“Dendrimers and Branched Molecules by Isocyanide-Based Multicomponent Reactions”

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Supervisor and thesis editor: Prof. Dr. Ludger Wessjohann

Declaration

"I declare that I have completed this dissertation without unauthorized help of a second party and only with the assistance acknowledged therein. I have appropriately acknowledged and referenced all text passages that are derived literally from or based on the content of published or unpublished work of other authors."

Nalin de Seixas Borges

1. Gutachter: Prof. Dr. Ludger Wessjohann

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To my family and friends

"There is nothing like looking, if you want to find something.
You certainly usually find something, if you look,
But it is not always quite the something you were after."

J. R. R. Tolkien

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Nalin de Seixas Borges

Halle (Saale), 2018

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List of abbreviations

Å	ångström	FRAC	fungicide-resistance action committee
Ac	acetyl	FTIR	Fourier transform infrared spectroscopy
Bn	benzyl	FTMS	Fourier transform mass spectrometry
Boc	*tert*-butoxycarbonyl	G	generation
°C	degrees Celcius (centigrade)	g	gram
calc.	calculated	h	hour (s)
CFSE	5(6)-carboxyfluorescein diacetate *N*-succinimidylester	HEPES	4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid
CLB	chlorambucil	HPLC	high performance liquid chromatography
3CR	three-component reaction	HRMS	high resolution mass spectrometry
4CR	four-component reaction	Hz	Hertz
CV	crystal violet	IC_{50}	median inhibitory concentration
d	doublet in NMR	IMCR	isocyanide multicomponent reaction
Đ	dispersity	*J*	coupling constant (in NMR)
DCC	*N*,*N*'-dicyclohexylcarbodiimide	KDa	kilodalton
DHR	dihydrorhodamine	LC	liquid chromatography
DIPEA	*N,N*-diisopropiletilamina	M	molar
DMAc	dimethylacetamide	m	milli
DMF	*N,N*-dimetilformamida	m	multiplet (in NMR)
DMSO	dimethylsulfoxide	MALDI	matrix-assisted laser desorption/ionization
DOPA	3,4-dihydroxyphenylalanine	MCR	multicomponent reaction
DPBS	Dulbecco's phosphate-buffered saline	Me	methyl
DSP	dithiobis(succinimidyl propionate)	min	minute (s)
EDC	*N*,*N*'-1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride	Mn	number average molecular weight
EDTA	ethylenediaminetetraacetic acid	MS	mass spectrometry
e.g.	exempli gratia (for example)	MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
ESI	electrospray ionization	Mw	weight average molecular weight
equiv.	equivalent	nm	nanometer
FDA	Food and Drug Administration	NMWL	nominal molecular weight limit
FRET	fluorescent resonance energy transfer analysis	NMR	nuclear magnetic resonance

NO	nitric oxide
NSAID	non-steroidal anti-inflammatory drug
OD	optical density
p.	page
PAMAM	polyamidoamine dendrimer
P-3CR	Passerini three-component reaction
PEG	polyethylene glycol
PPI	polypropylenimine dendrimer
ppm	parts per milion
QD	quantum dot
r.t.	room temperature
s	singlet (in NMR)
s	second (s)
SEC	size exclusion chromatography
SPDP	*N*-succinimidyl-3-(2-pyriidyldithio)propionate
Sulfo-SMCC	sulfosuccinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate
t-	*tert*- (tertiary)
TBAB	tetrabutylammonium bromide
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	tetramethylsilane
TOF	time-of-flight mass spectrometer
TOP	*N*-octylphosphine
TOPO	tri-*N*-octylphosphine oxide
U-4CR	Ugi four-component reaction

Chapter 1

Isocyanide-based multicomponent reaction as a strategy towards dendrimers and branched molecules

Abstract

Dendrimers are versatile synthetic highly branched polymers with well-defined molecular weight. The chemical composition of these compounds can be tailored, allowing predictable tuning of physical and chemical properties. As consequence of their unique behaviour, dendrimers are suitable for several industrial and biological applications. Quantum dots are nanocrystals with noteworthy optical, electrical, and mechanical properties that can be shaped to attend a desirable application. The traditional syntheses and surface functionalization of both macromolecules exhibit some challenges, including large number of reaction steps to achieve the desired features. With the purpose of eliminate such limitations, a new strategy based on isocyanide-based multicomponent reactions (IMCRs) can be developed. Thereby, the aim of this chapter is to give a brief overview on the main aspects of dendrimers, quantum dots, and IMCRs, focusing in their application in the synthesis of macromolecules so far.

1.1 Dendrimers

1.1.1 Definition and properties

The first low molecular weight branched molecule was synthesized by Vögtle and co-workers in 1978 by performing several Michael-type reactions of acrylonitrile with an amine followed by reduction of the nitrile group.[1] Three years later, Denkewalter and co-workers described high molecular weight dendritic poly(lysine).[2] However, it was not until 1985, when Tomalia and co-workers published the synthesis and characterization of a group of dendritic polyamidoamines (PAMAMs)[3] that the name "dendrimers" (from Greek language: dendrons = tree, meros = part) started to be related with this new class of highly branched polymers.

Dendrimers are three dimensional synthetic compounds with well-defined molecular structure and molecular weight, and low polydispersity in comparison with traditional linear polymers.[4] A typical dendrimer is constituted of three parts: a central core, branches or interior, and surface or periphery. The central core determines the orientation of the dendrimer. The formation of inner layers occurs by the addition of repetitive units, called generations. In the first stage, the addition of a repeating unit to the central core produces the first generation (G1). The successive cycle of reactions creates larger generations of the dendrimer (G2, G3, G4, …). At the end, an outer layer is formed containing the terminal functional groups (**Figure 1**).[5,6]

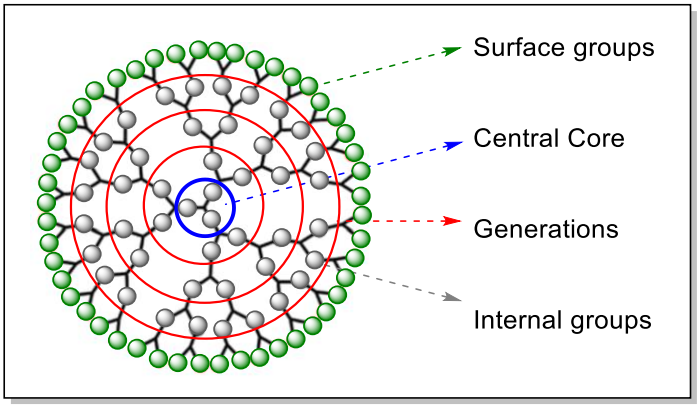


Figure 1. Parts of the dendrimers.

The properties of dendrimers are governed by both the internal and terminal functional groups. The solubility is highly influenced by the structure of the end groups. Dendrimers with hydrophilic groups on the surface are soluble in polar solvent, while dendrimers terminated with hydrophobic groups are soluble in nonpolar solvents.[7]

Dendrimers of lower generation normally have asymmetrical shape, which becomes globular with the growth of the dendrimer.[5,8] The properties of the internal groups, combined with the globular shape of dendrimers of higher generations, make possible, for example, the encapsulation of guest molecules in the macromolecules interior void space.[8,9]

1.1.2 Synthetic strategies

The conventional strategies for the syntheses of this class of compounds are based on convergent and divergent syntheses. In the divergent approach, the dendrimer is synthesized from the central core and built up generation by generation, as if the dendrimer was being constructed "from the inside out". On the other hand, in convergent methodologies, the synthesis begins from what will become the periphery of the dendrimer. The principle of this method involves the construction of small units called dendrons. These dendrons are then coupled together through a focal point, in order to accomplish the construction of the central core and, consequently, the formation of the dendrimer (**Figure 2**).[10]

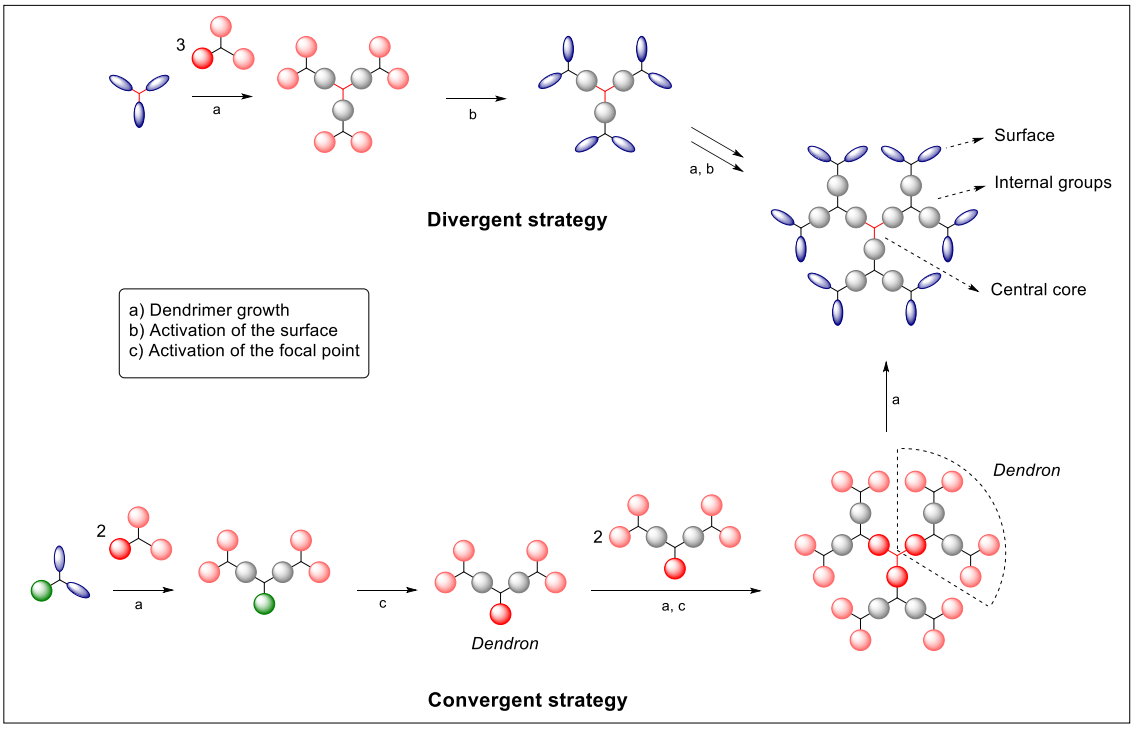
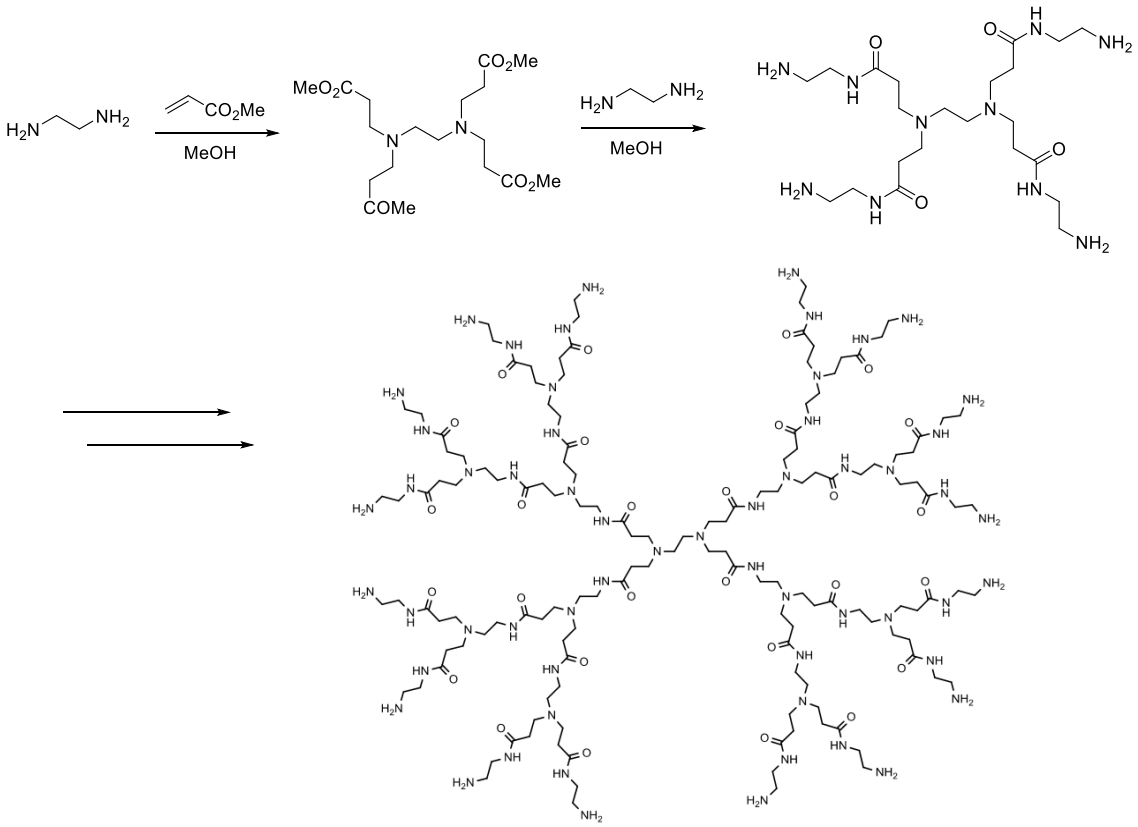


Figure 2. Divergent and convergent strategies for the synthesis of dendrimers.

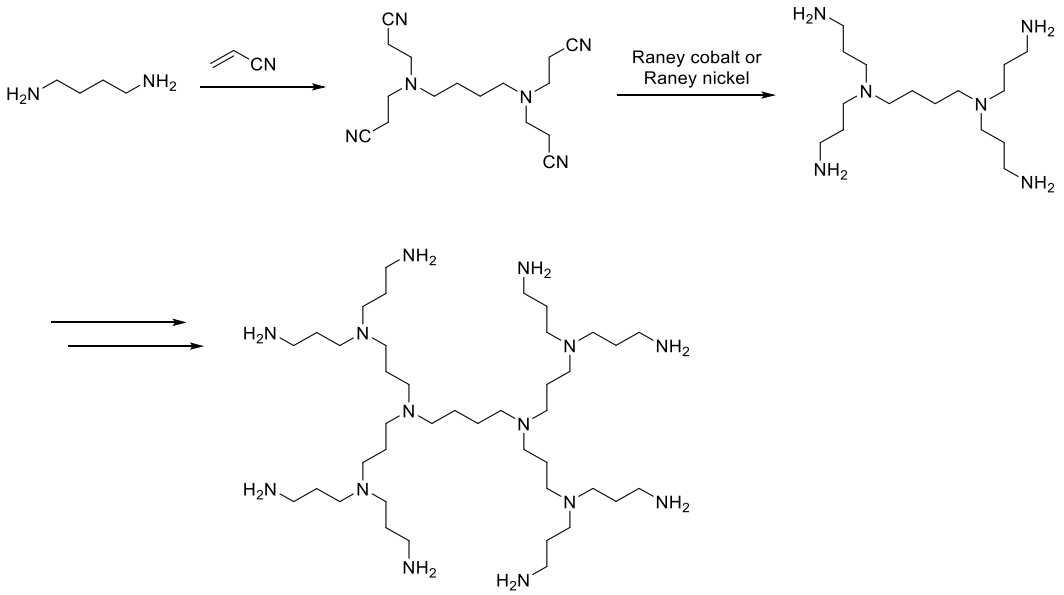
The remarkable example of a dendrimer synthesized through the divergent approach is the commercially available polyamidoamine dendrimer (PAMAM). The

synthesis involves two iterative reactions sequences: a Michael-type addition of an amino group (normally ethylenediamine or ammonia) to methyl or ethyl acrylate followed by aminolysis of the methyl or ethyl ester (**Scheme 1**).[3,11]



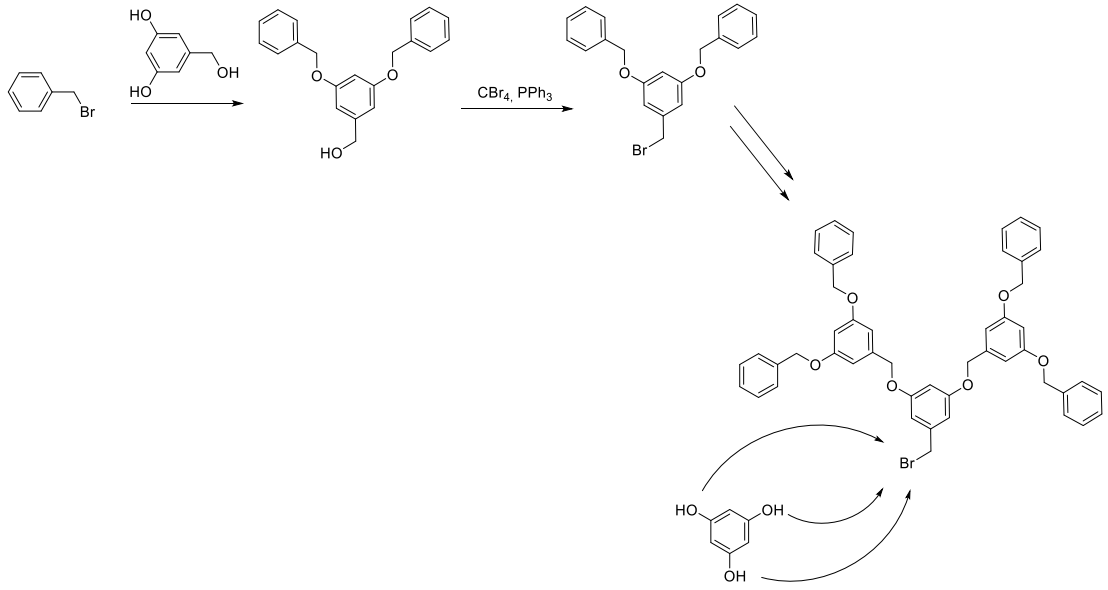
Scheme 1. Divergent synthesis of PAMAM dendrimer.

Another well-known example of divergent approach is employed in the synthesis of the commercially available polypropylenimine dendrimer (PPI). The first step consists of repeated double alkylation of primary amine with acrylonitrile by Michael-type reaction, followed by heterogeneous hydrogenation of the nitrile groups using Raney nickel or Raney cobalt as catalyst (**Scheme 2**).[12]



Scheme 2. Divergent synthesis of PPI dendrimer.

Fréchet and co-workers were the responsible for the first dendrimer synthesized *via* a convergent approach in 1990. Poly(aryl ether) dendrimers can be prepared initially by the Williamson etherification between two benzyl bromide and 2,5-dihydroxybenzyl alcohol. Subsequently, the benzylic hydroxyl group is converted into the corresponding bromide. This first generation dendron can again react with 2,5-dihydroxybenzyl alcohol in order to produce higher-generations. In the last step, the dendrons are coupled to phloroglucinol, which works as a trifunctional core (**Scheme 3**).[13]



Scheme 3. Convergent synthesis of poly(aryl ether) dendrimer.

1.1.3 Classification

Dendrimers can be classified according to the number of different functionalities and building blocks used during the synthetic approach. Traditional dendrimers are those synthesized from one type of monomer and have the same type of functionalization (monofunctional) on the periphery.[14] Heterofunctional dendrimers have different functional groups or building blocks and are classified according to the location of the different functionalities with respect to each other: block, alternating, or random.[15]

The first block dendrimers were synthesized by Fréchet and co-workers in 1993 and can be classified in three different subgroups: layer-block, segment-block, and surface-block. In the first one, the dendrimers have different internal and external branching units. Layer-block dendrimers can be synthesized by both convergent and divergent methods. Segment-block dendrimers are normally synthesized *via* convergent methodology considering that different types of dendrons are present in the final dendrimer. Segment-block dendrimers have different surface groups depending on the number of segments. On the other hand, surface-block dendrimers have the same dendritic skeleton or internal branches, but different surface groups. These last two types of heterofunctional dendrimers are also known as "Janus dendrimers" (**Figure 3**).[15]

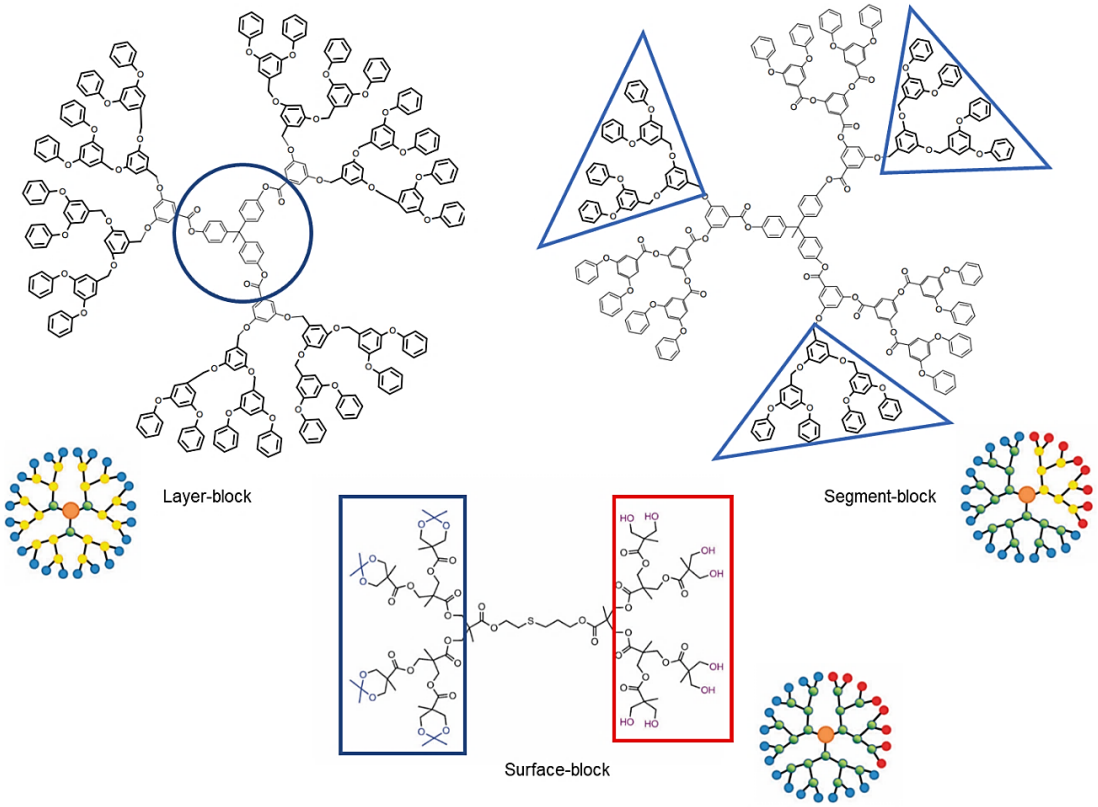


Figure 3. Classification of the block dendrimers.

1.1.4 Applications

After the first dendritic molecule designed by Vögtle and co-workers, many other examples have been synthesized with a variety of properties. Thanks to the feasible modification of surface, interior groups and central core, tailor dendrimers for different types of industrial and biomedical applications can be constructed.

Dendrimers can be employed in both homogeneous or heterogeneous catalysis due to their high surface area and solubility. They can act as catalytically active species or as soluble supports to catalysts, which can be chemically attached to the surface or interior of the dendrimer or even encapsulated.[16] In the field of liquid crystal chemistry, dendrimers have also been used due to the possibility of introducing mesogenic units and mesomorphic properties.[17]

Dendrimers can be applied in light harvesting devices (mainly layer-block dendrimers, **Figure 4a**)[18] or in pigment chemistry as additive for printing inks, photographies and paints. Additionally, they can act as chelating agents or nanofiltration membranes[19] for the removal of some types of metal ions or impurities from waste water and contaminated soil.

In the biomedical field, dendrimers have been studied as contrast agent for magnetic resonance because of their potential of combining several targeting and imaging compounds in one single molecule.[20] Dendrimers can also act as antiviral drugs when, generally, anionic groups, such as sulfonate groups, are attached to the surface (**Figure 4b**),[21] and as antibacterial drugs when cationic groups, such as amines or ammonium groups, are present in the periphery (**Figure 4c**).[22] Nevertheless, apparently the most promising potential of dendrimers in the biomedical field is in their ability to perform drug delivery, where drug molecules can be chemically linked to the surface of the dendrimer or loaded in its interior.

Hydrophilic dendrimers with nonpolar interior can encapsulate hydrophobic drugs in the interior void space and increase their solubility for drug delivery (**Figure 4d**).[23] This can improve the bioavailability of drugs.[24,25] Dendrimers can also minimize drug side effects and improve efficacy by incorporating on the surface targeting compounds such as folic acid, biotin, monoclonal antibodies, and peptides, that are capable of selectively deliver the drugs to desired specific site. These strategies have been commonly used for the effectively delivery of anticancer drugs to targeted tumor tissues.[26]

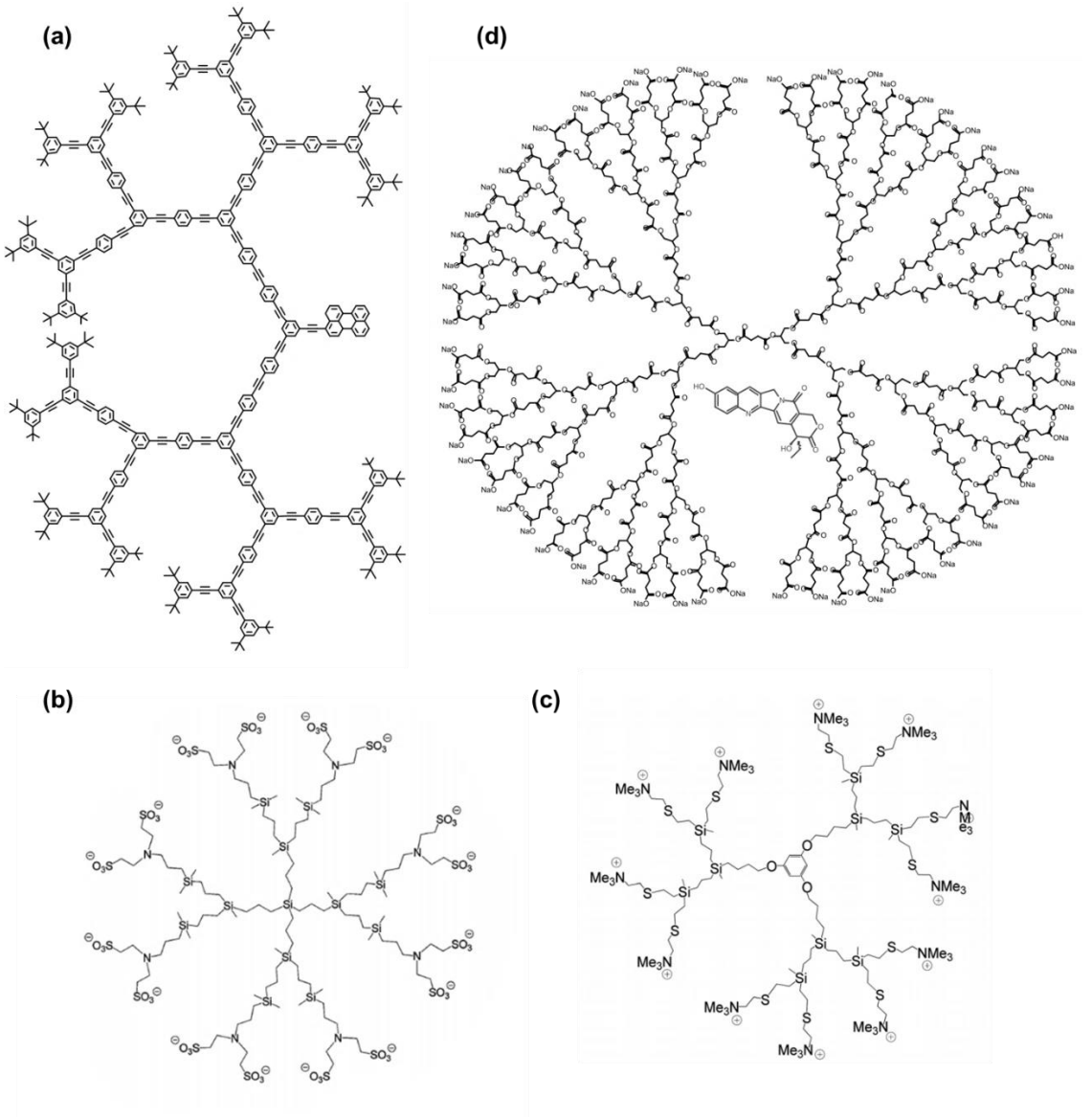


Figure 4. (a) Layer-block dendrimers used as light harvesting devices;[18c] (b) anionic dendrimers used as antiviral drugs;[21] (c) cationic dendrimer used as antimicrobial drug;[22] (d) dendrimer-encapsulation of the anticancer compound camptothecin.[23c]

1.2 Branched molecules: Quantum dots

1.2.1 Definition, properties, and applications

Quantum dots (QDs) are inorganic semiconductor nanocrystals (approx. 2 - 10 nm) with exceptional optical, electrical, and mechanical properties. QDs are composed of a metalloid core, often a shell that shields the core and a capping layer that allows further functionalization (**Figure 5a**).[27] QDs core are prepared with a variety of metal complexes such as semiconductors, noble metals and magnetic transition metals from the groups

II-VI (e.g. cadmium-selenium CdSe, cadmium-tellurium CdTe), III-V (e.g. indium phosphate, InP), or IV-VI (e.g. lead selenide PbSe).[28]

Quantum dots are mainly synthesized *via* high temperature organometallic processes, where the connection of a metal (e.g. Cd or Pb) with a corresponding chalcogen precursor (e.g. Se or Te) takes place in a solvent such as tri-*N*-octylphosphine oxide (TOPO), *N*-octylphosphine (TOP) or hexadecylamine at high temperatures. This synthetic approach relies on the principle that the organic solvents have molecules that can interact with the surface of the precipitated quantum dots. The same type of reaction can be used to "shield" the core with wider bandgap semiconductor such as ZnS or CdS, generating core/shell type quantum dots (e.g. CdSe/ZnS). Further, the hydrophobic coating created by the solvent can be modified to a water-soluble layer by the exchange with bifunctional molecules or by surface modification with silica compounds, usually alkoxysilanes.[29]

The optical properties of the nanocrystals ("dots") are governed by quantum mechanics, hence the name "quantum dot".[30] Due to QDs small size, normally smaller or equal than the exciton Bohr radius, the electrons are confined in a limited space and this quantum mechanical confinement effect causes the shift of the energy to higher levels (blue wavelength). As result, large QDs nanocrystals absorb and emit in the red wavelength which becomes blue with the decrease of the quantum dots size (**Figure 5b**).[30,31]

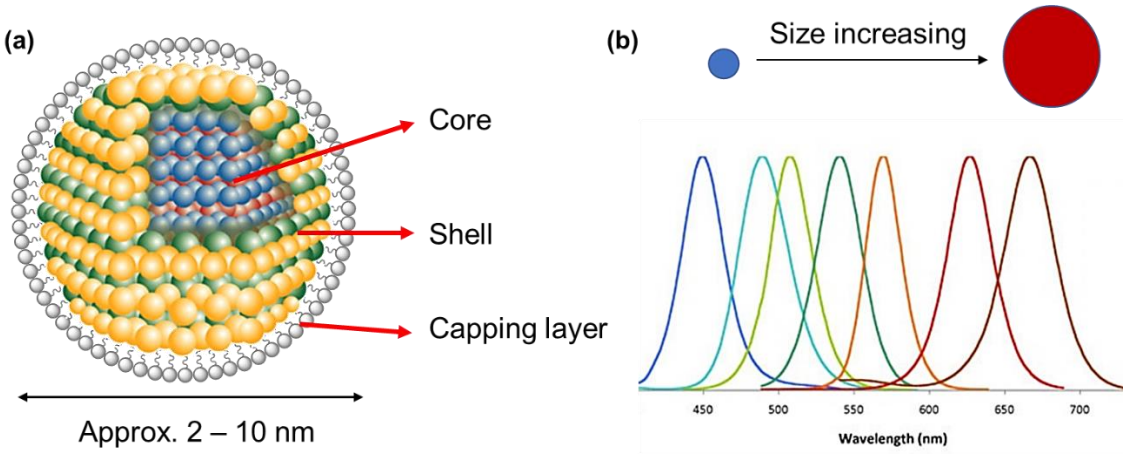


Figure 5. (a) Parts of the quantum dots; (b) Quantum dots size and colours.

This size-dependent and interchangeable photoluminescence properties together with the high photostability against photobleaching, high quantum yield, broad absorption and narrow, symmetric emission spectra, and the possibility of conjugation with other

molecules makes the QDs a special class of inorganic luminophores with remarkable chemical and biological application.[31,32]

The surface modification of QDs with biomolecules to increase biocompatibility and water solubility is normally required for biological applications and bioconjugated quantum dots have become popular for drug delivery,[33] photodynamic therapy,[34] fluorescent labelling,[35] and fluorescent resonance energy transfer analysis (FRET) for monitoring proteins interactions and assaying of enzyme activity.[36]

1.2.2 Synthetic strategies for the conjugation of biomolecules

There are two methodologies to conjugate biomolecules such as enzymes, antibodies, peptides, and small molecules on the quantum dots surface: covalent linkage and non-covalent conjugation.[37]

Non-covalent conjugation of a biomolecule on the QDs surface does not require any functional attachment and it is based on hydrophobic interactions of amphiphilic molecules, electrostatic interaction between positive and negative charged molecules, or strong bio-affinity interactions (e.g. biotin/(strept)avidin). Even though this strategy is simple and fast, it has a lot of disadvantages mainly because of the weak coordination in the presence of competitive molecules.[37]

The covalent linkage, on the other hand, creates highly stable covalent bonds between the functional group present on the QDs surface and the functional group of the biomolecules. Covalent conjugation of QDs containing amino, carboxyl and sulfhydryl functionalizations on the surface can be accomplished using functional crosslinkers such as EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide), DCC (*N*,*N*'-dicyclohexylcarbodiimide), DSP (dithiobis(succinimidylpropionate), SPDP (*N*-succinimidyl-3-(2-pyriidyldithio)propionate), sulfo-SMCC (sulfosuccinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate), and maleimido succinimide.[37]

Quantum dots have also been functionalized *via* click chemistry, however, the presence of copper ions strongly inhibit the luminescence of QDs. For this reason, copper-free 1,3-dipolar cycloaddition (copper-free click chemistry), in which the reaction is promoted by cyclic strain in a cyclooctyne molecule (strain-promoted alkyne-azide cycloaddition) instead of copper, has been applied for bioconjugation.[38]

1.3 Multicomponent reactions

Multicomponent reactions (MCRs) are one pot procedures in which three or more reactants are combined to generate a new single product incorporating the essential parts of all starting materials.[39] These types of reactions are among the most powerful processes in organic chemistry, because they are environment-friendly, normally quick, simple to set up and present high atom economy, diversity, and overall yields. These features are regarded as essential in the development of an ideal synthesis.[39,40]

In a multicomponent reaction, the starting materials are combined to produce a highly complex product. Thus, the starting materials do not react in one single step, but in a sequence of mono and bimolecular events until an irreversible final step to yield the product is fulfiled.[39]

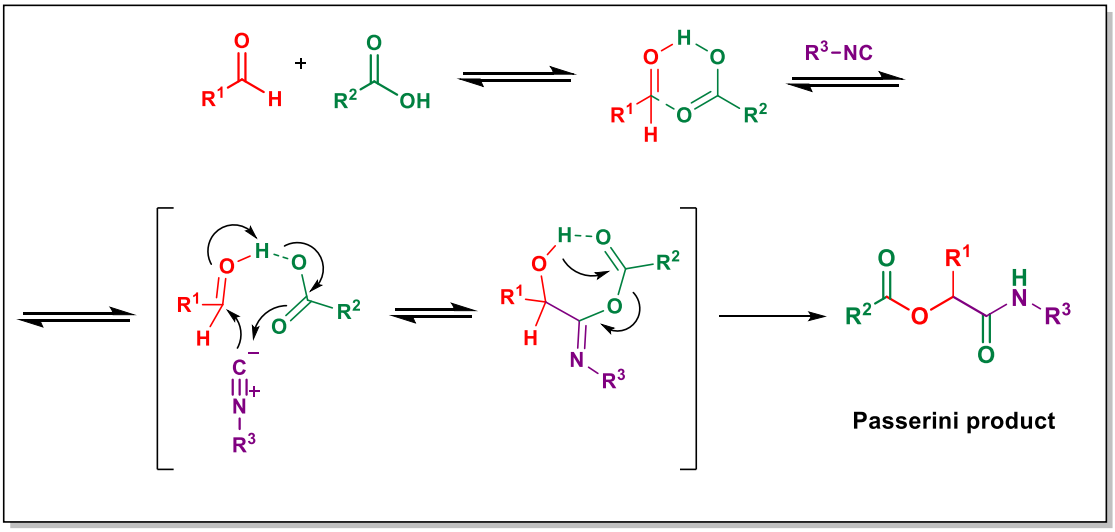
The first idea of a reaction with the previous characteristics was described in 1838 when Laurent and Gerhard reacted bitter almond oil, ammonia, and hydrogen cyanide.[41] However, the first officially recognized MCRs are dated from 1850, when Strecker synthesized α-aminocyanides from ammonia, carbonyl compounds, and hydrogen cyanide.[42] After that, many other MCRs were developed, among them the Hantzsch synthesis of 1,4-dihydropyridines (1882),[43] the Biginelli dihydropyrimidine synthesis (1891),[44] the Mannich reaction (1912),[45] and the Asinger reaction (1956) to name a few.[46]

In 1921, Passerini developed the first isocyanide-based multicomponent reaction (IMCR). Nowadays, the condensation between an isocyanide, carboxylic acid, and aldehyde became known as Passerini three-component reaction (P-3CR). In 1959, Ugi and co-workers introduced a new IMCR with isocyanides, carboxylic acids, amines and aldehydes, later named Ugi four-component reaction (U-4CR), opening a new chapter in the history of organic synthesis. The Ugi reaction became by far the most important multicomponent reaction because it leads to peptide derivatives.[47-51]

1.3.1 Passerini three-component reaction

The Passerini reaction is a three-component reaction between a carboxylic acid, an oxo compound such as an aldehyde or ketone, and an isocyanide to afford α-acyloxy-carboxamides in one-step procedure. This reaction is accelerated in aprotic solvents at room temperature and preferable performed with high concentration of the starting materials. Because of these characteristics, it is assumed that the Passerini reaction follows a non-ionic pathway. The mechanism has been debated many times, but it has not yet been clarified, however, the most accepted one involves the formation of a cyclic

transition state. The accepted P-3CR mechanism is based on the isocyanide insertion in the loosely hydrogen-bonded adduct formed from the interaction between the carboxylic acid and the carbonyl compound. The cyclic transition state with all the three components cannot be isolated and immediately undergoes an intramolecular rearrangement to afford the stable Passerini product (**Scheme 4**).[39a,47,52]



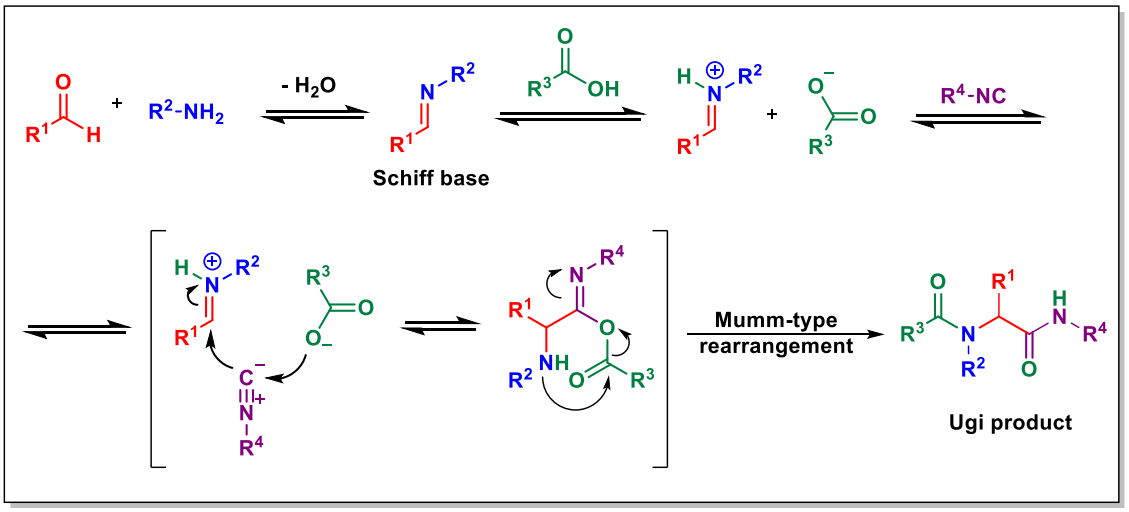
Scheme 4. Suggested mechanism of the Passerini reaction.

1.3.2 Ugi four-component reaction

The Ugi reaction is a four-component reaction between an amine compound, an oxo component, an acid, and an isocyanide to afford a peptoid-like structure. The reagents and products of the Ugi reaction are variable, and the skeletons of the products are defined mainly by the acid and amine components. The most frequently used acid component is a carboxylic acid, but hydrazoic acid, azides, cyanates, thiocyanates, water, salts of secondary amines, hydrogen sulfide, and hydrogen selenide can also be used. The amine component is also variable, in principle any compound having a sufficiently nucleophilic NH group such as ammonia, primary and secondary amine, hydrazines, diaziridines and hydroxylamines can be used. The major skeleton of the Ugi product is an α-acylaminocarboxamide, but also carbamates, α-acylaminothio-carbonamides and α-aminothioamides can be generated.[39a,50,51,53]

The most accepted mechanism for the Ugi reaction consists first of the condensation of the oxo component and the amine to generate the imine, also known as Schiff base. The acid component protonates the nitrogen of the imine providing an iminium ion. Next, an α-addition of the electrophilic iminium cation and the nucleophilic

carboxylate anion to the carbon of the isocyanide takes place to afford the α-adduct. After an intramolecular acyl rearrangement (Mumm-type rearrangement), the highly stable Ugi product is obtained. The first three steps of this reaction are in equilibrium, leading to the final irreversible rearrangement step (**Scheme 5**).[50,53]

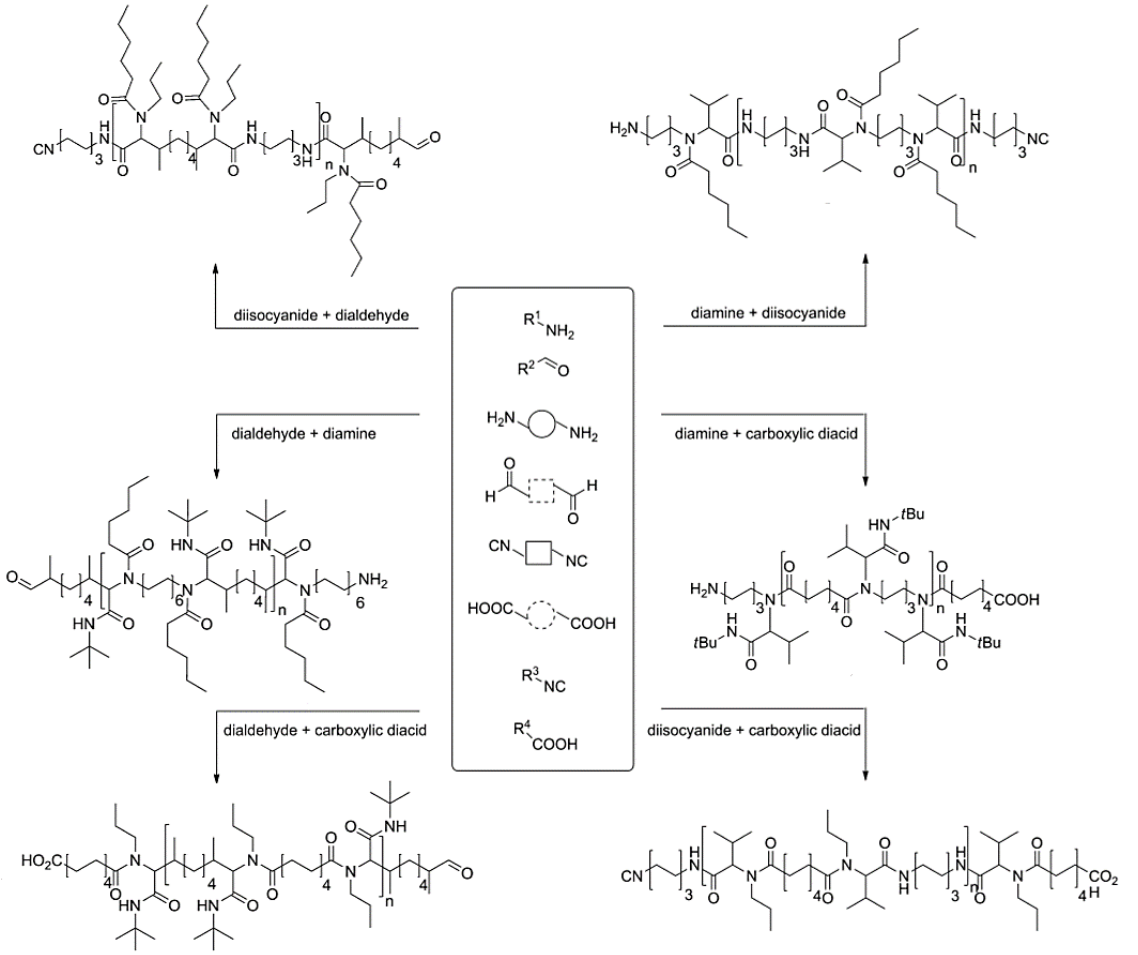


Scheme 5. Suggested mechanism of the Ugi reaction.

1.3.3 Application of IMCR in the synthesis of dendrimers and branched molecules

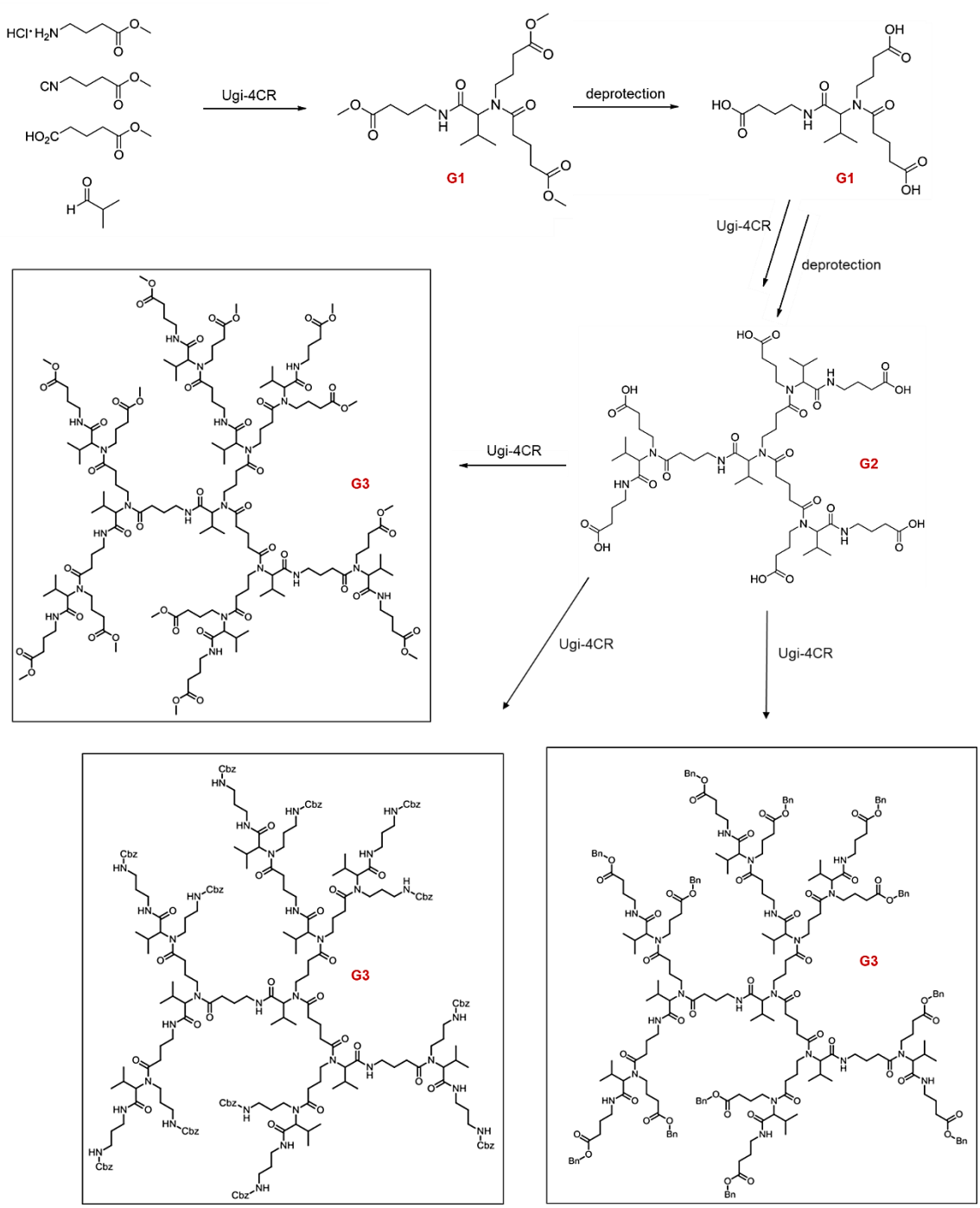
Isocyanide-based multicomponent reactions have recently been applied in the synthesis of branched molecules such as polymers and dendrimers due to the fast construction of structurally and functionally complex molecules. The first idea of IMCR based polymers was already described by Ivar Ugi between 1962 and 1967.[39] However, only in 2003, Wright and co-workers used the Ugi reaction for the preparation of norbornenyl carboxylic acid or aldehyde monomers for the syntheses of libraries of polymers.[54] The first example of a monomer synthesized using Passerini reaction was reported by Yang and co-workers in 2010.[55]

In 2011, Meier and co-workers reported the polymerization by Passerini reaction using bifunctional carboxylic acid and aldehyde monomers (AA-type) in combination with isocyanides.[56] In 2014, the same research group reported a very efficient and modular polycondensation approach to synthesize a collection of polyamides polymers *via* Ugi reaction. All six possible monomer combinations of two bifunctional compounds (AA-type) and two monofunctional components were applied (**Scheme 6**).[57]



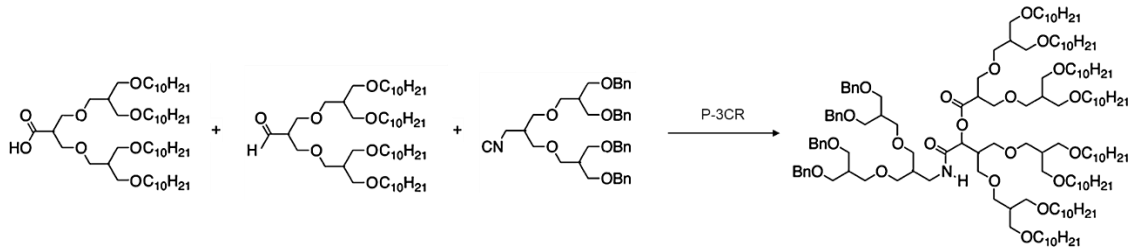
Scheme 6. All six possible monomer combinations of two bifunctional compounds for the syntheses of polymers *via* Ugi reaction.[57]

In a similar approach, in 2003-2011, Wessjohann and co-workers reported the first divergent synthesis of structurally diverse peptide-peptoidic dendrimers *via* Ugi reaction, while also demonstrating the possibility of a similar protocol for the Passerini reaction. Compared with the traditional methodologies employed for the synthesis of dendrimers, this strategy brings the advantage of highly flexible and structurally diversified composition, since it is possible to introduce independently internal and external functionalities into the dendrimer. The basic approach to obtain such dendrimers is the use of bifunctional monoprotected molecules that allow further Ugi or Passerini reaction after the deprotection. A collection of traditional and Janus dendrimers with a variety of end groups was synthesized *via* Ugi reaction up to the 4th generation (**Scheme 7**). One example of traditional dendrimer was also synthesized *via* Passerini reaction up to the 3rd generation.[58]



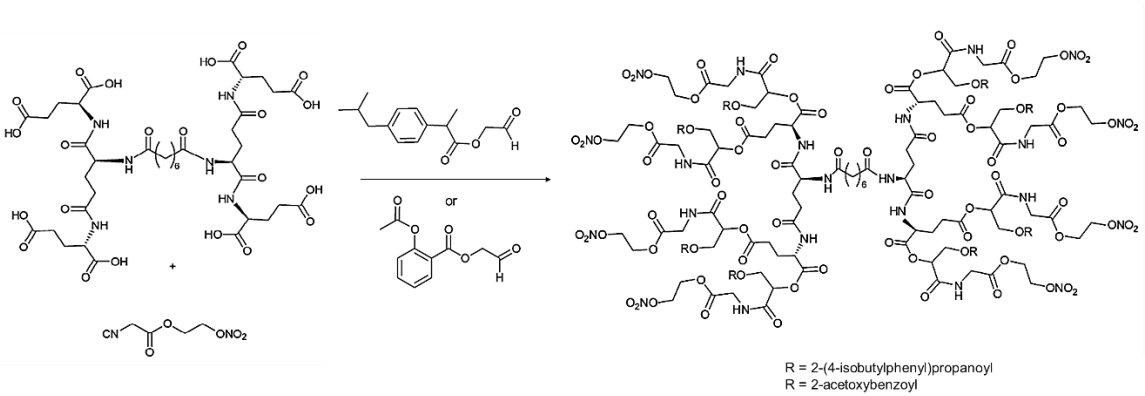
Scheme 7. Divergent synthesis of structurally diverse peptide-peptoidic dendrimers *via* Ugi reaction.[58]

In 2012, Rudick and co-workers reported the convergent synthesis of dendrimers *via* Passerini reaction. In their work, three dendrons were synthesized, each containing one functionality, applying the strategy introduced by Fréchet and co-workers, followed by the coupling of the dendrons by Passerini reaction (**Scheme 8**).[59]



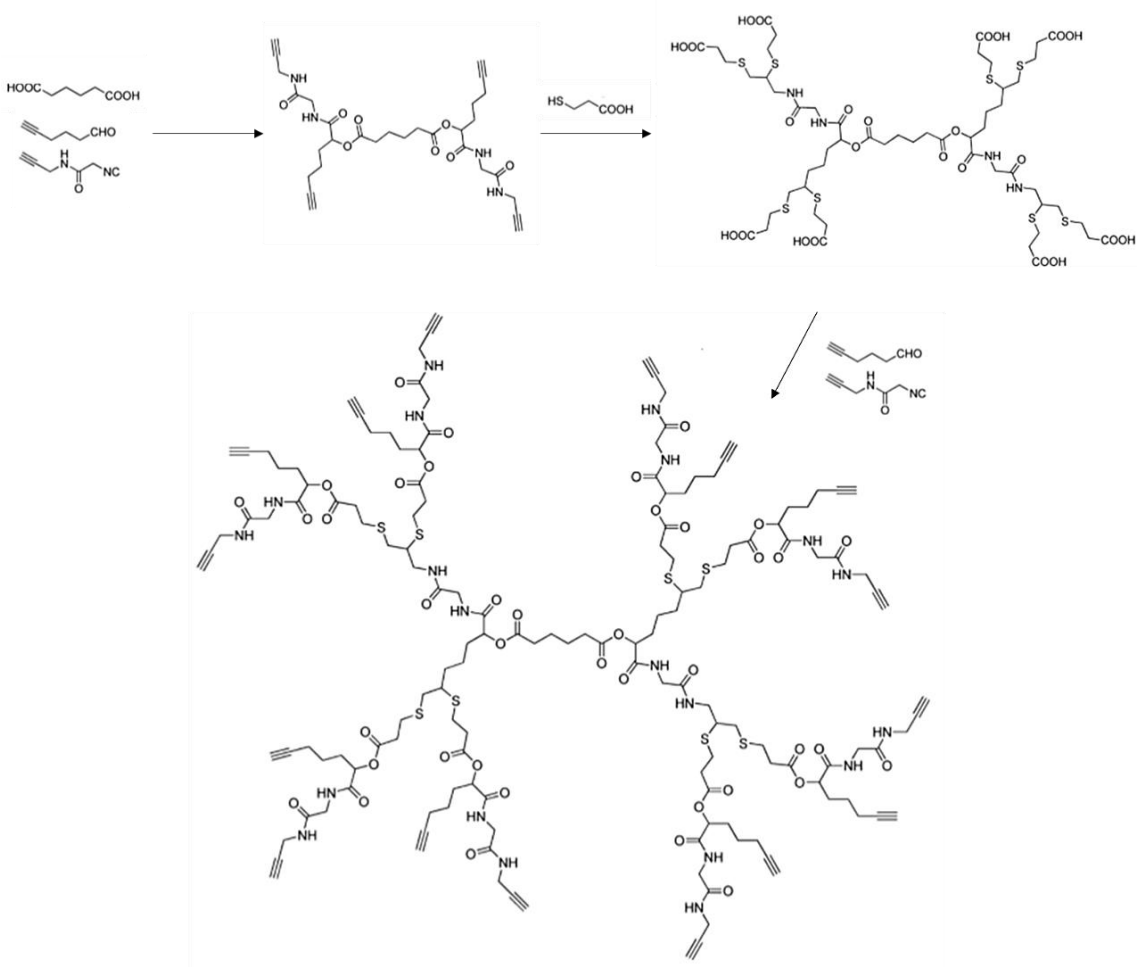
Scheme 8. Convergent synthesis of dendrimers *via* Passerini reaction.[59]

One year later, Gao and co-workers synthesized an octacarboxyl core derived from L-glutamic acid that was submitted to Passerini reaction with an isocyanide containing nitric oxide (NO) and an aldehyde derived from ibuprofen or aspirin (NSAIDs - non-steroidal anti-inflammatory drugs) to yield a new dendrimer drug delivery system for NO-releasing NSAID (**Scheme 9**).[60]



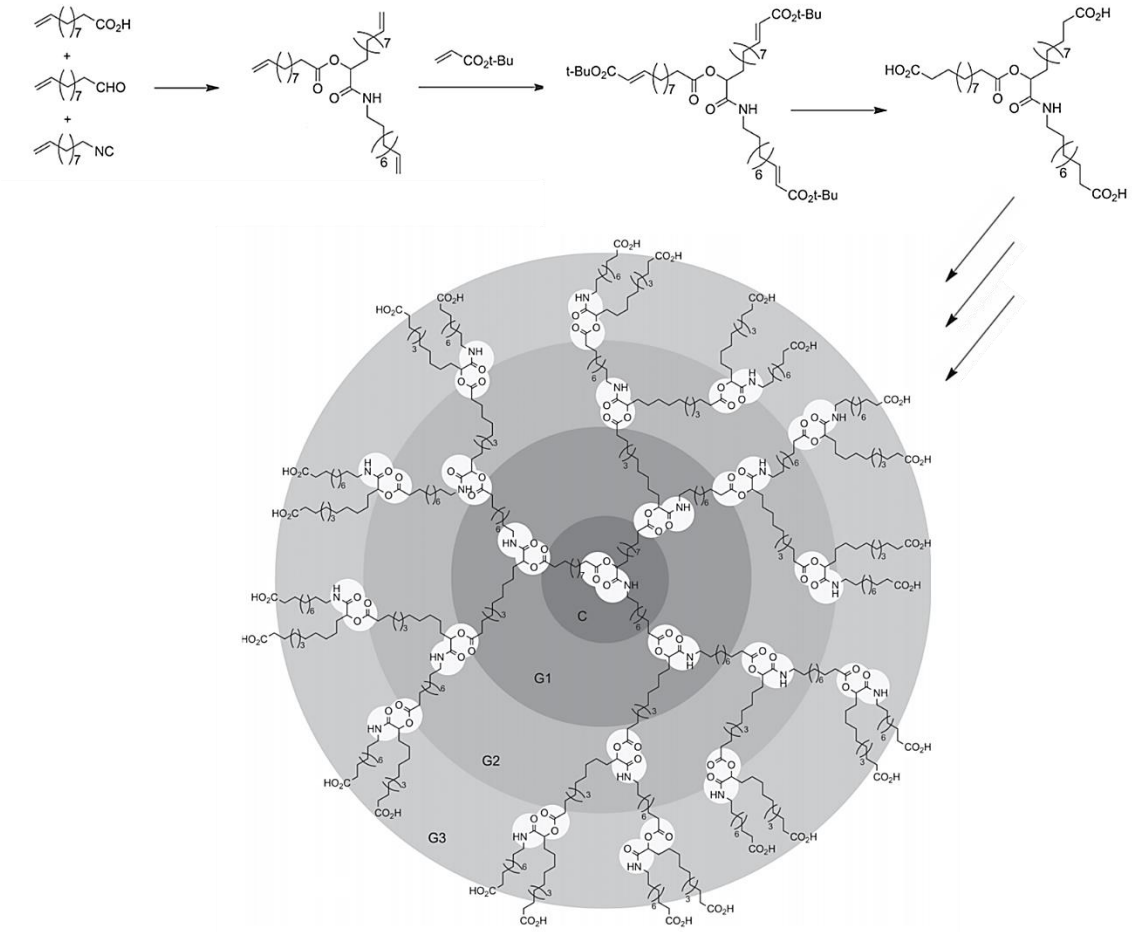
Scheme 9. NO-NSAIDs dendrimers synthesized *via* Passerini reaction.[60]

In 2014, Li and co-workers described the divergent synthesis of dendrimers with both ABC and ABB branching structures from nonbranching monomers up to the second generation by combination of efficient orthogonal ABB thiol-yne reaction and ABC Passerini reaction (**Scheme 10**).[61]



Scheme 10. Divergent synthesis of dendrimers by combination of ABB thiol-yne reaction and ABC Passerini reaction.[61]

Also in 2014, Meier and co-workers reported the divergent synthesis of dendrimers by the combination of Passerini reaction and olefin cross-metathesis. The strategy included a Passerini reaction with castor oil-derived platform chemicals, such as 10-undecenoicacid and 10-undecenal, with unsaturated isocyanide and aldehyde to obtain a central core with three terminal double bonds that allowed olefin cross-metathesis with *tert*-butyl acrylate. Subsequent hydrogenation and hydrolysis of the *tert*-butyl ester led to a molecule with three carboxylic acid groups that can be used for further Passerini reactions (**Scheme 11**).[62]



Scheme 11. Divergent synthesis of dendrimers by the combination of Passerini reaction and olefin cross-metathesis reaction.[62]

1.4 General goal

The general goals of this Ph.D. thesis are:

- Investigate a methodology to divergently synthesize structurally diverse traditional and Janus dendrimers, as well as polyvalent molecules using exclusively Passerini reaction;
- Synthesis of biotinylated PAMAM dendrimers with a chemotherapeutical drug by Ugi reaction and comparative studies of the anticancer activity;
- Synthesis of PPI dendrimers and polymers with catechol moieties by Ugi reaction and evaluation of the antimicrobial activity;
- Development of a new efficient methodology to functionalize quantum dots by isocyanide-based multicomponent reaction.

1.5 References

(1) Buhleier, E.; Wehner, W.; Vögtle, F. *Synthesis* **1978**, 1978, 155-158.

(2) Denkewalter, R. G.; Kolc, J.; Kukasavage, W. J. U.S. *Patent 4289872*, **1981**.

(3) Tomalia, D. A.; Baker, H.; Dewald, J.; Hall, M.; Kallos, G.; Martin, S.; Roeck, J.; Ryder, J.; Smith P. *Polym. J*. **1985**, 17, 117-132.

(4) (a) Boas, U.; Christensen, J. B.; Heegaard, P. M. H. *J. Mater. Chem*. **2006**, 16, 3785-3798. (b) Gao, C.; Yan, D. *Prog. Polym. Sci.* **2004**, 29, 183-275.

(5) (a) Twibanire, J. K.; Grindley, T. B. *Polymers* **2012**, 4, 794-879. (b) Boas, U.; Heegaard, P. M. H. *Chem. Soc. Rev*. **2004**, 33, 43-63.

(6) Lee, C. C.; MacKay, J. A.; Fréchet, J. M. J.; Szoka, F. C. *Nat. Biotechnol.* **2005**, 23, 1517-1526.

(7) Klajnert, B.; Bryszewska, M. *Acta Biochim Pol*. **2001**, 48, 199-208.

(8) Jang W.; Kamruzzaman Selim, K.; Lee, C.; Kang I. *Prog. Polym. Sci.* **2009**, 34, 1-23.

(9) Hecht, S.; Fréchet, J. M. J. *Angew. Chem. Int. Edn. Engl.* **2001**, 40, 74-91.

(10) Walter, M. V.; Malkoch, M. *Chem. Soc. Rev*. **2012**, 41, 4593-4609.

(11) (a) Tomalia, D. A.; Naylor, A. M.; Goddard, W. A. *Angew. Chem. Int. Ed*. **1990**, 29, 138-175. (b) Tomalia, D. A.; Durst, H. D.; *Top. Curr. Chem*. **1993**, 165, 193-313.

(12) Brabander-van den Berg, E. M. M.; Meijer, E. W. *Angew. Chem*. **1993**, 32, 1308-1311.

(13) (a) Hawker, C. J.; Frechet, J. M. J. *J. Chem. Soc., Chem. Commmun*. **1990**, 1010-1013. (b) Hawker, C. J.; Frechet, J. M. J. *J. Am. Chem. Soc*. **1990**, 112, 7638-7647. (c) Wooley, K. L.; Hawker, C. J.; Frechet, J. M. J. *J. Am. Chem. Soc*. **1991**, 113, 4252-4261.

(14) (a) Vögtle, F.; Richardt, G.; Werner, N. (2009). Chapter 3. Functional Dendrimers. *Functional Dendrimers, in Dendrimer Chemistry: Concepts, Syntheses, Properties, Applications,* pp. 49-79, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany. (b) Sowinska, M.; Urbanczyk-Lipkowska, Z. *New J. Chem*. **2014**, 38, 2168-2203.

(15) (a) Hawker, C. J.; Wooley, K. L.; Fréchet, J. M. *J. Macromol. Symp*. **1994**, 77, 11-20. (b) Wooley, K. L.; Hawker, C. J.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **1993**, 115, 11496-11505. (c) Maraval, V.; Laurent, R.; Donnadieu, B.; Mauzac, M.; Caminade, A. M.; Majoral, J. P. *J. Am. Chem. Soc*. **2000**, 122, 2499-2511. (d) Sharma, A.; Kakkar, A. *Molecules* **2015**, 20, 16987-17015.

(16) (a) Astruc, D.; Chardac, F. *Chem. Rev*. **2001**, 101, 2991-3024. (b) Alonso, E.; Astruc, D. *J. Am. Chem. Soc*. **2000**, 122, 3222-3223. (c) de Groot, D.; Reek, J. N. H.; Kamer, P. C. J.; van Leeuwen, P. W. N. M. *Eur. J. Org. Chem*. **2002**, 1085-1095. (d) Knapen, J. W. J.; van der Made, A. W.; de Wilde, J. C.; van Leeuwen, P. W. N. M.; Wijkens, P.; Grove, D. M.; van Koten, G. *Nature* **1994**, 372, 659-663.

(17) (a) Percec, V.; Chu, P.; Ungar, G.; Zhou, J. *J. Am. Chem. Soc*. **1995**, 117, 11441-11454. (b) Busson, O.; Ortegren, J.; Ihre, H.; Gedde, U. W.; Hult, A.; Andersson, G.; Eriksson, A.; Lindgren, M. *Macromolecules* **2002**, 35, 1663.

(18) (a) Bergamini, G.; Ceroni, P.; Maestri, M.; Balzani, V.; Lee, S.-K.; Vögtle, F. *Photochem. Photobiol. Sci*. **2004**, 3, 898-905. (b) Zeng. Y.; Li, Y. Y.; Chen, J.; Yang, G.; Li, Y. *Chem Asian J*. **2010**, 5, 992-1005. (c) Xu, Z.; Moore, J. S. *Acta Polym.* **1994**, 45, 83-87.

(19) (a) Xu, Y.H.; Zhao, D.Y. *Environ. Sci. Technol*. **2005**, 39, 2369-2375. (b) Cohen, S. M.; Petoud, S.; Raymond, K. N.; *Chem. Eur. J.* **2001**, 7, 272-279.

(20) (a) Kobayashi, H.; Brechbiel, M. W. *Curr. Pharm. Biotechnol*. **2004**, 5, 539-549. (b) Svenson, S.; Tomalia, D. A. *Adv. Drug Deliv. Rev*. **2005**, 57, 2106-2129. (c) Kobayashi, H.; Brechbiel, M. W. *Mol. Imaging* **2003**, 2, 1-10.

(21) Briz, V.; Sepúlveda-Crespo, D.; Diniz, A. R.; Borrego, P.; Rodes, B.; de la Mata, F. J.; Gómez, R.; Taveirad, N.; Muñoz-Fernández, A. *Nanoscale*. **2015**, 7, 14669-14683.

(22) Fuentes-Paniagua, E.; Hernández-Ros, J. M.; Sánchez-Milla, M.; Camero, M. A.; Maly, M.; Pérez-Serrano, J.; Copa-Patino, J. L.; Sánchez-Nieves J.; Soliveri, J.; Gómez, R.; de la Mata, F. J. *RSC Adv.* **2014**, 4, 1256-1265.

(23) (a) Morgan, M. T.; Nakanishi, Y.; Kroll, D. J.; Griset, A. P.; Carnahan, M. A.; Wathier, M.; Oberlies, N. H.; Manikumar, G.; Wani, M. C.; Grinstaff, M. W. *Cancer Res*. **2006**, 66, 11913-11921. (b) Baars, M. W. P. L.; Kleppinger, R.; Koch, M. H. J.; Yeu, S. L.; Meijer, E. W. *Angew. Chem. Int. Ed*. **2000**, 39, 1285-1288. (c) Haag, R. *Angew. Chem. Int. Ed*. **2004**, 43, 278-282. (d) Kojima, C.; Kono, K.; Maruyama, K.; Takagishi, T. *Bioconjug. Chem*. **2000**, 11, 910-917. (e) Medina, S. H.; El-Sayed, M. E. *Chem. Rev*. **2009**, 109, 3141-3157.

(24) Liu, M.; Kono, K.; Fréchet, J. M. J. *J. Control. Release* **2000**, 65, 121-131.

(25) Allen, T. M.; Cullis, P. R. *Science*, **2004**, 303, 1818-1822.

(26) (a) Dai, L.; Liu, J.; Luo, Z.; Li, M.; Cai, K. *J. Mater. Chem. B,* **2016**, 4, 6758-6772. (b) Soliman, G. M.; Sharma, A.; Maysinger, D.; Kakkar, A. *Chem. Commun*. **2011**, 47, 9572-9587.

(27) (a) Algar, W. R.; Tavares, A. J.; Krull, U. J. *Anal. Chim. Acta* **2010**, 673, 1-25. (b) Bilan, R.; Fleury, F.; Nabiev, I.; Sukhanova, A. *Bioconjugate Chem*. **2015**, 26, 609−624. (c) Bera, D.; Qian, L.; Tseng, T-K.; Holloway, P. H. *Materials* **2010**, 3, 2260-2345.

(28) Hardman, R*. Environ. Health Perspect*. **2006**, 114, 164-172.

(29) (a) Murray, C. B.; Noms, D. J.; Bawendi, M. G. *J. Am. Chem. Soc*. **1993**, 115, 8706-8715. (b) Esteve-Turrillas, F. A.; Abad-Fuentes, A. *Biosens. Bioelectron*. **2013**, 41, 12-29. (c) Sapsford, K. E.; Pons, T.; Medintz, I. L.; Mattoussi, H. *Sensors* **2006**, 6, 925-953.

(30) Rosenthal, S. J.; Chang, J. C.; Kovtun, O.; McBride, J. R.; Tomlinson, I. D. *Chem Biol*. *2011*, 18, 10-24.

(31) (a) Rogach, A. L. (2008) *Semiconductor Nanocrystal Quantum Dots.* Wien, Austria: Springer-Verlag/Wien. (b) Trindade, T.; O'Brien, P.; Pickett, N. L. *Chem. Mater.* **2001**, 13, 3843-3858.

(32) (a) Frigerio, C.; Ribeiro, D. S. M.; Rodrigues, S. S. M.; Abreu, V. L. R. G.; Barbosa, J. A. C.; Prior, J. A. V.; Marques, K. L.; Santos, J. L. M. *Anal. Chim. Acta* **2012**, 735, 9-22. (b) Jamiesona, T.; Bakhshia, R.; Petrovaa, D.; Pococka, R.; Imanib, M.; Seifalian, A. M. *Biomaterials* **2007**, 28, 4717-4732.

(33) Qi, L.; Gao, X. *Expert. Opin. Drug Deliv*. **2008**, 5, 263-267.

(34) Biju, V.; Mundayoor, S.; Omkumar, R. V.; Anas, A.; Ishikawa, M. *Biotechnol. Adv*. **2010**, 28, 199-213.

(35) Hoshino, A.; Fujioka, K.; Oku, T.; Nakamura, S.; Suga, M.; Yamaguchi, Y.; Suzuki, K.; Yasuhara, M.; Yamamoto, K. *Microbiol. Immunol.* **2004**, 48, 985-994.

(36) (a) Heyduk, T. *Curr. Opin. Biotechnol*. **2002**, 13, 292-296. (b) Day, R. N.; Periasamy, A.; Schaufele, F. *Methods* **2001**, 25, 4-18.

(37) Foubert, A.; Beloglazova, N. V.; Rajkovic, A.; Sas, B.; Madder, A.; Goryacheva, I. Y.; De Saeger, S. *Trends Anal. Chem.* **2016**, 83, 31-48.

(38) Bernardin, A.; Cazet, A.; Guyon, L.; Delannoy, P.; Vinet, F.; Bonnaffé, D.; Texier, I. *Bioconjugate Chem*. **2010**, 21, 583-588.

(39) (a) Dömling, A.; Ugi, I. *Angew. Chem. Int. Ed.* **2000**, 39, 3168-3210. (b) Dömling, A.; Wang, W.; Wang, K. *Chem Rev*. **2012**, 112, 3083-3135. (c) Dömling, A. *Chem. Rev*. **2006**, 106, 17-89.

(40) Wender, P. A. *Nat. Prod. Rep*. **2014**, 31, 433-440.

(41) (a) Laurent, A.; Gerhardt, C. F. *Ann. Chimi. Phys*. **1838**, 66, 181. (b) Laurent, A.; Gerhardt, C. F. *Liebigs Ann. Chem*. **1838**, 28, 265.

(42) Strecker, A. *Liebigs Ann. Chem*. **1850**, 75, 27-45.

(43) Hantzsch, A. *Liebigs Ann. Chem*. **1882**, 215, 1-82.

(44) Biginelli, P. *Gazz. Chim. Ital*. **1893**, 23, 360-413.

(45) Mannich, C.; Krösche, W. *Arch. Pharm*. **1912**, 250, 647-667.

(46) Asinger, A. *Angew. Chem*. **1956**, 68, 413.

(47) (a) Passerini, M. *Gazz. Chim. Ital*. **1921**, 51, 126-129. (b) Passerini, M. *Gazz. Chim. Ital.* **1921**, 51, 181-188.

(48) Lieke, W. *Liebigs Ann. Chem*. **1859**, 112, 316-321.

(49) Ugi, I.; Meyr, R. *Angew. Chem*. **1958**, 70, 702-703.

(50) (a) Ugi, I.; Meyr, R.; Fetzer, U.; Steinbrücker, C. *Angew. Chem*. **1959**, 71, 386. (b) Ugi, I.; Steinbrücker, C. *Angew. Chem*. **1960**, 72, 267-268.

(51) (a) Ugi, I.; Werner, B.; Dömling, A. *Molecules* **2003**, 8, 53-66. (b) Ugi, I. *Pure Appl. Chem*. **2001**, 73, 187-191.

(52) (a) Passerini, M.; Ragni, G. *Gazz. Chim. Ital*. **1931**, 61, 964-69. (b) Ugi, I. *Angew. Chem. Int. Ed*. **1962**, 1, 8-21. (c) Koopmanschap, G.; Ruijter, E.; Orru, R. V. A. *Beilstein J. Org. Chem*. **2014**, 10, 544-598.

(53) (a) El Kaim, L.; Grimaud, L. *Tetrahedron* **2009**, 65, 2153-2171. (b) Mumm, O. *Ber. Dtsch. Chem. Ges.* **1910**, 43, 886-893. (c) Mumm, O.; Hesse, H.; Volquartz, H. *Ber. Dtsch. Chem. Ges*. **1915**, 48, 379-391.

(54) Robotham, C. V.; Baker, C.; Cuevas, B.; Abboud, K.; Wright, D. L. *Mol. Divers*. **2003**, 6, 237-44.

(55) Rubinshtein, M.; James, C. R.; Young, J. L.; Ma, Y. J.; Kobayashi, Y.; Gianneschi, N. C.; Yang, J. *Org. Lett*. **2010**, 12, 3560-3563.

(56) Kreye, O.; Tóth, T.; Meier, M. A. R. *J. Am. Chem. Soc*. **2011**, 133, 1790-1792.

(57) Sehlinger, A.; Dannecker, P-K.; Kreye, O.; Meier, M. A. R. *Macromolecules* **2014**, 47, 2774−2783.

(58) Wessjohann, L. A.; Henze, M.; Kreye, O.; Rivera, D. G. DE 10 2010 018 882 A1 (03.11.**2011**), WO 2011/134607 A1 (03.11.**2011**).

(59) Jee, J-A.; Spagnuolo, L. A.; Rudick, J. G. *Org. Lett.* **2012**, 14, 3292-3295.

(60) Du, Z.; Lu, Y.; Dai, X.; Zhang-Negrerie, D.; Gao, Q. *J. Chem. Research* **2013**, 37, 181-185.

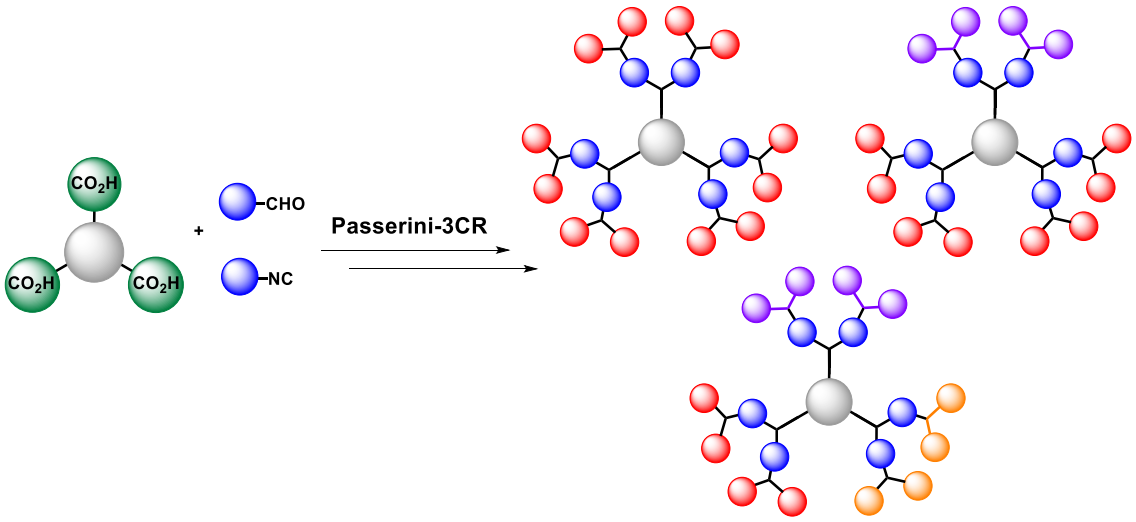
(61) Deng, X-X.; Du, F-S.; Li, Z-C. *ACS Macro Lett*. **2014**, 3, 667−670.

(62) Kreye, O.; Kugele, D.; Faust, L.; Meier, M. A. R. *Macromol. Rapid Commun*. **2014**, 35, 317−322.

Chapter 2

Development of a new methodology to synthesize polyvalent molecules and dendrimers by Passerini reaction using a divergent approach

Abstract



In this chapter the Passerini three-component reaction is investigated as a powerful strategy for the divergent synthesis of traditional and Janus dendrimers up the third generation, as well as polyvalent molecules with three different types of functional groups on the surface.

2.1 Introduction

In order to obtain more sophisticated dendrimers, in the end of 1990´s a new concept to synthesize these molecules with different building blocks was developed.[1,2] Following this trend, many dendrimers with at least two distinct functionalities on the surface were synthesized. These Janus dendrimers can distinguish themselves by having multiples properties in one molecule. In this context, small polyvalent molecules or “miktoarm star” molecules with three different functional groups on the surface also attract considerable attention due to their compact architecture.[3,4]

However, one of the limitations of almost all the syntheses of dendrimers and polyvalent molecules is, independent of the approach used, the routes have a minimal scope of variation in the construction, since the reagents used cannot be modified in their basic structure owing to their chemical reaction characteristics.[5-7] To avoid such limitations, in 2003-2011 Wessjohann and co-workers[8] developed a new divergent strategy to synthesize peptoid-based dendrimers using IMCRs, especially Ugi and Passerini reactions. IMCRs are very versatile reactions and can independently introduce internal and external functional groups into dendrimers.[9-11]

Currently, some research groups are applying Passerini reactions to connect dendrons or for one stage of the dendrimer synthesis, but so far, no dendrimers with diversified structures or Janus dendrimers synthesized entirely by Passerini reactions have been reported.[12-15] Therefore, the goal of this work is the divergent synthesis of polyvalent molecules, traditional and Janus dendrimers exclusively *via* Passerini reaction until the second and third generation.

2.2 Synthetic Strategy

The synthetic strategy to construct dendrimers *via* Passerini reaction is based on reaction branching, rather than branched building blocks. Thereby monoprotected bifunctional molecules allow further Passerini reactions after deprotection, as illustrated in **Figure 1**. Janus dendrimers and polyvalent molecules can be synthesized using the same approach but with different orthogonal protecting groups in the reagents. This allows to independently grow the dendrimer with two or three different functionalities

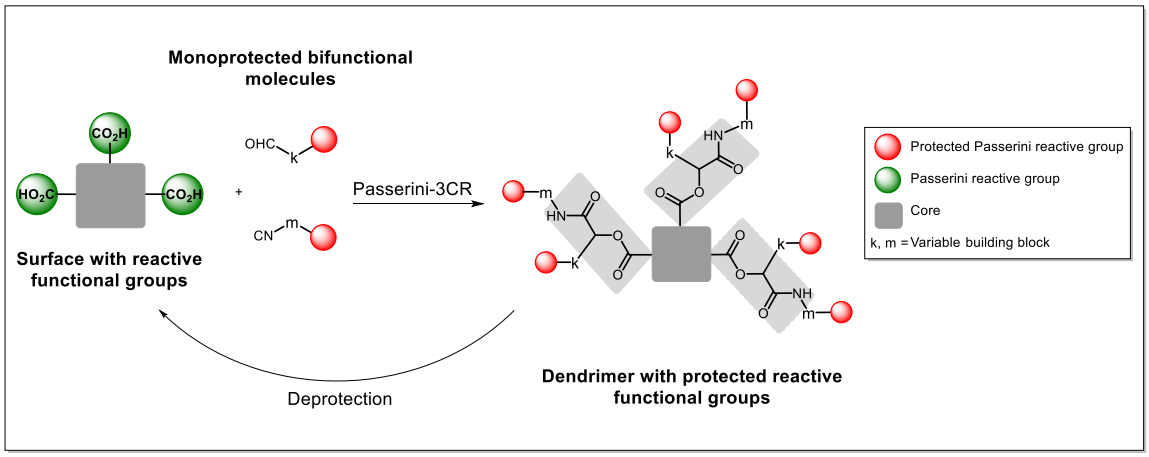


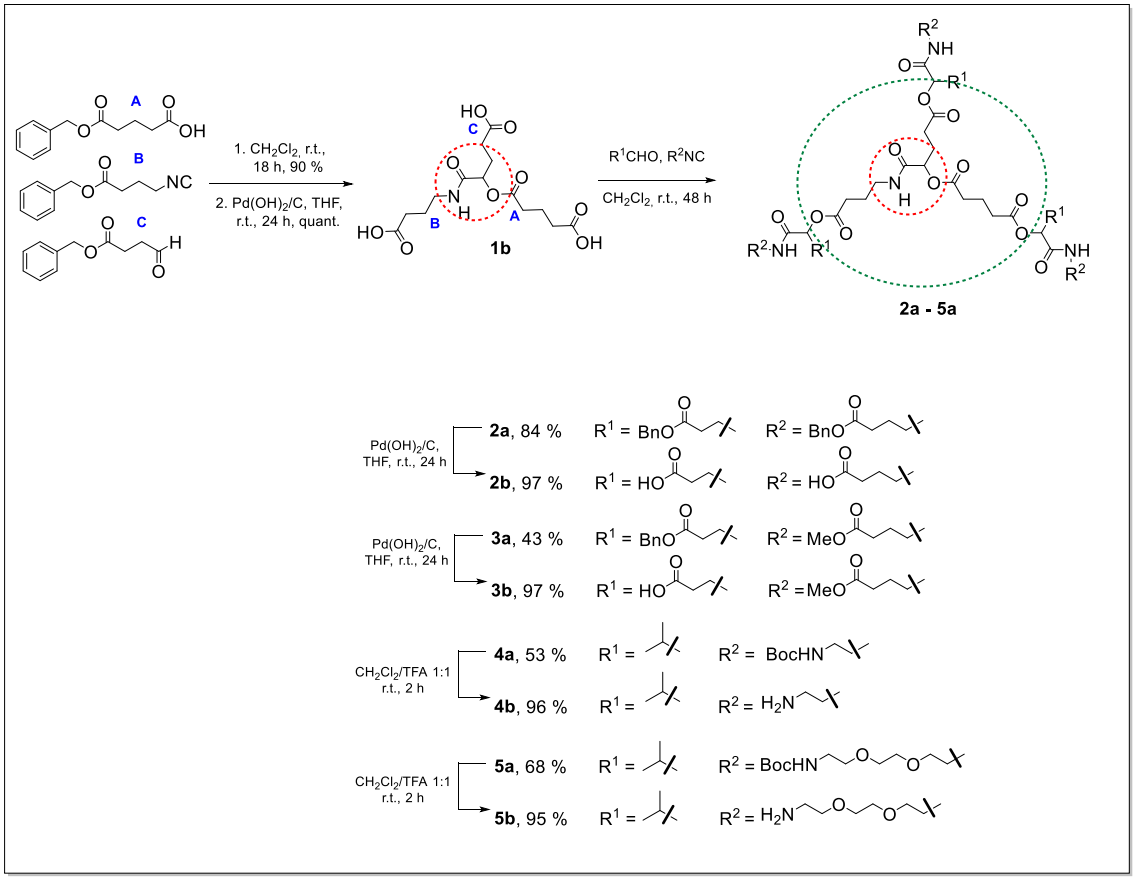
Figure 1. Synthetic strategy to synthesize dendrimers and polyvalent molecules *via* Passerini reaction.

2.3 Synthesis of polyvalent molecules and dendrimers by Passerini reaction

2.3.1 Synthesis of traditional dendrimers

Initially, a Passerini reaction between equimolar quantities of monoprotected bifunctional compounds benzyl 4-isocyanobutanoate, benzyl 4-oxobutanoate, and 5-benzyloxy-5-oxopentanoic acid in CH_2Cl_2 at room temperature for 18 hours afforded the first generation (G1) of the dendrimer (**1a**) with 90 % yield. After the deprotection of the three terminal carboxylic acid units (**1b**) another Passerini reaction afforded the second generation (G2). In order to investigate the scope of this protocol we used four different isocyanides and three different aldehydes to obtain the second generation of the dendrimers **2a** - **5a** with good yields and high purity after column chromatography (**Scheme 1**).

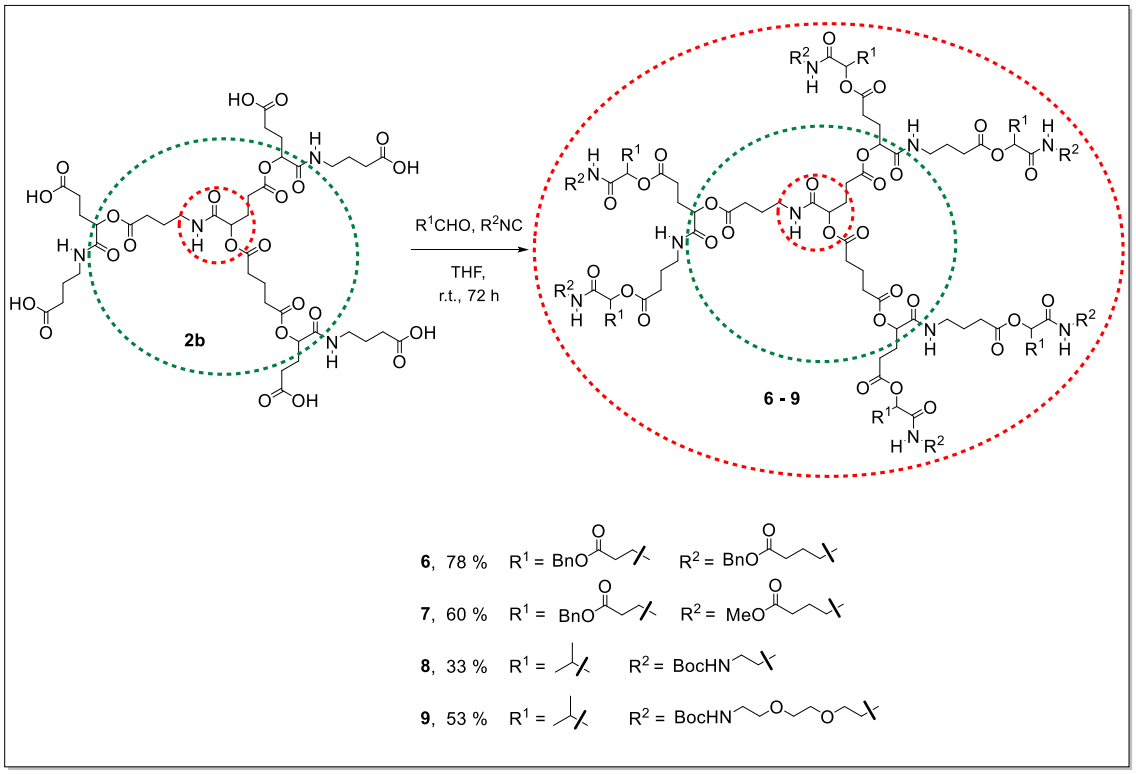
Dendrimers **2b** and **3b** were obtained by removal of the benzyl protecting groups which led to a dendrimer with six carboxylic acids and a dendrimer with three carboxylic acids and three methyl esters on the surface, respectively. Compounds **4b** and **5b** were achieved by deprotection of the amino groups. These four dendrimers have on the surface reactive functional groups that allow further modifications by Passerini reaction or any other desirable chemical reaction.



Scheme 1. Synthesis of the first and second generation Passerini dendrimers **2a** - **5b**.

Employing the same methodology, reaction conditions, and purification process as before, four dendrimers up to the third generation (**6** - **9**) were obtained *via* Passerini reaction between the compound **2b**, which contains six carboxylic acid groups available for the reaction, four different isocyanides, and two different aldehydes, with yields between 33 % and 78 % (**Scheme 2**).

To verify the identity and purity of the dendrimers, they were subjected to ^{1}H NMR, ^{13}C NMR, and mass spectrometry analyses. The signals in the NMR spectra can be very well assigned to the respective protons and carbon atoms. By measuring high-resolution ESI-MS or MALDI-TOF, the masses of the synthesized macromolecules could be clearly detected. The dispersity of the compounds was determined by size-exclusion chromatography (SEC). **Figure 2** shows the size-exclusion chromatogram of the traditional dendrimer **6** (G3). It can be seen from the diagram that there is only one signal in the chromatogram, which indicates the expected monodispersity of the synthesized dendrimers (Đ = 1.02).



Scheme 2. Synthesis of the third generation Passerini dendrimers **6** - **9**.

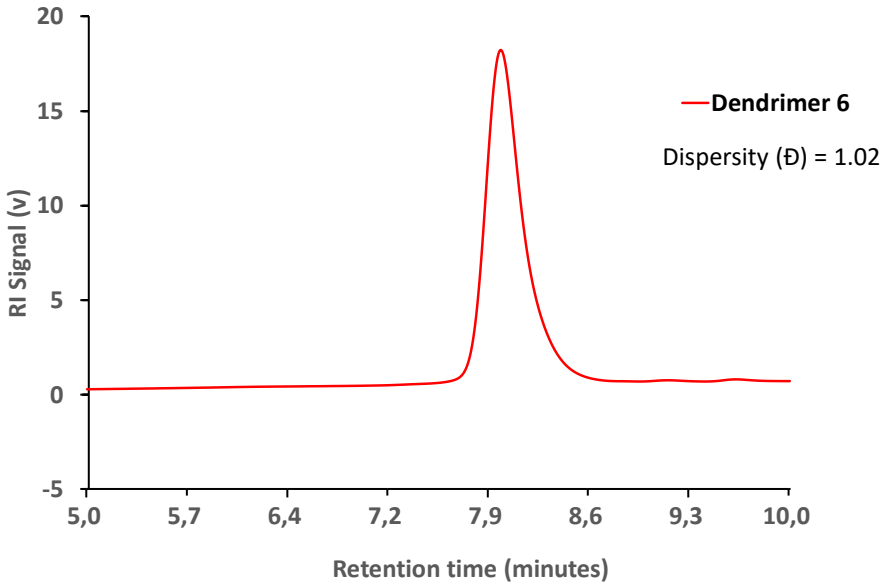


Figure 2. Size-exclusion chromatogram of dendrimer **6** (G3).

Applying this approach, twelve traditional polyester dendrimers were synthesized up to the second and third generation in a simple, divergent, and efficient procedure. Dendrimers with a variety of terminal groups, including carboxylic acids, methoxycarbonyl groups, amino groups, and PEG chains were obtained as a viscous colorless oil in high purity.

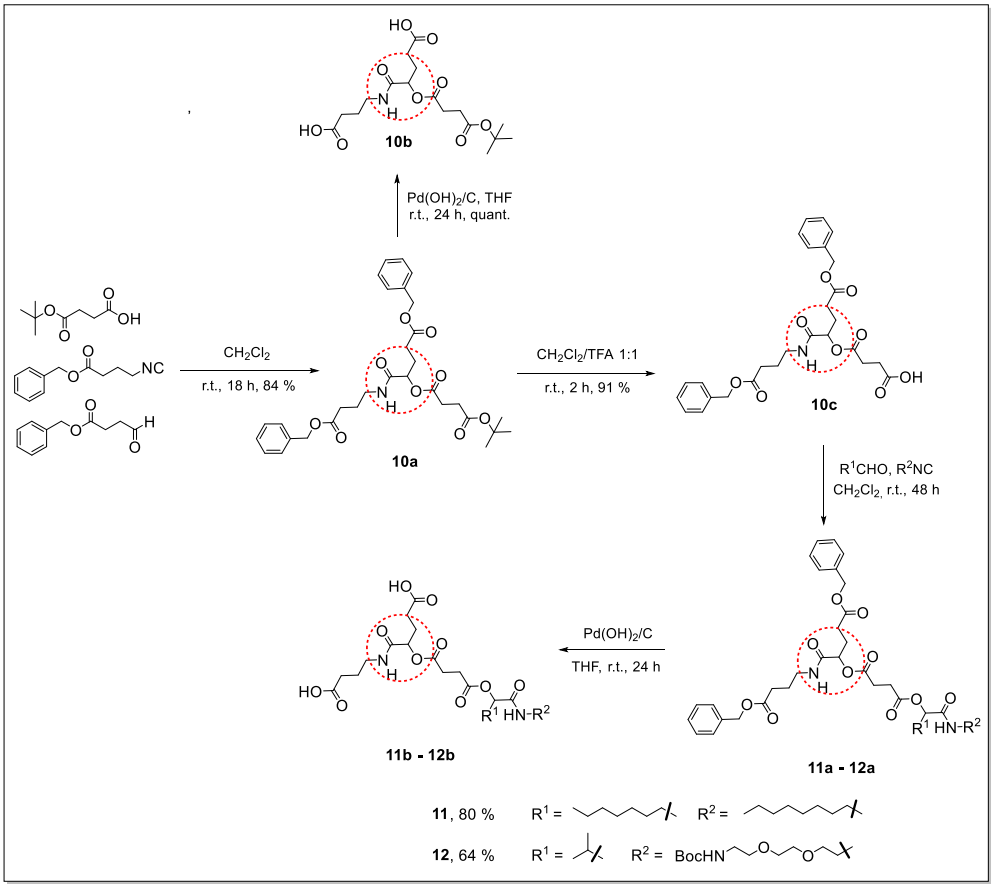
The solubility of the final dendrimers was improved with the increase of the generations (G1 normally soluble in CH_2Cl_2 and G3 in THF or MeOH) and the number of polar groups on the surface. The compounds proved to be stable, since analytical analysis after one year of storage showed no signs of decomposition. The synthesis of higher dendrimer generation was not successfully achieved by Passerini reaction possibly due to steric hindrance.

2.3.2 Synthesis of polyvalent molecules and Janus dendrimers

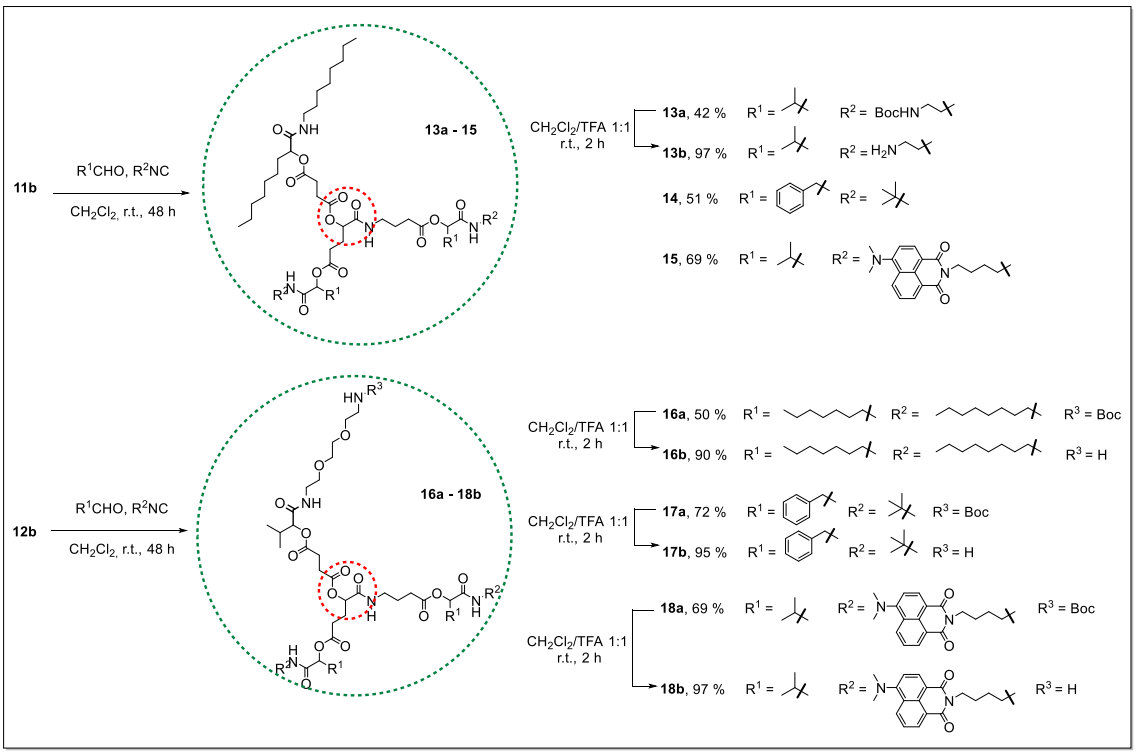
Surface-diblock dendrimers and polyvalent molecules were synthesized using different protecting groups on the monoprotected bifunctional starting materials. The first generation (**10a**) was synthesized between equimolar quantities of benzyl 4-isocyanobutanoate, benzyl 4-oxobutanoate, and mono-*tert*-butyl succinate in CH_2Cl_2 at room temperature for 18 hours and 84 % yield. The protecting group for the carboxylic acid is a *tert*-butyl group and for the isocyanide and aldehyde is a benzyl group which allow orthogonal removal (**Scheme 3**).

In the same way as previously described for the traditional dendrimers, the second generation of the Janus dendrimers (polyvalent molecules) was synthesized by elimination of the *tert*-butyl group (**10c**). Two distinct Passerini reaction between this deprotected carboxylic acid and two different sets of aldehydes and isocyanides yielded the dendrimer intermediates **11a** and **12a** (**Scheme 3**). The deprotection of the reminiscent two carboxylic acids, protected with benzyl groups, enabled another two identical Passerini reactions. Starting from **11b**, dendrimers **13a**, **14** and **15** were synthesized, and starting from **12b**, dendrimers **16a** - **18a** were prepared (**Scheme 4**).

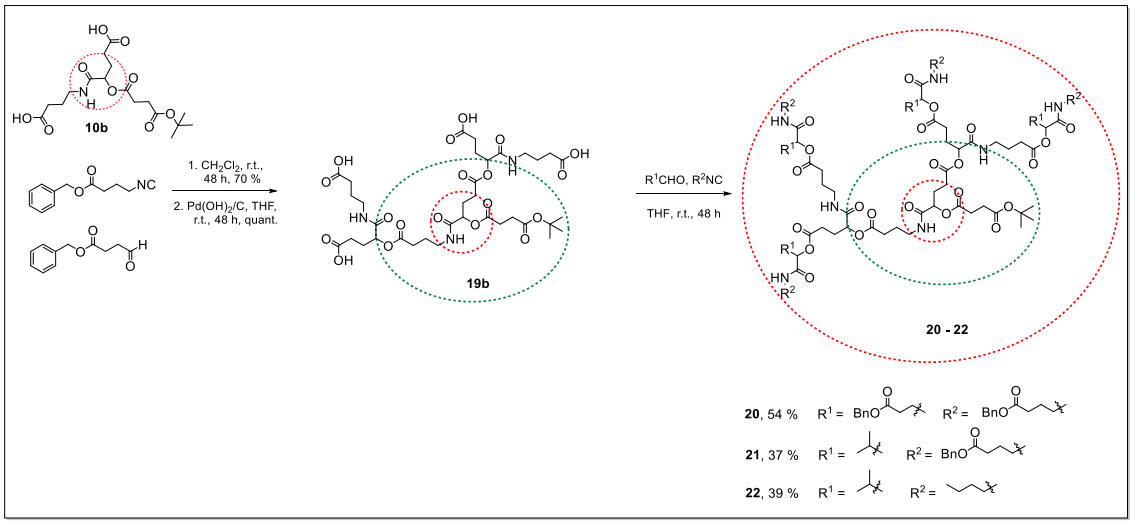
The third generation of the surface-diblock dendrimers was constructed first by a Passerini reaction between first generation dendrimer **10b**, benzyl 4-isocyanobutanoate, and benzyl 4-oxobutanoate, followed by the removal of the benzyl groups. A subsequent Passerini reaction provided the third generation of the dendrimers **20**, **21**, and **22** in good yields and high purity (**Scheme 5**).



Scheme 3. Synthesis of the dendrimer intermediates **11b** and **12b**.



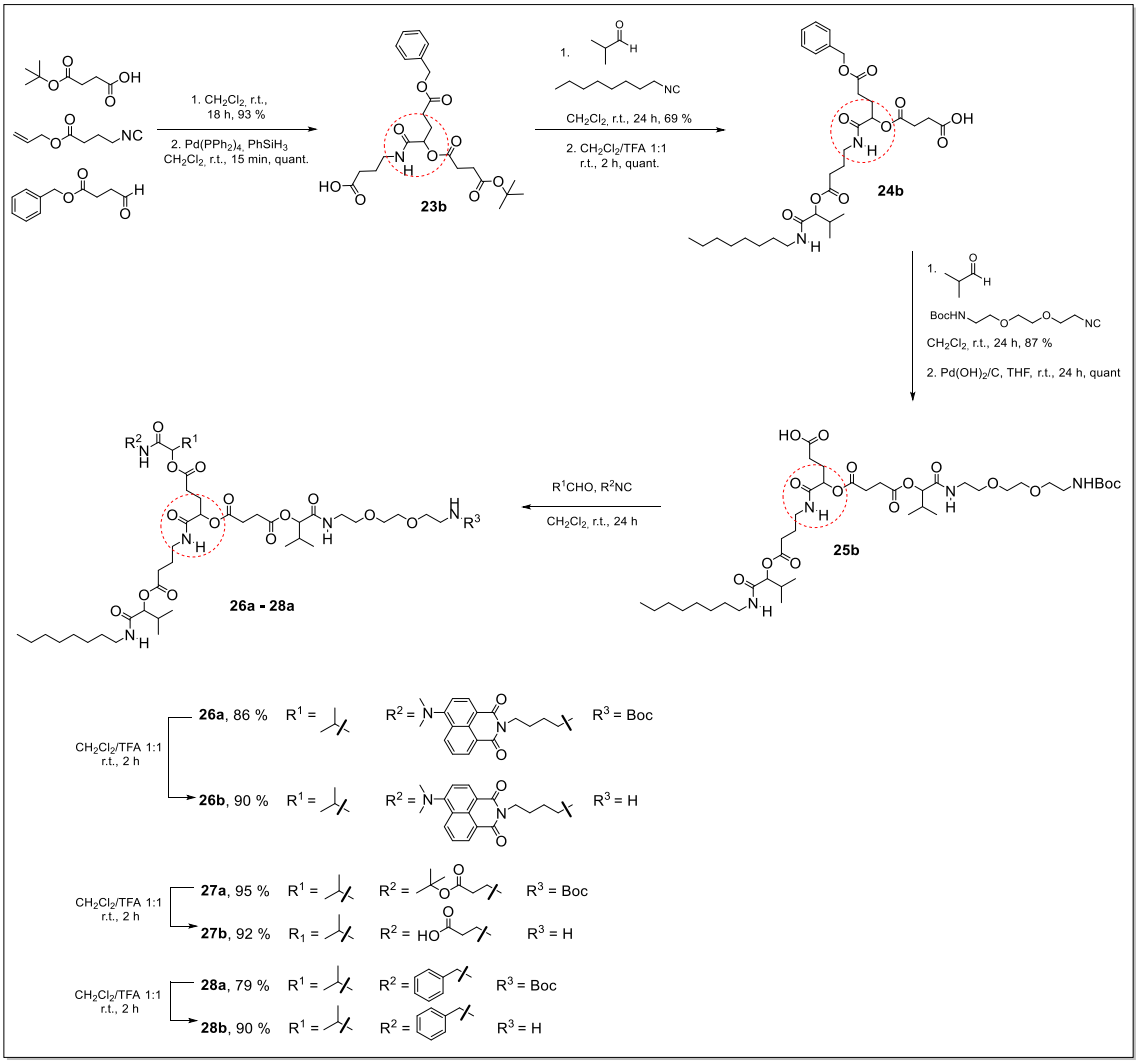
Scheme 4. Synthesis of the dendrimers **13a** - **18b**.



Scheme 5. Synthesis of the dendrimers **20**, **21**, and **22**.

Polyvalent molecules with three different types of end groups were synthesized in a similar manner as before using three distinct protecting groups, one for each component of the Passerini reaction. The three protecting groups can be removed orthogonally allowing the growth of the molecule in three separate parts.

The first generation G1 (**23a**) was synthesized between equimolar quantities of allyl 4-isocyanobutanoate, benzyl 4-oxobutanoate, and mono-*tert*-butyl succinate with 93 % yield and high purity after column chromatography. The elimination of the allyl group and a second Passerini reaction between 1-isocyanooctane and isobutyraldehyde, followed by the removal of the *tert*-butyl group yielded **24b** with 69 % yield. A subsequent Passerini reaction using *tert*-butyl (2-(2-(2-isocyanoethoxy)ethoxy) ethyl)carbamate and isobutyraldehyde, and the elimination of the benzyl group afforded **25b** with 87 % yield. Finally, a Passerini reaction between three different isocyanides and isobutyraldehyde gave the compounds **26a**, **27a**, and **28a**, respectively. The compounds **26b**, **27b**, and **28b** were obtained by the elimination of the respective protecting groups (**Scheme 6**).



Scheme 6. Synthesis of the dendrimers **26a** - **28b**.

Following the Passerini reaction for the synthesis of polyester Janus dendrimers and polyvalent molecules, we were able to synthesize a small library of complex, branched compounds with multifunctional surface and unique properties (e.g. amphiphilic dendrimers) up to the second and third generation. Dendrimers with two or three types of functional groups on the surface were obtained by combining different sets of aldehydes and isocyanides, including PEG and aliphatic chains, amino groups, carboxylic acids, aromatic groups, and fluorescent dye (maximum emission wavelength of 516 nm in THF).

Similarly to traditional dendrimers, the identity, purity, and monodispersity of the compounds were confirmed by ^{1}H NMR, ^{13}C NMR, and mass spectrometry analyses. All compounds were obtained as a viscous colorless oil, with exception of compounds **15**, **18a**, **18b**, **26a**, and **26b** which have an intense orange colour due to the presence of the

fluorescent dye moiety. Also, the solubility of the final dendrimers was improved with the increase of the generations and the number of polar groups on the surface.

2.4 Conclusion

In conclusion, we have described a new method to synthesize branched polyesters. The traditional and surface-block dendrimers were divergently assembled exclusively by Passerini reactions until the second and third generation with good yields. The key advantage of this protocol includes the possibility to use different types of functional groups in the internal and end groups without loss of yields and in a simple manner. This opens the opportunity to create new, complex branched molecules, linking desirable features, such as fluorescent dyes, lipophilic and hydrophilic areas, and relevant biomolecules.

2.5 Experimental Part

2.5.1 General information

All commercial reagents were purchased from Sigma-Aldrich (Germany), Alfa Aesar (Germany) or Iris Biotech (Germany) and were used without further purification. Column chromatography was carried out using Merck silica gel 60 (0.040-0.063 mm) with approximately 35 g of silica gel per gram of crude product. Analytical thin-layer chromatography (TLC) was performed using Merck silica gel 60 F254 aluminium sheets. ^{1}H NMR and ^{13}C NMR spectra were recorded at 25 °C in the respective solvents on an Agilent DD2 400 spectrometer at 400 MHz and 101 MHz, respectively. Chemical shifts (δ) are reported in ppm relative to TMS (^{1}H NMR) and to residual solvent signal (^{13}C NMR). High resolution ESI mass spectra were obtained with an Orbitrap Elite mass spectrometer (Thermo Fisher Scientific, Germany) equipped with HESI electrospray ion source (spray voltage 4.0 kV; capillary temperature 275 °C, source heater temperature 40 °C; FTMS resolution 60.000). Electrospray ionization mass spectra (ESI-MS) were recorded on a API 3200 system, Triple Quadrupole MS (AB Sciex) equipped with ESI electrospray ion source (positive spray voltage 5.5 kV, negative spray voltage 4.5 kV, and source heater temperature 400 °C). MALDI-TOF mass spectra were acquired on a Bruker Ultraflex III-MALDI-TOF/TOF mass spectrometer (Bruker Daltonics, Bremen, Germany). The samples (1 µL) were mixed with the equal volume of 4 mg/mL α-cyano-4-hydroxycinnamic acid solution in 50 % v/v acetonitrile/0.1 % v/v TFA (matrix) on a

stainless steel target and dried under air. The analysis was performed in a reflector positive ion mode, using the source and reflector voltages of 25 and 26.3 kV, respectively. Desorption/ionization of the analytes was achieved by a YAG 354 nm laser. Size exclusion chromatography was performed in LC 1100/1200 Agilent technologies with refractive index detector. The column was 1PL oligopore (300 x 7.5 mm ID) and the eluent DMAc + 2 vol % water + 3 g/L LiCl, 1 mL/min. The calibration standard used was polymethylmethacrylate. The samples were dissolved in the eluent (2.5 mg/mL).

2.5.2 Synthesis

Synthesis of starting materials

5-Benzyloxy-5-oxopentanoic acid (S1). To a solution of glutaric anhydride (11.4 g, 100 mmol) in DMF (40 mL) was added benzyl alcohol (9.40 mL, 100 mmol) and DIPEA (19.3 mL, 110 mmol) at 0 °C. The mixture was stirred at room temperature overnight and evaporated under reduced pressure. The residue was dissolved in ethyl acetate (200 mL) and washed with saturated aqueous NaCl (50 mL x 2). The organic phase was extracted with saturated aqueous $NaHCO_3$ (50 mL x 3) and the aqueous fractions were combined, acidified to pH 4 with citric acid (5 M), and extracted with ethyl acetate (100 mL x 3). The organic layers were combined, washed with saturated aqueous NaCl, dried over Na_2SO_4, and concentrated under reduced pressure to yield a yellow oil. The product was purified by column chromatography (SiO_2, CH_2Cl_2). Yield: 20.8 g (94 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 - 7.30 (m, 5H), 5.12 (s, 2H), 2.48 - 2.40 (m, 4H), 1.99 (q, *J* = 7.3 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.89, 172.65, 135.81, 128.55, 128.24, 128.19, 126.98, 66.33, 33.12, 32.89, 19.75.

Benzyl 4-isocyanobutanoate (S2). A solution of benzyl 4-aminobutanoate (17.9 g, 48 mmol) in ethyl formate (250 mL) was refluxed for 20 hours, followed by the evaporation of all volatiles to yield the corresponding formamide as a colorless oil. A solution of the formamide (10.6 g, 48 mmol) in CH_2Cl_2 (150 mL) was added in a round-bottom flask under nitrogen atmosphere. Diisopropylamine (20 mL, 144 mmol) was added and the mixture cooled to 0 °C. After the dropwise addition of phosphorus oxychloride (5.36 mL, 57.6 mmol), the mixture was warmed to room temperature and stirred for 2 hours. The mixture was quenched with saturated aqueous Na_2CO_3. The resulting suspension was stirred further 30 minutes, diluted with water, and the organic layer was separated. The aqueous phase was extracted with CH_2Cl_2 (100 mL x 3), the combined organic layers

dried over Na_2SO_4, and the organic solvent removed under reduced pressure. The product was purified by column chromatography (SiO_2, *n*-hexane/ethyl acetate 3:1). Yellow oil. Yield: 3.22 g (33 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 - 7.29 (m, 5H), 5.12 (s, 2H), 3.45 (t, *J* = 6.5 Hz, 2H), 2.53 (t, *J* = 7.1 Hz, 2H), 2.05 - 1.93 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.73, 156.75, 135.48, 128.45, 128.20, 128.07, 66.39, 40.58, 30.29, 24.12.

Benzyl 4-hydroxybutanoate (S3). A mixture of oxolan-2-one (51.0 g, 592 mmol) and NaOH (23.7 g, 592 mmol) in H_2O (590 mL) was heated to 70 °C overnight. The clear solution was then cooled, concentrated under reduced pressure, and lyophilized. The product was obtained as a white solid. The salt sodium 4-hydroxybutanoate (74.6 g, 592 mmol) was suspended in acetone (600 mL) along with TBAB (9.54 g, 29.6 mmol) and benzyl bromide (122 g, 711 mmol) and heated at reflux for 24 hours. The mixture was cooled, concentrated, and the residue partitioned between 750 mL of ethyl acetate and 250 mL of aqueous 1M $NaHSO_4$ solution. The organic phase was washed with saturated aqueous $NaHCO_3$ (250 mL) and saturated aqueous NaCl (250 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The product was used in the next step without further purification. Colorless oil.

Benzyl 4-oxobutanoate (S4). To a solution of DMSO (10.9 mL, 154 mmol) in CH_2Cl_2 (495 mL), in a round-bottom flask under nitrogen atmosphere, was added oxalyl chloride (6.6 mL, 77.2 mmol) at - 78 °C. The resultant mixture was stirred for 15 minutes followed by the dropwise addition of benzyl 4-hydroxybutanoate (**S3**, 10.0 g, 51.5 mmol) in CH_2Cl_2. After stirring for 1 hour at - 78 °C, triethylamine (35.9 mL, 257.4 mmol) was added, and the resulting mixture was allowed to warm to room temperature. After 30 minutes, the reaction was quenched with aqueous 10 % HCl solution and the resulting mixture washed with saturated aqueous Na_2CO_3 and brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The product was purified by column chromatography (SiO_2, *n*-hexane/ethyl acetate 3:7). Light yellow oil. Yield: 7.0 g (71 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.79 (s, 1H), 7.42 - 7.28 (m, 5H), 5.12 (s, 2H), 2.79 (t, *J* = 6.6 Hz, 2H), 2.67 (t, *J* = 6.6 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 199.78, 171.97, 135.63, 128.47, 128.44, 128.33, 128.20, 128.11, 66.53, 38.39, 26.49.

Methyl 4-aminobutanoate hydrochloride (S5). A mixture of 4-aminobutyric acid (2.0 g, 19.3 mmol) in methanol (30 mL) was cooled to 0 °C followed by the dropwise addition of thionyl chloride (2.3 g, 19.3 mmol). The ice bath was removed, and the mixture stirred

at room temperature overnight. The solution was concentrated and washed with diethyl ether to afford the product as a colorless solid. The product was used in the next step without further purification.

Methyl 4-isocyanobutanoate (S6). Methyl 4-isocyanobutanoate was prepared by the same method as **S2** starting from methyl 4-aminobutanoate hydrochloride (**S5**, 2.96 g, 19.3 mmol). Light yellow oil. Yield: 1.59 g (65 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.71 (s, 3H), 3.50 (ddt, *J* = 8.6, 3.9, 2.0 Hz, 2H), 2.52 (t, *J* = 7.1 Hz, 2H), 2.00 (dqt, *J* = 11.9, 4.9, 2.4 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.49, 156.72, 51.79, 40.72, 30.10, 24.21.

1-Isocyanooctane (S7). 1-Isocyanooctane was prepared by the same method as **S2** starting from *N*-octylamine (5.0 g, 38.7 mmol). Brown oil. Yield: 4.83 g (89 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.43 - 3.34 (m, 2H), 1.73 - 1.63 (m, 2H), 1.50 - 1.39 (m, 2H), 1.36 - 1.22 (m, 8H), 0.92 - 0.86 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.56, 41.54, 31.68, 29.10, 29.00, 28.64, 26.30, 22.57, 14.03.

tert-Butyl (2-aminoethyl)carbamate (S8). A mixture of ethylenediamine (2.0 g, 33.2 mmol) in 20 mL dioxane, 20 mL H_2O, and 30 mL NaOH 1N was cooled to 0 °C. Di-*tert*-butyl dicarbonate (1.44 g, 6.6 mmol) dissolved in 5 mL of dioxane was added dropwise. The ice bath was removed, and the mixture stirred at room temperature for 18 hours. Dioxane was evaporated, the residue extracted with ethyl acetate (75 mL x 3), and the solvent removed under reduced pressure. The product was used in the next step without further purification. Colorless oil.

tert-Butyl (2-isocyanoethyl)carbamate (S9). *tert*-Butyl (2-isocyanoethyl)carbamate was prepared by the same method as **S2** starting from *tert*-butyl (2-aminoethyl)carbamate (**S8**, 1.0 g, 6.25 mmol). Brown oil. Yield: 520 mg (52 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.54 - 3.52 (m, 2H), 3.45 - 3.32 (m, 2H), 1.45 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.61, 155.54, 80.18, 42.11, 39.85, 28.30.

tert-Butyl (2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate (S10). *tert*-Butyl (2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate was prepared by the same method as **S8** starting from 2,2′-(ethylenedioxy)bis(ethylamine) (1.0 g, 6.74 mmol). The product was used in the next step without further purification. Yellow oil.

tert-Butyl (2-(2-(2-isocyanoethoxy)ethoxy)ethyl)carbamate (S11). *tert*-Butyl (2-(2-(2-isocyanoethoxy)ethoxy)ethyl)carbamate was prepared by the same method as **S2**

starting from *tert*-butyl (2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate (**S10**, 1.5 g, 6.04 mmol). Brown oil. Yield: 1.23 g (79 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.76 - 3.53 (m, 10H), 3.34 - 3.30 (m, 2H), 1.45 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.45, 155.89, 79.21, 70.77, 70.29, 70.13, 68.62, 41.69, 40.29, 28.37.

4-N,N-Dimethylamino-1,8-naphthalic anhydride (S12). 4-Bromo-1,8-naphtalic anhydride (14.0 g, 50.4 mmol) and 170 mL *n*-butanol were added to a round-bottom flask equipped with a reflux condenser. The solution was brought to reflux followed by the slow addition of 11 mL of 3-*N,N*-dimethylaminopropionitrile. The mixture was stirred at reflux overnight and then cooled with an ice bath until the formation of a yellow precipitate. The solid was filtered and washed with *n*-hexane to give the desired product as a yellow solid. Yield: 10.7 g (89 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (dd, *J* = 7.3, 1.2 Hz, 1H), 8.51 - 8.45 (m, 2H), 7.69 (dd, *J* = 8.5, 7.3 Hz, 1H), 7.12 (d, *J* = 8.3 Hz, 1H), 3.18 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.68, 160.44, 134.92, 133.13, 132.89, 132.72, 124.99, 124.90, 119.28, 113.22, 44.58.

tert-Butyl (4-(6-(dimethylamino)-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)yl)butyl) carbamate (S13). 4-*N,N*-Dimethylamino-1,8-naphthalic anhydride (**S12**, 6.06 g, 25.1 mmol) and 400 mL absolute ethanol were added to a round-bottom flask equipped with a reflux condenser. *N*-(*tert*-Butoxycarbonyl)-1,4-diaminobutane was added to the mixture and the solution was brought to reflux. After 30 minutes, the solvent was removed under reduced pressure and the product purified by column chromatography (SiO_2, ethyl acetate). The product was isolated as an orange solid. Yield: 5.58 g (54 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (d, *J* = 7.2, 1.2 Hz, 1H), 8.48 - 8.41 (m, 2H), 7.65 (t, *J* = 8.5, 7.3 Hz, 1H), 7.11 (d, *J* = 8.2 Hz, 1H), 4.22 - 4.15 (m, 2H), 3.24 - 3.17 (m, 2H), 3.11 (s, 6H), 1.81 - 1.71 (m, 2H), 1.66 - 1.59 (m, 2H), 1.43 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.55, 164.01, 156.92, 155.88, 132.60, 131.14, 130.98, 130.19, 125.25, 124.84, 123.00, 114.92, 113.27, 78.93, 44.74, 40.22, 39.61, 28.38, 27.51, 25.46.

2-(4-Aminobutyl)-6-(dimethylamino)-1H-benzo[de]isoquinoline-1,3(2H)-dione (S14). *tert*-Butyl (4-(6-(dimethylamino)-1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)yl) butyl) carbamate (**S13**, 1.08 g, 2.45 mmol) and 40 mL of a CH_2Cl_2/TFA (1:1) solution were added to a round-bottom flask. The mixture was stirred at room temperature for 2 hours. The solvent was removed under reduced pressure to yield the corresponding product as a yellow solid. Yield: 723 mg (95 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (dd, *J* = 7.3, 1.2 Hz, 1H), 8.32 - 8.26 (m, 2H), 7.52 (dd, *J* = 8.5, 7.3 Hz, 1H), 6.98 (d, *J* = 8.3

Hz, 1H), 4.10 (t, *J* = 6.6 Hz, 2H), 3.13 (s, 2H), 3.05 (s, 6H), 1.89 - 1.75 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.67, 164.19, 157.06, 132.82, 131.39, 131.07, 130.05, 124.81, 124.65, 122.39, 113.97, 113.09, 44.64, 39.63, 38.98, 24.81, 24.72.

6-(Dimethylamino)-2-(4-isocyanobutyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (S15). 6-(Dimethylamino)-2-(4-isocyanobutyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione was prepared by the same method as **S2** starting from 2-(4-aminobutyl)-6-(dimethylamino)-1*H*-benzo[*de*]isoquinoline-1,3(*2H*)-dione (**S14**, 1.0 g, 3.21 mmol). Orange solid. Yield: 550 mg (54 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 (d, *J* = 7.3, 1.2 Hz, 1H), 8.50 - 8.42 (m, 2H), 7.66 (t, *J* = 8.5, 7.3 Hz, 1H), 7.12 (d, *J* = 8.2 Hz, 1H), 4.22 (t, *J* = 6.9 Hz, 2H), 3.48 (t, *J* = 6.6, 1.9 Hz, 2H), 3.12 (s, 6H), 1.94 - 1.76 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.63, 164.06, 157.07, 156.02, 132.76, 131.32, 131.11, 130.26, 125.23, 124.86, 122.86, 114.67, 113.28, 44.75, 41.23, 38.80, 26.66, 25.10.

4-Formamidobutanoic acid (S16). A mixture of acetic anhydride (57.1 g, 560 mmol) and formic acid (38.6 g, 840 mmol) was heated at 60 °C for 3 hours. After, the mixture was cooled and dissolved in THF. 4-Aminobutyric acid (5.8 g, 56 mmol) was added and the mixture stirred for further 12 hours at room temperature. The solvent was removed under reduced pressure to yield the corresponding product. The product was used in the next step without further purification. White solid.

Allyl 4-formamidobutanoate (S17). A mixture of 4-formamidobutanoic acid (**S16**, 4.8 g, 36.6 mmol), allyl bromide (6.24 g, 52 mmol), and K_2CO_3 (6.1 g, 44 mmol) in DMF was stirred at room temperature for 16 hours. Then, ethyl acetate was added, and the organic layer washed with small portions of water (20 mL). The solvent was removed under reduced pressure to yield the corresponding product. The product was used in the next step without further purification. Brown oil.

Allyl 4-isocyanobutanoate (S18). Allyl 4-isocyanobutanoate was prepared by the same method as **S2** starting from allyl 4-formamidobutanoate (**S17**, 4.5 g, 26 mmol). Brown oil. Yield: 2.7 g (68 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.92 (ddt, *J* = 17.2, 10.4, 5.8 Hz, 1H), 5.38 - 5.24 (m, 2H), 4.61 (dt, *J* = 5.8, 1.4 Hz, 2H), 3.51 (ddt, *J* = 8.6, 3.9, 1.9 Hz, 2H), 2.54 (t, *J* = 7.1 Hz, 2H), 2.02 (tdd, *J* = 8.9, 4.9, 2.4 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.73, 156.86, 131.82, 118.57, 65.40, 40.74, 30.32, 24.26.

tert-Butyl 3-isocyanopropanoate (S19). *tert*-Butyl 3-isocyanopropanoate was prepared by the same method as **S2** starting from β-alanine *tert*-butyl ester hydrochloride

(2.0 g, 11.0 mmol). Yellow oil. Yield: 954 mg (56 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.57 (t, *J* = 6.8 Hz, 2H), 2.56 (t, *J* = 6.8 Hz, 2H), 1.40 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.59, 156.16, 80.86, 36.40, 34.24, 27.00.

Synthesis of dendrimers

Dendrimer 1a. A mixture of 5-benzyloxy-5-oxopentanoic acid (**S1**, 880 mg, 4.0 mmol), benzyl 4-isocyanobutanoate (**S2**, 810 mg, 4.0 mmol), and benzyl 4-oxobutanoate (**S4**, 770 mg, 4.0 mmol) in CH_2Cl_2 was stirred at room temperature for 18 hours. The solvent was removed under reduced pressure and the product was purified by column chromatography (SiO_2, *n*-hexane/ethyl acetate 2:1). Colorless oil. Yield: 2.22 g (90 %). MS (ESI) of $C_{35}H_{39}NO_9$ $[M+Na]^+$ calc. 640.2, obs. 640.1; HRMS (ESI) of $C_{35}H_{39}NO_9$ $[M+H]^+$ calc. 618.26, obs. 618.27; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 - 7.28 (m, 15H), 5.18 (dd, *J* = 7.0, 4.8 Hz, 1H), 5.10 (d, *J* = 1.5 Hz, 6H), 3.29 (dt, *J* = 8.6, 6.9 Hz, 2H), 2.47 - 2.37 (m, 8H), 2.29 - 2.11 (m, 2H), 2.03 - 1.93 (m, 2H), 1.86 - 182 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.17, 172.68, 172.23, 171.56, 169.04, 135.70, 128.51, 128.22, 128.18, 128.15, 128.11, 72.70, 66.37, 66.36, 66.33, 38.76, 33.00, 32.82, 31.65, 29.69, 26.90, 24.21, 19.84.

Dendrimer 1b. To a solution of dendrimer **1a** (950 mg, 1.54 mmol) in THF was added $Pd(OH)_2/C$ (100 mg) under H_2 atmosphere. The mixture was stirred at room temperature for 24 hours. Afterwards, the mixture was filtered through a celite pad, and the organic phase concentrated under reduced pressure to yield the product as a colorless oil. Yield: 530 mg (99 %). MS (ESI) of $C_{14}H_{21}NO_9$ $[M+Na]^+$ calc. 370.0, obs. 370.1; HRMS (ESI) of $C_{14}H_{21}NO_9$ $[M+H]^+$ calc. 348.12, obs. 348.13; ^{1}H NMR (400 MHz, MeOD) δ 5.00 (dd, *J* = 7.8, 4.8 Hz, 1H), 3.25 (t, *J* = 6.9 Hz, 2H), 2.51 (td, *J* = 7.4, 4.3 Hz, 2H), 2.42 - 2.30 (m, 6H), 2.18 - 2.01 (m, 2H), 1.90 (q, *J* = 7.3 Hz, 2H), 1.79 (p, *J* = 7.2 Hz, 2H); ^{13}C NMR (101 MHz, MeOD) δ 176.96, 176.84, 176.24, 173.92, 172.42, 74.27, 39.61, 33.85, 33.72, 32.12, 30.54, 28.37, 25.63, 21.10.

Dendrimer 2a. See procedure **1a**: dendrimer **1b** (550 mg, 1.58 mmol), benzyl 4-isocyanobutanoate (**S2**, 1.0 g, 5.7 mmol), and benzyl 4-oxobutanoate (**S4**, 1.15 g, 5.7 mmol) in CH_2Cl_2. The product was purified by column chromatography (SiO_2, *n*-hexane/ethyl acetate 1:1). Colorless oil. Yield: 1.29 g (84 %). MS (ESI) of $C_{83}H_{96}N_4O_{24}$ $[M+Na]^+$ calc. 1555.6, obs. 1555.7; HRMS (ESI) of $C_{83}H_{96}N_4O_{24}$ $[M+2H]^{2+}$ calc. 767.32, obs. 767.33; ^{1}H NMR (400 MHz, MeOD) δ 7.35 - 7.25 (m, 30H), 5.08 (d, *J* = 1.6 Hz,

12H), 4.98 (dt, *J* = 7.6, 3.5 Hz, 4H), 3.21 (t, *J* = 6.9 Hz, 8H), 2.50 - 2.41 (m, 12H), 2.40 - 2.35 (m, 8H), 2.15 - 2.03 (m, 8H), 1.87 (m, 2H), 1.79 (p, *J* = 6.2 Hz, 8H); ^{13}C NMR (101 MHz, MeOD) δ 174.58, 173.93, 173.91, 173.86, 173.84, 173.82, 173.31, 173.29, 173.28, 172.26, 172.23, 172.20, 172.12, 172.09, 137.63, 129.57, 129.54, 129.25, 129.23, 129.16, 74.28, 67.44, 39.53, 39.49, 39.11, 33.60, 33.55, 33.49, 32.31, 32.24, 32.21, 31.62, 31.57, 30.89, 30.78, 30.69, 30.67, 30.37, 30.31, 28.19, 28.05, 27.99, 25.57, 25.52, 20.85.

Dendrimer 2b. See procedure **1b**: dendrimer **2a** (1.3 g, 0.85 mmol), $Pd(OH)_2/C$ (130 mg) in THF. The product was obtained as a colorless oil. Yield: 810 mg (97 %). MS (ESI) of $C_{41}H_{60}N_4O_{24}$ $[M+Na]^+$ calc. 1015.3, obs. 1015.2; HRMS (ESI) of $C_{41}H_{60}N_4O_{24}$ $[M+H]^+$ calc. 993.35, obs. 993.37; ^{1}H NMR (400 MHz, MeOD) δ 5.07 - 4.96 (m, 4H), 3.26 (td, *J*= 7.5, 6.8, 2.4 Hz, 8H), 2.60 - 1.75 (m, 38H); ^{13}C NMR (101 MHz, MeOD) δ 180.80, 178.62, 176.90, 176.79, 176.19, 175.35, 174.69, 174.01, 174.00, 173.93, 173.38, 172.38, 172.31, 172.28, 74.32, 39.66, 39.62, 39.34, 39.22, 33.58, 33.54, 32.09, 31.67, 30.88, 30.51, 28.62, 28.35, 28.05, 27.98, 27.05, 25.88, 25.62, 25.54, 25.49, 23.09, 20.84.

Dendrimer 3a. See procedure **1a**: dendrimer **1b** (100 mg, 0.28 mmol), methyl 4-isocyanobutanoate (**S6**, 127 mg, 1.0 mmol), and isobutyraldehyde (192 mg, 1.0 mmol) in CH_2Cl_2. The product was purified by column chromatography (SiO_2, *n*-hexane/ethyl acetate 50:50 to *n*-hexane/ethyl acetate 0:100). Colorless oil. Yield: 157 mg (43 %). MS (ESI) of $C_{65}H_{84}N_4O_{24}$ $[M+Na]^+$ calc. 1327.5, obs. 1327.4; HRMS (ESI) of $C_{65}H_{84}N_4O_{24}$ $[M+H]^+$ calc. 1305.54, obs. 1305.56; ^{1}H NMR (400 MHz, MeOD) δ 7.38 - 7.26 (m, 15H), 5.11 (s, 6H), 5.02 - 4.95 (m, 4H), 3.64 (t, *J* = 5.6 Hz, 9H), 3.26 - 3.20 (m, 8H), 2.54 - 2.31 (m, 20H), 2.19 - 2.06 (m, 6H), 1.95 - 1.74 (m, 12H); ^{13}C NMR (101 MHz, MeOD) δ 176.68, 175.31, 175.28, 175.15, 174.72, 173.93, 173.91, 173.83, 173.30, 172.26, 172.23, 172.14, 172.09, 137.52, 129.56, 129.50, 129.25, 129.17, 129.14, 74.27, 74.17, 74.14, 71.75, 67.44, 67.44, 67.27, 52.11, 52.08, 52.06, 39.51, 39.48, 39.21, 38.17, 32.03, 31.92, 30.90, 30.78, 30.69, 30.67, 28.21, 28.07, 28.02, 25.77, 25.69, 25.68, 25.62, 25.60, 25.58, 25.58, 25.56, 20.88, 20.85.

Dendrimer 3b. See procedure **1b**: dendrimer **3a** (60 mg, 0.06 mmol), $Pd(OH)_2/C$ (7 mg) in THF. The product was obtained as a colorless oil. Yield: 57 mg (97 %). MS (ESI) of $C_{44}H_{66}N_4O_{24}$ $[M-H]^-$ calc. 1033.3, obs. 1033.7; HRMS (ESI) of $C_{44}H_{66}N_4O_{24}$ $[M+H]^+$ calc. 1035.4147, obs. 1035.4129; ^{1}H NMR (400 MHz, acetone-D_6) δ 5.24 - 4.93 (m, 4H), 3.62 (s, 9H), 3.33 - 3.24 (m, 8H), 2.60 - 2.32 (m, 20H), 1.98 - 1.59 (m, 18H); ^{13}C NMR (101 MHz, acetone-D_6) δ 178.23, 178.15, 177.96, 174.74, 174.74, 174.22, 174.15, 173.95,

173.87, 173.85, 173.79, 172.95, 172.83, 172.76, 73.82, 73.77, 73.70, 71.48, 51.58, 51.55, 51.52, 38.88, 38.86, 38.71, 38.59, 33.74, 33.22, 31.67, 31.59, 30.83, 30.43, 30.24, 30.10, 30.05, 28.03, 26.97, 26.81, 26.68, 25.75, 25.75, 25.60, 25.51, 25.48, 20.63.

Dendrimer 4a. See procedure **1a**: dendrimer **1b** (20 mg, 0.05 mmol), t*ert*-butyl (2-isocyanoethyl) carbamate (**S9**, 34 mg, 0.2 mmol), and isobutyraldehyde (14 mg, 0.2 mmol) in CH_2Cl_2. The product was purified by column chromatography (SiO_2, ethyl acetate/methanol 100:0 to ethyl acetate/methanol 80:20). Colorless oil. Yield: 28 mg (53 %). MS (ESI) of $C_{50}H_{87}N_7O_{18}$ $[M+Na]^+$ calc. 1096.6, obs. 1096.6; HRMS (ESI) of $C_{50}H_{87}N_7O_{18}$ $[M+H]^+$ calc. 1074.6188, obs. 1074.6157; ^{1}H NMR (400 MHz, MeOD) δ 5.12 - 4.93 (m, 4H), 3.23 - 3.08 (m, 14H), 2.61 - 2.46 (m, 8H), 2.20 - 2.14 (m, 4H), 1.99 - 1.81 (m, 5H), 1.43 (s, 27H), 1.02 - 0.91 (m, 18H); ^{13}C NMR (101 MHz, MeOD) δ 174.21, 173.66, 173.56, 173.27, 172.54, 172.44, 172.41, 172.35, 164.09, 80.20, 80.19, 80.18, 79.58, 79.49, 79.46, 77.08, 40.85, 40.81, 40.77, 39.27, 39.23, 39.17, 39.05, 33.70, 33.67, 31.81, 31.78, 31.77, 31.74, 28.78, 28.71, 27.21, 25.64, 20.97, 19.60, 19.21, 19.19, 17.69, 17.68, 17.60.

Dendrimer 4b. Dendrimer **4a** (25 mg, 0.02 mmol) and 2 mL of a CH_2Cl_2/TFA (1:1) solution were mixed in a round-bottom flask. The mixture was stirred at room temperature for 2 hours. The solvent was removed under reduced pressure to yield the corresponding product as a colorless oil. Yield: 15 mg (96 %). MS (ESI) of $C_{35}H_{63}N_7O_{12}$ $[M+H]^+$ calc. 774.4, obs. 774.2; HRMS (ESI) of $C_{35}H_{63}N_7O_{12}$ $[M+H]^+$ calc. 774.4630, obs. 774.4601; ^{1}H NMR (400 MHz, acetone-D_6) δ 4.98 - 4.74 (m, 4H), 3.95 - 3.89 (m, 3H), 3.34 - 3.23 (m, 5H), 2.65 - 2.13 (m, 13H), 2.09 - 2.07 (m, 7H), 1.98 - 1.88 (m, 3H), 1.01 - 0.78 (m, 18H); ^{13}C NMR (101 MHz, acetone-D_6) δ 173.64, 173.24, 173.00, 172.04, 171.45, 170.95, 170.64, 170.45, 78.95, 78.64, 78.60, 76.46, 42.12, 40.71, 40.67, 36.51, 36.47, 36.12, 35.95, 33.41, 33.24, 31.29, 31.16, 31.02, 31.00, 27.89, 25.39, 23.28, 20.63, 19.13, 19.09, 19.09, 17.44, 17.39, 17.31.

Dendrimer 5a. See procedure **1a**: dendrimer **1b** (100 mg, 0.28 mmol), *tert*-butyl (2-(2-(2-isocyanoethoxy)ethoxy)ethyl)carbamate (**S11**, 238 mg, 0.92 mmol), and isobutyraldehyde (66 mg, 0.92 mmol) in CH_2Cl_2. The product was purified by column chromatography (SiO_2, *n*-hexane/ethyl acetate 50:50 to *n*-hexane/ethyl acetate 0:100). Colorless oil. Yield: 254 mg (68 %). MS (ESI) of $C_{62}H_{111}N_7O_{24}$ $[M+Na]^+$ calc. 1360.7, obs. 1360.6; HRMS (ESI) of $C_{62}H_{111}N_7O_{24}$ $[M+Na]^+$ calc. 1360.75, obs. 1360.76; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.09 - 4.96 (m, 4H), 3.65 - 3.41 (m, 32H), 3.35 - 3.29 (m, 6H), 2.54 - 2.20

(m, 11H), 2.02 - 1.68 (m, 8H), 1.44 (s, 27H), 0.99 - 0.91 (m, 16H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.45, 171.89, 171.72, 171.58, 169.92, 169.53, 169.51, 169.45, 156.22, 156.05, 156.00, 79.34, 79.30, 79.28, 78.31, 78.24, 78.22, 72.60, 70.17, 40.39, 40.31, 40.21, 39.00, 38.93, 38.84, 38.40, 32.82, 32.75, 31.24, 30.52, 30.50, 30.44, 28.40, 27.59, 27.19, 24.93, 18.74, 17.14, 17.09, 17.03, 17.01, 16.96, 16.91.

Dendrimer 5b. See procedure **4b**: dendrimer **5a** (15 mg, 0.01 mmol) and 1 mL of a CH_2Cl_2/TFA (1:1) solution. The product was obtained as a colorless oil. Yield: 10 mg (95 %). MS (ESI) of $C_{47}H_{87}N_7O_{18}$ $[M+H]^+$ calc. 1038.6, obs. 1038.7; HRMS (ESI) of $C_{47}H_{87}N_7O_{18}$ $[M+H]^+$ calc. 1038.6188, obs. 1038.6178; ^{1}H NMR (400 MHz, MeOD) δ 5.17 - 4.96 (m, 4H), 3.73 - 3.31 (m, 30H), 3.29 - 3.10 (m, 8H), 2.65 - 2.32 (m, 10H), 2.19 - 2.11 (m, 3H), 2.00 - 1.77 (m, 6H), 1.01 - 0.83 (m, 16H); ^{13}C NMR (101 MHz, MeOD) δ 174.20, 174.18, 174.13, 173.61, 173.44, 172.45, 172.34, 172.30, 79.76, 79.75, 79.63, 71.37, 71.29, 70.48, 70.47, 67.90, 40.69, 39.93, 31.82, 31.36, 30.43, 30.14, 29.79, 29.58, 28.92, 25.73, 23.75, 21.03, 19.15, 19.13, 19.11, 17.78, 17.76, 17.70.

Dendrimer 6. See procedure **1a**: dendrimer **2b** (100 g, 0.1 mmol), benzyl 4-isocyanobutanoate (**S2**, 130 mg, 0.65 mmol), and benzyl 4-oxobutanoate (**S4**, 120 mg, 0.65 mmol) in THF. The product was purified by column chromatography (SiO_2, *n*-hexane/ethyl acetate 100:0 to ethyl acetate/methanol 50:50). Colorless oil. Yield: 262 mg (78 %). MS (MALDI-TOF) of $C_{179}H_{210}N_{10}O_{54}$ $[M+Na]^+$ calc. 3386.398, obs. 3386.654; ^{1}H NMR (400 MHz, acetone-D_6) δ 7.44 - 7.22 (m, 60H), 5.13 - 5.04 (m, 34H), 3.32 - 3.17 (m, 20H), 2.54 - 2.36 (m, 46H), 2.22 - 2.08 (m, 20H), 1.86 - 1.75 (m, 20H); ^{13}C NMR (101 MHz, acetone-D_6) δ 173.40, 173.36, 172.95, 172.93, 172.90, 172.86, 172.43, 170.73, 170.68, 170.04, 169.96, 137.51, 137.49, 137.38, 137.36, 129.27, 129.27, 129.25, 128.88, 128.80, 128.75, 128.74, 73.61, 69.15, 66.60, 39.08, 39.02, 38.54, 31.89, 31.88, 31.60, 31.40, 31.31, 31.21, 31.02, 28.96, 28.77, 28.58, 27.96, 25.51.

Dendrimer 7. See procedure **1a**: dendrimer **2b** (100 mg, 0.1 mmol), methyl 4-isocyanobutanoate (**S6**, 82 mg, 0.65 mmol), and benzyl 4-oxobutanoate (**S4**, 120 mg, 0.65 mmol) in THF. The product was purified by column chromatography (SiO_2, *n*-hexane/ethyl acetate 100:0 to ethyl acetate/methanol 50:50). Colorless oil. Yield: 174 mg (60 %). MS (MALDI-TOF) of $C_{143}H_{186}N_{10}O_{54}$ $[M+Na]^+$ calc. 2930.200, obs. 2930.443; ^{1}H NMR (400 MHz, MeOD) δ 7.39 - 7.24 (m, 30H), 5.13 - 5.09 (m, 12H), 5.06 - 4.86 (m, 10H), 3.63 (s, 18H), 3.26 - 3.18 (m, 20H), 2.57 - 2.31 (m, 54H), 2.16 - 2.06 (m, 12H), 1.83 - 1.77 (m, 20H); ^{13}C NMR (101 MHz, MeOD) δ 175.30, 175.27, 173.93, 173.92, 173.84, 173.32, 172.56, 172.47, 172.28, 172.22, 137.53, 129.58, 129.58, 129.55,

129.28, 129.25, 74.29, 74.26, 74.24, 74.19, 74.17, 67.51, 67.43, 67.43, 67.38, 67.36, 67.27, 52.18, 52.14, 52.12, 52.08, 52.06, 52.06, 39.85, 39.84, 39.81, 39.58, 39.53, 39.50, 39.47, 39.22, 39.18, 38.17, 32.10, 32.03, 31.93, 31.71, 30.96, 30.90, 30.78, 30.70, 30.68, 30.68, 30.65, 30.55, 28.34, 28.31, 28.25, 28.24, 28.21, 28.20, 28.16, 27.10, 27.06, 27.05, 25.77, 25.75, 25.73, 25.72, 25.68, 25.66, 25.58, 25.57, 25.56, 25.54, 25.51, 25.47, 25.33, 25.28, 25.26, 25.26, 24.95, 24.94, 24.93, 24.92.

Dendrimer 8. See procedure **1a**: dendrimer **2b** (100 g, 0.1 mmol), t*ert*-butyl (2-isocyanoethyl) carbamate (**S9**, 110 mg, 0.65 mmol), and isobutyraldehyde (47 mg, 0.65 mmol) in THF. The product was purified by column chromatography (SiO_2, ethyl acetate/methanol 100:0 to ethyl acetate/methanol 70:30). Colorless oil. Yield: 80 g (33 %). MS (MALDI-TOF) of $C_{113}H_{192}N_{16}O_{42}$ $[M+Na]^+$ calc. 2468.327, obs. 2468.118; ^{1}H NMR (400 MHz, MeOD) δ 5.06 - 4.88 (m, 7H), 4.82 - 4.81 (m, 3H), 3.29 - 3.24 (m, 18H), 3.18 - 3.14 (m, 14H), 2.63 - 2.35 (m, 28H), 2.19 - 2.14 (m, 8H), 1.85 (q, *J* = 6.1, 5.4 Hz, 8H), 1.43 (s, 54H), 1.02 - 0.92 (m, 36H); ^{13}C NMR (101 MHz, MeOD) δ 174.21, 173.57, 172.54, 172.36, 164.09, 80.19, 79.60, 79.48, 40.83, 40.41, 39.05, 31.79, 30.47, 28.81, 28.75, 28.71, 25.64, 19.24, 19.16, 17.66.

Dendrimer 9. See procedure **1a**: dendrimer **2b** (100 mg, 0.1 mmol), *tert*-butyl (2-(2-(2-isocyanoethoxy)ethoxy)ethyl)carbamate (**S11**, 168 mg, 0.65 mmol), and isobutyraldehyde (47 mg, 0.65 mmol) in THF. The product was purified by column chromatography (SiO_2, ethyl acetate/methanol 100:0 to ethyl acetate/methanol 80:20). Colorless oil. Yield: 157 mg (53 %). MS (MALDI-TOF) of $C_{137}H_{240}N_{16}O_{54}$ $[M+Na]^+$ calc. 2996.641, obs. 2996.450; ^{1}H NMR (400 MHz, MeOD) δ 5.08 - 4.97 (m, 5H), 4.82 - 4.80 (m, 5H), 3.60 - 3.49 (m, 58H), 3.41 - 3.39 (m, 8H), 3.24 - 3.20 (m, 18H), 2.61 - 2.46 (m, 22H), 2.19 - 2.13 (m, 10H), 1.88 - 1.82 (m, 8H), 1.43 (s, 54H), 1.00 - 0.94 (m, 36H); ^{13}C NMR (101 MHz, MeOD) δ 176.86, 176.83, 174.20, 174.17, 174.16, 174.13, 174.08, 173.53, 173.50, 172.96, 172.91, 172.90, 172.32, 172.30, 172.27, 172.25, 172.17, 172.13, 172.12, 172.11, 158.43, 158.40, 158.40, 158.38, 158.36, 158.35, 80.09, 79.53, 77.05, 71.29, 71.08, 70.59, 70.44, 41.26, 40.07, 39.69, 32.92, 31.83, 30.54, 28.81, 28.31, 27.10, 19.61, 19.22, 17.79.

Dendrimer 10a. See procedure **1a**: mono-*tert*-butyl succinate (696 mg, 4.0 mmol), benzyl 4-isocyanobutanoate (**S2**, 760 mg, 4.0 mmol), and benzyl 4-oxobutanoate (**S4**, 800 mg, 4.0 mmol) in CH_2Cl_2. The product was purified by column chromatography (SiO_2, *n*-hexane/ethyl acetate 60:40). Colorless oil. Yield: 1.91 g (84 %). MS (ESI) of $C_{31}H_{39}NO_9$

$[M+Na]^+$ calc. 592.2, obs. 592.3; HRMS (ESI) of $C_{31}H_{39}NO_9$ $[M+H]^+$ calc. 570.26, obs. 560.27; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (d, *J* = 4.9 Hz, 10H), 5.24 (dd, *J* = 7.3, 4.5 Hz, 1H), 5.11 (d, *J* = 1.6 Hz, 4H), 3.30 (dp, *J* = 19.5, 6.6 Hz, 2H), 2.70 - 2.59 (m, 1H), 2.58 - 2.38 (m, 7H), 2.35 - 2.14 (m, 2H), 1.89 (q, *J* = 7.1 Hz, 2H), 1.41 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.94, 172.37, 172.27, 171.35, 169.12, 135.83, 135.77, 128.50, 128.48, 128.18, 128.15, 128.11, 81.46, 72.93, 66.32, 66.24, 38.68, 31.61, 30.33, 29.92, 29.26, 27.97, 26.85, 24.40.

Dendrimer 10b. See procedure **1b**: dendrimer **10a** (110 mg, 0.19 mmol), $Pd(OH)_2$/C (10 mg) in THF. The product was obtained as a colorless oil. Yield: 73 mg (99 %). MS (ESI) of $C_{17}H_{28}NO_9$ $[M+Na]^+$ calc. 412.1, obs. 412.6; HRMS (ESI) of $C_{17}H_{28}NO_9$ $[M+H]^+$ calc. 390.16, obs. 390.18; ^{1}H NMR (400 MHz, MeOD) δ 5.04 (dd, *J* = 7.9, 4.7 Hz, 1H), 3.25 (t, *J* = 6.9 Hz, 2H), 2.66 (dt, *J* = 6.5, 5.7 Hz, 2H), 2.60 - 2.55 (m, 2H), 2.42 - 2.37 (m, 2H), 2.33 (t, *J* = 7.4 Hz, 2H), 1.88 - 1.75 (m, 4H), 1.44 (s, 9H); ^{13}C NMR (101 MHz, MeOD) δ 176.81, 176.11, 173.66, 173.33, 172.23, 82.10, 74.37, 39.63, 32.09, 31.09, 30.44, 29.97, 28.32, 26.47, 25.61.

Dendrimer 10c. See procedure **4b**: dendrimer **10a** (1.79 g, 3.21 mmol) and 48 mL of a CH_2Cl_2/TFA (1:1) solution. The product was obtained as a colorless oil. Yield: 1.50 g (91 %). MS (ESI) of $C_{27}H_{31}NO_9$ $[M+H]^+$ calc. 514.1, obs. 514.1; HRMS (ESI) of $C_{27}H_{31}NO_9$ $[M+H]^+$ calc. 514.19, obs. 514.21; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 - 7.29 (m, 10H), 5.30 (dd, *J* = 7.6, 4.2 Hz, 1H), 5.16 - 5.08 (m, 4H), 3.63 (q, *J* = 7.1 Hz, 2H), 3.41 - 3.31 (m, 1H), 3.29 - 3.18 (m, 1H), 2.83 - 2.14 (m, 10H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.56, 175.00, 172.74, 171.30, 171.14, 135.44, 128.64, 128.58, 128.53, 128.51, 128.38, 128.29, 128.22, 72.72, 67.19, 66.82, 39.22, 31.83, 29.81, 28.88, 28.81, 26.79, 23.77.

Dendrimer 11a. See procedure **1a**: dendrimer **10c** (100 mg, 0.19 mmol), 1-isocyanooctane (**S7**, 40 mg, 0.29 mmol), and 1-octanal (37 mg, 0.29 mmol) in CH_2Cl_2. The product was purified by column chromatography (SiO_2, *n*-hexane/ethyl acetate 60:40 to *n*-hexane/ethyl acetate 0:100). Colorless oil. Yield: 112 mg (74 %). MS (ESI) of $C_{44}H_{64}N_2O_{10}$ $[M+Na]^+$ calc. 803.4, obs. 803.4; HRMS (ESI) of $C_{44}H_{64}N2O_{10}$ $[M+H]^+$ calc. 781.45, obs. 781.47; ^{1}H NMR (400 MHz, MeOD) δ 7.40 - 7.23 (m, 10H), 5.12 - 5.09 (m, 4H), 5.05 - 4.93 (m, 2H), 3.25 - 3.14 (m, 4H), 2.77 - 2.67 (m, 4H), 2.51 - 2.44 (m, 2H), 2.41 - 2.36 (m, 2H), 2.29 - 2.07 (m, 4H), 1.84 - 1.78 (m, 2H), 1.50 - 1.47 (m, 2H), 1.30 (s, 20H), 0.91 - 0.87 (m, 6H); ^{13}C NMR (101 MHz, MeOD) δ 174.63, 173.61, 173.35, 173.34, 172.56, 172.02, 137.53, 129.55, 129.53, 129.25, 129.23, 129.15, 75.59, 74.45, 67.43,

67.29, 40.20, 39.52, 32.98, 32.89, 32.22, 30.60, 30.40, 30.37, 30.34, 30.22, 30.10, 29.78, 28.17, 27.92, 26.15, 25.99, 25.52, 23.72, 23.68, 23.65, 14.44, 14.42.

Dendrimer 11b. See procedure **1b**: dendrimer **11a** (87 mg, 0.11 mmol), $Pd(OH)_2/C$ (2 mg) in THF. The product was obtained as a colorless oil. Yield: 67 mg (99 %). MS (ESI) of $C_{30}H_{52}N_2O_{10}$ $[M+Na]^+$ calc. 623.2, obs. 623,3; HRMS (ESI) of $C_{30}H_{52}N_2O_{10}$ $[M+H]^+$ calc. 601.3702, obs. 601.3686; ^{1}H NMR (400 MHz, MeOD) δ 5.07 - 4.93 (m, 2H), 3.27 - 3.15 (m, 4H), 2.85 - 2.71 (m, 4H), 2.44 - 2.25 (m, 4H), 2.22 - 1.97 (m, 2H), 1.85 - 1.74 (m, 4H), 1.54 - 1.45 (m, 2H), 1.30 (q, *J* = 5.7, 4.2 Hz, 20H), 0.95 - 0.85 (m, 6H); ^{13}C NMR (101 MHz, MeOD) δ 177.65, 177.13, 173.62, 173.43, 172.62, 172.20, 75.54, 74.71, 40.20, 39.80, 33.04, 32.98, 32.90, 31.32, 30.39, 30.39, 30.37, 30.33, 30.30, 30.22, 29.80, 28.72, 27.92, 25.98, 25.96, 25.81, 23.72, 23.68, 14.43, 14.42.

Dendrimer 12a. See procedure **1a**: dendrimer **10c** (100 mg, 0.19 mmol), *tert*-butyl (2-(2-(2-isocyanoethoxy)ethoxy)ethyl)carbamate (**S11**, 51 mg, 0.19 mmol), and isobutyraldehyde (14 mg, 0.19 mmol) in CH_2Cl_2. The product was purified by column chromatography (SiO_2, *n*-hexane/ethyl acetate 60:40 to *n*-hexane/ethyl acetate 0:100). Colorless oil. Yield: 104 mg (64 %). MS (ESI) of $C_{43}H_{61}N_3O_{14}$ $[M+Na]^+$ calc. 866.3, obs. 866.2; HRMS (ESI) of $C_{43}H_{61}N_3O_{14}$ $[M+H]^+$ calc. 844.41, obs. 844.42; ^{1}H NMR (400 MHz, MeOD) δ 7.36 - 7.27 (m, 10H), 5.11 (s, 4H), 5.05 - 5.00 (m, 2H), 3.57 (s, 4H), 3.53 - 3.47 (m, 4H), 3.42 - 3.35 (m, 2H), 3.25 - 3.19 (m, 4H), 2.82 - 2.65 (m, 5H), 2.49 - 2.44 (m, 2H), 2.39 (t, *J*= 7.3 Hz, 2H), 2.19 - 2.10 (m, 2H), 1.82 (q, *J* = 7.1 Hz, 2H), 1.43 (s, 9H), 0.97 - 0.93 (m, 6H); ^{13}C NMR (101 MHz, MeOD) δ 174.57, 173.86, 173.62, 173.56, 173.28, 172.01, 158.37, 137.62, 137.52, 129.55, 129.53, 129.25, 129.24, 129.23, 129.21, 129.20, 129.17, 129.15, 129.15, 80.07, 79.74, 74.43, 71.25, 71.24, 71.03, 70.39, 67.41, 67.27, 41.21, 40.05, 39.50, 32.20, 31.77, 30.61, 30.58, 29.74, 28.77, 28.16, 25.52, 19.11, 17.62.

Dendrimer 12b. See procedure **1b**: dendrimer **12a** (50 mg, 0.06 mmol), $Pd(OH)_2/C$ (2 mg) in THF. The product was obtained as a colorless oil. Yield: 39 mg (99 %). MS (ESI) of $C_{29}H_{49}N_3O_{14}$ $[M+Na]^+$ calc. 686.3, obs. 686.2; HRMS (ESI) of $C_{29}H_{49}N_3O_{14}$ $[M+H]^+$ calc. 664.32, obs. 664.33; ^{1}H NMR (400 MHz, MeOD) δ 5.06 - 4.97 (m, 2H), 3.60 (s, 4H), 3.56 - 3.49 (m, 4H), 3.41 - 3.37 (m, 2H), 3.26 - 3.20 (m, 4H), 2.86 - 2.72 (m, 5H), 2.36 - 2.26 (m, 4H), 2.19 - 2.04 (m, 2H), 1.80 (q, *J* = 7.1 Hz, 2H), 1.43 (s, 9H), 1.00 - 0.94 (m, 6H); ^{13}C NMR (101 MHz, MeOD) δ 178.59, 178.27, 173.58, 173.43, 172.31, 172.17, 158.45,

80.08, 79.74, 74.94, 71.28, 71.27, 71.07, 70.40, 41.23, 40.04, 39.94, 33.61, 32.24, 31.79, 29.80, 29.76, 29.13, 28.76, 26.08, 19.10, 17.62.

Dendrimer 13a. See procedure **1a**: dendrimer **11b** (23 mg, 0.03 mmol), *tert*-butyl (2-isocyanoethyl)carbamate (**S9**, 14 mg, 0.08 mmol), and isobutyraldehyde (6 mg, 0.08 mmol) in CH_2Cl_2. The product was purified by column chromatography (SiO_2, *n*-hexane/ethyl acetate 60:40 to *n*-hexane/ethyl acetate 0:100). Colorless oil. Yield: 15 mg (42 %). MS (ESI) of $C_{54}H_{96}N_6O_{16}$ $[M+Na]^+$ calc. 1107.5, obs. 1107.6; HRMS (ESI) of $C_{54}H_{96}N_6O_{16}$ $[M+H]^+$ calc. 1085.6963, obs. 1085.6944; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.20 - 5.01 (m, 4H), 3.44 - 3.24 (m, 12H), 2.80 - 2.68 (m, 4H), 2.58 - 2.42 (m, 4H), 2.32 - 2.21 (m, 4H), 1.95 - 1.79 (m, 4H), 1.50 - 1.47 (m, 2H), 1.44 (s, 18H), 1.32 - 1.24 (m, 20H), 0.98 - 0.90 (m, 12H), 0.90 - 0.85 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.92, 172.61, 172.38, 172.29, 172.14, 171.60, 171.45, 171.42, 158.49, 157.20, 79.57, 79.52, 74.98, 74.92, 73.24, 73.08, 40.44, 40.25, 40.19, 40.06, 39.53, 39.33, 31.77, 31.71, 30.52, 30.48, 30.44, 29.68, 29.51, 29.46, 29.31, 29.21, 29.16, 29.07, 28.90, 28.37, 28.32, 26.86, 24.90, 22.62, 22.59, 18.84, 18.80, 16.86, 16.83, 16.80, 16.79, 14.07, 14.05.

Dendrimer 13b. See procedure **4b**: dendrimer **13a** (10 mg, 9 µmol) and 500 µL of a CH_2Cl_2/TFA (1:1) solution. The product was obtained as a colorless oil. Yield: 8 mg (97 %). MS (ESI) of $C_{44}H_{80}N_6O_{12}$ $[M+H]^+$ calc. 885.5, obs. 885.4; HRMS (ESI) of $C_{44}H_{80}N_6O_{12}$ $[M+H]^+$ calc. 885.5914, obs. 885.5899; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.07 - 4.76 (m, 4H), 3.70 - 3.51 (m, 4H), 3.25 - 3.14 (m, 4H), 2.81 - 1.75 (m, 20H), 1.52 - 1.45 (m, 2H), 1.31 - 1.24 (m, 20H), 1.02 - 0.76 (m, 18H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.26, 172.97, 172.51, 171.99, 171.40, 171.22, 171.11, 170.62, 79.26, 78.92, 73.83, 73.56, 40.27, 39.49, 39.38, 39.35, 39.27, 39.19, 31.75, 31.69, 30.31, 29.68, 29.64, 29.59, 29.51, 29.39, 29.35, 29.33, 29.19, 29.10, 29.04, 28.91, 26.82, 24.80, 22.60, 22.57, 18.61, 18.45, 17.05, 17.03, 16.85, 16.83, 14.04, 14.02.

Dendrimer 14. See procedure **1a**: dendrimer **11b** (23 mg, 0.03 mmol), *tert*-butyl isocyanide (7 mg, 0.08 mmol), and phenylacetaldehyde (10 mg, 0.08 mmol) in CH_2Cl_2. The product was purified by column chromatography (SiO_2, *n*-hexane/ethyl acetate 60:40 to *n*-hexane/ethyl acetate 0:100). Colorless oil. Yield: 17 mg (51 %). MS (ESI) of $C_{56}H_{86}N_4O_{12}$ $[M+Na]^+$ calc. 1029.5, obs. 1029.4; HRMS (ESI) of $C_{56}H_{86}N_4O_{12}$ $[M+H]^+$ calc. 1007.6322, obs. 1007.6293; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 - 7.16 (m, 10H), 5.25 - 4.98 (m, 4H), 3.37 - 3.05 (m, 8H), 2.85 - 2.62 (m, 4H), 2.50 - 2.28 (m, 4H), 2.24 - 2.10 (m, 4H), 1.85 - 1.76 (m, 2H), 1.50 - 1.46 (m, 2H), 1.44 - 0.94 (m, 38H), 0.91 - 0.83 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.11, 172.40, 172.28, 171.34, 171.09, 170.05,

169.28, 167.91, 136.42, 135.92, 129.71, 129.66, 129.63, 128.43, 128.39, 128.37, 128.33, 126.93, 126.92, 126.90, 79.54, 79.22, 74.99, 74.80, 51.37, 51.29, 39.31, 39.26, 37.86, 37.81, 31.78, 31.72, 30.91, 29.55, 29.47, 29.26, 29.22, 29.21, 29.18, 29.07, 28.52, 28.50, 28.44, 26.89, 26.85, 24.88, 24.18, 22.63, 22.59, 14.08, 14.05.

Dendrimer 15. See procedure **1a**: dendrimer **11b** (20 mg, 0.03 mmol), 6-(dimethyl amino)-2-(4-isocyanobutyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**S15**, 19 mg, 0.06 mmol), and isobutyraldehyde (4 mg, 0.06 mmol) in CH_2Cl_2. The product was purified by column chromatography (SiO_2, *n*-hexane/ethyl acetate 50:50 to *n*-hexane/ethyl acetate 0:100). Orange oil. Yield: 27 mg (69 %). MS (ESI) of $C_{76}H_{106}N_8O_{16}$ $[M+Na]^+$ calc. 1409.6, obs. 1409.8; HRMS (ESI) of $C_{76}H_{106}N_8O_{16}$ $[M+H]^+$ calc. 1387.7807, obs. 1387.7787; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 - 8.49 (m, 2H), 8.46 - 8.39 (m, 4H), 7.68 - 7.61 (m, 2H), 7.11 - 7.07 (m, 2H), 5.17 - 4.99 (m, 4H), 4.18 - 4.11 (m, 4H), 3.43 - 3.20 (m, 8H), 3.10 (s, 12H), 2.83 - 2.15 (m, 16H), 1.77 - 1.61 (m, 10H), 1.31 - 1.19 (m, 20H), 0.96 - 0.83 (m, 18H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.69, 171.60, 171.32, 169.60, 169.46, 169.41, 169.31, 169.10, 164.57, 164.55, 164.02, 164.01, 157.02, 156.99, 132.62, 132.60, 131.27, 131.26, 130.99, 130.97, 130.21, 130.20, 125.20, 125.20, 124.87, 124.84, 122.93, 122.90, 114.76, 114.70, 113.29, 113.26, 78.32, 78.31, 74.87, 73.20, 44.75, 39.48, 39.42, 39.32, 38.86, 38.80, 38.77, 32.00, 31.94, 31.75, 31.71, 31.29, 30.55, 30.52, 29.67, 29.50, 29.46, 29.21, 29.21, 29.10, 29.07, 26.85, 26.69, 26.59, 26.56, 25.51, 25.48, 24.88, 24.38, 22.60, 22.57, 18.87, 18.85, 18.83, 18.80, 14.06, 14.04.

Dendrimer 16a. See procedure **1a**: dendrimer **12b** (20 mg, 0.03 mmol), 1-isocyanooctane (**S7**, 10 mg, 0.08 mmol), and 1-octanal (9 mg, 0.08 mmol) in CH_2Cl_2. The product was purified by column chromatography (SiO_2, *n*-hexane/ethyl acetate 60:40 to *n*-hexane/ethyl acetate 0:100). Colorless oil. Yield: 18 mg (50 %). MS (ESI) of $C_{63}H_{115}N_5O_{16}$ $[M+Na]^+$ calc. 1220.8, obs. 1220.8; HRMS (ESI) of $C_{63}H_{115}N_5O_{16}$ $[M+H]^+$ calc. 1198.83, obs. 1198.85; ^{1}H NMR (400 MHz, MeOD) δ 5.09 - 4.90 (m, 4H), 3.60 (s, 4H), 3.56 - 3.49 (m, 4H), 3.42 - 3.37 (m, 2H), 3.27 - 3.15 (m, 8H), 2.85 - 2.70 (m, 5H), 2.57 - 2.41 (m, 4H), 2.24 - 2.06 (m, 4H), 1.81 - 1.75 (m, 4H), 1.52 - 1.47 (m, 4H), 1.43 (s, 9H), 1.37 - 1.25 (m, 40H), 0.98 (dd, *J* = 6.9, 3.7 Hz, 6H), 0.90 (t, *J* = 6.5 Hz, 12H); ^{13}C NMR (101 MHz, MeOD) δ 174.08, 174.06, 173.61, 173.59, 173.38, 173.35, 173.28, 172.80, 172.05, 80.09, 79.80, 75.38, 75.29, 74.37, 71.30, 71.08, 70.44, 70.43, 41.25, 40.21, 40.10, 39.93, 39.30, 33.13, 33.04, 32.99, 32.92, 31.83, 30.46, 30.40, 30.34, 30.25, 29.84, 28.79, 27.95, 26.07, 26.06, 26.04, 25.98, 25.64, 25.63, 23.74, 23.74, 23.71, 23.70, 19.17, 17.68, 14.48, 14.46, 14.45, 14.41.

Dendrimer 16b. See procedure **4b**: dendrimer **16a** (12 mg, 0.01 mmol) and 500 µL of a CH_2Cl_2/TFA (1:1) solution. The product was obtained as a colorless oil. Yield: 10 mg (90 %). MS (ESI) of $C_{58}H_{107}N_5O_{14}$ $[M+H]^+$ calc. 1098.7, obs. 1098.8; HRMS (ESI) of $C_{58}H_{107}N_5O_{14}$ $[M+H]^+$ calc. 1098.78, obs. 1098.80; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.18 - 4.78 (m, 4H), 3.86 - 3.12 (m, 18H), 2.79 - 2.03 (m, 13H), 1.88 - 1.77 (m, 4H), 1.57 - 1.45 (m, 4H), 1.39 - 1.18 (m, 40H), 0.99 - 0.92 (m, 6H), 0.91 - 0.82 (m, 12H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.75, 171.98, 171.88, 171.83, 171.20, 170.12, 170.03, 169.89, 77.19, 74.39, 70.09, 70.03, 69.63, 66.79, 66.53, 66.47, 39.72, 39.43, 39.11, 38.73, 38.54, 32.06, 32.00, 31.91, 31.77, 31.72, 30.40, 30.33, 30.30, 30.26, 29.68, 29.64, 29.57, 29.46, 29.45, 29.43, 29.34, 29.31, 29.23, 29.21, 29.15, 29.07, 26.90, 26.88, 26.86, 24.95, 24.92, 24.89, 22.62, 22.62, 22.58, 22.58, 18.52, 17.06, 14.10, 14.07, 14.06, 14.04.

Dendrimer 17a. See procedure **1a**: dendrimer **12b** (20 mg, 0.03 mmol), *tert*-butyl isocyanide (7 mg, 0.08 mmol), and phenylacetaldehyde (9 mg, 0.08 mmol) in CH_2Cl_2. The product was purified by column chromatography (SiO_2, *n*-hexane/ethyl acetate 60:40 to *n*-hexane/ethyl acetate 0:100). Colorless oil. Yield: 23 mg (72 %). MS (ESI) of $C_{55}H_{83}N_5O_{16}$ $[M+Na]^+$ calc. 1092.4, obs. 1092.6; HRMS (ESI) of $C_{55}H_{83}N_5O_{16}$ $[M+H]^+$ calc. 1070.5915, obs. 1070.5899; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 - 7.27 (m, 2H), 7.26 - 7.16 (m, 8H), 5.23 - 5.17 (m, 2H), 5.10 - 5.00 (m, 2H), 3.60 - 3.52 (m, 10H), 3.35 - 3.26 (m, 4H), 3.17 - 3.10 (m, 4H), 2.80 - 2.63 (m, 5H), 2.46 - 2.34 (m, 4H), 2.18 - 2.12 (m, 2H), 1.81 - 1.74 (m, 2H), 1.44 (s, 9H), 1.25 (s, 18H), 0.97 - 0.94 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.09, 171.36, 171.31, 171.11, 169.05, 169.02, 167.94, 167.89, 155.99, 135.97, 135.94, 129.68, 129.66, 128.36, 128.33, 126.91, 126.89, 126.87, 126.87, 126.17, 126.14, 79.30, 78.89, 74.79, 74.64, 73.02, 70.17, 70.16, 69.74, 69.59, 51.34, 51.27, 40.31, 38.93, 38.42, 37.83, 37.81, 31.38, 30.49, 29.81, 29.13, 28.52, 28.40, 26.87, 24.32, 18.75, 17.06, 16.91.

Dendrimer 17b. See procedure **4b**: dendrimer **17a** (14 mg, 0.01 mmol) and 500 µL of a CH_2Cl_2/TFA (1:1) solution. The product was obtained as a colorless oil. Yield: 9 mg (95 %). MS (ESI) of $C_{50}H_{75}N_5O_{14}$ $[M+H]^+$ calc. 970.5, obs. 970.6; HRMS (ESI) of $C_{50}H_{75}N_5O_{14}$ $[M+H]^+$ calc. 970.5391, obs. 970.5372; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 - 7.26 (m, 5H), 7.26 - 7.15 (m, 5H), 5.23 - 5.00 (m, 4H), 3.79 - 3.09 (m, 18H), 2.74 - 2.12 (m, 13H), 1.24 (s, 18H), 0.95 - 0.86 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.95, 172.75, 172.36, 171.82, 171.61, 170.29, 169.59, 168.33, 135.74, 135.67, 129.64, 129.61, 128.45, 128.40, 127.06, 127.04, 127.01, 126.98, 125.49, 125.48, 79.29, 74.94, 74.74, 72.83, 70.00, 69.65, 66.80, 66.55, 51.64, 51.43, 39.73, 39.04, 38.72, 38.44, 37.90, 31.91, 30.90, 30.30, 29.68, 28.90, 28.42, 24.46, 22.67, 18.56, 18.34.

Dendrimer 18a. See procedure **1a**: dendrimer **12b** (20 mg, 0.03 mmol), 6-(dimethylamino)-2-(4-isocyanobutyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**S15**, 10 mg, 0.06 mmol), and isobutyraldehyde (5 mg, 0.06 mmol) in CH_2Cl_2. The product was purified by column chromatography (SiO_2, ethyl acetate/methanol 100:0 to ethyl acetate/methanol 90:10). Orange oil. Yield: 30 mg (69 %). MS (ESI) of $C_{75}H_{103}N_9O_{20}$ $[M+Na]^+$ calc. 1472.6, obs. 1472.7; HRMS (ESI) of $C_{75}H_{103}N_9O_{20}$ $[M+H]^+$ calc. 1450.7399, obs. 1450.7386; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (d, *J* = 7.5 Hz, 2H), 8.42 (d, *J* = 8.3 Hz, 4H), 7.64 (t, *J* = 7.9 Hz, 2H), 7.10 (d, *J* = 8.3 Hz, 2H), 5.14 - 4.98 (m, 4H), 4.18 - 4.11 (m, 4H), 3.61 - 3.29 (m, 18H), 3.10 (s, 12H), 2.81 - 2.50 (m, 8H), 2.33 - 2.13 (m, 7H), 1.79 - 1.72 (m, 4H), 1.66 - 1.61 (m, 4H), 1.44 (s, 9H), 0.97 - 0.88 (m, 18H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.71, 171.69, 171.64, 171.49, 171.40, 169.39, 169.36, 169.30, 164.57, 164.54, 164.53, 164.02, 164.00, 163.99, 156.99, 132.62, 132.61, 131.25, 131.24, 130.98, 130.97, 130.21, 130.20, 125.23, 125.22, 124.85, 124.81, 122.96, 122.95, 114.80, 114.78, 113.29, 113.28, 78.31, 78.31, 78.26, 77.19, 73.14, 70.19, 70.18, 70.16, 70.14, 44.76, 39.51, 39.49, 39.45, 38.87, 38.82, 38.80, 33.26, 30.59, 30.56, 30.53, 30.52, 30.49, 29.68, 29.13, 29.10, 28.40, 26.75, 26.72, 26.59, 25.54, 18.87, 18.86, 18.84, 18.74, 18.73, 18.67.

Dendrimer 18b. See procedure **4b**: dendrimer **18a** (10 mg, 7 µmol) and 500 µL of a CH_2Cl_2/TFA (1:1) solution. The product was obtained as an orange oil. Yield: 9 mg (97 %). MS (ESI) of $C_{70}H_{95}N_9O_{18}$ $[M+H]^+$ calc. 1350.6, obs. 1350.7; HRMS (ESI) of $C_{70}H_{95}N_9O_{18}$ $[M+H]^+$ calc. 1350.6875, obs. 1350.6863; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 - 8.50 (m, 2H), 8.45 - 8.39 (m, 4H), 7.64 (t, *J* = 7.9 Hz, 2H), 7.10 (d, *J* = 7.9 Hz, 2H), 5.00 - 4.85 (m, 4H), 4.18 - 4.10 (m, 4H), 3.79 - 3.26 (m, 18H), 3.11 (s, 12H), 2.95 - 2.85 (m, 10H), 2.28 - 2.18 (m, 5H), 1.88 - 1.80 (m, 3H), 1.68 - 1.59 (m, 5H), 0.96 - 0.90 (m, 18H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.95, 171.75, 171.72, 171.41, 171.29, 169.76, 169.69, 169.58, 164.62, 164.44, 164.36, 164.21, 164.15, 164.10, 132.85, 132.74, 131.75, 131.43, 131.07, 130.98, 130.22, 129.93, 125.12, 125.11, 124.84, 124.82, 122.84, 122.80, 114.52, 114.48, 113.29, 113.25, 79.57, 79.46, 79.38, 73.56, 73.24, 70.32, 70.13, 69.98, 44.74, 39.49, 39.46, 39.41, 38.93, 38.61, 38.53, 38.42, 33.60, 30.60, 30.58, 30.52, 30.31, 30.26, 29.74, 29.68, 29.57, 26.68, 26.59, 26.56, 25.46, 18.99, 18.86, 18.76, 18.71, 18.54, 18.45.

Dendrimer 19a. See procedure **1a**: dendrimer **10b** (110 mg, 0.33 mmol), benzyl 4-isocyanobutanoate (**S2**, 136 mg, 0.65 mmol), and benzyl 4-oxobutanoate (**S4**, 125 mg, 0.65 mmol) in CH_2Cl_2. The product was purified by column chromatography (SiO_2, *n*-

hexane/ethyl acetate 60:40 to *n*-hexane/ethyl acetate 0:100). Colorless oil. Yield: 269 mg (70 %). MS (ESI) of $C_{63}H_{77}N_3O_{19}$ $[M+Na]^+$ calc. 1202.5, obs. 1202.6; HRMS (ESI) of $C_{63}H_{77}N_3O_{19}$ $[M+Na]^+$ calc. 1202.51, obs. 1202.51; ^{1}H NMR (400 MHz, MeOD) δ 7.35 - 7.26 (m, 20H), 5.11 - 5.08 (m, 8H), 5.06 - 4.97 (m, 3H), 3.26 - 3.16 (m, 6H), 2.64 - 2.58 (m, 2H), 2.54 - 2.43 (m, 8H), 2.40 - 2.35 (m, 6H), 2.17 - 2.04 (m, 6H), 1.84 - 1.74 (m, 6H), 1.41 (s, 9H); ^{13}C NMR (101 MHz, MeOD) δ 174.59, 173.90, 173.63, 173.56, 173.48, 173.40, 173.26, 172.95, 172.27, 172.20, 172.12, 137.64, 137.63, 137.53, 137.52, 129.56, 129.56, 129.56, 129.56, 129.54, 129.54, 129.53, 129.53, 129.26, 129.26, 129.26, 129.25, 129.23, 129.22, 129.22, 129.22, 129.16, 129.16, 129.15, 129.15, 82.09, 82.06, 74.26, 74.18, 67.43, 67.27, 67.26, 61.52, 39.51, 39.49, 39.09, 32.23, 32.20, 31.59, 31.07, 30.69, 30.28, 29.98, 28.37, 28.21, 28.06, 28.03, 25.56, 25.50, 20.84.

Dendrimer 19b. See procedure **1b**: dendrimer **19a** (150 mg, 0.13 mmol), $Pd(OH)_2$/C (15 mg) in THF. The product was obtained as a colorless oil. Yield: 102 mg (99 %). MS (ESI) of $C_{35}H_{53}N_3O_{19}$ $[M+Na]^+$ calc. 842.3, obs. 842.5; HRMS (ESI) of $C_{35}H_{53}N_3O_{19}$ $[M+H]^+$ calc. 820.32, obs. 820.34; ^{1}H NMR (400 MHz, MeOD) δ 5.10 - 4.99 (m, 3H), 3.28 - 3.22 (m, 6H), 2.58 (t, *J* = 5.5 Hz, 4H), 2.52 - 2.46 (m, 4H), 2.41 - 2.31 (m, 8H), 2.28 - 2.21 (m, 2H), 1.87 - 1.77 (m, 10H), 1.44 (s, 9H); ^{13}C NMR (101 MHz, MeOD) δ 180.78, 177.10, 176.46, 173.97, 173.67, 173.63, 173.51, 173.44, 173.35, 172.43, 172.29, 82.11, 74.45, 74.35, 74.22, 39.69, 39.37, 39.23, 33.17, 32.28, 31.10, 30.89, 30.75, 30.31, 30.00, 28.62, 28.46, 28.36, 28.28, 25.94, 25.68, 25.62, 25.50, 24.40, 23.10.

Dendrimer 20. See procedure **1a**: dendrimer **19b** (130 mg, 0.16 mmol), benzyl 4-isocyanobutanoate (**S2**, 138 mg, 0.68 mmol), and benzyl 4-oxobutanoate (**S4**, 130 mg, 0.68 mmol) in THF. The product was purified by column chromatography (SiO_2, ethyl acetate/methanol 100:0 to ethyl acetate/methanol 70:30). Colorless oil. Yield: 205 mg (54 %). MS (MALDI-TOF) of $C_{127}H_{153}N_7O_{39}$ $[M+Na]^+$ calc. 2423.020, obs. 2423.030; ^{1}H NMR (400 MHz, MeOD) δ 7.42 - 7.17 (m, 40H), 5.10 - 5.07 (m, 16H), 5.04 - 4.95 (m, 7H), 3.26 - 3.16 (m, 14H), 2.55 - 2.35 (m, 31H), 2.19 - 2.03 (m, 15H), 1.84 - 1.73 (m, 15H), 1.41 (s, 9H); ^{13}C NMR (101 MHz, MeOD) δ 174.58, 173.93, 173.30, 172.24, 137.64, 129.58, 129.55, 129.26, 129.23, 129.17, 82.11, 74.20, 67.44, 39.51, 32.22, 31.64, 31.10, 30.71, 30.31, 28.43, 28.23, 25.58.

Dendrimer 21. See procedure **1a**: dendrimer **19b** (28 mg, 0.03 mmol), benzyl 4-isocyanobutanoate (**S2**, 26 mg, 0.13 mmol), and isobutyraldehyde (10 mg, 0.13 mmol) in THF. The product was purified by column chromatography (SiO_2, ethyl acetate/methanol 100:0 to ethyl acetate/methanol 70:30). Colorless oil. Yield: 23 mg (37

%). MS (ESI) of $C_{99}H_{137}N_7O_{31}$ $[M+Na]^+$ calc. 1942.9, obs. 1943.0; MS (MALDI-TOF) of $C_{99}H_{137}N_7O_{31}$ $[M+Na]^+$ calc. 1942.924, obs. 1943.022; ^{1}H NMR (400 MHz, MeOD) δ 7.34 - 7.29 (m, 22H), 5.14 - 5.05 (m, 13H), 4.80 - 4.75 (m, 3H), 3.27 - 3.13 (m, 16H), 2.57 - 2.32 (m, 26H), 2.18 - 2.09 (m, 6H), 1.84 - 1.77 (m, 14H), 1.42 (s, 8H), 0.96 - 0.76 (m, 24H); ^{13}C NMR (101 MHz, MeOD) δ 174.56, 174.45, 174.43, 174.39, 174.21, 174.16, 174.14, 173.57, 173.55, 173.53, 172.70, 172.64, 172.62, 172.32, 172.27, 172.23, 172.15, 172.13, 172.07, 137.64, 129.54, 129.52, 129.33, 129.20, 129.16, 82.09, 79.60, 74.20, 67.27, 39.54, 39.51, 39.44, 39.41, 39.28, 39.23, 39.20, 32.96, 32.36, 32.28, 32.24, 31.77, 31.73, 31.10, 30.36, 30.03, 30.01, 29.85, 29.75, 29.01, 28.42, 28.14, 28.13, 28.12, 26.03, 25.67, 25.50, 20.13, 20.06, 20.03, 19.60, 19.20, 19.18, 19.14, 17.78, 17.78, 17.78, 17.77, 17.72, 17.71, 17.70, 17.69.

Dendrimer 22. See procedure **1a**: dendrimer **19b** (28 mg, 0.04 mmol), *n*-butyl isocyanide (11 mg, 0.13 mmol), and isobutyraldehyde (10 mg, 0.13 mmol) in THF. The product was purified by column chromatography (SiO_2, ethyl acetate/methanol 100:0 to ethyl acetate/methanol 70:30). Colorless oil. Yield: 18 mg (39 %). MS (ESI) of $C_{71}H_{121}N_7O_{23}$ $[M+Na]^+$ calc. 1462.9, obs. 1463.0; MS (MALDI-TOF) of $C_{71}H_{121}N_7O_{23}$ $[M+Na]^+$ calc. 1462.840, obs. 1462.967; ^{1}H NMR (400 MHz, MeOD) δ 5.10 - 4.98 (m, 4H), 4.80 - 4.75 (m, 3H), 3.29 - 3.14 (m, 14H), 2.68 - 2.11 (m, 26H), 1.88 - 1.80 (m, 6H), 1.53 - 1.46 (m, 8H), 1.44 (s, 9H), 1.38 - 1.30 (m, 8H), 1.00 - 0.77 (m, 36H); ^{13}C NMR (101 MHz, MeOD) δ 174.18, 174.16, 174.12, 173.92, 173.89, 173.62, 173.59, 173.54, 173.52, 173.49, 172.07, 172.05, 171.96, 171.91, 171.88, 82.10, 79.72, 79.64, 79.59, 77.04, 74.25, 74.20, 71.78, 40.04, 39.93, 39.91, 39.62, 39.32, 39.27, 39.23, 33.12, 32.91, 32.71, 32.53, 32.30, 31.82, 31.79, 31.11, 30.66, 30.55, 30.37, 30.03, 28.41, 28.35, 28.27, 28.13, 25.88, 25.75, 25.73, 25.71, 25.69, 25.58, 21.32, 21.08, 21.07, 21.05, 20.01, 19.61, 19.19, 19.17, 19.13, 17.75, 17.69, 17.68, 17.66, 14.11, 14.09, 14.07, 14.06.

Dendrimer 23a. See procedure **1a**: mono-*tert*-butyl succinate (100 mg, 0.57 mmol), allyl 4-isocyanobutanoate (**S18**, 87 mg, 0.57 mmol), and benzyl 4-oxobutanoate (**S4**, 110 mg, 0.57 mmol) in CH_2Cl_2. The product was purified by column chromatography (SiO_2, *n*-hexane/ethyl acetate 100:0 to *n*-hexane/ethyl acetate 50:50). Colorless oil. Yield: 275 mg (93 %). MS (ESI) of $C_{27}H_{37}NO_9$ $[M+Na]^+$ calc. 542.1, obs. 542.4; HRMS (ESI) of $C_{27}H_{37}NO_9$ $[M+H]^+$ calc. 520.2548, obs. 520.2531; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 - 7.29 (m, 5H), 5.95 - 5.85 (m, 1H), 5.35 - 5.20 (m, 3H), 5.11 (s, 2H), 4.57 (dt, *J* = 5.7, 1.4 Hz, 2H), 3.37 - 3.23 (m, 2H), 2.66 - 2.20 (m, 11H), 1.88 (p, *J* = 7.1 Hz, 2H), 1.43 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.83, 172.43, 172.33, 171.40, 169.16, 135.80, 132.10,

128.54, 128.23, 118.23, 81.52, 77.19, 72.98, 66.38, 65.15, 38.74, 31.59, 30.38, 29.97, 29.31, 28.03, 26.89, 24.43.

Dendrimer 23b. To a stirred solution of dendrimer **23a** (200 mg, 0.38 mmol) in CH_2Cl_2 were added $Pd(PPh_3)_4$ (11 mg, 0.04 mmol) and $PhSiH_3$ (62 mg, 2.28 mmol). The contents were stirred at room temperature for 15 minutes. After completion of the reaction, the mixture was washed with saturated aqueous $NaHCO_3$ (25 mL x 2), the solvent was removed, and the product used in the next step without further purification. Brown oil. MS (ESI) of $C_{24}H_{33}NO_9$ $[M+Na]^+$ calc. 502.1, obs. 502.2; HRMS (ESI) of $C_{24}H_{33}NO_9$ $[M+Na]^+$ calc. 502.2045, obs. 502.2048.

Dendrimer 24a. See procedure **1a**: dendrimer **23b** (150 mg, 0.31 mmol), 1-isocyanooctane (**S7**, 43 mg, 0.31 mmol), and isobutyraldehyde (22 mg, 0.31 mmol) in CH_2Cl_2. The product was purified by column chromatography (SiO_2, *n*-hexane/ethyl acetate 100:0 to *n*-hexane/ethyl acetate 50:50). Colorless oil. Yield: 148 mg (69 %). MS (ESI) of $C_{37}H_{58}N_2O_{10}$ $[M+H]^+$ calc. 691.4, obs. 691.3; HRMS (ESI) of $C_{37}H_{58}N_2O_{10}$ $[M+H]^+$ calc. 691.4171, obs. 691.4164; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 - 7.30 (m, 5H), 5.25 - 5.21 (ddd, *J* = 7.4, 5.8, 4.6 Hz, 1H), 5.12 (d, *J* = 1.6 Hz, 2H), 5.06 (t, *J* = 3.9 Hz, 1H), 3.51 - 3.14 (m, 4H), 2.73 - 2.14 (m, 13H), 1.47 (m, 2H), 1.44 (s, 9H), 1.30 - 1.22 (m, 10H), 0.95 - 0.85 (m, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.79, 172.26, 172.21, 171.49, 169.66, 169.28, 135.73, 128.57, 128.30, 128.24, 81.74, 78.32, 73.08, 66.44, 39.26, 37.90, 31.78, 30.82, 30.45, 30.39, 29.96, 29.52, 29.33, 29.21, 28.05, 26.90, 25.05, 22.62, 18.88, 16.74, 14.08.

Dendrimer 24b. See procedure **4b**: dendrimer **24a** (150 mg, 0.21 mmol) and 10 mL of a CH_2Cl_2/TFA (1:1) solution. The product was obtained as a colorless oil. Yield: 131 mg (99 %). MS (ESI) of $C_{33}H_{50}N_2O_{10}$ $[M+H]^+$ calc. 635.3, obs. 635.4; HRMS (ESI) of $C_{33}H_{50}N_2O_{10}$ $[M+H]^+$ calc. 635.3545, obs. 635.3535; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 - 7.28 (m, 5H), 5.29 - 5.21 (m, 2H), 5.14 - 5.10 (m, 2H), 3.45 - 3.16 (m, 4H), 3.01 - 2.09 (m, 13H), 1.53 - 1.43 (m, 2H), 1.31 - 1.23 (m, 10H), 1.01 - 0.83 (m, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.02, 173.64, 173.34, 172.37, 170.88, 169.45, 135.79, 128.55, 128.26, 128.24, 79.22, 78.94, 72.95, 66.38, 39.85, 38.14, 31.73, 30.59, 30.22, 30.08, 29.71, 29.42, 29.18, 29.13, 29.04, 26.75, 24.24, 23.35, 22.59, 18.49, 17.86, 14.06.

Dendrimer 25a. See procedure **1a**: dendrimer **24b** (120 mg, 0.19 mmol), *tert*-butyl (2-(2-(2-isocyanoethoxy)ethoxy)ethyl)carbamate (**S11**, 49 mg, 0.19 mmol), and isobutyraldehyde (13 mg, 0.19 mmol) in CH_2Cl_2. The product was purified by column

chromatography (SiO_2, ethyl acetate/methanol 100:0 to ethyl acetate/methanol 80:20). Colorless oil. Yield: 159 mg (87 %). MS (ESI) of $C_{49}H_{80}N_4O_{15}$ $[M+Na]^+$ calc. 987.5, obs. 987.6; HRMS (ESI) of $C_{49}H_{80}N_4O_{15}$ $[M+H]^+$ calc. 965.5700, obs. 965.5688; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 - 7.26 (m, 5H), 5.22 - 5.18 (m, 1H), 5.14 - 4.98 (m, 4H), 3.60 - 3.17 (m, 18H), 2.76 - 2.15 (m, 10H), 1.95 - 1.71 (m, 4H), 1.45 (s, 9H), 1.34 - 1.19 (m, 8H), 1.06 - 1.02 (m, 3H), 0.97 - 0.84 (m, 12H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.36, 172.29, 172.25, 172.22, 172.19, 171.50, 171.45, 169.25, 155.98, 135.70, 128.57, 128.31, 128.22, 78.96, 78.28, 77.31, 77.20, 76.99, 76.67, 76.21, 73.39, 73.31, 70.16, 69.74, 66.46, 40.45, 40.30, 39.27, 38.70, 31.76, 30.88, 30.46, 29.90, 29.85, 29.53, 29.23, 29.19, 29.14, 29.05, 28.38, 26.90, 26.85, 24.73, 22.60, 19.17, 18.85, 18.73, 18.66, 14.06.

Dendrimer 25b. See procedure **1b**: dendrimer **25a** (140 mg, 0.15 mmol) and $Pd(OH)_2/C$ (2 mg) in THF. The product was obtained as a colorless oil. Yield: 129 mg (99 %). MS (ESI) of $C_{42}H_{74}N_4O_{15}$ $[M+Na]^+$ calc. 897.5, obs. 897.5; HRMS (ESI) of $C_{42}H_{74}N_4O_{15}$ $[M+H]^+$ calc. 875.5231, obs. 875.5220; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.29 - 5.21 (m, 1H), 5.08 - 4.99 (m, 2H), 3.58 - 3.24 (m, 16H), 2.94 - 2.07 (m, 14H), 1.45 (s, 18H), 1.28 (s, 8H), 1.04 (d, *J* = 7.3 Hz, 3H), 0.99 - 0.86 (m, 12H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.69, 173.49, 172.80, 172.63, 172.26, 172.06, 171.51, 169.43, 78.30, 77.19, 76.22, 70.33, 70.24, 70.15, 70.07, 69.72, 69.56, 40.43, 39.30, 38.88, 38.71, 31.77, 30.47, 30.46, 29.52, 29.50, 29.22, 29.19, 29.14, 29.11, 28.36, 26.90, 22.60, 19.16, 18.85, 18.81, 18.74, 14.06.

Dendrimer 26a. See procedure **1a**: dendrimer **25b** (29 mg, 0.03 mmol), 6-(dimethylamino)-2-(4-isocyanobutyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**S15**, 11 mg, 0.03 mmol), and isobutyraldehyde (3 mg, 0.03 mmol) in CH_2Cl_2. The product was purified by column chromatography (SiO_2, ethyl acetate). Orange oil. Yield: 36 mg (86 %). MS (ESI) of $C_{65}H_{101}N_7O_{18}$ $[M+Na]^+$ calc. 1290.7, obs. 1290.9; HRMS (ESI) of $C_{65}H_{101}N_7O_{18}$ $[M+H]^+$ calc. 1268.7283, obs. 1268.7279; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 (d, *J* = 7.7 Hz, 1H), 8.45 (dd, *J* = 8.4, 2.6 Hz, 2H), 7.66 (dd, *J* = 8.5, 7.3 Hz, 1H), 7.12 (d, *J* = 8.3 Hz, 1H), 5.12 - 4.95 (m, 4H), 3.62 - 3.53 (m, 13H), 3.47 - 3.30 (m, 7H), 3.12 (s, 6H), 2.83 - 2.12 (m, 15H), 1.79 - 1.74 (m, 6H), 1.45 (s, 9H), 1.31 - 1.19 (m, 10H), 1.06 - 0.84 (m, 22H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.38, 172.46, 172.41, 171.67, 171.62, 171.42, 169.27, 164.60, 164.05, 157.06, 156.17, 156.02, 132.68, 131.31, 131.02, 130.24, 128.32, 125.23, 124.86, 122.94, 113.27, 78.88, 78.32, 78.25, 77.19, 76.23, 70.35, 70.23, 70.16, 69.73, 44.75, 40.44, 40.30, 39.43, 39.29, 38.85, 38.71, 31.78, 31.75, 30.51, 29.55, 29.53, 29.22, 29.18, 29.11, 29.07, 28.38, 28.36, 26.90, 26.68, 25.52, 25.48, 24.60, 24.57, 22.59, 19.17, 18.83, 18.73, 18.66, 14.06.

Dendrimer 26b. See procedure **4b**: dendrimer **26a** (15 mg, 0.01 mmol) and 780 µL of a CH_2Cl_2/TFA (1:1) solution. Orange oil. Yield: 11 mg (90 %). MS (ESI) of $C_{60}H_{93}N_7O_{16}$ $[M+H]^+$ calc. 1168.6, obs. 1169.0; HRMS (ESI) of $C_{60}H_{93}N_7O_{16}$ $[M+H]^+$ calc. 1168.6759, obs. 1168.6738; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60 - 8.54 (m, 1H), 8.46 - 8.39 (m, 2H), 8.06 (s, 4H), 7.69 - 7.61 (m, 1H), 7.15 - 7.11 (m, 1H), 4.97 - 4.81 (m, 6H), 3.78 - 3.73 (m, 4H), 3.67 - 3.65 (m, 2H), 3.62 - 3.57 (m, 6H), 3.50 - 3.38 (m, 4H), 3.23 - 3.18 (m, 4H), 3.12 (s, 6H), 2.77 - 2.08 (m, 15H), 1.86 - 1.73 (m, 4H), 1.52 - 1.43 (m, 2H), 1.29 - 1.21 (m, 10H), 0.99 - 0.84 (m, 21H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.93, 172.78, 172.72, 172.03, 170.04, 169.74, 164.69, 164.16, 161.70, 161.34, 157.17, 132.77, 131.47, 131.09, 130.25, 125.15, 124.85, 122.81, 114.48, 113.23, 79.07, 78.48, 78.36, 76.31, 70.08, 69.99, 69.92, 69.66, 66.55, 44.73, 39.75, 39.71, 39.40, 39.04, 38.90, 38.67, 31.73, 31.70, 30.90, 30.56, 30.31, 29.67, 29.43, 29.19, 29.16, 28.98, 26.88, 26.53, 25.46, 25.44, 24.39, 24.36, 22.58, 18.93, 18.70, 18.48, 17.10, 17.03, 16.95, 14.04.

Dendrimer 27a. See procedure **1a**: dendrimer **25b** (29 mg, 0.03 mmol), *tert*-butyl 3-isocyanopropanoate (**S19**, 5 mg, 0.03 mmol), and isobutyraldehyde (3 mg, 0.03 mmol) in CH_2Cl_2. The product was purified by column chromatography (SiO_2, ethyl acetate/methanol 100:0 to ethyl acetate/methanol 80:20). Colorless oil. Yield: 34 mg (95 %). MS (ESI) of $C_{54}H_{95}N_5O_{18}$ $[M+Na]^+$ calc. 1124.6, obs. 1124.4; HRMS (ESI) of $C_{54}H_{95}N_5O_{18}$ $[M+H]^+$ calc. 1102.6752, obs. 1102.6737; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.09 - 4.94 (m, 4H), 3.62 - 3.53 (m, 14H), 3.31 (m , 4H), 2.92 - 2.12 (m, 17H), 1.97 - 1.81 (m, 2H), 1.45 - 1.44 (m, 18H), 1.32 - 1.20 (m, 10H), 1.05 - 0.85 (m, 21H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.78, 172.57, 172.51, 171.78, 171.49, 171.45, 171.45, 169.83, 169.55, 169.23, 156.00, 81.36, 81.06, 78.93, 78.26, 78.15, 77.84, 77.19, 76.23, 73.09, 70.16, 69.73, 40.44, 39.28, 38.93, 38.70, 38.26, 34.90, 34.84, 31.78, 31.75, 31.12, 30.50, 29.67, 29.54, 29.22, 29.19, 29.12, 28.39, 28.37, 28.03, 26.90, 24.60, 22.60, 19.17, 18.87, 18.83, 18.73, 18.65, 16.92, 16.87, 14.06.

Dendrimer 27b. See procedure **4b**: dendrimer **27a** (15 mg, 0.01 mmol) and 780 µL of a CH_2Cl_2/TFA (1:1) solution. Colorless oil. Yield: 11 mg (92 %). MS (ESI) of $C_{45}H_{79}N_5O_{16}$ $[M+H]^+$ calc. 946.5, obs. 946.7; HRMS (ESI) of $C_{45}H_{79}N_5O_{16}$ $[M+H]^+$ calc. 946.5602, obs. 946.5588; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.72 (s, 3H), 5.19 - 4.84 (m, 4H), 3.77 - 3.74 (m, 2H), 3.67 - 3.58 (m, 8H), 3.50 - 3.41 (m, 4H), 3.30 - 3.19 (m, 4H), 2.84 - 1.83 (m, 17H), 1.46 (d, J = 25.4 Hz, 2H), 1.33 - 1.23 (m, 10H), 1.01 - 0.81 (m, 21H).); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.01, 174.89, 172.85, 172.76, 172.00, 171.97, 170.26, 169.98, 169.77, 78.99, 78.70, 78.45, 78.43, 77.31, 77.19, 76.28, 70.06, 69.93, 69.64, 39.76, 39.43, 39.08,

38.68, 38.48, 34.79, 33.66, 31.74, 31.72, 30.57, 30.30, 29.67, 29.42, 29.19, 29.16, 28.97, 26.87, 24.27, 22.59, 18.94, 18.69, 18.66, 18.55, 18.50, 16.98, 16.85, 14.04.

Dendrimer 28a. See procedure **1a**: dendrimer **25b** (29 mg, 0.03 mmol), benzyl isocyanide (4 mg, 0.03 mmol), and isobutyraldehyde (3 mg, 0.03 mmol) in CH_2Cl_2. The product was purified by column chromatography (SiO_2, ethyl acetate/methanol 100:0 to ethyl acetate/methanol 80:20). Colorless oil. Yield: 28 mg (79 %). MS (ESI) of $C_{54}H_{89}N_5O_{16}$ $[M+Na]^+$ calc. 1086.5, obs. 1086.3; HRMS (ESI) of $C_{54}H_{89}N_5O_{16}$ $[M+H]^+$ calc. 1164.6384, obs. 1164.6377; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 - 7.27 (m, 5H), 5.15 - 4.97 (m, 4H), 4.54 - 4.42 (m, 2H), 3.61 - 3.52 (m, 10H), 3.47 - 3.41 (m, 2H), 3.32 - 3.25 (m, 4H), 2.82 - 2.13 (m, 15H), 1.55 - 1.48 (m, 2H), 1.44 (s, 9H), 1.32 - 1.19 (m, 10H), 1.05 - 0.85 (m, 21H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.92, 172.81, 172.60, 172.44, 171.62, 171.39, 171.24, 169.28, 155.99, 138.10, 128.68, 128.58, 127.78, 127.70, 127.34, 78.93, 78.34, 78.25, 77.20, 76.25, 73.01, 70.37, 70.16, 69.73, 43.16, 40.45, 40.31, 39.29, 38.71, 31.92, 31.78, 31.76, 30.58, 30.51, 29.55, 29.22, 29.19, 29.10, 29.09, 28.38, 27.33, 26.90, 24.47, 22.60, 19.17, 19.16, 18.89, 18.83, 18.73, 18.66, 14.07.

Dendrimer 28b. See procedure **4b**: dendrimer **28a** (10 mg, 9 µmol) and 780 µL of a CH_2Cl_2/TFA (1:1) solution. Colorless oil. Yield: 8 mg (90 %). MS (ESI) of $C_{49}H_{81}N_5O_{14}$ $[M+Na]^+$ calc. 986.4, obs. 986.6; HRMS (ESI) of $C_{49}H_{81}N_5O_{14}$ $[M+H]^+$ calc. 964.5860, obs. 964.5851; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 - 7.27 (m, 5H), 5.21 - 4.81 (m, 4H), 4.52 - 4.38 (m, 2H), 3.74 - 3.16 (m, 16H), 2.83 - 2.12 (m, 15H), 1.49 (s, 2H), 1.30 - 1.25 (m, 10H), 1.07 - 0.82 (m, 21H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.96, 172.69, 172.18, 172.10, 171.03, 170.62, 169.61, 169.61, 157.58, 138.24, 128.71, 128.64, 128.60, 127.72, 127.41, 78.42, 77.20, 76.40, 70.15, 70.01, 69.94, 69.71, 43.20, 39.70, 39.37, 39.09, 38.66, 31.94, 31.75, 31.71, 30.91, 30.58, 30.33, 29.68, 29.47, 29.31, 29.21, 29.18, 27.21, 26.89, 24.32, 22.60, 19.14, 18.96, 18.73, 18.59, 17.10, 17.04, 14.06.

2.6 References

(1) James, T. D.; Shinmori, H.; Takeuchi, M.; Shinkai, S. *Chem. Commun*. **1996**, 0, 705-706.

(2) Lartigue, M.-L.; Slany, M.; Caminade A.-M.; Majoral, J.-P. *Chem. Eur. J*. **1996**, 2,1417-1426.

(3) Caminade, A. M.; Laurent, R.; Delavaux-Nicot, B.; Majoral, J-P. *New J. Chem*., **2012**, 36, 217-226.

(4) Saez, I. M.; Goodby, J. W. *Chem. Eur. J*. **2003**, 9, 4869-4877.

(5) Tomalia, D. A.; Baker, H.; Dewald, J.; Hall, M.; Kallos, G.; Martin, S.; Roeck, J.; Ryder, J.; Smith, P. *Polymer J.* **1985**, 17, 117-132.

(6) Tomalia, D. A.; Baker, H.; Dewald, J.; Hall, M.; Kallos, G.; Martin, S.; Roeck, J.; Ryder, J.; Smith, P. *Macromolecules* **1986**, 19, 2466-2468.

(7) Tomalia, D. A.; Naylor, A. M.; Goddard III, W. A. *Angew. Chem. Int. Ed.* **1990**, 29, 138-175; *Angew. Chem.* **1990**, 102, 119-157.

(8) Wessjohann, L. A.; Henze, M.; Kreye, O.; Rivera, D. G. DE 10 2010 018 882 A1 (03.11.**2011**), WO 2011/134607 A1 (03.11.**2011**).

(9) Wessjohann, L. A.; Neves Filho, R. A. W.; Rivera, D. G. In Isocyanide Chemistry: Applications in Synthesis and Materials Science; Nenajdenko, V., Ed.; Wiley-VCH: Weinheim, Germany, **2012**, Chapter 7, 233.

(10) Diaz-Mochon, J. J.; Fara, M. A.; Sanchez-Martin, R. M.; Bradley, M. *Tetrahedron Lett.* **2008**, 49, 923-926.

(11) Shah, N. H.; Butterfoss, G. L.; Nguyen, K.; Yoo, B.; Bonneau, R.; Rabenstein, D. L.; Kirshenbaum, K. *J. Am. Chem. Soc*. **2008**, 130, 16622-16632.

(12) Jee, J. A.; Spagnuolo, L. A.; Rudick, J. G. *Org. Lett*. **2012**, 14, 3292-3295.

(13) Kreye, O.; Kugele, D.; Faus, L.; Meier, M. A. R. *Macromol. Rapid Commun*. **2014**, 35, 317-322.

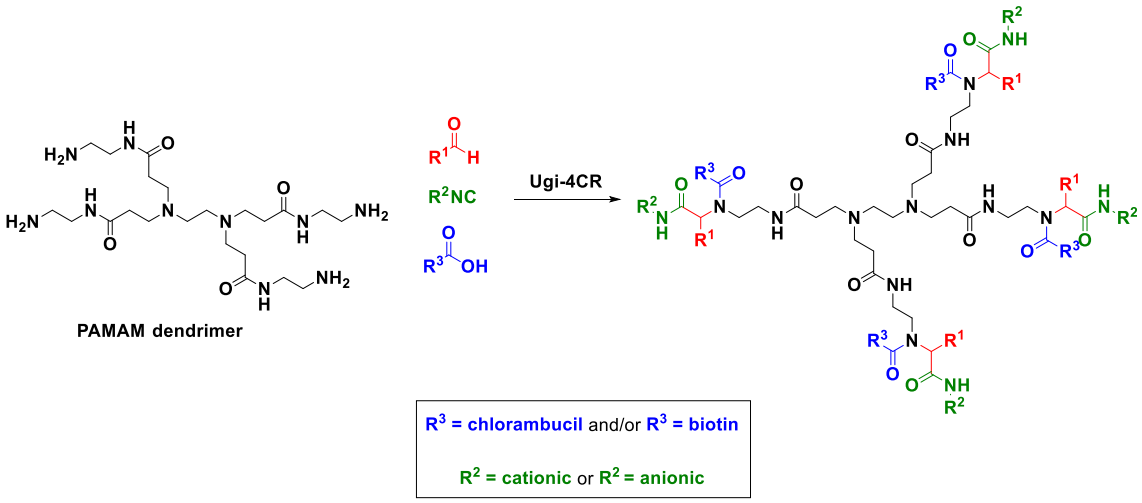
(14) Du, Z.; Lu, Y.; Dai, X.; Negrerie, D. Z.; Gao, Q. *J. Chem. Res*. **2013**, 181-185.

(15) Deng, X.; Du, F.; Li, Z. *ACS Macro Lett*. **2014**, 2, 667-670.

Chapter 3

Functionalization of PAMAM-NH_2 dendrimer with chlorambucil by Ugi reactions: a new approach to improve anticancer activity and selectivity

Abstract



A new synthetic strategy to improve water solubility of the anticancer drug chlorambucil and to enhance its efficacy as chemotherapeutic agent is described. The surface functionalization of PAMAM-NH_2 dendrimer with chlorambucil and with -NH_2 or -COOH groups on the surface, or with the targeting compound biotin was achieved by Ugi multicomponent reaction. The synthesized dendrimers were evaluated for their cytotoxic activity against PC-3 prostate and HT-29 colon cancer cell lines as well as normal mouse NIH3T3 fibroblasts employing MTT and CV assays.

3.1 Introduction

Chlorambucil (CLB) is an alkylating agent derived from nitrogen mustard and was used as chemotherapeutic drug in the treatment of some types of cancer, mainly chronic lymphocytic leukemia, Hodgkin lymphoma, and non-Hodgkin lymphoma (FDA approved drug).[1-4] Due to severe side effects (bone marrow suppression, nausea, anemia, infertility, etc.) as result of its poor specificity towards cancer cells and low solubility in water, chlorambucil has been replaced by other chemotherapeutic medications.[5-7] Currently, research is focused on strategies to enhance water solubility, improve bioavailability, and decrease the adverse effects of anticancer drugs by using nanosized drug carriers, such as dendrimers and polymers.[8-9]

Another approach to improve specificity of anticancer drugs is the targeted delivery of chemotherapeutics with cancer cell-specific compounds.[10-12] One of the most studied compounds for targeted drug delivery is biotin, an essential macronutrient regulating several cellular functions.[13] Biotin receptors are overexpressed during fast cellular division, which normally happens during tumor proliferation.[14,15] Thus, biotinylation has been used as a promising strategy to deliver chemotherapeutics to cancer cells.[14-21]

In 2015, Singh and co-workers synthesized a star shaped 4-arm PEG containing coumarin-chlorambucil and biotin in a two-step procedure and showed that the accumulation of the macromolecule was higher in the HeLa cell line than in non-cancerous cells.[22] Furthermore, in 2016, Bielawski and co-workers synthesized a G3 PAMAM-NH_2 dendrimer-chlorambucil conjugate *via* amide linkage and reported that the conjugate was a more potent antiproliferative agent than chlorambucil using MTT assays against both MDA-MB-231 and MCF-7 breast cancer cells. They also reported that the conjugate inhibits the proliferation with an increase of apoptotic and necrotic cells, which was higher than caused by CLB alone.[23] Also, in 2016, Assadi and co-workers synthesized a chlorambucil conjugated with anionic linear-globular dendrimer (G2) through an ester linkage in order to reduce the insolubility of CLB in water and improve the anticancer activity *in vitro* and *in vivo*.[24]

Herein, we report a powerful new synthetic strategy to improve water solubility and enhance the efficacy of chlorambucil as anticancer drug through the conjugation with polyamidoamine (PAMAM-NH_2) dendrimer by Ugi reaction. In this study, the anticancer activity among a collection of CLB-dendrimers with -NH_2 or -COOH on the surface, or with the target compound biotin was evaluated for the cytotoxic activity against tumor PC-3 prostate and HT-29 colon cell lines and non-cancerous mouse NIH3T3 fibroblasts using MTT and CV assays.

3.2 Synthetic Strategy

The synthetic strategy for the covalent surface functionalization of PAMAM-NH_2 dendrimer is based on the versatility of the Ugi four-component reaction that allows the addition of a new generation with three different features simultaneously introduced in a one-step procedure. This brings many advantages when compared with current methods available for the synthesis of dendrimers, e.g., with anticancer compounds containing different surface groups. This normally requires at least a two-step procedure and/or previous modification of the starting materials.[22]

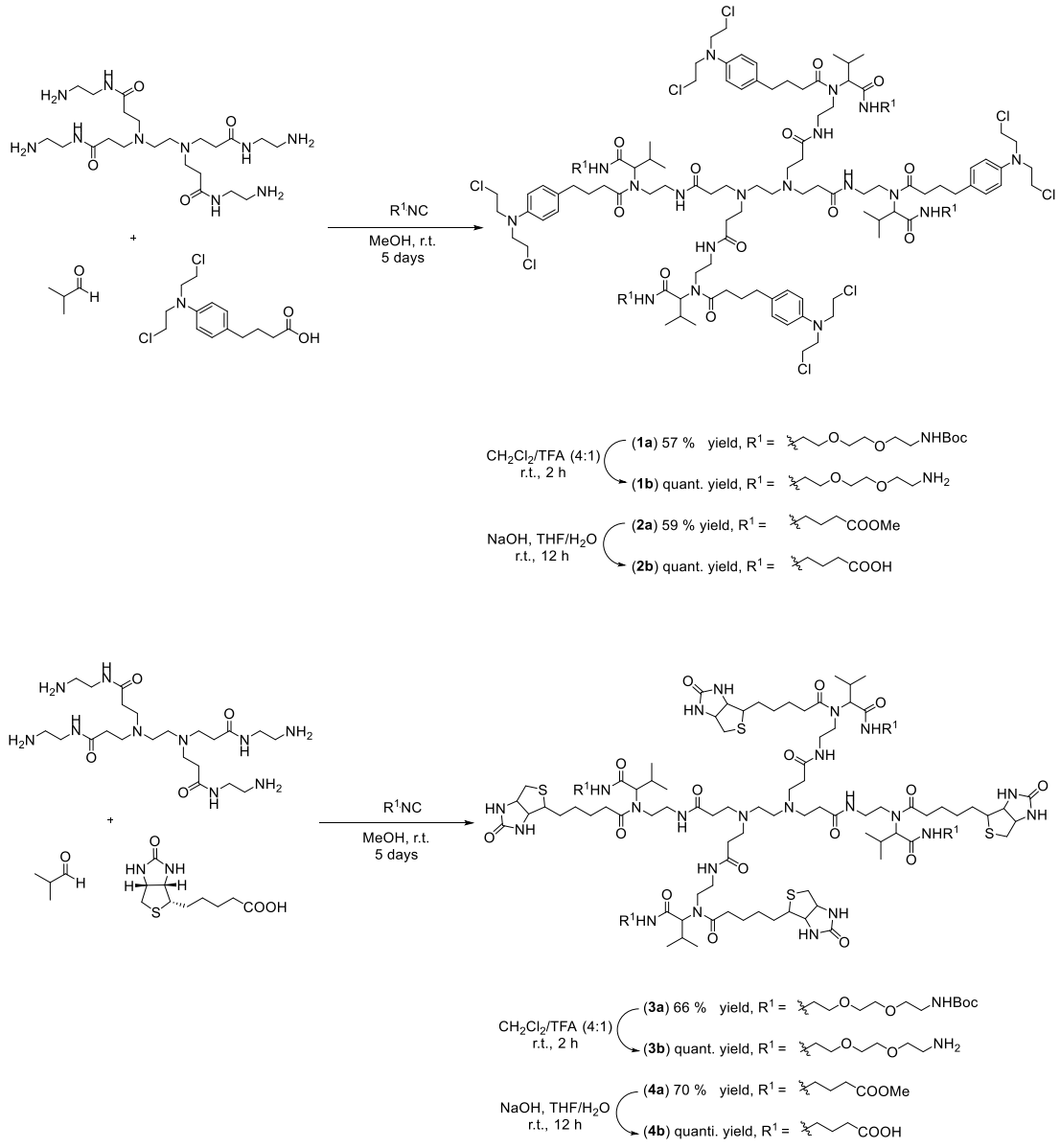
3.3 Surface functionalization of PAMAM-NH_2 dendrimer by Ugi reaction

As depicted in **Scheme 1**, we initially investigated if the commercially available PAMAM-NH_2 dendrimer generation 0 could be used as a component for the Ugi reaction and as a water-soluble nanocarrier for chlorambucil. To this end, two different reactions were performed using PAMAM-NH_2 dendrimer as the amine component of the Ugi reactions, isobutyraldehyde as aldehyde and chlorambucil as carboxylic acid. Two different isocyanides were used in order to obtain dendrimers with cationic (NH_3^+) or anionic (COO^-) properties. The reactions were performed at room temperature for 5 days in methanol, followed by purification by normal-phase column chromatography and deprotection of the amino groups with dichloromethane/trifluoroacetic acid (4:1) or hydrolysis of the methyl ester moieties with sodium hydroxide, which led to the desired water-soluble dendrimers with high purity and 57 % and 59 % overall yields, respectively. Applying the same approach as before, two biotinylated dendrimers were also synthesized with cationic and anionic surface, ratifying the Ugi reaction as an effective tool for the surface functionalization of PAMAM dendrimers.

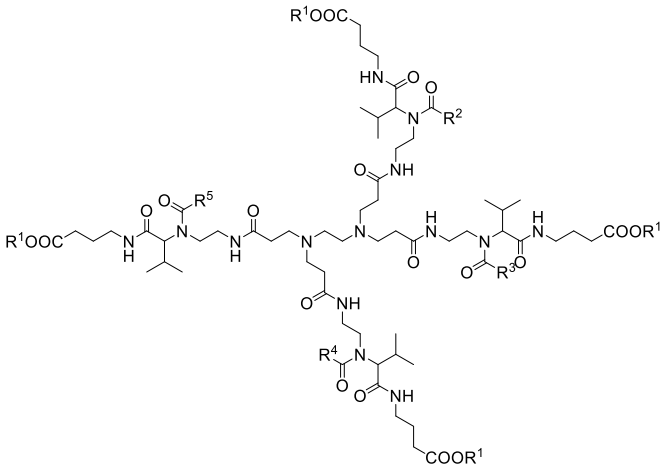
Taking advantage of the reaction potential, three water-soluble dendrimers containing both biotin and chlorambucil in the same molecule were synthesized for further evaluation of the anticancer activity. The one pot procedure was performed employing the same aldehyde as before, and both biotin and chlorambucil as the carboxylic acid component. Methyl 4-isocyanobutanoate was used as the isocyanide due to the slightly higher yields compared with the cationic isocyanide. After stirring for 5 days in methanol, the mixture was purified by normal-phase column chromatography followed by preparative HPLC. Five dendrimers were isolated according to their polarity: PAMAM-4-chlorambucil (**4a**), PAMAM-3-chlorambucil-1-biotin (**5a**), PAMAM-2-chlorambucil-2-

biotin (**6a**), PAMAM-1-chlorambucil-3-biotin (**7a**), and PAMAM-4-biotin (**2a**) (**Figure 1**). After purification, the dendrimers containing both biotin and chlorambucil were submitted to hydrolysis with sodium hydroxide in THF/H_2O overnight. The identity and purity of the dendrimers were confirmed by ^{1}H NMR, ^{13}C NMR, and MALDI-TOF analyses.

All dendrimers showed high solubility in water, thereby increasing the solubility of chlorambucil itself, which is essential for the improvement of the bioavailability and therapeutic efficiency.



Scheme 1. Functionalization of PAMAM dendrimers (**1a** - **4b**) by Ugi reaction.



(**5a**) R^1 = Me R^2 = biotin $R^3 = R^4 = R^5$ = chlorambucil

(**5b**) R^1 = H R^2 = biotin $R^3 = R^4 = R^5$ = chlorambucil

(**6a**) R^1 = Me $R^2 = R^3$ = biotin $R^4 = R^5$ = chlorambucil

(**6b**) R^1 = H $R^2 = R^3$ = biotin $R^4 = R^5$ = chlorambucil

(**7a**) R^1 = Me $R^2 = R^3 = R^4$ = biotin R^5 = chlorambucil

(**7b**) R^1 = H $R^2 = R^3 = R^4$ = biotin R^5 = chlorambucil

Figure 1. Structure of the dendrimers **5a** - **7b**.

3.4 Evaluation of anticancer activity

To evaluate anticancer activity of synthesized dendrimers they were tested against colon HT-29 and prostate PC-3 cancer cell lines (48 h of treatment). Biotin, targeting compound, and chlorambucil, anticancer drug, were used as controls (**Figure 2**). Fast screening of dendrimers, as well as biotin alone (all in concentrations 0.01 and 10 μM), demonstrated that those dendrimers decorated only with biotin (**3a** - **4b**) are not active in the investigated concentration range. This is reasonable since biotin, as folic acid, riboflavin and vitamin B12, are essential for cell division and not toxic to the cells.[25] Analogous dendrimers (**1a** - **2b**) containing chlorambucil instead of biotin depending on functional group on the dendrimer surface showed different activity. Namely, those containing -NHBoc (**1a**), -COOMe (**2a**) or -COOH (**2b**) were found inactive. In contrast to this, the dendrimer containing -NH_2 (**1b**) selectively inhibited growth by more than 50 % of the PC-3 cells, compared to the HT-29 cell line, at a concentration of 10 μM. The control compound chlorambucil was found ineffective against both cancer cell lines, what is in accordance with the literature.[26,27] Dendrimers decorated with different ratios of targeting agent biotin and anticancer drug chlorambucil (3:1 → 1:3; **5a** - **7a** and **5b** - **7b**) did not have any effect on cell survival, probably because they were not amino-substituted. Results from the CV and MTT assays are in a good agreement.

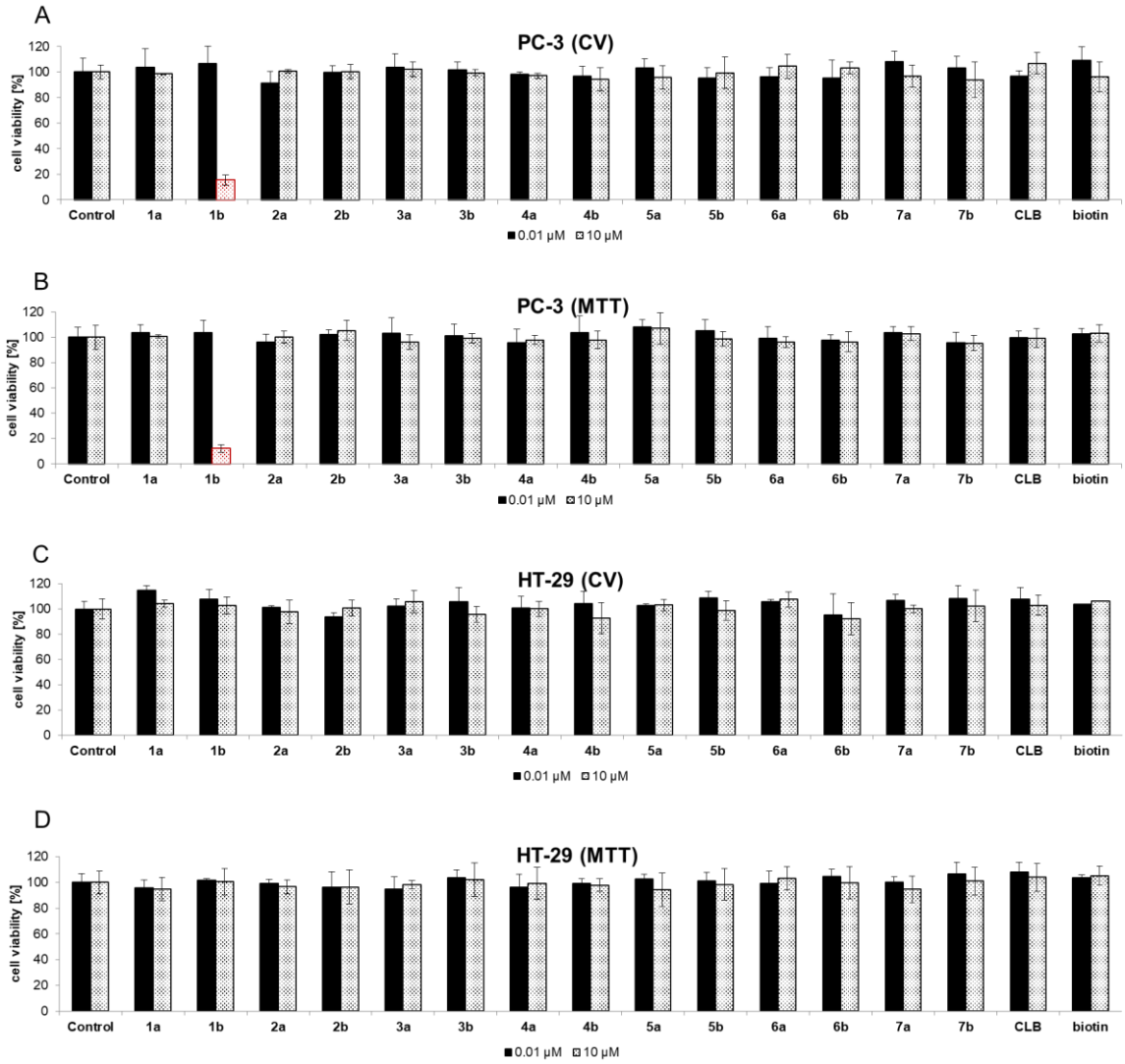


Figure 2. Fast screening of **1a** - **7b**, biotin and chlorambucil assessed with CV and MTT assays against HT-29 and PC-3 cancer cell lines (48 h of action).

For the active dendrimer **1b**, as well as chlorambucil, CV and MTT assays were performed to determine the IC_{50} against PC-3 prostate cell line (**Figure 3**). As shown recently,[27] chlorambucil has almost no effect of PC-3 cells. On the contrary, **1b** exhibits significant cytotoxicity (IC_{50} values, CV: 3.65 ± 0.6 µM; MTT: 7.29 ± 1.2 µM) compared to chlorambucil alone (CV, MTT: IC_{50} > 100 µM).

Additionally, the effect of dendrimer **1b** and chlorambucil alone were tested against non-cancerous mouse NIH3T3 fibroblasts (**Figure 4**). At IC_{50} value observed on PC-3 cell line, **1b** does not disturb viability of NIH3T3 cells at all. MTT and CV assays with dendrimer **1b** on non-cancerous cells revel an IC_{50} which is 10-20 times higher (IC_{50} values, CV: 70.21 ± 1.1 µM; MTT: 74.37 ± 2.3 µM) than that of PC-3 cancer cell line. Thus, dendrimer **1b**, decorated with four chlorambucil moieties and four amino

groups, not only increases cytotoxicity but also enhances selectivity toward PC-3 prostate tumor cells.

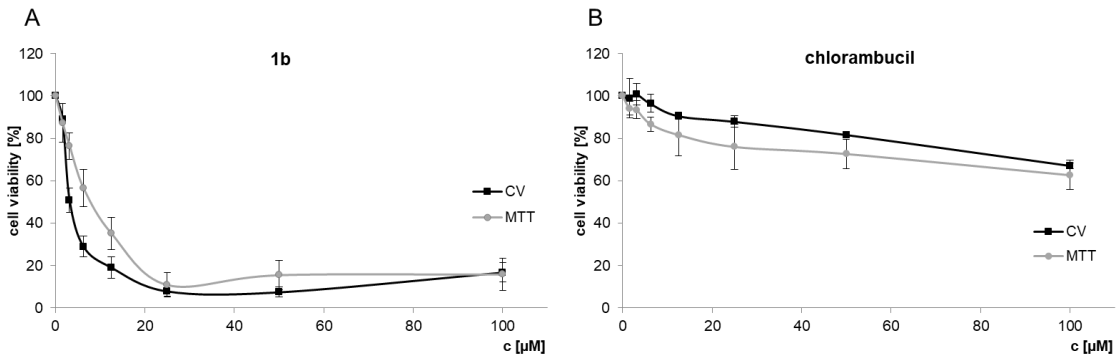


Figure 3. Survival of PC-3 cells after treatment with **1b** and chlorambucil (CV and MTT assays, after 48 h of action).

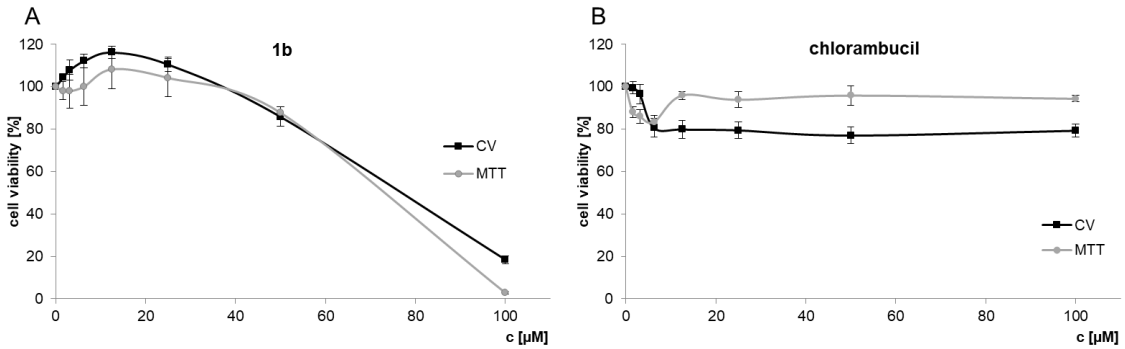


Figure 4. Effect of the active dendrimer **1b** and chlorambucil against non-cancerous mouse NIH3T3 fibroblasts (CV and MTT assays, after 48 h of action).

3.5 Conclusion

In conclusion, MCR-strategy was used to enhance activity and selectivity of the anticancer drug chlorambucil and expand PAMAM-NH_2 dendrimer with a new Ugi-branching generation with -COOH groups, -NH_2 groups, or biotin. It could also be shown that the anticancer activity against PC-3 prostate cancer cell line was improved when four chlorambucil units were present in the dendrimer containing -NH_2 groups on the surface (**1b**). However, the activity of **1b** is not due to additive effect of four chlorambucil present in dendrimer as chlorambucil itself was found inactive even at 4-fold concentration. Additionally, **1b** was found to act selectively not only between the two tumor cell lines tested (inactive against colon adenocarcinoma HT-29 cancer cell line) but more importantly, **1b** is also inactive against the non-cancerous mouse fibroblasts.

Furthermore, taking in consideration toxicity of chlorambucil alone and **1b**, inverse selectivity is achieved.

Polycationic macromolecules have the ability to interact with the negatively charged phospholipid bilayers present on cell membranes and allow the permeability of small compounds into cells. In severe cases, this interaction can lead to ruptures on the cell membrane and high cytotoxicity.[28,29] In our study, the ammonium groups in dendrimer **1b** (positively charged at physiological pH) appears to favour cellular uptake into PC-3 prostate cancer cells. The results also suggest that the biological nature of colon HT-29 cancer cells may be less susceptible to interaction with polycationic CLB-dendrimer, while prostate PC-3 cancer cells may be more responsive to this type of compound.

3.6 Experimental Part

3.6.1 General information

PAMAM-NH_2 dendrimer generation 0 (20 wt. % solution in methanol) was purchased from Sigma-Aldrich (manufactured by Dendritech). Chlorambucil was purchased from Alfa Aesar (Germany). D-Biotin was purchased from Iris Biotech (Germany). All commercial reagents and solvents were used without further purification. Column chromatography was carried out using Merck silica gel 60 (0.040-0.063 mm) with approximately 35 g of silica gel per gram of crude product. Analytical thin-layer chromatography (TLC) was performed using Merck silica gel 60 F254 aluminium sheets. Preparative RP18 HPLC was carried out with a Knauer system equipped with a WellChrom K-1001 pump and a WellChrom K-2501 UV detector using a preparative column (polymeric RP, 9 × 250 mm internal diameter, 300 Å, 8 µm, VYDAC, USA). ^{1}H NMR and ^{13}C NMR spectra were recorded at 25 °C in the respective solvents on an Agilent DD2 400 spectrometer at 400 MHz and 101 MHz, respectively. Chemical shifts (δ) are reported in ppm relative to TMS (^{1}H NMR) and to residual solvent signal (^{13}C NMR). High resolution ESI mass spectra were obtained with an Orbitrap Elite mass spectrometer (Thermo Fisher Scientific, Germany) equipped with HESI electrospray ion source (spray voltage 4.0 kV; capillary temperature 275 °C, source heater temperature 40 °C; FTMS resolution 60.000). MALDI-TOF mass spectra were acquired on a Bruker Ultraflex III-MALDI-TOF/TOF mass spectrometer (Bruker Daltonics, Bremen, Germany). The samples (1 µL) were mixed with the equal volume of 4 mg/mL α-cyano-4-hydroxycinnamic acid solution in 50 % v/v acetonitrile/0.1 % v/v TFA (matrix) on a

stainless steel target and dried under air. The analysis was performed in a reflector positive ion mode, using the source and reflector voltages of 25 and 26.3 kV, respectively. Desorption/ionization of the analytes was achieved by a YAG 354 nm laser.

Digitonin (Riedel De Haen, Seelze, Germany), crystal violet, paraformaldehyde, MTT, acridine orange, DAPI (Sigma-Aldrich, Germany), RPMI medium 1640, tryban blue stain 0.4 % (Life Technologies, Germany), DPBS, trypsin EDTA (PAN Biotech, Germany), fetal calf serum, penicillin/streptomycin, HEPES (PAA laboratories, Germany), DMSO (Duchefa Biochemie, Germany), acetic acid 33 % (Roth, Germany), DHR and CFSE (DB Biosciences, USA) were obtained commercially. Plate reader (Spectramax from Molecular Devices) and FACSAria III (DB Biosciences) were used for the anticancer experiments.

3.6.2 Cell lines and culture conditions

Colon adenocarcinoma cells (HT-29) were kindly provided by Prof. B. Seliger, Immunology Department, Martin-Luther-University Halle-Wittenberg, Germany. Human refractory prostate cancer cells (PC-3) and mouse fibroblasts (NIH3T3) were purchased from the German Collection of Microorganisms and Cell Cultures (Leibniz-DSMZ, Germany). The cell lines were grown in RPMI 1640 medium supplemented with 10 % fetal calf serum (FCS, Sigma-Aldrich) and 1 % penicillin/streptomycin (Sigma-Aldrich) and incubated at 37 °C and 5 % CO_2. Stock solutions of the investigated dendrimers (**1a**-**7b**) and compounds (biotin and chlorambucil) were prepared at 20 mM concentration in DMSO. The number of cells seeded per wells differs according to the plate type used. For 96 wells plate, 1000 PC-3 cells and 1500 HT-29 cells were seeded per well. While for the 6 wells plate, 1×10^5 PC-3 cells and 1.5×10^5 HT-29 cells were seeded per well.

3.6.3 MTT and CV assays

For fast screening, HT-29 (1500 cells/well) and PC-3 (1000 cells/well) cells were seeded in 96 well plate and treated, after 24 h of incubation, with dendrimers (**1a** - **7b**) and compounds (biotin and chlorambucil) in two concentrations (0.01 µM and 10 µM) in quadruplicate. MTT and CV assays were performed upon 48 h of treatment as described in the literature.[30] For IC_{50} value determination a dilution series was prepared (1.56, 3.12, 6.25, 12.50, 25.00, 50.00 and 100.00 µM) from the stock solution of **1b** and chlorambucil. For the most active dendrimer **1b** as well as chlorambucil CV and MTT assays were performed on NIH3T3 cells (5000 cells/well) analogously. Digitonin (125 µM) was used as control. Each experiment was performed in triplicate. IC_{50} values (concentrations of

the compound at which 50 % of cell growth inhibition occurs) were calculated using a four-parameter logistic function and presented as mean of three independent experiments. The absorbance of the dissolved dyes was measured in an automated microplate reader at 540 nm (reference wavelength 670 nm). The results are presented as a percentage of control values obtained from untreated cultures.

3.6.4 Synthesis

General procedure for the functionalization of PAMAM by Ugi reaction. In a round-bottom flask were added PAMAM dendrimer generation 0 (81.2 µmol, 1.0 equiv.) and the aldehyde (0.32 mmol, 4.0 equiv.) in dry methanol. The mixture was stirred at room temperature overnight to enable imine formation. The carboxylic acid (0.32 mmol, 4.0 equiv.) and the isocyanide (0.32 mmol, 4.0 equiv.) were added and the contents were stirred for 5 days. The volatiles were removed under reduced pressure in a rotary evaporator and the crude material purified by flash column chromatography using ethyl acetate/methanol as eluent.

General procedure for synthesis of dendrimers 5a, 6a, and 7a. In a round-bottom flask were added PAMAM dendrimer generation 0 (0.32 mmol, 1.0 equiv.) and the aldehyde (1.29 mmol, 4.0 equiv.) in dry methanol. The mixture was stirred at room temperature overnight in order to accomplish imine formation. Then, both biotin (0.65 mmol, 2.0 equiv.) and chlorambucil (0.65 mmol, 2.0 equiv.) were added followed by the isocyanide (1.29 mmol, 4.0 equiv.). The contents were stirred for 5 days at room temperature. The volatiles were removed under reduced pressure in a rotary evaporator and the three products formed pre-purified by flash column chromatography (SiO_2, ethyl acetate/methanol 100:0 to ethyl acetate/methanol 0:100) followed by preparative RP-HPLC (AcN:H_2O + 0.1 % FA. 35 % > 15 min > 80 % > 1 mim > 100 %).

General procedure for the deprotection of the amino group. In a round-bottom flask were added the suitable dendrimer and a CH_2Cl_2/TFA (4:1) solution (75 mL/mmol). The solution was stirred for two hours at room temperature. The volatiles were removed under reduced pressure in a rotary evaporator and the crude material washed several times with diethyl ether.

General procedure for the hydrolysis of methyl ester. In a round-bottom flask were added the dendrimer in a solution of THF/water 1:1 (5 mL/mmol) and NaOH (1 g/mmol). The mixture was stirred overnight at room temperature and then acidified with aqueous

HCl 5 % solution. The salt formed was filtered off and the solution concentrated under reduced pressure in a rotary evaporator.

Dendrimer 1a. PAMAM dendrimer (43 mg, 81.2 µmol), isobutyraldehyde (22 mg, 0.32 mmol), chlorambucil (98 mg, 0.32 mmol), and *tert*-butyl (2-(2-(2-isocyanoethoxy) ethoxy)ethyl)carbamate (46 mg, 0.32 mmol) were reacted in MeOH (20 mL) according to the general procedure for the functionalization of PAMAM by Ugi reaction (Section 3.6.4, p. 65). The product was purified by column chromatography (SiO_2, ethyl acetate/methanol 100:0 to ethyl acetate/methanol 50:50). Colorless oil. Yield: 138 mg (57 %). HRMS (ESI) of $C_{142}H_{236}Cl_8N_{22}O_{28}$ $[M+3H]^{3+}$ calc. 993.5156, obs. 993.8508; ^{1}H NMR (400 MHz, MeOD) δ 7.06 (d, 8H, *J* = 7.8 Hz), 6.66 (d, 8H, *J* = 12.8 Hz), 4.52 (d, 4H, *J* = 11.0 Hz), 3.76 - 3.32 (m, 88H), 3.22 - 3.17 (m, 8H), 2.91 - 2.78 (m, 8H), 2.62 - 2.16 (m, 32H), 1.94 - 1.85 (m, 8H), 1.42 (s, 36H), 0.95 - 0.79 (m, 24H); ^{13}C NMR (101 MHz, MeOD) δ 176.90, 172.68, 158.34, 145.99, 131.80, 130.67, 113.60, 80.06, 71.29, 71.08, 70.44, 54.55, 54.53, 48.85, 41.79, 41.27, 41.21, 40.38, 40.29, 40.03, 35.20, 35.06, 34.98, 33.66, 28.97, 28.84, 28.43, 28.28, 20.15, 19.88, 19.80, 19.41.

Dendrimer 1b. Dendrimer **1a** (30 mg, 0.01 mmol) and 750 µL of a CH_2Cl_2/TFA (4:1) solution were reacted according to the general procedure for deprotection of the amino group (Section 3.6.4, p. 65). Yield: 25 mg (quantitative). MS (MALDI-TOF) of $C_{122}H_{204}Cl_8N_{22}O_{20}$ $[M+Na]^+$ calc. 2600.302, obs. 2603.291; ^{1}H NMR (400 MHz, MeOD) δ 7.05 (d, 8H, *J* = 8.0 Hz), 6.66 (d, 8H, *J* = 7.2 Hz), 4.47 (d, 4H, *J* = 11.2 Hz), 3.73 - 3.32 (m, 88H), 3.24 - 3.09 (m, 20H), 2.61 - 2.48 (m, 16H), 2.27 - 2.19 (m, 4H), 1.92 - 1.83 (m, 8H), 1.34 - 1.28 (m, 8H), 0.96 - 0.79 (m, 24H); ^{13}C NMR (101 MHz, MeOD) δ 176.96, 176.26, 172.79, 172.15, 146.07, 131.73, 130.76, 130.67, 130.65, 130.56, 126.11, 113.75, 113.72, 113.63, 113.56, 71.34, 71.29, 71.24, 70.44, 67.91, 54.50, 54.32, 49.84, 41.79, 40.68, 40.57, 40.23, 40.18, 35.14, 35.10, 35.05, 34.96, 33.65, 30.89, 28.91, 28.44, 28.23, 20.07, 19.75, 19.67, 19.65, 19.42.

Dendrimer 2a. PAMAM dendrimer (80 mg, 0.16 mmol), isobutyraldehyde (47 mg, 0.65 mmol), chlorambucil (197 mg, 0.65 mmol), and methyl 4-isocyanobutanoate (83 mg, 0.65 mmol) were reacted in MeOH (30 mL) according to the general procedure for the functionalization of PAMAM by Ugi reaction (Section 3.6.4, p. 65). The product was purified by column chromatography (SiO_2, ethyl acetate/methanol 100:0 to ethyl acetate/methanol 50:50). Colorless oil. Yield: 235 mg (59 %). HRMS (ESI) of $C_{118}H_{184}Cl_8N_{18}O_{20}$ $[M+2H]^{2+}$ calc. 1227.5801, obs. 1228.5787; ^{1}H NMR (400 MHz, MeOD) δ 7.07 - 7.03 (m, 8H), 6.68 - 6.64 (m, 8H), 4.50 (d, 4H, *J* = 11.0 Hz), 3.74 - 3.32 (m, 64H),

3.28 - 3.13 (m, 12H), 2.97 - 2.88 (m, 4H), 2.57 - 2.21 (m, 36H), 1.90 - 1.75 (m, 16H), 0.97 - 0.75 (m, 24H); ^{13}C NMR (101 MHz, MeOD) δ 179.58, 176.89, 176.12, 175.09, 175.04, 172.60, 171.92, 146.01, 145.91, 132.13, 131.85, 131.79, 131.77, 130.68, 130.64, 130.58, 113.72, 113.61, 113.59, 113.55, 113.53, 113.37, 67.71, 54.61, 54.54, 54.52, 52.19, 52.15, 49.84, 41.78, 41.76, 41.70, 40.09, 39.75, 39.58, 35.90, 35.31, 35.15, 35.03, 33.61, 32.07, 32.02, 29.42, 28.87, 28.76, 28.38, 28.28, 27.85, 25.55, 20.09, 19.85, 19.66, 19.37.

Dendrimer 2b. Dendrimer **2a** (100 mg, 0.04 mmol) and NaOH (40 mg) were reacted according to the general procedure for the hydrolysis of methyl ester (Section 3.6.4, p. 65). Yield: 96 mg (quantitative). MS (MALDI-TOF) of $C_{114}H_{176}Cl_8N_{18}O_{20}$ $[M+2H+Na]^{3+}$ calc. 807.026, obs. 807.704; ^{1}H NMR (400 MHz, MeOD) δ 7.10 (d, 8H, *J* = 8.6 Hz), 6.77 (d, 8H, *J* = 8.4 Hz), 4.58 - 4.46 (m, 3H), 3.78 - 3.60 (m, 52H), 3.29 - 3.17 (m, 13H), 2.90 - 2.85 (m, 4H), 2.63 - 2.24 (m, 36H), 1.90 - 1.77 (m, 17H), 0.96 - 0.78 (m, 24H); ^{13}C NMR (101 MHz, MeOD) δ 177.44, 176.76, 176.75, 176.70, 176.68, 176.61, 176.15, 176.03, 172.61, 172.56, 171.95, 171.90, 145.20, 145.20, 145.12, 131.60, 131.53, 130.78, 130.74, 130.69, 114.41, 114.39, 114.36, 114.34, 114.28, 114.24, 114.22, 114.18, 68.84, 67.50, 65.38, 55.07, 54.93, 49.84, 41.54, 41.37, 39.78, 39.68, 35.39, 35.05, 35.01, 34.22, 34.10, 33.81, 33.63, 32.17, 32.08, 32.00, 28.42, 28.03, 27.92, 26.48, 25.60, 20.11, 19.79, 19.46, 19.39, 19.21.

Dendrimer 3a. PAMAM dendrimer (43 mg, 81.2 µmol), isobutyraldehyde (22 mg, 0.32 mmol), biotin (79 mg, 0.32 mmol), and *tert*-butyl (2-(2-(2-isocyanoethoxy) ethoxy)ethyl)carbamate (46 mg, 0.32 mmol) were reacted in MeOH (20 mL) according to the general procedure for the functionalization of PAMAM by Ugi reaction (Section 3.6.4, p. 65). The product was purified by column chromatography (SiO_2, ethyl acetate/methanol 100:0 to ethyl acetate/methanol 0:100). Colorless oil. Yield: 147 mg (66 %). MS (MALDI-TOF) of $C_{126}H_{224}N_{26}O_{32}S_4$ $[M+Na]^+$ calc. 2764.547, obs. 2764.468; ^{1}H NMR (400 MHz, MeOD) δ 4.59 - 4.47 (m, 8H), 4.36 - 4.30 (m, 4H), 3.61 - 3.35 (m, 54H), 3.25 - 3.19 (m, 14H), 2.97 - 2.92 (m, 4H), 2.82 - 2.56 (m, 24H), 2.42 - 2.28 (m, 12H), 1.75 - 1.62 (m, 12H), 1.53 - 1.49 (m, 4H), 1.43 (s, 36H), 1.15 - 0.81 (m, 32H); ^{13}C NMR (101 MHz, MeOD) δ 177.97, 176.16, 175.53, 173.53, 166.82, 159.22, 80.92, 72.17, 71.95, 71.30, 64.35, 64.23, 62.48, 62.42, 58.01, 57.95, 57.77, 53.38, 50.71, 42.11, 41.98, 41.23, 41.14, 40.92, 35.40, 35.33, 35.10, 34.84, 34.70, 30.81, 30.68, 30.54, 30.31, 29.70, 29.20, 28.01, 27.89, 27.29, 26.76, 20.98, 20.73, 20.65, 20.17.

Dendrimer 3b. Dendrimer **3a** (30 mg, 0.01 mmol) and 750 µL of a CH_2Cl_2/TFA (4:1) solution were reacted according to the general procedure for deprotection of the amino group (Section 3.6.4, p. 65). Yield: 25 mg (quantitative). MS (MALDI-TOF) of $C_{106}H_{192}N_{26}O_{24}S_4$ $[M+Na]^+$ calc. 2364.338, obs. 2364.354; ^{1}H NMR (400 MHz, MeOD) δ 4.51 (s, 8H), 4.33 (d, 4H, *J* = 12.2 Hz), 3.73 - 3.35 (m, 48H), 3.27 - 3.12 (m, 20H), 3.03 - 2.17 (m, 38H), 1.73 - 1.49 (m, 18H), 1.32 (d, 8H, *J* = 4.6 Hz), 1.07 - 0.84 (m, 24H); ^{13}C NMR (101 MHz, MeOD) δ 177.91, 173.82, 173.54, 166.83, 72.18, 71.95, 71.29, 64.36, 64.26, 64.16, 62.49, 62.42, 62.38, 58.04, 57.97, 57.89, 45.59, 42.12, 41.99, 41.15, 40.96, 34.80, 34.67, 30.88, 30.82, 30.64, 30.52, 30.37, 29.69, 29.22, 28.01, 27.84, 21.71, 21.03, 20.97, 20.72, 20.71, 20.17, 15.32.

Dendrimer 4a. PAMAM dendrimer (80 mg, 0.16 mmol), isobutyraldehyde (47 mg, 0.65 mmol), biotin (158 mg, 0.65 mmol), and methyl 4-isocyanobutanoate (83 mg, 0.65 mmol) were reacted in MeOH (30 mL) according to the general procedure for the functionalization of PAMAM by Ugi reaction (Section 3.6.4, p. 65). The product was purified by column chromatography (SiO_2, ethyl acetate/methanol 100:0 to ethyl acetate/methanol 0:100). Colorless oil. Yield: 251 mg (70 %). MS (MALDI-TOF) of $C_{102}H_{172}N_{22}O_{24}S_4$ $[M+Na]^+$ calc. 2240.169, obs. 2240.119; ^{1}H NMR (400 MHz, MeOD) δ 4.52 (td, 8H, *J* = 8.7, 7.8, 4.8 Hz), 4.36 - 4.31 (m, 4H), 3.66 (d, 12 H, *J* = 1.3 Hz), 3.43 - 3.34 (m, 8H), 3.25 - 3.19 (m, 12H), 2.97 - 2.91 (m, 4H), 2.82 - 2.59 (m, 20H), 2.43 - 2.23 (m, 24H), 1.91 - 1.41 (m, 40H), 0.97 - 0.81 (m, 24H); ^{13}C NMR (101 MHz, MeOD) δ 177.15, 177.14, 177.10, 177.10, 176.19, 176.13, 175.35, 175.34, 175.12, 174.70, 172.58, 172.57, 171.95, 171.92, 166.03, 165.98, 67.88, 63.48, 63.39, 61.63, 61.57, 57.13, 52.49, 52.18, 51.20, 41.23, 41.12, 40.09, 39.74, 39.57, 39.22, 34.47, 34.28, 33.96, 33.83, 32.12, 32.03, 29.94, 29.79, 29.70, 29.64, 29.41, 28.31, 27.15, 27.04, 26.43, 25.55, 20.08, 19.87, 19.70, 19.29.

Dendrimer 4b. Dendrimer **4a** (82 mg, 0.04 mmol) and NaOH (30 mg) were reacted according to the general procedure for the hydrolysis of methyl ester (Section 3.6.4, p. 65). Yield: 77 mg (quantitative). MS (MALDI-TOF) of $C_{98}H_{164}N_{22}O_{24}S_4$ $[M+Na]^+$ calc. 2184.117, obs. 2184.022; ^{1}H NMR (400 MHz, MeOD) δ 4.62 - 4.33 (m, 12H), 3.97 - 3.86 (m, 8H), 3.66 - 3.46 (m, 16H), 3.28 - 3.20 (m, 12H), 2.96 - 2.30 (m, 40H), 1.84 - 1.50 (m, 32H), 0.99 - 0.82 (m, 24H); ^{13}C NMR (101 MHz, MeOD) δ 177.02, 176.32, 176.15, 175.25, 174.52, 172.60, 171.99, 165.87, 67.50, 63.69, 61.98, 61.85, 57.16, 52.27, 49.84, 41.19, 41.02, 40.07, 39.67, 39.58, 34.94, 32.13, 32.04, 31.41, 30.28, 29.87, 29.75, 29.56, 29.43, 28.25, 25.54, 23.71, 20.07, 19.76, 19.42, 19.29, 19.24.

Dendrimer 5a. Dendrimer **5a** was synthesized according to the general procedure for synthesis of dendrimers 5a, 6a, and 7a (Section 3.6.4, p. 65). Yield: 23.3 mg. MS (MALDI-TOF) of $C_{114}H_{181}Cl_6N_{19}O_{21}S$ $[M+Na]^+$ calc. 2417.142, obs. 2419.247; ^{1}H NMR (400 MHz, MeOD) δ 7.09 - 7.03 (m, 6H), 6.67 (dd, 6H, *J* = 8.5, 4.2 Hz), 4.52 - 4.45 (m, 4H), 4.33 - 4.27 (m, 2H), 3.73 - 3.60 (m, 36H), 3.43 - 3.34 (m, 8H), 3.29 - 3.11 (m, 16H), 2.85 - 2.20 (m, 57H), 1.91 - 1.88 (m, 4H), 1.81 - 1.77 (m, 6H), 1.29 - 1.28 (m, 3H), 0.94 - 0.79 (m, 24H); ^{13}C NMR (101 MHz, MeOD) δ 177.01, 176.98, 175.11, 175.07, 172.61, 172.56, 146.02, 131.81, 130.65, 113.62, 54.57, 52.52, 52.18, 52.14, 51.28, 51.24, 51.20, 41.79, 40.10, 40.02, 39.58, 39.53, 35.16, 34.52, 33.63, 33.61, 32.10, 32.03, 28.92, 28.35, 28.28, 25.56, 20.10, 19.88, 19.71, 19.36.

Dendrimer 5b. Dendrimer **5a** (9.3 mg, 4 µmol) and NaOH (3 mg) were reacted according to the general procedure for the hydrolysis of methyl ester (Section 3.6.4, p. 65). Yield: 9 mg (quantitative). MS (MALDI-TOF) of $C_{110}H_{173}Cl_6N_{19}O_{21}S$ $[M+Na]^+$ calc. 2361.079, obs. 2363.169; ^{1}H NMR (400 MHz, MeOD) δ 7.06 (d, 6H, *J* = 8.2 Hz), 6.70 - 6.64 (m, 6H), 4.56 - 4.47 (m, 4H), 4.34 - 4.26 (m, 2H), 3.72 - 3.62 (m, 24H), 3.44 - 3.34 (m, 8H), 3.24 - 3.08 (m, 16H), 2.60 - 1.74 (m, 63H), 1.32 - 1.29 (m, 6H), 0.95 - 0.79 (m, 24H); ^{13}C NMR (101 MHz, MeOD) δ 176.72, 176.65, 176.22, 172.66, 146.06, 131.84, 131.75, 130.68, 130.63, 113.62, 54.59, 54.54, 41.80, 41.78, 39.87, 39.72, 35.12, 35.02, 33.66, 33.58, 32.24, 32.18, 30.75, 28.77, 28.41, 28.33, 25.63, 23.72, 20.85, 20.73, 20.06, 19.84, 19.61, 19.37.

Dendrimer 6a. Dendrimer **6a** was synthesized according to the general procedure for synthesis of dendrimers 5a, 6a, and 7a (Section 3.6.4, p. 65). Yield: 26.3 mg. MS (MALDI-TOF) of $C_{110}H_{178}Cl_4N_{20}O_{22}S_2$ $[M+Na]^+$ calc. 2358.151, obs. 2360.288; ^{1}H NMR (400 MHz, MeOD) δ 7.08 - 7.04 (m, 4H), 6.70 - 6.65 (m, 4H), 4.56 - 4.45 (m, 6H), 4.33 - 4.27 (m, 2H), 3.75 - 3.57 (m, 36H), 3.41 - 3.34 (m, 6H), 3.24 - 3.16 (m, 12H), 2.93 - 2.83 (m, 8H), 2.73 - 2.55 (m, 16H), 2.45 - 2.15 (m, 24H), 1.91 - 1.76 (m, 14H), 1.68 - 1.47 (m, 10H), 0.95 - 0.79 (m, 24H); ^{13}C NMR (101 MHz, MeOD) δ 177.11, 177.06, 176.92, 175.10, 175.06, 172.60, 172.56, 166.01, 165.95, 146.03, 146.01, 131.86, 131.78, 130.69, 130.65, 113.73, 113.60, 54.55, 54.52, 52.20, 52.17, 52.16, 41.81, 41.79, 41.24, 41.10, 40.12, 40.03, 39.75, 39.57, 35.15, 35.02, 33.91, 33.76, 33.62, 32.11, 32.08, 32.03, 29.93, 29.77, 29.68, 29.61, 29.41, 28.92, 28.38, 28.29, 27.15, 27.04, 25.55, 20.10, 20.06, 19.87, 19.69, 19.37, 19.27.

Dendrimer 6b. Dendrimer **6a** (18.8 mg, 8 µmol) and NaOH (6.4 mg) were reacted according to the general procedure for the hydrolysis of methyl ester (Section 3.6.4, p.

65). Yield: 18 mg (quantitative). MS (MALDI-TOF) of $C_{106}H_{170}Cl_4N_{20}O_{22}S_2$ $[M+K]^+$ calc. 2318.197, obs. 2318.053; ^{1}H NMR (400 MHz, MeOD) δ 7.08 - 7.04 (m, 4H), 6.70 - 6.66 (m, 4H), 4.59 - 4.42 (m, 6H), 4.35 - 4.27 (m, 2H), 3.72 - 3.62 (m, 24H), 3.42 - 3.33 (m, 6H), 3.26 - 3.05 (m, 20H), 2.60 - 2.25 (m, 30H), 1.92 - 1.59 (m, 24H), 1.47 - 1.29 (m, 10H), 0.98 - 0.77 (m, 24H); ^{13}C NMR (101 MHz, MeOD) δ 176.87, 146.06, 130.68, 130.63, 130.57, 130.11, 113.62, 113.56, 54.54, 51.26, 41.79, 41.69, 39.91, 39.71, 35.79, 35.20, 35.04, 34.24, 33.64, 32.32, 30.76, 29.74, 28.34, 28.05, 25.69, 20.05, 19.84, 19.52, 19.36.

Dendrimer 7a. Dendrimer **7a** was synthesized according to the general procedure for synthesis of dendrimers 5a, 6a, and 7a (Section 3.6.4, p. 65). Yield: 16 mg. MS (MALDI-TOF) of $C_{106}H_{175}Cl_2N_{21}O_{23}S_3$ $[M+Na]^+$ calc. 2299.159, obs. 2300.261; ^{1}H NMR (400 MHz, MeOD) δ 7.09 - 7.04 (m, 2H), 6.70 - 6.66 (m, 2H), 4.56 - 4.46 (m, 6H), 4.36 - 4.29 (m, 4H), 3.73 - 3.61 (m, 20H), 3.42 - 3.33 (m, 8H), 3.29 - 3.13 (m, 16H), 2.96 - 2.90 (m, 3H), 2.80 - 2.57 (m, 18H), 2.41 - 2.13 (m, 22H), 1.88 - 1.28 (m, 36H), 0.97 - 0.80 (m, 24H); ^{13}C NMR (101 MHz, MeOD) δ 177.17, 177.13, 175.36, 175.13, 172.59, 172.57, 166.04, 165.99, 146.04, 131.82, 130.66, 113.62, 64.36, 63.48, 61.58, 57.12, 54.58, 52.17, 41.82, 41.22, 40.10, 40.02, 39.76, 39.58, 35.16, 34.65, 34.50, 33.96, 32.13, 32.04, 29.93, 29.80, 29.70, 29.63, 28.29, 27.12, 27.04, 25.56, 20.07, 19.88, 19.70, 19.36, 19.31, 19.27.

Dendrimer 7b. Dendrimer **7a** (8 mg, 3 µmol) and NaOH (3 mg) were reacted according to the general procedure for the hydrolysis of methyl ester (Section 3.6.4, p. 65). Yield: 7 mg (quantitative). HRMS (ESI) of $C_{102}H_{167}Cl_2N_{21}O_{23}S_3$ $[M+Na+K]^{2+}$ calc. 1141.097, obs. 1141.092; ^{1}H NMR (400 MHz, MeOD) δ 7.27 - 7.24 (m, 2H), 7.11 - 7.06 (m, 2H), 4.62 - 4.31 (m, 10H), 3.92 - 3.85 (m, 8H), 3.67 - 3.59 (m, 14H), 3.26 - 3.19 (m, 10H), 2.97 - 2.24 (m, 43H), 1.94 - 1.30 (m, 36H), 0.97 - 0.78 (m, 24H); ^{13}C NMR (101 MHz, MeOD) δ 176.97, 176.62, 175.21, 172.60, 171.94, 165.86, 138.67, 131.20, 129.66, 128.85, 117.83, 67.52, 65.36, 63.67, 57.18, 56.84, 52.22, 49.84, 46.05, 41.03, 40.37, 39.71, 39.58, 35.27, 34.54, 32.03, 29.91, 29.67, 29.43, 28.27, 25.89, 25.54, 20.12, 19.77, 19.42, 19.34.

3.7 References

(1) Vidal, L.; Gurion, R.; Ram, R.; Raanani, P.; Bairey, O.; Robak, T.; Gafter-Gvili, A.; Shpilberg, O. *Leuk. Lymphoma* **2016**, 57, 2047-2057.

(2) Salmelin, C.; Hovinen, J.; Vilpo, J. *Mutat. Res.* **2000**, 467, 129-138.

(3) Omoomi, F. D.; Siadat, S. D.; Nourmohammadi, Z.; Tabasi, M. A.; Pourhoseini, S.; Babaei, R. A.; Saffari, M.; Ardestani, M. S. *J. Cancer Sci. Ther*. **2013**, 5, 075-084.

(4) Nicolle, A.; Proctor, S. J.; Summerfield, G. P. *Leuk. Lymphoma* **2004**, 45, 271-275.

(5) Rai, K. R.; Peterson, B. L.; Appelbaum, F. R.; Kolitz, J.; Elias, L.; Shepherd, L.; Hines, J.; Threatte G. A.; Larson, R. A.; Cheson, B. D.; Schiffer, C. A. *N. Engl. J. Med.* **2000**, 343, 1750-1757.

(6) Hillmen, P.; Skotnicki, A. B.; Robak, T.; Jaksic, B.; Dmoszynska, A.; Wu, J.; Sirard, C.; Mayer, J. *J. Clin. Oncol.* **2007**, 25, 5616-5623.

(7) Knauf, W. U.; Lissichkov, T.; Aldaoud, A.; Liberati, A.; Loscertales, J.; Herbrecht, R.; Juliusson, G.; Postner, G.; Gercheva, L.; Goranov, S.; Becker, M.; Fricke, H. J.; Huguet, F.; Del Giudice, I.; Klein, P.; Tremmel, L.; Merkle, K.; Montillo, M. *J. Clin. Oncol*. **2009**, 27, 4378-4384.

(8) (a) Madaan, K.; Kumar, S.; Poonia, N.; Lather, V.; Pandita, D. *J. Pharm. Bioallied Sci.* **2014**, 6, 139-150. (b) Kesharwani, P.; Jain, K.; Jain, N. K. *Prog. Polym. Sci.* **2014**, 39, 268-307.

(9) (a) Gao, Z.; Lukyanov, A. N.; Singhal, A.; Torchilin, V. P. *Nano Lett.* **2002**, 2, 979-982. (b) Anitha, A.; Maya, S.; Deepa, N.; Chennazhi, K. P.; Nair, S. V.; Tamura, H.; Jayakumar, R. *Carbohydr. Polym.* **2011**, 83, 452-561.

(10) (a) Zhu, J.; Shi, X. *J. Mater. Chem. B* **2013**, 1, 4199-4211. (b) Cho, K.; Wang, X.; Nie, S.; Chen, Z.; Shin, D. M. *Clin. Cancer Res*. **2008**, 14, 1310-1316.

(11) (a) Yuan, H.; Luo, K.; Lai, Y.; Pu, Y.; He, B.; Wang, G.; Wu, Y.; Gu, Z. *Mol. Pharmaceutics* **2010**, 7, 953-962. (b) Dai, L.; Liu, J.; Luo, Z.; Li, M.; Cai, K. *J. Mater. Chem. B* **2016**, 4, 6758-6772.

(12) Sunoqrot, S.; Bae, J. W.; Pearson, R. M.; Shyu, K.; Liu, Y.; Kim, D. H.; Hong, S. *Biomacromolecules* **2012,** 13, 1223-1230.

(13) Nicolas, J.; Mura, S.; Brambilla, D.; Mackiewicz, N.; Couvreur, P. *Chem. Soc. Rev*. **2013**, 42, 1147-1235.

(14) Manthey, K. C.; Griffin, J. B.; Zempleni, J. *J. Nutr*. **2002**, 132, 887-892.

(15) Russell-Jones, G.; McTavish, K.; McEwan, J.; Rice, J.; Nowotnik, D. *J. Inorg. Biochem*. **2004**, 98, 1625-1633.

(16) Kim, J. H.; Li, Y.; Kim, M. S.; Kang, S. W.; Jeong, J. H.; Lee, D. S. *Int. J. Pharm*. **2012**, 427, 435-442.

(17) Chen, W-H.; Luo, G-F.; Lei, Q.; Jia, H-Z.; Hong, S.; Wang, Q-R.; Zhuo, R-X.; Zhang, X-Z. *Chem. Commun*. **2015**, 51, 465-468.

(18) Chen, S.; Zhao, X.; Chen, J.; Chen, J.; Kuznetsova, L.; Wong, S. S.; Ojima, I. *Bioconjugate Chem*. **2010**, 21, 979-987.

(19) Hemmer, R.; Hall, A.; Spaulding, R.; Rossow, B.; Hester, M.; Caroway, M.; Haskamp, A.; Wall, S.; Bullen, H. A.; Morris, C.; Haik, K. L. *Molecules* **2013**, 18, 11537-11552.

(20) Minko, T.; Paranjpe, P. V.; Qiu, B.; Lalloo, A.; Won, R.; Stein, S.; Sinko, P. J. *Cancer Chemother. Pharmacol.* **2002***,* 50, 143-150.

(21) Shi, J. F.; Wu, P.; Jiang, Z. H.; Wei, X. Y. *Eur. J. Med. Chem*. **2014**, 71, 219-228.

(22) Gangopadhyay, M.; Singh, T.; Behara, K. K.; Karwa, S.; Ghosh, S. K.; Singh, N. D. P. *Photochem. Photobiol. Sci*. **2015**, 14, 1329-1336.

(23) Bielawski, K.; Bielawska, A.; Muszynska, A.; Popławska, B.; Czarnomysy, R. *Environ. Toxicol. Pharmacol.* **2011**, 32, 364-372.

(24) Assadi, A.; Najafabadi, V. S.; Shandiz, S. A. S.; Boroujeni, A. S.; Ashrafi, S.; Vaziri, A. Z.; Ghoreishi, S. M.; Aghasadeghi, M. R.; Ebrahimi, S. E. S.; Pirali-Hamedani, M.; Ardestani, M. S. *Onco Targets Ther.* **2016**, 9, 5531-5543.

(25) Tripodo, G.; Mandracchia, D.; Collina, S.; Rui, M.; Rossi, D. *Med. Chem.* **2014**, S1.

(26) Pedersen, P. J.; Christensen, M. S.; Ruysschaert, T.; Linderoth, L.; Andresen, T. L.; Melander, F.; Mouritsen, O. G.; Madsen, R.; Clausen, M. H. *J. Med. Chem*. **2009**, 52, 3408-3415.

(27) Idowu, T.; Samadder, P.; Arthur, G.; Schweizer, F. *Chem. Phys. Lipids* **2016**, 194, 139-148.

(28) Mecke, A.; Majoros, I. J.; Patri, A. K.; Baker, J. R.; Holl, M. M. B.; Orr, B. G. *Langmuir* **2005**, 21, 10348-10354.

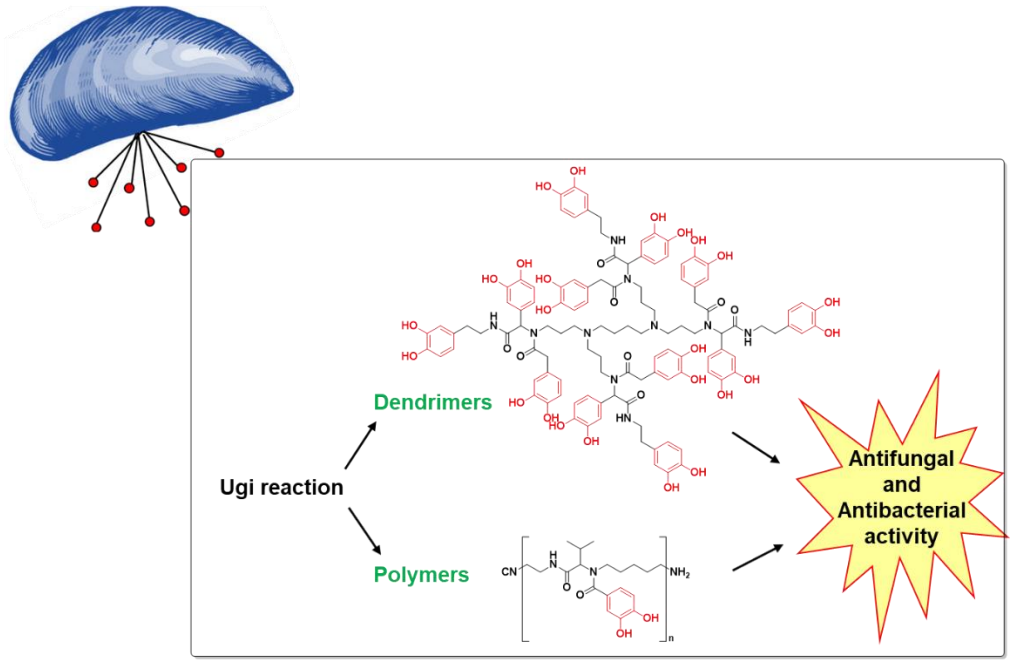
(29) Yellepeddi, V. K.; Pisal, D. S.; Kumar, A.; Kaushik, R. S.; Hildreth, M. B.; Guan, X.; Palakurthi, S. *Int. J. Pharm*. **2008**, 350, 113-121.

(30) Krajnović, T.; Kaluđerović, G. N.; Wessjohann, L. A.; Mijatović, S.; Maksimović-Ivanić, D. *Pharmacol. Res*. **2016**, 105, 62-73.

Chapter 4

Ugi reaction for the synthesis of catechol-functionalized dendrimers and polymers with adhesive and antimicrobial properties

Abstract



The adhesive protein secreted by mussels forms a strong and reversible glue underwater. It was found that the catechol moiety from the amino acid DOPA is the responsible for this adhesion, making catechols an important and versatile building block for the construction of mussel-inspired synthetic compounds. In view of this, we describe a new synthetic approach for the surface functionalization of the commercial dendrimer PPI and the synthesis of polymers with multiple catechol units by Ugi reaction and further evaluation of their potential as antibacterial and antifungal agents.

4.1 Introduction

In 1981, Waite and Tanzer discovered that the amino acid DOPA (3,4-dihydroxyphenyl-L-alanine), which contains the catechol moiety (**Figure 1**), is responsible for the strong and reversible underwater adhesion of mussels to rocks.[1] Later, innumerable studies report the synthesis of new compounds containing catechol groups, other studies cover the adhesion mechanism. Among these mussel-inspired compounds, polymers, hyperbranched polymers, and hydrogels using hyaluronic acid, heparin, chitosan, poly(ethylene glycol), and poly(acrylate/methacrylate) have been reported.[2-11]

Besides the adhesive and the intrinsic antioxidant ability of catechol groups,[12,13] the chelating property of catechols with various di- and trivalent metal ions can cause *in situ* deposition of silver nanoparticles,[12,13] and consequently the compounds can show antimicrobial activities against several bacteria and fungi.[14-16] Antimicrobial activity has also been described for catechol-based compounds linked with quaternary ammonium groups.[17,18] However, in both cases, the antimicrobial activity is not related with the presence of catechol groups itself but with the presence of well-known antimicrobial groups or particles.

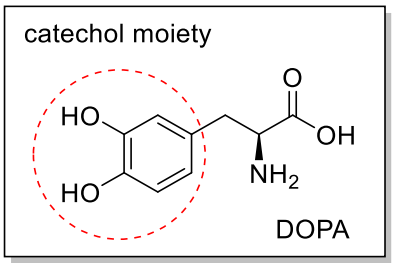


Figure 1. Amino acid 3,4-dihydroxyphenyl-L-alanine.

Herein, we report the synthesis of a collection of dendrimers and polymers with several catechol groups by Ugi multicomponent reaction and the investigation of the antifungal activity against *Botrytis cinerea*, *Septoria tritici*, and *Phytophthora infestans* (Oomycete) and antibacterial activity against *Aliivibrio fischeri* and *Bacillus subtilis*, focusing on the catechol moiety as the source of activity.

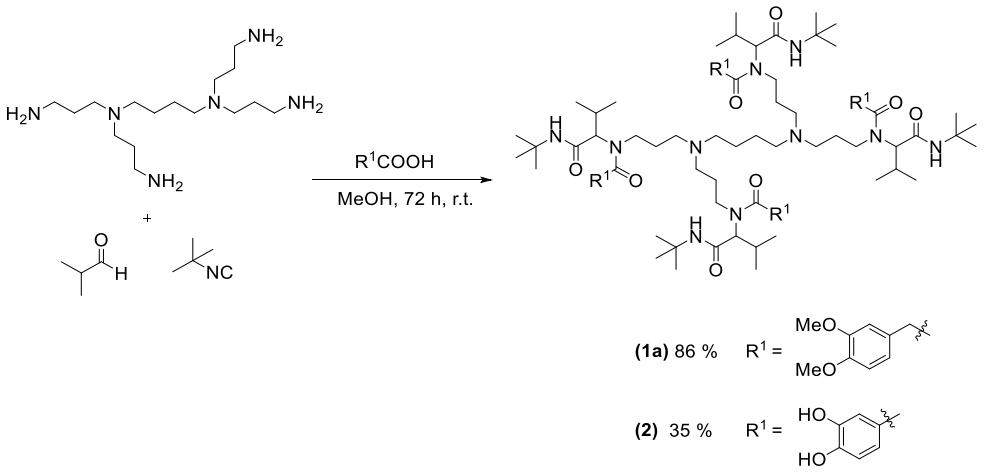
4.2 Synthetic Strategy

The synthetic approach normally used to achieve mussels-inspired polymers or branched molecules is based on amide coupling reactions in which one catechol group is incorporated as a terminal group on each polymer terminal or side chain.[7, 19-22] Another common option is the self-polymerization of dopamine or the use of catechol-based monomers, which has the limitation of the inhibition of the free-radical polymerization by catechol groups in the presence of air.[20, 23-27]

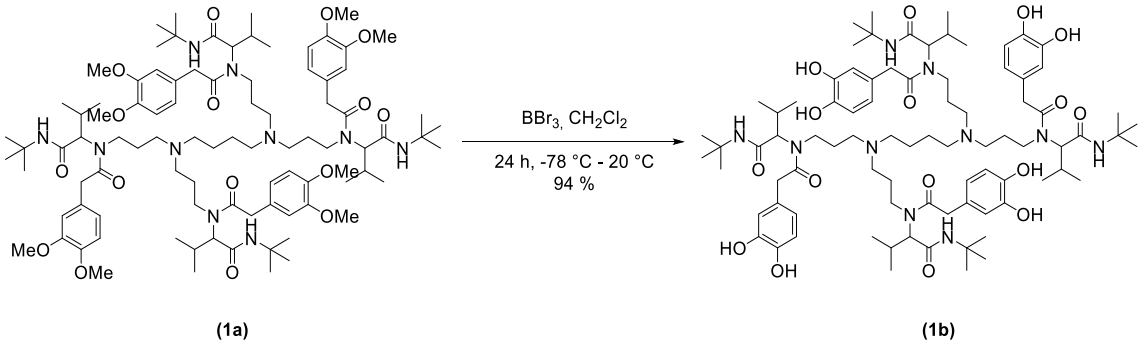
With the purpose of circumventing such limitation and since our research group has previously reported a new strategy to synthesize dendrimers *via* isocyanide-based multicomponent reaction,[28] we decided to expand our approach for the surface functionalization of the commercial dendrimer polypropylenimine tetramine (PPI) of first generation and for the synthesis of polymers containing catechol groups.

4.3 Synthesis of dendrimers with multiple catechol moieties

We started our research by performing an Ugi reaction with the commercial polypropylenimine tetramine dendrimer (PPI) of first generation, with four amino groups on the surface, isobutyraldehyde as the aldehyde component, (3,4-dimethoxyphenyl)acetic acid as the carboxylic acid, and *tert*-butyl isocyanide as the isocyanide at room temperature for 72 hours (**Scheme 1**). We chose to start the synthesis with the (3,4-dimethoxyphenyl)acetic acid considering it is commercially available and also when the reaction was performed with 3,4-dihydroxybenzoic acid the yield dropped significantly, from 86 % to 35 % (**Scheme 1**). Isobutyraldehyde and t*ert*-butyl isocyanide were initially used because they are readily available, highly reactive in the Ugi reaction and can provide better NMR control. After the Ugi reaction, the removal of the methyl groups was accomplished by aryl ether cleavage using 1M BBr_3 in dichloromethane for 24 hours (**Scheme 2**).[29] The final product with four catechol units (**1b**) on the surface of the dendrimer was obtained with 81 % overall yield and high purity.



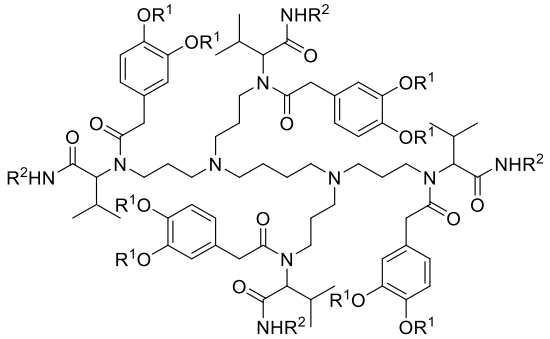
Scheme 1. Synthesis of the dendrimers **1a** and **2** starting from the commercial PPI dendrimer.



Scheme 2. Demethylation of the dendrimer **1a** to afford the catechol derivative **1b**.

Taking advantage of this characteristic, we also synthesized dendrimers with four catechol derivatives, like the examples synthesized earlier, but this time employing different isocyanides: an aliphatic one (**3a**), a bifunctional one with protected carboxylic acid (**4a**), and a bifunctional one with protected amino group (**5a**). The reactions were performed using the same approach as before and afforded the desired products in 83 %, 80 %, and 69 % yield, respectively. Dendrimer **3a** was submitted to demethylation with 1M BBr_3 in dichloromethane to afford the product **3b** with 79 % overall yield. The derivatives **4a** and **5a** were subjected to demethylation and removal of the protecting groups in a one pot procedure with the same protocol as before to yield the products **4b** and **5b** with 71 % and 65 % (overall yields after two steps), respectively (**Figure 2**).

The aim of this part is to have different catechol-based dendrimers with lipophilic (**3b**) and hydrophilic (**4b** - **5b**) chains, as well as positive and negative charges, in order to evaluate their antimicrobial activity.



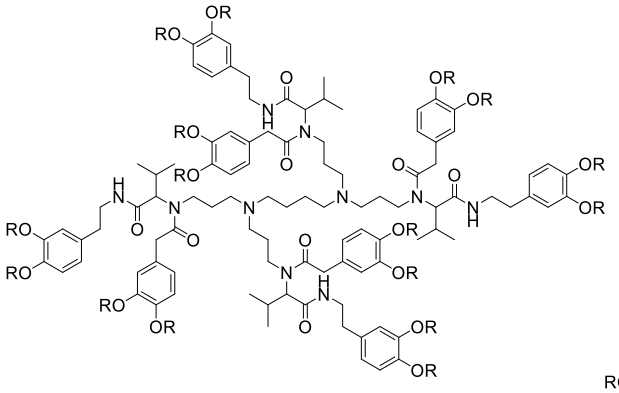
(3a) R^1 = Me, R^2 = $(CH_2)_7CH_3$ 83 %
(3b) R^1 = H, R^2 = $(CH_2)_7CH_3$ 96 %

(4a) R^1 = Me R^2 = $(CH_2)_3COOMe$ 80 %
(4b) R^1 = H R^2 = $(CH_2)_3COOH$ 89 %

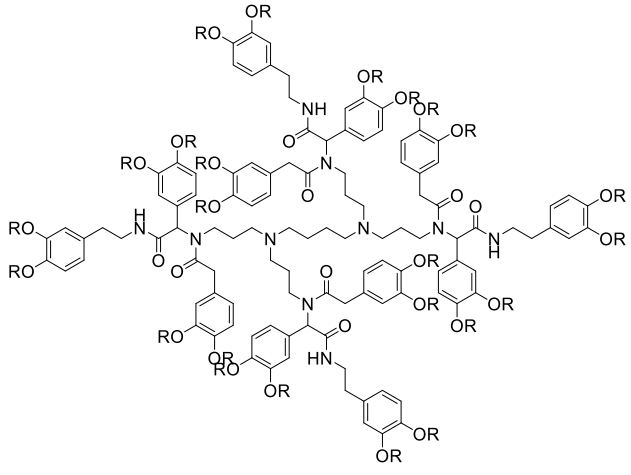
(5a) R^1 = Me R^2 = $(CH_2)_4NHCbz$ 69 %
(5b) R^1 = H R^2 = $(CH_2)_4NH_2$ 94 %

Figure 2. Structure of the dendrimers **3a** - **5b**.

In the wake of this results we proceeded to synthesize dendrimers with eight and twelve catechol moieties in their structures. For the first option, an Ugi reaction between PPI of first generation, isobutyraldehyde, (3,4-dimethoxyphenyl)acetic acid, and 2-(3,4-dimethoxyphenyl)ethyl isocyanide was performed using methanol as solvent at room temperature for 72 hours. This was followed by demethylation with 1M BBr_3 in dichloromethane for 48 hours. The protocol afforded the dendrimer **6b** in 67 % overall yield. The dendrimer with twelve catechol units (**7b**) was achieved in 54 % overall yield, after two reaction steps, by using PPI G1 and the same conditions as before, but with the commercially available 3,4-dimethoxybenzaldehyde instead of isobutyraldehyde (**Figure 3**).



(6a) R = Me 69 %
(6b) R = H 96 %



(7a) R = Me 58 %
(7b) R = H 93 %

Figure 3. Structure of the dendrimers **6a** - **7b**.

Finally, in order to investigate if the results obtained for the antimicrobial activity were not restricted to catechol-based compounds, we also synthesized a dendrimer with four pyrogallol (2,3,4-trihydroxyphenyl) units. Dendrimer **8b** was obtained after two reaction steps applying a similar protocol as before, employing commercially available 2,3,4-trimethoxybenzaldehyde, in 47 % of overall yield (**Figure 4**).

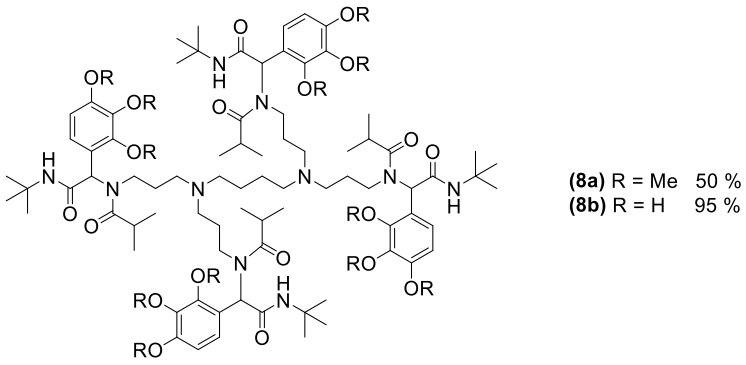


Figure 4. Structure of the dendrimers **8a** and **8b**.

The identity and purity of the dendrimers were confirmed by ^{1}H NMR, ^{13}C NMR, and MALDI-TOF analyses. The catechol-based and pyrogallol-based dendrimers were obtained, after the demethylation, in high purity as light yellow amorphous solids (when completely dry) with low solubility in water and high solubility in organic solvents such as dichloromethane and methanol. In the presence of small amounts of solvent, the synthesized dendrimers are very sticky and act, as expected, as glue/adhesives.

4.4 Synthesis of polymers with multiple catechol moieties

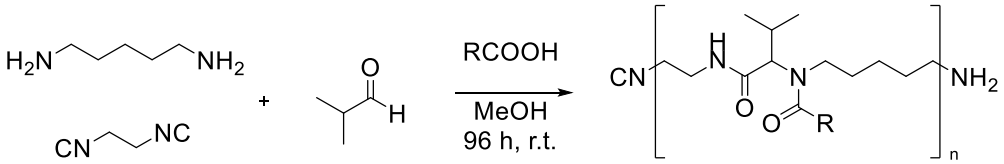
The polymerization *via* Ugi reaction using two bifunctional AA-type components was described, among others, by Meier and co-workers.[30] For our purpose, bifunctional AA-type monomers with 1,5-diaminopentane as the amine component and 1,2-diisocyanoethane as the isocyanide have been used. The employed components were isobutyraldehyde and 3,4-dihydroxybenzoic acid, (3,4-dimethoxyphenyl)acetic acid or 3,4-diacetoxybenzoic acid (**Table 1**). The reactions were performed in methanol as solvent, at room temperature for 96 hours and the product purified by precipitation with cold diethyl ether (5x). The polymerization and the molecular weight of the synthesized polymers were confirmed by ^{1}H NMR and size exclusion chromatography (SEC) analyses.

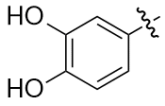
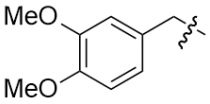
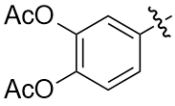
Three different polymers were synthesized, two (**9** and **10**) with the same carboxylic acid as used for the dendrimer functionalization and one (**11**) as a new type

of monomer containing 3,4-acetoxybenzyl groups, in order to verify if the presence of hydroxy, methoxy or acetoxy groups in the compounds have influence in the antimicrobial activity.

Similar to dendrimers, the synthesized polymers were obtained as light yellow amorphous solids with low solubility in water and high solubility in organic solvents.

Table 1. Polymerization *via* Ugi reaction.



Polymer	R	Mn [g/mol]*[a]	Mw [g/mol]*[b]	Đ*[c]
9		4900	7400	1.51
10		5500	8900	1.62
11		5400	8200	1.52

*Determined by SEC. a) Mn: Number average molecular weight; b) Mw: Weight average molecular weight; c) Đ: dispersity.

4.5 Evaluation of the antimicrobial activity

All synthesized dendrimers and polymers were submitted to a 96-well microtiter plate assays on antifungal and antibacterial activity. For the antifungal activity, the phytopathogenic ascomycetous fungi *Botrytis cinerea* and *Septoria tritici*, as well as the Oomycete *Phytophthora infestans* were tested. For the antibacterial activity, one Gram-negative (*Aliivibrio fischeri)* and one Gram-positive (*Bacillus subtilis*) bacterium each were evaluated.

4.5.1 Evaluation of the antifungal activity

The polycatechol dendrimers (**1b** - **7b**) show admirable growth inhibition of the Oomycete *Phytophthora infestans*, when compared to the reference fungicide oxocrotonate acid C16 (**Figure 5a**). An increase of the number of catechol units in the

dendrimer also increased the growth-inhibitory effect, primarily at a concentration of 14 µM with inhibitions of 81 %, 83 % and 100 % of growth for the dendrimers **1b**, **6b**, and **7b**, respectively.

The dendrimers with four catechol units and aliphatic chain, negative or positive charge also show high growth-inhibitory activity, between 89 % and 100 % at the concentrations of 125 µM and 41 µM, but without differentiation. At the concentration of 14 µM it is possible to observe that the positively charged dendrimer (**5b**) had the best result with 96 % of inhibition, followed by the negatively charged one (**4b**) with 72 % and the dendrimer with aliphatic chains (**3b**) with 55 % of inhibition.

The *O*-methylated polycatechol dendrimers show growth-inhibitory effect between 58 % and 8 %, especially the dendrimer **5a** that inhibits the growth by 47 % at the lowest concentration tested (1.5 µM). The dendrimer with four pyrogallol units shows no inhibition of the Oomycete *Phytophthora infestans* in any concentration tested.

Compounds with a median of inhibition above 50 % were submitted for determination of IC_{50} and the results are depicted in **Table 2**. The results obtained ratify the previously statement, the increase of catechol groups on the periphery of the dendrimer improves the inhibition of the Oomycete *Phytophthora infestans*. The positively charged dendrimer (**5b**), as well as the dendrimer with *tert*-butyl groups on the surface (**1b**), are more potent compared with the negatively charged one (**4b**) and the dendrimer with aliphatic chains (**3b**).

Almost all dendrimers show minimal or no inhibition of the fungal strains *Botrytis cinerea* and *Septoria tritici*, with exception of the dendrimers **6a** and **7a** with 68 % and 69 %, respectively, against *Botrytis cinerea*, and the dendrimers **5a**, **6a**, **7a**, **7b** and **8a** with 54 %, 98 %, 100 %, 53 %, and 49 %, respectively, against *Septoria tritici* at 125 µM. (**Figure 5b** and **5c**).

Almost all polycatechol polymers show growth-inhibitory effect between 100 % and 60 % against *Phytophthora infestans*, *Botrytis cinerea,* and *Septoria tritici* at the higher concentration tested (125 µM) (**Figure 5**).

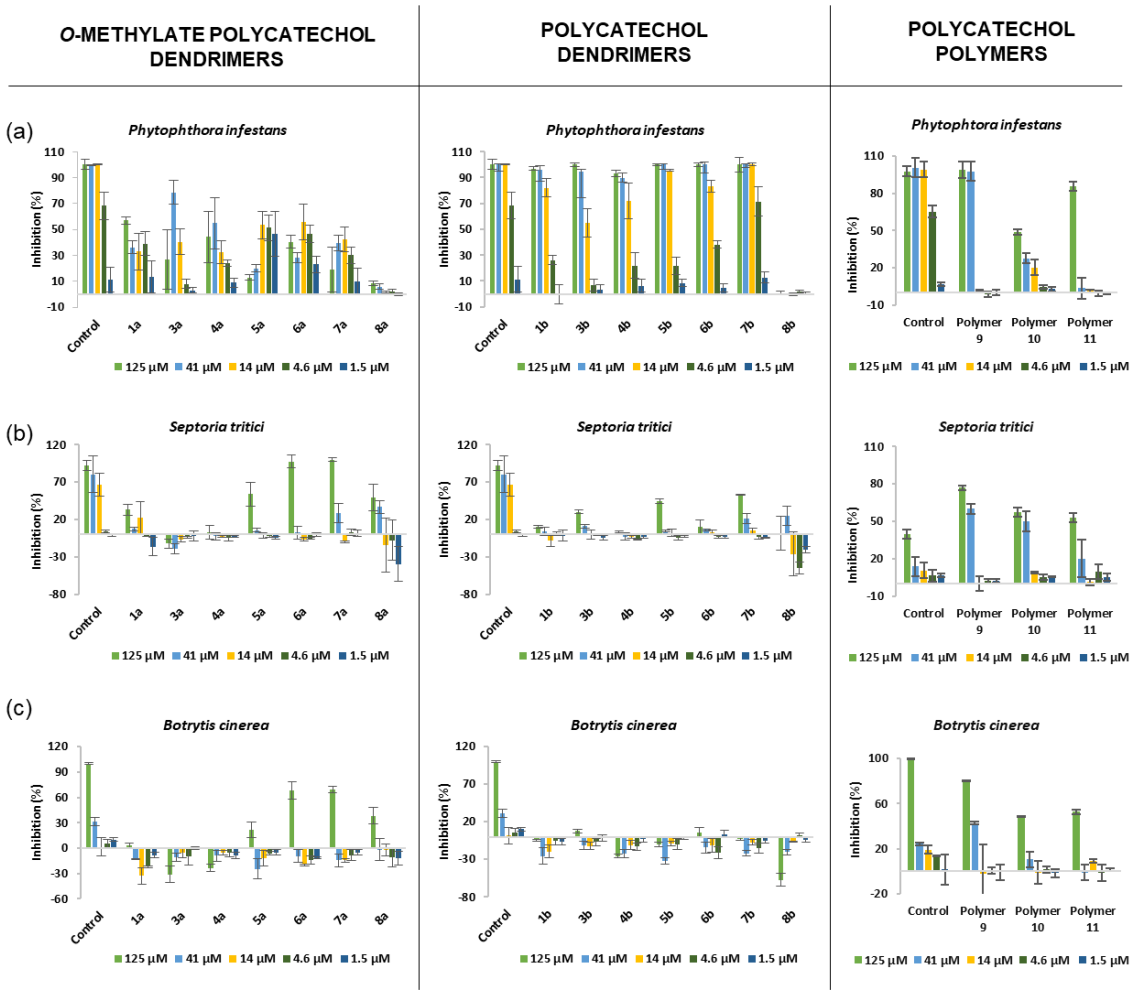


Figure 5. Antifungal growth inhibition assay at five different concentrations (after seven days of inoculation) against: (a) *Phytophthora infestans;* (b) *Septoria tritici;* (c) *Botrytis cinerea*. Control: reference fungicide oxocrotonate acid C16. Error bars represent standard deviation (SD) of the mean.

Table 2. Approximate IC_{50} values for selected dendrimers.

	Compound	IC_{50} (µM)[a]
Phytophthora infestans	**1b**	6.61 ± 3.7
	3b	13.26 ± 3.6
	4b	8.45 ± 4.9
	5b	6.98 ± 0.7
	6b	5.80 ± 2.8
	7b	3.96 ± 1.0
	oxocrotonate acid C16[b]	4.13 ± 3.9

[a]Values reported are as means ±SD.
[b]Used as reference compound.

4.5.2 Evaluation of the antibacterial activity

The antibacterial activity against the Gram-negative bacterium *Aliivibrio fischeri* show 100 % of growth inhibition for the dendrimer with four pyrogallol units (**8b**) at 100 µM, which is not observed for the *O*-methylated dendrimer (**8a**). The polycatechol dendrimers do not show any growth-inhibitory activity, on the contrary, it is possible to observe bacterial growth, which is higher with an increase of catechol units, suggesting that somehow catechol moieties are helping in the development of the microorganism. It is also noteworthy that the dendrimer with the aliphatic chain (**3b**) is responsible for the highest bacterial growth. The polycatechol polymers were also investigated and show only a minimal decrease or increase of the bacterial growth (**Figure 6a**).

Similar results showing the increase of the bacterial growth were also obtained for the dendrimers evaluated against *Bacillus subtilis*. It is possible to observe, once more, that the dendrimer with aliphatic chain (**3b**) is the responsible for the highest bacterial growth. On the contrary, the polymers **9** and **11** show growth inhibition around 70 % in both concentrations tested against the Gram-positive bacterium, while the polymer **10** exhibits inhibition of 40 % at 1 µM and 70 % at 100 µM (**Figure 6b**).

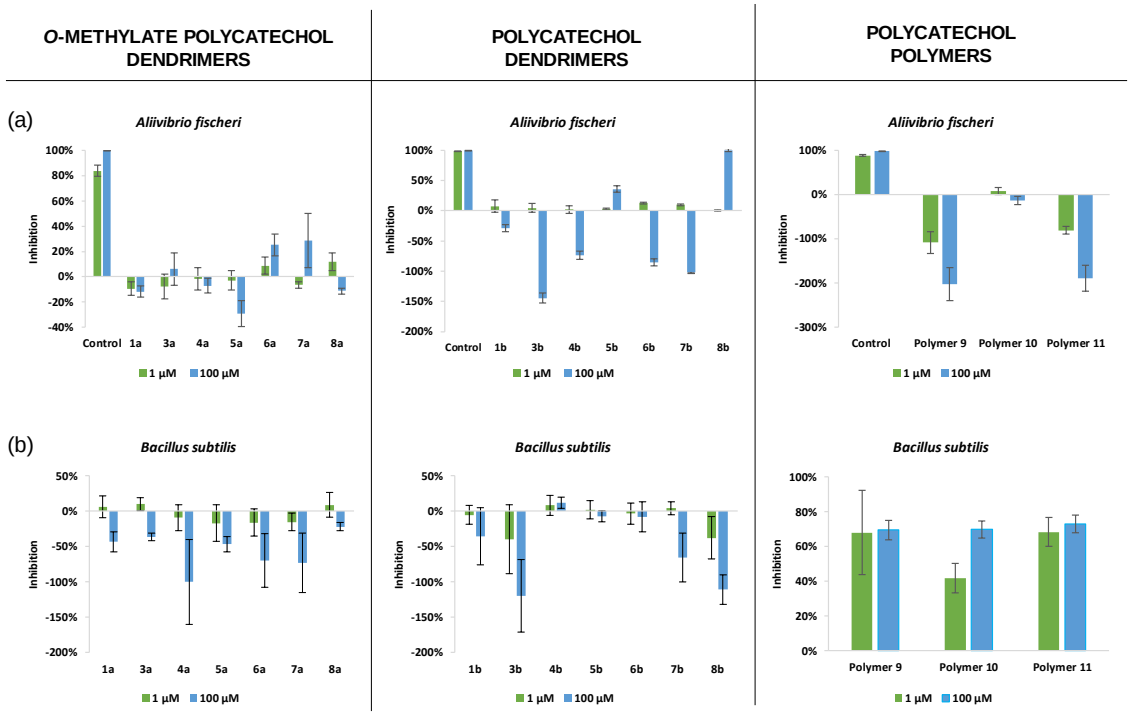


Figure 6. Antibacterial growth inhibition assay at two different concentrations (after 24 hours of inoculation) against: (a) *Aliivibrio fischeri.* Positive control: chloramphenicol; (b) *Bacillus subtilis.* Positive control: erythromycin (growth inhibition: 71 % at 1 µM). Error bars represent standard deviation (SD) of the mean.

4.6 Conclusion

In conclusion, we were able to synthesize a library of new PPI Ugi-chimeric dendrimers and Ugi-polymers containing several catechol moieties. This class of compounds with polyhydroxy phenyl groups are already well known for their adhesive properties, however, we could also demonstrate that they can act as antimicrobial agents.

The polycatechol dendrimers could be used as inhibitors of the Oomycete *Phytophthora infestans*. The increase of the number of catechol units on the surface of the dendrimer improves the growth-inhibitory effect, which suggests that the Oomycete may be hypersensitive to catechol groups. IC_{50} values of dendrimers with the same number of catechol groups on the surface but different types of functionalities show that the cationic dendrimer (**5b**) is a better antifungal agent than the anionic (**4b**) and aliphatic (**3b**) dendrimers. This can be explained based on the fact that polycationic molecules may interact with the negatively charged membrane components of the Oomycete cells and influence the membrane permeability.[31] Surprisingly, the dendrimer with *tert*-butyl groups on the surface (**1b**) show similar activity to the cationic dendrimer.

Oomycetes differ from true fungi in their cell wall composition, that contains cellulose but do not chitin, which is present in the fungal cell walls.[32] This can explain the difference in activity observed when the compounds where tested against the fungal strains *Botrytis cinerea* and *Septoria tritici.*

Dendrimer **8b**, with four pyrogallol moieties, is the only compound active against the Gram-negative bacterium *Aliivibrio fischeri*. This result should be further examined in the near future by the synthesis of new dendrimers with pyrogallol units. Additionally, the three polycatechol polymers show good inhibition of the Gram-positive bacterium *Bacillus subtilis* in both concentrations tested.

4.7 Experimental Part

4.7.1 General information

Polypropylenimine tetramine dendrimer generation 1 was purchased from Sigma-Aldrich (Germany). All commercial reagents were purchased from Sigma-Aldrich (Germany), Alfa Aesar (Germany) or Iris Biotech (Germany) and were used without further purification. Column chromatography was carried out using Merck silica gel 60 (0.040-0.063 mm) with approximately 35 g of silica gel per gram of crude product.

Analytical thin-layer chromatography (TLC) was performed using Merck silica gel 60 F254 aluminium sheets. ^{1}H NMR and ^{13}C NMR spectra were recorded at 25 °C in the respective solvents on an Agilent DD2 400 spectrometer at 400 MHz and 101 MHz, respectively. Chemical shifts (δ) are reported in ppm relative to TMS (^{1}H NMR) and to residual solvent signal (^{13}C NMR). MALDI-TOF mass spectra were acquired on a Bruker Ultraflex III-MALDI-TOF/TOF mass spectrometer (Bruker Daltonics, Bremen, Germany). The samples (1 µL) were mixed with the equal volume of 4 mg/mL α-cyano-4-hydroxycinnamic acid solution in 50 % v/v acetonitrile/0.1 % v/v TFA (matrix) on a stainless steel target and dried under air. The analysis was performed in a reflector positive ion mode, using the source and reflector voltages of 25 and 26.3 kV, respectively. Desorption/ionization of the analytes was achieved by a YAG 354 nm laser. Size exclusion chromatography (SEC) was performed in LC 1100/1200 Agilent technologies with refractive index detector. The column was 1PL oligopore (300 x 7.5 mm ID) and the eluent DMAc + 2 vol % water + 3 g/L LiCl, 1 mL/min. The calibration standard used was polymethylmethacrylate (PMMA). The samples were dissolved in the eluent (concentration: 2.5 mg/mL).

4.7.2 Antifungal activity assay

All the compounds were tested against *Botrytis cinerea* Pers., *Septoria tritici* Desm., and *Phytophthora infestans* (Mont.) de Bary, in a 96-well microtiter plate assay, according to the fungicide-resistance action committee (FRAC) with modifications.[33] Compounds were tested with five different concentrations in DMSO (125 µM, 41 µM, 14 µM, 4.6 µM, and 1.5 µM). The solvent DMSO was used as a negative control (max. concentration 2.5 %), and the fungicide compound oxocrotonate acid C16 used as reference compound. Seven days after inoculation, the pathogen growth was evaluated by measurement of the optical density (OD) at λ = 405 nm with a microtiter plate reader GENios Pro (Fa. Tecan, 5 measurements per well using multiple reads in a 3 x 3 square). Each experiment was carried out in triplicate. IC_{50} values were calculated from dose/response curves on the basis of sigmoidal curve fitting (four-parameter logistic) using the software SigmaPlot 12.0.

4.7.3 Antibacterial activity assay

***Aliivibrio fischeri* luminescence assay.** The antibacterial activity against *Aliivibrio fischeri* is based on the measurement of the inhibition of bacterial luminescence against a negative control. Bacteria were pregrown on a saline BOSS medium (3 % NaCl w/w) whereby at a certain population density, bacterial luminescence will start. The bacterial suspension was diluted, distributed into 96-well microtiter plates and the respective compounds were applied in two different concentrations (100 µM and 1 µM) in DMSO (2 % v/v) and mixed. The luminescence of bacteria treated with the respective compounds was measured after 24 h incubation at 23 °C in the dark, in relation to controls of untreated bacteria. Each experiment was carried out in triplicate. Chloramphenicol was used as reference compound.[34]

***Bacillus subtilis* fluorescence assay.** The antibacterial activity against *Bacillus subtilis* was determined with a fluorescence based antibacterial growth inhibition assay.[33] The fluorescence was measured on a microtiter plate reader GENios Pro (Fa. Tecan, excitation, 510 nm; emission, 535 nm). The *Bacillus subtilis* strain 168 (PAbrB-IYFP) was maintained on TY (tryptone-yeast extract) medium supplemented with 1 % Bacto-tryptone, 0.5 % Bacto-yeast extract, and 1 % NaCl and chloramphenicol (5 µg/ml). Each experiment was carried out in triplicate. Erythromycin was used as reference compound.[35]

4.7.4 Synthesis

General procedure for the functionalization of PPI by Ugi reaction. In a round-bottom flask were added PPI dendrimer (0.32 mmol, 1.0 equiv.) and the aldehyde (1.42 mmol, 4.5 equiv.) in dry methanol. The mixture was stirred at room temperature for one hour to enable imine formation. The carboxylic acid (1.42 mmol, 4.5 equiv.) and the isocyanide (1.42 mmol, 4.5 equiv.) were added and the mixture was stirred for 3 days. The volatiles were removed under reduced pressure in a rotary evaporator and the crude material purified by flash column chromatography using ethyl acetate/methanol as eluent.

General procedure for the demethylation of aryl methyl ethers. In a two-necks round-bottom flask a solution of the dendrimer in CH_2Cl_2 was cooled to - 78 °C under N_2 atmosphere. 1M BBr_3 in CH_2Cl_2 (1.5 equiv. per methyl ether) was added dropwise into the solution. The mixture was allowed to slowly reach room temperature for 24/48/72

hours. Water was added in the reactional mixture and the precipitated collected and washed several times with water. The product was dried under high vacuum overnight.

General procedure for the demethylation of aryl methyl ethers and deprotection of carboxylic acid or amino group in one pot. In a two-necks round-bottom flask a solution of the dendrimer in CH_2Cl_2 was cooled to - 78 °C under N_2 atmosphere. 1M BBr_3 in CH_2Cl_2 (1.5 equiv. per methyl ether plus 1.5 equiv. per carboxylic acid or amino group) was added dropwise into the solution. The mixture was allowed to slowly reach room temperature for 24/48/72 hours. Water was added in the reactional mixture and the precipitated collected and washed several times with water. The product was dried under high vacuum overnight.

General procedure for the synthesis of polymers by Ugi reaction. In a round-bottom flask were added 1,5-diaminopentane (0.49 mmol, 1.0 equiv.) and the aldehyde (1.22 mmol, 2.5 equiv.) in dry methanol. The mixture was stirred at room temperature for one hour to enable imine formation. The carboxylic acid (1.22 mmol, 2.5 equiv.) and 1,2-diisocyanoethane (0.49 mmol, 1 equiv.) were added and the mixture was stirred for 4 days. The solution was concentrated under reduced pressure in a rotary evaporator and precipitated into cold diethyl ether for three times. The obtained polymer was dried under high vacuum overnight.

***Dendrimer 1a*.** PPI dendrimer (100 mg, 0.32 mmol), isobutyraldehyde (102 mg, 1.42 mmol), (3,4-dimethoxyphenyl)acetic acid (273 mg, 1.42 mmol), and *tert*-butyl isocyanide (118 mg, 1.42 mmol) were reacted in MeOH (20 mL) according to the general procedure for the functionalization of PPI by Ugi reaction (Section 4.7.4, p. 85). Colorless oil. Yield: 448 mg (86 %). MS (MALDI-TOF) of $C_{92}H_{148}N_{10}O_{16}$ $[M+H]^+$ calc. 1650.115, obs. 1650.011; ^{1}H NMR (400 MHz, MeOD) δ 6.95 - 6.75 (m, 12H), 4.34 - 4.19 (m, 4H), 3.85 - 3.34 (m, 40H), 3.30 - 3.09 (m, 4H), 2.45 - 2.29 (m, 12H), 1.78 - 1.40 (m, 12H), 1.34 - 1.13 (m, 36H), 0.94 - 0.68 (m, 24H); ^{13}C NMR (101 MHz, MeOD) δ 175.31, 175.29, 175.18, 175.16, 171.60, 150.83, 150.60, 150.60, 150.56, 149.75, 149.67, 149.67, 149.63, 129.29, 129.23, 129.15, 129.15, 122.69, 122.57, 122.57, 122.18, 114.15, 114.12, 113.59, 113.38, 113.37, 113.13, 113.11, 68.27, 61.51, 56.56, 56.54, 56.53, 56.50, 56.41, 52.04, 41.40, 41.24, 41.18, 28.88, 28.74, 28.43, 28.39, 19.98, 19.95, 19.62, 19.59, 19.26, 19.22, 19.18, 18.77.

Dendrimer 1b. Dendrimer **1a** (100 mg, 0.06 mmol) and 1M BBr_3 in CH_2Cl_2 (0.73 mL, 0.73 mmol, 12 equiv.) were reacted in CH_2Cl_2 according to the general procedure for the

demethylation of aryl methyl ethers (Section 4.7.4, p. 85). Light yellow solid. Yield: 86.7 mg (94 %). MS (MALDI-TOF) of $C_{84}H_{132}N_{10}O_{16}$ $[M+H]^+$ calc.1537.990, obs. 1538.258; ^1H NMR (400 MHz, MeOD) δ 6.84 - 6.59 (m, 12H), 4.28 (s, 4H), 3.95 - 3.56 (m, 16H), 3.47 - 3.39 (m, 4H), 3.19 - 2.94 (m, 12H), 2.38 - 2.23 (m, 4H), 1.93 - 1.69 (m, 8H), 1.42 - 1.17 (m, 36H), 1.01 - 0.75 (m, 24H); ^{13}C NMR (101 MHz, MeOD) δ 175.47, 175.45, 175.42, 175.38, 170.69, 170.65, 170.63, 170.60, 146.96, 146.89, 146.82, 146.74, 145.74, 145.69, 145.50, 145.48, 127.85, 127.84, 127.68, 127.68, 121.35, 121.33, 121.32, 121.30, 117.13, 117.09, 117.05, 116.99, 116.96, 116.84, 116.83, 116.73, 68.29, 52.54, 52.21, 41.14, 40.97, 29.57, 28.89, 28.71, 19.89, 19.49, 19.23, 19.14, 19.12, 19.06, 19.00, 18.98.

Dendrimer 2*.* PPI dendrimer (100 mg, 0.32 mmol), isobutyraldehyde (102 mg, 1.42 mmol), 3,4-dihydroxybenzoic acid (218 mg, 1.42 mmol), and *tert*-butyl isocyanide (118 mg, 1.42 mmol) were reacted in MeOH (20 mL) according to the general procedure for the functionalization of PPI by Ugi reaction (Section 4.7.4, p. 85). Colorless oil. Yield: 163 mg (35 %). MS (MALDI-TOF) of $C_{80}H_{124}N_{10}O_{16}$ $[M+H]^+$ calc. 1481.928, obs. 1481.926;

Dendrimer 3a. PPI dendrimer (100 mg, 0.32 mmol), isobutyraldehyde (102 mg, 1.42 mmol), (3,4-dimethoxyphenyl)acetic acid (273 mg, 1.42 mmol), and 1-isocyanooctane (198 mg, 1.42 mmol) were reacted in MeOH (20 mL) according to the general procedure for the functionalization of PPI by Ugi reaction (Section 4.7.4, p. 85). Colorless oil. Yield: 492 mg (83 %). MS (MALDI-TOF) of $C_{108}H_{180}N_{10}O_{16}$ $[M+Na]^+$ calc. 1896.347, obs. 1896.688; ^1H NMR (400 MHz, MeOD) δ 6.92 - 6.77 (m, 12H), 4.51 - 4.40 (m, 4H), 3.89 - 3.31 (m, 44H), 3.28 - 2.94 (m, 12H), 2.42 - 2.26 (m, 12H), 1.85 - 1.61 (m, 8H), 1.46 - 1.23 (m, 48H), 0.94 - 0.59 (m, 36H); ^{13}C NMR (101 MHz, MeOD) δ 175.05, 174.93, 174.91, 173.75, 172.94, 172.26, 172.17, 172.16, 150.86, 150.58, 150.54, 149.78, 149.62, 149.58, 129.28, 129.22, 129.16, 129.15, 122.71, 122.70, 122.59, 122.42, 114.09, 113.89, 113.22, 113.05, 61.51, 56.54, 56.51, 56.49, 52.32, 45.10, 41.50, 41.14, 41.08, 40.47, 40.33, 33.02, 30.42, 30.41, 30.32, 30.25, 28.94, 28.39, 28.05, 23.75, 20.15, 20.10, 19.78, 19.23, 19.21, 18.80, 14.51.

Dendrimer 3b*.* Dendrimer **3a** (100 mg, 0.05 mmol) and 1M BBr_3 in CH_2Cl_2 (0.64 mL, 0.64 mmol, 12 equiv.) were reacted in CH_2Cl_2 according to the general procedure for the demethylation of aryl methyl ethers (Section 4.7.4, p. 85). Light yellow solid. Yield: 89.6 mg (96 %). MS (MALDI-TOF) of $C_{100}H_{164}N_{10}O_{16}$ $[M+Na]^+$ calc. 1784.222, obs. 1784.422; ^1H NMR (400 MHz, MeOD) δ 6.80 - 6.60 (m, 12H), 4.53 - 4.36 (m, 4H), 3.94 - 3.41 (m,

20H), 3.27 - 2.98 (m, 20H), 2.33 - 2.22 (m, 4H), 1.98 - 1.75 (m, 8H), 1.52 - 1.42 (m, 8H), 1.29 (s, 40H), 0.96 - 0.72 (m, 36H); ^{13}C NMR (101 MHz, MeOD) δ 175.31, 175.28, 175.25, 175.19, 172.47, 171.36, 171.33, 171.29, 146.89, 146.85, 146.82, 146.65, 145.72, 145.67, 145.63, 145.45, 127.73, 127.71, 127.65, 127.61, 121.62, 121.58, 121.55, 121.52, 117.38, 117.36, 117.27, 117.23, 116.70, 116.70, 116.67, 116.64, 68.84, 68.02, 52.51, 41.39, 41.23, 41.08, 40.63, 40.51, 32.98, 30.41, 30.36, 30.30, 30.27, 30.14, 29.27, 28.44, 28.12, 26.48, 23.72, 20.04, 19.66, 19.23, 19.01, 18.97, 14.47.

Dendrimer 4a. PPI dendrimer (100 mg, 0.32 mmol), isobutyraldehyde (102 mg, 1.42 mmol), (3,4-dimethoxyphenyl)acetic acid (273 mg, 1.42 mmol), and methyl 4-isocyanobutanoate (200 mg, 1.42 mmol) were reacted in MeOH (20 mL) according to the general procedure for the functionalization of PPI by Ugi reaction (Section 4.7.4, p. 85). Colorless oil. Yield: 461 mg (80 %). MS (MALDI-TOF) of $C_{96}H_{148}N_{10}O_{24}$ $[M+Na]^+$ calc. 1848.056, obs. 1848.389; ^{1}H NMR (400 MHz, MeOD) δ 6.91 - 6.78 (m, 12H), 4.47 (d, *J* = 11.1 Hz, 4H), 3.84 - 3.35 (m, 58H), 3.21 - 3.15 (m, 6H), 2.44 - 2.24 (m, 20H), 1.82 - 1.63 (m, 16H), 1.49 - 1.39 (m, 4H), 0.91 - 0.63 (m, 24H); ^{13}C NMR (101 MHz, MeOD) δ 175.03, 175.01, 175.00, 174.95, 173.88, 173.83, 172.49, 172.48, 172.39, 172.38, 171.32, 171.30, 150.80, 150.56, 150.54, 149.79, 149.76, 149.61, 149.58, 129.24, 129.19, 122.66, 122.57, 122.49, 122.48, 114.13, 114.10, 113.99, 113.97, 113.20, 113.10, 112.98, 68.00, 65.00, 56.51, 52.28, 52.12, 45.03, 42.91, 41.39, 41.09, 41.04, 39.75, 39.56, 32.02, 29.02, 28.33, 25.63, 25.48, 20.12, 20.08, 19.75, 19.21, 18.80.

Dendrimer 4b. Dendrimer **4a** (137 mg, 0.08 mmol) and 1M BBr_3 in CH_2Cl_2 (1.35 mL, 1.35 mmol, 18 equiv.) were reacted in CH_2Cl_2 according to the general procedure for the demethylation of aryl methyl ethers and deprotection of carboxylic acid or amino group in one pot (Section 4.7.4, p. 86). Light yellow solid. Yield: 110 mg (89 %). MS (MALDI-TOF) of $C_{84}H_{124}N_{10}O_{24}$ $[M+H]^+$ calc. 1657.887, obs. 1658.196; ^{1}H NMR (400 MHz, MeOD) δ 6.82 - 6.61 (m, 12H), 4.56 - 4.35 (m, 4H), 3.95 - 3.65 (m, 16H), 3.23 - 3.02 (m, 16H), 2.40 - 1.67 (m, 36H), 0.98 - 0.67 (m, 24H); ^{13}C NMR (101 MHz, MeOD) δ 175.36, 175.24, 175.16, 172.68, 172.65, 171.57, 171.54, 171.50, 146.87, 146.83, 146.79, 146.61, 145.68, 145.63, 145.58, 145.42, 127.75, 127.67, 127.64, 127.62, 121.66, 121.60, 121.58, 121.24, 117.39, 117.23, 117.18, 117.16, 116.77, 116.70, 116.67, 116.60, 67.95, 52.25, 41.28, 41.22, 41.16, 39.79, 39.65, 32.06, 29.21, 28.27, 25.58, 25.45, 25.43, 20.00, 19.63, 19.13, 18.89, 18.86.

Dendrimer 5a. PPI dendrimer (100 mg, 0.32 mmol), isobutyraldehyde (102 mg, 1.42 mmol), (3,4-dimethoxyphenyl)acetic acid (273 mg, 1.42 mmol), and benzyl (4-

isocyanobutyl)carbamate (330 mg, 1.42 mmol) were reacted in MeOH (20 mL) according to the general procedure for the functionalization of PPI by Ugi reaction (Section 4.7.4, p. 85). Colorless oil. Yield: 490 mg (69 %). MS (MALDI-TOF) of $C_{124}H_{176}N_{14}O_{24}$ $[M+Na]^+$ calc. 2268.287, obs. 2268.095; ^{1}H NMR (400 MHz, MeOD) δ 7.38 - 7.23 (m, 20H), 6.90 - 6.78 (m, 12H), 5.09 - 5.02 (m, 8H), 4.46 (d, *J* = 11.0 Hz, 4H), 3.82 - 3.37 (m, 44H), 3.18 - 3.05 (m, 16H), 2.55 - 2.25 (m, 12H), 1.75 - 1.38 (m, 28H), 0.90 - 0.62 (m, 24H); ^{13}C NMR (101 MHz, MeOD) δ 174.99, 174.93, 173.98, 173.93, 172.34, 172.28, 171.37, 171.22, 158.81, 158.80, 158.77, 158.75, 150.76, 150.74, 150.53, 149.79, 149.75, 149.60, 138.43, 129.47, 129.21, 129.18, 129.07, 128.97, 128.96, 128.95, 128.79, 122.70, 122.63, 122.59, 114.32, 114.17, 114.14, 114.10, 113.25, 113.13, 113.10, 112.98, 68.00, 67.31, 56.63, 56.54, 56.35, 52.15, 45.22, 41.44, 41.36, 41.10, 40.13, 39.96, 29.09, 28.35, 27.51, 27.40, 20.11, 19.77, 19.23, 18.86.

Dendrimer 5b. Dendrimer **5a** (200 mg, 0.09 mmol) and 1M BBr_3 in CH_2Cl_2 (1.6 mL, 1.6 mmol, 18 equiv.) were reacted in CH_2Cl_2 according to the general procedure for the demethylation of aryl methyl ethers and deprotection of carboxylic acid or amino group in one pot (Section 4.7.4, p. 86). Light yellow solid. Yield: 134 mg (94 %). MS (MALDI-TOF) of $C_{84}H_{136}N_{14}O_{16}$ $[M+Na]^+$ calc. 1620.014, obs. 1619.917; ^{1}H NMR (400 MHz, MeOD) δ 6.86 - 6.54 (m, 12H), 4.41 (d, *J* = 11.0 Hz, 4H), 4.02 - 3.33 (m, 24H), 3.25 - 2.92 (m, 24H), 2.05 - 1.45 (m, 28H), 1.12 - 0.58 (m, 24H); ^{13}C NMR (101 MHz, MeOD) δ 175.40, 175.35, 175.30, 171.58, 171.55, 171.49, 146.67, 146.65, 146.55, 146.51, 145.58, 145.55, 145.43, 145.38, 127.88, 127.82, 127.76, 127.71, 121.86, 121.82, 121.62, 121.50, 117.34, 117.32, 117.25, 117.22, 116.82, 116.76, 116.65, 116.30, 67.93, 67.89, 52.51, 41.39, 41.34, 41.16, 40.45, 40.21, 40.21, 40.11, 39.89, 39.73, 29.30, 29.28, 28.34, 27.22, 27.11, 26.04, 25.97, 25.60, 20.23, 19.74, 19.61, 19.37, 19.00, 18.96.

***Dendrimer 6a*.** PPI dendrimer (100 mg, 0.32 mmol), isobutyraldehyde (102 mg, 1.42 mmol), (3,4-dimethoxyphenyl)acetic acid (273 mg, 1.42 mmol), and 4-(2-isocyanoethyl)-1,2-dimethoxybenzen (271 mg, 1.42 mmol) were reacted in MeOH (20 mL) according to the general procedure for the functionalization of PPI by Ugi reaction (Section 4.7.4, p. 85). Colorless oil. Yield: 454 mg (69 %). MS (MALDI-TOF) of $C_{116}H_{164}N_{10}O_{24}$ $[M+Na]^+$ calc. 2104.181, obs. 2104.541; ^{1}H NMR (400 MHz, MeOD) δ 6.88 - 6.65 (m, 24H), 4.46 - 4.30 (m, 4H), 3.80 - 3.73 (m, 48H), 3.72 - 3.32 (m, 24H), 2.71 - 2.23 (m, 24H), 1.66 - 1.40 (m, 12H), 0.83 - 0.56 (m, 24H); ^{13}C NMR (101 MHz, MeOD) δ 174.88, 174.81, 172.95, 172.29, 172.19, 150.80, 150.77, 150.54, 150.47, 149.83, 149.78, 149.61, 149.24, 149.22, 149.13, 133.25, 133.03, 129.14, 129.13, 122.76, 122.66, 122.27, 122.18, 114.28, 114.26, 114.18, 114.14, 114.09, 113.92, 113.83, 113.24, 113.12,

113.08, 67.99, 61.52, 56.58, 56.53, 56.50, 52.29, 44.97, 41.93, 41.42, 41.39, 41.21, 41.06, 41.03, 36.00, 28.35, 28.18, 20.85, 20.09, 19.73, 19.16, 18.78.

Dendrimer 6b. Dendrimer **6a** (100 mg, 0.05 mmol) and 1M BBr_3 in CH_2Cl_2 (1.52 mL, 1.52 mmol, 24 equiv.) were reacted in CH_2Cl_2 according to the general procedure for the demethylation of aryl methyl ethers (Section 4.7.4, p. 85). Light yellow solid. Yield: 85.6 mg (96 %). MS (MALDI-TOF) of $C_{100}H_{132}N_{10}O_{24}$ $[M+Na]^+$ calc. 1879.931, obs. 1880.226; ^{1}H NMR (400 MHz, MeOD) δ 6.81 - 6.46 (m, 24H), 3.87 - 3.41 (m, 20H), 3.05 - 1.64 (m, 44H), 0.90 - 0.60 (m, 24H); ^{13}C NMR (101 MHz, MeOD) δ 175.25, 175.23, 175.15, 175.12, 172.40, 171.27, 171.26, 171.20, 146.83, 146.72, 146.53, 146.26, 146.20, 146.17, 145.67, 145.60, 145.41, 145.38, 144.74, 144.69, 132.19, 132.14, 131.85, 131.81, 127.69, 127.68, 127.59, 126.92, 121.61, 121.46, 117.33, 117.26, 117.21, 117.05, 116.68, 116.48, 116.43, 116.30, 67.94, 52.36, 49.84, 41.97, 41.89, 41.73, 41.23, 41.15, 35.72, 35.56, 29.07, 29.05, 28.33, 28.31, 23.69, 20.07, 19.97, 19.58, 19.03, 18.73, 18.71.

Dendrimer 7a. PPI dendrimer (100 mg, 0.32 mmol), 3,4-dimethoxybenzaldehyde (236 mg, 1.42 mmol), (3,4-dimethoxyphenyl)acetic acid (273 mg, 1.42 mmol), and 4-(2-Iiocyanoethyl)-1,2-dimethoxybenzen (271 mg, 1.42 mmol) were reacted in MeOH (20 mL) according to the general procedure for the functionalization of PPI by Ugi reaction (Section 4.7.4, p. 85). Colorless oil. Yield: 451 mg (58 %). MS (MALDI-TOF) of $C_{136}H_{172}N_{10}O_{32}$ $[M+Na]^+$ calc. 2480.203, obs. 2480.613; ^{1}H NMR (400 MHz, MeOD) δ 6.96 - 6.61 (m, 36H), 5.73 (d, *J* = 5.5 Hz, 4H), 3.97 - 3.33 (m, 100H), 2.77 - 2.62 (m, 8H), 2.11 - 1.84 (m, 8H), 1.42 - 0.94 (m, 12H); ^{13}C NMR (101 MHz, MeOD) δ 174.37, 174.34, 174.32, 174.30, 172.04, 172.02, 171.15, 171.10, 150.86, 150.80, 150.55, 150.44, 150.35, 150.17, 149.82, 149.47, 149.15, 148.98, 148.90, 133.32, 133.11, 131.71, 129.28, 127.77, 123.82, 123.80, 123.78, 123.76, 122.91, 122.88, 122.62, 122.58, 122.56, 122.50, 122.48, 122.41, 122.36, 122.31, 122.20, 122.09, 114.64, 114.61, 114.59, 114.34, 114.12, 113.93, 113.85, 113.85, 113.31, 113.15, 113.10, 112.98, 112.78, 112.76, 112.63, 112.09, 65.37, 63.92, 56.54, 56.52, 56.49, 56.41, 56.36, 56.33, 51.77, 51.44, 46.28, 45.02, 44.98, 42.09, 41.91, 41.71, 41.08, 41.02, 35.89, 35.76.

Dendrimer 7b. Dendrimer **7a** (100 mg, 0.04 mmol) and 1M BBr_3 in CH_2Cl_2 (1.46 mL, 1.46 mmol, 36 equiv.) were reacted in CH_2Cl_2 according to the general procedure for the demethylation of aryl methyl ethers (Section 4.7.4, p. 85). Light yellow solid. Yield: 79 mg (93 %). MS (MALDI-TOF) of $C_{112}H_{124}N_{10}O_{32}$ $[M+H]^+$ calc. 2121.846, obs. 2122.139; ^{1}H NMR (400 MHz, MeOD) δ 6.78 - 6.38 (m, 36H), 5.71 - 5.57 (m, 4H), 3.79 - 3.32 (m,

28H), 2.81 - 2.56 (m, 16H), 1.51 - 1.17 (m, 12H); ^{13}C NMR (101 MHz, MeOD) δ 176.03, 175.98, 175.74, 175.72, 174.77, 174.75, 171.51, 171.49, 147.21, 147.05, 146.73, 146.68, 146.65, 146.62, 146.23, 146.13, 145.56, 145.32, 144.77, 144.69, 131.99, 131.79, 127.74, 127.20, 127.16, 126.86, 126.69, 126.39, 122.49, 121.57, 121.57, 121.28, 117.74, 117.69, 117.66, 117.10, 116.96, 116.84, 116.77, 116.42, 65.48, 63.94, 51.68, 44.64, 42.53, 42.48, 42.45, 42.15, 42.14, 41.50, 35.76, 35.75, 35.65, 24.22, 24.19, 24.18.

***Dendrimer 8a*.** PPI dendrimer (100 mg, 0.32 mmol), 2,3,4-trimethoxybenzaldehyde (278 mg, 1.42 mmol), isobutyric acid (125 mg, 1.42 mmol), and *tert*-butyl isocyanide (118 mg, 1.42 mmol) were reacted in MeOH (20 mL) according to the general procedure for the functionalization of PPI by Ugi reaction (Section 4.7.4, p. 85). Yellow oil. Yield: 271 mg (50 %). MS (MALDI-TOF) of $C_{92}H_{148}N_{10}O_{20}$ $[M+H]^+$ calc. 1714.095, obs. 1714.389; ^{1}H NMR (400 MHz, MeOD) δ 7.03 - 6.98 (m, 4H), 6.86 - 6.80 (m, 4H), 5.88 (s, 4H), 3.96 - 3.79 (m, 36H), 3.24 - 2.42 (m, 24H), 1.33 (dd, *J* = 9.9, 1.8 Hz, 36H), 1.25 - 1.17 (m, 12H), 1.15 - 1.08 (m, 24H); ^{13}C NMR (101 MHz, MeOD) δ 183.17, 180.93, 180.70, 180.01, 171.91, 171.57, 171.48, 171.44, 156.33, 156.27, 156.14, 155.86, 153.94, 153.89, 153.85, 153.83, 143.08, 143.06, 143.04, 142.97, 125.47, 125.39, 125.05, 125.02, 122.17, 122.13, 121.97, 121.96, 108.29, 108.28, 108.27, 108.24, 61.51, 61.47, 61.21, 60.38, 60.27, 56.68, 56.63, 52.32, 52.19, 51.72, 36.24, 32.15, 32.04, 31.91, 28.92, 28.81, 24.83, 24.63, 20.43, 20.15, 19.92.

Dendrimer 8b. Dendrimer **8a** (10 mg, 5 µmol) and 1M BBr_3 in CH_2Cl_2 (100 µL, 100 µmol, 18 equiv.) were reacted in CH_2Cl_2 according to the general procedure for the demethylation of aryl methyl ethers (Section 4.7.4, p. 85). Light yellow solid. Yield: 5.4 mg (64 %). MS (MALDI-TOF) of $C_{80}H_{124}N_{10}O_{20}$ $[M+H]^+$ calc. 1545.907, obs. 1546.200; ^{1}H NMR (400 MHz, MeOD) δ 6.74 - 6.41 (m, 8H), 6.04 (s, 4H), 3.28 - 2.46 (m, 24H), 2.03 - 1.49 (m, 12H), 1.36 (s, 36H), 1.33 - 0.99 (m, 24H).

Polymer 9. 1,5-Diaminopentane (50 mg, 0.49 mmol), isobutyraldehyde (88 mg, 1.22 mmol), 3,4-dihydroxybenzoic acid (188 mg, 1.22 mmol), and 1,2-diisocyanoethane (39 mg, 0.49 mmol) were reacted in MeOH according to the general procedure for the synthesis of polymers by Ugi reaction (Section 4.7.4, p. 86). Light yellow solid. SEC: M_n = 4900, M_w = 7400, Đ = 1.51; ^{1}H NMR (400 MHz, MeOD) δ 7.42 - 6.76 (m, 3H), 4.31 (s, 1H), 4.02 - 3.33 (m, 5H), 3.30 - 2.98 (m, 2H), 2.58 - 1.93 (m, 2H), 1.65 - 0.58 (m, 12H).

***Polymer 10*.** 1,5-Diaminopentane (50 mg, 0.49 mmol), isobutyraldehyde (88 mg, 1.22 mmol), (3,4-dimethoxyphenyl)acetic acid (240 mg, 1.22 mmol), and 1,2-diisocyanoethane (39 mg, 0.49 mmol) were reacted in MeOH according to the general procedure for the synthesis of polymers by Ugi reaction (Section 4.7.4, p. 86). Light yellow solid. SEC: M_n = 5500, M_w = 8900, Đ = 1.62; ^{1}H NMR (400 MHz, MeOD) δ 6.91 - 6.77 (m, 3H), 4.42 (s, 1H), 3.89 - 3.33 (m, 12H), 3.29 - 3.09 (m, 3H), 2.52 - 2.10 (m, 2H), 1.57 - 0.59 (m, 12H).

***Polymer 11*.** 1,5-Diaminopentane (50 mg, 0.49 mmol), isobutyraldehyde (88 mg, 1.22 mmol), 3,4-diacetyloxybenzoic acid (291 mg, 1.22 mmol), and 1,2-diisocyanoethane (39 mg, 0.49 mmol) were reacted in MeOH according to the general procedure for the synthesis of polymers by Ugi reaction (Section 4.7.4, p. 86). Light yellow solid. SEC: M_n = 5400, M_w = 8200, Đ = 1.52; ^{1}H NMR (400 MHz, MeOD) δ 7.34 - 6.76 (m, 3H), 4.39 (s, 1H), 3.85 - 3.31 (m, 5H), 3.28 - 3.12 (m, 1H), 2.65 - 1.79 (m, 8H), 1.49 - 0.76 (m, 12H).

4.8 References

(1) Waite, J. H.; Tanzer, M. L. *Science* **1981**, 212, 1038-1040.

(2) Hong, S.; Yang, K.; Kang, B.; Lee, C.; Song, I. T.; Byun, K. I. P.; Cho, S.; Lee, H. *Adv. Funct. Mater*. **2013**, 23, 1774-1780.

(3) You, I.; Kang, S. M.; Byun, Y.; Lee, H. *Bioconjugate Chem*. **2011**, 22, 1264-1269.

(4) Wang, R.; Xie, Y.; Xiang, T.; Suna, S.; Zhao, C. *J. Mater. Chem. B* **2017**, 5, 3035-3046.

(5) Lee, B. P.; Dalsin, J.; Messersmith, P. B. *Biomacromolecules* **2002**, 3, 1038-1047.

(6) Guvendiren, M.; Messersmith, P. B.; Shull, K. R. *Biomacromolecules* **2008**, 9, 122-128.

(7) Guvendiren, M.; Brass, D. A.; Messersmith, P. B.; Shull, K. R. *J. Adhes*. **2009**, 85, 631-645.

(8) Wang, J.; Tahir, M. N.; Kappl, M.; Tremel, W.; Metz, N.; Barz, M.; Theato, P.; Butt, H-J. *Adv. Mater*. **2008**, 20, 3872-3876.

(9) Krysiak, S.; Wei, Q.; Rischka, K.; Hartwig, A.; Haag, R.; Hugel, T. *Beilstein J. Org. Chem*. **2015**, 11, 828-836.

(10) Kinugawa, S.; Wang, S.; Taira, S.; Tsuge, A.; Kaneko, D. *Polymer J.* **2016**, 48, 715-721.

(11) Das, P.; Reches, M. *Nanoscale* **2016**, 8, 15309-15316.

(12) Forooshani, P. K.; Lee, B. P. *J. Polym. Sci., Part A: Polym. Chem*. **2017**, 55, 9-33.

(13) Schweigert, N.; Zehnder, A. J. B.; Eggen, R. I. L. *Environ. Microbiol.* **2001**, 3, 81-91.

(14) Nie, C.; Cheng, C.; Peng, Z.; Ma, L.; He, C.; Xiaa, Y.; Zhao, C. *J. Mater. Chem. B* **2016**, 4, 2749-2756.

(15) Fullenkamp, D. E.; Rivera, J. G.; Gong, Y.; Lau, K. H. A.; He, L.; Varshney, R.; Messersmith, P. B. *Biomaterials* **2012**, 33, 3783-3791.

(16) Nguyen, H.-L.; Jo, Y. K.; Cha, M.; Cha, Y. J.; Yoon, D. K.; Sanandiya, N. D.; Prajatelistia, E.; Oh, D. X.; Hwang, D. S. *Polymers* **2016**, 8, 102-114.

(17) Oh, Y. J.; Jeong, C. J.; Sharker, S. M.; Lee, S. Y.; In, I.; Park, S. Y. *Surf. Interface Anal*. **2015**, 47, 259-264.

(18) Liu, S. Q.; Yang, C.; Huang, Y.; Ding, X.; Li, Y.; Fan, W. M.; Hedrick, J. L.; Yang, Y. Y. *Adv Mater*. **2012**, 24, 6484-6489.

(19) Kim, H. S.; Ham, H. O.; Son, Y. J.; Messersmith, P. B.; Yoo, H. S. *J. Mater. Chem. B* **2013**, 1, 3940-3949.

(20) Brubakera, C. E.; Kisslerc, H.; Wangc, L.; Kaufmanb, D. B.; Messersmith, P. B.

Biomaterials **2010**, 31, 420-427.

(21) Faure, E.; Falentin-Daudré, C.; Jérôme, C; Lyskawa, J.; Fournier, D.; Woisel, P.; Detrembleur, C. *Prog. Polym. Sci.* **2013**, 38, 236-270.

(22) Su, J.; Chen, F.; Cryns, V. L.; Messersmith, P. B. *J. Am. Chem. Soc*. **2011**, 133, 11850-11853.

(23) Zhang, H.; Bré, L.; Zhao, T.; Newland, B.; Da Costa, M.; Wang, W. *J. Mater. Chem. B*, **2014**, 2, 4067-4071.

(24) Lee, S-B.; González-Cabezas, C.; Kim, K-M.; Kim, K-N.; Kuroda, K. *Biomacromolecules* **2015**, 16, 2265−2275.

(25) Lee, B. P.; Huang, K.; Nunalee, F. N.; Shull, K.; Messersmith, P. B. *J. Biomater. Sci. Polym. Ed*. **2004**, 15, 449-464.

(26) Matos-Pérez, C. R.; Wilker, J. J. *Macromolecules* **2012**, 45, 6634-6639.

(27) Postma, A.;Yan, Y.; Wang, Y.; Zelikin, A. N.; Tjipto, E.; Caruso, F. *Chem. Mater.* **2009**, 21, 3042-3044.

(28) Wessjohann, L. A.; Henze, M.; Kreye, O.; Rivera, D. G. DE 10 2010 018 882 A1 (03.11.**2011**), WO 2011/134607 A1 (03.11.**2011**).

(29) McOmie, J. F. W.; Watts, M. L.; West, D. E. *Tetrahedron* **1968**, 25, 2289-2292.

(30) Sehlinger, A.; Dannecker, P-K.; Kreye, O.; Meier, M. A. R. *Macromolecules* **2014**, 47, 2774−2783.

(31) Liao, L.; Zhou, J.; Wang, H.; He, F.; Liu, S.; Jiang, Z.; Chen, S.; Zhang, L-H. *Sci. Rep.* **2015**, 5, 1-11.

(32) Badreddine, I.; Lafitte, C.; Heux, L.; Skandalis, N.; Spanou, Z.; Martinez, Y.; Esquerré-Tugayé, M-T.; Bulone, V.; Dumas, B.; Bottin, A. *Eukaryot. Cell* **2008**, 7, 1980-1993.

(33) Otto, A.; Laub, A.; Porzel, A.; Schmidt, J.; Wessjohann, L.; Westermann, B.; Arnold, N. *Eur. J. Org. Chem*. **2015**, 7449-7459.

(34) Neves Filho, R. A. W.; Stark, S.; Westermann, B.; Wessjohann, L. A. *Beilstein J. Org. Chem*. **2012**, 8, 2085-2090.

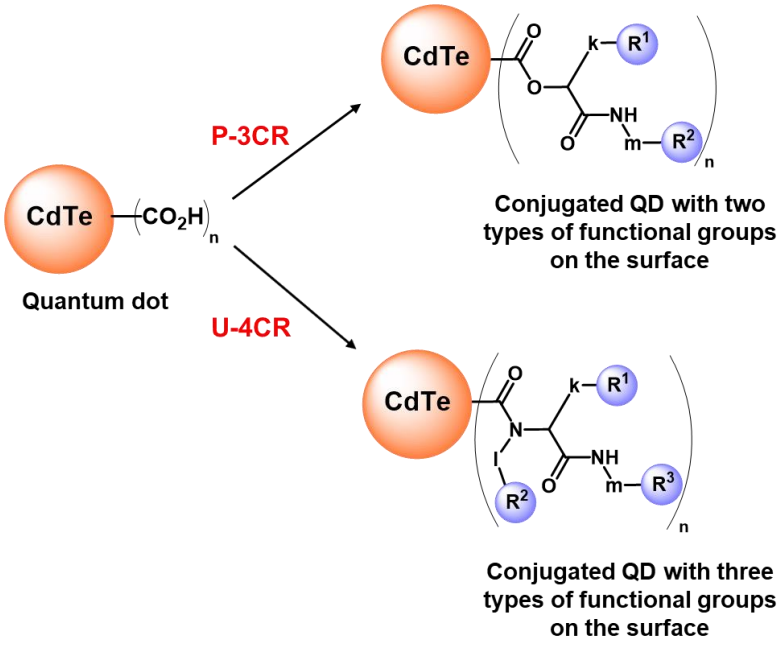
(35) Veening, J. W.; Smits, W. K.; Hamoen, L. W.; Jongbloed, J. D. H.; Kuipers, O. P. *Appl. Environ. Microbiol*. **2004**, 70, 6809-6815.

(36) Heinke, R.; Franke, K.; Porzel, A.; Wessjohann, L. A.; Ali, N. A. A.; Schmidt, J. *Phytochemistry* **2011**, 72, 929-934.

Chapter 5

Isocyanide-based multicomponent reaction as a powerful tool for the covalent surface functionalization of CdTe quantum dots

Abstract



Surface functionalizations of quantum dots are essential for the development of fluorescent probes with biological and chemical applications. The prospect of adding up to three different functionalities in one single MCR procedure will allow to tune all the desired properties by programming the surface functional group composition. Isocyanide-based multicomponent reactions have all the requirements for the covalent conjugation to quantum dots. Thus, herein we reported the development of a new versatile strategy for the functionalization of QDs *via* Passerini (P-3CR) and Ugi (U-4CR) reactions.

5.1 Introduction

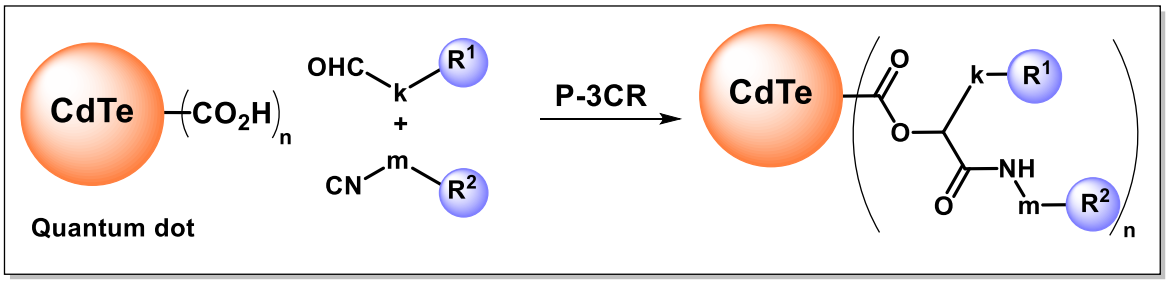
In the last decade, the fluorescent nanocrystal quantum dots have attracted great interest in the chemical and biomedical fields, mostly due to their unique optical, electrical and mechanical properties, associated with the possibility of targeting the desired applications by selecting the molecules conjugated with them.[1-3] As a result of this conjugation, a new exclusively nanomolecular structure is obtained assembling the properties and applications of both compounds.[4-7]

One of the main procedures for QD functionalization is the covalent conjugation using zero-length crosslinkers (e.g. carbodiimides) that connect a carboxyl group to an amino group without adding additional atoms or spacers.[8,9] Other common reagents used for the covalent conjugation are homobifunctional (e.g. dithiobis(succinimidylpropionate) and heterobifunctional (e.g. *N*-succinimidyl-3-(2-pyridyldithio)propionate and sulfosuccinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate) crosslinkers that, unlike zero-length crosslinkers, add an additional spacer between the QD and the molecule.[10] Homobifunctional crosslinkers have the same functional group on both ends of an alkyl spacer and can connect two molecules with the same reactive groups.[11] Heterobifunctional crosslinkers have two different functionalities and can connect two molecules with different functional groups in the quantum dot.[12]

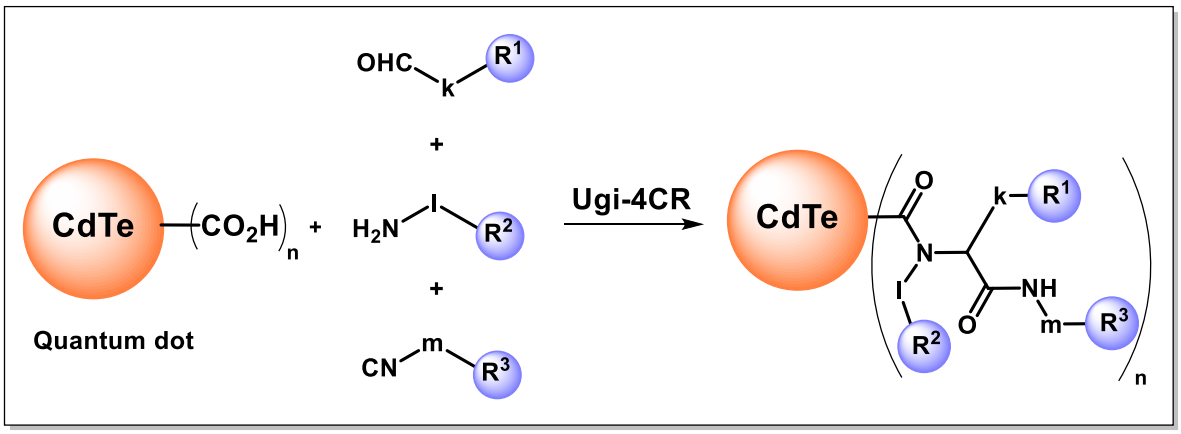
However, independently which type of crosslinker is selected for the covalent conjugation of QDs, side reactions and often the need of more than two-step procedures to achieve the desired product,[13,14] renders the development of new strategies for assembling complex quantum dots coated with multiples biomolecules extremely important. With the aim of covering this limitation, isocyanide-based multicomponent reactions are a powerful synthetic alternative, since they can introduce up to three different functionalities in the quantum dot in a simple, fast, and one pot procedure. This allows to decrease the chances of secondary product formation, lowers reaction time, reduces purification steps, and as consequence, increases the overall yields.[15,16]

5.2 Synthetic Strategy

Herein, we report a new powerful strategy for the covalent surface functionalization of CdTe quantum dots with carboxyl groups on the surface by Passerini three-component (**Scheme 1**) and Ugi four-component reactions (**Scheme 2**). A key feature of this methodology is the simultaneous incorporation of two or three different functional groups in one pot procedures. Our initial proof-of-concept experiments establish a new strategy for the functionalization of QDs.



Scheme 1. Strategy for the covalent functionalization by Passerini reaction. k, m = spacer (e.g. aliphatic chains); R^1, R^2 = functional groups (e.g. amino groups).



Scheme 2. Strategy for the covalent functionalization by Ugi reaction. k, i, m = spacer (e.g. aliphatic chains); R^1, R^2 = functional groups (e.g. amino groups).

5.3 Covalent surface functionalization of quantum dots by IMCR

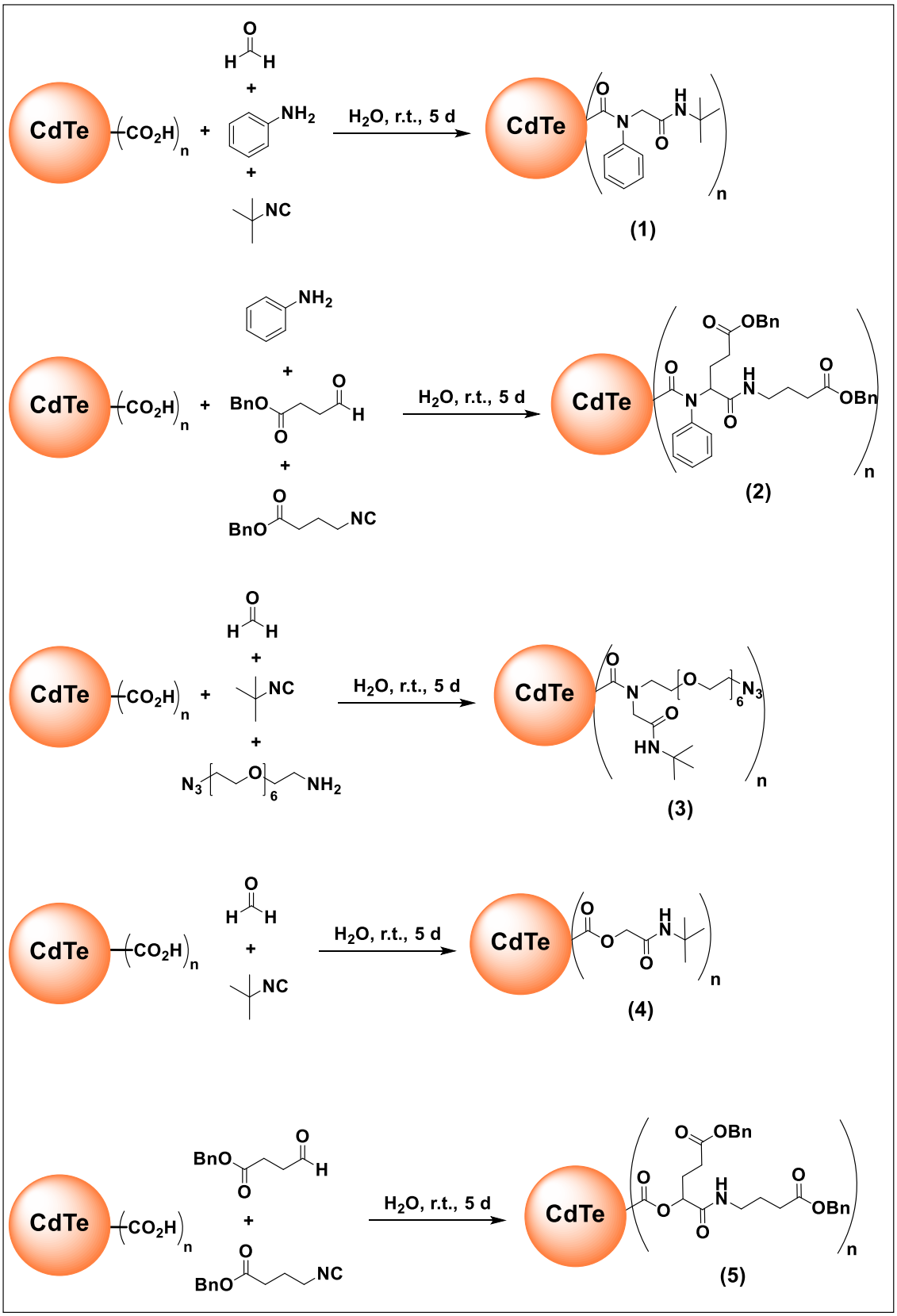
In order to establish the use of Passerini and Ugi reactions for the covalent surface functionalization of QDs, we selected the commercially available core-type carboxyl functionalized CdTe quantum dots (purchased from Sigma-Aldrich) with fluorescence λem at 570 nm and 36 % of quantum yield as a representative case study material.

For the Ugi reactions, three examples were synthesized using the QD-COOH and different amines, aldehydes, and isocyanides. The reactions were performed at room temperature for 5 days in water, followed by purification to remove unreacted amines, aldehydes and isocyanide, using ultracentrifugation filter units with ultracel, regenerated cellulose, and a nominal molecular weight limit (NMWL) of 30 kDa (10 times with water and 5 times with water/methanol 1:1) to afford the conjugated quantum dots **1** - **3** (**Scheme 3**). For the Passerini reaction, two reactions were carried out with different aldehydes and isocyanides, under the same conditions and purification method as for the Ugi reaction to yield the conjugated QDs **4** and **5** (**Scheme 3**).

After the purification, all the functionalized quantum dots (**1** - **5**) were analysed by Fourier transform infrared spectroscopy (FTIR spectroscopy) in comparison to the unreacted QD. In order to confirm that no unreacted amine, aldehyde or isocyanide remained in the analysed samples, TLC and ESI-MS were performed after the last ultracentrifugation, also in comparison with the starting materials.

Depicted in **Figure 1** are the infrared spectra of the conjugates **2**, **3**, **5**, and the commercial quantum dot. It is possible to observe in the spectrum of compound **2** (**Figure 1a**) the presence of a strong absorption at 1729 cm^{-1} characteristic for carbonyl (C=O) stretch, some bands between 1255 - 1068 cm^{-1} characteristic for C-O stretch, and multiple bands in the area of 1600 - 1500 cm^{-1} characteristic for aromatic (C=C) stretch, which is not present in the spectrum of the commercial QD (**Figure 1d**). In the spectrum of compound **3** (**Figure 1b**) two new strong infrared bands appear at 2103 cm^{-1} and 1066 cm^{-1}, being the first one related with the presence of the azide group (N=N=N stretch) and the second one with the presence of ethers (C-O stretch) in the quantum dot after the Ugi reaction. The Passerini reaction with an aldehyde and an isocyanide containing carbonyl groups caused an appearance of a new infrared band at 1733 cm^{-1} (weak) related with C=O stretch in the spectrum of compound **5** (**Figure 1c**).

Another important fact to consider is that the quantum dot COOH functionalized is not soluble in organic solvent, but after the Ugi reaction the compound **2** became completely soluble in methanol and the compound **3** slightly soluble in the same solvent, ratifying the occurrence of the reactions.



Scheme 3. Surface modified QDs **1** - **3** from Ugi reaction and **4** - **5** from Passerini reaction.

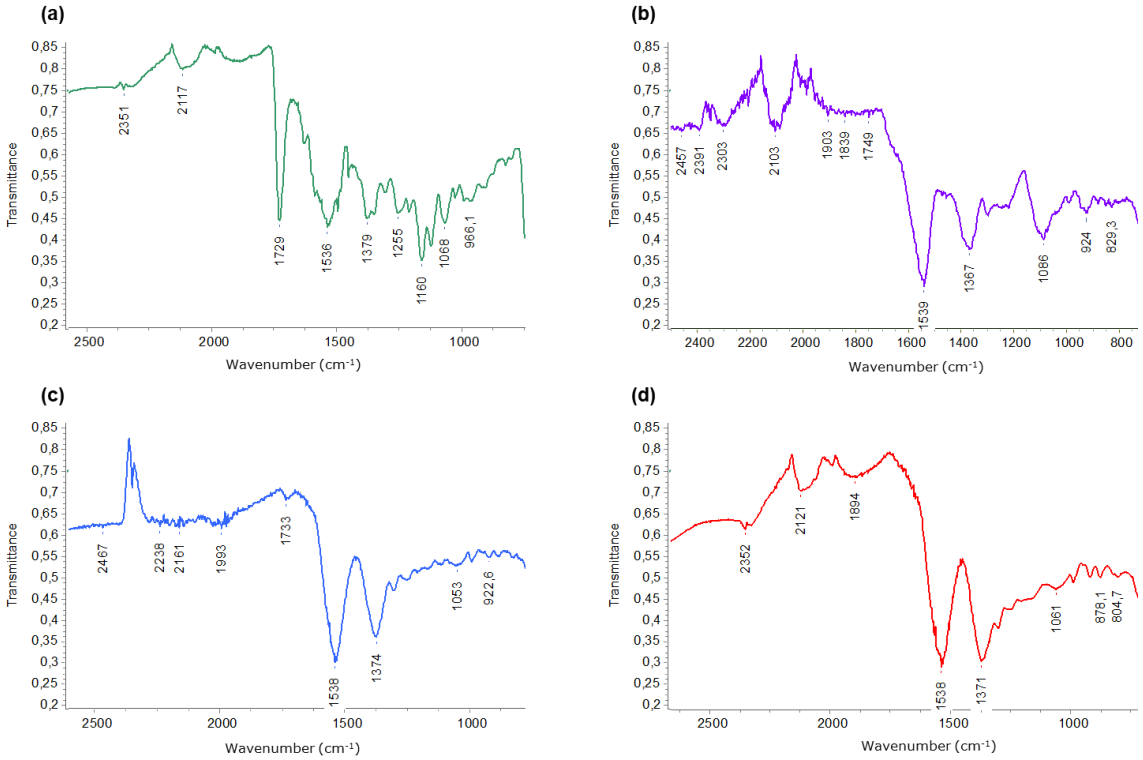


Figure 1. FTIR of (a) QD **2**, (b) QD **3**, (c) QD **5**, (d) unreacted quantum dot.

As a representative example, the fluorescence emission spectra (excitation wavelength at 530 nm) of quantum dot **2** and unreacted quantum dot is depicted in **Figure 2**. The fluorescence intensity of unreacted QD and product **2** were measured relatively at the same concentration (0.5 mg/mL) in the wavelength range from 450 to 750 nm. There is a significant decrease after the Ugi reaction (approx. 50 %) in fluorescence at 570 nm wavelength, which serves as further proof that the conjugation by Ugi multicomponent reaction occurred. Additionally, measurements performed at different concentrations followed the same pattern (see **Attachment S31**).

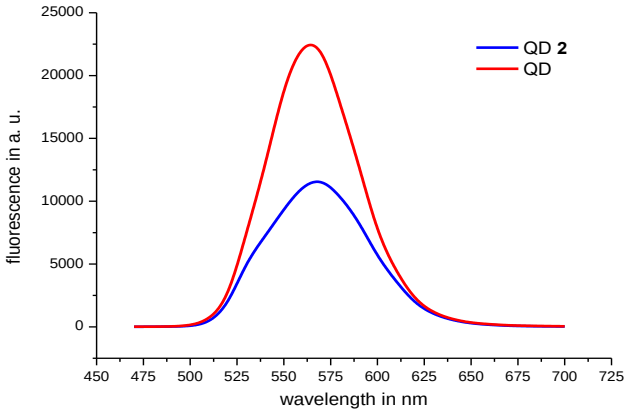


Figure 2. Fluorescence emission spectra (λexc = 530 nm) of quantum dot **2** and unreacted quantum dot (0.5 mg/mL).

5.4 Conclusion

In our proof-of-concept study, core-type carboxyl functionalized CdTe quantum dots was successfully conjugated with two or three different types of functional groups in one-step procedure by Ugi and Passerini multicomponent reactions. Further studies will be performed to determine the loading density of the conjugation and the scope of the method. With the development of this approach, we expect to facilitate conjugations with bioactive molecules, as well as the tuning of desired properties.

5.5 Experimental part

5.5.1 General information

Core-type carboxyl functionalized CdTe quantum dots with fluorescence λem at 570 nm was purchased from Sigma-Aldrich (Germany). Formaldehyde, *tert*-butyl isocyanide, and aniline were purchased from Sigma-Aldrich (Germany) or Alfa Aesar (Germany) and were used without further purification. Amicon Ultra 0.5 mL centrifugal filter units with ultracel, regenerated cellulose NMWL of 30 kDa were purchased from Sigma-Aldrich (Germany). FTIR spectra were measured from 250 to 4000 cm^{-1} with a Thermo Nicolet 5700 FT-IR spectrometer with diamond ATR. Analytical thin-layer chromatography (TLC) was performed using Merck silica gel 60 F254 aluminium sheets. Electrospray ionization mass spectra (ESI-MS) were recorded on a API 3200 system, Triple Quadrupole MS (AB Sciex) equipped with ESI electrospray ion source (positive spray voltage 5.5 kV, negative spray voltage 4.5 kV, and source heater temperature 400 °C). Spectramax from Molecular Devices was used to acquire absorbance and fluorescence spectra using acrylic semi-micro cuvette (1.6 mL, 10 x 4 x 45 mm) at 25°C.

5.5.2 Synthesis

General procedure for the covalent functionalization of QDs by Ugi reaction. In an Eppendorf were added 1 mg of QD-COOH (approx. 0.017 µmol) and the amine (1.7 µmol, 100 equiv.) in water as solvent (500 µL). The mixture was stirred at room temperature for 15 minutes. Then, the aldehyde (1.7 µmol, 100 equiv.) and the isocyanide (1.7 µmol, 100 equiv.) were added and the contents stirred for 5 days at room temperature in the dark. After this time, the unreacted and reacted quantum dots were

purified using ultracentrifugation filter units with NMWL of 30 kDa at 12000 rpm for 5 minutes (10 times with distilled water and 5 times with water/methanol 1:1).

General procedure for the covalent functionalization of QDs by Passerini reaction. In an Eppendorf were added 1 mg of QD-COOH (approx. 0.017 µmol), the aldehyde (1.7 µmol, 100 equiv.), and the isocyanide (1.7 µmol, 100 equiv.). The mixture was stirred in water (500 µL) for 5 days at room temperature in the dark. After this time, the unreacted and reacted quantum dots were purified using ultracentrifugation filter units with NMWL of 30 kDa at 12000 rpm for 5 minutes (10 times with distilled water and 5 times with water/methanol1:1).

Quantum dot 1. QD-COOH (1 mg, approx. 0.017 µmol), aniline (0.16 mg, 1.7 µmol), formaldehyde (0.06 mg, 1.7 µmol), and *tert*-butyl isocyanide (0.14 mg, 1.7 µmol) were reacted in water (500 µL) according to the general procedure for the covalent functionalization of QDs by Ugi reaction (Section 5.5.2, p. 101).

Quantum dot 2. QD-COOH (1 mg, approx. 0.017 µmol), aniline (0.16 mg, 1.7 µmol), benzyl 4-oxobutanoate (0.32 mg, 1.7 µmol), and benzyl 4-isocyanobutanoate (0.34 mg, 1.7 µmol) were reacted in water (500 µL) according to the general procedure for the covalent functionalization of QDs by Ugi reaction (Section 5.5.2, p. 101).

Quantum dot 3. QD-COOH (1 mg, approx. 0.017 µmol), 2-[2-(2-azidoethoxy)ethoxy]ethanamine (0.58 mg, 1.7 µmol), formaldehyde (0.06 mg, 1.7 µmol), and *tert*-butyl isocyanide (0.14 mg, 1.7 µmol) were reacted in water (500 µL) according to the general procedure for the covalent functionalization of QDs by Ugi reaction (Section 5.5.2, p. 101).

Quantum dot 4. QD-COOH (1 mg, approx. 0.017 µmol), formaldehyde (0.06 mg, 1.7 µmol), and *tert*-butyl isocyanide (0.14 mg, 1.7 µmol) were reacted in water (500 µL) according to the general procedure for the covalent functionalization of QDs by Passerini reaction (Section 5.5.2, p. 102).

Quantum dot 5. QD-COOH (1 mg, approx. 0.017 µmol), benzyl 4-oxobutanoate (0.32 mg, 1.7 µmol), and benzyl 4-isocyanobutanoate (0.34 mg, 1.7 µmol) were reacted in water (500 µL) according to the general procedure for the covalent functionalization of QDs by Passerini reaction (Section 5.5.2, p. 102).

5.6 References

(1) Medintz, I. L.; Uyeda, H. T.; Goldman, E. R.; Mattoussi, H. *Nat. Mater*. **2005**, 4, 435-446.

(2) Algar, W. R.; Tavares, A. J.; Krull, U. J. *Analytica Chimica Acta* **2010**, 673, 1-25.

(3) Hildebrandt, N.; Spillmann, C. M.; Algar, W. R.; Pons, T.; Stewart, M. H.; Oh, E.; Susumu, K.; Díaz, S. A.; Delehanty, J. B.; Medintz, I. L. *Chem. Rev***. 2017**, 117, 536−711.

(4) (a) Johari-Ahar, M.; Barar, J.; Alizadeh, A. M.; Davaran, S.; Omidi, Y.; Rashidi, M-R. *J. Drug Target*. **2016**, 24, 120-133. (b) Birma, I.; Bwatanglang; Mohammad, F.; Yusof, N. A.; Abdullah, J.; Hussein, M. Z.; Alitheen, N. B.; Abu, N. *Int. J. Nanomed.* **2016**, 11, 413-428.

(5) (a) Liua, J. L.; Walpera, S. A.; Turnera, K. B.; Leeb, A. B.; Medintza, I. L.; Susumuc, K.; Ohc, E.; Zabetakisa, D.; Goldmana, E. R.; Anderson, G. P. *Biotechnol. Rep.* **2016**, 10, 56-65. (b) Wang, Y.; Cai, E.; Rosenkranz, T.; Ge, P.; Teng, K. W.; Lim, S. J.; Smith, A. M.; Chung, H. J.; Sachs, F.; Green, W. N.; Gottlieb, P.; Selvin, P. R. *Bioconjugate Chem*. **2014**, 25, 2205−2211.

(6) (a) Tsay, J. M.; Trzoss, M.; Shi, L.; Kong, X.; Selke, M.; Jung, M. E.; Weiss, S. *J. Am. Chem. Soc***. 2007**, 129, 6865-6871. (b) Morosini, V.; Bastogne, T.; Frochot, C.; Schneider, R.; François, A.; Guilleminac, F.; Barberi-Heyob, M. *Photochem. Photobiol. Sci*. **2011**, 10, 842-851.

(7) (a) Liu, J.; Chen, H.; Lin, Z.; Lin, J-M. *Anal. Chem*. **2010**, 82, 7380-7386. (b) Ren, S.; Chang, L-Y.; Lim, S-K.; Zhao, J.; Smith, M.; Zhao, N.; Bulović, V.; Bawendi, M.; Gradečak, S. *Nano Lett*. **2011**, 11, 3998-4002.

(8) Yang, H.; Li, D.; He, R.; Guo, Q.; Wang, K.; Zhang, X.; Huang, P.; Cui, D. *Nanoscale Res. Lett*. **2010**, 5, 875-881.

(9) (a) Dvorakova, V.; Cadkova, M.; Datinska, V.; Kleparnik, K.; Foret, F.; Bilkova, Z.; Korecka, L. *Anal. Methods* **2017**, 9, 1991-1997. (b) Aga, D.; Bongartz, R.; Doganc, L. E.; Seleci, M.; Walter, J-G.; Demirkol, D. O.; Stahl, F.; Ozcelikc, S.; Timur, S.; Scheper, T. *Colloids Surf. B Biointerfaces* **2014**, 114, 96-103. (c) Tiwari, D. K.; Tanaka, S-I.; Inouye, Y.; Yoshizawa, K.; Watanabe, T. M.; Jin, T. *Sensors* **2009**, 9, 9332-9354.

(10) Foubert, A.; Beloglazova, N. V.; Rajkovic, A.; Sas, B.; Madder, A.; Goryacheva, I. Y.; De Saeger, S. *Trends Anal. Chem.* **2016**, 83, 31-48.

(11) Lim, I-I. S.; Njoki, P. N.; Park, H-Y.; Wang, X.; Wang, L.; Mott, D.; Zhong, C-J. *Nanotechnology* **2008**, 19, 305102-3051113.

(12) (a) Pereira, M.; Lai, E. P. C. *J. Nanobiotechnology* **2008**, 6, 10. (b) Liu, B. R.; Huang, Y-W.; Chiang, H-J.; Lee, H-J. *J. Nanosci. Nanotechnol*. **2010**, 10, 7897-7905. (c) Derfus, A. M.; Chen, A. A.; Min, D-H.; Ruoslahti,E.; Bhatia, S. N. *Bioconjugate Chem*. **2007**, 18, 1391-1396.

(13) Hermanson, G. T. (**2008**) *Bioconjugate Techniques, 2nd ed.,* Academic Press, 1143 Illinois.

(14) Sapsford, K. E.; Algar, W. R.; Berti, L.; Gemmill, K. B.; Casey, B. J.; Oh, E.; Stewart, M. H.; Medintz, I. L. *Chem. Rev*. **2013**, 113, 1904-2074.

(15) (a) Dömling, A.; Ugi, I. *Angew. Chem. Int. Ed.* **2000**, 39, 3168-3210. (b) Dömling, A.; Wang, W.; Wang, K. *Chem Rev*. **2012**, 112, 3083-3135. (c) Dömling, A. *Chem. Rev*. **2006**, 106, 17-89.

(16) Wender, P. A. *Nat. Prod. Rep*. **2014**, 31, 433-440.

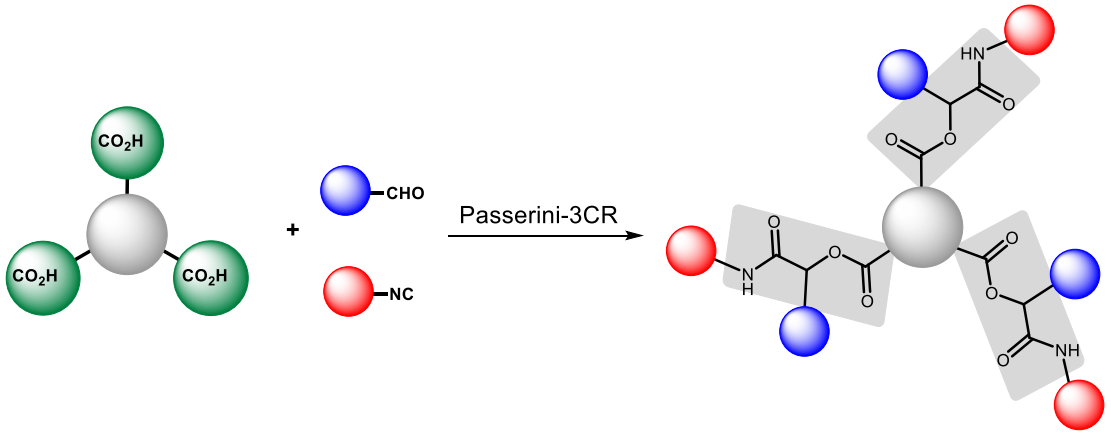
Summary and Outlook

Dendrimers and branched molecules, such as polymers and quantum dots, are extraordinary classes of compounds with unique properties and a variety of industrial and biomedical applications. Currently, new synthetic strategies have been the focus of research to overcome some of the limitations present in almost all the synthetic routes and to facilitate the design of new molecules with desirable features. One approach perfectly suited to accomplish these requirements is the use of isocyanide-based multicomponent reactions (IMCRs), which are able to efficiently create new complex compounds in one pot procedures.

The goal of this research project was to develop new synthetic strategies based on IMCRs towards the synthesis and functionalization of dendrimers and branched molecules.

Chapter 1 highlights the general aspects of dendrimers and quantum dots and their most common applications as well as a brief description of the Passerini and Ugi multicomponent reactions, emphasizing their previous use in the synthesis of macromolecules.

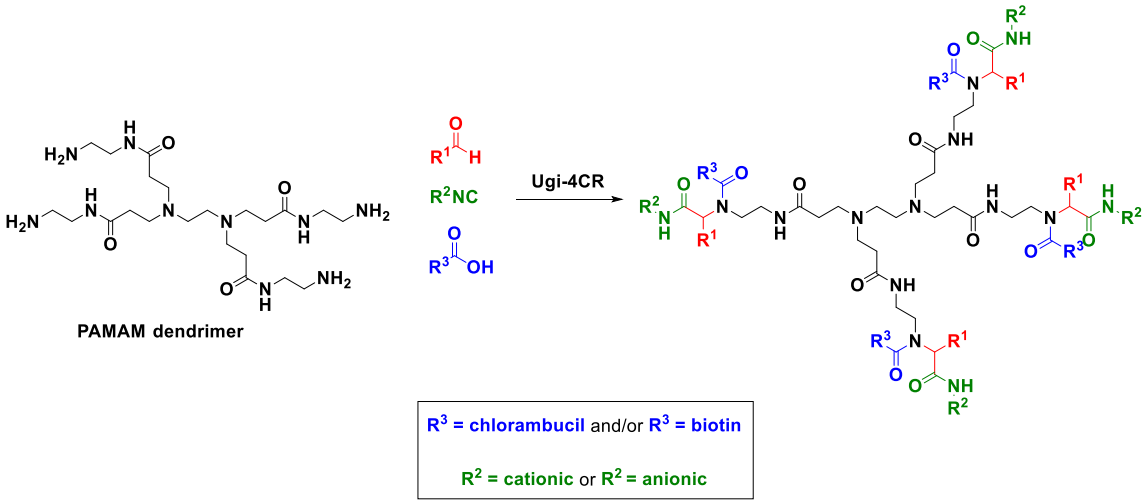
Chapter 2 describes the use of the Passerini three-component reaction for the divergent synthesis of structurally diverse traditional and Janus dendrimers, as well as polyvalent molecules, based on the use of reaction branching, rather than branched building blocks (**Scheme 1**).



Scheme 1. Divergent synthesis of dendrimers by Passerini reaction.

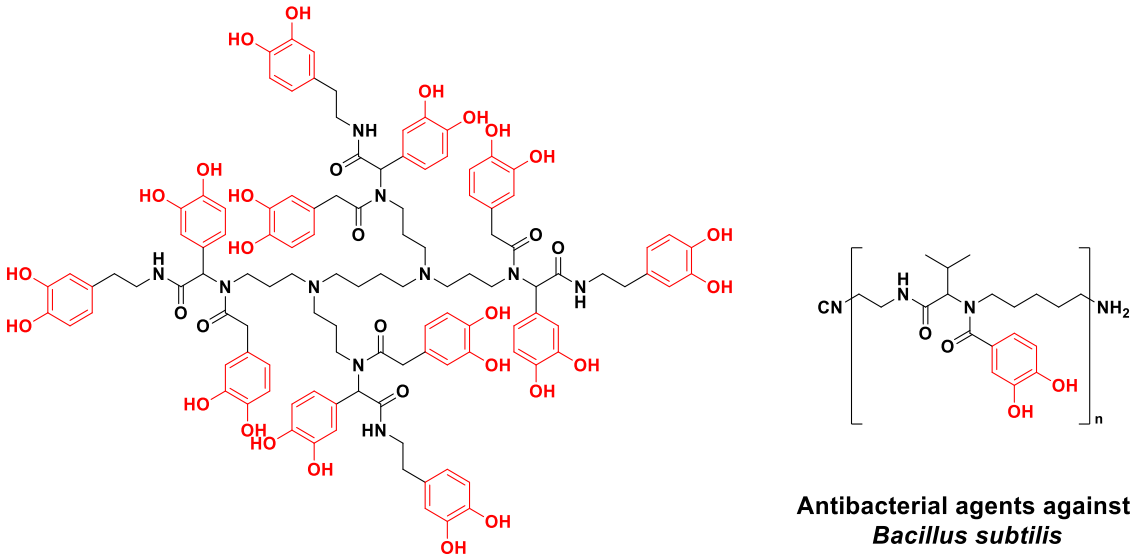
In **Chapter 3**, a new synthetic strategy to improve water solubility and enhance the efficacy of the chemotherapeutic drug chlorambucil by conjugation with PAMAM-NH_2 dendrimer and -NH_2 or -COOH groups, or the targeting compound biotin by Ugi reaction

was reported. The cytotoxic activity of all synthesized compounds was also evaluated against PC-3 prostate and HT-29 colon cancer cell lines as well as non-cancerous mouse NIH3T3 fibroblasts employing MTT and CV assays. In this study, we could show that the dendrimer with four chlorambucil units containing -NH_2 groups on the surface increased cytotoxicity and also enhanced selectivity toward PC-3 prostate tumor cells (**Scheme 2**).



Scheme 2. Synthesis of PAMAM dendrimers containing biotin and/or chlorambucil by Ugi reaction.

Chapter 4 describes the use of Ugi multicomponent reaction for the functionalization of polypropylenimine dendrimers (PPI dendrimers) with several catechol moieties on the surface as well as one dendrimer with pyrogallol units. Polymers with catechol groups were also synthesized applying the same method. Catechol-based compounds are well known for their adhesive properties, however, in this study we were able to demonstrate that they can act as antifungal compounds against *Phytophthora infestans* when present in dendrimers and as antibacterial agents against the Gram-positive bacterium *Bacillus subtilis* when connected to polymers (**Scheme 3**).

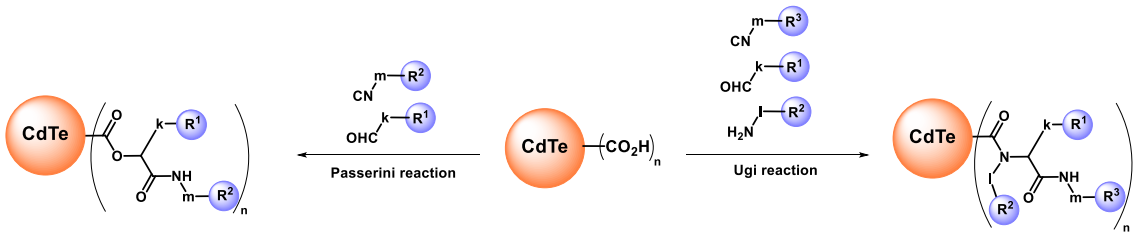


Antifungal compounds against *Phytophthora infestans*

Antibacterial agents against *Bacillus subtilis*

Scheme 3. Example of a dendrimer with antifungal properties and a polymer with antibacterial activity synthesized by Ugi reaction.

Finally, in **Chapter 5**, the use of Passerini and Ugi multicomponent reactions were described for the covalent modification of core-type carboxyl functionalized CdTe quantum dots. This proof-of-concept study will be continued to evaluate the potential and scope of the conjugation method (**Scheme 4**).



Scheme 4. Covalent conjugation of CdTe quantum dots by Passerini and Ugi reactions.

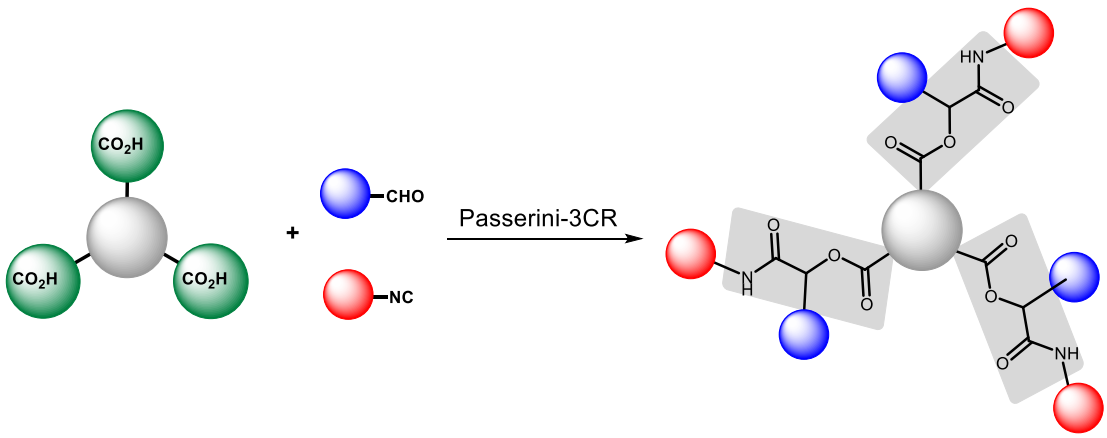
Zusammenfassung und Ausblick

Dendrimere und verzweigte Moleküle, wie Polymere und Quantenpunkte, sind ungewöhnliche Verbindungsklassen mit einzigartigen Eigenschaften und weisen eine Vielfalt an kommerziellen und biomedizinischen Anwendungen auf. Derzeit liegt der Schwerpunkt der Wissenschaft auf neuen Synthesestrategien, um die Grenzen in nahezu allen gegenwärtigen Synthesewegen zu überwinden und das Design neuer Moleküle zu ermöglichen. Ein geeigneter Ansatz um diese Anforderungen zu bewerkstelligen, ist die Nutzung von Isocyanid-basierenden Multikomponentenreaktionen (IMCRS), welche in der Lage sind neue komplexe Verbindungen effizient in einem „Ein-Topf"-Verfahren zu synthetisieren.

Um die Synthese und die Funktionalisierung von Dendrimeren und anderen verzweigten Molekülen zu ermöglichen, ist das Ziel dieses wissenschaftlichen Projektes neue Synthesestrategien auf der Grundlage von IMCRS zu entwickeln.

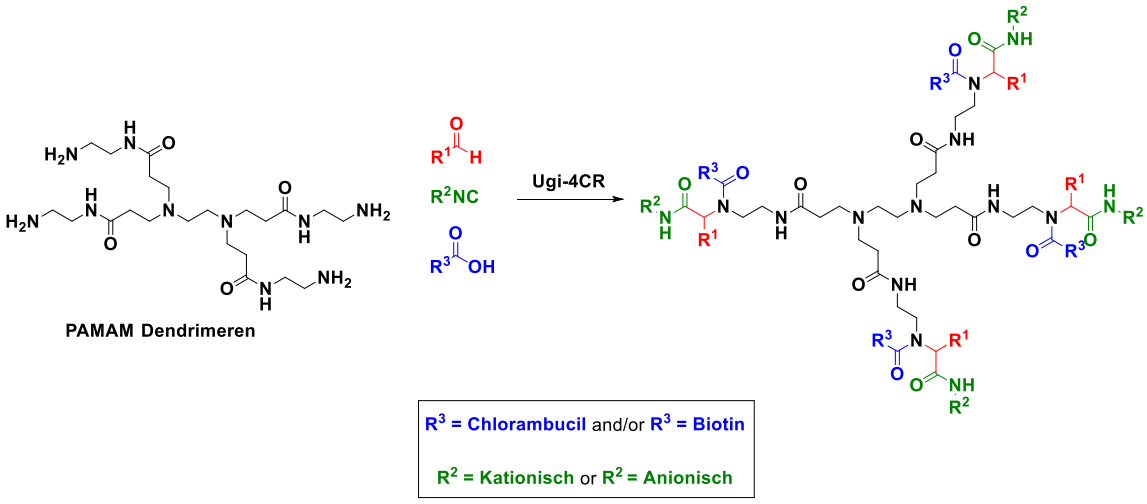
Kapitel 1 hebt die grundlegenden Aspekte von Dendrimeren und Quantenpunkten und deren Anwendungen hervor und fokussiert sich auf die Passerini und Ugi Multikomponentenreaktion und deren bisheriger Einsatz in der Synthese von Makromolekülen.

Kapitel 2 beschreibt die Anwendung der Passerini Drei-Komponenten Reaktion für die divergente Synthese von strukturell vielfältigen traditionellen und Janus Dendrimeren, sowie von polyvalenten Molekülen basierend auf Verzweigungsreaktionen, anstatt verzweigte Grundbausteine zu verwenden (**Schema 1**).



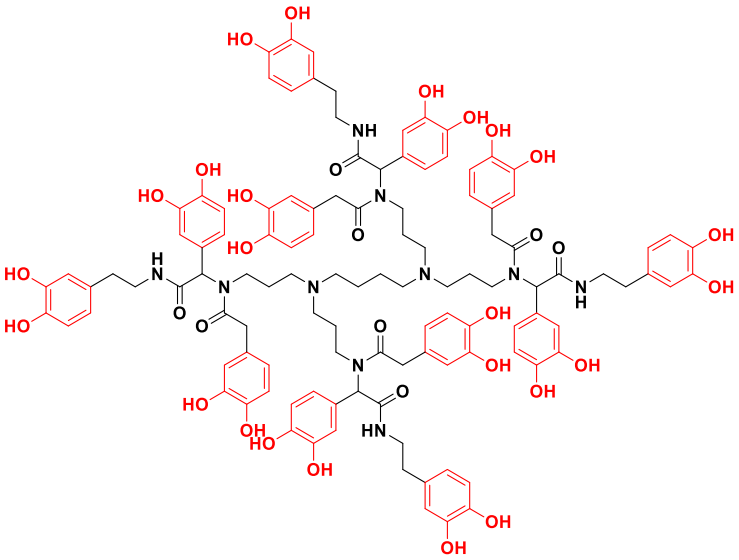
Schema 1. Divergent Synthese von Dendrimeren mittels Passerini-Reaktion.

In **Kapitel 3** wird von einer neuen Synthesestrategie zur Verbesserung der Wasserlöslichkeit und Effizienzförderung des chemotherapeutischen Arzneistoffes Chlorambucil durch Verknüpfung mit PAMAM-NH_2 Dendrimeren und -NH_2 oder -COOH Gruppen oder der Zielverbindung Biotin mit einer Ugi-Reaktion berichtet. Die zytotoxische Aktivität von allen synthetisierten Verbindungen wurde in den Krebszelllinien PC-3 (Prostata) und HT29 (Dickdarm) mittels MTT und CV Assay getestet und als Kontrolle dienten Maus-3T3-Zelllinien. Innerhalb dieser Arbeit konnte gezeigt werden, dass das Dendrimer mit vier Chlorambucil-Einheiten und den oberflächlichen -NH_2 Gruppen eine erhöhte Zytotoxizität und eine verbesserte Selektivität hinsichtlich der PC-3 Prostatatumorzelllinie aufweist (**Schema 2**).

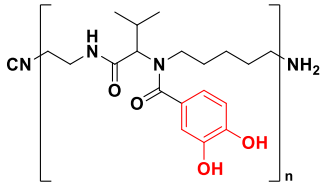


Schema 2. Synthese von PAMAM Dendrimeren mit einer eingebauten Biotin- und/oder Chlorambucileinheit mittels Ugi-Reaktion.

Kapitel 4 beschreibt die Anwendung von Ugi Mutlikomponentenreaktionen für die Funktionalisierung von Poly(Propylenimin) Dendrimeren (PPI Dendrimeren) mit verschiedenen oberflächlichen Catecholkomponenten, sowie ein Dendrimer mit einer Pyrogallol-Einheit. Polymere mit Catechol-Einheiten wurdenentsprechend des gleichen Protokolls synthetisiert. Obwohl Catechol-basierende Verbindungen sind für ihre adhäsiven Eigenschaften bekannt, konnte innerhalbdieser Studie zeigt werden, dass sie als antimykotische Verbindungen gegen *Phytophthora infestans* in Anwesenheit von Dendrimeren und als antibakterielle Verbindungen gegen das grampositive Bakterium *Bacillus subtilis* in Anwesenheit von Polymeren fungieren (**Schema 3**).



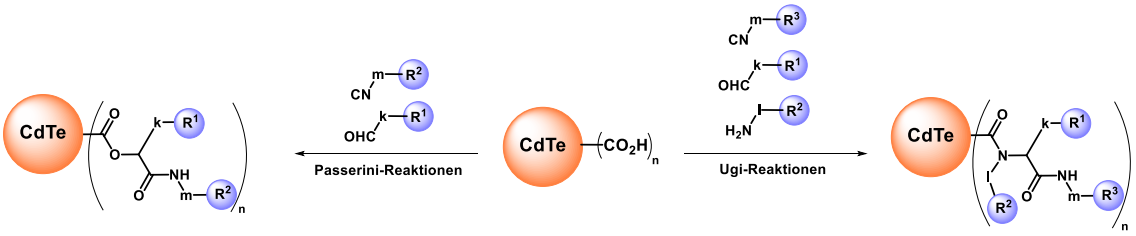
Antimykotische Verbindungen gegen
Phytophthora infestans



Antibakterielle Verbindungen gegen
Bacillus subtilis

Schema 3. Beispiel eines mittels Ugi-Reaktion hergestellten Dedrimers mit antimykiotischen Eigenschaften und eines Polymers mit antibakterieller Aktivität.

Abschließend wird in **Kapitel 5** die Anwendung der Passerini und Ugi Multikomponentenreaktion für kovalente Modifikationen von kernbasierenden Carboxyl-funktionalisierten CdTe Quantenpunkten beschrieben. Mit Hilfe dieser Konjugationsmethode konnte die Studie fortgesetzt werden, um das Potenzial und den Umfang zu untersuchen (**Schema 4**).



Schema 4. Kovalent Konjugation von CdTe Quantenpunkten mittels Passerini und Ugi-Reaktionen.

Attachments

Figure S1 - ^{1}H NMR (400 MHz, acetone-D6) spectrum of dendrimer **6** (Chapter 2).

Figure S2 - ^{13}C NMR (101 MHz, acetone-D6) spectrum of dendrimer **6** (Chapter 2).

Figure S3 - MALDI-TOF-MS spectrum of dendrimer **6** (Chapter 2).

Figure S4 - ^{1}H NMR (400 MHz, MeOD) spectrum of dendrimer **20** (Chapter 2).

Figure S5 - ^{13}C NMR (101 MHz, MeOD) spectrum of dendrimer **20** (Chapter 2).

Figure S6 - MALDI-TOF-MS spectrum of dendrimer **20** (Chapter 2).

Figure S7 - ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of dendrimer **26a** (Chapter 2).

Figure S8 - ^{13}C NMR (101 MHz, $CDCl_3$) spectrum of dendrimer **26a** (Chapter 2).

Figure S9 - HRMS (ESI) spectrum of dendrimer **26a** (Chapter 2).

Figure S10 - ^{1}H NMR (400 MHz, MeOD) spectrum of dendrimer **1b** (Chapter 3).

Figure S11 - ^{13}C NMR (101 MHz, MeOD) spectrum of dendrimer **1b** (Chapter 3).

Figure S12 - MALDI-TOF-MS spectrum of dendrimer **1b** (Chapter 3).

Figure S13 - ^{1}H NMR (400 MHz, MeOD) spectrum of dendrimer **3a** (Chapter 3).

Figure S14 - ^{13}C NMR (101 MHz, MeOD) spectrum of dendrimer **3a** (Chapter 3).

Figure S15 - MALDI-TOF-MS spectrum of dendrimer **3a** (Chapter 3).

Figure S16 - ^{1}H NMR (400 MHz, MeOD) spectrum of dendrimer **5a** (Chapter 3).

Figure S17 - ^{13}C NMR (101 MHz, MeOD) spectrum of dendrimer **5a** (Chapter 3).

Figure S18 - MALDI-TOF-MS spectrum of dendrimer **5a** (Chapter 3).

Figure S19 - ^{1}H NMR (400 MHz, MeOD) spectrum of dendrimer **1a** (Chapter 4).

Figure S20 - ^{13}C NMR (101 MHz, MeOD) spectrum of dendrimer **1a** (Chapter 4).

Figure S21- MALDI-TOF-MS spectrum of dendrimer **1a** (Chapter 4).

Figure S22 - ^{1}H NMR (400 MHz, MeOD) spectrum of dendrimer **7b** (Chapter 4).

Figure S23 - ^{13}C NMR (101 MHz, MeOD) spectrum of dendrimer **7b** (Chapter 4).

Figure S24- MALDI-TOF-MS spectrum of dendrimer **7b** (Chapter 4).

Figure S25 - Size exclusion chromatogram of polymers **9**, **10**, and **11** (Chapter 4).

Figure S26 - ^{1}H NMR (400 MHz, MeOD) spectrum of polymer **9** (Chapter 4).

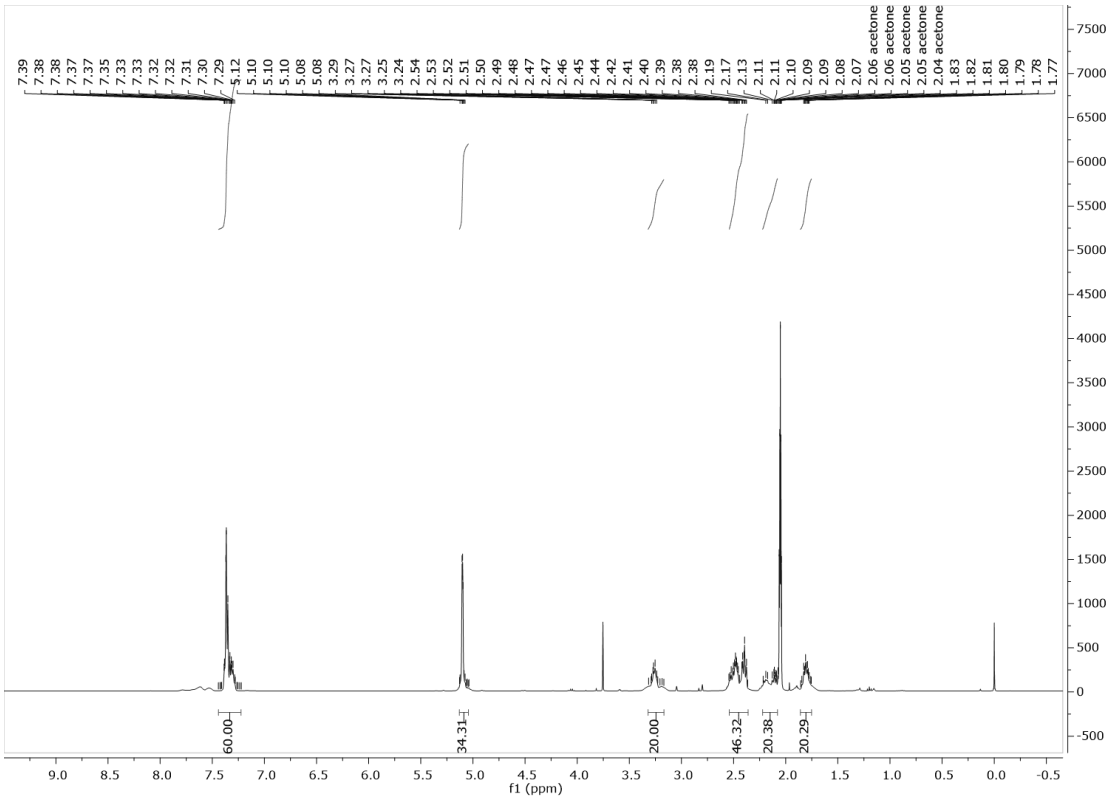


Figure S1 - ^{1}H NMR (400 MHz, acetone-D6) spectrum of dendrimer **6** (Chapter 2).

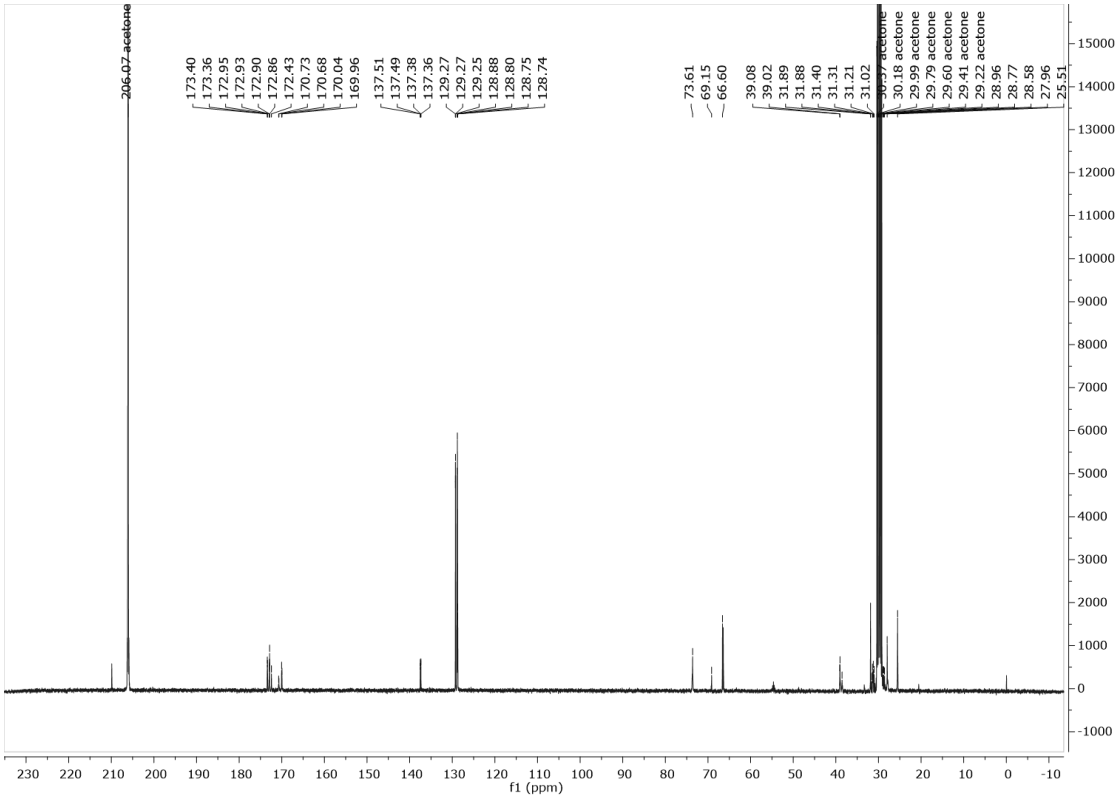


Figure S2 - ^{13}C NMR (101 MHz, acetone-D6) spectrum of dendrimer **6** (Chapter 2).

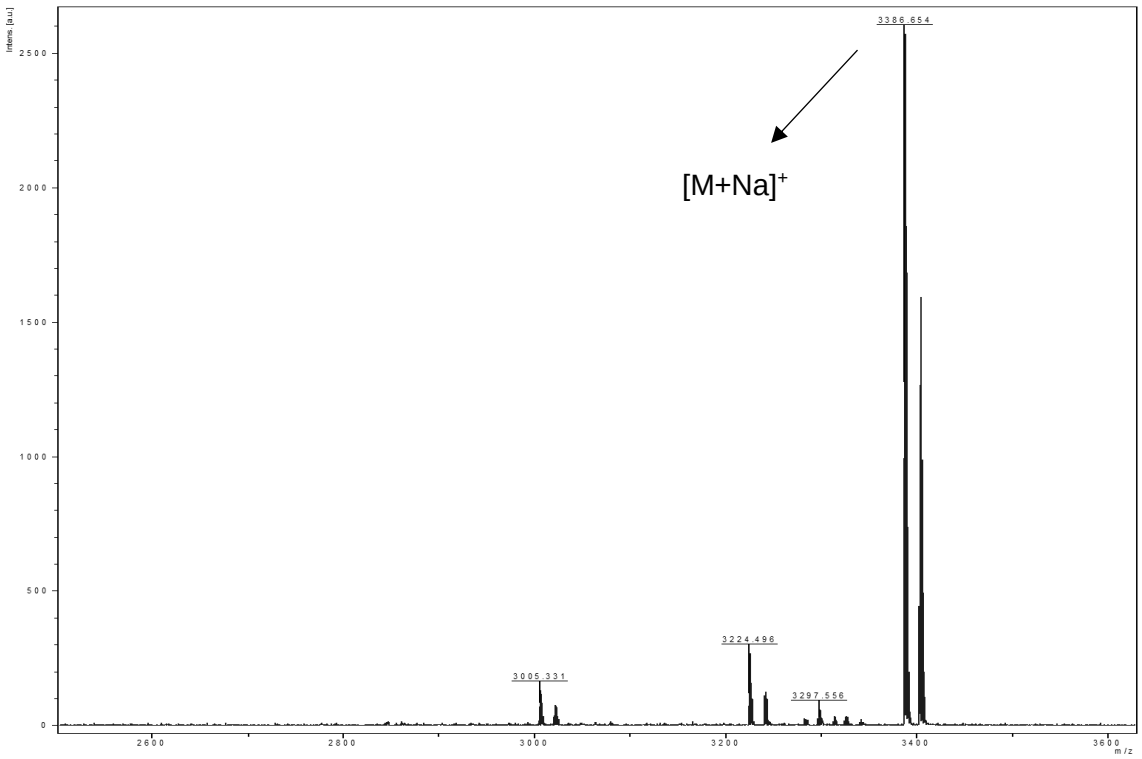


Figure S3 - MALDI-TOF-MS spectrum of dendrimer **6** (Chapter 2).

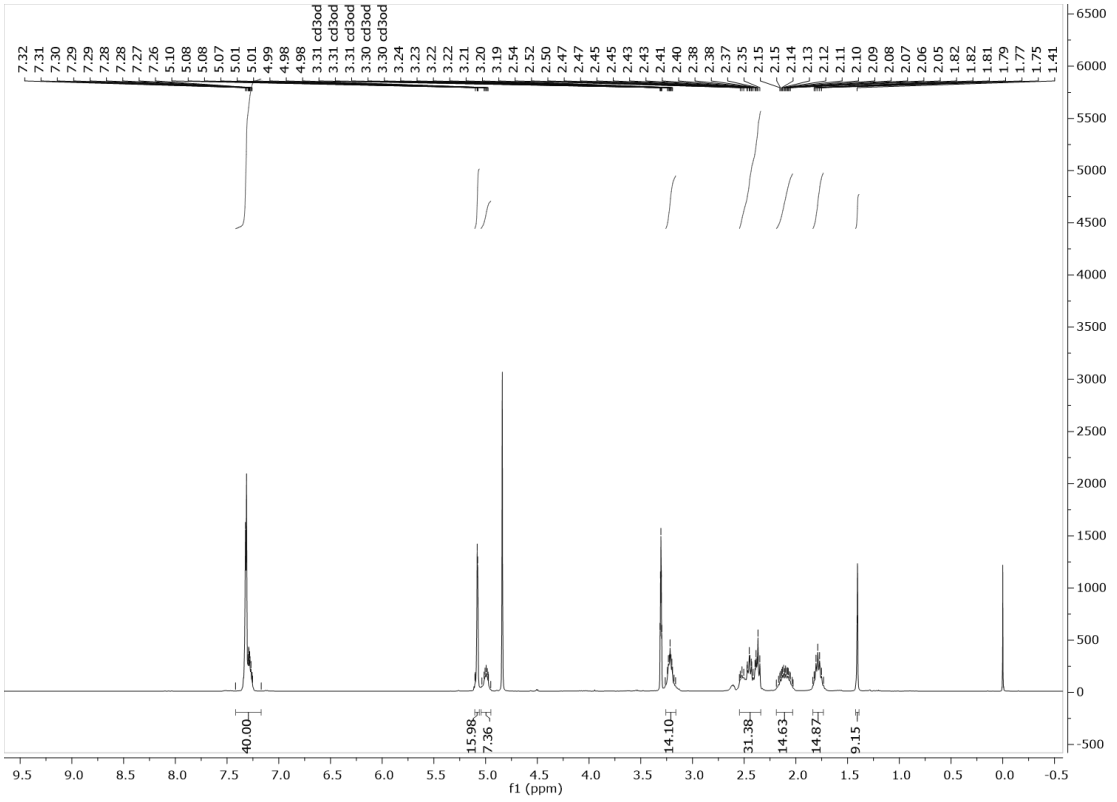


Figure S4 - ^{1}H NMR (400 MHz, MeOD) spectrum of dendrimer **20** (Chapter 2).

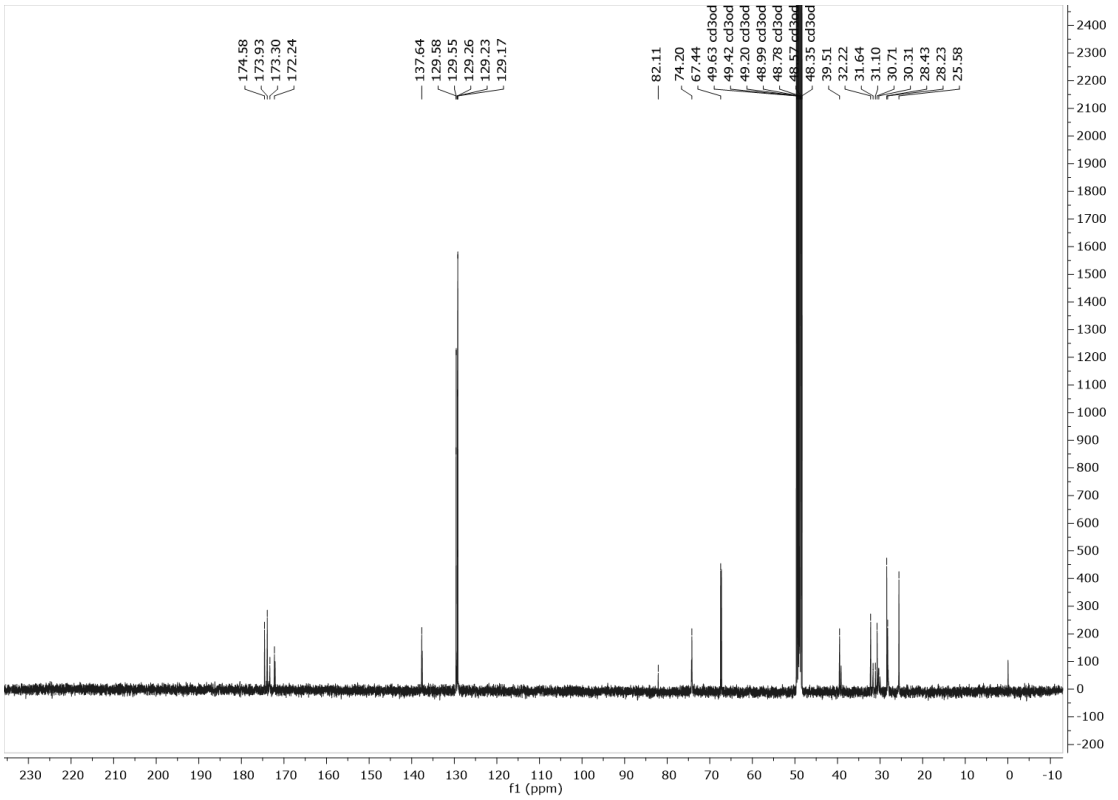


Figure S5 - ^{13}C NMR (101 MHz, MeOD) spectrum of dendrimer **20** (Chapter 2).

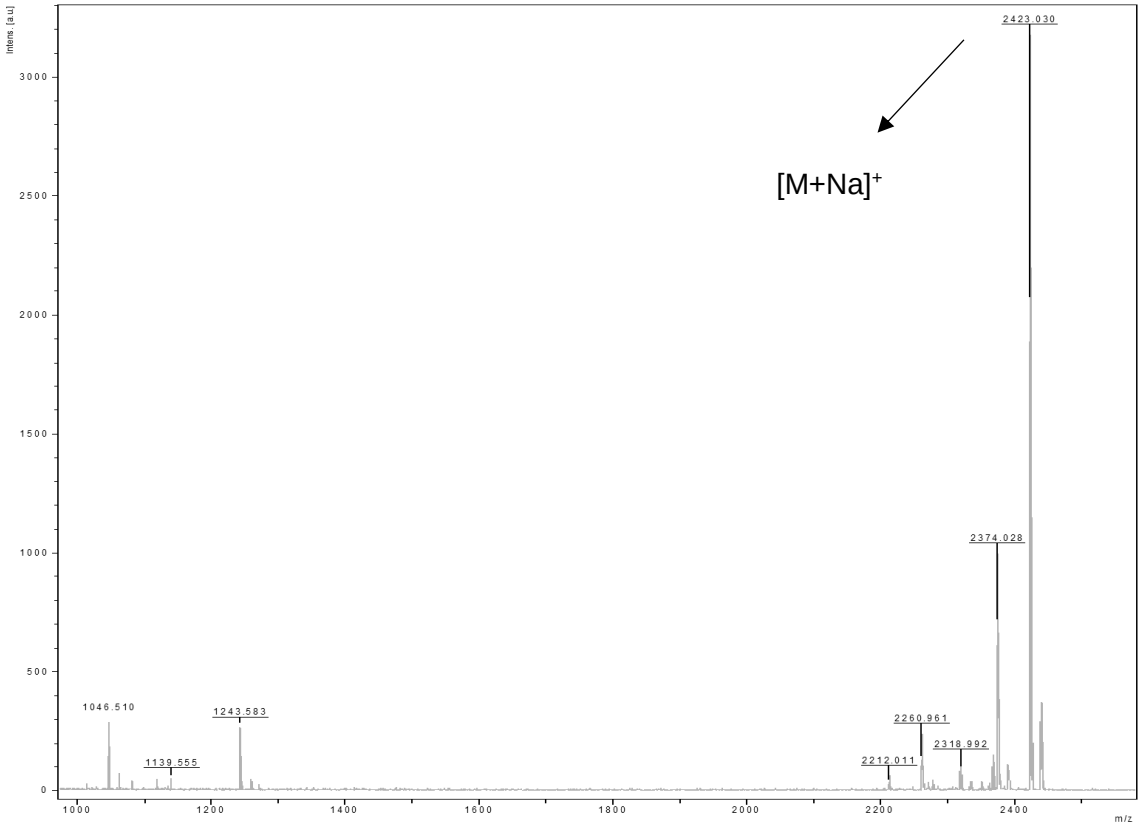


Figure S6 - MALDI-TOF-MS spectrum of dendrimer **20** (Chapter 2).

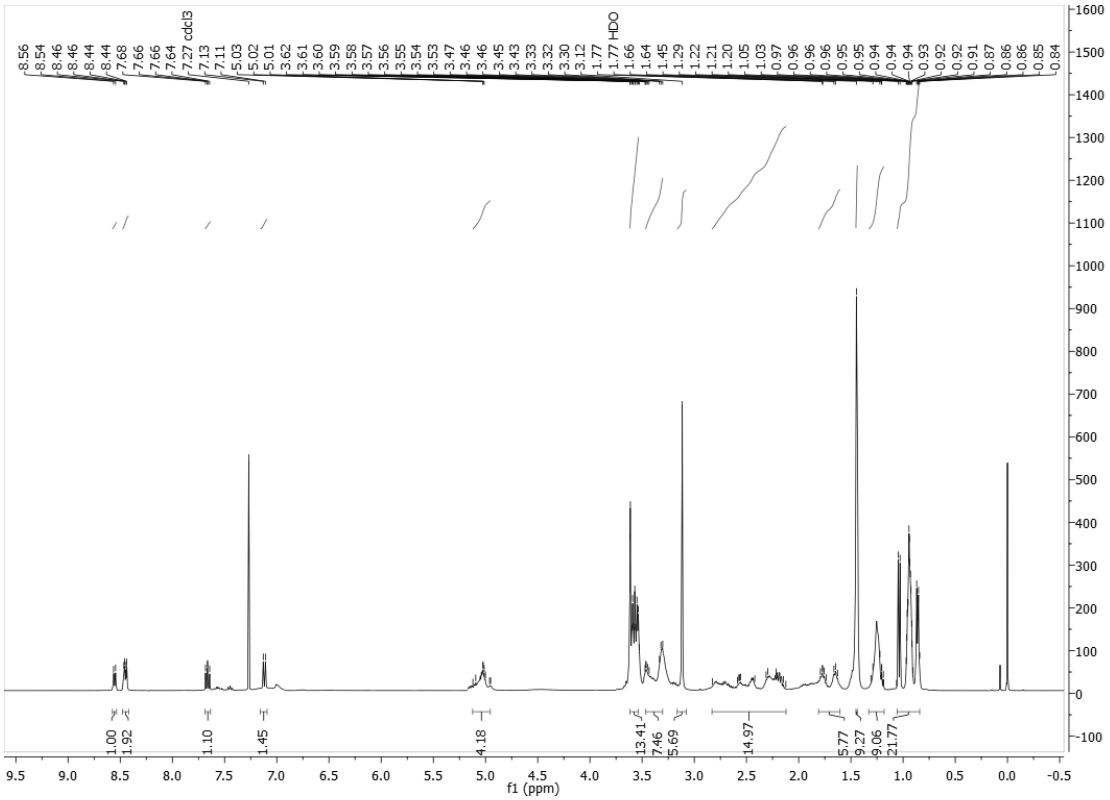


Figure S7 - ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of dendrimer **26a** (Chapter 2).

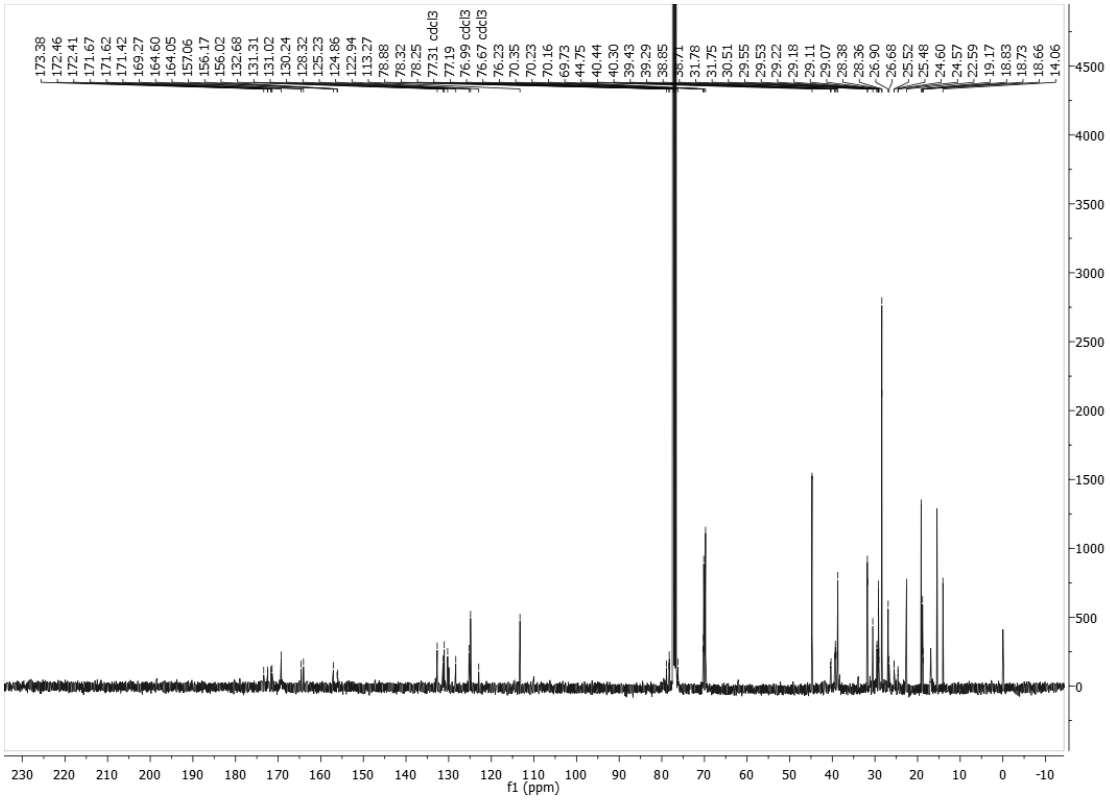


Figure S8 - ^{13}C NMR (101 MHz, $CDCl_3$) spectrum of dendrimer **26a** (Chapter 2).

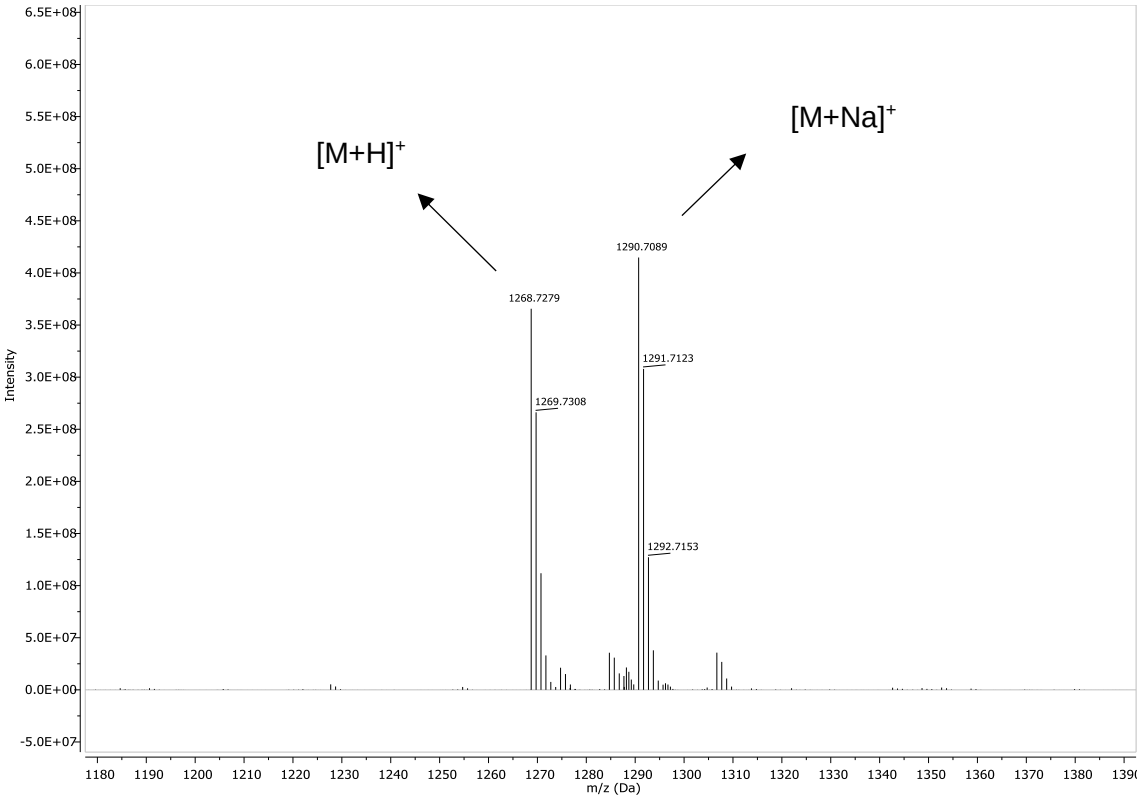


Figure S9 - HRMS (ESI) spectrum of dendrimer **26a** (Chapter 2).

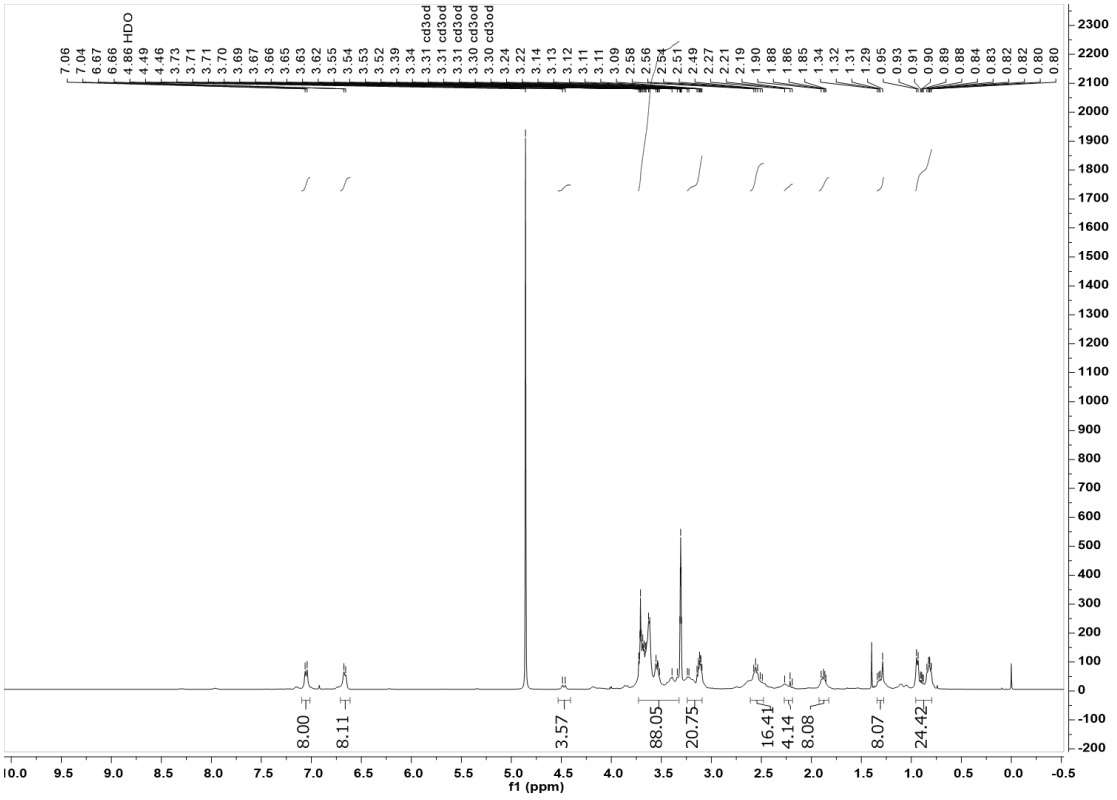


Figure S10 - ^{1}H NMR (400 MHz, MeOD) spectrum of dendrimer **1b** (Chapter 3).

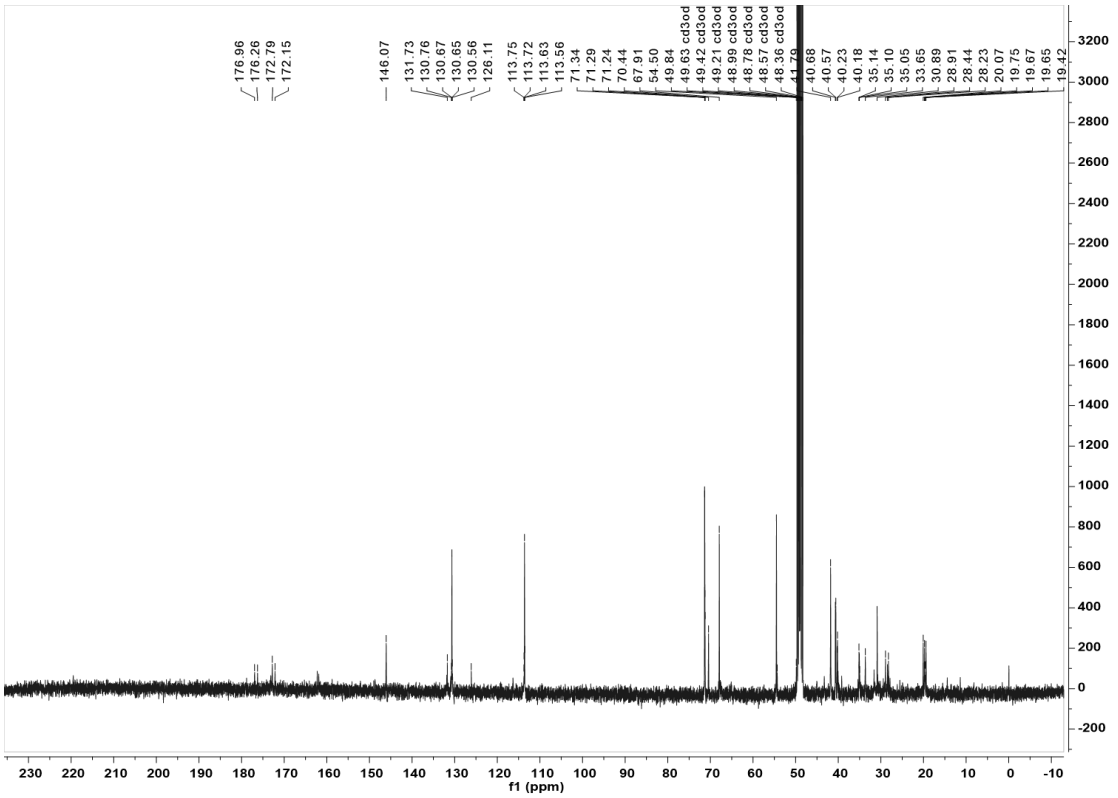


Figure S11 - ^{13}C NMR (101 MHz, MeOD) spectrum of dendrimer **1b** (Chapter 3).

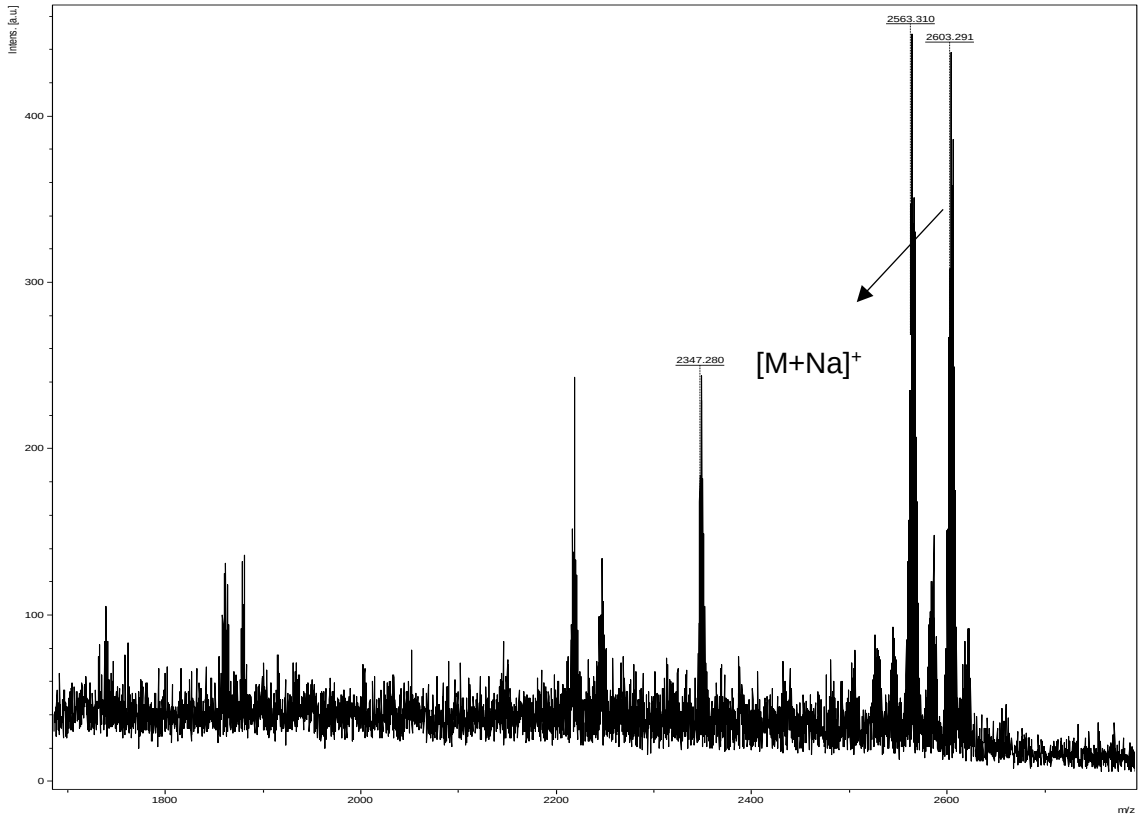


Figure S12 - MALDI-TOF-MS spectrum of dendrimer **1b** (Chapter 3).

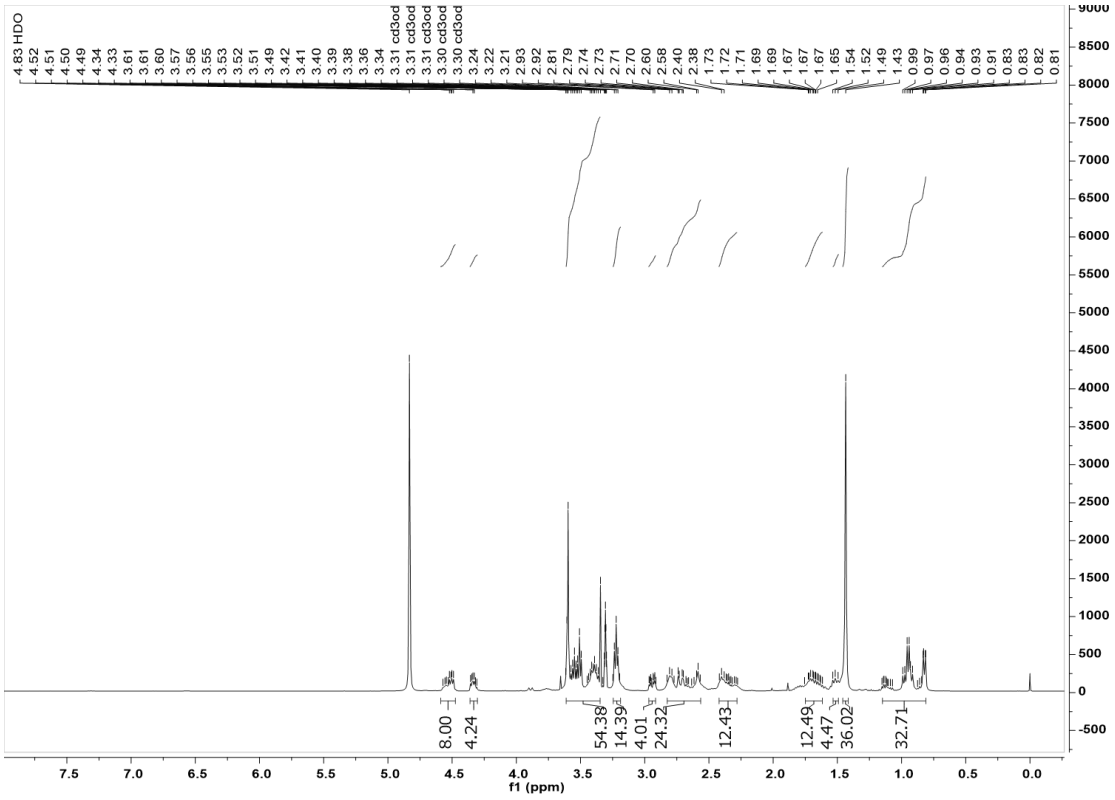


Figure S13 - ^{1}H NMR (400 MHz, MeOD) spectrum of dendrimer **3a** (Chapter 3).

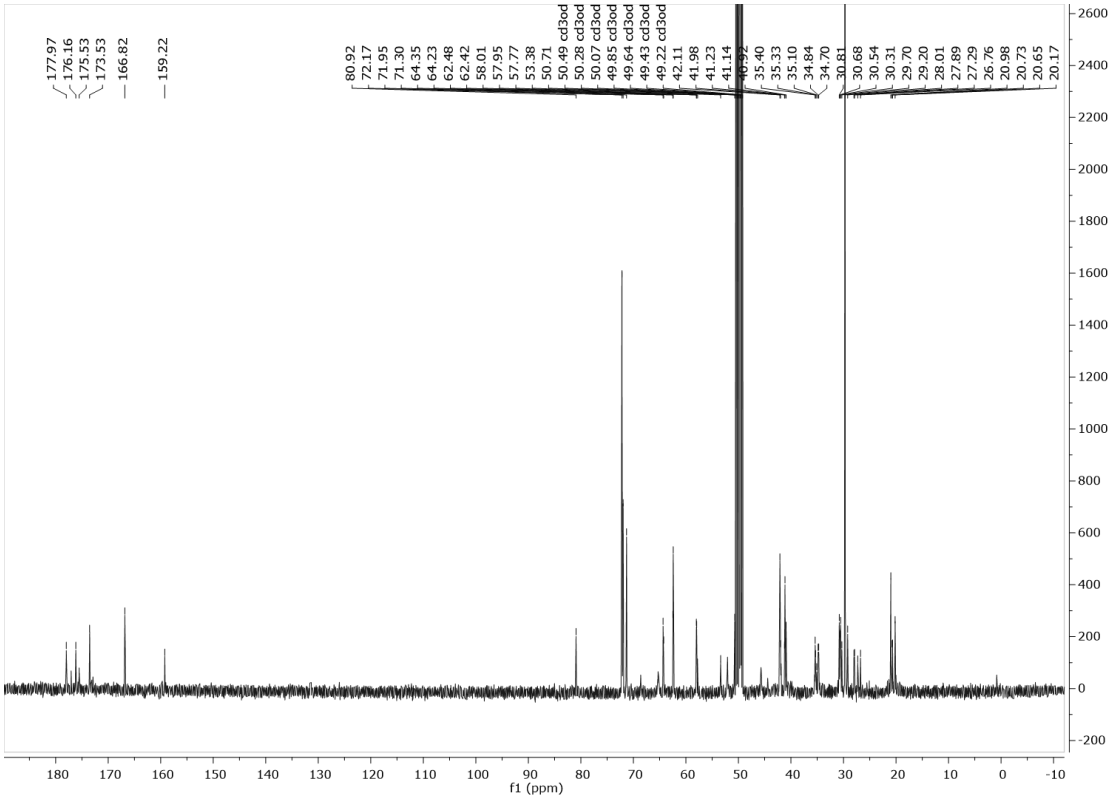


Figure S14 - ^{13}C NMR (101 MHz, MeOD) spectrum of dendrimer **3a** (Chapter 3).

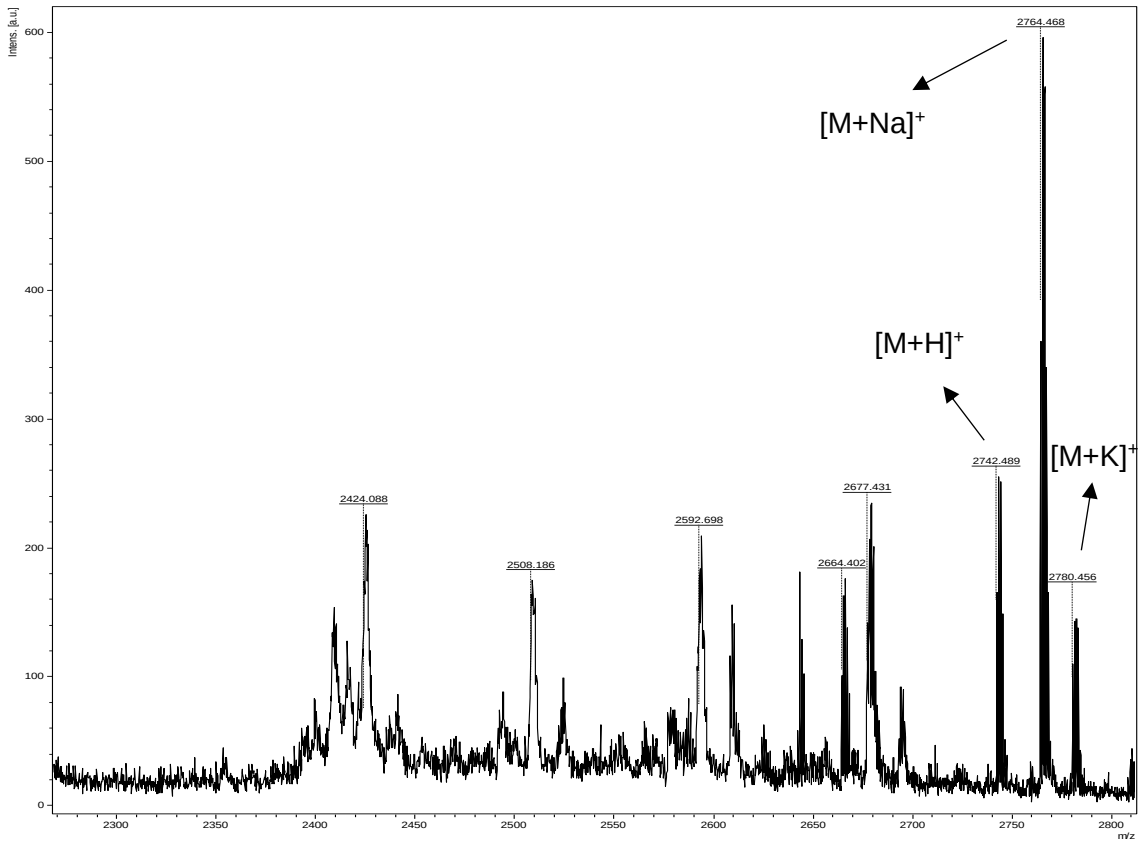


Figure S15 - MALDI-TOF-MS spectrum of dendrimer **3a** (Chapter 3).

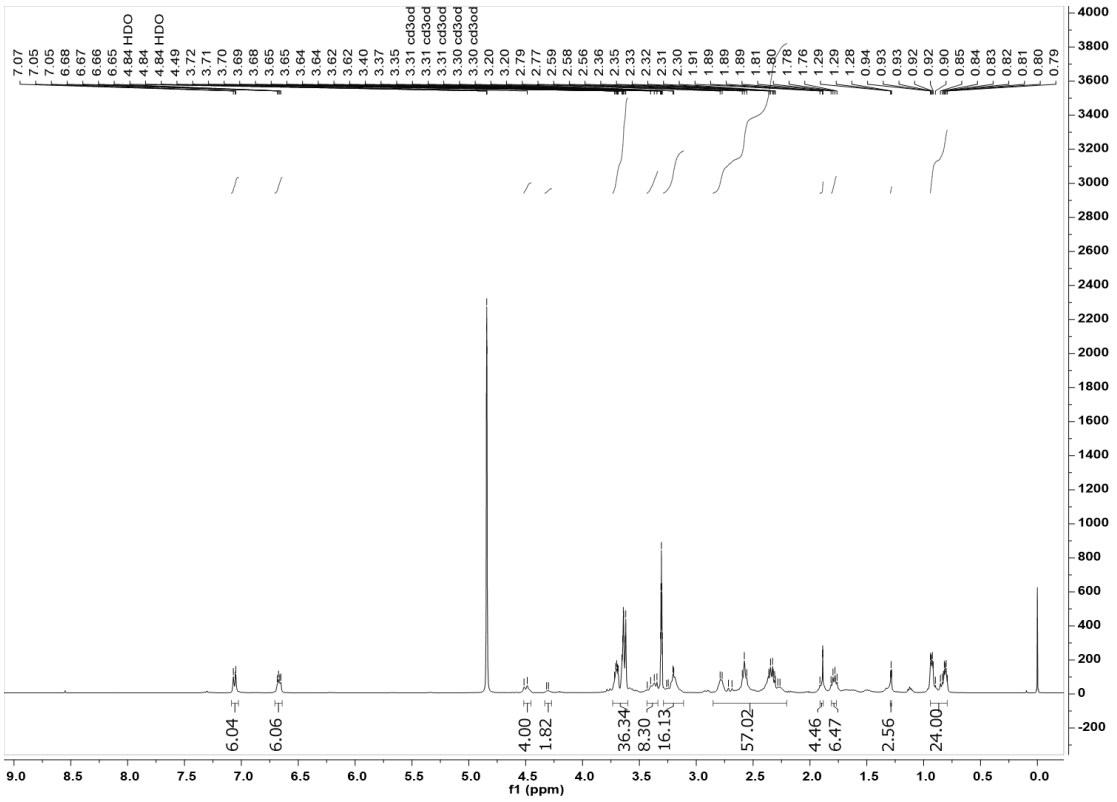


Figure S16 - ^{1}H NMR (400 MHz, MeOD) spectrum of dendrimer **5a** (Chapter 3).

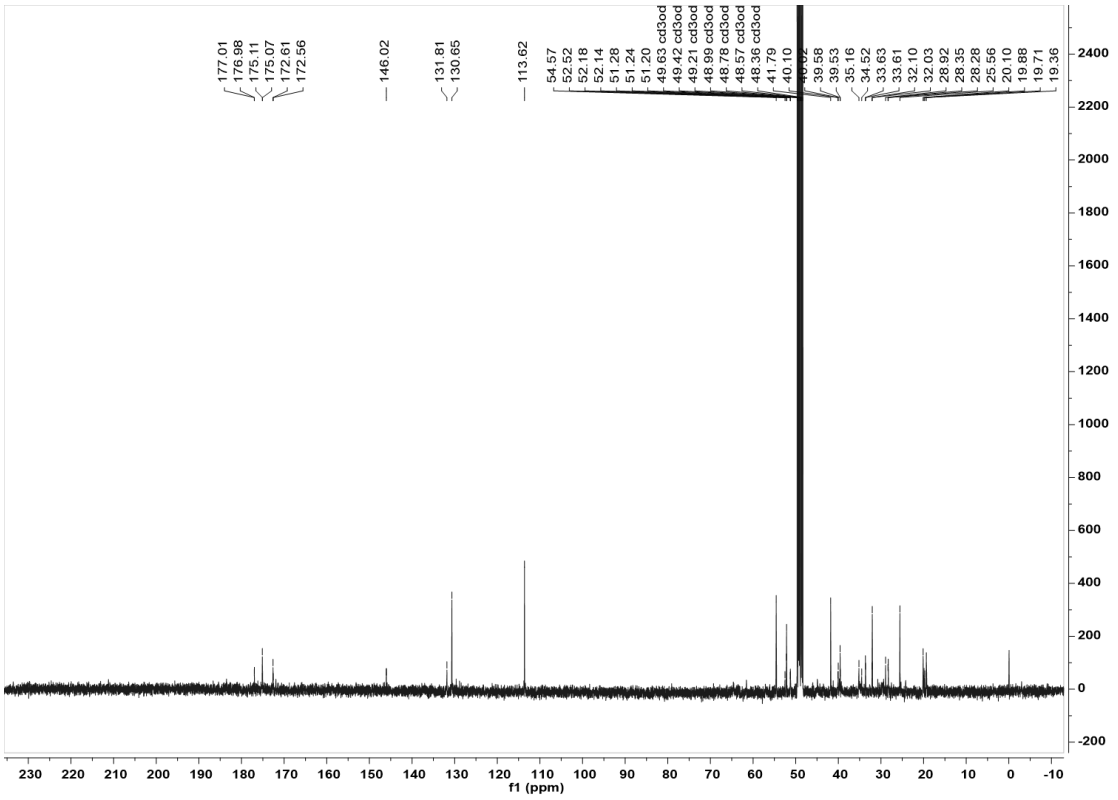


Figure S17 - ^{13}C NMR (101 MHz, MeOD) spectrum of dendrimer **5a** (Chapter 3).

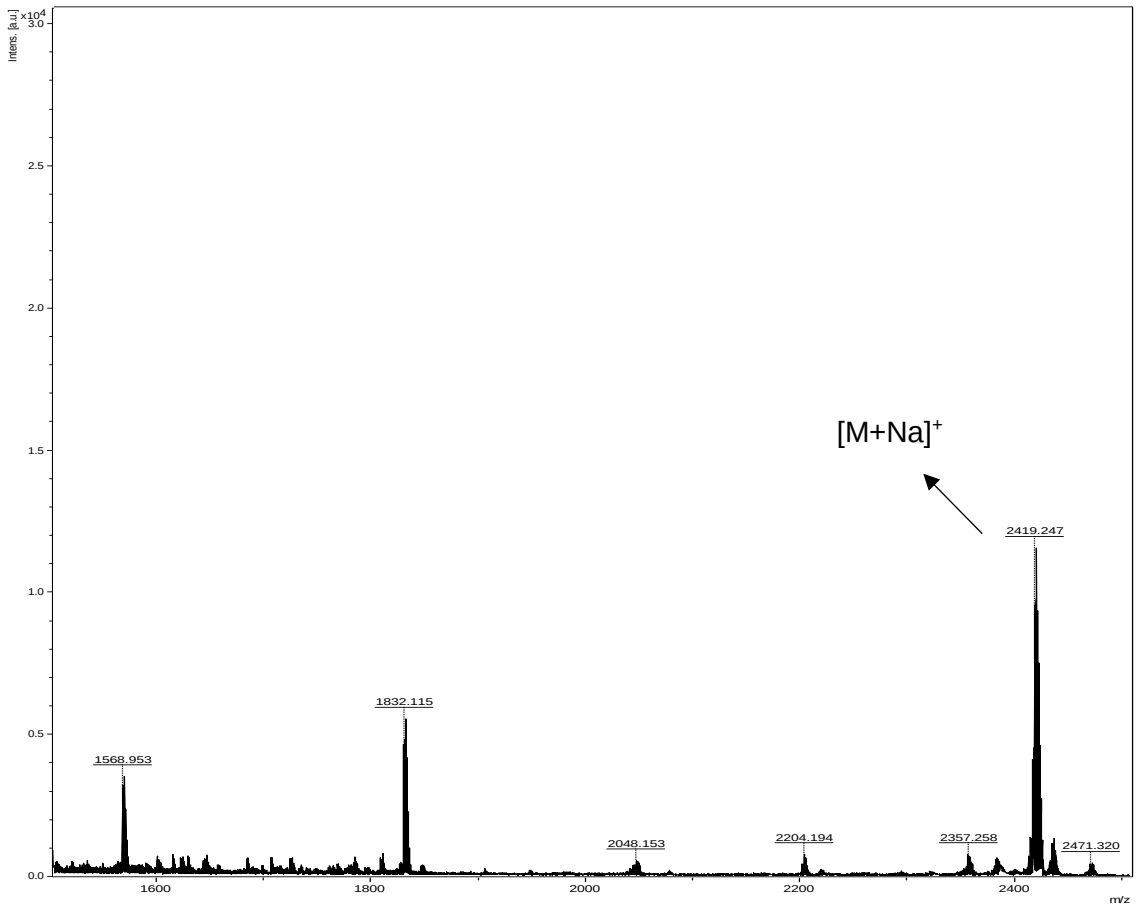


Figure S18 - MALDI-TOF-MS spectrum of dendrimer **5a** (Chapter 3).

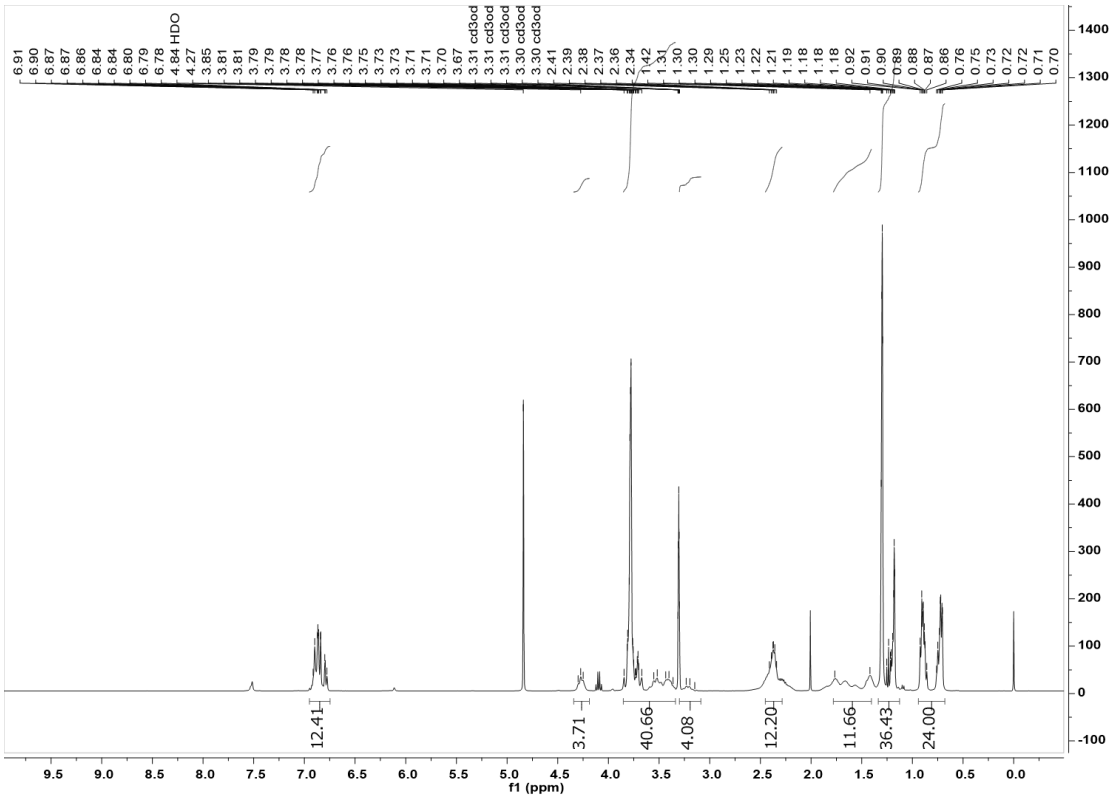


Figure S19 - ^{1}H NMR (400 MHz, MeOD) spectrum of dendrimer **1a** (Chapter 4).

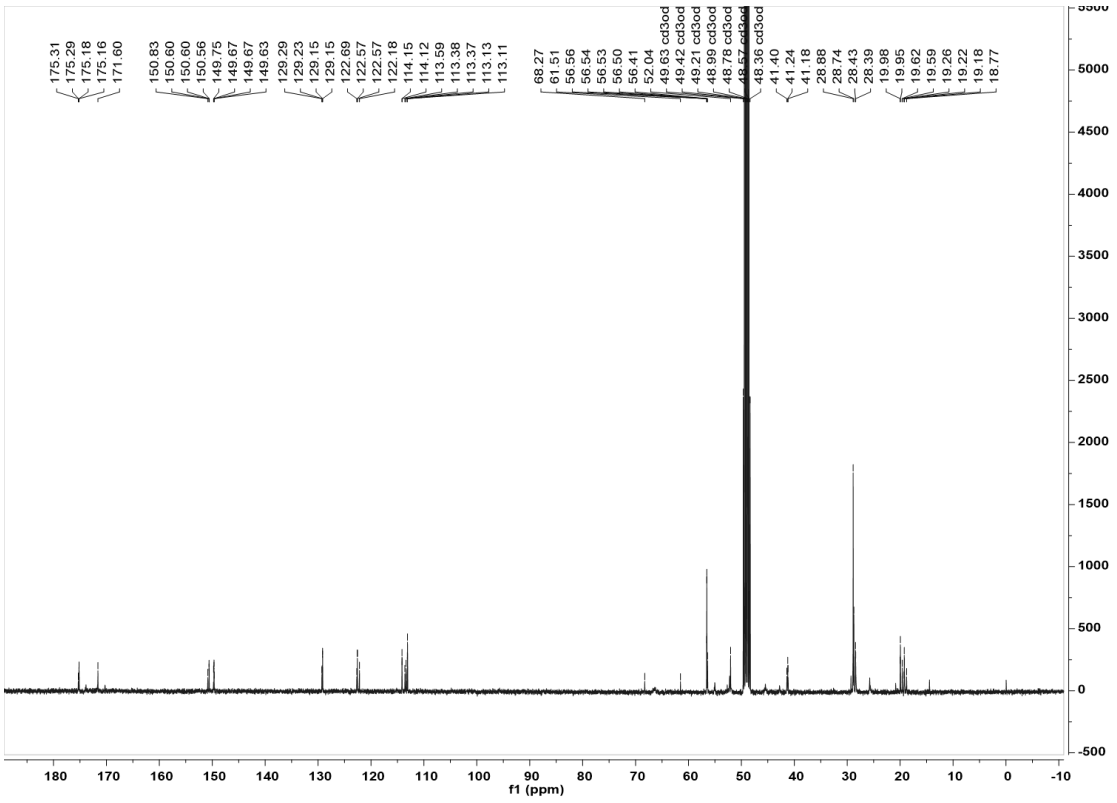


Figure S20 - ^{13}C NMR (101 MHz, MeOD) spectrum of dendrimer **1a** (Chapter 4).

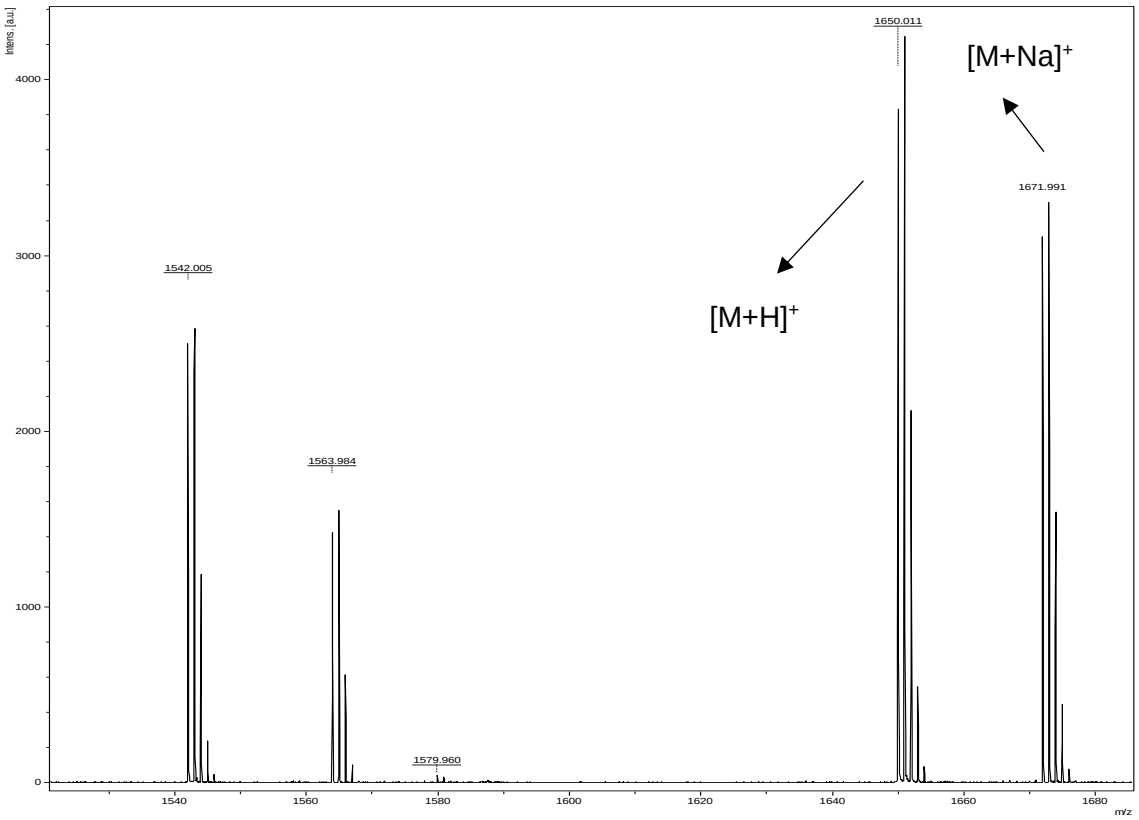


Figure S21 - MALDI-TOF-MS spectrum of dendrimer **1a** (Chapter 4).

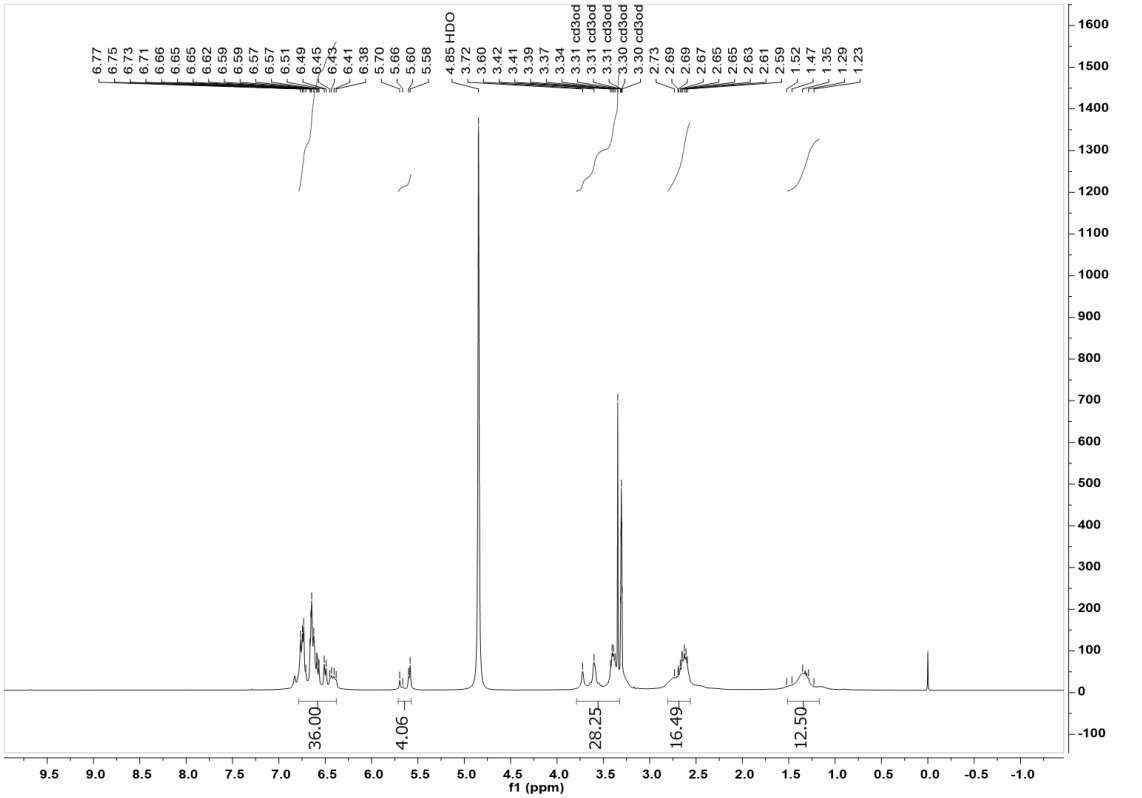


Figure S22 - ^{1}H NMR (400 MHz, MeOD) spectrum of dendrimer **7b** (Chapter 4).

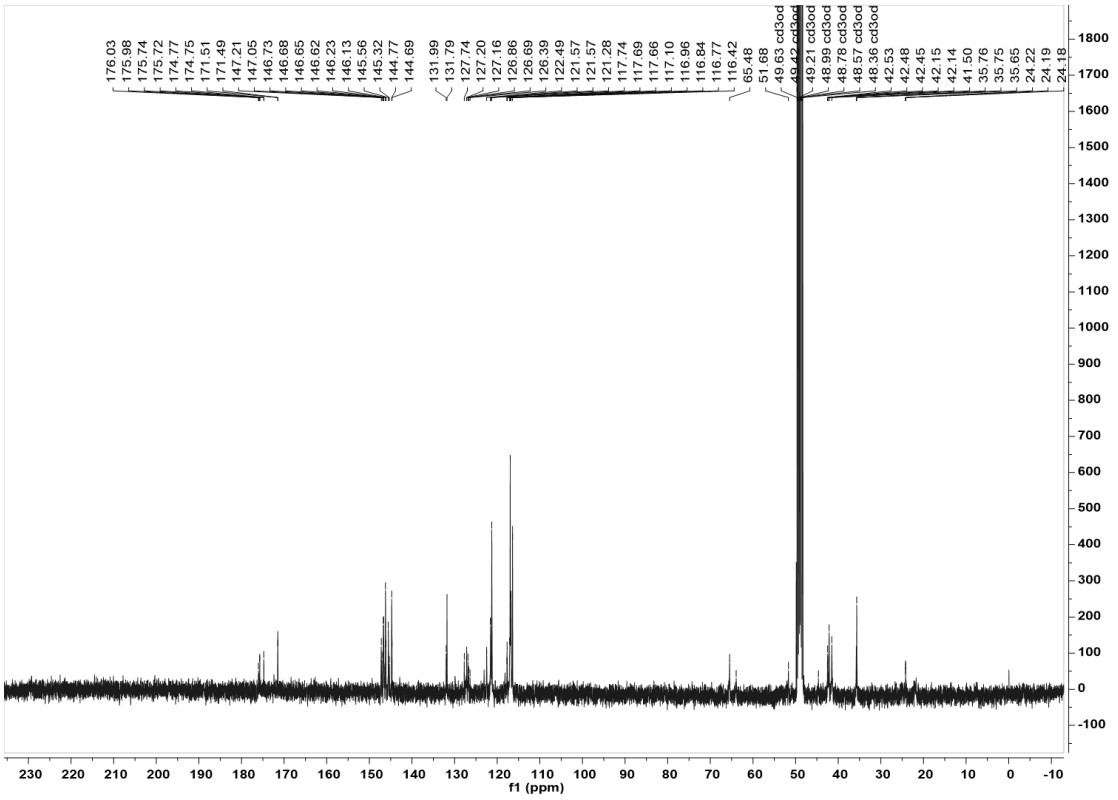


Figure S23 - ^{13}C NMR (101 MHz, MeOD) spectrum of dendrimer **7b** (Chapter 4).

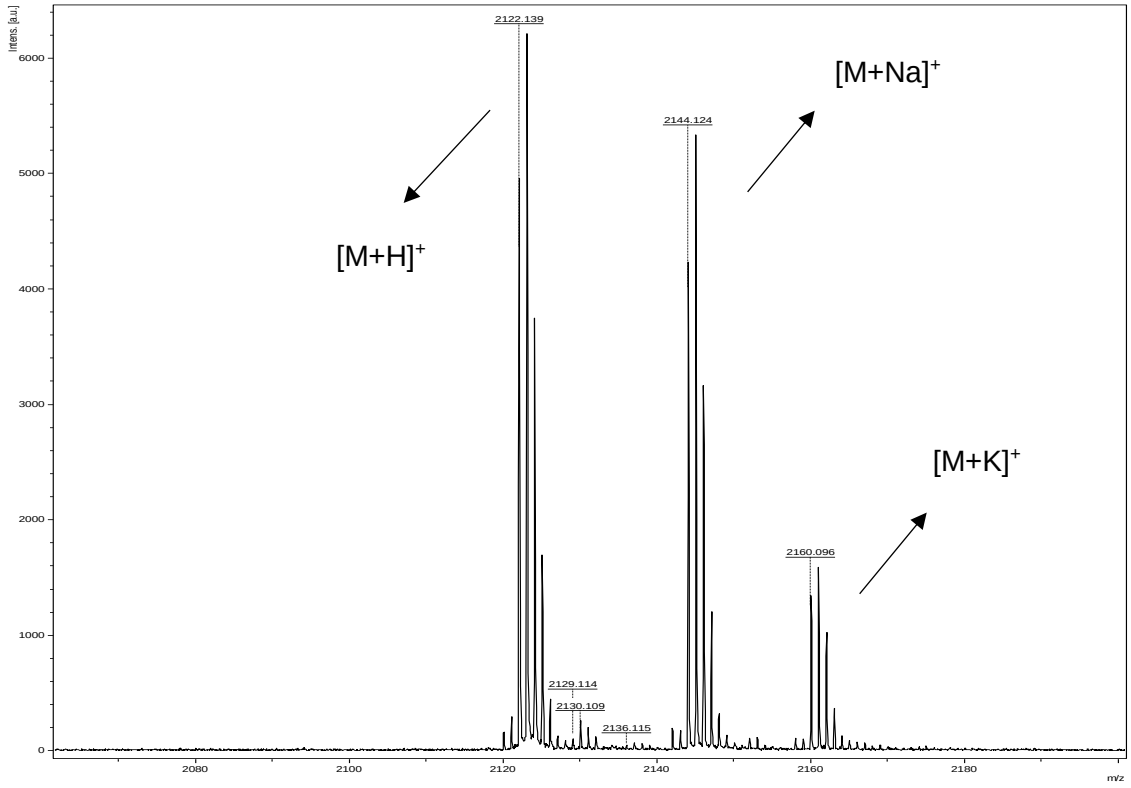


Figure S24 - MALDI-TOF-MS spectrum of dendrimer **7b** (Chapter 4).

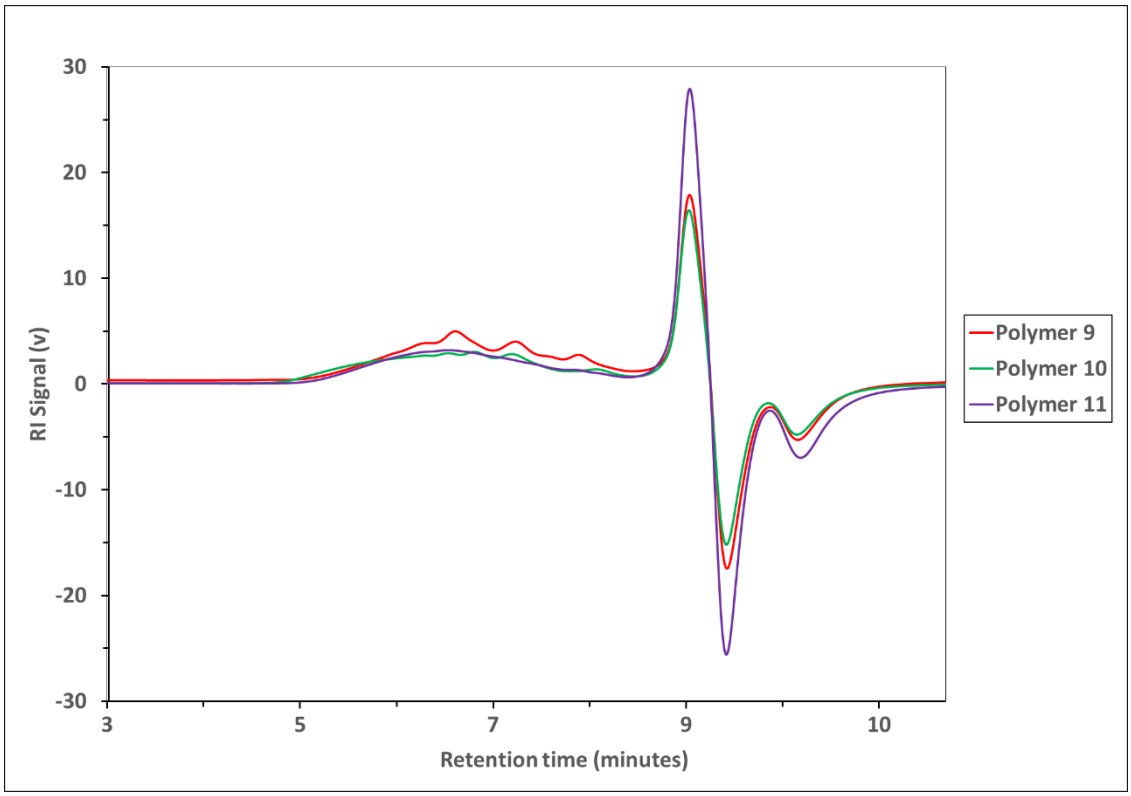


Figure S25 - Size exclusion chromatogram of polymers **9**, **10**, and **11** (Chapter 4).

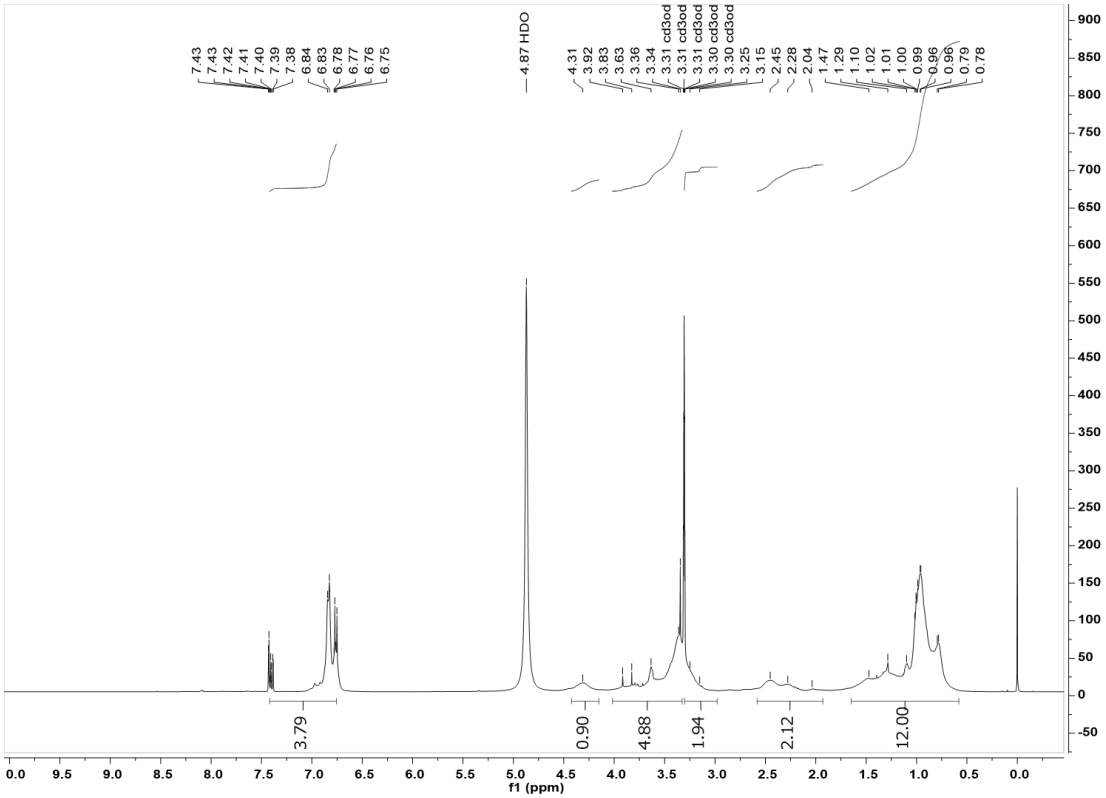


Figure S26 - ^{1}H NMR (400 MHz, MeOD) spectrum of polymer **9** (Chapter 4).

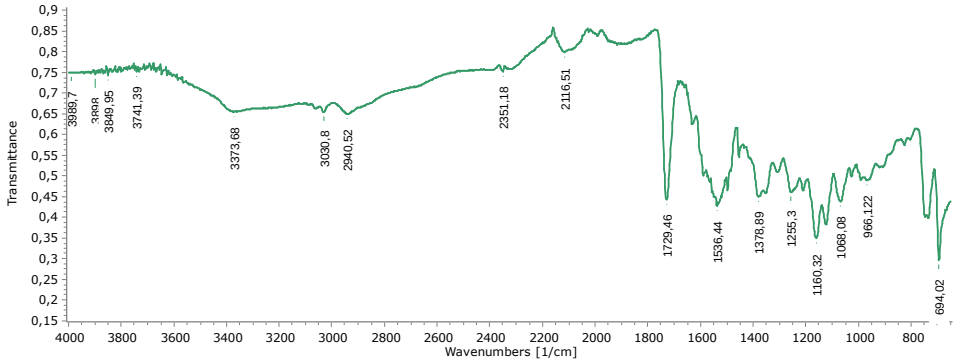


Figure S27 - FTIR spectrum of quantum dot **2** (Chapter 5).

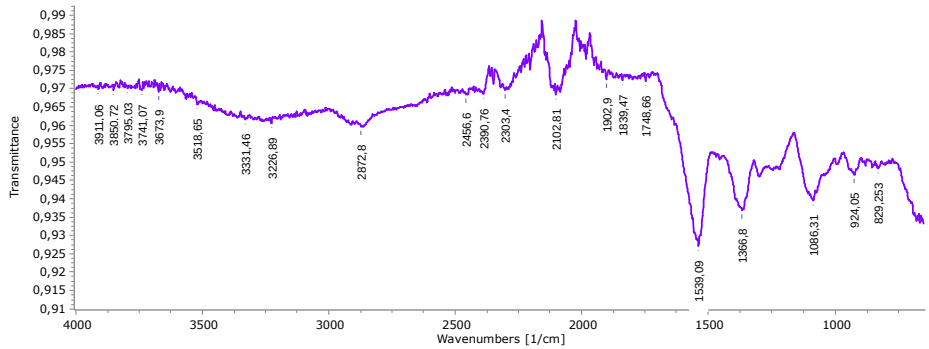


Figure S28 - FTIR spectrum of quantum dot **3** (Chapter 5).

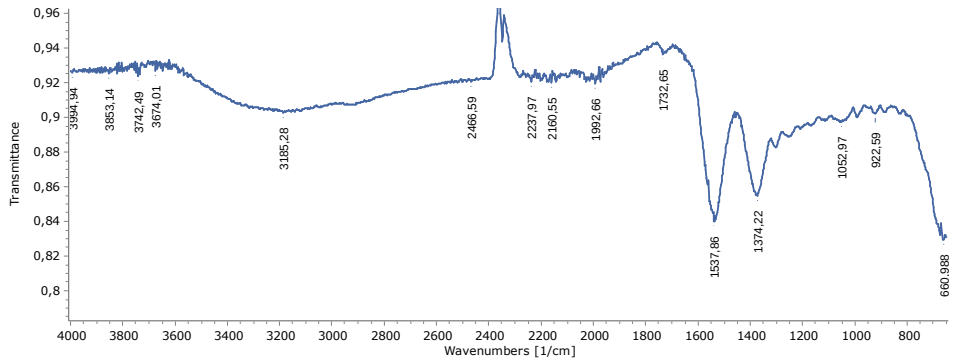


Figure S29 - FTIR spectrum of quantum dot **5** (Chapter 5).

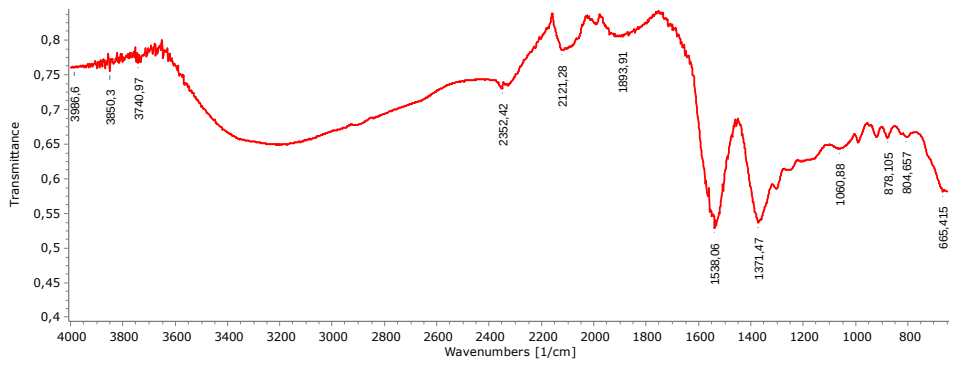


Figure S30 - FTIR spectrum of commercial quantum dot (Chapter 5).

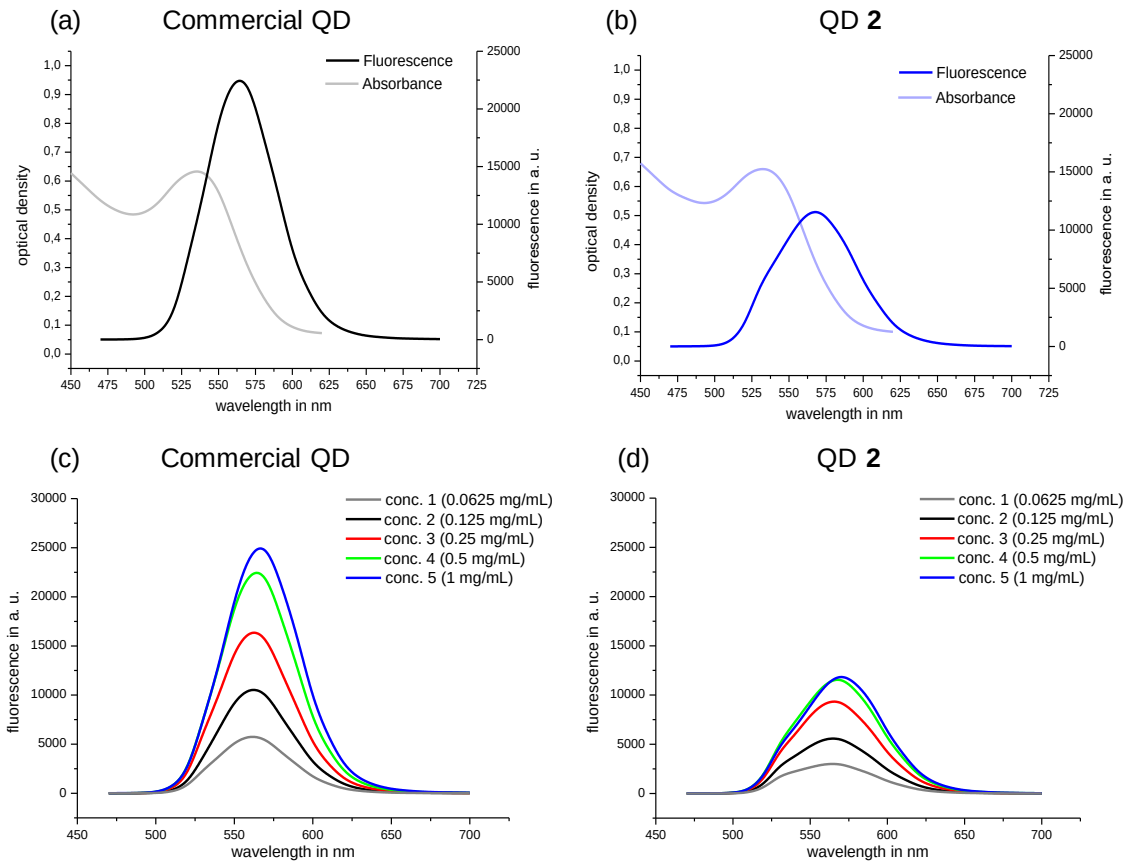


Figure S31 – (a) UV-Vis absorption and fluorescence emission spectra of commercial quantum dot (0.5 mg/mL); (b) UV-Vis absorption and fluorescence emission spectra of quantum dot **2** (0.5 mg/mL); (c) Fluorescence emission spectra of commercial quantum dot at different concentrations; (d) Fluorescence emission spectra of quantum dot **2** at different concentrations. (Chapter 5).

Institute internal substance assignments

Chapter 2

Product number	Three letter code SBD	Product number	Three letter code SBD
1a	63	**15**	291
1b	66	**16a**	239
2a	69	**16b**	250
2b	72	**17a**	252
3a	220	**17b**	258
3b	262	**18a**	289
4a	219	**18b**	290
4b	261	**19a**	70
5a	229	**19b**	73
5b	233	**20**	31
6	30	**21**	75
7	224	**22**	76
8	223	**23a**	274
9	230	**23b**	275
10a	64	**24a**	276
10b	67	**24b**	277
10c	200	**25a**	278
11a	207	**25b**	280
11b	226	**26a**	281
12a	228	**26b**	285
12b	238	**27a**	282
13a	227	**27b**	286
13b	259	**28a**	283
14	256	**28b**	287

Chapter 3

Product number	Three letter code SBD	Product number	Three letter code SBD
1a	339	**4b**	373
1b	349	**5a**	422_3
2a	383	**5b**	440
2b	384	**6a**	422_5
3a	308	**6b**	441
3b	351	**7a**	422_6
4a	372	**7b**	442

Chapter 4

Product number	Three letter code SBD	Product number	Three letter code SBD
1a	434	**6a**	435
1b	444	**6b**	445
2	433	**7a**	439
3a	443	**7b**	446
3b	449	**8a**	447
4a	438	**8b**	451
4b	450	**polymer 9**	519
5a	452	**polymer 10**	520
5b	456	**polymer 11**	521

Chapter 5

Product number	Three letter code SBD
1	517
2	524
3	525
4	529
5	528

M.Sc. Nalin de Seixas Borges

1. General Informations

Place and Date of Birth: São Sepé, Rio Grande do Sul, Brazil, 5th October 1990

Office Address:

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Department of Bioorganic Chemistry

Weinberg 3, 06120

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2. Education

2014 - currently	PhD Student at the Leibniz Institute of Plant Biochemistry Department of Bioorganic Chemistry, IPB Halle (Saale), Germany. Recipient of a Ph.D. Fellowship of the science without borders program (Brazil), 2014-2018. Supervisor: Prof. Dr. L.A. Wessjohann
2012-2014	*M. Sc.* in Organic Chemistry, Federal University of Santa Maria, UFSM, Santa Maria, RS, Brazil. "Synthesis of derivatives of stigmasterol and ursolic acid and evaluation of their biological activities." Mention: excellent. Supervisor: Dr. Ionara Irion Dalcol. Recipient of a Master Fellowship of CAPES program.
2008-2011	Diploma thesis at the Federal University of Santa Maria, UFSM, Santa Maria, RS, Brazil. Project: "Synthesis of stigmasterol derivatives, a metabolite isolated from the *Buddleja brasiliensis* plant, and its evaluation as an inhibitor of the enzymes acetylcholinesterase, prolyl oligopeptidase and dipeptidylpeptidase-4." Grade: 10.0 (0 - 10.0). Supervisor: Dr. Ionara Irion Dalcol. Recipient of a Fellowship of FAPERGS program.
2008-2011	*B.Sc.* in Chemistry, Federal University of Santa Maria, UFSM, Santa Maria, RS, Brazil.

3. Languages

Portuguese, English, Spanish

4. Complementary Training

2010 "Chromatography and analytical validation of chromatographic methods."
Workload: 20 hours.
Federal University of Santa Catarina, UFSC, Florianópolis, SC, Brazil.

2018 "Medicinal Chemistry: The Molecular Basis of Drug Discovery"
Workload: 56 hours
Davidson College through edX.

5. Selected Conferences

2017 De Seixas Borges, N.; Wessjohann, L. A.; Development of a new methodology to synthesize dendrimers *via*
Passerini reaction using a divergent approach. At Modern trends in dendrimer chemistry and applications, Moscow, Russia. Poster presentation.

2013 Seixas, N.; Bender, V.; Pereira, A.; Dalcol I.; Morel A.; Tarragó T.; Girald, E. In Vitro Evaluation of stigmasterol derivatives as potential prolyl oligopeptidase and acetylcholinesterase inhibitors. At 4th Brazilian Conference on Natural Products, XXX RESEM, Natal, Brazil. Poster presentation.

Pereira, A.; de Ávila, J.; Neto, A.; Seixas, N.; Dalcol, I.; Morel, A. In vitro antimicrobial evaluation of *Erythrina crista-galli* from Southern Brazil. At 4th Brazilian Conference on Natural Products, XXX RESEM, Natal, Brazil. Poster presentation.

2011 Seixas, N.; Riveiro, A. C.; Adolpho, L. Antimicrobial and acetylcholinesterase activities of the essential oil from *Chenopodium ambrosioides*. At 3rd Brazilian Conference on Natural Products, XXIX RESEM and VII SFL, Ouro Preto, Brazil. Poster presentation.

Seixas, N.; Adolpho, L.; Gitzel Filho, A. Biological activity of compounds isolated from *Buddleja brasiliensis*. At 3rd Brazilian Conference on Natural Products, XXIX RESEM and VII SFL, Ouro Preto, Brazil. Poster presentation.

Riveiro, A. C.; Gitzel Filho, A; Seixas, N. a-cadinol: acetylcholinesterase inhibitor isolated from the essential oil of *Artemisia verlotorum*. At 8ª International Congress of

Pharmaceutical Sciences - CIFARP, Ribeirão Preto, Brazil. Poster presentation.

2010 Adolpho, L.; Seixas, N.; Gitzel Filho, A. Activity of *Buddleja brasiliensis* Jacq. ex Spreng against POP and DDP-IV enzymes. At XXI Symposium of Medicinal Plants of Brazil, Joao Pessoa, Brazil. Poster presentation

2009 Maldaner, G.; Maragon, P.; Illha, V.; Dalcol, I. I.; Morel, A.; Adolpho, L.; Seixas, N. Natural acetylcholinesterase inhibitors isolated from *Scutia buxifolia*. At 32nd Annual Meeting of the Brazilian Chemical Society, Fortaleza, Brazil. Poster presentation.