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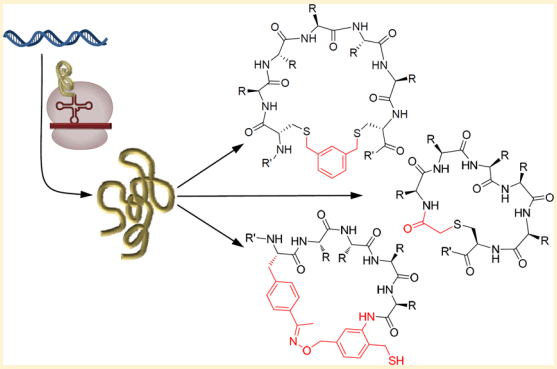
Emerging Strategies to Access Peptide Macrocycles from Genetically Encoded Polypeptides

Jessica M. Smith, John R. Frost, and Rudi Fasan*

Department of Chemistry, University of Rochester, Rochester, New York 14627, United States

ABSTRACT: Macrocyclic peptides have emerged as attractive molecular scaffolds for the development of chemical probes and therapeutics. In this synopsis, we highlight contemporary strategies to access peptide macrocycles from ribosomally produced polypeptides. Challenges that have been tackled in this area involve orchestrating the desired macrocyclization process in the presence of unprotected polypeptide precursors and expanding the functional space encompassed by these molecules beyond that of canonical amino acid structures. Applications of these methodologies for the discovery of bioactive molecules are also discussed.



Macrocyclic peptides have recently attracted increasing attention as molecular scaffolds for the development of therapeutics and chemical probes to interrogate biological systems.[1−4] Interest in this structural class has been stimulated by the biomedical value and breadth of biological activities presented by macrocyclic peptides isolated from nature, including cyclosporine A (immunosuppressant), caspofungin (antifungal), and polymixin (antibiotic).[5] Furthermore, conformational restriction of peptidic structures via backbone/side-chain cyclization has often resulted in enhanced protein binding affinity,[6,7] selectivity,[8] membrane permeability,[9−11] and/or proteolytic stability[12,13] over linear peptides, all of which are desirable features in the context of in vivo applications and therapeutic development.

Important advances in peptide cyclization strategies have enriched the portfolio of synthetic methods to afford macrocyclic peptides.[14] Parallel efforts have focused on developing methodologies to access peptide macrocycles via chemical modification and/or engineering of ribosomally produced polypeptides, which constitutes the object of this synopsis. Research in this area is largely driven by the potential advantages the latter approaches can provide over purely synthetic methods. Among these, there is the high combinatorial potential inherent to the ribosomal synthesis of genetically encoded polypeptides, which enables the rapid generation of vast chemical libraries (10^7−10^{12} members). In addition, these methods may be coupled to powerful, high-throughput platforms (e.g., yeast, phage, mRNA display) or genetic selection systems for the rapid functional screening of these libraries. Finally, there is the ease by which these libraries can be deconvoluted to elucidate the structure of the members that exhibit the desired functional properties, namely via sequencing of the peptide-encoding gene. At the same time, challenges in this area are concerned with (a) the need to orchestrate the desired macrocyclization in the presence of fully unprotected polypeptides (and other biomolecules) and (b) the desire to overcome the limitations imposed by the restricted building block repertoire given by the 20 canonical amino acids. As discussed below, a number of creative approaches have been devised over the past few years to achieve this goal and tackle these challenges. These include the polypeptide-driven or chemically induced cyclization of linear precursor polypeptides, cyclopeptide synthesis through in vitro translation and genetic code reprogramming methods, and the creation of hybrid organo-peptide macrocycles via embedding of synthetic scaffolds into genetically encoded peptidic backbones. Exemplary structures of peptide-based macrocycles accessible through these methodologies are provided in Figure 1. Representative applications of these strategies toward the discovery of bioactive compounds are also discussed.

■ PEPTIDE MACROCYCLES VIA CHEMICAL CROSS-LINKING

The first strategy introduced to generate ribosomal libraries of constrained peptides has involved rigidifying genetically randomized peptide sequences via a disulfide bridge. This approach has been successfully applied in combination with phage display[15] to identify binders for a variety of protein targets.[16−21] A major advantage of the strategy is its technical simplicity, as the structural elements necessary for peptide cylization are defined at the genetic level. An inherent drawback, however, is the chemical instability of the disulfide bond which remains susceptible to exchange and/or reduction in vivo. Thus, functional/isosteric replacement of this linkage (e.g., via dipeptide templates,[22,23] triazole groups,[24] thioether/

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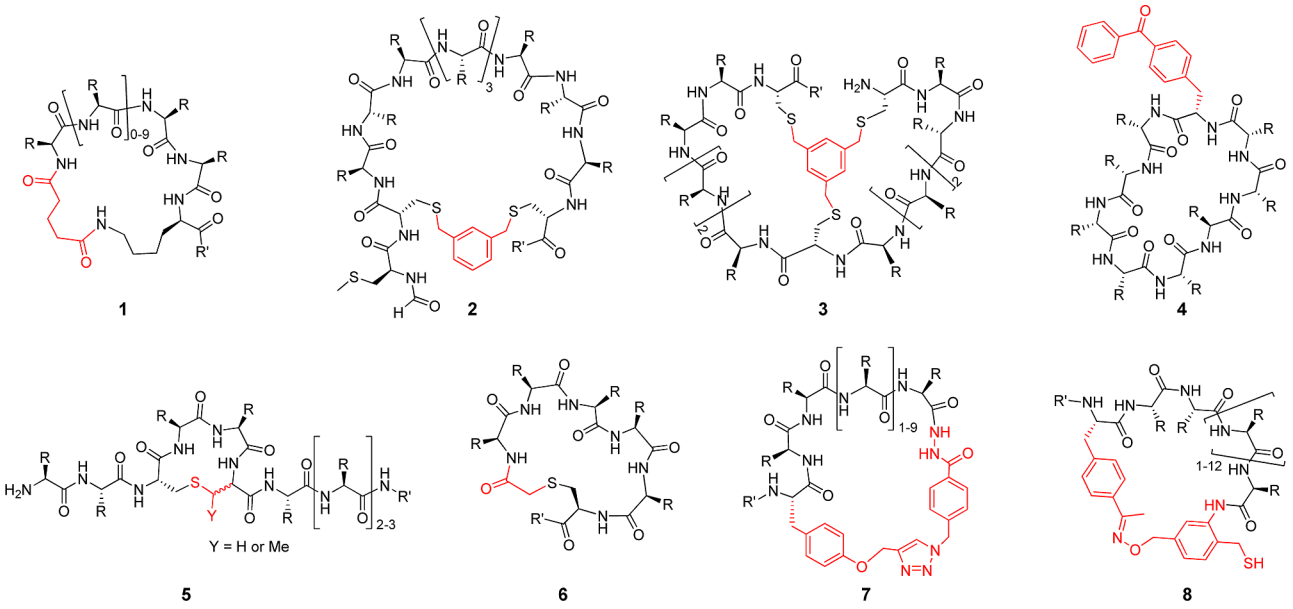

Figure 1. Representative macrocyclic peptide structures obtained via the methodologies discussed in the text, with nonproteinogenic moieties highlighted in red.

alkyl linkers[23,25]) has been typically necessary to convert these disulfide-constrained peptide ligands into more stable counterparts.

To overcome this issue, researchers have investigated amine- or thiol-reactive cross-linking reagents to constrain ribosomal peptides via redox-stable covalent linkages. A first example was provided by Roberts and co-workers, who demonstrated the formation of cyclic peptides of structure **1** by exposing mRNA-displayed peptides (Met-$(AA)_n$-Lys-mRNA; n = 3−11) to the diacylating reagent disuccinimidyl glutarate (DSG).[26] DSG cross-links the N-terminal amino group with the side-chain amino group from a fixed lysine at the C-terminus of the target sequence. Cyclization efficiency was found to depend on the length of the intervening amino acid sequence, decreasing from 55% for pentamer sequences (n = 3) to 31% for 13mer sequences (n = 11).[26] Using this chemistry in conjunction with mRNA display,[27] the group later constructed and screened a 10^{12}-member dodecamer macrocycle library (M$(AA)_{10}$K) for binders to the signaling protein Gαi1, from which a high-affinity Gαi1-targeting cyclopeptide (cycGiBP, K_D = 2.1 nM) was successfully isolated.[28] Compared to its linear counterpart, cycGiBP exhibits a 15-fold higher affinity and 3-fold longer half-life in the presence of proteases, demonstrating the importance of the conformational constraint for these properties.

Other groups have exploited cysteine-reactive cross-linking reagents for constructing macrocyclic peptide libraries.[29−31] Studies by Timmerman and co-workers provided a methodological basis for this approach, showing how synthetic peptides containing two and three cysteine residues undergo rapid and efficient cyclization (85−95% yield, 15 min) upon reaction with 1,3-bis- and 1,3,5-tris(bromomethyl)aryl compounds to yield mono- or bicyclic peptides, respectively.[32]

Application of this cyclization strategy in the context of ribosomally produced peptides was first demonstrated by the Szostak group,[29] which also exploited this method to generate macrocyclic peptides of structure **2** containing unnatural amino acids as discussed below.[30] In another example, Winter and Heinis utilized a trifunctional cysteine-reactive reagent to create a large library (>10^9 members) of bicyclic peptides of structure **3** displayed on the surface of M13 phage particles.[31] Here, randomized polypeptides with intercalating cysteines (Cys-$(Xxx)_6$-Cys-$(Xxx)_6$-Cys) were fused to a cysteine-free variant of phage pIII protein and cyclized upon incubation with 1,3,5-tris(bromomethyl)benzene (TBMB). Upon library panning, a very potent inhibitor of human plasma protease kallikrein (PK15, K_i = 1.5 nM) was isolated. Although the bicyclic peptide was found by NMR to be devoid of a well-defined structure in solution, the 6000-fold higher IC_{50} exhibited by its acyclic version clearly supported the critical role of the organic linker for function. In a subsequent study,[33] screening of the bicyclic peptide library yielded a nanomolar inhibitor (K_i = 53 nM) of the human urokinase-type plasminogen activator. Also in this case, the bicyclic structure was crucial for full enzyme inhibitory activity, as indicated by the 7- and 320-fold higher K_i value displayed by a monocyclic and linear variants of the peptide, respectively. X-ray crystallography revealed that an extended contact interface (700 Å^2) and 14 intermolecular H-bonds lie at the basis of the tight peptide/enzyme interaction.[33]

Overall, the aforementioned cross-linking strategies provide a straightforward approach to cyclization of ribosomal peptide sequences. An absolute requirement of these methods is the use of reagents with a 2-fold (or 3-fold) rotational symmetry to avoid the formation of multiple regioisomers upon (bi)-cyclization. While this aspect limits to a certain extent the spectrum of organic scaffolds amenable to these strategies. Several reagents that meet this requirement can be envisioned, as shown in a recent contribution from Heinis and co-workers, in which two reagents alternative to TBMB were applied to obtain PK15 structural analogues.[34]

"NATURAL PRODUCT-LIKE" PEPTIDE MACROCYCLES

Other groups have focused on developing methods to generate macrocyclic peptides that reproduce ring topologies found in natural cyclopeptides. A notable contribution in this area is the SICLOPPS method, reported by Benkovic and co-workers, to produce head-to-tail cyclopeptides.[35] Here, a target peptide sequence is introduced between the C-terminal (In_C) and the

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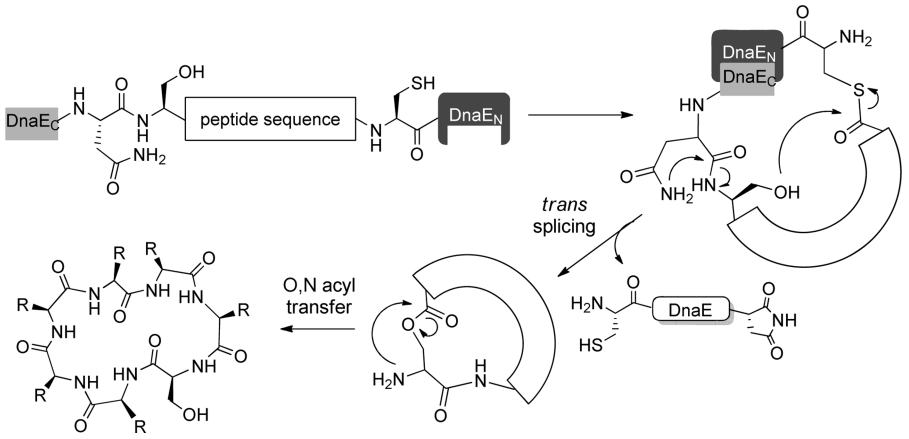

Figure 2. Cyclopeptide formation via split-intein-mediated splicing (SICLOPPS).

N-terminal (In_N) domain of natural split intein DnaE. Upon expression, the In_C and In_N domains associate, resulting in a trans-splicing event leading to the formation of a cyclopeptide encompassing the target sequence (Figure 2).[35] Although sequence-dependent factors were found to largely affect both the efficiency of peptide cyclization and the rate of this process in vivo,[36] a key advantage of this strategy is that the cyclopeptide products can be produced directly inside a living cell. As such, SICLOPPS library generation could be coupled to genetic selection[37−39] or intracellular reporter systems[40] to identify cyclopeptide inhibitors for a variety of target proteins/enzymes, including ribonucleotide reductase (IC_{50} = 2 μM),[37] AICAR transformylase (K_i = 17 μM),[41] ClpXP protease (K_i = 8 μM),[40] Dam methyltransferase (IC_{50} = 50 μM),[38] and HIV Gag protein (IC_{50} = 7 μM).[42] In all these cases, linearization of these compounds resulted in a 2- to 100-fold reduction in potency, which demonstrated the functional advantage of the cyclic backbone. More recently, the functional space accessible in SICLOPPS libraries has been expanded via the incorporation of unnatural amino acids using amber stop codon suppression with evolved tRNA/tRNA-aminoacyl synthetases (AARSs).[43] In this study,[39] *p*-benzoylphenylalanine (*p*BzF)-containing cyclopeptides (structure **4**) capable of inhibiting HIV protease were discovered (IC_{50} = 0.85−1 μM). Interestingly, *p*BzF played a crucial role in enzyme inhibition, engaging Lys14 in the protease in a destabilizing covalent imine bond.

Methods to generate macrocyclic peptides reproducing the structural features of lantipeptides (structure **5**) have also been investigated.[44−46] Lantipeptides are natural polycyclic peptides characterized by the presence of dehydroamino acids (dehydroalanine (Dha); dehydrobutyrine (Dhb)) and (methyl)lanthionine bridges, which are formed during biosynthesis via enzyme-assisted Michael addition of cysteine thiols onto Dha or Dhb residues generated via enzymatic dehydration of serine or threonine.[47,48]

In a first approach, Suga and co-workers utilized codon reprogramming/in vitro translation to incorporate vinylglycine into two short peptide sequences encompassing the B- and C-ring of the lantibiotic nisin. The desired methyllanthionine-containing peptides were then obtained via heat-induced (95 °C) isomerization of vinylglycine into (*Z*)-dehydrobutyrine, followed by an intramolecular attack by a cysteine within the sequence.[44] An alternative strategy was reported by the Szostak group (Figure 3).[45,46] Here, structural mimics of lysine (4-seleno-Lys) and isoleucine (4-seleno-Ile) were incorporated

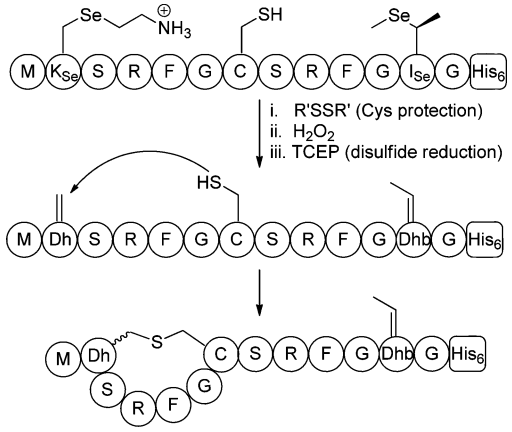

Figure 3. Synthesis of lantipeptide-like macrocyclic peptides.

into a precursor polypeptide via in vitro translation and then converted into Dha and (*E*)-Dhb, respectively, via H_2O_2-induced oxidative β-elimination. Lantipeptides containing one or two lanthionine bridges were then obtained by virtue of the higher reactivity of Dha to cysteine-mediated β-addition over Dhb.[45] Compared to the former, the latter approach involves milder reaction conditions, but it requires protection/deprotection of the cysteines to prevent their oxidation during Dha/Dhb formation. In contrast to lantipeptide biosynthesis,[47,48] neither of the methods provides stringent control on the stereoselectivity of (methyl)lanthionine bond formation and thus mixtures of stereoisomeric products are typically obtained. This notwithstanding, Seebeck et al. recently demonstrated the possibility to combine the latter method with mRNA display to discover bioactive lantipeptides.[46] Here, screening of a 10^{11}-member library of lanthionine (Lan)-constrained peptides enabled the isolation of a low-micromolar binder for Sortase A (LWY-Lan-LS-Lan-WGRI; K_d = 3 μM). Although this molecule failed to inhibit the enzyme, binding studies revealed the critical importance of the Lan bridge and of its stereochemical configuration for interaction with the protein.[46]

IN VITRO SYNTHESIS OF UAA-CONTAINING PEPTIDE MACROCYCLES

In vitro translation[49] methods in combination with natural amino acid analogues[50,51] or artificially made aminoacylated-tRNAs[52−54] have provided a viable route to incorporate

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multiple unnatural amino acids (UAAs) into ribosomal peptides. Combining these procedures with cyclization strategies have recently enabled the production of cyclopeptides containing various nonproteinogenic amino acids such as N-methylated or α,α-disubstituted amino acids.[51,53,55]

In recent work, Szostak and co-workers reported the in vitro translation of a 10^{10}-member library of highly modified cyclopeptides with sequence Met-Cys-$(X)_{10}$-Cys-mRNA, where 12 of the 20 natural amino acids were substituted with structural analogs, including alkyne-, thiazolidine-, and α,α-cyclopentyl-containing amino acids and halogenated Tyr/Phe derivatives.[30] These peptides were then cyclized via 1,3-dibromomethyl-benzene-mediated cross-linking of the two flanking cysteines. Screening of the mRNA-display library enabled the selection of a potent thrombin inhibitor (U1, K_i = 20 nM) containing four unnatural amino acids. Replacement of these UAAs with canonical ones resulted in negligible enzyme binding whereas a linear analogue of U1 exhibited a 78-fold lower binding affinity, highlighting the functional importance of the UAA constituents and cyclic backbone, respectively.[30]

Utilizing in vitro translation systems supplemented with artificial aminoacylated tRNAs, Suga and co-workers reported various strategies for ribosomal synthesis of cyclic peptides.[56] In one case, peptides constrained by an inter-side-chain thioether linkage were obtained via ribosomal incorporation of 4-(2-chloroacetyl)aminobutyric acid (Cab) into a cysteine-containing peptide followed by spontaneous cysteine-mediated substitution of Cab α-halo amide moiety.[57] This strategy was subsequently combined with Cu^{I}-catalyzed azide−alkyne cycloaddition to yield bicyclic peptides.[58] Exploiting a similar chemistry, head-to-side-chain cyclopeptides (4−14 residues) of structure **6** could be efficiently obtained by using an initiator tRNA ($tRNA^{fMet}_{CAU}$) precharged with *N*-(2-chloroacetyl)-Phe in a reconstituted translation system lacking methionine.[59] This cyclization approach was later applied in conjunction with mRNA display to isolate inhibitors of ubiquitin ligase E6AP.[55] Here, a 10^{12}-member library of 10-mer to 15-mer macrocyclic peptides containing a D-Trp and various *N*-methyl-AAs was prepared by initiating the translated peptide sequence with *N*-(2-chloroacetyl)-D-tryptophan ($ClAc^{D}W$) and reassigning four codons to N-methylated Phe, Gly, Ser, and Ala, followed by cyclization via attack of a C-terminal cysteine onto $ClAc^{D}W$. From library screening, a potent binder of E6AP was isolated (K_D: 0.6 nM), which was capable of inhibiting p53 ubiquitinylation in vivo. The N-methylated backbone and cyclic structure of the peptide were found to be essential for enzyme binding.[55]

Inspired by a previously reported peptide ligation methodology,[60] the Suga group recently developed another interesting strategy for in vitro synthesis of head-to-tail cyclopeptides, which involves the attack of the peptide N-terminal amine onto a C-terminal diketopiperazine thioester formed via spontaneous rearrangement of a Cys-Pro-glycolic acid moiety (Figure 4).[61] This protocol is compatible with 14 different amino acids at the N-terminal ligation point, as dictated by the specificity of the enzymes (peptide deformylase and methionine aminopeptidase) required to unmask the N-terminal amino group prior to cyclization.[61] This methodology was subsequently extended to enable the synthesis of cyclopeptides containing γ-amino acids via initiator tRNAs charged with a Phe-γ-AA dipeptide.[62] Cyclization efficiency was affected in this case by the type of γ-amino acid employed, proceeding well in the presence of unhindered residues but being compromised when large groups

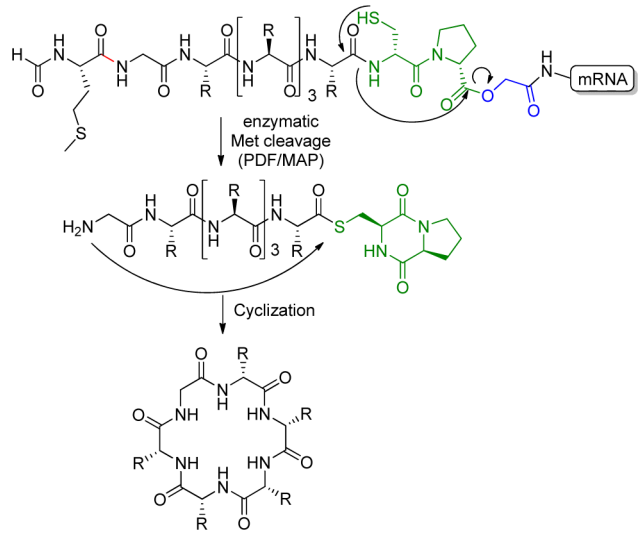

Figure 4. Head-to-tail cyclopeptide formation via diketopiperazine thioester-mediated cyclization.

at the γ-carbon were present.[62] The requirement for the C-terminal glycolic acid moiety could be eliminated by adopting conditions that promote peptidyl-tRNA drop-off during in vitro translation, with the C-terminal ester-linked tRNA serving as the leaving group to mediate diketopiperazine-thioester formation.[63] Finally, successful formation of cyclopeptides via a $K_3Fe(CN)_6$-catalyzed oxidation of in vitro translated peptides containing a 5-hydroxy-indole and benzylamine moieties was also described.[64]

The in vitro translation strategies described above nicely complement each other (and amber stop codon suppression methods[43]) in expanding the pool of UAA structures amenable to ribosomal incorporation into peptides and cyclized variants thereof. In terms of relative advantages, the use of preformed aminoacylated-tRNAs is not subject to the recognition requirements and potential cross-reactivity of AARS enzymes,[51] but the need to prepare these reagents makes this approach less straightforward than the simple use of amino acid analogues during in vitro translation. Open challenges in this area include the decreasing yield of the desired peptide product as more UAAs are incorporated into the sequence[51,65] and the incompatibility of certain UAAs (e.g., β- and γ-amino acids) with peptide chain elongation at the ribosomal level.[51,62] Strategies to address these issues have yet begun to emerge,[62,66] and more are expected to appear in the future.

■ MACROCYCLIC ORGANO-PEPTIDE HYBRIDS

The aforementioned strategies have permitted the incorporation of various nonproteinogenic structures into macrocyclic peptides, but such structures have mainly consisted of UAA or amino acid analogues. Methodologies introduced by the Fasan group have recently enabled the synthesis of hybrid macrocycles in which a variety of synthetic, amino acid-unrelated scaffolds are embedded within a peptidic framework (structures **7** and **8**).[67−69] This capability provides the opportunity to modulate the topology, ring size, and functionalization pattern of the macrocyclic products through variation of the synthetic moiety of these molecules in addition to modification of the genetically encoded peptide sequence.

These so-called macrocyclic organo-peptidic hybrids (MOrPHs) are generated via a dual, bio-orthogonal ligation

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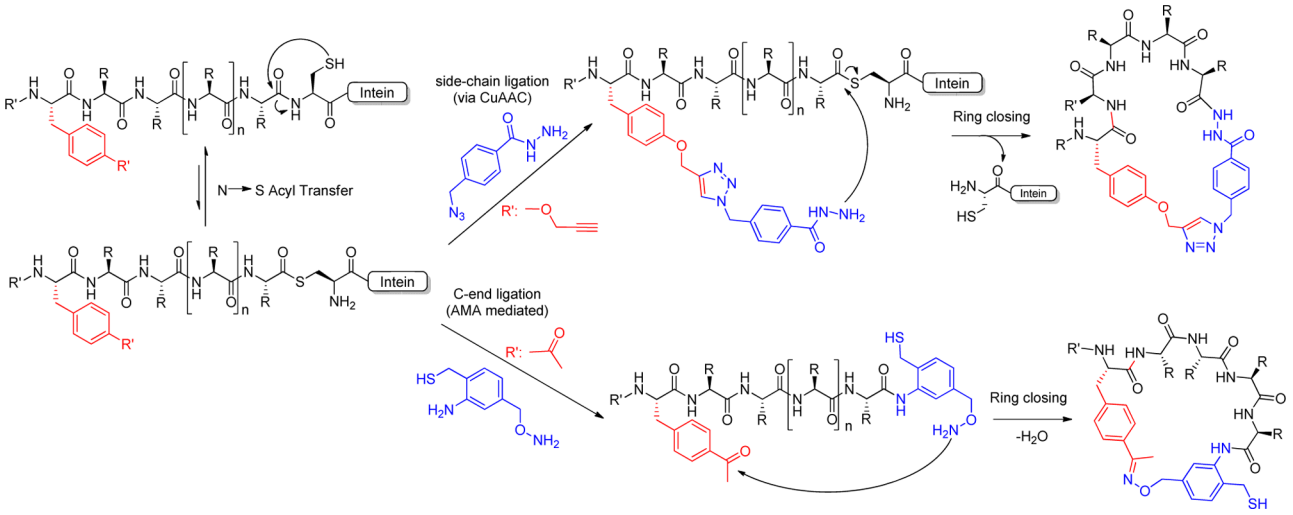

Figure 5. Synthesis of macrocyclic organo-peptide hybrids (MOrPHs).

between bifunctional synthetic precursors (SPs) and recombinant protein precursors (BPs), in which a variable peptide target sequence is framed between an unnatural amino acid and an engineered intein (GyrA) lacking C-terminal splicing ability. In a first implementation of this concept, azide/hydrazide-based SPs were used in combination with protein precursors bearing an alkyne-containing tyrosine derivative (OpgY) to generate macrocycles via a side-chain Cu^{I}-catalyzed azide/alkyne cycloaddition (CuAAC) followed by ring closure via SP-hydrazide attack onto the intein-thioester (Figure 5, top route).[67] This cyclization strategy proved viable across variable target sequence lengths (4mer to 12mer) and structurally different, phenyl/biphenyl-based SPs, resulting in rapid formation (2−3 h) of the desired MOrPHs as the predominant product (80−100%). More modest yields (50−60%) were observed in the presence of the 4mer target sequence and/or SPs with short azido/hydrazide distances (<6 Å), possibly due to increased conformational strain during cyclization. Furthermore, cyclic, lariat-shaped, and protein-fused MOrPHs were efficiently obtained through variation of the N-terminal tail preceding the UAA in the protein precursor.[67]

An alternative approach to access MOrPHs was later implemented that involves a dual oxime/intein-mediated ligation using an oxyamino/1,3-amino-thiol-aryl reagent and a biosynthetic precursor bearing a side-chain keto group provided by the UAA *p*-acetyl-Phe (Figure 5, bottom route).[68] Compared to the previous approach, this MOrPH-forming strategy does not require any catalyst. Moreover, organo-peptide macrocycles of varying size, i.e., encompassing 4−12 amino acid residues, were obtained in good yields (50−80%), with no byproducts, and within relatively short times (<5 h). Using this approach, MOrPH libraries with randomized 5mer and 8mer peptide sequences could be produced.[68]

The scope of this methodology was further investigated in a more recent study. Here, more conformationally rigid SPs containing the critical 2-amino-mercaptomethyl-aryl (AMA) moiety[68] were found to induce MOrPH formation across 4 to 15 amino acid long target sequences with even higher efficiency (80−95% yield in 5 h).[69] Systematic mutagenesis studies evidenced the importance of the amino acid residue preceding the intein-thioester for cyclization, with 12 out of the 20 possible substitutions at this site being compatible with MOrPH formation and a subset of them (Phe, Tyr, Ala, Thr) furnishing the highest overall yields (70−90%). Other interesting insights were gained from mechanistic investigation of MOrPH macrocyclization via CuAAC/hydrazide-mediated versus oxime/amino-thiol-mediated ligation. Whereas the former was determined to proceed exclusively via side-chain ligation followed by C-terminal ring closure,[67] the latter was found to involve primarily a C-end → side-chain tandem ligation mechanism.[69]

Overall, these studies introduced highly modular and bioorthogonal strategies to access hybrid peptide macrocycles, which are rather tolerant to structural variations in the synthetic/peptidic moieties.[69] These features are expected to facilitate future application toward the identification of bioactive macrocycles via the combinatorial assembly and screening of large libraries of diverse MOrPH structures.

■ CONCLUSIONS

As highlighted above, a variety of strategies have emerged over the past few years to enable the synthesis of conformationally constrained, macrocyclic peptides from ribosomally derived polypeptides. In particular, opportunities have been made available toward accessing different ring topologies, incorporating nonproteinogenic elements, and generating large collections of these compounds, which will aid in the search for novel functional entities. Owing to their macrocylic structure, medium size (500−2000 Da), and high functional/stereochemical density, molecules accessible through these methodologies occupy a highly relevant region of the chemical space, in between that encompassed by small molecules and biologics (e.g., antibodies).[1,3] These features make them particularly well suited to interact with extended biomolecular interfaces and thus address historically challenging targets in chemical biology and drug discovery, such as protein−protein and protein−nucleic acid interactions. Further development, application, and integration of these strategies with high-throughput screening platforms are therefore expected to open exciting opportunities in the future toward the discovery of biologically active molecules, in particular against targets which have so far remained elusive or inaccessible to conventional classes of probes and therapeutics.

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AUTHOR INFORMATION

Corresponding Author

*E-mail: fasan@chem.rochester.edu.

Notes

The authors declare no competing financial interest.

Biographies

Jessica Smith completed her undergraduate education at the Rochester Institute of Technology before joining the lab of Rudi Fasan for her graduate studies.

John Frost completed his undergraduate education at Canisius College before joining the lab of Rudi Fasan for his graduate studies.

Rudi Fasan received his Ph.D. in Prof. John Robinson's group at the University of Zurich and worked as a postdoctoral fellow in Prof. Frances Arnold's group at Caltech. He joined the faculty at the University of Rochester in 2008, and his research program focuses on the development and application of methodologies for synthesis of organo-peptide macrocycles for modulating protein−protein interactions and P450-mediated chemoenzymatic strategies for C−H functionalization in complex molecules.

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REFERENCES

(1) Driggers, E. M.; Hale, S. P.; Lee, J.; Terrett, N. K. *Nat. Rev. Drug Discov.* **2008**, *7*, 608.

(2) Obrecht, D.; Robinson, J. A.; Bernardini, F.; Bisang, C.; DeMarco, S. J.; Moehle, K.; Gombert, F. O. *Curr. Med. Chem.* **2009**, *16*, 42.

(3) Marsault, E.; Peterson, M. L. *J. Med. Chem.* **2011**, *54*, 1961.

(4) Katsara, M.; Tselios, T.; Deraos, S.; Deraos, G.; Matsoukas, M. T.; Lazoura, E.; Matsoukas, J.; Apostolopoulos, V. *Curr. Med. Chem.* **2006**, *13*, 2221.

(5) Nolan, E. M.; Walsh, C. T. *ChemBioChem* **2009**, *10*, 34.

(6) Tang, Y. Q.; Yuan, J.; Osapay, G.; Osapay, K.; Tran, D.; Miller, C. J.; Ouellette, A. J.; Selsted, M. E. *Science* **1999**, *286*, 498.

(7) Dias, R. L. A.; Fasan, R.; Moehle, K.; Renard, A.; Obrecht, D.; Robinson, J. A. *J. Am. Chem. Soc.* **2006**, *128*, 2726.

(8) Henchey, L. K.; Porter, J. R.; Ghosh, I.; Arora, P. S. *ChemBioChem* **2010**, *11*, 2104.

(9) Walensky, L. D.; Kung, A. L.; Escher, I.; Malia, T. J.; Barbuto, S.; Wright, R. D.; Wagner, G.; Verdine, G. L.; Korsmeyer, S. J. *Science* **2004**, *305*, 1466.

(10) Rezai, T.; Bock, J. E.; Zhou, M. V.; Kalyanaraman, C.; Lokey, R. S.; Jacobson, M. P. *J. Am. Chem. Soc.* **2006**, *128*, 14073.

(11) Rezai, T.; Yu, B.; Millhauser, G. L.; Jacobson, M. P.; Lokey, R. S. *J. Am. Chem. Soc.* **2006**, *128*, 2510.

(12) Fairlie, D. P.; Tyndall, J. D. A.; Reid, R. C.; Wong, A. K.; Abbenante, G.; Scanlon, M. J.; March, D. R.; Bergman, D. A.; Chai, C. L. L.; Burkett, B. A. *J. Med. Chem.* **2000**, *43*, 1271.

(13) Wang, D.; Liao, W.; Arora, P. S. *Angew. Chem., Int. Ed.* **2005**, *44*, 6525.

(14) White, C. J.; Yudin, A. K. *Nat. Chem.* **2011**, *3*, 509.

(15) Sidhu, S. S.; Lowman, H. B.; Cunningham, B. C.; Wells, J. A. *Methods Enzymol.* **2000**, *328*, 333.

(16) Wrighton, N. C.; Farrell, F. X.; Chang, R.; Kashyap, A. K.; Barbone, F. P.; Mulcahy, L. S.; Johnson, D. L.; Barrett, R. W.; Jolliffe, L. K.; Dower, W. J. *Science* **1996**, 458.

(17) DeLano, W. L.; Ultsch, M. H.; de Vos, A. M.; Wells, J. A. *Science* **2000**, *287*, 1279.

(18) Livnah, O.; Stura, E. A.; Johnson, D. L.; Middleton, S. A.; Mulcahy, L. S.; Wrighton, N. C.; Dower, W. J.; Jolliffe, L. K.; Wilson, I. A. *Science* **1996**, 464.

(19) Skelton, N. J.; Chen, Y. M.; Dubree, N.; Quan, C.; Jackson, D. Y.; Cochran, A.; Zobel, K.; Deshayes, K.; Baca, M.; Pisabarro, M. T.; Lowman, H. B. *Biochemistry* **2001**, *40*, 8487.

(20) Pasqualini, R.; Koivunen, E.; Ruoslahti, E. *J. Cell Biol.* **1995**, *130*, 1189.

(21) Meyer, S. C.; Gaj, T.; Ghosh, I. *Chem. Biol. Drug Des.* **2006**, *68*, 3.

(22) Dias, R. L.; Fasan, R.; Moehle, K.; Renard, A.; Obrecht, D.; Robinson, J. A. *J. Am. Chem. Soc.* **2006**, *128*, 2726.

(23) Foister, S.; Taylor, L. L.; Feng, J. J.; Chen, W. L.; Lin, A.; Cheng, F. C.; Smith, A. B.; Hirschmann, R. *Org. Lett.* **2006**, *8*, 1799.

(24) Empting, M.; Avrutina, O.; Meusinger, R.; Fabritz, S.; Reinwarth, M.; Biesalski, M.; Voigt, S.; Buntkowsky, G.; Kolmar, H. *Angew. Chem., Int. Ed.* **2011**, *50*, 5207.

(25) Pero, S. C.; Oligino, L.; Daly, R. J.; Soden, A. L.; Liu, C.; Roller, P. P.; Li, P.; Krag, D. N. *J. Biol. Chem.* **2002**, *277*, 11918.

(26) Millward, S. W.; Takahashi, T. T.; Roberts, R. W. *J. Am. Chem. Soc.* **2005**, *127*, 14142.

(27) Roberts, R. W.; Szostak, J. W. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 12297.

(28) Millward, S. W.; Fiacco, S.; Austin, R. J.; Roberts, R. W. *ACS Chem. Biol.* **2007**, *2*, 625.
(29) Seebeck, F. P.; Szostak, J. W. *J. Am. Chem. Soc.* **2006**, *128*, 7150.
(30) Schlippe, Y. V.; Hartman, M. C.; Josephson, K.; Szostak, J. W. *J. Am. Chem. Soc.* **2012**, *134*, 10469.
(31) Heinis, C.; Rutherford, T.; Freund, S.; Winter, G. *Nat. Chem. Biol.* **2009**, *5*, 502.
(32) Timmerman, P.; Beld, J.; Puijk, W. C.; Meloen, R. H. *ChemBioChem* **2005**, *6*, 821.
(33) Angelini, A.; Cendron, L.; Chen, S. Y.; Touati, J.; Winter, G.; Zanotti, G.; Heinis, C. *ACS Chem. Biol.* **2012**, *7*, 817.
(34) Chen, S.; Morales-Sanfrutos, J.; Angelini, A.; Cutting, B.; Heinis, C. *ChemBioChem* **2012**, *13*, 1032.
(35) Scott, C. P.; Abel-Santos, E.; Wall, M.; Wahnon, D. C.; Benkovic, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 13638.
(36) Scott, C. P.; Abel-Santos, E.; Jones, A. D.; Benkovic, S. J. *Chem. Biol.* **2001**, *8*, 801.
(37) Horswill, A. R.; Savinov, S. N.; Benkovic, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 15591.
(38) Naumann, T. A.; Tavassoli, A.; Benkovic, S. J. *ChemBioChem* **2008**, *9*, 194.
(39) Young, T. S.; Young, D. D.; Ahmad, I.; Louis, J. M.; Benkovic, S. J.; Schultz, P. G. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 11052.
(40) Cheng, L.; Naumann, T. A.; Horswill, A. R.; Hong, S. J.; Venters, B. J.; Tomsho, J. W.; Benkovic, S. J.; Keiler, K. C. *Protein Sci.* **2007**, *16*, 1535.
(41) Tavassoli, A.; Benkovic, S. J. *Angew. Chem., Int. Ed.* **2005**, *44*, 2760.
(42) Tavassoli, A.; Lu, Q.; Gam, J.; Pan, H.; Benkovic, S. J.; Cohen, S. N. *ACS Chem. Biol.* **2008**, *3*, 757.
(43) Liu, C. C.; Schultz, P. G. *Annu. Rev. Biochem.* **2010**, *79*, 413.
(44) Goto, Y.; Iwasaki, K.; Torikai, K.; Murakami, H.; Suga, H. *Chem. Commun.* **2009**, 3419.
(45) Seebeck, F. P.; Ricardo, A.; Szostak, J. W. *Chem. Commun.* **2011**, *47*, 6141.
(46) Hofmann, F. T.; Szostak, J. W.; Seebeck, F. P. *J. Am. Chem. Soc.* **2012**, *134*, 8038.
(47) Knerr, P. J.; van der Donk, W. A. *Annu. Rev. Biochem.* **2012**, *81*, 479.
(48) Oman, T. J.; van der Donk, W. A. *Nat. Chem. Biol.* **2010**, *6*, 9.
(49) Shimizu, Y.; Inoue, A.; Tomari, Y.; Suzuki, T.; Yokogawa, T.; Nishikawa, K.; Ueda, T. *Nat. Biotechnol.* **2001**, *19*, 751.
(50) Josephson, K.; Hartman, M. C.; Szostak, J. W. *J. Am. Chem. Soc.* **2005**, *127*, 11727.
(51) Hartman, M. C.; Josephson, K.; Lin, C. W.; Szostak, J. W. *PLoS One* **2007**, *2*, e972.
(52) Forster, A. C.; Tan, Z.; Nalam, M. N.; Lin, H.; Qu, H.; Cornish, V. W.; Blacklow, S. C. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 6353.
(53) Ohta, A.; Yamagishi, Y.; Suga, H. *Curr. Opin. Chem. Biol.* **2008**, *12*, 159.
(54) Ohuchi, M.; Murakami, H.; Suga, H. *Curr. Opin. Chem. Biol.* **2007**, *11*, 537.
(55) Yamagishi, Y.; Shoji, I.; Miyagawa, S.; Kawakami, T.; Katoh, T.; Goto, Y.; Suga, H. *Chem. Biol.* **2011**, *18*, 1562.
(56) Hipolito, C. J.; Suga, H. *Curr. Opin. Chem. Biol.* **2012**, *16*, 196.
(57) Sako, Y.; Goto, Y.; Murakami, H.; Suga, H. *ACS Chem. Biol.* **2008**, *3*, 241.
(58) Sako, Y.; Morimoto, J.; Murakami, H.; Suga, H. *J. Am. Chem. Soc.* **2008**, *130*, 7232.
(59) Goto, Y.; Ohta, A.; Sako, Y.; Yamagishi, Y.; Murakami, H.; Suga, H. *ACS Chem. Biol.* **2008**, *3*, 120.
(60) Kawakami, T.; Aimoto, S. *Chem. Lett.* **2007**, *36*, 76.
(61) Kawakami, T.; Ohta, A.; Ohuchi, M.; Ashigai, H.; Murakami, H.; Suga, H. *Nat. Chem. Biol.* **2009**, *5*, 888.
(62) Ohshiro, Y.; Nakajima, E.; Goto, Y.; Fuse, S.; Takahashi, T.; Doi, T.; Suga, H. *Chembiochem* **2011**, *12*, 1183.
(63) Kang, T. J.; Hayashi, Y.; Suga, H. *Angew. Chem., Int. Ed.* **2011**, *50*, 2159.
(64) Yamagishi, Y.; Ashigai, H.; Goto, Y.; Murakami, H.; Suga, H. *ChemBioChem* **2009**, *10*, 1469.
(65) Kawakami, T.; Murakami, H.; Suga, H. *J. Am. Chem. Soc.* **2008**, *130*, 16861.
(66) Subtelny, A. O.; Hartman, M. C.; Szostak, J. W. *Angew. Chem., Int. Ed.* **2011**, *50*, 3164.
(67) Smith, J. M.; Vitali, F.; Archer, S. A.; Fasan, R. *Angew. Chem., Int. Ed.* **2011**, *50*, 5075.
(68) Satyanarayana, M.; Vitali, F.; Frost, J. R.; Fasan, R. *Chem. Commun.* **2012**, *48*, 1461.
(69) Frost, J. R.; Vitali, F.; Jacob, N. T.; Brown, M. D.; Fasan, R. *ChemBioChem* **2013**, *14*, 147.