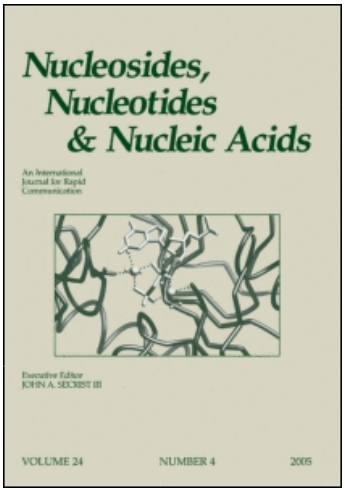

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THE MOX/SUC PRECURSOR STRATEGIES: ROBUST WAYS TO CONSTRUCT FUNCTIONALIZED OLIGONUCLEOTIDES

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THE MOX/SUC PRECURSOR STRATEGIES: ROBUST WAYS TO CONSTRUCT FUNCTIONALIZED OLIGONUCLEOTIDES

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ABSTRACT

The use of phosphoramidites bearing one or more methoxyoxalamido (MOX) or succinimido (SUC) reactive groups for construction of functionalized oligonucleotides is described. The efficiency of the new precursor strategy was demonstrated in the synthesis of oligonucleotide containing up to 16 imidazole residues.

Oligonucleotides bearing different reporting groups and other functional entities have become a commonplace tool in many diagnostic and therapeutic applications. Generally, synthesis of functionalized oligonucleotides is implemented by direct introduction of a protected functionality via phosphoramidite solid phase chemistry. This approach necessitates the preparation of the corresponding monomer for every new functionality to be incorporated. A more productive way would be to first form a precursor oligonucleotide by introduction of a heterobifunctional reagent (first modifier) bearing, along with a phosphoramidite moiety, an orthogonal reactive group. The reactive group of the precursor is then post-synthetically derivatized with an appropriate functional additive (second modifier).

We are using our proprietary methoxyoxalamido (MOX) and succinimido (SUC) precursor strategies to construct functionalized oligonucleotides (1–4). These strategies are based on the high reactivity of MOX and SUC groups towards primary aliphatic amines. Phosphoramidites bearing MOX/SUC moieties are introduced during automated solid-phase synthesis and the precursor oligonucleotides are then post-synthetically treated with desirable amines (second modifiers). Schematically this concept is outlined in Fig. 1. We synthesized a number of

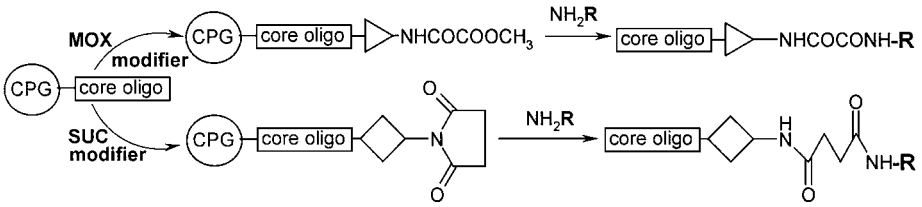

Figure 1. Schematic representation of the MOX/SUC precursor strategies.

nucleosidic and non-nucleosidic phosphoramidites bearing one or more precursor groups. All monomers could be divided into two major groups: terminus and extending modifiers (Fig. 2 and 3). Coupling efficiencies are different among prepared monomers. Phosphoramidites **1**–**4** couple with efficiencies similar to that of conventional phosphoramidites. Larger quadruple MOX modifiers **5** and 2′-substituted monomers **6**–**10** necessitate prolonged, 10–15 min., coupling time and the use of 5-ethylthio-1H-tetrazole (ETT) to secure an efficient (>97%) incorporation. The same coupling conditions were applied for introduction of branching unit **11**. All

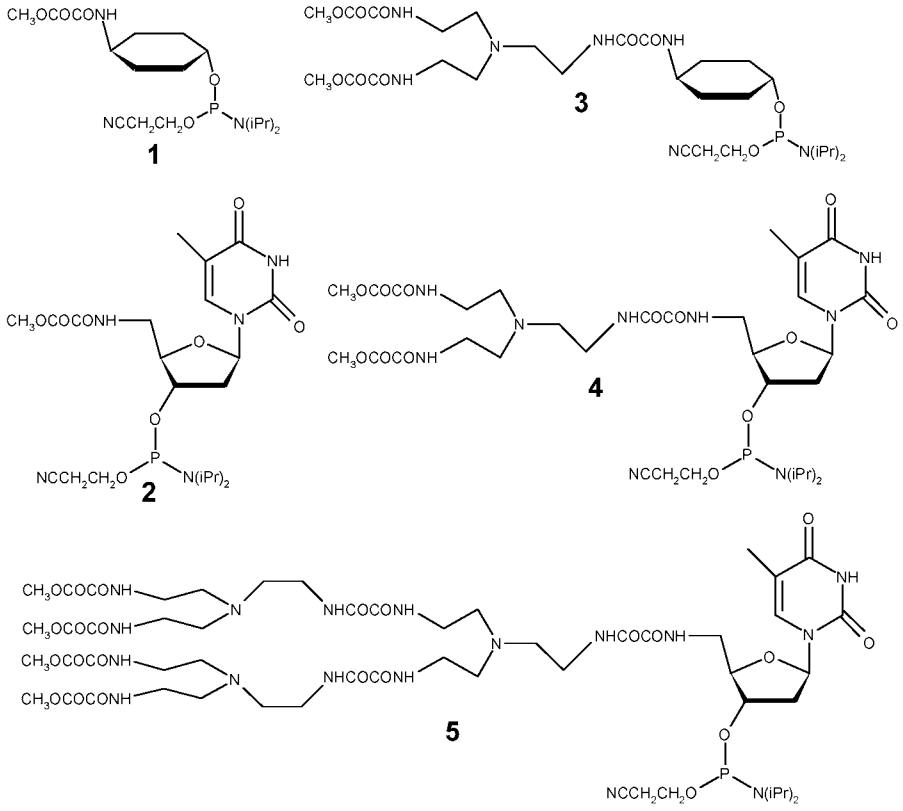

Figure 2. Terminus MOX modifiers.

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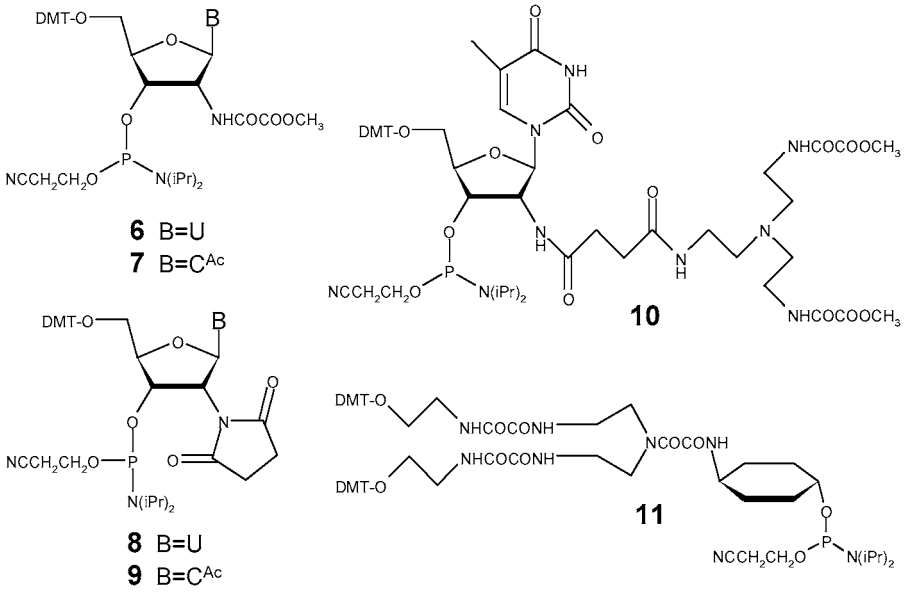

Figure 3. Extending MOX/SUC modifiers and a branching unit.

phosphoramidites were used as 0.1 M acetonitrile solutions except for **5**, which used as 0.2 M solution.

The efficiency of the MOX/SUC precursor strategies was demonstrated in the synthesis of oligonucleotides containing up to 16 imidazole residues (Table 1, entries 1–3). Such oligonucleotides are of interest as potential artificial ribonucleases. The core deoxyoligonucleotide 5′-ATC GAA CAC AGG ACC T-3′ (A), that is a complement to the loop region of $tRNA^{Phe}$, was built up with modifying units **3–5** and **10**, and a branching unit **11**, used in different combinations. The prepared multiple MOX precursors were then functionalized with histamine (2M solution in DMF, shaking for 2–6 hrs at r.t.) and, finally, deprotected in a usual way. Some of the synthesized ribonuclease mimics showed a high efficiency in cleaving the RNA target.

Direct functionalization of a MOX/SUC precursor oligonucleotide with bulky amines is problematic due to their decreased reactivity and, generally, limited

Table 1. Yields and MS Data of the Functionalized Oligonucleotides

Entry	Core Oligo	Modifying Units	Second Modifier	Reporter Molecule	Number of Functions	Yield, % (by CGE)	MW (exper.)	MW (calc.)
1	**A**	**11** × 2, **3**	$NH_2(CH_2)_2Im$	none	8	54	9389,2	9385,2
2	**A**	**10** × 2, **11**, **5**	$NH_2(CH_2)_2Im$	none	12	48	10277	10280
3	**A**	**11** × 2, **5**	$NH_2(CH_2)_2Im$	none	16	45	12793	12811
4	**T7**	**8** × 2	$NH_2(CH_2)_2NH_2$	**13**	2	71	7698,5	7692,5
5	**T7**	**8** × 2	**12**	**13**	2	63	8018,1	8012,9

solubility. Thus, to introduce a bulky reporter molecule, the precursor oligonucleotide was first treated with a suitable diamine linker to form an amino derivative to which the reporter molecule was conjugated in the standard fashion. T7 promoter containing 2′-succinimido-2′-deoxy-uridine residues (introduced with the use of phosphoramidite **8**) instead of thymidines in the fifth and seventeenth positions was synthesized. The T7-derivatized CPG was split in two portions and treated with ethylenediamine (20 min, 70°C) and 4,7,10-trioxa-1,13-tridecanediamine (**12**, 40 min, 70°C). Amino oligonucleotides were twice precipitated into ethyl alcohol and conjugated with succinimidyl ester of fluorescein-5-EX (**13**, Molecular Probes) in accordance with the manufacture's protocol. The experimental data are given in Table 1 (entries 4,5).

In conclusion, the developed MOX/SUC precursor strategies provide a robust way to construct functionalized oligonucleotides.

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