Application of a Semi-Automated Solid-Phase Synthesis Platform for the Discovery of Macrocyclic Peptide Cyclin A RxL Inhibitors

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BACKGROUND

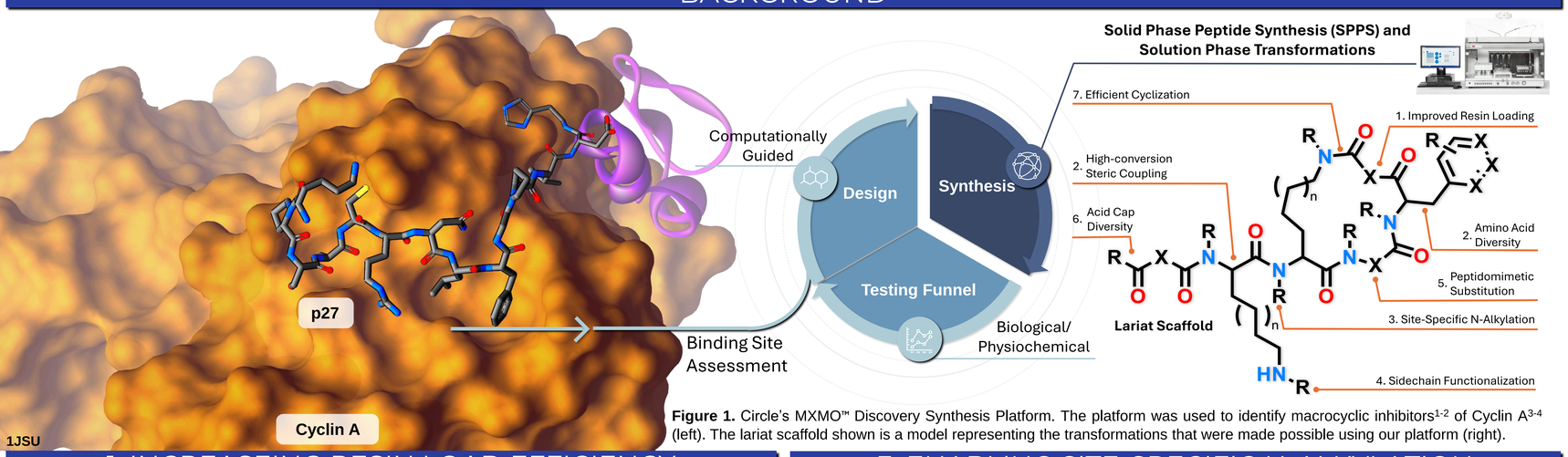
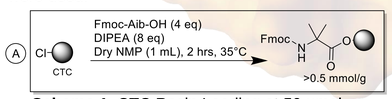


Figure 1. Circle's MXMO™ Discovery Synthesis Platform. The platform was used to identify macrocyclic inhibitors[1-2] of Cyclin A[3-4] (left). The lariat scaffold shown is a model representing the transformations that were made possible using our platform (right).

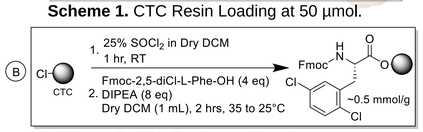
1. INCREASEING RESIN LOAD EFFICIENCY

2-chlorotrityl Chloride (CTC) Resin. CTC resin was widely utilized for routine SPPS due its versatility.

- Simplicity of racemization-free resin loading through pretreatment of AA with DIPEA prior to addition to resin was excellent for automated SPPS.
- Cleavage with mild acid condition allowed retention of acid-labile PG for selective cyclizations.
- The steric bulk of CTC resin combined with shortened FMOC deprotection times helped prevent premature cleavage during FMOC deprotection via DKP formation when β turn-inducers were used.



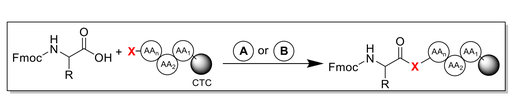
Scheme 1. CTC Resin Loading at 50 µmol.



Scheme 2. CTC Resin Loading of Functionalized Phe Derivatives at 50 µmol.[5]

Table 1. CTC Resin Loading Efficiency Relative to AA Type. Synthesis scale at 50 µmol. Conditions A and B are detailed in Scheme 1 and 2 respectively.

2. STERICALLY HINDERED AMIDE COUPLING



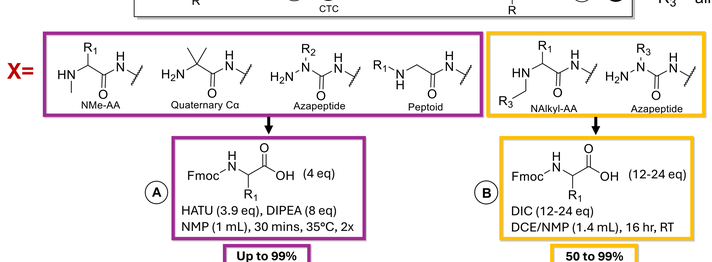
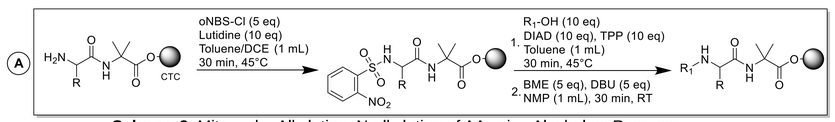


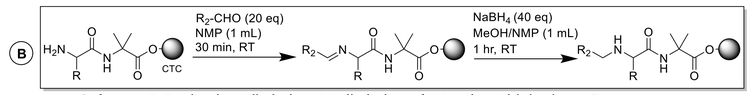
Figure 2. Coupling Condition Decision Schematic. Choice **A** or **B** is relative to steric hindrance of the preceding AA.

HATU/DIPEA served as our main coupling reagent for routine SPPS as it consistently produced low racemization AA couplings at high conversions. Also, it dependably coupled AAs onto NMe-AA species or other 2º amines if reacted twice at 50 µmol scale.

3. ENABLING SITE-SPECIFIC N-ALKYLATION



Scheme 3. Mitsunobu Alkylation. N-alkylation of AA using Alcohol as R source.

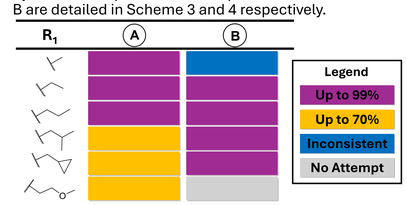


Scheme 4. Reductive Alkylation. N-alkylation of AA using Aldehyde as R source.

Fukuyama-Mitsunobu[6] (A). This method was applied to satisfy on-demand alkylation of key residues. Notably, it was our core method for ubiquitous N-methylation of peptide backbones and certain amine sidechains.

Reductive Alkylation[7] (B). In scenarios where Mitsunobu alkylation was ineffective, reductive alkylation was employed to fulfill design demands. However, attaining mono-methylation of peptide backbone was inconsistent and the incompatibility of formaldehyde or 'cracked' paraformaldehyde posed difficulty in automatable on-resin peptide synthesis settings.

Table 2. N-Alkylation Efficiency Relative to R Groups. Synthesis was performed at 50 µmol. Conditions A and B are detailed in Scheme 3 and 4 respectively.



4. ON-RESIN SIDECHAIN FUNCTIONALIZATION

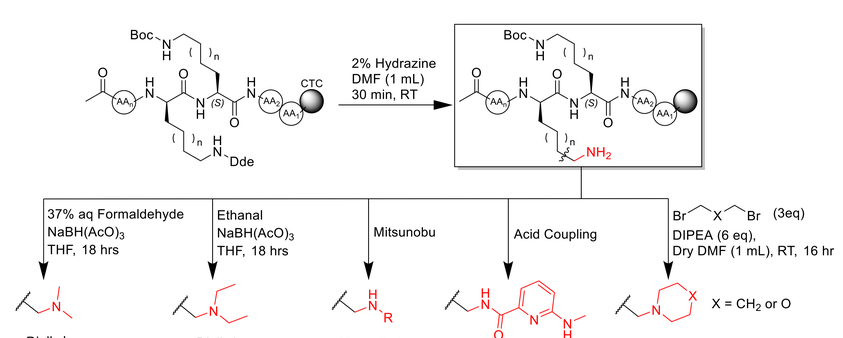
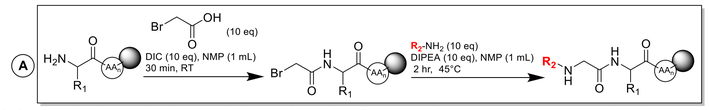
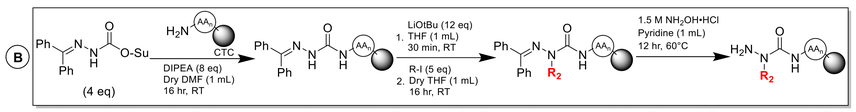


Figure 3. Late-stage On-resin Sidechain Amine Functionalization. Dde protected Lys derivative were used for PG orthogonality with Boc protected Lys derivatives. Various methods were applied to attain diverse and on-demand sidechain functionalization at 50 µmol.

5. PEPTIDOMEMETIC SUBSTITUTIONS



Scheme 5. On-resin Peptoid Formation Via Classical Sub-monomer Approach[8]. Example R_2 sidechains are displayed in Figure 15. Peptoids were incorporated on lariat scaffold at 50 µmol scale.



Scheme 6. On-resin Sub-monomer Azapaptide Synthesis[9]. Starred compounds in Figure 15 are example R_2 sidechains that were applied in lariat scaffold at 50 µmol scale.

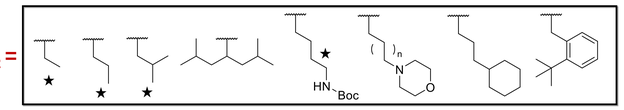
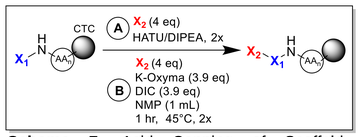
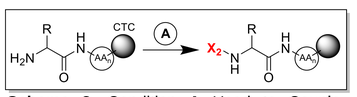


Figure 4. Example Sidechains that were Applied in **A** and **B**. Starred compounds were used for both A and B, unstarred compounds were used for A only.

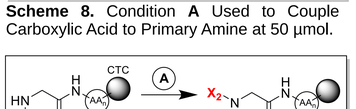
6. N-TERMINAL CARBOXYLIC ACID DIVERSITY



Scheme 7. Acid Capping of Scaffold. Condition **A** or **B** usage was determined based on the identify of (X_2) and the preceding residue (X_1) in accordance to Figure 5.



Scheme 8. Condition **A** Used to Couple Carboxylic Acid to Primary Amine at 50 µmol.



Scheme 9. Condition **A** Used to Couple Carboxylic Acid to Peptoid at 50 µmol.

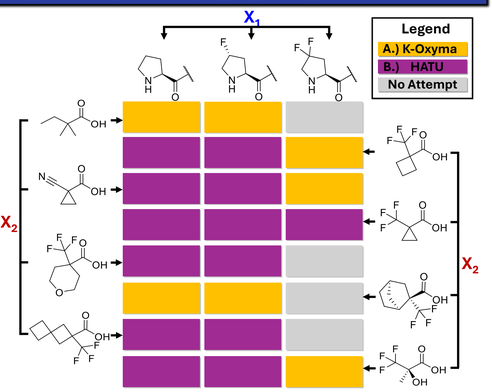
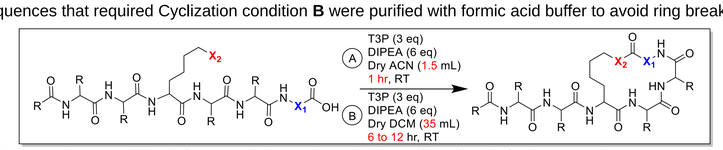


Figure 5. Acid (X_2) Capping of Pro Derivatives (X_1). Acid coupling via HATU (**A**, mauve) achieved 70 to 99% conversion. K-Oxyma (**B**, gold) was used in cases where HATU failed. Acid Capping with K-Oxyma achieved 50 to 70% conversion. Up to 30% Oxyma byproduct observed with use of K-Oxyma conditions.

7. LOW-VOLUME CYCLIZATION

Figure 6. Post-SPPS Workflow. Elimination of linear RP-LC purification step allows rapid progression. Sequences that required Cyclization condition **B** were purified with formic acid buffer to avoid ring breakage.



Scheme 10. Lariat Scaffold Macrolactamization at 50 µmol Scale. Cyclization condition is dependent on nature of reacting moieties, X_1 and X_2. Condition **A** and **B** usage is detailed in Figure 7.

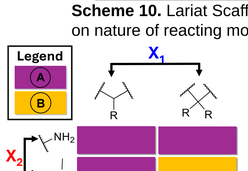


Figure 7. Cyclization Condition Relative to Cyclization Point, X_1 and X_2.

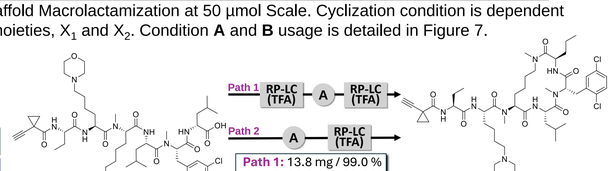


Figure 8. Crude Linear Low-volume Cyclization of **3**[10] at 50 µmol Scale. Workup free cyclization, Path 2, achieved acceptable yield and purity in shorter time.

REFERENCES: 1.) Andrews, M. J. I.; McInnes, C.; Kontopidis, G.; Innes, L.; Cowan, A.; Plater, A.; Fischer, P. M. Design, Synthesis, Biological Activity and Structural Analysis of Cyclic Peptide Inhibitors Targeting the Substrate Recruitment Site of Cyclin-Dependent Kinase Complexes. Org Biomol Chem 2004, No. 19, 2735. https://doi.org/10.1039/b409157d. **2.)** Buyanova, M.; Pei, D. Targeting Intracellular Protein–Protein Interactions with Macrocyclic Peptides. Trends Pharmacol Sci 2022, No. 3, 234–248. https://doi.org/10.1016/j.tips.2021.11.008. **3.)** Crystal structure of the p27^{Kip1} cyclin-dependent-kinase inhibitor bound to the cyclin A/CDK2 complex. Russo, A. A. et al. Nature, 1996, 382, 325-331, DOI: 10.1038/382325a0 **4.)** Chen, Y.-N. P.; Sharma, S. K.; Ramsey, T. M.; Jiang, L.; Martin, M. S.; Baker, K.; Adams, P. D.; Bair, K. W.; Kaelin, W. G. Selective Killing of Transformed Cells by Cyclin/Cyclin-Dependent Kinase 2 Antagonists. Proc Natl Acad Sci U S A 1999, No. 8, 4325–4329. https://doi.org/10.1073/pnas.96.8.4325. 5.) Román T, Acosta G, de la Torre BG, Cárdenas C, Guzmán F, Albericio F. Improving 2-Chlorotrityl Chloride (2-CTC) Resin Activation. Methods Protoc. 2023 Sep 8;6(5):82. doi: 10.3390/mps6050082. **6.)** Chatterjee, J.; Laufer, B.; Kessler, H. Synthesis of N-Methylated Cyclic Peptides. Nat Protoc 2012, No. 3, 432–444. https://doi.org/10.1038/nprot.2011.450. **7.)** Pels, K.; Kodadek, T. Solid-Phase Synthesis of Diverse Peptide Tertiary Amides by Reductive Amination. ACS Comb Sci 2015, No. 3, 152–155. https://doi.org/10.1021/acscombsci.5b00007. **8.)** Zuckermann, R. N.; Kerr, J. M.; Kent, S. B. H.; Moos, W. H. Efficient Method for the Preparation of Peptoids [oligo(N-Substituted Glycines)] by Submonomer Solid-Phase Synthesis. J Am Chem Soc 1992, No. 26, 10646–10647. https://doi.org/10.1021/ja00052a076. **9.)** Chingle, R.; Proulx, C.; Lubell, W. D. Azapeptide Synthesis Methods for Expanding Side-Chain Diversity for Biomedical Applications. Acc Chem Res 2017, No. 7, 1541–1556. https://doi.org/10.1021/acs.accounts.7b00114. **10.)** Cyclin Inhibitors, W0 2024/086814 A2, International Publication Date 25 April 2024.