadducts.

However, this method resulted into very low quantities of bisadducts with yields between 0.4-2% (see experimental part). At this point, the acquired knowledge on the control on bisaddition (see Paragraph 2.1.2) was of extreme utility in order to obtain good quantities of bisadducts 11a-e (Scheme 2.5). Thus, when the reaction was done under MW irradiation and using a mixture OMIM[BF₄]:o-DCB= 3:1, a very much simpler mixture of C₆₀, monoadduct, *trans-1, trans-2, trans-3* and *equatorial* was obtained. By combining both methods, we were able to isolate reasonable quantities of five different bisadducts: *trans-1, trans-2, trans-3, trans-4* and *equatorial* (Scheme 2.5, 11a-e). They have been characterized by UV-Vis absorption measurements, which are in perfect agreement with the previous works,³⁵ and by ¹H and ¹³C NMR.

![Scheme 2.5](image)

**Scheme 2.5.** Optimized conditions to obtain fulleropyrrolidine bisadducts in classical conditions (i) o-DCB, p-formaldehyde, reflux, 45 min or (ii) MW, OMIM[BF₄]:o-DCB=3:1, 120ºC, 10 min, 20 W.
Also in this case, the pyrrolidine proton NMR spectra, together with the number of the carbon signal $s$ from the $^{13}$C spectra, confirm the identity of the isomers. In fact, as shown in Figure 2.11 the trans-1 presents only one singlet (8H) while in the carbon sp$^2$ region only 8 signals of the fullerene cage are present, as expected for a $D_{2h}$ geometry.

![UV spectra, pyrrolidinic $^1$H signals and fullerene sp$^2$ $^{13}$C signals of bisadducts 11a-e.](image)

**Figure 2.11.** UV spectra (left), pyrrolidinic $^1$H signals (centre) and fullerene sp$^2$ $^{13}$C signals. signals of bisadducts 11a-e.
In the case of \( C_2 \) symmetry, as for \( \text{trans-2} \) and \( \text{trans-3} \), the pyrrolidine protons resound as 4 doublets of intensity 2H while the \( \text{sp}^2 \) C are 28. Although the \( \text{trans-4} \) and the \textit{equatorial} belong to the \( C_s \) group, characterized by the presence of a plane of symmetry, in the case of \( \text{trans-4} \) the pyrrolidine proton signals are still 4 doublets while in the \textit{equatorial} they resound as two singlets and two doublets. Also they differ in the \( \text{sp}^2 \) carbon region of the NMR spectrum where the \( \text{trans-4} \) present 30 peaks and the \textit{equatorial}'s signals are 29.

Monoadduct 10 and bisadducts 11a-e can be easily deprotected from the \( t \)-butyl group in acidic condition (mixture of DCM/TFA 1:1) to obtain 12 and 13a-e respectively (Scheme 2.6). In the case of bisadducts, longer time of reaction was needed than for the monoadduct, in order to achieve the complete deprotection of both carboxylic acid functions.

Scheme 2.6. Deprotection of monoadduct 10 and bisadducts 11a-e. (i) TFA/DCM, r.t., 2-24 h.

### 2.2.2 Synthesis of 0.5, 1, 1.5 and 2 G PAMAM dendrons

To prepare PAMAM dendron, we have firstly developed the synthesis to afford straightaway the first generation of dendron 18, as sketched in Scheme 2.7.
Scheme 2.7. Synthesis of the first generation of PAMAM dendron. (i) CHCl₃, r.t, overnight (ii) MeOH, r.t, Ar, 24 h, methyl acrylate (iii) MeOH, r.t., Ar, 7 days, ethylenediamine (iv) DCM, r.t, 48 h, Boc₂O (v) MeOH, r.t, Pd/C (10%), 24 h.

Commercially available ethylenediamine was used as the central core for the growing of the dendron. In this fashion, one of the two amines was protected with a CBz group by treatment with N-(benzyloxycarbonyloxy)succinimide. Thus, a large excess of ethylenediamine was used in order to avoid the double protection, obtaining compound 14. Consecutively, the very reactive methyl acrylate was added to 14 for a contemporary double aza-Michael reaction, resulting into bis-methyl ester derivate 15. The attempts of reacting compound 16 with N-Boc ethylenediamine to afford 17 resulted in very low yields of the desired product, despite various conditions were studied, by changing temperature, number of reagent equivalents, and time of reaction.

Thus, an excess of ethylenediamine was added to a solution of 15 and stirred for five days, when eventually the presence of a new compound (the desired product 16) was revealed. After two more days no further progression was observed and the reaction was stopped. Terminal amines were protected by treatment with di-tert-butyl dicarbonate leading to a mixture of unreacted, mono and bis-protected dendron, efficiently separated by chromatography to afford pure 17. Last, benzyl group was easily removed by hydrogenation using Pd/C as catalyst giving 18 quantitatively.

2.2.1.1 Synthesis of the 0.5 G generation

The dendron 0.5 G generation, containing two terminal carboxylic acids protected with methyl group, can be easily achieved by following the same pathway described in the Scheme 2.7, to achieve 15. After hydrogenation, the CBz group was selectively removed from compound 15 to obtain compound 19 (Scheme 2.8).

However, we decided to synthesize the tert-butyl analogue of compound 15, since the hydrolysis of methyl esters coupled on to the fullerene has been only reported in a few cases, using harsh conditions. For this purpose, tert-butyl acrylate was efficiently used.
instead of methyl acrylate in order to induce the double Michael addition to the amine. Longer time of reactions, with lower yields, were needed to obtain 20, since tert-butyl acrylate is much less reactive than the methyl analogue. Once more, catalytic hydrogenation permitted to obtain quantitatively compound 21.

Scheme 2.8. Synthesis of dendron 0.5 generation. (i) MeOH, r.t., Pd/C (10%), 24 h (ii) MeOH, r.t., Ar, 24 h, tert-butyl acrylate (iii) (i) MeOH, r.t., Pd/C (10%), 24 h.

2.2.1.2 Synthesis of the 1.5 and 2 G generation

Motivated by the facility to obtain the first generation by the synthetic strategies described before, we decide to face the synthesis of the second generation by following the same protocol, as reported in Scheme 2.9. In this fashion, consecutive addition of methyl acrylate to 17 yielded 22, further treated with an excess of ethylenediamine to afford 23, the main core of the second generation. As for the 1G derivative, addition of a solution of Boc anhydride was performed, but eventually, even if the presence of the bis Boc-protected derivative was revealed by mass spectroscopy, the complexity of the mixture (extremely rich in mono-Boc protected compound), did not allow the separation of the mixture. Several conditions were tested in order to optimise the reaction conditions, including classical and MW-assisted heating up to 80°C and addition of water, among others, but no significant changes were observed. This fact can be possibly explained by the formation of intramolecular hydrogen bonding that partially cover one of the amine groups as reported from other groups.155
In order to achieve the second generation of the dendron and to maintain the consistence with the protective group strategy (other protecting groups as acetyl worked with success on compound 23), we proceed as depicted in the Scheme 2.10. Compound 17 was reacted with *tert*-butyl acrylate to afford 24 that was successfully hydrolyzed in acidic media to obtain 25. Thus, a very much reactive derivative towards the amide-bond formation was obtained, that was successfully reacted with N-Boc ethylenediamine. This reaction was further optimized using distilled N-Boc ethylenediamine. By using hydrogenation in presence of catalyst, the benzyl group was selectively removed in compounds 24 and 26 to
obtain the 1.5 and 2 generations of the PAMAM dendron (27 and 28 respectively) (Scheme 2.11).

2.2.1.3 Coupling reactions with $C_{60}$ monoadduct

Having the fullerene mono and bisadduct derivatives bearing carboxylic functionalities and the four different generations of dendron derivatives with an unprotected amine, we proceeded to the their coupling via amidation. In principle, the activation of the carboxylic groups by using conventional activating agent procedures followed by the addition of the amine would make possible the formation of the desired derivatives.

The coupling reaction was firstly optimized using the first generation dendron 12 and the monoadduct 12 (Scheme 2.12). It was observed that the use of a 10 equivalents of EDC and DMAP and the use of pyridine as solvent resulted into a better performance of the reaction. In addition, when very strict anhydrous conditions were used the yield boosted up. As a result, a satisfactory 80% of yield was obtained, recovering in a good quantity the first generation dendrofullerene 29.

![Scheme 2.12. Coupling reaction between deprotected fullerene monoadduct 12 and first generation dendron 18 to afford 29. (i) EDC, DMAP, Ar, anhydrous pyridine, r.t., 12 h.](image)

These experimental conditions were applied to the coupling between the monoadduct 12 and the other generations of the PAMAM dendron, as reported in Scheme 2.13, obtaining dendrofullerenes 30, 31 and 32. Noteworthy, the coupling of 12 with 0.5 G (21) and 2G (28) generations gave the low yields, probably due for 21 to its low solubility in pyridine, and, in the case of 28, to the formation of intramolecular hydrogen bonds inducing steric effects in the proximity of the free amine.
Scheme 2.13. Coupling between 0.5G, 1.5 and 2G dendrons and monoadduct 12. (i) 21, EDC, DMAP, anhydrous DMF, 80°C, 12 h (ii) 27, EDC, DMAP, anhydrous pyridine, room temperature, 36 h (iii) 28, EDC, DMAP, anhydrous pyridine/CHCl₃, 0°C, 12 h.

In Figure 2.12, the ¹H-NMR spectra between 2-4 ppm of 29, 30, 31, 32 are reported. This part of the spectra present the PAMAM signals and the differences in spectrum complexity are evident, starting from a the very simple spectrum for 29, in which only five triplets are observed, to a the very complex multiplet region for 32.
Results and discussion

Figure 2.12. From bottom to top, $^1$H-NMR spectra between 2 and 4 ppm of 29 (a), 30 (b), 31 (c), 32 (d), showing the signals corresponding to the dendron moiety.
All the purified bisadducts were successfully coupled to the first generation dendron, giving derivatives 33a-e (Scheme 2.14).

**Scheme 2.14.** Coupling between bisadducts 13a-e and first generation PAMAM dendron. (i) 18, EDC, DMAP, HOBr, Ar, anhydrous DMF/CHCl$_3$, 50°C, 12-60 h.

The deprotection of all mono and bis dendrofullerenes was performed by TFA treatment, obtaining 34, 36 and the trifluoroacetic salts of 35, 37, 38a-e (Scheme 2.15).
2.2.2 Aggregation studies

Dynamic Light Scattering (DLS) has been used to determine the presence and the size of aggregates in water solution, at neutral pH, of monoadduct 35 and bisadducts 38d and 38e, which resulted highly soluble in aqueous media (Figure 2.13).
Monoadduct 35 presented already aggregation at a concentration of $10^{-5}$ M as detected by the UV spectra and DLS, with an almost identical curve profile at $10^{-4}$ and $10^{-3}$ M, concentration at which part of 35 precipitated and should be removed by centrifugation.

Figure 2.13. Water solution of 35, 38d and 38e ($10^{-3}$M) after centrifugation.
Bisadducts solutions at $10^{-6}$, $10^{-5}$, $10^{-4}$ M exhibited a non aggregation profile in the UV spectra, confirmed by DLS where only temporally small aggregates were observed with an average hydrodynamic ratio of <10 nm in which no Gaussian curve could be satisfactorily fitted, meaning the absence of relevant aggregation in the solution.

To find aggregation in bisadducts 38d and 38e it was necessary to increase the concentration up to $10^{-3}$ M. The two isomers behave differently as expected considering the different distribution of the hydrophilic and hydrophobic portions on the fullerene surface.

Indeed, the equatorial isomer presents two types of clusters, the smallest with average hydrodynamic ratio of 7 nm (32.7%) while the largest has an average hydrodynamic ratio of 300 nm (Figure 2.14).

Trans-4 isomer presents only larger aggregates with an average hydrodynamic ratio of 192 nm (60%) and 807 nm (38%) (Figure 2.14).

These results perfectly match with what observed in the UV spectra, (Figure 2.15) where the UV features of the trans-4 isomer appear broad, specially the two typical small bands at 620 and 710 nm, while the equatorial’s were still perfectly define.

Figure 2.14 DLS spectra of monoadduct 35 and bisadducts 38d and 38e.
These considerations were further demonstrated by analytic measurement of the maximum solubility of these derivatives, obtained by the UV of the saturated solution in neutral water. Monoadduct 35 presents a discrete solubility of 1.02 mg/mL while 10.02 mg of trans-4 isomer 38d can be solved in 1 mL of neutral water. Interestingly, the equatorial isomer 38e is much more soluble than its trans-4 analogue with a remarkable solubility value of 23.90 mg/mL, confirming in this manner the precedent observations.

2.2.3 Complexation studies

As already discussed in the section 1.3.2, cationic fullerene derivatives possess the ability to efficiently condensate nucleic acids and deliver it into the cell.

In this part, we have focused on proving the great potential of these derivatives as siRNA transfection vectors by performing complexation studies with siRNA, eventually analyzed by agarose gel electrophoresis as imaged in Figure 2.16.

Although all the tested compounds are able to complete complex siRNA, remarkable differences can be found among them. Thus, as expected, monoadduct 35 needed the highest N/P ratio (15) to achieve full complexation. In addition, the different distribution of the positive charges around the apolar carbon cage, strongly influenciates the performance of the different bisadducts. Trans-1 and trans-2 isomers
fully complex siRNA with a N/P ratio of 1 while \textit{trans-3} requires a higher value (2.5). In addition, \textit{trans-4} and \textit{equatorial} presented the worst values among the bisadducts isomers since N/P of 5 and 7.5, respectively, were needed.

Figure 2.16 Electrophoretic motility of dendrofullerenes 35 and 38a-e, reconstituted at 1 mg/mL in 5% dextrose complexed with siRNA. Various N/P (+/-) ratios of the four conjugates were complexed to a fixed 0.5 μg of siRNA. Full complexation for every fullerene derivative is highlighted in white.
2.2 Synthesis of dendrofullerene hexakisadducts for gene and drug delivery

After successfully synthesizing the series of dendritic monoadducts 34-37 and bisadducts 38a-e, we have focused our attention on the synthesis of Th-symmetrical hexakisadduct, herein referred as hexakisadduct for brevity (Figure 2.17), incorporating the 1G dendron 18 into its structure. From the practical point of view, the hexakisadduct of fulleropyrrolidine has been never reported as pure compound, since it would demand an enormous effort in order to separate it from the reaction mixture. Thus, we have decided to switch to the Bingel-Hirsch reaction, where several examples of Th-symmetrical fullerene hexakisadducts have been reported. The preparation of fullerene hexakisadducts has been introduced and largely developed by Prof. Hirsch, but the original procedure was conveniently modified by Sun et al., who described how the use of a large excess of the bromination agent (normally CBr₄) and the base (DBU) resulted into simpler mixtures of isomers and increased yields.

To prepare the hexakisadduct coupled to 1G PAMAM dendron 18, two different strategies were proposed, (i) the earlier attachment to six malonyl units containing a linker and two terminal carboxyl acids and subsequent coupling of the desired dendron moiety and (ii) the conjugation of the dendron moiety to the malonyl unit and subsequent nucleophilic addition to the fullerene.

In a first stage, we face the synthesis of a proper linker between the C₆₀ and the dendron moieties (Scheme 2.16). Thus, when a mixture of succinic anhydride, N-hydroxsuccinimide, DMAP and tert-butyl alcohol was refluxed in toluene for 24 hours in the presence of NEt₃, compound 39 was easily obtained and crystalized. It was further reduced by using BH₃-DMS to obtain 40.

![Figure 2.17. Structure of the fullerene cage with the Th-symmetrical hexakis positions in red.](image)

Scheme 2.16. Synthesis of 42. (i) DMAP, t-BuOH, toluene, NEt₃, reflux, 24 h (ii) BH₃-DMS, THF, N₂, r.t., 0ºC (iii) malonyl dichloride, anhydrous DCM, 12 h (iv) formic acid, DCM, 6 h.
Compound 40 was easily reacted with the malonyl chloride in anhydrous conditions to give 41, later deprotected by formic acid to yield 42.

To find the most appropriate conditions for the coupling, we started the exploration using, instead of 1G PAMAM dendron, the 2,2’-(ethylenedioxy)bis(ethylamine). Its monoBoc derivative 43 was easily obtained by using Boc anhydride. Several conditions for the next step were tested, varying temperature and number of equivalents of DCC and DMAP. The optimum was achieved when 42 (1 eq), 43 (2.2 eq) and DMAP (2.2 eq) were dissolved in anhydrous DCM, cooled to 0°C and subsequently DCC was added in small portions, to obtain 44. The Bingel-Hirsch reaction on fullerene was performed following the conditions described by Sun: a large excess of CBr₄ and DBU was used to induce nucleophilic addition of 43 to the C₆₀ in the presence of DMA, used as templating agent. The mixture was allowed to react for 5 days to successfully obtain 44 (Scheme 2.17). When scale-up reactions were performed (more than 10 mg of fullerene), the purification process was remarkably difficult and the isolation of the hexakisadduct was only possible using preparative HPLC technique.
Scheme 2.17. Synthesis of hexakisadduct 45. (i) MeOH, r.t, 24 h, Boc₂O (ii) EDC, DMAP, CHCl₃, 24 h, 0°C (iii) CBr₄, DMA, DBU, anhydrous o-DCB, r.t., Ar, 5 days.

We decided to explore also the second strategy. Thus compound 41 was allowed to react for 3 days with C₆₀ using the conditions described before, to afford 46 as a pale yellow solid. In this case, both small-scale and large-scale nucleophilic cyclopropanation permitted the isolation of 46 by chromatographic silica column.
The 12 tert-butyl ester groups were successfully hydrolyzed by addition of TFA to the pure solid of 46 to afford 47, which contains 12 carboxylic acid residues and, from the following coupling reaction using 43, a very complex mixture could be expected. Indeed, only the use of 100 equivalents of EDC and DMAP permitted to properly activate all 12 carboxylic acids and to obtain compound 45 in good yields (Scheme 2.18).

Scheme 2.18. Synthesis of hexakisadduct 45 via coupling. (i) CBr₄, DMA, DBU, anhydrous o-DCB, r.t., Ar, 3 days (ii) TFA/DCM, 1 day (iii) 43, EDC, DMAP, N₂, anhydrous DCM, 0°C, 12 h.

So, both strategies were satisfactory when applied with the 2,2'-(ethylenedioxy)bis(ethyamine). Taking into account this results, a simply coupling between dendron 18 and the hexakisadduct 47 was performed using a large excess of coupling agent to obtain compound 49. However, although the presence of 49 was detected by MALDI spectroscopy, the reaction crude consisted of a very complex mixture of compounds, highly difficult to purify.
This remarkable difference on the reactivity between dendron 18 and 43 can be ascribed to a combination of steric factors and formation of intramolecular hydrogen bonds.

On the other hand, these steric factors were not relevant in the coupling between 42 and the first generation dendron 18, resulting into compound 48. Sun’s procedure was again used to afford the hexakisadduct containing 12 units of dendron 18. In addition to longer reaction times, a lower yield (15% ) for 49 compared to 45 was obtained (Scheme 2.20).

Scheme 2.19. Attempt of coupling between f1G dendron 18 and 47. (i) EDC, DMAP, anhydrous DCM, 0°C, 12 h.

(i)
Scheme 2.20. Synthesis of the symmetric hexakisadduct containing 12 units of 1G PAMAM dendron (i) 18, EDC, DMAP, r.t., 0°C, 24 h (ii) 47, CBr₄, DMA, DBU, Ar, anhydrous o-DCB, r.t., 8 days.

The ¹H-NMR and ¹³C spectra of 49 in CDCl₃ are reported in Figure 2.18 with their respective assignments for each signal. Interestingly, in the ¹³C-NMR spectra only two sp² carbon signals are observed as expected for a D₂h symmetry. Noteworthy, it is also possible to observe the quaternary equivalent sp³ carbons of the fullerene at 79 ppm (Figure 2.18, bottom, q) and the one corresponding to the malonyl portion (Figure 2.18, bottom, p).
The use of mild acid media provided by the addition of TFA to 45 and 49 resulted into hexakisadduct 50, containing 12 positive charges, and dendron hexakisadduct 51, with 36 positive charges respectively (Scheme 2.21).

Figure 2.18. $^1$H-NMR spectra (top) and $^{13}$C-NMR spectra (bottom) of 48 in CDCl$_3$. 
Scheme 2.21. Deprotection of hexakisadducts 45 and 49 (i) TFA/DCM, r.t., 24-48 h.
2.3 Loading chromophores on dendrofullerene derivatives

The attachment of chromophores, as porphyrins, to fullerene derivatives with an enhanced solubility in water, as dendrofullerenes, is of interest not only for biological purposes, i.e. DNA photocleavage, but also for materials science, for example with the possibility to build dyads apt to give electron transfer and charge separation states in polar media, resembling processes that can be found in nature, as the photosynthesis.

With this aim, we started with the synthesis of an asymmetric porphyrin where three meso positions were occupied by pyridine substituents while the fourth meso position contained a methyl ester group, potential anchor point for the conjugation. This structure provides three potential positive charges conferring additional solubility to the final derivative.

The synthesis of the mentioned porphyrin has already been described in literature, and we used the classic Rothemund method. The use of pyrrole and two aldehydes gave a statistic mixture of the possible six isomers 52a-f (Scheme 2.22) which were efficiently separated by column chromatography. The major drawback of this approach is the low yield (2%) obtained for porphyrin 52e, reason why large scale-up reactions were mandatory to obtain a good quantity of 52e to work with.

Scheme 2.22. Synthesis of porphyrin 52. (i) propionic acid, reflux, 90 min.

The ester function of porphyrin 52e was then easily converted into the corresponding carboxylic acid (compound 53, Scheme 2.23) by hydrolysis in strong basic media. Derivative 52e was also reduced by using LiAlH₄ to yield 54 and a subsequent Swern oxidation afforded the aldehyde porphyrin 55. So, derivatives 54 and 55 can be used respectively in a coupling reaction with a fullerene derivative bearing amine or...
hydroxyl groups and in the cycloaddition reaction, in presence of C$_{60}$ and a proper amino acid.

Thus, to prepare an analogue of 1G dendrofullerene 29 bearing the tris(4-pyridyl) porphyrin we performed the cycloaddition with amino acid 9, C$_{60}$ and p-nitrobenzaldehyde to obtain 56. This compound presents two potential anchor points, since the deprotection of the tert-butyl group will afford a free carboxylic acid and the nitro group can be reduced to amine. The reduction of the nitro group has been widely describe in literature, where three main procedures has been reported: (i) reflux in acetic acid in the presence of iron (ii) use Samarium diiodide as selective reductive agent (iii) catalytic hydrogenation in the presence of PtO$_2$ or Nickel Raney. The first procedure was immediately disregarded due to the required harsh conditions, while samarium diiodide seemed to not work properly with fullerene derivatives. On the other hand, it has been reported that catalytic hydrogenation in presence of fullerene can reduce the fullerene double bonds. In order to find a convenient nitro reduction protocol, we used a simpler fullerene derivative (compound 57, scheme 2.24), prepared using sarcosine instead of amino acid 9, p-nitrobenzaldehyde and C$_{60}$.

Compound 57 was hydrogenated in the presence of PtO$_2$ as catalyst, and the reaction was monitored by TLC. After three hours the amine fulleropyrrolidine 57 were required to obtain 58 in good yields (80%, see scheme inserted into Figure 2.19), while longer times of reaction led to the formation of unknown fullerenic species. In the $^1$H-NMR spectra (Figure 2.19) it is possible to appreciate the differences of the signals corresponding to the phenyl ring of the nitro derivative of 57 and the amino analogue 58, whose spectrum shows also a broad singlet at 3.8 ppm, integrating two protons, corresponding to the amine group.
Scheme 2.24. 1,3-dipolar cycloadditions to afford fulleropyrrolidines 56 (top) and 59 (bottom) with a lateral p-nitro group. (i) toluene, reflux, 1 h.

Figure 2.19. $^1$H-NMR spectra of nitro derivative 57 (bottom) and amine derivative 58 (top). (i) toluene, H$_2$, Pd/C, r.t., 3 h.
Thus, compound 56 was first hydrolyzed in acidic media to give 59 that was successfully coupled with the first generation PAMAM dendron 18, obtaining 60. The reduction of the nitro group to amine was performed following the previously described procedure obtaining the amine derivative 61 (Scheme 2.25). The latter was coupled with porphyrin 53 in the presence of EDC and DMAP as activating agents, but derivative 62 was obtained in very low yields in all the tested conditions. The best result (yield 10%) was get using 10 equivalents of both EDC and DMAP.

Scheme 2.25. Synthetic strategy to obtain for the porphyrin attachment to dendrofullerene 61 via amide linkage. (i) TFA/DCM, r.t., 2h (ii) 18, EDC, DMAP, Ar, anhydrous pyridine, r.t., 12h (iii) toluene, H₂, Pd/C, r.t., 12h (iv) EDC, DMAP, Ar, anhydrous DMF, 24h.
Compound 63 was characterized by both $^1$H and $^{13}$C-NMR. In the $^1$H-NMR spectrum reported in Figure 2.20, the signals corresponding to the porphyrin can be clearly observed between 7 and 9 ppm, the fulleropyrrolidine ring protons are at 4.3 and 5.2 ppm, and from 2-4 ppm it is possible to observe the dendron signals. At -3 ppm there is also a broad singlet corresponding to the characteristic protons at the endo- positions of the porphyrin (2 H).

![Figure 2.20. $^1$H-NMR spectrum of derivative 63 in CDCl$_3$.](image)

Even though the preparation of the dendrofullerene dyad was achieved, the low yield obtained in the process induced us to explore a more appealing synthetic strategy.

In this second approach, derivative 55 was allowed to react with amino acid 9 and C$_{60}$ in a mixture of toluene/DMF to obtain compound 63. The advantage of this procedure was clearly to recover the unreacted porphyrin by simple column chromatography.

Furthermore, the hydrolysis of the $t$-butyl ester yielded 64 that was efficiently coupled with the first generation PAMAM dendron 19, obtaining 65.
Thus, with two different synthetic paths, the tris(4-pyridyl)porphyrin was successfully linked to a first generation dendrofullerene to obtain derivatives 62 and 65, which differ in the presence of a short linker present in the case of 62, while in the case of 65 the porphyrin is direct attached to the fullerene functionalization.

As last stage, we have methylated both P-C<sub>60</sub> dyad 62 and 65. Firstly, we treated both compounds with a large excess of CH<sub>3</sub>I using a small quantity of DMF until their complete solubilization leaving by reflux under stirring. The reaction was screened by reversed-phase HPLC, observing no further progression after 72 hours within the presence of a single peak in the chromatogram. When the reaction is performed at room temperature, it was possible to observe the presence of an additional peak in the chromatogram, the not methylated N-pyrrolidinic compound, as revealed by ESI. Compounds 66 and 67 were not isolated, but to the crude of reaction was directly passed an HCl gas flow for 5 minutes to obtain 68 and 69. The final compounds presented a discrete solubility in DMSO that permitted to characterize them by <sup>1</sup>H-NMR technique and confirm the grade of methylation, as sketched on Scheme 2.27.
Surprisingly, global yields in both synthetic pathways are very similar, 7 synthetic steps are needed in the case of 69 with 9% yield, while 5 synthetic steps were required for 68, with a 8.9% yield. However, it has to be considered the possibility to simply recovered the unreacted porphyrin 55 on the crude of 63, that eventually confirms the second pathway as the most advantageous one.

As expected, both compounds 68 and 69 were found to be highly soluble in water, presenting very low aggregation as revealed by the UV-Vis spectrum (Figure 2.21). Although broader, the absorption profile of 68 and 69 in neutral water present the same profile of its precursor when dissolved in CHCl₃ being perfectly distinguishable the Soret Band at 420 nm and the Q bands region from 470 to 650 (Figure 2.21).
Figure 2.21. Comparison between UV spectra of compound 62 and 65 in CHCl$_3$ and 68 and 69 in neutral water.
Chapter 3

Conclusions

“Science is built up with facts, as a house is with stones. But a collection of facts is no more a science than a heap of stones is a house.”

Henri Poincaré
In conclusion, during this thesis we have synthesized, isolated and characterized a broad range of fullerene derivatives containing dendron moieties that can found potential uses in biological applications such as drug and gene delivery. In concrete, we have focused most of our efforts on to the obtention of bisadducts of fulleropyrrolidine incorporating two units of the dendron moiety, which implies a substantial decreasing in the aggregation behavior and an increased biocompatibility. In order to achieve this, we have firstly studied the 1,3-dipolar cycloaddition in special conditions: using a combination of ionic liquids and MW irradiation, obtaining remarkable differences into the reactivity and selectivity as compared with the classic conditions.

Thanks to this, we have been able to obtain five bisadducts isomers that were further conjugated with a first generation PAMAM dendron, obtaining five analogue systems with a completely different distribution of the polar appendage around the carbon cage. This could have several implications, reason why we have performed solubility and aggregations studied by DLS technique confirming different aggregation and solubility behaviours. Furthermore, the ability of these derivatives to complex siRNA has been demonstrated by electrophoretic analysis concluding very satisfactory results for all the compounds, revealing at the same time interesting differences between the dendrofulleropyrrolidine bisadducts.

Motivated by the chance to enrich this library of compounds, we have synthesized a $T_5$-symmetric a dendritic hexakisadduct by using the Bingel-Hirsch procedure. In this case, an homogeneous distribution of the positively charged dendritic appendage around the fullerene sphere is obtained, that could involve different results as a transfection vector.

The third part of this thesis has been devoted to the attachment of a porphyrin unit to a monoadduct dendrofullerene by two different pathways: including a short amide linker or not. In addition to the synthetic exercise, the final structures obtained are porphyrin-fullerene dyads, multipositively charged with a remarkable solubility in water and the possibility to assemble with other complementary negatively charged structures in order to further develop more complex systems.
Chapter 4

Experimental Part

“Facts are the air of scientists. Without them you can never fly.”
Linus Pauling
4.1 Materials and methods

Reagents and solvents

All reagents have been purchased from Sigma-Aldrich, Fluka and J. T. Backer. Solvents were used as received without further purification, unless specified. Deuterated solvents were purchased from Cambridge Isotope Laboratories. Fullerene C_{60} has been acquired from Bucky-USA.

NMR experiments

$^1$H-NMR and $^{13}$C-NMR spectra were recorded on a Varian Gemini-200 (200 MHz) or a Varian INOVA (500 MHz) at Università degli Studi di Trieste or on a Bruker Avance-300 (300 MHz) or a Bruker Avance-400 (400 MHz) at Friedrich-Alexander-University Erlangen-Nuremberg.

Chemical shifts are expressed as ppm (parts per million) with respect to the solvent signal and multiplicity of the peaks with the following abbreviations: s (singlet), d (doublet), dd (doublet of doublets), t (triplet), m (multiplet), q (quadruplet), b (broad).

Mass spectroscopy

Electrospray (ES-MS) experiments were recorded on a Perkin-Elmer APIII at 5600 eV at University of Trieste and MALDI-TOF or HR-MALDI-TOF measurements were made with an AXIMACFR plus instrument (Kratos Analytical) or a Shimadzu AXIMA Confidence at Friedrich-Alexander-University Erlangen-Nuremberg.

HPLC analyses

HPLC analyses of section 3.1 were conducted at Università degli studi di Padova with a Cosmosil Buckyprep column (5 µm particles) and toluene as mobile phase (flow = 1 mL min).

HPLC analyses of Section 3.2 were conducted with a Phenomenex Prodigy 5 µm silica or a Luna 5 µm silica using the following program for bisadducts 10a-e:

A: t=0 min, toluene 100%, flow 0 mL/min;
B: t =3 min, toluene 100%, flow 1 mL/min;
C: t = 33 min, toluene/ethyl acetate 90/10, flow 1 mL/min;
D: t =83 min, toluene 100%, flow 1 mL/min;
E: t =85 min, toluene 100%, flow 0 mL/min.

Purity of final or key compounds was checked using Phenomenex Prodigy 5 µm silica or a Luna 5 µm silica columns for normal phase and a Luna 5 µm column for reverse phase using adapted elution programs in each case.
MW synthesis

MW synthesis with ionic liquids were carried at Università degli studi di Padova in a CEM-Discover-Coolmate monomode microwave apparatus with simultaneous monitoring of irradiation power, pressure and temperature. During the high-power experiments, interruption of MW irradiation and sonication was necessary to homogenize the sample and improve the yields.

Dynamic light scattering measurements

DLS measurements were performed in a Malvern ZS apparatus with the following specifications: sampling time=120 s, refractive index of medium (water)= 1.33, refractive index of particles= 1.40, viscosity= 1.0150 cP, T=20ºC.

UV Measurements

UV-Vis-NIR measurements were recorded on a Varian 5000 UV-Vis-NIR spectrometer.

IR Spectroscopy

FT-IR spectra have been registered with a FT-IR Perkin Elmer system 2000..

Thin Layer chromatography (TLC)

Reactions have been followed by silica gel thin layer chromatography 60 F254 purchased from Merck. Non-colored compounds were detected by UV lamp or by oxidation with a solution of KMnO₄.

Chromatographic separation

Chromatographic separations have been performed with silica gel 40-63 µm from Merck, unless differently specified.

Gel electrophoresis

siRNA-dendrofullerene complexes were prepared by mixing 0.5 mg of siRNA in 30 mL of 5% dextrose with the dendrofullerene at different charges ratio. 0.5 mg of free siRNA was used as a control. Complexes were incubated for 30 minutes at room temperature to allow complete complexation to occur. siRNA complexes or siRNA alone were mixed with orange dye solution (1:10 dilution) and loaded onto 1% w/v agarose/TBE gel containing ethidium bromide (0.5 mg/mL). The gel was run for 45 min at 70 mV (BioRad, UK). The gel was then photographed under UV light using GeneGenius system (PerkinElmer Life and Analytical Sciences, USA)
4.2 Synthetic procedures

Synthesis of 1

In a 50 mL flask, fullerene $C_{60}$ (87 mg, 0.12 mmol), formaldehyde (3 mg, 0.12 mmol) and sarcosine (11 mg, 0.12 mmol) were solved in 10 mL of toluene and sonicated for 15 minutes. The mixture was refluxed for 30 min and the solvent removed under reduced pressure. The solid sample was separated on silica chromatographic column by using toluene to remove first the unreacted fullerene, and then toluene/ethyl acetate 95:5 to isolate the monoadduct. The solvent of the collected fractions was evaporated at reduced pressure, then 1 mL of chloroform was added to the solid residue, the solution was filtrated to remove the solid impurities and precipitated using distilled methanol to afford 1 as a brown solid (18 mg, yield 84% on recovered $C_{60}$).

Characterizations were in accordance with literature.\textsuperscript{14,15}

$^1$H-NMR (200 MHz, CDCl$_3$): $\delta$ 4.73 (s, 4H), 2.82 (s, 3H).

Synthesis of 2

In a 50 mL flask, fullerene $C_{60}$ (87 mg, 0.12 mmol), heptaldehyde (25 mg, 0.22 mmol) and sarcosine (53 mg, 0.60 mmol) were solved in 10 mL of toluene and sonicated for 15 minutes. The mixture was refluxed for 20 min and the solvent removed under reduced pressure. The solid sample was dissolved in toluene and separated by silica chromatographic column by using toluene to remove first the unreacted fullerene and

\textsuperscript{14,15}
then toluene/ethyl acetate 9:1 to isolate the monoadduct. The solvent of the collected fractions was evaporated at reduced pressure, the residue was dissolved in 1 mL of CS$_2$, filtrated to remove the solid residues and precipitated using distilled methanol and petroleum ether to afford 2 as a brown solid (22 mg, 92% expressed on recovered C$_{60}$).

Characterizations were in accordance with literature.$^{14,15}$

$^1$H-NMR (200 MHz, CDCl$_3$): δ 4.72 (d, $J$=9.0 Hz, 1H), 4.53 (d, $J$=9.0 Hz, 1H), 4.20 (s, 1H), 2.80 (s, 3H), 2.50 (t, $J$=7.4 Hz, 2H), 2.25-1.80 (m, 8H), 1.75 (t, $J$=7.0 Hz, 2H).

Synthesis of 3

![Reaction Scheme]

$C_{60}$ (87 mg, 0.12 mmol), $p$-methoxybenzaldehyde (27 µL, 0.22 mmol) and sarcosine (53 mg, 0.60 mmol) were solved in 10 mL of toluene and sonicated for 15 minutes. The mixture was refluxed for 30 min and the solvent removed under reduced pressure. The reaction mixture was separated by silica chromatographic column using toluene to remove first the unreacted fullerene and then toluene/ethyl acetate 9:1 to isolate the monoadduct. The solvent of the collected fractions was evaporated at reduced pressure, the residue was dissolved in 1 mL of CHCl$_3$, filtrated to remove the solid residues and precipitated using distilled methanol and petroleum ether to afford 3 as a brown solid.

Characterizations were in agreement with literature.$^{14,15}$

$^1$H-NMR (200 MHz, CDCl$_3$): δ 7.52 (d, $J$=2.1 Hz, 2H), 7.30 (d, $J$=2.0 Hz, 2H), 4.81 (d, $J$=8.9 Hz, 1H), 4.60 (d, $J$=8.9 Hz, 1H), 4.18 (s, 1H), 3.20 (s, 3H), 2.81 (s, 3H).
Synthesis of 4

In a 50 mL flask, fullerene C_{60} (87 mg, 0.12 mmol), a 50% solution of ethyl glyoxalate in toluene (48 µL, 0.22 mmol) and sarcosine (53 mg, 0.60 mmol) were solved in 10 mL of toluene and sonicated for 15 minutes. The mixture was refluxed for 3 h and the solvent removed under reduced pressure. The solid sample was separated on silica column chromatography by using toluene to remove first the unreacted fullerene and then toluene/ethyl acetate 95:5 to isolate the monoadduct. The solvent of the collected fractions was evaporated at reduced pressure, added 1 mL of CHCl₃, filtrated to remove the solid residues and precipitated in distilled methanol and petroleum ether to afford 4 as a brown solid (15 mg, 91% yield on recovered C_{60}).

Characterizations were in agreement with literature.¹⁴,¹⁵

¹H-NMR (200 MHz, CDCl₃): δ 4.81 (d, J=8.9 Hz, 1H), 4.72 (d, J=8.9 Hz, 1H), 4.33 (s, 1H), 3.22 (s, 3H), 2.80-2.73 (m, 5H).

Synthesis of 5

In a 50 mL flask, fullerene C_{60} (87 mg, 0.12 mmol), isonicotinaldehyde (62 µL, 0.22 mmol) and sarcosine (53 mg, 0.60 mmol) were solved in 10 mL of toluene and sonicated for 15 minutes. The mixture was refluxed for 3 h and the solvent removed under reduced pressure. The solid sample was separated on silica column chromatography by using toluene to remove first the unreacted fullerene and then toluene/ethyl acetate 95:5 to isolate the monoadduct. The solvent of the collected fractions was evaporated at reduced pressure, added 1 mL of CHCl₃, filtrated to remove the solid residues and precipitated in distilled methanol and petroleum ether to afford 5 as a brown solid (15 mg, 91% yield on recovered C_{60}).

Characterizations were in agreement with literature.¹⁴,¹⁵

¹H-NMR (200 MHz, CDCl₃): δ 4.81 (d, J=8.9 Hz, 1H), 4.72 (d, J=8.9 Hz, 1H), 4.33 (s, 1H), 3.22 (s, 3H), 2.80-2.73 (m, 5H).
chromatography by using toluene to remove first the unreacted fullerene and then toluene/ethyl acetate 90:1 to isolate the monoadduct. The solvent of the collected fractions was evaporated at reduced pressure, added 1 mL of CHCl$_3$, filtrated to remove the solid residues and precipitated in distilled methanol and petroleum ether to afford 5 as a brown solid (15 mg, 80% on recovered C$_{60}$).

Characterizations were in agreement with literature.$^{14,15}$

$^1$H-NMR (200 MHz, CDCl$_3$): δ 8.82 (m, 2H), 8.20 (m, 2H), 5.2 (d, $J$=8.9 Hz, 1H), 4.92 (d, 8.9 Hz, 1H), 4.50 (s, 1H), 3.28 (s, 3H).

Synthesis of 6

In a 50 mL flask, fullerene C$_{60}$ (87 mg, 0.12 mmol), formaldehyde (3 mg, 0.12 mmol) and N-(4-hydroxyphenyl)glycine (20 mg, 0.12 mmol) were solved in 10 mL of toluene and sonicated for 15 minutes. The mixture was refluxed for 30 min and the solvent removed under reduced pressure. The solid sample was separated on silica column chromatography by using toluene to remove first the unreacted fullerene, and then toluene/ethyl acetate 9:1 to isolate the monoadduct. The solvent of the collected fractions was evaporated at reduced pressure, added 1 mL of CS$_2$, filtrated to remove the solid residues and precipitated in distilled methanol to afford 1 as a brown solid (15 mg, 77% expressed on recovered C$_{60}$).

Characterizations were in agreement with literature.$^{14,15}$

$^1$H-NMR (200 MHz, CDCl$_3$): δ 7.76 (d, 2H, $J$= 1.5 Hz), 7.52 (d, 2H, $J$= 1.5 Hz), 4.32 (s, 4H).
Synthesis of 7

In a 50 mL flask, fullerene C$_{60}$ (87 mg, 0.12 mmol), 1-pyrenecarboxaldehyde (28 mg, 0.12 mmol) and sarcosine (53 mg, 0.60 mmol) were solved in 10 mL of o-dichlorobenzene and sonicated for 15 minutes. The mixture was refluxed for 2 h and the solvent removed under reduced pressure. The solid sample was separated on silica column chromatography by using toluene to remove first the unreacted fullerene, and then toluene/ethyl acetate 9:1 to isolate the monoadduct. The solvent of the collected fractions was evaporated at reduced pressure and 1 mL of CS$_2$ was added to the solid residue, filtrated to remove the solid particles and precipitated in distilled methanol to afford 1 as a brown solid. (10 mg, 67% expressed on recovered C$_{60}$).

Characterizations were in agreement with literature.$^{14,15}$

$^1$H-NMR (200 MHz, CDCl$_3$): $\delta$ 9.29 (m, 2H), 8.32 (m, 4H), 8.10 (m, 3H), 5.41 (d, $J$=9.1 Hz, 1H), 5.01 (d, 9.1 Hz, 1H), 4.91 (s, 1H), 3.30 (s, 3H).

Synthesis of 8

A solution of benzylbromoacetate (5.58 mL, 35.3 mmol) in MeOH (20 mL) was added via addition funnel to a solution of tert-buthyl-3-amminopropanoate clorhydrate (8.0 g, 44.0 mmol) and NEt$_3$ (12.2 mL, 88.0 mmol) in MeOH (300 mL). The mixture was leaved under magnetic stirring at room temperature for 12 hours. The solvent was removed under reduced pressure, the crude was ridisolved in 100 mL of CHCl$_3$ and extracted with brine (x3). The organic phase was dried by adding anhydrous Na$_2$SO$_4$. Column
chromatography in silica gel was required using first Petroleum ether and then Ethyl acetate/Petroleum ether (1:1), to obtain 8 as a pale yellow oil. (4.2 g, yield 40.5 %)

Characterizations were in agreement with literature.\textsuperscript{154}

$^1\text{H}-\text{NMR}$ (200 MHz, CDCl$_3$): $\delta$ 7.37-7.33 (m, 5H, CHAr); 5.17 (s, 2H, Ar-CH$_2$); 3.48 (s, 2H, CO-CH$_2$-NH); 2.87 (t, $J=6.0$ Hz, 2H), CH$_2$-CH$_2$-CO); 2.45 (t, 2H, $J=6.0$ Hz, NH-CH$_2$-CH$_2$); 2.39 (bs, 1H, NH); 1.44 (s, 9H, Boc).

Synthesis of 9

\begin{center}
\includegraphics[width=0.3\textwidth]{synthesis_9}
\end{center}

To a solution of 8 (4.2 g) in MeOH (150 mL) was added a catalytic amount (400 mg) of Pd/C (10% w/w). The solution was leaved by magnetic stirring overnight in an hydrogen atmosphere. The mixture was then filtrated under celite and the solvent removed under reduced pressure to obtain 9 as a white solid (2.9, quantitative yield).

Characterizations were in agreement with literature.\textsuperscript{154}

$^1\text{H}-\text{NMR}$ (200 MHz, CD$_3$OD): $\delta$ 3.50 (s, 2H, NH-CH$_2$-CO); 3.23 (t, $J=7.1$ Hz, 2H, CO-CH$_2$-CH$_2$); 2.70 (t, $J=7.1$ Hz, 2H, CH$_2$-CH$_2$-NH); 1.47 (s, 9H, Boc).

Synthesis of 10 and 11a-e (classic conditions).
A mixture of C\textsubscript{60} (2.00 g, 2.80 mmol), 6-\textit{tert}-butyl hydrogen 3-azahexanedioate (1.13 g, 5.60 mmol) and \(p\)-formaldehyde (0.42 g, 14 mmol) were dissolved in 500 mL of orthodichlorobenzene and heated to reflux for 45 minutes. The solvent was removed by reduced pressure obtaining a brown solid as a mixture of unreacted C\textsubscript{60}, monoadduct and bisadducts, separated by column chromatography on silica gel using, as eluent, toluene / ethyl acetate mixtures: 100/0 (C\textsubscript{60}), 99/1 (monoadduct, \textit{trans}-1), 98/2 (\textit{trans}-2, \textit{trans}-3 and \textit{cis}-1), 97/3 (\textit{trans}-4), 96/4 (equatorial). \textit{Trans}-3 was further separated from \textit{cis}-1 with a second chromatographic column using toluene/ethylacetate/cyclohexane (98/1/1) as eluent. Precipitation of the isolated bisadducts from a highly concentrated CHCl\textsubscript{3} solution with distilled MeOH gave products 10 and 11\textit{a-e} as brown solids.

### Synthesis of 10 and 11\textit{a-e} (MW conditions).

A mixture of C\textsubscript{60} (20 mg, 28 \mu mol), 6-\textit{tert}-butyl hydrogen 3-azahexanedioate (11.3 mg, 56 \mu mol) and \(p\)-formaldehyde (5 mg, 120 \mu mol) were suspended with a Vortex on a mixture of 2 mL of \(o\)-DCB/OMIM [BF\textsubscript{4}] 3:1 and sonicated for 20 min. Then, the reaction was heated under MW irradiation at 120\(^\circ\)C for 10 min. The resulting crude was extracted with water (5mLx3) to remove the ionic liquid and purified by column chromatography on silica gel using as eluent toluene / ethyl acetate mixtures: 100/0 (C\textsubscript{60}), 99/1 (monoadduct, \textit{trans}-1), 97/3 (\textit{trans}-4), 96/4 (equatorial). Precipitation of the isolated bisadducts from a highly concentrated CHCl\textsubscript{3} solution with distilled MeOH gave products 10 and 11\textit{a-e} as brown solids.

#### 10 monoadduct: classic: Yield 1.11 g (45%). MW: 40 mg (60 %)

\textsuperscript{1}H-NMR (200 MHz, toluene[\textsubscript{d8}]): \(\delta\) 3.92 (s, 4H, CH\textsubscript{2} pyrrolidine), 3.10 (t, \(J = 8.9\) Hz, 2H, N-CH\textsubscript{2}), 2.69 (t, \(J = 8.9\) Hz, 2H, CH\textsubscript{2}-CO), 1.51 (s, 9H, \textit{t}-Bu).

\textsuperscript{13}C-NMR (50 MHz, CDCl\textsubscript{3} + CS\textsubscript{2}): \(\delta\) 171.22, 154.68, 147.12, 146.07, 145.85, 145.51, 145.25, 145.10, 144.37, 142.92, 142.45, 142.01, 141.90, 141.71, 139.78, 139.99, 136.10, 80.65, 70.46, 67.62, 50.25, 35.40, 28.17.

MS ESI m/z: found 892.1 (M+1), 914.1 (M+Na); calculated 891.88.

UV/vis (toluene, \(\lambda\)/nm): 325, 433, 704.

FT-IR (KBr): \(\nu\) (cm\textsuperscript{-1}) 2971, 2922, 2776, 2327, 1726, 1628, 1454, 1427, 1398, 1364, 1344, 1247, 1219, 1150, 1115, 1043, 895, 846, 768, 703, 597, 574, 561, 553, 542, 526, 478.

#### 11\textit{a} (\textit{trans}-1): classic: Yield 13.00 mg (0.5%). MW: Yield 5 mg, 15 %

\textsuperscript{1}H-NMR (400 MHz, CDCl\textsubscript{3}): \(\delta\) 4.70 (s, 8H, CH\textsubscript{2} pyrrolidine), 3.53 (t, \(J = 8.9\) Hz, 4H, N-CH\textsubscript{2}), 2.97 (t, \(J = 8.9\) Hz, 4H, CH\textsubscript{2}-CO), 1.61 (s, 18H, Boc).
$^{13}$C-NMR (125 MHz, CDCl$_3$): δ 171.93, 153.78 (8C), 147.90 (4C), 146.38 (8C), 145.66 (8C), 144.43 (8C), 142.53 (4C), 140.95 (8C), 136.73 (8C), 81.13, 68.63 (4C), 68.14 (4C), 50.58 (2C), 35.52 (2C), 28.49 (6C).

MS ESI m/z: found 1064.5 (M+1), calculated 1063.76.

UV/vis (toluene, λ/nm): 320, 458, 491, 668.

FT-IR (KBr): ν (cm$^{-1}$) 2971, 2816, 1721, 1553, 1534, 1470, 1453, 1424, 1391, 1343, 1142, 1022, 768.

11b (trans-2): classic: Yield 48.10 mg (1.6%).

$^1$H-NMR (400 MHz, CDCl$_3$): δ 4.67 (d, $J = 9.1$ Hz, 2H, CH$_2$ pyrrolidine), 4.49 (d, $J = 9.1$ Hz, 2H, CH$_2$ pyrrolidine), 4.36 (d, $J = 9.1$ Hz, 2H, CH$_2$ pyrrolidine), 4.33 (d, $J = 9.1$ Hz, 2H, CH$_2$ pyrrolidine), 3.43 (t, $J = 6.9$ Hz, 4H, CH$_2$-N), 2.90 (t, $J = 6.9$ Hz, 4H, CH$_2$-CO), 1.58 (s, 18H, Boc).

$^{13}$C-NMR (75 MHz, CDCl$_3$): δ 171.50, 158.48 (2C), 152.81 (2C), 152.00 (2C), 147.97 (2C), 147.26 (2C), 146.65 (2C), 146.58 (2C), 145.97 (2C), 145.83 (2C), 145.60 (2C), 145.21 (2C), 145.19 (2C), 144.89 (2C), 144.70 (2C), 143.75 (2C), 143.34 (2C), 143.20 (2C), 142.12 (2C), 142.08 (2C), 142.03 (2C), 141.93 (2C), 141.07 (2C), 141.05 (2C), 139.17 (2C), 139.12 (2C), 134.09 (2C), 133.32 (2C), 80.42, 68.76 (2C), 68.59 (2C), 67.32 (2C), 67.12 (2C), 49.85 (2C), 35.05 (2C), 27.81 (6C).

MS ESI m/z: found 1063.6 (M+1), calculated 1063.76.

UV/vis (Toluene, λ/nm): 431, 477, 724.

FT-IR (KBr): ν (cm$^{-1}$) 2976, 2359, 1715, 1555, 1455, 1420, 1390, 1350, 1266, 1141, 1031, 811, 762

11c (trans-3): classic: Yield 53.20 mg (1.8%).

$^1$H-NMR (500 MHz, CDCl$_3$): δ 4.42 (d, $J = 9.0$ Hz, 2H, CH$_2$ pyrrolidine), 4.37 (d, $J = 9.0$ Hz, 2H, CH$_2$ pyrrolidine), 4.15 (d, $J = 9.0$ Hz, 2H, CH$_2$ pyrrolidine), 4.09 (d, $J = 9.1$ Hz, 2H, CH$_2$ pyrrolidine), 3.30 (t, $J = 7.0$ Hz, 4H, CH$_2$-N), 2.81 (t, $J = 7.0$ Hz, 4H, CH$_2$-CO), 1.58 (s, 18H, Boc).

$^{13}$C-NMR (50 MHz, CDCl$_3$): δ 170.89, 153.30 (2C), 152.11 (2C), 152.92 (2C), 152.16 (2C), 149.04 (2C), 148.63 (2C), 148.16 (2C), 148.11 (2C), 147.8 (2C), 147.80 (2C), 145.86 (2C), 145.11 (2C), 145.06 (2C), 144.99 (2C), 144.74 (2C), 144.47 (2C), 143.84 (2C), 143.76 (2C), 143.73 (2C), 143.33 (2C), 142.32 (2C), 141.57 (2C), 141.11 (2C), 140.86 (2C), 140.69 (2C), 139.15 (2C), 136.23 (2C), 135.62 (2C), 81.72, 68.22 (2C), 67.91 (2C), 65.67 (2C), 64.96 (2C), 50.01 (2C), 32.75 (2C), 27.92 (6C).
MS ESI m/z: found 1063.6 (M+1), calculated 1063.76.

UV/vis (toluene, λ/nm): 414, 462, 700.

FT-IR (KBr): ν (cm⁻¹) 2933, 2359, 1731, 1674, 1558, 1506, 1463, 1367, 1147, 1108, 769.

11d (trans-4): classic: Yield 26.00 mg (0.9%). MW: Yield 8 mg, 22%

1H-NMR (400 MHz, CDCl₃): δ 4.33 (d, J = 8.8 Hz, 2H, CH₂ ppyrrolidine), 4.19 (d, J = 9.1 Hz, 2H, CH₂ ppyrrolidine), 4.11 (m, 4H, CH₂ ppyrrolidine), 3.27 (t, J = 7.0 Hz, 4H, CH₂-N), 2.77 (t, J = 7.0 Hz, 4H, CH₂-CO), 1.58 (s, 18H, Boc).

13C-NMR (125 MHz, CDCl₃): δ 171.90, 155.04 (2C), 153.04 (2C), 151.83 (2C), 151.29 (1C), 151.11 (2C), 149.99 (1C), 149.65 (2C), 148.71(2C), 148.34 (2C), 148.05(1C), 147.87 (2C), 146.63 (2C), 146.60 (2C), 146.51 (2C), 145.95 (2C), 145.40 (2C), 145.35 (2C), 145.04 (2C), 143.16 (1C), 143.01 (2C), 142.52 (2C), 142.17 (2C), 142.13 (2C), 141.80 (2C), 141.64 (2C), 139.52 (2C), 139.07 (2C), 136.52 (2C), 135.84 (2C), 131.63 (2C), 81.19, 69.75(2C), 69.52(2C), 68.17(2C), 67.53 (2C), 50.63 (2C), 35.79 (2C), 28.62 (6C).

MS ESI m/z: found 1063.6 (M+1), calculated 1063.76.

UV/vis (toluene, λ/nm): 416, 452, 705.

FT-IR (KBr): ν (cm⁻¹) 2924, 2359, 1732, 1559, 1453, 1366, 1258, 1157, 1032, 798.

11e (equatorial): classic: Yield 41.06 mg (1.4%) MW: Yield 9 mg, 23%

1H-NMR (500 MHz, CDCl₃): δ 4.08 (m (d+s), 4H, CH₂ ppyrrolidine), 4.01 (d, J = 9.8 Hz, 2H, CH₂ ppyrrolidine), 3.91 (s, 2H, CH₂ ppyrrolidine), 3.19 (m, 4H, CH₂-N), 2.71 (m, 4H, CH₂-CO), 1.58 (s, 18H, Boc).

13C-NMR (75 MHz, CDCl₃): δ 171.92, 171.90, 159.42 (2C), 153.96 (2C), 153.42 (2C), 153.13 (2C), 150.23 (1C), 149.29 (2C), 148.46 (2C), 148.17(1C), 148.16 (2C), 147.64 (2C), 147.63 (2C), 147.16 (2C), 147.01 (2C), 146.27 (2C), 145.57 (2C), 145.51 (2C), 145.04 (2C), 144.79 (2C), 144.10 (2C), 143.62 (2C), 142.68 (2C), 142.21 (2C), 142.20 (2C) 142.00 (2C), 141.84 (2C), 141.16 (2C), 139.60 (2C), 137.12 (2C), 136.00 (2C), 81.18, 81.14, 70.13 (2C), 69.89 (1C), 69.81 (1C), 67.99 (2C), 67.65 (1C), 67.03 (1C), 50.65 (1C), 50.50 (1C), 35.73 (1C), 35.72 (1C), 28.63 (6C).

MS ESI m/z: found 1063.6 (M+1), calculated 1063.76.

UV/vis (toluene, λ/nm): 399, 423, 750.

FT-IR (KBr): ν (cm⁻¹) 2949, 2361, 1714, 1502, 1451, 1363, 1260, 1150, 1036, 842, 757.
Synthesis of 12 and 13a-e

10.00 mg (11.22 and 9.40 mmol for 12 and 13a-e respectively) of compound were solved in 10 mL of a mixture DCM/TFA (1:1) and stirred for 24 h. The reaction crude was diluted with additional 10 mL of DCM and extracted with brine (x2) to remove the excess of TFA. The organic phase containing a brown solid in suspension was recovered and the solvent removed by reduced pressure obtaining the desired unprotected derivative in quantitative yields.

12 (monoadduct): 9.35 mg,

$^1$H-NMR (200 MHz, pyridine[d$_5$]): δ 4.51 (s, 4H, CH$_2$pyrrolidine), 3.68 (t, $J = 7.0$ Hz, 2H, N-CH$_2$), 3.30 (t, $J = 7.0$ Hz, 2H, CH$_2$-CO).

The low solubility of 12 did not allow to record the $^{13}$C spectrum.

MS ESI m/z: found 834.9; calculated 835.77.

UV/vis (THF, λ/nm): 333, 433, 706.

FT-IR (KBr): ν (cm$^{-1}$) 2919, 2360, 1747, 1642, 1429, 1386, 1359, 796, 768, 724, 553, 527.

13a (trans-1): 8.80 mg, $^1$H-NMR (200 MHz, pyridine[d$_5$]): δ 4.50 (s, 8H, CH$_2$ pyrrolidine), 3.42 (t, $J = 7.0$ Hz, 4H, N-CH$_2$), 2.60 (t, 4H, $J = 6.0$ Hz, CH$_2$-CO).

$^{13}$C-NMR (50 MHz, pyridine[d$_5$]): δ 174.90, 154.48 (8C), 149.82 (4C), 148.13 (8C), 145.50 (8C), 144.62 (8C), 141.50 (4C), 140.92 (8C), 136.74 (8C), 68.43(4C), 67.96 (4C), 50.20 (2C), 36.20 (2C).

MS ESI m/z: found 951.4 (M+1), calculated 950.81. UV/vis (pyridine, λ/nm): 318, 450, 480, 666.

FT-IR (KBr): ν (cm$^{-1}$) 2969, 2805, 1720, 1550, 1532, 1470, 1453, 1434, 1386, 1340, 768.
13b (trans-2): 8.83 mg,

$^1$H-NMR (200 MHz, pyridine[d$_5$]): $\delta$ 4.67 (d, $J = 9.0$ Hz, 2H, CH$_2$ pyrrolidine), 4.49 (d, $J = 9.2$ Hz, 2H, CH$_2$ pyrrolidine), 4.36 (d, $J = 9.2$ Hz, 2H, CH$_2$ pyrrolidine), 4.33 (d, $J = 9.1$ Hz, 2H, CH$_2$ pyrrolidine), 3.70 (m, $J = 7.0$ Hz, 4H, CH$_2$-N), 3.03 (m, $J = 7.1$ Hz, 4H, CH$_2$-CO).

$^{13}$C-NMR (75 MHz, pyridine[d$_5$]): $\delta$ 173.30, 159.02 (2C), 152.65 (2C), 152.58 (2C), 152.02 (2C), 149.25 (2C), 147.26 (2C), 146.45 (2C), 146.18 (2C), 145.92 (2C), 145.76 (2C), 145.68(2C), 145.21 (2C), 145.19 (2C), 144.99 (2C), 144.69 (2C), 143.44 (2C), 143.36 (2C), 143.31 (2C), 142.12 (2C), 142.08 (2C), 142.02 (2C), 141.89 (2C), 141.27 (2C), 141.15 (2C), 139.87 (2C), 139.10 (2C), 133.99 (2C), 133.32 (2C), 68.76 (2C), 68.59 (2C), 67.32 (2C), 65.12 (2C), 50.05(2C), 36.15 (2C).

MS ESI m/z: found 951.2 (M+1), calculated 950.81.

UV/vis (pyridine, $\lambda$/nm): 421, 457, 724.

FT-IR (KBr): $\nu$ (cm$^{-1}$) 2966, 2459, 1715, 1565, 1453, 1420, 1390, 1350, 1264, 801, 760.

13c (trans-3): 8.82 mg,

$^1$H-NMR (200 MHz, pyridine[d$_5$]): $\delta$ 4.51 (d, $J = 9.0$ Hz, 2H, CH$_2$ pyrrolidine), 4.45 (d, $J = 9.0$ Hz, 2H, CH$_2$ pyrrolidine), 4.10 (m, 4H, CH$_2$ pyrrolidine), 3.5 (t, $J = 7.2$ Hz, 4H, CH$_2$-N), 2.91 (t, $J = 7.2$ Hz, 4H, CH$_2$-CO).

$^{13}$C-NMR (50 MHz, pyridine[d$_5$]): $\delta$ 171.52, 154.33 (2C), 153.51 (2C), 152.44 (2C), 152.22 (2C), 150.87 (2C), 150.62 (2C), 150.43 (2C), 149.62 (2C), 148.92 (2C), 147.24 (2C), 147.05 (2C), 146.92 (2C), 146.67 (2C), 146.13 (2C), 145.82 (2C), 145.25 (2C), 145.14 (2C), 144.68 (2C), 143.34 (2C), 142.89 (2C), 142.46 (2C), 142.33 (2C), 142.00 (2C), 141.46 (2C), 140.28 (2C), 140.00 (2C), 69.58 (2C), 69.30 (2C), 67.00 (2C), 66.29 (2C), 51.02 (2C), 36.82 (2C).

MS ESI m/z: found 951.2 (M+1), calculated 950.81.

UV/vis (pyridine, $\lambda$/nm): 420, 452, 702.

FT-IR (KBr): $\nu$ (cm$^{-1}$) 2930, 2370, 1731, 1674, 1558, 1505, 1463, 1367, 769.

13d (trans-4): 8.78 mg

$^1$H-NMR (200 MHz, pyridine[d$_5$]): $\delta$ 4.43 (d, $J = 9.1$ Hz, 2H, CH$_2$ pyrrolidine), 4.26 (d, $J = 9.0$ Hz, 2H, CH$_2$ pyrrolidine), 4.12 (m, 4H, CH$_2$ pyrrolidine), 3.27 (t, $J = 7.2$ Hz, 4H, CH$_2$-N), 2.77 (t, $J = 7.1$ Hz, 4H, CH$_2$-CO).
\[ ^{13}\text{C-NMR (100 MHz, pyridine[d5]): } \delta \ 173.40, 153.24 (2C), 153.02 (2C), 151.63 (2C), 151.19 (2C), 150.96 (1C), 149.79 (1C), 148.95 (2C), 148.70 (2C), 148.43 (2C), 148.00 (1C), 147.67 (2C), 147.37 (2C), 146.92 (2C), 146.88 (2C), 144.85 (2C), 144.34 (2C), 144.12 (2C), 144.01 (2C), 141.06 (1C), 141.01 (2C), 141.25 (2C), 141.17 (2C), 141.09 (2C), 139.27 (2C), 139.02 (2C), 137.27 (2C), 136.04 (2C), 134.52 (2C), 133.99 (2C), 131.33 (2C), 67.55 (2C), 66.52 (2C), 66.17 (2C), 66.22 (2C), 50.44 (2C), 35.42 (2C). \]

MS ESI m/z: found 951.6 (M+1), calculated 950.81

UV/vis (pyridine, \( \lambda / \text{nm} \)): 414, 452, 705.

FT-IR (KBr): \( \nu (\text{cm}^{-1}) \) 2925, 2360, 1722, 1559, 1453, 1366, 1258, 799.

13e (equatorial): 8.84 mg,

\[ ^1\text{H-NMR (500 MHz, pyridine[d5]): } \delta \ 4.12 (m, 4H, CH}_2\text{pyrrolidine), 3.91 (s, 4H, CH}_2\text{pyrrolidine), 3.88 (t, } J = 9.0 \text{ Hz, 4H, CH}_2\text{-N), 3.16 (t, } J = 9.0 \text{ Hz, 4H, CH}_2\text{-CO) \]

\[ ^{13}\text{C-NMR (50 MHz, pyridine[d5]): } \delta \ 176.22, 154.32 (2C), 154.11 (2C), 153.96 (2C), 154.00 (2C), 152.01 (1C), 149.28 (2C), 148.25 (2C), 148.17 (1C), 148.15 (2C), 147.93 (2C), 147.65 (2C), 147.20 (2C), 146.74 (2C), 145.97 (2C), 145.41 (2C), 145.01 (2C), 144.92 (2C), 144.99 (2C), 144.29 (2C), 143.88 (2C), 142.77 (2C), 142.50 (2C), 142.46 (2C), 141.80 (2C), 141.63 (2C), 141.15 (2C), 139.45 (2C), 137.22 (2C), 135.64 (2C), 70.00 (2C), 69.96 (1C), 69.80 (1C), 67.87 (2C), 67.34 (1C), 67.22 (1C), 50.59 (1C), 30.46 (1C), 30.44 (1C). \]

MS ESI m/z: found 951.4 (M+1), calculated 950.81.

UV/vis (pyridine, \( \lambda / \text{nm} \)): 389, 423, 726.

FT-IR (KBr): \( \nu (\text{cm}^{-1}) \) 2946, 2361, 1722, 1500, 1451, 1373, 1268, 842, 760.

**Synthesis of 14**

A solution of N-(benzyloxycarbonyloxy) succinimide (3.00 g, 12 mmol) in CHCl\( _3 \) (20 mL) was added drop by drop via syringe pump in 5 h to a solution of ethylenediamine (20 mL, 300 mmol) in 150 mL of CHCl\( _3 \), then the mixture was stirred for 8 hours. The crude was washed with 50 mL of brine (x3), 50 mL of saturated NaHCO\( _3 \) (x3) and finally 50 mL of water (x3). The organic phase was dried on Na\( _2\)SO\( _4 \), filtered and the
solvent removed under pressure obtaining a white solid (1.75 g, 75% yield). The characterization was in agreement with literature data.³

¹H-NMR (200 MHz, CDCl₃): δ 7.38-7.34 (m, 5H, CHAr); 5.10 (s, 2H, Ar-CH₂); 3.33 (bs, 1H, NH); 3.24 (q, J=6.0 Hz, 2H, NH-CH₂); 2.82 (t, J=6.0 Hz, 2H, CH₂-NH₂); 1.18 (bs, 2H, CH₂-NH₂).

Synthesis of 15

To a solution of 14 (1.75 g, 9 mmol) in MeOH (50 mL), degassed by bubbling Ar for 15 minutes, was added drop by drop methylacrylate (2 mL, 22 mmol). The mixture was stirred for 24 hours under Ar atmosphere. The yellow oil obtained after evaporation of the solvent was diluted in CHCl₃, washed extensively with 50 mL of brine (x10), dried on Na₂SO₄, filtered, and the solvent removed under reduced pressure obtaining a colorless oil (3.05 g, 90% yield).

¹H-NMR (200 MHz, CD₃OD): δ 7.38-7.34 (m, 5H, CHAr), 4.90 (s, 2H, Ar-CH₂), 3.11 (m, 4H, NH-CH₂-CH₂), 2.80 (s, 6H, O-CH₃), 2.63 (m, 8H, N-CH₂-CH₂-CO).

¹³C-NMR (50 MHz, CD₃OD): δ 175.46, 158.78, 138.41, 129.58, 129.13, 128.99, 70.2, 65.60, 52.58, 51.40, 51.11, 48.38.

MS ESI m/z: found 367.2 (M+1), calculated 366.18.

FT-IR (KBr): ν (cm⁻¹) 3500, 2910, 2852, 2525, 2335, 1694, 1240, 950, 796, 755, 695.
Synthesis of 16

A solution of 15 (3.00 g, 8 mmol) in 50 mL of MeOH was added via addition funnel to a cooled solution (0°C) of ethylenediamine (3.50 mL, 50 mmol) in MeOH (10 mL), and stirred for 7 days at room temperature under Ar. The crude was washed with 50 mL of brine (x5) and 50 mL of saturated NaHCO$_3$ (x5) until removal of the ethylenediamine excess. The organic solvent was distilled obtaining a pale-yellow oil (2.36 g, 65% yield) used without further purification. An aliquot was purified by silica gel chromatography for characterization (CHCl$_3$/MeOH 9:1).

$^1$H-NMR (200 MHz, CD$_3$OD): δ 7.38-7.34 (m, 5H, CHAr), 4.90 (s, 2H, Ar-CH$_2$), 3.11 (t, $J$=6.0 Hz, 6H, NH-C$_2$H$_4$), 2.63 (m, 8H, N-CH$_2$-CH$_2$-CO and CH$_2$-NH$_2$), 2.44 (t, $J$=6.0 Hz, 2H, N-CH$_2$-CH$_2$-NH), 2.23 (t, $J$=6.0 Hz, 4H, CH$_2$-CO).

$^{13}$C-NMR (50 MHz, CD$_3$OD): δ 175.46, 158.78, 138.41, 129.58, 129.13, 128.99, 67.60, 53.78, 51.11, 42.41, 41.97, 39.91, 34.82.

MS ESI m/z: found 423.3 (M+1), 445.3 (M+Na), calculated 422.52.

FT-IR (KBr): ν (cm$^{-1}$) 3419, 2954, 2926, 2852, 2525, 2238, 2075, 1694, 1635, 1553, 1456, 1265, 1117, 973, 775, 745, 700.

Synthesis of 17
To a cooled solution (0°C) of 16 (100.00 mg, 0.25 mmol) in CHCl₃ (5 mL) a solution of Boc₂O (285.00 mg, 1.25 mmol) in DCM (5 mL) was added drop by drop and the resulting solution was stirred for 2 days. The organic solvent was removed under reduced pressure obtaining a mixture of not protected, mono and bis-protected derivative that was purified by silica gel chromatography (CHCl₃/MeOH 95:5) obtaining 6 as a white oil (70.20 mg, 45% yield).

¹H-NMR (400 MHz, CDCl₃): δ 7.35-7.31 (m, 5H, CH₆Ar), 7.13 (bs, 2H, CH₂-CO-NH), 5.82 (bs, 1H, Cbz-NH), 5.31 (bs, 2H, NH-Boc), 5.07 (s, 2H, Ar-CH₂), 3.34-3.06 (m, 10H, NH-CH₂), 2.66 (t, J=6.0 Hz, 4H, CH₂-N-(CH₂)₂), 2.49 (t, J=6.1 Hz, 2H, CH₂-N-(CH₂)₂), 2.28 (t, J=6.1 Hz, 4H, CH₂-CO), 1.41 (s, 18H, NH-Boc).

¹³C-NMR (50 MHz, CDCl₃): δ 172.87, 156.41, 136.48, 128.43, 128.13, 128.09, 79.34, 66.57, 52.67, 50.07, 40.30, 39.77, 38.74, 34.08, 28.40.

MS ESI m/z: found 623.4 (M+1), 645.3 (M+Na), calculated 622.75.

FT-IR (KBr): ν (cm⁻¹) 3327, 3068, 3036, 2976, 2934, 2849, 2828, 1695, 1653, 1539, 1438, 1392, 1366, 1339, 1272, 1253, 1170, 1039, 1027, 1011, 862, 778, 754, 737, 698, 460.

Synthesis of 18

To a solution of 17 (70.00 mg, 0.112 mmol) in MeOH, previously degassed for 10 minutes by bubbling N₂, a catalytic amount of Pd/C (10% w/w) was added. The reaction was stirred for 24 hours under H₂ atmosphere, then filtered on celite and the solvent was removed under reduced pressure, obtaining a white solid in quantitative yield (55 mg).

¹H-NMR (500 MHz, CD₃OD): δ 3.23 (m, 4H, CH₂-NH), 3.16 (m, 4H, CH₂-NHBoc), 2.99 (m, 2H, CH₂-NH₂), 2.72 (m, 6H, CH₂-N), 2.36 (t, J=5.0 Hz, 4H, CH₂-CO), 1.44 (s, 18H, NH-Boc).
\(^{13}\text{C-NMR}\) (500 MHz, \(\text{CD}_3\text{OD}\)): \(\delta\) 175.12, 52.65, 51.13, 43.91, 40.88, 38.80, 34.26.

MS ESI m/z: found 489.4 (M+1); calculated 488.6.

FT-IR (KBr): \(v\) (cm\(^{-1}\)) 3302, 3072, 2977, 2931, 2872, 2852, 1703, 1694, 1657, 1651, 1538, 1455, 1392, 1366, 1275, 1252, 1171, 1040, 1027, 1013, 861, 781, 757, 590, 465.

Synthesis of 19

![Chemical structure of 19](image)

To a solution of 15 (250 mg, 0.273 mmol) in MeOH previously degassed for 10 minutes by bubbling \(\text{N}_2\) was added a catalytic amount of Pd/C (10% w/w). The reaction was leaved for 12 hours under \(\text{H}_2\) atmosphere to finally filtered it in celite and removed the solvent under reduced pressure, obtaining a pale yellow oil in quantitative yield (62 mg).

\(^1\text{H-NMR}\) (200 MHz, \(\text{CD}_3\text{OD}\)): \(\delta\) 7.42 (m, 5H, \(\text{CH}_2\text{Ar}\)), 4.77 (s, 2H, \(\text{Ar-CH}_2\)), 3.22-3.06 (m, 4H, \text{NH-(CH}_2)_2\)), 2.82 (s, 3H, \(\text{O-CH}_3\)), 2.60-2.40 (m, 8H, \(\text{N-(CH}_2)_2\text{-CO}\)).

Synthesis of 20

![Chemical structure of 20](image)

To a solution of 14 (2.00 g, 10 mmol) in MeOH (50 mL) was bubbled Ar for 15 minutes. Later on, \(\text{tert-buthyl}\) acrylate was added in excess drop by drop (5 mL, 34 mmol) leaving it stirring for 72 hours under Ar. The yellow oil obtained was rediluted in CHCl\(_3\) washed extensively with water (x3), saturated solution of NaHCO\(_3\) (x3), brine (x3) dried with Na\(_2\)SO\(_4\), filtered, and the solvent removed under reduced pressure obtaining a colorless oil (2.72 g, 60% yield).
**Experimental Part**

\[^1\text{H-}N^\text{MR} (200 \text{ MHz, } \text{CD}_3\text{OD})\]: \(\delta\) 7.38-7.34 (m, 5H, CHAr), 4.78 (s, 2H, Ar-CH\(_2\)), 3.14-3.08 (m, 4H, NH-(CH\(_2\))\(_2\)), 2.58-2.34 (m, 8H, N-(CH\(_2\))\(_2\)-CO), 1.78 (s, 18H, tBu).

\[^{13}\text{C-NMR} (50 \text{ MHz, } \text{CD}_3\text{OD})\]: \(\delta\) 176.02, 158.38, 129.28 (Ar), 129.22 (Ar), 128.79 (Ar), 128.50 (Ar), 71.03, 60.42 (1C), 50.96 (1C), 48.76 (2C), 47.95 (2C), 27.99 (6C).

MS ESI m/z: found 451.30 (M+1), calculated 450.27.

FT-IR (KBr): \(v\) (cm\(^{-1}\)) 3487, 2853, 2512, 2295, 1674, 1200, 786, 755, 689.

**Synthesis of 21**

\[
\begin{align*}
\text{20} & \quad \rightarrow \quad \text{21}
\end{align*}
\]

To a solution of 20 (1 g, 2.85 mmol) in 20 mL of CHCl\(_3\) previously degassed for 10 minutes by bubbling N\(_2\) was added a catalytic amount of Pd/C (10% w/w). The reaction was leaved for 18 hours under H\(_2\) atmosphere to finally filtered it in celite and removed the solvent under reduced pressure, obtaining a colorless oil (602 mg, 89% yield).

\[^1\text{H-NMR} (200 \text{ MHz, } \text{CD}_3\text{OD})\]: \(\delta\) 3.14 (m, 4H, NH-(CH\(_2\))\(_2\)), 2.82 (t, \(J=6.4\) Hz, 4H, N-CH\(_2\)), 2.63 (t, \(J=6.4\) Hz, 4H, CH\(_2\)-CO) 1.79 (s, 18H, tBu).

\[^{13}\text{C-NMR} (50 \text{ MHz, } \text{CD}_3\text{OD})\]: \(\delta\) 175.82, 158.36, 71.00, 50.89 (1C), 48.73 (2C), 47.94 (2C), 27.89 (6C).

MS ESI m/z: found 233.3 (M+1), calculated 232.14.

FT-IR (KBr): \(v\) (cm\(^{-1}\)) 3486, 2851, 1680, 1203, 784, 772, 753, 688.

**Synthesis of 22**
A solution of 17 (2g, 4.72 mmol) in 50 mL of MeOH was added drop by drop via addition funnel to a solution of methyl acrylate (2.5 mL, 17 mmol) leaving it under stirring for 72 hours in Ar atmosphere. The solvent was removed and the excess of methylacrylate removed by coevaporation with toluene (20 mL, x3) giving a pale yellow oil (2.42 g, 75% yield).

$^1$H-NMR (200 MHz, CD$_3$OD): $\delta$ 3.25-3.18 (m, 12H, NH-(CH$_2$)$_2$), 2.82 (s, 12H, OMe) 2.64-2.48 (m, 24H, N-(CH$_2$)$_2$-CO and N-(CH$_2$)$_2$-CONH).

$^{13}$C-NMR (50 MHz, CD$_3$OD): $\delta$ 174.92, 158.92, 71.99, 61.20 (4C) 52.67 (1C), 51.08 (2C), 42.39(1C), 41.52 (2C), 40.91 (2C), 40.48 (2C) 39.67 (4C), 39.24 (2C), 34.77 (2C),

MS ESI m/z: found 736.55 (M+1) calculated 735.42.

FT-IR (KBr): v (cm$^{-1}$) 3448, 3440, 2967, 2818, 2506, 2491, 2132, 1072, 1046, 780, 744, 443.

Synthesis of 23

A solution of 22 (1g, 1.36 mmol) in 10 mL of MeOH was added via addition funnel to a cooled solution (0°C) of ethylendiamine (7.0 mL, 100 mmol) in MeOH (10mL), and leaved by stirring for 7 days under Ar. The crude of reaction was washed with brine (x5) and saturated NaHCO$_3$ (x5) until remotion of the excess of ethylendiamine. The
organic solvent was removed under pressure obtaining a yellow oil further purified by silica chromatographic column using DCM /MeOH (9:1) as eluent mixture (691 mg, 60% yield).

$^1$H-NMR (200 MHz, MeOD): $\delta$ 7.30-7.28 (m, 5H, CHAr), 5.10 (s, 2H, Ar-CH$_2$), 3.43-3.26 (m, 12H, NH-(CH$_2$)$_2$) 3.19-2.88 (m, 16H, CH$_2$-NHBOc) 2.59-2.44 (m, 24H, N-(CH$_2$)$_2$-CO and N-(CH$_2$)$_2$-CONH).

$^{13}$C-NMR (100 MHz, MeOD): $\delta$ 176.13, 138.41 (Ar), 129.36 (Ar), 129.09 (Ar), 128.97 (Ar), 67.19 (1C), 53.45 (1C), 51.20 (2C), 42.40(1C), 41.78 (2C), 41.44 (2C), 41.42 (2C), 40.90 (2C), 40.84 (2C), 40.25 (2C), 40.65 (2C) 39.90 (4C), 39.73 (2C), 34.82 (2C).

MS ESI m/z: 874.6 (M+Na) calculated 851.52.

FT-IR (KBr): $\nu$ (cm$^{-1}$) 3436, 2965, 2819, 2031, 1552, 1538, 1059, 1078 1016, 746, 733, 452.

**Synthesis of 24**

A solution of 17 (1g, 2.36 mmol) in 20 mL of DCM (HPLC grade, 99.9 %) was added drop by drop via syringe pump to a solution of tert-buthyl acrylate (2.5 mL, 17 mmol) leaving it under stirring for 72 hours under Ar atmosphere. The solvent was removed under reduced pressure to give a brown oil, that was firstly filtrated in a silica plug using DCM and then purified by cromatographic column on silica using DCM/MeOH 9:1 as eluent to give a colorless oil ( 876.24 mg, 40% yield)

$^1$H-NMR (500 MHz, CD$_3$OD): $\delta$ 7.30-7.28 (m, 5H, CHAr), 5.11 (s, 2H, Ar-CH$_2$), 3.20-3.15 (m, 12H, NH-(CH$_2$)$_2$), 2.60-2.45 (m, 24H, N-(CH$_2$)$_2$-CO and N-(CH$_2$)$_2$-CONH), 1.77 (s, 36H, tBu).
\(^{13}\)C-NMR (125 MHz, CD\(_3\)OD): \(\delta\) 175.50, 159.61, 138.40 (Ar), 129.37 (Ar), 129.10 (Ar), 128.98 (Ar), 72.12, 67.10 (1C), 53.78 (1C), 51.11 (2C), 42.41 (1C), 41.97 (2C), 40.62 (2C), 40.60 (2C), 39.91 (4C), 39.80 (2C), 34.82 (2C), 28.21 (12C).

MS HR-MALDI-TOF (matrix: THAP) found: 921.5943 M+1) calculated 920.58.

FT-IR (KBr): \(\nu\) (cm\(^{-1}\)) 3456, 2987, 2830, 2510, 2488, 2120, 1060, 1025, 786, 752, 480.

**Synthesis of 25**

A solution of 24 (100 mg, 0.11 mmol) in 5 mL of DCM/TFA 1:1 was stirred for 4 h until no trace of 25 was observed by TLC. The solvent was removed under reduced pressure and rests of TFA were removed by addition of 10 mL MeOH and coevaporation. As a result, a pale yellow oil was obtained in quantitative yield (75 mg).

\(^{1}\)H-NMR (200 MHz, CD\(_3\)OD): \(\delta\) 7.31-7.28 (m, 5H, CHAr), 5.09 (s, 2H, Ar-CH\(_2\)), 3.30-3.18 (m, 12H, NH-(CH\(_2\))\(_2\)), 2.60-2.48 (m, 24H, N-(CH\(_2\))\(_2\)-CO and N-(CH\(_2\))\(_2\)-CONH).

\(^{13}\)C-NMR (50 MHz, CD\(_3\)OD): \(\delta\) 175.30, 138.42 (Ar), 129.35 (Ar), 129.08 (Ar), 128.98 (Ar), 67.09 (1C), 53.77 (1C), 51.11 (2C), 42.40 (1C), 41.89 (2C), 40.68 (2C), 40.56 (2C), 39.93 (4C), 39.79 (2C), 34.81 (2C).

MS ESI found: 697.5 (M+1) calculated 696.33

FT-IR (KBr): \(\nu\) (cm\(^{-1}\)) 3453, 2988, 2829, 2510, 2488, 1058, 1029, 785, 751, 478.
Synthesis of 26

To a solution of 25 (500 mg, 0.70 mmol) in 10 mL of MeOH was added dropwise distilled, filtered a solution of N-Boc ethylenediamine (4.70 g, 29 mmol) in 10 mL of MeOH. The resulting mixture was left under stirring at room temperature for 7 days under Ar. The solvent was removed under reduced pressure and the excedent of N-Boc ethylenediamine removed by coevaporation with toluene. A final purification using a Sephadex 6-100 resin column yielded 26 as a white sticky solid (200 mg, 22%).

\[ \text{Experimental Part} \]

$^1$H-NMR (500 MHz, D$_2$O): δ 7.31-7.28 (m, 5H, CHAr), 5.09 (bs, 2H, Ar-CH$_2$), 3.42-3.25 (m, 12H, NH-(CH$_2$)$_2$) 3.18-2.80 (m, 16H, CH$_2$-NHBoc) 2.58-2.42 (m, 24H, N-(CH$_2$)$_2$-CO and N-(CH$_2$)$_2$-CONH), 1.80 (bs, 36H, Boc).

$^{13}$C-NMR (100 MHz, D$_2$O): δ 176.20, 159.22 138.42 (Ar), 129.35 (Ar), 129.08 (Ar), 128.98 (Ar), 70.77, 67.19 (1C), 53.47 (1C), 51.21 (2C), 42.38 (1C), 41.76 (2C), 41.44 (2C), 41.43 (2C), 40.89 (2C), 40.86 (2C), 40.27 (2C), 40.64 (2C) 39.89 (4C), 39.70 (2C), 34.80 (2C), 27.02 (12C).

MS MALDI-TOF (matrix: DCTB) m/z: 1265.9 (M+1) calculated 1264.78.

FT-IR (KBr): ν (cm$^{-1}$) 3446, 2967, 2820, 2500, 2448, 203, 1552, 1538, 1060, 1098 1018, 766, 742, 478.
Synthesis of 27

To a solution of 24 (500 mg, 0.54 mmol) in 5 mL of MeOH previously degassed for 10 minutes by bubbling N₂ was added a catalytic amount of Pd/C (10% w/w). The reaction was leaved for 12 hours under H₂ atmosphere to finally filtered it in celite and removed the solvent under reduced pressure, obtaining a colorless oil (341 mg, 70% yield).

¹H-NMR (200 MHz, CD₃OD): δ 3.32-3.19 (m, 12H, NH-(CH₂)₂), 2.67-2.48 (m, 24H, N-(CH₂)₂-CO and N-(CH₂)₂-CNH), 1.90 (s, 36H, tBu).

¹³C-NMR (75 MHz, CD₃OD): δ 175.50, 159.61, 72.12, 52.80 (1C), 51.09 (2C), 42.39 (1C), 42.00 (2C), 40.15 (2C), 40.58 (2C) 39.22 (4C), 39.67 (2C), 34.64 (2C), 28.15 (12C).

MS ESI found: 809.2 (M+Na) calculated 786.55.

FT-IR (KBr): ν (cm⁻¹) 3339, 2965, 2829, 2507, 1059, 1012, 784, 749, 482.
Synthesis of 28

To a solution of 26 (100 mg, 0.07 mmol) in 5 mL of MeOH previously degassed for 10 minutes by bubbling N₂ was added a catalytic amount of Pd/C (10% w/w). The reaction was leaved for 12 hours under H₂ atmosphere to finally filtered it in celite and removed the solvent under reduced pressure, obtaining a white sticky solid in quantitative yield (79 mg).

¹H-NMR (200 MHz, D₂O): δ 3.50-3.24 (m, 12H, NH-(CH₂)₃) 3.06-2.82 (m, 16H, CH₂-NHBoc) 2.60-2.44 (m, 24H, N-(CH₂)₂-CO and N-(CH₂)₂-CONH), 1.79 (bs, 36H, Boc).

¹³C-NMR (50 MHz, D₂O): δ 176.22, 159.20, 67.20 (1C), 53.43 (1C), 51.18 (2C), 42.08 (1C), 41.66 (2C), 41.43 (4C), 40.85 (2C), 40.81 (2C), 40.44 (2C), 40.61 (2C) 39.79 (4C), 39.70 (2C), 34.92 (2C), 27.25 (12C).

MS ESI m/z: 1131.2 (M+1), 1154.4 (M+23) calculated 1130.74.

FT-IR (KBr): ν (cm⁻¹) 3436, 2492, 2445, 2031, 1563, 1537, 1100, 1095 1020, 740, 468.
General procedure for synthesis of 29, 30, 31 and 32

To a solution of 10 mg of fullerene derivative (0.01 mmol) and EDC (0.10 mmol) in 2 mL of anhydrous pyridine was added drop by drop a mixture of 18, 21, 27 and 28 (0.10 mmol) for 29, 30, 31, 32 respectively and DMAP (0.10 mmol) and the reaction was stirred under Ar atmosphere for 12 hours. The solvent was removed under reduced pressure to obtain a brown oil that was purified by column chromatography with silica using CHCl₃/MeOH (9:1) as eluent. The product was precipitated from a highly concentrated solution in CHCl₃ by adding distilled MeOH to give a brown solid.
29: (11.50 mg, 70% yield)

$^1$H-NMR (500 MHz, CDCl$_3$): $\delta$ 8.19 (bs, 1H, NH-CO); 7.20 (bs, 2H, NH-CO), 5.53 (bs, 2H, NH-Boc), 4.49 (s, 4H, CH$_2$ pyrrolidine), 3.45 (t, $J$ = 6.5 Hz, 2H, CH$_2$-N(CH$_2$)$_2$); 3.37 (t, $J$ = 6.3 Hz, 6H, NH-CH$_2$); 3.27 (t, $J$ = 5.0 Hz, 4H, CH$_2$-NHboc); 2.86 (t, $J$ = 6.5 Hz, 2H, CH$_2$N pyrrolidine), 2.74 (bs, 4H, N-(CH$_2$)$_2$), 2.65 (bs, 2H, N$_{pyrrolidine}$-CH$_2$-CH$_2$), 2.35 (bs, 4H, CH$_2$-CONH), 1.43 (s, 18H, NH-Boc).

$^{13}$C-NMR (125 MHz, CDCl$_3$): $\delta$ 173.02, 156.74, 147.33, 146.29, 146.11, 145.91, 145.48, 145.31, 144.54, 143.14, 142.67, 142.04, 141.89, 140.22, 135.98, 79.48 (2C), 70.52 (2C), 67.42 (2C), 53.07 (1C), 50.82 (2C), 40.45 (1C), 40.15 (2C), 38.10 (1C), 35.06 (2C), 34.20 (1C), 28.45 (6C).

MS ESI m/z: found 1306.3 (M$^+$), 1328.3 (M+Na), calculated 1306.38.

UV/vis (DCM, $\lambda$/nm): 226, 254, 313, 691.

FT-IR (KBr): $\nu$ (cm$^{-1}$) 3370, 2925, 1691, 1648, 1538, 1455, 1365, 1250, 1169, 527.

30: (2.60 mg, 23% yield)

$^1$H-NMR (200 MHz, CDCl$_3$/CS$_2$): $\delta$ 5.11 (s, 4H, CH$_2$ pyrrolidine), 3.62 (t, $J$=6.5 Hz, 4H, NH-(CH$_2$)$_2$), 3.02 (t, $J$=9.1 Hz, 2H, CH$_2$N pyrrolidine), 2.95 (t, $J$=6.3 Hz, 4H, N-(CH$_2$)$_2$), 2.88 (t, $J$=9.1 Hz, 2H, N$_{pyrrolidine}$-CH$_2$-CH$_2$), 2.49 (t, $J$= 6.3 Hz, 4H, CH$_2$-CO), 1.75 (s, 18H, tBu).

The low solubility of this derivative did not allow to record $^{13}$C-NMR spectra.

UV/vis (CHCl$_3$, $\lambda$/nm): 230, 262, 316, 690.

FT-IR (KBr): $\nu$ (cm$^{-1}$) 3370, 2920, 1635, 1533, 1342, 1289, 1169, 527.

31: (12.94 mg, 84% yield)

$^1$H-NMR (500 MHz, CDCl$_3$): $\delta$ 4.80 (s, 4H, CH$_2$ pyrrolidine), 3.62-3.40 (m, 12H, CH$_2$N(CH$_2$)$_2$ and NH-CH$_2$), 3.38-2.99 (m, 18H, CH$_3$N pyrrolidine and N-(CH$_2$)$_2$), 2.96-2.80 (m, 10H, CH$_2$-CO and N$_{pyrrolidine}$-CH$_2$-CH$_2$), 1.72 (s, 36H, tBu).

$^{13}$C-NMR (125 MHz, CDCl$_3$): $\delta$ 173.00, 156.74, 156.72, 147.31, 146.22, 146.08, 146.05, 145.54, 145.35, 145.06, 144.87, 143.12, 142.45, 142.10, 142.04, 141.76, 140.18, 135.95, 79.52 , 71.34 , 67.40, 53.05 (1C), 50.82 (2C), 50.62 (2C), 50.30 (2C), 40.43 (1C), 40.13 (2C), 40.10 (2C), 40.08 (2C), 38.10 (1C), 35.07 (2C), 35.03 (2C), 34.20 (1C), 28.84 (12C).

UV/vis (CHCl$_3$, $\lambda$/nm): 229, 260, 316, 698.
FT-IR (KBr): ν (cm⁻¹) 3312, 3300, 2943, 2922, 1680, 1635, 1533, 1350, 1311, 1289, 1246, 1169, 649, 523.

32: (3.94 mg, 22% yield)

¹H-NMR (500 MHz, CDCl₃): δ 4.82 (s, 4H, CH₂ pyrrolidine), 3.62-3.41 (m, 20H, CH₂-N(CH₂)₂ and NH-CH₂), 3.38 (t, J=6.5 Hz, 2H, CH₂-N pyrrolidine), 3.31-2.98 (m, 24 H, N-(CH₂)₂ 2.96-2.40 (m, 10H, CH₂-NHBoc and N₄ pyrrolidine-CH₂-CH₂) 1.45 (s, 36H, NH-Boc).

The low quantity obtained did not permit to record ¹³C-NMR spectra.

MS MALDI (matrix: THAP) found 1963.1 (M⁺), 1328.3 (M+Na), calculated 1961.88.

UV/vis (CHCl₃, λ/nm): 228, 260, 315, 695.

FT-IR (KBr): ν (cm⁻¹) 3360, 3357, 3349, 2923, 2918, 1865, 1853, 1672, 1648, 1630 1534, 1422, 1353, 1310, 1296 1250, 1168, 753, 650, 527.

**Synthesis of 33a-e**

To a solution of 10.00 mg of 13a-e (0.010 mmol for 12) and EDC (0.10 mmol) in mixture of 1mL of CHCl₃/pyridine, was added drop by drop a mixture of 1G-PAMAM 18 (0.10 mmol) and DMAP (0.100 mmol) and the reaction was stirred under Ar atmosphere for 12 hours. The solvent was removed under reduced pressure to obtain a brown oil that was purified by column chromatography with Al₂O₃ using CHCl₃/MeOH (99:1) as
eluent. The product was precipitated from a highly concentrated solution in CHCl₃ by adding separately ethanol, ethyl ether and cyclohexane to give a brown solid.

**33a (trans-1)**

Yield 82% (16.62 mg)

$^1$H-NMR (400 MHz, CDCl₃): δ 4.70 (s, 8H, CH₂ pyrrolidine), 3.80 (t, J = 6.5 Hz, 4H, CH₂-N(CH₂)₂); 3.62 (t, J = 6.3 Hz, 12H, NH-CH₂); 3.57 (t, J = 5.0 Hz, 8H, CH₂-NHBoc); 3.01 (t, J = 6.5 Hz, 4H, CH₂-N pyrrolidine), 2.96 (bs, 8H, N-(CH₂)₂), 2.52 (bs, 4H, N_pyrrolidine-CH₂-CH₂), 2.41 (bs, 8H, CH₂-COHN), 1.38 (s, 36H, NH-Boc).

$^{13}$C-NMR (100 MHz, CDCl₃): δ 173.89, 154.62 (8C), 148.88 (8C), 144.32 (8C), 144.89 (8C), 143.02 (4C), 142.77 (8C), 136.63 (8C), 80.00, 68.59 (4C), 68.12 (4C), 52.98 (2C), 50.46 (4C), 40.42 (2C), 40.10 (4C), 37.99 (2C), 35.16 (4C), 35.46 (2C), 28.02 (12C).

MS MALDI-TOF (matrix: (DCTB)) m/z: found 1891.8 (M+1), calculated: 1890.82.

UV/vis (CHCl₃, λ/ nm): 325, 460, 491, 667.

FT-IR (KBr): ν (cm⁻¹) 3365, 2898, 1701, 1650, 1453, 1372, 1259, 770, 530.

**33b (trans-2)**

Yield 41% (8.22 mg)

$^1$H-NMR (400 MHz, CDCl₃): δ 4.70 (bb, 2H, CH₂ pyrrolidine), 4.61 (d, J = 9.1 Hz, 2H, CH₂ pyrrolidine), 4.42 (bb, 2H, CH₂pyrrolidine), 4.38 (bb, 2H, CH₂ pyrrolidine), 3.63-3.51 (m, 16H, CH₂-N(CH₂)₂ and NH-CH₂), 3.20-3.12 (m, 24H, CH₂-NHBoc, CH₂-NPyrrolidine and N-(CH₂)₂), 2.30 (m, 8H, CH₂-COHN), 1.46 (s, 36H, Boc).

The low quantity obtained did not permit to record $^{13}$C.

MALDI-TOF (no matrix) m/z: found 1891.7 (M+1), calculated: 1890.82. UV/vis (CHCl₃, λ/ nm): 441, 480, 723.

FT-IR (KBr): ν (cm⁻¹) 3070, 2969, 2930, 2816, 2849, 2361, 1700, 1697, 1660, 1649, 1550, 1549, 1250, 1152, 1000, 795, 563, 452.

**33c (trans-3)**

Yield 75% (15.20 mg)

$^1$H-NMR (400 MHz, CDCl₃): δ 4.60 (d, 2H, J = 9.3 Hz, CH₂ pyrrolidine), 4.51 (d, J = 9.3 Hz, 2H, CH₂ pyrrolidine), 4.45 (d, J = 9.9 Hz, 2H, CH₂ pyrrolidine), 4.23 (d, J = 9.4 Hz, 2H, CH₂ pyrrolidine) 3.82-3.71 (m, 16H, CH₂-N(CH₂)₂ and NH-CH₂), 3.46-3.35 (m, 24H, CH₂-NHBoc, CH₂-NPyrrolidine and N-(CH₂)₂), 2.56 (m, 8H, CH₂-COHN), 1.72 (s, 36H, Boc).
13C-NMR (100 MHz, CDCl3): δ 173.21, 154.12 (2C), 153.06 (2C), 152.88 (2C), 152.33 (2C), 149.76 (2C), 148.99 (2C), 148.34 (2C), 148.10 (2C), 147.61 (2C), 147.52 (2C), 145.92 (2C), 145.20 (2C), 145.00 (2C), 144.76 (2C), 144.54 (2C), 144.38 (2C), 143.79 (2C), 143.53 (2C), 143.47 (2C), 143.33 (2C), 142.28 (2C), 141.74 (2C), 141.10 (2C), 140.91 (2C), 140.68 (2C), 139.26 (2C), 136.23 (2C), 135.11 (2C), 82.42, 69.35 (2C), 68.01 (2C), 66.49 (2C), 63.89 (2C), 50.11 (2C), 50.08 (4C), 49.78 (4C), 44.18 (4C), 41.23 (4C), 37.21 (2C), 34.11 (2C), 33.02 (2C), 28.26 (12C).

MALDI-TOF (matrix: pivalic acid) m/z: found 1891.8 (M+1), calculated: 1890.82.

UV/vis (toluene, λ/nm): 418, 461, 700.

FT-IR (KBr): ν (cm⁻¹) 3069, 2975, 2982, 2900, 2852, 2378, 1742, 1697, 1660, 1617, 1558, 1368, 1243, 1150, 1061, 698, 540, 498.

33d (trans-4)

Yield 60% (12.00 mg).

1H-NMR (400 MHz, CDCl3): δ 6.08 (bs, 2H, NH-Boc), 4.35 (dd, J = 9.0 Hz, 4H, CH₂-pyrrolidine), 4.18 (s, 4H, CH₂-pyrrolidine), 3.55-3.48 (m, 16H, CH₂-N(CH₂)₂ and NH-CH₂), 3.21 (m, 8H, CH₂-NHBoc), 3.05 (m, 12H, CH₂-Pyrrolidine and N-(CH₂)₂), 2.72 (m, 4H, NPyrrolidine-CH₂-CH₂), 2.48 (m, 8H, CH₂-CONH), 1.45 (s, 36H, NH-Boc).

13C-NMR (125 MHz, CDCl3): δ 171.95, 150.78 (2C), 149.55 (2C), 149.52 (2C), 149.02 (2C), 148.19 (1C), 147.69 (1C), 147.64 (2C), 147.41 (2C), 147.26 (2C), 147.23 (1C), 146.10 (2C), 145.99 (2C), 145.97 (2C), 145.42 (2C), 144.75 (2C), 144.71 (2C), 144.42 (2C), 142.66 (2C), 142.55 (1C), 142.47 (2C), 141.97 (2C), 141.66 (2C), 141.63 (2C), 141.23 (2C), 141.13 (2C), 139.02 (2C), 138.14 (2C), 135.71 (2C), 134.99 (2C), 130.84 (2C), 77.53, 69.09 (2C), 68.87 (2C), 67.14 (2C), 66.47 (2C), 53.23 (2C), 49.77 (4C), 49.76 (4C), 44.79 (4C), 40.06 (4C), 36.00 (2C), 34.64 (2C), 34.60 (2C) 28.24 (12C).

MS MALDI-TOF (matrix: DCTB)) m/z: found 1891.9 (M+1), calculated: 1890.82.

UV/vis (CHCl₃, λ/nm): 418, 452, 710.

FT-IR (KBr): ν (cm⁻¹) 3069, 2975, 2982, 2900, 2852, 2378, 1742, 1697, 1660, 1617, 1558, 1368, 1243, 1150, 1061, 698, 540, 498.

33e (equatorial)

Yield 71% (14.00 mg)

1H-NMR (400 MHz, CDCl₃): δ 4.35 (d, J = 9.0 Hz, 2H, CH₂ Pyrrolidine), 4.24 (d, J = 9.0 Hz, 2H, CH₂ Pyrrolidine), 4.13 (bb, 4H, CH₂ Pyrrolidine), 3.52-3.21 (m, 36H, CH₂-NPyrrolidine, N-(CH₂)₂, CH₂-N(CH₂)₂, NH-CH₂ and CH₂-NHBoc), 2.72 (bt, 4H, NPyrrolidine-CH₂-CH₂), 2.42 (bb, 8H, CH₂-CONH), 1.45 (s, 36H, Boc).
$^{13}$C-NMR (125 MHz, CDCl$_3$): $\delta$ 173.36, 159.30 (2C), 157.07 (2C), 153.36 (2C), 153.11 (2C), 150.24 (1C), 149.34 (2C), 148.53 (2C), 148.16 (1C), 147.66 (2C), 147.50 (2C), 147.06 (2C), 146.19 (2C), 146.16 (2C), 145.65 (2C), 145.46 (2C), 145.14 (2C), 145.11 (2C), 145.06 (2C), 144.88 (2C), 144.84 (2C), 144.22 (2C), 143.55 (2C), 142.68 (2C), 140.90 (2C), 140.87 (2C), 139.60 (2C), 139.45 (2C), 137.11 (2C), 135.76 (2C), 79.86, 79.83, 79.80, 70.18 (1C), 70.09 (2C), 70.06 (2C), 69.77 (1C), 69.68 (1C), 67.71 (1C), 52.38 (1C), 52.30 (1C), 51.23 (2C), 51.20 (1C), 51.18 (1C), 40.84 (2C), 40.81 (1C), 40.78 (1C), 40.54 (1C), 40.52 (2C), 40.50 (1C), 38.42 (1C), 38.35 (2C), 38.20 (1C), 35.29 (2C), 35.22 (1C), 35.19 (1C), 34.29 (1C), 34.26 (1C), 28.89 (12 C).

MS-MALDI-TOF (matrix: (DCTB)) $m/z$: found 1891.9 (M+1); calculated: 1890.82.

UV/vis (CHCl$_3$, $\lambda$/nm): 390, 424, 720

FT-IR (KBr): $\nu$ (cm$^{-1}$) 3365, 2910, 1688, 1678, 1645, 1530, 1449, 1365, 1248, 1168, 526.
General procedure for 34, 35, 36, 37 and 38a-e

5.00 mg of compound (4.2, 3.8, 3.0, 2.5 and 2.6 µmol for 30, 29, 31, 32 and 33a-e respectively) were solved in 5 mL of a mixture DCM/TFA (1:1) and stirred for 12 h to 48 h. The reaction crude was diluted with additional 10 mL of DCM and extracted with a solution of saturated NaHCO₃ and brine (x2) to remove the excess of TFA. The organic phase containing a brownish solid in suspension was recovered and the solvent removed by reduced pressure obtaining the desired unprotected derivative in quantitative yields.

34: 4.28 mg

¹H-NMR (200 MHz, pyridine[d₅]): δ 5.11 (s, 4H, CH₂ pyrrolidine), 3.80 (t, J=6.5 Hz, 4H, and NH-(CH₂)₂), 3.25 (t, J=9.4 Hz, 2H, CH₂-N pyrrolidine), 3.11 (t, J=6.3 Hz, 4H, N-(CH₂)₂), 2.82 (t, J=9.4 Hz, 2H, N_pyrrolidine-CH₂-CH₂), 2.52 (t, J= 6.3 Hz, 4H, CH₂-CO).
The low solubility of this derivative did not allow to record $^{13}$C-NMR spectra.

MS ESI: found 1020.0 (M-1); calculated 1021.16.

UV/vis (Pyridine, $\lambda$/nm): 240, 318, 680.

35: 2.80 mg,

MS ESI m/z : found 368.9 (M+); calculated 369.43.

UV/vis (H$_2$O, $\lambda$/nm) = 260.

36: 4.18 mg

$^1$H-NMR (200 MHz, CDCl$_3$/pyridine[d$_5$]): $\delta$ 5.10 (s, 4H, CH$_2$ pyrroldine), 3.82-3.56 (m, 12H, CH$_2$-N(CH$_2$)$_2$ and NH-CH$_2$), 3.42-3.02 (m, 18H, CH$_2$-N pyrrolidine and N-(CH$_2$)$_2$), 3.01-2.92 ( m, 10H, CH$_2$-CO and N-pyrrolidine-CH$_2$-CH$_2$).

The small quantity obtained did not permit to record $^{13}$C spectra.

MS ESI: found 1393.2 (M-1); calculated 1394.35 UV/vis (CHCl$_3$, $\lambda$/nm): 240, 320, 700.

38a: 3.39 mg,

MS MALDI (no matrix) m/z : found 254.7 (M+); calculated 254.61.

UV/vis (H$_2$O, $\lambda$/nm): 320, 459, 489, 665.

38b: 3.38 mg,

MS MALDI (no matrix) m/z : found 254.6 (M+); calculated 254.61.

UV/vis (H$_2$O, $\lambda$/nm): 417 (broad), 461, 708.

38c: 3.38 mg,

MS MALDI (no matrix) m/z : found 254.4 (M+); calculated 254.61.

UV/vis (H$_2$O, $\lambda$/nm): 415, 450, 705.

38d: 3.39 mg,

MS MALDI (no matrix) m/z : found 254.6 (M+); calculated 254.61.

UV/vis (H$_2$O, $\lambda$/nm) = 415, 450, 705.
38e: 3.40 mg.

MS MALDI (no matrix) m/z : found 254.6. (M+); calculated 254.61.

UV/vis (H₂O, λ/nm) = 390, 422, 749.

Synthesis of 39 Tert-butyl 4-hydroxybutanoate

![Chemical structure](image)

To a mixture of succinic anhydride (30 g, 0.30 mol), N-hydroxysuccinimide (0.30 equiv, 10 g, 0.09 mol), and DMAP (0.10 equiv, 3.5 g, 0.03 mol) in toluene (150 mL) were added tert-butyl alcohol (1.3 equiv, 35 mL, 0.37 mol) and Et₃N (0.3 equiv, 0.09 mol, 12.5 mL). The suspension was refluxed for 24 h. The solution was cooled and diluted with EtOAc (150 mL). The reaction mixture was washed with 10% citric acid and brine, dried over Na₂SO₄, and concentrated to give a brown oil. The oil was recrystallized with ether and petroleum ether at -20 °C to give 40 as a white crystal (41 g, 78%).

Characterizations were in accordance with literature.¹⁵⁶a

¹H-NMR (300 MHz, CDCl₃): δ 2.65-2.50 (m, 4 H), 1.45 (s, 9 H).

Synthesis of 40 4-Hydroxybutanoic Acid tert-Butyl Ester

![Chemical structure](image)

To a solution of acid 4 (21 g, 0.12 mol) in dry THF (183 mL) at 0 °C was added BH₃·Me₂S (2.0 M, 65.5 mL, 0.131 mol) dropwise. After being stirred at room temperature for 24 h, the mixture was cooled to 0 °C. Water (100 mL) and solid K₂CO₃
were added to the reaction mixture. The reaction mixture was extracted with Et₂O (200 mL × 3), and the combined organic extracts were washed with brine, dried over Na₂SO₄, and concentrated in vacuo. The residue was distilled to give a colorless liquid (14.50 g, 74%).

Characterizations were in accordance with literature.¹⁵⁶a

¹H-NMR (300 MHz, CDCl₃): δ 3.70 (t, J = 6.0 Hz, 2 H), 2.40-2.30 (m, 3 H), 1.85 (m, 2 H), 1.45 (s, 9 H).

Synthesis of 41

Tert-butyl 4-hydroxybutanoate 39 (2.00 g, 12.5 mmol) and pyridine (1.0 mL, 12.5 mmol) were dissolved in dry CH₂Cl₂ (20mL) under nitrogen atmosphere and cooled to 0 °C. Subsequently, a solution of malonyl dichloride (0.61 mL, 6.22 mmol) in dry CH₂Cl₂ (20 mL) was added dropwise under vigorous stirring over a period of 2 hours. The reaction mixture was stirred for additionally 2 hours at 0 °C, followed by stirring at room temperature overnight. After filtering through a silica gel plug with hexanes/EtOAc 50:50, the solution was extracted with a saturated solution of NH₄Cl (2 x 100 mL) and NaHCO₃ (2 x 100 mL). Purification was achieved by flash chromatography on silica gel using a mixture of hexanes/EtOAc = 5:1 as eluent. Finally the product fractions were evaporated in vacuum to isolate 41 as a colorless oil. (1.47 g, 61% based on malonyl dichloride).

Characterizations were in accordance with literature.¹⁵⁶b

¹H-NMR (300 MHz, CDCl₃): δ 4.43 (t, J = 6.4 Hz, 4H, OCH₂) 3.31 (s, 2H, O₂CCH₂CO₂), 2.24 (t, J = 7.4 Hz, 4H, CH₂CO₂Bu), 1.87 (tt, J = 6.9 Hz, J =6.8 Hz, 4H, CH₂CH₂CH₂), 1.38 (s, 18H, C(CH₃)₃).

¹³C-NMR (75 MHz, CDCl₃): δ 172.01 (2C, CO₂Bu) 166.42 (2C, O₂CCH₂CO₂) 80.51 (2C, C(CH₃)₃), 64.63 (2C, OCH₂),41.48 (1C, O₂CCH₂CO₂)31.82 (2C, CH₂CO₂Bu), 28.17 (6C, C(CH₃)₃),24.03 (2C, CH₂CH₂CH₂).

IR (diamond) ν (cm⁻¹) =2980, 2937, 1725, 1459, 1420, 1393, 1366, 1328, 1254, 1212, 1146, 1096, 1031, 953, 884, 845, 753

Synthesis of 42

To a solution of 40 (500 mg, 1.3 mmol) in DCM (20 mL) was added dropwise formic acid until the obtention of a pH=3 solution and leaved by stirring for 12 h. After a first addition of water (20 mL) the reaction was extracted with brine (x3) to obtain a colorless oil in quantitative yield (350 mg).

Characterizations were in accordance with literature.¹⁵⁶b

¹H-NMR (300 MHz, CDCl₃): δ 10.02 (bs, COOH) 4.42 (t, J = 6.4 Hz, 4H, OCH₂) 3.20 (s, 2H, O₂CCH₂CO₂), 2.24 (t, J = 7.4 Hz, 4H, CH₂COOH), 1.87 (tt, J = 6.9 Hz, J =6.8 Hz, 4H, (CH₂CH₂CH₂).

Synthesis of 43

A solution of Boc anhydride (29.45 g, 0.14 mol) in dioxane (150 mL) was added dropwise to a solution of 2,2-(ethylent-dioxy) bis(ethylamine) (40.00 g, 0.27 mol) in dioxane (150 mL). After 12 h, the solvent was removed under reduced pressure and water was added to give a semisolid that was filtered over celite to remove the
bisprotected amine. The resulting oil was extracted from the filtrated aqueous solution with ethyl acetate (x3) and dried over Na$_2$SO$_4$ to give 43 (18.83 g, 56%).

Characterizations were in accordance with literature.$^{157}$

$^1$H-NMR (300 MHz, CDCl$_3$): δ 5.15 (bs, 1H), 3.65-3.45 (m, 8H), 3.31 (t, $J$=5.2 Hz, 2H), 2.85 (t, $J$=5.2 Hz, 2H), 1.42 (s, 9H).

**Synthesis of 44**

\[
\begin{align*}
\text{NH}_2 & \text{O} \quad \text{O} \quad \text{NHBoc} + \text{HOOC} & \quad \text{O} \quad \text{O} \quad \text{COOH} \\
\text{BocHN} & \quad \text{O} \quad \text{O} \quad \text{NHBoc} & \quad \text{O} \quad \text{O} \quad \text{NH} \\
\text{O} & \quad \text{O} \quad \text{O} \quad \text{O} & \quad \text{O} \quad \text{O} \quad \text{NHBoc} \\
\text{BocHN} & \quad \text{O} \quad \text{O} \quad \text{NHBoc} & \quad \text{O} \quad \text{O} \quad \text{NH} \\
\text{O} & \quad \text{O} \quad \text{O} \quad \text{O} & \quad \text{O} \quad \text{O} \quad \text{NHBoc} \\
\text{BocHN} & \quad \text{O} \quad \text{O} \quad \text{NHBoc} & \quad \text{O} \quad \text{O} \quad \text{NH} \\
\text{O} & \quad \text{O} \quad \text{O} \quad \text{O} & \quad \text{O} \quad \text{O} \quad \text{NHBoc} \\
\end{align*}
\]

To a cooled solution of 42 (100 mg, 0.72 mmol), 43 (360 mg, 1.44 mmol), and DMAP (439 mg, 3.60 mmol) in DCM anhydrous under Ar atmosphere was added in one pot (742 mg, 3.60 mmol) and leaved by stirring for 2 hours at 0°C and 12 additional hours at room temperature. The solvent was removed under reduced pressure to give a yellow oil that was purified by using silica column chromatography and an eluent mixture DCM/MeOH 9:1 to afford 44 as a colorless oil (317 mg, 60% yield).

$^1$H-NMR (300 MHz, CDCl$_3$): δ 4.56 (t, $J$= 6.4 Hz, 4H, OCH$_2$), 3.76-3.62 (m, 16H), 3.36 (t, $J$=5.5 Hz, 4H, CH$_2$-HNCO), 3.18 (s, 2H, O$_2$CCH$_2$CO$_2$), 2.88 (t, $J$=5.5 Hz, 4H, BocHN-CH$_2$) 2.42 (t, $J$= 7.8 Hz, 4H, CH$_2$COONH), 1.87 (tt, $J$= 6.9 Hz, $J$=6.8 Hz, 4H, (CH$_2$CH$_2$CH$_2$), 1.60 (s, 18H, Boc).

$^{13}$C-NMR (75 MHz, CDCl$_3$): δ 173.02 (2C, CO$_2$Bu) 156.42 (2C, OOCCH$_2$COO), 155.91 (2C, OCONH) 78.92 (2C, C(CH$_3$)$_3$), 68.06 (2C), 66.66 (2C) 64.63 (2C, OOC-CH$_2$-COOCH$_3$), 50.77 (2C), 48.81 (2C), 41.51 (1C, O$_2$CCH$_2$CO$_2$), 40.39 (2C), 40.31 (2C), 31.77 (2C, CH$_3$CONH), 28.46 (6C, C(CH$_3$)$_3$), 24.03 (2C, CH$_2$CH$_2$CH$_2$).

MS-MALDI-TOF (matrix: (DCTB)): found 737.8 (M+1), expected 736.40.
IR (diamond): ν (cm⁻¹) 3347, 2980, 2937, 2871, 1720, 1712, 1512, 1458, 1420, 1399, 1368, 1248, 1175, 966, 865, 747, 752, 701.

Synthesis of 45

A solution of C₆₀ (14 mg, 0.02 mmol) in anhydrous o-dichlorobenzene (50 mL) was prepared and to the solution were added 44 (125 mg, 0.16 mmol), CBr₄ (550 mg, 33.0 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (45 µL, 0.33 mmol). The mixture was stirred under N₂ for 72 hours and purified by column chromatography on silica gel using CHCl₃/pyridine 97:3, to provide the corresponding hexakisadduct 45 (34 mg, 40%).

¹H-NMR (400 MHz, CDCl₃): δ 4.86 (t, J = 6.4 Hz, 24H, OCH₂), 4.02-3.83 (m, 96H), 3.42 (bb, 24H, BocHN-CH₂), 2.421 (t, J = 8.0 Hz, 24H, CH₂COONH), 1.92 (tt, J = 6.9 Hz, J = 6.8 Hz, 24H, (CH₂CH₂CH₂), 1.60 (s, 108H, Boc).

¹³C-NMR (100 MHz, CDCl₃): δ 169.05 (12C, CO₂Bu) 168.40 (12C, OOCCH₂COO), 154.92 (12C, OCONH), 145.83 (sp² C₆₀), 141.03 (sp² C₆₀), 80.12 (12C, C(CH₃)₃), 70.01 (sp³ C₆₀), 68.13 (12C), 66.91 (12C) 64.72 (12C, OOC-CH₂-COOCH₂), 51.86 (12C), 48.89 (12C), 41.57 (6C, O₅CCH₂CO₂), 40.62 (12C), 39.98 (12C), 32.63 (12C, CH₂CONH), 28.92 (36C, C(CH₃)₃), 26.25 (12C, CH₂CH₂CH₂).
Synthesis of 46

A solution of C₆₀ (370 mg, 0.51 mmol) in anhydrous o-dichlorobenzene (250 mL) was prepared and to the solution were added 41 (2 g, 5.15 mmol), CBr₄ (17 g, 51.5 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (155 µL, 10.3 mmol). The mixture was stirred under N₂ for 24 hours and purified by column chromatography on silica gel using CHCl₃/MeOH 95:5, to provide the corresponding hexakisadduct 46 (541 mg, 35%).

¹H-NMR (400 MHz, CDCl₃): δ 4.77 (t, J = 6.8 Hz, 24H, OCH₂), 2.80 (t, J = 8.1 Hz, 48H, CH₂CO₂Bu), 1.92 (tt, J = 7.9 Hz, J = 7.8 Hz, 24H, CH₂CH₂CH₂), 1.89 (s, 108H, C(CH₃)).

¹³C-NMR (100 MHz, CDCl₃): δ 168.09 (12C, CO₂Bu) 166.42 (12C, OOCCH₂COO), 145.81 (sp² C₆₀), 141.18 (sp² C₆₀), 79.21 (12C, C(CH₃)₃), 70.11 (sp³ C₆₀) 65.68 (12C, OCH₂), 33.02 (12C, CH₂CO₂Bu), 29.40 (36C, C(CH₃)₃), 24.04 (12C, CH₂CH₂CH₂).

MS-MALDI-TOF (matrix: pivalic acid): found 3059.82 (M+Na), expected 3036.11
Synthesis of 47

A solution of 46 (500 mg, 0.16 mmol) in 20 mL of a mixture of DCM/TFA 1:1 was stirred for 12 hours, the solvent removed under reduced pressure to give 47 in quantitative yield (375 mg).

$^1$H-NMR (400 MHz, MeOD): $\delta$ 10.06 (bb, 1H, COOH), 4.55 (bb, 24H, OCH$_2$), 2.67 (t, $J$ = 7.7 Hz, 48H, CH$_2$CO$_2$H), 1.90 (tt, $J$ = 7.5 Hz, $J$ = 7.4 Hz, 24H, CH$_2$CH$_2$CH$_2$)

$^{13}$C-NMR (100 MHz, MeOD): $\delta$ 164.12 (12C, OOCCCH$_2$COO), 143.26 (sp$^2$ C$_{60}$), 141.10 (sp$^2$ C$_{60}$), 69.86 (sp$^3$ C$_{60}$) 63.22 (12C, OCH$_2$), 32.96 (12C, CH$_2$CO$_2$H), 23.99 (12C, CH$_2$CH$_2$CH$_2$).

MS-MALDI-TOF (matrix: CHCA): found 2363.86 (M-1H), expected 2364.65.

Synthesis of 48
To a cooled solution of 42 (40 mg, 0.14 mmol), 18 (140 mg, 0.28 mmol), and DMAP (34 mg, 0.28 mmol) in DCM anhydrous under Ar atmosphere was added in one pot EDC (53 mg, 0.28 mmol) and leaved by stirring for 2 hours at 0°C and 36 additional hours at room temperature. The solvent was removed under reduced pressure to give a yellow oil that was purified by using silica column chromatography and an eluent mixture DCM/MeOH 8:2 to afford 48 as a white solid (102 mg, 61% yield).

$^1$H-NMR (300 MHz, CDCl$_3$): $\delta$ 3.78-3.62 (m, 20H, CH$_2$-N-(CH$_2$)$_2$ HNCO-CH$_2$ and tBuOCONH-CH$_3$), 3.50 (t, $J = 6.0$ Hz, 8H, N-(CH$_3$)$_2$), 3.22 (s, 2H, O$_2$CCCH$_2$CO$_2$), 2.98-2.88 (m, 8H, CH$_2$CONH and CH$_2$-CO-CH$_2$-CO), 2.31 (t, $J = 7.2$ Hz, 4H, CH$_2$(CH$_2$)$_2$CO-CH$_2$-CO), 1.80 (tt, 4H, CH$_2$-CH$_2$-CH$_2$-CO-CH$_2$-CO), 1.61 (s, 36 H, Boc).

$^{13}$C-NMR (75 MHz, CDCl$_3$): $\delta$ 170.06, 162.76 (2C, O$_2$C-CH$_2$-CO), 157.99 (4C, OCONH), 79.94 (24C, C-(CH$_3$)$_3$), 65.73 (OOC-CH$_2$-COO), 64.86, 52.99, 52.95, 48.21, 46.92, 28.72 (12C, Boc), 23.62, 20.03.

MALDI-TOF (no matrix): found 1218.2 (M+1H), expected 1216.73
Synthesis of 49

A solution of C$_{60}$ (15 mg, 0.02 mmol) in anhydrous o-dichlorobenzene (20 mL) was prepared and to the solution were added 48 (260 mg, 0.21 mmol), CBr$_4$ (706 mg, 2.13 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU)(62 µL, 0.42 mmol). The mixture was stirred under N$_2$ for 8 days, and purified by column chromatography on silica gel using CHCl$_3$/Pyridine 97:3, to provide the corresponding hexakisadduct 49 (24 mg, 15%).
1H-NMR (400 MHz, CDCl₃): δ 5.26 (s, 24H, 'BuOCONH), 3.68 (t, J = 6.6 Hz, 24H, CH₂-N-(CH₂)₃), 3.66 (t, J = 6.6 Hz, 48H, HNCO-CH₂), 3.64-3.62 (m, 48H, 'BuOCONH-CH₂), 3.60 (t, t, J = 6.5 Hz 48H, N-(CH₂)₃), 3.02-2.96 (m, 48H, CH₂CONH and CH₂-CO-CH₂-CH₂-CO), 2.31 (t, J = 7.9 Hz 24H, CH₂(CH₂)₂-CO-CH₂-CO), 2.28 (bs, 24H, CH₂-CH₂-CH₂-CO-CH₂-CO), 1.52 (s, 216H, Boc).

13C-NMR (100 MHz, CDCl₃): δ 164.92, 162.86, 161.13, 158.20 (24 C, OCONH), 146.30 (sp² C₆₀), 138.61 (sp² C₆₀), 79.94 (24C, C-(CH₃)₃), 73.21 (sp³ C₆₀), 71.70, 64.92, 54.86, 51.12, 48.52, 46.02, 41.03 (OOC-C-COO), 28.72 (72C, Boc), 24.85, 19.92.

MALDI-TOF (matrix: pivalic acid): found 8005.7 (M+H), expected 8004.16.

Synthesis of 50

A solution of 45 (10 mg, 2.0 μmol) in 5 mL of a mixture of DCM/TFA 1:1 was stirred for 12 hours, the solvent removed under reduced pressure to give 50 in quantitative yield (9 mg).
1H-NMR (400 MHz, D2O): δ 4.50 (bb, 24H, OCH2), 4.17-3.88 (m, 96H), 3.45 (bb, 24H, CH2-HNCO), 3.21 (bb, 24H, BocHN-CH2) 2.26 (bb, 24H, CH2-COONH), 1.88 (m, 24H, (CH2CH2CH2).

MS-MALDI-TOF (no matrix): found 269.1, expected 269.19

Synthesis of 51

A solution of 49 (10 mg, 1.2 µmol) in 5 mL of a mixture of DCM/TFA 1:1 was stirred for 12 hours and the solvent removed under reduced pressure to give 51 in quantitative yield (8 mg).

1H-NMR (400 MHz, D2O): δ 3.82-3.66 (m, 72H, CH2-N-(CH2)2 and HNCO-CH2), 3.34-3.22 (m, 48H, tBuOCONH-CH2), 3.20 (m, 96H, N-(CH2)2), CH3CONH and CH2-CO-CH2-CO), 2.31-2.28 (m, 48H, CH2(CH2)2-CO-CH2-CO and CH2-CH2-CH2-CO-CH2-CO).

MS-MALDI-TOF (no matrix): found 137.2, expected 137.09
Synthesis of 52a-f

A solution of methyl 4-formylbenzoate (1.13 g, 6.89 mmol) and distilled isonicotinaldehyde (2.03 mL, 2.10 mmol) in propionic acid (100 mL) was heated to 130 °C and subsequently added drop by drop pyrrole (1.94 mL, 27.99 mmol). The resulting dark solution was refluxed for 2 hours under darkness, when the solvent was removed to give a dark oil. When a minimum quantity of NET₃ and excess of MeOH was added a dark violet precipitated was observed after 2 days at -20°C. Thus, the crude was filtered and extensively washed with cooled MeOH to give a violet solid containing mixture of all the isomers 55a-f. This mixture can be efficiently separated by using chromatographic column using as eluent CHCl₃/MeOH (99:1). Each isolated isomer was finally precipitated from a saturated solution in THF and addition of petroleum ether.

Characterizations were in agreement with literature.¹⁵⁸

52a

¹H-NMR (200 MHz, CDCl₃): δ 8.85 (s, 8H), 8.48 (d, J =8.3 Hz, 8H) 8.32 (d, J =8.0 Hz, 8H), 4.14 (s, 12H, CH), 2.80 (s, 2H).

52b

¹H-NMR (200 MHz, CDCl₃): δ 9.07 (d, J =5.4 Hz, 2H) 8.85 (m, 8H) 8.48 (d, J =8.3) 8.32 (d, J =8.0 Hz, 6H), 8.18 (d, J =5.9 Hz, 2H), 4.14 (s, 9H), -2.83 (s, 2H).
52c and 52d were obtained as a mixture, their separation is beyond the scope of this thesis.

52e

$^1$H-NMR (200 MHz, CDCl$_3$): $\delta$ 9.06, 8.86 (8H, m), 8.47 (2H, d, $J$=8.1Hz) 8.30 (2H, d, $J$=8.1Hz), 8.30 (2H, d, $J$=8.1Hz), 8.16 (6H, m), 4.12 (3H, s), -2.89 (2H, s).

52f was obtained in very small quantities since quickly decomposes in silica, but its obtention is beyond the scope of this thesis.

Synthesis of 53

![Chemical structure of 52e and 53]

To a solution of 52e (50 mg, 0.06 mmol) in 24 mL of THF/MeOH 2:1 were added 3 mL of a 40% (w/v) solution of KOH in water. The mixture was heated to 40°C and leaved under stirring for 40 hours. The reaction can be monitored by means of TLC using CHCl$_3$/EtOH 98:2 until the no trace of the ester is detected. The mixture was acidified with concentrated HCl to pH 5 and then 20 mL of water were added to extract the organic phase with chloroform (20 mL). The organic phase obtained was further washed with 20 mL of water (x3). Later, the organic solvent was removed under reduced pressure to obtain the desired acid 53 (24 mg, 51%).

The characterizations were in accordance with literature.$^{159}$

$^1$H-NMR (200 MHz, DMSO [d$_6$]): $\delta$ 13.31 (bs, 3H, COO$_3$H), 9.05 (m, 2H, H$_{2,6}$-pyridine), 8.89 (m, 8H, H$_8$), 8.39 (m, 12H, H$_{2,6}$ and H$_{3,5}$-phenyl), 8.29 (m, 2H, H$_{3,5}$-pyridine), -2.97 (s, 2, NH).
Synthesis of 54

To a solution of 52e (710 mg, 1.05 mmol) in 100 mL of anhydrous THF was added LiAlH₄ (319 mg, 80.4 mmol) at room temperature. After being stirred for 30 min, 10 mL of AcOEt was added and then poured into H₂O (100 mL). The aqueous suspension was extracted with CHCl₃ (300 mL), washed with brine, dried over MgSO₄, and evaporated under reduced pressure to yield 54 (360 mg, 53%).

^1H-NMR (200 MHz, CDCl₃): δ 9.06 (d, 6H, J=4.4 Hz, 2,6-pyridyl), 8.86 (m, 8H, pyrrole ß), 8.19 (m, 8H, 2,6-phenyl and 3,5-pyridyl), 7.80 (d, J=7.6 Hz, 2H, 3,5-phenyl), 5.10 (s, 2H, -CH₂OH), -2.88 (s, 2H, internal pyrrole)

Characterizations were in accordance with literature.¹⁵⁹

Synthesis of 55
To a solution of (COCl)$_2$ (0.5 mL, 5.85 mmol) in anhydrous DCM (5 mL) was dropped DMSO (0.5 mL, 7.05 mmol) for 2 min at -60°C. After being stirred for 15 min at the same temperature, 54 (99.4 mg, 0.15 mmol) in DCM (50 mL) was dropped into the above mixture for 10 min. After being further stirred for 20 min at the same temperature, the reaction was completed by the addition of triethylamine (2.5 mL, 17.9 mmol) and was stirred for 30 min longer at the same temperature. After being warmed to room temperature, the organic layer was washed with water, saturated NaHCO$_3$, and then brine. After being dried over MgSO$_4$ and evaporated under reduced pressure, the crude 55 was filtered through a plug of silica using CHCl$_3$/MeOH 95:5 to afford 55 as a purple solid (65.6 mg, 66%).

Characterizations were in accordance with literature.$^{159}$

$^1$H NMR (200 MHz, CDCl$_3$): $\delta$ 10.43 (s, 1H, CHO), 9.05 (bs, 6H, 2,6-pyridyl), 8.84 (m, 2H, pyrrole $\beta$), 8.40 (m, 2H, 2,6-phenyl), 8.30 (d, $J=8.2$ Hz, 2H, 3,5-phenyl), 8.18 (d, 6H, $J=4.8$ Hz, 3,5-pyridyl) -2.88 (s, 2H, internal pyrrole).

**Synthesis of 56**

![chemical structure](image)

A solution of 9 (200 mg, 0.98 mmol), p-nitro benzaldehyde (148 mg, 0.98 mmol) and C$_{60}$ (708 mg, 0.98 mmol) in 100 mL of $o$-dichlorobenzene previously sonicated for 10 minutes until complete dissolution was refluxed for 30 min. The monoadduct was recovered by silica chromatographic column with toluene/ethylacetate 9:1. After precipitation with the addition of Petroleum eter to a saturated solution 56 was afforded as a brown solid. (100 mg, 86% yield respect to recovered C$_{60}$).

$^1$H-NMR (200 MHz, CDCl$_3$): $\delta$ 7.92 (d, $J=1.2$ Hz, 2H, p-Ar), 7.01 (d, $J=1.2$ Hz, 2H, p-Ar), 5.36 (d, $J=8.9$ Hz, 1H, CH$_2$pyrrolidine), 4.96 (s, 1H, CH$_2$pyrrolidine), 4.72 (d, $J=8.9$ Hz,
1H, CH$_2$pyrrolidine), 3.90 (m, 2H, N-CH$_2$), 3.22 (m, 2H, N-CH$_2$-CH$_2$-COO), 1.89 (s, 9H, tBu).

$^{13}$C-NMR (50 MHz, CDCl$_3$): δ 170.23, 155.64, 154.42, 150.02 (Ar), 148.96, 147.23, 145.11, 145.03, 144.96, 143.32, 143.06, 142.87, 142.55, 142.30, 141.89, 141.35, 140.65, 140.33, 136.62 (Ar) 125.03 (Ar), 124.98 (Ar), 78.03(=CH$_3$)3, 72.62 (1C), 70.45 (1C), 68.62, 68.61, 36.40, 29.10, 28.11.

MS ESI m/z : found 1013.2 (M+1); calculated 1012.14.

UV/vis (DCM, λ nm) = 225, 250, 310, 689.

FT-IR (KBr): ν (cm$^{-1}$) 3370, 2925, 2920, 2896, 1815, 1805, 1798, 1690, 1644, 1536, 1450, 1410, 1363, 1200, 1159, 750 527.

**Synthesis of 57**

A solution of sarcosine (62 mg, 0.69 mmol), $p$-nitro benzaldehyde (104 mg, 0.69 mmol) and C$_{60}$ (500 mg, 0.609 mmol) previously sonicated for 10 minutes was refluxed in o-dichlorobenzene for 20 min. The monoadduct was recovered by silica chromatographic column with toluene/ethylacetate 9:1. After precipitation by addition of Petroleum eter to a saturated solution 57 was afforded as a brown solid. (80 mg, 78% yield respect to recovered C$_{60}$).

$^1$H-NMR (200 MHz, CDCl$_3$): δ 8.22 (d, $J$=1.1 Hz, 2H, $p$-Ar), 8.11 (d, $J$=1.1 Hz, 2H, $p$-Ar), 5.20-5.16 (m, 2H, CH$_2$pyrrolidine), 4.46, $J$=8.9 Hz, 1H, CH$_2$pyrrolidine),2.98 (s, 3H, OCH$_3$).

$^{13}$C-NMR (50 MHz, CDCl$_3$): δ 169.11, 155.62, 153.97, 151.62 (Ar), 148.85, 140.21, 145.10, 144.96, 144.93, 142.99, 142.96, 142.86, 142.61, 142.04, 141.92, 141.26, 140.85, 140.22, 137.52
(Ar) 132.08 (Ar), 125.55 (Ar), 79.22 (C(CH$_3$)$_3$), 73.06 (1C), 70.45 (1C), 70.36 (1C), 68.61, 52.60.

MS ESI m/z : found 899.02 (M+1); calculated 897.07, UV/vis (DCM, $\lambda$ nm) = 226, 252, 309, 690.

FT-IR (KBr): $\nu$ (cm$^{-1}$) 3369, 2920, 1814, 1806, 1790, 1690, 1644, 1452, 1411, 1364, 1199, 1159, 732 525.

**Synthesis of 58**

To a solution of 59 (50 mg, 0.05 mmol) in 10 mL of toluene previously degassed for 10 minutes by bubbling N$_2$ was added a catalytic amount of Pd/C (10% w/w). The reaction was leaved for 6 hours under H$_2$ atmosphere to finally filtered it in celite and removed the solvent under reduced pressure, obtaining a brown solid in quantitative yield (42 mg).

$^1$H-NMR (200 MHz, CDCl$_3$): $\delta$ 7.74 (d, $J$=1.0 Hz, 2H, p-Ar), 6.95 (d, $J$=1.0 Hz, 2H, p-Ar), 5.10 (d, $J$=9.1 Hz, 1H, CH$_2$pyrrolidine), 4.96 (s, 1H, CH$_2$pyrrolidine), 4.45 (d, $J$=9.1 Hz, 1H, CH$_2$pyrrolidine), 3.76 (bb, 2H, O-CH$_3$), 2.96 (s, 3H, OCH$_3$).

MS-ESI: found 869.20 (M+1), expected: 868.10.
Synthesis of 59

A solution of 59 (25 mg, 0.02 mmol) in 5 mL of a mixture of DCM/TFA 1:1 was stirred for 12 hours, the solvent removed under reduced pressure to give 62 in quantitative yield (23 mg).

\[ \text{1H-NMR (200 MHz, pyridine \([d_5]\]):} \delta 7.76 (bb, 2H, p-Ar), 7.65 (bb, 2H, p-Ar), 5.36 (m 2H, CH\textsubscript{2}pyrrolidine), 4.81 (d, J=8.8 Hz, 1H, CH\textsubscript{2}pyrrolidine), 3.62 (bb, 2H, N-CH\textsubscript{2}), 3.30 (m, 2H, N-CH\textsubscript{2}-CH\textsubscript{2}-COO). \]

\[ \text{13C-NMR (50 MHz, CDCl\textsubscript{3}):} \delta 173.23, 152.60, 151.23, 150.00 (Ar), 148.95, 147.22, 146.84, 145.01, 144.85, 144.02, 143.92, 142.86, 142.55, 142.30, 141.7, 141.02, 140.55, 140.11, 134.23 (Ar) 125.46 (Ar), 124.21 (Ar), 73.00 (1C), 71.95 (1C), 69.03, 69.02, 36.12, 29.20. \]

\[ \text{MS ESI m/z : found 925.0 (M-1); calculated 926.11.} \]

\[ \text{UV/vis (CHCl\textsubscript{3}, } \lambda \text{ nm)} = 230, 252, 311, 689. \]

\[ \text{FT-IR (KBr): } \nu (\text{cm}^{-1}) 3400, 2986, 2915, 2920, 1801, 1760, 1686, 1632, 1525, 1406, 1400, 1363, 740. \]
Synthesis of 60

To a solution of 59 (10 mg, 0.01 mmol) and EDC (19 mg, 0.10 mmol) in 1mL of CHCl₃, was added drop by drop a mixture of 1G-PAMAM 18 (48.8 mg, 0.10 mmol) and DMAP (12 mg, 0.100 mmol) and the reaction was stirred under Ar atmosphere for 12 hours. The solvent was removed under reduced pressure to obtain a brown solid that was purified by silica column chromatography CHCl₃/MeOH (90:10) as eluent. The product was precipitated from a highly concentrated solution in CHCl₃ by adding ethyl ether to give 60 as a brown solid (13 mg, 90% yield)

¹H-NMR (500 MHz, pyridine[de₅]): δ 8.92 (bb, 1H, NH-CO), 8.46 (d, J=1.0 Hz, 2H, p-Ar), 7.62 (d, J=1.0 Hz, 2H, p-Ar), 5.61 (d, 1H, J=9.2 Hz, 1H, CH₂pyrrolidine), 5.42 (s, 1H, CH₂pyrrolidine), 4.36 (d, 1H, J=9.2 Hz, 1H, CH₂pyrrolidine), 4.01-3.42 (m, 12H, NH(CH₂), CH₂-NHBoc, CH₂-N(pyrrlidade) 3.08-3.01 (m, 4H, N-(CH₃)₂) 3.00-2.96 (m, 2H, N(pyrrlidade)-CH₂-CH₂) 2.86-2.42 (m, 4H, CH₂-CONH), 1.51 (s, 18H, Boc).

¹³C-NMR (125 MHz, CDCl₃): δ 172.62, 155.78, 153.69, 149.99 (Ar), 148.26, 146.52, 145.08, 144.94, 144.23, 143.57, 143.05, 142.67, 142.14, 142.01, 141.90, 141.26, 140.46, 140.33, 136.28 (Ar) 124.92 (Ar), 124.00 (Ar), 79.06 (C-(CH₃)₃), 71.58 (1C), 70.12 (1C), 68.76 (1C), 68.72 (1C), 51.22 (1C), 50.77 (2C), 40.32 (1C), 40.01 (2C), 37.97 (1C), 35.02 (2C), 34.10 (1C), 28.10 (6C).

MS ESI m/z : found 1397.5(M+1); calculated 1396.43.
UV/vis (CHCl₃, λ nm) = 231, 253, 311, 690.

FT-IR (KBr): ν (cm⁻¹) 3369, 3320, 3108, 2924, 1690, 1582, 1496, 1455, 1430, 1364, 1250, 1210, 1169, 723, 525.

Synthesis of 61

To a solution of 60 (10 mg, 7 µmol) in 5 mL of toluene previously degassed for 10 minutes by bubbling N₂ was added a catalytic amount of Pd/C (10% w/w). The reaction was leaved for 36 hours under H₂ atmosphere to finally filtered it and removed the solvent under reduced pressure, obtaining 61 as a brown solid in quantitative yield (10 mg). When the solid is filtered on celite, the dendrofullerene 64 tends to adsorb on it.

¹H-NMR (200 MHz,CDCl₃): δ 7.86 (d, J=1.0 Hz, 2H, p-Ar), 7.72 (d, J=1.0 Hz, 2H, p-Ar), 5.23 (d, J=9.2 Hz, 1H, CH₂pyrrolidine), 5.32 (s, 1H, CH₂pyrrolidine), 4.51 (d, 1H, d, J=9.2 Hz, 1H, CH₂pyrrolidine), 4.22-3.42 (m, 15H, NH-C₃H₂, CH₂-NHBoc, CH₂-N pyrrolidine, NH₂) 3.26-3.12 (m, 4H, N-(CH₂)₂) 2.95-2.46 (m, 6H, Npyrrolidine-CH₂-CH₂ and CH₂-CONH), 1.51 (s, 18H, Boc).

¹³C-NMR (50 MHz,CDCl₃): δ 172.59, 155.77, 153.62, 148.92 (Ar+1C), 146.32, 146.01, 145.63, 145.00, 144.32, 143.26, 142.96, 142.67, 141.88, 141.24, 140.96, 140.35, 140.2, 138.12 (Ar), 128.13 (Ar), 123.99 (Ar), 80.02 (C-(CH₃)₃), 71.23 (2C), 68.73 (2C), 51.50 (1C), 50.50 (1C), 40.48 (1C), 40.12 (2C), 37.99 (1C), 35.00 (2C), 34.62(1C), 28.90 (6C)
UV/vis (CHCl₃, λ nm) = 231, 254, 311, 690.

FT-IR (KBr): ν (cm⁻¹) 3420, 3369, 3321, 3108, 2924, 1689, 1582, 1496, 1455, 1430, 1364, 1210, 1168, 722, 523.

**Synthesis of 62**

To a solution of 53 (10 mg, 15 µmol) and EDC (21 mg, 0.15 mmol) in 1mL of DMF anh, was added drop by drop a mixture of 61 (21 mg, 15 µmol) and DMAP (12 mg, 0.15 mmol) and the reaction was stirred under Ar atmosphere for 36 hours at 60°C. The solvent was removed under reduced pressure to obtain a brown solid that was purified by silica column chromatography CHCl₃/MeOH (95:5) as eluent. The product was precipitated from a highly concentrated solution in CHCl₃ by adding petroleum ether to give 62 as a brown redish solid (3.8 mg, 12% yield).

¹H-NMR (500 MHz, CDCl₃): δ 9.1 (m, 6H, 2,6-pyridil), 8.91 (m, 8H, pyrrole β), 8.25 (m, 4H, Ar-P), 8.10 (m, 2H, 3,5-pyridyl ), 8.01 (bb, Npyrr-(CH₂)₂CONH), 7.72 (m, 4H, CHPyrr-Ar), 7.01 (bb, 3H, CONH), 5.31 (bb, 2H, NHBOC), 5.25 (m, 2H, CH₂pyrrolidine), 3.86-3.22 (m, NH-CH₂, CH₂-NHBoc and CH₂-N(CH₂)₂, 2.98-2.44 (m, CH₂-N(CH₂)₂ and Npyrrolidine-CH₂-CH₂), (m, 4H, CH₂CONH), 1.49 (s, 18H, Boc), -2.98 (s, 2H, internal pyrrole).

The low quantity obtained did not permit to record ¹³C-NMR spectra.

MS-MALDI-TOF (no matrix): found 2057.2, expected 2056.64.

UV/vis (CHCl₃, λ nm) = 417, 512, 547, 590, 645.
Synthesis of 63

A solution of 9 (10 mg, 49 µmol), 55 (104 mg, 49 µmol) and C₆₀ (500 mg, 0.609 mmol) previously sonicated for 10 minutes was refluxed in o-dichlorobenzene anhydrous/DMF anhydrous 1:1 for 2 hours under Ar. After removing the unreacted C₆₀ by silica chromatographic column with only toluene, the monoadduct was recovered with toluene/ethylacetate 9:1. After precipitation by addition of petroleum ether to a saturated solution 63 was afforded as a brown solid. (22 mg, 15% yield respect to recovered C₆₀).

¹H-NMR (500 MHz,CDCl₃): δ 9.12 (m, 6H, 2,6-pyridil), 8.86 (m, 8H, pyrrole β), 8.30 (m, J = 1.0 Hz, 10H, Ar and 3,5-pyridyl ), 5.42 (m, 2H, CH₂pyrrolidine), 4.40 (d, J = 9.2 Hz 1H, CH₂pyrrolidine), 3.45 (t, J = 9.0 Hz 2H, N-CH₂), 3.18 (t, J = 9.0 Hz, 2H, CH₂-CO), 1.51 (s, 9H, tBu), -2.90 (s, 2H, internal pyrrole).

¹³C-NMR (100 MHz,CDCl₃): δ 172.60, 167.31, 155.76, 153.62, 150.22 (4'-py), 148.90, 148.51 (2',6' pyr), 147.20 (broad, Cα pyrrol), 146.40 (Ar-P), 146.32, 146.12, 145.60, 144.99, 144.23, 143.18, 142.95, 142.66, 141.78, 141.15, 140.89, 140.33, 140.18, 138.23 (Ar), 134.61 (Ar-P), 131.99 (broad, β-pyrrole), 130.06 (Ar-P), 129.55 (3',5'-pyr), 128.11, 128.08 (Ar-P), 123.90, 120.00 (Cmeso), 118.25 (Cmeso), 79.82 (C-(CH₃)₃), 71.06 (CH₂-CO), 68.88 (CH₂-CO), 51.30 (1C), 50.49 (1C), 41.46 (1C), 41.02 (2C), 38.02 (1C), 34.62 (2C), 34.22(1C), 28.88 (6C).

MS-ESI found 1513.0 (M+4H), expected 1508.36

UV/vis (CHCl₃, λ nm) = 420, 510, 545, 588, 643.
Synthesis of 64

A solution of 63 (20 mg, 13 µmol) in 5 mL of a mixture of DCM/TFA 1:1 was stirred for 1 hour, the solvent removed under reduced pressure to give 64 in quantitative yield (18 mg).

$^1$H-NMR (200 MHz, DMF [d$_7$]): δ 9.31 (bb, 6H, 2,6-pyridil), 8.72 (bb, 8H, pyrrole β), 8.05 (bb, overlaped with DMF signal, 10H, Ar and 3,5-pyridyl), 5.50 (m, 2H, CH$_2$pyrrolidine), 4.48 (bb, 1H, CH$_2$pyrrolidine), 3.52 (bb, 2H, N-CH$_2$), 3.18 (bb, 2H, CH$_2$-CO), -2.90 (s, 2H, internal pyrrole).

$^{13}$C-NMR spectra could not be recorded due to the low solubility of 64.

MS-ESI found 1436.8 (M+4H), expected 1432.30

UV/vis (DMF, λ nm) = 520 (broad), 543, 590, 643.
Synthesis of 65

A solution of 64 (10 mg, 7 µmol) and EDC (13 mg, 70 µmol) in 5 mL of DMF anh (pH of the resultant solution= 8.2), was added drop by drop a mixture of 18 (35 mg, 70 µmol) and DMAP (8 mg, 70 µmol) and the reaction was stirred under Ar atmosphere for 36 hours at r.t. The solvent was removed under reduced pressure to obtain a red oil that was purified by silica column chromatography CHCl₃/MeOH (98:2) as eluent. The product was precipitated from a highly concentrated solution in CHCl₃ by adding petroleum ether to give 65 as a dark red solid (16 mg, 62% yield).

¹H-NMR (400 MHz,CDCl₃): δ 9.06 (m, 6H, 2,6-pyridil), 8.83 (m, 8H, pyrrole β), 8.10-7.89 (m,10H, Ar and 3,5-pyridyl ), 5.10 (bb, 1H, CH₂pyrrolidine), 5.00 (s, 1H, CH₂pyrrolidine), 4.80 (d, J=8.9 Hz, 1H, CH₂pyrrolidine), 4.10-3.26 (m, 15H, NH-C₆H₄, C₆H₄-NHBoc, C₆H₄-N pyrrolidine, NH₂) 3.05-2.80 (m, 10H, N-(C₆H₄)₂ and Npyrrolidine-CH₂-CH₂ and CH₂-CNH), 1.48 (s, 18H, Boc), -2.92 (s, 2H, internal pyrrole).

¹³C-NMR (75 MHz,CDCl₃): δ 172.60, 167.28, 155.77, 153.60, 150.30 (4'-py), 148.99, 148.62 (2',6' pyr), 146.52 (Ar-P), 146.41, 146.20, 145.66, 144.89, 144.22, 143.6, 142.93, 142.80, 141.79, 141.25, 140.89, 140.32, 140.26, 138.57 (Ar), 134.86 (Ar-P), 132.00 (broad, β-pyrrole), 130.02 (Ar-P), 129.56 (3’,5’-pyr), 128.10, 128.18 (Ar-P), 123.89, 120.06 (Cmeso), 117.10 (Cmeso), 79.99 (C-(CH₃)₃), 71.22 (2C), 69.25 (2C), 51.48 (1C), 50.46 (1C), 40.21 (1C), 40.13 (2C), 37.52 (1C), 34.96 (2C), 33.78(1C), 28.88 (6C).

MS-ESI found 1927.8 (M+H), expected 1926.62.

UV/vis (CHCl₃, λ nm) = 421, 510, 540, 580, 640.
General procedure of methylation to obtain 66 and 67

In a sealed vial, 5 mg of 62 (2.4 µmol) or 65 (2.5 µmol) were solved in the minimum volume of chloroform (1 mL) and then added an excess (4 mL) of methyl iodide. The mixture was refluxed for 72 hours and the reaction followed by reversed-face HPLC until observation of a single peak. Thus, the reaction was stopped, cooled to 0ºC, and the solvent removed by reduced pressure to obtain a brown-redish solid in quantitative yield. Crude of reactions were used for the next step without further purification.
Synthesis of 68 and 69

![Chemical structures 66 and 67](image)

To a suspension of the crudes of 66 and 67 in DMF was bubbled HCl gas for 5 minutes and leaved under stirring for additional 30 minutes. The solvent was removed and the crude was washed with distilled chloroform (x5) to obtain a brown-redish solid.

68 (6 mg, >99% yield respect to 62)

$^1$H-NMR (500 MHz, DMSO): $\delta$ 9.14 (bb, 6H, 2,6-pyridil), 8.88 (bb, 8H, pyrrole β), 8.32 (m, 4H, Ar-P), 8.24 (m, 2H, 3,5-pyridyl), 7.88 (m, 4H, CH$_{\text{pyrr}}$-Ar), 5.30-5.20 (m, 3H, CH$_2$pyrrolidine), 4.82 (s, 9H, quaternary ammonium methyl), 4.18 (s, 3H, N$_{\text{pyrr}}$-Me).
3.86-3.22 (m, NH-CH$_2$, CH$_2$-NHBoc and CH$_2$-N(CH$_2$)$_2$, 3.12-2.77 (m, CH$_2$-N(CH$_2$)$_2$ and Npyrrolidine-CH$_2$-CH$_2$), (m, 4H, CH$_2$CONH), 2.79 (s, 3H, N-CH$_3$, partially overlapped)

UV/vis (H$_2$O, \( \lambda \) nm) = 425, 510, 545, 588, 640

MS-ESI found 374.8 (M/7), expected 374.71

69 (6 mg, 92% yield respect to 65)

$^1$H-NMR (500 MHz, DMSO): \( \delta \) 9.15-8.80 (bb, 14H, 2,6-pyridil and pyrole ß), 8.20-8.06 (m, 10H, Ar and 3,5-pyridyl), 5.06 (s, 1H, CH$_2$pyrrolidine), 4.84 (bb, 2H, CH$_2$pyrrolidine), 4.81(s, 9H, quaternary ammonium methyl), 4.20(s, 3H, Npyr-Me), 4.15-3.28 (m, 15H, NH-CH$_2$, CH$_2$-NHBoc, CH$_2$-N pyrrolidine, NH$_2$), 3.25-2.98 (m, 10H, N-(CH$_2$)$_2$ and Npyrrolidine-CH$_2$-CH$_2$ and CH$_2$-CONH), 2.85 (s, 3H, N-CH$_3$)

UV/vis (H$_2$O, \( \lambda \) nm) = 423, 508, 539, 577, 639

MS-ESI found 357.6 (M/7), expected 357.44
References


References


75. A. Markham and H. Bryson, *Drugs*, 1995, **49**, 232


83. X. Gao, K.-S. Kim and D. Liu, *The AAPS Journal*, 2007, **9**, E921


References


References


References


