Expanding Circle Pharma's Discovery Synthesis Toolbox for Permeable Macrocycles With Automatable Bioisoteric Cyclization Chemistries

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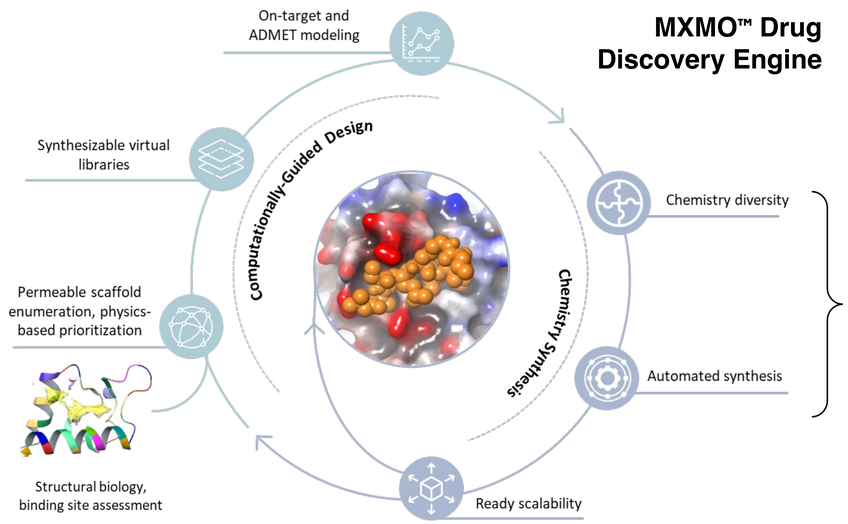


MXMO™ Drug Discovery Engine

MXMO™ Macrocycle Discovery Synthesis Workflow

SPPS/on-resin cyclization

Optional:
Functionalization
Cyclization
Deprotection

Purification

Sufficient quantity and quality material for:
- Biochemical potency
- Physicochemical properties
- Cell proliferation
- *In vitro* ADME
- *In vivo* ADME
- X-ray Crystallography
- NMR spectroscopy

Scale	Efficiency	Quality	Turnaround
▪ Discovery: 5-30 mg per peptide ▪ Lead optimization: 200 mg to grams	▪ 48 macrocycles per automated synthesis instrument per cycle	▪ >95% purity for reliable data generation	▪ Workflow cycle: 1-2 weeks from design to assay

Background

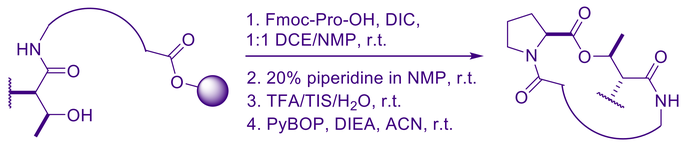
Circle Pharma Inc. is a leader in the development of macrocyclic peptides for treatment of diseases traditionally thought to be "undruggable", bridging the gap between small molecules and monoclonal antibodies. Our proprietary MXMO™ platform is a robust semi-automated system that seamlessly combines computationally guided design with well executed wet lab macrocycle synthesis. Our drug discovery engine is adaptable, agile, and highly iterative, allowing us to hone onto advantageous molecular, structural, and conformational motifs for target engagement and passive permeability after only a few design cycles.

A major hurdle to the development of macrocyclic peptides is enabling passive permeability. One approach is to reduce the number of available hydrogen bond donors, using strategies such as the replacement of backbone amides with N-alkylation, the synthesis of peptoids, and other bioisosteres. The incorporation of increasing structural complexity into these molecules poses many challenges for traditional solution phase synthetic approaches, which can be addressed via solid-phase peptide synthesis (SPPS).

To enable rapid exploration of chemical space, Circle Pharma has adapted a variety of cyclization strategies, diversifying our access to unique macrocyclic peptides, as well as enriching our chemical toolbox; herein, we showcase synthetic features of our discovery engine, encompassing cyclization methods such as lactonization, lactamization, Click chemistry, cyclic thioethers, and ring-closing metathesis, most of which are amenable to on-resin automation (Schemes 1-7.)

MXMO™ Generates Diverse Macrocycles Enabling Rapid Exploration of Vast Chemical Space

De facto Lactonization



Scheme 1. Representative reaction for *de facto* lactonization,[1] circumventing challenges inherent in other macrolactonization methods e.g., Yamaguchi Lactonization.

On-resin Lactamization

Scheme 2. Representative reaction for *on-resin* lactamization,[2] which can be carried out on automation.

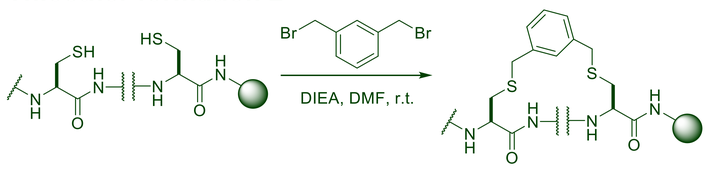
Aminothiol Click chemistry

Scheme 3. Representative reaction for *on-resin* aminothiol click reaction,[3] which can be carried out on automation allowing access to N-terminal lariats.

Thioether formation 1

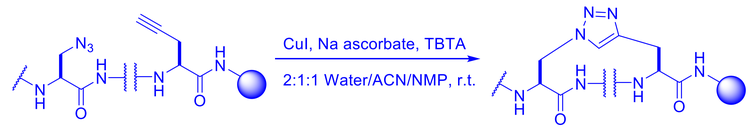
Scheme 4. Representative reaction for *on-resin* thioether formation between cysteine and chloroacetic acid,[4] which can be carried out on automation allowing access to N-terminal lariats.

Thioether formation 2



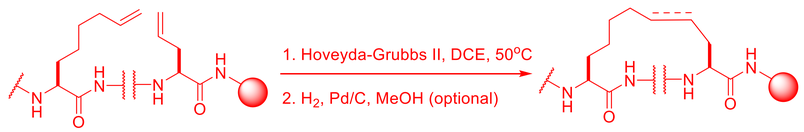
Scheme 5. Representative reaction for *on-resin* thioether formation via *m*-xylene linkage,[5] which can be carried out on automation.

Click chemistry (1,4-triazole)



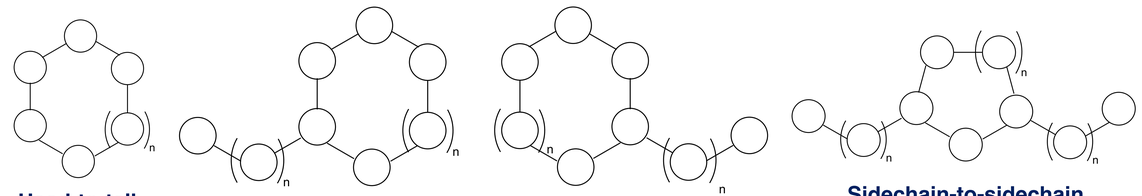
Scheme 6. Representative reaction for *on-resin* copper-assisted azide/alkyne cyclization (CuAAC)[6]; the position of the 1,4-triazole is tunable via switching positions of the relevant building blocks.

Ring-Closing Metathesis



Scheme 7. Representative reaction for *on-resin* ring-closing metathesis (RCM)[7], which can be carried out on automation; hydrogenation can also be carried out on-resin.

Scaffold Topologies Enabled by and Accessible via MXMO™



Head-to-tail | **N- and C-terminal Lariats** | **Sidechain-to-sidechain**

References

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