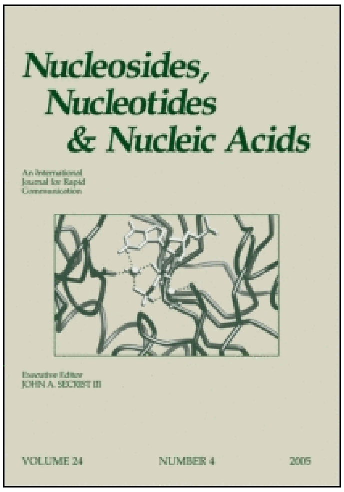

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Synthesis of Fluorescent Adenosine Analogues[1]

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SYNTHESIS OF FLUORESCENT ADENOSINE ANALOGUES[1]

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Abstract: In the synthesis of a transition state inhibitor of the enzyme adenylosuccinate lyase two different [2,1-i]-pyrimido-purine derivatives are formed, of which one might be very interesting for cell biological research, due to its strong fluorescent properties.

Adenylosuccinate lyase is an essential enzyme catalysing two steps of the biosynthesis of adenosine nucleotides. In the conversion IMP to AMP it facilitates the elimination of fumaric acid from adenylosuccinate[2], via transelimination of the pro-R proton (FIG.1).

The enzyme can be used as a tumor marker, because the activity in malignant tumors is significantly enhanced[3] as compared to benign tumors. Our reasoning is, that tumor cells require this higher activity and that inhibitors would do more harm to the malignant tumors than to the corresponding normal tissue.

Earlier work in our group on inhibitors of adenylosuccinate lyase resulted in the synthesis of suicide inhibitors **1**[4], which are able to liberate very reactive ketenes ($O{=}C{=}C(R)COO^-$, R=H, Me) in the active site of the enzyme. Furthermore, compound **2** was devised as a new type of inhibitor, as a so called pre-transition state analogue,

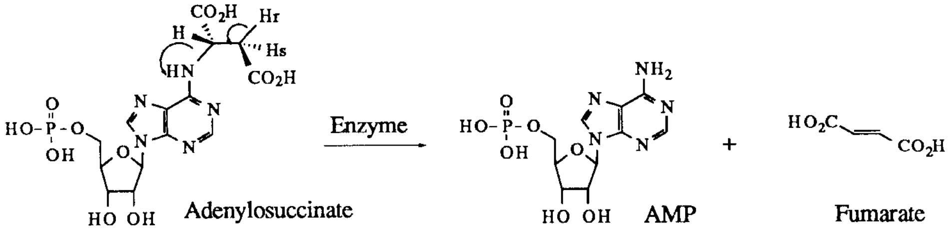

FIG. 1

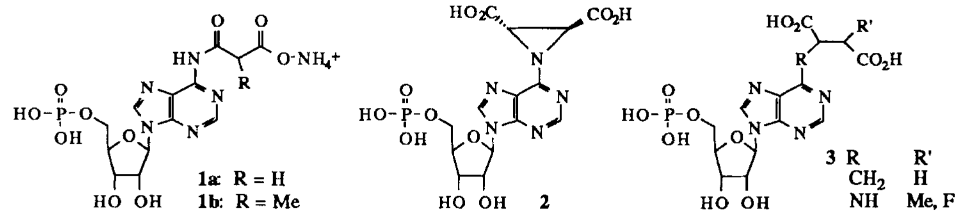

FIG. 2

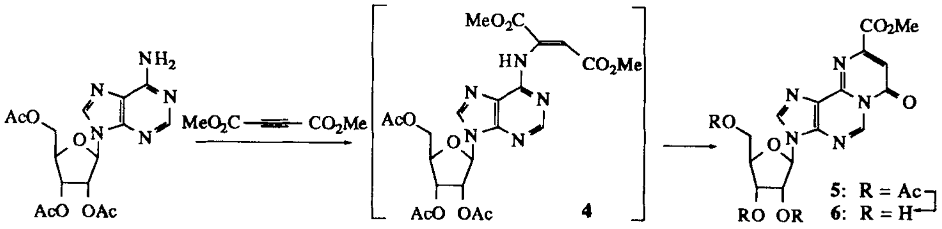

FIG. 3

because the enzyme might convert this substrate into its own transition state. Also some substituted analogues of general structure 3 have been synthesized before[5] (FIG. 2).

One of our goals was to synthesise transition state inhibitor 4 by a Michael addition of protected adenosine to methyl acetylene dicarboxylate. Due to attack of the N-1 nitrogen atom on the carbonyl of the ester function, the cyclized [2,1-i]-pyrimido-purine derivative 5 is formed (FIG. 3), as previously described for the carba-analogues[6]. The same cyclization occurred with the benzyl- and *t*-butyl-acetylenic esters, despite the steric hindrance of the latter. Compound 5 was deprotected with NaOMe to give the free sugar-derivative 6.

These derivatives might be very interesting for biological applications, due to their high fluorescent properties in the UV/VIS region ($\lambda_{em.}$ = 420 nm, quantum yield ± 50%).

Another [2,1-i]-pyrimido-purine isomer (7) is formed when the unprotected sugar is used in this Michael addition. This product is formed in a higher yield (78 %) than described by Roques et al. (FIG 4.)[7]. This cyclization is initiated by addition of the N1-ring nitrogen atom to the triple bond of the acetylene followed by cyclization of the ester to the exocyclic amine function of the purine[8].

Due to the lower solubility of this compound it crystallizes from the reaction medium. Both isomers have been characterized by their spectral properties and by NOE-experiments.

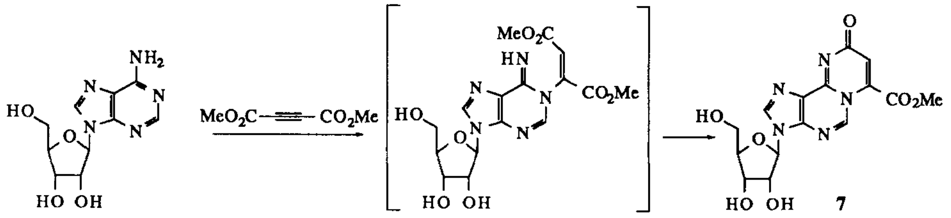

FIG. 4

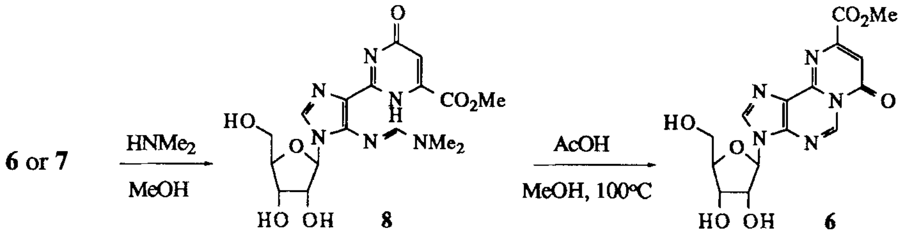

FIG. 5

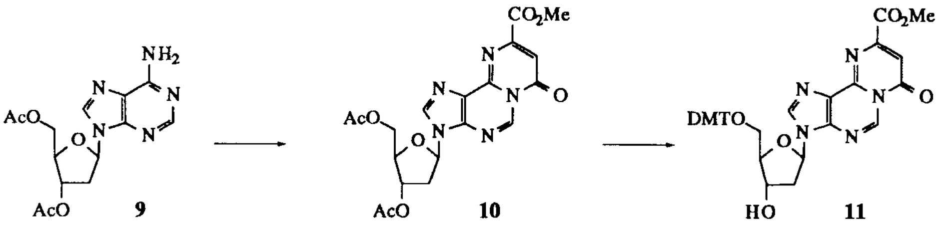

FIG. 6

Carbon atom 5 of both isomers is very sensitive to nucleophilic attack. Stirring with dimethylamine, both **6** and **7** give the product **8**, which can easily be cyclized under acidic conditions, to give only the thermodynamic product **6** (FIG.5).

A similar conversion was observed when the free riboside (**7**) was acetylated.

Currently the synthesis of some fluorescent DNA-fragments is in progress. Therefore compound **11** was synthesized in four steps starting from 2'-deoxyadenosine (FIG. 6). First, the 2'-deoxyadenosine had to be acetylated (**9**) to obtain after the Michael addition the fluorescent isomer **10**. This compound was deacetylated and the 5'-hydroxyl was selectively protected with DMTCl.

DISCUSSION

The Michael addition of adenosine to methyl acetelene dicarboxylate is complex, due to the different equilibria present in the reaction mixture. The primary adduct is in equilibrium with the starting materials and formation of the product may be strongly dependent on the presence of proton donors in the reaction medium. If the products don't dissolve in the reaction medium, the kinetically favored product **7** precipitates. The thermodynamically stable derivatives of general structure **6** are only formed when the products and intermediates are fully soluble in the reaction medium, thus giving rise to the next equilibrium **7** to **8** to **6**. The isomers of general structure **6** show strong blue fluorescence, which might be very useful for biological applications.

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