

5. Synthesis of higher generation dendronized polystyrene by attach-to route

5.1. Some general comments

An aspect which is of interest are the structural requirements rendering a dendronized polymer into a rigid nanoscale cylindrical object whose shape is more or less independent of the respective environment. Important factors here are: the space demand (generation) of the dendrons, the distance between consecutive sites at which the dendrons are connected to the backbone, the intrinsic backbone rigidity and attractive interactions (e.g. hydrogen bonding) between consecutive dendrons. Recently a poly(*para*-phenylene) with fourth generation (G4) dendrons was synthesized using Suzuki polycondensation (SPC). Although this polymer carries spatially very demanding substituents-they are actually the largest dendrons ever attached to a polymer- and has a backbone which is commonly considered a rigid-rod, computer generated space-filling modes show that this polymer is unlikely to be highly rigid. There is still considerable space between dendrons and this will allow the backbone to bend. Responsible for this space is the relatively large distance of approximately 8.5 Å between the dendron anchor sites along the backbone. From a computer model of a polystyrene with third generation (G3) dendrons, a much more compact structure is obtained although the dendrons are less demanding. Besides some possible backbone contraction the respective distance of only 2.5 Å seems to be responsible for this although these factors cannot yet be differentiated. This computer model suggests how to proceed on the way to absolutely rigid structures. A vinyl polymer, which all have the 2.5 Å repeat unit, should be prepared with G4 dendrons on every repeat unit.

From the obtained data for the degree of polymerisation of G1, G2 and G3 (styrene-type) monomers, It can be seen that the molar masses of resulting polymers decrease with the increase of the monomer generation. This observation rendered the preparation of G4 polymer (styrene type) unattractive by monomer route, although there has been a successful example of G4 polymer.

Up to date, the attach-to strategy in which dendrons of the final size were attached to the anchor groups of mostly poly(para-phenylene) type backbones met only part success. The limitation of this method, however, becomes visible when the same reaction is carried out with G3 dendron. Here the maxim achievable "magic" coverage is 90-92%. Though this number would be considered more than sufficient for many chemical reactions, it cannot obscure the fact that the coverage is incomplete.

To avoid the steric hindrance especially associated with large dendrons, the mixed attach-to strategy was devised as follows: small dendrons (G1 or G2) were used to attach the polymeric core with low generation's dendrons. Further increase of generations can be achieved by repetitions of this method by using the resulted polymers (after deprotection) as starting material. For example:



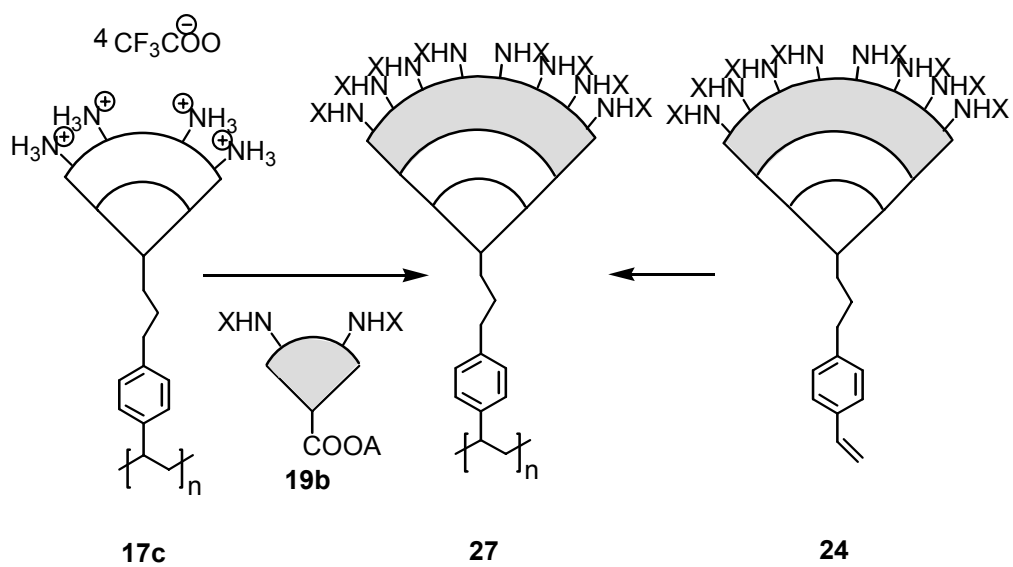
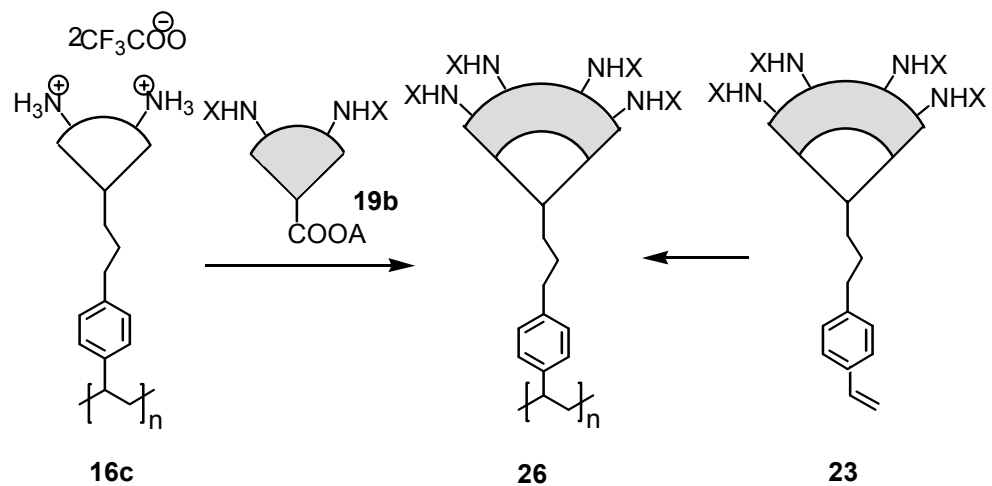
By using this modified method, it should be expected that steric congestion can be reduced to some extent. This should favour the achievement of higher coverage of reactive points on polymers.

The obtained unpolar dendronized polymers G1 (**16c**) and G2 (**17c**) with amino functional groups were an ideal (simple structure) starting materials for the synthesis of higher generation dendrinized polymers by the attach-to strategy. The polymers prepared in chapter 3 were expected to make structure characterisation of the resulting polymers especially easy.

5.2. Synthesis and characterization of G2 and G3 dendronized Polystyrene by the attach-to route

Deprotected polymers **16c** and **17c**, which are fully soluble in methanol and water, were used for the dendronization process (Scheme 14). To bring about the dendronization, the methanolic solutions containing triethylamine used to deprotonate the ammonium functions were treated at room temperature with a 2-fold excess per ammonium function of the dendritic building block **19b**, which has an activated ester at its focal point (Scheme 14). The decreasing solubility of the polymers in methanol in the course of the dendronization caused by their decreasing polarity was accounted for by the addition of methylene chloride after a few hours of reaction (MeOH:CH₂Cl₂ = 1:1). After removal of the solvents and the base in vacuum, the remaining material, with the proposed structures **26** or **27**, respectively, were precipitated into methanol/water (4:1) whereby the accompanying salts dissolved. This process had to be repeated several times in order to completely remove excess **19b** or its derivatives. The losses of polymer associated with this somewhat tedious procedure fortunately remained below some 20-25%. To determine the degree of dendronization, polymers **26** and **27** were investigated by high-field ¹H and ¹³C NMR spectroscopy. All signals appeared at the expected shifts and with the ¹H integration ratios required for a complete coverage. Because of the considerable widths at half-height of the signals involved and the maximally achievable yet relatively low concentrations, the accuracy of NMR integration was considered insufficient to prove this important point. Therefore, polymers **26** and **27** were prepared independently from their corresponding macromonomers **23** and **24** respectively. They carry the same G2 and G3 dendrons as in **26** and **27**.

To differentiate between the polymers of proposedly the same structure but different synthetic origin, the prefixes *at* for attach to and *mm* for macromonomer are used in the following. The ¹H NMR spectra of *at*-**26** and *mm*-**26** (Figure 15a) as well as the ones of *at*-**27** and *mm*-**27** (Figure 15b) are virtually superimposable except for the peaks



Scheme 14: Synthesis of G2 polymer **26** and G3 polymer **27** by two different routes.

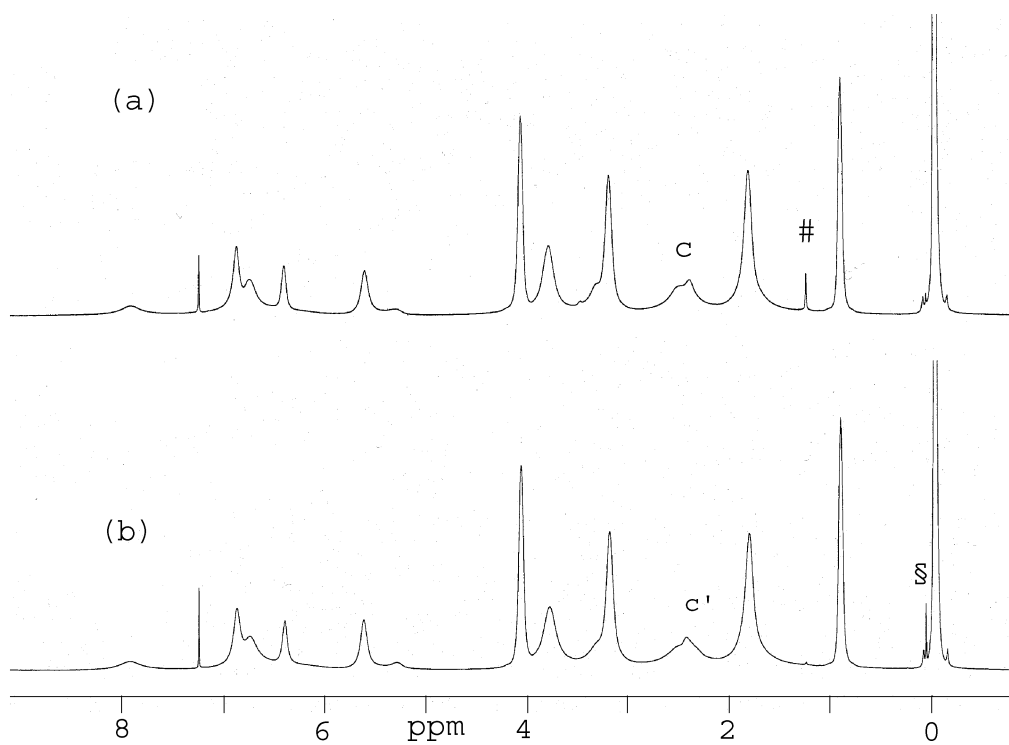


Fig. 15a. ¹H NMR (500MHz) spectra of at-26 (a), mm-26 (b). (peak # is the remained solvent, and peak § is originated from grease)

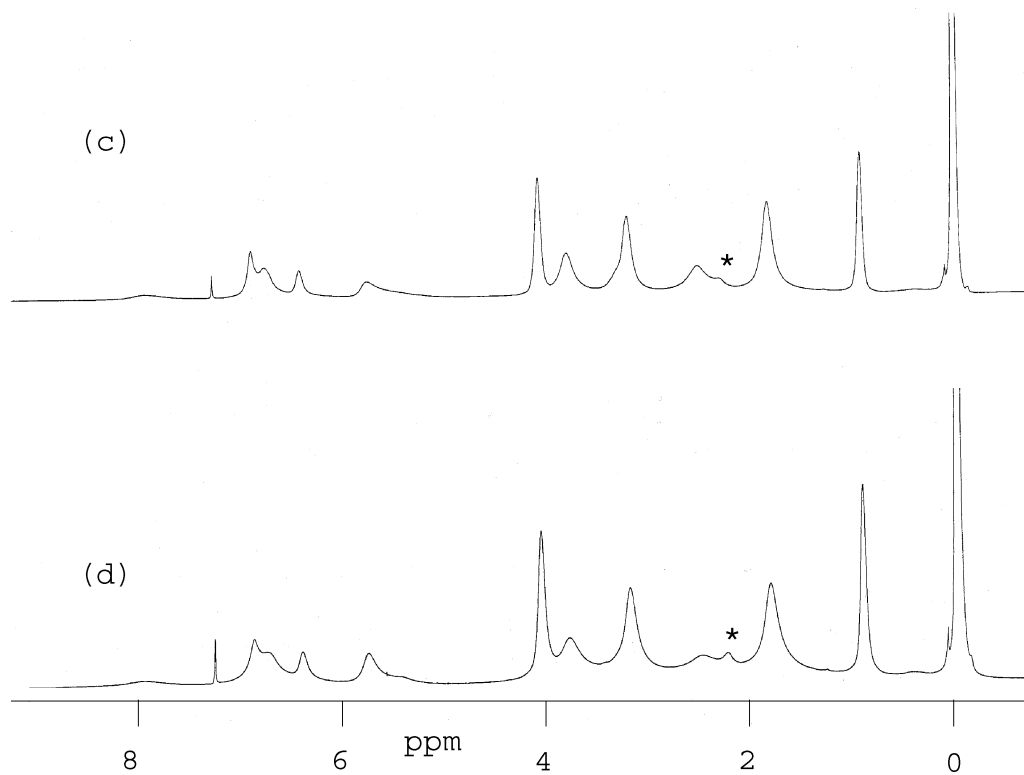


Fig. 15b. ¹H NMR (500MHz) spectra of at-27 (c) and mm-27 (d). (peak * is the proton of water)

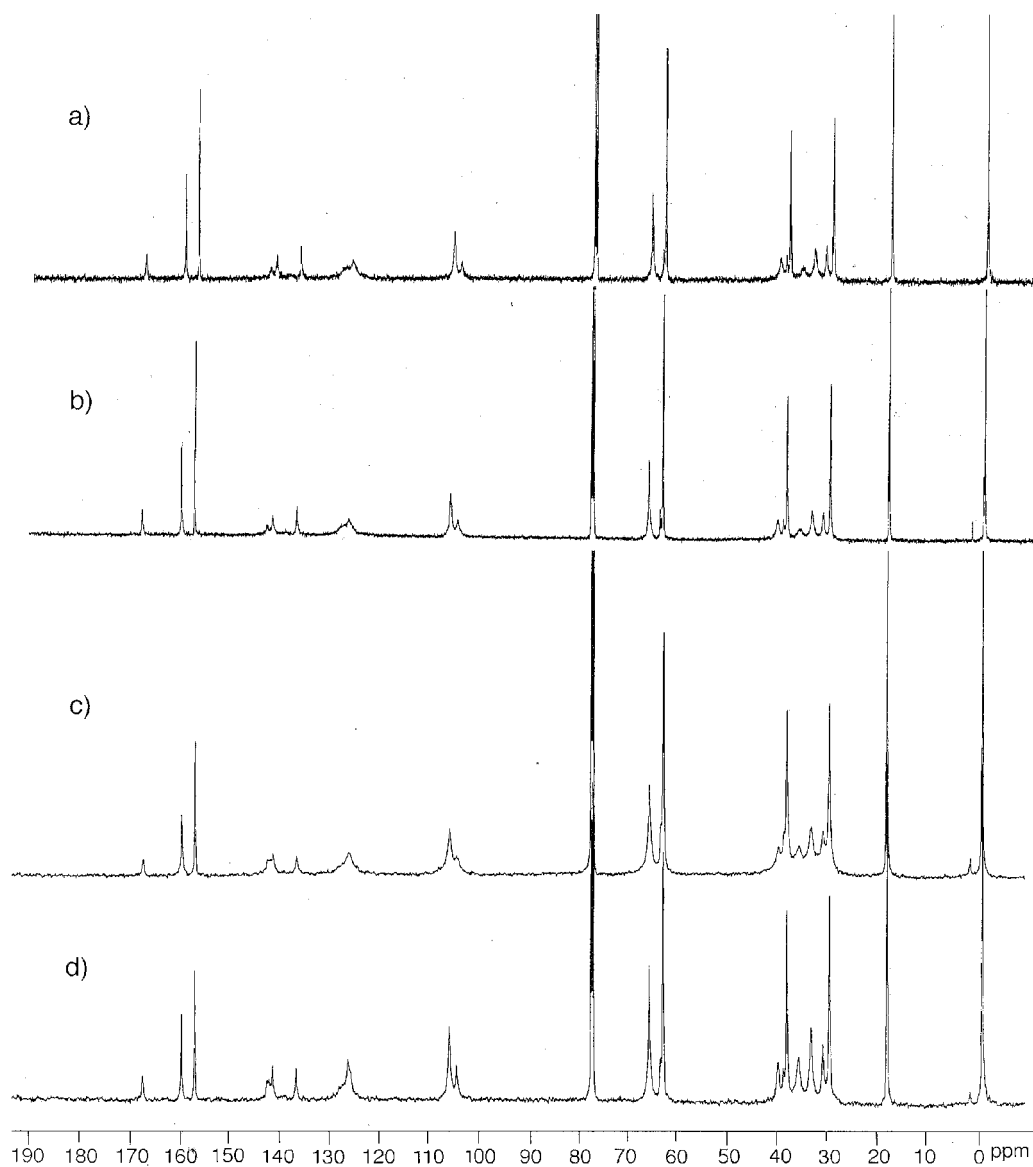
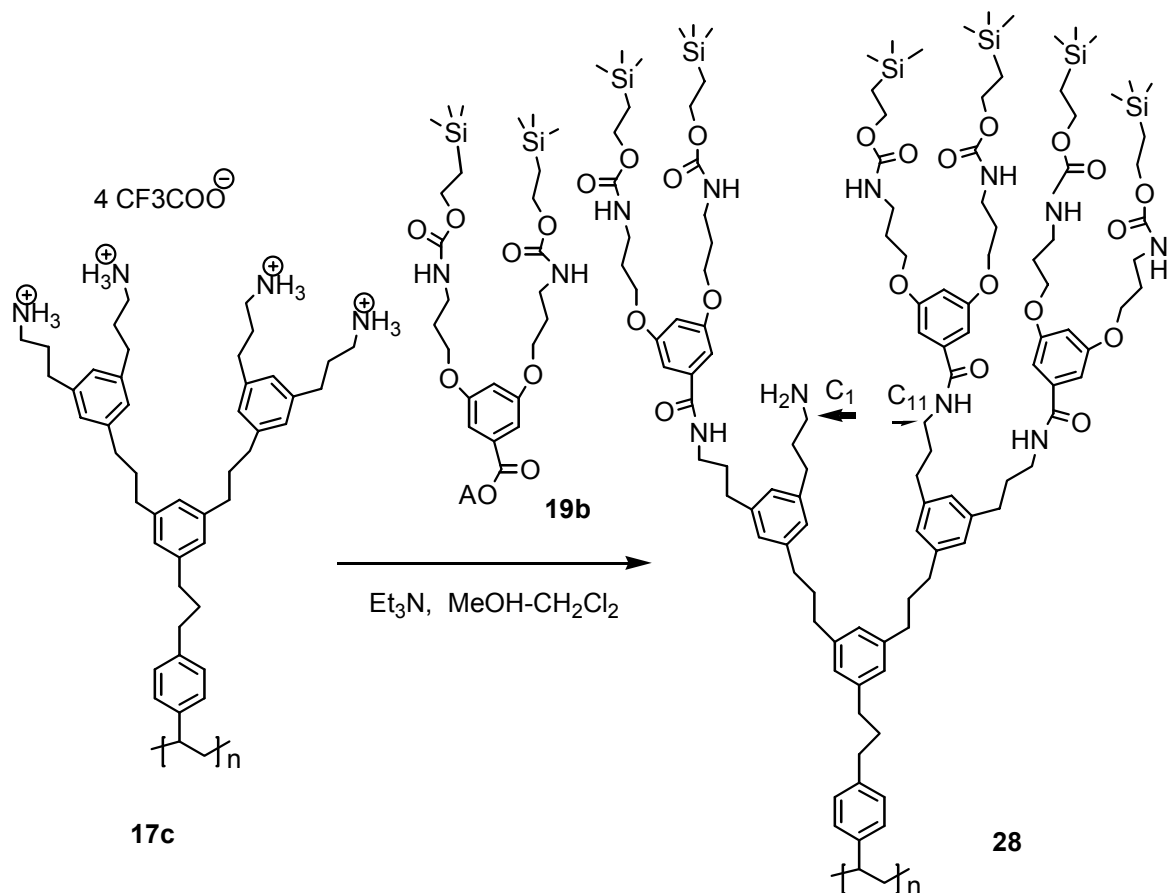


Fig. 16. ^{13}C NMR (125MHz) of polymer at-26 (a), mm-26 (b), at-27 (c) and at-28 (d).

between 2.2 and 2.8 ppm, which belong to the resonance of protons of methylene groups connected to benzene rings and that of protons of water. Due to the shift of the absorption of protons from water, the shape of ^1H NMR are not superimposable in this

range. Figure 16 shows the ^{13}C NMR spectra of at-**26**, mm-**26**, at-**27** and mm-**27**. As can be seen, these spectra are also pairwise and virtually superimposable except for the fact that the line shapes of the latter two differ somewhat presumably due to a resolution problem. Most importantly, there is no indication of the obvious defection.



Scheme 15. Synthesis of G3 polymer **28** with approximately 70 % coverage.

Amino functionalized methylene carbons typically absorb at $\delta = 29\text{-}42$ ppm.^[37] This shift is very close to the signals absorbing in the range $\delta = 29\text{-}40$ ppm that, a confirmation seemed therefore necessary that free amines can be unambiguously detected. Polymer **17c** was therefore reacted with less than stoichiometric amounts of

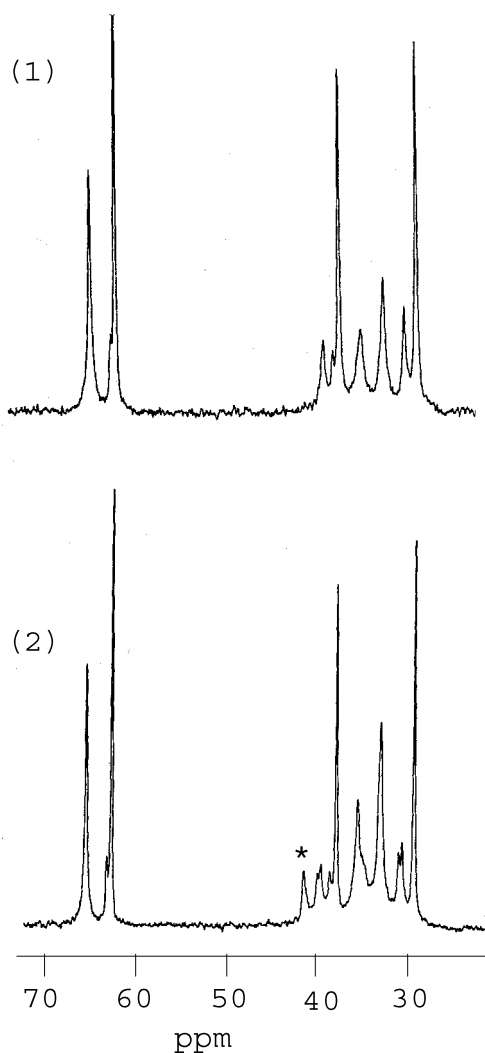


Fig. 17. Parts of ^{13}C NMR spectra of at-**27** (1) and at-**28** (2). (peak * corresponds to the absorption of methylene (C_1) linked with amine group)

19b (0.70 equiv. per amine function) in order to deliberately create free, non-dendronized amines. According to the TLC and GPC of the raw material, all of the **19b** could be brought to reaction, and it is therefore reasonable to assume that the resulting at-**28** contains roughly 30% of the unreacted amines.

Figure 17 compares part of the ^{13}C NMR spectrum of this incompletely covered at-**28** (2) with one which has presumably the maximum achievable coverage (1). The bottom spectrum(2) shows clearly a signal well-separated off at $\delta = 42$, which raised from C_1 , whereas nothing is to be seen in the top spectrum(1).

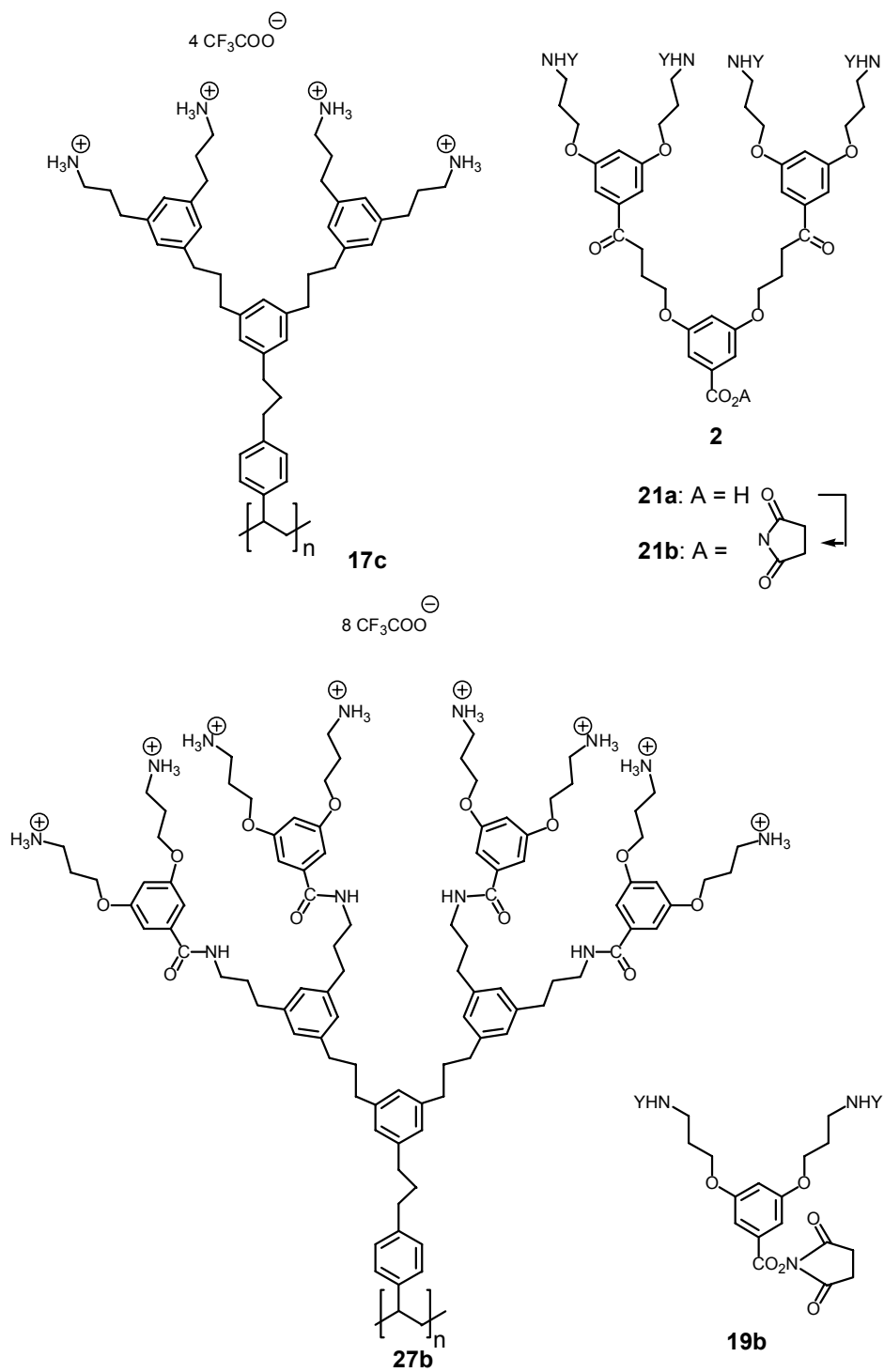
Besides this spectra evidence, the completeness of the coverage is also indicated by combustion analysis. The data obtained are in excellent agreement with the calculated ones not only for mm-**26** and mm-**27** but also for at-**26** and at-**27**. The calculated values are quite sensitive to structural imperfections. Assuming that 5%, 10%, or 15% of the amine anchor groups of **17c** did not react with **19b**, the value for carbon increases from 62.12 for the structurally perfect material to 62.39, 62.67 and 62.97, respectively. The observed values for carbon from two independent determinations are 61.94 and 61.71.

Though each of the points mentioned has its individual weakness, together they provide convincing evidence for the proposed complete coverage of polymers **26** and **27** with the G1 dendritic building block **19b**. Interestingly, the GPC molecular weights decrease through the dendronization.

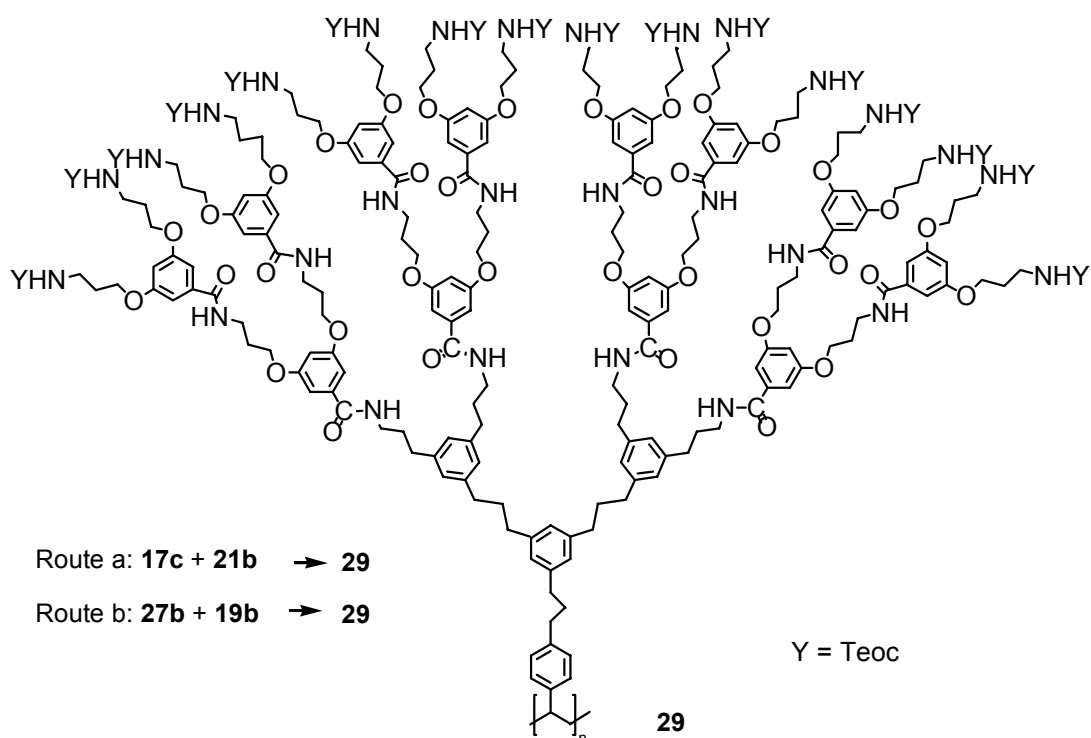
5.3. Synthesis and characterization of G4 dendronized polystyrene by attach-to route

By the method described above for the preparation of G2 and G3 dendronized polymers by the attach-to route, G4 dendronized polystyrene may be prepared by two routes: a) G3 polymer + G1 dendrons, and b) G2 polymer + G2 dendrons. Dendronizations of **17c** and **27b** with **21b** and **19b**, respectively, were carried out at room temperature. In order to drive the reaction to maximum extent, two consecutive stages are followed. For the preparation of Polymers **29e** and **29f**. First, the methanolic solution of **17c** or **27b** containing triethylamine were treated with a 1.2 equiv. of activated ester per ammonium function. With the dendronization going on, the solubility of polymers became poor due to the decreasing polarity, which can be overcome by addition of methylene chloride after a few hours of reaction.

Secondly, after removal of solvents and base, the crude material was dissolved in methylene chloride and the remained 0.6 equiv. of activated ester was added. The reaction was continued for another two days. After removal of the solvents and base in vacuum, the residue was dissolved in THF, and were precipitated into methanol/water



Scheme 16. Polymers and compounds used for the preparation of G4 polymer **29**. (Y = Teoc protecting groups)



Scheme 17. Synthesis of G4 polymer **29** by two routes.

(4:1), whereby the accompanying salts dissolved. This process had to be repeated several times in order to completely remove excess reagents. The loss of polymer associated with this procedure remained below some 15%. The resulting polymers could be lyophilised from benzene. For the preparation of **29a**, **29b** and **29c**, the activated ester was added at the first step. High field ^1H NMR (500 MHz) spectra were recorded for polymers **29e** and **29f**. All signals appear as expected. Due to the considerable widths at half height of the signals involved, it is difficult to characterize the polymer by integration. Similar spectra were obtained for these two polymers. To acquire better ^{13}C NMR (125 MHz) spectrum, larger NMR tube (1 cm in diameter) was used. Figure 18 exhibits the deprotected G-3 polymer **27b** and the corresponding dendronized G-4 polymer **29e**. As can be seen, the noise level is low, all the peaks correspond to the

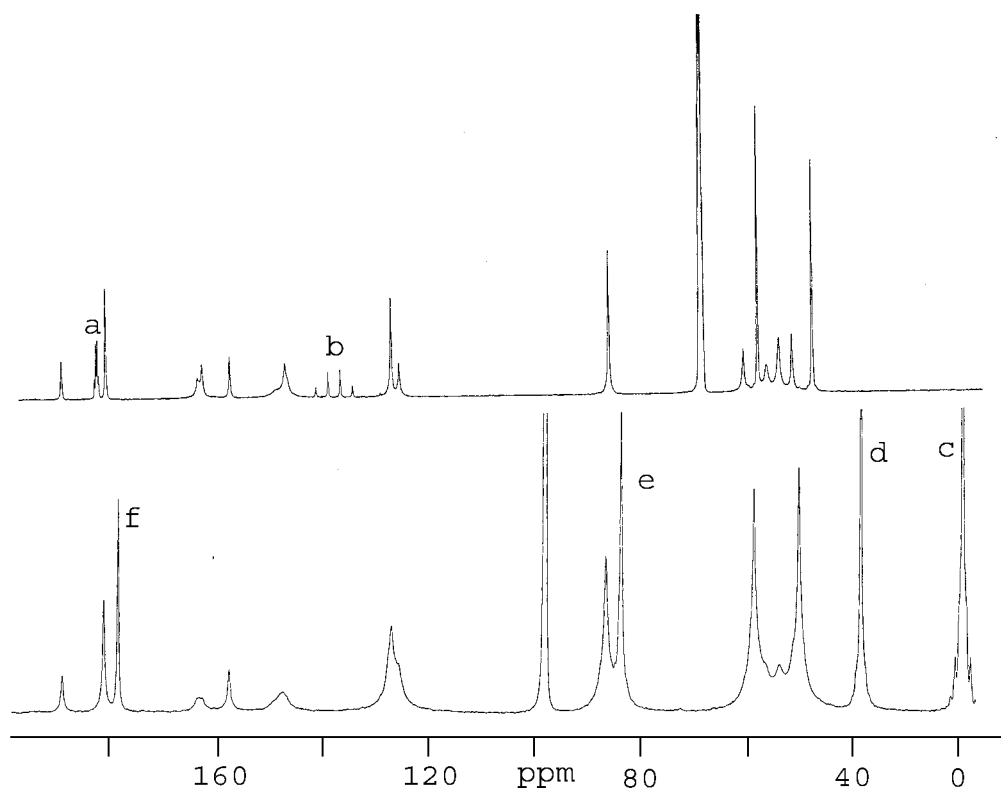
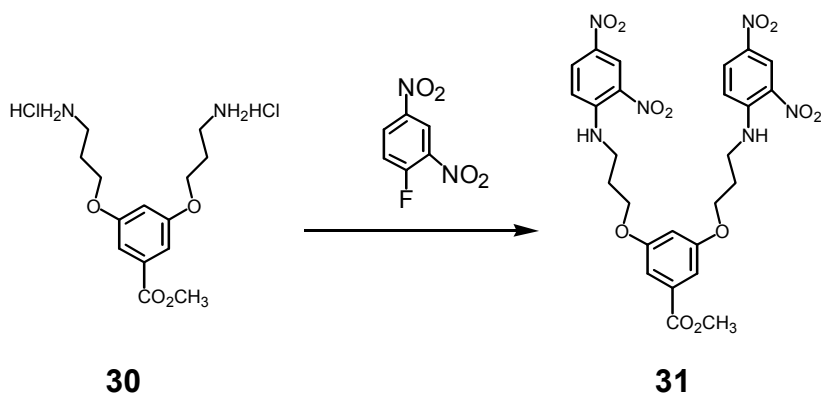
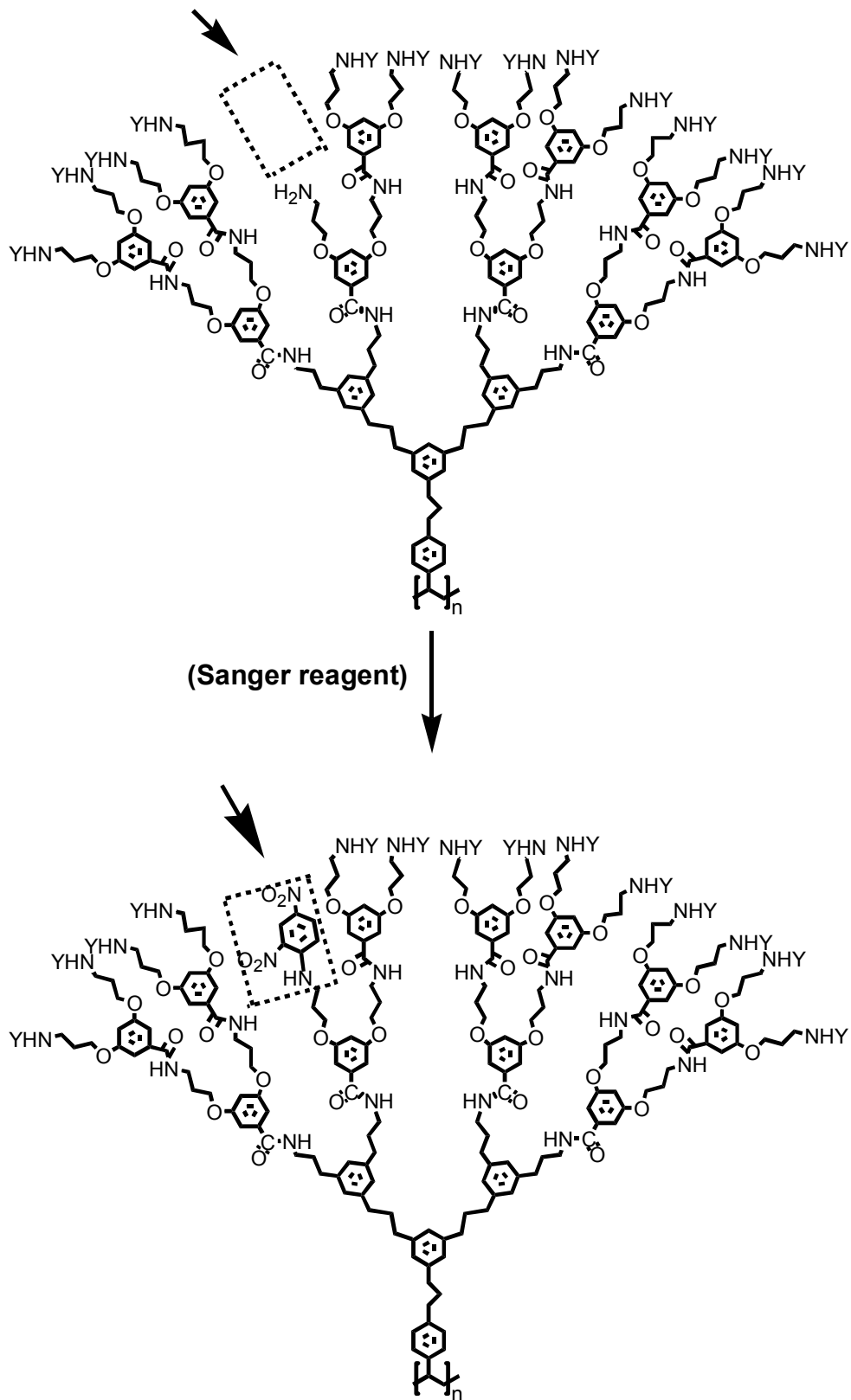


Fig. 18: ^{13}C NMR spectra of G4 polymer **29** and polymer **23b**. Peaks a and b belong to CF_3COOH . Peaks c, d, e and f are from Teoc.



Scheme 20. Synthesis of UV-standard **31**.



Scheme 21. Labelling of unreacted free amino groups with Sanger's reagent.

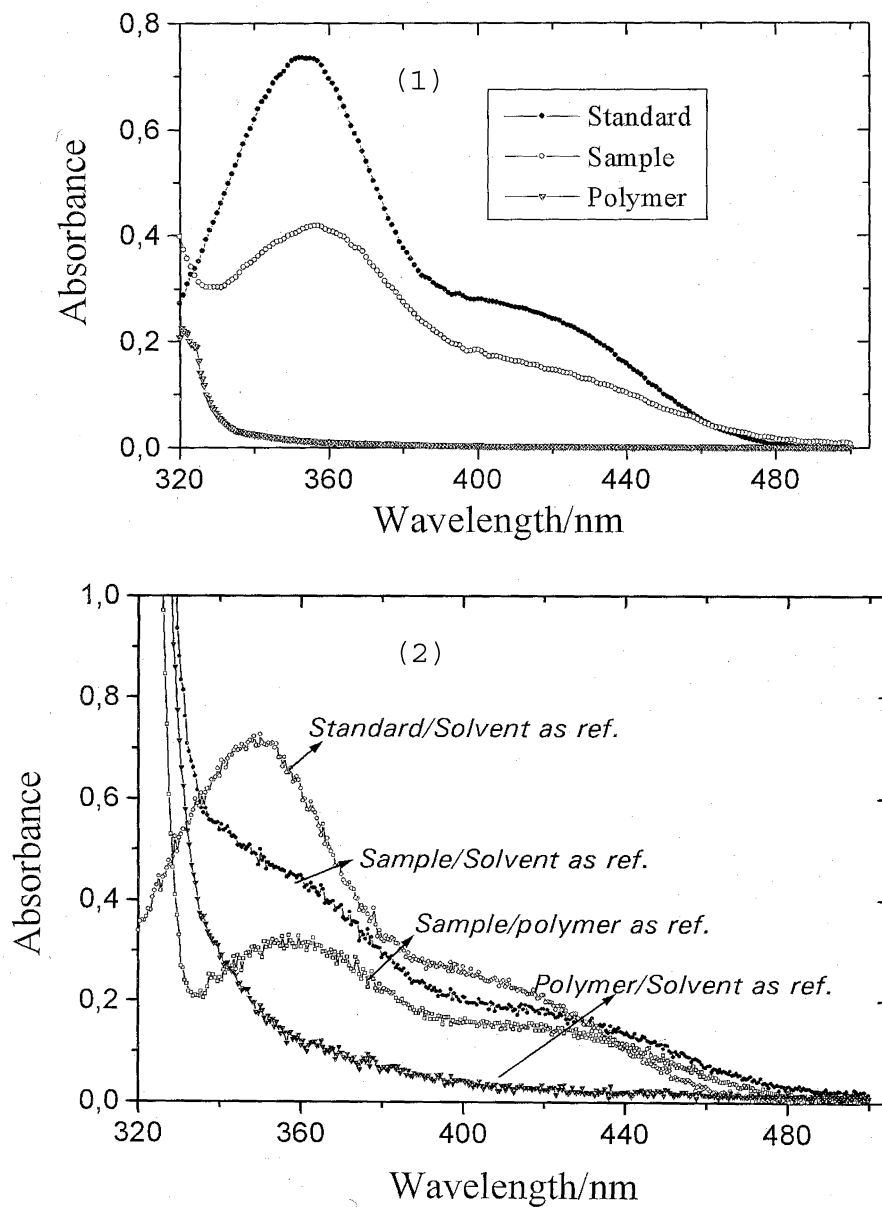


Fig. 19. UV spectra of standard and labelled polymers. (1) $C_{\text{polymer}} = 2.0 \times 10^{-4} \text{ M}$, $C_{\text{standard}} = 2.4 \times 10^{-5} \text{ M}$; (2) $C_{\text{polymer}} = 4.7 \times 10^{-4} \text{ M}$, $C_{\text{standard}} = 2.4 \times 10^{-5} \text{ M}$.

polymers respectively. By comparison of the two spectra, it is obvious that dendronization is successful from the four strong peaks (-1.5, 17.7, 62.7, 157.0) corresponding to the Teoc protecting groups of the attached dendrons. Unfortunately, with the broadening of peaks, it's difficult to observe the fine structures.

To determine quantitatively the coverage of the attachment, 1-Fluoro-2, 4-dinitrobenzene was used here. This reagent has been applied extensively as a

quantitative reagent for labelling the free amine groups of proteins and peptides.^[38-41] The method consists of reacting the amine groups with 1-Fluoro-2,4-dinitrobenzene, separating the excess reagents and measuring the quantity of the 2, 4-dinitrophenylamine by its light absorption. To determine the coverage, structurally similar compound **31** was synthesized (Scheme 20) and used as a standard. The absorbance of the solution of the 2, 4-dinitrophenylamine is a linear function of the quantity of amine present. Figure 19 depicts the UV spectrum of the standard with maximum absorption at 355 nm. The extinction coefficient of the standard should be calculated according to Beer's law: $\epsilon = A/(Cl)$, where A is the absorption of standard at 355 nm, l is the length of the UV cell, c is the concentration of amino-2,4-dinitrobenzene of the standard, which should be expressed as follow:

$$C = 2w/(VM) \text{ (w: weight, V: solution volume, M: molecular weight of the standard)}$$

So, the extinction coefficient of the standard should be expressed as follows:

$$\epsilon = A/(Cl) = 2Aw/(VMI).$$

For one of the measurements, $w = 3.7 \times 10^{-3}$ g, $V = 0.25$ L, $l = 1.0$ cm, $A = 0.75$.

After calculation, $\epsilon = 1.64 \times 10^4$.

Reaction of resulting polymers with 1-Fluoro-2,4-dinitrobenzene was carried out according to literature procedure with some modifications. To prevent polymers from precipitating from the reaction medium, tetrachloroethane was used as solvent for polymers. The labelling reaction was brought about by adding estimated 5 times in excess of amine groups and 0.1 M sodium bicarbonate as weak base at 60 °C for 1 hour. After reaction, two methods were tried to separate the excess reagents. The first one involves converting the excess reagent to sodium salt of 2, 4-dinitrophenol and separating the excess reagents by two methods. The first one involves converting the excess reagent to sodium salt of 2,4-dinitrophenol and separating this by extraction. Unfortunately, it was found that the excess reagent can't be totally converted to the corresponding salts (From the ratio of absorbance at 360 to 390 nm). The second method consists of separating the aqueous phase, removing tetrachloride ethane under vacuum, dissolving the residue in THF and precipitating the polymer in MeOH/H₂O (v:v/4:1). This precipitation procedure had to be repeated 4 times to ensure the totally removal of excess reagent.

The labelled polymer has yellow colour and can be lyophilised from benzene. Figure 19 shows the UV spectra of 2,4-dinitrophenyl-labelled polymer (**29d**) and polymer **29d** in Tetrachloride ethane. The shape of UV absorbance of standard and labelled polymer is in good agreement when the absorption of polymer itself was taken into account. The maximum absorbance of sample and standard is at 355 nm whereas the polymer itself has absorbance below 340 nm. That means the polymer itself at this concentration does not interfere the measurement of labelled polymer. With the increasing of coverage, in order to achieve the reasonable value of absorbance, The concentration of polymer should be increased. From Figure 19 (2). it is obvious at 355 nm, the polymer itself has absorbance (about 0.1). This interference can be counteracted by using virtually the same concentration of polymer solution.

The percentage of unreacted amine groups in the polymer can be expressed by the following equation:

$$\text{molarity of unreacted NH}_2 \% = (\text{molarity of unreacted amine groups}) / (\text{molarity of total NH}_2 \text{ groups in the starting polymers})$$

Because the reaction of NH₂ group with 1-Fluoro-2,4-dinitrobenzene is almost complete, molarity of unreacted amine groups is nearly equal to that of dinitrophenyl derivative in the labelled polymers. The above equation can be transformed into:

$$\text{molarity of unreacted NH}_2 \% = (\text{molarity of dinitrophenyl derivative}) / (\text{molarity of total ammonium groups in the starting polymers}) \times 100.$$

In order to make deduction simple, the following signs are introduced:

X: Percentage of unreacted NH₂ groups in the resulted polymers (after dendronization)

C: Molar concentration of dinitrophenylamine derivative

V: Volume of solution of labelled polymer in tetrachloroethane (L)

A: Absorbance of dinitrophenylamine derivative at 355 nm

ε: Extinction coefficient of dinitrophenylamine standard

C_{ru}: Concentration of repeating unite of resulted polymer

N: Number of amine groups per repeating unite in the starting polymers (before dendronization)

M: Molecular weight of repeating unite of resulted polymer

W: Weight of labelled polymer (g) used for the UV measurement

Consequently, molarity of dinitrophenylamine derivative = $C \times V$, molarity of total NH_2 groups in the starting polymer = wN/M

$X = (CV)/(wN/M) \times 100$, according to Beer's law, $C = A / (\epsilon l)$.

Then $X = (AMV)/(\epsilon WNl)$. The corresponding coverage is $Y = (100-X)\%$.

For G-2 and G-3 polymers, N is 4 and 8, respectively.

The results of the UV measurement for the coverage of polymer **29** are displayed in Table 6 ($\epsilon = 1.64 \times 10^4$, which is calculated from standard **31**).

Table 6. Solubility and UV measurements of different samples of Polymer **29**.^a

Entry	Route	R	Solubility			W·10 ³ (g)	V·10 ³ (L)	A	Y (%)
			THF	CH ₂ Cl ₂	DMF				
1	b	0.83	-	-	-	/	/	/	/
2	b	0.93	-	-	-	/	/	/	/
3	b	1.00	+	+	-	/	/	/	/
4	b	1.20	++	++	+	8.6	3.0	0.42	93.3
5	b	1.80	++	++	++	12.0	6	0.33	99.2
6	b	1.80	++	++	++	11.0	3	0.50	99.4
7	b	1.80	++	++	++	12.2	3	0.48	99.4
8	a	1.80	++	++	++	10.8	9	0.76	94.2

a) R is the molar ratio of activated Ester to amine. Other symbols are for weight of dinitrophenyl labelled polymer (W), used volume of labelled polymer **29** solution in 1,1,2,2-tetrachloroethane (V), absorption (A), and coverage Y, not soluble (-), partially soluble (+), and fully soluble (++)

For example, for G4 polymer **29** obtained by (G3 polymer + G1 dendrons), in one of the UV measurement, $w = 0.012$ g, $V = 0.003$ L, $M = 5997$, $A = 0.48$, $l = 1.0$ cm (cell length), $N = 8$ (for G3 starting polymer). After calculation, $Y = 99.4\%$.

The data in Table 6 show that (a) high coverage between 93% and over 99% can be reached, (b) the highest coverage are reproducibly reached for 1.8 equivalents of active Ester dendron per amine group, and (c) the highest coverage are obtained for route b.

The degree of polymerization (DP) of polymer **17c** and **27b** used for the coverage experiments amounted to $DP = 40$ according to GPC versus polystyrene standard. By this method molar masses of dendronized polymers are grossly underestimated by a factor of 1.5-4.5. This is due to the unprecedentedly high mass per unit length encountered in these polymers. According to molecularly resolved SFM experiments (not shown), many chains of this polymer have at least $DP = 200$. For a chain with $DP = 100$, for example, a coverage of 99.3% means that 794 of 800 amine groups were successfully reacted with the G1 fragment **19b**, which nicely underlines the potential of this method.

At present, SFM investigations with polymer **29** and related ones are in progress. In this context, it is interesting to note that the corresponding G1 polystyrene with unprotected and protonated amine groups (structure not shown) has been individualized on HOPG by spin-coating techniques and moved about with the SFM tip. These results will be discussed later.

5.4. Synthesis of dendronized polystyrene with higher molar mass

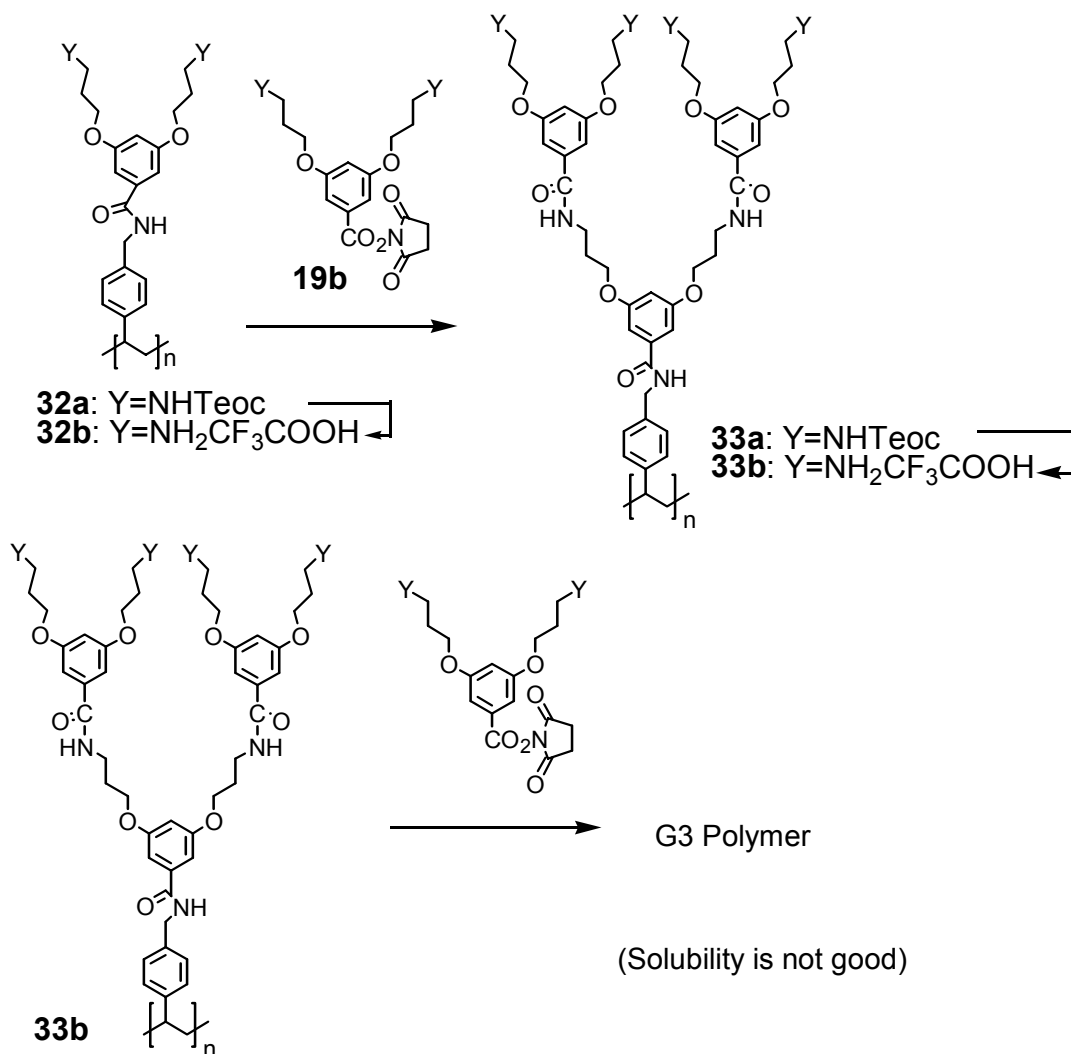
One of the main goals of this thesis was to prepare higher generation dendronized polystyrene with functional groups at the periphery. According to the above preliminary results, higher generation dendronized polystyrene could be furnished by attach-to strategy. These resulting polymers could be characterized by high field NMR spectra (for lower than 3 generation) and UV labelling method (for low and higher generations). This opened an avenue for the synthesis of high molecular weight dendronized polymer with higher generations.

Preliminary investigations of polymer conformation of deprotected polymers **29** by SFM (which will be discussed later) show that this polymer is rigid. The main problem associated with SFM experiments was that the polymer length is not long enough for the manipulation. It would be urgent to obtain dendronized polymers with higher molar mass. According to simple calculation, if we need a 125 nm polymer chain in length, at least 500 repeat units are needed.

From our previous experiment, G1 dendronized polymer is easy to have higher molar mass. Here, polymer **32a** was prepared according to methods described by Ingo Neubert^[32] with some adaptation. After removal of monomer and oligomers, polymer having $DP_n = 430$ and $PD = 1.77$ was available. Deprotection of this polymer by treatment with trifluoacetic acid gave polymer **32b**, which is water and methanol soluble. The completeness of this deprotection step was confirmed by the 1H NMR and ^{13}C NMR spectra with the disappear of Teoc protecting groups.

The conversion of deprotected G1 polymer **32b** to G2 polymer **33a** was carried out by the method described above (Scheme 19). Polymer **33a** is soluble in THF and methylene

dichloride, rendering the determination of polymer structure possible. Treatment of **33a** with trifluoacetic acid furnished water and methanol soluble **33b**. The completeness of the removal of Teoc was proved by NMR spectra. Further increase of generation was tried by using **33b** as raw material. Unfortunately, The solubility of the resulting G3 polymer was not good in THF and chloroform (only partly dissolved).



Scheme 21: Synthesis of G2 and G3 polymers by attach to route.

The main reasons may be attributed to the slight increase of polarity of the resulting polymer after dendroniation. Considering that, a little more unpolar dendron (Scheme 20) was prepared accordingly. Compound **35c** is two oxygen less than **19b**.

The attachment of **35c** was brought about by applying the same procedure as before. G3 polymer **36a** is soluble in THF and methylene dichloride, and can be deprotected completely to give polymer **36b**. G4 polymer **37a** and **37b** was obtained by using the same procedure as for **36a**.

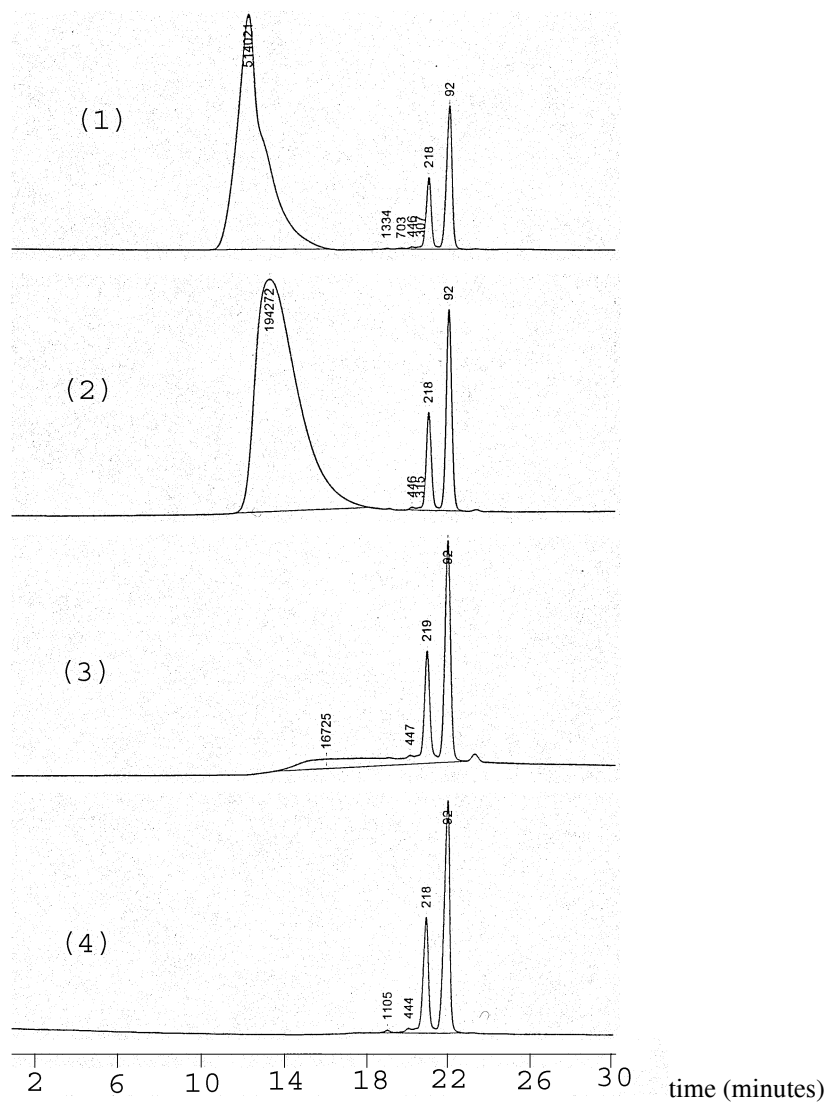


Fig. 20. GPC curves of polymers **32a** (1), **33a** (2), **36a** (3) and **37a** (4).

The coverage of the resulting polymers **33a**, **36a** and **37a** was measured by UV-labelling method described in 5.3.2. The results is summarized in Table 7. From the data of coverage given in the Table 7, it is clear that virtually 100 % coverage were achieved by this amide formation reaction.

Table 7. Results of the measurement of the coverage of G2-G4 polymers(33a, 36a and 37a) by attach-to route

polymer	W×10 ³ (g)	V×10 ³ (L)	A	Y(%)
33a	11.6	3.0	0.177	99.83
36a	12.0	3.0	0.20	99.75
37a	11.7	3.0	0.36	99.69

note: (V) is used volume of labelled polymer solution in 1,1,2,2-tetrachloroethane, absorption (A), and coverage (Y), weight of labelled polymer (w).

The molecular weights of resulting polymers were measured by GPC with Polystyrenes as standards and THF as eluent. Figure 20 presents the GPC curves of polymers **32a** (1), **33a** (2), **36a** (3) and **37a** (4). It can be seen that, with increasing generations, the molar masses of the polymers decrease sharply. In the case of G4 polymers, almost nothing can be eluted. The reasons for this has been discussed in chapter 2, and further investigation is needed to uncover the behaviour of polymers. It should be mentioned that molar masses of dendronized polymers measured by GPC need to be treated with care, especially for higher (above generation 3) generation dendronized polymers.