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The solid-phase synthesis of dendritic polyamides

K. E. Uhrich[1], S. Boegeman[1], J. M. J. Fréchet[1,*], and S. R. Turner[2]

[1]Department of Chemistry, Baker Laboratory, Cornell University, Ithaca, NY 14853, USA
[2]Corporate Research Laboratories, Eastman Kodak Company, Rochester, NY 14650-2110, USA

Summary

The solid-phase synthesis of hyperbranched dendritic polyamides was attempted using two different monomers based on 3,5-diaminobenzoic acid. Growth of the dendritic molecules past the fourth generation was extremely sluggish. The hyperbranched molecules were cleaved from the solid support and examined by gel permeation chromatography, and other spectroscopic methods.

Introduction

The synthesis and properties of dendritic macromolecules have received a considerable amount of attention in the past few years. The "Starburst" polymers of Tomalia [1] have been the impetus for a number of synthetic efforts by workers such as Hall [2]) and Kim and Webster [3] to build new examples of these dense, compact molecules. Synthetic routes to these structures have for the most part been based on divergent schemes that involve step growth reactions from a polyfunctional core [1], although a novel convergent route to these hyperbranched macromolecules has recently been developed [4]. In all reported cases, the synthesis involves the step-wise build-up of the macromolecule, with purification (such as fractional distillation, crystallization, or chromatography) required after each step to remove unreacted starting material and reagents from the desired product.

In the divergent method of synthesis, as the macromolecule grows, there is a rapid increase in the number of chain ends, and complete reaction becomes extremely difficult. Incomplete reaction of the chain ends leads to failure sequences in the next generation, with the probability of failure sequences increasing with the size of the macromolecule. More significantly, the "Starburst" synthesis suffers from the activation step in which a difunctional reagent is used. The use of this reagent may lead to "interstarburst" reactions, [5] linking the dendrimers together into one, thereby destroying the size monodispersity of the sample. As a result, larger and larger excesses of reagents are necessary to ensure complete reaction of the chain ends and to avoid the "interstarburst" coupling. For example, over 10,000 molecules of activator for each molecule of starburst are required at the fourth generation. Since complete removal of these reagents, particularly the difunctional activator, are required to avoid the initiation of growth of a new dendrimer, the usual problems associated with purification of dendritic polymers are even more severe at higher generations.

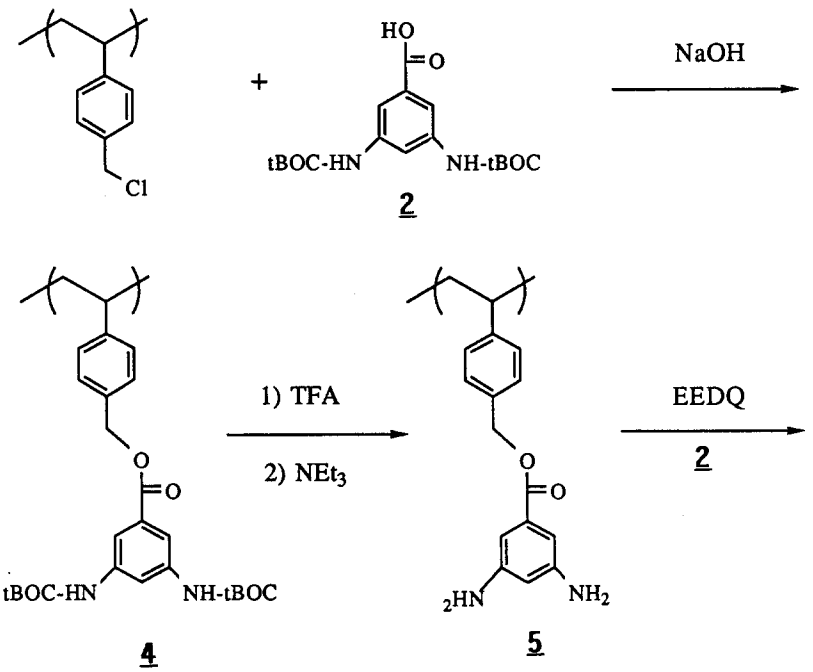
The synthesis of polypeptides requires several, repetitious, step-wise reactions in which large excesses of reagents are used. The solid support method of peptide synthesis, pioneered by Merrifield [6] has proven to be a powerful tool in making linear, amide-linked polymers. Merrifield successfully simplified and automated the reactions necessary to produce polypeptides. In principle, the requirements for growth of hyperbranched macromolecules by the divergent methodology are very similar to those for the growth of peptides. Therefore, we have attempted to use Merrifield's approach to build dendritic macromolecules. The solid support technique simplifies the purification of each generation since the excess reagents used to ensure complete reaction of the terminal functional groups can be easily removed by washing of the polymer-bound dendrimer. Once the reaction is complete, the final product is isolated by cleavage of the macromolecule from the solid support.

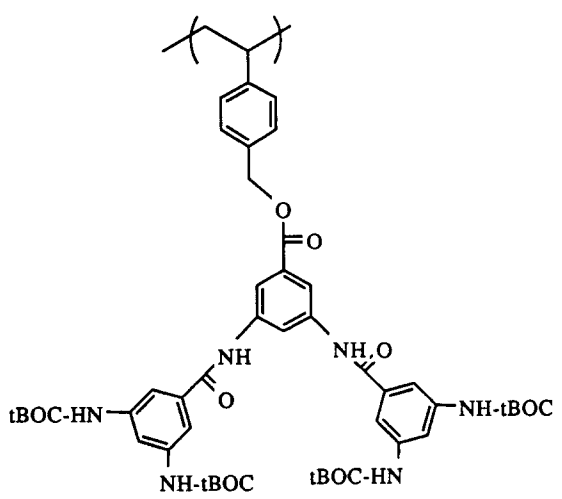
*To whom offprint requests should be sent

We now wish to report our attempted synthesis of hyperbranched polyamides using the solid phase technique.

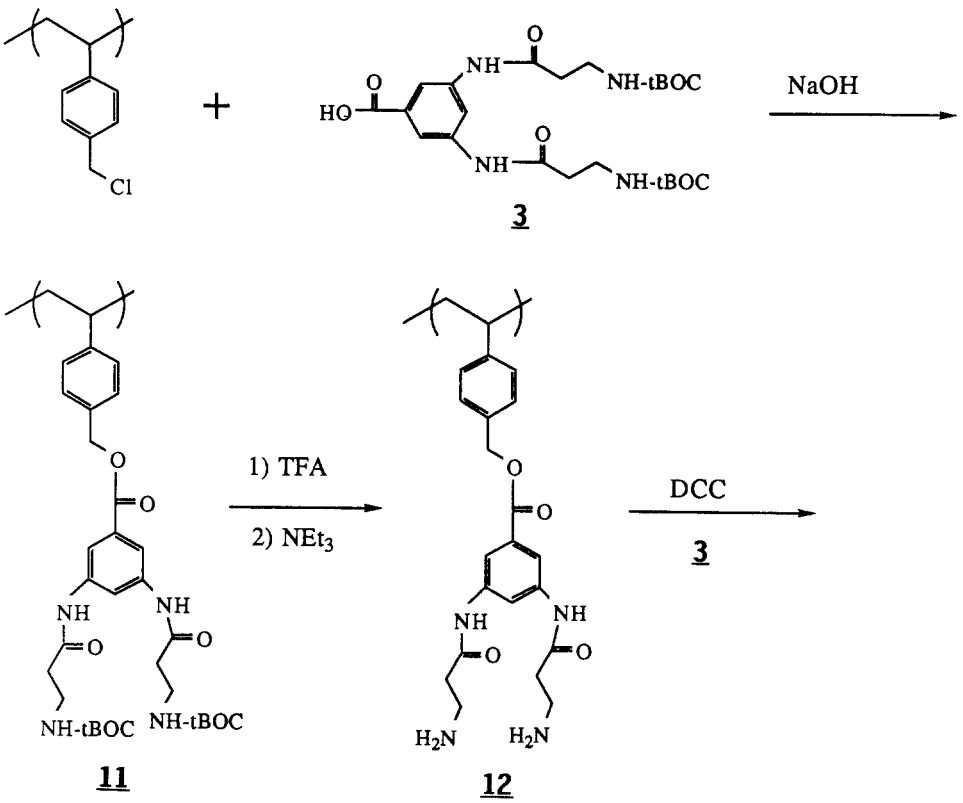
Results and Discussion

To study the feasibility of the solid phase technique, several dendritic macromolecules were synthesized using 3,5-diaminobenzoic acid, 1, as the basic monomer building block in two separate approaches. In one approach, the monomer, 2, is used to generate a fully aromatic dendrimer as shown in **Scheme I**. In the second approach, an aliphatic spacer group is added to 1 to afford monomer 3, and eventually an aralkyl dendrimer as shown in **Scheme II**.





Scheme I



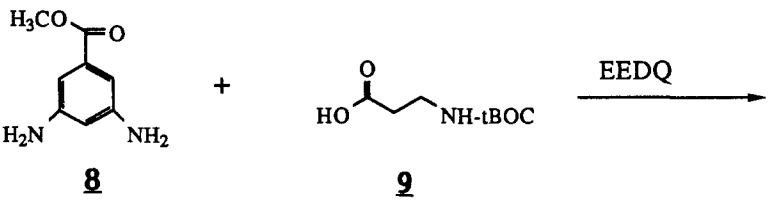
Scheme II

With both monomers, the amino groups were protected using the *t*-butyloxycarbonyl (t-BOC) group while the carboxylic acid functionality was left unprotected. The polymer chosen as the solid support for the synthesis was Merrifield's original chloromethylated polystyrene (2% crosslinked, 1.23 meq of chlorine/gram resin). As can be seen in Scheme I, coupling to the chloromethyl groups of the polymer is accomplished by a simple nucleophilic displacement using the sodium salt of 2 to afford the protected, first generation compound, 4, in 89% yield. Any residual chloromethyl groups on the resin were then acetylated with sodium acetate to prevent the formation of unwanted products in subsequent coupling steps.

Stirring 4 in a solution of dichloromethane:trifluoroacetic acid:acetic acid (5:3:2), followed by treatment with triethylamine gave the diamine, 5, in quantitative yield. A peptide coupling agent, 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ), was used in the coupling of 5 with an excess of monomer 2 to give the protected second generation polymer-bound dendrimer in 57% yield after two successive coupling procedures achieved in 48 and 9% conversion respectively. Deprotection of 6, followed by condensation with monomer 2, gave the protected, third generation dendrimer, 7, in only 14% coupling yield.

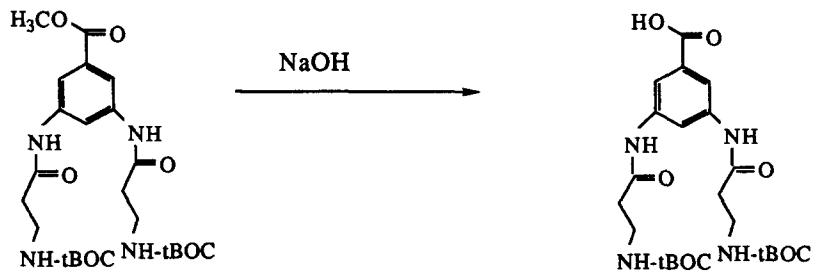
Therefore, after only three generations, it appears that further growth is stunted as it has already become difficult to couple monomer 2 with the polymer-bound, second generation dendritic amine to give 7. In view of these results, monomer 3 which contains a short aliphatic spacer group and a somewhat more reactive amino functionality, was tested as it was expected that these structural modifications contributing both to steric and electronic factors, would facilitate the growth of larger dendrimers.

Monomer, 3, was prepared as outlined in **Scheme III**. Methyl 3,5-diaminobenzoate, 8, was condensed with t-BOC protected alanine [7], 9, in the presence of EEDQ to give the diamide 10 in 78% yield. Hydrolysis of the ester group of 10 gave the desired compound 3 in 94% yield.



8 9

Scheme III



10 3

Treatment of 2% crosslinked chloromethylated polystyrene resin with the sodium salt of 3, followed by acetylation of any remaining chloromethyl groups, gave the second generation compound, 11, in 77% coupling yield (Scheme II). The procedures for coupling and

deprotection were the same as those previously described for monomer 2, except that dicyclohexylcarbodiimide (DCC) was used in place of EEDQ. Deprotection of 11 gave 12, the second generation amine, in quantitative yield. Monomer 3 was condensed with 12 to give the second generation compound, 13, in 72% coupling yield. This was followed by quantitative deprotection to give the free amine, 14. Coupling yields were 49% and 31% in two consecutive condensation experiments for the preparation of the third generation protected compound, 15 for a total coupling yield of 80%. Quantitative deprotection of 15, followed by repeated condensations in 37%, 18% and 13% coupling yields, gave the protected fourth generation compound, 16, in an overall coupling yield of only 68%. After deprotection to give the fourth generation free amine, 17, condensation with monomer 3 gave the fifth generation compound, 18 in only 10% coupling yield. Repeated condensation attempts did not lead to improved coupling yields in the preparation of the fifth generation dendrimer 18.

For both approaches, there was a dramatic decrease in the coupling yields as the size of the polymer-bound hyperbranched macromolecule increased. This may be due to a confinement problem whereby the polymer support restricts accessibility to the chain ends of the increasingly large polymer-bound dendritic macromolecule. Alternately, this growth inhibition may be analogous to that encountered by Hall et al. [2] in the conventional synthesis of highly compact dendritic macromolecules.

In an attempt to solve this problem of growth inhibition, a flexible aliphatic chain was attached to the resin to provide a spacer group between the point of polymer attachment and the core of the growing dendritic macromolecule. Therefore, using methods previously described, three β-alanine units were linked consecutively and in linear fashion onto the chloromethylated solid support with a coupling efficiency exceeding 90%. Coupling of the aralkyl monomer 3 to the resin-bound β-alanine chain, in the presence of DCC, gave the first generation macromolecule, 19, in 73% coupling yield. Deprotection of 19, followed by condensation with monomer 3, gave the second generation compound, 20 in 80% coupling yield. Condensation yields for the third generation dendrimer were 61%, 12%, and 15%, to afford 21, in a total coupling yield of 88%. The fourth generation molecule, 22, was obtained similarly but with coupling yields of only 11% and 2% in two successive attempts for an overall coupling yield of 13%.

Cleavage of the dendritic macromolecules from the polymer support was accomplished using hydrogen bromide generated in situ [8]. For example, the fifth generation compound, 18, and the fourth generation compound, 22, which was attached to the resin via the β-alanine trimer chain, were cleaved to give compounds, 23 and 24, respectively.

The infrared spectra of compounds 23 and 24 were similar to that of the starting monomer 3, although the peaks were significantly broader. The predominant features were the carbonyl stretches at 1673 cm^{-1} for the aliphatic amides, at 1650 cm^{-1} for the aromatic amides and at 1560 cm^{-1} for the amide II stretch.

The ^{1}H-NMR spectra of the cleavage products were also similar with the spectrum of the monomer. Compound 23 displayed peaks at 2.6-3.1 ppm for methylene protons α to the carbonyl, 3.4-3.8 ppm for methylene protons α to the nitrogen, 7.9-8.7 ppm for the aromatic protons, 9.6-9.7 ppm for amines associated with TFA and at 10.2-10.6 ppm for the acidic protons. Compound 24, which contained the β-alanine chain, had peaks at 2.3-3.1 ppm for methylene protons α to a carbonyl, 3.3-3.8 ppm for methylene protons α to nitrogen, 7.9-8.7 ppm for aromatic protons, 9.5-9.7 ppm for amines associated with TFA, and at 10.2-10.6 for carboxylic acidic protons.

Size exclusion chromatography (with polystyrene standards) showed a broad molecular weight distribution. Dendrimer 23 had Mw=2250, Mn=898, for a polydispersity of 2.5, while dendrimer 24 had Mw=3600, Mn=1000 and a polydispersity of 3.6. The broad molecular weight distribution confirmed that the solid support method did not provide access to high purity fourth or fifth generation macromolecules. The relatively high molecular weights that are obtained suggest that a significant proportion of the isolated products is made up of third or fourth generation dendrimers with a varying number of failure sequences. This result was not unexpected in view of the low coupling yields obtained in the preparation of the dendrimers.

Conclusion

Overall, the use of the solid support method for the preparation of dendritic polyamides based on 3,5-diaminobenzoic acid has been found to have severe limitations. The condensation reaction which is necessary for each generation growth could not be driven to completion, as indicated by the modest yields obtained for each condensation step. The incomplete reaction of the dendritic chain ends gives failure sequences in the next generation, as was verified by size exclusion chromatography of the products obtained after cleavage from the solid support. In addition to difficulties inherent to the aromatic polyamide chemistry selected for this study, these incomplete reactions may result in part from the lack of accessibility of the dendritic chain ends within the solid support which inhibit subsequent condensation reactions. Overall, this approach does not rival Tomalia's "starburst" approach [1], or the versatile "convergent-growth" approach we have introduced recently [4]. Another synthetic procedure for the preparation of small dendritic aromatic polyamides of a size comparable to those reported in this work has been described recently by Miller and Neenan [9].

Experimental

Merrifield's peptide resin was chloromethylated divinylbenzene 2% cross-linked polystyrene, Sigma No. M-2728, 1.23 mmol chlorine/g resin. All yields are based on gravimetric analysis, with the assumption of no material loss. Infrared spectra were recorded on a Nicolet FT IR/44 spectrometer as thin films on KBr disks. ^{1}H-NMR spectra were recorded in solutions of $CDCl_3$, DMF-d_6, and DMSO-d_6 on a Brücker WM 300 (300 MHz) spectrometer using the solvent proton(s) as the standard. Melting points are uncorrected. Size exclusion chromatography was carried out on three 10 μm PL Gel mixed-bed columns in dimethylformamide. Calibration of the GPC columns was with polystyrene standards.

Synthesis of monomer **2**.

Di-t-butyl dicarbonate (80 mL, 0.35 mol, 2.2 eq) was added dropwise to a solution of 3,5-diaminobenzoic acid (23.8 g, 0.16 mol) in 1N NaOH (172 mL, 1.1 eq), and t-butanol (110 mL). The reaction mixture was stirred at room temperature under nitrogen for 24 hours, diluted with ethyl acetate (150 mL), chilled on ice and neutralized with a chilled solution of 1N $KHSO_4$ to pH=2,upon which a black solid was formed and filtered off. The aqueous phase was extracted with ethyl acetate (3x), the combined organic layers were washed with water (2x), and evaporated to dryness. The crude product was purified by flash chromatography with ether:hexanes:acetic acid (1:1:0.03) as the eluent and evaporated to dryness to obtain a white solid (39.9 g, 72.0%). Mp 128°C (dec).
^{1}H-NMR ($CDCl_3$): δ = 1.53 (s,18H,t-BOC), 7.72 (t,2H,ArH), 7.91 (t,1H,ArH). ^{13}C-NMR (CD_3OD): 28.7 (CH_3), 81.1 (t-BOC), 114.3 (Ar), 115.3 (Ar), 132.8 (Ar), 141.3 (Ar), 155.1 (C=O), 169.6 (COOH). IR (cm-^{1}): 3350 (NH), 1705 (br, C=O).

Methyl 3,5-diaminobenzoate **8**.

Thionyl chloride (32 mL, 0.439 mol) and 3,5-diaminobenzoic acid (20.4 g, 0.134 mol) were added to ice-chilled methanol (500 mL). The solution was warmed to room temperature, heated to 50°C for 38 hours, and concentrated to ca 50 mL. The residue was diluted with water (400 mL), neutralized with ammonium hydroxide and stirred for one hour. The precipitate was filtered, dried, redissolved in hot methanol and refiltered to remove impurities. The crude product was recrystallized from methanol/toluene to give 16.4 g (74%) of **8**. Mp 131-132°C.
^{1}H-NMR (CD_3OD): δ = 3.81 (s,3H,-OCH_3), 6.31(d,1H,ArH), 6.74 (s,2H,ArH).
IR(cm-^{1}): 3460(NH), 3370(OH), 1715(C=O), 1200(C-O).

Coupling to form diamide **10**.

To a mixture of BOC-alanine, **9**, (31.6 g, 0.167 mole) and methyl 3,5-diaminobenzo-

ate, 8, (9.3 g, 0.056 mole) dissolved in freshly distilled tetrahydrofuran (250 mL) was added EEDQ (41.3 g, 0.167 mole), and the reaction mixture was stirred at room temperature, under nitrogen. After 20 hours, the reaction mixture was evaporated to dryness, the viscous oil dissolved in ethyl acetate (20 mL), and washed with water (2x), saturated $NaHCO_3$ solution (3x), 1.1M $KHSO_4$ solution (3x) and brine (1x). The organic portion was dried over $MgSO_4$, evaporated to dryness, and the crude product recrystallized from ethyl acetate/hexane to give 22.1 g (78% yield) of white powder.
^{1}H-NMR (acetone-d_6): δ = 1.39 (s,18H,t-BOC), 2.63 (t,4H,CH_2), 3.41 (t,4H,CH_2), 3.85 (s,3H,-OCH_3), 8.03 (d,2H,ArH), 8.25 (t,1H,ArH). IR (cm-^{1}): 3325 (NH), 1710 (br, C=O).

Hydrolysis of compound 10 *to form monomer* 3.

A 1.0N NaOH solution (24 mL, 0.024 mole) was added dropwise to compound 10 (9.8 g, 0.019 mole) dissolved in methanol (150 mL), heated to reflux under nitrogen for 3.5 hours, neutralized to pH=7 with 1N HCl solution and evaporated to dryness. The residue was diluted with water (200 mL) and acidified to pH=3 with 1N HCl solution. The precipitate was filtered and dried to give 8.9 g (94% yield) of white solid. Mp 180-181 (dec).
^{1}H-NMR (DMSO-d_6): δ = 1.38 (s,18H,t-BOC), 2.50 (t,4H,CH_2), 3.23 (t,4H,CH_2), 7.93 (s,2H,ArH), 8.16 (s,1H,ArH), 10.15 (s,COOH).
IR (cm-^{1}): 3336 (NH), 3005 (t-BOC), 1690 (C=O).

Attachment of 3 *to the polystyrene resin.*

Monomer 3 (3.8 g, 11 mmol) was dissolved in methanol (50 mL) and treated with one equivalent of 1N aqueous NaOH (11 mL). The mixture was evaporated to dryness and dried in a vacuum oven for 4 hours at 40°C. Merrifield's peptide resin (1.23 mmol Cl/g resin, 1.91 g, 0.22 eq) and the dry salt were swollen in dimethylformamide (12 mL), heated at 80°C for 68 hours, then heated for another 66 hours after the addition of sodium acetate (26.1 mmol, 11 eq). The polymer was washed with dimethylformamide (2x), 5:1 dioxane/water (3x), methanol (1x) and dried to give 2.58 g of resin (89%).
IR (cm-^{1}): 3396, 3353 (NHC=O), 2978 (t-BOC), 1700 (C=O).

Deprotection of the resin-bound compound.

A mixture of trifluoroacetic acid (8 mL) and acetic acid (5 mL) was added dropwise to the resin (2.6458 g) swollen in dichloromethane (13 mL). After five hours, the resin was washed with dichloromethane (1x), dimethylformamide (1x), 20% triethylamine in dimethylformamide (2x), dimethylformamide (1x), 80% aqueous dioxane (2x), dioxane (1x) and dried in a vacuum oven overnight to give 2.4694 g of resin.
IR (cm-^{1}): disappearance of 2978 (t-BOC).

Coupling of the polymer supported macromolecule with 3, *using DCC.*

Monomer 3 (1.7484 g, 3.54 meq) and DCC (0.463 g, 2.24 meq) were dissolved in dimethylformamide (10 mL) and transferred to the polystyrene resin (2.1623 g) swollen in dichloromethane (7.5 mL). After 12 hours, the resin was washed with dichloromethane (3x), ethanol (3x), acetic acid (3x), 80% aqueous dioxane (2x), dioxane (2x) and dried in a vacuum oven overnight to give 2.6907 g resin.
IR (cm-^{1}): 3390, 3310 (NH), 2980 (t-BOC), 1690 (C=O).

Coupling of polymer supported macromolecule with 2, *using EEDQ.*

EEDQ (2.0 g, 2.2 eq) was added to a solution of polystyrene resin (2.12 g, 2.0 mmol/g) and monomer 2 (2.63 g, 2.0 eq) in freshly distilled tetrahydrofuran (8 mL). After 14 hours, the polymer was washed with tetrahydrofuran (2x), dichloromethane (2x), 5:1

tetrahydrofuran:methanol (1x), methanol (1x), and dried overnight in a vacuum oven to give 2.79 g (48%).
IR (cm^{-1}): 3330 (NH), 2978 (t-BOC), 1710 (br, C=O).

Cleavage of the hyperbranched polymer from the resin.

The resin (1.0311 g) was slurried in trifluoroacetic acid (20 mL), and HBr was bubbled into the mixture. After 100 minutes, the resin was washed with trifluoroacetic acid (3x). The solution was evaporated to dryness, washed with 1:1 methanol:water (3x), and evaporated to dryness. The polystyrene resin and the residue were dried in a vacuum oven to give 0.689 g of the resin and 0.3845 g of the macromolecule.
IR (cm^{-1}): 3290 (br, NH), 1670 (br, C=O).

Acknowledgements

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