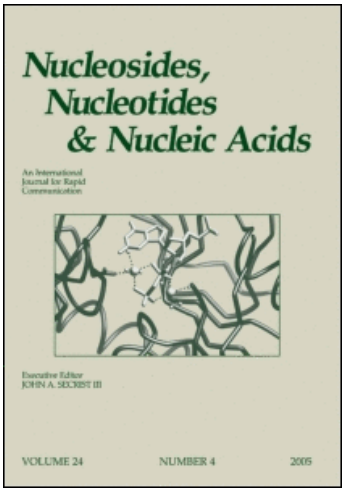

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New Substrate Analogues as Inhibitors of S-Adenosylmetfflonine Decarboxylase

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NEW SUBSTRATE ANALOGUES AS INHIBITORS OF S-ADENOSYLMETHIONINE DECARBOXYLASE

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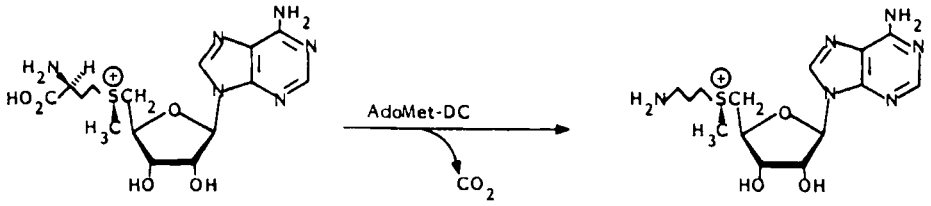
Summary: Analogues of S-adenosylmethionine with modifications in the 5'-group were prepared as potential inhibitors of S-adenosylmethionine decarboxylase (AdoMet-DC). These new analogues contained carbonyl-reactive end groups in the 5'-side chain, designed to interact favorably with the pyruvate prosthetic group of AdoMet-DC. Several of the analogues proved to be outstanding inhibitors of the enzyme. The analogues were also evaluated for their activity against human cytomegalovirus in vitro.

In recent years there has been considerable research carried out that relates to the polyamine biosynthetic pathway. The polyamines putrescine, spermidine, and spermine are known to be required for normal cell growth and differentiation,[1] and it has been shown that interference with polyamine metabolism can lead to effects of possible therapeutic utility.[2,3] For example, certain types of human cancers have shown some response to the S-adenosylmethionine decarboxylase (AdoMet-DC) inhibitor MGBG [methylglyoxal bis(guanylhydrazone)] when treated on a schedule that minimized toxicity problems.[4-9] Synergism has been seen between 2-difluoromethylornithine (DFMO), an enzyme-activated irreversible inhibitor of ornithine decarboxylase (ODC), and MGBG in patients with childhood leukemias.[10,11] DFMO has also been shown to be synergistic with other anticancer agents in several rat models.[12-15] DFMO administration to mice with the 3LL tumor also showed decreased secondary pulmonary metastases relative to the number that are seen normally.[16-17] A comparable effect was seen in mice after subcutaneous inoculation of adenocarcinoma cells.[18] Pronounced activity has also been seen with DFMO against certain parasitic diseases in humans.[19,20]

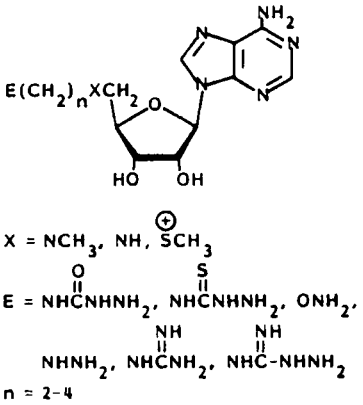
Though ODC is the rate-limiting enzyme on the polyamine pathway, cells are able to compensate for ODC inhibition in a number of ways, and it appears that in order to most effectively reduce polyamine pools, more than one enzyme on the pathway will need to be inhibited.[21,22] The next likely target for the design of a chemotherapeutic agent appears to be AdoMet-DC. Some inhibitors of this enzyme have been synthesized,[23-26] but with the exception of MGBG and related compounds, none of them has particularly promising inhibitory activity. Compounds such as MGBG have significant toxicity that makes them undesirable for many applications, and, in addition, they seem to affect other processes besides the polyamine pathway. Thus, new potent and specific inhibitors of AdoMet-DC are needed.

Our goal was to design and synthesize useful inhibitors of AdoMet-DC and to examine them alone and in combination with ODC inhibitors or other appropriate drugs as chemotherapeutic agents. Such a combination should dramatically reduce the polyamine pools in treated cells. Specifically, our initial research has dealt with the development of a new drug that might be used alone or in an appropriate combination for the treatment of human cytomegalovirus (CMV) infections. Human CMV infection is a particularly severe problem for immune-suppressed patients such as those with AIDS, with organ transplants, or those undergoing cancer chemotherapy. Polyamine biosynthesis has been found to be essential for the replication of human CMV as well as vaccinia virus.[27,28] Infection of mammalian cells with either vaccinia virus or human CMV results in the stimulation of polyamine biosynthesis.[28-31] Both MGBG and DFMO have been reported to inhibit replication of CMV in vitro.[27,28] Thus, we felt that the development of an effective inhibitor of AdoMet-DC might allow the development of a chemotherapeutic regimen effective against human CMV. Such a compound might also be useful against other diseases, including certain parasitic diseases, where the polyamine pathway plays an important role.

The process catalyzed by AdoMet-DC is shown below. The decarboxylated AdoMet serves as the aminopropyl donor in the synthesis of both spermidine and spermine. The activity of the enzyme is dependent upon a covalently bound pyruvoyl residue.[32] This prosthetic group reversibly forms a Schiff base with the amino group of the methionine portion of the substrate during the decarboxylation reaction. Various substrate analogues have been synthesized and evaluated as inhibitors of the enzyme, but none of them was nearly as good an inhibitor as MGBG.[23-26] The inhibitory activity that such compounds possessed, however, was encouraging enough to use them as a basis for designing potentially more effective inhibitors. Analysis of the known inhibitors resulted in the following list

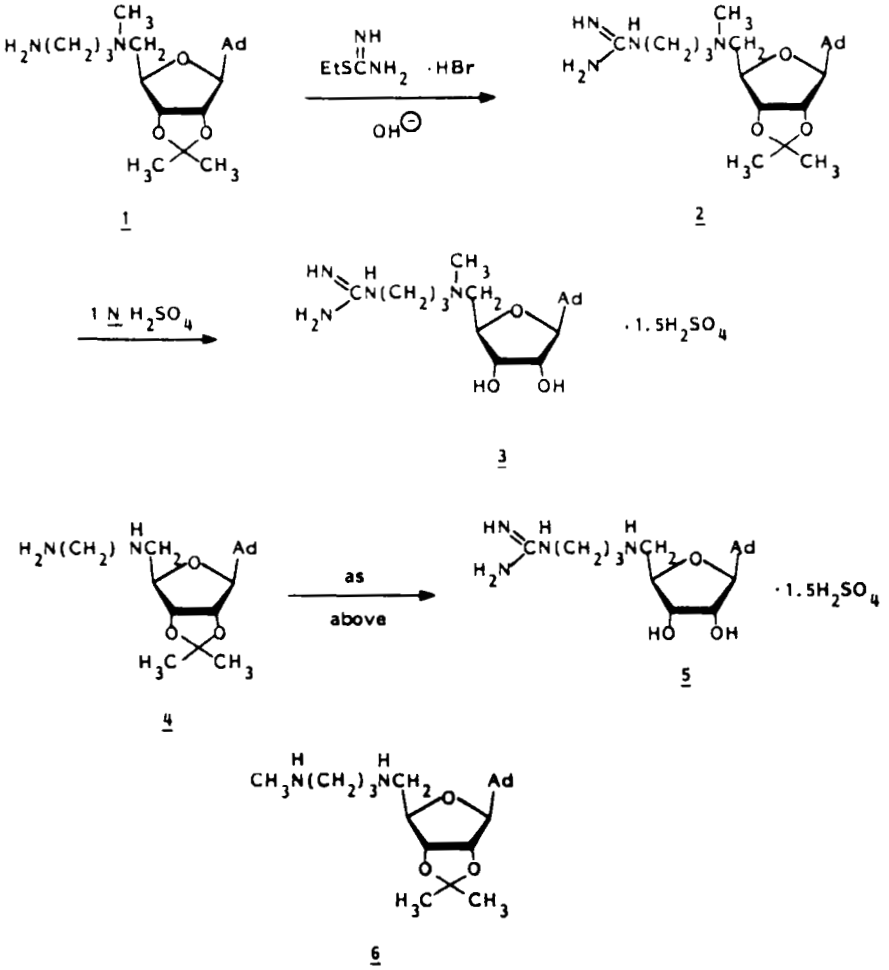


of features that we wanted to include in our set of potential inhibitors: 1) An AdoMet structural framework; 2) An atom capable of protonation at the site of the sulfur in AdoMet (or a positively-charged atom at that site); 3) A side chain long enough to allow condensation of a terminal group with the carbonyl group of the pyruvate prosthetic group; 4) An end group that will react to form a more stable linkage with the carbonyl than a simple primary amine does. Our 5'-atom might be a nitrogen or a positively-charged sulfur, but the lability of the sulfonium salts as well as their likely reduced transport into cells seemed to make nitrogen the preferred choice. As end groups we wanted to examine a series of carbonyl-reactive groups as well as the groups present in MGBG and MBAG (guanidine and aminoguanidine). Attractive end groups aside from guanidino and aminoguanidino were semicarbazide, thiosemicarbazide, aminooxy, and hydrazino. The end group would be attached to the 5'-atom by an alkyl chain of variable length, but typically two to four carbons in length. Thus, the structures below summarize our initial target molecules. Recently, two groups have reported the synthesis of aminooxy compounds from this list, aminooxy compounds with a 5'-NH[33] or a 5'-$\overset{\oplus}{S}CH_3$ and n = 3.[34] The latter compound has been shown to be an excellent inhibitor of E. coli AdoMet-DC.



CHEMISTRY

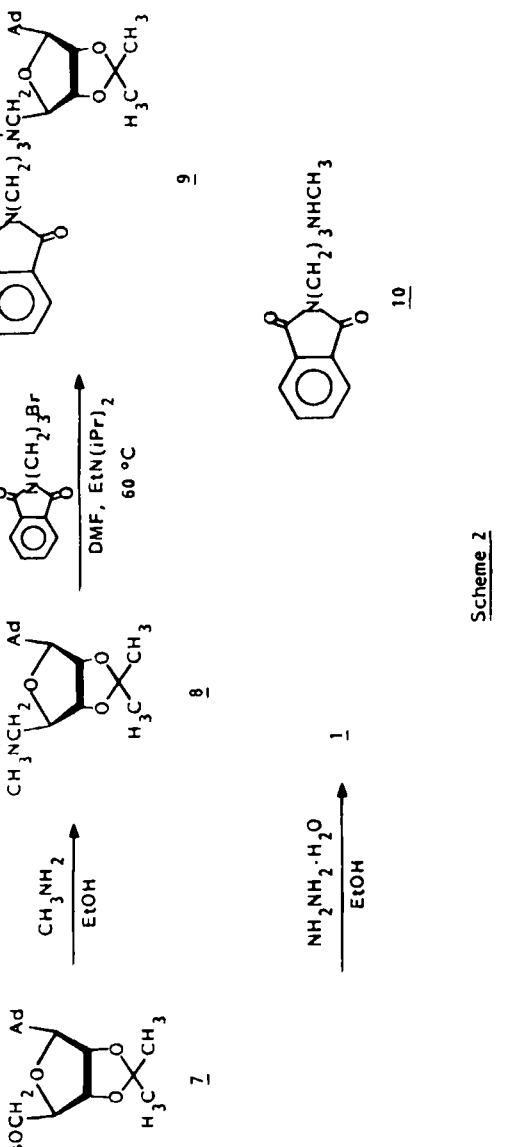
The starting material for all of the target compounds that we have thus far prepared is adenosine. Incorporation of a leaving group at the 5'-position allows displacement with a nucleophile suitable for development into the proper side



Scheme 1

chain. Two of our initial targets were the guanidines **3** and **5**, which were prepared as shown in Scheme 1. The amines **1** and **4** were prepared similarly to the unblocked compounds, which are reported in the literature,[23] with **1** being isolated as a mixture with **6**. Though we prepared **3** from this mixture, we also developed a synthesis of **1** that avoided such problems, shown in Scheme 2.

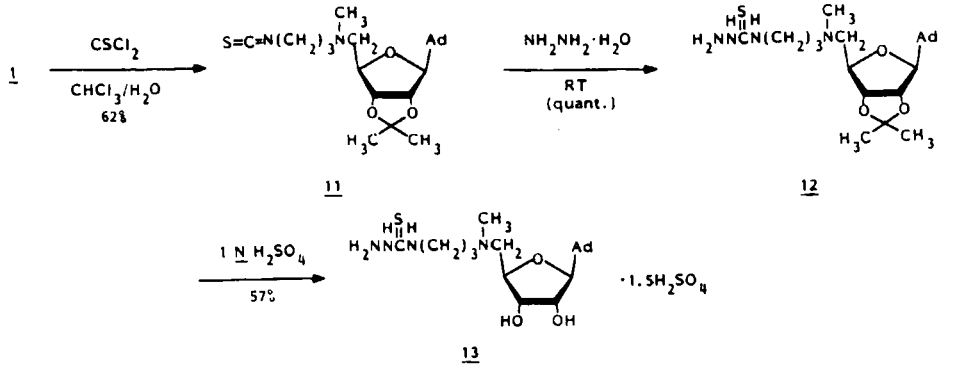
Displacement of the tosylate in **7** with methylamine produced **8**,[35] which was alkylated with 3-bromopropylphthalimide to produce **9**. Hydrazine treatment then gave the desired **1**. Attempted displacement of the tosylate in **7** with 3-(methylamino)propylphthalimide (**10**) failed, presumably because the extra bulk of



Scheme 2

the reagent slowed down the displacement sufficiently that internal cyclization became the predominant reaction pathway.

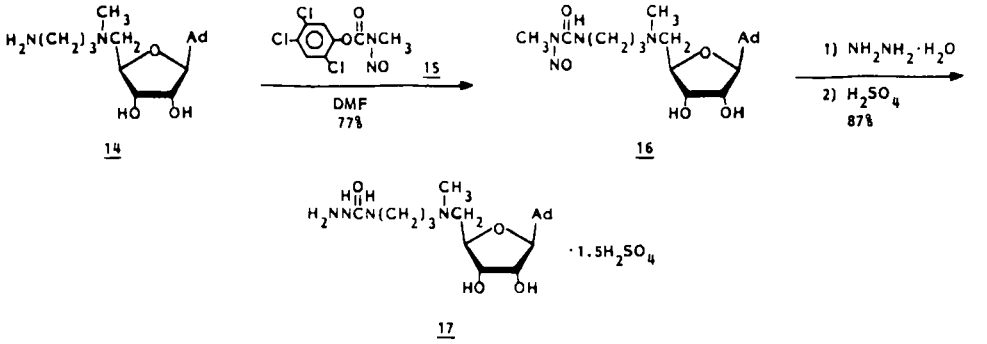
The thiosemicarbazide **13** was readily prepared from **1** also. Formation of the isothiocyanate **11** by standard means was followed by hydrazine addition across the isothiocyanate and then deblocking. The semicarbazide **17** was not as simple to prepare because of the considerable reactivity of the proposed isocyanate precursor. The successful route involved treatment of **14,** formed by



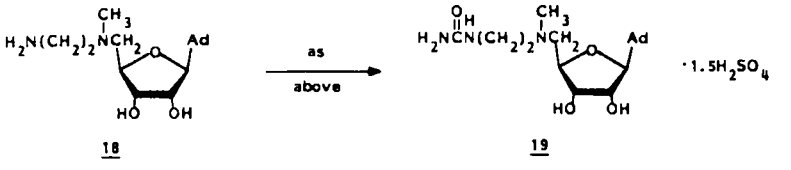
acidic deblocking of **1,** with nitrosourethane **15** to afford the nitrosourea **16.** Treatment of **16** with hydrazine resulted in the formation of the desired target structure **17,** which was isolated as the sulfate salt. A comparable sequence was used to prepare the semicarbazide **19,** with one less carbon in the side chain, starting from **18.**

Aminooxy compounds **22a** and **22b** were prepared from the alcohols **20a** and **20b.** These alcohols were prepared by direct displacement of the tosylate of **7** with the amino alcohols **23a** and **23b.** Treatment of **20a** and **20b** with N-hydroxyphthalimide, diethyl azodicarboxylate, and triphenylphosphine under the Mitsunobu conditions[38] afforded the blocked phthalimides **21a** and **21b.** These were converted directly to the desired target structures **22a** and **22b** by warming with 1 N sulfuric acid.

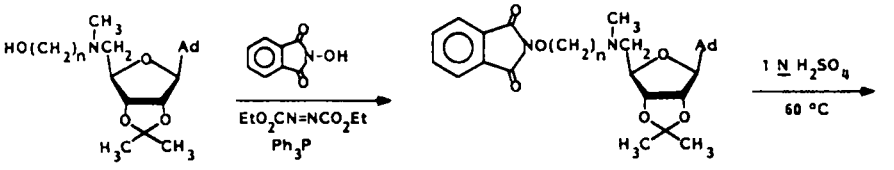
The aminoguanidine **26** was prepared as shown in Scheme 3. Our only preparation of this material was contaminated with a minor amount of what we believe is **27.** This slightly contaminated material was examined for inhibition of AdoMet-DC.



14 → (15, DMF, 77%) → 16 → (1) $NH_2NH_2 \cdot H_2O$ (2) H_2SO_4, 87%) → 17 $\cdot 1.5H_2SO_4$



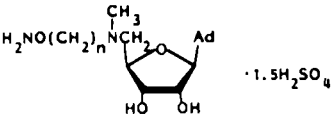
18 → (as above) → 19 $\cdot 1.5H_2SO_4$



20 → ($EtO_2CN{=}NCO_2Et$, Ph_3P) → 21 → (1 N H_2SO_4, 60 °C)

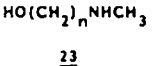
20: a) n = 3; b) n = 2

21: a) n = 3; b) n = 2



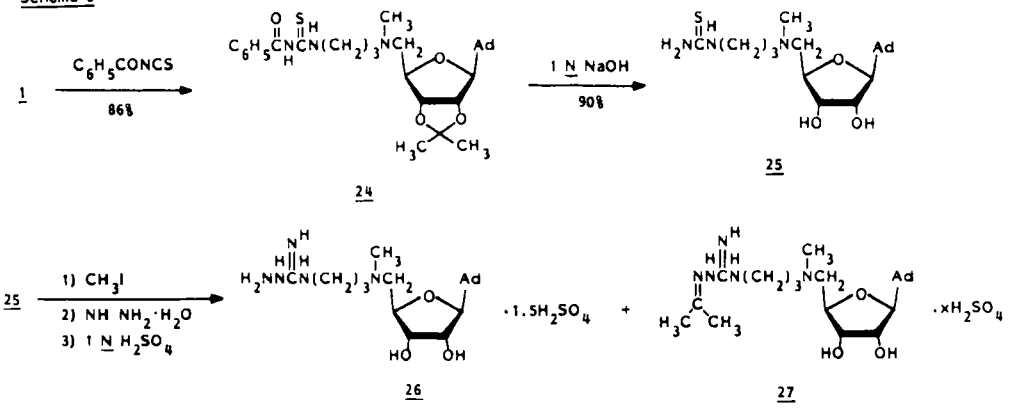
22 $\cdot 1.5H_2SO_4$

22: a) n = 3 (66%); b) n = 2 (68%)



23: a) n = 3; b) n = 2

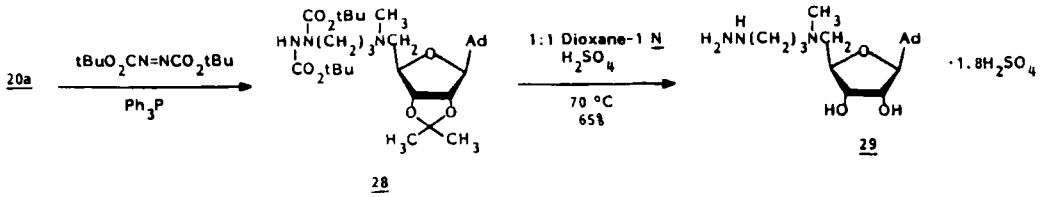
Scheme 3



1 → (C_6H_5CONCS, 86%) → 24 → (1 N NaOH, 90%) → 25

25 → (1) CH_3I (2) $NH_2NH_2 \cdot H_2O$ (3) 1 N H_2SO_4) → 26 $\cdot 1.5H_2SO_4$ + 27 $\cdot xH_2SO_4$

The last compound that to be discussed herein is the hydrazino compound **29**. This material was not available by several of the standard methods for the preparation of substituted hydrazines, but a modification of the Mitsunobu reaction proved successful. Treatment of alcohol **20a** with di-tert.-butyl azidocarboxylate and triphenylphosphine in the absence of another alcohol component allowed formation of the substituted hydrazine **28**,[39] which was deblocked to **29** by warming in a dioxane-1 N sulfuric acid mixture. The initial step also was successful with diethyl azodicarboxylate, but we were not able to decarboxylate the product in our system.



BIOLOGICAL EVALUATION

Initially we wished to examine the ability of each of the target compounds to inhibit AdoMet-DC. Because our virology experiments were being conducted with MRC 5 cells, we chose to evaluate compounds against the crude enzyme isolated from uninfected MRC 5 cells and human CMV-infected MRC 5 cells. Table 1 lists the ID_{50} values for each potential inhibitor with both enzyme preparations. The values determined in our laboratory for MGBG are also included in the Table. It can be seen that the two guanidines **3** and **5** and the aminoguanidine **26** were either extremely poor inhibitors or not inhibitory at all at the levels that we examined. The semicarbazides **17** and **19** and the thiosemicarbazide **13** were reasonable inhibitors, though still about tenfold poorer than MGBG. The two aminooxy compounds **22a** and **22b** and the hydrazino compound **29** were outstanding inhibitors of the enzyme, significantly better than MGBG. The hydrazino compound had an ID_{50} about thirtyfold lower than MGBG. The data in the table do not allow us to state whether or not there is a virus-encoded enzyme separate from the enzyme from normal cells, and we are currently looking into this question. The trend seen in the table has been confirmed with AdoMet-DC from another source, as well.

Briefly, initial examination of the activity of the target inhibitors against human CMV in vitro has shown that several of the compounds have activity when tested alone. The best compound appears to be the hydrazino compound **29**, with the semicarbazide **17** next, and modest activity found for the aminooxy compounds

Table 1. Effect of Target Compounds on AdoMet-DC from MRC 5 Cells

Compound	ID_{50} (μM) Uninfected	ID_{50} (μM) CMV-Infected
29	0.0075	0.006
22b	0.04	0.009
22a	0.12	0.022
MGBG	0.20	0.19
19	0.6	0.6
13	1.8	1.7
17	2.5	2.3
16	50	50
26	>100	95
3	>100	>100
5	>100	>100

Uninfected or CMV-infected MRC 5 cells are disrupted in a minimum amount of pH 7.5 buffer containing 25 mM Tris, 0.1 mM EDTA, 2 mM β-mercaptoethanol, 25 mM putrescine, 0.2 mM pyridoxal phosphate, and 0.5% Triton X-100. The suspension is clarified by centrifugation and the supernatant used for analysis.

22a and **22b** as well as the thiosemicarbazide **13** and the nitrosourea **16.** No activity was found for the guanidino compounds **3** and **5.** The others have not yet been evaluated. The degree of antiviral activity very roughly corresponds to the ability of the compounds to inhibit AdoMet-DC, with the exception that the aminooxy compounds have less activity than might be expected. A possible explanation for these results was found by monitoring the amount of **22a** in the medium upon incubation with MRC 5 cells. Over a period of hours the aminooxy compound is converted to a new, less polar metabolite. Presumably the aminooxy compounds are gradually inactivated by this conversion, thus accounting for their reduced antiviral activity. Further evaluation of the best of these compounds in combination with other appropriate drugs is currently taking place.

To summarize, we have developed a series of compounds that might be inhibitors of AdoMet-DC, and we have found that the best compounds from this

series are outstanding inhibitors of the enzyme. In addition, several of the compounds that show inhibitory activity also have antiviral activity against human CMV. Our initial results with these compounds have encouraged us to undertake examination of their properties in many biological systems. We are also in the process of synthesizing various other related compounds that might have improved properties.

ACKNOWLEDGMENT

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