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Thermal Decomposition of 5-(Hydroxymethyl)-2-furaldehyde (HMF) and Its Further Transformations in the Presence of Glycine

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ABSTRACT: Thermal decomposition of HMF has been so far studied indirectly through carbohydrate degradation reactions assuming HMF as the main product. Such studies, however, do not necessarily generate relevant information on HMF decomposition because many other products are generated simultaneously. Direct thermal decomposition using different concentrations of HMF in silica gel was studied using pyrolysis-GC-MS. Undiluted HMF generated four peaks corresponding to 5-methylfurfural, 2,5-furandicarboxaldehdye, HMF, and a major unknown peak at retention time of 20.73 min. The diluted HMF in silica gel (15-fold) generated only the first three peaks. The generation of the unknown peak was dependent on the concentration of HMF, indicating the possibility of a dimeric structure; furthermore, when HMF was generated from [U-$^{13}C_6$]glucose in the reaction mixture, the highest mass in the spectrum of the unknown peak showed the incorporation of 11 carbon atoms from the glucose. Thermal decomposition studies of HMF have also indicated that in the absence of amino acids it can mainly dimerize and the initially formed dimer can degrade to generate 5-methylfurfural and 2,5-furandicarboxaldehyde. On the other hand, thermal degradation of HMF in the presence of glycine generated Schiff base adducts of HMF, 5-methylfurfural, and 2,5-furandicarboxaldehdye in addition to 2-acetyl-5-methylfuran and a newly discovered adduct, 5-[(dimethylamino)methyl]-2-furanmethanol.

KEYWORDS: HMF dimers, glycine, 5-methylfurfural, 2, 5-furandicarboxaldehyde, 2-acetyl-5-methylfuran, 5-[(dimethylamino)methyl]-2-furanmethanol, Schiff base adducts of HMF with amino acids and N-methylation of amino acids

INTRODUCTION

Most of the studies on the thermal decomposition products of 5-(hydroxymethyl)-2-furaldehyde (HMF) have been performed utilizing different carbohydrates[1−3] as precursors instead of HMF itself. Such studies have indicated the decomposition of HMF into levulinic acid, formic acid, formaldehyde, 2,5-furandialdehyde (FDA), and 5-methylfurfural (MF). Under autoclaving conditions, Durham et al.[4] also observed the formation of 5-hydroxymethylfuroic acid and furan-2,5-dicarboxylic acid from glucose solutions and attributed their presence to the formation of HMF. On the other hand, Chambel et al.,[5] utilizing HMF as a precursor, observed its complete decomposition when heated alone at 210 °C and the formation of four decomposition products using liquid chromatography; however, they identified the structure of only one of the four products as a symmetric ether formed through dehydration of two HMF molecules, giving rise to 5,5′-oxydimethylenebis(2-furaldehyde). The same dimer was also identified earlier by Popoff and Theander[6] in slightly aqueous fructose and glucose solutions. Relative to aliphatic aldehydes, HMF is less volatile and chemically more stable and able to accumulate and persist longer in food to undergo polymerization. Its concentrations have been shown to vary over time, experiencing continuous upward and downward fluxes, especially during storage.[7] Such changes over time can indicate the occurrence of polymerization−depolymerization, degradation,[1] or other reactions with *N*-nucleophiles such as amino acids. The ability of HMF to undergo reversible and covalent bond formation with primary and secondary amino acids, respectively,[8] and with human hemoglobin has been also demonstrated.[9] Although HMF is one of the most abundant furfural derivatives in food, its polymerization mechanism and its chemical interaction with amino acids have not been explored in detail. One of the drawbacks of using carbohydrate degradation to study HMF decomposition is that such studies do not necessarily generate relevant information on HMF decomposition because many other products are formed simultaneously. The aim of this investigation was to explore direct degradation of HMF in the presence and absence of amino acids.

MATERIALS AND METHODS

Materials. Glycine (98%), 5-hydroxymethylfurfural (99%), furfural (99%), 5-methylfurfural (99%), methylamine hydrochloride, sodium glycinate (98%), 2-acetyl-5-methylfuran (98%), sarcosine (98%), paraformaldehyde (95%), furfuryl alcohol (99%), and 5-[(dimethylamino)methyl]-2-furanmethanol hydrochloride (98%) were purchased from Aldrich Chemical Co. (Milwaukee, WI). [U-$^{13}C_6$]Glucose, [^{13}C-1]glucose, [^{13}C-6]glucose, [^{13}C-1]glycine, [^{13}C-2]glycine, and [^{15}N]glycine were all >98% enriched and purchased from CIL (Andover, MA). D-Glucose (99%, BDH, Toronto, Canada) and 2,5-furandicarboxaldehyde (98%, TRC, Toronto, Canada) were purchased as indicated. The ^{13}C and ^{1}H NMR spectra were acquired in CD_3OD on a Varian VNMRS 500 MHz spectrometer. Infrared spectra were recorded on a Bruker Alpha-P spectrometer (Bruker Optic GmbH, Ettlingen, Germany) equipped with a deuterated triglycine sulfate

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Table 1. Composition of Model Systems Analyzed by Py-GC-MS

target ions/ product	ratio	model system
m/z 111,		[U-$^{13}C_6$]glucose
15.452 min	1:7	HMF + [U-$^{13}C_6$]glucose
	1:1	[U-$^{13}C_6$]glucose + methylamine·HCl
		5-methylfurfural
m/z 124,		[U-$^{13}C_6$]glucose
17.820 min	1:7	HMF + [U-$^{13}C_6$]glucose
	1:1	[U-$^{13}C_6$]glucose + methylamine·HCl
		2,5-furandicarboxaldehyde
m/z 126,	1:15	HMF + silica gel [U-$^{13}C_6$]glucose
20.577 min	1:7	HMF + [U-$^{13}C_6$]glucose
m/z 189,	1:7	HMF + [U-$^{13}C_6$]glucose
20.705mn	1:7	HMF + [^{13}C-1 or 6]glucose
	1:1	[U-$^{13}C_6$]glucose + methylamine·HCl
	2:1:1	[U-$^{13}C_6$]glucose + methylamine·HCl + glycine
Schiff base adducts		
m/z 123,	3:1	HMF + [^{13}C-1 or 2]glycine
6.568 min	3:1	HMF + [^{15}N]glycine
	3:1	HMF + glycine
m/z 137,	2:1:1	[U-$^{13}C_6$]glucose+ methylamine-HCl + glycine
0.125 min	1:1	[U-$^{13}C_6$]glucose + methylamine-HCl
	2:1:1	glucose + methylamine·HCl + glycine
		glucose + methylamine·HCl
m/z 139,	1: 1	HMF + methylamine·HCl
1.401 min		{[(5-methylfuran-2-yl)methylidene]amino}acetic acid sodium salt (**7**)
m/z 150,		2,2′-{furan-2,5-diylbis[methylylidenenitrilo]}diacetic acid disodium salt (**9**)
21.864 min		
chain elongation adducts		
m/z 124,	1:3	MF[a]/HMF + [^{13}C-1 or 2]glycine
16.907 min	1:3	MF[a]/HMF + [^{15}N]glycine
	1:3	MF[a]/HMF + glycine
m/z 140,		2-acetyl-5-methylfuran[a] (**12**)
21.746 min		
N-methylation of glycine		
m/z 155,	1:3	HMF + [^{13}C-1 or 2]glycine
0.674 min	1:3	HMF + [^{15}N]glycine
	1:3	HMF + glycine
m/z 153,	1:3	HMF + sarcosine
0.674 min	1:3:3	HMF + glycine + paraformaldehyde
	1:1.5:1.5	HMF + glycine + sarcosine
		5-[(dimethylamino)methyl]-2-furanmethanol hydrochloride (**16**)

[a] MF, 5-methylfurfural used only for *m/z* 124 adduct.

Table 2. Percent Distribution of HMF and Its Degradation Products[a]

HMF/silica	MF[b] (15.32 min)	FDA[c] (17.8 min)	HMF[d] (20.55 min)	HMF dimer[e] (20.73 min)
1:0	13.3	18.7	8.5	22.6
1:10	3.4	29.9	6.78	10.2
1:15	1.5	29.6	3.2	0

[a] Pyrolysis at 250 °C for 20 s. [b] MF, 5-methylfurfural. [c] FDA, 2,5-furandicarboxaldehyde. [d] HMF, 5-(hydroxymethyl)-2-furaldehyde. [e] *m/z* (%): 39 (11.5), 81 (0.23), 109 (22.4), 126 (36.4%), 127 (100), 189 (22.3); see also Figure 4 (structure **5**).

(DTGS) detector, a temperature-controlled single-bounce diamond attenuated total reflectance (ATR) crystal, and a pressure application device for solid samples.

Pyrolysis-GC-MS. Analyses were conducted using a Varian CP-3800 GC coupled with a Saturn 2000 ion trap mass spectrometer (Varian, Walnut Creek, CA). The pyrolysis unit included a CDS Pyroprobe 2000 and a CDS 1500 valved interface (CDS Analytical, Oxford, PA) installed onto the GC injection port. Between 0.5 and 1.5 mg of a sample mixture (see Table 1) was packed inside a quartz tube (0.3 mm thickness), plugged with quartz wool, inserted inside the coil probe, and pyrolyzed for 20 s at a temperature of 250 °C. The sample separation was carried out on a DB-5MS (5% diphenyl, 95% dimethyl polysiloxane) capillary column with dimensions of 50 m length by 0.2 mm internal diameter and 0.33 μm film thickness (J&W Scientific, ON, Canada), using helium as the carrier gas. The GC column flow rate was regulated by an electronic flow controller (EFC) and set at a pressure pulse of 70 psi for the first 4 min and later maintained with a constant flow of 1.5 mL/min for the remainder of the run. The GC oven temperature was set at −5 °C for 5 min using CO_2 as the cryogenic cooling source. The temperature was increased to 50 °C at a rate of 50 °C/min and then to 270 °C at a rate of 8 °C/min and kept at 270 °C for 5 min. The samples were detected by using an ion trap mass spectrometer with a scan range of *m/z* 20−650. The MS transferline temperature was set at 250 °C, the manifold temperature was set at 50 °C, and the ion trap temperature was set at 175 °C. An ionization voltage of 70 eV was used, and EMV was set at 1700 V. Compound identification was performed using AMDIS (ver. 2.65) and NIST Standard Reference Databases (data version 05 and software ver. 2.0d) to compare the target compounds with the existing mass spectral libraries or by injecting commercially available standards. The reported percent label incorporation values (corrected for natural abundance and for percent enrichment) are the average of duplicate analyses and are rounded off to the nearest multiple of 5%. The reported percent peak areas in Tables 2 and 4 are the averages of duplicate analyses with percent standard deviation of <10%.

Synthesis of {[(5-Methylfuran-2-yl)methylidene]amino} acetic Acid Sodium Salt (7). 5-Methyfurfural (124 mg) and sodium glycinate (97 mg) were intimately mixed at room temperature in a mortar without a solvent until a homogeneous yellow solid was produced (15 min): ^{1}H NMR, δ 2.35 (s, 3H, H-6), 4.16 (s, 2H, H-2′), 6.18 (d, 1H, H-3), 6.80 (d, 1H, H-4), 7.96 (s, H-1); ^{13}C NMR, δ 176.3 (C-1′), 155.8 (C-5), 152.2 (C-1), 149.9 (C-2), 116.9 (C-4), 108.0 (C-3), 63.5 (C-2′), 12.3 (C-6); FTIR (solid), 3116 cm^{-1} (Ar−H stretch), 2925 and 2889 cm^{-1} (−CH_3 and −CH_2− stretch), 1644 cm^{-1} (C═N stretch), 1583 cm^{-1} (COO^- antisymmetrical stretch), 1391 cm^{-1} (COO^- symmetrical stretch); MS, *m/z* (% abundance) 39 (12.7), 42 (14.5), 50 (15.2), 51 (16.3), 53 (26.8), 67 (14.6), 79 (11.1), 80 (11.6), 81 (11.7), 95 (100), 107 (6.8), 122 (79.9), 123 (97.4), 124 (14.8).

Synthesis of 2,2′-{Furan-2,5-diylbis[methylylidenenitrilo]} diacetic Acid Disodium Salt (9). Furan-2,5-dicarboxaldehyde (124

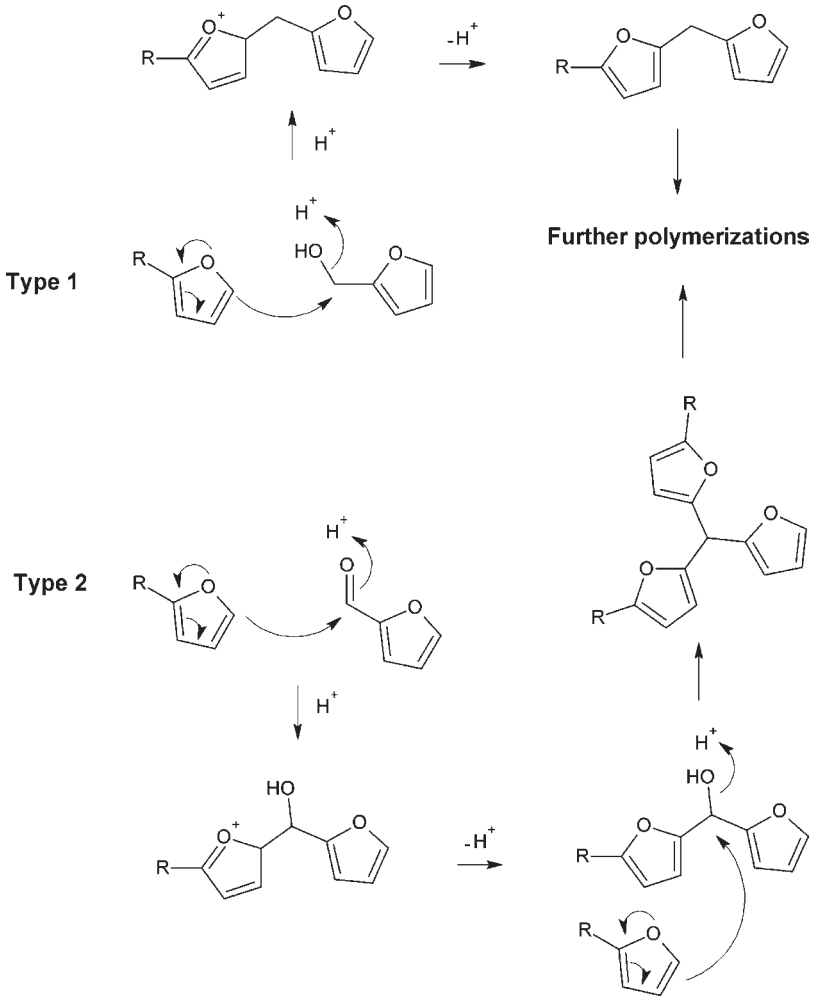

Figure 1. Type 1 and 2 polymerization reactions proposed by Tressl et al.[13,14]

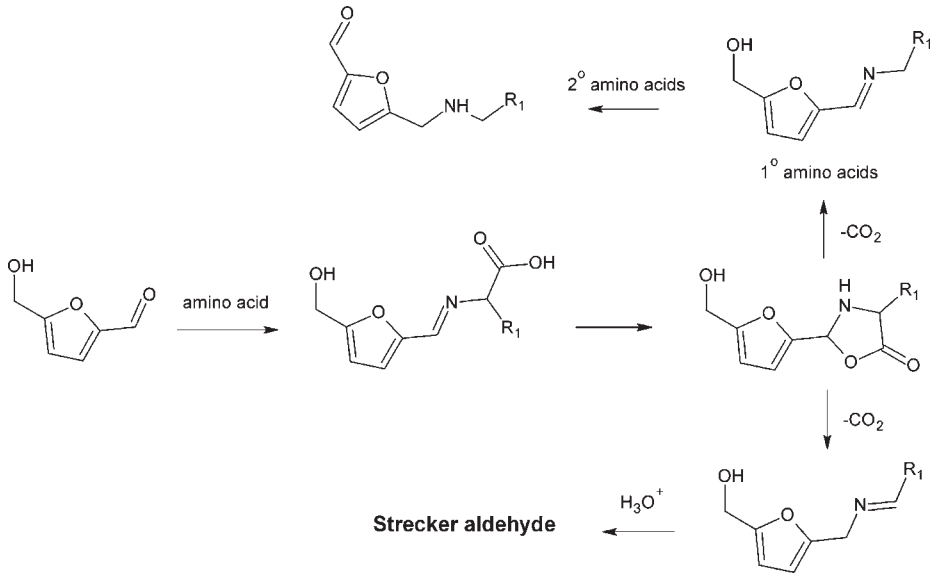

Figure 2. HMF reactions with amino acids according to Nikolov and Yaylayan.[8]

mg) and sodium glycinate (97 mg) were intimately mixed at room temperature in a mortar without a solvent until a homogeneous yellow solid was produced (15 min): ^{1}H NMR, δ 4.25 (d, 4H, H-2′,2″), 7.01 (s, 2H, H-3,4), 8.122 (t, 2H, H-1,6); ^{13}C NMR, δ 175.9 (C-1′,1″), 152.6

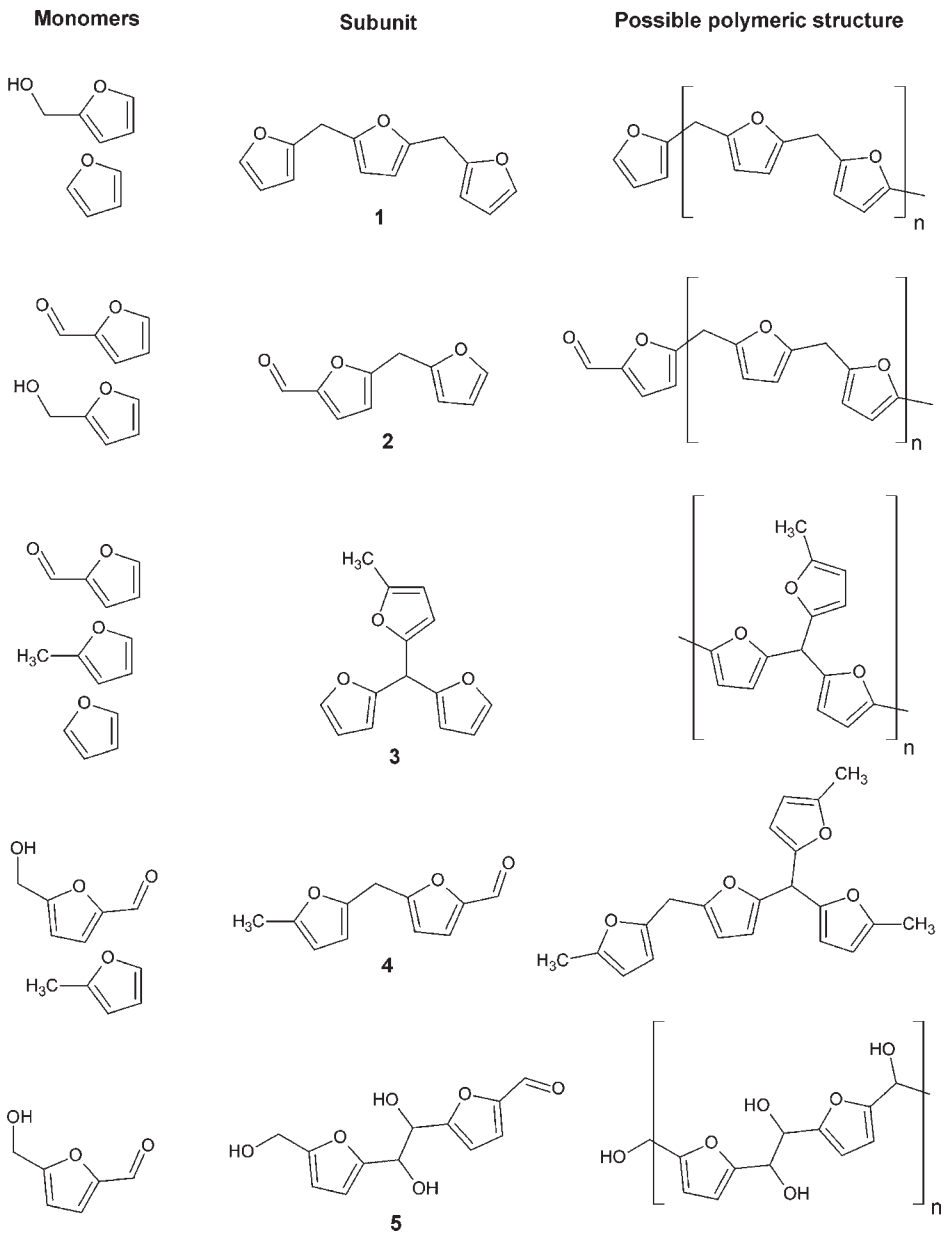

Figure 3. Characteristic polymeric subunits showing the monomers and possible polymeric structures.

Table 3. Number of Glucose Carbon Atom Incorporation into the Mass Spectral[a] Fragments of the Proposed HMF Dimer (5, t_R 20.734 min)

fragment ion	*m/z* 189	*m/z* 127	*m/z* 109	*m/z* 97	*m/z* 81
[U-$^{13}C_6$]glucose	11	6	6	5	5
[^{13}C-1]glucose	1	1	1	0	0
[^{13}C-6]glucose	2	1	1	1	1

[a] See Figure 4.

(C-2,5), 151.7 (C-1,6), 116.8 (C-3,4), 64.0 (C-2′,2″); FTIR (solid), 3140 and 3107 cm^{-1} (Ar−H stretch), 2901 cm^{-1} (−CH_2− stretch), 1641 cm^{-1} (C═N stretch), 1582 cm^{-1} (COO^- antisymmetrical stretch), 1392 cm^{-1} (COO^- symmetrical stretch); MS, *m/z* (% abundance) 42 (24.4), 51 (10.3), 80 (15.7), 81 (11.3), 94 (15.6), 108 (30.1), 122 (10.5), 123 (67.5), 149 (39.9), 150 (100), 151 (27.5).

RESULTS AND DISCUSSION

Different furfural derivatives, such as HMF, can serve as building blocks for the construction of polymeric structures (Figure 1) or can be transformed into amino acid adducts[8] through carbonyl−amine reactions (Figure 2) during the Maillard reaction. Due to the complexity of the resulting polymers, structural elucidation often relies on the identification of the subunits released during degradation of isolated polymers[10,11] or identification of the structure of the smaller building blocks such as dimers or oligomers having the potential for polymerization.[12a] The applicability of the concept of "polymer generating subunits" as indicators of the structure of polymers has been demonstrated by the detection of dimers such as Blue-M1, which polymerizes readily to form a yellow polymer.[12b] Furan or pyrrole derivatives possessing a hydroxymethyl group as a side chain can undergo type 1 polymerization through electrophilic

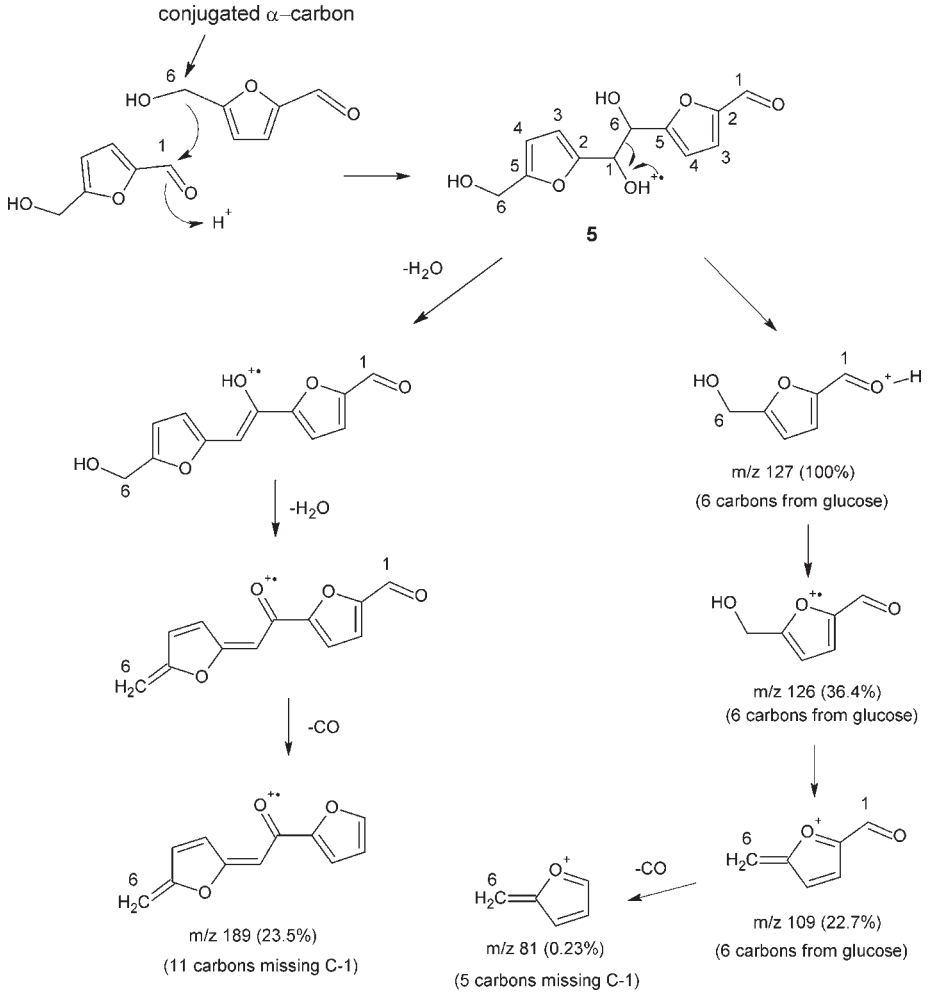

Figure 4. Formation of proposed HMF dimer (**5**) through vinylogous aldol addition of two HMF molecules and its mass spectral fragmentations indicating the incorporation of glucose carbon atoms.

aromatic substitution to generate a linear polymer;[13] on the other hand, if they possess carbonyl groups as side chains, they can undergo type 2 polymerization[14] through electrophilic aromatic addition to generate a branched polymer (Figure 1). Linear oligomers of up to 12 *N*-methylpyrrole subunits were detected, whereas some branched oligomers were found to contain >30 pyrrolyl-2-furylmethyl units. One strategy to postulate possible molecular structures of polymeric material based on the mechanisms depicted in Figure 1 is to identify initially formed dimeric or oligomeric structures capable of polymerization. To confirm the ability of pyrolysis to generate such dimeric subunits, furfuryl alcohol, for example, was pyrolyzed, and structures **1**, **2**, and **3** shown in Figure 3 were identified through NIST library searches. Structures **1**−**3** are known to be fragments of larger polymeric material. Pyrolysis of HMF, on the other hand, generated two dimeric structures, one minor (structure **4**) and the other major (structure **5**). Structure **4** was identified through a NIST library search, and it can be envisaged to be formed through a type 1 mechanism involving HMF and 2-methylfuran; however, structure **5** was a major peak comprising almost 23% of the total area of all the peaks generated from HMF degradation (see Table 2).

Thermal Degradation of HMF. To study the thermal degradation of HMF and its potential to generate polymeric subunits, different concentrations of HMF in silica gel were prepared and pyrolyzed under the same conditions. Undiluted HMF generated four peaks (see Table 2) corresponding to 5-methylfurfural, 2,5-furandicarboxaldehdye, HMF, and an unknown peak at a retention time of 20.73 min. On the other hand, the diluted HMF in silica gel (15-fold) generated only the first three peaks (Table 2). The generation of the unknown peak was dependent on the concentration of HMF, indicating the possibility of having oligomeric structure; furthermore, when HMF was generated from [U-$^{13}C_6$]glucose in the reaction mixture, the highest mass in the spectrum of the unknown peak showed the incorporation of 11 carbon atoms from the glucose (Table 3). Because HMF cannot undergo electrophilic substitution or addition reactions to form type 1 or 2 polymers depicted in Figure 1 due to the presence of substituents at positions 2 and 5 of the furan ring, vinylogous aldol addition could be considered a possible mechanism that can generate the dimeric structure **5**, which is consistent not only with the observed mass spectral fragmentation pattern but also with the label incorporation in these fragments (see Figure 4). HMF has been shown to undergo other vinylogous reactions such as Amadori rearrangement with secondary amino acids or amines,[8] and more importantly a structure based on an identical HMF dimer was recently also identified as a building block for a melanoidin generated from glucose.[15] The highest mass spectral ion at m/z 189 generated

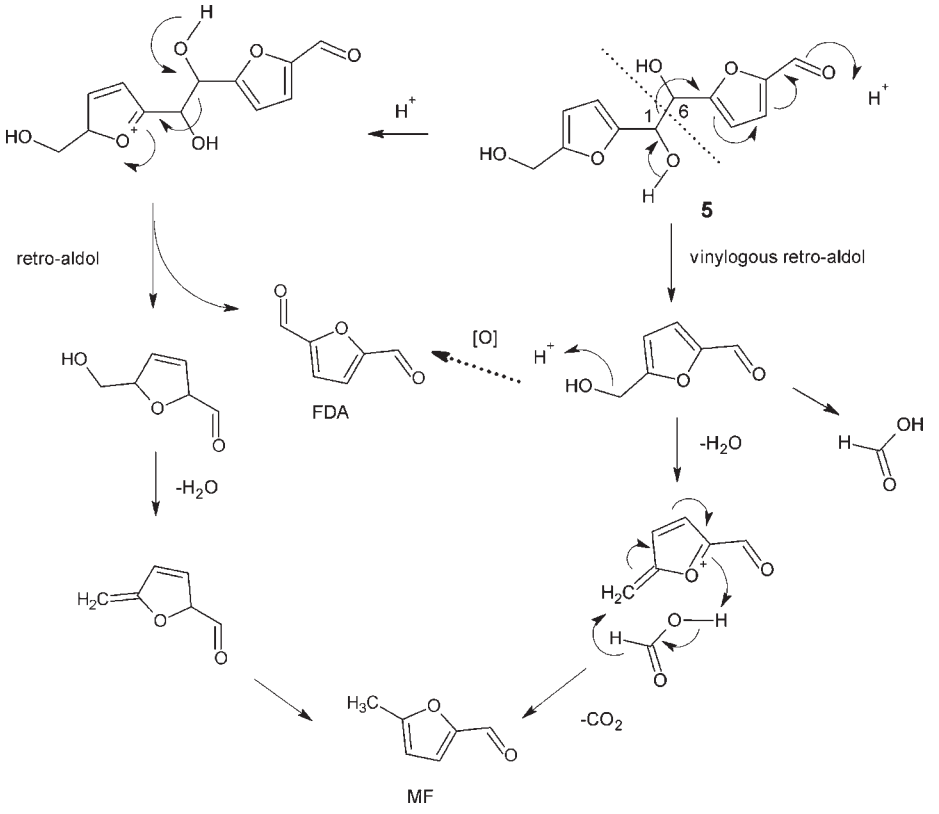

Figure 5. Retro-aldol reaction of protonated HMF dimer and formation of 5-methylfurfural (MF) and 2,5-furandicarboxaldehyde (FDA).

from the proposed dimeric structure shown in Figure 4 can be easily rationalized to be formed by the loss of two water molecules followed by the loss of a CO molecule from the parent ion; furthermore, the ion at m/z 189 exhibited double incorporation of all the glucose carbon atoms with the exception of C-1 (lost as CO) (see Table 3), consistent with the mechanism and the structure shown in Figure 4. This fact also confirms the formation of a C-1−C-6 bond during dimerization, which can be achieved only through aldol-type interaction. The proposed dimeric structure **5**, although subject to volatilization under the experimental conditions, being a reactive molecule cannot be isolated from the reaction mixtures due to its ability to undergo type 1 polymerization at the hydroxymethyl terminal or type 2 polymerizations at the carbonyl terminal of the molecule or continue polymerization with other HMF molecules through a series of vinylogous aldol condensations.[15] As a result, very complex and nonhomogenous polymers can be generated. In addition, formation of MF and FDA in the thermal degradation mixtures of HMF (see Table 2), which has been speculated to be formed from HMF,[16,17] can be rationalized through the proposed degradation mechanism of the proposed dimer shown in Figure 5. Carbon−carbon bond cleavage of the diol group can occur through two pathways: a vinylogous retro-aldol, which can generate HMF, and a retro-aldol reaction after a protonation step that can generate FDA and 5-MF without the need for oxidation and reduction steps required for the direct conversion from HMF. FDA and MF were identified by comparison of their retention times and their mass spectra to those of commercial standards and through NIST library matches in addition to isotope labeling data.

Table 4. Percent Distribution of HMF and Its Degradation Products in the Presence of Glycine[a]

compound	MF[b]	FDA[c]	HMF[d]	HMF dimer[e]
HMF + glycine (3:1)	33.8	19.2	10.9	31.0
HMF + glycine (2.5:1)	14.3	14.3	9.4	26.3
HMF + glycine (1.5:1)	15.3	10	8.3	18.4
HMF + glycine (1:1)	3.6	2.4	2.5	0
HMF + glycine (1:3)	2	0.15	0	0

[a] Pyrolysis at 250 °C for 20 s (percentages exclude other products). [b] MR, 5-methylfurfural. [c] FDA, 2,5-furandicarboxaldehyde. [d] HMF, 5-(hydroxymethyl)-2-furaldehyde. [e] m/z (%): 39 (11.5), 81 (0.23), 109 (22.4), 126 (36.4%), 127 (100), 189 (22.3); see also Figure 4.

Thermal Degradation of HMF in the Presence of Glycine. Glycine is known to form a Schiff base adduct with HMF[8] and consequently block the carbonyl group and prevent its dimerization; similarly, glycine can react with MF and FDA formed from its degradation through similar Schiff base formation. Table 4 indicates that percent distribution of HMF, HMF dimer, and its degradation products, changes depending on the ratio of HMF to glycine; increasing the ratio of glycine to HMF not only prevents dimerization but also reduces the intensity of MF, HMF, and FDA peaks. Analysis of the new peaks that formed after the addition of glycine indicated the formation of the corresponding decarboxylated Schiff base adducts of MF, HMF, and FDA (both mono- and disubstituted) as shown in Figure 6. The Schiff base adduct of HMF (**6**) had been identified earlier;[8] however, FDA and MF adducts (**8**, **10**, and **11**) were confirmed through synthesis and pyrolysis of their corresponding glycine adducts

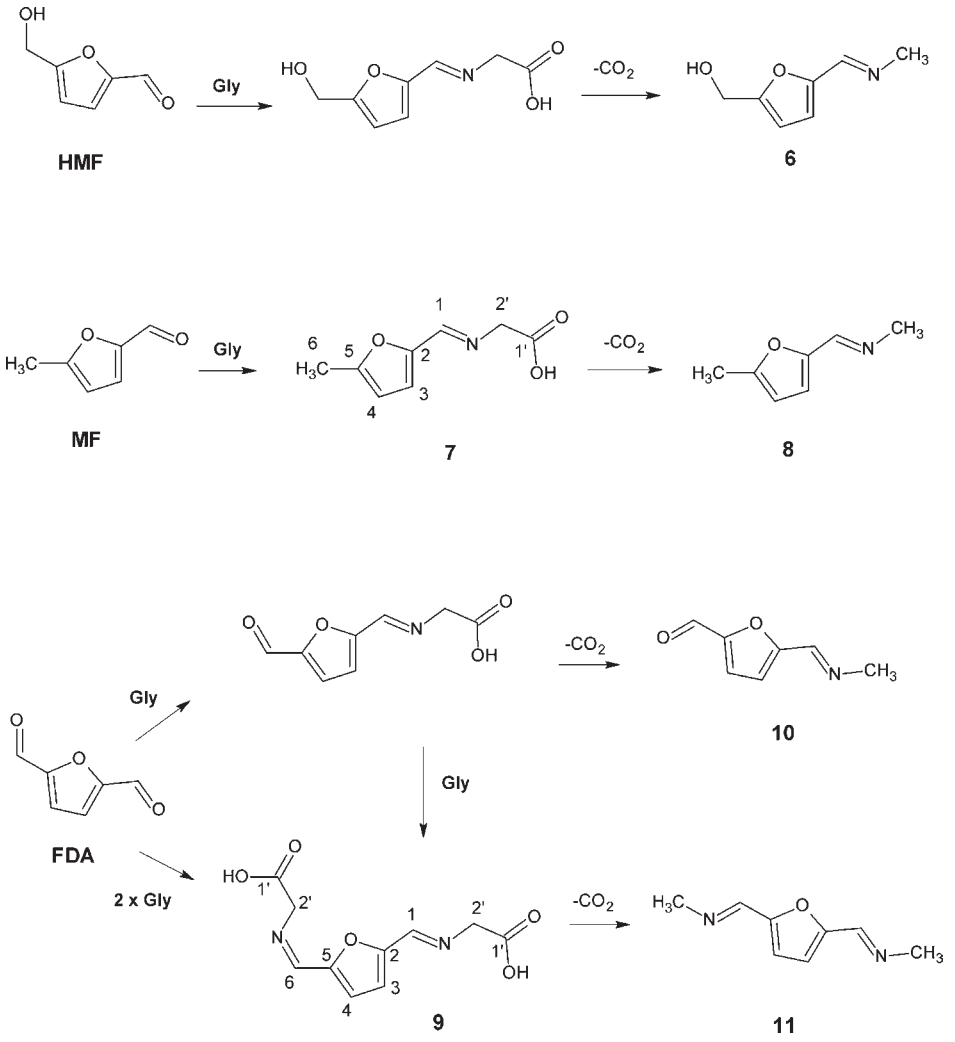

Figure 6. Schiff base adducts of HMF, 5-methylfurfural (MF), and 2,5-furandicarboxaldehyde (FDA) with glycine.

(**7** and **9**), generating peaks with identical retention times and mass spectra. In addition, isotope labeling experiments with glycine (Table 5) indicated the incorporation of one nitrogen and one C-2 atom of glycine in HMF and MF Schiff base adducts, whereas in the double-substituted FDA adduct (**11**) two C-2 atoms and two nitrogen atoms were detected. In situ generation of a labeled HMF moiety through the use of [U-$^{13}C_6$]-glucose/methylamine and [U-$^{13}C_6$]-glucose/methylamine/glycine models confirmed the presence of six carbon atoms from glucose (see Table 5) in each of the above adducts (**6**, **8**, **10**, and **11**). Furthermore, glycine reaction with FDA and MF also generated compounds that eluted at the same retention times and had mass spectra identical to those of **8**, **10**, and **11**. Increasing the glycine to FDA ratio from 1:1 to 3:1 resulted in a 20-fold increase of FDA−diglycine adduct relative to FDA−monoglycine adduct, indicating that FDA−diglycine is formed from the interaction of glycine with FDA−monoglycine.

Formation of 2-Acetyl-5-methylfuran (12) and 5-[(Dimethylamino)methyl]-2-furanmethanol (16). In addition to the observed Schiff base adducts of glycine with HMF, FDA, and MF that showed one C-2 atom incorporation for every nitrogen atom originating from glycine, a peak incorporating only one C-2 atom from glycine without showing any incorporation of nitrogen atom was also identified. NIST library searches indicated the identity of this peak to be 2-acetyl-5-methylfuran (**12** in Figure 7), a known Maillard reaction product; however, to the best of our knowledge its origin has not been reported yet. The structure of **12** was confirmed by comparing its retention time and mass spectrum to those generated from a commercially available standard. Because the compound

Table 5. Incorporation of Total Number of Glucose and Glycine Labeled Atoms into the Schiff Base Adducts from Specifically Labeled Precursors Observed in the HMF/Glycine Model System

compound	MF[a] (**8**)	HMF[b] (**6**)	FDA[c] (**10**)	FDA[d] (**11**)
[^{13}C-1]glycine	0	0	0	0
[^{13}C-2]glycine	1	1	1	2
[^{15}N]glycine	1	1	1	2
[U-$^{13}C_6$]glucose	6	6	6	6

[a] MF, 5-methylfurfural (t_R = 16.58 min). [b] HMF, 5-(hydroxymethyl)-2-furaldehyde (t_R = 20.13 min). [c] FDA, 2,5-furandicarboxaldehyde single Schiff base (t_R = 21.44 min). [d] FDA, furandicarboxaldehyde double Schiff base (t_R = 21.85 min).

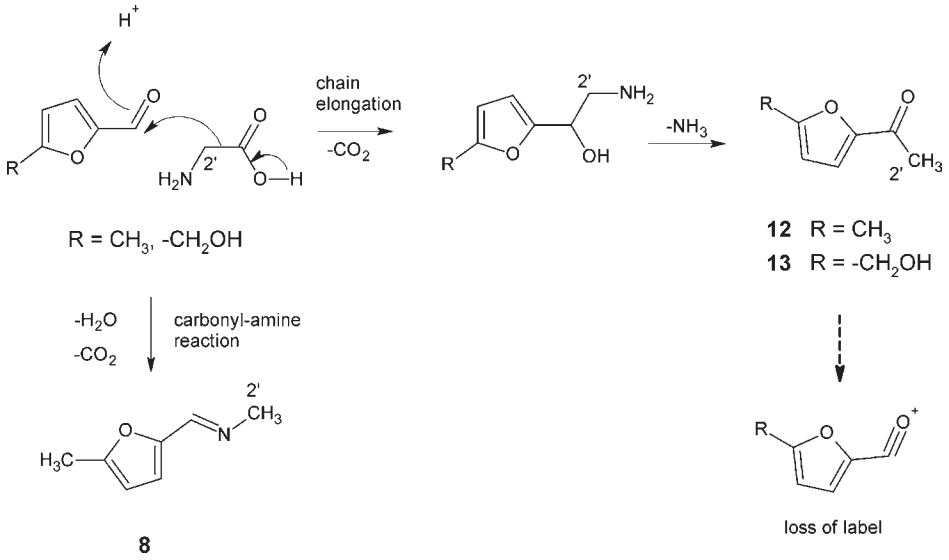

Figure 7. Chain elongation reactions of furfural derivatives. Dotted arrow indicates mass spectral fragmentation steps.

Table 6. Number of Glycine Atom Incorporations in Structures 12, 13, 15, and 16 Generated from the Glycine/HMF Model System and Shown in Figures *7* and *8*

structure	**12**[a]	**13**[b]	**15**[c]	**16**[d]
[^{15}N]glycine	0	0	1	1
[^{13}C-1]glycine	0	0	0	0
[^{13}C-2]glycine	1	1	2[e]	2[e]

[a] 2-Acetyl-5-methylfuran (MW 124 amu, t_R = 16.907 min). [b] MW 140 amu, t_R = 21.746 mi. [c] MW 153 amu, t_R = 20.728 min. [d] MW 155 amu, t_R = 20.654 min. [e] Incorporation of only one C-2 is observed if excess paraformaldehyde is added to the model.

incorporated only one carbon atom from glycine, the remaining six carbon atoms must have originated from HMF or MF. Analysis of the mass spectral fragmentation pattern confirmed the location of the C-2 label from glycine as the methyl group in the acetyl side chain (Figure 7 and Table 6). This type of amino acid C-2 atom incorporation can be achieved through the chain elongation mechanism[18] shown in Figure 7. HMF also undergoes similar chain elongation to produce the corresponding structure **13**, which incorporates only one atom from glycine (C-2) and shows the expected molecular mass at *m*/*z* 140. However, no standards were available to confirm this proposal. When the temperature of the reaction was raised from 250 to 350 °C, a 20-fold increase was observed in the ion count of **12** relative to its corresponding Schiff base adduct **8**, indicating the chain elongation process is highly temperature dependent. The potential of furfurals to participate in chain elongation reactions has not been documented, and it can provide one possible mechanism for the formation of 2-acetyl-5-methylfuran in the Maillard reaction mixtures.

Another chromatographic peak that deviated from the expected pattern of one C-2 atom incorporation for every nitrogen atom characteristic of the Schiff base adducts had the highest intensity in the HMF/glycine chromatogram and showed the incorporation of two C-2 atoms of glycine and only one nitrogen atom. The NIST library search indicated the possible structure of 5-[(dimethylamino)methyl]-2-furanmethanol (**16**) shown in Figure 8 with a high confidence. When commercially available standard was analyzed, it generated a peak having the same retention time and mass spectrum as those of the target compound, confirming the proposed structure. The labeling studies (Tables 6 and 7) clearly indicated incorporation of two C-2 and one nitrogen atom from glycine; however, when excess formaldehyde was added to the HMF/glycine model, only one C-2 atom from glycine was found to be incorporated in **16**, indicating formaldehyde as a possible source of the N-methyl group. The N-methylation of amino acids through reduction of Schiff base adducts of formaldehyde has already been reported,[19] and in Figure 8 we propose formic acid as the reducing agent[20] originating from the degradation of HMF[3] and converting the formaldehyde Schiff base adduct of glycine into sarcosine. The HMF/sarcosine model, as expected, also generated a peak with a retention time and mass fragmentation profile matching those of the commercial standard. Although formaldehyde can be generated as the Strecker aldehyde of glycine, the reaction of HMF with glycine known to proceed with the formation of oxazolidinone intermediate[8] can also generate formaldehyde as shown in Figure 2. The Schiff base adduct **14**, being a secondary amine, is expected to also undergo vinylogous Amadori rearrangement[8] to form structure **15**. A peak eluting at a retention time of 20.73 min and corresponding to the specifications of **15** with a consistent mass spectrum (Table 8) was observed; as shown in Figure 8, the characteristic peak at *m*/*z* 109 (55.7%) was completely absent from **16**, and the peak at *m*/*z* 111 (65.5%) characteristic of **16** was absent from **15**. The identity of **15**, however, remains to be confirmed.

Thermal decomposition studies of HMF have indicated that in the absence of amino acids it can mainly dimerize and the initially formed dimer can degrade to generate 5-methylfurfural and 2,5-furandicarboxaldehyde. On the other hand, in the presence of amino acids such as glycine, HMF and its degradation products can form Schiff base adducts. In addition, glycine not only can undergo carbonyl amine reactions with HMF but also chain elongation reactions in the presence of furfural derivatives to generate 2-acetyl furan derivatives. The abilities of HMF and glycine to generate formic acid during the Maillard reaction can provide the reducing agent needed to justify many of the

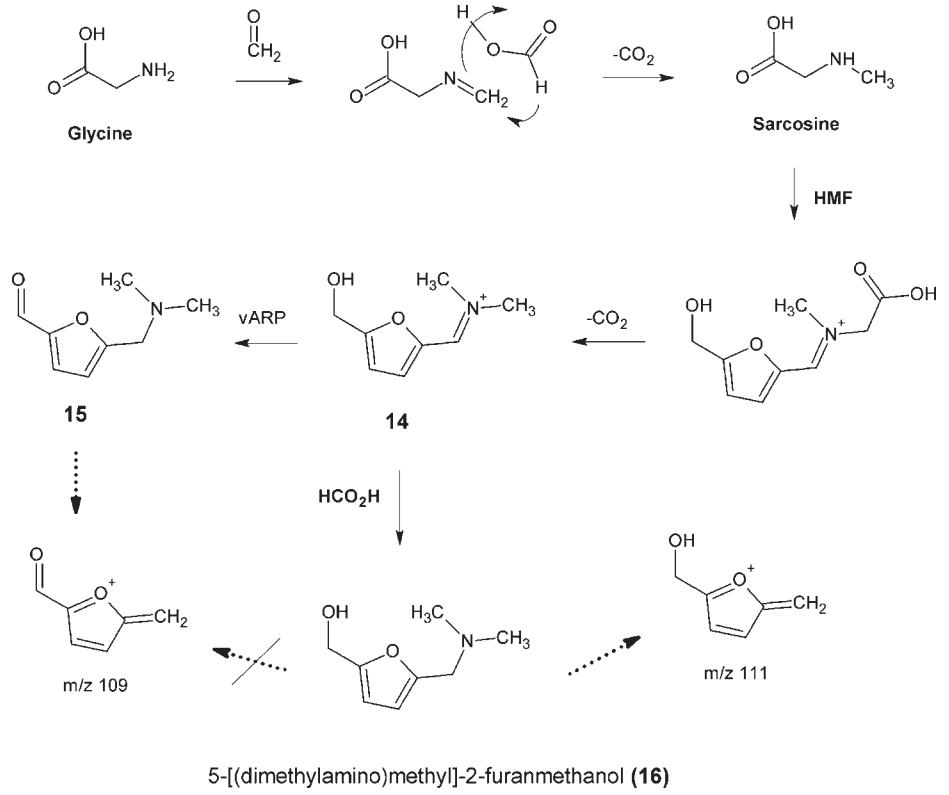

Figure 8. N-Methylation of glycine and subsequent formation of Schiff base adduct (**14**) followed by either a reduction to form **16** or vinylogous Amadori rearrangement to form **15**. Dotted arrows indicate mass spectral fragmentation steps.

Table 7. Number of Glycine Atom Incorporations into the Mass Spectral Fragments of 5-[(Dimethylamino)methyl]-2-furanmethanol (16, t_R 20.65 min) in the Glycine/HMF Model System

	m/z					
	155[a]	138	124	111	94	83
[^{15}N]glycine	1	1	1	0	1	0
[^{13}C-1]glycine	0	0	0	0	0	0
[^{13}C-2]glycine	2	2	2	0	1	0

[a] Molecular ion.

Table 8. Number of Glycine Atom Incorporations into the Mass Spectral Fragments of the Proposed Structure 15 (t_R 20.73 min) in the Glycine/HMF Model System

	m/z			
	153[a]	124	109	81
[^{15}N]glycine	1	1	0	0
[^{13}C-1]glycine	0	0	0	0
[^{13}C-2]glycine	2	2	0	0

[a] Molecular ion.

products observed during the Maillard reaction,[20] such as the formation of sarcosine from glycine.

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