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Dendrimers in solution can have their remote catalytic groups folded back into the core: Enantioselective transaminations by dendritic enzyme mimics-II

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ABSTRACT

PAMAM dendrimers with double thioether arms have been synthesized with a pyridoxamine core and terminal chiral amino groups. Transamination to afford natural isomers of phenylalanine and alanine induced enantioselectivity by the peripheral chiral caps, supporting a computer model that indicates folding of dendrimer chains back into the core.

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When dendrimers were first introduced as catalysts and mimics of enzymes, it was common to take the analogy to trees seriously and to assume that groups on the outside of the dendrimers would be unable to reach the center of the dendrimer to catalyze processes at the core. As part of our general study of mimics of transaminase enzymes,[1] we studied some dendrimers in which a pyridoxamine unit was at the core and PAMAM dendrimers were grown from C-3 of the pyridoxamine.[2] We used them to perform the transamination of the pyridoxamine with α-ketoacids, forming amino acids and a core pyridoxal unit. We found that the reactions in water were speeded when the terminal groups at the end of the dendrimer chains were tertiary amines, while no such rate increase was seen when the terminal amino groups were acylated and thus non-basic.

Our molecular modeling, using a water continuum, indicated that the dendrimer chains could curl back to bring the terminal amino groups next to the core pyridoxamine, and furthermore that the core was partially exposed to solvent so that substrates could enter and products could leave. We proposed such a model for dendrimer conformations. In this model the dendrimer is not a simple outward branching tree-like species. Instead, the branches can curl back to fill the empty inner spaces. However, we wanted more direct proof that terminal groups on the dendrimer chains could directly interact with the core, so we turned to chiral induction.

A number of research groups worldwide have been focused on affording chiral amino acids in transaminase mimics. Models including catalytic antibodies,[3] polypeptide–pyridoxamine complexes,[4] an adipocyte lipid binding protein,[5] metal-chiral ligand systems,[6] and more recently a molecularly imprinted polymer[7] have been developed as transaminase mimics.

In our group, a high degree of chiral induction in transamination has been achieved by using a pyridoxamine derivative with a rigidly attached chiral base species.[8] A pyridoxamine attached to a chiral amine, derived by reduction of a polypeptide, afforded amino acids with modest to high chirality.[9] Recently, isotactic polyethylenimines with (*S*)-benzyl side chains, and a pyridoxamine cofactor with a hydrophobic chain, induced the formation of some chirality in transamination reactions, but it was followed by racemization of amino acid.[10] However, the racemization was suppressed by attaching the pyridoxamine cofactor directly to the polymer.[11] In recent transamination studies, we reported that turnover catalysis of the transaminations could be achieved by decarboxylative transamination of a pyridoxal with an α,α-disubstituted glycine derivative.[12,13] We will use this reaction again in the current paper.

In contrast to linear or branched polyamines, dendrimers are three-dimensional macromolecules with defined structures, so they have interesting potential as enzyme mimics.

We first synthesized PAMAM dendrimers from a single branch point at a pyridoxamine core, and found that a chiral terminal group—*N*,*N*-dimethylphenylalanine—could induce chiral preferences in the product amino acids.[14] By molecular mechanics calculations study we found that the pyridoxamine was not located in an enclosed core area, even in as large as generation three (G-3). Instead, it rested at the surface with the solvent, with easy access by the 'remote' chiral amine species. This directly supported the picture of dendrimers as curled up like proteins, rather than with

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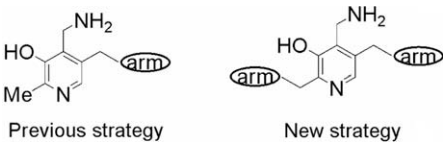


Figure 1.

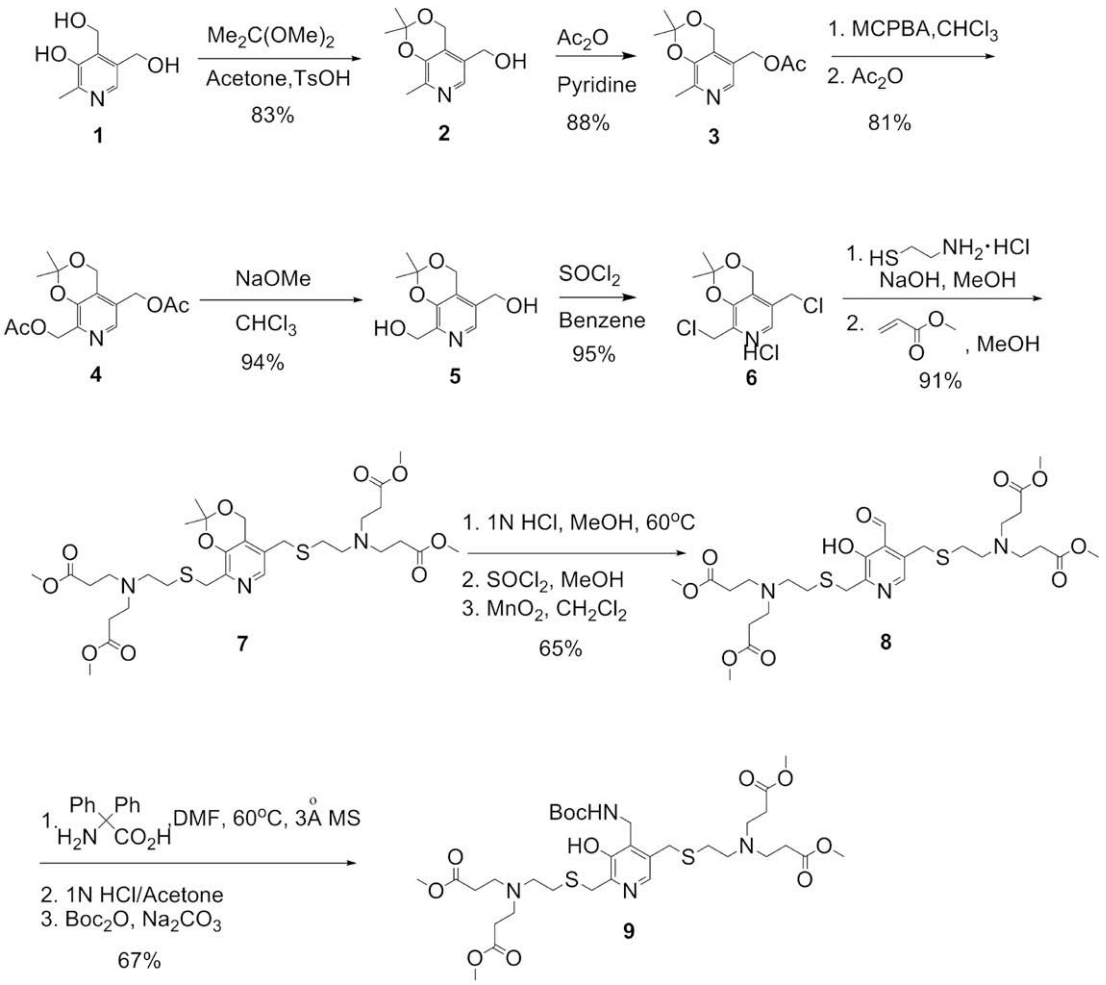
arms projecting outward like tree branches. However, we were concerned that this group of dendrimers, branching from a single point on the pyridoxamine core species, might not be typical relative to dendrimers with a growth from more than one point of the core. Therefore we have now synthesized new dendrimers with two opposite starting points from the pyridoxamine, to see whether this more enclosed pyridoxamine would still be accessible to terminal chiral amine groups (Fig. 1). The results confirm our previous picture.

Our new dendrimers were synthesized from key intermediate **6**, which has two benzylic chlorides. The preparation of **6** starting with the isopropylidenylation of pyridoxine **1** is outlined in Scheme 1.[15] The primary alcohol in protected pyridoxine **2** was first converted to acetate ester **3**. Compound **3** was oxidized by *m*-chloroperoxybenzoic acid (MCPBA), and the resulting N-oxide was acetylated with acetic anhydride. The product rearranged on heating to afford diester **4**. After deacetylation with sodium methoxide, the diol **5** was transformed to the reactive dichloride **6**.

The two chloride groups in **6** were replaced by cysteamine hydrochloride under basic conditions, and then the two free amino groups were trapped by slightly excess methyl acrylate to give compound **7**. After acidic deprotection and recovery of some methyl esters, the intermediate was oxidized to the aldehyde **8** with manganese(IV) oxide.

Transforming the pyridoxal **8** to the Boc-protected pyridoxamine **9** or its free amine form turned out to be a problem. We first tried traditional approaches, such as direct reductive amination,[16] condensation with hydroxylamine or its oxygen protected derivatives followed by reduction, and one pot reductive N-alkylation with carbamate.[17] Not one of those approaches was successful. The failure prompted us to review our previous way to regenerate the pyridoxamine from its aldehyde by using a sacrificial α,α-disubstituted amino acid.[12,13] After screening with different solvents, sacrificial amino acids and aryl aldehydes, the optimized reaction conditions and preliminary scope were established. Further study on this novel decarboxylative transamination is still underway. The pyridoxal **8** was treated with 2,2-diphenylglycine in DMF solution at 60 °C under argon, followed by transamination with acetone under acid conditions, and finally converted to the protected pyridoxamine **9** with di-*tert*-butyl dicarbonate.[18]

The four methyl ester groups in **9** were utilized as the starting points to synthesize PAMAM dendrimers, following the typical two-step iterative sequences by aminolysis of each ester group with excess ethylenediamine, then attachment of two molecules of methyl acrylate to each new NH_2 group, G′-1 to G′-4 were synthe-



Scheme 1.

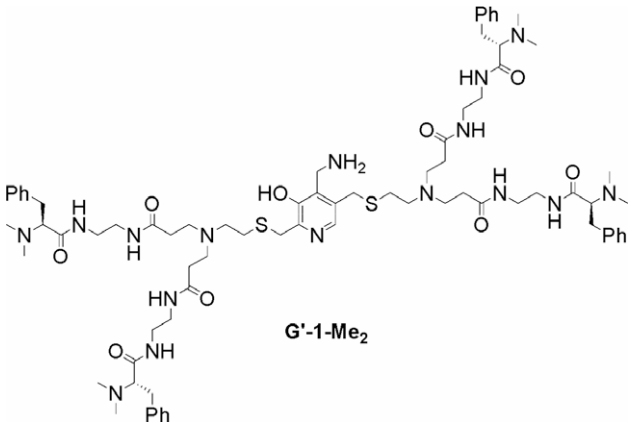


Figure 2.

sized.[19] Following our previous procedure, the terminal free amines were coupled with *N*,*N*-dimethyl-L-phenylalanine using water soluble 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) as a coupling reagent.[20] Finally, the Boc group was removed by 1 N HCl, and the resulting free pyridoxamine was subjected to the next transamination studies. All the final pyridoxamine dendrimers were completely water soluble. The pyridoxamine's characteristic peak at 318 nm in the UV–vis spectrum was observed for all the deprotected dendrimers. The structure of the first generation (Fig. 2) is shown as G′-1-Me_2.

The concentrations of the dendrimers' water solutions were adjusted to ca. 5.0×10^{-3} M by comparing the intensity of the UV–vis spectrum at 318 nm with the absorption of a standard solution of pyridoxamine. The transamination reactions were run between dendrimers (ca. 5.0×10^{-3} M) and α-keto acids (5.0×10^{-2} M) at pH 7.5, t = 20–25 °C. The resulting amino acids, after derivatization with *o*-phthalaldehyde and *N*-Ac-L-cysteine, were analyzed with reverse-phase HPLC. The final results are summarized in Table 1.

It is striking that the largest effects were seen with the dendrimers having the longest arms and therefore in principal with chiral catalytic groups furthest from the core (but not if they fold back!).

Molecular modeling studies were performed to further understand the enantioselectivity of transamination within the dendrimers. The calculations were executed using MMFF's force field in the presence of the GB/SA continuum water model to simulate the real aqueous system. The resulting structure of G′-4-Me_2 is depicted in Figure 3, in which tight packing was observed near the pyridoxamine residue. The strong hydrophobic interaction among the side chains as well as multiple intramolecular hydrogen bonds lock the two thioether arms mostly on one side of the pyridoxamine core. Also, the combination of these interactions holds the pyridoxamine residue within the chiral environment generated by the proximate terminal *N*,*N*-dimethyl-L-phenylalanine, and

Table 1
The % ee values of the L-amino acids formed in the transamination reactions between dendrimers and phenylpyruvic acid or pyruvic acid in aqueous media[a–c]

Dendrimer	Phenylpyruvic acid	Pyruvic acid
G′-1-Me_2	20.3	16.7
G′-2-Me_2	24.9	27.1
G′-3-Me_2	26.7	29.7
G′-4-Me_2	34.5	33.2

[a] All% ee values are averaged from two or three trials, and agreed within ca. 3%.
[b] Transamination reaction conditions: dendrimers (ca. 5.0×10^{-3} M), α-keto acids (5.0×10^{-2} M), EDTA (1.0×10^{-2} M), pH 7.5, t = 20–25 °C.
[c] The amino acid products were derivatized by *o*-phthalaldehyde and *N*-Ac-L-cysteine before analysis by reverse-phase HPLC.

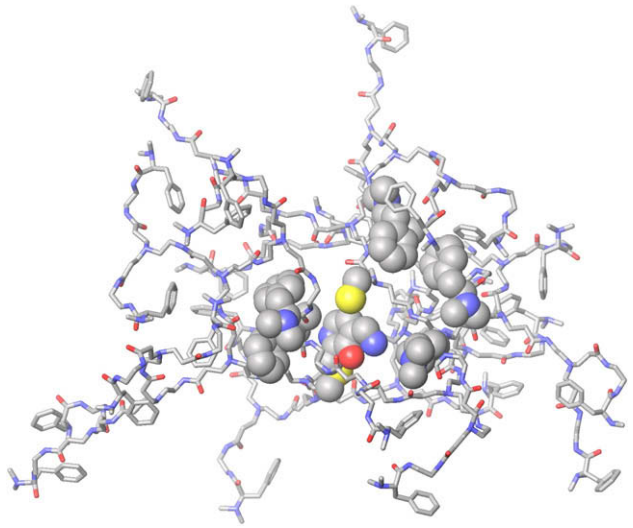


Figure 3. Calculated structure of the G′-4-Me_2 dendrimer in tube version which indicates the tight packing around the pyridoxamine group. The pyridoxamine and surrounding *N*,*N*-dimethyl-L-phenylalanine residues are presented as space-filling models. As can be seen, the amino and hydroxyl groups are exposed to solvent, and to dimethylamino groups.

therefore promotes asymmetrical transamination favoring the formation of L-isomers. In comparison to the single thioether dendrimers,[14] a slight decrease in the ee value of transamination reaction was observed.

Our new dendrimers synthesized from two starting points support the dense core model of dendrimer structures in solution, where the flexible chains can fold back to fill otherwise empty space. The observed catalysis and chiral induction by formally further away residues, coupled with our previous results, demonstrate that even a dendrimer with catalytic groups formally remote from the pyridoxamine unit is a good mimic of some aspects of transaminase enzymes.

This shows how flexible dendrimers are, and how far from the formal tree structure they really can exist. They share this flexible folding with proteins. Thus it greatly expands the potential for dendrimers to serve as multifunctional catalysts and reactants.

Acknowledgments

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18. *Typical procedure*: A suspension of aldehyde **8** (160 mg, 0.25 mmol) and 2,2-diphenylglycine (124 mg, 0.55 mmol) in 2.5 mL DMF was stirred at 60 °C with 3 Å MS for 6 h. Remove DMF and the residue was stirred with 2 mL 1 N HCl and 4 mL acetone overnight. Two milliliters H_2O were added and the resulting solution was turned basic with solid Na_2CO_3. Di-*tert*-butyl dicarbonate (273 mg, 1.25 mmol) was added and the solution was kept stirring for 6 h. Extract with CH_2Cl_2 and after removal of CH_2Cl_2, the residue was purified by chromatography (CH_2Cl_2: MeOH = 20:1 with 2% TEA) to give slightly yellow oil **9** (115 mg, 67% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.88 (s, 1H), 4.31 (s, 2H), 3.89 (s, 2H), 3.65 (s, 14H), 2.96–2.35 (m, 24H), 1.44 (s, 9H). CI-MS(M+1): 747.2.
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