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AN OPEN ACCESS JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

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Article

Solid-Phase Synthesis of Diverse Macrocycles by Regiospecific 2-Pyridone Formation: Scope and Applications

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Cite This: *JACS Au* 2024, 4, 3018−3027
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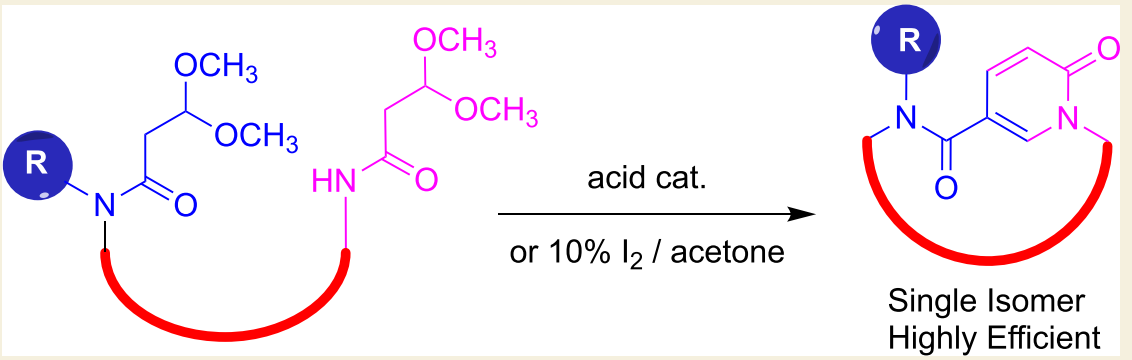


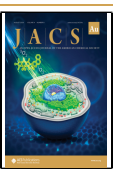
ABSTRACT: This study introduces a novel solid-phase macrocyclization method generating 2-pyridone rings. This method relies on the intramolecular condensation between secondary and tertiary dimethoxy-propionic amide units. This selective reaction leads to the formation of a single well-defined regioisomer. The method demonstrates remarkable efficiency in producing diverse peptidic and nonpeptidic bioactive targets, paving the way for the development of innovative macrocycle libraries featuring the 2-pyridone unit.

KEYWORDS: *pyridone, macrocycle, peptide, antibacterial, stapled peptide*

Many disease-causing proteins lack deep, readily ligandable pockets. Macrocyclic peptides and peptidomimetic compounds[1,2] are one class of molecules that have shown promise in engaging these difficult targets.[3−6] Macrocyclization limits the conformational flexibility of a molecule, often resulting in higher affinity binding to proteins and RNA targets of interest. In the case of peptide ligands, macrocyclization provides opportunities for the formation of intramolecular hydrogen bonds that can have profound effects on cell permeability and bioavailability.[7−11] Therefore, the development of new methods for the efficient creation of macrocycles from linear precursors is an important goal. This is particularly true for the synthesis of libraries of macrocycles using diversity-oriented synthesis[12] or split and pool strategies.[13−17] Common methods for the macrocyclization of peptides or peptidomimetic compounds, such as amide bond formation, are complicated by intermolecular dimerization and often must be carried out under high dilution conditions to achieve a high yield of the desired products.[18] Many fail to provide high yields of smaller macrocycles such as those of tetrapeptides. As a result, there has been a surge of interest in more efficient methods to close macrocycles,[19−22] especially those that provide a high level of selectivity for intramolecular coupling.[23]

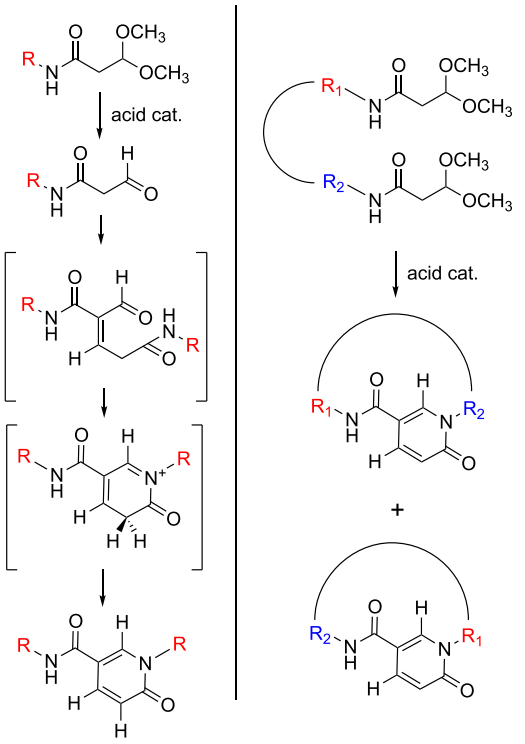
We previously reported that the acid-catalyzed coupling of 3,3-dimethoxy-propionic amide [dimethoxy-propionic amide (DMPA)]-containing compounds to form 2-pyridones (Scheme 1) is an unusually clean and efficient method for the creation of homodimeric compounds, both in solution and on resin.[24,25] Given the efficiency of this chemistry, we were intrigued by the idea that the same reaction could be employed for the closure of macrocyclic rings. However, a complication of this scheme is that two regioisomeric macrocycles would likely be produced (Scheme 1). As is shown below, this is indeed the case, although the ratios of the two products vary dramatically depending on the substrate. In this study, we report a simple strategy to avoid this problem, in which one end of the linear precursor contains a tertiary DMPA unit and the other a secondary DMPA unit. This blocks the formation of the regioisomer, in which the nitrogen in the tertiary amide is incorporated into the pyridone ring. These starting materials produce a single macrocyclic product. We anticipate that this

Received: April 20, 2024
Revised: July 3, 2024
Accepted: July 3, 2024
Published: August 12, 2024



https://doi.org/10.1021/jacsau.4c00352
JACS Au 2024, 4, 3018−3027

Scheme 1. Synthesis of Dimeric Compounds on Resin via Self-Condensation of DMPA-Equipped Molecules[a]



[a]Left: mechanism of the 2-pyridone-forming dimerization reaction. Right: expected production of regioisomers when this reaction is applied to macrocyclization.

methodology will be useful for the construction of high-quality libraries of novel macrocycles.

RESULTS AND DISCUSSION

On-Resin Synthesis of 2-Pryidone-Containing Macrocycles

We showed previously that TentaGel resin-bound molecules terminating in a DMPA unit can be efficiently dimerized by treatment with either neat trifluoroacetic acid (TFA) or 10% iodine in acetone.[26] These protocols result in the exposure of the formyl group, which is followed by spontaneous heterocycle formation (Scheme 1). To investigate the utility of this reaction for the on-resin synthesis of macrocycles, we created the simple compound shown in Figure 1A, in which DMPA units placed at the N-terminus of the molecule and on a lysine side chain are linked by an ethylene glycol unit. 5 μmol 90 μm TentaGel RAM resin displaying this starting material was incubated in a 10% iodine in acetone solution (5 mL volume) for 2 h. The beads were then washed and treated with 93% TFA for 2 h to release the bound material, which was analyzed by liquid chromatography−mass spectrometry (LC−MS). Note that we demonstrated in our previous report that the 2-pyridone ring is formed during the iodine treatment prior to TFA cleavage.[25] As shown in Figure 1A-2, two peaks with the masses expected for the regioisomeric 2-pyridone-containing macrocycles were the major products, produced in an approximately 83:17 ratio. Importantly, we did not detect any product peaks with masses corresponding to the products of the intermolecular coupling reactions. To probe the generality of this reaction, we utilized the same protocol to synthesize the diverse cyclic structures shown in Figure 1B. Gratifyingly, LC−MS analysis of the crude material after release from the resin showed that all of these reactions, including those that formed relatively small rings, proceeded efficiently. The ratio of the regioisomeric products varied dramatically from 99:1 to 80:20. We did not attempt to determine which peak represents which regioisomer since, as described below, we developed a strategy to limit the reaction to the creation of a single product. We also created a variety of macrocycles by using this chemistry to stitch together DMPA-modified side chains, as shown in Figure 2. Specifically, we introduced two Lysine residues into the sequence. The side chains of these Lysine residues were differentially protected using *N*-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl] (Dde) and 4-methyltrityl (Mtt) groups. These protective groups were then selectively removed using 2% hydrazine in *N*-methyl-2-pyrrolidone (NMP) and 20% hexafluoroisopropanol (HFIP) in NMP, respectively, to prepare the amine side chains of the lysines for the addition of the two DMPA groups. Gratifyingly, the cyclization proceeded successfully with all of the target compounds (Figure 2). Again, a range of regioselectivities was observed, ranging from 95:5 to 55:45. The size, flexibility, and presence of specific functional groups within the macrocycle, particularly those neighboring the DMPA units, are parameters that might favor one isomer over the other due to steric interactions or hydrogen bonding patterns.

We conclude from the data shown in Figures 1 and 2 that the macrocyclization of diverse compounds is quite efficient and generally clean using this chemistry, but as expected, mixtures of regioisomers will often be produced.

Production of a Single Regioisomer

Based on the mechanism depicted in Scheme 1, we hypothesized that the production of only a single 2-pyridone-linked macrocycle could be achieved by using a substrate in which one end of the molecule is a DMP ester rather than an amide. To test this idea, we synthesized a model compound (**14**; Figure 3A-1) in which DMPA units were attached to a serine side chain and a terminal amine. When the resin was treated with neat TFA and the crude product was analyzed by LC−MS, we observed none of the desired macrocycles. Instead, the intermolecular dimer formed by the condensation of two DMPA units and hydrolysis of the DMP esters was the major product. The same result was obtained using the 10% I_2 in acetone protocol (Figure 3A-2).

Somewhat different results were obtained when the same reaction was attempted using a substrate (**17**, Figure 3B-1) in which a tyrosine rather than a serine DMP ester was present. In this case, the desired 2-pyridone was formed upon treatment with TFA but the ester bond hydrolyzed sometime after macrocycle formation, resulting in the production of compound **18** (Figure 3B-2). In contrast, when bead-bound **17** was treated with 10% I_2 in acetone, the desired macrolactone was detected by LC−MS after release from the beads. However, the reaction was not clean, and a variety of uncharacterized products were also formed (Figure 3B-3). Significant attempts at optimizing this process failed to provide a better result (see Supporting Information, Section 2-3-2-i).

An alternative strategy would be to use a substrate containing one secondary DMPA unit and one tertiary DMPA unit. The nitrogen in the tertiary amide cannot become a part of the 2-pyridone ring, thus preventing the formation of this regioisomer. To explore this idea, we synthesized substrate **22** using the route shown in Figure 4A-1. When beads displaying this compound were treated with neat TFA or 10% I_2 in acetone, essentially quantitative conversion into desired macrocycle **23** was

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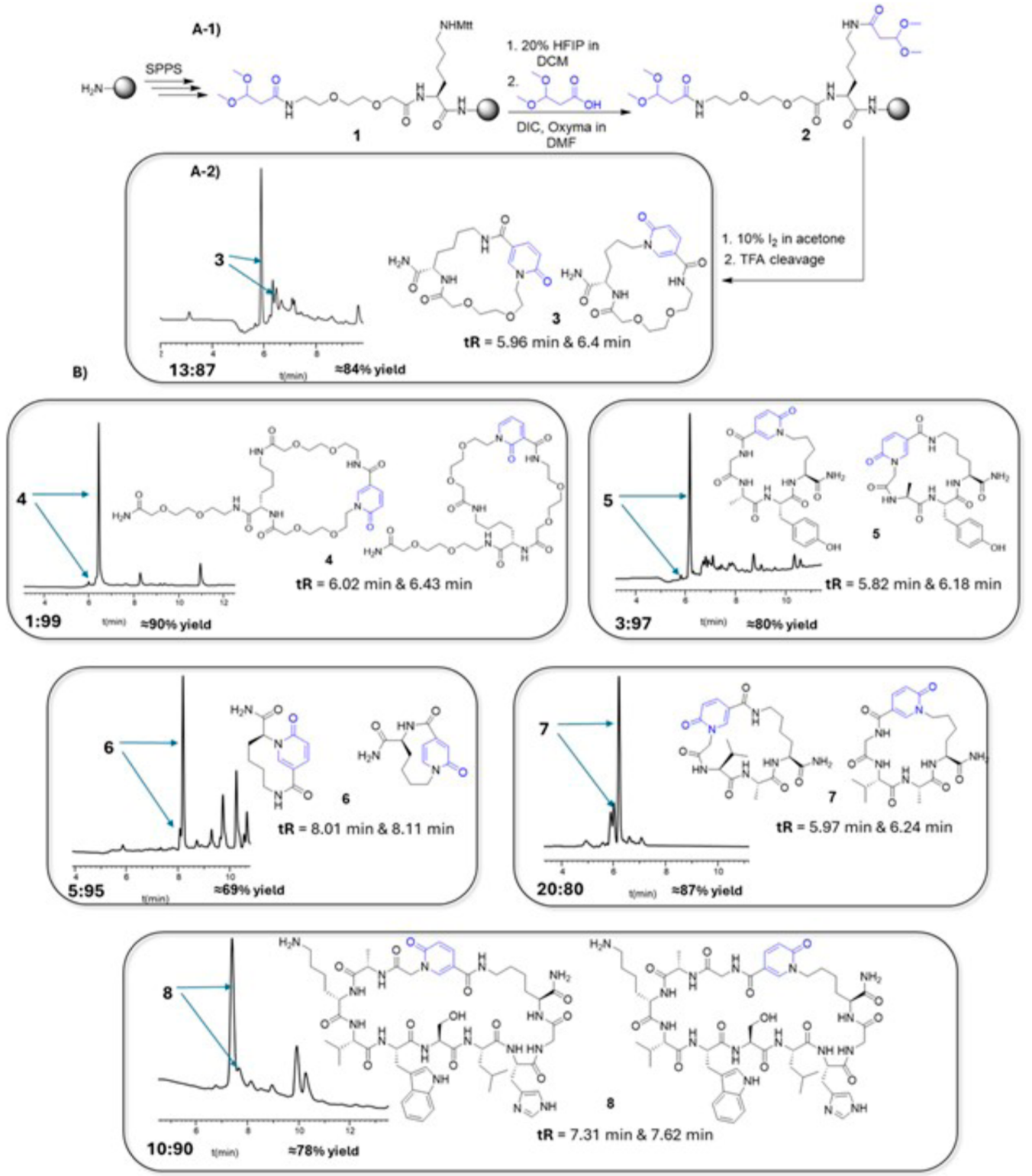

Figure 1. (A) General approach for synthesizing cyclic compounds. (B) HPLC chromatograms of head-to-tail cyclized crude compounds.

observed by LC−MS after release from the resin. Encouraged by this result, we created another linear molecule containing one secondary and one tertiary DMPA (**26**, Figure 4B-1). Again, treatment with neat TFA or 10% I_2 in acetone produced a high yield of the desired macrocycle **27**, albeit somewhat less cleanly that was the case for **23**. We conclude that the macrocyclization of linear precursors containing one secondary and one tertiary DMPA unit is a promising approach to create a single regioisomer of 2-pyridone-linked macrocycles. Note that using proline to generate a tertiary DMPA also resulted in the formation of the desired macrocycle, but with a very low conversion. (see Supporting Information, Section 2-3-5-ii).). Furthermore, to investigate the influence of resin loading on the formation of the desired macrocycle, we resynthesized

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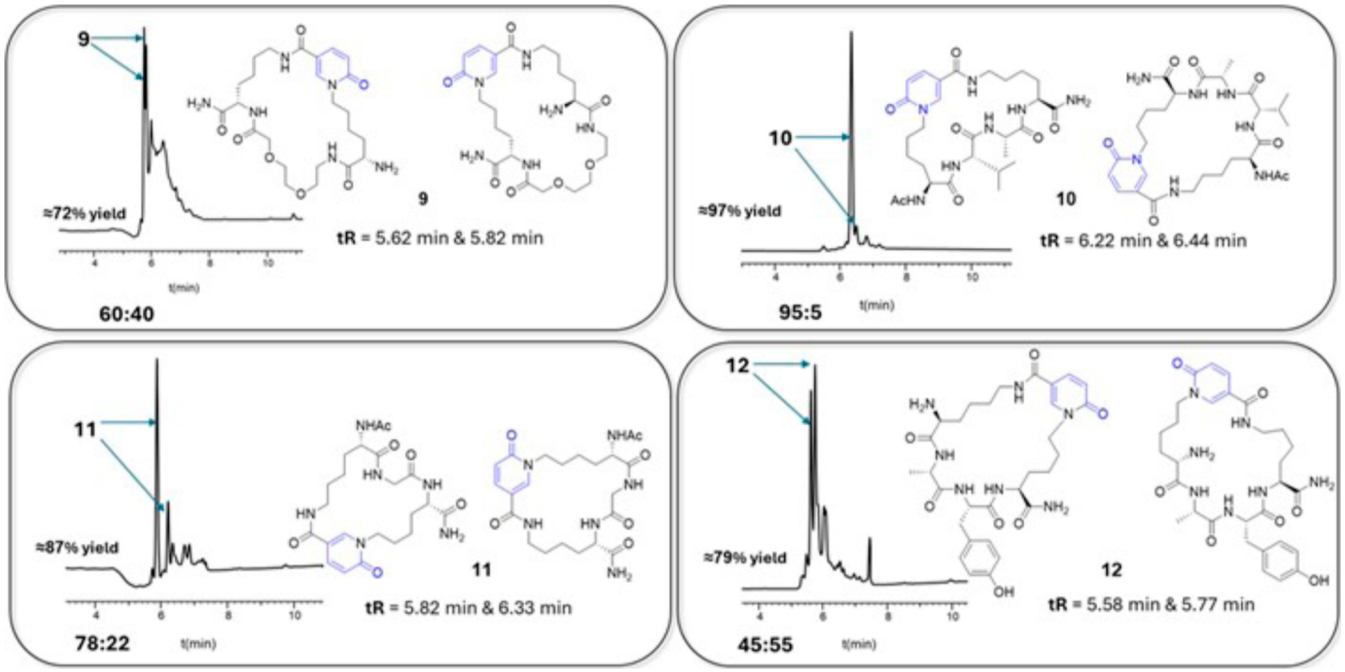

Figure 2. HPLC chromatograms of side chain to side chain cyclized crude compounds.

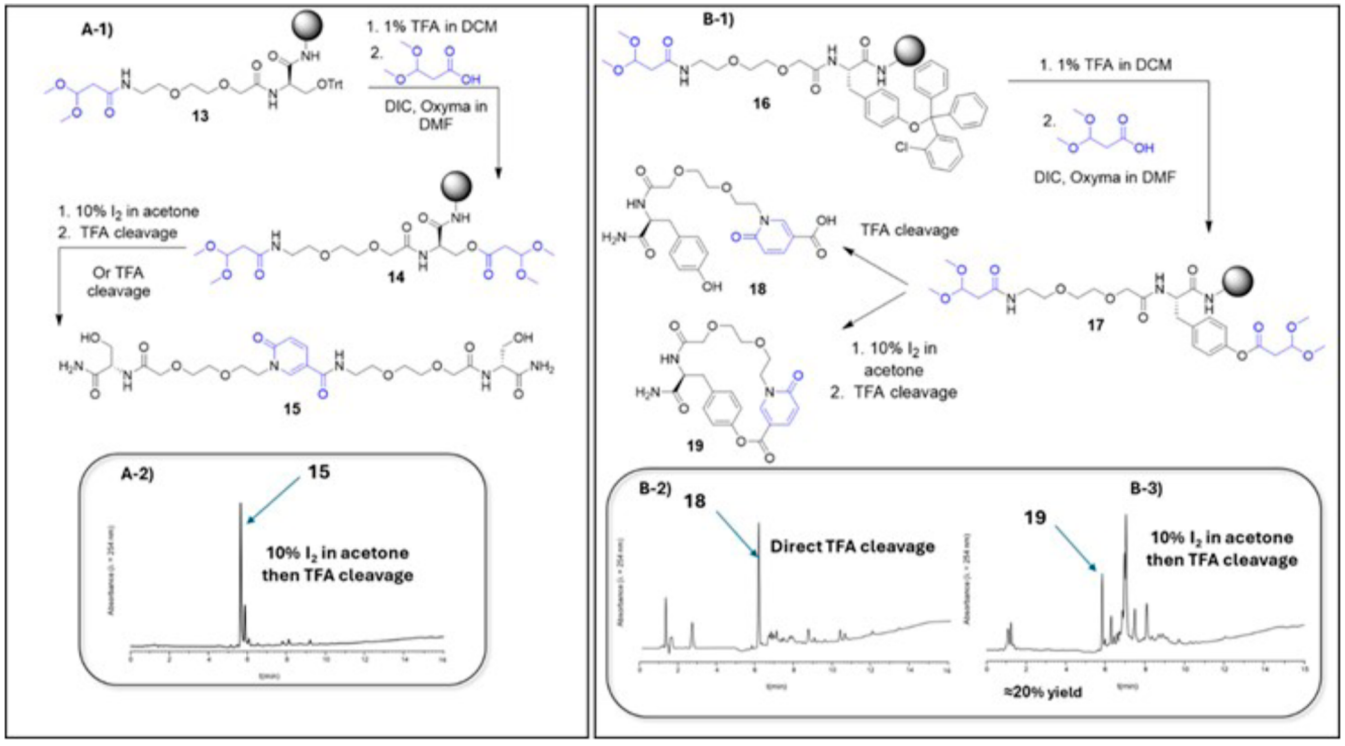

Figure 3. Attempted macrocyclization between DMPA and DMP ester moieties. (A-1) Addition of DMPA on serine side chain. (A-2) HPLC chromatogram of crude compound 15 after treatment with 10% iodine in acetone followed by TFA cleavage. (B-1) Addition of DMPA on tyrosine side chain. (B-2) HPLC chromatogram of crude compound 18 upon resin treatment with TFA. (B-3) HPLC chromatogram of crude compound 19 upon resin treatment with 10% iodine in acetone followed by TFA cleavage.

compound **23** using high-loading TentaGel and polystyrene resins. The cyclization reaction yielded the desired product in each case but with different conversion efficiencies (detailed comparison in Supporting Information, 2-3-4).

2-Pyridone-Based Macrocyclization to Create Bioactive Macrocyclic Peptides

Antimicrobial peptides (AMPs)[27] are characterized by their positive charge and amphiphilic helical structure. AMPs exhibit broad-spectrum activity against various microorganisms including bacteria, fungi, and viruses. Their mode of action involves

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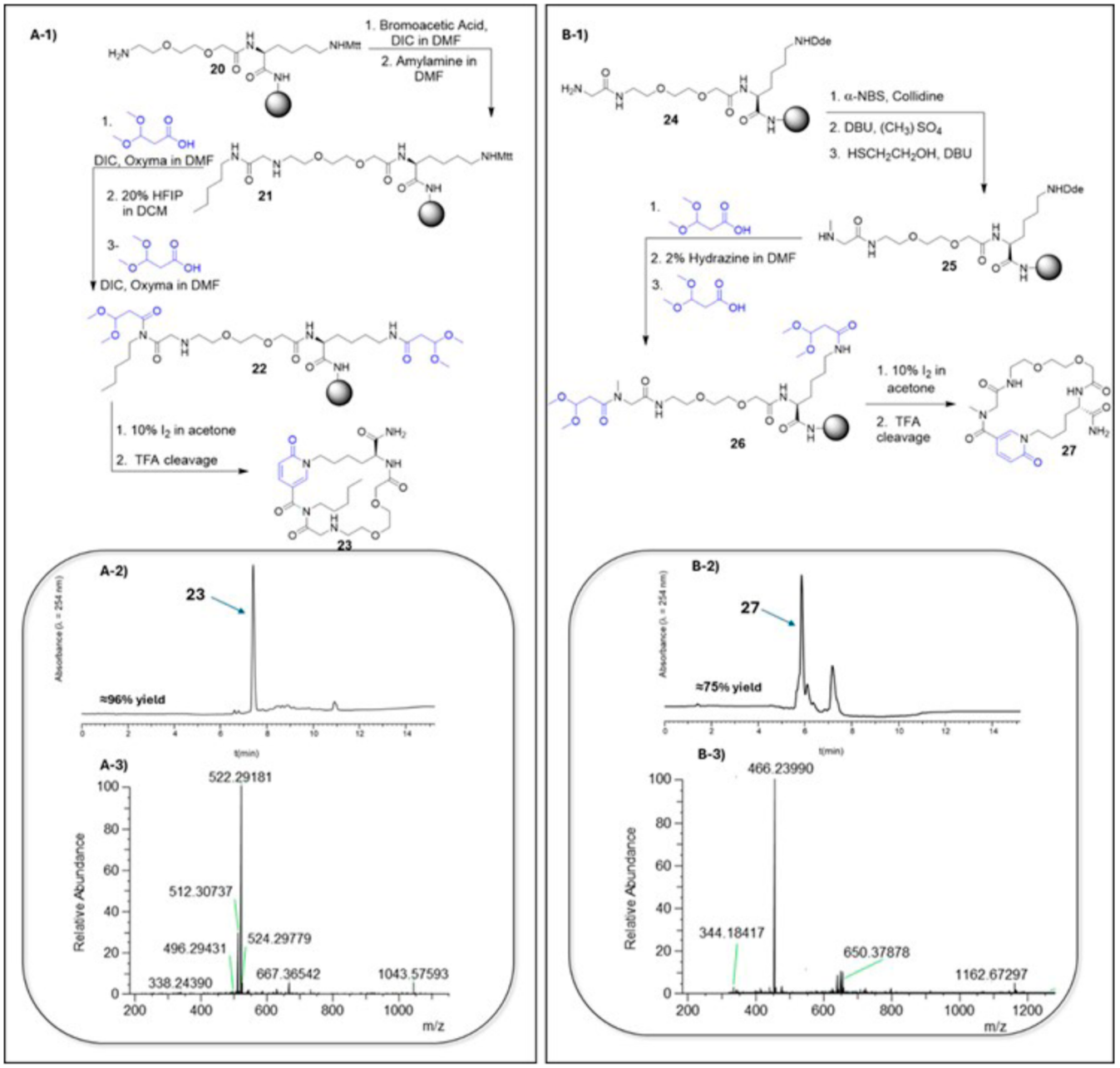

Figure 4. Regiospecific macrocyclization between secondary and tertiary DMPA moieties. (A-1) Addition of DMPA on a secondary amide. (A-2) HPLC chromatogram of crude compound **23**. (A-3) High-resolution spectra of purified **23**. (B-1) Addition of DMPA on a secondary amine. (B-2) HPLC chromatogram of crude compound **27**. (B-3) High-resolution spectra of purified **27**.

binding to microbial cell membranes, inducing structural changes that compromise membrane integrity, leading to cell lysis. Numerous studies have demonstrated that introducing conformational rigidity through cyclization is an effective method for enhancing the activity of AMPs. For example, Phe-Trp-Lys-Phe-Lys-NH_2 is a small peptide that has modest antimicrobial activity against *Escherichia coli* and *Bacillus subtilis*.[28] To probe the utility of 2-pyridone-linked macrocyclization to create bioactive macrocyclic peptides, we synthesized the linear bis-DMPA-containing precursor **30** using the route shown in Figure 5. The resin was then treated with 10% I_2 in acetone, followed by TFA-mediated release from the bead. As shown in Figure 5, an approximately 75% yield of the desired "*i* to *i* + 4" 2-pyridone-stapled peptide **31** was indicated by LC−MS analysis. The crude product was readily purified to apparent homogeneity by HPLC.

Linear peptide **28** and macrocyclic peptide **31** were then assessed for their antibacterial activity against *E. coli* and *B. subtilis*. The 2-pyridone-stapled macrocycle inhibited growth of both Gram negative and Gram-positive bacteria (*E. coli* and *B. subtilis*, respectively) with an IC_{90} of 125 μg/mL. The activity of the linear peptide was about 4-fold worse against *E. coli* and unmeasurable against *B. subtilis*.

As a second example, we investigated the utility of the 2-pyridone-forming macrocyclization reaction for creating a stapled derivative of the wasp venom peptide Anoplin, a 10 amino acid linear peptide with modest antibacterial activity.[29] Again, an established approach to enhance the bactericidal properties of Anoplin is to stabilize its helical structure. For

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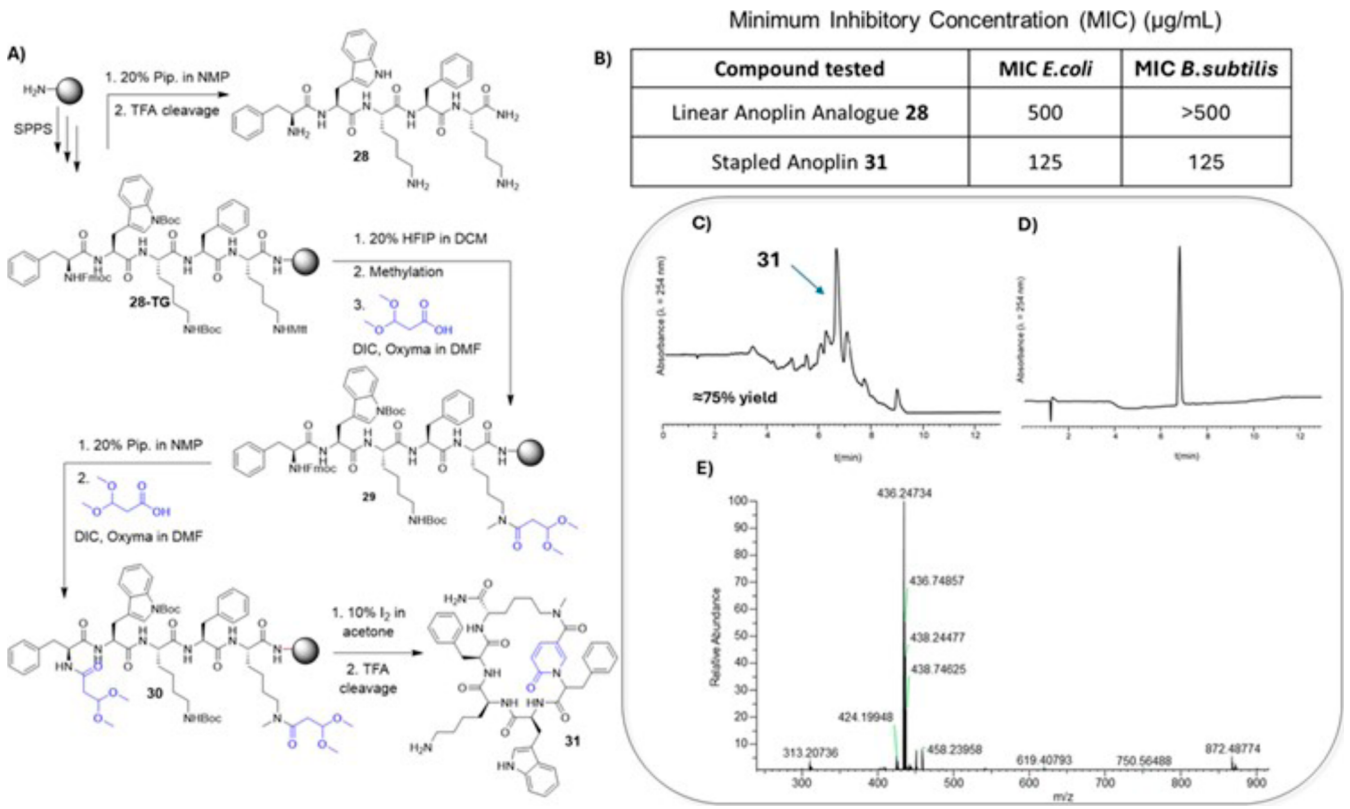

Figure 5. Comparison of the anti-bacterial activity of linear and 2-pyridone-cyclic peptides (A) solid-phase synthesis of cyclic peptide **31**. (B) Minimum inhibitory concentration (MIC) assay results. (C,D) HPLC chromatograms of crude and purified **31**. (E) High-resolution MS spectra of purified **31**.

example, Zhang[30] and Trylska[31] groups have used a ring closing metathesis reaction to generate stapled versions of Anoplin with improved activity.

We synthesized a 2-pyridone-stapled analogue of the Anoplin peptide using the route shown in Figure 6A. Arg 6 and Ile 2 were replaced with Dab(Mtt) and Dap(Dde) residues, respectively, to maintain the same number of atoms in the staple ring as in the previously mentioned studies. The side chain of the Dap residue was then exposed, and the resulting free amine was methylated and subsequently acylated to establish a tertiary DMPA unit at this site. The Mtt protecting group was then removed, and the resulting amine was acylated to create a secondary DMPA unit. Finally, the resin was treated with 10% iodine in acetone and, after washing, dilute TFA was used to release the products from the resin.

As shown in Figure 6B, a peak with the correct mass for the expected macrocycle **35** was the major product observed in the LC−MS trace of the crude reaction product (≈83% purity). This was readily purified to homogeneity by HPLC (Figure 6C,D). As anticipated, the stapling of Anoplin increased its antibacterial potency approximately twofold against *E. coli* and fourfold against *B. subtilis*. (Figure 6E). Finally, comparison of the circular dichroism (CD) spectra of linear Anoplin and the stapled macrocycle **35** in a membrane-mimicking solvent (50% 2,2,2-trifluoroethanol in water) demonstrated the latter has a much higher propensity to adopt a helical conformation [35 was 76% helical vs 19% for linear Anoplin (Figure 6F,G)]. We conclude that the 2-pyridone-forming macrocyclization chemistry is an efficient method to create bioactive stapled peptides.

Replacement of a Thioether Linkage with a 2-Pyridone Ring in a Streptavidin-Binding Macrocycle

We recently reported the synthesis of a one bead one compound DNA-encoded library of nonpeptidic macrocycles of macrocycles in which the ring was closed by thioether formation and showed that this library could be mined for high affinity streptavidin (SA)-binding macrocycles.[15] However, the thioether unit is a potential metabolic liability because it is relatively easily oxidized. Therefore, we were curious whether this linkage could be replaced by the 2-pyridone unit. Thus, macrocycle **39** was synthesized using the route shown in Figure 7A. Compound **36** was synthesized using the previously reported method.[15] Subsequently, a second DMPA group was added to yield compound **37**, which was then treated with 10% iodine in acetone to generate macrocycle **38**. Finally, the Dde protecting group was removed, and fluorescein was attached to the peptide using *N*-α-Fmoc-*N*-ε-(5/6-carboxyfluorescein)-L-lysine. After Fmoc removal, the resin was treated with TFA, resulting in the release of cyclic compound **39** (≈65% yield). The affinity of this macrocycle for streptavidin was measured by titration of the labeled compound with streptavidin followed by the increase in fluorescence polarization (FP),[32] yielding a K_D of approximately 38 μM. This value is much higher than that measured for the thioether-linked macrocycle-streptavidin complex (K_D = 0.5 μM).[15] The ring sizes of the 2-pyridone- and thioether-linked macrocycles are slightly different, and the two linkages likely result in different conformational preferences in the two molecules; therefore, this result is not surprising and highlights the fact that one cannot confidently "swap out" macrocycle linkages without expecting significant changes in the properties of the ligand. The important result here is that the efficiency of

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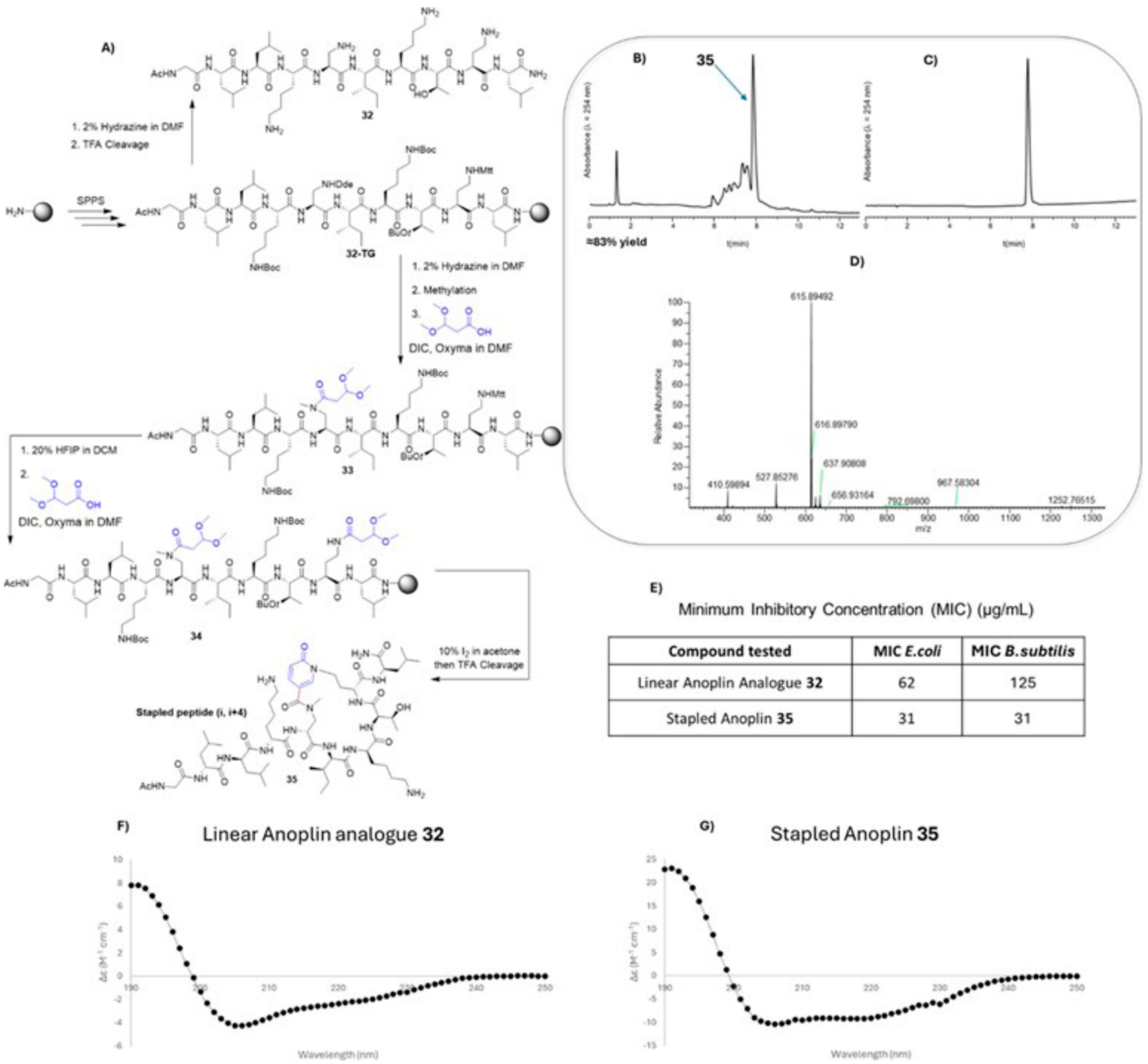

Compound tested	MIC *E.coli*	MIC *B.subtilis*
Linear Anoplin Analogue **32**	62	125
Stapled Anoplin **35**	31	31

Figure 6. Synthesis, characterization, and activity of 2-pyridone-stapled Anoplin peptide. (A) solid-phase synthesis of stapled Anoplin **35**. (B,C) HPLC chromatograms of crude and purified 35. (D) High-resolution MS spectra of purified **35**. (E) Minimum inhibitory concentration (MIC) assay results. (F,G) CD spectra of **32** and **35**.

the 2-pyridone-linked, nonpeptidic macrocycle is efficient enough to support the creation of libraries that can be screened directly for binding to proteins of interest.

CONCLUSIONS

In conclusion, we show here that the intramolecular condensation of two DMPA units to form a 2-pyridone ring is a highly efficient method for the solid-phase synthesis of macrocycles. Linear precursors that contain two secondary DMPA units can form a dimer free mixture of two regioisomers, the ratio of which varies substantially depending on the particular substrate. A key finding presented here is that efficient macrocyclization leading to a single 2-pyridone-linked product can be achieved if one of the DMPA units is a tertiary amide, blocking that nitrogen from incorporation into the heterocyclic ring. Using such starting materials, we show that this chemistry is useful for the construction of several bioactive macrocycles. These findings set the stage for the synthesis and screening of novel libraries of macrocycles containing the 2-pyridone unit.

Compared with traditional peptide macrocyclization methods, the 2-pyridone linkage offers significant advantages. It requires fewer steps, achieving a high efficiency under mild conditions, making it simpler to implement. Unlike amide bond formation, which can suffer from intermolecular dimerization and necessitates high dilution, the 2-pyridone method is particularly suitable for smaller macrocycles like tetrapeptides. DMPA condensation exhibits a high selectivity, leading to high yields with minimal intermolecular side products. These findings collectively highlight the robustness and efficiency of the 2-pyridone linkage for peptide macrocyclization.

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JACS Au 2024, 4, 3018−3027

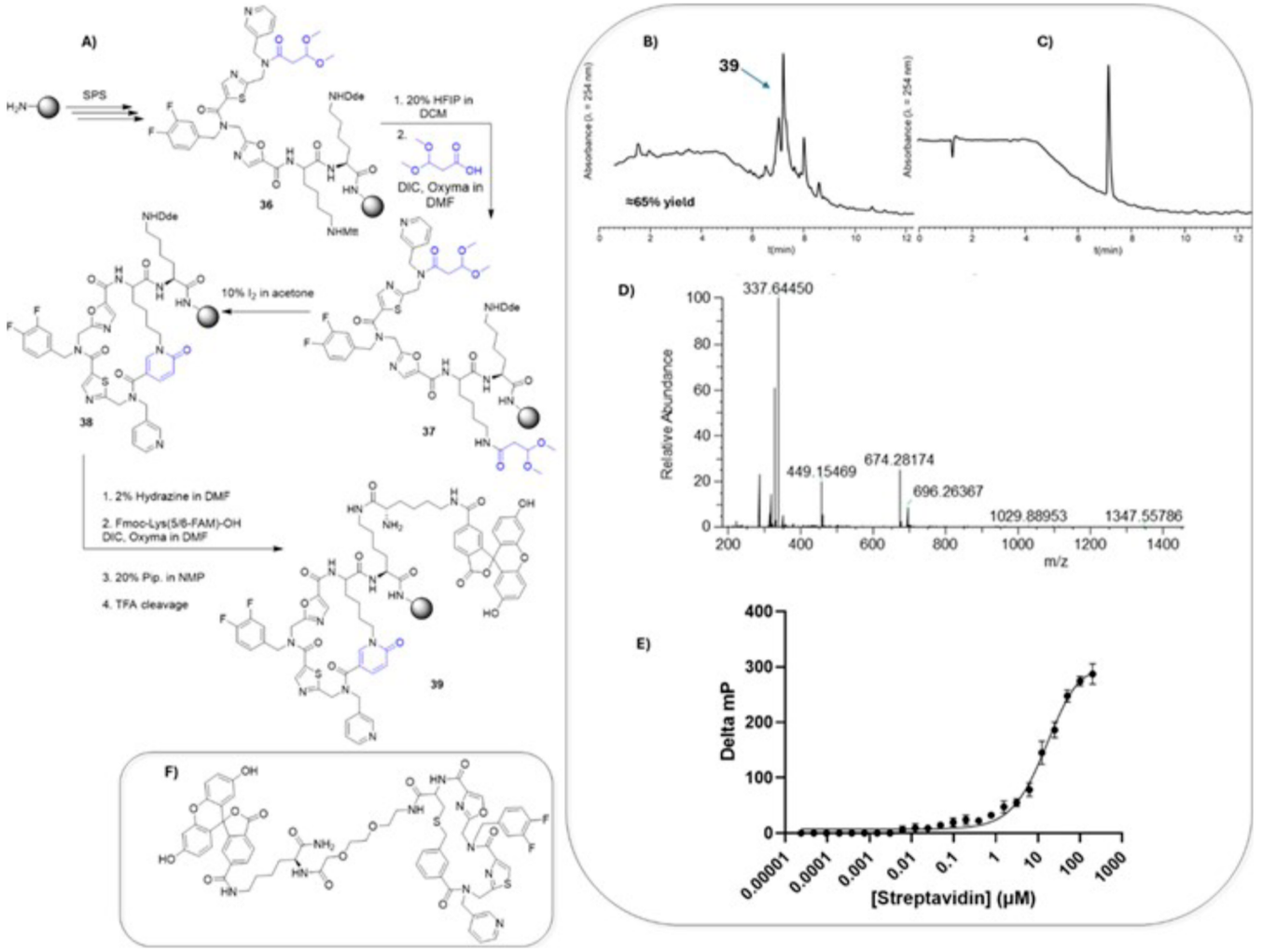

Figure 7. Synthesis and characterization of a non-peptidic Streptavidin-binding macrocycle including a 2-pyridone linkage. (A) Synthesis route to the labelled macrocycle. (B,C) HPLC chromatograms of crude and purified **39**. (D) High-resolution MS spectra of purified **39**. (E) Binding data. (F) Structure of the original thioether macrocycle.

METHODS

General Procedure for Peptides Synthesis (P1)

Syntheses were performed on a 0.05 mmol-per-reactor scale. Protected amino acids (6 equiv, 0.35 M) were coupled using Oxyma 0.5 M in DMF (5 equiv) and DIC 0.5 M in DMF (5 equiv) for 1 h at 37 °C. The capping of eventual unreacted amine groups was achieved by treatment with acetic anhydride (5 M in DMF) and DIEA (2 M in DMF) for 5 min. The Fmoc group was deprotected by two successive treatments with 20% piperidine in DMF for 3 and 5 min, respectively. DMPA addition was carried out following this protocol (P1).

General Procedure for TFA Cleavage (P2)

Peptidyl resin was washed with dichloromethane (DCM) and then dried under an air flow. The crude peptide was deprotected and cleaved from the resin by treatment with TFA/H_2O/iPr_3SiH (93:5:2 v/v/v) for 2 h, and the peptide was precipitated by dilution into an ice-cold diethyl ether, recovered by centrifugation and washed twice with diethyl ether.

General Procedure for On-Resin Cyclization and Stapling

Cyclization and stapling were carried out by treatment with 10% I_2 in acetone or TFA [1 mM] for 2 h at room temperature.

PICCO Synthesis

The synthesis was performed on a 0.05 mmol-per-reactor scale. The deprotected N-terminus was acylated by preparing a solution of halide displaying backbone (80 mM) and coupling reagents DIC/Oxyma (125, 80 mM, added to resin and incubated for 2 h at 37 °C. The resin was washed, and amine containing solution (1 M, DMF) was added and incubated for 3 h at 37 °C. These two steps were repeated until the peptoid synthesis was complete.

Minimum Inhibitory Concentration Assay

Cultures were grown to mid logarithmic phase and diluted to 10^5−10^6 cfu/mL in MH broth. The concentrations of peptides **28**, **31**, **32**, **35**, and kanamycin (used here as positive control) were present at a concentration of 1−500 μg/mL. All samples were loaded in triplicate, and the average OD_{600} value was taken for calculating the minimum inhibitory concentration (MIC). Each assay was repeated twice. Stock solutions were prepared in H_2O. Plates were loaded with a 95 μL bacterial suspension in MH broth and 5 μL aliquots of twofold serial dilutions of **28**, **31**, **32**, **35**, and kanamycin. Plates were then incubated at 37 °C for 20 h with gentle shaking. The MIC was defined as the lowest concentration of peptide that inhibited 90% of bacterial growth.

CD Measurements

The CD spectra of all peptides were measured by using a JACSO-(J-810) spectrophotometer (Japan). All samples (66 μM) were dissolved in a 1:1 mixture of water and trifluoroethanol (TFE). The following instrument parameters were employed: 50 nm/min scanning speed, 0.1 nm step size, 0.5 s response time, 0.1 cm path-length cell, and the scanning wavelength from 190 to 250 nm. The CD curves were smoothed by using standard parameters. Data were expressed as delta epsilon $\Delta\varepsilon$ (cm^{-1} M^{-1}). $\Delta\varepsilon$ was calculated as $[\theta \times (MRW \times 0.1)/(C \times$

https://doi.org/10.1021/jacsau.4c00352
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L)/3298], where θ is the ellipticity (in millidegrees), C is the concentration (in mg/mL), L is the path length (in cm), and MRW is the mean residue weight.

Fluorescence Polarization Assay

The concentrations of the labeled molecules were determined using the absorbance of carboxyfluorescein at 493 nm (ε493 = 83,000 M^{-1} cm^{-1}) using a Nanodrop UV−vis spectrophotometer (Thermo Scientific). FP binding saturation were performed in 384-well, medium bind black microtiter plates (Greiner Bio-One). Serial dilutions of streptavidin (238 nM to 200 μM) were titrated into PBS containing the fluorescent probe (10 nM final concentration, 10 μL final volume). Plates were incubated (30 min, RT, shaking) and read on an infinite M1000 ProPlate Reader (Tecan) using 470 nm excitation and 517 nm emission filters. The K_D value was obtained using Prism (GraphPad Software, Inc.) with a nonlinear regression with a one site total. The data shown are averages of technical triplicates.

More detailed procedures and characterization data are provided in the Supporting Information.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacsau.4c00352.

Detailed experimental and synthetic protocols and characterization of compounds (PDF)

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Author Contributions

S.A.A. conducted all experiments and data analysis and wrote the first draft of the manuscript. S.A.A. and T.K. conceptualized the work and participated in writing the final draft. T.K. guided and supervised the findings of this work. CRediT: **Skander A. Abboud** conceptualization, data curation, formal analysis, investigation, methodology, software, validation, writing-original draft, writing-review & editing; **Thomas Kodadek** conceptualization, funding acquisition, project administration, supervision, writing-review & editing.

Notes

The authors declare the following competing financial interest(s): T.K. is a significant shareholder in Triana Biomedicines and Deluge Biotechnologies.

■ ACKNOWLEDGMENTS

This work was funded by the National Cancer Institute (R21CA273954).

■ REFERENCES

(1) Yudin, A. K. Macrocycles: lessons from the distant past, recent developments, and future directions. *Chem. Sci.* **2015**, *6*, 30−49.

(2) Villar, E. A.; Beglov, D.; Chennamadhavuni, S.; Porco, J. A., Jr.; Kozakov, D.; Vajda, S.; Whitty, A. How proteins bind macrocycles. *Nat. Chem. Biol.* **2014**, *10*, 723−731.

(3) Boehm, M.; Beaumont, K.; Jones, R.; Kalgutkar, A. S.; Zhang, L.; Atkinson, K.; Bai, G.; Brown, J. A.; Eng, H.; Goetz, G. H.; Holder, B. R.; Khunte, B.; Lazzaro, S.; Limberakis, C.; Ryu, S.; Shapiro, M. J.; Tylaska, L.; Yan, J.; Turner, R.; Leung, S. S. F.; Ramaseshan, M.; Price, D. A.; Liras, S.; Jacobson, M. P.; Earp, D. J.; Lokey, R. S.; Mathiowetz, A. M.; Menhaji-Klotz, E. Discovery of Potent and Orally Bioavailable Macrocyclic Peptide-Peptoid Hybrid CXCR7Modulators. *J. Med. Chem.* **2017**, *60*, 9653−9663.

(4) Morgan, M.; Ikenoue, T.; Suga, H.; Wolberger, C. Potent macrocycle inhibitors of the human SAGA deubiquitinating module. *Cell Chem. Biol.* **2022**, *29*, 544−554.e4.

(5) Usanov, D. L.; Chan, A. I.; Maianti, J. P.; Liu, D. R. Second-generation DNA-templated macrocycle libraries for the discovery of bioactive small molecules. *Nat. Chem.* **2018**, *10*, 704−714.

(6) Hazama, D.; Yin, Y.; Murata, Y.; Matsuda, M.; Okamoto, T.; Tanaka, D.; Terasaka, N.; Zhao, J.; Sakamoto, M.; Kakuchi, Y.; Saito, Y.; Kotani, T.; Nishimura, Y.; Nakagawa, A.; Suga, H.; Matozaki, T. Macrocyclic Peptide-Mediated Blockade of the CD47-SIRPα Interaction as a Potential Cancer Immunotherapy. *Cell Chem. Biol.* **2020**, *27*, 1181−1191.e7.

(7) Beck, J. G.; Chatterjee, J.; Laufer, B.; Kiran, M. U.; Frank, A. O.; Neubauer, S.; Ovadia, O.; Greenberg, S.; Gilon, C.; Hoffman, A.; Kessler, H. Intestinal permeability of cyclic peptides: common key backbone motifs identified. *J. Am. Chem. Soc.* **2012**, *134*, 12125−12133.

(8) Whitty, A.; Zhong, M.; Viarengo, L.; Beglov, D.; Hall, D. R.; Vajda, S. Quantifying the chameleonic properties of macrocycles and other high-molecular-weight drugs. *Drug Discovery Today* **2016**, *21*, 712−717.

(9) Matsson, P.; Doak, B. C.; Over, B.; Kihlberg, J. Cell permeability beyond the rule of 5. *Adv. Drug Delivery Rev.* **2016**, *101*, 42−61.

(10) Over, B.; Matsson, P.; Tyrchan, C.; Artursson, P.; Doak, B. C.; Foley, M. A.; Hilgendorf, C.; Johnston, S. E.; Lee, M. D.; Lewis, R. J.; McCarren, P.; Muncipinto, G.; Norinder, U.; Perry, M. W. D.; Duvall, J. R.; Kihlberg, J. Structural and conformational determinants of macrocycle cell permeability. *Nat. Chem. Biol.* **2016**, *12*, 1065−1074.

(11) Ahlbach, C. L.; Lexa, K. W.; Bockus, A. T.; Chen, V.; Crews, P.; Jacobson, M. P.; Lokey, R. S. Beyond cyclosporine A: conformation-dependent passive membrane permeabilities of cyclic peptide natural products. *Future Med. Chem.* **2015**, *7*, 2121−2130.

(12) Mortensen, K. T.; Osberger, T. J.; King, T. A.; Sore, H. F.; Spring, D. R. Strategies for the Diversity-Oriented Synthesis of Macrocycles. *Chem. Rev.* **2019**, *119* (17), 10288−10317.

(13) Connors, W. H.; Hale, S. P.; Terrett, N. K. DNA-encoded chemical libraries of macrocycles. *Curr. Opin. Chem. Biol.* **2015**, *26*, 42−47.

(14) Roy, A.; Koesema, E.; Kodadek, T. High-Throughput Quality Control Assay for the Solid-Phase Synthesis of DNA-Encoded Libraries of Macrocycles*. *Angew. Chem., Int. Ed. Engl.* **2021**, *60*, 11983−11990.

(15) Koesema, E.; Roy, A.; Paciaroni, N. G.; Coito, C.; Tokmina-Roszyk, M.; Kodadek, T. Synthesis and Screening of a DNA-Encoded Library of Non-Peptidic Macrocycles. *Angew. Chem., Int. Ed. Engl.* **2022**, *61*, No. e202116999.

(16) Stress, C. J.; Sauter, B.; Schneider, L. A.; Sharpe, T.; Gillingham, D. A DNA-Encoded Chemical Library Incorporating Elements of Natural Macrocycles. *Angew. Chem., Int. Ed.* **2019**, *58*, 9570−9574.

(17) Buyanova, M.; Pei, D. Targeting intracellular protein-protein interactions with macrocyclic peptides. *Trends Pharmacol. Sci.* **2022**, *43*, 234−248.

(18) White, C. J.; Yudin, A. K. Contemporary strategies for peptide macrocyclization. *Nat. Chem.* **2011**, *3*, 509−524.

(19) Bechtler, C.; Lamers, C. Macrocyclization strategies for cyclic peptides and peptidomimetics. *RSC Med. Chem.* **2021**, *12*, 1325−1351.

(20) Wills, R.; Adebomi, V.; Raj, M. Site Selective Peptide Macrocyclization. *ChemBioChem* **2021**, *22*, 52−62.
(21) Nitsche, C.; Onagi, H.; Quek, J.-P.; Otting, G.; Luo, D.; Huber, T. Biocompatible Macrocyclization between Cysteine and 2-Cyanopyridine Generates Stable Peptide Inhibitors. *Org. Lett.* **2019**, *21*, 4709−4712.
(22) Spokoyny, A. M.; Zou, Y.; Ling, J. J.; Yu, H.; Lin, Y.-S.; Pentelute, B. L. A Perfluoroaryl-Cysteine SNAr Chemistry Approach to Unprotected Peptide Stapling. *J. Am. Chem. Soc.* **2013**, *135*, 5946−5949.
(23) Adebomi, V.; Cohen, R. D.; Wills, R.; Chavers, H. A. H.; Martin, G. E.; Raj, M. CyClick Chemistry for the Synthesis of Cyclic Peptides. *Angew. Chem., Int. Ed.* **2019**, *58*, 19073−19080.
(24) Gui, W.; Kodadek, T. Facile Synthesis of Homodimeric Protein Ligands. *Chembiochem* **2023**, *24*, No. e202300392.
(25) Abboud, S.; Kodadek, T. 2-pyridone formation: An efficient method for the solid-phase synthesis of homodimers. *Chem. - Eur. J.* **2024**, *30*, No. e2023029.
(26) Sun, J.; Dong, Y.; Cao, L.; Wang, X.; Wang, S.; Hu, Y. Highly Efficient Chemoselective Deprotection of O,O-Acetals and O,O-Ketals Catalyzed by Molecular Iodine in Acetone. *J. Org. Chem.* **2004**, *69*, 8932−8934.
(27) Moretta, A.; Scieuzo, C.; Petrone, A. M.; Salvia, R.; Manniello, M. D.; Franco, A.; Lucchetti, D.; Vassallo, A.; Vogel, H.; Sgambato, A.; Falabella, P. Antimicrobial Peptides: A New Hope in Biomedical and Pharmaceutical Fields. *Front. Cell. Infect. Microbiol.* **2021**, *11*, 668632.
(28) Zhang, R.; Yan, H.; Wang, X.; Cong, H.; Yu, B.; Shen, Y. Screening of a short chain antimicrobial peptide-FWKFK and its application in wound healing. *Biomater. Sci.* **2023**, *11*, 1867−1875.
(29) Jindrichova, B.; Burketova, L.; Novotna, Z. Novel properties of antimicrobial peptide anoplin. *Biochem. Biophys. Res. Commun.* **2014**, *444*, 520−524.
(30) Wu, Y.; Lu, D.; Jiang, Y.; Jin, J.; Liu, S.; Chen, L.; Zhang, H.; Zhou, Y.; Chen, H.; Nagle, D. G.; Luan, X.; Zhang, W. Stapled Wasp Venom-Derived Oncolytic Peptides with Side Chains Induce Rapid Membrane Lysis and Prolonged Immune Responses in Melanoma. *J. Med. Chem.* **2021**, *64*, 5802−5815.
(31) Macyszyn, J.; Burmistrz, M.; Mieczkowski, A.; Wojciechowska, M.; Trylska, J. Conjugates of Aminoglycosides with Stapled Peptides as a Way to Target Antibiotic-Resistant Bacteria. *ACS Omega* **2023**, *8*, 19047−19056.
(32) Heyduk, T.; Ma, Y.; Tang, H.; Ebright, R. H. Fluorescence anisotropy: Rapid, quantitative assay for protein-DNA and protein-protein interaction. *Methods Enzymol.* **1996**, *274*, 492−503.

https://doi.org/10.1021/jacsau.4c00352
JACS Au 2024, 4, 3018−3027