New and efficient Synthesis of Nucleoside Polyphosphates and Nucleoside Monophosphate Sugars

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ABSTRACT

A new and efficient method for the synthesis of nucleoside di- and triphosphates, dinucleoside tetraphosphates and nucleoside monophosphate sugars is described. This new route is based on cycloSal-nucleosyl-phosphate triesters as active ester that underlie fast conversion to the corresponding products.

INTRODUCTION

Progress in biochemical research has clearly pointed out the unique importance of phosphorylated nucleosides in biological systems. Nucleoside triphosphates serve, in addition to the well-known role of adenosine triphosphate as the primary energy source in many systems, as direct precursors of both ribonucleic and deoxyribonucleic acids. Modified nucleoside triphosphates have received much attention in the development of potential therapeutic and diagnostic agents, that are needed for studies of numerous biochemical and pharmacological processes. Dinucleoside polyphosphates (Np,N) have been proposed as signaling and regulatory molecules for many different biological functions in most forms of life. Two examples with important therapeutic potential are inhibition of platelet aggregation and regulation of vasoactivity. Equally important is CMP-N-acetylneuraminic acid as activated form of neuraminic acid. Generally sialic acids (derivatives of neuraminic acids) are compounds of glycoproteins and glycolipids. They play an important role in biological recognition. Biological syntheses of sialylated glycoproteins and glycolipids follow the Leloir pathway. The involved sialyl transferases require the activated form of neuraminic acid, CMP-NeuAc, to attach NeuAc to a nascent glycoconjugate. A universal and efficient method for the synthesis of all of these classes of compounds and their analogues would facilitate studies of their possible medical applications.

RESULTS AND DISCUSSION

We have developed a new synthetic approach for the synthesis of nucleoside di- and triphosphates, dinucleoside tetraphosphates and nucleoside monophosphate sugars using cycloSal activated nucleotides that undergo fast and efficient coupling with the corresponding nucleophiles. The cycloSal-technique has been primarily developed as a prodrug concept to deliver biologically active nucleotides into cells. The purely pH-dependent cleavage is initialized by a nucleophilic attack of water or hydroxide on the neutral phosphate triester 1 leading to an intermediate benzyl phosphate diester 2. This diester is spontaneously cleaved to yield the desired nucleotide and the masking unit, a salicyl alcohol (Figure 1).

However, the same type of compounds can also be used as active esters for synthetic application. Here, phosphate, pyrophosphate, nucleoside triphosphates or glycosyl-1-oxides were used as nucleophiles to cleave the cycloSal nucleotides yielding the corresponding products (Figure 1).

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Fig. 1 Concept of cycloSal-triester cleavage

As starting material 5-nitro-cycloSal-3'-O-acetylthymidin 6 was synthesized. Therefore 5-nitro-2-hydroxybenzylalcohol 4 was prepared from the corresponding salicylic aldehyde derivative 3 by reduction with sodium borohydride. The alcohol 4 was then converted into 5-nitrophosphorochloridite 5 using phosphorus trichloride and pyridine. Finally, the conversion of phosphorochloridite 5 into the target triester 6 was achieved by reaction of the 3'-protected nucleoside with subsequent oxidation using oxone (2KHSO₅,KHSO₄,K₂SO₄) in 80 – 90 % yield (Scheme 1). The product is pure enough to be used directly in the following conversions.
However, if oxone is replaced by the originally used t-butylhydroperoxide the yields were found to be significantly lower and sometime the reaction failed completely. Alternatively, also an iodine/pyridine/THF/water-solution as used in oligonucleotide-synthesizers can be used.

![Chemical structure](image)

**Scheme 1** Reagents and Conditions: i) NaBH₄, EtOH, rt, 20h; ii) PCl₅, pyridin, Et₂O, -20°C-rt, 4h; iii) 1. 3°-OAcdT, MeCN, DIPEA, -20°C-rt, 2h. 2. Oxone, H₂O₂, -10°C-rt, 15min.

The commercially available sodium phosphates (Na₂HPO₄, Na₃H₂P₂O₇, Na₃H₂ATP) were converted into their corresponding (nBu₄)N⁺ salts 7-9 by ion exchange chromatography (Dowex 50WX8) to increase their lipophilicity and the solubility in organic solvents. To remove remaining moisture from the hygroscopic solids they were dried by azotroporic removal of acetonitrile and further dried over molecularsieve (4 Å). Best results for the coupling of the cycloSal-nucleotides and the corresponding nucleophiles 7-9 were obtained using DMF as solvent. The cycloSal-triester was completely converted after 2-4 h at room temperature (Scheme 2).

![Chemical structure](image)

**Scheme 2** Reagents and Conditions: i) 1.Dowex (H⁺), 2.NBu₄OH to pH 6.7; ii) 1.DMF, 4Å, rt, 2-5h, 2. NEt₃/MeOH/H₂O (1:7.3), rt, 20h, 3.Dowex (NH₄⁺).

D-Glucose, D-mannose and D-galactose were applied as their 2,3,4,6-O-tetraacetates. Deprotonation with sodium hydride gave the nucleophilic pyranosyl-1-oxides 13-15. Reaction with triester 6 was carried out in situ in DCM within 2 h (Scheme 3).

Before purification the protecting groups were cleaved. The acetyl groups were cleaved by treating the crude product with a mixture of NEt₃/MeOH/H₂O 1:7.3. Due to the difficult chromatographic properties of tetra-n-butyrammonium salts, they were replaced by their ammonium counterparts. Ion exchange was efficiently achieved with Dowex cation-exchange resin. Nucleoside polyphosphates 10-12 and NMP-sugars 16-18 were finally purified by chromatography on RP-18 silica gel. Yields after purification were found to be between 55 % and 85 %.

![Chemical structure](image)

**Scheme 3** Reagents and Conditions: i) NaH (60%), DCM, rt, 15min; ii) 1.DCM, rt, 2h, 2. NEt₃/MeOH/H₂O (1:7:3), rt, 20h.

The presented method has been successfully applied to the synthesis of NDP and NTP analogues of thymidine and uridine. We prepared carba-DTTP as well as the di-, and triphosphate of BVdU using the corresponding cycloSal-nucleotides.

**CONCLUSION**

In summary, this report confirms that the cycloSal-technique can not only be used as nucleotide delivery system (pronucleotides) but is also applicable as a strategy for the synthesis of nucleoside polyphosphates and biomolecule conjugates linked by a phosphate group.

**REFERENCES**


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