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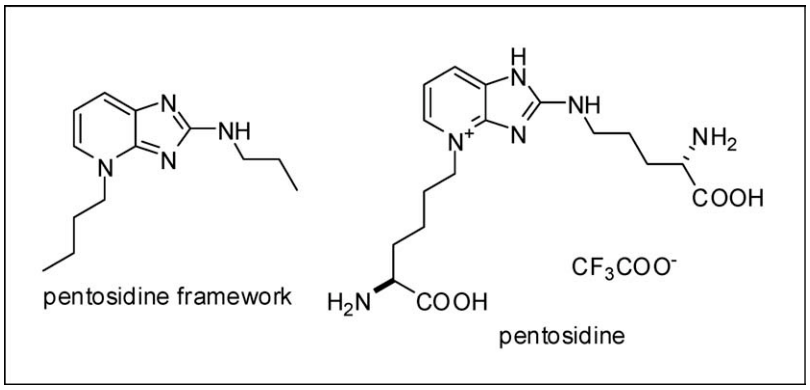
This work was performed under Professor Sayre's direction. Professor Lawrence M. Sayre passed away May 8, 2009, following an incapacitating stroke. His impact on the lives of his colleagues, students, and friends was profound and he is deeply missed [3]. We dedicate this article to him as a great mentor and brilliant scientist.



Pentosidine framework 4-butyl-2-propyl-4*H*-aminoimidazo[4,5-*b*]pyridine (**2**) was synthesized through a five steps reaction sequence. The regiochemistry of **2** was confirmed by an unambiguous synthesis, and the UV absorption and fluorescent properties of **2** were examined.

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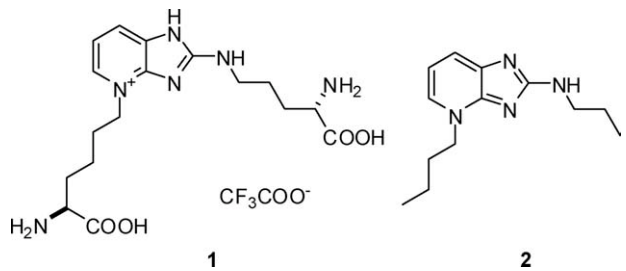
INTRODUCTION

Pentosidine (**1**) is one of the major fluorescent advanced glycation end products that have been isolated and structurally characterized. It was isolated in trifluoroacetic acid salt form from dura mater by Sell and Monnier in 1989 [4]. It was postulated that pentosidine forms from a lysine residue and an arginine residue crosslinked by a pentose [4–6]. The fluorescent properties of pentosidine (ex/em 335/385 nm) make it an oxidative biomarker in many clinical conditions such as diabetes, kidney disease, and other vascular illnesses [7].



1 **2**

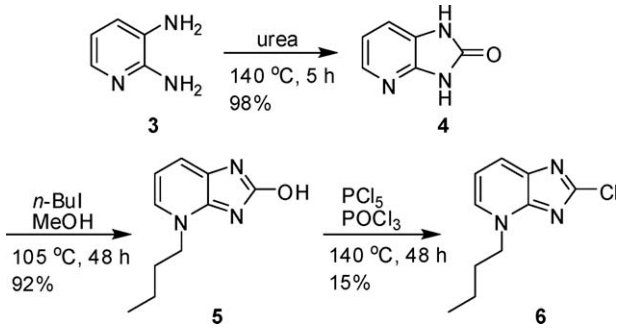
There has been only one total synthesis of pentosidine so far [8]. Shioiri and coworkers obtained 5.1 mg TFA salt of pentosidine (**1**) from their synthesis that involves an asymmetric alkylation of a chiral Schiff base to provide a lysine-like fragment, the intramolecular guanylation with mercury chloride, and the quaternization accompanied by the removal of trityl group as key steps [8].

In the investigation of the fluorescence properties of the pentosidine, one of our tasks was to evaluate the fluorescence property of the core of pentosidine, 4-alkyl-2-amino-4*H*-imidazo[4,5-*b*]pyridine, without the interference of amino acid chains. 2-Aminoimidazo[4,5-*b*]pyridine derivatives are important in medicinal chemistry because of their biological and pharmacological properties such as antihistamines, antibacterial agents, and integrin $\alpha_v\beta_3$ antagonists [9]. Therefore, a concise synthesis of the pentosidine framework 4-butyl-2-propyl-4*H*-aminoimidazo[4,5-*b*]pyridine (**2**) is of important for both biochemistry research of pentosidine and medicinal chemistry research. Herein, we report a straightforward synthesis of the pentosidine framework **2**.

RESULTS AND DISCUSSION

Our synthesis of **2** commenced from 2,3-diaminopyridine **3** (Scheme 1). The reaction of **3** with urea furnished 1*H*-imidazo[4,5-*b*]pyridin-2(3*H*)-one (**4**) [10]. Alkylation of **4** gave 4-butyl-2-hydroxyimidazo[4,5-*b*]pyridine (**5**) in 92% yield. The transformation from **5** to **6** failed with most usual chlorodehydroxylation reagents such as thionyl chloride and phosphorus oxychloride,

Scheme 1

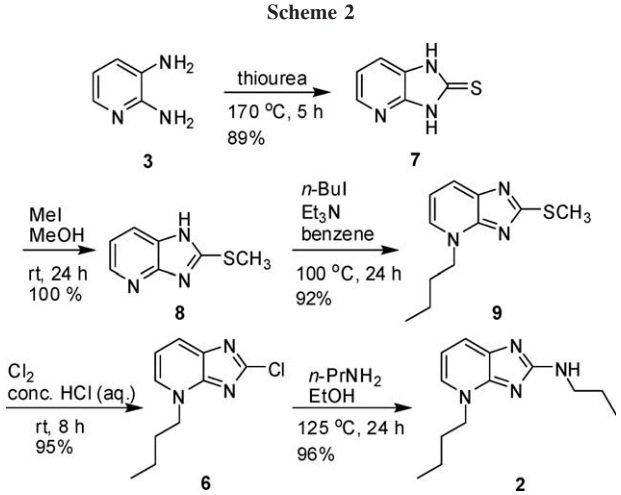


but compounds **6** could be obtained albeit in low yield (15%) after heating for 24 h in phosphorus oxychloride in the presence of phosphorus pentachloride [10].

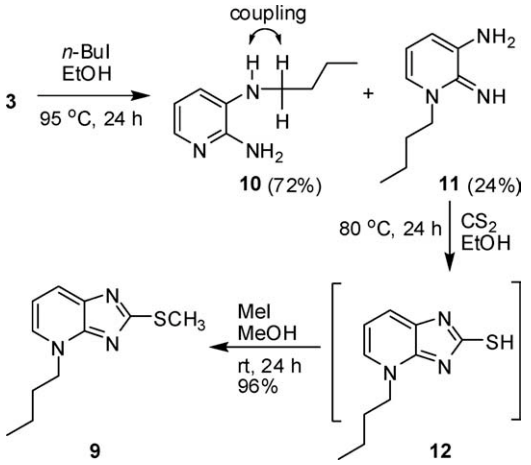
Our improved synthesis (Scheme 2) started with the reaction of 2,3-diaminopyridine **3** with thiourea, which afforded 1*H*-imidazo[4,5-b]pyridine-2(3H)-thione (**7**) [11]. Compound **7** is different from **4** in that alkylation of **7** would give an *S*-alkylated product instead of a pyridine *N*-alkylated product. Therefore, the sulfur in **7** was masked by prior methylation to give 2-(methylthio)imidazo[4,5-*b*]pyridine (**8**), which was alkylated by *n*-BuI regioselectively at the pyridine nitrogen to furnish **9** in good yields (78%) [12]. Compound **6** was obtained in good yield (96%) by bubbling Cl_2 through a concentrated hydrochloric acid solution of **9** followed by neutralization [13]. The reaction of **6** with *n*-$PrNH_2$ gave us the final target in good yields (97%) [14].

Although target compound **2** could be obtained according to the synthesis shown in Scheme 2 with satisfactory overall yields (75%), evidence for the regiochemistry of alkylation in **2**, **5**, **6**, and **9** was not fully discussed in the above description. We assigned both **5** and **9** as pyridine *N*-alkylated products based on the

Scheme 2



Scheme 3



expected greater nucleophilicity of the pyridine nitrogen relative to the imidazole nitrogens at positions **1** or **3** due to resonance [12]. To provide conclusive evidence for our structural assignment for **5** and **9**, as well as for compounds **6** and **2** that were obtained in subsequent steps, an unambiguous synthesis was used (Scheme 3). The synthesis started with alkylation of **3**, which was known to give only two alkylated products **10** and **11** [15]. The distinction of **11** from **10** could be made on the basis of both proton chemical shift and characteristic couplings. The chemical shift for the methylene group neighboring nitrogen in **10** is δ 3.08, whereas δ 4.22 in the case of **11** [15]. Furthermore, in **10**, the $HNCH_2$ vicinal coupling could be observed clearly in DMSO-d_6, whereas no such coupling was seen for **11**. The reaction of **11** with carbon disulfide and further methylation of sulfur could only provide 4-alkylated imidazo[4,5-*b*]pyridine structure **9** [16]. Compound **9** obtained through

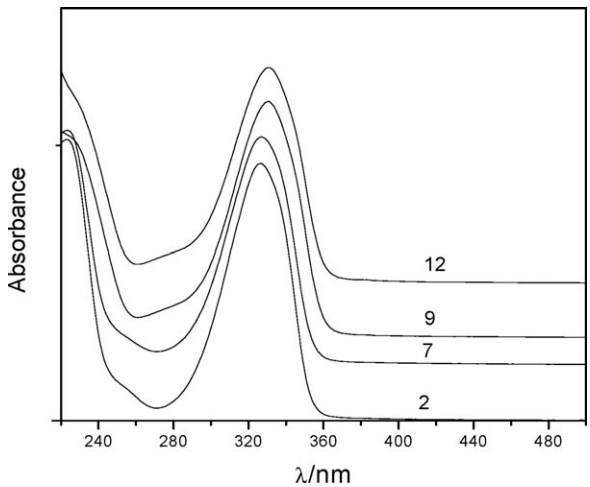


Figure 1. UV spectrum of **2** at pH = 2, 7, 9, and 12.

Table 1

Fluorescence property of pentosidine framework **2**.

pH	Excitation λ_{max} (nm)	Emission λ_{max} (nm)	Relative fluorescence intensity[a]
2	332	382	55
7	335	386	43
9	338	389	68
12			None

[a] When excitation λ_{max} is 335 nm.

this route was found to be identical with the product obtained from the synthesis shown in Scheme 2. This conclusion was based not only by direct comparison of their NMR spectra run separately but also by the NMR spectrum of a 1:1 mixture.

The absorption spectra for the pentosidine framework **2** are shown in Figure 1, and its fluorescence properties (ex/em 335/385 nm) are listed in Table 1. The shifts of the λ_{max} in different pH buffers were consistent with those of pentosidine **1** [4]. However, the variation of fluorescence intensity with pH values for **2** differed somewhat from that of **1**. Although we observed a decrease in fluorescence intensity upon increasing pH above 2, we did not observe the regain in fluorescence intensity observed for pentosidine at pH 12. As **2** lacks the free α-amino acid groups of pentosidine itself, we suggest that this is the basis of the different behavior.

In summary, pentosidine framework **2** was synthesized from readily commercially available 2,3-diaminopyridine in five steps, and the regiochemistry of the target was established by an unambiguous synthesis. This method is of material accessibility and operational simplicity and also serves as a general method to construct the 4-alkylated-2-amino-4*H*-imidazo[4,5-*b*]pyridines.

EXPERIMENTAL

^{1}H-NMR (300 or 200 MHz) and ^{13}C-NMR (75.1 or 50 MHz) were recorded on Varian Gemini 300 or Gemini 200 instruments. In the ^{13}C-NMR line listings, attached proton test 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. UV–visible spectra were obtained with a Perkin-Elmer model Lambda 3B spectrophotometer, and fluorescence was determined using an Aminco-Bowman spectrofluorometer. TLC analysis was carried out on silica gel 60 F254 precoated aluminum sheets, and UV light was used for detection. Flash column chromatography was done using E. Merck silica gel 60 (230–400 mesh). Solvent removal was accomplished by a rotary evaporator operating at vacuum (40–50 Torr).

1*H*-imidazo[4,5-*b*]pyridin-2(3*H*)-one (4). A mixture of 2,3-diaminopyridine (**3**, 763.9 mg, 7.0 mmol) and urea (1.40 g, 23.3 mmol) was heated at 140°C for 5 h. The cooled reaction mixture was extracted with boiling ethanol (5 × 6 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 **4** was 929.7 mg (98%). **4**: ^{1}H-NMR (DMSO-d_6) δ 6.93 (dd, J = 7.1, 5.9 Hz, 1H), 7.21 (d, J = 7.1 Hz, 1H), 7.85 (d, J = 5.8 Hz, 1H), 10.82 (s, 1H, NH), 11.29 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6) δ 114.4 (−), 116.6 (−), 123.6 (+), 139.6 (−), 144.8 (+), 154.4 (+). HRMS calcd for $C_6H_5N_3O$ (M^+) 135.0433, found 135.0428.

4-Butyl-2-hydroxy-4*H*-imidazo[4,5-*b*]pyridine (5). A mixture of **3** (810.7 mg, 6.0 mmol) and *n*-butyl iodide (5.52 g, 30.0 mmol) in MeOH (8 mL) was sealed in a Teflon-screwed high-pressure vessel and stirred at 105°C for 48 h and concentrated. The resulting residue was diluted with a mixed solvent of CH_2Cl_2/MeOH (9:1), basified with 28% aqueous NH_4OH solution, and subjected to chromatography (CH_2Cl_2/MeOH/28% aqueous NH_4OH = 90:10:1) to afford **5** (1.055 g, 92%): ^{1}H-NMR (CD_3OD) δ 0.98 (t, J = 7.1 Hz, 3H), 1.41 (m, 2H), 1.92 (m, 2H), 4.42 (t, J = 7.5 Hz, 2H), 7.06 (dd, J = 7.5, 6.8 Hz, 1H), 7.45 (d, J = 7.5 Hz, 1H), 7.84 (d, J = 6.7 Hz, 1H); ^{13}C-NMR (CD_3OD) δ 14.1 (−), 20.8 (+), 32.3 (+), 54.7 (+), 115.2 (−), 116.3 (−), 131.8 (−), 132.6 (+), 152.1 (+) (one quaternary peak is missing); HRMS calcd for $C_{10}H_{13}N_3O$ (M^+) 191.1059, found 191.1056.

4-Butyl-2-chloro-4*H*-imidazo[4,5-*b*]pyridine (6). A suspension of **5** (956.2 mg, 5.0 mmol) and PCl_5 (1.20 g, 5.40 mmol) in $POCl_3$ (120 mL) was sealed in a Teflon-screwed high-pressure vessel and stirred at 140°C for 48 h. Then, $POCl_3$ was removed under reduced pressure, and the residue was dissolved in water (10 mL) and basified to pH = 8 with 2*N* aqueous solution of NaOH. The aqueous solution was extracted with CH_2Cl_2, and the combined organic layers were dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (CH_2Cl_2/MeOH, 5:1) to afford **6** (157.3 mg, 15%). ^{1}H-NMR ($CDCl_3$) δ 0.93 (t, J = 7.3 Hz, 3H), 1.36 (m, 2H), 1.98 (m, 2H), 4.57 (t, J = 7.5 Hz, 2H), 7.09 (dd, J = 7.5, 6.5 Hz, 1H), 7.68 (d, J = 6.5 Hz, 1H), 8.05 (d, J = 7.6, 1H); ^{13}C-NMR ($CDCl_3$) δ 13.6 (−), 19.8 (+), 31.7 (+), 54.2 (+), 113.5 (−), 127.7 (−), 130.1 (−), 144.1 (+), 152.5 (+), 160.7 (+); HRMS calcd for $C_{10}H_{12}N_3Cl$ (M^+) 209.0720, found 209.0724.

1*H*-imidazo[4,5-*b*]pyridine-2(3*H*)-thione (7). A mixture of 2,3-diaminopyridine (**3**, 2.18 g, 20 mmol) and thiourea (7.6 g, 100 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 **7** was 2.7 g (89%). **7**: ^{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 (8). A solution of **7** (2.26 g, 15.0 mmol) and methyl iodide (8.52 g, 60 mmol) in MeOH (30 mL) was stirred at room temperature for 24 h. Evaporation of the solvent and CH_3I under reduced pressure gave pure **8** (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.

4-Butyl-2-(methylthio)-4*H*-imidazo[4,5-*b*]pyridine (9). A suspension of **8** (1.65 g, 10.0 mmol) in a solution of *n*-butyl iodide (2.76 g, 15.0 mmol) and triethylamine (1.52 g, 15.0 mmol) in benzene (150 mL) was sealed in a Teflon-screwed high-pressure vessel and stirred at 100°C for 24 h and concentrated. The residue was diluted with mixed solvent of CH_2Cl_2/MeOH (9:1), basified with 28% aqueous NH_4OH solution, and subjected to chromatography (EtOAc/acetone/MeOH/28%NH_4OH, 140:60:10:1) to afford **9** (2.03 g, 92%): ^{1}H-NMR ($CDCl_3$) δ 0.97 (t, J = 7.3 Hz, 3H), 1.38 (m, 2H), 2.01 (m, 2H), 2.78 (s, 3H), 4.60 (t, J = 7.3 Hz, 2H), 7.07 (dd, J = 7.7, 6.7 Hz, 1H), 7.45 (d, J = 6.6 Hz, 1H), 7.84 (d, J = 7.7 Hz, 1H); ^{13}C-NMR ($CDCl_3$) δ 13.6 (−), 14.6 (−), 19.8 (+), 31.6 (+), 53.9 (+), 113.5 (−), 124.0 (−), 129.2 (−), 143.2 (+), 151.8 (+), 172.1 (+); HRMS calcd for $C_{11}H_{15}N_3S$ (M^+) 221.0987, found 221.0982.

Preparation of 6 from 9. Chlorine was introduced into a solution of **9** (664.0 mg, 3.0 mmol) in hydrochloric acid (12*N*, 300 mL) with stirring at room temperature for 8 h. After standing overnight, the reaction mixture was concentrated under reduced pressure. The resulting residue was diluted with ice water (400 mL), basified with 2*N* aqueous solution of NaOH, and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated resulting in a crude, which was subjected to chromatography (CH_2Cl_2/CH_3OH, 5:1) to afford **6** (597.5 mg, 95%). ^{1}H-NMR, ^{13}C-NMR, and HRMS data were identical with those of **6** prepared from **5**.

4-Butyl-2-(propylamino)-4*H*-imidazo[4,5-*b*]pyridine (2). A mixture of **6** (524.2 mg, 2.50 mmol) and propylamine (1.18 g, 20.0 mmol) in EtOH (30 mL) was sealed in a Teflon-screwed high-pressure vessel and stirred at 125°C for 24 h and was evaporated to result in a residue, which was subjected to chromatography (CH_2Cl_2/MeOH, 5:1) to afford **2** (557.2 mg, 96%). ^{1}H-NMR (CD_3OD) δ 0.98 (t, J = 7.5 Hz, 3H), 1.01 (t, J = 7.3 Hz, 3H), 1.38 (m, 2H), 1.72 (m, 2H), 1.96 (m, 2H), 3.46 (t, J = 6.9, 2H), 4.56 (t, J = 7.3, 2H), 7.16 (dd, J = 7.6, 6.6 Hz, 1H), 7.75 (d, J = 7.6 Hz, 1H), 8.00 (d, J = 6.6, 1H); ^{13}C-NMR ($CDCl_3$) δ 11.5 (−), 13.6 (−), 19.6 (+), 22.8 (+), 31.4 (+), 44.9 (+), 53.3 (+), 114.2 (−), 118.1 (−), 129.9 (−), 152.6 (+), 156.7 (+), 161.8 (+); HRMS calcd for $C_{13}H_{20}N_4$ (M^+) 232.1688, found 232.1685.

2-Amino-3-(butylamino)pyridine (10) and 1-butyl-3-amino-2-pyridone imine (11). A mixture of **4** (1.09 g, 10.0 mmol) and *n*-butyl iodide (7.36 g, 40.0 mmol) in EtOH (60 mL) was sealed in a Teflon-screwed high-pressure vessel and stirred at 95°C for 24 h and concentrated. The resulting residue was dissolved in a mixed solvent of MeOH and water (1:1) (160 mL), adjusted to pH 10 with 2*N* aqueous solution of NaOH, and evaporated ending in a crude, which was subjected to chromatographic (EtOAc/MeOH, 4:1) separation to afford **10** (1.19 g, 72%) and **11** (396.6 mg, 24%). **10**: ^{1}H-NMR ($CDCl_3$) δ 0.97 (t, J = 7.2 Hz, 3H), 1.45 (m, 2H), 1.64 (m, 2H), 3.08 (t, J = 6.7 Hz, 2H), 6.70 (dd, J = 7.6, 4.8 Hz, 1H), 6.80 (d, J = 7.6 Hz, 1H), 7.57 (d, J = 4.8 Hz, 1H); ^{13}C-NMR ($CDCl_3$) δ 14.0 (−), 20.4 (+), 31.5 (+), 43.6 (+), 115.8 (−), 116.7 (−), 132.5 (+), 135.8 (−), 148.7 (+); HRMS calcd for $C_9H_{15}N_3$ (M^+) 165.1266, found 165.1264. **11**: ^{1}H-NMR (CD_3OD) δ 0.98 (t, J = 7.3 Hz, 3H), 1.42 (m, 2H), 1.79 (m, 2H), 4.22 (t, J = 7.6 Hz, 2H), 6.77 (dd, J = 7.7, 6.5 Hz, 1H), 7.13 (d, J = 7.7 Hz, 1H), 7.37 (d, J = 6.5 Hz, 1H); ^{13}C-NMR (CD_3OD) δ 14.1 (−), 20.5 (+), 30.8 (+), 55.4 (+), 115.3 (−), 120.9 (−), 128.8 (−), 135.8 (+), 146.4 (+); HRMS calcd for $C_9H_{15}N_3$ (M^+) 165.1266, found 165.1265.

Preparation of 9 from 11. A solution of **11** (165.2 mg, 1.0 mmol) and CS_2 (5 mL) in EtOH (8 mL) in a sealed Teflon-screwed high-pressure vessel was stirred at 80°C for 24 h and concentrated. The residue was mixed with MeI (1.41 g, 10.0 mmol) in MeOH (6 mL), stirred at room temperature for 24 h, and concentrated resulting in a crude mixture, which was subjected to chromatography (EtOAc/MeOH, 4:1) to afford **9** (179.3 mg, 81%). ^{1}H-NMR, ^{13}C-NMR, and HRMS data were identical with those of **9** prepared from **8**.

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