Claims

1. A method for the preparation of a modified nucleic acid molecule comprising a nucleic acid moiety and a non-nucleic acid moiety by reacting a first reactant and a second reactant, wherein the first reactant comprises the non-nucleic acid moiety and a carboxyl group, wherein said non-nucleic acid moiety comprises a polyalkoxy compound and wherein the second reactant comprises an amino-modified nucleic acid molecule comprising the nucleic acid moiety and an amino modification comprising an amino group which is attached to the nucleic acid moiety, wherein the method comprises the following steps:

a) activating the carboxyl group of the first reactant by a condensation reagent in a water miscible organic solvent, and

b) reacting the activated carboxyl group of step a) and the amino group of the amino modification of the amino-modified nucleic acid molecule of the second reactant, dissolved in water or in a mixture of a water miscible organic solvent and water,

wherein the modified nucleic acid molecule is formed.

2. The method of claim 1, wherein the amino-modified nucleic acid molecule is dissolved in a mixture of water and a water miscible organic solvent in the presence of a quaternary ammonium salt and/or, wherein the activated first reactant of step a) is added to the amino-modified nucleic acid molecule dissolved in water or in a mixture of a water miscible organic solvent and water.

3. The method of claim 1, wherein

the amino-modified nucleic acid molecule comprises amino-modified aptamers, amino-modified Spiegelmers, amino-modified immunostimulatory nucleic acids, amino-modified siRNA, amino-modified miRNA molecules and/or amino-modified nucleic acid antisense molecules, and/or

the nucleic acid moiety comprises aptamers, Spiegelmers, immunostimulatory nucleic acids, siRNA’s, miRNA molecules and nucleic acid antisense molecules.

4. The method of claim 1, wherein in step b), an excess of molecules of the activated first reactant over the amino-modified nucleic acid molecules is used.

5. The method of claim 4, wherein the excess is expressed as a molar ratio of molecules of the activated first reactant and the amino-modified nucleic acid molecules, wherein the molar ratio is from about 1.1 to about 10.

6. The method of claim 1, wherein for activating the first reactant according to step a), the first reactant is dissolved in a water miscible organic solvent, and a condensing agent, and subsequently a base is added thereto.

7. The method of claim 6, wherein the base is a non-nucleophilic base selected from the group consisting of diisopropylethylamine (DIPEA), trimethylamine and DBU.

8. The method of claim 6, wherein the condensing agent is selected from the group consisting of a phosphonium salt, a uronium salt and a carbodiimide.

9. The method according to claim 8,
wherein the phosphonium salt is selected from the group consisting of BOP, PyBOP, PyBrop, AOP, PyAOP, BrOP and PyClOP;
the uronium salt is selected from the group consisting of HCTU, TCTU, TBTU, HBTU, HATU, TOTU and COMU; and
the carbodiimide is selected from the group consisting of DCC (N,N'-dicyclohexylcarbodiimide), EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) and DIC (N,N'-diisopropylcarbodiimide).

10. The method of claim 1, wherein the water miscible organic solvent is selected from the group consisting of methanol, ethanol, n-propanol, isopropanol, n-butanol, sec-butanol, tert-butanol, dimethyl sulfoxide, diethyl sulfoxide, methyl ethyl sulfoxide, formamide, methyl formamide, dimethyl formamide, ethyl formamide, ethyl methyl formamide, diethyl formamide, 2-pyrrolidone, N-methyl pyrrolidone, N-ethylpyrrolidone, acetonitrile, acetone, ethyl methyl ketone, methyl propyl ketone, diethyl ketone, methyl isopropyl ketone, methyl formate, ethyl formate, propyl formate, isopropyl formate, methyl acetate, ethyl acetate, methyl propanoate, tetrahydrofuran and dioxan.

11. The method of claim 6, wherein a molar ratio of the base to the first reactant is equal to or greater than 1.

12. The method of claim 1, wherein in step b), the activated first reactant of step a) is added to the amino-modified nucleic acid molecules until 80% to 100% or 90% to 100% of the amino-modified molecules are reacted with the first reactant.

13. The method of claim 1, wherein after completion of step b), any non-reacted first reactant is separated by ultrafiltration and/or chromatography.

14. The method of claim 13, wherein the separated first reactant is recycled and used in step a).

15. The method of claim 1, wherein the polyalkoxy compound is a straight or a branched polyalkoxy compound.

16. The method of claim 1, wherein the polyalkoxy compound is selected from the group consisting of polyethylene glycol, polypropylene glycol, poly butylene glycol and polyglycerol.

17. The method of claim 1, wherein the polyalkoxy compound is polyethylene glycol.

18. The method of claim 1, wherein the polyalkoxy compound comprises a molecular weight of 5,000 Da to 100,000 Da, 20,000 Da to 80,000 or 40,000 Da.

19. A modified nucleic acid molecule obtained by the method of claim 1.

20. The method of claim 1, wherein said nucleic acid moiety comprises an aptamer comprising L-nucleotides and/or D-nucleotides.

21. The method of claim 6, wherein said base comprises DIPEA.

22. The method of claim 1, wherein said water miscible organic solvent comprises dimethyl formamide, acetonitrile or dimethyl sulfoxide.

23. The method of claim 1, wherein after completion of step b), any non-reacted first reactant is separated by ion exchange chromatography.