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New *S*-Adenosyl-L-methionine Analogues: Synthesis and Reactivity Studies

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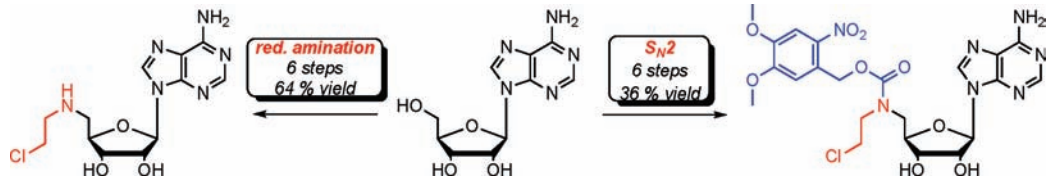
ABSTRACT



Two new and complementary synthetic strategies for 5′-*N*-chloroethylamino-5′-deoxyadenosines are presented. Additionally, the reaction kinetics of their conversion into aziridines under typical enzyme assay conditions is reported using time-resolved NMR spectroscopy. A stable photocaged derivative of 5′-*N*-chloroethylamino-5′-deoxyadenosine has also been synthesized, and its stability and activation in aqueous solution at physiological pH have been examined.

S-Adenosyl methione (SAM) is the second most abundant coenzyme in the human body and often referred to as "mother nature's methyl iodide". Methyltransferases (MTs) transfer the activated methyl group from the sulfur center to specific positions in a variety of substrates, e.g., DNA, RNA, proteins, and secondary metabolites.[1] In recent years, increasing interest in these enzymes has led to the design of two classes of SAM analogues: Aziridinoadenosines ($\mathbf{A_1}$) and double-activated SAM analogues (**B**) (Figure 1).[2] These compounds have been used to label a variety of biopolymers and secondary metabolites using MTs. Other interesting applications of these coenzyme analogues include their use as inhibitors of specific enzymes[3] or their use as chemical tools for the identification of methylation targets.[4] Aziridinoadenosines are highly reactive and unstable compounds; therefore, 5′-*N*-halogenoethylamino-5′-deoxyadenosines ($\mathbf{A_0}$) have been proposed as synthetic precursors, as they can form the aziridine ring in situ (Figure 1).[5] To date, only iodo compounds have been synthesized and successfully reported to alkylate DNA molecules via intermediate aziridine formation in reactions catalyzed by *Thermus aquaticus* (*MTaq*.1) and *E. coli* (EcoR1) DNA methylases,[5,6] while the 5′-*N*-

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(1) For reviews, see: (a) Fontecave, M.; Atta, M.; Mulliez, E. *Trends Biochem. Sci.* **2004**, *29*, 243–249. (b) Chiang, P. K.; Gordon, R. K.; Tal, J.; Zeng, G. C.; Doctor, B. P.; Pardhasaradhi, K.; McCann, P. P. *FASEB J.* **1996**, *10*, 471–480. (c) Frey, P. A.; Magnusson, O. T. *Chem. Rev.* **2003**, *103*, 2129–2148.

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(5) Weller, R. L.; Rajski, S. R. *Org. Lett.* **2005**, *5*, 2141–2144.

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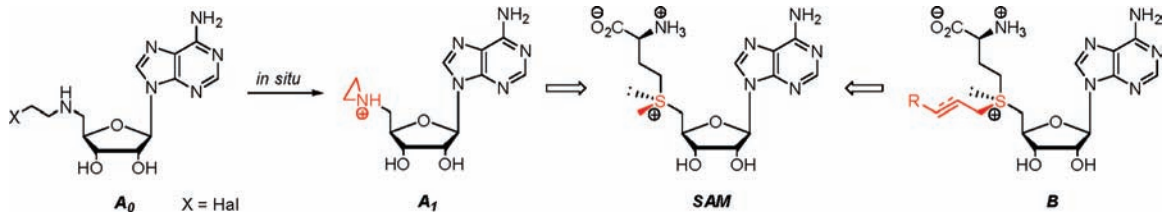


Figure 1. *S*-Adenosylmethionine and currently available analogues. $\mathbf{A_0}$: Halogenoethylamino precursor. $\mathbf{A_1}$: Aziridinoadenosine. **B**: Double-activated analogues.

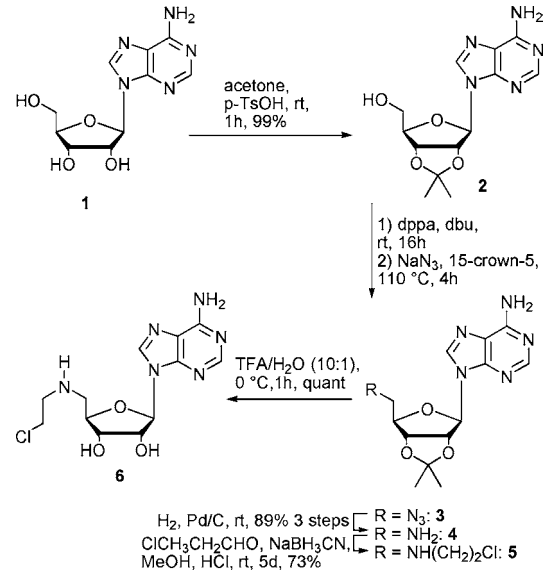
homoalanine derivative of $\mathbf{A_0}$ has been shown to alkylate the arginine side chain of a peptide corresponding to the N-terminal of the H4-histone in a PRMT1-mediated reaction.[7]

These compounds have been used increasingly as biological tools; however, their take up is hampered by the long, inflexible, and low-yielding syntheses that have been reported to date even following some optimization.[5,6] Even more surprising, currently there are no substantive studies on the reaction kinetics for the aziridine formation and breakdown of these nucleosides.[8] Previous studies on simple alkyl nitrogen mustards suggest that the iodo compounds are highly reactive electrophiles themselves[9] and could therefore compete with the in situ formed aziridines for available nucleophiles, opening the opportunity for undesired nonspecific and nonenzyme mediated alkylations.

Here, we report two new and efficient routes to *N*-chloromustard-substituted adenosines, the synthesis of a photocaged version, and kinetic studies on the formation and chemical behavior of the aziridine compounds in a phosphate buffer at physiological pH. In previously reported syntheses, the installation of the 5′-nitrogen proved to be a major difficulty which led to lengthy and inefficient synthetic routes.

We proposed that the introduction of the nitrogen in a simpler fashion into standard protected adenosine derivatives would decrease the length and improve the yield of a synthetic route immensely. We therefore chose reductive amination[10] as the key reaction to introduce the complete *N*-chloromustard substituent on the 5′-position in a single step. Starting from commercially available adenosine (**1**), the 2′- and 3′-hydroxyl groups were selectively protected in quantitative yields as the corresponding acetonide **2**.[11] The 5′-nitrogen was introduced using diphenylphosphoryl azide (dppa) and subsequent treatment with sodium azide, followed by reduction to give amine **4** in 89% yield over the three steps. Amine **4** was then subjected to a direct reductive amination with chloroacetic aldehyde, and pleasingly, the corresponding *N*-chloromustard was installed in 73% yield. Subsequent acid-mediated deprotection of the acetonide gave the desired compound (**6**) in five steps and 64% overall yield (Scheme 1). This provides the first reported synthesis of this compound and is one of the shortest and highest yielding syntheses of a nitrogen-containing SAM analogue to date.

Scheme 1. Synthesis of 5′-*N*-Chloroethylamino-5′-deoxyadenosine (**6**)



In order to gain a deeper insight into the reactivity and chemical behavior of these compounds, we investigated the kinetics of the transformation of the chloromustard adenosine **6** as well as the in situ formed aziridinoadenosine **7** by time-resolved ^{1}H NMR spectroscopy (Figure 2). As expected, the conversion of the chloromustard side chain into the aziridine

(6) (a) Pignot, M.; Siethoff, C.; Linscheid, M.; Weinhold, E. *Angew. Chem., Int. Ed.* **1998**, *37*, 2888–2891. (b) Pljevaljcic, G.; Schmidt, F.; Weinhold, E. *ChemBioChem* **2004**, *5*, 265–269. (c) Comstock, L. R.; Rajski, S. R. *Nucleic Acids Res.* **2005**, *33*, 1644–1652. (d) Weller, R. J.; Rajski, S. R. *ChemBioChem* **2006**, *7*, 243–245.

(7) Osborne, T.; Weller Roska, R. L.; Rajski, S. R.; Thompson, P. R. *J. Am. Chem. Soc.* **2008**, *130*, 4574–4575.

(8) Note that there has been an investigation with respect to the in situ formation of the aziridine by Rajski et al.; however, this was just a preliminary investigation and did not lead to the determination of a half-life: Petersen, S. G.; Rajski, S. R. *J. Org. Chem.* **2005**, *70*, 5833–5839.

(9) Jenkins, T. C.; Naylor, M. A.; O'Neill, P.; Threadgill, M. D.; Cole, S.; Stratford, I. J.; Adams, G. E.; Fielden, E. M.; Suto, M. J.; Stier, M. A *J. Med. Chem.* **1990**, *33*, 2603–2610.

(10) For a review on reductive aminations, see: Baxter, E.; Reitz, A. B. *Org. Reactions* **2002**, *59*, 1–714.

(11) Note that 2′,3′-*O*-isopropylideneadenosine is also commercially available, i.e., from Sigma-Aldrich.

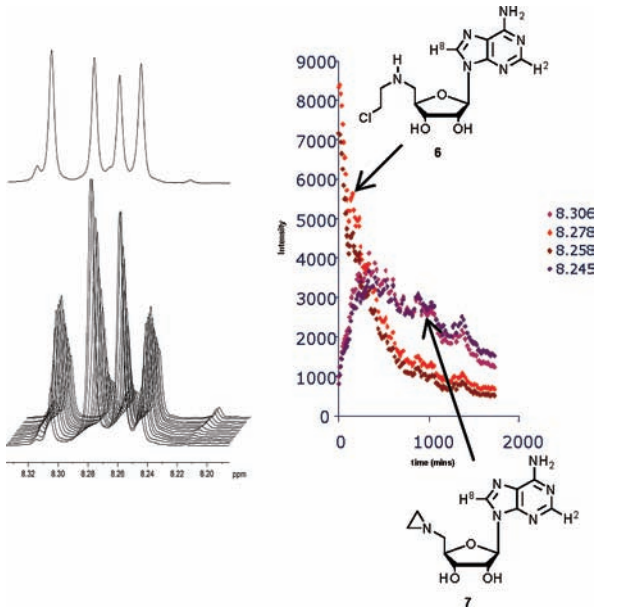


Figure 2. Half-life studies of **6**. Peaks: 8.31 (H-2, **7**), 8.28 (H-2, **6**), 8.26 (H-8, **6**), 8.25 (H-8, **7**). (Note that the 5′-nitrogen of **7** would be partially protonated.)

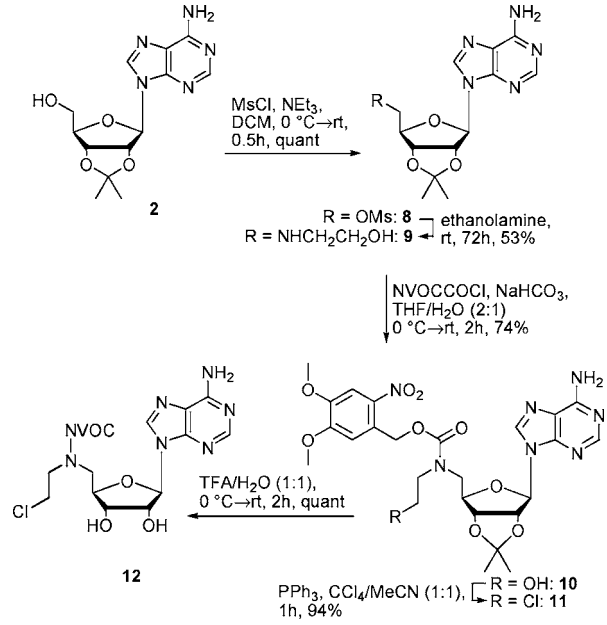
ring followed typical first-order reaction kinetics, and the half-life ($t_{1/2}$) of the parent compound was determined to be $t_{1/2} = 390$ min in 25 mM sodium phosphate buffer at pH = 7.4 and 25 °C.

Furthermore, we observed that the concentration of aziridine **7** formed, reached a maximum, and then began to decrease after approximately half of the chloromustard had been converted to it, producing several products. Investigation of the NMR solution with electrospray mass spectrometry, revealed the major components to be products of aziridine ring-opening by nucleophiles present in the buffer system (hydroxide and phosphate ions). Based on these findings, we concluded that there seems to be an "optimal time-frame" for the use of *N*-chloromustard-substituted SAM analogues in biological assays, and ideally, a mainly non-nucleophilic buffer system should be employed.[12] Due to the low stability of the chloromustard-substituted compounds in aqueous solutions and on prolonged storage at −20 °C, we decided to synthesize a photocaged derivative of the parent chloromustard-substituted adenosine **6**. Photocleavable protecting groups have previously been used in various biological applications and have proved to be extremely useful in controlling the reactivity of highly reactive probes, thus allowing the storage of the compounds not only in pure form on the bench but even in the buffer necessary for the biological assays without any reaction occurring.[13] We chose the 6-nitroveratryl group (NVOC), as this group can be cleanly removed by exposure of the "caged" compound to UV radiation of wavelength of >350 nm. NVOC has been used for the protection of nucleoside-based drugs and probes before.[14] For the synthesis, we decided to apply a second approach to install the 5′-nitrogen, a nucleophilic displacement of a leaving group at the 5′-position, which would be complementary to the reductive amination route.

The leaving group was introduced by transforming the free 5′-hydroxy group of the 2′,3′-*O*-isopropylideneadenosine (**2**) into a mesylate (**8**). Subsequent nucleophilic displacement could only be achieved using 2-aminoethanol, both as reactant and as solvent. All other reaction conditions and nucleophiles tested led primarily to a previously described intramolecular cyclization product.[15]

Pleasingly, using 2-aminoethanol provided the corresponding 5′-hydroxyethylaminoadenosine **9** in 53% yield without the formation of the cyclized side product.[16] The NVOC-protecting group was installed as its carbamate using the corresponding commercially available chloroformate, and the free hydroxy group was transformed subsequently into the chloride by an Appel chlorination. After acid-catalyzed deprotection, the photocaged nucleoside **12** was obtained in 36% overall yield in six steps from adenosine (Scheme 2).

Scheme 2. Synthesis of a Photocaged SAM Analogue (**12**)



To our surprise, the photocaged compound (**12**) could be stirred unchanged at room temperature in daylight for 12 h

(12) Kinetic studies involving *N*-chloromustards bearing a second nitrogen substituent are ongoing and will be reported in due course.

(13) For a review on photolabile protecting groups, see: Bochet, C. G. *J. Chem. Soc., Perkin Trans. 1* **2002**, 125–142.

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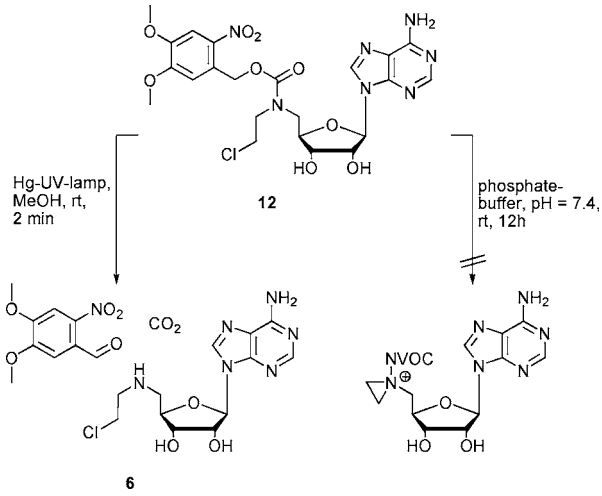
(15) The intramolecular cyclization product results of an attack of N-9 on the 5′-carbon to form a tetracyclic salt; see: (a) Liu, F.; Austin, D. J. *J. Org. Chem.* **2001**, *66*, 8643–8645. (b) Liu, F.; Austin, D. J. *Tetrahedron Lett.* **2001**, *42*, 3153–3154. (c) Ashton, T. D.; Scammells, P. J. *Bioorg. Med. Chem. Lett.* **2006**, *42*, 4564–4566.

(16) It should be noted that this yield is that after a double purification by column chromatography. The overall yield can be increased by submitting the crude product directly to the next step.

Scheme 3. Activation and Reactivity Studies of Photocaged Analogue **12**



without any reaction in the same buffer system as previously used for the NMR studies of compound **6**. Activation of the compound by removal of the photocaging protecting group was possible with just 2 min irradiation with a standard laboratory Mercury UV lamp (Scheme 3).

In conclusion, we presented two new, highly efficient, versatile, and complementary approaches for the synthesis of 5′-*N*-chloroethylamino-5′-deoxyadenosines for applications as nitrogen-containing SAM analogues. For the first time, a detailed investigation of the reaction kinetics of the aziridine formation and the chemical behavior of the aziridine has been conducted using time-resolved NMR spectroscopy and electrospray mass spectrometry. In addition, a photocaged derivative has been synthesized and found to be stable in solid form, in aqueous buffer solution, and in methanol, in which its activation by UV irradiation was studied.

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Supporting Information Available: Experimental procedures, characterization data, ^{1}H and ^{13}C NMR spectra for new compounds as well as details on the kinetic studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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