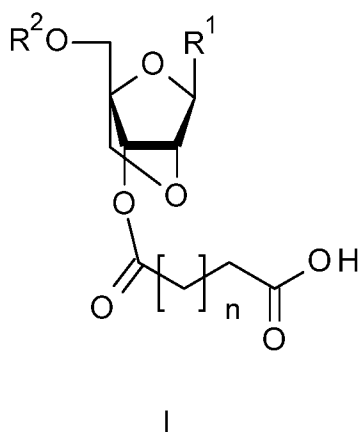


LNA-dicarboxylic acid derivatives and process for their preparation

The invention relates to LNA-dicarboxylic acid derivatives of the formula I



or salts thereof,

wherein

5 R^1 is a nucleobase or a modified nucleobase

R^2 is a hydroxy protecting group and

n is an integer from 1 to 5

to a process for their preparation, to its use for the preparation of a LNA-preloaded solid support containing the LNA-dicarboxylic acid derivatives of the formula I, to a LNA-preloaded solid support and its use in the oligonucleotide synthesis.

Oligonucleotides are generally prepared on a solid support medium. In general a first synthon (e.g. a monomer, such as a nucleoside) is first attached to the solid support medium,

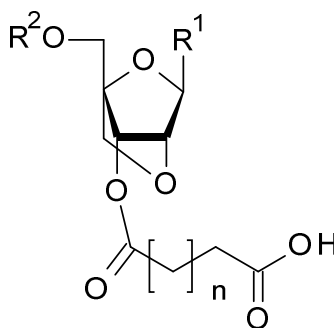
and the oligonucleotide is then synthesized by sequentially coupling monomers to the solid support-bound synthon. This iterative elongation eventually results in a final oligonucleotide compound which it is cleaved from the support and, if necessary further worked up to produce the final oligonucleotide compound.

5 In a further development linker molecules attached to the solid support have been introduced for carrying out the oligonucleotide synthesis more efficiently, particularly on the automated process synthesizers nowadays used. For instance the Int. Patent Publication WO 2005/049621 or WO 2006/029023 discloses such linker compounds. Oligonucleotide elongation usually starts from an O-dimethoxytrityl (O-DMT) on the linker molecule.

10 However, in the synthesis of LNA oligonucleotides, it was found in that to a certain extent the first coupling on the linker molecule failed, resulting in final oligonucleotides with a substantial amount of (N-1) impurities, i.e. oligonucleotides with one lacking nucleoside at the 3'-end.

Object of the present disclosure therefore was to reduce the amount of 3'-end (N-1)
15 impurities to a great extent, as such impurities can hardly be removed by state-of-the-art chromatographic purification methods and cause thereof a substantial loss in yield, e.g. by tight fraction pooling in the preparative chromatography step and/or an unsatisfying impurity profile in the isolated LNA oligonucleotide.

In one aspect, there is provided LNA-dicarboxylic acid derivatives of the formula I



20

I

or salts thereof,

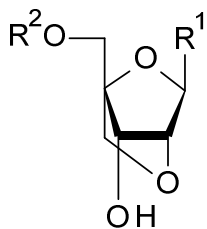
wherein

R^1 is an optionally modified or an amino group protected adenine (A), cytosine (C), guanine (G) or thymine (T);

R^2 is a hydroxy protecting group; and

n is an integer from 1 to 5.

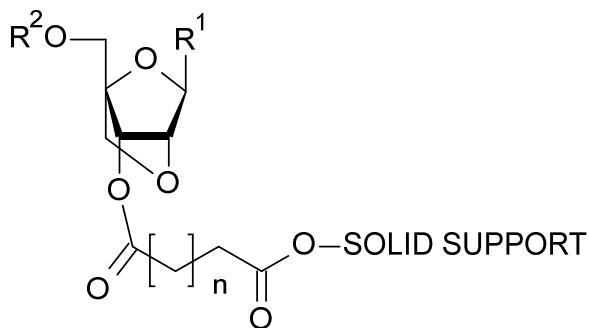
In another aspect, there is provided a process for the preparation of LNA-dicarboxylic acid derivatives of formula I comprising the reaction of an LNA alcohol of formula II



II

wherein R^1 and R^2 are as above, with a C_{2-6} -dicarboxylic acid anhydride, in the presence of a base and an organic solvent to form the LNA-dicarboxylic acid derivatives of formula I.

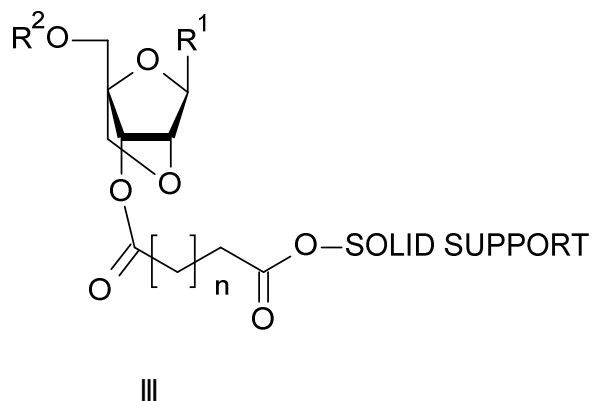
In another aspect, there is provided use of the LNA-dicarboxylic acid derivatives of the formula I as defined herein, for the preparation of a preloaded solid support of the formula III



III

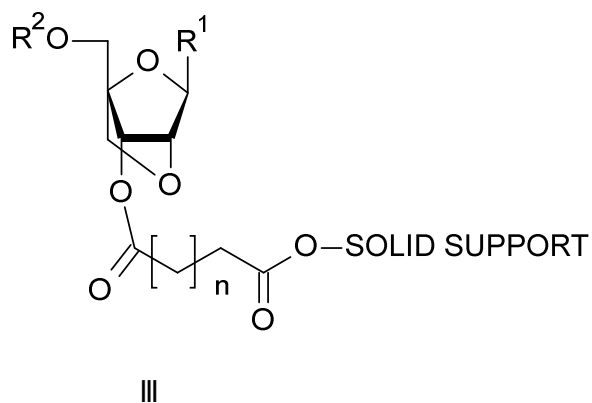
wherein R^1 , R^2 and n are as above and SOLID SUPPORT is a solid support material suitable for oligonucleotide synthesis.

In another aspect, there is provided a preloaded solid support of the formula III



wherein R¹, R² and n are as above and SOLID SUPPORT is a solid support material suitable for oligonucleotide synthesis.

- 5 In another aspect, there is provided use of the preloaded solid support of the formula III



wherein R¹, R² and n are as above and SOLID SUPPORT is a solid support material suitable for oligonucleotide synthesis as start material in oligonucleotide synthesis.

- 10 It was found that with LNA-dicarboxylic acid derivatives of the formula I as described above, which can be attached to a solid resin (Int. Patent Publication WO 1992/006103) and act as pre-loaded resin the N-1 failures can be reduced to great extent.

The following definitions are set forth to illustrate and define the meaning and scope of the various terms used to describe the invention herein.

The term “C₁₋₆-alkyl” denotes a monovalent linear or branched saturated hydrocarbon group of 1 to 6 carbon atoms, and in a more particular embodiment 1 to 4 carbon atoms. Typical examples include methyl, ethyl, propyl, isopropyl, n-butyl, *i*-butyl, sec-butyl, or *t*-butyl, preferably methyl or ethyl.

- 5 The term “C₁₋₆-alkyoxy” denotes a C₁₋₆-alkyl group, more preferably a C₁₋₄-alkyl group, as defined above attached to an oxygen atom. Typical examples include methoxy, ethoxy, propoxy, isopropoxy, n-butoxy, *i*-butoxy, *t*-butoxy, preferably methoxy or ethoxy.

The term “C₁₋₆-alkanoyl” denotes a C₁₋₆-alkyl group, more preferably a C₁₋₄-alkyl group, as defined above attached to a carbonyl group. Typical examples include acetyl, ethanoyl, propanoyl, i-propanoyl, n-butanoyl, i-butanoyl or t-butanoyl, preferably acetyl.

5 The term “hydroxy-protecting group as used for R²” denotes groups which intended to protect a hydroxy group and include ester- and ether-forming groups, in particular modified trityl groups, tetrahydropyranyl, acyl groups, carbamoyl, benzyl and silyl ethers (e.g. TBS, TBDPS) groups. Further examples of these groups are found in T. W. Greene and P. G. M. Wuts, “Protective Groups in Organic Synthesis”, 2nd ed., John Wiley & Sons, Inc., New York, NY, 1991, chapters 2-3; E. Haslam, “Protective Groups in Organic Chemistry”, J. G. W. McOmie, Ed., Plenum Press, New York, NY, 1973, Chapter 5, and T.W. Greene,
10 “Protective Groups in Organic Synthesis”, John Wiley and Sons, New York, NY, 1981.

Preferred are acid sensitive hydroxyl groups, more preferred alkoxy-modified trityl groups. Modified in this context mean the substitution of one, two or three of the phenyl rings by one or more C₁₋₆-alkoxy groups, preferably methoxy groups.

15 The most preferred hydroxy protecting group is 4,4'-dimethoxytrityl (DMT).

The term “amino-protecting group” denotes groups intended to protect an amino group and includes C₁₋₆-alkanoyl, benzoyl, benzyloxycarbonyl, carbobenzyloxy (CBZ or Z), 9-fluorenylmethyloxycarbonyl (Fmoc), *p*-methoxybenzyloxycarbonyl, *p*-nitrobenzyloxycarbonyl, *t*-butoxycarbonyl (BOC), and trifluoroacetyl, dimethylformamidine
20 (dmf). Further examples of these groups are found in T. W. Greene and P. G. M. Wuts, “Protective Groups in Organic Synthesis”, 2nd ed., John Wiley & Sons, Inc., New York, NY, 1991, chapter 7; E. Haslam, “Protective Groups in Organic Chemistry”, J. G. W. McOmie, Ed., Plenum Press, New York, NY, 1973, Chapter 5, and T.W. Greene, “Protective Groups in Organic Synthesis”, John Wiley and Sons, New York, NY, 1981.

25 Preferred amino protecting groups are selected from C₁₋₆-alkanoyl, benzoyl (bz) or dimethylformamidine (dmf).

The term “nucleobase” or “modified nucleobase” used for R¹ stands for the five nucleobases adenine (A), cytosine (C), guanine (G), thymine (T) and Uracil (U) and for modifications thereof.

30 The term „salt“ in the context of the LNA-dicarboxylic acid derivatives of the present invention of the formula I the present invention stands for a salt from an inorganic or organic base.

Inorganic salts typically are alkali- or earth alkali salts such as sodium-, potassium-, magnesium- or calcium salts, preferably sodium or potassium salts from an inorganic alkali – or earth alkali hydroxide base or from an organic alkali- or earth alkali alcoholate.

Organic salts typically are ammonium salts from an organic amine, typically from an aliphatic amine or an aromatic amine, preferably from a tertiary amine preferably a tri-C₁₋₄-alkylamine or pyridine more preferably triethylamine or pyridine

Organic salts are preferred over the inorganic salts.

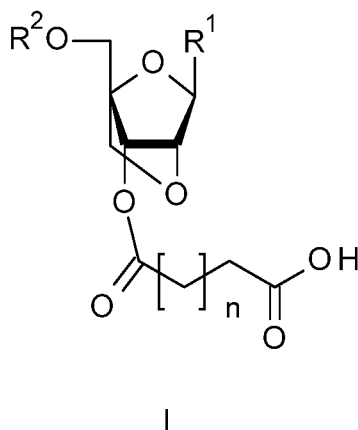
The LNA-dicarboxylic acid derivatives of formula I can occur in the form of any mixture of the free acid and the salt from the inorganic or organic base.

A typical modified nucleobase is 5-methyl cytosine (5-MeC).

In a preferred embodiment R¹ is an optionally modified and / or an amino group protected adenine (A), cytosine (C), 5-methyl cytosine (5-MeC), guanine (G) or thymine (T), preferably an amino group protected adenine (A), an amino group protected cytosine (C), an amino group protected 5-methyl cytosine (5-MeC), an amino group protected guanine (G) or thymine (T).

While the typical amino protecting groups have been defined above, the preferred amino protecting group for adenine (A), cytosine (C) and 5-methyl cytosine (5-MeC) is benzoyl, for guanine (G) is isobutanoyl (isobutyryl) or dimethylformamidine (dmf).

In a preferred embodiment the LNA-dicarboxylic acid derivatives of the present invention have the formula I



or ammonium salts from an organic amine thereof,

wherein

R^1 is an optionally modified and / or an amino group protected adenine (A), cytosine (C), 5-methyl cytosine (5-MeC), guanine (G) or thymine (T), preferably an amino group protected adenine, an amino group protected cytosine (C), an amino group protected 5-methyl cytosine (5-MeC), an amino group protected guanine (G) or thymine (T).

R^2 is an acid sensitive hydroxy protecting group selected from an alkoxy-modified trityl group;

n is an integer from 1 to 5.

In a further preferred embodiment the LNA-dicarboxylic acid derivatives of the present invention have the formula I

or ammonium salts from tertiary amines selected from a tri- C_{1-4} -alkylamine, more preferably triethylamine or from pyridine thereof;

wherein;

R^1 is benzoyl protected adenine (A), benzoyl protected cytosine (C), benzoyl protected 5-methyl cytosine (5-MeC), isobutanoyl or dimethylformamide protected guanine (G) or thymine (T);

R^2 is 4,4'-dimethoxytrityl (DMT);

n is 1.

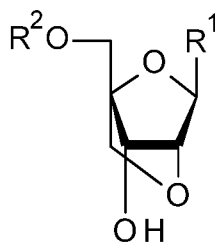
The most preferred LNA-dicarboxylic acid derivatives of the present invention have the formula I and are characterized by the following definitions

- R^1 = benzoyl protected adenine (A); R^2 = 4,4'-dimethoxytrityl (DMT); n = 1 ; in the form of the triethylammonium or pyridinium salt
- R^1 = benzoyl protected cytosine (C); R^2 = 4,4'-dimethoxytrityl (DMT); n = 1 ; in the form of the triethylammonium or pyridinium salt
- R^1 = benzoyl protected 5-methyl cytosine (5-MeC); R^2 = 4,4'-dimethoxytrityl (DMT); n = 1 ; in the form of the triethylammonium or pyridinium salt
- R^1 = isobutanoyl protected guanine (G); R^2 = 4,4'-dimethoxytrityl (DMT); n = 1 ; in the form of the triethylammonium or pyridinium salt

- R^1 = dimethylformamidine protected guanine (G); R^2 = 4,4'-dimethoxytrityl (DMT); $n = 1$; in the form of the triethylammonium or pyridinium salt
- R^1 = thymine (T); R^2 = 4,4'-dimethoxytrityl (DMT); $n = 1$ in the form of the triethylammonium or pyridinium salt.

5 The preferred LNA-dicarboxylic acid derivatives of the present invention as outlined above can either occur as free acids, or in the form of the ammonium salts as described above or also as any mixture of the ammonium salts and the free acid.

The process for the preparation of LNA-dicarboxylic acid derivatives of formula I comprises the reaction of an LNA alcohol of formula II



II

10 wherein R^1 and R^2 are as above, with a C_{2-6} -dicarboxylic acid anhydride to form the LNA-dicarboxylic acid derivatives of formula I in the presence of a base and an organic solvent.

LNA alcohols of formula II are as a rule commercially available or can be prepared according to Wengel et al. Tetrahedron 54 (1998) 3607-3630.

15 Likewise the C_{2-6} -dicarboxylic acid anhydrides are commercial available compounds. Typical C_{2-6} -dicarboxylic acid anhydride is succinic anhydride.

Usually, in an initial step the starting material is freed from residual water under azeotropic conditions with a suitable organic solvent such as with toluene.

Suitable bases have been described above, but preferably organic amines are used.

20 Preferably a tertiary amine, more preferably a tri- C_{1-4} -alkylamine or pyridine even more preferably triethylamine or pyridine is the base typically applied.

Suitable organic solvent is a halogenated hydrocarbon such as dichloromethane.

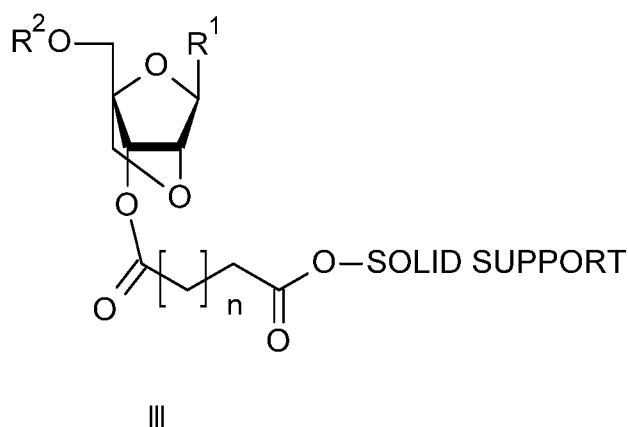
The reaction is conveniently performed under inert gas atmosphere at a reaction temperature of 10°C to 40°C.

Upon completion of the reaction, typically after about 2h, the ammonium salt can be obtained via an extraction process, suitably applying the solvent used for the reaction.

- 5 Removal of the solvent from the combined organic phases and optional further purification by chromatography the respective ammonium salt of the LNA-dicarboxylic acid derivatives of formula I can be delivered in a yield of 94-99.9% and a HPLC purity of 97-99.5 area-% (excluding residual toluene).

- 10 The LNA-dicarboxylic acid derivatives of formula I can also be obtained in the form of the free acid. This can be accomplished by converting a salt of the LNA-dicarboxylic acid derivatives of the formula I according to the present invention with hydrochloric acid or an organic acid such as with citric acid in a suitable organic solvent such as in dichloromethane or ethylacetate.

- 15 In a further embodiment of the invention the LNA-dicarboxylic acid derivatives of the formula I can be used for the preparation of a preloaded solid support of the formula III

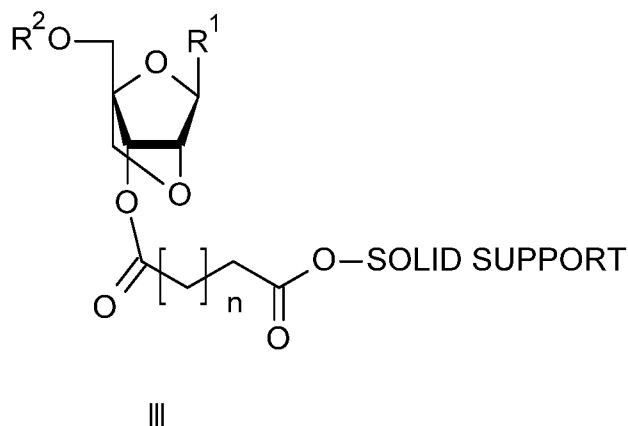


wherein R¹, R² and n are as above and SOLID SUPPORT is a solid support material suitable for oligonucleotide synthesis.

- 20 Suitable solid support materials are well described for instance in Guzaev, A. P. Solid-phase supports for oligonucleotide synthesis. In: Current protocols in nucleic acid chemistry. (John Wiley & Sons, Inc.) (2013), Chapter 3, Unit 3.1., pp. 3.1.1-3.1.60. Typical commercial solid supports are the Primer Support 5G series from GE Healthcare or the NittoPhase®HL solid supports from Nitto Denko.

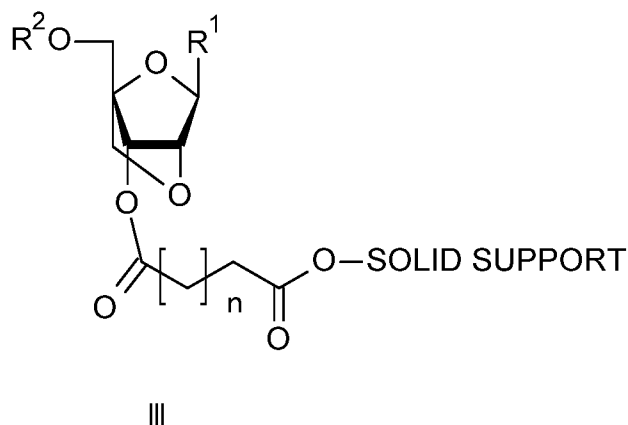
The definitions and preferences provided above for R^1 , R^2 and n likewise apply for the preloaded solid support of the formula III.

In another embodiment the invention relates to preloaded solid support of the formula III



- 5 wherein R^1 , R^2 , n and SOLID SUPPORT are as defined above. The preferences provided above for R^1 , R^2 , n and SOLID SUPPORT likewise apply for the preloaded solid support of the formula III.

In still another embodiment of the invention the preloaded solid support of the formula III



- 10 wherein R^1 , R^2 , n and SOLID SUPPORT are as above can be used as start material for the oligonucleotide synthesis. The definitions and preferences provided above for R^1 , R^2 , n and SOLID SUPPORT likewise apply for the preloaded solid support of the formula III.

The LNA-dicarboxylic acid derivatives of formula I can be linked to the solid support by methods known to the skilled in the art and for instance as described in the Int. Patent

- 15 Publication WO 1992/006103.

The principles of the oligonucleotide synthesis are well known in the art und well described in literature and public fora like Wikipedia (see e.g. Oligonucleotide synthesis; Wikipedia, the free encyclopedia; https://en.wikipedia.org/wiki/Oligonucleotide_synthesis, of March 15, 2016).

5 Larger scale oligonucleotide synthesis nowadays is carried automatically using computer controlled synthesizers.

As a rule oligonucleotide synthesis is a solid-phase synthesis, wherein the oligonucleotide being assembled is covalently bound, *via* its 3'-terminal hydroxy group, to a solid support material and remains attached to it over the entire course of the chain assembly.
10 Suitable solid supports are described above.

Preferably the oligonucleotide consists of optionally modified DNA or LNA nucleoside monomers or combinations thereof and is 10 to 25 nucleotides in length.

In view of the fact that the preloaded solid support of the formula III comprises a LNA nucleoside the 3' terminal nucleoside of the oligonucleotide chain produced is always
15 an LNA nucleoside.

The oligonucleotide synthesis in principle is a stepwise addition of nucleotide residues to the 5'-terminus of the growing chain until the desired sequence is assembled.

As a rule each addition is referred to as a synthetic cycle and in principle consists of the chemical reactions

20 a₁) de-blocking the protected hydroxyl group on the pre-loaded solid support of formula III,

a₂) coupling the first nucleoside as activated phosphoramidite with the free hydroxyl group on the solid support,

a₃) oxidizing or sulfurizing the respective P-linked nucleoside to form the respective
25 phosphotriester (P=O) or the respective phosphorothioate (P=S);

a₄) optionally, capping any unreacted hydroxyl groups on the solid support;

a₅) de-blocking the 5' hydroxyl group of the second nucleoside attached to the solid support;

a₆) coupling the third nucleoside as activated phosphoramidite to form the respective P-linked trimer;

a₇) oxidizing or sulfurizing the respective P-linked di-nucleoside to form the respective phosphotriester (P=O) or the respective phosphorothioate (P=S);

5 a₈) optionally, capping any unreacted 5' hydroxyl groups;

a₉) repeating the previous steps a₅ to a₈ until the desired sequence is assembled;

a₁₀) global deprotection to obtain the crude oligonucleotide which can be further purified by typical oligonucleotide purification techniques such as with chromatography and/or ultrafiltration and / or lyophilization

10 The following examples shall illustrate the invention without limiting it.

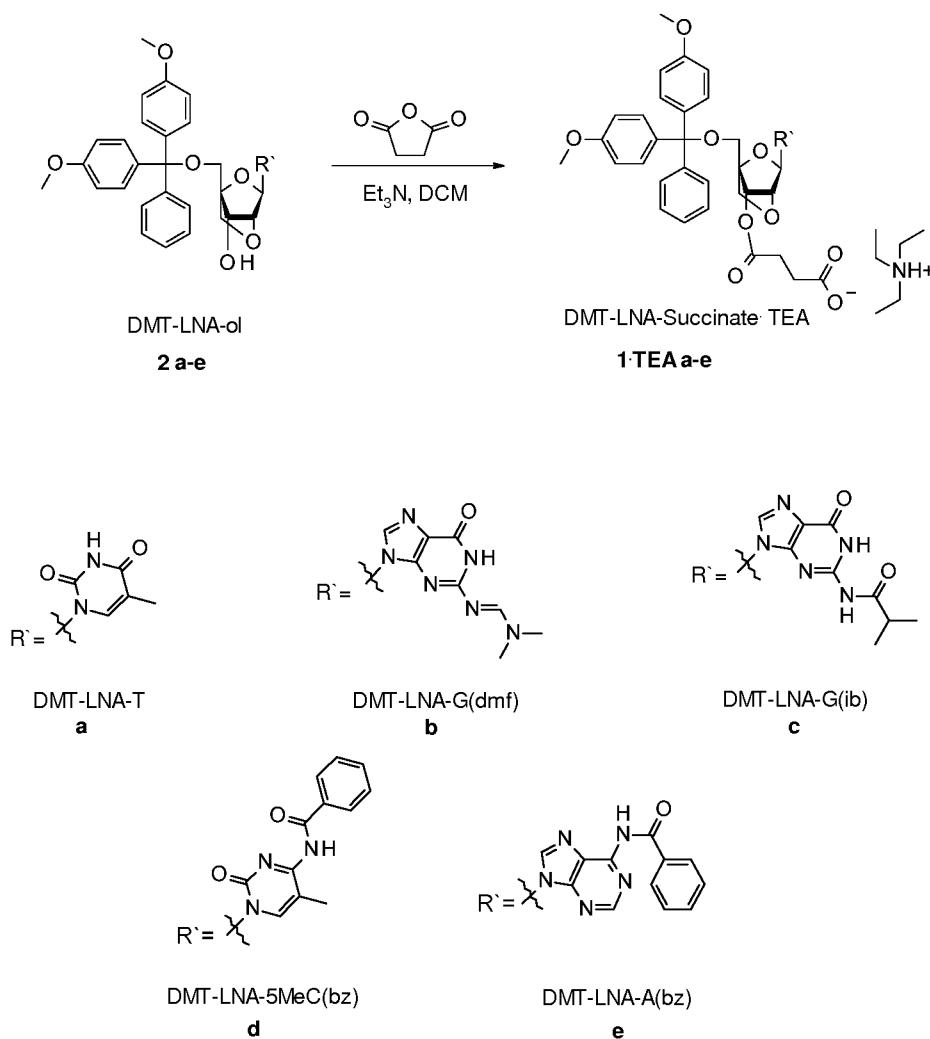
Examples

Abbreviations:

5	EtOAc	= Ethylacetate
	MeCN	= Acetonitrile
	MeOH	= Methanol
	DCM	= Dichloromethane
	DMAP	= 4-Dimethylaminopyridine
10	TEA	= Triethylamine
	THF	= Tetrahydrofuran
	TLC	= Thin layer chromatography
	rt	= Room temperature
	eq	= Equivalent

15

Process Scheme



General Procedure

- A round-bottom flask was charged with nucleoside **2** (1 eq) and succinic anhydride (1.5 eq).
 5 For azeotropic removal of residual water, the mixture was suspended in abs. toluene (20 mL per 1 g of **2**) at rt. Next volatiles were stripped off *in vacuo*. This procedure was repeated once, then toluene was stripped off to yield a solid but not fully dried residue. Next operations were all carried out under argon atmosphere. To the above's formed solid, abs. DCM (18 mL per 1 g of **2**) was added. Then TEA (3.0 eq rel to **2a**, 5.0 eq rel to **2b**) was
 10 added in one portion and mixture was stirred at rt whereby after 30 min a clear reaction mixture was obtained. The conversion was determined by TLC or LC-MS (see Example 1, Table 1 for **1a.TEA** and Example 2, Table 2 for **1b.TEA**).

Usually the conversions were complete after 2 h, but in all cases mixtures were additionally stirred for additional 1 h at rt. The reaction mixture was then poured to a triethylammonium

phosphate buffer solution (15 mL of buffer per 1 g of **2**. Buffer solution preparation: Mixing 85% aq. H₃PO₄ (8.5 mL, 1 eq), TEA (26 mL, 1.5 eq) and water (465.5 mL)). The formed layers were separated and the aqueous layer was additionally extracted with DCM (2x10 mL per 1 g of **2**). The combined organic layers were filtered through a pad of anhydrous Na₂SO₄ and concentrated under vacuum. The crude product, obtained as a white foam, was further purified by filtration through a silica column (20 g of silica per 1 g of the crude product; eluent: EtOH in DCM from 2.5% to 10%, the mixture is further supplemented with 3 vol-% of TEA). The target fractions were concentrated to dryness, immediately suspended in abs. toluene and co-evaporated *in vacuo*. Co-evaporation was repeated one more time.

Example 1: Preparation of **1a.TEA**

8.6 g of **2a** was converted into **1a.TEA** as described in the *General Procedure* to yield 11.3 g of **1a.TEA** in 96% yield and 99.1 HPLC area-% purity (excluding 0.67 mol eq residual toluene) (column: Apollo C-18 (10 µm) (4,6x250 mm) 10.65 min; mobile phase: gradient 40% MeCN + 60% of 0,1% aq. H₃PO₄; flow rate 1.0 mL/min; column temperature 40 °C; detection: 210 nm, 254 nm; sample concentration: 1 mg/mL in MeCN; injection volume: 0.002 mL).

LC-MS ESI (m/z): 671.2 [M-H]

¹H-NMR (D₄-MeOH, 400 MHz): δ 7.77-7.73 (m, 1H), 7.50-7.43 (m, 2H), 7.38-7.28 (m, 6H), 7.26-7.21 (m, 1H), 6.91-6.85 (m, 4H), 5.62 (s, 1H), 5.21 (s, 1H), 4.56 (s, 1H), 3.89-3.79 (m, 2H), 3.78 (s, 6H), 3.57 (d, J=11.2Hz, 1H), 3.47 (d, J=11.2Hz, 1H), 2.94 (q, J=7.3Hz, 6H), 2.64-2.46 (m, 2H), 2.46-2.36 (m, 2H), 1.61 (d, J=1.2Hz, 3H), 1.29 (t, J=7.3Hz, 9H).

Table 1

Method	Sorbent	Eluent	Retention Time	Detection
TLC	Merck silica gel TLC plates UV254	DCM/MeOH 9:1 incl one drop TEA	R _f = 0.55 (2a) R _f = 0.25 (1a.TEA)	UV 254 nm heating to yellow stain
LC-MS	Acquity UPLC BEH C ₁₈	MeCN in 0.1% aq. HCO ₂ H from 5% to 100%	R _t = 3.62 min (1a.TEA)	UV 244 nm ESI: 1a.TEA [M-H]=671.2 m/z

Example 2: Preparation of 1b.TEA

8.9 g of **2b** was converted into **1b.TEA** as described in the *General Procedure* to yield 11.0 g of **1b.TEA** in 94% yield and 97.2 HPLC area-% purity (excluding 0.75 mol eq residual toluene) (column: Apollo C-18 (10 μ m) (4,6x250 mm) 9.18 min; mobile phase: gradient 40% MeCN + 60% of 0,1% aq. H₃PO₄; flow rate 1.0 mL/min; column temperature 40 °C; detection: 210 nm, 254 nm; sample concentration: 1 mg/mL in MeCN; injection volume: 0.002 mL). LC-MS ESI (m/z): 753.3 [M+H]⁺

¹H-NMR (D₄-MeOH, 400 MHz): δ 8.70 (s, 1H), 7.98 (s, 1H), 7.44-7.39 (m, 2H), 7.32-7.15 (m, 7H), 6.87-6.81 (m, 4H), 6.00 (s, 1H), 5.85 (s, 1H), 4.80 (s, 1H), 4.01-3.89 (m, 2H), 3.76 (s, 6H), 3.53-3.74 (m, 2H), 3.15 (q, J=7.3Hz, 6H), 3.09 (s, 3H), 3.07 (s, 3H), 2.63-2.46 (m, 2H), 2.45-2.33 (m, 2H), 1.26 (t, J=7.3Hz, 9H).

Table 2

Method	Sorbent	Eluent	Retention Time	Detection
TLC	Merck silica gel TLC plates UV254	DCM/MeOH 9:1 incl one drop TEA	R _f = 0.55 (2b) R _f = 0.25 (1b.TEA)	UV 254 nm heating to yellow stain
LC-MS	Acquity UPLC BEH C ₁₈	MeCN in 0.1% aq. HCO ₂ H from 5% to 100%	R _t = 3.40 min (1b.TEA)	UV 303 nm ESI ⁺ : 1b.TEA [M+H]=753.3 m/z ESI ⁻ : 1b.TEA [M-H]=751.3 m/z

Example 3: Preparation of 1c.TEA

10 g of **2c** was converted into **1c.TEA** as described in the *General Procedure* to yield 13.6 g of **1c.TEA** in 98% yield and 98.6 HPLC area-% purity (excluding 1.20 mol eq residual toluene) (column: Apollo C-18 (10 μ m) (4,6x250 mm) 11.00 min; mobile phase: gradient 40% MeCN + 60% of 0,1% aq. H₃PO₄; flow rate 1.0 mL/min; column temperature 40 °C; detection: 210 nm, 254 nm; sample concentration: 1 mg/mL in MeCN; injection volume: 0.002 mL).

LC-MS ESI (m/z): 768.5 [M-TEA+H]⁺

¹H-NMR (D₄-MeOH, 400 MHz): δ 8.11 (s, 1H), 7.46-7.40 (m, 2H), 7.34-7.26 (m, 4H), 7.25-7.06 (m, 5H), 6.90-6.82 (m, 4H), 5.99 (s, 1H), 5.35 (s, 1H), 4.85 (s, 1H), 3.96 (q,

J=8.3Hz, 2H), 3.76 (s, 6H), 3.56–3.47 (m, 2H), 3.16 (q, J=7.3Hz, 6H), 2.71 (hept, J=6.8Hz, 1H), 2.61–2.38 (m, 4H), 1.27 (t, J=7.3Hz, 9H), 1.21 (d, J=6.9Hz, 6H).

Example 4: Preparation of 1d.TEA

- 5 10 g of **2d** was converted into **1d.TEA** as described in the *General Procedure* to yield 14.0 g of **1d.TEA** in >99% yield and 99 HPLC area-% purity (excluding 0.50 mol eq residual toluene) (column: Apollo C-18 (10 μ m) (4,6x250 mm) 12.89 min; mobile phase: gradient 40% MeCN + 60% of 0,1% aq. H₃PO₄; flow rate 1.0 mL/min; column temperature 40 °C; detection: 210 nm, 254 nm; sample concentration: 1 mg/mL in MeCN; injection volume:
10 0.002 mL).

LC-MS ESI (m/z): 775.5 [M-TEA+H]⁺

- ¹H-NMR (D₄-MeOH, 400 MHz): δ 8.22 (s, 1H), 8.19 (s, 1H), 7.98 (s, 1H), 7.59–7.52 (m, 1H), 7.51–7.42 (m, 4H), 7.40–7.32 (m, 4H), 7.28–7.09 (m, 5H), 6.95–6.84 (m, 4H), 5.69 (s, 1H), 5.23 (s, 1H), 4.64 (s, 1H), 3.87 (dd, J=15.6Hz, J=8.2Hz, 2H), 3.79 (s, 6H), 3.55 (dd, J=34.1Hz, J = 11.3Hz, 2H), 3.13 (q, J = 7.3 Hz, 6H), 2.67–2.37 (m, 4H), 1.83 (s, 3H), 1.27 (t, J=7.3Hz, 9H).

Example 5: Preparation of 1e.TEA

- 10 g of **2c** was converted into **1c.TEA** as described in the *General Procedure* to yield 14.2 g of **1c.TEA** in >99% yield and 99.2 HPLC area-% purity (excluding 1.00 mol eq residual toluene) (column: Apollo C-18 (10 μ m) (4,6x250 mm) 5.17 min; mobile phase: gradient 40% MeCN + 60% of 0,1% aq. H₃PO₄; flow rate 1.0 mL/min; column temperature 40 °C; detection: 210 nm, 254 nm; sample concentration: 1 mg/mL in MeCN; injection volume:
20 0.002 mL).

LC-MS ESI (m/z): 786.5 [M-TEA+H]⁺

- 25 ¹H-NMR (D₄-MeOH, 400 MHz): δ 8.73 (s, 1H), 8.55 (s, 1H), 8.11–8.05 (m, 2H), 7.68–7.61 (m, 1H), 7.59–7.52 (m, 2H), 7.47–7.42 (m, 2H, Ar H), 7.37–7.26(m, 5H), 7.25–7.07 (m, 3H, Ar H), 6.91–6.82 (m, 4H), 6.24 (s, 1H), 5.44 (s, 1H), 4.93 (s, 1H), 4.02 (dd, 2H, J=16.5Hz, J=8.3 Hz), 3.77 (s, 6H), 3.57 (br, 2H), 3.12 (q, J=7.3Hz, 6H), 2.65–2.35 (m, 4H), 1.25 (t, J=7.3Hz, 9H).

Example 6: Preparation of 1e.TEA and 1e (acid form).

Compound **2e** (6.71 g, 9.80 mmol) was dissolved in dry THF (70 mL) and concentrated under vacuum at 35 °C. The solid was then further dried under vacuum for 4 h at ambient temperature and dissolved in dry EtOAc (50 mL) and TEA (1.60 mL, 11.7 mmol), DMAP (0.30 g, 2.4 mmol) and succinic anhydride (1.37 g, 13.7 mmol) were sequentially added to the nucleoside solution. The reaction mixture was then heated at 50 °C for 16 hours. The reaction mixture was diluted with EtOAc (100 mL) and hexane (5 mL) and extracted with 10% citric acid solution (3 x 50 mL). The organic phase was extracted with 5% NaHCO₃ solution (4 x 50 mL), diluted with EtOAc (150 mL) and further extracted with 10% citric acid solution (2 x 75 mL) and water (2 x 75 mL). The combined citric acid and water fractions were back extracted with EtOAc (2 x 50 mL). The combined organic phases were dried (Na₂SO₄) and concentrated under reduced pressure to obtain the succinate **1e** (acid form) as a white foam.

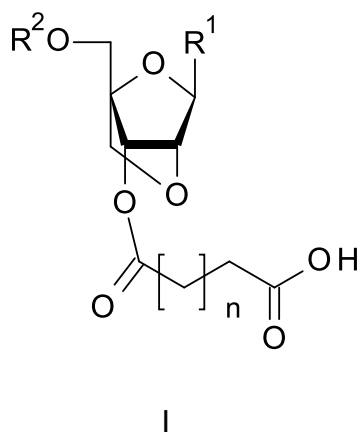
¹H NMR (600 MHz, DMSO-d₆) δ ppm 12.27 (br s, 1 H), 11.28 (s, 1 H), 8.79 (s, 1 H), 8.51 (s, 1 H), 8.06 (br d, J=7.5 Hz, 2 H), 7.64 -7.70 (m, 1 H), 7.53 - 7.61 (m, 2 H), 7.39 (br d, J=7.6 Hz, 2 H), 7.32 (br t, J=7.7 Hz, 2 H), 7.23 - 7.28 (m, 4 H), 7.24 (br s, 1 H), 6.90 (br d, J=8.6 Hz, 4 H), 6.23 (s, 1 H), 5.58 (s, 1 H), 4.93 (s, 1 H), 4.02 - 4.07 (m, 2 H), 3.89 (br d, J=8.5 Hz, 1 H), 3.74 (s, 6 H), 3.53 (br d, J=11.2 Hz, 1 H), 3.41 (br d, J=11.2 Hz, 1 H), 2.40 - 2.45 (m, 2 H).

The solid was redissolved in MeCN (70 mL) and TEA (6.8 mL, 49 mmol) was added. After stirring 10 minutes, the mixture was concentrated under vacuum at 35 °C for 10 minutes and the remainder was dried under vacuum at ambient temperature overnight to obtain **1e.TEA** salt (7.45 g of succinate x 0.6 TEA, 90% yield, HPLC purity >98%).

Analytical data for **1e.TEA** were in agreement with those reported in Example 5

Claims:

1. LNA-dicarboxylic acid derivatives of the formula I



5 or salts thereof,

wherein

R¹ is an optionally modified or an amino group protected adenine (A), cytosine (C), guanine (G) or thymine (T);

R² is a hydroxy protecting group; and

10 n is an integer from 1 to 5.

2. LNA-dicarboxylic acid derivatives of claim 1, wherein R¹ is an amino group protected adenine (A), an amino group protected cytosine (C), an amino group protected 5-methyl cytosine (5-MeC), an amino group protected guanine (G) or is thymine.

3. LNA-dicarboxylic acid derivatives of claim 2, wherein the amino protecting group is
15 selected from C₁₋₆-alkanoyl, benzoyl (bz) or dimethylformamidine (dmf).

4. LNA-dicarboxylic acid derivatives of anyone of claims 1 to 3, wherein R² is an acid sensitive hydroxyl protecting group selected from an alkoxy-modified trityl group, preferably from 4,4'-dimethoxytrityl.

5. LNA-dicarboxylic acid derivatives of anyone of claims 1 to 4, wherein n is 1.

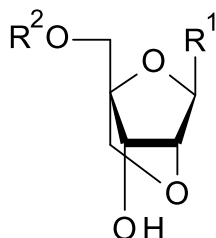
20 6. LNA-dicarboxylic acid derivatives of any one of claims 1 to 5, in the form of a salt from an inorganic or organic base.

7. LNA-dicarboxylic acid derivatives of any one of claims 1 to 6, in the form of an ammonium salt of an organic amine.

8. LNA-dicarboxylic acid derivatives of claim 7, in the form of an ammonium salt of a tertiary amine, preferably a tri-C₁₋₄-alkylamine or pyridine, more preferably triethylamine or pyridine.

9. LNA-dicarboxylic acid derivatives of any one of claims 1 to 8, in the form of any mixture of the free acid and the salt from the inorganic or organic base.

10. Process for the preparation of LNA-dicarboxylic acid derivatives of formula I comprising the reaction of an LNA alcohol of formula II



II

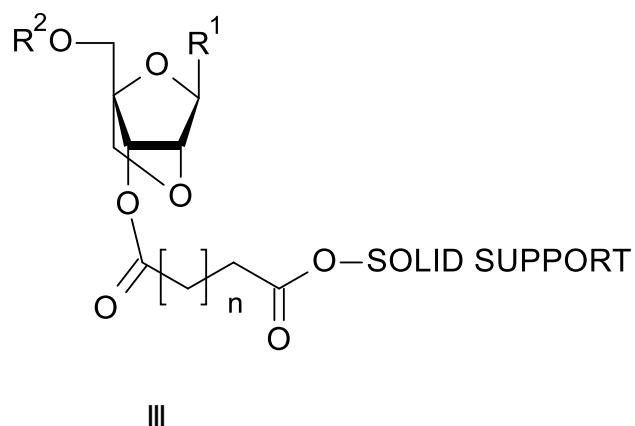
wherein R¹ and R² are as above, with a C₂₋₆-dicarboxylic acid anhydride, in the presence of a base and an organic solvent to form the LNA-dicarboxylic acid derivatives of formula I.

11. Process of claim 10, wherein the C₂₋₆-dicarboxylic acid anhydride is succinic anhydride.

12. Process of claims 10 or 11, wherein the base is an organic amine, preferably a tertiary amine, more preferably a tri-C₁₋₄-alkylamine or pyridine even more preferably triethylamine or pyridine.

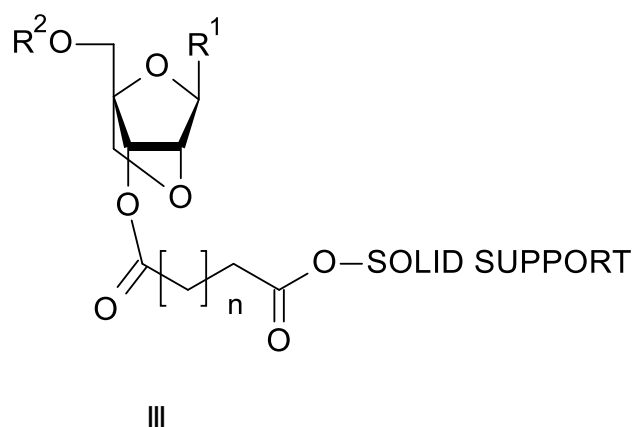
13. Process of any one of claims 10 to 12, wherein the organic solvent is a halogenated hydrocarbon.

14. Use of the LNA-dicarboxylic acid derivatives of the formula I of claims 1 to 9, for the preparation of a preloaded solid support of the formula III



wherein R^1 , R^2 and n are as above and SOLID SUPPORT is a solid support material suitable for oligonucleotide synthesis.

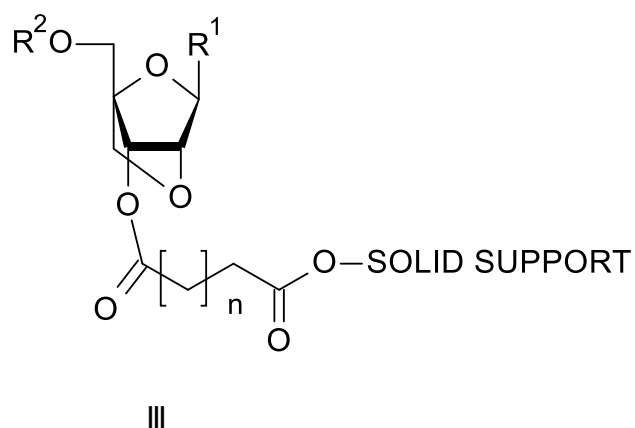
15. Preloaded solid support of the formula III



5

wherein R^1 , R^2 and n are as above and SOLID SUPPORT is a solid support material suitable for oligonucleotide synthesis.

16. Use of the preloaded solid support of the formula III



wherein R^1 , R^2 and n are as above and SOLID SUPPORT is a solid support material suitable for oligonucleotide synthesis as start material in oligonucleotide synthesis.