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Efficient Total Synthesis of Pentosidine, an Advanced Glycation Endproduct

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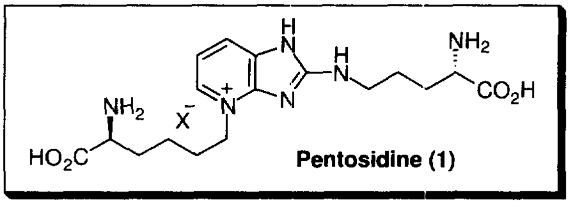
Abstract: The efficient total synthesis of pentosidine (**1**), an advanced glycation endproduct, was achieved using the asymmetric alkylation of a chiral schiff base (**2**), the intramolecular guanylation with mercury (II) chloride, and the quaternization accompanied by removal of the trityl group as key steps.

Keywords: pentosidine, an advanced glycation endproduct, asymmetric synthesis, cyclization, amino acids and derivatives

Pentosidine (**1**) was discovered as a fluorescent protein cross-link isolated from the human extracellar matrix by Monnier *et al.* in 1989.[1] Pentosidine (**1**) contains lysine and arginine as side chains connected to an imidazo[4,5-b]pyridinium ring. The special significance is the attachment of lysine as a quaternary ammonium salt at the 7-position on the imidazo[4,5-b]pyridinium ring. Pentosidine (**1**) is one of the AGEs (Advanced Glycation Endproducts) which are the endproducts of the Maillard reaction (nonenzymatic glycosylation reaction) that occurs between reducing sugars and amino groups on proteins in connection with the aging and the complications of diabetes. The pentosidine content of collagen-rich tissues has been demonstrated to increase with age. Both the skin and plasma contents further increase in the presence of diabetes, where levels are found to correlate with the severity of the complications. The highest levels of pentosidine are found on tissue and circulating proteins from patients with renal failure.[2]



Pentosidine (1)

Recently, the measurement of AGEs has been attempted by building up the antibodies to pentosidine (**1**). Although pentosidine (**1**) has already been synthesized by just mixing ribose, lysine and arginine[1] like biosynthesis, several complicated purification steps were necessary and the overall yield was also very low (0.23%). Consequently, the development of a more efficient supply of pentosidine (**1**) is needed to facilitate the

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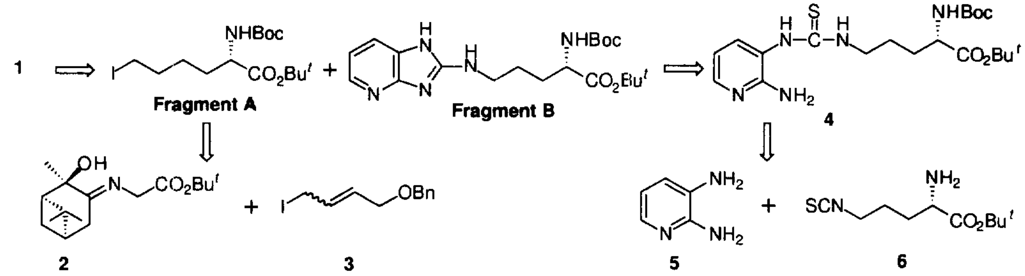
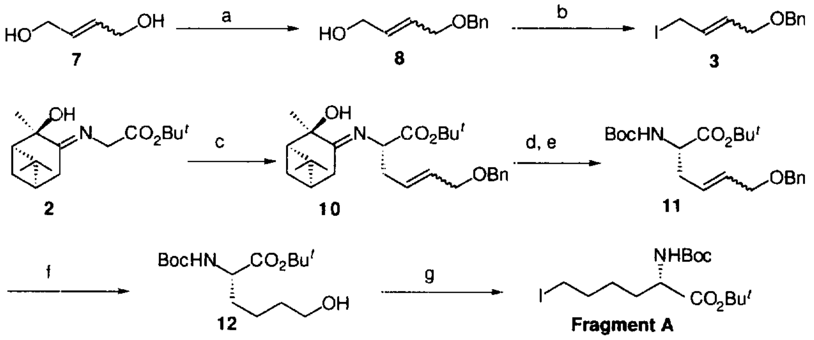
further investigation of it's biological role. In this paper, we describe the efficient total synthesis of pentosidine (1).

Our synthetic strategy is shown in Scheme 1. Pentosidine (1) could be constructed by the quaternization of the imidazo[4,5-b]pyridine ring (fragment B) with alkyl iodide (fragment A). The unusual amino acid fragment A would be obtained by the asymmetric alkylation of the chiral Schiff base 2 derived from (+)-2-hydroxy-3-pinanone ((+)-HyPN) (9)[3] with allyl iodide 3. Fragment B would be synthesized by the intramolecular guanylation[4] of 4, which is obtained by coupling of 2,3-diaminopyridine 5 with the isothiocyanate 6 derived from ornithine.



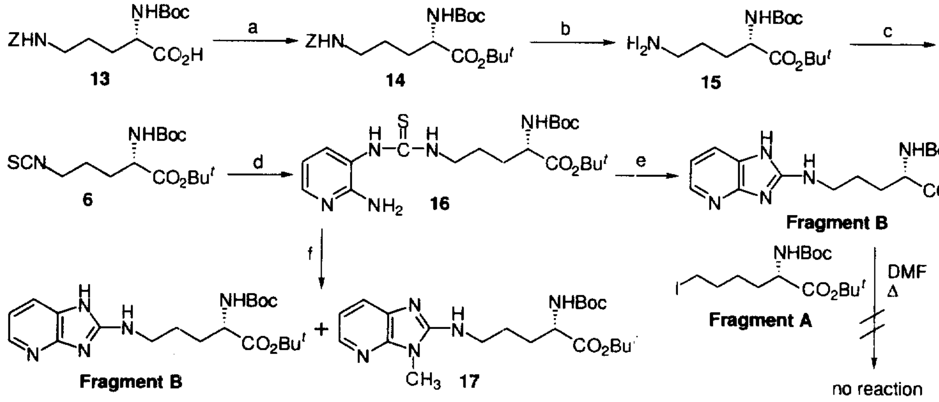
Scheme 1

The synthesis of fragment A began with 7 which is commercially available as a cis and trans mixture (Scheme 2).[5] Monoprotection of the hydroxyl function of 7 with benzyl bromide,[6] followed by iodination of the free hydroxyl group afforded the labile iodide 3, which was immediately used for the alkylation. According to our asymmetric alkylation procedure,[3] deprotonation of the chiral Schiff base 2 with lithium diisopropylamide (LDA) followed by the addition of the iodide 3 gave the imine 10 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 11 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 Pd/C to give the alcohol 12 in 77% yield. The enantiomeric excess of the alcohol 12 was determined to be 92% based on HPLC analysis of the corresponding MTPA ester of 12. Conversion of the primary alcohol to the iodide was achieved with iodine and triphenylphosphine to give fragment A in 99% yield.



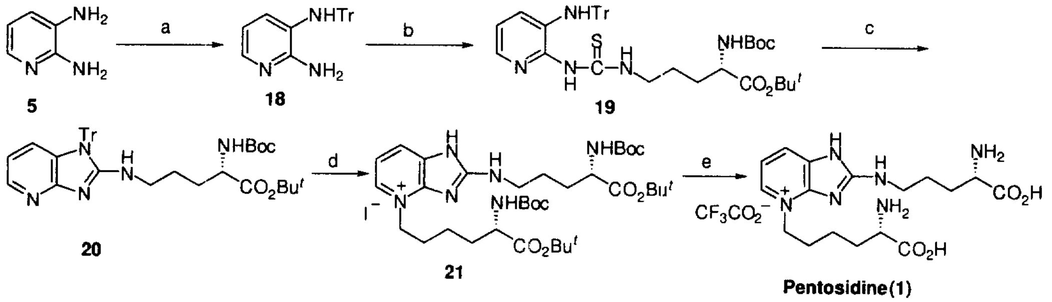
Scheme 2. a) NaH (0.5eq), BnBr (0.5eq), DMF, rt, 1 h, 36%. b) I_2, Ph_3P, imidazole, PhH, rt, 0.5 h, 64%. c) LDA, THF, -78 °C, then, 3, -78 °C, 2 h, 87%. d) 15% citric acid/THF, rt. e) Boc_2O, dioxane, rt, 80% in 2 steps. f) H_2, 5% Pd/C, EtOAc, rt, 77%. g) I_2, Ph_3P, imidazole, PhH, rt, 0.5 h, 99%.

The synthesis of fragment B is summarized in Scheme 3.[5] After Boc-Orn(Z)-OH was converted to the *tert*-butyl ester **14** with O-*tert*-butyl-*N*,*N'*-diisopropyl isourea,[7] deprotection of the carbobenzyloxy (Z) group gave the amine **15**. Treatment of this amine **15** with triethylamine, carbondisulfide and 35% aqueous hydrogen peroxide[8] afforded the isothiocyanate **6** in 87% yield (2 steps). Coupling of **6** with 2,3-diaminopyridine **5** in the presence of Et_3N gave the thiourea **16**, which was converted to fragment B having the imidazo[4,5-b]pyridine ring by intramolecular guanylation with lead (II) acetate trihydrate.[4] Alternatively, the treatment of **16** with methyl iodide gave fragment B accompanied by the undesired product **17**, which was obtained by additional methylation at the 1 or 3 position of the imidazole ring. This result suggested that regioselective quaternization at the pyridine ring of fragment B with alkyl iodide would be impossible. Moreover, fragment B did not react with fragment A at all. Therefore, 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.[9]



Scheme 3. a) O-*tert*-butyl-*N*,*N'*-diisopropyl isourea, *t*-BuOH, CH_2Cl_2, 50 °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 35% aq. H_2O_2, 0 °C, 87% in 2 steps. d) 5, Et_3N, THF, reflux, 14 h, 76%. e) $Pb(OAc)_2{\bullet}3H_2O$, Et_3N, MeOH, reflux, 2 d, 80%. f) CH_3I, Et_3N, MeOH, reflux, 2 d, 35% and **17**, 41%

Monotritylation of **5** followed by coupling with the isothiocyanate **6** gave the thiourea **19** in 54% yield (Scheme 4).[5] Intramolecular guanylation of **19** by mercury (II) chloride[10] rapidly proceeded to quantitatively



Scheme 4. 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, 96%

afford the trityl protected imidazo[4,5-b]pyridine derivative **20**. As expected, the quaternization of **20** with fragment A proceeded with concomitant deprotection of the trityl group to provide the quaternary salt **21** in 81% yield. Finally, the cleavage of all the protective groups of **21** by treatment with trifluoroacetic acid (TFA) quantitatively furnished pentosidine (**1**) as the TFA salt. 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[11] based on several criteria[12]: ^{1}H-NMR, ^{13}C-NMR, FAB-HRMS, and HPLC.

In conclusion, we have accomplished the total synthesis of pentosidine (**1**). Our efficient synthesis will provide a large quantity of pentosidine for making antibodies on a large scale.

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12. Synthetic pentosidine(**1**); $[\alpha]_D^{24}$+16.48 (c, 0.26 in MeOH). ^{1}H-NMR(270MHz, D_2O): δ 1.3~1.5(m, 2H), 1.6~2.0(m, 8H), 3.45(t, 2H, J=6.6Hz), 3.95(t, 1H, J=6.3Hz), 4.03(t, 1H, J=6.0Hz), 4.42(t, 2H, J=7.0Hz), 7.09(t, 1H, J=6.6Hz), 7.65(d, 1H, J=7.6Hz), 7.80(d, 1H, J=6.6Hz). ^{13}C-NMR(67.8MHz, D_2O): δ 21.99, 25.19, 27.85, 28.78, 29.99, 42.62, 53.36, 53.81, 114.91($CF_3CO_2^-$), 116.01, 119.20($CF_3CO_2^-$), 120.48, 132.39, 132.79, 152.05, 160.28, 163(q, $CF_3CO_2^-$), 172.45, 172.52. FAB-HRMS calcd for $C_{17}H_{27}N_6O_4$: 370.2094, found: 370.2096.