REVIEW

The 8 year thicket of triazine dendrimers:
strategies, targets and applications

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This manuscript focuses on the routes, methods and reagents used to synthesize triazine-based dendrimers. Our pursuit of macromolecular architectures for drug delivery—dendrimers based on triazines—has been an ongoing effort for 8 years. To date, we have produced complex dendrimers with diverse peripheries as proof-of-concept, less complex molecules tailored for specific applications including DNA and RNA delivery and drug-decorated dendrimers for potential therapeutic applications including infectious disease and cancer. These syntheses have been executed at scales that range from high milligrams to over a kilogram. The routes, reagents and diversity displayed by a target anchors it in time. Early targets derive from convergent synthetic routes while later targets are prepared using divergent syntheses. The core of early dendrimers was a simple diamine, including piperazine, yielding the so-called bow-tie structures, middle period targets boast either a trispiperazinyltriazine core or a ‘super-core’ with six piperazine groups. Later targets return to the trispiperazinyltriazine core. The choice of linking diamine has also changed. Over time, p-aminobenzylamine was replaced by piperazine and then by aminomethylpiperidine with more exotic diamines sprinkled in throughout. Peripheral group choice has undergone similar variations: from AB$_2$ to AB$_4$ to, more recently, AB$_3$. The diversity communicated by these groups yields dendrimers ranging from those with a common surface to examples where two groups were presented to those where four orthogonally reactive groups appear. Over time, these groups have grown in complexity from protected amines to tags for biodistribution and drugs like paclitaxel. Herein, strategies adopted and lessons learned are reviewed, intuitions relayed and future directions forecast.

Keywords: triazine; melamine; dendrimers; drug delivery; synthesis; polymer

1. The seed: the motivation for triazine-based dendrimers

From a synthetic standpoint, dendrimers represent an interesting challenge. Any number of strategies can be employed in their synthesis. Syntheses are judged by

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The authors dedicate this manuscript to the memory of Mr Suhyung Park, a second-year graduate student in the group who passed away in 2008 during treatment for Ewing’s sarcoma.

One contribution of 7 to a Special feature ‘Current research trends in dendritic materials’.
both the complexity of the target and the ease of its preparation. The synthetic efficiency of a reaction can be evaluated in terms of reagent costs and stabilities, conditions, yields, isolation protocols and waste streams. For us, synthesis is a stimulating task given the wealth of reactions and reagents to choose from. Sometimes, the consideration dealt to these factors is transparent. Other times, less so. Our synthetic approaches, like most, are weighted by the biases and intuitions garnered along the process. Herein, we aim to provide some clarity towards our choices in efforts to produce dendrimers based on triazines and communicate the lessons we have learned over the last 8 years. For us, however, the area started in the early 1990s with a mentor’s mandate to ‘make something big’ from triazines (Mathias et al. 1993) and the report of a convergent dendrimer synthesis from Hawker & Frechet (1990). A year later, with encouragement from Professor Jeffrey Moore, who was engaged in arylacetylene dendrimers, the seed was buried (Xu & Moore 1993).

Before germination of our seed, other triazine-based architectures were reported (Steffensen et al. 2006). Many employed a nitrile cyclization to obtain the triazine ring, a method useful for synthesizing symmetric molecules (Hamerton 1994; Maciejewski 1995; Meijer et al. 1996; Maciejewski & Janiszewski 1999). To obtain asymmetric architectures, others described the chemoselective reactivity of cyanuric chloride using nucleophilic aromatic substitution. These chemistries find origins in the work of Fries in the late 1800s (Fries 1886a, b). The ‘ease’ of these reactions has precedent: substitution of cyanuric chloride with isopropylamine and ethylamine affords the asymmetric monochlorotriazine atrazine, a broadleaf herbicide, at 98.6 per cent yield (Gysin & Knusli 1958; Colby et al. 1979) in volumes of hundreds of millions of pounds each year (Kiely et al. 2004).

Cyanuric chloride is an attractive molecule for dendrimer synthesis owing to its low cost and chemoselective reactivity. The generally accepted reactivity trend is shown in scheme 1. The first substitution proceeds at 0°C, the second at 25°C and the third at 70°C (Steffensen & Simanek 2003).

While this scheme communicates the pronounced electrophilic differences between trichlorotriazines, dichlorotriazines and monochlorotriazines, abbreviating the nucleophile with an R hides elements of the design. The choice of diamine used to link these triazines into dendrimers plays a critical role. Differences in amine reactivity enable further control over the generalized reactivity trend and allow for more efficient and robust syntheses of dendrimers. To develop an understanding of the relative reactivity of different amines, we made monochlorotriazines to react with a variety of amines in competition experiments. The product distributions were quantified using nuclear magnetic resonance (NMR) to generate the series with associated relative reactivity values shown in figure 1 (Steffensen & Simanek 2003; Moreno & Simanek 2008a).
The reactivity trend follows the pattern of cyclic secondary amines > primary amines > anilines. This reactivity series can be rationalized using $p\text{Ka}$ data as well as steric and electronic arguments. Invoking $p\text{Ka}$ differences is useful for distinguishing the sluggish reactivity of anilines ($p\text{Ka}$ approx. 5.0) with most other amines used ($p\text{Ka}$’s approx. 8.0–12). However, this argument is lacking as the fourfold difference in reactivity between pyrrolidine ($p\text{Ka} = 11.27$) and piperidine ($p\text{Ka} = 11.22$) would not be expected (Moreno & Simanek 2008 $a$). Cyclic secondary amines are generally more reactive owing to a combination of effects. As the ring size decreases, the ring strain and the s-orbital character increases, which is hypothesized to cause an increase in orbital overlap with the electrophile and therefore increase the reaction rate. The 1.5-fold difference in reaction rates between diethylamine ($p\text{Ka} = 10.64$) and butylamine ($p\text{Ka} = 10.59$) is ascribed to steric. The difference in reactivity from the cyclic to the linear form is apparent. By combining amines with markedly different reactivity, we obtain linkers that are useful in dendrimer synthesis in that the chemistries could conceivably proceed without the use of protecting groups. Table 1 captures both data and intuition with respect to differences in reactivity for a range of amines with triazines. Reactions proceed when indicated in white, but not when indicated in dark grey. Light grey indicates partial reaction or more subtle factors. The table also reveals the extent of a reaction under given conditions. By reading down a column, one can infer that at room temperature (RT) aniline will react to form largely dichlorotriazine with trace conversion to monochlorotriazine. However, no trisubstituted product should be expected. Solvent, base choice and sterics play roles too, as will be addressed throughout the following pages.

2. Germination: breaking ground with convergent routes and $p$-aminobenzylamine

Early efforts in our laboratory focused almost exclusively on the convergent approach to dendrimer synthesis wherein the peripheral groups were elaborated and ultimately attached to the dendrimer core (Zhang & Simanek 2000). $p$-Aminobenzylamine ($p$-ABA) was pervasively used as a peripheral group (tert-butoxycarbonyl (BOC) protected) and as a linker in our early efforts: while it is prone to discolour owing to oxidation, $p$-ABA is commercially available, inexpensive and has a useful reactivity difference between the two amine groups. Scheme 2 shows the reaction of a peripheral group through the first of two iterative cycles with $p$-ABA and cyanuric chloride in atomic detail. The syntheses were convergent and divergent routes employed to the generation 3 dendrimer are
Table 1. A starting reference for the reactivity of amines with triazines as a function of temperature based on both experimental data and intuition. 

\(T\), temperature; \(t\) time (in h); TCT, trichlorotriazines; DCT, dichlorotriazines; MCT, monochlorotriazines; white, reaction proceeds to completion; light grey, partial reaction; dark grey, no reaction. Reading down a column allows prediction of product distributions.

<table>
<thead>
<tr>
<th></th>
<th>aniline</th>
<th>diethylamine</th>
<th>benzyl, butyl, azacyclononane</th>
<th>piperazine, piperidine, azetidine, pyrrolidine</th>
<th>water, methanol</th>
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<tr>
<td></td>
<td>T(^{\circ})C</td>
<td>0</td>
<td>25</td>
<td>70</td>
<td>0</td>
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<td>(t) (h)</td>
<td>1</td>
<td>6</td>
<td>12</td>
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also shown using a colour scheme that is conserved throughout the manuscript (linking diamines in blue distinguished by shape; triazines as a dot). Yields for each step (after chromatography) hovered between 89 and 98 per cent. In general, addition of cyanuric chloride is done at 0°C and warmed to RT. Typically, this reaction is stirred for 24 h at RT. Addition of \( p \)-ABA at 70°C in tetrahydrofuran (THF) led to a single regioisomer in times varying from 12 to 30 h depending on the starting material: longer reaction times are required for larger generation reagents because of steric congestion. At 100°C in dioxane, evidence for substitution with the aniline nitrogen was observed.

To complete the convergent reaction sequence to form generation 1, 2 (not shown) or 3 dendrimers, two monochlorotriazines can be reacted with a one-half equivalent of ethylenediamine in a procedure that requires 100°C dioxane and 36 h for generation 3; 80°C dioxane and 24 h for generation 2; and 80°C dioxane and 12 h for generation 1. Although these reactions are not fully optimized, the yields are consistent with this trend: generation 1–3 dendrimers are isolated in 80, 70 and 60 per cent, respectively. Our initial report also elaborated the BOC protected, generation 1 dendrimer in a divergent sense to the same generation 2 and 3 products under similar conditions (24 h at 80°C in dioxane), but at low yields for the monochlorotriazine addition steps (<10%). The divergent route to the generation 3 target is schematically shown in the bottom panel of scheme 2.

The convergent route was subsequently extended to generation 5 dendrons (Zhang & Simanek 2001). Chart 1 shows the fifth-generation target. The synthesis extended the convergent iteration described above. Throughout the third generation, yields ranged from 89 to 96 per cent for both the cyanuric chloride substitution steps (generally higher yields) and the \( p \)-ABA addition
steps (generally lower yields). Iteration to the fourth generation led to lower, but acceptable, yields for both steps (89%; 85%). Reaction with cyanuric chloride proceeded in 71 per cent to yield the product shown, although rigorous purification from the aniline-bearing dendron precursor proved impossible. The iterative nature of the synthesis was readily reflected in the $^1$H NMR traces. The chemical shift of the aromatic protons of the free aniline derivatives is distinct from the triazine-substituted derivative. These traces appear in the original scientific manuscript along with size exclusion chromatography traces that reveal that these materials are prone to aggregation. We attribute aggregation to the presence of multiple hydrogen bond donor and acceptor groups within the molecules. This aggregation can be reduced by adding copper (II).

The first hint at the versatility of triazine chemistry from our laboratory appeared later in 2001 (Zhang et al. 2001). Taking advantage of the stepwise substitution of cyanuric chloride, generation 3 dendrimers bearing either one or two protected amines on the periphery in a sea of butyl groups were prepared (scheme 3; aliphatics are shown in purple). The convergent approach required that in addition to the convergent elaboration of the amine dendron (top path), all-butyl dendrons (bottom path) were also prepared and utilized as reagents at the appropriate times. The targets shown in scheme 3 could be prepared in five or six linear steps (11 or 12 total steps) with an approximately 40 per cent overall yield.
Scheme 4. Dendrimers with piperazine and p-ABA linkers show different propensity for gelation in acidic chloroform. Reproduced with permission from Zhang et al. (2002).

Throughout, the synthesis of the all-butyl dendrons, yields ranged between 89 and 95 per cent. For the dendrons comprising a single, protected amine, yields for the p-ABA step averaged approximately 10 per cent higher (93%) than reactions with cyanuric chloride. Completion of this synthesis featured the use of the more reactive diamine, piperazine, as the central core in yields of approximately 80 per cent based on 36 h reaction at 80°C. To make the monofunctionalized dendrimer, the all-butyl dendrimer was first elaborated with an excess of piperazine in 92 per cent yield before reacting with the monochlorotriazine dendron bearing the single, protected amine. More than 250 mg of each product was obtained and characterized by mass spectrometry, NMR spectroscopy and atomic force microscopy. Characterization data derived from combustion analysis could only be reconciled with the inclusion of one or two solvent (THF or MeOH) molecules or multiple trifluoroacetic acid (TFA) molecules (seven or nine) when the protected amines were unmasked.

3. Exploring new leaves and branches

The transition from p-ABA to other linking diamines started when we pursued the molecular basis for gelation of the targets shown in scheme 3 (Zhang et al. 2002). Piperazine was chosen as a linker because it does not provide hydrogen bond donors (scheme 4). Generation 1 and 2 dendrimers derived from p-ABA did not form gels. Generation 3 dendrimers with no piperazine linkers formed gels at concentrations > 2 mM. Dendrimers wherein four or six linking p-ABA groups were replaced with piperazine did not gel until concentrations exceeded 30 mM. Dendrimers wherein all the p-ABA groups were replaced or all the peripheral butyl groups were substituted with piperidine groups did not form gels. Traces of ethanol rapidly dissolved the gels as did methanol, isopropanol and DMSO. These materials were accessed using a convergent synthetic approach. The synthesis was executed for long reaction times (up to 36 h at 80°C) that were not optimized with excellent yields for most steps except the final dimerization reaction around the

Scheme 6. An orthogonally reactive dendrimer with 4 hydroxyl groups, 4 silyl ethers, 4 esters, 4 disulphides and 16 BOC-protected amines. Reproduced with permission from Steffensen & Simanek (2004).

piperazine core. These reactions proceeded with yields of 70–80% for generation 3 dendrimers.

Over time, we invested further energies towards identifying other linking diamines besides p-ABA and piperazine. Our first studies of chemoselectivity culminated in the convergent synthesis of the dendron shown in scheme 5 (Steffensen & Simanek 2003). The unprotected alcohols (illustrated throughout in pink) were installed at low temperature. In the same pot, p-ABA was reacted at 70°C to provide the peripheral group in 92 per cent yield. Iteration with cyanuric chloride, then aminomethylpiperidine (AMP), at RT proceeded without any undesirable isomer in 85 per cent overall yield. The sequence of p-ABA and AMP additions can be reversed without penalty. Iteration with cyanuric chloride and then isonicotinic acid with ammonium hydroxide at 50°C led to the desired product in 87 per cent yield. Pilot studies also confirmed that aminoethylpiperazine reacts with dichlorotriazines through the constrained secondary amine. Although the hydroxyl groups suggested opportunities for purification by precipitation, conventional silica gel chromatography was used in most cases.

While linking groups are important for efficient synthesis, we recognized that peripheral group diversity was critical as we considered function. The peripheral group diversity accessible in triazine dendrimers is reflected in a target referred to as the fruit salad tree (scheme 6), a namesake chosen owing to its similarity to trees bearing grafts derived from multiple fruiting trees (Steffensen & Simanek 2004). Our tree bears 16 BOC-protected amines, four free hydroxyls, four pyridyl disulfides and four levulinic acid groups. The reagents, conditions and yields serve
as benchmarks for many of these transformations. A common monochlorotriazine peripheral group presenting two BOC-protected amines and an alcohol is available in quantitative yields from the protected triamine and aminoethoxyethanol in 10 g batches. This alcohol can be elaborated to levulinic ester or silyl ether in 96 per cent or 95 per cent yields in 8 h at RT with the appropriate reagents (dicyclohexylcarbodiimide coupling or silylchloride, respectively). Elaboration to the AMP derivatives proceeds in 100 per cent (alcohol), 96 per cent (silyl ether) or 94 per cent (ester) yields by treatment with AMP for 8 h at RT. Preparation of the disulphide-containing groups proceeds in one pot at 93 per cent yield.

Coupling reactive peripheral groups with cyanuric chloride (2 h at 0°C for the first group; 24 h at RT for the second group) followed by reaction with AMP (18 h, RT) provided the generation 2 dendrons. Note the longer—doubled—reaction time for generation 2 compared with generation 1. Lower yields for the disulphide/levulinic acid dendron (79%) might be attributed to loss of either the disulphide or ester groups: indeed, we cannot definitively say. The ether/alcohol dendron was available in 99 per cent yield. Reacting both fragments in a stepwise fashion with cyanuric chloride under the previous conditions yielded the generation 3 dendron in high yield (95%) that could be dimerized with piperazine in a reaction that yielded 89 per cent of the desired product after 10 days at RT. Acylation of the pendant alcohols (90%) followed by desilylation (96%) followed by ester hydrolysis (92%) and thiol–disulphide exchange with a biotin derivative (88%) served as proof-of-concept for manipulating these groups. These reactions were affected in <4 h at RT.

The preceding synthesis anchored our first serious efforts in drug conjugation chemistry (Lim & Simanek 2005). Both the lower yields of the levulinic/disulphide intermediates and our belief that this level of diversity was excessive led us to pursue the generation 4 target shown in scheme 7. Using conditions similar to those previously described, this material is available in seven linear steps at 55 per cent overall yield. Yields for the peripheral groups proceed above 90 per cent with reaction times that vary with the step: reactions of amine groups with cyanuric chloride proceed in 2 h at 0°C, then 24 h at RT; the third chloride substitution with AMP proceeds in 8–24 h at RT with increasing times required with increasing generation. Iteration to the product proceeds in yields between 83 and 87 per cent. The synthesis features the isolation of a structurally sophisticated dichlorotriazine as the penultimate intermediate before dimerization with piperazine. This dichlorotriazine is afforded after 5 h at 0°C.

Dimerization proceeds in 48h at RT. Overall, the entire synthesis can be executed in 7–8 days to yield 20g of the product. As a prelude to biodistribution studies, the product was reacted with a derivative of the Bolton–Hunter reagent with a reactive, constrained piperazine group (panel 3 of scheme 7). In a 1:2 mixture of CH₂Cl₂ : methanol, the reaction proceeds quantitatively after 7 days, although no effort was made to optimize this reaction. Evidence for solvolysis of the chlorotriazine groups was not observed in this case.

The linking diamine of choice clearly has evolved over time—from p-ABA to piperazine to AMP—as schematically reflected in the preceding figures. Although published more recently, the target shown in scheme 8 was prepared in 2004/2005, as might be predicted based on the choice of piperazine linkers (Umali et al. 2007). The primary motivation for synthesizing this molecule was to explore strategies for incorporating orthogonally protected amines. The product is available in four linear steps in 43 per cent overall yield in 5g batches. Preparation of the suitably protected Dde (2-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl) and BOC-protected amines proceeds in 85 and 88 per cent yield. Reaction with cyanuric chloride takes place in 75 per cent yield overnight. Piperazine reaction and subsequent elaboration to yield the generation 2 monochlorotriazine dendrimer both require 12h reaction times at RT. The synthesis is intercepted with a super-core displaying six piperazinyl groups because the more common trispiperazinyltriazine core did not yield a product upon reaction with the requisite generation 3 dendrons. The protected target was prepared with the polymer-supported base TBD (1,5,7-triazabicyclo[4.4.0]dec-5-ene) (rather than the typical diisopropylethylamine) in 66 per cent yield after 48h. Low yields were attributed to difficulties in handling the Dde group. The use of dichlorotriazines as intermediates was also investigated in this synthesis, because these materials allowed greater confidence when characterizing these materials. Indeed, during the synthesis of the heterofunctionalized peripheral group, traces of homofunctionalized materials derived from a two-step/one-pot procedure could pass through undetected by thin-layer chromatography (TLC), NMR and mass spectrometry, but only to be detected in the mass spectra of more sophisticated intermediates. Finally, Dde groups could be cleaved with hydrazine (85% yield) and the resulting amines acylated with mercaptopropionic acid groups bearing a pyridyldisulphide (60% yield). We conclude that it is
more effective carrying disulphides through the entire synthesis than appending through this strategy. Subsequent deprotection of the BOC-protected amine and polyethylene glycol (PEG)ylation yields constructs that are water soluble and reminiscent of drug-delivery vehicles.

The studies of different linking diamines were punctuated last year with the exploration of an additional three diamine linkers that contain both a constrained cyclic secondary amine and an exocyclic primary amine shown in scheme 9 (Moreno & Simanek 2008b). The synthesis relied on the preparation of generation 3 dendrons and their attachment to a core that displayed three azetidine groups. These selections were judicious in that they provided (i) proof-of-concept for new linkers, (ii) unique $^1$H NMR signatures, and (iii) further insight into the reactivity/steric congestion conflict that arose in previous failed attempts using the trispiperazinyltriazine core with trisubstituted (AB$_2$B$'$) peripheral groups. To prepare the target, BOC–azetidine was reacted with cyanuric chloride for 7 days at 70°C, isolated and deprotected. The first reaction proceeds in 95 per cent yield. The second is assumed quantitative to yield the core that appears in the dashed circle of scheme 9. Reaction with the generation 3 monochlorotriazine dendrons required 7 days at 70°C yet yielded the desired dendrimer in 35 per cent after purification by silica gel chromatography. We would predict that the trispiperazinyltriazine core with a four times lower reactivity (figure 1) would not have provided a product in any satisfactory period. The growing steric congestion of the dendrons was reflected in increased reaction times and temperatures as the synthesis progressed—from overnight at RT to 2 days at 40°C. Conformational analysis on the dendrimer and other models shown led to the picture that the peripheral groups are rapidly moving and folding backward into the dendrimer, whereas the core moves more sluggishly. Additional observations are reported in the paper.

4. Starting to bear fruit: strategies for decorating trees

The ability of these materials to engage in host–guest chemistry was probed very early with a generation 3 dendrimer comprising triazines linked by piperazine derived using a convergent approach (scheme 10; Zhang et al. 2003a). This target was obtained by reacting generation 3 dendrons with what would become
a commonly used trispiperazinyltriazine core. Water solubility was conveyed by the oligo(ethyleneglycol)diamines on the periphery. Yields that hovered at approximately 80 per cent for all these reactions were attributed to the undesirable dimerization event that occurred on elaborating a monochlorotriazine dendron with piperazine. Reactions were performed at 70–80° C for 12–24 h. Lower temperatures and longer reaction times would ultimately improve these yields. Ultimately, this shortcoming of piperazine (dimerization) would lead to its replacement with AMP.

The iterative nature of the chemistry was revealed using 1H NMR by the extent of substitution of piperazine groups from 3.6 ppm (disubstituted interior) to 3.9 and 3.5 ppm (monosubstituted focal) and also using 13C NMR by the chlorotriazine line in the 13C NMR (approx. 168 ppm). These traces appear in the paper. Comparison of pyrene solubilization between this triazine dendrimer and representative polyamidoamine (PAMAM), poly(propyleneimine) (PPI), and Fréchet benzylethers confirmed the hydrophobic nature of the interior. That is, triazines on average solubilized 0.2 molecules of pyrene/dendrimer whereas benzylethers solubilized 0.5 molecules/dendrimer, PAMAM solubilized 0.01 molecules/dendrimer and PPI dendrimers solubilized 0.03 molecules/dendrimer. The dendrimer also enhanced the solubility of 10-hydroxycamptothecin (3.7 molecules/dendrimer) and a bisindolemethane (4.5 molecules/dendrimer), but did not affect the solubility of more hydrophilic compounds like methotrexate. Preliminary biological evaluation in vitro and in vivo bolstered our enthusiasm towards the use of these materials in drug delivery (Neerman et al. 2004a).

We reported on DNA–dendrimer constructs in 2003 (Bell et al. 2003). These targets shown in scheme 11 were arrived at by the convergent route and incorporated cationic amines, and one or more pyridyl disulphide groups as well. The enhanced reactivity of piperazine allowed for reactions to

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Scheme 10. A host of guests derived convergently using piperazine linkers and a ‘common’ core. Reproduced with permission from Zhang et al. (2003a).

Scheme 11. Targets for DNA conjugation. Reproduced with permission from Bell et al. (2003).
be conducted at RT over 14h. Installation of the peripheral disulphide relied on the use of the cysteamine derivative as the second substitution following the BOC-protected diamine (illustrated in green). To install the focal disulphide, the monochlorotriazine dendrimers were reacted with an excess of piperazine at RT for 14h followed by the commercially available NHS-ester derivative of 3-S-thiopyridylmercaptopropanoic acid. Disulphide exchange with commercially synthesized oligonucleotides bearing a 3′-thiol was followed using mass spectrometry and polyacrylamide gel electrophoresis. The low reaction yields for many of the steps (30–80%) led us to further explore the compatibility of disulphides with triazine chemistry. Extensive efforts for characterizing these adducts are described in greater detail in the manuscript.

Scheme 12 shows the targets of interest that were ultimately elaborated with the angiotensin-converting enzyme inhibitor (and thiol containing) captopril and cysteine peptides containing the T-antigen for K99, a colonization factor antigen responsible for bacterial infection in cattle (Umali & Simanek 2003). The tetravalent and octavalent constructs were available in six or eight steps in 17 and 10 per cent overall yields, respectively. In general, reaction with cyanuric chloride proceeded in good yields (>85%), but elaboration with piperazine proceed in poor yields over the first two iterations (86%, then 53%). Dimerization to yield the octamer proceeded in 37 per cent yield. Exchange with captopril proceeded to completion in methanol at RT. The process can be monitored with mass spectrometry and high-performance liquid chromatography (HPLC). Exchange with the peptide (CLKKDDRA) proceeded to completion with the tetravalent platform, but not with the octavalent construct. The hexavalent peptide construct is evident within 2 days, but further incubation up to 10 days did not lead to complete reaction of the octamer. Importantly, peptide exchange is accomplished in dimethylformamide with guanidinium chloride. In a pilot study, the octavalent display elicited a weak immune response in a rabbit with the addition of Freund’s adjuvant (Neerman et al. 2005). Unlike bovine serum albumin, there was no immune response to the naked dendrimer carrier. Perhaps more interesting, however, was the observation that these constructs proved very useful for ELISAs.

One of the earliest initiated efforts with disulphide-containing dendrimers—revealed by the reliance on the p-ABA linker—was designed to probe whether reductive cleavage of the disulphide bond might be both cooperative (owing to intramolecular macrocyclization) and subject to manipulation of reaction rates owing to their placement within the construct (Zhang et al. 2003b).
The structures shown in scheme 13 were obtained through iterative convergent synthesis. Yields for individual steps ranged from 70 to 90 per cent. Typical reaction times for iteration with cyanuric chloride (0°C at RT), p−ABA (80°C) and final dimerization with piperazine (80°C) were 24, 18 and 24 h, respectively. Release of the dansyl chromophore was monitored spectrophotometrically using dithiothreitol as a model reductant that might emulate the higher intracellular concentrations of reducing agents compared with the vasculature. As the size of the construct increases, the rate of release decreases, albeit slightly. Mass spectrometry was used to obtain a more elaborate model of release in one construct. Kinetic analysis of the data showed that cleavage rates increased slightly as the reaction progressed from tetra(disulphide) towards tetra(thiol).

5. Planting trees on solid supports

One of the first examples of nanocomposite materials that we described grew from a collaboration with investigators in the Department of Soil and Crop Sciences at Texas A&M University (Acosta et al. 2003). The goal was to derivatize a layered smectite clay, montmorillonite, with triazine dendrimers by replacing advantageous adsorbed cations with cationic amine anchors at our ‘tree’s trunk’. Incorporating hydrophobic groups on the periphery was intended to make the materials compatible with a polymer matrix. The iterative convergent synthesis of the targets shown in panel 2 of scheme 14 proceeded in yields that varied from 60 to 90 per cent generally. Iteration with cyanuric chloride and diamines were accomplished in 24 h at 0°C at RT or 100°C, respectively. Using X-ray powder diffraction, thermal gravimetric analysis and IR spectroscopy, we concluded that only the generation 1 dendron (leftmost) and linear analogue of the generation 2 dendron (rightmost) intercalate. In subsequent studies, the ability of these materials to sequester atrazine was examined (Neitsch et al. 2006).
Triazine dendrons could also be attached to, or grown from, inorganic supports including mesoporous silica (Acosta et al. 2004a). Scheme 15 shows the strategy employed to decorate mesoporous SBA-15. The reactions with cyanuric chloride or AMP were executed in a cold room on a shaker at 4°C for 24 h by incubating wafers in THF solutions at 0.3 or 0.4 M, respectively. Organic content increased from 3 per cent to a maximum of 35 per cent for the fourth generation, values consistent with reaction yields of 70–80 per cent per step. Nitrogen adsorption isotherms are consistent with decreasing pore volume with increasing dendrimer size. Calculated effective pore diameters drop from 8.3 nm for the unmodified substrate to 4.5 nm for those modified with the generation 4 dendron. Physisorption of these materials to inorganic supports has also been examined (Javaid et al. 2006).

Silica gels (Acosta et al. 2005) and polystyrene resins (Acosta et al. 2004b) have also been used as solid supports, according to the previously described divergent growth strategy and an attach-to strategy. The attach-to strategy used dendrons comprising piperazine and triazines that were prepared convergently in solution and then reacted with the surface through the surface-pendant piperazinyl group. Reaction with the chlorotriazine dendrons in refluxing THF took 12 h. This latter approach yields composites that were approximately 20 per cent organic content independent of whether generation 1–3 materials were used. The divergent growth approach led to materials with organic content that increased with increasing generation based on thermal gravimetric analysis. Instead of the two-step iterative approach with piperazine and cyanuric chloride, a monochlorotriazine monomer displaying two BOC-protected piperazines was used. The iterative divergent approach then required both reaction and TFA-deprotection steps. Disappearance of the carbonyl line in the IR spectrum in iterative cycles served as a useful handle for monitoring the extent of the deprotection. The materials were also

characterized by X-ray photoelectron spectroscopy and mass spectrometry of organic residue derived after dissolution of the silica support with HF. The ability of these materials with reactive piperazine groups to sequester the monochlorotriazine, atrazine, was also probed. Like similar constructs prepared divergently on polystyrene, the ability to react with atrazine correlated with the density of piperazine groups (Hollink et al. 2005a). Extending these efforts to make regenerable resins by acidic cleavage of dimethoxybenzylamines was also probed (Hollink et al. 2005b). Other studies include the derivatization of nanoporous membranes for the separation of volatile organics (Javaid et al. 2006; Yoo et al. 2006, 2009).

6. Moving forward with biological purpose

To further our evaluation of the role of these materials in drug delivery, we prepared seven generation 3 dendrimers that varied in the peripheral group (Chen et al. 2004). These materials were prepared by multi-day reactions of a pure parent (R=H; scheme 16) with a variety of reagents. The resulting materials are most certainly mixtures deriving from substoichiometric (incomplete), desired and superstoichiometric (quarternization) reaction; descriptions that vary with reagent. Two elements of the convergent synthesis are notable. First, the peripheral groups represent AB₄ building blocks derived from reaction of diBOC-protected triamine. Interestingly, the barrier for triazine-exocyclic amine rotation is high enough in the monochlorotriazine intermediates that BOC groups and methylenes of the linking propyl group are chemically unique. Additionally, the synthesis employed a super-core, a generation 1 dendrimer displaying six piperazine groups. A survey of bases revealed a preference for this reaction: Instead of the commonly used Hunig’s base, solid phase BEMP led to the highest conversions and fewest side products during the coupling of the super-core with the generation 2 dendrons. Yields for the preparation of the parent dendrimer reflect our improved understanding and design (86–98%). Overall, the parent is available in five steps at 56 per cent overall yield at 5 g scale. Biological evaluation of these materials reveals a dose-dependent toxicity that decreased from the toxic cations to the more biocompatible PEG derivative. The PEG derivative was well tolerated in vivo. The ability of one material (R=H) to attenuate hepatotoxicity of mercaptopurine and methotrexate was probed using alanine transaminase levels as reporter. Toxicity was reduced by 27 and 36 per cent, respectively (Neerman et al. 2004b).

The target shown in scheme 17 closes what we consider the first chapter of our efforts in this area (Lim & Simanek 2008). The synthesis used to achieve the unmodified dendrimer reagent proceeded in six linear steps from the protected triamine in 64 per cent overall yield (all reactions ≥90%). Most reactions in this convergent synthesis are executed over 24 h at RT with the exception of the final dimerization with piperazine that requires 2 days at 40°C. After quantitative deprotection, the polyamine is elaborated in four steps to a candidate vehicle for drug delivery, a PEGylated dendrimer displaying 16 (hypothetically) molecules of paclitaxel and an identical number (hypothetically) of PEG-2 kDa chains. At this point, in our experience, we hesitate to either over- or under-dramaticize the importance of the last four steps shown. The success we have
had with these four steps clearly shapes our current approach to many targets (although arrival at the dendrimer reagent has undergone dramatic revision, vide infra). We find four points noteworthy. First, paclitaxel was installed through the use of a dichlorotriazine linker onto a poly(amine) dendrimer. While the reaction clearly can proceed to completion, the final product here appears to be a mixture comprising primarily both 13–16 paclitaxel groups. Second, the resulting poly(monochlorotriazine) dendrimer can be ‘quenched’ with AMP without significant loss of paclitaxel groups, which is ameliorated, in part, by immediate PEGylation. Third, minimum PEG size to convey water solubility appears to be 2 kDa. PEGylation with 0.6 or 1.2 kDa PEG yielded products that were not water soluble. Fourth, the drug loading achieved after 2 kDa PEG (46 kDa total mass) or 5 kDa PEG (77 total mass) is 30 or 18 wt%, respectively. PEG content is 52 or 71 wt%, respectively. Triazine dendrimer represents the smallest composition component at 18 or 11 wt%, respectively. These concentrations place us above the clinically relevant range for Taxol (delivered in Cremophore EL) before the dilution step. A construct with very similar composition (without paclitaxel) was evaluated in vivo for biodistribution (Lim et al. 2008).
Scheme 18. A divergent route to diversity with a representative dichlorotriazine monomer that was added to the trispiperazinyltriazine core, capped with aminoethoxyethanol and elaborated. Reproduced with permission from Hollink & Simanek (2006).

7. Going out on a new limb: solution phase divergent syntheses

When we ventured into this field, we recognized that the primary limitations were the lack of scalable syntheses that produced pure materials with orthogonally reactive groups. Our efforts with convergent routes to these triazine dendrimers represent solutions to these challenges at some level. Most certainly we can make multigram (i.e. 20 g) batches of small generation dendrimers that are pure owing to, in large part, chromatographic purification after every synthetic step. The strategies could take us to the doorstep of pre-clinical evaluation if one was willing to invest in the effort. However, these routes still leave room for improvement.

One stick by which we measure our progress is PAMAM dendrimers. As the products of divergent syntheses, these materials can reach high generations at the cost of the moniker ‘single chemical entity’ as well as the need for statistical decoration of either the poly(amine) or poly(acid) surface. Still, the divergent route communicates clear advantages, not the least of which is the opportunity for ‘mole conservation’. That is, convergent routes suffer, in general, from the need for dimerizations (for AB₂ building blocks) at each generation. If a mole of peripheral groups is fed into the synthesis, a theoretical one-half mole of product is available for a generation 1 construct (or less if not a dimerization product), a one-fourth mole for generation 2, a one-eighth mole for generation 3, etc. This strategy communicates the need to carry almost all of the mass through the synthesis from the initial stage. These characteristics are in stark contrast to a divergent route wherein if 1 mol of core is started with, 1 mol of dendrimer is theoretically available. Given the relative mass of core (MW 100s Da) to a dendrimer of even modest generation (3 has an MW exceeding 6 kDa), most of the mass is added at the end of the synthesis. Depending on the chemistries employed, the divergent route could reduce solvent demands at reaction, workup and purification stages. Starting in 2005/2006, we re-examined the role that divergent routes might play in the preparation of triazine dendrimers.
Our first report described a range of targets produced from the common trispiperazinyltriazine core shown within the dashed circle in scheme 18 (Hollink & Simanek 2006). Four of these targets relied on the initial reaction with the dichlorotriazine shown. Implicit in the design is the recognized importance of a reactive, constrained secondary amine, piperazine, on the core and a reactive electrophile. The pronounced reactivity differences between monochlorotriazines and dichlorotriazines (illustrated by our first effort in 2000) biased us towards the latter. This approach also afforded us the opportunity for a capping step which, to us, represented an opportunity for the introduction of compositional/functional diversity elements. In the end, the top three dendrimers shown in scheme 18 were prepared in four steps from the monomer and core, required two chromatographic purifications and were obtained in overall yields ranging from 65 to 75 per cent. Reactions with dichlorotriazine monomers were initiated at 0°C and then stirred at RT for 16 h. Capping steps with aminoethoxyethanol required 24 h at 65°C. The deprotection required a 1:2 mixture of conc. HCl: MeOH and 12 h reaction time. The poor solubility of the bottom two targets provided enough material for characterization. Mass spectra revealed that the dichlorotriazine can engage in over reaction (with an alcohol) and incomplete reaction.
While certainly dendritic, the molecules of scheme 18 might also be considered ‘star-like’ owing to the dichlorotriazine monomer used. Indeed, for dendrimers to result, the dichlorotriazine must either provide a substituent with two reactive nucleophiles the capping step must install a nucleophilic group. We pursued the former and instead of the triamine used in the original study, H₂NCH₂CH₂NHCH₂CH₂NH₂, we used one with propyl spacers. In our experience, materials derived from the latter longer triamine were much more soluble in a range of organic solvents than those derived from the former shorter triamine with ethyl spacers. The iterative divergent synthesis we envisioned and ultimately executed using piperidine as the capping reagent is shown in scheme 19.

The strategy outlined works well. Scheme 20 shows the generation 1–5 products obtained by this strategy along with the key monomer (Crampton et al. 2007). In addition to common reagents, the yields and conditions remain largely universal across the range of targets. Capping with piperidine and deprotection proceed quantitatively in all but one case. The capping step is affected at RT over 12–16 h. The deprotection is accomplished in 5 N methanolic HCl at RT over 15 h. Addition of monomer for generations 1–4 occurs in 15 h at RT (although the initial addition is done at 0°C). Solubility challenges led us to move from THF to THF–EtOAc mixtures to ultimately chloroform for generation 5 in a reaction that took 5 days at RT. The yields of these monomer additions dropped over the generations from 93 per cent at generations 1 and 2 to 78 per cent, then 69 per cent and finally 25 per cent for generation 5. Only the
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products of monomer addition, the poly(monochlorotriazine) dendrimers, were subjected to chromatography. Through generation 3, the materials appeared to be pure based on TLC, NMR spectroscopy, mass spectrometry, gel permeation chromatography and HPLC. Impurities arising from incomplete reaction are present in the generation 4 and 5 materials. These efforts are scalable. Two contributions to Organic Synthesis will appear in 2009 that describe preparation of monomer and core as well as the generation 1 dendrimer (Chouai et al. in press a,b). With a single chromatographic step, we have prepared a kilogram of generation 2 dendrimer in our lab at a purity of approximately 95 per cent (Chouai & Simanek 2008).

8. A new spring: conclusions and future directions

A rapid iconic review of the figures reveals synthetic trends anchored in lessons we have learned in the preparation of these molecules. The routes, whether convergent or divergent, rarely yield products greater than generation 3 compared with PAMAM strategies that yield generations up to 10. Both the divergent and convergent routes are successful, yielding materials that are accurately described as single-chemical entities. Both routes require longer reaction times and increased temperatures as size increases. Convergent routes rely on reaction of monochlorotriazines that become increasingly congested sterically as generation increases. Divergent routes require more reactions per synthetic step as generation increases. To accelerate reaction, more reactive amines are employed in the convergent strategy while dichlorotriazines are utilized in the divergent route. During a convergent synthesis, dimerization around a central core appears to work so long as the branching and peripheral groups are AB2 type. If AB3 type groups (like the AB2B’ described) are used, trimerization around a trispiperazinyltriazine core is successful, but a larger hexavalent core is required if AB4-type groups are employed. The diamine of choice for convergent iteration has evolved from p-ABA to piperazine to AMP and remains as such. However, divergent routes relying on a diprotected triamine appended to a dichlorotriazine are emerging as routes of great interest. Propyl spacers provide solubility and reactivity advantages over ethyl spacers in this triamine. Regardless of route, dispersity is usually introduced on post-synthetic (in terms of dendrimer) manipulations whether it be on PEGylation or functionalization.

Perhaps not surprisingly, predicting what will happen with our efforts over the next 8 years is difficult. We can predict with some reasonable certainty that the lessons we learn in characterization, the preparation of disulphide core dendrimers (Zhang et al. in press), and the application of click chemistry will appear in the near future. Fruits of collaboration that further our efforts in drug delivery—from the synthesis of new paclitaxel constructs or those containing other drugs to more intensive biological investigations—will also appear. Indeed, we hope that part of the story turns sharply into applications. We hold firm to our belief that these materials may indeed play a role in human health at some point in the future. We take consolation in the belief that targets selected and pursued for these ends will push the chemistry forward. We have intentionally not focused our discussions heavily in these areas of application: while multiple saplings are evident to us, we are far short of a thicket. Chemical
challenges still exist. Cost-effective surrogates for the BOC group, shorter reaction times, easier purification strategies and more rigorous evaluation of the potential of the iterative, divergent route to dendrimers will occupy some of our energies.

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