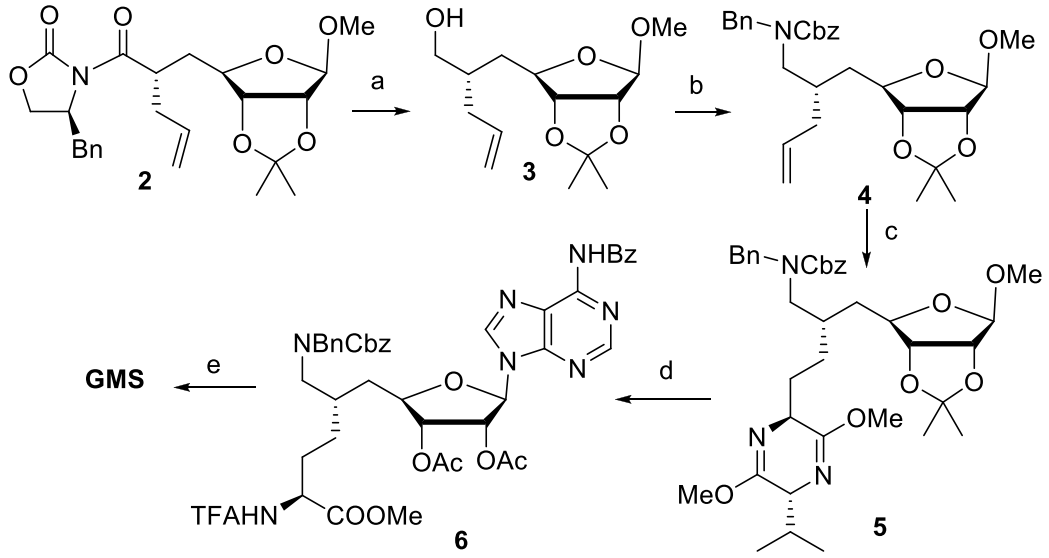
Supplementary Methods

Synthesis of Sinefungin Analogue GMS.



Reagents and conditions: (a) Dess-Martin periodinane, $NaHCO_3$, CH_2Cl_2; (b) 1) $BnNH_2$, $NaBH(OAc)_3$, $ClCH_2CH_2Cl$, rt; 2) CbzCl, Sat. $NaHCO_3$, THF, 0°C-rt.75% over 3 steps; (c) 1) O_3, CH_2Cl_2, -78°C,then PPh_3, rt, 78%; 2) $NaBH_4$, EtOH, 0°C; 3) MsCl, TEA, CH_2Cl_2, 0°C; 4) NaI, Na_2SO_3, $NaHCO_3$, acetone, 40°C, 82% over 3 steps; 5) n-BuLi, CuCN, (R)-2,5-Dihydro-3,6-dimethoxy-2-isopropylpyrazine THF, -20°C, 20h, 82%; (d) 1) 0.25M HCl/MeCN, rt; 2) TFAA, Py, CH_2Cl_2, 0 °C. 75% over 2 steps 3) HCl/dioxane, rt; 4) Ac_2O, Py, 0 °C-rt, 76% over 2 steps; 5)silyl N^6-Bz-adenine, TMSOTf, $ClCH_2CH_2Cl$, 45 °C, 92%; (e) 1), H_2, 20% $Pd(OH)_2$ CF_3CH_2OH; 2) 1,3-Di-Boc-2-methylisothiourea, triethylamine, $HgCl_2$, THF/CH_2Cl_2, rt , 72% over 2 steps. (c) 1) 0.2M LiOH, MeOH, rt; 2) TFA: H_2O = 9:1, rt. 28% over 2 steps.

6′-Methyleneamine sinefungin analogues GMS was synthesized from a known oxazolidinone imide precursor 2[1] (Scheme 1). The reduction of the chiral auxiliary imide of 2 led to the primary alcohol 3 with the desired steric center maintained at the C6 position of D-ribose ring. The approach was then implemented to introduce the amine functionality via 4 by Dess-Martin oxidation of the primary alcohol 3 and then reductive amination with benzyl amine, followed by carbobenzyloxy (Cbz) protection. The terminal alkene of the intermediate 4 was readily converted into the protected amino acid 5 as described previously[2]. After acidic deprotection of its amin and N-trifuoroacetylation (TFA), the protecting groups on glycoside were exchanged with acetyl to facilitate the following adenosylation. The β-ribosyl adenine in 6 was installed through Vorbrüggen glycosylation of bis-silyl-N-benzoyladenine[2]. Here the amino acid in 6 was

masked with N-trifuoroacetyl group rather than the previously-reported Cbz or acetyl group[1, 3] for its orthogonality to hydrogenolysis and ease of removal. The hydrogenolysis condition set the primary amine on 6' position free for further guanidylation . Trifluoroethanol was used as solvent to avoid N-alkylation in alcoholic solvent[4]. At last, global deprotection of base-labile blocking groups in the present of lithium hydroxide, followed by acidic removal of t-butoxycarbonyl completed the synthesis of target compound GMS. (see Method and Supporting Information).

(1) Ghosh, A. K.; Liu, W. *J. Org. Chem.* 1996, *61*, 6175.

(2) Zheng, W.; Ibanez, G.; Wu, H.; Blum, G.; Zeng, H.; Dong, A.; Li, F.; Hajian, T.; Allali-Hassani, A.; Amaya, M. F.; Siarheyeva, A.; Yu, W.; Brown, P. J.; Schapira, M.; Vedadi, M.; Min, J.; Luo, M. *J. Am. Chem. Soc.* 2012, *134*, 18004.

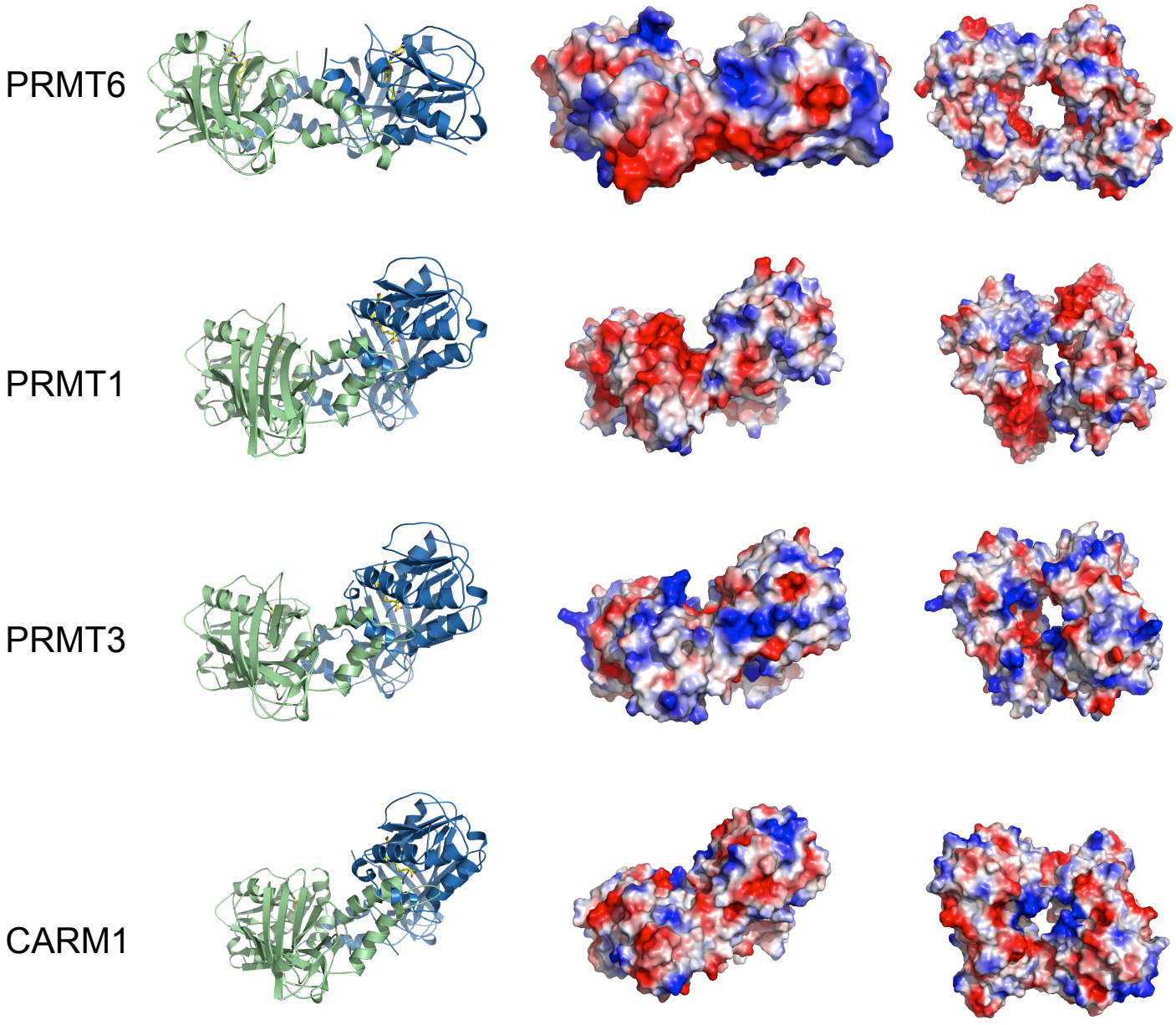
(3) Maria, E. J.; Da, S. A. D.; Fourrey, J.-L. *Eur. J. Org. Chem.* 2000, 627.

(4) Bailey, P. D.; Beard, M. A.; Dang, H. P. T.; Phillips, T. R.; Price, R. A.; Whittaker, J. H. *Tetrahedron Lett.* 2008, *49*, 2150.

Supplementary Table 1: Methyltransferase selectivity profile of GMS.

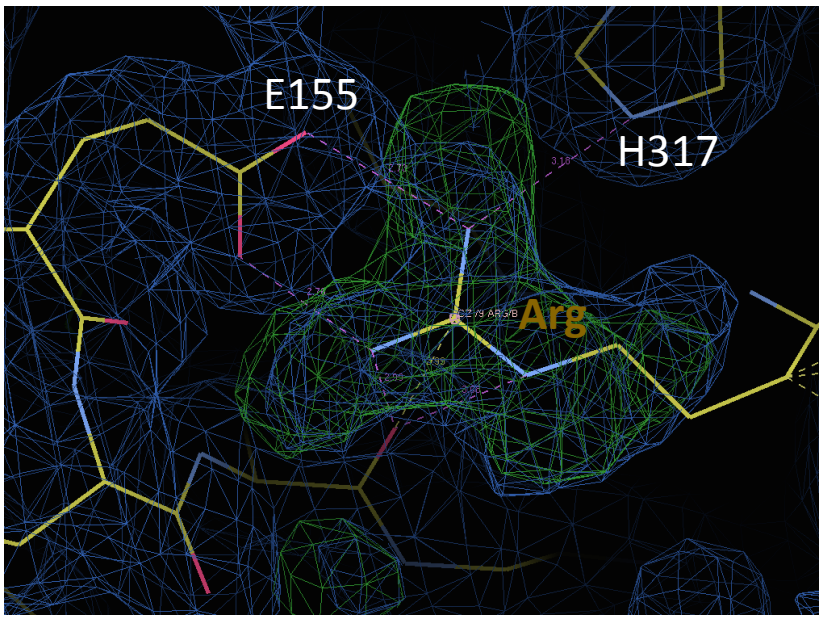
Protein	IC_{50} (nM)	Hill Slope
PRMT1	500 ± 30	1.1
PRMT3	700 ± 50	1.2
CRAM1	< 15	1.1
PRMT5	1600 ± 300	0.9
PRMT6	90 ± 3	1.1
PRMT8	11 ± 0.6	1
DOT1L	1500 ± 200	0.9
G9a	> 50000	NA
SETD7	> 45000	NA
PRDM9	>50000	NA

NA: not applicable



Supplementary Figure 1: The dimerization arm in PRMT6 (PDB: 4HC4) has a different conformation, which leads to a flat dimer ring structure with a wide central cavity, whereas the two monomers in the PRMT1 (PDB: 1OR8), PRMT3 (PDB: 2FYT) and CARM1 (PDB: 3B3F) dimers form concave surfaces. The two molecules in the dimer are colored in green and blue, and the cofactor product SAH is shown in stick figures in the cartoon structures in the left panels. Surfaces with positive and negative electrostatic potentials are shown in blue and red, respectively. The center panel has the same view as the left panel and the right panels is a top view related to the left panel.

A



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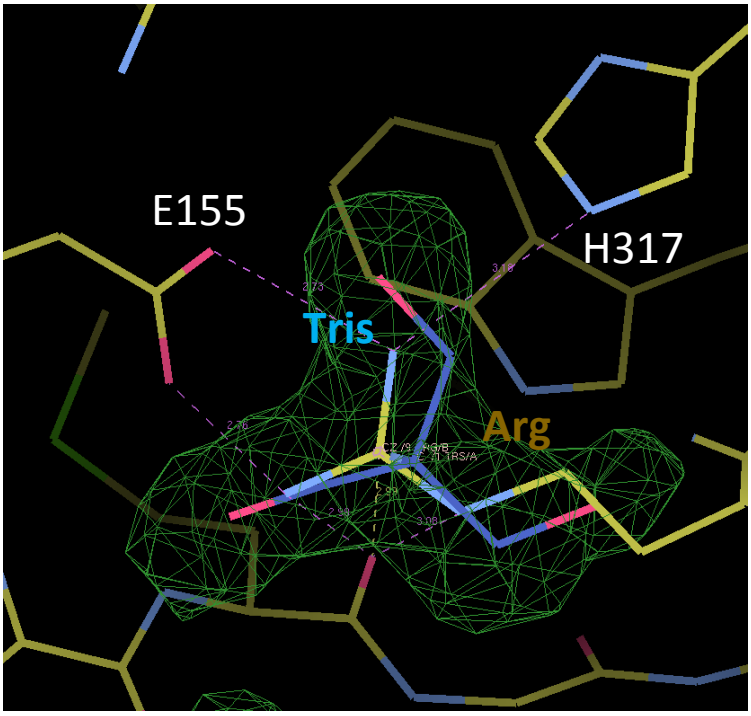
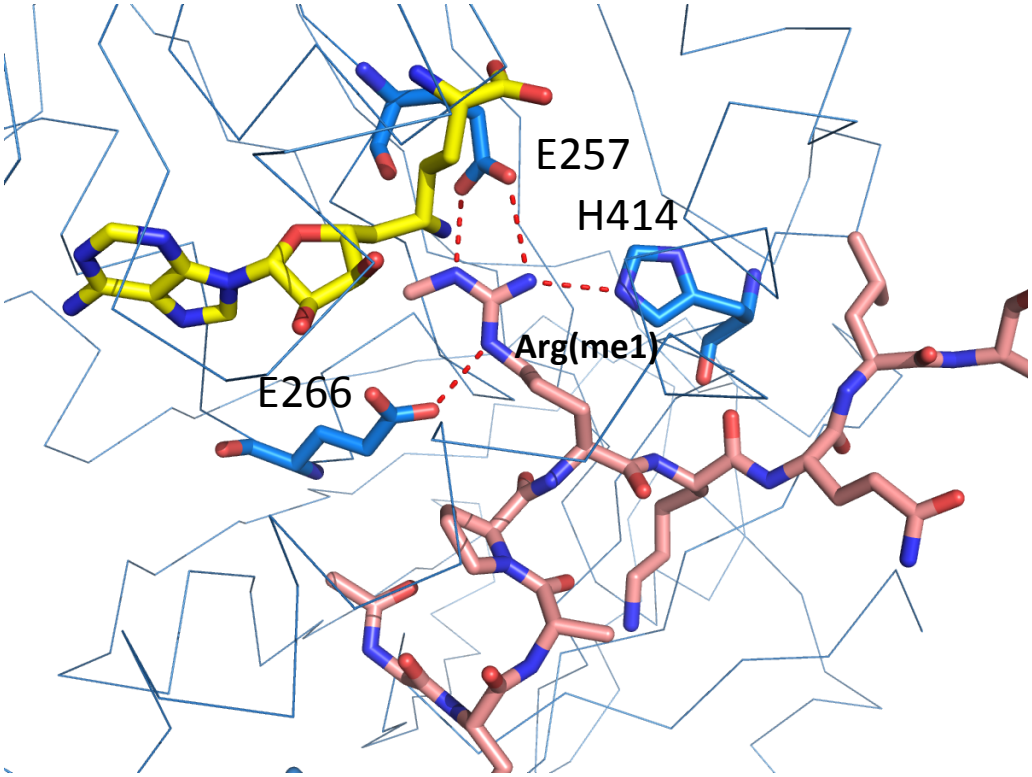
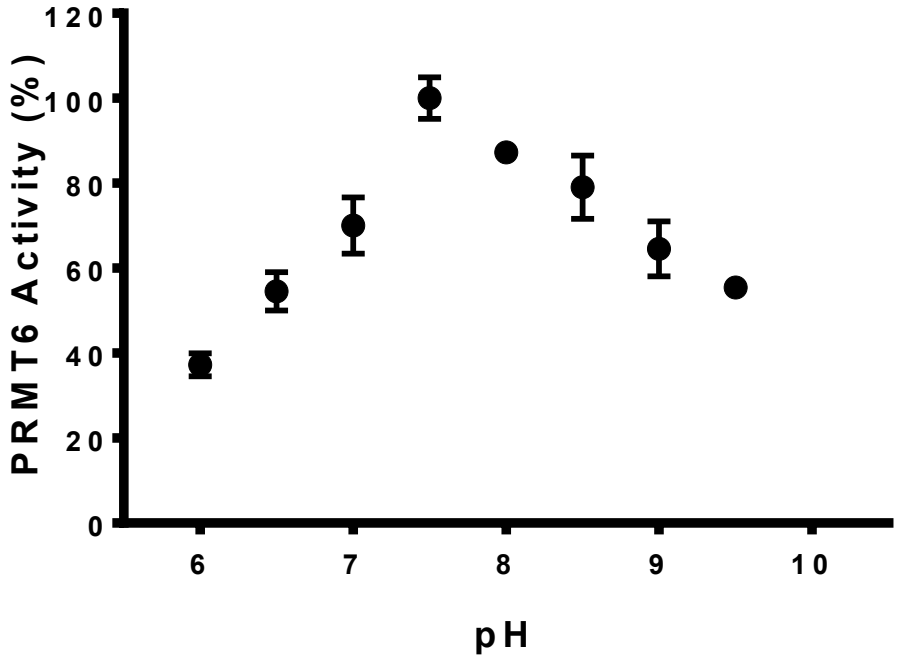


Supplementary Figure 2: Electron density map for the PRMT6-SAH-arginine peptide complex (5HZM). (A) The extra electron density is modeled as a arginine residue. The difference map is colored in green and the 2Fo-Fc map is in blue. (B) The extra electron density is modeled as a Tris molecule (blue) with the arginine residue (yellow) overlayed with it.



Supplementary Figure 3: Detailed interaction of the target arginine residue with CARM1 in the CARM1-SNF-H3R17 peptide complex (PDB: 5DWQ). SAH is in yellow, peptide is in pink and CARM1 residues are in blue.



Supplementary Figure 4: pH profile of PRMT6. Activity of PRMT6 was determined at saturation of both substrates (25 µM SAM and 5 µM of biotinylated H4(1-24) peptide) in 20 mM Bis-tris-propane buffer at various pH values. Data are plotted as mean ± SD of three replicates.