Syntheses of novel O^6-substituted guanine-derivatives for the investigation of the MGMT-status of tumors with PET

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Introduction

The resistance of tumor cells to the cytostatic activity of methylating and chloroethylating chemotherapeutics is determined by the level of the DNA repair protein O^6-methylguanine-DNA methyl-transferase (MGMT). The protein MGMT binds to both O^6-methylguanine and O^6-chloroethylguanine of the DNA at a fast rate. MGMT transfers the methyl or chloroethyl moiety to its own cysteine acceptor site. Because of the irreversible nature of this binding process, the enzyme remains inactivated. Thus, the amount of MGMT per cell is a direct measure of the cell's capability to repair O^6-guanine alkylation damage in DNA. Some types of tumor tissues do not display any detectable MGMT activity at all. Especially those tumors would lend themselves to a therapy with cytostatic drugs. It would therefore be valuable to develop a method which allows to determine the MGMT status of a given tissue *in vivo*.

O^6-benzylguanine was found to be a potent inhibiting agent of MGMT, successfully depleting the existing amount of MGMT in a cell, while being non-toxic on the level of biologically effective doses. The synthesis of a labeled 9-substituted O^6-benzylguanine has been demonstrated [1]. The necessary prerequisites would be a synthesis of a ^{18}F-labeled compound, whose ED_{50} would prove to be comparable to that of O^6-benzylguanine, and a synthesis of the analogue ^{19}F-compound for further toxicological evaluation.

2-amino-6-benzyloxy-9-[^{18}F]fluoroethylpurine was chosen as a first compound for proving the concept but did not qualify itself for further investigations [1]. Recently, the synthesis of 6-(4-[^{18}F]fluorobenzyloxy)-)-9H-purin-2-ylamine has been reported and the compound was evaluated successfully [2]. However, the multistep synthetic way was troublesome and gave only poor yields.

Syntheses

We developed the prerequisites for new compounds bearing the radioactive tag at the 6-substituend residue. The advantage of our planned synthesis is the direct one step ^{18}F-fluorination of the precursor while avoiding too many steps. For that purpose we synthesized the respective 6-substituted guanine derivative 6-(2-Chloro-pyridin-4-ylmethoxy)-9H-purin-2-ylamine (1) as a possible precursor for the radioactive labeling with [^{18}F]fluoride (fig. 1). The synthesis started from 2-amino-N,N,N-trimethyl-1H-purin-6-amonium chloride (4) which has a better leaving group than the commonly used 2-amino-6-chloroguanine (3).

Nucleophilic displacement of the trimethylamino-leaving group with alcohols is easier to accomplish than for chloride. The disadvantage of this compound is its synthesis, starting from highly volatile trimethylamine, which is condensed at low temperature [3]. We found an easier synthetic route to avoid this step [4].

The methanol derivative (2-chloro-pyridin-4-yl)-methanol (5) for the reaction with the guanine salt was synthesized via a multistep synthetic way (fig. 1). The respective fluorine-standard compound was not yet prepared because in first experiments the coupling of the fluoride bearing alcohol (6) did not work, however we are currently investigating.

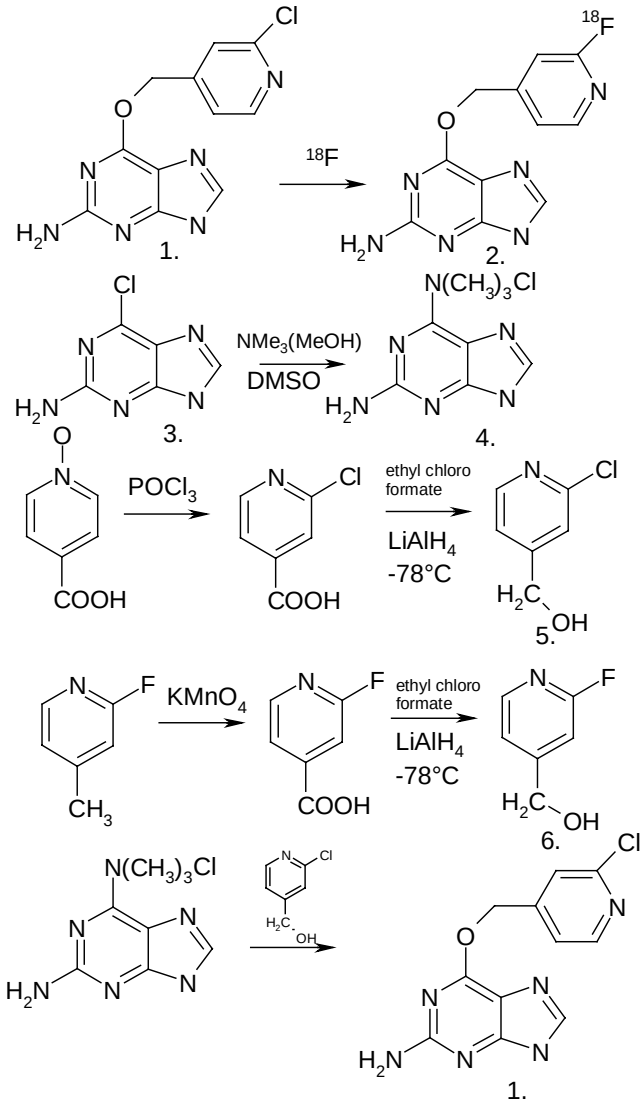

fig. 1: planned organic syntheses and radiolabeling

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