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5'-O-Alkylpyridoxamines: Lipophilic Analogs of Pyridoxamine are Potent Scavengers of 1,2-Dicarbonyls

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Abstract

Pyridoxamine (PM) is a prospective drug for treatment of diabetic complications. In order to make zwitterionic PM more lipophilic and improve its tissue distribution, PM derivatives containing medium length alkyl groups on the hydroxymethyl side-chain were prepared. The synthesis of these alkylpyridoxamines (alkyl-PMs) starting from pyridoxine offers high yields and is amenable to bulk preparations. Interestingly, alkyl-PMs were found to react with methylglyoxal (MGO), a major toxic product of glucose metabolism and autoxidation, several orders of magnitude faster than PM. This suggests the formation of non-ionic pyrido-1,3-oxazine as the key step in the reaction of PM with MGO. Since the primary target of MGO in proteins is the guanidine side-chain of arginine, alkyl-PMs were shown to be more effective than PM in reducing modification of *N*- α -benzoylarginine by MGO. Alkyl-PMs in the presence of MGO also protected the enzymatic activity of lysozyme that contains several arginine residues next to its active site. Alkyl-PMs can be expected to trap MGO and other toxic 1,2-carbonyl compounds more effectively than PM, especially in lipophilic tissue environments, thus protecting macromolecules from functional damage. This suggests potential therapeutic uses for alkyl-PMs in diabetes and other diseases characterized by the elevated levels of toxic dicarbonyl compounds.

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Supporting Information

Identification of the major product of SA and MGO, chromatograms of reaction between bzArg and MGO and oxidation products of glucose and a scheme showing proposed reaction pathways between PM and MGO. The material is available free of charge via the Internet at <http://pubs.acs.org>.

Notes

The authors declare no competing financial interest.

Graphical abstract

INTRODUCTION

One of the ways high level of glucose in the body causes damage to vascular, renal, and ocular tissues is through metabolic and oxidative generation of reactive carbonyl compounds.¹ The latter mainly consist of 1,2-dicarbonyls - glyoxal (GO), methylglyoxal (MGO), 3-deoxyglucosone (3-DG) and several others.² MGO, that can also be formed during cooking and that is an environmental pollutant, has attracted most attention. It can chemically modify both proteins³ and DNA.⁴ Although MGO may react readily with cysteinyl thiols resulting in *S*-(1-hydroxy-2-oxoprop-1-yl) modification and more slowly with ϵ -amino groups of lysines to form Schiff bases rearranging to *N*- ϵ -carboxyethyllysine derivatives, modification of protein arginine residues is the most damaging. The reaction of bifunctional MGO with guanidine side chain of arginine residues is selective and rapid, and the resulting adductions are quite stable.⁵ These modifications have been shown to cause loss of protein functionality, such as, enzyme activity or receptor-ligand interactions.^{6–9} The amino groups of adenine and guanine in DNA are targets for MGO resulting in *N*-carboxyethyl adducts.⁴ MGO can also form lysine-lysine and lysine-arginine protein cross-links¹⁰ and lysine-guanosine cross-link between proteins and DNA.¹¹

Pyridoxamine (PM, **1**), a member of vitamin B6 family, is a safe drug showing promise in treatment of diabetic nephropathy.^{12, 13} Proposed mechanisms of PM action include sequestration of redox-active metal ions and scavenging of hydroxyl radical and reactive carbonyl species^{14, 15} One limitation of PM is that it exists mostly in zwitterionic form (**2**) in aqueous solutions near physiological pH (Figure 1). This prevents PM from partitioning into hydrophobic environments,¹⁶ thereby hindering its access to lipophilic biomolecules and hydrophobic regions of proteins. To circumvent this potential shortcoming, we prepared 5'-*O*-alkyl derivatives of PM (alkyl-PMs, **6**) that possess improved lipophilicity, while retaining the chemical reactivity of PM. When we compared the reactivity of PM and alkyl-PMs with MGO, we found that by making PM more lipophilic we also dramatically increased its ability to scavenge MGO. This unexpected but highly desirable property of alkyl-PMs was manifested in the improved protection of free arginine from MGO modification and of lysozyme activity from inhibition by MGO. We propose a pathway for the reaction between

PM and 1,2-dicarbonyls and a simple and scalable method for the synthesis of 5'-*O*-alkylpyridoxamines.

EXPERIMENTAL PROCEDURES

Synthesis of 5'-*O*-Pentylpyridoxamine

Powdered KOH (6.5 g) was stirred with DMSO (30 mL) cooled in a water bath (< 20 °C). After 5 min, 3,4'-*O*-isopropylidenepyridoxine¹⁷ (**3**, 5 g, 24 mmol) was added. 1-Iodopentane was added dropwise (6 g immediately and 2 g after 15 min, 40 mmol total). After stirring for additional 15 min, the reaction mixture was poured into 50 mL each of saturated ammonium chloride, ice, and ethyl acetate. The layers were separated and the aqueous phase was extracted with ethyl acetate (3 × 15 mL). The extracts were combined, washed with brine (25 mL), dried and evaporated. The crude product from three batches was purified by column chromatography (silica, 2:1 hexane-ethyl acetate) to a thick liquid, 18 g (90%). Isopropylidene group was removed quantitatively in 1:1 formic acid-water (70 mL) at 50–55 °C (4 h) to give 5'-*O*-pentylpyridoxine (**4a**) as white solid.

5'-*O*-Pentylpyridoxine (4.8 g, 20 mmol) was dissolved in an aqueous solution (60 mL) consisting of 0.33 M HCl and 0.33 M H₂SO₄. MnO₂ (2.2 g, 24 mmol) was added and the suspension was heated at 40–42 °C for 40 min. The off-white solid was filtered through a bed of Celite on a fritted funnel, and rinsed with 1 M HCl (10 mL) and ethyl acetate (50 mL). The aqueous layer of the filtrate was extracted with CH₂Cl₂ (5 × 10 mL). The combined extracts from two batches were dried and concentrated to ~10 mL. The residue was stirred in ethanol (75 mL) with NH₂OH.HCl (3.3 g, 48 mmol) and CH₃CO₂Na (4.6 g, 56 mmol) for 30 min. Ethanol was removed and the residue was mixed with water (40 mL) and ethyl acetate (80 mL). The aqueous phase was extracted with CH₂Cl₂ (2 × 15 mL). The extracts were dried and evaporated to obtain the oxime (**5a**) as white solid; 7.2 g (70%).

The oxime (6.5 g, 25 mmol) was dissolved in acetic acid (70 mL) and stirred with zinc powder (7.5 g) at 13–18 °C for 90 min and at ambient temperature for 45 min. The reaction mixture was filtered through Celite and the filtrate was evaporated and co-evaporated with toluene (2 × 15 mL) and ethanol (15 mL). The residue was purified by flash chromatography (10–30 % methanol in ethyl acetate). The free amine was stirred with acetic acid (15 mL) for 10 min and evaporated. After removal of excess acid by co-evaporation with toluene (2 × 10 mL), the residue was mixed with ethyl acetate and filtered to obtain a white fluffy solid (**6a**); mp 188–190 °C; 5 g (65%). It was chromatographically identical to the hydrochloride salt prepared previously.¹⁸

5'-*O*-Hexylpyridoxamine (**6b**) [MS *m/z* 253 (*M* + 1); ¹H NMR (600 MHz, DMSO) δ 0.8 (3H, t, CH₂CH₃), 1.2–1.3 (6H, m), 1.4 (2H, m, OCH₂CH₂), 1.85 (3H, s, CH₃CO₂⁻), 2.2 (3H, s, 2-CH₃), 3.3 (2H, t, OCH₂CH₂), 4.0 (2H, 5-CH₂), 4.3 (2H, 4-CH₂), 7.8 (1H, 6-H)] and 5'-*O*-heptylpyridoxamine (**6c**) [MS *m/z* 267 (*M* + 1); ¹H NMR (600 MHz, DMSO) δ 0.8 (3H, t, CH₂CH₃), 1.2–1.3 (8H, m), 1.4 (2H, m, OCH₂CH₂), 1.85 (3H, s, CH₃CO₂⁻), 2.2 (3H, s, 2-CH₃), 3.3 (2H, t, OCH₂CH₂), 4.0 (2H, 5-CH₂), 4.3 (2H, 4-CH₂), 7.8 (1H, 6-H)] were similarly prepared.

2-(1-(3,4-dihydro-2*H*-benzo-1,3-oxazin-2-yl)ethylidene)-3,4-dihydro-2*H*-benzo-1,3-oxazine (19)

A reaction mixture containing salicylamine (SA, **17**, 185 mg, 1 mmol of acetic acid salt) and K₂HPO₄ (1.34 g, 10 mmol) were dissolved in water (~95 mL), and pH adjusted to 7.4. MGO (0.3 g of 40% solution, 15 mmol) was added and the mixture was stirred at 37 °C for 16 h. It was washed with ethyl acetate (3 × 10 mL) and evaporated. The residue was dissolved in methanol (4 mL) and chromatographed twice (10–40% methanol in ethyl acetate); 35 mg. MS *m/z* 295 (*M* + 1); NMR data are given in the Supporting Information (Table S1).

Lipophilicity

A 2 mM solution of PM or PM analog in 0.1 M phosphate, pH 7.4 (2 mL) was vigorously mixed with an equal volume of ethyl acetate for 1 min. The phases were separated and the concentration of PM or PM analog in the aqueous phase was determined spectrophotometrically.

Rates of reaction with MGO

The reaction mixture containing 5 mM PM, PM analog or SA (**17**), 5 mM MGO or GO, and 1.2 mM pentylpyridoxine (internal standard) in 0.1 M phosphate buffer (pH 7.4) was mixed at 37 °C. At various time points including at the start of the reaction, an aliquot of 10 µL was diluted to 200 µL of solvent system A and analyzed by Waters liquid chromatograph 2695 with photo-diode detector 996. The column (Phenomenex Synergi, 150 × 2 mm, 4 µm) was held at 30 °C. Solvent system A (20% methanol in 5 mM formic acid) was held for 1 min, changed linearly to solvent system B (100% methanol with 5 mM formic acid) in 7 min and held at system B for 10 min. From Max-plot, peak areas of the reactant and the internal standard were obtained and the ratios were used to calculate the concentrations of PM or PM analog and subsequently the rate constants.

Formation of pyrido-1,3-oxazine with dimethoxyacetaldehyde

Solutions of PM or PM analog (0.1 mM) and dimethoxyacetaldehyde (0, 5, 10, 15 and 20 mM) in 0.1 M phosphate buffer (pH 7.4) were mixed and let stand for more than 30 min to reach equilibrium. From the spectral scans (380 to 230 nm), absorbances at 323 and 280 nm were obtained for calculating the relative concentrations of the dipolar ionic species and pyrido-1,3-oxazine.

When pentyl-PM (or hexyl-PM) was mixed with equimolar dimethoxyacetaldehyde in ethyl acetate, a new spot was detected immediately by TLC (silica, 10% methanol-ethyl acetate, *R_F* 0.6–0.7); MS *m/z* 347 (*M* + Na⁺), 325 (*M* + H⁺), 293 (*M* – CH₃O). It was ascribed to 2-dimethoxymethyl-5-pentylloxymethyl-8-methyl-3,4-dihydro-2*H*-pyrido[4,3-*e*]oxazine.

Competing Experiments

N-α-benzoylarginine, (bzArg, 5 mM) and MGO or GO (5 mM) were mixed at 37 °C in phosphate buffer (0.1 M, pH 7.4) for 2 h, either by themselves or with added PM or PM analog (5 mM). Aliquots were diluted 20 times and analyzed by LC. The solvent system A was 10% methanol-water with 5 mM formic acid. Column (held at 40 °C) and solvent

system B were the same as above. Solvent system A was maintained at 100% for 12 min when bzArg and its products were eluted. The column was flushed with solvent system B before the next run.

Glucose (100 mM) and bzArg (2 mM) were incubated at 37 °C in the dark with 2 mM PM, pentyl-PM, hexyl-PM or none for 40 d. The samples were diluted 5 times and analyzed by LC using the above conditions. In the absence of scavengers, extra peaks besides the peak for bzArg were attributed to its reaction with GO and MGO that were shown to be formed under these conditions.⁹ Earlier peaks were assigned to isomers of dihydroxyimidazolidine adduct of bzArg.¹⁹ The peak eluting immediately after bzArg was assigned to tetrahydropyrimidine derivative identified as the stable adduct when arginine is exposed to MGO over several days.²⁰

Modification of Lysozyme by MGO

Lysozyme from chicken egg white was purchased from Sigma. All incubations were carried out in 0.15 M sodium phosphate buffer, pH 7.5, containing 0.02% sodium azide to prevent bacterial growth. Solutions were incubated in the dark at 37 °C for 20 h. Lysozyme (0.5 mg/mL) was incubated either with or without 0.5 mM MGO in the presence or absence of various amounts of PM, SA, pentyl-PM or hexyl-PM.

The enzymatic activity of lysozyme was determined by measuring the rate of lysis of *Micrococcus lysodeikticus* cells according to Shugar.²¹ Samples containing lysozyme were diluted to 17 µg/mL in distilled water and kept on ice. An aliquot of 180 µL of diluted lysozyme was mixed with a 900 µL aliquot of a fresh suspension of *Micrococcus lysodeikticus* cells (0.3 mg/mL) in 0.1 M potassium phosphate buffer, pH 7.0. The rate of lysis was determined by measuring the change in absorbance at 450 nm every 15 seconds for two minutes (A_{450}/min).

RESULTS AND DISCUSSION

Synthesis of Alkylpyridoxamines

Hydroxymethyl side-chain of PM is the ideal site for introducing an alkyl group without disturbing the aminomethyl and phenolic groups that were found critical for reacting with dicarbonyls.²² We chose medium chain lengths over methyl or ethyl for their ability to overcome the ionic nature of PM and over longer chains for the ready availability of 1-iodoalkanes ($\text{CH}_3(\text{CH}_2)_n\text{I}$, $n = 4, 5, 6$) needed for substitution. Starting with 3,4'-*O*-isopropylidenepyridoxine (**3**) the new synthesis (Scheme 1) almost doubled the yield of pentyl-PM (**6a**) compared to the previous method¹⁸ while reducing the total synthesis time by half. Hexyl-PM (**6b**) and heptyl-PM (**6c**) were prepared with similar results.

Lipophilicity and reactivity with MGO

As expected the alkyl-PMs were more lipophilic than the parent compound as measured by the relative solubilities in ethyl acetate and buffered (pH 7.4) aqueous phases (Table 1). With the introduction of alkyl chain on the alcoholic group, the non-ionic **1** is likely to be favored over **2**. Although in aqueous solution alkyl-PMs exhibit UV spectra characteristic of the

zwitterionic structure, it is reasonable to expect that they are readily converted to the neutral form that moves into ethyl acetate phase. As the chain lengthens the conversion to the neutral species is so much favored that only about 1% of heptyl-PM remains in the aqueous solution.

As MGO and other 1,2-dicarbonyls are targets for PM, the rate of reaction with MGO was measured and the second-order rate constants were calculated (Table 1). Unexpectedly, the rate of reaction with MGO increased dramatically with alkylation of the hydroxyl and the increase was commensurate with the length of the alkyl chain: PM $\ll$ pentyl-PM < hexyl-PM < heptyl-PM. For comparison, salicylamine (SA, **17**), a benzene analog of PM, showed only moderate reactivity (Table 1).

Mechanism of reaction

The adduction of MGO by PM has been known for a long time, but the complex reaction mechanism is not well understood. Several possible pathways for this reaction are outlined in Scheme S1. The addition of the amino group to the more reactive aldehyde results in hemiaminal and the subsequent loss of water results in the Schiff base. As expected, for this step, the reactivity of PM is on par with other amino groups, such as, in Ac-Phe-Lys or 4-picolylamine.²³ Since PM is known to react with 1,2-dicarbonyls much faster than lysine, participation of the adjacent phenolic group in subsequent steps seems important. The formation of reaction products between PM and 1,2-dicarbonyls that do not involve the phenolic group, such as, methylimidazolium cross-link²⁴ or *N*-acyl derivatives,²⁵ is rather slow and is not considered here.

Rearrangement of Schiff base **9** to **12** (Scheme 2) followed by hydrolysis to pyridoxal (**14**) and amino ketone (a characteristic reaction of vitamin B6) was found to be only a minor pathway. Also, we were unable to find evidence for the hemiaminal (**7**) undergoing addition of phenol to the other carbonyl group in MGO followed by dehydration resulting in pyrido-1,4-oxazepine (**11**). Formation of a seven-membered ring is unlikely to compete with the formation of pyrido-1,3-oxazine (**13**) through attack of the phenol on the imine (**10**).

Very few carbonyl compounds with amino and hydroxyl groups at the 2-position are known and they rapidly undergo further reactions.²⁶ SA (**17**) has been shown to form stable benzo-1,3-oxazines with aldehydes (**15**) and ketones (Scheme 3).²⁷ They are stable in organic solvents and are in equilibrium with a small amount of the open-chain imine. When treated with half equivalent of α -dicarbonyls, including MGO, in methanol, SA forms *bis*-benzo-1,3-oxazines (**16**) by reacting with both the carbonyl groups. *bis*-Benzo-1,3-oxazines were found to be thermally unstable and on exposure to water hydrolyzed to salicylaldehyde and aminocarbonyls.²⁸ Reaction between SA and MGO at equimolar concentrations has not been reported and when attempted in methanol it led to colored products.

In the present study, when the rate of the reaction between SA and MGO was followed by LC, a major peak along with a few minor ones were observed later in the chromatogram (Figure S1A). The product corresponding to the major peak was isolated and based on the molecular and fragmented ions (Figure S1B), a dimeric structure **19** containing an ethylidene bridge was proposed and confirmed by NMR (Table S1). The initial product, 2-

acetyl-3,4-dihydro-2*H*-benzo(1,3)oxazine (**18**), very likely underwent aldol condensation to form **20**, and subsequent loss of acetic acid led to **19**.

In the case of PM and glyoxal (GO) at pH 7.5, the dimer **22** has been isolated and characterized²⁹ supporting the intermediacy of the bicyclic aldehyde **21** that undergoes mutual addition of the amino group of one adduct to the carbonyl of the other (Scheme 4). For this reaction also the alkyl-PMs held substantial advantage over PM (Table 1).

From the reaction solutions of alkylpyridoxamines (**6**) and MGO, a mixture of products was extracted into ethyl acetate, but could not be separated into dimers resembling either **19** or **22** that would arise from **13**. Therefore, the formation of pyridoxazine ring during the reaction was inferred indirectly. Previously, the presence of pyrido-1,3-oxazine has been detected by UV and NMR spectra in aqueous solutions of PM and greater than 10 times excess of propanal (or formaldehyde).³⁰ In order to compare PMs in their ability to form pyrido-1,3-oxazine, we used dimethoxyacetaldehyde (Scheme 5) as it is less volatile than propanal. In ethyl acetate solution of a 1:1 mixture of pentyl-PM (or hexyl-PM) and dimethoxyacetaldehyde, pyrido-1,3-oxazine (**24**, R = pentyl) was identified by mass spectrometry. It reverted to starting compounds when dissolved in water.

Aqueous solutions of PM or alkyl-PM with excess dimethoxyacetaldehyde at pH 7.4 were studied spectrophotometrically. In accordance with the previous observation,³⁰ as the concentration of the aldehyde increased, the equilibrium shifted through the neutral form of the Schiff base (**25**) to pyrido-1,3-oxazine (**24**) as indicated by the increase in absorbance at 280 nm and concomitant decrease at 323 nm. From the absorbance values at those two wavelengths and the reported ϵ values for neutral (6 300)³⁰ and zwitterionic (7 900)³¹ forms of PM, the percentage of **24** in equilibrium with **23** was calculated (Figure 2).

While for PM or each of the alkyl-PMs, the neutral species increased with the aldehyde concentration, at the same aldehyde concentration, a shift in the equilibrium away from the pyridinium phenoxide zwitterion and towards **24** was proportional to the increase in the lipophilicity of the compound. This equilibrium shift is similar to the one observed by Metzler³¹ with PM or pyridoxine solutions in a series of mixtures of water (pH 6.8) and dioxane. As the proportion of less polar dioxane increased, the spectral maxima at 324 and 253 nm decreased while a new maximum at 282 nm appeared. The change is attributed to the conversion of the dipolar ionic form **2** to the neutral non-ionic form **1**.³¹

Based on this evidence, we propose the addition of phenolic group to the imine leading to neutral and highly reactive pyrido-1,3-oxazine (**13**) to be the key step, followed by rapid oligomerization (Scheme 2). The hydrophobic interaction of the alkyl substituent of PM derivatives with the methyl group of MGO bringing the reactants close to each other in the aqueous environment does not fully explain the high reactivity of alkyl-PMs towards MGO, as they also react almost as rapidly with GO, that lacks the methyl group (Table 1). The loss of the zwitterionic structure of PM, that is stable over a wide pH range,¹⁶ may play a significant role. The conversion of PM and alkyl-PMs to **13** corresponds to the distribution of these compounds across the aqueous and ethyl acetate phases, and alkylation of 5-hydroxyl group was shown to facilitate this process. Hence, introduction of alkyl moiety at

the 5-hydroxymethyl side-chain of PM would be expected to favor the formation of pyrido-1,3-oxazine, thus resulting in higher rate for the over-all reaction. The increase in the rate of the reaction would then be proportional to the length of the alkyl chain.

Protection of arginine

Since arginine residues are abundant within protein receptor binding domains, protein-nucleotide binding sites, and active sites of enzymes,⁷⁻⁹ protecting these residues from modification by MGO and other 1,2-dicarbonyls is an important goal.³² Therefore, the ability of various alkyl-PMs to provide this protection was studied. The reaction of *N*- α -benzoylarginine (bzArg) with MGO was followed by LC. After a 2 h incubation, the chromatogram of the reaction mixture showed unreacted bzArg and peaks corresponding to three initial products identified previously (Figure 3 and Figure S2).²⁰ Supporting the kinetic data, hexyl-PM was more effective than PM in competing with bzArg for MGO (Figure 4). The reaction of bzArg with GO was slower than its adduction with MGO,^{19, 20} only to the extent of $12.1 \pm 0.19\%$ during the same time period. However, this was reduced to $7.8 \pm 0.8\%$ and $6.0 \pm 0.16\%$ in the presence of PM and HxPM, respectively.

In the next set of experiments, bzArg was exposed to 100 mM glucose undergoing autoxidation for 40 days. It has been previously demonstrated that under these experimental conditions there is a slow accumulation of reactive dicarbonyl compounds GO and MGO.⁶⁻⁸ When incubated with glucose without any additives (Figure S3A) bzArg was modified to the extent of $5.0 \pm 0.05\%$ by GO (peaks in chromatogram preceding bzArg peak¹⁹) and $3.6 \pm 0.40\%$ by MGO (later product appearing as a shoulder immediately following bzArg peak²⁰). PM as well as pentyl-PM or hexyl-PM effectively scavenged the dicarbonyl products of glucose autoxidation, thus leaving bzArg unscathed (Figure S3B).

Protection of lysozyme activity

In order to demonstrate the ability of alkyl-PMs to protect proteins from damage by MGO, lysozyme was chosen. Lysozyme has several arginine residues that are critical for its activity and 1,2-dicarbonyls from glucose oxidation has been shown to reduce its ability to degrade bacterial cell wall.⁹ Consistent with protection of bzArg, pentyl-PM and hexyl-PM significantly protected lysozyme activity from MGO-induced inhibition (Figure 5). Importantly, the degree of protection by these alkyl-PMs was proportional to their respective reactivities with MGO (Table 1). Hexyl-PM at five times molar excess of MGO preserved almost 75% of the activity of lysozyme, while PM at the same concentration offered only marginal protection (Figure 5). These data demonstrate that alkyl-PMs can protect protein functionality from damage by reactive carbonyl compounds such as MGO much better than PM. Because arginine is among the most abundant residues within protein functional sites,⁷⁻⁹ many proteins can potentially benefit from this functional protection by alkyl-PMs. Although lysine residues react with MGO more slowly compared with arginine residues, those at the active sites of enzymes, such as Lys¹⁸² of glucose-6-phosphate dehydrogenase, may have lower pK_a and consequently enhanced reactivity towards MGO.³³ As biogenic amines were found ineffective in preventing inactivation of glucose-6-phosphate dehydrogenase by MGO,³³ alkyl-PMs may be able to compete favorably with reactive lysyl residues for adduction of 1,2-dicarbonyl.

CONCLUSIONS

The lipophilicity of zwitterionic PM was increased by converting the alcoholic side-chain to pentyl, hexyl, or heptyl ether. Compared with PM the resulting alkyl-PMs exhibited much greater ability to react with MGO, a toxic diacarbonyl compound that can cause pathogenic protein damage. During a slow release of MGO and other 1,2-dicarbonyl compounds, such as, during glucose autooxidation in aqueous environments, PM as well as alkyl-PMs can effectively protect arginine residues from modification. However, when there is a large influx of MGO or other 1,2-dicarbonyls, alkyl-PMs have distinct advantage over PM. This advantage of alkyl-PMs may be critical for protection of hydrophobic pockets of proteins, membrane proteins within lipid bilayer, or nuclear proteins.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

GO	glyoxal
MGO	methylglyoxal
PM	pyridoxamine
SA	salicylamine
bzArg	<i>N</i> - α -benzoylarginine

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Figure 1.

The equilibrium between neutral and zwitterionic structures of PM in solution.

Figure 2.

The percentage of oxazine in solutions of PM and analogs (0.1 mM) with increasing concentration of dimethoxyacetaldehyde at pH 7.4. The concentrations were calculated from the UV spectra of the mixtures. PM (○), pentyl-PM (▲), hexyl-PM (◆), heptyl-PM (■).

Figure 3.
Reaction of MGO with guanidine side-chain of bzArg.

Figure 4.

The inhibition of MGO reaction with bzARG by SA, PM or hexyl-PM (5 mM). Shown are the percentage of bzARG (5 mM) reacted with MGO (5 mM) in 0.1 M phosphate buffer, pH 7.4 at 37 °C in 2 h.

Figure 5.

Protective effect of SA, PM, pentyl-PM or hexyl-PM on lysozyme activity in the presence of MGO. Lysozyme was incubated alone (), without MGO (open symbols) or with 0.5 mM MGO (filled symbols) in the presence of the indicated concentrations of PM ($\circ$, $\bullet$), SA (∇ , $\blacktriangledown$), pentyl-PM ($\diamond$, $\blacklozenge$) or hexyl-PM ($\square$, $\blacksquare$). Incubations were carried out in 0.15 M phosphate buffer at 37 °C for 20 h. Lysozyme activity was measured as described under Materials and Methods.

Scheme 1.
Synthesis of 5'-O-Alkylpyridoxamines

Scheme 2.
Reaction between Pyridoxamines and Methylglyoxal

Scheme 3.
Reaction of Salicylamine with Aldehydes and Methylglyoxal

Scheme 4.

Reaction of Pyridoxamine and Glyoxal forming a Dimer

Scheme 5.

Pyridoxamine and Alkyl-PMs form Pyridoxazines with excess Dimethoxyacetaldehyde

Table 1

Lipophilicity and the second-order rate constants for reaction of PM and PM analogs with MGO or GO

amine	lipophilicity ^a	reaction with MGO $k^b \times 10^3 \text{ sec}^{-1}\text{M}^{-1}$ (relative rate)	reaction with GO $k^b \times 10^3 \text{ sec}^{-1}\text{M}^{-1}$ (relative rate)
SA	0.18 ¹⁶	1.5 ± 0.20 (0.5)	–
PM	0.08 ± 0.03	3.1 ± 0.16 (1.0)	7.1 ± 1.2 (1.0)
pentyl-PM	2.22 ± 0.14	62.8 ± 7.6 (20.3)	33.0 ± 3.5 (4.6)
hexyl-PM	10.90 ± 0.83	272 ± 21.6 (88)	141.1 ± 13.2 (19.9)
heptyl-PM	92.6 ± 4.3	948 ± 45 (306)	–

^aThe ratio of concentrations of the amine in ethyl acetate and in water (buffered at pH 7.4) after equilibration at ambient temperature (24 °C).^bSecond-order rate constant for the disappearance of the amine, at pH 7.4 and 37 °C.