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Toward the development of potent and selective bisubstrate inhibitors of protein arginine methyltransferases

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ABSTRACT

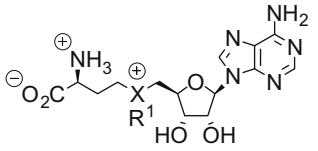
Prototype inhibitors of protein arginine methyltransferases (PRMTs) have been constructed by attaching guanidine functionality via a variable linker to non-reactive amine analogues of the cellular co-factor (*S*)-adenosyl methionine (AdoMet). Potent inhibition of PRMT1 (IC_{50} of ~3–6 μM) combined with weak inhibition of the lysine methyltransferase SET7 (~50% of activity at 100 μM) was observed for two such compounds.

Understanding post-translational modification of proteins is fundamental to the study and control of the dynamic range of cells. Resurgent interest in protein methylation has renewed demand for compounds that can be used to control the action of methyltransferases responsible for such site-specific alkylation.[1,2]

Methylation at arginine residues is of interest because it can affect gene transcription, or signal transduction by modulating protein–protein interactions.[3] In mammalian cells methylation of arginine residues is catalysed by a family of at least nine protein methyl transferases (PRMTs, E.C. 2.1.1.125).[4] Most PRMTs precisely orchestrate arginine side chains and the highly reactive methyl sulfonium of the co-factor (*S*)-adenosyl methionine (AdoMet) **1** (Fig. 1) to bring about alkylation at a guanidinium nitrogen.[5] Subsequent methylation may occur, leading to either asymmetrical or symmetrical substitution by alkylation of the same or adjacent nitrogen under the control of Type I or II PRMTs, respectively.[6]

Since the first identification of PRMT inhibitors from random screening,[7] the number of publications that describe small molecule inhibitors of PRMTs has grown steadily.[8] A number of possible lead structures have emerged,[8,9] amongst which pyrazoles have received significant attention as inhibitors of coactivator associated arginine methyltransferase 1 (CARM1, or PRMT4).[10] Such compounds show much promise but have shown limited in vivo utility so far. Very recently, analogues of the first arginine methyltransferase inhibitor ('AMI-1', not shown)[7] have been developed that show good potency for PRMTs in vitro and in vivo and that appear to be inactive against the lysine methyltransferase SET7/9.[11] Much remains to be discovered regarding the function of the remainder of the PRMT family for which a repertoire of chemical probes will be required.[11]

The goal of developing inhibitors for each PRMT may be achieved by exploring structures based on the structure of AdoMet and the target arginine-bearing substrates, but this hypothesis has not been widely explored. Expanded structures on AdoMet **1** or its de-methylated product (*S*)-adenosylhomocysteine (AdoHcy) **2** featuring N-6 adenine modifications, have been reported to respectively act as specific co-factors or inhibitors of yeast RMT1 with complementary mutations at the AdoMet binding site, but they do not appear to have received further attention.[12]



1 AdoMet - X = S, R^1 = Me
2 AdoHcy - X = S, R^1 = H
3 AzaAdoMet - X = N, R^1 = Me
4 Sinefungin - X = CH, R^1 = NH_2

Figure 1. Structure of AdoMet and related compounds.

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The inherent reactivity of the AdoMet **1** sulfonium centre delivers nature with a broad repertoire of possible reactions,[13] but makes synthesis of its analogues particularly challenging. The slow pyramidal inversion of the sulfonium centre is a further complicating factor for synthesis. AzaAdoMet **3** analogues that replace the sulfonium centre with nitrogen have been reported as potent but non-selective inhibitors.[14–17]

In principle, bisubstrate inhibitors[18] that combine structural elements of the AdoMet **1** with the arginine-bearing sequence should provide high levels of selectivity.[19,20] Recently, PRMT1 was used to direct a mustard containing AdoMet **1** analogue to modify a peptide substrate in vitro.[21] Good levels of PRMT1 inhibition were observed in the presence of the resulting adduct mixture (IC_{50} ~12 μM), although incomplete conversion (estimated at 22%) and difficulties in separating adduct from substrate protein made it impossible to report a precise value.[21] Such highly reactive AdoMet **1** analogues are particularly interesting, not least for substrate identification, but the resulting peptide conjugates are of limited value for cellular studies. Furthermore, the opportunity for design variation at the linker is limited by the requirement for a reactive aziridinium species.

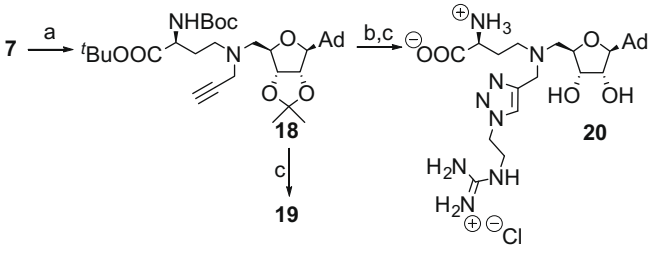
This paper describes a new, simple and expedient synthesis that provides general access to AzaAdoMet **3** analogues in which the methyl group is extended to accommodate functionality that may target the substrate binding pocket of target enzymes. This route has the benefit of being readily amenable to structural variation and consequently, optimisation of selectivity and potency for target proteins. The prototype inhibitors described herein are designed to carry guanidine functionality via a variable linker with the aim of inhibiting PRMTs.

AzaAdoMet **3** has previously been reported as potent but promiscuous methyltransferase inhibitor when protonated or methylated at nitrogen.[14,15] We sought to exploit and extend the structure at the secondary nitrogen toward the envisaged inhibitors. Crystal structures and homology modelling reveal two conserved glutamate residues at the active site which are believed to be essential for catalysis (E144 and E153 for PRMT1),[22] which may be viable targets for guanidine isosteres covalently linked to AzaAdoMet **3** via an appropriate linker. A simple flexible synthesis was therefore sought that would allow evaluation of the design while providing a clear route for future optimisation of potency and selectivity.

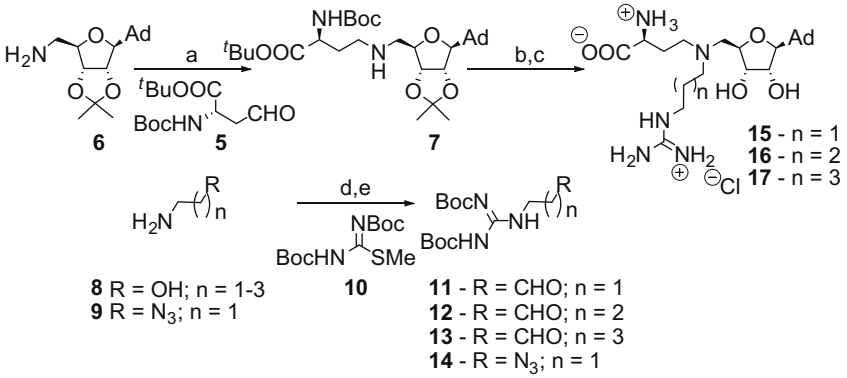
Previous synthetic routes to AzaAdoMet **3** have utilised an alkylation strategy,[14–17] but we envisaged sequential reductive amination as a convenient means of achieving modular installation of various functional groups using readily accessible aldehyde precursors (Scheme 1). 5-Aza-5-deoxy-adenosine **6**[23] was condensed with aspartic acid derived aldehyde **5**[24] to produce the protected AzaAdoMet precursor **7** in good, scalable yield. Guanidylation of amino alcohols **8** promoted by mercury (II) chloride proceeded in excellent yields, with oxidation of the remaining alcohol functionality leading to the respective aldehydes **11**–**13** with variable efficiency. The prototype inhibitors were then constructed by a second reductive amination between these aldehydes **11–13** with the protected precursor **7** in generally good yields. Finally, deprotection using aqueous TFA solution removed all protecting groups and the resulting trifluoroacetate salts were subject to anion exchange chromatography to deliver the putative inhibitors **15**–**17** as their hydrochloride salts. A further AzaAdoMet derivative **19** obtained by alkylation of precursor **7** with propargyl bromide was attractive because the copper catalysed Huisgen reaction offers an alternative high-yielding reaction for achieving linkage.[25,26] Indeed, the triazole bearing compound **20** was synthesised in high yield by combining the propargyl compound **7** with azide **14** (Scheme 1) and subsequent deprotection of the resulting adduct under standard conditions (Scheme 2).[25] Furthermore, the structural similarity between the alkyne analogue **19** and AzaAdoMet **3** meant that it could be conveniently used to determine the contribution of the additional guanidine functionality borne by the putative inhibitors to the level of PRMT1 inhibition.

Standard in vitro methylation assay conditions were used to compare the compounds.[7] To this end we expressed human recombinant PRMT1 as well as SET domain lysine methyl transferase, SET7, with the aim of establishing whether the inhibitors were selective. Methyl transfer from [^{3}H]AdoMet to peptides P3, derived from Sam68, and histone H3 was measured by scintillation counting after separation by SDS–PAGE and transfer to nitrocellulose membrane. IC_{50} values were obtained by comparing the degree of methylation over a range of concentrations of inhibitor and are representative of values obtained over at least three experiments (Table 1).

We chose to use sinefungin **4**, a potent but promiscuous inhibitor of AdoMet **1** utilising proteins, as a benchmark for inhibi-



Scheme 2. Synthesis of propargyl **19** and triazole **20** AdoMet analogues. Reagents and conditions: (a) **7**, propargyl bromide, K_2CO_3, DMF, rt, 3 h, 71%; (b) azide **14**, $CuSO_4$, sodium ascorbate, 3:1 MeOH/H_2O, rt, 70%; (c) 50:1 TFA/H_2O, rt, 91%.



Scheme 1. Synthesis of inhibitors. Reagents and conditions: (a) $NaBH(OAc)_3$, $ClCH_2CH_2Cl$, rt, 2 h, 73%; (b) $NaBH(OAc)_3$, $ClCH_2CH_2Cl$, rt, 2 h, 34–89%; (c) 9:1 TFA/H_2O, rt, 4 h, 67–90%; (d) Et_3N, $HgCl_2$, DMF, rt, 1.5 h, 92–99%; (e) DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2, −78 °C, 1.5 h, 40–71% for **11**–**13**.

Table 1
Evaluation of AdoMet analogues

Compound	PRMT1[a]	SET7[a]
4	<1	2.5
15	6.2 ± 3.9	>100[b]
16	2.9 ± 0.8	>50[b]
17	5.6 ± 4.5	>100[b]
19	>100[b]	>100[b]
20	>100[b]	>100[b]

[a] Mean IC_{50} ± standard deviation (μM) based on triplicate experiments.
[b] All compounds >50% enzyme activity remaining at 100 μM.

tion.[27–29] As expected, sinefungin **4** was observed to be a potent inhibitor of both arginine and lysine methyltransferases with IC_{50} values of <1 and ~3 μM for PRMT1 and SET7, respectively; these values compare well with previous measurements.[7] The alkyne analogue **19** showed very weak inhibition of both enzymes (~77% and 63% inhibition at 100 μM vs PRMT1 and SET7, respectively), presumably because the secondary amine is not protonated in the assay conditions (pH 7.4). It has previously been reported that AzaAdoMet **3** has a pK_a of ~7 and therefore requires low pH in order to afford the ammonium species presumed to be the inhibitory species,[15] but PRMT1 is not active at low pH.[22]

The same assay conditions revealed that AzaAdoMet analogues **15**, **16** and **17**, bearing guanidines attached via 3, 4 and 5 carbon alkyl linkers, respectively, inhibited PRMT1 with good potency (IC_{50} ~3–6 μM). Neither of these analogues had significant inhibitory activity against the lysine methyl transferase SET domain, showing at least 20-fold discrimination between these proteins. Interestingly, the triazole linked inhibitor was an equally poor inhibitor of both arginine and lysine methyltransferases (IC_{50} >50% at 100 μM), presumably because the rigid triazole part of the linker prevents binding at the target sub-site.

The notion of joining structural components from both co-factor and substrate peptides offers a powerful route to achieve potent and selective enzyme inhibition is reinforced by these promising results for the prototype design presented herein. Whereas the unadorned alkyne derived AzaAdoMet **19** is a weak inhibitor at neutral pH, extension of this structure to display guanidine functionality via an alkyl linker appears to significantly improve potency to approach that of non-specific sinefungin **4**. Presumably, the guanidine functionality gains favourable binding interactions within the enzyme binding site, possibly at the conserved glutamate residues required for catalysis (E144 or E153 in PRMT1).[22] Certainly, decoration of AzaAdoMet **3** appears to confer specificity, since poor activity was observed against lysine methyltransferase. The basis of this selectivity remains to be clarified, but significant differences between the conformation of AdoMet **1** at the active sites of arginine and lysine methyltransferases have previously been noted.[1] The weak inhibition of either arginine or lysine methyltransferases observed for the triazole derived compound **20** suggests that an optimum arrangement of linker is required for activity, which reinforces the idea that the observed potencies are indeed the result of specific interactions at the active site. This offers the prospect of further optimisation to increase potency and selectivity by further development of structure.

In summary, we have described a novel design for inhibitors of protein arginine methyl transferases based on non-reactive amine analogues of AdoMet **1**. An expedient synthesis of prototype compounds has been described using successive reductive aminations to install functional variation at the amine analogous to the sulfonium of AdoMet **1**. The synthetic route is well suited to further investigation of related structures. Production of related inhibitor designs for other AdoMet utilising enzymes can be easily realised using this route. In vitro assay of a small array of prototype compounds revealed potent and selective PRMT1 inhibitors that reinforce the potential of the design and encourage further investigation. Detailed kinetic analysis will be required in order to confirm whether the reported compounds compete at both co-factor and substrate peptide binding sites. Like AzaAdoMet **3**, it is unlikely that the tertiary amine is protonated at neutral pH, thus future synthesis of the equivalent methyl ammonium species may provide even more potent inhibitors. In any case, these promising results justify more detailed investigation of a broader range of structures to optimise potency and selectivity, with the ultimate goal of designing compounds that can be tuned to inhibit the function of individual members of the PRMT family. While the prototype compounds are far from ideal therapeutic compounds, their structures will provide inspiration for the development of more drug-like molecules.

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Supplementary data

Supplementary data (spectral data of compounds and procedures) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.02.069.

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