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An expeditious synthesis of pentosidine, an advanced glycation end product

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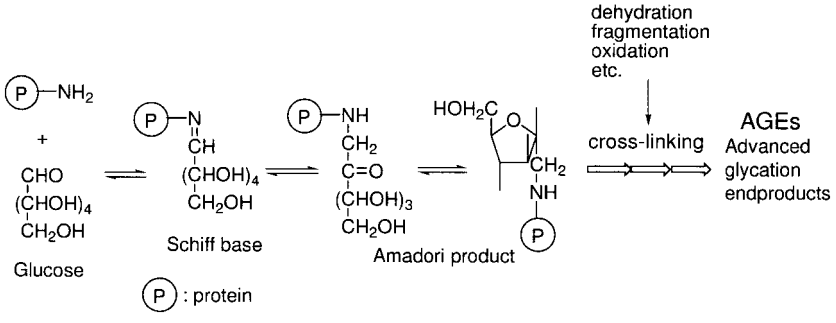
Abstract—The chemical synthesis of pentosidine (**1**), an advanced glycation end product, was achieved via the asymmetric alkylation of the chiral schiff base derived from (+)-2-hydroxy-3-pinanone ((+)-HyPN) and glycine *tert*-butyl ester, the mercury salt mediated intramolecular guanylation, and the regioselective alkylation of imidazo[4,5-*b*]pyridine ring. This reliable synthetic achievement will promise availability of pentosidine (**1**) in quantities.

1. Introduction

Diabetes has been estimated to afflict more than one hundred million people in the world, and the contribution of the diabetic complications to the overall mortality of heart disease and stroke is a particularly significant problem.[1] The reaction between reducing sugars and amino structures in amino acids or proteins (also called Maillard reaction) has been shown to proceed in living systems and to have a role in pathophysiology of aging and diabetic complications.[2] As shown in Scheme 1, the Maillard reaction is initiated by the formation of a schiff base adduct between the carbonyl and the amine moiety, and then the aldimine rearranges to a more stable ketoamine (Amadori product). Subsequently, enolization, dehydration, cyclization, fragmentation, and oxidation reactions form reactive intermediates that ultimately lead to stable end-products, termed AGEs (advanced glycation end products). The features of AGEs are fluorescent, browning, and highly reactive with nearby amino groups to produce both intra and intermolecular crosslinks. AGEs increase in collagen and lens proteins with age and the accelerated accumulation of these products in tissues of diabetic patients, particularly of patients with complications.[3] Accordingly, AGEs are expected to be clinically useful marker for diabetic complications, and some attempt to build up the antibodies of AGEs are performed.

Since enormous efforts were devoted to elucidate the nature of these protein modifications, so far some structures of AGEs have been established as shown in Fig. 1.[4]

Pentosidine (**1**) is one of the AGEs and was discovered as a fluorescent protein crosslink isolated from the human



Keywords: pentosidine; an advanced glycation end product; asymmetric alkylation; imidazo[4,5-*b*]pyridine; quaternization.

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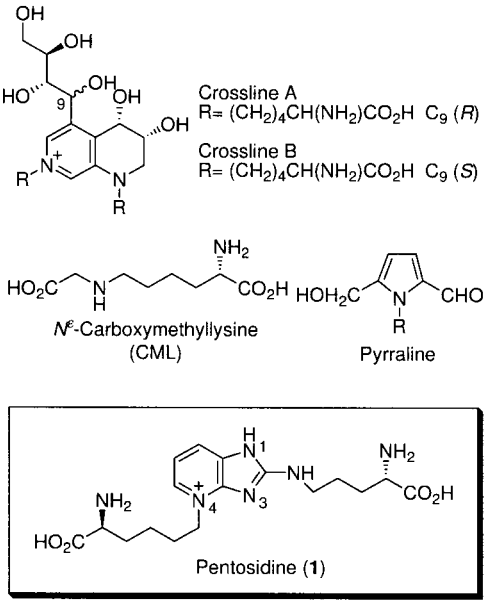


Figure 1. Proposed AGEs compounds.

extracellar matrix by Monnier et al. in 1989.[5] Pentosidine (**1**) contains lysine and arginine as side chains connected to an imidazo[4,5-*b*] pyridine ring. The special significance is the attachment of lysine as a quaternary ammonium salt at the 4-position on the imidazo[4,5-*b*]pyridine ring. While pentosidine (**1**) has already been synthesized by just mixing ribose, lysine and arginine like biosynthesis, several complicated purification steps were necessary and the overall yield was also very low (0.23%).[5] Currently, pentosidine (**1**) is available in very low yield at a high cost by this method. Therefore, pentosidine (**1**) is an attractive synthetic target due to its novel structural features and practical applications in diabetic complications. In this paper, we describe a reliable and efficient total synthesis of pentosidine (**1**).[6]

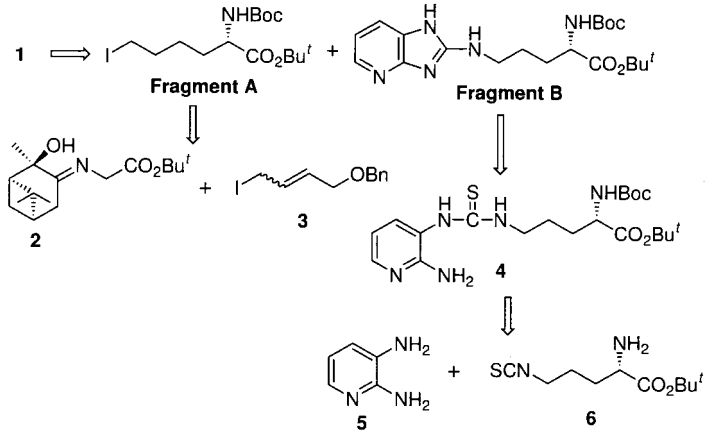
2. Results and discussion

2.1. Retrosynthesis of pentosidine

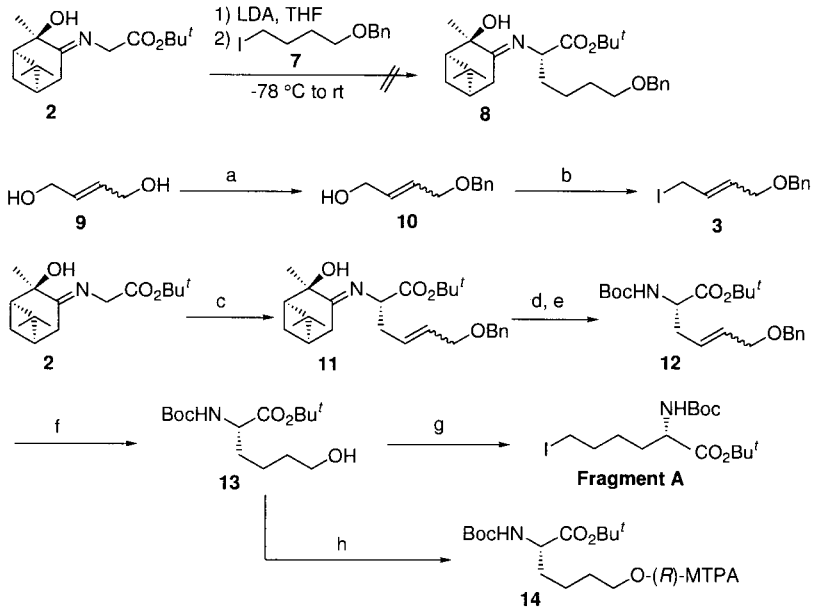
Our synthetic plan is shown in Scheme 2. Pentosidine (**1**) can be disconnected into alkyl iodide (fragment A) and imidazopyridine fragment (fragment B).[7] Because Yutilov has reported that the alkylation of imidazo[4,5-*b*]pyridine with methyl iodide predominantly occurred at the nitrogen of pyridine ring,[8] the regioselective alkylation of fragment B with fragment A can be expected. The unusual amino acid fragment A can be obtained by the asymmetric alkylation of the chiral schiff base **2** derived from (+)-2-hydroxy-3-pinanone ((+)-HyPN)[9] with the allyl iodide **3**. Fragment B can be synthesized by the intramolecular guanylation of the amino thiourea **4**, which is obtained by coupling of 2,3-diaminopyridine (**5**) with the isothiocyanate **6** derived from ornithine.

2.2. Synthesis of fragment A

The preparation of fragment A is shown in Scheme 3. We have previously demonstrated the asymmetric alkylation of a chiral schiff base derived from (+)- or (−)-HyPN, and applied the natural product synthesis.[9] Therefore, we employed this methodology for the preparation of fragment A. Although we initially attempted the alkylation of the chiral schiff base **2**[9] with the benzyl protected alkyl iodide **7**, no alkylated product **8** was obtained due to low reactivity of the alkyl iodide **7**. Accordingly, we next tried to use the allyl iodide **3** in order to increase the reactivity of electrophile. Monoprotection of the hydroxyl function of commercially available **9** with benzyl bromide,[10] followed by iodination of the free hydroxy group afforded the labile iodide **3**, which was immediately used for the alkylation. Deprotonation of the chiral schiff base **2** with lithium diisopropylamide (LDA) followed by the addition of the iodide **3** gave the imine **11** in 87% yield. After removal of the chiral auxiliary under mild acidic conditions, protection of the resulting amino group with Boc_2O provided the amino ester **12** in 80% yield. Simultaneous hydrogenation of the double bond and deprotection of the benzyl group were carried out under hydrogen in the presence of a catalytic amount of palladium on carbon to give the alcohol **13** in 77% yield. The enantiomeric excess of the alcohol **13** was determined to be 92% based on HPLC analysis of the corresponding MTPA ester **14**. Conversion of the primary alcohol group to the iodide was achieved with iodine and triphenylphosphine to give fragment A in 99% yield.[11]



Scheme 2. Retrosynthetic analysis of pentosidine.



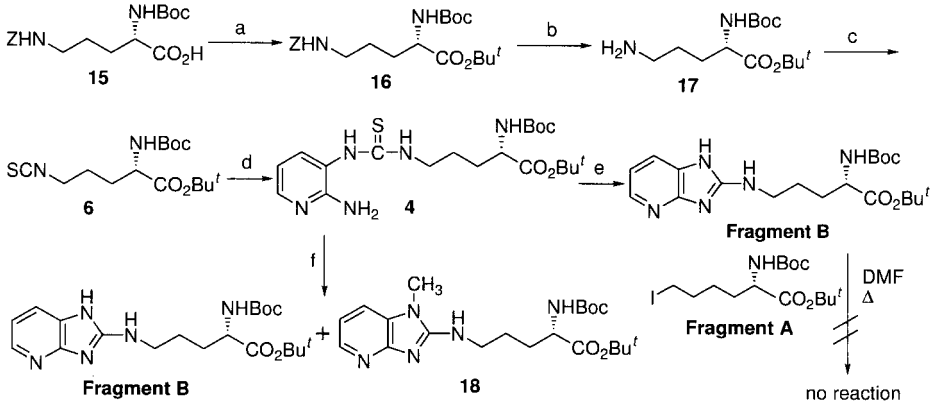
Scheme 3. Synthesis of fragment A (a) NaH (0.5 equiv.), BnBr (0.5 equiv.), DMF, rt, 1 h, 36%. (b) I_2, Ph_3P, imidazole, PhH, rt, 0.5 h. (c) LDA, THF, −78°C, then, **3**, −78°C, 2 h, 87%. (d) 15% citric acid/THF, rt. (e) Boc_2O, dioxane, rt, 80% in 2 steps. (f) H_2, 5% Pd/C, EtOAc, rt, 77%. (g) I_2, Ph_3P, imidazole, PhH, rt, 0.5 h, 99%.

2.3. Synthesis of fragment B

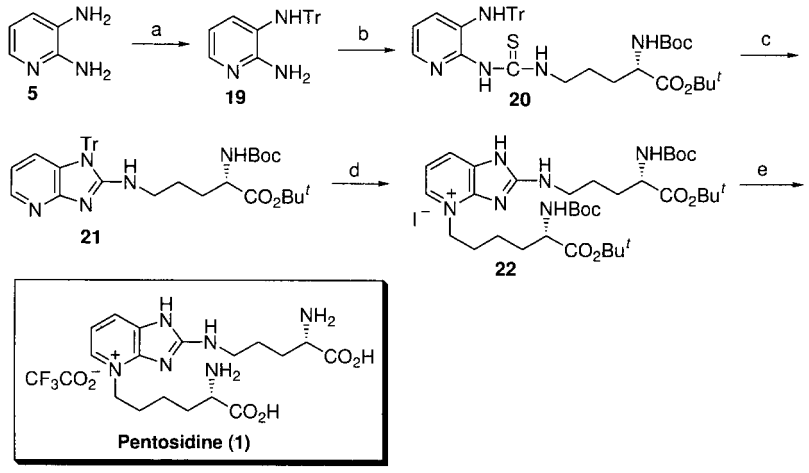
The synthesis of fragment B is summarized in Scheme 4. After Boc-L-Orn(*Z*)-OH (**15**) was converted to the *tert*-butyl ester **16** with *O*-*tert*-butyl-*N*,*N*′-diisopropyl isourea,[12] deprotection of the carbobenzyloxy (*Z*) group gave the amine **17**. Treatment of the amine **17** with triethylamine, carbondisulfide and 30% aqueous hydrogen peroxide[13] afforded the isothiocyanate **6** in 87% yield (2 steps). Under careful optimized reaction conditions, coupling of **6** with 2,3-diaminopyridine (**5**) in the presence of triethylamine cleanly proceeded to give the thiourea **4**, which was converted to fragment B having the imidazo[4,5-*b*]-pyridine ring by intramolecular guanylation with lead(II) acetate trihydrate.[14] Alternatively, treatment of **4** with methyl iodide gave fragment B accompanied by the undesired product **18**, which was obtained by additional methylation at the 1-position of the imidazole ring.[14] This result suggested that regioselective quaternization at the pyridine ring of fragment B with alkyl iodide was problematic. Moreover, alkylation of fragment B with fragment A did not proceed at all. To overcome this problem we attempted to introduce the electron donating group at the 1-position of the imidazole moiety for activation of the imidazo[4,5-*b*]-pyridine ring and regioselective quaternization at the pyridine ring.[15] The group we chose was the trityl group.

2.4. Total synthesis of pentosidine

As shown in Scheme 5, monotritylation of **5** followed by coupling with the isothiocyanate **6** gave the labile thiourea **20**. Intramolecular guanylation of **20** by mercury(II) chloride[16] rapidly proceeded to afford the trityl protected imidazo[4,5-*b*]pyridine derivative **21** in 54% yield from **19**. As expected, the quaternization of **21** with fragment A proceeded with concomitant deprotection of the trityl group to provide the quaternary salt **22** in 81% yield.[17] Finally, the cleavage of all the protective groups of **22** by treatment with



Scheme 4. Synthesis of fragment B (a) *O*-*tert*-butyl-*N*,*N*′-diisopropyl isourea, *t*-BuOH, CH_2Cl_2, 51°C, 20 h, quant. (b) H_2, 5% Pd/C, EtOAc, rt, 12 h. (c) Et_3N, CS_2, THF, 0°C, 40 min. then, 30% aq. H_2O_2, 0°C, 87% in 2 steps. (d) **5**, Et_3N, THF, reflux, 14 h, 76%. (e) $Pb(OAc)_2{\cdot}3H_2O$, Et_3N, MeOH, reflux, 2d, 80%. (f) CH_3I, Et_3N, MeOH, reflux, 2 d, 35% and **18**, 41%.



Scheme 5. Total synthesis of pentosidine (a) TrCl, Et_3N, THF, rt, 1 h, 58%. (b) **6**, Et_3N, THF, reflux, 4 d. (c) $HgCl_2$, Et_3N, MeOH, 0°C, 10 min, 54% in 2 steps. (d) Fragment A, THF, reflux, 48 h, 81%. (e) TFA/$CHCl_3$, rt, 11 h, quant.

trifluoroacetic acid (TFA) furnished pentosidine (**1**) as its TFA salt quantitatively. HPLC purification of the crude product afforded pure pentosidine (**1**) in 96% yield. The synthetic pentosidine obtained by this procedure was identical to the authentic sample[18] based on several criteria: ^{1}H NMR, ^{13}C NMR, FAB-HRMS, and HPLC.

In conclusion, a reliable and efficient total synthesis of pentosidine (**1**) was developed from commercially available compounds. According to our strategy, we have synthesized approximately 1 g of pentosidine (**1**). Our synthetic pentosidine is currently employed for the biochemical investigations.

3. Experimental

3.1. General information

Melting points were determined on a YANAGIMOTO micro melting point apparatus (hot plate) and uncorrected. Infrared (IR) spectra were measured with a SHIMADZU FTIR-8100 spectrometer. Optical rotations were measured on a JASCO DIP-1000 digital polarimeter with sodium lamp (λ=589 nm, D line) and were recorded as follows: $[\alpha]_D^T$ (*c* g/100 mL, solvent). ^{1}H- or ^{13}C NMR spectra were recorded on a JEOL EX-270 spectrometer in deuterio solvents using tetramethylsilane or $CHCl_3$ as an internal standard. Mass spectra were obtained on a JEOL JMS-SX 102A (EI) and JMS-AX 505HA (FAB) spectrometer. Analytical thin layer chromatography (TLC) was performed on a Merck Art. 5715, Kieselgel 60 F_{254}/0.25 mm thickness plates. Visualization was accomplished with UV light, phosphomolybdic acid, or ninhydrin solution followed by heating. Column chromatography was performed with silica gel BW-820MH or BW-200 (Fuji Davison Co.). HPLC was carried out with a JASCO UV-970 (detector) and PU-980 (pump) high pressure liquid chromatography. Reverse phase HPLC was performed with a SHIMADZU SCL-6A system and JASCO FP-110 (fluorescence detector). Solvents for extraction and chromatography were reagent grade and distilled from the indicated drying agents: Tetrahydrofuran (THF) was dried by distillation from sodium/benzophenone ketyl. Dichloromethane (CH_2Cl_2) was dried by distillation from calcium hydride. Other solvents were dried and stored over molecular sieves (3 or 4 Å).

3.1.1. (2*EZ*)-4-Benzyloxy-2-butenol (10). To a stirred suspension of NaH (60% oil dispersion, 200 mg, 5.0 mmol) in DMF (20 mL) was added dropwise a solution of *cis* and *trans*-2-butene-1,4-diol (**9**) (881 mg, 10 mmol) in DMF (10 mL) at −10°C under argon. The mixture was stirred at −10°C for 1 h under argon, and benzyl bromide (0.6 mL, 5 mmol) was added dropwise to the mixture at −10°C. After stirring at −10°C for 1 h, the reaction was quenched with 1 M aqueous $KHSO_4$ (150 mL). After salting-out, the mixture was extracted with Et_2O (50 mL×4). The extracts were washed with saturated brine, and dried over Na_2SO_4. Filtration and concentration in vacuo gave the crude residue, which was purified by column chromatography (silica gel BW-820MH, 150 g, hexane–EtOAc= 10:1 to 1:1) to give the monobenzylated product **10** (645 mg, 36%) as a colorless oil and the dibenzylated product (192 mg, 7%) as a colorless oil.

Monobenzylated product **10**: IR ν_{max}^{neat} cm^{-1}: 3390, 1496, 1454, 1095. ^{1}H NMR ($CDCl_3$) δ: 1.60 (1H, brt, disappeared with D_2O, O*H*), 4.05 (2H, dd, *J*=4.5, 6.0 Hz, CH═CHCH_2OH), 4.18 (2H, d, *J*=4.5 Hz, CH_2OBn), 4.53 (2H,s×2, C$H_2C_6H_5$), 5.70–6.00 (2H, m, C*H*═C*H*), 7.28–7.35 (5H, m, C_6H_5).

Dibenzylated product: IR ν_{max}^{neat} cm^{-1}: 1496, 1454, 1093. ^{1}H NMR ($CDCl_3$) δ: 4.04–4.07 (4H, m, (═CHCH_2OBn)$_2$), 4.50 (4H, s×2, C$H_2C_6H_5$×2), 5.77–5.80 (1H, m, C*H*═CH), 5.87–5.89 (1H, m, C*H*═CH), 7.3 (10H, m, C_6H_5×2).

3.1.2. (2*EZ*)-4-Benzyloxy-1-iodo-2-butene (3). To a stirred solution of the monobenzylated alcohol **10** (300 mg, 1.68 mmol) in benzene (17 mL) were added successively imidazole (252 mg, 4.2 mmol), triphenylphosphine (1.10 g, 4.2 mmol) and iodine (850 mg, 4.2 mmol) at room temperature. After being stirred for 30 min, the reaction was quenched with saturated aqueous $Na_2S_2O_3$ (20 mL) and extracted with ether (50 mL). The extracts were washed with saturated aqueous $Na_2S_2O_3$ (20 mL×2) and saturated

brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography (silica gel BW-820MH, 100 g, hexane–EtOAc=10:1) to give the iodide **3** (310 mg, 64%) as a pale yellow oil: IR ν_{max}^{neat} cm^{-1}: 1496, 1454, 1360, 1100. ^1H NMR ($CDCl_3$) δ: 3.90 (2H, d, J=7.5 Hz, ICH_2CH), 4.00 (2H, d, J=1.0 Hz, BnOCH_2CH), 4.50 (2H,s, C$H_2C_6H_5$), 5.81–5.87 (1H, m, CH=CH), 5.95–6.07 (1H, m, CH=CH), 7.28–7.38 (5H, m, C_6H_5). This compound was immediately used for the next reaction.

3.1.3. (2*S*,4*EZ*)-(+)-*tert*-Butyl 6-benzyloxy-2-*N*-((1*R*,2*R*, 5*R*)-2-hydroxy-3-pinanylidene)imino-4-hexenoate (11). To a stirred solution of LDA (prepared from *i*-Pr_2NH (1.54 mL, 10.7 mmol) and *n*-BuLi (1.60 M in hexane, 7.33 mL, 10.7 mmol)) in THF (5 mL) was added dropwise a solution of the chiral schiff base **2**[9a] (1.50 g, 5.33 mmol) in THF (5 mL plus 2 mL of rinse) at −78°C, and the mixture was stirred at −78°C for 1 h. A solution of the iodide **3** (3.07 g, 10.7 mmol) in THF (5 mL plus 2 mL of rinse) was added to the mixture at −78°C. The resulting mixture was stirred at −78°C, for 2 h and quenched with saturated aqueous NH_4Cl (15 mL). After dilution with EtOAc (100 mL), the mixture was washed with H_2O (30 mL) and saturated brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by column chromatography (silica gel BW-820MH, 150 g, hexane–EtOAc=3:1) to give **11** (2.05 g, 87%) as a yellow oil: $[\alpha]_D^{26}$=−46.5 (*c* 1.0, $CHCl_3$). IR ν_{max}^{neat} cm^{-1}: 3440, 1732, 1651, 1454, 1367, 1155. ^1H NMR ($CDCl_3$) δ: 0.81 (3H, s, CH_3), 1.31 (3H, s, CH_3), 1.47 (9H, s, *Bu*^{t}), 1.50–1.63 (5H, m, decrease 1H with D_2O, CH_3, pinene-4-*H*, O*H*), 2.00–2.09 (2H, m, pinene-3, 5-*H*), 2.25–2.35 (1H, m, pinene-4-*H*), 2.42–2.69 (4H, m, CHCH_2CH=CH, pinene-6-CH_2), 3.94 (2H, d, J=4.6 Hz, CH=CHCH_2OBn), 4.12–4.16 (1H, m, α-C*H*), 4.49 (2H, s×2, C$H_2C_6H_5$), 5.64–5.68 (2H, m, C*H*=C*H*), 7.31–7.38 (5H, m, C_6H_5). Anal. calcd for $C_{27}H_{39}NO_4$; C, 73.44;H, 8.90; N, 3.17. Found; C, 73.18; H,8.85; N, 2.85.

3.1.4. (2*S*,3*EZ*)-*tert*-Butyl 6-benzyloxy-2-*N*-(*tert*-butoxycarbonyl)amino-4-hexenoate (12). To a stirred solution of **11** (1.69 g, 4.44 mmol) in THF (12 mL) was added 15% aqueous citric acid (12 mL) and the mixture was stirred at room temperature for 12 h. After the solvent was removed, the residue was dissolved in benzene (30 mL), and the mixture was extracted with 15% aqueous citric acid (10 mL×3).The combined aqueous layer was basified to pH 9 with potassium carbonate, salted out, and extracted with ether (20 mL×8). The ethereal extracts were dried over $MgSo_4$, filtered, and concentraded in vacuo. To a solution of the residue in dioxane (10 mL) was added a solution of Boc_2O (2.2 g, 10 mmol) in dioxane (4 mL). After being stirred at room temperature for 10 h, the reaction was quenched with H_2O (10 mL). After dilution with ether (80 mL), the mixture was washed with H_2O (20 mL) and saturated brine (20 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified with column chromatography (silica gel BW-820MH, 35 g, hexane–EtOAc=20:1 to 10:1) to give **12** (1.39 g, 80%) as a colorless oil: $[\alpha]_D^{22}$=+18.7 (*c* 1.0, $CHCl_3$). IR ν_{max}^{neat} cm^{-1}: 3360, 1780, 1716, 1496, 1367, 115. ^1H NMR ($CDCl_3$) δ: 1.43 (9H, s, *Bu*^{t}), 1.45 (9H, s, *Bu*^{t}), 2.40–2.59 (2H, m, CHCH_2CH=CH), 4.00 (2H, dd, J= 5.9, 7.4 Hz, CH=CHCH_2OBn), 4.25 (1H, brd, α-C*H*), 4.50 (2H, s×2, C$H_2C_6H_5$), 5.10 (1H, b, N*H*), 5.56–5.82 (2H, m, C*H*=C*H*), 7.28–7.34 (5H, m, C_6H_5). Anal. calcd for $C_{22}H_{33}NO_5$; C, 67.49; H, 8.50; N, 3.58. Found; C, 67.40; H, 8.57; N, 3.54.

3.1.5. (2*S*)-*tert*-Butyl 2-*N*-(*tert*-butoxycarbonyl)amino-6-hydroxyhexanoate (13). To a solution of **12** (1.39 g, 3.56 mmol) in EtOAc (25 mL) was added 5% Pd/C (400 mg) under argon, and hydrogen gas was introduced (balloon with needle through septum). The black slurry was stirred under 1 atm of H_2 at room temperature for 10 h. The mixture was filtered through the pad of Celite® and the filtrate was concentrated in vacuo. The residue was purified by column chromatography (silica gel BW-820MH, 50 g, hexane:EtOAc=3:2) to give the alcohol **13** (938 mg, 87%) as a colorless oil. $[\alpha]_D^{27}$=+7.0 (*c* 1.0, $CHCl_3$). IR ν_{max}^{neat} cm^{-1}: 3390, 1719, 1700, 1367, 1157. ^1H NMR ($CDCl_3$) δ: 1.44 (9H, s, *Bu*^{t}), 1.46 (9H, s, *Bu*^{t}), 1.49–1.86 (7H, m, decrease 1H with D_2O, CH_2×3, O*H*), 3.64 (2H, t, J=6.0 Hz, CH_2OH), 4.18 (1H, brs, α-C*H*), 5.05 (1H, brd, N*H*Boc), ^{13}C NMR ($CDCl_3$) δ: 21.35 (CH_2), 27.88 (CH_3), 28.21 (CH_3), 32.04 (CH_2), 32.54 (CH_2), 53.75 (CH), 62.12 (CH_2), 79.48 (C), 81.06 (C), 155.40 (C=O), 171.93 (C=O). Anal. calcd for $C_{15}H_{29}NO_5$; C, 59.38; H, 9.63; N, 4.62. Found; C, 59.26; H, 9.62; N, 4.36. The enantiomeric purity (92% ee) was determined as its MTPA ester **14** (see below).

3.1.6. (2*S*)-*tert*-Butyl 2-*N*-(*tert*-butoxycarbonyl)amino-6-iodohexanoate (fragment A). To a solution of the alcohol **13** (62 mg, 0.2 mmol) in benzene (2 mL) were added successively imidazole (30 mg, 0.5 mmol), triphenylphosphine (131 mg, 0.5 mmol) and iodine (102 mg, 0.4 mmol), and the mixture was stirred at room temperature for 0.5 h. The reaction was quenched with saturated aqueous $Na_2S_2O_3$ (2 mL). After dilution with EtOAc (20 mL), the mixture was washed with saturated aqueous $Na_2S_2O_3$ (5 mL×3), H_2O (5 mL), saturated brine (5 mL), and dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by column chromatography (silica gel BW-200, 20 g, hexane: EtOAc=20:1) to give fragment A (83 mg, 99%) as a colorless oil: $[\alpha]_D^{22}$=+14.2 (*c* 1.0, $CHCl_3$). IR ν_{max}^{neat} cm^{-1}: 3387, 1738, 1714, 1504, 1367, 1155. ^1H NMR ($CDCl_3$) δ: 1.44 (9H, s, *Bu*^{t}), 1.46 (9H, s, *Bu*^{t}), 1.56–1.87 (6H, m, CH_2×3), 3.18 (2H, t, J=7.0 Hz, CH_2I), 4.14–4.23 (1H, m, α-C*H*), 5.05 (1H, brd, N*H*Boc). ^{13}C NMR ($CDCl_3$) δ: 6.31 (CH_2I), 25.90 (CH_2), 27.94 (CH_3), 28.25 (CH_3), 31.75 (CH_2), 32.78 (CH_2), 53.57 (CH), 79.53 (C), 81.82 (C), 155.22 (C=O), 171.63 (C=O). Anal. calcd for $C_{15}H_{28}INO_4$; C, 43.59; H, 6.83; N, 3.39. Found; C, 43.87; H, 7.19; N, 3.22.

3.1.7. (2*S*)-*tert*-Butyl 2-*N*-(*tert*-butoxycarbonyl)amino-6-(α-methoxy-α-trifluoromethyl-acetoxy)-hexanoate (14). To a solution of **13** (18 mg, 0.059 mmol) in CH_2Cl_2 (1.0 mL) at 0°C were added (*R*)-MTPA (14 mg, 0.06 mmol), DCC (20 mg, 0.08 mmol) and DMAP (2.5 mg, 0.02 mmol). The reaction mixture was stirred at 0°C for 0.5 h and at room temperature for 11.5 h. After addition of Et_2O (10 mL), the mixture was filtered through the pad of Celite® and the filtrate was successively washed with 15% aqueous citric acid, water, saturated aqueous

$NaHCO_3$, water, saturated brine, and dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography (silica gel BW-200, 10 g, hexane–EtOAc=10:1) to give the ester **14** (32 mg, quant.) as a colorless oil: IR ν_{max}^{neat} cm^{-1}: 3395, 1748, 1714, 1505, 1455, 1368, 1234, 1157, 1024. ^1H NMR ($CDCl_3$) δ: 1.44 (9H, s, *Bu*^{t}), 1.45 (9H, s, *Bu*^{t}), 1.53–1.79 (6H, m, C*H*$_2$×3), 3.54 (3H, s, OC*H*$_3$), 4.13 (1H, brt, α-C*H*), 4.28–4.34 (2H, m, C*H*$_2$OMTPA), 5.00 (1H, brd, N*H*Boc), 7.39–7.49 (3H, m, Ph (*o*, *p*)), 7.50–7.52 (2H, m, Ph (*m*)). HPLC analysis of **14** was carried out as followed; Column: Daicel Chiralcel OD; Solvent: Hexane–*i*-PrOH=20:1; Flow Rate: 0.5 mL/min; Detector: 254 nm; Retention Time: 11.5 (minor isomer) and 12.5 min (major isomer). The enantiomeric purity of **14** was 92% ee.

3.1.8. Boc-L-Orn(*Z*)-OBu^t (16). To a stirred solution of Boc-L-Orn(*Z*)-OH (**15**) (3.66 g, 10 mmol) in *t*-BuOH/CH_2Cl_2 (1:1, 40 mL) was added *N*,*N*′-diisopropyl-*O*-*t*-butylisourea (12 mL, 50 mmol). The mixture was stirred at 50°C for 20 h. The mixture was filtered through the pad of Celite® and the filtrate was concentrated in vacuo. The residue was purified by column chromatography (silica gel BW-820MH, 150 g, hexane–EtOAc=5:2) to give Boc-L-Orn(*Z*)-OBu^t (**16**) (4.92 g, quant.) as a colorless oil: $[\alpha]_D^{23}$=+9.6 (*c* 1.1, $CHCl_3$). IR ν_{max}^{neat} cm^{-1}: 3357, 1738, 1696, 1537, 1456, 1367, 1252, 1153. ^1H NMR ($CDCl_3$) δ: 1.43 (9H, s, *Bu*^{t}), 1.45 (9H, s, *Bu*^{t}), 1.49–1.67 (3H, m, β-C*H*$_2$, C*H*$_2$), 1.80 (1H, m, β-C*H*$_2$), 3.20–3.26 (2*H*, m, ZNHC*H*$_2$), 4.12–4.17 (1H, m, α-C*H*), 4.86 (1H, brs, ZN*H*), 5.09 (3H, s, N*H*Boc, C*H*$_2$C$_6$H$_5$), 7.30–7.36 (5H, m, C$_6$*H*$_5$). Anal. calcd for $C_{22}H_{34}N_2O_6$; C, 62.54; H, 8.11; N, 6.63. Found; C, 62.46; H, 8.18; N, 6.37.

3.1.9. (2*S*)-*tert*-Butyl 2-*N*-(*tert*-butoxycarbonyl)amino-5-isothiocyanatopentanoate (6). To a stirred solution of Boc-L-Orn(*Z*)-OBu^t (**16**) (14.25 g, 33.7 mmol) in EtOAc (100 mL) was added 5% Pd/C (2.8 g) under argon, and hydrogen gas was introduced (balloon with three way cock). The black slurry was stirred under 1 atm of H_2 at room temperature for 5 h. The mixture was filtered through the pad of Celite® and the filtrate was concentrated in vacuo.

The crude amine **17** was dissolved in THF (160 mL), and triethylamine (9.4 mL, 67.4 mmol) and carbon disulfide (10.1 mL, 168.5 mmol) were added to the mixture at 0°C. After being stirred at 0°C for 40 min, 30% aqueous H_2O_2 (23 mL, 202.2 mmol) was added dropwise at 0°C, and then the mixture was diluted with ether (300 mL). The mixture was washed with 1 M aqueous $KHSO_4$ (100 mL), H_2O (100 mL), saturated brine (100 mL), dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by column chromatography (silica gel BW-820MH, 300 g, hexane–EtOAc=20:1 to 5:1) to give the isothiocyanate **6** (9.66 g, 87%) as a pale yellow oil: $[\alpha]_D^{22}$=+17.2 (*c* 1.1, $CHCl_3$). IR ν_{max}^{neat} cm^{-1}: 3330, 2187, 2110, 1738, 1714, 1504, 1454, 1367, 1250, 1155, 1059. ^1H NMR ($CDCl_3$) δ: 1.44 (9H, s, *Bu*^{t}), 1.48 (9H, s, *Bu*^{t}), 1.65–1.94 (4H, m, C*H*$_2$×2), 3.56 (2H, t, *J*=6.0 Hz, SCNC*H*$_2$), 4.16–4.24 (1H, m, α-C*H*), 5.08 (1H, brd, N*H*Boc). ^{13}C NMR ($CDCl_3$) δ: 25.79 (CH_2), 27.80 (CH_3), 28.12 (CH_3), 29.92 (CH_2), 44.46 (CH_2), 52.94 (CH), 79.62 (C), 82.12 (C), 130.44 (SCN), 155.20 (C=O), 171.12 (C=O). Anal. calcd for $C_{15}H_{26}N_2O_4S$; C, 54.52; H, 7.93; N, 8.48. Found; C, 54.61; H, 7.86; N, 8.30.

3.1.10. (2*S*)-*tert*-Butyl 2-*N*-(*tert*-butoxycarbonyl)amino-5-*N*′-(3-(2-aminopyrydinyl)ureido)-pentanoate (4). To a stirred solution of the isothiocyanate **6** (3.62 g, 10.95 mmol) in THF (50 mL) were added 2,3-diaminopyridine (**5**) (1.31 g, 12.04 mmol) and Et_3N (3 mL, 21.9 mmol). The mixture was heated under reflux for 14 h and concentrated in vacuo. The residue was purified by column chromatography (silica gel BW-820MH, 250 g, hexane–EtOAc=1:4 to EtOAc:MeOH=10:1) to give the thiourea **4** (3.64 g, 76%) as a pale brown amorphous solid: $[\alpha]_D^{22}$=+13.7 (*c* 1.1, $CHCl_3$). IR ν_{max}^{neat} cm^{-1}: 3379, 3332, 1738, 1714, 1614, 1537, 1456,1367, 1153. ^1H NMR ($CDCl_3$) δ: 1.41 (9H, s, *Bu*^{t}), 1.45 (9H, s, *Bu*^{t}), 1.63–1.81 (4H, m, C*H*$_2$×2), 3.64 (2H, m, C(S)NHC*H*$_2$) 4.04–4.19 (1H, m, α-C*H*), 4.83 (2H, brs, disappeared with D_2O, N*H*$_2$), 5.11 (1H, brd, N*H*Boc), 6.07 (1H, brs, disappeared with D_2O, C(S)N*H*CH$_2$), 6.71 (1H, dd, *J*=5.0, 7.6 Hz, pyridine-5), 7.37 (2H, dd, *J*=1.7, 7.6 Hz, pyridine-4 and brs, decrease 1H with D2O, N*H*C(S)NH), 8.08 (1H, dd, *J*=1.7, 5.0 Hz, pyridine-6). ^{13}C NMR ($CDCl_3$) δ: 24.64 (CH_2), 27.77 (CH_3), 28.09(CH_3), 29.89 (CH_2), 44.55 (CH_2), 53.41 (CH), 79.52 (C), 81.83 (C), 113.98 (CH), 116.70 (C), 136.64 (CH), 147.58 (CH), 155.33 (C), 155.74 (C=O), 171.50 (C=O), 180.97 (C=S). HRMS (EI) calcd for $C_{20}H_{33}N_5O_4S$; 439.2253. Found; 439.2260.

3.1.11. (2*S*)-*tert*-Butyl 2-*N*-(*tert*-butoxycarbonyl)amino-5-*N*-(2-imidazo[4,5-*b*]pyridinyl)amino-pentanoate (fragment B). To a stirred solution of the thiourea **4** (200 mg, 0.46 mmol) in MeOH (4.5 mL) were added $Pb(OAc)_2{\cdot}3H_2O$ (436.2 mg, 1.14 mmol) and Et_3N (63 μL, 0.46 mmol), and the mixture was heated under reflux for 45 h. After dilution with MeOH, the mixture was filtered through the pad of Celite® and the filtrate was concentrated in vacuo. The residue was purified by column chromatography (silica gel BW-820MH, 50 g, $CHCl_3$–MeOH=13:1) to give fragment B (148 mg, 80%) as a white solid: mp 195–196°C (MeOH). $[\alpha]_D^{25}$=−15.3 (*c* 0.1, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3389, 1734, 1699, 1651, 1628, 1504, 1417, 1363, 1259, 1180, 1153. ^1H NMR (CD_3OD) δ: 1.41 (9H, s, *Bu*^{t}), 1.42 (9H, s, *Bu*^{t}), 1.72–1.94 (4H, m, C*H*$_2$×2), 3.43 (2H, t, *J*=6.0 Hz, NHC*H*$_2$), 3.99 (1H, brs, α-C*H*), 6.97 (1H, dd, *J*=5.0, 7.5 Hz, pyridine-5-*H*), 7.48 (1H, d, *J*=7.5 Hz, pyridine-4-*H*), 7.91 (1H, brs, pyridine-6-*H*). HRMS (EI) calcd for $C_{20}H_{31}N_5O_4$; 405.2376. Found; 405.2369. Anal. calcd for $C_{20}H_{31}N_5O_4{\cdot}1/2MeOH$; C, 58.41; H, 7.89; N, 16.61. Found; C, 58.72; H, 7.59; N, 16.96.

3.1.12. 2-Amino-3-triphenylmethylaminopyridine (19). To a stirred solution of 2,3-diaminopyridine (**5**) (110 mg, 1.01 mmol) in THF (3 mL) were added TrCl (306.7 mg, 1.1 mg) and Et_3N (280 μl, 2 mmol). After being stirred at room temperature for 1 h, the mixture was concentrated in vacuo. The residue was purified by column chromatography (silica gel BW-200, 10 g, $CHCl_3$–MeOH=50:1) to give **19** (207 mg, 58%) as a purple amorphous powder: IR ν_{max} $CHCl_3$ cm^{-1}: 3359, 1614, 1597, 1574, 1487, 1458, 1448, 1215, 756. ^1H NMR ($CDCl_3$) δ: 4.22 (2H, brs, disappeared with D_2O, N*H*$_2$), 4.76 (1H, s N*H*Tr), 6.21–6.30 (2H, m, pyridine-4, 5-*H*), 7.19–7.34 (15H, m, *Ph*$_3$C), 7.44 (1H, dd,

J=1.5, 4.5 Hz, pyridine-6-*H*). ^{13}C NMR ($CDCl_3$) δ: 71.41 (C), 115.71(CH), 122.43 (CH), 127.39 (CH), 128.41 (CH), 129.36 (CH), 130.64 (C), 136.59 (CH), 145.12 (C), 148.79 (C). HRMS (EI) calcd for $C_{24}H_{21}N_3$; 351.1735. Found; 351.1737.

3.1.13. (2*S*)-*tert*-Butyl 2-*N*-(*tert*-butoxycarbonyl)amino-5-*N'*-(2-(3-*N*-(triphenylmethyl)amino-pyridinyl)ureido)-pentanoate (20). To a stirred solution of **19** (2.95 g, 8.39 mmol) in THF (25 mL) were added the isothiocyanate **10** (2.77 g, 8.39 mmol) and Et_3N (2.34 mL, 16.78 mmol). The mixture was refluxed for 87 h, and concentrated in vacuo. The residue was purified by column chromatography (silica gel BW-820MH, 280 g, hexane–EtOAc=4:1 to 1:1) to give the thiourea **20** (3.06 g) as a greenish brown amorphous solid. Because **20** was highly unstable, this material was immediately used for the next step without further purification.

3.1.14. (2*S*)-*tert*-Butyl 2-*N*-(*tert*-butoxycarbonyl)amino-5-*N*-(2-1-triphenylmethyl)-1*H*-imidazo-[4,5-*b*]pyridinyl)-aminopentanoate (21). To a stirred solution of the thiourea **20** (1.8 g, 2.64 mmol) in MeOH (9 mL) at 0°C were added Et_3N (1.2 mL, 8.61 mmol) and $HgCl_2$ (1.08 g, 3.98 mmol). After being stirred at 0°C for 10 min, the mixture was diluted with $CHCl_3$ (20 mL) and filtered through the pad of Celite® (rinsed with $CHCl_3$), and the filtrate was concentrated in vacuo. The residue was purified by column chromatography (silica gel BW-820MH, 150 g, $CHCl_3$–MeOH=50:1 to 30:1) to give **21** (1.766 g, 54% from **19**) as a pale brown amorphous solid: $[\alpha]_D^{22}$=+9.8 (*c* 1.1, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3440, 1713, 1605, 1549, 1495, 1410, 1367, 1153, 731. ^{1}H NMR ($CDCl_3$) δ: 1.18–1.27 (4H, m, C*H*$_2$×2), 1.42 (9H, s, *Bu*^{t}), 1.43 (9H, s, *Bu*^{t}), 3.29–3.36 (2H, m, NHC*H*$_2$), 3.76 (1H, brt, N*H*CH$_2$), 4.00 (1H, brs, α-C*H*), 4.85 (1H, brd, N*H*Boc), 5.63 (1H, dd, J=1.3, 8.3 Hz, pyridine-5-*H*), 6.46 (1H, dd, J=5.0, 8.0 Hz), 7.34 (15H, brs, *Ph*$_3$C), 8.08 (1H, dd, J=1.0, 5.0 Hz, pyridine-6-*H*). ^{13}C NMR ($CDCl_3$) δ: 25.11 (CH_2), 27.87 (CH_3), 28.23(CH_3), 29.98 (CH_2), 42.97 (CH_2), 53.51 (CH), 74.86 (C), 79.44 (C), 81.65 (C), 113.75 (CH), 118.98 (CH), 128.25 (CH), 130.0 (CH), 140.93 (C), 141.89 (CH), 155.20 (C), 155.99 (C=O), 157.05 (C), 171.52 (C=O). Anal. calcd for $C_{39}H_{45}N_5O_4$·7/20 $CHCl_3$; C, 71.01; H, 6.87; N, 10.52. Found; C, 70.68; H, 6.98; N, 10.53. ($CHCl_3$, which was used for column chromatography, tenaciously stuck to **21** and was hard to remove.) HRMS (EI) calcd for $C_{20}H_{31}N_5O_4$ (M+H–Ph_3C); 405.2376. Found; 405.2376. FAB-MS (NBA) *m*/*z* 648 (M^+).

3.1.15. Protected pentosidine (22). To a stirred solution of **21** (55.5 mg, 0.0857 mmol) in THF (1 mL) was added fragment A (38.9 mg, 0.0941 mmol) at room temperature. The mixture was heated under reflux for 48 h, and concentrated in vacuo. The residue was purified by column chromatography (silica gel BW-200, 8 g, $CHCl_3$–MeOH–Et_3N=100:2:1) to give **22** (57 mg, 81%) as a pale brown amorphous solid: $[\alpha]_D^{22}$=+13.9 (*c* 1.6, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3439, 1709, 1651, 1554, 1498, 1369, 1155,908, 735. ^{1}H NMR ($CDCl_3$) δ: 1.43 (9H, s, *Bu*^{t}), 1.44(18H, s, *Bu*^{t}), 1.45 (9H, s, *Bu*^{t}), 1.60–2.10 (4H, m, C*H*$_2$×2), 3.60 (2H, brs, NHC*H*$_2$), 4.13–4.24 (2H, brm, α-C*H*×2), 4.40 (2H, brt, N$^+$C*H*$_2$), 5.14 (1H, brd, N*H*Boc), 5.25 (2H, brt, N*H*Boc, N*H*CH$_2$), 6.20 (1H, brs, disappeared with D_2O, imidazole-N*H*), 6.83 (1H, t, J=7.0 Hz, pyridine-5-*H*), 7.28–7.32 (1H, m, pyridine-4-*H*), 7.53 (1H, d, J=7.0 Hz, pyridine-6-*H*). ^{13}C NMR ($CDCl_3$) δ: 21.98 (CH_2), 25.75 (CH_2), 27.59 (CH_3), 27.96 (CH_3), 28.38 (CH_2), 29.60 (CH_2), 31.93 (CH_2), 42.16 (CH_2), 52.35 (CH_2), 53.34 (CH), 35.60 (CH), 79.01 (C), 79.14 (C), 81.24 (C), 81.44 (C), 111.54 (CH), 116.75 (CH), 124.94 (CH), 143.76 (C), 154.82 (C=O), 155.13 (C=O), 168.50 (C), 171.37 (C=O), 171.55 (C=O). FAB-MS (glycerin) *m*/*z* 692 (M−I).

3.1.16. Pentosidine (1). To a solution of **22** (33 mg, 0.04 mmol) in $CHCl_3$ (0.2 mL) at 0°C was added TFA (0.2 mL). After being stirred at room temperature for 11 h, the mixture was concentrated in vacuo. Azeotropic removal of the excess TFA in the residue with toluene gave the crude pentosidine (**1**) (28 mg, quant.) as a pale brown amorphous solid. The crude product (5.3 mg) was purified by reverse phase C_{18} preparative column (Wako sil II 5C-18HG (20×250 mm), linear gradient of 0–10% of acetonitrile from 0–40 min with TFA (0.075–0.01%) as a counter ion at a flow rate of 9.5 mL/min) gave pentosidine (**1**) (5.1 mg, 96%) as a pale brown amorphous solid: $[\alpha]_D^{24}$=+16.5 (*c* 0.3, MeOH). ^{1}H NMR (D_2O) δ: 1.30–1.50 (2H, m), 1.60–2.00 (8H, m), 3.45 (2H, t, J=6.5 Hz), 3.95 (1H, t, J=6.5 Hz), 4.03 (1H, t, J=6.0 Hz), 4.42 (2H, t, J=7.0 Hz), 7.09 (1H, t, J=6.5 Hz), 7.65 (1H, d, J=7.5 Hz), 7.80 (1H, d, J=6.5 Hz). ^{13}C NMR (D_2O) δ: 21.99 (CH_2), 25.19 (CH_2), 27.85 (CH_2), 29.99 (CH_2), 42.62 (CH_2), 53.36 (CH), 53.81 (CH_2), 116.01 (CH), 120.48 (CH), 132.39 (C), 132.79 (CH), 152.05 (C), 160.28 (C), 172.45 (C=O), 172.52 (C=O), 114.94, 119.20 ($CF_3CO_2^-$, J=35.4 Hz), 162.40, 162.92, 163.46, 163.98 ($CF_3CO_2^-$, J=291.8 Hz). HRMS (FAB) (glycerin) calcd for $C_{17}H_{27}N_6O_4$ (M−$CF_3CO_2^-$); 370.2094. Found; 370.2096. HPLC analysis of pentosidine (**1**) was carried out as followed; (1) Column: TSK-GEL ODS-80TM (4.6×250 mm); Solvent: linear gradient; (liquid A; H_2O (0.1% TFA). Liquid B; acetonitrile (0.075% TFA) Time (concentration of liquid B); 0 (0)–40 (10)); Flow Rate: 1.0 mL/min; Detector: fluorescence (exicitation/emission: 335/385 nm); Retention Time: 30.1 min. (2) Column: Vydac 208TP5415 (4.6×150 mm); Solvent: linear gradient; (liquid A; H_2O (0.1% TFA). liquid B; acetonitrile (0.075% TFA) Time (concentration of liquid B); 0 (0)–40 (10)); Flow Rate: 1.0 mL/min; Detector: fluorescence (exicitation/emission:335/385 nm); Retention time: 12.0 min.

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