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Development of Polymer-supported synthetic procedure for Heyns Rearrangement Products

by

Miho Tateyama

Department of Food Science and Agricultural Chemistry

Macdonald Campus, McGill University,

Montreal, Quebec

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ABSTRACT

Amadori and Heyns rearrangement products are currently produced as crude Maillard reaction mixtures, due to lack of convenient procedures for their synthesis. The objective of this study was to develop a synthetic strategy based on polymer-supported synthesis that allows the production of different derivatives of these sugar-amino acid conjugates. The strategy involved coupling of poly(ethyleneglycol) mono methyl ether (PEGME) with N-protected (t-BOC) amino acid through catalysis by DCC (dicyclohexylcarbodimide) to form a polymer bound ester of the amino acid (89 % yield with β-alanine), followed by deprotection of the t-BOC group through a novel microwave-assisted hydrolysis process in high yields (98 %). After the deprotection, the polymer-based amino acid was neutralized by triethyl amine treatment, followed by room temperature reaction with selected reducing sugars or sugar analogs, for 48 hrs to produce polymer bound ARP in 75 % yield (using acetol). The final product was cleaved by methanolysis using sodium methioxide in 65 % yield. The reactions were monitored by spectroscopic analysis and the intermediates were identified by FTIR, Py/GC/MS and ^{1}H NMR.

RÉSUMÉ

Les molécules de type réarrangement Amadori (ARP) et Heyns sont obtenues de nos jours comme composantes impures de mélanges réactionnels de Maillard, à défault de méthodes de synthèse adéquates. L'objectif de la présente étude était le développement d'une stratégie de synthèse sur support polymérique qui permet la préparation de divers dérivés de ces conjuguats de glucides-acides aminés. La stratégie comprend le couplage de l'éther monométhylique de poly(ethyleneglycol) (= PEGME) avec un acide aminé N-protégé (par t-BOC); ceci est effectué par voie catalytique à l'aide de DCC (dicyclohexylcarbodiimide) qui forme alors un ester d'acide aminé lié au polymère (rendement de 89 % avec ß-alanine dans ce cas), le tout suivi par la libération du groupe t-BOC via un nouveau procédé d'hydrolyse par micro-ondes à haut rendement (98 %). Après libération du groupe protecteur, l'acide aminé sur polymère est neutralisé en traitant avec triéthylamine, et la réaction à température ambiante avec certains glucides réducteurs ou gluco-analogues permet alors d'obtenir après 48 heures un rendement de 75 % en ARP/polymère (en utilisant l'acétol). Le produit final est scindé via méthanolyse par méthioxyde de sodium, avec rendement de 65 %. Les diverses réactions ont été suivies par analyse spectroscopique, et les espèces moléculaires intermédiaires ont été identifiées par Fourier Transformation Infrared Spectroscopy, Py/GC/MS et Résonance Magnétique Nucléaire H^1 .

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Chapter 1

INTRODUCTION

Many unpleasant tasting raw foods can be transformed into desirable products through thermal processes such as baking and roasting. Flavors of cooked foods depend in part on the aroma compounds present in the raw material and in part on how the food was prepared. Heat treatment of foods rich in reducing sugars and amino acids, peptides, or proteins can initiate Mailllard reaction, (non-enzymatic browning reaction). The reaction results in the formation of the brown color and flavors and has a great effect on changes in functional properties, and nutritional value of the food.

Amadori and Heyns rearrangement products (ARPs and HRPs) are important Maillard reaction intermediates. They are the precursors to the various compounds formed during the Maillard reaction and have unique industrial implications not only in food but also in cosmetics and pharmaceutical industries. ARPs or HRPs could be added to food products to enhance roasted and cooked flavors, especially in packaged foods. However, facile methods for their preparation have not been developed yet due to the many side-reactions encountered during classical synthesis such as oxidations and decompositions.

Development of a simple and efficient synthesis methods for ARPs or HRPs are needed. The availability of synthetic ARPs and HRPs can be a valuable tool for a variety of applications, providing useful information towards the understanding of the Maillard reactions.

Chapter 2

OBJECTIVES

The objectives of this study were (1) To develop a liquid phase polymer-supported synthesis of Amadori or Heyns rearrangement products. (2) To optimize the yield of each step. (3) To characterize the intermediates and final products by different spectroscopic means.

Chapter 3

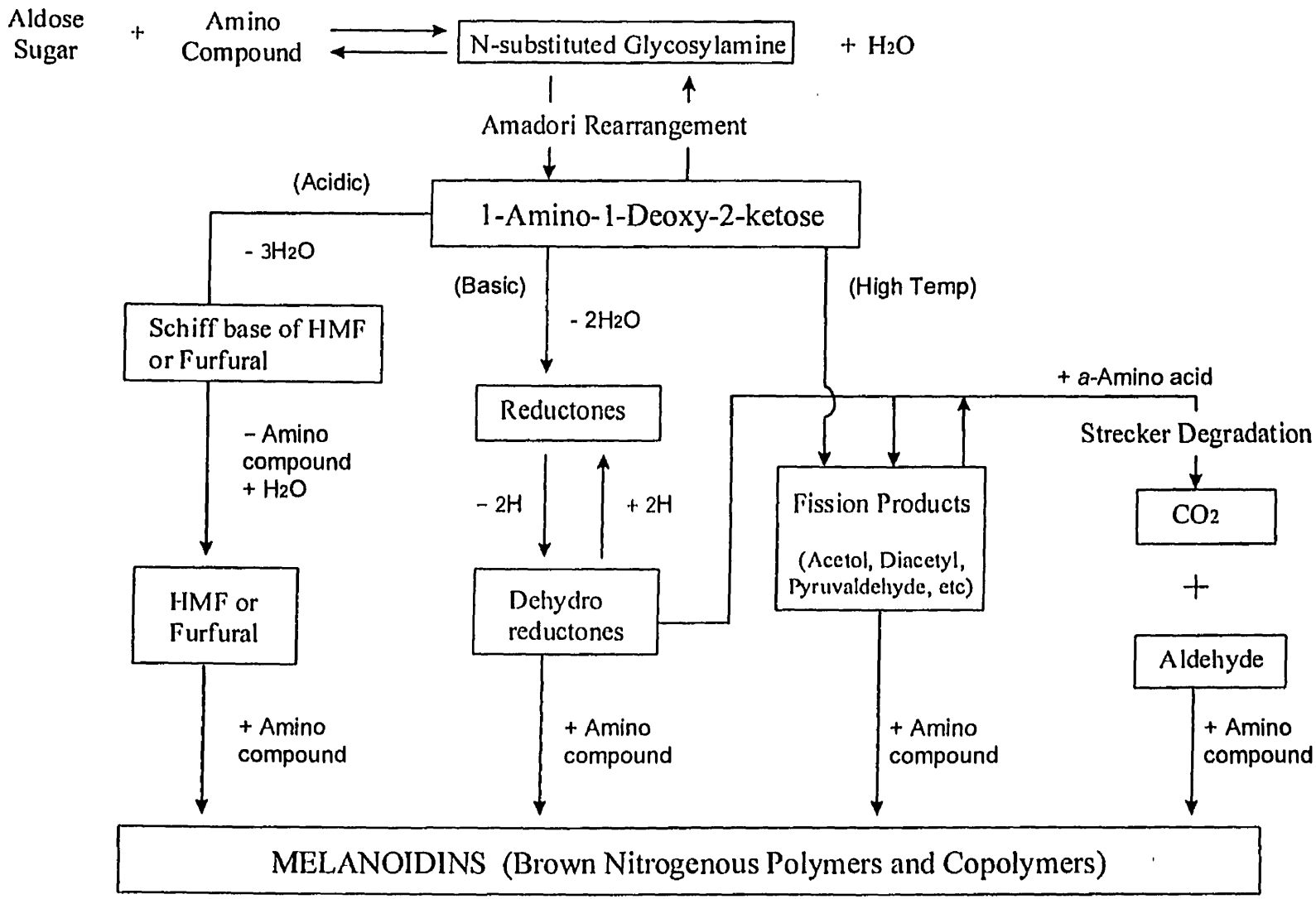
LITERATURE REVIEW

3.1. Maillard reaction

The Maillard reaction is named after the French chemist, Louis Camille Maillard, who examined the reacion between glycine and glucose and observed the formation of melanoidins - brown-black pigments and CO_2 generation during the reaction (Maillard, 1912). Although more than 80 years have passed since the Maillard reaction was first investigated and many results have been gathered, however; it is still not possible to present a complete reaction scheme. The first coherent scheme of the complex series of reactions was proposed by Hodge in 1953 (Scheme 1). The whole network of reactions can be divided into three stages.

3.1.1. Early stage

First step of the reaction is the condensation of the amine with carbonyl compounds present in foods. As shown in Scheme 2, the reaction of the open chain form of reducing sugars (1) with the amino group of amino acid or protein produces the addition compound 2 which rapidly loses water to form a Schiff base (3) followed by cyclization to the corresponding N-substituted glycosylamine (4). Glycosylamines of amino acids or aliphatic amines, which are strongly basic, can quickly undergo further reactions and rearrange into relatively stable derivatives. Other amino groups such as amides or secondary amines, can also undergo similar reactions, however, increasing nucleophilic strength of the amine will increase the rate of carbonyl-amine reaction. The type of derivative formed depends on the reducing sugar; aldoses undergo Amadori rearrangement (Scheme 3) to produce 1-amino-1-deoxy-2-ketoses (5), and ketoses undergo Heyns rearrangement (Scheme 4) to produce 2-amino-2-deoxy-1-aldoses (13).

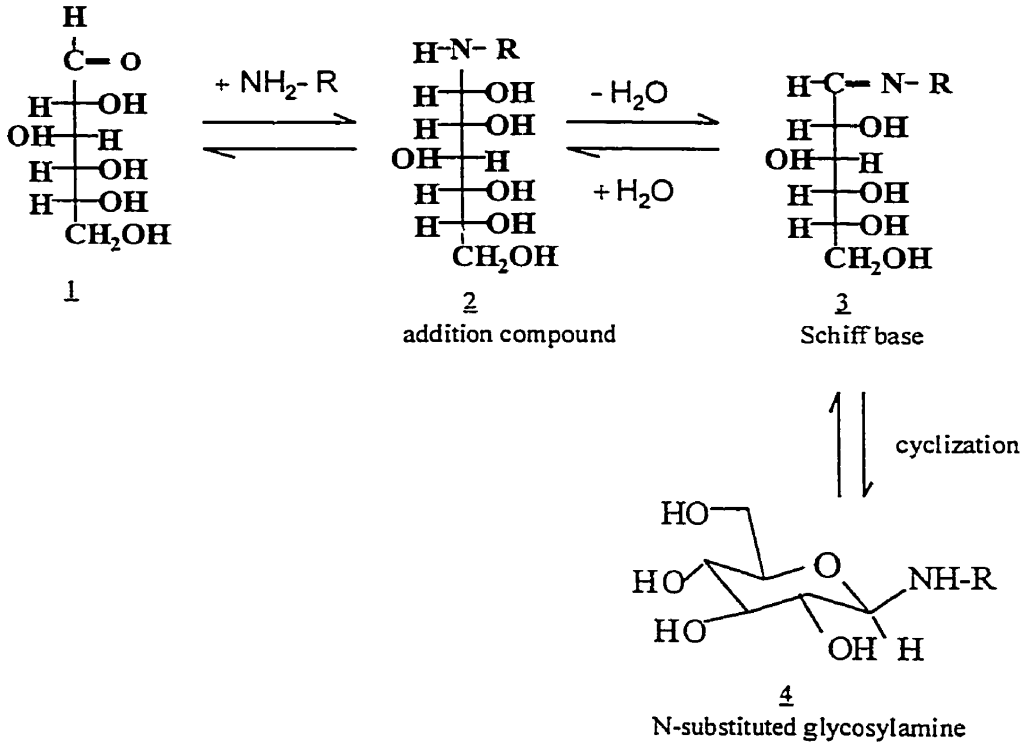


Scheme 1: Maillard Reaction Scheme according to Hodge (1953)

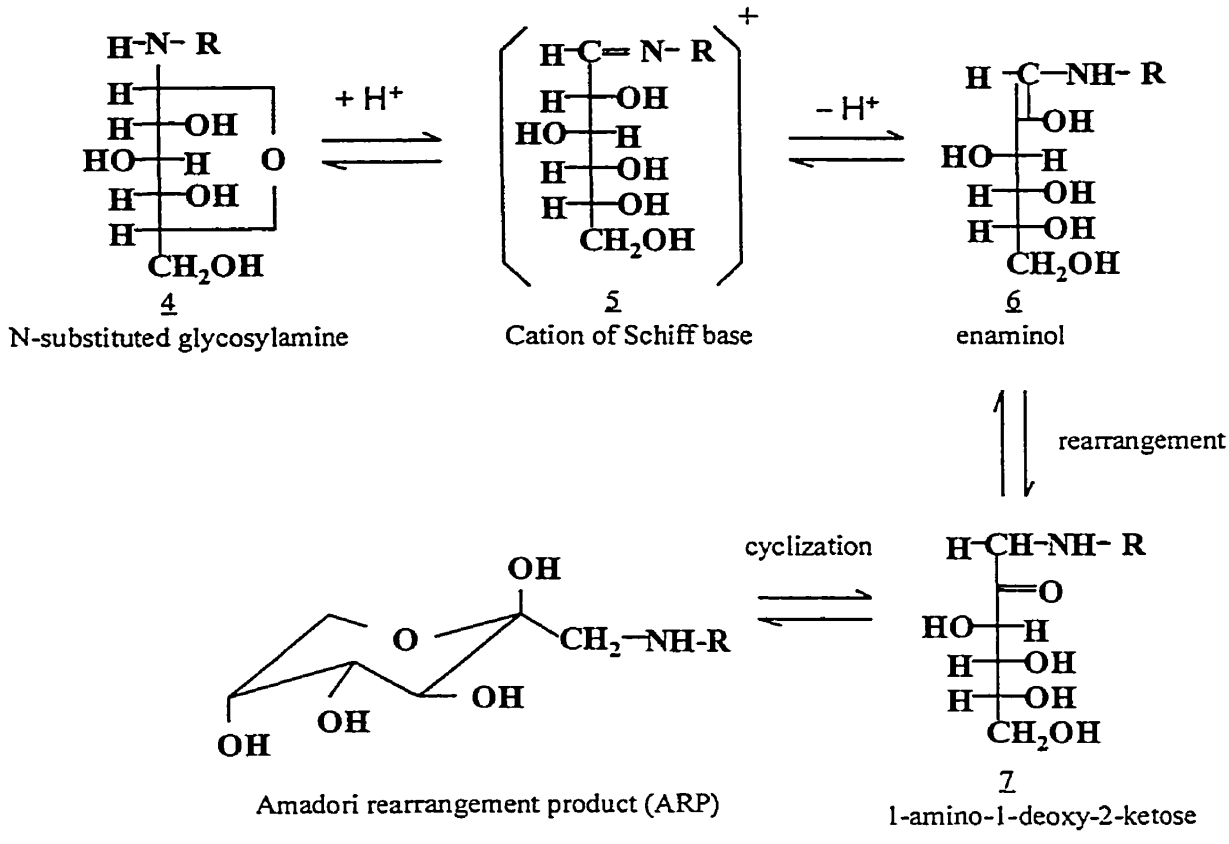
3.1.1.1. Mechanism of the carbonyl-amine reaction

The initial interaction products of the reducing sugars with amino acids have been extensively reviewed in the literature (Yaylayan and Huyghues-Despointes, 1994).

The open chain form of sugar molecules present in the solution is very low (in case of glucose, the open chain form present is less than 1 % of the total sugar). It is therefore likely that the ring opening reaction is initiated by the presence of amino acids. As nucleophiles, amino acids can initiate the formation of the Amadori rearrangement product. After the formation of cyclic glycosylamine (4), the next step involves protonation of the nitrogen at C-1 of the cyclic glycosylamine.

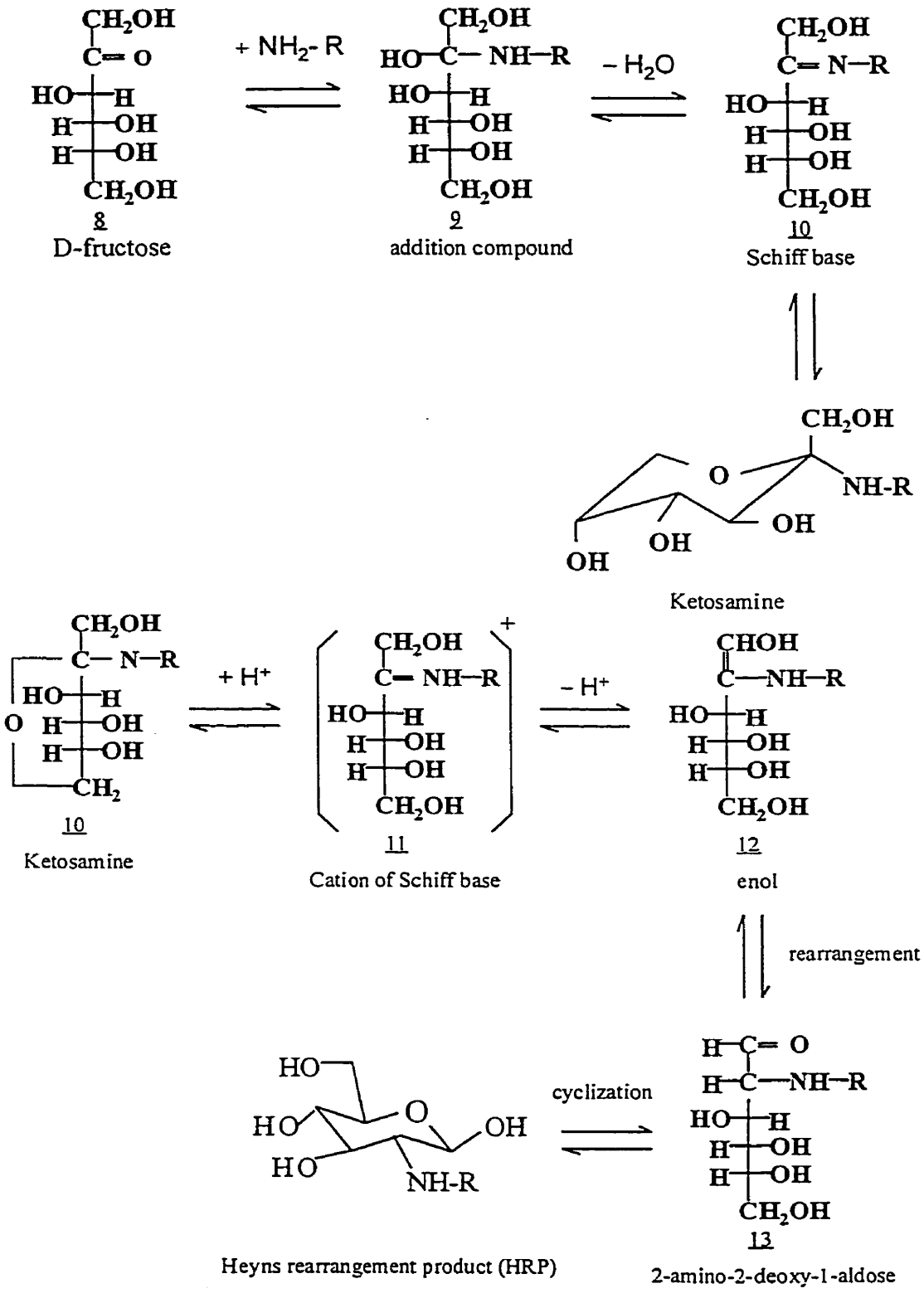


Scheme 2: Molecular events in the initial stages of the Maillard reaction



Scheme 3: Amadori rearrangement

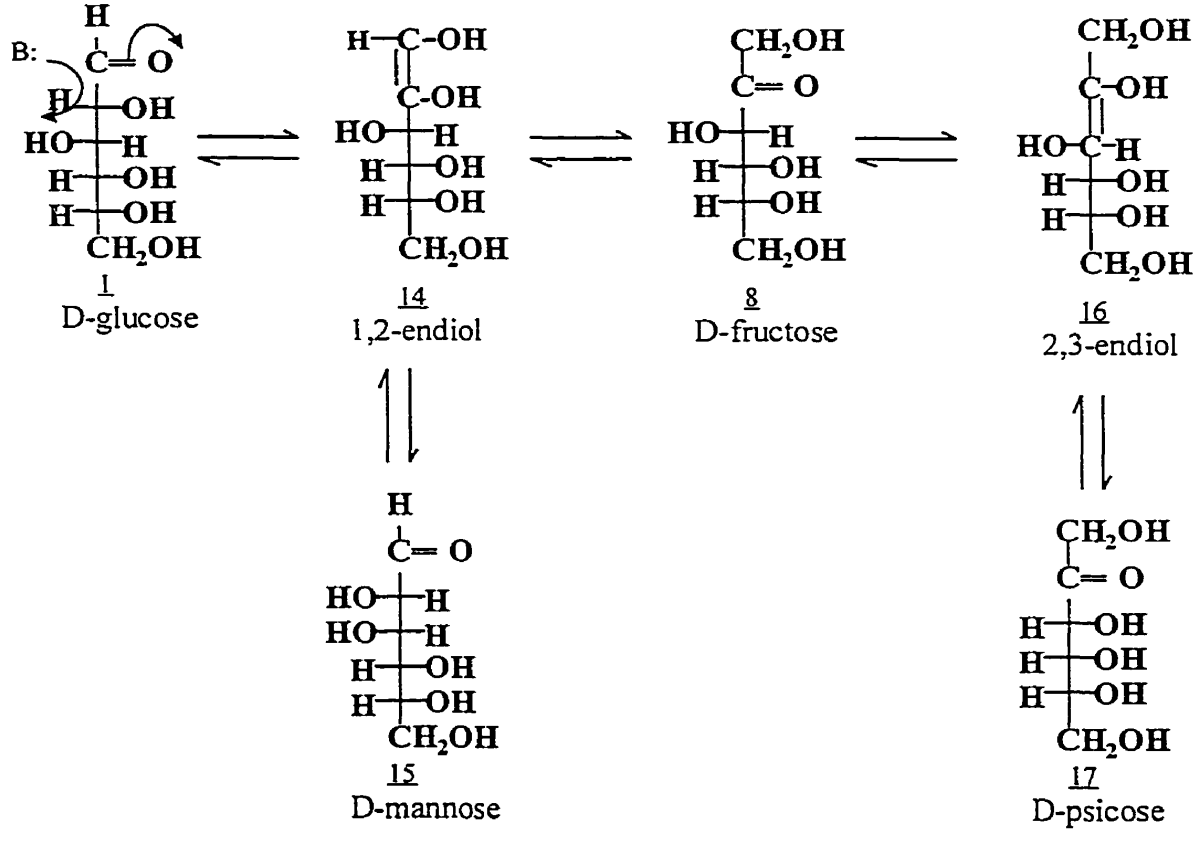
The carboxyl group of the amino acid provides the necessary protons for this reaction. After protonation and the corresponding ring opening, the Schiff's base (5) is enolized and is quickly converted to relatively stable 1-amino-1-deoxy-2-ketose (7). The reactions leading up to this step are all reversible and the Amadori rearrangement product in turn can decompose into α-dicarbonyl compounds.



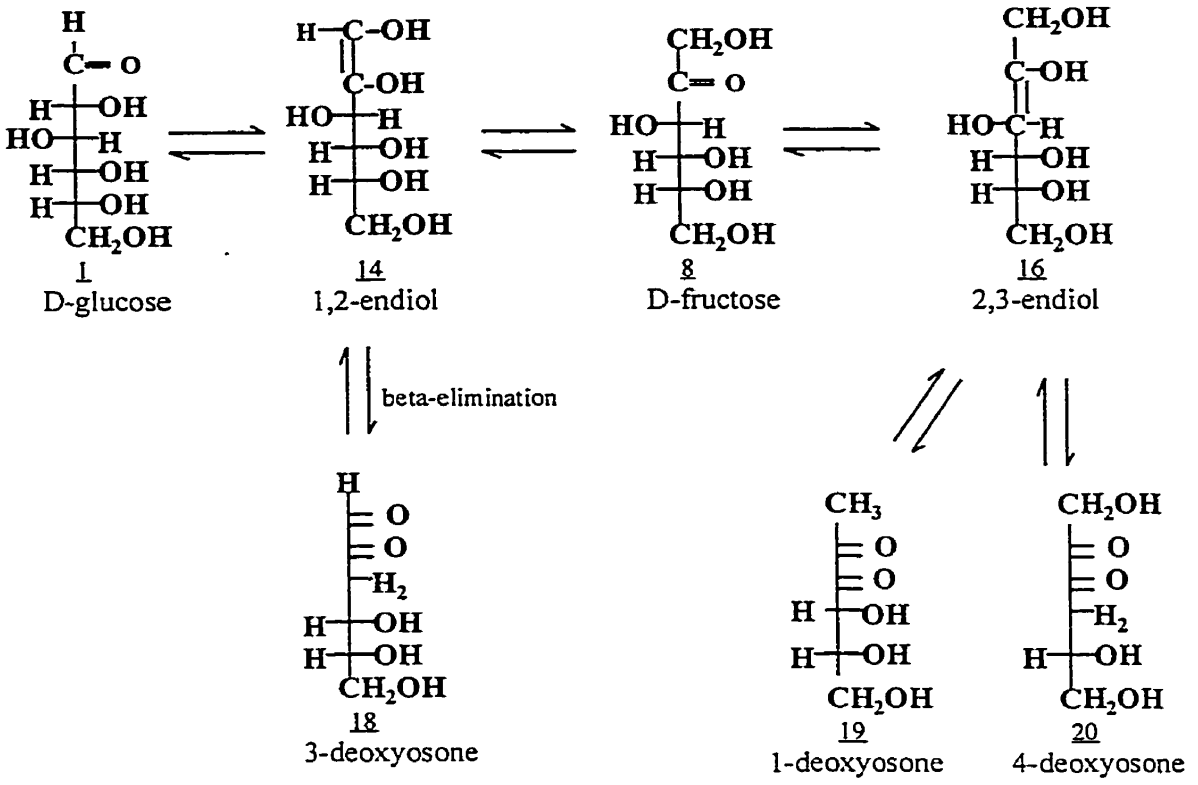
Scheme 4: Heyns rearrangement

3.1.1.2. Amino acids as bases or acids

Depending on the prevailing pH, amino acids can act either as acid or base catalysts. As acids or bases, they can catalyze the sugar transformation reactions *via* the Lobry de Bruyn-Alberda van Ekenstein transformation (Scheme 5). Through loss of water, the endiols produce reactive α-dicarbonyl compounds (Scheme 6) which subsequently can react with the amino acids. Through the sugar transformation reactions, aldoses are converted into ketoses and vise versa. Similarly, an aminoketose (7) is converted into aminoaldose (13) in the presence of free amino acids as shown in Scheme 7.

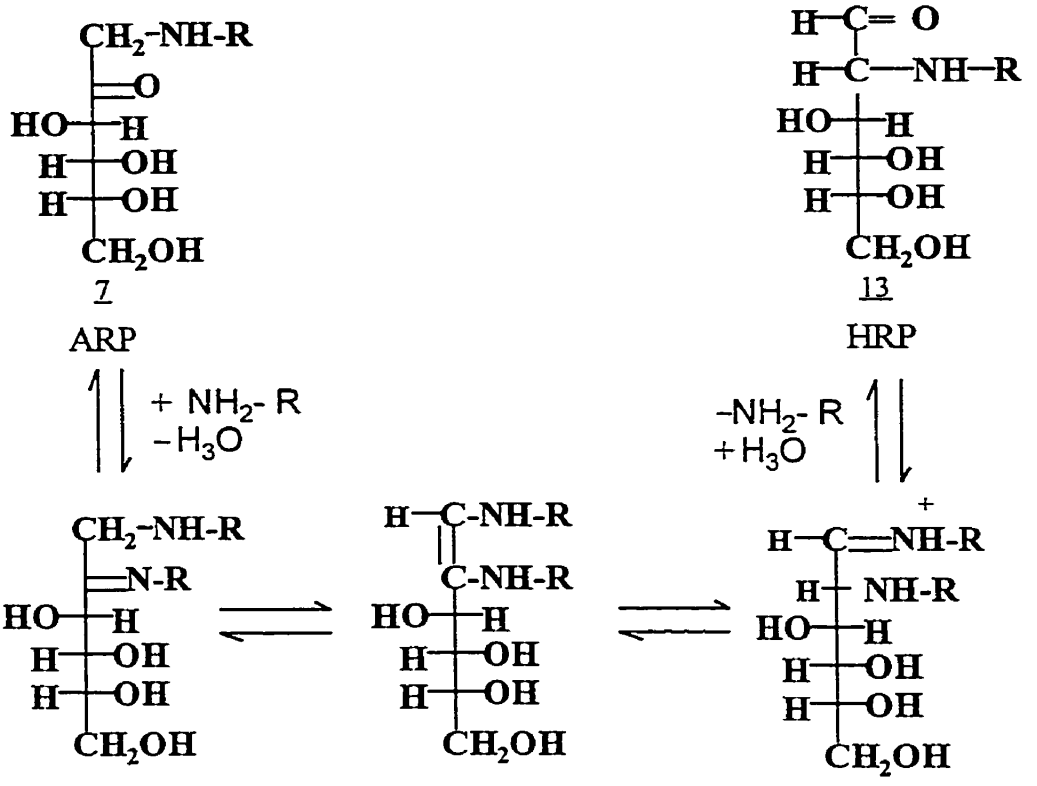


Scheme 5: Lobry de Bruyn-Alberda van Ekenstein transformation



Scheme 6: Formation of α-dicarbonyl compounds

It is also known that sugars alone can undergo similar transformations at higher temperatures. On heating sugars in the absence of amines, sugar transformation reactions occurs at extreme pH value (pH<3; pH>8), or temperatures above 130°C, a condition known as caramerization. Therefore, the significance of amines in the formation of the α-dicarbonyl compounds lies in their ability to catalyze these sugar rearrangements under pH condition normally encountered in foods or living organisms (pH 4-7). However, the enolization process requires more basic conditions.

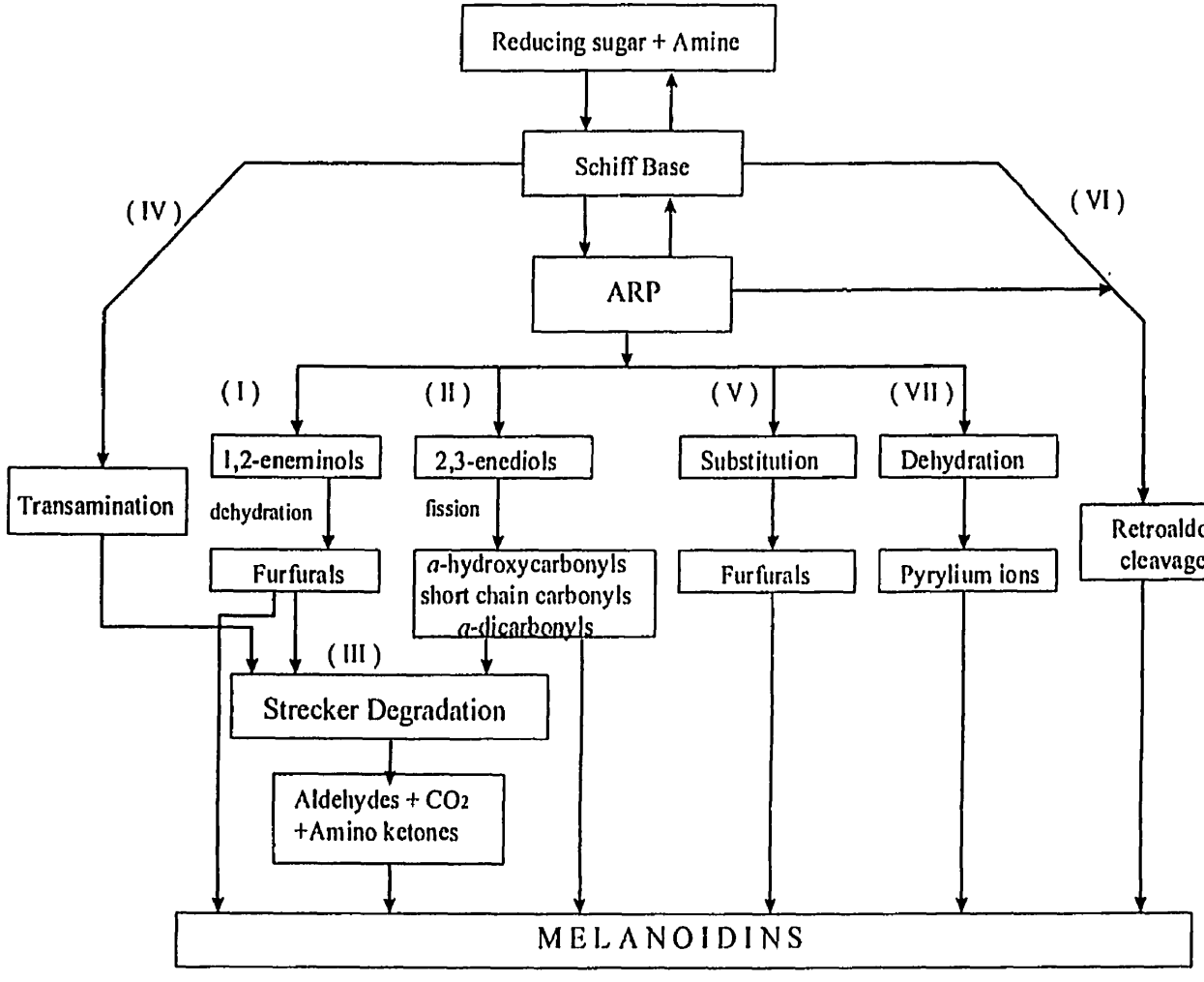


Scheme 7: Interconversion of ARP and HRP

Many reactions that occur during the thermal degradation of sugars (caramerization) are also observed in the Maillard reaction. However, those chemical reactions that occur in sugars alone at high temperatures, take place at much lower temperatures once the sugars have reacted with amino acids.

3.1.2. Advanced stage

Depending on the reaction conditions, such as pH and temperature, various volatile compounds are formed through different pathways of the advanced Maillard reaction as shown in Figure 1.



A. Directry via ARP

Pathway (I): 2,3 enolization
Pathway (II): 1,2 enolization
Pathway (V): Di-substitution of ARP
Pathway (VII): Dehydration of ARP

B. Indirectly via dicarbonyls of pathways (I) +(II)

Pathway (III): Strecker Degradation

C. By-passing ARP stage

Pathway (IV): Transamination of sciff base
Pathway (VI): Retroaldol cleavage

D. Later stage : Formation of heterocyclics (N,O,S)

Pyrazines
Pyrroles, Pyrrolidines, Pyridines
Oxazolines
Thiazoles
Maltols, Isomaltols
Dehydrofuranones, Dehydropyrones
Cyclopentenolones

Fig. 1: Advanced Maillard Reactions

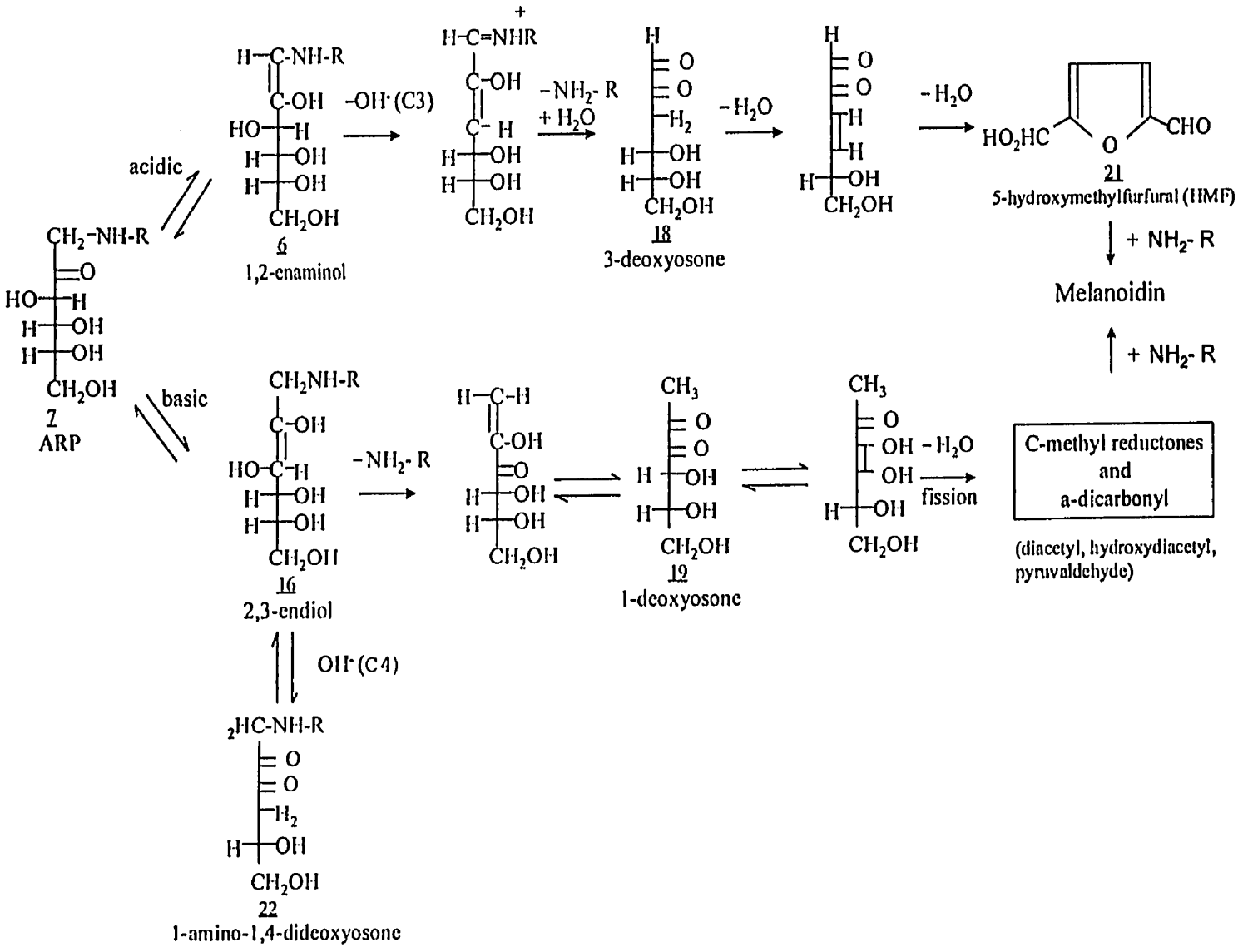
3.1.2.A. Pathways via Amadori compound

ENOLIZATION (PATHWAYS I AND II)

Degradation of ARPs (or HRPs) may occur *via* two main pathways, depending on the pH of the system (see Scheme 8). At acidic conditions, enolization involving the C-1 atom of the sugar is favored to form a 1,2-eneminol, whereas at basic conditions, enolization involves the C-3 atom to form a 2,3-enediol. In the 1,2-eneminol pathway, the 1,2-eneminol form of ARP loses the hydroxyl group at C-3 with subsequent deamination at C-1. Following dehydration, the 3-deoxyosone thus formed, readily loses another water molecule and cylcizes to form furfural derivatives. In the 2,3-enediol pathway, 2,3-enediol is deaminated to yield a 1-deoxyosone, or can eliminate the C-4 hydroxyl group to produce 1-amino-1,4-dideoxyosone. Further degradation of 1-deoxyosone leads to the formation of fission products, including reductones, maltol derivatives and other dicarbonyls such as 4-deoxyosones. In both pathways, the reactions that follow the formation of the α-dicarbonyl compounds are very complex. The further reactions of these interemediates eventually result in the production of dark-brown nitrogen-containing pigments. Aldol condensations and further amino carbonyl reactions lead also to the formation of various heterocyclic compounds such as pyrazines, pyrrols and pyridines which appears to be largely responsible for the roasted, bready and nutty flavors of heated foods.

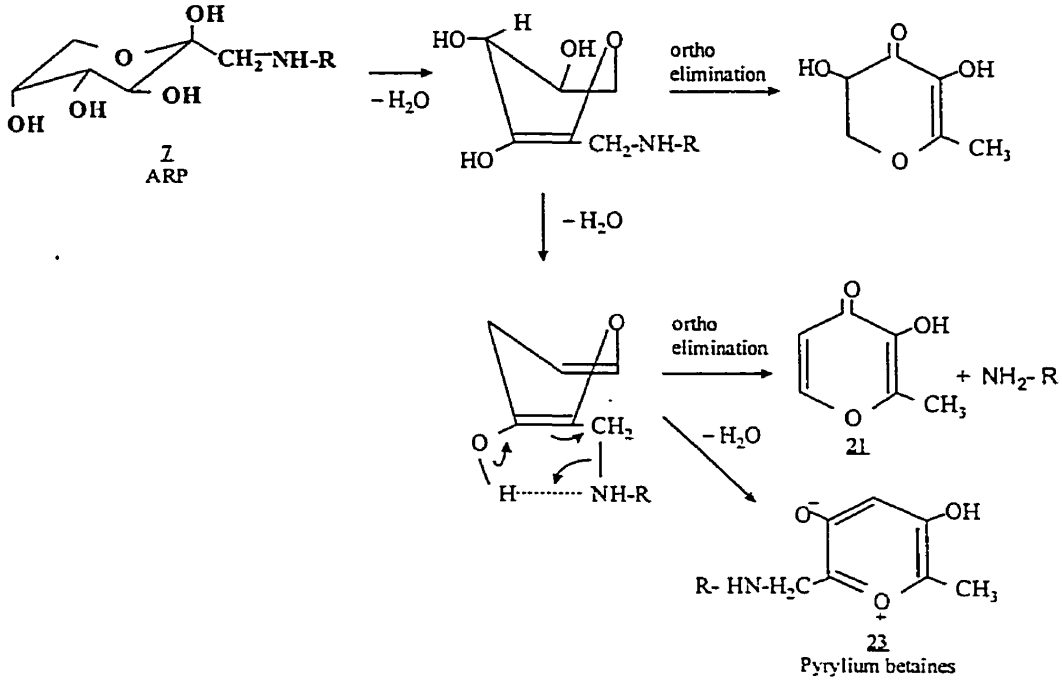
DEHYDRATION OF ARP (PATHWAY VII)

Most products formed through the degradation of ARPs are mainly rationalized through the enolization of the open chain forms, however; open chain forms represent only 1 to 7 % of the total concentration of ARPs, depending on the temperature. Yaylayan (1990) suggested alternative routes of decomposition of ARPs based on dehydrations directly from the most abundant cyclic forms. A possible mechanism for such dehydrations from the cyclic ARP (the chair conformation of the fructopyranose) is shown in Scheme 9. Dehydration from cyclic forms involves *trans*-elimination of the anomeric hydroxyl group and the C-3



Scheme 8: Degradation of ARP (pathways I and II)

hydrogen to form boat conformation and a double bond. Further loss of two water molecules can produce pyrylium ions (or pyrylium betaines). Both intermediates formed during the dehydration process can also undergo *ortho*-elimination to produce maltol and its derivatives. The presence of reactive pyrylium ions can provide a pathway not only for the formation of different heterocycles responsible for the flavor production but also for the formation of brown polymers.

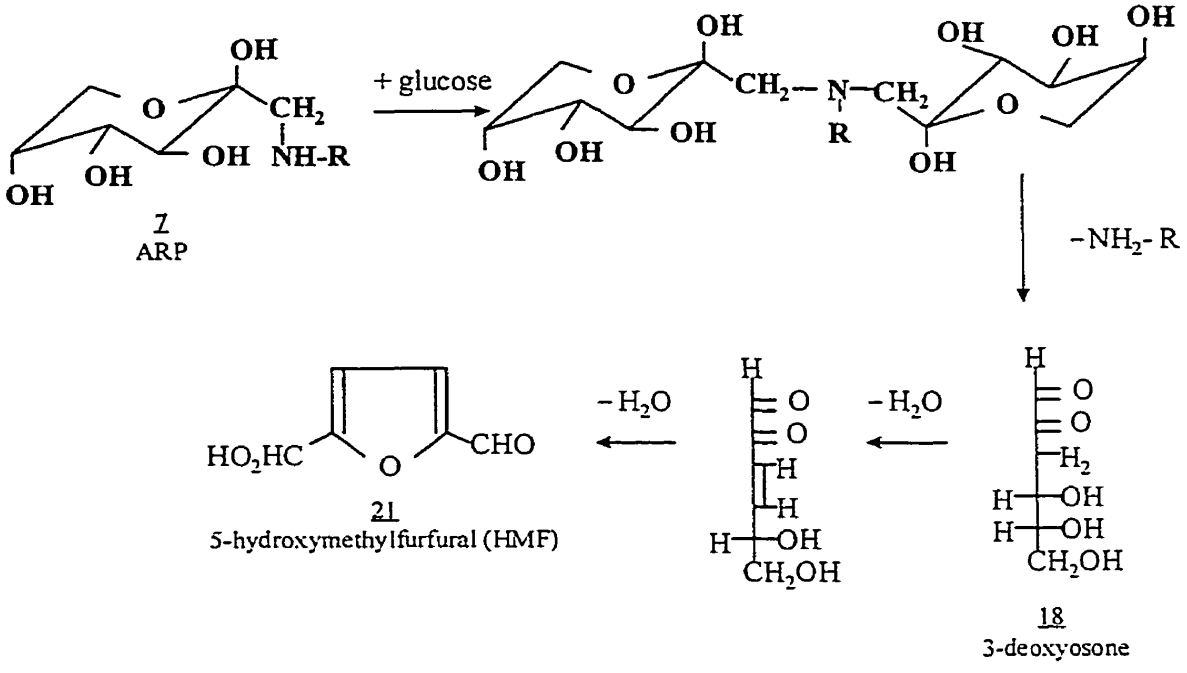


Scheme 9: Dehydration reactions from cyclic forms of ARP

DI-SUBSTITUTION OF ARP (PATHWAY V)

Another pathway proposed earlier by Burton and McWeeney (1964), is based on the reaction of a second sugar molecule with ARP to form a diketo amino compound. The

disubstituted ARP undergoes decomposition to form 3-deoxyosone and 5-HMF (5-hydroxymethylfurfural) by dehydration as shown in Scheme 10.



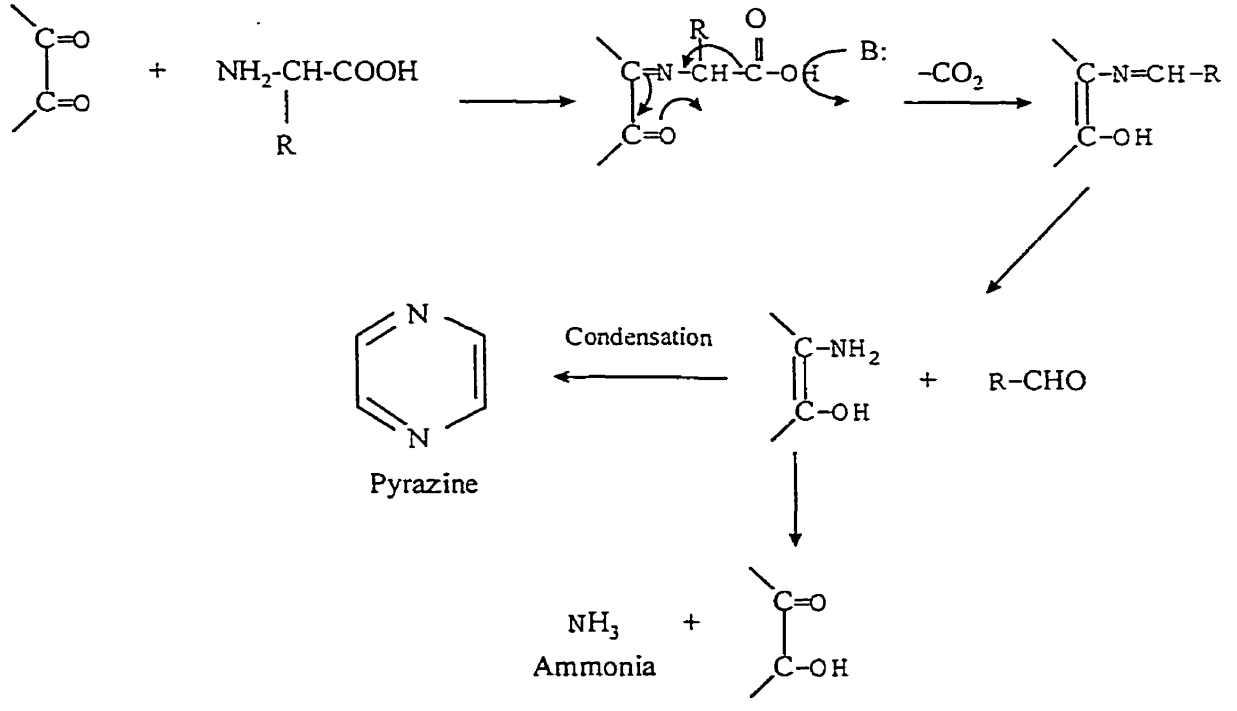
Scheme 10: Di-substitution of ARP

3.1.2.B. Pathway via Strecker Degradation

This pathway mainly involves oxidative degradation of amino acids in the presence of α-dicarbonyl or other conjugated dicarbonyl compounds. The reaction is not directly concerned with pigment formation but provides reducing compounds essential for its formation.

STRECKER DEGRADATION (PATHWAY III)

The initial reaction involves the formation of a Schiff base with the amino acid (Scheme 11). The enolized form then decarboxylates to produce a new Schiff base with one carbon atom less. Hydrolysis of this intermediate generates a 1-amino-2-*keto* derivative and an aldehyde which corresponds to the side chain of the original amino acid with one carbon atom less. It appears that most of the carbon dioxide released during Maillard reaction is derived from the carboxyl group of amino acids during Strecker degradation. The Strecker aldehyde appears to be very important auxiliary aroma compound. Condensation of the intermediates formed through Strecker degradation produces many heterocyclic compounds such as pyrazines, pyrrolines, oxazoles, oxazolines, and thiazole derivatives responsible for the flavor of heated foods.



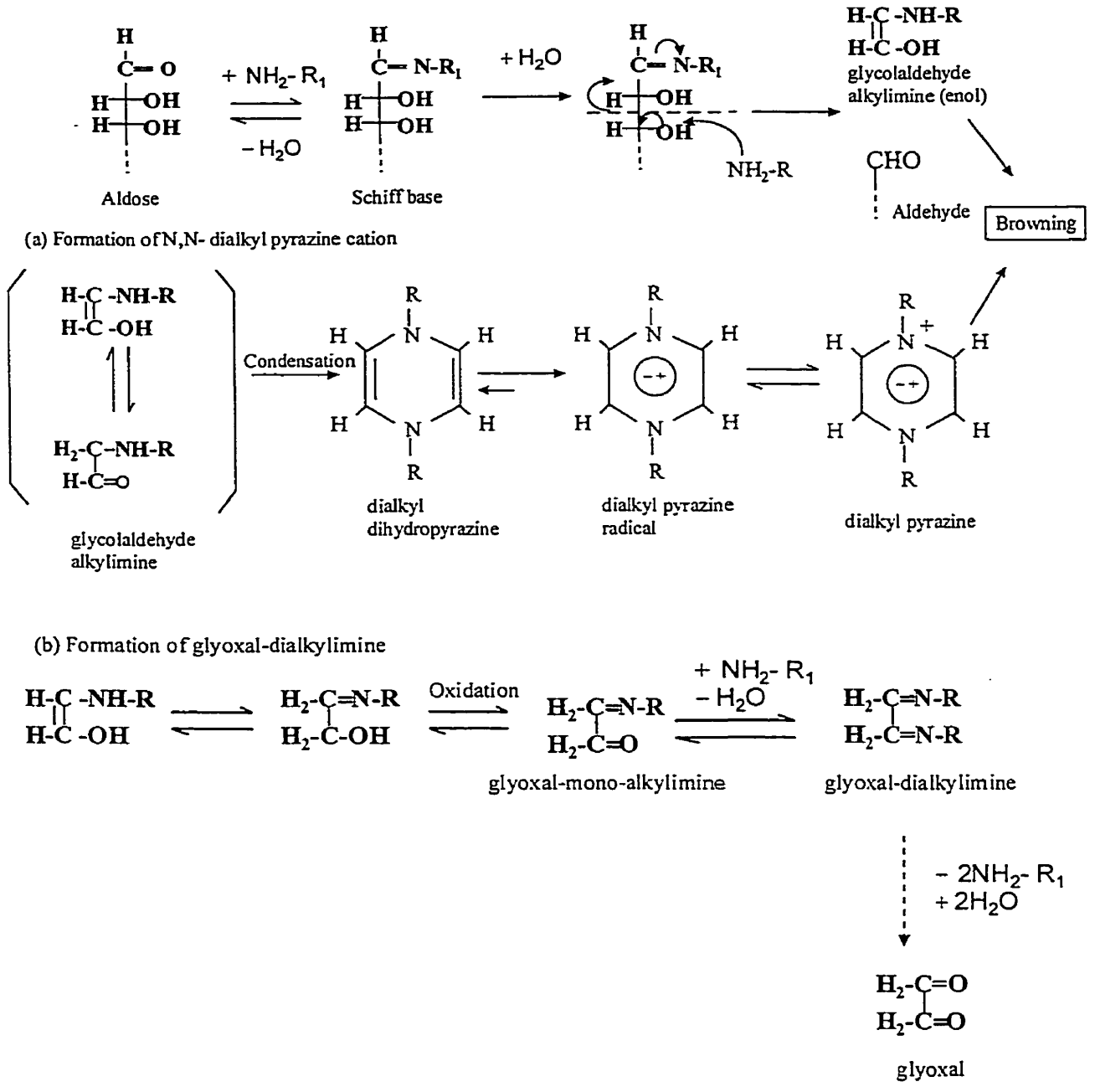
Scheme 11: Strecker Degradation

3.1.2.C. Pathways by-passing ARP stage

Several alternative pathways which by-pass the formation of ARP were also suggested as follows.

RETROALDOL CLEAVAGE (PATHWAY VI)

The formation of free radicals in browning mixtures of carbonyl compounds and amines or amino acids was first proposed by Namiki et al. (1973). The same researchers later reported that model systems with alanine and arabinose gave distinctive signals in ESR (electron spin resonance) spectra which were attributed to the presence of *N,N*-dialkyl pyrazine cation radicals (Scheme12 a), these signals were detected prior to the formation of ARP (Hayashi et al., 1977). They also proposed the formation of a C_2 sugar fragment as the precursor of these radicals, The presence of this fragment was confirmed by isolation and identification of glyoxal dialkylimine (Scheme12 b) (Namiki and Hayashi, 1983; Hayashi and Namiki, 1986). This finding pointed to an alternative pathway for browning in which the sugar moiety of the Schiff base was cleaved prior to the formation of ARP, leading to the formation of glycolaldehyde alkylamine or its corresponding eneminol. Under acidic conditions, the traditionally accepted pathway involves osone formation (Pathways I and II) *via* ARP. Under basic conditions, however, the increase in browning, was largely attributed to the fragmentation of sugar into C_2 and C_3 fragments. In addition, a recent study based on rat tail collagen model system, suggested that glyoxal is the major oxidation product of glucose. It was proposed that glyoxal then reacts directly with lysine resulting in the formation of carboxymethyllysine *via* a Cannizano reaction. It has been suggested that Amadori route might contribute less than 10 % of the glycoxidation products in Maillard reaction systems (Baynes et al., 1995).



Scheme 12: Formation of *N,N*-dialkyl pyrazine cation radicals and glyoxal dialkylimine

TRANSAMINATON (PATHWAY IV)

Another possible pathway is the transamination reaction of the Schiff bases derived from sugar and amino acids. Holtermand (1966) proposed migration of a C=N double bond in the Schiff base, which when hydrolysed can release an oxo acid and a nonreducing amino sugar. The oxo acid could react with an amino acid and liberate an aldehyde by the Strecker degradation (pathway III)

3.1.3. Final stage

The final stage of Maillard browning reaction involves the conversion of low molecular weight precursors, such as furfurals, fission products, and reductones into high molecular weight pigments. The pigments isolated from the reaction between aldoses and amines contain nitrogen, some are readily soluble in water, some slightly soluble and others are insoluble. Soluble pigments were found to be undialysable.

The melanoidin formation is the result of the polymerization of the many reactive compounds that are formed during the advanced stage of Maillard reaction such as dicarbonyl compounds and furfural. These precursors are similar to those formed in the caramelization of sugars, containing one or more carbonyl groups, but in the Maillard reaction they are formed at lower temperatures than those required for caramelization. A complete picture of melanoidin structure is still not well defined mainly due to the complex and inhomogeneous nature of the brown polymers. Recently some type of cross-linked biopolymers, pyrrole cross-linked, and pyrazinium radical cation cross-linking of 2-carbon fragments were found as components of food melanoidins (Namiki, 1988). In addition to the brown color formation, these polymerization reactions definitely lead to toughing of stored food.

3.2. Factors affecting the rate of Maillard reaction

3.2.1. pH

Both the initial pH of the products and the buffering capacity of the system greatly affect the rate and direction of the reaction. Several model studies have discovered that as the pH

increases (from 3 up to 8) the rate of the overall Maillard reaction (Lee et al., 1984) and the quantities of colored and polymeric compounds also increase (Ashoor and Zent, 1984). The rate of browning increases with increasing pH to a maximum at pH of ~10. This may be explained by the inhibition of melanoidin formation *via* transamination at lower pH values due to protonation of the amino groups. In general, Maillard reaction is favored under alkaline pH, however; the reaction rate decreases at extreme alkaline conditions (pH value higher than 10) due to the requirement of protons to catalyze both Amadori and Heyns rearrangements. In addition, the degradation of the Amadori products through enolization pathways is pH dependent. For example, degradation takes place *via* 1,2,eneminol pathway at acidic pH, while the 2,3-endiol route is favored at alkaline pH. However, it should be noted that during reaction, the pH of the system decreases due to the disappearance of basic amino groups. In terms of flavor generation, even small changes in pH can have marked effect on the formation of certain classes of volatiles (Farmer and Mottram, 1990). Therefore, in unbuffered model systems, pH changes of 3 or more pH units may occur during heating, and this may affect both the rate and the pathway by which volatiles and colored products are formed (Whitfield et al., 1988) (Wong and Bernhard, 1988).

3.2.2. Temperature and heating time

The effect of temperature and heating time was first studied by Maillard (1921) who reported that the rate of the reaction increases with temperature. Many researchers have since confirmed this observation (Lea and Hannan, 1949; Hurrell and Carpenter, 1974, 1977). At lower temperatures, the Maillard reaction can proceed at slower rate, and typically yielding off-flavors. For example, mixtures of amino acids and sugars stored at refrigerated temperatures can show signs of Maillard browning on prolonged storage. However, only at elevated temperatures, the desirable flavors and brown color associated with cooking are formed. In addition, a kinetic study showed that one can not typically produce the same flavor profile at a lower temperature by simply increasing processing time (Leahy and Reineccius, 1989).

3.2.3. Moisture content

Water in food systems has significant influence on the Maillard reaction through control of the liquid phase viscosity or by dissolution, concentration and dilution of reactants

(Warmbier et al., 1976; Labuza, 1980). Although water is necessary for the initial reaction to take place, however, it inhibits the browning reaction which comprises of a series of dehydration reactions (Wolfrom and Rooney, 1953). This may be due to dilution effect or to decreased rate of reaction at high moisture levels since water is produced in a series of condensation reactions (Eichner and Karel, 1972). It is generally recognized that the reaction proceeds more readily at low moisture levels, the brown colors and characteristic flavors are generated particularly at the outside areas of roasted or baked foods, where dehydration has occurred. It is believed that sufficient browning take place in most foods at the level of water activity between 0.3 and 0.7. However, water influence is particularly dependent on the temperature since high temperatures dehydrate the surface in processes such as baking and frying and accelerate the browning. Therefore, especially in food processing, the temperature - water content relationship is found to be of primary importance for controlling Maillard reaction. For example, extensive lysine losses during the severe conditions of extrusion cooking can be reduced by changing the parameter, decreasing temperature and increased water content.

In terms of water binding and its availability, the physicochemical state of food system may also affect the Maillard reaction. The moisture sorption isotherm of a food depends on whether the system is crystalline or amorphous and on other factors, such as fat percentage (Supplee, 1926). At low water activity (a_w), an amorphous food system absorbs much water in the spaces between the molecules, while in a crystalline system, absorption of water can take place only at the surface of the crystal lattice. In some dried food systems (e.g., dried milk), a shift from the amorphous to the crystalline state occurs at a_w values in the region of 0.6 to 0.7 (Troy and Sharp, 1930; Sharp and Doob, 1941). The amorphous phase absorbs water until the molecules acquire sufficient mobility and space to form a crystal lattice. As crystallization is initiated, water is expelled and may become trapped in localized areas within the food (Saltmarch et al, 1981). This water is then available for interaction with other food components and affects the rate of the reaction unless it evaporates.

3.2.4. The nature of the reactants

Low molecular weight compounds tend to be more reactive than high molecular weight compounds due to greater steric hindrance in the latter. Accordingly, aldopentoses are generally more reactive than aldohexoses and monosaccharides are more reactive than di-or oligosaccharides. Aldoses in general appear to be more reactive than ketoses as a consequence of the more sterically hindered carbonyl group. Thus, glucose is more reactive than lactose and the rate of browning in lactose-hydrolyzed milk powder is greater than in a control powder. Accordingly, the rate of lysine destruction is more rapid in milk products in which the lactose has been hydrolyzed or replaced by glucose.

The nature of amino compounds also affects the rate of Maillard browning. For example, lysine is more reactive than other amino acids due to the presence of the ε-amino group. In unbuffered systems, the amino compound may also influence the rate of browning through its effect on pH. In protein systems, the concentration of lysine in the protein is closely related to the propensity to brown. Thus, milk proteins that are rich in lysine tend to brown more readily than proteins low in lysine, such as soy protein.

The reactivity of aliphatic aldehydes with amino acids in the Maillard reaction has long been recognized. Thus, aldehydes produced due to lipid oxidation may also undergo Maillard reaction with amino compounds. Interestingly, limiting the autooxidation of lipids in lipid- protein systems by introducing antioxidants may also limit the degree of browning observed. Aldehyde produced during Maillard reaction may also react with amino compounds. In this respect, furfural has been reported to be particularly reactive.

It is generally agreed that an excess of reducing sugars over amino compounds promotes the rate of Maillard browning. This may be due to mechanistic differences in the destruction of sugars compared with amino acids. Browning in amino acid/sugar systems also appears to be maximal when sugar is in excess.

3.3. Significance of the Maillard reaction

The significance of the Maillard reaction in foods can be summarized by at least five aspects:

- *Production of color.* This may be desirable, as in coffee and bread crust, or undesirable as in glucose syrup and in many intermediate moisture food products.

- *Flavor generation or off-flavors.* Flavors and off-flavors are due to volatile products, e.g. fission products and Strecker aldehydes. Substances tasting sweet or bitter may be involved, as well as effects on texture.

- *Reduction of nutritional value.* By involvement of ascorbic acid (a reductone) and of lysine (free or bound), an essential and often limiting amino acid. Metal-chelating properties may also be significant.

- *Toxicity.* Through the possible formation of mutagens such as imidazoquinolins (IQs) and N-nitroso derivatives of Amadori compounds.

- *Antioxidant properties and antimicrobial potencies.* These are thought to be due to the reductones formed and chelating properties of melanoidins.

3.3.1. Medical aspects

The Maillard reaction also occurs *in vivo*, specially in diabetic patients. Recent studies have correlated the formation of advanced glycation end products (AGEs) to early aging as well as to complications of diabetes. AGEs are continuously formed in tissues both in healthy individuals and in diabetics; macropharges are responsible for recognizing and removing the glycated proteins and for initiating the process of tissue remodeling. According to Dunn et al. (1989) and Sell and Monnier (1989), proteins are glycated (nonenzymatically glucosylated) in *vivo*, forming fructosyllysine derivatives which can be transformed oxidatively to N-ε-(carboxymethyl) lysine and hydrolytically to furosine. Levels of fructosyllysine in hemoglobin, plasma proteins, collagen, hair and lens, increase in direct proportion to the degree of hyperglycemia in diabetic patients. Dunn et al (1990) showed that carboxymethyllysine rather than furosine may be the major glycation end products in adult human lens protein during aging. According to Knecht et al. (1992), glucose causes browning and protein cross-linking at a rate 10 times greater than fructose. Both fructose- and glucose-derived Maillard reaction products are potential precursors for dicarbonyl compounds such as 3-deoxyglucosone. This compound appears to be a potent cross-linking

agent and diabetes-related changes in tissue proteins. The ability to detoxify 3-deoxyglucosone may provide a genetic basis for differences in age-related pathologies and complications of diabetes. Moreover, Shaw et al (1995) reported that nonspecific binding of Maillard products to macropharges might be more important than specific receptor binding in cellular recognition of such products. This finding implies that food derived Maillard products may compromise the effectiveness of binding and removal by macropharges of Maillard products formed *in vivo* in individuals of older age or suffering from diabetes.

3.3.2. Nutritional aspects

Protein bioavailability is also affected by cross-linking phenomena similar to that observed in *vivo* (Pongor et al., 1984). Cross-linking of 2-carbon fragments or further reactions of pendant ARPs or pyrraline can lead to indigestible forms of protein (Klein et al., 1992). Other *in vivo* results suggest that multiple amino acid residues i.e. lysine plus arginine can be involved in cross-linked proteins *via* pentosidine formation. Maillard reaction products exibit antinutritive effects through mechanisms involving complexation with micronutrients, destruction of vitamines or by acting as inhibitors of digestive enzymes. Lysinoalanine is known to form stable metal complexes, similar complexes with Maillard reaction product may be the cause of observed urinary excretion of zinc, copper and iron. Vitamines with reactive functionality i.e. NH_2 in thiamine or CHO in pyridoxal can be destroyed in thermally induced amine/carbonyl reactions. The inhibitory effect of Maillard reaction products on digestive enzymes such as trypsin, carboxypeptidases A and B, aminopeptidase N and intestinal dissaccharidases has been reported.

3.3.3. Toxicological aspects

A high correlation between color and mutagenicity was found in the crust of baked meat loaves by Holtz et al (1985). Concern over mutagenicity in cooked foods originated when mutagenic substances were observed in pyrolyzed proteins and amino acids. Cooked muscle foods seem to contain more mutagens than other foods. A particularly potent class of mutagens, imidazoquinolins (IQs) were first observed in broiled fish and were isolated from heated beef extracts and hamburger. IQ compounds are moderately carcinogenic *in vivo*, but their relevance to human carcinogenesis is unknown. A mechanism for IQ formation was proposed involving creatinine and Maillard reaction products (Jägerstad et

al., 1983; Milic et al., 1993). Interestingly, it has been reported that the formation of IQ compounds in model systems can also be suppressed by the addition of pre-formed Maillard reaction products (Kim et al., 1986; Yen and Chau, 1993).

3.4. Synthesis of Amadori and Heyns rearrangement products

3.4.1. Commercial applications of Amadori and Heyns compounds

Amadori and Heyns products (ARPs and HRPs) have unique industrial implications and are commercially important sugar derivatives that can be used in a wide range of applications such as cosmetics and pharmaceuticals and as anticancer and antileprosy drugs. In food industry, ARPs are used as flavoring and browning agents. With the advent of new food processes (extrusion cooking, microwave heating, infrared heating etc.), a new way of flavor delivery system known as 'dynamic flavors" has been developed. Using mixtures rich in specific ARPs as flavor precursors, these "dynamic flavor systems" are able to generate desirable brown color and mimic the final flavor profile of cooked foods. They enhance intrinsic flavor of the food products, or compensate for the flavor losses resulting from the process in order to obtain products of the desired quality. In addition, ARPs are reported to affect the adhesion and aggregation properties of cancer cells. There is an increasing interest for the synthesis of variety of amino-sugar derivatives for biological evaluation and chemotherapeutic applications.

However, the commercial availability of ARPs is still limited due to lack of efficient methods for their synthesis in large scale. The problems associated with the current techniques of preparation of ARPs are the low yields and the difficulty of separation from the unreacted starting materials and from the decomposition products formed during the reaction. Development of simple and efficient synthesis methods for ARPs is an area of much interest to the food, pharmaceutical, biomedical-related industries.

3.4.2. Classical synthesis

Synthetic approaches for ARPs or HRPs have been reviewed by Yaylayan and Huyghues-Despointes (1994). Generally, ARPs are prepared by refluxing a solution of glucose in the presence of amino acid in anhydrous methanol for several hours. Subsequently, the product

is isolated and purified by ion-exchange column chromatography, using cellulose, silica gel, or sephadex as stationary phases. The main limitation of this classical synthesis method is poor product yields due to air oxidation and side-reactions associated with glucose and ARPs.

In 'fusion' method, dried glucose and aromatic amines are reacted together at 70-80°C for 2 hours. Subsequently, the obtained product is crystalized from hot ethanol, and further purified by recrystalization. The solvent-free 'fusion' method was used by Amadori (1931) and improvement of the yield by adding acid catalyst was reported later (Inoue and Onodera, 1948). Similar 'syrup' synthesis has been conducted by Anet and Reynolds (1957) using sodium bisulfite. Mixtures of aldoses and amino acids were heated, and concentrated into syrup with 10-20% water. With or without sodium bisulfite, carboxylic group of amino acids provided internal acid catalyst for Amadori rearrangement. The 'fusion' and 'syrup' methods are suitable for aromatic amines or amino acids. However, the most used synthesis for ARPs is 'reflux' method followed by purification through ionic exchanger. The elution with concentrated acid improves the isolation of pure ARPs but only in 20-30% yield or lower.

It was found that protected sugars in the presence of primary amines readily formed the corresponding glycosylamine in the absence of acid catalyst and do not undergo rearrangement into ARPs. With oxalic acid as catalyst, the protected glycosylamines easily rearrange into ARPs. The protected C-6 group of glucose can be cleaved by oxalic acid to give the salt form of ARPs. Another modified approach, using protected glucose is the activation of anomeric carbon. Anomeric carbon is the most reactive atom in reducing sugars, however it is less susceptible to nucleophilic attack than carbonyl carbon. The activation of the anomeric carbon can be achieved by two ways: either converting the hydroxyl group into a better leaving group or replacing it by a good leaving group. The affinity of anomeric carbon atoms towards nucleophiles depends on the nature of the leaving group. The nucleophillic substitution of the activated anomeric group of glucose by amino acids is usually proceed in the presence of catalyst and with protection of other hydroxyl group. After the substitution, deprotection will generate the ARPs. In case of

fructose, Walton et al (1987) successfully used a protected fructose intermediate to synthesize ARPs.

3.4.3. Microwave-assisted synthesis (MAS) of Maillard reaction products (MRPs)

There are publications on the preparation of Maillard reaction products (MRPs) by microwave irradiation. Steinke et al. (1989) observed the generation of Strecker aldehydes from a sample of amino acid and diacetyl in aqueous solution with microwave heating for 4 minutes. Compared with the conventional heating at the same temperature with longer heating time, significantly higher concentrations of aldehydes were measured in the microwaved sample. Recently, a focused microwave system under atmospheric pressure condition was used to synthesize and extract selected MRPs (Yaylayan et al., 1997). Phenylalanine or glycine / glucose mixtures were sequentially treated with microwave irradiation in an aqueous medium to initiate the synthesis step followed by irradiation in an microwave transparent solvent to perform selective extraction of the products formed. The ability of focused microwave system under atmospheric pressure conditions to selectively synthesize and quantitatively separate MRPs was investigated using a two-stage microwave assisted process (MAP). The first stage - MAS (Microwave assisted synthesis) could be carried out in a microwave active solvent such as water, ethanol, or water-ethanol mixtures depending on the energy requirements of the reaction. The second stage - MASE (Microwave assisted selective extraction) could be carried out in an microwave transparent solvent such as petroleum ether, hexane or mixtures of hexane and acetone to selectively extract minimum number of products formed in the first stage. After evaporation of the solvent, the residue could be further purified, if necessary, by chromatography and characterized by spectroscopic techniques. By controlling the irradiation time and temperature during MAS stage, certain products could be made to be formed preferentially thus producing mixtures rich in specific products. Further selectivity could be obtained during MASE stage whereby controlling the solvent polarity and extraction time, specific products formed in the MAS stage could be extracted sequentially, by varying solvent composition.

3.5. Polymer-supported synthesis

Since solid phase peptide synthesis method was introduced by R. B. Merrifield in 1963, insoluble polymer supports have been incorporated into numerous synthetic methodologies to facilitate product purification (Gallop et al., 1994; Gold, et al., 1995). Although highly successful, solid-phase synthesis still exhibits several shortcomings due to the nature of heterogeneous reaction conditions. Nonlinear kinetic behavior, unequal distribution and/or access to the chemical reaction, solvation problems and pure synthetic problems associated with solid-phase synthesis have led several groups to pursue alternative methodologies to restore homogeneous reaction conditions. By replacing insoluble cross-linked resins with soluble polymer supports, the familiar reaction conditions of classical organic chemistry are reinstated, and yet product purification is still facilitated through application of macromolecular properties. This methodology, termed 'liquid-phase' synthesis, in essence avoids the difficulties of solid-phase synthesis while preserving its positive aspects.

3.5.1. Properties of Poly ethylene glycol (PEG) polymer

A number of polymeric reagents have been used for the simplification of organic synthesis. The liquid phase method of peptide synthesis on polyetylene glycol (PEG) was first introduced in 1971 and the PEG method has emerged as one of the most effective supports for the synthesis of diverse structures of organic molecules as well as for the construction of combinatorial libraries (Han et al., 1995; Pillai and Mutter, 1980). Employed as a protecting group, this linear homopolymer exhibits solubility in a wide range of organic solvents and water. PEG is insoluble in hexane, diethyl ether and *tert*-butyl methyl ether, and these solvents have been used to induce PEG precipitation. Careful precipitation conditions or cooling of polymer solutions in ethanol or methanol yields crystalline PEG due to the helical structure of the polymer that produces a strong propensity to crystallize. Thus, as long as the polymer backbone remains unaltered during liquid-phase synthesis, then purification by crystallization can be utilized at each reaction step. Furthermore, the solubilizing power of PEG not only allows homogeneous reactions under numerous reaction conditions, but these solubility properties permit individual reaction steps to be monitored without requiring cleavage of product from the polymer support. The characterization of PEG-bound organic moieties is often straightforward as the polymer

does not interfere with spectroscopic or chemical methods of analysis. In addition, MeO-PEG (PEGME: polyetylene glycol monomethyl ether) contains a single methoxy group (δ = 3.38 ppm) and ethyl protons of PEG backbone (δ= 3.64 ppm) that provide internal standards for easy monitoring of reactions by ^{1}H NMR spectroscopy (Han et al., 1995).

Depending on polymerization conditions, PEG terminal may consist of a hydroxyl group or may be selectively functionalized. Commercially available PEG is produced through anionic polymerization of ethylene oxide to yield a polyether structure possessing either hydroxyl groups at both ends or a methoxy group at one end and a hydroxyl group at the other (MeO-PEG). The polymer MeO-PEG is considered monofunctional, as typically the methoxy group of MeO-PEG remains unchanged throughout chemical manipulations. For identical chain lengths, the loading capacity of PEG is twice that of MeO-PEG as two hydroxyl groups serve as anchoring sites on PEG. Recently, varieties of PEG derivatives have been developed and are commercially available (Shearwater Polymers, Inc., 1997).

3.5.2. Applications of PEG in biological studies

A key property of PEG is that attachment to other molecules and surfaces provides a bio-compatible, protective coating. This protective coating slows rejection of materials in biological systems (such as the human body), greatly reduces protein, cell and bacterial adsorption, and reduces the rate of kidney clearance (because of larger size). PEG also is nontoxic and has been approved by the FDA for topical and internal use in humans. PEG is soluble in water and many organic solvents, and it forms aqueous two-phase systems when paired with certain other polymers (such as dextran). It is insoluble in ethyl ether and hydrocarbons such as hexane. The water solubility, lack of toxicity, high flexibility and well-defined chemistry of difunctional PEG makes it ideally suited for many crosslinking or tethering applications. Seven technologies that have resulted from use of these properties are: (1) PEG-proteins for pharmaceutical use; (2) PEG-surfaces for electrically-controlled, nonfouling materials; (3) PEG-liposomes for drug delivery; (4) molecule-molecule or molecule-surface coupling for drug and materials applications; (5) PEG-molecules for biological purifications; (6) biopolymer synthesis on PEG supports; and (7) PEG attachment for control of solubility (e.g., enzymes into organic solvents or water

solubilization of enzyme substrates, dyes, flavors and chemotherapeutic agents) (Harris, 1992).

3.5.2.1. PEG-proteins for pharmaceutical use

It has been demonstrated that proteins with PEG attached remain active and have a greatly diminished or negligible immune response. The result is that these PEG-proteins have greatly increased serum lifetimes. Examples include PEG-SOD, PEG-asparaginase, PEG-IL-2 and PEG-hemoglobin. In addition, PEG attachment makes proteins much larger and thus reduces their rate of clearance through the kidney. PEG has also been attached to many small molecules (such as vitamin E, cholesterol, fluoro-uracil, etc.). The goal here is to reduce rate of kidney clearance and impart water solubility.

3.5.2.2. PEG-surfaces

In addition to the molecular modifications, PEG can also be attached to surfaces to form protective, bio-compatible coatings. A variety of applications result, including PEG-coatings for arterial replacements, diagnostic apparatus and blood contacting devices. Similarly, capillary zone electrophoresis has emerged as an important new analytical technique in biochemistry, and PEG coatings on the capillaries prevent protein adsorption and provide critical control of electroosmosis.

3.5.2.3. PEG-liposomes

There has been intense interest in use of liposomes for controlled-release and selective-delivery of drugs. A problem with this application is that liposomes, especially larger ones, are quickly attacked and cleared from the body. Recent research has shown that incorporation of PEG into the outer coating of liposomes can greatly increase serum lifetime, thus solving a critical problem blocking application of this promising drug delivery technique.

3.5.2.4. Molecule-molecule and molecule-surface coupling

The hydrophilic, biocompatible nature of PEGs and their mild, well-defined chemistry makes them ideal for coupling or tethering molecules to molecules or molecules to surfaces. This technology is critical for the next generation of drugs and biomaterials.

Research has shown that use of PEG as a coupler to bind molecules to other molecules and surfaces provides highly active materials.

3.5.2.5. Biological Purification

The genetic engineering revolution has led to methods for production of a variety of physiologically active proteins. There is, however, a critical need in this industry for improved methods for isolation of the proteins produced. An approach to this problem that has recently received much interest is purification by partitioning in aqueous two-phase systems (analogous to oil and water) made by solution of PEG, other polymers and salts in water. In this approach, a PEG-ligand is made (such as a PEG-antibody), which binds specifically with the desired protein and pulls the protein into the PEG-rich phase.

3.5.2.6. Biopolymer synthesis

The three bio-oligomers (peptides, oligonucleotides, and oligosaccharides) can all be grown on PEG as a soluble carrier. The PEG-oligomer is precipitated after each step to isolate the product, which can then be cleaved or taken to the next addition step. Advantages of this method are that fewer errors result, chemistry is faster, and large quantities of materials can be handled. A variation on this theme is to build the bio-oligomer on a PEG chain that is bound to a solid polystyrene particle. This approach apparently provides advantages of both solid-phase and liquid-phase synthesis.

3.5.2.7. Solubilization of insoluble molecules

PEG is soluble both in water and in many organic solvents. This property has been utilized to solubilize other molecules by PEG attachment. An interesting biotechnical application is solubilization of enzymes in organic solvents such as chlorinated hydrocarbons. Additionally, water-insoluble materials may become water-soluble after PEG attachment. Examples here include dyes, flavors, substrates for enzymes, cofactors, pharmaceuticals, etc.

Chapter 4

MATERIALS AND METHODS

4.1. Materials

4.1.1. Reagents and chemicals

Poly (ethylene glycol) methyl ether (MW~2000), acetol (tech, 90%), L-ribulose hydrate (95%), DOWEX®50WX2-100 (hydrogen, strongly acidic cation) ionic exchange resin, ammonium hydroxide (28 % NH_3 , ACS certified reagent) were purchased from Aldrich Chemical Company (Milwaukee, WI). N-t-BOC-β-alanine was purchased from Sigma Chemical Co. (St-Louis, MO). Dichloromethane (ACS certified reagent), methanol (ACS certified reagent), ethyl ether (ACS certified reagent), trifluoroacetic acid (Reagent grade) Sodium methioxide were purchased from Fisher Scientific (Fair Lawn, NJ). DOWEX®1X2-100 (strongly basic anion) ionic exchange resin was purchased from Acros Organic (NJ, USA) Hydrochloric acid (33.5-38%, analyzed reagent) was purchased from J.T.Baker (Phillipsburg NJ). 1,3-Dicyclohexylcarbodiimide was purchased from ACP Chemical Inc. (Montreal, QC).

4.1.2. Instrumentation

Melting points were determined on a Fisher Scientific melting point apparatus (Fair Lawn, NJ) and are uncorrected.

4.1.2.1. Microwave

The irradiation was carried out with a domestic microwave and a focused microwave system SYNTHEWAVE™ 402. Domestic microwave with an energy output of 700W full power was purchased from a local retail store. A SYNTHEWAVE™ 402 (focused microwave system at atomospheric pressure) was obtained from Prolabo (Fontenay-Sous-Bois Cedex, France), operating at an emission frequency of 2450 MHz, and a 300 W full power.

4.1.2.2. FTIR

Infrared spectra were recorded on CaF_2 IR cell with 25μ Teflon spacer, on a Nicolet 8210 Fourier-transform spectrometer equipped with a deuterated triglycine sulphate (DTGS) detector. Processing of the FTIR data was performed using the GRAMS / 386 version 3.01 (Galactic Industries, 1994).

4.1.2.3. Pyrolysis/GC/MS

A Hewlett-Packard GC/mass selective detector (5890 GC/5971B MSD) interfaced to a CDS Pyroprobe 2000 unit, was used for the Pyrolysis/GC/MS analysis. 1-5 mg solid samples were introduced inside the coil probe with a Total heating time of 20 seconds. The GC column flow rate was 0.8 ml/min. for a split ration of 92:1 and a septum purge of 3 ml/min. The Pyroprobe interface was set at the temperature at which the sample was to be pyrolyzed and the Pyroprobe was set at the desired temperature at a rate of 50°C /ms. Capillary direct MS interface temperature was 180°C; ion source temperature was 280°C. The ionization voltage was 70 eV and the electron multiplier was 1494 volts. The mass range analyzed was 35-350 amu. The column was a fused silica DB-5 column (30 m length x 0.25 i.d. x 0.25 μm film thickness; Supelco, Inc.). The column initial temperature was -5°C for 3 min. and was increased to 50 °C at a rate of 30°C/min.; immediately the temperature was further increased to 270 °C at a rate of 8°C/min. and kept at 270°C for five minutes.

4.1.2.4. NMR

^{1}H-NMR spectra were recorded in D_2O or $CDCl_3$ at 300 MHz using DSS as internal reference on a Varian Anova Instrument.

4.1.3. Experimental condition and procedures

4.1.3.1. Synthesis of t-BOC-β-alanine-PEGME (26)

PEGME (24, Polyethylene glycol monomethyl ether, average MW 2,000; 20 g, 10 mmol) and t-BOC β-alanine (2.02 g, 10 mmol) were dissolved in 100 mL of dichloromethane. Dicyclohexylcarbodiimide (DCC; 2.3 g, 11 mmol) was then added to the above solution. The mixture was stirred at room temperature for 24 h. The precipitated dicyclohexylurea (DCU) was removed by filtration and additional t-BOC-β-alanine (0.4 g, 2 mmol) and DCC

(0.43 g, 2 mmol) were added to the filtrate. The stirring was continued for another 4-5 h to complete the reaction. The precipitated DCU was again filtered and the filtrate was concentrated under vacuum to 20 mL. Diethyl ether was then added slowly to the solution and cooled to facilitate the precipitation of the polymer. The precipitate was filtered, washed with ether, collected, and again dissolved in CH_2CL_2. The precipitation and filtration was repeated. The collected precipitate was dried on a filter paper to a constant mass. Final white solid gave 18.08 g of Boc-β-alanine-PEGME (26). Calculated product recovery was 89 %. Melting point 35°C, FTIR (CH_2CL_2), 1733 cm^{-1}(ester COOPEG), 1710 cm^{-1}(t-BOC), ~2900 cm^{-1}(methylene, broad). ^{1}H-nmr (D_2O) δ: (ppm) 1.45 (9H, s, t-BOC); 3.95 (2H, m, CH_2CO), 3.35 (3H, s, CH_3O); 3.60-3.70 (180 H, s, CH_2, PEG); 3.75 (2H, m, CH_2N); 4.28 (2H, m, CH_2OCO); 2.6 (1H, m, NH).

4.1.3.2. Deprotection and neutralization of t-BOC-β-alanine-PEGME

Boc-β-alanine-PEGME (26, 3 g, 1 mmol) was placed in a glass vial (i.d. 2.3 cm x length 8.5 cm) and irradiated in a domestic microwave oven (700 W) for 35 sec to melt the solid completely. Then, trifluoroacetic acid (TFA, 140 μL) was added and the solution was vortexed and immediately irradiated in a domestic microwave oven (700 W full power) for 20 seconds. The viscous yellow polymer solution was cooled and dissolved in methanol and the pH was adjusted to 5 by addition of triethyl amine (TEA). The same procedure was repeated to deprotect the rest of the sample. The product was precipitated from the pooled and cooled solution by addition of diethyl ether. The solid precipitate was filtered and washed by ether several times. Finally, the collected precipitate was dried on a filter paper to a constant mass of 14.5 g (~ 98 %) of deprotected product (27). Alternatively, the pH of the solution can be adjusted by stirring the solution with a pre-washed Dowex 1X2-100 strongly basic resin in a beaker. Adding fresh resin until the pH is 5. The product β-alanine-PEGME (27) was precipitated by addition of ether and cooling as mentioned above. FTIR (CH_2CL_2), 1733 cm^{-1} (ester COOR), ~2900 cm^{-1} (alkyl, broad). ^{1}H-nmr (D_2O) δ: (ppm) 3.95 (2H, m, CH_2CO); 3.35 (3H, s, CH_3O); 3.60-3.70 (180 H, s, CH_2, PEG); 3.75 (2H, m, CH_2N); 4.3-4.4 (2H, m, CH_2OCO); 2.9 (2H, m, NH_2).

4.1.3.3. Attachment of sugar analogs to β-alanine-PEGME

4.1.3.3.1. Synthesis of acetol-β-alanine-PEGME (28)

Slightly acidic β-alanine-PEGME (7 g, 3.4 mmol) obtained from the previous step (pH adjusted to 5) was dissolved in 20 mL of methanol and 2-fold exess of acetol (0.5 g, 6.7 mmol). The reaction mixture was stirred for 48 hours continuously at room temperature. At the completion of the coupling reaction, the product was precipitated, filtered and washed several times with diethyl ether. Collected precipitate was dried on a filter paper to a constant mass of 5.4 g (yield 75 %) of the title compound. Melting point 35°C. FTIR (CH_2CL_2), 1733 cm^{-1} (ester COOR); 1645 cm^{-1} (enediol). ^{1}H-nmr (D_2O) δ: 3.95 (2H, m, CH_2CO); 3.35 (3H, s, CH_3O); 3.60-3.70 (180 H, s, CH_2, PEG); 3.75 (2H, m, CH_2N); 4.3-4.4 (2H, m, CH_2OCO); 2.85 (1H, m, NH); 1.4 (3H, d, CH_3 acetol); 3.3 (1H, NCH, acetol); 3.45 (1H, m, $HC(OD)_2$).

4.1.3.3.2. Synthesis of ribulose-β-alanine-PEGME

The coupling reaction was accomplished in the same way as acetol attachment. β-alanine-PEGME (7 g, 3.4 mmol) was dissolved in 20 mL methanol and 2-fold excess of ribulose (1 g, 6.8 mmol). The reaction mixture was stirred for 48 hours continuously at room temperature. At the completion of the coupling reaction, the product was precipitated, and filtered, followed by washing several times with diethyl ether. The collected precipitate was dried on a filter paper to a constant mass of 6.4 g (yield 86 %) of the title compound. Melting point 35°C. FTIR (CH_2Cl_2), 1733 cm^{-1} (ester COOR). ^{1}H-nmr (D_2O) δ: 3.95 (2H, m, CH_2CO); 3.35 (3H, s, CH_3O); 3.60-3.70 (180 H, s, CH_2, PEG); 3.75 (2H, m, CH_2N); 4.3-4.4 (2H, m, CH_2OCO); 2.85 (1H, m, NH); 3.30 (1H, NCH); 3.45 (1H, m, $HC(OD)_2$); 1.4-1.5 (1H, m CH ribulose); 1.2 (2H, m, CH_2 ribulose); 0.9 (1H, m, CH ribulose).

4.1.3.4. Cleavage reaction and purification

4.1.3.4.1. Cleavage of Heyns product of β-alanine and acetol from PEGME

β-alanine-acetol-PEGME (5.4 g, 2.5 mmol) was stirred overnight in 50 mL of methanol and 2-fold excess of sodium methoxide (0.27 g, 5 mmol). At the completion of the reaction, one drop of water was added to convert excess methoxide to methanol. The solution was

concentrated and the cleaved polymer was precipitated by the addition of diethyl ether to the cooled solution. The precipitate was filtered and washed several times with diethyl ether. The filtrate from all washings were carefully collected and evaporated at room temperature. The precipitation procedure was repeated one more time to remove all the cleaved polymer. After the precipitate was removed the combined filtrates were evaporated to give slightly yellowish viscous oil. Approximate product yield was 0.20 g (64 %). FTIR (neat), 1736 cm^{-1}(C=O, ester), 1690 cm^{-1} (C=O, aldehyde), 1530 cm^{-1} (N-H, broad), 1445, 1380 cm^{-1}(-CH_3, weak), 1190 cm^{-1}(C-O-C, strong) ^{1}H-nmr ($CDCl_3$) δ: 3.75 (2H, m, CH_2CO); 3.4 (3H, s, CH_3O ester); 2.4 (2H, m, CH_2N); 1.4 (3H, d, CH_3 acetol); 3.3 (1H, NCH, acetol); 3.7 (3H, m, HC(OH)(OCH_3)); 3.8 (0.8 H, m, HC(OH)(OCH_3)); 8.2 (0.2 H, s, CHO). EIMS m/z (relative intensity) 41 (25), 43 (15), 57 (100), 59 (33), 70 (11), 88 (18), 98 (32), 102 (12), 104 (19), 116 (45), 130 (33), 146 (11), 147 (54).

4.1.3.4.2. β-alanine-ribulose-PEGME

The same procedure as above was repeated using β-alanine-ribulose-PEGME (6.4 g, 3.4 mmol) and 0.36 g (6.8 mmol) of sodium methoxide in 50 mL of methanol. After all precipitate was removed, finally all filtrate were collected, evaporated to gave slightly brown viscous solution. Approximate product yield was 0.368 g (63 %) of ribulose-β-alanine. FTIR (neat), 1736 cm^{-1}(C=O, strong), 1690 cm^{-1}(C=C, strong), 1530 cm^{-1}(N-H, broad), 1445, 1380 cm^{-1}(-CH_3, weak), 1190 cm^{-1}(C-O-C, strong).

Chapter 5

RESULTS AND DISCUSSION

5.1. Introduction

Polymer-supported synthesis has been demonstrated to be extremely valuable technique for routine preparation of peptides. In addition, it has been also applied for the synthesis of small organic molecules in combinatorial chemistry, owing to the ease of work-up, purification, and to the ease of adaptation into repetitive coupling schemes. Although solid-phase synthesis exhibits several shortcomings, due to the nature of heterogeneous reaction conditions, the liquid-phase method using soluble polymers, such as poly (ethylene glycol) methyl ether (PEGME), combines the strategic features of both liquid and solid phases. In essence it avoids the difficulties of solid-phase synthesis while preserving its positive aspects. In this thesis, a general procedure using polymer supports, was developed and applied to the synthesis of Heyns rearrangement products (HRPs). β-Alanine-acetol was prepared by liquid-phase polymer supported synthesis using PEGME (average molecular weight of 2000 amu). The experimental strategy, including selection of reagents and substrates, reaction conditions, and product recovery were followed by different spectroscopic means.

5.2. Choice of Polymer, Amino acids and Sugars

The design of the synthesis strategy included the selection of suitable polymeric supports, solvents, reactants and reagents. These factors are the most important to optimize the yield and minimize unwanted side reactions.

5.2.1. Choice of Poly ethylene glycol (PEG) as the polymer support

Polyethylene glycols are commonly used in liquid phase synthesis and commercially available in different molecular weights and in differently substituted functional groups. Difunctional PEGs contain hydroxyl groups at both ends of the molecule and

monofunctional methoxy PEGs (PEGME) contains one hydroxyl group and one methoxy terminal group. PEGME with average molecular weight of 5000 or 6000 are commonly used for polymer-supported peptide syntheses. However; to increase loading capacity and to avoid oxidative chain cleavage reactions and the formation of crosslinked side products, monofunctional methoxy derivative with average molecular weight of 2000 was selected as the polymer of choice. In our experience, difunctional PEG with average molecular weight of 2000 was found to be unstable and more susceptible to degradation, resulting in cleavage of the PEG backbone chain during the chemical manipulations.

5.2.2. Choice of Amino acids and sugars

Factors involved in the choice of suitable amino acids and sugars are their reactivity and solubility in the organic solvents used during the synthesis steps.

5.2.2.1. Amino acid

Several types of N-protected amino acids are commercially available. t-BOC protected amino acids can be easily deprotected by treatment with acids such as trifluoroacetic acid (TFA). In addition, they are soluble in non-polar organic solvents such as dichloromethane, and are economically feasible for the syntheses. Among commercially available t-BOC-protected amino acids, t-BOC-β-alanine was chosen to develop the synthetic strategy. It has no reactive side chains and is sterically less hindered compared to α-alanine. Those structural characteristics makes β-alanine a suitable amino acid to avoid side reactions that may complicate the development of synthesis conditions.

5.2.2.2. Sugars

Sugars were mainly selected based on their reactivity and solubility in methanol. It is known that most hexoses have poor solubility in methanol and low reactivity due to the formation of cyclic acetals. To overcome these problems, reactive sugars analogs such as acetol and soluble sugars such as ribulose were chosen for the development of the synthesis procedure. Especially, acetol, which has a three-carbon backbone, can not cyclize, and therefore is much more reactive than hexoses.

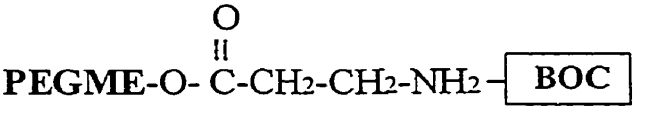
5.2.3. Choice of Solvents and reagents

The solvents and reagents used for synthesis must not react with the polymer and should not interfere with the coupling reaction. Reaction solvents must be capable to precipitate the polymer by the addition of diethyl ether.

5.3. Synthetic strategies

Two polymer-supported strategies (see Schemes 13 and 14) were explored for the synthesis of Heyns products. The two approaches differed from each other in the order of attachment of the amino acid. In one approach (Scheme 13; strategy A) t-BOC amino acid was first attached through an ester linkage to the polymer and in the second approach (Scheme 14; strategy B) sugar was attached to the polymer with a spacer arm. Strategy A successfully produced the target compound, whereas strategy B was not adopted due to the reasons discussed in Chapter 7.

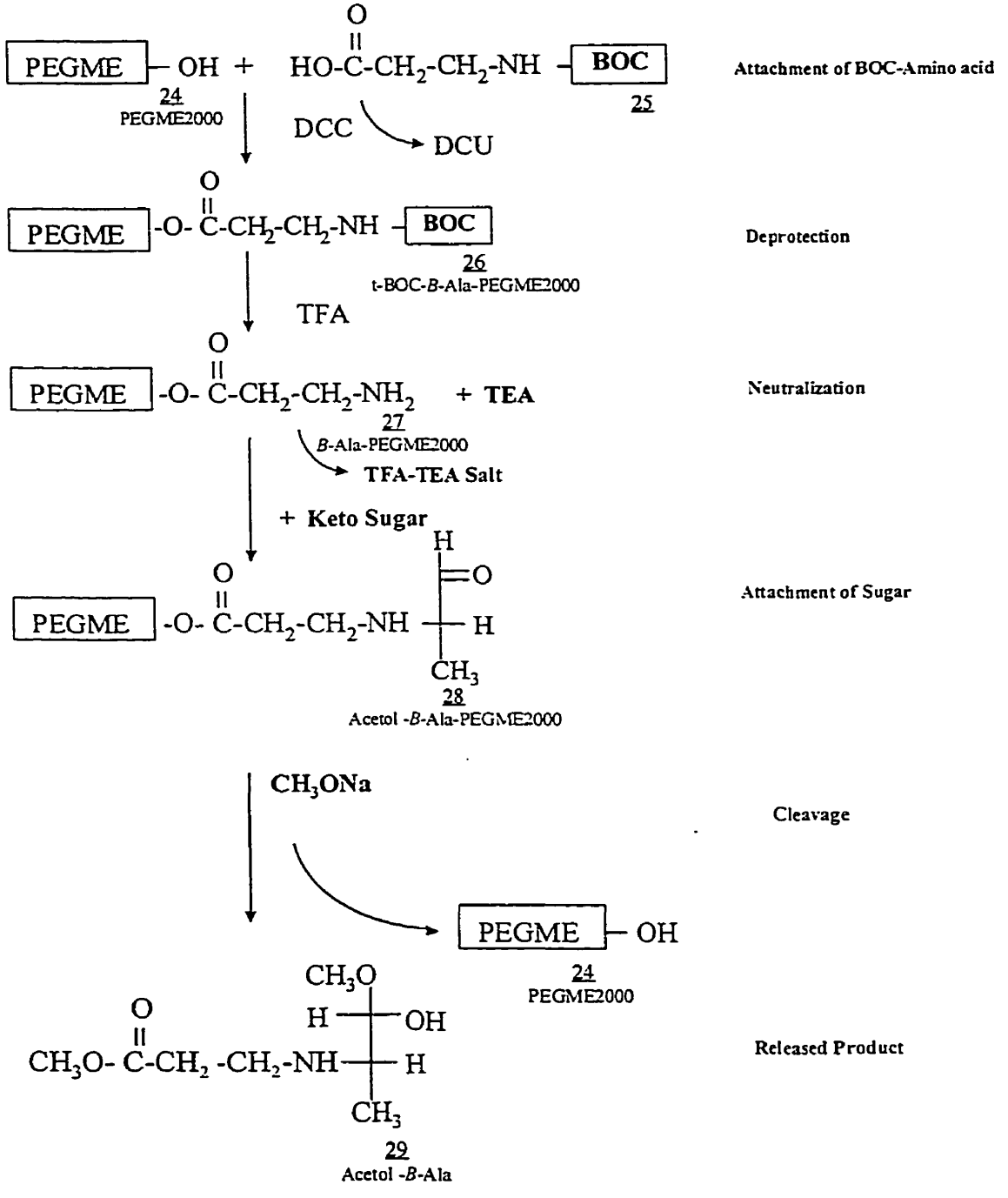
5.3.1. Coupling reaction of t-BOC protected amino acid with PEGME



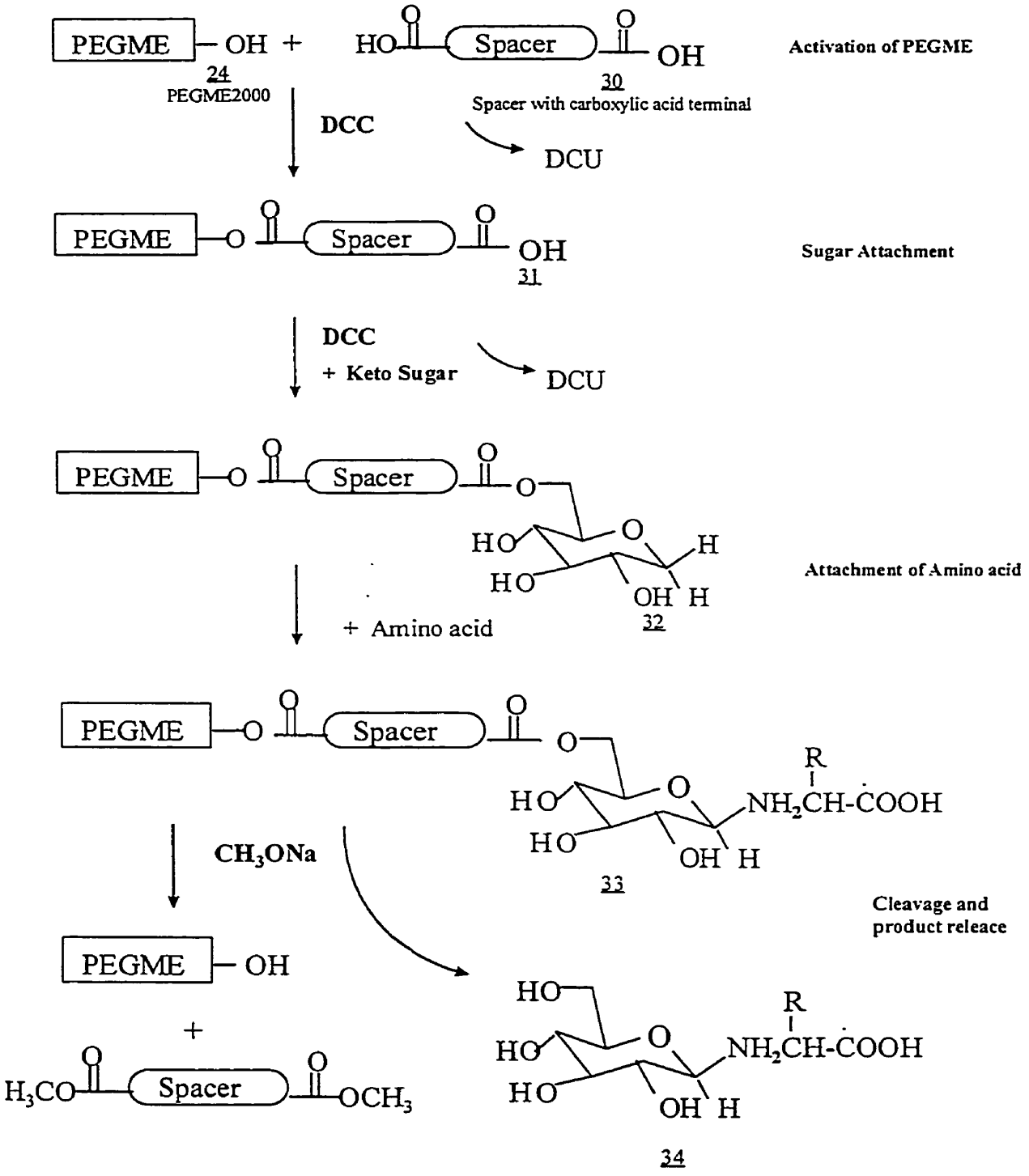
t-BOC protected β-alanine ester of PEGME (26)

As shown in Scheme 13 (strategy A), the first step of the synthesis involves the preparation of amino acid ester of PEGME. The formation of active ester linkage was accomplished by dicyclohexylcarbodiimide (DCC) method, the most often applied condensing agent in peptide synthesis (Rich and Singh, 1979) (see Scheme 15).

Scheme 13: Strategy A



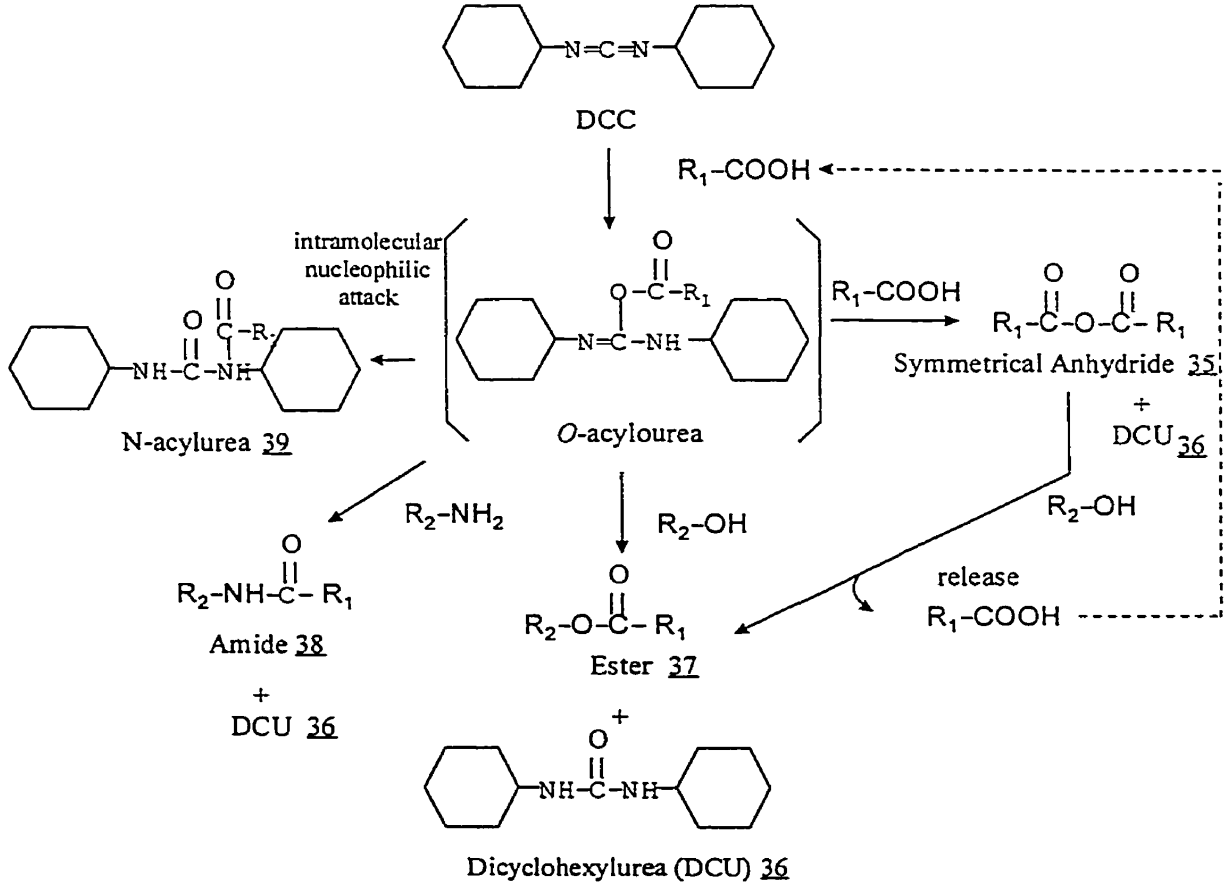
Scheme 14: Strategy B



A typical DCC coupling reaction, known as "pre-mix" procedure, uses 2:1 ratio of t-BOC-amino acid and DCC. The t-BOC-amino acid / DCC mixture was stirred at 0°C for 30 min before addition of coupling substrate since the preparation of highly reactive symmetrical anhydrides (35) gives higher yields and purer product (Rich and Singh, 1979). Under our experimental conditions, however, the coupling accomplished by use of 1:1 ratio produced higher yields than the use of a 2:1 ratio.

5.3.1.1. Possible side reactions associated with DCC method

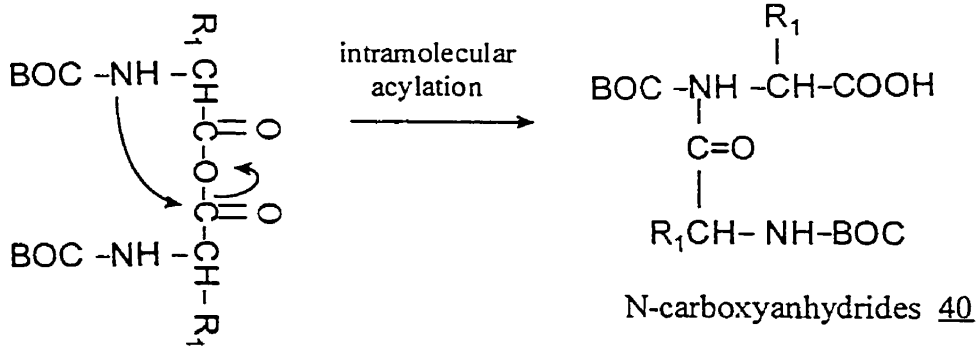
For the preparation of ester 37, the reactive nitrogen group of the amino acid should be protected in order to avoid the formation of amide bonds (38) by reacting with the carboxylic group of another amino acid. Most of the side reactions encountered during the preparation of active esters of protected amino acids are associated with functional groups



Scheme 15. Reactions of DCC

in the side chain. In the absence of amines or phenols, the N-protected amino acids in general will react rapidly ($t_{1/2}$ ~1-5 min) with DCC in non polar, low dielectric constant solvents (CCl_4, CH_2Cl_2, C_6H_6, etc.) to form symmetrical anhydrides in good yields. However, it has been reported that excess DCC (which has about 1/5 the basicity of pyridine) may catalyze decomposition of symmetrical anhydride *via* intramolecular acylation and form N-acylurea (39). Moreover, reaction with side chain groups of certain amino acids (imidazole, NH_2, β-,γ- COOH, ε-NH_2, SH_2, etc.) may also lead to insertion by-products. In addition, the coupling efficiency may be suppressed by steric hindrance whenever there is a stretch of vicinal β-branched amino acids or amino acids with bulky protecting groups close to the α- carbon atoms (Rich and Singh, 1979).

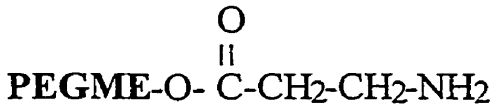
Additionally, DCC has been reported to react with acid labile N-protected amino acids (t-BOC, etc) in CH_2Cl_2 to form N-carboxyanhydrides (40) (Scheme 16); however, the extent of this undesired side reaction appears to be very small.



Scheme 16: Decomposition of symmetric anhydrides by intramolecular acylation

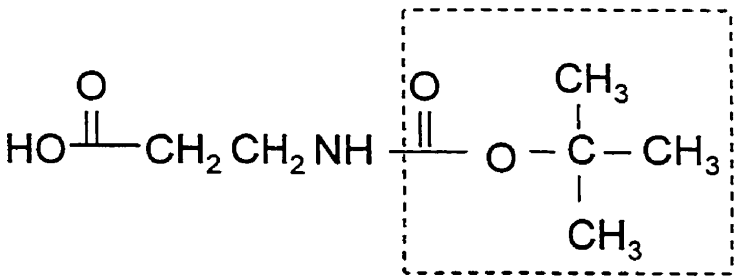
5.3.2. Deprotection and neutralization of t-BOC

Different deprotection techniques of t-BOC protecting groups are available in the literature. Trifluoroacetic acid (TFA), which is a relatively weak acid when compared with HF and trifluoromethanesulfonic acid (TFMSA) is often used in synthesis strategies and is therefore considered to be a desirable alternative to HF or TFMSA (Barany and Merrifield, 1980).



Deprotected β-alanine esters of PEGME (27)

Deprotection reaction was first initiated by stirring the polymer-bound t-BOC-β-alanine at room temperature with a suspension of TFA in CH_2CL_2, for 24-48 h. This initial cleavage conditions were not satisfactory due to the slow rate and the low yields. The presence of solvent might have interfered with the acid hydrolysis due to solvent coating of the polymer surface and thus restricting access of reagent to the site of t-BOC group. In addition, the longer reaction times may have also resulted in the hydrolysis of the ester linkage. The problem was solved when the polymer-bound t-BOC-β-alanine was irradiated by microwave for a short period of time, in the presence of TFA without addition of any solvent.



tert-butoxycarbonyl (t-BOC)

Scheme 17. t-BOC protected-β-alanine (25)

5.3.2.1. Possible side reactions of TFA cleavage

It has been reported (Lundt et al., 1978) that during TFA catalyzed cleavage of t-BOC groups, t-butyl cations and t-butyl trifluoroacetate are also produced as side products. The reactive cations can bring about undesirable t-butylation of some amino acids such as tryptophan, tyrosine and methionine. No such t-butylation however, was observed using β-alanine as a coupling substrate. In our experience, it was found that minimum 2-fold excess of TFA is required to achieve complete deprotection. However, it was also found that excess TFA can induce the formation of trifluoroacetate salts with the amino groups of the amino acids. Addition of triethyl amine until pH of the solution drops to a value between 5 and 6, can prevent this reaction by forming triethyl ammonium trifluoro acetate (TFA-TEA). The salt produced (TFA-TEA) remains soluble in methanol and the product can be separated by precipitation with diethyl ether. Repeating the precipitation and washing processes few times removes most of the salt. The salt shows a strong absorption peak in the IR spectrum centered at 1680 cm^{-1}. To eliminate the salt formation, basic ionic exchange resin (DOWEX®1X2-100, strongly basic anion) can be applied as an alternative method for the removal of trifluoroacetate ion.

5.3.3. Attachment of sugar derivatives

After deprotection and neutralization, free amino terminal of PEGME-bound amino acid can readily undergo addition reaction with the carbonyl groups of reducing sugars followed by Amadori or Heyns rearrangements, depending on the nature of the reducing sugar.

5.3.3.1. Acetol (1-hydroxy-2-propanone)

Acetol is a reactive sugar analogue. In the Maillard reaction, it has been reported that the reactivity of acetol with β-alanine, especially the rate of browning, is much faster (approximately 2000 times) than that of glucose (Hayashi and Namiki, 1986). After adjusting the pH of the solution of PEGME-β-alanine to slightly acidic conditions (pH ~5), the coupling was accomplished by continuous stirring of the reaction mixture (acetol and PEGME-β-alanine in 2:1 ratio dissolved in methanol) at room temperature for 48 hours. Acetol was readily reacted with the amino acid and subsequently formed corresponding Heyns rearrangement product. At the completion of the coupling reaction, the polymer-bound β-alanine-acetol product was precipitated, washed with diethyl ether several times to

remove excess liquid acetol, and the precipitate was dried to a constant mass. This coupling reaction was monitored by IR followed by Py-GC/MS analysis.

5.3.3.2. Ribulose

Ribulose is a five-carbon ketose available as liquid. The coupling reaction was accomplished in a similar manner described for acetol.

5.3.4. Cleavage

Having completed the construction of β-alanine-sugar sequence, PEGME was cleaved by sodium methoxide treatment. According to the literature, ionic exchange resin and acidic clays are also effective in hydrolyzing the ester linkage; however, those trials with polymer-bound products were not successful. The problem may have resulted from the inaccessibility of the reagents to the polymer matrix. The aliphatic ester linkage between the polymer and the amino acid residue was hydrolyzed by sodium methoxide in methanol, releasing the original PEGME polymer and methyl ester of alanine-sugar derivative. Liberation of PEGME polymer was monitored by the disappearance of IR band attributed to the characteristic ester absorption at 1733 cm^{-1}.

5.4. Spectroscopic monitoring of the synthesis steps

Each product collected during reaction was analyzed by FTIR, Py/GC/MS, and ^{1}H-NMR.

5.4.1. β - alanine-PEGME

5.4.1.1. FTIR analysis

Infrared analysis is a simple, rapid, non-destructive method to monitor synthesis reactions. FTIR analysis of the polymer bound product was accomplished by placing approximately 1~2 mg of the dried solid between two CaF_2 windows, dissolving the solid by adding a drop of dichloromethane and immediately recording the spectrum. The carbonyl absorption region can provide sufficient information regarding the success of each step. The starting polymer does not possess any functional groups that absorb in this region. However, esterification of the polymer with t-BOC-β-alanine should produce two carbonyl absorption peaks; one due to the ester (1733 cm^{-1}) and the other due to the carbamate (t-BOC) group at 1710 cm^{-1} (Figure 2).

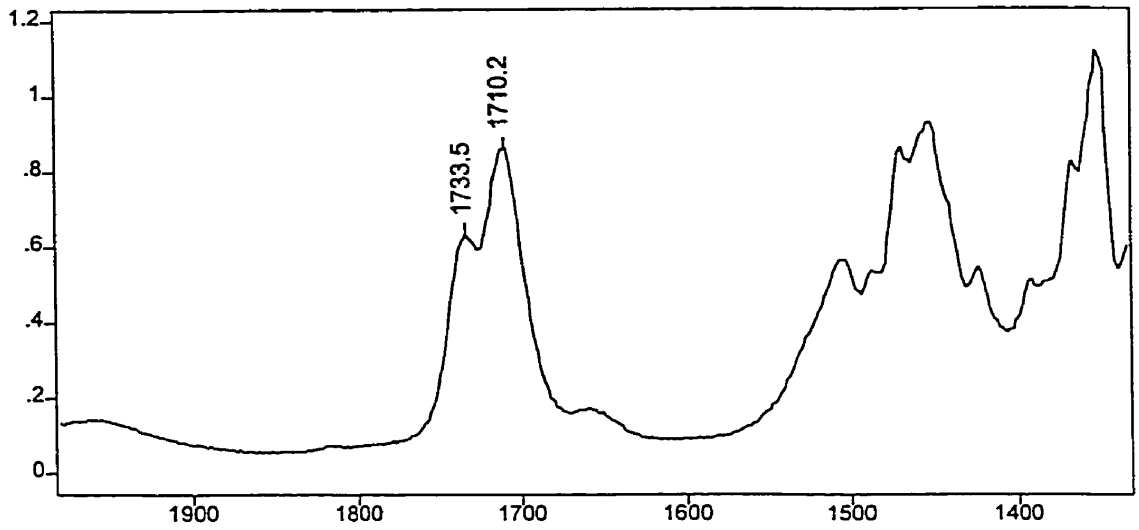


Figure 2: IR spectrum of t-BOC-β-alanine-PEGME (26) dissolved in dichloromethane

Subsequent deprotection step eliminated the peak centered at 1710 cm^{-1} that was attributed to the carbamate group (see Figure 3).

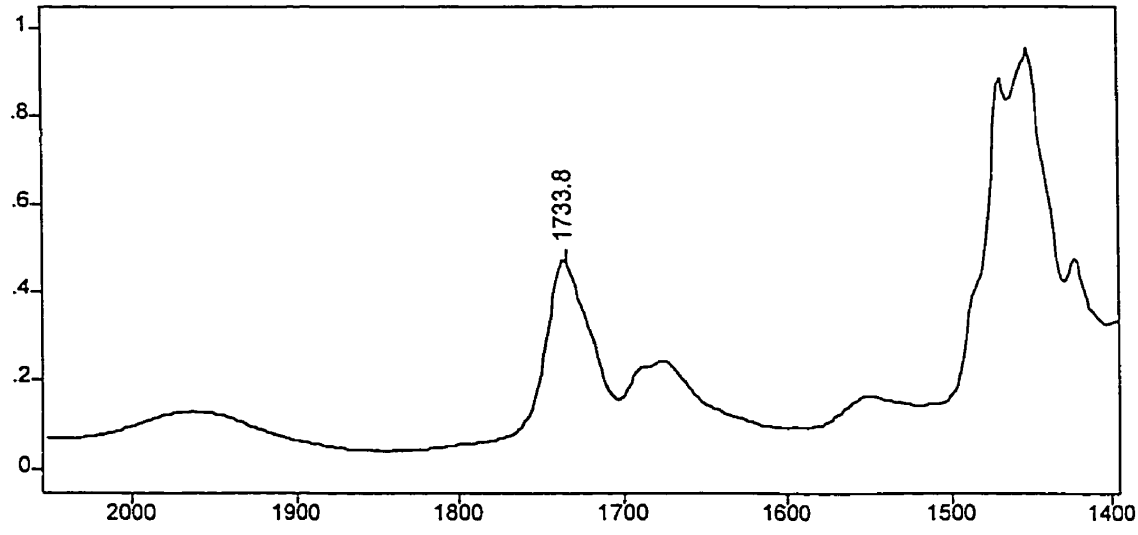
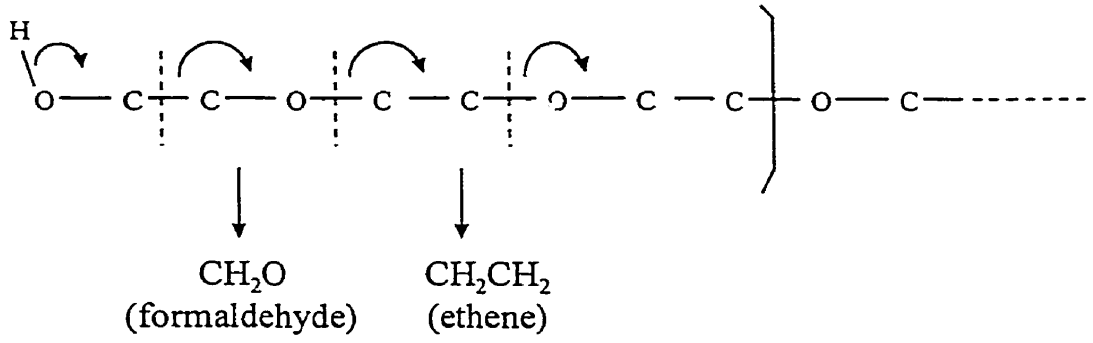


Figure 3: IR spectrum of β-alanine-PEGME (27) dissolved in dichloromethane

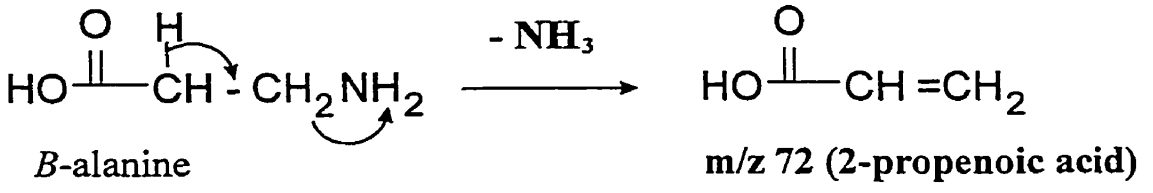
5.4.1.2. Py/GC/MS analysis

Pyrolysis is the most common technique of polymer analysis. Controlled degradation can produce fragments characteristic of the parent polymer. When original PEGME 2000 was pyrolyzed at 250°C, it degraded into small molecular weight, volatile fragments not retained on the GC column. A proposed degradation mechanism is shown in Scheme 18. According to this proposal, PEGME could be degraded into ethene (MW 28) and formaldehyde (MW 30). However, the intermediate β-alanine-PEGME (27) produced an unknown pyrolysis product with the following major fragments at m/z of 153, 72 and 55. Since the starting polymer did not produce this product, this fragment should contain an β-alanine moiety plus a fragment from PEGME.

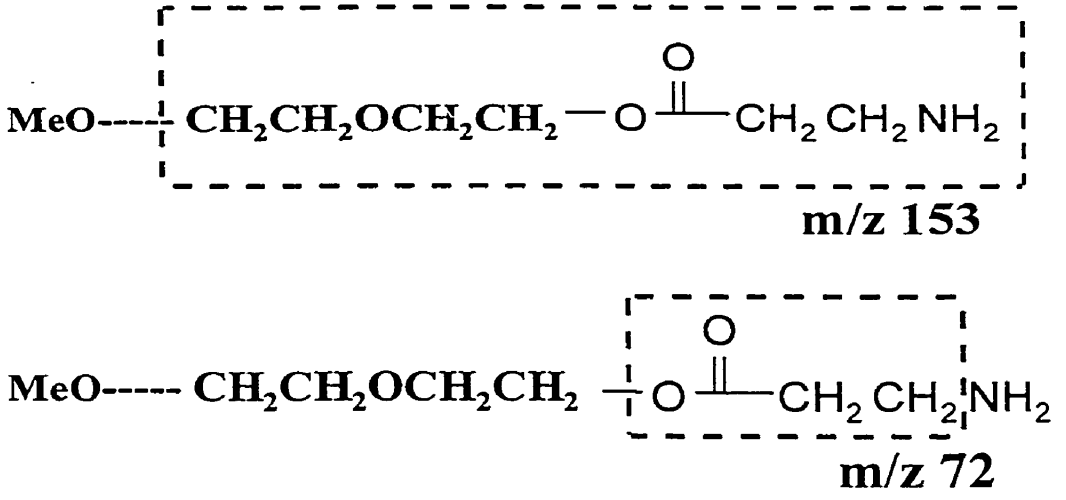


Scheme 18. Degradation of $PEGME_{2000}$ during pyrolysis

The known fragmentation of β-alanine which produces a fragment at m/z 72 supports this assumption. (see Schemes 19 and 20). Major indicator fragments produced during Py/GC/MS analysis of synthetic intermediates are shown in Table 1.



Scheme 19. Loss of ammonia from pure β-alanine during pyrolysis/GC-MS analysis



Scheme 20. Proposed origin of fragment at m/z 153 during pyrolysis/GC-MS analysis

Table 1. Major fragments produced during pyrolysis/GC/MS analysis of synthetic intermediates and reactants.

Compound	Indicator fragments produced during pyrolysis
Acetol	Furaneol (m/z 112), acetol (m/z 74)
β-alanine	2-propenoic acid (m/z 72), 2-propenamide (m/z 71)
Ribulose	Furancarboxylaldehyde, 2-cyclopenten-1,4-dione (20:1)
PEGME $_{2000}$	No detectable fragments
β-alanine-PEGME	Unknown fragment (m/z 153, 72, 55)

5.4.1.3. ^{1}H-NMR analysis

^{1}H-NMR analysis of the intermediates confirmed the conclusions of FTIR data. The starting unreacted polymer showed the presence of three types of protons; methoxy at 3.37 ppm, methylene at 3.69-3.65 ppm and the terminal hydroxy methylene at 3.63 ppm. Attachment of t-BOC-β-alanine was indicated by the presence of methyl protons of t-BOC at 1.45 ppm. However, the t-BOC methyl protons disappeared completely after the subsequent deprotection step.

5.4.2. Acetol-β-alanine-PEGME

5.4.2.1. FTIR analysis

The addition of acetol to β-alanine-PEGME can be followed by FTIR analysis either through monitoring the carbonyl or the enol region (1600-1700 cm^{-1}). Literature data indicated that enol and enediol moieties have strong absorption bands in the alkene region (Yaylayan and Ismail, 1995). Due to an overlapping peak of TFA-TEA salt, which appears around 1690 cm^{-1}, the carbonyl signal was difficult to identify. However, a strong absorption peak in the enol region centered at 1645 cm^{-1} has confirmed the presence of a

sugar moiety (see Figure 4). In addition, the enol peak diminished in intensity when the spectrum was acquired in D_2O due the hydration of the aldehyde.

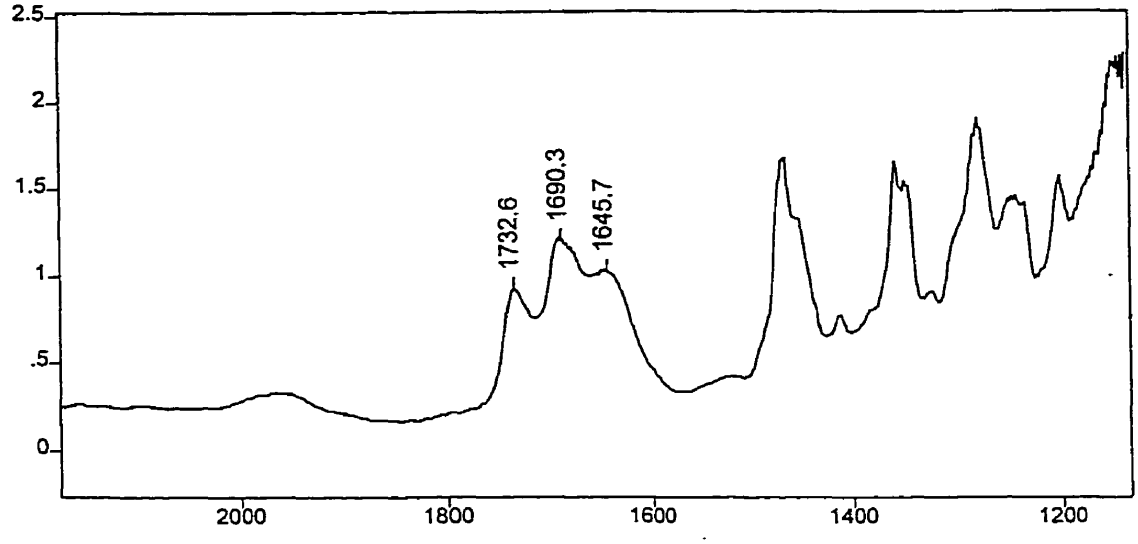


Figure 4: FTIR spectrum of acetol-β-alanine-PEGME (in dichloromethane)

5.4.2.2. Py/GC/MS analysis

Pyrolysis products of acetol-β-alanine-PEGME are listed in Table 2. The observed fragment from β-alanine-PEGME, at m/z 153 was also present in low intensity.

Table 2: Major fragments produced during pyrolysis/GC/MS analysis of acetol-β-alanine-PEGME.

Compound	**Indicator fragments produced during pyrolysis**
acetol-β-alanine-PEGME	acetic acid, m/z 153, other unknown degradation products

5.4.2.3. ^{1}H-NMR analysis

The ^{1}H-NMR analysis clearly indicates the presence of acetol protons on the rearrangement product. The solvent (D_2O) however, caused the formation of acetal bonds with the aldehyde group thus moving the aldehyde proton to 3.45 ppm, whereas methyl protons absorbed at 1.4 ppm and the methine proton at 3.3 ppm.

5.4.3. Ribulose-β-alanine-PEGME

5.4.3.1. FTIR analysis

Ribulose-β-alanine-PEGME showed similar ester and enediol absorption bands to that of acetol derivative, at 1732 and 1635 cm^{-1} respectively. A broad OH absorption band also appeared around 3500 cm^{-1} after ribulose attachment (see Figure 5).

5.4.3.2. Py/GC/MS analysis

Pyrolysis of ribulose-(β)-alanine- PEGMEshows indicator fragments as listed in Table 3. Furancarboxyaldehyde and 2-cyclopenten-1,4-dione are characteristic fragments derived from ribulose molecule. An indicator fragment from PEGME attached-β-alanine, m/z 153 has also been observed, which indicate the presence of β-alanine moiety.

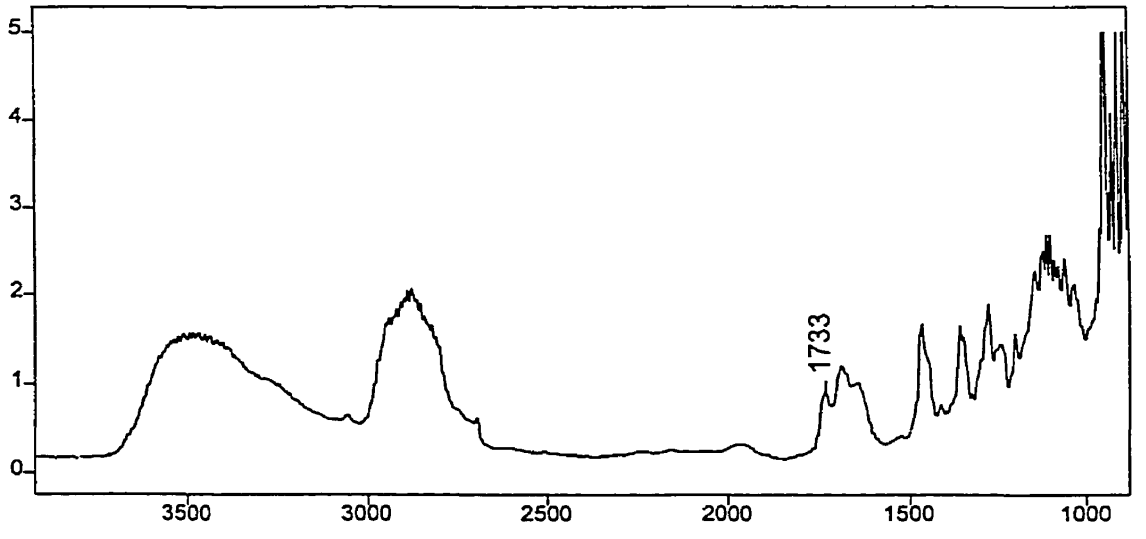


Figure 5. FTIR spectrum of ribulose-β-alanine-PEGME (in dichloromethane)

Table 3: Major fragments produced during pyrolysis/GC/MS analysis of ribulose-β-alanine-PEGME.

Compound	Indicator fragment produced during pyrolysis
Ribulose-β-alanine-PEGME	Furancarboxaldehyde, 2-cyclopenten-1,4-dione, m/z 153

5.4.3.3. ^{1}H-NMR analysis

The ^{1}H-NMR analysis clearly indicated the presence of ribulose protons on the rearrangement product. The solvent (D_2O) however, caused the formation of acetal bonds with the aldehyde group thus moving the aldehyde proton to 3.45 ppm. Other ribulose protones absorbed at 0.9, 1.2 and 1.4 ppm.

5.4.4. β-alanine-acetol (29)

The final Heyns rearrangement product (29) was cleaved from the polymer using sodium methoxide in methanol. As a result the methyl ester was formed as a yellow viscous solution. In addition, the aldehyde group was also expected to react with methanol to form an equilibrium mixture with corresponding hemiacetal (see Scheme 21).

5.4.4.1. FTIR analysis

The infrared spectrum (see Figure 6) of the final product (29) shows the characteristic bands of the functional groups present. The ester carbonyl stretching frequency appeared at 1736 cm^{-1} (strong), the aldehyde band at 1690 cm^{-1}, N-H bending at 1526 cm^{-1} (weak, broad), methoxycrbonyl band at 1445 cm^{-1} (weak), methyl group band around 1380 cm^{-1} (weak) and C-O-C asymmetric stretching band around 1190 cm^{-1} (strong).

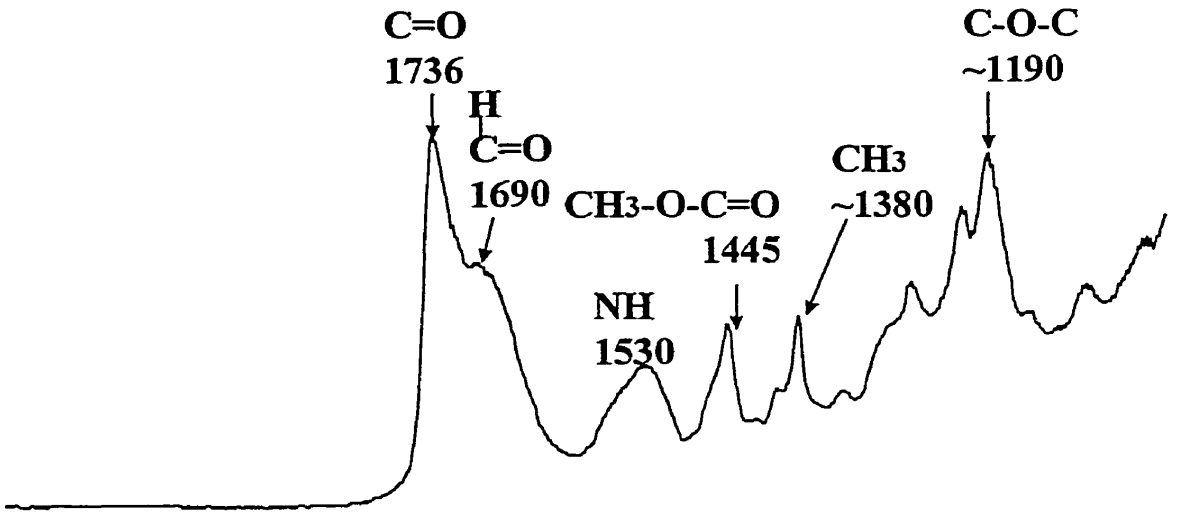
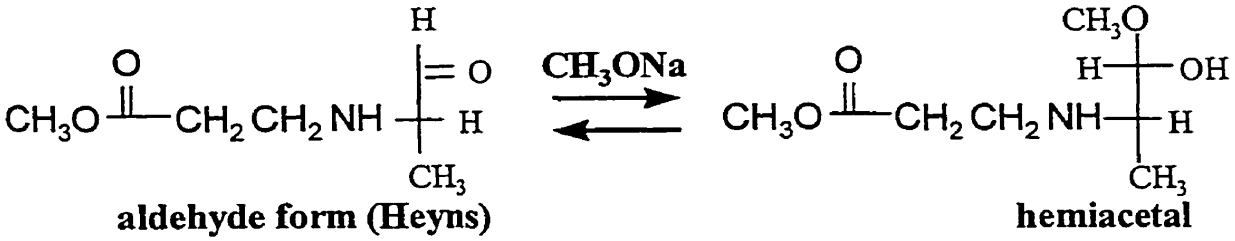


Figure 6. FTIR spectrum of β-alanine-acetol (neat)

5.4.4.2. Py/GC/MS analysis

It was found that this molecule was volatile enough not to decompose completely during pyrolysis, and reach the mass detector intact. The proposed EI mass spectral fragmentation pattern is shown in Scheme 22. The hemiacetal structure was therefore confirmed by the characteristic fragmentation patterns observed during MS analysis. Some of the relevant pyrolysis products observed included 2-propenoic acid methyl ester, 2,3-dimethylpyrazine and 2,3,5-trimethylpyrazine known Maillard reaction products.

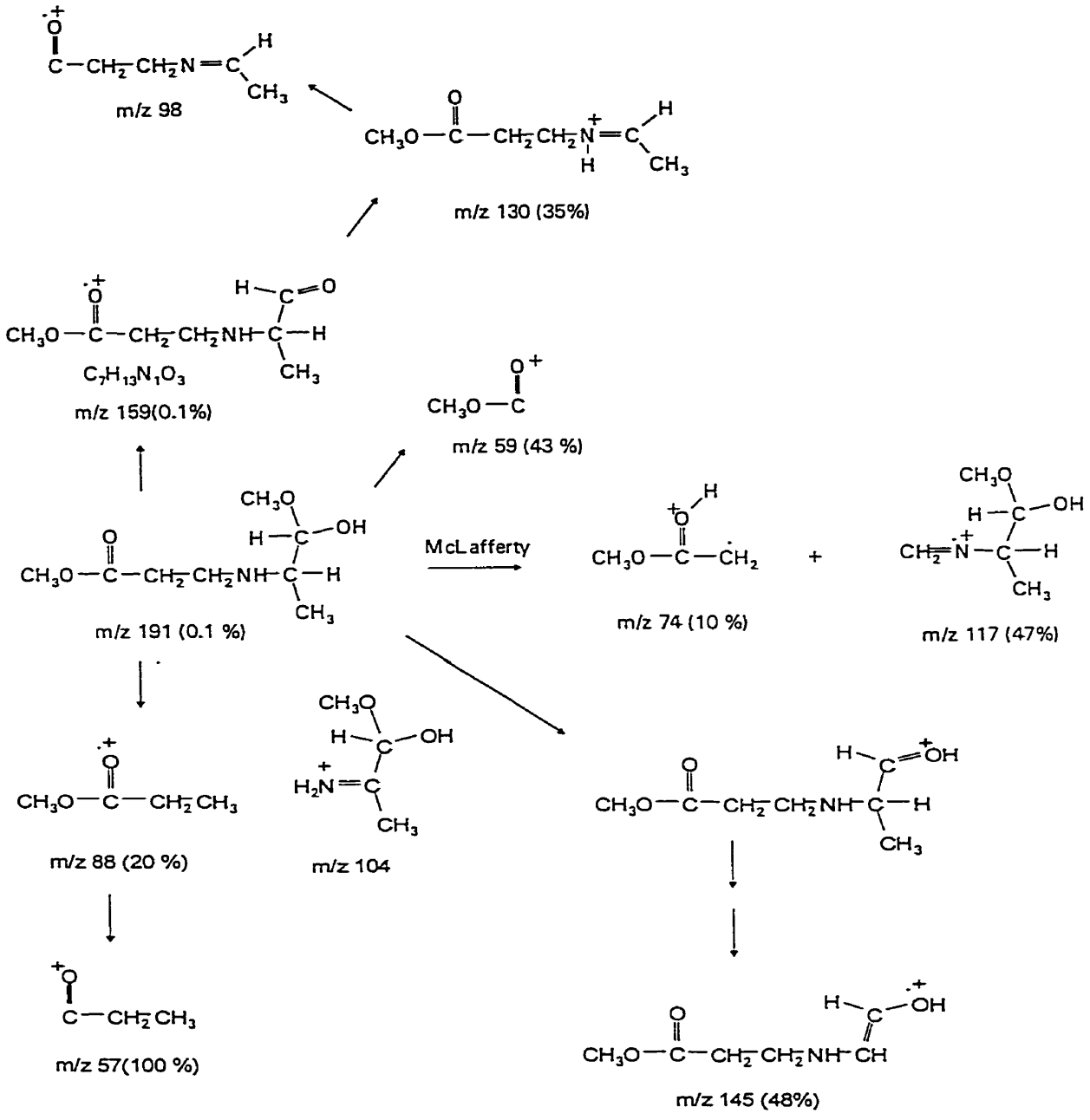


aldehyde form (Heyns) **hemiacetal**

Scheme 21. Equilibrium between aldehyde and hemiacetal forms of β-alanine-acetol

5.4.4.3. ^{1}H-NMR analysis

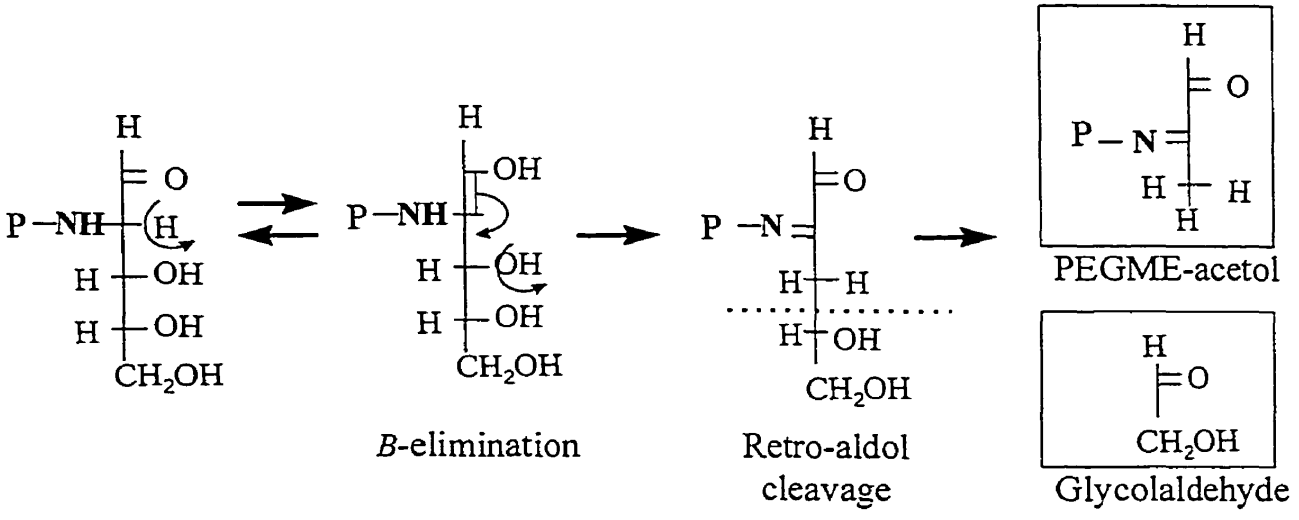
The important feature of the ^{1}H-NMR spectrum was the presence of an aldehyde proton at 8.2 ppm. The integrated intensity indicated that only 20 % of the product existed as free aldehyde in $CDCl_3$ solution at room temperature and 80 % as hemiacetal.



Scheme 22. Proposed EI fragmentation pattern of β-alanine-acetol

5.4.5. β-alanine-ribulose

FTIR and Py/GC/MS analysis revealed that final product isolated was spectroscopically similar to that of β-alanine-acetol. This observation could be attributed to the degradation of the molecule under basic conditions of cleavage reaction (methoxide). The proposed degradation process is shown in Scheme 23. According to this scheme, the base catalyzed enolization can initiate a β-elimintaion followed by retro aldol reaction to produce a product similar to β-alanine-acetol.



Scheme 23: Suggested degradation process of β-alanine-ribulose under basic conditions

Chapter 6

ATTEMPTED SYNTHETIC APPROACHES

6.1. Synthesis *via* initial sugar attachment to the polymer

As mentioned in Chapter 5, two approaches were proposed for the synthesis of Amadori or Heyns rearrangement products. These approaches differed from each other with respect to the sequence of attachment of the amino acid and the sugar (see Schemes 13 and 14). The strategy described in Chapter 5 (Strategy A shown in Scheme 13) involved the initial coupling of the polymer with amino acid and subsequent attachment of the sugar residue. In the alternate method (Strategy B shown in Scheme 14), it was attempted to attach the sugar to the polymer through a bifunctional spacer arm. The working assumption being the most reactive primary alcohol (C-6) will react predominantly during this reaction. The spacer arms, in addition to their function as linkages between the polymer and the reactants, they can also direct the bulk of the growing product away from the polymer matrix to minimize the interaction between polymer and products. Oxalyl chloride, oxalic acid and succinic anhydride were tried as anchoring groups to attach the sugar residue to the polymer followed by the reaction of the polymer adduct with different amino acids. The following synthetic attempts were discarded due to complications and low yields of the reactions.

6.1.1. Attempted reaction of oxalyl chloride with PEG

Activation of PEG with oxalyl chloride should produce a highly reactive polymer with a terminal acid chloride that subsequently can react with the primary alcohol group of glucose to produce an ester linkage. Specific procedure attempted is described below;

PEGME (40.0 g, 0.008 moles, MW 5000) was dissolved in 400 mL of dichloromethane under an inert atmosphere. Oxalylchloride (12 mL, 0.24 moles) and triethylamine (1.12 mL, 0.008 mol, d 0.726 g/mL) were subsequently added to the above solution and the mixture was stirred overnight in the presence of activated molecular sieves. The Molecular

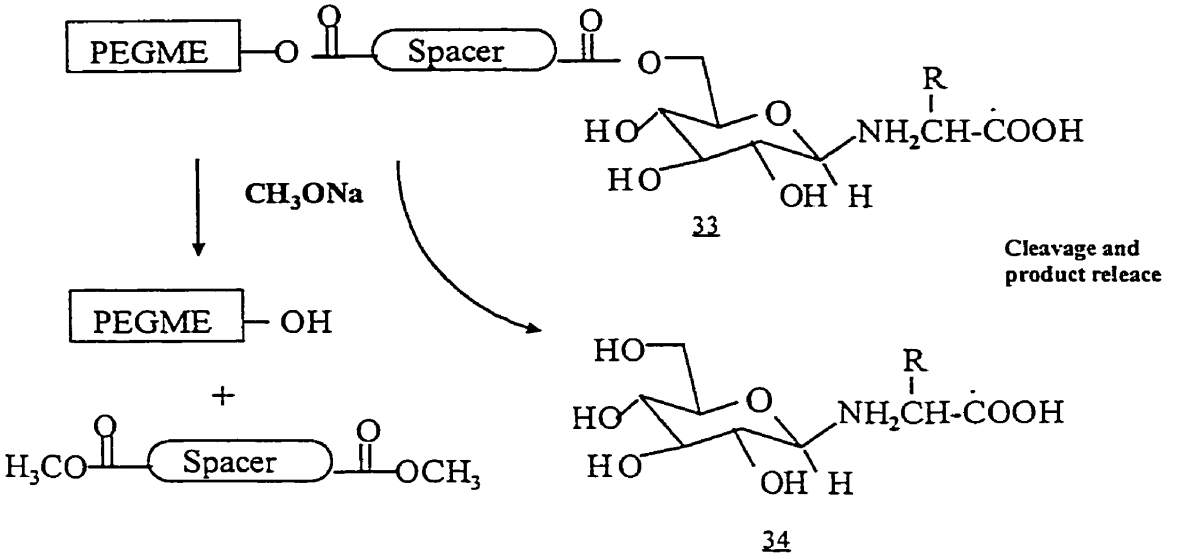
sieves were filtered and removed. The filtrate was concentrated, and the product was precipitated by the addition of ether and cooling. The solid precipitate was immediately dissolved in dichloromethane (400 mL) and reacted with glucose (4.24 g, 0.0235 mol) in the presence of triethylamine (1.12 mL). The mixture was stirred overnight.

Several problems were encountered in this procedure. One was the reactivity of the terminal acid chloride group. Although molecular sieves were used to absorb the moisture to prevent the conversion of acid chloride to carboxylic acid, however, the effectiveness was not sufficient. The presence of moisture seemed to convert acid chloride to the unreactive carboxylic acid. It should be also noted that some dimerization might have occurred between the reacted and unreacted polymers. Another complication was the formation of HCl as a side product. Although trietylamine (TEA) was added to neutralize the acid, however, it was found that excess TEA eventually made the reaction mixture dark brown. Subsequently, TEA was replaced with DMF to trap the chloride ions but in both cases, the removal of the resulting salts was difficult, resulting in significant loss of the product. The major problem however was the solubility of glucose in non-polar dichloromethane. Although PEGME has remarkable solubilizing effect, however, it did not aid in solubilizing glucose effectively. DMF was also used as a solvent to dissolve glucose, however; the higher boiling point and the polarity was not convenient for the subsequent precipitation steps.

6.1.2. Preparation of PEG-succinate

PEG-Succinate is a versatile derivative bearing a terminal carboxyl group and it is commercially available (Shearwater Polymers, Inc., Huntsville, AL). The presence of cleavable (e.g. by alkaline hydrolysis or reaction with hydrazine) succinate ester in such conjugates can be advantageous in some synthesis (Abuchowski et al., 1984; Douglas et al.,1991). In our laboratory, difunctional PEG with an average molecular weight of 2000 was used instead of the monofunctional PEGME (average molecular weight 5000) to increase the loading of the polymer. Specific procedure attempted is described below.

Difunctional PEG 2000 (10.0 g, 0.005 moles) was dissolved in acetonitrile (100 mL) and succinic anhydride (1.0 g, 0.01 moles, MW 100.07) was added to the solution. Small amount of anhydrous oxalic acid can be added as a catalyst. The reaction mixture was refluxed for 24 hrs. After reflux, product was precipitated by addition of ether and cooling.



The dry product (PEG-succinate) was again dissolved in acetonitrile (100 mL) followed by addition of DCC (2.06 g, 0.01 moles MW 206) and dihydroxyacetone (0.9 g, 0.005 moles, MW180). The solution was refluxed for 3 hrs. After the reaction, dicyclohexylurea (DCU, mp. 232-233) was precipitated and filtered. The filtrate was concentrated and the product was precipitated by addition of ether and cooling.

PEG-succinate was identified only by its characteristic IR absoption band at 1735 cm^{-1}. The very low yields of this product could be attributed to the increased susceptibility of difunctional PEG to degradation compared to monofunctional PEG, under the experimental conditions. In addition, it seemed difunctional PEG underwent more dimerization reactions specially during the DCC addition. It should be noted that, for reaction of monofunctional PEGs, small amounts of impurities are generally not of a concern if the impurities are

chemically inert and can be removed in subsequent steps. This statement does not hold when difunctional derivatives are used since an inert end group leads to monosubstituted product that can be difficult to remove. In addition, sugar attachment under the basic condition might accelerate the degradation of DHA, eventually turning the reaction mixture into a brown solution.

6.1.3. Attempted preparation of PEG-oxalate

Oxalic acid was also used to functionalize PEG through DCC method, however, the DCC was quite reactive with oxalic acid, degrading it to produce CO_2 and DCU.

Chapter 7

CONCLUSIONS

The development of a liquid-phase method is the first step toward the combinatorial synthesis of Heyns rearrangement products (HRPs). This is the first report to describe the attachment of sugar-amino acid conjugates to the PEGME support. The main advantage of using polymer-supported synthesis is the ease of separation of intermediates and higher purity of the products. Heyns rearrangement product (HRPs) of acetol-alanine was synthesized on PEGME and collected after cleavage/precipitation of PEGME. Ribulose-alanine however was degraded during basic cleavage with sodium methoxide. Spectroscopic analysis was a useful strategy for tracking each step during synthesis. Esterification, removal of t-BOC group and hydrolysis of the ester linkage have been successfully monitored by FTIR. In addition, Py/GC/MS revealed a characteristic fragment liberated from β-alanine-PEGME. Assessed by FTIR, Py/GC/MS, and ^{1}H-NMR, the complete structure of synthesized final acetol-alanine product was elucidated.

Employing simplified procedures relative to classical methods, acetol-alanine was synthesized in gram quantities without the need for further chromatographic purification. Due to the hemiacetal formation with methanol, the product was quite stable at the room temperature. Upon heating, the Heyn's product browned quickly with the formation of characteristic roasted, bread-like aroma. In addition, the polymer attached acetol-alanine, can also release aroma compounds upon heating in the presence of water. Furthermore, it exhibited great stability at room temperature and high solubility in non-polar solvents. Since food-grade PEG polymers are non-toxic and allowed to be used in food products; such functional derivatives can be used as value-added ingredients for food products. They can provide various advantages such as (1) stabilization of low molecular weight flavor precursors; (2) solubilization of non-polar flavors into high moisture systems, such as liquid beverages; (3) alteration of the texture and viscosity of the food systems.

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