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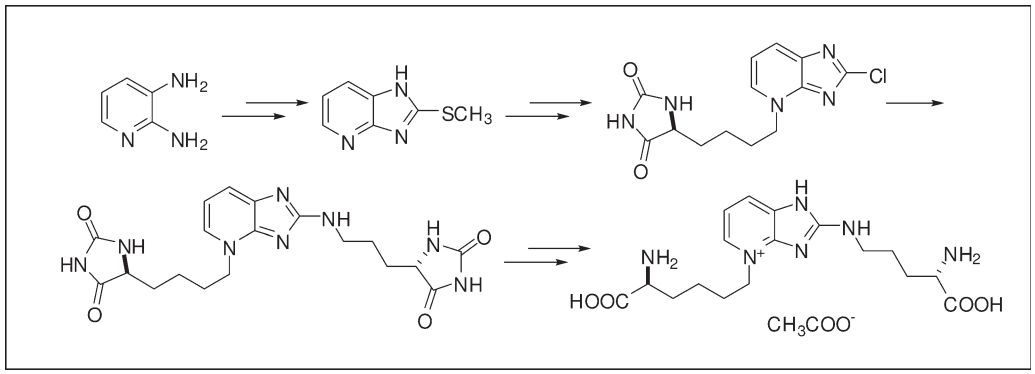
This work is dedicated to Professor Lawrence M. Sayre.



Pentosidine, a fluorescent advanced glycation endproduct that serves as a biomarker of diabetic complications, kidney dysfunction, oxidative stress, and aging and age-related diseases, was synthesized from 2,3-diaminopyridine and benzyloxycarbonyl (Cbz) protected chiral amino acids N^{α}-Cbz-lysine and N^{δ}-Cbz-ornithine. Regioselective alkylation of 2-(methylthio)imidazo[4,5-*b*]pyridine, chlorination of methylthio group, and amination of 2-chloro-imidazo[4,5-*b*]pyridine are the key steps. Hydantoin protection of amino acids was used and the deprotection under acidic condition was achieved in the presence of glycine.

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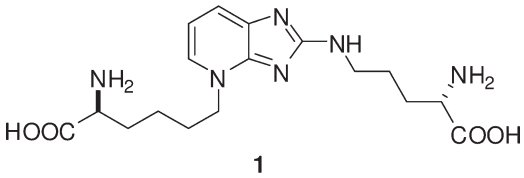
INTRODUCTION

Pentosidine (**1**) is one of the fluorescent advanced glycation endproducts (AGEs) that have been isolated and structurally characterized [3]. It was reported that pentosidine elevates in diabetes mellitus, and in patients with end-stage renal failure and kidney dysfunction [4]. A significant elevation of pentosidine was found in rheumatoid arthritis and in osteoarthritis as well [5]. Carbonyl stress, oxidative stress and aging progress the formation and accumulation of pentosidine [6]. During the aging process, pentosidine accumulates in the extracellular matrix and causes insolubility and protease resistance of extracellular matrix proteins [7]. Pentosidine level increases in disorders of pentose metabolism during aging and in age-related diseases such as Alzheimer's disease [8]. Ophthalmology research shows that pentosidine elevates in the aging vitreous body of eyes and in diabetic retinopathy [9]. Emerging evidence shows that pentosidine increases along with the deterioration of bone quality in terms of collagen crosslink formation in bone collagen and proteoglycan, and the stiffness of the cartilage [10]. Recent research shows that pentosidine levels may be associated with heart failure [11], alcohol misuse [12], and other drug abuse [13]. Because its characteristic fluorescence allows it to be seen and measured easily, pentosidine has become an indispensable biomarker in kidney dysfunction, end-stage renal disease, diabetes, and diabetic complications (including diabetic eye complications, such as diabetic retinopathy), rheumatoid arthritis, bone collagen deterioration, oxidative stress, and age-related diseases [14].



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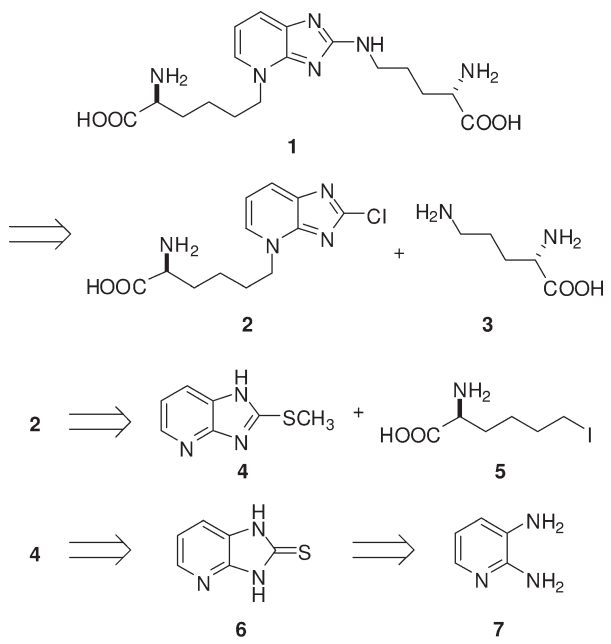
1a, CF_3CO_2H salt of **1**
1b, HCl salt of **1**
1c, CH_3CO_2H salt of **1**

In contrast to its extensive applications as a biomarker in biological and clinical research, synthesis of pentosidine has received little attention. Until now only two synthetic approaches have been reported. The first synthesis of pentosidine, reported by Monier and Sell, was

Scheme 1



achieved biochemically through incubation of ribose, lysine, and arginine [3a,15]. The synthesis served to confirm its structure and propose possible mechanism of its formation, but suffered complicated purification and extremely low yield (0.02%). Shioiri and coworkers obtained 5.1 mg of pentosidine trifluoroacetic acid salt (**1a**) using asymmetric alkylation of a chiral Schiff base to provide a lysine-like fragment, intramolecular guanylation with mercury (II) chloride, and quaternization accompanied by removal of the trityl group as key steps [16]. Because of the increasing importance of pentosidine in biological and clinical research, we have developed an alternate entry to this biomarker. In this article, we describe the details of our approach to this unique AGE crosslink.

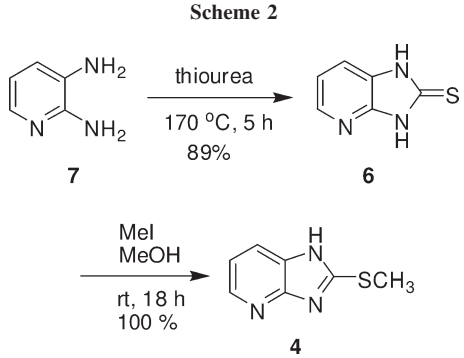
RESULTS AND DISCUSSION

Based on the synthesis of pentosidine framework we have previously reported [17], our plan for the synthesis of pentosidine is shown in retrosynthetic format in Scheme 1 and is centered on the construction of the key intermediate imidazo[4,5-*b*]pyridine core **4.** Pentosidine (**1**) should be available by consecutive introduction of amino acid subunit **3** (for lysine portion of pentosidine) and subunit **5** (for arginine portion of pentosidine) to the central piece **4** by a regioselective alkylation and an amination, respectively. We anticipated that the two subunits **3** and **5** could be obtained from chiral amino acids.

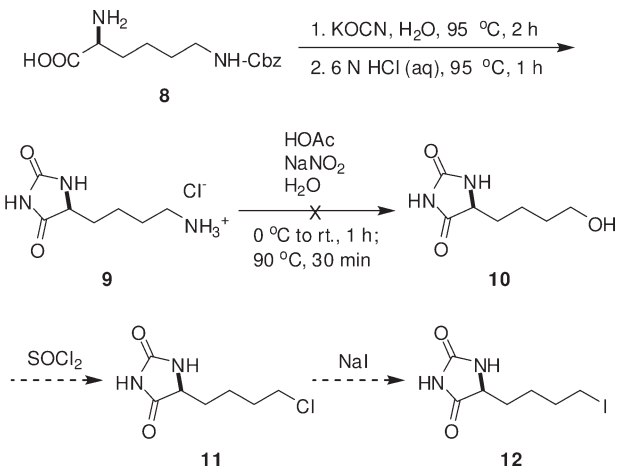
Chiral fragments developed from natural amino acids have been used in many asymmetric syntheses of natural products [18]. We envisioned that the amino acid subunit **3** could be generated from lysine and the subunit **5** from onithine. To make amino acid subunit fragments which could be incorporated through either an alkylation reaction or an amination reaction, the α-amino and carboxy groups of the amino acids needed to be protected. Of the several methods available for protecting amino acids, we decided to employ hydantoin derivatization. Hydantoins have been used as precursors of amino acids in many syntheses and serve as important amino acid surrogates for the production of pharmaceuticals (e.g., semisynthetic penicillins and cephalosporins), agrochemicals, and fine chemicals [19]. The conversion of hydantoins to amino acids could be achieved through hydrolysis [20,21] or biocatalysis [22]. Another reason for selecting hydantoin protection is that our synthesis involves some harsh reaction conditions, such as chlorination in 12*N* HCl [17], and hydantoin is the only protecting group that can survive all the reaction conditions involved in the synthesis.

As planned in Scheme 1, our synthesis began with the preparation of the key intermediate imidazo[4,5-*b*]pyridine core **4** (Scheme 2) [17]. The reaction of 2,3-diaminopyridine (**7**) with thiourea afforded 1*H*-imidazo[4,5-*b*]pyridine-2(3H)-thione (**6**) [17,23] and S-methylation of **6** gave 2-(methylthio)imidazo[4,5-*b*]pyridine (**4**) [17].

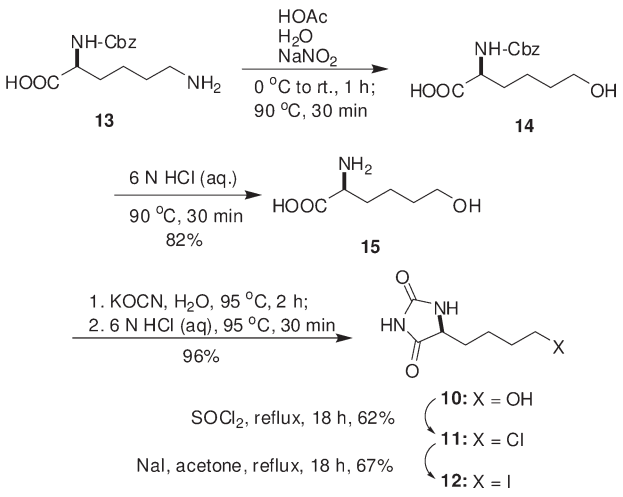
Scheme 2



Scheme 3



Scheme 4

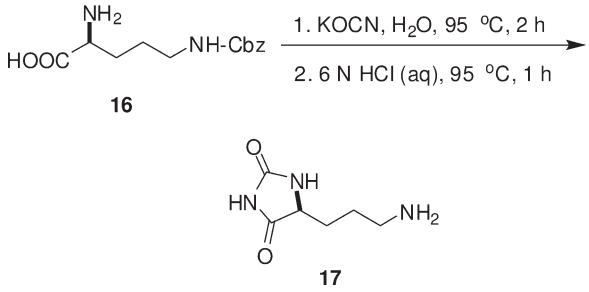


It was conjectured that the preparation of the hydantoin protected lysine subunit fragment **12** could be fulfilled by conversion of commercially available N^{ε}-Cbz-lysine (**8**) to the hydantoin compound **9** through Urech hydantoin synthesis (Scheme 3) [24], followed by a key conversion of **9** to **10** through diazotization and hydroxylation [25], a nucleophilic substitution of alcohol **10** to form **11** [26] and an alteration of chloride **11** into iodide **12** [27]. Although transformation of alkylamines into alcohols through diazonium intermediates has been reported to be achievable in good yield [25], the transformation of 5-(4-aminobutyl)hydantoin (**9**) to 5-(4-hydroxybutyl)-hydantoin (**10**) was not successful, with recovery of unchanged amino compound **9**.

We chose an alternate strategy involving transformation of amino to hydroxy group prior to hydantoin derivatization (Scheme 4) [25]. The conversion of N^{α}-Cbz-lysine (**13**) into alcohol **14** through diazotization and hydroxylation was accomplished in good yield [25]. L-6-hydroxynorleucine (**15**), obtained from **14** after deprotection, was then transformed into the hydantoin **10** through Urech hydantoin synthesis [24]. The alcohol **10** was converted into the chloride **11** via a nucleophilic substitution using $SOCl_2$ [26], and then into iodide **12** by refluxing with NaI in acetone [27]. It is worth mentioning that although the yields of both the conversion of **10** to **11** using thionyl chloride and the conversion of **11** to **12** were moderate, the both conversions could be forced to quantitative by prolonged refluxing (>168 h).

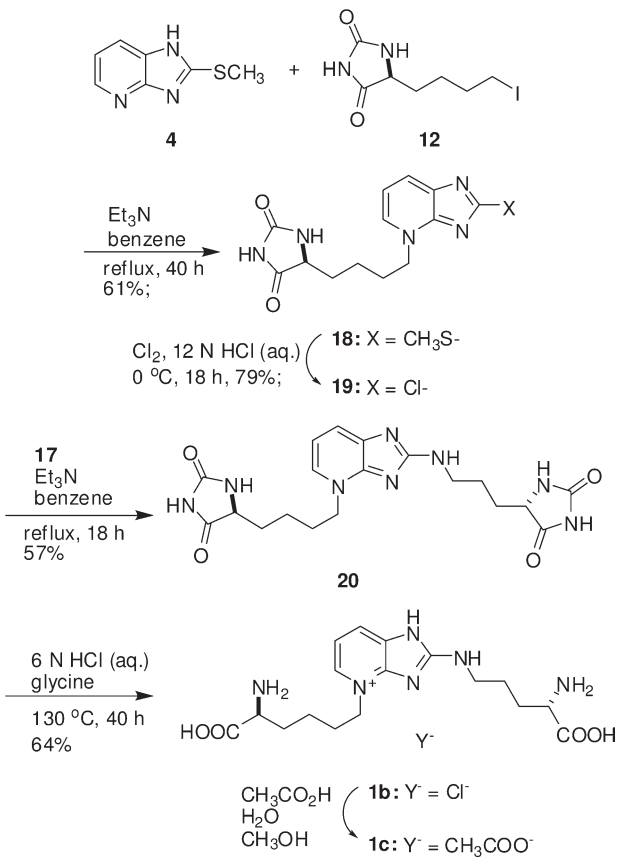
The amination reagent required for elaboration of the arginine portion of pentosidine corresponds to the ornithine subunit fragment **17**, which was prepared from N^{δ}-Cbz-ornithine (**16**) through Urech hydantoin synthesis followed by the removal of Cbz protective group (Scheme 5) [24].

Scheme 5

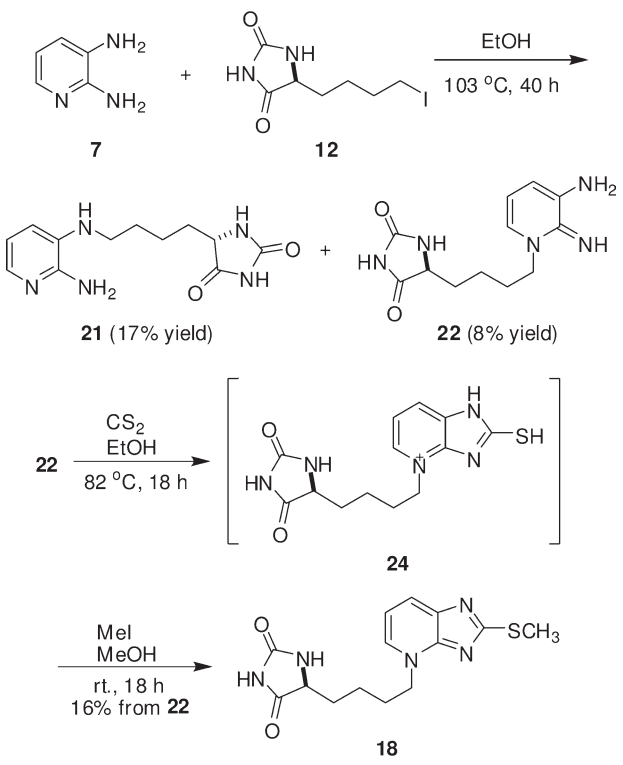


After we had prepared the key intermediate **4** and the amino acid subunit fragments **12** and **17**, their assembly started from the alkylation of imidazo[4,5-*b*]pyridine core **4** with iodide **12** in benzene in the presence of Et_3N to give pyridine *N*-alkylated imidazopyridine **18** [28] for pyridine nitrogen is more nucleophilic than the imidazole nitrogens at positions 1 or 3 (Scheme 6) [28]. Compound **18** was then transformed into 2-chloroimidazopyridine **19** through chlorination in hydrochloric acid [29]. The ornithine subunit fragment **17** was then introduced through amination of chloride **19** in the presence of Et_3N, resulting in the dihydantoin-protected pentosidine (**20**) [30].

Scheme 6



Scheme 7



Deprotection of hydantoins to amino acids could be achieved through basic hydrolysis [20], acidic hydrolysis [21], and biocatalytic means [22]. Although hydrolysis of the hydantoin protecting group under basic conditions is straightforward [20], it can lead to unwanted racemization. Acidic hydrolysis is favored because hydantoins and amino acids do not undergo racemization under such conditions [21]. The biocatalytic conversion of hydantoins to amino acids has been recognized in recent decades for potential application in the industrial production of amino acids [22]. The biocatalysis reaction involves two consecutive hydrolysis steps, catalyzed by both a hydantoinase and an *N*-carbamoylamino acid amidohydrolase (NCAAH). During the process, an L-selective hydantoinase converts a 5-monosubstituted hydantoin to an L-*N*-carbamoylamino acid and an L-selective NCAAH then converts the *N*-carbamoylamino acid to an L-amino acid. It seemed to us that the biocatalytic conversion is the most favored for the deprotection of dihydantoin-protected pentosidine **20**.

Because our work was halted due to the death of Professor Sayre and the consequent laboratory closing [31], we were unable to explore the preparation of amino acids via biocatalysis with hydantoinase and NCAAH. Prior to that we found removal of the hydantoin protecting groups required refluxing in 6*N* HCl in the presence of glycine. Pentosidine is stable in concentrated acid and was first obtained after refluxing in 6*N* HCl for 36 h [3a]. Only small amount of pentosidine formed during hydrolysis of **20** when glycine was absent. We still do not know how glycine facilitates the hydrolysis, a hydantoin change reaction could be a plausible explanation [32]. Purification of crude HCl salt of pentosidine (**1b**) with a mixed eluent of $CH_3OH/H_2O/CH_3CO_2H$ afforded its CH_3CO_2H salt (**1c**). Comparison of NMR, FAB-MS, and LC-MS data of our synthesized **1c** with the pentosidine CF_3CO_2H salt (**1a**) isolated by Sell and Monnier showed the consistency of synthesized and isolated compounds [3a]. We have also noted that the specific rotation value of our pentosidine CH_3CO_2H salt (**1c**) is $[\alpha]_D^{24} = +11.5°$ (c 0.2, CH_3OH), which differs from the value $[\alpha]_D^{24} = +16.5°$ (c 0.3, CH_3OH) of its CF_3CO_2H salt (**1a**) [15]. Considering UV and fluorescent properties of pentosidine are highly dependent on pH value [3,17], the difference of specific rotation values could be attributed to the different counterions in the two salt forms.

While pentosidine **1** could be obtained from the synthesis shown in Scheme 6, evidence for the regiochemistry of alkylation in **18**, and for structural assignment of **19**, **20**, and **1** that were obtained in subsequent steps, was not fully discussed. We assigned **18** as pyridine *N*-alkylated product on the basis of the regiochemistry of the alkylation reaction used to synthesize pentosidine framework [17]. To provide conclusive evidence for our structural assignment of **18**, **19**, **20**, and **1**, an unambiguous synthesis was used (Scheme 7). The regiochemistry of intermediate **18** in Scheme 6 was proven through comparison of its NMR spectrum to the spectrum of **18** obtained according to Scheme 7. In Scheme 7, direct alkylation of diaminopyridine (**7**) with iodide **12** gave a mixture of two isomeric products **21** and **22** [33]. The distinction of **22** from **21** could be made on the basis of both proton chemical shift and characteristic couplings. The chemical shift for the methylene group neighboring N in **21** is δ 3.17, but δ 4.22 in the case of **22.** Transformation of the pyridine *N*-alkylated isomer **22** afforded intermediate **18** [17,23,34]. The consistency of NMR spectra for the two individual samples of **18**, and the NMR spectrum of a mixture of the two samples (in 1:1 ratio), added conclusive evidence that imidazopyridine **18** obtained through this route is identical with the product obtained from the synthesis shown in Scheme 6.

In summary, pentosidine, an important biological marker for diabetic complications and oxidative damage has been synthesized. The regiochemistry of the targets and intermediates was established by an unambiguous synthesis. Our approach to the biomarker is more efficient than those reported in literature, especially in obtaining lysine subunit fragment through a procedure with fewer steps. The hydantoin-protected pentosidine

provides a platform for the future research on biocatalysis to generate pentosidine enantiomerically.

EXPERIMENTAL

All commercial reagents and solvents were used without purification. ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75.1 MHz) were recorded on Varian Gemini 300 or Gemini 200 instruments using TMS as internal standard. In the ^{13}C-NMR data, attached proton test (APT) designations are given as (+) or (−) following the chemical shift. High-resolution mass spectra (HRMS) were obtained at 20 eV on a Kratos MS-25A instrument. TLC analysis was carried out on silica gel 60 F254 precoated aluminum sheets, and UV light was used for visualization. Flash column chromatography was done using E. Merck silica gel 60 (230–400 mesh). Solvent removal was accomplished with a rotary evaporator operating at vacuum (40–50 Torr).

1*H*-imidazo[4,5-*b*]pyridine-2(3*H*)-thione (6). A mixture of 2,3-diaminopyridine (**3**) (2.18 g, 20.0 mmol) and thiourea (7.6 g, 100.0 mmol) was heated at 170 °C for 5 h. The reaction mixture was extracted with boiling ethanol (5 × 20 mL), leaving crystals that were collected by filtration. A second crop of crystals was obtained from the cooled filtrate after standing overnight. The combined yield of pure **6** was 2.7 g (89%). **6**: ^{1}H-NMR (DMSO-d_6) δ 7.13 (dd, J = 7.9, 5.0 Hz, 1H), 7.46 (d, J = 7.9 Hz, 1H), 8.09 (d, J = 5.0 Hz, 1H), 12.70 (s, 1H, NH), 13.12 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6) δ 116.2 (−), 118.1 (−), 125.4 (+), 142.3 (−), 146.4 (+), 169.8 (+). HRMS calcd for $C_6H_5N_3S$ (M^+) 151.0204, found 151.0204.

2-(Methylthio)-1*H*-imidazo[4,5-*b*]pyridine (4). A solution of **6** (2.26 g, 15.0 mmol) and methyl iodide (8.52 g, 60.0 mmol) in MeOH (30.0 mL) was stirred at room temperature for 18 h. Evaporation of the solvent and CH_3I under reduced pressure gave pure **4** (3.32 g, 100 %): ^{1}H-NMR (CD_3OD) δ 2.75 (s, 3H), 7.20 (dd, J = 7.9, 4.9 Hz, 1H), 7.82 (d, J = 7.9 Hz, 1H), 8.21 (d, J = 4.9 Hz, 1H); ^{13}C-NMR (CD_3OD) δ 15.0 (−), 119.1 (−), 127.4 (−), 134.1 (+), 134.5 (−), 150.3 (+), 166.4 (+); HRMS calcd for $C_7H_7N_3S$ (M^+) 165.0361, found 165.0363.

5-(4-Aminobutyl)hydantoin hydrochloride (9). A mixture of *N*ε-Cbz-L-lysine **8** (841.0 mg, 3.0 mmol) and potassium cyanate (260.0 mg, 3.20 mmol) in water (20.0 mL) was stirred at 95°C for 2 h, then cooled to room temperature, acidified by adding 12*N* HCl (20.0 mL), and then stirred at 95°C for 1 h. Evaporation to dryness under reduced pressure afforded crude product **9** which was sufficiently pure for the next reaction step: ^{1}H-NMR (D_2O) δ 1.46 (m, 2H), 1.70 (m, 2H), 1.84 (m, 2H), 2.99 (t, J = 7.5 Hz, 2H), 4.29 (dd, J = 6.3, 4.8 Hz, 1H); ^{13}C-NMR (D_2O) δ 20.9 (+), 26.5 (+), 30.0 (+), 39.3 (+), 58.6 (−), 159.4 (+), 178.9 (+); FAB HRMS calcd for $C_7H_{14}N_3O_2$ (M + H) $^+$ 172.1086, found 172.1093.

L-6-Hydroxynorleucine (15). To a solution of N^{α}-Cbz lysine (**13**) (5.60 g, 20.0 mmol) in a mixed solvent of acetic acid (20.0 mL) and water (4.0 mL) was added a solution of $NaNO_2$ (2.76 g, 40.0 mmol) in water (4.0 mL) at 0°C dropwise. The mixture was stirred at room temperature for 1 h, at 90°C for 30 min, and then cooled to room temperature. After an aqueous 6*N* HCl (120.0 mL) was added, the reaction mixture was stirred at 90°C for another 30 min, and evaporated to give a residue which was diluted with a mixed solvent of (MeOH/ H_2O = 4:1, 40.0 mL), neutralized to pH 7.5 with 2*N* NaOH solution, and concentrated. The resulting crude was subjected to chromatography (DCM/MeOH = 1:5) to provide **15** (2.40 g, 82%): ^{1}H-NMR (D_2O) δ 1.46 (m, 2H), 1.62 (m, 2H), 1.91(m, 2H), 3.64 (t, J = 6.3 Hz, 2H), 3.76 (t, J = 4.1 Hz, 1H); ^{13}C-NMR (D_2O) δ 21.8 (+), 31.2 (+), 31.9 (+), 55.7 (−), 62.2 (+), 175.7 (+); $[\alpha]_D^{20}$ = + 6.35° (c 0.2, H_2O); HRMS calcd for $C_7H_{12}N_2O_3$ (M^+) 147.0895, found 172.0889.

5-(4-Hydroxybutyl)hydantoin (10). A mixture of L-6-hydroxynorleucine (**15**) (2.38 g, 16.23 mmol) and potassium cyanate (1.76 g, 22.0 mmol) in water (100 mL) was stirred at 90°C for 2 h, then cooled to room temperature, acidified by adding 12*N* HCl (100.0 mL), and then stirred at 95°C for 30 min. The reaction mixture was concentrated, diluted with a mixed solvent of (MeOH/ H_2O = 4:1, 60.0 mL), neutralized to pH 7.5 with 2*N* NaOH solution, and concentrated to give a residue which was subjected to chromatography (CH_2Cl_2/CH_3OH = 6:1) to afford **10** (2.68 g, 96%): ^{1}H-NMR (CD_3OD) δ 1.40–1.88 (m, 6H), 3.56 (t, J = 6.4 Hz, 2H), 4.09 (dd, J = 6.9, 5.1 Hz, 1H); ^{13}C-NMR (CD_3OD) δ 22.1 (+), 32.5 (+), 33.2 (+), 59.8 (−), 62.6 (+), 160.0 (+), 178.3 (+); HRMS calcd for $C_7H_{12}N_2O_3$ (M^+) 172.0848, found 172.0868.

5-(4-Chlorobutyl)hydantoin (11). A solution of **10** (2.66 g, 15.45 mmol) in thionyl chloride (100 mL) was heated at reflux for 18 h and evaporated to result in a residue which was diluted with a mixed solvent of (MeOH/ H_2O = 4:1, 40.0 mL), neutralized to pH 7.5 with 2 N NaOH solution, and concentrated. The residual crude was subjected to chromatography (CH_2Cl_2/CH_3OH = 6:1) to give **11** (1.83 g, 62%): ^{1}H-NMR (CD_3OD) δ 1.42 (m, 2H), 1.58 (m, 2H), 1.81 (m, 2H), 3.57 (t, J = 6.4 Hz, 2H), 4.10 (dd, J = 6.9, 5.1 Hz, 1H); ^{13}C-NMR (CD_3OD) δ 23.1 (+), 32.0 (+), 33.3 (+), 45.5 (+), 59.7 (−), 160.0 (+), 178.1 (+); HRMS calcd for $C_7H_{11}N_2O_2Cl$ (M^+) 190.0509, found 190.0507.

5-(4-Iodobutyl)hydantoin (11). A solution of 5-(4-chlorobutyl)hydantoin (**11**) (1.79 g, 9.40 mmol) and NaI (4.68 g, 31.2 mmol) in acetone (100 mL) was refluxed for 18 h and then concentrated to give a residue which was subjected to chromatography (CH_2Cl_2/CH_3OH = 9:1) to afford **12** (1.78 g, 67%): ^{1}H-NMR (CD_3OD) δ 1.51 (m, 2H), 1.70 (m, 2H), 1.82 (m, 2H), 3.23 (t, J = 6.9 Hz, 2H), 4.29 (dd, J = 6.7, 4.7 Hz, 1H); ^{13}C-NMR (CD_3OD) δ 6.4 (+), 26.6 (+), 31.5 (+), 34.3 (+), 59.7 (−), 160.0 (+), 178.1 (+); FAB HRMS calcd for $C_7H_{11}IN_2NaO_2$ ($(M + Na)^+$ 304.9763, found 304.9763.

5-(3-Aminopropyl)hydantoin (17). A suspension of N^{δ}-Cbz-L-ornithine (**16**) (266.3 mg, 1.0 mmol) and potassium cyanate (87.0 mg, 1.07 mmol) in water (10 mL) was stirred at 95°C for 2 h, then cooled to room temperature, acidified by adding 12*N* HCl (20 mL), then stirred at 95 °C for 1 h. The reaction mixture was concentrated, diluted with a mixed solvent of (MeOH/ H_2O = 4:1, 20.0 mL), neutralized to pH 7.5 with 2*N* NaOH solution, and concentrated to afford a residue which was subjected to chromatography (CH_2Cl_2/MeOH = 3:2) to provide **17** (151 mg, 96%): ^{1}H-NMR (CD_3OD) δ 1.75–1.93 (4H), 3.00 (t, J = 6.7 Hz, 2H), 4.70 (dd, J = 6.3, 4.7 Hz, 1H); ^{13}C-NMR (D_2O) δ 22.7 (+), 28.0 (+), 39.7 (+), 58.6 (−), 159.5 (+), 178.5 (+); HRMS calcd for $C_6H_{11}N_3O_2$ (M^+) 157.0851, found 157.0835.

4-[4-(5-Hydantoin)butyl]-2-methylthio-4*H*-imidazo[4,5-*b*] pyridine (18). A suspension of 5-(4-iodobutyl)hydantoin (**12**) (1.69 g, 6.0 mmol) and 2-(methylthio)-1*H*-imidazo[4,5-

b]pyridine (**4**) (495.1 mg, 3.0 mmol) in benzene (60 mL) with triethylamine (0.5 mL, 3.6 mmol) was stirred and heated in an oil bath (90°C) for 40 h. After removing the solvent under reduced pressure, the residue was dissolved in a mixture of MeOH (20 mL) and H_2O (20 mL) and adjusted to pH 9.5. Upon evaporation of the solvent under reduced pressure, flash chromatography (EtOAc/MeOH = 3:1) of the residue afforded **18** (584.0 mg, 61%): ^{1}H-NMR (CD_3OD) δ 1.45 (m, 2H), 1.75 (m, 2H), 1.92 (m, 2H), 2.67 (s, 3H), 4.09 (dd, J = 6.8, 4.6 Hz, 1H), 4.53 (t, J = 7.2 Hz, 2H), 7.09 (dd, J = 7.7, 6.7 Hz, 1H), 7.89 (d, J = 6.7 Hz, 1H), 7.91 (d, J = 7.7 Hz, 1H); ^{13}C-NMR (CD_3OD) δ 14.6 (−), 22.9 (+), 30.2 (+), 32.1 (+), 54.8 (+), 59.5 (−), 114.6 (−), 125.6 (−), 131. 4 (−), 145.3 (+), 154.3 (+), 159.9 (+), 173.1 (+), 178.0 (+); FAB HRMS calcd for $C_{14}H_{18}N_5O_2S$ $(M+1)^+$ 320.1181, found 320.1175.

4-[4-(5-Hydantoin)butyl]-2-chloro-4*H*-imidazo[4,5-*b*]pyridine (19). Chlorine was introduced into a solution of **18** (200.0 mg, 0.62 mmol) in 12*N* HCl (60 mL) with stirring at 0°C for 18 h. After evaporation to dryness under reduced pressure, the residue was dissolved in water (20 mL) and neutralized to 7.8 with NH_4OH. Water was removed under reduced pressure and the residue was extracted with CH_2Cl_2/MeOH (8:1). The organic extract was dried (Na_2SO_4) and evaporated to give a residue, which was subjected to chromatography (CH_2Cl_2/CH_3OH= 8:1) to furnish **19** (151.0 mg, 79%): ^{1}H-NMR (CD_3OD) δ 1.49 (m, 2H), 1.79 (m, 2H), 2.00 (m, 2H), 4.09 (dd, J = 6.9, 4.7 Hz, 1H), 4.64 (t, J = 7.5 Hz, 2H), 7.32 (dd, J = 7.8, 6.3 Hz, 1H), 8.16 (d, J = 7.9 Hz, 1H), 8.19 (d, J = 6.3 Hz, 1H); ^{13}C-NMR (CD_3OD) δ 22.6 (+), 30.3 (+), 32.0 (+), 55.3 (+), 59.5 (−), 116.0 (−), 129.7 (−), 134.1 (−), 143.9 (+), 152.6 (+), 159.7 (+), 159.9 (+), 178.0 (+); FAB HRMS calcd for $C_{13}H_{15}N_5O_2Cl$ $(M+1)^+$ 308.0914, found 308.0917.

4-[4-(5-Hydantoin)butyl]-2-[3-(5-hydantoin)propylamino]-4*H*-imidazo[4,5-*b*]pyridine (20). A suspension of **19** (138.4 mg, 0.48 mmol) and **17** (151.0 mg, 0.96 mmol) in benzene (20 mL) with triethylamine (0.334 mL, 242 mg g, 2.40 mmol) was stirred and heated in an oil bath (90°C) for 18 h. After removing solvent under reduced pressure, the residue was dissolved in MeOH (20 mL) and H_2O (20 mL) and adjusted to pH 9.5. Concentration under reduced pressure left behind a residue which was purified by chromatography (CH_2Cl_2/MeOH = 3:1) to provide **24** (117.2 mg, 57%): ^{1}H-NMR (CD_3OD) δ 1.40–1.98 (10H), 3.46 (t, J = 4.9 Hz, 2H), 4.08 (dd, J = 6.8, 5.2 Hz, 1H), 4.15 (dd, J = 5.9, 4.7 Hz, 1H), 4.41 (t, J = 4.9 Hz, 2H), 6.89 (dd, J = 6.4, 5.9 Hz, 1H), 7.42 (d, J = 5.9 Hz, 1H), 7.54 (d, J = 6.4 Hz, 1H); ^{13}C-NMR (DMSO-d_6) δ 21.4 (+), 25.1 (+), 28.2 (+), 28.9 (+), 30.7 (+), 41.6 (+), 51.8 (+), 57.5 (2C) (−), 110.6 (−), 115.4 (−), 124.9 (−), 145.9 (+), 155.5 (+), 157.6 (2C) (+), 170.2 (+), 176.1 (+), 176.2 (+) (two peaks are missing due to overlap); FAB HRMS calcd for $C_{19}H_{25}N_8O_4$ $(M + 1)^+$ 429.2000, found 429.2008.

Acetic acid salt of pentosidine (1c). A mixture of **20** (107.5 mg, 0.25 mmol) and glycine (75.1 mg, 1.0 mmol) in 6 N HCl (45 mL) was refluxed under N_2 for 40 h and evaporated under reduced pressure to result in crude HCl salt of pentosidine (**1a**) which was subjected to chromatography (CH_3OH/H_2O/HOAc = 7:2:1) to provide **1c** (70.2 mg, 64%): $[\alpha]_D$ = +11.5° (c 0.2, CH_3OH); ^{1}H-NMR (D_2O) δ 1.4–1.6 (m, 2H), 1.8–2.1 (m, 8H), 2.08 (s, 3H, CH_3COO^-), 3.57 (t, J = 6.1 Hz, 2H), 3.96 (t, J = 6.3 Hz, 1H), 4.02 (t, J = 6.3 Hz, 1H), 4.55 (t, J = 7.1 Hz, 2H), 7.22 (dd, J = 7.7, 6.5 Hz, 1H), 7.78 (d, J = 7.7 Hz. 1H), 7.93 (d, J = 6.5 Hz, 1H); ^{13}C-NMR δ 21.0(+) (CH_3COO^-), 22.0 (+), 25.1(+), 28.3(+), 28.8(+), 30.5(+), 39.5(+), 42.6(+), 53.7(−), 53.8(−), 115.8(−), 120.2(−), 132.4(+), 132.7(−), 152.2(+), 160.3(+), 171.7(+), 172.3(+), 177.0(−) (CH_3COO^-); FAB HRMS calcd for $C_{17}H_{27}N_6O_4$ $(M + 1)^+$ 379.2094, found 379.2083.

2-Amino-3-[4-(5-hydantoin)butyl]aminopyridine (21) and 3-amino-1-[4-(5-hydantoin)butyl]-2-pyridone imine (22). A mixture of **7** (436.0 mg, 4 mmol) and **12** (1.35 g, 4.8 mmol) in EtOH (16.0 mL) was stirred at 100°C in a high pressure reaction vessel for 40 h, and then concentrated under reduced pressure to result in a residue which was diluted with a mixed solvent of MeOH (30 mL) and H_2O (30 mL), basified to pH 10 with 2*N* aqueous solution of NaOH, and concentrated. The residual crude was subjected to chromatography (EtOAc/MeOH = 4:1) to afford **21** (180.1 mg, 17% yield) and **22** (84.2 mg, 8%). **21**: ^{1}H-NMR (CD_3OD) δ 1.55 (m, 2H), 1.75 (m, 2H), 1.85 (m, 2H), 3.17 (t, J = 6.7 Hz, 2H), 4.12 (dd, J = 6.2, 5.6 Hz, 1H), 6.77 (dd, J = 7.7, 6.0 Hz, 1H), 6.91 (d, J = 7.7 Hz, 1H), 7.21 (d, J = 5.9 Hz, 1H); **22**: ^{1}H-NMR (CD_3OD) δ 1.50 (m, 2H), 1.75 (m, 2H), 1.85 (m, 2H), 4.12 (dd, J = 6.2, 5.6 Hz, 1H), 4.22 (t, J = 7.3 Hz, 2H), 6.77 (dd, J = 7.2, 6.4 Hz, 1H), 7.10 (d, J = 7.2 Hz, 1H), 7.34 (d, J = 6.3 Hz, 1H).

Preparation of 18 from 22. A mixture of **22** (71.8 mg, 0.27 mmol) in EtOH (4 mL) and CS_2 (4 mL) was stirred at 80°C in for 18 h and concentrated under reduced pressure. The resulting residue was mixed with a solution of methyl iodide (428 mg, 3 mmol) in MeOH (2 mL), stirred at room temperature for 18 h, evaporated to dryness under reduced pressure. The crude residue was dissolved in MeOH (10 mL) and H_2O (10 mL), basified to pH 9.5 with 2*N* aqueous solution of NaOH, and concentrated to furnish a residue which was subjected to chromatography (EtOAc/MeOH = 3:1) to furnish **18** (13.8 mg, 16%). ^{1}H-NMR, ^{13}C-NMR, and HRMS are identical with those of **18** prepared from **4** and **15**.

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