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Hydrophobic and electronic factors in the design of dialkylglycine decarboxylase mimics

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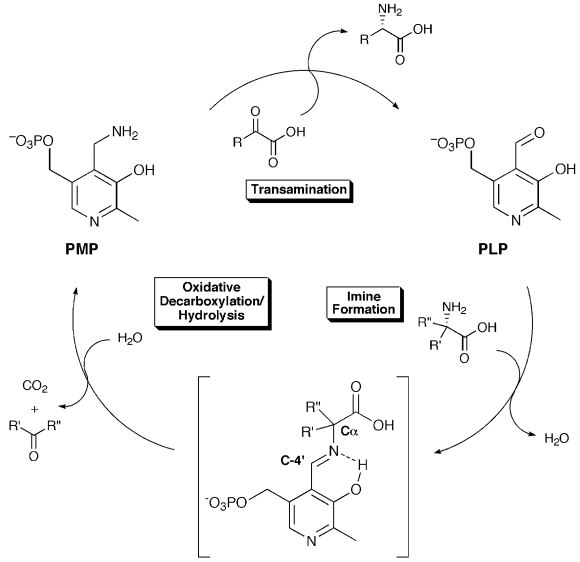
Abstract—The first functional catalytic mimic of the enzyme dialkylglycine decarboxylase is described. This system utilizes a hydrophobically modified polyethylenimine polymer, a pyridoxamine cofactor, and a 2-aryl-2-alkylglycine sacrificial amine source to convert α-keto acids to α-amino acids at biologically relevant temperatures with multiple turnovers of the pyridoxamine catalyst. The effects of hydrophobic and electronic factors in the 2,2-disubstituted sacrificial amine source and the pyridoxamine catalyst on turnover frequency and turnover number are explored.

1. Introduction

Dialkylglycine decarboxylase (DGD) is unique among pyridoxamine 5′-phosphate (PMP)-dependent transaminase and decarboxylase enzymes. Unlike typical transaminase enzymes, which utilize α-monosubstituted amino acids as sacrificial amine sources, DGD regenerates the PMP cofactor from the corresponding aldehyde, pyridoxal 5′-phosphate (PLP), by an oxidative decarboxylation of a 2,2-dialkylglycine substrate (Scheme 1).[1] In contrast to normative decarboxylase enzymes, in which Cα protonation is greatly favored, protonation in DGD occurs preferentially at the benzylic C-4′ position, to afford a ketone and PMP after hydrolysis or transimination. Given its unique activity, DGD has experienced significant mechanistic and crystallographic examination in the past 10 years.[2] Distinctive to DGD among other PMP/PLP-dependent enzymes is the lack of an observable quinoid intermediate throughout the catalytic cycle.[3]

As part of a long-lived initiative toward the mimicry of various pyridoxamine-dependent enzymatic systems (e.g., transaminases,[4] racemases,[4e,5] tryptophan synthases,[6] and decarboxylases[4e,7]), we recently reported the first functional mimic of DGD utilizing a laurylated polyethylenimine polymer catalyst [PEI-C_{12} (8.7%)] and a hydrophobically modified pyridoxamine cofactor (**1**) in aqueous reaction conditions (Scheme 2).[8] Employing



Scheme 1. PMP/PLP catalytic cycle in dialkylglycine decarboxylase.

2-phenyl-2-methylglycine (Pmg) as a sacrificial amine source, we obtained record rate accelerations (up to 725,000-fold) and turnover numbers (up to 100 turnovers) for a non-enzymatic pyridoxamine-mediated transamination.

While our initial studies with the polymer-catalyzed, **1**-mediated transamination of α-keto acids to α-amino acids demonstrated remarkably fast reaction rates in

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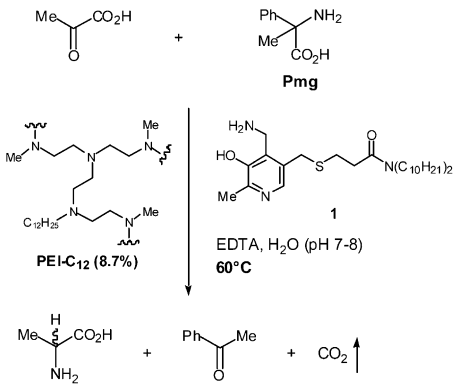


Scheme 2. DGD mimetic system exploiting hydrophobic interactions. PEI-C12 (8.7%) is commercial branched polyethylenimine[22] in which 8.7% of the amines are laurylated and the remaining amines are permethylated.[8]

comparison to pyridoxamine in water alone, the corresponding reverse reaction did not proceed appreciably. Other groups have reported similar difficulties in regenerating the pyridoxamine cofactor from pyridoxal using monosubstituted α-amino acids under aqueous conditions.[9] This reverse reaction can be promoted, with varying degrees of success, by very high concentrations (1 M!) of the α-monosubstituted sacrificial amine source,[13a] high temperatures,[10] the addition of metal[10,11] or imidazolium salts,[12] incorporation of the pyridoxamine cofactor into a protein environment,[13] or quaternization of the pyridoxamine/pyridoxal cofactor pyridine nitrogen.[14,23] Presumably, all of these strategies act to reduce the resonance stabilization of the pyridoxal aldehyde/imine with the pyridine ring of the cofactor. Utilizing α, α-disubstituted amino acid, as in our DGD-mimetic system, represents a unique strategy to regenerate the pyridoxamine cofactor and obtain a truly catalytic reaction.

Given our initial success, we sought to improve our DGD mimic protocol to achieve faster turnover rates at lower, more biologically relevant temperatures, potentially with higher turnover numbers. In this paper, we describe the influence of electronic and hydrophobic factors in the 2-aryl-2-alkylglycine sacrificial amine source on turnover rate and number in our system. Furthermore, the importance of a cationic pyridoxamine cofactor is explored. These studies indicate that enhancing hydrophobic interactions between the sacrificial amine source and the polymer–cofactor diad leads to greater fidelity and rate acceleration in our DGD-mimetic protocol over strictly electronic modifications. Additionally, quaternization of the pyridine nitrogen of the hydrophobically modified pyridoxal cofactor by alkylation greatly increases the rate of cofactor-mediated decarboxylation.

2. Results and discussion

2.1. Synthesis of 2-aryl-2-alkylglycines

The rate determining step for our new catalytic system is the decarboxylation of the putative internal aldimine

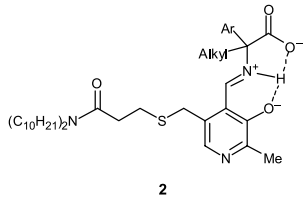
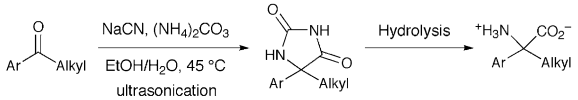


Figure 1. Putative internal aldimine intermediate **2**.

intermediate **2** (Fig. 1); therefore, increasing the rate of decarboxylation should lead to higher catalyst turnover frequencies. Electronic factors in the sacrificial amine source and pyridoxal cofactor, as well as hydrophobic interactions between the reagents and the polymeric cocatalyst in the aqueous reaction mixture should have a dramatic impact on the rate of decarboxylation.

We previously observed[8] that electron-rich 2,2-diphenylglycine is too reactive in the polymer-buffered reaction conditions, reacting directly with the α-keto acid substrate and obviating the hydrophobically modified pyridoxamine catalyst.[8] 2-Aminoisobutyric acid (2,2-dimethylglycine), in contrast, did not decarboxylate appreciably under our reaction conditions. Given our success with Pmg as a sacrificial amino source, we synthesized a small set of 2-aryl-2-alkylglycines to further investigate electronic and hydrophobic influences on the rate of pyridoxal-mediated decarboxylation. These α,α-disubstituted amino acids were obtained in short order from the corresponding ketones employing a modified Bucherer–Bergs reaction,[15] followed by hydrolysis of the resulting hydantoins (Scheme 3). The amino acid products were purified, generally by ion-exchange chromatography, and dissolved in slightly acidic deionized water to afford 0.1 M stock solutions.[16]

Attempts to synthesize either 2-(2-pyridyl)-2-methylglycine or the 4-pyridyl derivative from the corresponding hydantoins were unsuccessful, as these amino acids rapidly decomposed under both basic and acidic hydrolytic conditions. 2-(3-Pyridyl)-2-methylglycine (3-Pmg)[17] was successfully obtained following the above protocol, but it was indistinguishable from Pmg in both initial turnover frequency and turnover number when used as a sacrificial amine source for our DGD-mimetic system (data not shown).



Amino Acid	Ar	Alkyl	Yield (%)	Yield (%)
4-Mmg	4-MeOPh	Me	69	33 (A)
4-Nmg	4-NO_2Ph	Me	92	12 (C)
4-Tmg	4-F_3CPh	Me	90	9 (A)
Pxg	Ph	Hexyl	45	81 (A)
1-Aic			83	83 (B)

Scheme 3. Synthesis of 2-aryl-2-alkylglycines. Hydrolysis conditions: (A) 2 N aqueous NaOH, Δ; (B) $Ba(OH)_2$, H_2O, Δ; (C) 6 N aqueous HCl, Δ.

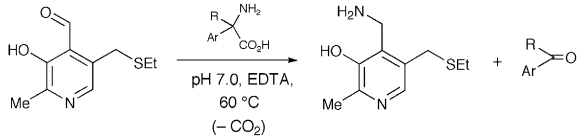
2.2. Rate of decarboxylation of 2-aryl-2-alkylglycines and influence on catalyst turnover frequency and number

The 2-aryl-2-alkylglycines described above were designed to explore electronic and hydrophobic factors in the rate of pyridoxal-mediated decarboxylation. Whether decarboxylation and C-4′ protonation proceed in a step-wise fashion through the intermediacy of a resonance-stabilized anion or represent a less likely concerted event in our DGD mimic, electron-withdrawing substituents on the sacrificial amine source (e.g., 4-NO_2Ph or 4-CF_3Ph) are expected to increase the rate of decarboxylation for intermediate **2**. Increasing the electron-withdrawing potential of the amino acid α-substituents, however, could have deleterious consequences. For example, the aryl methyl ketones produced from the PLP-mediated oxidative decarboxylation of the 2,2-disubstituted amino acids will be more activated toward aldol condensation with the pyridoxal intermediate, thus killing the catalytic cycle; we have observed that the hydrophobically modified polyethylenimine polymers can accelerate such aldol condensations (data not shown). Electron-donating substituents (e.g., 4-MeOPh), on the other hand, should retard the rate of decarboxylation, but exhibit longer-lived catalytic cycles due to slower ketone-induced catalyst decomposition.

Hydrophobic factors are also expected to play a significant role. Lengthening the alkyl substituents on the sacrificial amine source should increase hydrophobic interactions with the modified polymer, and the resulting increase in local concentration should afford faster rates of decarboxylation. A similar phenomenon was observed in the rate of pyridoxamine-mediated transaminations when the hydrophobicity of either the pyridoxamine cofactor or α-keto acid substrate was increase, with the former being more influential.[8] Furthermore, increasing the length, and thus steric bulk, of the alkyl substituent should *reduce* the rate of aldol condensation between the resulting ketone byproduct and the pyridoxal cofactor.

Preliminary amino acid decarboxylation studies were conducted in the absence of the modified PEI polymer employing the fully water-soluble pyridoxal 5′-ethyl thioether[4a] (Table 1). The reactions were monitored by UV–vis spectroscopy at 60 °C,[18] and pseudo-first-order rates of decarboxylation ($k_{decarboxylation}$) were computed following the exponential decay of the characteristic pyridoxal/pyridoximine absorbance at 400 nm.[4a] To prevent any adventitious metal ion catalysis, the decarboxylation reactions were performed in the presence of 2.0×10^{-3} M EDTA. As predicted, 2-aryl-2-alkylglycines with electron-withdrawing groups on the aromatic ring (i.e., 4-Tmg and 4-Nmg) showed marked increases in $k_{decarboxylation}$ over Pmg, while an electron-donating methoxy moiety (i.e., 4-Mmg) retarded the rate of decarboxylation. The strength of the electron-withdrawing aromatic substituent directly translates into increases in $k_{decarboxylation}$; a *para*-trifluoromethyl group (i.e., 4-Tmg) affords a nearly twofold increase in $k_{decarboxylation}$, while the stronger electron-withdrawing *para* nitro group (i.e., 4-Nmg) exhibits over a 17-fold effect. Interestingly, replacing the 2-methyl substituent with an electron-withdrawing trifluoromethyl group[19] resulted in almost no observed decarboxylation, presumably due to the significant decrease in amine nucleophilicity and thus slower imine formation between the amino acid and pyridoxal reagents (data not shown). In the absence of the modified PEI polymer, increasing the hydrophobicity of the amino acid was not expected to greatly influence the rate of decarboxylation. Indeed, the more hydrophobic 2-phenyl-2-hexylglycine (Pxg) decarboxylated at a rate nearly identical to Pmg. Joining the 2-phenyl and 2-alkyl groups into a constrained five-membered ring (i.e., 1-Aic) also displayed little effect on the apparent $k_{decarboxylation}$.

Table 1. Rate of 2-aryl-2-alkylglycine decarboxylation by pyridoxal 5′-ethylthioether in water and 34.5% aqueous acetonitrile



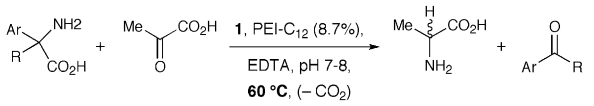
Amino acid	k ($\times 10^{-3}$ min^{-1})	$k_{relative}$	w/34.5% MeCN k ($\times 10^{-3}$ min^{-1})	k_{MeCN}/k
Pmg	3.2 ± 1.4	1.0	39.5 ± 0.7	12.3
4-Tmg	6.2 ± 4.3	1.9	66.8 ± 1.4	10.8
4-Nmg	57.0 ± 1.7	17.3	123.3 ± 1.0	2.2
4-Mmg	1.2 ± 4.1	0.4	24.5 ± 0.8	20.4
Pxg	4.6 ± 3.5	1.4	47.4 ± 1.3	10.3
1-Aic	2.6 ± 1.3	0.8	46.5 ± 1.8	17.9

Reaction conditions: 1.0×10^{-4} M pyridoxal 5′-ethylthioether, 1.0×10^{-3} M amino acid, 2.0×10^{-3} M EDTA, 2.5×10^{-2} M Hepes, 2.5×10^{-2} M KCl, T = 60 °C, pH 7.0.

Given previous studies indicating that reducing the polarity of the reaction medium can greatly enhance decarboxylation reactivity,[8,20] we also determined $k_{decarboxylation}$ of the 2-aryl-2-alkylglycines in 34.5% aqueous acetonitrile. This relatively modest reduction in solvent polarity afforded an average 12-fold increase in $k_{decarboxylation}$. By far, 2-(4-nitropheny)-2-methylglycine (4-Nmg) decarboxylated fastest of all the amino acids studied. In fact, the $k_{decarboxylation}$ for 4-Nmg in 34.5% acetonitrile [$(123.3 \pm 1.0) \times 10^{-3}$ min^{-1}] is nearly identical to the fastest rate of pyruvate transamination observed for our **1**/PEI-C_{12} (8.7%) system [$(3.9 \pm 0.2) \times 10^{-2}$ min^{-1}].[8]

The above decarboxylation studies strongly support the proposal that incorporation of electron-withdrawing groups on the aromatic substituent of the 2-aryl-2-alkylglycine sacrificial amine source should increase the turnover frequency of the DGD mimic catalytic cycle. To test this hypothesis, the conversion of pyruvate (5.0×10^{-3} M) to alanine in the presence of PEI-C_{12} (8.7%) (2.5×10^{-5} M), EDTA (2.0×10^{-3} M), a catalytic amount of **1** (2.5×10^{-5} M), and either Pmg, 4-Nmg, or 4-Mmg (1.0×10^{-2} M) at pH 7–8 and 60 °C was monitored by previously reported HPLC techniques (Table 2).[8] The initial turnover frequency (TOF), the number

Table 2. Catalytic turnover frequency and number with pyridoxamine **1** and 2-aryl-2-alkylglycines



Amino acid	TOF (h^{-1})[a]	$TOF_{relative}$	TON	$TON_{relative}$
Pmg	0.83	1.0	41.2 ± 7.5	1.0
4-Nmg	⩾1.3	⩾1.6	7.2 ± 1.9	0.2
4-Mmg	0.78	0.9	40.3 ± 0.3	1.0

[a] Initial turnover frequency (TOF) = number of turnovers in 2 h/2 h. Reaction conditions: 2.5×10^{-5} M **1**, 2.5×10^{-5} M PEI-C_{12} (8.7%), 5.0×10^{-3} M pyruvic acid, 1.0×10^{-2} M amino acid, 2.0×10^{-3} M EDTA, $T = 60$ °C, pH 7.5.

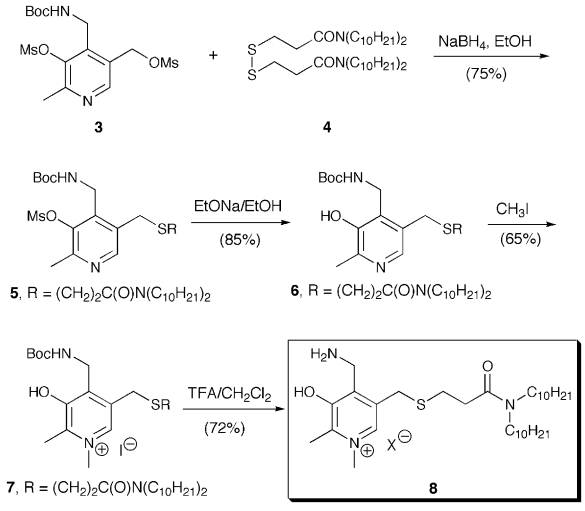
of turnovers after 2 h of reaction divided by 2 h, employing 4-Nmg as a sacrificial amine source, was markedly faster than that observed with Pmg or 4-Mmg, but the overall number of turnovers (TON) was significantly less than that obtained from Pmg (7.2 ± 1.9 vs 41.2 ± 7.5). Furthermore, a large amount of unidentified peaks rapidly accumulated on the HPLC trace, complicating the ability to determine accurately the TOF value. As expected, similar results were observed when enantiopure (*S*)-4-Nmg[21] was employed. The multiple side products and relatively low TON observed with 4-Nmg, despite the very rapid initial turnover of the pyridoxamine catalyst, indicates that, in the presence of the polymer, the highly active 4-nitro-acetophenone produced from the oxidative decarboxylation of 4-Nmg is reacting with the pyridoxal catalytic intermediate, thus killing the catalytic cycle. The opposite phenomenon was observed employing 4-Mmg as the sacrificial amine source; the TOF was slightly less in comparison to Pmg (0.78 vs 0.83 h^{-1}), but the TON was nearly identical and very consistent. Importantly, for all three amino acids tested, the corresponding phenethylamines were not observed throughout the entirety of the reaction, suggesting that oxidative decarboxylation (i.e., C-4′ protonation), and thus regeneration of catalyst **1**, is preferred exclusively over non-oxidative decarboxylation (i.e., Cα protonation) for this system.

It should be noted that the observed TOF and TON values are highly dependent on the particular batch of synthetic PEI-C_{12} (8.7%) utilized, presumably due to the inherent difficulties of producing identical batches of modified polymers from the highly polydisperse unfunctionalized commercial PEI.[22] All of the results reported in this paper were determined using the same batch of PEI-C_{12} (8.7%).

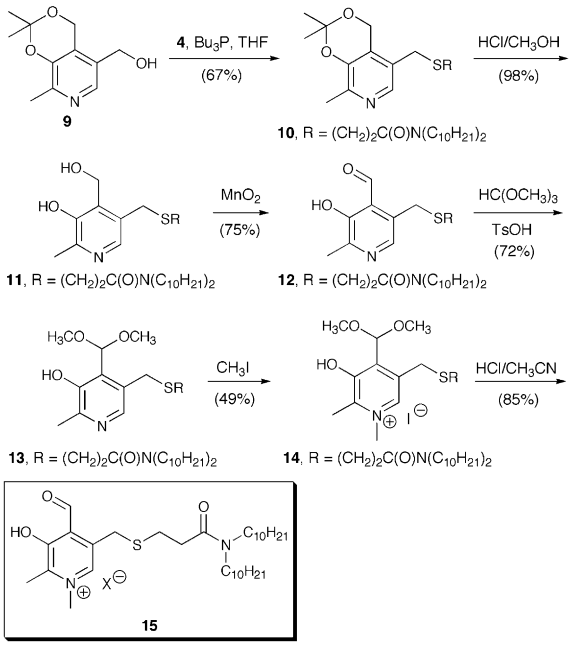
2.3. Synthesis and evaluation of quaternized, hydrophobically modified pyridoxamine and pyridoxal cofactors 8 and 15

In enzymatic systems, the pyridine nitrogen of the PLP cofactor is in close proximity to an appropriately placed acidic amino acid side chain in the cofactor binding pocket, suggesting that the cofactor may exist in or convert to a protonated, that is pyridinium, form. Common arguments dictate that this pyridinium formation may serve as an 'electron-sink' to accelerate decarboxylation. However, such a directed protonation is not feasible under the polymer-buffered reaction conditions of the DGD mimic described above. A similar stable pyridinium species can be generated, though, by alkylation of the pyridine nitrogen. To this end, Murakami and coworkers reported catalytic turnover for a transaminase mimic composed of a quaternized pyridoxamine in a lipid micelle at room temperature, though in the presence of copper salts.[23] Furthermore, 10-fold increases in enzymatic activity have been reported by Gong and coworkers when employing *N*-methyl-PLP as a catalyst for decarboxylases.[24] On the other hand, Bach and coworkers, following high-level enzyme active-site computational modeling, found no computational evidence for an increase in $k_{decarboxylation}$ due to pyridinium formation by protonation or methylation.[25] To further explore empirically the effect of pyridinium formation in our DGD-mimetic system, we synthesized the pyridinium salts **8** and **15**, representing quaternized versions of **1** and the corresponding pyridoxal cofactor, respectively.

Pyridoxamine **8** was synthesized starting from nucleophilic substitution of mesylate **3** by the thiolate generated from disulfide **4** and $NaBH_4$ (Scheme 4). Deprotection of the resulting phenol sulfate ester **5** afforded free phenol **6**, which was then selectively N-methylated with MeI. While phenolic O-methylation may have occurred, we have observed that substituents on the pyridoxamine/pyridoxal phenolic oxygen are highly susceptible toward hydrolysis under standard work-up conditions in the pyridinium species. Similarly, phenolic O-alkylation of the pyridinium salts has proven extremely difficult. Accordingly, Boc-protected pyridinium salt **7** was obtained in good purity by simple evaporation of the solvent and excess iodomethane. Removal of the Boc group with TFA followed by preparative reverse-phase HPLC provided the fully elaborated hydrophobic quaternized pyridoxamine **8**.



Scheme 4. Synthesis of pyridoxamine **8**.



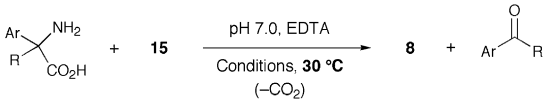
Scheme 5. Synthesis of pyridoxal **15**.

Synthesis of the quaternized pyridoxal **15** proved to be more complicated (Scheme 5). Thioether **10** was obtained from benzyl alcohol **9** and dithiane **4** employing PBu_3 in THF. The acetal protecting group was then hydrolyzed under acidic conditions to afford benzyl alcohol **11**. After we screened a variety of oxidative conditions, MnO_2 proved to be the best selective reagent to convert alcohol **11** to pyridoxal **12**. Attempts to oxidize the benzylic alcohol after pyridinium formation led to an intractable mixture of compounds. Likewise, reacting pyridoxal **12** with MeI to directly form the pyridinium salt **15** afforded only complex mixtures. Therefore, a protection–alkylation–deprotection strategy was employed. Specifically, the aldehyde moiety of **12** was masked as the corresponding dimethylacetal **13**, which was then N-methylated with MeI. Unlike the pyridoxamine derivative **6**, a significant amount of S-methylation accompanied formation of pyridinium **14**, necessitating purification of the protected aldehyde by reverse-phase preparative HPLC. Conversion of the dimethylacetal to the aldehyde with HCl in acetonitrile, followed by another round of HPLC purification provided quaternized hydrophobic pyridoxal **15**. The identity of the counteranions for both **8** and **15** was not rigorously determined. Like their non-ionic counterparts, pyridinium salts **8** and **15** exhibit poor water-solubility. They were therefore dissolved in acetonitrile to afford ca. 5.0×10^{-3} M solutions.

Pyridinium **8** converted pyruvic acid to alanine at a rate ($k_{transamination}$) only slightly faster than the non-quaternized pyridoxamine **1** [$(5.2 \pm 1.4) \times 10^{-2}$ min^{-1} at 20 °C vs $(3.5 \pm 0.2) \times 10^{-2}$ min^{-1}]. Furthermore, competition reactions run with 30:30:1 (pyruvic acid–phenylpyruvic acid–**8**) ratios at 20 °C, pH 7.5, and in the presence of PEI-C_{12} (8.7%) in water revealed alanine:phenylalanine products ratios similar to those observed with **1** [1:(11 ± 2) vs 1:(14 ± 2)].

While the effect of pyridine quaternization on $k_{transamination}$ was slight, pyridoxal **15** exhibited marked increases in $k_{decarboxylation}$ over a non-quaternized version, permitting the decarboxylation reactions to proceed smoothly at 30 °C. This apparently contrasts with the computational predictions of Bach and coworkers,[25] and represents the first time we have observed substantial cofactor-mediated amino acid decarboxylation at a biologically relevant temperature. To insure that reagent **15** was fully solubilized, initial decarboxylation studies were performed in 58% aqueous acetonitrile (Conditions A, Table 3). Heterogeneous reaction mixtures were still observed, however, when Pxg and 4-Tmg were utilized as sacrificial amine sources, possibly accounting for the lower than expected $k_{decarboxylation}$ values observed for these two amino acids. Regardless, $k_{decarboxylation}$

Table 3. Rate of 2-aryl-2-alkylglycine decarboxylation



Amino acid	Conditions	k ($\times 10^{-3}$ min^{-1})	$k_{relative}$	Relative rate to *S*-Et-pyridoxal; at 60 °C
Pmg	A	29.1 ± 1.2	1.2	9.1
Pmg	B	24.4 ± 2.3	1.0	7.6
4-Tmg	A	31.9 ± 3.3	1.3	5.1
4-Tmg	B	83.3 ± 5.4	3.4	13.4
4-Mmg	A	24.1 ± 1.1	1.0	20.1
4-Mmg	B	19.7 ± 2.3	0.8	16.4
Pxg	A	24.0 ± 0.7	1.0	5.2
Pxg	B	67.6 ± 5.0	2.8	14.7
1-Aic	A	42.1 ± 1.2	1.7	16.2
1-Aic	B	30.5 ± 2.7	1.3	11.7

Reaction conditions A: 1.0×10^{-4} M **15**, 1.0×10^{-3} M amino acid, 2.0×10^{-3} M EDTA, 1.3×10^{-2} M Hepes, 1.3×10^{-2} M KCl in 58% aqueous MeCN, $T = 30$ °C, pH 7.0. Reaction conditions B: 1.0×10^{-4} M **15**, 1.0×10^{-3} M amino acid, 1.25×10^{-5} M PEI-C_{12} (8.7%), 2.0×10^{-3} M EDTA, 2.5×10^{-2} M Hepes, 2.5×10^{-2} M KCl, $T = 30$ °C, pH 7.0.

values for the 2-aryl-2-alkylglycines with **15** at 30 °C were an average ninefold faster than those observed with pyridoxal 5′-ethyl thioether at 60 °C. Pyridinium **15** rapidly forms the corresponding hydrate of the aldehyde in aqueous conditions, and thus does not exhibit the characteristic absorption peak at 400 nm in the UV–vis spectrum. Therefore, decarboxylation reactions with **15** were monitored by HPLC following the generation of the ketone corresponding to amino acid oxidative decarboxylation. As before, none of the corresponding phenethylamines were detected throughout the entirety of the reactions.

Even more remarkable results were obtained when the decarboxylation reactions were carried out in the presence of PEI-C_{12} (8.7%) in water (Conditions B, Table 3). Unlike the results in 58% aqueous acetonitrile, all of the reaction mixtures were apparently homogeneous in the presence of the polymer. Furthermore, the observed $k_{\text{decarboxylation}}$ values at 30 °C were an average 11-fold greater than those observed with pyridoxal 5′-ethyl thioether at 60 °C. 4-Tmg, which possesses an electron-withdrawing trifluoromethyl group on the aromatic substituent, decarboxylated fastest under these reaction conditions [$(83.3 \pm 5.4) \times 10^{-3}$ min^{-1}]. However, in the presence of the hydrophobically modified polymer, the hydrophobic amino acid Pxg also decarboxylated at a remarkably fast rate [$(67.6 \pm 5.0) \times 10^{-3}$ min^{-1}], supporting the previous hypothesis that hydrophobic interactions between the polymer catalyst, the sacrificial amine source, and the pyridoxal intermediate would increase the local concentration of the reagents and thus increase the reaction rate.

It is important to note that the $k_{\text{decarboxylation}}$ values observed for 4-Tmg and Pxg are close to, if not greater than the $k_{\text{transamination}}$ value observed for pyruvic acid with quaternized pyridoxamine **8**, implying that decarboxylation may no longer be a rate limiting step, and that faster catalytic turnover should be observed. Indeed, the average initial turnover frequencies observed with pyridinium **8** at 30 °C is faster than with non-quaternized cofactor **1** at 60 °C (Table 4). Quaternization of the pyridoxamine cofactor also appears to accelerate catalyst decomposition, as evidenced by the lower TON values observed for **8** versus **1**. The highest TOF and TON values were observed utilizing the hydrophobic sacrificial amine source Pxg. As with 4-Nmg and catalyst **1**, 4-Tmg displayed the lowest TON value with catalyst **8**, along with the marked production of catalyst decomposition products. These turnover studies demonstrate the particular advantage that hydrophobic effects can have over electronic factors.

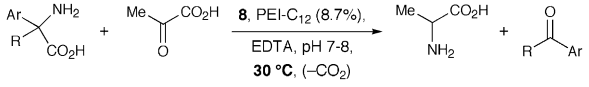
3. Conclusions

An excellent mimic of the enzyme dialkylglycine decarboxylase has been created using a pyridoxal derivative and a modified polyethylenimine. While standard transaminations are used in both directions by transaminase enzymes, our work and that of others indicates that simple transaminations in one direction—converting a pyridoxal and an amino acid to a pyridoxamine and a keto acid—are not efficient in model systems. The decarboxylation process with disubstituted glycines substitutes nicely for this inefficient process, and its conversion of a pyridoxal to a pyridoxamine then couples with our previous studies of highly efficient reactions of a pyridoxamine derivative with an α-keto acid to form the α-amino acid and the pyridoxal derivative.

In our present study, the other product of the decarboxylation process, besides the pyridoxamine, is a ketone. This is the same ketone that is used to synthesize the disubstituted glycine by reaction with NaCN and ammonium carbonate, followed by hydrolysis of the resulting hydantoin. Thus, the ketone can be considered a catalyst that is regenerated, and the overall reductive amination of the pyridoxal is formally accomplished with the consumption of ammonium cyanide and aqueous base; indeed the ammonia is even regenerated in the hydrolysis. The overall result is the catalytic conversion of a keto acid to an amino acid with the consumption of these simple inorganic materials. The pyridoxamine derivative and the polymer are simply catalysts, but for a very effective process. In addition, both parts of the overall cycle mimic well-established biochemical reactions.

We recently reported that covalent linkage of a pyridoxamine to peptide-derived homochiral oligoamines affords an effective reagent for the conversion of various α-keto acids to the corresponding α-amino acids in moderate to good enantiomeric excesses even though the reactions were performed under aqueous conditions.[26] However, chiral oligoamine-cofactor studies have not yet been reported. Since peptide-derived chiral polyamines can be

Table 4. Catalytic turnover frequency and number with pyridoxamine **8** and 2-aryl-2-alkylglycines



Amino acid	TOF (h^{-1})[a]	$TOF_{relative}$	TON	$TON_{relative}$
Pxg	1.14	1.00	14.6 ± 7.9	1.0
4-Tmg	0.97	0.85	9.4 ± 0.1	0.6
4-Mmg	0.81	0.71	12.3 ± 0.2	0.8

[a] Initial turnover frequency (TOF) = number of turnovers in 2 h/2 h. Reaction conditions: 2.5×10^{-5} M **8**, 2.5×10^{-5} M PEI-C_{12} (8.7%), 5.0×10^{-3} M pyruvic acid, 2.0×10^{-3} M EDTA, 1.0×10^{-2} M amino acid, $T = 60$ °C, pH 7.5.

synthesized in a defined and homochiral fashion,[26] they could serve as replacements for PEI-C_{12} (8.7%) in our DGD mimetic system, potentially alleviating the current polymer batch-dependence issues and allowing for the catalytic, biomimetic, and *enantioselective* synthesis of α-amino acids from α-keto acids, 2-aryl-2-alkylglycine sacrificial amines, and a hydrophobically modified pyridoxamine cofactor. These studies are currently underway, and the results will be reported in due course.

4. Experimental

4.1. Synthesis

All reagents were purchased from commercial sources and utilized as received unless otherwise noted. DMF, THF, CH_2Cl_2, and CH_3CN were dried through individual alumina-based solvent purification columns. 2-Amino-2-phenylpropionic acid (Pmg), purchased from Aldrich Co., was purified by preparative RP-HPLC with a Vydac Protein & Peptide C18 column. All reactions were carried out under atmospheric conditions, unless otherwise noted. Flash chromatography was performed on 230–400 mesh silica (Silica Gel 60) from EM Science. NMR spectra were obtained on a Bruker DPX 300 MHz 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 using either glycerol or *p*-nitrobenzyl alcohol as matrices.

4.1.1. Representative procedure for 5-aryl-5-alkylhydantoin synthesis;[15] 5-(4-nitrophenyl)-5-methylhydantoin.[27] A 100 mL flask was charged with 4-nitroacetophenone (0.8 g, 5.0 mmol), $(NH_4)_2CO_3$ (3.6 g, 37.9 mmol), EtOH (7.5 mL), H_2O (7.5 mL), and a 32.6% (w/v) aqueous solution of NaCN (0.8 mL, 6.5 mmol). The resulting mixture was ultrasonicated in a 45 °C water bath for 5 h, then cooled in an ice-bath and brought to neutral pH with 1 N HCl. The resulting white precipitate was obtained by filtration and washed with copious amounts of 1:1 EtOH/H_2O to afford the desired hydantoin as a white powdery solid (1.1 g, 4.6 mmol, 92%) in spectroscopically pure form: ^{1}H NMR (d_6-DMSO, 300 MHz) δ: 10.92 (br s, 1H), 8.77 (s, 1H), 8.25 (d, J = 9.0 Hz, 2H), 7.76 (d, J = 9.0 Hz, 2H), 1.70 (s, 3H).

4.1.2. 5-(4-Trifluoromethylphenyl)-5-methylhydatoin. White solid; mp 165.0–169.0 °C; HRMS (FAB+) m/z 259.0681 [$(M+H)^+$; calcd for $C_{11}H_{10}O_2N_2F_3^+$: 259.0694]; ^{1}H NMR (d_6-DMSO, 300 MHz) δ: 10.89 (s, 1H), 8.74 (s, 1H), 7.76 (d, J = 8.5 Hz, 2H), 7.69 (d, J = 8.4 Hz, 2H), 1.68 (s, 3H); ^{19}F NMR (d_6-DMSO, 282.4 MHz) δ: –60.15 (s, 3F); ^{13}C NMR (d_6-DMSO, 100 MHz) δ: 176.37, 156.19, 144.48, 128.52 (q, J_{C-C-F} = 31.9 Hz), 126.39, 124.16 (q, J_{C-F} = 272.9 Hz), 125.46, 63.90, 25.14.

4.1.3. 5-(4-Methoxyphenyl)-5-methylhydantoin.[15]. White solid; MS (CI+) m/z 221.15 $(M+H)^+$; ^{1}H NMR (d_6-DMSO, 300 MHz) δ: 10.64 (br s, 1H), 8.52 (s, 1H), 7.35 (d, J = 8.9 Hz, 2H), 6.93 (d, J = 8.9 Hz, 2H), 3.74 (s, 3H), 1.62 (s, 3H)

4.1.4. 5-Phenyl-5-hexylhydantoin.[28] White solid; MS (CI+) m/z 261.03 $(M+H)^+$; ^{1}H NMR (d_6-DMSO, 300 MHz) δ: 10.76 (s, 1H), 8.66 (s, 1H), 7.50–7.29 (m, 5H), 2.05–1.84 (m, 2H), 1.22 (br s, 6H), 0.84 (t, J = 6.5 Hz, 3H).

4.1.5. Spiro[imidazolidine-4,1′-indan]-2,5-dione.[29] White solid; MS (CI+) m/z 203.14 $(M+H)^+$; ^{1}H NMR (d_6-DMSO, 300 MHz) δ: 9.39 (br s, 1H), 8.41 (s, 1H), 7.30–7.02 (m, 4H), 3.06–2.96 (m, 2H), 2.56–2.46 (m, 1H), 2.13 (dt, J = 13.2 and 8.3 Hz, 1H).

4.1.6. 2-(4-Nitrophenyl)-2-methylglycine (4-Nmg).[23] 5-(4-Nitrophenyl)-5-methylhydantoin (517 mg, 2.2 mmol) and 6 N HCl (22 mL) were combined in a sealed high-pressure tube and the resulting slurry was stirred at 130 °C for 67 h. After cooling to ambient temperature, the mixture was filter through glass wool, concentrated, and dried azeotropically from $PhCH_3$. The resulting yellow solid was purified by reverse-phase HPLC (80 mg, 0.3 mmol, 15%): ^{1}H NMR (d_4-MeOH, 300 MHz) δ: 8.32 (d, J = 8.9 Hz), 7.75 (d, J = 8.9 Hz), 1.97 (s, 3H).

4.1.7. 2-(4-Trifluoromethylphenyl)-2-methylglycine (4-Tmg). 5-(4-Trifluoromethylphenyl)-5-methylhydantoin (536 mg, 2.1 mmol) and 2 N NaOH (8.2 mL) were combined in a sealed high-pressure tube, and the resulting solution was stirred at 115 °C overnight. After cooling to 0 °C, the resulting solution was acidified with concentrated HCl, and the resulting white precipitate was removed by filtration. The resulting filtrate was passed through a column containing Dowex 50x-8 ion exchange resin, (eluted with 1.7 M aqueous NH_4OH) to afford, after lyophilization, a white solid (46 mg, 0.2 mmol, 9%): ^{1}H NMR (d_6-DMSO, 300 MHz) δ: 7.74 (d, J = 6.7 Hz, 2H), 7.56 (d, J = 6.9 Hz, 2H), 1.50 (s, 3H); ^{19}F NMR (d_6-DMSO, 282.4 MHz) δ: −59.63 (s, 3F).

4.1.8. 2-(4-Methoxyphenyl)-2-methylglycine (4-Mmg).[30] 5-(4-Methoxyphenyl)-5-methylhydantoin (495 mg, 2.3 mmol) was dissolved in 2 N NaOH (6 mL) and stirred at reflux (125 °C) for 24 h. After cooling to ambient temperature and filtration through glass wool, the resulting solution was cooled to 0 °C and brought to neutral pH with 6 N HCl. The resulting precipitate was removed by filtration, and the filtrate was concentrated in vacuo to a white solid. This solid was extracted with MeOH, and the resulting extract was concentrated to a white solid which was recrystallized from EtOH/H_2O (144 mg, 0.7 mmol, 33%): ^{1}H NMR (d_6-DMSO, 300 MHz) δ: 7.84 (br s, 3H), 7.44 (d, J = 6.7 Hz, 2H), 6.87 (d, J = 6.7 Hz, 2H), 3.73 (s, 3H), 1.62 (s, 3H).

4.1.9. 2-Phenyl-2-hexylglycine (Pxg). 5-Phenyl-5-hexylhydantoin (260 mg, 1.0 mmol) was dissolved in 2 N NaOH (4.0 mL) and stirred at reflux (120 °C) for 24 h, then cooled to 0 °C, and acidified with 6 N HCl. The resulting precipitate was removed by filtration and the filtrate was passed through a column containing Dowex 50x-8 ion exchange resin (eluted with 1.7 M aqueous NH_4OH) to afford, after lyophilization, a white solid (191 mg, 0.8 mmol, 81%): ^{1}H NMR (d_6-DMSO, 300 MHz) δ: 7.47 (d, J = 7.6 Hz, 2H), 7.30–7.17 (m,

3H), 5.56 (br s, 3H), 2.06–1.93 (m, 2H), 1.21 (br s, 6H), 0.84 (t, $J = 6.6$ Hz, 3H).

4.1.10. 1-Aminoindane-1-carboxylic acid (1-Aic).[31] Spiro[imidazolidine-4,1′-indan]-2,5-dione (407 mg, 2.0 mmol), $Ba(OH)_2{\cdot}8H_2O$ (1.0 g, 3.2 mmol), and H_2O (8.0 mL) were combined in a sealed high-pressure tube, and the solution was stirred at 150 °C overnight, then cooled to ambient temperature and filtered. To the resulting filtrate was added $(NH_4)_2CO_3$ (514 mg, 2.9 mmol), and the resulting precipitate was removed by centrifugation following by decanting the supernatant, which was evaporated to a white solid. This solid was dissolved in 0.3 N HCl and passed through a column containing Dowex 50x-8 ion exchange resin (eluted with 1.7 M aqueous NH_4OH), to afford, after lyophilization, a white solid (296 mg, 1.7 mmol, 84%): ^{1}H NMR (d_6-DMSO, 300 MHz) δ: 7.92 (br s, 3H), 7.36 (d, $J = 7.3$ Hz, 1H), 7.23–7.13 (m, 3H), 3.07–2.89 (m, 2H), 2.68–2.59 (m, 1H), 2.04–1.95 (m, 1H).

4.1.11. 3-[2-(Bisdecylcarbamoyl)ethyldisulfanyl]-*N*,*N*-bisdecylpropionamide (4). To a solution of 3-(2-carboxy-ethyldisulfanyl)propionic acid (2.1 g, 10.0 mmol) in benzene (20 mL) was added $SOCl_2$ (10.0 mL, 137.1 mmol). The reaction was stirred at reflux for 2 h, after which the solution became clear. The solvent then was removed by distillation and the resulting acid chloride was dissolved in CH_2Cl_2 (20 mL). This CH_2Cl_2 solution was added dropwise to a flask containing $NH(C_{10}H_{21})_2$ (6.0 g, 20.1 mmol), and Et_3N (3.0 mL, 21.5 mmol) in CH_2Cl_2 (20 mL), at 0 °C, and the resulting reaction mixture was stirred overnight. The reaction mixture was washed sequentially with 1 N HCl and 1 N NaOH, then concentrated in vacuo to afford the title compound. The resulting product was carried on without further purification: MS (CI+) *m*/*z* 770 $(M+H)^+$; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.34 (m, 8H) 3.05 (t, $J = 7.1$ Hz, 4H), 2.81 (t, 4H), 1.65 (m, 8H), 1.40 (m, 56H) 0.98 (t, 12H).

4.1.12. Methanesulfonic acid 5-[2-(bisdecyl-carbamoyl)ethylsulfanylmethyl]-4-(*tert*-butoxycarbonyl-aminomethyl)-2-methylpyridin-3-yl ester (5). To a solution of 3-hydroxy-5-hydroxymethyl-2-methylpyridin-4-ylmethyl) carbamic acid *tert*-butyl ester[8] (1.5 g, 3.5 mmol) in CH_2Cl_2 (20 mL) at 0 °C, was added Et_3N (1.6 mL, 11.5 mmol) followed by MsCl (0.87 mL, 11.2 mmol). The reaction mixture was stirred for 15 min at 0 °C, and then washed with saturated aqueous NH_4Cl. Evaporation of the resulting organic layer afforded a solid product. This product (i.e., **3**) was then dissolved in EtOH (10 mL). Meanwhile, in a separate flask containing **4** (2.1 g, 2.7 mmol) in EtOH (20 mL), $NaBH_4$ (1.0 g, 26.4 mmol) was added. After the mixture was stirred for 20 min, the previous ethanolic solution of **3** was added, and the mixture was stirred for 2 h. The solvent was removed by rotary evaporation and the resulting product was purified by flash chromatography to afford a waxy solid (1.6 g, 2.6 mmol, 75%): R_f (20:1 CH_2Cl_2:MeOH) = 0.31; MS (CI+) *m*/*z* 712.8 $(M+H)^+$; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.49 (s, 1H), 5.72 (br s, 1H), 4.71 (d, $J = 6.2$ Hz, 2H), 4.03 (s, 2H), 3.60 (s, 3H), 3.30 (m, 4H), 2.95–2.76 (m, 7H), 1.85–1.32 (m, 41H), 1.00 (t, $J = 6.5$ Hz, 6H).

4.1.13. {5-[2-(Bisdecylcarbamoyl)ethylsulfanylmethyl]-3-hydroxy-2-methylpyridin-4-ylmethyl} carbamicacid *tert*-butyl ester (6). To a solution of **5** (1.0 g, 1.7 mmol) in EtOH (10 mL) was added a 21% solution of EtONa in EtOH (10.0 mL), and the reaction mixture was stirred for 12 h at ambient temperature. The solvent was then removed by rotary evaporation, and the mixture was neutralized with saturated aqueous NH_4Cl. The product was extracted from the resulting aqueous solution with $CHCl_3$ (150 mL), and the resulting organic extract was concentrated in vacuo to afford a waxy solid which was purified by flash chromatography (740 mg, 1.4 mmol, 85%): R_f (20:1 CH_2Cl_2:MeOH) = 0.30; MS (CI+) *m*/*z* 634.8 $(M+H)^+$; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 11.49 (s, 1H), 8.25 (s, 1H), 4.69 (d, $J = 6.3$ Hz, 2H), 4.09 (s, 2H), 3.34 (m, 4H), 2.95 (m, 7H), 1.89–1.39 (m, 41H), 1.02 (t, $J = 6.4$ Hz, 6H).

4.1.14. 5-[2-(Bisdecylcarbamoyl)ethylsulfanylmethyl]-4-(*tert*-butoxycarbonylaminomethyl)-3-hydroxy-1,2-di-methylpyridinium iodide (7). To a solution of **6** (0.3 g, 0.6 mmol) in CH_3CN (10 mL) CH_3I (2.0 mL, 32.1 mmol) was added. The reaction was heated to reflux (80 °C) and stirred for 30 min. The solvent was removed by rotary evaporation affording an yellow waxy solid (247 mg, 0.4 mmol, 65%). The resulting product was obtained with sufficient purity for further reaction: MS (CI+) *m*/*z* 650.5 $(M-I)^+$; ^{1}H NMR (CD_3OD, 300 MHz) δ: 8.53 (s, 1H), 4.61 (s, 2H), 4.37 (s, 3H), 4.11 (s, 2H), 3.00–2.80 (m, 7H), 1.90–1.41 (m, 41H), 1.03 (t, $J = 6.5$ Hz, 6H).

4.1.15. 4-Aminomethyl-5-[2-(bisdecylcarbamoyl)ethylsulfanylmethyl]-3-hydroxy-1,2-dimethylpyridinium halide (8). To a solution of **7** (0.2 g, 0.4 mmol) in CH_2Cl_2 (10 mL), TFA (5 mL) was added, and the resulting mixture was stirred at ambient temperature overnight. The solvent was removed by rotary evaporation and the resulting product was purified using preparative reverse-phase HPLC [50% to 100% aqueous MeOH (0.1% TFA buffer) over 50 min, rt = 26–45 min] to afford a yellow waxy solid (164 mg): MS (CI+) *m*/*z* 550.6 $(M-X)^+$; ^{1}H NMR (CD_3OD, 400 MHz) δ: 8.45 (s, 1H), 4.43 (s, 2H), 4.31 (s, 3H), 4.02 (s, 2H), 2.72 (m, 7H), 1.65 (m, 4H), 1.39 (br s, 28H), 0.98 (t, $J = 6.6$ Hz, 6H).

4.1.16. *N*,*N*-Bisdecyl-3-(2,2,8-trimethyl-4*H*-[1,3]-dioxino[4,5-*c*]pyridin-5-ylmethylsulfanyl)propionamide (10). To a solution of (2,2,8-Trimethyl-4*H*-[1,3]dioxino-[4,5-*c*]pyridin-5-yl)methanol[8] (**9**, 1.1 g, 5.3 mmol) and **4** (1.9 g, 2.5 mmol) in THF (20 mL), Pbu_3 (2.5 g, 10.0 mmol) was added, and the mixture was stirred under argon at ambient temperature for 10 h. After evaporation of the solvent, the resulting product was purified using flash chromatography to afford a waxy solid (2.0 g, 3.4 mmol, 67%): MS (CI+) *m*/*z* 579 $(M+H)^+$; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.96 (s, 1H), 5.03 (s, 2H), 3.71 (s, 2H), 3.35 (m, 4H), 2.89–2.51 (m, 7H), 1.79 (s, 6H), 1.75–1.23 (m, 32H), 1.01 (t, $J = 6.6$ Hz, 6H).

4.1.17. *N,N*-Bisdecyl-3-(5-hydroxy-4-hydroxymethyl-6-methylpyridin-3-ylmethylsulfanyl)propionamide (11). To a solution of **10** (1.5 g, 2.6 mmol) in MeOH (20 mL) was added concentrated aqueous HCl (20 mL), and the resulting mixture was stirred overnight. The solvent was evaporated, and the resulting residue was dissolved in CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$. The organic layer was dried (Na_2SO_4), filtered, and concentrated to afford a spectroscopically pure waxy solid (1.4 g, 2.5 mmol, 98%): MS (CI+) *m/z* 538 $(M+H)^+$; ^1H NMR ($CDCl_3$, 300 MHz) δ: 7.99 (s, 1H), 5.16 (s, 2H), 3.87 (s, 2H), 3.40 (m, 4H), 2.90–2.59 (m, 7H), 1.95–1.01 (m, 38H).

4.1.18. *N,N*-Bisdecyl-3-(4-formyl-5-hydroxy-6-methylpyridin-3-ylmethylsulfanyl) propionamide (12). To a solution of **10** (1.5 g, 2.5 mmol) in CH_2Cl_2 (50 mL), MnO_2 (5.0 g, 57.5 mmol) was added, and the reaction was stirred at ambient temperature for 3 h. The solution was then filtered through Celite and concentrated to a spectroscopically pure yellow waxy solid. (1.0 g, 1.8 mmol, 70%). MS (CI+) *m/z* 536 $(M+H)^+$; ^1H NMR (CD_3CN, 300 MHz) δ: 10.56 (s, 1H), 8.09 (s, 1H), 4.13 (s, 2H), 3.30 (m, 4H), 2.82 (t, J = 6.9 Hz, 2H), 2.61 (t, 2H), 2.56 (s, 3H), 1.70–1.25 (m, 32H), 1.01 (t, J = 6.9 Hz, 6H).

4.1.19. *N,N*-Bisdecyl-3-(4-dimethoxymethyl-5-hydroxy-6-methylpyridin-3-ylmethylsulfanyl)propionamide (13). To a solution of **12** (2.0 g, 3.7 mmol) in $HC(OCH_3)_3$ (20.0 mL) and CH_3OH (10 mL), TsOH (1.0 g, 5.8 mmol) was added, and the reaction was heated to 110 °C and stirred at that temperature for 3 h. After evaporation of the solvent, the product was purified using flash chromatography to obtain a waxy solid (1.6 g, 2.7 mmol, 72%): R_f (20:1 CH_2Cl_2:MeOH) = 0.09; MS (CI+) *m/z* 580.3 $(M+H)^+$; ^1H NMR ($CDCl_3$, 300 MHz) δ: 8.92 (s, 1H), 7.95 (s, 1H), 6.18 (s, 1H), 3.88 (s, 2H), 3.56 (s, 6H), 3.41–3.26 (m, 4H), 2.95 (t, J = 7.2 Hz, 2H), 2.62 (t, J = 7.2 Hz, 2H), 2.53 (s, 3H), 1.73 (br s, 4H), 1.40 (br s, 28H), 0.99 (t, J = 6.1 Hz, 6H).

4.1.20. 5-[2-(Bisdecylcarbamoyl)ethylsulfanylmethyl]-4-dimethoxymethyl-3-hydroxy-1,2-dimethylpyridinium iodide (14). To a solution of **13** (0.5 g, 0.9 mmol) in CH_3CN (20 mL), CH_3I (2.0 mL, 32.1 mmol) was added, and the reaction was stirred at ambient temperature for 5 h, monitoring by TLC [MeOH, R_f (starting material) = 0.85, R_f (product) = 0.75]. After evaporation of the solvent, the resulting product was purified using preparative reverse-phase HPLC [50% to 100% aqueous MeOH (0.1% TFA buffer) over 40 min, then 100% MeOH (0.1% TFA buffer) for 20 min, rt = 41–49 min] to afford a yellow waxy solid: MS (CI+) *m/z* 594.4 $(M-I)^+$; ^1H NMR (CD_3OD, 300 MHz) δ: 7.82 (s, 1H), 6.08 (s, 1H), 4.19 (s, 3H), 4.11 (s, 2H), 3.40 (m, 4H), 2.80 (m, 4H), 2.67 (s, 3H), 1.70–1.35 (m, 32H), 1.01 (t, J = 6.8 Hz, 6H).

4.1.21. 5-[2-(Bisdecylcarbamoyl)ethylsulfanylmethyl]-4-formyl-3- hydroxy-1,2-dimethylpyridinium halide (15). To a solution of **14** (0.2 g, 0.3 mmol) in CH_3CN (10 mL) was added 1 N aqueous HCl (10 mL), and the reaction was stirred at ambient temperature for 12 h. After evaporation of the solvent, the resulting product was purified using preparative reverse-phase HPLC [50 to 100% aqueous CH_3CN (0.1% TFA buffer) over 45 min, rt = 24–32 min] to afford a deep red waxy solid (145 mg): R_f (CH_3OH) = 0.76; MS (CI+) *m/z* 548.0 $(M-X)^+$; ^1H NMR (CD_3CN, 400 MHz) δ: 10.58 (s, 1H), 8.39 (s, 1H), 4.22 (s, 3H), 4.18 (s, 2H), 3.30 (m, 4H), 2.75–2.62 (m, 7H), 1.50 (m, 4H), 1.43 (br s, 28H), 0.96 (t, J = 6.8 Hz, 6H).

4.2. Decarboxylation and Catalyst Turnover Studies

The concentrations of PEI-C_{12} (8.7%), pyridoxal 5′-ethylthioether, **8**, and **15** solutions were determined by ^1H NMR analysis using terephthalic acid disodium salt as an internal standard. UV–vis spectra and kinetics were taken on a Varian Cary IE UV–vis spectrometer with accompanying kinetics-calculating software. Analytical HPLC was run on an HP1090 liquid chromatography (series II) equipped with a DR5 pumping system, a temperature-controlled autosampler, and a diode-array UV–vis detector. Vydac Protein & Peptide C18 reverse phase analytical columns (4.6 × 150 mm) were used exclusively. Derivatization Reagent: A solution of *N*-Boc-L-cysteine (219 mg, 1.0 mmol) and *o*-phthalyldialdehyde (140 mg, 1.0 mol) in MeOH (5 mL). Derivatization Buffer: 1.0 M KH_2PO_4, pH 8.0, buffer solution.

4.2.1. General procedure for determining $k_{decarboxylation}$ of 2-aryl-2-alkylglycines with pyridoxal 5′-ethylthioether. Three UV cuvettes charged with pyridoxal 5′-ethylthioether (1.0×10^{-4} M), amino acid (1.0×10^{-3} M), EDTA (2.0×10^{-3} M), pH 7.0, Hepes buffer (2.5×10^{-2} M), and KCl (2.5×10^{-2} M) in either pure deionized H_2O or 34.5% aqueous acetonitrile were heated to 60 °C and monitored by UV–vis spectroscopy at 400 nm every 5 s for 4–24 h, depending on the reaction rate. The resulting exponential curves were converted into pseudo-first-order rate constants using the spectrometer software and the average value ± standard deviation was reported as $k_{decarboxylation}$ in Table 1.

4.2.2. General procedure for determining $k_{decarboxylation}$ of 2-aryl-2-alkylglycines with 15.

4.2.2.1. Conditions A. A capped HPLC autosampler vial was charged with **15** (1.0×10^{-4} M), amino acid (1.0×10^{-3} M), EDTA (2.0×10^{-3} M), pH 7.0, Hepes buffer (2.5×10^{-2} M), and KCl (2.5×10^{-2} M) in 58% aqueous acetonitrile. The reaction mixture was heated to 30 °C and resulting ketone production (see below) was monitored by analytical HPLC/UV–vis (250 nm). Pseudo-first-order rate constants were calculated from the resulting exponential area/time curves. A minimum of three runs were performed and the average value ± standard deviation are reported as $k_{decarboxylation}$ in Table 3.

4.2.2.2. Conditions B. A capped HPLC autosampler vial was charged with **15** (1.0×10^{-4} M), amino acid (1.0×10^{-3} M), EDTA (2.0×10^{-3} M), PEI-C_{12} (8.7%) (1.25×10^{-5}), pH 7.0, Hepes buffer (2.5×10^{-2} M), and KCl (2.5×10^{-2} M). The reaction mixture was heated to 30 °C. Analysis and $k_{decarboxylation}$ determination were performed as per Conditions A.

4.2.2.3. Sample intervals, ketone elution gradient conditions, and retention times. Acetophenone (from Pmg): 10 min intervals, 45 to 55% aqueous MeOH (0.1% TFA buffer) over 8 min at 1.0 mL/min, rt = 5.92 min; 4-trifluoro-methylacetophenone (from 4-Tmg): 15 min intervals, 50 to 65% aqueous MeOH (0.1% TFA buffer) over 12 min at 1.0 mL/min, rt = 9.38 min; 4-methoxyacetophenone (from 4-Mmg): 10 min intervals, 50% to 58.8% aqueous MeOH (0.1% TFA buffer) over 7 min at 1.0 mL/min, rt = 5.57 min; heptanophenone (from Pxg): 10 min intervals, 75.8 to 84.5% aqueous MeOH (0.1% TFA buffer) over 7 min at 1.0 mL/min, rt = 6.44 min; 1-indanone (from 1-Aic): 10 min intervals, 45% to 55% aqueous MeOH (0.1% TFA buffer) over 8 min at 1.0 mL/min, rt = 5.88 min.

4.2.3. General procedure for determining TOF and TON for 2-aryl-2-alkylglycines with PEI-C_{12} (8.7%) and 1 or 8. A tightly capped vial was charged with **1** or **8** (2.5×10^{-5} M), sodium pyruvate (5.0×10^{-3} M), amino acid (1.0×10^{-2} M), EDTA (2.0×10^{-3} M), and PEI-C_{12} (8.7%) (2.5×10^{-5} M). The reaction mixture was brought to pH 7.5 with 1 N HCl or 1 N NaOH and heated to 60 °C for **1** or 30 °C for **8**. Two separate controls reactions, one in which the pyridoxamine catalyst **1** or **8** was excluded (no turnover, control #1) and another in which the amino acid was excluded (one turnover, control #2) were run simultaneously. At regular intervals, 10 μL aliquots from the reaction mixture and the two controls were removed and individually combined with 80 μL Derivatization Reagent and 10 μL Derivatization Buffer. The resulting three solutions of 1-thioisoindoles were analyzed by HPLC with UV–vis detection at 344.5 nm. Retention time: DL-alanine = 17–18 min. Turnover was calculated by dividing the area under the DL-alanine peak for the reaction mixture by that of control #2. A minimum of three runs were performed and the average TOF and TON values ± standard deviation are reported in Tables 2 and 4.

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