Exploiting dense shell/packing principles to invoke stereoselectivity in a reaction accelerated by a chiral dendrimer†

Adam Ellis, Melanie Wallace and Lance J. Twyman*

As dendrimers approach their dense shell or dense packed limit, a certain amount of conformational organization exists. Any substrate binding within the dendrimer’s external layer will experience the same organizational effects. This paper describes how these effects can be exploited towards stereocontrol with respect to binding and reactivity.

Our attempts to mimic the reaction rates of enzymes fall into two main classes. The dominant mimetic group consists of small molecules (usually organic soluble), designed to bind substrates and influence reactions through geometric constraints. A second approach attempts to mimic the encapsulation behaviour of enzymes using larger polymeric molecules. This approach enables careful control of internal functionality and more importantly, internal environment. As well as providing specific internal environments, these bulk molecules are often very soluble in water. Many types and classes of polymers have been used to mimic enzyme behaviour. However, the best of these are probably dendrimers. These globular polymers possess precisely controlled monodispersed structures. As a result of this control relatively complicated molecules can be created.

We have previously used the terminal amine groups from a series of dendrimers to accelerate a simple aminolysis reaction in water. As part of this study we noticed that the size of the dendrimer had a significant effect on the rate of reaction. This was due to the specific encapsulation mechanism of the accelerated reaction, which involves hydrophobic binding of the substrate within the outer sterlic layer of the dendrimer. As such, the substrate is held in close proximity to the terminal (reactive) amine groups. The fastest reaction was observed for the dendrimer with 32 terminal groups.7,8 Peripheral functionalization of the DAB dendrimer with \( \text{L-phenylalanine} \) was performed in two steps as shown in Scheme 1. The DAB-Am-16 dendrimer 1 was reacted with a 10% excess (per terminal amine) of the Fmoc-L-phenylalanine \( N \)-hydroxysuccinimide,11 to produce a protected amino acid terminated DAB dendrimer. The Fmoc protecting groups were removed giving the desired chiral DAB dendrimer 2 in a reasonable 27% overall yield. Mass spectrometry, along with 1H and \( ^{13} \text{C} \) NMR confirmed that reactions had taken place with complete saturation of the terminal groups. Specifically, the MALDI-TOF mass spectrum of the Fmoc protected intermediate generated a molecular ion peak at 7598 (M+), which corresponds to a DAB dendrimer with 16 terminal Fmoc groups. Similarly, the 1H and \( ^{13} \text{C} \) NMR spectra of the dendrimers confirmed aromatic functionality on the intermediate and final structures. Comparisons of the aromatic and aliphatic...
integration ratios confirmed the correct number of protons for the 16 terminal Fmoc groups in the intermediate, as well as the 16 terminal benzyl groups on the DAB-AM-16 α-phenylalanine terminated dendrimer 2.

The next step was to select the substrates. In an effort to maximize host-substrate interactions, the p-nitrophenyl active esters of Boc protected α- and β-phenylalanine were chosen (compounds 4 and 5 respectively) (Scheme 2). The functional groups match the terminal amino acid functionalities on the dendrimer. In aqueous solution, any hydrophobically driven inter-penetration or surface complex will be accompanied by intermolecular hydrogen bonds and π-π stacking interactions, leading to the formation of a diastereotopic complex, with the polar NO₂ groups orientating themselves towards the bulk aqueous solvent. When the 4 substrate 4 is used, a matched isomeric complex can form between the L terminated dendrimer and the L substrate. Alternatively, a mismatched isomeric complex forms when substrate 5 is used (the D dendrimer and the D substrate). After substrate penetration and complex formation, the dendrimer’s terminal amine groups are then free to attack the substrate’s active ester and for each reaction two energetically different transition states and products are possible (the L,L or L,D α-amino acid products 6 and 8 respectively). Due to the chirality on the dendrimer’s terminal groups, these combined interactions may be optimized for one particular diastereotopic pair, leading to a faster (and selective) aminolysis reaction.

The reactions were carried out as previously reported, with reactions performed at pH 8.5 using the α-phenylalanine terminated DAB-Am-16 2, and either the L or D substrate (compounds 4 and 5 respectively). For all reactions, p-nitrophenolate is liberated during aminolysis, which is UV active and enables the concentration of product to be calculated using UV spectrophotometry (by following the increase in the signal intensity at 410 nm). Our first control experiment simply measured the background reaction of the substrates in buffer (6.0 × 10⁻⁵ M solution of substrate 4 or 5 in 0.1 M, pH 8.5 Tris). The initial rate was determined as 3.11 × 10⁻⁹ M s⁻¹ and was subsequently subtracted from all future (aminolysis) experiments. A second set of controls designed to measure any difference in rate between the D/L substrates and the chiral amine α-phenylalanine methylster (4, 5 and 3 respectively). The amino acid 3 is an analogue of the dendrimers terminal groups and represents a useful control for the non-encapsulated reactions. Specifically, a 6.4 mM solution of α-phenylalanine methylster 3 was prepared in Tris buffer (0.1 M, pH 8.5). Separate concentrated acetonitrile solutions were made up for the D and the L substrates (compounds 4 and 5 respectively). The D and the L reactions were studied independently by placing a solution of dendrimer 2 into a 1 cm UV cuvette and inserting this into the UV machine. A small aliquot of the concentrated D or the L substrate solution was then added (giving a final substrate concentration of 6.0 × 10⁻⁵ M in the cuvette) and the absorption intensity at 410 nm recorded every 10 seconds for a total of 1200 seconds. The experiments were repeated six times. The absorption data was then averaged and plotted against time. The plots were linear over the first 60 seconds and the gradients used to calculate the initial rates for the D and the L substrate (after subtracting the initial rate of the background hydrolysis reaction), Table 1. The rate for the L substrate 4 was slightly faster than the corresponding reaction with the D substrate 5. Although these rates are within the error range of the experiments, all six sets of reactions showed a similar preference for the L substrate. As such we can conclude that the fastest control reaction occurs between the L amino acid 3 and the L substrate 4 (via a lower energy diastereomeric transition state).
Having carried out the controls and determined the reactive pair preferences, we then proceeded to react the same d and l substrates (4 and 5 respectively) with the l-amino acid dendrimer 2. The reactions were carried out in the same way, except that a 0.4 mM solution of l-phenyl terminated dendrimer 2 was used (giving the same 6.4 mM amine concentration used in the controls). On this occasion the initial rate for the matched system (the l substrate 4 and l terminated dendrimer 2) was much faster than the mismatched pair (the d substrate 5 and l terminated dendrimer 2) – Table 1. If we look at the dendrimer data in more detail and compare the rates to those obtained for the control reactions, we observe relative rate accelerations of 101.9 and 163.7 for the D and L terminated dendrimer respectively. Table 1 also shows the differences in the initial rate concentrations of the reactive partners, leading to a substantial increase in rate. Table 1 also shows the differences in the initial rate for the d and l substrates after reaction with either the single amino acid 3 or the multi amino acid terminated dendrimer 2. It is clear that there is a significant increase in selectivity for the dendrimer-based reaction. Specifically, the initial rate for the aminolysis reaction is 61% faster when the l substrate reacts with the amino acids on the dendrimer’s periphery. This compares to a modest 10% increases for the control reaction.

We suggest that the increased selectivity is due to the onset of a dense shell structure. At this point the dendrimer’s terminal groups will be able to interact with each other and adopt a minimum energy conformation. Due to the relative proximity and chiral nature of the terminal groups, it is sensible to predict a conformation possessing a certain amount of organizational symmetry.14 Therefore, when the hydrophobic substrates are added to an aqueous solution of the dendrimer, it is equally sensible to suggest that hydrophobic binding will occur in a similarly organized manner (via interpenetration at the surface). As such, it will be energetically more favourable for one enantiomer to bind with respect to the other, leading to a faster reaction. A similar argument can be made for the energy of the diastereomeric transition states.15 In our case, the functionalized dendrimer exhibited a credible degree of diastereoselectivity in favour of the matched system, involving the l-dendrimer 2 and the l-substrate 4.

In conclusion we have presented the synthesis of a chiral dendrimer possessing 16 terminal amines. This dendrimer is capable of accelerating the aminolysis reaction between its terminal amine groups and the active esters of d and l phenyl aniline.13 Furthermore, the dendrimer displayed chiral selectivity with respect to the initial rates of reaction, with the l-substrate reacting 61% faster than the d stereoisomer. The increased rate and chiral selectivity are a result of steric crowding just below the dense shell/packing limit, leading to an organized and low energy diastereomeric conformation.

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### Notes and references

13. Due to the small amounts of starting material we were not able to synthesise the dipeptide products 6 and 7, or to specifically identify them during the aminolysis reaction. However, based on our recent studies using dendrimers (ref. 6 and 7 above) and other work on polymer based aminolysis (see for example: D. J. Evans, A. Kanagasooriam, A. Williams and R. J. Pryce, J. Mol. Catal., 1993, 85(1), 21), we are confident that aminolysis and not hydrolysis is occurring. More evidence comes from LC-MS, which could not detect any of the N-Boc-phenylane during the dendrimer catalyzed reactions (i.e. the hydrolysis by-product). Nevertheless, we do acknowledge that the dendrimer reactions maybe more complex and a number of cleavage processes may be occurring/competing including aminolysis and possibly hydrolysis. Nevertheless, the overall outcome and conclusions remain the same. That is, the dendrimer reactions are rate enhanced and demonstrate chiral selectivity based on a dense shell structure.

### Table 1

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Initial rate $v_0$ (M s$^{-1}$)</th>
<th>Relative rate$^a$</th>
<th>$v_0$, relative to $v_{0,d}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 3 + d substrate 4</td>
<td>$3.71 \times 10^{-9}$</td>
<td>1.00</td>
<td>19</td>
</tr>
<tr>
<td>Control 3 + l substrate 4</td>
<td>$(\pm 0.45 \times 10^{-9})$</td>
<td>1.19</td>
<td>19</td>
</tr>
<tr>
<td>Dendrimer 2 + d substrate 5</td>
<td>$3.77 \times 10^{-7}$</td>
<td>101.9</td>
<td>61</td>
</tr>
<tr>
<td>Dendrimer 2 + l substrate 5</td>
<td>$(\pm 0.42 \times 10^{-8})$</td>
<td>163.7</td>
<td></td>
</tr>
</tbody>
</table>

$^a v_0/(v_0$ control 3 + d). $^b$ For the control and dendrimer systems respectively $v_0 = 100 \times (v_{0,l} - v_{0,d})/v_{0,d}$. |