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S-Adenosylhomocysteine Analogues with the Carbon-5′ and Sulfur Atoms Replaced by a Vinyl Unit

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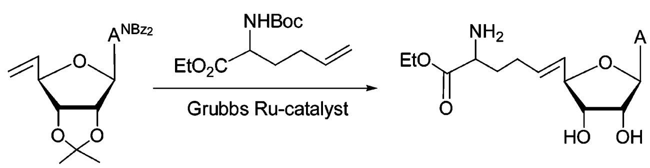
ABSTRACT



Cross-metathesis of suitably protected 5′-deoxy-5′-methyleneadenosines with racemic and chiral *N*-Boc-protected six-carbon amino acids bearing a terminal double bond in the presence of the Hoveyda−Grubbs catalyst gave adenosylhomocysteine analogues with the C5′−C6′ double bond. Bromination with pyridinium tribromide and dehydrobromination with DBU followed by standard deprotections yielded the 5′-(bromo)vinyl analogue.

The enzyme *S*-adenosyl-L-homocysteine (AdoHcy) hydrolase (EC 3.3.1.1) effects hydrolytic cleavage of AdoHcy to adenosine (Ado) and L-homocysteine (Hcy).[1] The cellular levels of AdoHcy and Hcy are critical because AdoHcy is a potent feedback inhibitor of crucial transmethylation enzymes.[1,2] Also, elevated plasma levels of Hcy in humans have been shown to be a risk factor in coronary artery disease.[3]

The X-ray crystal analysis of human AdoHcy hydrolase inactivated with 9-(dihydroxycyclopentene)adenine[4a] and neplanocin A,[4b] as well as of AdoHcy hydrolase from rat liver,[4c] established the presence of a water molecule in the active site of the enzyme. This observation made it a high priority to prepare analogues of AdoHcy that closely resemble the natural substrate that binds tightly to the enzyme. Such compounds should form “stable” complexes with the enzyme that would help to identify key binding groups at the active site of the enzyme that interact with the Hcy moiety and participate in subsequent elimination and “hydrolytic” activity steps.

On the basis of the previous finding that AdoHcy hydrolase is able to add the enzyme-sequestered water molecule across the 5′,6′-double bond of 5′-deoxy-5′-(halo or dihalomethylene)adenosines causing covalent binding inhibition,[5,6] we now describe the synthesis of AdoHcy analogues **A** (X = H) with the 5′,6′-olefin motif incorporated in place of the carbon-5′ and sulfur atoms (Figure 1). The analogues **A** or **B** (X = halogen) should be substrates for the oxidative activity of the enzyme, and the resulting 3′-keto products

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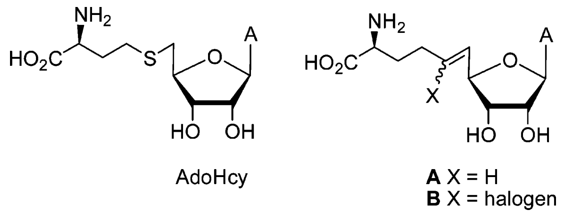
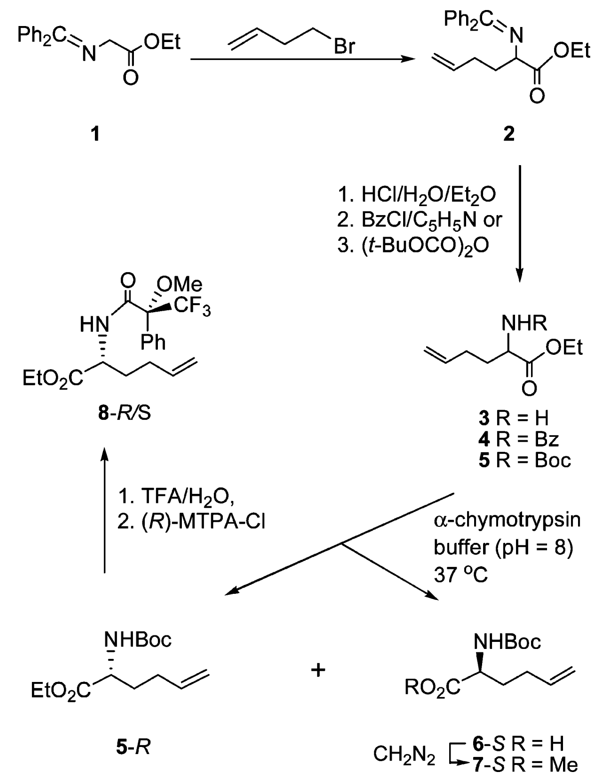


Figure 1. *S*-Adenosyl-L-homocysteine and analogues with the sulfur atom replaced by the vinyl unit.

might be substrates for the hydrolytic activity. Enzyme-mediated addition of water might occur at C5′ or C6′ of **A** or **B** to generate new species with hydroxyl or keto (after β-elimination of HBr) binding sites within the enzyme. X-ray structures of such oxidation and/or hydrolytic activity-bound products might provide important information regarding key residues in the protein and their interactions with substrates (Hcy unit) and/or the sequestered water molecule.

Retrosynthetic analysis indicates that AdoHcy analogue **A** (X = H) can be prepared by construction of a new C5′−C6′ double bond via Wittig or metathesis reactions. For example, condensation of adenosine 5′-aldehyde with a Wittig-type reagent or cross-metathesis between 5′-deoxy-5′-methyleneadenosine and the appropriate amino acid-derived terminal alkenes should give **A**. Because nucleoside 5′-aldehydes are unstable in the presence of strong bases required for the generation of nonstabilized phosphorane−Wittig reagents,[7] we decided to target an AdoHcy analogue of type **A** via the cross-metathesis reaction. Another possibility is Pd-catalyzed cross-coupling approaches between sp^2 and sp^3 hybridized carbons to form a new C6′−C7′ single bond as a key step.[8,9]

Scheme 1



Alkylation of protected glycine **1** with 4-bromo-1-butene followed by hydrolysis of the resulting Schiff base derivative[10] **2** yielded racemic 2-amino-5-hexenoate **3** (Scheme 1). Attempted cross-metathesis[11] between 5′-deoxy-2′,3′-*O*-isopropylidene-5′-methyleneadenosine **9a**[5a,7] and *N*-benzoyl **4** or *N*-Boc **5** protected amino acids bearing a terminal double bond in the presence of first and second (2-imidazolidinylidene-Ru) generation Grubbs catalysts[11c,e] failed to give the desired products **10a** or **11a** (Scheme 2). Also, metathesis of the 6-*N*-benzoyl adenosine substrate **9b** with **4** or **5** was unsuccessful. It is noteworthy that metathesis between 5′-deoxy-2′,3′-*O*-isopropylidene-5′-methyleneuridine[12] and **4** (CH_2Cl_2/second generation Grubbs catalyst) afforded the desired product of type **10** (i.e., B = U; 62%)[13] in addition to two dimers resulting from the self-metathesis of nucleoside[14] and amino acid[15] (e.g., **17**) substrates.

We found however that treatment of **9b** with **4** in the presence of the Hoveyda−Grubbs catalyst[16] (*o*-isopropoxyphenylmethylene-Ru) led to the formation of metathesis product **10b** (51%) in addition to dimer **17** (11%), and self-metathesis of **9b** was not observed. Metathesis of the 6-*N*,*N*-

(6) For other examples on the hydrolytic activity of AdoHcy hydrolase, see: (a) Ref 1c. (b) Yang, X.; Yin, D.; Wnuk, S. F.; Robins, M. J.; Borchardt, R.T. *Biochemistry* **2000**, *39*, 15234−15241. (4′-Haloacetylene adenosine analogues). (c) Guillerm, G.; Guillerm, D.; Vandenplas-Witkowski, C.; Roginaux, H.; Carte, N.; Leize, E.; Van Dorsselaer, A.; De Clercq, E.; Lambert, C. *J. Med. Chem.* **2001**, *44*, 2743−2752. (5′-*S*-Allenyl-5′-thioadenosine). (d) Jeong, L. S.; Yoo, S. J.; Lee, K. M.; Koo, M. J.; Choi, W. J.; Kim, H. O.; Moon, H. R.; Lee, M. Y.; Park, J. G.; Lee, S. K.; Chun, M. W. *J. Med. Chem.* **2003**, *46*, 201−203. (Fluoro-neplanocin A). (e) Wnuk, S. F.; Lewandowska, E.; Sacasa, P. R.; Crain, L. N.; Zhang, J.; Borchardt, R. T.; De Clercq, E. *J. Med. Chem.* **2004**, *47*, 5251−5257. (4′-Enyne adenosine analogues). (f) Guillerm, G.; Muzard, M.; Glapski, C. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5799−5802. (Haloethyl esters of homoadenosine-6′-carboxylic acid). (g) Guillerm, G.; Muzard, M.; Glapski, C.; Pilard, S.; De Clercq, E. *J. Med. Chem.* **2006**, *49*, 1223−1226. [5′-Deoxy-5′-(cyanomethylene)adenosine].

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(8) For example couplings between 5′-deoxy-5′-(iodomethylene)-adenosine[5a] and suitable alkylzinc bromide produced analogues of type **A**, see: Wnuk, S. F.; Lalama, J.; Andrei, D.; Garmendia, C.; Robert, J. *S*-Adenosylhomocysteine and *S*-ribosylhomocysteine analogues with sulfur atom replaced by the vinyl unit. *Abstracts of Papers, Carbohydrate Division*, 229th National Meeting of the American Chemical Society, San Diego, CA, March 13−17, 2005; American Chemical Society: Washington, DC, 2005; CARB-035.

(9) (a) Pd-catalyzed alkylation of the 5′-deoxy-5′-(dihalomethylene)-adenosine[5b] precursors employing recently reported selective monoalkylation of the unactivated 1,1-dichloro-1-alkenes[9b] or 1-fluoro-1-halo-1-alkenes[9c] might give direct access to analogues **B**. (b) Tan, Z.; Negishi, E.-I. *Angew. Chem., Int. Ed.* **2006**, *45*, 762−765. (c) Andrei, D.; Wnuk, S. F. *J. Org. Chem.* **2006**, *71*, 405−408.

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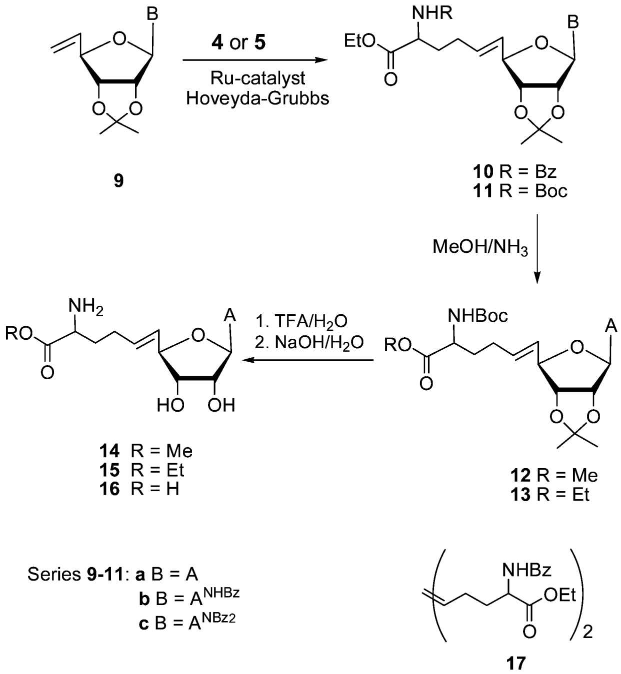
Scheme 2



dibenzoyl **9c** with **4** gave **10c** in 60% yield in addition to dimer **17** (18%). The protection of the 6-amino group of the adenine ring seems to be necessary because metathesis between **9a** and **4** or **5** in the presence of the Hoveyda–Grubbs catalyst did not yield the corresponding product **10a** or **11a**.

Metathesis of **9b** and **9c** with *N*-Boc-protected **5** gave **11b** (61%) and **11c** (76%) in higher isolated yields. Moreover, byproducts of the self-metathesis of amino acid or nucleoside substrates were not isolated. The cross-metathesis products **10** and **11** were found to be predominantly trans isomers.[17] Purification on a silica gel column afforded **10** and **11** as 5′*E* isomers of a ~1:1 mixture of 9′*R/S* diastereomers. The *E* stereochemistry for **10** and **11** was established from ^{1}H NMR spectra based on the magnitude of $J_{H5'-H6'}$. For example, the 5′ proton in **11c** appears at δ 5.58 (dd, $J_{H5'-H4'} =$ 7.3 Hz and $J_{H5'-H6'} =$ 15.2 Hz) and the 6′ proton resonates at δ 5.73 (dt, $J_{H6'-H7'/7''} =$ 6.5 Hz and $J_{H5'-H6'} =$ 15.2 Hz).

Deprotection of **10** or **11** turned out to be more challenging than we expected. Thus, treatment of **11c** (or **11b**) with a 1:1 mixture of saturated (at ~0 °C) methanolic ammonia solution and methanol for 48 h at ~5 °C removed the 6-*N*-benzoyl group(s) and produced a partially separable mixture of methyl **12** and ethyl **13** esters (~3:2, ~92% total yield). Using diluted NH_3/MeOH minimized the formation of the amidation byproducts (~5%). Acid-catalyzed deprotection of **12** and **13** with an aqueous solution of trifluoroacetic acid (TFA) effected the removal of both the Boc and the isopropylidene protection groups to give **14** and **15** in high yields. It is important to perform debenzoylation of **11c** (or **11b**) as the initial deprotection step because treatment of **11c** (or **11b**) with TFA/H_2O resulted in the substantial cleavage of the glycosylic bond. Saponification of **14** and **15** with NaOH in H_2O/MeOH solution and purification on RP-HPLC afforded the sodium salt of **16** [67%; *E*, 9′*R/S* (~1:1)].

Because the separation of 9′*R/S* diastereomers in products **10**–**16** was difficult, we attempted the synthesis of analogue **A** with a 9′*S* configuration employing a chiral amino acid precursor, e.g., (*S*)-homoallylglycine. Given that the methods available for the preparation of enantiomerically pure unnatural amino acids usually require multistep synthesis,[18] we chose the enantioselective hydrolysis of racemic **5** as a way to provide chiral (*S*)-homoallylglycine. Thus, treatment of **5** with α-chymotrypsin in phosphate buffer (24 h, 37 °C)[19] gave the unreacted (*R*)-ester **5** (~50%) and (*S*)-acid **6** (~50%, Scheme 1). Enantiomeric purity of **5**-*R* was established using the Mosher test.[20a] Thus, treatment of **5**-*R* with TFA/H_2O followed by acylation with (*R*)-2-methoxy-2-trifluoromethyl-2-phenylacetyl chloride (MTPA-Cl)[20] gave **8**-*R/S*. (Note that the absolute configuration at the chiral carbon in the Mosher reagent is the same, but the *R/S* descriptors change owing to the change in Cahn–Ingold–Prelog priority.) Analysis of the ^{19}F NMR spectra [δ −69.16 (s, 0.02F) and −69.55 (s, 0.98F)] established the stereochemistry for **5** as *R* (ee 96%) in agreement with Mosher's empirical formula.[20a] Because metathesis of the "free" carboxylic acid precursor **6**-*S* with **9b** or **9c** in the presence of the Hoveyda–Grubbs catalyst failed, **6**-*S* was converted into the methyl ester **7**-*S* with diazomethane.

Cross-metathesis of **9c** with **7**-*S* afforded **18**-*S* (77%; Scheme 3). Sequential deprotections of **18**-*S* with NH_3/MeOH (to give **12**-*S*, 91%) and TFA/H_2O gave the enantiomerically pure **14**-*S* (90%) as the single *E* isomer (Scheme 3). On the other hand, metathesis of **9c** with **5**-*R* gave ethyl ester **11c**-*R*. Contrary to products **10**–**16** obtained from racemic homoallylglycine, the ^{13}C NMR spectra for the products obtained from (*S*)- and (*R*)-homoallylglycine substrates showed a single set of peaks.

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(17) ^{1}H NMR analysis of the crude reaction mixtures showed the presence of other isomers in variable quantities of ~2–8%.

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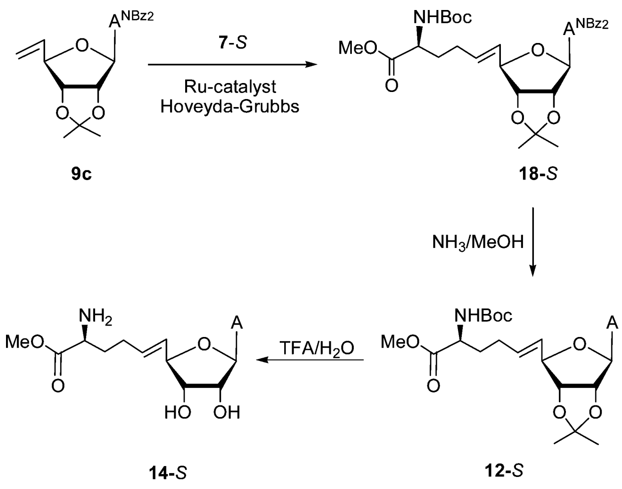
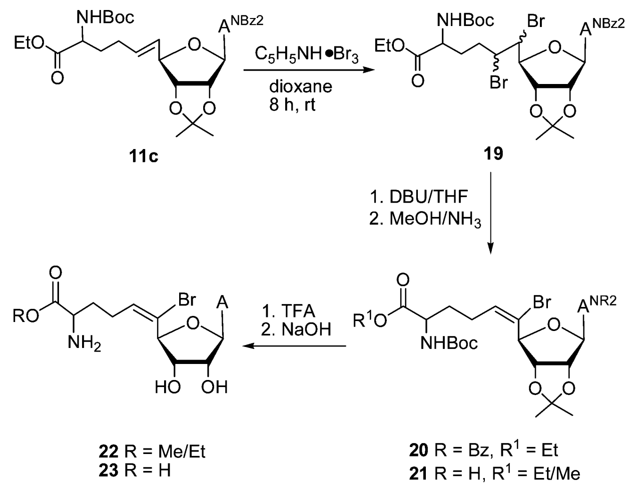
Scheme 3



Scheme 4



Finally, we attempted the synthesis of bromovinyl analogue **B** by the bromination−dehydrobromination strategy. Treatment of **11c** with pyridinium tribromide[21] gave the 5′,6′-dibromo diastereomers **19** which were dehydrobrominated with 1,8-diazobicyclo[5.4.0]undec-7-ene (DBU) to yield **20** as a single isomer (one of the 6-*N*-benzoyl protective groups was also partially cleaved) in 70% yield (Scheme 4). Standard deprotections with NH_3/MeOH and TFA/H_2O followed by saponification with NaOH and HPLC purification gave **23** (*E*, 54% overall).

The regioselectivity of HBr elimination and the position of bromine (at 5′) were assigned on the basis of the presence of a triplet signal for the olefinic hydrogen (H6′) in ^{1}H NMR spectra [δ 6.40 (t, $J_{6'-7'/7''} = 7.6$ Hz) for **23**]. This assignment was also supported by a COSY experiment. The product *E* configuration is expected from a specific antiaddition in the pyridinium tribromide bromination of the *E* alkene **11c** followed by an E2 (antielimination) process. This was also supported by NOESY analysis of **23** in which the cross-peaks between H4′ and H7′/7″ were observed.

In summary, we have developed a synthesis of AdoHcy analogues in which the carbon-5′ and sulfur atoms are replaced by a vinyl unit utilizing cross-metathesis reactions between 5′-deoxy-5′-methyleneadenosine analogues and homoallylglycine in the presence of the Hoveyda−Grubbs catalyst. The 5′-(bromo)vinyl AdoHcy analogue has been prepared via the bromination−dehydrobromination strategy. Enzymatic studies with AdoHcy hydrolase and our attempts to synthesize 6′-(halo)vinyl analogues **B** via cross-coupling approaches will be published elsewhere.

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Supporting Information Available: Experimental procedures and characterization data for all compounds (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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