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Macrocyclizing DNA-Linked Peptides via Three-Component Cyclization and Photoinduced Chemistry

Yandan Bao, Minyan Xing, Naylor Matthew, Xiaohua Chen, Xuan Wang,* and Xiaojie Lu*

Cite This: *Org. Lett.* 2024, 26, 2763−2767 Read Online

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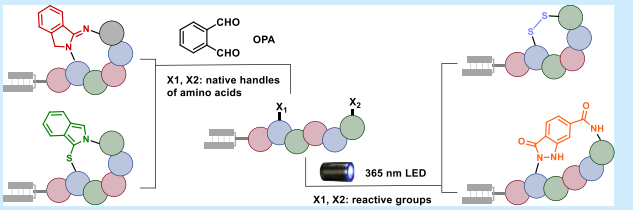
ABSTRACT: While DNA-encoded macrocyclic libraries have gained substantial attention and several hit compounds have been identified from DNA-encoded library technology, efficient on-DNA macrocyclic methods are also required to construct DNA-linked libraries with a high degree of cyclization and DNA integrity. In this paper, we reported a set of on-DNA methodologies, including the use of an OPA-mediated three-component cyclization with native handles of amino acids and photoredox chemistries. These chemistries proceed smoothly under mild conditions in good to excellent conversions, successfully generating novel isoindole, isoindoline, indazolone, and bicyclic scaffolds.



Cyclic peptides hold a significant position due to their unique properties and diverse therapeutic applications.[1] In recent years, there has been a notable increase in the number of approved macrocyclic drugs,[2] and the methods employed for drug discovery continue to advance and evolve.[3] Various discovery modalities,[4] including phage display,[5] mRNA display,[6] SOCLOPPS,[7] and DNA-encoded library technology (DELT),[8] have played pivotal roles in generating macrocyclic candidates. Each technology possesses distinct strengths and weaknesses, and DNA-encoded library technology stands out as a powerful tool for generating and screening large-scale libraries against multiple therapeutic targets in a time-saving and cost-effective manner. In recent years, the DELT screening platform has undergone rapid development, leading to a surge in publications and the identification of numerous macrocyclic hit compounds in both academic and industrial settings. These achievements demonstrate its immense potential for drug development.[8]

The chemical synthesis of DNA-linked cyclic peptides typically involves the sequential connection of amino acids, followed by a critical cyclization step. Researchers have dedicated numerous efforts to developing highly efficient cyclization methods that can be applied to constructing DELs. However, only a limited number of reactions, such as Wittig olefination,[9] CuAAc reaction,[10] amidation,[11] and S-arylation,[12] have been reported for producing solution DELs. The need for new on-DNA cyclization chemistries persists. One crucial aspect in developing new cyclization chemistries is the implementation of mild reaction conditions that do not require transition metal catalysts. Such conditions help minimize damage to DNA tags. Furthermore, utilizing endogenous handles for cyclization can eliminate the incorporation of nonessential components into the macrocycles.[3a] Moreover, the motifs formed by cyclization chemistry also could increase the molecular diversity in DNA-linked compounds.[13] Consequently, continued exploration and development of new on-DNA cyclization strategies are essential to advance the field of DNA-encoded libraries.

In this study, we present novel on-DNA macrocyclization strategies using *ortho*-phthalaldehyde (OPA) to react with native amine−amine[14] or amine−thiol[15] to generate isoindoline- or isoindole-bridged cyclic peptides, respectively. In addition, we developed photoinduced cyclization methods that produce disulfide bond[16] or indazolone[17] moieties under mild conditions without the need for transition metal catalysts (Figure 1). These newly developed methods exhibited high chemical reactivity and broad substrate compatibility with satisfactory results obtained without a tedious optimization process.

As illustrated in Scheme 1A, we commenced our investigation with DNA-linked lysine **A1** as the model starting material, in which the ε-amino of the side chain and the N-terminus would be clamped with OPA. As the previously published literature reports,[14] this reaction is heteroselective. The ε-NH_2 of Lys is more reactive than the α-NH_2 of α-AA and will first condense with the OPA to form the intermediate, then followed by the cross-linking with the α-NH_2. This three-component chemistry is highly effective when only 5 equiv of OPA was added under a base condition, and the desired product **A2-1** was observed with 51% conversion, along with

Special Issue: Organic Chemistry Driven by Academic-Industrial Collaborations

Received: June 5, 2023
Published: June 29, 2023

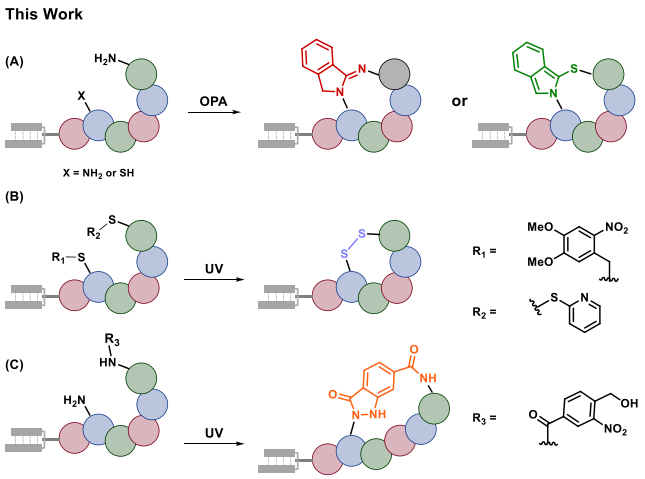


Figure 1. Newly developed macrocyclic methods revealed in this manuscript. (A) OPA-mediated three-component cyclization. (B) Photoinduced disulfide bond formation. (C) Photoinduced indazolone formation.

the additional condensation byproducts **A2-2** and **A2-3** (entry 1). Pleasingly, decreasing the usage of OPA exactly avoids the occurrence of aldol condensation (entry 2). Further testing indicated a base condition is required to afford the desired product with satisfactory condition (entries 4−5).

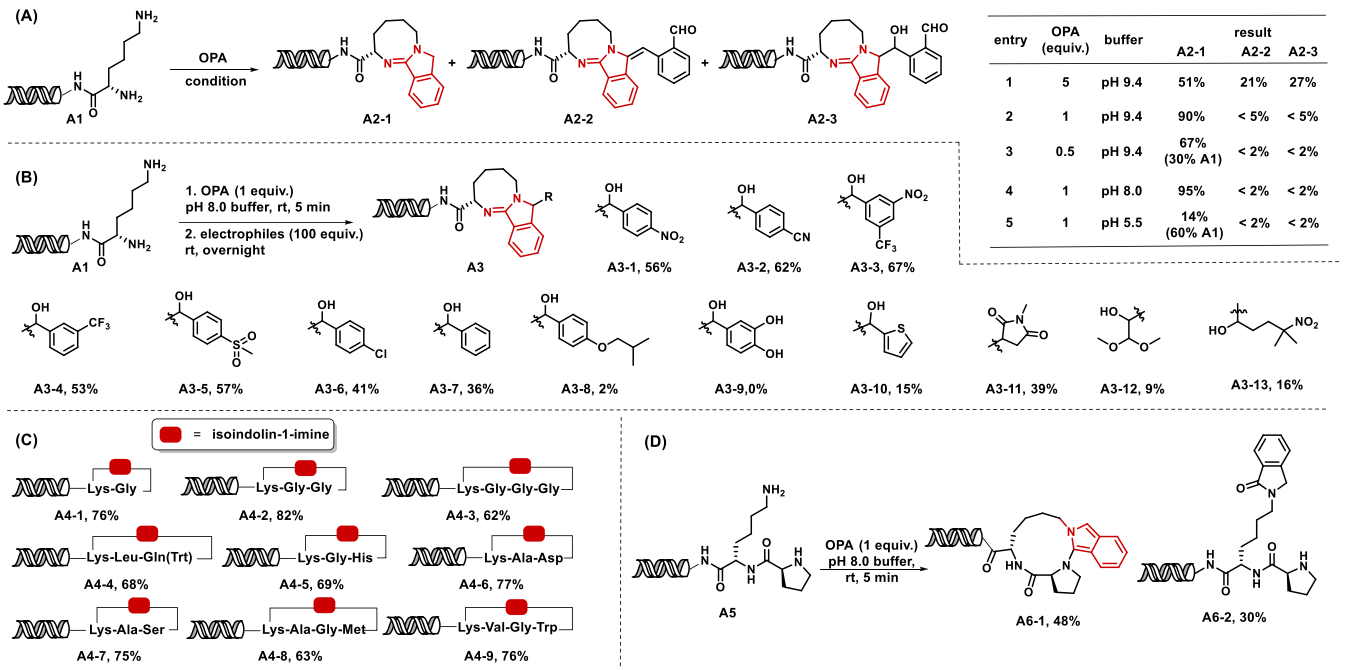
The successful formation of **A2-2** and **A2-3** inspired us to investigate a one-pot, two-step protocol aimed at achieving successive condensation with different aldehyde reagents (Scheme 1B). Following the three-component reaction with 1 equiv of OPA, the unpurified intermediate was directly subjected to aldol condensation with 100 equiv of electrophiles. Notably, both the aromatic and alkyl aldehydes could afford the corresponding aldol products, with the electron-deficient groups showing greater reactivity (**A3-1**∼**A3-6**) in comparison to the electron-donating groups (**A3-7**∼**A3-8**) and the alkyl electrophiles (**A3-11**∼**A3-13**). However, it is worth mentioning that reagents containing a phenolic hydroxyl group exhibited a tendency for self-condensation under these reaction conditions, leading to failure of the formation of the desired aldol product (**A3-9**).

With the optimized OPA-mediated DNA-compatible protocol in hand, we next tested different DNA-linked peptide sequences. As outlined in Scheme 1C, DNA-linked cyclic peptides **A4-1**∼**A4-3** with varied ring sizes were obtained with good efficiency. Several residues, such as imidazole (**A4-5**), carboxylic acid (**A4-6**), hydroxyl (**A4-7**), S-methyl thioether (**A4-8**), and indole (**A4-9**), were untouched. The occurrence of the aldol byproduct or remaining starting material cannot be avoided because it is too difficult to accurately quantify the DNA-linked samples.

We also explored the cross-linking potential between the secondary amino group of proline and the primary amino group with OPA, aiming to generate the isoindole-1-amine scaffold. As shown in Scheme 1D, DNA-linked substrate **A5** was subjected to the optimized three-component condition and afforded the desired product **A6-1** with 48% conversion and 30% byproduct arising from the condensation between the primary amine and the OPA. This observation demonstrates that the secondary amine is less reactive than the primary amine. Based on these findings, we propose employing two primary amines in conjunction with OPA for subsequent library construction, expecting improved macrocyclization efficiency.

The thiol group of cysteine is a good nucleophile and could engage in an OPA-mediated three-component reaction. While Li et al. have previously published this on-DNA methodology,[18] our work places greater emphasis on its application in macrocyclization rather than intermolecular cross-linking. It is worth noting that in our study we utilize the thiol group derived from the native amino acid instead of an aromatic thiol group, as we have observed its greater preference and suitability for our purposes.

Scheme 1. OPA-Mediated Three-Component Cyclization with Two Amino Groups



entry	OPA (equiv.)	buffer	result A2-1	A2-2	A2-3
1	5	pH 9.4	51%	21%	27%
2	1	pH 9.4	90%	< 5%	< 5%
3	0.5	pH 9.4	67% (30% A1)	< 2%	< 2%
4	1	pH 8.0	95%	< 2%	< 2%
5	1	pH 5.5	14% (60% A1)	< 2%	< 2%

https://doi.org/10.1021/acs.orglett.3c01817
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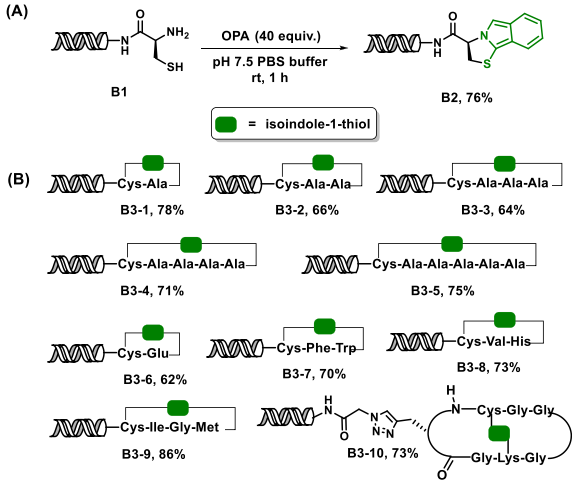
This work is illustrated in Scheme 2, where the clamping of the thiol and amino groups of **B1** proceeded smoothly under mild aqueous conditions, yielding the desired cyclic product **B2** in 76% conversion. This chemistry is effective, as demonstrated by the accomplishment of macrocyclization between 1-mer and 6-mer linear peptides with good to excellent conversions. Several residues, such as carboxylic acid, indole, imidazole, and S-methyl thioether, are untouched (Scheme 2B).

Scheme 2. OPA-Mediated Three-Component Cyclization with One Amino and One Thiol Group

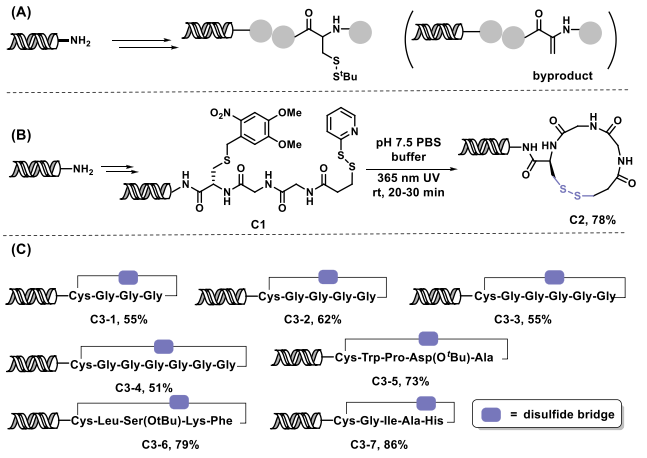


We then investigated its applicability in generating bicyclic scaffolds. A model cyclic peptide substrate was readily prepared via the two-directional synthetic strategy, and the Lys and Cys monomers were preinstalled into the peptide sequence (see Supporting Information for detail). When this substrate was subjected to the cyclization condition with 1 equiv of OPA, it successfully yielded the desired bicyclic product **B3-10** with 73% conversion. Aldehyde reagent 2,3-thiophenedicarboxaldehyde also could successfully provide the bicyclic product in 68% conversion (see Supporting Information for detail). This is the first example of constructing a bicyclic scaffold in solution-phase DNA-encoded libraries, suggesting a new avenue of great importance to identify bicyclic hit compounds.

In addition, we conceived whether the thiol and amine could be cross-linked with only one aldehyde functional group to cyclize the linear peptide. The intermolecular clamping of the thiol and amine groups with aryl aldehydes proceeded smoothly and gave the desired products with good conversions. However, we encountered challenges in achieving cyclization due to the unavailability of suitable orthogonal DNA-compatible protection groups (see Supporting Information for detail).

Both thiol and amino groups possess remarkable nucleophilic properties and can participate in various chemical transformations. In addition to exploring the three-component reaction, we also investigated alternative chemical reactions involving thiol or amino groups. Heinis et al. published a valuable DNA-compatible methodology to generate macrocycles cyclized by disulfide and thioether, in which *tert*-butylthio (S-tBu) was used as the protection group for cysteine. We replicated this transformation in our laboratory and successfully obtained the DNA-linked disulfide- and thioether-cyclized peptides. However, we observed that the S-tBu group was not sufficiently stable under amidation conditions and could undergo elimination to form an electrophilic alkene moiety, which subsequently reacted with piperidine, leading to the formation of undesired byproducts (Scheme 3A). To address this issue, we changed our protection strategy by introducing a photocleavable 2-nitroveratryl (oNv) group as a new orthogonal protecting group, ensuring improved stability and control over the desired reactions.

Scheme 3. On-DNA Photoinduced Disulfide Formation



As illustrated in Scheme 3B, the DNA-linked peptide **C1** combined with complementary S-pyridinesulfenyl group on the N-terminus, and the disulfide bond are generated rapidly via photoinduced thiolysis. The photoredox reaction was performed using a 365 nm LED in an aqueous solution without any additives. To validate the efficacy and versatility of this approach, cyclization of a number of DNA-linked substrates with varied sizes and functionalized residues was performed. Pleasingly, the macrocyclization of ring sizes between 17 (**C3-1**) and 26 (**C3-4**) can be generated in satisfactory conversions, and photosensitive residues, such as tryptophane (**C3-5**), were untouched.

Photoinduced chemistry is inherently mild and able to occur efficiently under aqueous conditions without reagents harmful to the DNA tags. It also has the potential to activate the amino group to cyclize the peptide product. As shown in Scheme 4A, the DNA-linked peptide was capped with the *o*-nitrobenzyl alcohol (*o*-Nba) on the N-terminus to generate the precursor **D1**. Upon activation of the 365 nm light, the *o*-nitrobenzyl alcohol moiety was spontaneously converted to the reactive intermediate **D2** and then rapidly condensed with the ε-amino group of lysine to form indazolone moiety **D3** in 80% conversion. The indazolone moiety is a privileged scaffold and exhibits a wide range of biological and pharmaceutical activities such as antiviral, antibacterial, anticancer, and so on. A highly potent CDK inhibitor illustrated the importance of indazolone incorporated into a cyclic peptide.[19] This straightforward protocol also performed smoothly to generate cyclic peptides with varied ring sizes and has good functional compatibility (Scheme 4B).

In summary, we have developed a set of efficient macrocyclic protocols for constructing DNA-encoded libraries. The OPA-mediated cyclization reaction leveraged the endogenous nucleophilic handles of natural amino acids such as Lys and Cys. It avoids the introduction of exogenous reactive handles,

https://doi.org/10.1021/acs.orglett.3c01817
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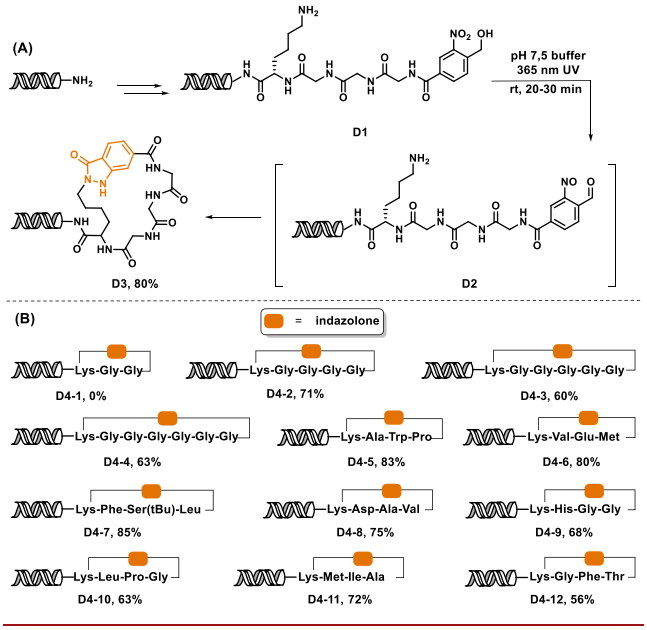
Scheme 4. On-DNA Photoinduced Indazolone Formation



decreasing the number of synthetic steps and improving the overall conversions. Meanwhile, the OPA-mediated methodology is first engaged in the construction of DNA-linked bicyclic compounds in solution-phase reaction conditions, establishing a more convenient way to construct bicyclic libraries. We also first introduce the photoinduced methodology into on-DNA macrocyclization to produce the disulfide bond and indazolone scaffold. We expect these methods could pave the way to preparing large-scale macrocyclic libraries with functional features for hit identification against biological targets of interest.

■ ASSOCIATED CONTENT

Data Availability Statement

The data underlying this study are available in the published article and its Supporting Information.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.3c01817.

Materials and methods; UV/mass spectra for DNA-linked compounds (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Xuan Wang − *State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China;* orcid.org/0000-0003-4986-0231; Email: wangxuan2@simm.ac.cn

Xiaojie Lu − *School of Chinese Materia Medica, Nanjing University of Chinese Medicine, Nanjing 210023, China; State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China; University of Chinese Academy of Sciences, Beijing 100049, China;* orcid.org/0000-0002-3600-288X; Email: xjlu@simm.ac.cn

Authors

Yandan Bao − *School of Chinese Materia Medica, Nanjing University of Chinese Medicine, Nanjing 210023, China; State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China*

Minyan Xing − *School of Pharmacy, China Pharmaceutical University, Nanjing 211198, China*

Naylor Matthew − *UCB, Cambridge, Massachusetts 02140, United States*

Xiaohua Chen − *State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China; University of Chinese Academy of Sciences, Beijing 100049, China;* orcid.org/0000-0003-3031-7095

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.orglett.3c01817

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Xiaojie Lu thanks NSFC-91953203 and NSFC-92253305 for financial support of this work. Xuan Wang thanks Lingang Laboratory (Grant No. LG-QS-202206-09) and National Key Research and Development Program of China (2022YFC2804400) for financial support of this work.

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