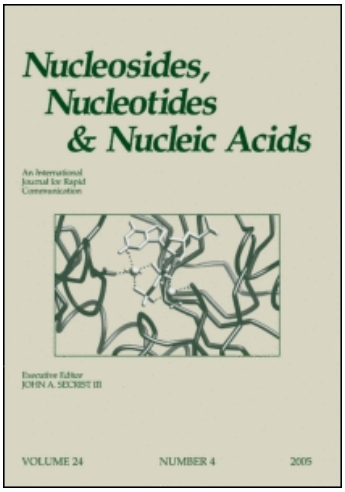

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NOVEL PROPARGYLAMINE-LINKED NUCLEOSIDES FOR HIGH THROUGHPUT SNP GENOTYPING BY MALDI-TOF MS

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ABSTRACT

The synthesis of positively charged and mass tagged nucleosides containing a quaternary ammonium functionality within the penultimate position of a primer is described. Neutralization of the sugar/thiophosphate backbone by alkylation increases the detection sensitivity in the mass spectrometric analysis by a factor of at least 100. The variable introduction of these novel compounds within the extension primers enables flexible design of multiplex genotyping reactions.

INTRODUCTION

Matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI- TOF MS) is one of the highly sophisticated methods for high throughput screening of single nucleotide polymorphisms (SNPs) because of its accuracy, cost-effectiveness, and facile capability for automation. Recently, Sauer et al. have established a novel procedure for genotyping SNPs; they have named this procedure the GOOD assay (1). The main advantage of the GOOD assay over other MALDI MS based genotyping strategies is that the assay consists only of pipetting and

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Table 1. Charge Tagged and Methylated Trimers, Extension Products, Molecular Masses, and Respective Genotypes for SNP Determination. In Each Case, the Charge Tagged Nucleosides Are Written in Bold Letters.

Sequence ID	Sequence	Molecular Mass [Da]	SNP type
SNP 1	5′-d(Gpt**C**^{ct}ptA)-3′	1140.6	
	5′-d(Gpt**C**^{ct}ptAptdA)-3′	1468.8	A
	5′-d(Gpt**C**^{ct}ptAptdG)-3′	1484.8	G
SNP 2	5′-d(Apt**A**^{ct}ptC)-3′	1124.6	
	5′-d(Apt**A**^{ct}ptCptdA)-3′	1428.7	A
	5′-d(Apt**A**^{ct}ptCptdG)-3′	1443.3	G
SNP 3	5′-d(Gpt**U**^{ct}ptG)-3′	1157.6	
	5′-d(Gpt**U**^{ct}ptGptdC)-3′	1461.7	C
	5′-d(Gpt**U**^{ct}ptGptdG)-3′	1501.8	G
SNP 4	5′-d(Gpt**G**^{ct}ptC)-3′	1156.6	
	5′-d(Gpt**G**^{ct}ptCptdA)-3′	1484.8	A
	5′-d(Gpt**G**^{ct}ptCptdG)-3′	1500.8	G

pt = methylated phosphothioate bridge; ^{ct} = charge tag.

incubation steps. Purification steps of the products prior to mass spectrometric analysis are not required. Therefore, the GOOD assay is a highly reliable SNP genotyping technique that fulfils all criteria for high throughput analysis.

As the assay did not allow full flexibility due to the limited availability of mass and charge tagged nucleosides within the primers, we provide a set of propargylamine-linked nucleosides that can be easily converted into positively charged amides of different masses on the oligomeric level allowing unambiguous differentiation of different primers and extension products.

RESULTS AND DISCUSSION

The β-cyanoethyl phosphoramidites containing phthaloyl-protected propargylamine side chains (2–5) were incorporated at the penultimate position of the 3′-end of the primer using phosphorothioate chemistry. To obtain coupling yields of the modified building blocks greater than 96%, we have increased the coupling step by a factor of 10. After removal of the amino protecting groups (25% aqueous NH_3, 8h, 60°C) and OPC purification the propargylamine linked nucleosides were treated with several trimethylammonium alkyryl-N-hydroxysuccinimidyl esters (n = 2–5, depending on the structure of the primer) which were synthesized according to the method of Bartlet-Jones (6). The reaction exclusively takes place on aliphatic amino groups whereas those of the nucleobases were completely inert under the chosen reaction conditions. Following primer extension and PDE digestion, the negatively charged sugar/thiophosphate backbone of the products was alkylated using methyl iodide in a mixture of acetonitrile/2M aqueous triethyl ammonium hydrogen carbonate buffer (pH 7.7–8.3).

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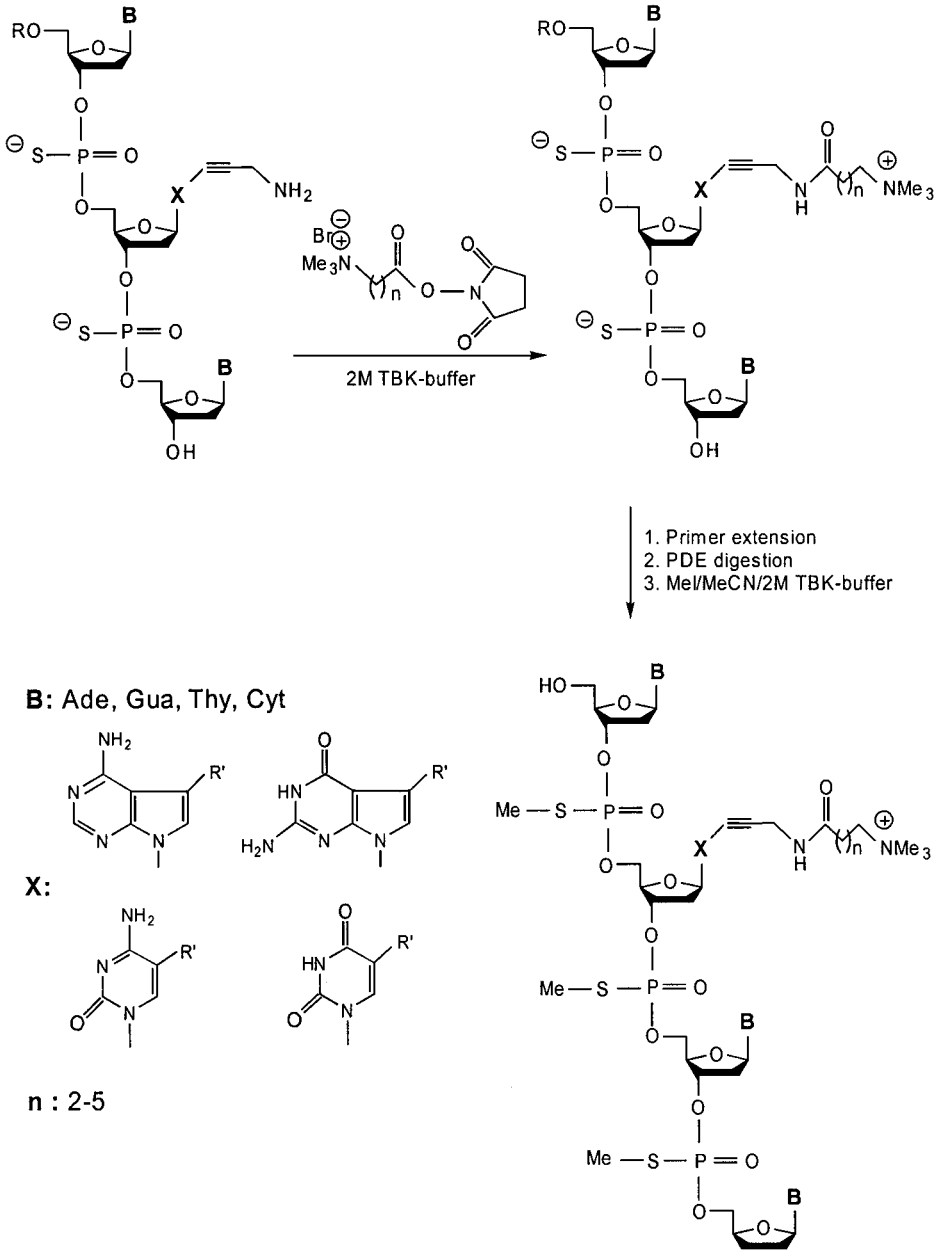

Scheme 1. Two-step synthesis of charge tagged and backbone alkylated DNA. In the first step, the quaternary ammonium charge tag is attached to the oligonucleotide via a primary aliphatic amino group. Subsequently, without purification of the intermediate, the backbone is alkylated.

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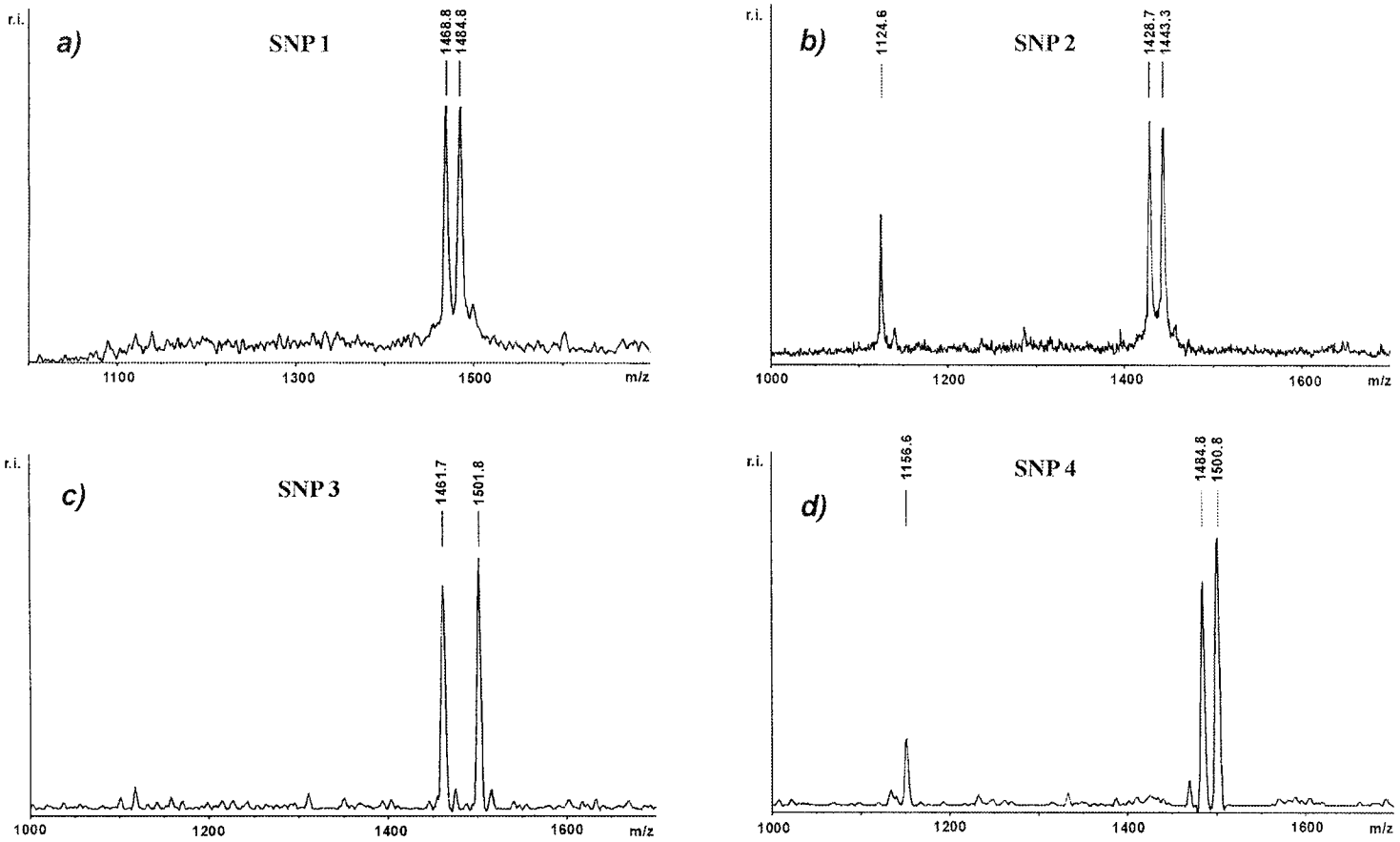

Figure 1. MALDI-MS spectra of genotyping four human SNPs. Spectra for the heterozygous genotypes are shown. Charge tagged dC (a), dA (b), dU (c) and dG (d) derivatives for introduction of the positive charge have been used; details see Table 1.

As a demonstration of the successful incorporation of the propargylamine linked phos-phoramidites, we present their application in the GOOD assay for several SNPs that occur in the human genome.

The combination of charge tagging, sugar/thiophosphate backbone neutralization and the use of a non-protonating matrix significantly improves the detectability of small oligonucleotides by MALDI-TOF MS. The alkali-ion adduct formation of DNA, a severe problem for MALDI MS analysis, could be excluded. Furthermore, the variable introduction of trimethylammonium alkyryl (3-aminopropynyl) side chains within the extension primers enables flexible design of multiplex genotyping reactions resulting in a well spaced pattern of different allelic variants.

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