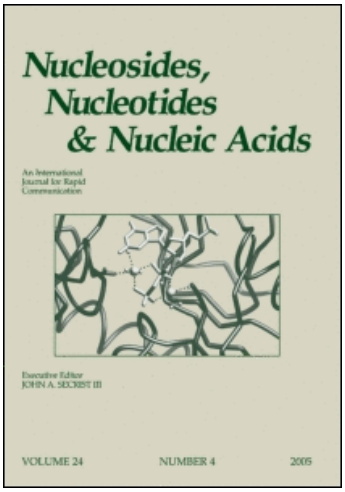

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NOVEL CoA-POLYAMINE CONJUGATES FOR EFFECTIVE INHIBITION OF SPERMINE/SPERMIDINE-N^1-ACETYLTRANSFERASE

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□ *New mimics of the transition state of spermine/spermidine-N^1-acetyltransferase reaction were prepared starting from aminooxy analogues of spermidine or spermine and SH-CoA. The activity depended on the structure of polyamine fragment of the conjugate and best of the synthesized compounds were active at micromolar concentrations.*

Keywords Polyamines; coenzyme A; SSAT; coenzyme-substrate inhibitors

INTRODUCTION

The biogenic polyamines spermidine (Spd) and spermine (Spm) occur in significant amounts in all mammalian cells and are essential for their normal growth. Key enzymes of polyamine biosynthesis are

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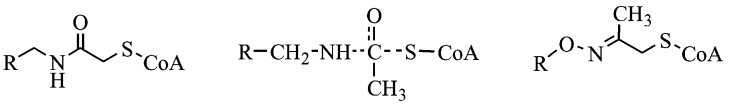


Amide as an analogue of transition state of SSAT-reaction[2,3]

Transition state of SSAT-reaction

Oxime as an analogue of transition state of SSAT-reaction

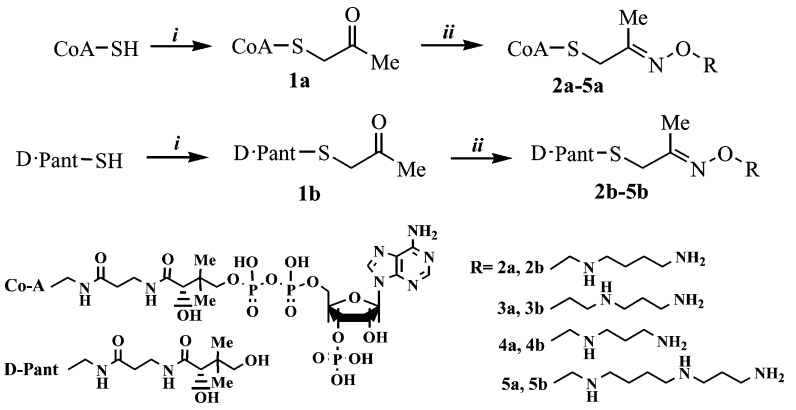
FIGURE 1 Mimic of the transition state of spermine/spermidine-N^1-acetyltransferase reaction.

ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylase (AdoMetDC), while acetyl-CoA-dependent spermine/spermidine-N^1-acetyltransferase (SSAT) is the rate-controlling enzyme of polyamine catabolism. Tumor cells have elevated levels of polyamines as compared with normal cells and this stimulated design of specific and powerful inhibitors of ODC and AdoMetDC. However, the most efficient strategy to deplete the polyamine pool is the activation of SSAT. Biosynthesis and activity of SSAT is regulated by intracellular content of polyamines and/or their properly designed analogues. Hence, a big family of terminally *bis*-alkylated derivatives of Spm and Spd, which turned to be effective inductors of the enzyme in vitro and in vivo, were synthesized.[1] However, the family of SSAT inhibitors is far too limited.[2–5]

Spm/Spd-acetyl transferase reaction does not involve an acetylated enzyme intermediate and this was the reason to design CoA-substrate conjugates derived from Spm, *nor*-Spd or their symmetric homologues which turned to be effective (IC_{50}) toward isolated enzyme at 0.5–5 μM concentrations.[2] Later, regioselective, but overly complicated, synthesis of the adducts of CoA to either N^1 or N^8 positions of Spd were suggested.[3] Here we present a simple synthesis of two novel types of the analogues of transition acetyl-CoA-substrate complex derived either from HS-CoA or D-N-pantothenylamidoethyl mercaptane (Figure 1).

RESULTS AND DISCUSSION

Having hydroxylamine-containing analogues of spermine and spermidine with a terminal aminooxy group,[6] it was obvious to use an acetone linker to bind the polyamine analogue together and CoA. This was the key point of a simple and convenient two-step synthesis of the required conjugates derived either from HS-CoA or D-N-pantothenylamidoethyl mercaptane (D-Pant) and afforded an opportunity to attach the CoA easily to the required terminus of the polyamine analogue (Scheme 1). At the first step of the synthesis the free thiol group was alkylated with equimolar amount of chloroacetone at slightly basic pH. Thus obtained ketone was reacted with



SCHEME 1 i - $ClCH_2COCH_3$; ii - $RONH_2$.

equimolar amount of corresponding O-substitution hydroxylamines that resulted in required conjugates **2a–5a** and **2b–5b** with quantitative yield.

Compound **(3a)** turned to be the most effective inhibitor of SSAT (Table 1). This substance (IC_{50} 1 μM) formally may be considered as an analogue of the intermediate of N^8-acetyltransferase reaction, while compound **2a**, which initially was design as the most exact mimic of the intermediate of N^1-acetyltransferase reaction was found to be less active (IC_{50} 6 μM). The observed dependence of the activity of the conjugate with oxime linker on the structure of the polyamine group of the conjugate was quite opposite as compared with that for earlier inhibitors with the acetate linker.[2] Hence, the nature of the linker at least must be taken into consideration. The significant contribution of the polyamine fragment into the inhibition was proved with lower activity (IC_{50} 22 μM) of the ketone **1a**. The existence of adenosine group was crucial for effective inhibition, since none of the conjugates of D-N-pantothenylamidoethyl mercaptane family exhibited IC_{50} better than 100 μM.

Synthesized set of novel effective inhibitors of SSAT is of obvious interest for x-ray investigations of the enzyme and also for studying the peculiarities of the organization of the active sites of different forms of SSAT.

TABLE 1 Inhibition of SSAT by the synthesized coenzyme-substrate conjugates. Substrate mixture was the same as published [7] but contained 3 μM [1-^{14}C]-Acetyl-CoA

Compound	**1a**	**2a**	**3a**	**4a**	**5a**	**2b**	**3b**	**4b**	**5b**
IC_{50}, μM	22	6	1	17	5	>100	>100	>100	>100

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