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

ARTICLE *in* ORGANIC LETTERS · NOVEMBER 2006

Impact Factor: 6.36 · DOI: 10.1021/ol062026m · Source: PubMed

2 AUTHORS:

Daniela Andrei
Dominican University
9 PUBLICATIONS **140** CITATIONS
SEE PROFILE

Stanislaw F Wnuk
Florida International University
162 PUBLICATIONS **2,125** CITATIONS
SEE PROFILE

Published in final edited form as:
Org Lett. 2006 October 26; 8(22): 5093–5096. doi:10.1021/ol062026m.

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

Daniela Andrei and **Stanislaw F. Wnuk***
Department of Chemistry and Biochemistry, Florida International University, Miami, Florida 33199.

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 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 bind 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.

Based on 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 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 Wittig-type reagent or cross-metathesis between 5'-deoxy-5'-methyleneadenosine and the appropriate amino acid-derived terminal alkenes

wnuk@fiu.edu.

should give **A**. Since nucleoside 5'-aldehydes are unstable in the presence of strong bases required for the generation of non-stabilized 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-sp^3 hybridized carbons to form a new C6'–C7' single bond as a key step.[8,9]

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] with *N*-benzoyl **4** or *N*-Boc **5** protected amino acids bearing terminal double bond in the presence of 1st and 2nd (2-imidazolidinylidene-Ru) generation Grubbs catalysts[11c,e] failed to give 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_2/2nd 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 Hoveyda-Grubb's catalyst[16] (*o*-isopropoxy-phenylmethylene-Ru) led to the formation of metathesis product **10b** (51%) in addition to dimer **17** (11%) while self-metathesis of **9b** was not observed. Metathesis of the 6-*N*,*N*-dibenzoyl **9c** with **4** gave **10c** in 60% yield in addition to dimer **17** (18%). The protection of 6-amino group of the adenine ring seems to be necessary because metathesis between **9a** and **4** or **5** in the presence of 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, by-products 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 the *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) while 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 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 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 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)].

Since separation of 9'*R/S* diastereomers in products **10–16** was difficult, we attempted the synthesis of analogue **A** with 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 the **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 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] Since metathesis of the "free" carboxylic acid precursor **6**-*S* with **9b** or **9c** in the presence of Hoveyda-Grubbs catalyst failed, the **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.

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 group 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 based on the presence of a triplet signal for the olefinic hydrogen (H6') in ^1H NMR spectra [δ 6.40 (t, $J_{6'-7'/7''}$ = 7.6 Hz) for **23**]. This assignment was also supported by COSY experiment. The product *E* configuration is expected from a specific anti-addition in the pyridinium tribromide bromination of the *E* alkene **11c** followed by an E2 (anti elimination) 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 is replaced by a "vinyl unit" utilizing cross-metathesis reactions between 5'-deoxy-5'-methyleneadenosine analogues and homoallylglycine in the presence of 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgment

We thank NIH/NIGMS program (S06 GM08205) for supporting this research, US ARO (W911NF-04-1-0022) for financial help to purchase 600 MHz NMR spectrometer and Materia Company for a gift of Hoveyda-Grubbs catalyst.

References

1. Yuan, C-S.; Liu, S.; Wnuk, SF.; Robins, MJ.; Borchardt, RT. Advances in Antiviral Drug Design. De Clercq, E., editor. Vol. 2. Greenwich: JAI Press; 1996. p. 41-88. (b) Turner MA, Yang X, Yin D,

Kuczera K, Borchardt RT, Howell PL. Cell Biochem. Biophys 2000;33:101–125. [PubMed: 11325033] (c) Wnuk SF. Mini-Rev. Med. Chem 2001;1:307–316. [PubMed: 12369977]

2. (a) Ueland PM. Pharmacol. Rev 1982;34:223–253. [PubMed: 6760211] (b) Chiang PK. Pharmacol. Ther 1998;77:115–134. [PubMed: 9578320]
3. (a) Nehler MR, Taylor LM, Porter JM. Cardiovasc. Surgery 1997:559–567. (b) Refsum H, Ueland PM, Nygard O, Vollset SE. Annu. Rev. Med 1998;49:31–62. [PubMed: 9509248] (c) Schynder G, Roffi M, Pin R, Flammer Y, Lange H, Eberli FR, Meier B, Turi ZG, Hess OM. N. Eng. J. Med 2001;345:1593–1600.
4. (a) Turner MA, Yuan C-S, Borchardt RT, Hershfield MS, Smith GD, Howell PL. Nature Struct. Biol 1998;5:369–376. [PubMed: 9586999] (b) Yang X, Hu Y, Yin DH, Turner MA, Wang M, Borchardt RT, Howell PL, Kuczera K, Schowen RL. Biochemistry 2003;42:1900–1909. [PubMed: 12590576] (c) Hu Y, Komoto J, Huang Y, Gomi T, Ogawa H, Takata Y, Fujioka M, Takusagawa F. Biochemistry 1999;38:8323–8333. [PubMed: 10387078]
5. (a) Wnuk SF, Yuan C-S, Borchardt RT, Balzarini J, De Clercq E, Robins MJ. J. Med. Chem 1994;37:3579–3587. [PubMed: 7932585] (b) Wnuk SF, Mao Y, Yuan C-S, Borchardt RT, Andrei J, Balzarini J, De Clercq E, Robins MJ. J. Med. Chem 1998;41:3078–3083. [PubMed: 9685247] (c) Yuan C-S, Wnuk SF, Robins MJ, Borchardt RT. J. Biol. Chem 1998;273:18191–18197. [PubMed: 9660780]
6. For other examples on the hydrolytic activity of AdoHcy hydrolase see(a) Ref 1c (b) Yang X, Yin D, Wnuk SF, Robins MJ, Borchardt RT. Biochemistry 2000;39:15234–15241. [PubMed: 11106503](4'-haloacetyleneadenosine 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. [PubMed: 11495586](5'-*S*-allenyl-5'-thioadenosine) (d) Jeong LS, Yoo SJ, Lee KM, Koo MJ, Choi WJ, Kim HO, Moon HR, Lee MY, Park JG, Lee SK, Chun MW. J. Med. Chem 2003;46:201–203. [PubMed: 12519056](fluoro-neplanocin A) (e) Wnuk SF, Lewandowska E, Sacasa PR, Crain LN, Zhang J, Borchardt RT, De Clercq E. J. Med. Chem 2004;47:5251–5257. [PubMed: 15456269](4'-enyne adenosine analogues) (f) Guillerm G, Muzard M, Glapski C. Bioorg. Med. Chem. Lett 2004;14:5799–5802. [PubMed: 15501043](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. [PubMed: 16480257][5'-deoxy-5'-(cyanomethylene)adenosine]
7. Wnuk SF, Robins MJ. Can. J. Chem 1991;69:334–338.
8. For example couplings between 5'-deoxy-5'-(iodomethylene)-adenosine[5a] and suitable alkylzinc bromides produced analogues of type **A**:WnukSFLalamaJAndreiDGarmendiaCRobertJ*S*-Adenosylhomocysteine and *S*-ribosylhomocysteine analogues with sulfur atom replaced by the vinyl unit. Abstracts of Papers, Carbohydrate Division2005March229th National Meeting of the American Chemical SocietySan Diego, CA1317CARB-035
9. (a) Pd-catalyzed alkylation of the 5'-deoxy-5'-(dihalomethylene)-adenosine[5b] precursors employing recently reporting 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 SF. J. Org. Chem 2006;71:405–408. [PubMed: 16388671]
10. (a) O'Donnell MJ, Wojciechowski K. Synthesis 1984:313–315. (b) O'Donnell MJ, Polt RL. J. Org. Chem 1982;47:2663–2666.
11. (a) Grubbs RH, Chang S. Tetrahedron 1998;54:4413–4450. (b) Fürstner A. Angew. Chem., Int. Ed 2000;39:3012–3043. (c) Trnka TM, Grubbs RH. Acc. Chem. Res 2001;34:18–29. [PubMed: 11170353] (d) Chatterjee AK, Choi T-L, Sanders DP, Grubbs RH. J. Am. Chem. Soc 2003;125:11360–11370. [PubMed: 16220959] (e) Nicolaou KC, Bulger PG, Sarlah D. Angew. Chem., Int. Ed 2005;44:4490–4527.
12. Wnuk SF, Robins MJ. Can. J. Chem 1993;71:192–198.
13. Sacasa, PabloR. M.Sc. Thesis. Florida International University; 2003.
14. (a)For recent reviews on application of metathesis towards synthesis of nucleoside analogues see (a) Agrofoglio LA, Nolan SP. Curr. Top. Med. Chem 2005;5:1541–1558. [PubMed: 16378491]Amblard F, Nolan SP, Agrofoglio LA. Tetrahedron 2005;61:7067–7080.(b)For an example on self metathesis reaction of carbohydrate derived terminal olefins (e.g., 5,6-dideoxy-1,2-*O*-isopropylidene-α-D-*ribo*-hex-5-enofuranose) see:HadwigerPStützAESynlett199917871789

15. (a) Gibson SE, Gibson VC, Keen SP. Chem. Commun 1997:1107–1108. (b) Biagini SCG, Gibson SE, Keen SP. J. Chem. Soc., Perkin Trans. 1 1998:2485–2499. (c) Vasbinder MM, Miller SJ. J. Org. Chem 2002;67:6240–6242. [PubMed: 12182670]

16. (a) Garber SB, Kingsbury JS, Gray BL, Hoveyda AH. J. Am. Chem. Soc 2000;122:8168–8179. (b) Gessler S, Randl S, Blechert S. Tetrahedron Lett 2000;41:9973–9976.

17. ^{1}H NMR analysis of the crude reaction mixtures showed the presence of other isomers in variable quantities of ~2–6%.

18. (a) Dunn MJ, Jackson RFW, Pietruszka J, Turner D. J. Org. Chem 1995;60:2210–2215. (b) Waelchli R, Beerli C, Meigel H, Revesz L. Bioorg. Med. Chem. Lett 1997;7:2831–2836. (c) Löhr B, Orlich S, Kunz H. Synlett 1999:1139–1141. (d) Bachmann S, Knudsen KR, Jorgensen KA. Org. Biomol. Chem 2004;2:2044–2049. [PubMed: 15254632]

19. Schricker B, Thirring K, Berner H. Bioorg. Med. Chem. Lett 1992;2:387–390.

20. (a) Sullivan GR, Dale JA, Mosher HS. J. Org. Chem 1973;38:2143–2147. (b) Oh SS, Butler WM, Koreeda M. J. Org. Chem 1989;54:4499–4503.

21. Husstedt U, Schäfer HJ. Tetrahedron Lett 1981:623–624.

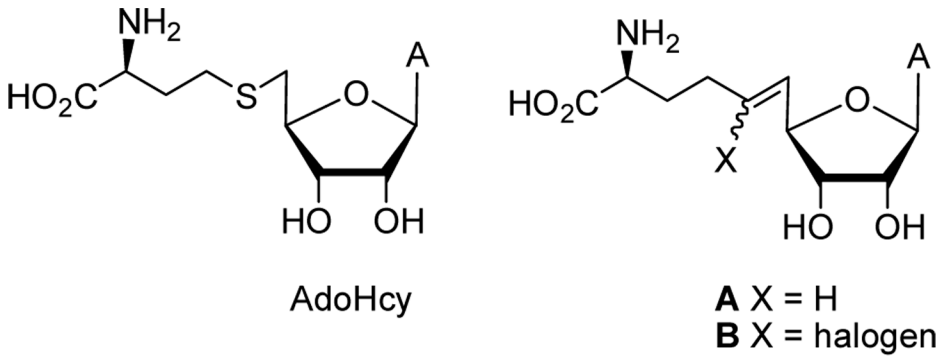
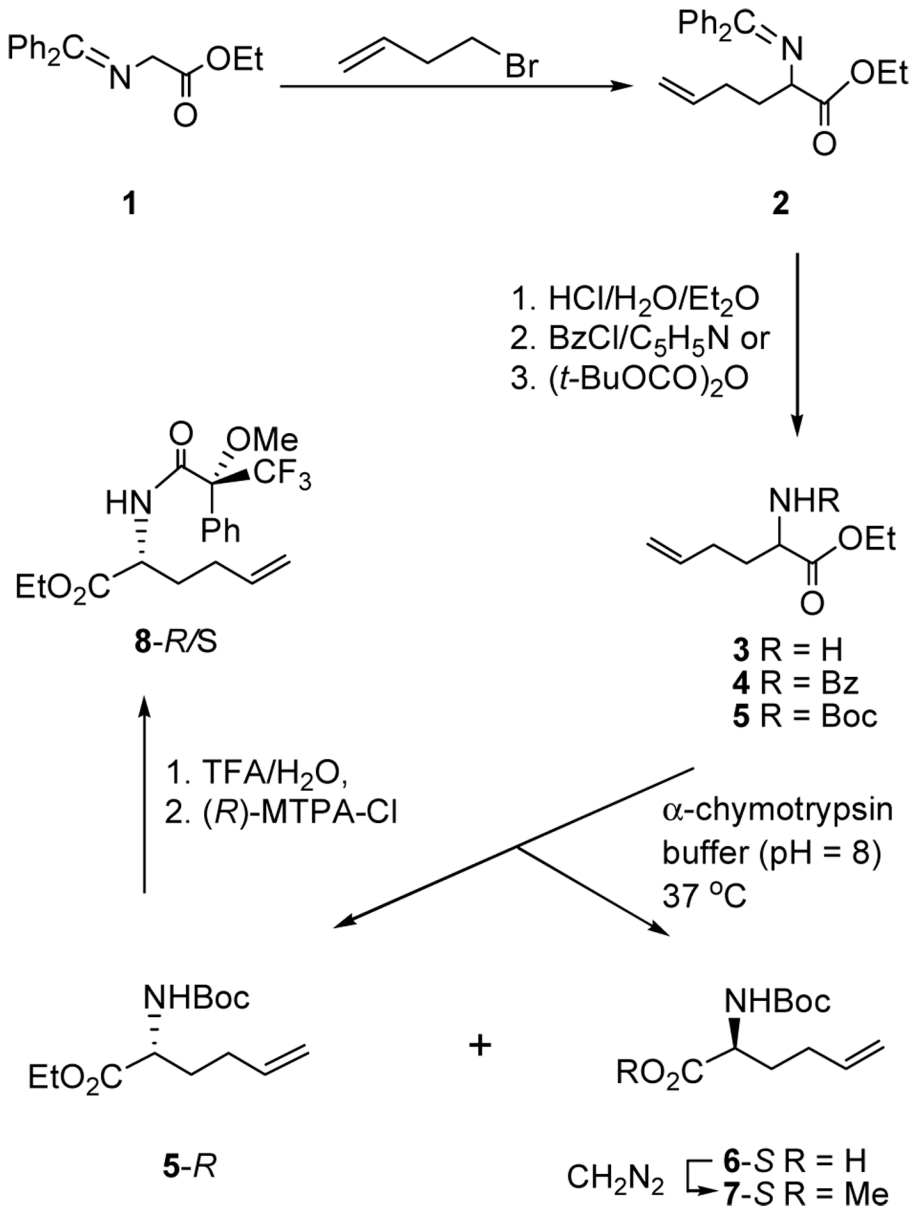
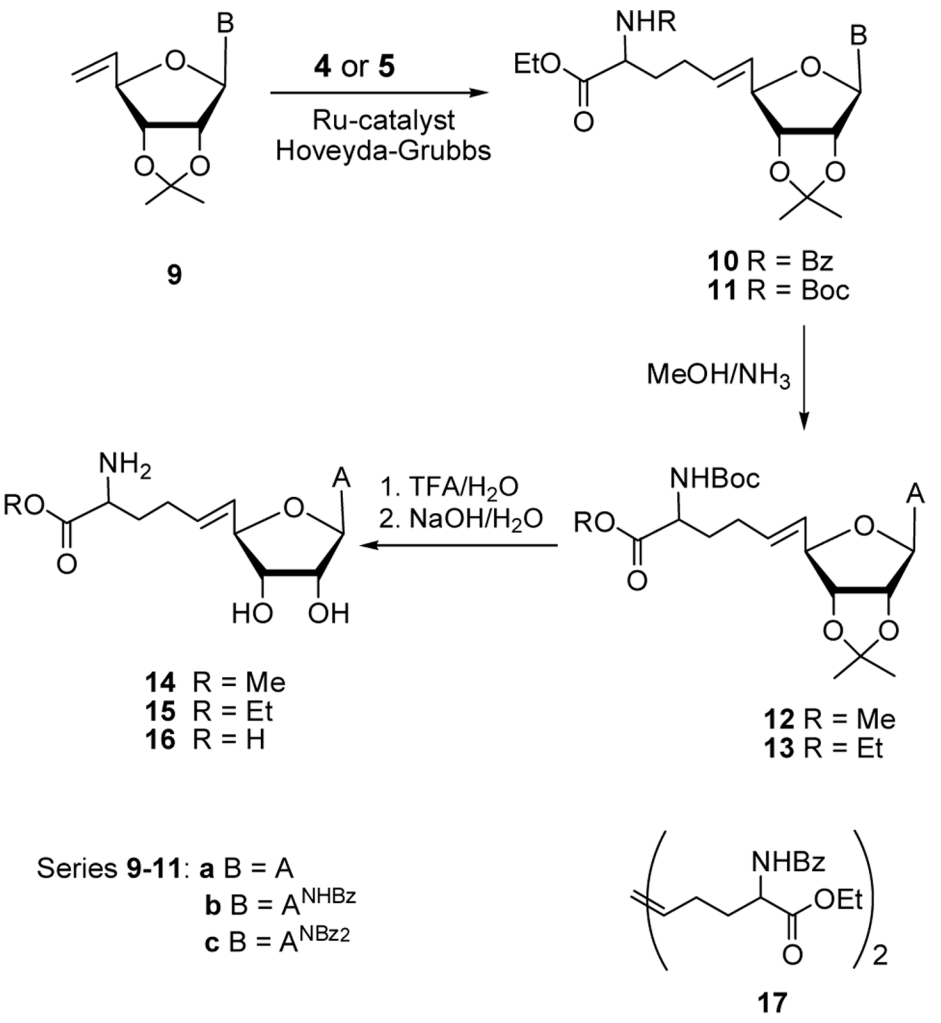


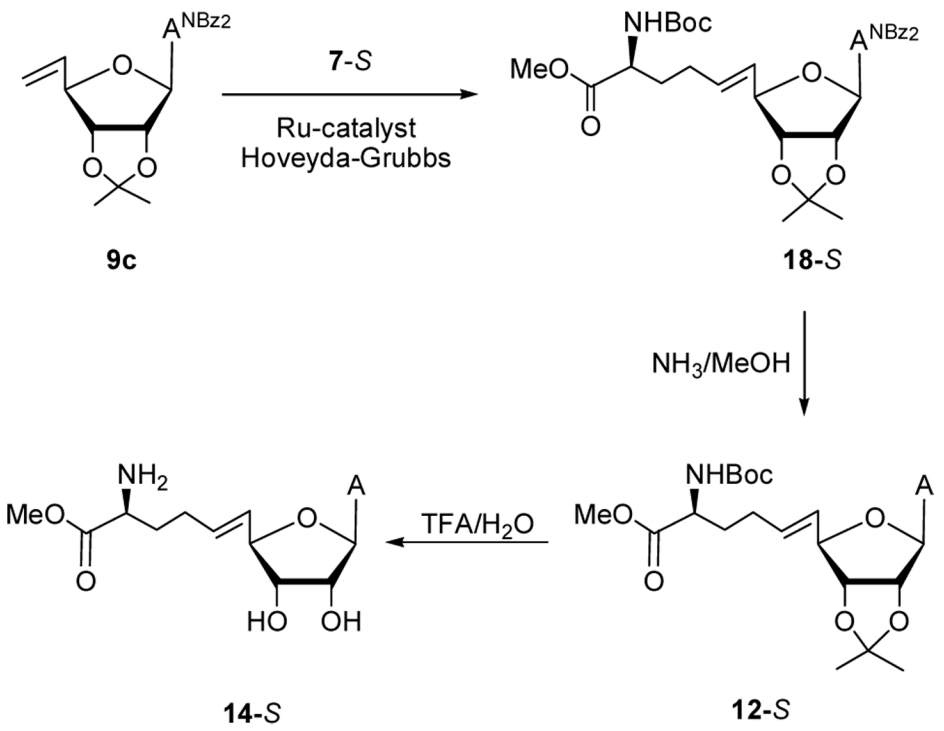
Figure 1.
S-Adenosyl-L-homocysteine and analogues with sulfur atom replaced by the "vinyl unit".



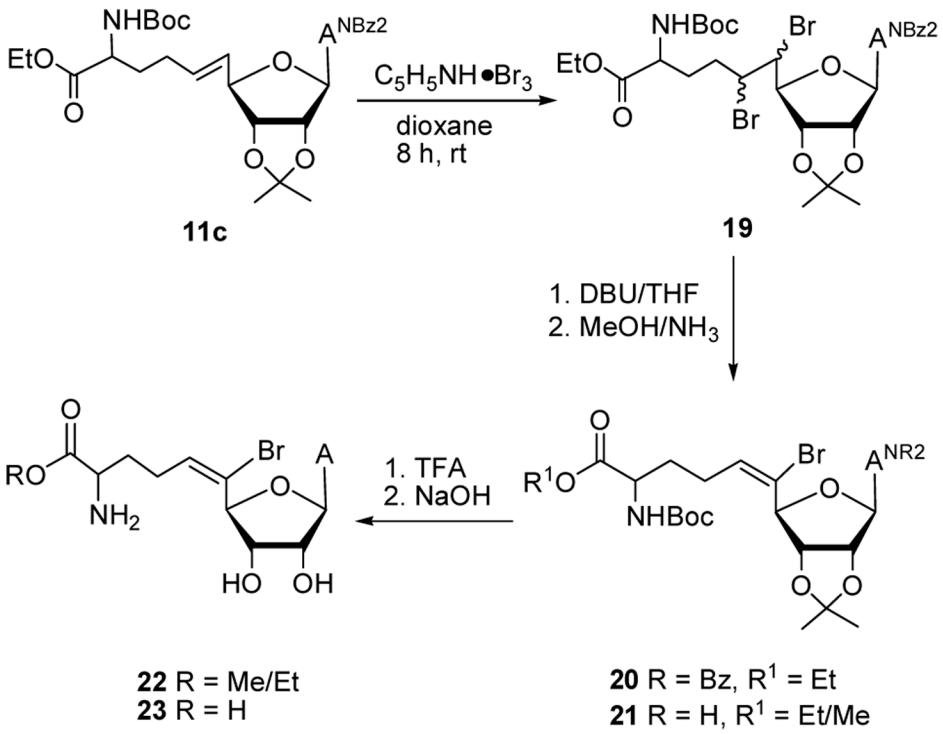
Scheme 1.



Scheme 2.



Scheme 3.



Scheme 4.