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A Simple Method for N-Acylation of Adenosine and Cytidine Nucleosides using Carboxylic Acids Activated *In-Situ* with Carbonyldiimidazole

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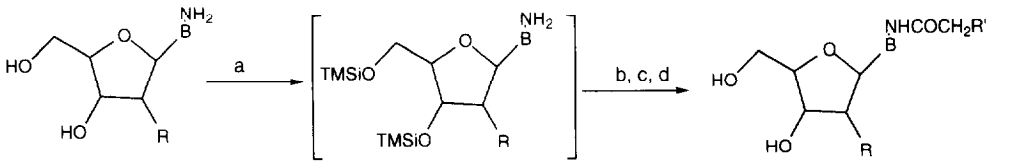
Summary: Carboxylic acids are activated with 1,1'-carbonyldiimidazole in acetonitrile to form N-acylimidazoles which are then treated with per-trimethylsilyl ethers of nucleosides adenosine or cytidine at ambient temperature to generate exclusively N-acylated-Adenosine or N-acylated-Cytidine derivatives.

Protection of exocyclic amino group of nucleosides is one of the important steps in the synthesis of oligonucleotides. Although there are various protecting groups, the main strategy to protect this reactive amine function was developed by Khorana and co-workers,[2] where nucleoside is per-acylated and subsequently deacylated selectively with base to result in the desired N-acylated nucleoside. This method was utilized successfully until 1982 when the reaction was simplified by Jones and co-workers,[3] using transient protection of hydroxyl groups with trimethylsilyl chloride followed by treatment with excess acid chloride or acid anhydride in pyridine. This modification eliminated the need for isolation of peracylated nucleoside and selective de-acylation with base, as silyl ethers are deprotected with pyridine and water during workup. Presently this is the method of choice for the protection of exocyclic amine group of nucleosides.

In connection with the synthesis of oligoribonucleotides and modified oligonucleotides with either base labile nucleosides such as dihydrothymidine[4] and 4-thio-thymidine[5] or modified internucleotide linkages[6,7,8,9] such as methylphosphonate diester and phosphate triester, milder or faster de-N-acylation of exocyclic amino groups have been suggested in order to minimize chain cleavage or base modification. Various acyl protecting groups such as PAC = phenoxyacetyl,[4] t-BPA = t-butylphenoxyacetyl,[7] IPAC = isopropylphenoxyacetyl, and IPOAc = isopropoxyacetyl[6] have been introduced to achieve milder and faster deprotection. Incorporation of these groups on to the exocyclic amine of nucleosides has been achieved using Jones's transient protection strategy with a 3-5 fold excess of anhydride or chloride of the above acids. There can be several drawbacks to using this approach. First, the above anhydrides or acid chlorides are not readily available and have to be synthesized. For example, isopropoxyacetic anhydride[6] is synthesized in a two step process from isopropoxyacetic acid chloride and its trimethylsilyl derivative. Secondly, since excess acylating agent is generally required there is a wastage of valuable free acid. Furthermore, because some of the acids listed above are insoluble in water they can interfere in the isolation of N-acylated nucleoside. In our work with labile N-protection and faster de-N-acylation, we were concerned with the wastage of valuable acid anhydride used in the N-acylation step. The acid anhydrides we were interested in were either expensive or they required a time consuming process to prepare them. Our goal was to introduce the labile protection on the exocyclic amine by a procedure that was both suitable to multigram scales and that uses the valuable acid as economically as possible. In this communication, we would like to report our

success in acylating the exocyclic amine of adenosine and cytidine (deoxyribo-, ribo- or modified nucleosides)with N,N'-carbonyldiimidazole.[10] Our method follows Jones's strategy to some extent, where the nucleoside is treated with excess trimethylsilyl chloride (5 eq.) in pyridine to protect the hydroxyl groups as the O-silyl ethers. The solution of O-silyl-nucleoside is then allowed to react with pre-activated N-acylimidazole (1.1-1.2 eq.) solution in acetonitrile instead of acid anhydride or acid chloride. Finally, the O-silyl ether of N-acylated nucleoside is converted into N-acylated nucleoside with methanol/ethanol and water. The steps involved in our strategy are given in the scheme below.

Scheme: Steps involved in the N-Acylation of exocyclic amine of Adenosine and Cytidine



R = H, OH, OCH_3
B = Adenine, Cytosine

R = H, OTMSi, OCH_3

R = H, OH, OCH_3;
R' = Phenyl, Phenoxy, Isopropylphenoxy or Isopropoxy;
B = Adenosine or Cytosine

Reagents and Conditions: (a) 5 equivalents of TMSiCl, pyridine, 0°C then warmed to RT for 1-2 h; (b) Add a premixed solution of $R'CH_2COOH$ and N,N'-Carbonyldiimidazole in CH_3CN; (c) Stir for 12-36 h at RT; (d) EtOH and H_2O at 0°C.

General method (50 to 250 mmol synthesis scales) for N-acylation consists of the following steps:

a) Transient protection of hydroxyl groups of nucleoside:

The dried nucleoside (deoxy, ribo or modified) is suspended in anhydrous pyridine (3-5 ml/mmol). To this trimethylsilyl chloride (5.0 eq. of nucleoside) is added slowly at 0°C under constant stirring, and the reaction mixture is further stirred for 1-2 h at RT.

b) Conversion of free carboxylic acid into reactive N-acylimidazole derivative:

The vacuum dried or distilled acid (1.1-1.2 eq.) is dissolved in anhydrous acetonitrile (2-4 ml/mmol). To this 1,1'-carbonyldiimidazole (1.1-1.2 eq.) is added as a solid portionwise at a rate slow enough to prevent excessive gas formation. The reaction mixture is stirred for an additional 30-60 min at RT under inert atmosphere for complete conversion of carboxylic acid into N-acylimidazole derivative.

c) N-acylation of exocyclic amine group of nucleoside:

The N-acylimidazole solution generated from step (b) is added to O-silylated nucleoside derived from step (a). The reaction mixture is stirred for another 12-36 h at RT. In the case of adenosine, certain acids require stirring at a slightly elevated temperature for complete conversion to the protected amine.

d) Work-up of the reaction mixture:

After complete N-acylation, the reaction mixture is quenched by stirring with ethanol (5.0 to 7.0 eq.) for 15 min at 0°C followed by addition of cold water (5-10 eq.) and stirring for another 15 min at 0°C. The reaction mixture is concentrated under reduce pressure to remove pyridine and other volatile materials at 30-35°C to give a viscous residue. The desired N-acyl-nucleoside is isolated in good yield (60-85%) and in pure form (≥ 95%) by either of two methods: (I) The viscous residue is triturated with ether/hexane, organic layer is discarded and traces of volatile solvent is removed. The residue is treated with ice-cold water whereby N-acyl-nucleoside separates out as a white solid material or viscous oil which solidifies on cooling. The filtered solid is washed with water to remove any residual unreacted nucleoside. The solid thus obtained can be crystallized from an appropriate solvent. (II) Alternatively, especially in the case of water soluble N-acylated nucleoside, the viscous material is taken up in chloroform (0.5 to 1.0 L) and washed with cold brine (2 x 250-500 mL). The aqueous layer is back extracted once with chloroform (250 mL). The combined organic extracts are dried over anhydrous sodium sulfate and concentrated to a foam. Tritutration with 1:1 ether/hexane gives N-acyl-nucleoside as a solid.

Using the above procedure we have successfully prepared N-acylated derivatives of adenosine and cytidine (deoxy-, ribo-, 2'-O-methyl-2'-deoxy-)nucleosides using phenoxyacetic acid, isopropylphenoxyacetic acid, isopropoxyacetic acid, and phenylacetic acid. This method not only works with acetic acid derivatives but can also be used to acylate the exocyclic amine function with benzoic acid derivatives. Some representative examples are given in the Table below.

Table: N-acylation of adenosine (A) and cytidine (C) nucleosides with activated acids:

Acids	Reaction condition	Work-up method	% of N-acyl-adenosine	% of N-acyl-cytidine
Phenylacetic acid (1.2 eq.)	8 h at RT for C overnight at 40°C for A	method (I)	60% for A 65% for 2'-O-Me-adenosine	85% for C 90% for 2'-O-Me-cytidine
Phenoxyacetic acid (1.2 eq.)	8 h at RT for C overnight at RT for A	method (I) for C and(I) and (II) for A.	55-65% for A 60-70% for 2'-O-Me-adenosine	70-75% for C
Isopropoxy acetic acid (1.2 eq.)	overnight at RT for C 50°C for A (with 20% DMF as a co-solvent)	method (I) for C and only method (II) for A since N-isopropoxy-A is water soluble	65-68% for A (this yield may vary).	80-85% for 2'-O-Me-cytidine
4-Isopropyl-phenoxyacetic acid (1.2 eq.)	overnight at RT for A	method (I)	70% for A 70-75% for 2'-O-Me-adenosine	Not performed

Synthesized N-acyl-nucleoside products were characterized by ^{1}H-NMR and compared to samples that were synthesized according to literature methods using acid anhydrides. With the use of in-situ generated N-acylimidazole derivatives, we do not see either the formation of di-N-acylated-adenosine derivatives or the formation of N-acylated-O-acylated nucleoside. This may be attributed to the fact that a large excess of N-acylimidazole is not used. We also found that under the above reaction conditions, the imidazolide reacts exclusively with the more nucleophilic amino group as demonstrated by the reaction of 6-amino-hexanol. With 1.1 equivalent of N-phenacyl-imidazole in acetonitrile 90% of N-phenacyl-aminohexanol was observed. It is possible that with this method the initial protection of hydroxyl groups may not be needed when there is a strong nucleophilic amine present as in the case of cytidine. We do believe, however, that the formation of the O-silyl with trimethylsilyl chloride does serve to solubilize the nucleoside in pyridine and acetonitrile. Our attempt to use N,N'-carbonyldiimidazole for the protection of guanosine nucleosides was found to be not very practical presumably because of the poor nucleophilicity of the exocyclic amino group. Only 40-50% of N-acylated product was obtained after heating the reaction mixture at 50°C for 7-10 days.

In summary, we have shown that in combination with Jones's transient hydroxyl protection using trimethylsilyl chloride, in-situ generated N-acylimidazoles can be used successfully to protect the exocyclic amino group of adenosine and cytidine nucleosides or their 2'-O-alkyl-modified forms. The utility of this method for N-acylation of other molecules with both amino and hydroxyl functions is currently being examined.

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