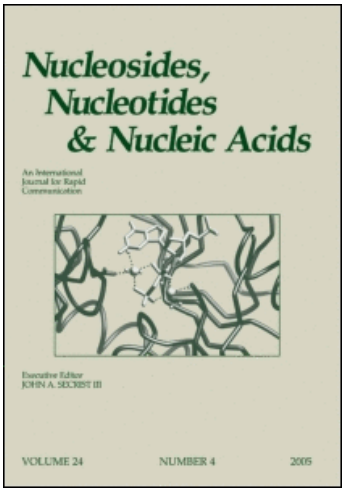

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A Novel Amino-ON CPG-Support for the Synthesis of 3'-Aminoalkylated Oligonucleotides

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A NOVEL AMINO-ON CPG-SUPPORT FOR THE SYNTHESIS OF 3′-AMINOALKYLATED OLIGONUCLEOTIDES

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▫ *The synthesis of a novel amino-ON CPG support and its application in the synthesis of 3′-aminoalkylated oligonucleotides is reported. The release of oligonucleotides with free 3′-amino groups is accomplished by treatment with concentrated ammonia for 2 h at 55°C.*

Keywords Amino-ON CPG, 3′-Amino-Modifier, 3′-Aminoalkylated Oligonucleotide

INTRODUCTION

The increased demand of chemically modified oligonucleotides in molecular biology and DNA-based diagnostics applications led to the development of novel processes for their synthesis. 3′-Aminoalkylated oligonucleotides are particularly useful for the preparation of 3′,5′-doubly labeled detection probes, such as the Taqman probes or molecular beacons employed in common RT-PCR assays. 3′-Amino modified oligonucleotides are conveniently prepared using amino-ON CPG supports containing an immobilized amino protective group. The amino-oligonucleotide is released during the ammonia deprotection process, which usually requires long deprotection times. We report here the development of a novel amino-ON CPG support (Figure 1), which enables the release of 3′-aminoalkylated oligonucleotides within 2 h at 55°C in concentrated aqueous ammonia.

The amino-ON CPG support **1** was prepared in four steps from the DMT-protected aminolinker and 6-nitrophthalide as described previously.[1] The loading of **1** was determined by a photometric DMT-assay to be 36 μmol/g. The amino-ON support **1** was employed in solid-phase oligonucleotide synthesis applying the

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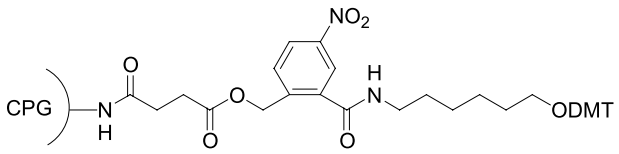
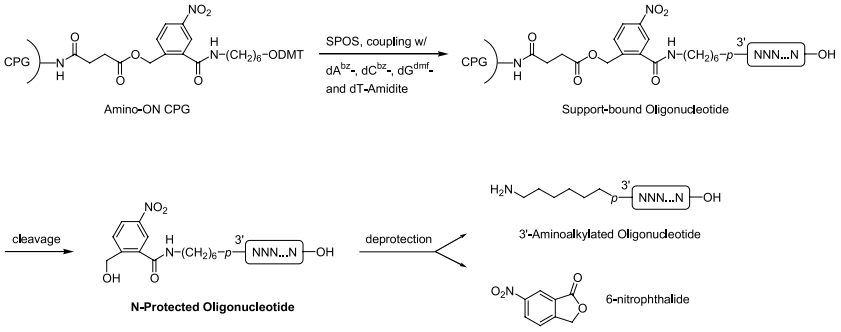


FIGURE 1 Structure of amino-ON CPG **1**.

phosphoramidite approach (Scheme 1). Cleavage of the oligonucleotide from the support was accomplished by treatment with concentrated ammonia for 40 min or AMA for 5 min at ambient temperature, respectively. Cleavage and complete deprotection of the amino group was achieved by treatment with concentrated ammonia for 2 h at 55°C or 24 h at ambient temperature, respectively. Alternatively, application of AMA reagent for 30 min at 65°C led to complete deprotection.

The deprotection reaction conditions are compatible with the fast deprotection schemes employing a set of dA^{bz}-, dC^{bz}-, dG^{dmf}- and dT-phosphoramidites. For validation, the model sequence 3′-aminohexyl-AAC TCC GAG CGA CTC TC **2** was synthesized on amino-ON support **1**. Complete deprotection was achieved after treatment with concentrated ammonia for 2 h at 55°C. Anion-exchange HPLC revealed that the 3′-aminoalkylated sequence **2** was obtained in 80% purity. The structure of **2** was confirmed by MALDI-TOF MS analysis: 5294.5 (calcd); 5294.2 (found) (Figure 2).

The performance of the novel support was demonstrated by a competition experiment. A synthesizer was programmed to prepare a dT_{15}-sequence on a 1:1 mixture of commercially available phthalimidyl-CPG (to give H_2N-dT_{14}) and standard CPG-dT (to give dT_{15}) employing the standard synthesis protocol. After deprotection with concentrated ammonia (15 h, 55°C), the products were analyzed by anion-exchange HPLC (Figure 3A). The experiment was repeated exactly using a 1:1 mixture of CPG support **1** and standard CPG-dT (Figure 3B). The approximately 1:1 ratio of oligonucleotide products obtained in the example where support **1** was employed demonstrates that the efficiency of oligonucleotide synthesis with **1** is comparable to syntheses with standard CPG supports.



SCHEME 1

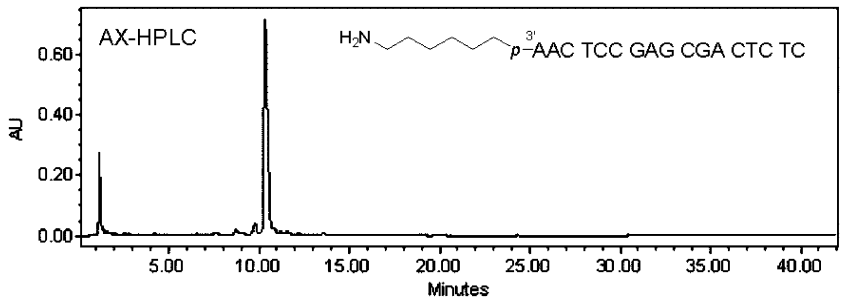


FIGURE 2 Anion-exchange HPLC-chromatogram of model sequence **2** prepared on amino-ON CPG support **1** employing a fast deprotection protocol.

In a second experiment, the deprotection efficiency was investigated. Therefore, a 1:1 mixture of dT_8 bound to phthalimidyl-CPG and dT_{10} bound to CPG support **1**, respectively, was treated with concentrated ammonia for 24 h at ambient temperature (Figure 4A) and 2 h at 55°C (Figure 4B), respectively. Anion-exchange analysis of the products revealed that the release of oligonucleotides bound to phthalimidyl-CPG was far from being complete under the deprotection reaction conditions applied in the experiment.

The application of a post-synthetic labeling reaction of 3′-aminoalkyated oligonucleotides prepared on amino-ON support **1** with dye NHS-esters was exemplified by the synthesis of a dT_{10} model sequence on 0.2 μmol scale. After cleavage and deprotection with concentrated ammonia the crude oligonucleotide was dissolved in 500 μL of $NaHCO_3/Na_2CO_3$ buffer, pH 9, and treated with a mixture of TAMRA NHS-ester (6 equivalents) in DMSO at 37°C overnight. Subsequently, the product was separated by size exclusion chromatography using NAP-columns and analyzed by anion-exchange HPLC. The purity of the obtained oligonucleotide was 80.6%. The product was further characterized by MALDI-TOF MS: 3571.6 (calcd.); 3575.2 (found) (Figure 5).

In conclusion, the novel amino-ON CPG support **1** is compatible with standard solid-phase synthesis conditions employing the phosphoramidite method. The observed coupling efficiencies and yields are comparable to those obtained with

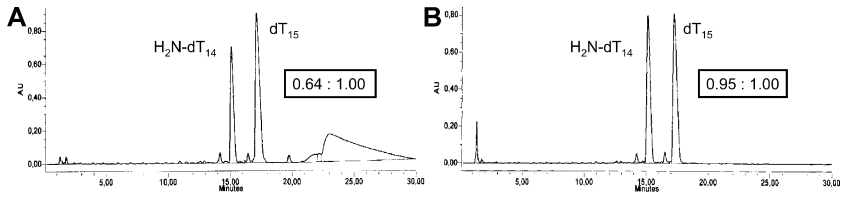


FIGURE 3 Anion-exchange HPLC-chromatograms of oligonucleotide products obtained from a competition experiment. A: dT_{15}-Synthesis on a 1:1 mixture of phthalimidyl-CPG and standard CPG-dT. B: dT_{15}-Synthesis on a 1:1 mixture of amino-ON CPG **1** and standard CPG-dT.

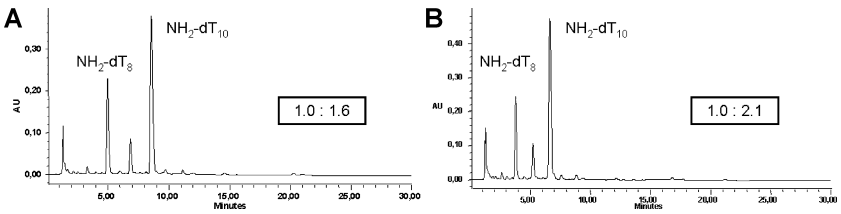


FIGURE 4 Anion-exchange HPLC-chromatograms of oligonucleotide products obtained from the concentrated ammonia treatment of a 1:1 mixture of dT_8 bound to phthalimidyl-CPG and dT_{10} bound to CPG support **5**, respectively. A: 24 h at ambient temperature. B: 2 h at 55°C.

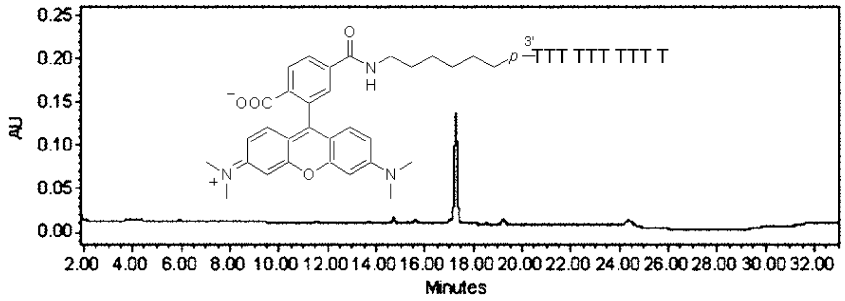


FIGURE 5 Anion-exchange HPLC-chromatogram of the TAMRA-labeled dT_{10}-sequence obtained after oligonucleotide synthesis on support **1** and post-synthetic TAMRA-labeling.

standard nucleoside-loaded CPG supports. The application of mild cleavage/deprotection conditions allows the use of CPG **1** in high throughput synthesis protocols and the on-support preparation of labile 5′-modified oligonucleotides with 3′-aminoalkyl chains.

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