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S-Homoadenosyl-L-cysteine and *S*-Homoadenosyl-L-homocysteine. Synthesis and Binding Studies of Non-Hydrolyzed Substrate Analogues with *S*-Adenosyl-L-homocysteine Hydrolase[1]

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Abstract: Treatment of homoadenosine [9-(5-deoxy-β-D-*ribo*-hexofuranosyl)adenine] with thionyl chloride and pyridine in acetonitrile gave 6′-chloro-6′-deoxyhomoadenosine, which underwent nucleophilic displacement with L-cysteine or L-homocysteine to give homologated analogues of *S*-adenosyl-L-homocysteine. Each amino acid in aqueous sodium hydroxide at 60 °C gave excellent conversion from the chloronucleoside, and adsorption on Amberlite XAD-4 resin provided more convenient isolation than prior methods. Weak binding of these non-hydrolyzed analogues to *S*-adenosyl-L-homocysteine hydrolase was observed.

Biomethylation is a key step in regulation of a number of cellular processes, and *S*-adenosyl-L-methionine (AdoMet) serves as methyl donor in the majority of cases. AdoMet-dependent transferases catalyze methyl transfer to a wide range of substrates including DNA, RNA, proteins, hormones, and neurotransmitters. The byproduct, *S*-adenosyl-L-homocysteine (AdoHcy), is a potent feedback inhibitor of these enzymes. Therefore, cellular concentrations and ratios of AdoMet and AdoHcy are stringently regulated. AdoHcy levels in eukaryotes are controlled mainly by cleavage into Ado and Hcy, and this reversible process is catalyzed solely by AdoHcy hydrolase (EC 3.3.1.1). Although the thermodynamic equilibrium favors AdoHcy synthesis, processing of Ado and Hcy by other enzymes drives the reaction in the hydrolytic direction in vivo.[2,3] Inhibition of AdoHcy hydrolase results in elevated levels of AdoHcy, which adversely affects methylation steps required for cellular division and viral multiplication. Consequently, there has been great interest in the design and study of inhibitors of AdoHcy hydrolase,[4−6] and activity against a range of viruses and tumor cells has been found.[5,7]

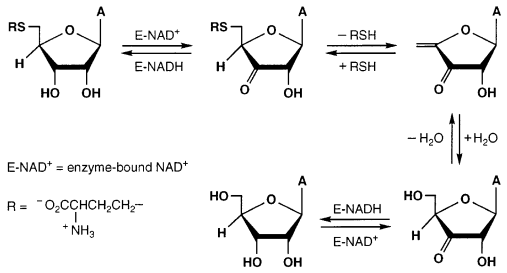

FIGURE 1. Proposed mechanism for *S*-adenosyl-L-homocysteine hydrolase.[8]

Palmer and Abeles[8] proposed the currently accepted enzyme mechanism illustrated in Figure 1. Enzyme-bound NAD^+ first effects oxidation of the 3′-hydroxyl function to a ketone, which activates the α-hydrogen on C4′ for base-promoted removal. Enolate-stabilized elimination of Cys (or water in the synthetic direction) then generates an enone substrate for Michael addition of H_2O (or Cys). X-ray crystallographic structures of a human AdoHcy hydrolase with a bound cyclopentenyladenine inhibitor analogue,[9] and of a rat enzyme (97% sequence homology) without a substrate surrogate,[10] were published recently. These studies provide important data for rational design of new inhibitors, but the significant structural differences[9,10] indicate the need for further crystallographic work with better substrate analogues containing an amino acid moiety to probe interactions of AdoHcy with residues at the binding site.

Relatively few methods have been reported for coupling nucleosides and thiol-containing amino acids. The first reported syntheses of AdoHcy (with 2′,3′-*O*-isopropylidene-5′-*O*-tosyladenosine and *S*-benzyl-L-homocysteine)[11] and a number of analogues[12] employed Na/NH_3

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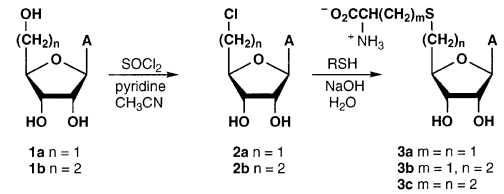
SCHEME 1



(liquid). Later studies used 5′-halo-5′-deoxynucleosides, which avoided the need for hydroxyl group protection.[13] More convenient base/solvent conditions were developed,[14] and a Mitsunobu-type synthesis from a cystine derivative was reported.[15] However, moderate yields or more complex reaction parameters or purification procedures make a number of these approaches unattractive.

We now describe syntheses (and preliminary enzyme-binding studies) of two close analogues of AdoHcy that are not susceptible to enzymatic cleavage of the "adenosine-amino acid" bond. Diffusion of such a ground-state analogue into a crystal of the enzyme (or cocrystallization) might produce a complex in which oxidation to a 3′-ketone and proton abstraction from C4′ remain possible, but the absence of a leaving group at C5′ would preclude cleavage of the resulting enolate (stable C5′-C6′ bond). Homoadenosyl-L-cysteine (**3b**) should be essentially isopolar/isosteric with AdoHcy, differing only by a formal transposition of CH_2 and S. The CH_2-extended homoadenosyl-L-homocysteine (**3c**) was designed to probe the flexibility of hydrogen bonding among enzyme residues and the zwitterionic terminus of AdoHcy (Scheme 1).

Heating an aqueous solution of 5′-chloro-5′-deoxyadenosine[16] (**2a**)/Cys/NaOH at 100 °C resulted in complete conversion of **2a** into AdoCys (**3a**) and a minor byproduct (TLC). At least 3 equiv of Cys was required for rapid reaction, and less than 2 equiv of NaOH was used to ensure that the Cys anion would be the strongest base present. Strong base promotes amino acid racemization[17] as well as glycosyl bond cleavage and purine ring decomposition.[18] Precipitation of analytically pure **3a**·H_2O (61%) occurred upon cooling and neutralization (HOAc) of the solution, and no attempt was made to recover further material from the filtrate.

Treatment of homoadenosine [9-(5-deoxy-β-D-*ribo*-hexofuranosyl)adenine][19] (**1b**) with thionyl chloride/pyridine/acetonitrile [as described for the conversion of adenosine (**1a**) → **2a**[16]] gave 6′-chloro-6′-deoxyhomoadenosine (**2b**) (88%). Heating a solution of **2b**/Cys/NaOH/H_2O at 60 °C for 17 h gave **3b**. Although the lower temperature (60 versus 100 °C) required longer reaction times for complete conversion of **2b** → **3b**, much less decomposition or byproduct formation was observed (TLC). Precipitation of **3b** upon cooling and neutralization was poorly reproducible, so a general purification procedure that gave complete separation of product from unreacted amino acid was devised. Direct application of the reaction mixture to a column of polystyrene resin (Amberlite XAD-4) was followed immediately by washing the column with NH_4OAc buffer (pH ∼5) (good differential adsorption of the neutralized nucleoside conjugate relative to the aliphatic amino acid). Buffer and Hcy were removed by washing with H_2O until the wash was ninhydrin negative, and **3b** (89%) was then eluted (MeOH). The same reaction and purification conditions gave **3c** (quantitative) from **2b** and Hcy.

Montgomery et al. had noted epimerization of the amino acid moiety of AdoHcy synthesized with excess NaOH in aqueous solution at reflux.[13d] We also observed significant decomposition upon heating AdoCys (**3a**) in 1 M NaOH/H_2O for 2 h at 100 °C. However, a synthesis of AdoHcy with the weakly basic disodium salt of Hcy in hot aqueous solution gave product with conserved stereochemistry.[14c] The specific rotation of our **3a** [$[\alpha]^{23}_D$ +20.1° (*c* 0.48, 1 M HCl/H_2O)] was within experimental error of that of a commercial sample (Sigma) [$[\alpha]^{23}_D$ +19.5° (*c* 0.26, 1 M HCl/H_2O)] and greater than that reported for **3a** [$[\alpha]^{20}_D$ +16.9° (*c* 0.5, 1 M HCl)] synthesized by a different method.[12d] Compounds **3b** and **3c** were prepared by analogous (and even milder, 60 °C) conditions and should also be stereochemically homogeneous.

Standard assays with a human placental AdoHcy hydrolase[20] confirmed that neither **3b** nor **3c** was cleaved, whereas AdoCys (**3a**) was hydrolyzed at ∼10% of the rate of the natural substrate. Incubation of **3a**−**c** (100 μM) with AdoHcy hydrolase (420 nM) at 37 °C for 20 min followed by assay of the enzyme activity (hydrolytic direction with 210 nM AdoHcy hydrolase, 10 μg of Ado deaminase, and 1 mM AdoHcy at 37 °C for 5 min)[20] gave residual activities of 86% (**3a**), 90% (**3b**), and 92% (**3c**). The observed ∼10% inhibition by **3a**−**c** at 1/10 of the molar concentration of AdoHcy is consistent with competitive binding with similar affinity to that of the natural substrate in agreement with their nearly isopolar/isosteric structures.

In conclusion, 6′-chloro-6′-deoxyhomoadenosine (**2b**) was prepared in high yield and underwent clean displacement with Cys and Hcy in aqueous base at 60 °C. Convenient and complete separation of the homologated AdoHcy analogues **3b**,**c** from amino acid species and/or byproducts was effected by selective adsorption on a polystyrene resin (Amberlite XAD-4). AdoCys (**3a**) was hydrolyzed by human placental AdoHcy hydrolase at a

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rate ~10% that of the natural substrate, whereas cleavage of the homoadenosine analogues **3b**,**c** was not detected. Analogues **3a**−**c** function as competitive inhibitors of the enzyme with binding comparable to that of AdoHcy in harmony with their isopolar/isosteric structures. Attempts to obtain suitable crystals of enzyme−analogue complexes are in progress.

Experimental Section

Uncorrected melting points were determined with a capillary apparatus. UV spectra were determined with solutions in MeOH. NMR spectra were determined with solutions in DMSO-d_6 (^{1}H at 500 and ^{13}C at 125 MHz) unless otherwise indicated. MS were determined in the FAB mode (glycerol). TLC was performed with silica gel on aluminum plates [EtOAc/H_2O/*i*-PrOH, 4:2:1 (upper phase), neutral compounds; or BuOH/H_2O/AcOH, 60:25:15, ionic compounds].

6′-Chloro-6′-deoxyhomoadenosine (2b). Freshly distilled $SOCl_2$ (37 μL, 60.3 mg, 0.54 mmol) was added to a cold (0 °C) stirred suspension of **1b** (50.2 mg, 0.18 mmol) in MeCN (0.5 mL). The mixture was cooled (0 °C), pyridine (29 μL, 28.4 mg, 0.36 mmol) was added, and stirring was continued at 0 °C for 4 h and overnight at ambient temperature. MeOH (0.5 mL), NH_3/H_2O (29%, 0.5 mL), and H_2O (0.5 mL) were added to the yellow solution, and stirring was continued for 1 h. The solution was applied to a column (1.5 × 13.5 cm) of Dowex 1 × 2 (OH^-) resin, and the column was washed with H_2O (40 mL). Elution [H_2O/MeOH (1:1, 40 mL), MeOH] gave **2b**·0.5H_2O (49.2 mg, 88%) as a white solid: mp ~63 °C (softening), ~178−180 °C dec; UV max 259 nm (ϵ 14 700), min 227 (ϵ 2700); ^{1}H NMR δ 2.06−2.12 (m, 1H), 2.17−2.24 (m, 1H), 3.60−3.65 (m, 1H), 3.69−3.74 (m, 1H), 4.01−4.05 (m, 1H), 4.12−4.15 (m, 1H), 4.69−4.72 (m, 1H), 5.29 (d, J = 4.9 Hz, 1H, exch), 5.49 (d, J = 5.9 Hz, 1H, exch), 5.87 (d, J = 4.9 Hz, 1H), 7.30 (br s, 2H, exch), 8.14 (s, 1H), 8.34 (s, 1H); ^{13}C NMR δ 156.2, 153.3, 149.8, 140.9, 119.5, 88.3, 81.5, 73.5, 73.3, 42.5, 36.5; MS *m*/*z* 300.0846/302.0840 ([$^{35/37}$Cl] M + 1 = 300.0863/302.0838). Anal. Calcd for $C_{11}H_{14}ClN_5O_3$·0.5H_2O (308.7): C, 42.80; H, 4.90; N, 22.69. Found: C, 43.09; H, 4.96; N, 22.79.

***S*-Adenosyl-L-cysteine (3a).** Compound **2a** (100.5 mg, 0.350 mmol) was added to a deoxygenated (N_2/30 min) solution of L-Cys (150.4 mg, 1.24 mmol) in NaOH/H_2O (1.0 M, 2.15 mL, 2.21 mmol), and the mixture was heated at 100 °C (a clear solution resulted in ≤10 min). No **2a** was detected after 2 h (TLC). The solution was cooled to ambient temperature, and HOAc was added dropwise (to pH 5−6). The precipitate was filtered, washed (H_2O, cold MeOH), and dried (P_2O_5/78 °C/48 h) to give **3a** (79.2 mg, 61%) as a white solid: mp ~217 °C dec (lit.[13b] mp 215−218 °C); UV max 260 nm (ϵ 13 500), min 228 nm (ϵ 1300); ^{1}H NMR (300 MHz) δ 2.77−3.12 (m, 4H), 3.40−3.50 (m, 1H), 4.05 (q, J = 5.9 Hz, 1H), 4.19 (t, J = 4.4 Hz, 1H), 4.68 (t, J = 5.5 Hz, 1H), 5.90 (d, J = 5.6 Hz, 1H), 7.32 (br s, 2H), 8.16 (s, 1H), 8.39 (s, 1H); MS *m*/*z* 371.1123 ([MH^+ = 371.1138). Anal. Calcd for $C_{13}H_{18}N_6O_5S$·H_2O (388.4): C, 40.20; H, 5.19; N, 21.64. Found: C, 40.25; H, 5.09; N, 21.25.

***S*-Homoadenosyl-L-cysteine (3b).** L-Cys (61.3 mg, 0.51 mmol) was added to a deoxygenated (N_2/30 min) suspension of **2b** (42.9 mg, 0.14 mmol) in NaOH/H_2O (1.0 M, 0.86 mL, 0.88 mmol), and the mixture was heated at 60 °C (a clear solution resulted after 5−10 min). Heating was continued for 17 h, and the cooled solution was applied to a column (3.1 × 28 cm) of Amberlite XAD-4 resin. The column was washed [NH_4OAc/H_2O, 0.2 M, pH 5 (200 mL); H_2O (300 mL)], and elution [H_2O/MeOH (1:1, 200 mL); MeOH (~250 mL)] gave **3b**·2H_2O (39.5 mg, 68%) [plus 12.3 mg (21%) of slightly contaminated product in later fractions] as a white solid: mp ~176−184 °C (softening), 197−201 °C dec; UV max 260 nm (ϵ 14 900), min 228 nm (ϵ 3000); ^{1}H NMR (D_2O) δ 2.06−2.19 (m, 2H), 2.70−2.75 (m, 1H), 2.78−2.83 (m, 1H), 3.05−3.09 (dd, J = 7.3, 14.7 Hz, 1H), 3.15−3.19 (dd, J = 4.4, 14.6 Hz, 1H), 3.93−3.96 (dd, J = 4.2, 7.6 Hz, 1H), 4.28−4.34 (m, 2H), 4.75−4.88 (m, HDO overlap), 6.07 (d, J = 4.9 Hz, 1H), 8.27 (s, 1H), 8.33 (s, 1H); ^{13}C NMR (D_2O) δ 173.2, 155.9, 153.1, 149.2, 140.3, 119.1, 87.6, 83.3, 73.6, 73.2, 53.8, 32.7, 32.4, 27.7; MS *m*/*z* 407.1117 [(MH + Na) = 407.1113]. Anal. Calcd for $C_{14}H_{20}N_6O_5S$·2H_2O (420.4): C, 39.99; H, 5.75; N, 19.99. Found: C, 40.18; H, 5.96; N, 19.86.

***S*-Homoadenosyl-L-homocysteine (3c).** A suspension of L-Hcy (72.3 mg, 0.53 mmol) and **2b** (45.4 mg, 0.15 mmol) in NaOH/H_2O (1.0 M, 0.85 mL, 0.87 mmol) was treated as for **2b** → **3b**. Elution gave **3c** (60.4 mg, quant) as a white solid: mp ~181−214 °C (softening, dec), ~214−217 °C dec; UV max 259 nm (ϵ 14 500), min 228 nm (ϵ 3800); ^{1}H NMR δ 1.91−2.06 (m, 4H), 2.52−2.64 (m, 4H), 3.70 (dd, J = 5.4, 6.8 Hz, 1H), 4.12−4.19 (m, 2H), 4.60−4.74 (m, H_2O overlap), 6.08 (d, J = 5.4 Hz, 1H), 8.28 (s, 1H), 8.33 (s, 1H); ^{13}C NMR δ 174.4, 155.9, 153.1, 149.2, 140.3, 119.1, 87.6, 83.4, 73.5, 73.2, 54.1, 32.8, 30.5, 27.1, 27.0; MS *m*/*z* 399.1452 (MH^+ = 399.1450). Anal. Calcd for $C_{15}H_{22}N_6O_5S$ (398.4): C, 45.22; H, 5.57; N, 21.09. Found: C, 45.30; H, 5.63; N, 20.96.

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