Synthesis and characterization of bis-MPA based branched polymers with thymine core

Dissertation

zur Erlangung des akademischen Grades
Doktor rerum naturalium
(Dr. rer. nat.)

Vorgelegt

der Fakultät Mathematik und Naturwissenschaften der Technischen Universität Dresden

von

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geboren am 22.10.1983 in Lenigrad (USSR)

In memory of my granny

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1 Introduction

The branched systems were getting a lot of attention through the last decades due to their unique properties and interest is not lost but only increasing. This statement is equally true for both perfect structures (dendrimers and dendronized polymers) and imperfect ones (hyperbranched polymers and their derivatives).

Dendrimers were first synthesised in a research group at DuPont [1]. From the first moment it was obvious that it was some kind of breakthrough. This could be explained by dramatic difference between their physical properties and the ones possessed by linear polymers of the same molecular weight. It is well-known that the globular shape of these molecules obtained at a certain point is the major cause of the "special" behaviour. Another point is the number and the distribution of active groups, which are observed on the focal point and/or outer shell. The main problem was and still is a complicated synthesis, including repeating protection/deprotection steps and rather complex working-up and/or purification procedures. Through application of new methods and strategies, the number of steps could be slightly reduced, but it is still hard to go further to high generations. It all makes this material very expensive and not much appealing for manufacturing, though there are some positive examples.

Due to the obvious problems with dendrimers the need to prepare some kind of similar materials using some simple synthetic methods was growing. Here the simplest idea was to prepare imperfect structures by on-pot strategy, though in theory these hyperbranched (hb) polymers were not really considered promising. Despite this, hb polymers are more than just fascinating. In 1999 Vogl and Jaycox [2] said that a new area of nonlinear polymers could be much more explored in the next years and that this kind of materials would find there unique applications, though considered a bit strange at the time of invention. Hyperbranched (hb) polymers are considered to have a random geometry and to be cheaper in preparation and more likely to be suitable in the cost-to-performance balance [2]. One-pot or pseudo-one-step synthesis are fast, precipitation is an easy purification method, though drawbacks include not fully controllable structure, side reactions, problem with scaling up, but with an appropriate approach all these problems can be more or less overcome. Imperfect branched structures, compared to perfect ones, are characterized by a lower degree of branching (DB) [3-7], but still possess a non-linear architecture and high number of potentially reactive end groups.

For all kind of branched structures the fact of existing of a free focal point and/or periphery end-groups gives scientists room for wide range of modifications that in turn lead to the formation of materials with new properties, sometimes completely different from those of known starting materials. That is why macromolecular engineering of complex molecular architectures through the introduction of controlled branching has become an increasingly important theme in polymer science. Many research groups are working with branched molecular architectures, such as dendrimers, hyperbranched or dendronized polymers, and stars. There are several examples that these materials were transferred from chemical laboratories to the manufactures.

Dendrimers and hyperbranched polymers, which were a novelty in 1990s, still receive a lot of attention. Scientists all over the World are looking for new methods of synthesis and modification of such materials in order to find new applications. They are known to be useful as coating, additives and resins, but it cannot be all.

Nowadays the biochemistry and medicine related fields are growing fast and this tendency is expected even to speed up. This includes synthesis of artificial analogous to natural enzymes and receptors, molecular recognition, molecular imprinting, drug delivery and more. For such applications more and more artificial substances are used and branched structures are considered to have a very high potential. For example, PPI dendrimer have been already used to improve solubility, biocompatibility and also to reduce toxicity of some drugs.

Molecular recognition, molecular imprinting as well as drug deliver can be based on the physical associations between targeting molecule and material. For that perfectly/randomly branched structures with their active focal point and high amount of active end-groups together with possibility to modify them are promising candidates.

There are only a couple of investigations including branched structures desired for molecular recognition, which were completed until now. So it is very interesting to try known monomers in synthesis of these materials and to explore the natural phenomena in order to increase their selectivity.

The incorporation of natural DNA or RNA nucleic bases or their artificial analogues into a polymer structure and exploring their abilities to form stable complementary bonds was done for linear polymers via either incorporation of the active unit into monomer or modification of a final polymer. This strategy was once applied to hyperbranched polymer based on 2,2-bix(hydroxymethyl)propionic acid and the preliminary results were quite good [8]. That is the reason to go on in this direction and perform extensive synthesis and characterization not only for hyperbranched polyesters but also for corresponding dendrimers.

2   Literature overview

2.1   Dendritic polymers

2.1.1   Perfectly branched structures

Perfectly branched structures include dendrons, dendrimers, linear-dendritic hybrids and dendronized polymers. The first two are synthesized in the same manner with or without core molecule. The third case is when dendrons are coupled to a linear polymer chain "as end groups", while in the last dendrons are attached to active groups on a polymer backbone. All of them are quite interesting and promising for different applications. As more than half of the work was focused on dendrimers they will be paid a special attention

Vögtle was the first one to publish an article on dendrimer synthesis [9], it was followed by articles by Tomalia [3] and Fréchet [4]. Dendrimers are practically monodisperse, well-defined materials. The hydrodynamic volume of these compact molecules has a different relationship with respect to the molecular weight and seems to be smaller in size in comparison to the linear polymers. The difference between the hydrodynamic volume of linear end dendritic macromolecule depends on the generation and solvents [10]. Low viscosity, small hydrodynamic volume and better solubility of dendrimers comparing to the linear analogous of the same molecular weight led to an exponential development of this area in the end of 20th century.
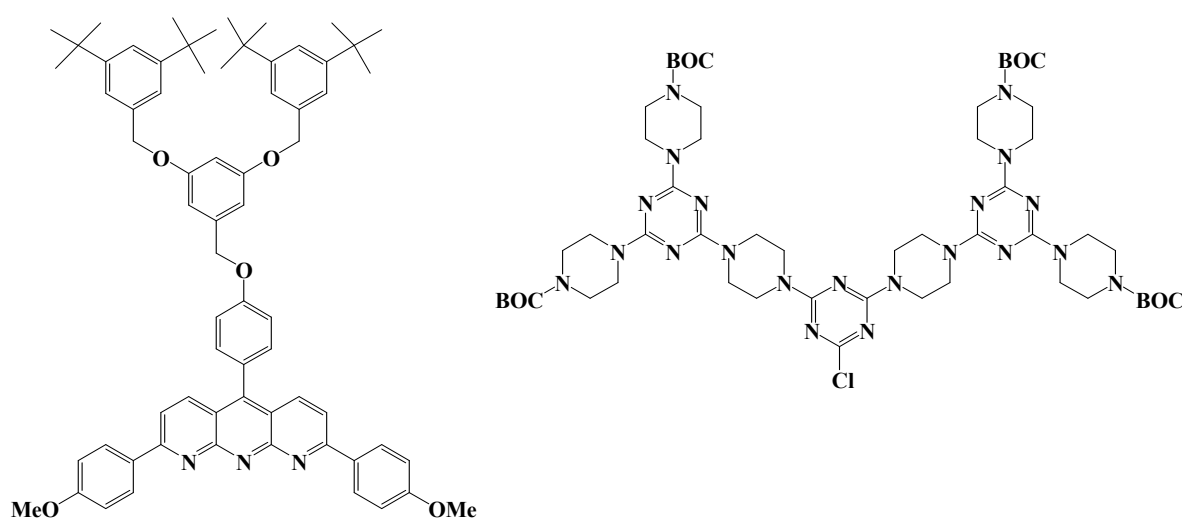
Figure 1 Examples of dendritic structures especially suited for physical interactions [11, 12]

Many different types of dendritic structures are known and investigated. Among them are poly(ether amide) [13], polyamidoamine [14-16], polyhydroxycarbosilane [17, 18], melamine [11], aromatic ether [12] and aliphatic ester based dendrimers [19-30], dendrigrafts and dendronized polymers. A lot of work is in progress in this area, so more and more new materials are produced and for some of them new applications have been found. One of the fast developing areas is molecular recognition (MR), because it could be considered a starting point for the preparation of materials suitable for drug/dye delivery and biomedical applications. Some examples of structures containing active group for specific physical binding, which could be useful in MR, are presented in **Figure 1.**

2.1.1.1   General approaches in dendrimer synthesis

From the beginning it was obvious that dendrimers can be obtained only through a rather complicated set of reactions including protection/deprotection steps. Mono- or multi-functional cores and AB_x monomer are used, depending on the synthetic way either focal or shell protected monomer derivatives are also implied. Preferably AB_2 are applied, though x = 3,4 are also known (**Figure 2**).
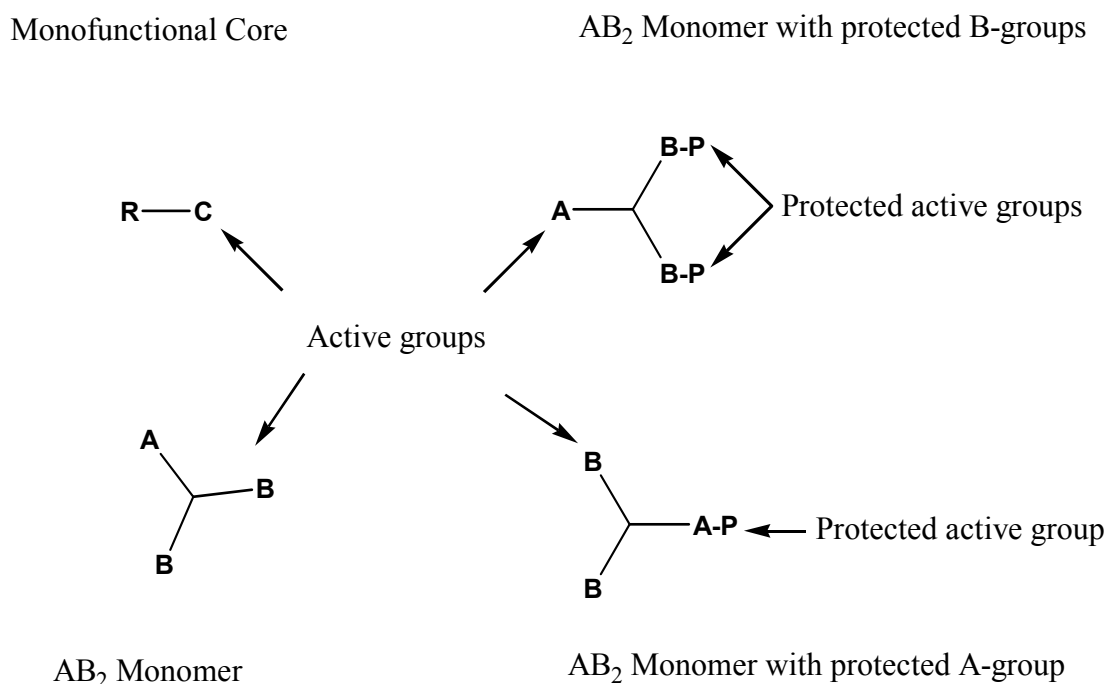
Figure 2 Monomers and core moiety applied in dendrimer synthesis

Through years spent on synthesis of perfect structures two general synthetic approaches have been developed [31]. Divergent and convergent routes (**Figure 3 - 4**) are the main strategies used in dendrimer synthesis; in order to reduce a number of steps some groups switched to their combinations, that means preparation of low generations (up to second) using divergent approach and switching to the convergent one for the higher generations (usually from third). The mixed-approach helps not only to reduce the number of steps, but also to increase the chances of complete substitution, so it is a powerful tool.

Only two approaches, divergent and convergent, will be discussed in details, because on every particular step the mixed one is not different from the corresponding step in either approach.

Divergent approach

Coupling of the protected AB$_2$ monomer with core, followed by deprotection

First generation dendrimer
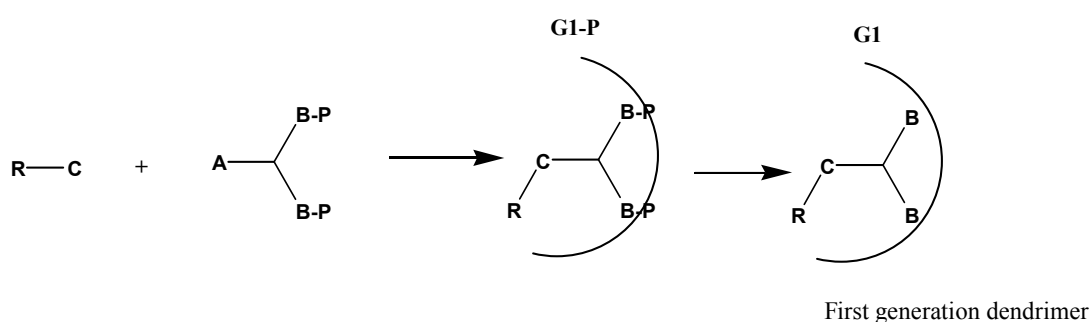
Synthesis of the second generation dendrimer

Second generation dendrimer
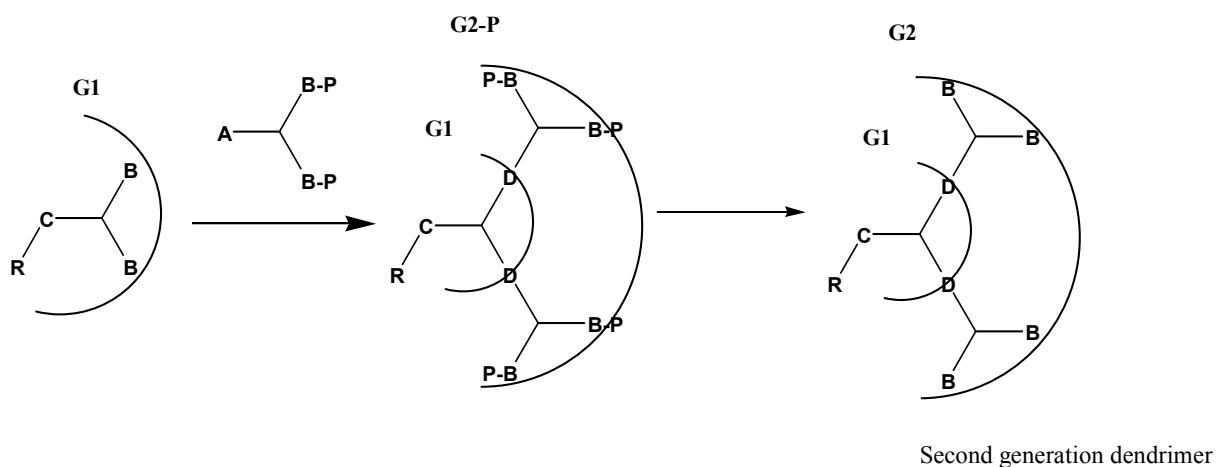
Figure 3 General scheme for divergent approach

The divergent approach includes stepwise increase in number of generations starting from a core and using protected monomers [32]. At first B-groups in monomer are protected, but the active focal point is left untouched. (AB$_2$ Monomer with protected B-groups in **Figure 2**) Then this protected monomer is coupled to a core molecule and B-groups are deprotected. From this point to prepare a dendrimer of higher generation the coupling/deprotection cycle has to be reproduced. The amount of dendritic points (**D**) raises exponential, while the number of end groups only doubles. In most of the cases column chromatography is needed to obtain a clean product. Basic scheme is presented in **Figure 3**. The most obvious drawback is that the higher the generation the more sterical hindrance and the greater the chance of imperfect structure of a product due to incomplete reactions at the B functions.

Convergent approach

Synthesis of dendrons

First generation dendron
with protected focal point

Second generation dendron
with protected active groups
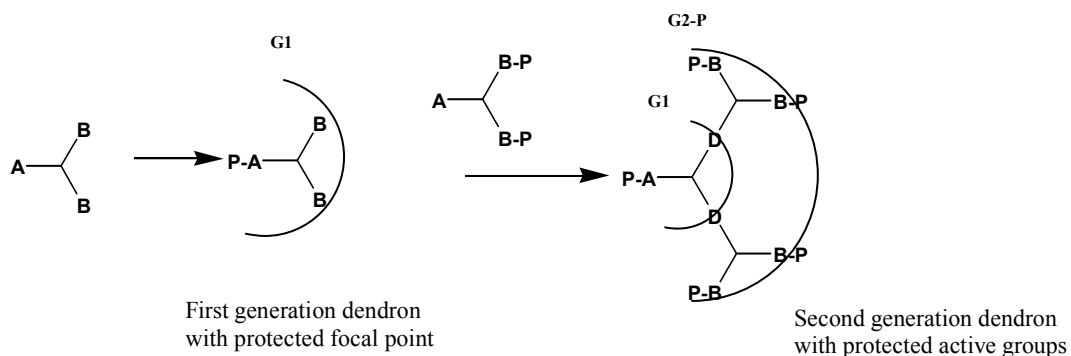
Deprotection of active group in focal point

Second generation dendron
with protected end/groups and free acive focal point
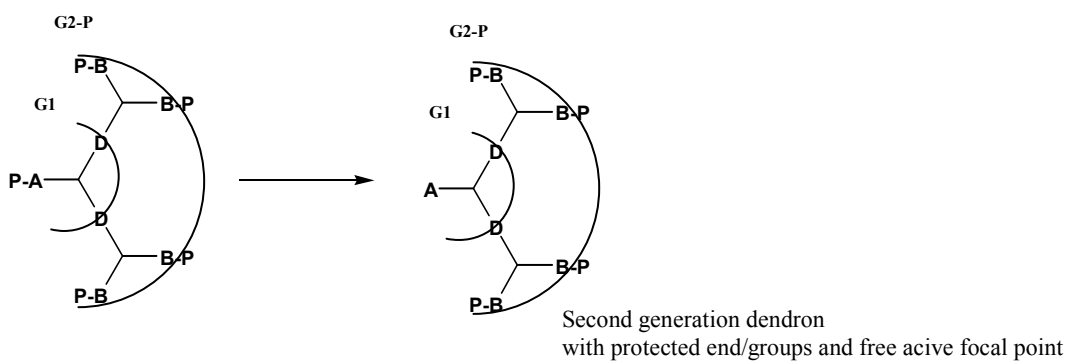
Synthesis of dendrimer via coupling with core molecule

Second generation dendrimer
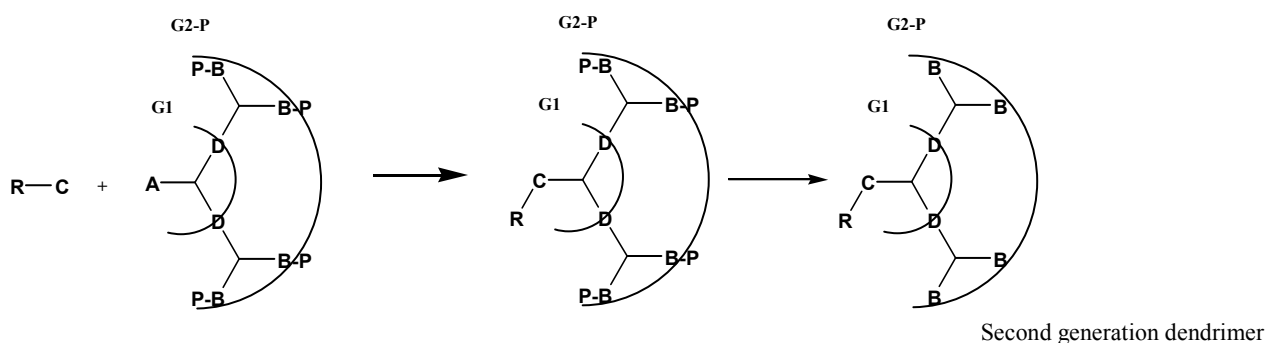
Figure 4 General scheme for convergent approach

In the convergent approach two kinds of protected monomers: AB_2 monomer with protected focal point A and AB_2 monomer with protected B-groups are synthesized. Synthesis starts from coupling of a first derivative with protected focal unit A and a standard B-protected monomer (somehow it resembles the divergent approach); molecules obtained here are called dendrons. When finished they are deprotected on the focal unit and coupled to a core (**Figure**

4). It looks like arms were attached to a core. The multi-functional core could be a problem; here one can face sterical problems during incorporation. It leads again to defects in structure, in this case this defects mean that one or more dendrons are missing.

More detailed description and definition, as well as examples of structures and synthetic schemes could be found in the work of Tomalia [33-35], Fréchet [23, 32] and Schlüter [31, 36].

2.1.1.2 Synthesis of dendrimers based on 2,2-bis(hydroxymethyl)propionic acid

As the present work is based mainly on the AB$_2$ monomer 2,2-bis(hydroxymethyl)propionic acid (bis-MPA), it may be useful to have a close look on related dendrimer examples described in literature. In the mid-1990s, Hult et al. presented dendrimers up to fourth generation based on bis-MPA [20]. Dendrimers were achieved by introducing the acetonide-protecting group and by use of N,N'-dicyclohexylcarbodiimid (DCC) as the dehydration agent. Now dendritic structures based on bis-MPA are synthesized in several groups, and activating agent, core molecule and protecting groups have been varied. Through the years esterification was optimized and now some "typical procedures", that will be discussed later, are used nearly by everyone interested in the synthesis of such structures.
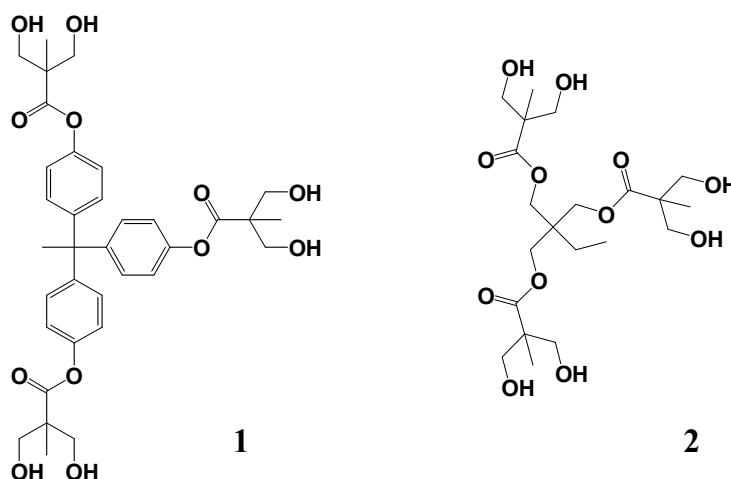
Figure 5 Examples of first generation dendrimers on bis-MPA basis as AB$_2$ monomer

As for any dendritic structure synthesis of bis-MPA dendrimers could be accomplished via divergent or convergent routes [21]. It would be wrong to say that one of them is favourable, so in every case it is up to the scientist to chose. The starting core (**Figure 5**) could be an aromatic **1** [22, 23] or an aliphatic one **2** [19], as it is shown above for the first generation structures described by Hult et al. They were pioneers in this area and put a lot of work into

the development of the fastest and most effective synthesis, and they were the first ones to explore the DCC/DPTS method as well as acetonide protected anhydride of bis-MPA in esterification. Protection/deprotection could be done also in several ways. All possible methods are summarized in **Figure 6** and **Figure 7**.

Second generation dendron   **1,1,1-tris(hydroxyphenyl)ethane**
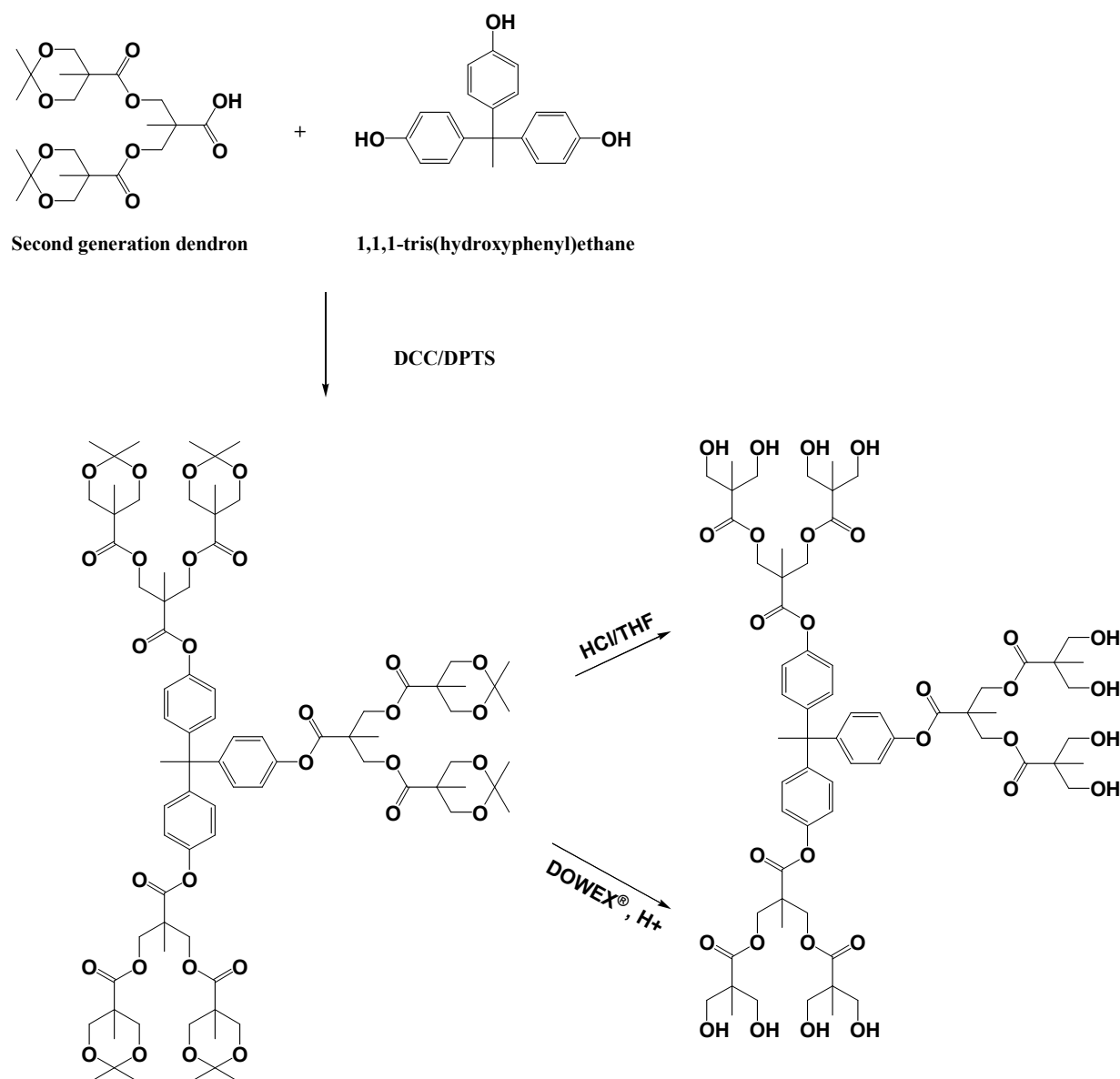
DCC/DPTS

Figure 6 Convergent synthesis of a second generation bis-MPA based dendrimer via the DCC/DPTS method

As shown above acetonide-protection could be cleaved either by 2M aqueous solution HCl mixed with THF (1:1), which gives the desired product in 2 h with 92% yield [37], or by simple stirring with DOWEX® suspension in MeOH, which gives quantitative yields. The first way of deprotection could also lead to partial decomposition of the dendrimer and was never described for branched structures with aliphatic cores.
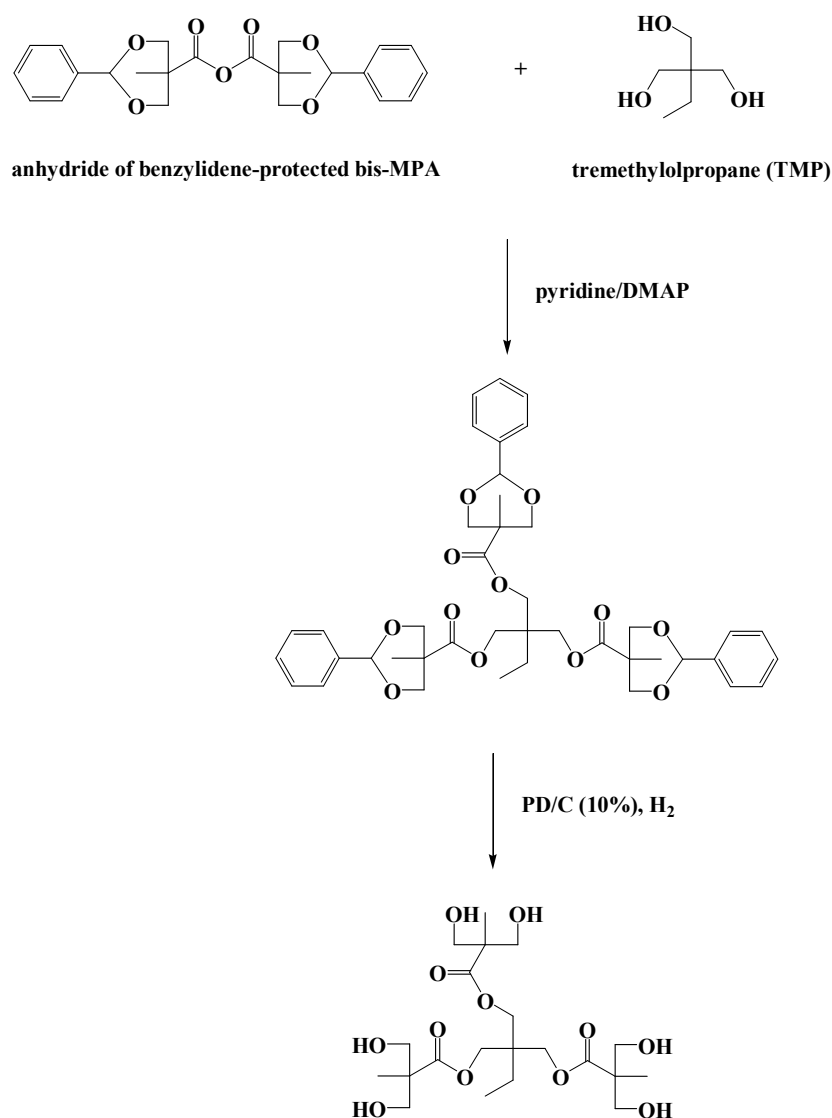
Figure 7 Divergent synthesis of a first generation bis-MPA based dendrimer via benzylidene protected anhydride

Some scientists are still using the DCC/DPTS method of the free acid [24] but in the majority of cases the anhydride method is referred as "typical procedure" (**Figure 8**), though column chromatography for purification in every step is hard and time consuming [25, 26, 38]. Here the anhydride of acetonide protected bis-MPA is used for divergent dendron growth and followed by deprotection of the acetonide protective group by stirring the monomer in methanol with acidic DOWEX-50-X2 resin. The coupling reactions proved to give high yields, facilitating dendrimer synthesis on a large scale [26].

Benzylidene-protection is also good, and can be easily cleaved, but still acetonide-protected anhydride seemed somehow better.
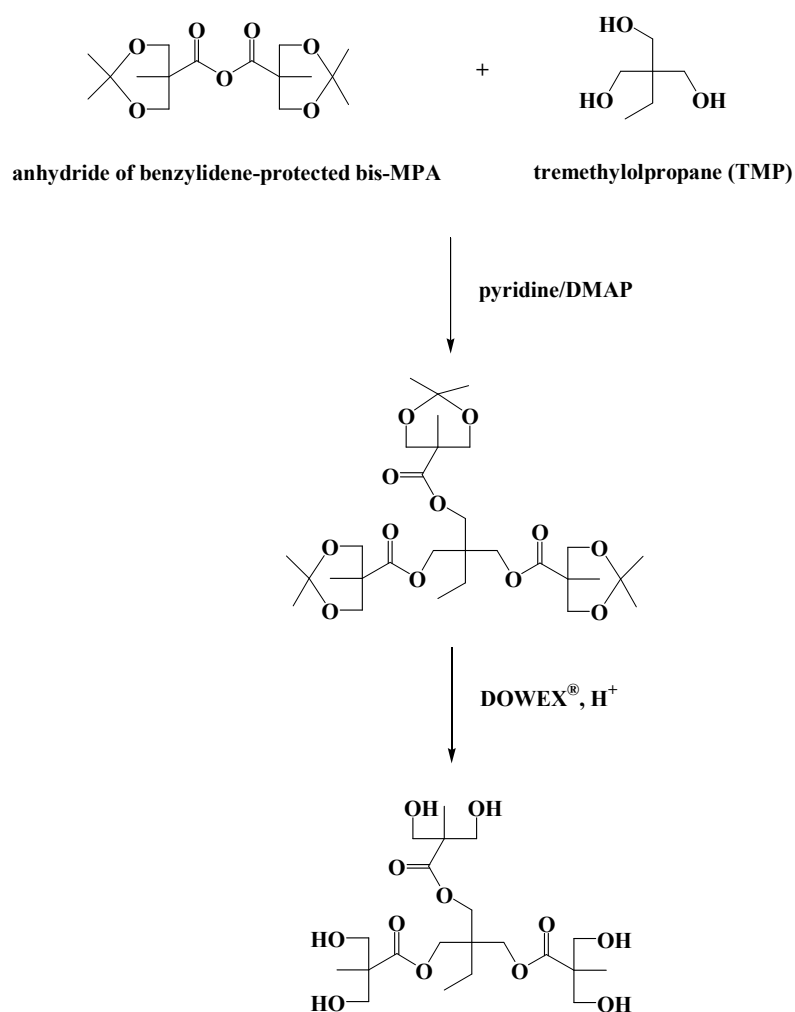
Figure 8 Most often used divergent synthesis of bis-MPA based dendrimers

Usually couplings were monitored by ^{13}C NMR that gives information about the conversion and helps to determine the end of the esterification.

2.1.2   Synthesis of linear-dendritic hybrids

Linear-dendritic hybrids or dendronized polymers are of a great interest for preparation of thin layers, modification of surfaces [27, 31] and preparation of star polymers [28, 29]. Despite the fact that they were not prepared during this work, still the information about their synthesis could be useful for the preparation of dendrimers that is why such structures with bis-MPA monomer will be discussed.

Two different synthetic approaches exist for dendrigrafts preparation. One of them, the so called "grafting from" approach used by Benhabbour et al. [30] is shown in **Figure 9**. They have prepared dendronized surfaces by chemisorption of poly(ethylene glycol) monothiol (Hs-PEG$_{650}$-OH) onto gold-coated silicon wafers and then modified surface OH-groups with

PEG, to see the difference in the protein absorption of such materials [30]. Dendrons of G1-G4 were synthesised according to divergent route using standard methods developed for the simple dendrimer synthesis. The same principle as for convergent approach could be used in so called "grafting to" approach.
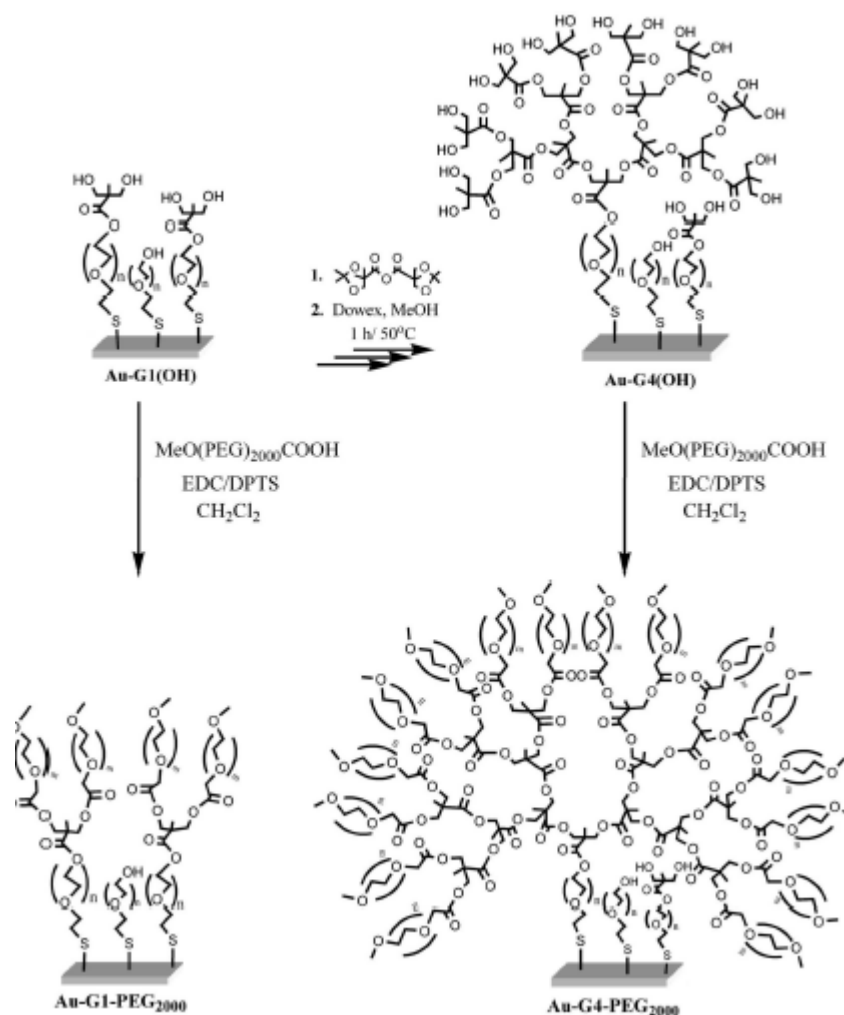
Figure 9 Synthesis and modification of dendronized surfaces [30]

Fictionalisation of different surfaces could be performed in other manner, via dendronization [27, 31].

Dendronized polymers carry dendrons coupled to the main skeleton; here also two ways of synthesis exist (**Figure 10**): coupling of the prepared dendrons to the polymer backbone ("grafting-onto" route) [28] or polymerization of a monomer bearing a small dendron

(macromonomer approach) [10, 25, 39, 40]. In later case, only small dendrons are used and the divergent growth could be applied to prepare higher generations, when it is needed.
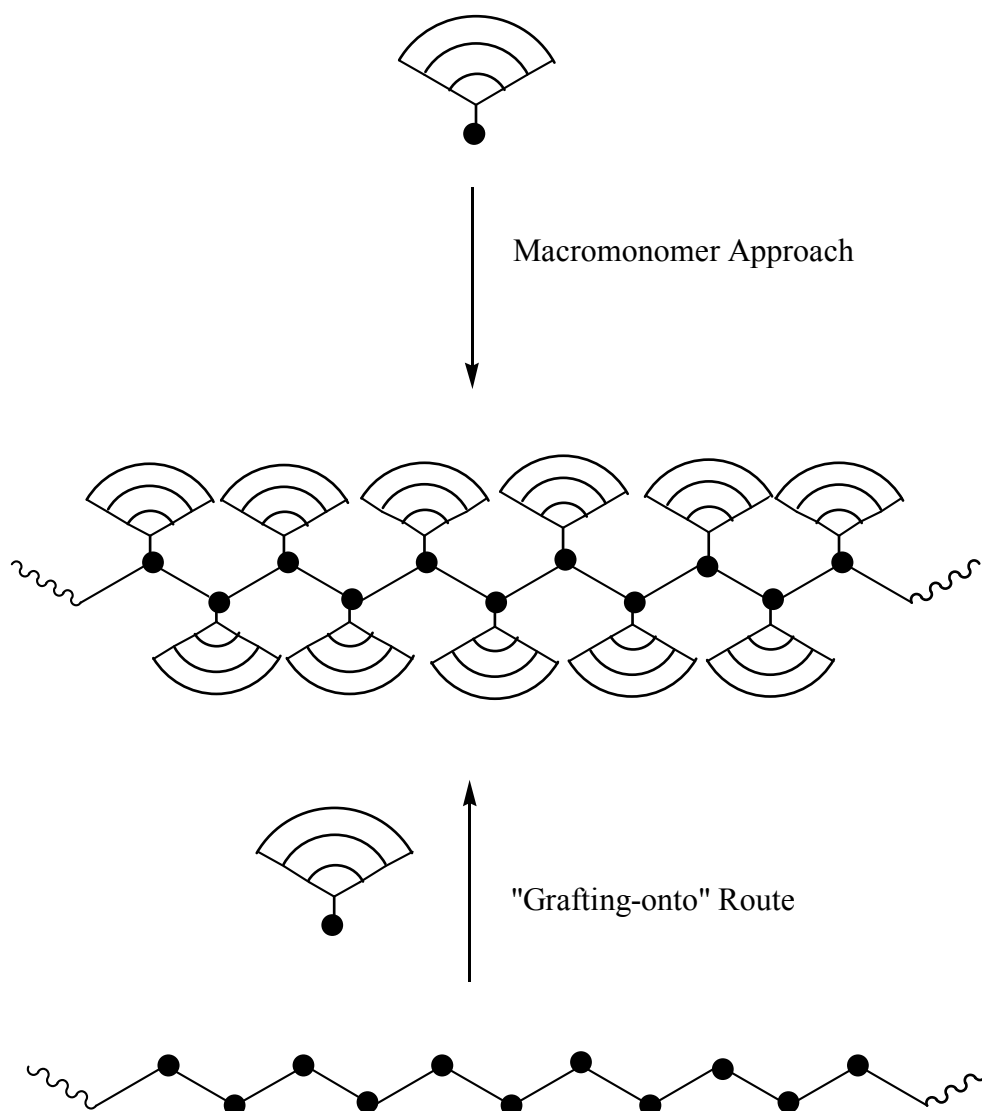
Figure 10 Different approaches to prepare dendronized polymers

Through the generation number physical properties of dendronized polymers could be varied. For example, there is a general tendency of T_g increasing simultaneously with generation when bis-MPA was used as monomer [40]. Further modification of end groups is also a possible way to control the physical properties of final materials.

2.1.3 Hyperbranched polymers

When Flory wrote his famous book Principles of Polymer Chemistry in 1952, he indicated an alternative scheme for the synthesis of hyperbranched structures [41]. He theorized about

synthesizing polymers from multifunctional monomers (AB_x) via condensation. These polymers were predicted to have a broad molecular weight distribution and to be non-entangled and non-crystalline due to their highly branched structure. However, they were considered to be less interesting since they would provide materials with poor mechanical strength, and at that time Flory did not feel it was worthwhile pursuing this line of research. Despite such predictions hyperbranched polymers turned out to be interesting and useful [2, 42]. Of course, in some case problems arise from crosslinking and gelation, but with variation of monomers and synthetic approaches drawbacks could be minimized.

First synthesis of hyperbranched polymer was performed in the early 20th century [43] and it took nearly 80 years till the first intended synthesis of such materials was reported by Kim and Webster. There are several very good, informative and complex reviews in this area published by Voit [44, 45], Gao [46] and others[47-49].

As well as dendrimers, hyperbranched macromolecules are also prepared from multifunctional monomers but not limited to AB_2 ones and not involving protection/deprotection steps. In contrast to perfect dendrimers, hb polymers are prepared in single-step [50] or in pseudo-step-by-step polymerizations [51], which greatly facilitates there availability but leads to polymers with irregular branching and broad molecular weight distribution. Now a broad range of hb polymers including polyamides [52, 53], polyamidoamine [54], polyimides [55], polyimines [56], poly(amine-ester)s [57], polyethers [58-65], polyesters [66-71], polyarylene [72], polystyrenes [73, 74], polyphenylenes [75, 76], poly(ether imide)s [77] and poly(urea urethane)s [78] exist.

The different synthetic approaches for dendritic and hb materials lead to significantly different structures, though they are much closer to one another than to linear ones. It is well-know that hb structures posses dendritic (**D**), linear (**L**) and terminal units (**T**) (**Figure 11**), while perfectly branched molecules have only first and last ones. Both the dendritic and terminal units contribute to the "perfect" or "fully" hyperbranched character of the molecule, while the linear units decrease the degree of branching.
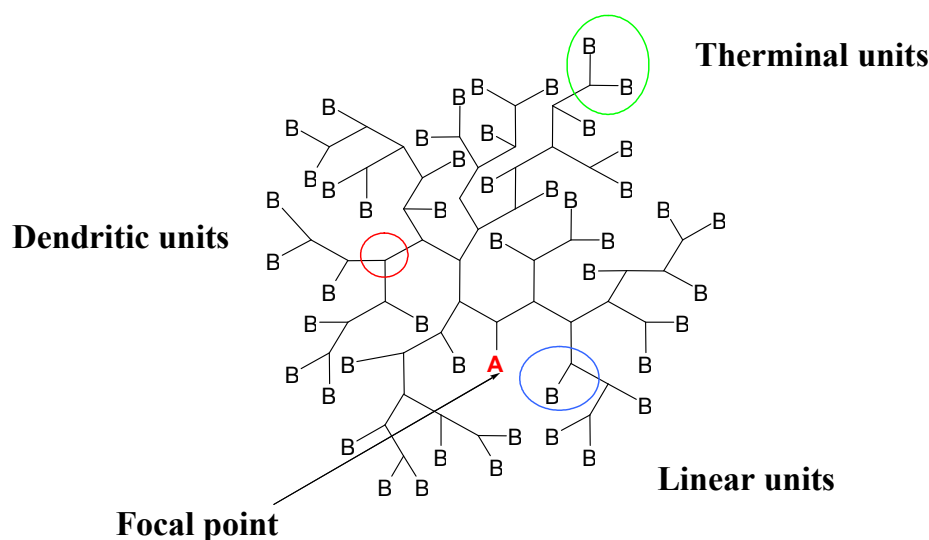
Figure 11 Structural units in hyperbranched polymers

To make characterisation easier the term "degree of branching" (DB) was introduced by Fréchet et al. [50]; it is a proportion of branched units in comparison to the perfectly branched structure. Simultaneously its mathematical equivalent was derived (**Equation 1**).

$$DB = \frac{D+T}{D+L+T}$$

Equation 1 Degree of branching according to Fréchet

Frey et al. have followed with further theoretical excavations and were able to find a more general mathematical form for DB, which could be personalized in every particular case [5-7]. During calculations equal reactivity for all units was assumed as well as the single monomer unit at the focal point was ignored since it may be difficult to be observed for high molecular weight polymers and its influence becomes insignificant as molecular weight increases. For example, DB for hb polymer based on an AB_2 monomer is the following:

$$DB = \frac{2D}{2D+L}$$

Equation 2 Degree of branching according to Frey

Whatever equation would be taken degree of branching for dendritic structures is equal to 1 and for hyperbranched structures is lower than that, for AB_2 monomer it usually lays between 40 and 60%. Though imperfect in structures hb molecules become globular at some point like

15

their dendritic analogues. This, together with good solubility and low viscosity and a less complicated synthesis overcomes obvious drawbacks of the imperfect structures and makes highly branched structures promising for a number of applications.

2.1.3.1 Methods of synthesis

As shown above there are a lot of different hyperbranched polymers with completely different structures that also means considerable number of reactions for their preparation. In general, the synthetic approaches can be divided into two major categories: the AB_x approach and the $A_x + B_y$ approach [46]. Among the examples of the first one are reactions with different mechanisms: (1) step-growth polycondensation of AB_x or latent AB_x monomer [50, 51, 60, 79], (2) self-condensing vinyl polymerization [80, 81], and (3) self-condensing ring-opening polymerization [82, 83] and (4) proton-transfer polymerization [84, 85]. In the $A_x + B_y$ approach two monomers or a monomer pair are polymerized together [55, 59, 69, 71, 72, 74, 86].

In the majority of cases the amount of side reactions is quite high, that can lead to crosslinking and formation of gels that in turn can cause low solubility in common organic solvents. It was a especially true for the first attempt in preparation of hb structures on the 2,2-bis(hydroxymethyl)propionic acid. Of course, modification of reaction conditions, monomer ratios and activating agent, precise observation and stopping of the reactions before gelation has led to the preparation of many different samples. A variation could also show the way to obtain materials with improved or new properties.

It is understandable that the controlled synthesis of high molecular weight hb polymers requires the careful selection of the reaction conditions. Through all years of synthesis scientists were trying to prepare more or less controllable hyperbranched structures and even to reach 100% degree of branching [87, 88]. It was not until the application of specific type of polymerization, that someone was able to completely overcome the drawbacks. In this case Maier and co-workers were using a 'criss-cross' cycloaddition, which gives us only dendritic and terminal units. Still controllable structures are rather an exception than a rule.

2.1.3.2 Synthesis of hyperbranched polymers based on 2,2-bis(hydroxymethyl)propionic acid

Hb polyesters were usually synthesized via one-pot approach by either AB_x [50] or $A_x + B_y$ [86]. The AB_x polycondensation reactions generally led to the formation of highly irregular structures. Groups all over the world put lot of effort into developing accurate method of

characterization, still there is a lot of work to be done to modify methods and adjust them to the specificity of the hb materials.

Hyperbranched polyesters based on 2,2-bis(hydroxymethyl)propionic acid (bis-MPA) are usually synthesized by acid-catalyzed polycondensation in melt. This standard way of synthesis includes at first stirring of the reaction mixture under Ar flow and then under vacuum. Exceptionally, for vary small amount of monomer, the step under vacuum could be omitted [89]. Polycondensation conditions often lead to high degree of side reactions, crosslinking, followed in the worst case by gelation. Still every parameter (from temperature to stirring speed) can be varied in order to get soluble products possessing needed molecular mass, T_g and to reduce the side reactions.
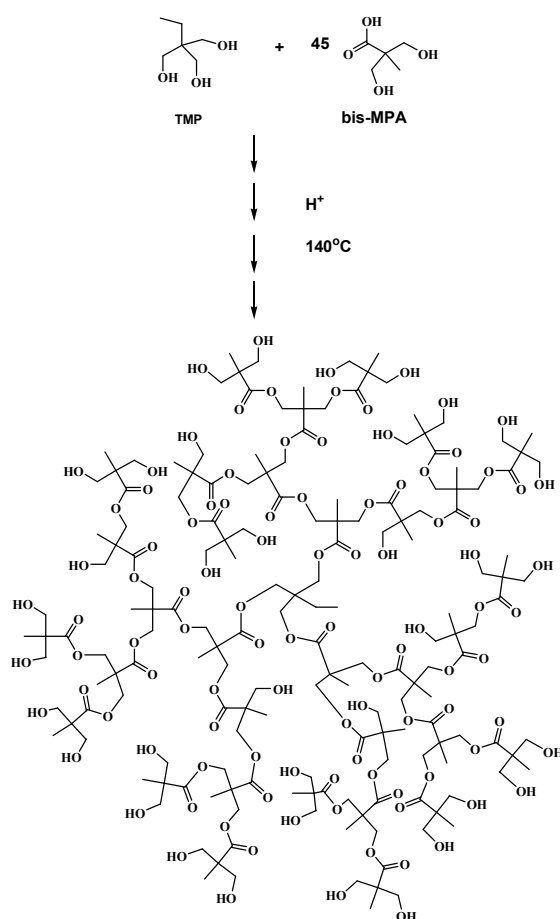
Figure 12 Theoretical model for synthesis of pseudo 4-th generation hyperbranched polyester with DB of 50% with 2-ethyl-2(hydroxymethyl)-1,3-propandiol as core [51]

These materials were hard to prepare until 1995 when Hults with his co-workers announced the synthesis of hyperbranched polymers through slow addition approach where during each step of polycondensation amounts of monomer corresponding to the following generation were added [51]. [51]. It leads to a narrower molecular weight distribution. Here not only the approach was revised but also a core molecule was introduced to improve solubility and to reduce the number of side reactions. These two "simple" changes gave an astonishing result and made possible a large-scale manufacturing, though scaling up is not easy to do. **Figure 12** shows the first reported structure prepared by this method.

Since that mainly three- [51] and tetra-functional aliphatic [90] core moieties were tried.

Through the incorporation of different core units it is possible to prepare new material which can be tested for supramolecular organization or for different applications like nanocarriers and molecular imprints. These materials can be later applied in the fast growing areas of biochemistry and those related to medical investigations. Still not that much is done, because of the irregular structure of the hb polymers and inability to achieve the full control of the incorporation of desired core. The modification goes usually via end-groups and can be achieved easier, though not all difficulties are overcome.

Despite existing Hults' method later scientist came back to the synthesis of aliphatic polyesters without any cores. A lot of work was done to optimize it, some positive results were observed after careful investigation and picking up of the reaction conditions, such as temperature, vacuum and of course catalyst. The role of the later has been extensively studied. The degree of side reactions has been shown to depend on a catalyst. For hb polyesters under consideration it generally follows the trend $Ti(^tPrO)_4 > H_2SO_4 > $ p-toluenesulphonic acid (pTSA) [91]. According to publications nowadays pTSA is used in all groups.

A short overview of the conditions for esterification implying bis-MPA monomer is presented in **Table 1**.

Bis-MPA polycondensation in solution was not reported until 2007, when Liu has described preparation of hyperbranched aliphatic polyesters grafted attapulgite [92]. Here solution polycondensation was carried out under drastic conditions: reaction mixture was first irradiated ultrasonically for 30 min and then refluxed at 140ºC for 4h under N_2 flow. This new way is interesting, but still polycondensation in melt is much easier and gives in this particular case better results.

Table 1 Monomer proportions and conditions of hb polyester synthesis

Reagents	Ratio mmol	Method of polymerization	T ºC	Reaction time		Reference
				Vacuum	Ar	
Bis-MPA	1.0	bulk	140	-	4	[89]
DMPA	8.0					
p-TSA	0.021					
Dodecanoic acid	8.0					
Bis-MPA	470.0	Step-by-step addition (Hult's method)	140	0.5	2	[93]
TMP	22.4					
pTSA	c.a.[1]					
Bis-MPA	116.7		140	2	1	[51]
TMP	5.55					
pTSA	c.a[1]					

[1]c.a.-catalytic amounts

2.1.4   Modification of end groups in branched structures based on bis-MPA

Modification of end groups in hb polymers as well as dendrimers means not only different chemical structures, but a dramatic effect on physical properties. This is a powerful technique. Some tendencies in modification of end-groups in bis-MPA based structures have been observed and seemed to resemble the ones for other polymers; nevertheless, the whole field is not fully explored. Besides the fulfilling of the aims in generating new properties it gives useful general information on reactivity of groups.

Speaking about reactivity it should be stated, that despite the assumption that it is all the same for any group no matter linear or terminal, it was shown that the reactivity of the terminal units are significantly greater [29]. Hult et al. observed complete disappearance of the terminal units during coupling, while unreacted linear units were still presented. A combination of steric and electronic effects is the reason of this. For example, steric factors make some of the "buried" hydroxyl groups not available and prevent molecules from taking conformation favourable to the reaction.
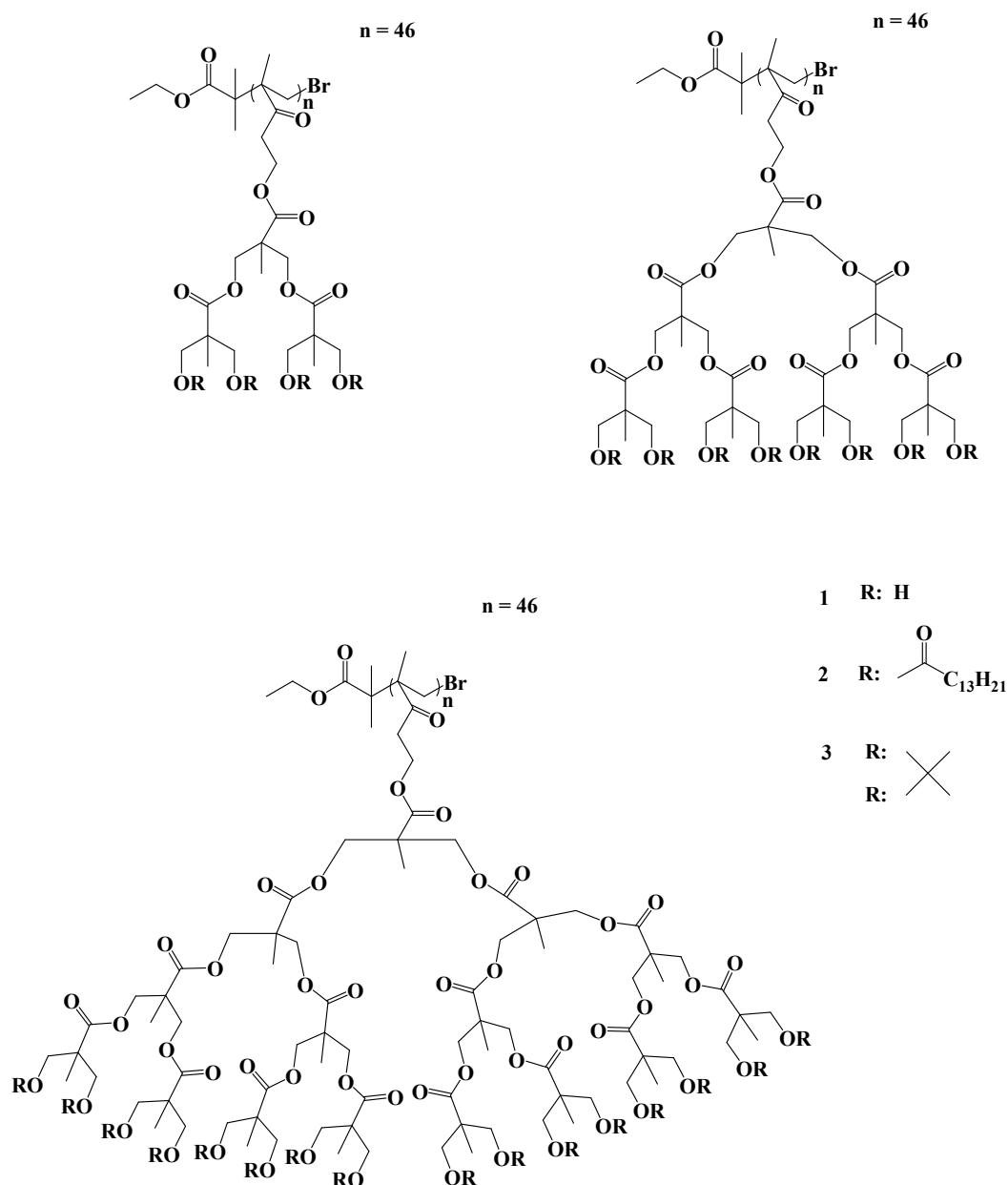
Figure 13 Structure of the dendronized polymers with hydroxyl end-groups, aliphatic hexadecyl chains (C16), and acetonide end-groups [40]

Investigations performed in the same group of Hult revealed a crucial role of end groups as well as generation in thermal properties of dendronized polymers (**Figure 13**). The higher the generation the higher T_g is, vice versa for the temperature of crystallisation, which could be found only for low generation samples with hexadecyl modified end groups [40]. An explanation could be the dendritic architecture, which prevents close packing of the various polymer segments and crystallization. Confirmation of this result could be found in later work

published by Qiu at al. [94]. Here end-groups of bis-MPA based hb polyester were modified with either phthalic anhydride or trimellitic anhydride. Prepared samples with 65% or 55% conversion of OH-groups have shown improved solubility in nonpolar solvents and T_g, which has raised from 37.6ºC to 54.8ºC.

Modification of end groups could be made not only with small molecules but also with polymers, giving star polymers [30]. Incorporation is done via "grafting to" and "grafting from" routes similar to the ones used in preparation of dendrigrafts. In the first case the already prepared polymer is coupled to the terminal groups, while in the second case polymer chains grow directly from groups in branched unit. Perfect and imperfect branched structures could be applied as shown by Johansson et al., though difference in reacting could be significant [20, 89]. For bis-MPA based branched structures activity of end groups in dendrimers is higher than in the isomeric hyperbranched structures. It could be because of the steric hindrance of some groups that are trapped inside of the imperfect structure.

2.1.5 Methods of characterization

There are three main characteristic of hyperbranched polymers: molecular weight, polydispersity index (PDI) and degree of branching (DB). It is also useful to estimate amount of side reactions, but this is not always possible. Below methods to determine all these parameters will be discussed.

There are several ways to measure molecular weight for all kind of polymers like GPC/SEC, NMR and MALDI-TOF analysis; any of these methods is not ideal and has limitations, some times combination of two works better.

GPC/SEC method, which is the standard one in investigations of molecular weight and PDI for linear polymers, is much more complicated to perform and to interpret for branched structures. The experimentally measured molecular weights are systematically lower than the theoretical values [95]. It is not surprising as polystyrene standards or any other linear standards used for a calibration of SEC equipment have different properties than branched structures. Later have a smaller hydrodynamic volume in comparison to linear ones that leads to observed underestimation of the molecular weight of branched macromolecules. Therefore, molecular weight obtained by SEC should be used only for comparison. SEC with light scattering detector avoids problems with calibration, but its limitation is inability to register molecules with a very small molar mass and so overestimation of M_w may results.

In SEC/GPC method nature of terminal groups plays a significant role as interactions between material under investigation and chromatographic columns are strong enough to

effects the final result. This conclusion comes from the comparison of the theoretical prediction of the molecular weight of the protected samples based on the data obtained for their unprotected precursors. Partial or full protection could be performed to reduce or eliminate such side effects and to measure molecular weight and distribution closer to reality.

NMR spectroscopy is useful to estimate M_n, but not so easy to fulfil. Good resolution of signals and absence of overlapping signals should be checked prior to use. Data calculated here could be considered close to reality unless the molecular weight is not too high. The limit is individual for every polymer and usually is bellow 12000 g/mol.

MALDI-TOF method usually gives accurate results only for dendrimers and usually has nearly the same value as the one from SEC with light scattering, if no unexpected interactions occurred. Spectra of hb polymers are more complicated, because of the irregular structure and lager number of signals from different units. The amount of signals could be so high that it is impossible to assign them and this analysis is not informative anymore. Sometimes another problem appears and prevents analysis; this is low tendency of macromolecules to desorb from the matrix. MALDI-TOF spectroscopy can also be used to verify the structure and purity of dendrimers, for which NMR spectroscopy failed due to peak overlapping [19].

Now speaking about the degree of branching, two different determination techniques have been used. The first method was presented by Fréchet at al. [50] and involves the synthesis of low molecular weight model compounds resembling the repeat units to be found in the hyperbranched skeleton. The model compounds are characterized with ^{13}C NMR spectra to find characteristic signals for different structures. DB is calculated from the integrals of the corresponding signals in the spectrum of polymers using one of the equations (**Equation 1** and **2**). A problem emerges when analysis is made for high molecular weight molecules, because of broading and overlapping of signals.

During analysis attention should be paid to the conditions, as they could be misleading like in the case of Hults group. Here DB for these hb polyesters based on bis-MPA was reported to be close to 80%, as determined by ^{13}C NMR in acetonide-d_6 [51]. However, DB calculations were incorrect, since the ^{13}C NMR study was performed in acetone and since small traces of pTSA were present, most end-groups were transformed into acetonide end groups. When DB was re-estimated from spectra recorded in DMSO-d_6, it turned out to be close to 50%, as could be expected for hb polymers with AB_2 monomer unit.

NMR method cannot be applied every time, so a second method, based on the degradation of the hyperbranched backbone, was presented by Hawker and Kambouris [96]. Chemical modification of the chain ends was followed by full degradation of the hyperbranched

skeleton by hydrolysis. The products of degradation were identified using capillary chromatography. This method is more complicated.

All these methods give the opportunity to a more or less good characterization of branched materials, though some data, especially from SEC measurements, should be handled with care.

2.1.6   Applications of branched structures

Dendritic structures, though known for quite a while, have not spread wildly as could be expected. They have unique architecture and novel properties, which distinguish them from their linear analogues, and could find many applications ranging from coatings to medicine. A strong competition between two types of branched materials is observed.

As it was discussed earlier the perfect structure of dendrimers is very promising, but the synthetic steps, taken to prepare them, are too complicated. From other point of view, the "easier" one-pot or step-by-step approaches which are used to obtain hyperbranched polymers are more appealing.   Though, the disadvantages of irregular structure and problematic scaling-up can exceed the advantage of the simplicity in synthetic methods.

The number of application for dendrimers and hyperbranched polymers can be lower than expected, but this area is still unexplored and tests are run continuously. The main restriction here is the costs of manufacturing and a possibility for reactions made in the laboratory in small scale to be transferred to plants.

All applications (already existing and potential) could be divided into following categories:

- Coatings [42, 97]
- Resins [42, 98]
- Additives [99]
- Analytical techniques[100]
- Medicine [101, 102]
- Drug and dye delivery [103, 104]
- Molecular imprinting [105]
- Organic and polymer synthesis and catalysis [21, 29]
- Optoelectronic applications [106]

Bellow detailed description is done for some latest interesting examples.

Hyperbranched polymers are used as coating components for quite some time, several big companies including Peterstop, DSM and BASF own patents or have submitted patent

applications in this area. Still research has not stopped and the latest results published by Andreeva and Schukin show the ability of hb polymer to serve as smart self-repairing protective coatings, which can respond quickly to changes in the coating environment or coating's integrity. The main idea is shown below **Figure 14** [107]. Such possibility is interesting and has potential to be called-for in industrial market.
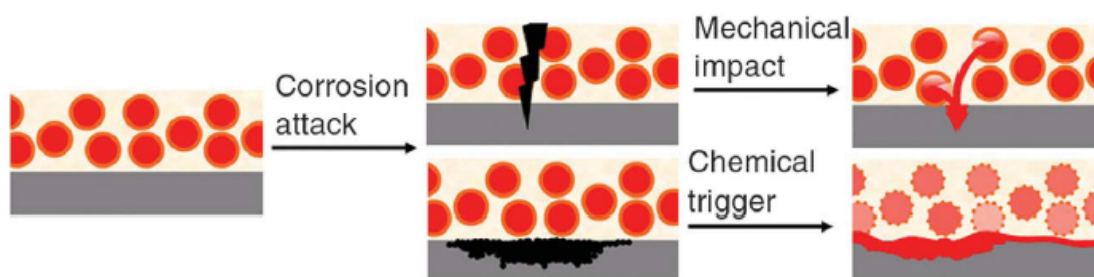
Figure 14 The mechanism of self-healing action of a 'smart' anticorrosion coating [107]

Hb polymers can near by act as nanocontainers carrying inhibitors (e.g. antibacterial, anticorrosion or antistatic). The stimuli for release can be pH, ionic strength, temperature, ultrasonic, magnetic or electromagnetic fields.

Biomedicine and related applications are a fast growing area where nearly every material was once tested for its ability to be used there and dendrimers are no exception. They are tried as additives to optimize solubility of drugs, reduce their toxicity and increase efficiency. The compact structure and large amounts of active groups in the outer shell are in charge of success of dendrimers in this application field.

The only limitation is the rather high toxicity of materials to cell membranes which can prevent them from reaching the desired target. Progress in overcoming this problem is fast, several examples were described in the review published by Svenson [108]. In some cases the their toxicity can be useful. In communication published by Chen, Tan and Cooper [102], they reported the modification of readily available polypropylene imine (PPI) dendrimers to dimethyldodexcyl ammonium chloride PPI dendrimers (**Figure 15**). These dendrimers modified with biocides proved to be effective against Gram-positive bacteria. It is suggested that other modification reactions can lead to a broad range of possible antibacterial and/or antiviral products.

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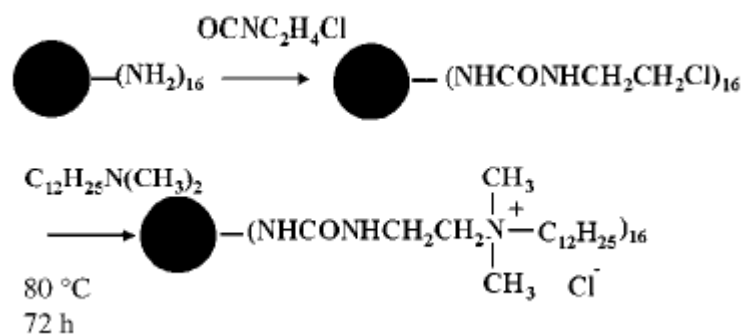
Figure 15 Synthesis of dimethyldodecylammonium chloride functionalized generation 3 PPI dendrimers (the circle represents a complete dendrimer molecule except for the end groups) [102]
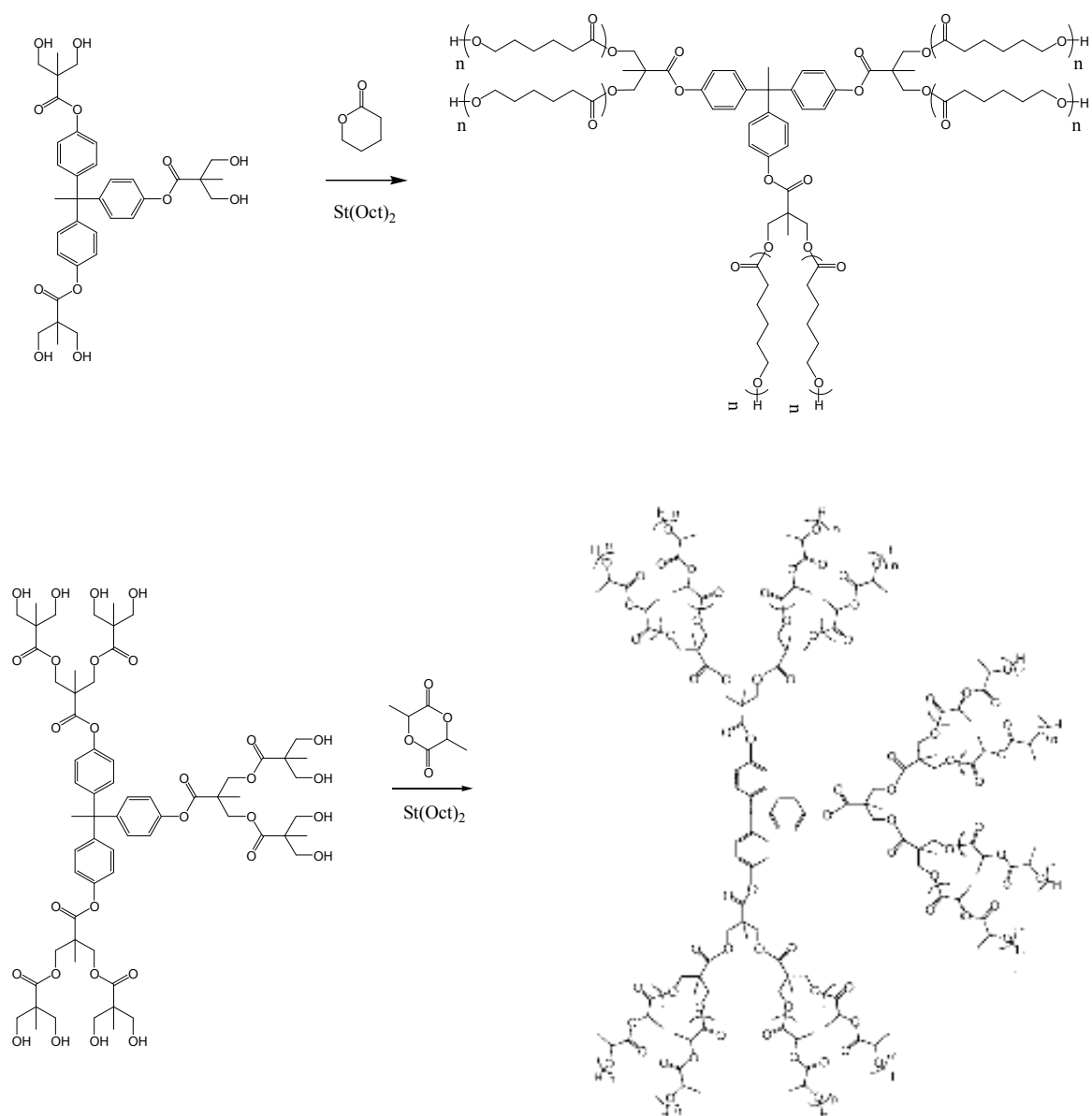
Figure 16 Examples of bis-MPA dendrimers used as a macromolecular cores for polymerisations [22]

Branched materials could be used as macroinitiators in chemical reactions in the synthesis of star macromolecules. For example, the living ring-opening polymerization (ROP) of caprolactone was performed with dendritic initiators based on bis-MPA (**Figure 16**) [22]. Through this way a new hybrid dendritic-linear copolymer was prepared. The dendritic cores were giving polymers with controlled structure and low polydispersity, meanwhile the hyperbranched structures led to higher polydispersity due to the polydispercity of the starting initiators and the different reactivity of terminal and linear hydroxyl groups, though it was still lower than for a linear homopolymer, generating one arm of the star.

Poly(lactides) with controlled molecular architecture initiated from hydroxyl functional dendrimers were reported by Hendrick et al. in the same manner as shown for ε-caprolactone. (**Figure 16**) [21]. The bis-MPA derivates acted as efficient initiators for ROP of lactide. During these experiments two interesting things were observed: the "living" nature of polymerization and the lag between increase in the hydrodynamic volume and molecular weight. The same behaviour has been observed for the star with polylactone arms. Such materials could be considered for biomaterial applications and as drug delivery systems and much more similar applications like for the poly(lactide) precursors.

2.1.6.1 Possible applications of branched structures based on bis-MPA

Branched structures of bis-MPA are considered very successful considering their application possibilities. Significantly, commercially available hyperbranched polyesters, derived from 2,2-bis(hydroxymethyl)propionic acid (bis-MPA), have been introduced by Perstorp under trade mark BOLTORN® and are attractive for a number of technological applications: coatings, additives and so on [42, 109]. The exclusive right to the production and marketing of the well-defined dendrimers and dendrons based on bis-MPA belongs to Polymer Factory [110].

Recently Wei et al. have applied these hb polyesters in preparation of nanofiltration (NF) membranes [100]. Such membranes are designed for selective separation of solvent from solvent and solute mixture and could be an alternative to reverse osmosis (RO) membranes in production of pure water. The majority of commercial NF membranes are limited to polyamide, although some other polymer materials such as sulfonated polyether-sulfone, polyvinyl alcohol, and chitosane derivatives were tried. Wei and his co-workers have used crosslinked BOLTORN® 40 as an active layer. The NF membranes were successfully prepared and after preliminary tests, they were pronounced stable and rejecting salts, so the overall result was positive.

High availability of monomer, simple synthetic procedure and growing application fields make structures based on bis-MPA interesting and worth trying in new areas.

2.2   Polymers for the formation of assemblies

Over past decade there has been considerable interest in structure with nanometre dimension. Self-assemblies are of a particular interest, because such macrostructures could be used in biochemistry, molecular recognition and as a precursor for molecular imprinting.
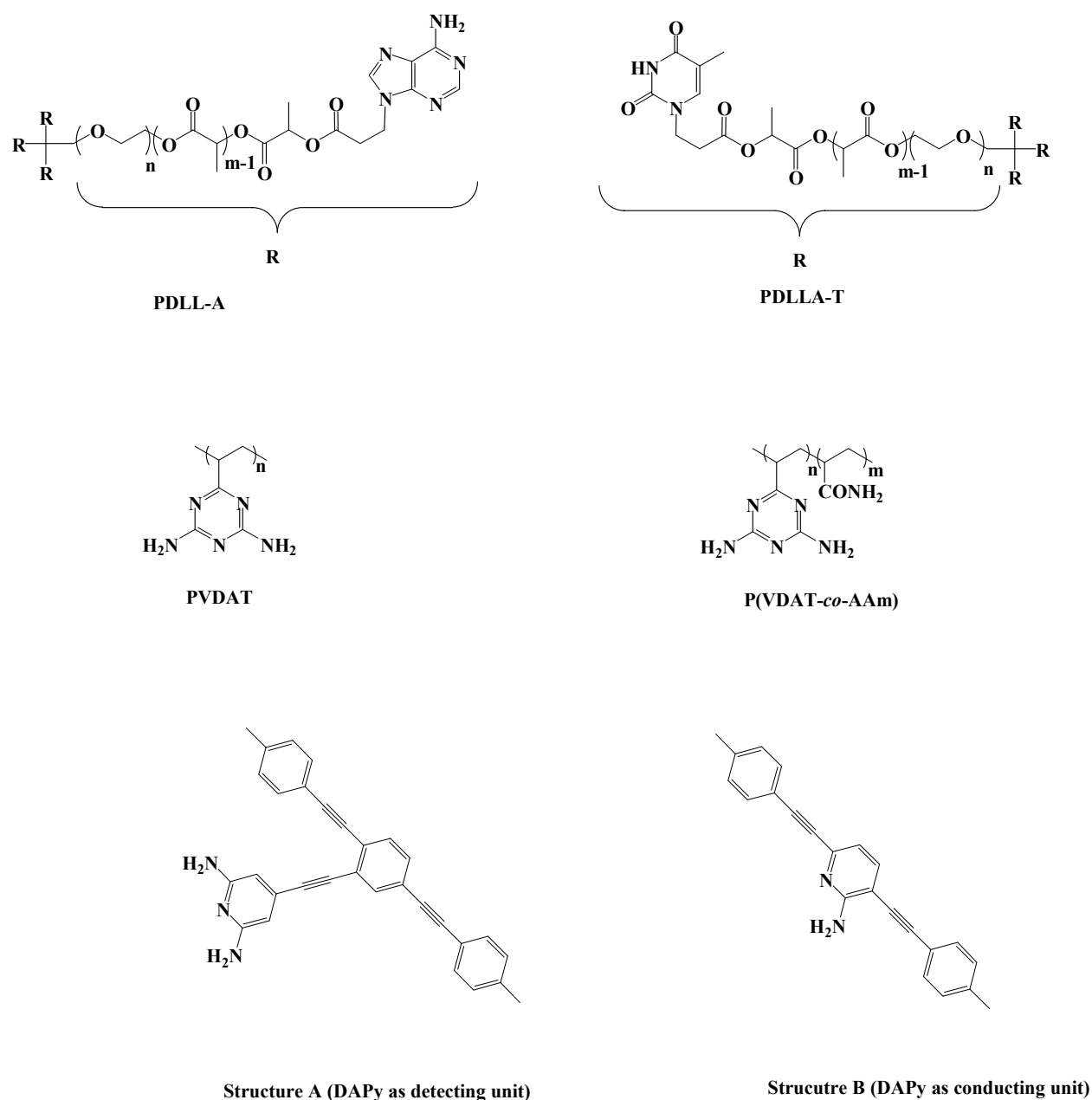
Figure 17 Examples of linear polymers containing active units for complexation [111-113]

Molecular self-assembly is an aggregating process of molecules in which molecules or parts of molecules spontaneously organize into highly ordered objects and no human intervention is involved. The interactions among self-assembled molecules are usually noncovalent ones, including van der Waals interactions and Coulomb interactions, hydrophobic interactions and π-π stack, etc. [114].

Artificial assemblies could be produced via synthesis of molecules bearing an active group and introduction of corresponding template into the system. Following organization of molecules with active group around the template proceeds according to the same laws as the spontaneous one [115]. Many such assemblies described in the literature are based upon H-bonding interactions between the host and the guest molecules.

These interactions were observed for linear [116, 117] and star polymers [112], some examples are shown in **Figure 17**. Molecular sensors were also studied theoretically via modulation of their structures and interactions with template, like it was done for sensors containing 2,6-diaminopyridine (DAPy) and the nucleic bases. The simulations for Structure A and Structure B were presented by Abe et al. [113]. One of the first assemblies including branched structures was reported by Zimmerman et al. [12, 118] and overall there are not that much of them [119].

It is beyond any doubt that structure of the molecules has a great effect on the abilities of the substance to interact with the template or to form self-assemblies. Changes in end-groups, protection/deprotection of the functionalities can result in disappearance of the complexation abilities. For example, benzoyl-modified bis-MPA hyperbranched polymers with TMP core shows self-assembly, while for non-modified structures nothing of this kind was observed. In this case no special techniques were needed to show assemblies while they were big enough to be seen with bar eyes and photo documented [95].

2.2.1   Multifunctional templates based on nucleic bases

As it was discussed before templates can be used to form three-dimensional structures. The higher is the functionality of the template the more complex assemblies would be prepared. Selectivity tests could be also made using different templates, the number of active groups and their disposition in the molecule are considered to be very important for the recognition process. H-bonding interactions are one of the often used methods to generate complexes, which in turn can lead to the preparation of the systems for molecular recognition.

H-bonds between natural nucleic bases are universally acknowledged and are based on the complimentary donor (D) and acceptor (A) canters possessed by them. Below **Figure 18**

illustrates nature and situation of active centres in RNA nucleic bases: adenine (A), cytosine (C), guanine (G) and uracil (U).
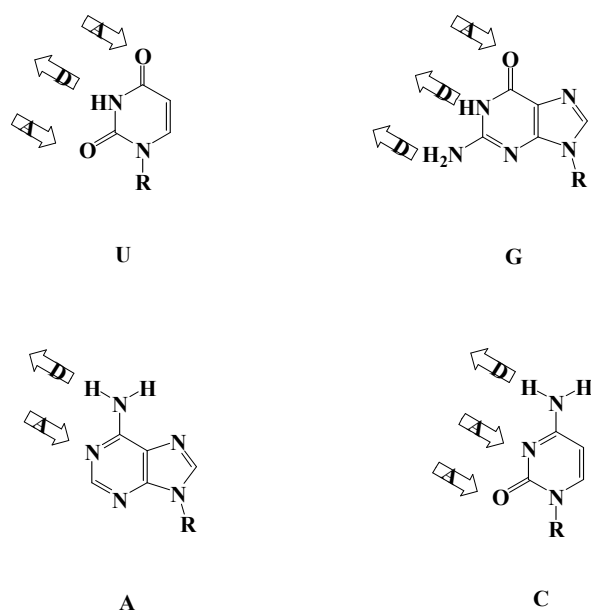
Figure 18 Principals of Watson-Crick hydrogen-bonding for natural RNA bases [120]

In this work H-bonds formed by thymine were to be explored to form different assemblies and to prepare molecular recognition structures. It was interesting not only to use a single-site modified adenine, but also to prepare the di- and three-functional templates. That is why only the multifunctional templates possessing nucleic bases as active groups will be discussed bellow. There is limited amount of literature references reporting synthesis of low molecular weight molecules possessing two or three nucleic base residues [121]. Examples of multifunctional cores synthesis are presented in **Figure 19**.
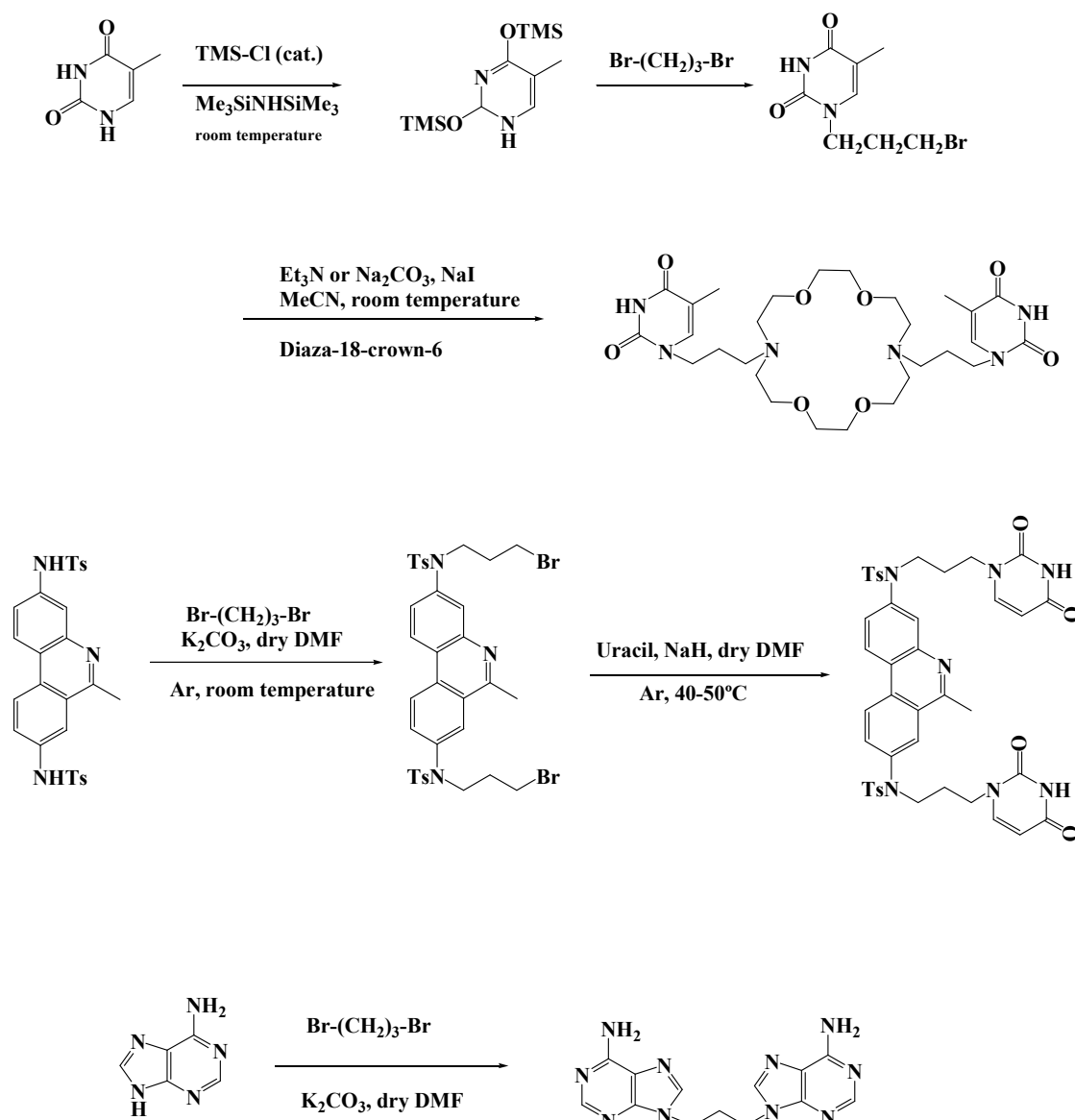
Figure 19 Synthesis of di-adenine, di-thymine and di-uracil derivatives

The di-thymine derivative [122-124] and di-uracil [125, 126] synthesises are too complicated for being paractical. The last one consists of more than five steps, though only two are shown. Only by using the method developed by Itahara low molecular weight di- and three- functional templates (**Figures 19**, last example, **20**) with adenine and thymine could be synthesized in a very easy manner and with moderate yields [127-129].
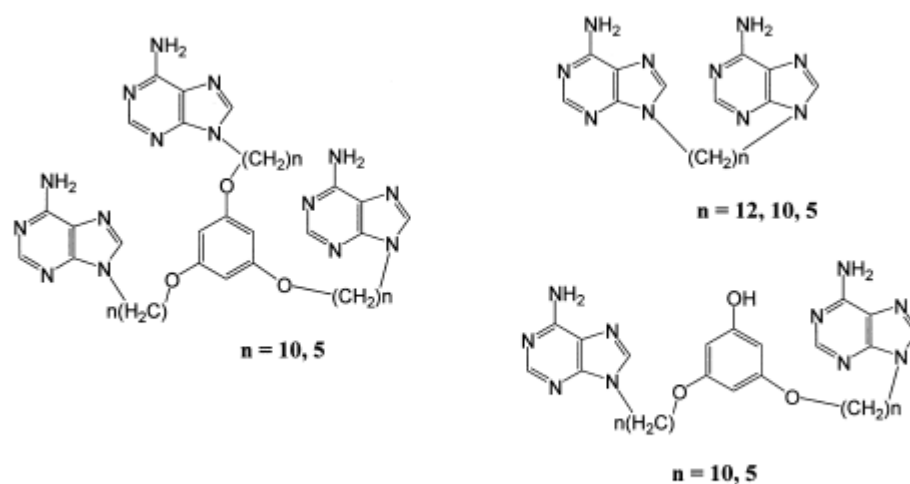
Figure 20 Di- and tri-adenine derivatives synthesized by Itahara [127]

So there are possibilities to produce multifunctional molecules bearing natural nucleic bases, but for most of them it was not stated if they are good for future applications as templates for H-bonding investigations.

2.2.2   Investigation of assemblies based on H-bonding interactions

Presence or absence of macromolecular organisation in compounds is usually determined analytically by techniques depending on the nature of interactions. In rare cases they could be observed without any help as was mentioned before. H-bonding could be investigated by several powerful analytical techniques: NMR [112, 130, 131] and IR [132-136], as well as UV-vis spectroscopy [137, 138] and DSC [139, 140]. Only one of all methods will be discussed in detail as all of them though different are working according to the same rule, results are obtained after comparison between data for free templates and for their mixtures.

NMR analysis is an easy and powerful tool not only for confirmation of existences of H-bonds but also calculation of parameters of complexes. This analysis involves comparison between spectra of the starting templates and their mixture in different solvents, the signals for the groups involved in H-bonds formation shift and the higher is the difference the stronger are the interactions. **Figure 21** illustrates investigation of bemegride (**b**) complexation with DAPy derivative (**a**) made by Feibush et al. [141].
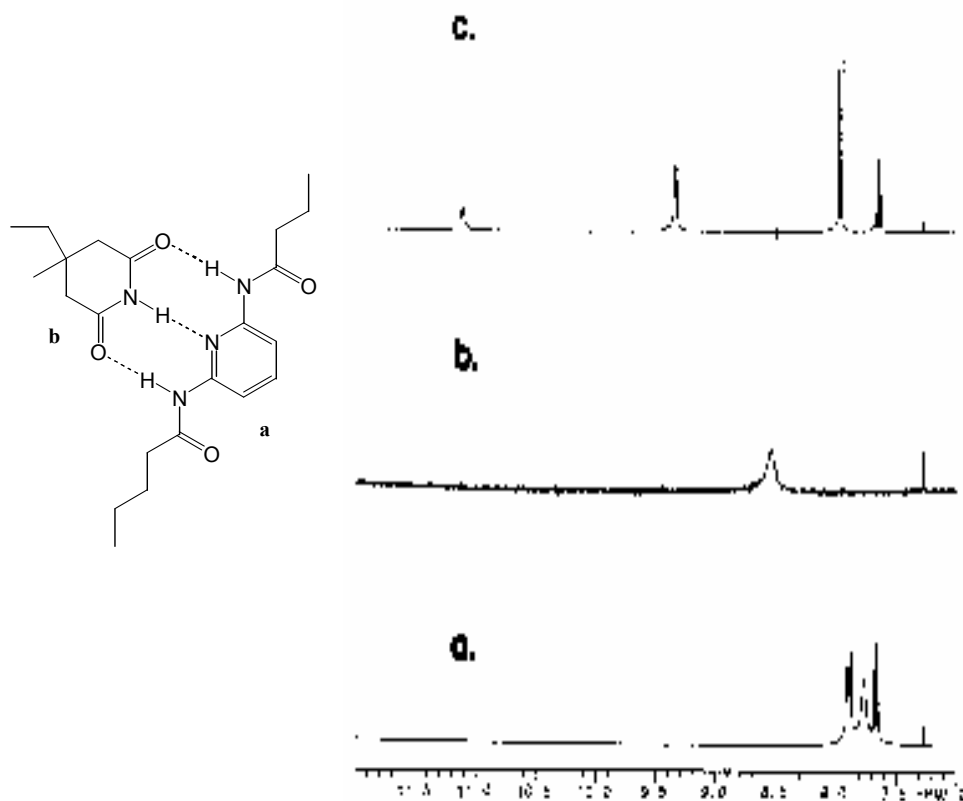
Figure 21 Chemical shifts of the protons participating in the formation of a triple hydrogen bonded complex. Conditions: 0.20 M CDCl$_3$ solution, 19.5±0.5°C. (a) Spectrum of *N,N'-2,6-pyridinediylbis[butanamide]*, δ 7.75 ppm (2 NH); (b) spectrum of bemegride, 8.57 ppm (NH); (c) the 1:1 mixtures, δ 9.38 ppm (2 NH), 11.15 ppm (NH) [141]

Using this method Zimmerman et al. have confirmed complex formation, got information about its stoichiometry by Job's analysis and calculated association constant values, using ^{1}H NMR dilution method [12].

$$A + kB \Leftrightarrow D$$
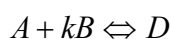
Equation 3 Formation of the complex between molecules A and B

Job's method (the method of continuous variations) is one of the methods used to characterize complexes in solution. **Equation 3** shows the complexation of two substances. It is based on the following fact: if a series of solutions is prepared, each containing the same total number of mols of A and B, but a different ratio (R) of moles B to moles A, the

maximum amount of complex, D, is obtained in the solution in which R = k (the stoichiometric ratio) [142].

Comparable results for this method could be made only if all the parameters stay constant during all tests. Variations in concentration, temperature, solvents and of course in templates can have dramatic effect. For example, a change in the chemical shifts of the nucleic acids at different concentrations was observed by Itahara. Here the proton signals in the base moved to higher fields simultaneously with increase in concentrations [128]. The situation with solvents is quite simple, absences of other possible noncovalent interactions between solvent and solute is essential for the formation of stable H-bonds between two templates. Methanol in this case should be considered as very bad choice, while all nonpolar organic solvents are welcome. In principle there are no restrictions, but in fact experiments are carried out in dichloromethane or chloroform, as long as all components are soluble in either of them.

During all experiment one should not forget that in some cases polymers do form H-bonds in a free state, without template presence, that could complicate experiments or make analysis of data nearly impossible. To reduce all minor effects it is important to find a proper system and method of investigation or slightly modify the conditions. Doping with deuterium as well as increasing or decreasing temperature can increase resolution [143].

Usually the existence of all noncovalent interactions is proved by two independent techniques, in order to eliminate the mistakes coming from impurities or other factors. It was done by Itahara for multifunctional adenine template with thymine derivative [139]. Evidences of macroscopic structures could be obtained from viscosimetry, SEC data. Self-assembly of polymers was depicted by digital photos , AFM and TEM microscopic methods [95].

In some cases it is interesting to perform molecular modelling and then compare the theoretical and practical results [144].

2.2.3 Hydrogen bonding and molecular recognition/molecular imprinting

The abilities of substances to form hydrogen bonds could be used to prepare artificial molecules capable to recognize and bond different natural or artificial templates [141]. The main idea is to introduce an active group into the molecule with the needed number of donor and acceptor groups in the structure to form stable interactions with potential template. Besides the needed number of functionalities their position should enable a stable interaction with target. (**Figure 22**)
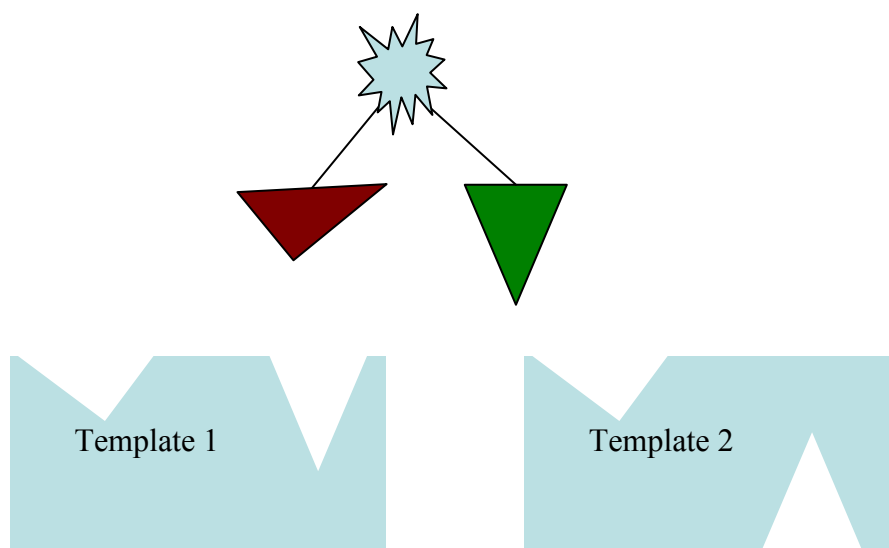
Figure 22 Schematic representation of molecule with active centres and two different templates

Specific molecular recognition of compounds containing nitrogen, in particular N-heterocycles, is of fundamental importance in biological chemistry and is particularly interesting for the separation techniques and pharmaceuticals. That is the reason why a lot of work is dealing with the design of receptors for recognition and selective complexation of nucleic bases and their derivatives via non-covalent interactions. Template structures varied from simple low molecular derivatives to macrocycles [145] and even polymers [116]. In principle active receptor possessing hydrogen bonding based complementarily units can some times be not that much selective and interact with either desired target or some other guests. Progress in this area is obvious, so materials become more perfect in structure and selectivity.

Design of artificial receptor molecules varies. Active group could be situated on the outer shell [144, 146] and be readily available or incorporated inward of a cavity of defined geometry, the last version should lead to strong and selective binding to the substrates with complementary shape and H-bonding characteristics [147].

From the last idea comes an alternative approach to artificial molecular recognition named molecular imprinting [148], also an attractive and not always simple way of incorporation of recognition sites in synthetic polymers. In some cases they are more appealing considering analytical applications, as these materials offer the combination of selectivity towards target compounds, matrix robustness and ability to be produced in a variety of forms, such as beads [149], membranes [150] or column packing each of which may be required for a particular detection system [151-153]. Molecular imprinted polymers

can find their application not only in analysis, but also in drug delivery [154] and catalysis [155].
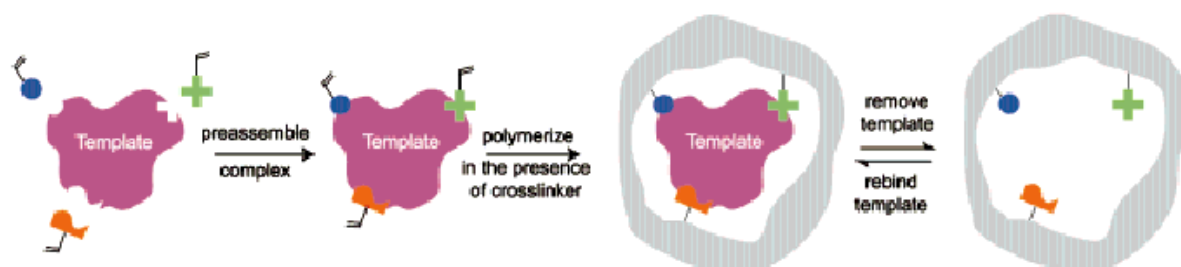
Figure 23 Schematic representation of the imprinting process: A template is complexed, either covalently or noncovalnetly with functional monomers. The complex is polymerized with an excess of crosslinker to form a rigid porous shell around the template. Removal of the template creates a recognition site or activity capable of reversibly rebinding the template [156].

Figure 23 presents the general approach to synthesize molecular imprints. Imprinting can be made via covalent, non-covalent, and their hybrids approaches [156-158]. In the first case, the template covalently incorporated into the structure of the polymer is removed usually by hydrolysis what leads to the formation of the cavity [159-162]. In the second case, the formation of the noncovalent bonds between the template and monomers is followed by polymerization and extraction of the template [163-167]. Both strategies have their advantages and disadvantages, so for every target molecule the methodology is chosen separately according to its properties. Now scientists are trying to find new systems [93, 168], investigating the already existing ones [169-171], improving their selectivity [172, 173] and adjusting them to different conditions [174].

There are many examples of systems designed for nucleotide recognition, including the ones for thymine. Through the last years 2,6-diaminopyridine (DAPy) became very popular as a detecting unit among scientist searching for the thymine template detecting unit. The model compounds used by Tsuzuki et al. bind only one nucleic acid base [113]. Yano et al. have prepared molecular imprinted polymers, which mimic multiple hydrogen bonds between nucleic bases, using DAPy derivative [175]. Hamilton and Van Engen introduced a macrocycle based on bis(acrylamino)pyridine receptor [176], capable of binding thymine through hydrogen bonding and stacking forces [177]. Beijer et al. have studied the hydrogen bonding complexation of diaminopyridines and diaminotriazines and their acylated derivatives with both uracil and thymine derivatives [178]. Slinchenko and co-workers have

used 2-vinyl -4,6-diamino-1,3,5- triazine as a functional monomer for the preparation of DNA-imprinted polymers [131]. Despite all this interest not that much groups were using dendritic polymers in molecular imprinting like it was in the molecular recognition. So this field is opened for investigations.

3   Aims of the work

Two fields of specific polymer chemistry, branched structures and molecular recognition, are very interesting and fast developing. To the first one particular attention is payed due to some specific properties of these branched polymer structures and large room for modification. The second draws interest because it makes use of non-covalent interactions, which can be considered key-points for different applications including ones in biochemistry. Molecular recognition and preparation of molecular imprints usually starts with the formation of assemblies via specific interactions. There is not that much work so far describing investigations where both of these fields, highly branched polymer structures and molecular recognition, come together and thus, the potential of branched structures in that area is not yet revealed.

The main aim of the work is to synthesize randomly and perfectly branched structures bearing an active unit and to investigate the specific non-covalent interactions with different templates. (**Figure 24**) Two natural principles were to be used to accomplish it: the complementarity of nucleic bases in DNA or RNA and the specific recognition which can be found between a cell membrane and a virus. Thymine was chosen as a starting nucleic base.

As monomer for the build-up of dendritic structures 2,2-bis(hydroxymethyl)propionic acid was chosen, which is a known compound used for the synthesis of

Figure 24 Schematic representation of the aims of the work

dendritic polyesters that are used as coating and additives and are explored in other fields. The first commercial success among hyperbranched materials is based on that easily available monomer which can be very important if the preparation of materials for molecular recognition or molecular imprints shows to be successful. Many groups are working with the resulting aliphatic dendritic polyester either trying to analyze or modify the readily available samples or to prepare new ones. Despite all this interest bis-MPA based branched materials have been only once tried as a precursor for molecular recognition. This work is the continuation of that preliminary investigation carried out by Griebel [179], who used hyperbranched bis-MPA polyester and thymine derivative to prepare molecular imprints.

The different aspects of the work are listed bellow:

- Preparation of branched and low molecular weight precursors
  - ✓ Hyperbranched polyesters with thymine core
  - ✓ Dendrimers with thymine core
  - ✓ Multifunctional templates

- Analysis of hyperbranched polyesters
  - ✓ Estimation of molecular weight and polydispersity
  - ✓ Estimation of the degree of incorporation of thymine derivative
  - ✓ Estimation of the degree of side reactions

- Preparation and analysis of assemblies
  - ✓ Preliminary tests of H-bonding activity on low molecular weight templates
  - ✓ Test of branched structures

- Simulation of some dendritic structures and their potential non-covalent interactions

4   Results and discussion

4.1   General strategy

The aim of this work was the synthesis of branched structures and the investigation of their non-covalent interaction. For that monomer, active unit and the way of its incorporation, as well as templates should be picked up

There are a lot of aliphatic and aromatic monomers, which could be used to prepare hyperbranched and dendritic structures. No less is the number of possible reactions ranging from a polycondensation to ATRP. As discussed earlier, the monomer and templates choices were dictated by their availability and existing methods for manufacturing. Bis-MPA was taken because of several reasons: availability of the monomer, existing methods and conditions for synthesis.
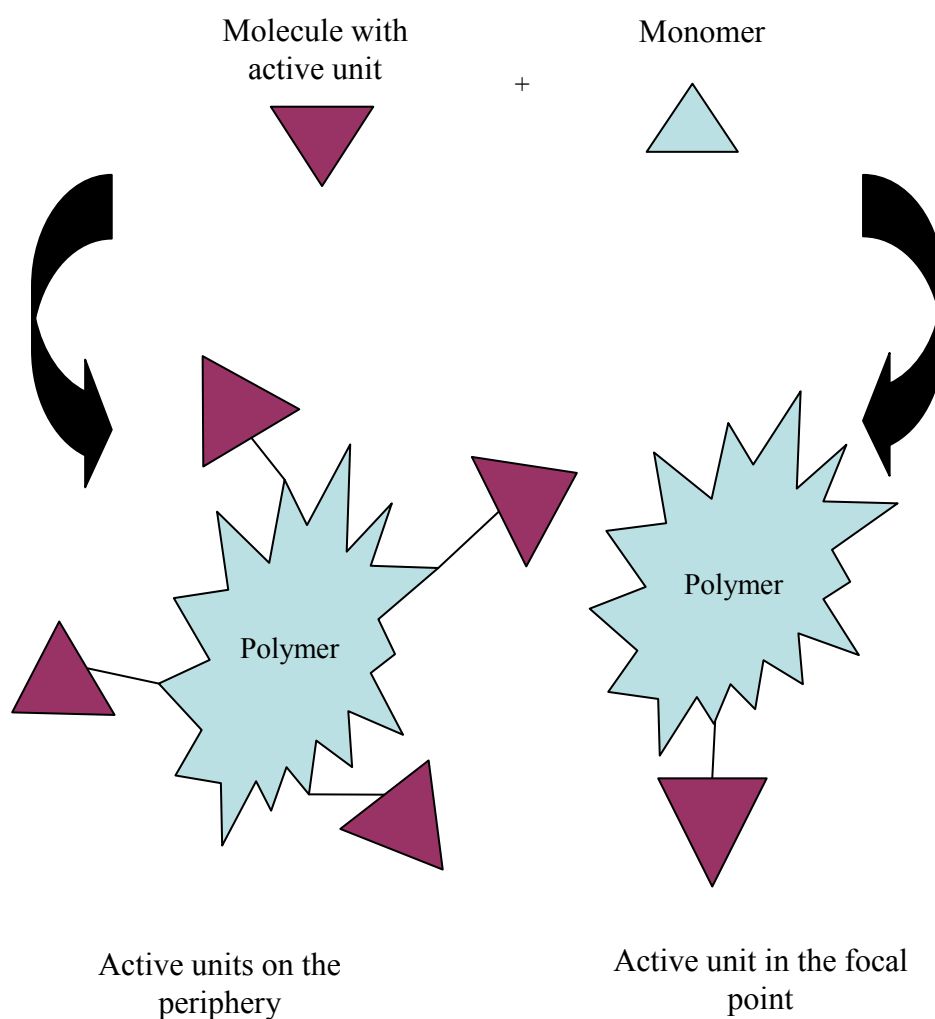
Figure 25 Schematic representation of the ways of incorporation of active unit(s) into the polymer backbone

As for the active unit it should posses H-bonding abilities, because this kind of interactions was to be the driving force for the formation of assemblies and molecular recognition. These non-covalent interactions should be also specific where the final materials are to be explored in molecular recognition. The simplest way is to use one of the five nucleic bases. In our case the choice falls on thymine because of some preliminary investigations described in literature.

Not only the active unit is important, but also the way of its incorporation into the polymer/dendrimer backbone. Generally it could be done in two ways (**Figure 25**); modification in the focal point/usage of a core moiety [105] and modification of the end-groups [16]. Through these two methods either mono-functional or multifunctional polymer templates can be prepared. For this work the first way has been chosen.

To test the complexation abilities of the materials different templates (with one or more active units) are to be prepared. Adenine or 2,6-diaminopyridine can be used as an active units. The detailed discussion and explanation of this choice will be given in the corresponding chapter.

This work is focused on synthesis and investigation of bis-MPA aliphatic dendrimers and hyperbranched polymers with a thymine derivative as a core moiety and their extensive investigation using different analytic techniques. Degree of branching (DB), degree of incorporation (DI) of thymine derivative and the amount of side reactions will be determined. It will be followed by the tests and evaluation of complexation abilities with different templates.

Modelling was made for several molecules and their interactions, in order to support the practical investigations. These studies can also help to understand and explain some problems which could arise during the analysis of prepared materials.

4.2   Synthesis of core molecules

The reason to use a core molecule is coming from the final aim of the work, the formation of assemblies through molecular recognition. Self-assembly processes can be based on different effects from electrostatic forces to donor/acceptor interactions. More and more work is described using H-bonding abilities of different molecules to prepare complex structures, starting from the natural complementary nucleic base pairs and going to a variety of synthetical materials. The idea of incorporation of the nucleic bases into the polymer materials via their modification seems to be the simplest. To accomplish the aims of this work thymine and 2,6-diaminopyridine derivatives were chosen to incorporate the active group/groups capable of H-bonding.

Two ways of incorporation of active centres into the hb structures were outlined earlier. There are several reasons why the incorporation as a core was chosen. The first reason was that Griebel in his work has briefly discussed the possibility for the thymine derivative to be incorporated into the hb polyesters and the results seemed promising [8, 179]. The second one was that the application of a core can help to control the reaction, reducing the number of side reactions. A well-known successful application of a core in order to get ride of by-products resulted in polyesters produced under BOLTORN® trade mark.

Though the thymine derivative was already approved as core, it was interesting to modulate a reverse situation, where it would act as a template. A literature search was made to find a suitable second precursor. It was shown that 2,6-diaminopyridine is quite known for its abilities to interact with natural nucleic bases [177]. It has been also incorporated into linear polymer structures as active centre for selective complexation [116, 180].

Thym-C5-NH2   **Thym-C3-OH**

DAPy-OH
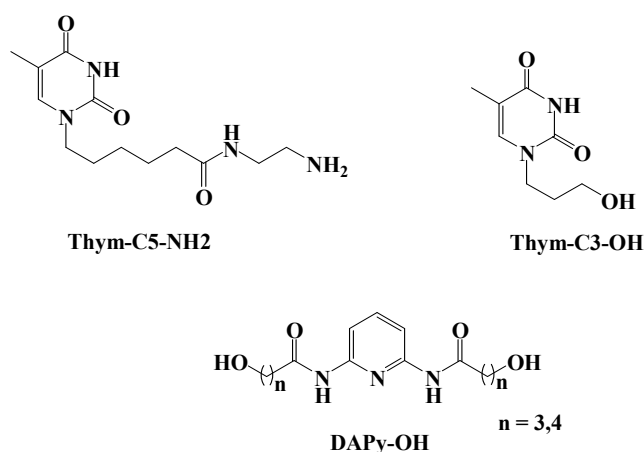
Figure 26 The three types of potential core molecules

So in the end it was confirmed that starting templates should either have a thymine or a 2,6-diaminopyridine active unit. Two different derivatives of each kind were to be prepared (**Figure 26**), because it was essential to understand the effect of the spacer length and the type of bond on complexation abilities. Until now multifunctional cores, applied in the polycondensation of bis-MPA monomer, were usually three- [51] or four-functional aliphatic or aromatic alcohols. The incorporation was successful and could reach 100%. So there was a good chance for success of this approach.

4.2.1 Synthesis of thymine derivatives

Thymine derivatives with either hydroxyl or amine end-group were prepared. Some general methods were explored. For further potential applications in molecular recognition it was essential that synthesis of a low molecular weight precursor was going with high yields, in the best case quantitative, and pure products can be isolated. The polycondensation is very sensitive towards any impurities, and they can accelerate side reactions or even stop the polymerization process.

4.2.1.1   N-(3-hydroxypropyl) thymine (Thym-C3-OH)

The first scheme of three-step synthesis of N-(3-hydroxypropyl)thymine, shown in **Figure 27** was proposed by Griebel in the course of his PhD work [179]. The overall synthesis looked rather easy and the yields were high.
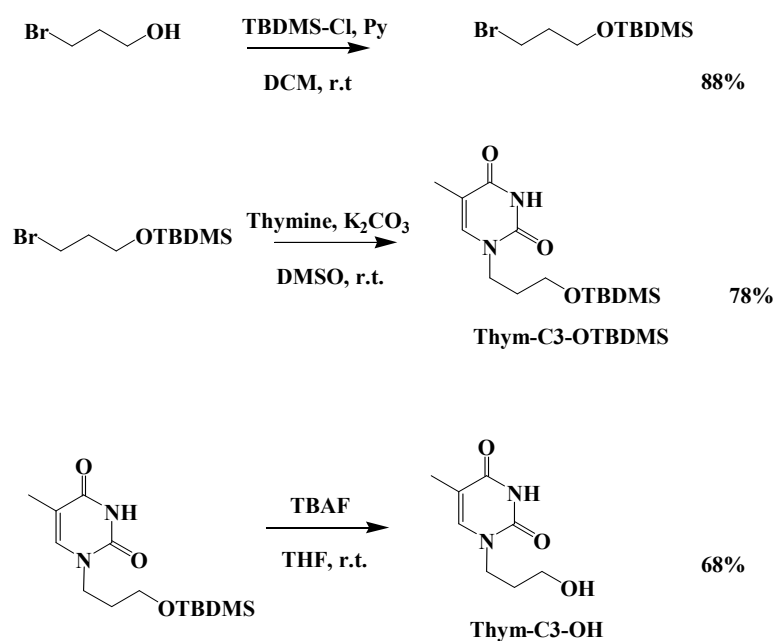
Figure 27 Synthesis of N-(3-hydroxypropyl) thymine according to Griebel [179]

According to this scheme the first step is the protection of hydroxyl group in 3-bromopropanol. The protection is a well known reaction and can be performed in many ways including conversion into tetrahydropyranyl (THP) ether or t-butyldimethylsilyl (TBDMS) ether. The later has been widely used for protecting hydroxyl groups, probably because TBDMS-protecting group could be introduced in many ways [181]. An alcohol reacts with t-butyldimethylchlorsilane in DMF in the presence of imidazole [182] or in CH_2Cl_2 using DBU as a base [183], but nowadays method with pyridine could be considered the simplest one. All reactions are going fast with high yields, and it can be cleaved in many ways. Preparation of 3-brompropyl-t-butyldimethylsilyl ether was going as expected.

The second step is N-alkylation reaction, which is often explored in order to modify nucleic bases [184, 185]. The different substances possessing bromine on the end of the chain are coupled to the desired nucleic base [186]. This reaction is well-known, often described in literature and usually is performed in DMF or DMSO in the presence of potassium carbonate. Another method was found for adenine; here sodium hydride is used as a catalyst. Unfortunately this kind of activation is not applicable for thymine, because it can facilitate the disubstitution instead of the needed monosubstitution.

Synthesis of N-(3-t-butyldimethylsiloxypropyl)thymine was going without any complications. Only one deviation from standard procedure was made in order to decrease the time of working up procedures. For that the reaction was run in the DMF/DMSO mixture, though the yield was 5% lower than described in DMSO.

The third and the last reaction is the cleavage of TBDMS-protection. It is more stable to hydrolysis than for example trimethylsilyl ether, but is still readily cleaved by a variety of selective conditions [181, 187-190]. The deprotection is usually performed under mild acidic conditions[191] or with a fluoride ion like in TBAF [182, 192, 193]. In the last years a method for more sensitive substances was developed, here the deprotection is performed under mild conditions, using acetone/H_2O (95/5) mixture containing 5 mmol % of $CuCl_2 \cdot 2H_2O$. This reaction works perfectly well and usually is completed within 5 hours. The yields for the aliphatic structures are reported to vary form 90 to 99% and there had been no signs of contamination with the deprotecting agent or by-products [194]. This appealing cleavage procedure is hard to perform for aromatic structures.

Table 2 shows the conditions and final results of several deprotection attempts. At first the deprotection was performed with TBAF in THF. In this particular case the deprotection turned out to be troublesome. The deprotection itself was going well, but it was not possible to clean the row product using column chromatography. Traces of deprotecting agent were

found despite the variation of conditions and the number of columns used for the purification. Unfortunately, though such a problem is widely acknowledged, there has not been found any real solution to the problem. Taking into account that the final yield after the TBAF-deprotection does not exceed 70%, it was decided to try another method. .

Table 2 Reaction conditions for deprotection of *tert*-butyldimethylsilyl protected hydroxyl groups

Deprotecting agent(DA)	Molar ratio DA : Thym-C3-OTBDMS	Temperature ºC	Reaction time	Solvent	Yield
TBAF	1:1	20	24h	THF	-
TBAF[a]	15:1	20	24h	THF	-
TBAF[a]	3:1	20	48h	THF	-
CuCl$_2$	1:20	33/50	3.5h/14h	Acetone/H$_2$O (95:5)	30%[b]
CuCl$_2$	1:20	50	2.5h	Acetone/H$_2$O (95:5)	70%[c]
CuCl$_2$	1:20	50	3h	Acetone/H$_2$O (95:5)	95%[c]

a – 1M solution of TBAF in THF by Aldrich

b - column chromatography with THF

c - column chromatography with MeOH

It was decided to test the deprotection of N-(3-t-butyldimethylsiloxypropyl)thymine with CuCl$_2$ (5mmol% in acetone/water solution), which was first described by Tan et al. [194]. The clean N-(3-hydroxypropyl)thymine is isolated after column chromatography with 95-98% yield. Extra column chromatography can be used to clean the final product from CuCl$_2$, because the contamination with salt can effect future polymerisations.

As future polymerisations were to be made in melt at 140-185ºC, the thermal stability was tested to make sure that this thymine derivative would not decompose under the high temperature. The sample was heated up to 185ºC on the air for 150 minutes The NMR spectra were taken before and after thermal treatment. (**Figure 28**) They showed no change in structure after thermal treatment.
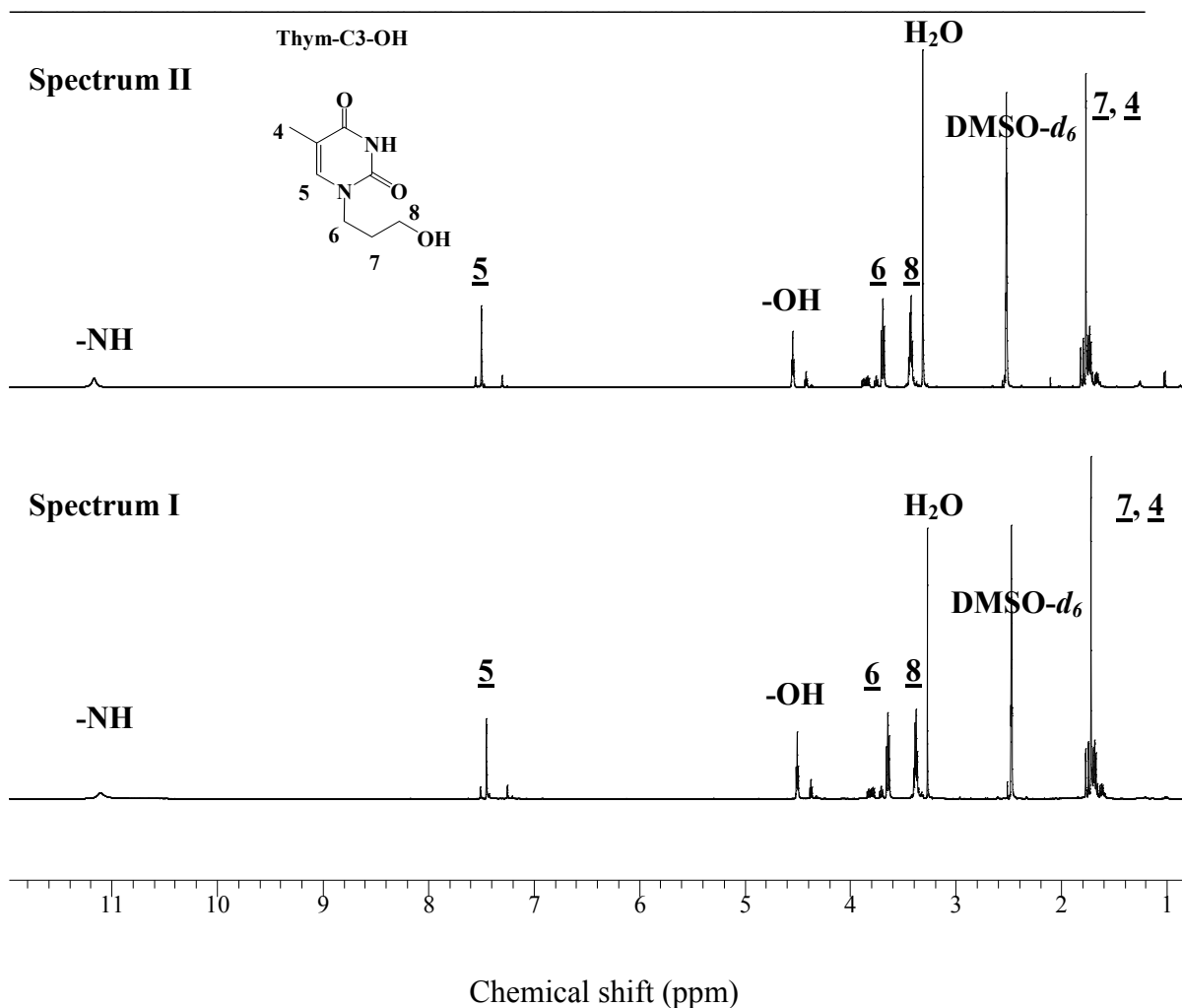
Figure 28 ^{1}H NMR for N-(3-hydroxypropyl)thymine (Thym-C3-OH) in DMSO-d_6 before (**Spectrum I**) and after (**Spectrum II**) thermal treatment

4.2.1.2 Synthesis of Thym-C5-NH$_2$

The need to prepare different thymine derivative was caused by interest in the potential effect of the structure of the core on the synthesis procedures (side reactions, degree of incorporation and so on) and physical properties of hyperbranched polyesters. In principle the nature of core stays constant, just a new spacer is introduced. Its length between core and the branched part could be of a great importance, because it affects mobility of the active group. The later is in charge of ability to adopt the most adequate position to form stable H-bonds with templates. The nature of such a spacer and the coupling bonds should not be forgotten, because it will be later introduced into the polycondensation.

It was decided to make the spacer longer and to replace the ester bond by amide one. Synthesis of this new thymine derivative, Thym-C5-NH$_2$, is presented in **Figure 29**. Now the

45

spacer was altogether nearly three times longer and possessed only the amide bonds. Of course, there was some risk that this substance possessing free amine group will not be stable.
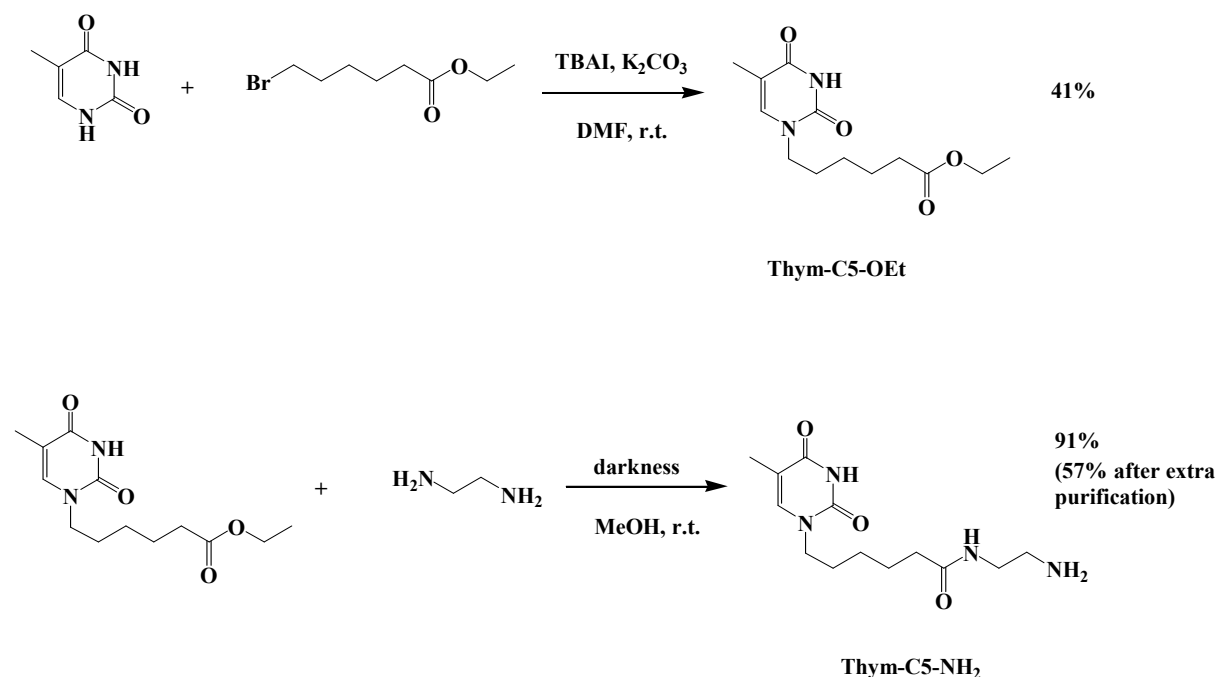
Figure 29 Synthesis of Thym-C5-NH$_2$ derivative

The two-steps synthesis includes the coupling of 6-bromoethylhexanoate to thymine followed by reaction with excess of ethylenediamine. The reactions are going smoothly with good yields. For the first stage, NMR spectra showed that monosubstitution was suppressing disubstitution, though it still occurred in less than 1% of the cases. In the second stage, ethylenediamine was taken in excess to avoid the reaction on both amino groups.

The final product is clean enough after standard washing-up procedure as could be seen on ^{1}H and 13C NMR spectra. (**Figure 30**) Of course, it is possible to remove some traces of a di-substituted thymine derivative or oxidation products by column chromatography. This extra purification step causes a 35% decrease in yield. In principle such impurities do not play any considerable role in further reaction, so extra purification should be done only for material stored for a very long time.

The thermal stability tests were made for this derivative in the same manner as it was describe for the Thym-C3-OH. The decomposition was observed at about 130ºC, when the material from white powder turned into the brown resin. No ^{1}H NMR spectrum could be measured on that, because after the thermal treatment samples were not soluble in DMSO.
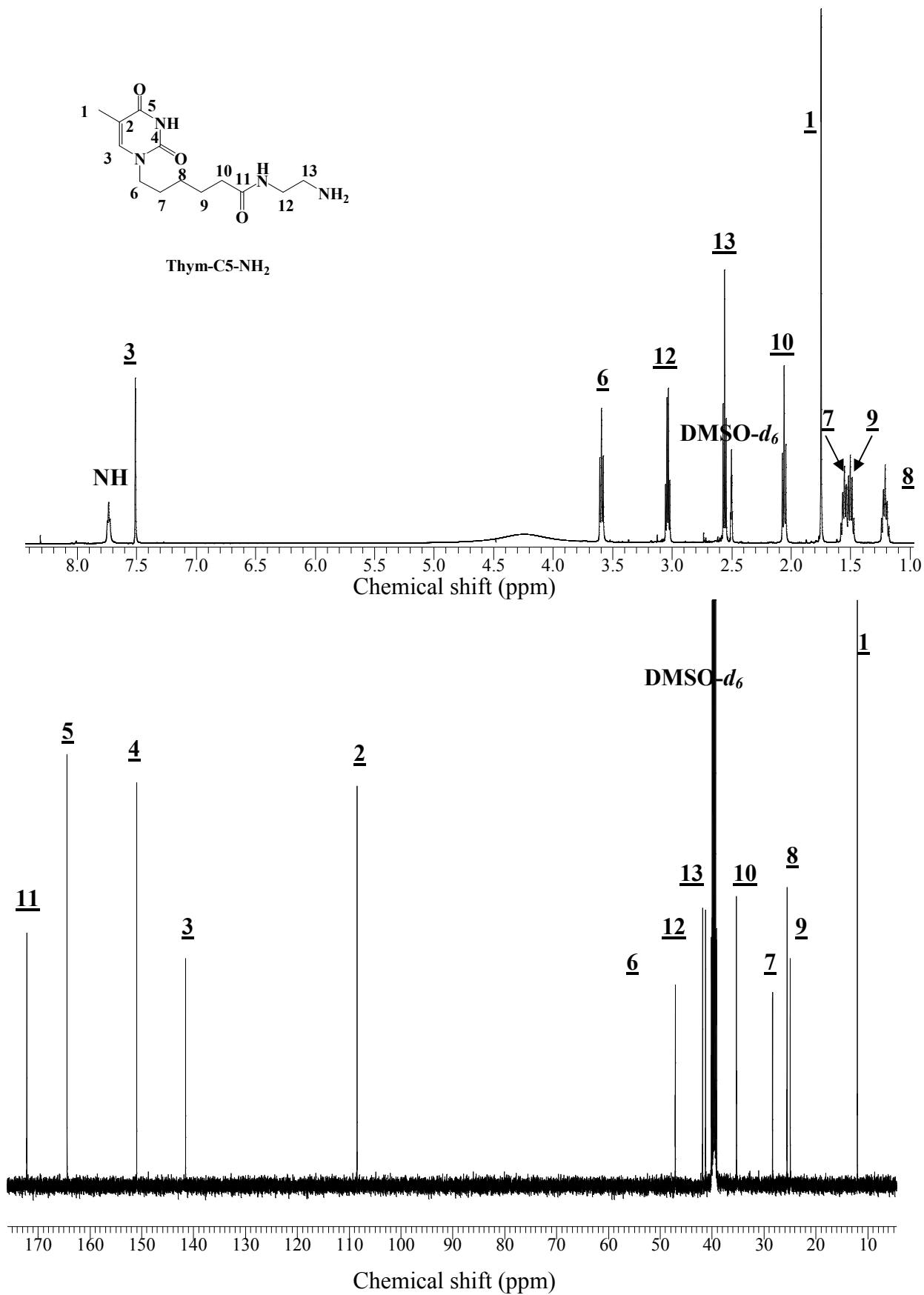
Figure 30 ^{1}H and ^{13}C NMR spectra of Thym-C5-NH$_2$

4.2.2 Synthesis of 2,6-diaminopyridine derivatives

As discussed earlier 2,6-diaminopyridine (DAPy) and its derivatives are readily used as templates for complexation of DNA [177, 180, 195] and RNA bases [184, 196], as well as barbiturate [176]. First observation of H-bonding between DAPy derivatives and the amide group of thymine dates back to 1986 [141]. In this work it was decided to substitute the naturally complementary nucleic base, adenine, by artificial analogues. Synthetic approaches for the preparation of DAPy derivatives done in this PhD work are presented in **Figure 31**.
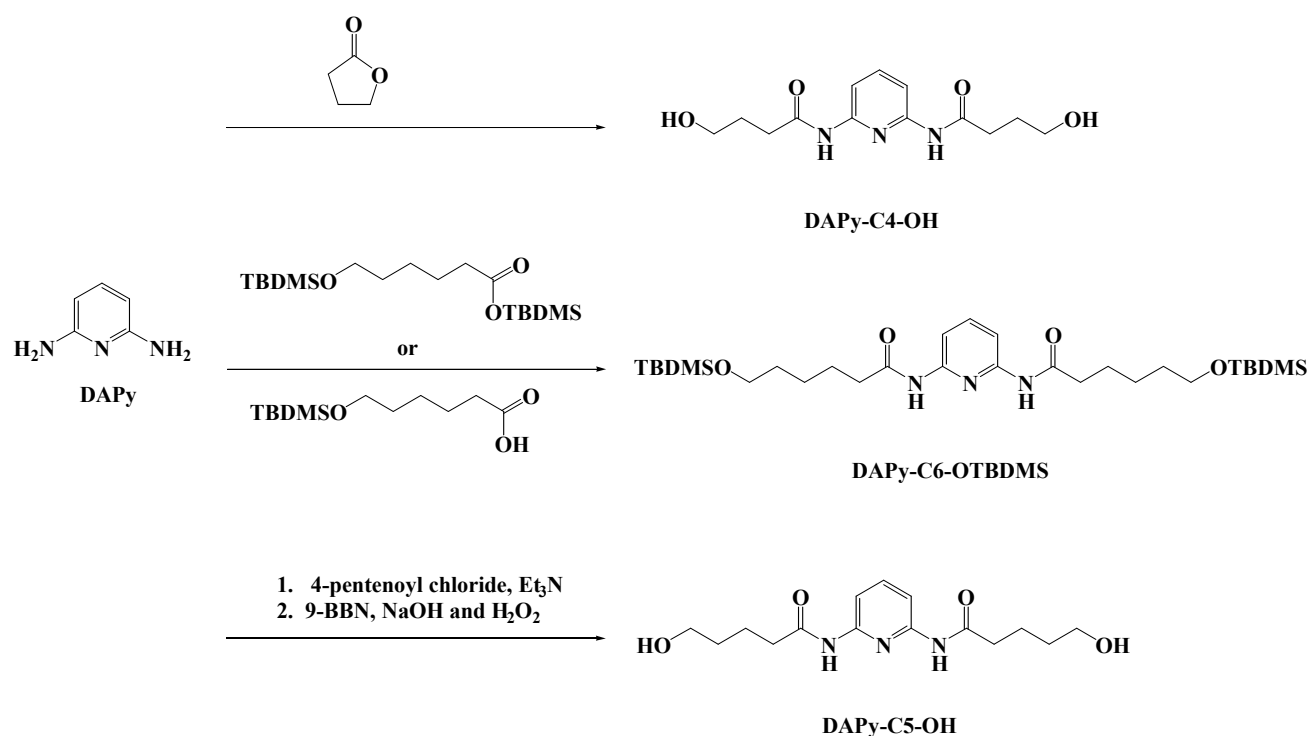
Figure 31 Synthesis of 2,6-diaminopyridine derivatives with end hydroxyl groups

The reason, why it was decided to reproduce the three-step synthesis and to apply aminolysis to synthesize DAPy derivative with free hydroxyl groups, is that in principle such derivatives could be either incorporated into the structure of hyperbranched polyesters as a core moiety or used as a simple template.

4.2.2.1   Synthesis of N,N'-(4-pentenoyl)-2,6-diaminopyridine and N,N'-(5-hydroxypentanoyl)-2,6-diaminopyridine

The three-step synthesis starting from 2,6-diaminopyridine (DAPy) and leading to N,N'-(4-pentenoyl)-2,6-diaminopyridine and N,N'-(5-hydroxypentanoyl)-2,6-diaminopyridine was described by Das et al. [116]. The first two steps give DAPy derivative with unsaturated end-

groups. Following hydroboration with 9-BBN should lead to the final product. (**Figure 32**) The last step is known to be tricky, because the hydroboration agent is very sensitive to many conditions and the amounts of reagent should be taken in strictly defined proportions, otherwise the reaction would not go [197]. There is also a chance for side reactions under oxidation, which would lead to undesired products or full decomposition of starting material.
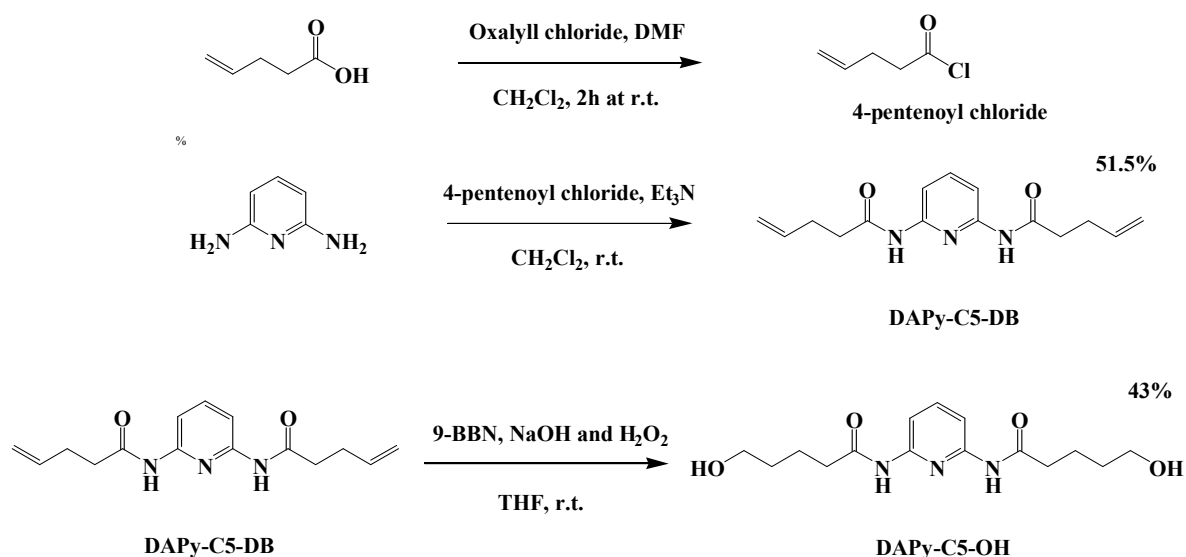
Figure 32 Three step synthesis of N,N'-(5-hydroxypentanoyl)-2,6-diaminopyridine (DAPy-C3-OH)

As it was supposed, the first two conversion steps are easy-going and give N,N'-(4-pentenoyl)-2,6-diaminopyridine. The yields were improved from 40% described in literature to 51.5% and the pure product was isolated. Detailed analysis of the by-products showed that about 10-15% of monosubstituted DAPy was formed.

The final hydroboration using well-known 9-BBN reagent [197-201] was hard to complete. Probably, due to instability of DAPy derivative at reaction conditions and the fact that too many factors may have here a crucial effect [202]; it was possible only once to get the desired product. In other cases the reaction was going by the way of decomposition of DAPy-C5-DB to the starting DAPy and other materials. The amount of synthesized N,N'-(5-hydroxypentanoyl)-2,6-diaminopyridine was just enough to perform some analytical tests.

So in the end it was possible to synthesize two derivates, which can be tested as H-bonding agents. Unfortunately, DAPy-C5-OH could not be used as a core moiety in polycondensation because of the unreliable synthesis.

4.2.2.2 Aminolysis of γ-butyrolactone

Aminolysis of lactones is an established and well-known reaction. As it was described in literature, reaction of lactones with amines is very sensible to the nature of both reagents [203]. Unfortunately, it is going rather easy only for the aliphatic amines [204] though even in this case reaction could go through the formation of either amino acids or hydroxyamides [203]. Speaking about aromatic structures in the majority of cases it was shown that they are inactive or pure products could not be isolated. Still in some cases amides were prepared in rather low yields through the variation of conditions and methods of synthesis. Temperature, catalyst, and method of coupling (in bulk or in solution) need to be adjusted individually, so it is hard to find the right combination.

DAPy-C4-OH
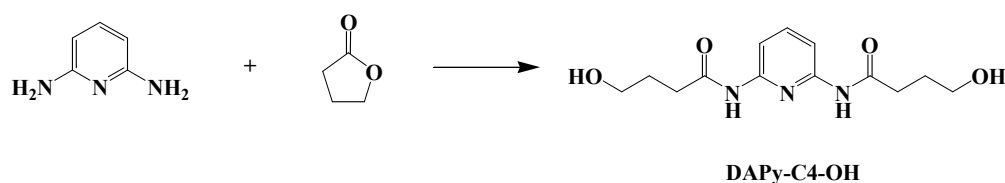
Figure 33 General scheme of aminolysis of gamma-butyrolactone with 2,6-diaminopyridine

From the literature it was known that β-lactones react with aliphatic diamines in aqueous solution within 2-3 h giving good yields. Room and higher temperatures have no significant affect, while decrease of it down to 5- 10ºC increases conversion of diamine. To perform aminolysis of less active structures Levit et al. have been using a catalyst [205]. They prepared γ- and δ-hydroxyamides by ring opening of aromatic γ- and δ-lactones with potassium amide in THF and with hydrazine, though lithium methylamide was also tested in some cases. In principle, the ring opening was going in all cases fast and with rather good yields except for the ones where structures on cyclohexanone or fluorenone basis were used. It is also possible to perform aminolysis of δ-lactones by direct reaction with diamine in dry DMF at room temperature, but these reactions were described only for aliphatic amines. Sudha and Pillai have used sodium hydride to activate aromatic diamines [206].

All the possible ways were analyzed and the best suited were chosen. It was obvious that this reaction could be difficult to perform, but still some trials have been started. The reaction conditions and general scheme of reaction are shown in **Figure 33** and **Table 3**.

To begin with a rather common aminolysis in bulk at the melting temperature of DAPy was performed. Here the traces of the probable final product could be seen using thin layer

chromatography (TLC), and NMR, but the amount of by-products and unreacted starting material prevented further analysis. The 5-10% yield was estimated on ^{1}H NMR data basis.

Though the reaction in melt was disappointing and generally aromatic diamines in solution are pronounced to be inactive in aminolysis of lactones, a set of reaction in DMF was performed. As it is shown in **Table 3**, the reaction conditions (room temperature and DMF as a solvent), which are usually applied for aliphatic diamines did not work. Though some traces of product were seen on TLC plates it could not be isolated. Even increase in temperature up to 100ºC has not made much difference. All this corresponds to the literature data. The last synthesis was carried out in DMF solution using potassium hydride as an activating agent, but still it was not possible to isolate the final product.

Table 3 Reaction conditions of aminolysis of γ-butyrolactone with 2,6-diaminopyridine

Solvent	Activating agent	Time	Temperature ºC	Yield
-	-	19h	120	Some unclean product was isolated after column chromatography with the yield of about 5-10%
DMF abs.	-	1d	20	-
DMF abs.	-	1d/1d	20/65	-
DMF abs.	-	1d/1d/1d	20/65/100	Product, seen on TLC, could not be separated
DMF abs.	NaH	20h	140	Crude product could not be purified. Potential yield could be at about 30%

The reasons for the misfortune could be that the equilibrium in the reaction is shifted toward the starting materials and that DAPy undergoes decomposition at high temperature even under constant protecting atmosphere.

In the end one can conclude that DAPy is passive in aminolysis, as all aromatic diamines are. No further tests were made in order to optimize the conditions.

4.2.2.3 Synthesis of N,N'-(6-hydroxyhexanoyl)-2,6-diaminopyridine

Three-step synthesis of DAPy-C$_6$-OH was planed as shown in **Figure 34**. The main idea was to couple the acid possessing protected hydroxyl functionality to DAPy.
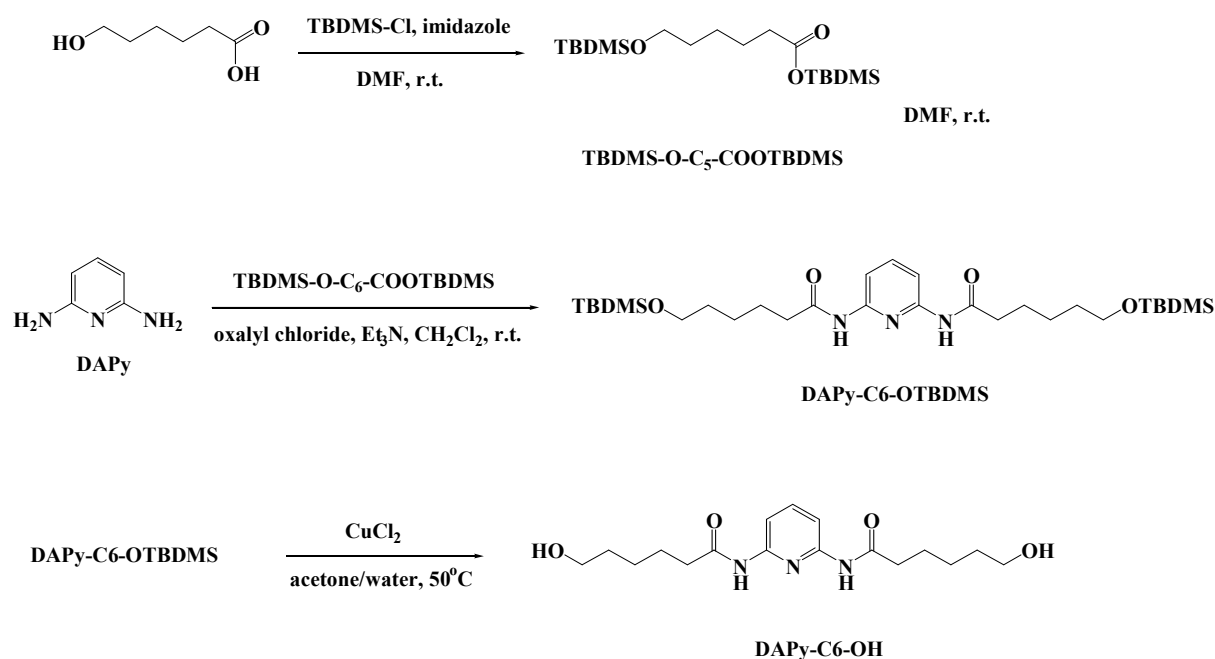
Figure 34 Scheme for the three-step synthesis of DAPy-C6-OH

At first hydroxyl and carboxyl group in 6-hydroxycaproic acid were protected using tert-butyldimethylsilyl chloride. The protection of both functionalities was done, because the second step included conversion of the acid into chloroanhydride. If this conversion is performed using 6-(tert-butyldimethylsilyloxy)hexanoic acid, TBDMS protecting group will be cleaved and oligomers will be formed. This methodology has been explored by Mourey and co-workers in the synthesis of aromatic monomer with TMS protected hydroxyl groups for the synthesis of hyperbranched polyesters [207].

Unfortunately, despite the full deprotection it was not possible to avoid the undesired cleavage of the protection of hydroxyl group and to prepare the desired product. The lower stability of aliphatic tert-butyldimethylsilyl ethers compared to the aromatic ones explains this fact.

4.3 Synthesis of multifunctional templates

As shown above self-assembly and molecular recognition could be based on the noncovalent interactions between two templates. Van der Waals, ionic forces and H-bonds are the most common ones. For such applications templates should posses some specific properties apart from selective interaction with one another. For example, to be useful in building of molecular organizations formed via H-bonding interactions, they should be soluble in so called "good" solvents, which are generally nonpolar ones.

One of the aims of the work was to test the ability of the branched structures in molecular recognition. For that low molecular weight templates capable to form stable complexes with thymine tails were prepared. It was also interesting to see the difference in the polymer behaviour, when macromolecules are forced to organise around the multifunctional template. It would be also an example of the formation of rather big assemblies.

From the wide range of the possible pairs for thymine adenine and 2,6-diaminopyridine derivatives would be particularly interesting for this work. In this case one should be careful, because, though modification of adenine can be made in position six [208, 209] or nine [210-212], only 9-substituted derivatives can be applied for the H-bonding investigations. Simple modification can be accomplished in several ways, but it is not the same for the multifunctional templates. There are only several examples of di-adenine [213, 214] and di-2,6-diaminopyridine derivatives [146, 147, 176, 215] (**Figure 18**), though not that much of them will fit in the solubility limitations. Unfortunately, authors were mostly interested in synthesis and have performed only basic analysis to confirm the structure, so nearly no information about solubility of these substances could be found in their publications. To save the time it was decided to try only the simplest synthetic ways. The work of Itahara et al. seemed to be the most useful, presenting an easy way to prepare bis- and thri-adenine and thymine derivates. They were the only ones to report about tri-adenine samples soluble in chloroform and pre-investigations of their H-bonding activity [127].

4.3.1 Synthesis of di-diaminopyridine derivatives

The examples of di-2,6-diaminopyridine found in literature are not that good in our case, because active groups in them a turned inwards of their structure in order to generate stable bond with the desired target [216]. The bifunctional derivatives where two DAPy units would be connected via an aliphatic chain were not described before. This kind of molecules would be very interesting, because here both units can complex separate molecules. It was decided

to use methods close to the formation of the polymer containing diaminopyridine in order to form the "linear" di-DAPy derivatives.

Two routes are proposed for the synthesis, both involving reaction with adipoyl chloride and other chloroanhydride. The formation of amide bonds via reaction between the amino groups of DAPy and acid chlorides was described in literature [116, 184, 217].

The first one starts with the reaction of DAPy with adipoyl chloride and is followed by reaction of the intermediate with 2,2-dimethylpropanoyl chloride or acetyl chloride (**Figure 32**).
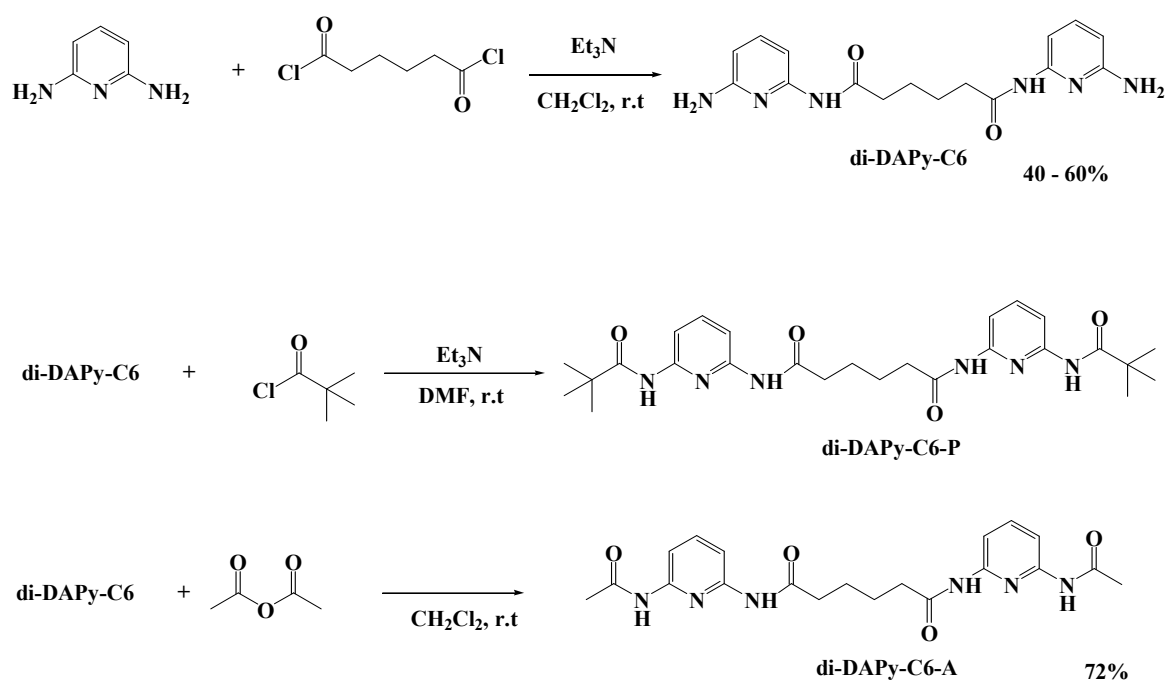
Figure 35   Synthesis of di-DAPy via first route

The main challenge here was to suppress the formation of oligomers and to synthesize only the disubstituted adipoyl amide. Taking into account high reactivity of adipoyl chloride and the average one of DAPy, the later was taken in excess as it was done in the reaction of Thym-C5-OEt and ethylenediamine. This can slightly prevent the formation of by-products. The addition of acid chloride was performed slowly under vigorous stirring, but it has not helped that much. Row products were purified by simple washing up with water. No column chromatography was possible as all the oligomers were moving together. Overall yields of oligomers in all reactions were between 40 and 60%. The pure content of di-DAPy-C6 would not exceed 27%.

All products were only soluble in polar solvents such as MeOH and DMSO. In order to improve solubility in chloroform the protection of free amino-group was needed. Since the

protection of amino group with 2,2-dimethylpropanoyl chloride did not go well, acetide protected derivative (di-DAPy-C6-A) was synthesized. This phenomenon can be explained by the sterical hindrance. The final product has up to 10% of oligomers. Its solubility in unpolar solvents is just good enough to use it as template in H-bonding investigations.

To increase the solubility and to eliminate the formation of oligomers, the second way of synthesis goes through the preparation of mono-substituted DAPy followed by the coupling with adipoyl chloride. (**Figure 36**)
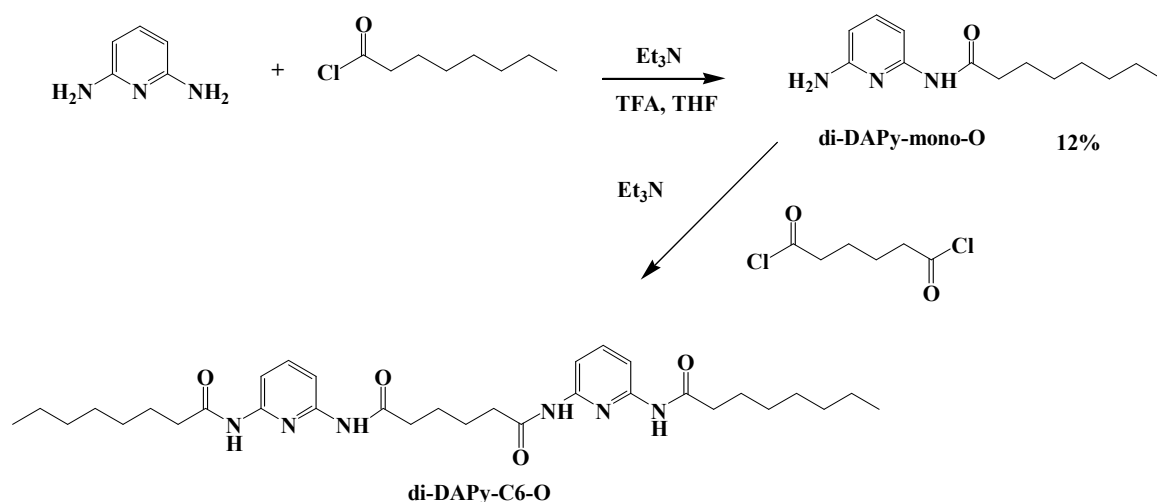
Figure 36 Synthesis of di-DAPy via route II

The substitution of DAPy was carried out in the THF/TFA solution as described in the literature [218]. The di-DAPy-mono-O has been received with 12% yield after purification by means of column chromatography. The following amidation was going slower compared to the speed of the reaction between DAPy and adipyl chloride and in the end it was not possible to isolate the di-DAPy-C6-O derivative.

4.3.2 Synthesis of the templates on the adenine basis

Besides DAPy derivatives adenine derivatives were prepared to compare the stability of the complexes. Thymine and adenine are the natural complementary bases and their interactions are considered to be the most stable ones. The single-site modified adenine and multifunctional derivatives were prepared. The former was to be used in the preliminary test to define the conditions for the formation of stable H-bonds.

Despite many literature references, it was not that easy to prepare multifunctional molecules with adenine as an end-group. Until 2007 the only information about solubility

came from NMR spectra, which were usually taken in DMSO-d_6. Some of the syntheses were reproduced to perform the solubility test.

4.3.2.1 Synthesis of mono- and di-adenine derivatives

Monosubstituted and disubstituted adenine derivatives were prepared according to the standard methods described in literature [139]. Derivative with different spacer length were synthesized simultaneously, in order to determine their solubility in chloroform and dichloromethane.
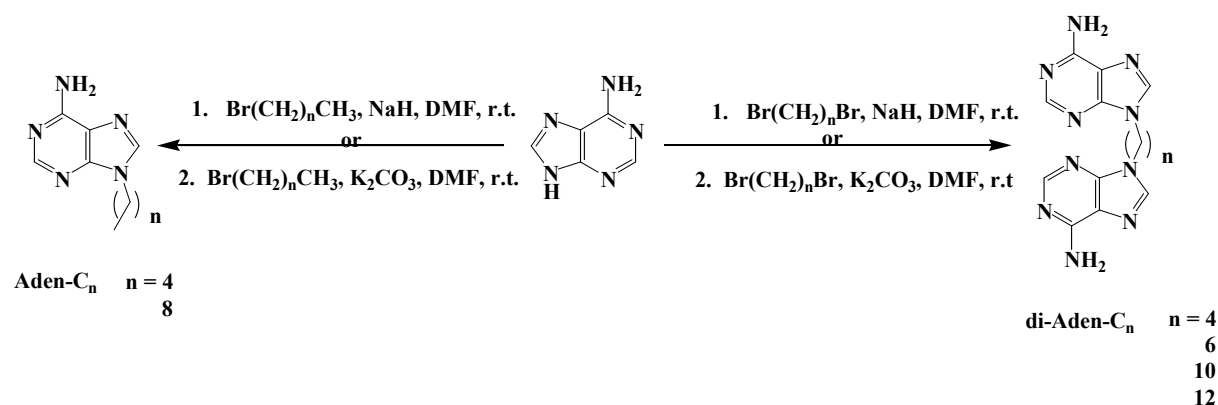
Figure 37 General scheme of synthesis of mono- and di-adenine templates

The coupling of bromides or dibromides to the adenine in the presence of K_2CO_3 is a well-known reaction. [128]. The reaction is rather time consuming, 40h or more, and that is the major disadvantage. But the 90% theoretical yield and absence of washing up procedures are very appealing. Reactions performed in the course of this work were no exception. They proceeded at room temperature for 48h and the yields were 92-95%.

There is also another less common way, where to accelerate the N-alkylation sodium hydride is used as a catalyst [185, 210]. The reaction with NaH activation is completed within 12-24h depending on the bromine containing reagent. The yields here were about 50% after purification, but could be improved up to 80%. So both methods lead to the same result, but speaking about time the one with activation is favourable.

Among the multifunctional templates here only 9,9'-undecamethylene di-adenine was slightly soluble in chloroform, the other products were only soluble in DMF and the solubility increased with the increase in the spacer length. This, unfortunately, it is not possible to make test to determine the correlation between the H-bonding interactions and the length of the spacer.

Here computer modeling for ideal systems without considering any physical properties of starting substances, which is discussed later, was of a particular interest and help.(**Chapter 4.10**, p.109)

4.3.2.2 Synthesis of tri-adenine derivative

Three-functional templates were synthesized in order to be able to generate bigger complexes during the investigation of H-bonding interactions. Tri-adenine derivative was synthesized according to Itahara et al [139].
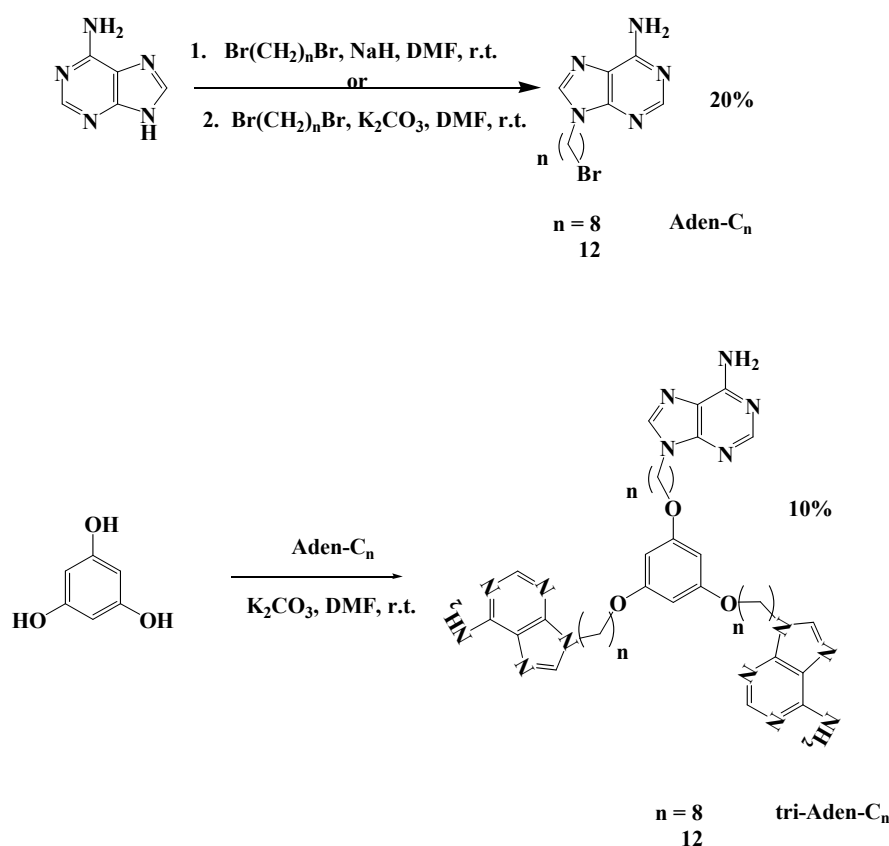
Figure 38 Synthesis of tri-adenine derivative

Synthesis of monosubstituted di-bromoalkane has been tricky and hard to accomplish. In the literature such reaction was performed in DMF with K_2CO_3 [139]. This synthesis was successfully reproduced. The same reaction was performed using sodium hydride as an activating agent. During this reaction only the disubstituted derivative has been formed due to high reactivity of the activated adenine. The slow addition of reagents has not made much

change. So it is clear that N-alkylation of nucleic bases with this activating agent leads only to the formation of di-adenine derivatives

The following reaction of Aden-C_n-Br with phloroglucinol was performed in the similar condition to the ones described by Itahara [139]. The reaction was going slowly, so the final product could be isolated in the synthesis of tri-Aden-C_{12} only with low yield. The misfortune in the case of three-Aden-C_8 can be explained by either slow reactivity of the reagents or sterical hindrance.

4.4 Polycondensation using thymine derivatives as core moieties

2,2-Bis(hydroxymethyl)propanoic acid (bis-MPA), which is widely used, was chosen as a monomer. Nowadays, there is a lot of work describing various polycondensations with this AB_2 monomer. On the theoretical basis there are two polycondensation approaches, one-pot and step-by-step syntheses. The main requirements for the final polyester were a molecular weight in the range of 5000-50000 with quite low polydispersity index (PDI). In order to achieve it vacuum, time distribution, temperature and even the starting core moiety have been varied.

Two different thymine derivatives Thym-C3-OH and Thym-C5-NH$_2$ as core moieties were synthesized in advance. The complications during the modification of DAPy prevented its usage as a multifunctional core moiety in polycondensation.

4.4.1 Polycondensation with N-(3-Hydroxypropyl) thymine core moiety

The polymerisations were made in batch. The main reaction conditions are presented in **Table 4** and the more complete overview is shown in Appendix. The polycondensations without core and with TMP core moiety were used as references. The later synthesis is a well-known reaction; the former one was also investigated. The main change was to prepare the material with needed characteristic in a small scale; in every attempt the final amount of bis-MPA monomer did not exceed 7g.

First step-by-step approach (Hult's method) was applied, the conditions were adopted from the ones used by Hult's group for the synthesis of bis-MPA polyester with TMP core [51]. This methodology has not been working properly. All products were highly crosslinked and thus, hardly soluble in any organic solvent including DMSO.

There are explanations for this. The inability of scaling up or down in such polymerizations and application of the same conditions after the change in a core is a known fact. The amount of monomer used in Hult's group was bigger. Still the published results were useful for the preparation of the standard sample with TMP core, p.60.1. Still further work proceeded in the direction of one-pot synthesis.

Conditions for polymerizations in melt (one-pot approach) were chosen among the ones described in literature [91, 179]. Purification for all samples was done by precipitation from THF into cold diethyl ether. Shortly after the first test it was clear that optimization was needed in order to prepare materials with properties close to the needed parameters.

Table 4 Reaction conditions used for the polycondensations

Sample	Core	Gen.	T°C	Time/h		Vacuum
				Ar	Vacuum	mbr
p.7.1		4	150/185	1	10	15
p.11.1		5	185	1	8	14
p.13.1		5	166	1	15	14
p.13.2		5	185	1	15	14
p.14.1		5	185	0.5	8	12-15-25
p.14.2	Thym-C3-OH	5	160	0.5	8	12-25
p.16.1		6	185	1	8	14
p.17.1		6	185	1	10	15
p.18.1		6	185	1	15	12-18
p.20.1		6	185	0.5	10	14-25
p.21.1	(HO)$_2$- [G#1]-O-Thym	6	185	0.5	8	14
p.60.1[1]	TMP	6	140	2	1	12
p.62.1	-	-	185	2	6	2
p.65.1	-	-	185	2	8	2.6

[1] synthesized according to Hult's method

At first temperature of the synthesis was varied. This step was done before the SEC analysis of polyesters, because gelation and high crosslinking have appeared during the polymerizations, if the reaction was carried out at temperatures less than 185ºC. For the second generation polycondensation has not started at all. In synthesis, described by Hult or other scientists, the low temperature used was determined by the melting point of the core molecule. After all, it was concluded that reactions should be performed at around 185 ºC, which is the same as for the polycondensation of bis-MPA without addition of a core. From observations during synthesis, purification and preliminary analysis of ^{1}H NMR spectra the conditions in sample p.20.1 were considered to be the most suitable ones. Here the final product was a solid, what indicates the high molecular weight, but soluble in THF, that means rather low content of ether groups (side reaction which leads to crosslinking). This sample has readily precipitated in diethyl ether.

From literature the tendency for the better incorporation of multifunctional cores is known. It is understandable, the more functionalities the higher the probability of its reaction

with bis-MPA. For the further application in molecular recognition a very high incorporation of the core is important, because the thymine functional unit would be responsible for the formation of H-bonds. In order to investigate the influence of the number of hydroxyl groups in the core on its incorporation into hb polyester the first generation dendrimer modified with thymine (see p.75) was used (p.21.1). Here the polycondensation conditions were at first the same as from the best attempt with Thym-C3-OH and were later modified.

For the future experiments it was essential to be able to reproduce the polycondensation. After optimization, a couple of syntheses were done several times to confirm reproducibility of the results. Prepared polyesters were characterized via different techniques. For all chosen samples molecular weight and PDI are as the ones expected for materials prepared in such a small scale. The detailed result of polymer characterization and effects of different parameters on the structure are discussed later.

4.4.2 Polycondensation with Thym-C5-NH$_2$ core moiety

The Thym-C3-OH core moiety is connected to the polymer via the ester bond that is why it can take part in transesterification reactions happening in the main polyester structure. Such phenomenon would lead to the lower incorporation of the thymine derivative. The way to avoid this is to synthesize another core with different functional active group, which could be coupled to the bis-MPA via more stable bond like amide.

The other core moiety, Thym-C5-NH$_2$ possessing only amide bonds was synthesized and applied for the polycondensation. The amines are known for their instability at high temperatures, so the thermal tests were run. From the preliminary investigations it was not clear weather real melting or decomposition has occurred. The residue after thermal treatment was not soluble in common organic solvents and it was not possible to analyze it with NMR spectroscopy. Without extra experiments under protecting atmosphere it was not possible to find out whether oxidation or decomposition took place and to pronounce instability of this derivative.

The first trial polycondensation was made before the thermal analysis was completed. For the pilot reaction conditions were applied like for the sample p.20.1 (**Table 4**). Only half an hour after start the dark colour of the melt was indicating that something went wrong, while, usually during polycondensation it varies from total absence of colour to slightly yellowish, becoming dark yellow or even brownish only after cooling down to the room temperature. The ^{1}H NMR analysis showed no signs of thymine core in the polymer.

It was only later, when Thym-C5-NH$_2$ was proved to be unstable at high temperatures. Its decomposition takes place at about 130°C while the best result for the polycondensation with the other core moieties based on thymine was obtained at 185°C and 140°C was the lowest reaction temperature repeated in literature.

The only possibility to prepare the desired polyester was the polycondensation in solution, where one reagent would be used for both amidation and esterification. Polymerization of bis-MPA in solution was not described in literature until 2007 [92]. This method is considered to be not promising because of low molecular weights of final polyester.

In this case low molecular weight would not be that problematic, because oligomers with core coupled via amide bond could be later on used as a starting material. The amide bonds are much more stable and will not decompose in the usual polycondensation conditions.

There are not so much activating agents that work well for both esterification and amidation. After careful consideration, benzotriazole-1-yl-uloxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (BOP) was chosen, because it is a good reagent for peptide synthesis [219-224] and also can be used as an agent to prepare esters under mild conditions [225], though this kind of reaction is not that common.

Thym-C5-NH$_2$ was led to react with the bis-MPA in the presence of benzotriazole-1-yl-uloxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (BOP) and N,N-diisopropyethylamine (DIEA) in DMF at room temperature. (**Figure 39**)
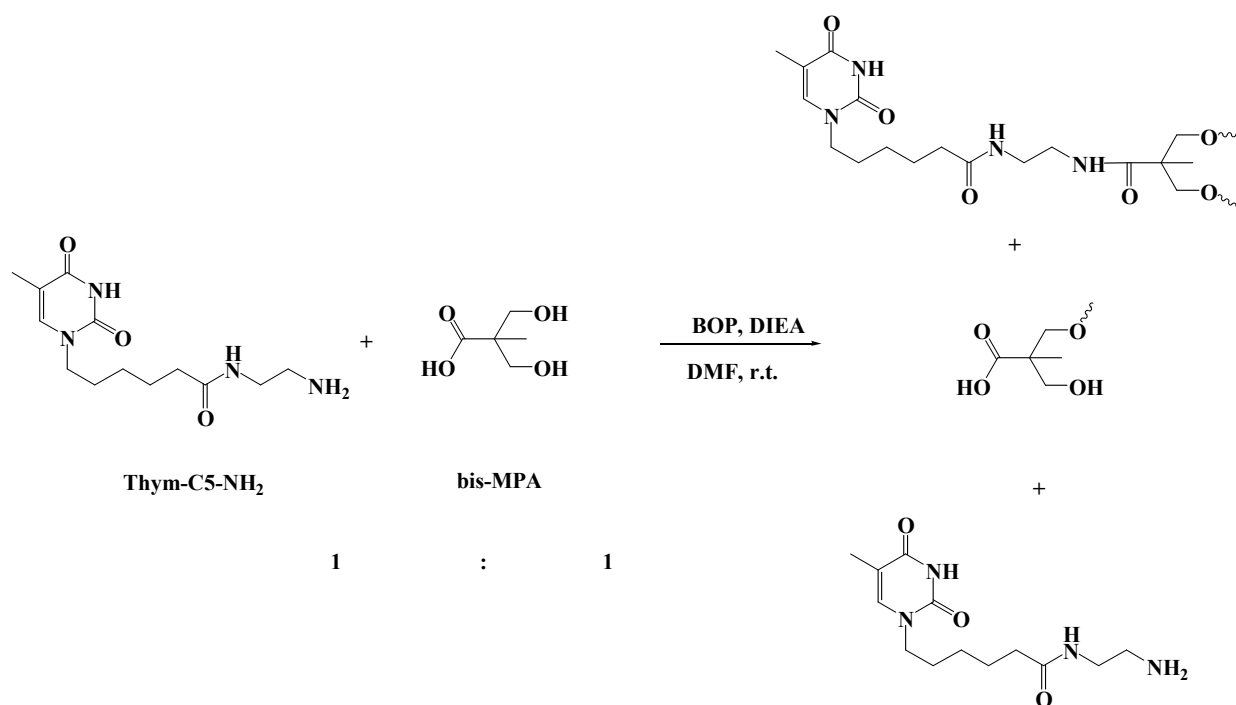
Figure 39 General scheme for coupling of the Thym-C5-NH$_2$ and bis-MPA

As it was expected for bis-MPA monomer polymerization was not working well. The incorporation of a core and chain growth were going too slow, so in the end it was a low molecular weight product (oligomers from 2 to 4 bis-MPA units) with about 10% conversion of focal carboxyl groups. With such low degree of incorporation it could not be used in further polymerizations. If it was applied, the incorporation of thymine derivative in the final polymer would be not more than 2.5%. This is too low and this way of synthesis was put aside.

Despite first setback the work went on in the direction of dendrimer synthesis in order to prepare the first generation. This material could have been used as a starting core molecule, but synthesis turned out to be time-consuming and with low yield as is shown in **Chapter 4.6.1**. So this way was also closed.

4.4.3   Modification of terminal hydroxyl groups in polyesters

Right after purification hyperbranched polyesters are only soluble in polar organic solvents such as DMF, DMSO or THF. For the investigation of H-bonding abilities, it is better to dissolve samples in one of the so-called "good" solvents such as CH$_2$Cl2 or CHCl$_3$. The detailed explanation of the solvents choice will be given later in **Chapter 4.7**.

Solubility of hb polyester in unpolar solvents could be enhanced by the protection or modification of hydroxyl groups. There are several methods for partial and full modification of end-groups in bis-MPA polyester [29, 95, 179, 226-229]. Usually modification is made with acid chlorides, because it is a fast and efficient method. Some methods are not that convenient like partial modification of hydroxyl groups in bulk [89] and are not often used. After all such modifications it is not possible to go back to the starting hb polyester without full decomposition of the material.
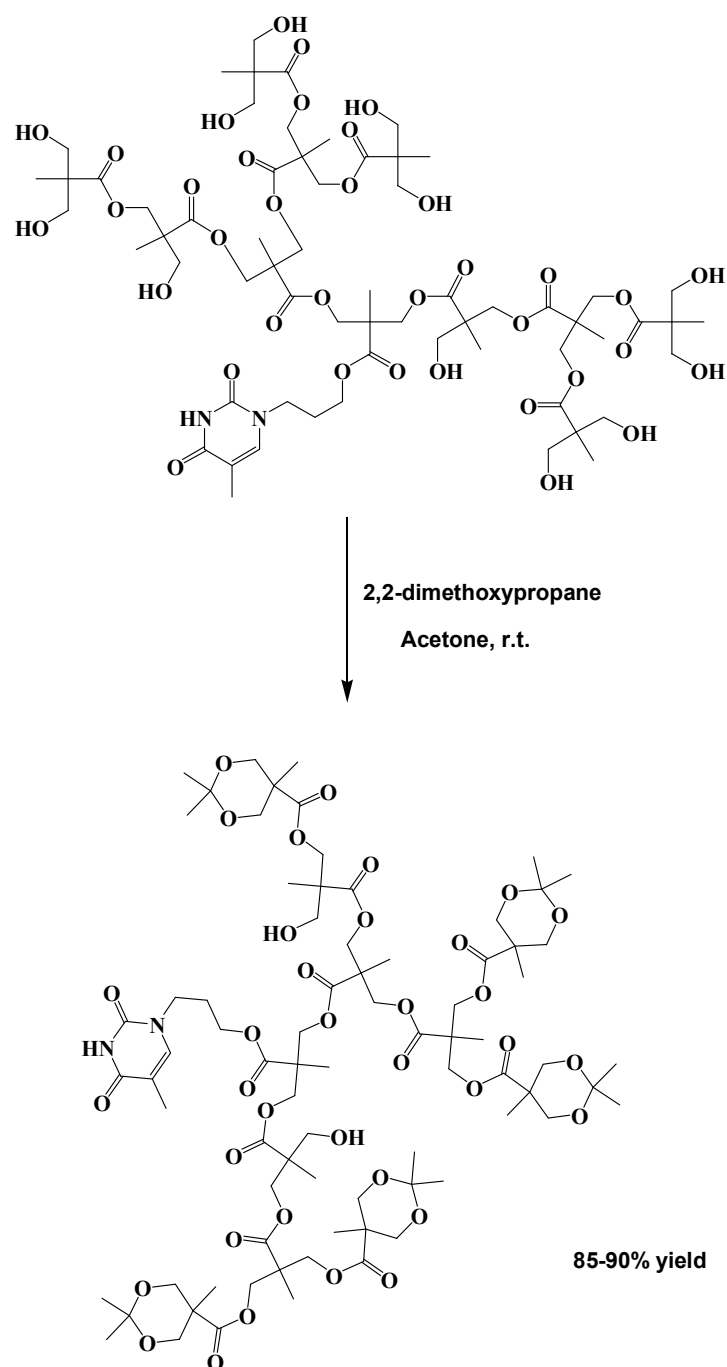
Figure 40 Selective protection of terminal hydroxyl groups in hb polyester

For this work it was better to protect and not to modify hydroxyl groups, because in this case it would be possible to deprotect them and return to the starting material when needed. The number of protecting groups is limited, because they should be incorporated and cleaved in easy manner and under mild conditions, so no decomposition of polyester backbone appears. From the known methods acetonide and acetyl protections, which are performed in solution, could be used [181, 230].

The acetonide protection was found to be selective toward the terminal OH-groups [230]. Simple deprotection using DOWEX resin, that does not involve extra purification, makes it even more appealing.
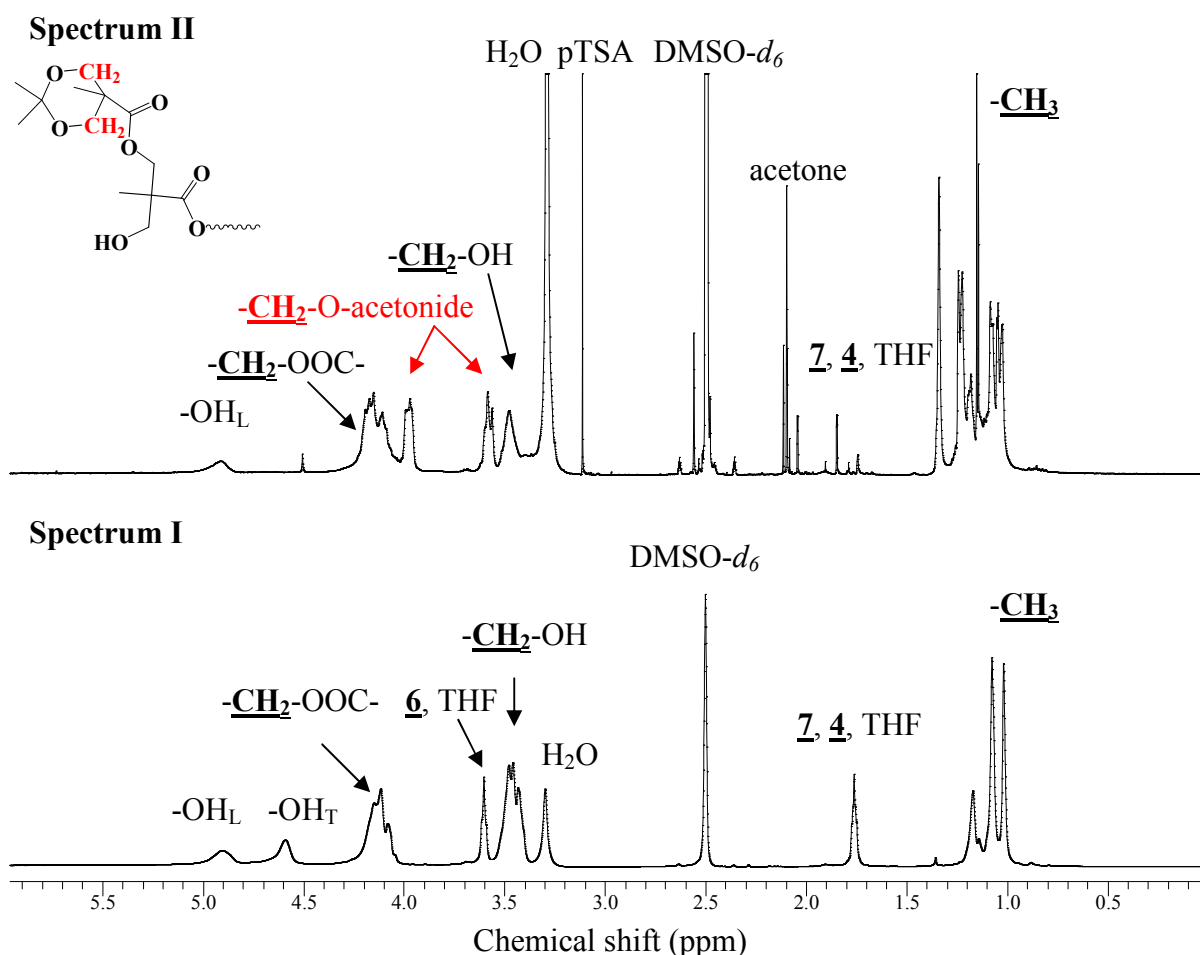
Figure 41 ^{1}H NMR spectra for hb polyesters before (Spectrum I) and after (Spectrum II) selective protection of terminal groups

Nearly all prepared polyesters were protected in this way. (**Figure 40**) The protection of terminal hydroxyl groups was proved using ^{1}H NMR spectroscopy. The signal from terminal hydroxyl coming at 4.59 ppm disappeared while signals at 3.6 ppm and 3.9 ppm coming from methylene groups in protected terminal monomer chains were clearly seen. According to the spectra all terminal groups were converted (**Figure 41**). For all samples even this partial modification was enough to make them soluble in CH_2Cl_2 and $CHCl_3$.
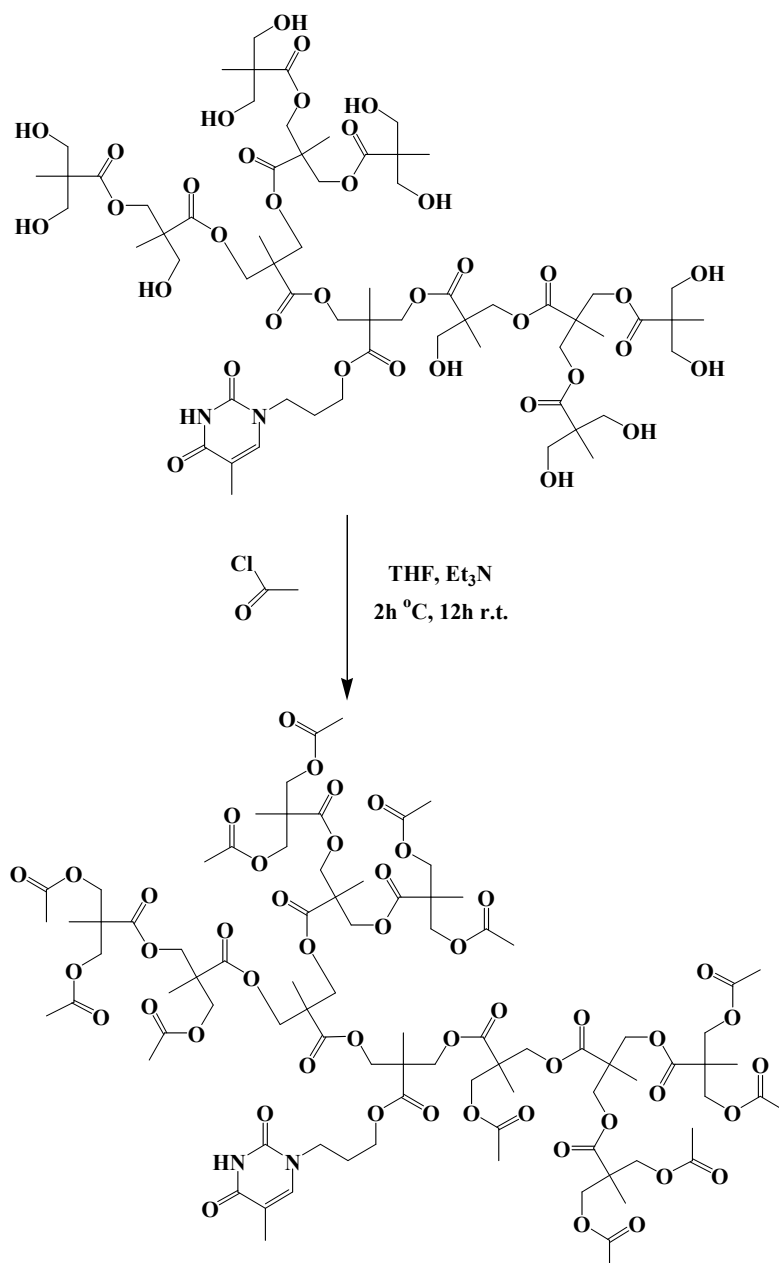
Figure 42 Full protection of hydroxyl groups in hb polyester

After the successful selective protection of terminal groups the full protection was not needed that much. Still this kind of modification was used for several samples to see if there was any difference in solubility of the protected hb polyesters. This could be done by modification with acetyl chloride.

Such modification leads to materials which are also soluble in unpolar solvents. As for the solubility, it was nearly the same in both cases and rather low, but still enough for the H-bonding analysis.

4.4.4 General conclusions about synthesis of hb polymers

Even before the analysis of materials could be made some general tendencies were obvious. In this case all is based on observations during polycondensations and purification of the final products. From the data obtained following conclusions could be drawn:

1. Polycondensations were possible only when Thym-C3-OH was used as a core moiety, because of instability of Thym-C5-NH$_2$ at high temperatures

2. Predictably, a polymerisation in solution failed

3. The Hult's method turned out to be inappropriate for synthesis of hb polyesters with thymine core moiety in the needed scale

4. Selective and full protection of terminal hydroxyl groups was done, which allowed solubility of the hb polyesters in chlorinated solvents necessary for H-bonding studies.

4.5   Synthesis of dendrimers

Analysis of hyperbranched polymers is a very complicated process. The perfectly branched analogous can be used as references during NMR and SEC analysis.   Thus, synthesis of dendrimers with thymine core moieties was performed following the divergent route. There is much information about the synthesis of such structures with different multifunctional aliphatic or aromatic cores. The synthetic approach, which was developed by Hult, could be considered the most used one. It is rather fast, but involves purification via column chromatography, which is a little bit inconvenient.

As in the case of hyperbranched polymer two kind of thymine derivatives were used: Thym-C3-OH and Thym-C5-NH$_2$.

4.5.1   Synthesis of first generation dendrimer with Thym-C5-NH$_2$ core moiety

It was reported already that Thym-C5-NH$_2$ derivative is not stable at the high temperature during polycondensations. Obviously, the only right way was to synthesize the more stable derivative with amide bond. In this case synthesis of the first generation dendrimer seemed the easiest solution.

As it was discussed before (**Chapter 4.4.2**) the use of bis-MPA in the synthesis with BOP reagent led to the formation of oligomers. So to complete the synthesis of the dendrimer it was necessary to use the protected bis-MPA in order to eliminate the esterification. Therefore, bis-MPA was protected by the conversion with 2,2-dimethoxypropane and with acetyl chloride. The protecting groups are readily incorporated and could be cleaved in an easy manner. The two kind of protecting groups were tested, because the acetonide-protection is easier to remove, but could be less stable than acetate-protection.

Figure 43 shows the proposed reactions, where protected monomers were explored. The activation agent (BOP) was not changed, and all other reaction conditions were also maintained as it was for the polymerisation of bis-MPA in solution (see p.68).
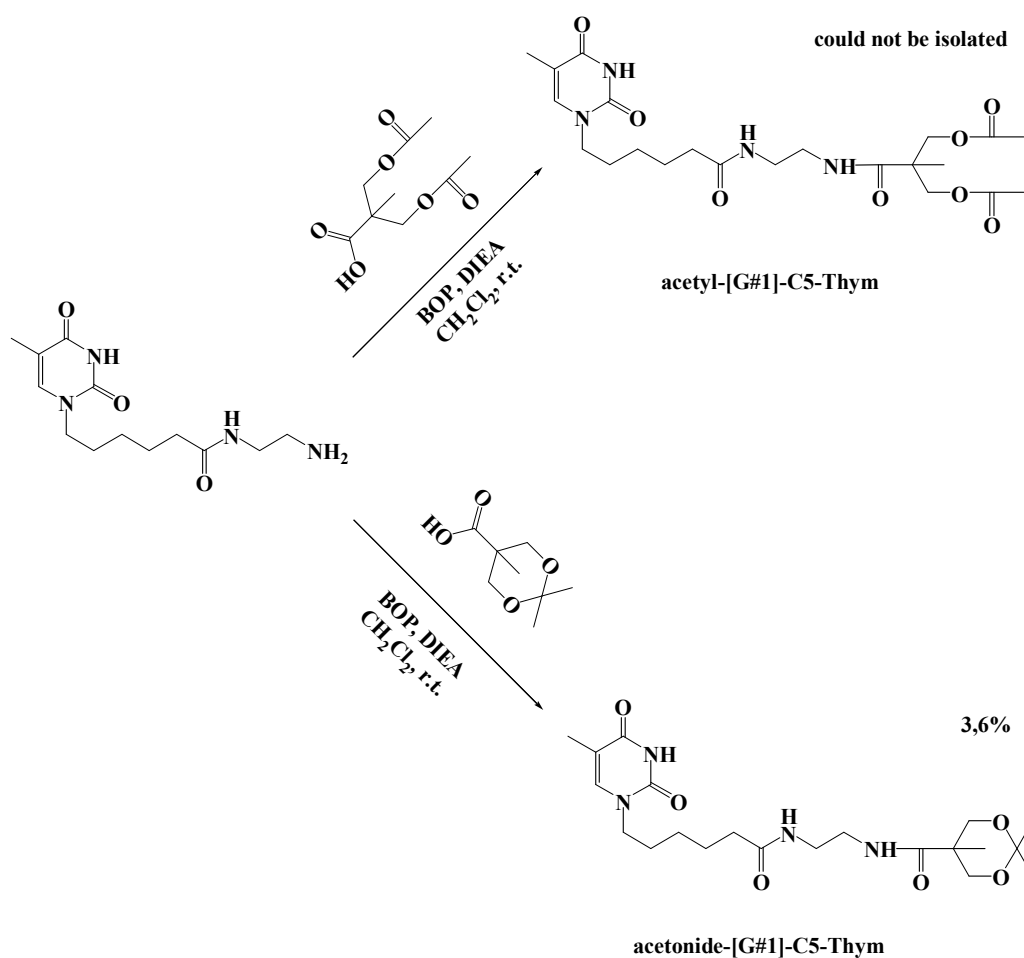
Figure 43 Synthesis of G1 dendrimer with Thym-C5-NH$_2$

Unfortunately, the synthesis of dendritic structures turned out to be going only with very small yields so it was left behind. The possible reason could be that BOP can act as a deprotecting agent due to its hexafluorophosphate anion and its ability to participate in the reactions not only as the reagent for peptide synthesis but also as a catalyst for esterification. The mechanism of BOP activation is presented in **Figure 44**. The intermediate A is converted to intermediate B within 15 min at room temperature. The formation of ester goes only within a quarter of an hour; afterwards the activated acid can react only with amine. Still anions, formed during this activation, though should be bonded to the DIEA, are rather active.
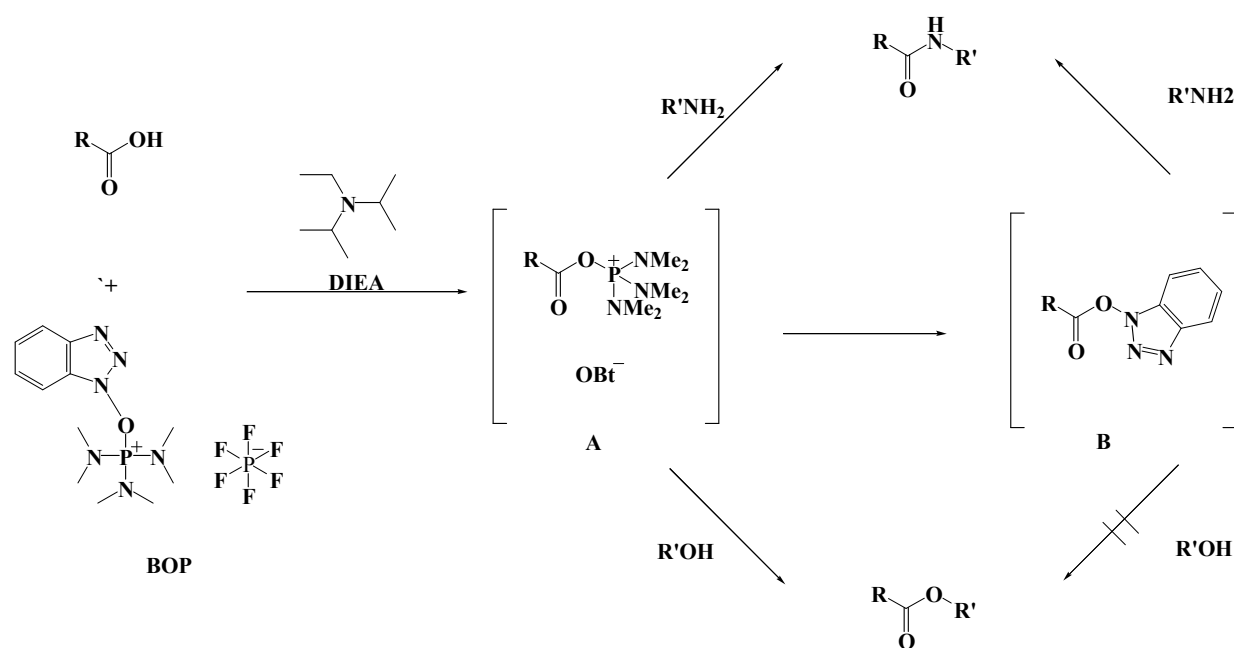
Figure 44 Mechanism of coupling with BOP activation [225, 231]

So it is probable that after partial deprotection not only amide bonds are formed, but also ester ones. Therefore, only a mixture of product and by-products was isolated after first column chromatography. A very small fraction of pure acetonide-[G#1]-C5-Thym was obtained after third column chromatography. It was not possible to get any acetyl-[G#1]-C5-Thym. NMR spectroscopy confirmed the presence of bis-MPA oligomers, but the spectra were too complex to be fully analyzed.

There is no clear explanation, why the reaction with acetyl protected bis-MPA was not going, may be the same problem as in previous case appeared, though this protecting group was expected to be more stable.

The coupling with acetonide protected acid was also tried with CDI as an activating agent, but no desired product was formed.

Taking into account all results this route was put aside. In the future the work can go in this direction using new more appropriate activating agents.

4.5.2 Synthesis of dendrimers with Thym-C3-OH core moiety

In the case of Thym-C3-OH core moiety everything was considered to be easier. Esterification leading to the formation of the bis-MPA dendrimers is a well explored process. These structures should be very helpful in the analysis of hb polyester with the same core

moiety. From the very beginning acetonide-protected bis-MPA was used in order to avoid the problem with formation of oligomers.

The synthesis of isopropylidene-2,2-bis(methoxy)propionic acid and of isopropylidene-2,2-bis(methoxy)propionic anhydride, which are used as general building blocks for divergent growth, was conducted according to literature procedures [25].

The procedure developed by Hult is good, but still the purification is time-consuming and application of DCC is not that favorable in this case. Thymine is capable of forming rather stable H-bonds. So urea formed during synthesis could be complexed that in turn will make purification harder or even impossible. In the search for an easier method CDI/DMAP activating method was considered to be a good alternative. (**Figure 45**)

acetonide-[G#1]-O-Thym

(OH)₂-[G#1]-O-Thym

(OH)₈-[G#3]-O-Thym
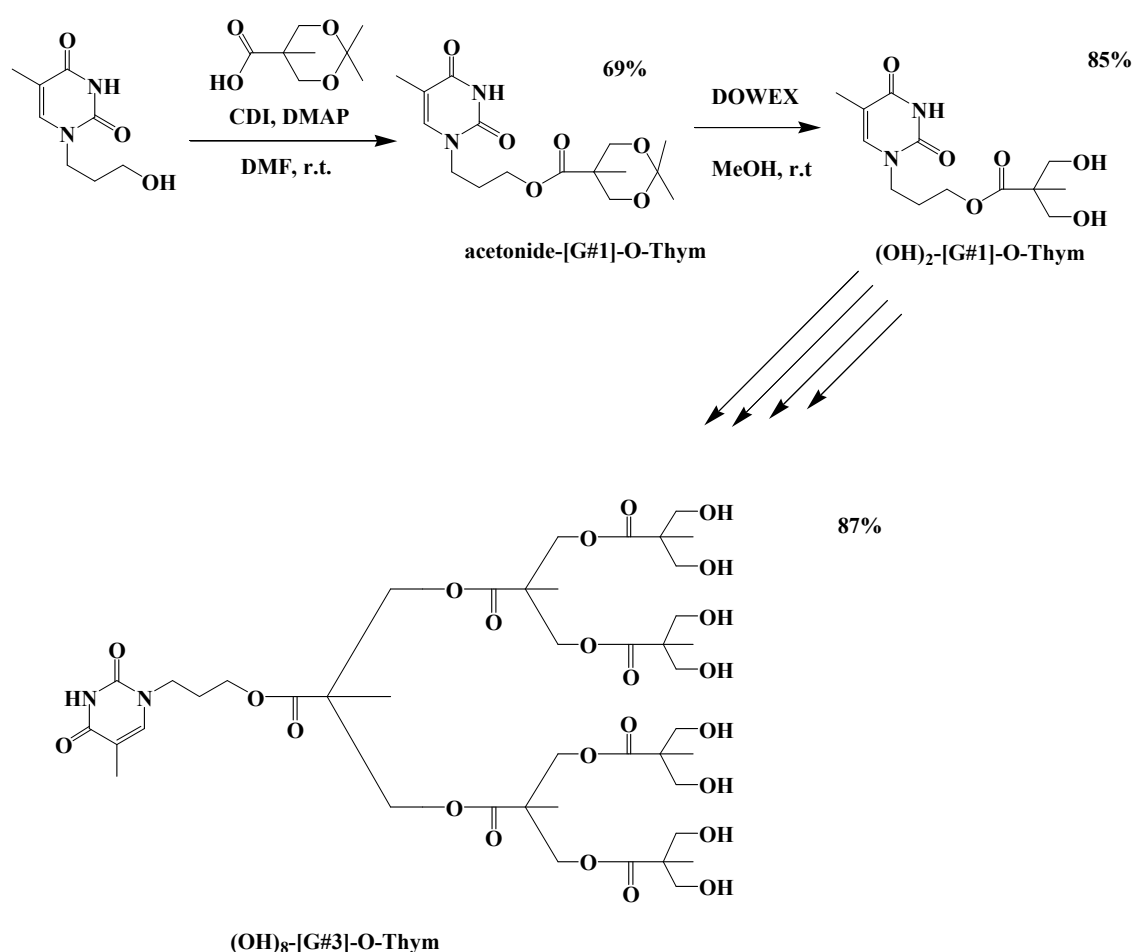
Figure 45 Synthesis of dendrimers via CDI/DMAP activation

Standard methodology was explored. At first the bis-MPA protected acid was activated with CDI in the presence of DMAP, then Thym-C3-OH or corresponding dendrimer was added. The final reaction mixture was stirred overnight, then working up procedures

followed. At first purification was made using column chromatography and the yield was so low, that it was not possible to estimate it. Later on it was made by simple washing with brine, saturated Na_2CO_3 and 2% $KHSO_4$ solutions. It could be accomplished within minutes.

The esterification was going rather well with moderate yields. They were in the 60-70% range. For comparison, the first generation obtained via coupling of acetonide protected bis-MPA with DCC/DPTS was going with 39% yield.

This reaction has one drawback, it is time-consuming. The higher generation the longer should be the reaction time, for the third generation it reached 5 days. This result could not be compared to the literature because all authors have not presented details, just mentioned that esterification was monitored by ^{13}C NMR [26].

For the better analysis of hyperbranched polyesters the partly converted dendrimers acetonide-[G#1/2]-O-Thym were prepared. In those only 75% of hydroxyl end-groups in $(HO)_2$-[G#1]-O-Thym were converted.

Thymine-core modified polyester dendrimers up to third generation have been prepared. In principle it is possible to go to higher generation dendrimers but it will be time-consuming. The modification of 60% of hydroxyl groups in the acetonide-[G#3]-O-Thym took 7d; to complete it one more week or even more would needed. The purification can still be performed by simple washing with different solutions.

All structures were confirmed using NMR and ESI-MS spectroscopy and some spectra are presented in **Chapter 4.6.1.1**, ESI-MS and SEC analysis (**Chapter 4.6.1.2** and **4.6.1.3**)

4.5.3 General conclusions about synthesis of dendrimers

Synthesis of the dendritic structures was accomplished and conclusions are as follows:

1. Synthesis of dendrimers with Thym-C5-NH_2, though possible, is inefficient

2. Synthesis of dendrimers with Thym-C3-OH has been successfully achieved up to third generation, as well as the partly substituted structures.

3. A new effective methodology for the synthesis of bis-MPA based dendrimers has been developed.

4.6 Characterization of branched structures

Both perfectly and randomly branched materials were characterized by means of NMR, SEC, UV-vis and IR-spectrosocopy, ESI-MS. The complex structure makes some experiments very time-consuming, so sometimes only selected samples were analyzed. Due to the specificity of the branched structures some data presented could not be referred as absolute results, and are used only for comparison.

4.6.1 Analysis of dendrimers

4.6.1.1 ^{1}H and ^{13}C NMR analysis

Structure of all dendrimers have been confirmed by ^{1}H and ^{13}C NMR spectroscopy (**Figure 46** and **Figure 47**), and the spectra were than compared with each other and the ones for the starting Thym-C3-OH. This comparison is done in order to detect any change in the placement of signals which can be useful in the future analysis of the degree of incorporation of thymine in hyperbranched polyesters.

The analysis of ^{1}H NMR comparison shows shifting of a signal for the methylene at C-7. In the starting Thym-C3-OH it comes around 1.7 ppm and overlaps with the signal coming from the thymine methyl group, while for all dendrimers it is observed as quintet at 1.9 ppm. (**Figure 47**) It is a clear indication of the incorporation of the thymine derivative in the dendritic structure. Signals at H-8 and H-6 were also moving, but in the spectra of hb polyesters those overlap with signals coming from the polyester backbone and so could not be used as references for the comparison.
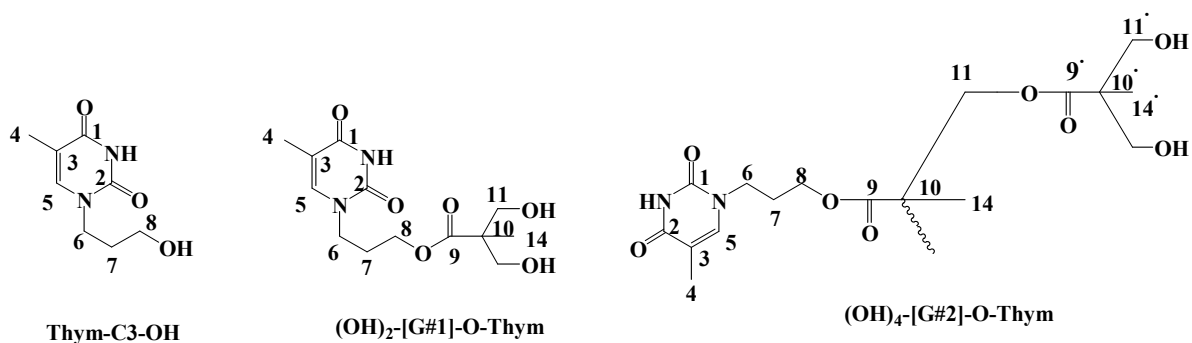
Figure 46 Dendritic structures and Thym-C3-OH core
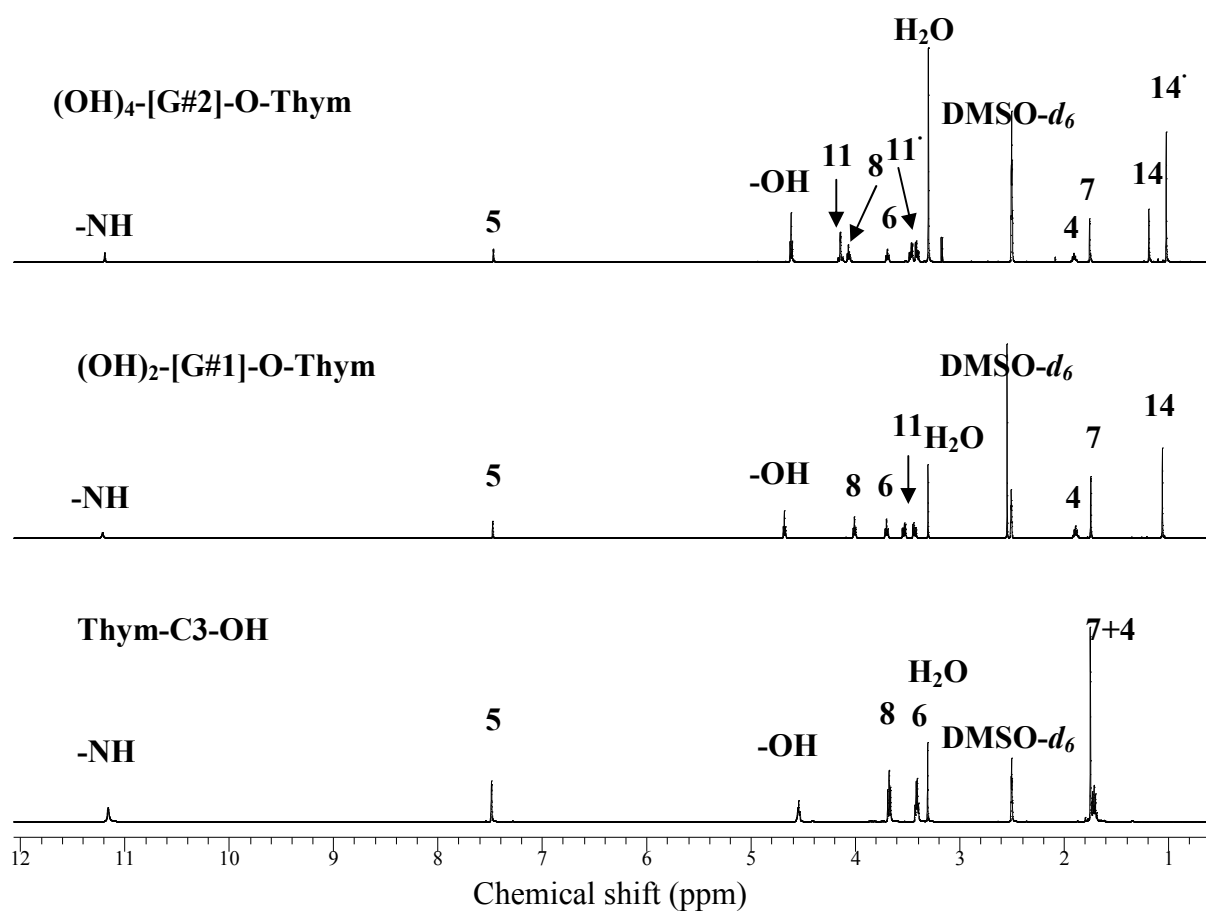
Figure 47 Comparison of ^{1}H NMR spectra of the thymine core and dendrimers (assignment see **Figure 46**)

Analysis of ^{13}C NMR spectra showed that signals coming from carbons number 6, 7 and 8 are shifted. (**Figure 48**) This is the second way to determine the degree of incorporation of the thymine core into the hb backbone. This method could be more accurate than ^{1}H NMR, due to less chance for overlapping signals.
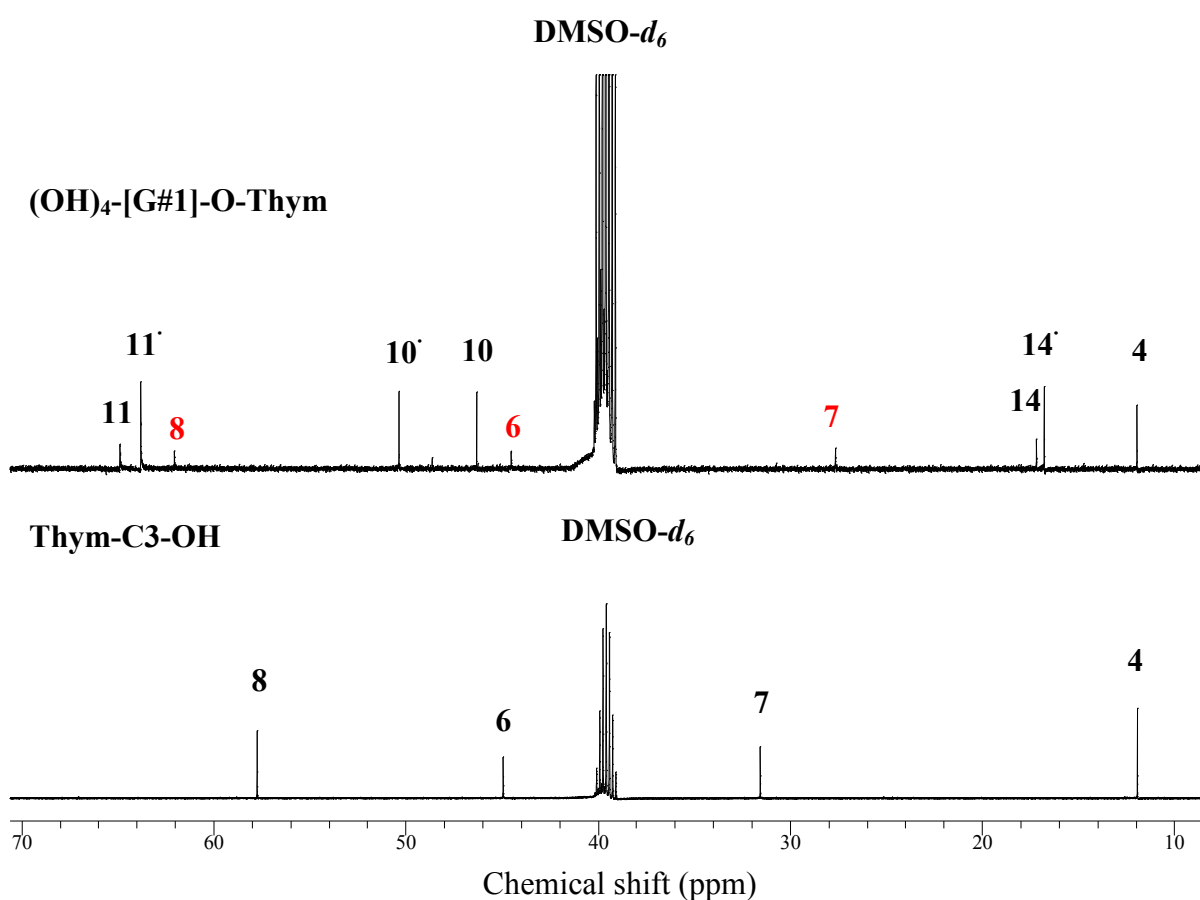
Figure 48 ^{13}C NMR spectra of Thym-C3-OH and dendrimer of the second generation (assignment see **Figure 46**)

For the better understanding of the spectra of hb polyesters another comparison was made between perfectly branched structures and those having partly converted OH-groups. Here a situation with linear and terminal groups was simulated, so close attention was paid to signals coming from the methylene groups in bis-MPA building blocks and OH-groups.

The ^{1}H NMR spectra of the (HO)$_2$-[G#1]-O-Thym, acetonide-[G#1/2]-O-Thym and acetonide-[G#2]-O-Thym (**Figure 49** and **Figure 50**) as well as between (HO)$_8$-[G#3]-O-Thym and acetonide-[G#3/4]-O-Thym were investigated. It is clear that spectra of imperfect structures have a more complicated nature in the region 3.9-4.3 ppm where signals from methylene protons should appear. Very interesting in this case was analysis of the disposition of free hydroxyl groups, because it could be useful for the rough estimation of DB. So, the signal at 4.67 ppm for (HO)$_2$-[G#1]-O-Thym shifted in the case of acetonide-[G#1/2]-O-Thym to the 4.98 ppm. Nearly the same picture was observed for (HO)$_8$-[G#3]-O-Thym and acetonide-[G#3/4]-O-Thym, shift from 4.59 ppm to 4.93 ppm, accordingly.
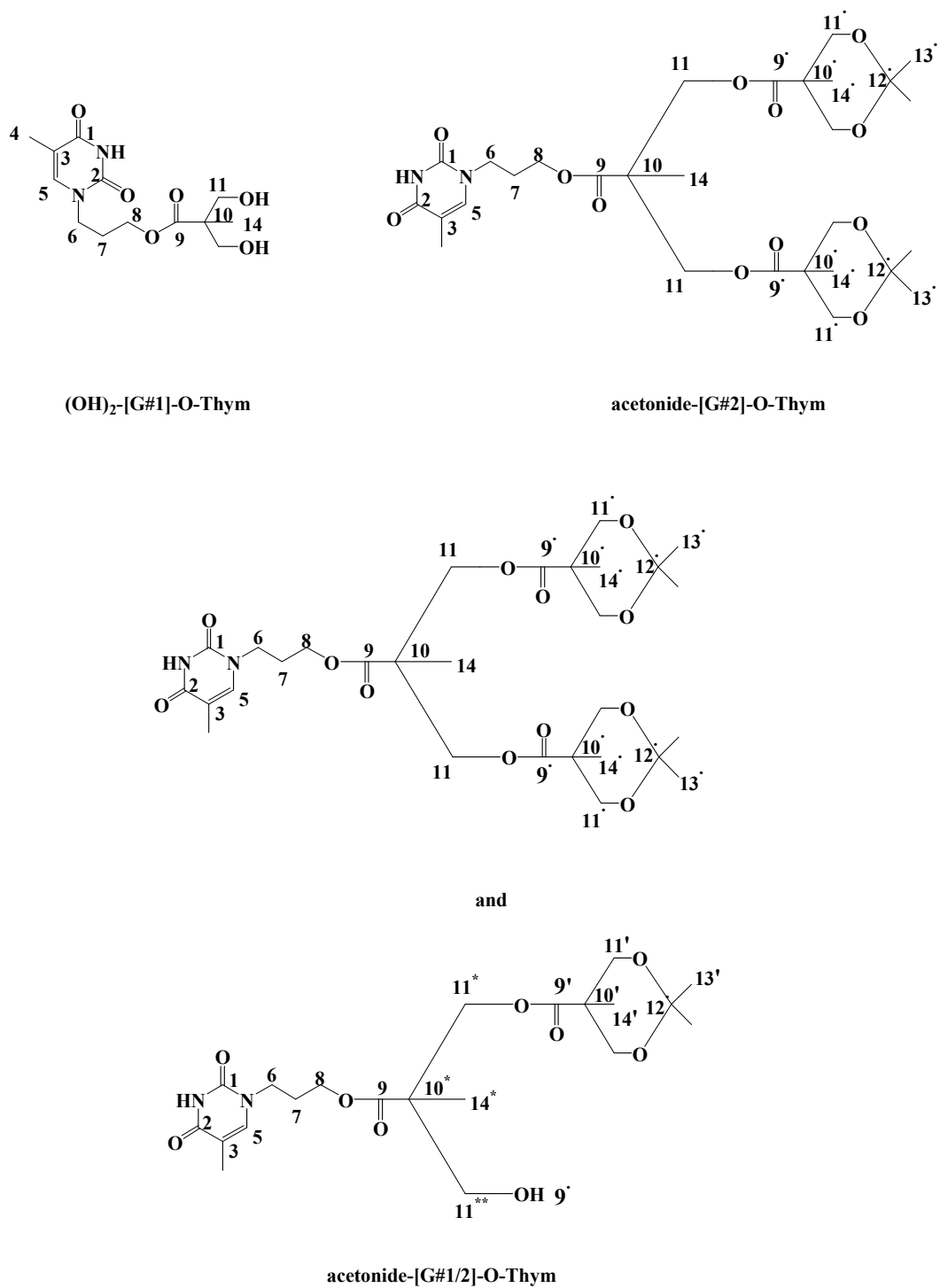
Figure 49 Dendritic structures as analyzed in **Figure 50**
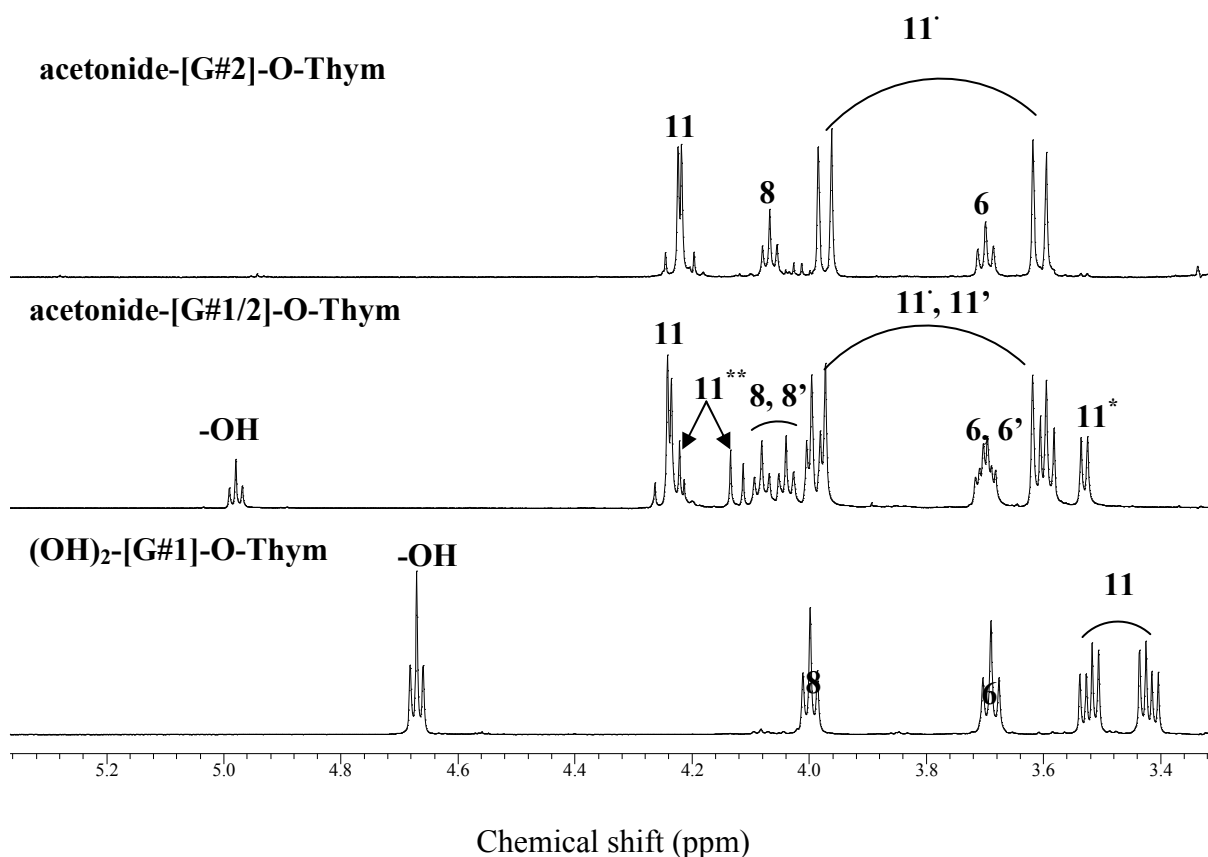
Figure 50 Parts of ^{1}H NMR spectra of different dendritic structures

The differences in the placement of signals in different structures will be helpful during analysis of the degree of core incorporation of thymine derivative into hyperbranched polyesters, of course, the data obtained by this method contain a high error, but they are still giving tendencies.

4.6.1.2 ESI-MS analysis

NMR spectroscopy has surely confirmed the structure of dendrimers, but still it would be interesting to obtain information about structure from other sources. Mass spectrometry has become a powerful analytical technique for the determination of the molecular weights [90, 232]. In the best cases it shows the signal coming from the molecules itself and then from different fragments.

Thymine-core dendrimers of first and second generation with and without acetonide protection were submitted to the analysis and successfully analysed, while it was not possible to obtain any data for the third generation dendrimer. This method has not worked for the

third generation. The results are summarized in **Table 5**, where only the most characteristic signals are listed.

Table 5 Results of the ESI-MS analysis of dendrimers

Sample	Theoretical molecular weight g/mol	Signals in ESI-MS spectra	Explanation
(HO)$_2$-[G#1]-O-Thym	300.13	301.13	$(M+H)^+$
		318.16	$(M+NH_4)^+$
acetonide-[G#1]-O-Thym	340.37	341.1	$(M+H)^+$
		358.41	$(M+NH_4)^+$
(HO)$_4$-[G#2]-O-Thym	532.54	458.17	$(M-G+OAc)^{+}$ [1]
acetonide-[G#2]-O-Thym	612.29	630.3	$(M+NH_4)^+$

[1] G = bis-MPA building block ($C_5O_4H_5$)

From the table it is clear that desired substances were obtained. Overall analysis of the ESI-MS spectra showed that destruction appeared for all samples, but the unprotected dendrimers were more sensible. The bis-MPA building blocks separated during decomposition can couple with dendritic structures leading to the "higher generation", the percentage of these signals rises with the time. In the acetonide protected samples deprotection happens or sometimes it is substituted with acetyl fragment coming from the starting solution, NH_4OAc in MeOH.

In general analysis was successful but the number of side by-products was increasing considerably with generation as well as the time spent on the ionization. So it is highly probable that for the dendrimer of third generation molecules are decomposed before the ionization. So the amount of signals and side reactions would be too high and no certain confirmation of the structure would be obtained.

To avoid the fast decomposition of the sample MALDI-TOF spectrometry can be used. It was tried for both hyperbranched and perfectly branched dendritic structures, but without any good outcome.

4.6.1.3   SEC analysis

Molecular weight could be analysed a using SEC method. In principle for the dendritic structures this method is not that much help because of their compact structure. For the bis-MPA dendrimers another problem could arise because of the high content of OH-groups, that

is why all data obtained from this method could be considered relative and are used only for the comparison.

Dendrimers of first and second generation were under consideration. All experiments were performed to use the results (**Table 6**) in future analysis of chromatograms of hyperbranched structures.

Table 6 SEC results for dendrimers

Sample	Theoretical molecular weight g/mol	SEC with PS standards		
		M_w	M_n	PDI
(HO)$_2$-[G#1]-O-Thym	300.13	430	400	1,08
(HO)$_4$-[G#2]-O-Thym	532.54	847	819	1.03
acetonide-[G#1]-O-Thym	340.37	390	370	1,05
acetonide-[G#2]-O-Thym	612.29	400	360	1,14

It is clearly seen that OH-groups play a great role during analysis, the numbers for the unprotected dendrimers of the first and second generation exceed the theoretical molecular weight. But what is more important the experimental data for the G1 dendrimer with protected end-groups is also somewhat higher then the detected one for the G2 dendrimer. This is an acknowledged effect for such smaller dendritic structure.

It is well-known that from some generation dendrimers have a more compact structure than any linear macromolecule, while for dendrimers with generations lower than that the hydrodynamic volume is exceeding the one of linear polymer standards. So data from SEC investigations could be not that accurate. The influence of the shape of the dendrimer is clearly seen for the second generation dendrimer with protected groups, here nearly no change in molecular mass was observed comparing it to the corresponding acetonide-[G#1]-O-Thym. That is why polystyrene (PS) standards can be considered not that good for calibration in the case of branched structures based on bis-MPA. For the third generation it was not possible to complete analysis, because the signal was out of the calibration range.

From all said above it is clear that SEC with PS standards overestimates molecular weight for the small branched samples with free hydroxyl groups and underestimates it for the acetonide protected ones, as could be expected on theoretical basis. The first phenomenon is explained by either the interactions between branched sample and chromatographic column or solvent, the second one by the compact packing of molecules.

4.6.2 Analysis of hyperbranched structures

In general hyperbranched structures are characterized by their molecular weight, polydispersity index, degree of branching, number of side reactions and T_g. In this work the degree of incorporation of starting thymine core moiety was added. All data could be obtained from TGA, NMR, SEC, UV-vis and IR-spectroscopy analysis. The complex structure of these materials makes quantitative ^{13}C NMR experiments time-consuming (it took up to 72h), so this method was applied only in some cases

4.6.2.1 Molecular weight analysis

Molecular weight is one of the most important characteristics of polymers. It can be determined using viscosimetry, size exclusion chromatography (SEC), light scattering or mass spectrometry. M_n can be also calculated from NMR data. These methods are working quite well as long as only linear polymers were tested. For all branched structures investigation with relative methods are much more complicated, because they possess smaller hydrodynamic volume and lower viscosity compared to the linear analogues of the same molecular mass. Commercially available hb bis-MPA polyesters were extensively studied by the Žagar group [203-205].

Hb polyesters, prepared in the course of this work, were submitted to SEC. The smaller hydrodynamic volume of branched polyesters plays crucial role, when linear calibration standards are used, meaning underestimation of the molecular weight and therefore these data can only be used for comparison between samples of the same nature. Unfortunately, up to now there is no standard dendrimer or just branched structure used as calibration standards for such system, though scientists are working in this direction. Changing the detector can make some improvements. For example, light scattering detector gives more reliable M_w results, but it is not sensitive towards molecules with low molecular weight. If the ratio of low molecular weight products is pretty high it will obviously overestimated the molecular mass. So the SEC results should be interpreted with care in order to avoid misunderstanding.

In **Table 7** some SEC results are presented. Some samples were not soluble in THF, so the water/DMAc system with LiCl was also applied. Though the LiCl was aggregation has still occurred leading to high M_w and PDI numbers observed during experiments. So these measurments were performed in order to be able to compare all samples not taking in account their solubility in THF.

For THF soluble samples more precise investigations were made by the SEC in THF with either with PS standards or LS-detector.

Table 7 Results of SEC analysis for hyperbranched polyesters with and without thymine core

Sample	Theor. M_n[1]	SEC in DMAc/2% water/ 3g/L LiCl PVP standard			SEC with LS detector			SEC with PS standards		
		M_n	M_w	PDI	M_n	M_w	PDI	M_n	M_w	PDI
p.7.1[2]		1600	2400	1.5	too low M_w			600	900	1.5
p.7.2[2]		1700	2400	1.4	insoluble in THF			insoluble in THF		
p.9.1[2]	1910.78	-	-	-	1500	2100	1.46	600	800	1.33
p.11.1[2]		-	-	-	4500	6200	1.36	1700	2300	1.35
p.12.1[2]		3800	6000	1.6	insoluble in THF			insoluble in THF		
p.13.1[2]		insoluble in mixture			too low M_w			300	500	1.67
p.13.2[2]		9700	49000	5.1	20100	33800	1.68	1900	4600	2.42
p.14.1[2]		insoluble in mixture			9900	24400	2.47	2200	3900	1.77
p.14.2[2]		2000	3000	1.5	insoluble in THF			insoluble in THF		
p.16.2[2]		5100	23000	4.5	7000	13100	1.86	2300	5600	2.43
p.17.1[2]		6500	30000	4.6	13600	23200	1.71	2200	5200	2.36
p.17.2[2]		insoluble in mixture			9400	19000	2.02	1900	3800	2.00
p.18.1[2]		insoluble in mixture			11800	19100	1.63	1500	2900	1.93
p.18.2[2]		insoluble in mixture			11800	19100	1.63	1600	2500	1.56
p.20.1[2]		insoluble in mixture			13100	17100	1.3	2000	4200	2.10
p.21.1[3]	3767.54	-	-	-	8400	11600	1.37	1500	2600	1.73
p.60.1	22080	4500	9700	2.2	-	-	-	-	-	-
p.65.1	-	3100	4900	1.6	insoluble in THF			insoluble in THF		
p.68.1	-	2800	4700	1.7	insoluble in THF			insoluble in THF		
p.69.1	-	3000	5100	1.7	-[5]			1100	2100	1ю91
p.70.1[2]	1910.78	7100	54000	7.6	50270	144400	2.87	3400	12200	3.59
p.71.1[2]	3767.54				10030	20370	2.03			
p.72.1[2]	7485.42	6000	30000	4.8	14650	32850	2.24	2800	7000	2.50

[1] molecular weight of the perfect dendrimer of the corresponding generation

[2] hb polymers synthesized with Thym-C3-OH core

[3] hb polymers synthesized with (HO)$_2$-[G#1]-O-Thym

[4] hb polymer synthesized with TMP core

[5] molecular weight was too small to be detected

PDI under 2.00 is a very good result, because reactions were made in small scale. They correlates with the ones reported by Hult [51] for the step by step approach, though here it was a one-pot methodology. Samples p.70.1, p.71.1 and p.72.1 were synthesized step-by-step and the higher PDI observed for them is explained by higher amount of side reactions. This

again confirmed that polycondensation could not be scaled up without any change in reaction conditions.

No results are presented for the materials with theoretical generation lower than four, due to low molecular weight or high percentage of crosslinking via cyclization, that make them completely insoluble in common organic solvents.

Table 8 SEC results for the hb polyesters with protected hydroxyl groups

Sample	Starting hb polyester	SEC with LS detector			SEC with PS standards		
		M_n	M_w	PDI	M_n	M_w	PDI
p.41.1	p.14.1	10100	24500	2.43	2300	5900	2.56
p.42.1	p.14.2	9900	22800	2.30	2900	11300	3.90
p.42.2	p.16.1	9000	12100	1.35	1900	4300	2.26
p.43.1	p.16.2	7400	13300	1.81	1600	2900	1.81
p.44.1	p.20.1	35300	241500	6.84	2500	128800	51.25
p.45.1	p.21.1	28800	81300	2.83	2800	25400	9.07
p.46.1	p.17.1	3500	5000	1.46	1300	3700	2.85
p.47.1	p.17.2	12500	20700	1.66	2800	9600	3.43
p.48.1	p.18.1	12100	27300	2.26	2200	24400	11.09
p.49.1	p.18.2	12100	23900	1.98	3000	11600	3.87

Table 8 presents the results of SEC measurements performed for hb polyesters with acetonide protected terminal hydroxyl groups. In the majority cases the PDI is in the same region as for the starting material. Also, molecular weights are lower than expected taking into account the starting ones. The situation with p.44.1 can be explained by the formation of aggregates or some kind of interaction with the system.

4.6.2.2 ^{1}H and ^{13}C NMR analysis

NMR is an established method to determine the degree of side reactions and incorporation of core moieties into the branched structures.
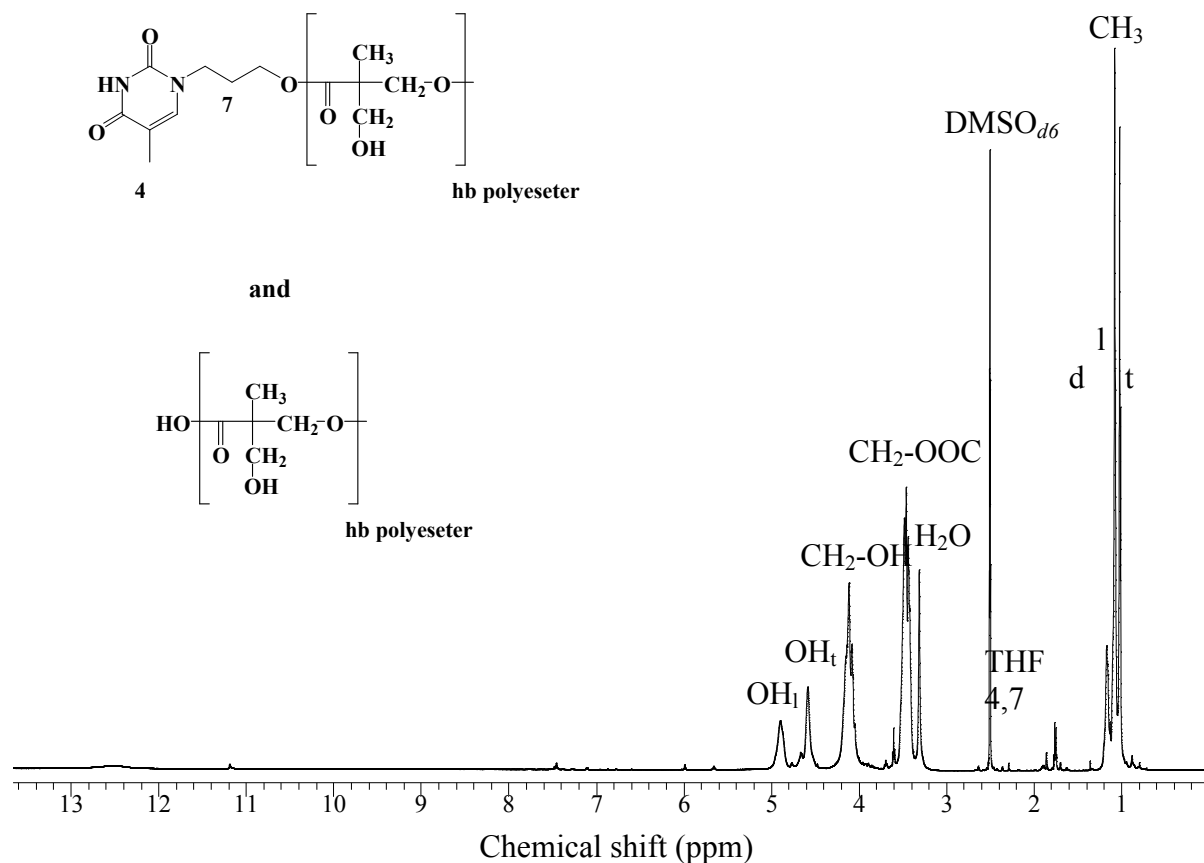
Figure 51 ^{1}H NMR spectrum of p.21.1

^{1}H NMR spectra of hyperbranched polymers have often complex signal patterns which are difficult to analyse. The ones obtained in the course of this work were no exception in the region, where all signals coming from the aliphatic backbone appear. (**Figure 51**) On the contrary the region, where signals from aromatic structures come, was nearly empty, which allowed possible the determination of the residue of the activating agent pTSA. **Figure 52** represents part of the ^{1}H NMR spectrum of p.21.1 showing the presence of pTSA which is either coupled to the backbone or stays free in the polymer backbone. The coupled activating agent could not be washed out during standard working up procedure like precipitation. Signals of pTSA were observed in all spectra of polyesters. This will affect future analysis.
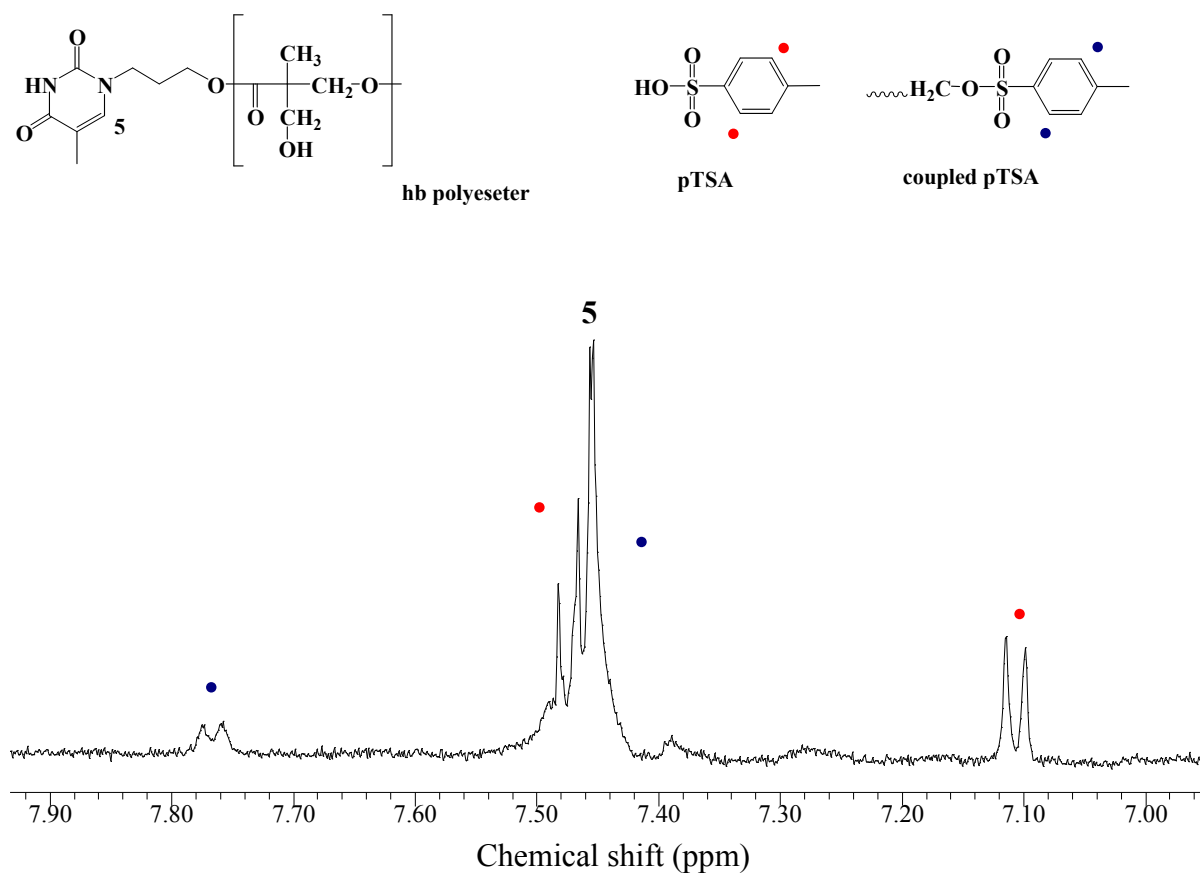
Figure 52 Part of the ^{1}H NMR spectrum of p.21.1 showing presence of pTSA

The degree of branching (DB) and, sometimes, M_n are calculated on the basis of results of ^{13}C spectra [21], due to better resolution and separation of the signal patterns [22]. Usually to enable this analysis model compounds are synthesized, where corresponding terminal, linear and dendritic units are present. The model compound could help to determine the signals coming form groups resulting from side reactions, as well as the defect units in the hb structure.
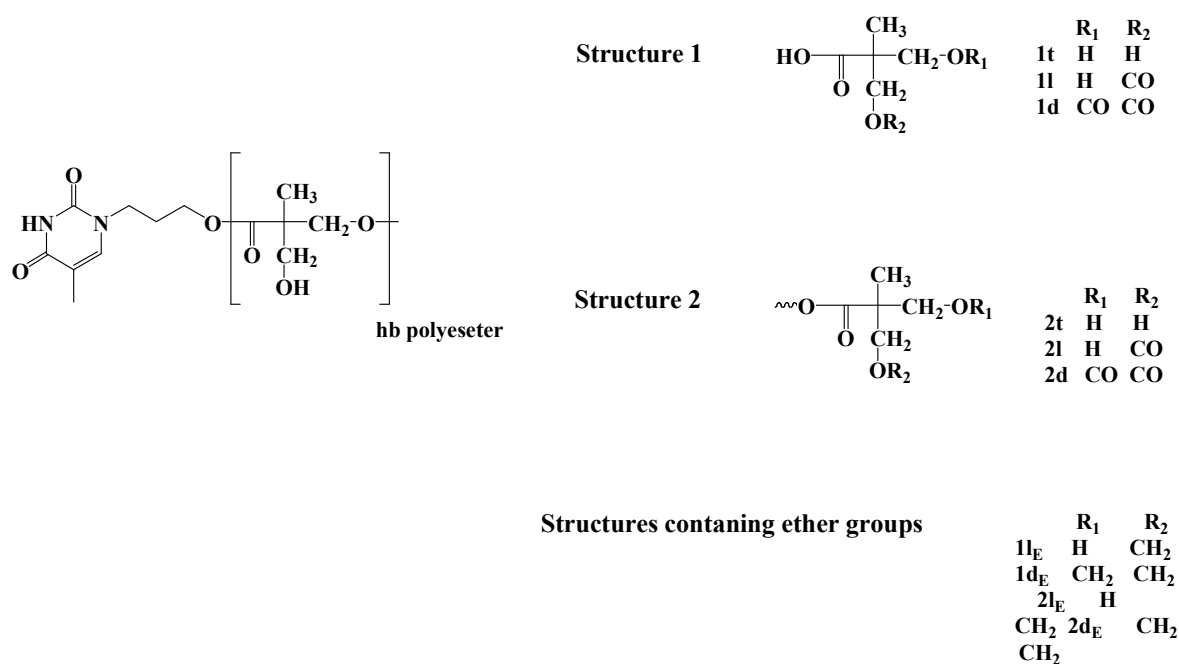
Figure 53 Possible variations in bis-MPA building blocks

Bis-MPA based hyperbranched polymers were already studied by NMR in other groups, so there was no urgent need to prepare low molecular weight model compounds. Their spectra were only compared to the ones for perfectly branched dendrimers in order to calculate the degree of incorporation of Thym-C3-OH. References for the signal of all types of bis-MPA building blocks were taken from literature [91]. The references for quaternary carbons in ^{13}C NMR spectroscopy are dendritic repeating units (D) at 41.8 ppm, terminal (T) at 50.2 ppm and linear (L) at 48.4 ppm. DB was calculated using **Equation 1** (p. 19).

In the synthesized hyperbranched structures there are ten possible variations of the quaternary carbon in bis-MPA building block, which are presented in **Figure 53** as substructures in hb polyester. As a core moiety was used, final material is a mixture of molecules containing the desired core (included into **Structure 2** with 2l, 2d and 2t) and the ones, which have not reacted with it (**Structure 1** with 1l, 1d, 1t). In both cases bis-MPA building blocks could be incorporated in three major ways as D, L or T units, this fact is reflected by the additional letter in the structure name. Index E means ether bond in units, where side reactions took place. Not all of the quaternary carbons can be seen in every spectrum. For example, the bis-MPA monomer named as 1t should not be present at all. The amount of side reactions could be too low to be detected, so no signal for $1l_E$, $1d_E$, $2l_E$ and $1d_E$ could be identified e.g. in sample p.60.1 (**Table 9**, p. 91).
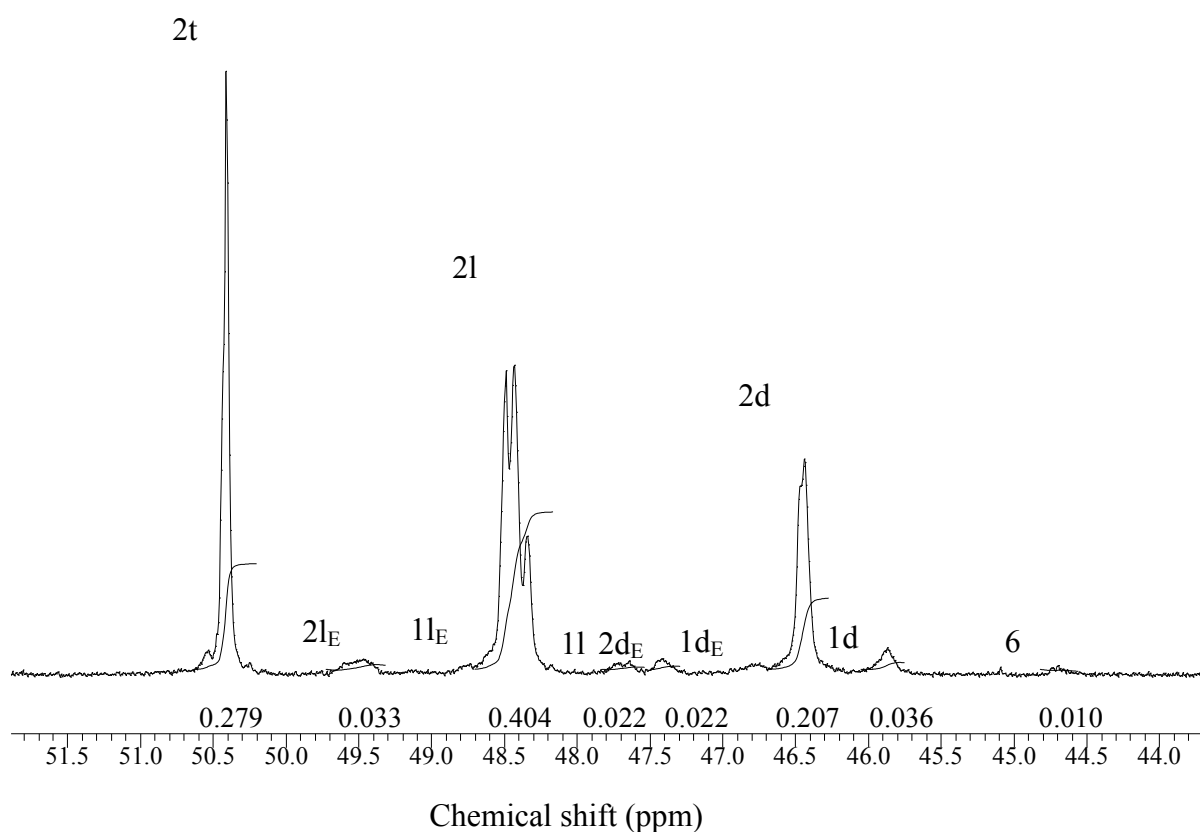
Figure 54 a) Signals in the ^{13}C NMR spectrum of p.20.1 used to calculate DB

Figure 54 a) and **b)** (p. 89-90) present parts of ^{13}C NMR spectrum of p.20.1 (**Table 4**, p.64) which was used to identify DB and the amount of ether groups as well as the presence of incorporated thymine core. The signals are rather broad because of the polymer structure. Here it is clearly seen that thymine was incorporated into the structure, signal 6 coming from C-6, but incorporation was low, because the integral number of the corresponding signal low. The overlapping of signals like for $2d/1d_E$, $2l/1l_E$ and $2t/1d$ makes estimation of the degree of side reactions harder; still it was possible to calculate DB and the amount of side reactions.
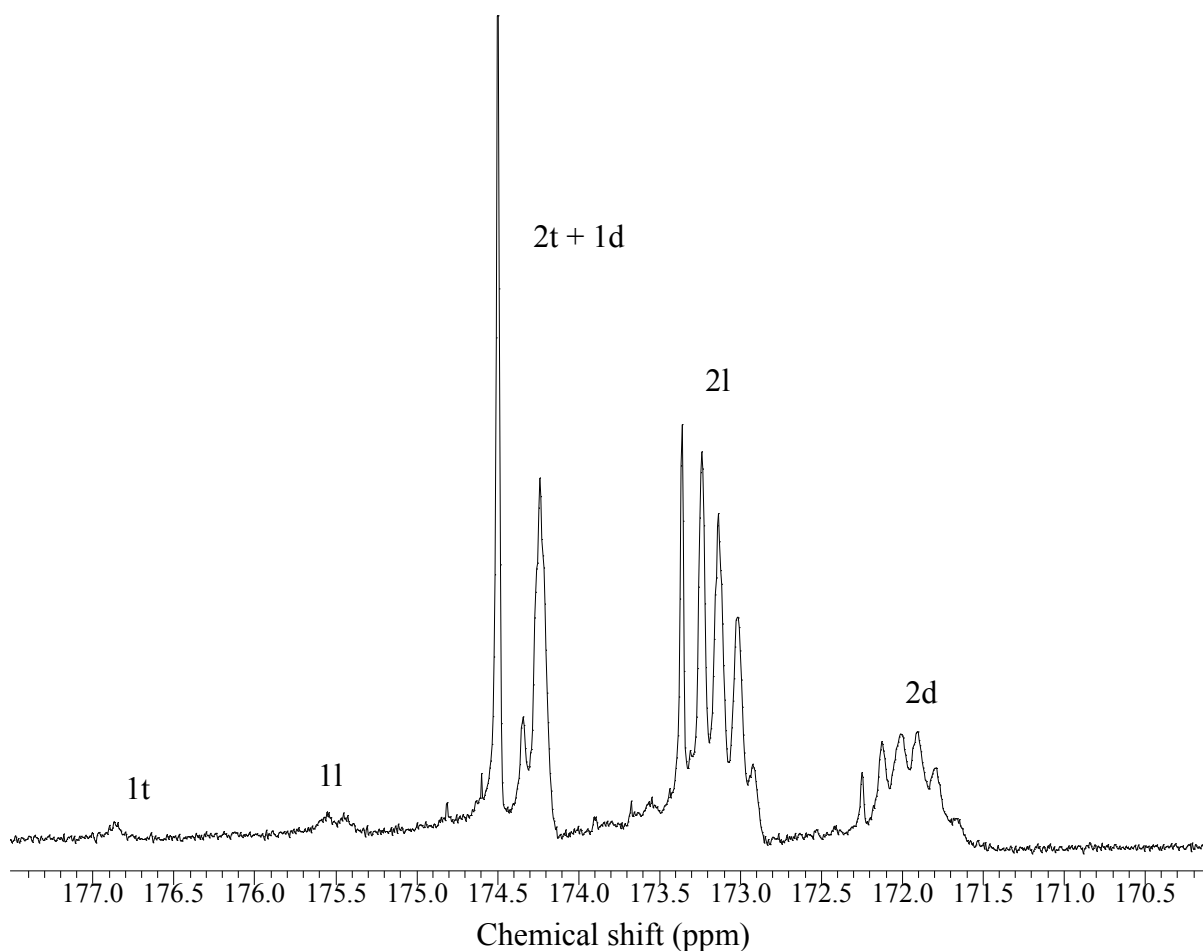
Figure 53 b) Signals in the ^{13}C NMR spectrum of p.20.1 used to calculate DB

Table 9 represents overall data obtained after detailed analysis of NMR spectra. All samples, except for p.21.1, contain starting thymine core, however, the opposite was observed for remaining unreacted monomer. As for the ether groups content it is rather stable for the so-called fifth generation hb polyester and close to 3%, and is similar for the six one like for sample p.20.1a. Still if variation of reaction conditions made it rise up to 9%. M_n values are lower than the ones obtained from SEC measurements (**Table 7**, p.85), the difference could be expected, as SEC is a relative method. But also NMR has limitations especially for higher molecular mass samples. Degree of branching is at about 50% for the samples with thymine core and is higher than for the sample p.60.1 with TMP core. These numbers are average for such hb polyesters. The degree of incorporation of Thym-C3-OH will be discussed in detail in the next chapter.

Table 9 NMR analysis data for hb polymers with different core moieties

Sample	Mn	DB %	Ratio –COOH: -COO-Core	Incorp. of core %	Ether groups %	Starting core	Starting bis-MPA
p.21.1[1]	940	[3]	10:1	9	8-9	-	+
p.20.6[1]	940	50,5	10.1	9	9	+	-
p.20.1[1]	1800	54.7	6:1	14.2	3	+	-
p.20.1a[1]	1500	54.5	7:1	12.5	3	+	-
p.18.1[1]	1350	55.0	10:1	9	3	+	-
p.16.2[1]	1350	50.5	10:1	9	3	+	-
p.11.1[1]	1500	54.5	6:1	14.2	4-5	-	-
p.60.1[2]	4550	47.4	1:3	75	0[4]	-	-

[1] polyester with Thym-C3-OH or $(HO)_2$-[G#1]-O-Thym core

[2] polyester with TMP core

[3] it was not possible to calculate DB because of overlapping of signals

[3] it was not possible to determine signals of these groups

Synthesis of hyperbranched polyesters was performed using N-(3-hydroxypropyl) thymine as a core, so it is important to know how much of the thymine groups are covalently incorporated into the hb polyester backbone. It is assumed that this molecule can be only incorporated as a starting core and cannot participate in any side reactions. For the future investigations it is also interesting to know the overall amount of thymine, bonded and free.

4.6.2.3   Analysis of the degree of incorporation of N-(3-hydroxypropyl) thymine in hyperbranched polyesters

Synthesis of hyperbranched polyester was performed using N-(3-hydroxypropyl) thymine as a core, so it is important to know how much of the thymine groups are covalently incorporated into the hb polyester backbone. It is assumed that this molecule can be only incorporated as a starting core and cannot participate in any side reactions. For the future investigations it is also interesting to know the overall amount of thymine, bonded and free.
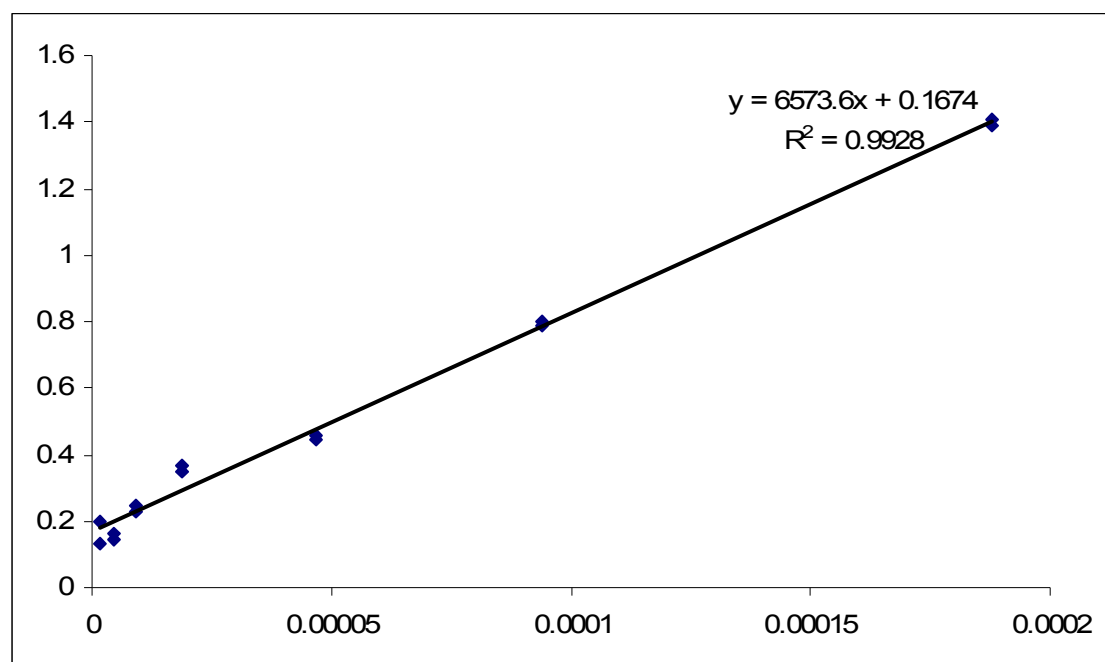
Figure 55 Calibration curve (UV absorption versus concentration) for Thym-C3-OH for the UV-vis analysis at 269 nm

This could be accomplished using two analytical technique: NMR and UV-vis spectroscopy. Both methods have their limitations so it is better to use both of them to be sure about the final results.

UV-vis analysis could be considered the easiest one with high sensitivity. To use it calibration was performed using starting Thym-C3-OH or acetonide-[G#1]-O-Thymine. In both cases the same calibration curve was observed. (**Figure 55**) Concentrations of thymine between 5^{-8} and 2^{-7} mol/ml could be determined using this plot, but it is not possible to differentiate between the coupled and free thymine cores.

NMR method allowed based on the determination of the ratio between free acid groups as focal unit and amount of coupled thymine, which can be distinguished due to the difference in shifts of free and covalently coupled thymine groups. This method is less sensible to the low concentrations and is time-consuming as ^{13}C NMR spectra are used, but is more precise and selective in the end.

Table 10 summarizes the results from both methods. It is clearly seen that UV-vis spectroscopy overestimates the degree of incorporation of thymine core (DI). Nearly for all samples it exceeds 100%. The reason for this is contamination of hb polymers with activating agent, pTSA, which also show UV absorption in the same range as thymine. The fact, that one part of catalyst stays in polymer either in free state or covalently coupled, has been discussed earlier. After partial correction, which could be done after performing additional

calibration for this catalyst, recalculation gives better values but still they are at least 3.5 times higher than the ones in NMR analysis. Only for the sample p.11.1 they come close together and difference is only 3.5%. So UV-vis method was pronounced to be useful only for the comparison of samples, though still very unreliable.

Table 10 Incorporation of N-(3-hydroxypropyl) thymine into the hb polyester

Sample	NMR results %		UV-vis spectroscopy results %	
	Coupled	Overall content	Before correction %	After correction %
p.21.1[1]	9	9	130	64.4
p.20.6[2]	9	14.1	-	-
p.20.1[2]	14.2	17.0	121	69.5
p.20.1a[2]	12.5	15.0	-	63.2
p.18.2[2]	-	-	164	57.6
p.18.1[2]	9	10.6	210	55.4
p.17.2[2]	-	-	243	46.9
p.16.2[2]	9	10.2	98	35.6
p.13.2[2]	-	-	377	27.6
p.11.2[2]	-	-	50	34.8
p.11.1[2]	14.2	14.2	30	17.7

[1] the polyester synthesised starting from $(HO)_2$-[G#1]-O-Thym

[2] the polyester synthesised starting from Thym-C3-OH

As was already mentioned the $(HO)_2$-[G#1]-O-Thym was tried for the synthesis of hb polyesters(sample p.21.1) in order to understand the influence of the number of OH-groups on the incorporation of thymine derivative. Theoretically the incorporation should increase with the functionality of the starting core. But in fact, in p.21.1 it is 9 %, what is the same as for the average sample prepared using Thym-C3-OH. The transesterification explains this phenomenon. This was proved by the investigation of the $(HO)_2$-[G#1]-O-Thym before and after thermal treatment at the same conditions like in polycondensation. After heating up to 180ºC for 2 h ^{1}H NMR analysis has confirmed the formation of the Thym-C3-OH and probable oligomers. The transesterification phenomenon for TMP core was not reported by any group, where the incorporation is rather high and reaches at least 75 %.

UV-spectra of modified samples show the same tendency, so they were not considered for NMR. Their incorporation should not be that much different from the one in corresponding precursors.

4.6.2.4   Thermal analysis (TGA)

Bis-MPA hyperbranched polymers are known for their rather low glass transition temperature (T_g) and thermal stability. It was shown that end groups and core molecules have great influence on the thermal properties [233], so it was interesting to perform TGA and DSC experiments. For comparison dendrimers and hyperbranched polyesters synthesized with or without thymine core moiety were chosen.

Some results of TGA analysis are presented in **Table 11** and the full report can be found in Appendix. After pre-heating weight loss can be caused by contamination of samples with traces of solvents. All phenomena observed in the temperature range from 95 to 200ºC could be referred to THF or water evaporation, melting of residual bis-MPA monomer in the range 170-185ºC, melting of residual pTSA (103-106ºC), breacking of H-bonds and starting of the polycondensation or cross-linking. The form of TGA curves (**Figure 56-59**) allows the assumption, that the weight loss happening at 70ºC is either evaporation of THF or breaking of the H-bond in bis-MPA backbone.

Eight of eleven samples seemed to undergo final decomposition from 330 to 420 ºC, that correlates with the literature data for the bis-MPA hb polymer with di-trimethylolpropane core [233]. In some cases it was followed by another process, which could be the decomposition of thymine core. Higher amount of ether groups could be the reason of increased stability of p.21.1 and p.26.1 (see Appendix). The last sample (p.26.1) is insoluble in THF, what can only be due to cross-linked structure. In general, hb polyesters with thymine core tend to be less stable than analogues dendrimers and hb polymers without core.

Table 11 Selected TGA results

sample	weight loss after isoth. 10 min at 40°C (%)	$T_{max.}$ dec.step (°C)	T_{final} dec.step (°C)	weight loss dec. step (%)
p.11.1	0.0	70	110	0.5
		300	400	97.5
p.20.1	0.2	73	90	0,5
		135	195	2.5
		308	400	94.6
p.21.1	0.2	73	110	0,6
		343	450	97.0
p.65.1	0.1	90	130	0.9
		s165	200	0.9
		325	435	96.8
		460	500	0.5
acetonide-[G#1]-O-Thym	0.1	80	95	0.1
		150	167	1.5
		298	395	92.3
		455	500	5.4
(OH)$_2$-[G#1]-O-Thym	0.2	70	90	0.1
		120	145	0.4
		314	415	86.2
		448	545	11.6

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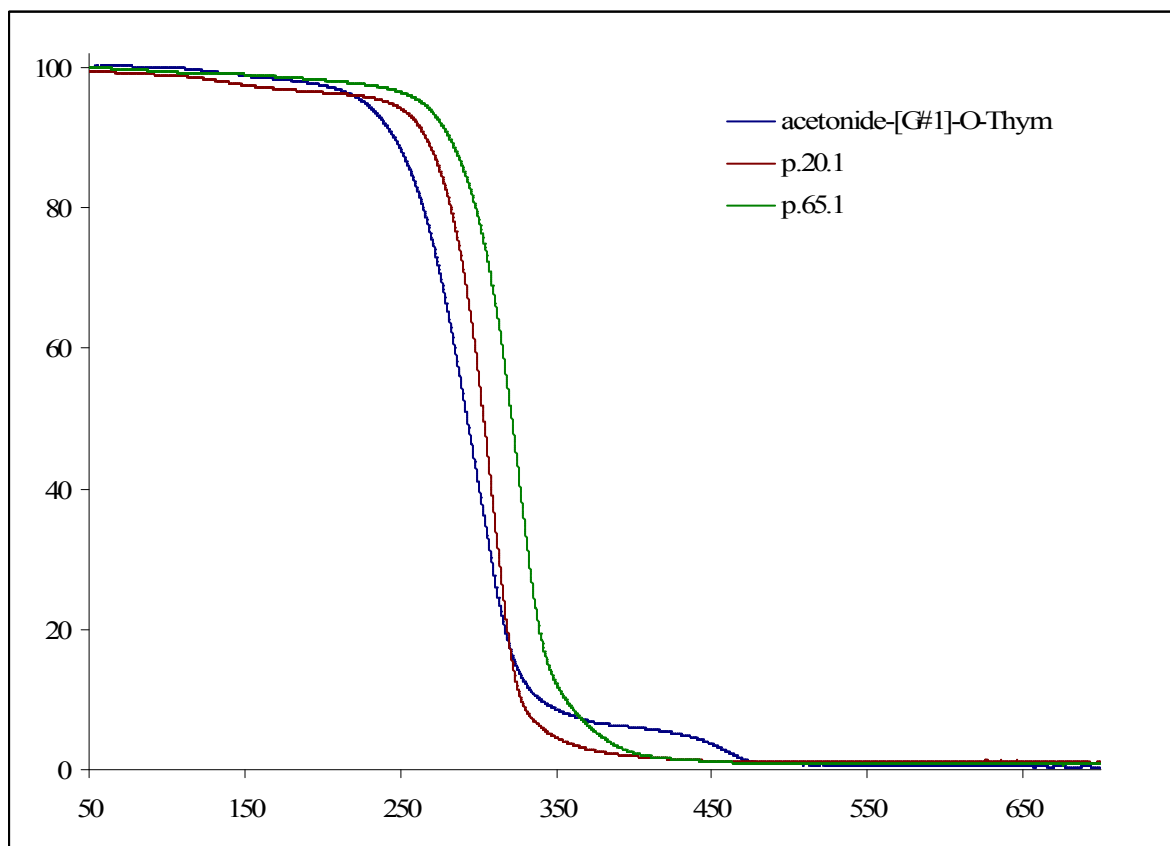
Figure 56 TGA curves for selected dendritic polyesters
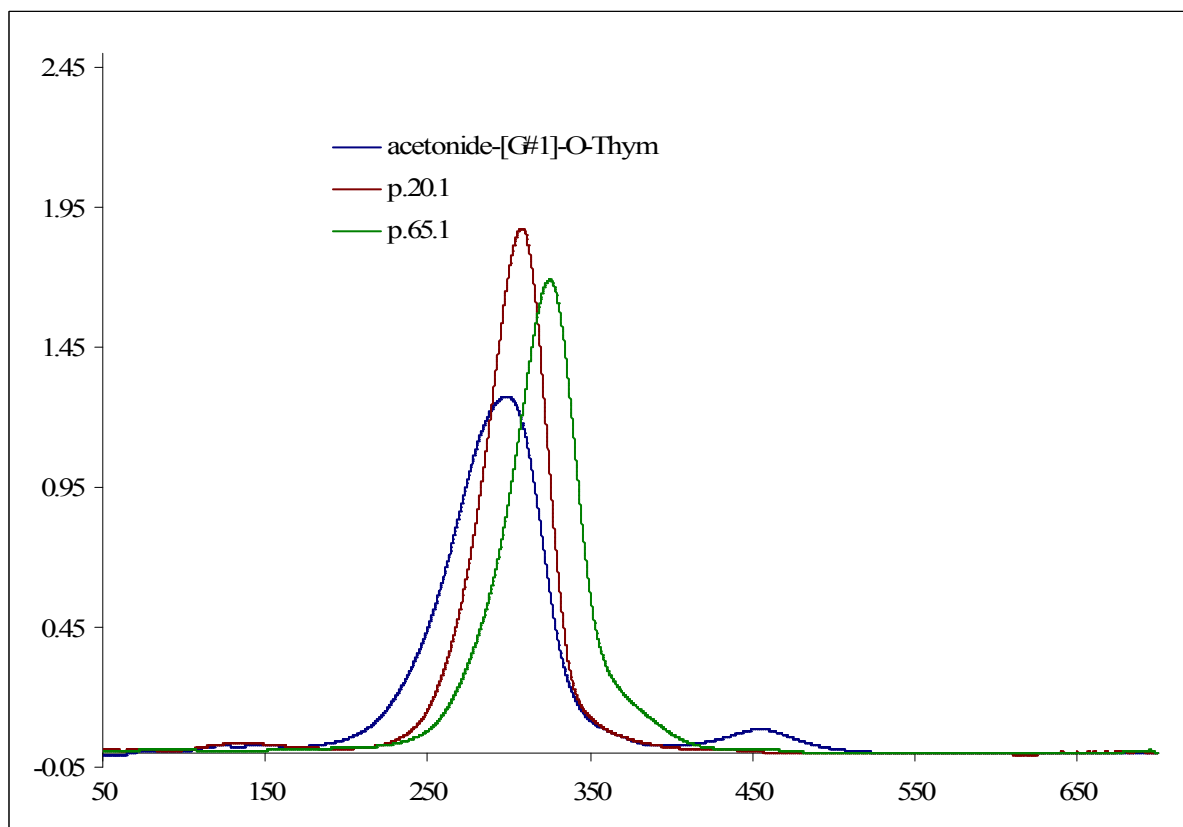
Figure 57 Derivative weight loss (%/°C) curves for selected dendritic polyesters
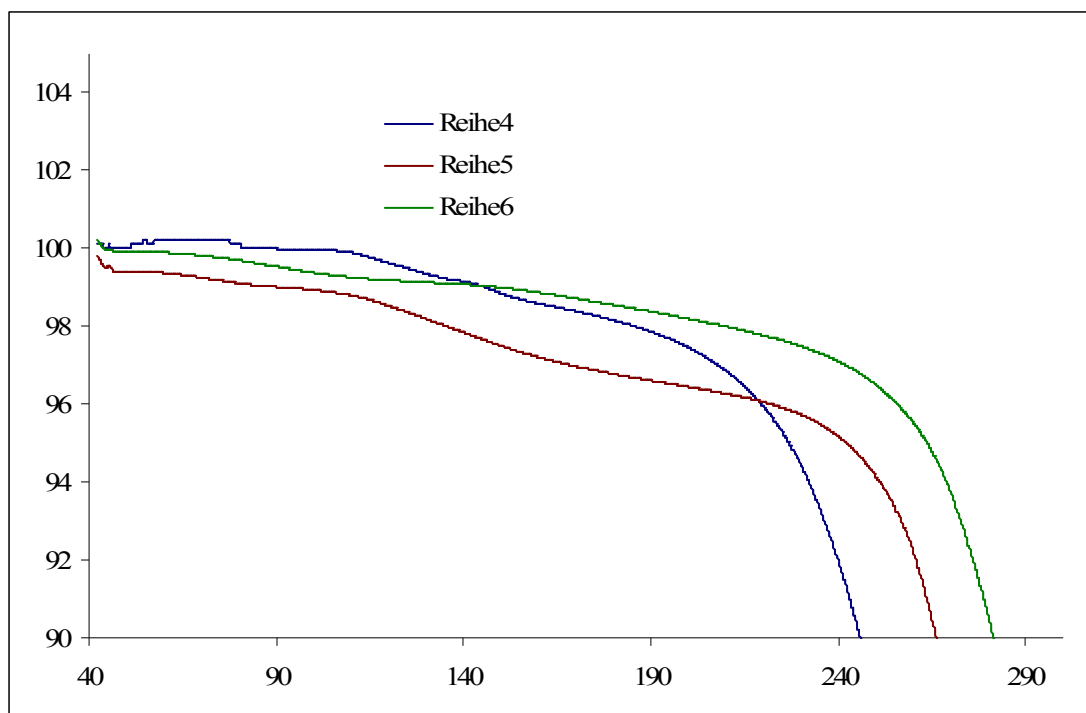
Figure 58 Detailed TGA curves for selected dendritic polyesters (weight loss up to 300ºC)
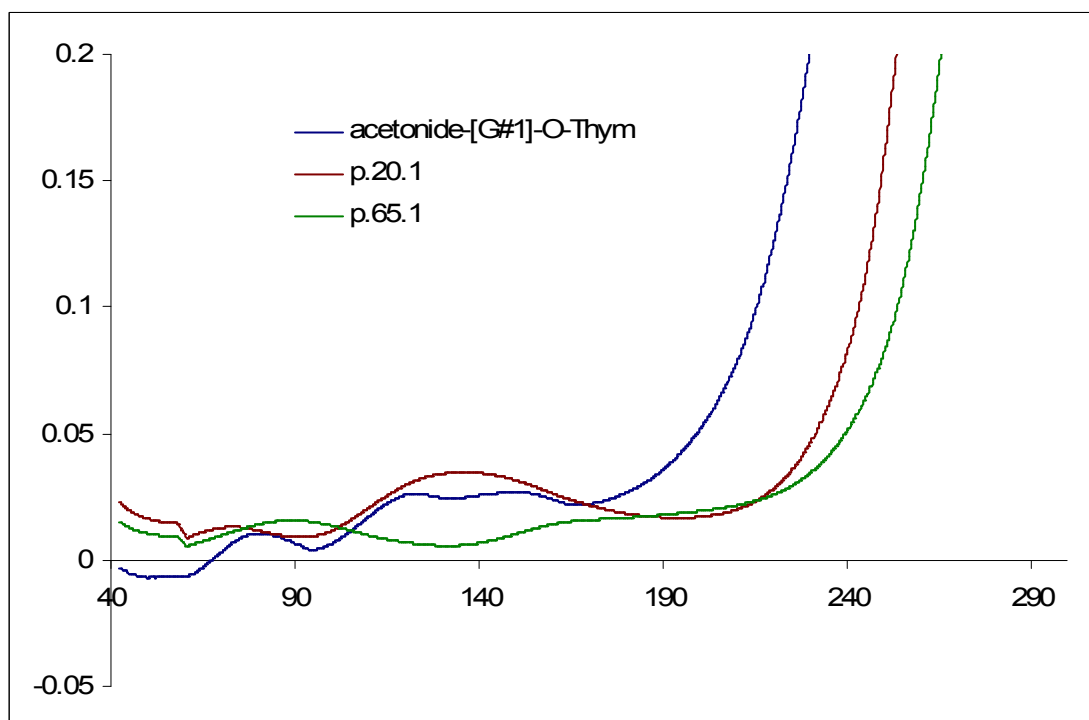
Figure 59 Detailed derivative weight loss (%/ºC) curves for selected dendritic polyesters (weight loss up to 300ºC)

DSC analysis revealed glass transition temperatures around 35.5ºC that are typical for structures of this type. Differences between the numbers obtained during first and second

heating and cooling could be referred to the changes happening in materials, which were discussed earlier. The observation of "T_m" can be explained by the same reasons, as it does not appear during the second cycle. Crystallisation of polyester p.65.1 synthesized without core is explained by its higher molecular weight and crosslinking. Some data are presented in **Table 12** and **Figures 60 – 62**. (for more see Appendix)

Table 12 Selected DSC results

sample	mode	Tg (°C)	Δc_p (J/gK)	Int. limit (°C)	ΔH (J/g)	T_m or $T_{c,m}$ (°C)
p.11.1	1st heating	18.2	0.54	35-125	16.4	87.6
	2nd heating	35.4	0.41	-	-	-
	cooling	33.1	0.36	-	-	-
p.20.1	1st heating	12.4	0.52	35-100	18.0	75.3
	2nd heating	34.4	0.41	-	-	-
	cooling	32.1	0.37	-	-	-
p.21.1	1st heating	24.7	0.44	40-140	35.0	74.8/104.7/ 123.6
	2nd heating	37.8	0.52	-	-	-
	cooling	35.9	0.40	-	-	-
p.65.1	1st heating	15.8	0.29	35-175	84.9	84.7
	2nd heating	29.2	0.51	40-180		
				p.40-85	-15.2	67.0
				p.85-140	22.8	117.5
	cooling	26	0.42	40-85	-5.5	63.4
acetonide-[G#1]-O-Thym	1st heating	-8.4	0.2	40-165	79.5	81/122/148 ,8
	2nd heating	5.7	0.48	-	-	-
	cooling	4,6	0,34	-	-	-
acetonide-[G#1]-O-Thym	1st heating	-16,5	0,2	25-145	92,2	123,5
	2nd heating	8,3	0,53	-	-	-
	cooling	7,1	0,39	-	-	-
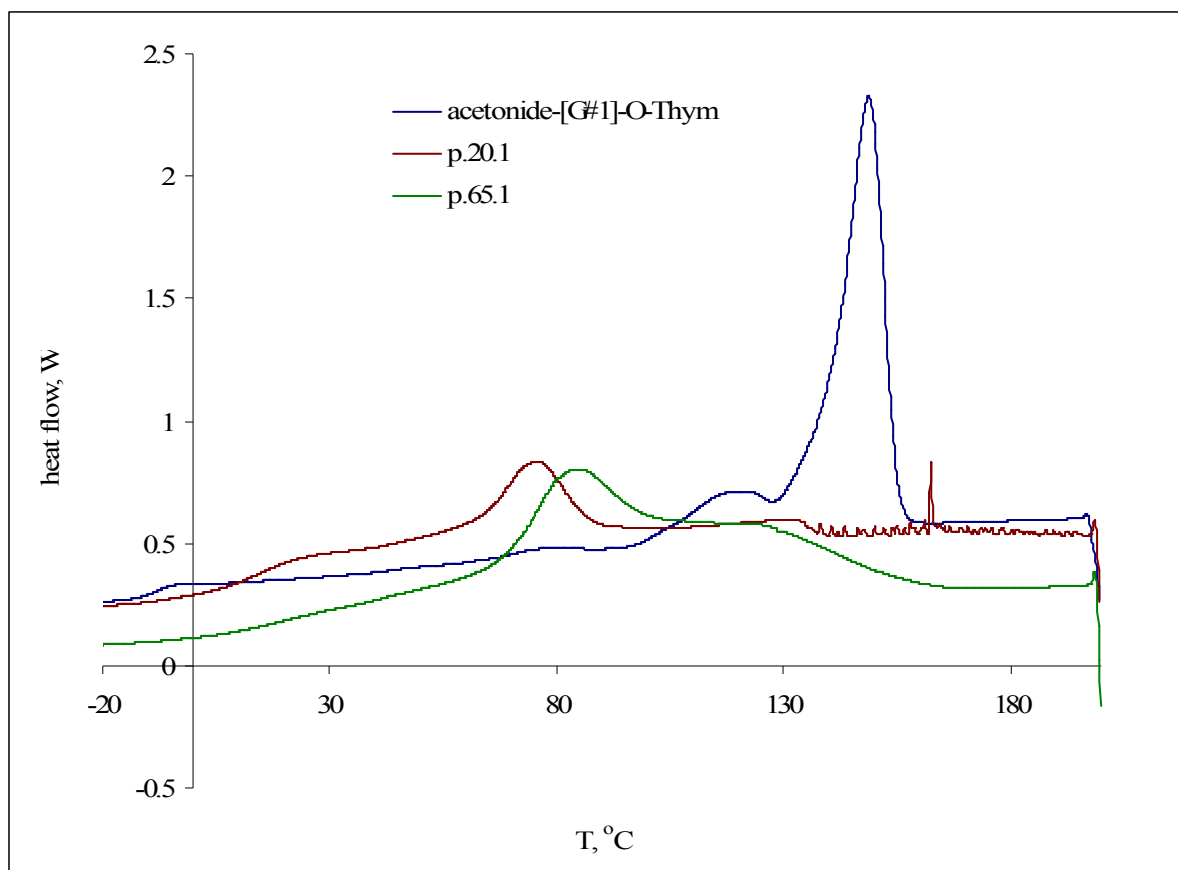
Figure 60 First heating curves for selected branched polyester
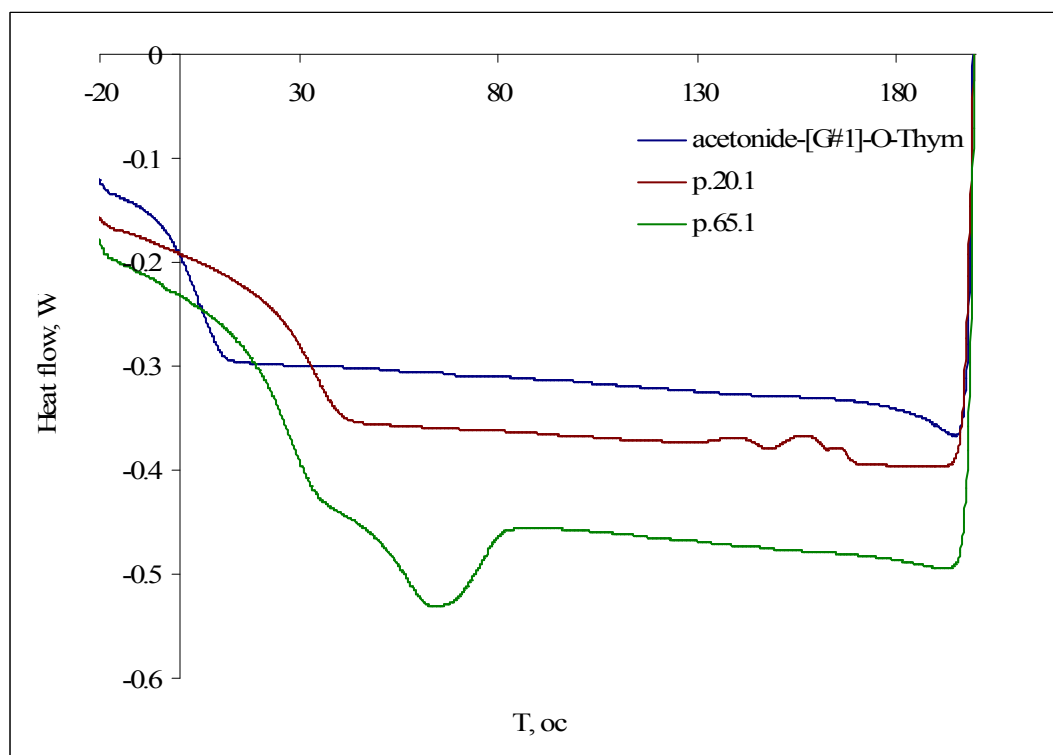
Figure 61 Cooling curves for selected hb polyesters
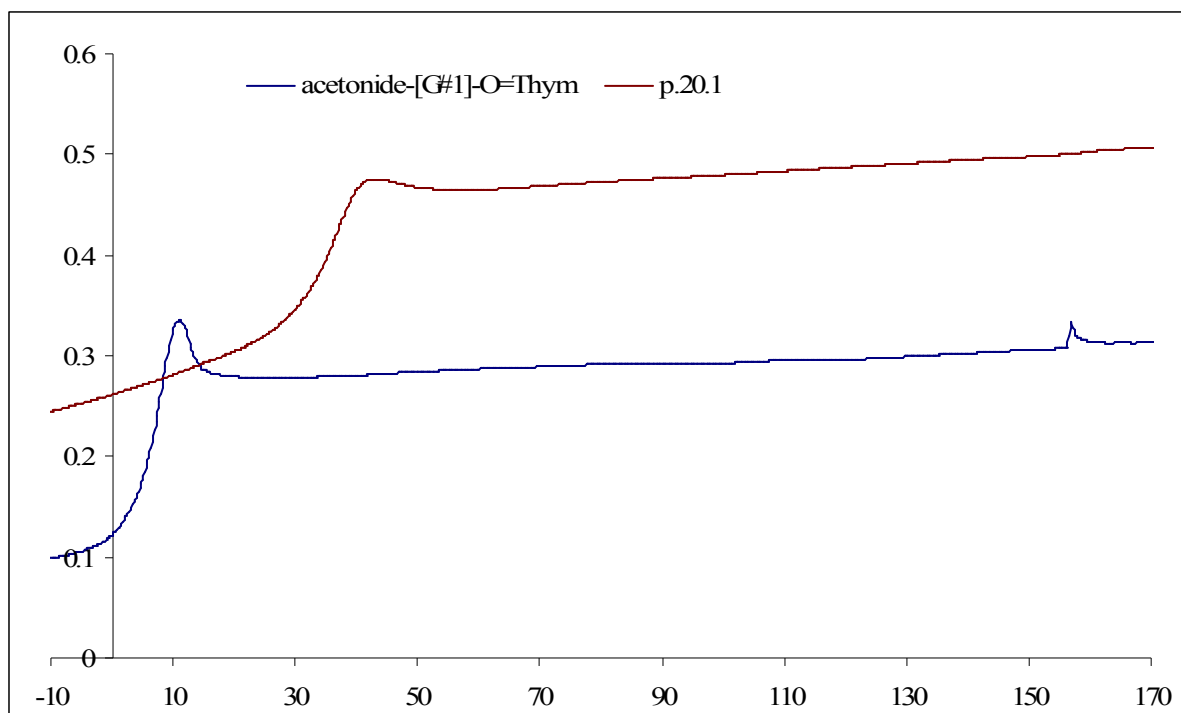
Figure 62 Second heating curves for selected hb polyesters

4.7   Investigation of noncovalent interactions

H-bonding is the main driving power for the formation of aggregates for branched structures synthesized in this work. Several methods for the determination of these non-covalent interactions were described in literature. NMR, IR or UV-vis spectrosocpopy in solution and viscosimetry can be chosen. All of them are rather sensitive and reliable, though some limitations do exist.

4.7.1   H-bonding test with low molecular weight templates

The basic idea for the formation of the assemblies and first steps toward molecular recognition was to use the H-bonding abilities of the active core moiety towards templates. Before proceeding with synthesis of polymer structures preliminary investigations of the H-bonds formation were made. It was needed to verify that the chosen pairs are working at all. Tests with low molecular weight substances can also give useful information about the crucial moments for successful formation of H-bonds and can help to determine the best conditions needed for future investigation.

From all methods mentioned above NMR analysis seemed to be the fastest and easiest one. It is understandable that to enable a comparison of the results all test should be performed under the same conditions. There is no standard procedure, because concentration of templates depends on their solubility.

In the course of this work a standard procedure was developed. First of all, 0.025M solutions of needed substances were prepared and mixed in appropriate ratios, then sample staied overnight at room temperature. The next day ^{1}HNMR spectra were measured and chemical shift differences analyzed, this is a measure of H-bond's stability. The higher is the shifting value the more stable is the interaction.

The Aden-C5/Thym-C5 (**Figure 64**) pair will be considered as a reference for the determination of the H-bonds stability. They are a natural complementary pair and do possess a stable interaction in natural or artificial receptors. Detailed analysis of the spectra of Aden-C5/Thym-C5 (**Figure 63** and **Table 13**) confirmed the theoretical predictions. The more nonpolar is the solvent the more stable is the formed H-bond. It is particularly obvious when the CDCl$_3$:DMSO-d_6 mixture was tested: concentration of chloroform has been gradually increased and the difference in shifts has been followed. Adenine seems to be not that much sensitive, compared to the thymine derivative. When concentration of CDCl$_3$ in

CDCl$_3$:DMSO-d_6 rise from 50% to 100%, shift in Aden-C5 had a 25% rise, while for the thymine derivative this number is nearly ten times higher.

Table 13 Shifting of signals in adenine/thymine mixture in different solvents

Experiment		Shifts of the signals	
Sample	Solvent	Aden-C5	Thym-C5
S1	CDCl$_3$:DMSO-d_6 (1:1)	0.0057	0.106
S2	CDCl$_3$:DMSO-d_6 (3:1)	0.400	0.255
S3	CDCl$_3$	0.51	2.58
S4	DMSO-d_6	0.005	0.026
S5	MeOH-d_3	0.007	0.100
S6	THF-d_8	0.102	0.457
S7	Acetonitrile-d_3	0.174	0.775
S8	1,4-Dioxane-d_8	0.055	0.278
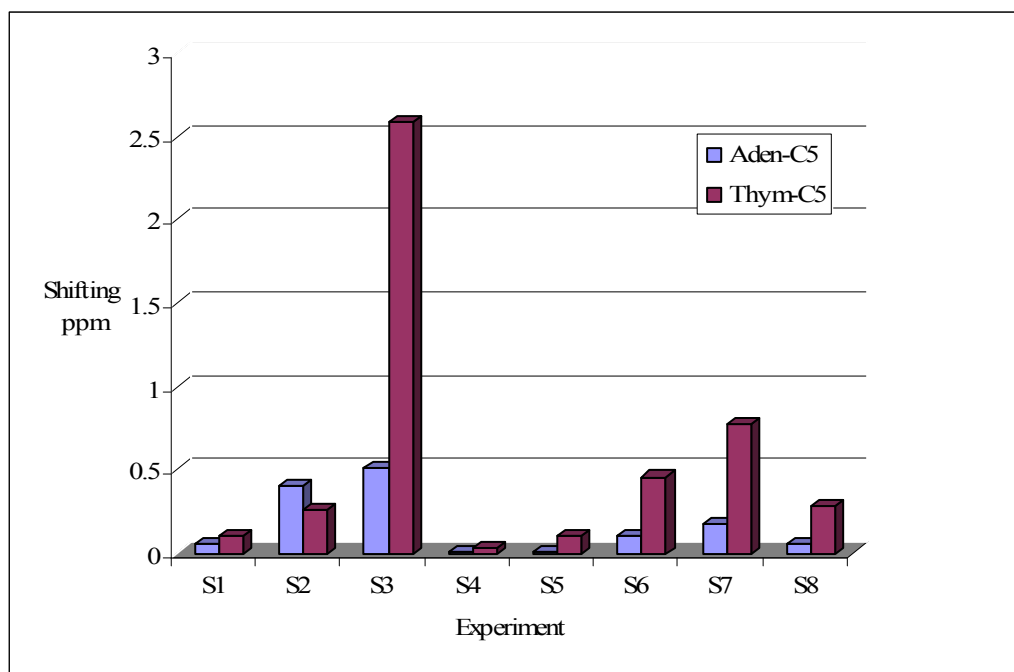
Figure 63 Shifting of signals in adenine/thymine mixture in different solvents

It is clearly seen that chloroform, THF and acetonitrile can be named "good" solvents for the H-bonding investigations. This means that all templates should be soluble in one of them, in the best case chloroform. Such restriction has limited the number of potential templates, especially on the site of the adenine base.

After preliminary investigation the artificial DAPy base receptors were subjected to the analysis. All tests were started with DAPy-C5-DB because of the limited amount of DAPy-C5-OH.

At first DAPy-C5-DB/Aden-C5 pair was tested in DMSO and CDCl₃. (**Figure 64**) These particular solvents represent a "bad" and a "good" solvent, so the decision about presence or absence of H-bonds could be made after the comparison of the shift differences in both of them.
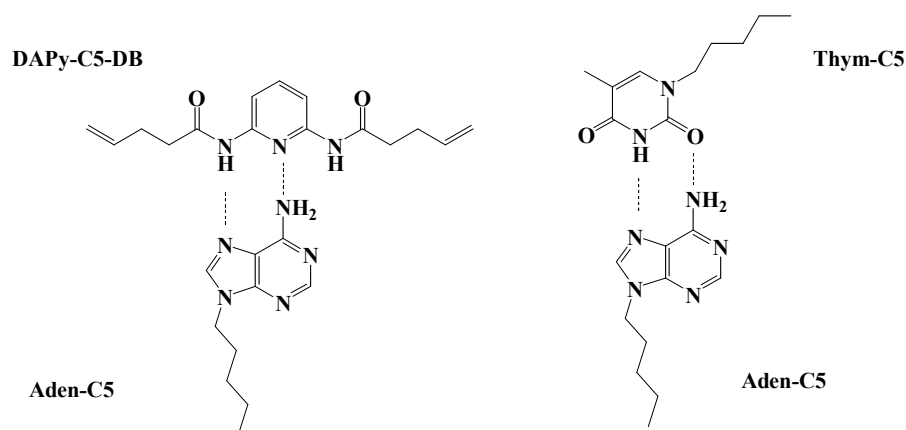
Figure 64 Possible interactions of 9-pentyladenine template

Table 14 shows the results. Compression of Thym-C5/Aden-C5 and DAPy-C5-DB/Aden-C5 pairs led to the conclusion, that in the later H-bonds are hardly formed. On this evidence no further experiments with aden-C5 template were made for either DAPy-C5-DB or DAPy-C5-OH. Of course even in this vary bad example one sees that signal difference in less polar solvents is much more significant.

Table 14 Shifting of signals in 1H NMR spectra in the tests with adenine template

Solvent	NH- (ppm) for Thymine-C5	NH2-(ppm) for Adenine-C5	NH- (ppm) for DAPy-C5-DB
CDCl₃	2.580	0.510	
		0.326	0.000
DMSO-d_6	0.026	0.005	
		0.004	0.032

Results and discussion

The second part of experiments was made using thymine derivative (Thym-C5). After first positive results obtained from tests in CDCl$_3$ (**Figure 66**) and DMSO-d_6 it was decided to perform a full set of experiments for both 2,6-diaminopyridine derivatives. (**Figure 65**) Unfortunately, the low solubility of DAPy-C5-OH has caused exclusion of some solvents.
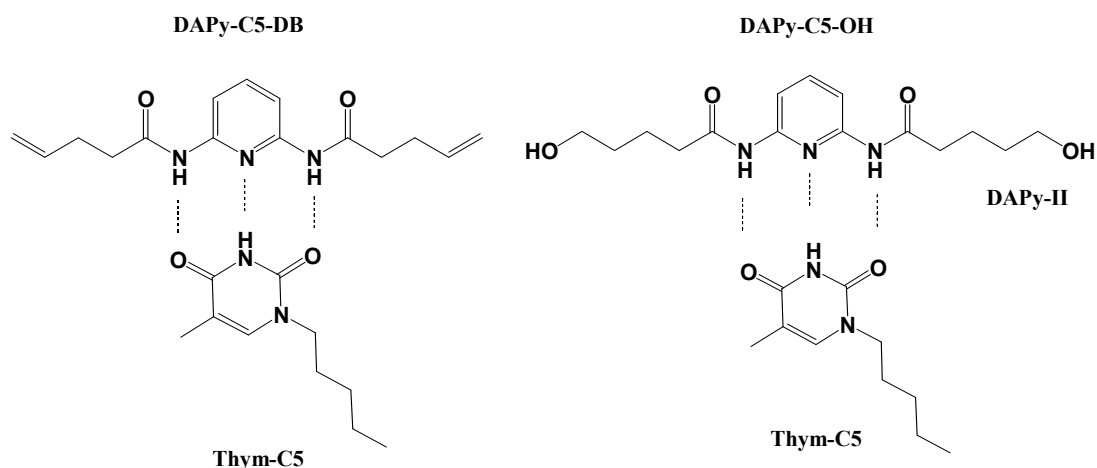
Figure 65 Interactions of 2,6-diaminopyridine derivative with thymine derivative
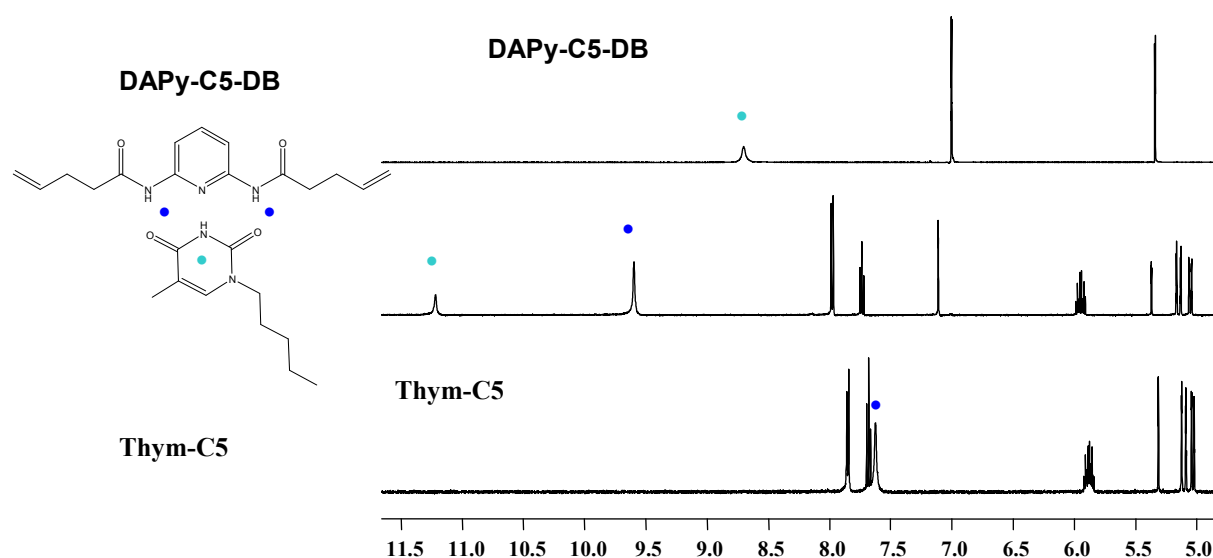
Figure 66 Confirmation of H-bonding interaction in Thym-C5/DAPy-C5-DB pair (^{1}H NMR spectra measured in CDCl$_3$)

Table 15 presents the results of ^{1}H NMR analysis of spectra for thymine derivative and tree different templates. No matter which template was used, shifts were more pronounced in less polar solvent. It means that more stable H-bonds were formed in less polar solvents; on the contrary practically no difference in signals was observed in methanol.

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Table 15 Results of H-bond formation for Thym-C5 with DAPy derivatives and Aden-C5

Solvent	NH2-(ppm) for Aden-C5	NH-(ppm) for Thym_C5	NH-(ppm) for DAPy-C5-DB	NH-(ppm) for DAPy-C5-OH
CDCl$_3$/DMSO-d_6 (3:1)	0.400	0.225 0.1566 0.0328	0.012	0
CDCl3/DMSO-d6 (1:1)	0.057	0.106 0.0279 0.1156	0.0392	0.5050
CDCl$_3$	0.510	2.580 2.8247 -	0.1265	-
DMSO-d_6	0.005	0.026 0.0137 0.0002	0.0278	0.0000
MeOH-d_3	0.000	0.010 0.010 0.0000	0.000	0.0060
THF-d_6	0.102	0.457 0.358 0.3197	0.2656	0.1626
Acetonitrile-d_3	0.174	0.7746 0.6599 0.6699	0.4136	0.3897
1,4-Dioxane-d_6	0.055	0.2777 0.242 -	0.151	-
CD$_2$Cl$_2$	-	- 2.4916 -	1.9384	-

In the majority of cases interactions between DAPy-C5-DB and Thym-C5 are stronger than between DAPy-C5-OH, except for the CDCl$_3$/DMSO-d_d (1:1) mixture and acetonitrile-

d_3. The adenine derivative, which is an analogous natural complementary partner, is more effective in all solvents.

In order to understand better the influence of the polar solvents on the formation of H-bonds, and due to the possibility to use mixtures for samples with low solubility, the effect of DMSO-d_6 content in the DMSO-d_6/CDCl$_3$ was investigated. From **Figure 67** it is clear that overall tendency is the same, the more polar is the mixture the weaker are the H-bonds. Apparently it is no good to use mixtures, where the DMSO-d_6 content exceeds 50%, because of less pronounced shifting of the signals.
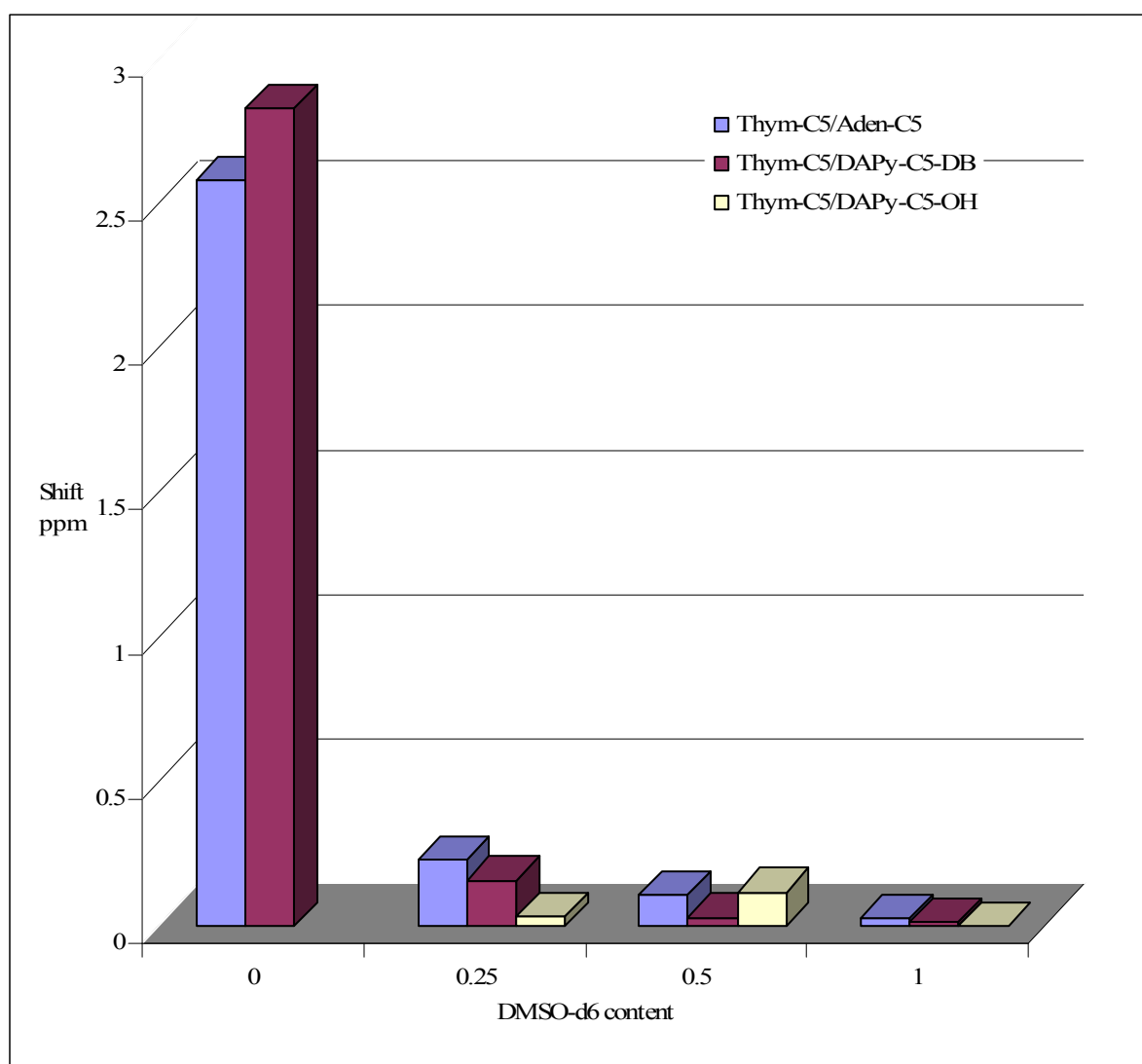
Figure 67 Effect of the DMSO-d_6 content in the DMSO-d_6/CDCl$_3$ on the H-bonds formation in different pairs of templates (shifting of the signal from NH in thymine)

4.7.2   Investigations of H-bonding interactions of branched structures

After the tests with low molecular weight precursors it was clear that noncovalent interactions implying adenine, thymine and 2,6-diaminopyridine derivative are stable. So it would be right to predict stability of such interactions in the systems, where branched structures are used as one of the templates. Problems can arise from the complex structure of such molecules. It has been shown earlier that incorporation of the thymine core moiety into the hyperbranched polyesters is low and does not exceed 20%. So, all signals coming from it are weak and hard to distinguish. This problem does not appear in the case of dendrimers, so they could be expected to be more effective. To prove it several methods were explored: ^{1}H NMR spectroscopy, IR-spectroscopy in solution and viscosimetry.

Investigation has started with ^{1}H NMR analysis, first of all because of all preliminary work with low molecular weight templates has been done by it. The set-up procedure developed on the stage of preliminary research was used here again. It was modified only if the amount of substance or its solubility were not sufficient for the accurate analysis.

For example, at least 300g of the protected hyperbranched polyester sample p.20.1a must be dissolved in 1mL of $CHCl_3$ in order to reach the standard concentration of thymine ends. So here the concentration was scaled down, that led to decrease in intensity of signals of reference groups, so in the end it was not possible to get any information out of the spectra. It does not postulate absence of the H-bonding interaction, but only shows the limited sensitivity of this method. Due to this problem only a couple of tests were performed on hyperbranched polymers. The experiments with hb materials, however, were not giving positive information due to the low content of the thymine core.

As for the perfectly branched dendritic structures, here there were no complications. Dendrimers with acetonide protected end groups are rather well soluble in non-polar solvents like dichloromethane or chloroform. The first test performed in standard conditions has shown that their complexes with adenine or DAP-C5-DB template are nearly as stable as the ones observed during preliminary tests.

When the existence of complexes was confirmed, the ratio between the template and a dendritic molecule bearing thymine in the focal point was of particular interest. From the chemical structures it could be assumed to be 1:1, while in reality it could be a bit different, due to some reasons. One of them is possible sterical hindrance caused by the complex structure of the dendrimer, here modeling was performed in order to prove or disprove this theory.

While the modeling was running, the Job's plot analysis was performed. Its principles were discussed earlier. **Figure 68** presents the results of this investigation performed at the constant concentration of DAPy-C5-DB and varied one of acetonide-[#G1]-O-Thym. Both curves have one significant transition.
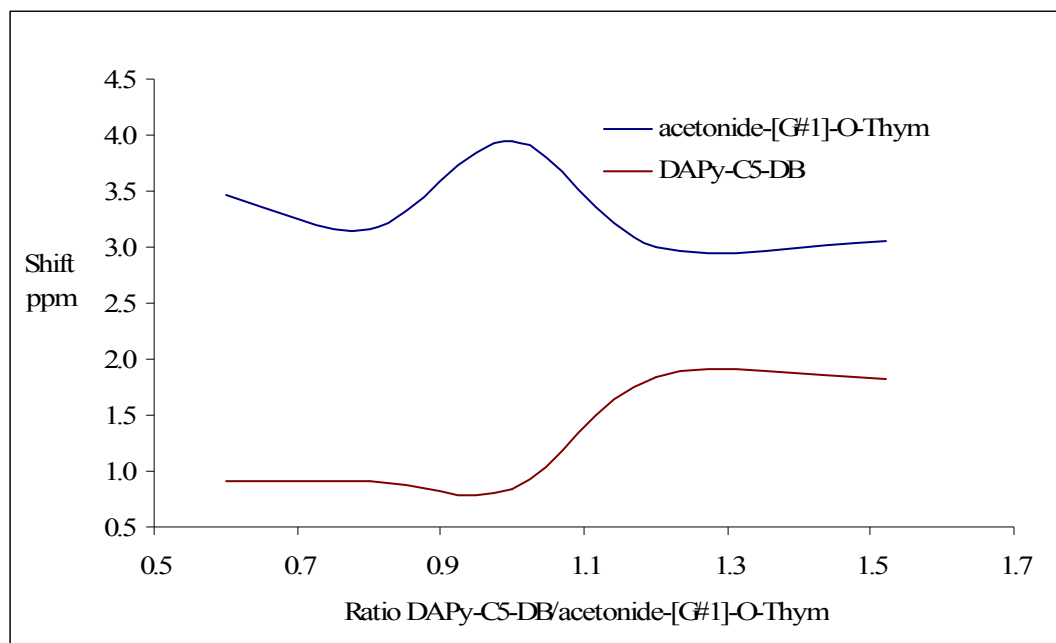
Figure 68 Job's plot for the DAPy-C5-DB/acetonide-[#G1]-O-Thym complex

In the case of the dedndrimer acetonide-[#G1]-O-Thym a maximum was recorded at equimolar ratio with DAPy-C5-DB. Meantime, the curve for DAPy-C5-DB has a minimum in the same point. So it was concluded that the complex between the two of them is a 1:1 complex.

As in the case of the determination of the degree of incorporation of thymine core it is decided to apply another methods. The second choice falls on viscosimetry. It is well-known that viscosity depends on the molecular weight of the substance, for linear polymers the one increases simultaneously with another. The outflow time should also change when complexation happens; of course there is a chance that this difference would not be big enough to be detected. The results of the viscosimetry tests are listed bellow. (**Figure 69**)

The changes though moderate were enough to confirm the complexation. These tests were made according to the standard methodology developed for the viscosimeter applied, where concentration of every investigated substance in solution is 1 mg/ 1 mL. So the ratios between templates were not corresponding to the 1:1 complex, therefore the response from the system with concentration ratio 1:1 can be even higher.
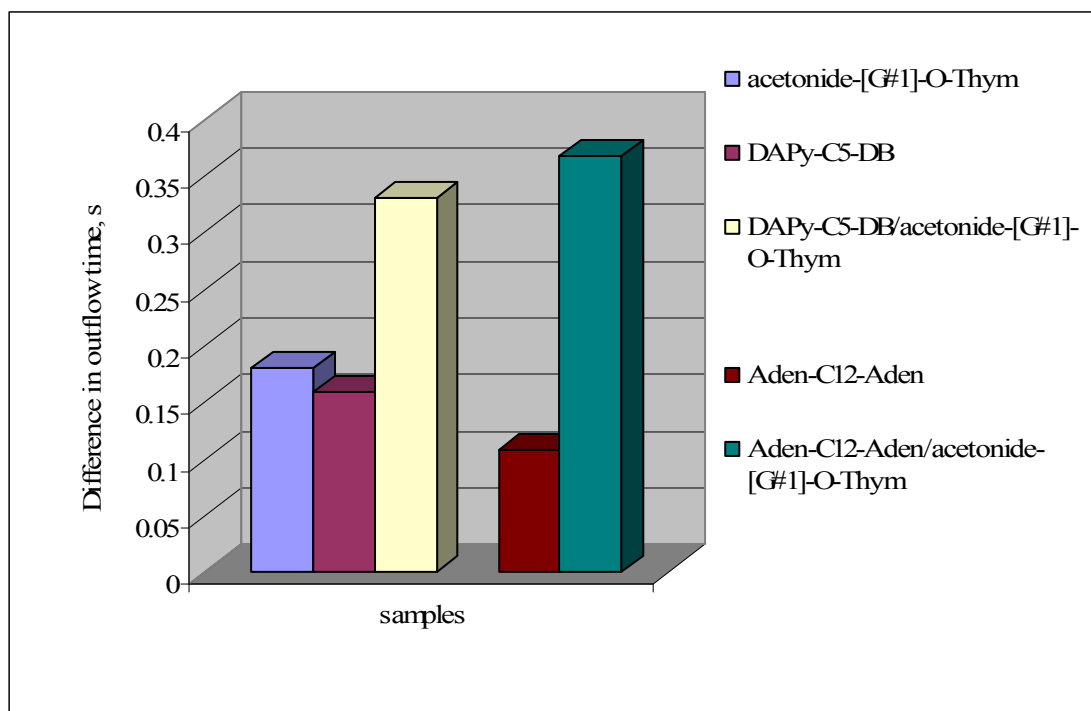
Figure 69 Results of viscosimetry in CHCl$_3$ for the pairs with acetonide[G#1]-O-Thym
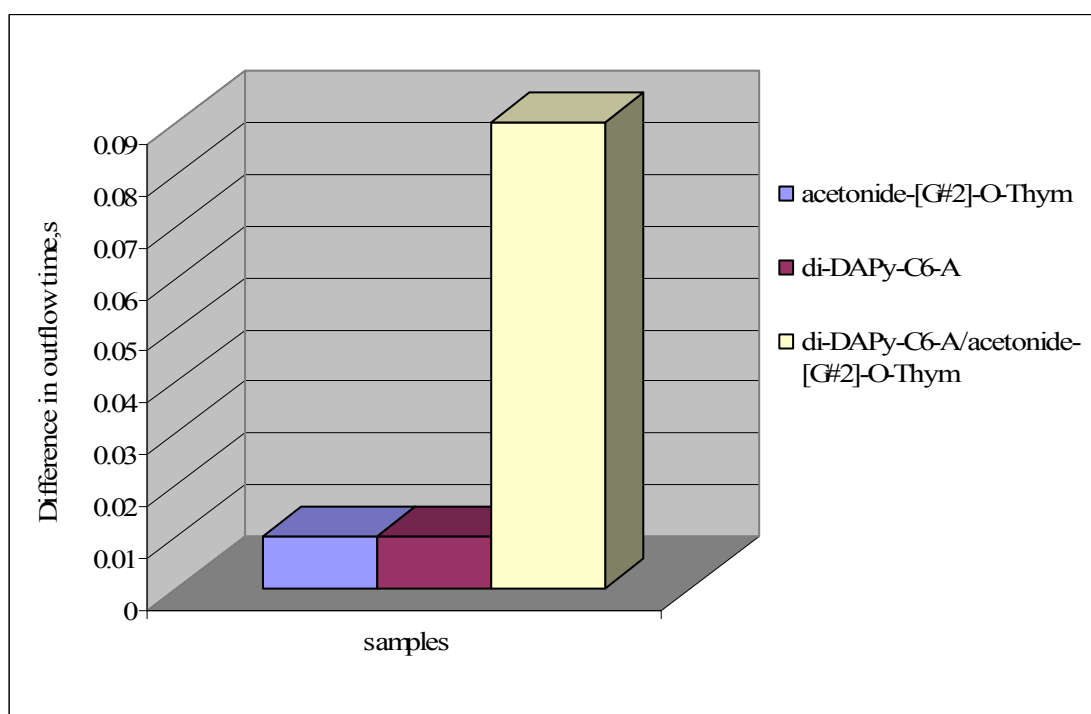
Figure 70 Results of viscosimetry in CHCl$_3$ for the pairs with acetonide[G#1]-O-Thym

For the dendrimer of second generation it was possible only to perform the viscosimetry experiments with a less concentrated solution (2.5 mg/mL), due to the lower solubility. Here,

the di-DAPy derivative was used as a template. Though the concentration was lower it was possible to observe quite high difference between the outflow times for the solution of templates and their mixture. (**Figure 70**) The formation of H-bonds was confirmed.

The last experiments were made using IR-spectroscopy. This method was once applied in investigations of aliphatic hb polymers by Žagar and Grdadolnik, who used it to confirm intra- or inter-molecular H-bonding interactions in aliphatic hyperbranched bis-MPA polyester itself. [234]

Here all experiments were made in $CDCl_3$ solution with the same concentration like in standard ^{1}H NMR measurements. Through this method it was not possible to determine any kind of H-bonding interactions in mixtures of any of the core moieties and the hyperbranched samples, due to the low incorporation of the starting core moiety. As for the perfectly branched structures this method was working perfectly. Complexes of acetonide-[G#1]-O-Thym with either DAPy-C5-DB or Aden-C12-Aden were confirmed. In their spectra differences comparing to the ones for starting materials were obvious. For example, in the spectra of acetonide-[G#1]-O-Thym and DAPy-C5-DB the region 3100-3350 cm^{-1} is empty, while in their mixture there are signals at 3230 cm^{-1} and 3100 cm^{-1} , but no signal coming from dendrimer at 3400 cm^{-1} were detected. (**Figure 71**)
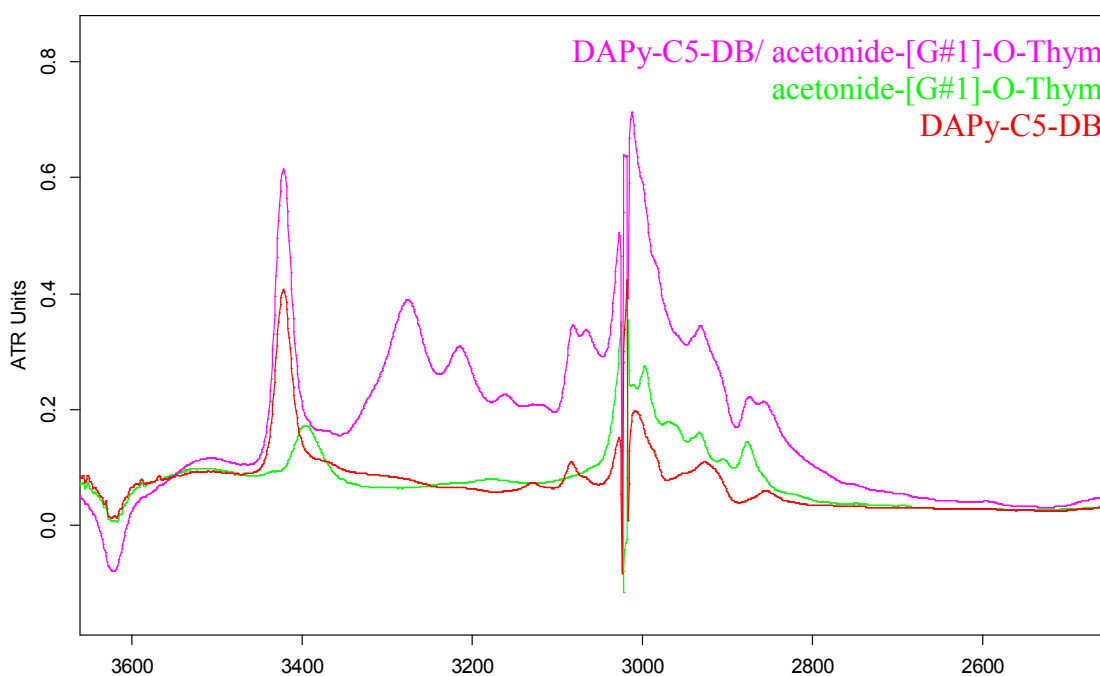
Figure 71 IR-spectra of the DAPy-C5-DB, acetonide-[G#1]-O-Thym and DAPy-C5-DB/ acetonide-[G#1]-O-Thym solutions in CHCl$_3$

So it was confirmed that dendrimers can form H-bonds with the desired templates.

4.8 Molecular modelling of the branched structures and their interactions

Molecular modelling was performed to get more information about packing of the structures and possibility for H-bonding interactions. Simulations were made only for the perfect dendritic structures bearing thymine derivative in the focal point, because of all problems with the incorporation of the core moiety into hyperbranched polyester.

Modelling was considered for the dendrimers of the second and the fourth generation both with and without acetonide protection. It was interesting to see possible difference in the packing of the dendrimer and further possibilities for its interaction with chosen template, Aden-C5. **Figures 72-78** show all of the modulated structures.
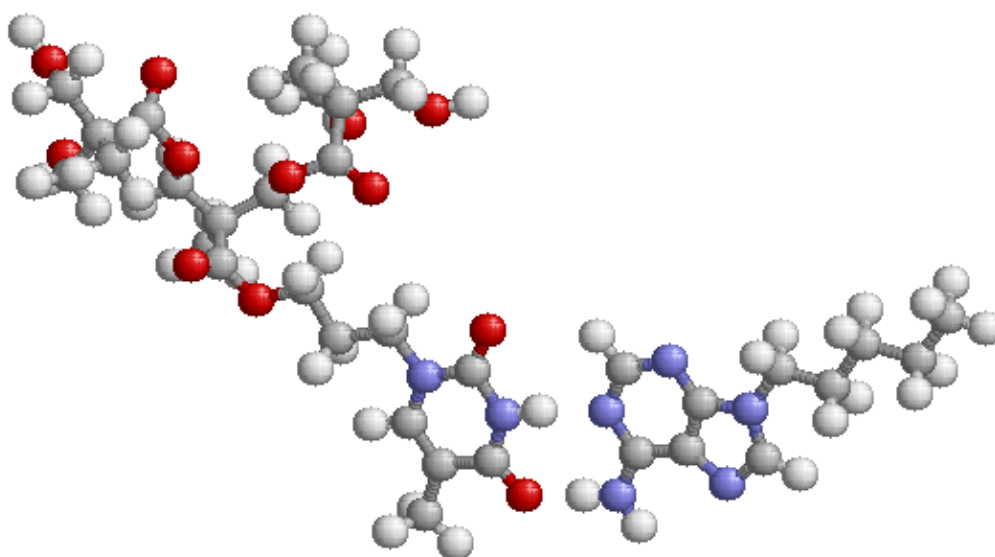
Figure 72 Simulation of the H-bonding interactions between Ad-C5 and $(OH)_4$-[G#2]-O-Thym
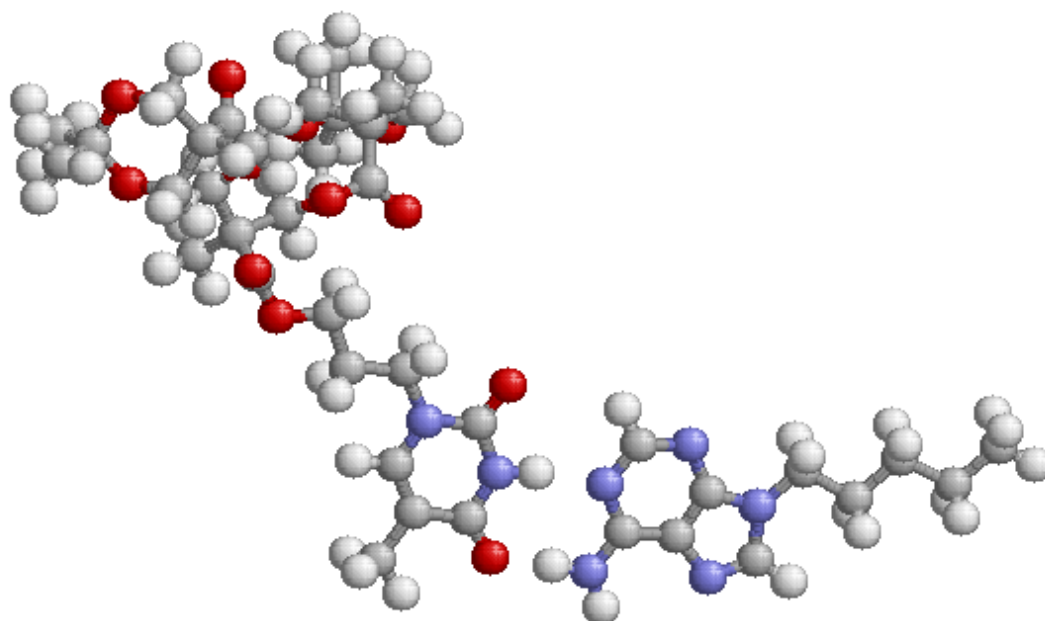
Figure 73 Simulation of the H-bonding interactions between Ad-C5 and acetonide-[G#2]-O-Thym
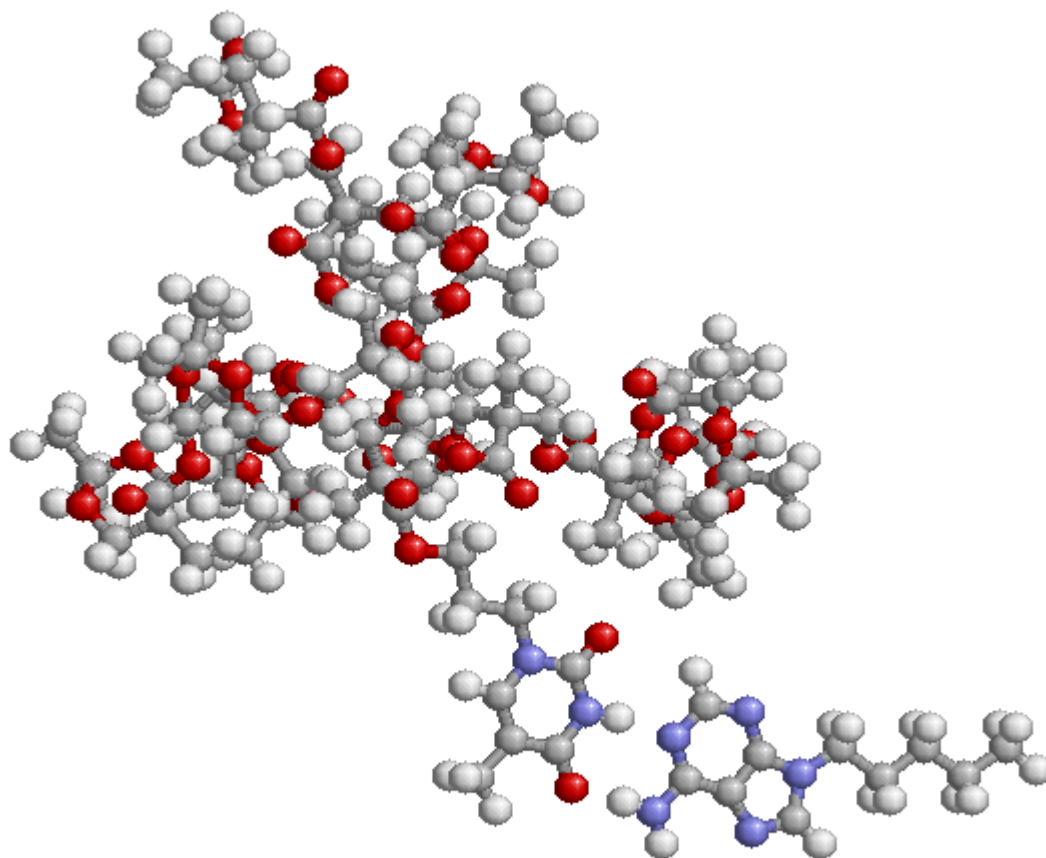
Figure 74 Simulation of the H-bonding interactions between Ad-C5 and acetonide-[G#4]-O-Thym

It should not be forgotten that all simulation were performed assuming that interactions take place in the absence of solvent. This means that the solubility of these all presented substances was not taken into account as well as influence of solvent on the formation and stability of noncovalent interactions.

From the models above it is seen that, though the volume of the dendrimer of fourth generation is quite big, the thymine focal group is still available for the complexation. It means that theoretically H-bonds should be formed by any dendrimer from second till fourth generation if the proper template is applied. The character of the end groups was not playing a significant role.
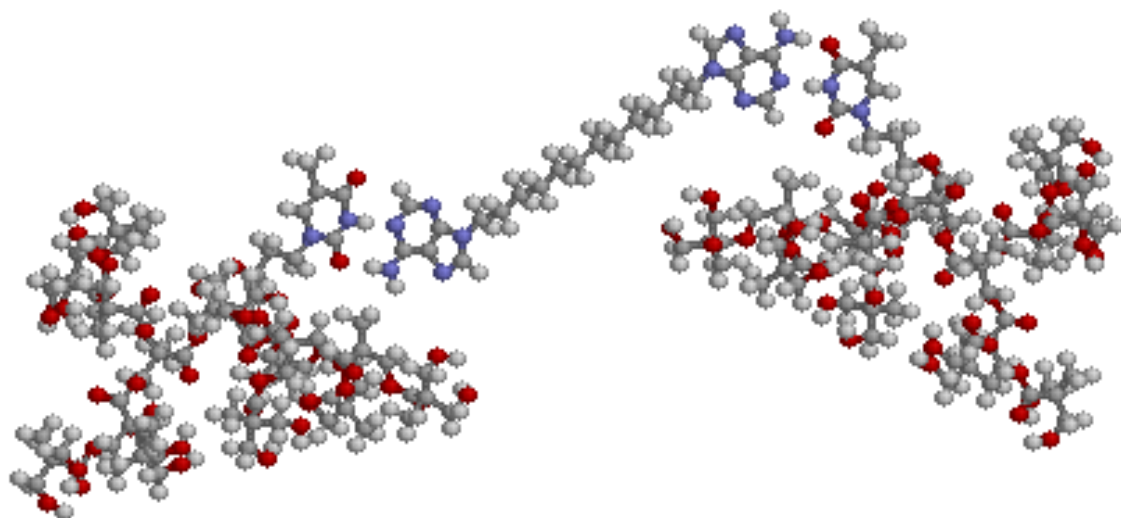
Figure 75 Simulation of the H-bonding interactions between Ad-C6-Ad and $(OH)_{16}$-[G#4]-O-Thym
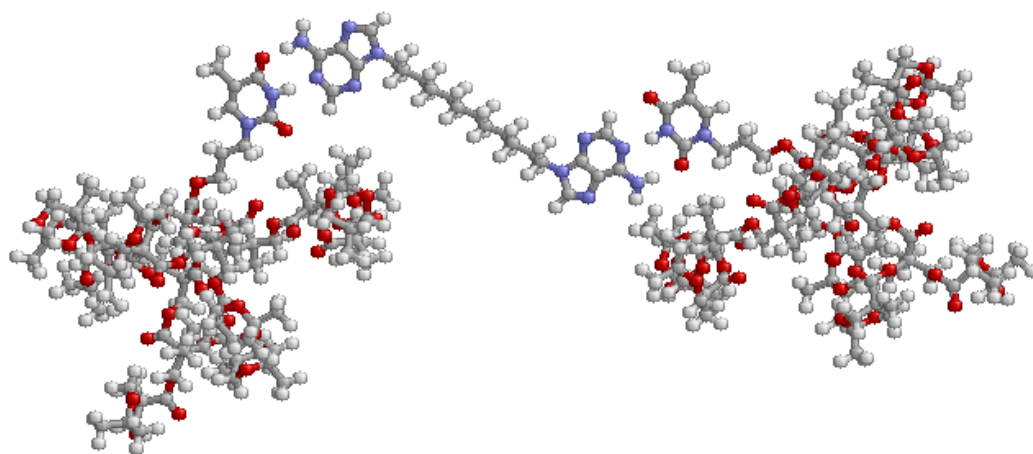
Figure 76 Simulation of the H-bonding interactions between Ad-C6-Ad and acetonide-[G#4]-O-Thym
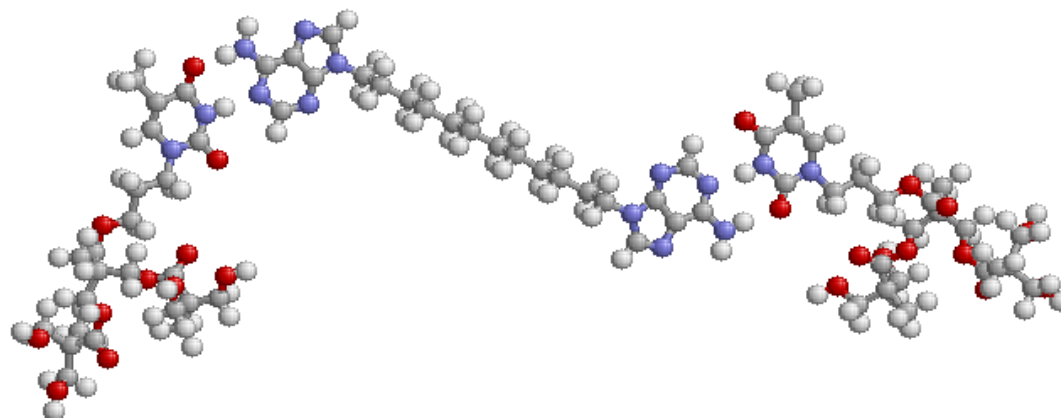
Figure 77 Simulation of the H-bonding interactions between Ad-C6-Ad and (OH)$_4$-[G#2]-O-Thym
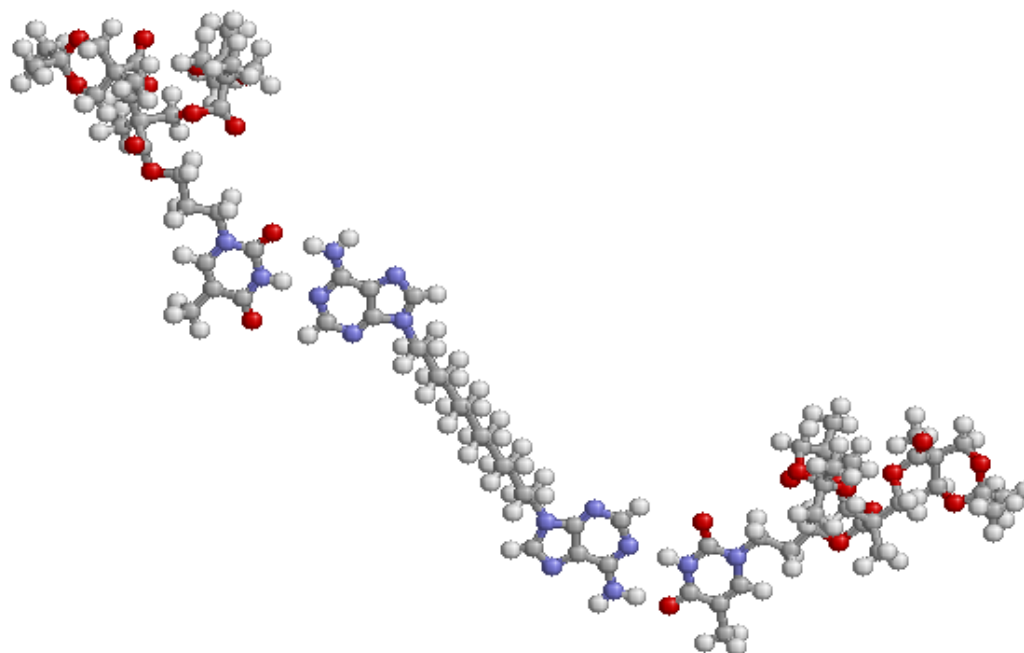
Figure 78 Simulation of the H-bonding interactions between Ad-C6-Ad and acetonide-[G#2]-O-Thym

More complex structures were simulated using the same dendrimers and di-adenine derivative (Ad-C6-Ad) template. Though the spacer between two adenine active groups is rather short, it is big enough to enable the possibility of interactions from both sides for all presented branched structures. Here again, the same picture was observed for polymers with and without acetonide protection.

These theoretical results were very helpful and could be partly proved by the first preliminary experiments.

Unfortunately, it was not possible to perform test for the same substances as described above. As it was discussed earlier in the real systems solubility of the templates was the main restricting factor. It was possible to perform investigations only in nonpolar solvents. Only acetonide protected dendrimers were soluble in them, the di-adenine derivative has been prepared with the spacer twice as much in order to make it useful in chloroform. For the dendrimer of the first and second generation it was possible to confirm the formation of the H-bonds using several independent methods. The investigation of the systems is tricky because reference signals can be to weak due to the limited solubility of templates.

5   Conclusions and outlook

5.1   Conclusions

✓ Different low molecular weight templates on the thymine, adenine and 2,6-diaminopyridine basis were prepared. Their solubility in nonpolar solvents was investigated. From the number of prepared molecules only Thym-C3-OH and Thym-C5-NH$_2$ were used as starting core moieties in polymerizations and synthesis of perfectly branched dendrimers, and DAPy-C5-DB, DAPy-C5-OH, Thym-C5, Aden-C5, Aden-C5-Aden and di-DAPy-C6-A were used later one as templates in the investigation of H-bonds formation and molecular recognition.

✓ Hb polyesters were synthesized in melt using only Thym-C3-OH as a starting core and bis-MPA as monomer. Thym-C5-NH$_2$ was excluded due to its instability at high temperatures. After optimization of conditions hb polymers with molecular weight and PDI close to the desired ones were obtained. Polycondensations in solution for both core moieties were proved to be ineffective.

✓ All hb materials were fully characterized using different techniques: NMR, UV-vis spectroscopy, TGA, DSC and SEC. The degree of incorporation of thymine derivative was not exceeding 15%, as shown by NMR analysis. The UV-vis data were not accurate because of the contamination of polyesters with the starting Thym-C3-OH and pTSA used as catalyst. So low degree of incorporation can be explained by the transesterification happening at the reaction conditions.

✓ The low degree of incorporation of thymine core prevented use of hb polyesters in molecular recognition. The applied methods were not sensitive enough to show any change in signals happening due to the formation of H-bonds.

✓ The perfectly branched dendrimers were synthesized with both core moieties. The new methodology for the synthesis of bis-MPA dendrimers (activation with CDI/DMAP, **Figure 79**) was applied during synthesis with Thym-C3-OH core.
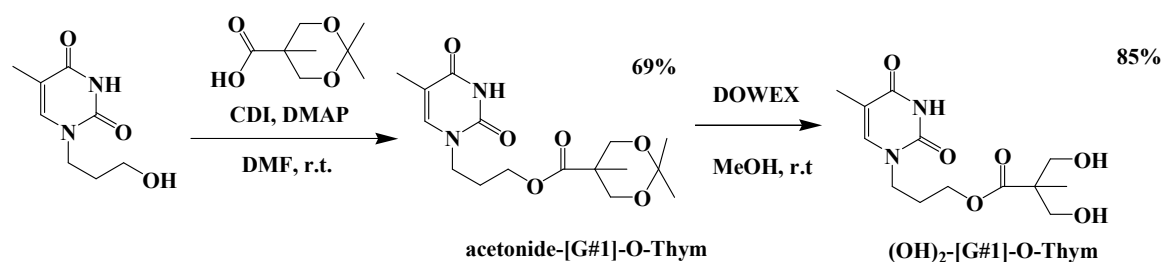
Figure 79 Synthesis of dendron via CDI/DMAP activation

Here it was possible to go up to the third generation, though it is possible to go further to the higher generation, but these syntheses will be time-consuming. For the other core though acetonide-[G#1]-NH-Thym was synthesized, the procedure was ineffective. It is highly probable that this setback is caused by the application of BOP reagent as a catalyst.

✓ Different templates with one, two or three active centres were successfully synthesized. Their solubility was checked and three of them (two on 2,6-diaminopyridine and one on adenine basis) were used later on in the H-bonding investigations.

✓ Preliminary investigations of H-bonding abilities were performed for dendrimers and some of the available cores. Different methods indicated the formation of H-bonds so these materials were pronounced promising for application in molecular recognition.

5.2 Outlook

➢ The selectivity of dendritic molecules towards different templates can be investigated. For that tests can be made with the mixture of the templates used alone to confirm the H-bonding activity of the dendrimers. For example, it can be the mixture of DAPy-C5-DB and Aden-C12-Aden.

➢ The degree of incorporation of the thymine core can be enhanced via either application of a new effective method for the polycondensation in solution or preparation of a derivative which will not be able to participate in the transesterification, after coupling to the polyester backbone. It could be either a simple

core moiety or the first generation dendrimer with available hydroxyl groups on its outer-shell.

➢ The modification of hb polyester can be done on its outer-shell using either thymine or adenine. Final polyester will be multifunctional and a good candidate for the molecular recognition on the surface.

6   Experimental part

6.1   Materials and methods

6.1.1 Materials

When not specified the chemicals that have been used in the course of the work have been purchased from Fluka, Aldrich or Acros Organics and used without further purification. The inhibitor was removed from Aldrich's THF by distillation under reduced pressure.

6.1.2 Methods of investigation

Nuclear Magnetic Resonance (NMR)

The NMR experiments were performed in 5 mm diameter tubes with a Bruker DRX 500 NMR spectrometer at 500.13 MHz (1H NMR) and 125.75 MHz (13C NMR). Deuterated solvents: DMSO-d_6, CDCl3, CD$_2$Cl$_2$, THF-d_8, Acetonitril-d_3, 1,4-Dioxane-d_8 were used to dissolve samples and also as internal standards for the spectra calibration. All information about signal of the solvents was found in the literature [235].

Size exclusion chromatography (SEC)

SEC was made with two Zobrax PSM Trimodale-S columns using DMAc with 2% of 3g/L LiCl water solution. Speed was set to 0.5mL/min and IR detector was applied. For every sample 2mg/mL solution was prepared and filtrated before the measurement. PVP standards were used for calibration.

SEC in THF was made using P2Mixed-C column, Q-2010 GPC-pump from Bures. Speed was set to 1.0mL/min. ETA-2020 Visc/RI detector from Bures and MALLS detector from Wyatt were applied. Here PS standards were taken for calibration.

Viscosimetry

Viscosimetry was performed at 25ºC. A Thermo Schott CT52 thermostat was applied together with ViscoSystem Shott AVS 470 viscosimetry pump. Ubbelohde-Viscosimetry

K=0.03029 was purchased from Schott-Geräte GmbH. Pure solvents were used as reference solutions.

Molecular modelling

Using ab initio quantum mechanical methods based directly on the solution of the time-free Schr"odinger equation, one may be able to optimize the geometry of molecular units and determine, for example, electro\-statical properties like the charge distribution, dipole moments and vibrational properties [236]. Here, the method of Restricted Open Shell Hartree Fock (ROHF) method was applied using the the basis set 6-31G to different chemical units like N-hydroxypropyl-thymine, methyl-dihydroxy-neopentanic acid, acetonyl-(methyl-dihydroxy-neopentanic acid)-acetal, 6-amino-9-n-pentyl-purin, 6-amino-9-n-dodecyl-purin, 6-amino-9-n-(6-amino-purinyl-dodecyl)-purin.

Furthermore, the own written tool RESMAIN [237] was applied to connect the different monomer units to the corresponding dendritic adenine-thymine complex structures.

Additionally, for graphical representations the RASMOL2.7 package [238] was used.

Differential Scanning Calorimetry (DSC)

The DSC experiments have been made with DSC 7 from Perkin Elmer endowed with a Q-Advantage Software 4.5.2. On the basis of the TGA results, the samples are subjected to two heating-cool-heat cycles between -60ºC and 150ºC, or -60ºC and 200ºC with heating and cooling rate 20 K/min. All samples were dried under vacuum at 50ºC for 72h before the submission to the analysis.

Thermal Gravimetric Analysis (TGA)

TGA were measured on TGA Q 5000 of TA Instruments in the nitrogen atmosphere. At first samples were isothermal heated up to 40ºC for 10 min and than temperature was raised up to 700ºC with the rate of 10 ºC/min. All samples were dried under vacuum at 50ºC for 72h before the submission to the analysis.

UV-vis spectroscopy

All UV-vis experiments were made on the Specord 40 spectrometer, Analytic Jena in a special room with constant temperature of about 22ºC. 1cm quartz cells were used. Pure solvent was taken as a reference. Measurements were made for some samples in the waive length region from 200nm to 700nm, and for others only in the region from 200nm to 400nm.

Infrared spectroscopy in solution

IR spectroscopy in solution was performed on the Tensor 27 spectrometer, Bruker. All experiments were made at the mode of 120 scans in the waive length region from $400sm^{-1}$ to $3700sm^{-1}$. A special sell with NaCl/KBr glass and different machetes were purchased from Specac. Pure solvents were used as reference solutions and data were analysed with Brucker OPUS program.

6.2   Synthesis

6.2.1   Synthesis of low molecular weight precursors

6.2.1.1   Synthesis of core moiety on thymine basis

6.2.1.1.1   Synthesis of 3-bromopropyl-t-butyldimethylsilyl ether [179]
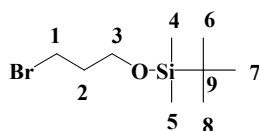
15.2 g (100.8 mmol) of t-butylchlorodimethylsilane (TBDMS-Cl) was dissolved in 100 mL dry CH_2Cl_2 in 250 mL round-bottom flask under argon atmosphere. 8.9 mL (102.4 mmol) of 3-bromoropanol was added under constant stirring, the reaction mixture was cooled in ice-base and 9.66 mL (118.5 mmol) of pyridine was poured in. After 10 minutes cooling the ice-bad was removed and the mixture was stirred overnight at room temperature. The precipitated pyridine chloride was filtrated of and washed with CH_2Cl_2 .Organic phase was washed with 2x50 mL of water. Water phase was extracted with 50 mL of CH_2Cl_2. The organic fractions were collected and dried over $MgSO_4$. Inorganic solid was filtrated and the excess of solvent was evaporated under vacuum. The final product was dried in a vacuum oven at 40°C overnight.

Yield: 12.33 g (48.3%) of a colourless liquid

^{1}H NMR (CDCl$_3$): δ [ppm] = 3.72 (t, 2H, H-3,), 3.50 (t, 2H, H-1), 2.02 (q, 2H, H-2), 0.89 (s, 9H, H-6, H-7, H-8), 0.06 (s, 6H, H-4,H-5).

^{13}C NMR (CDCl$_3$): δ [ppm] = 59 (C-3), 37 (C-2), 28 (C-1), 25 (C-6, C-7, C-8), 15 (C-6), -6 (C-4, C-5).

6.2.1.1.2 Synthesis of 1-tert-butyldimethylsiloxypropyl thymine
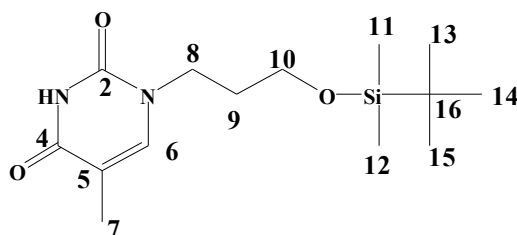
In DMF/DMSO mixture:

2.138 g (17 mmol) of thymine were dissolved in 65mL of DMF/DMSO mixture (55:10) and 1.624 g (6.4 mmol) of 3-bromopropyl-t-butyldimethylsilyl ether together with 2.364 g (17.1 mmol) of potassium carbonate were added to the resulting solution. Reaction mixture was stirred for 2d, the precipitate was filtrated and solution evaporated to dryness under vacuum. Resulting solid was suspended in 130 mL of distilled water and then extracted with 50 mL of CHCl$_3$. Organic phase was collected, dried over anhydrous Na$_2$SO$_4$ and evaporated.

Yield: 1.067 g (55.7%) as a white solid

In DMSO [179]:

15 g (118.9 mmol) of thymine were dissolved in 390 mL of DMSO and 11.43 g (45.2 mmol) of 3-bromporopyl-t-butyldimethylsilyl ether together with 16.52 g (119.5 mmol) of potassium carbonate were added to the resulting solution. Reaction mixture was let to stir for 2d, the precipitate was filtrated and solution evaporated to dryness under vacuum. Resulting solid was suspended in 300 mL of distilled water and then extracted with 5x100 mL of CHCl$_3$. Organic phase was collected, dried over anhydrous Na$_2$SO$_4$ and evaporated.

Yield: 9.41g (69.7%) of a white solid

^{1}H NMR (CDCl$_3$): δ [ppm] = 9.30 (1H, NH), 7.04 (s, 1H, H-6), 3.79 (t, 2H, H-10), 3.61 (t, 2H, H-8), 1.88 (m, 5H, H-7, H-9), 0.89(s, 9H, H-13,H-14, H-15), 0.04(s, 6H, H-11, H-12).

^{13}C NMR (CDCl$_3$): δ [ppm] = 164 (C-4), 151 (C-2), 142 (C-6), 109 (C-5), 60 (C-10), 45 (C-8), 32 (C-9), 26 (C-13,C-14, C-15), 18 (C-16), 12 (C-7), -5 (C-11, C-12).

6.2.1.1.3 General procedure for 1-tert-butyldimethylsiloxypropyl thymine deprotection using TBAF / Synthesis of N-(3-hydroxypropyl) thymine [179]

0.5 g (1.7 mmol) of 1-tert-butyldimethylsiloxypropyl thymine was stirred overnight in corresponding amount of 1M solution of tert-butylammonium fluoride in THF (**Table 2**, p. 49). Then THF was evaporated under reduced pressure. Row material was purified twice by column chromatography using 50 mL silica gel and THF as an eluent.   For all fractions NMR analysis has been performed. Recrystallization from i-propanol did not work, because product has not precipitated.

6.2.1.1.4 General procedure 1-tert-butyldimethylsiloxypropyl thymine deprotection using CuCl₂ / Synthesis of N-(3-hydroxypropyl) thymine
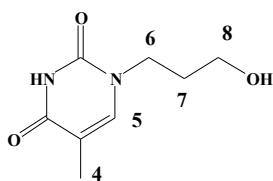
0.2975 g (1 mmol) of 1-tert-butyldimethylsiloxypropyl thymine was dissolved in 10 mL of acetone/water mixture (95:5) and 0.096 g (0.7 mmol) of $CuCl_2$ was added. The reaction mixture was heated up to 50ºC and let to stir overnight, afterwards solvents were evaporated to dryness under reduced pressure. Crude product was purified by column chromatography on silica gel with methanol as an eluente.

Yield: 0.1745 g (95%) of a white solid

^{1}H NMR (DMSO-d_6): δ [ppm] = 11.15 (s, 1H, NH), 7.48 (s, 1H, H-5), 4.54 (t, 1H, OH), 3.67 (t, 2H, H-6), 3.41 (q, 2H, H-8), 1.75 (s, 3H, H-4), 1.71 (partly overlapped with s from H-4, 2H, H-7).

^{13}C NMR (DMSO-d_6): δ [ppm] = 164.37 (C-2), 150.94 (C-1), 141.75 (C-5), 108.32 (C-3), 57.77 (C-8), 44.97 (C-6), 31.59 (C-7), 11.95 (C-4).

6.2.1.1.5 Synthesis of 6-(tetrahydro-5-methyl-2,4-dioxopyrimidine-1(2H-yl)hexane acid ethyl ester(Thym-C5-OEt)
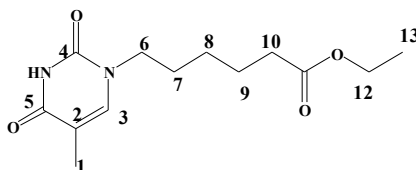
Under inert atmosphere 2.0 g (15.9 mmol) of thymine, 2.18 g (15.8 mmol) of potassium carbonate, 0.6g (1.6 mmol) of TBAI were mixed and then dissolved in 200 mL DMF containing less than 0.1% of water. After stirring for 1 h 1.2 mL (7 mmol) of 6-bromohexanoic acid ethyl ester was added. Reaction mixture was stirred for 6 days under argon atmosphere, and then poured into 600 mL of brine. It was extracted 4x100 mL with ethyl acetate. Inorganic residue was filtrated off; organic phase was dried over $MgSO_4$ and evaporated. Crude product was purified by column chromatography using n-hexane/ethyl acetate mixture as a gradient eluent varied from 50:1 to 1:2.

Yield: 0.7416 g (40.98%) of a white solid

^{1}H NMR (DMSO-d_6): δ [ppm] = 11.15 (s, 1H, NH), 7.51 (s, 1H, H-3), 4.04 (q, 2H, H-12), 3.60 (t, 2H, 6), 2.27 (t, 2H, H-10), 1.51 – 1.58 (m, 4H, H-7, H-9), 1.25 (m, 2H, H-8), 1.17 (t, 3H, H-13).

6.2.1.1.6 Synthesis of N-(2-aminoethyl)-6-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)hexanamide(Thym-C5-NH₂)
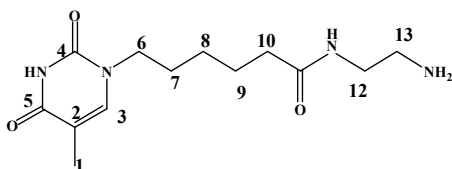
0.64 g (2.5 mmol) of Thym-C5-OEt was dissolved in 10 mL MeOH and 8 mL (0.118 mmol) of 1,2-diaminoethane was added. The resulting solution was stirred at room temperature for 5d under protection from light. The solvent and excess of 1,2-diaminoethane were evaporated

under reduced pressure. The crude product was washed two times with hot ethyl acetate (70 mL and 120 mL) in flask. The solid collected after filtration was pure enough to be used further. Still as extra purification can be performed using column chromatography on silica gel with CH_2Cl_2/MeOH (5:1) as an eluent. Thym-C5-NH$_2$ was obtained after evaporation of solvents.

Yield: 0.615 g (91.5%) of an yellowish solid or 0.404 g (56.8%) of a white solid

^{1}H NMR (DMSO-d_6): δ [ppm] = 7.74 (s, 1H, NH), 7.51 (s, 1H, H-3), 3.59 (t, 2H, H-6), 3.03 (m, 2H, H-12), 2.56 (t, 2H, 13), 2.06 (t, 2H, H-10), 1.75 (s, 3H, 1), 1.50-1.55 (m, 4H, H-7, H-9), 1.21 (m, 2H, H-8).

^{13}C NMR (DMSO-d_6): δ [ppm] = 172.12 (C-11), 164.35 (C-5), 150.92 (C-4), 141.49 (C-3), 108.42 (C-2), 47.06 (C-6), 41.79 (C-12), 41.22 (C-13), 35.25 (C-10), 28.29 (C-7), 25.50 (C-8), 24.88 (C-9), 11.94 (C-1).

6.2.1.1.7 Synthesis of N-pentyl thymine
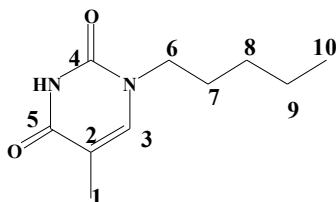
To a solution of 1g (7.9 mmol) of thymine and 0.3g of TBAI (0.8 mmol) in 130 mL of anhydrous DMF, 1.095 g (7.9 mmol) of K_2CO_3 and 2.395 g (15.9 mmol) of 1-bromopentane were added. The reaction mixture was stirred under Ar atmosphere for 3d and then the solution was poured into 300 mL of distilled water and extracted 3x50 mL of ethyl acetate. Organic phase was evaporated under reduced pressure. Crud product was purified by column chromatography on silica gel with gradient eluent mixture n-hexane/ethyl acetate varied from 3:2 to 2.1. Finally it was recrystallized from toluene and dried at 50ºC under vacuum.

Yield: 50% of white solid

^{1}H NMR (CDCl$_3$): δ [ppm] = 9.54 (1H, NH), 6.96 (s, 2H, H-3), 3.66 (t, 2H, H-6), 1.93 (m, 3H, H-1), 1.65-1.58 (m, 2H, H-7), 1.31 (m, 4H, H-8, H-9), 0.88 (t, 3H, H-10)

6.2.1.1.8 Synthesis of N-undecanoyl thymine
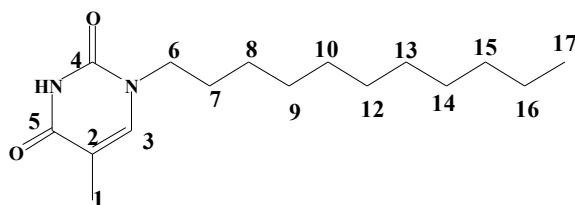
To a solution of 5.3 g (42 mmol) of thymine in 150 mL of anhydrous DMF, 7 g (50.6 mmol) of K$_2$CO$_3$ and 2.34 g (10 mmol) of 1-bromoundecane were added. The reaction mixture was stirred under Ar atmosphere for 5d, and then the solvent was evaporated under vacuum. Crude product was suspended in 200 mL of distilled water, precipitate was filtrated and dissolved in 250 mL of CHCl$_3$. Organic phase was washed 3x50 mL distilled water, dried over sodium sulphate and evaporated.

Yield: 2.8 g (51.6%) of a white solid

^{1}H NMR (DMSO-d_6): δ [ppm] = 11.14 (1H, NH), 7.51 (s, 1H, H-3), 3.58 (t, 2H, H-6), 1.74 (m, 3H, H-5), 1.57 (m, 2H, H-7), 1.23-1.27 (br, 16H, H-8, H-9, H-10, H-11, H-12, H-13, H-14, H15), 1.75 (s, 3H, 1), 0.85 (t, 3H, H-16).

6.2.1.2   Synthesis of templates on 2,6-diaminopyridine basis

6.2.1.2.1 Synthesis of N,N'-(4-pentenoyl)-2,6-diaminopyridine [116]
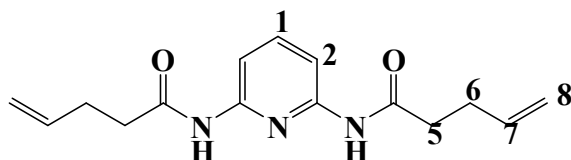
2.1 mL (20.6 mmol) of 4-pentenoic acid was stirred in 80 mL of dry dichloromethane under argon atmosphere and then 2.0 mL (23.2 mmol) of oxalyl chloride and 0.1 mL DMF were injected. After 2 h stirring reaction mixture was added dropwise to the solution of 1,108 g

(10.1 mol) of 2,6-diaminopyridine and 3.1 mL (22 mmol) of triethylamine in 130 mL of dry dichloromethane. This mixture was stirred overnight under argon atmosphere. Organic phase was washed 3×50 mL of brine and dried over Na_2SO_4. Solvent was evaporated, and crude product was purified by column chromatography. The eluent, a n-hexane/ethyl acetate mixture, was varied from 100:1 to 2:1.

Yield: 1.42 g (51.46%) of a white solid

Melting point: 98.5ºC

^{1}H NMR (DMSO-d_6): δ [ppm] = 9.99 (s, 1H, NH), 7.71 (s, 3H, H-1, H-2), 5.84 (m, 2H, H-7), 5.06 and 4.97 (dd, 4H, H-8), 2.50 overlapped with DMSO-d_6, H-5), 2.33 (q, 4H,H-6).

^{13}C NMR (DMSO-d_6): δ [ppm] = 171.42 (C-4), 150.33 (C-3), 139.83 (C-1), 137.50 (C-7), 115.21 (C-8), 109.06 (C-2), 35.24 (C-5), 28.90 (C-6).

6.2.1.2.2 Synthesis N,N'-(5-hydroxypentanoyl)-2,6-diaminopyridine [116]
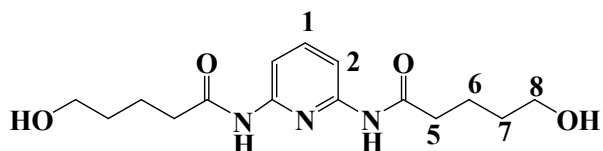
18 mL of 0.5 mol L^{-1} solution of 9-BBN in THF was added dropwise to a solution of 0.54 g (1.98 mmol) N,N'-4-dipentenoyl-2,6-diamiopyridine in 4 mL of dry THF at room temperature. After stirring for 1 h at room temperature 5 mL H_2O was added dropwise, followed by 10 mL of 3 M solution of NaOH in water. Then 10 mL of 30% H_2O_2 solution was added carefully to maintain the reaction temperature between 30ºC and 50ºC. The resulting mixture was stirred overnight and than heated up to 50ºC in water bath for 4h. The precipitate was filtered, and the filtrate was evaporated and purified with flash chromatography on silica gel with CH_2Cl_2/MeOH (20:1 volume). Product was dried under vacuum for 20 h after the evaporation of solvents.

Yield: 0.213 g (34.8%) of a white solid

Melting point: 125-126ºC

^{1}H NMR (DMSO-d_6): δ [ppm] = 9.93 (s, 1H, NH), 7.70 (s, 3H, H-1, H-2), 4.36 (s, 2H, OH), 3.40 (t, 4H, H-8), 2.39 (t, 4H, H-5), 1.69 (p, 4H,H-6), 1.44 (p, 4H,H-7).

^{13}C NMR (DMSO-d_6): δ [ppm] = 172.21 (C-4), 150.37 (C-3), 139.82 (C-1), 109.01 (C-2), 60.47 (C-8), 35.98 (C-5), 32.02 (C-7), 21.72 (C-6).

6.2.1.3 Synthesis of N,N'-(pyridine-2,6-diyl)bis(6-(tert-butyldimethylsilyloxy)hexanamide (DAPy-C5-OTBDMS)
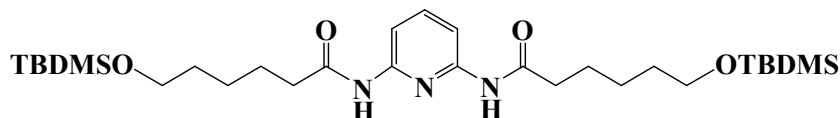
1.69 g (4.7 mmol) of TBDMS-O-C5-COOTBDMS was dissolved in18 mL of CH$_2$Cl$_2$ and resulting solution was cooled down to 0ºC, then 0.45 mL (5.2 mmol) of oxalyl chloride was added together with one drop of DMF. Cooling was maintained for 1.5 h. The resulting mixture was added dropwise to the solution of 0.25 g (2.3 mmol) of DAPy and 2 mL (14 mmol) of Et$_3$N in 40 mL of CH$_2$Cl$_2$. The reaction mixture was stirred overnight at room temperature, and then it was washed 3×25 mL of brine. The organic phase was collected and dried over Na$_2$SO$_4$, and then solvent was evaporated under reduced pressure.

Yield: no desired product was isolated

6.2.1.4 Synthesis N,N'-(4-hydroxybutanoyl)-2,6-diaminopyridine via aminolysis of gamma-butyrolactone

6.2.1.4.1 Aminolysis of γ-butyrolactone in melt

1.10 g (10 mmol) of 2,6–diaminopyridine was dissolved in 15.4 mL (124.2 mmol) of γ-butyrolactone under argon atmosphere. Reaction mixture was heated up to 120ºC, and stirred

overnight. As reaction proceeded TLC were made. After the reaction reached equilibrium, excess of γ-butyrolactone was evaporated under reduced pressure at 80ºC. The crude product was applied to column chromatography on silica gel with CH_2Cl_2:MeOH (4:1) mixture as an eluent. It was not possible to isolate the desired product; potential yield was estimated using ^{1}H NMR spectroscopy

Yield: 5-10% of a desired product in a crude product

6.2.1.4.2 Aminolysis of γ-butyrolactone in solution

5.46 g (50 mmol) of 2,6–diaminopyridine was dissolved in 100 mL of dry DMF under argon atmosphere. To this mixture 12.6 mL (101.6 mmol) of γ-butyrolactone was added dropwise and the reaction mixture was at first let to react overnight at room temperature, then placed for 1d into an oil bath heated up to 65ºC, then temperature was raised up to 100ºC and reaction proceeded for 5 more days.

Yield: no desired product was isolated

6.2.1.4.3 Aminolysis of γ-butyrolactone in solution with NaH catalysis

5.52 g (50.6 mmol) of 2,6–diaminopyridine was dissolved in 250 mL of dry DMF under argon atmosphere. To this mixture 12.4 mL (100 mmol) of γ-butyrolactone was added dropwise followed by 2.43 g (100 mmol) of NaH. The reaction mixture was left to react overnight at 140ºC and was followed by TLC. Excess of γ-butyrolactone and solvent were distilled of under reduced pressure. The crude product was purified by column chromatography on silica gel using CH_2Cl_2:MeOH (4:1) mixture as an eluent.

Yield: no desired product was isolated

6.2.2   Synthesis of di-DAPy derivatives

6.2.2.1   Synthesis of N,N-bis(6-aminopyridin-2-yl)adipamide. Synthesis of di-DAPy with adipoyl chloride (di-DAPy-C6)
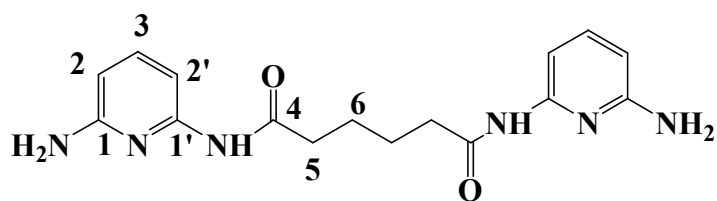
Corresponding amounts of DAPy and triethylamine (**Table 16**) were dissolved in THF. Reaction mixture was cooled down and corresponding amount of adipoyl chloride was added dropwise. Reaction mixture was stirred overnight at room temperature. Then it was washed with distilled water. Organic phase was collected, dried over magnesium sulphate and evaporated. The purification with column chromatography was not possible due to the too close R_f of all oligomers.

Ratios of starting materials and yields could be found in **Table 16**.

Table 16 Reagents ratios and results for the reaction of DAPy with adipoyl chloride

Amount				Yield	NMR cooments
DAPy	Adipoyl chloride	Et$_3$N	Solvent		
4.37 g 40 mmol	1.5 mL 13.5 mmol	2.78 mL 20 mmol	THF 50 mL	2.24 g (55.6%)	Oligomers
2.4 g 22 mmol	1.25 mL 8.5 mmol	1.8 mL 13 mmol	CH$_2$Cl$_2$ 200 mL	1.55 g (55%)	Oligomers
4.9 g 45 mmol	0.16 mL 1 mmol	13 mL 93 mmol	CH$_2$Cl$_2$ 350 mL	1.77 g (40%)	Oligomers

^{1}H NMR (DMSO-d_6): δ [ppm] = 9.745 (s, NH), 7.70 (s, H-3), 7.54 (t, H-3), 7.21 (d, H-2'), 6.13 (d, H-2), 5.63 (s, NH$_2$), 2.33 (br, H-5), 1.54 (br, H-6).

6.2.2.2   Modification of di-DAPy-C6 with 4-pentenoic acid. Synthesis of N,N-bis(6-pent-4-enamidopyridin-2-yl)adipamide
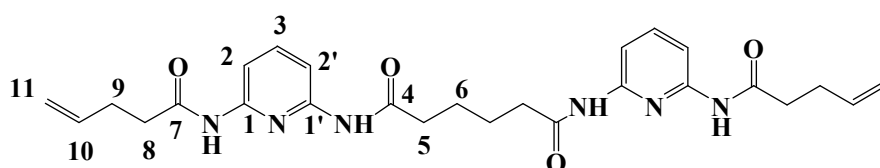
0.208 mL (2 mmol) of 4-pentenoic acid was mixed with 0.198 mL (2.3 mmol) of oxaly chloride in 10 mL of CH_2Cl_2, one drop of DMF was used as a catalyst. Reaction mixture was stirred for 2h and then was added dropwise to a cold solution of 0.4 g (1.2 mmol) of di-DAPy-C6, 0.307 mL (2.2 mmol) of Et_3N in 15 mL of CH_2Cl_2. Reaction was stirred at room temperature for 24h. The excess of solvent was evaporated and crude product was submitted to ^1H NMR spectroscopy. No further purification was done, because the reaction has not gone through.

Yield: no desired product was isolated

6.2.2.3 Modification of di-DAPy-C6 with pivaloyl chloride. Synthesis of N,N-bis(6-pivalamidopyridin-2-yl)adipamide
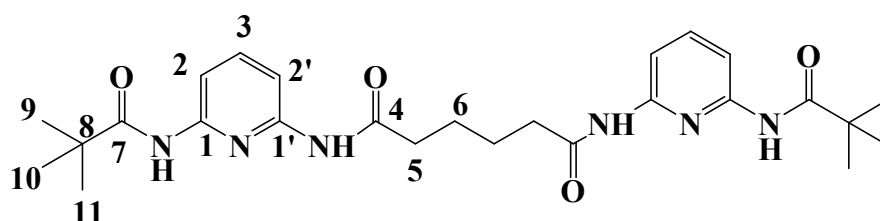
To a suspension of 0.2 g (0.61 mmol) of di-DAPy-C6 in 5 mL of pyridine 0.2 mL (1.6 mmol) of pivaloyl chloride and 1 mL of anhydrous DMF were added. Reaction mixture was led to react at room temperature for 24h. Then solvent was evaporated and crude material was purified by column chromatography on silica gel with hexane/ethyl acetate (10:1) mixture as an eluent.

Yield: no desired product was isolated

6.2.2.4 Modification of di-DAPy-C6 with acetic acid anhydride. Synthesis of N,N-bis(6-acetamidopyridin-2-yl)adipamide
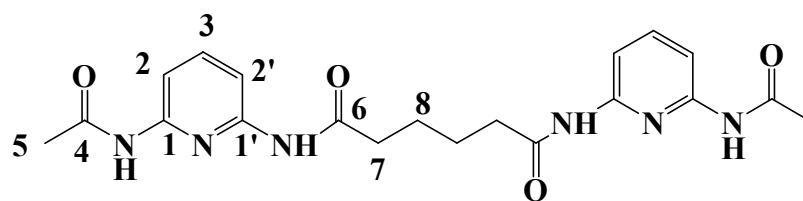
0.1 g (0.3 mmol) of di-DAPy-C6 was suspended in 50 mL of CH_2Cl_2 then it was cooled down to 0°C and 5.7 mL (60.4 mmol) of acetic anhydride was slowly added. The reaction mixture was stirred at room temperature overnight. After the reaction was completed, the precipitate was filtered off and washed with 100 mL water and 2×50 mL ethyl acetate. It was dried under vacuum.

Yield: 0.09 g (72.4 %) of a cream solid

¹H NMR (DMSO-d_6): δ [ppm] = 9.99 and 10.03 (d, NH), 7.70 (s, 6H, H-2, H-2', H-3), 2.42 (4H, H-7), 2.1 (4H, H-5), 1.61 (m, 4H, H-8).

¹³C NMR (DMSO-d_6): δ [ppm] = 172.16 (C-4), 169.41 (C-6), 150.26 and 150.18 (C-1 and C-1'), 140.07 (C-3), 109.03 and 108.91 (C-2 and C-2'), 36.06 (C-7), 24.73 (C-8), 24.04 (C-5).

6.2.2.5 Synthesis of DAPy-mono-C8
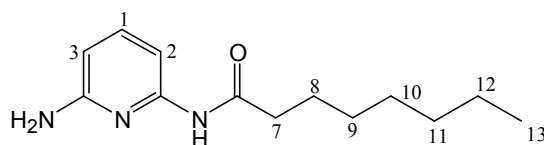
2 g (18.3 mmol) of 2,6-diaminopyridine and 1.95 g (19.2 mmol) of triethylamine were mixed together in 37 mL/12.3 mL of TFA/ THF mixture , the resulting solution was cooled down to -3°C, then 2.95 g (18.1 mmol) of octanoyl chloride was added dropwise during 30 min. The reaction mixture was kept cold for three more hours and then stirred overnight at room temperature. The excess of TFA was distilled off under reduced pressure and crude product was purified by column chromatography on silica gel using ethyl acetate as an eluent.

Yield: 0.5 g (11.6%) of a white solid

^{1}H NMR (DMSO-d_6): δ [ppm] = 9.7 (s, 1H, NH), 7.30 (t, 1H, H-1) 7.21 (d, 1H, H-2), 6.1 (d, 1H, H-3), 5.65 (s, 2H, NH$_2$), 3.40 (t, 4H, H-8), 2.31 (t, 2H, H-7), 1.53 (m, 2H,H-8), 1.24 (p, 8H, H-9, H-10, H-11, H-12), 0.857 (t, 3H, H-13).

6.2.2.6   Synthesis of N-(6-aminopyridin-2-yl)octanamide (DAPy-mono-C8)

2 g (18.3 mmol) of 2,6-diaminopyridine and 1.95 g (19.2 mmol) of triethylamine were mixed together in 37 mL/12.3 mL of TFA/ THF mixture , the resulting solution was cooled down to -3ºC, then 2.95 g (18.1 mmol) of octanoyl chloride was added dropwise during 30 min. The reaction mixture was kept cold for three more hours and then stirred overnight at room temperature. The excess of TFA was distilled off under reduced pressure and crude product was purified by column chromatography on silica gel using ethyl acetate as an eluent.

Yield: 0.5 g (11.6%) of a white solid

^{1}H NMR (DMSO-d_6): δ [ppm] = 9.7 (s, 1H, NH), 7.30 (t, 1H, H-1) 7.21 (d, 1H, H-2), 6.1 (d, 1H, H-3), 5.65 (s, 2H, NH$_2$), 3.40 (t, 4H, H-8), 2.31 (t, 2H, H-7), 1.53 (m, 2H,H-8), 1.24 (p, 8H, H-9, H-10, H-11, H-12), 0.857 (t, 3H, H-13).

6.2.2.7   Synthesis of N,N-bis(6-octanamidopyridin-2-yl)adipamide (di-C6-DAPy-C8)
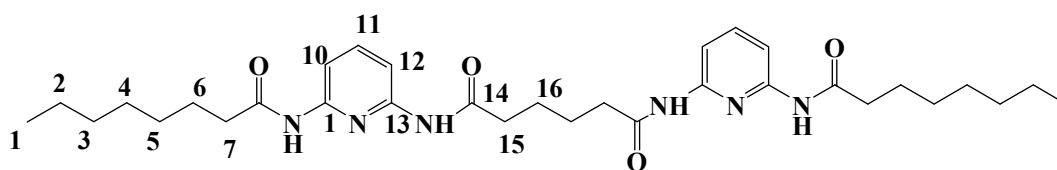
0.2 g (mmol) of DAPy-mono-C8 and 5 mL (62 mmol) of pyridine were mixed together, the resulting solution was cooled down to -3ºC, then 0.2 g (1.1 mmol) of adipoyl chloride was

added dropwise. The reaction mixture was kept cold for one more hours and then stirred overnight at room temperature. Precipitate was filtrated off. The crude product was applied to the column chromatography on silica gel with CH_2Cl_2:MeOH (1:1) as an eluent. The excess of solvent was distilled off and crude product was subjected to ^{1}H NMR analysis.

Yield: no clean product was isolated

^{1}H NMR (DMSO-d_6): δ [ppm] = 9.796 and 9.92 (ds, NH), 7.69 (s, H-10, H-11, H-12), 6.61(H-3 in di-DAPy-mono-C8), 5.3 (NH$_2$ in Di-DAPy-mono-C8), 2.4 (m, H-7, H-15), 2.21 (t, H-7, adipoyl chloride), 2.05 (m, H-6 adipoyl chloride), 1.57 (m, H-6, H-16), 1.25 (br, H-2, H-3, H-4, H-5), 0.85 (t, H-1).

6.2.3 Synthesis of adenine derivatives

6.2.3.1 Synthesis of 9,9'-(Hexane-1,6-dyl)diadenine and general procedure of synthesis of di-adenine with NaH as a catalyst [185]
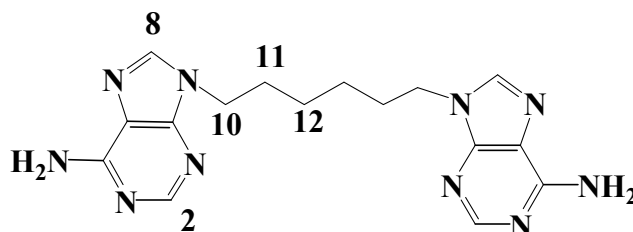
10.1 g (74.8 mmol) of adenine was suspended in 300 mL of dry DMF and 1.976 g (82.3 mmol) of sodium hydride was added. In 1h 6 mL (36.9 mmol) of 1,6-dibromohexane was injected into the reaction mixture, which was stirred overnight at room temperature. Precipitate was filtrated and DMF solution was washed 3x50 mL of water, 2x80 mL of acetone and 2x80 mL of chloroform, and then evaporated under reduced pressure. Product was dried in the oven at 50ºC under vacuum for 2d. Recrystallization was not possible, though described in literature. No further purification was needed according to the ^{1}H NMR.

Yield: 6.568 g (50.5%) of a white solid.

¹H NMR (DMSO-*d₆*): δ [ppm] = 8.81 (d, 4H, H2, H-8), 7.12 (s, 4H, NH$_2$), 4.09 (t, 4H, H-10), 1.78 (t, 4H, H-11), 1.26 (br, 4H, H-12).

All other di-adenine and tri-adenine derivatives were synthesized either according to this methodology [185] or to the one used by Itahara [128]. In the later cases NMR spectra and yields were similar to the literature data.

6.2.3.2   Synthesis of 9-pentyladenine
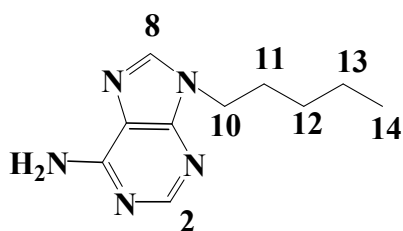
1 g (7.4 mmol) of adenine was dissolved in 130 mL of dried DMF under Ar atmosphere. 1.02 g (7.4 mmol) of K$_2$CO$_3$, 2.235 g (14.8 mmol) of 1-bromopentane and finally 0.3 g (0.8 mmol) TBAI were added. The reaction mixture was stirred at room temperature for 3d. Solution was then poured into 300 mL of distilled water and extracted with 2x50 mL of ethyl acetate. The excess of ethyl acetate was evaporated under reduced pressure. No further purification was needed.

Yield: 1.36 g (90%) of a white solid

¹H NMR (DMSO-*d₆*): δ [ppm] = 8.37 (s, 1H, H-2), 7.79 (s, 1H, H-8), 5.61 (s, 2H, NH$_2$), 4.19 (t, 2H, H-10), 1.90 (q, 2H, H-11), 1.34 (m, 4H, H-12, H-13), 0.9 (t, 3H, H-14).

6.2.4   Synthesis of dendritic polymers

6.2.4.1   Synthesis of dendritic structures

6.2.4.1.1   Synthesis of isopropylidene-2,2-bis(methoxy)propionic acid [23]
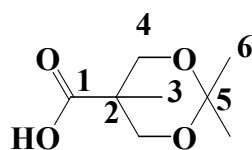
5.1 g (38 mmol) of bis-MPA was suspended in 35 mL of acetone. After adiition of 0.37g (1.9 mmol) of pTSA, 7.1 mL (57.9 mmol) of DMP reaction mixture has cleared and was stirred for at room temperature 4h. Reaction was stopped and 7 mL of 0.1M NH_3 in ethanol solution was added to neutralize pTSA, then solvents were evaporated under reduced pressure. Crude product was dissolved in 110 mL of CH_2Cl_2 and washed with 2x20 mL of distilled water. Organic phase was evaporated after drying over Na_2SO_4. No additional purification was needed.

Yield: 5.0 g (75.4%) of a white or transparent glass-like solid

^{1}H NMR (DMSO-d_6): δ [ppm] = 2.35 (s, 1H, COOH), 4.01 and 3.55 (dd, 4H, H-4$_{ax, eq}$), 1.34 and 1.27 (ds, 6H, H-6$_{ax, eq}$), 1.08 (s, 3H, H-1).

^{13}C NMR (DMSO-d_6): δ [ppm] = 175.53 (C-1), 97.29 (C-5), 65.24 (C-4), 40.76 (C-2), 24.59 and 22.87 (C-6$_{ax, eq}$), 18.44 (C-3).

6.2.4.1.2 Synthesis of 2,2-bis(acetoxymethyl)propionic acid [239]
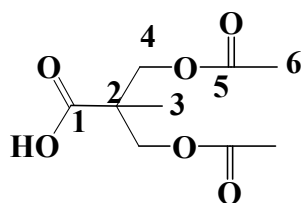
15 g (111.8 mmol) of bis-MPA , 0.69 g (5.6 mmol) of DMAP and 39 mL (265.4 mmol) of triethylamine were mixed in 200 mL of CH_2Cl_2. Reaction mixture was cooled down to 0ºC and 13.4 mL (188.8 mmol) of acetyl chloride was added dropwise. Reaction flask was stirred at room temperature for 4h and then precipitate was filtrated off. Organic phase was evaporated and product dried under vacuum.

Yield: 14.6 g (60%) of a white solid

^{1}H NMR (DMSO-d_6): δ [ppm] = 4.25 (m, 4H, CH_2), 2.06 (s, 6H, CH_3COO), 1.28 (s, 3H, CH_3).

^{13}**C NMR (DMSO-d_6)**: δ [ppm] = 177.96 (C-1), 170.84 (C-5), 65.15 (C-4), 45.66 (C-2), 20.60 (C-6), 17.63 (C-3).

6.2.4.1.3 Synthesis of 3-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)propyl 2,2-dimethyl-1,3-dioxane-5-carboxylate (acetonide-[G#1]-O-Thym) and general procedure for synthesis of acetonide-protected dendrimers
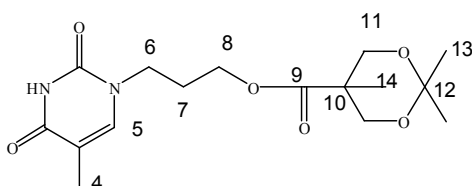
0.1 g (0.57 mmol) of isopropylidene-2,2-bis(methoxy)propionic acid was suspended in 3 mL of CH$_2$Cl$_2$, 0.112 g (0.69 mmol) of CDI together with catalytic amounts of DMAP were first dissolved in 2 mL of CH$_2$Cl$_2$ and then injected into the reaction mixture. Activation was going for 1h and then suspension of 0.147 g (0.8 mmol) of Thym-C3-OH in 2 mL CH$_2$Cl$_2$ was added, followed by several DMF drops in order to improve solubility. In 2d the reaction has been completed at room temperature and solvent was evaporated. Crude product was purified by column chromatography on silica gel with CH$_2$Cl$_2$/MeOH (3:1) mixture as an eluent, followed by another one with CH$_2$Cl$_2$/MeOH (10:1) mixture as an eluent.

Yield: was too low to be estimated

1.8 g (10.3 mmol) of isopropylidene-2,2-bis(methoxy)propionic acid, 1.86 g (11.5 mmol) of CDI and 0.1464 g (1.2 mmol) of DMAP were dissolved in 60 mL of anhydrous DMF. Activation was going for 2h and then 1.5 g (8.1 mmol) of Thym-C3-OH in 10 mL of DMF was added. Reaction mixture was stirred overnight at room temperature, then solvent was evaporated. Crude product was dissolved in 20 mL of CH$_2$Cl$_2$ and washed with 2x50 mL 1% solution of KHSO$_4$, 2x50 mL of saturated NaHCO$_3$ solution and 2x50 mL of brine. Organic phase was collected, dried over MgSO$_4$ and evaporated.

Yield: 1.9 g (68.6%) of a white solid

ESI-MS (theor. 340.37 g/mol): 341.1 $(M+H)^+$, 358.41 $(M+NH_4)^+$

^{1}H NMR (DMSO-d_6): δ [ppm] = 11.19 (s, 1H, NH), 7.47 (s, 1H, H-5), 4.09 (t, 2H, H-8), 4.04 and 3.60 (dd, 4H, H-11$_{ax, eq}$), 3.71 (t, 2H, H-6), 1.92 (t, 2H, H-7), 1.75 (s, 2H, H-4), 1.35 and 1.26 (ds,6H, H-13$_{ax, eq}$), 1.07 (s, 3H, H-14).

^{13}C NMR (DMSO-d_6): δ [ppm] = 173.68 (C-9), 164.29 (C-2), 150 (C-1), 141.36 (C-5), 108.59 (C-3), 97.45 (C-12), 65.09 (C-11), 61.67(C-8), 44.60 (C-6), 41.32 (C-10), 27.62 (C-7), 25.17 and 22.26 (C-13$_{ax, eq}$), 18.11 (C-14), 11.95 (C-4).

6.2.4.1.4 Synthesis of 75% modified (OH)$_2$-[G#1]-O-Thym ((acetonide-[G#1/2]-O-Thym)

00.134 g (0.77 mmol) of isopropylidene-2,2-bis(methoxy)propionic acid was mixed with 0.146 g (0.9 mmol) of CDI and catalytic amounts of DMAP in 5 mL of dry DMF. In 2h solution of 0.1917 g (0.64 mmol) of (OH)$_2$-[G#1]-O-Thym in 2 mL of dry DMF was added. The reaction mixture was stirred for 24h and then it was worked up according to the standard procedure.

Yield: 0.2148 g (58.5%) of glass-like solid.

^{1}H NMR (DMSO-d_6): δ [ppm] = 11.19 (s, 0.918H, NH), 7.49 (s, 0.438H, H-5)and 7.47 (s, 0.498H, H-5'), 4.98 (t, 0.475H, OH), 4.24 and 4.23(d,H, H11 G#1), 4.12 (11'' G#1), 4.08 (t,

1.013H, H-8), 4.04 (t, 1.040H, H-8'), 3.99 and 3.60 (dm, 2.738H and 2.821H, H-11 G#2 and H-11' G#2), 3.70 (t, 1.920H, H-6 and H-6'), 3.53 (d, 1.007H, H-11' G#1), 1.90 (m, 2H, H-7 and H-7'), 1.75 t(s, H-4 and H-4'), 1.35, 1.24 and 1.22 (s, H-13 and H-13'), 1.225, 1.12, 1.04 and 1.02 (qs, H-14 G#1, H-14' G#1, H-14 G#2, H-14' G#2).

6.2.4.1.5 Synthesis of acetonide-[G#2]-O-Thym
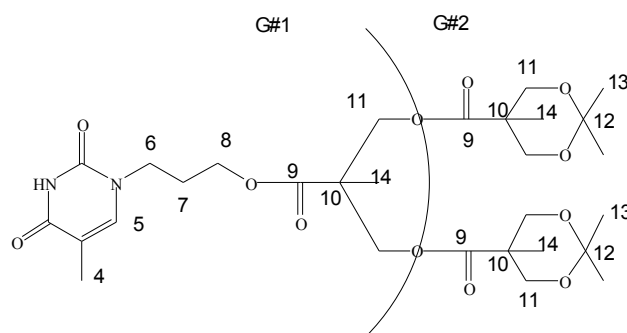
0.051 g (0.3 mmol) of isopropylidene-2,2-bis(methoxy)propionic acid was mixed with 0.0567 g (0.35 mmol) CDI and catalytic amounts of DMAP in 5 mL of dry DMF. In 2h solution of 0.09 g of partly modified (OH)$_2$-[G#1]-O-Thym in 5 mL of dry DMF was added. The reaction mixture was stirred for 24h and then it was worked up according to the standard procedure.

Yield: 0.1881g (88.3%)

ESI-MS (theor. 612.29 g/mol): 630.3 (M+NH$_4$)$^+$

^{1}H NMR (DMSO-d_6): δ [ppm] = 11.2 (s, 1H, NH), 7.49 (s, 1H, H-5), 4.24 and 4.23 (d, 4H, H-11 G#1), 4.08 (t, 2H, H-8), 3.98 and 3.61 (dd, 8H, H-11 G#2), 3.70 (t, 2H, H-6), 1.91 (p, 2H, H-7), 1.75 (s, 3H, H-4), 1.35 and 1.21 (ds, 12H, H-13$_{ax,eq}$), 1.22 (s, 3H, H-14 G#1), 1.02 (s, 6H, H-14 G#2).

^{13}C NMR (DMSO-d_6): δ [ppm] = 173.22 (C-9 G#2), 172.14 (C-9 G#1), 164.26 (C-2), 150.90 (C-1), 141.25 (C-5), 108.63 (C-3), 97.45 (C-12),65.12 and 65.10 (C-11 G#2), 64.92 (C-11 G#1), 62.19 (C-8), 46.33 (C-10 G#1), 44.39 (C-6), 41.60 (C-10 G#1), 27.64 (C-7), 25.86 and 21.39 (C-13$_{ax,eq}$), 17.95 (C-14 G#2), 17.16 (C-14 G#1), 11.93 (C-4).

6.2.4.1.6 Synthesis of acetonide-[G#3]-O-Thym
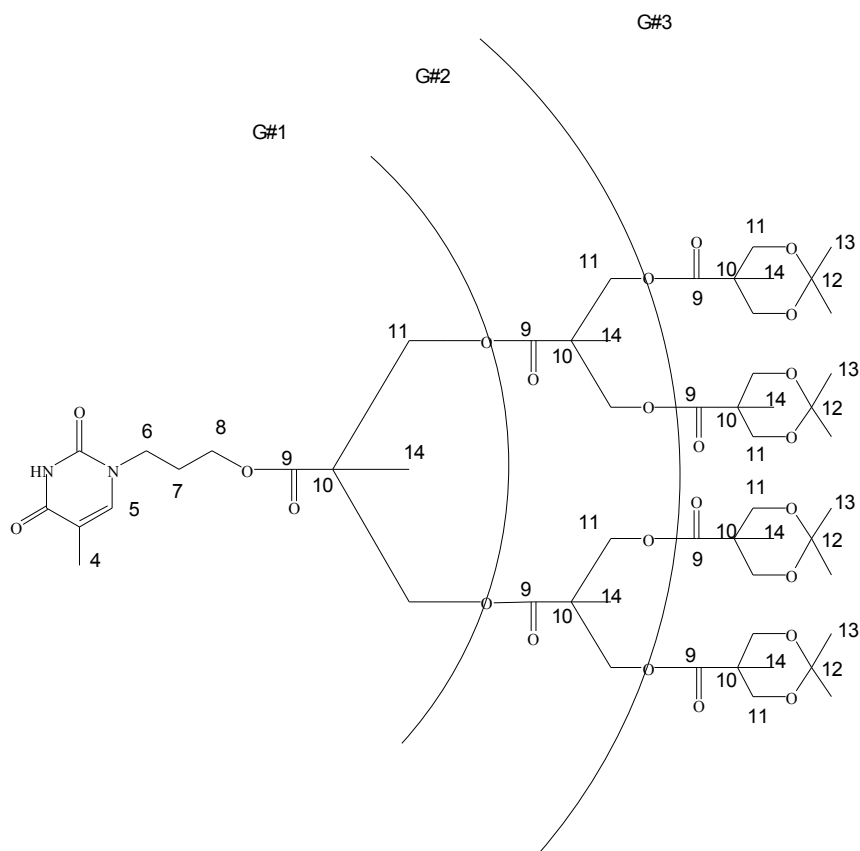
0.159 g (0.91 mmol) of isopropylidene-2,2-bis(methoxy)propionic acid was mixed with 0.173 g (1.07 mmol) of CDI and catalytic amounts of DMAP in 5 mL of dry DMF. In 2h solution of 0.09 mg (0.17 mmol) of $(OH)_4$-[G#2]-O-Thym in 7 mL of dry DMF was added. The reaction mixture was stirred for 5d and then it was worked up according to the standard procedure.

Yield: 0.154 g (71.8%)

^{1}H NMR (DMSO-d_6): δ [ppm] = 11.17 (s, 1H, NH), 7.48 (s, 1H, H-5), 4.24 and 4.20 (d, 4H, H-11 G#1), 4.21 (s, 8H, H-11 G#2), 4.08 (t, 2H, H-8), 3.98 and 3.60 (dd, 16H, H-11 G#3), 3.71 (t, 2H, H-6), 1.92 (p, 2H, H-7), 1.75 (s, 3H, H-4), 1.34 and 1.22 (ds, 24H, H-13$_{ax,eq}$), 1.21 (s, 3H, H-14 G#1), 1.20 (s, 6H, H-14 G#2), 1.02 (s, 12H, H-14 G#3).

^{13}C NMR (DMSO-d_6): δ [ppm] = 173.20 (C-9 G#3), 171.86 (C-9 G#2), 171.69 (C-9 G#3), 164.24 (C-2), 150.98 (C-1), 141.19 (C-5), 108.65 (C-3), 97.46 (C-12), 65.76 (C-11 G#1), 65.10 and 65.06 (C-11 G#3), 64.71 (C-11 G#2), 62.32 (C-8), 46.44 (C-10 G#2), 46.18 (C-10

G#1), 44.33 (C-6), 41.57 (C-12), 27.65 (C-7), 25.72 and 21.52 (C-13$_{ax,eq}$), 17.93 (C-14 G#3), 17.14 (C-14 G#2), 16.94 (C-14 G#1), 11.94 (C-4).

6.2.4.1.7 Synthesis of acetonide-[G#3/4]-O-Thym
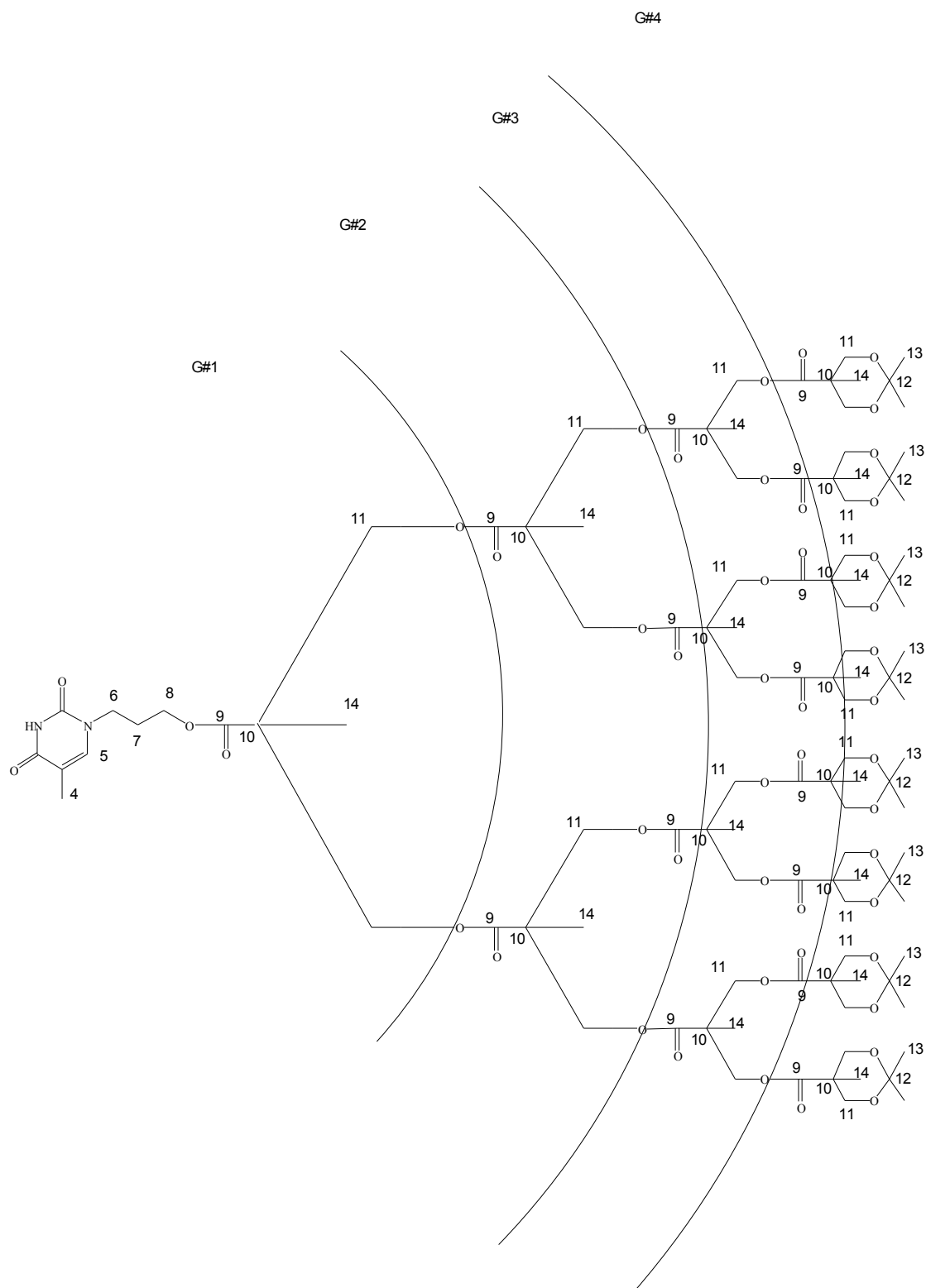
0.227 g (1.3 mmol) of isopropylidene-2,2-bis(methoxy)propionic acid was mixed with 0.247 g (1.5 mmol) of CDI and catalytic amounts of DMAP in 5 mL of dry DMF. In 2h solution of 0.118 g (0.12 mmol) of (OH)$_8$-[G#3]-O-Thym in 3 mL of dry DMF was added. The reaction mixture was stirred for 7d and then it was worked up according to the standard procedure.

Yield: 0.14 g of substance with only 60% conversion of end groups

^{1}H NMR (DMSO-d_6): was to complex to be fully analyzed

6.2.4.2   Synthesis of dendrimers with deprotected end-groups

6.2.4.2.1 Synthesis of 3-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)propyl 3-hydroxy-2-(hydroxymethyl)-2-methylpropanoate ((HO)$_2$-[G#1]-O-Thym) and general procedure for deprotaction of acetonide-protected dendrimers
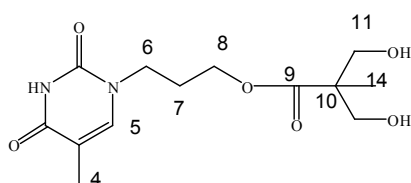
0.1038 g of DOWEX 50WX2 was added to a solution of 0.3044 g (0.89 mmol) of acetonide-[G#1]-O-Thym in 5 mL of MeOH. The mixture was stirred at 50ºC and deprotection was followed with TLC in THF. Once the reaction was complete, the resin was filtrated off and washed with MeOH. The filtrate was evaporated, and product dried overnight under vacuum.

Yield: 0.2293 g (85.4%) of a colourless glass-like solid.

ESI-MS (theor. 300.13 g/mol): 301.13 (M+H)$^+$, 318.16 (M+NH$_4$)$^+$

^{1}H NMR (DMSO-d_6): δ [ppm] = 11.19 (s, 1H, NH), 7.46 (s, 1H, H-5), 4.67 (s, 2H, OH), 4.00 (t, 2H, H-8), 3.69 (t, 2H, H-6), 3.53 and 3.42 (dq, 4H, H-11), 1.89 (p, 2H, H-7), 1.74 (s, 3H, H-4), 1.06 (s, 3H, H-14).

^{13}C NMR (DMSO-d_6): δ [ppm] = 174.66 (C-9), 164.32 (C-2), 150.90 (C-1), 141.52 (C-5), 108.55 (C-3), 64.02 (C-11), 60.73 (C-8), 50.36 (C-10), 44.58 (C-6), 27.56 (C-7), 16.89 (C-14), 11.92 (C-4).

6.2.4.2.2 Synthesis of (HO)$_4$-[G#2]-O-Thym
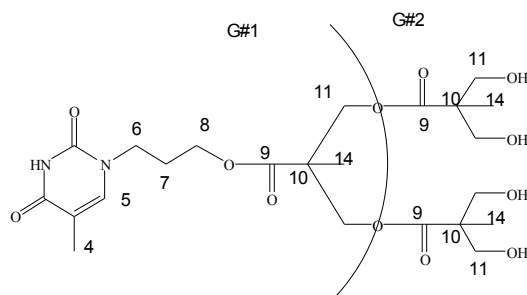
0.12 g (0.23 mmol) of acetonide-[G#2]-O-Thym, 0.13 g of DOWEX 50WX2 and 5 mL of MeOH were allowed to react according to the general deprotection procedure to give (HO)$_4$-[G#2]-O-Thym.

Yield: 0.0936 g (90.86%) of a colourless glass-like solid

ESI-MS (theor. 532.54 g/mol): 458.17 (M-G+Ac)$^+$

^{1}H NMR (DMSO-d_6): δ [ppm] = 11.19 (s, 1H, NH), 7.47 (s, 1H, H-5), 4.61 (t, 4H, OH), 4.15 and 4.14 (d, 4H, H-11 G#1), 4.08 (t, 2H, H-8), 3.69 (t, 2H, H-6), 3.47 and 3.41 (dm, 8H, H-11 G#2), 1.91 (p, 2H, H-7), 1.75 (s, 3H, H-4), 1.19 (s, 3H, H-14 G#1), 1.02 (s, 6H, H-14 G#2).

^{13}C NMR (DMSO-d_6): δ [ppm] = 174.14 (C-9 G#2), 172.37 (C-9, G#1), 164.30 (C-2), 150.92 (C-1), 141.31 (C-5), 108.66 (C-3), 64.88 (C-11 G#1), 63.80 (C-11 G#2), 62.05 (C-8), 50.36 (C-10 G#2), 46.31 (C-10 G#1), 44.52 (C-6), 27.64 (C-7), 17.18 (C-14 G#1), 11.77 (C-14 G#2), 11.95 (C-4).

6.2.4.2.3 Synthesis of (HO)$_8$-[G#3]-O-Thym
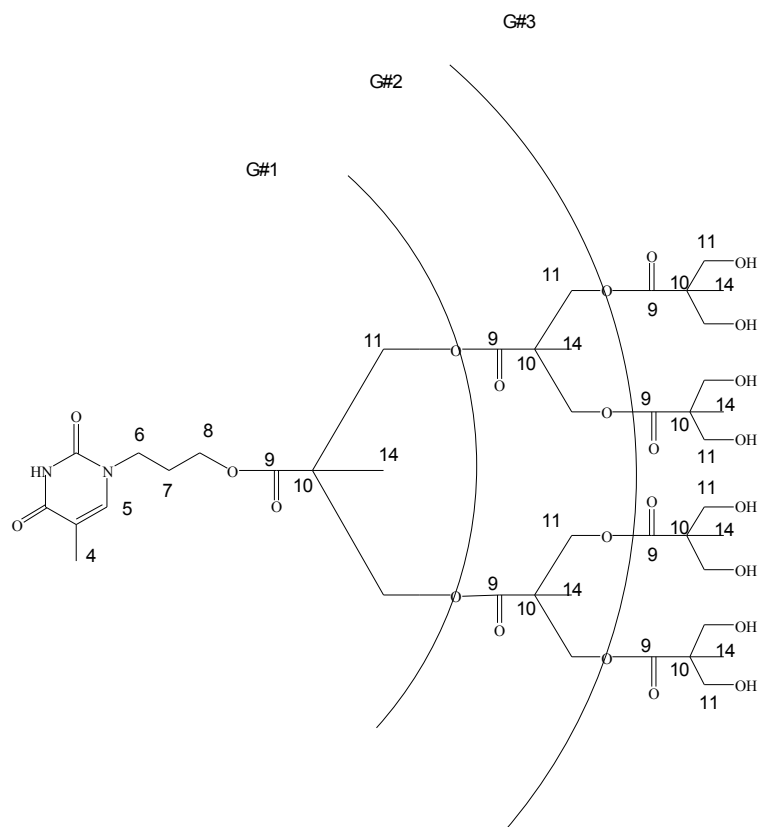
0.15 g (0.136 mmol) acetonide-[G#3]-O-Thym, 0.18 g of DOWEX 50WX2 and 10 mL of MeOH were allowed to react according to the general deprotection procedure to give $(HO)_8$-[G#3]-O-Thym.

Yield: 0.129 mg (95%) of an yellowish glass-like solid

¹H NMR (DMSO-d_6): δ [ppm] = 11.17 (s, 1H, NH), 7.45 (s, 1H, H-5), 4.59 (s, 8H, OH), 4.24 and 4.18 (dd, 4H, H-11 G#1), 4.11 (d, 8H, H-11 G#2), 4.08 (t, 2H, H-8), 3.70 (t, 2H, H-6), 3.46 and 3.41 (dd, 16H, H-11 G#3), 1.92 (p, 2H, H-7), 1.75 (s, 3H, H-4), 1.20 (s, 3H, H-14 G#1), 1.16 (s, 6H, H-14 G#2), 1.01 (s, 12H, H-14 G#3).

¹³C NMR (DMSO-d_6): δ [ppm] = 174.10 (C-9 G#3), 171.92 (C-9, G#1), 171.88 (C-9 G#2), 164.28 (C-2), 150.92 (C-1), 141.24 (C-5), 108.70 (C-3), 65.73 (C-11 G#1), 64.56 (C-11 G#2), 63.74 (C-11 G#3), 62.29 (C-8), 50.30 (C-10 G#3), 46.37 (C-10 G#2), 46.19 (C-10 G#1), 44.39 (C-6), 27.66 (C-7), 16.94 (C-14 G#1), 17.17 (C-14 G#2), 16.74 (C-14 G#3), 11.95 (C-4).

6.2.4.3   Synthesis of dendrimers with Thym-C5-NH$_2$ core

6.2.4.3.1   Synthesis of 2-methyl-2-(2-(6-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)hexanamido)ethylcarbamoyl)propane-1,3-diyl diacetate (acetyl-[G#1]-NH-Thym)
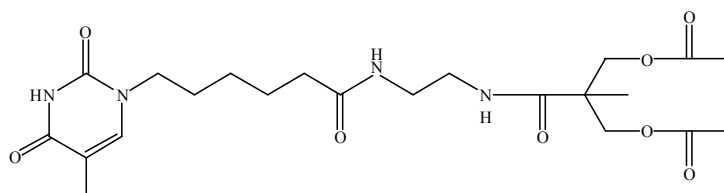
Under inert atmosphere 0.058 g (0.26 mmol) of 2,2-bis(acetoxymethyl)propionic acid, 0.3 mL (1.7 mmol) of DIEA and 0.1 g (0.35 mmol) of Thym-C5-NH$_2$ were mixed in dichloromethane/DMF mixture (20 mL/5 mL). To the suspension 0.14 mg (0.32 mmol) of BOP reagent was added. Reaction mixture was stirred for 4d at room temperature. Then it was diluted with 50 mL CH$_2$Cl$_2$ and washed with 2x20 mL of 2% solution of KHSO$_4$, 2x20 mL of saturated NaHCO$_3$ and 2x20 mL of brine. Organic phase was collected, dried over magnesium sulphate and evaporated.

Yield: no desired product was isolated

6.2.4.3.2   Synthesis of 2,2,5-trimethyl-N-(2-(6-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)hexanamido)ethyl)-1,3-dioxane-5-carboxamide (acetonide-[G#1]-NH-Thym) in dichloromethane
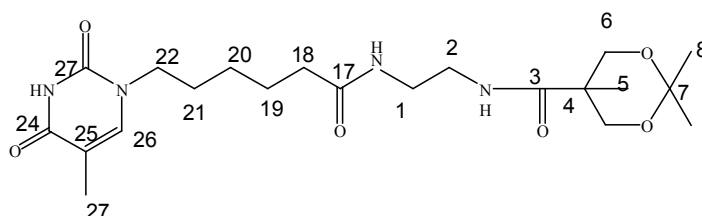
Under inert atmosphere 0.097 mg (0.55 mmol) of isopropylidene-2,2-bis(methoxy)propionic acid, 0.096 mg (0.59 mmol) of CDI, catalytic amounts of DMAP and 0.102 g (0.36 mmol) of

Thym-C5-NH$_2$ were mixed in 20 mL of CH$_2$Cl$_2$. Reaction mixture was stirred for 3d at room temperature. After the reaction has been completed, the reaction mixture was diluted with 50 mL CH$_2$Cl$_2$ and washed with 2x20 mL of 1% solution of KHSO$_4$, 2x20 mL of saturated NaHCO$_3$ and 2x20 mL of brine. Organic phase was collected, dried over magnesium sulphate and evaporated.

Yield: no desired product was isolated

6.2.4.3.3 Synthesis of 2,2,5-trimethyl-N-(2-(6-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)hexanamido)ethyl)-1,3-dioxane-5-carboxamide (acetonide-[G#1]-NH-Thym) in DMF

Under inert atmosphere 0.0464 g (0.26 mmol) of isopropylidene-2,2-bis(methoxy)propionic acid, 0.3 mL (1.7 mmol) of DIEA and 0.1 g (0.35 mmol) of Thym-C5-NH$_2$ were mixed in 7 mL of DMF. To the suspension 0.141 g (0.32 mmol) of BOP reagent were added. Reaction mixture was stirred for 4d at room temperature. After the reaction has been completed, the reaction mixture was diluted with 50 mL CH$_2$Cl$_2$ and washed with 2x20 mL of 1% solution of KHSO$_4$, 2x20 mL of saturated NaHCO$_3$ and 2x20 mL of brine. Organic phase was collected, dried over MgSO$_4$ and evaporated. Crude product was purified by column chromatography on silica gel with CH$_2$Cl$_2$/MeOH (1:1) mixture as an eluent, followed by the another one with CH$_2$Cl$_2$/MeOH (10:1) mixture as an eluent.

Yield: 0.0092 g (3.6%) of an yellow-brown solid

^{1}H NMR (DMSO-d_6): δ [ppm] = 11.14 (s, 1H, NH), 7.76 (s, 1H, NH 17'), 7.56 (s, 1H, NH 3'), 7.50 (s, 1H, H-26), 3.94 and 3.57 (dd, 4H, H-6$_{ax, eq}$), 3.59 (t, overlapping of sH-6$_{ax, eq}$ and 2H, H-22), 3.13 and 3.10 (p, 4H, H-1 and H-2), 2.05 (t, 2H, H-18), 1.55 and 1.51 (m, 4H, H-21 and H-29), 1.33 and 1.28 (ds, 6H, H-8$_{ax, eq}$), 1.21 (p, 2H, H-20), 1.04 (s, 3H, H-5).

^{13}C NMR (DMSO-d_6): δ [ppm] =   173.66 (C-3), 172.33 (C-17), 164.30 (C-24), 150.88 (C-23), 141.44 (C-26), 108.40 (C-25), 97.42(C-7), 65.56 (C-6), 47.03 (C-22), 40.39 (C-4), 38.71 (C-2), 38.20 (C-1), 35.26 (C-18), 28.29 (C-21), 25.50 (C-20), 24.80 (C-19), 23.98 and 23.52 (C-8$_{ax, eq}$), 18.64 (C-5), 11.93 (C-27).

6.2.4.4 Synthesis of hyperbranched polymers with Thym-C3-OH core and their modification

6.2.4.4.1 General procedure for one-pot synthesis of hyperbranched polyester with core moiety

Bis-MPA , Thym-C3-OH or $(OH)_2$-[G#1]-O-Thym in stoichiometric correspondence to a perfect generation of needed number and p-TSA (**Table 17**) were carefully mixed in a three-necked flask equipped with an argon inlet, a drying $CaCl_2$ tube, and mechanical stirrer. The flask was placed in a previously heated oil bath. The mixture was left to react under stream of argon, removing the water formed during the reaction. After the argon stream was turned off and the flask connected to a vacuum line. After that the reaction mixture was removed from the flask by dissolving in THF. The polyester was precipitated in cold diethylether (the temperature varied from -3ºC to -78ºC acoording to the precipitation) and then was dried under vacuum. The ratios between time under argon and vacuum, temperature of oil bath, and vacuum pressure can be found in **Table 18**.

6.2.4.4.2 One-pot synthesis of hyperbranched polyester of pseudo fourth generation (p.7.1)

1.29g (9.6 mmol) of bis-MPA, 0.117 g (0.64 mmol) of Thym-C3-OH (in stoichiometric correspondence to a perfect fourth generation) and p-TSA (catalytic amounts) were carefully mixed in three-necked flask equipped with an argon inlet, a drying $CaCl_2$ tube, and placed on magnetic bar. The flask was placed into an oil bath previously heated up to 185ºC. The mixture was stirred under stream of argon for 1h. After the argon stream was turned off and the reaction proceeded under vacuum (15 mbr) for 10h. The reaction mixture was partly dissolved in THF. Attempt to precipitate the polyester in cold diethylether was unsuccessful.

^{1}H NMR (DMSO-*d_6*): spectrum was too complicated to be analyzed

6.2.4.4.3 Step-by-step synthesis of hyperbranched polyester of pseudo fourth generation (p.70.1)

0.219 g (1.64 mmol) of bis-MPA, 0.1 g (0.55 mmol) of Thym-C3-OH (in stoichiometric correspondence to a second generation) and pTSA (catalytic amount) were carefully mixed in three-necked flask equipped with an argon inlet, a drying $CaCl_2$ tube, and placed on magnetic bar. The flask was placed in oil bath previously heated up to 140ºC. The mixture was stirred under stream of argon, removing the water formed during the reaction. After 2 h, the argon

stream was turned off and the flask connected to a vacuum line (12 mbar, cooling trap) for 1 h. After the pressure was increased to atmospheric, 0.292 g (2.17 mmol) of bis-MPA corresponding to the third generation and pTSA (catalytic amount) were added and the argon flow was started. After 2 h of reaction at normal pressure, vacuum was applied for 1 h. After the pressure was again increased to atmospheric, 0.583 g (4.35 mmol) of bis-MPA corresponding to the fourth generation and pTSA (catalytic amount) were added and the argon flow was started. After 2 h more of reaction at normal pressure, vacuum was applied for 1 h before the reaction was removed from the flask. The crude product was dissolved in THF with 3 drops of DMF at 60ºC (though, it was more like suspension). The polyester was precipitated in cold diethylether (-3ºC) and than dried under vacuum.

Yield: 1 g (91.5%) of a white solid

SEC with LS-detector: M_w 144400 g/mol, M_n 50270 g/mol, PDI 2.87

^{1}H NMR (DMSO-d_6): δ δ [ppm] = 12.84 and 12.5 (s, -COOH), 11.14 (NH thymine core), 7.75 and 7.45 (dd, pTSA bonded), 7.47 and 7.11 (pTSA), 7.47 (CH thymine core) 4.90 (br, -OH l),4.58 (br, -OH t) 4.11 (br m, CH$_2$-OOC-), 3.46 (br m, CH$_2$-OH), 3.30 (m, -COO-CH$_2$-C), 1.7 and 1.9 (CH$_3$ and –CH$_2$-), 1.45 and 1.34 (C-CH$_2$-CH$_3$), 1.17 (CH$_3$ d), 1.02 (CH$_3$ l), 1.02 (CH$_3$ t), 0.85 (m, CH$_2$-CH$_3$).

6.2.4.4.4 Step-by-step synthesis of hyperbranched polyester of pseudo fifth generation (p.71.1)

0.219 g (1.63 mmol) of bis-MPA, 0.1013 g (0.55 mmol) of Thym-C3-OH (in stoichiometric correspondence to a perfect second generation) and pTSA (catalytic amount) were carefully mixed in three-necked flask equipped with an argon inlet, a drying CaCl$_2$ tube, and placed on magnetic bar. The flask was placed in an oil bath previously heated up to 140ºC. The mixture was stirred under stream of argon, removing the water formed during the reaction. After 2 h, the argon stream was turned of and the flask connected to a vacuum line (12 mbar, cooling trap) for 1 h. After the pressure was increased to atmospheric, 0.292 g (2.174 mmol) of bis-MPA corresponding to the third generation and pTSA (catalytic amount) were added and the argon flow was started. After 2 h of reaction at normal pressure, vacuum was applied for 1 h.

After the pressure was again increased to atmospheric, 0.583 mg (4.34 mmol) of bis-MPA corresponding to the fourth generation and pTSA (catalytic amount) were added and the argon flow was started. After the pressure was increased to atmospheric, 1.1651 g (8.686 mmol) of bis-MPA corresponding to the fifth generation and pTSA (catalytic amount) were added and the argon flow was started. After 2 h of reaction at normal pressure, vacuum was applied for 1 h before the reaction was removed from the flask. Partly crude product was dissolved in hot THF, rest was filtrated off. The polyester was precipitated in cold diethylether and than dried under vacuum.

Yield: 0.635 g (28.1%) of a white solid

SEC with LS-detector: M_w 20370 g/mol, M_n 10030 g/mol, PDI 2.03

^{1}H NMR (DMSO-d_6): δ δ [ppm] = 12.84 and 12.5 (s, -COOH), 11.14 (NH thymine core), 7.75 and 7.45 (dd, pTSA bonded), 7.47 and 7.11 (pTSA), 7.47 (CH thymine core) 4.90 (br, -OH l),4.58 (br, -OH t) 4.11 (br m, $\underline{CH_2}$-OOC-), 3.46 (br m, $\underline{CH_2}$-OH), 3.30 (m, -COO-$\underline{CH_2}$-C), 1.7 and 1.9 (CH$_3$ and –CH$_2$-), 1.45 and 1.34 (C-CH$_2$-CH$_3$), 1.17 (CH$_3$ d), 1.02 (CH$_3$ l), 1.02 (CH$_3$ t), 0.85 (m, CH$_2$-$\underline{CH_3}$).

6.2.4.4.5 Step-by-step synthesis of hyperbranched polyester of pseudo six generation (p.72.1)

0.219 g (1.63 mmol) of bis-MPA, 0.104 g (0.564 mmol) of Thym-C3-OH (in stoichiometric correspondence to a perfect second generation) and pTSA (catalytic amount) were carefully mixed in three-necked flask equipped with an argon inlet, a drying CaCl$_2$ tube, and placed on magnetic bar. The flask was placed in an oil bath previously heated up to 140ºC. The mixture was left to react under stream of argon, removing the water formed during the reaction. After 2 h, the argon stream was turned of and the flask connected to a vacuum line (12 mbar, cooling trap) for 1 h. After the pressure was increased to atmospheric, 0.292 g (2.18 mmol) of bisMPA corresponding to the third generation and p-TSA (catalytic amount) were added and the argon flow was started. After 2 h of reaction at normal pressure, vacuum was applied for 1 h. After the pressure was again increased to atmospheric, 0.583 g (4.346 mmol) of bis-MPA corresponding to the fourth generation and pTSA (catalytic amount) were added and the argon flow was started. After the pressure was increased to atmospheric, 1.1678 g (8.7 mmol) of bis-MPA corresponding to the fifth generation and pTSA (catalytic amount) were added

and the argon flow was started. After 2 h reaction under normal pressure was followed by 1h under one vacuum. After the pressure was increased to atmospheric, 2.3301 g (17.37 mmol) of bis-MPA corresponding to the six generation and 0.012 g (0.07 mmol) of pTSA were added and the argon flow was started. After 2 h of reaction at normal pressure, vacuum was applied for 1 h before the reaction was partly removed from the flask by dissolving in hot THF. The polyester was precipitated in cold diethyl ether, filtrated and then dried under vacuum.

Yield: 1.399 g (30.5%) of a white solid

SEC with LS-detector: M_w 32850 g/mol, M_n 14650 g/mol, PDI 2.24

^{1}H NMR (DMSO-d_6): δ δ [ppm] = 12.84 and 12.5 (s, -COOH), 11.14 (NH thymine core), 7.75 and 7.45 (dd, pTSA bonded), 7.47 and 7.11 (pTSA), 7.47 (CH thymine core) 4.90 (br, -OH l),4.58 (br, -OH t) 4.11 (br m, CH$_2$-OOC-), 3.46 (br m, CH$_2$-OH), 3.30 (m, -COO-CH$_2$-C), 1.7 and 1.9 (CH$_3$ and –CH$_2$-), 1.45 and 1.34 (C-CH$_2$-CH$_3$), 1.17 (CH$_3$ d), 1.02 (CH$_3$ l), 1.02 (CH$_3$ t), 0.85 (m, CH$_2$-CH$_3$).

6.2.4.4.6   Synthesis of p.60.1 (General procedure for the polycondensation of bis-MPA with TMP core)

2.22 g (16.5 mmol) of bis-MPA, 0.245 g (1.8 mmol) of TMP (in stoichiometric correspondence to the perfect second generation), and 0.011 g (0.06 mmol)of pTSA were mixed in a three-necked flask under Ar flow. The flask was placed in an oil bath previously heated to 140ºC. The mixture was left to react under the stream of argon, for 2h, followed one more hour under vacuum (14 mbar, cooling trap). Then flask was filled with Ar and 2.89g (21.5 mmol) of bis-MPA corresponding to the third generation and 0.0144 g (0.076 mmol) of pTSA were added and the polycondensation cycle was repeated. Then flask was again filled with Ar and 5.80 g (43.2 mmol) of bis-MPA corresponding to the fourth generation and 0.0297 g (0.16 mmol) of pTSA were added and the polycondensation cycle was repeated, followed by addition of 11.59 g (86.3 mmol) of bis-MPA corresponding to the fifth generation and 0.058 g (0.3 mmol) of pTSA and repeating of the polycondensation cycle. Then flask was again filled with Ar and 23.15 g (173.5 mmol) of bis-MPA corresponding to the six generation and 0.1393 g (0.73 mmol) of pTSA were added and the polycondensation cycle was repeated. After the reaction was stopped, reaction mixture was left to cool down to

the room temperature and then dissolved in THF. This solution was precipitated into the cold diethyl ether (-78ºC) in three fractions.

Yield: 33 g (72.3%) of a white solid

SEC (DMAc/2% water/ 3g/L LiCl PVP standard): first fraction M_w 9700 g/mol, M_n 4500 g/mol, PDI 2.2; second fraction M_w 10000 g/mol, M_n 4900 g/mol, PDI 2.0; third fraction M_w 10000 g/mol, M_n 4700 g/mol, PDI 2.1

^{1}H NMR (DMSO-d_6): δ [ppm] = 12.84 and 12.4 (s, -COOH), 4.90 (br, -OH l),4.58 (br, -OH t) 4.11 (br m, $\underline{CH_2}$-OOC-), 3.46 (br m, $\underline{CH_2}$-OH), 3.30 (m, -COO-$\underline{CH_2}$-C-), 1.45 and 1.34 (C-CH_2-CH_3), 1.17 (CH_3 d), 1.02 (CH_3 l), 1.02 (CH_3 t), 0.85 (m, CH_2-$\underline{CH_3}$). First fraction has DB 47.4 %; second fraction - DB 45.7 %; third fraction - DB 45.4%.

6.2.4.4.7 General Procedure for selective acetonide protection of terminal groups in hyperbranched polyester

Under inert atmosphere 0.1 g of corresponding polyester was dissolved in 5 mL of acetone, corresponding amounts of 2,2-DMP and p-TSA were added. Reaction mixture was stirred under room temperature for 24h, and then catalyst was neutralized with 2 mL of mixture NH_3/EtOH (1:1) and evaporated. The crude product was dissolved in 5 mL dichloromethane and washed with 2x5 mL of distilled water. The organic phase was collected and dried over K_2SO_4. Solvent was evaporated and products were used without further purification.

Yield: 85-90% of a white solid or glass-like solid

6.2.4.5   Polymerization in solution

6.2.4.5.1 Synthesis of hyperbranched polyester with Thym-C3-OH core moiety in solution

Under inert atmosphere, 0.1 g (0.54 mmol) of Thymine-C3-OH, 1.7486 g (5.98 mmol) of DPTS and 4.59g (34.2 mmol) of bis-MPA were mixed in 45 mL of DMF. After all components have fully dissolved 9.33 g (45.2 mmol) of DCC were added together with catalytic amount of DPTS. Reaction mixture was stirred for 4d at room temperature, and then

solvent was evaporated under vacuum. The crude product was dissolved in THF, but could not be precipitated in cold diethyl ether.

Yield: no desired product was isolated

6.2.4.5.2 Synthesis of branched structures with Thym-C5-NH$_2$ core moiety in solution

Under inert atmosphere 0.1017 g (0.76 mmol) of bis-MPA, 0.259 g (0.92 mmol) of Thym-C5-NH$_2$ and 0.62 mL (3.6 mmol) of DIEA were suspended in 40 mL of CH$_2$Cl$_2$. 0.398 g (0.9 mmol) of BOP was added to this suspension together with 0.1 mL of methanol. Solution has cleared and was left to stir at room temperature. After 48h reaction mixture was washed with 30 mL of distilled water and 30 mL of 1M KHSO$_4$ solution. Organic phase was dried over sodium sulphate and evaporated. Crude product was purified by column chromatography on silica gel with CH$_2$Cl$_2$/MeOH (1:5) mixture as eluent. Final product was dissolved in THF and precipitated in cold diethyl ether (-3ºC).

Yield: 183.7 mg (61.5%) of transparent crystals before precipitation
112.6 mg (37.7%) of transparent glass-like solid after precipitation.

7 Appendix

Table 17 Ratios of the reagents in polycondensations

Sample	Core	Generation	Final amounts g		
			Core	bis-MPA	p-TSA
p.7.1	Thym-C3-OH	4	0.117	1.290	c.a.
p.7.2		4	0.1185	1.299	c.a.
p.11.1	Thym-C3-OH	5	0.1181	2.6588	c.a.
p.11.2		5	0.1175	2.6693	c.a
p.13.1		5	0.1176	2.6599	c.a
p.13.2		5	0.1176	2.6608	c.a
p.14.1		5	0.1176	2.6561	c.a
p.14.2		5	0.1180	2.6684	c.a.
p.15.1		5	0.118	2.6617	c.a
p.15.2		5	0.1175	2.6591	c.a
p.16.1	Thym-C3-OH	6	0.1177	5.3963	c.a.
p.16.2		6	0.1175	5.3951	c.a.
p.17.1		6	0.1201	5.4006	c.a
p.17.2		6	0.1185	5.3958	a.c.
p.18.1		6	0.1179	5.3963	a.c.
p.20.1		6	0.1180	5.3990	c.a.
p.21.1	(HO)$_2$-[G#1]-O-Thym	6			
p.60.1	TMP	4	0.245	45.6449	0.2524
p.62.1	-	-	-	33.5647	0.1301
p.63.1	-	-	-	61.6402	0.2291
p.67.1	-	-	-	6.7082	0.0124
p.68.1	-	-	-	6.7072	0.0125
p.69.1	Thym-C3-OH	-	0.1848	6.7023	c.a.
p.70.1		4	0.1000	1.094	c.a
p.71.1		5	0.1013	2.2591	c.a
p.72.1		6	0.104	4.5919	c.a

c.a. – catalytic amounts

Table 18 Reaction conditions for the polycondensation with Thym-C3-OH as a core unit

Sample	Method	T ºC	Ttime		Vacuum mbr
			Ar	Vacuum	
p.7.1	batch	150	1	10	15
p.7.2		150	1	10	16
p.11.1	batch	185	1	8	14
p.11.2		185	1	8	14
p.13.1		166	1	15	14
p.13.2		185	1	15	14
p.14.1		185	0.5	8	12-15-25
p.14.2		160	0.5	8	12-15-25
p.15.1		185	0.5	10	14-25
p.15.2		185	0.5	10	14-25
p.16.1	batch	185	1	8	14
p.16.2		185	1	8	14
p.17.1		185	1	10	15
p.17.2		185	1	10	15
p.18.1		185	1	15	12-18
p.21.1		185	1	15	12-15-25
p.20.1		185	0.5	10	14-25
p.21.1		185	0.5	8	12
p.60.1	Hult	140	2^1	1^1	12
p.61.1	batch	140	2	6	12
p.62.1		185	2	6	2-3
p.63.1		185	2	4	7
p.67.1		185	2	8	3.2
p.68.1		145	2	8	1.9
p.69.1		185	2	9	8
p.70.1	Hult	140	2^1	1^1	12
p.71.1		140	2^1	1^1	12
p.72.1		140	2	1^1	12

[1] for every generation of step-by-step synthesis

Table 19 Yields and information about purification of some polyesters

Sample	Yield %[1]	Purification by precipitation from THF into Et$_2$O	
		Solubility in THF	Precipitation in Et$_2$O
p.7.1	-	+	-
p.11.1	29.2	+	+
p.11.2	53.5	+	+
p.13.1	64.6	+	+
p.13.2	49.5	+	+
p.14.1	27.5	+	+
p.14.2	26	+	+
p.16.1	24.3	+	+
p.16.2	37.9	+	+
p.17.1	41.4	+	+
p.17.2	26.3	+	+
p.18.1	29.5	+	+
p.20.1	68.1	+	+
p.21.1	40.2	+	+
p.60.1	83.15	+	+
p.61.1	-	-	-
p.62.1	20.1	±	+
p.63.1	15.7	±	+
p.69.1	65.7	+	+

[1] for the clean polyester after the precipitation

[2] Several drops of DMSO were added

± rather low solubility

Table 20 SEC results for hb polyesters synthesized wit core moiety

Sample	Theor. M_n^1	SEC in DMAc/2% water/ 3g/L LiCl PVP standard			SEC with LS detector			SEC with PS standards		
		M_n	M_w	PDI	M_n	M_w	PDI	M_n	M_w	PDI
p.7.1		1600	2400	1.5	too low M_w			600	900	1.5
p.7.2	1910.78	1700	2400	1.4	insoluble in THF			insoluble in THF		
p.9.1		-	-	-	1500	2100	1.46	600	800	1.33
p.11.1		-	-	-	4500	6200	1.36	1700	2300	1.35
p.12.1		3800	6000	1.6	insoluble in THF			insoluble in THF		
p.13.1	3767.54	insoluble in mixture			too low M_w			300	500	1.67
p.13.2		9700	49000	5.1	20100	33800	1.68	1900	4600	2.42
p.14.1		insoluble in mixture			9900	24400	2.47	2200	3900	1.77
p.14.2		2000	3000	1.5	insoluble in THF			insoluble in THF		
p.16.1		3100	5300	1.7	insoluble in THF			insoluble in THF		
p.16.2		5100	23000	4.5	7000	13100	1.86	2300	5600	2.43
p.17.1		6500	30000	4.6	13600	23200	1.71	2200	5200	2.36
p.17.2		insoluble in mixture			9400	19000	2.02	1900	3800	2.00
p.18.1		insoluble in mixture			11800	19100	1.63	1500	2900	1.93
p.18.2	7485.42	insoluble in mixture			11800	19100	1.63	1600	2500	1.56
p.20.1		insoluble in mixture			13100	17100	1.3	2000	4200	2.10
p.21.1		-	-	-	8400	11600	1.37	1500	2600	1.73
p.22.1		-	-	-	14500	18700	1.29	1600	2700	1.69
p.25.1		-	-	-	4300	6400	1.48	1200	1800	1.50
p.26.1		-	-	-	8300	10500	1.26	1700	2800	1.65
p.60.1	22080	4500	9700	2.2	-	-	-	-	-	-
p.60.1.2	22080	4900	10000	2.0	-	-	-	-	-	-
p.60.1.3	22080	4700	10000	2.1	-	-	-	-	-	-
p.70.1	1910.78	7100	54000	7.6	50270	144400	2.87	3400	12200	3.59
p.71.1	3767.54				10030	20370	2.03			
p.72.1	7485.42	6000	30000	4.8	14650	32850	2.24	2800	7000	2.50

Table 21 SEC results for hb polyesters synthesized without core moiety

Sample	Theor. M_n[1]	SEC in DMAc/2% water/ 3g/L LiCl PVP standard			SEC with LS detector			SEC with PS standards		
		M_n	M_w	PDI	M_n	M_w	PDI	M_n	M_w	PDI
p.61.1.1	-	-	-	-	4600	7000	1.52	8800	16200	1.84
p.61.1.2	-	-	-	-	2100	2600	1.24	6400	8800	1.38
p.62.1.1	-	-	-	-	3300	6300	1.91	7700	16000	2.08
p.62.1.2	-	-	-	-	3300	6700	2.03	7900	15800	2.00
p.63.1.1	-	-	-	-	3900	6600	1.69	8500	16200	1.90
p.64.1.1	-	-	-	-	4100	6200	1.51	8600	13300	1.55
p.64.1.2	-	-	-	-	3300	5800	1.76	6300	12800	2.03
p.65.1	-	3100	4900	1.6	insoluble in THF			insoluble in THF		
p.68.1	-	2800	4700	1.7	insoluble in THF			insoluble in THF		
p.69.1	-	3000	5100	1.7	-[1]			1100	2100	1.91

[1]signal was too low to be detected

Table 22 Results of TGA investigations for the dendrimers

sample	weight (mg)	weight loss after isoth. 10 min at 40°C (%)	$T_{max.}$ dec.step (°C)	T_{final} dec.step (°C)	weight loss dec. step (%)	residue at 700°C
acetonide-[G#1]-O-Thym	7,501	0,1	80 150 298 455	95 167 395 500	0,1 1,5 92,3 5,4	0,4
(OH)$_2$-[G#1]-O-Thym	5,234	0,2	70 120 314 448	90 145 415 545	0,1 0,4 86,2 11,6	0,1

Table 23 Results of TGA investigations for the hb polyesters

sample	weight (mg)	weight loss after isoth. 10 min at 40°C (%)	$T_{max.}$ dec.step (°C)	T_{final} dec.step (°C)	weight loss dec. step (%)	residue at 700°C
p.11.1	5.505	0.0	70	110	0.5	1,2
			300	400	97.5	
p.20.1	5.093	0.2	73	90	0.5	1,2
			135	195	2..5	
			308	400	94.6	
p.21.1	5.576	0.2	73	110	0.6	1,1
			343	450	97.0	
p.22.1	4.357	0.0	90	100	0.1	1,7
			145	210	2.6	
			s310		(12.5)	
			s365		(29.5)	
			402	450	(53.5)95.5	
p.25.1	8.082	0.1	75	95	0.3	1,1
			135	190	1.1	
			317	425	96.3	
p.26.1	4.443	0.3	70	105	0.4	1,5
			318	350	64.8	
			s380	450	32.0	
p.65.1	7.030	0.1	90	130	0.9	0,7
			s165	200	0.9	
			325	435	96.8	
			460	500	0.5	
p.68.1	7.050	0.4	75	110	0.3	0,7
			306	400	96,5	
p.69.1	5.442	0.1	73	87	0.5	1,8
			133	190	3.4	
			296	380	90.5	
			400	420	1.6	
			s440	500	1.6	

Table 24 Results of DSC investigations for dendritic polymers

sample	weight (mg)	weight loss (%)	mode	Tg (°C)	Δc$_p$ (J/gK)	Int. limit (°C)	ΔH (J/g)	T$_m$ or T$_{c,m}$ (°C)	T$_{c,0}$ (°C)
p.11.1	5.637	1.1	1st heating	18.2	0.54	35-125	16.4	87.6	
			2nd heating	35.4	0.41	-	-	-	-
			cooling	33.1	0.36	-	-	-	-
p.20.1	5.315	1.0	1st heating	12.4	0.52	35-100	18.0	75.3	
			2nd heating	34.4	0.41	-	-	-	-
			cooling	32.1	0.37	-	-	-	-
p.21.1	5.381	0.6	1st heating	24.7	0.44	40-140	35.0	74.8/104.7/123.6	
			2nd heating	37.8	0.52	-	-	-	-
			cooling	35.9	0.40	-	-	-	-
p.22.1	5.455	0.8	1st heating	14.0	0.44	70-110	7.1	87.3	
				54.2	0.39	110-140	4.2	126.7	
			2nd heating	37.7	0.42	-	-	-	-
			cooling	35.1	0.40	-	-	-	-
p.25.1	5.156	0.2	1st heating	9.8	0.48	35-100	14.4	75.4	
			2nd heating	27.4	0.44	-	-	-	-
			cooling	25.2	0.43	-	-	-	-
p.26.1	5.434	---	1st heating	26.0	0.45	40-100	31.0	81.9	
						100-140	1.1	112.6/127.6	
						40-140	36.0		
			2nd heating	40.7	0.44	-	-	-	-
			cooling	36.2	0.42	-	-	-	-
p.65.1	5.504	1.3	1st heating	15.8	0.29	35-175	84.9	84.7	
			2nd heating	29.2	0.51	40-180			
						p.40-85	-15.2	67.0	
						p.85-140	22.8	117.5	
			cooling	26	0.42	40-85	-5.5	63.4	80.5

p.68.1	5.290	1.8	1st heating	14.2	0.34	35-155	58.0	76.6/113.0	
			2nd heating			45-170			
						p.45-92	-7.2	77.1	
						p.92-170	9.5	109.2	
			cooling	24.4	0.48	-	-	-	-
p.69.1	5.520	1.1	1st heating	7.0	0.46	25-120	25.5	74.5/115.6	
			2nd heating	24.6	0.41	-	-	-	-
			cooling	23.2	0.42	-	-	-	-
aetonide-[G#1]-O-Thym	5.170	1.6	1st heating	-8.4	0.2	40-165	79.5	81/122/148.8	
			2nd heating	5.7	0.48	-	-	-	-
			cooling	4.6	0.34	-	-	-	-
(OH)-[G#1]-O-Thym	5.198	0.3	1st heating	-16.5	0.2	25-145	92.2	123.5	
			2nd heating	8.3	0.53	-	-	-	-
			cooling	7.1	0.39	-	-	-	-
aetonide-[G#2-O-Thym	5.440	9.0	1st heating	-8.7	0.8	-	-	-	-
			2nd heating	17.6	0.67	-	-	-	-
			cooling	17.7	0.55	-	-	-	-

8 Abbreviations

Ar	argon
9-BBN	9-borabicyclo[3.3.1]nonane
BOP	benzotriazole-1-yl-uloxy-tris-(dimethylamino)-phosphonium hexafluorophosphate
bis-MPA	2,2'-bis(hydroxymethyl)propionic acid
ºC	temperature in degrees Celsius
c.a.	catalytic amounts
Cat.	catalyst
CDI	N,N'-carbonyldiimidazole
$CHCl_3$	chlorophorm
CH_2Cl_3	dichloromethane
con.	concentrated
$CuCl_2$	cupper (II) chloride
DA	deprotecting agent
DAPy	2,6-diaminopyridine
DB	degree of dranching
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	N,N'-dicyclohexylcarbodimimide
DCM	dichloromethane
dec.	decomposition
DIEA	N,N-diisopropyethylamine
DMAP	4-dimethyl aminopyridine
DMF	dimethylformamide
DMP	2,2-dimethoxypropane
DMSO	dimethylsulfoxide
DSC	differential scanning calorimetry
Gen.	generation
GPC	gel permeation chromatography
IR	Infrared
h	hour
hb	hyperbranched
mbar	millibar

MeOH	methanol
min	minute
mmol	millimole
ml	millilitre
M_n	number average molecular weight
mol	mole
MR	molecular recognition
M_w	weight average molecular weight
NaH	sodium hydride
NMR	nuclear magnetic resonance
PDI	polydispersity index
ppm	parts per million
PS	polystyrene
pTSA	p-toluenesulphonic acid
Py	pyridine
PVP	poly(vinyl pyridine)
r.t.	room temperature
T	temperature
TBAF	tetra-*n*-butylammonium fluoride
TBAI	tetra-*n*-butylammonium iodide
TBDMS	*tert*-butyldimethyl silyl group
THF	tetrahydrofuran
TMP	2-ethyl-2-(hydroxymethyl)-1,3-propanediol or trimethylolpropane
TMS	trimethylsilane
SEC	size exclusion chromatography
UV	ultraviolet

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