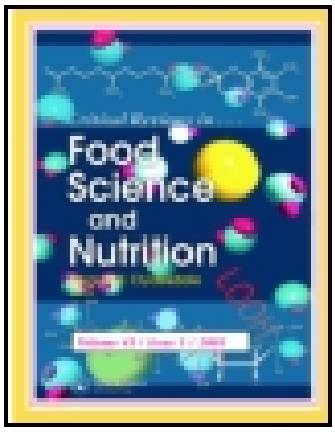

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2,5-Diketopiperazines in Food and Beverages: Taste and Bioactivity

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2,5-Diketopiperazines in Food and Beverages: Taste and Bioactivity

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2,5-Diketopiperazines (2,5-DKPs) have been found to occur in a wide range of food and beverages, and display an array of chemesthetic effects (bitter, astringent, metallic, umami) that can contribute to the taste of a variety of foods. These smallest cyclic peptides also occur as natural products and have been found to display a variety of bioactivities from antibacterial, antifungal to anthroprotective effects and have the potential to be used in the development of new functional foods. An overview of the synthesis of these small chiral molecules and their molecular properties is presented. The occurrence, taste and bioactivity of all simple naturally occurring 2,5-DKPs to date have been reviewed and those found in food from yeasts, fungi and bacteria that have been used in food preparation or contamination, as well as metabolites of sweeteners and antibiotics added to food are also reviewed.

Keywords: Flavor, Cyclic dipeptides, Piperazine-2,5-diones, Maillard reaction

CONTENTS

1. INTRODUCTION

2,5-Diketopiperazines (2,5-DKPs), also known as cyclic dipeptides, 2,5-dioxopiperazines and dipeptide anhydrides were first discovered as natural products in the early 20th century. The first one synthesized was cyclo(Gly-Gly) by Curtius and Gloebel (1888). They are found in fermentation broths and yeast cultures as well as in a variety of natural products from fungi, bacteria, plants, and mammals, and their core structure occurs in several drugs (Borthwick, 2012). The first biologically active compound cyclo(L-His-L-Pro) was found in human urine by Perry et al., in 1965.

2,5-Diketopiperazines are often the unwanted side reactions, products or degradation products of oligo- and polypeptides in processed food and beverages (Prasad, 1995) that are often formed during chemical and thermal processing. They have been detected in beef (Chen et al., 2009), beer (Gautschi et al., 1997), bread (Ryan et al., 2009), Awamori spirits (Takaya et al., 2007), cocoa (Stark et al., 2005), chicken essence (Chen et al., 2004), roasted coffee (Ginz et al., 2000; Ginz et al., 2001), Comte cheese (Roudot-Agaron et al., 1993), dried squid (Kawai et al., 1991), aged saki (Takahashi et al., 1974) and autolyzed yeast extract (Da Costa et al., 2010).

There are four ways 2,5-DKPs can occur in food. There is contamination of food in its production and storage by fungi, yeasts and bacteria that produce 2,5-diketopiperazines. They are also found in foods as natural products from yeast used for fermentation in brewing and lactic acid bacteria for fermentation in bread, and from fungi (e.g. *Penicillium roqueforti*) used in the maturation of blue-veined cheese. In addition they are found as degradation products of certain additives to food, such as the artificial sweetener aspartame or the antibiotic Amoxicillin added

to the feeds of animals that ends up in meat. However the major route for 2,5-DKP occurrence in foods is probably through formation from peptides and protein by chemical reaction during the thermal processing of food.

In food systems 2,5-diketopiperazines have been shown to be important sensory compounds contributing to the taste of the final products and being perceived as astringent, salty, grainy, metallic or bitter (Tables 1-5). They have also been shown to have a range of other bioactivities (Borthwick, 2012) and are widespread in nature. The dietary origin of simple neuroactive 2,5-diketopiperazines has recently been reviewed (Prasad, 2005).

The objective of the present review is to comprehensively cover the literature from 1989-until the end of 2013 on the occurrence and flavour of 2,5-diketopiperazines in food and beverages, and their corresponding biological activity. In this review the numbering of 2,5-DKPs is as shown in (Figure 1.) and when referred to as a cyclodipeptide the nomenclature is indicated by the three letter code for each of the two amino acids plus a prefix to designate the absolute configuration, e.g. cyclo(L-Xaa-L-Yaa). Compounds are referred to with bold numbers, these can be found in the various Figures, and Tables 1 - 5 summarise the data.

[Figure 1 near here]

2. FORMATION AND MOLECULAR PROPERTIES

Formation in Foods

In 1989 Rizzi described much experimental work on 2,5-diketopiperazine formation in foods. Their formation was described as thermally dependant under acid conditions (Figure 2.) and

several experiments were conducted to prove this. It was shown that heating equimolar amounts of amino acids did not produce cyclic dipeptides. However, heating acyclic dipeptides under acidic conditions did produce the corresponding cyclic dipeptides. Also heating a tripeptide such as Ala-Leu-Gly led to the formation of cyclo(L-Ala-L-Leu).

[Figure 2 near here]

Many 2,5-diketopiperazines are also formed as a side reaction of the Maillard reaction which is a well known source of aroma and taste compounds in processed foods (Ho, 1996). The Maillard reaction is the thermal reaction of amino acids / peptides with reducing sugar /carbohydrates leading to a multitude of flavor compounds. It is also part of non-enzymatic browning which occurs in roasting meats, baking bread, French fries, etc. The reactive carbonyl of the sugar/ carbohydrates reacts with the nucleophilic amino group of the amino acid/peptide and forms a complex mixture responsible for a wide range of aromas and flavors. This process can be accelerated under alkaline conditions as the amino groups are deprotonated and hence have an increased nucleophilicity. The type of the amino acid /peptide and sugar /carbohydrate determines the resulting flavor. This reaction is the basis of the savory flavoring industry (Ho, 1996).

However, it has been shown that the yield of 2,5-diketopiperazines formed by the reaction of peptides with reducing sugars is very similar to that obtained by the thermal generation of 2,5-diketopiperazines from peptides without the intervention of other reactive species (Jakas and Horvat, 2003).

Molecular Properties

The 2,5-diketopiperazines (2,5-DKPs) are the smallest possible cyclic peptides, which are peptidomimetic in nature and bear resemblance with a constrained protein β-turn. These cyclic dipeptides incorporate both donor and acceptor groups for hydrogen bonding and are small, conformationally constrained heterocyclic scaffolds in which diversity can be introduced at up to six positions and stereochemistry controlled at up to four positions, and they are stable to proteolysis. These characteristics enable them to bind to a wide range of receptors which result in a widespread class of biologically active natural compounds. They have a rigid backbone, which can mimic a preferential peptide conformation and contain constrained amino acids imbedded within their structures without the unwanted physical and metabolite properties of peptides. This improves their bioavailability and resistance to enzymatic degradation in comparison to their linear analogues.

These conformationally constrained heterocycles are flexible because the six-membered ring can exist in an essentially flat conformation or a slightly puckered boat form, with only a few kcal/mol difference in energy between the boat and planar forms. In addition, the 2.5-DKPs can be configured as both *cis* and *trans* isomers (Eguchi and Kakuta, 1974). As a consequence of their predominant biosynthetic origin from L-α-amino acids most naturally occurring 2.5-DKPs are *cis* configured as the cyclo(L-Xaa-L-Yaa) isomers. However, 2,5-DKP's epimerize under basic (Eguchi and Kakuta, 1974), acidic (Steinberg and Bada, 1981)

and thermal (Eguchi and Kakuta, 1974) conditions (Figure 3.). The composition of the *cis* and *trans* isomers in the equilibrium state varies widely depending on the bulk of the side chains, if a ring (e.g. proline) is present, or if the nitrogen atoms are alkylated (Anteunis, 1978).

[Figure 3 near here]

Although epimerizisation was historically an issue in the synthesis of 2,5-DKPs, several mild methods of synthesis of 2,5-DKPs have been developed recently that avoid epimerizisation (see Synthetic Methods section).

The structurally simple 2,5-DKPs cyclo(L-Xaa-L-Yaa) commonly found in food have characteristic mass spectra where the dominant features are fragments corresponding to cleavages of side chains and the rupture of the six-membered 2,5-DKP ring (Szafranek et al., 1976), and this has been developed into a rapid method for determining their structure.

Table 1 shows a fairly comprehensive list of naturally occurring cyclic dipeptides identified by GC-MS analysis, in non-polar elution order using Kovats indices, plus their occurrence in foods and beverages. Note that often more than one isomer was observed. Without specific stereochemical synthesis, the exact *cis* or *trans* confirmation cannot be positively confirmed.

[Table 1 near here]

Synthetic Methods

In the synthesis of 2,5-diketopiperazines there are essentially three methods of ring closure: Amide bond formation **1,** N-alkylation **2** and C-acylation **3** (Figure 4.).

[Figure 4. near here]

These three methods used for the synthesis of 2,5-diketopiperazines in solution are outlined below. For a more detailed description of the various other methods used in solution and on

solid phase (Chen et al., 2009) to make the 2,5-diketopiperazine ring system, including Aza-Wittig Cyclization, Aza-Michael Addition, Diels-Alder Reaction, Tandem Cyclization, and Radical-Mediated *C-C* Cyclization see a recent comprehensive review (Borthwick, 2012).

Via Amide Bond Formation

Dipeptide Formation and Cyclization

The most commonly used synthesis of 2,5-diketopiperazines **3** is amide bond formation by cyclisation of a dipeptide **1** or **2**. This dipeptide can be formed (Figure 5) by coupling two amino acids together using route **A** or by using the Ugi reaction of an isonitrile, acid (or amino acid), aldehyde, and amine; route **B**,

[Figure 5. near here]

Dipeptide Ester Cyclization

Dipeptides substituted with an amine at one terminus and an ester at the other can spontaneously cyclize to form a 2,5-diketopiperazine at a range of pH's. However, careful selection of reaction conditions is required to limit racemization. This was recently achieved in a general, efficient, and environmentally benign solution-phase synthesis of 2,5-diketopiperazines using microwave-assisted heating in water (Tullberg et al., 2006), which in contrast to other published methods is independent of the amino acid sequence, and no epimerization was seen. Evaluation of solvent, reaction times, temperatures and comparison with classic thermal heating showed that microwave-assisted heating for 10 min using water as solvent was the most efficient method of

cyclization giving moderate to excellent yields (63−97%) of 2,5-diketopiperazines. Both cyclo(L-Phe-L-Pro) **21** and cyclo(L-Leu-L-Phe) **36**, were prepared by this method. This route has been shortened by using the intermediate protected *N*-Boc dipeptide esters directly. Because *N*-Boc protecting groups are unstable at temperatures higher than 90 °C, conditions were investigated that would deprotect the amines facilitating the spontaneous intramolecular aminolysis. This was achieved by microwave irradiation of *N*α-Boc-dipeptidyl-tert-butyl esters in water (MW conditions 250 °C, 250W and 150psi) for 10min. to give 2,5-diketopiperazines in excellent yields (Pérez-Picaso et al., 2009). The following were prepared by this method: cyclo(L-Phe-L-Phe) **32**, cyclo(L-Val-L-Phe) **39**, cyclo(Gly-L-Phe) **41**, cyclo(L-Val-L-Val) **47** and cyclo(Gly-L-Val) **57**. This cyclization was also achieved thermally with *N*α-Boc-dipeptidyl methyl esters in water at 130°C for 4 hr (Thajudeen et al., 2010). The following proline 2,5-diketopiperazines were prepared by this method: cyclo(L-Pro-L-Pro) **11**, cyclo(L-Tyr-L-Pro) **13**, cyclo(L-Phe-L-Pro) **21**, cyclo(L-Leu-L-Pro) **22**, cyclo(L-Ile-L-Pro) **23**, cyclo(L-Val-L-Pro) **24**, cyclo(L-Ala-L-Pro) **25**, cyclo(Gly-L-Pro) **26**, cyclo(L-Thr-L-Pro) **27**, cyclo(L-Ser-L-Pro) **28**, cyclo(L-Asn-L-Pro) **29** and also the non proline derivatives cyclo(L-Ala-L-Ala) **48**, cyclo(Gly-Gly) **49** and cyclo(Gly-L-Trp). Similarly cyclo(L-Trp-L-Pro) **19**, Brevianamide F was prepared (in 63% yield) by heating its *N*α-Boc-dipeptidyl methyl ester precursor at 200°C without solvent for 4hr; however, some epimerisation occurred with the preparation of its (3*R*, 8a*S*) diasteroisomer (25%) (Caballero et al., 2003).

Ugi Chemistry

The Ugi reaction using an isonitrile, acid (or amino acid), aldehyde and amine, can produce a dipeptide in equally high yield and optical purity to that formed by standard peptide couplings

(Figure 5) (Dömling, 2006). Commonly, an isonitrile is chosen to give a labile terminal amide to enable cyclization. For example, the direct 2,5-DKP ring formation via such an activated leaving group using the stable, easily accessible and versatile convertible isonitrile 1-isocyano-2-(2,2-dimethoxyethyl)-benzene **4** gave a one-pot synthesis of *N*-substituted 2,5-diketopiperazine's **7** (Figure 6.) (Rhoden et al., 2009).

[Figure 6. near here]

The mild acidic and chemoselective post Ugi activation of **5** involving simultaneous indolamide formation and Boc removal gives the active amide **6,** which allows cyclization to **7** without affecting other peptidic or even ester moieties, and with stereochemical retention of the chiral centers.

Amino Acid Condensation

Direct condensation of two amino acids **8** to form an *N*-substituted 2,5-diketopiperazine **9** (Figure 7.) most often suffers from a poor yield of product (Falorni et al., 2000). The use of peptide coupling reagents has been reported to facilitate 2,5-diketopiperazine formation (e.g. glycine polypeptide dimerization) (Cavelier et al., 2001).

[Figure 7. near here]

A recent improvement in this chemistry was reported by Santagada et al. who used microwaves in the condensation of two amino acids. They used amide bond coupling reagents (HBTU/DMAP) with 400W irradiation in DMF to produce yields of 85-95% in short reaction times (5mins) without epimerization (Santagada et al., 2003). Improvement has been made to this one pot reaction by using small amounts of ionic liquid in the presence of microwaves

(Jainta et al., 2008) and the reaction has been broadened to include chiral components which allowed the stereoselective preparation of optically pure symmetrical 2,5-diketopiperazines as well as unsymmetrical 2,5-diketopiperazines from unprotected amino acids. For example the preparation of **11** from **10** (Jainta et al., 2008), and **13** from **10** and **12** (Friedrich et al., 2007) (Figure 8.).

[Figure 8 near here]

Via N-Alkylation

N-alkylation offers the second most common way to form a 2,5-diketopiperazine ring. Marcaccini et al., (2001) used an Ugi-4CR between amines, aldehydes, isocyanides and chloroacetic acid to get the α-haloacetamide amide adducts **14**. Treatment of **14** with ethanolic potassium hydroxide using ultrasonication led to intramolecular amide *N*-alkylation, giving 2,5-diketopiperazines **15** (Figure 9.). However, this route is limited by epimerization at the stereogenic centre and failure to obtain the 2,5-diketopiperazine ring if R_1 = Alkyl.

[Figure 9 near here]

Via C-Acylation

Formation of the 2,5-diketopiperazine ring by enolate acylation was used by Peng and Clive (2008) in their asymmetric synthesis of the ADB tricyclic ring system **18** of the anticancer, antibiotic MPC1001 (Figure 10.).

[Figure 10 near here]

Construction of the 2,5-diketopiperazine ring in **17** was achieved by intramolecular cyclization of the enolate of **16** onto the carbonyl of the phenyl carbamate to give **17** in 90% yield.

3. FLAVOR, OCCURRENCE AND BIOACTIVITY

Of the five basic taste attributes, sweet and umami are generally regarded as positive signs in foods such as increased nutrition and higher caloric availability. Bitterness, however, is generally seen as a warning against toxicity in most foods. Despite this, bitterness is still desirable in some foods like beer, chocolate, green and black tea, red wine and coffee. There are 25 different human bitter receptors, mainly in the TAS2R's class, linked to G protein-coupled receptors GPCRs. Less is known about bitter receptors than the others as they have received less attention in research, plus there are many more to be studied. Recently Kohl et al., (2012) studied them in more detail and found them to be even more complex than they had anticipated.

Bitter peptides are a structurally diverse group of compounds. The bitter taste of protein-rich foods usually resides in these peptide fractions (Machasashi and Huang, 2009). Hydrolysis of proteins with proteolytic enzymes is very often accompanied by the formation of bitter substances. Initial work was done on hydrolysed casein by Murray and Baker (1952). Mercier et al., (1971) studied the bitterness of cyclo(Leu-Trp), followed by Takahashi et al., (1974) with cyclo(Pro-Leu) in saké. In 1974 Pickenhagen reported twenty cyclic dipeptides in cocoa. They are formed during the roasting process of cocoa and exhibit a synergistic increase in bitterness in the presence of theobromine. Thus it is possible that they potentiate bitterness in other compounds and vice-versa. Theobromine has a low threshold of 10mg/L in water and is present in cocoa beans in concentrations between 1.8-3.8g/100g (Stark and Hofmann, 2005).

Current evidence clearly indicates that bitterness increases with the hydrophobicity of the peptide (Guigoz and Solms, 1976; Kim and Li-Chan, 2006). Ney, (1971) developed Q values – the average amount of free energy needed for the transfer of amino acid chains from ethanol to water - for amino acids, peptides and proteins. He found that bitter peptides have a Q value greater than 1400 kcal/mol, whereas non-bitter peptides have values less than 1300 kcal/mol. He also showed (Ney, 1986) that the bitterness of 2,5-DKPs generally followed the so-called "Q rule", which relates perceived bitterness to amino acid composition. However, exceptions to this rule have been subsequently discovered as it does not take steric parameters into account (Toelstede and Hofmann, 2008). Recently Quantitative Structure Bitter taste Relationships (QSBR) models have been used by Yin et al., (2010) to more accurately predict bitterness in di and tripeptides.

Cyclic dipeptides are generally regarded as more bitter than linear peptides. This partly because they have often been described in the literature as bitter, drying, astringent, umami, metallic and little else. Ishibashi et al., (1988) conducted an organoleptic comparison study of 23 synthesized cyclic and their corresponding linear dipeptides, respectively. They confirmed that the cyclic dipeptides were more bitter and this correlated well with the presence of hydrophobic amino acids. They reported that neutral amino acids had no taste. Linear peptides display a wider variety of taste descriptors such as bitter, acidic, sweet, brothy and flavour-enhancing like monosodium glutamate. Bitterness in linear peptides can be enhanced by acetylating or esterifying the amino acid and/or carboxy groups of the chain according to Kohl et al., (2012).

Shiba et al., (1981) used affinity chromatography with cyclo(Leu-Trp) as a bitter ligand to indicate that phosphoglycerides of lipids tested showed a particular affinity to the bitter diketopiperazines. All 4 stereoisomers of cyclo(Leu-Trp) L-L, D-D, L-D, D-L exhibited

comparable bitterness with threshold values of 0.03-0.06mol/L. Thus they theorized that bitter taste receptors do not recognize chirality. However linear peptides such as Asp-Phe-OMe did display differing chirality tastes where the R configuration was sweet and S configuration was bitter (Goodman and Temussi, 1985).

An alternative hypothesis to explain this discrepancy was described by Goodman and Temussi, (1985). They developed a protein receptor site model whose topology is consistent with several classes of bitter compounds. They theorized that the discrepancy is related to a given molecule's spatial orientation in the cavities of the receptor model. The depth of the cavity is such that any group pointing downward with a steric encumbrance of 0.4nm can be accommodated by the site, whereas larger groups are prevented from binding. In diketopiperazines the chiral center parts of the molecules are far from the critical region around the moiety that interacts with the receptor cavity, and thus have no or little influence on the bitter taste.

Ishibashi et al., (1988) and Temura et al., (1990) developed an improved theory of bitter receptor activation and the model of the receptor cavity, which is 1.5nm wide. The bitter peptide cavity is theorized to have a "binding unit" and a "stimulating unit", optimally 0.41 nm apart, located at the bottom. The size of the cavity allows peptides of 8 amino acids or fewer to bind. In addition the receptor cavity has a "hydrophobicty recognition zone" around the walls of the cavity which stimulates the various bitter potencies.

2,5-DKPs and some of their constituent amino acids are both known to be bitter. For example cyclo(Leu-Trp) is bitter, and tryptophan on its own is also bitter and hydrophobic. Otagiri et al., (1985) and Ishibashi et al., (1987) studied the taste of individual amino acids and found that proline, phenylalanine, glycine, leucine, tyrosine, and arginine are all similarly bitter tasting. In

addition, as the peptide length increases so does bitterness. Bitter linear peptides usually contain 8 or fewer amino acids. More than 8 amino acids in chain length have little impact on increased bitterness (Machasashi and Huang, 2009).

3.1 Proline Derivatives

The simple proline cyclodipeptides cyclo(L-Xaa-L-Pro), including the natural products cyclo(L-Pro-L-Pro), cyclo(L-His-L-Pro), cyclo(L-Phe-L-Pro), cyclo(L-Tyr-L-Pro), cyclo(L-Leu-L-Pro) and cyclo(L-Val-L-Pro), are found widespread in food and beverages (Table 2). Also the aromatic and aliphatic analogues cyclo(L-Phe-L-Pro), cyclo(L-Tyr-L-Pro), cyclo(L-Leu-L-Pro) and cyclo(L-Val-L-Pro) often occur together in a variety of bacterial and fungal cultures (Prasad, 2005; Park et al., 2006; Mitova, et al., 2004; Jayatilake, et al., 1996). The associated biological activity exhibited by this class of 2,5-diketopiperazines is extensive and has been reviewed (Borthwick, 2012). The proline 2,5-diketopiperazines are the most abundant and structurally diverse 2,5-diketopiperazines found in food because the proline amino acid residue adopts a *cis*-conformation about the Xaa-Pro tertiary amide bond and hence makes the Xaa-Pro sequences prone to 2,5-diketopiperazine formation (Yaron and Naider, 1993; Rose, et al., 1985). The ease with which L-Pro-2,5-diketopiperazines epimerize at the proline ring junction under either mild basic or acidic conditions, suggested that natural products derived from D-Pro-L-Xaa-2,5-diketopiperazines are in fact artefacts resulting from non-enzymatic epimerization of the corresponding parent L-Pro-L-Xaa-2,5-diketopiperazine natural products. This was shown to be the case in several examples (Bull, et al., 1998) where the L-proline to D-proline epimerization process occurs in vivo or during the isolation procedure.

Symmetrical Cyclo(L-Pro-L-Pro).

The symmetrical cyclo(L-Pro-L-Pro) **11** (Figure 11.) is the major 2,5-diketopiperazine in stewed beef (55ppm) (Chen et al., 2009), but is only a minor 2,5-diketopiperazine in cocoa (6.3ppm, 32.5 μmol/kg) where it is present well below its individual bitter taste threshold concentration (DoT, dose-over-threshold <0.1) (Stark and Hofmann, (2005). It also occurs in other beverages and foods: beer (Gautschi et al., 1997), bread (Ryan et al., 2009), chicken essence (Chen et al., 2004), coffee (Ginz and Engelhardt, 2000; 2001) and Comte cheese (Roudot-Agaron et al., 1993). It has a weak, slightly bitter, grainy taste at 50 ppm (Gautschi et al., 1997), a metallic taste at 760μmol/L (threshold concentration, TC), a bitter taste at 2580μmol/L (TC) (Stark and Hofmann, 2005) and a slightly brothy, metallic taste at 200ppm (Chen et al., 2009). It has also been isolated from the Antarctic psychrophilic bacterium *Pseudoalteromonas haloplanktis* TAC125, (Mitova et al., 2005) and shown to have weak antibacterial activity against *Pseudomonas aeruginosa* and *M. luteus* (Huberman et al., 2007). In addition it has been identified as a sex pheromone in the unicellular photosynthetic organism diatom *Seminavis robusta* (Gillard et al., 2013).

Aromatic Proline Derivatives

Cyclo(L-Trp-L-Pro)

The tryptophan-proline 2,5-diketopiperazine cyclo(L-Trp-L-Pro) **19** has been found as a minor 2,5-diketopiperazine (8.2ppm) in autolyzed yeast extract (Da Costa et al., 2010). It is also known as brevianamide F and is the simplest member of a large family of biologically active

tryptophan-proline 2,5-diketopiperazines that are produced by the fungi *A. fumigates* (Nierman et al., 2005) and *Aspergillus sp*. (Ding et al., 2010). It has also been isolated from the bacterium *Streptomyces sp. strain TN58* and shown to possess activity against two Gram-positive bacteria, *S.aureus* and *Micrococcus luteus* (Ben Ameur Mehdi et al., 2009). Although **19** initially showed potential for use in the treatment of cardiovascular dysfunction (Jamie et al., 2002a), it was later shown to be hepatoxic (Jamie et al., 2002b).

Cyclo(L-His-L-Pro)

The most studied of all the simple 2,5-diketopiperazines is the histidyl-proline 2,5-diketopiperazine **20** cyclo(L-His-L-Pro) (Minelli et al., 2008) (Figure 11.) which is found in a variety of foods, meat, wheat, eggs and dairy products with particularly high concentrations in fish and fish products (>2000pmol/g food) (Hilton et al., 1992). It is thought to occur in foods by the thermal manipulation of hydrolysed protein which occurs during food processing. It is well absorbed orally, and crosses the blood−brain barrier via a non-saturable mechanism (Minelli et al., 2008). It also occurs in humans (Prasad, 1995) as a metabolite from the thyrotropin-releasing hormone (TRH) and exhibits a wide variety of central nervous system, endocrine, electrophysiological, and cardiovascular effects (Minelli et al., 2008). Derivatives of cyclo(L-His-L-Pro) have been studied extensively to develop therapeutic agents for neuronal degeneration (Cornacchia et al., 2012).

Cyclo(L-Tyr-L-Pro)

The tyrosine derivative cyclo(L-Tyr-L-Pro) **13** is a very minor 2,5-diketopiperazine in cocoa (1.0ppm, 4.0µmol/kg) where it is present well below its individual bitter taste threshold concentration (DoT <0.1) (Stark and Hofmann, 2005). It has also been found in autolyzed yeast extract at 8.7ppm (Da Costa et al., 2010). It has a metallic taste at 190µmol/L (TC) and a bitter taste at 480µmol/L(TC) (Stark and Hofmann, 2005). The tyrosine derivative cyclo(L-Tyr-L-Pro) **13** also known as maculosin-1, is a fungal host-specific phytotoxin and together with cyclo(L-Phe-L-Pro) **21** maculosin-2, is produced by *Alternaria alternata* on spotted knapweed (*Centaurea maculosa*) (Stierle et al., 1988). These 2,5-diketopiperazines have been investigated (Bobylev et al., 1999) as possible templates for creating a safe and environmentally friendly specific bioherbicide.

Cyclo(L-Phe-L-Pro)

The other well characterised aromatic proline 2,5-diketopiperazine cyclo(L-Phe-L-Pro) **21** is found as a major 2,5-diketopiperazine in a variety of foods. It is one of the two major proline derivatives together with **22** cyclo(L-Leu-L-Pro) (Figure 11.) found in wheat sourdough and bread (Ryan et al., 2009). The lactic acid bacteria *Lactobacillus plantarum* FST 1.7 produces low levels of both antifungal 2,5-diketopiperazine's **21** and **22,** and has been used to ferment wheat sourdough which resulted in improved shelf life of wheat bread. Although both are formed during sourdough fermentation, temperature was found to be the main causative agent of 2,5-diketopiperazine formation during the baking process. It dramatically increased the levels of

both diketopiperazine's, with bread crumb and crust containing almost 100 and 2000 times respectively the levels found in dough prior to baking.

Both **21** and **22** were also found to be at sensorially active levels in bread crust, however both 2,5-diketopiperazines were found to be below the minimum inhibitory concentration for antifungal activity in bread (Ryan et al., 2009). Cyclo(L-Phe-L-Pro) **21** has also been found as the major 2,5-diketopiperazine in autolyzed yeast extract (133ppm) (Da Costa et al., 2010), and was the major 2,5-diketopiperazine (24ppm) together with six other proline-based diketopiperazines, **11, 22, 23, 24, 25,** and **29** identified in a range of commercial beers from different countries using distinctly different raw materials and brewing styles (Gautschi et al., 1997). Also cyclo(L-Phe-L-Pro) **21** is the second major diketopiperazine (36ppm) found to be of particular organoleptic interest among ten 2,5-diketopiperazines found in stewed beef (Chen et al., 2009), and has also been identified in roasted coffee along with the six other proline 2,5-diketopiperazines **11, 22 – 26** (Ginz and Engelhardt, 2000; 2001).

Cyclo(L-Phe-L-Pro) **21** has a metallic and bitter taste at the taste threshold concentration of 131µmol/L and 1020µmol/L respectively. However, in roasted cocoa nibs cyclo(L-Phe-L-Pro) **21** was present at 15.7ppm (64.3µmol/kg) but did not exceed its individual bitter taste threshold concentration (DoT 0.1) (Stark and Hofmann, (2005). It was described as drying, slightly astringent at 50 ppm (Gautschi et al., 1997), while being fishy, bitter, meaty and savory at 200ppm (Chen et al., 2009).

[Figure 11 near here]

The scavenging effect of cyclic dipeptides from the distillation residue of Awamori spirits (Takaya et al., 2007) showed that cyclo(L-Phe-L-Pro) had a moderate inhibitory effect against

the hydroxyl radical OH· (64.9% at 2.5 x 10^{-3}M). Cyclo(L-Phe-L-Pro) **21** has also been identified as an active quorum sensing signal molecule controlling the expression of genes important for the pathogenicity of the certain bacteria (Park, et al., 2006). In addition to *L. plantarum* (Stro□m, et al., 2002) it is also produced by the lactic acid bacteria *L. coryniformis* (Magnusson, et al., 2003) and has been shown to have antifungal activity against *A. fumigatus* and *P. Roquefort*. It was also found to have antibacterial activity against a range of bacteria including vancomycin-resistant enterococci (VRE) (Graz, et al., 1999; Rhee, et al., 2001) as well as inhibiting DNA topoisomerase I activity (Rhee, 2002a) and showing antimutagenic activity (Rhee, 2004).

Aliphatic Proline Derivatives

Cyclo(L-Leu-L-Pro)

The leucine derivative cyclo(L-Leu-L-Pro) **22** (Figure 11.) was found as one of the major 2,5-diketopiperazines in wheat sourdough and bread. It reached levels of 23mg/kg in bread crust (Ryan et al., 2009), is one of the major 2,5-diketopiperazines in cocoa (149ppm, 771µmol/kg) where it is present below its individual bitter taste threshold concentration (DoT 0.60) (Stark and Hofmann, (2005), and is the second most prominent proline DKP found in a range of beers (Gautschi et al., 1997). It has also been found in cooked beef (20.6ppm) (Chen et al., 2009), autolyzed yeast extract (19.2ppm) (Da Costa et al., 2010) and in coffee (Ginz and Engelhardt, 2000). It has a slightly bitter, salty character at 30 ppm (Gautschi et al., 1997), a vegetative, green, green beans, rare beef taste at 100 ppm (Chen et al., 2009) and a metallic taste at 120µmol/L (TC) and a bitter taste at 1190µmol/L (TC) (Stark and Hofmann, (2005). Cyclo(L-

Leu-L-Pro) **22** is an antifungal agent isolated from *Streptomyces sp.* and the bacterium *Achromobacter xylosoxidans* and has been identified as active against the rice blast fungus P. oryzae and a range of other fungi (Rhee, 2003). Remarkably **22** inhibits the production of the highly carcinogenic and teratogenic aflatoxins by the fungus *Aspergillus parasiticus* (Yan et al., 2004). SAR studies showed that the trans-isomers were less active, other cyclodipeptides without proline did not show the same activity and open chain dipeptides were inactive. The D-Leu isomer cyclo(D-Leu-L-Pro) of **22** was also shown (Nishanth Kumar et al., 2013) to be a potential biopreservative by strongly inhibiting the mycelia growth of fungus affecting aflatoxin production on soybeans and peanuts and was nontoxic to two normal human cell lines (FS normal fibroblast and L231 lung epithelial) up to 200µg/ml. In addition **22** was found to be active against 12 vancomycin-resistant enterococci (VRE) strains of bacteria and was also effective against three leukemic cell lines showing that it is a potential antileukemic and anti-VRE agent (Rhee, 2002). The combination of cyclo(L-Leu-L-Pro) **22** and cyclo(L-Phe-L-Pro) **21** appears to be synergistic. It exhibits strong activity against anaerobic Gram-negative and Gram-positive bacteria and had a radioprotective effect on five normal human lung fibroblast cells, showing survival rates higher than 90% (Rhee, 2006).

Cyclo(L-Ile-L-Pro)

The isoleucine derivative cyclo(L-Ile-L-Pro) **23** (Figure 11.) was the second most prominent proline 2,5-diketopiperazine found in cocoa (113ppm, 537µmol/kg) where it is present above its individual bitter taste threshold concentration (DoT 1.1) (Stark and Hofmann, (2005). It was the third most prominent proline 2,5-diketopiperazine found in a range of beers, but was present in a

concentration below its taste threshold (30ppm in water) (Gautschi et al., 1997). It was a minor component in cooked beef (12.7 ppm) (Chen et al., 2009) and was also found in bread (Ryan et al., 2009), chicken (Chen et al., 2004), and in coffee (Ginz and Engelhardt, 2000). It has a lingering, salty taste at 30 ppm (Gautschi et al., 1997), a metallic taste at 120µmol/L (TC) and a bitter taste at 480µmol/L (TC) (Stark and Hofmann, 2005). Cyclo(L-Ile-L-Pro) **23** was isolated from the fungus *Rhizoctonia solani* (Pedras et al., 2005), but was not phytotoxic to the leaves of canola or mustard. Cyclo(L-Ile-L-Pro) **23** was shown to have antifungal activity against *A. fumigatus* (MIC = 20mg/mL) and was isolated from the cultures of dairy propionibacteria (Lind, et al., 2007). However further investigations showed that (L-Ile-L-Pro) **23**, and cyclo(L-Phe-L-Pro) **21** were present in the non-innoculated growth medium probably formed from peptides present in the medium by the heat treatment used in its preparation.

Cyclo(L-Val-L-Pro)

The valine derivative **24** cyclo(L-Val-L-Pro) at a concentration of 1742 ppm, (8878µmol/kg) was identified as the most important bitter 2,5-diketopiperazine contributing to the bitter taste of roasted cocoa (Stark and Hofmann, 2005), where it is present well above its individual bitter taste threshold concentration (DoT 6.9). Out of twenty five 2,5-diketopiperazines found in roasted cocoa **24** together with the other proline 2,5-diketopiperazine **23** were two of five 2,5-diketopiperazines cyclo(L-Val-L-Pro) **24**, cyclo(L-Val-L-Leu) **26**, cyclo(L-Ala-L-Leu) **27**, cyclo(L-Ala-L-Ile) **28** and cyclo(L-Ile-L-Pro) **23**, identified as being present above their individual bitter taste threshold concentrations which contribute to the cocoa taste. The valine derivative **24** has also been found as one of the major 2,5-diketopiperazines in autolyzed yeast

extract at 120ppm (Da Costa et al., 2010), and one of the major 2,5-diketopiperazines in stewed beef at 24ppm (Chen et al., 2009). It is also present in chicken essence (Chen et al., 2004) and coffee (Ginz and Engelhardt, 2000). It has a lingering, metallic, salty taste a 10ppm (Gautschi et al., 1997), a bitter taste at 500ppm (Chen et al., 2009) and a metallic taste at 320μmol/L (TC) and a bitter taste at 1280μmol/L (TC) (Stark and Hofmann, (2005).

The valine 2,5-diketopiperazine cyclo(L-Val-L-Pro) **24** has been isolated from the fungus *Aspergillus fumigate* (Furtadoa et al., 2005) together with **11**, **21**, **22** and **26**, but all have very weak antibacterial activity, inhibiting the growth of *Staphylococcus aureus* and *Micrococcus luteus* only at the concentration of 2.9mmolL^{-1}. It has also been isolated from a variety of marine microorganisms (Borthwick, 2012), and as well as **13** and **21** it has been identified as an active LasI quorum-sensing signal molecule important for the plant growth promotion by *Pseudomonas aeruginosa* (Ortiz-Castro et al., 2011).

Cyclo(L-Ala-L-Pro)

The 2,5-diketopiperazine cyclo(L-Ala-L-Pro) **25** is the third most prominent proline 2,5-diketopiperazine found in cocoa, (228ppm, 1357μmol/kg) where it is present just below its individual bitter taste threshold concentration (DoT 0.9) (Stark and Hofmann, 2005) and it is found in a range of beers at a concentration below its taste threshold (30ppm) in water (Gautschi et al., 1997). It is also found in autolyzed yeast extract at 32ppm (Da Costa et al., 2010), in stewed beef at 11ppm (Chen et al., 2009) and as a minor component in bread (Ryan et al., 2009), in chicken essence (Chen et al., 2004) and in coffee (Ginz and Engelhardt, 2001). It has a weak, drying and very slightly astringent taste at 50 ppm (Gautschi et al., 1997), a metallic taste at

387μmol/L (TC) and a bitter taste at 1490μmol/L (TC) (Stark and Hofmann, 2005). Cyclo(L-Ala-L-Pro) **25** has been isolated from the sponge *Callyspongia sp*.(Chen et al., 2012) and the mangrove endophytic fungus *Penicillium thomi* obtained from the root of *Bruguiera gymnorrhiza* (Chen et al., 2007), and shown to moderately upregulated the three cytokines (IL10 1.56 fold, IFN-γ 1.36 fold and MCP-1 1.47 fold) in macrophage cells (Chen et al., 2012). It also exhibited cytotoxicity against three different cancer cell lines (A549, HepG2 and HT29) with IC50's of 9.6, 13.6 and 20.1μM respectively (Chen et al., 2007).

Cyclo(Gly-L-Pro)

The glycine derivative cyclo(Gly-L-Pro) **26** is a minor component in bread (Ryan et al., 2009), and in cocoa (0.3ppm, 2.1μmol/kg) where it is present well below its individual bitter taste threshold concentration (DoT <0.1) (Stark and Hofmann, 2005). It also occurs in chicken essence (Chen et al., 2004). It has a metallic taste at 384μmol/L (TC) and a bitter taste at 3250μmol/L (TC) (Stark and Hofmann, 2005). This endogenous 2,5-diketo-piperazine **26** occurs in rat brain (Gudasheva et al., 1996) and has been reported to enhance memory (Samonina et al., 2002), and have a neuroprotective effect (Guan et al., 2007). Together with the proline 2,5-diketopiperazines **21** and **25** cyclo(Gly-L-Pro) **26**, was isolated from the sponge *Callyspongia sp*. (Chen et al., 2012) and shown to regulate cytokines in macrophage cells. It moderately upregulates MCP-1(1.86-fold) and significantly upregulates IFN-γ (3.50-fold), but has no effect on TNF-α secretion.

Polar Proline Derivatives

Cyclo-(L-Thr-L-Pro)

The threonine derivative cyclo(L-Thr-L-Pro) **27** is a minor 2,5-diketopiperazine in cocoa (4.5ppm, 22.9µmol/kg) where it is present well below its individual bitter taste threshold concentration (DoT <0.1) (Stark and Hofmann, 2005). It has also been found in bread (Ryan et al., 2009), and chicken essence (Chen et al., 2004). It has a metallic taste at 631µmol/L (TC) and a bitter taste at 1281µmol/L (TC) (Stark and Hofmann, 2005). Cyclo(L-Thr-L-Pro) **27** was isolated from the fermentation broth of *Kribbella yunnanensis* (Liu et al., 2011) and from the endophytic fungus *Penicillium sp*.0935030 (Hai-bin et al., 2008) obtained from the mangrove plant *Acrostichum aureurm*. It showed an inhibitory effect on *S. aureus* and MRSA (Hai-bin et al., 2008).

Cyclo(L-Ser-L-Pro)

The serine-proline 2,5-diketopiperazine cyclo(L-Ser-L-Pro) **28** has been found in chicken essence (Chen et al., 2004). Cyclo(L-Pro-L-Ser**) 28** has been isolated from the fermentation broth of *Kribbella yunnanensis* (Liu et al., 2011) and from the culture of an endophytic bacterium *Pseudomonas brassicacearum* subsp. *Neoaurantiaca* in *Salvia miltiorrhiza* Bunge (Li et al., 2013), where it showed good antifungal activity against *F. solani*, *F. oxysporum f. sp. vasinfectum* and *F. oxysporum*.with MIC values ranging from 25 to 50 µg mL^{-1}. It has also been shown to have neuroprotective activity (Faden et al., 2005).

Cyclo-(L-Asn-L-Pro)

The asparagine derivative cyclo(L-Asn-L-Pro) **29** has been found in chicken essence (Chen et al., 2004) and cocoa (Rizzi, 1989). Cyclo(L-Asn-L-Pro) **29** was isolated from the fermentation broth of the aerobic actinomycete yeast *Sphaerisporangium* album (Liu et al., 2011) and showed cytotoxicity towards the chronic myeloid leukemia cell line k562 (IC_{50} = 0.26μmol·mL^{-1}).

Cyclo(L-Asp-L-Pro)

The aspartic acid 2,5-diketopiperazine cyclo(L-Asp-L-Pro) **30** was shown to be as effective as the pentapeptide enterostatin (Val-Pro-Asp-Pro-Arg) in inhibiting the caloric intake and dietary fat in rats (Lin et al., 1994), whereas the linear peptide Asp-Pro was inactive.

Cyclo(L-Arg-L-Pro)

The arginine derivative cyclo(L-Arg-L-Pro) **31** has a bitter taste at a threshold concentration of 4.5mM (Ishibashi et al., 1988). Cyclo(L-Arg-L-Pro) **31** also known as verpacamide A, has been isolated from the marine sponges *Axinella vaceleti* (Menna et al., 2012) and *Axinella polypoides* (Vergne et al., 2006) and is known to inhibit the growth of the fungus *Saccharomyces cerevisae* by inhibiting family 18 chitinases (Houston et al., 2004).

[Table 2 near here]

3.2. Aromatic Derivatives

Symmetrical Cyclo(L-Phe-L-Phe)

The symmetrical aromatic 2,5-diketopiperazine cyclo(L-Phe-L-Phe) **32** (Figure 12.) occurs in a variety of beverages and foods: beef, cheddar cheese, cocoa, white wine, yeast extract (13.1ppm) (Da Costa et al., 2010), and it is also present abundantly in some foods such as chicken essence (Chen et al., 2004). It has been shown to be a naturally occurring dual inhibitor that inhibited both serotonin transporter (SERT) and acetylcholinesterase (AChE) in vitro (Tsuruoka et al., 2012). Animal tests confirmed that oral administration of cyclo(L-Phe-L-Phe) increased cerebral monoamine levels and significantly improved depressive behavior in mice, and hence may abrogate the onset of depression and contribute to preventing the development of dementia (Tsuruoka et al., 2012). Cyclo(L-Phe-L-Phe) **32** originally isolated from *P. nigricans* (Birkenshaw and Mohammed, 1962) has also been isolated from a marine mangrove endophytic fungus (Zhu et al., 2003) and exhibits good anthelmintic activity against *H. nana* and *Schistosoma mansoni* in mice (Walchshofer et al., 1997).

Non-Symmetrical Aromatic Derivatives

Cyclo(L-Leu-L-Trp)

Cyclo(L-Leu-L-Trp) **33** (Figure 12.) occurs in chicken, cocoa and yeast extract (2.0ppm) (Da Costa et al., 2010). Cyclo(L-Leu-L-Trp) and cyclo(L-Phe-L-Trp) were isolated from the marine fungus *Acremonium strictum*, obtained from the Choristida sponge (Julianti et al., 2011), and cyclo(L-Leu-L-Trp) **33** has been isolated from the sponge *Callyspongia* sp. (Chen et al., 2012) and has been shown to upregulate the cytokines MCP-1 (2.24-fold) significantly and IFN-γ

(1.82-fold) moderately (Chen et al., 2012). The scavenging effect of cyclic dipeptides showed that cyclo(L-Leu-L-Trp) had an inhibitory effect against the hydroxyl radical OH· (66.0% at 2.5 x 10^{-5}M) (IC_{50} 1.8 x10^{-3}) (Furukawa et al., 2012) which is a higher antioxidant activity than vitamin E (37%).

Cyclo(L-Val- L-Tyr)

The valine derivative cyclo(L-Val-L-Tyr) **34** is a minor 2,5-diketopiperazine in cocoa (2.3ppm, 8.8µmol/kg) where it is present well below its individual bitter taste threshold concentration (DoT <0.1) (Stark and Hofmann, 2005), and also occurs in bread (Ryan et al., 2009). It has a metallic taste at 100µmol/L (TC) and a bitter taste at 190µmol/L (TC) (Stark and Hofmann, 2005). It was also isolated from the tubers of *Gymnadenia conopsea* (Zi et al., 2010) and from the halophilic actinomycete Nocardiopsis gilva YIM 90087 (Tian et al., 2013).

[Figure 12. near here]

Cyclo(L-Ala-L-Tyr)

The alanine 2,5-diketopiperazine cyclo(L-Ala-L-Tyr) **35** is a minor 2,5-diketopiperazine in cocoa (0.4ppm, 1.6µmol/kg) where it is present well below its individual bitter taste threshold concentration (DoT <0.1) (Stark and Hofmann, 2005) and it also occurs in bread (Ryan et al., 2009). It has a metallic taste at 430µmol/L (TC) and a bitter taste at 530µmol/L (TC) (Stark and Hofmann, 2005). It was isolated from the terrestrial endophytic bacterium *Pseudomonas brassicacearum subsp. Neoaurantiaca* present in the herbal plant *Salvia miltiorrhiza* Bunge (Li et al., 2013) and from *Bacillus subtilis* (Lu et al., 2009) as well as from the marine endophytic

fungus 1893# (Chen et al., 2006). It has an inhibitory effect (MIC = 50μgmL^{-1}) against the pathogenic fungi, *Fusarium oxysporum f. sp.* and an LC_{50}=71.0μg /mL against brine shrimp (Li et al., 2013).

Cyclo(L-Leu-L-Phe)

The leucine derivative cyclo(L-Leu-L-Phe) **36** is a major 2,5-diketopiperazine in yeast extract (49.4ppm) (Da Costa et al., 2010), but only a minor 2,5-diketopiperazine in cocoa (12.5ppm, 47.9μmol/kg) (Stark and Hofmann, 2005) where it is present below its individual bitter taste threshold concentration (DoT 0.3). It is also present in Awamori spirit (Takaya, et al., 2007), beef, beer, bread (Ryan et al., 2009), cheddar cheese, chicken (Chen et al., 2004), coffee, roast pork, red wine, white wine and balsamic vinegar. It has a metallic taste at 40μmol/L (TC) and a bitter taste at 190μmol/L (TC) (Stark and Hofmann, 2005). Cyclo(L-Leu-L-Phe) **36** was isolated from the marine bacteria *Sulfitobacter* strain M44 (Long et al., 2011). An investigation of the scavenging effect of cyclic dipeptides showed that cyclo(L-Leu-L-Phe) had a inhibitory effect against the hydroxyl radical OH· (23.5% at 2.5 x 10^{-5}M) (Furukawa et al., 2012), but this was a lower level of antioxidant activity than vitamin E (37%).

Cyclo(L-Ile-L-Phe)

The isoleucine derivative cyclo(L-Ile-L-Phe) **37** was present as only a very minor 2,5-diketopiperazine in yeast (0.01ppm) while its isomer *trans*-cyclo(Ile-Phe) **38** was present as a major 2,5-diketopiperazine (65.2ppm) (Da Costa et al., 2010). The *cis* isoleucine **37** is a significant 2,5-diketopiperazine in cocoa (16.0ppm, 61.5μmol/kg) but it is present below its

individual bitter taste threshold concentration (DoT 0.3) (Stark and Hofmann, 2005). It is also present in chicken (Chen et al., 2004), coffee (Ginz and Engelhardt, 2001), and bread (Ryan et al., 2009). It has a metallic taste at 40µmol/L (TC) and a bitter taste at 190µmol/L (TC) (Stark and Hofmann, 2005). Cyclo(L-Ile-L-Phe) **37** was isolated from the mangrove fungus Penicillium oxalicum (Liu et al., 2009) and the marine bacteria *Sulfitobacter* strain M44 (Long et al., 2011).

Cyclo(L-Val-L-Phe)

The valine 2,5-diketopiperazine cyclo(L-Val-L-Phe) **39** is a significant 2,5-diketopiperazine in yeast extract (24.3ppm) (Da Costa et al., 2010), but only a minor 2,5-diketopiperazine in beef (2.0ppm) (Chen et al., 2009) and in cocoa (14.3ppm, 58.0µmol/kg) where it is present well below its individual bitter taste threshold concentration (DoT <0.1) (Stark and Hofmann, 2005). It is also present in bread (Ryan et al., 2009), cheddar cheese, chicken (Chen et al., 2004), coffee, Parma ham, roast pork, balsamic vinegar, red wine and white wine. It has a metallic taste at 40µmol/L (TC) and a bitter taste at 1000µmol/L (TC) (Stark and Hofmann, 2005). Cyclo(L-Val-L-Phe) **39** has antimalarial activity against *Plasmodium berghei* schizont cultures with an IC_{50} of 3.89µM (Pérez-Picaso et al., 2012), and was isolated from the marine bacteria *Pseudoalteromonas* sp. NJ6-3-1 associated with the sponge *Hymeniacidon perieve*. It was identified as an autoinducer that could induce NJ6-3-1 to produce antibacterial substances under low cell density as part of a chemical defense system based on quorum sensing (Guo et al., 2011).

Cyclo(L-Ala-L-Phe)

The alanine derivative cyclo(L-Ala-L-Phe) **40** is a minor 2,5-diketopiperazine in yeast (4.2ppm) (Da Costa et al., 2010) and in cocoa (15.7ppm, 72.0µmol/kg) where it is present below its individual bitter taste threshold concentration (DoT 0.1) (Stark and Hofmann, 2005). It is also present in beef, bread (Ryan et al., 2009), chicken, red wine, and white wine. It has a metallic taste at 144µmol/L (TC) and a bitter taste at 570µmol/L (TC) (Stark and Hofmann, 2005). Cyclo(L-Ala-L-Phe) **40** is produced by the marine actinobacteria *Streptomyces praecox* 291-11 isolated from the seaweed, *Undaria pinnatifida*, rhizosphere and exhibits antifouling activity against the marine seaweed, *Ulva pertusa*, therapeutic ratio (LC_{50}/EC_{50} = 17.7) and fouling diatom, *Navicula annexa*. therapeutic ratio (LC_{50}/EC_{50} = 263) (Cho et al., 2012). This is ~9 fold and 40 fold respectively more potent than the commercial compound Irgarol which has a therapeutic ratio (LC_{50}/EC_{50}) of 2 and 6 respectively against these algae.

Cyclo(Gly-L-Phe)

The glycine 2,5-diketopiperazine cyclo(Gly-L-Phe) **41** is a minor 2,5-diketopiperazine in yeast (3.9ppm) (Da Costa et al., 2010) and in cocoa (2.3ppm, 11.3µmol/kg) where it is present well below its individual bitter taste threshold concentration (DoT <0.1) (Stark and Hofmann, 2005), and also occurs in bread (Ryan et al., 2009). It has a metallic taste at 50µmol/L (TC) and a bitter taste at 610µmol/L (TC) (Stark and Hofmann, 2005). Cyclo(L-Gly-L-Phe) **41** has antimalarial activity against *Plasmodium berghei* schizont cultures with an IC_{50} of 4.26µM (Pérez-Picaso et al., 2012), and was isolated from the marine actinobacteria *Streptomyces* sp. KMM 7210 and shown to exhibit cytotoxicity against the sea urchin *S.nudus* sperm (IC_{50} = 56µg /mL)

(Zhuravleva et al., 2011). It also exhibits 22% inhibition of the SKBR3 cancer cell line at a concentration of 100μM (Coursindel et al., 2010).

Polar Aromatic Derivatives

Cyclo(L-Ser-L-Phe)

The serine derivative cyclo(L-Ser-L-Phe) **42** (Figure 12.) is a minor 2,5-diketopiperazine in cocoa (0.7ppm, 3.0μmol/kg,) where it is present well below its individual bitter and taste threshold concentration (DoT <0.1) (Stark and Hofmann, 2005) and it also occurs in bread (Ryan et al., 2009). It has a metallic taste at 40μmol/L (TC) and a bitter taste at 210μmol/L (TC) (Stark and Hofmann, 2005). The cyclic serine-phenylalanine **42** has neuroprotective activity (Faden et al., 2005), and has been isolated from the insect pathogenic fungus *Verticillium hemipterigenum* (Isaka et al., 2005).

Cyclo(L-Asn-L-Phe)

The asparagine 2,5-diketopiperazine cyclo(L-Asn-L-Phe) **43** is a minor 2,5-diketopiperazine in cocoa **(**3.8ppm, 14.6μmol/kg,) where it is present well below its individual bitter and taste threshold concentration (DoT <0.1) (Stark and Hofmann, 2005) and it also occurs in bread (Ryan et al., 2009). It has a metallic taste at 380μmol/L (TC) and a bitter taste at 960μmol/L (TC) (Stark and Hofmann, 2005).

Cyclo(L-Asp-L-Phe)

The aspartic acid cyclo(L-Asp-L-Phe) **44** is a minor 2,5-diketopiperazine in cocoa **(**0.2ppm, 0.7μmol/kg) where it is present well below its individual bitter and taste threshold concentration (DoT <0.1) (Stark and Hofmann, 2005), and it also occurs in bread (Ryan et al., 2009). It has a metallic taste at 3810μmol/L (TC) and a bitter taste at 3810μmol/L (TC) (Stark and Hofmann, 2005). The 2,5-diketopiperazine cyclo(L-Asp-L-Phe) **44** is a degradation product of the artificial sweetener aspartame (see Section 4.1).

[Table 3 near here]

3.3. Aliphatic Derivatives

Symmetrical Aliphatic Derivatives

Cyclo(L-Leu-L-Leu)

The symmetrical aliphatic 2,5-diketopiperazine cyclo(L-Leu-L-Leu) **45** (Figure 13.) occurs in chicken essence (Chen et al., 2004), and has a bitter taste at a threshold concentration of 1.2mM (Li et al., 2013). It has been isolated from the marine microorganism *Bacillus subtilis* (Lu et al., 2009) and has been shown to be the intermediate that Cytochrome P450 CypX (CYP134A1) converts to the extracellular iron chelator pulcherrimin (Cryle et al., 2010).

Cyclo(L-Ile-L-Ile)

The symmetrical aliphatic 2,5-diketopiperazine cyclo(L-Ile-L-Ile) **46** occurs in chicken essence(Chen et al., 2004), and has a bitter taste at a threshold concentration of 0.6mM (Ishibashi et al., 1988). It has been isolated from the entomopathogenic fungus *Beauveria bassiana* (Grove and Pople, 1981).

Cyclo(L-Val-L-Val)

The symmetrical aliphatic 2,5-diketopiperazine cyclo(L-Val-L-Val) **47** is a minor 2,5-diketopiperazine in cocoa (47.1ppm, 237.6μmol/kg) where it is present below its individual bitter taste threshold concentration (DoT 0.2) (Stark and Hofmann, 2005). It also occurs in beef, bread (Ryan et al., 2009), chicken, and yeast extract (12.6ppm) (Da Costa et al., 2010). It has a metallic taste at 510μmol/L(TC) and a bitter taste at 1260μmol/L(TC) (Stark and Hofmann, 2005). It has been isolated from the marine microorganism *Bacillus subtilis* (Lu et al., 2009) and has been shown to have antimalarial activity (IC_{50} = 2.45μM) *P. berghei schizonts* (Pérez-Picaso et al., 2012).

Cyclo(L-Ala-L-Ala)

The symmetrical aliphatic 2,5-diketopiperazine cyclo(L-Ala-L-Ala) **48** occurs in cocoa (Stark and Hofmann, 2005) and chicken essence (Chen et al., 2004), and has a metallic and bitter taste at a threshold concentration of >7000μmol/L (Stark and Hofmann, 2005). It has been isolated

from the endophytic bacterium *Pseudomonas brassicacearum subsp.Neoaurantiaca* in *Salvia miltiorrhiza* Bunge (Li et al., 2013).

Cyclo(Gly-Gly)

The symmetrical aliphatic 2,5-diketopiperazine cyclo(Gly-Gly) **49** occurs in cocoa (Stark and Hofmann, 2005) bread (Ryan et al., 2009), and in the Maillard reaction products from the hydrolysate of the warm water fish tilapia (Huang et al., 2007), and has a metallic and bitter taste at a threshold concentration of >8000μmol/L (Stark and Hofmann, 2005). It also inhibits quorum sensing-dependent factor production in *Pseudomonas aeruginosa PAO1* both in vitro and in vivo (Musthafa et al., 2012).

[Figure 13. near here]

Non Symmetrical Aliphatic Derivatives

Cyclo(L-Leu-L-Ile)

The aliphatic 2,5-diketopiperazine cyclo(L-Ile-L-Leu) **50** (Figure 13.) is a significant 2,5-diketopiperazine in yeast extract (16.3ppm) (Da Costa et al., 2010) and also occurs in beef, beer, chicken (Chen et al., 2004), coffee, cocoa and white wine. It has been isolated from the marine microorganism *Bacillus subtilis* (Lu et al., 2009) and from the intertidal marine fungus *Paecilomyces marquandii* strain (Cabrera et al., 2006).

Cyclo(L-Val-L-Ile)

The aliphatic 2,5-diketopiperazine cyclo(L-Val-L-Ile) **51** occurs in chicken (Chen et al., 2004), and has also been isolated from the entomopathogenic fungus *Beauveria bassiana* (Grove and Pople, 1981).

Cyclo(L-Ala-L-Ile)

The aliphatic 2,5-diketopiperazine cyclo(L-Ala-L-Ile) **52** is a major 2,5-diketopiperazine in cocoa (117.8ppm, 639.5µmol/kg) where it is present above its individual bitter taste threshold concentration (DoT 1.2) (Stark and Hofmann, 2005). It also occurs in bread (Ryan et al., 2009) and chicken (Chen et al., 2004). It has a metallic taste at 168µmol/L (TC) and a bitter taste at 540µmol/L (TC) (Stark and Hofmann, 2005). It has been isolated from the Gram-positive actinobacteria *Nocardia alba* sp. nov (YIM 30243T) (Ding et al., 2009).

Cyclo(L-Val-L-Leu)

The aliphatic 2,5-diketopiperazine cyclo(L-Val-L-Leu) **53** is a major 2,5-diketopiperazine in cocoa (173.5ppm, 817.1µmol/kg) where it is present above its individual bitter taste threshold concentration (DoT 1.7) (Stark and Hofmann, 2005), and it also occurs in bread (Ryan et al., 2009) and in chicken essence (Chen et al., 2004). It has a metallic taste at 120µmol/L (TC) and a bitter taste at 470µmol/L (TC) (Stark and Hofmann, 2005). It has also been isolated from the marine microorganism *Bacillus subtilis* (Lu et al., 2009).

Cyclo(L-Ala-L-Leu)

The aliphatic 2,5-diketopiperazine cyclo(L-Ala-L-Leu) **54** is a major 2,5-diketopiperazine in cocoa (135.8ppm, 734.0µmol/kg) where it is present above its individual bitter taste threshold concentration (DoT 1.1) (Stark and Hofmann, 2005). It also occurs in bread (Ryan et al., 2009), chicken (Chen et al., 2004), roast pork, white wine, and yeast extract (10.4ppm) (Da Costa et al., 2010). It has a metallic taste at 30µmol/L (TC) and a bitter taste at 680µmol/L (TC) (Stark and Hofmann, 2005). It has also been isolated from the Gram-positive bacteria *Streptomyces* sp. (YIM 56130) a plant endophyte from *Drymaria diandra* (Yang et al., 2011). It is also produced by the marine actinobacteria *Streptomyces praecox* 291-11 isolated from the seaweed, *Undaria pinnatifida*, rhizosphere and exhibits antifouling activity against the marine seaweed, *Ulva pertusa*, therapeutic ratio (LC_{50}/EC_{50} = 21) and fouling diatom, *Navicula annexa*. therapeutic ratio (LC_{50}/EC_{50} = 120.2) (Cho et al., 2012). This is ~10 fold and 20 fold respectively more potent than the commercial compound Irgarol which has a therapeutic ratio (LC_{50}/EC_{50}) of 2 and 6 respectively against these algae.

Cyclo(Gly-L-Leu)

The aliphatic 2,5-diketopiperazine cyclo(Gly-L-Leu) **55** is a very minor 2,5-diketopiperazine in cocoa (<0.2ppm, <1.2 µmol/kg) where it is present well below its individual bitter taste threshold concentration (DoT <0.1) (Stark and Hofmann, 2005), and a minor 2,5-diketopiperazine in beef (2.7ppm) (Chen et al., 2009). It is also present in bread (Ryan et al., 2009), and yeast extract. It has a chalky taste at 250ppm, chalky and dirty taste at 500ppm (Gautschi et al., 1997). It also has a metallic taste at 529µmol/L (TC), a bitter taste at 590µmol/L (TC) (Stark and Hofmann,

2005), and a dirty, asparagus, isopropanol taste at 1000ppm (Gautschi et al., 1997). It has been isolated from the lactic acid bacteria *Lactobacillus plantarum* VTT E-78076 (Niku-Paavola et al., 1999) and exhibits 27% inhibition of the MCF-7 cancer cell line at a concentration of 100 μM (Coursindel et al., 2010).

Cyclo(L-Ala-L-Val)

The aliphatic 2,5-diketopiperazine cyclo(L-Ala-L-Val) **56** is a major 2,5-diketopiperazine in cocoa (107.8ppm, 633.5μmol/kg) where it is present below its individual bitter taste threshold concentration (DoT 0.4) (Stark and Hofmann, 2005). It also occurs in bread (Ryan et al., 2009), chicken (Chen et al., 2004), roast pork, balsamic vinegar and yeast extract (2.5ppm) (Da Costa et al., 2010). It has a metallic taste at 406μmol/L (TC) and a bitter taste at 1470μmol/L (TC) (Stark and Hofmann, 2005). It was isolated from the Gram-positive actinobacteria *Nocardia alba* sp. nov (YIM 30243T) (Ding et al., 2009), and from a marine-derived fungus *Aspergillus fumigatus* Fres (Zhao et al., 2010).

Cyclo(Gly-L-Val)

The aliphatic 2,5-diketopiperazine cyclo(Gly-L-Val) **57** occurs in chicken (Chen et al., 2004), and has a bitter taste at a threshold concentration of 5mM (Ishibashi et al., 1988). It has antimalarial activity against *Plasmodium berghei* schizont cultures with an IC_{50} of 3.23μM (Pérez-Picaso et al., 2012).

Cyclo(Gly-L-Ala)

The aliphatic 2,5-diketopiperazine cyclo(Gly-L-Ala) **58** occurs in cocoa (0-12ppm) (Rizzi, 1989), and has a metallic taste at 1950µmol/L (TC) and a bitter taste at 3910µmol/L (TC) (Stark and Hofmann, 2005). It has been isolated from the endophytic bacterium *Pseudomonas brassicacearum subsp. Neo-aurantiaca* in *Salvia miltiorrhiza* Bunge (Li et al., 2013), and exhibits 30% inhibition of the SKBR3 cancer cell line at a concentration of 100µM (Coursindel et al., 2010).

Polar Aliphatic Derivatives

Cyclo(L-Leu-L-Glu)

The aliphatic 2,5-diketopiperazine cyclo(L-Leu-L-Glu) **59** occurs in Awamori spirit (Takaya, et al., 2007). The scavenging effect of cyclic dipeptides showed that cyclo(L-Leu-L-Glu) **59** had an inhibitory effect against the hydroxyl radical OH· (97.8% at 2.5 x 10^{-5} M) (IC_{50} 8.0 x10^{-5}) (Furukawa et al., 2012), which is a higher antioxidant activity than vitamin E (37%).

Cyclo(Gly-L-Ser)

The aliphatic 2,5-diketopiperazine cyclo(Gly-L-Ser) **60** occurs in chicken (Table 4).

[Table 4 near here]

3.4. Methionine and Cysteine Derivatives

There are two naturally occurring sulfur containing amino acids that can form cyclic dipeptides; namely methionine and cysteine. The latter is potentially highly labile and thus few cyclic dipeptides have been reported from it. Methionine containing cyclic dipeptides were first reported in beer (Gautschi et al., 1997). They usually occur at trace concentrations in foods and beverages (Table 5) compared to many of the other 2,5-DKPs. Autolyzed yeasts are used in hydrolyzed vegetable proteins (HVP's) and are rich sources of these types of compounds according to Da Costa et al., in 2010. They reported the synthesis of eight methionine containing compounds; most were found in autolyzed yeasts, except cyclo(L-Met-L-Met) which is yet to be found in nature. Most cyclic dipeptides have no aroma where as methionine containing diketopiperazine derivatives have been described as being mildly vegatative or rotten. Sulfur containing compounds usually have a high impact and in this case this is displayed more in taste than aroma.

Proline Derivative Cyclo(L-Met-L-Pro)

The proline derivative cyclo(L-Met-L-Pro) **61** (Figure 14.) was the first methionine containing diketopiperazine to be reported and since then has been widely reported in a wide variety of foods and beverages. Gautschi et al., (1997) found seven proline-based diketopiperazines in beer including cyclo(L-Met-L-Pro). They evaluated them sensorially in water at concentrations from 10 to 50ppm, and these compounds were described as having flavor characteristics such as bitter, mouth coating, drying and astringent. More recently, cyclo(L-Met-L-Pro) was also found in yeast extracts used in food products such as Marmite, Bovril, Vegamite and Oxo. The taste of the synthesized molecule was evaluated in 0.30% salt water and was bitter and chalky at 10 and

50ppm (Da Costa et al., 2010). Cyclo(L-Pro-L-Met) **61** was first isolated from *P. aeruginosa* (Jayatilake et al., 1996) and later shown to have activity against *B. subtilis* and *S. aureus* with an MIC of 64 and 32µg/mL, as well as exhibiting strong inhibition towards fungi *R. solani, P. expansum*, and *F. oxysporum* (Kumar et al., 2013).

[Figure 14. near here]

Aromatic Derivative Cyclo(L-Met-L-Phe)

The phenylalanine derivative cyclo(L-Met-L-Phe) **62** has been reported in yeast extracts (2.8ppm) (Da Costa et al., 2010) and chicken. The taste of the synthesized molecule was evaluated in 0.5% ethanol/water and was described as creamy, vegetative, and metallic at 20ppm. The cyclodipeptide synthase recombinant pSHaeC06 (encoded in the genome of *Staphylococcus heamolyticus* JCSC1435) expressed in *E.coli* produced the major 2,5 diketopiperazine cyclo(L-Phe-L-Met) in 117.5mg/L of culture supernatant (Gondry et al., 2009).

Aliphatic Derivatives

Cyclo(L-Leu-L-Met)

The leucine derivative cyclo(L-Leu-L-Met) **63** has been reported in yeast extracts (4.1ppm) (Da Costa et al., 2010) and chicken. The taste of the synthesized molecule was evaluated in 1% ethanol/water and at 100ppm was described as weak, little taste and in 0.30% salt water at 200ppm as weak, vegetative, creamy and metallic. The scavenging effect of cyclic dipeptides showed that cyclo(L-Leu-L-Met) had an inhibitory effect against the hydroxyl radical OH·

(79.0% at 2.5 x 10^{-5}M) (IC_{50} 8.0 x10^{-4}) (Furukawa et al., 2012), which is a higher antioxidant activity than vitamin E (37%).

Cyclo(L-Ile-L-Met)

The isoleucine derivative cyclo(L-Ile-L-Met) **64** has been reported in yeast extracts (11.5ppm) (Da Costa et al., 2010), beef and chicken. The taste of the synthesized molecule was evaluated in plain water and at 100ppm was described as vegetable, celery and creamy.

Cyclo(L-Met-L-Val)

The valine derivative cyclo(L-Met-L-Val) **65** has been reported in yeast extracts (3.9ppm) (Da Costa et al., 2010) and chicken. The taste of the synthesized molecule was evaluated in 1% ethanol/water and at 50ppm was described as vegetative, metallic and creamy

Cyclo(L-Ala-L-Met)

The alanine derivative cyclo(L-Ala-L-Met) **66** has been reported in yeast extracts (0.02ppm) (Da Costa et al., 2010). The taste of the synthesized molecule was evaluated in 0.30% salt water, and at 1000ppm was described as creamy cheese, milky, and at 400ppm and 200ppm as creamy and milky.

Cyclo(Gly-L-Met)

The glycine derivative cyclo(Gly-L-Met) **67** has been reported in yeast extracts (0.09ppm) (Da Costa et al., 2010) and chicken. The taste of the synthesized molecule was evaluated in 0.30% salt water, and at 1000ppm was described as bitter, rotten, garbage, and at 400ppm as creamy, cheesy, melted cheese, and at 200ppm as vegetable, creamy. Cyclo(Gly-L-Met) displayed antibacterial activity against the Gram negative bacteria *E. coli* and *P. aeruginosa* (Pitchen, 2002).

Cysteine Derivative Cyclo(L-Cys-Gly)

The only cysteine 2,5-DKP reported in the literature so far is cyclo(L-cysteine-glycine). It was identified as a by-product of the Maillard reaction between glutathione/xylose by Wang et al., (2012) and gluthathione/ribose by Ho et al., (2007) respectively. Glutathione is a naturally ocurring linear tripeptide (γ–L-glutamyl-L-cysteinyl-glycine), which when reacted with a reducing sugar, can break down to the linear dipeptide (L-cysteine-glycine), followed by intramolecular cyclization to the 2,5-DKP. This cyclic dipeptide is unstable and can break down to various smaller sulfur-containing molecules. While no sensory data has been reported for the cyclic dipeptide, its is interesting to note that glutathione has been described as tasting Kokumi "mouthfilling and thickness of taste".

[Table 5 near here]

4. OTHER OCCURRENCE IN FOOD

Diketopiperazines are often found in food because they occur in a variety of fungal species, yeasts and bacteria that are used in the preparation of food. For example; from yeasts used for fermentation in brewing, from lactic acid bacteria used for fermentation in bread and from fungi (*Penicillium roqueforti*) used as a source of enzymes during maturation of blue-veined cheeses. They are also formed as metabolites of some sweeteners added to food, and metabolites of antibiotics added to the feeds of animals that end up in meat.

Roquefortine C

Roquefortine C **68** (Figure 15.), found in the blue vein of Roquefort and other blue cheeses, is a cyclodipeptide mycotoxin derived from the diketopiperazine cyclo(Trp-dehydro-His) and is a relatively common fungal metabolite produced by a number of Penicillium species. First isolated from *Penicillium roqueforti*, a fungi used as a source of proteolytic and lipolytic enzymes during maturation of blue-veined cheeses, roquefortine C **68** is also considered as one of the most important fungal contaminants of carbonated beverages, beer, wine, meats, cheese and bread. At high doses roquefortine C **68** is classified as a toxic compound (Aninat et al., 2001). The mechanisms underlying its toxicity and metabolism have been investigated by studying its interaction with mammalian cytochromes P450 (Aninat et al., 2001). In addition to these toxic properties, roquefortine C **68** reportedly possesses bacteriostatic activity against gram-positive bacteria (Kopp-Holtwiesche and Rehm, 1990), but only in those organisms containing haemoproteins (Aninat et al., 2001; 2005). Roquefortine C **68** contains the unusual E-dehydrohistidine moiety, a system that typically undergoes facile isomerization under acidic,

basic, or photochemical conditions to isoroquefortine C **69**, the 3,12 double-bond Z isomer of roquefortine C (Shangguan et al., 2008). However isoroquefortine C **69** is not a natural product and in contrast to roquefortine C **68** does not bind iron. Both have been synthesised (Shangguan et al., 2008).

[Figure 15. near here]

Antifungal Cyclo(L-Phe-trans-4-hydroxy-L-Pro) and analogues

Lactic acid bacteria (LAB) occur naturally in various foods such as dairy and meat products as well as vegetables and have long been used in food fermentation. Recently interest has increased in antifungal lactic acid bacteria as biopreservatives (Schnürer and Magnusson, 2005). Several antifungal cyclic dipeptides are produced by various strains of lactic acid bacteria. Cyclo(L-Phe-trans-4-hydroxy-L-Pro) **70** (Figure 15.) has been produced by the strains *Lactobacillus plantarum* MiLAB 393 (Ström et al., 2002), *P. pentosaceus*, *L. sakei* and *L. coryniformis* (Schnürer and Magnusson, 2005). Also cyclo(L-Phe-4R-HO-L-Pro) **70** has been isolated from the marine bacterium *Pseudoalteromonas luteoviolacea* (Jiang et al., 2000) and the marine yeast *Aureobasidium pullulans* (Shigemori et al., 1998), and has been shown to have weak antibacterial activity against *Staph Aureus* (Bertinetti et al., 2009; Jhaumeer-Laulloo et al., 2003) and *P.aeruginosa* (Jhaumeer-Laulloo et al., 2003).

Other simple cyclic dipeptides discussed earlier have also been produced by various strains of lactic acid bacteria. Cyclo(L-Phe-L-Pro) has been produced by the same strains as **70** (Schnürer and Magnusson, 2005; Ström et al., 2002) and from *L. plantarum* FST 1.7 (Ryan et al., 2009). Cyclo(Gly-L-Leu) from *L. plantarum* VTT E-78076 (Niku-Paavola et al., 1999). Cyclo(L-Leu-

L-Leu) from *L. plantarum* AF1 (Yang and Chang, 2010). Cyclo(L-Leu-L-Pro) from *L. plantarum* FST 1.7 (Ryan et al., 2009), *L. casei* AST18 (Li et al., 2012) and *L.amylovorus* DSM 19280 (Ryan et al., 2011). Cyclo(L-Pro-L-Pro), Cyclo(L-Tyr-L-Pro), Cyclo(L-Met-L-Pro), Cyclo(L-His-L-Pro) from *L.amylovorus* DSM 19280 (Ryan et al., 2011).

2,5-Diketopiperazine Siderophores

Siderophores are high-affinity chelating agents for ferric iron, produced by bacterial and fungal phytopathogens and also by graminaceous plants, for scavenging iron from the environment (Hider and Kong, 2010). The 2,5-diketopiperazine hydroxamate family of siderophores are outlined in Figure 16. Fungi produce the 2,5-diketopiperazine hydroxamates such as rhodotorulic acid **71**, dimerumic acid **72** and the coprogen B **73**, which may be absorbed following the ingestion of food containing such fungi (Pócsi et al., 2008). The smallest is Rhodotorulic acid **71** which is the diketopiperazine of N-d-acetyl-L-N d-hydroxyornithine (Figure 16.) which has been found mainly in basidiomycetous yeasts (van der Helm and Winkelmann, 1994). Rhodotorulic acid **71** was found to retard the spore germination of the fungus *Botrytis cinerea* and in combination with yeast. *R. glutinis* found to be effective in the biocontrol (Sansone et al., 2005) of iprodione-resistant *B. cinerea* of apple wounds caused by the disease.

[Figure 16. near here]

Dimerumic acid **72** (Figure 16.) has been isolated from the fermented food products of the molds *Monascus anka* and *Monascus pilosus* which are traditionally used for fermentation of

food in Asia. It has been shown to be an antioxidant with hepatoprotective actions against chemically induced liver injuries (Aniya, et al., 2000), as well as protecting against oxidative stress-induced cytotoxicity in isolated rat hepatocytes (Yamashiro et al., 2008). Further studies have shown that **72** exerts a chemoprotective effect on liver damage by Nrf2 upregulation. It inhibits inflammation by free radical down regulation and suppresses cancer cell invasion caused by oxidative stress (Lee et al., 2013). High concentrations of hexadentate fungal siderophore Coprogen B **73** was detected in penicillia-ripened Camembert- and Roquefort-type cheeses (up to 38±8 mg kg−1) and also in some fermented meat (sausage 1, 23±5 mg kg−1; salami 1, 2.0±0.5mg kg−1) (Emri et al., 2013). Because fungal siderophores are likely to possess atheroprotective effects in humans, optimisation of coprogen production in submerged cultures of Penicillium nalgiovense has been investigated to enable the potential development of siderophore-rich food additives or functional foods to increase the siderophore uptake in people prone to cardiovascular diseases (Emri et al., 2013).

Amoxicillin-2,5-diketopiperazine

Amoxicillin-2,5-diketopiperazine **76** (Figure 17.) occurs in food originating from animals treated with the beta-lactam antibiotic Amoxicillin **74**, which has been used to treat and prevent respiratory, gastrointestinal, urinary and skin bacterial infections in a variety of food animals including chickens, pigs, goats, sheep, calves and cattle (Ramos et al., 2012). Amoxicillin **74** is metabolized to the two major metabolites amoxicilloic acid **75** and amoxicillin-2,5-diketopiperazine **76** (Figure 17), but no antibacterial activity is recognized for these metabolites. In pigs, amoxicillin is rapidly metabolized to amoxicillin-2,5-diketopiperazine **76** after

intravenous, oral and subcutaneous administrations, but the metabolite was found in low concentrations and had nearly disappeared in all tissues within 36 hr after administration (<LOQ) (De Baere et al., 2002). It has also been detected in groundwater at a concentration of 0.03 µg/L (Gozlan et al., 2013). As a result **76** is found in the environment and especially in various water sources and food.

[Figure 17. near here]

Aspartame-2,5-diketopiperazine

The 2,5-diketopiperazine **78** cyclo(L-Asp-L-Phe) is a degradation product of the artificial sweetener aspartame (N-L-α-Asp-L-Phe-1-methyl ester) **77** (Figure 18.) which breaks down at the high temperatures and extremes of pH that can occur during food preparation. Also after 6 months in carbonated beverages, 25% of the aspartame **77** had been converted to the 2,5-diketopiperazine **78** (Tsang et al.,1985).

It has a bitter taste in contrast to the sweet taste of aspartame. There are very few human studies on the effects of 2,5-diketopiperazine **78** cyclo(L-Asp-L-Phe). However, a (one-day) exposure study showed that the 2,5-diketopiperazine **78** was tolerated without adverse effects (Geha et al., 1993). Although aspartame **77** had been implicated in causing brain tumors (Anonymous et al., 1980) after administering a large dosage for 2 years to rats, a detailed toxicology study of **78** did not reveal an increased incidence of cancer with the use of this peptide (Ishii et al., 1981a; 1981b; Geha et al., 1993), which was confirmed in a recent meta-analysis of 10 rodent studies (Mallikarjun and Sieburth, 2013).

[Figure 18. near here]

5. CONCLUSIONS

2,5-Diketopiperazines (cyclic dipeptides) are found in wide variety of foods and beverages and can be expected to be found in an ever-increasing number of foods. They are prevalent in protein-rich processed foods and are degradation products of polypeptides, produced as a thermal reaction by-product accompanying the Maillard reaction. They are also found in foods as natural products of yeast and bacteria used in fermentation of beer and bread and from fungi (e.g. *Penicillium roqueforti*) in the maturation of blue-veined cheese. They are present in a range of concentrations and display a variety of chemesthetic effects (bitter, astringent, umami, grainy, chalky, vegetative and metallic) that can contribute to the taste of a variety of foods and, although present at low concentrations in certain foods, their taste can be augmented by the synergistic effect of purines (e.g. theobromine in cocoa).

These smallest possible cyclic peptides are also found as natural products in yeasts, fungi, bacteria, plants, and animals and have a range of bioactivities from antitumor, antiviral, antifungal and antibacterial activities to anthroprotective effects. These simple chiral molecules are easily synthesised and have the potential to augment the taste of a variety of foods and to construct new functional foods to contribute to human health.

ABBREVIATIONS:

2,5-DKP	2,5-diketopiperazine;
DoT	dose-over-threshold, the ratio of the concentration and the threshold concentration of a compound;
TC	threshold concentration, the concentrations at which the typical taste qualities of the compounds were just detectable; The solvent of choice for tastings was water.
MW	microwave
Ugi-4CR	Ugi four component reaction
MIC	minimum inhibitory concentration; the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation.
LC_{50}	(lethal concentration, 50%) is the dose in toxicology required to kill half the members of a tested population after a specified test duration
ppm	part per million, one part in 10^6 defined as being from weight/weight units
Pg	Protecting group
LOQ	limit of quantification. The detection limit where the lowest quantity of a substance that can be distinguished from the absence of that substance within a stated confidence limit (generally 1%)
Boc	Di-*tert*-butyl dicarbonate,
HBTU	O-benzotriazol-1-yl-tetramethyluronium
DMAP	4-Dimethylaminopyridine

TAS2R's	bitter taste receptors
GPCR's	G protein-coupled receptors
TRH	thyrotropin-releasing hormone
VRE	vancomycin-resistant enterococci
IFN-γ	Interferon gamma
TNF-α	Tumor necrosis factor alpha
MRSA	Methicillin-resistant Staphylococcus aureus
SERT	serotonin transporter
AChE	acetylcholinesterase
HVP's	hydrolyzed vegetable proteins
Nrf2	Nuclear factor (erythroid-derived 2)

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Table 1. Cyclic Dipeptides, Kovats and Natural Occurrence

Cyclic Dipeptide	Kovats: Non-Polar	Occurrence in Food and Beverages
Cyclo(L-Ala-L-Val)	1563	Bread, chicken, cocoa, roast pork, balsamic vinegar, yeast extract
Cyclo(L-Ala-L-Pro) I	1609	Açai, beef, beer, bread, cheddar cheese, chicken, chipotle chilli,
Cyclo(L-Ala-L-Pro) II	1633	cocoa, Parma ham, malt extract, morel mushroom, porcini mushroom, pecans, roast pork, balsamic vinegar, red wine, white wine, yeast extract
Cyclo(Gly-L-Pro)	1651	Beef, bread, chicken, cocoa, yeast extract
Cyclo(L-Ala-L-Leu) I	1649	Bread, chicken, cocoa, roast pork, white wine, yeast extract
Cyclo(L-Ala-L-Leu) II	1654	
Cyclo(L-Val-L-Val)	1668	Beef, bread, chicken, cocoa, yeast extract
Cyclo(Gly-L-Leu)	1670	Beef, bread, cocoa, yeast extract
Cyclo(L-Pro-L-Val) I	1748	Awamori spirit, beef, beer, bread, cheddar cheese, chicken, cocoa,
Cyclo(L-Pro-L-Val) II	1794	coffee, corn extract, Parma ham, malt extract, roast pork, salami, balsamic vinegar, port wine, red wine, white wine, yeast extract, Greek yogurt
Cyclo(L-iLe-L-Pro) I	1837	Awamori spirits, beef, beer, chicken, cocoa, coffee, bread, yeast
Cyclo(L-iLe-L-Pro) II	1851	extract
Cyclo(L-Pro-L-Pro)	1845	Almonds, beef, beer, bread, cheddar cheese, comte cheese chicken, cocoa, coffee, corn extract, malt extract, pecans, tamarind, red wine, balsamic vinegar, yeast extract
Cyclo(L-Leu-L-Pro) I	1853	Almonds, Awamori spirit, beef, beer , bread, chicken, cocoa, coffee,
Cyclo(L-Leu-L-Pro) II	1867	corn extract, Parma ham, malt extract, porcini mushroom, roast pork, sake, salami, vanilla, balsamic vinegar, port wine, red wine, white wine, yeast extract, Greek yogurt
Cyclo(L-iLe-L-Leu) I	1855	Beef, beer, chicken, coffee, cocoa, white wine, yeast extract
Cyclo(L-iLe-L-Leu) II	1878	
Cyclo(Gly-L-Ser)	1866	Chicken
Cyclo(L-Leu-L-Leu) I	1896	Chicken
Cyclo(L-Leu-L-Leu) II	1901	
Cyclo(L-Ala-L-Met)	1901	Yeast extract
Cyclo(Gly-L-Met)	1938	Yeast extract
Cyclo(L-Ala-L-Phe)	2008	Beef, bread, chicken, cocoa, red wine, white wine, yeast extract
Cyclo(L-Met-L-Val) I	2010	Chicken, yeast extract
Cyclo(L-Met-L-Val) II	2021	
Cyclo(L-Leu-L-Met)	2117	Chicken, yeast extract
Cyclo(L-iLe-L-Met)	2131	Beef, chicken, yeast extract
Cyclo(L-Phe-L-Val) I	2136	Beef, bread, cheddar cheese, chicken, cocoa, coffee, Parma ham, roast
Cyclo(L-Phe-L-Val) II	2175	pork, balsamic vinegar, red wine, white wine, yeast extract
Cyclo(L-Met-L-Pro) I	2143	Beef, beer, cheddar cheese, chicken, cocoa, Parma ham, malt extract,
Cyclo(L-Met-L-Pro) II	2153	roast pork, balsamic vinegar, white wine, yeast extract
Cyclo(L-Phe-L-Pro) I	2208	Awamori spirit, beef, beer, bread , cheddar cheese, chicken, cocoa,
Cyclo(L-Phe-L-Pro) II	2256	coffee, corn extract, Parma ham, roast pork, red wine, white wine, balsamic vinegar, yeast extract
Cyclo(L-Leu-L-Phe) I	2233	Awamori spirit, beef, beer, bread, cheddar cheese, chicken, cocoa,
Cyclo(L-Leu-L-Phe) II	2249	coffee, roast pork, red wine, white wine, balsamic vinegar, yeast extract
Cyclo(L-Met-L-Met)	2384	Yeast extract

Cyclo(L-Met-L-Phe)	2529	Chicken, yeast extract
Cyclo(L-His-L-Pro)	2569	Meat, wheat, eggs, dairy products, fish
Cyclo(L-Pro-L-Tyr) I	2601	Bread, chicken, cocoa, yeast extract
Cyclo(L-Pro-L-Tyr) II	2616	
Cyclo(L-Phe-L-Phe) I	2624	Beef, cheddar cheese, chicken, cocoa, white wine, yeast extract
Cyclo(L-Phe-L-Phe) II	2684	
Cyclo(L-Pro-L-Trp) I	2868	Beef, chicken, cocoa, yeast extract
Cyclo(L-Pro-L-Trp) II	3244	
Cyclo(L-Leu-L-Trp) I	2873	Chicken, cocoa, yeast extract
Cyclo(L-Leu-L-Trp) II	2905	

Kovats indices are standard retention data using a homologous series of aliphatic alkanes on a non-polar column (50m, OV1 capillary column, 50m x 0.32mm i.d., 0.5µm film thickness, Restek, Bellefonte, PA).

All unreferenced sources of diketopiperazines are from a proprietary IFF database 2014.

Table 2. Proline Derivatives

Cyclic Dipeptide	Food Source (Conc.)	Flavour (Conc.)	Other Sources	Biological Activity
Cyclo(L-Pro-L-Pro)	Beef (55ppm),[a] cocoa (6.3ppm, 32.5 µmol/kg),[e] almonds, beer,[b] bread, [c] cheddar cheese, comte cheese,[h] chicken,[f] coffee,[g] corn extract, malt extract, pecans, tamarind, red wine, balsamic vinegar, yeast extract.	Brothy, metallic, green (200-1000 ppm),[a] Metallic (760 µmol/L), Bitter (2580 µmol /L).[e*]	*Pseudoalteromonas haloplanktis*TA C125.[m]	Weak antibacterial: Pseudomonas aeruginosa and M. luteus.[n, o]
Cyclo(L-Trp-L-Pro)	Yeast extract(8.2ppm), [k] beef, chicken, [f] cocoa.	-	*A. fumigates*[p] and *Aspergillus sp.*,[q]	Antibacterial: *S.aureus* and *Micrococcus luteus.*[r]
Cyclo(L-His-L-Pro)	Meat, wheat, eggs dairy products, fish.[t]	-	Human blood, brain, and gastrointestinal tract.[s]	CNS, endocrine, electrophysiological, and cardiovascular effects.[s]
Cyclo(L-Tyr-L-Pro)	Yeast extract(8.7ppm),[k] cocoa (1.0ppm, 4.0µmol/kg),[e] bread,[c] chicken.[f]	Metallic (190µmol/L), Bitter (480µmol/L).[e*]	Fungi: *Alternaria alternate.*[u]	Bioherbicide.[v]
Cyclo(L-Phe-L-Pro)	Yeast extract(133.4 ppm), [k] beef(36 ppm),[a] beer(24 ppm),[b] cocoa(15.7ppm, 64.3 µmol/kg),[e] (0-26ppm),[l] chicken(1.38ppm),[f] bread(0.025ppm),[c] awamori spirit,[d] cheddar cheese, coffee,[g] corn extract, Parma ham, roast pork, red wine, white wine, balsamic vinegar.	Metallic (131µmol/L),[e*] Fishy, bitter, meaty, savory (200 ppm).[a] Bitter (1020µmol/L).[e*]	Lactic acid bacteria: *L. plantarum*[w] *and L. coryniformis.*[x]	Antifungal,[w, x] Antibacterial.[y]
Cyclo(L-Leu-L-Pro)	Cocoa (149ppm,771µmol/kg),[e] (39-115ppm), [l] beer (30ppm),[b] beef (20.6ppm),[a] yeast extract (19.2ppm),[k] sake(15.7ppm),[j] chicken (3.55ppm), [f] bread (0.035ppm),[c] almonds, Awamori spirit,[d] coffee [g] corn extract, Parma ham, malt extract, porcini mushroom, roast pork,salami, vanilla, balsamic vinegar, port wine, red wine, white wine, Greek yogurt.	Slightly bitter, salty character (30 ppm) [b] Vegetative, green, green beans, rare beef (100 ppm) [a] Metallic (120µmol/L), Bitter (1190µmol/L).[e*]	*Streptomyces sp.*[z] *Achromobacter xylosoxidans.*[aa]	Antifungal,[z,aa] Antibacterial,[bb, cc] Antileukemic,[bb] Radioprotective.[cc]
Cyclo(L-Ile-L-Pro)	Cocoa (113ppm,537µmol/kg),[e] yeast extract (29.5ppm), [k] beef (12.7ppm),[a] Awamori spirits,[d] beer,[b] bread,[c] chicken,[f] coffee.[g]	Salty taste (30 ppm) [b] Metallic (120µmol/L), Bitter (480µmol/L).[e*]	Fungus *Rhizoctonia solani.*[dd]	Not phytotoxic.[dd]

Cyclo(L-Val-L-Pro)	Cocoa(1742ppm,8878µmol/kg),[e] yeast extract (120ppm),[k] beef (24ppm).[a] beer (10ppm),[b] chicken (3.39ppm),[f] Awamori spirit,[d] bread [c] cheddar cheese, coffee,[g] corn extract, Parma ham, malt extract, roast pork, salami, balsamic vinegar, port wine, red wine, white wine, Greek yogurt.	Lingering, metallic, salty taste (10 ppm),[b] Bitter (500ppm),[a] Metallic (320µmol/L), Bitter (1280µmol/L).[e*]	Fungi: *Aspergillus fumigates.*[ee]	Very weak antibacterial.[ee]
Cyclo(L-Ala-L-Pro)	Cocoa(228ppm,1357µmol/kg)[e] (19-44ppm),[l] yeast extract(31.5ppm),[k] beef (11.3ppm),[a] Açai, beer,[b] bread,[c] cheddar cheese, chicken,[f] chipotle chilli, Parma ham, malt extract, morel mushroom, porcini mushroom, pecans, roast pork, balsamic vinegar, red wine, white wine.	Weak,drying,very slightly astringent (50 ppm),[b] beefy, slightly bloody (200ppm), bitter (400ppm),[a] Metallic (387µmol/L), Bitter (1490µmol/L).[e*]	Fungus *Penicillium thom,I*[gg] sponge *Callyspongia sp.*[ff]	Regulates cytokines in macrophage cells.[ff] Cytotoxic against cancer cell lines.[gg]
Cyclo(Gly-L-Pro)	Beef (2.0ppm),[a] chicken (1.66ppm),[f] cocoa (0.3ppm,2.1µmol/kg)),[e] (2-7ppm),[l] bread,[c] yeast extract.	Metallic (384µmol/L), Bitter (3250µmol/L).[e*]	Occurs in rat brain [hh] sponge: *Callyspongia sp.*[ff]	Regulates cytokines in macrophage cells.[ff]
Cyclo(L-Thr-L-Pro)	Cocoa (4.5ppm,22.9µmol/kg),[e] chicken (0.16ppm),[f] bread.[c]	Metallic (631µmol/L), Bitter (1281µmol/L).[e*]	Fungus *Penicillium sp.*0935030 [jj] and *Kribbella yunnanensis.*[ii]	Inhibits *Staph.A* and *MRSA.*[jj]
Cyclo(L-Ser-L-Pro)	Chicken (minor component).[f]	-	Bacteria: *Pseudomonas brassicacearum* subsp.[kk] and *Kribbella yunnanensis.*[ii]	Neuroprotective activity.[ll]
Cyclo(L-Asn-L-Pro)	Chicken (minor component),[f] cocoa.[l]	-	Yeast: *Sphaerisporangium album.*[ii]	Cytotoxic against leukemia cell line k562.[ii]
Cyclo(L-Asp-L-Pro)	-	-	-	Inhibits caloric intake and dietary fat in rats.[mm]
Cyclo(L-Arg-L-Pro) Verpacamide A	-	Bitter (4.5mM).[kk*]	Marine sponges: *Axinella vaceleti.*[nn] and *Axinella polypoides.*[oo]	Inhibits chitinases of fungus *Saccharomyces cerevisae.*[pp]

All unreferenced sources of diketopiperazines are from a proprietary IFF database 2014.

[*] taste threshold concentrations

[a] Chen et al. (2009), [b] Gautschi et al. (1997), [c] Ryan et al. (2009), [d] Takaya et al. (2007), [e] Stark and Hofmann, (2005). [f] Chen et al. (2004). [g] Ginz and Engelhardt, (2000; 2001),[h]Roudot-Agaron et al. (1993),[i] Kawai et al. (1991), [j] Takahashi et al. (1974) [k] Da Costa et al. (2010), [l] Rizzi et al. (1989), [m]Mitova et al. (2005), [n] Huberman et al.(2007), [o] Gillard et al. (2013), [p] Nierman et al. (2005), [q]Ding et al. (2010), [r] Ben Ameur Mehdi et al. (2009), [s] Minelli et al. (2008), [t] Hilton et al. (1992),[u] Stierle et al. (1988), [v] Bobylev et al. (1999), [w] Stro□m et al. (2002), [x] Magnusson et al. (2003), [y] Rhee et al. (2001),[z] Rhee, (2003), [aa] Yan, et al. (2004), [bb] Rhee, (2002), [cc] Rhee, (2006), [dd] Pedras, et al. (2005), [ee] Furtadoa et al. (2005), [ff] Chen et al. (2012), [gg] Chen et al. (2007), [hh] Gudasheva et al. (1996), [ii] Liu et al. (2011), [jj] Hai-bin et al. (2008), [kk] Li et al. (2013), [ll] Faden et al. (2005), [mm] Lin et al. (1994), [nn] Menna et al. (2012), [oo] Vergne C et al. (2006), [pp] Houston et al. (2004),

Table 3. Aromatic Derivatives

Cyclic Dipeptide	Food Source	Flavour Conc.	Other Sources	Biological Activity
Cyclo(L-Phe-L-Phe)	Yeast extract (13.1ppm),[k] chicken,[f] beef, cheddar cheese, cocoa, white wine.	-	Bacteria: *P. nigricans*,[rr] mangrove endophytic fungus.[ss]	Inhibitor of acetylcholinesterase (AChE) in vitro.[qq] anthelmintic activity against *H. nana* and *Schistosoma mansoni* in mice.[tt]
Cyclo(L-Leu-L-Trp)	Yeast extract (2.0ppm),[k] chicken, cocoa.	-	Fungus: *Acremonium strictum.*[uu]	Upregulated cytokines MCP-1 (2.24-fold) and IFN-γ (1.82-fold),[ff] antioxidant activity > vitamin E.[vv]
Cyclo(L-Val-L-Tyr)	Cocoa (2.3ppm,8.8µmol/kg),[e] Bread.[c]	Metallic (100µmol/L), Bitter (190µmol/L).[e*]	Tubers: *Gymnadenia conopsea*,[ww] halophilic actinomycete Nocardiopsis gilva YIM 90087.[xx]	-
Cyclo(L-Ala-L-Tyr)	Cocoa (0.4ppm, 1.6µmol/kg),[e] bread.[c]	Metallic (430µmol/L), Bitter (530µmol/L).[e*]	Bacteria: *P. brassicacearum subsp.Neoaurantiac*[kk] and *Bacillus subtilis.*[yy]	Antifungal activity *Fusarium oxysporum f. sp* (MIC=50 $\mu g mL^{-1}$).[kk]
Cyclo(L-Leu-L-Phe)	Yeast extract (49.4ppm),[k] cocoa (12.5ppm, 47.9µmol/kg),[e] coffee, Awamori spirit,[d] beef, beer, bread,[c] cheddar cheese, chicken,[f] roast pork, red wine, white wine, balsamic vinegar.	Metallic (40µmol/L), Bitter (190µmol/L).[e*]	Marine bacteria: *Sulfitobacter* strain M44.[zz]	-
Cyclo(L-Ile-L-Phe)	Yeast extract (65.2ppm),[k] cocoa (16.0ppm, 61.5µmol/kg),[e] chicken,[f] coffee,[g] bread.[c]	Metallic (40µmol/L), Bitter (190µmol/L).[e*]	Fungus: *Penicillium oxalicum.*[aaa] Bacteria: *Sulfitobacter* strainM44.[zz]	-
Cyclo(L-Val-L-Phe)	Yeast extract (24.3ppm),[k] cocoa (14.3ppm, 58.0µmol/kg),[e] beef (2.0ppm),[a] bread,[c] chicken,[f] cheddar cheese, coffee, Parma ham, roast pork, balsamic vinegar, red wine, white wine.	Phenolic, band-aid, sweaty, formaldehyde (100 ppm)[a] Metallic (40µmol/L), Bitter (1000µmol/L).[e*]	Bacteria: *Pseudoalteromonas* sp. NJ6-3-1.[ccc]	Antimalarial activity *Plasmodium berghei* IC_{50} 3.89µM.[bbb]
Cyclo(L-Ala-L-Phe)	Cocoa (15.7ppm, 72.0µmol/kg),[e] yeast extract (4.2ppm),[k] beef, bread,[c] chicken, red wine, white wine.	Metallic (144µmol/L), Bitter (570µmol/L).[e*]	Bacteria: *Streptomyces praecox* 291-11.[ddd]	Antifouling activity (LC_{50}/EC_{50} = 17.7).[ddd]

Cyclo(Gly-L-Phe)	Cocoa(2.3ppm, 11.3μmol/kg),[e] yeast extract (3.9ppm),[k] bread.[c]	Metallic (50μmol/L), Bitter (610μmol/L).[e*]	Bacteria: *Streptomyces* sp. KMM 7210.[eee]	Antimalarial activity *Plasmodium berghei* IC_{50} 4.26μM.[bbb]
Cyclo(L-Ser-L-Phe)	Cocoa (0.7ppm, 3.0μ mol/kg)[e] bread.[c]	Metallic (40μmol/L), Bitter (210μmol/L).[e*]	Fungus: *Verticillium hemipterigenum.*[ggg]	Neuroprotective activity.[ll]
Cyclo(L-Asn-L-Phe)	Cocoa (3.8ppm, 14.6μmol/kg),[e] bread.[c]	Metallic (380μmol/L), Bitter (960μmol/L).[e*]	-	-
Cyclo(L-Asp-L-Phe)	Cocoa (0.2ppm, 0.7μmol/kg),[e] bread.[c]	Metallic (3810μmol/L), Bitter (3810μmol/L).[e*]	-	-

All unreferenced sources of diketopiperazines are from a proprietary IFF database 2014.

[*] taste threshold concentrations

[a-k, ff, kk, ll] See Table 2, [qq] Tsuruoka et al. (2012), [rr] Birkenshaw et al. (1962),[ss] Zhu et al. (2003),[tt] Walchshofer, et al. (1997),[uu] Julianti et al. (2011),[vv] Furukawa et al. (2012),[ww] Zi et al. (2010),[xx] Tian et al. (2013),[yy] Lu et al. (2009),[zz] Long et al. (2011),[aaa] Liu et al. (2009),[bbb] Pérez-Picaso et al. (2012),[ccc] Guo et al. (2011), [ddd] Cho et al. (2012),[eee] Zhuravleva et al. (2011), [ggg] Isaka et al. (2005),

Table 4. Aliphatic Derivatives

Cyclic Dipeptide	Food Source (Conc.)	Flavour (Conc.)	Other Sources	Biological Activity
Cyclo(L-Leu-L-Leu)	Chicken.[f]	Bitter (1.2 mM).[hhh*]	Bacteria:*Bacillus subtilis.*[yy]	Intermediate for iron chelator pulcherrimin.[jjj]
Cyclo(L-Ile-L-Ile)	Chicken.[f]	Bitter (0.6 mM).[hhh*]	fungus: *Beauveria bassiana.*[kkk]	-
Cyclo(L-Val-L-Val)	Cocoa (47.1ppm,237.6µmol/kg)),[e] yeast extract (12.6ppm),[k] beef, bread, [c] chicken.	Metallic (510µmol/L), Bitter (1260µmol/L).[e*]	Bacteria: *Bacillus subtilis.*[yy]	Antimalarial activity (IC_{50} 2.45µM) *P. berghei schizonts.*[bbb]
Cyclo(L-Ala-L-Ala)	Cocoa (>7000µmol/L) [e] chicken.[f]	Metallic and Bitter at >7000µmol/L.[e]	Bacteria: *Pseudomonas brassicacearum* subsp.[kk]	-
Cyclo(Gly-Gly)	Cocoa (>8000µmol/L),[e] bread,[c] tilapia fish.[lll]	Metallic and Bitter at >8000µmol/L.[e]	-	Inhibits quorum sensing in *P.aeruginosa* PAO1 in vitro and in vivio. [mmm]
Cyclo(L-Leu-L-Ile)	Yeast extract (16.3ppm)[k] chicken,[f] beef, beer, coffee, cocoa, white wine.	-	Bacteria: *Bacillus subtilis,*[yy] fungus: *Paecilomyces marquandii*. [nnn]	-
Cyclo(L-Val-L-Ile)	Chicken.[f]	-	Fungus: *Beauveria bassiana.*[kkk]	-
Cyclo(L-Ala-L-Ile)	Cocoa (117.8ppm,639.5µmol/kg), [e] chicken,[f] bread. [c]	Metallic (168µmol/L), Bitter (540µmol/L).[e]	Bacteria: *Nocardia alba* sp. nov (YIM 30243T).[ooo]	-
Cyclo(L-Val-L-Leu)	Cocoa (173.5ppm, 817.1µmol/kg)),[e] bread,[c] chicken.[f]	Metallic (120µmol/L), Bitter (470µmol/L).[e]	Bacteria: *Bacillus subtilis.*[yy]	-
Cyclo(L-Ala-L-Leu)	Cocoa (135.8ppm, 734.0µmol/kg), [e] yeast extract (10.4ppm),[k] bread,[c] chicken,[f] roast pork, white wine.	Metallic (30µmol/L), Bitter (680µmol/L).[e]	Bacteria: *Streptomyces* sp. (YIM 56130).[ppp]	Antifouling activity (LC_{50}/EC_{50} = 21) [ddd]
Cyclo(Gly-L-Leu)	Beef (2.7ppm),[a] cocoa (<0.2ppm, <1.2 µmol/kg),[e] bread, [c] yeast extract.	Chalky at 250ppm, Chalky and dirty at 500ppm,[b] Metallic(529µmol/L) ,Bitter (590µmol/L),[e] and dirty, asparagus, isopropanol (1000ppm).[b]	Bacteria: *Lactobacillus plantarum* VTT E-78076.[qqq]	Inhibition MCF-7 cancer cells (27% at 100 µM).[iii]
Cyclo(L-Ala-L-Val)	Cocoa (107.8ppm,	Metallic	Bacteria:	-

	633.5µmol/kg),[e] yeast extract (2.5ppm),[k] bread,[c] chicken,[f] roast pork, balsamic vinegar.	(406µmol/L), Bitter (1470µmol/L).[e]	*Nocardia alba* sp. nov (YIM 30243T).[ooo] Fungus: *Aspergillus fumigates* Fres.[rrr]	
Cyclo(Gly-L-Val)	Chicken[f]	Bitter (5 mM).[hhh]	-	Antimalarial activity (IC_{50} 3.23µM) against *Plasmodium berghei*.[bb]
Cyclo(Gly-L-Ala)	Cocoa (0-12ppm).[l, e]	Metallic (1950µmol/L), Bitter (3910µmol/L).[e]	Bacteria: *Pseudomonas brassicacearum subsp. Neoaurantiaca.* [kk]	Inhibition of SKBR3 cancer cells (30% at 100 µM).[iii]
Cyclo(L-Leu-L-Glu)	Awamori spirit.[d]	-	-	Inhibitory (IC_{50} 8.0 $x10^{-5}$) against the hydroxyl radical OH·.[vv]
Cyclo(L-Glu-L-Leu) ethyl ester	Awamori spirit.[d]			
Cyclo(L-Glu-L-Leu) benzyl ester	Awamori spirit.[d]			
Cyclo(L-Glu-L-Val) ethyl ester	Awamori spirit.[d]			
Cyclo(Gly-L-Ser)	Chicken.	-	-	-

All unreferenced sources of diketopiperazines are from a proprietary IFF database 2014.

[*] taste threshold concentrations

[a-k, ff, kk] See Table 2, [vv, yy, bbb, ddd] See Table 3, [hhh]Ishibashi et al. (1988), [iii] Coursindel et al. (2010), [jjj]Cryle et al. (2010), [kkk] Grove and Pople, (1981), [lll] Huang et al. (2007),[mmm] Musthafa et al. (2012),[nnn] Cabrera et al. (2006),[ooo]Ding et al. (2009),[ppp] Yang et al. (2011), [qqq] Niku-Paavola et al. (1999),[rrr] Zhao et al. (2010),

Table 5. Methionine and Cysteine Derivatives

Cyclic Dipeptide	Food Source (Conc.)	Flavour (Conc.)	Other Sources	Biological Activity
Cyclo(L-Met-L-Pro)	Yeast extract (60.1ppm),[k] beer (30ppm),[b] beef (3.3ppm),[a] cheddar cheese, chicken, cocoa, Parma ham, malt extract, roast pork, balsamic vinegar, white wine.	Bitter and chalky (10 and 50 ppm).[k]	Bacteria: *P. aeruginosa*.[sss]	Antibacterial activity against *B. subtilis*, *S. aureus* and *E. coli*, inhibition of fungi *R. solani*, *P.expansum*, and *F. oxysporum*.[ttt]
Cyclo(L-Phe-L-Met)	Yeast extract (2.8ppm),[k] chicken.	Creamy, vegetative and metallic (20ppm).[k]	Recombinant pSHaeC06 expressed in *E.coli*.[uuu]	-
Cyclo(L-Leu-L-Met)	Yeast extract (4.1ppm),[k] chicken.	Weak, vegetative, creamy, metallic (200ppm).[k]	-	Antioxidant activity > vitamin E.[vv]
Cyclo(L-Ile-L-Met)	Yeast extract (11.5ppm),[k] beef, chicken.	Creamy, vegetable celery (100ppm).[k]	-	-
Cyclo(L-Met-L-Val)	Yeast extract (3.9ppm),[k] chicken.	Vegetative, metallic, creamy (50ppm).[k]	-	-
Cyclo(L-Ala-L-Met)	Yeast extract (0.02ppm).[k]	Creamy, milky (200ppm), creamy cheese, milky (1000ppm).[k]	-	-
Cyclo(Gly-L-Met)	Yeast extract (0.09ppm).[k]	Vegetable, creamy (200ppm), creamy, cheesy, melted cheese (400ppm), bitter, rotten, garbage (1000ppm).[k]	-	Antibacterial activity against *E. coli* and *P. aeruginosa*.[vvv]
Cyclo(L-Cys-Gly)	Gluthathione/xylose or ribose breakdown product.			

All unreferenced sources of diketopiperazines are from a proprietary IFF database 2014.

[a, b, k,] See Table 2, [vv] See Table 3,[sss] Jayatilake et al. (1996),[ttt] Kumar et al. (2013),[uuu] Gondry et al. (2009),[vvv] Pitchen, (2002).

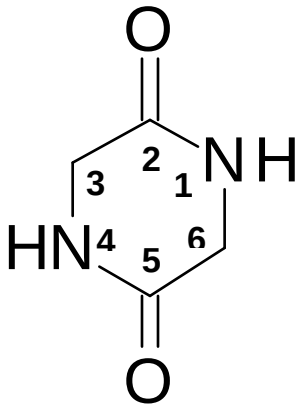


Figure 1.

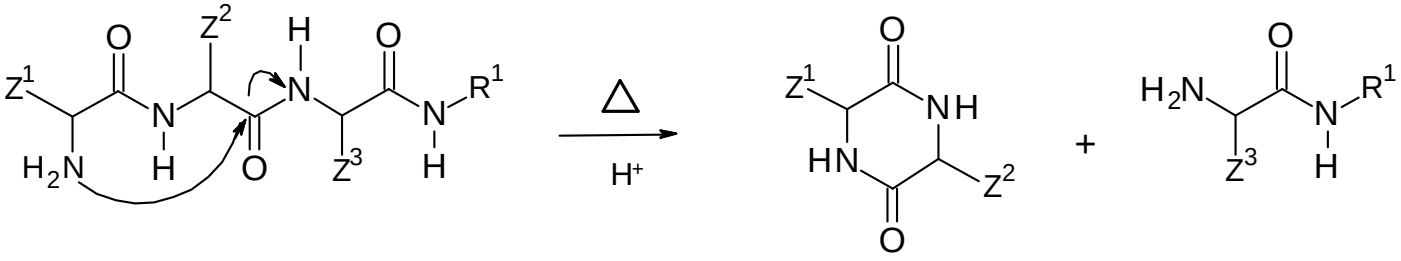


Figure 2. Proposed formation of cyclic dipeptides (Rizzi 1989).

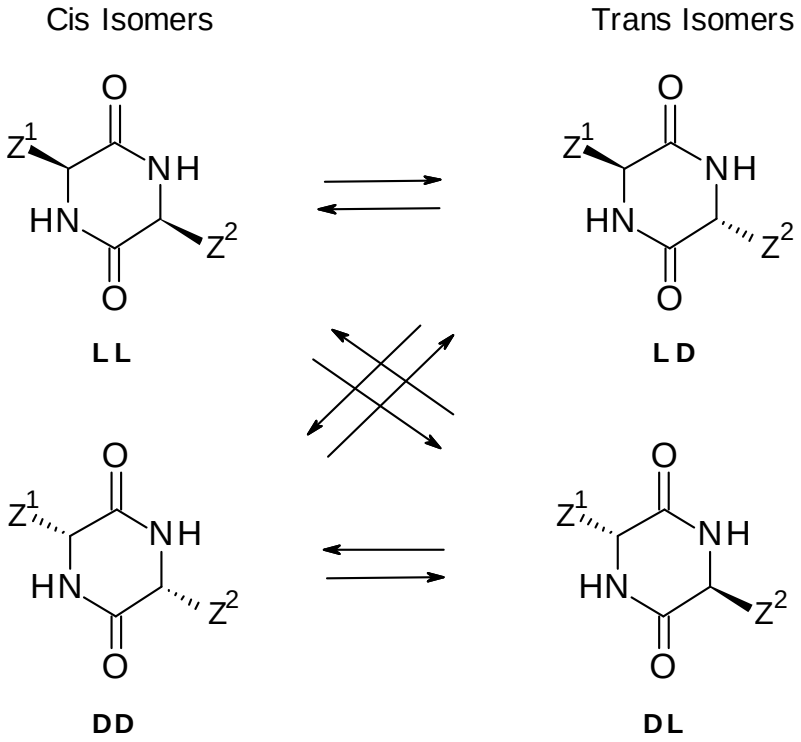


Figure 3. *Cis* and *Trans* isomers.

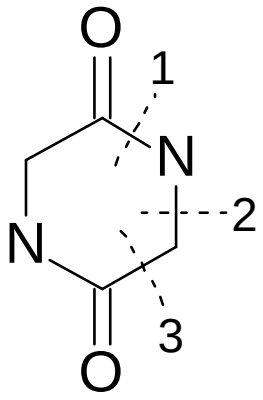


Figure 4.

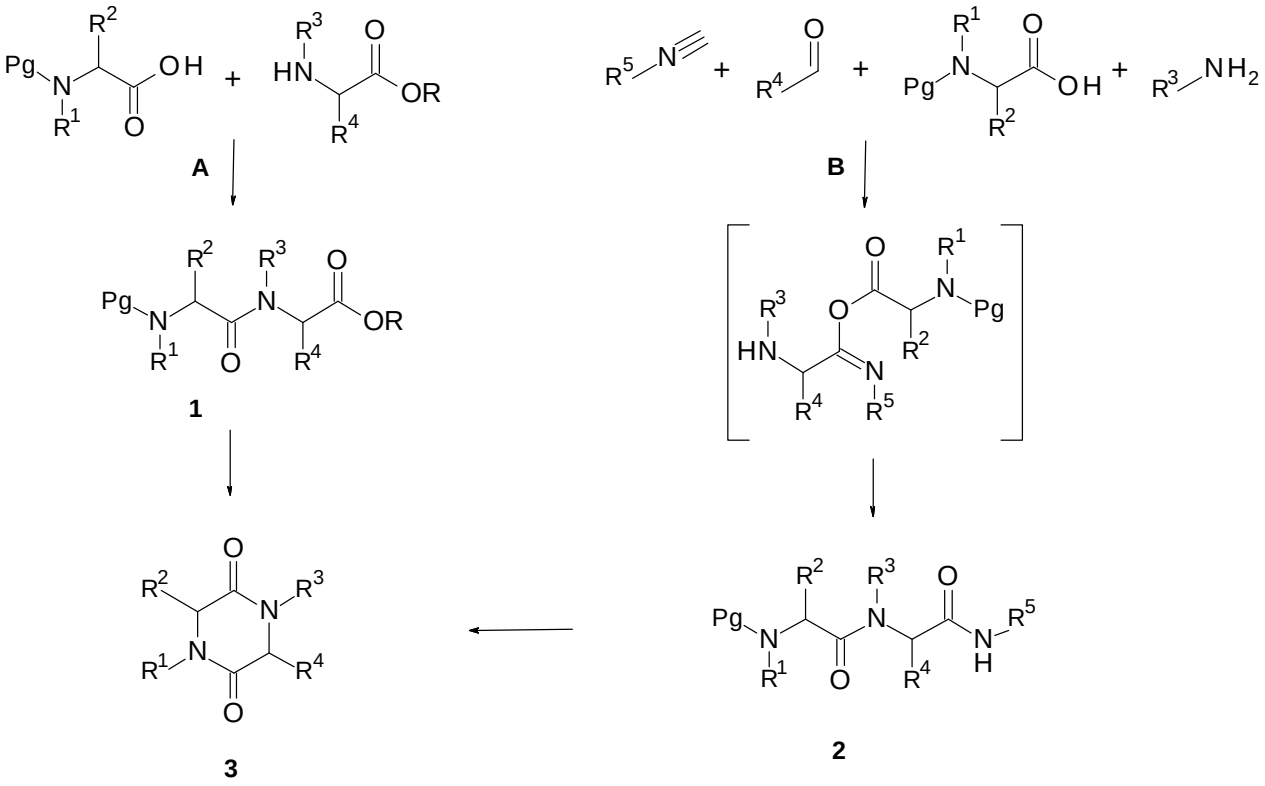


Figure 5. Dipeptide Cyclization. (Pg = protecting group)

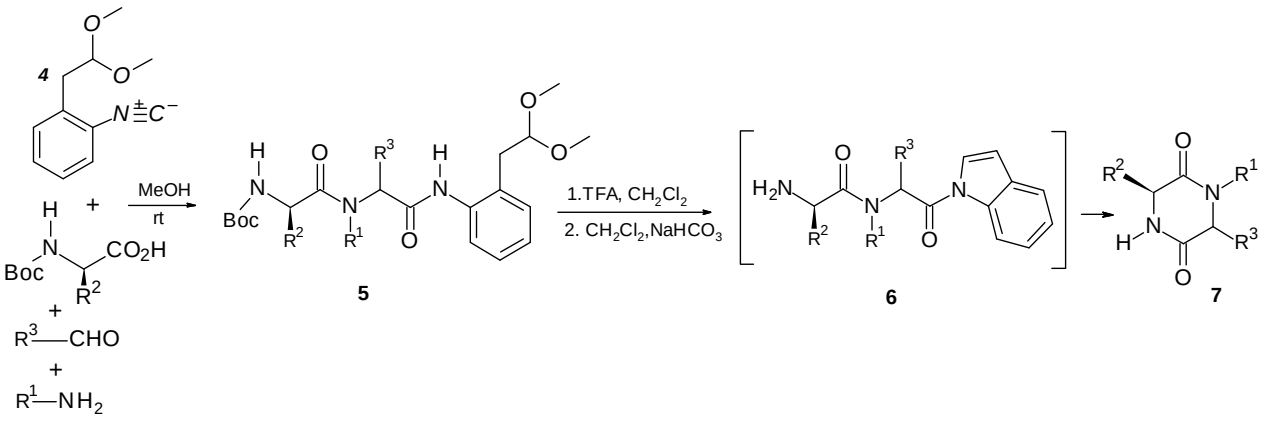


Figure 6. Cyclization via Intermediate Indolamide.

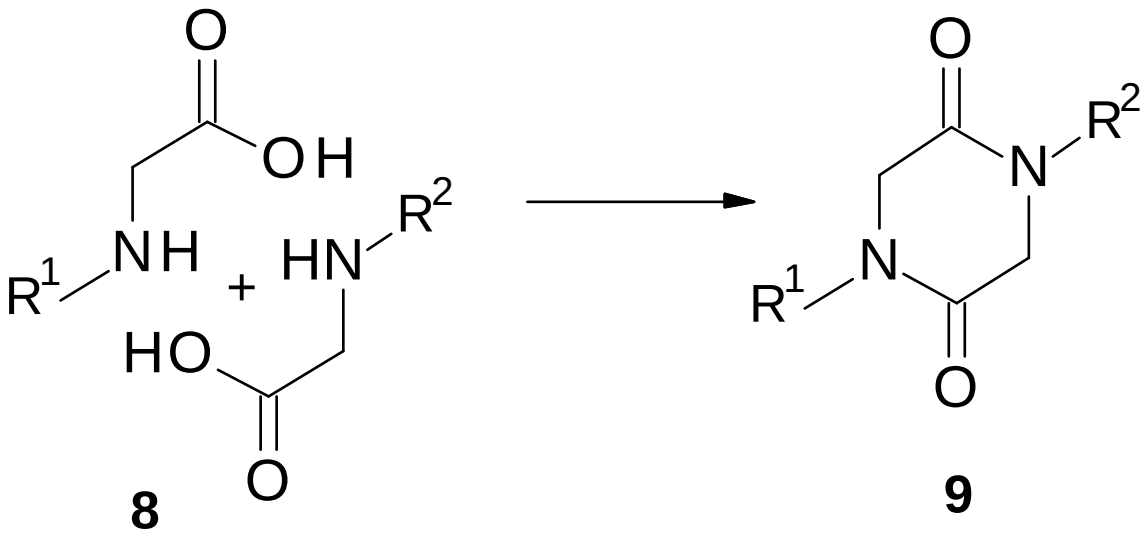


Figure 7. Amino Acid Condensation.

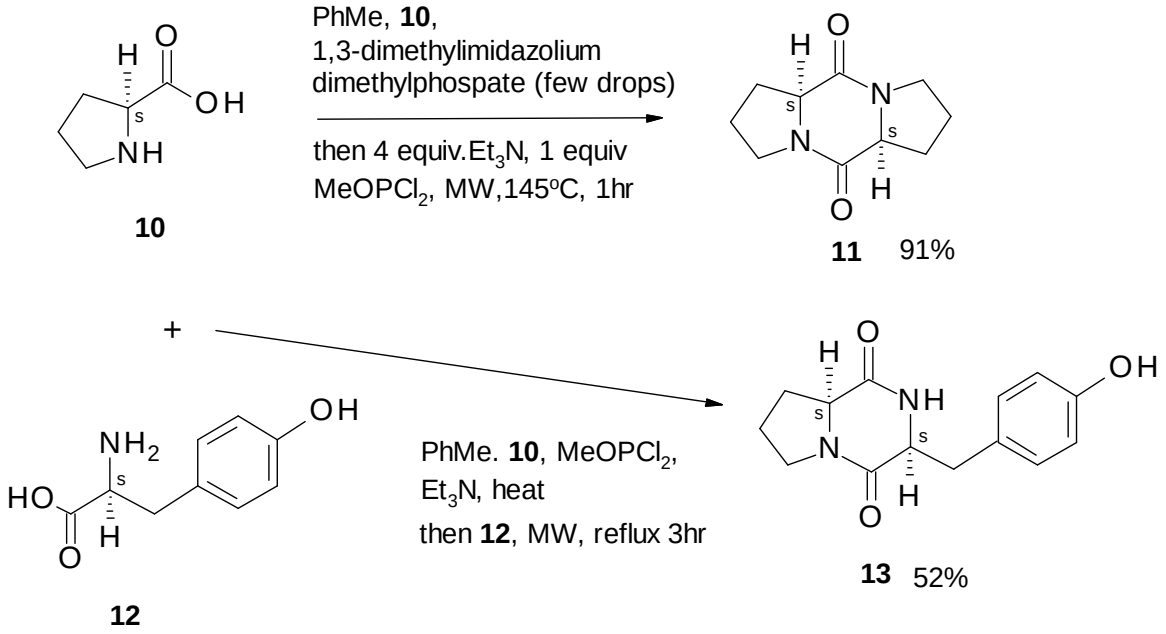


Figure 8. Symmetrical and Unsymmetrical 2,5-Diketopiperazine's via Amino acid Condensation.

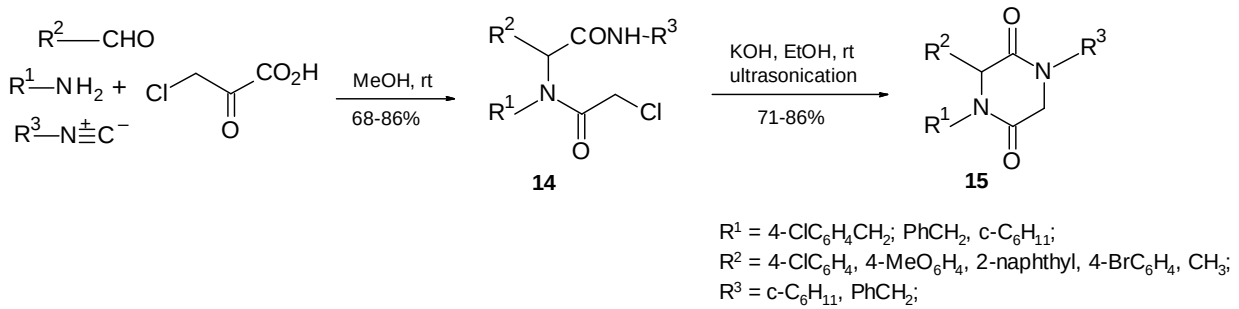


Figure 9. Synthesis of 2,5-Diketopiperazines via Ugi-4CR/Intramolecular *N*-Alkylation.

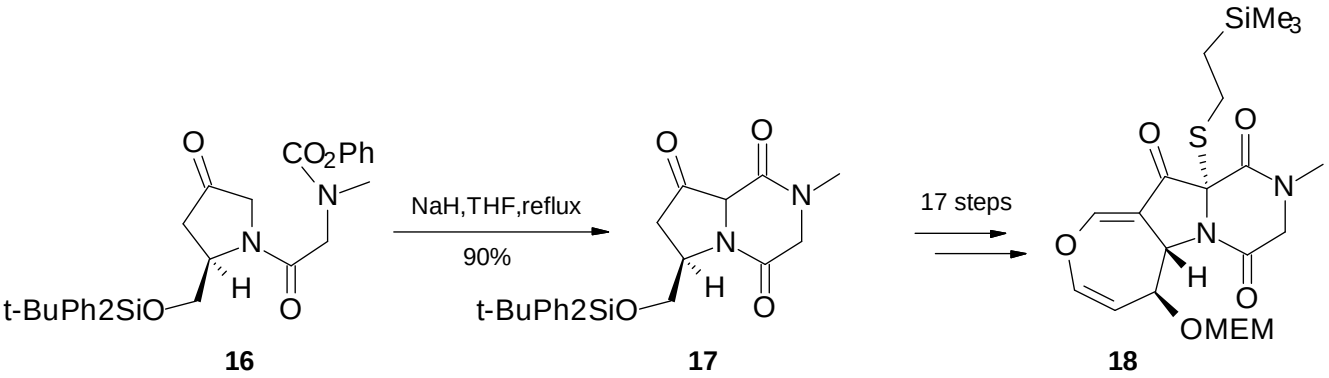
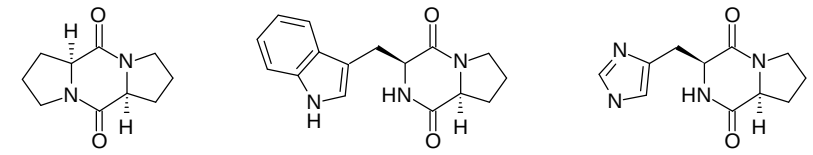


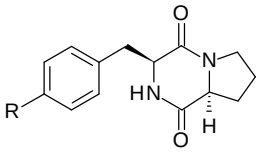
Figure 10. Cyclization via Enolate Acylation.



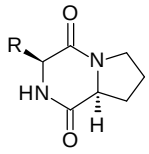
11
cyclo [L-Pro-L-Pro]

19
cyclo [L-Trp-L-Pro]

20
cyclo [L-His-L-Pro]

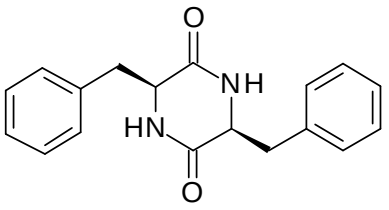


13 R = OH cyclo [L-Tyr-L-Pro]
21 R = H cyclo [L-Phe-L-Pro]



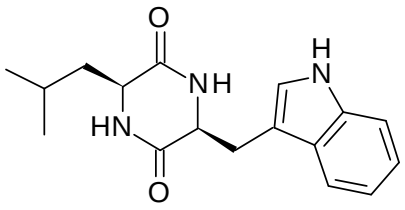
22 **R = CH_2CHMe_2** cyclo[L-Leu-L-Pro]
23 **R = $CHMeCH_2Me$** cyclo[L-Ileu-L-Pro]
24 **R = $CHMe_2$** cyclo[L-Val-L-Pro]
25 **R = Me** cyclo[L-Ala-L-Pro]
26 **R = H** cyclo[Gly-L-Pro]
27 **R = CHMeOH** cyclo[L-Thr-L-Pro]
28 **R = CH_2OH** cyclo[L-Ser-L-Pro]
29 **R = CH_2CONH_2** cyclo[L-Asn-L-Pro]
30 **R = CH_2CO_2H** cyclo[L-Asp-L-Pro]
31 **R = $CH_2CH_2CH_2$ $NH(C=NH)NH_2$** cyclo[L-Arg-L-Pro]

Figure 11. Proline Derivatives.



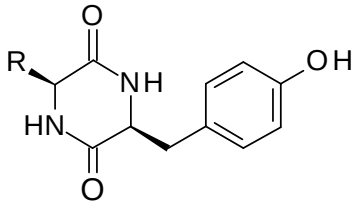
32

cyclo [L-Phe-L-Phe]

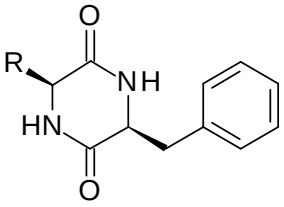


33

cyclo [L-Leu-L-Trp]

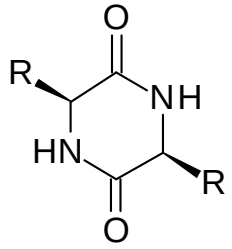


34 R = $CHMe_2$ cyclo[L-Val-L-Tyr]
35 R = Me cyclo[L-Ala-L-Tyr]

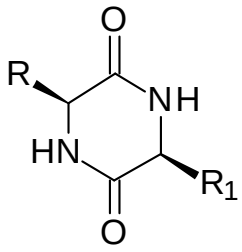


36 R = CH_2CHMe_2 cyclo[L-Leu-L-Phe]
37 R = $CHMeCH_2Me$ cyclo[L-Ileu-L-Phe]
38 R = $CHMeCH_2Me$ cyclo[L-Ileu-L-Phe] trans isomer
39 R = $CHMe_2$ cyclo[L-Val-L-Phe]
40 R = Me cyclo[L-Ala-L-Phe]
41 R = H cyclo[Gly-L-Phe]
42 R = CH_2OH cyclo[L-Ser-L-Phe]
43 R = CH_2CONH_2 cyclo[L-Asn-L-Phe]
44 R = CH_2CO_2H cyclo[L-Asp-L-Phe]

Figure 12. Aromatic Derivatives.

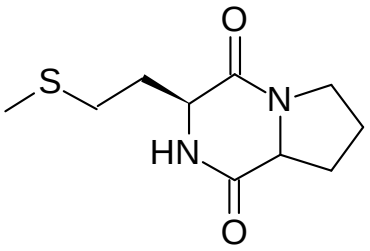


45	**R = CH_2CHMe_2**	cyclo[L-Leu-L-Leu]
46	**R = $CHMeCH_2Me$**	cyclo[L-Ileu-L-Ileu]
47	**R = $CHMe_2$**	cyclo[L-Val-L-Val]
48	**R = Me**	cyclo[L-Ala-L-Ala]
49	**R = H**	cyclo[Gly-L-Gly]



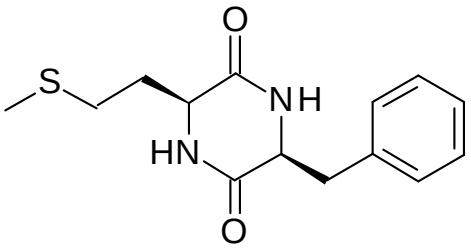
50	**R = CH_2CHMe_2**	**R_1= $CHMeCH_2Me$**	cyclo[L-Leu-L-Ileu]
51	**R = $CHMe_2$**	**R_1= $CHMeCH_2Me$**	cyclo[L-Val-L-Ileu]
52	**R = Me**	**R_1= $CHMeCH_2Me$**	cyclo[L-Ala-L-Ileu]
53	**R = $CHMe_2$**	**R_1= CH_2CHMe_2**	cyclo[L-Val-L-Leu]
54	**R = Me**	**R_1= CH_2CHMe_2**	cyclo[L-Ala-L-Leu]
55	**R = H**	**R_1= CH_2CHMe_2**	cyclo[Gly-L-Leu]
56	**R = Me**	**R_1= $CHMe_2$**	cyclo[L-Ala-L-Val]
57	**R = H**	**R_1= $CHMe_2$**	cyclo[L-Gly-L-Val]
58	**R = H**	**R_1= Me**	cyclo[L-Gly-L-Ala]
59	**R = CH_2CHMe_2**	**R_1= $CH_2CH_2CO_2H$**	cyclo[L-Leu-L-Glu]
60	**R = H**	**R_1= CH_2OH**	cyclo[L-Gly-L-Ser]

Figure 13. Aliphatic Derivatives.



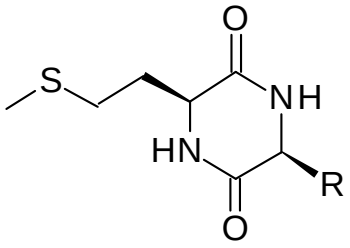
61

cyclo [L-Met-L-Pro]



62

cyclo [L-Met-L-Phe]



63	**R = CH_2CHMe_2**	cyclo[L-Leu-L-Met]
64	**R = $CHMeCH_2Me$**	cyclo[L-Ileu-L-Met]
65	**R = $CHMe_2$**	cyclo[L-Val-L-Met]
66	**R = Me**	cyclo[L-Ala-L-Met]
67	**R = H**	cyclo[Gly-L-Met]

Figure 14. Methionine Derivatives.

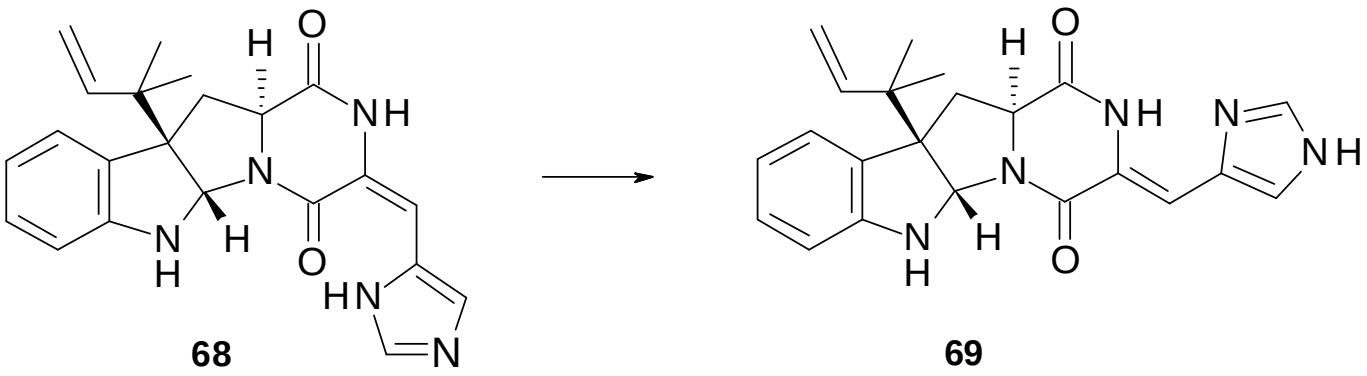


Figure 15. Roquefortine C, Isoroquefortine C and Cyclo(L-Phe-trans-4-hydroxy-L-Pro).

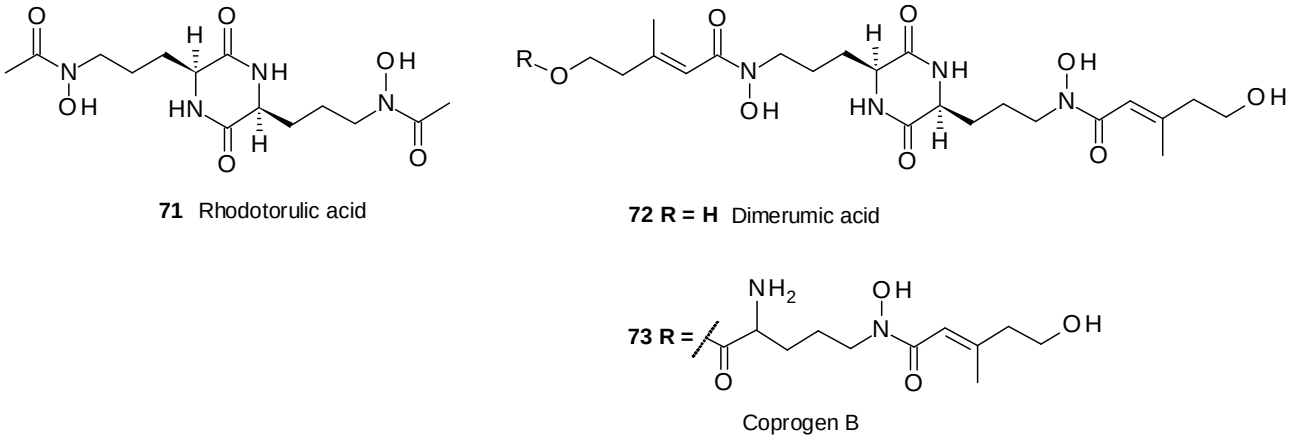


Figure 16. 2,5-Diketopiperazine Siderophores.

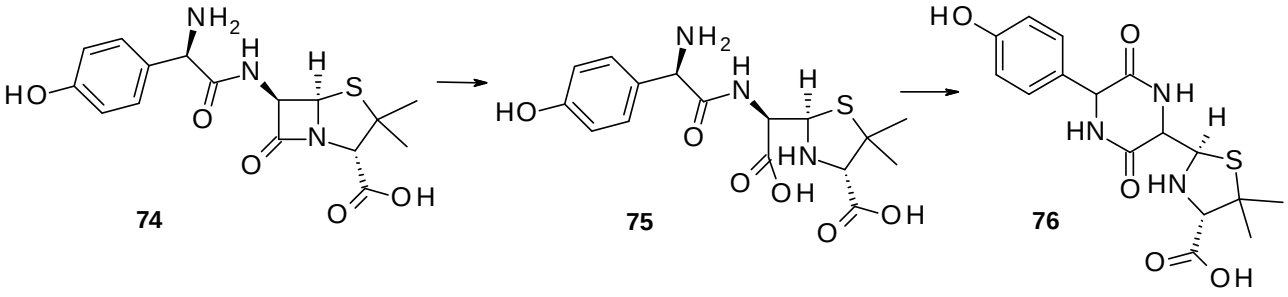


Figure 17. Amoxicillin, amoxicilloic acid and amoxicillin-2,5-diketopiperazine.

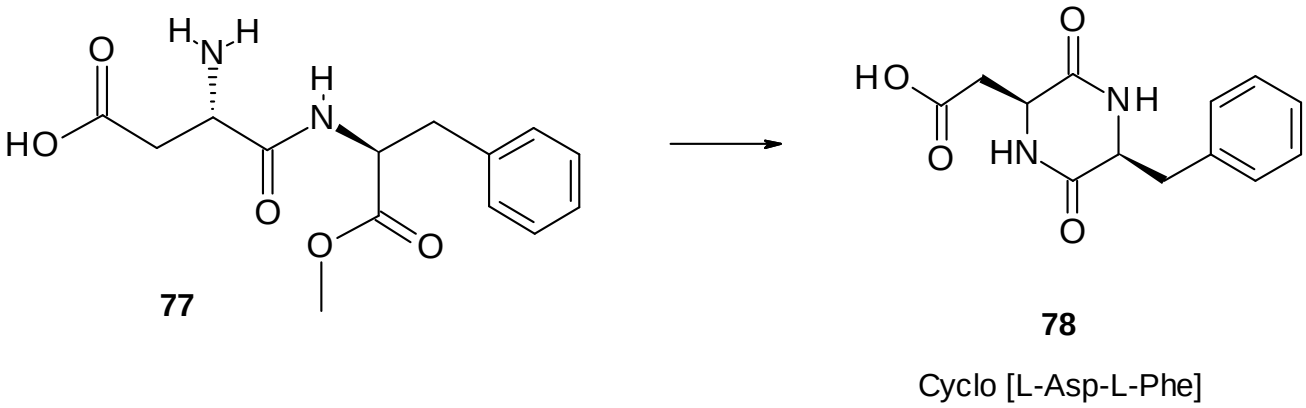


Figure 18. Aspartame and Aspartame-2,5-diketopiperazine.