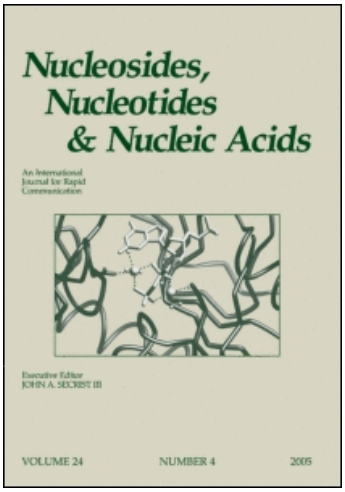

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A NEW APPROACH TO OLIGONUCLEOTIDE N3′→P5′ PHOSPHORAMIDATE BUILDING BLOCKS

Daria Zielinska, Krisztina Pongracz, and Sergei Gryaznov ▫ *Geron Corporation, Menlo Park, California, USA*

▫ *A new synthetic approach to 5′-phosphoramidites of 3′-aminonucleosides was developed. The methodology relies upon the use of 3′-amino-2′,3′-dideoxy nucleosides as the key starting materials. The final phosphoramidite products were obtained with high yields via 2–3-step efficient chemical transformations using selective introduction of orthogonal protective groups to the 3′-aminonucleoside sugar and base moieties.*

Keywords 3′-Aminonucleosides, Oligonucleotide Phosphoramidates

RESULTS AND DISCUSSION

Oligonucleotide N3′→P5′ *thio*-phosphoramidates (NPS) are currently under development as telomerase-addressed potential anti-cancer agents.[1] At the same time oligonucleotide N3′→P5′ phosphoramidates (NP) have been successfully used as FISH probes.[2] These oligonucleotide analogues are currently prepared using an amidite transfer method of choice that utilizes key 3′-aminonucleoside-5′-phosphoramidite building blocks (**I**) (Figure 1). Hence, the availability and accessibility of these monomer compounds may play an important role in successful development of the oligonucleotide phosphoramidates as therapeutic and diagnostic agents.

The earlier described synthesis of these monomers, unfortunately, is a highly complex, labor-intensive, low-yielding multi-step process, especially for purine nucleosides (10 steps for purines and 6–7 steps for pyrimidines).[3] The total yield of the phosphoramidite products (on multi-gram to ~kg scale) reaches only ~5% for purines and ~15–20% for pyrimidines. The natural 2′-deoxynucleosides with 3′-hydroxyl group are used as starting materials for these procedures.

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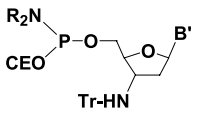


1a,g,c,t

B' = *Thy, Gua*^{ibu}, *Ade*^{bz}, Cyt^{bz}; R = iPr; CE = 2-cyanoethyl

FIGURE 1 General structure of 3′-aminonucleoside-5′-phosphoramidites.

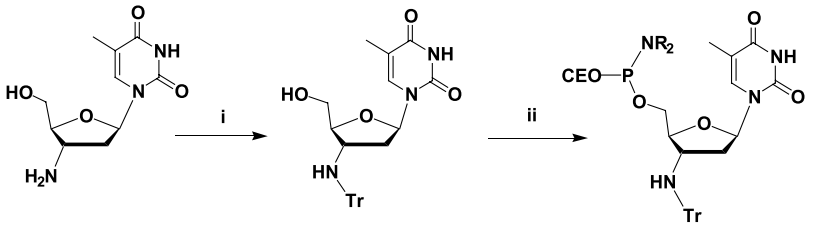
Here we present a new synthetic approach to the purine (**Ade**^{bz} and **Gua**^{ibu}) and thymidine (**Thy**) phosphoramidites (**1a**,**g**,**t**, respectively). The developed methodology relies upon the application of 3′-amino-2′,3′-dideoxynucleosides as the key starting materials. The final phosphoramidite products were obtained, with high yields, via 2–4-step efficient chemical transformations using a selective introduction of orthogonal protective groups to the 3′-aminonucleoside sugar and base moieties.

The 3′-amino purine nucleosides, 3′-amino-2′, 3′-dideoxyadenosine, and 3′-amino-2′, 3′-dideoxyguanosine, were acquired from Metkinen Oy Company (Finland). These compounds were prepared by an enzymatic trans-glycosylation process starting from 3′-amino-3′-deoxythymidine, which was obtained via reduction of readily available 3′-azido-3′-deoxythymidine (AZT). The performed chemical reactions are outlined in Schemes 1–3.

Thus, preparation of the thymidine monomer **1t** involves only two chemical steps: 3′-NH-selective (3′-amino- vs. 5′-hydroxyl group) tritylation in pyridine of the nucleoside 3′-amino group (yield ~95%). Importantly, in general, the regioselective 3′-NH-tritylation is a key step enabling this new approach to phosphoramidites **1a**,**g**,**t**. The tritylation reaction was subsequently followed by a standard 5′-O-phosphitylation of the 5′-OH-3′-NH-Tr thymidine precursor, resulting in **1t**. The total yield of the phosphoramidite product **1t** was ~70% based on the starting 3′-amino-3′-deoxythymidine (Scheme 1).

The adenosine and guanosine-based monomers **1a**,**g** were prepared in a similar manner (Schemes 2 and 3). However, presence of the second reactive heterocyclic amino group at purine bases (N^6 or N^2), as well as the low solubility of these nucleosides in anhydrous organic solvents (particularly 3′-aminoguanosine), required the use of different tritylation conditions.

Hence, adenosine phosphoramidite **1a** was prepared as follows: 3′-amino-adenosine was 3′-NH-tritylated in a mixture of DMF and pyridine in the presence of triethylamine. The 3′-NH-tritylated intermediate was isolated via aqueous wash and extraction procedure with >90% yield, and it was taken to the next N^6-benzoylation step without any further purification (Scheme 2). Formation of only small amounts (<5%) of the bis-5′-O-, 3′-NH-trityl byproduct was detected. The subsequent N^6 benzoylation (using either a per-benzoylation process or TMS-Cl based transient protection protocols) resulted in 5′-OH-3′-NH-Tr-adenosine precursor (Scheme 2). This compound was purified by silica gel column chromatography (isolated yield

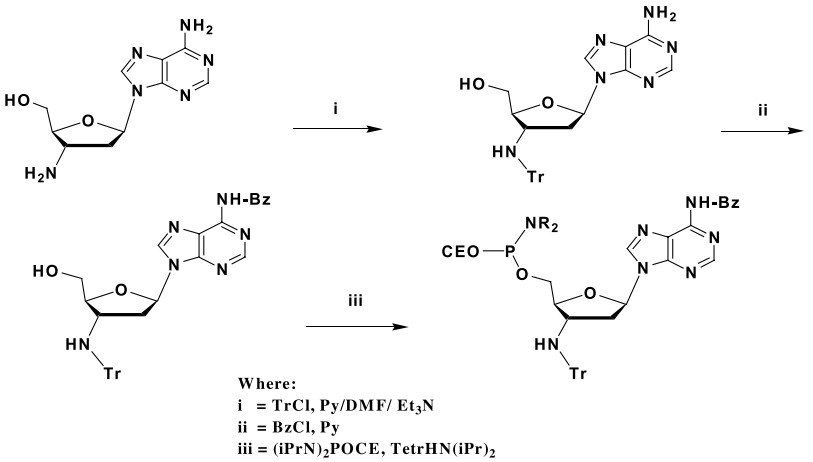


SCHEME 1 Synthesis of thymidine phosphoramidite **1t**.

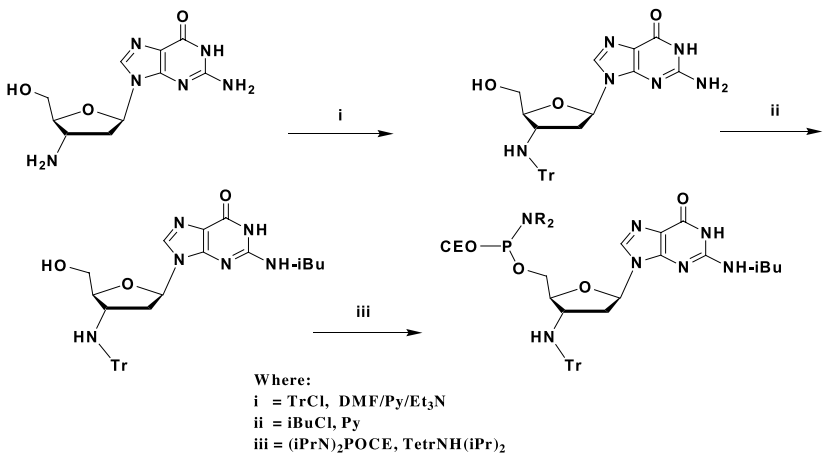
of ~70%), and then 5′-O-phosphitylated, resulting in the desirable phosphoramidite **1a** with total yield of ~60% based on the starting amino nucleoside.

Alternatively, using a one-pot synthetic approach, 3′-amino-2,′3′-deoxyadenosine was 3′-NH-tritylated in a solution of pyridine in the presence of triethylamine, and the reaction mixture was N^6-benzoylated further (without isolation of the 5′-tritylated product) using TMS-Cl-based transient protection methodology. The total yield of the 5′-OH-3′-NH-Tr-adenosine intermediate was ~70% after silica gel column chromatography. This synthetic route resulted in generation of a by-product (<10% by weight), which was readily separated from the desirable compound during silica gel chromatography. The exact chemical structure of the byproduct is yet unknown (ESI MH + 1382.8, 691.38, possible M.W. 1381 D, main fragment 690 D) and ^{1}H NMR spectrum of this compound is markedly different from the main product. However, it appears that its formation is caused by the presence of triethylamine during the N6-benzoylation step.

Synthesis of guanosine phosphoramidite **1g** was also performed in three steps as follows, (Scheme 3). First, 3′-amino-2′,3′-dideoxyguanosine was selectively



SCHEME 2 Synthesis of adenosine phosphoramidite **1a**.



SCHEME 3 Synthesis of guanosine phosphoramidite **1g**.

3′-NH-tritylated in the mixture of DMF and pyridine at 50°C. Formation of relatively small amounts of a single impurity *bis*-3′-NH,-N^2-trityl-guanosine (<5%) was detected. The desirable product, 5′-hydroxy-3′-NH-trityl guanosine, was isolated by precipitation from dichloromethane with yield of ~95%. Further, this compound was reacted with *iso*-butyryl chloride (iBu-Cl) to form the phosphoramidite precursor N^2-iBu-protected 3′-NH-Tr-guanosine. Either N,O per-acylation with iBu-Cl, or transient protection with TMS-Cl followed by iBu-Cl acylation was used for the N^2-iso-butyrylation step, resulting in product yields of 60 and 53%, respectively, after crystallization from acetonitrile. Finally, the phosphoramidite **1g** was formed using common phosphitylation procedure with bis-diisopropyl phosphoramidite, resulting in the final product **1g** (yield 80%).

Interestingly, if isobutyric anhydride (rather than iBu-Cl) was used for N^2-amino group protection, then formation of primarily 5′-O-iBu-3′-NH-Tr guanosine with unacylated N^2 group was observed. This compound was isolated with ~90% yield via crystallization from dichloromethane/acetonitrile. Moreover, the quality of iBu-Cl plays an important role in achieving good yield of the product. Apparently, the presence of products of hydrolysis of iBu-Cl in the reaction mixture led to formation of significant amounts (up to ~50%) of a major byproduct: 3′-NH-iBu-N^2-Tr guanosine (the molecular weight is the same as for the desirable product) during the N^2 protection reaction.

In conclusion, we developed an efficient and relatively simple method for preparation of 3′-aminonucleoside-5′-phosphoramidites, key building blocks used for assembly of oligonucleotide N3′→P5′ phosphoramidates and thio-phosphoramidates.

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