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Improving binding affinity through cyclization

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1. Introduction

Background and Significance

Most cancer chemotherapy makes patients sick because the drugs used are toxic to normal tissues as well. If one could selectively sensitize a cancer cell to chemotherapy or radiation, patients could receive lower doses of the chemotherapy, which in turn would reduce this unwanted toxicity. Since many types of cancer chemotherapy and radiation cause DNA damage, one way to sensitize a cancer cell to therapy is by interfering with the mechanisms by which it repairs DNA. Work from the Hartman lab developed a peptide that binds with moderate affinity (K_D of 1 μM) to the C-terminal $(BRCT)_2$ domain of breast cancer associated protein (BRCA1).[1] This protein is a critical component of DNA repair by homologous recombination.

2. Hypothesis

The crystal structure of peptide 8.6 (MCTIDFDEYRFRKT) bound to BRCA1 $(BRCT)_2$ domain shows that the side chains of the underlined tyrosine and threonine residues are close together (Fig. 1). Our hypothesis is that by cyclizing these positions, the peptide may become constrained into its bound conformation.

We used a bis-cysteine mutant of our peptide MCTIDFNECRFRKC that will mimic the bound conformation when cyclized. A series of bis(bromomethyl)naphthalene linkers (Table 1) will be used for cyclization in order to vary and optimize the end-to-end distance of the cysteine residues.

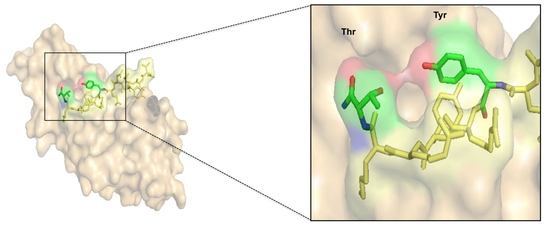


Figure 1. Crystal Structure of bound peptide 8.6 on BRCA1

3. Methods

Synthesis of linkers (Fig. 2)
-monitored by thin layer chromatography
-purified through column chromatography
-final products confirmed by ^{1}H and ^{13}C NMR.

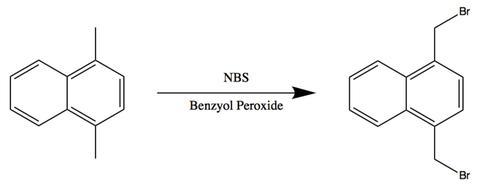
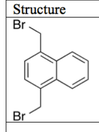
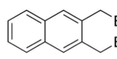
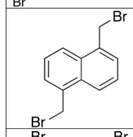
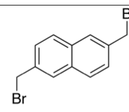
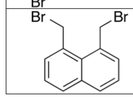
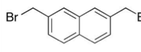


Figure 2. Synthesis of 1,4-bis(bromo)methylnaphthalene

Table 1. Synthesized and commercially purchased linkers used to cyclize

Structure	Name	Structure	Name
	1,4- bis(bromomethyl) naphthalene		2,3- bis(bromomethyl) naphthalene
	1,5- bis(bromomethyl) naphthalene		2,6- bis(bromomethyl) naphthalene
	1,8- bis(bromomethyl) naphthalene		2,7- bis(bromomethyl) naphthalene

Synthesis of peptide 8.6 (Fig. 3 A)
-automated microwave-enhanced solid-phase peptide synthesis (SPPS)

Deprotection of s-t-butyl and FMOC groups (Fig. 3 B)
- In DTT and ammonium bicarbonate

Cyclization with linker (Fig. 3 C)
- In TCEP and ammonium carbonate

Fluorescence labeling (Fig. 4 D)
-5-FAM with DCC and HOBt

Methods cont.

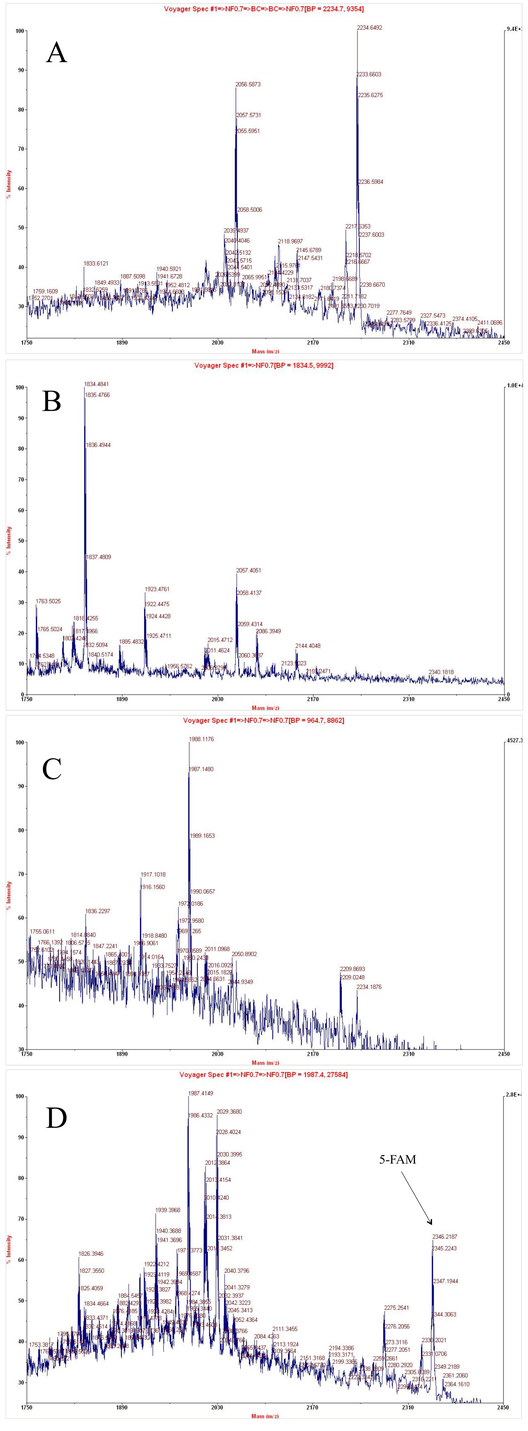


Figure 3. MALDI-MS of peptide 8.6 at synthesis (A), deprotection (B), cyclization (C), fluorescence labeling (D)

4. Discussion

Why use dibromomethylnaphthalene linkers?

- Very robust, easy to cyclize with cysteines[2]
- mRNA display compatible (can use this cyclization strategy for future targets)
- Attaching the bromomethyl groups at different locations of the naphthalene ring provides variability in size

The 7th residue in the sequence was changed from aspartate to asparagine to avoid aspartimide formation during synthesis.

5. Future Direction

The binding of the cyclic peptides with BRCA1 (BRCT)2 domain will be compared to peptide 8.6 through the use of fluorescence polarization.

The Hartman lab is also working to increase binding affinity through various mutations to the peptide sequence.

6. References and Recognition

[1]White, E. R.; Sun, L.; Ma, Z.; Beckta, J. M.; Danzig, B. A.; Hacker, D. E.; Huie, M.; Williams, D. C.; Edwards, R. A.; Valerie, K.; Glover, J. N. M.; Hartman, M. C. T. Peptide Library Approach to Uncover Phosphomimetic Inhibitors of the BRCA1 C-Terminal Domain. ACS Chem. Biol. 2015, 10 (5), 1198–1208.

[2]Dewkar, G. K.; Carneiro, P. B.; Hartman, M. C. T. Org. Lett. 2009, 11, 4708. (b) Smeenk, L. E. J.; Dailly, N.; Hiemstra, H.; van Maarseveen, J. H.; Timmerman, P. Org. Lett. 2012, 14, 1194

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