A facile solid phase synthesis of 2′- and 3′-aminonucleoside triphosphates

Thomas Schoetzau, Torsten Holletz and Dieter Cech*

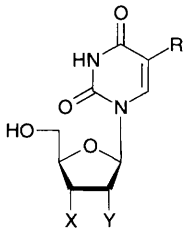
Institut für Chemie, Humboldt-Universität zu Berlin, Hessische Str. 1-2, 10099 Berlin, Germany

A convenient and time-saving solid phase synthesis of different 2′- and 3′-amino-functionalized nucleoside-5′-triphosphates using polymer bounded triphenylphosphine is described.

Chemically or enzymatically synthesized nucleoside 5′-triphosphates have found numerous applications for the structural investigations of nucleic acids, as substrates for DNA and RNA sequencing and for labelling of hybridisation probes. Both natural and modified triphosphates have been utilised in this regard, and the synthesis of modified derivatives and their application in different fields of molecular biology has received notable attention.[1] Recently, there has been a growing interest in 2′- or 3′-amino-functionalised nucleoside 5′-triphosphates,[2] because of their use as terminators for DNA and RNA sequencing[3] and their applicability for studying the mechanisms of ribozymes.[4] In addition, amino modifications have been described that prolong the lifetime of oligonucleotides by protecting them against degradation by nucleases, which is beneficial when using modified RNA or DNA sequences in the antisense strategy or for *in vitro* selections.[5,6]

We report here a rapid preparation of amino nucleoside 5′-triphosphates based on a solid-support approach. Ludwig *et al.*[7] have improved the procedure for the synthesis of nucleoside 5′-triphosphates[8,9] using 2-chloro-4*H*-1,2,3,-benzodioxaphosphorin-4-one (salicyl phosphorochloridite–pyrophosphate. The new method is a rapid one-pot reaction that does not require protection of the nucleotide bases. A further improvement has been described by Sproat[10] who performed the Ludwig–Eckstein procedure on a solid phase.

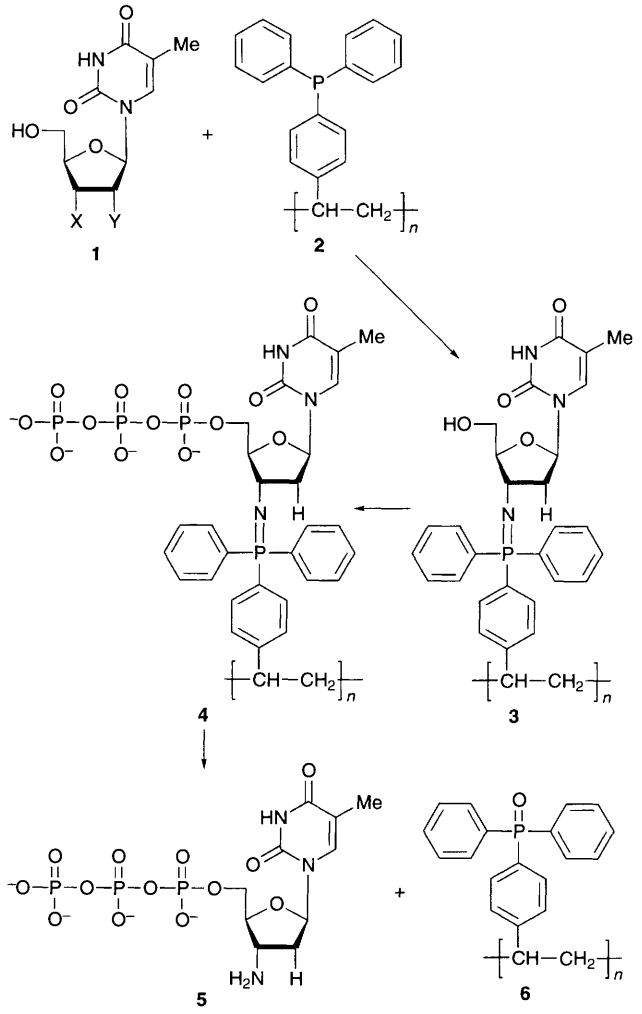
Recently we have shown that polymer-bounded triphenylphosphine is suitable for reduction of 2′- or 3′-azidonucleosides to the corresponding aminonucleosides.[11] Since the azidonucleosides are fixed to the polymer support *via* a stable phosphinimine linkage, chemical manipulations can be performed while the nucleoside is attached to the polymer. We have used this technique to transform different 2′- and 3′-azidonucleoside derivatives **1** into the corresponding 2′- and 3′-aminonucleoside 5′-triphosphates **5**. Thus, the 5′-triphosphates of 3′-amino-2′,3′-dideoxythymidine **5a**, 3′-amino-2′,3′-dideoxyuridine **5b** 2′-amino-2′,3′-dideoxyuridine **5c** and 2′-amino-2′-deoxyuridine **5d–e**, respectively, can be isolated from the corresponding azidonucleosides without any intermediate isolation, protection or purification of the aminonucleosides in a one step procedure.



	R	X	Y
a	Me	N_3	H
b	H	N_3	H
c	H	H	N_3
d	H	OH	N_3
e	H	OAc	N_3

A general synthesis for 3′-amino-2′,3′-deoxythymidine 5′-triphosphate **5a** is illustrated in Scheme 1. In the first step the support is loaded with 3′-azido-2′,3′-deoxythymidine **1a**. A commercially available triphenylphosphine-containing polymer (Fluka) with a given loading of 3 mmol PPh_3 per 1 g support is used. It is recommended that supports are used which are not loaded with more than 0.7 mmol nucleoside per 1 g support because any greater loading leads to lower yields of triphosphates and considerable amounts of side products. The low yields are probably caused by the formation of 5′,5′-dinucleotides. The 5′,5′-dinucleotides were presumably formed by reaction of activated and unreacted nucleosides (**3**) due to high loading.[7]

Typically, the optimal loading is adjusted so that 0.2 mmol nucleoside **1a** is treated with 1 mmol (330 mg) support **2** in dry



Scheme 1

dioxan for 2 h at room temperature. By measuring the UV maximum of the azido nucleoside at 260 nm, an approximate quantitation of the extent of loading is possible. The loading is determined by observing the loss of the UV activity of the solution.

Next, the polymer bound nucleoside-3′-phosphinimine **3a** is subjected to phosphorylation with salicyl phosphorochloridite in a simple batch procedure. A known amount of support derivatised by the nucleoside, typically 100 μmol bound to the triphenylphosphine resin, is placed in a small two-necked flask fitted with a septum. All chemical manipulations are carried out under argon. The support is allowed to swell by shaking in 4 cm^3 dioxan–pyridine (3 : 1, *v*/*v*) for 10 min. After swelling, 1.1 cm^3 of freshly prepared 0.5 mol dm^{-3} solution of salicyl phosphorochloridite in dry dioxan is added and the vessel agitated for 25 min. The solvent is removed and the support washed extensively with dioxan–pyridine. The linkage of the nucleoside to the polymer is stable under these conditions, and is also not cleaved by treatment with bis(tributylammonium) pyrophosphate in DMF in the presence of tributylamine. For that bis(tributylammonium) pyrophosphate solution in dry DMF (3 cm^3 1 mol dm^{-3}) and tributylamine (1 cm^3) are added and the solution is shaken for 30 min. Excess pyrophosphate is removed by washing with DMF followed by dioxan–pyridine. The oxidation of the cyclic intermediate to **4a** is performed with a 2% solution of iodine in pyridine–water (98/2, *v*/*v*). Cleavage of the final amino product **5a** from the support is performed by treatment with conc. ammonia at 50 °C for 2 h.

The resulting mixture is lyophilised, and the final product purified by chromatography on a DEAE-Sephadex column at 4 °C using a linear gradient of 0.05 to 1 mol dm^{-3} triethylammonium bicarbonate buffer (pH 7.5) over 8.5 h. Further isolation is straightforward[10] and is therefore not dealt with here. In contrast to the Ludwig method, the ^{31}P NMR spectroscopy of the final product does not show side-products such as pyrophosphate or inorganic cyclic triphosphate, which are difficult to remove by conventional chromatography.

To test the generality of this method, we converted the azidonucleosides **1b–e** into the corresponding aminonucleoside triphosphates **5b–e**. The procedure for loading of **1a** so far described is readily applicable to **1b–e**, and yields of loading are similar to those obtained with **1a**. The transformation into the amino derivatives is performed as described above with satisfactory yields.†

It has been reported by Ludwig[7] that phosphitylation does not require protection of the base functional groups. In addition, the method described here does not require any protection of the 2′- or 3′-OH functions. In an attempt to determine whether the yield might be higher using protected azidonucleosides, we carried out the reaction with 3′-acetyl-2′-azido-2′-deoxyuridine **1e**. However, the yield of **5e** is similar to those of unprotected derivatives. We assume that the steric hindrance caused by the polymer-bound triphenylphosphine prevents the initial phosphitylation at the 2′- or 3′-positions.

Since the described strategy is compatible to standard solid phase synthesis of oligonucleotides on automated comercially available DNA synthesizers, we performed the procedure on a Gene-Assembler (Pharmacia). The standard cycle of the synthesiser was modified to the conditions of triphosphate synthesis according to Scheme 1. Syntheses of triphosphates were carried out on either 0.2 or 1.3 μmole scale. The reaction was complete within 80 min and the triphosphates were obtained in yields of about 70%.

Product purity was checked by ^{31}P NMR spectroscopy, and in D_2O showed distinct singlets for α-P, β-P and γ-P, respectively.‡ These figures are similar to those given for natural triphosphates in the same solvent.[7,10] The absence of any other resonance signals in the spectra indicates the above stated purity.

The 2′-aminonucleoside triphosphate **5d** can be incorporated into RNA transcripts using a DNA template and T7 RNA polymerase.[5]

The synthetic method described here affords triphosphates in overall yields of 70–75% based on azidonucleosides as starting materials, and represents an improvement both in time-efficiency and simplicity over the synthesis previously described for these compounds. The synthesis of the corresponding purine derivatives is in progress.

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Footnotes

† Yields were calculated by integration of HPLC peak areas; **5a** 70%, **5b** 75%, **5c** 74%, **5d** 70% and **5e** 71%, respectively.

‡ ^{31}P NMR shifts of the triethylammonium salts in ppm (recorded in D_2O, 85% H_3PO_4 as external standard): **5a**: −10.82 (α-P), −22.12 (β-P), −09.20 (γ-P). For **5b**: −10.48 (α-P), −21.53 (β-P), −07.86 (γ-P). For **5c**: −10.75 (α-P), −22.32 (β-P), −08.86 (γ-P). For **5d**: −10.92 (α-P), −22.52 (β-P), −09.00 (γ-P).

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