

Review

# Diketopiperazine Gels: New Horizons from the Self-Assembly of Cyclic Dipeptides

Marco Scarel<sup>1</sup> and Silvia Marchesan<sup>1,2,\*</sup> 

<sup>1</sup> Chemical and Pharmaceutical Sciences Department, University of Trieste, Via Giorgieri 1, 34127 Trieste, Italy; marco.scarel2@studenti.units.it

<sup>2</sup> National Interuniversity Consortium of Materials Science and Technology (INSTM), University of Trieste, 34127 Trieste, Italy

\* Correspondence: smarchesan@units.it; Tel.: +39-040-558-3923

**Abstract:** Cyclodipeptides (CDPs) or 2,5-diketopiperazines (DKPs) can exert a variety of biological activities and display pronounced resistance against enzymatic hydrolysis as well as a propensity towards self-assembly into gels, relative to the linear-dipeptide counterparts. They have attracted great interest in a variety of fields spanning from functional materials to drug discovery. This concise review will analyze the latest advancements in their synthesis, self-assembly into gels, and their more innovative applications.

**Keywords:** cyclopeptides; diketopiperazines; gels; hydrogels; peptides; amino acids; self-assembly; supramolecular; nanomaterials; microwave



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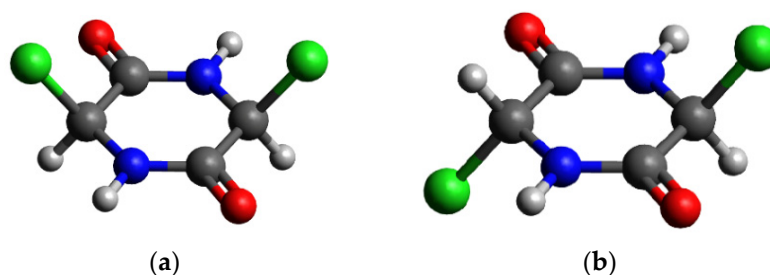


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## 1. Introduction

Gels based on self-assembling biomolecules have become a popular research topic [1]. Amongst their attractive features, there is also good potential as greener alternatives to traditional synthetic polymers. They do not persist in the environment and are not derived from oil-industry raw materials. In particular, self-assembling short-peptides represent cheap building blocks for large-scale production, and with overall promising properties in terms of bioactivity [2] and biocompatibility to achieve functional materials [3,4]. The large chemical diversity they provide ensures continuous advancements, and even considering just the publications in the last couple of years, numerous regard self-assembling tripeptides [5–15] and dipeptides [16–25]. Furthermore, the study of such simple systems could advance the understanding of prebiotic chemistry, relative to the potential role of peptide-based organized structures in the emergence of catalytic activity [26].

Linear dipeptides can easily cyclize into 2,5-diketopiperazines (DKPs) or cyclodipeptides (CDPs), which are widely reported as self-assembling building blocks in virtue of their enhanced rigidity and hydrogen-bonding into defined networks, both of which can be advantageous for self-organization into gels [27]. The orientation of the amino acid lateral chains (Figure 1) could be on the same side of the ring plane for the homochiral CDPs (LL/DD), or on the opposite side for the heterochiral stereoisomers (LD or DL). The ring structure increases the resistance against proteolytic degradation compared to linear peptides and allows them to cross the blood-brain barrier, both of which are important aspects, for instance, for the development of neuroprotective agents for oral administration [28]. CDPs are indeed attracting interest as drug carriers, thanks to their demonstrated ability to cross membranes and penetrate cells [29]. Their aggregates can display fluorescence [30], and they are widely studied for their diverse biological activities [31–38], which make them promising for biomedical applications, as recently reviewed [39]. Further, they are being investigated also for optoelectronic [40] and catalytic [41] applications, as biodegradable and environmentally-friendly substitutes of more traditional options.



**Figure 1.** 3D Chemical structure of generic cyclodipeptides (CDPs) obtained from the cyclization of L-homochiral (a) or D,L-heterochiral (b) dipeptides. Side chains are represented as a generic green sphere and the rest of the atom types are represented using the standard CPK model.

All these features make CDPs an attractive choice as biocompatible, green, functionalizable, and cheap building blocks for smart materials with a wide scope of applications from industry to medicine. The basic design is very simple: two amino acids bound together in a cyclic structure with a defined stereochemistry (i.e., LL, LD/DL, or DD) and the possibility to further derivatize the side chains [42–46] of selected amino acids [47–50], as widely applied in nature [51–56].

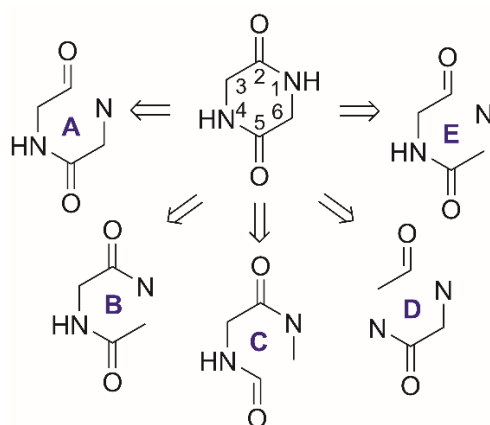
CDPs are synthesized by microorganisms [57,58], they are found in food as tasting agents, and can be the product of fermentation processes that are particularly relevant to the food and beverage industry [59]. A dipeptide is a relatively simple structure, and it is easy to synthesize for modern organisms, thanks to the complex molecular machinery provided by ribosomes and enzymes. CDPs can also form spontaneously without enzymes, and therefore interest in their role as prebiotic-chemistry building blocks is growing [60]. The nature of the side chains of CDPs is not only involved in the self-assembly process but they could also provide catalytic functional groups. Thus, materials obtained by these molecules could be versatile [41]. There is growing interest in CDPs, and recent reviews exist that discuss their bioactivity [58,61,62], supramolecular organization [31,40], and use as scaffolds for drug discovery [52,63–66].

Therefore, this concise review will focus on gels obtained through the self-assembly of CDPs formed by unprotected and underivatized amino acids. Hydrogels and organogels are the two types of materials that are mostly studied; xerogels are obtained upon drying gels, and often their purpose is the characterization of the system whenever it is not possible to use a hydrogel, for example in transmission-electron microscopy (TEM) and infrared (IR) spectroscopy techniques. Conversely, studies of aerogels and ionogels based on CDPs are still scarce [67], therefore these two types of materials will not be discussed.

## 2. Synthesis of CDPs

### 2.1. Organic Synthesis of CDPs in Solution

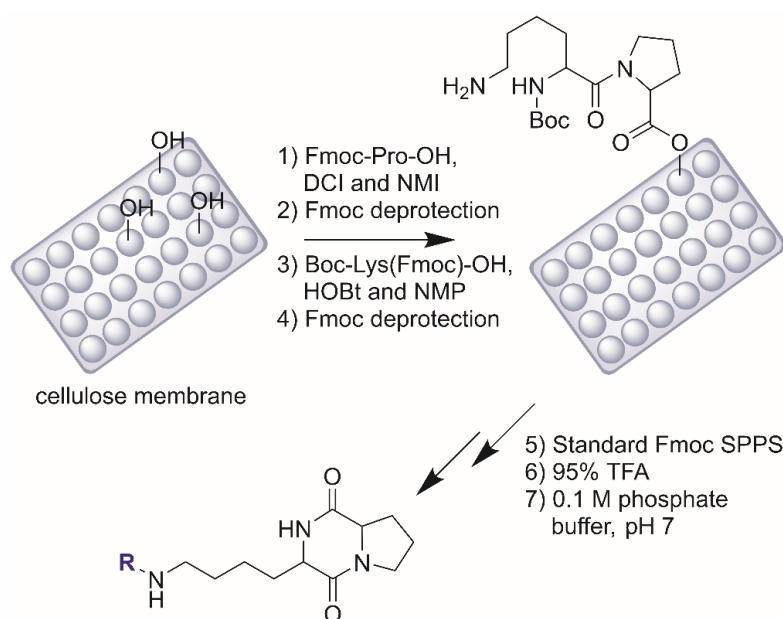
Retrosynthetic analysis of the CDP ring (Scheme 1) reveals several possible disconnections with routes A–B being far more popular than tandem cyclizations (D–E). The most popular route, which can be easily performed also in water or solvent-free through green approaches, goes through the disconnection of the amide bond (Scheme 1, A), and several methods to cyclize dipeptides for this approach are described further below. An aza-Wittig cyclization will also go through disconnection A, as will do Ugi chemistry using an isonitrile, an acid, an aldehyde, and an amine. The C–N disconnection (Scheme 1, B) is another quite popular alternative that involves intramolecular N-alkylation. Also, in this case, 4-component Ugi-type chemistry can be employed, in tandem with an Aza-Michael. Alternatively, the use of a Diels–Alder allows to achieve CDPs with a high level of structural complexity. The C–C disconnection (Scheme 1, C) often involves enolate acylation, albeit radical routes were also described to attain the C–C cyclization [63]. Despite the various synthetic routes available, the search is still active in this area with new developments pertaining to CDP synthesis appearing also in the most recent literature [68–76].



**Scheme 1.** Retrosynthetic analysis reveals five disconnections (A–E) to yield the CDP ring [63].

## 2.2. Organic Synthesis of CDPs in Solid Phase

Solid-phase synthesis of CDPs [77,78] offers the possibility to rapidly gain access to a plethora of structurally complex products [79]. Solid-phase methods can be very convenient for the rapid screening of arrays of libraries obtained through the parallel synthesis of CDPs, for instance via the SPOT method on cellulose membranes (Scheme 2) [80]. This type of approach to preparing CDPs is very convenient on a small scale when the priority is time. Conversely, it is rather costly and poses challenges for scale-up, so it will not be described further here, since several convenient liquid-phase alternatives are available.



**Scheme 2.** CDP-array synthesis on cellulose membranes via SPOT method for rapid screening [80]. Boc = *tert*-butyloxycarbonyl; DIC = *N,N'*-diisopropylcarbodiimide; Fmoc = fluorenylmethoxycarbonyl; HOBt = Hydroxybenzotriazole; Lys = lysine; NMI = *N*-methylimidazole; NMP = *N*-methyl-2-pyrrolidone; Pro = proline; SPPS = solid-phase peptide synthesis; TFA = trifluoroacetic acid.

## 2.3. Dipeptide Cyclization to CDPs

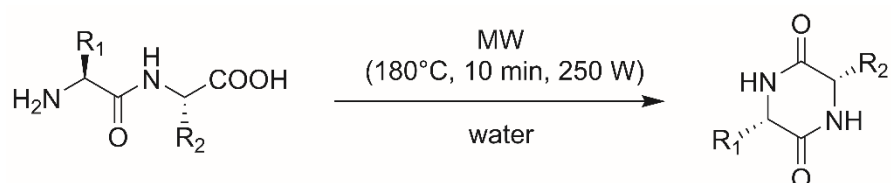
### 2.3.1. Liquid-Phase Cyclization

Dipeptide cyclization is certainly the most widely used approach and can be performed starting with a C-terminally protected dipeptide, both in organic solvent and water. The reaction is an aminolysis, therefore the N-terminus must be deprotected either before the reaction or during the cyclization in a one-pot fashion. The C-terminal protecting group

is usually a methyl ester so that the N-terminal amine acts as the nucleophile and the methoxy functionality is the leaving group. For example, the cyclization of aspartame (L-Asp-L-Phe-OMe) was studied in an organic solvent. The reaction was performed in dimethylsulfoxide (DMSO), by stirring the solution for 8 h at 80 °C with a final yield after workup of 88% [81]. Dipeptides featuring a methoxy group at the C-terminus could also spontaneously cyclize in water via aminolytic reaction [82].

### 2.3.2. Microwave-Assisted Cyclization

Microwave-assisted cyclization in water is an interesting technique that allows one to make CDPs in high-yield and within a short time. In the case of hydrophobic CDPs, the workup is straightforward due to the precipitation of the product. With this technique, it is possible to perform a greener synthesis of CDPs because more steps are merged in one, avoiding expensive workups and petroleum-derived solvents. Microwave heating was studied in many different solvents for dipeptide methyl esters and only in water it is possible to reach high yields [83]. It is possible to remove the Boc (*tert*-butyloxycarbonyl) protecting group through microwave heating in water when amino acids or peptides display an unprotected C-terminus [84]. However, N-Boc deprotection and cyclization can also occur in one-pot, as demonstrated on C-terminal *tert*-butyl and methyl esters [85]. Recently, the green synthesis of CDPs was obtained in high-to-quantitative yield by microwave-assisted cyclization in water (Scheme 3), also without the methyl ester at the C-terminus, and with no need for work-ups for hydrophobic CDPs that precipitate as white solids, leaving any unreacted dipeptide in solution [86].



**Scheme 3.** Microwave-assisted cyclization of terminally unprotected dipeptides in water occurs in nearly quantitative yield with no need for workups [86]. R<sub>1</sub> = benzyl; R<sub>2</sub> = benzyl; *iso*-propyl.

### 2.3.3. Cyclization Using Vapor Deposition

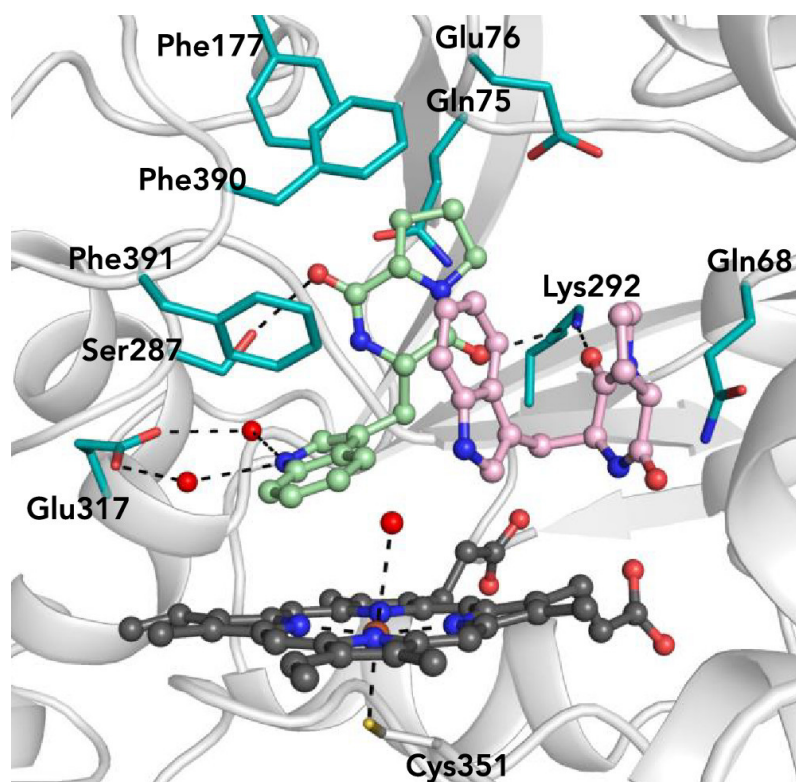
A peculiar case of dipeptide cyclization was described using vapor deposition [87]. Diphenylalanine (Phe-Phe) was heated at 220 °C and thus evaporated in a vacuum chamber. When the peptide reached a cooler surface (i.e., 80 °C), it cyclized and formed nanotubes. The stacking interactions between the aromatic rings of the side chains were the key for inter-molecular recognition and templating of the cyclization to CDPs, as well as the growth of their self-assembled nanotubes.

### 2.3.4. Cyclization in the Solid State

CDPs can be synthesized also in the solid state, through the heating of a powder sample [88,89]. The appropriate reaction temperature depends on the nature of the peptide sequence. For example, Gly-Gly needs 230 °C, while Phe-Phe 147 °C [90] or 125 °C [91], suggesting a correlation between the reaction minimum temperature and the amino acid side-chain steric hindrance. Cyclization indeed requires molecular mobility, which is governed by the nature of the side chains in the solid state. The melting points of Gly-Gly and Phe-Phe are respectively 262–264 °C and 288–290 °C, thus confirming that the reaction occurred in a solid state and probably above the glass transition temperature ( $T_g$ ). The latter depends on the sample preparation when manufactured; from that point onwards, CDP molecules do not maintain random conformations and, instead, they start to reorganize in the solid structure, since rotations and translations are possible [90].

#### 2.4. Enzymes and Biotechnological Tools for CDP Synthesis

Finally, biotechnological tools are also being developed to prepare CDPs. To this end, cyclodipeptide synthases were recently reported to catalyze the cyclization of aminoacylated-tRNA substrates [92,93]. Installation of C-C double bonds to attain dehydrogenated CDPs can be achieved through oxidase-mediated catalysis [94], while N-alkylation is catalyzed by methyltransferases [95]. Interestingly, a cytochrome P450 has been identified that can catalyze both the CDP dimerization and cyclization towards bioactive CDP-derived natural products with interesting bioactivities (Figure 2) [96]. Mutagenesis of other cytochrome P450s gave access to catalysts for CDP synthesis with good regio- and stereo-specificity, and chemical versatility [97]. Whole-cell biocatalysts were used to attain heterodimeric CDPs, with *Mycobacterium smegmatis* demonstrating to be a more robust and efficient organism, than the commonly used *Escherichia coli* or *Streptomyces* systems [98]. The large variety of reactions catalyzed by cytochrome P450s to synthesize and modify CDPs is gaining momentum and has just been reviewed [99].



**Figure 2.** Active site of cytochrome P450 NzeB showing the concave substrate (pink) bound in the “cyclization site”, and the extended substrate (green) bound in the “dimerization site”. Reproduced with permission from [96], copyright © 2020, American Chemical Society.

### 3. Self-Assembly of CDPs into Gels

#### 3.1. Gels from Unprotected-Dipeptide Derived CDPs

##### 3.1.1. Non-Covalent Interactions Responsible for Gelation

Supramolecular gels are very interesting because of their self-organized structure via non-covalent interactions. This feature allows one to make dynamic materials with self-healing properties and thermal reversibility avoiding any by-products, which are net advantages over classic covalent interactions. The driving force for gel transition is non-covalent, therefore it is important to evaluate the CDP structure in terms of available interactions, such as hydrogen bonding,  $\pi$ - $\pi$  stacking, CH- $\pi$ , or dipole-dipole interactions, etc. Numerous examples of gels formed by unprotected-dipeptide derived CDPs have been reported (Table 1), although the number of studies involving modified CDPs is far greater, as discussed in Section 3.2.

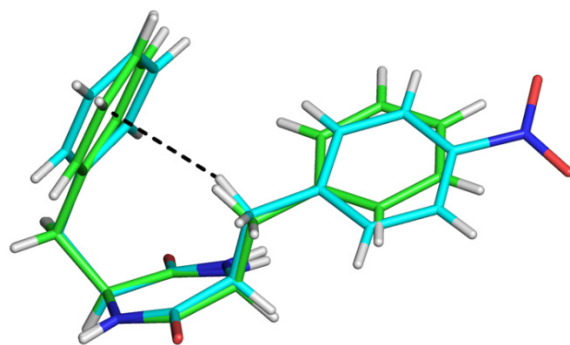
**Table 1.** Gelling CDPs obtained from unprotected dipeptides.

CDP Sequence	Gel Type	Reference
Cyclo(Ala-Gly)	Organogel	[100]
Cyclo(Val-Gly)	Organogel	[100]
Cyclo(Leu-Gly)	Organogel	[100]
Cyclo(Leu-Val)	Organogel	[100]
Cyclo(Leu-Ala)	Organogel	[100]
Cyclo(Leu-Leu)	Organogel	[100]
Cyclo(Phe-Gly)	Organogel	[100]
Cyclo(Phe-Gly)	Hydrogel	[41]
Cyclo(Phe-Val)	Hydrogel	[86]
Cyclo(Phe-Leu)	Hydrogel	[82,101]
Cyclo(Phe-Leu)	Organogel	[100,102]
Cyclo(Phe-Phe)	Hydrogel	[86]
Cyclo(Phe-Phe)	Organogel	[100]
Cyclo(Phe-Cys)	Hydrogel	[103]
Cyclo(Phe-Ser)	Hydrogel	[103]
Cyclo(Phe-Glu)	Hydrogel	[103]
Cyclo(Phe-His)	Hydrogel	[103]
Cyclo(Phe-Lys)	Hydrogel	[103]
Cyclo(Trp-Trp)	Organogel	[104]
Cyclo(Trp-Tyr)	Hydrogel	[105]
Cyclo(Tyr-Tyr)	Hydrogel	[104]
Cyclo(Tyr-Tyr)	Organogel	[104]
Cyclo(Tyr-Lys)	Hydrogel	[106]
Cyclo(Tyr-Lys)	Organogel	[106]
Cyclo(Lys-Glu)	Organogel	[107]

The role of hydrogen-bond interactions between CDP rings was studied on organogels made by cyclo(Leu-Leu) [108]. This compound is a very interesting organogelator, which self-assembles into aromatic solvents, such as benzene, xylene, toluene, and ethylbenzene, whilst not in aliphatic solvents. Surprisingly, it was noted that a small amount of water addition, not only improved the kinetics of the gelation process (that was accelerated from two days to six hours in toluene) but also allowed to attain organogels with aliphatic hydrocarbons, such as cyclohexane, hexane, and heptane. The cases of the latter three are very interesting because, in the absence of any functional group in the solvent molecules, Van der Waals forces are the only possible interactions available [108]. Hydrogen bonds play an important scaffolding role in the assemblies, while van der Waals forces are typically the driving force in the initial stages to generate the hydrophobic core of the supramolecular structures [109].

The nature of the side chains of CDPs plays a role in the final structure of the supramolecular system. The presence of different functional groups allows one to expand the non-covalent interaction toolkit of CDPs. Further, they have a great influence on the solubility of CDPs in one particular solvent. Short and hydrophilic side chains are not suitable for hydrogels as seen in the case of the aspartame CDP [82]. Phenylalanine, tyrosine, tryptophan, and histidine are the four natural aromatic amino acids; the latter is also a base and, thus, it is easily ionized at different pH values. Phe, Tyr, and Trp are hydrophobic, and they could be a good choice for a potential hydrogelator. All three derived CDPs were studied as gelling agents in water and many organic solvents [104]. In particular, cyclo(Tyr-Tyr) is a robust hydrogelator and it is also an organogelator for alcohol-based solvents. Cyclo(Phe-Phe) is an organogelator, whilst cyclo(Trp-Trp) showed a weak gelling ability only in chloroform. Conversely, another study reported cyclo(Tyr-Trp) as a robust hydrogelator [105]. All these findings raise the question about the possible driving force embodied in non-covalent interactions of aromatic moieties, such as  $\pi$ - $\pi$  stacking and CH- $\pi$  interactions. Recently, a study about cyclo(Phe-Phe) and cyclo(*p*-nitro-Phe-Phe) highlighted the role of this kind of interaction with the aid of single-crystal XRD data [86]. Both CDPs displayed the same conformation in the crystal state with an intramolecular CH- $\pi$

interaction (Figure 3). However, the structure of cyclo(Phe-Phe) showed intermolecular  $\pi$ - $\pi$  stacking and intra-intermolecular CH- $\pi$  interactions. Surprisingly, the introduction of the nitro group did not affect the molecular conformation, rather, it resulted in the rigid translation of the molecule into another position, thus creating a new crystal packing, with loss of an intermolecular CH- $\pi$  interaction. Nevertheless, cyclo(*p*-nitro-Phe-Phe) demonstrated to be a better hydrogelator than cyclo(Phe-Phe), thus the different electronic density between the two aromatic rings could play an important role.



**Figure 3.** Superimposition of the single-crystal XRD structures of cyclo(Phe-Phe) (green carbon atoms) and cyclo(*p*-nitro-Phe-Phe) (cyan carbon atoms) reveal the same conformation with an intramolecular CH- $\pi$  interaction (dashed line). Nitrogen, oxygen, and hydrogen atoms are colored in blue, red, and white, respectively. Reproduced with permission from [86], Copyright © 2019, Thieme Verlag, Germany.

### 3.1.2. Spectroscopic Characterization Methods to Monitor Gelation

The CDP basic scaffold is a six-membered ring with two amide groups. N-H and C=O can engage in hydrogen bonding. Fourier-transformed infrared (FT-IR) spectroscopy is a good technique to observe if there are any hydrogen-bond interactions. Generally, the C=O stretching signal for an amide group falls around  $1690\text{ cm}^{-1}$ , while the N-H stretching and bending signals appear approximately at  $3400$  and  $1500\text{ cm}^{-1}$ , respectively. As a result of the transition from solution to gel, it is possible to observe a shift of these signals because of the hydrogen-bonding interaction. For instance, FT-IR of cyclo(Val-Leu) in chloroform solution shows a shift of  $1690$  to  $1640\text{ cm}^{-1}$  for the C=O group, and from  $3400$  to  $3320\text{ cm}^{-1}$  for the N-H group, as a result of gelation [102]. FT-IR of cyclo(Glu-Lys) in DMSO before and after transition also shows a more complex situation. The C=O signal is shifted from  $1687$ – $1682\text{ cm}^{-1}$  to  $1670$ – $1677\text{ cm}^{-1}$ , the N-H stretching from  $3515$ – $3500\text{ cm}^{-1}$  to  $3320$ – $3200\text{ cm}^{-1}$ , and the N-H bending from  $1500$ – $1510\text{ cm}^{-1}$  to  $1520$ – $1538\text{ cm}^{-1}$  [107]. Stretching signals are shifted towards lower wavenumbers when hydrogen bonding occurs.

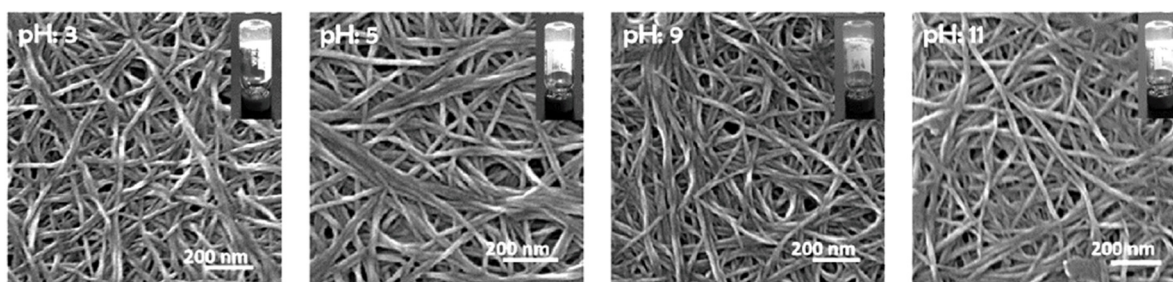
<sup>1</sup>H-NMR is another useful spectroscopic technique to study hydrogen bonding in gels. Amide signals are shifted downfield when this interaction occurs [100]. It is not possible to measure signals from a gelling agent in a rigid packing because of the long correlation time needed. However, it is possible to study small aggregates in a solution that are representatives of the material. <sup>1</sup>H-NMR spectra of cyclo(Tyr-Lys) in DMF-*d*<sub>7</sub> showed the situation in solution at  $60\text{ °C}$  of pre-aggregated gelators that gelled at room temperature [106]. Amide signals were broadened and shifted from  $7.78$  (at  $65\text{ °C}$ ) to  $8.10\text{ ppm}$  (at  $25\text{ °C}$ ) as a result of hydrogen bonding.

### 3.1.3. Oscillatory Rheometry to Monitor Gelation

Rheometry is the technique of election to quantify the viscoelastic properties pertaining to gels and their precursor solutions. When the elastic or storage modulus ( $G'$ ) is significantly higher than the viscous or loss modulus ( $G''$ ), the system is defined as a gel. With this technique, it is possible also to study the thermo-reversibility and self-healing properties of these systems, as well as the shear-thinning behavior that is desirable for injectability. For instance, cyclo(Phe-Xaa) (Xaa = Gly, Ser, Cys, Glu, His, Lys) CDPs were

characterized by rheometry, and all the soft materials displayed thermo-reversibility [103]. All these compounds formed stable hydrogels at 4 wt%. Cyclo(Phe-Cys) was found to be the best hydrogelator of the series with as little as 0.25 wt% needed for gelation, and a remarkably high gel-sol transition temperature of 100 °C when formulated at 1 wt%. Interestingly, despite the fact that serine is isoelectronic with cysteine, cyclo(Phe-Ser) was the worst gelling agent of this series with a gel-sol transition temperature of 12 °C also with high loading (4 wt%). All these compounds, except for cyclo(Phe-Ser), showed a thixotropic and self-healing behavior. Understandably, ionizable side chains revealed a pH dependency of the gel-sol transition. For instance, cyclo(Phe-Glu) formed stable hydrogels at pH 6, while cyclo(Phe-Lys) at pH 8–11. The blended hydrogel obtained mixing these two CDPs showed remarkably wider pH stability from 2 to 11. The gel-to-sol transition temperature was dependent on the pH value, with a maximum corresponding to 79 °C at pH 2, and a minimum corresponding to 27 °C at pH 7 [103].

Cyclo(Phe-Leu) is a strong hydrogelator with self-healing ability and rheological stability in the temperature range corresponding to 20–60 °C [101]. The stability against heating highlighted the different behavior of CDPs relative to linear short peptides, whose gels remained stable at analogous conditions. Temperature changes can sometimes be accompanied by crystallization, especially if gels are a metastable phase. Cyclo(Phe-Leu) is not ionizable, and thus it showed a gelling ability through a wide range of pH values, from 3 to 11 (Figure 4). Another interesting feature of this compound was its demonstrated resistance against protease-catalyzed hydrolysis [101]. It is apparent that CDPs can be a good choice to make hydrogels suitable for medical applications, and offer various advantages over the linear-peptide analogs.



**Figure 4.** SEM images and photographs (insets) of the gels formed by cyclo(Phe-Leu) at various pH values. Adapted with permission from [101], copyright © 2019, Elsevier.

### 3.2. Gels from Protected-Dipeptide Derived CDPs

As can be seen from Table 1, the vast majority of reported CDP gelators based on unprotected dipeptides features hydrophobic amino acids. To further expand the chemical diversity of the building blocks and attain CDP hydrogelators also from hydrophilic amino acids, a popular strategy employs the use of hydrophobic protecting groups. These include Fmoc (fluorenylmethyloxycarbonyl) [107,110–112], *O*<sup>t</sup>Bu (*tert*-butoxy) [113,114], Boc [111], and, generally, alkyl-chain derivatizations [106,115], for instance through formation of amide bonds with lipophilic carboxylic acids, as described more in detail below.

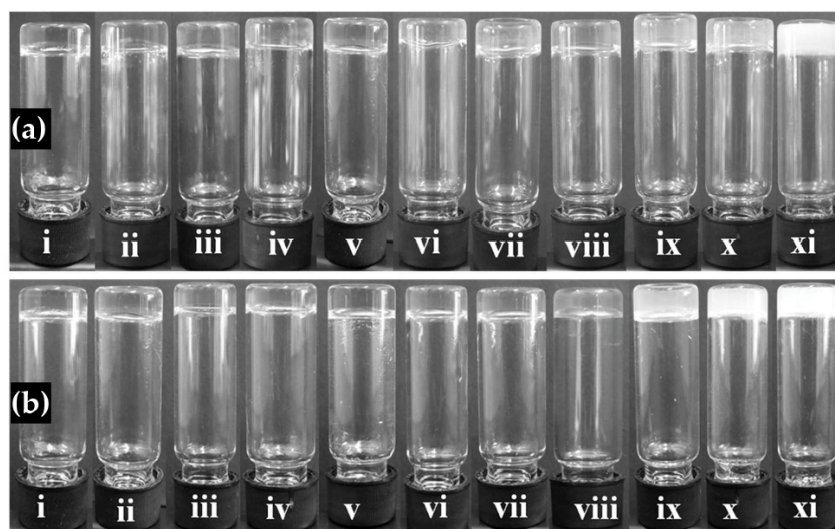
#### 3.2.1. Protected CDPs with Glu or Asp Acidic Groups

Cyclo(Glu-Glu) is a hydrophilic CDP, featuring two ionizable carboxylic groups of the side chains of the glutamic acid residues. These functional groups were reacted with Fmoc-Cl as a protection strategy. In particular, the Fmoc mono-substituted CDP revealed an interesting gelation ability [110]. Without the Fmoc protecting group, the CDP was too hydrophilic to yield hydrogels. Instead, with the Fmoc mono-substitution, it gained ability as superhydrogelator, superorganogelator in aromatic and chlorinated solvents, and also as organogelator in alcohols. The racemate formed hydrogels with a slightly higher concentration (0.6 wt%). XRD data showed a “pseudoracemate” non-crystalline



self-assembly, and this system showed a faster thixotropic recovery time than the enantiopure analogs. The recovery time was also tunable with the concentration (using 0.6 to 1.2 wt%) from 4 to 10 min [110].

A series of four CDPs with Glu and Asp were protected with  $O^t$ Bu and studied for the influence of the length of the side chain of Glu and Asp on their gelation ability [113]. The four compounds were thus cyclo(Tyr-Glu( $O^t$ Bu)), cyclo(Phe-Glu( $O^t$ Bu)), cyclo(Phe-Asp( $O^t$ Bu)), and cyclo(Leu-Glu( $O^t$ Bu)). The first two were compared to understand the role, if any, of the *para*-hydroxyl substitution on the aromatic side chain. Both were found to be super hydro- and organo-gelators in many solvents (Figure 5), therefore it seemed that the OH group of Tyr was not involved in the main driving-force interactions of the self-assembly process. The latter two were compared to elucidate the effect, if any, of the differing number by one carbon atom in the side chain. This small difference was sufficient to lead to two completely divergent supramolecular behaviors. In particular, the CDP featuring Glu was a super hydro- and organo-gelator, whilst that featuring Asp was almost insoluble in all the solvents, with organogels being formed only in 1,2-dichlorobenzene, and at high concentration (4.6 wt%), relative to the Glu-analogue in the same solvent (0.1 wt%). This latter CDP demonstrated to be a hydro- and organo-gelator, although it required higher concentrations to gel, relative to the aromatic analogs cyclo(Tyr-Glu( $O^t$ Bu)) and cyclo(Phe-Glu( $O^t$ Bu)), presumably due to the loss of the possibility to engage in  $\pi$ - $\pi$  stacking and CH- $\pi$  intermolecular interactions [113].



**Figure 5.** Photographs of gels formed by (a) cyclo(Tyr-Glu( $O^t$ Bu)) and (b) cyclo(Phe-Glu( $O^t$ Bu)) in various solvents: (i) toluene, (ii) chloroform, (iii) ethyl acetate, (iv) dichloromethane, (v) 1,2-dichlorobenzene, (vi) *tert*-butanol, (vii) *sec*-butanol, (viii) acetonitrile, (ix) 50% glycerol, (x) water, (xi) phosphate buffered saline (PBS, 10 mM, pH 7.4). Reproduced with permission from [113], copyright © 2017 American Chemical Society.

Cyclo(Glu-Glu) is functionalizable as a symmetric or asymmetric compound, thanks to the presence of two carboxylic groups in the side chains of the symmetric scaffold. A series of cyclo(Glu-Glu) derivatives were studied to understand the difference in supramolecular behavior between symmetric and asymmetric building blocks [114]. Tyramine, phenylalanine, tyrosine, and  $O^t$ Bu-protection were the four variable structural components of this study; tyramine was linked to the side chain of Glu through the amino group, thus forming an amide bond. Phe or Tyr instead was derivatized through the  $\alpha$ -amino group.  $O^t$ Bu was added to the C-terminal carboxylic group of Phe or Tyr. All these building blocks were studied as asymmetric compounds (with functionalization occurring on only one of the Glu side chains) or symmetric (with both Glu being bonded to Tyramine, Phe, or Tyr). Tyramine demonstrated not to be a good choice, as both mono- and di-substituted CDPs did not gel. Without  $O^t$ Bu on the carboxylic group of Phe or Tyr, no gels were formed

either, the only exception being in acetone but only for two particular CDPs. The only hydrogelator was the Tyr-*O*<sup>t</sup>Bu mono-substituted CDP, while all the other compounds were good organogelators, although each one in different solvents. The presence of *O*<sup>t</sup>Bu seemed to play an important role in organogel formation in this series of analogs. As in the study described in the previous paragraph, mono-substitution at one Glu with a hydrophobic group enabled gelation. In particular, in this study, when cyclo(Glu-Glu) was mono-substituted with Phe or Tyr, it yielded a gelling agent as long as *O*<sup>t</sup>Bu was present on the free carboxylic group of Phe or Tyr [114].

### 3.2.2. Protected CDPs with Lys Amino Groups

Lysine is another convenient amino acid to functionalize through the  $\epsilon$ -amino group of the side chain. Protection with the Fmoc group increases the hydrophobicity and provides an aromatic character, which is useful to engage in non-covalent interactions for self-assembly. As an example, the amino acidic cyclo(Glu-Lys) was capped with *O*<sup>t</sup>Bu (Glu) and Fmoc (Lys) and compared against the unprotected analog [107]. With the capping of either one or both polar groups, all CDPs gained organogelation ability. When the *O*<sup>t</sup>Bu protecting group was present on the carboxylic moiety, the corresponding CDP became a superorganogelator for aromatic solvents, such as benzene, xylene, or toluene, with the possibility for the amine group to be either free or linked to Fmoc. When the carboxylic group was free and the Fmoc was installed on the amine, the CDP became a hydrogelator and an organogelator for chlorinated solvents [107].

Besides Fmoc, Boc is another convenient protecting group for the Lys  $\epsilon$ -amine. A similar study on the influence of Boc and Fmoc groups showed that cyclo(Lys-Lys) did not form hydrogels, while the corresponding Fmoc and Boc derivatives were organogelators, although none of these compounds showed a minimum gelling concentration <1 wt% [111]. Another study showed the importance of the Fmoc-protecting group in the organogelation of cyclo(Lys-Lys) derivatives. The CDPs were functionalized with cysteine on both Lys-side chains through amide bonding, with the  $\alpha$ -amino group of Cys being either Fmoc-protected or free. Only the derivatives with the Fmoc group were able to yield organogels, while thiols could be either in the reduced form or oxidized in a bicycle [112].

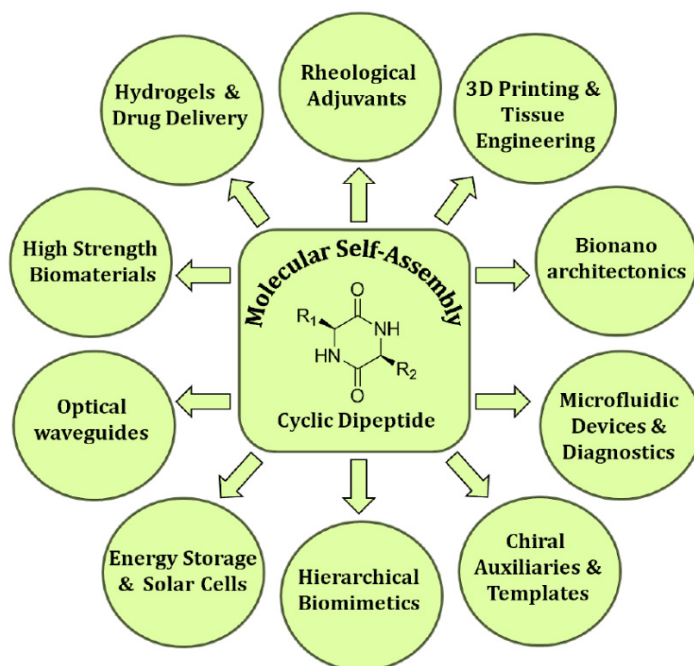
Alkyl chains provide another strategy to increase the hydrophobicity of polar side chains of CDPs. Lysine could be acylated with anhydrides or coupled with an aliphatic carboxylic acid through an amide bond. A study of these kinds of derivatives, using cyclo(Tyr-Lys) as CDP scaffold, showed the change in hydrophobicity was related to the different gelling behavior [106]. In particular, linear carboxylic acids with an aliphatic chain composed of 1, 2, 3, 5, 7, 11, or 17 carbon atoms were chosen as acyl functional groups. Low-molecular-weight acids (with 1–4 carbon-atom chains) rendered CDPs hydrogelators, with the lighter compound being also an organogelator for polar solvents, such as dimethylformamide, DMSO, and piperidine. The use of alkyl chains composed of 2–17 carbon atoms provided organogelation ability in alcohols [106].

## 4. Applications

Self-assembled gels are attractive systems to design innovative materials. As discussed in this review, CDPs can be biocompatible, cheap, and easy to synthesize, and natural CDPs provide a great source of inspiration to develop green solutions for many of the challenges of our century. As CDPs are a popular topic, recent reviews already exist on CDP applications in medicine [39] and optoelectronics [40]. A comprehensive review of CDP uses (Figure 6) was published in 2017 [27], therefore here we will focus on the latest advancements in the field.

One research area that is gaining momentum is bioinspired enzyme mimicry through the design of supramolecular catalysts based on minimalistic peptides [116] and their derivatives, CDPs included. For instance, His and Cys residues are catalytically active residues of various hydrolases. Cyclo(Phe-His) and cyclo(Phe-Cys) successfully self-assembled into a hydrogel for esterase mimicry [41]. Cyclo(Phe-Leu) formed a hydrogel in

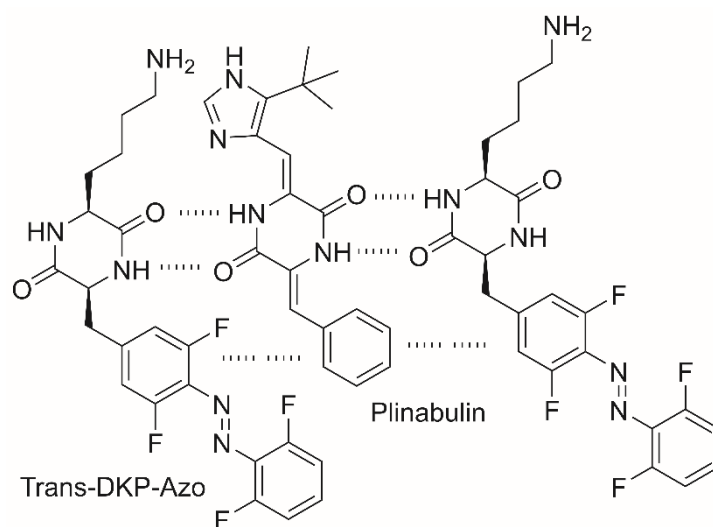
the presence of tetramethylpyridylporphyrin iron complex (Fe(III)-TMPyP) as a catalyst for peroxidase reactions [82]. The hydrogel not only provided a scaffold but also enhanced the catalyst acceleration of pyrogallol oxidation, relative to the free catalyst in solution [82].



**Figure 6.** Various applications are envisaged for self-assembled CDPs. Reproduced with permission from [27], © 2017 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

Another area that is very promising for its innovative potential consists of the development of smart materials, which respond to a variety of stimuli, with light being particularly attractive for spatio-temporal control. For instance, a biocompatible non-natural CDP equipped with an azobenzene (Scheme 4) provided an interesting hydrogelator that responded to a light switch through sol-gel transitions [117]. In particular, green-light irradiation promoted *trans*-to-*cis* isomerization, which could be reversed upon appropriate change of the light wavelength. Since only the *trans* isomer was a gelling agent, it was thus possible to switch from the gel to a solution upon green-light irradiation. The compound was studied to carry a possible anticancer drug into a living system and then release it with an external-light stimulus [117].

Finally, organic electronics is one of the most researched areas as finding new technologies in this field could translate into great innovations in the electronic market. Recent advancements include organic micro-devices that can be printed avoiding traditional silicon-based components, although at present this is sustainable only in batch [118]. For large-scale production, the key is finding a few versatile organic materials to reduce the number of components needed for the printing process and to optimize the number of reagents needed to print a complex circuit. The high stability provided by CDPs, together with their easy low-cost production on a large scale, indeed makes them attractive to develop organic electronics [40]. For example, cyclo(Tyr-Trp) is a hydrogelator with good resistance to harsh environments. This compound was tested at different pH and temperature values, as well as in the presence of charged biopolymers, and the noticeable resistance suggested the potential for electronic applications. Cyclic voltammetry studies showed a supercapacitor behavior and good electrochemical stability, thus this material could be a good candidate for an organic supercapacitor component [105].



**Scheme 4.** Chemical structures of the photo-responsive trans isomer of an azobenzene-derivative of a DKP (Trans-DKP-Azo) that self-assembles into a hydrogel and can be used to carry the anticancer drug plinabulin (center) through non-covalent interactions [117].

## 5. Conclusions

The area of self-assembling minimalistic systems composed of di- and tri-peptides has attracted great interest in the last decade, showing promising potential for a variety of applications. Recently, we have witnessed a renaissance of their cyclic derivatives, CDPs, in virtue of their higher stability against physico-chemical harsh conditions as well as biological degradation. The rigid structure provided by the CDP ring is particularly attractive to impart various bioactivities, to allow to cross the blood-brain barrier and reach otherwise difficult pathological targets to treat, and also to favor self-assembly into hydro- and organo-gels. As new bioactive CDPs continue to be identified from natural sources, and their synthesis can be easily attained also through the development of biotechnological and microwave-assisted green methods, it appears evident that the field is well-set to keep growing and bring further innovation in all the areas where greener alternatives are highly sought after.

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## References

- Sharifzadeh, G.; Hosseinkhani, H. Biomolecule-Responsive Hydrogels in Medicine. *Adv. Healthc. Mater.* **2017**, *6*, 1700801. [CrossRef]
- Apostolopoulos, V.; Bojarska, J.; Chai, T.T.; Elnagdy, S.; Kaczmarek, K.; Matsoukas, J.; New, R.; Parang, K.; Lopez, O.P.; Parhiz, H.; et al. A Global Review on Short Peptides: Frontiers and Perspectives. *Molecules* **2021**, *26*, 430. [CrossRef]
- Ni, M.; Zhuo, S. Applications of self-assembling ultrashort peptides in bionanotechnology. *RSC Adv.* **2019**, *9*, 844–852. [CrossRef]
- Gupta, S.; Singh, I.; Sharma, A.K.; Kumar, P. Ultrashort Peptide Self-Assembly: Front-Runners to Transport Drug and Gene Cargos. *Front. Bioeng. Biotechnol.* **2020**, *8*, 504. [CrossRef]
- Sequeira, M.A.; Herrera, M.G.; Dodero, V.I. Modulating amyloid fibrillation in a minimalist model peptide by intermolecular disulfide chemical reduction. *Phys. Chem. Chem. Phys.* **2019**, *21*, 11916–11923. [CrossRef]

6. Reddy, S.M.M.; Raßlenberg, E.; Sloan-Dennison, S.; Hesketh, T.; Silberbush, O.; Tuttle, T.; Smith, E.; Graham, D.; Faulds, K.; Ulijn, R.V.; et al. Proton-Conductive Melanin-Like Fibers through Enzymatic Oxidation of a Self-Assembling Peptide. *Adv. Mater.* **2020**, *32*, e2003511. [[CrossRef](#)] [[PubMed](#)]
7. Xiong, Q.; Liu, Z.; Han, W. Sequence-Dependent Nanofiber Structures of Phenylalanine and Isoleucine Tripeptides. *Int. J. Mol. Sci.* **2020**, *21*, 8431. [[CrossRef](#)] [[PubMed](#)]
8. Saddik, A.A.; Chakravarthy, R.D.; Mohammed, M.; Lin, H.C. Effects of fluoro substitutions and electrostatic interactions on the self-assembled structures and hydrogelation of tripeptides: Tuning the mechanical properties of co-assembled hydrogels. *Soft Matter* **2020**, *16*, 10143–10150. [[CrossRef](#)] [[PubMed](#)]
9. Criado-Gonzalez, M.; Wagner, D.; Iqbal, M.H.; Ontani, A.; Carvalho, A.; Schmutz, M.; Schlenoff, J.B.; Schaaf, P.; Jierry, L.; Boulmedais, F. Supramolecular tripeptide self-assembly initiated at the surface of coacervates by polyelectrolyte exchange. *J. Coll. Interface Sci.* **2021**, *588*, 580–588. [[CrossRef](#)]
10. Vardhishna, M.V.; Srinivasulu, G.; Hari Krishna, A.; Thakur, S.S.; Chatterjee, B. Simultaneous Occurrence of Nanospheres and Nanofibers Self-Assembled from Achiral Tripeptides. *ChemistryOpen* **2019**, *8*, 266–270. [[CrossRef](#)]
11. Nir, S.; Zanuy, D.; Zada, T.; Agazani, O.; Aleman, C.; Shalev, D.E.; Reches, M. Tailoring the self-assembly of a tripeptide for the formation of antimicrobial surfaces. *Nanoscale* **2019**, *11*, 8752–8759. [[CrossRef](#)]
12. Kumar, S.; Bera, S.; Nandi, S.K.; Haldar, D. The effect of amide bond orientation and symmetry on the self-assembly and gelation of discotic tripeptides. *Soft Matter* **2021**, *17*, 113–119. [[CrossRef](#)] [[PubMed](#)]
13. Ozawa, Y.; Sato, H.; Kayano, Y.; Yamaki, N.; Izato, Y.I.; Miyake, A.; Naito, A.; Kawamura, I. Self-assembly of tripeptides into  $\gamma$ -turn nanostructures. *Phys. Chem. Chem. Phys.* **2019**, *21*, 10879–10883. [[CrossRef](#)] [[PubMed](#)]
14. Cringoli, M.C.; Romano, C.; Parisi, E.; Waddington, L.J.; Melchionna, M.; Semeraro, S.; De Zorzi, R.; Grönholm, M.; Marchesan, S. Bioadhesive supramolecular hydrogel from unprotected, short D,L-peptides with Phe-Phe and Leu-Asp-Val motifs. *Chem. Commun.* **2020**, *56*, 3015–3018. [[CrossRef](#)] [[PubMed](#)]
15. Garcia, A.M.; Melchionna, M.; Bellotto, O.; Kralj, S.; Semeraro, S.; Parisi, E.; Iglesias, D.; D'Andrea, P.; De Zorzi, R.; Vargiu, A.V.; et al. Nanoscale Assembly of Functional Peptides with Divergent Programming Elements. *ACS Nano* **2021**, *15*, 3015–3025. [[CrossRef](#)] [[PubMed](#)]
16. Kralj, S.; Bellotto, O.; Parisi, E.; Garcia, A.M.; Iglesias, D.; Semeraro, S.; Deganutti, C.; D'Andrea, P.; Vargiu, A.V.; Geremia, S.; et al. Heterochirality and Halogenation Control Phe-Phe Hierarchical Assembly. *ACS Nano* **2020**, *14*, 16951–16961. [[CrossRef](#)] [[PubMed](#)]
17. Bellotto, O.; Kralj, S.; De Zorzi, R.; Geremia, S.; Marchesan, S. Supramolecular hydrogels from unprotected dipeptides: A comparative study on stereoisomers and structural isomers. *Soft Matter* **2020**, *16*, 10151–10157. [[CrossRef](#)] [[PubMed](#)]
18. Nikoloudakis, E.; Mitropoulou, K.; Landrou, G.; Charalambidis, G.; Nikolaou, V.; Mitraki, A.; Coutsolelos, A.G. Self-assembly of aliphatic dipeptides coupled with porphyrin and BODIPY chromophores. *Chem. Commun.* **2019**, *55*, 14103–14106. [[CrossRef](#)]
19. Hu, T.; Zhang, Z.; Hu, H.; Euston, S.R.; Pan, S. A Comprehensive Study on Self-Assembly and Gelation of C(13)-Dipeptides-From Design Strategies to Functionalities. *Biomacromolecules* **2020**, *21*, 670–679. [[CrossRef](#)]
20. Zhang, H.; Lou, S.; Yu, Z. Polar- $\pi$  Interactions Promote Self-assembly of Dipeptides into Laminated Nanofibers. *Langmuir* **2019**, *35*, 4710–4717. [[CrossRef](#)]
21. Rissanou, A.N.; Simatos, G.; Siachouli, P.; Harmandaris, V.; Mitraki, A. Self-assembly of Alanine-Isoleucine and Isoleucine-Isoleucine Dipeptides through Atomistic Simulations and Experiments. *J. Phys. Chem. B* **2020**, *124*, 7102–7114. [[CrossRef](#)] [[PubMed](#)]
22. Zhang, L.; Lin, S.; Tong, Q.; Li, Y.; Wang, Y.; Li, Y.; Li, B.; Yang, Y. Helicity of perfluoroalkyl chains controlled by the self-assembly of the Ala-Ala dipeptides. *Chirality* **2019**, *31*, 992–1000. [[CrossRef](#)]
23. Gil, A.M.; Casanovas, J.; Mayans, E.; Jiménez, A.I.; Puiggali, J.; Alemán, C. Heterochirality Restricts the Self-Assembly of Phenylalanine Dipeptides Capped with Highly Aromatic Groups. *J. Phys. Chem. B* **2020**, *124*, 5913–5918. [[CrossRef](#)]
24. Kumar, M.; Sementa, D.; Narang, V.; Riedo, E.; Ulijn, R.V. Self-Assembly Propensity Dictates Lifetimes in Transient Naphthalimide-Dipeptide Nanofibers. *Chem. Eur. J.* **2020**, *26*, 8372–8376. [[CrossRef](#)]
25. Anand, B.G.; Prajapati, K.P.; Dubey, K.; Ahamad, N.; Shekhawat, D.S.; Rath, P.C.; Joseph, G.K.; Kar, K. Self-Assembly of Artificial Sweetener Aspartame Yields Amyloid-like Cytotoxic Nanostructures. *ACS Nano* **2019**, *13*, 6033–6049. [[CrossRef](#)]
26. Frenkel-Pinter, M.; Samanta, M.; Ashkenasy, G.; Leman, L.J. Prebiotic Peptides: Molecular Hubs in the Origin of Life. *Chem. Rev.* **2020**, *120*, 4707–4765. [[CrossRef](#)]
27. Manchineella, S.; Govindaraju, T. Molecular Self-Assembly of Cyclic Dipeptide Derivatives and Their Applications. *ChemPlusChem* **2017**, *82*, 88–106. [[CrossRef](#)] [[PubMed](#)]
28. Cornacchia, C.; Cacciatore, I.; Baldassarre, L.; Mollica, A.; Feliciani, F.; Pinnen, F. 2,5-Diketopiperazines as Neuroprotective Agents. *Mini-Rev. Med. Chem.* **2012**, *12*, 2–12. [[CrossRef](#)]
29. Feni, L.; Jütten, L.; Parente, S.; Piarulli, U.; Neundorff, I.; Diaz, D. Cell-penetrating peptides containing 2,5-diketopiperazine (DKP) scaffolds as shuttles for anti-cancer drugs: Conformational studies and biological activity. *Chem. Commun.* **2020**, *56*, 5685–5688. [[CrossRef](#)] [[PubMed](#)]
30. Yang, J.; Li, Y.; Wang, X.; Wang, D.; Sun, Y.; Wang, J.; Xu, H. Self-Assembly of Cyclic Dipeptides and Their Fluorescent Properties. *Acta Chim. Sin.* **2019**, *77*, 1279–1286. [[CrossRef](#)]

31. Zhao, K.; Xing, R.; Yan, X. Cyclic dipeptides: Biological activities and self-assembled materials. *Pept. Sci.* **2021**, *113*, e24202. [[CrossRef](#)]
32. Sittmann, J.; Bae, M.; Mevers, E.; Li, M.; Quinn, A.; Sriram, G.; Clardy, J.; Liu, Z. Bacterial diketopiperazines stimulate diatom growth and lipid accumulation. *Plant. Phys.* **2021**, *80*. [[CrossRef](#)]
33. Zink, K.E.; Ludvik, D.A.; Lazzara, P.R.; Moore, T.W.; Mandel, M.J.; Sanchez, L.M. A Small Molecule Coordinates Symbiotic Behaviors in a Host Organ. *mBio* **2021**, *12*, e03637-20. [[CrossRef](#)]
34. Ding, Y.; Zhu, X.; Hao, L.; Zhao, M.; Hua, Q.; An, F. Bioactive Indolyl Diketopiperazines from the Marine Derived Endophytic *Aspergillus versicolor* DY180635. *Mar. Drugs* **2020**, *18*. [[CrossRef](#)]
35. André, A.; Touré, A.K.; Stien, D.; Eparvier, V. 2,5-diketopiperazines mitigate the amount of advanced glycation end products accumulated with age in human dermal fibroblasts. *Int. J. Cosm. Sci.* **2020**, *42*, 596–604. [[CrossRef](#)]
36. Deigin, V.; Ksenofontova, O.; Yatskin, O.; Goryacheva, A.; Ignatova, A.; Feofanov, A.; Ivanov, V. Novel platform for the preparation of synthetic orally active peptidomimetics with hemoregulating activity. II. Hemosuppressor activity of 2,5-diketopiperazine-based cyclopeptides. *Int. Immunopharmacol.* **2020**, *81*, 106185. [[CrossRef](#)]
37. Guo, X.-C.; Zhang, Y.-H.; Gao, W.-B.; Pan, L.; Zhu, H.-J.; Cao, F. Absolute Configurations and Chitinase Inhibitions of Quinazoline-Containing Diketopiperazines from the Marine-Derived Fungus *Penicillium polonicum*. *Mar. Drugs* **2020**, *18*. [[CrossRef](#)]
38. Yu, X.; Li, L.; Sun, S.; Chang, A.; Dai, X.; Li, H.; Wang, Y.; Zhu, H. A Cyclic Dipeptide from Marine Fungus *Penicillium chrysogenum* DXY-1 Exhibits Anti-quorum Sensing Activity. *ACS Omega* **2021**, *6*, 7693–7700. [[CrossRef](#)]
39. Chibh, S.; Mishra, J.; Kour, A.; Chauhan, V.S.; Panda, J.J. Recent advances in the fabrication and bio-medical applications of self-assembled dipeptide nanostructures. *Nanomedicine* **2021**, *16*, 139–163. [[CrossRef](#)]
40. Yu, C.; Kai, T.; Wei, J.; Pandeewar, M.; Sigal, R.-L.; Ehud, G. Self-Assembly of Cyclic Dipeptides: Platforms for Functional Materials. *Prot. Pept. Lett.* **2020**, *27*, 688–697. [[CrossRef](#)]
41. Kleinsmann, A.J.; Nachtsheim, B.J. A minimalistic hydrolase based on co-assembled cyclic dipeptides. *Org. Biomol. Chem.* **2020**, *18*, 102–107. [[CrossRef](#)]
42. Maisto, S.K.; Leersnyder, A.P.; Pudner, G.L.; Scheerer, J.R. Synthesis of Pyrrolopyrazinones by Construction of the Pyrrole Ring onto an Intact Diketopiperazine. *J. Org. Chem.* **2020**, *85*, 9264–9271. [[CrossRef](#)]
43. Chen, X.; Chen, X.; Steimbach, R.R.; Wu, T.; Li, H.; Dan, W.; Shi, P.; Cao, C.; Li, D.; Miller, A.K.; et al. Novel 2, 5-diketopiperazine derivatives as potent selective histone deacetylase 6 inhibitors: Rational design, synthesis and antiproliferative activity. *Eur. J. Med. Chem.* **2020**, *187*, 111950. [[CrossRef](#)]
44. Takahashi, S.; Kimishima, A.; Hirose, T.; Yamada, T.; Sugawara, A.; Shirahata, T.; Noguchi, Y.; Iwatsuki, M.; Hokari, R.; Ishiyama, A.; et al. Unified enantioselective total synthesis of 3,6-dioxygenated diketopiperazine natural products, diatretole and lepidistamides A, B and C. *Tet. Lett.* **2021**, *67*, 152895. [[CrossRef](#)]
45. Olsson, C.R.; Payette, J.N.; Cheah, J.H.; Movassaghi, M. Synthesis of Potent Cytotoxic Epidithiodiketopiperazines Designed for Derivatization. *J. Org. Chem.* **2020**, *85*, 4648–4662. [[CrossRef](#)]
46. Ganesh, A.; Chaturvedi, P.; Karkara, B.B.; Chatterjee, I.; Datta, D.; Panda, G. One pot synthesis of N-monoalkylated plinabulin derivatives via multicomponent protocol and their application as anticancer agents. *J. Mol. Struct.* **2021**, *1229*, 129830. [[CrossRef](#)]
47. Saavedra, C.J.; Cuevas, F.; Romero-Estudillo, I.; Boto, A. Synthesis of Diketopiperazine Scaffolds with Tailored N- and  $\alpha$ -Chains by Selective Modification of Customizable Units. *Adv. Synth. Catal.* **2020**, *362*, 3158–3169. [[CrossRef](#)]
48. Bonneure, E.; De Baets, A.; De Decker, S.; Van den Berge, K.; Clement, L.; Vyverman, W.; Mangelinckx, S. Altering the Sex Phorene Cyclo(l-Pro-l-Pro) of the Diatom *Seminavis robusta* towards a Chemical Probe. *Int. J. Mol. Sci.* **2021**, *22*. [[CrossRef](#)]
49. Yu, H.; Zong, Y.; Xu, T. Total synthesis of (–)-penicimutanin A and related congeners. *Chem. Sci.* **2020**, *11*, 656–660. [[CrossRef](#)]
50. Khopade, T.M.; Ajayan, K.; Joshi, S.S.; Lane, A.L.; Viswanathan, R. Bioinspired Brønsted Acid-Promoted Regioselective Tryptophan Isoprenylations. *ACS Omega* **2021**, *6*, 10840–10858. [[CrossRef](#)] [[PubMed](#)]
51. Gomes, N.G.M.; Pereira, R.B.; Andrade, P.B.; Valentão, P. Double the Chemistry, Double the Fun: Structural Diversity and Biological Activity of Marine-Derived Diketopiperazine Dimers. *Mar. Drugs* **2019**, *17*, 551. [[CrossRef](#)] [[PubMed](#)]
52. Zhao, P.; Xue, Y.; Li, J.; Li, X.; Zu, X.; Zhao, Z.; Quan, C.; Gao, W.; Feng, S. Non-lipopeptide fungi-derived peptide antibiotics developed since 2000. *Biotechnol. Lett.* **2019**, *41*, 651–673. [[CrossRef](#)] [[PubMed](#)]
53. Long, S.; Sousa, E.; Kijjoa, A.; Pinto, M.M. Marine Natural Products as Models to Circumvent Multidrug Resistance. *Molecules* **2016**, *21*, 892. [[CrossRef](#)]
54. Kim, S.-W.; Hume, P.A.; Sperry, J. Bioinspired Synthesis of the Furopyrazine Alkaloid Hyrtioseragine A. *J. Org. Chem.* **2021**, *86*, 4779–4785. [[CrossRef](#)]
55. Harizani, M.; Katsini, E.; Georgantea, P.; Roussis, V.; Ioannou, E. New Chlorinated 2,5-Diketopiperazines from Marine-Derived Bacteria Isolated from Sediments of the Eastern Mediterranean Sea. *Molecules* **2020**, *25*, 1509. [[CrossRef](#)]
56. Nies, J.; Li, S.-M. Prenylation and Dehydrogenation of a C2-Reversely Prenylated Diketopiperazine as a Branching Point in the Biosynthesis of Echinulin Family Alkaloids in *Aspergillus ruber*. *ACS Chem. Biol.* **2021**, *16*, 185–192. [[CrossRef](#)]
57. Borgman, P.; Lopez, R.D.; Lane, A.L. The expanding spectrum of diketopiperazine natural product biosynthetic pathways containing cyclodipeptide synthases. *Org. Biomol. Chem.* **2019**, *17*, 2305–2314. [[CrossRef](#)]
58. Wang, X.; Li, Y.; Zhang, X.; Lai, D.; Zhou, L. Structural Diversity and Biological Activities of the Cyclodipeptides from Fungi. *Molecules* **2017**, *22*, 2026. [[CrossRef](#)]

59. Borthwick, A.D.; Da Costa, N.C. 2,5-diketopiperazines in food and beverages: Taste and bioactivity. *Crit. Rev. Food Sci. Nutr.* **2017**, *57*, 718–742. [[CrossRef](#)]
60. Ying, J.; Lin, R.; Xu, P.; Wu, Y.; Liu, Y.; Zhao, Y. Prebiotic formation of cyclic dipeptides under potentially early Earth conditions. *Sci. Rep.* **2018**, *8*, 936. [[CrossRef](#)]
61. Ma, Y.-M.; Liang, X.-A.; Kong, Y.; Jia, B. Structural Diversity and Biological Activities of Indole Diketopiperazine Alkaloids from Fungi. *J. Agric. Food Chem.* **2016**, *64*, 6659–6671. [[CrossRef](#)] [[PubMed](#)]
62. Ortiz, A.; Sansinenea, E. Cyclic Dipeptides: Secondary Metabolites Isolated from Different Microorganisms with Diverse Biological Activities. *Curr. Med. Chem.* **2017**, *24*, 2773–2780. [[CrossRef](#)]
63. Borthwick, A.D. 2,5-Diketopiperazines: Synthesis, Reactions, Medicinal Chemistry, and Bioactive Natural Products. *Chem. Rev.* **2012**, *112*, 3641–3716. [[CrossRef](#)]
64. Regenass, P.; Riché, S.; Péron, F.; Rognan, D.; Hibert, M.; Girard, N.; Bonnet, D. A step-economical multicomponent synthesis of 3D-shaped aza-diketopiperazines and their drug-like chemical space analysis. *Org. Biomol. Chem.* **2016**, *14*, 8859–8863. [[CrossRef](#)] [[PubMed](#)]
65. Rahim, A.; Sahariah, B.; Sarma, B.K. N, N'-Di(acylamino)-2,5-diketopiperazines: Strategic Incorporation of Reciprocal  $n \rightarrow \pi^*$  Interactions in a Druglike Scaffold. *Org. Lett.* **2018**, *20*, 5743–5746. [[CrossRef](#)]
66. Al-Wahaibi, L.H.; Gouda, A.M.; Abou-Ghadir, O.F.; Salem, O.I.A.; Ali, A.T.; Farghaly, H.S.; Abdelrahman, M.H.; Trembleau, L.; Abdu-Allah, H.H.M.; Youssif, B.G.M. Design and synthesis of novel 2,3-dihydropyrazino [1,2-a]indole-1,4-dione derivatives as antiproliferative EGFR and BRAFV600E dual inhibitors. *Bioorg. Chem.* **2020**, *104*, 104260. [[CrossRef](#)]
67. Hanabusa, K.; Fukui, H.; Suzuki, M.; Shirai, H. Specialist Gelator for Ionic Liquids. *Langmuir* **2005**, *21*, 10383–10390. [[CrossRef](#)]
68. Zhang, D.; Wang, W. A facile synthesis of cysteine-based diketopiperazine from thiol-protected precursor. *R. Soc. Open Sci.* **2018**, *5*, 180272. [[CrossRef](#)]
69. Mieczkowski, A.; Speina, E.; Trzybiński, D.; Winiewska-Szajewska, M.; Wińska, P.; Borsuk, E.M.; Podsiadła-Białoskórska, M.; Przygodzki, T.; Drabikowski, K.; Stanczyk, L.; et al. Diketopiperazine-Based, Flexible Tadalafil Analogues: Synthesis, Crystal Structures and Biological Activity Profile. *Molecules* **2021**, *26*, 794. [[CrossRef](#)]
70. Garrido González, F.P.; Mancilla Percino, T. Synthesis, docking study and inhibitory activity of 2,6-diketopiperazines derived from  $\alpha$ -amino acids on HDAC8. *Bioorg. Chem.* **2020**, *102*, 104080. [[CrossRef](#)]
71. Ramírez-López, S.C.; Rentería-Gómez, M.A.; Alvarado, C.R.S.; Gámez-Montaño, R. Synthesis of Peptidomimetics via IMCR/Post-Transformation Strategy. *Chem. Proc.* **2021**, *3*, 8396. [[CrossRef](#)]
72. Ryan, J.S.; Schindler, C.S. Total Synthesis of Mycrocyclosin and the Herquelines. *Synthesis* **2021**, *53*. [[CrossRef](#)]
73. García-Mingüens, E.; Selva, V.; Larrañaga Agirre, O.; Nájera, C.; Sansano, J.M.; Cózar Ruano, A.d. Nitroprolinates as Nucleophiles in Michael-type Additions and Acylations. Synthesis of Enantiomerically Enriched Fused Amino-pyrrolidino-[1,2-a]pyrazinones and -diketopiperazines. *ChemCatChem* **2020**, *12*, 2014–2021. [[CrossRef](#)]
74. Perić, B.; Juraj, N.P.; Szalontai, G.; Veličković, S.R.; Veljković, F.M.; Vikić-Topić, D.; Kirin, S.I. A crystallographic and solid-state NMR study of 1,4-disubstituted 2,5-diketopiperazines. *J. Mol. Struct.* **2021**, *1234*, 130157. [[CrossRef](#)]
75. Pound, S.M.; Underwood, S.J.; Douglas, C.J. Studies towards the Total Synthesis of Drimentine C. Preparation of the AB and CDEF Ring Fragments. *Eur. J. Org. Chem.* **2020**, *2020*, 2448–2453. [[CrossRef](#)] [[PubMed](#)]
76. Magriotis, P.A. Recent progress toward the asymmetric synthesis of carbon-substituted piperazine pharmacophores and oxidative related heterocycles. *RSC Med. Chem.* **2020**, *11*, 745–759. [[CrossRef](#)]
77. Rahim, A.; Sahariah, B.; Baruah, K.; Deka, J.K.R.; Sarma, B.K. Solid-Phase Synthesis of Hybrid 2,5-Diketopiperazines Using Acylhydrazide, Carbazate, Semicarbazide, Amino Acid, and Primary Amine Submonomers. *J. Org. Chem.* **2020**, *85*, 2927–2937. [[CrossRef](#)]
78. Hickey, J.L.; Lin, S. One-pot peptide cleavage and macrocyclization through direct amidation using triazabicyclodecene. *Pept. Sci.* **2020**, *112*, e24161. [[CrossRef](#)]
79. Rivera, D.G.; Ricardo, M.G.; Vasco, A.V.; Wessjohann, L.A.; Van der Eycken, E.V. On-resin multicomponent protocols for biopolymer assembly and derivatization. *Nat. Prot.* **2021**, *16*, 561–578. [[CrossRef](#)]
80. Li, W.; Wade, J.D.; Reynolds, E.; O'Brien-Simpson, N.M. Chemical Modification of Cellulose Membranes for SPOT Synthesis. *Aust. J. Chem.* **2020**, *73*, 78–84. [[CrossRef](#)]
81. Yin, H.; Takada, K.; Kumar, A.; Hirayama, T.; Kaneko, T. Synthesis and solvent-controlled self-assembly of diketopiperazine-based polyamides from aspartame. *RSC Adv.* **2021**, *11*, 5938–5946. [[CrossRef](#)]
82. Pappas, C.G.; Wijerathne, N.; Sahoo, J.K.; Jain, A.; Kroiss, D.; Sasselli, I.R.; Pina, A.S.; Lampel, A.; Ulijn, R.V. Spontaneous Aminolytic Cyclization and Self-Assembly of Dipeptide Methyl Esters in Water. *ChemSystemsChem* **2020**, *2*, e2000013. [[CrossRef](#)]
83. Tullberg, M.; Grøtli, M.; Luthman, K. Efficient synthesis of 2,5-diketopiperazines using microwave assisted heating. *Tetrahedron* **2006**, *62*, 7484–7491. [[CrossRef](#)]
84. Thaqi, A.; McCluskey, A.; Scott, J.L. A mild Boc deprotection and the importance of a free carboxylate. *Tet. Lett.* **2008**, *49*, 6962–6964. [[CrossRef](#)]
85. Pérez-Picaso, L.; Escalante, J.; Olivo, H.F.; Rios, M.Y. Efficient microwave assisted syntheses of 2,5-diketopiperazines in aqueous media. *Molecules* **2009**, *14*, 2836–2849. [[CrossRef](#)] [[PubMed](#)]

86. Kurbasic, M.; Semeraro, S.; García, A.M.; Kralj, S.; Parisi, E.; Deganutti, C.; Zorzi, R.D.; Marchesan, S. Microwave-Assisted Cyclization of Unprotected Dipeptides in Water to 2,5-Piperazinediones and Self-Assembly Study of Products and Reagents. *Synthesis* **2019**, *51*, 2829–2838. [[CrossRef](#)]
87. Adler-Abramovich, L.; Aronov, D.; Beker, P.; Yevnin, M.; Stempler, S.; Buzhansky, L.; Rosenman, G.; Gazit, E. Self-assembled arrays of peptide nanotubes by vapour deposition. *Nat. Nanotechnol.* **2009**, *4*, 849–854. [[CrossRef](#)] [[PubMed](#)]
88. Ziganshin, M.A.; Safiullina, A.S.; Gerasimov, A.V.; Ziganshina, S.A.; Klimovitskii, A.E.; Khayarov, K.R.; Gorbachuk, V.V. Thermally Induced Self-Assembly and Cyclization of L-Leucyl-L-Leucine in Solid State. *J. Phys. Chem. B* **2017**, *121*, 8603–8610. [[CrossRef](#)]
89. Safiullina, A.S.; Buzyurov, A.V.; Ziganshina, S.A.; Gerasimov, A.V.; Schick, C.; Gorbachuk, V.V.; Ziganshin, M.A. Using fast scanning calorimetry to study solid-state cyclization of dipeptide L-leucyl-L-leucine. *Thermochim. Acta* **2020**, *692*, 178748. [[CrossRef](#)]
90. Ziganshin, M.A.; Larionov, R.A.; Gerasimov, A.V.; Ziganshina, S.A.; Klimovitskii, A.E.; Khayarov, K.R.; Mukhametzyanov, T.A.; Gorbachuk, V.V. Thermally induced cyclization of (L)-isoleucyl-(L)-alanine in solid state: Effect of dipeptide structure on reaction temperature and self-assembly. *J. Pept. Sci.* **2019**, *25*, e3177. [[CrossRef](#)]
91. Pérez-Mellor, A.; Le Barbu-Debus, K.; Zehnacker, A. Solid-state synthesis of cyclo LD-diphenylalanine: A chiral phase built from achiral subunits. *Chirality* **2020**, *32*, 693–703. [[CrossRef](#)]
92. Canu, N.; Moutiez, M.; Belin, P.; Gondry, M. Cyclodipeptide synthases: A promising biotechnological tool for the synthesis of diverse 2,5-diketopiperazines. *Nat. Prod. Rep.* **2020**, *37*, 312–321. [[CrossRef](#)] [[PubMed](#)]
93. Zhang, Y.; Yao, T.; Jiang, Y.; Li, H.; Yuan, W.; Li, W. Deciphering a Cyclodipeptide Synthase Pathway Encoding Prenylated Indole Alkaloids in *Streptomyces leeuwenhoekii*. *Appl. Environ. Microbiol.* **2021**, *87*, e02525-20. [[CrossRef](#)] [[PubMed](#)]
94. Mikulski, L.; Schäfer, J.; Brockmeyer, K.; Kraut, R.; Li, S.-M. Comparative studies on similarities and differences of cyclodipeptide oxidases for installation of C–C double bonds at the diketopiperazine ring. *Appl. Microbiol. Biotechnol.* **2020**, *104*, 2523–2536. [[CrossRef](#)] [[PubMed](#)]
95. Yao, T.; Liu, J.; Jin, E.; Liu, Z.; Li, H.; Che, Q.; Zhu, T.; Li, D.; Li, W. Expanding the Structural Diversity of Drimentines by Exploring the Promiscuity of Two N-methyltransferases. *iScience* **2020**, *23*, 101323. [[CrossRef](#)] [[PubMed](#)]
96. Shende, V.V.; Khatri, Y.; Newmister, S.A.; Sanders, J.N.; Lindovska, P.; Yu, F.; Doyon, T.J.; Kim, J.; Houk, K.N.; Movassaghi, M.; et al. Structure and Function of NzeB, a Versatile C–C and C–N Bond-Forming Diketopiperazine Dimerase. *J. Am. Chem. Soc.* **2020**, *142*, 17413–17424. [[CrossRef](#)]
97. Sun, C.; Luo, Z.; Zhang, W.; Tian, W.; Peng, H.; Lin, Z.; Deng, Z.; Kobe, B.; Jia, X.; Qu, X. Molecular basis of regio- and stereo-specificity in biosynthesis of bacterial heterodimeric diketopiperazines. *Nat. Commun.* **2020**, *11*, 6251. [[CrossRef](#)]
98. Sun, C.; Peng, H.; Zhang, W.; Zheng, M.; Tian, W.; Zhang, Y.; Liu, H.; Lin, Z.; Deng, Z.; Qu, X. Production of Heterodimeric Diketopiperazines Employing a Mycobacterium-Based Whole-Cell Biocatalysis System. *J. Org. Chem.* **2021**. [[CrossRef](#)]
99. Harken, L.; Li, S.-M. Modifications of diketopiperazines assembled by cyclodipeptide synthases with cytochrome P450 enzymes. *Appl. Microbiol. Biotechnol.* **2021**, *105*, 2277–2285. [[CrossRef](#)]
100. Hanabusa, K.; Matsumoto, M.; Kimura, M.; Kakehi, A.; Shirai, H. Low Molecular Weight Gelators for Organic Fluids: Gelation Using a Family of Cyclo(dipeptide)s. *J. Coll. Interface Sci.* **2000**, *224*, 231–244. [[CrossRef](#)]
101. Yang, M.; Xing, R.; Shen, G.; Yuan, C.; Yan, X. A versatile cyclic dipeptide hydrogelator: Self-assembly and rheology in various physiological conditions. *Coll. Surf. A* **2019**, *572*, 259–265. [[CrossRef](#)]
102. Hanabusa, K.; Matsumoto, Y.; Miki, T.; Koyama, T.; Shirai, H. Cyclo(dipeptide)s as low-molecular-mass gelling agents to harden organic fluids. *J. Chem. Soc. Chem. Commun.* **1994**, 1401–1402. [[CrossRef](#)]
103. Kleinsmann, A.J.; Nachtsheim, B.J. Phenylalanine-containing cyclic dipeptides—the lowest molecular weight hydrogelators based on unmodified proteinogenic amino acids. *Chem. Commun.* **2013**, *49*, 7818–7820. [[CrossRef](#)] [[PubMed](#)]
104. Lan, Y.; Lv, M.; Guo, S.; Nasr, P.; Ladizhansky, V.; Vaz, R.; Corradini, M.G.; Hou, T.; Ghazani, S.M.; Marnangoni, A.; et al. Molecular motifs encoding self-assembly of peptide fibers into molecular gels. *Soft Matter* **2019**, *15*, 9205–9214. [[CrossRef](#)] [[PubMed](#)]
105. You, Y.; Xing, R.; Zou, Q.; Shi, F.; Yan, X. High-tolerance crystalline hydrogels formed from self-assembling cyclic dipeptide. *Beilstein J. Nanotechnol.* **2019**, *10*, 1894–1901. [[CrossRef](#)]
106. Xie, Z.; Zhang, A.; Ye, L.; Feng, Z.-g. Organo- and hydrogels derived from cyclo(L-Tyr-L-Lys) and its  $\epsilon$ -amino derivatives. *Soft Matter* **2009**, *5*, 1474–1482. [[CrossRef](#)]
107. Geng, H.; Ye, L.; Zhang, A.Y.; Li, J.; Feng, Z.G. Low-Molecular-Weight Organo- and Hydrogelators Based on Cyclo(L-Lys-L-Glu). *Langmuir* **2016**, *32*, 4586–4594. [[CrossRef](#)] [[PubMed](#)]
108. Safiullina, A.S.; Ziganshina, S.A.; Lyadov, N.M.; Klimovitskii, A.E.; Ziganshin, M.A.; Gorbachuk, V.V. Role of water in the formation of unusual organogels with cyclo(leucyl-leucyl). *Soft Matter* **2019**, *15*, 3595–3606. [[CrossRef](#)]
109. Sun, J.; Zhang, H.; Guo, K.; Yua, S. Self-assembly of dipeptide sodium salts derived from alanine: A molecular dynamics study. *RSC Adv.* **2015**, *5*, 102182–102190. [[CrossRef](#)]
110. Wang, L.; Jin, X.; Ye, L.; Zhang, A.-y.; Bezuidenhout, D.; Feng, Z.-g. Rapidly Recoverable Thixotropic Hydrogels from the Racemate of Chiral OFm Monosubstituted Cyclo(Glu-Glu) Derivatives. *Langmuir* **2017**, *33*, 13821–13827. [[CrossRef](#)]
111. Zong, Q.; Geng, H.; Ye, L.; Zhang, A.; Shao, Z.; Feng, Z. Synthesis and gelation capability of Fmoc and Boc mono-substituted cyclo(L-Lys-L-Lys)s. *Chem. Res. Chin. Univ.* **2016**, *32*, 484–492. [[CrossRef](#)]



112. Geng, H.; Zong, Q.; You, J.; Ye, L.; Zhang, A.; Shao, Z.; Feng, Z. Gelation capability of cysteine-modified cyclo(L-Lys-L-Lys)s dominated by Fmoc and Trt protecting groups. *Sci. China Chem.* **2016**, *59*, 293–302. [[CrossRef](#)]
113. Manchineella, S.; Murugan, N.A.; Govindaraju, T. Cyclic Dipeptide-Based Ambidextrous Supergelators: Minimalistic Rational Design, Structure-Gelation Studies, and In Situ Hydrogelation. *Biomacromolecules* **2017**, *18*, 3581–3590. [[CrossRef](#)] [[PubMed](#)]
114. Wang, L.; Hui, X.; Geng, H.; Ye, L.; Zhang, A.-y.; Shao, Z.; Feng, Z.-g. Synthesis and gelation capability of mono- and disubstituted cyclo(L-Glu-L-Glu) derivatives with tyramine, tyrosine and phenylalanine. *Coll. Polym. Sci.* **2017**, *295*, 1549–1561. [[CrossRef](#)]
115. Hoshizawa, H.; Minemura, Y.; Yoshikawa, K.; Suzuki, M.; Hanabusa, K. Thixotropic Hydrogelators Based on a Cyclo(dipeptide) Derivative. *Langmuir* **2013**, *29*, 14666–14673. [[CrossRef](#)] [[PubMed](#)]
116. Zozulia, O.; Dolan, M.A.; Korendovych, I.V. Catalytic peptide assemblies. *Chem. Soc. Rev.* **2018**, *47*, 3621–3639. [[CrossRef](#)] [[PubMed](#)]
117. Karcher, J.; Kirchner, S.; Leistner, A.-L.; Hald, C.; Geng, P.; Bantle, T.; Gödtel, P.; Pfeifer, J.; Pianowski, Z.L. Selective release of a potent anticancer agent from a supramolecular hydrogel using green light. *RSC Adv.* **2021**, *11*, 8546–8551. [[CrossRef](#)]
118. Berggren, M.; Nilsson, D.; Robinson, N.D. Organic materials for printed electronics. *Nat. Mater.* **2007**, *6*, 3–5. [[CrossRef](#)] [[PubMed](#)]