

REVIEW

[View Article Online](#)
[View Journal](#) | [View Issue](#)Cite this: *RSC Med. Chem.*, 2021, 12, 1325Received 10th March 2021,
Accepted 1st June 2021

DOI: 10.1039/d1md00083g

rsc.li/medchem

Macrocyclization strategies for cyclic peptides and peptidomimetics†

Clément Bechtler and Christina Lamers *

Peptides are a growing therapeutic class due to their unique spatial characteristics that can target traditionally “undruggable” protein–protein interactions and surfaces. Despite their advantages, peptides must overcome several key shortcomings to be considered as drug leads, including their high conformational flexibility and susceptibility to proteolytic cleavage. As a general approach for overcoming these challenges, macrocyclization of a linear peptide can usually improve these characteristics. Their synthetic accessibility makes peptide macrocycles very attractive, though traditional synthetic methods for macrocyclization can be challenging for peptides, especially for head-to-tail cyclization. This review provides an updated summary of the available macrocyclization chemistries, such as traditional lactam formation, azide–alkyne cycloadditions, ring-closing metathesis as well as unconventional cyclization reactions, and it is structured according to the obtained functional groups. Keeping peptide chemistry and screening in mind, the focus is given to reactions applicable in solution, on solid supports, and compatible with contemporary screening methods.

1. Introduction

Macrocyclic peptides are an interesting molecular format for drug discovery,¹ combining the advantages of small-molecule

and biological therapeutics: synthetic accessibility, low immunogenicity and toxicity, high binding affinity and selectivity, and the ability to target protein surfaces traditionally considered “undruggable”.^{2–5} Furthermore, macrocyclization renders peptides more stable and can increase membrane permeability,⁶ making it an important medicinal chemistry strategy in peptide drug development.⁷ Advances in high-throughput *in vitro* screening techniques have accelerated the identification of biologically potent

Department Pharmaceutical Sciences, University of Basel, Klingelbergstr. 50, 4056 Basel, Switzerland. E-mail: christina.lamers@unibas.ch

† Electronic supplementary information (ESI) available. See DOI: 10.1039/d1md00083g



Clément Bechtler

Clément Bechtler graduated in 2015 from LMU Munich in Pharmacy. Afterwards, he moved to the University College London, working on small molecule inhibitors of the Nrf2-Keap1 interaction in the research group of Dr. Geoffrey Wells and obtaining an MPhil in Medicinal Chemistry. He started then his PhD in 2017 in Prof Daniel Ricklin's group at the University of Basel, where his work is focused on peptide macrocycles, aimed at reducing undesired complement system activity. His interests include the design and synthesis of peptide macrocycles as well as their biophysical and functional characterization.



Christina Lamers

Dr. Christina Lamers received her PhD from the Pharmaceutical Chemistry Department of the Goethe-University (Frankfurt/Main, Germany) in 2014. From 2015–2017 she was a post-doctoral fellow in the laboratory of Prof. Christian Heinis at EPFL (Lausanne, Switzerland), focusing on bicyclic peptides as therapeutics and their identification with phage display. During this postdoc she won a Marie-Curie individual fellowship. In November 2017 she joined the group of Prof. Daniel Ricklin as senior post-doctoral fellow at the Molecular Pharmacy Group, University of Basel (Switzerland), developing cyclic peptides as therapeutics to target the innate immune system.

macrocyclic peptides,^{3,8,9} and the field of macrocyclization is developing quickly to match.

The synthesis of cyclic peptides can be difficult to achieve by traditional methods, such as amide formation, because a defined pre-cyclization conformation must be formed, an entropically unfavorable process, before the desired intramolecular reaction can occur. This is especially true for head-to-tail cyclization, involving the cyclization of the C-terminus of the peptide with its N-terminus, because the preferred confirmation of amide bonds is all-*trans*, which leads to an extended peptide precursor.¹⁰ The introduction of turn-inducing elements is a strategy to circumvent this.^{11,12} Furthermore, most cyclizations need to be conducted in dilute solutions to favor the intramolecular reaction over intermolecular oligomerization.¹² A pseudo-dilution effect can also be achieved by anchoring the peptide to an insoluble polymer, though this requires three-dimensional orthogonality in the protecting group strategy and anchoring *via* a sidechain when a head-to-tail cyclization is the goal.

Besides head-to-tail, peptides can also be cyclized head-to-sidechain, sidechain-to-tail or sidechain-to-sidechain. In particular, sidechain-to-sidechain cyclization has been extensively used to stabilize secondary structures, such as α -helices and β -sheets, yielding so-called 'stapled peptides',^{13,14} or to generate protein epitopes^{15,16} and antibody CDR mimetics.¹⁷

In recent years, great progress has been made in identifying new cyclization strategies for peptide macrocyclization, spanning a wide range of chemistries from cross-coupling and photochemical reactions¹⁸ to enzymatic macrocyclization.¹⁹ Chemoselective reactions,²⁰ reactions introducing orthogonality²¹ and diversity, are pushing the chemical space of macrocyclic peptides to new, more drug-like modalities. The chemoselective approaches in particular, such as ligations,^{22,23} will accelerate the increasing interest in peptide macrocycles since they allow peptide macrocyclization without the need for tedious protecting group strategies and are applicable to *in vitro* selection systems,²⁴ accelerating lead identification.

In this review, we compiled the most important and modern organic chemistry macrocyclization strategies, structured by the produced connectivity. With this, we provided a concise overview for how to choose the appropriate reaction for peptide macrocyclization based on desired functional group. Finally, we summarized the different approaches in ESI† Table S1 to give the reader a short guide for selecting suitable reactions based on their specific requirements. To underline the importance of peptide macrocyclization in medicinal chemistry we highlight some applied examples and their bioactivities in ESI† Table S2.

2. Amide bond formation

2.1 Traditional amide cyclization

Many naturally occurring pharmacologically active peptides are cyclized head-to-tail, rendering them more resistant to

hydrolysis by exopeptidases due to the absence of an N- and C-terminus. To cyclize a linear peptide precursor by amide bond formation, traditionally the same coupling chemistry is used as in linear peptide bond formation.⁸ However, conventional head-to-tail amide formation is non-trivial. Especially for head-to-tail cyclization of peptides shorter than seven residues, cyclodimerization and C-terminal epimerization can occur. In the retrosynthetic planning the ring disconnection must be chosen carefully, as for example, sterically hindered amino acids at the side of cyclization can reduce yields.²⁵ To improve yields and reduce side product formation, preorganization of the peptide backbone can create a high effective molarity of the reaction partner. This can be done through turn-inducing elements such as proline, D-amino acids, or N-methylation.¹² Conformational elements to pre-organize peptides for head-to-tail cyclization have been reviewed in detail.^{12,26}

For amide formation, three main classes of peptide coupling reagents are used: carbodiimides, phosphonium reagents, and aminium-/uronium-iminium reagents.²⁷ The careful choice of coupling reagent and additives can reduce epimerization.^{9,28} For example, PyBOP was used to complete the synthesis of cyclomarin C,²⁹ whereas for teixobactin, a mixture of HATU/Oxyma Pure/HOAt/DIEA was preferred.³⁰

Amide bond formation is not chemoselective, and in-solution cyclization requires sidechain-protected peptides, often rendering them poorly soluble. By forming the amide on solid support, the pseudo-dilution effect helps to reduce intermolecular reactions. In principle, two strategies have been used to achieve a head-to-tail cyclization on solid support: anchoring the peptide to the resin *via* the sidechain of a trifunctional amino acid³¹ or *via* the N- α atom of the C-terminus;³² the C-terminal carboxylate can react after orthogonal deprotection to form the cyclized product. Notable applications of head-to-tail and sidechain-to-sidechain lactam formation aim at the stabilization of secondary structures.³³ For example, the design of a β -hairpin generated a protein-protein-interaction (PPI) inhibitor of the oncotarget p53-HDM2 that was smaller and had a higher activity compared to an α -helix (IC₅₀ 0.53 μ M *vs.* 1.1 μ M, ESI† Table S2).³⁴

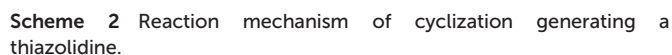
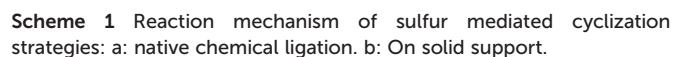
2.2 Amide formation – sulfur mediated

In the last two decades, chemoselective reactions became the prevailing strategy for the synthesis of amide head-to-tail cyclized peptides.²² A multitude of different synthesis strategies employ S-to-N transfer in so-called ligation reactions, which have been recently reviewed.^{35,36}

Native chemical ligation (NCL) was introduced as a mild and site-selective amide-formation reaction for synthesizing proteins from peptide fragments,³⁷ and it was extended to the cyclization of peptides by Tam and coworkers.³⁸ Here, an N-terminal cysteine reacts with a C-terminal thioester in neutral, aqueous solution. The reversibility of the transthioesterification step ensures chemoselectivity, since



A different chemoselective reaction uses the 1,2-aminothiol of an N-terminal cysteine that readily condenses with an aldehyde to form a thiazolidine ring. By incorporating the aldehyde as an oxidized C-terminal glycolaldehyde ester, a head-to-tail cyclized peptide can be



Other chemoselective ligation reactions have been reported where the cyclization occurs by the attack of a nucleophilic amine at the mildly activated C-terminus. Houghten *et al.* reported an aminolysis of a C-terminal thioester in the presence of imidazole in an aqueous solution. However, this reaction is not chemoselective over the ϵ -amino group of Lys and shows epimerization.⁴⁶ The use of other mildly activated esters (*e.g.*, selenoester,⁴⁷ 2-formylthiophenol,⁴⁸ selenobenzaldehyde⁴⁹) can increase the reaction speed of the aminolysis, though their use has not yet been reported for peptide macrocyclizations (Scheme 4, a).²² Similarly, a C-terminal 9-fluorenylmethyl (Fm)-thioester reacts with the N-terminus when activated *in situ* by Sanger's reagent (1-fluoro-2,4-dinitrobenzene). The linear precursor can be synthesized in solution using Boc-chemistry except for the last amino acid, whose Fmoc protection will be removed simultaneously with the cleavage of the Fm-protected thioester to facilitate the reaction with Sanger's reagent and subsequent aminolysis (Scheme 4, a).⁵⁰ By linking a linear peptide to a solid support using a diaminobenzoyl (Dbz) linker, a macrocyclization by aminolysis can be achieved that is analogous to the NCL described above. The linear precursor is synthesized by Fmoc-SPPS, and the Dbz linker is subsequently activated by nitrite-mediated acyl benzotriazole formation to generate an activated C-terminus. This macrocyclization can be achieved under mild acidic conditions with the addition of HOAt and HOBt and in moderate to good yields (Scheme 4, b).⁵¹ The advantage of these aminolysis strategies over NCL is their



a

$\text{H}_2\text{N}-\text{PEPTIDE}-\text{C}(=\text{O})\text{X} \rightleftharpoons \text{H}_2\text{N}-\text{PEPTIDE}-\text{C}(=\text{O})\text{X} \rightleftharpoons \text{PEPTIDE}-\text{HN}-\text{C}(=\text{O})\text{X}$

x = activated C-terminus

Chemical structures of Sanger's reagent and its derivatives are shown below the text:

- $\text{S}-\text{C}_6\text{H}_5$
- $\text{Se}-\text{C}_6\text{H}_5$
- $\text{S}-\text{C}_6\text{H}_4-\text{C}(=\text{O})\text{NH}_2$
- $\text{Se}-\text{C}_6\text{H}_4-\text{C}(=\text{O})\text{NH}_2$
- $\text{S}-\text{C}_6\text{H}_3(\text{NO}_2)_2-\text{C}(=\text{O})\text{NH}_2$

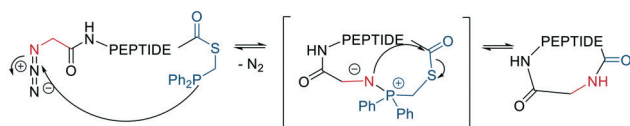
Sanger's reagent

b

$\text{H}_2\text{N}-\text{PEPTIDE}-\text{C}(=\text{O})\text{NH}-\text{C}_6\text{H}_3(\text{NO}_2)_2-\text{C}(=\text{O})\text{NH}_2 \xrightarrow{\text{nitrite, H}^+} \text{H}_2\text{N}-\text{PEPTIDE}-\text{C}(=\text{O})\text{NH}-\text{C}_6\text{H}_3(\text{NO}_2)_2-\text{C}(=\text{O})\text{NH}_2 \rightleftharpoons \text{PEPTIDE}-\text{HN}-\text{C}(=\text{O})\text{NH}-\text{C}_6\text{H}_3(\text{NO}_2)_2-\text{C}(=\text{O})\text{NH}_2$

Finally, a traceless Staudinger ligation can be used to head-to-tail cyclize a peptide in a chemoselective fashion. The C-terminal phosphino-thioester reacts with an N-terminal azide introduced by the noncanonical azidoglycine to yield a cyclic iminophosphorane, which collapses to an amide bond by eliminating the thiophosphorane (Scheme 5).⁵²

Ser/Thr ligation has been developed for the synthesis of proteins through the ligation of peptide fragments containing a serine or threonine.⁵⁷ The C-terminal ester is activated as a salicylaldehyde ester, which is generated by the on-resin phenolysis of an *N*-acyl-benzimidazolinone (Nbz) linker with salicylaldehyde dimethyl acetal in Na₂CO₃, DCM/THF. This produces macrocycles without C-terminal epimerization.⁵⁷ Ser/Thr ligation technology was extended to the backbone cyclization of tetrapeptides containing an N-terminal serine or threonine and C-terminal

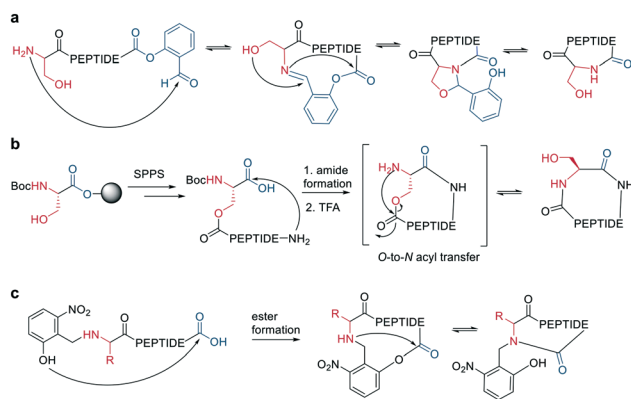


a

b

salicylaldehyde ester. The ligation of the unprotected peptide occurred in pyridine/acetic acid (1:2). After acidolysis with TFA/H₂O, the cyclic peptides were obtained with no epimerization (Scheme 7, a).⁵⁸

The usage of auxiliary groups is another strategy for cyclizing difficult sequences. For example, 2-hydroxy-6-



This journal is © The Royal Society of Chemistry 2021

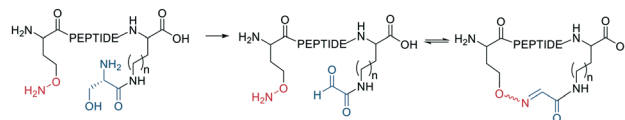
RSC Med. Chem., 2021, 12, 1325–1351 | 1329

Another strategy to trap an imine is by a boronic acid, which can be introduced in the peptide to allow the cyclization to proceed rapidly and spontaneously under physiologic conditions. Interestingly, this cyclization can be rapidly reversed in response to acids, oxidation, and α -nucleophiles (hydrazine and amino alcohols). At neutral pH (6.8), the macrocycle is stable, while acidic conditions hydrolyze the iminoboronate (Scheme 10, b). When reduced (NaCNBH_3), the iminoboronate can be trapped irreversibly as aminoboronate in two diastereomers.⁶⁴

A recent strategy traps the imine in a 1,3-dipolar cycloaddition, generating fused and spiro-ring systems that are frequently found in pharmacologically active natural products. The imine is generated on solid support by reacting 4-carboxybenzaldehyde with the primary amine of an N-terminal glycine (Scheme 10, c).⁶⁵

Native peptides reacted with formaldehyde to form an imine with the ϵ -amino group of Lys, which can crosslink to nearby tyrosine or arginine residues. Tyrosine reacts in the *ortho* position *via* a C-nucleophilic attack of the iminium ion intermediate and a subsequent re-aromatization (Scheme 11).⁶⁶

Schiff bases (imines, hydrazones, and oximes) are used in dynamic covalent chemistry approaches due to their hydrolytic reversibility, with oximes being generally the most stable. Side-chain cyclization *via* oxime formation is achieved using noncanonical amino acids containing a 1,2-aminoalcohol, which is oxidized by NaIO_4 to an aldehyde (Scheme 12). The aldehyde reacts with an noncanonical amino acid containing an aminoxy-sidechain to form an oxime in phosphate buffer (pH 7).⁶⁷ Oxime formation is thermodynamically favored but kinetically slow at neutral pH. It can be accelerated by acidic conditions or nucleophilic catalysts. Importantly, oxime formation generates two isomers (*E* and *Z* oximes). When using aminoxy noncanonical amino acids, ethanedithiol should be added as a nucleophilic scavenger during cleavage to prevent the peptide from reattaching to the resin. Furthermore, aminoxy amino acids have been reported to bind irreversibly to the stationary phase of some C_{18} columns.⁶⁷ Oxime formation has also been used to stabilize α -helical conformations (*i, i + 4* spacing),⁶⁸ and oxime chemistry can be applied for stapling peptides using noncanonical amino acids with amino-alcohol or hydrazine sidechains. By adding commercially available di-



Scheme 12 Macrocyclization by oxime formation.

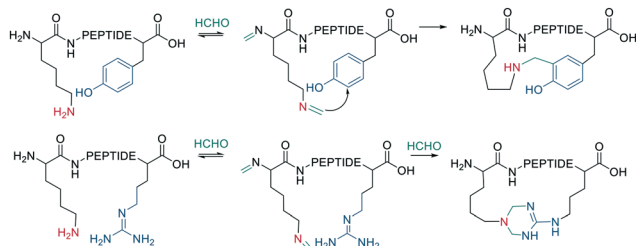
aldehyde scaffolds, the cyclization proceeds in phosphate buffer at pH 7.⁶⁹

The noncanonical amino acid furanyllalanine can be oxidized by *N*-bromosuccinimide (NBS) to form a ketoenal, which can react with nucleophilic sidechains (*e.g.*, Lys) to cyclize (Scheme 13). The reaction is irreversible after imine trapping by reduction with NaCNBH_3 and can be applied as a one-pot reaction, though cysteine and tyrosine were not tolerated.⁷⁰

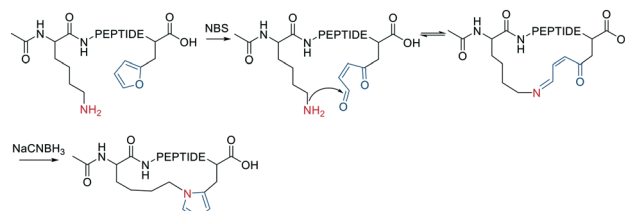
2.5 Amine-reactive stapling

A diverse set of amide-generating scaffolds for reactions with amines has been reported (Fig. 1). To cyclize peptides of an mRNA display library, a bifunctional NHS-scaffold (*e.g.*, disuccinimidyl glutarate) was used.⁷¹ Di-NHS scaffolds (1–8) generate crosslinked peptides stabilizing α -helical structures with different residue spacings (*i, i + 4* ≈ 5 Å, *i, i + 7* ≈ 11 Å, and *i, i + 11* ≈ 16 Å).⁷² Using benzene-1,3,5-tricarboxylic acid (9) as an organic, planar, and tri-reactive scaffold, a bicyclic one-bead-two-compounds library was generated by amide formation to sidechain amino groups using PyBOP/HOBT/DIPEA. The library was applied to identify TNF α inhibitors with nanomolar affinities (450 nM), and the scaffold was essential to that affinity (linear sequence >10 μM , ESI† Table S2).⁷³ Bicyclic scaffolds can also be used to target intracellular targets (*e.g.*, tyrosine phosphatase 1B), by encompassing a cell-permeable sequence in one cycle of the bicyclic peptides.⁷⁴

Nucleophilic aromatic substitution and palladium-catalyzed arylation chemistry for peptide stapling was first introduced to react with the highly nucleophilic thiolate of cysteines (see below and Fig. 2) and was subsequently adapted to react with amine sidechains. As electrophiles for the nucleophilic aromatic substitution, perfluoroaryl-, perfluorodiphenylsulfone-, and dichlorotriazine-derived scaffolds are used.⁷⁵ Installing an electron-withdrawing group at the *para* position of the electrophilic arene increases the



Scheme 11 Cooperative macrocyclization of iminium with nearby arginine or tyrosine sidechain.

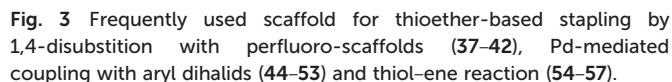


Scheme 13 Oxidation of furan ring and subsequent cyclization with amino sidechain of lysine.



This journal is © The Royal Society of Chemistry 2021

For linear peptides with more than two cysteines several scaffolds have been reported able to bridge multiple cysteines. For example, three cysteines can be bridged using 2,4,6-tris(bromomethylene)benzene (**30**), and four cysteines can be bridged using 1,2,4,5-tetrabromodurene (**31**).¹⁰² However, organic scaffolds with three or four spatially isometric thiol-reactive groups yield a mixture of regioisomers. 2,3,5,6-tetrafluoro-1,4-dicyanobenzene (**43**, Fig. 3) was proposed to reduce regioisomers due to a drastically changed reactivity of the remaining C-F sites after the first substitution, resulting in a stepwise crosslinking process.¹¹³ However, two regioisomers are still generated, except when bridging two cysteines and one penicillamine, where one specific bicyclic structure is yielded.¹¹⁴



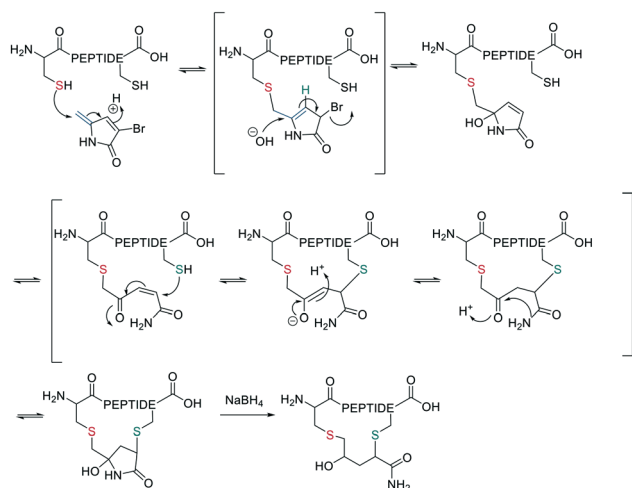
Dichlorotetrazine was suggested as a reversible cyclization scaffold, which can be released by a photochemical trigger to generate thiocyanates and molecular nitrogen. The thiocyanates can be converted back to sulfhydryl groups (Cys) by reaction with cysteine. To tackle the low solubility of dichlorotetrazine in water, it was dissolved in chloroform and mixed vigorously with the peptide in phosphate buffer (pH 5).¹¹⁵

An emerging technique to promote cysteine stapling is the use of reactive aromatic linkers containing electron-withdrawing and activating moieties, such as perfluoroarenes (39–44), that result exclusively in a 1,4-disubstitution.¹¹⁶ Increased helicity, stability, and cellular permeability can be obtained by stapling with perfluoroaryl scaffolds, and multiple scaffolds for different cysteine distances have been reported.¹¹⁷

Furthermore, aryl dihalides (45–53) have been shown to react with cysteines of an unprotected peptide in the presence of palladium under mild aqueous conditions (pH 5.5–8.5, small amounts DMF, DMSO, or MeCN).¹¹⁸ The careful choice of palladium ligand (RuPhos) led to a selective and fast C–S bond formation, though previous reports had shown that free thiols could inhibit palladium-catalyzed cross-coupling reactions¹¹⁹ and that Pd(II) complexes could exhibit protease-like activity.¹²⁰ This approach is chemoselective over serine, in contrast to palladium-mediated allylation,¹²¹ and the required bis-palladium crosslinking reagents can be generated in one-step from commercially available aryl dihalides.¹²² The *S*-arylated peptide was shown to be stable towards acids, bases, and thiol nucleophiles.¹¹⁸ However, cysteine-aryl homologues can be eliminated under basic conditions to form dehydroalanine or can be subject to oxidation.⁷⁵

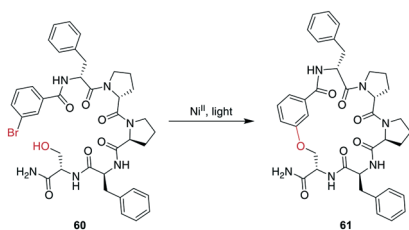
The thiol-ene reaction has also been applied for stapling and is especially suitable for certain bis-electrophilic linkers

4,5-Dibromo-1,2-dihydro-pyridazine-3,6-dione has been derivatized with TCEP to work as a reversible thiol-reactive



5.1.1. Scaffold thioether reactions on *in vitro* selection systems. To obtain bicyclic peptides on disulfide-free gIII phage, Heinis *et al.* applied the tribromomethylenebenzene (TBMB, **30**) scaffold to cyclize a peptide library containing three cysteines. The reaction proceeded with 10 μ M TBMB in 20 mM NH_4HCO_3 , 5 mM EDTA, pH 8 for 1 h.¹³² The phages were still sufficiently infective, which enabled the phage display screening of bicyclic peptides. Further scaffolds suitable for building bicyclic peptides on phage by thioether formation have been reported.¹³³ To increase the diversity of phage displayed libraries, linear peptides containing four cysteines were cyclized on phage with a bi-reactive scaffold to generate three isomers with different conformations per linear peptide sequence. Twelve different scaffolds have been applied.¹³⁴ Similarly, mRNA-displayed peptides containing multiple noncanonical amino acids and two cysteines were also cyclized with dibromoxylene.¹³⁵ Further scaffolds applied on phages are decafluoro-diphenylsulfone (**41**, Fig. 3)¹³⁶ and 2,4-difluoro-6-hydroxy-1,3,5-benzenetricarbonitrile.¹³⁷ The latter scaffold is soluble in buffer (pH 7.4), in contrast to previously reported perfluoroaryl scaffolds, and is chemoselective for cysteine in neutral conditions. It reacts with primary amines under basic conditions (*e.g.*, pH 9.2).¹³⁷

Scaffolds with two electrophilic groups have been used to bridge a cysteine thiolate with the N-terminal amino group.^{138,139} Kubota *et al.* introduced a stapling scaffold that can connect Cys and Lys sidechains on an unprotected peptide *via* Pd-mediated *S*-arylation and subsequent reaction of a tethered electrophile to the Lys sidechain.¹⁴⁰ Another chemoselective cyclization on unprotected peptides generates isoindole-bridged cyclic peptides *via* the reaction of a lysine or the N-terminus and cysteine thiolate with *ortho*-phthalaldehyde (OPA) in aqueous buffer (PBS pH 7.4).



Scheme 21 Ni^{II}/photoredox-catalyzed ether formation between a bromo-benzoyl moiety and serine side chain.

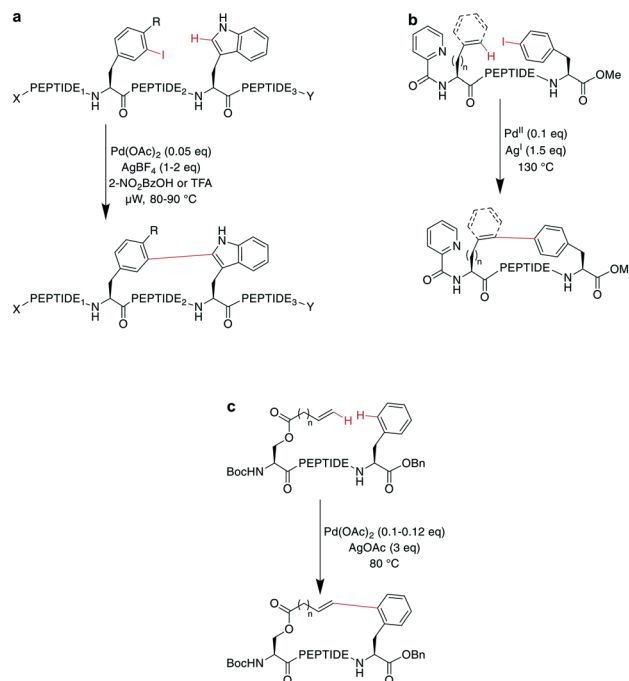
organometallic compound leads to two organic fragments bound to the catalyst. Formation of the new carbon–carbon bond occurs by reductive elimination. The various cross couplings differ mainly in substrates and catalysts, but all require both reaction partners to be pre-functionalized, excepting the Sonogashira coupling and Heck reaction.^{166,167}

Most of the typical C–C cross couplings, namely the Suzuki, Stille, Negishi, Tsuji–Trost, Heck, and Sonogashira couplings, have been used to generate peptide macrocycles, although less often than other metal-catalyzed reactions, such as the copper-catalyzed azide–alkyne cycloaddition (CuAAC) or ring-closing metathesis (RCM).¹⁶⁰ The use of the Suzuki coupling for amino acid modifications and peptides has been reviewed, including examples of macrocyclizations.^{168,169} Overall, it has been used in solution¹⁷⁰ and on-resin to form five- and six-residue macrocycles.^{171,172}

7.2 CH-Activation

In contrast to traditional cross couplings, couplings mediated by CH activation do not require the introduction of an organometal in the substrate, which is an important advantage as these usually strongly basic groups are problematic for peptides.¹⁶⁰ In these reactions, one partner is a CH-group that typically reacts as a nucleophile with an organic halide, although more recent examples in which the CH group acts as an electrophile have also emerged. Typically, directing groups support the process by laying out the spatial arrangement and fine-tuning the electronic environment. The coupled CH-groups can be sp-, sp²- or sp³-hybridized centers, and for the latter group, β, γ, and δ modifications have been described.¹⁷³ Despite the versatility of CH-activation, the reaction is usually performed on protected peptides, which contrasts with other metal-catalyzed macrocyclizations, such as RCM and CuAAC.^{174–177} CH activations on peptides, more generally as well as focused on macrocyclizations, have been reviewed recently.^{160,173,178,179}

CH activation reactions can be used to selectively modify the C2 of the indole of Trp. These reactions have been used to successfully couple aryl halides to an assembled peptide using Pd-catalysis.¹⁸⁰ In a series of intramolecular cross couplings between Trp and *m*-iodotyrosine and *m*-iodophenylalanine, Mendive-Tapia *et al.* achieved up to 100% conversions of linear precursors by linking positions from *i*, *i* + 1 to *i*, *i* + 5, though overall isolated yields were low (Scheme 22a). The cyclized

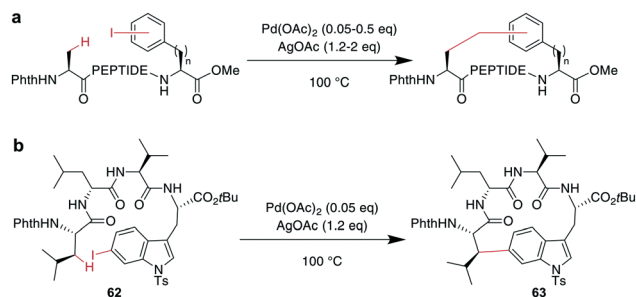


Scheme 22 a: Peptides macrocyclized through their Trp-C2 and an iodophenyl residue from Phe or Tyr; X = H, Ac; Y = OH, NH₂; R = H, OH; PEPTIDE₁ = 0–1 amino acids; PEPTIDE₂ = 0–3 amino acids; PEPTIDE₃ = 0–1 amino acids; b: a typical substrate for a macrocyclization between a sp²-CH₂ group and a phenyl iodide; *n* = 1, 2; PEPTIDE = 2, 3, 4 or 6 amino acids; c: oxidative cross-coupling between an alkenyl ester and a phenyl group to obtain macrocyclic peptides; *n* = 0, 2; PEPTIDE = 1–4 amino acids.

peptides were stable against proteolytic degradation,¹⁸¹ and most amino acids, including His, Tyr, and Lys were tolerated, though Met was not compatible.^{179,181} Recently, Han *et al.* showed that this type of reaction can also be used to cross couple phenyl iodides with sp²-CH groups in the γ or δ position at the N-terminus when acylated with picolinic acid (Scheme 22b).¹⁸² Removing the necessity to introduce an aryl halide, the Wang group coupled phenyl residues, including on the phenylalanine sidechain, to terminal alkenes under Pd-catalysis with added AgOAc (Scheme 22c).^{174,183,184} Although most amino acids are tolerated in this reaction, sulfur-containing amino acids are not.¹⁶⁰

Phthaloyl-protection of the N-terminus leads to modified acidity which was employed to specifically activate the β-hydrogens on aliphatic sidechains to couple to aromatic halides under Pd-catalysis for macrocyclization (Scheme 23a).^{175,176} No epimerization occurs under the reaction conditions and cyclization between *i*, *i* + 4 and *i*, *i* + 3 residues was successful, but not for *i*, *i* + 2 residues. The reaction could be applied to the ring system A of the natural product celogentin C (Scheme 23b).^{175,176} Conveniently, the procedure could also be performed on-resin with continued C-to-N elongation after the cyclization and phthaloyl group removal. The products showed massively increased tryptic stability,¹⁷⁶ and when applied to peptides with the integrin-binding motif





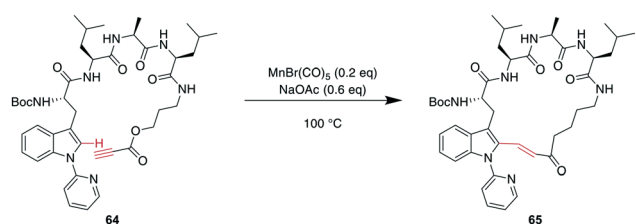
Scheme 23 a: General reaction scheme for the oxidative cross coupling between β -Hs and *meta*- or *para* phenyl iodides; PEPTIDE = 1 or 2 amino acids; $n = 1, 3$. b: The application of the method to the A ring of celogentin C.

RGD, binding to $\alpha v \beta 3$ integrin-overexpressing cells was strongly increased (ESI† Table S2).¹⁷⁵

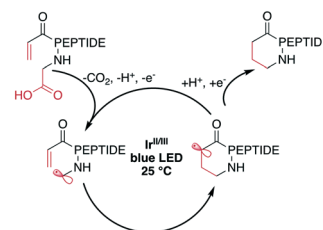
More recently, transition metals other than Pd have been reported for CH activations. For example, Mn was shown to successfully alkylate the indole-C2 of *N*-pyridine Trp, where pyridine is essential as a directing group, providing an opportunity for selectivity when multiple Trp are present.¹⁶⁰ Combining this CH-activation with the introduction of a propargylic ester in the peptide yielded α,β -unsaturated esters, which are versatile handles that can be further derivatized using, for example, cyclo- or conjugate additions (Scheme 24).¹⁸⁵ Given the high temperatures generally employed for CH activations, this can also be achieved using Rh catalysis with acryl instead of propargylic esters with the addition of AgSbF₆ and Cu(OAc)₂ at 37 °C, an advantage for temperature-sensitive materials.¹⁸⁶

7.3 Photocatalyzed reactions

Peptide macrocyclization can also be performed using unfunctionalized iridium (Ir)- or other transition-metal-catalyzed photoredox reactions, which have been recently reviewed.¹⁸ Upon light irradiation, the Ir-catalyst promotes radical formation on the C-terminal carboxylate, leading to decarboxylation. The remaining carboradical undergoes a 1,4-addition with Michael acceptors, such as acrylates or malonates (Scheme 25). An impressive application of this methodology is the selective modification of the C-terminus of insulin, despite the presence of sidechain carboxylic acids



Scheme 24 Propargylic esters can be hydroarylated with *N*-(2-pyridyl)-derivatised Trp under Mnl catalysis to yield macrocyclic 3-indolyl acrylates.



Scheme 25 Simplified reaction mechanism for the Ir-photocatalyzed macrocyclization of *N*-acroyl peptides.

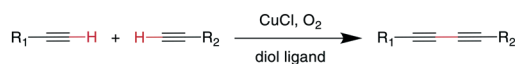
and disulfides.¹⁸⁷ The reaction was successfully applied to form rings ranging from 11 to 47 atoms in size from protected peptides and tolerated all tested residues, including His, Met, Arg, and Tyr.¹⁷⁷ Similar efforts have been made using Ni-catalysts with phthalimide esters on the C-terminus.^{160,188}

7.4 Glaser–Hay coupling

The Glaser reaction couples two alkynes to a dialkyne, creating a very rigid and extended bridging group (Scheme 26). This reaction occurs in the presence of O₂ under Cu-catalysis, involving Cu^I/Cu^{II} and possibly Cu^{III} oxidation states within the catalytic cycle.^{189,190} Modifications including Ni²⁺ salts have also been described, and the addition of diol ligands seems to be beneficial for oxidation-sensitive molecules, such as peptides, as the diols remove Cu^{II} species by gel formation.^{190,191} The reaction was employed successfully with *N*-, *O*-, and *C*-propargyl groups at *i*, *i* + 3 through *i*, *i* + 7 positions and could stabilize secondary structure motifs such as β-turns and α-helices.^{192,193} The resulting bis-alkyne can be reduced by catalytic hydrogenation.¹⁹¹

8. C-C double bond formation: alkene metathesis

Alkene metathesis, the reaction between two alkenes to form two new alkenes, was propelled into popularity in the 1990s by the development of chemoselective and stable molybdenum (Mo)- and ruthenium (Ru)-based catalysts.^{194–198} The reaction is most prominently applied to intramolecular reactions to generate cyclic systems, and labeled ring-closing metathesis (RCM) for this application. Its high tolerance for most functional groups as well as its usually high yields make it well suited for cyclizing relatively large, functionally diverse molecules. It has therefore been employed on a large number of complex structures, such as peptides and peptidomimetics. It



Scheme 26 General reaction scheme for the Glaser–Hay reaction applied to peptides.

can be performed in solution as well as on solid phase and has also been used in the synthesis of DNA-encoded libraries.^{199,200}

Mechanistically, the catalyst-bound carbene or alkylidene first undergoes a [2 + 2]-cycloaddition with an alkene, leading to a metallacyclobutane intermediate. The subsequent ring opening can either revert unproductively to the starting materials or bind the catalyst to the substrate, which causes the release of another alkene (ethene for a terminal alkene substrate). This release is followed by a second [2 + 2] cycloaddition with the second alkene on the substrate, and finally, the formed metallacyclobutane opens to release the bridged product and the catalyst (Scheme 27). As all steps are in principle reversible, the reaction is under thermodynamic control such that the most stable product is the one predominantly formed, which is the *E*-alkene in an unstrained system. However, for macrocyclic peptides, mixtures of *E/Z*-alkenes are often obtained. By using two terminal alkenes the equilibrium is shifted towards the product side due to the released ethene gas, which is easily removed from the reaction mixture, leading to a high entropic contribution. However, in case of strained cyclic systems, RCM can still be challenging.¹⁹⁵

The most common catalysts are Ru-based Grubbs 67–71 or, less frequently, Mo-based Schrock 66 catalysts. The Schrock catalysts are more reactive but less chemoselective and are water- and air-sensitive. Second (II) 68 and third (III) generation Grubbs 69 and Hoveyda–Grubbs 70–71 catalysts were developed to increase reactivity and thermostability (Fig. 4). For the Ru catalysts, unprotected amines are problematic though oxygen-bearing groups are tolerated, whereas the opposite is true for the Mo-catalysts.^{195,197,201–203}

Typical reaction conditions can vary substantially for different applications. Generally for peptide macrocyclizations, in-solution reactions tend to have lower concentrations (<10 mM), as high concentrations favor dimer over monomer formation, and catalyst loadings (<20 mol%). For on-resin reactions, MBHA or NovaPEG resins are considered superior to Wang and Tentagel, and the reaction is usually performed under microwave irradiation at elevated temperatures. A selection of reaction conditions can be found in the ESI† Tables S3 and S4, and publications containing comprehensive, general protocol descriptions are available.^{204,205}

This reaction is among the most commonly applied to peptides for stabilizing secondary structures, in particular for

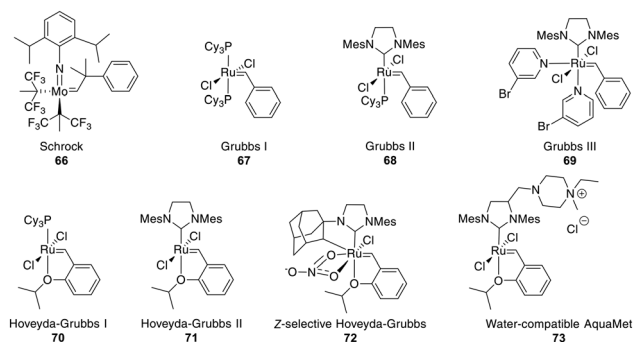


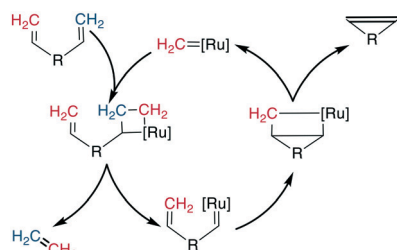
Fig. 4 Frequently employed catalysts for metathesis reactions (Cy = cyclohexyl, Mes = mesityl = 2,4,6 trimethylphenyl).

α -helices.^{206–209} Furthermore, it has also been employed to stabilize or mimic other motifs, such as β -sheets,²¹⁰ β -turns,^{211,212} polyproline II helices,²¹³ 3_{10} helices,^{214,215} N-capping boxes,²¹⁶ and disulfide bridges.^{217,218} The generated alkenes are generally more conformationally restricted than disulfides, but this is not always the case.²¹⁷ Usually, the stapled peptides display improved proteolytic stability and cell permeability,^{208,219,220} with some exceptions.²²¹ The effect on affinity, either through a *de novo* staple or when using it as surrogate for another functional group, is highly dependent on the specific peptide-target interaction as well as the alkene stereoisomer (*E/Z*) and stereochemistry, typically requiring an empirical approach.^{13,217,218,222,223}

A successful example of the application of RCM to a peptide was provided by Song *et al.* Here, the affinity of a peptide sequence based on the natural binding partner of initiation factor eIF4E could be increased six-fold (ESI† Table S2).²²³ Furthermore, van Lierop *et al.* developed an insulin analogue in which the A6-A11 disulfide was replaced with an alkene. The *cis*-alkene analogue maintained affinity to the insulin receptors and showed improved efficacy in mice, whereas the *trans*-alkene had a 50-fold reduced binding affinity (ESI† Table S2).²²⁴ The application of RCM to peptides has been reviewed previously,^{209,219,225} including a perspective discussing all-hydrocarbon-stapled α -helical peptides in general.²⁰⁸

Alkenes for peptide stapling have been introduced as modified sidechains on carbon^{13,204,226–230} as well as on the α -N,^{231–234} side-chain aliphatic alcohols^{206,229} and phenols,^{235,236} C-terminal or side-chain acids,^{46,237,238} N-terminal carbamates,²³⁷ and cysteine thiol groups.²³⁹ Often, when the alkene is introduced as a modified C-bound chain, α -methyl- α -alkenyl sidechains are used for additional helix stabilisation.¹³

Depending on the strategy, it can be advantageous to have access to *Z*- and *E*-isomers in one step. These can mostly be separated by HPLC,^{218,240,241} though occasionally no separation is achieved.²⁴² Ru-Catalysts selectively forming *Z*-alkenes 72 have been developed (Fig. 4), including some for more challenging substrates, such as those containing steric hindrance or polar groups near the reaction center.^{242,243}



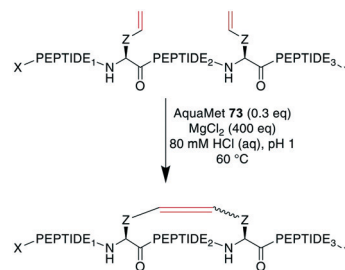
Scheme 27 Reaction scheme for the Ru-catalyzed RCM.



Available strategies for *Z*-selective RCM have been recently reviewed.²⁴⁴ Substrates undergoing selective *E*-alkene formation have been reported, such as those containing α,α -disubstituted amino acids between the bridging amino acids.²¹⁴

Demonstrating the impact of this reaction, chemists at Boehringer Ingelheim used RCM on a large industrial scale for the formation of a 15-membered macrocycle to produce an anti-hepatitis C peptidomimetic **76** (Scheme 28).^{202,245,246} Notably, when switching from a Grubbs I **67** to a Hoveyda-Grubbs I **70** catalyst, the reaction rate decreased but exclusively yielded the desired product **75** without concomitant isomeric or epimerized compounds.²⁴⁵ In contrast, switching to a Hoveyda-Grubbs II catalyst **71** drastically accelerated the reaction yet also increased the amount of dimers, emphasizing that the optimally balanced catalyst needs to be chosen carefully for specific reactivity requirements.²⁴⁶

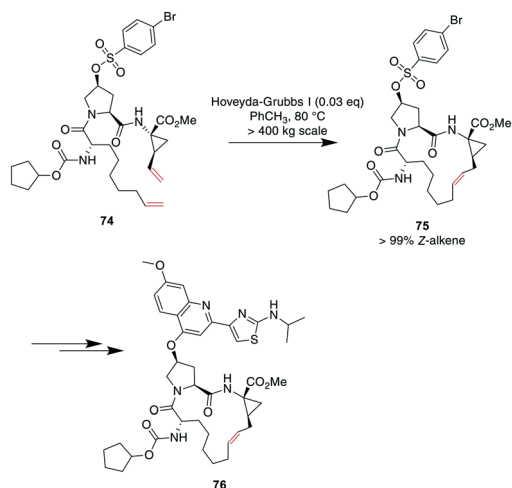
To demonstrate the flexibility of RCM, Gleeson *et al.* exploited the fact that Brønsted acids can mask free amines by protonation. They applied RCM to otherwise unprotected oxytocin, octreotate, two α -conotoxins, and an insulin fragment. The choice of solvent here was crucial, as the conversion of oxytocin proceeded quantitatively in DMF and with 84% and 66% conversion in MeOH and EtOH, respectively, while no product formation was observed in DMSO, MeCN, or solvent mixtures containing water.²⁴⁷ Cochrane *et al.* showed that the cyclization of unprotected peptides through allyl cysteinyl residues in solution with *t*BuOH/H₂O as the solvent could be achieved by adding 5000 eq. of MgCl₂,²³⁹ which was thought to act as a mild Lewis acid to effectively block potential peptide coordination sites to the Ru catalyst.²⁰⁰ Combining those previous findings, Masuda *et al.* were able to perform RCM on an unprotected model decapeptide in aqueous medium using the water-soluble Ru catalyst AquaMet **73**.^{248,249} The use of



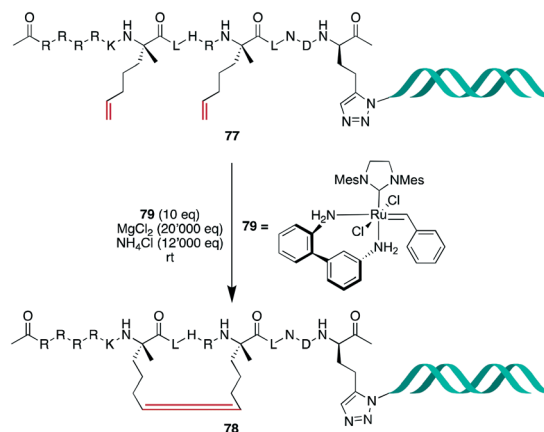
Scheme 29 Synthesis of unprotected alkene-bridged cyclic peptides under aqueous conditions with AquaMet **73** as Ru-catalyst. AA = amino acid, X = H, Ac; Y = OH, NH₂, Z = CH₂, (CH₂)₃, CH₂SCH₂, PEPTIDE₁ = 1 or 5 amino acids, PEPTIDE₂ = 2 or 5 amino acids, PEPTIDE₃ = 1 amino acid.

either acidic or neutral conditions in water or phosphate buffer containing MgCl₂ allowed the synthesis of an octreotide analogue from different alkenes in yields of 53–64%. Amine-containing buffers were not tolerated, and the addition of a chaotropic agent such as guanidinium-HCl improved the yields substantially in neutral conditions (Scheme 29). Importantly, changing the peptide sequence decreased yields under neutral conditions. Conversely, the acidic conditions proceeded consistently satisfyingly with yields from 48–81%, suggesting the broader scope of these conditions.²⁴⁹

Based on this work, Monty *et al.* embarked on the challenging task to optimize RCM for DNA-encoded libraries. Rationalizing that previously reported conditions¹⁹⁹ could be further improved by maintaining acidic conditions to mask coordinating groups in the substrate and that improved solvent composition was needed to prevent phase separation between *t*BuOH and high ionic strength water, NH₄Cl was added to the reaction conditions, and a mixture of H₂O:EtOH:MeOAc (5:4:1) was used as the solvent. Diverse sets of simple substrates were tested, and generally robust conversion could be observed, although certain functional groups were not or poorly tolerated, such as 1,1-substituted



Scheme 28 Synthesis of peptidomimetic **76** on an industrial scale with RCM as a key transformation for closing the macrocycle.



Scheme 30 Synthetic scheme for the synthesis of an alkene-bridged α -helical cyclic peptide with a DNA tag.



alkenes, pyridines, and sulfonamides. Finally, they could obtain an α -helical stapled peptide **78** with 52–65% conversion, depending on the linker length between the peptide and DNA tag (Scheme 30).²⁰⁰

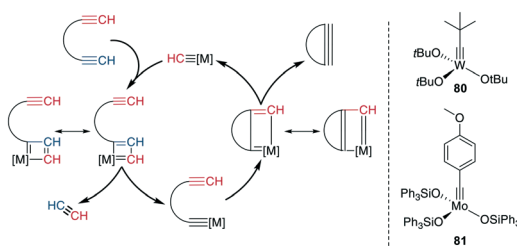
9. C–C triple bond formation: ring-closing alkyne metathesis

The tungsten (W)- or Mo-catalyzed ring-closing alkyne metathesis (RCAM) produces a new alkyne in a similar fashion to alkene metathesis. An obvious difference to RCM is the resulting extended conformation and lack of isomers. The employed catalysts are high-valency W- and Mo-based complexes, particularly $(t\text{BuO})_3\text{W}\equiv\text{CtBu}$ **80** and $(\text{Ph}_3\text{SiO})\text{Mo}\equiv\text{CPhOMe}$ **81** (Scheme 31, right).

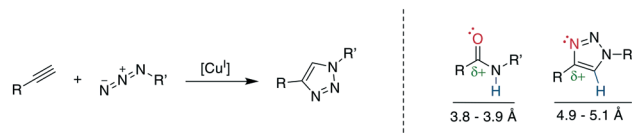
Mechanistically, an alkyne is first [2 + 2]-cycloadded to the catalyst-bound alkylidene to form a metallacyclobutadiene. The complex undergoes ring opening, binding the substrate to the catalyst. Upon cycloaddition of another alkyne and formation of a new metallacycle, the product is liberated after another ring opening (Scheme 31, left). Alkyne metathesis in general has already been comprehensively reviewed by Fürstner.²⁵⁰

Despite the similarities, the application of RCAM to peptides is less widespread than RCM.^{251–255} An early application of RCAM to a peptide cyclization of the A, B, C and E rings of nisin was published in 2005 by Ghalit *et al.* The N- and C-terminal-protected tetra- to heptapeptides were cyclized in solution using $(t\text{BuO})_3\text{W}\equiv\text{CtBu}$ **80** as a catalyst with 18–82% yield, with smaller cycles producing better results.²⁵³ In a further application, Cromm *et al.* synthesized bicyclic inhibitors against Rab8, a GTPase, using a combination of RCM and RCAM. The alkynes were successfully installed at position *i*, *i* + 3, *i*, *i* + 4, and *i*, *i* + 7 with $(\text{Ph}_3\text{SiO})\text{Mo}\equiv\text{CPhOMe}$ **81** as the catalyst, yielding compounds with improved affinities (ESI† Table S2). Nicely, the work also demonstrated that RCM and RCAM could be performed selectively with both alkynes and alkenes present and in both possible orders.²⁵⁴

The advantages of introducing alkynes include the possibility to further modify those, such as to Z-alkenes using Lindlar's catalyst,²⁵⁶ to E-alkenes by hydrosilylation,²⁵⁷ or to dibromoalkenes with CuBr_2 .²⁵⁴



Scheme 31 Reaction mechanism for the RCAM (left) and commonly used catalysts (right), [M] = metal catalyst.



Scheme 32 Left: reaction scheme of the CuI-catalyzed azide-alkyne cycloaddition. Right: comparison of electronic and steric properties of the Z-amide and the 1,4-disubstituted 1,2,3-triazole. Red: H-bond acceptor position, blue: H-bond donor position.

10. Triazole formation

10.1 1,4-Disubstituted triazole formation: the copper-catalyzed azide-alkyne cycloaddition

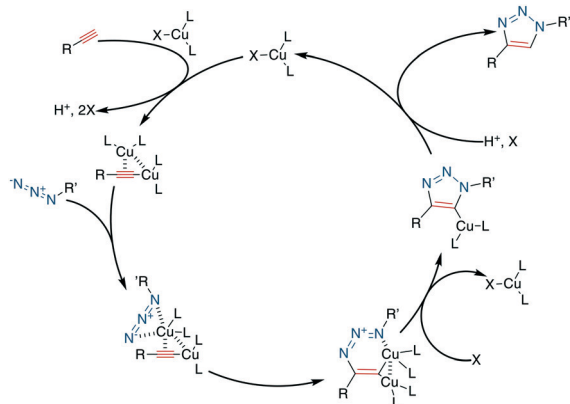
The copper (Cu)-catalyzed azide-alkyne cycloaddition (CuAAC) is an advancement of the Huisgen 1,3-dipolar cycloaddition and is the exemplary “click reaction” as established by Kolb, Finn, and Sharpless in 2001.²⁵⁸ Cu^I-Mediated catalysis provides the robust, selective, and water-compatible formation of 1,4-disubstituted 1,2,3-triazoles from a terminal alkyne and an azide (Scheme 32, left).^{259,260} The 1,5-regioisomer is accessible through Ru-catalysis (*vide infra*), whereas traditional Huisgen cycloaddition conditions at high temperatures tend to give mixtures of isomers.²⁶¹ The resulting 1,2,3-triazole is metabolically stable and mimics a Z-amide in terms of position of H-bond acceptors and donors, though there are somewhat different distances for the substituents. Furthermore, the dipolarity of the triazole compares well to the amide's (Scheme 32, right).^{262–264} The 1,2,3-triazole has been used to stabilize secondary structures or other motifs, including α -helices,^{265,266} 3_{10} -helices,^{262,267} β -hairpins,²⁶⁸ and disulfides.^{21,269} The reaction has been used extensively for, among others, bioconjugation and materials science, with comprehensive reviews available.^{12,263,264,270}

Despite its widespread usage, the detailed reaction mechanism has been challenging to establish. The current consensus²⁶⁰ is that the catalytic cycle is initiated by the coordination of the Cu^I-species to the alkyne, followed by the tethering of the azide to the complex. The addition of the internal alkyne-carbon to the terminal azide-nitrogen forms a six-membered metallacycle. Reductive ring contraction and copper elimination releases the triazole (Scheme 33).

Many azides are commercially available, but can also be accessed by diazo transfer to primary amines, such as the ϵ -amino group of Lys.²⁷¹ Likewise, alkynes are commercially available or can be synthetically accessed by, for instance, the Corey–Fuchs reaction or Seyferth–Gilbert homologation.²⁶⁴ When a short spacer on the C-terminus is desired for head-to-tail cyclization, it is possible to introduce a C-terminal propargylamine by using a silyl-based alkyne modifying (SAM)-resin.^{272,273}

CuAAC does not require protecting groups and can be performed on-resin^{267,271,274–278} as well as in solution,^{262,265–269,279–295} which is more common and proceeds under mild conditions. Cu salts most frequently used are CuSO_4 with sodium ascorbate, or Cu^I salts such as CuI and





Scheme 33 Reaction mechanism of the CuAAC, X: mostly SO_4^{2-} , I^- , Br^- , Cl^- , PF_6^- , L = ligand such as THPTA, TBTA.

$\text{Cu}(\text{MeCN})_4\text{PF}_6$. Where on-resin efforts fail, in solution approaches might still be successful, arguably due to the higher conformational flexibility of the starting material in solution.²⁸²

Cu species can oxidize His and Cys sidechains. In particular, Asp residues were shown to promote this by chelating Cu^{II} centers. The use of tris(triazolylmethyl)amine-based ligands, such as tris(3-hydroxypropyltriazolylmethyl)amine (THTPA) or tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA), can substantially reduce this oxidative damage.²⁹⁶ Furthermore, by stabilizing the Cu^{I} species, the ligands accelerate the reaction.^{297,298} A selection of reported reaction conditions can be found in ESI† Tables S5 and S6.

Monitoring the reaction of an intramolecular CuAAC can be a challenge, as the starting material and product have the same molecular weight. To therefore indicate the reaction progress, one can use LCMS monitoring, the disappearance of the azide stretch at 2100 cm^{-1} in the IR,²⁸⁷ or a modified Kaiser reaction.²⁹⁹

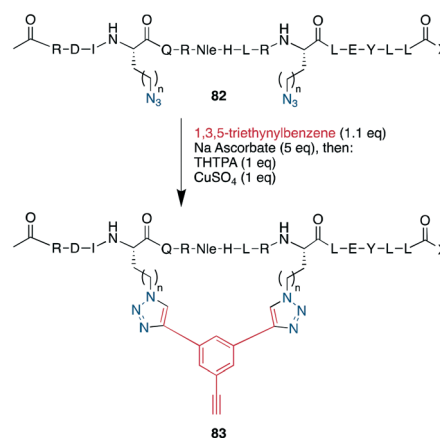
As for other macrocyclizations, dimerization (*i.e.* an inter- instead of intramolecular reaction) is a major issue for the CuAAC,³⁰⁰ but can be reduced with higher Cu-concentrations³⁰¹ and tends to be less problematic for on-resin-CuAAC of peptide macrocycles with less than six amino acids.³⁰⁰ Furthermore, for on-resin cyclization, this problem can frequently be addressed by changing the resin or solvent, particularly by using H-bond-disrupting solvents such as DMF or DMSO.^{300,302,303} Jagasia *et al.* extensively discussed the different parameters that affect the preference for mono- versus dimeric macrocyclization.³⁰² Very recently, Kandler *et al.* performed an in-depth analysis of the parameters for on-resin CuAAC and found that when the macrocycle comprises six, seven, or eight amino acids, the monomeric form is predominantly obtained. DMF seemed to be the optimal solvent, and including 20% piperidine improved the monomer to dimer ratio.³⁰³

As an example of CuAAC in peptide therapeutics, Gori used it to replace one of the two disulfides in the α -conotoxin MriA, yielding compounds as efficacious as the native disulfides in a rat model for neuropathic pain while

strongly increasing the plasma stability.²¹ By introducing two ω -azido amino acids within a peptide, the macrocyclization can also be done by two CuAACs employing a bis-alkynyl linker.^{265,266,269,288–290,304} This has been used as a stapling approach at the *i, i + 7* position for an α -helix^{265,266} and to successfully develop a peptidomimetic to allosterically target the kinase CK2, showing the potential of macrocyclic peptides even for a classical small-molecule target.²⁶⁹ This scaffold approach to bridge a peptide by a bis-alkynyl linker was further advanced by Tran *et al.* who used triethynylbenzene as linker. In their explorative study on the C-terminal α -helix from the Gs protein, this allowed them to introduce further functionality at the remaining free alkyne, such as a dye or biotin, after having stapled the peptides either at positions *i, i + 7* or *i, i + 9* (Scheme 34).²⁸⁶ Finally, the reaction was also used successfully in the synthesis of a DNA-encoded peptidomimetic library with 10^6 members to identify ligands against several targets with K_{D} s in the μM range.²⁹¹ Showcasing the versatility of having a set of chemoselective reactions, several efforts have successfully obtained multicyclic peptides by combining different cyclisation strategies, such as CuAAC, enzymatically-mediated lactamization, oxime ligations, or thioether formation on scaffolds, yielding structurally unique moieties.^{305–307}

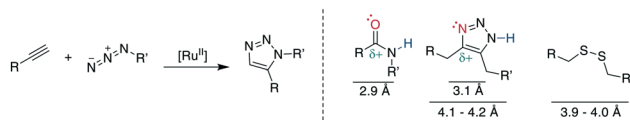
10.2 1,5-Disubstituted 1,2,3-triazole formation: ruthenium-catalyzed azide-alkyne cycloaddition

A 1,5-disubstituted 1,2,3-triazole is similar to an *E*-amide bond in terms of H-bond acceptor and donor positions and can function as a bioisostere for it. Additionally, as the $\text{C}_\alpha\text{--C}_\alpha$ distance in a 1,5-substituted triazole formed between β -azidohomoalanine and propargyl glycine is similar to the $\text{C}_\alpha\text{--C}_\alpha$ distance in a cysteine disulfide ($4.1\text{--}4.2\text{ \AA}$ versus $3.9\text{--}4.0\text{ \AA}$), it has also attracted substantial interest as a disulfide mimetic (Scheme 35, right).^{308–310}



Scheme 34 Performing CuAAC on helical peptides like **82** with triethynylbenzene allowed Tran *et al.* to additionally to stapling them to introduce further functionality on the cyclisation products such as **83**, $n = 1\text{--}3$.



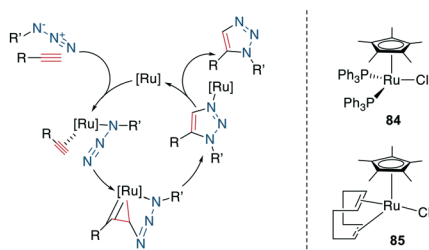


Scheme 35 Left: reaction scheme for the Ru^{II} -catalysed RuAAC, right: comparison of electronic and steric properties of the 1,4-substituted 1,2,3-triazole and the *E*-amide as well as a disulfide bond. Red: H-bond acceptor position, blue: H-bond donor position.

Similarly to the 1,4-disubstituted triazoles, their 1,5-disubstituted counterparts can be obtained regioselectively by a transition-metal catalyzed cycloaddition, although by Ru instead of Cu-catalysis (RuAAC) (Scheme 35, left).³¹¹ In contrast to the CuAAC, internal alkynes can also undergo RuAAC, allowing the introduction of additional substituents.^{311,312} Here, the regioisomer obtained depends on the steric and electronic properties of the alkyne substituents.³¹² Common catalysts include $[\text{Cp}^*\text{RuCl}]$, $\text{Cp}^*\text{RuCl}(\text{PPh}_3)_2$, **84** and $\text{Cp}^*\text{RuCl}(\text{COD})$ **85** (Scheme 36, right), the latter reported to work particularly well for secondary azides³¹³ and reacts at room temperature.³¹⁴ With Ru-catalysis, carboxylic acids and sidechains need to be protected,^{308,310,315} though the reaction can still be used in solution^{310,316–318} as well as on solid support,^{287,304,308,309,319} usually with 15–50 mol% catalyst loading at elevated temperatures and in DMF. An overview of reaction conditions can be found in ESI† Tables S7 and S8.

Mechanistically, the catalytic cycle begins with the displacement of Ru-ligands and coordination of the azide and alkyne. Oxidative coupling forms a metallacycle *via* a new bond between the less sterically hindered, more electronegative carbon of the alkyne and the terminal nitrogen of the azide. After reductive elimination, this produces the 1,5-disubstituted 1,2,3-triazole for terminal alkynes (Scheme 36, left).³¹⁴ Under high temperatures reminiscent of classical Huisgen conditions, the 1,4-substitution regioisomer can also be obtained.³¹⁶ Shortly after its discovery, this reaction was already applied as a turn inducer in a peptoid³²⁰ and as a replacement of an *E*-amide in RNase A.³¹³ It was later used as a disulfide substitute in the sunflower trypsin inhibitor 1 (SFTI-1).³²¹

Multiple examples underline the higher bioisostery for disulfides of 1,5-sbstituted triazoles over 1,4-substituted triazoles. For example, the affinity was retained after the replacement of a disulfide in urotensin-II analogues by a



Scheme 36 Left: mechanism of the RuAAC, right: structures of commonly used catalysts for RuAAC. [Ru] = ruthenium catalyst.

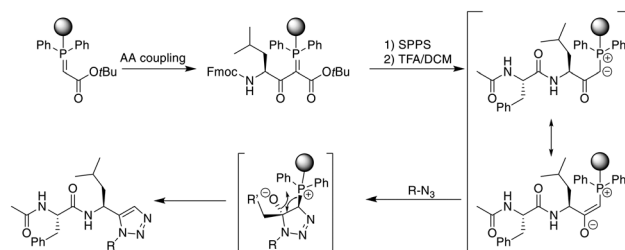
1,5-triazole, whereas the affinity was reduced or completely lost for 1,4-triazoles, which could be linked to structural reasons.²⁸⁷ Similar observations were made for SFTI-1 (ESI† Table S2).^{308,310} Further examples demonstrate the impressive potential of 1,5-disubstituted triazoles as disulfide isosteres by improving pharmacokinetic or creating changes in the pharmacodynamic profile.^{304,309,319}

10.3 1,5-Disubstituted 1,2,3-triazole formation: alternative approaches

As an alternative approach to the metal-catalyzed formation of 1,5-disubstituted triazoles, the Rademann group published an elegant method to introduce a 1,5-disubstituted 1,2,3-triazole on-resin. A resin-bound phosphoranylidene acetate reacts with the carboxyl group of an Fmoc-amino acid, which can subsequently be elongated using standard SPPS. Treatment with acid liberates an α -carbonyl phosphorous ylide that, upon treatment with an azide, directly yields a 1,5-disubstituted triazole without metal-catalysis (Scheme 37).³²² This was subsequently transferred to the on-resin cyclization of peptides. For dipeptide synthesis, only dimeric products were obtained, while tri- and tetrapeptides yielded mixtures of dimeric and monomeric products. A penta- and an octapeptide were obtained exclusively as monomeric products, making the method more attractive for larger macrocycles.³²³

11. Macrocyclization *via* multicomponent reactions

In recent years, the interest in multicomponent reactions for peptide macrocyclization is increasing and has been recently reviewed in detail.³²⁴ The Ugi reaction, which is a 4-component reaction combining isocyanide, amino, carboxylic acid, and aldehyde functionalities, yields *N*-methylated amides. The advantage of this reaction for peptide cyclization is the possibility of increasing molecular diversity during the ring closing step through the use of non-amino-acid building blocks. It was first applied in the context of peptide synthesis to obtain linear peptide esters³²⁵ and peptidomimetics,³²⁶ while cyclic peptides were generated though several subsequent Ugi reactions.³²⁷ The Ugi reaction has been used in the synthesis of head-to-tail cyclic peptides from linear peptides (amine and carboxylic acid) and conventional



Scheme 37 Synthetic scheme for the metal-free synthesis of 1,5-substituted triazole peptides. AA = amino acid.

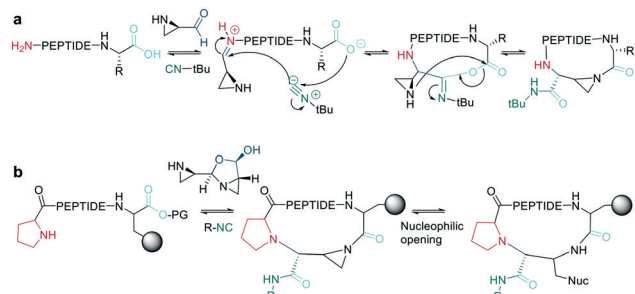


aldehydes. Due to the lack of diastereoselectivity³²⁸ and cyclodimerization, which can form the dominant product, low yields are achieved. The low selectivity can be explained by a kinetically competitive intermolecular process due to the slow transannular attack of the amine onto the mixed anhydride, which Hili *et al.* solved using *tert*-butyl-isocyanide and an aziridine aldehyde to obtain a cyclic piperazinone in high yields as a single diastereoisomer.³²⁹ With an amino aldehyde, the attack of the exocyclic nucleophilic aziridine is fast. A non-nucleophilic solvent, such as trifluoroethanol, prevents the premature solvolysis of the mixed anhydride (Scheme 38, a).³²⁹ For later bioconjugation, the activated aziridine ring within the cyclic peptide can be used *via* nucleophilic ring opening (Scheme 38, b).

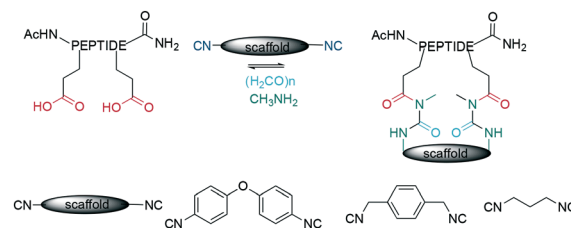
Ugi-mediated head-to-tail cyclization was adapted to on-resin macrocyclization using side-chain attachment of the C-terminal amino acid and an orthogonal protecting group strategy (allyl ester) to selectively deprotect the C-terminal COOH on resin followed by Fmoc removal and reaction with an aziridine aldehyde dimer and *tert*-butyl isocyanide in equal parts DCM/TFE (Scheme 38, b).³³⁰

The Ugi reaction is also suitable for sidechain-to-sidechain and sidechain-to-tail/head cyclization, which was shown to be faster and more efficient than head-to-tail cyclization, probably due to the higher flexibility of the sidechains. By carefully choosing the isocyanide component, the diversity of the peptide scaffold can be increased *via* *N*-substitution of the newly formed amide.³³¹ This approach was applied to stabilize secondary structures and simultaneously functionalize the sidechain-tethering lactam. To achieve this on-resin, a peptide was built using three dimensional orthogonal protecting groups for asparagine and lysine (alloc, allyl). Condensation of paraformaldehyde with pyrrolidine generated a pyrrolidinium ion, which is crucial for complete conversion to the imine by aminocatalysis mediated transamination, since on-resin imine formation is difficult to achieve with paraformaldehyde.³³² Careful washing removes any remaining base before isonitrile is added to finally cyclize the peptide.³³³

As for many cyclization chemistries, the scaffold strategy has also been applied for Ugi multi component reactions (MCR), where linear peptides containing two acidic amino



Scheme 38 Mechanism of Ugi reaction using aziridine aldehyde a: in solution and b: on resin.

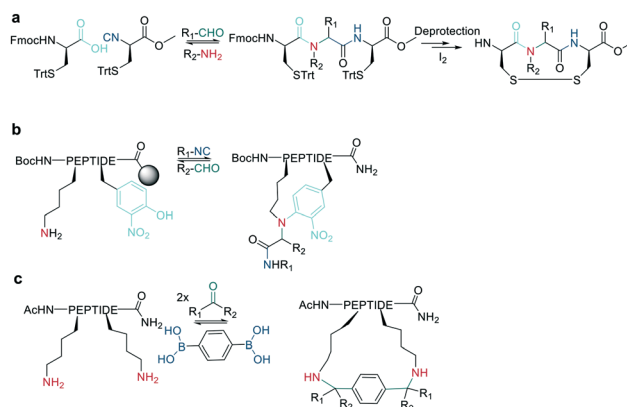


Scheme 39 Synthetic scheme for the four component Ugi reaction.

acids are coupled with diisocyanide scaffolds to generate sidechain-to-sidechain cyclized peptides (Scheme 39). Cyclization here is achieved in a pseudo-dilution protocol by slowly adding the peptide diacid and the diisocyanide to a mixture containing the preformed imine. However, this is slow, with reaction times of 96 h.³³⁴ A recent report highlighted the diversity achieved by distinct combinations of amino and isocyanide components, identifying stapled peptides that inhibited p53/MDM2/X.³³⁵

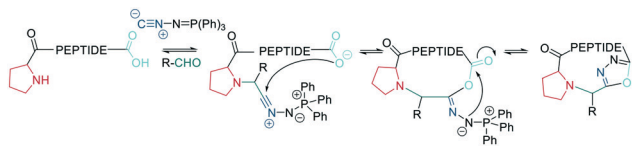
Macrocyclization *via* Ugi MCR can be combined with subsequent disulfide formation to generate bicyclic peptides.³³⁶ A similar approach called the sulfur-switch Ugi reaction was proposed to synthesize disulfide-linked cyclic peptides *de novo* from four components, followed by oxidative cyclization of the two cysteines with I₂ to form disulfides (Scheme 40, a). The double mercapto input that is possible on each Ugi component yields six topologically possible combinations.³³⁷ To generate macrocyclic peptides in solution and on resin, the carboxylic acid in the classic four-component Ugi is replaced by an electron-poor phenol (such as 3-nitrotyrosine) in the Ugi-smiles reaction to yield tertiary nitroanilines (Scheme 40, b).³³⁸

The Petasis reaction, also known as the borono-Mannich reaction,³³⁹ was reported for late-stage diversification (on-resin) and stapling (in solution, Scheme 40, c) of peptides. This three-component condensation of an aldehyde/ketone, an amine (*e.g.*, Lys sidechain), and an aryl/vinyl boronic



Scheme 40 a: Sulfur-Ugi-switch reaction of an *in situ* assembled peptide with successive disulfide formation. b: Ugi-smiles reaction with nitro-tyrosine as acidic component. c: Petasis reaction for peptide scaffolding.





Scheme 41 Multicomponent reaction for peptide macrocyclization employing an aldehyde and (*N*-isocyanimino)triphenylphosphorane to form a 1,3,4-oxadiazole.

ester/boronic acid depends on imine formation *via* transamination, as described for the on-resin Ugi reaction above, and the addition of boronic acid. The outcome and conversion rate of the Petasis reaction depends on the reactivity of the boronic acid, leading to singly and doubly modified products. To achieve stapling in solution, pseudo-dilution conditions were used by the slow addition of boronic acid and peptide to a solution of oxo-component.³⁴⁰

A head-to-tail peptidomimetic can be generated in one step *via* the multicomponent reaction of an aldehyde, linear peptide, and (*N*-isocyanimino)triphenylphosphorane.⁶ The resulting backbone contains a 1,3,4-oxadiazole, which was shown to stabilize a unique intramolecular hydrogen-bond network and enable a high passive membrane permeability in contrast to the analogous homodetic macrocycles. Oxadiazoles have also been a focus of medicinal chemistry for being a proteolytically stable isostere of amides. The aldehyde component serves as a linker between the N-terminus, and oxadiazole and can mimic amino acid sidechains based on aldehyde substituents; for example, phenylacetaldehyde mimics phenylalanine and isovaleraldehyde mimics leucine. However, the reaction produces both diastereomers. For efficient cyclization, this macrocyclization approach uses a zwitterionic control element, which prevents oligomerization even for more constrained 4-*mer* sequences and at high concentrations (5–100 mM peptide). It was proposed that the positively charged triphenylphosphonium ion augments interaction between the chain termini, leading to a more efficient macrocyclization (Scheme 41).

Conclusions

The favorable properties of peptidic macrocycles and peptidomimetics as potential drug leads have led to the rapid evolution of peptide chemistry beyond traditional amide formation. This is especially true for chemistries that expand the chemical space, such as derivatizing natural linear peptides with organic scaffolds, as these are expected to further improve the drug-like properties of peptides and peptidomimetics and extend the chemical space towards new therapeutic chimeric modalities. With more and more mild, specific, and mutually orthogonal cyclization methods at hand, the possibility for synthesizing highly conformationally constrained, more chemically diverse peptides and peptidomimetics in a controlled manner is continuously increasing. Given that modifying the peptide structure post-discovery can reduce the binding affinity compared to the

initial hit, the increasing number of reactions compatible with *in vitro* selection systems and encoded combinatorial libraries will accelerate drug discovery efforts of those new macrocyclic peptidomimetic modalities, opening a great future for peptide-derived drug discovery.

Author contributions

CB and CL have both conceptualized and written the manuscript.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

We thank Prof. Daniel Ricklin for his support and guidance and Dr. Kaycie Butler (Butler SciComm) for language editing. Funding from the SNF (No. 31003A_176104) for CB and from University Basel Fund for Excellent Junior Researcher for CL is gratefully acknowledged.

References

- 1 A. Zorzi, K. Deyle and C. Heinis, *Curr. Opin. Chem. Biol.*, 2017, **38**, 24–29.
- 2 T. Passioura, *Biochemistry*, 2020, **59**, 139–145.
- 3 A. A. Vinogradov, Y. Yin and H. Suga, *J. Am. Chem. Soc.*, 2019, **141**, 4167–4181.
- 4 P. G. Dougherty, Z. Qian and D. Pei, *Biochem. J.*, 2017, **474**, 1109–1125.
- 5 N. S. Robertson and D. R. Spring, *Molecules*, 2018, **23**, 959.
- 6 J. R. Frost, C. C. G. Scully and A. K. Yudin, *Nat. Chem.*, 2016, **8**, 1105–1111.
- 7 M. Muttenthaler, G. F. King, D. J. Adams and P. F. Alewood, *Nat. Rev. Drug Discovery*, 2021, **20**, 309–325.
- 8 A. Bhat, L. R. Roberts and J. J. Dwyer, *Eur. J. Med. Chem.*, 2015, **94**, 471–479.
- 9 X. Jing and K. Jin, *Med. Res. Rev.*, 2020, **40**, 753–810.
- 10 V. Martí-Centelles, M. D. Pandey, M. I. Burguete and S. V. Luis, *Chem. Rev.*, 2015, **115**, 8769–8834.
- 11 J. M. Humphrey and A. R. Chamberlin, *Chem. Rev.*, 1997, **97**, 2243–2266.
- 12 C. J. White and A. K. Yudin, *Nat. Chem.*, 2011, **3**, 509–524.
- 13 C. E. Schafmeister, J. Po and G. L. Verdine, *J. Am. Chem. Soc.*, 2000, **122**, 5891–5892.
- 14 T. A. Hill, N. E. Shepherd, F. Diness and D. P. Fairlie, *Angew. Chem., Int. Ed.*, 2014, **53**, 13020–13041.
- 15 A. Luther, K. Moehle, E. Chevalier, G. Dale and D. Obrecht, *Curr. Opin. Chem. Biol.*, 2017, **38**, 45–51.
- 16 P. Timmerman, W. C. Puijk and R. H. Melen, *J. Mol. Recognit.*, 2007, **20**, 283–299.
- 17 P. Timmerman, R. Barderas, J. Desmet, D. Altschuh, S. Schochat, M. J. Hollestelle, J. W. M. Höppener, A. Monasterio, J. I. Casal and R. H. Melen, *J. Biol. Chem.*, 2009, **284**, 34126–34134.



- 18 L. Raynal, N. C. Rose, J. R. Donald and C. D. Spicer, *Chem. – Eur. J.*, 2021, **27**, 69–88.
- 19 M. Schmidt, A. Toplak, P. J. L. M. Quaedflieg, J. H. van Maarseveen and T. Nuijens, *Drug Discovery Today: Technol.*, 2017, **26**, 11–16.
- 20 R. Wills, V. Adebomi and M. Raj, *ChemBioChem*, 2020, **21**, 52–62.
- 21 A. Gori, C. I. A. Wang, P. J. Harvey, K. J. Rosengren, R. F. Bhola, M. L. Gelmi, R. Longhi, M. J. Christie, R. J. Lewis, P. F. Alewood and A. Brust, *Angew. Chem., Int. Ed.*, 2015, **54**, 1361–1364.
- 22 H. Y. Chow, Y. Zhang, E. Matheson and X. Li, *Chem. Rev.*, 2019, **119**, 9971–10001.
- 23 V. Agouridas, O. El Mahdi, V. Diemer, M. Cargoët, J. C. M. Monbaliu and O. Melnyk, *Chem. Rev.*, 2019, **119**, 7328–7443.
- 24 C. Sohrabi, A. Foster and A. Tavassoli, *Nat. Rev. Chem.*, 2020, **4**, 90–101.
- 25 J. M. Humphrey and A. R. Chamberlin, *Chem. Rev.*, 1997, **97**, 2243–2266.
- 26 J. Blankenstein and J. Zhu, *Eur. J. Org. Chem.*, 2005, 1949–1964.
- 27 J. W. Taylor, *Biopolymers*, 2002, **66**, 49–75.
- 28 A. El-Faham and F. Albericio, *Chem. Rev.*, 2011, **111**, 6557–6602.
- 29 S. J. Wen and Z. J. Yao, *Org. Lett.*, 2004, **6**, 2721–2724.
- 30 K. Jin, I. H. Sam, K. H. L. Po, D. Lin, E. H. G. Zadeh, S. Chen, Y. Yuan and X. Li, *Nat. Commun.*, 2016, 12364, DOI: 10.1038/ncomms12394.
- 31 P. Rovero, L. Quartara and G. Fabbri, *Tetrahedron Lett.*, 1991, **32**, 2639–2642.
- 32 G. T. Bourne, W. D. F. Meutermans, P. F. Alewood, R. P. McGeary, M. Scanlon, A. A. Watson and M. L. Smythe, *J. Org. Chem.*, 1999, **64**, 3095–3101.
- 33 N. E. Shepherd, H. N. Hoang, G. Abbenante and D. P. Fairlie, *J. Am. Chem. Soc.*, 2005, **127**, 2974–2983.
- 34 R. Fasan, R. L. A. Dias, K. Moehle, O. Zerbe, J. W. Vrijbloed, D. Obrecht and J. A. Robinson, *Angew. Chem., Int. Ed.*, 2004, **43**, 2109–2112.
- 35 H. Y. Chow, Y. Zhang, E. Matheson and X. Li, *Chem. Rev.*, 2019, **119**, 9971–10001.
- 36 K. Shinbara, W. Liu, R. H. P. van Neer, T. Katoh, H. Suga, R. Herman, P. Van Neer, T. Katoh, R. H. P. van Neer, T. Katoh and H. Suga, *Front. Chem.*, 2020, **8**, 1–19.
- 37 P. E. Dawson, T. W. Muir, I. Clark-Lewis and S. B. H. Kent, *Science*, 1994, **266**, 776–779.
- 38 L. Zhang and J. P. Tam, *J. Am. Chem. Soc.*, 1997, **119**, 2363–2370.
- 39 J. P. Tam, Y. A. Lu and Q. Yu, *J. Am. Chem. Soc.*, 1999, **121**, 4316–4324.
- 40 J. A. Camarero, G. J. Cotton, A. Adeva and T. W. Muir, *J. Pept. Res.*, 1998, **51**, 303–316.
- 41 J. Tulla-Puche and G. Barany, *J. Org. Chem.*, 2004, **69**, 4101–4107.
- 42 B. H. Gless and C. A. Olsen, *J. Org. Chem.*, 2018, **83**, 10525–10534.
- 43 L. Z. Yan and P. E. Dawson, *J. Am. Chem. Soc.*, 2001, **123**, 526–533.
- 44 P. Botti, T. D. Pallin and J. P. Tam, *J. Am. Chem. Soc.*, 1996, **118**, 10018–10024.
- 45 V. J. Thombare and C. A. Hutton, *Angew. Chem., Int. Ed.*, 2019, **58**, 5052–5056.
- 46 Y. Li, A. Yongye, M. Giulianotti, K. Martinez-Mayorga, Y. Yu and R. A. Houghten, *J. Comb. Chem.*, 2009, **11**(6), 1066–1072.
- 47 J.-J. Du, L.-M. Xin, Z. Lei, S.-Y. Zou, W.-B. Xu, C.-W. Wang, L. Zhang, X.-F. Gao and J. Guo, *Chin. Chem. Lett.*, 2018, **29**, 1127–1130.
- 48 C. L. Tung, C. T. T. Wong and X. Li, *Org. Biomol. Chem.*, 2015, **13**, 6922–6926.
- 49 M. Raj, H. Wu, S. L. Blosser, M. A. Vittoria and P. S. Arora, *J. Am. Chem. Soc.*, 2015, **137**, 6932–6940.
- 50 K. Sasaki and D. Crich, *Org. Lett.*, 2010, **12**, 3254–3257.
- 51 T. Ohara, M. Kaneda, T. Saito, N. Fujii, H. Ohno and S. Oishi, *Bioorg. Med. Chem. Lett.*, 2018, **28**, 1283–1286.
- 52 R. Kleineweischede and C. P. R. Hackenberger, *Angew. Chem., Int. Ed.*, 2008, **47**, 5984–5988.
- 53 J. W. Bode, R. M. Fox and K. D. Baucom, *Angew. Chem., Int. Ed.*, 2006, **45**, 1248–1252.
- 54 T. Fukuzumi, L. Ju and J. W. Bode, *Org. Biomol. Chem.*, 2012, **10**, 5837–5844.
- 55 Y. L. Huang, R. Frey, M. E. Juarez-Garcia and J. W. Bode, *Heterocycles*, 2012, **84**, 1179–1191.
- 56 V. R. Pattabiraman, A. O. Ogunkoya and J. W. Bode, *Angew. Chem., Int. Ed.*, 2012, **51**, 5114–5118.
- 57 Y. Zhang, C. Xu, H. Y. Lam, C. L. Lee and X. Li, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, **110**, 6657–6662.
- 58 C. T. T. Wong, H. Y. Lam, T. Song, G. Chen and X. Li, *Angew. Chem., Int. Ed.*, 2013, **52**, 10212–10215.
- 59 J. Lécaillon, P. Gilles, G. Subra, J. Martinez and M. Amblard, *Tetrahedron Lett.*, 2008, **49**, 4674–4676.
- 60 W. D. F. Meutermans, S. W. Golding, G. T. Bourne, L. P. Miranda, M. J. Dooley, P. F. Alewood and M. L. Smythe, *J. Am. Chem. Soc.*, 1991, **113**, 9790–9796.
- 61 A. A. Vinogradov, Z. N. Choo, K. A. Totaro and B. L. Pentelute, *Org. Lett.*, 2016, **18**, 1226–1229.
- 62 L. R. Malins, J. N. Degruyter, K. J. Robbins, P. M. Scola, M. D. Eastgate, M. R. Ghadiri and P. S. Baran, *J. Am. Chem. Soc.*, 2017, **139**, 5233–5241.
- 63 V. Adebomi, R. D. Cohen, R. Wills, H. A. H. Chavers, G. E. Martin and M. Raj, *Angew. Chem.*, 2019, **131**, 19249–19256.
- 64 A. Bandyopadhyay and J. Gao, *J. Am. Chem. Soc.*, 2016, **138**, 2098–2101.
- 65 S. M. Guéret, S. Thavam, R. J. Carbajo, M. Potowski, N. Larsson, G. Dahl, A. Dellsén, T. N. Grossmann, A. T. Plowright, E. Valeur, M. Lemurell and H. Waldmann, *J. Am. Chem. Soc.*, 2020, **142**, 4904–4915.
- 66 B. Li, H. Tang, A. Turlik, Z. Wan, X.-S. Xue, L. Li, X. Yang, J. Li, G. He, K. N. Houk and G. Chen, *Angew. Chem.*, 2020, 2–9.
- 67 C. M. Haney, M. T. Loch and W. S. Horne, *Chem. Commun.*, 2011, **47**, 10915–10917.



- 68 C. M. Haney and W. S. Horne, *Chem. – Eur. J.*, 2013, **19**, 11342–11351.
- 69 C. M. Haney and W. S. Horne, *J. Pept. Sci.*, 2014, **20**, 108–114.
- 70 K. W. Decoene, W. Vannecke, T. Passioura, H. Suga and A. Madder, *Biomedicines*, 2018, **6**, 1–12.
- 71 S. W. Millward, T. T. Takahashi and R. W. Roberts, *J. Am. Chem. Soc.*, 2005, **127**, 14142–14143.
- 72 K. Fujimoto, M. Kajino and M. Inouye, *Chem. – Eur. J.*, 2008, **14**, 857–863.
- 73 W. Lian, P. Upadhyaya, C. A. Rhodes, Y. Liu and D. Pei, *J. Am. Chem. Soc.*, 2013, **135**, 11990–11995.
- 74 W. Lian, B. Jiang, Z. Qian and D. Pei, *J. Am. Chem. Soc.*, 2014, **136**, 9830–9833.
- 75 G. Lautrette, F. Touti, H. G. Lee, P. Dai and B. L. Pentelute, *J. Am. Chem. Soc.*, 2016, **138**, 8340–8343.
- 76 H. G. Lee, G. Lautrette, B. L. Pentelute and S. L. Buchwald, *Angew. Chem.*, 2017, **129**, 3225–3229.
- 77 D. P. Fairlie and A. D. de Araujo, *Biopolymers*, 2016, **106**, 843–852.
- 78 D. Y. Jackson, D. S. King, J. Chmielewski, S. Singh and P. G. Schultz, *J. Am. Chem. Soc.*, 1991, **113**, 9392–9394.
- 79 A. M. Almeida, R. Li and S. H. Gellman, *J. Am. Chem. Soc.*, 2012, **134**, 75–78.
- 80 C. M. B. K. Kourra and N. Cramer, *Chem. Sci.*, 2016, **7**, 7007–7012.
- 81 S. Lindman, G. Lindeberg, P. A. Frändberg, F. Nyberg, A. Karlén and A. Hallberg, *Bioorg. Med. Chem.*, 2003, **11**, 2947–2954.
- 82 M. Ueki, T. Ikeo, K. Hokari, K. Nakamura, A. Saeki and H. Komatsu, *Bull. Chem. Soc. Jpn.*, 1999, **72**, 829–838.
- 83 M. Uekia, T. Ikeoa, M. Iwadateb, T. Asakurab, M. P. Williamsonc and J. Slaninová, *Bioorg. Med. Chem. Lett.*, 1999, **9**, 1767–1772.
- 84 H. I. Mosberg, J. R. Omnaas and A. Goldstein, *Mol. Pharmacol.*, 1987, **31**, 599–602.
- 85 N. Zheng, P. Karra, M. A. Vandenberg, J. H. Kim, M. J. Webber, W. L. Holland and D. H.-C. Chou, *J. Med. Chem.*, 2019, **62**, 11437–11443.
- 86 R. Mousa, S. Lansky, G. Shoham and N. Metanis, *Chem. Sci.*, 2018, **9**, 4814–4820.
- 87 J. P. Fischer, R. Schönauer, S. Els-Heindl, D. Bierer, J. Koebberling, B. Riedl and A. G. Beck-Sickinger, *J. Pept. Sci.*, 2019, **25**, 1–13.
- 88 S. S. Chavali, S. M. Mali, J. L. Jenkins, R. Fasan and J. E. Wedekind, *J. Biol. Chem.*, 2020, **295**, 16470–16486.
- 89 C. E. Murar, M. Ninomiya, S. Shimura, U. Karakus, O. Boyman and J. W. Bode, *Angew. Chem.*, 2020, **132**, 8503–8507.
- 90 F. M. Brunel and P. E. Dawson, *Chem. Commun.*, 2005, 2552–2554.
- 91 N. Bionda, A. L. Cryan and R. Fasan, *ACS Chem. Biol.*, 2014, **9**, 2008–2013.
- 92 Y. Goto, A. Ohta, Y. Sako, Y. Yamagishi, H. Murakami and H. Suga, *ACS Chem. Biol.*, 2008, **3**, 120–129.
- 93 X. S. Wang, P. H. C. Chen, J. T. Hampton, J. M. Tharp, C. A. Reed, S. K. Das, D. S. Wang, H. S. Hayatshahi, Y. Shen, J. Liu and W. R. Liu, *Angew. Chem., Int. Ed.*, 2019, **58**, 15904–15909.
- 94 A. A. Aimetti, R. K. Shoemaker, C. C. Lin and K. S. Anseth, *Chem. Commun.*, 2010, **46**, 4061–4063.
- 95 Y. Tian, D. Yang, X. Ye and Z. Li, *Chem. Rec.*, 2017, **17**, 874–885.
- 96 B. P. Sutherland, B. M. El-Zaatari, N. I. Halaszynski, J. M. French, S. Bai and C. J. Kloxin, *Bioconjugate Chem.*, 2018, **29**, 3987–3992.
- 97 Y. Tian, J. Li, H. Zhao, X. Zeng, D. Wang, Q. Liu, X. Niu, X. Huang, N. Xu and Z. Li, *Chem. Sci.*, 2016, **7**, 3325–3330.
- 98 J. M. Chalker, S. B. Gunnoo, O. Boutureira, S. C. Gerstberger, M. Fernández-González, G. J. L. Bernardes, L. Griffin, H. Hailu, C. J. Schofield and B. G. Davis, *Chem. Sci.*, 2011, **2**, 1666–1676.
- 99 J. M. Chalker, L. Lercher, N. R. Rose, C. J. Schofield and B. G. Davis, *Angew. Chem., Int. Ed.*, 2012, **51**, 1835–1839.
- 100 A. Muppidi, Z. Wang, X. Li, J. Chen and Q. Lin, *Chem. Commun.*, 2011, **47**, 9396–9398.
- 101 D. S. Kemp and P. McNamara, *Tetrahedron Lett.*, 1981, **22**, 4571–4574.
- 102 P. Timmerman, J. Beld, W. C. Puijk and R. H. Meloen, *ChemBioChem*, 2005, **6**, 821–824.
- 103 F. Zhang, O. Sadovski, S. J. Xin and G. A. Woolley, *J. Am. Chem. Soc.*, 2007, **129**, 14154–14155.
- 104 S. Sun, I. Compañón, N. Martínez-Sáez, J. D. Seixas, O. Boutureira, F. Corzana and G. J. L. Bernardes, *ChemBioChem*, 2018, **19**, 48–52.
- 105 N. Martínez-Sáez, S. Sun, D. Oldrini, P. Sormanni, O. Boutureira, F. Carboni, I. Compañón, M. J. Deery, M. Vendruscolo, F. Corzana, R. Adamo and G. J. L. Bernardes, *Angew. Chem., Int. Ed.*, 2017, **56**, 14963–14967.
- 106 N. Assem, D. J. Ferreira, D. W. Wolan and P. E. Dawson, *Angew. Chem., Int. Ed.*, 2015, **54**, 8665–8668.
- 107 G. K. Dewkar, P. B. Carneiro and M. C. T. Hartman, *Org. Lett.*, 2009, **11**, 4708–4711.
- 108 I. Ramos-Tomillero, G. Perez-Chacon, B. Somovilla-Crespo, F. Sanchez-Madrid, J. M. Domínguez, C. Cuevas, J. M. Zapata, H. Rodríguez and F. Albericio, *Bioconjugate Chem.*, 2018, **29**, 1199–1208.
- 109 L. E. J. Smeenk, N. Dailly, H. Hiemstra, J. H. Van Maarseveen and P. Timmerman, *Org. Lett.*, 2012, **14**, 1194–1197.
- 110 G. A. Woolley, *Acc. Chem. Res.*, 2005, **38**, 486–493.
- 111 M. Blanco-Lomas, S. Samanta, P. J. Campos, G. A. Woolley and D. Sampedro, *J. Am. Chem. Soc.*, 2012, **134**, 6960–6963.
- 112 S. Samanta, C. Qin, A. J. Lough and G. A. Woolley, *Angew. Chem.*, 2012, **124**, 6558–6561.
- 113 W. Liu, Y. Zheng, X. Kong, C. Heinis, Y. Zhao and C. Wu, *Angew. Chem., Int. Ed.*, 2017, **56**, 4458–4463.
- 114 J. Wang, M. Zha, Q. Fei, W. Liu, Y. Zhao and C. Wu, *Chem. – Eur. J.*, 2017, **23**, 15150–15155.
- 115 S. P. Brown and A. B. Smith, *J. Am. Chem. Soc.*, 2015, **137**, 4034–4037.
- 116 A. M. Spokoiny, Y. Zou, J. J. Ling, H. Yu, Y. Lin and B. L. Pentelute, *J. Am. Chem. Soc.*, 2013, **135**, 5946–5949.



- 117 Y. Zou, A. M. Spokoyny, C. Zhang, M. D. Simon, H. Yu, Y. S. Lin and B. L. Pentelute, *Org. Biomol. Chem.*, 2014, **12**, 566–573.
- 118 E. V. Vinogradova, C. Zhang, A. M. Spokoyny, B. L. Pentelute and S. L. Buchwald, *Nature*, 2015, **526**, 687–691.
- 119 D. T. Bong and M. R. Ghadiri, *Org. Lett.*, 2001, **3**, 2509–2511.
- 120 E. N. Korneeva, M. V. Ovchinnikov and N. M. Kostić, *Inorg. Chim. Acta*, 1996, **243**, 9–13.
- 121 S. D. Tilley and M. B. Francis, *J. Am. Chem. Soc.*, 2006, **128**, 1080–1081.
- 122 A. J. Rojas, C. Zhang, E. V. Vinogradova, N. H. Buchwald, J. Reilly, B. L. Pentelute and S. L. Buchwald, *Chem. Sci.*, 2017, **8**, 4257–4263.
- 123 Y. Wang and D. H. C. Chou, *Angew. Chem., Int. Ed.*, 2015, **54**, 10931–10934.
- 124 T. Wang, A. Riegger, M. Lamla, S. Wiese, P. Oeckl, M. Otto, Y. Wu, S. Fischer, H. Barth, S. L. Kuan and T. Weil, *Chem. Sci.*, 2016, **7**, 3234–3239.
- 125 O. Brun, L. J. Archibald, J. Agramunt, E. Pedroso and A. Grandas, *Org. Lett.*, 2017, **19**, 992–995.
- 126 M. E. B. Smith, F. F. Schumacher, C. P. Ryan, L. M. Tedaldi, D. Papaioannou, G. Waksman, S. Caddick and J. R. Baker, *J. Am. Chem. Soc.*, 2010, **132**, 1960–1965.
- 127 C. M. Grison, G. M. Burslem, J. A. Miles, L. K. A. Pilsl, D. J. Yeo, Z. Imani, S. L. Warriner, M. E. Webb and A. J. Wilson, *Chem. Sci.*, 2017, **8**, 5166–5171.
- 128 Y. Zhang, C. Zang, G. An, M. Shang, Z. Cui, G. Chen, Z. Xi and C. Zhou, *Nat. Commun.*, 2020, **11**, 1–10.
- 129 X. Zheng, Z. Li, W. Gao, X. Meng, X. Li, L. Y. P. Luk, Y. Zhao, Y. H. Tsai and C. Wu, *J. Am. Chem. Soc.*, 2020, **142**, 5097–5103.
- 130 V. Chudasama, M. E. B. Smith, F. F. Schumacher, D. Papaioannou, G. Waksman, J. R. Baker and S. Caddick, *Chem. Commun.*, 2011, **47**, 8781–8783.
- 131 M. T. W. Lee, A. Maruani, J. R. Baker, S. Caddick and V. Chudasama, *Chem. Sci.*, 2016, **7**, 799–802.
- 132 C. Heinis, T. Rutherford, S. Freund and G. Winter, *Nat. Chem. Biol.*, 2009, **5**, 502–507.
- 133 S. Chen, D. Bertoldo, A. Angelini, F. Pojer and C. Heinis, *Angew. Chem., Int. Ed.*, 2014, **53**, 1602–1606.
- 134 S. S. Kale, C. Villequey, X. D. Kong, A. Zorzi, K. Deyle and C. Heinis, *Nat. Chem.*, 2018, **10**, 715–723.
- 135 Y. V. Guillen Schlippe, M. C. T. Hartman, K. Josephson and J. W. Szostak, *J. Am. Chem. Soc.*, 2012, **134**, 10469–10477.
- 136 S. Kalhor-Monfared, M. R. Jafari, J. T. Patterson, P. I. Kitov, J. J. Dwyer, J. M. Nuss and R. Derda, *Chem. Sci.*, 2016, **7**, 3785–3790.
- 137 X. Zheng, W. Liu, Z. Liu, Y. Zhao and C. Wu, *Bioconjugate Chem.*, 2020, **31**, 2085–2091.
- 138 J. R. Frost, J. Z. Essman, C. Huang, N. A. Pierson, N. Pissarnitski and T. Meng, *Pept. Sci.*, 2020, **112**, e24160.
- 139 S. S. Kale, M. Bergeron-Brelek, Y. Wu, M. G. Kumar, M. V. Pham, J. Bortoli, J. Vesin, X. D. Kong, J. F. Machado, K. Deyle, P. Gonschorek, G. Turcatti, L. Cendron, A. Angelini and C. Heinis, *Sci. Adv.*, 2019, **5**, 1–10.
- 140 K. Kubota, P. Dai, B. L. Pentelute and S. L. Buchwald, *J. Am. Chem. Soc.*, 2018, **140**, 3128–3133.
- 141 Y. Zhang, Q. Zhang, C. T. T. Wong and X. Li, *J. Am. Chem. Soc.*, 2019, **141**, 12274–12279.
- 142 M. Todorovic, K. D. Schwab, J. Zeisler, C. Zhang, F. Bénard and D. M. Perrin, *Angew. Chem., Int. Ed.*, 2019, **58**, 14120–14124.
- 143 Q. Luo, Y. Tao, W. Sheng, J. Lu and H. Wang, *Nat. Commun.*, 2019, **10**, 1–9.
- 144 R. Zhao, P. Shi, J. Chen, S. Sun, J. Chen, J. Cui, F. Wu, G. Fang, C. Tian, J. Shi, D. Bierer, L. Liu and Y.-M. Li, *Chem. Sci.*, 2020, **11**, 7927–7932.
- 145 K. C. Nicolaou, N. F. Jain, S. Natarajan, R. Hughes, M. E. Solomon, H. Li, J. M. Ramanjulu, M. Takayanagi, A. E. Koumbis and T. Bando, *Angew. Chem., Int. Ed.*, 1998, **37**, 2714–2716.
- 146 S. C. Stolze and M. Kaiser, *Synthesis*, 2012, **44**, 1755–1777.
- 147 S. Shabani and C. A. Hutton, *Org. Lett.*, 2020, **22**, 4557–4561.
- 148 P. Cristau, J.-P. Vors and J. Zhu, *Org. Lett.*, 2001, **3**, 4079–4082.
- 149 M. Bois-Choussy, P. Cristau and J. Zhu, *Angew. Chem., Int. Ed.*, 2003, **42**, 4238–4241.
- 150 A. T. Londregan, K. A. Farley, C. Limberakis, P. B. Mullins and D. W. Piotrowski, *Org. Lett.*, 2012, **14**, 2890–2893.
- 151 J. Zhu, *Chimia*, 2013, **67**, 916–920.
- 152 K. Burgess, D. Lim, M. Bois-Choussy and J. Zhu, *Tetrahedron Lett.*, 1997, **38**, 3345–3348.
- 153 C. Fotsch, G. Kumaravel, S. K. Sharma, A. D. Wu, J. S. Gounarides, N. R. Nirmala and R. C. Petter, *Tetrahedron Lett.*, 1999, **9**, 2125–2130.
- 154 A. J. Pearson, G. Bignan, P. Zhang and M. Chelliah, *J. Org. Chem.*, 1996, **3263**, 3940–3941.
- 155 S. J. Lee, M. M. Joullie, J. Lee and M. M. Joullie, *Chem. Sci.*, 2018, **9**, 2432–2436.
- 156 Q. Cai, B. Zou and D. Ma, *Angew. Chem., Int. Ed.*, 2006, **45**, 1276–1279.
- 157 C. P. Decicco, Y. Song and D. A. Evans, *Org. Lett.*, 2001, **3**, 1029–1032.
- 158 C. W. West and D. H. Rich, *Org. Lett.*, 1999, **1**, 1819–1822.
- 159 K. V. Lawson, T. E. Rose and P. G. Harran, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, **110**, E3753–E3760.
- 160 D. G. Rivera, G. M. Ojeda-Carralero, L. Reguera and E. V. Van Der Eycken, *Chem. Soc. Rev.*, 2020, **49**, 2039–2059.
- 161 H. Lee, N. C. Boyer, Q. Deng, H.-Y. Kim, T. K. Sawyer and N. Sciammetta, *Chem. Sci.*, 2019, **10**, 5073–5078.
- 162 Y. A. Kim, H. N. Shin, M. S. Park, S. H. Cho and S. Y. Han, *Tetrahedron Lett.*, 2003, **44**, 2557–2560.
- 163 Q. Cai, G. He and D. Ma, *J. Org. Chem.*, 2006, **71**, 5268–5273.
- 164 M. Toumi, F. Couty and G. Evano, *J. Org. Chem.*, 2008, **73**, 1270–1281.
- 165 G. Cao, K. Yang, Y. Li, L. Huang and D. Teng, *Molecules*, 2016, **21**, 212.
- 166 J. Choi and G. C. Fu, *Science*, 2017, **356**, eaaf7230.
- 167 C. C. C. J. Seechurn, M. O. Kitching, T. J. Colacot and V. Snieckus, *Angew. Chem., Int. Ed.*, 2012, **51**, 5062–5085.



- 168 T. Willemse, W. Schepens, H. W. T. Van Vlijmen, B. U. W. Maes and S. Ballet, *Catalysts*, 2017, **7**, 74.
- 169 H. Grubbs and N. Sewald, *Chem. – Eur. J.*, 2020, **26**, 5328–5340.
- 170 M. Pérez-González and R. F. W. Jackson, *Chem. Commun.*, 2000, 2423–2424.
- 171 A. Afonso, L. Feliu and M. Planas, *Tetrahedron*, 2011, **67**, 2238–2245.
- 172 I. Ng-Choi, À. Oliveras, L. Feliu and M. Planas, *Beilstein J. Org. Chem.*, 2019, **15**, 761–768.
- 173 B.-B. Zhan, M.-X. Jiang and B.-F. Shi, *Chem. Commun.*, 2020, **56**, 13950.
- 174 Z. Bai, C. Cai, Z. Yu and H. Wang, *Angew. Chem., Int. Ed.*, 2018, **57**, 13912–13916.
- 175 J. Tang, Y. He, H. Chen, W. Sheng and H. Wang, *Chem. Sci.*, 2017, **8**, 4565–4570.
- 176 A. F. M. Noisier, J. García, I. A. Ionuț and F. Albericio, *Angew. Chem., Int. Ed.*, 2017, **56**, 314–318.
- 177 S. J. McCarver, J. X. Qiao, J. Carpenter, R. M. Borzilleri, M. A. Poss, M. D. Eastgate, M. M. Miller and D. W. C. MacMillan, *Angew. Chem.*, 2017, **129**, 746–750.
- 178 A. F. M. Noisier and M. A. Brimble, *Chem. Rev.*, 2014, **114**, 8775–8806.
- 179 W. Wang, M. M. Lorion, J. Shah, A. R. Kapdi and L. Ackermann, *Angew. Chem., Int. Ed.*, 2018, **57**, 14700–14717.
- 180 J. Ruiz-Rodríguez, F. Albericio and R. Lavilla, *Chem. – Eur. J.*, 2010, **16**, 1124–1127.
- 181 L. Mendive-Tapia, S. Preciado, J. García, R. Ramón, N. Kielland, F. Albericio and R. Lavilla, *Nat. Commun.*, 2015, **6**, 7160.
- 182 B. Han, B. Li, L. Qi, P. Yang, G. He and G. Chen, *Org. Lett.*, 2020, **22**, 6879–6883.
- 183 J. Tang, H. Chen, Y. He, W. Sheng, Q. Bai and H. Wang, *Nat. Commun.*, 2018, **9**, 3383.
- 184 J. Tan, J. Wu, S. Liu, H. Yao and H. Wang, *Sci. Adv.*, 2019, **5**, eaaw0323.
- 185 N. Kaplaneris, F. Kaltenhäuser, G. Sirvinskaitė, S. Fan, T. De Oliveira, L.-C. Conradi and L. Ackermann, *Sci. Adv.*, 2021, **7**, eabe6202.
- 186 J. Liu, X. Liu, F. Zhang, J. Qu, H. Sun and Q. Zhu, *Chem. – Eur. J.*, 2020, **26**, 16122–16128.
- 187 S. Bloom, C. Liu, D. K. Kölmel, J. X. Qiao, Y. Zhang, M. A. Poss, W. R. Ewing and D. W. C. MacMillan, *Nat. Chem.*, 2018, **10**, 205.
- 188 T. Qin, L. R. Malins, J. T. Edwards, R. R. Merchant, A. J. E. Novak, J. Z. Zhong, R. B. Mills, M. Yan, C. Yuan, M. D. Eastgate and P. S. Baran, *Angew. Chem., Int. Ed.*, 2017, **56**, 260–265.
- 189 M. H. Vilhelmsen, J. Jensen, C. G. Tortzen and M. B. Nielsen, *Eur. J. Org. Chem.*, 2013, **3**, 701–711.
- 190 A. P. Silvestri, P. A. Cistrone and P. E. Dawson, *Angew. Chem., Int. Ed.*, 2017, **56**, 10438–10442.
- 191 S. Verlinden, N. Geudens, J. C. Martins, D. Tourwé, S. Ballet and G. Verniest, *Org. Biomol. Chem.*, 2015, **13**, 9398–9404.
- 192 N. Auberger, M. Di Pisa, M. Larregola, G. Chassaing, E. Peroni, S. Lavielle, A. M. Papini, O. Lequin and J. M. Mallet, *Bioorg. Med. Chem.*, 2014, **22**, 6924–6932.
- 193 P. A. Cistrone, A. P. Silvestri, J. C. J. Hintzen and P. E. Dawson, *ChemBioChem*, 2018, **19**, 1031–1035.
- 194 R. H. Grubbs and S. Chang, *Tetrahedron*, 1998, **54**, 4413–4450.
- 195 A. Fürstner, *Angew. Chem., Int. Ed.*, 2000, **39**, 3012–3043.
- 196 R. H. Grubbs, *Tetrahedron*, 2004, **60**, 7117–7140.
- 197 A. Gradillas and J. Pérez-Castells, *Angew. Chem., Int. Ed.*, 2006, **45**, 6086–6101.
- 198 A. H. Hoveyda and A. R. Zhugralin, *Nature*, 2007, **450**, 243–251.
- 199 X. Lu, L. Fan, C. B. Phelps, C. P. Davie and C. P. Donahue, *Bioconjugate Chem.*, 2017, **28**, 1625–1629.
- 200 O. B. C. Monty, P. Nyshadham, K. M. Bohren, M. Palaniappan, M. M. Matzuk, D. W. Young and N. Simmons, *ACS Comb. Sci.*, 2020, **22**, 80–88.
- 201 T. M. Trnka and R. H. Grubbs, *Acc. Chem. Res.*, 2001, **34**, 18–29.
- 202 A. H. Hoveyda and A. R. Zhugralin, *Nature*, 2007, **450**, 243–251.
- 203 E. C. Gleeson, W. R. Jackson and A. J. Robinson, *Tetrahedron Lett.*, 2016, **57**, 4325–4333.
- 204 Y.-W. Kim, T. N. Grossmann and G. L. Verdine, *Nat. Protoc.*, 2011, **6**, 761–771.
- 205 F. Bernal and S. G. Katz, *Methods Mol. Biol.*, 2014, **1176**, 107–114.
- 206 H. E. Blackwell and R. H. Grubbs, *Angew. Chem., Int. Ed.*, 1998, **37**, 3281–3284.
- 207 Y. S. Chang, B. Graves, V. Guerlavais, C. Tovar, K. Packman, K. H. To, K. A. Olson, K. Kesavan, P. Gangurde, A. Mukherjee, T. Baker, K. Darlak, C. Elkin, Z. Filipovic, F. Z. Qureshi, H. Cai, P. Berry, E. Feyfant, X. E. Shi, J. Horstick, D. A. Annis, A. M. Manning, N. Fotouhi, H. Nash, L. T. Vassilev and T. K. Sawyer, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, **110**, E3445–E3454.
- 208 L. D. Walensky and G. H. Bird, *J. Med. Chem.*, 2014, **57**, 6275–6288.
- 209 Y. H. Lau, P. De Andrade, Y. Wu and D. R. Spring, *Chem. Soc. Rev.*, 2015, **44**, 91–102.
- 210 T. D. Clark, M. Reza Ghadiri and D. E. Angew Chem, *Angew. Chem.*, 1995, **117**, 201–203.
- 211 J. Pernerstorfer, *Chem. Commun.*, 1997, 1949–1950.
- 212 B. E. Fink, P. R. Kym and J. A. Katzenellenbogen, *J. Am. Chem. Soc.*, 1998, **120**, 4334–4344.
- 213 F. Liu, A. Giubellino, P. C. Simister, W. Qian, M. C. Giano, S. M. Feller, D. P. Bottaro and T. R. Burke, *Biopolymers*, 2011, **96**, 780–788.
- 214 A. K. Boal, I. Guryanov, A. Moretto, M. Crisma, E. L. Lanni, C. Toniolo, R. H. Grubbs and D. J. O'Leary, *J. Am. Chem. Soc.*, 2007, **129**, 6986–6987.
- 215 Ø. Jacobsen, J. Klaveness, O. Petter Ottersen, M. R. Amiry-Moghaddam and P. Rongved, *Org. Biomol. Chem.*, 2009, **7**, 1599–1611.
- 216 T. K. Pham and Y. W. Kim, *Bioorg. Chem.*, 2020, **101**, 104024.



- 217 P. Martín-Gago, R. Ramón, E. Aragón, J. Fernández-Carneado, P. Martín-Malpartida, X. Verdager, P. López-Ruiz, B. Colás, M. A. Cortes, B. Ponsati, M. J. Macias and A. Riera, *Bioorg. Med. Chem. Lett.*, 2014, **24**, 103–107.
- 218 B. Van Lierop, S. Chee Ong, A. Belgi, C. Delaine, S. Andrikopoulos, N. L. Haworth, J. G. Menting, M. C. Lawrence, A. J. Robinson and B. E. Forbes, *Sci. Rep.*, 2017, **7**, 17239.
- 219 Ø. Jacobsen, J. Klaveness and P. Rongved, *Molecules*, 2010, **15**, 6638–6677.
- 220 G. H. Bird, N. Madani, A. F. Perry, A. M. Princiotto, J. G. Supko, X. He, E. Gavathiotis, J. G. Sodroski, L. D. Walensky and S. H. Snyder, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 14093–14098.
- 221 M. A. Hossain, L. M. Haugaard-Kedström, K. J. Rosengren, R. A. D. Bathgate and J. D. Wade, *Org. Biomol. Chem.*, 2015, **13**, 10895–10903.
- 222 Z. Amso, R. Kowalczyk, Y.-E. Park, M. Watson, J.-M. Lin, D. S. Musson, J. Cornish and M. A. Brimble, *Org. Biomol. Chem.*, 2016, **14**, 6231–6243.
- 223 J. M. Song, E. E. Gallagher, A. Menon, L. D. Mishra and A. L. Garner, *Org. Biomol. Chem.*, 2019, **17**, 6414–6419.
- 224 B. Van Lierop, S. Chee Ong, A. Belgi, C. Delaine, S. Andrikopoulos, N. L. Haworth, J. G. Menting, M. C. Lawrence, A. J. Robinson and B. E. Forbes, *Sci. Rep.*, 2017, **7**, 1–13.
- 225 P. M. Cromm, J. Spiegel and T. N. Grossmann, *ACS Chem. Biol.*, 2015, **10**, 1362–1375.
- 226 S. J. Miller, H. E. Blackwell and R. H. Grubbs, *J. Am. Chem. Soc.*, 1996, **118**, 9606–9614.
- 227 R. J. Platt, T. S. Han, B. R. Green, M. D. Smith, J. Skalicky, P. Gruszczynski, H. S. White, B. Olivera, G. Bulaj and J. Gajewiak, *J. Biol. Chem.*, 2012, **287**, 20727–20736.
- 228 Y.-W. Kim, P. S. Kutchukian and G. L. Verdine, *Org. Lett.*, 2010, **12**, 3046–3049.
- 229 M. N. Islam, M. S. Islam, M. A. Hoque, T. Kato, N. Nishino, A. Ito and M. Yoshida, *Bioorg. Med. Chem.*, 2014, **22**, 3862–3870.
- 230 A. M. Heapy, G. M. Williams, J. D. Fraser and M. A. Brimble, *Org. Lett.*, 2012, **14**, 878–881.
- 231 J. F. Reichwein, B. Wels, J. A. W. Kruijtzter, C. Versluis and R. M. J. Liskamp, *Angew. Chem., Int. Ed.*, 1999, **38**, 3684–3687.
- 232 J. F. Reichwein, C. Versluis and R. M. J. Liskamp, *J. Org. Chem.*, 2000, **65**, 6187–6195.
- 233 J. Lawrence, M. Jourdan, Y. Vallée and V. Blandin, *Org. Biomol. Chem.*, 2008, **6**, 4575–4581.
- 234 F. Liu, A. G. Stephen, A. A. Waheed, E. O. Freed, R. J. Fisher and T. R. Burke, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 318–321.
- 235 W. J. Fang, T. F. Murray and J. V. Aldrich, *Bioorg. Med. Chem.*, 2018, **26**, 1157–1161.
- 236 S. A. Gisemba and J. V. Aldrich, *J. Org. Chem.*, 2020, **85**, 1407–1415.
- 237 U. Kazmaier, C. Hebach, A. Watzke, S. Maier, H. Mues and V. Huch, *Org. Biomol. Chem.*, 2005, **3**, 136–145.
- 238 I. W. Hamley, G. Cheng, V. Castelletto, S. Handschin and R. Mezzenga, *Chem. Commun.*, 2012, **48**, 3757–3759.
- 239 S. A. Cochrane, Z. Huang and J. C. Vederas, *Org. Biomol. Chem.*, 2013, **11**, 630–639.
- 240 J. L. Stymiest, B. F. Mitchell, S. Wong and J. C. Vederas, *Org. Lett.*, 2003, **5**, 47–49.
- 241 F. J. Dekker, N. J. De Mol, M. J. E. Fischer, J. Kemmink and R. M. J. Liskamp, *Org. Biomol. Chem.*, 2003, **1**, 3297–3303.
- 242 S. L. Mangold, D. J. O'Leary and R. H. Grubbs, *J. Am. Chem. Soc.*, 2014, **136**, 12469–12478.
- 243 C. Xu, X. Shen and A. H. Hoveyda, *J. Am. Chem. Soc.*, 2017, **139**, 10919–10928.
- 244 K. M. Dawood and K. Nomura, *Adv. Synth. Catal.*, 2021, **363**, 1970–1997.
- 245 M. Poirier, N. Aubry, C. Boucher, J.-M. Ferland, S. Laplante and Y. S. Tsantrizos, *J. Org. Chem.*, 2005, **70**, 10765–10773.
- 246 N. K. Yee, V. Farina, I. N. Houpi, N. Haddad, R. P. Frutos, F. Gallou, X.-J. Wang, X. Wei, R. D. Simpson, X. Feng, V. Fuchs, Y. Xu, J. Tan, L. Zhang, J. Xu, L. L. Smith-Keenan, J. Vitous, M. D. Ridges, E. M. Spinelli, M. Johnson, K. Donsbach, T. Nicola, M. Brenner, E. Winter, P. Kreye and W. Samstag, *J. Org. Chem.*, 2006, **71**, 7133–7145.
- 247 E. C. Gleeson, W. R. Jackson and A. J. Robinson, *Chem. Commun.*, 2017, **53**, 9769–9772.
- 248 K. Skowerski, G. Szczepaniak, C. Wierzbicka, Ł. Guśajski, M. Ś Bieniek and K. Grela, *Catal. Sci. Technol.*, 2012, **2**, 2424–2427.
- 249 S. Masuda, S. Tsuda and T. Yoshiya, *Org. Biomol. Chem.*, 2018, **16**, 9364–9367.
- 250 A. Fürstner, *Angew. Chem., Int. Ed.*, 2013, **52**, 2794–2819.
- 251 B. Aguilera, L. B. Wolf, P. Nieczypor, F. P. J. T. Rutjes, H. S. Overkleeft, J. C. M. van Hest, H. E. Schoemaker, B. Wang, J. C. Mol, A. Fürstner, M. Overhand, G. A. van der Marel and J. H. van Boom, *J. Org. Chem.*, 2001, **66**, 3584–3589.
- 252 M. Ijsselstijn, B. Aguilera, G. A. Van Der Marel, J. H. Van Boom, F. L. Van Delft, H. E. Schoemaker, H. S. Overkleeft, F. P. J. T. Rutjes and M. Overhand, *Tetrahedron Lett.*, 2004, **45**, 4379–4382.
- 253 N. Ghalit, A. J. Poot, A. Fürstner, D. T. S. Rijkers and R. M. J. Liskamp, *Org. Lett.*, 2005, **7**, 2961–2964.
- 254 P. M. Cromm, S. Schaubach, J. Spiegel, A. Fürstner, T. N. Grossmann and H. Waldmann, *Nat. Commun.*, 2016, **7**, 11300.
- 255 P. M. Cromm, K. Wallraven, A. Glas, D. Bier, A. Fürstner, C. Ottmann and T. N. Grossmann, *ChemBioChem*, 2016, **17**, 1915–1919.
- 256 K. R. Campos, D. Cai, M. Journet, J. J. Kowal, R. D. Larsen and P. J. Reider, *J. Org. Chem.*, 2001, **66**, 3634–3635.
- 257 A. Fürstner and K. Radkowski, *Chem. Commun.*, 2002, 2182–2183.
- 258 H. C. Kolb, M. G. Finn and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2001, **40**, 2004–2021.
- 259 C. W. Tornøe, C. Christensen and M. Meldal, *J. Org. Chem.*, 2002, **67**, 3057–3064.
- 260 V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chem.*, 2002, **114**, 2708–2711.



- This journal is © The Royal Society of Chemistry 2021

- 306 G. J. J. Richelle, M. Schmidt, H. Ippel, T. M. Hackeng, J. H. van Maarseveen, T. Nuijens and P. Timmerman, *ChemBioChem*, 2018, **19**, 1934–1938.
- 307 D. E. Streefkerk, M. Schmidt, J. H. Ippel, T. M. Hackeng, T. Nuijens, P. Timmerman and J. H. Van Maarseveen, *Org. Lett.*, 2019, **21**, 2095–2100.
- 308 M. Empting, O. Avrutina, R. Meusinger, S. Fabritz, M. Reinwarth, M. Biesalski, S. Voigt, G. Buntkowsky and H. Kolmar, *Angew. Chem., Int. Ed.*, 2011, **50**, 5207–5211.
- 309 A. Knuhtsen, C. Whitmore, F. S. Mcwhinnie, L. McDougall, R. Whiting, B. O. Smith, C. M. Timperley, A. C. Green, K. I. Kinnear and A. G. Jamieson, *Chem. Sci.*, 2019, **10**, 1671–1676.
- 310 A. M. White, S. J. de Veer, G. Wu, P. J. Harvey, K. Yap, G. J. King, J. E. Swedberg, C. K. Wang, R. H. P. Law, T. Durek and D. J. Craik, *Angew. Chem., Int. Ed.*, 2020, **59**, 11273–11277.
- 311 L. Zhang, X. Chen, P. Xue, H. H. Y. Sun, I. D. Williams, K. B. Sharpless, V. V. Fokin and G. Jia, *J. Am. Chem. Soc.*, 2005, **127**, 15998–15999.
- 312 M. M. Majireck and S. M. Weinreb, *J. Org. Chem.*, 2006, **71**, 8680–8683.
- 313 A. Tam, U. Arnold, M. B. Soellner and R. T. Raines, *J. Am. Chem. Soc.*, 2007, **129**, 12670–12671.
- 314 B. C. Boren, S. Narayan, L. K. Rasmussen, L. Zhang, H. Zhao, Z. Lin, G. Jia and V. V. Fokin, *J. Am. Chem. Soc.*, 2008, **130**, 8923–8930.
- 315 O. Kracker, J. Góra, J. Krzciuk-Gula, A. Marion, B. Neumann, H. G. Stammler, A. Nieß, I. Antes, R. Latajka and N. Sewald, *Chem. – Eur. J.*, 2018, **24**, 953–961.
- 316 M. R. Krause, R. Goddard and S. Kubik, *J. Org. Chem.*, 2011, **76**, 7084–7095.
- 317 J. Zhang, J. Kemmink, D. T. S. Rijkers and R. M. J. Liskamp, *Org. Lett.*, 2011, **13**, 3438–3441.
- 318 J. Zhang, J. Kemmink, D. T. S. Rijkers and R. M. J. Liskamp, *Chem. Commun.*, 2013, **49**, 4498–4500.
- 319 S. Tomassi, A. M. Trotta, C. Ieranò, F. Merlino, A. Messere, G. Rea, F. Santoro, D. Brancaccio, A. Carotenuto, V. M. D'Amore, F. S. Di Leva, E. Novellino, S. Cosconati, L. Marinelli, S. Scala and S. Di Maro, *Chem. – Eur. J.*, 2020, **26**, 10113–10125.
- 320 J. K. Pokorski, L. M. M. Jenkins, H. Feng, S. R. Durell, Y. Bai and D. H. Appella, *Org. Lett.*, 2007, **9**, 2381–2382.
- 321 M. Tischler, D. Nasu, M. Empting, S. Schmelz, D. W. Heinz, P. Rottmann, H. Kolmar, G. Buntkowsky, D. Tietze and O. Avrutina, *Angew. Chem., Int. Ed.*, 2012, **51**, 3708–3712.
- 322 Ahsanullah, P. Schmieder, R. Kühne and J. Rademann, *Angew. Chem., Int. Ed.*, 2009, **48**, 5042–5045.
- 323 Ahsanullah and J. Rademann, *Angew. Chem., Int. Ed.*, 2010, **49**, 5378–5382.
- 324 L. Reguera and D. G. Rivera, *Chem. Rev.*, 2019, **119**, 9836–9860.
- 325 A. Demharther, W. Hörl, E. Herdtweck and I. Ugi, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 173–175.
- 326 C. Hebach and U. Kazmaier, *Chem. Commun.*, 2003, 596–597.
- 327 O. E. Vercillo, C. K. Z. Andrade and L. A. Wessjohann, *Org. Lett.*, 2008, **10**, 205–208.
- 328 A. Dömling and I. Ugi, *Angew. Chem., Int. Ed.*, 2000, **39**, 3168–3210.
- 329 R. Hili, V. Rai and A. K. Yudin, *J. Am. Chem. Soc.*, 2010, **132**, 2889–2891.
- 330 A. P. Treder, J. L. Hickey, M. C. J. Tremblay, S. Zaretsky, C. C. G. Scully, J. Mancuso, A. Doucet, A. K. Yudin and E. Marsault, *Chem. – Eur. J.*, 2015, **21**, 9249–9255.
- 331 A. V. Vasco, C. S. Pérez, F. E. Morales, H. E. Garay, D. Vasilev, J. A. Gavín, L. A. Wessjohann and D. G. Rivera, *J. Org. Chem.*, 2015, **80**, 6697–6707.
- 332 F. E. Morales, H. E. Garay, D. F. Muñoz, Y. E. Augusto, A. J. Otero-González, O. Reyes Acosta and D. G. Rivera, *Org. Lett.*, 2015, **17**, 2728–2731.
- 333 A. V. Vasco, Y. Méndez, A. Porzel, J. Balbach, L. A. Wessjohann and D. G. Rivera, *Bioconjugate Chem.*, 2019, **30**, 253–259.
- 334 M. G. Ricardo, F. E. Morales, H. Garay, O. Reyes, D. Vasilev, L. A. Wessjohann and D. G. Rivera, *Org. Biomol. Chem.*, 2015, **13**, 438–446.
- 335 M. G. Ricardo, A. M. Ali, J. Plewka, E. Surmiak, B. Labuzek, C. G. Neochoritis, J. Atmaj, L. Skalniak, R. Zhang, T. A. Holak, M. Groves, D. G. Rivera and A. Dömling, *Angew. Chem., Int. Ed.*, 2020, **59**, 5235–5241.
- 336 B. K. W. Chung, J. L. Hickey, C. C. G. Scully, S. Zaretsky and A. K. Yudin, *MedChemComm*, 2013, **4**, 1124–1128.
- 337 T. M. Vishwanatha, E. Bergamaschi and A. Dömling, *Org. Lett.*, 2017, **19**, 3195–3198.
- 338 M. C. Morejón, A. Laub, B. Westermann, D. G. Rivera and L. A. Wessjohann, *Org. Lett.*, 2016, **18**, 4096–4099.
- 339 N. A. Petasis, I. A. Zavialov and L. Angeles, *J. Am. Chem. Soc.*, 1997, **7863**, 445–446.
- 340 M. G. Ricardo, J. F. Marrero, O. Valdés, D. G. Rivera and L. A. Wessjohann, *Chem. – Eur. J.*, 2019, **25**, 769–774.

