Identification of Histone H4-Based Peptoids as Inhibitors of PRMT1

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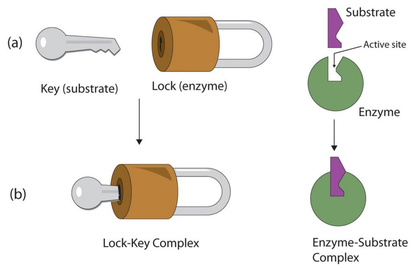
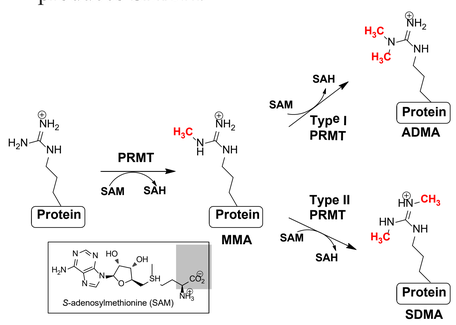
Protein Arginine Methyltransferases (PRMTs) are a family of 11 mammalian enzymes characterized by the post-translational methylation of arginine residues in the histone tail. The majority of the 11 members of the PRMT family are divided into two main types, Type I and Type II. PRMT1, the major Type I isozyme, catalyzes the formation of asymmetrically dimethylated arginine (ADMA). PRMT1 activates transcription of cancer genes. Peptoids, or poly-N-substituted glycine's are a class of oligomers whose side chains are appended to the nitrogen atom of the peptide backbone rather than the alpha carbon. Kinetic parameters were conducted for both peptide and peptoid sequences. The K_{cat}/K_m and IC_{50} values determined that peptoids show inhibition activity. The specificity and location of these interactions are currently being determined by altering the residues of a known peptoid sequence that has these interactions.

Background

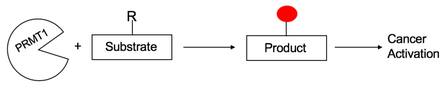
- Enzymes bind with substrates in the active site. The active site is composed of unique amino acid residues which create a specific, ideal environment. This enzyme-substrate complex is formed via a lock and key model shown below.



- PRMTs post translationally modify arginine residues by methylating its histone tails. PRMT1 produces ADMA, while PRMT5 produces SMDA.



- PRMT1 can activate transcription of cancer-related genes. Inhibition of PRMT1 would be beneficial to repress activation of these cancer-related genes



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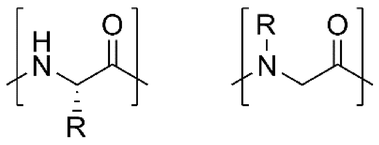
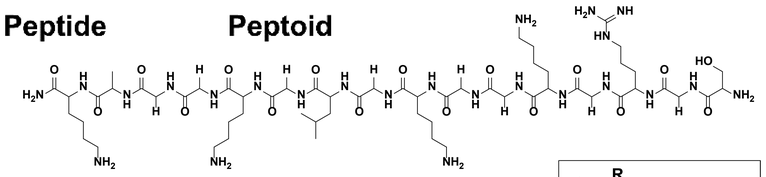
General Peptidomimetics

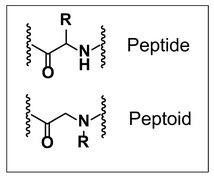


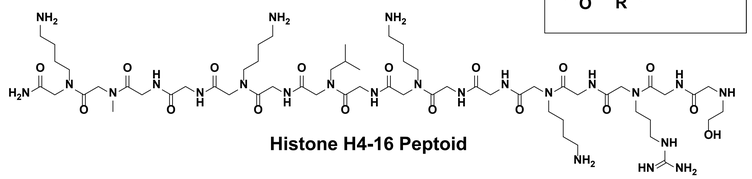
- Peptoids are similar to peptides but differ in the location of the side chain. Peptoids have high bio stability because they are not susceptible to proteases.

Peptide **Peptoid**



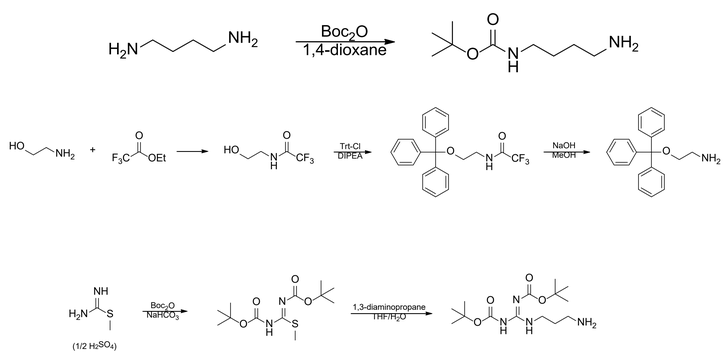
Histone H4-16 Peptide

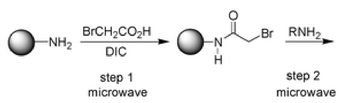




Histone H4-16 Peptoid

Peptoid Monomer Synthesis





- The amines that were not commercially available were synthesized. These include N-Lysine, N-Serine, N-Arginine and N-Leucine, whereas both N-alanine and N-glycine were readily available.

Kinetic Parameters and IC_{50} values of Peptides and Peptoids

Kinetic parameters of selected peptides

Peptide	k_{cat}(min^{-1})	K_m(μM)	K_{cat}/K_m(M^{-1}min^{-1})
AcH4-21	3.55 ± 0.68	0.39 ± 0.01	1.10×10^5
AcH4-16	152 ± 14	0.28 ± 0.01	1.82×10^3
AcH4-13	365 ± 174	0.18 ± 0.05	4.94×10^2

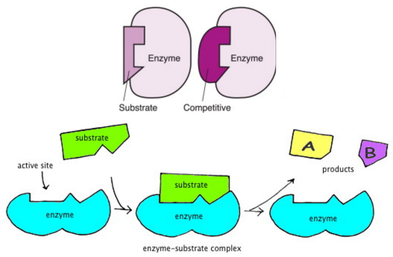
[a]These values were reported in Osborne et al[1]. The SAM concentration for determining these values was 15 μM.

Kinetic parameters of peptoids

Peptoid	k_{cat}(min^{-1})	K_m(μM)	K_{cat}/K_m(M^{-1}min^{-1})
AcH4-16	nd[a]	nd[a]	nd[a]
AcH4-13	nd[a]	nd[a]	nd[a]
AcH4-8	nd[a]	nd[a]	nd[a]

[a]The kinetic parameters for these compounds were not determined due to a lack of product formation, SAM concentration was 40 μM.

Substrate vs Inhibitor



IC_{50} values of peptoids

Acetylated Peptoids		Unacetylated Peptoids	
Peptoid	IC_{50} (μM)	Peptoid	IC_{50}(μM)
AcH4-16	916 ± 19.5	H4-16	396 ± 31.4
AcH4-13	>1000	H4-13	898±93.5
AcH4-8	>1000	H4-8	>1000

[a]SAM concentration was 40 μM.

Future Directions

Peptoid	Sequence
H4-16 K12A	KAGGAGLGKGGKGRGS
H4-16 K16A	AAGGKGLGKGGKGRGS
H4-16 K12A K16A	AAGGAGLGKGGKGRGS

- Determine the kinetic parameters and IC_{50} values for the three H4-16 peptoids.
- We also plan on testing the H4-16 peptoid as a possible inhibitor for PRMT5
- Determine the the type of inhibition
- Determine the binding site for the H4-16 peptoid

Conclusions

We were able to identify a novel PRMT inhibitor. Advantages of this inhibitor are that it is resistant to proteases due to the peptoid structure and the fact that it lacks peptide bonds. This inhibitor is likely a competitive inhibitor and may be specific to PRMT1.

Acknowledgements

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