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Synthesis of aromatic Amadori compounds

Mercedes G. López and Dieter W. Gruenwedel*
Department of Food Science and Technology, University of California, Davis, CA 95616 (USA)

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ABSTRACT

A series of *O*-isopropylidene protected aromatic Amadori compounds [*N*-(1-deoxy-D-fructos-1-yl)-L-amino acid esters] were synthesized by reacting 2,3:4,5-di-*O*-isopropylidene-1-*O*-(trifluoromethanesulfonyl)-D-fructopyranose with alkyl esters of L-phenylalanine, L-tyrosine, and L-tryptophan. The Amadori compounds were obtained in much higher yields than those previously reported due to utilizing triflate as an extremely reactive leaving group at C-1 of the sugar reagent. The triflate derivative was prepared in high yield using the sterically hindered, non-nucleophilic base, 2,6-di-*tert*-butyl-4-methylpyridine, as proton acceptor. The Amadori compounds were characterized by ^{1}H- or ^{13}C-n.m.r. spectroscopy, as well as by i.r. and mass spectroscopy. ^{13}C-n.m.r. spectroscopy was used to study the equilibria among the various ring forms of the free-hydroxy D-fructose–tryptophan compound.

INTRODUCTION

L-Amino acids react readily with D-hexoses upon heating to yield 1-L-carboxy-aralkylamino-1-deoxy-D-hexuloses[1] (Amadori compounds or "fructose–amino acid" conjugates). The compounds are key intermediates of the so-called Maillard (non-enzymic browning) reaction of foods, which is a series of amino acid–carbonyl group condensation and rearrangement steps that gives rise to compounds that contribute to the aroma and color of many foods[2–4]. Recently, Amadori compounds have been found to form *in vivo*[5].

Amadori compounds are secondary amines and, as such, are susceptible to nitrosation reactions. In fact, as shown by a number of workers[6–10], they undergo a surprisingly easy and quantitative conversion to their *N*-nitroso derivatives when incubated with sodium nitrite under mildly acidic conditions (pH 4) and at physiological temperatures (37°). Several of them, notably those containing aromatic amino acid residues, have been found to behave as direct-acting mutagens in *S. typhimurium* his^- strains[6,11–14] (*i.e.*, in the Ames test), and two (*e.g.*, nitrosated fructose–tryptophan and fructose–serotonin compounds) are known to induce DNA repair synthesis in cells of the human HeLa S3 cell line[8–10].

Amadori compounds are traditionally prepared by heating the amino acids in question under reflux for extended periods of time with an excess of D-glucose in absolute methanol, then isolating and purifying the rearrangement products *via* column

* Author to whom reprint requests should be addressed.

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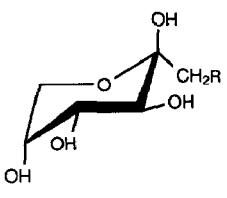
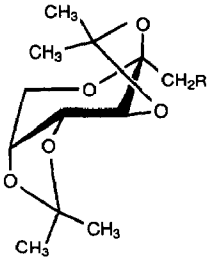
chromatography[7,15]. One drawback of this approach is that unprotected D-glucose, in view of its polyfunctional nature, participates in many side reactions in addition to the desired Amadori rearrangement step. The requisite elaborate purification procedures generally result in very poor yields of products.

Since we were in need of larger quantities of pure Amadori compounds for biological studies[8–10] as well as for physiochemical studies designed to monitor the binding *via* intercalation of aromatic Amadori compounds to DNA[16], we decided to synthesize such compounds by starting with a properly protected β-D-fructose and reacting it with the N-terminal side of the amino acid esters question under an atmosphere of nitrogen. This approach should circumvent side reactions, particularly those due to air oxidation. The sugar was protected in positions C-2–C-5 by condensing it with acetone, and it was activated at C-1 by a triflate group, a procedure which is a modification of the technique employed by Xenakis *et al.*[17] The major modification concerns the activation step at C-1. We have prepared the Amadori compounds fructose–phenylalanine[4], fructose–tyrosine[5], and fructose–tryptophan[6] (in the form of their esters with the sugar moiety still protected) and characterized them *via* i.r., and m.s., as well as by ^{1}H- or ^{13}C-n.m.r. spectroscopy. We have also prepared the unprotected fructose–tryptophan compound by hydrolytically removing the isopropylidene groups, and we have studied the distribution of the tautomeric sugar forms using ^{13}C-n.m.r. spectroscopy.

RESULTS AND DISCUSSION

Synthesis. — The preparative steps employed by us are as follows: the first intermediate, 2,3:4,5-di-*O*-isopropylidene-β-D-fructopyranose (**2**), was synthesized ac-





1 R = OH

2 R = OH

3 R = OSO_2CF_3

4 R = HN–CH(COOCH$_2$CH$_3$)–CH$_2$–(C_6H_5)

5 R = HN–CH(COOCH$_3$)–CH$_2$–(C_6H_4)OH

6 R = HN–CH(COOCH$_3$)–CH$_2$–(C_8H_6N)

7 R = HN–CH(COOCH$_3$)–CH$_2$–(C_8H_6N)

cording to the method of Brady[18]. It was then activated at for nucleophilic displacement at C-1 by preparing the triflate derivative **3**. Base–adduct formation during sulfonation has been noted on numerous occasions, as the reaction is independent of the nature of the sulfonating agent (*e.g.*, it is known to occur with either methane- or *p*-toluenesulfonic halides or with anhydrides)[19]. We have circumvented this side reaction by employing the sterically hindered base, 2,6-di-*tert*-butyl-4-methylpyridine, as a non-nucleophilic proton acceptor. Although sterically hindered bases such as 2,4,6-collidine[20] or 2,6-lutidine[21] have also been used[20], the presence of the bulky *tert*-butyl group in the 2- and 6-positions would be particularly effective in preventing the formation of the triflate–pyridine salt[21]. Sulfonation in the presence of this type of base should not only improve the yield of the reaction product, but also result in a clean, homogeneous reaction[21,22].

Triflate **3** was reacted with the appropriate amino acid ester to yield the protected Amadori compounds **4**, **5**, and **6**. The correctness of the above statement concerning the advantages of the synthetic route *via* **3** is borne out by the fact that we have obtained the protected Amadori compounds in yields significantly higher than those reported in the literature for free-hydroxy Amadori compounds[23] (see Table I). Removal of the *O*-isopropylidene groups of **6** yielded the end-product **7** which was identical with that previously reported[23]. Preparative details are presented in the Experimental section. M.s., i.r., and n.m.r. data of the various reaction products are presented in Tables II–V.

Mass-spectral data. — Due to the mode of ionization employed in the analysis [fast-atom bombardment (f.a.b.)], we find the $[M + 1]^+$ *m/z* peaks 261, 393, 461, and 381 of **2**, **3**, **6**, and **7**, respectively, and not the molecular ion peaks $[M]^+$. Our m.s. data for **2** (Table II) are in good agreement with published values[24,25]. Compound **3** (Table II) gave a fragmentation pattern similar to that of **2**. We assign fragment *m/z* 113 as the 3,5-dihydroxypyrylium cation.

The m.s. data for protected **6**, as well as that for the unprotected fructose–tryptophan methyl ester **7**, are listed in Tables III and IV. We have no explanation for the production of fragment *m/z* 393 originating with **6**. Fragment C_9H_8N (Tables III and IV) is the skatolyl residue, a major breakdown product of tryptophan. Ring fragment *m/z* 149 (Table IV) is fructose with its hydroxymethyl group removed, and we assign *m/z* 57 as the cyclopropanol cation.

Infrared spectral data. — The i.r. data of **2** (Table II) agree fully with those published[26]. Card and Hitz[27] provide i.r. information on **3**, but they did not give assignments for the various i.r. bands. The absence of the hydroxyl band in **3** is noted (Table II), as the OH is replaced by the triflate group. The assignment of the various triflate bands is based on literature data[22,28].

The i.r. bands of the Amadori compounds **4**, **5**, and **6** are given in Table III. The stretching frequencies for the carbonyl and ester groupings, as well as the Me deformation bands, are in full agreement with the information provided by Xenakis *et al.*[17] for their aliphatic Amadori compounds. We give additional information on the stretching frequencies of the α-amino groups, the aromatic carbons of Phe, Tyr, and Trp, and also of the aromatic hydroxyl group of Tyr. These are all in regions well established from the

literature[28]. Lastly, Table IV contains the i.r. data of **7**, which are similar to those of **6**.

^{1}H- and ^{13}C-N.m.r. spectral data. — Assignment of the ^{1}H-n.m.r. chemical shifts is straightforward and follows established rules[29]. Chemical-shift values (δ, p.p.m.), shift splitting patterns, and assignment of protons to their respective attachment sites are presented in Tables II and III for compounds **3–6**. The chemical shift data for **3** are indeed very close to those reported by Card and Hitz[27]. Regarding **4** (Table III), the

TABLE I

Yield (%) of aromatic Amadori compounds **4–6**

Compound	*Reaction time (min)*	*Röper* et al.[a,b] *(%)*	*This work*[c] *(%)*
4 (0.80 mmol **3**, 2.19 mmol Phe)[d]	30	24.0	76.2
5 (1.44 mmol **3**, 3.05 mmol Tyr)[d]	25	2.0	63.0
6 (14.08 mmol **3**, 79.28 mmol Trp)[d]	25	5.4	46.3

[a] See ref. 23. These data are for the free-hydroxy *N*-(1-deoxy-D-fructos-1-yl)amino acids. [b] The amino acid reagent is the limiting reagent. [c] Sugar–triflate is the limiting reagent. Yields are for the synthesis of the *N*-(1-deoxy-2,3:4,5-di-*O*-isopropylidene-β-D-fructose-1-yl)-L-amino acid alkyl esters. Compound **6** was deprotected to give the free-hydroxyl *N*-(1-deoxy-D-fructos-1-yl) amino acid methyl ester (**7**) in 69.6% yield (see Experimental section). [d] Amounts of reactants appear in parentheses. Phe = phenylalanine ethyl ester; Tyr = tyrosine methyl ester; Trp = tryptophan methyl ester.

TABLE II

M.s., i.r., and ^{1}H-n.m.r. spectral data of **2** and **3**

Compound	*m.s.* m/z *(% base peak)*	*i.r.*[a] *(cm^{-1})*	*^{1}H-n.m.r.*[b] *(p.p.m.)*
2[c]	261 $[M + 1]^+$ (71), 245 (22), 229 (3), 203 (100), 171 (5), 135 (37), 127 (31), 103 (32), 101 (31), 85 (36), 59 (64)	3325 $(OH)_{st}$, 3000–2800 $(CH)_{st}$, 1379 $(CMe_2)_{asy}$	1.32, 1.37, 1.45, 1.51 (4 s, 3 H each, Me_2C), 2.26 (m, 2 H, H-1), 3.63 (m, 1 OH, H-1), 3.76 (d, 1 H, H-6_{eq}), 3.86 (dd, 1 H, H-6_{ax}), 4.22 (d, 1 H, H-5), 4.32 (d, 1 H, H-3), 4.57 (dd, 1 H, H-4)
3[d]	393 $[M + 1]^+$ (77), 377 (73), 334 (61), 259 (19), 233 (23), 229 (8), 171 (12), 156 (33), 127 (45), 113 (33), 103 (23), 69 (23), 59 (100)	no (OH), 3000–2884 $(CH)_{st}$, 1381 $(CMe_2)_{asy}$, 1423, 1190, and 1135 all (OSO_2CF_3): CF_{st}, $SO_{2\ as,\ sy\ st}$	1.35, 1.40, 1.48, 1.54 (4 s, 3 H each, Me_2C), 3.75 (d, 1 H, H-6_{eq}), 3.88 (dd, 1 H, H-6_{ax}), 4.21 (dd, 1 H, H-5), 4.27 (d, 1 H, H-3), 4.37 (1/2 AB, 1 H, H-1), 4.48 (1/2 AB, 1 H, H-1), 4.61 (dd, 1 H, H-4)

[a] I.r. designations: st = stretch; asy = asymmetric; sy = symmetric. [b] In $CDCl_3$; p.p.m. measured relative to solvent signal. [c] See refs. 24 and 25 for chemical composition of fragments. [d] F.a.b.-m.s. Peaks (*m/z*) denoted as follows: 377 $[M - CH_3]^+$, 334 $[M - (CH_3COCH_3)]^+$, 259 $[M - (SO_2CF_3)]^+$, 233 [?], 229 $[M - (CH_2OSO_2CF_3)]^+$, 171 $[229 - (CH_3COCH_3)]^+$, 156 $[171 - CH_3]^+$, 127 $[C_6H_7O_3$ (ring fragment)$]^+$, 113 $[C_5H_5O_3$ (ring fragment)$]^+$, 103 $[CH(OH)–C(OH)CH(OH)–CH_2]^+$, 69 $[CF_3]^+$, and 59 $[CH_3C(OH)CH_3]^+$.

δ-value of the protons of the pH-ring, giving rise to the multiplet at 7.24 p.p.m., agrees extremely well with data given by Röper *et al.*[23] [7.26 p.p.m. (m)]. Otherwise, the fructose protons at C-3–C-6 are shifted somewhat further downfield than reported due no doubt to the presence of the *O*-isopropylidene groups in **4**, which were not present in the corresponding Amadori compound[23]. Similarly, ignoring differences due to the presence of the *O*-isopropylidene residues in **5** or **6**, our δ data for these two compounds essentially follow the chemical shift sequences listed by Röper *et al.*[23]

Since the ^{1}H-n.m.r. spectrum for **7** was difficult to evaluate due to the presence of the tautomeric forms of the fructose residue, we used ^{13}C-n.m.r. spectroscopy to characterize the unprotected compound in greater detail (Table IV).[13] Both the data for the sugar residue and for the amino acid component are in excellent agreement with the published data[23,30]. We have further evaluated the ^{13}C-n.m.r. spectra regarding the existence of **7** in α- and β-furanose form (see Table V). Again, there is excellent agreement with published information concerning the tautomeric equilibrium of fructose at room temperature[23,30–34]. From the distribution of the relative peak intensities, we find the tautomeric composition as follows: β-fructopyranose:β-fructofuranose:α-fructofuranose = 65:20:15. Since the ranges for the three tautomers have been listed as

TABLE III

I.r. and ^{1}H-n.m.r. spectral data of compounds **4–6**[a]

Compound	*I.r.*[b] *(cm^{-1})*	*^{1}H-n.m.r.*[c] *(p.p.m.)*
4	3345 $(NH)_{st}$, 1734 $(C=O)_{st}$, 1605 $(C=C)_{st}$, 1381 $[C(CH_3)_2]_{asy}$, 1315 $(CH_3)_{asy}$, 1252 $(C–O–C)_{st}$	1.09 (t, 3 H, CH_3-ester); 1.45, 1.48, 1.50, 1.55 (4 s, 3 H each, Me_2C); 2.89 (m, 2 H, H-1); 2.98 (m, 2 H, H_β); 3.62 (m, 2 H, CH_2-ester); 3.70 (m, 1 H, H_α); 3.86 (d, 1 H, H-6_{eq}); 3.90 (d, 1 H, H-6_{ax}); 4.20 (dd, 1 H, H-5); 4.37 (d, 1 H, H-3); 4.59 (dd, 1 H, H-4); 7.24 (m, 5 H, Ph).
5	3389 (NH, $OH)_{st}$, 1736 $(C=O)_{st}$, 1614 $(C=C)_{st}$, 1373 $[C(CH_3)_2]_{asy}$, 1315 $(CH_3)_{asy}$, 1251 $(C–O–C)_{st}$	1.41, 1.47, 1.52, 1.57 (4 s, 3 H each), Me_2C); 2.93 (m, 2 H, H-1); 3.61 (s, 3 H, CH_3-ester); 3.68 (t, 1 H, H_α); 3.75 (m, 2 H, H_β); 3.90 (d, 1 H, H-6_{eq}), 3.95 (d, 1 H, H-6_{ax}); 4.26 (dd, 1 H, H-5); 4.40 (d, 1 H, H-3); 4.64 (dd, 1 H, H-4); 6.72 (dd, 2 H, *o*-Ph); 7.01 (dd, 2 H, *m*-Ph).
6[d,e]	3373 $(NH)_{st}$, 1736 $(C=O)_{st}$, 1620 $(C=O)_{st}$, 1372 $[C(CH_3)_2]_{asy}$, 1317 $(CH_3)_{asy}$, 1252 $(C–O–C)_{st}$	1.36, 1.43, 1.52, 1.54 (4 s, 3 H each, Me_2C), 2.91 (d, 1 H, H-1); 3.18 (d, 1 H, H-1); 3.62 (s, 3 H, CH_3-ester); 3.80 (t, 1 H, H_α); 3.88 (d, 2 H, H_β); 3.92 (d, 1 H, H.6_{eq}); 4.15 (dd, 1 H, H-6_{ax}); 4.25 (d, 1 H, H-5); 4.41 (d, 1 H, H-3); 4.62 (dd, 1 H, H-4); 6.98 (s, 1 H, $H_{2'}$); 7.09 (dd, 1 H, $H_{5'}$); 7.15 (dd, 1 H, $H_{6'}$); 7.32 (d, 1 H, $H_{7'}$); 7.58 (d, 1 H, $H_{4'}$); 8.33 (s, 1 H, N–H_{indole}).

[a] Esterified compounds: **4**, Et ester; **5** and **6**, Me esters. [b] I.r. designations: st = stretch; asy = asymmetric. [c] In $CDCl_3$; p.p.m. measured relative to solvent signal. [d] F.a.b.-m.s. data are as follows *m/z* (% of base peak): 461 $[M + 1]^+$ (100), 402 $[M - (CH_3–CO–CH_3)]^+$ (4), 393 [?] (10), 330 $[M - C_9H_8N]^+$ (22), 272 $[330 - (CH_3–CO–CH_3)]^+$ (5), 202 $[(C_9H_8N–CH–COOCH_3)]^+$ (7), 159 $[(C_9H_8N–CH–NH_2)]^+$ (5), 144 $[(C_9H_8N–CH_2)]^+$ (6), 130 $[C_9H_8N]^+$ (34), 103 [Ph–CH–CH]$^+$ (3), and 59 $[CH_3–C(OH)–CH_3]^+$ (15). [e] Primed nos. refer to carbons of the indole ring system.

TABLE IV

M.s., i.r., and ^{13}C-n.m.r. spectral data of compound 7.

Compound	*M.s.* m/z *(% base peak)*	*I.r.* *(cm^{-1})*	*^{13}C-n.m.r.*[a] *(p.p.m.)*
7[b]	381 $[M + 1]^+$ (47), 363 (8), 320 (2), 232 (7), 219 (2), 202 (9), 155 (45), 149 (8), 135 (53), 130 (33), 119 (100), 103 (59), 85 (90), 77 (15), 57 (25)	3600–3110 $(NH,OH)_{st}$, 3000–2817 $(CH)_{st}$, 1682 $(C=O)_{st}$, 1598 $(C=C)_{st}$, 1213 $(C–O–C)_{st}$	C_β, 29.63; C_α, 53.82; C-6, 63.01; CH_3, 63.57; C-1, 64.39; C-3, 70.69; C-5, 70.99; C-4, 71.16; C-2, 98.80; C-3′, 110.05; C-7′, 113.05; C-6′, 119.60; C-4′, 120.41; C-5′, 123.32; C-2′, 125.63; C-9′, 128.08; C-8′, 137.46; C=O, 177.90

[a] In dioxane-d_8; p.p.m. measured relative to solvent signal. Chemical shift values are given only for fructose in the β-D-pyranose form. The primed numbers refer to the carbons of the indole ring. C_α and C_β are, respectively, the α- and β-protons of Trp. [b] F.a.b.-m.s. peaks denoted as follows: 363 $[M - OH]^+$, 232 $[(C_9H_8N–CH(NH–CH_3)–COOCH_3\cdot)]$, 219 $[(C_9H_8N–CH(NH_3)COOCH_3)]^+$, 202 $[(C_9H_8N–CH–(CO)–O–CH_3)]^+$, 155 $[C_9H_8N–C\equiv CH)\cdot]$, 149 $[C_5H_9O_5$ (ring fragment)$]^+$, 135 [?], 130 $[C_9H_8N]^+$, 119 $[CH(OH)=C(OH)–CH(OH)–CH(OH)]^+$, 103 $[Ph–CH=CH]^+$, 85 $[C_5H_9O]^+$, 77 $[Ph]^+$, 57 $[C_3H_4(OH)$ (ring fragment)$]^+$.

TABLE V

^{13}C-N.m.r. data[a] for the tautomeric forms of 7[b]

Carbon	*β-D-Pyranose* *(p.p.m.)*	*β-D-Furanose* *(p.p.m.)*	*α-D-Furanose* *(p.p.m.)*
C-1	64.39	63.21	64.10
C-2	98.80	102.75	105.09
C-3	70.69	78.20	84.34
C-4	71.16	75.71	78.35
C-5	70.99	82.01	82.62
C-6	63.01	62.89	62.51

[a] In dioxane-d_8; p.p.m. measured relative to solvent signal. [b] ^{13}C-Chemical shifts (in p.p.m.) for the carbons of the amino acid moiety were unchanged irrespective of the tautomeric forms of the sugar. These chemical shifts are listed in Table IV.

(57–75), (21–31), and (4–10) for the β-pyranose, β-furanose, and α-furanose forms, respectively, (all based on the results of ^{13}C-n.m.r. measurements[34]) we feel that our data are in agreement with the information provided by Röper *et al.*[23] (*β-p:β-f:α-f* = 64:15:21). Although these authors state that the various Amadori compounds studied by them show the α-pyranose form in about 6% abundance, they do not seem to have quantitatively evaluated the amount of this particular tautomeric form of 7. We were unable to detect ^{13}C-n.m.r. signals attributable to the α-pyranose form of 7. D-Fructose is said to exist as in the α-pyranose form in amounts ranging from traces to about 5% (ref. 34). Thus, there can be little doubt that this sugar predominantly exists in the pyranose form, as either the free monosaccharide or bound in an Amadori compound.

Conclusions. — The success of selectively attaching the *N*-terminus side of an (aromatic) amino acid to the C-1 carbon of fructose involves three crucial steps: (1) protection of the functional hydroxyl groups at C-2–C-5; (2) use of a highly reactive leaving group at C-1 such as the triflate; and (3) use of a non-nucleophilic base sufficiently hindered so that it does not react with the leaving group. Last but not least, the approach used here is much less time-consuming (*c.f.*, Table I) than the classical procedure, which consists of performing the Amadori rearrangement *via* refluxing D-glucose in the presence of amino acids for extended periods of time[8,15].

EXPERIMENTAL

General methods. — ^{1}H-n.m.r. spectra were recorded in $CDCl_3$ with a General Electric QE-300 (300 MHz) instrument. ^{13}C-n.m.r. spectra were collected at 90 MHz with the Nicolet Technology Corporation F.t.-n.m.r. 1180 spectrometer. I.r. spectra were taken with an IBM IR/32 F.t.-i.r. spectrophotometer, and m.s. spectra were obtained by using VG Analytical ZAB-HS-2F mass spectrometer. Silica Gel-60 (E. Merck, 230–400 mesh, 60 Å) was used in column chromatography. Progess of the reaction steps was monitored by t.l.c. on silica gel plates that contained a fluorescent indicator. Ultraviolet light and/or iodine were used for detection. Solvents were distilled and stored over 4Å molecular sieves (8–12 mesh). All reactions were carried out in an atmosphere of purified, dry nitrogen. Dichloromethane was predried over $CaCl_2$, then heated under reflux for 6–8 h and distilled from CaH_2. Trifluoromethanesulfonic anhydride (Aldrich) was distilled according to the procedure of Stang and Dueber[35], except that the amount of P_2O_5 was limited to less than 50% of that recommended.

Preparation of 2,3:4,5-di-O-isopropylidene-β-D-fructopyranose (**2**). — According to the method of Brady[18], **1** was reacted with acetone in the presence of sulfuric acid to give **2** as colorless needles: m.p. 94° (ether–pentane); lit.[18,26] m.p. 97°; for i.r., m.s., and ^{1}H-n.m.r. data, see Table II.

Preparation of 2,3:4,5-di-O-isopropylidene-1-O-(trifluoromethanesulfonyl)-β-D-fructopyranose (**3**). — This procedure was a modification of that reported by Card and Hitz[27]. To a solution of 2,6-di-*tert*-butyl-4-methylpyridine (1.13 g, 5.50 mmol, Aldrich) in dry dichloromethane (20 mL), maintained under dry conditions at −10°, was added dropwise, under stirring, distilled trifluoromethanesulfonic anhydride (1.44 g, 5.10 mmol), which resulted in the formation of a precipitate. To this stirred mixture was added dropwise a solution of **2** (0.663 g, 2.55 mmol) in dichloromethane (10 mL). The resulting slurry was stirred, and the reaction was monitored at 5-min intervals by t.l.c. (1:1 hexane–ethyl acetate). After 30 min when all of **2** had reacted, ice-cold water (30 mL) was added, and the mixture was extracted with dichloromethane (8 × 20 mL). The combined organic extracts were dried ($MgSO_4$), and the solvent was evaporated at 40° *in vacuo*. Column chromatography of the crude product (silica gel, 8:2 hexane–ethyl acetate) and evaporation of the solvent from the appropriate fractions gave **3** (0.761 g, 76%) as a clear, yellow syrup; lit.[27] m.p. 36–38.5°. For m.s., i.r., and ^{1}H-n.m.r. data, see Table II.

Preparation of N-*(1-deoxy-*D*-fructos-1-yl)-*L*-amino acid ethyl* **4** *and methyl esters* **5** and **6**. — Triflate **3**, in the amount listed in Table I, was dissolved in anhydrous *N,N*-dimethylformamide (DMF, 20 mL), and the appropriate amino acid ester was added to the solution. The mixture was then heated under reflux until the reaction was shown to be complete by t.l.c. (conditions as for **3**, above). The reaction was terminated by adding water (25 mL), and the DMF–water phase was extracted with dichloromethane (8 × 20 mL). The combined extracts were washed with water (6 × 20 mL), dried over $MgSO_4$, and the solvent was evaporated under reduced pressure. The crude Amadori compounds were purified by column chromatography (silica gel, 6:4 hexane–ethyl acetate). See Table III for I.r. and ^{1}H-n.m.r. data of compounds **4**, **5**, **6** and Table I for yields and reaction times.

Removal of the O-*isopropylidene groups from* **6**. — The *O*-isopropylidene groups were removed by dissolving **6** (1.0 g, 2.17 mmol) in 9:1 (v/v) trifluoroacetic acid–water[36] (15 mL) and stirring the mixture for ~2 h at room temperature, with monitoring by t.l.c. The reagent and solvent were evaporated under reduced pressure, and the methyl ester of the Amadori compound was obtained as a syrup. Ether was added to the syrup to initiate crystallization, and the resulting beige crystals were washed repeatedly with ether and dried to give **7** (0.574 g, 69.6%). For m.s., i.r., and ^{13}C-n.m.r. data, see Table IV; for ^{13}C-n.m.r. data on the tautomeric forms of **7**, see Table V. These data for **7** compared favorably with those previously reported[23].

*Recovery of 2,6-di-*tert*-butyl-4-methyl pyridine*. — The aqueous solution from the preparation of **3** was treated with a mixture of 50% aqueous NaOH and pentane (100 mL). The layers were separated, the aq. layer was removed and was back-extracted with pentane (2 × 50 mL), and the combined pentane extracts were evaporated. Column chromatography of the residue (non-activated silica gel, pentane) gave the free base in ~90% yield.

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