Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry 12 (2004) 3277–3287
Bioorganic & Medicinal Chemistry

Polymeric and dendrimeric pyridoxal enzyme mimics ☆

Lei Liu and Ronald Breslow*

Department of Chemistry, Columbia University, New York, NY 10027, USA

Received 5 February 2004; accepted 29 March 2004
Available online 6 May 2004

Abstract—Pyridoxal was covalently attached to polyethylenimine polymers, but the resulting materials were found to degrade rapidly. In comparison, the dendrimeric pyridoxals, which possess only one pyridoxal unit at the core of every dendrimer molecule were found to be relatively stable compounds. A total of 12 poly(amidoamine) type dendrimers were synthesized. They range from G1 to G6 with either NMe_2 or NHAc termini. The NMe_2-terminated pyridoxal dendrimers racemize α-amino acids 50–100 times faster than does simple pyridoxal, while the NHAc-terminated pyridoxal dendrimers racemize α-amino acids only 3–5 times faster than does simple pyridoxal. Both the NMe_2- and NHAc-terminated pyridoxal dendrimers decarboxylate 2-amino-2-phenyl-propionic acid 1–3 times faster than simple pyridoxal. The interior polarity in the pyridoxal dendrimers is similar to that of 85:15 water–DMF solution. Furthermore, we successfully incorporated eight lauryl groups to the G5 pyridoxal dendrimer at known positions. The laurylated dendrimer exhibits lower racemization and decarboxylation rates than do the unlaurylated ones, in contrast to the positive rate effects of laurylation in polyethylenimine–pyridoxamines in our previous transamination studies.

1. Introduction

Natural enzymes are macromolecules. The macromolecular structures offer ideal frames for construction of versatile and robust catalytic sites. Strong and selective binding of the substrate is attained through a combination of the hydrophobic effect and various specific substrate–enzyme interactions such as hydrogen bonding and salt bridge. The macromolecular structure can also create regions in which the catalyzed reactions occur in a less than fully aqueous medium.

In comparison with natural enzymes, most artificial synthesized enzyme mimics are small molecules. Although many features of the real enzymes have been well mimicked by these models, it remains to mimic the role of the macromolecular character of enzymes in catalysis. For this reason, there has been interest in examining synthetic polymers as enzyme mimics.

Irving Klotz pioneered the use of polyethylenimine (PEI) derivatives as enzyme mimics.[1–7] He showed that attachment of dodecyl groups and methyleneimidazole groups to PEI yielded a catalyst that was very effective in hydrolyzing activated esters such as 4-nitrophenyl acetate and in the decarboxylation of isoxazole derivatives. He called these catalysts 'synzymes'. His reference included Junghun Suh as a co-author,[3,7] and Suh has pursued this area further. Suh summarized his work in a recent account,[8] and has used not only PEI but also polystyrene with appropriate attached basic groups.[9,10] Recently, Kirby and co-workers have described studies on the 'synzymes' derived from PEI, catalyzing the ring opening of benzisoxazoles.[11,12]

There were no previous examples in which a cofactor such as pyridoxamine was incorporated into polymeric enzyme mimics, so we recently undertook such a study. We reported a great increase of transamination rate for the pyridoxamine–keto acid system when we attached pyridoxamine to PEI carrying some attached lauryl groups.[13] We reported that hydrophobic effects exert profound effects on rates and substrate selectivities in the PEI–pyridoxamine transaminase mimics.[14] We also reported that the catalytic effects are seen with PEIs ranging from $M_n = 600$ up to 60,000, and the variation in polymer size does not strongly affect the polymer-induced acceleration.[15]

We saw that the excellent general acid–base catalysis exerted by the polyamines is one of the reasons for the large rate enhancement. We also saw that the lauryl chains attached to the polymers create regions in which

☆ Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2004.03.062

* Corresponding author. Tel.: +1-212-854-2170; fax: +1-212-854-2755; e-mail: rb33@columbia.edu

0968-0896/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2004.03.062

the rate constants are substantially increased and into which hydrophobic keto acid substrates selectively bind. Nevertheless, the PEI polymeric enzyme models do not have a well-defined structure, and each polymer molecule contains more than one coenzyme unit. Thus, we recently synthesized a number of poly(amidoamine) (PAMAM) dendrimers in the core of which a single pyridoxamine was covalently attached.[16] We found that the G6-PAMAM–pyridoxamine dendrimer is comparable to the PEI–pyridoxamine polymer in transaminating pyruvic and phenylpyruvic acids to alanine and phenylalanine, but it is less effective than the laurylated PEI–pyridoxamine.

Both the polymeric and dendrimeric pyridoxamines show great rate enhancement in transaminating keto acids to amino acids. However, in both the polymeric and dendrimeric pyridoxamines, we see less than five turnovers using sacrificial amino acids such as phenylglycine.[13,16] There is probably some problem in the back-transamination reaction between the sacrificial amino acids and polymeric or dendrimer pyridoxal that is formed in situ from the transamination between keto acids and polymeric or dendrimeric pyridoxamine. Thus, we decided to prepare polymeric and dendrimeric pyridoxals and study their reactivities in transaminating amino acids to keto acids. In addition to the turnover problem, we are also interested in the polymer or dendrimer effects on the many other reactions that can be catalyzed by pyridoxal but not pyridoxamine, for instance, racemization reaction and decarboxylation reaction.

2. Results and discussion

2.1. Synthesis of PEI–pyridoxals

In comparison with pyridoxamine polymers, it is much more challenging to prepare pyridoxal polymers. The free aldehyde group in pyridoxal forms an imine readily if any primary or secondary amino group is left on the PEI polymers. Therefore, an attempt to ligate a pyridoxal compound carrying a COOH side chain and PEIs using EDC did not yield any desired aldehyde. Another attempt to attach pyridoxine to PEI at first and then oxidize pyridoxine to pyridoxal was also unsuccessful, because all the oxidizing reagents that we tried (MnO_2, CrO_3/pyridine, and DMSO/$(COCl)_2$) failed to work in the presence of strongly hygroscopic polyamines.

A successful approach to make the PEI–pyridoxal involves the protection of the aldehyde group as dimethoxyl ketal, the protection of the phenolic hydroxyl group with MOM, and the activation of the side chain using *N*-hydroxy-succinimide (see Fig. 1). For the PEI part, we laurylated about 10% of the amino groups on the PEI with lauryl iodide. Then we used $(Boc)_2O$ to protect about 2% of the primary or secondary amino groups on the laurylated PEI. The remaining primary and secondary amino groups on PEI were fully methylated using HCHO/$NaBH_3CN$. After TFA treatment that removes the Boc protection, we obtained the PEI polymers with about 10% lauryl groups and only about 2% primary or secondary amino groups (see Fig. 2).

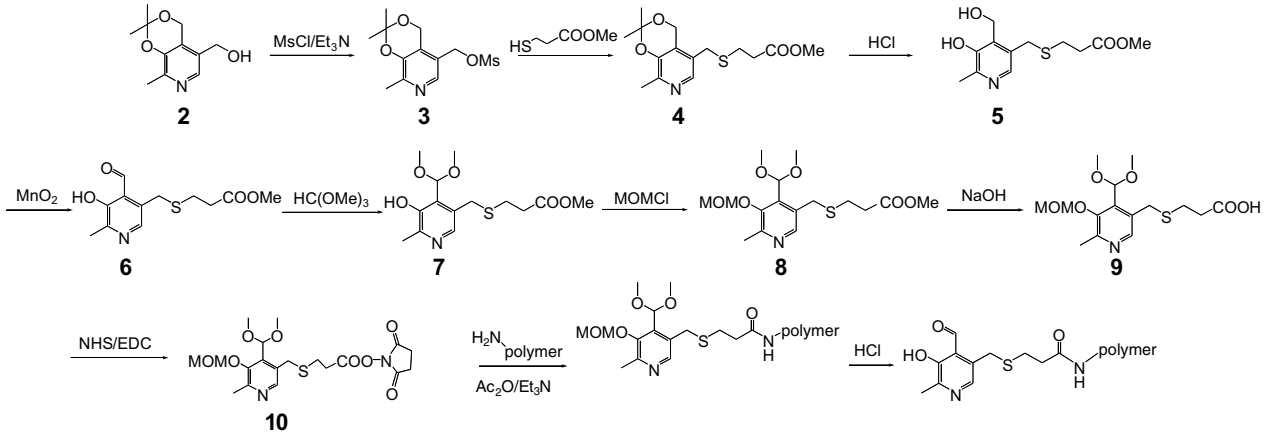

Figure 1. The synthesis of polymeric PEI–pyridoxals.

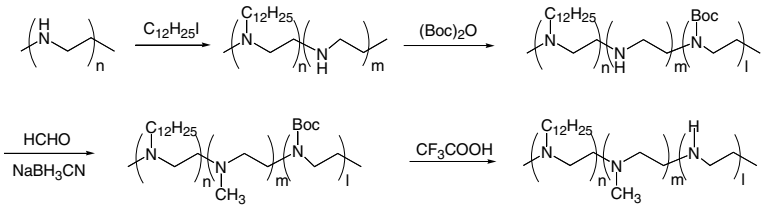

Figure 2. The synthesis of PEI polymers with only about 2% primary or secondary amino groups.

The PEI polymer with only about 2% primary or secondary amino groups can easily react with compound **10**. An important step in the next is to fully remove any remaining primary or secondary amino groups on the PEI polymer using Ac_2O/Et_3N. Without any primary or secondary amino groups left, we can use 1 M HCl (aq) to remove the dimethoxyl and MOM protections liberating the CHO and phenolic OH groups. Proton NMR of the final product clearly shows the free aldehyde C–H. The UV spectrum of the final product shows the characteristic pyridoxal absorption at 400 nm (see Fig. 3).

Unfortunately, we found that the PEI–pyridoxal reagent is not a stable species. When it is dissolved in HEPES buffer at pH = 7.5, the UV spectrum of the solution exhibits significant variation during a relatively short period of time. The absorption at 400 nm decreases while a new peak is formed at about 325 nm, following first-order kinetics (see the supporting information). This indicates that the aldehyde sp^2 carbon is irreversibly converted to some sp^3 carbon.

We measured the degradation rate by monitoring the decrease of the absorption at 400 nm. We found that the degradation rate for the laurylated PEI–pyridoxal is $(1.4 \pm 0.2) \times 10^{-2}\,min^{-1}$ at pH = 7.5 and $t = 60\,^{\circ}C$.[17] The degradation rate the unlaurylated PEI–pyridoxal under the same conditions is slightly slower, that is $(7.1 \pm 0.3) \times 10^{-3}\,min^{-1}$. Addition of alanine ($2.5 \times 10^{-3}$ M) to the PEI–pyridoxal solution does not change the degradation rate within the experimental errors. Therefore, using the UV method we could not determine the rate of the transamination reaction between the PEI–pyridoxal and alanine.

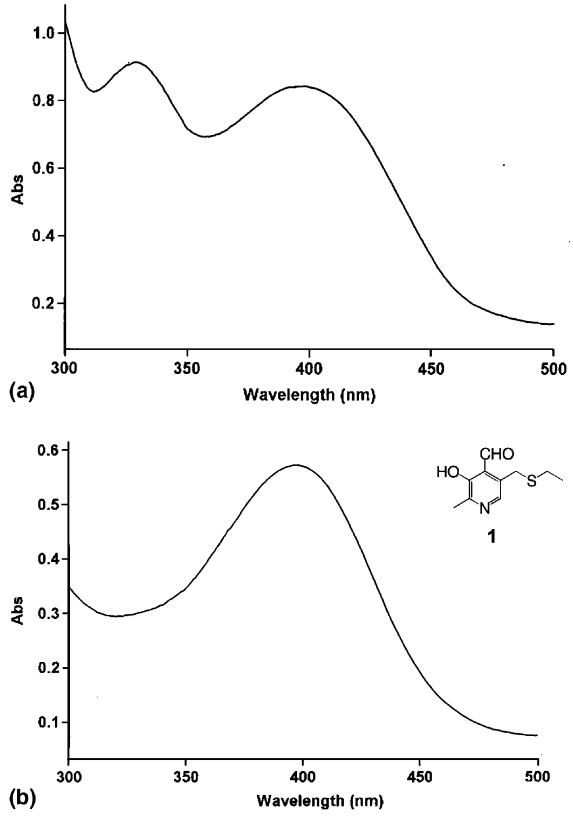

Figure 3. The UV spectrum of (a) the laurylated polymeric PEI–pyridoxal, and (b) of a simple pyridoxal compound, that is 5-[(ethylthio)methyl-3-hydroxy-2-methyl-4-pyridinecarbaldehyde] (**1**).

2.2. Synthesis of dendrimeric pyridoxals

At present we do not know the exact reason for the rapid degradation of the PEI–pyridoxal species. Nonetheless, as each PEI polymer carries about 28 pyridoxal units in average,[18] we speculate that the pyridoxals neighboring to each other on the same polymer molecule may react with each other readily in the presence of polyamines. An ideal solution to the problem would be to attach only one pyridoxal unit to each polymer molecule, but this is difficult to achieve with the randomly distributed PEI polymers. We decided at this point to synthesize pyridoxal dendrimers, where we can design to put only one pyridoxal unit inside each dendrimer molecule.

The synthesis of the dendrimers began with the dimethoxyl-ketal and phenolic-*O*-MOM protected pyridoxal (see Fig. 4), to which a spacer was covalently attached. The $COOCH_3$ group of the spacer was then used as the starting point for the PAMAM dendrimer synthesis, which basically involves a two-step iterative sequence[19]: (a) amidation of the terminal esters with excess ethylenediamine followed by (b) branching double alkylation of the terminal NH_2 groups with methyl acrylate. The ester-terminated, which are referred to as 'half-generations', were converted to the NMe_2-terminated full generations using excess *N*,*N*-dimethylethylenediamine. We can also cap the NH_2-terminated full

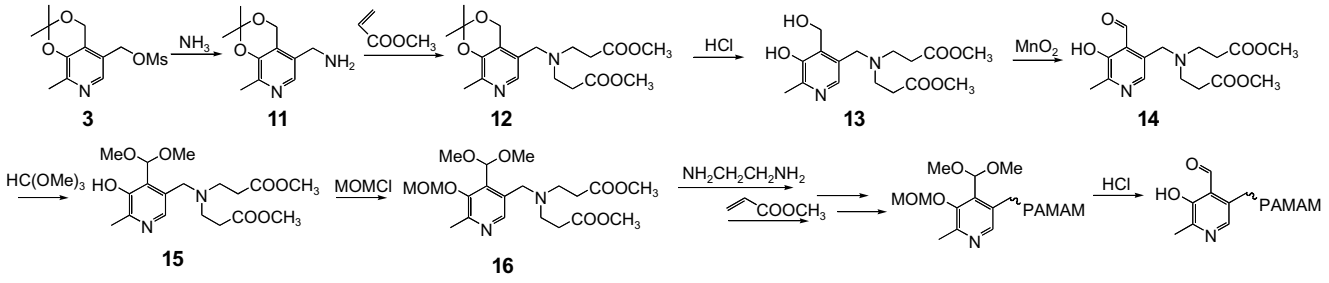

Figure 4. Synthesis of dendrimeric PAMAM–pyridoxals.

generations with acetyl groups using Ac_2O. The last step in the synthesis is the deprotection of both dimethoxyl and MOM groups using HCl (1 N).

The NMe_2 or NHAc-terminated full generations are referred to as **Gn-Me** or **Gn-Ac**. Figure 5 shows **G6-Me** as an example. Gn possesses $2n$ terminal NMe_2 or NHAc groups. According to Tomalia's studies,[20] the diameters of the PAMAM dendrimers without any pyridoxal are about 1.5, 2.2, 2.9, 3.6, and 4.5 nm for G2, G3, G4, G5, and G6, respectively. In comparison with these values, the respective diameters of insulin, cytochrome *c*, and hemoglobin are about 3.0, 4.0, and 5.5 nm. The sizes of the G4–6 pyridoxal dendrimers are close to those of natural globular enzymes.

The stable dimethoxyl and MOM-protected dendrimers were characterized with ^{1}H NMR and FAB or MALDI MS (the broad MALDI spectra indicate some defects in the dendrimers). After HCl treatment, the resulting pyridoxal dendrimers are mixtures of free aldehyde and hydrated aldehyde forms. They are used in the catalysis studies directly without further characterization (see the supporting information). The UV–vis spectrum of the HCl-treated **G5-Me** dendrimer shows the characteristic absorption of pyridoxal at 400 nm, suggesting the success of the deprotection.

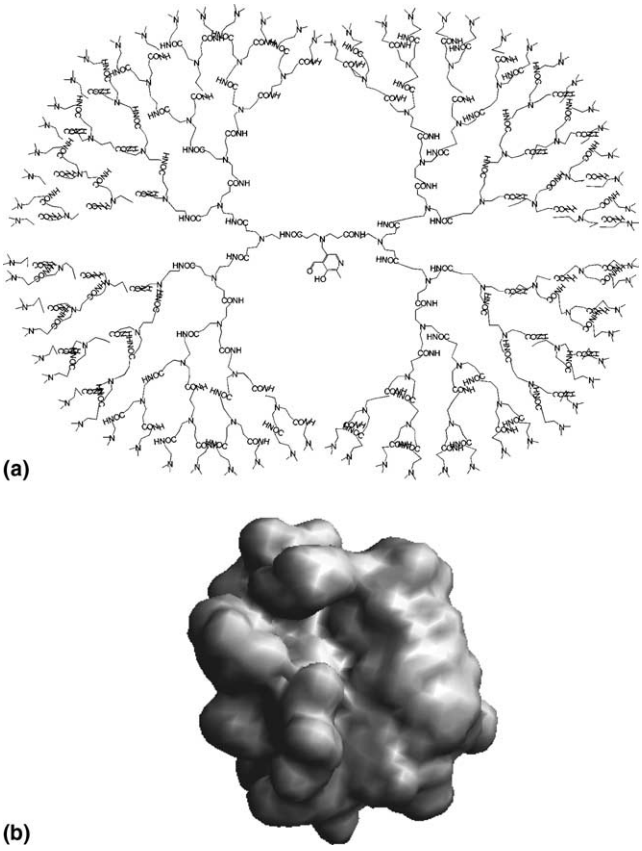

Figure 5. Chemical structure (a) and three-dimensional view (b) of the **G6-Me** PAMAM–pyridoxal molecule.

2.3. Racemization and decarboxylation reactions associated with dendrimeric pyridoxals

In comparison with the polymeric PEI–pyridoxals, the dendrimeric PAMAM–pyridoxals are much more stable compounds. Their degradation rates, as measured from the disappearance of the UV–vis absorption at 400 nm, are about 10–100 times slower than the PEI–pyridoxals (see Table 1).

When alanine (2.5×10^{-3} M) is added to the dendrimeric PAMAM–pyridoxal solutions at pH = 7.5, the absorption at 400 nm disappears significantly faster than in the absence of alanine (see Table 2). Meanwhile a new peak at 324 nm appears at the same rate as the disappearance of the 400 nm absorption. This might indicate that transamination occurs between the pyridoxal dendrimers and aniline. However, earlier experiments by Auld and Bruice,[21] Vazquez et al.,[22] and Imperiali and Ranabir,[23] showed that a great excess of alanine (e.g., 1.0 M) must be added to the reaction solution in order to see the transamination between pyridoxal and alanine. Zabinski and Toney also reported that decarboxylation reaction can take place between 2-aminoisobutyrate and pyridoxal phosphate yielding acetone and pyridoxamine.[24] Thus we worried that the disappearance of pyridoxal in our experiments may be due to both transamination and decarboxylation.

Table 1. Degradation rates of dendrimeric PAMAM–pyridoxals (min^{-1})[a]

Dendrimer	$k_{degradation}$
G1-Me	$(7.5\pm0.4)\times10^{-5}$
G2-Me	$(1.1\pm0.2)\times10^{-4}$
G3-Me	$(1.7\pm0.2)\times10^{-4}$
G4-Me	$(3.2\pm0.3)\times10^{-4}$
G5-Me	$(5.1\pm0.4)\times10^{-4}$
G6-Me	$(6.2\pm0.3)\times10^{-4}$
G1-Ac	$(4.2\pm0.3)\times10^{-5}$
G2-Ac	$(6.7\pm0.4)\times10^{-5}$
G3-Ac	$(7.4\pm0.3)\times10^{-5}$
G4-Ac	$(7.8\pm0.2)\times10^{-5}$
G5-Ac	$(8.1\pm0.3)\times10^{-5}$
G6-Ac	$(8.3\pm0.2)\times10^{-5}$

[a] For detailed experimental conditions see Ref. 17.

Table 2. The rates for the disappearance of pyridoxal in the reaction between alanine and dendrimeric PAMAM–pyridoxals (min^{-1})[a]

Dendrimer	k
G1-Me	$(2.8\pm0.2)\times10^{-3}$
G2-Me	$(3.3\pm0.1)\times10^{-3}$
G3-Me	$(3.8\pm0.2)\times10^{-3}$
G4-Me	$(4.0\pm0.3)\times10^{-3}$
G5-Me	$(4.4\pm0.2)\times10^{-3}$
G6-Me	$(4.7\pm0.2)\times10^{-3}$
G1-Ac	$(4.0\pm0.3)\times10^{-4}$
G2-Ac	$(6.9\pm0.4)\times10^{-4}$
G3-Ac	$(7.4\pm0.2)\times10^{-4}$
G4-Ac	$(7.8\pm0.1)\times10^{-4}$
G5-Ac	$(7.5\pm0.2)\times10^{-4}$
G6-Ac	$(7.9\pm0.4)\times10^{-4}$

[a] Reaction condition: 2.5×10^{-3} M alanine, 1.0×10^{-4} M PAMAM–pyridoxal, 0.25 M HEPES, 0.1 M KCl, 0.01 M EDTA, pH = 7.5, $t = 60$ °C.

Without a clear conclusion about the transamination reaction, we turned to the racemization reaction of α-amino acid. In this reaction pyridoxal is a real catalyst (see Fig. 6). In addition, we can unambiguously monitor both the L- and D-amino acid product using the HPLC method. As shown in Table 3, we indeed found that the PAMAM–pyridoxals can significantly catalyze the racemization reactions.

We found that the control compound (**1**) can also catalyze the racemization reaction of alanine and phenylalanine. The reaction rate shows strong buffer dependence when we change the buffer (CHES) concentration from 0.18 to 0.06 M. At zero buffer condition (extrapolated), the racemization rate for alanine is slightly higher than that for phenylalanine.

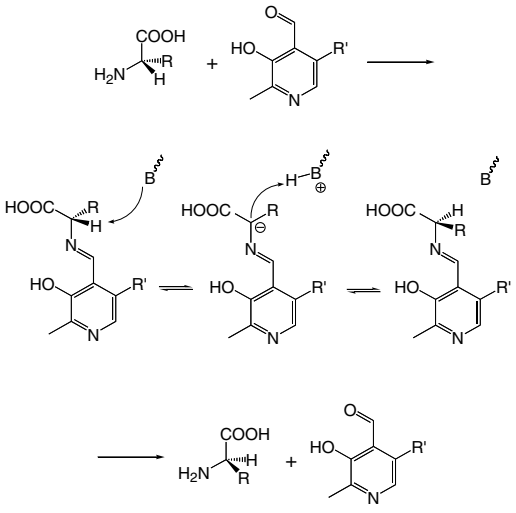

Figure 6. Racemization of α-amino acid catalyzed by pyridoxals.

Table 3. The rates for the racemization reactions between alanine or phenylalanine and dendrimeric PAMAM–pyridoxals (min^{-1})[a]

Dendrimer	Buffer concentration (M)	$k_{racemization}$	
		Alanine	Phenylalanine
1	0.18	$(3.0\pm0.2)\times10^{-5}$	$(2.5\pm0.2)\times10^{-5}$
1	0.14	$(2.3\pm0.1)\times10^{-5}$	$(1.8\pm0.2)\times10^{-5}$
1	0.10	$(1.4\pm0.1)\times10^{-5}$	$(1.1\pm0.1)\times10^{-5}$
1	0.06	$(8.6\pm0.5)\times10^{-6}$	$(7.5\pm0.4)\times10^{-6}$
1	0.00[b]	3.1×10^{-6}	2.5×10^{-6}
G1-Me	0.10	$(1.5\pm0.1)\times10^{-4}$	$(1.7\pm0.2)\times10^{-4}$
G2-Me	0.10	$(1.7\pm0.2)\times10^{-4}$	$(1.9\pm0.1)\times10^{-4}$
G3-Me	0.10	$(2.0\pm0.1)\times10^{-4}$	$(2.6\pm0.2)\times10^{-4}$
G4-Me	0.10	$(2.3\pm0.2)\times10^{-4}$	$(3.2\pm0.3)\times10^{-4}$
G5-Me	0.10	$(2.7\pm0.2)\times10^{-4}$	$(3.9\pm0.2)\times10^{-4}$
G6-Me	0.10	$(3.0\pm0.1)\times10^{-4}$	$(4.6\pm0.3)\times10^{-4}$
G6-Me	0.15	$(3.1\pm0.2)\times10^{-4}$	$(4.5\pm0.2)\times10^{-4}$
G6-Me	0.05	$(3.0\pm0.1)\times10^{-4}$	$(4.6\pm0.2)\times10^{-4}$
G1-Ac	0.10	$(1.0\pm0.1)\times10^{-5}$	$(1.1\pm0.1)\times10^{-5}$
G2-Ac	0.10	$(1.1\pm0.1)\times10^{-5}$	$(1.1\pm0.2)\times10^{-5}$
G3-Ac	0.10	$(1.3\pm0.1)\times10^{-5}$	$(1.5\pm0.1)\times10^{-5}$
G4-Ac	0.10	$(1.4\pm0.2)\times10^{-5}$	$(1.7\pm0.1)\times10^{-5}$
G5-Ac	0.10	$(1.6\pm0.1)\times10^{-5}$	$(2.0\pm0.2)\times10^{-5}$
G6-Ac	0.10	$(1.6\pm0.2)\times10^{-5}$	$(2.1\pm0.1)\times10^{-5}$
G6-Ac	0.15	$(1.6\pm0.1)\times10^{-5}$	$(2.2\pm0.2)\times10^{-5}$
G6-Ac	0.05	$(1.5\pm0.1)\times10^{-5}$	$(2.1\pm0.2)\times10^{-5}$

[a] Reaction condition: 2.5×10^{-3} M alanine or phenylalanine, 1.0×10^{-4} M PAMAM–pyridoxal, certain concentration of CHES buffer, 0.1 M KCl, 0.01 M EDTA, pH = 9.0, $t = 20\,^{\circ}C$.

[b] Linear extrapolation to zero buffer condition.

In comparison with **1**, the dendrimers show less buffer dependence in the racemization. For **G6-Me** and **G6-Ac**, we observed almost no buffer effects on the racemization rate. These observations suggest that that the amino groups of the PAMAM moiety are performing the catalytic proton transfers in the racemization process. Due to this internal general acid/base catalysis, the NMe_2-terminated pyridoxal dendrimers show about 50–100-fold rate enhancements in the racemization reaction. With only the interior tertiary amines as bases, NHAc-terminated dendrimers show only about 3–5-fold rate enhancements in the racemization reaction compared to **1** under zero buffer condition (Table 4).

Previous studies have demonstrated that PAMAM dendrimers can selectively bind hydrophobic guest molecules.[25] Similar hydrophobic binding effects are indicated by our results concerning the racemization reactivities between the dendrimeric pyridoxals and phenylalanine substrate. Although the control compound (**1**) shows slightly less reactivity toward phenylalanine than toward alanine, the dendrimers show slightly higher reactivities with phenylalanine than with alanine.

In addition to racemization, another pyridoxal-related reaction is amino acid decarboxylation (Fig. 7). In an earlier study by Janda and co-workers, a catalytic antibody was developed to model pyridoxal-dependent decarboxylases.[26] It was proposed that an apolar environment could greatly enhance the decarboxylation reactivity. Thus we were interested to know how 'apolar' our dendrimers' core could be.

We chose 2-amino-2-phenyl-propionic acid as the substrate for decarboxylation reactions. This substrate does not have any α-proton so that only decarboxylation can take place. Furthermore, the product of the decarboxylation reaction, acetophenone, can be easily monitored using HPLC.

Table 4. Relative racemization rates of dendrimeric pyridoxals[a]

Dendrimer	$k_{relative}$	$k_{alanine}$:$k_{phenylalanine}$
1	1.00	1:0.8
G1-Me	48	1:1.1
G2-Me	55	1:1.1
G3-Me	64	1:1.3
G4-Me	74	1:1.4
G5-Me	87	1:1.4
G6-Me	97	1:1.5
G1-Ac	3.2	1:1.1
G2-Ac	3.5	1:1.0
G3-Ac	4.2	1:1.2
G4-Ac	4.5	1:1.2
G5-Ac	5.2	1:1.2
G6-Ac	5.2	1:1.3

[a] Relative rates are calculated using the racemization rate of **1** at zero buffer condition.

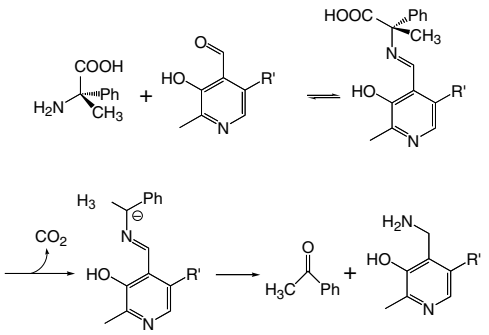

Figure 7. Decarboxylation of 2-amino-2-phenyl-propionic acid mediated by pyridoxals.

In agreement with previous findings,[27] we found that the pyridoxal-dependent decarboxylation reaction has a strong rate dependence on the polarity of the medium (see Table 5). From pure aqueous solution to 40:60 water–DMF solution, the decarboxylation rate of **1** increases by 83-folds. Similar rate enhancement effect is also seen by addition of acetonitrile.

Despite the strong solvent effect on **1**, all of the dendrimers do not show high decarboxylation rate. In purely aqueous solution, the highest decarboxylation rate is seen with **G6-Me** and **G6-Ac**, and this rate is only about 3 times faster than that of compound **1**. By comparison to the decarboxylation rates of **1** in different water–DMF solutions, one may predict that the interior polarity in our pyridoxal dendrimers is close to that of 85:15 water–DMF solution.

Table 5. Rates of the decarboxylation reactions between dendrimeric pyridoxals and 2-amino-2-phenyl-propionic acid[a]

Dendrimer	$k_{decarboxylation}$	$k_{relative}$
1 (100% water)	$(3.5\pm0.1)\times10^{-3}$	1.00
1 (85:15 water–DMF)	$(1.2\pm0.1)\times10^{-2}$	3.4
1 (70:30 water–DMF)	$(5.6\pm0.2)\times10^{-2}$	16
1 (70:30 water–MeCN)	$(5.9\pm0.1)\times10^{-2}$	17
1 (55:45 water–DMF)	$(1.5\pm0.2)\times10^{-1}$	43
1 (40:60 water–DMF)	$(2.9\pm0.2)\times10^{-1}$	83
1 (70:30 water–MeCN)	$(2.7\pm0.2)\times10^{-1}$	77
G1-Me (100% water)	$(4.0\pm0.2)\times10^{-3}$	1.1
G2-Me (100% water)	$(5.5\pm0.3)\times10^{-3}$	1.6
G3-Me (100% water)	$(8.1\pm0.6)\times10^{-3}$	2.3
G4-Me (100% water)	$(8.7\pm0.5)\times10^{-3}$	2.5
G5-Me (100% water)	$(1.0\pm0.1)\times10^{-2}$	2.8
G6-Me (100% water)	$(1.2\pm0.1)\times10^{-2}$	3.4
G6-Me (70:30 water–DMF)	$(3.4\pm0.3)\times10^{-2}$	9.7
G6-Me (40:60 water–DMF)	$(5.3\pm0.3)\times10^{-2}$	15
G1-Ac (100% water)	$(3.8\pm0.1)\times10^{-3}$	1.1
G2-Ac (100% water)	$(6.1\pm0.2)\times10^{-3}$	1.7
G3-Ac (100% water)	$(7.5\pm0.5)\times10^{-3}$	2.1
G4-Ac (100% water)	$(9.2\pm0.7)\times10^{-3}$	2.6
G5-Ac (100% water)	$(1.1\pm0.1)\times10^{-2}$	3.1
G6-Ac (100% water)	$(1.3\pm0.1)\times10^{-2}$	3.7

[a] Reaction condition: 2.0×10^{-3} M 2-amino-2-phenyl-propionic acid, 1.0×10^{-4} M PAMAM–pyridoxal, 0.25 M HEPES, 0.1 M KCl, 0.01 M EDTA, pH = 7.5, $t = 60\,^{\circ}C$.

Nonetheless, our results show that increasing the dendrimer size has a positive effect on the decarboxylation rate. The decarboxylation is not buffer catalyzed so that the decarboxylation rate of **Gx-Me** is close to that of **Gx-Ac**. Furthermore, we measured the decarboxylation rates of **G6-Me** dendrimer in different media. We found that in 40:60 water–DMF the decarboxylation rate of **G6-Me** is much lower than the that of **1** under the same condition. This may suggest that the polarity of the dendrimer interior is not fully controlled by the solution.

2.4. Synthesis of dendrimeric pyridoxals with internal lauryl groups

In the previous studies we found that attaching lauryl groups to PEI–pyridoxamines increases the transamination rate significantly.[13,14] We suggested that a medium effect exerted by the lauryl groups may cause this rate enhancement. In the present study we were interested to incorporate lauryl groups in the pyridoxal dendrimers and see if the laurylation exerts any effect on the racemization and decarboxylation reactions.

Random alkylation of the PAMAM dendrimers at the terminal amino groups has been reported before.[16] Herein we hope to get more well-defined materials. Thus we fully capped the G3 dendrimer, which contains eight terminal NH_2 groups, using activated L-aspartic acid β-methyl ester each carrying one lauryl group. The product was treated iteratively with ethylenediamine and methyl acrylate, and finally terminated with *N*,*N*-dimethylethylenediamine (see Fig. 8).

Using the above procedure we successively obtained **G5-Me-lauryl** pyridoxal carrying eight internal lauryl groups at known positions. This compound (in its protected form) has a fairly clean ^1H NMR, although its MALDI mass (obs: 8500–9600; calcd: 10,908) is slightly smaller than expected (see the supporting information). The eight lauryl groups may impede the dendrimer growth so that defects occur. Moreover, the **G6-Me-lauryl** dendrimer was obtained as a much less reliable material (unclean NMR and poor MALDI mass).

We studied the racemization and decarboxylation reactions associated with **G5-Me-lauryl**. Under the same condition as reported in Table 3, the rate of the racemization reaction between alanine and **G5-Me-lauryl** is $(1.5\pm0.1)\times10^{-4}\,min^{-1}$. For **G5-Me** without any lauryl group, this rate is $(2.7\pm0.2)\times10^{-4}\,min^{-1}$. Therefore, laurylation decreases the racemization rate.

Under the same condition as described in Table 5, the rate of the decarboxylation reaction between 2-amino-2-phenyl-propionic acid and **G5-Me-lauryl** in purely aqueous solution is $(8.5\pm0.4)\times10^{-3}\,min^{-1}$. The rate for **G5-Me** under this condition is $(1.0\pm0.1)\times10^{-2}\,min^{-1}$. Thus laurylation also reduces the decarboxylation rate.

Currently we do not have a firm explanation for the rate reduction in both the racemization and decarboxylation reactions associated with **G5-Me-lauryl**. As a likely

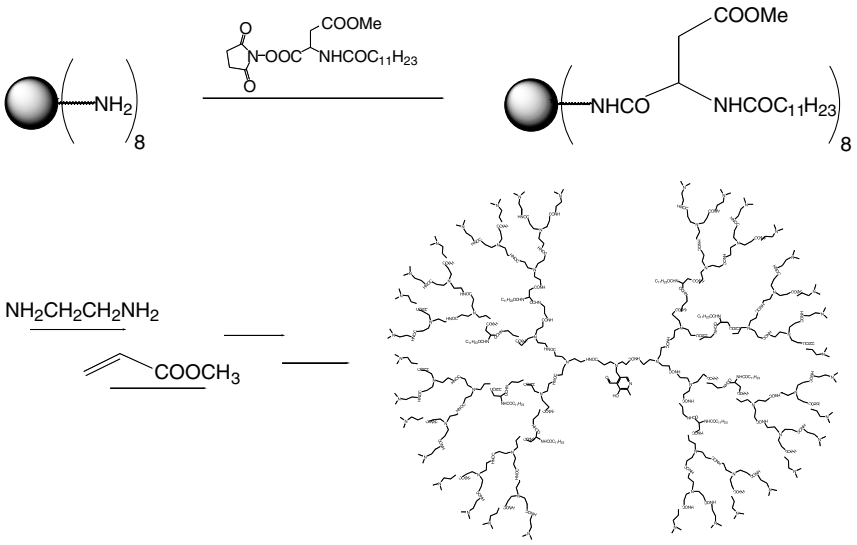

Figure 8. Synthesis of dendrimeric PAMAM–pyridoxals carrying eight internal lauryl groups.

possibility, the steric crowding may interfere with the geometry needed for Schiff base formation with the pyridoxals. However, the contrast of this slowing effect of the lauryl groups with our previously reported dramatic rate increasing effect of laurylation in transaminations by PEI–pyridoxamine is certainly striking.

3. Conclusions

To summarize, we covalently attached pyridoxal to polyethylenimine polymers, but found the resulting materials to be highly unstable. Thus we turned to synthesize dendrimeric pyridoxals that possess only one pyridoxal unit at the core of every dendrimer molecule. Totally 12 poly(amidoamine) type dendrimers were made in this work. They range from G1 to G6 with either NMe_2 or NHAc termini. The NMe_2-terminated pyridoxal dendrimers racemize α-amino acids 50–100 times faster than simple pyridoxal, while the NHAc-terminated pyridoxal dendrimers racemize α-amino acids only 3–5 times faster than simple pyridoxal. Both the NMe_2- and NHAc-terminated pyridoxal dendrimers decarboxylate 2-amino-2-phenyl-propionic acid 1–3 times faster than simple pyridoxal. It was predicted that the interior polarity in the pyridoxal dendrimers is close to that of 85:15 water–DMF solution. Furthermore, we successfully incorporated eight lauryl groups to the G5 pyridoxal dendrimer at known positions. The laurylated dendrimer exhibits lower racemization and decarboxylation rates than do the unlaurylated ones, probably because of steric crowding in the reaction zone.

4. Experimental

4.1. General

All the chemicals were purchased from commercial resources and used without further purification. Merck pre-coated 0.25 mm silica plates containing a 254 nm fluorescence indicator were used for analytical thin-layer chromatography. Flash chromatography was performed on 230–400 mesh silica from EM Science. Size-exclusion chromatography was performed using Sephadex®G10–G75 from Pharmacia Biochemtech. NMR spectra were obtained on a Bruker DPX 300 or 400 MHz spectrometer. UV–vis spectra were taken on a Varian Cary IE UV–vis spectrometer. CI MS spectra were taken on a Nermag R-10-10 instrument. FAB MS spectra were taken on a JEOL JMS-DX-303 HF instrument. MALDI mass spectra were acquired using a Bruker Biflex-III time-of-flight mass spectrometer.

4.2. Kinetics

Analytical HPLC was run on a HP1090 liquid chromatography (series II) equipped with a DR5 pumping system, a temperature-controlled autosampler and a diode-array UV–vis detector. C-18 reverse-phase analytical columns were used as solid phase.

The rates of racemization reactions were monitored with HPLC by detecting the formation of the L- and D-amino acid products. For this purpose, a derivatizing solution (OPA/NBC) of 0.2 M *o*-phthalaldehyde and 0.2 M *N*-Boc-cysteine in methanol as well as an aqueous buffer solution (KHP) of 1.0 M pH 8.0 K_2HPO_4 were prepared. The amino acid solutions were derivatized by mixing 10 μL of KHP, 10 μL of OPA/NBC and 80 μL of reaction mixture. A mixture of methanol and water with 1% TFA was used as eluent. The reaction was monitored at 344 nm, bandwidth 4 with the baseline taken at 450 nm, bandwidth 40.

The rates of decarboxylation reactions were monitored with HPLC by detecting the formation of acetophenone product. No derivatization was needed. The reaction was monitored at 244 nm, bandwidth 8 with the baseline taken at 550 nm, bandwidth 40.

4.2.1. PEI-Boc. Two milliliters (0.023 mol residue of monomers) of commercial polyethylenimine was dissolved in 10 mL of water, to which a solution of 0.10 g (0.00046 mol) of $(Boc)_2O$ in 1 mL of CH_3CN was added dropwise. The reaction was stirred at room temperature for an hour. The product was purified by dialysis against water several times overnight. ^1H NMR (300 MHz, D_2O): 3.0–2.5, 237.9H; 1.5, 9.0H. From NMR, it was calculated that about 1.7% of nitrogens on PEI were protected with Boc group.

4.2.2. PEI-Boc-Me. One gram of PEI-Boc as prepared above was dissolved in 20 mL of HCHO (37%, aq) solution. After 20 min, 2.5 g of $NaBH_3CN$ was added in several portions. The reaction was stirred at room temperature overnight. The product was purified by dialysis against aqueous solution of NH_4Cl and water. ^1H NMR (300 MHz, D_2O): 3.2–2.2, 330.6H; 1.6, 9.0H.

4.2.3. PEI-Me-Ac. PEI-Boc-Me (1.2 g) was dissolved in 20 mL of ethanol. Two milliliters of triethylamine was added. Then 2 mL of acetic anhydride was added. The reaction was stirred at room temperature for 1 h. The product was dialyzed against ethanol and water for 24 h. ^1H NMR (300 MHz, D_2O): 3.6–2.3, 324.1H; 2.0–1.8, 17.5H; 1.6, 9.0H.

4.2.4. PEI-Me-Ac. PEI-Boc-Me-Ac (1.5 g) prepared above was dissolved in 20 mL of trifluoroacetic acid (TFA). The reaction was stirred at room temperature for 4 h. TFA was evaporated then. The residue was redissolved in aqueous solution of $NaHCO_3$. The product was purified by dialysis against aqueous solution of HCl and water. ^1H NMR (300 MHz, D_2O): 4.2–3.5 (nitrogen protonated), 57.8H; 2.0–1.7, 3.0H.

4.2.5. PEI-lauryl. Four milliliters of commercial PEI was dissolved in 50 mL of ethanol. Lauryl iodide (1.4 g) was added, followed by the addition of 2 mL of diisopropylethylamine. The reaction was refluxed at 90 °C under Ar protection for 8 h. The product was dialyzed against water and water/ethanol solution. ^1H NMR (300 MHz, D_2O): 4.0–2.6, 40.2H; 2.2–1.0, 23.0H. From NMR, it was calculated that 10.5% of nitrogens on PEI were alkylated.

4.2.6. PEI-lauryl-Boc. All product obtained in the above step was dissolved in 25 mL of water/25 mL of ethanol. Two milliliters of triethylamine was added to the solution. $(Boc)_2O$ (0.20 g), dissolved in 1 mL of acetonitrile, was added. The reaction was stirred at room temperature for an hour. The product was dialyzed against ethanol and water. ^1H NMR (300 MHz, D_2O): 3.8–2.5, 40.0H; 2.0–0.9, 24.9H.

4.2.7. PEI-lauryl-Boc-Me. One gram of PEI-lauryl-Boc was dissolved in 15 mL of HCHO (aq 37%). Five grams of $NaBH_3CN$ was added then. The reaction was stirred at room temperature for 2 h. The product was dialyzed against water and ethanol. ^1H NMR (300 MHz, D_2O): 4.0–2.2, 67.4H; 2.1–0.9, 23.2H.

4.2.8. PEI-lauryl-Boc-Me-Ac. PEI-lauryl-Boc-Me (1.5 g) was dissolved in 20 mL of ethanol. 2 mL of triethylamine was added. Then 2 mL of acetic anhydride was added. The reaction was stirred at room temperature for 1 h. The product was dialyzed against water and ethanol for 24 h. ^1H NMR (300 MHz, D_2O): 3.8–2.2, 63.2H; 1.9, 3.5H; 1.8–0.8, 23.0H.

4.2.9. PEI-lauryl-Me-Ac. PEI-lauryl-Boc-Me-Ac (1.2 g) was dissolved in 15 mL of trifluoroacetic acid. The reaction was stirred at room temperature for 4 h. The product was dialyzed against water and ethanol for 24 h. ^1H NMR (300 MHz, D_2O): 4.0–2.8, 58.9H; 2.0–0.8, 23.0H.

4.2.10. Methanesulfonic acid 2,2,8-trimethyl-4*H*-[1,3]dioxino-[4,5-*c*]pyridin-5-ylmethyl ester (3). Five grams of (2,2,8-trimethyl-4*H*-[1,3]dioxino[4,5-*c*]pyridin-5-yl)methanol (**2**) was dissolved in 50 mL of anhydrous CH_2Cl_2. The solution was cooled to 0 °C. Then 3.1 g triethylamine was added, followed by the addition of 1.9 mL MsCl. The reaction was stirred for 20 min. The mixture was washed with NH_4Cl aqueous solution. TLC (ethyl acetate) $R_f = 0.52$. Yield: quantitative. CI MS: 288 (M+1). ^1H NMR (300 MHz, $CDCl_3$): 8.02, s, 1H; 5.18, s, 2H; 4.96, s, 2H; 3.02, s, 3H; 2.46, s, 3H; 1.61, s, 6H.

4.2.11. 3-(2,2,8-Trimethyl-4*H*-[1,3]dioxino[4,5-*c*]pyridin-5-yl-methylsulfanyl)-propionic acid methyl ester (4). Compound **3** (6.6 g) was dissolved in 50 mL of DMF. This solution was added slowly to a mixture of 3.4 g of $HS–CH_2CH_2COOCH_3$ and 1.2 g of NaH in 50 mL of DMF. The reaction was stirred at room temperature for 1 h. The product was purified using flash chromatography (3:1 hexane–ethyl acetate). TLC (ethyl acetate) $R_f = 0.72$. Yield: 74%. CI MS: 312 (M+1). ^1H NMR (400 MHz, $CDCl_3$): 7.93, s, 1H; 5.02, s, 2H; 3.78, s, 3H; 3.68, s, 2H; 2.78, t, 2H; 2.68, t, 2H, $J = 6.9$ Hz; 2.51, s, 3H; 1.62, s, 6H.

4.2.12. 4-(5-Hydroxy-4-hydroxymethyl-6-methyl-pyridin-3-yl-methylsulfanyl)-butyric acid methyl ester (5). Two grams of **4** was dissolved in 10 mL of methanol. Twenty milliliters of HCl (aq, 37%) was added. The reaction was stirred at room temperature for 15 h. The solution was neutralized using $NaHCO_3$ aqueous solution. The product was extracted using $CHCl_3$. TLC (ethyl acetate) $R_f = 0.42$. Yield: 81%. CI MS: 272 (M+1). ^1H NMR (300 MHz, $CDCl_3$): 7.81, s, 1H; 5.10, s, 2H; 3.72, s, 3H; 3.65, s, 2H; 2.68, t, 2H; 2.51, t, 2H, $J = 6.9$ Hz; 2.45, s, 3H.

4.2.13. 4-(4-Formyl-5-hydroxy-6-methyl-pyridin-3-yl-methyl-sulfanyl)-butyric acid methyl ester (6). Compound

5 (1.3 g) was dissolved in 50 mL of CH_2Cl_2. Activated MnO_2 (4.5 g) was added. The reaction was stirred at room temperature under Ar protection for 6 h. The solution was filtered through a pad of Celite. The product was purified using flash chromatography (1:1 ethyl acetate–hexane). TLC (ethyl acetate): $R_f = 0.84$. Yield: 77%. CI MS: 270 (M+1). ^{1}H NMR (300 MHz, $CDCl_3$): 10.58, s, 1H; 8.02, s, 1H; 4.02, s, 2H; 3.82, s, 3H; 3.65, s, 2H; 2.80, t, 2H; 2.66, t, 2H, $J = 6.9$ Hz; 2.62, s, 3H.

4.2.14. 3-(4-Dimethoxymethyl-5-hydroxy-6-methyl-pyridin-3-ylmethylsulfanyl)-propionic acid methyl ester (7). Compound **6** (1.0 g) was dissolved in 10 mL of methanol. Ten milliliters of $HC(OMe)_3$ was added. Then 0.1 g of *p*-toluenesulfonic acid was added. The reaction mixture was refluxed under Ar protection for 12 h. After removal of the solvent, the product was redissolved in $CHCl_3$, which was washed with aqueous solution of $NaHCO_3$. TLC (1:1 hexane–ethyl acetate): $R_f = 0.40$. Yield: 81%. CI MS: 316 (M+1). ^{1}H NMR (300 MHz, $CDCl_3$): 8.88, s, 1H; 7.89, s, 1H; 6.04, s, 1H; 3.76, s, 2H; 3.70, s, 3H; 3.46, s, 6H; 2.76, t, 2H; 2.65, t, 2H, $J = 6.9$ Hz; 2.48, s, 3H.

4.2.15. 3-(4-Dimethoxymethyl-5-methoxymethoxy-6-methyl-pyridin-3-ylmethylsulfanyl)-propionic acid methyl ester (8). Compound **7** (1.5 g) was dissolved in 20 mL of DMF. NaH (0.22 g) was added. Then 0.4 mL of MOMCl was added. The reaction was stirred at room temperature overnight. The product was purified using flash chromatography (2:1 AcOEt–hexanes). TLC (ethyl acetate): $R_f = 0.47$. Yield: 62%. CI MS: 360 (M+1). ^{1}H NMR (300 MHz, $CDCl_3$): 8.52, s, 1H; 5.81, s, 1H; 5.08, s, 2H; 4.12, s, 2H; 3.81, s, 3H; 3.70, s, 3H; 3.59, s, 6H; 2.95–2.69, m, 7H.

4.2.16. 3-(4-Dimethoxymethyl-5-methoxymethoxy-6-methyl-pyridin-3-ylmethylsulfanyl)-propionic acid (9). Compound **8** (0.8 g) was dissolved in 10 mL of MeOH and 10 mL of water. One gram of NaOH was added. After reaction was stirred for 1 h, the pH value of the mixture was adjusted to 5. The product was extracted using chloroform and purified using flash chromatography (10:1 CH_2Cl_2–MeOH). TLC (ethyl acetate): $R_f = 0.18$. Yield: quantitative. CI MS: 346 (M+1). ^{1}H NMR (300 MHz, $CDCl_3$): 8.60, s, 1H; 5.78, s, 1H; 5.12, s, 2H; 4.18, s, 2H; 3.70, s, 3H; 3.58, s, 6H; 3.00–2.68, m, 7H.

4.2.17. 3-(4-Dimethoxymethyl-5-methoxymethoxy-6-methyl-pyridin-3-ylmethylsulfanyl)-propionic acid 2,5-dioxo-pyrrolidin-1-yl ester (10). Compound **9** (0.7 g) was dissolved in 10 mL of CH_2Cl_2. EDC (0.40 g) and 0.23 g of *N*-hydroxy-succinimide was added. The reaction was stirred at room temperature for 1 h. The product was purified using flash chromatography (100% ethyl acetate). TLC (ethyl acetate): $R_f = 0.52$. Yield: quantitative. CI MS: 444 (M+1). ^{1}H NMR (300 MHz, $CDCl_3$): 8.50, s, 1H; 5.70, s, 1H; 5.05, s, 2H; 4.15, s, 2H; 3.73, s, 3H; 3.52, s, 6H; 3.00–2.82, m, 8H, 2.68, s, 3H.

4.2.18. PEI-Me-Ac-protected-pyridoxal. Compound **10** (0.05 g) was dissolved in 1 mL THF. This solution was added to a solution of 0.5 g of PEI-Me-Ac in water (pH = 8.0). The reaction was stirred for 1 h. Then 0.2 mL of acetic anhydride and 0.2 mL of triethylamine were added to the reaction mixture in order to completely remove the free amine groups. After 1 h, the product was purified by dialysis against ethanol, aqueous solution of NaCl, and water several times for 24 h. ^{1}H NMR (300 MHz, D_2O): 8.3, 1H; 5.7, 1H; 4.0–2.0, 350H.

4.2.19. PEI-Me-Ac-pyridoxal. PEI-Me-Ac-protected-pyridoxal (0.1 g) was dissolved in 5 mL of 1 N HCl. The reaction was stirred overnight under Ar protection at room temperature. The product was purified by dialysis against ethanol, aqueous solution of NaCl, and water several times for 24 h. ^{1}H NMR (300 MHz, D_2O): 8.1, 1H; 5.7, 1H; 4.5–2.0, 319H. UV–vis (pH = 7.5 in water): 398 nm, broad.

4.2.20. PEI-lauryl-Me-Ac-protected-pyridoxal. The synthesis is similar to that for PEI-Me-Ac-protected-pyridoxal. ^{1}H NMR (300 MHz, D_2O): 8.2, 1.0H; 5.7, s, 1H; 4.0–2.0, 396H; 1.9–0.9, 122H.

4.2.21. PEI-lauryl-Me-Ac-protected-pyridoxal. The synthesis is similar to that for PEI-Me-Ac-pyridoxal. ^{1}H NMR (300 MHz, D_2O): 8.3, 1.0H; 4.5–2.0, 351H; 1.9–0.9, 116H. UV–vis: (pH = 7.5 in water) 400 nm, broad.

4.2.22. C-(2,2,8-Trimethyl-4*H*-[1,3]dioxino[4,5-*c*]pyridin-5-yl)-methylamine (11). Compound **3** (7.7 g) was dissolved in 60 mL of 7 N ammonia in methanol. The solution was refluxed for 1 h. The solvent was removed. The product was purified using flash chromatography. $R_f = 0.15$ (10:1 methanol–chloroform, 2% ammonia). Yield: 66%. CI MS: 208.5 (M+1). ^{1}H NMR ($CDCl_3$, 300 MHz): 8.02, s, 1H; 5.02, s, 2H; 3.90, s, 2H; 2.52, s, 3H; 1.73, s, 6H.

4.2.23. 3-[(2-Methoxycarbonyl-ethyl)-(2,2,8-trimethyl-4*H*-[1,3]-dioxino[4,5-*c*]pyridin-5-yl-methyl)-amino]-propionic acid methyl ester (12). Compound **11** (5.3 g) was dissolved in 50 mL of methanol. Fifty milliliters of methyl acrylate was added. Five milliliters of triethylamine was also added. The reaction was refluxed overnight. Yield: quantitative. CI MS: 379.8 (M+1). ^{1}H NMR (CD_3OD, 300 MHz): 7.90, s, 1H; 5.02, s, 2H; 3.80, s, 6H; 3.73, s, 2H; 2.89, t, 4H, $J = 6.8$ Hz; 2.58, t, 4H; 2.43, s, 3H; 1.60, s, 6H.

4.2.24. 3-[(5-Hydroxy-4-hydroxymethyl-6-methyl-pyridin-3-ylmethyl)-(2-methoxycarbonyl-ethyl)-amino]-propionic acid methyl ester (13). Compound **12** (6.2 g) was dissolved in 20 mL of methanol. Twenty milliliters HCl (37%) was added. The reaction was stirred overnight at room temperature. After the solvent was removed, the

residue was re-dissolved in 100 mL of methanol. Two milliliters of H_2SO_4 (concd) was added. The reaction was refluxed for 2 h. The solvent was removed. The solution was neutralized using $NaHCO_3$ (aq) solution. The product was extracted out using methylene chloride. Yield: quantitative. CI MS: 339.9 (M+1). ^{1}H NMR (CD_3OD, 300 MHz): 7.80, s, 1H; 4.92, s, 2H; 3.72, s, 6H; 3.69, s, 2H; 2.82, t, 4H, $J = 6.8$ Hz; 2.58, t, 4H; 2.49, s, 3H.

4.2.25. 3-[(4-Formyl-5-hydroxy-6-methyl-pyridin-3-yl-methyl)-(2-methoxycarbonyl-ethyl)-amino]-propionic acid methyl ester (14). Two grams of **13** was dissolved in 100 mL of methylene chloride. Ten grams of MnO_2 was added. The reaction was stirred at room temperature for 4 h. The product was purified using flash chromatography. Yield: 86%. CI MS: 337.9 (M+1). ^{1}H NMR ($CDCl_3$, 300 MHz): 11.57, s, 1H; 10.47, s, 1H; 8.02, s, 1H; 3.91, s, 2H; 3.70, s, 6H; 2.91, t, 4H, $J = 6.8$ Hz; 2.61, s, 3H; 2.54, t, 4H.

4.2.26. 3-[(4-Dimethoxymethyl-5-hydroxy-6-methyl-pyridin-3-ylmethyl)-(2-methoxy-carbonyl-ethyl)-amino]-propionic acid methyl ester (15). Compound **14** (1.8 g) was dissolved in 20 mL of methanol. Twenty milliliters of $HC(OCH_3)_3$ was added. One gram Me–C_6H_4–SO_3H was added. The reaction was refluxed overnight. The product was purified using flash chromatography. Yield: 86%. CI MS: 383.9 (M+1). ^{1}H NMR ($CDCl_3$, 300 MHz): 8.95, s, 1H; 7.98, s, 1H; 6.30, s, 1H; 3.95, s, 6H; 3.82, s, 2H; 3.70, s, 6H; 2.91, t, 4H, $J = 6.8$ Hz; 2.61, s, 3H; 2.54, t, 4H.

4.2.27. 3-[(4-Dimethoxymethyl-5-methoxymethoxy-6-methyl-pyridin-3-ylmethyl)-(2-methoxycarbonyl-ethyl)-amino]-propionic acid methyl ester (16). Compound **15** (1.2 g) was dissolved in 40 mL of DMF. NaH (0.14 g) was added. After 30 min, 0.25 mL of $MeOCH_2Cl$ was added. The reaction was stirred at room temperature overnight. The product was purified using flash chromatography. Yield: 86%. CI MS: 428.8 (M+1). ^{1}H NMR (CD_3OD, 300 MHz): 8.48, s, 1H; 5.79, s, 1H; 5.13, s, 2H; 3.99, s, 2H; 3.71, s, 6H; 3.68, s, 3H; 3.52, s, 6H; 2.89, t, 4H, $J = 6.8$ Hz; 2.60, m, 7H.

4.2.28. G1-Me (protected). Compound **15** (0.08 g) was dissolved in 2 mL of MeOH. One milliliter of *N*,*N*-dimethylethylenediamine was added. The reaction was stirred at 60 °C for 3 days. The product was purified using flash chromatography. CI MS: 541.3 (M+1). ^{1}H NMR (D_2O, 300 MHz): 8.31, s, 1H; 5.70, s, 1H; 5.08, s, 2H; 3.80, s, 2H; 3.61, s, 3H; 3.41, s, 6H; 3.30, m, 4H; 2.85–2.25, m, 27H.

4.2.29. G1-Ac (protected). One gram of **15** was dissolved in 20 mL of methanol. Forty milliliters of ethylenediamine was added. The reaction was stirred at room temperature for 48 h. The product was purified using flash chromatography. CI MS: 483.6 (M+1). ^{1}H NMR (CD_3OD, 300 MHz): 8.43, s, 1H; 5.75, s, 1H; 5.11, s, 2H; 3.99, s, 2H; 3.76, s, 3H; 3.62, s, 6H; 3.32, m, 4H; 2.95, m, 8H; 2.59, s, 3H; 2.48, m, 4H. This product (0.12 g) was dissolved in 2 mL of methanol. Triethylamine (0.4 mL) was added. Then 0.4 mL of acetic anhydride was added. The reaction was stirred at room temperature for 0.5 h. The product was purified using flash chromatography. CI MS: 567.3 (M+1). FAB MS+: 569.41 (M+1). ^{1}H NMR (CD_3OD, 300 MHz): 8.47, s, 1H; 5.78, s, 1H; 5.13, s, 2H; 3.99, s, 2H; 3.78, s, 3H; 3.62, s, 6H; 3.40, m, 8H; 2.85, m, 4H; 2.59, s, 3H; 2.48, m, 4H, 2.05, s, 6H.

4.2.30. G2-Ac (protected). FAB MS+: 1109.9. (Exact mass for $C_{50}H_{88}N_{14}O_{14}$: 1108.7.) ^{1}H NMR (CD_3OD, 300 MHz): 8.38, s, 1H; 5.78, s, 1H; 5.12, s, 2H; 3.98, s, 2H; 3.70, s, 3H; 3.55, s, 6H; 3.28, m, 20H; 2.95–2.38, m, 31H; 2.02, s, 12H.

4.2.31. G2-Me (protected). FAB MS+: 1053.3 (M+1). 1075.2 (M+Na). (Exact mass for $C_{50}H_{96}N_{14}O_{10}$: 1052.7.) ^{1}H NMR (D_2O, 300 MHz): 8.29, s, 1H; 5.73, s, 1H; 5.05, s, 2H; 3.79, s, 2H; 3.54, s, 3H; 3.40, s, 6H; 3.20, m, 12H; 2.75–2.20, m, 39H; 2.11, s, 24H.

4.2.32. G3-Ac (protected). MALDI MS: 2211.0 (M+23). (Exact mass for $C_{98}H_{176}N_{30}O_{26}$: 2189.3.) ^{1}H NMR ($D_2O$, 300 MHz): 8.31, s, 1H; 5.81, s, 1H; 5.12, s, 2H; 4.28, s, 2H; 3.62, s, 3H; 3.55, s, 6H; 3.45–2.39, m, 115H; 1.98, s, 24H.

4.2.33. G3-Me (protected). MALDI MS: 2076.4. (Exact mass for $C_{98}H_{192}N_{30}O_{18}$: 2077.5). ^{1}H NMR ($D_2O$, 300 MHz): 8.29, s, 1H; 5.72, s, 1H; 5.03, s, 2H; 3.78, s, 2H; 3.64, s, 3H; 3.45, s, 6H; 3.35–2.18, m, 28H; 2.80–2.30, m, 84H; 2.25, s, 48H.

4.2.34. G4-Ac (protected). MALDI MS: 4360.24 (M+1), 4383.69 (M+23). (Exact mass for $C_{194}H_{352}N_{62}O_{50}$: 4350.7.) ^{1}H NMR ($D_2O$, 300 MHz): 3.50, sb, 92H; 3.00–2.50, m, 151H; 1.98, s, 48H.

4.2.35. G4-Me (protected). MALDI MS: 4150.0 (M+23). (Exact mass for $C_{194}H_{384}N_{62}O_{34}$: 4127.0.) ^{1}H NMR ($D_2O$, 300 MHz): 8.28, s, 1H; 5.69, s, 1H; 5.05, s, 2H; 3.79, s, 2H; 3.60, s, 3H; 3.48, s, 6H; 3.28, m, 60H; 2.95–2.25, m, 183H; 2.22, s, 96H.

4.2.36. G5-Ac (protected). MILDI MS: 7600–9300. (Exact mass for $C_{386}H_{704}N_{126}O_{98}$: 8673.4.) ^{1}H NMR ($D_2O$, 300 MHz): 3.50, sb, 188H; 3.00–2.50, m, 311H; 1.98, s, 96H.

4.2.37. G5-Me (protected). MILDI MS: 6000–8000. (Exact mass for $C_{386}H_{768}N_{126}O_{66}$: 8226.1.) ^{1}H NMR ($D_2O$, 300 MHz): 8.24, s, 1H; 5.61, s, 1H; 5.01, s, 2H; 3.56, s, 3H; 3.38, s, 6H; 3.25, m, 124H; 2.95–2.25, m, 375H; 2.22, s, 192H.

4.2.38. G6-Ac (protected). ^{1}H NMR (D_2O, 300 MHz): 3.50, mb, 380H; 3.00–2.50, m, 631H; 1.98, s, 192H. MALDI MS: 14,800–17,500. (Exact mass of $C_{770}H_{1408}N_{254}O_{194}$: 17318.8.)

4.2.39. G6-Me (protected). ^{1}H NMR (D_2O, 300 MHz): 3.50, mb; 2.90–2.18, m. Ratio: 1.00:4.29 (Theoretical ratio: 268:1143). MALDI MS: 13,000–17,000. (Exact mass of $C_{770}H_{1536}N_{254}O_{130}$: 16424.1.)

4.2.40. 2-Dodecanoylamino-succinic acid 4-methyl ester (16). L-Aspartic acid β-methyl ester hydrochloride (1.84 g) was dissolved in 20 mL of DMF. Two milliliters of triethylamine was added. Then 3.0 g of dodecanoic acid 2,5-dioxo-pyrrolidin-1-yl ester (made from dodecanoic acid and *N*-hydroxy-succinimide) was added. The reaction was stirred for 2 h. The solvent was removed. Then 100 mL $CHCl_3$ was added. The solution was washed with 50 mL of HCl (4 N). After $CHCl_3$ was removed, pure product was obtained as white wax. CI MS: 330.6 (M+1). ^{1}H NMR ($CDCl_3$, 300 MHz): 10.96, s, 1H; 6.81, d, 1H, $J = 7.6$ Hz; 4.92, m, 1H; 3.78, s, 3H; 2.30, t, 2H, $J = 7.6$ Hz; 1.69, m, 2H; 1.40, m, 18H; 0.95, t, 3H, $J = 6.9$ Hz.

4.2.41. 2-Dodecanoylamino-succinic acid 4-(2,5-dioxo-pyrrolidin-1-yl) ester 4-methyl ester (17). Compound **16** (3.1 g) was dissolved in 50 mL of CH_2Cl_2. *N*-hydroxy-succinimide (1.2 g) was added. Then 2.1 g of EDC was added. The reaction was stirred at room temperature for 2 h. The solution was washed with NH_4Cl (aq) solutions. CI MS: 427.8 (M+1). ^{1}H NMR ($CDCl_3$, 300 MHz): 6.72, d, 1H, $J = 8.2$ Hz; 5.47, m, 1H; 3.92, s, 3H; 2.89, s, 4H; 2.36, m, 2H; 1.74, m, 2H; 1.40, m, 18H; 1.00, t, 3H, $J = 6.8$ Hz.

4.2.42. G5-Me-lauryl (protected). MILDI MS: 8500–9600. (Exact mass for $C_{529}H_{1028}N_{150}O_{89}$: 10,908.) ^{1}H NMR ($D_2O$, 300 MHz): 8.39, s, 1H; 5.75, s, 1H; 5.07, s, 2H; 3.40, sb, 132H; 3.00–2.30, mb, 570H; 1.75, sb, 20H; 1.35, 3b, 154H; 0.90, s, 29H.

Supplementary data

Supplementary data are available for the detailed spectra of all the dendrimer compounds.

Acknowledgements

This work has been supported by the NSF and the NIH.

References and notes

1. Klotz, I. M.; Royer, G. P.; Scarpa, I. S. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 263.
2. Johnson, T. W.; Klotz, I. M. *Macromolecules* **1974**, *7*, 149.
3. Suh, J.; Scarpa, I. S.; Klotz, I. M. *J. Am. Chem. Soc.* **1976**, *98*, 7060.
4. Johnson, R. S.; Walder, J. A.; Klotz, I. M. *J. Am. Chem. Soc.* **1978**, *100*, 5159.
5. Hierl, M. A.; Gamson, E. P.; Klotz, I. M. *J. Am. Chem. Soc.* **1979**, *101*, 6020.
6. Delaney, E. J.; Wood, L. E.; Klotz, I. M. *J. Am. Chem. Soc.* **1982**, *104*, 799.
7. Suh, J.; Klotz, I. M. *J. Am. Chem. Soc.* **1984**, *106*, 2373.
8. Suh, J. *Synlett* **2001**, 1343.
9. Suh, J.; Lee, S. H. *J. Org. Chem.* **1998**, *63*, 1519.
10. Suh, J.; Oh, S. *J. Org. Chem.* **2000**, *65*, 7534.
11. Hollfelder, F.; Kirby, A. J.; Tawfik, D. S. *J. Am. Chem. Soc.* **1997**, *119*, 9578.
12. Hollfelder, F.; Kirby, A. J.; Tawfik, D. S. *J. Org. Chem.* **2001**, *66*, 5866.
13. Liu, L.; Breslow, R. *J. Am. Chem. Soc.* **2002**, *124*, 4978.
14. Liu, L.; Rozenman, M.; Breslow, R. *J. Am. Chem. Soc.* **2002**, *124*, 12660.
15. Zhou, W.; Liu, L.; Breslow, R. *Helv. Chim. Acta* **2003**, *86*, 3560.
16. Liu, L.; Brelsow, R. *J. Am. Chem. Soc.* **2003**, *125*, 12110.
17. Detailed experimental conditions: 1.0×10^{-4} M pyridoxal derivative (as determined by the concentration of the pyridoxal units by UV–vis absorption at 400 nm), 0.25 M of buffer (HEPES), 0.1 M KCl, 0.01 M EDTA, pH = 7.5, $t = 60$ °C.
18. From NMR analysis, we found that about 2% nitrogen atoms in each PEI molecule are attached with pyridoxal. Since each PEI molecule has 1400 monomers in average, there are about 28 pyridoxal units on each PEI molecule.
19. Tomalia, D. A.; Baker, H.; Hall, M.; Kallos, G.; Rock, J.; Smith, P. *Polym. J.* **1985**, *17*, 117.
20. Tomalia, D. A.; Huang, B.; Swanson, D. R.; Brothers, H. M., II; Klimash, J. W. *Tetrahedron* **2003**, *59*, 3799.
21. Auld, D. S.; Bruice, T. C. *J. Am. Chem. Soc.* **1967**, *89*, 2090.
22. Vazquez, M. A.; Munoz, F.; Donoso, J. *J. Mol. Catal.* **1991**, *68*, 105.
23. Imperiali, B.; Ranabir, S. B. *J. Org. Chem.* **1995**, *60*, 1891.
24. Zabinski, R. F.; Toney, M. D. *J. Am. Chem. Soc.* **2001**, *123*, 193.
25. Beezer, A. E.; King, A. S. H.; Martin, I. K.; Mitchel, J. C.; Twyman, L. J.; Wain, C. F. *Tetrahedron* **2003**, *59*, 3873.
26. Askley, J. A.; Lo, C.-H. L.; McElhaney, G. P.; Wirsching, P.; Janda, K. D. *J. Am. Chem. Soc.* **1993**, *115*, 2515.
27. Taylor, P. J. *J. Chem. Soc., Perkin Trans. 2* **1972**, 1077.