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Peptide-based probes for protein N-Methyltransferases

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Chapter 1

Introduction

1. Introduction to protein methyltransferases

Protein methyltransferases (PMTs) are responsible for the methylation of amino acid residues in a number of proteins including histones. PMTs that act on histones are in turn highly involved in the regulation of gene expression and transcription.¹ Protein methylation can occur on lysine, arginine, histidine, or glutamine side chains as well as on α -N-terminal residues.² All PMTs use a common mechanism of catalysis, in which the universal methyl donor S-adenosyl-L-methionine (AdoMet) and the target protein substrate bind the enzyme to form a ternary complex and, after transfer of the methyl group, the methylated protein substrate and the demethylated cofactor S-adenosyl-L-homocysteine (AdoHcy) are released from the active site. Methyltransferase enzymes share high homology in the active site residues that interact with the AdoMet cofactor, but can be distinguished by their ability to methylate specific amino acids of a small selection of protein substrates.

The methylation of lysine side chains and N-terminal amino acids result in mono-, di and trimethylated products,³ while the arginine side chain can be monomethylated or symmetrically or asymmetrically dimethylated on its guanidyl group.⁴ Histidine can be monomethylated on its imidazole nitrogen atom⁵ and glutamine can be monomethylated to N⁵-methylated glutamine (Figure 1).⁶ Histone methylation has been extensively investigated since it was first recognized in the early 2000s and it has since been shown to be vital in mediating cell signalling and a range of cellular functions.⁷⁻¹⁰ For example, histone H3 methyltransferases control the DNA methylation¹¹ and the lysine methyltransferase Dot1p is involved in telomere silencing.¹² Aberrant expression of PMTs and their concomitant deleterious effects can be observed in cancer¹³, diabetes,¹⁴ and neurological diseases.¹⁵ Due to their role in a variety of key cellular functions, the discovery of selective inhibitors of PMTs has increasingly become an avenue of interest for therapeutic development^{16, 17}. Inhibitors of protein lysine methyltransferases, such as UNC0642 (G9a/GLP) against breast cancer,¹⁸ EPZ005687 (EZH2) against lymphoma cells,¹⁹ and CPI-1205 (EZH2) against B-cell lymphoma²⁰ have been investigated in preclinical trials. In addition, protein arginine methyltransferases inhibitors, MS049 (PRMT4/6),²¹ TP-064 (CARM1),²² and EPZ020411 (PRMT6),²³ are highly selective chemical tools for inhibiting specific protein arginine methyltransferases (PRMTs). The different histone protein methylations regulate gene expression and transcription through a dynamic interplay of chromatin readers, writers, and erasers.^{24, 25} To gain a deeper understanding of the mechanistic processes related to histone methylation, peptide-based probes are valuable tools for understanding the roles of specific enzymes in the complexity of epigenetics. In this thesis, the development of peptide-based probes specific for the PRMT family of methyltransferases is discussed. Inspired by naturally occurring PRMT substrates, the general

methodology to design the peptide probes is based on the substitution and modification of the guanidine moiety of the target arginine residue. Through this methodology, the peptide based probes retain the selectivity and specificity of the peptide substrate, while the guanidine side chain modification mimics the methyltransferase transition state, turning a substrate into an inhibitor. These tool compounds provide detailed insight into the interactions of peptide substrate and AdoMet cofactor in the active site of the targeted methyltransferase.

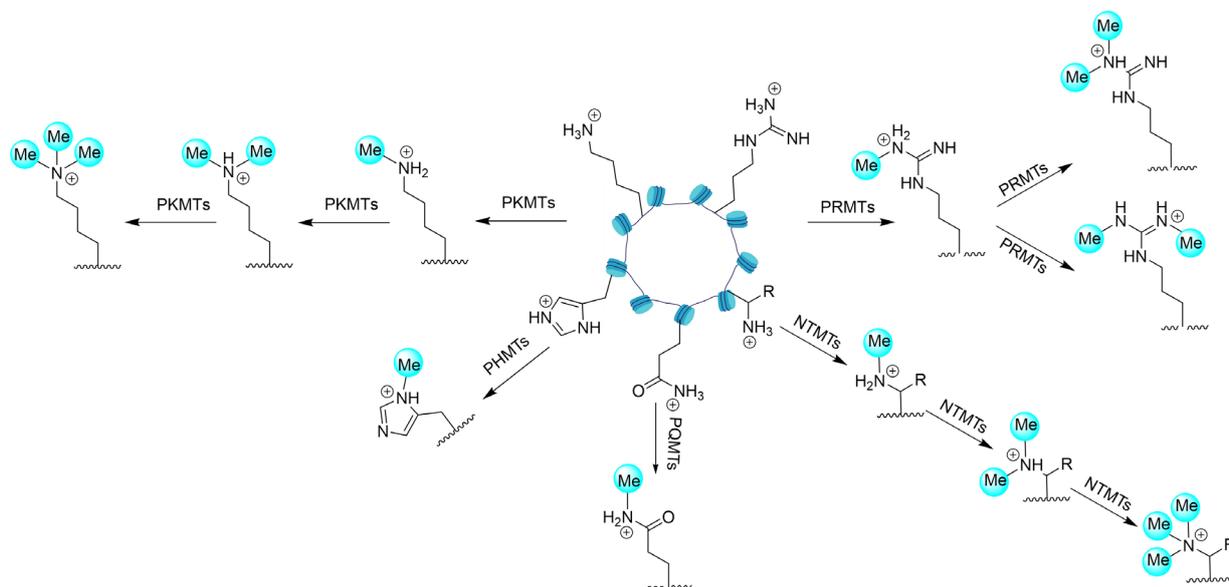


Figure 1. The different types of histone protein methyltransferases and their products. Protein lysine methyltransferases (PKMTs) form mono-, di- or tri-methylated lysine, Protein arginine methyltransferases (PRMTs) form mono-, asymmetrically or symmetrically dimethylated arginine; Protein histidine methyltransferases (PHMTs) form monomethylated histidine; Protein glutamine methyltransferase (PQMTs) form N⁵-methylated glutamine; and Protein N-terminal methyltransferases (NTMTs) form mono-, di-, or tri-methylated N-terminal.

2. Peptide Based Inhibitors and Mechanistic Probes for Protein Arginine Methyltransferases

Protein arginine methyltransferases catalyse the transfer of the methyl group from cofactor AdoMet to the guanidine moiety in the sidechain of arginine residues of protein substrates. After binding of the substrate and the cofactor to the catalytic domain of the PRMT, the methyl group is transferred from AdoMet to the arginine side chain following an S_N2-like mechanism, to yield the products S-adenosyl-L-homocysteine (AdoHcy) and the methylated protein substrate (Figure 2). There are three distinct types of PRMTs found in mammalian cells,⁴ classified by their methylated product resulting in either ω-N^G-monomethylarginine (MMA), symmetric ω-N^G,N^G-dimethylarginine (sDMA), or asymmetric ω-N^G,N^G-dimethylarginine (aDMA).^{26, 27} The type-I PRMTs catalyze the formation of MMA

and aDMA, and include PRMT1, PRMT3, PRMT4 (CARM1), PRMT6, or PRMT8⁴. The type II PRMT5 and PRMT9 form MMA and sDMA,^{4, 27} while PRMT7 is the only type-III PRMT known to date that exclusively produces MMA. PRMT1 was the first mammalian PRMT discovered and is responsible for about 85% of total protein arginine methylation activity.²⁸ Type-I and type-II PRMTs are responsible for the majority of arginine methylation in humans and their aberrant expression has been linked to different cancers, such as prostate cancer²⁹ and leukemia^{29, 30} as well as other pathologies including cardiovascular disease.^{31, 32} Considering the impact of histone methylation on gene regulation and by extension epigenetic processes, PRMTs have been most heavily researched as potential therapeutical targets in a variety of cancers.³³⁻³⁵

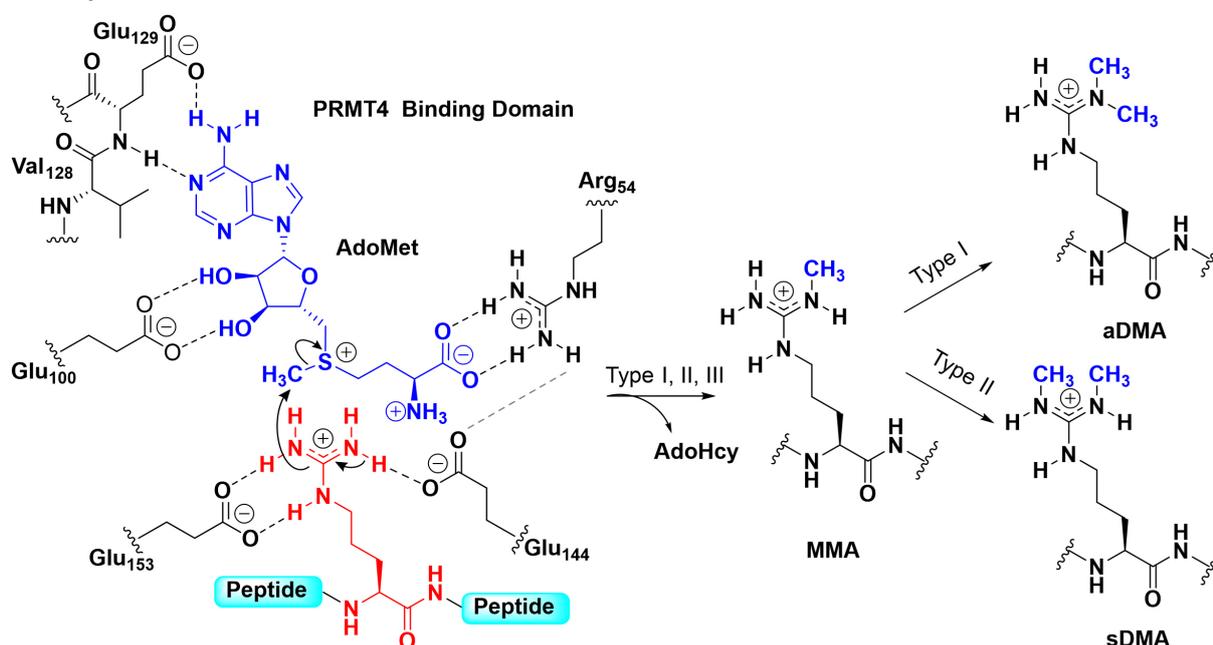


Figure 2. The overview of the PRMT binding site (residue numbering shown for PRMT4) bound to cofactor AdoMet in blue and a protein substrate in red indicating the interactions with the active site residues and the formation of monomethylated arginine (MMA) and subsequently asymmetrically or symmetrically dimethylated arginine (aDMA and sDMA) produced by the different types of PRMTs.

2.1 Peptide probes with substituted guanidino groups

The first peptide-based probes targeting PRMTs focussed on the substitution of the guanidine moiety in the arginine side chain in the context of a peptide fragment of a protein substrate.³⁶⁻³⁸ Starting from the sequence of a known PRMT substrate to achieve selectivity, a variety of different substitution of the ω -nitrogen of the target arginine residue were explored to evaluate the effects on the methylating activity of the target PRMT. The first generation N^{η} -substituted arginyl peptides prepared in our group investigated the effects of substituent electronics and sterics on the second methylation step performed by PRMT1. The introduction of

ethyl, or mono-, di- and tri-fluoroethyl groups on one of the terminal nitrogens of the target arginine in the R1 peptide resulted in the inhibition of PRMT1 with IC_{50} values in the micromolar range (13-29 μ M) (Figure 3A).³⁶ Peptides R1-1, -2, -3, and -4 demonstrated a 5- to 24-fold increase in potency compared to the products of the methylation process (i.e. the aDMA or sDMA-containing peptides). Notably, the potency of the probes against PRMT1 increased with an increasing number of fluorine atoms. In addition, comparable activity was observed against PRMT6, but only weak inhibition of CARM1 was found. Inspired by these results, a second generation of N^{η} -substituted PRMT probes were explored that expanded the range of substitutions on target arginine specifically examining steric, electronic, and pKa and effects (Figure 3B). PRMT1 demonstrated methylating activity towards R4, R5, R6, and R7, but only low levels of methylated product were found for R1 and R3 and no product was observed for R2 in which the arginine residue was modified with a strongly electron withdrawing nitro group. A similar approach was subsequently applied to another PRMT substrate, the HIV-Tat protein. A variety of modifications were introduced onto arginine residue 52 in the HIV-Tat⁴⁸⁻⁶⁰ peptide sequence (Figure 3C) and their effects on enzyme activity analysed. These peptide probes were found to be substrate inhibitors of PRMT1 and PRMT6 and the results obtained demonstrated that a wide range of substitutions were accepted by the PRMTs.

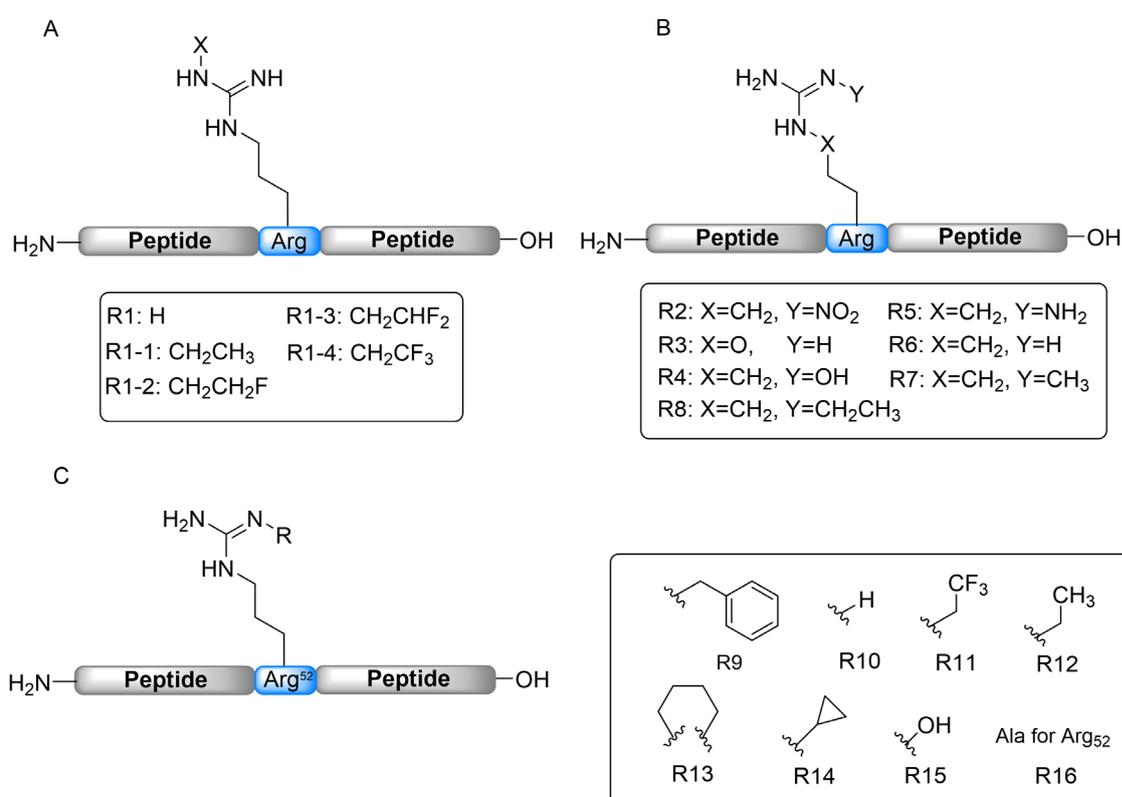


Figure 3. The peptide-based N^{η} -substituted arginine analogues. A and B showed the guanidino modified peptide inhibitors; C. HIV-Tat⁴⁸⁻⁶⁰Arg⁵² guanidino modified peptide inhibitors.

2.2 Chloroacetylated arginines in peptide-based covalent inhibitors of PRMT1

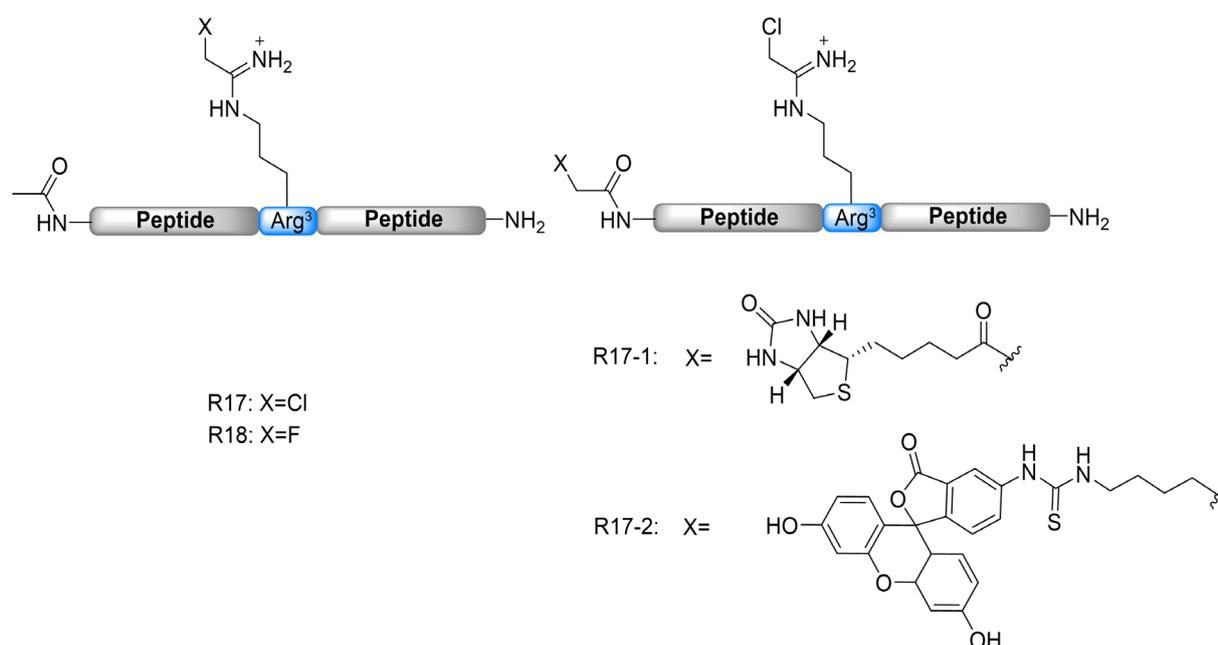


Figure 4. A. Structure of H4 peptide based covalent inhibitors R17 and R18. B. Structure of H4 activity-based probes (ABPs) R17-1 (biotin-conjugated R17) and R18-1 (fluorescein-conjugated R18).

Covalent inhibitors have been increasingly considered a viable option in clinic and more frequently enter the market.^{39, 40} The covalent interaction with its target blocks the enzyme permanently and the enzyme's activity can be recovered only through de novo protein production. While this can be a downside when de novo production is fast or when prolonged effects are undesirable, covalent inhibitors do have the potential to alter disease pharmacology.³⁹ Recently, several covalent inhibitors of PRMT5 and PRMT6 have been described, showing promising activity outperforming the most potent competitive inhibitors.^{41, 42} The crystal structure of the target enzyme plays a vital role in the design of the covalent inhibitors to select the correct spacers and warheads to evaluate. The work on covalent PRMT inhibitors was initiated by the group of Thompson who designed and synthesized two histone H4-based peptides consisting of the first 21 amino acids of the H4 tail and contained a chloroacetamide warhead on Arg¹⁷ or a fluoroacetamide on Arg¹⁸ (Figure 4). To investigate their potencies, the chloroacetamide peptides were incubated with PRMT1 showing that R17 (IC_{50} $1.8 \pm 0.1 \mu\text{M}$) is about 52-fold more potent than R18 (IC_{50} $94 \pm 17 \mu\text{M}$). Also of note, while R17 exhibited irreversible inactivation of PRMT1, the fluoroacetamide in R18 was found to be a competitive inhibitor.⁴³

The covalent chloroacetamide-containing H4 peptide R17 was subsequently conjugated at the N-terminus to biotin (R17-1) or fluorescein (R17-2) turning the

peptides into PRMT1-targeting activity-based probes (ABPs) (Figure 4).⁴⁴ ABPs can be used as chemical tools for the investigation of novel functions, binding partners, expression levels or cellular localisation of enzymes as well as for the screening of inhibitors.⁴⁵⁻⁴⁷ The results indicated that N-terminal labelling of R17 did not affect the compound's inhibition of PRMT1. Both ABPs showed good labelling of PRMT1 in MCF-7 cells and R17-2 was found efficient to enrich and isolate the PRMT1.⁴⁴

2.3 Peptide-based probes mimicking the methyltransferase transition state

In an attempt to develop more potent PRMT inhibitors, our group reported a series of small bisubstrate molecules with a guanidinium group attached to the adenosine unit of cofactor AdoMet with different linker lengths.⁴⁸ While this approach resulted in potent inhibitor with somewhat surprising selectivity, the bisubstrate inhibitors were subsequently optimized towards further enhancing their selectivity through the incorporation of a PRMT-specific peptide sequence.⁴⁹ This strategy was initially applied on PRMT4 (also known as coactivator-associated arginine methyltransferase 1 (CARM1)). The CARM1 transition state (TS) mimics were designed and prepared based on the peptide sequence of its known substrate poly(A)-binding protein 1 (PABP1) (Figure 5). Different linkers between the adenosine and guanidinium moieties were also evaluated revealing the three-carbon linker to most closely mimic the distance between the AdoMet adenosine moiety and the substrate arginine guanidino group. The PABP1⁴⁴⁷⁻⁴⁵⁹ and PABP1⁴⁵⁶⁻⁴⁶⁶ TS mimics showed a nanomolar range of inhibition against CARM1 with up to ~300-fold selectivity over PRMT1.⁴⁹

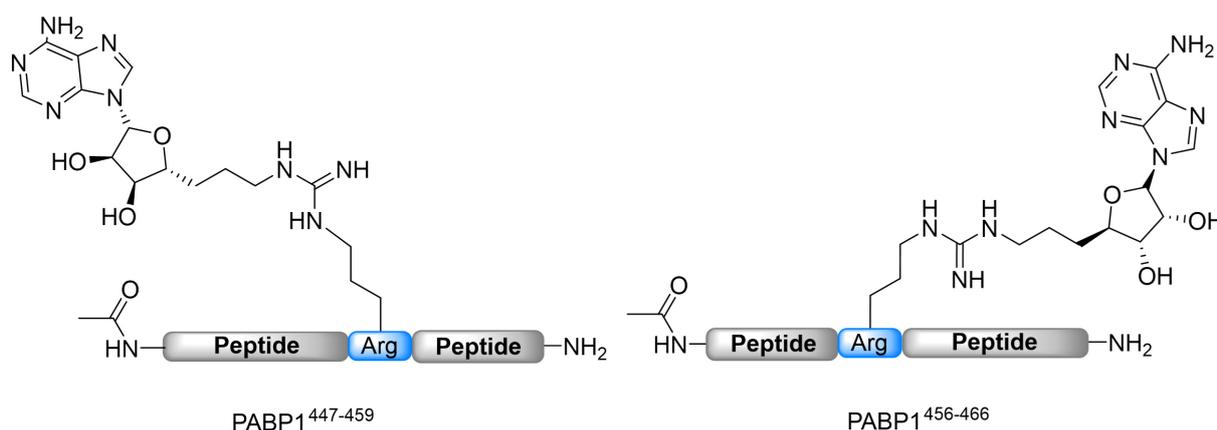


Figure 5. The structure of transition state mimic peptide inhibitors based on CARM1 substrate poly(A)-binding protein 1 (PABP1).

Co-crystal structures obtained with CARM1 and these PABP1 inspired adenosine-peptide conjugates (Figure 6) showed that they effectively mimic the TS of the first methylation step performed by PRMTs.⁴⁹ These structural results provided new insights into the binding interactions of the PRMTs with the specific peptide

substrate. This approach to generating PRMT-specific TS mimics is in principle also applicable to all PRMTs. In this regard, part of the work contained in this thesis was aimed at extending this approach to other PRMTs.

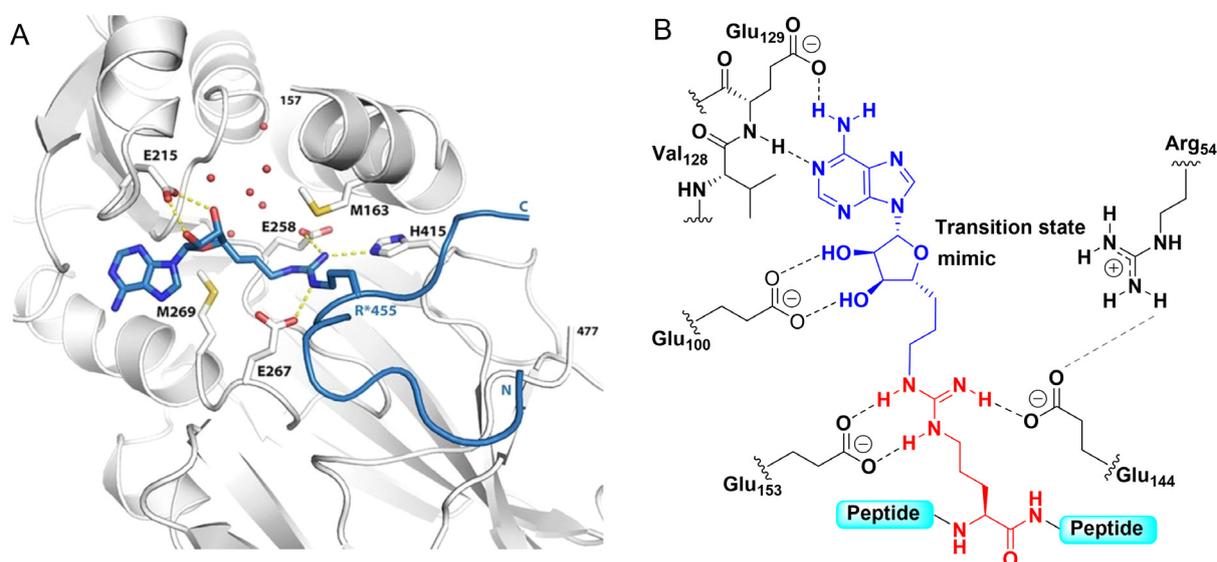


Figure 6. A. The crystal structure of CARM1 and PABP1 peptide substrates. B. Transition state mimic bind to CARM1 catalytic pocket.

3. Thesis Outline

In Chapter 2, the general applicability of the PRMT TS mimic methodology is reported. Specifically, TS mimicking adenosine-peptide conjugates were designed and synthesized to target PRMT1.⁵⁰ Based on the previous work targeting CARM1,⁴⁹ the three-carbon spacer was selected to covalently connect arginine side chains in histone H4¹⁻⁷ and H4¹⁻⁸ peptides to the adenosine moiety. Both sequences were evaluated with a free and an acetylated N-terminus and the ability of the corresponding TS mimics to inhibit PRMT1 compared to the asymmetrically dimethylated arginine (aDMA) product. In all cases the H4 transition state mimics showed inhibition of PRMT1 and PRMT6 with micromolar IC₅₀ values. Interestingly, the extra C-terminal lysine present in H4¹⁻⁸ decreased the selectivity of the compounds towards PRMT1 over PRMT6.

Chapter 3 describes the development of a new series of CARM1 TS mimics based on the sequence of histone H3 around target arginine R¹⁷. To examine the impact of neighbouring lysine acetylation in histone H3 on the recognition of arginine residues by CARM1, adenosine-linked peptides were synthesized with the neighbouring lysine residue K¹⁸ present as either the free amine or in its acetylated form. The potent inhibition observed for both H3¹⁰⁻²⁵ and H3¹⁰⁻²⁵(K¹⁸Ac) TS mimics, led to further evaluation of shorter peptidomimetics by sequentially omitting N- and C-terminal residues to generate the corresponding deca-, octa-

, hexa-, and tetra-peptide analogues 7–14 (See Chapter 3, Table 1). Each of these truncated peptidomimetics were prepared with and without acetylation of the neighboring Lys¹⁸ residue to probe the interplay between peptide sequence and lysine acetylation on recognition by and inhibition of CARM1. Inhibition studies subsequently showed the most potent inhibition for the hexapeptide with nanomolar IC₅₀ values. Structural studies performed with the H3-based TS mimics provide insight into the effect of acetylation on CARM1-binding. The findings point to the intriguing possibility that crosstalk between lysine acetylation and arginine methylation may also serve to reinforce PRMT specificity beyond the primary sequence of the peptide substrate.

The development of a direct, specific, and convenient analytical method for measuring the activity of CARM1 is described in Chapter 4. The LC-MS based method we developed applies multiple reaction monitoring (MRM) for the detection and quantification of a methylated peptide substrate. The assay presents a significant simplification over existing ELISA and radiometric methods while benefitting from high sensitivity and convenient sample preparation. The application of the MRM LC-MS method has been demonstrated by assaying the inhibitory activity of a selection of known CARM1 inhibitors, showing good comparability with previously published data.

Chapter 5, reports the development of peptide-based inhibitors for a different class of methyltransferase, nicotinamide N-methyltransferase (NNMT). NNMT is an AdoMet-dependent small molecule methyltransferase responsible for the methylation of pyridinyl compounds including nicotinamide (vitamin b3) and is implicated as a driver of both metabolic disease and many cancers. Macrocyclic peptide-based NNMT inhibitors were identified using an mRNA-peptide display technique known as the random nonstandard peptides integrated discovery (RaPID) system. The most highly enriched cyclic peptides from both L-tyrosine and D-tyrosine initiating libraries were synthesized using Fmoc- solid phase peptide synthesis (SPPS) and subsequently evaluated for their inhibitory activity against NNMT. Interestingly, while good inhibition of NNMT was observed, none of the macrocyclic peptides identified in our study exhibit significant competition with the substrates of NNMT; AdoMet or nicotinamide. These findings indicate that rather than binding in the active site, the macrocyclic peptides may instead bind at an allosteric site on the enzyme. Furthermore, in cell-based assays, administration of our macrocyclic peptides was found to result in a significant reduction in the production of MNA by endothelial HAEC cells and A549 lung carcinoma cells.

References

1. Schneider, R.; Bannister, A. J., Protein N-methyltransferase assays in the study of gene transcription. *Methods* 2002, 26 (3), 226-32.
2. Huang, R., Chemical Biology of Protein N-Terminal Methyltransferases. *Chembiochem* 2019, 20 (8), 976-984.
3. Murray, K., The Occurrence of ϵ -N-Methyl Lysine in Histones. *Biochemistry* 1964, 3 (1), 10-15.
4. Bedford, M. T.; Clarke, S. G., Protein arginine methylation in mammals: who, what, and why. *Molecular cell* 2009, 33 (1), 1-13.
5. Kwiatkowski, S.; Drozak, J., Protein Histidine Methylation. *Current protein & peptide science* 2020, 21 (7), 675-689.
6. Tessarz, P.; Santos-Rosa, H.; Robson, S. C.; Sylvestersen, K. B.; Nelson, C. J.; Nielsen, M. L.; Kouzarides, T., Glutamine methylation in histone H2A is an RNA-polymerase-I-dedicated modification. *Nature* 2014, 505 (7484), 564-8.
7. Briggs, S. D.; Xiao, T.; Sun, Z. W.; Caldwell, J. A.; Shabanowitz, J.; Hunt, D. F.; Allis, C. D.; Strahl, B. D., Gene silencing: trans-histone regulatory pathway in chromatin. *Nature* 2002, 418 (6897), 498.
8. Frederiks, F.; Tzouros, M.; Oudgenoeg, G.; van Welsem, T.; Fornerod, M.; Krijgsveld, J.; van Leeuwen, F., Nonprocessive methylation by Dot1 leads to functional redundancy of histone H3K79 methylation states. *Nature structural & molecular biology* 2008, 15 (6), 550-7.
9. Pokholok, D. K.; Harbison, C. T.; Levine, S.; Cole, M.; Hannett, N. M.; Lee, T. I.; Bell, G. W.; Walker, K.; Rolfe, P. A.; Herbolsheimer, E.; Zeitlinger, J.; Lewitter, F.; Gifford, D. K.; Young, R. A., Genome-wide map of nucleosome acetylation and methylation in yeast. *Cell* 2005, 122 (4), 517-27.
10. Rea, S.; Eisenhaber, F.; O'Carroll, D.; Strahl, B. D.; Sun, Z.-W.; Schmid, M.; Opravil, S.; Mechtler, K.; Ponting, C. P.; Allis, C. D.; Jenuwein, T., Regulation of chromatin structure by site-specific histone H3 methyltransferases. *Nature* 2000, 406 (6796), 593-599.
11. Tamaru, H.; Selker, E. U., A histone H3 methyltransferase controls DNA methylation in *Neurospora crassa*. *Nature* 2001, 414 (6861), 277-283.
12. van Leeuwen, F.; Gafken, P. R.; Gottschling, D. E., Dot1p Modulates Silencing in Yeast by Methylation of the Nucleosome Core. *Cell* 2002, 109 (6), 745-756.
13. Han, D.; Huang, M.; Wang, T.; Li, Z.; Chen, Y.; Liu, C.; Lei, Z.; Chu, X., Lysine methylation of transcription factors in cancer. *Cell Death & Disease* 2019, 10 (4), 290.
14. Sun, G. D.; Cui, W. P.; Guo, Q. Y.; Miao, L. N., Histone lysine methylation in diabetic nephropathy. *Journal of diabetes research* 2014, 2014, 654148.
15. Arrowsmith, C. H.; Bountra, C.; Fish, P. V.; Lee, K.; Schapira, M., Epigenetic protein families: a new frontier for drug discovery. *Nature Reviews Drug Discovery* 2012, 11 (5), 384-400.

16. Schapira, M.; Arrowsmith, C. H., Methyltransferase inhibitors for modulation of the epigenome and beyond. *Current Opinion in Chemical Biology* 2016, 33, 81-87.
17. Schapira, M., Chemical Inhibition of Protein Methyltransferases. *Cell Chemical Biology* 2016, 23 (9), 1067-1076.
18. Liu, X. R.; Zhou, L. H.; Hu, J. X.; Liu, L. M.; Wan, H. P.; Zhang, X. Q., UNC0638, a G9a inhibitor, suppresses epithelial-mesenchymal transition-mediated cellular migration and invasion in triple negative breast cancer. *Molecular medicine reports* 2018, 17 (2), 2239-2244.
19. Knutson, S. K.; Wigle, T. J.; Warholic, N. M.; Sneeringer, C. J.; Allain, C. J.; Klaus, C. R.; Sacks, J. D.; Raimondi, A.; Majer, C. R.; Song, J.; Scott, M. P.; Jin, L.; Smith, J. J.; Olhava, E. J.; Chesworth, R.; Moyer, M. P.; Richon, V. M.; Copeland, R. A.; Keilhack, H.; Pollock, R. M.; Kuntz, K. W., A selective inhibitor of EZH2 blocks H3K27 methylation and kills mutant lymphoma cells. *Nat Chem Biol* 2012, 8 (11), 890-6.
20. Harb, W.; Abramson, J.; Lunning, M.; Goy, A.; Maddocks, K.; Lebedinsky, C.; Senderowicz, A.; Trojer, P.; Bradley, W. D.; Flinn, I., A phase 1 study of CPI-1205, a small molecule inhibitor of EZH2, preliminary safety in patients with B-cell lymphomas. *Annals of Oncology* 2018, 29, iii7.
21. Shen, Y.; Szewczyk, M. M.; Eram, M. S.; Smil, D.; Kaniskan, H.; de Freitas, R. F.; Senisterra, G.; Li, F.; Schapira, M.; Brown, P. J.; Arrowsmith, C. H.; Barsyte-Lovejoy, D.; Liu, J.; Vedadi, M.; Jin, J., Discovery of a Potent, Selective, and Cell-Active Dual Inhibitor of Protein Arginine Methyltransferase 4 and Protein Arginine Methyltransferase 6. *J Med Chem* 2016, 59 (19), 9124-9139.
22. Nakayama, K.; Szewczyk, M. M.; Dela Sena, C.; Wu, H.; Dong, A.; Zeng, H.; Li, F.; de Freitas, R. F.; Eram, M. S.; Schapira, M.; Baba, Y.; Kunitomo, M.; Cary, D. R.; Tawada, M.; Ohashi, A.; Imaeda, Y.; Saikatendu, K. S.; Grimshaw, C. E.; Vedadi, M.; Arrowsmith, C. H.; Barsyte-Lovejoy, D.; Kiba, A.; Tomita, D.; Brown, P. J., TP-064, a potent and selective small molecule inhibitor of PRMT4 for multiple myeloma. *Oncotarget* 2018, 9 (26), 18480-18493.
23. Mitchell, L. H.; Drew, A. E.; Ribich, S. A.; Rioux, N.; Swinger, K. K.; Jacques, S. L.; Lingaraj, T.; Boriack-Sjodin, P. A.; Waters, N. J.; Wigle, T. J.; Moradei, O.; Jin, L.; Riera, T.; Porter-Scott, M.; Moyer, M. P.; Smith, J. J.; Chesworth, R.; Copeland, R. A., Aryl Pyrazoles as Potent Inhibitors of Arginine Methyltransferases: Identification of the First PRMT6 Tool Compound. *ACS medicinal chemistry letters* 2015, 6 (6), 655-659.
24. Greer, E. L.; Shi, Y., Histone methylation: a dynamic mark in health, disease and inheritance. *Nature Reviews Genetics* 2012, 13 (5), 343-357.
25. Taverna, S. D.; Li, H.; Ruthenburg, A. J.; Allis, C. D.; Patel, D. J., How chromatin-binding modules interpret histone modifications: lessons from professional pocket pickers. *Nature structural & molecular biology* 2007, 14 (11), 1025-1040.

26. Pahlich, S.; Zakaryan, R. P.; Gehring, H., Protein arginine methylation: Cellular functions and methods of analysis. *Biochimica et biophysica acta* 2006, 1764 (12), 1890-903.
27. Wolf, S. S., The protein arginine methyltransferase family: an update about function, new perspectives and the physiological role in humans. *Cellular and molecular life sciences : CMLS* 2009, 66 (13), 2109-21.
28. Tang, J.; Frankel, A.; Cook, R. J.; Kim, S.; Paik, W. K.; Williams, K. R.; Clarke, S.; Herschman, H. R., PRMT1 is the predominant type I protein arginine methyltransferase in mammalian cells. *The Journal of biological chemistry* 2000, 275 (11), 7723-30.
29. Hong, H.; Kao, C.; Jeng, M. H.; Eble, J. N.; Koch, M. O.; Gardner, T. A.; Zhang, S.; Li, L.; Pan, C. X.; Hu, Z., Aberrant expression of CARM1, a transcriptional coactivator of androgen receptor, in the development of prostate carcinoma and androgen - independent status. *Cancer: Interdisciplinary International Journal of the American Cancer Society* 2004, 101 (1), 83-89.
30. So, C. W.; Caldas, C.; Liu, M.-M.; Chen, S.-J.; Huang, Q.-H.; Gu, L.-J.; Sham, M. H.; Wiedemann, L. M.; Chan, L. C., EEN encodes for a member of a new family of proteins containing an Src homology 3 domain and is the third gene located on chromosome 19p13 that fuses to MLL in human leukemia. *Proceedings of the National Academy of Sciences* 1997, 94 (6), 2563-2568.
31. Couto, E. S. A.; Wu, C. Y.; Citadin, C. T.; Clemons, G. A.; Possoit, H. E.; Grames, M. S.; Lien, C. F.; Minagar, A.; Lee, R. H.; Frankel, A.; Lin, H. W., Protein Arginine Methyltransferases in Cardiovascular and Neuronal Function. *Molecular neurobiology* 2020, 57 (3), 1716-1732.
32. Cai, S.; Liu, R.; Wang, P.; Li, J.; Xie, T.; Wang, M.; Cao, Y.; Li, Z.; Liu, P., PRMT5 Prevents Cardiomyocyte Hypertrophy via Symmetric Dimethylating HoxA9 and Repressing HoxA9 Expression. *Frontiers in Pharmacology* 2020, 11 (2140).
33. Elakoum, R.; Gauchotte, G.; Oussalah, A.; Wissler, M. P.; Clément-Duchêne, C.; Vignaud, J. M.; Guéant, J. L.; Namour, F., CARM1 and PRMT1 are dysregulated in lung cancer without hierarchical features. *Biochimie* 2014, 97, 210-8.
34. Jarrold, J.; Davies, C. C., PRMTs and Arginine Methylation: Cancer's Best-Kept Secret? *Trends in molecular medicine* 2019, 25 (11), 993-1009.
35. Wang, S. M.; Dowhan, D. H.; Muscat, G. E. O., Epigenetic arginine methylation in breast cancer: emerging therapeutic strategies. *Journal of molecular endocrinology* 2019, 62 (3), R223-r237.
36. Lakowski, T. M.; t Hart, P.; Ahern, C. A.; Martin, N. I.; Frankel, A., N η -substituted arginyl peptide inhibitors of protein arginine N-methyltransferases. *ACS chemical biology* 2010, 5 (11), 1053-63.
37. t Hart, P.; Thomas, D.; van Ommeren, R.; Lakowski, T. M.; Frankel, A.; Martin, N. I., Analogues of the HIV-Tat peptide containing N η -modified arginines as potent inhibitors of protein arginine N-methyltransferases. *MedChemComm* 2012, 3

- (10), 1235-1244.
38. Thomas, D.; Koopmans, T.; Lakowski, T. M.; Kreinin, H.; Vhuiyan, M. I.; Sedlock, S. A.; Bui, J. M.; Martin, N. I.; Frankel, A., Protein Arginine N-Methyltransferase Substrate Preferences for Different N η -Substituted Arginyl Peptides. *ChemBioChem* 2014, 15 (11), 1607-1613.
39. Strelow, J. M., A Perspective on the Kinetics of Covalent and Irreversible Inhibition. *SLAS DISCOVERY: Advancing the Science of Drug Discovery* 2016, 22 (1), 3-20.
40. Ghosh, A. K.; Samanta, I.; Mondal, A.; Liu, W. R., Covalent Inhibition in Drug Discovery. *ChemMedChem* 2019, 14 (9), 889-906.
41. Lin, H.; Wang, M.; Zhang, Y. W.; Tong, S.; Leal, R. A.; Shetty, R.; Vaddi, K.; Luengo, J. I., Discovery of Potent and Selective Covalent Protein Arginine Methyltransferase 5 (PRMT5) Inhibitors. *ACS Medicinal Chemistry Letters* 2019, 10 (7), 1033-1038.
42. Shen, Y.; Li, F.; Szewczyk, M. M.; Halabelian, L.; Park, K.-s.; Chau, I.; Dong, A.; Zeng, H.; Chen, H.; Meng, F.; Barsyte-Lovejoy, D.; Arrowsmith, C. H.; Brown, P. J.; Liu, J.; Vedadi, M.; Jin, J., Discovery of a First-in-Class Protein Arginine Methyltransferase 6 (PRMT6) Covalent Inhibitor. *Journal of Medicinal Chemistry* 2020, 63 (10), 5477-5487.
43. Obiany, O.; Causey, C. P.; Osborne, T. C.; Jones, J. E.; Lee, Y. H.; Stallcup, M. R.; Thompson, P. R., A chloroacetamide-based inactivator of protein arginine methyltransferase 1: design, synthesis, and in vitro and in vivo evaluation. *Chembiochem* 2010, 11 (9), 1219-23.
44. Obiany, O.; Causey, C. P.; Jones, J. E.; Thompson, P. R., Activity-based protein profiling of protein arginine methyltransferase 1. *ACS chemical biology* 2011, 6 (10), 1127-35.
45. Greenbaum, D.; Baruch, A.; Hayrapetian, L.; Darula, Z.; Burlingame, A.; Medzhradszky, K. F.; Bogoy, M., Chemical approaches for functionally probing the proteome. *Molecular & cellular proteomics : MCP* 2002, 1 (1), 60-8.
46. Berger, A. B.; Vitorino, P. M.; Bogoy, M., Activity-based protein profiling: applications to biomarker discovery, in vivo imaging and drug discovery. *American journal of pharmacogenomics : genomics-related research in drug development and clinical practice* 2004, 4 (6), 371-81.
47. Leung, D.; Hardouin, C.; Boger, D. L.; Cravatt, B. F., Discovering potent and selective reversible inhibitors of enzymes in complex proteomes. *Nature biotechnology* 2003, 21 (6), 687-91.
48. van Haren, M.; van Ufford, L. Q.; Moret, E. E.; Martin, N. I., Synthesis and evaluation of protein arginine N-methyltransferase inhibitors designed to simultaneously occupy both substrate binding sites. *Organic & Biomolecular Chemistry* 2015, 13 (2), 549-560.
49. van Haren, M. J.; Marechal, N.; Troffer-Charlier, N.; Cianciulli, A.; Sbardella, G.;

1
Cavarelli, J.; Martin, N. I., Transition state mimics are valuable mechanistic probes for structural studies with the arginine methyltransferase CARM1. *Proceedings of the National Academy of Sciences of the United States of America* 2017, 114 (14), 3625-3630.

50. Zhang, Y.; van Haren, M. J.; Martin, N. I., Peptidic transition state analogues as PRMT inhibitors. *Methods* 2020, 175, 24-29.