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α-Cyano-Substituted Analogues of Decarboxylated S-Adenosylmethionine as Enzyme Activated, Irreversible Inhibitors of S-Adenosylmethionine Decarboxylase

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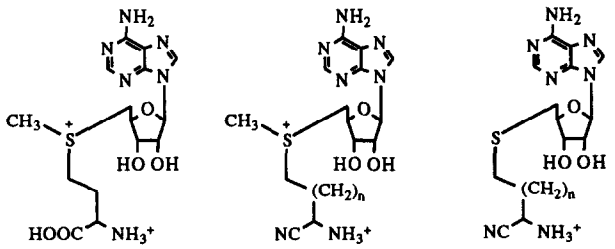
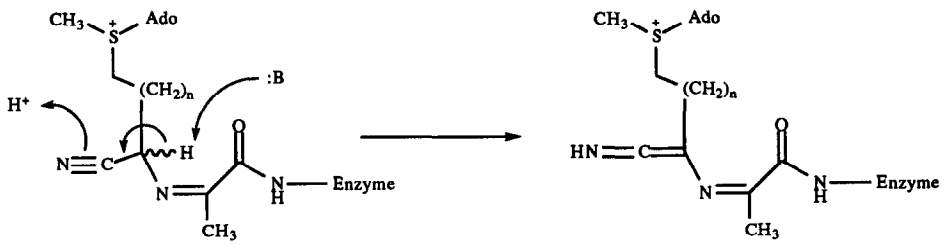
Abstract: A pair of α-cyano analogues of decarboxylated S-adenosylmethionine (**2a** and **2b**) were synthesized as potential enzyme activated, irreversible inhibitors of the pyruvoyl enzyme S-adenosylmethionine decarboxylase (AdoMet-DC). Each of these analogues acts as an irreversible inactivator of AdoMet-DC from *Escherichia coli* (IC_{50} values of 9 and 50 μM, respectively). These analogues also inactivate human AdoMet-DC, with K_I values of 246.6 and 7.2 μM, and k_{inact} values of 0.29 and 0.03 min^{-1}, respectively.

The enzyme S-adenosylmethionine decarboxylase (AdoMet-DC, EC 4.1.1.50) is an essential enzyme in the polyamine biosynthetic pathway, and as such has become an important target for the design of potential antitumor and antiprotozoal agents.[1] All of the known forms of AdoMet-DC contain a covalently bound pyruvate cofactor at the amino terminus within the catalytic site.[1-3] The substrate AdoMet, **1**, must form an imine linkage with this



AdoMet, **1** — **2a** n = 1, **2b** n = 2 — nor-**2a** n = 1, nor-**2b** n = 2

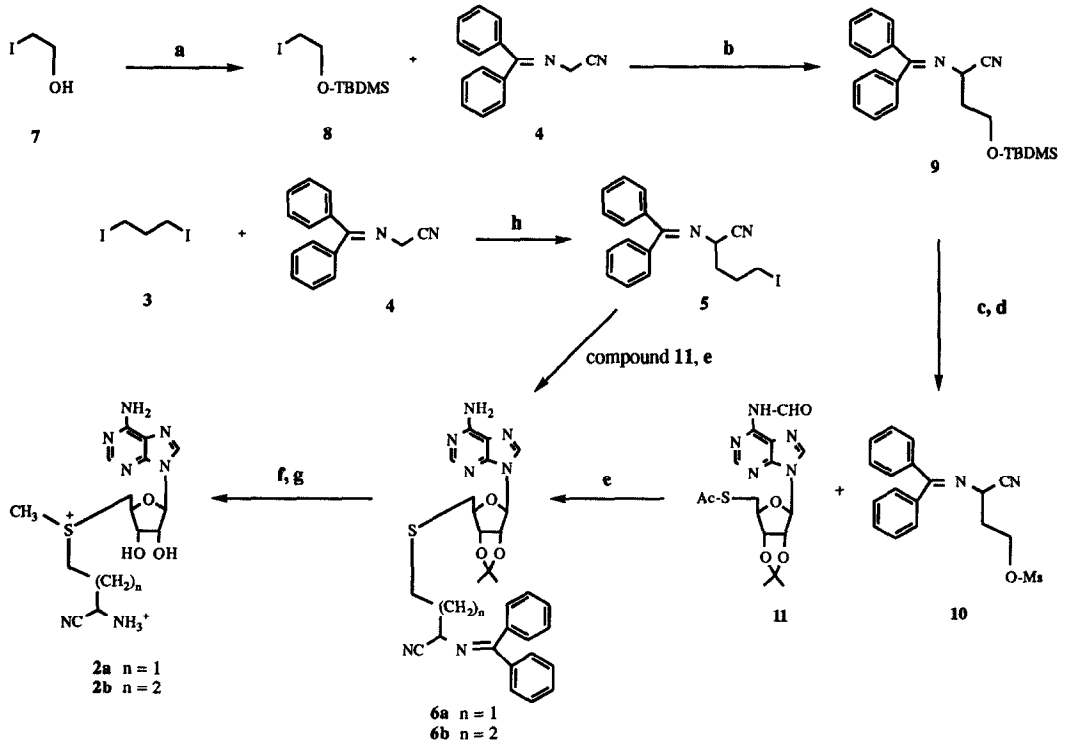
pyruvate residue prior to the enzyme-assisted decarboxylation which produces the product, decarboxylated S-adenosylmethionine (dc-AdoMet). This mechanistic feature has recently been exploited to produce a series of product analogs which act as effective inhibitors of AdoMet-DC.[4-7] Along the same lines, the α-cyano-substituted AdoMet analogues **2a** and **2b** have been proposed as irreversible, enzyme activated inhibitors of AdoMet-DC. In theory, formation of an imine adduct between **2a** or **2b** and the terminal pyruvate of AdoMet-DC could activate the α-proton to abstraction by general base catalysis, followed by formation of a reactive ketenimine within the active site, as shown in the figure below. A reaction between this intermediate and a nucleophilic residue within



the catalytic site could then be envisioned, resulting in irreversible inactivation of the enzyme. In order to test this hypothesis, compounds **2a** and **2b** have now been synthesized and evaluated as enzyme activated, irreversible inhibitors of purified AdoMet-DC.

The synthetic pathway leading to the potential irreversible AdoMet-DC inhibitors **2a** and **2b** is outlined in Scheme 1. Thus, 1,3-diiodopropane **3** was treated with the imine-protected glycine equivalent **4**[8,9] under phase transfer

Scheme 1



a) TBDMS-Cl, DMAP, triethylamine, CH_2Cl_2, R.T., 87%; b) LDA, HMPA, THF, -78°C, 55%;
c) TBAF, THF, R.T., 63%; d) MsCl, triethylamine, CH_2Cl_2, R.T., 81%; e) $NaOCH_3$, DMF/MeOH 1:1, R.T., 64%; f) 88% HCOOH, 48 hrs., R.T., 92%; g) CH_3I, $AgClO_4$, HCOOH/AcOH 1:1, R.T., 64%;
h) 10% NaOH/toluene, benzyltributylammonium chloride, R.T., 56%.

conditions to afford the corresponding cyanoiodide **5**, which was coupled to N^6-formyl-5'-deoxy-5'-thioacetyl-2',3'-isopropylideneadenosine **11** as previously described[10] to produce thionucleoside **6b**. Simultaneous acid catalyzed removal of the acetonide protecting group and cleavage of the imine linkage, followed by methylation of the resultant thioether[11] then afforded the desired target compound **2b**. Surprizingly, our attempts to extend this chemistry to the production of **6a** were unsuccesful, since we were unable to convert diiodoethane to an intermediate corresponding to **5**. Therefore, 2-iodoethanol **7** was converted to the corresponding *tert*-butyldimethylsilyl (TBDMS) ether[12] **8**, which was then coupled to glycine equivalent **4**[8,9] to afford the protected cyanoalcohol **9**. Removal of the TBDMS protecting group[12] followed by mesylation[13] of the intermediate alcohol

then yielded synthon **10**, which was coupled to N^6-formyl-5'-deoxy-5'-thioacetyl-2',3'-isopropylideneadenosine **11** as previously described[10] to produce thionucleoside **6a**. Removal of the acetonide protecting group and cleavage of the imine linkage, followed by methylation[11] then afforded target compound **2a**.

Compounds **2a** and **2b** , as well as the corresponding thioether precursors (nor-**2a** and nor-**2b**), were initially screened as inhibitors of AdoMet-DC using the bacterial form of the enzyme. AdoMet-DC was isolated from *Escherichia coli* using an MGBG-Sepharose affinity column prepared by a modification of the method of Anton and Kutny[14] as previously described,[7] and AdoMet-DC activity was monitored by following the evolution of ^{14}C-CO_2 from S-adenosyl-L-[^{14}C-COOH]-methionine according to a modification of the method of Markham.[7,15] The target compounds **2a** and **2b** each acted as inactivators of AdoMet-DC from *Escherichia coli*, exhibiting IC_{50} values of 9 and 50 μM, as shown in Figure 1. The corresponding unmethylated analogues nor-**2a** and nor **2b**

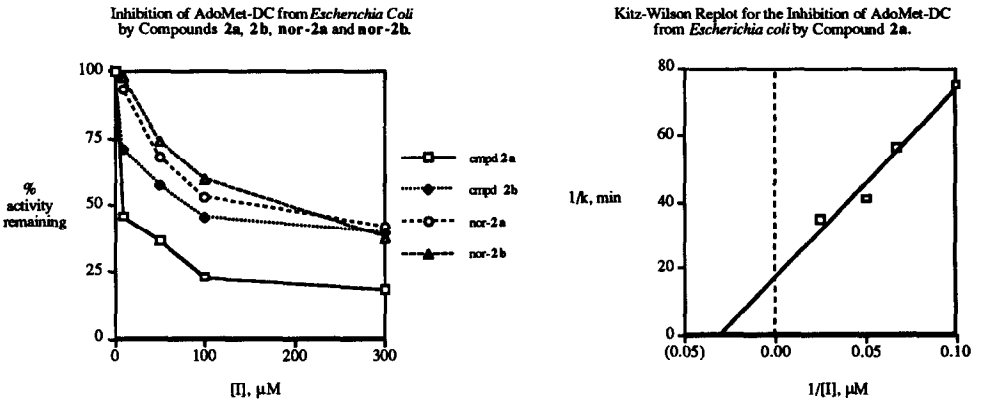


Figure 1. Inhibition of AdoMet-DC from *Escherichia coli* by compounds **2a**, **2b**, **nor-2a** and **nor-2b**.

were much less effective inactivators, with IC_{50} values of 178.6 and 204.8 μM, respectively. The most active inhibitor molecule, compound **2a**, was further evaluated using the method of Kitz and Wilson,[16] and was found to inactivate the bacterial form of AdoMet-DC in a time-dependent manner with a K_I of 31.1 μM, and a k_{inact} value of 0.06 min^{-1}. AdoMet-DC could be protected from inactivation by both **2a** and **2b** when the enzyme was preincubated with the known competitive inhibitor MGBG. In addition, the inhibition produced by each analogue was irreversible, as demonstrated by the inability to dialyze away the inhibitor following binding to AdoMet-DC. AdoMet-DC was incubated with 50 μM of **2a** or **2b** for 30 minutes, and then assayed (average of 15% activity remaining). The sample was then dialyzed overnight (62.5 mM Tris-HCl/100 mM $MgSO_4$, pH 7.4, 4 buffer changes) and reassayed. There was no significant change in the activity level of AdoMet-DC following dialysis, suggesting that the inhibition produced by each analogue was irreversible. When subjected to the same dialysis conditions in the absence of inhibitor, AdoMet-DC retained greater than 95% of its pre-treatment level of activity.

Compounds **2a** and **2b** were next evaluated as inhibitors of the human form of AdoMet-DC. As was the case with the *Escherichia coli* form of the enzyme, both analogues served as time-dependent inactivators. The inhibition produced by **2a** and **2b** was analyzed using the method of Kitz and Wilson,[16] and these compounds were found to exhibit K_I values of 246.6 and 7.2 μM, and k_{inact} values of 0.29 and 0.03 min^{-1}, respectively, as shown in Figure 2. In addition, the inhibition produced by both **2a** and **2b** was shown to be active site-directed and irreversible using the MGBG protection and dialysis experiments described above.

Our laboratories have recently reported that the conformationally restricted S-adenosylmethionine analogue AdoMac {S-(5'-deoxy-5'-adenosyl)-1-ammonio-4-methylsulfonio-2-cyclopentene} acts as an enzyme activated, irreversible inhibitor of the *Escherichia coli* form of AdoMet-DC.[7] Each of the four possible diastereomers of

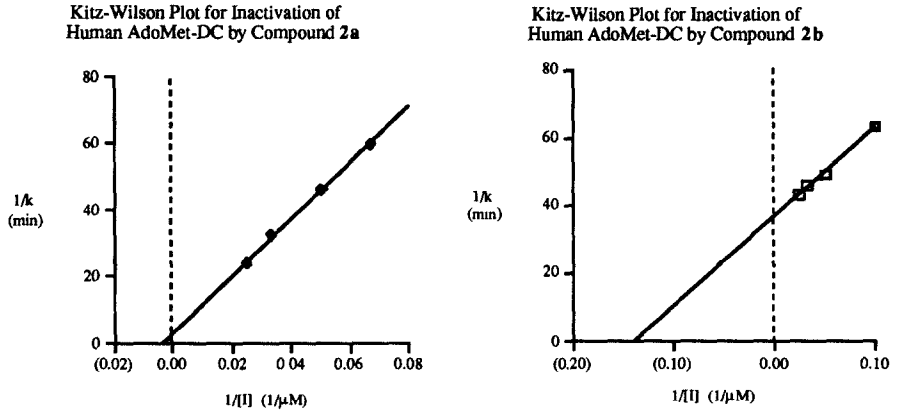
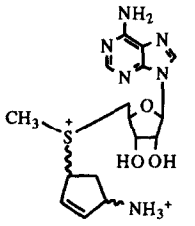


Figure 2. Inactivation of human AdoMet-DC by compounds **2a** and **2b**.

AdoMac represents a distinct conformational mimic exhibiting restricted sidechain rotation, and as such may be useful as conformational probes for the catalytic site of AdoMet-DC. To this end, AdoMac has now been resolved



AdoMac

into its four pure diastereomeric forms, and each diastereomer has been individually evaluated as an irreversible inactivator of the *Escherichia coli* form of AdoMet-DC. The K_I values for the individual diastereomers range between 3.8 and 39.6 μM, with the *cis*-1S,4R diastereomer being the most potent inactivator (unpublished data). When evaluated against the human form of AdoMet-DC, the pure diastereomeric forms of AdoMac exhibited K_I values ranging between 10.7 and 62.7 μM. However, in this case, the *cis*-1R,4S diastereomer, which assumes a significantly different least energy conformation from the *cis*-1S,4R diastereomer, was the most potent inactivator (unpublished data). In the present study, the two forms of the enzyme also appear to differ in their preference for optimal sidechain length, since **2a** is significantly more potent than **2b** against the *Escherichia coli* form of AdoMet-DC, and the reverse is true for the human form of the enzyme. These findings are not surprizing in light of the lack of sequence homology between the human and *Escherichia* coli forms of AdoMet-DC,[17] and suggest that analogues of AdoMet may be used to discover and exploit the differences in catalytic site shape and dimension

between the human and bacterial forms of the enzyme. In addition, compounds **2a** and **2b** may be useful in identifying specific residues which are present in the active site of the various forms of AdoMet-DC by isolating and sequencing peptide fragments derived from proteolysis of the inhibitor-AdoMet-DC adduct formed during inactivation. These experiments, as well as the synthesis and biological evaluation of additional AdoMet analogues, are ongoing concerns in our laboratories.

Acknowledgement

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