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# Enantioselective transaminations by dendrimeric enzyme mimics

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**Abstract**—PAMAM dendrimers have been constructed with a pyridoxamine core and chiral capping amino groups. Transamination to form phenylalanine and alanine from their related keto acids produced enantioselectivities induced by the formally remote chiral caps, supporting computer models that indicate folding of the dendrimer chains back into the core region.


## 1. Introduction

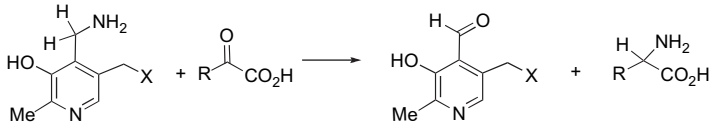
We have reported mimics of transamination,[1] both simple pyridoxamine derivatives[2–8] and enzyme mimics with a pyridoxamine attached to cyclodextrins,[9–13] to a synthetic macrocycle,[14] to polyethyleneimines of various sizes,[15–17] and to dendrimers.[18] The process led to the formation of amino acids from keto acids, while converting the pyridoxamine to its pyridoxal form (Scheme 1). Turnover was achieved by a process of decarboxylative transamination to regenerate the pyridoxamine from the pyridoxal.[19,20] With a modified polyethyleneimine, having a hydrophobic core, we were able to use the pyridoxamine as a reversibly bound cofactor rather than a covalently attached functional group, and achieved accelerations of transamination as large as 725,000-fold relative to pyridoxamine itself.[19]

Transamination produces an amino acid with a new chiral center, so synthesis with enantioselectivity is an important goal. We were able to achieve considerable enantioselectivity with a rigid pyridoxamine derivative carrying a chirally mounted amino group for proton transfers.[2] We also saw enantioselective transamination with a pyridoxamine covalently attached to a chiral triamine that was derived by reduction of a tripeptide (but not with the unreduced tripeptide).[21]

We then wished to create a polyamine catalyst that could produce enantioselective transamination by the simple action of the chirality of the polymer itself, as happens in natural enzymes.

We have described elsewhere the enantioselective transaminations by pyridoxamines attached to a linear polyamine derived by the polymerization of a chirally substituted oxazoline.[22] Further work on this system is underway. However, dendrimers are three-dimensional macromolecules with defined structures—in contrast with polyimines—some as large as small proteins, so they have good potential as enzyme mimics.

In our previous study, we saw that a PAMAM dendrimer grown from a pyridoxamine core performed transaminations when basic amino groups were linked to the *outside* of the dendrimer, formally far from the pyridoxamine unit.[18] Without those remote amino groups little catalysis was seen. This reflects the fact that dendrimers can be flexible species in which the chains fold back into the otherwise empty space near the core, as our computer model of the pyridoxamine-dendrimer species indicated.[23] The tertiary amino groups that are part of the core of a PAMAM dendrimer are not effective catalysts, presumably because




**Scheme 1**.

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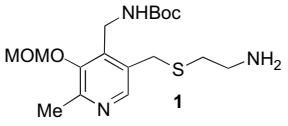
they cannot reach the core pyridoxamine. Thus it seemed likely that we would see chiral induction in transamination with a pyridoxamine-dendrimer species carrying chirally mounted amino groups on their periphery, as terminators of the dendrimer chains. This is what we have now observed.

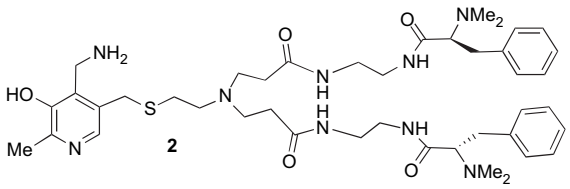
## 2. Results and discussion

### 2.1. Synthesis of the dendrimers




The synthesis of our dendrimers started from protected pyridoxamine with an attached 2-aminoethanethiol spacer (**1**). The pyridoxamine nitrogen carried a *tert*-butoxycarbonyl (Boc) protecting group, while the phenolic oxygen was protected with a methoxymethyl (MOM) group. The $NH_2$ group on the spacer was used as a starting point to synthesize PAMAM dendrimers. Following the typical two-step iterative sequences[24]—attachment of two molecules of methyl acrylate to each $NH_2$ group, then aminolysis of each ester group with excess ethylenediamine—generations G-1 to G-4 were synthesized (Scheme 2).

The amino groups on the periphery of each generation were modified with *N*,*N*-dimethyl-L-phenylalanine using water soluble 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM)[25] as a coupling reagent. The extent of coverage of the periphery was indicated by comparing the NMR integrated areas of the phenyl protons to those of Boc groups in the pyridoxamine core, and further confirmed by mass spectra. The series of dendrimers was referred as G-*n*-$Me_2$ (*n* refers to generation). We saw that up to generation 3 the materials were reasonably pure, but at generation 4 the mass spectrum indicated a range of masses, reflecting incomplete attachment of all 16 dimethylphenylalanine groups. Finally, the Boc and the MOM groups on the pyridoxamine were removed with 1 N HCl. An example for the first generation is shown as structure **2**.




### 2.2. Transamination studies

The transamination reactions transforming α-keto acids to amino acids were performed as indicated in the legend of Table 1 (see also Section 4), and monitored by HPLC. The % ee values are summarized in Table 1. For comparison, a pyridoxamine derivative with a rigidly attached chiral base species afforded amino acids with ee values as high as 90%.[3] Also, a pyridoxamine attached to a chiral amine derived by reduction of a polypeptide afforded amino acids with ee values as high as 85%.[21] Thus our new ee values are not impressive, except to the extent that they give insight into the structures of the dendrimers involved.

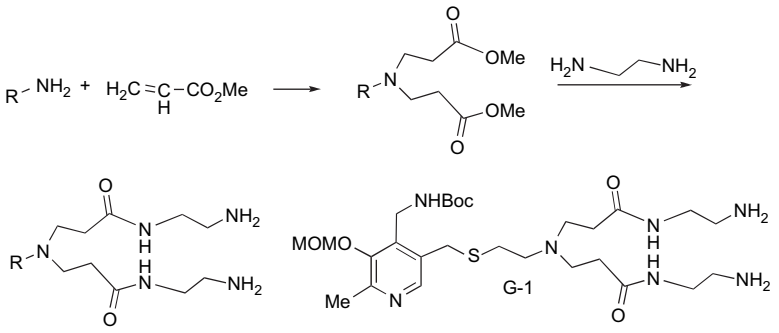
The reactions were quite slow relative to those we have seen with polyamine catalysts having a hydrophobic core.[19] However, in the dendrimer we do have a defined covalent structure with a single pyridoxamine unit, so the results can be more easily interpreted.

To further understand the role of the chiral unit on the periphery, *N*-acetyl-L-phenylalanine was attached to G-2 (we refer it as G-2-Ac). After the removal of Boc and MOM groups in pyridoxamine, this molecule also transaminated

**Table 1**. The % ee values of the L enantiomer products in the transamination reactions between the dendrimers and phenylpyruvic acid or pyruvic acid in aqueous media[a–c]

| Dendrimer | Phenylpyruvic acid | Pyruvic acid |
|---|---|---|
| G-1-Me2 | 23.9±0.7 | 21.3±0.4 |
| G-2-Me2 | 42.8±0.2 | 39.4±0.9 |
| G-3-Me2 | 45.4±1.2 | 42.2±3.7 |
| G-4-Me2 | 38.9±0.4 | 42.4±2.3 |

[a] All % ee values are averaged from two or three trials.
[b] Transamination reaction condition: dendrimers (ca. $5.0\times10^{-3}$ M), α-keto acids ($5.0\times10^{-2}$ M), EDTA ($1.0\times10^{-2}$ M), pH=7.5, $T$=20–25 °C.
[c] The amino acid products were derivatized with *o*-phthalaldehyde and *N*-acetyl-L-cysteine before analysis by reverse-phase HPLC, as described elsewhere [Ref. 8, and Section 4].




**Scheme 2**.

phenylpyruvic acid to phenylalanine, but the product was racemic. Therefore, the chiral units on the periphery did not simply create a chiral environment; the basic dimethylamino group is also necessary. This observation is consistent with our previous findings that peripheral dimethylamino groups on a PAMAM dendrimer with a pyridoxamine core reach the core and catalyze transaminations, but *N*-acetyl terminated dendrimers do not significantly catalyze the transaminations.

We also examined dendrimers capped with phenylalanine and with *N*-methylphenylalanine instead of the *N*,*N*-dimethyl derivative. Interestingly, these performed transaminations but the products were racemic. Probably the conformations differ with these more hydrophilic caps, bringing the chiral centers away from the pyridoxamine. Reactions of the primary or secondary amino groups of these derivatives with the keto acid substrates are also possible.

### 2.3. Molecular mechanics calculations

To gain a better understanding of the stereoselectivity of the transaminations taking place within the chiral environment of the dendrimers, we performed molecular mechanics calculations (MacroModel 9.1) of the pyridoxamine-dendrimer complexes. Calculations were executed using the MMFFs (Merck Molecular Force Field) and a water GBSA (Generalized Born Surface Area) solvent continuum to promote the hydrophobic packing of the dendrimer arms, as is expected in the real aqueous system. The resulting structure of the G3 pyridoxamine-dendrimer is shown in Figure 1 in two different forms. In the tube version it is clear that the pyridoxamine unit is solvent exposed, and that some of the formally remote phenyl rings are in fact packed close to the pyridoxamine. This explains their ability to induce enantioselectivity in the products. In the space-filling structures with van der Waals radii added it is clear that the computed structures are solidly packed. The structure on the left has the same view as that of the tube version, while the one on the right has this rotated by 90° to show how solid the packing is. Thus the dendrimer is hardly 'tree-like' unless the branches and trunk of the tree are as flexible as spaghetti. The computed structures of the other dendrimers are shown in Supplementary data. In all cases the computation was for the neutral compounds, rather than partially protonated species with counterions. Of course the terminal amino groups will be protonated at pH 7.5, producing a charged surface that will diminish any tendency for aggregation of the dendrimers themselves in water.

We examined the situation in G3 computationally when the two methyl groups were removed from the phenylalanine units. There was not a large change in geometry, but the primary amino groups were somewhat more free and could adopt geometries where they were further from the pyridoxamine units. This may account for our findings that they did not induce chirality, although it is still possible that they simply added to the keto acids, greatly changing their nature.

The G1 and G2 complexes had significant conformational flexibility, with no well-defined three-dimensional structure. However, both pyridoxamine complexes possessed intramolecular hydrogen bonds between the pyridine nitrogen and the amide NH groups. Such a hydrogen bond holds the pyridoxamine residue in the proximity of a chiral terminal group and promotes asymmetry in the transamination.

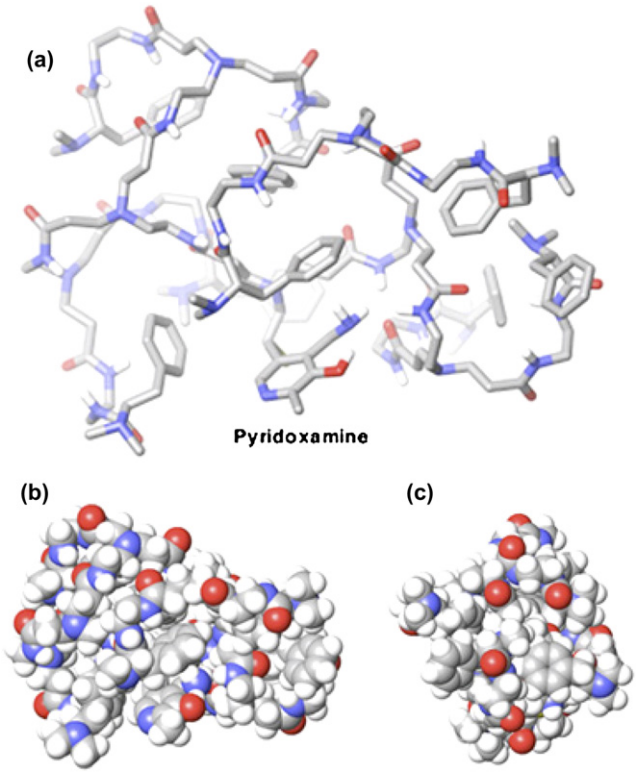



**Figure 1**. The calculated structures of the G-3 dendrimer with an attached pyridoxamine in water solution. Structure a, the tube version, shows how close the pyridoxamine is to some of the dimethylphenylalanine units that are attached to the ends of the dendrimer chains, explaining why the transaminations are enantioselective. Structure b, the space-filling version of structure a, shows that the dendrimer is tightly packed, with no interior space. Structure c is the same space-filling version rotated 90°, to show again the tight packing.

The G3 dendrimer differs from the smaller dendrimers in the existence of a well-defined structure stabilized by the hydrophobic packing of dendrimer appendages, and intramolecular hydrogen bonds between amide groups. Unlike the G1 and G2 structures, with dendrimer arms radiating outward from the pyridoxamine core, the outermost arms of the G3 dendrimer fold backward into the interior, presumably due to the polar solvation medium. In this structure, the pyridoxamine residue exists on the surface of the dendrimer, reflecting the length of the thioethoxy linker and the backward folding of the dendrimer arms. This arrangement of the pyridoxamine residue in proximity to the chiral terminal groups explains why the G3 compound shows the best enantioselectivity in these transaminations.

Knowing that the pyridoxamine residue rested on the surface of the G3 dendrimer, we reasoned that elaboration of the system to a fourth generation could encapsulate the catalytic core residue fully within the chiral hydrophobic environment. Upon simulation of the G4 dendrimer, we were surprised to find that the pyridoxamine residue did not reside within the interior of the complex. Instead, aggregation of the hydrophobic groups to form an interlocking network of dendrimer arms actually withdrew the chiral termini somewhat

from the pyridoxamine, which, being the most hydrophilic region of the molecule, had a significant degree of aqueous solvation. This observation may account for the slight loss of enantioselectivity obtained with G4 relative to G3. Of course our mass spectrum indicates that the G4 system does not have its full complement of phenylalanine units, in contrast to G3, which may contribute to the experimental finding of slight loss in enantioselectivity.

## 3. Conclusions

Our results, in combination with our previous findings, are strong experimental evidence to support the dense core model of dendrimer structures in solution, where the flexible chains can fold back to fill otherwise empty space, as indicated in our computer models. We observe catalysis and chiral induction by groups that are formally far from the pyridoxamine core, in topological terms, but are clearly nearby in terms of their real geometry.

Furthermore, our dendrimeric enzyme mimics have achieved significant enantiomeric excesses in the transamination products, induced by this formally remote chirality. Coupled with our previous demonstration that decarboxylative transaminations can be used to promote turnovers in such models,[19,20] the dendrimeric mimics are a real advance in the quest to achieve enzyme mimics with potency and utility.

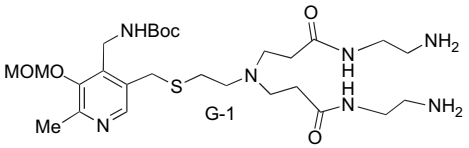
## 4. Experimental

### 4.1. General

Solvents, inorganic salts, and organic reagents were purchased from commercial resources and used without further purification unless otherwise noted. Merck pre-coated 0.25 mm silica plates coating a 254 nm fluorescence indicator were used for analytical thin-layer chromatography. Flash chromatography was performed on 230–400 mesh silica (Silica Gel 60) from EM Science. Analytical HPLC was run on a Waters 600 liquid chromatography with a pumping system, a temperature controlled autosampler (Waters 717), and a diode-array UV–vis detector (Waters 2996). Waters Sunfire C18 reverse-phase analytical columns (particle size 5 μm, 4.6×150 mm) were used as the solid phase. NMR spectra were obtained on a Bruker DPX 300 MHz spectrometer. UV–vis spectra were taken on a Varian Cary IE UV–vis spectrometer. CIMS spectra were taken on Nermag R-10-10 instrument. FABMS spectra were taken on a JEOL JMS-DX-303 HF instrument using either glycerol or *p*-nitrobenzyl alcohol as matrices. Matrix assisted laser desorption ionization (MALDI) mass spectra were taken on a VOYAGER DE instrument with α-cyano-4-hydroxycinnamic acid as a matrix. Positive ion mass spectra were acquired in the linear mode.

### 4.2. Synthesis of the dendrimers

The dendrimers were grown from a protected pyridoxamine using methyl acrylate and ethylenediamine, as described in Refs. 18 and 23. They were terminated with ethylenediamine, and the terminal amino groups were then acylated with phenylalanine derivatives and the protecting groups were then removed as described below.

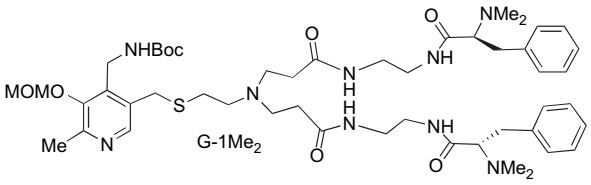



**4.2.1. G-1.** $^1$H NMR (300 MHz, $D_2O$): 8.15, s, 1H; 5.09, s, 2H; 4.45, s, 2H; 3.89, s, 2H; 3.62, s, 3H; 3.29–3.19, m, 4H; 2.79–2.66, m, 8H; 2.66–2.52, m, 4H; 2.47, s, 3H; 2.49–2.29, m, 4H; 1.41, s, 9H. CIMS: 600.51 (M+1).

**4.2.2. G-2.** $^1$H NMR (300 MHz, $D_2O$): 8.15, s, 1H; 5.08, s, 2H; 4.44, s, 2H; 3.89, s, 2H; 3.62, s, 3H; 3.31–3.17, m, 12H; 2.80–2.53, m, 28H; 2.50–2.26, m, 15H; 1.41, s, 9H. FAB$^+$ MS: 1056.4 (M+1).

**4.2.3. G-3.** $^1$H NMR (300 MHz, $D_2O$): 8.20, s, 1H; 5.12, s, 2H; 4.48, s, 2H; 3.93, s, 2H; 3.66, s, 3H; 3.32–3.21, m, 28H; 2.95–2.58, m, 60H; 2.54–2.35, m, 31H; 1.45, s, 9H. MALDI MS$^+$: 1970.49.

**4.2.4. G-4.** $^1$H NMR (300 MHz, $D_2O$): 8.21, s, 1H; 5.13, s, 2H; 4.49, s, 2H; 3.94, s, 2H; 3.67, s, 3H; 3.32–3.16, m, 60H; 2.95–2.57, m, 124H; 2.54–2.37, m, 63H; 1.46, s, 9H. MALDI MS$^+$: 3792.76.




**4.2.5. G-1-Me$_2$.** All modified dendrimers were prepared analogous to the following procedure: G-1 (0.1017 g, 0.17 mmol) and *N*,*N*-dimethyl-L-phenylalanine (0.0721 g, 0.374 mmol) were dissolved in 2 ml $CH_3OH$/2 ml $CH_3CN$. After 5 min, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (0.1032 g, 0.374 mmol) was added into the solution. The mixture was stirred overnight. After removal of the solvent, the residue was dissolved in $CH_2Cl_2$, which was washed successively with saturated aqueous $Na_2CO_3$ solution and brine. Removal of the solvent gave the product as a slightly yellow solid. Yield: 50%. $^1$H NMR (300 MHz, $CD_3OD$): 8.13, s, 1H; 7.30–7.15, m, 10H; 5.03, s, 2H; 4.46, s, 2H; 3.92, s, 2H; 3.58, s, 3H; 3.57–3.51, dd, 2H; 3.25–2.94, m, 12H; 2.90–2.73, m, 6H; 2.72–2.54, m, 14H; 2.46, s, 3H; 2.43–2.28, m, 4H; 1.41, s, 9H. CIMS: 982 (M+33).

**4.2.6. G-2-Me$_2$.** Yield: 42%. $^1$H NMR (300 MHz, $CD_3OD$): 8.13, s, 1H; 7.37–7.08, m, 20H; 5.03, s, 2H; 4.45, s, 2H; 3.89, s, 2H; 3.54, s, 3H; 3.25–2.86, m, 32H; 2.84–2.61, m, 16H; 2.60–2.46, m, 7H; 2.45–2.20, m, 36H; 1.42, s, 9H. FAB$^+$ MS: 1758.06 (M+1), 1780.09 (M+Na).

**4.2.7. G-3-Me$_2$.** Yield: 37%. $^1$H NMR (300 MHz, $CD_3OD$): 8.13, s, 1H; 7.37–7.08, m, 40H; 5.03, s, 2H; 4.45, s, 2H; 3.91, s, 2H; 3.59, s, 3H; 3.32–2.85, m, 70H; 2.84–2.61, m, 34H;

2.60–2.46, m, 11H; 2.45–2.18, m, 76H; 1.42, s, 9H. MALDI $MS^+$: 3372.54 (M+2).

**4.2.8. G-4-$Me_2$.** Yield: 30%. $^1H$ NMR (300 MHz, $CD_3OD$): 8.15, s, 1H; 7.32–7.10, m, 80H; 5.05, s, 2H; 4.47, s, 2H; 3.91, s, 2H; 3.59, s, 3H; 3.32–2.84, m, 144H; 2.83–2.61, m, 72H; 2.60–2.46, m, 19H; 2.45–2.16, m, 156H; 1.44, s, 9H. MALDI $MS^+$: 5000–7000. Expected mass: 6885.6.

### 4.3. Deprotection of the dendrimers

All modified dendrimers were deprotected similar to the following procedure:

G-1-$Me_2$ of 50 mg was dissolved in 1 mL of HCl (1 N). The reaction mixture was stirred at room temperature overnight. The solvent was removed by vacuum evaporation. The residue was dissolved in deionized water directly for the transamination study without further purification.

### 4.4. Transaminations by the dendrimers

Aqueous dendrimer solution of 100 μl (ca. $5.0\times10^{-3}$ M), 100 μl of aqueous α-keto acids solution ($5.0\times10^{-2}$ M), and 25 μl of aqueous EDTA solution ($1.0\times10^{-2}$ M) were added to a vial. The pH of the solution was adjusted to 7.5 with NaOH ($1.0\times10^{-2}$ M) and HCl ($1.0\times10^{-2}$ M). The reaction at room temperature usually took 6–24 h.

### 4.5. Assay of the amino acid products

The reaction mixture from transamination was extracted with $CH_2Cl_2$, and then the water layer containing the amino acid was collected and the volume of the aqueous media was reduced to 80 μl. A derivatizing solution of 10 μl containing 0.2 M *o*-phthalaldehyde and 0.2 M *N*-acetyl-L-cysteine in methanol, and 10 μl of an aqueous buffer solution of 1.0 M $K_2HPO_4$ (pH 8.0) were added. The resulting solutions of the diastereomeric 1-thioisoindoles were analyzed by reverse-phase HPLC using aqueous buffer/THF/acetonitrile with UV–vis detection at 344 nm.

The retention time for the derivatized D-phenylalanine, L-phenylalanine, D-alanine, and L-alanine was ~21.1, ~22.3, ~16.5, and ~18.8 min, respectively.

## Acknowledgements

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## Supplementary data

Supplementary data include the mass spectra and NMR spectra of the compounds synthesized and the computed structures of dendrimers G1, G2, and G4. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2007.02.052.

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