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Directed Approaches to Reactive Maillard Intermediates: Formation of a Novel 3-Alkylamino-2-hydroxy-4-hydroxymethyl-2-cyclopenten-1-one ("Cypentodine")

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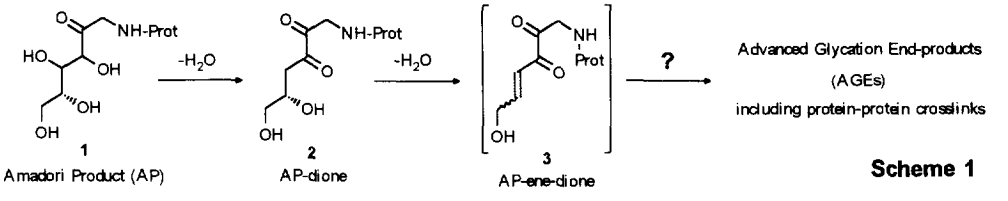
Abstract: Dehydration of the 4- and 5-hydroxyls of an Amadori product would lead to a reactive potential cross-linker, 1,4,5-trideoxy-1-alkylamino-2,3-hexulos-4-ene (AP-ene-dione) which has as yet only been isolated in low yield as a triacetylated 1,2-enol. We have prepared a 4,5-di-*O*-tosyl Amadori product as an activated precursor to AP-ene-dione. Incubation of this precursor under physiological conditions yields a novel cyclopentenone aminoreductone, 3-alkylamino-2-hydroxy-4-hydroxymethyl-2-cyclopenten-1-one, presumably derived from internal cyclization of AP-ene-dione.

Glucose can react with an amino group such as the ε-amine of a protein-bound lysine residue to form an aldimine, or Schiff base, which can then undergo Amadori rearrangement to *N*ε-(1-deoxy-1-fructosyl)lysine, an Amadori product (AP) (**1**).[1] This process is called protein glycation and occurs in vivo or in vitro. Hemoglobin A_{1c} is an example of a glycated protein found in vivo; it is used as a marker of diabetic glucose control indicating the average glucose level over the preceding several weeks.[2] Further poorly understood reactions of AP lead to the formation of advanced glycation end-products (AGEs) which can be colored or fluorescent and which can contain electrophilic centers which can cross-link two proteins together.[3] Substantial evidence exists that such processes contribute to the pathology of diabetes and aging.[4] We have been investigating the possibility that AP-ene-dione (**3**), a double dehydration product of **1**, may play an important role in mediating the formation of AGEs, including glucose-derived random protein-protein cross-links.



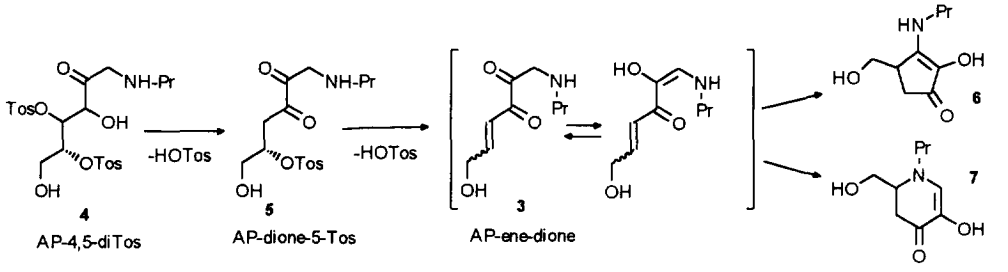
1 Amadori Product (AP) **2** AP-dione **3** AP-ene-dione

Scheme 1

Much previous work at elucidating the chemistry of biologically relevant advanced glycation processes has involved isolation of AGE-derived products from in-vivo tissues, or from model reactions of glucose or

other more reactive sugars with amines, α-protected lysine derivatives, other amino acids, peptides, proteins, DNA, or DNA bases. The products usually formed only in tiny amounts relative to starting materials or early glycation products from which they must be separated. Often, arbitrary distinguishing criteria such as fluorescence or color must be used to determine what components may be of interest. However, in recent years it has become apparent that protein cross-linking caused by advanced glycation is due primarily to unknown structures which are not highly colored or fluorescent.

In light of these limitations of earlier methods, we have decided instead to employ a directed approach to identifying glycation-derived cross-linking species or immunoactive moieties. We chose to use this approach to investigate the AGE formation pathway shown in Scheme 1, in which dehydration of propyl-AP at the 4-position produces the 2,3-dione **2**, and subsequent dehydration at the 5-position gives the enedione **3**. This AGE formation pathway has been little studied, and **3** has only been isolated in low yield as a tri-acetylated derivative.[5] However, **3** should be a highly reactive electrophile; its γ-hydroxy α,β-unsaturated ketone moiety bears structural analogy to the lipid peroxidation product, 4-hydroxy-2-nonenal, which is known to be capable of Michael addition at the β-ene position by nucleophilic amino acid residues of proteins.[6] Therefore, we designed a synthetic precursor of **3**, the Amadori product 4,5-ditosylate **4**. Through successive β-eliminations of tosylate from **4** under neutral to basic conditions, **3** would be readily available *in situ*

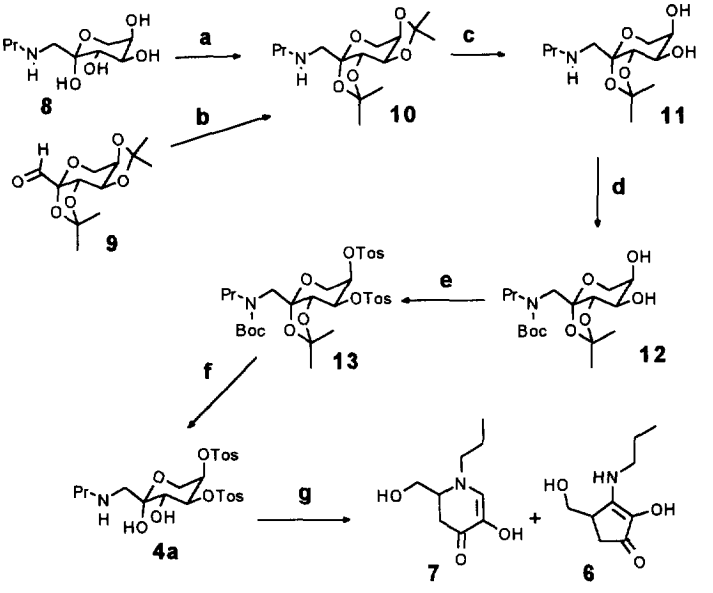


Scheme 2

In the absence of other nucleophiles, **3a** could be expected to undergo internal β-addition reactions of C-1 or the 1-amine to the C-5 position to form two products: a 3-alkylamino-2-hydroxy-4-hydroxymethyl-2-cyclopenten-1-one **6** (the N-propyl derivative of a new type of aminoreductone for which we propose the trivial name "cypentodine"), and the known 2-(hydroxymethyl)-4,5-piperidinedione enol **7** (Scheme 2). We have indeed found that solvolysis of **4** leads to formation of these compounds.

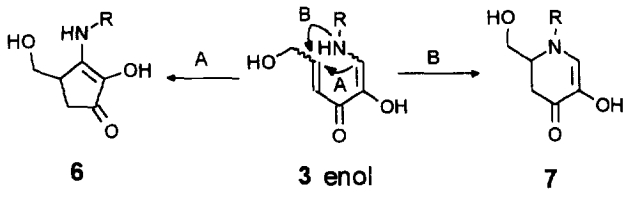
We have synthesized **4a**, the pyranose form of **4**, from the propylamine Amadori product 1-deoxy-1-propylamino-β-D-fructopyranose hydrogen oxalate[7] (**8**) or 2,3:4,5-di-*O*-isopropylidene-*aldehydo*-β-D-*arabino*-hexos-2-ulo-2,6-pyranose[8] (**9**) in 77% or 81% overall yield, respectively. Reductive amination[9] of protected aldehyde **9** with propylamine gave **10** in 95% yield. Compound **10** was also obtained by treating propylamine Amadori product **8** with conc. H_2SO_4 in acetone in 90% yield. Selective removal of the 4,5-isopropylidene group to afford **11** was achieved by treating **10** with 1N HCl in a mixture of methanol and water (1:1) in 90% yield. Subsequent protection of the amine by reaction with di-t-butyl dicarbonate gave t-BOC derivative **12** in quantitative yield. Treatment of **12** with *p*-toluenesulfonylchloride in anhydrous pyridine gave the ditosylate **13** quantitatively. Removal of t-BOC and 2,3-isopropylidene groups in 1N HCl at 50°C gave target compound **4a**[10] in 95% yield. The novel **6** as well as the known **7**[11] were formed in 3:2 ratio (est. total yield 90% by

HPLC) after incubation of **4a** in phosphate buffer (0.5 M, pH 7.4) and methanol (1:1) under nitrogen (Scheme 3). The major product (**6**)[12] with UV absorption maximum at 295 nm and the minor product (**7**)[13] with UV absorption maximum at 360 nm were isolated by semi-preparative HPLC and the structures were assigned on the basis of spectral data. The piperdinedione enol **7** has been reported to be formed in minor amount along with **2** in the degradation of a maltose-derived Amadori product at 100°C in phosphate buffer.[11]



Scheme 3: Reagents and conditions: a) H_2SO_4/acetone. b) n-$PrNH_2$, MeOH, reflux; $NaBH_4$. c) 1N HCl, H_2O/MeOH, r.t. d) $(t\text{-BOC})_2O$, $KHCO_3$, H_2O/CH_2Cl_2. e) TosCl, pyridine, r.t. f) 1N HCl, H_2O/MeOH, 50°C. g) phosphate buffer, H_2O/MeOH, 37°C, N_2, 3 hr.

Scheme 4 shows how the enol of AP-ene-dione **3** can undergo internal cyclization in two ways: via attack of C-1 of the enol (path A) or the amine nitrogen (path B) at C-5 of the enone carbon-carbon double bond, to give **6** and **7**, respectively. Although an alternative pathway involving internal S_N2 displacement of tosylate in intermediate **5** cannot be ruled out at this time, it seems likely that β-elimination of the tosylate of **5** would be a more rapid process. Moreover, the reported isolation[11] of **7** along with **2** (as its enol triacetyl derivative) from the degradation products of an Amadori product of maltose on heating at neutral pH lends support to the intermediacy of AP-ene-dione **3** in the formation of cyclization products **6** and **7** from the ditosylate **4**.



Scheme 4

To further elucidate the mechanism of these reactions, we are synthesizing the 4-*O*-mono-tosylate of Amadori product **8**. In order to clarify the cross-linking potential of AP-ene-dione **3**, studies of model cross-linking reactions of **3** (via solvolysis of **4**), **6**, and **7** with various nucleophiles are also currently underway. A further interest will be to establish the presence of cypentodine (**6**) among the various solvolysis products which slowly form during incubation of propyl Amadori product **8** by itself under physiological conditions, and in the complex mixtures which form in the the classical Maillard reaction conditions of incubation of glucose with amines.

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10. **4a**: ^{1}H NMR δ 0.87 (t, 3H, CH_3CH_2), 1.33 (s, 3H, CH_3CMe), 1.40 (s, 3H, CH_3CMe), 1.42 (s, 9H, t-Bu), ca. 1.3-1.5 (m, 2H, CH_3CH_2), 2.46 (s, 6H, $CH_3C_6H_4$), ca. 3.15 (m, 2H, H(1a) + $CH_2CH^aH^bN$), ca. 3.35 (m, 1H, $CH_2CH^aH^bN$), 3.60 (dd, 1H, H(6a)), 3.9-4.3 (complex m, 3H, H(1b)) + H(3) + H(6b)), 4.69 (m, 1H, H(5)), 4.87 (br m, 1H, H(4)), ca. 7.3 (2d, 4H, meta to SO_2), 7.60 (d, 2H, ortho to SO_2), 7.81 (d, 2H, ortho to SO_2).
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12. N-Propyl cypentodine (**6**): ^{1}H-NMR (CD_3OD) : 0.95 (t, 3H, CH_2CH_3), 1.60 (q, 2H, $NHCH_2CH_2CH_3$), 2.08 (dd, 17.5Hz, 1.7Hz, 1H $CHaH_bCO$), 2.38 (dd, 17.5Hz, 6.7Hz, 1H, $CHaH_bCO$), 2.80 (m, 1H, $COCH_2CH$), 3.5 (t, $NHCH_2CH_2$), 3.59 (dd, 10.8Hz, 5.9Hz, 1H, CH_aH_b OH), 3.69 (dd, 10.8Hz, 5.4Hz, 1H, $CHaH_bOH$).
    ^{13}C-NMR (CD_3OD): 10.5 (CH_2CH_3), 24.3 (CH_2CH_3), 33.6 ($COCH_2$), 37.8 (CH_2CH), 45.2 (NHC), 63.8(CH_2OH), 128.5 ($NH_2C=COH$), 160.4 ($NH_2C=COH$), 193.2 (C=O)
    MS (FAB+) m/z: 186 (MH^+, 100%). HRMS (FAB+) m/z: 186.1128 (calcd for $C_9H_{15}NO_3 + H^+$, 186.1130).
13. UV, ^{1}H NMR, and ^{13}C NMR spectroscopic properties were in accord with literature precedent for the N-butyl analogue of compound **7** (Ref. 11).

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