Pentoses and Hexoses as Sources of New Melanoidin-like Maillard Polymers

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N-Substituted pyrroles (**1**), 2-furaldehyde (**2**), and N-substituted 2-formylpyrroles (**3**), formed in pentose (hexose) Maillard systems, were identified as components of extraordinary polycondensation activity. The polycondensation was studied in model experiments with *N*-methylpyrrole (**1a**)/*N*-methyl-2-formylpyrrole (**3a**), *N*-(2-methoxycarbonylethyl)pyrrole (**1b**)/*N*-(2-methoxycarbonylethyl)-2-formylpyrrole (**3b**), *N*-methyl-2-formylpyrrole (**3a**), *N*-methylpyrrole (**1a**)/2-furaldehyde (**2**), and *N*-(2-methoxycarbonylethyl)pyrrole (**1b**)/2-furaldehyde (**2**), respectively**.** MALDI-TOF-MS spectra indicated regular oligomers of up to 15−30 methine-bridged *N*-methyl(or *N*-2-methoxycarbonylethyl)-pyrroles. With participation of aldehyde **2**, furan rings instead of pyrrole rings were incorporated. The oligomers **5**−**11** were isolated and identified by MS and NMR techniques. A complementary experiment with *N*-methyl-2-[^{13}C]formylpyrrole ([^{13}CHO]-**3a**)/*N*-methylpyrrole (**1a**) was performed. The relevance of the new (type II) melanoidin-like oligomers/polymers in Maillard reactions is discussed and, in conclusion, a corresponding structure for native melanoidins is proposed. The oligomers **5**, **6**, **8**, and **9** were tested for antioxidative activity in an iron(III) thiocyanate assay.

Keywords: *Model compounds for melanoidins; pyrroles from pentoses and hexoses; β-dicarbonyl pathway of the Maillard reaction; polycondensation of N-methylpyrrole with N-methyl-2-formylpyrrole (N-methyl-2-[^{13}C]formylpyrrole) or 2-formylfuran; polycondensation of N-(2-methoxycarbonylethyl)-pyrrole with N-(2-methoxycarbonylethyl)-2-formylpyrrole or 2-formylfuran; MALDI-TOF-MS analysis of melanoidin-like oligomers/polymers; antioxidative activity of oligomeric model compounds*

INTRODUCTION

In the Maillard reaction the formation of macromolecular compounds, generally referred to as melanoidins (Maillard, 1912), is predominant (>95% p.w.). In a previous paper Tressl et al. (1998a) reported on the formation and characterization of linear (type I) melanoidin-like polymers from *N*-methyl-2-(hydroxymethyl)pyrrole, especially important in 2-deoxy-D-ribose as well as DNA−Maillard systems (Wondrak et al., 1997). This route cannot explain the extensive formation of melanoidins from pentoses as well as from hexoses: According to Tressl et al. (1993a), 2-deoxypentoses arise from hexoses by fragmentation only in minor yield. Thus, up to now the structures of the low molecular weight intermediates as well as the structure of the macromolecular melanoidins formed from pentoses and hexoses are still unknown.

From the various published data on the spectroscopic properties of melanoidins we expected certain pyrroles and furans as key intermediates of melanoidin formation (Benzing-Purdie et al., 1983; Feather and Huang, 1986; Hayase et al., 1986; Ledl and Schleicher, 1990). As proven by extensive labeling experiments (Tressl et al., 1993a,b, 1995), several C_6-, C_5-, and C_4-pyrroles and -furans (C_n with respect to the number of C atoms incorporated from the sugar molecule) are formed during the Maillard reaction with either an intact or a fragmented sugar skeleton. Because of the superior browning activity of pentoses and tetroses as compared to hexoses, we focused on C_4- and C_5-pyrroles and -furans as most suitable precursors of melanoidins and started a series of model reactions exploring their polycondensation potential.

EXPERIMENTAL PROCEDURES

Materials and Methods. *N*-Methyl-2-[^{13}C]formylpyrrole (2-[^{13}C]-**3a**) was prepared according to the method of Tressl et al. (1998a). Other reagents were from Fluka AG, Neu-Ulm, Germany. Silica gel 60 (Merck Chemical Co., Darmstadt, Germany) was used for LC. Autoclaving was done in a stainless steel laboratory autoclave (Roth, I series) equipped with a 100 mL duran glass tube and heated by an electric heater with magnetic stirrer. During autoclaving the peak temperature (120 °C) was reached after 45 min.

***N*-(2-Methoxycarbonylethyl)pyrrole (1b).** According to the method of Blume and Lindwall (1945), *N*-(2-cyanoethyl)-pyrrole was converted into the free acid, which was methylated with BF_3 (10% in methanol) according to the standard procedure of Metcalfe and Schmitz (1961) (yield = 74%).

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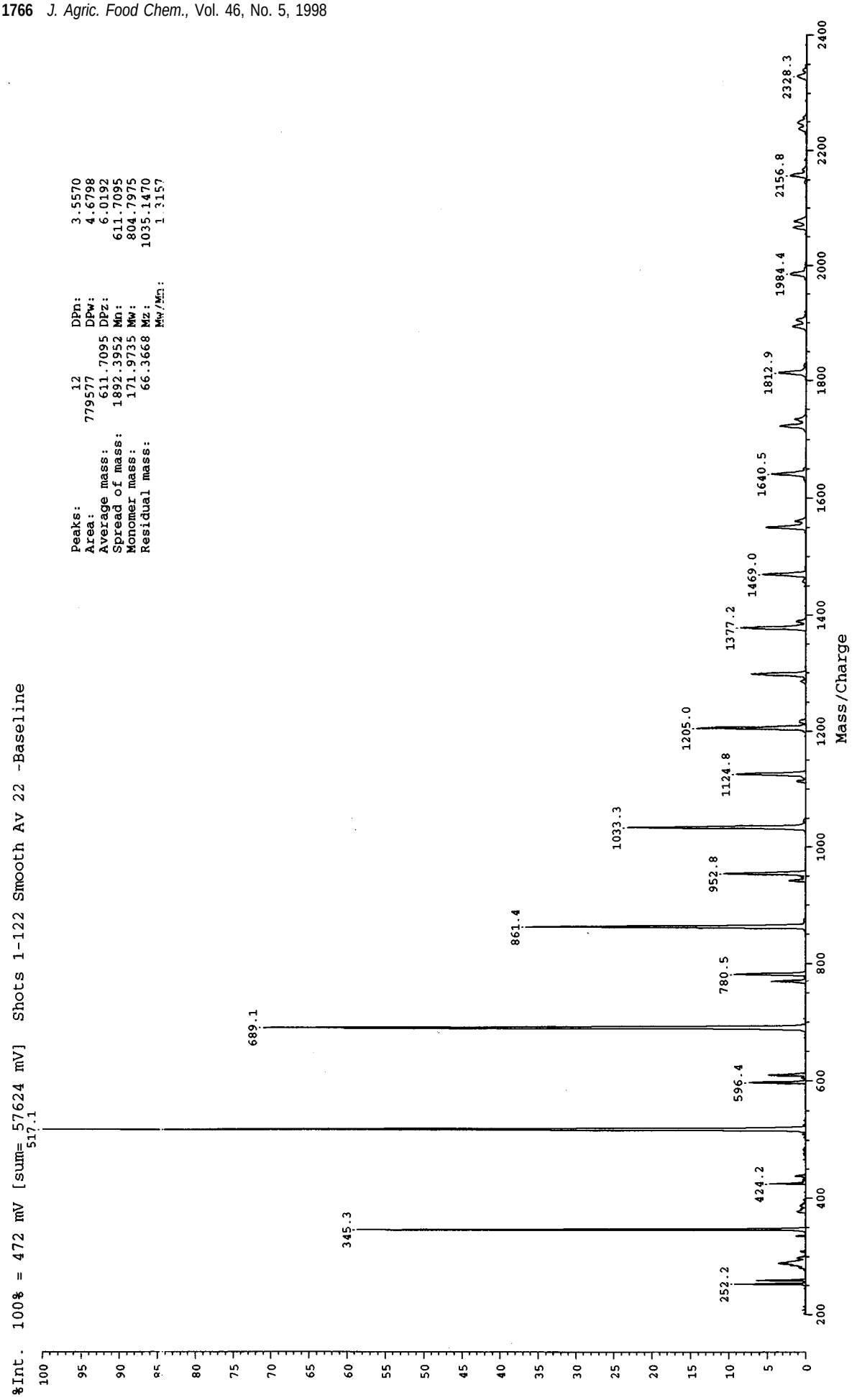
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1033.3
424.2
596.4
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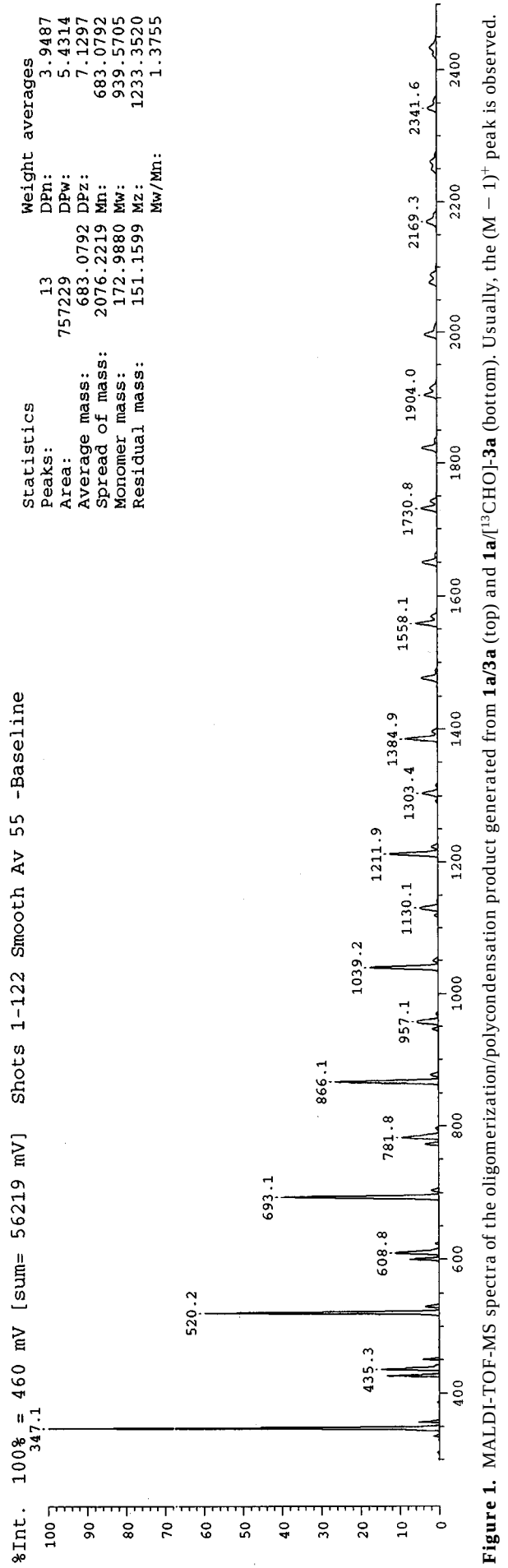


Figure 1. MALDI-TOF-MS spectra of the oligomerization/polycondensation product generated from **1a/3a** (top) and **1a**/[^{13}CHO]-**3a** (bottom). Usually, the $(M - 1)^+$ peak is observed.

***N*-(2-Methoxycarbonylethyl)-2-formylpyrrole (3b).** According to the method of Chan and Lee (1983), $TiCl_4$ (0.21 g, 1.2 mmol) was dropped into 0.127 g (1.20 mmol) of trimethyl orthoformate in 5 mL of dry dichloromethane at −40 °C under nitrogen. Pyrrole **1b** (0.15 g, 1 mmol) in dry dichloromethane (2 mL) was added dropwise over 5 min. After 90 min at −40 °C, the mixture was allowed to come to 20 °C. After addition of water (1 mL), the mixture was extracted three times with diethyl ether (each 20 mL). The combined organic phases were dried over Na_2SO_4. The diethyl ether was evaporated, and **3b** was isolated by column chromatography (silica gel; hexanes/ethyl acetate = 7:3) as a colorless oil (95 mg, 53%).

Sample Preparation. Acid-Catalyzed Polycondensations. *(A) N-Methylpyrrole (**1a**) and N-Methyl-2-formylpyrrole (**3a**).* A solution of **1a** (1 mL) and **3a** (1 mL) in methanol (40 mL) was stirred for 10 min at 20 °C after addition of 1 N HCl (100 μL). The solution became red within seconds. After evaporation of the solvent, the deep red residue was redissolved in trichloromethane and an aliquot was analyzed by GC/MS and MALDI-TOF-MS (see Figure 1): UV−VIS (0.1 mg/mL in $CHCl_3$) λ_{max} (E) = 245 (2.6), 514 (0.45).

*(A.1) Isolation of the Oligomers **5**−**7**.* An aliqout (1.5 mL) of the reaction mixture was fractionated by TLC (1 mm silica gel; petroleum ether/ethyl acetate = 3:1, + 1% NH_3). Tris(*N*-methyl-2-pyrryl)methane (**5**) (6 mg; R_f = 0.4), *N*-methyl-2,5-bis[bis(*N*-methyl-2-pyrryl)methyl]pyrrole (**6**) (2 mg; R_f = 0.21), and [*N*-methyl-2-pyrryl]bis{5-[bis(*N*-methyl-2-pyrryl)methyl]-*N*-methyl-2-pyrryl}methane (**7**) (2 mg; R_f = 0.12) were isolated and characterized (see Table 1).

*(B) N-(2-Methoxycarbonylethyl)pyrrole (**1b**) and N-(2-Methoxycarbonylethyl)-2-formylpyrrole (**3b**).* The components (each 100 μL) were reacted and analyzed as described above (A).

*(C) N-Methyl-2-formylpyrrole (**3a**) and 2-Methylfuran.* The components (each 1 mL) were reacted and analyzed as described above.

*(C.1) Isolation of the Trimer **11**.* An aliquot (1 mL) of the reaction mixture was fractionated by preparative TLC (1 mm silica gel, petrolether/ethyl acetate = 1:1). [*N*-methyl-2-pyrryl]-bis[(*N*-methyl-2-formyl-5-pyrryl]methane (**11**)] (4 mg; R_f = 0.4) was isolated and characterized (see Table 1).

*(D) N-Methylpyrrole (**1a**) and 2-Furaldehyde (**2**).* The components (each 1 mL) were reacted as described above. A white solid was recovered by filtration and redissolved in diethyl ether. An aliquot was analyzed by GC/MS and MALDI-TOF-MS (see Figure 3). *(D.1) Isolation of the Oligomers **8**−**10**.* An aliquot of the reaction mixture (1.5 mL) was fractionated by TLC (1 mm silica gel, petroleum ether/ethyl acetate = 3:1). (2-Furyl)bis(*N*-methyl-2-pyrryl)methane (**8**) (5 mg; R_f =0.44), *N*-methyl-2,5-bis[(2-furyl)(*N*-methyl-2-pyrrolyl)]-pyrrole (**9**) (4 mg; R_f = 0.37), and [2-furyl]bis{5-[(2-furyl)(*N*-methyl-2-pyrrolyl)methyl]-*N*-methyl-2-pyrrolyl}methane (**10**) (3 mg; R_f = 0.29) were isolated and characterized (see Table 1).

*(E) N-(2-Methoxycarbonylethyl)pyrrole (**1b**) and 2-Furaldehyde (**2**).* The components (each 100 μL) were reacted and analyzed as described above (A).

Gel Filtration Conditions: column, Ultra-Styragel (100000 nm + 10000 nm + 1000 nm); Waters HPLC pump 150C; Waters multiwavelength detector M 490; eluent, tetrahydrofuran (1.5 mL/min); UV detection (λ = 254 nm).

Gas Chromatography (GC)/Mass Spectrometry (MS). The extracts prepared were analyzed by GC/MS using a 60 m × 0.32 mm i.d. DB-1 fused silica capillary column coupled with a double-focusing mass spectrometer CH 5-DF (Varian MAT), ionization voltage 70 eV, and resolution 2000 (10% valley). Temperature was programmed from 80 to 280 °C at 4 °C/min.

UV−Vis Spectrometry. UV−vis spectra were recorded with a Uvikon 922 spectrophotometer (Kontron Instruments).

FAB-Mass Spectrometry. The fast atom bombardment mass spectra (8 keV, xenon) were recorded on the CH5-DF spectrometer (Varian MAT) using glycerol as matrix.

MALDI-TOF Mass Spectrometry. Measurements were carried out on a Kratos Kompact MALDI III, Shimadzu. Half a microliter of the sample solution (1 mg/mL in $CHCl_3$ or tetrahydrofuran) and 0.5 μL of matrix solution (25 mg/mL of

Table 1. MS and NMR Spectra of Selected Products

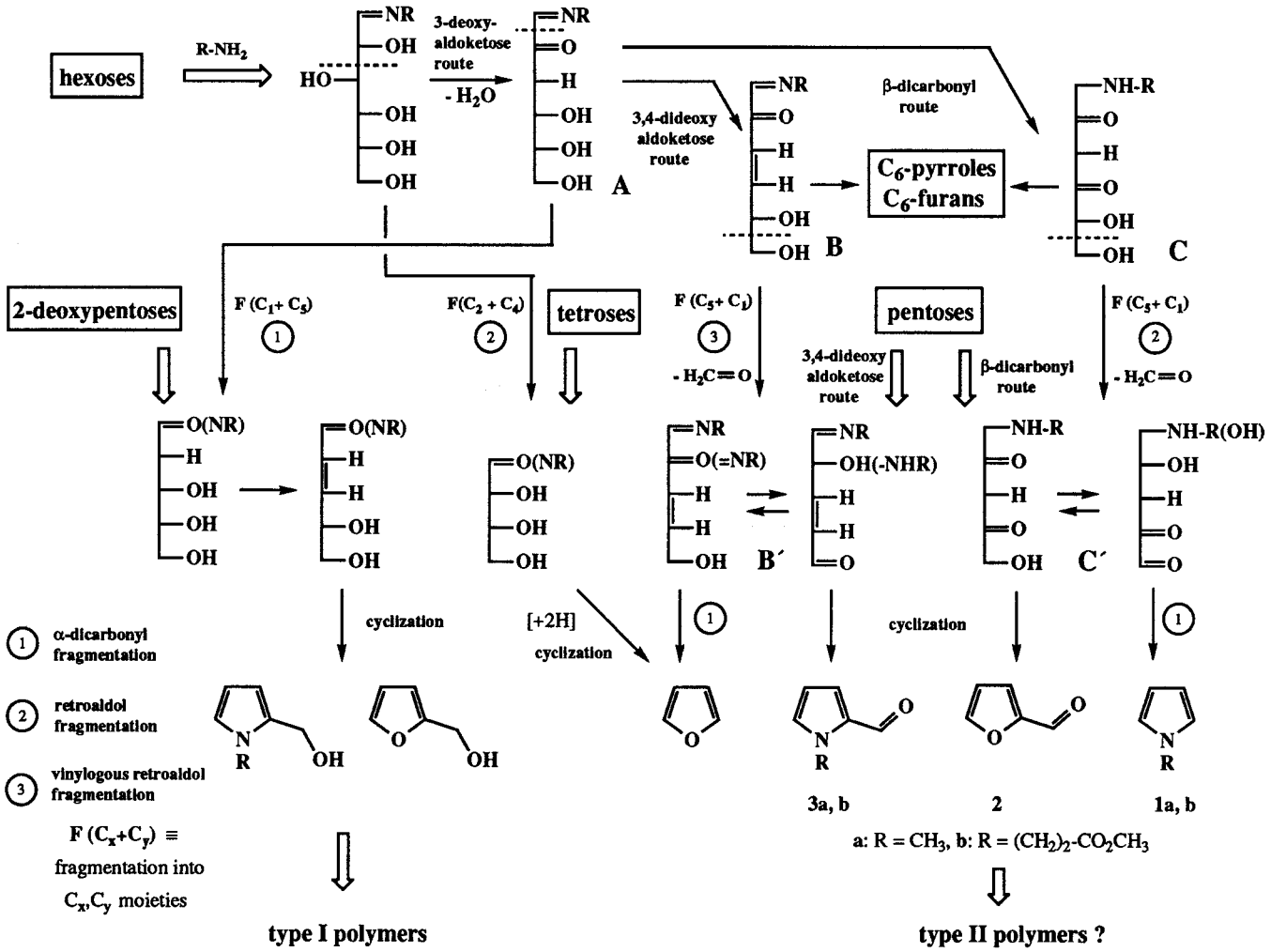
compound	MS and NMR data
N-(2-methoxycarbonylethyl)-2-formylpyrrole **(1b)**	MS (EI, 70 eV), *m/z* 181 (30), 153 (75), 138 (21), 122 (100), 108 (43), 94 (78), 80 (62), 67 (28), 55 (58), 39 (47) ^{1}H NMR 2.80 (t, 2H, J = 6.5 Hz, RO_2C-CH_2), 3.63 (s, 3H, CH_3), 4.55 (t, 2H, J = 6.5 Hz, $N-CH_2$), 6.18 (dd, 1H, J = 4,0, 2.6 Hz, H-4), 6.93 (dd, 1H, J = 4.0, 1.7 Hz, H-3), 7.02 (br s, 1H, H-5), 9.50 (s, 1H, CHO)
N-(2-methoxycarbonylethyl)-pyrrole **(3b)**	MS (EI, 70 eV), *m/z* 153 (47), 122 (9), 94 (55), 80 (100), 67 (20), 53 (18), 39 (23) ^{1}H NMR 2.76 (t, 2H, J = 7.0 Hz, RO_2C-CH_2), 3.67 (s, 3H, CH_3), 4.20 (t, 2H, J = 7.0 Hz, $N-CH_2$), 6.12 (mc, 2H, H-3,4), 6.65 (mc, 2H, H-2,5)
tris(*N*-methyl-2-pyrrolyl)methane (**5**) (trimer)	MS, *m/z* 253 (100), 173 (39), 172 (26), 171 (76), 94 (28), 42 (6) ^{1}H NMR 3.395 (s, 9H, $N-CH_3$); 5.20 (s, 1H, sp^3-CH); 5.53 (mc, 3H, H-3), 5.995 (mc, 3H, H-4), 6.555 (mc, 3H, H-5)
tris(*N*-methyl-2-pyrrolyl)[^{13}C]methane ([^{13}C]-**5)** (trimer)	MS, *m/z* 254 (100), 253 (39), 174 (32), 173 (21), 172 (70), 94 (19), 42 (7)
N-methyl-2,5-bis[bis(*N*-methyl-2-pyrrolyl)methyl]pyrrole (**6**) (pentamer)	FAB-MS, *m/z* 425 (M^+) ^{1}H NMR 3.17 (s, 3H, central $N-CH_3$), 3.396 (s, 12H, peripheral $N-CH_3$), 5.176 (s, 2H, sp^3-CH), 5.412 (s, 2H, H-3,4 of central pyrrole), 5.524 (mc, 4H, H-3 of peripheral pyrroles); 5.996 (mc, 4H, H-4 of peripheral pyrroles), 6.556 (mc, 4H, H-5 of pyrroles) ^{13}C NMR 30.53 (sp^3-*C*H), 33.80, 34.80 (N*C*H_3), 106.44 (ring *C*H), 107.40 (C-3,4 of central pyrrole), 108.52 (ring *C*H), 121.90 (5-*C*H of peripheral pyrroles), 131.94 (quart. C of pyrroles)
[*N*-methyl-2-pyrrolyl]bis{5-[(bis-*N*-methyl-2-pyrrolyl)methyl]-*N*-methyl-2-pyrrolyl}methane (**7**) (heptamer)	FAB-MS, *m/z* 597 (M^+) ^{1}H NMR 3.16 (s, 6H, $N-CH_3$), 3.368, 3.384 (s, 12H, peripheral $N-CH_3$), 3.390 (s, 3H, central N-CH_3), 5.14(s, 1H, central sp^3-CH), 5.175 (s, 2H, peripheral sp^3-CH), 5.395 (s, 4H, H-3,4 of disubstituted pyrroles), 5.515 (mc, 5H, H-3 of monosubstituted pyrroles), 5.98 (mc, 5H, H-4 of monosubstituted pyrroles), 6.55 (mc, 5H, H-5 pyrroles) ^{13}C NMR 29.70, 30.57 (sp^3-*C*H), 33.81, 34.81 (N*C*H_3), 106.46 (ring *C*H), 107.32 (C-3,4 of disubstituted pyrrole), 108.52 (ring *C*H), 122.33 (5-*C*H of pyrroles), 131.04, 131.97 (quart. C of pyrroles)
(2-furyl)bis(*N*-methyl-2-pyrrolyl)-methane (**8**) (trimer)	MS 240 (100), 211 (25), 196 (10), 173 (12), 160 (30), 159 (25), 131 (22), 130 (27), 94 (33), 42 (25) ^{1}H NMR 3.41 (s, 6H, NCH_3), 5.36 (s, 1H, sp^3-CH), 5.65 (mc, 2H, pyrrole H-3), 5.89 (dd, 1H, J = 3.2, <1 Hz, furan H-3), 6.02 (t, 2H, J = 3.2 Hz, pyrrole H-4), 6.30 (dd, 1 H, J = 3.0, 1.9 Hz, furan H-4), 6.57 (t, 2H, J = 2.2 Hz, pyrrole H-5), 7.35 (mc, 1H, furan H-5)
N-methyl-2,5-bis[(2-furyl)(*N*-methyl-2-pyrrolyl)]pyrrole (**9**) (pentamer, mixture of two diastereomers)	FAB-MS, *m/z* 399 (M^+) ^{1}H NMR 3.179, 3.184 (each s, 3H, central N-CH_3), 3.413, 3.420 (each s, 6H, peripheral $N-CH_3$), 5.34(br s, 2H, sp^3-CH), 5.540, 5.545 (each s, 2H, H-3,4 of central pyrrole), 5.63, 5.64 (each mc, 2H, H-3 of peripheral pyrroles), 5.86, 5.89 (each dd, 2H, J = 3.0, <1 Hz, H-3 of the furans), 6.02 (mc, 2H, H-4 of peripheral pyrroles), 6.30 (mc, 2H, H-4 of furans), 6.57 (mc, 2H, H-5 of pyrroles), 7.35 (mc, 2H, H-5 of furans) ^{13}C NMR 30.46, 30.52 (sp^3*C*-H), 33.75 (central N*C*H_3), 36.22, 36.24 (peripheral N*C*H_3), 106.54, 107.02, 107.54, 108.20, 108.23, 110.26 (ring *C*H), 122.18, 122.22 (5-*C*H of peripheral pyrroles), 130.80, 130.84, 131.12, 131.14 (quart. C of pyrroles), 141.59 (5-*C*H of furans), 154.26, 154.36 (quart. C of furans)
[2-furyl]bis{5-[(2-furyl)(*N*-methyl-2-pyrrolyl)methyl]-*N*-methyl-2-pyrrolyl}methane (**10**) (heptamer, mixture of three diastereomers)	FAB-MS, *m/z* 558 (M^+) ^{1}H NMR 3.155, 3.165, 3.176 (each s, 3H, central $N-CH_3$-groups), 3.380, 3.390, 3.401 (each s, 6H, peripheral $N-CH_3$), 5.295 (br s, 1H, sp^3-CH), 5.322 (br s, 2H, sp^3-CH), 5.495−5.545 (4H, H-3,4 of central pyrroles), 5.590−5.635 (2H, H-3 of peripheral pyrroles), 5.815−5.895 (3H, H-3 of the furans), 5.99−6.03 (2H, H-4 of peripheral pyrroles), 6.270−6.305 (3H, H-4 of furans), 6.54−6.56 (2H, H-5 of pyrroles), 7.34 (3H, H-5 of furans) ^{13}C NMR 30.49 (sp^3*C*-H), 33.78 (central N-*C*H_3-groups), 36.24, 36.60 (peripheral N-*C*H_3− groups), 106.56, 107.03, 107.56, 107.67, 108.20, 110.28 (ring *C*H), 122.22 (5-*C*H of peripheral pyrroles), 130.85, 131.11(quart. C of pyrroles), 141.61 (5-*C*H of furans), 154.26, 154.34 (quart. C of furans)
[*N*-methyl-2-pyrrolyl]bis[(*N*-methyl-2-formyl-5-pyrrolyl]methane (**11**) (trimer)	MS, *m/z* 309 (65), 294 (6), 280 (31), 201 (19), 199 (51), 187 (15), 171 (20), 94 (100), 42 (22) ^{1}H NMR 3.39 (s, 3H, $N-CH_3$ of monosubstituted pyrrole), 3.78 (s, 6H, $N-CH_3$ of disubstituted pyrroles), 5.23 (s, 1H, sp^3-CH), 5.52 (mc, 1H, of monosubstituted pyrrole), 5.66 (d, J = 4.25 Hz, 2H, H-4 of disubstituted pyrroles), 6.02 (mc, 1H, H-4 of monosubstituted pyrrole), 6.62 (mc, 1H, H-5 of monosubstituted pyrrole), 6.84 (d, J = 4.25 Hz, 2H, H-3 of disubstituted pyrroles), 9.50 (s, 2H, CHO)

2,5-dihydroxybenzoic acid or 2,4,6-trihydroxyacetophenone in ethanol) were mixed on the stainless steel sample slide and the solvent was evaporated. Bovine insulin was used for calibration (molar mass 5734.5 g/mol). The following conditions were applied: polarity positive, flight path reflection, 20 kV acceleration voltage, nitrogen laser 337 nm; the spectra were smoothed.

^{1}H/^{13}C NMR Spectroscopy. ^{1}H NMR spectra were recorded at 270 (500 MHz) on Bruker WH 270 and AMX 500 NMR spectrometers in $CDCl_3$. Chemical shifts (parts per million) are referenced to tetramethylsilane (TMS) as internal standard. Coupling constants (J) are in hertz.

Iron(III) Thiocyanate Antioxidative Assay (Inatani et al., 1983). To 2 (20) mg of trimer (two types) and pentamer (two types) or other antioxidants in 2 mL of 99.5% ethanol were added 2.052 mL of linoleic acid (2.51% in 99.5% ethanol), 4 mL of phosphate buffer (0.05 M, pH 7), and 1.948 mL of distilled water. The solution was kept at 40 °C for 2 weeks.

Scheme 1. Transformation of Hexoses and Pentoses to C_5- and C_4-Pyrroles and -Furans



Every 24 h an aliquot of this solution (0.1 mL) was added to 9.7 mL of ethanol (75%, v/v) plus 0.1 mL of NH_4SCN (30%, w/v). Exactly 3 min after addition of 0.1 mL of $FeCl_2$ (2×10^{-2} M in 3.5% HCl), the absorption was measured at $\lambda = 500$ nm. The antioxidative activities (in percent $= [1 - A_{probe}/A_{sample}] \times 100$) measured at $\lambda = 500$ nm for two different amounts of test compounds (2 mg/ 20 mg) are as follows: octylgallate, 96 (88)%; L-ascorbic acid, 94 (89)%; bilirubine, 94 (92)%; *N*-methylpyrrole (**1a**), 11 (15)%; *N*-methyl-2-formylpyrrole (**3a**), 19 (17)%; **5**, 94 (87)%; **6**, 68(−13)%; **8**, 80 (90)%; **9**, 7 (80)%.

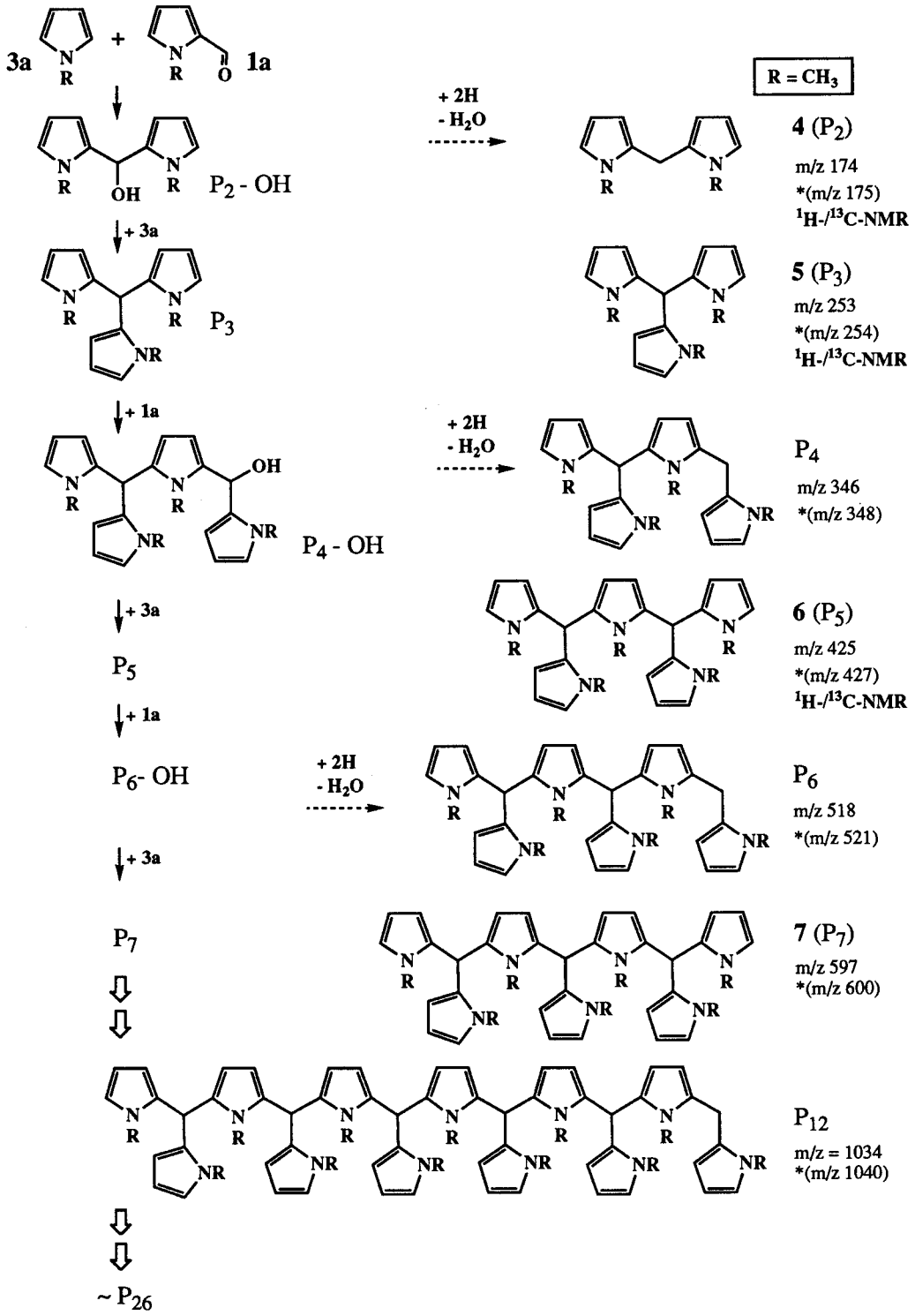
RESULTS AND DISCUSSION

On the basis of extensive ^{13}C- and ^{2}H-labeling experiments, a detailed Maillard reaction scheme of hexoses and pentoses was proposed by Tressl et al. (1995). Thus, formation of various C_6-, C_5-, and C_4-pyrroles and -furans from intact as well as from fragmented hexoses and amines or ω-aminoalkanoic acids could be unambiguously attributed to distinct reaction pathways via the intermediates **A**−**C** (see Scheme 1). The novel β-dicarbonyl route via **C** (from hexoses) or **C′** (from pentoses) competes with the well-known 3,4-dideoxyaldoketose route (via **B** or **B′**). These two routes result in different isomeric C_6 products from hexoses and in identical (but isotopomeric) C_5 products from (labeled) pentoses. As summarized in Scheme 1, the C_5- and C_4-pyrroles and -furans, of special interest with respect to the formation of melanoidins, are also easily formed from hexoses via different fragmentation reactions (Hayashi and Namiki, 1986; Hayase and Kato, 1985; Severin and Sengl, unpublished results, 1988; Tressl et al., 1998c).

Whereas the C_6-pyrroles and -furans represent compounds of low polymerizing, but high cross-linking, activity, the N-substituted C_5- and C_4-pyrroles, either alone [for example, *N*-methyl-2-(hydroxymethyl)pyrrole] or in combination, will represent species with at least two sites of reactivity and, therefore, suitable precursors for the formation of macromolecules. The extraordinary polycondensation potential of *N*-methyl-2-(hydroxyalkyl)pyrroles even under mild reaction conditions was demonstrated by Tressl et al. (1998a) as well as by Hidalgo and Zamora (1993). Now we report on corresponding experiments using N-substituted pyrroles (**1**) (or furan) and N-substituted 2-formylpyrroles (**3**) (or furfural **2**) as components.

Under very mild conditions (20 °C, H^+ catalysis) new colored polymers (type II polymers) were formed from N-substituted pyrrole/N-substituted 2-formylpyrrole (or furfural) combinations and from N-substituted 2-formylpyrroles alone. A systematic structural investigation by GC/MS, FAB-MS, MALDI-TOF-MS, and $^{1}H/^{13}C$ NMR spectroscopy of single oligomers isolated by TLC and of the polymers revealed the structure of the new melanoidin-like polymers. The MALDI-TOF-MS method (Krüger, 1995; Bahr et al., 1992; Siuzdak, 1994)

Scheme 2. Generation of Polymers of Type II in N-Substituted Pyrrole/N-Substituted 2-Formylpyrrole Systems



proved to be an especially very powerful tool in the structure elucidation of the formed complex oligmer/polymer mixtures.

***N*-Methylpyrrole (1a)/*N*-Methyl-2-formylpyrrole (3a) System.** N-Substituted 2-formylpyrroles as well as N-substituted pyrroles are common products in amino acid/hexose or pentose Maillard reactions. First, we studied **3a** (or 2-[^{13}CHO]-**3a**) in combination with **1a** as simple model systems and observed extensive polycondensation within minutes. Oligomeric species of up to ~25 methine-bridged pyrroles were detected by MALDI-TOF-MS (Figure 1), which also clearly demonstrates their regular linear or branched structure. The monomeric unit of these oligomers is a bis(*N*-alkyl-2-pyrrolyl)methyl unit (Δm = 172 and 173, respectively). The peaks of the even-numbered species are much more intense than those of odd-numbered species. The oligomers **5**−**7** (Scheme 2) could be isolated and characterized by spectroscopic methods (Table 1). The labeled oligomer [^{13}CH]-**5** was studied by GC/MS.

Obviously, the +I-effect of the *N*-alkyl group strongly activates the pyrroles in electrophilic substitution reactions. In a reaction cascade shown in Scheme 2 the intermediate carbinols are either reduced to the even-

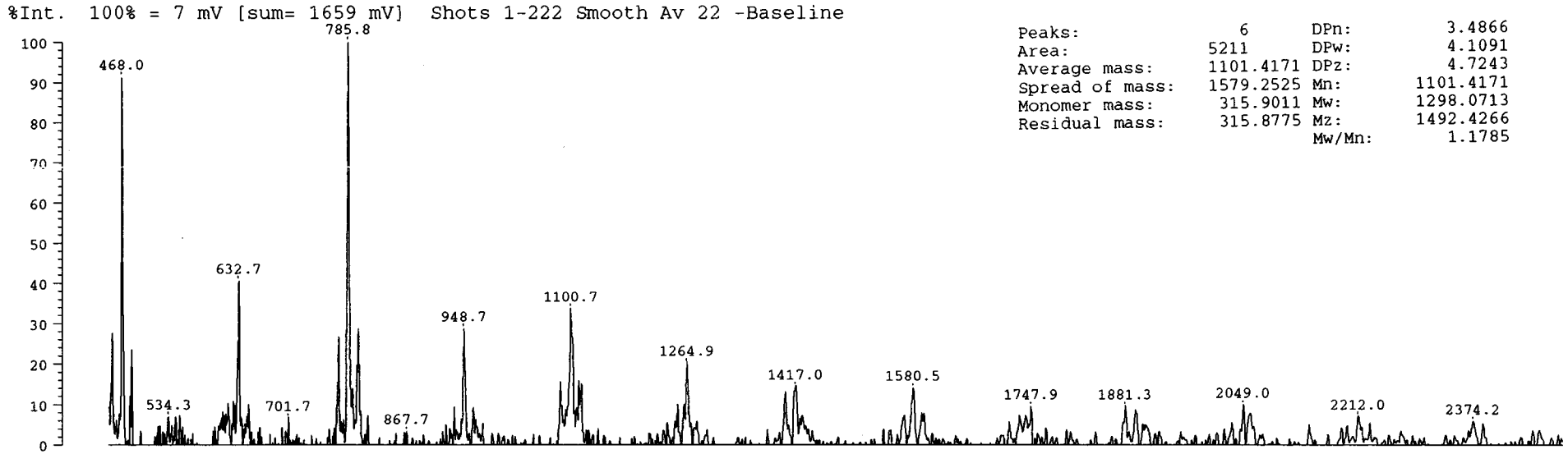


Figure 2. MALDI-TOF-MS spectra of the oligomerization/polycondensation product generated from **1b/3b**. Usually, the $(M - 1)^+$ peak is observed.

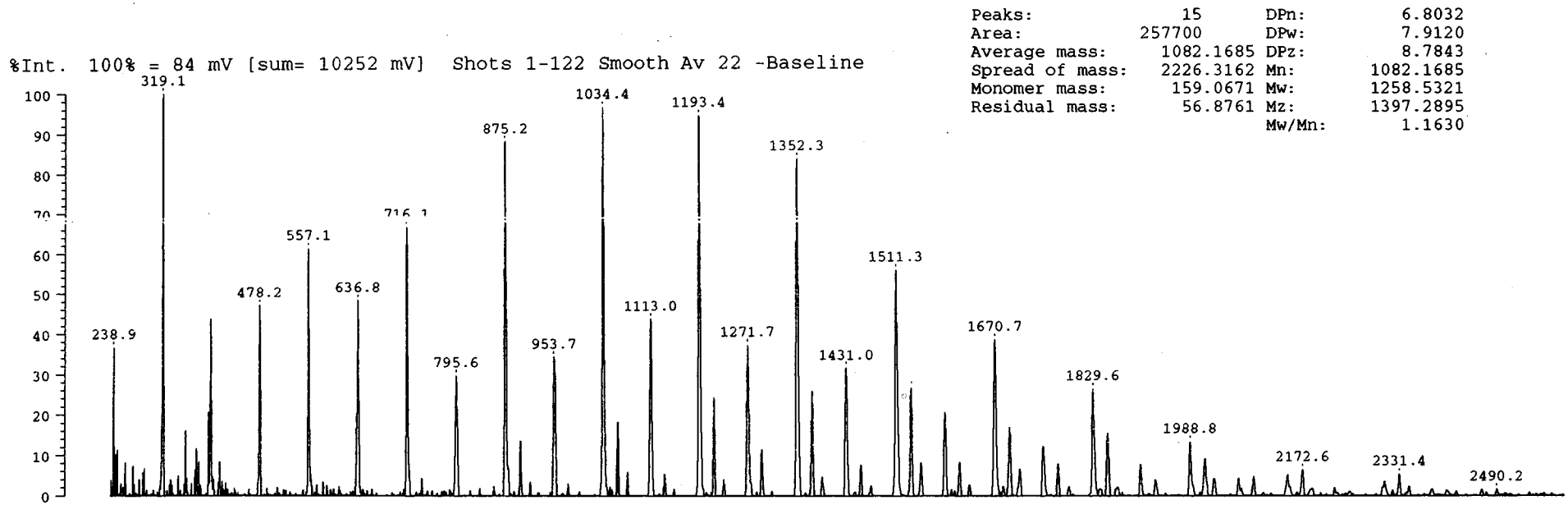


Figure 3. MALDI-TOF-MS spectra of the oligomerization/polycondensation product generated from **1a/2**. Usually, the $(M - 1)^+$ peak is observed.

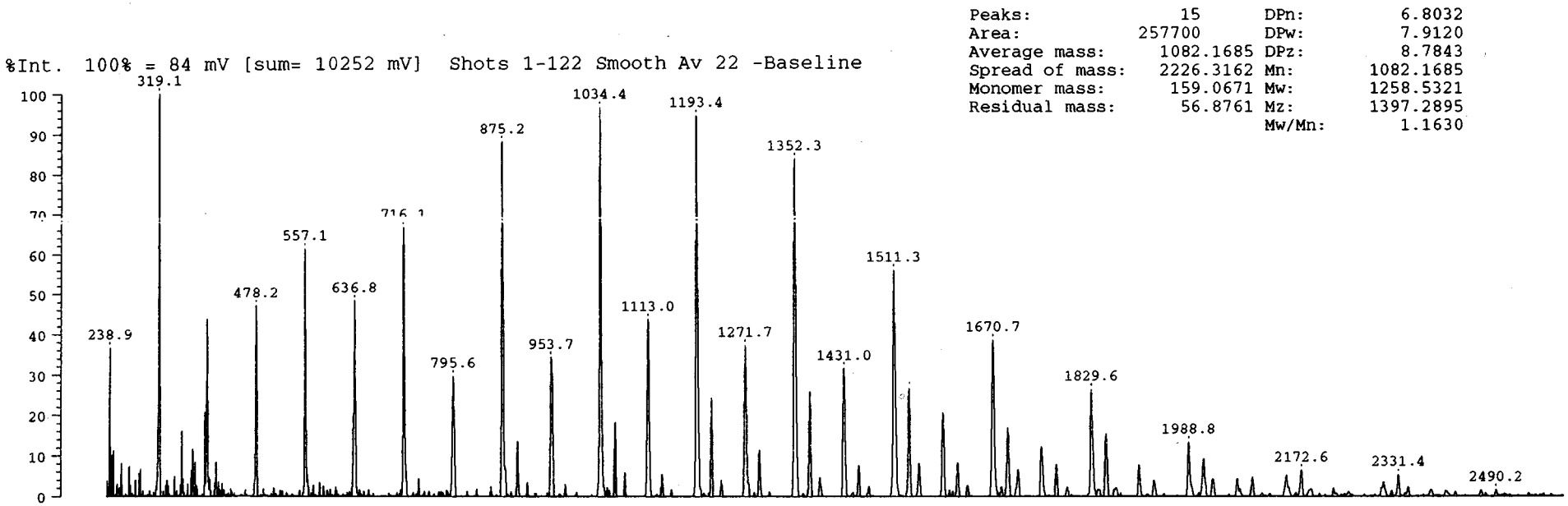


Figure 4. MALDI-TOF-MS spectra of the oligomerization/polycondensation product generated from **1b/2**. Usually, the $(M - 1)^+$ peak is observed.

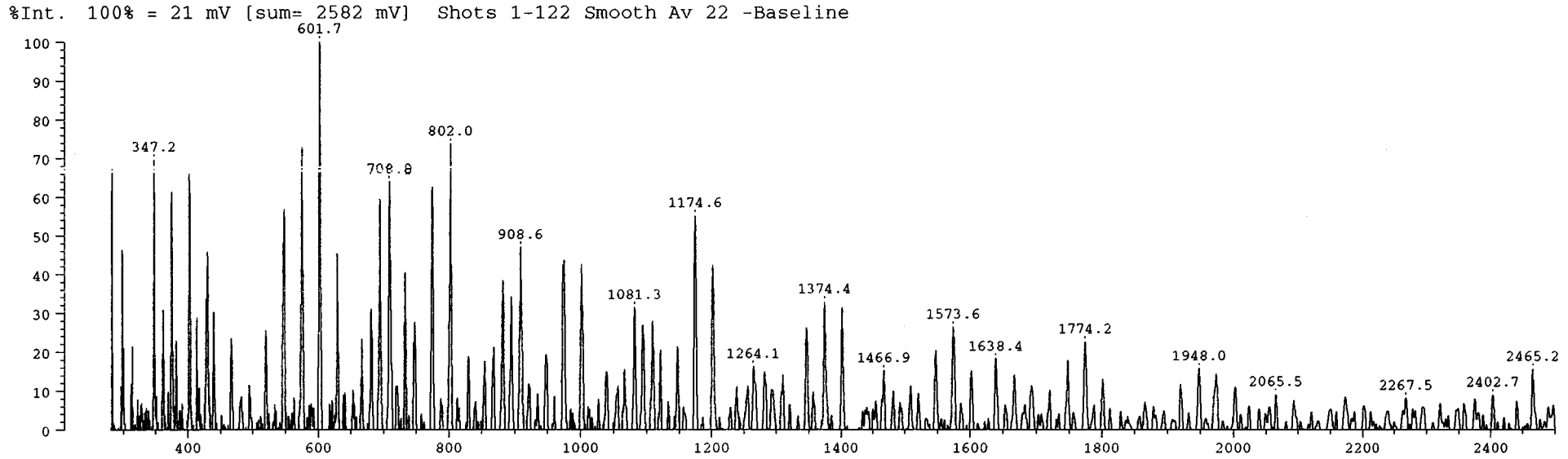
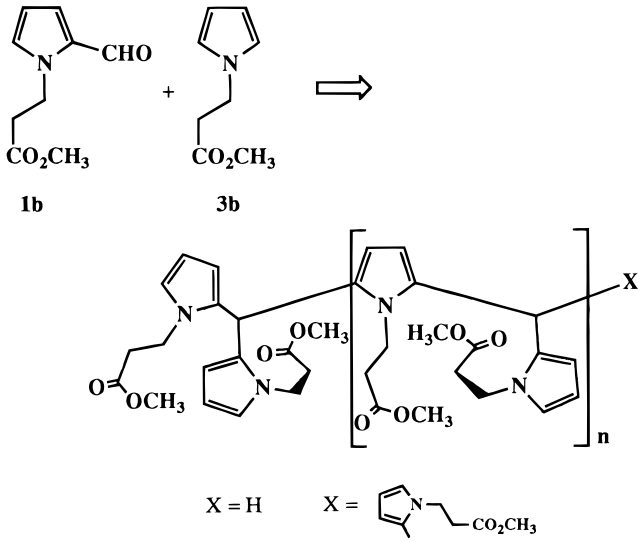


Figure 5. MALDI-TOF-MS spectra of the oligomerization/polycondensation product generated from **3a**. Usually, the $(M - 1)^+$ peak is observed.

numbered series of oligomers or combine with *N*-alkylpyrrole to the odd-numbered series. The oligomers/polymers are expected to undergo dehydrogenation/oxidation upon exposure to air, generating polyconjugated domaines as chromophors: The intense red color of the reaction mixture (λ_{max} = 515 nm, in $CHCl_3$) turned to brown/black.

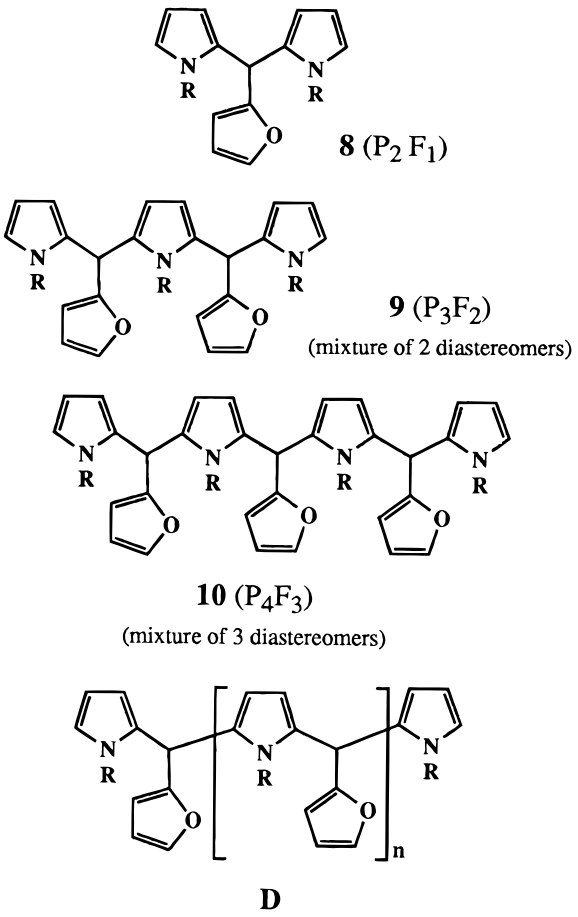
***N*-(2-Methoxycarbonylethyl)pyrrole (1b)/*N*-(2-Methoxycarbonylethyl)-2-formylpyrrole (3b) System.** Of course, the model experiment described above only represents a methylamine Maillard system. As an approach to more relevant Maillard systems, corresponding pyrroles with an amino acid derived substituent were investigated. Therefore, we synthesized the pyrroles **1b** and **3b** derived from β-alanine as a non-Strecker active amino acid. The analogous experiment results in corresponding red colored oligomers/polymers



up to ~14 pyrrole moieties (m/z = 2212) of the expected monomeric unit Δm = 316 (Figure 2).

***N*-Methylpyrrole (1a) or *N*-(2-Methoxycarbonylethyl)pyrrole (1b)/2-Furaldehyde (2) System.** As one of the main products in common Maillard systems, **2** should be a suitable substitute for the aldehyde components in the experiments described above. Indeed, rapid acid-catalyzed polycondensation takes place between the pyrrole and the furan components, resulting in a white precipitate, which changes to black only after prolonged exposure to air. The MALDI-TOF-MS (Figure 3) shows oligomers of up to >30 heterocycles with a (2-pyrrolyl)-2-(furyl)methyl moiety as monomeric unit (Δm = 159). Obviously, an analogous reaction cascade as shown in Scheme 2 leads to polymers of structure **D**. This structure was verified by isolation of the oligomers **8**−**10**, which were characterized by FAB-MS and ^1H and ^{13}C NMR. Because of the lower symmetry of compounds **9** and **10**, centers of chirality are generated and, therefore, the NMR spectra reveal mixtures of the expected number of diastereomers.

Furthermore, the MALDI-TOF-MS clearly demonstrates the regular linear structure of the polymer: Otherwise, in the case of branching along the furan nucleus, the $\Delta m/z$ pattern should be variable instead of monoton. This result may be due to the lower reactivity of the incorporated furan ring as compared to the pyrrole ring toward electrophilic substitution. From the MALDI-TOF-MS data a weight-average molecular weight M_w = 1259 was calculated. This result was checked by size exclusion chromatography (M_w = 1383), which was possible because of the stability and solubility of this material.



8 (P_2F_1)

9 (P_3F_2)
(mixture of 2 diastereomers)

10 (P_4F_3)
(mixture of 3 diastereomers)

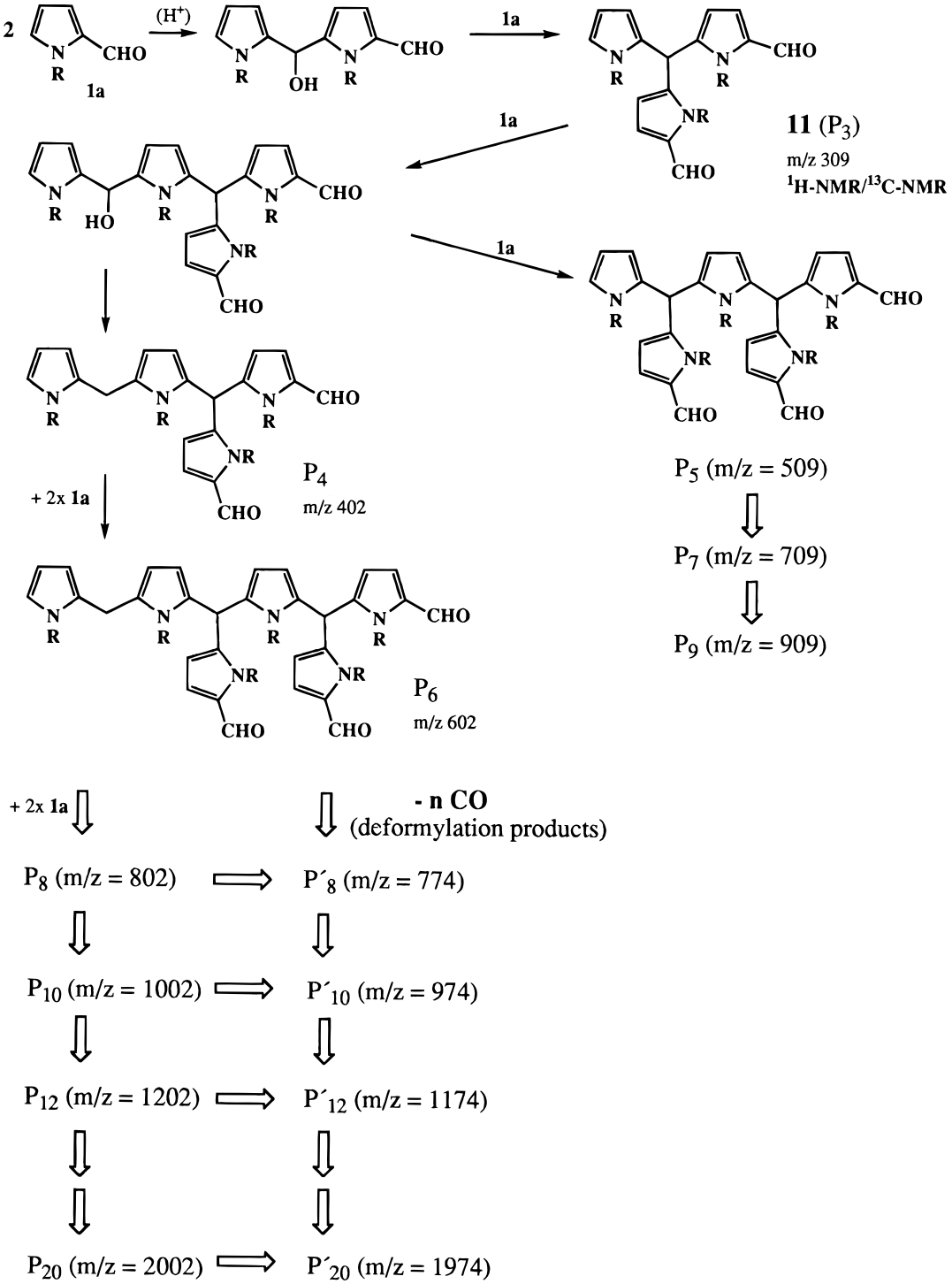
D

Substitution of **1a** by **1b** results in corresponding oligomers/polymers (**D**; R = $-(CH_2)_2CO_2CH_3$) up to detectable species with 25 heterocyclic rings and in an expected monomeric unit of Δm = 231 (Figure 4).

In contrast, by the complementary condensation of 2-formylpyrroles with furans no corresponding products were generated. Instead, because of the lower reactivity (compared to pyrroles) of furans in electrophilic substitution, the self-condensation of the *N*-alkylpyrrolaldehyde (**3**) is favored. Thus, in a **3a**/2-methylfuran system the furan is not incorporated: Via the trimer **11**, which was isolated and characterized, three homologous series of oligomeric compounds (Scheme 3) were formed (monomer unit Δm = 200), as clearly indicated by MALDI-TOF-MS analysis (Figure 5) of the deep red product. By trimerization of the aldehyde, a reactive pyrrole nucleus (without an electron-withdrawing CHO group) is generated, which leads either to a polyaldehyde system (up to P_9) or, accompanied by a hydrogenation step and subsequent deformylation, to detectable oligomers of up to 20 pyrrole rings.

Antioxidative Activity of Oligomers 5, 6, 8, and 9. With respect to the melanoidin character of our model compounds, we examined some of their functional qualities (Tressl et al., 1998b). Extended chromophors generated by dehydrogenation and/or the formation of corresponding delocalized radicals must be responsible for the red to black color of the polymers after exposure to air. The well-known antioxidative activity of native melanoidins (Hayase et al., 1989) stimulated us to study

Scheme 3. Generation of Polymers of Type II from N-Substituted 2-Formylpyrrole Systems



the oligomers **5**, **6**, **8**, and **9** in an iron(III) thiocyanate antioxidative assay. (Because of their very low solubility, the unfractionated polymer mixture could not be tested.) Compared to the corresponding monomers (**1a**, **3a**), a strong antioxidative activity could be observed in the cases of **5** and **8** in the inhibition of the linoleic acid autoxidation. Remarkably, the pentamer **6** showed an antioxidative activity in low concentration and a prooxidative activity in high concentration, whereas the oligomer **9** showed antioxidative activity only in the latter case.

Our model experiments demonstrate a novel route to melanoidin-like polymers from the predominant low molecular weight Maillard products formed from hexoses and pentoses. Scheme 4 summarizes the involved fragmentation pathways of different sugars leading to polymers of types I and II. The important disaccharide specific fragmentation pathway mentioned will be the subject of another paper (Tressl et al., in preparation).

The described structures of types I and II polymers can only represent distinct domains (or substructures) of native melanoidins. Of course, the different polycondensations will simultaneously occur during the complex Maillard process. Moreover, unsubstituted pyrroles (after Strecker degradation of α-amino acids) as well as Strecker aldehydes themselves may be integrated into

Scheme 4. Maillard Transformations of Hexoses, Pentoses, and Disaccharides into Melanoidin-like Polymers (R = Alkyl or Amino Acid Residue)

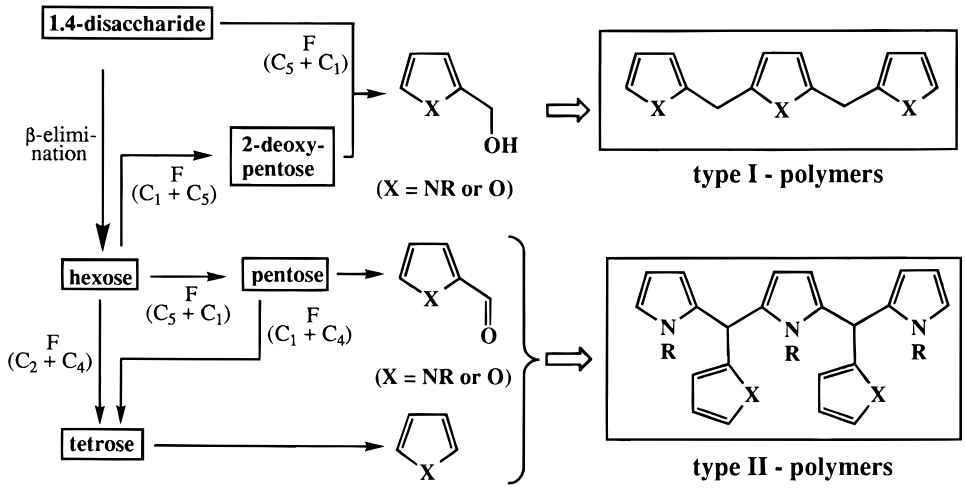
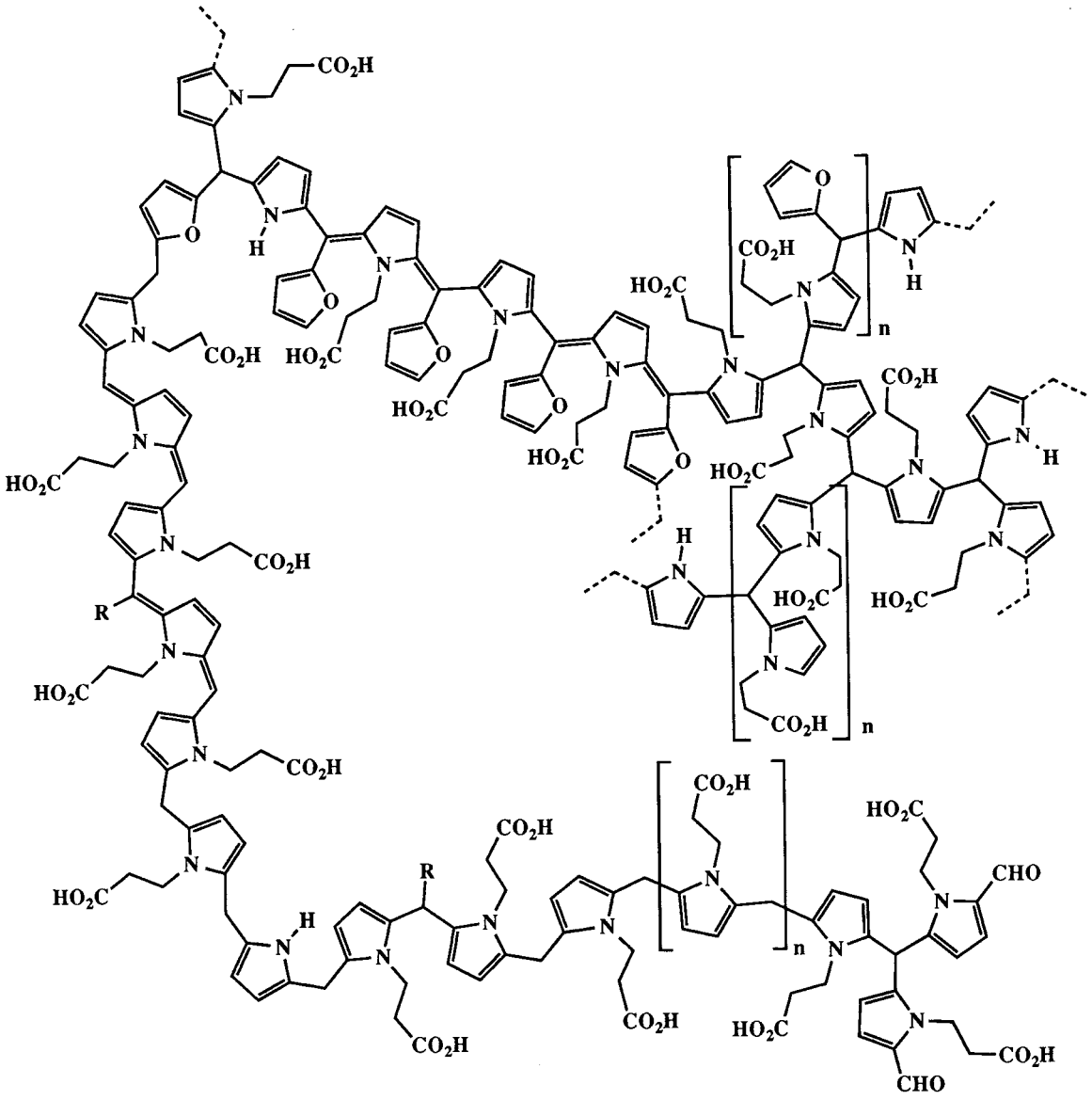


Chart 1



the melanoidin backbone. Therefore, instead of a regular polymer, a complex macromolecular melanoidin structure is very likely, as is schematically demonstrated in Chart 1.

The described oligomerization/polycondensation reactions are, up to now, the only experimentally established pathways by which simple Maillard end products (generated from hexoses and pentoses) are easily and

irreversibly transferred into macromolecules. In conclusion, the driving force for the transformation of sugar/amine systems into melanoidins seems to be strongly linked to the formation of C_5-/C_4-pyrroles (furans) suitable for a spontaneous polyreaction. Of course, an extensive investigation of native melanoidins, generated from selectively labeled precursors, is necessary to establish the proposed melanoidin structure.

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