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RESTRICTED ROTATION ANALOGS OF DECARBOXYLATED S-ADENOSYLMETHIONINE AS INHIBITORS OF POLYAMINE BIOSYNTHESIS.[1]

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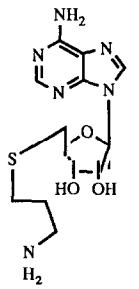
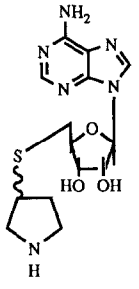
Abstract: The synthesis and preliminary biological evaluation of decarboxylated S-adenosylmethionine analogs **1** and **2** is described. In an L1210 cultured cell assay, these compounds, designed as conformationally restricted active site probes for spermidine and spermine synthase, exhibit LD_{50} values of 0.1 and 1.0 mM, respectively. In addition, these compounds have significant effects on levels of cellular polyamines and S-adenosylmethionine.

The polyamine biosynthetic pathway represents a logical target for chemotherapeutic intervention, since depletion of polyamines results in disruption of a variety of cellular functions, and ultimately in cell death.[2] Such agents also play a critical role as research tools to elucidate the cellular functions of the naturally occurring polyamines. Although a number of compounds have been developed,[3-6] there is still an ongoing need for specific inhibitors of the enzymes involved in polyamine biosynthesis. Compounds **1** and **2** are derivatives of decarboxylated S-adenosylmethionine (dc-AdoMet) which were designed as part of a program to synthesize conformationally restricted active site probes for enzymes involved in the polyamine pathway. Such compounds, if active, would represent a new class of polyamine biosynthesis inhibitor, and could prove valuable in determining the active site conformational requirements of the target enzymes. In particular, the compounds in this report are targeted to the aminopropyltransferases spermidine synthase and spermine synthase, for which dc-AdoMet is a substrate. The purpose of this preliminary study was to determine whether restricted rotation analogs would have significant effects on cell growth and polyamine levels, as well as to work out a chemical pathway which could be adapted to the synthesis of related, diastereomerically pure analogs. We now report the synthesis of compounds **1** and **2** in their diastereomeric forms, as well as the results of preliminary biological testing.



dc-AdoMet



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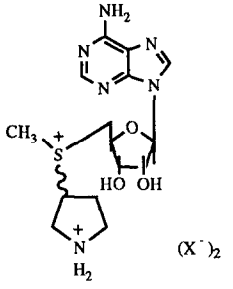


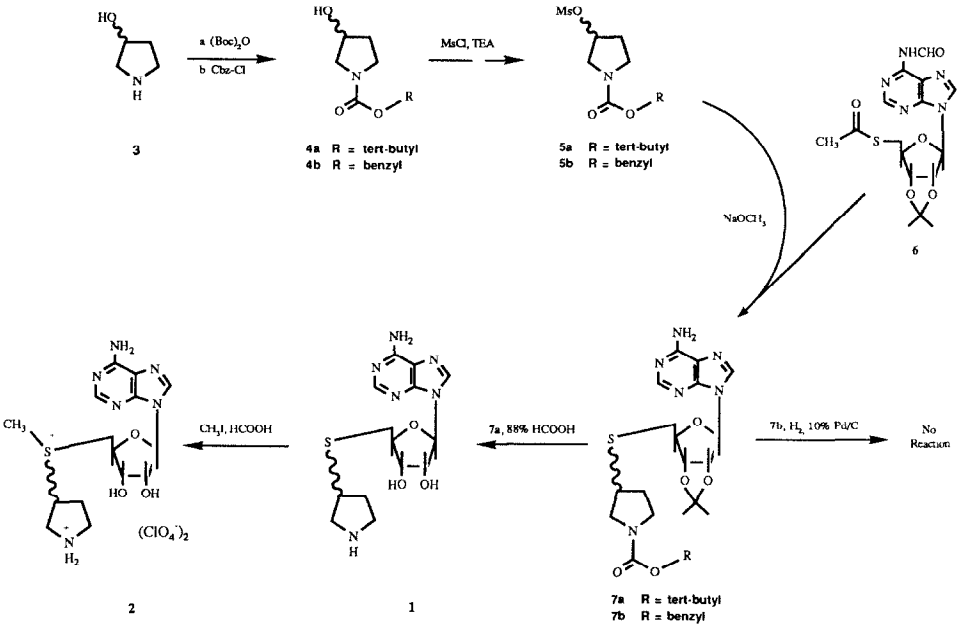
2

The synthetic route leading to dc-AdoMet analogs **1** and **2** is depicted in Scheme 1. Treatment of 3-pyrrolidinol (**3**) with di-*tert*-butyl dicarbonate in the presence of $NaHCO_3$ and NaCl[7] afforded the N-Boc-protected aminoalcohol **4a**, which was then mesylated (methanesulfonyl chloride, triethylamine, CH_2Cl_2)[8] to

produce the desired sidechain synthon **5a**. In similar fashion, treatment of **3** with CbzCl[9] followed by mesylation of the resulting N-Cbz-protected derivative **4b** afforded the corresponding Cbz-protected synthon **5b**.

Initially, we were concerned about the possible lack of reactivity towards nucleophilic substitution of the cyclic, secondary mesylates **5a** and **5b**. Early attempts to effect coupling of the adenosyl synthon **6** (synthesized in two steps from 2',3'-isopropylideneadenosine[6,10]) to either **5a** or **5b** in dry methanol[6] and other solvents resulted in yields of less than 10%. Coupling to the appropriate pyrrolidine ring synthon was ultimately accomplished by treating **6** with sodium methoxide in a 50:50 mixture of methanol/DMF in the presence of either mesylate **5a** or **5b**. These conditions resulted in the formation of the fully protected coupled products **7a** and **7b** in 69 and 81% yields, respectively. However, these yields could only be attained if the rigorous exclusion of oxygen from the reaction mixture was ensured, as previously described.[6]

Scheme 1



Hydrogenolysis of the Cbz protecting group in **7b** proved to be a significant problem. No N-Cbz cleavage could be detected by ^{1}H-NMR, even following prolonged treatment with hydrogen over 10% Pd/C in methanol (50 p.s.i., fresh catalyst every 24 hours). This lack of reactivity is most likely due to catalyst poisoning by the thioether linkage in **7b**.[6,11] Conversely, deprotection of the N-Boc group and the acetonide in **7a** proceeded smoothly in a single step (88% aqueous formic acid) to afford **1**, which was isolated as its free base following chromatography on silica gel. Methylation of **1** (formic acid, CH_3I[10]) then resulted in the formation of the methylsulfonium analog **2**, which was converted to the perchlorate salt using an anion exchange column (Dowex AG1 X 8, 100-200 mesh, 1 X 8 cm, ClO_4^{-1} form). Isolation of the pure diastereomeric forms of **1** and **2** or the

precursor **7b** by chromatography or other conventional means proved to be a difficult undertaking. Rather than pursue separation of these isomers, we have now undertaken the synthesis of diastereomerically pure **1** and **2** by a stereochemically unambiguous route.

Compounds **1** and **2** have now undergone preliminary screening in an L1210 cultured cell assay procedure.[12] The results of this assay appear in Table I. The IC_{50} values for compounds **1** and **2** were found to be 0.1 and 1.0 mM, respectively. Cells were then treated with **1** and **2** at the appropriate IC_{50} value for 48 hours prior to harvest. Ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylase (AdoMet-DC) activity were measured by following the enzymatic decarboxylation of L-[^{14}C-COOH]-ornithine or S-adenosyl-L-[^{14}C-COOH]-methionine, respectively, as nmol CO_2 produced per hour per mg of protein.[13] Intracellular polyamine levels were determined by HPLC as previously described by Porter *et al.*,[13] and are expressed as pmol/10^6 cells.

Table I: Effect of Analogs 1 and 2 on Cell Growth and Polyamine Biosynthesis in Cultured L1210 Cells

Treatment (IC_{50}, 48 hr.)	Growth (% of control)	Decarboxylase Activity ODC (nmol CO_2/mg/hr)	Decarboxylase Activity SAM-DC (nmol CO_2/mg/hr)	Polyamines PUT (pmol/10^6 cells)	Polyamines SPD (pmol/10^6 cells)	Polyamines SPM (pmol/10^6 cells)	AdoMet/AdoHcy (pmol/10^6 cells)
None	100	1.94	0.83	72	1260	415	29 05/<1.10
0 1 mM **1**	46	2.98	3 67	37	1000	410	45 88/7 60
1.0 mM **2**	58	6.16	5 00	170	1290	208	42 80/9 17

Each data point presented is the mean of two experimental determinations with agreement within ± 10%

Although no specific inferences may be made as to the site of action of compounds **1** and **2** from the data in Table I, and the possibility of non-specific effects on cell growth cannot be ruled out at the concentrations used, the results strongly suggest that the compounds have an effect on the polyamine pathway. Clearly, both compounds **1** and **2** are able to penetrate the cell in sufficient quantities to cause the observed changes in enzymatic activity and polyamine content. The increases observed in ODC activity following treatment with compounds **1** and **2** are probably not significant, since a 150 to 200-fold increase in ODC activity secondary to interruption of the pathway is more typical.[15] However, the 4.4 and 6.0-fold increases in AdoMet-DC activity following treatment with **1** and **2**, respectively, are more consistent with the degree of induction observed following interruption of the polyamine pathway. Compound **2** demonstrates a significant effect on cellular polyamine content, effecting a 2.4-fold increase in putrescine and a 50% reduction in spermine content. This effect suggests that compound **2** may interact specifically with the enzyme spermine synthase in L1210 cells. Both compounds **1** and **2** decrease the cellular ratio of AdoMet/S-adenosylhomocysteine (AdoHcy); the mechanism underlying this striking effect is as yet unclear. Purified enzyme studies involving enzymes in the polyamine pathway, as well as with enzymes involved in cellular methylation processes and AdoHcy metabolism, are now being conducted to pinpoint the enzymatic specificity and affinity of compounds **1** and **2**. Synthesis of

additional conformationally restricted analogs, of analogs **1** and **2** in diastereomerically pure form, and molecular modeling studies are all of ongoing concern in our laboratories.

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REFERENCES AND NOTES

1. Presented in part at the 198th National Meeting of the American Chemical Society, September 10-15, 1989, Miami Beach, FL; Abstract MEDI23.

2. Sunkara, P.S.; Baylin, S.B.; Luk, G.D. *Inhibition of Polyamine Metabolism: Biological Significance and Basis for New Therapies*; McCann, P.P.; Pegg, A.E.; Sjoerdsma, A., Eds.; Academic Press: New York, 1987; pp. 121-140.

3. Metcalf, B.W.; Bey, P.; Danzin, C.; Jung, M.J.; Casara, P.; Vevert, J.P. *J. Am. Chem. Soc.* **1978**, *100*, 2551.

4. a. Williams-Ashman, H.G.; Pegg, A.E. Polyamines in Biology and Medicine, Morris, D.R. and Marton, L.J., Eds.; Marcel Dekker: New York, 1981; pp. 43-73. b. Pegg, A.E.; Jones, D.B.; Secrist III, J.A. *Biochemistry* **1988**, *27*, 1408. c. Casara, P.; Marchal, P.; Wagner, J.; Danzin, C. *J. Am. Chem. Soc.* **1989**, *111*, 9111.

5. Tang, K.-C.; Mariuzza R.; Coward, J.K. *J. Med. Chem.* **1981**, *24*, 1277.

6. Woster, P.M.; Black, A.Y.; Duff, K.J.; Coward, J.K.; Pegg, A.E. *J. Med. Chem.* **1989**, *32*, 1300.

7. Keller, O.; Keller, W.E.; van Look, G.; Wersin, G. *Org. Syn.* **1985**, *63*, 160.

8. Crossland, R.K.; Servis, K.L. *J. Org. Chem.* **1970**, *35*, 3195.

9. Bergmann, M.; Zervas, L. *Chem Ber.* **1932**, *65*, 1192.

10. Coward, J.K.; Anderson, G.L.; Tang, K.-C. *Meth Enz.* **1983**, *94*, 286.

11. Rylander, P.N. *Hydrogenation Methods*; Academic Press: New York, 1985; pp. 44.

12. Kramer, D.L.; Khomutov, R.M.; Bukin, Y.V.; Khomutov, A.R.; Porter, C.W. *Biochem. J.* **1989**, *259*, 325.

13. Porter, C.W.; Cavanaugh, P.F.; Stolowich, N.; Ganis, B.; Kelly, E.; Bergeron, R. J. *Cancer Res.* **1985**, *45*, 2050.

14. Wagner, J.; Danzin, C.; Mamont, P. *J. Chromatog.* **1982**, *227*, 349.

15. Pegg, A.E. *Biochem. J.* **1986**, *234*, 249.