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RAPID SYNTHESIS OF 2'- AND 3'-FLUORINATED NUCLEOTIDES AND THEIR USE IN ^{19}F-NMR-SPECTROSCOPY OF NUCLEOTIDE-BINDING ENZYMES

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ABSTRACT

Improved methods have been developed for the syntheses of nucleoside analogs with fluorine atoms in the furanose moiety. 5'-O-mono- and triphosphates of these analogs were used in stuctural studies on adenylate kinase using ^{19}F-NMR.

Because of its high sensitivity, ^{19}F-NMR is a valuable technique for structural and mechanistic investigations of biological molecules. In general, the fluorine atom must be introduced into the sytem under investigation by synthetic methods. In the case of nucleotide-binding enzymes, the 2'- and 3'-hydroxyl groups of the nucleotide are often not involved in enzyme-substrate interactions, so that introduction of fluorine at these positions is potentially a promising approach.

2'-Fluoroadenosine (**1**) was first synthesized by Ranganathan in 1977 in a 9-step reaction scheme starting from arabinofuranothionoxazolidine[1]. In 1983, an 11 step synthesis was reported by Uesugi et al[2], this time starting from adenosine. Thus, a rapid and efficient synthesis for this interesting derivative was highly desirable.

Direct fluorination of 9-(3',5'-di-O-tetrahydropyranyl-ß-D-arabinofuranosyl)adenine (di-THP-araA) (conveniently obtained in a one-step synthesis from araA[3]) using diethylamino sulfur trifluoride (DAST) in methylene chloride at room temperature led to **1** in 3% yield. Using the monomethoxy trityl group to protect the 3'- and 5'-positions (44% from araA), this was increased to 16%. The yield is still low, but the unfavourable dipolar and steric interactions between the approaching nucleophile or the leaving groups and the anomeric and 3'-substituents in the S_N2 transition state make higher yields improbable.

9-(3'-Fluoro-3'-deoxy-ß-D-xylo- and -arabinofuranosyl)adenine (**2** and **3**) were prepared in two-step syntheses from adenosine and araA. A Mattock reaction using α-acetoxyisobutyryl bromide on adenosine led to 9-(2',3'-anhydro-ß-D-ribofuranosyl)adenine[4] (**4**) in 98.7% yield. An intramolecular Mitsunobu reaction on araA[5] gave a 94% yield of 9-(2',3'-anhydro-ß-D-lyxofuranosyl)adenine (**5**). In ca. 150 small-scale sealed glass-tube experiments, conditions were optimized for the ring-opening reactions of the 2',3',-anhydro compounds. A series of currently used fluorination reagents (tetraethylammonium fluoride, tetrabutylammonium fluoride (TBAF), TBAF on resin, xenon difluoride, tris-(dimethylamino)-sulfur-(trimethylsilyl)difluoride (TASF), potassium hydrogen fluoride, $BF_3{\cdot}Et_2O$, HF-DMF, HF-THF) were tested under a broad range of conditions (concentration: 4-20 equiv.; solvents: methylene chloride, chloroform,

tetrahydrofuran, dimethylformamide, acetonitrile, hexamethylphosportriamide; time: 1 hr.-7 days; temperature: 25-200°). In the nucleophilic attack of "naked" fluoride anion at the sterically less hindered 3'-carbon atom, best results were obtained using tetrabutylammonium fluoride or tris-(dimethylamino)-sulfur-(trimethylsilyl)difluoride in dry acetonitrile (10 ml/mMol epoxide) for 3hr. at 130-140°C; yield 32-41%. Base- and C-5'-protection was not necessary and did not improve the yield.

All fluorinated nucleosides were phosphorylated to mono- and triphosphates by chemical one-flask syntheses[6] or enzymatic phosphorylation (monophosphate plus adenylate kinase and pyruvate kinase with ATP and phosphoenol pyruvate as phosphate donors; lactate dehydrogenase and NADH to reduce pyruvate to lactate; yield >80%). Compounds **2-5** were evaluated for their inhibitory effect on the cytopathogenicity of HIV-1 in MT4 cells and compared to 3´-azido-3´-deoxythymidine[7]. They showed no antiviral activity up to 200μM.

Adenylate kinase (AK) catalyses the transfer of the γ-phosphate group of Mg·ATP to AMP. A point of controversy is whether the two known nucleotide binding sites on the enzyme can both bind ATP and AMP. It was shown by ^{19}F-NMR titration experiments using the mono- and triphosphates of **2** that no fluorine resonance of the triphosphate complex could be detected at the position of the corresponding signal of the monophosphate complex (and vice-versa) up to an 8-fold excess of the analogs over enzyme, indicating that the mono- and triphosphates do indeed bind to the enzyme in different modes (signals at -126.2 ppm for the free nucleotides, -131.6 ppm for the bound triphosphate and -125.0 ppm for the bound monophosphate; standard: trifluoracetic acid; Bruker AM500). ^{19}F-^{1}H Nuclear Overhauser Enhancement studies on the near stoichiometric complexes of the monophosphate of **2** with porcine AK and E.coli AK resulted in a decrease of intensity of the adenine H8 and xylose H1'resonances, both effects being of essentially equal magnitude. The situation is also seen in the free nucleotide, suggesting a similar geometry of bound and free nucleoside monophosphates (anti orientation; glycosidic torsion angle χ (O4'-C1'-N9-C4) = 200-220°).

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