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Synthesis of 5'-Functionalized Nucleosides: S-Adenosylhomocysteine Analogues with the Carbon-5' and Sulfur Atoms Replaced by a Vinyl or Halovinyl Unit

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Abstract

Adenosine and uridine analogues functionalized with alkenyl or fluoroalkenyl chain at C5' were prepared employing cross-metathesis, Negishi couplings and Wittig reactions. Metathesis of the protected 5'-deoxy-5'-methyleneadenosine or uridine analogues with six-carbon amino acids (homoallylglycines) in the presence of Grubbs catalysts gave nucleoside analogues with the C5'-C6' double bond. Alternatively, the Pd-catalyzed cross-coupling between the protected 5'-deoxy-5'-(iodomethylene) nucleosides and suitable alkylzinc bromides also provided analogues with alkenyl unit. Stereoselective Pd-catalyzed monoalkylation of 5'-(bromofluoromethylene)-5'-deoxyadenosine with alkylzinc bromides afforded adenosylhomocysteine analogues with a 6'-(fluoro)vinyl motif. The vinylic adenine nucleosides produced time-dependent inactivation of the S-adenosyl-L-homocysteine hydrolases.

Keywords

S-Adenosylhomocysteine hydrolase; cross-coupling; cross-metathesis; nucleosides; Wittig reaction

1. Introduction

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 since AdoHcy is a potent feedback inhibitor of crucial methylation enzymes,^{1,2} and elevated plasma levels of Hcy in humans have been shown to be a risk factor in coronary artery disease.³ Therefore, the design of inhibitors of AdoHcy hydrolase provides a rational approach to mechanism-based chemotherapy of cancer and viral diseases.^{1,2} Several inhibitors which function as substrates for the "3'-oxidative activity" of AdoHcy hydrolase and converts the enzyme from its active form (NAD⁺) to its inactive form (NADH) have been prepared.¹ Inhibitors which function as substrates for the "5'/6'-hydrolytic activity" include

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the vinyl fluorides [9-(5-deoxy-5-fluoro- β -D-*erythro*-pent-4-enofuranosyl)adenine],⁴ 5'-deoxy-5'-(halomethylene)adenosine,⁵ 5'-(bromofluoromethylene)-5'-deoxyadenosine (i.e., **A**; Figure 1),⁶ 5'-S-allenyl(or propynyl)-5'-thioadenosine analogues^{7a} and 6-fluoroneplanocin, **7b** among others.^{1c,7}

Addition of an enzyme-sequestered water molecule across the 5',6'-double bond of **A** followed by loss of bromide was proposed to generate the homoAdo 6'-carboxyl fluoride **B** at the active site of AdoHcy hydrolase.^{6b} Nucleophilic attack by proximal amino acid functionalities caused covalent binding inhibition of type **C**. Addition of water across the 5',6'-triple bond of haloacetylenes was postulated to generate similar reactive electrophiles at the enzyme active site.⁸

The X-ray crystallographic analysis of the human AdoHcy hydrolase inactivated with 9-(dihydroxycyclopentene)adenine,^{9a} and neplanocin **A**,^{9b} as well as AdoHcy hydrolase from rat liver^{9c} established the presence of a water molecule in the active site of the enzyme. This made it a 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. We now describe syntheses of the adenosine and uridine¹⁰ nucleosides modified with alkenyl or haloalkenyl chain at C5'. Adenosine analogues with the 5',6'-vinyl **D** or halovinyl **E** motif incorporated in place of the carbon-5' and sulfur atoms (Figure 2) are expected to be substrates for the "oxidative" and/or "hydrolytic" activity of AdoHcy hydrolase. Enzyme-mediated addition of water to **E** (X = F, Z = H or NH₂) might occur at C5' or C6'. This would generate new species bearing hydroxyl or keto (after β -elimination of HF) binding sites within the enzyme.

2. Chemistry

Retrosynthetic analysis indicated that AdoHcy analogue **D** can be targeted by the construction of new C5'–C6' double bond via either a Wittig reaction employing adenosine 5'-aldehyde **H** or cross-metathesis reaction utilizing 5'-deoxy-5'-methyleneadenosine **I** as nucleoside precursors (Figure 3). Subsequent bromination-dehydrobromination of **D** might afford bromovinyl analogue **E** (X = Br). It is also possible to use Pd-catalyzed cross-coupling approaches to form a new C6'–C7' bond in a key step. For example, alkylation of 5'-deoxy-5'-(iodomethylene)adenosine **G** with the suitable alkylzinc bromides should produce **D**. Alternatively, selective monoalkylation of 5'-deoxy-5'-(dihalomethylene)adenosine **F** should afford **E** directly. Coupling approaches were tested on model compounds¹¹ and reactions were performed on the pyrimidine¹⁰ and purine nucleosides to generalize synthetic approaches.

Moffatt oxidation¹² of 2',3'-*O*-isopropylideneuridine **1a** and treatment of the crude 5'-aldehyde with Wittig reagent, generated from the commercially available EtO₂C(CH₂)₃PPh₃•Br with lithium bis(trimethylsilyl)amide (LHMDS), gave ethyl 4(*Z*)-(5'-deoxy-2',3'-*O*-isopropylideneuridin-5'-ylidene)butanoate **2a** in 16% yield. Deprotections [trifluoroacetic acid (TFA)/H₂O] of **2a** yielded **2b** (Scheme 1). The vicinal coupling constant (**2b**; $J_{5'-6'} = 9.8$ Hz) was diagnostic for the *Z* stereochemistry, as expected for the Wittig product derived from the non-stabilized ylide.¹³ Analogous Wittig-treatment of 6-*N*-benzoyl-2',3'-*O*-isopropylideneadenosine **1b** gave **2c**. Treatment of crude **2c** with NH₃/MeOH removed the benzoyl group and converted the ethyl ester into a methyl ester **2d** (18% from **1b**). Deacetonization of **2d** and saponification of the resulting **2e** afforded **2f**; a nine-carbon analogue of AdoHcy with the vinyl unit between C5' and C6'. The low yields and notorious instability of 5'-aldehydes¹⁴ toward the experimental conditions, that is required for the

generation of non-stabilized Wittig reagents, prompted us to examine metathesis approach for the synthesis of **D**.

In order to explore the metathesis route,^{15,16} ethyl 2-amino-5-hexenoate (homoallylglycine) bearing a terminal double bond was prepared by alkylation of glycine with 4-bromo-1-butene followed by protection of the α -amino group with benzoyl or Boc group to give racemic **4a** or **4b**, respectively.¹⁷ Cross-metathesis between 2',3'-*O*-isopropylidene-5'-deoxy-5'-methyleneuridine¹⁸ **3** with **4a** in a CH₂Cl₂ solution containing 2nd generation (2-imidazolidinylidene-Ru) Grubbs catalyst¹⁵ gave the desired product **5a** (72%) in addition to two dimers **7a** (5%) and **6** (7%) resulting from the self-metathesis of nucleoside^{16,19} **3** and amino acid^{17,20} **4b** substrates, respectively (Scheme 2). Deacetonization of **5a** and HPLC purification yielded **5b** (66%) as 5'*E* isomers ($J_{5'-6'} = 15.3$ Hz) of a 1:1 mixture of 9'*R/S* diastereomers. Ratio of diastereomers was determined based on the NMR spectra where a double set of peaks for several protons (¹H) and carbons (¹³C) of similar intensities were observed. Analogous metathesis of **3** and **4b** gave **5c** as the sole product (74%). Aqueous TFA effected concomitant removal of Boc and isopropylidene protection groups to produce **5d**; a uracil analogue of AdoHcy with the amino group at C9' and the carboxylic group at C10'.²¹

As previously reported, metathesis of 6-*N,N*-dibenzoyl-2',3'-*O*-isopropylidene-5'-deoxy-5'-methylenadenosine with **4b** in the presence of Hoveyda-Grubb's catalyst (*o*-isopropoxy-phenylmethylene-Ru) gave the protected AdoHcy analogue **D** (Z = NH₂) in 76% yield.^{17,22} However, treatment of the latter with pyridinium tribromide followed by dehydrobromination with DBU yielded 5'-(bromo)vinyl AdoHcy analogue as a single isomer, which was deprotected to the esters **8a** and acid **8b**.¹⁷ Consequently, in order to prepare 6'-(halo)vinyl analogues **E**, we turned our attention to the Pd-catalyzed selective monoalkylation of 5'-deoxy-5'-(dihalomethylene)nucleoside precursors.

Although Pd-catalyzed cross-coupling reactions are powerful methods for the formation of carbon-carbon bonds under conditions that are compatible with a broad range of functional groups,^{23a} couplings involving C_{sp}³ centers^{23b} are less explored with the exception of couplings between C_{sp}² as electrophiles and C_{sp}³ as nucleophiles.^{23a,24} Therefore among other approaches,^{25,26} we began with exploring Negishi coupling.²⁴ Treatment of the protected 5'-deoxy-5'-(iodomethylene)adenosine^{5a} (*E*)-**10** with commercially available EtO₂C(CH₂)₃ZnBr produced ethyl 5-(5'-deoxy-2',3'-*O*-isopropylideneadenosin-5'-ylidene)pentanoate **12a** (68%) as a single *E* isomer ($J_{6'-5'} = 15.4$ Hz; Scheme 3). Deacetonization of **12a** with TFA/H₂O afforded ester **12b** (61%) and subsequent saponification gave **12c** with a six-atom alkenyl chain attached to the furanosyl C4' and the carboxylic group at C10' as in AdoHcy. Subjection of the 5'-deoxy-5'-(iodomethylene)uridine¹⁸ (*E*)-**9** to the analogous Negishi coupling and deacetonization of the resulting **11a** afforded **11b** (52% overall).

Encouraged by the results on the alkylation of iodo(vinyl) nucleosides **9** and **10** with alkylzinc bromides, we have explored selective monoalkylation of dihalo(vinyl) nucleoside substrates (e.g., **13**), although the cross-coupling reactions of 1,1-dihalo(vinyl) electrophiles with C_{sp}³ nucleophiles are very scarce.^{24c,27} We recently developed Pd-catalyzed Negishi coupling of 1-fluoro-1-(iodo, or bromo, or chloro)alkenes with alkylzincs, which provided stereoselective access to the internal fluoroalkenes.¹¹ We were gratified to find that treatment of the protected 5'-(bromofluoromethylene)-5'-deoxyadenosine^{6a} **13** (*E/Z*, 3:2) with EtO₂C(CH₂)₃ZnBr/Pd(PPh₃)₄ produced **14a** (56%) as a mixture²⁸ of the geometric isomers (*E/Z*, 2:3; Scheme 4).²⁹ Acid-catalyzed deprotection of **14a** gave ester **14b**, and saponification afforded **14c**; a ten-carbon AdoHcy analogue with C5' and sulfur atoms replaced by 6'-fluoro(vinyl) unit. Attempted monoalkylation of **13** with the zinc salt of enantiomerically pure *N*-Boc- γ -bromohomoalanine-OMe,³⁰ generated by Knochel's procedure³¹ utilizing a Zn/LiCl

combination, failed to give 6'-(fluoro)vinyl analogue **E** (X = F, Z = NH₂) bearing amino group at C9'.

3. Interactions with AdoHcy Hydrolase

The 5',6'-vinylc adenine nucleosides were tested against human AdoHcy hydrolase using a protocol involving a 0.5 h or a 4 h preincubation with the enzyme followed by a 12 min incubation that measures the residual enzyme activity (Table 1). For the esters **2e**, **8a**, **12b**, and **14b** only data for the 0.5 h preincubation are included since these esters are potentially unstable either chemically or enzymatically under the assay conditions. The compounds had similar activity toward the *Trypanosoma cruzi* AdoHcy hydrolase³² (data not shown). The most active inhibitors were found to be the nine-carbon **2e** and **2f** as well as the ten-carbon **12b** and **12c** vinylc analogues. With a 30 min preincubation, the esters **2e** and **12b** were found to be more active than the corresponding acids **2f** and **12c**. The 6'-fluoro vinylc analogues **14b** and **14c** showed a lower activity than the nonhalogenated derivatives **12b** and **12c** perhaps in accord with the fact that 5'-deoxy-5'-(fluoromethylene)adenosine showed the lowest enzyme inactivation efficiency among all 5'-halomethylene derivatives.^{5b,c} The 5'-(bromo)vinylc analogues **8a** and **8b** with the amino group at C9' also showed a lower enzyme inhibition. The acids **2f**, **8b**, **12c**, and **14c** produced similar time-dependent inactivation of the human (Table 1) and *T. cruzi* (data not shown) AdoHcy hydrolases. Since esters **2e** and **12b** are less active than structurally related dienes^{33a} (see Table 1, footnote *a*) and enynes,^{33b} and **8a** and **14b** are significantly less active no further enzymatic studies were performed.

4. Summary and Conclusions

We have developed synthesis of adenosine and uridine analogues functionalized at C5' with alkenyl or 6'-fluoroalkenyl unit employing metathesis, coupling and Wittig reactions. Cross-metathesis of the 5'-deoxy-5'-methyleneadenosine or uridine analogues with homoallylglycines gave a ten-carbon nucleoside analogues with the C5'-C6' double bond. The Pd-catalyzed selective monoalkylation of the 5'-(bromofluoromethylene)-5'-deoxyadenosine with EtO₂C(CH₂)₃ZnBr afforded AdoHcy analogues with 6'-(fluoro)vinyl unit. The vinylc adenine nucleosides were tested against human and *T. cruzi* AdoHcy hydrolases. The most active inhibitors were found to be ten- and nine-carbon nonhalogenated vinylc analogues. The free carboxylic acids derivatives produced time-dependent inactivation of the enzyme.

5. Experimental Section

UV spectra were measured with solutions in MeOH. ¹H (400 or 600 MHz) and ¹³C (100 MHz) NMR spectra were determined with solutions in CDCl₃ unless otherwise noted. Mass spectra (MS) were obtained with atmospheric pressure chemical ionization (APCI) technique and HRMS in AP-ESI mode. TLC was performed with Merck kieselgel 60-F₂₅₄ sheets with MeOH/CHCl₃ (1:19), EtOAc/hexane (2:1) or EtOAc/*i*-PrOH/H₂O (4:1:2, upper layer; S1) as developing systems and products were detected with 254 nm light. Merck kieselgel 60 (230–400 mesh) was used for column chromatography. HPLC purifications were performed using XTerra® preparative RP₁₈ OBD™ column (5μm 19 × 150 mm) with gradient program using CH₃CN/H₂O as a mobile phase. Elemental analyses were determined by Galbraith Laboratories, Knoxville, TN. Reagent grade chemicals were used, and solvents were dried by reflux over and distillation from CaH₂ (except THF/potassium) under argon.

Ethyl 4(Z)-(5'-Deoxy-2',3'-O-isopropylideneuridin-5'-ylidene)butanoate (2a). Procedure A

In flask A, Cl₂CHCO₂H (0.62 mL, 97 mg, 0.75 mmol) was added dropwise *via* syringe to a stirred solution of **1a** (426 mg, 1.5 mmol) and DCC (1.08g, 5.25 mmol) in DMSO (4 mL) at ambient temperature under N₂. The stirring was continued for an additional 1 h to produce the

corresponding aldehyde. In flask B, lithium bis(trimethylsilyl)amide (3.6 mL/1 M THF, 3.6 mmol) was added to a stirred solution of [3-(ethoxycarbonyl)propyl]triphenylphosphonium bromide (823 mg, 1.8 mmol) in anhydrous THF (6 mL) at ambient temperature under N₂ and stirring was continued until reaction turned red (~2 h). The content of flask A was then transferred to flask B *via* syringe and the resulting mixture was stirred overnight (TLC analysis showed the formation of a less polar product). Oxalic acid dihydrate (378 mg, 3 mmol) dissolved in MeOH (3 mL) was added and after 20 min the reaction mixture was concentrated (to ~1/3 volume). Dicyclohexylurea was filtered, washed with cold MeOH, and the combined filtrates were evaporated. The residue was partitioned (CHCl₃/NaHCO₃/H₂O) and the organic layer was washed (3 × H₂O, brine), dried (MgSO₄) and was evaporated. Column chromatography of the residue (40 → 60% EtOAc/hexane) gave **2a** (92 mg, 16%): ¹H NMR δ 1.23 (t, *J* = 7.1 Hz, 3, CH₃), 1.32 (s, 3, CH₃), 1.54 (s, 3, CH₃), 2.34–2.48 (m, 2, H7',7'',8',8''), 4.11 (q, *J* = 7.1 Hz, 2, CH₂), 5.14 (dd, *J* = 4.3, 6.3, Hz, 1, H3'), 4.85 (dd, *J* = 4.2, 8.5 Hz, 1, H4'), 4.98 (dd, *J* = 1.8, 6.3 Hz, 1, H2'), 5.51–5.68 (m, 3, H5,5',6'), 5.81 (d, *J* = 2.0 Hz, 1, H1'), 7.45 (d, *J* = 8.1 Hz, 1, H6); MS *m/z* 381 (100, MH⁺).

Ethyl 4(Z)-(5'-Deoxyuridin-5'-ylidene)butanoate (**2b**). Procedure B

A solution of **2a** (140 mg, 0.37 mmol) in TFA/H₂O (9:1, 5 mL) was stirred at 0 °C (ice bath) for 1 h. The volatiles were evaporated under *vacuum* (< 15 °C) and co-evaporated (3 ×) with toluene. The residue was column chromatographed (1 → 6% MeOH/CHCl₃) to give **2b** (48 mg, 38%): UV max 261 nm (ε 8800), min 230 (2200); ¹H NMR (MeOH-*d*₄, 600 MHz) δ 1.27 (t, *J* = 7.1 Hz, 3, CH₃), 2.41–2.58 (m, 4, H7',7'',8',8''), 3.95 (t, *J* = 5.8 Hz, 1, H3'), 4.15 (q, *J* = 7.2 Hz, 2, CH₂), 4.22 (dd, *J* = 3.7, 5.4 Hz, 1, H2'), 4.68 (dd, *J* = 6.6, 8.2 Hz, 1, H4'), 5.64 ("tt", *J*_{5'-4'/6'} = 9.8 Hz, *J*_{5'-7'/7''} = 1.2 Hz, 1, H5'), 5.73–5.78 (m, 2, H5,6'), 5.83 (d, *J* = 3.6 Hz, 1, H1'), 7.63 (d, *J* = 8.0 Hz, 1, H6); ¹³C NMR (MeOH-*d*₄, 600 MHz) δ 14.52 (CH₃), 24.45 (C7'), 35.08 (C8'), 61.61 (CH₂), 75.31 (C3'), 75.81 (C3'), 80.38 (C4'), 92.17 (C1'), 102.84 (C5), 129.41 (C5'), 134.87 (C6'), 142.57 (C6), 152.55 (C2), 166.21 (C4), 174.66 (C9'); MS *m/z* 341 (100, MH⁺). Anal. Calcd for C₁₅H₂₀N₂O₇•H₂O (358.35): C, 50.28; H, 6.19; N, 7.82. Found: C, 49.91; H, 5.82; N, 7.56.

Methyl 4(Z)-(5'-Deoxy-2',3'-O-isopropylideneadenosin-5'-ylidene)butanoate (**2d**)

Oxidation of **1b**¹⁴ (205 mg, 0.5 mmol) with DMSO (2 mL)/DCC (395 mg, 1.75 mmol)/Cl₂CHCO₂H (0.044 mL, 65 mg, 0.5 mmol) and treatment with Wittig reagent (274 mg, 0.6 mmol) by procedure A gave **2c** (~90 mg, ~36%; contaminated in ~20%, ¹H NMR): MS *m/z* 508 (100, MH⁺). This material was dissolved in NH₃/MeOH and was stirred overnight at ambient temperature. The volatiles were evaporated in *vacuo* and the residue was partitioned (CHCl₃/H₂O/NaHCO₃). The organic layer was washed (brine), dried (MgSO₄), evaporated and purified on column chromatography (50 → 70% EtOAc/hexane) to give **2d** (35 mg, 18% from **1b**): ¹H NMR δ 1.41 (s, 3, CH₃), 1.64 (s, 3, CH₃), 2.42 (t, *J* = 6.8 Hz, 2, H8',8''), 2.47–2.52 (m, 2, H7',7''), 3.68 (s, 3, CH₃), 4.95 (dd, *J* = 3.3, 6.1 Hz, 1, H3'), 5.06–5.09 (m, 1, H4'), 5.52–5.68 (m, 3, H2',5',6'), 5.81 (br s, 2, NH₂), 6.15 (d, *J* = 1.9 Hz, 1, H1'), 7.92 (s, 1, H2), 8.37 (s, 1, H8); MS *m/z* 390 (100, MH⁺). HRMS calcd for C₁₈H₂₄N₅O₅ [M + H]⁺ 390.1772, found 390.1782.

Methyl 4(Z)-(5'-Deoxyadenosin-5'-ylidene)butanoate (**2e**)

Treatment of **2d** (30 mg, 0.077 mmol) with TFA/H₂O (9:1, 5 mL) by procedure B gave **2e** (15 mg, 56%): ¹H NMR (MeOH-*d*₄) δ 2.35–2.58 (m, 4, H7',7'',8',8''), 3.67 (s, 3, CH₃), 4.22 (t, *J* = 5.3 Hz, 1, H3'), 4.72 ("t", *J* = 4.8 Hz, 1, H2'), 4.82–4.92 (m, 1, H4'), 5.62–5.78 (m, 2, H5',6'), 6.02 (d, *J* = 4.3 Hz, 1, H1'), 8.18 (s, 1, H2), 8.21 (s, 1, H8); ¹³C NMR (MeOH-*d*₄) δ 23.34 (C7'), 33.77 (C8'), 51.09 (CH₃), 74.27 (C3'), 75.23 (C2'), 80.19 (C4'), 89.42 (C1'), 119.63 (C5),

128.99 (C6'), 132.93 (C5'), 140.31 (C8), 149.57 (C4), 152.89 (C2), 156.33 (C6), 174.07 (C9'); MS m/z 350 (100, MH⁺). HRMS calcd for C₁₅H₂₀N₅O₅ [M + H]⁺ 350.1464, found 350.1460.

4(Z)-(5'-Deoxyadenosin-5'-ylidene)butanoic acid (2f)

NaOH/H₂O (0.2 mL, 1 M) was added to a stirred solution of **2e** (13 mg, 0.037 mmol) in MeOH (2 mL) at ambient temperature. After 24 h, volatiles were evaporated in vacuo and the residue was partitioned [EtOAc/H₂O/CH₃COOH (pH ~4)]. Aqueous layer was extracted 4 times with EtOAc and the combined organic layer was dried (MgSO₄), evaporated and purified on a column chromatography (EtOAc → 20% S1/EtOAc) to give **2f** (9 mg, 73%): mp 220–221 °C (MeOH); ¹H NMR (MeOH-*d*₄, 600 MHz) δ 1.92–2.18 (m, 4, H7',7'',8',8''), 3.91 (t, *J* = 4.9 Hz, 1, H3'), 4.46 (t, *J* = 4.9 Hz, 1, H2'), 4.53 (dd, *J* = 5.1, 8.0 Hz, 1, H4'), 5.39 (dt, *J* = 10.5, 7.9 Hz, 1, H6'), 5.44 (dd, *J* = 7.4, 10.6 Hz, 1, H5'), 5.71 (d, *J* = 4.7 Hz, 1, H1'), 7.99 (s, 1, H2), 8.08 (s, 1, H8); ¹³C NMR (Me₂SO-*d*₆) δ 22.30 (C7'), 33.51 (C8'), 73.99 (C3'), 75.45 (C2'), 80.36 (C4'), 88.64 (C1'), 120.06 (C5), 130.05, 132.99 (C5' & 6'), 140.78 (C8), 150.18 (C4), 153.50 (C2), 156.93 (C6), 175.89 (C9'), MS m/z 336 (100, MH⁺). Anal. Calcd for C₁₄H₁₇N₅O₅ (335.32): C, 50.15; H, 5.11; N, 20.89. Found: C, 50.45; H, 5.47; N, 20.45.

Ethyl 2(R/S)-Benzamido-5(E)-(5'-deoxy-2',3'-O-isopropylideneuridin-5'-ylidene)pentanoate (5a). Procedure C

Compounds **3**¹⁸ (90 mg, 0.3 mmol) and **4a**¹⁷ (78 mg, 0.3 mmol) were added to a stirred solution of [1,3-bis-(2,4,6-trimethylphenyl)-2-imidazolidinylidene]dichloro (phenylmethylene)(tricyclohexylphosphine)ruthenium (5 mg, 0.006 mmol) in dried CH₂Cl₂ (5 mL) at ambient temperature under an Ar atmosphere. The reaction mixture was stirred overnight at 55 °C in the Teflon cap closed vial, cooled down to ambient temperature and was partitioned (CHCl₃/H₂O). The organic layer was washed (brine), dried (MgSO₄), and evaporated to give brown foam. Column chromatography (1 → 3% MeOH/CHCl₃) gave **5a** (111 mg, 72%; *R/S*, ~1:1): ¹H NMR δ 1.26 (t, *J* = 7.1 Hz, 3, CH₃), 1.31 (s, 3, CH₃), 1.36 (s, 3, CH₃), 1.81–1.95 (m, 2, H8',8''), 2.02–2.25 (m, 2, H7',7''), 4.23 (q, *J* = 7.1 Hz, 2, CH₂), 4.47–4.52 (m, 1, H4'), 4.69–4.74 (m, 1, H3'), 4.84 ("q", *J* = 6.5 Hz, 1, H9'), 5.01 ("dm", *J* = 6.4 Hz, 1, H2'), 5.61 (d, *J* = 2.8 Hz, 0.5, H1'), 5.62 (d, *J* = 2.8 Hz, 0.5, H1') 5.64–5.75 (m, 2, H5,5'), 5.79–5.87 (m, 1, H6'), 6.84 (d, *J* = 7.8 Hz, 0.5, NH), 6.91 (d, *J* = 7.8 Hz, 0.5, NH), 7.26 (d, *J* = 8.1 Hz, 0.5, H6), 7.28 (d, *J* = 8.1 Hz, 0.5, H6), 7.45 (t, *J* = 7.7 Hz, 2, Ar), 7.52 (t, *J* = 7.9 Hz, 1, Ar), 7.82 (d, *J* = 8.0, 2, Ar), 9.38 (br s, 1, NH); ¹³C NMR δ 13.18 (CH₃), 24.27 & 26.11 (CMe₂), 26.98 (C7'), 30.75 (C8'), 51.06 (C9'), 60.72 (CH₂), 83.15 & 83.24 (C3'), 83.71, 83.78 (C2'), 87.45 & 87.50 (C4'), 93.51 & 93.38 (C1'), 101.50 (C5), 113.46 (CMe₂), 126.10, 127.59, 130.78, 133.03 (Bz), 127.12 & 127.13 (C5'), 132.84 & 132.86 (C6'), 141.36 (C6), 148.94 (C2), 162.28 (C4), 166.08 & 166.11 (Bz), 171.50 & 171.54 (C10'); MS m/z 514 (100, MH⁺).

Isolated also from column were self-metathesis dimers diethyl 2,9-bis(benzamido)dec-5-enedioate **6**¹⁷ (10 mg, 7%, 14% consumption of **4a**; less polar than **5a**) and **7a** (8 mg, 5%, 10% consumption of **3**; more polar than **5a**). Compound **7a** had: ¹H NMR: δ 1.34 (s, 3, CH₃), 1.56 (s, 3, CH₃), 4.54 ("dt", *J* = 2.1, 3.8 Hz, 1, H4'), 4.76 (dd, *J* = 4.0, 6.3 Hz, 1, H3'), 5.05 (dd, *J* = 1.8, 6.4 Hz, 1, H2'), 5.52 (d, *J* = 1.7 Hz, H1'), 5.83 (d, *J* = 8.0 Hz, 1, H5), 5.97 ("dd", *J* = 1.7, 3.7 Hz, 1, H5'), 7.19 (d, *J* = 8.0 Hz, 1, H6), 9.73 (br s, 1, NH); ¹³C NMR δ 25.63 & 27.49 (CMe₂), 84.88 (C2' & C3'), 87.71 (C4'), 95.92 (C1'), 102.95 (C5), 114.88 (CMe₂), 130.99 (C5'), 143.45 (C6), 150.18 (C2), 164.66 (C4); MS m/z 533 (100, MH⁺). Treatment of **7a** (8 mg, 0.015 mmol) with TFA/H₂O (9:1, 1.0 mL) by procedure B and HPLC purification (10 → 40% CH₃CN/H₂O for 50 min, *t*_R = 20 min) gave **7b** (4 mg, 60%): ¹H NMR (MeOH-*d*₄) δ 4.02 (t, *J* = 5.8 Hz, 1, H3'), 4.26 (dd, *J* = 3.8, 5.3 Hz, 1, H2'), 4.44 ("ddd", *J* = 1.8, 3.7, 5.8 Hz, 1, H4'), 5.75 (d, *J* = 8.1 Hz, 1, H5), 5.83 (d, *J* = 3.6 Hz, 1, H1'), 6.08 (dd, *J* = 1.8, 3.7 Hz, 1, H5'), 7.64 (d, *J* = 8.1 Hz, 1, H6); ¹³C NMR (MeOH-*d*₄) δ 74.93 (C2'), 75.11 (C3'), 84.68 (C4'), 92.45

(C1'), 102.95 (C5), 132.23 (C5'), 142.80 (C6), 152.23 (C2), 166.15 (C4). HRMS calcd for $C_{18}H_{21}N_4O_{10}$ $[M + H]^+$ 453.1252, found 453.1260.

Ethyl 2(*R/S*)-Benzamido-5(*E*)-(5'-deoxyuridin-5'-ylidene)pentanoate (5b)

Treatment of **5a** (25 mg, 0.05 mmol) with TFA/H₂O (9:1, 1.5 mL) by procedure B [column chromatography (CHCl₃/MeOH; 95:5)] gave **5b** (15 mg, 66%; *R/S*, ~1:1): UV max 260 nm (ϵ 14 500), min 233 nm (ϵ 9300); ¹H NMR (MeOH-*d*₄) δ 1.28 (t, *J* = 7.1 Hz, 3, CH₃), 1.93–2.22 (m, 2, H8',8''), 2.24–2.33 (m, 2, H7',7''), 3.92 ("q", *J* = 5.2 Hz, 1, H3'), 4.15–4.20 (m, 1, H2') 4.22 ("dq", *J* = 2.0, 7.0 Hz, 2, CH₂), 4.35 (t, *J* = 7.1, 1, H4'), 4.62 ("dd", *J* = 4.9, 9.8 Hz, 1, H9'), 5.62–5.76 (m, 2, H5,5'), 5.81 (d, *J* = 3.4 Hz, 0.5, H1'), 5.83 (d, *J* = 3.4 Hz, 0.5, H1'), 5.90 (dt, *J* = 15.3, 6.7 Hz, 0.5, H6'), 5.91 (dt, *J* = 15.3, 6.6 Hz, 0.5, H6'), 7.51 ("t", *J* = 7.8 Hz, 2, Ar), 7.53–7.66 (m, 2, H6 & Ar), 7.91 (d, *J* = 8.1 Hz, 2, Ar); ¹³C NMR (MeOH-*d*₄) δ 13.53 (CH₃), 28.82 & 28.89 (C7'), 30.45 & 30.48 (C8'), 52.69 (C9'), 62.44 (CH₂), 74.15 (C3'), 74.22 & 74.25 (C2'), 84.37 & 84.48 (C4'), 90.69 (C1'), 101.84 (C5), 127.54 & 127.56, 128.59, 131.95, 133.71 (Bz), 129.16 & 129.24 (C5'), 134.22 & 134.24 (C6'), 141.40 (C6), 151.25 (C2), 165.15 (C4), 169.51 (Bz), 172.85 (C10'); MS *m/z* 474 (100, MH⁺). HRMS calcd for $C_{23}H_{28}N_3O_8$ $[M + H]^+$ 474.1871, found 474.1880.

Ethyl 2(*R/S*)-*tert*-Butoxycarbonylamino-5(*E*)-(5'-deoxy-2',3'-O-isopropylideneuridin-5'-ylidene)pentanoate (5c)

Metathesis of **3**¹⁸ (40 mg, 0.14 mmol) and **4b**¹⁷ (35 mg, 0.14 mmol) by procedure C [column chromatography (CHCl₃ → 2% MeOH)] gave **5c** (73 mg, 74%, *R/S*, 1:1): ¹H NMR δ 1.28 (t, *J* = 7.1 Hz, 3, CH₃), 1.36 (s, 3, CH₃), 1.45 (s, 9, *t*-Bu), 1.59 (s, 3, CH₃), 1.69–2.19 (m, 4, H7', 7'',8',8''), 4.22 (q, *J* = 7.1 Hz, 2, CH₂), 4.27–4.34 ("br q", *J* = 8.0 Hz, 1, H9'), 4.51 (dd, *J* = 4.2, 8.1 Hz, 0.5, H4'), 4.52 (dd, *J* = 4.2, 8.1 Hz, 0.5, H4'), 4.75–4.80 (m, 1, H3'), 5.01–5.07 (m, 1, H2'), 5.15 ("t", *J* = 9.0 Hz, 1, NH), 5.58–5.85 (m, 4, H1',5, 5',6'), 7.25 (d, *J* = 8.0 Hz, 0.5, H6), 7.27 (d, *J* = 8.0 Hz, 0.5, H6), 9.45 (br s, 1, NH); MS *m/z* 510 (100, MH⁺), 410 (97, MH⁺-Boc). HRMS calcd for $C_{24}H_{36}N_3O_9$ $[M + H]^+$ 510.2452, found 510.2455.

Ethyl 2(*R/S*)-Amino-5(*E*)-(5'-deoxyuridin-5'-ylidene)pentanoate (5d)

Treatment of **5c** (45 mg, 0.088 mmol) with TFA/H₂O (9:1, 3.5 mL) by procedure B [column chromatography (5 → 12% MeOH/CHCl₃)] gave **5d** (20 mg, 61%; *R/S*, 1:1) as a white hygroscopic solid: UV max 260 nm (ϵ 10 500), min 233 nm (ϵ 3800); ¹H NMR (Me₂SO-*d*₆) δ 1.19 (t, *J* = 7.1 Hz, 3, CH₃), 1.48–1.56 (m, 1, H8'), 1.60–1.69 (m, 1, H8''), 2.10 ("q", *J* = 7.2 Hz, 2, H7',7''), 3.29–3.31 (m, 1, H9'), 3.79 (m, 1, H3'), 4.05–4.18 (m, 4, H2',4' & CH₂), 5.21 (br s, 2, OH2' & OH3'), 5.42–5.44 (m, 2, NH₂), 5.63 (dd, *J* = 7.8, 15.2 Hz, 1, H5'), 5.65 (d, *J* = 8.0 Hz, 1, H5), 5.72 (d, *J* = 4.1 Hz, 1, H1'), 5.74 (dt, *J* = 6.8, 15.2 Hz, 1, H6'), 7.59 (d, *J* = 8.0 Hz, 1, H6), 11.35 (br s, 1, NH); ¹³C NMR (Me₂SO-*d*₆) δ 15.00 (CH₃), 28.78 (C7'), 34.55 & 34.60 (C8'), 54.22 (C9'), 60.91 (CH₂), 73.72 & 73.75 (C3'), 74.37 & 74.39 (C2'), 84.82 & 84.87 (C4'), 89.60 (C1'), 102.79 (C5), 129.31 (C5'), 134.58 (C6'), 141.88 (C6), 151.48 (C2), 163.96 (C4), 176.35 (C10'); MS *m/z* 370 (100, MH⁺). HRMS calcd for $C_{16}H_{24}N_3O_7$ $[M + H]^+$ 370.1609, found 370.1618.

Ethyl 5(*E*)-(5'-Deoxy-2',3'-O-isopropylideneuridin-5'-ylidene)pentanoate (11a). Procedure D

Tris(dibenzylideneacetone)dipalladium (9.2 mg, 0.01 mmol) was added to a solution of (*E*)-**9**¹⁸ (40 mg, 0.1 mmol) in benzene (2 mL) followed by dropwise addition of EtO₂C (CH₂)₃ZnBr (0.5 M/THF, 0.3 mL, 0.15 mmol) via syringe at ambient temperature under N₂. The resulting mixture was heated at 55 °C for 8 h [additional EtO₂C(CH₂)₃ZnBr (0.1 mL, 0.05 mmol) was added after 5 h] and progress of the reaction was monitored (TLC) by the formation of slightly more polar compound. The volatiles were evaporated and the brown residue was partitioned (EtOAc/H₂O). The organic layer was washed (brine), dried (Na₂SO₄), evaporated

and the residue was column chromatographed (EtOAc/hexane, 85:15) to give **11a** (28 mg, 72%): ^1H NMR δ 1.25 (t, J = 7.1 Hz, 3, CH₃), 1.38 (s, 3, CH₃), 1.58 (s, 3, CH₃), 1.72 (quint, J = 7.6 Hz, 2, H8',8''), 2.12 (q, J = 7.1 Hz, 2, H7',7''), 2.32 (t, J = 7.6 Hz, 2, H9'), 4.14 (q, J = 7.1 Hz, 2, CH₂), 4.50 (dd, J = 4.3, 7.8 Hz, 1, H4'), 4.74 (dd, J = 4.4, 6.2 Hz, 1, H3'), 5.02 (dd, J = 1.7, 6.5 Hz, 1, H2'), 5.60 (d, J = 1.6 Hz, 1, H1'), 5.62 (dd, J = 8.0, 14.6 Hz, 1, H5'), 5.73 (d, J = 8.2 Hz, 1, H5), 5.82 (dt, J = 6.8, 14.6 Hz, 1, H6'), 7.28 (d, J = 8.2 Hz, 1, H6), 9.40 (s, 1, NH); ^{13}C NMR δ 14.25 (CH₃), 24.03 (C8'), 25.32 & 27.16 (CMe₂), 31.53 (C7'), 33.62 (C9'), 60.35 (CH₂), 84.22 (C3'), 84.84 (C2'), 88.63 (C4'), 94.29 (C1'), 102.45 (C5), 114.57 (CMe₂), 128.57 (C6'), 131.96 (C5'), 142.23 (C6), 149.77 (C2), 163.03 (C4), 173.45 (C10'); MS m/z 395 (100, MH⁺); HRMS calcd for C₁₉H₂₇N₂O₇ [M + H]⁺ 395.1818, found 395.1811.

Ethyl 5(*E*)-(5'-Deoxyuridin-5'-ylidene)pentanoate (**11b**)

Treatment of **11a** (70 mg, 0.78 mmol) with TFA/H₂O (9:1, 2.5 mL) by procedure B gave **11b** (45 mg, 72%): mp 102–104 °C dec; UV max 262 nm (ϵ 9200), min 230 nm (ϵ 2100); ^1H NMR (Me₂SO-*d*₆/D₂O) δ 1.18 (t, J = 7.1 Hz, 3, CH₃), 1.62 ("quint", J = 7.3 Hz, 2, H8',8''), 2.08 (q, J = 6.8 Hz, 2, H7',7''), 2.32 (t, J = 7.4 Hz, 2, H9',9''), 3.81 (t, J = 5.3 Hz, 1, H3'), 4.04–4.10 (m, 3, H2',CH₂), 4.15 ("t", J = 6.4 Hz, 1, H4'), 5.57–5.72 (m, 4, H1',5,5',6'), 7.58 (d, J = 8.1 Hz, 1, H6); ^{13}C NMR (Me₂SO-*d*₆) δ 14.91 (CH₃), 24.60 (C8'), 31.75 (C7'), 33.68 (C9'), 60.60 (CH₂), 73.73 (C3'), 74.40 (C2'), 84.81 (C4'), 89.62 (C1'), 102.79 (C5), 129.58 (C6'), 134.44 (C5'), 141.89 (C6), 151.49 (C2), 163.95 (C4), 173.61 (C10'); MS m/z 355 (100, MH⁺). Anal. Calcd for C₁₆H₂₂N₂O₇ (354.36): C, 54.23; H, 6.26; N, 7.91. Found: C, 54.34; H, 6.28; N, 7.68.

Ethyl 5(*E*)-(5'-Deoxy-2',3'-O-isopropylideneadenosin-5'-ylidene)pentanoate (**12a**)

Coupling of (*E*)-**10**^{5a} (41 mg, 0.1 mmol) with EtO₂C(CH₂)₃ZnBr (0.5 M/THF, 0.4 mL, 0.2 mmol) by procedure D [column chromatography (1 → 2% MeOH/EtOAc)] gave **12a** (27 mg, 68%): ^1H NMR (600 MHz) δ 1.25 (t, J = 7.2 Hz, 3, CH₃), 1.28 (s, 3, CH₃), 1.42 (s, 3, CH₃), 1.68 (quint, J = 7.5 Hz, 2, H8',8''), 2.04 (q, J = 7.2 Hz, 2, H7',7''), 2.28 (t, J = 7.5 Hz, 2, H9',9''), 4.08 (q, J = 7.1 Hz, 2, CH₂), 4.65 (dd, J = 3.3, 7.5 Hz, 1, H4'), 4.98 (dd, J = 3.5, 6.2 Hz, 1, H3'), 5.51 (dd, J = 1.9, 6.3 Hz, 1, H2'), 5.59 (ddt, J = 1.2, 7.6, 15.4 Hz, 1, H5'), 5.72 (dt, J = 15.4, 6.6 Hz, 1, H6'), 5.90 (br s, 2, NH₂), 6.09 (d, J = 1.9 Hz, 1, H1'), 7.91 (s, 1, H2), 8.38 (s, 1, H8); MS m/z 418 (100, MH⁺); HRMS calcd for C₂₀H₂₈N₅O₅ [M + H]⁺ 418.2090, found 418.2082.

Ethyl 5(*E*)-(5'-Deoxyadenosin-5'-ylidene)pentanoate (**12b**)

Treatment of **12a** (27 mg, 0.065 mmol) with TFA/H₂O by procedure B gave **12b** (15 mg, 61%). Purification on HPLC (10 → 60% CH₃CN/H₂O for 1 h, t_R = 43 min) and recrystallization (MeOH) gave white crystals: mp 91–93 °C dec; UV max 260 nm (ϵ 12 700), min 230 nm (ϵ 1840); ^1H NMR (MeOH-*d*₆, 600 MHz) δ 1.05 (t, J = 7.2 Hz, 3, CH₃), 1.55 (quint, J = 7.3 Hz, 2, H8',8''), 1.98 (q, J = 7.0 Hz, 2, H7',7''), 2.16 (t, J = 7.4 Hz, 2, H9',9''), 3.94 (q, J = 7.0 Hz, 2, CH₂), 4.04 (t, J = 5.2 Hz, 1, H3'), 4.25 (dd, J = 5.4, 6.6 Hz, 1, H4'), 4.59 (t, J = 4.8 Hz, 1, H2'), 5.58 (dd, J = 6.8, 15.4 Hz, 1, H5'), 5.65 (dt, J = 15.4, 6.4 Hz, 1, H6'), 5.84 (d, J = 4.5 Hz, 1, H1'), 8.08 (s, 1, H2), 8.10 (s, 1, H8); ^{13}C NMR (Me₂SO-*d*₆) δ 14.90 (CH₃), 24.46 (C8'), 31.57 (C7'), 33.62 (C9'), 60.74 (CH₂), 73.54 (C3'), 74.69 (C2'), 85.40 (C4'), 88.33 (C1'), 119.76 (C5), 129.64 (C6'), 133.98 (C5'), 140.95 (C8), 150.11 (C4), 153.53 (C2), 156.56 (C6), 173.97 (C10'); MS m/z 378 (100, MH⁺). Anal. Calcd for C₁₇H₂₃N₅O₅•H₂O (395.41): C, 51.64; H, 6.37; N, 17.71. Found: C, 51.53; H, 6.42; N, 17.37.

5(*E*)-(5'-Deoxyadenosin-5'-ylidene)pentanoic acid (**12c**)

NaOH/H₂O (1 M, 0.35 mL) was added to a solution of **12b** (10 mg, 0.026 mmol) in MeOH (2.5 mL) and stirring was continued at ambient temperature overnight. The resulting mixture

was neutralized with AcOH to pH ~7. Volatiles were evaporated and the residue was purified on HPLC (10 → 30% CH₃CN/H₂O for 50 min, *t_R* = 28 min) to give **12c** (7.5 mg, 82%): ¹H NMR (MeOH-*d*₄) δ 1.74 ("quint", *J* = 7.4 Hz, 2, H8',8"), 2.15 (q, *J* = 7.1 Hz, 2, H7',7"), 2.32 (t, *J* = 7.4 Hz, 2, H9',9"), 4.21 (t, *J* = 5.2 Hz, 1, H3'), 4.43 (dd, *J* = 5.5, 6.8 Hz, 1, H4'), 4.72 (t, *J* = 4.9 Hz, 1, H2'), 5.75 (dd, *J* = 7.0, 15.4 Hz, 1, H5'), 5.83 (dt, *J* = 15.5, 6.5 Hz, 1, H6'), 6.01 (d, *J* = 4.5 Hz, 1, H1'), 8.16 (s, 1, H2), 8.22 (s, 1, H8); ¹³C NMR (MeOH-*d*₄) δ 25.37 (C8'), 32.64 (C7'), 34.32 (C9'), 75.12 (C3'), 75.70 (C2'), 86.43 (C4'), 90.14 (C1'), 120.10 (C5), 129.76 (C6'), 135.18 (C5'), 141.28 (C8), 150.66 (C4), 153.93 (C2), 157.36 (C6), 177.59 (C10'); MS *m/z* 350 (100, MH⁺); HRMS calcd for C₁₅H₂₀N₅O₅ [M + H]⁺ 350.1464, found 350.1469.

Ethyl 5-Fluoro-5-(5'-deoxy-2',3'-O-isopropylideneadenosin-5'-ylidene)pentanoate (**14a**)

Pd(PPh₃)₄ (25.6 mg, 0.022 mmol) was added to a solution of **13**^{6a} (*E/Z*, ~60:40; 80 mg, 0.2 mmol) in benzene (5 mL) followed by dropwise addition of EtO₂C(CH₂)₃ZnBr (0.5M/THF, 0.88 mL, 0.44 mmol) via syringe at ambient temperature under N₂. The resulting mixture was heated at 55 °C for 8 h. The volatiles were evaporated and the brown residue was partitioned (EtOAc/NaHCO₃/H₂O). The organic layer was washed (brine), dried (Na₂SO₄), evaporated and the residue was column chromatographed (1 → 3% MeOH/CHCl₃) to give **14a** (49 mg, 56%; *E/Z* ~ 40:60): ¹⁹F NMR δ -95.78 ("q" *J_{F-H5'}* = 21.8 Hz, 0.4 F, *E*), -101.80 (dt, *J_{F-H5'}* = 35.5 Hz, *J_{F-H7',7''}* = 17.3 Hz, 0.6 F, *Z*); MS *m/z* 436 (MH⁺); HRMS calcd for C₂₀H₂₇FN₅O₅ [M + H]⁺ 436.1996, found 436.1991. (*Z*)-**14a** had: ¹H NMR δ 1.23 (t, *J* = 7.1 Hz, 3, CH₃), 1.36 (s, 3, CH₃), 1.61 (s, 3, CH₃), 1.84 ("quint", *J* = 7.3 Hz, 2, H8',8"), 2.15 (dt, *J* = 17.1, 7.7 Hz, 2, H7',7"), 2.30 (t, *J* = 7.5 Hz, 2, H9',9"), 4.09 (q, *J* = 7.2 Hz, 2, CH₂), 4.85 (dd, *J* = 9.2, 35.5 Hz, 1, H5'), 4.97 ("dd", *J* = 3.3, 6.2 Hz, 1, H3'), 5.15 (dd, *J* = 3.1, 9.2 Hz, 1, H4'), 5.54 (dd, *J* = 1.9, 6.2 Hz, 1, H2'), 5.88 (br s, 2, NH₂), 5.98 (d, *J* = 1.7 Hz, 1, H1'), 7.86 (s, 1, H2), 8.34 (s, 1, H8). (*E*)-**14a** had: ¹H NMR δ 1.21 (t, *J* = 7.1 Hz, 3, CH₃), 1.35 (s, 3, CH₃), 1.60 (s, 3, CH₃), 1.74 ("quint", *J* = 7.2 Hz, 2, H8',8"), 2.26 (t, *J* = 7.4 Hz, 2, H9',9"), 2.35 (dt, *J* = 23.3, 7.3 Hz, 2, H7',7"), 4.11 (q, *J* = 7.2 Hz, 2, CH₂), 4.75 (ddd, *J* = 1.8, 3.2, 10.1 Hz, 1, H4'), 4.97 ("dd", *J* = 3.3, 6.2 Hz, 1, H3'), 5.30 (dd, *J* = 10.1, 19.6 Hz, 1, H5'), 5.50 (dd, *J* = 1.7, 6.3 Hz, 1, H2'), 5.88 (br s, 2, NH₂), 5.97 (d, *J* = 1.5 Hz, 1, H1'), 7.84 (s, 1, H2), 8.34 (s, 1, H8).

Ethyl 5-Fluoro-5-(5'-deoxyadenosin-5'-ylidene)pentanoate (**14b**)

Treatment of **14a** (40 mg, 0.092 mmol; *E/Z*, 40:60) with TFA/H₂O by procedure B and HPLC purification (15 → 50% CH₃CN/H₂O, *t_R* = 49 min) gave **14b** (32 mg, 88%; *E/Z*, ~35:65): ¹⁹F NMR (MeOH-*d*₄) δ -94.73 ("q", *J_{F-H5'}* = 20.5 Hz, 0.35, *E*), -102.14 (dt, *J_{F-H5'}* = 35.9 Hz, *J_{F-H7',7''}* = 17.7 Hz, 0.65, *Z*); MS *m/z* 396 (100, MH⁺); HRMS calcd for C₁₇H₂₃FN₅O₅ [M + H]⁺ 396.1678, found 396.1680. (*Z*)-**14b** had: ¹H NMR (MeOH-*d*₄) δ 1.24 (t, *J* = 7.1 Hz, 3, CH₃), 1.86 ("quint", *J* = 7.4 Hz, 2, H8',8"), 2.32 ("dt", *J* = 17.6, 7.3 Hz, 2, H7',7"), 2.40 (t, *J* = 7.4 Hz, 2, H9',9"), 4.13 (q, *J* = 7.1 Hz, 2, CH₂), 4.23 (t, *J* = 4.7 Hz, 1, H3'), 4.86 ("t", *J* = 4.4 Hz, 1, H2'), 4.94 (dd, *J* = 4.5, 9.2 Hz, 1, H4'), 5.20 (dd, *J* = 9.2, 36.5 Hz, 1, H5'), 5.99 (d, *J* = 4.8 Hz, 1, H1'), 8.24 (s, 1, H2), 8.26 (s, 1, H8); ¹³C NMR (MeOH-*d*₄) δ 14.50 (CH₃), 22.34 (C8'), 31.99 (d, *J* = 27.2 Hz, C7'), 33.87 (C9'), 61.55 (CH₂), 74.89 (C2'), 76.29 (C3'), 79.34 (d, *J* = 6.5 Hz, C4'), 90.38 (C1'), 106.01 (d, *J* = 11.3 Hz, C5'), 120.82 (C5), 141.69 (C8), 150.62 (C4), 153.90 (C2), 157.37 (C6), 163.50 (d, *J* = 260.9 Hz, C6'), 174.86 (C10'). (*E*)-**14b** had: ¹H NMR (MeOH-*d*₄) δ 1.24 (t, *J* = 7.1 Hz, 3, CH₃), 1.84 ("quint", *J* = 7.4 Hz, 2, H8',8"), 2.39 (t, *J* = 7.3 Hz, 2, H9',9"), 2.43–2.55 (m, 2, H7',7"), 4.12 (q, *J* = 7.1 Hz, 2, CH₂), 4.28 (t, *J* = 5.5 Hz, 1, H3'), 4.60 (ddd, *J* = 1.9, 5.8, 9.8 Hz, 1, H4'), 4.72 (dd, *J* = 4.0, 5.1 Hz, 1, H2'), 5.44 (dd, *J* = 9.7, 19.9 Hz, 1, H5'), 6.00 (d, *J* = 3.6 Hz, 1, H1'), 8.25 (s, 1, H2), 8.23 (s, 1, H8); ¹³C NMR (MeOH-*d*₄) δ 14.50 (CH₃), 22.34 (C8'), 28.56 (d, *J* = 27.2 Hz, C7'), 33.78 (C9'), 61.55 (CH₂), 75.21 (C2'), 76.11 (C3'), 81.03 (d, *J* = 14.4 Hz, C4'), 90.57 (C1'), 106.83 (d, *J* = 24.4 Hz, C5'), 120.70 (C5), 141.31 (C8), 150.54 (C4), 153.94 (C2), 157.37 (C6), 165.42 (d, *J* = 255.3 Hz, C6'), 174.89 (C10').

5-Fluoro-5(*E*)-(5'-Deoxyadenosin-5'-ylidene)pentanoic acid (**14c**)

Treatment of **14b** (8 mg, 0.020 mmol; *E/Z*, 35:65) with NaOH as described for **12c** and HPLC purification (5 → 50% CH₃CN/H₂O for 1 h, *t_R* = 42 min) gave **14c** (6 mg, 82%; *E/Z*, 45:55): ¹⁹F NMR (MeOH-*d*₄) δ -97.8 ("q", *J*_{F-H5',7',9'} = 23.9 Hz, 0.45, *E*), -105.0 ("dt", *J*_{F-H5'} = 35.6 Hz, *J*_{F-H7',9'} = 17.6 Hz, 0.55, *Z*); MS *m/z* 368 (100, MH⁺); HRMS calcd for C₁₅H₁₉FN₅O₅ [M + H]⁺ 368.1370, found 368.1375. (*Z*)-**14c** had: ¹H NMR (MeOH-*d*₄) δ 1.85 ("quint", *J* = 7.4 Hz, 2, H8',8"), 2.32 (dt, *J* = 17.3, 7.4 Hz, 2, H7',7"), 2.38 (t, *J* = 7.3 Hz, 2, H9',9"), 4.23 (t, *J* = 4.8 Hz, 1, H3'), 4.83 ("t", *J* = 5.0 Hz, 1H, H2'), 4.92 (dd, *J* = 4.7, 9.3 Hz, 1, H4'), 5.20 (dd, *J* = 9.2, 36.0 Hz, 1, H5'), 6.01 (d, *J* = 5.2 Hz, 1, H1'), 8.24 (s, 1, H2), 8.27 (s, 1H, H8); ¹³C NMR (MeOH-*d*₄) δ 23.02 (C8'), 32.05 (d, *J* = 27.4 Hz, C7'), 33.82 (C9'), 74.91 (C2'), 76.27 (C3'), 79.34 (d, *J* = 6.6 Hz, C4'), 90.27 (C1'), 105.90 (d, *J* = 11.0 Hz, C5'), 120.46 (C5), 141.36 (C8), 150.60 (C4), 153.88 (C2), 157.34 (C6), 163.30 (d, *J* = 258.6 Hz, C6'), 177.09 (C10'). (*E*)-**14c** had: ¹H NMR (MeOH-*d*₄) δ 1.84 ("quint", *J* = 7.4 Hz, 2H, H8', 8"), 2.39 (t, *J* = 7.3 Hz, 2, H9',9"), 2.45 (dt, *J* = 23.7, 7.3 Hz, 1, H7'), 2.46 (dt, *J* = 23.7, 7.3 Hz, 1, H7"), 4.28 (t, *J* = 5.6 Hz, 1, H3'), 4.60 (ddd, *J* = 1.8, 5.8, 9.7 Hz, 1, H4'), 4.72 (dd *J* = 4.0, 5.2 Hz, 1, H2'), 5.44 (dd, *J* = 9.8, 19.9 Hz, 1, H5'), 6.00 (d, *J* = 3.7 Hz, 1, H1'), 8.23 (s, 1, H2), 8.25 (s, 1, H8); ¹³C NMR (MeOH-*d*₄) δ 22.45 (C8'), 28.65 (d, *J* = 26.9 Hz, C7'), 33.82 (C9'), 75.17 (C2'), 76.12 (C3'), 81.04 (d, *J* = 14.8 Hz, C4'), 90.58 (C1'), 106.73 (d, *J* = 24.7 Hz, C5'), 120.53 (C5), 141.68 (C8), 150.49 (C4), 153.91 (C2), 157.34 (C6), 165.88 (d, *J* = 258.6 Hz, C6'), 177.09 (C10').

Inactivation of AdoHcy Hydrolase

Recombinant human placental AdoHcy hydrolase (204 nM) was incubated with 281 μM of **2e**, **2f**, **8a**, **8b**, **12b**, **12c**, **14b**, and **14c** in potassium-phosphate buffer [50.5 μL; 50 mM, pH 7.2 containing 1 mM EDTA (Buffer A)] at 22 °C for 0.5 h or 4 h. The remaining enzyme activity was assayed in the synthetic direction essentially as previously described.^{8b} An aliquot (5 μL) of the enzyme/inhibitor was added to 995 μL of Buffer A containing 0.5 mM Ado and 0.5 mM Hcy in buffer A and then incubated at 37° C for 12 min followed by addition of HClO₄ (25 μL, 5 M) to terminate the reaction. The reaction product, AdoHcy, was quantitatively measured using RP-HPLC with the detector monitoring at 258 nm.^{8b}

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Figure 1. Generation of active intermediates from the (bromofluoro)vinyl analogue **A** by the "hydrolytic activity" of AdoHcy hydrolase.^{6b}

Figure 2.

S-Adenosyl-L-homocysteine (AdoHcy) and analogues with the carbon-5' and sulfur atoms replaced by a vinyl or halovinyl unit.

Figure 3. Retrosynthetic analysis for AdoHcy analogues with the carbon-5' and sulfur atoms replaced by a vinyl or halovinyl unit.

Scheme 1.

Reagents and conditions: (a) (i) DCC/DMSO/Cl₂CHCO₂H, (ii) EtO₂C(CH₂)₃PPh₃Br/LHMDS/THF; (b) TFA/H₂O; (c) NH₃/MeOH; (d) NaOH/H₂O/MeOH.

Scheme 2.

Reagents and conditions: (a) 2nd generation Grubbs catalyst/ $\text{CH}_2\text{Cl}_2/\Delta$; (b) TFA/ H_2O .

Scheme 3.

Reagents and conditions: (a) EtO₂C(CH₂)₃ZnBr/Pd₂(dba)₃/benzene/Δ; (b) TFA/H₂O; (c) NaOH/H₂O.

Scheme 4.

Reagents and conditions: (a) EtO₂C(CH₂)₃ZnBr/Pd(PPh₃)₄/benzene/Δ; (b) TFA/H₂O; (c) NaOH/H₂O.

Table 1

Inhibition of AdoHcy hydrolase by 5',6'-vinylc adenosine derivatives

Compound ^a	% of enzyme inhibition ^{b,c}	
	0.5 h ^d	4 h ^d
2e^e	68.1	
2f	50.5	95.5
8a^e	11.4	
8b	14.5	44.6
12b^e	81	
12c	28.5	92.4
14b^e	14.1	
14c	15.2	35.2

^a Dienes e.g., ethyl (*E/E*)- and (*Z/E*)-4-(5'-deoxyadenosin-5'-ylidene)but-2-enoate caused 96.4 and 98.2% of the enzyme inhibition under nearly identical conditions with 20 min incubation time.^{33a}

^b AdoHcy hydrolase (204 nM) was incubated with the inhibitors (281 μM) at 37 °C for 12 min. and the remaining enzyme activity was assayed as described in the Experimental Section.

^c Data are the average of duplicate determinations.

^d Preincubation time of the inhibitors with the enzyme.

^e Esters were not tested with 4 h preincubation time because of the chemical instability in the enzymatic assay.