

COMPOSITIONS FOR TRANSFECTING A NUCLEIC ACID MOLECULE INTO A CELL COMPRISING TRIAZOLE COMPOUNDS GRAFTED TO A CATIONIC POLYMER, AND THEIR APPLICATIONS

5 The present invention relates to compositions for transfecting a nucleic acid molecule into a cell comprising heterocyclic compounds, in particular triazole derivatives grafted to a cationic polymer, and their applications. The present invention is directed to a composition suitable for transfecting a nucleic acid molecule into a cell, preferably a eukaryotic cell, comprising (i) at least one compound of general formula (I), preferably at least one compound of general formula (III), or
10 a tautomer, mesomer, racemate, enantiomer, diastereomer, or mixture thereof, or an acceptable salt thereof, and (ii) an acceptable excipient, buffering agent, cell culture medium, or transfection medium, wherein Y^1 , Y^2 , Y^3 , Z^1 , Z^2 , Z^3 , X_1 , X_2 , R_3 , P^+ , R and V are as defined in the description. The present invention also relates to uses of said composition and to a method for *in vitro* or *ex vivo* transfection of live cells.

15 The gene transfer is the process of introducing copies of exogenous genes into living cells in order to induce synthesis of the gene's products. Transfection is the process of deliberately and artificially introducing nucleic acids (DNA or RNA) into eukaryotic cells, utilizing means of non-viral methods. The transfection is of fundamental importance to developments in modern biology and
20 medicine, and has provided much of our knowledge of gene function and regulation.

 The transfection according to the invention can be achieved in various cells, including mammalian and insect cells, in primary cells, cell lines, stable cells or tumoral cells. The transfection is a powerful tool for *in vitro* genomic studies by offering the possibility to express in cells new exogenous proteins or to over-express or silence naturally occurring proteins.

25 Transfection according to the invention can be applied in therapy through *ex vivo* or *in vivo* protocols. Nucleic acid-based therapy with non-viral vectors can target different diseases, genetic diseases, immune diseases, cancers or viral infections in various tissues/organs or tumors. The cell targeting is achieved through different mechanisms and depends on the nature and properties of the transfection reagent, method or protocol composition or formulation and the route of
30 administration (Kaestner *et al.*, 2015).

 In bioproduction, transfection according to the invention can be used to generate stable cell clones over-producing recombinant proteins, peptides or antibodies. More recently, the transfection allowing transient gene expression (TGE) is becoming a valuable method for the fast production of moderate level of recombinant proteins or antibodies useful for research and process

development phases. Transient gene expression processes are advantageously applied for the production of recombinant viruses such as adeno-associated viruses (AAV), lentiviruses (LV) or adenoviruses (Merten *et al.*, 2016; Van Der Loo and Wright, 2015). Such processes consist of transfecting many expression vectors (plasmids) expressing in cells the different components
5 necessary to produce the viruses including capsid proteins, helper proteins, envelop proteins, viral polymerase or regulators, or viral genomes. High producing cells are used in viral production such as HEK293 and derivative cells, HeLa, BHK-21, A549 or insect cells. The transfection can be achieved in adherent or suspension-adapted cells at high cell density cultured in media containing serum or in protein-free, chemically defined or completely synthetic media.

10 Transfection is a method to introduce the different components in cells necessary to induce genome modification, engineering or editing such as zing finger nucleases, CRE/LOX proteins or CRISPR Cas-9 proteins.

DNA transfection uses plasmid DNA which triggers the gene expression driven by a promoter of a protein or peptide and/or a nucleic acid such as messenger RNA, long RNA,
15 microRNA, short hairpin RNA, short interfering RNA, ...

In mainly all cases plasmid DNA has been used for transfection purposes because of its inherent stability and its ability to integrate into the host genome to produce stable gene expression or to remain in the nucleus under an episomal form providing transient gene expression. However, some cells, named 'hard to transfect' cells (HTT) are refractory to DNA transfection or exhibit low
20 levels of transfection and gene expression when compared to standard transformed cells lines routinely used in laboratory settings. These "hard to transfect" cells exhibit less than 50% transfection efficiency when transfected with the last generation of commercially available transfection reagents such as LipoFectAmine® 2000 & 3000 (ThermoFisher), TransIT reagents® (MirusBio), FuGene® (Promega), XtremeGene® (Roche), jetPRIME® (Polyplus-transfection) or
25 ViaFect® (Promega).

Recent progresses to improve the gene expression efficiency of HTT cells are the transfection with messenger RNA (mRNA) sequences rather than plasmid DNA constructs which showed significant increase of transfection and gene expression levels in a majority of cell types, and particularly in challenging HTT cells. The benefice is explained by the fact that the transfected
30 mRNA does not need to reach the nucleus for cellular action contrasting with DNA transfection where the major limitation is to reach and penetrate the nucleus. The plasmid DNA import is not well understood but an efficient DNA transfection is mainly correlated with an active proliferation rate of cells where the transfected DNA may diffuse in the nuclear space during the nuclear membrane breakdown. In most post-mitotic cells or non-dividing cells, DNA transfection is not

effective. Most of the HTT cells exhibits a low level or absence of mitosis such as neuronal cells or other cell types derived from neural tissue, primary blood cells like dendritic cells or macrophages, or primary hepatocytes. However, for other HTT cells, the low transfection efficiency might be explained by other factors such as the cell fragility, the low binding of
5 transfection material to the cell plasma membrane, the low endocytosis capacity or a non-efficient intracellular trafficking towards the nucleus of the transfected DNA.

Transfection of plasmid DNA is the most common method to overexpress proteins in cells grown in culture. Most of the methods to introduce genetic DNA material into cells include the use of reagents such as calcium phosphate, cationic liposomes, peptides or polymers. When the
10 transfection fails, the reagent is generally recognized as the culprit. There is still a need to improve the efficiency of transfection reagents particularly for the HTT cells, with new concepts and generation of reagents.

DNA transfection in eukaryotic cells involves combining or mixing the polyanionic DNA molecule with a reagent to form transfection complexes or aggregates. Among the most commonly
15 used reagents, cationic lipids, peptides or polymers are suitable to interact with the negatively charged DNA. If an excess of the cationic reagents is used, complexes or aggregates having a positive character are generated. Such complexes are able to interact with the negatively charged glycosaminoglycans such as heparan sulfates present on the cell plasma membranes (Labatmoleur *et al.*, 1996, Mislick and Baldeschwieler, 1996). The cell membrane binding of
20 complexes induces a cell internalization or uptake by endocytosis mechanism. Transfection complexes are transported into endosomes where transfection reagents exhibit membrane destabilization through fusogenic activity and/or endosomolysis to release DNA in the cytoplasm. Following the release from the endosomes, the transfected DNA has to diffuse towards the perinuclear space and penetrate in the nucleus. The nuclear import is a limiting step as plasmid
25 DNA is not able to diffuse through the nuclear pore complexes because of its large size.

Among the non-viral vectors for DNA transfection, cationic liposomes or aggregates are one of the major classes which consist of combining or formulating cationic lipids with other types of lipids, such as phospholipids or cholesterol, to generate positively charged liposomes, vesicles or micelles that can bind negatively charged DNA and bind negatively charged cell membranes
30 ending by cell transfection. In the prior art, the first synthetic cationic lipid is N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA) by Felgner *et al.* When combined with dioleoylphosphatidylethanolamine at a ratio of 1:1, DOTMA formed cationic liposomes that were able to transfect cells *in vitro*. Based on the positively charged trimethylammonium polar head other monocationic lipids were developed such as

1,2bis(oleoyloxy)-3,3-(trimethylammonium) propane chloride (DOTAP). Other prior art compounds are based on polycationic polar head such as lipids described by Behr *et al.*, 1989, dioctadecylamidoglycylspermine (DOGS) or dipalmitoyl phosphatidylethanolamidosperrmine (DPPEs) where the carboxyspermine was used instead of ammonium group or the phospholipid moiety was replaced by a cholesterol derivative (Gao & Huang, 1991) such as 3 β -[N-(N',N'-dimethylaminoethane)-carbamoyl]cholesterol hydrochloride (DC-CHOL). Since these pioneer works, many cationic lipids were designed with the goal to generate novel cationic lipid reagents with increased transfection efficiencies. Many of these reagents are commercially available and the recent LipoFectAmine3000® reagent represents the most advanced reagent of cationic lipids available on the market. However, limitations are still observed as transfection is not effective in all cell types and cell toxicity is still a major concern of cationic lipid systems.

Cationic polymers represent the second major class of transfection reagents with the advantage to offer a large density of charged amino groups in their backbones. Cationic polymers having a positive charge at physiological pH are able to complex DNA into particles or aggregates, initiate cell binding and trigger cell internalisation through endocytosis. Polylysine (PLL) was the first polymer used but showed very limited transfection efficiencies (Wu and Wu, 1987, Zenke *et al.*, 1990). Its efficiency can be improved when additives such as weak bases like chloroquine (Erbacher *et al.*, 1996) or fusogenic peptide like influenza peptides (Planck *et al.*, 1994) were added in order to buffer the acidic pH of destabilize endosomes, respectively, and induce more release of DNA in the cytoplasm. Behr *et al.* has showed that the polyethylenimine (PEI) was a more effective polymer than PLL in transfection (Boussif *et al.*, 1995). PEI has a high density of amino groups and is not fully protonated at physiological pH. After endocytosis of DNA complexed with PEI, the polymer has buffering capacity which induces a 'proton sponge' activity resulting in vesicles swelling and endosomolysis ending by the release of DNA in the cytoplasm without the help of additives (Boussif *et al.*, 1995; Sonawane *et al.*, 2003). Both branched and linear PEI are efficient in transfection but the linear topology was shown to be more efficient (Itaka *et al.*, 2004), not inhibited by the presence of serum and less toxic when compared to the branched form. Since two decades, many strategies were developed to increase the transfection efficiency of PEI, reduce its toxicity or propose alternative of biodegradable PEI-based polymers.

Many works were concentrated on the optimisation of the intrinsic proton-sponge endosomolytic activities of PEI by grafting histidyl or benzyl residues (US8658150, Chandrashekhhar *et al.*, 2012) to the polymers. Other modifications were explored like addition of hydrophilic groups (EP2070970) to increase the solubility of DNA/PEI complexes and reduce the cell toxicity. Hydrophobic functionalities were added to PEI to increase the biodegradability of the

polymer using N-acyl groups (EP0262641) or to generate lipopolymers (US20090022746, WO2006/041617). Higher gene transfection efficiencies were observed in various cell lines. However, the efficiency in "hard to transfect" cells remained very limited.

Other cationic polymers were described for DNA transfection such as chitosan (Erbacher *et al.*, 1998), polyamidoamine (PAMAM) dendrimers (Tomalia *et al.*, 1985, Haensler and Szoka, 2003), degraded or fractured dendrimers (Tang *et al.*, 1996), structurally flexible dendrimers (Liu *et al.*, 2011), polyaminoesters (Little *et al.*, 2004), poly(α [4-aminobutyl]-L-glycolic acid) (Akinc *et al.*, 2003), cationic cyclodextrin amphiphiles (Cryan *et al.*, 2004), poly(N-methylvinylamine) (Dréan *et al.*, 2018), poly(2-N-dimethylaminoethyl)methacrylate (PDMAEMA), polyallylamine (Boussif *et al.*, 1999), polyornithine (Dong *et al.*, 1993), polyarginine (Alhakamy *et al.*, 2013), polyhistidine (Putman *et al.*, 2003) and cell penetrating peptides (CPPs) (Gupta, 2005).

It was reported that cationic polymers such as PEI were able to transfect post-mitotic cells (Brunner *et al.*). However, in the absence of mitosis and the subsequent breakdown of the nuclear membrane, it was shown that plasmid DNA, because of its large size > 1 kbp, was not able to enter the nucleus through the nuclear pore complexes (Lukacs *et al.* 2000). Once released from endosomes, DNA was still associated with some cationic polymers which contributed to protect it against the nuclease degradation (Lechardeur *et al.*, 1999). It is known that DNA is able to interact with proteins present in the cytoplasm, particularly dynein, allowing a microtubule-based movement towards the nucleus or binding of transcription factors having NLS signals, which may direct DNA to the nuclear pore complexes through the importin pathway (Bai *et al.*, 2017).

Cationic polymers represent one class of delivery reagents suitable for in vivo applications for gene therapy approach where DNA/cationic polymer complexes are directly injected through different routes of administration, such as intravenous, intraperitoneal, intradermal, intratumoral or intracerebral injection. Cationic polymers formulated with an acceptable excipient and /or buffering agent are suitable for in vivo gene transfer. Particularly, PEI was reported as an efficient polymer for in vivo applications (Boussif *et al.*, 1995).

Due to their special structural features and electron-rich environment, heterocyclic compounds such as pyrazole, imidazole or triazole derivatives, in particular triazole derivatives exhibit a broad spectrum of bioactivities. Triazoles derivatives may have properties to influence the pH in endosomes. In addition, the triazole may contribute to hydrogen bonds with nucleic acids. The addition of cycloalkyl or aryl moieties to triazole may offer supplementary hydrophobic interactions such as π - π stacking with nucleobases. All together, these properties may fine-tune the interactions with nucleic acids and offer the possibilities to develop new DNA carriers.

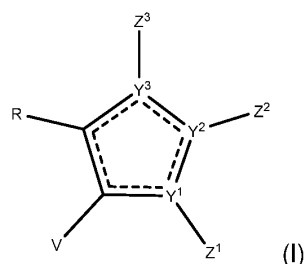
The inventors provide a way to improve transfection reagent by using aromatic heterocyclic compounds, in particular triazole derivatives to fine-tune the affinity and binding to a nucleic acid molecule, *e.g.* DNA, optimize the buffering capacity in acidic conditions and/or increase the diffusion, binding and uptake in the nucleus.

5 Thus it is an object of the present invention to provide a more efficient transfection composition or a formulation for transfecting a nucleic acid molecule into a cell.

It is another object of the present invention to provide a method for transfecting a nucleic acid molecule using said composition or formulation comprising such composition for administration to cells.

10 The inventors carried out a structure-based screening of substituted heterocyclic compounds, in particular imidazole, triazole, pyrazole derivatives, to improve the efficiency of transfection by cationic polymers. Such substituted heterocyclic compounds were grafted to cationic polymers, in particular polyethylenimine (PEI) polymers, of various molecular weight in order to fine-tune the conjugates. Many variations were proposed in order to define optimal
15 structures facilitating transfection of a nucleic acid molecule, *e.g.* DNA. Heterocycles exhibiting hydrophobic properties were developed and may represent binding motifs to cytoplasmic proteins promoting potentially the nuclear import.

20 The present invention relates to a composition suitable for transfecting a nucleic acid molecule into a cell, preferably a eukaryotic cell, comprising (i) at least one compound of general formula (I) or a tautomer, mesomer, racemate, enantiomer, diastereomer, or mixture thereof, or an acceptable salt thereof, and (ii) an acceptable excipient, buffering agent, cell culture medium, or transfection medium:



25 wherein:

- **Y¹, Y² and Y³**, which may be identical or different, represent C or N, with the proviso that at least two of Y¹, Y² and Y³ are N, and with the further proviso that at least one, but no more than two, of Y¹, Y² and Y³ are substituted by Z¹, Z² and Z³ respectively;

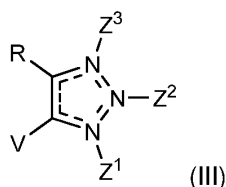
- **Z¹** represents H, X₁-R₃-X₂-P⁺, X₁-R₃-P⁺, X₁-X₂-P⁺, R₃-X₂-P⁺, X₁-P⁺, R₃-P⁺, or X₂-P⁺; or **Z¹** is absent;
- **Z²** represents H, a linear or branched, saturated or unsaturated C₁-C₁₈ alkyl, C₆-C₁₈ aryl, a linear or branched, saturated or unsaturated C₆-C₁₈ aryl-C₁-C₁₈ alkyl, a linear or branched, saturated or unsaturated C₂-C₁₈ heteroalkyl, C₅-C₁₀ heteroaryl, halogen, OH, a linear or branched, saturated or unsaturated C₁-C₁₈ alkylamine, a C₁-C₁₂ alkoxy, a linear or branched, saturated or unsaturated C₁-C₁₈ alkyl-C₁-C₁₂ alkoxy, X₁-R₃-X₂-P⁺, X₁-R₃-P⁺, X₁-X₂-P⁺, R₃-X₂-P⁺, X₁-P⁺, R₃-P⁺, or X₂-P⁺; or **Z²** is absent;
- **Z³** represents H, a linear or branched, saturated or unsaturated C₁-C₁₈ alkyl, C₆-C₁₈ aryl, a linear or branched, saturated or unsaturated C₆-C₁₈ aryl-C₁-C₁₈ alkyl, C₅-C₁₀ heteroaryl, a linear or branched, saturated or unsaturated C₂-C₁₈ heteroalkyl, C₂-C₁₈ alkylidene, OH, guanidine, halogen, X₁-R₃-X₂-P⁺, X₁-R₃-P⁺, X₁-X₂-P⁺, R₃-X₂-P⁺, X₁-P⁺, R₃-P⁺, or X₂-P⁺; or **Z³** is absent;
- **X₁** and **X₂**, which may be identical or different, represent CO or CH₂;
- **R₃** represents (CH₂)_m, (CH₂)_m-CHCH₃-(CH₂)_n-, (CH₂)_m-C(CH₃)₂-(CH₂)_n-, (CH₂)_m-O-(CH₂)_n-, (CH₂)_m-S-(CH₂)_n-, (CH₂)_m-CH₂-O-, with m representing an integer between 1 and 3, preferably m is equal to 2 and n representing an integer between 1 and 3;
- **P⁺** represents a graft cationic polymer, which is a polyamine comprising secondary amines, tertiary amines, a mixture of primary and secondary amines, a mixture of primary and tertiary amines, a mixture of secondary and tertiary amines, or a mixture of primary, secondary and tertiary amines;
- **R** or **V** represents H, a linear or branched, saturated or unsaturated C₁-C₁₈ alkyl or cycloalkyl, a C₆-C₁₈ aryl, a linear or branched, saturated or unsaturated C₆-C₁₈ aryl-C₁-C₁₈ alkyl, a linear or branched, saturated or unsaturated C₂-C₁₈ heteroalkyl, a linear or branched, saturated or unsaturated C₁-C₂₄ ester, a C₅-C₁₀ heteroaryl, a C₅-C₁₀ heterocyclyl, a linear or branched, saturated or unsaturated C₁-C₁₈ alkyl-C₅-C₁₀ heteroaryl, X₁-R₃-X₂-P⁺, X₁-R₃-P⁺, X₁-X₂-P⁺, R₃-X₂-P⁺, X₁-P⁺, R₃-P⁺, or X₂-P⁺;

with the proviso that:

- only one of **Z¹**, **Z²**, **Z³**, **R** or **V** represents X₁-R₃-X₂-P⁺, X₁-R₃-P⁺, X₁-X₂-P⁺, R₃-X₂-P⁺, X₁-P⁺, R₃-P⁺, or X₂-P⁺, preferably X₁-R₃-X₂-P⁺.

In a preferred embodiment of the invention, the composition suitable for transfecting a nucleic acid molecule into a cell, preferably a eukaryotic cell, comprises (i) at least one compound of general formula (III) or a tautomer, mesomer, racemate, enantiomer, diastereomer, or mixture

thereof, or an acceptable salt thereof, and (ii) an acceptable excipient, buffering agent, cell culture medium, or transfection medium:



wherein:

- 5 - **Z¹** represents H, X₁-R₃-X₂-P⁺, X₁-R₃-P⁺, X₁-X₂-P⁺, R₃-X₂-P⁺, X₁-P⁺, R₃-P⁺, or X₂-P⁺; or **Z¹** is absent;
- **Z²** represents H, a linear or branched, saturated or unsaturated C₁-C₁₈ alkyl, C₆-C₁₈ aryl, a linear or branched, saturated or unsaturated C₆-C₁₈ aryl-C₁-C₁₈ alkyl, a linear or branched, saturated or unsaturated C₂-C₁₈ heteroalkyl, C₅-C₁₀ heteroaryl, halogen, OH, a linear or branched, saturated or unsaturated C₁-C₁₈ alkylamine, a C₁-C₁₂ alkoxy, a linear or branched, saturated or unsaturated C₁-C₁₈ alkyl-C₁-C₁₂ alkoxy, X₁-R₃-X₂-P⁺, X₁-R₃-P⁺, X₁-X₂-P⁺, R₃-X₂-P⁺, X₁-P⁺, R₃-P⁺, or X₂-P⁺; or **Z²** is absent;
- 10 - **Z³** represents H, a linear or branched, saturated or unsaturated C₁-C₁₈ alkyl, C₆-C₁₈ aryl, a linear or branched, saturated or unsaturated C₆-C₁₈ aryl-C₁-C₁₈ alkyl, C₅-C₁₀ heteroaryl, a linear or branched, saturated or unsaturated C₂-C₁₈ heteroalkyl, C₂-C₁₈ alkylidene, OH, guanidine, halogen, X₁-R₃-X₂-P⁺, X₁-R₃-P⁺, X₁-X₂-P⁺, R₃-X₂-P⁺, X₁-P⁺, R₃-P⁺, or X₂-P⁺; or **Z³** is absent;
- 15 - **X₁** and **X₂**, which may be identical or different, represent CO or CH₂;
- **R₃** represents (CH₂)_m, (CH₂)_m-CHCH₃-(CH₂)_n-, (CH₂)_m-C(CH₃)₂-(CH₂)_n-, (CH₂)_m-O-(CH₂)_n-, (CH₂)_m-S-(CH₂)_n-, (CH₂)_m-CH₂-O-, with m representing an integer between 1 and 3, preferably m is equal to 2 and n representing an integer between 1 and 3;
- 20 - **P⁺** represents a graft cationic polymer, which is a polyamine comprising secondary amines, tertiary amines, a mixture of primary and secondary amines, a mixture of primary and tertiary amines, a mixture of secondary and tertiary amines, or a mixture of primary, secondary and tertiary amines;
- 25 - **R** or **V** represents H, a linear or branched, saturated or unsaturated C₁-C₁₈ alkyl or cycloalkyl, a C₆-C₁₈ aryl, a linear or branched, saturated or unsaturated C₆-C₁₈ aryl-C₁-C₁₈ alkyl, a linear or branched, saturated or unsaturated C₂-C₁₈ heteroalkyl, a linear or branched, saturated or unsaturated C₁-C₂₄ ester, a C₅-C₁₀ heterocyclyl, a C₅-C₁₀ heteroaryl, a linear or branched, saturated or unsaturated C₁-C₁₈ alkyl-C₅-C₁₀ heteroaryl, X₁-R₃-X₂-P⁺, X₁-R₃-P⁺, X₁-X₂-P⁺, R₃-X₂-P⁺, X₁-P⁺, R₃-P⁺, or X₂-P⁺;
- 30

with the provisos that:

- at least one of **Z**¹, **Z**² or **Z**³ is present, preferably **Z**¹ or **Z**³ is present; and
- only one of **Z**¹, **Z**², **Z**³, **R** or **V** represents X₁-R₃-X₂-P⁺, X₁-R₃-P⁺, X₁-X₂-P⁺, R₃-X₂-P⁺, X₁-P⁺, R₃-P⁺, or X₂-P⁺.

5

In a particular embodiment of the above composition, only one of **Z**¹, **Z**² or **Z**³ is present, preferably **Z**¹ or **Z**³ is present.

As defined herein, the term “*tautomer*” refers to structural isomers differing only in the positions of hydrogen atoms and electrons. Examples of tautomers include, but are not limited to, ketone-enol, enamine-imine, amide-imidic acid, lactam-lactim, nitroso-oxime, ketene-ynol, amino acid, or phosphite-phosphonate.

As defined herein, the term “*mesomer*” or “*meso compound*” refers to a stereoisomer that has two or more chiral centers but is optically inactive.

As defined herein, the term “*racemate*” or “*racemic mixtures*” refers to a mixture of two enantiomers in equal proportions.

As defined herein, the term “*enantiomer*” refers stereoisomers that are mirror images, *i.e.* mirror image isomers.

As defined herein, the term “*diastereomer*” refers to isomers of compounds with more than one chiral center that are not mirror images of one another.

As defined herein, the term “*acceptable excipient*” refers to a pharmaceutically acceptable vehicle, which is any substance or combination of substances physiologically acceptable *i.e.*, appropriate for its use in a composition in contact with a host, especially a human, and thus non-toxic. It can refer to a solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any conventional type. Examples of suitable acceptable excipients include, but are not limited to, glucose, galactose, lactose, dextrose, maltose, mannitol, sucrose, trehalose, polyethyleneglycol, or pluronic acid.

As defined herein, the term “*buffering agent*” refers to an agent that adjusts, maintains or controls the pH of a solution. Buffering agents can be either the weak acid or weak base that would comprise a buffer solution. Examples of suitable buffering agents include, but are not limited to, sodium carbonate, sodium bicarbonate, sodium hydroxide, calcium bicarbonate, calcium citrate, sodium citrate, magnesium hydroxide, magnesium bicarbonate, potassium acetate, Tris acetate, sodium acetate, potassium phosphate monobasic, potassium carbonate, potassium bicarbonate, potassium citrate, or magnesium oxide.

As defined herein, the term “*cell culture medium*” or “*transfection medium*” refers to a medium containing serum, synthetic medium, animal-free component medium or chemically defined medium, in particular medium for maintaining cells alive, or for growing, for differentiating or for expanding cells, or for enhancing transfection.

5 As defined herein, the term “*C₁-C₁₈ alkyl*” represents any monovalent radical of a linear or branched hydrocarbon chain comprising 1 to 18 carbon atoms. The term “*C₁-C₆ alkyl*” represents an alkyl group having 1 to 6 carbon atoms. Examples of suitable C₁-C₁₈ alkyl groups include, but are not limited to, C₁-C₄ alkyl groups such as methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, *i*-butyl, *s*-butyl or *t*-butyl, C₆-C₈ alkyl groups such as *n*-hexyl, *n*-heptyl or *n*-octyl, as well as *n*-pentyl, 2-ethylhexyl, 3,5,5-trimethylhexyl, *n*-nonyl, *n*-decyl, *n*-undecyl, *n*-dodecyl or *n*-octadecyl.

10 As defined herein, the term “*C₁-C₁₂ alkoxy*” represents a radical of formula –OR’, wherein R’ is a C₁-C₁₂ alkyl. Examples of suitable C₁-C₁₂ alkoxy groups include, but are not limited to, C₁-C₆ alkoxy groups such as methoxy (–OCH₃), ethoxy (–OCH₂CH₃), *t*-butoxy (–OC(CH₃)₃), or –O(CH₂)₅CH₃.

15 As defined herein, the term “*C₆-C₁₈ aryl*” represents any monovalent radical of an aromatic hydrocarbon comprising 6 to 18 carbon atoms. Examples of suitable C₆-C₁₈ aryl groups include, but are not limited to, phenyl, naphthyl, anthracenyl or phenanthrenyl.

As defined herein, the term “*C₆-C₁₈ aryl-C₁-C₁₈ alkyl*” represents an aryl group as defined herein combined to an alkyl group as defined herein. Examples of suitable C₆-C₁₈ aryl-C₁-C₁₈ alkyl groups include, but are not limited to, benzyl, phenylethyl (or phenethyl), phenylpropyl, phenylbutyl, phenylpentyl, phenylhexyl, naphthylmethyl, naphthylethyl, naphthylpropyl, naphthylbutyl, naphthylpentyl, naphthylhexyl, anthracenylmethyl, anthracenylethyl, anthracenylpropyl, anthracenylbutyl, anthracenylpentyl, anthracenylhexyl, phenanthrenylmethyl, phenanthrenylethyl, phenanthrenylpropyl, phenanthrenylbutyl, phenanthrenylpentyl or
25 phenanthrenylhexyl.

As defined herein, the term “*C₂-C₁₈ heteroalkyl*” represents an alkyl group as defined herein substituted by one or more heteroatoms such as O, N, or S.

As defined herein, the term “*C₅-C₁₀ heteroaryl*” represents any monovalent radical of a monocyclic or bicyclic 5 to 10 membered aromatic group comprising from 1 to 3 heteroatoms independently selected from oxygen, nitrogen and sulfur. Examples of suitable C₅-C₁₀ heteroaryl groups include, but are not limited to, furyl, thienyl, pyrrolyl, pyrazoyl, imidazolyl, isoxazolyl, isothiazoyl, thiazoyl, oxazolyl, 1,2,3-triazolyl, 1,2,4-triazolyl, 1-benzofuryl, 1-benzothienyl, indolyl, benzimidazolyl, indazolyl, 1,2-benzisoxazolyl, 2,1-benzisoxazolyl, 1,2-benzisothiazolyl, 2,1-benzisothiazolyl, benzothiazolyl, benzoxazolyl, benzotriazolyl, pyridyl, pyridinium, quinoliny,

quinolinium, isoquinolinyl, isoquinolinium, pyridazinyl, cinnolinyl, phthalazinyl, pyrimidinyl, quinazolinyl, pyrazinyl or quinoxalinyl.

As defined herein, the term "*C₁-C₁₈ alkylamine*" represents any monovalent radical of a linear or branched hydrocarbon chain comprising 1 to 18 carbon atoms, in which one of the hydrogen atom bonded to a carbon atom is replaced by an amino group. Examples of suitable *C₁-C₁₈ alkylamine* include, but are not limited to, $-(CH_2)_n-NH_2$, with *n* representing an integer between 1 and 18, $-CH_2NHCH_3$, $-CH_2CH(CH_3)-NH_2$, or $-(CH_2)_n-N(CH_3)_2$, with *n* representing an integer between 1 and 6.

As defined herein, the term "*C₁-C₁₈ alkyl-C₁-C₁₂ alkoxy*" represents an alkyl group as defined herein combined to an alkoxy group as defined herein.

As defined herein, the term "*C₂-C₁₈ alkylidene*" refers to a divalent group derived from an alkane by removal of two hydrogen atoms from the same carbon atom, the free valencies being part of a double bond ($=CR_2$). Examples of suitable *C₂-C₁₈ alkylidene* include, but are not limited to, $=CH_2$, $=CH(CH_2CH_3)$, or $=C(CH_3)_2$.

As defined herein, the term "*halogen*" represents an atom of F, Cl, Br or I.

As defined herein, the term "*C₁-C₂₄ ester*" represents a radical of formula $-C(O)OR$, wherein R" is a *C₁-C₂₄ alkyl*, in particular a *C₁-C₁₈ alkyl* as defined herein.

As defined herein, the term "*C₅-C₁₀ heterocyclyl*" refers to any monovalent radical of a monocyclic or bicyclic 5 to 10 membered ring containing one or more heteroatoms such as O, N, or S. Examples of suitable heterocyclyl groups include, but are not limited to, piperidinyl, piperazinyl, tetrahydropyranyl, morpholinyl, thiomorpholinyl or azepanyl.

Unless mentioned otherwise, the groups and radicals defined hereinabove may be unsubstituted or substituted by one or more substituents such as, for example, halogen, alkyl, alkoxy, aryl, heteroaryl, haloalkyl, haloalkoxy, alkoxycarbonyl, alkanoyl, aroyl, formyl, nitrile, nitro, amido, alkylthio, alkylsulfinyl, alkylsulfonyl, arylthio, arylsulfinyl, arylsulfonyl, amino, alkylamino, arylamino, dialkylamino and diarylamino.

In a particular embodiment of the invention, the composition further comprises at least one nucleic acid molecule to be transfected in a cell. Preferably said nucleic acid molecule is selected from the group consisting of a deoxyribonucleic acid (DNA), a ribonucleic acid (RNA), a DNA/RNA hybrid, a short interfering RNA (siRNA), a microRNA (miRNA), a short hairpin RNA (shRNA), a messenger RNA (mRNA), a CRISPR guide RNA, and an expression vector encoding said nucleic acid molecule, in particular a plasmid encoding said nucleic acid molecule, or a plasmid expressing said nucleic acid molecule such as siRNA, microRNA, shRNA, CRISPR guide RNA. Preferably said nucleic acid molecule is a DNA.

When distinct nucleic acids are provided in the composition of the invention, they may be all DNA molecules or all RNA molecules or may be mixtures of DNA and RNA molecules or molecules comprising an association of DNA and RNA strands.

Said nucleic acid molecule may be single stranded or double stranded, and may contain
5 modified or unmodified bases.

The terms "polynucleotide", "nucleic acid", "oligonucleotide", and "*nucleic acid molecule*" are used interchangeably herein to designate these nucleic acid molecules.

The composition according to the invention may be used as a formulation of the nucleic acid molecule with the at least one compound of general formula (I) (including any of its particular
10 embodiments disclosed herein), preferably the at least one compound of general formula (III), and the acceptable excipient, buffering agent, cell culture medium, or transfection medium, in accordance with the disclosure provided herein. It may alternatively be used as a cell culture or as expanded cells, wherein prior to being provided as a culture and/or as expanded cells, isolated cells have been treated with said formulation for transfection. Otherwise stated, the composition
15 of the invention encompasses, as an embodiment, a cell or a cell culture or expanded cells wherein said formulation has been introduced by transfection according to the invention. The cells are in particular mammalian cells, preferably human cells. The cells may be dividing cells or non-dividing cells.

In a particular embodiment of the invention, the composition according to the invention
20 comprises from 1 to 5, preferably at least two distinct compounds of general formula (I), preferably of general formula (III), or a tautomer, mesomer, racemate, enantiomer, diastereomer, or mixture thereof, or an acceptable salt thereof.

In a particular embodiment of the invention, the at least one preferred compound of general formula (I) as defined herein is one wherein: (i) $\mathbf{Y}^1$ and $\mathbf{Y}^3$ represent N, $\mathbf{Y}^2$ represents C; or (ii) $\mathbf{Y}^1$
25 and $\mathbf{Y}^2$ represent N, $\mathbf{Y}^3$ represents C; or (iii) $\mathbf{Y}^2$ and $\mathbf{Y}^3$ represent N, $\mathbf{Y}^1$ represents C; or (iv) $\mathbf{Y}^1$, $\mathbf{Y}^2$ and $\mathbf{Y}^3$ represent N. When (iv) $\mathbf{Y}^1$, $\mathbf{Y}^2$ and $\mathbf{Y}^3$ represent N, the at least one preferred compound of general formula (I) as defined herein corresponds to the compound of general formula (III).

The structure of the compounds of general formula (III) is symmetric so that $\mathbf{R}$ and $\mathbf{V}$ may be interchanged, and $\mathbf{Z}^1$ and $\mathbf{Z}^3$ may be interchanged. Thus the definitions directed to R also apply
30 to V, and the definitions directed to $\mathbf{Z}^1$ also apply to $\mathbf{Z}^3$.

In a particular embodiment of the invention, the at least one preferred compound of general formula (I), preferably of general formula (III), as defined herein is one wherein (i) $\mathbf{Z}^1$ represents H; or (ii) $\mathbf{Z}^1$ represents $\mathbf{X}_1\text{-R}_3\text{-X}_2\text{-P}^+$, $\mathbf{X}_1\text{-R}_3\text{-P}^+$, $\mathbf{X}_1\text{-X}_2\text{-P}^+$, $\mathbf{R}_3\text{-X}_2\text{-P}^+$, $\mathbf{X}_1\text{-P}^+$, $\mathbf{R}_3\text{-P}^+$, or $\mathbf{X}_2\text{-P}^+$, preferably $\mathbf{X}_1\text{-R}_3\text{-X}_2\text{-P}^+$, wherein $\mathbf{X}_1$, $\mathbf{X}_2$, $\mathbf{R}_3$ and $\mathbf{P}^+$ are as defined herein; more preferably $\mathbf{Z}^1$ represents $\mathbf{X}_1\text{-R}_3\text{-}$

X_2-P^+ , wherein X_1 represents CH_2 , X_2 represents CO , and R_3 represents $(CH_2)_m$, with m representing an integer between 1 and 3, preferably m is equal to 2.

In a particular embodiment of the invention, the at least one preferred compound of general formula (I), preferably of general formula (III), as defined herein is one wherein (i) Z^2 represents H, a C_1 - C_{12} alkoxy, or a linear or branched, saturated or unsaturated C_1 - C_{18} alkyl, preferably a linear or branched, saturated or unsaturated C_1 - C_6 alkyl; more preferably Z^2 represents H, CH_3 , CF_3 or OCH_3 ; even more preferably Z^2 represents CH_3 ; or (ii) Z^2 represents $X_1-R_3-X_2-P^+$, $X_1-R_3-P^+$, $X_1-X_2-P^+$, $R_3-X_2-P^+$, X_1-P^+ , R_3-P^+ , or X_2-P^+ , preferably $X_1-R_3-X_2-P^+$, wherein X_1 , X_2 , R_3 and P^+ are as defined herein; more preferably Z^2 represents $X_1-R_3-X_2-P^+$, wherein X_1 represents CH_2 , X_2 represents CO , and R_3 represents $(CH_2)_m$, with m representing an integer between 1 and 3, preferably m is equal to 2.

In a particular embodiment of the invention, the at least one preferred compound of general formula (I), preferably of general formula (III), as defined herein is one wherein (i) Z^3 represents H, a linear or branched, saturated or unsaturated C_1 - C_{18} alkyl, preferably a linear or branched, saturated or unsaturated C_1 - C_6 alkyl, or a linear or branched, saturated or unsaturated C_6 - C_{18} aryl- C_1 - C_{18} alkyl, preferably fluorobenzyl or 4-hydroxyphenethyl; or (ii) Z^3 represents $X_1-R_3-X_2-P^+$, $X_1-R_3-P^+$, $X_1-X_2-P^+$, $R_3-X_2-P^+$, X_1-P^+ , R_3-P^+ , or X_2-P^+ , preferably $X_1-R_3-X_2-P^+$, wherein X_1 , X_2 , R_3 and P^+ are as defined herein; more preferably Z^3 represents $X_1-R_3-X_2-P^+$, wherein X_1 represents CH_2 , X_2 represents CO , and R_3 represents $(CH_2)_m$, with m representing an integer between 1 and 3, preferably m is equal to 2.

In a preferred embodiment of the invention, if (i) Z^1 represents $X_1-R_3-X_2-P^+$, $X_1-R_3-P^+$, $X_1-X_2-P^+$, $R_3-X_2-P^+$, X_1-P^+ , R_3-P^+ , or X_2-P^+ , preferably $X_1-R_3-X_2-P^+$, wherein X_1 , X_2 , R_3 and P^+ are as defined herein; more preferably Z^1 represents $X_1-R_3-X_2-P^+$, wherein X_1 represents CH_2 , X_2 represents CO , and R_3 represents $(CH_2)_m$, with m representing an integer between 1 and 3, preferably m is equal to 2 then (ii) Z^2 represents H, a C_1 - C_{12} alkoxy, or a linear or branched, saturated or unsaturated C_1 - C_{18} alkyl, preferably a linear or branched, saturated or unsaturated C_1 - C_6 alkyl; more preferably Z^2 represents H, CH_3 , CF_3 or OCH_3 ; and/or (iii) Z^3 represents H, a linear or branched, saturated or unsaturated C_1 - C_{18} alkyl, preferably a linear or branched, saturated or unsaturated C_1 - C_6 alkyl, or a linear or branched, saturated or unsaturated C_6 - C_{18} aryl- C_1 - C_{18} alkyl, preferably fluorobenzyl or 4-hydroxyphenethyl; and/or (iv) R or V represents H, a linear or branched, saturated or unsaturated C_1 - C_{18} alkyl or cycloalkyl, a C_6 - C_{18} aryl, a linear or branched, saturated or unsaturated C_6 - C_{18} aryl- C_1 - C_{18} alkyl, a linear or branched, saturated or unsaturated C_2 - C_{18} heteroalkyl, a linear or branched, saturated or unsaturated C_1 - C_{24} ester, a C_5 - C_{10}

heterocyclyl, a C₅-C₁₀ heteroaryl, or a linear or branched, saturated or unsaturated C₁-C₁₈ alkyl-C₅-C₁₀ heteroaryl.

In another preferred embodiment of the invention, if (i) **Z**² represents X₁-R₃-X₂-P⁺, X₁-R₃-P⁺, X₁-X₂-P⁺, R₃-X₂-P⁺, X₁-P⁺, R₃-P⁺, or X₂-P⁺, preferably X₁-R₃-X₂-P⁺, wherein **X**₁, **X**₂, **R**₃ and **P**⁺ are as defined herein; more preferably **Z**² represents X₁-R₃-X₂-P⁺, wherein **X**₁ represents CH₂, **X**₂ represents CO, and **R**₃ represents (CH₂)_m, with m representing an integer between 1 and 3, preferably m is equal to 2 then (ii) **Z**¹ represents H; and/or (iii) **Z**³ represents H, a linear or branched, saturated or unsaturated C₁-C₁₈ alkyl, preferably a linear or branched, saturated or unsaturated C₁-C₆ alkyl, or a linear or branched, saturated or unsaturated C₆-C₁₈ aryl-C₁-C₁₈ alkyl, preferably fluorobenzyl or 4-hydroxyphenethyl; and/or (iv) **R** or **V** represents H, a linear or branched, saturated or unsaturated C₁-C₁₈ alkyl or cycloalkyl, a C₆-C₁₈ aryl, a linear or branched, saturated or unsaturated C₆-C₁₈ aryl-C₁-C₁₈ alkyl, a linear or branched, saturated or unsaturated C₂-C₁₈ heteroalkyl, a linear or branched, saturated or unsaturated C₁-C₂₄ ester, a C₅-C₁₀ heterocyclyl, a C₅-C₁₀ heteroaryl, or a linear or branched, saturated or unsaturated C₁-C₁₈ alkyl-C₅-C₁₀ heteroaryl.

In another preferred embodiment of the invention, if (i) **Z**³ represents X₁-R₃-X₂-P⁺, X₁-R₃-P⁺, X₁-X₂-P⁺, R₃-X₂-P⁺, X₁-P⁺, R₃-P⁺, or X₂-P⁺, preferably X₁-R₃-X₂-P⁺, wherein **X**₁, **X**₂, **R**₃ and **P**⁺ are as defined herein; more preferably **Z**³ represents X₁-R₃-X₂-P⁺, wherein **X**₁ represents CH₂, **X**₂ represents CO, and **R**₃ represents (CH₂)_m, with m representing an integer between 1 and 3, preferably m is equal to 2 then (ii) **Z**¹ represents H; and/or (iii) **Z**² represents H, a C₁-C₁₂ alkoxy, or a linear or branched, saturated or unsaturated C₁-C₁₈ alkyl, preferably a linear or branched, saturated or unsaturated C₁-C₆ alkyl; more preferably **Z**² represents H, CH₃, CF₃ or OCH₃; and/or (iv) **R** or **V** represents H, a linear or branched, saturated or unsaturated C₁-C₁₈ alkyl or cycloalkyl, a C₆-C₁₈ aryl, a linear or branched, saturated or unsaturated C₆-C₁₈ aryl-C₁-C₁₈ alkyl, a linear or branched, saturated or unsaturated C₂-C₁₈ heteroalkyl, a linear or branched, saturated or unsaturated C₁-C₂₄ ester, a C₅-C₁₀ heterocyclyl, a C₅-C₁₀ heteroaryl, or a linear or branched, saturated or unsaturated C₁-C₁₈ alkyl-C₅-C₁₀ heteroaryl.

In another preferred embodiment of the invention, if (i) **R** or **V** represents X₁-R₃-X₂-P⁺, X₁-R₃-P⁺, X₁-X₂-P⁺, R₃-X₂-P⁺, X₁-P⁺, R₃-P⁺, or X₂-P⁺, preferably X₁-R₃-X₂-P⁺, wherein **X**₁, **X**₂, **R**₃ and **P**⁺ are as defined herein; more preferably **Z**³ represents X₁-R₃-X₂-P⁺, wherein **X**₁ represents CH₂, **X**₂ represents CO, and **R**₃ represents (CH₂)_m, with m representing an integer between 1 and 3, preferably m is equal to 2 then (ii) **Z**¹ represents H; and/or (iii) **Z**² represents H, a C₁-C₁₂ alkoxy, or a linear or branched, saturated or unsaturated C₁-C₁₈ alkyl, preferably a linear or branched, saturated or unsaturated C₁-C₆ alkyl; and/or (iv) **Z**³ represents H, a linear or branched, saturated or unsaturated C₁-C₁₈ alkyl, preferably a linear or branched, saturated or unsaturated C₁-C₆ alkyl,

or a linear or branched, saturated or unsaturated C₆-C₁₈ aryl-C₁-C₁₈ alkyl, preferably fluorobenzyl or 4-hydroxyphenethyl.

In a particular embodiment of the invention, the at least one preferred compound of general formula (I), preferably of general formula (III), as defined herein is one wherein: (i) only one of **Z**¹, **Z**² or **Z**³ represents X₁-R₃-X₂-P⁺, X₁-R₃-P⁺, X₁-X₂-P⁺, R₃-X₂-P⁺, X₁-P⁺, R₃-P⁺, or X₂-P⁺, preferably X₁-R₃-X₂-P⁺, wherein **X**₁, **X**₂, **R**₃ and **P**⁺ are as defined herein; more preferably only one of **Z**¹, **Z**² or **Z**³ represents X₁-R₃-X₂-P⁺, wherein **X**₁ represents CH₂, **X**₂ represents CO, and **R**₃ represents (CH₂)_m, with m representing an integer between 1 and 3, preferably m is equal to 2; and/or (ii) **Z**¹ represents H; and/or (iii) **Z**² represents H, a C₁-C₁₂ alkoxy, or a linear or branched, saturated or unsaturated C₁-C₁₈ alkyl, preferably a linear or branched, saturated or unsaturated C₁-C₆ alkyl; more preferably **Z**² represents H, CH₃, CF₃ or OCH₃; and/or (iv) **Z**³ represents H, or a linear or branched, saturated or unsaturated C₁-C₁₈ alkyl, preferably a linear or branched, saturated or unsaturated C₁-C₆ alkyl.

In a particular embodiment of the invention, the at least one preferred compound of general formula (I), preferably of general formula (III), as defined herein is one wherein: if (i) **R** or **V** represents X₁-R₃-X₂-P⁺, X₁-R₃-P⁺, X₁-X₂-P⁺, R₃-X₂-P⁺, X₁-P⁺, R₃-P⁺, or X₂-P⁺, preferably X₁-R₃-X₂-P⁺, wherein **X**₁, **X**₂, **R**₃ and **P**⁺ are as defined herein; more preferably **Z**³ represents X₁-R₃-X₂-P⁺, wherein **X**₁ represents CH₂, **X**₂ represents CO, and **R**₃ represents (CH₂)_m, with m representing an integer between 1 and 3, preferably m is equal to 2 then (ii) **Z**³ is present and **Z**³ represents H, a linear or branched, saturated or unsaturated C₁-C₁₈ alkyl, preferably a linear or branched, saturated or unsaturated C₁-C₆ alkyl, or a linear or branched, saturated or unsaturated C₆-C₁₈ aryl-C₁-C₁₈ alkyl, preferably fluorobenzyl or 4-hydroxyphenethyl.

In a particular embodiment of the invention, the at least one preferred compound of general formula (I), preferably of general formula (III), as defined is one wherein the graft cationic polymer is selected from the group consisting of a linear or branched polyethyleneimine (PEI), PEI dendrimers, a polypropyleneimine (PPI), Poly(amidoamine) (PAA) and dendrimers (PAMAM), cationic cyclodextrin, polyalkylamine, a polyhydroxyalkylamine, poly(butyleneimine) (PBI), spermine, a N-substituted polyallylamine, N-substituted chitosan, a N-substituted polyornithine, a N-substituted polylysine (PLL), a N-substituted polyvinylamine, poly(β-amino ester), hyperbranched poly(amino ester) (h-PAE), networked poly(amino ester) (n-PAE), poly(4-hydroxy-1-proline ester) (PHP-ester) and a poly-β-aminoacid. Preferably the graft cationic polymer is a linear or branched PEI, more preferably a linear PEI.

The graft cationic polymer may have a grafting ratio ranging from 1 to 50%, preferably from 5 to 30%, more preferably is 20%.

As defined herein, the term “*grafting ratio*” refers to the number of grafted monomers on primary or secondary amino groups by side chains, divided by the number of total monomers present in the original cationic polymer. The grafting ratio will depend upon the molecular weight of the cationic polymer, the chemical reactivity of the grafted side chains onto the polymer, or the obtained biological effect. Said grafting ratio may be determined by a measurement method well known in the art, for example by NMR.

The graft cationic polymer may have an average molecular weight (Mw) ranging from 1 kDa to 500 kDa, preferably from 1 kDa to 50 kDa, more preferably from 5 kDa to 50 kDa or from 1 kDa to 15 kDa. In particular the graft cationic polymer may have an average molecular weight (Mw) of 6, 8, 10, 15, 22 or 30 kDa, preferably of 6, 8, 10, 15 or 30 kDa.

The graft cationic polymer can be associated with a counterion such as chloride, phosphate, citrate, acetate, propionate, carbonate, succinate, sulfonate, sulfate, or carboxylate.

In a particular embodiment of the invention, the at least one preferred compound of general formula (I), preferably of general formula (III), as defined herein is one wherein **Y¹**, **Y²**, **Y³**, **Z¹**, **Z²**, **Z³**, **X₁**, **X₂**, **R₃** and **P⁺** are as defined herein; and **R** or **V** represents H, a linear or branched, saturated or unsaturated C₁-C₁₈ alkyl or cycloalkyl, a C₆-C₁₈ aryl, a linear or branched, saturated or unsaturated C₆-C₁₈ aryl-C₁-C₁₈ alkyl, a linear or branched, saturated or unsaturated C₂-C₁₈ heteroalkyl, a linear or branched, saturated or unsaturated C₁-C₂₄ ester, a C₅-C₁₀ heterocyclyl, a C₅-C₁₀ heteroaryl, a linear or branched, saturated or unsaturated C₁-C₁₈ alkyl-C₅-C₁₀ heteroaryl, **X₁-R₃-X₂-P⁺**, **X₁-R₃-P⁺**, **X₁-X₂-P⁺**, **R₃-X₂-P⁺**, **X₁-P⁺**, **R₃-P⁺**, or **X₂-P⁺**.

Preferably, **R** or **V** represents H, methyl, ethyl, propyl, cyclopropyl, isopropyl, sec-butyl, cyclopentyl, phenyl, fluorophenyl, benzyl, pyridine, 2-pyridine, 3-pyridine, fluorobenzyl, substituted morpholinyl, substituted piperazinyl, 4-hydroxybenzyl, or 4-hydroxyphenethyl; more preferably **R** or **V** represents methyl, ethyl, propyl, cyclopropyl, isopropyl, sec-butyl, cyclopentyl, phenyl, benzyl, fluorobenzyl, 4-hydroxyphenethyl, 2-pyridine or 3-pyridine.

The most preferred embodiments for compounds of formula (III) in relation to **Z¹**, **Z²**, **Z³**, **X₁**, **X₂**, **R₃** and **P** are as defined herein for compounds of formula (I).

In a particular embodiment of the invention, preferred compounds are those wherein only one of **Z¹**, **Z²** or **Z³**, preferably **Z¹**, represents **X₁-R₃-X₂-P⁺**, **X₁-R₃-P⁺**, **X₁-X₂-P⁺**, **R₃-X₂-P⁺**, **X₁-P⁺**, **R₃-P⁺**, or **X₂-P⁺**, preferably **X₁-R₃-X₂-P⁺**, wherein **X₁**, **X₂**, **R₃** and **P⁺** are as defined in formula (I), preferably in formula (III).

In a particular embodiment of the invention, preferred compounds are those wherein only one of **R** or **V** represents **X₁-R₃-X₂-P⁺**, **X₁-R₃-P⁺**, **X₁-X₂-P⁺**, **R₃-X₂-P⁺**, **X₁-P⁺**, **R₃-P⁺**, or **X₂-P⁺**,

preferably $X_1-R_3-X_2-P^+$, wherein X_1 , X_2 , R_3 and P^+ are as defined in formula (I), preferably in formula (III).

In a particular embodiment of the invention, preferred compounds are those wherein Y^1 , Y^2 and Y^3 represent N. These compounds correspond to compounds of general formula (III).

5 In a particular embodiment of the invention, preferred compounds are those wherein Z^3 represents a linear or branched, saturated or unsaturated C_6-C_{18} aryl- C_1-C_{18} alkyl, preferably fluorobenzyl or 4-hydroxyphenethyl.

10 In a particular embodiment of the invention, preferred compounds are those wherein R represents H, methyl, propyl, isopropyl, cyclopropyl, benzyl, fluorobenzyl, pyridine, 2-pyridine, 3-pyridine, phenyl, fluorophenyl, substituted morpholinyl or substituted piperazinyl.

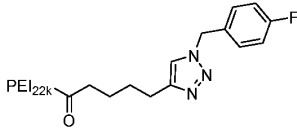
In a particular embodiment of the invention, preferred compounds are those wherein V represents H, $X_1-R_3-X_2-P^+$, $X_1-R_3-P^+$, $X_1-X_2-P^+$, $R_3-X_2-P^+$, X_1-P^+ , R_3-P^+ , or X_2-P^+ , preferably $X_1-R_3-X_2-P^+$, wherein X_1 , X_2 , R_3 and P^+ are as defined in formula (I), preferably in formula (III).

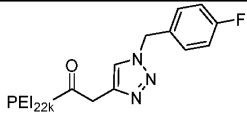
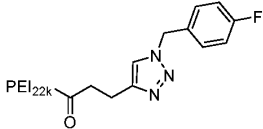
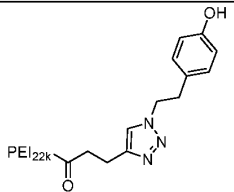
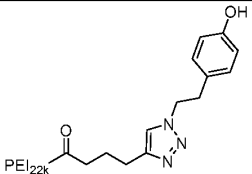
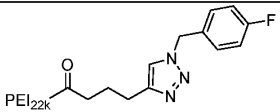
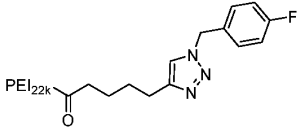
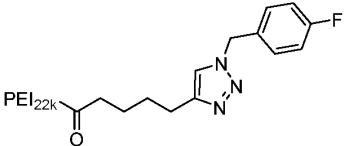
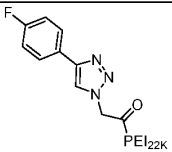
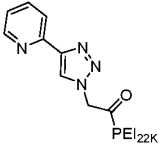
15 In a preferred embodiment of the invention, preferred compounds are those wherein (i) Y^1 , Y^2 and Y^3 represent N; and/or (ii) V represents $X_1-R_3-X_2-P^+$, $X_1-R_3-P^+$, $X_1-X_2-P^+$, $R_3-X_2-P^+$, X_1-P^+ , R_3-P^+ , or X_2-P^+ , preferably $X_1-R_3-X_2-P^+$, wherein X_1 , X_2 , R_3 and P^+ are as defined in formula (I); and/or (iii) R represents H; and/or (iv) Z^3 represents fluorobenzyl or 4-hydroxyphenethyl.

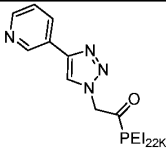
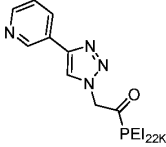
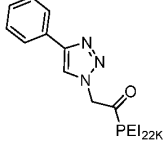
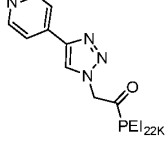
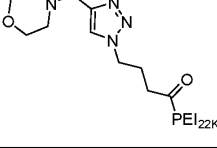
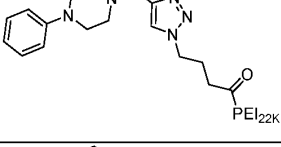
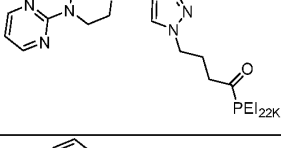
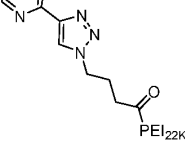
20 In a preferred embodiment of the invention, preferred compounds are those wherein (i) Y^1 , Y^2 and Y^3 represent N; and/or (ii) Z^1 represents $X_1-R_3-X_2-P^+$, $X_1-R_3-P^+$, $X_1-X_2-P^+$, $R_3-X_2-P^+$, X_1-P^+ , R_3-P^+ , or X_2-P^+ , preferably $X_1-R_3-X_2-P^+$; and/or (iii) V represents H; and/or (iv) R represents benzyl, fluorobenzyl, pyridine, 2-pyridine, 3-pyridine, methyl, propyl, isopropyl, cyclopropyl, phenyl, fluorophenyl, substituted morpholinyl or substituted piperazinyl.

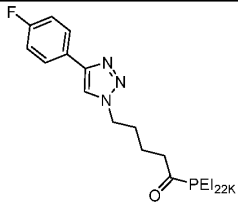
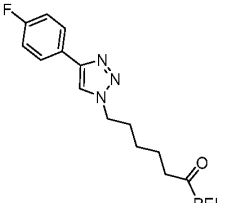
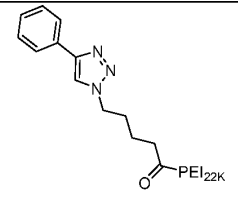
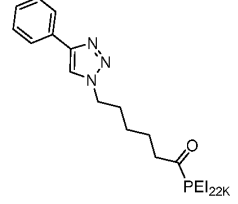
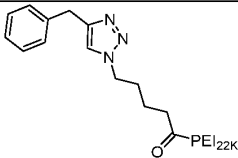
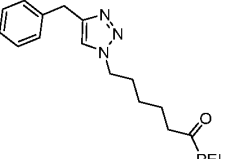
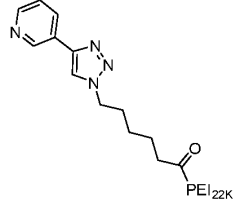
25 According to a particular embodiment of the invention, preferred compounds correspond to compounds 2.19 to 2.61, preferably compounds 2.19, 2.22, 2.23, 2.42, 2.43, 2.44, 2.46, 2.47, 2.54, 2.55, 2.56, 2.57, 2.58, 2.59, 2.60 and 2.61 as disclosed in Table 1.

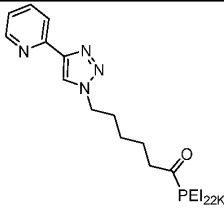
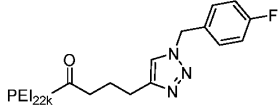
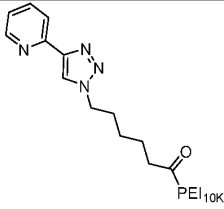
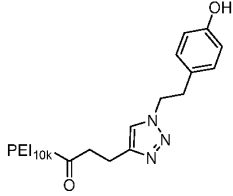
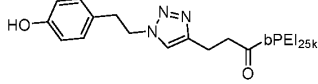
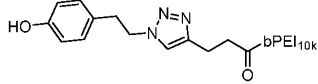
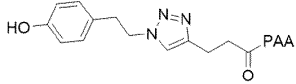
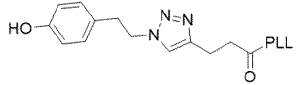
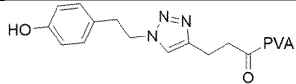
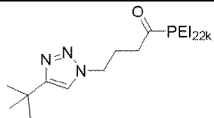
Table 1. Structures of preferred compounds of the invention

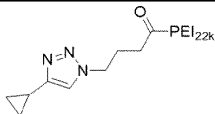
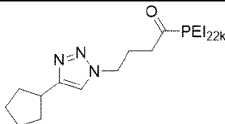
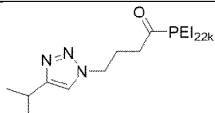
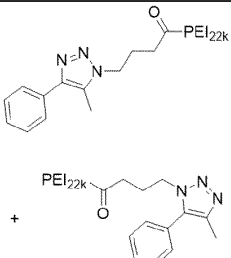
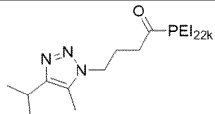
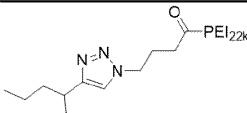
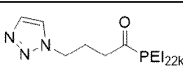
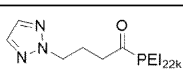
Compounds	Structures	Molecular weight	Heterocycle Grafting (%)
2.19		22K	24%

2.20		22K	16%
2.21		22K	13%
2.22		22k	18%
2.23		22k	15%
2.24		22K	11%
2.25		22K	19%
2.26		22K	25%
2.27		22K	9%
2.28		22K	20%

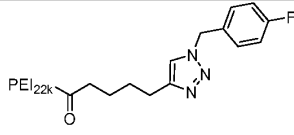
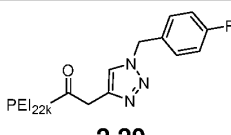
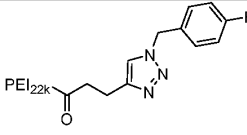
2.29		22K	6%
2.30		22K	21%
2.31		22K	13%
2.32		22K	7%
2.33		22K	25%
2.34		22K	19%
2.35		22K	18%
2.36		22K	31%

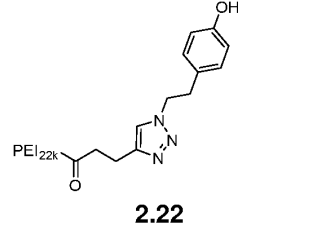
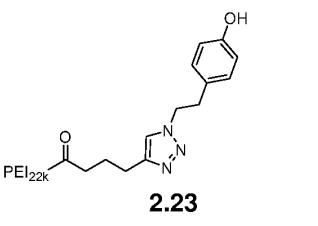
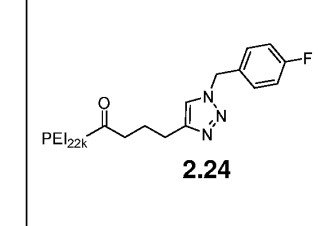
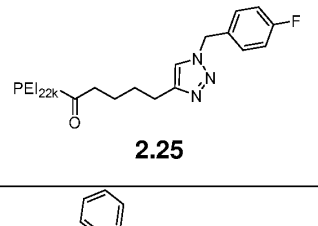
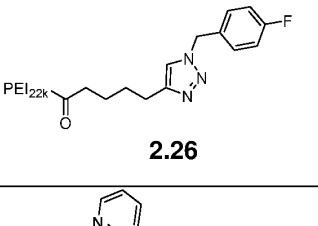
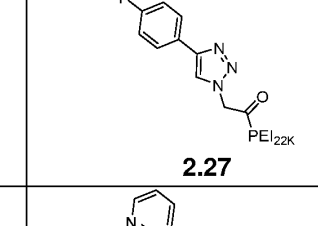
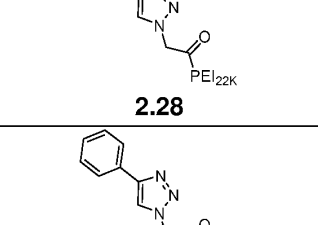
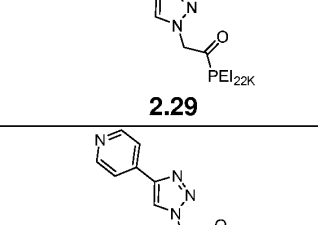
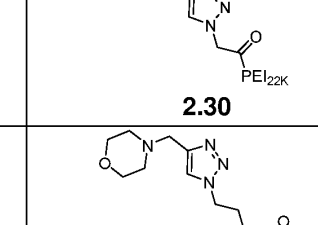
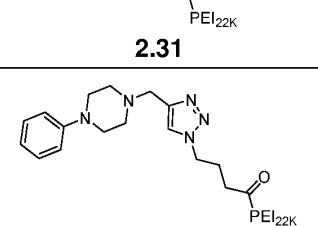
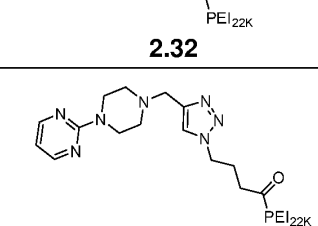
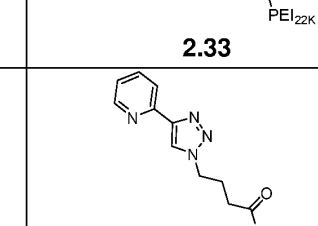
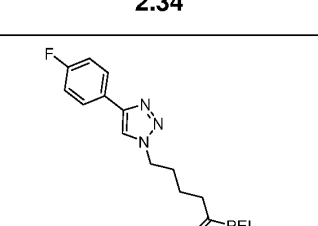
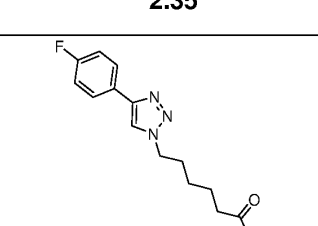
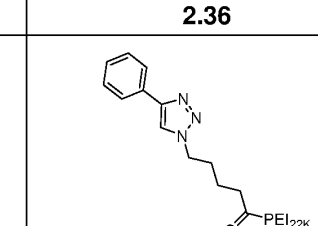
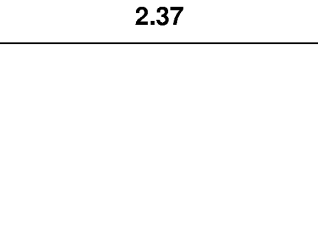
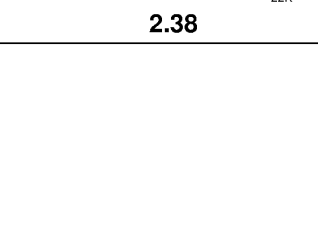
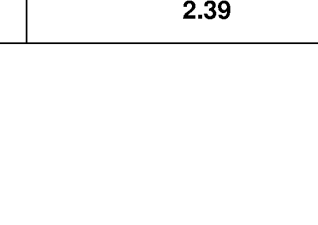
2.37		22k	25%
2.38		22k	24%
2.39		22k	21%
2.40		22k	23%
2.41		22k	19%
2.42		22k	17%
2.43		22k	18%

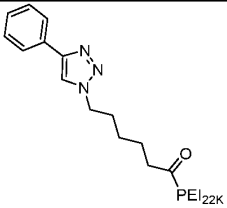
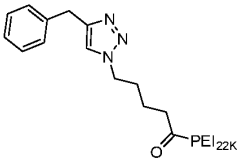
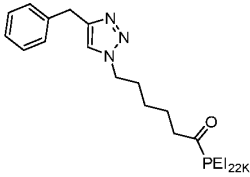
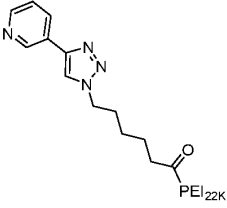
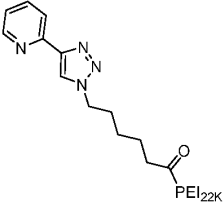
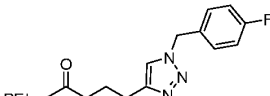
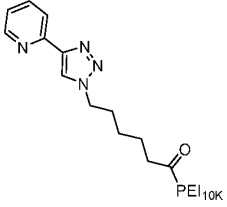
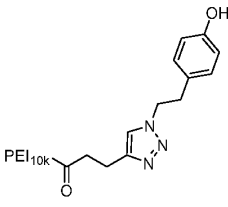
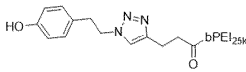
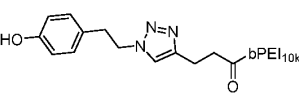
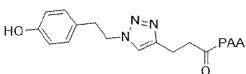
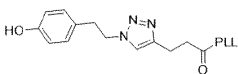
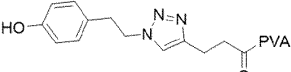
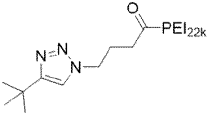
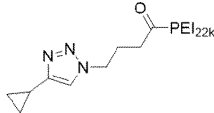
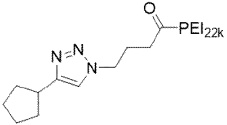
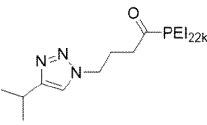
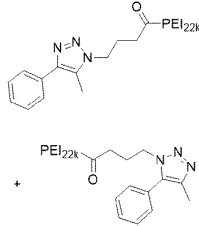
2.44		10k	18%
2.45		22K	7%
2.46		10k	22%
2.47		10k	16%
2.48		25k	21%
2.49		10k	19%
2.50		15k	25%
2.51		22k	17%
2.52		25k	16%
2.53		22k	16%

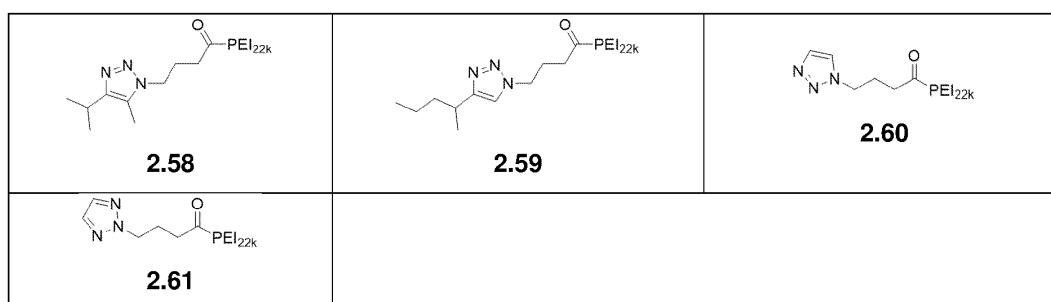
2.54		22k	19%
2.55		22k	17%
2.56		22k	19%
2.57		22k	22%
2.58		22k	21%
2.59		22k	20%
2.60		22k	25%
2.61		22k	25%

In a particular embodiment of the invention, the at least one compound of general formula (III) is selected from the group consisting of the following compounds:

 2.19	 2.20	 2.21
--	--	---

 2.22	 2.23	 2.24
 2.25	 2.26	 2.27
 2.28	 2.29	 2.30
 2.31	 2.32	 2.33
 2.34	 2.35	 2.36
 2.37	 2.38	 2.39

 2.40	 2.41	 2.42
 2.43	 2.44	 2.45
 2.46	 2.47	 2.48
 2.49	 2.50	 2.51
 2.52	 2.53	 2.54
 2.55	 2.56	 2.57



In a preferred embodiment of the invention, the at least one compound of general formula (III) is selected from the group consisting of the following compounds: **2.19, 2.22, 2.42, 2.43, 2.44, 2.46, 2.47, 2.54, 2.55, 2.56, 2.57, 2.58, 2.59, 2.60** and **2.61**, even more preferably is compound **2.22**.

The at least one compound of general formula (I), preferably of general formula (III), may be prepared according to various methods well known in the art.

The present invention is also directed to the composition according to the invention for use in *in vivo* applications for cell transformation by uptake of exogenous nucleic acid using the composition of the invention, for cell therapy or for gene therapy. The cells may be eukaryotic cells, in particular mammalian cells, especially human cells, in particular primary cells, either dividing or non-dividing cells.

The present invention also concerns a method for *in vitro* or *ex vivo* transfection of live cells comprising introducing in the cells the composition according to the invention. Said live cells may be provided or maintained in medium containing serum, synthetic medium, animal-free component medium or chemically defined medium.

The present invention also relates to the *in vitro* or *ex vivo* use of the composition according to the invention to transfect at least one nucleic acid molecule into a cell, cell line or cells, preferably a cell, cell line or cells selected from the group consisting of a mammalian cell, an insect cell, a primary cell, an adherent cell, a suspension cell, a dividing cell such as a stem cell, a non-dividing cell such as a neuronal cell, and a cancer cell, said cell, cell line or cells being optionally organized into spheroids, organoids, 2D or 3D cell culture, or provided as fibre or matrix culture, and/or within a bioreactor.

As defined herein, the term "*adherent cells*" refers to cells that need solid support for growth, and are thus anchorage-dependent. Examples of adherent cells include, but are not limited to, MRC-5 cells, HeLa cells, Vero cells, NIH-3T3 cells, L293 cells, CHO cells, BHK-21 cells,

MCF-7 cells, A549 cells, COS cells, HEK 293 cells, Hep G2 cells, SNN-BE(2) cells, BAE-1 cells or SH-SY5Y cells.

As defined herein, the term "*suspension cells*" refers to cells that do not need solid support for growth, and are thus anchorage-independent. Examples of suspension cells include, but are not limited to, NSO cells, U937 cells, Namalawa cells, HL60 cells, WEHI231 cells, Yac 1 cells, Jurkat cells, THP-1 cells, K562 cells or U266B1 cells.

As defined herein, the term "*spheroids*" refers to spherical, heterogenous aggregates of cells in culture that retain three-dimensional architecture.

As defined herein, the term "*organoids*" refers to three-dimensional structures made of collection of organ-specific cell types self-organized in a manner similar to *in vivo*.

As defined herein, the term "*fibre or matrix culture*" refers to three-dimensional cell culture support composed of insoluble elastic fibers or extracellular proteins self-organized into matrix.

Said transfection may be stable or transient, standard or reverse.

As disclosed herein, the composition according to the invention may comprise multiple distinct nucleic acids, in particular selected from the group consisting of multiple plasmid DNA, plasmid DNA and oligonucleotide, plasmid DNA and mRNA for co-transfection.

Said at least one nucleic acid molecule to be transfected may be a gene encoding a protein, a protein fragment, a peptide or an antibody or functional antigen-binding regions thereof, in particular VH and/or VL chains thereof. Said protein may be selected from the group consisting of a reporter protein, a fluorescent protein, an enzyme, a structural protein, a receptor, a transmembrane protein, a therapeutic protein, a cytokine, a toxin, an oncogenic protein, an anti-oncogene, a pro-apoptotic protein, an anti-apoptotic protein, a polymerase, a transcription factor and a capsid protein.

The present invention also relates to the *in vitro* or *ex vivo* use of the composition according to the invention for genome engineering, for cell reprogramming, in particular for the reprogramming of differentiated cells into induced pluripotent stem cells (iPCs), for differentiating cells, or for gene-editing. Such use may be carried out in a culture of cells *in vitro* or *ex vivo* for the production of biologics, for the preparation of cells for therapy purpose, or for the study of cell functions or behaviour in particular with a step of expansion of cells after their transfection or may be carried out *in vivo* for a therapeutic purpose in a host in need thereof.

The present invention also relates to the *in vitro* or *ex vivo* use of the composition according to the invention (i) in the production of biologics, in particular biologics encoding a recombinant protein, peptide or antibody; or (ii) in the production of recombinant virus, such as adeno-associated virus (AAV), lentivirus (LV), adenovirus, oncolytic virus, or baculovirus, said

composition comprising multiple nucleic acid molecules for co-transfection such as a plurality of plasmids; or (iii) in the production of viral or virus-like particles, said composition comprising multiple nucleic acid molecules for co-transfection such as a plurality of plasmids.

Thus the present invention also relates to a method for the production of (i) biologics, in particular biologics encoding a recombinant protein, peptide or antibody; or (ii) recombinant virus, such as adeno-associated virus (AAV), lentivirus (LV), adenovirus, oncolytic virus, or baculovirus, wherein the composition according to the invention comprises multiple nucleic acid molecules for co-transfection; or (iii) viral or virus-like particles, wherein the composition according to the invention comprises multiple nucleic acid molecules for co-transfection.

In a preferred embodiment of the method for the production of AAV, said composition comprises (i) at least one compound selected from the group consisting of compounds **2.22**, **2.23**, **2.43**, **2.44**, **2.47**, **2.54**, **2.57**, **2.60** and **2.61** and (ii) an acceptable excipient, buffering agent, cell culture medium, or transfection medium.

In a preferred embodiment of the method for the production of LV, said composition comprises (i) at least the compound **2.22**, and (ii) an acceptable excipient, buffering agent, cell culture medium, or transfection medium.

As defined herein, the term “*biologics*” refers to proteins or nucleic acids or combinations thereof, living entities such as cells or viruses, cell compartments, organoids, and tissues.

In a particular embodiment of the invention, said *in vitro* or *ex vivo* use of the composition or said method according to the invention is for the production of recombinant virus, said composition comprising a plurality of expression vectors such as plasmid vectors to transfect in an adherent or suspension cell, such as HEK293 and derivative cells, HeLa, BHK-21, A549 or insect cells, wherein said vectors, in particular plasmids, are construct expressing viral structural sequences and transfer vector genome for virus or virus-like production and optionally expressing molecules of interest encoded by the transfer vector genome.

In a particular embodiment of the invention, said recombinant virus is for use in *in vivo* applications for cell therapy or for gene therapy.

In a particular embodiment of the invention, the invention relates to the *in vitro* or *ex vivo* use of the composition according to the invention in the production of a recombinant virus, such as an adeno-associated virus (AAV) or a lentivirus (LV), said composition comprising (i) at least one compound selected from the group of compounds **2.22**, **2.23**, **2.42**, **2.43**, **2.44**, **2.46**, **2.47**, **2.54**, **2.57**, **2.60** and **2.61** and (ii) an acceptable excipient, buffering agent, cell culture medium, or transfection medium. Preferably, a composition comprising the compound **2.22** is used in the production of LV; and a composition comprising at least one compound selected from the group

consisting of compounds **2.22**, **2.23**, **2.43**, **2.44**, **2.47**, **2.54**, **2.57**, **2.60** and **2.61** is used in the production of AAV.

Unless otherwise stated, all the above-mentioned embodiments may be combined together. Thus features which are described in the context of separate embodiments may be
5 combined in a single embodiment.

Other features and advantages of the invention will be apparent from the examples which follow and will also be illustrated in the figures.

BRIEF DESCRIPTION OF THE FIGURES

10 **Figure 1. Chemical structure of a compound of general formula (I).**

Figure 2. Percentage of GFP expression after transfection of Caco-2, Hep G2, MDCK and MCF-10A with compounds of Example 3. The ratio 1:3 and 1:4 indicate the ratio of μg of DNA per μL of compound.

15

Figure 3. Production of AAV-2 from suspension HEK-293T cells. AAV-2 vectors expressing the GFP reporter gene were produced in HEK-293T cells grown in suspension in FreeStyle F17 media. Cells were seeded and cultured for 3 days before being transfected by 3 plasmids (pAAV-RC2 vector expressing Rep and Cap, pHelper vector expressing Adeno E2A, Adeno E4 and Adeno VA helper factors, and pAAV-GFP control vector expressing the GFP under
20 the control of a CMV promoter) with PEIpro® or various compounds at ratio 1:2 or 1:3 μg DNA/ μL reagent. AAV titers (transducing unit, TU/mL) were determined 72 hours post-transfection. The results are expressed as relative AAV-2 transducing Units/mL (TU/mL) in comparison to PEIpro® transfection at ratio 1:2 and 1:3.

25

Figure 4. Production of lentivirus particles from suspension HEK-293T cells. Lentivirus expressing the GFP reporter gene was produced in HEK-293T cells grown in suspension in FreeStyle F17 media. Cells were seeded and cultured for 3 days before being transfected by 4 plasmids with PEIpro® or compound **2.22** at ratio 1:2 μg total DNA/ μL reagent. Lentivirus titers
30 (transducing unit, TU/mL) were determined 72 hours post-transfection.

Figure 5. Chemical structure of a compound of general formula (III).

Figure 6. Percentage of GFP expression after transfection of Hep G2 cells with compounds 2.22 and 2.53 to 2.61. The ratio 1:3 and 1:4 indicate the ratio of μg of DNA per μL of compound.

5 **Figure 7. Production of AAV-2 from suspension HEK-293T cells with compounds 2.22 and 2.53 to 2.61.** AAV-2 vectors expressing the GFP reporter gene were produced in HEK-293T cells grown in suspension in FreeStyle F17 media. Cells were seeded and cultured for 3 days before being transfected by 3 plasmids (pAAV-RC2 vector expressing Rep and Cap, pHelper vector expressing Adeno E2A, Adeno E4 and Adeno VA helper factors, and pAAV-GFP control vector expressing the GFP under the control of a CMV promoter) with PEIpro® or various
10 compounds at ratio 1:2 μg DNA/ μL reagent. AAV titers (transducing unit, TU/mL) were determined 72 hours post-transfection. The results are expressed as relative AAV-2 transducing Units/mL (TU/mL).

15 **Figure 8. Influence of the amount of DNA transfected and the ratio of compound 2.22 per μg DNA on the production of AAV-2 from suspension HEK-293T cells.** AAV-2 vectors expressing the GFP reporter gene were produced in HEK-293T cells grown in suspension in FreeStyle F17 media. Cells were seeded and cultured for 3 days before being transfected by 3 plasmids (pAAV-RC2 vector expressing Rep and Cap, pHelper vector expressing Adeno E2A,
20 Adeno E4 and Adeno VA helper factors, and pAAV-GFP control vector expressing the GFP under the control of a CMV promoter) with compound 2.22 (formulated at 15 mM nitrogen concentration) at different ratio of μg DNA/ μL reagent (ratio 1:1.5 to 1:3). AAV titers (transducing unit, TU/mL) were determined 72 hours post-transfection. The results are expressed as relative AAV-2 transducing Units/mL (TU/mL). The cell viability was determined 72 hours post-transfection with a
25 Trypan blue assay.

30 **Figure 9. Influence of time of DNA complexation with compound 2.22 on the production of AAV-2 from suspension HEK-293T cells.** AAV-2 vectors expressing the GFP reporter gene were produced in HEK-293T cells grown in suspension in FreeStyle F17 media. Cells were seeded and cultured for 3 days before being transfected by 3 plasmids (pAAV-RC2 vector expressing Rep and Cap, pHelper vector expressing Adeno E2A, Adeno E4 and Adeno VA helper factors, and pAAV-GFP control vector expressing the GFP under the control of a CMV promoter) with compound 2.22 (formulated at 15 mM nitrogen concentration) at a ratio 1:2 of DNA/ μL reagent and with 1 μg DNA/million cells. AAV titers (transducing unit, TU/mL) were

determined 72 hours post-transfection. The results are expressed as relative AAV-2 transducing Units/mL (TU/mL).

EXAMPLES

5 Experimental section

Material and Methods

Cell culture

Caco-2 (ATCC® HTB-37™) human colon epithelial cells were grown in DMEM 4.5 g/L glucose with 20% FBS supplemented with 1% non-essential amino acids, 1 mM sodium pyruvate, 10 2 mM glutamine and 100 U/mL of penicillin and 100 µg/mL of streptomycin at 37°C in a 5% CO₂ in air atmosphere.

MCF 10A (ATCC® CRL-10317™) human mammary epithelial cells were grown in MEM (Lonza) supplemented with SingleQuots™ Supplements and Growth Factors (Lonza) and 100 ng/ml cholera toxin at 37°C in a 5% CO₂ in air atmosphere.

15 Hep G2 (ATCC® HB-8065™) human hepatocarcinoma cells were grown in MEM (Ozyme) with 10% FBS supplemented with 1% non-essential amino acids, 1 mM sodium pyruvate, 2 mM glutamine and 100 U/mL of penicillin and 100 µg/mL of streptomycin at 37°C in a 5% CO₂ in air atmosphere.

20 MDCK (ATCC® CCL-34™) Madin-Darby canine kidney epithelial cells were grown in MEM (Ozyme) with 10% FBS supplemented with 2 mM glutamine and 100 U/mL of penicillin and 100 µg/mL of streptomycin at 37°C in a 5% CO₂ in air atmosphere.

Primary human dermal fibroblasts were grown in DMEM (Ozyme) supplemented with 10% FBS, 1% non-essential amino acids, 1 mM sodium pyruvate, 2 mM glutamine and 100 U/mL of penicillin and 100 µg/mL of streptomycin at 37°C in a 5% CO₂ in air atmosphere.

25

Transfection assay (96-well format)

One day before transfection, Caco-2, MCF 10A, Hep G2 and MDCK Cells were seeded at 10 000, 25 000, 25 000, 10 000 cells per well (96-well plate format), respectively, in 125 µL of their respective complete medium and incubated at 37°C in a 5% CO₂ in air atmosphere. On the day 30 of transfection 200 ng of pCMV-EGFP_{Luc} DNA (Clontech) was added in 20 µL of OPTIMEM (Thermo Fisher), mixed with a vortex and incubated for 5 minutes at room temperature (rt). Then, 0.6 or 0.8 µL of a compound of general formula (I), preferably of general formula (III) (at 7.5 mM nitrogen concentration) were added onto the diluted DNA, mixed with a vortex and incubated for

10 minutes at rt. The transfection DNA solution (20 µL) was added into the well and the plate was incubated for 24 hours at 37°C in a 5% CO₂ in air atmosphere.

For the GFP expression analysis, one day post-transfection, the cell culture medium was removed and 50 µL of trypsin-EDTA (1x, Lonza) were added per well and the plate was incubated for 5 minutes at 37°C. 150 µL of complete medium were added to neutralize the trypsin, and the GFP expression was analysed (2000 events) by flow cytometry (Exc 488 nm, Em 520 nm) using a Guava easyCyte 6HT cytometer (Millipore).

Recombinant virus production

HEK-293T (ATCC® CRL-3216™): Human embryonic kidney cell is a highly transfectable derivative of human embryonic kidney 293 cells, and contains the SV40 T-antigen. HEK-293T cells are widely used for recombinant virus production, gene expression and protein production.

For adherent cells, HEK-293T cells were seeded at 5 x 10⁶ cells in 145 cm² petri dishes in 15 mL of DMEM 4.5 g/L glucose supplemented with 10% FBS, 2 mM glutamine and 100 U/mL of penicillin and 100 µg/mL of streptomycin, and incubated at 37°C in a 5% CO₂ in air atmosphere.

AAV-2 was produced in HEK-293 T cells using the AAV-2 Helper Free Packaging System (catalog number VPK-402, Cell BIALABS, INC.) by co-transfection of 3 plasmids, pAAV-RC2 vector expressing Rep and Cap, pHelper vector expressing Adeno E2A, Adeno E4 and Adeno VA helper factors, and pAAV-GFP control vector expressing the GFP under the control of a CMV promoter. Transfection complexes (10 µg total DNA per petri dish) were prepared with a ratio of 2:2:1 with pAAV-RC2, pHelper and pAAV-GFP, respectively. Plasmids were diluted in a total volume of 1.5 mL of OPTIMEM. Then, 20 or 30 µL of compounds were added onto the diluted DNA, mixed with a vortex and incubated for 10 minutes at rt. Transfection complexes were added onto the cells and the plate was incubated for 72 h at 37°C in a 5% CO₂ in air atmosphere.

For suspension cells, HEK-293T cells were seeded at 1 x 10⁶ cells/mL in 27 mL of FreeStyle F17 supplemented with 4% Glutamine, 100 U/mL of penicillin, 100 µg/mL of streptomycin and 0.1% Pluronic in 125 mL flask Erlenmeyer (Corning). Cells were incubated for 24 h at 37°C in an 8% CO₂ in air atmosphere under agitation (130 rpm). Plasmids (pAAV-GFP – pAAV-RC2 – pHelper at ratio 2:2:1) were diluted in 3 mL of FreeStyle F17. Then, compounds were added onto the diluted DNA (ratio 2 or 3 µL per µg of DNA), mixed with a vortex and incubated for 10 minutes at rt. Transfection complexes were added onto the cells (2 µg DNA per 1 x 10⁶ cells) and the plate was incubated for 72 h at 37°C in a 8% CO₂ in air atmosphere under agitation (130 rpm).

Lentivirus particles were produced using the ViraSafe™ Lentiviral Packaging System, Pantropic (Catalog Number VPK- 20, CELL BIOLABS INC.) containing pRSV-REV packaging vector, pCgpV Packaging Vector and pCMV-VSV-G Envelop Vector. pLenti6.3/V5-GW/EmGFP Expression Control Vector was from Thermo Fisher.

- 5 HEK-293T cells were seeded at 1×10^6 cells/mL in 27 mL of FreeStyle F17 supplemented with 4% Glutamine, 100 U/mL of penicillin, 100 µg/mL of streptomycin and 0.1% Pluronic in 125 mL flask Erlenmeyer (Corning). Cells were incubated for 24 h at 37°C in an 8% CO₂ in air atmosphere under agitation (130 rpm). Plasmids (pRSV-REV – pCgpV – pCMV-VSV-G - pLenti6.3 at ratio 1:1:1:3) were diluted in 3 mL of FreeStyle F17. Then, compounds were added onto the
- 10 diluted DNA (ratio 2 µL per µg of DNA), mixed with a vortex and incubated for 10 minutes at rt. Transfection complexes were added onto the cells (2 µg DNA per 1×10^6 cells) and the plate was incubated for 72 h at 37°C in an 8% CO₂ in air atmosphere under agitation (130 rpm).

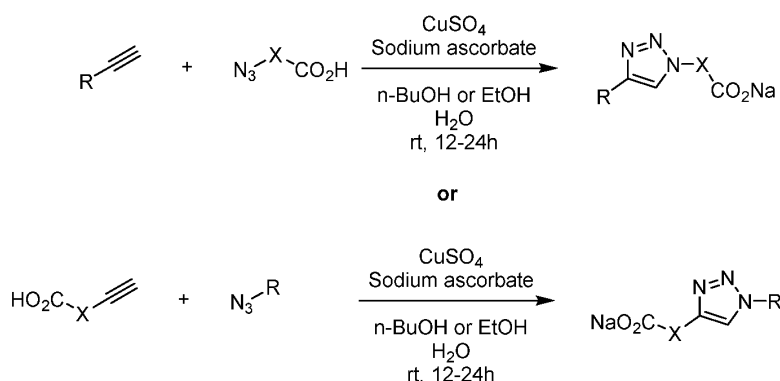
- The transducing unit (TU/mL) was determined by using virus vectors expressing the GFP reporter gene after infection of permissive HT 1080 cells for lentivirus vectors and HEK-293T cells
- 15 for AAV-2 vectors in 96-well and in presence of polybrene (8 µg/mL). The GFP expression was analysed by cytometry 72 h after transduction to determine the transducing units.

Example 1. General procedure for the preparation of grafted polymers

Step 1: Grafting

- 20 In a round-bottom flask was added the cationic polymer (1 equiv.) in water (4 mL/mmol of starting material) followed by N-methyl morpholine or NMM (2 equiv.). The carboxylate (0.3-1 equiv.) was added followed by MeOH (16 mL/mmol of polymer). After stirring 10 min, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride was added or DMTMM (0.6-2 equiv.) and the mixture was stirred 12-24h at room temperature. Then, MeOH was removed in vacuo, water (4 mL/mmol
- 25 of starting material) followed by a solution of 3M HCl (1 mL/mmol of starting material) were added. The residue was purified using a dialysis cassette in a 50mM HCl bath.

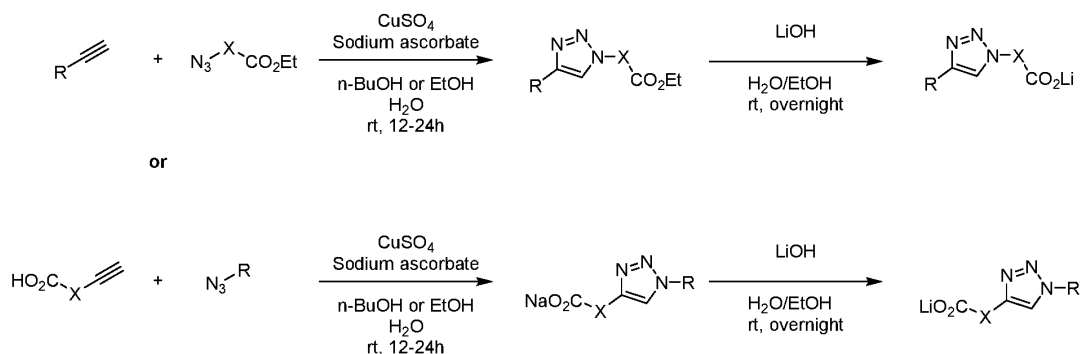
Step 2: Synthesis of triazole by « click » chemistry starting from an acid



Alkyne (1 equiv.), azide (1 equiv.), CuSO₄ (0.01 equiv) and sodium ascorbate (0.03 equiv) were added to a 2:1 (v/v) solution of nBuOH and water. The reaction was stirred at room temperature for 24 h. Then, NaOH (5M, 2 equiv.) was added and the organic solvent was removed in vacuo.

- 5 The residue was purified by reversed phase flash chromatography using 0 to 100% CH₃CN in water as eluant.

Step 3: Synthesis of triazole by « click » chemistry starting from an ester



- 10 Alkyne (1 equiv.), azide (1 equiv.), CuSO₄ (0.01 equiv) and sodium ascorbate (0.03 equiv) were added to a 2:1 (v/v) solution of nBuOH and water. The reaction was stirred at room temperature for 24 h. Then, NaOH (5M, 2 equiv.) was added and the organic solvent was removed in vacuo. The residue was purified by reversed phase flash chromatography using 0 to 100% CH₃CN in water as eluant.

15

Step 4: Saponification of the ester moiety

To a solution of ester in EtOH was added dropwise a 3M solution of LiOH, and the mixture was stirred at rt for the week-end. Then, the solvent was removed in vacuo and the residue was purified

by reverse phase FC on SiO₂ using H₂O/MeCN as eluant using a Biotage Flash purification system. The acid obtained was lyophilized to yield a solid.

Step 5: Synthesis of triazole by Ruthenium catalyzed « click » chemistry starting from an ester.

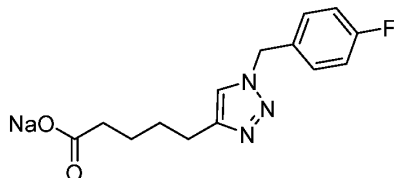
- 5 Cp*RuCl(cod) was added to a microwave vial. The vial was then evacuated and backfilled with Argon (3x). Alkyne (1,1eq.); alkyne (1eq.) and toluene were added to the vial under Ar and the mixture was stirred at rt overnight. Toluene was evaporated and the product was purified on reverse phase chromatography using H₂O and MeCN.
- The ester was retaken in EtOH and NaOH 1M (1,1eq.) and stirred until completion (followed by
- 10 HPLC). EtOH was evaporated and the product was purified by reverse phase chromatography using H₂O and MeCN. The product was lyophilized.

Step 6: Synthesis of 1,2,3-triazole.

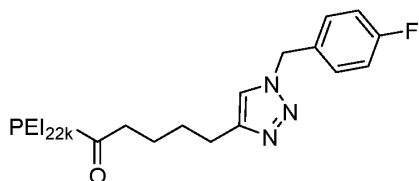
- 15 Triazole and K₂CO₃ in MeCN at 80°C. Add R-Br dropwise and stirred at 80°C overnight. Filtrate and washed the solid with MeCN. The filtrate was evaporated and purified by reverse phase chromatography (H₂O : MeCN). Two fractions were collected.
- The esters were retaken in EtOH and NaOH 1M (1,1eq) and stirred until completion. EtOH was evaporated and the product was purified by reverse phase chromatography H₂O:MeCN.

20 **Example 2. Syntheses of compounds of the invention**

- Synthesis of Product 2.19

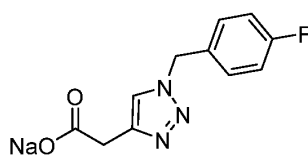


- Intermediate **2.19a** was prepared analogously to the general procedure, step 2 (Example 1). Yield= 87%; m= 520 mg; ¹H NMR (400 MHz, Deuterium Oxide) δ 7.75 (s, 1H), 7.34 – 7.25 (m, 2H), 7.18 – 7.04 (m, 2H), 5.50 (s, 2H), 2.65 (t, *J* = 7.2 Hz, 2H), 2.17 (t, *J* = 7.1 Hz, 2H), 1.55 (dq, *J* = 23.6, 7.8 Hz, 3H).
- 25

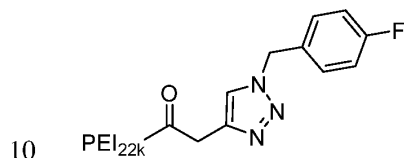


Product **2.19** was prepared analogously to the general procedure, step 1 (Example 1). Yield= 36%; m= 21 mg; ^1H NMR (400 MHz, Deuterium Oxide) δ 8.12 – 6.41 (m, 5H), 5.68 – 4.93 (m, 2H), 4.05 – 2.88 (m, 17H), 2.79 – 0.87 (m, 8H).

5 - **Synthesis of Product 2.20**

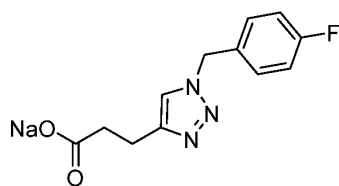


Intermediate **2.20a** was prepared analogously to the general procedure, step 2 (Example 1). Yield= 51%; m= 261mg; ^1H NMR (400 MHz, Deuterium Oxide) δ 7.75 (s, 1H), 7.34 – 7.25 (m, 2H), 7.18 – 7.04 (m, 2H), 5.50 (s, 2H), 2.65 (t, J = 7.2 Hz, 2H), 2.17 (t, J = 7.1 Hz, 2H), 1.64 – 1.45 (m, 3H).

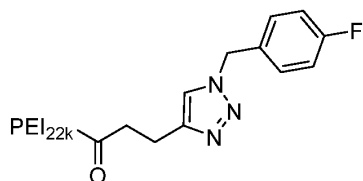


Product **2.20** was prepared analogously to the general procedure, step 1 (Example 1). Yield= 71%; m= 31 mg; ^1H NMR (400 MHz, Deuterium Oxide) δ 8.17 – 6.68 (m, 5H), 5.60 – 5.28 (m, 2H), 4.10 – 2.93 (m, 27H).

- **Synthesis of Product 2.21**

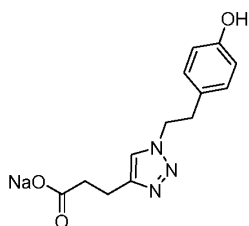


Intermediate **2.21a** was prepared analogously to the general procedure, step 2 (Example 1). Yield= 27%; m= 148 mg; ^1H NMR (400 MHz, Deuterium Oxide) δ 7.71 (s, 1H), 7.32 – 7.24 (m, 2H), 7.09 (td, J = 8.8, 2.0 Hz, 2H), 5.55 – 5.46 (m, 2H), 2.86 (t, J = 7.5 Hz, 2H), 2.45 (t, J = 7.5 Hz, 2H).

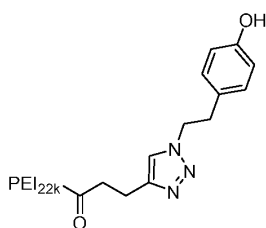


Product **2.21** was prepared analogously to the general procedure, step 1 (Example 1). Yield= 29%; m= 12 mg; ^1H NMR (400 MHz, Deuterium Oxide) δ 8.90 – 6.37 (m, 5H), 5.58 – 5.25 (m, 2H), 4.20 – 2.91 (m, 36H).

5 **- Synthesis of Product 2.22**

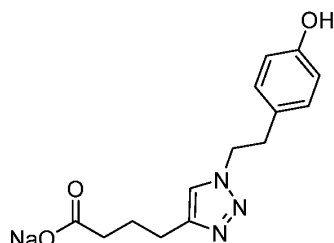


Intermediate **2.22a** was prepared analogously to the general procedure, step 2 (Example 1). Yield= 28%; m= 78 mg; ^1H NMR (400 MHz, Methanol- d_4) δ 7.57 (s, 1H), 6.93 – 6.84 (m, 2H), 6.68 – 6.60 (m, 2H), 4.54 – 4.45 (m, 2H), 3.04 (t, J = 7.4 Hz, 2H), 2.99 – 2.91 (m, 2H), 2.53 – 2.44 (m, 2H).



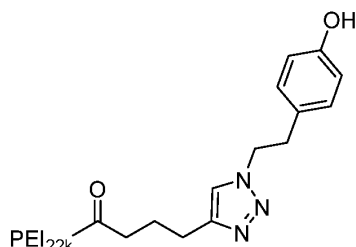
Product **2.22** was prepared analogously to the general procedure, step 1 (Example 1). Yield= 87%; m= 44 mg; ^1H NMR (400 MHz, Deuterium Oxide) δ 7.82 – 6.34 (m, 5H), 4.60 – 4.06 (m, 2H), 4.00 – 3.07 (m, 22H), 3.06 – 2.24 (m, 7H).

- Synthesis of Product 2.23



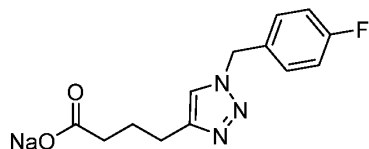
Intermediate **2.23a** was prepared analogously to the general procedure, step 2 (Example 1). Yield= 87%; m= 258 mg; ^1H NMR (400 MHz, Methanol- d_4) δ 7.53

(s, 1H), 6.98 – 6.87 (m, 2H), 6.75 – 6.63 (m, 2H), 4.53 (t, $J = 7.1$ Hz, 2H), 3.08 (t, $J = 7.1$ Hz, 2H), 2.69 (t, $J = 7.6$ Hz, 2H), 2.26 – 2.16 (m, 2H), 1.91 (tt, $J = 8.3, 6.9$ Hz, 2H).

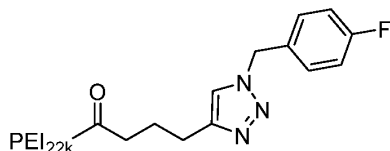


Product **2.23** was prepared analogously to the general procedure, step 1 (Example 1). Yield= 100%; m= 48 mg; ^1H NMR (400 MHz, Deuterium Oxide) δ 8.14 – 6.01 (m, 5H), 4.62 – 4.11 (m, 2H), 3.99 – 2.76 (m, 26H), 2.73 – 0.92 (m, 8H).

- Synthesis of Product 2.24

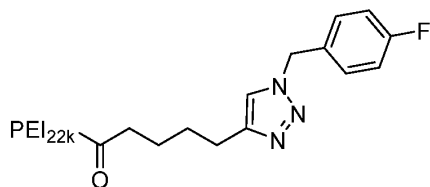


Intermediate **2.24a** was prepared analogously to the general procedure, step 2 (Example 1). Yield= 67%; m= 379 mg; ^1H NMR (400 MHz, Deuterium Oxide) δ 7.62 (s, 1H), 7.24 – 7.14 (m, 2H), 7.05 – 6.92 (m, 2H), 5.35 (s, 2H), 2.54 (t, $J = 7.6$ Hz, 2H), 2.08 (t, $J = 7.5$ Hz, 2H), 1.73 (tt, $J = 8.2, 7.0$ Hz, 2H).

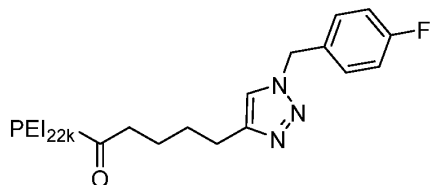


Product **2.24** was prepared analogously to the general procedure, step 1 (Example 1). Yield= 97%; m= 42 mg; ^1H NMR (400 MHz, Deuterium Oxide) δ 7.90 – 6.74 (m, 5H), 5.57 – 5.15 (m, 2H), 4.19 – 3.11 (m, 35H), 2.91 – 1.47 (m, 6H).

- Synthesis of Product 2.25

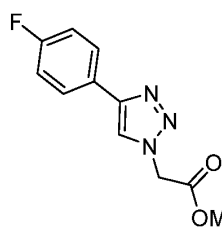


Product **2.25** was prepared analogously to the general procedure, step 1 (Example 1). Yield= 85%; m= 41 mg; ^1H NMR (400 MHz, Deuterium Oxide) δ 7.84 – 6.46 (m, 5H), 5.54 – 4.94 (m, 2H), 4.15 – 3.11 (m, 26H), 2.97 – 1.11 (m, 8H).

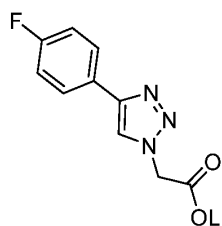
- Synthesis of Product 2.26

Product **2.26** was prepared analogously to the general procedure, step 1 (Example 1). Yield= 80%; m= 44 mg; ^1H NMR (400 MHz, Deuterium Oxide) δ 8.00 – 6.36 (m, 5H), 5.60 – 4.93 (m, 2H), 4.12 – 3.01 (m, 19H), 2.79 – 0.93 (m, 8H).

5

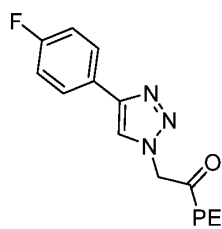
- Synthesis of Product 2.27

Intermediate **2.27a** was prepared analogously to the general procedure, step 3 (Example 1). Yield= 65%; m= 305 mg; ^1H NMR (400 MHz, Chloroform- d) δ 8.03 (s, 1H), 8.00 – 7.91 (m, 2H), 7.31 – 7.21 (m, 2H), 5.36 (s, 2H), 3.96 (s, 3H).



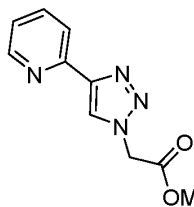
10

Intermediate **2.27b** was prepared analogously to the general procedure, step 4 (Example 1). Yield= 35%; m= 97 mg; ^1H NMR (400 MHz, Deuterium Oxide) δ 8.07 (s, 0H), 7.71 – 7.59 (m, 1H), 7.19 – 7.04 (m, 1H), 4.96 (s, 1H).

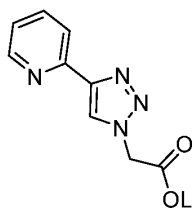


15

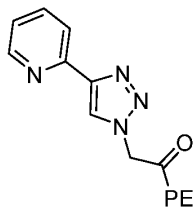
Product **2.27** was prepared analogously to the general procedure, step 1 (Example 1). Yield= 67%; m= 28 mg; ^1H NMR (400 MHz, Deuterium Oxide) δ 9.33 – 7.35 (m, 5H), 6.13 – 5.19 (m, 2H), 4.17 – 3.22 (m, 42H).

- Synthesis of Product 2.28

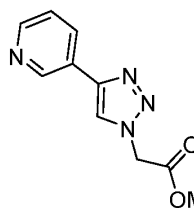
Intermediate **2.28a** was prepared analogously to the general procedure, step 3 (Example 1). Yield= 62%; m= 272 mg; ¹H NMR (400 MHz, Methanol-*d*₄) δ 8.59 (d, *J* = 5.0 Hz, 1H), 8.50 (s, 1H), 8.10 (dt, *J* = 7.9, 1.1 Hz, 1H), 7.93 (td, *J* = 7.8, 1.8 Hz, 1H), 7.38 (ddd, *J* = 7.6, 4.9, 1.2 Hz, 1H), 5.45 (s, 2H), 3.83 (s, 3H).



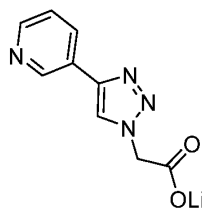
Intermediate **2.28b** was prepared analogously to the general procedure, step 4 (Example 1). Yield= 94%; m= 236 mg; ¹H NMR (400 MHz, Deuterium Oxide) δ 8.44 – 8.38 (m, 1H), 8.23 (s, 1H), 7.89 – 7.74 (m, 2H), 7.31 (ddd, *J* = 6.0, 5.0, 2.8 Hz, 1H), 5.02 (s, 2H).



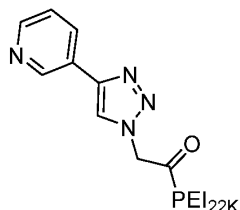
Product **2.28** was prepared analogously to the general procedure, step 1 (Example 1). Yield= 47%; m= 23 mg; ¹H NMR (400 MHz, Deuterium Oxide) δ 8.06 – 6.11 (m, 5H), 5.55 – 4.96 (m, 2H), 4.26 – 2.20 (m, 20H).

- Synthesis of Product 2.29

Intermediate **2.29a** was prepared analogously to the general procedure, step 3 (Example 1). Yield= 81%; m= 355 mg; ¹H NMR (400 MHz, Methanol-*d*₄) δ 9.08 (s, 1H), 8.64 – 8.46 (m, 2H), 8.28 (tt, *J* = 6.3, 1.6 Hz, 1H), 7.55 (dd, *J* = 8.0, 4.7 Hz, 1H), 5.44 (s, 2H), 3.84 (s, 2H).

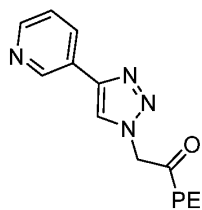


Intermediate **2.29b** was prepared analogously to the general procedure, step 4 (Example 1). Yield= 88%; m= 287 mg; ^1H NMR (400 MHz, Deuterium Oxide) δ 8.76 (dd, J = 2.3, 0.9 Hz, 1H), 8.40 (dd, J = 5.0, 1.6 Hz, 1H), 8.23 (s, 1H), 8.07 (ddd, J = 8.0, 2.3, 1.6 Hz, 1H), 7.42 (ddd, J = 8.0, 5.0, 0.9 Hz, 1H), 5.01 (s, 2H), 1.09 (t, J = 7.1 Hz, 2H).



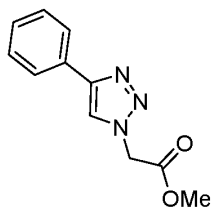
5 Product **2.29** was prepared analogously to the general procedure, step 1 (Example 1). Yield= 76%; m= 29 mg; ^1H NMR (400 MHz, Deuterium Oxide) δ 9.28 – 7.21 (m, 5H), 5.94 – 5.16 (m, 2H), 4.19 – 2.35 (m, 19H).

- Synthesis of Product 2.30



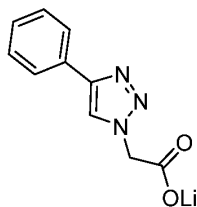
10 Product **2.30** was prepared analogously to the general procedure, step 1 (Example 1). Yield= 66%; m= 32 mg; ^1H NMR (400 MHz, Deuterium Oxide) δ 9.47 – 7.87 (m, 5H), 6.08 – 5.50 (m, 2H), 4.32 – 2.94 (m, 50H).

- Synthesis of Product 2.31

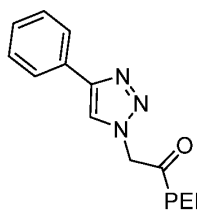


15 Intermediate **2.31a** was prepared analogously to the general procedure, step 3 (Example 1). Yield= 82%; m= 354 mg; ^1H NMR (400 MHz, Deuterium Oxide) δ 9.46 – 7.68 (m, 5H), 6.03 – 5.32 (m, 2H), 4.28 – 2.83 (m, 50H). ^1H NMR (400 MHz, Methanol- d_4)

δ 8.34 (s, 1H), 7.88 – 7.80 (m, 2H), 7.51 – 7.41 (m, 2H), 7.41 – 7.32 (m, 1H), 5.39 (s, 2H), 3.83 (s, 3H).

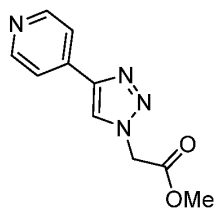


Intermediate **2.31b** was prepared analogously to the general procedure, step 4 (Example 1). Yield= 99%; m= 325 mg; ^1H NMR (400 MHz, Deuterium Oxide) δ 8.08 (s, 1H), 7.71 – 7.62 (m, 2H), 7.46 – 7.37 (m, 2H), 7.41 – 7.30 (m, 1H), 4.94 (s, 2H).

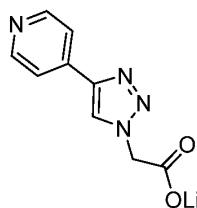


Product **2.31** was prepared analogously to the general procedure, step 1 (Example 1). Yield= 58%; m= 24mg; ^1H NMR (400 MHz, Deuterium Oxide) δ 8.60 – 6.52 (m, 6H), 5.90 – 5.15 (m, 2H), 4.23 – 2.90 (m, 32H).

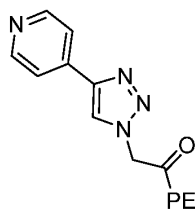
10 **- Synthesis of Product 2.32**



Intermediate **2.32a** was prepared analogously to the general procedure, step 3 (Example 1). Yield= 87%; m= 380 mg; ^1H NMR (400 MHz, Methanol- d_4) δ 8.82 – 8.45 (m, 3H), 7.92 (s, 2H), 5.45 (s, 2H), 3.84 (s, 3H).

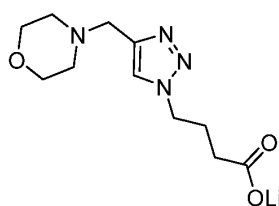


Intermediate **2.32b** was prepared analogously to the general procedure, step 4 (Example 1). Yield= 100%; m= 351 mg; ^1H NMR (400 MHz, Deuterium Oxide) δ 8.49 – 8.43 (m, 2H), 8.34 (s, 1H), 7.70 – 7.64 (m, 2H), 5.02 (s, 2H).

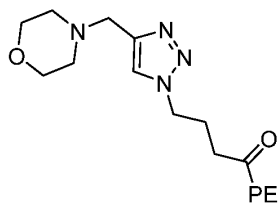


Product **2.32** was prepared analogously to the general procedure, step 1 (Example 1). Yield= 91%; m= 32 mg; ^1H NMR (400 MHz, Deuterium Oxide) δ 9.17 – 8.00 (m, 5H), 6.13 – 5.23 (m, 2H), 4.21 – 3.01 (m, 74H).

5 **- Synthesis of Product 2.33**

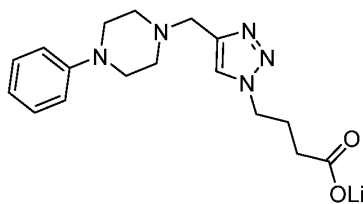


Intermediate **2.33a** was prepared analogously to the general procedure, step 2 (Example 1). Yield= 59%; m= 49 mg; ^1H NMR (400 MHz, Deuterium Oxide) δ 7.90 (s, 1H), 4.40 – 4.32 (m, 2H), 3.69 – 3.62 (m, 6H), 2.52 – 2.45 (m, 4H), 2.14 – 2.00 (m, 4H).

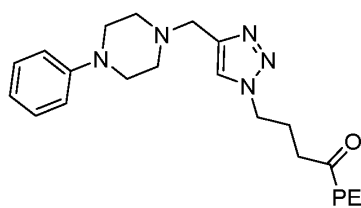


Product **2.33** was prepared analogously to the general procedure, step 1 (Example 1). Yield= 89%; m= 15 mg; ^1H NMR (400 MHz, Deuterium Oxide) δ 8.63 – 7.84 (m, 1H), 4.52 – 4.23 (m, 3H), 4.13 – 2.86 (m, 27H), 2.74 – 1.54 (m, 4H).

- Synthesis of Product 2.34



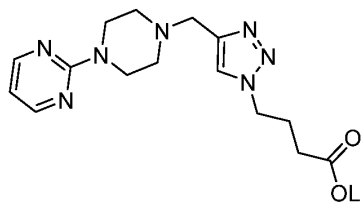
Intermediate **2.34a** was prepared analogously to the general procedure, step 2 (Example 1). Yield= 48%; m= 51mg; ^1H NMR (400 MHz, Deuterium Oxide) δ 7.92 (s, 1H), 7.35 – 7.26 (m, 2H), 7.09 – 7.01 (m, 2H), 6.98 (tt, J = 7.4, 1.1 Hz, 1H), 4.41 – 4.33 (m, 2H), 3.71 (s, 2H), 3.14 – 3.07 (m, 4H), 2.69 – 2.61 (m, 4H), 2.14 – 2.00 (m, 4H).



Product **2.34** was prepared analogously to the general procedure, step 1 (Example 1). Yield= 98%; m= 17mg; ^1H NMR (400 MHz, Deuterium Oxide) δ 8.67 – 7.86 (m, 1H), 7.64 – 6.61 (m, 5H), 4.66 – 4.19 (m, 3H), 4.11 – 3.09 (m, 31H), 2.80 – 1.74 (m, 4H).

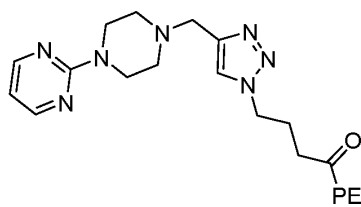
5

- Synthesis of Product 2.35



Intermediate **2.35a** was prepared analogously to the general procedure, step 2 (Example 1). Yield= 19%; m= 20mg; ^1H NMR (400 MHz, Deuterium Oxide) δ 8.24 (d, J = 4.9 Hz, 2H), 7.95 (s, 1H), 6.64 (t, J = 4.9 Hz, 1H), 4.40 – 4.33 (m, 2H), 3.81 – 3.77 (m, 2H), 3.67 – 3.60 (m, 4H), 2.66 – 2.58 (m, 4H), 2.12 – 2.00 (m, 4H).

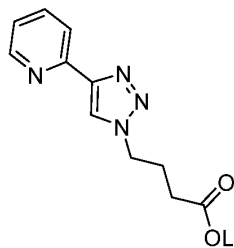
10



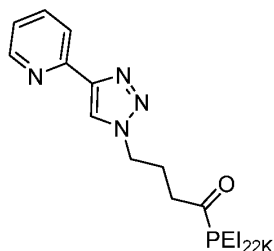
Product **2.35** was prepared analogously to the general procedure, step 1 (Example 1). Yield= 44 %; m= 7 mg; ^1H NMR (400 MHz, Deuterium Oxide) δ 8.58 – 7.90 (m, 3H), 7.07 – 6.43 (m, 1H), 4.57 – 4.19 (m, 3H), 4.23 – 2.99 (m, 32H), 2.83 – 1.68 (m, 4H).

15

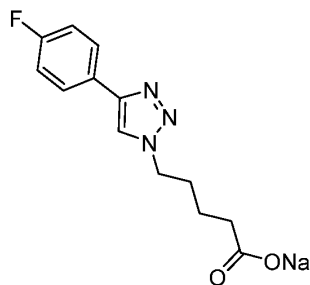
20

- Synthesis of Product 2.36

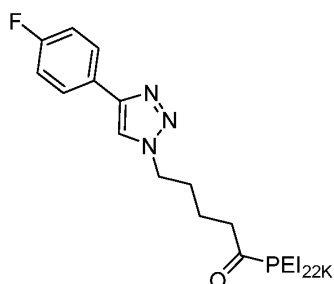
Intermediate **2.36a** was prepared analogously to the general procedure, step 2 (Example 1). Yield= 51%; m= 78mg; ¹H NMR (400 MHz, Deuterium Oxide) δ 8.39 (ddd, *J* = 5.0, 1.7, 1.0 Hz, 1H), 8.21 (s, 1H), 7.85 – 7.71 (m, 1H), 7.30 (ddd, *J* = 7.3, 5.0, 1.5 Hz, 1H), 4.43 – 4.35 (m, 2H), 2.20 – 2.03 (m, 4H).



Product **2.36** was prepared analogously to the general procedure, step 1 (Example 1). Yield= 77%; m= 14 mg; ¹H NMR (400 MHz, Deuterium Oxide) δ 9.15 – 7.11 (m, 5H), 4.57 – 4.15 (m, 1H), 4.07 – 2.86 (m, 13H), 2.74 – 1.68 (m, 4H).

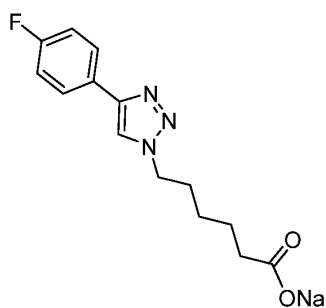
10 **- Synthesis of Product 2.37**

Intermediate **2.37a** was prepared analogously to the general procedure, step 2 (Example 1). Yield= 14%; m= 38 mg; ¹H NMR (400 MHz, Deuterium Oxide) δ 8.00 (s, 1H), 7.57 – 7.47 (m, 2H), 7.09 – 6.95 (m, 2H), 4.26 (t, *J* = 7.0 Hz, 2H), 2.08 (t, *J* = 7.5 Hz, 2H), 1.76 (p, *J* = 7.2 Hz, 2H), 1.49 – 1.35 (m, 2H).

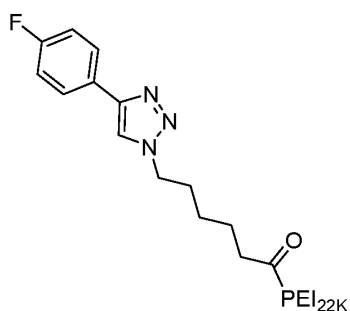


Product **2.37** was prepared analogously to the general procedure, step 1 (Example 1). Yield= 24%; m= 9 mg; ^1H NMR (400 MHz, Deuterium Oxide) δ 8.23 – 6.04 (m, 5H), 4.39 – 2.72 (m, 18H), 2.70 – 0.56 (m, 6H).

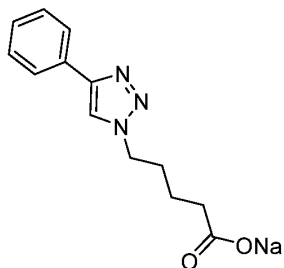
5 - Synthesis of Product 2.38



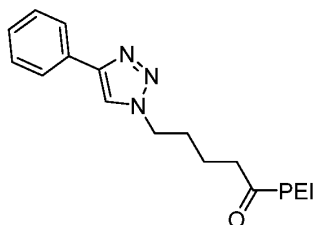
Intermediate **2.38a** was prepared analogously to the general procedure, step 2 (Example 1). Yield= 11%; m= 27 mg; ^1H NMR (400 MHz, Deuterium Oxide) δ 7.97 (s, 1H), 7.51 (dd, J = 8.7, 5.3 Hz, 2H), 7.02 (t, J = 8.9 Hz, 2H), 4.23 (t, J = 7.1 Hz, 2H), 2.04 (t, J = 7.5 Hz, 2H), 1.76 (p, J = 7.2 Hz, 2H), 1.45 (p, J = 7.6 Hz, 2H), 1.21 – 1.09 (m, 2H).



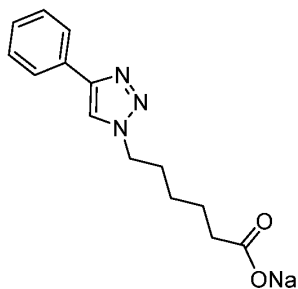
Product **2.38** was prepared analogously to the general procedure, step 1 (Example 1). Yield= 18%; m= 6 mg; ^1H NMR (400 MHz, Deuterium Oxide) δ 8.31 – 6.11 (m, 5H), 4.33 – 2.72 (m, 21H), 2.68 – 0.15 (m, 6H).

- Synthesis of Product 2.39

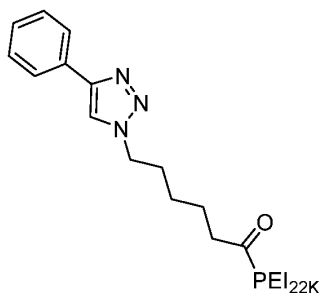
Intermediate **2.39a** was prepared analogously to the general procedure, step 2 (Example 1). Yield= 35%; m= 11 mg; ¹H NMR (400 MHz, Deuterium Oxide) δ 8.01 (s, 1H), 7.60 – 7.53 (m, 2H), 7.39 – 7.25 (m, 3H), 4.24 (t, *J* = 7.1 Hz, 2H), 2.09 (t, *J* = 7.5 Hz, 2H), 1.76 (p, *J* = 7.2 Hz, 2H), 1.48 – 1.35 (m, 2H).



Product **2.39** was prepared analogously to the general procedure, step 1 (Example 1). Yield= 53%; m= 133 mg; ¹H NMR (400 MHz, Deuterium Oxide) δ 8.49 – 6.15 (m, 6H), 4.52 – 2.83 (m, 21H), 2.66 – 0.54 (m, 6H).

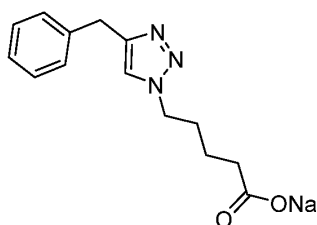
10 **- Synthesis of Product 2.40**

Intermediate **2.40a** was prepared analogously to the general procedure, step 2 (Example 1). Yield= 76%; m= 184 mg; ¹H NMR (400 MHz, Deuterium Oxide) δ 7.88 (s, 1H), 7.54 – 7.47 (m, 2H), 7.35 – 7.20 (m, 3H), 4.14 (t, *J* = 7.1 Hz, 2H), 2.04 (t, *J* = 7.5 Hz, 2H), 1.76 – 1.64 (m, 2H), 1.43 (p, *J* = 7.6 Hz, 2H), 1.21 – 1.06 (m, 2H).

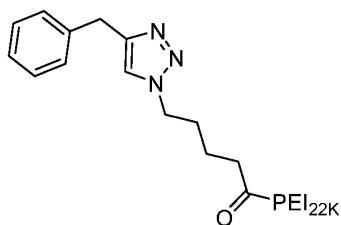


Product **2.40** was prepared analogously to the general procedure, step 1 (Example 1). Yield= 12%; m= 4 mg; ^1H NMR (400 MHz, Deuterium Oxide) δ 7.94 – 6.37 (m, 6H), 4.43 – 2.84 (m, 19H), 2.68 – 0.23 (m, 8H).

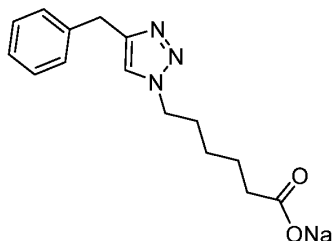
5 - Synthesis of Product 2.41



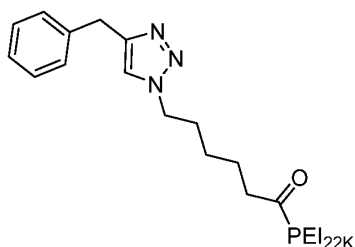
Intermediate **2.41a** was prepared analogously to the general procedure, step 2 (Example 1). Yield= 26%; m= 46 mg; ^1H NMR (400 MHz, Deuterium Oxide) δ 7.41 (s, 1H), 7.20 – 7.01 (m, 5H), 4.08 (t, J = 7.0 Hz, 2H), 3.78 (s, 2H), 2.00 (t, J = 7.5 Hz, 2H), 1.72 – 1.50 (m, 2H), 1.32 (tt, J = 15.0, 9.9 Hz, 2H).



Product **2.41** was prepared analogously to the general procedure, step 1 (Example 1). Yield= 75%; m= 26 mg; ^1H NMR (400 MHz, Deuterium Oxide) δ 7.99 – 6.38 (m, 6H), 4.32 – 1.58 (m, 30H).

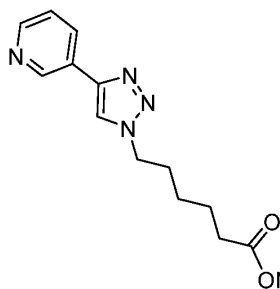
- Synthesis of Product 2.42

Intermediate **2.42a** was prepared analogously to the general procedure, step 2 (Example 1). Yield= 57%; m= 96 mg; ¹H NMR (400 MHz, Deuterium Oxide) δ 7.15 (s, 1H), 7.05 – 6.86 (m, 5H), 3.92 (t, *J* = 7.2 Hz, 2H), 3.67 (s, 2H), 1.97 (t, *J* = 7.6 Hz, 2H), 1.54 – 1.42 (m, 2H), 1.39 – 1.27 (m, 2H), 1.05 – 0.92 (m, 2H).



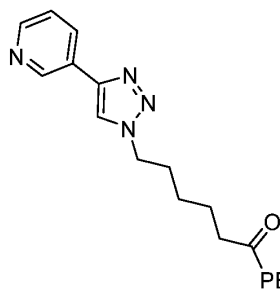
Product **2.42** was prepared analogously to the general procedure, step 1 (Example 1). Yield= 87%; m= 27 mg; ¹H NMR (400 MHz, Deuterium Oxide) δ 7.58 – 6.61 (m, 6H), 4.55 – 0.72 (m, 33H).

10 **- Synthesis of Product 2.43**



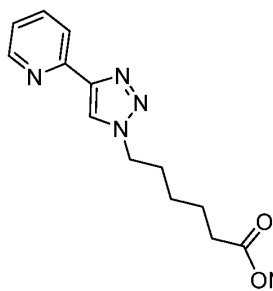
Intermediate **2.43a** was prepared analogously to the general procedure, step 2 (Example 1). Yield= 24%; m= 36mg; ¹H NMR (400 MHz, Deuterium Oxide) δ 8.78 (s, 1H), 8.43 – 8.37 (m, 1H), 8.29 (s, 1H), 8.09 (dt, *J* = 8.1, 1.9 Hz, 1H), 7.43 (ddd, *J* = 8.0, 5.0, 0.9 Hz, 1H), 4.38 (t, *J* = 7.0 Hz, 2H), 2.06 (t, *J* = 7.5 Hz, 2H), 1.91 – 1.79 (m, 2H), 1.54 – 1.42 (m, 2H), 1.26 – 1.14 (m, 2H).

15

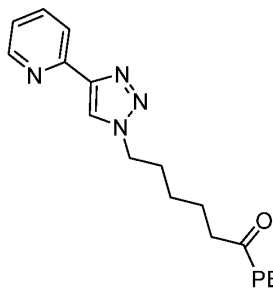


Product **2.43** was prepared analogously to the general procedure, step 1 (Example 1). Yield= 38%; m= 17 mg; ; ^1H NMR (400 MHz, Deuterium Oxide) δ 9.38 – 7.80 (m, 5H), 4.58 – 0.92 (m, 31H).

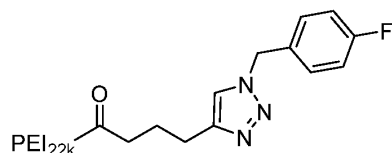
5 - Synthesis of Product 2.44



Intermediate **2.44a** was prepared analogously to the general procedure, step 2 (Example 1). Yield= 25%; m= 38 mg; ^1H NMR (400 MHz, Deuterium Oxide) δ 8.45 – 8.40 (m, 1H), 8.24 (s, 1H), 7.85 – 7.79 (m, 2H), 7.34 – 7.30 (m, 1H), 4.37 (t, J = 7.0 Hz, 2H), 2.05 (t, J = 7.4 Hz, 2H), 1.91 – 1.79 (m, 2H), 1.54 – 1.42 (m, 2H), 1.27 – 1.14 (m, 2H).

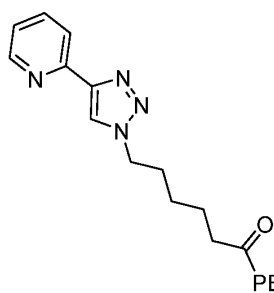


Product **2.44** was prepared analogously to the general procedure, step 1 (Example 1). Yield= 64%; m= 29 mg; ^1H NMR (400 MHz, Deuterium Oxide) δ 8.79 – 7.46 (m, 5H), 4.56 – 2.83 (m, 23H), 2.72 – 0.78 (m, 8H).

- Synthesis of Product 2.45

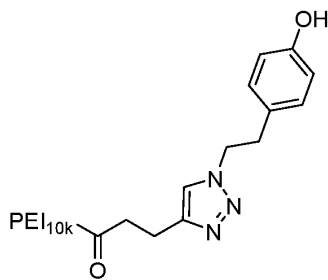
Product **2.45** was prepared analogously to the general procedure, step 1 (Example 1). Yield= 49%; m= 18 mg; ^1H NMR (400 MHz, Deuterium Oxide) δ 8.01 – 6.82 (m, 5H), 5.62 – 5.23 (m, 2H), 4.04 – 3.18 (m, 66H), 2.95 – 1.63 (m, 6H).

5

- Synthesis of Product 2.46

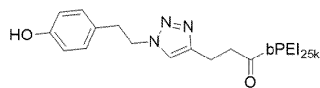
Product **2.46** was prepared analogously to the general procedure, step 1 (Example 1). Yield= 93%; m= 156 mg; ^1H NMR (400 MHz, Deuterium Oxide) δ 8.98 – 8.37 (m, 3H), 8.27 (s, 1H), 7.90 (s, 1H), 4.61 – 4.32 (m, 2H), 4.05 – 3.13 (m, 18.5H), 2.56 – 2.18 (m, 2H), 2.12 – 1.79 (m, 2H), 1.73 – 1.42 (m, 2H), 1.42 – 1.14 (m, 2H).

10

- Synthesis of Product 2.47

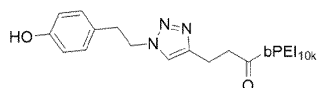
Product **2.47** was prepared analogously to the general procedure, step 1 (Example 1). Yield= 99%; m= 44 mg; ^1H NMR (400 MHz, Deuterium Oxide) δ 8.00 – 6.02 (m, 5H), 4.66 – 4.05 (m, 1H), 3.97 – 2.01 (m, 32H).

15

- Synthesis of Product 2.48

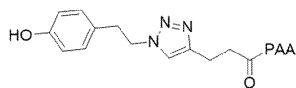
Product **2.48** was prepared analogously to the general procedure, step 1. Yield= 28% ; m= 83 mg ; ^1H NMR (400 MHz, Deuterium Oxide) δ 7.75 – 7.23 (s, 1H), 7.15 – 6.38 (m, 4H), 4.64 – 4.17 (m, 2H), 4.14 – 2.00 (m, 25H).

5

- Synthesis of Product 2.49

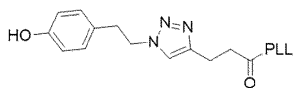
Product **2.49** was prepared analogously to the general procedure, step 1. Yield= 5% ; m= 13 mg ; ^1H NMR (400 MHz, Deuterium Oxide) δ 7.78 – 7.32 (s, 1H), 7.20 – 6.31 (m, 4H), 4.66 – 4.31 (m, 2H), 4.22 – 2.20 (m, 27H).

10

- Synthesis of Product 2.50

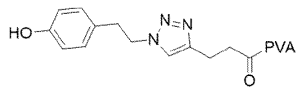
Product **2.50** was prepared analogously to the general procedure, step 1. Yield= 18% ; m= 29 mg ; ^1H NMR (400 MHz, Deuterium Oxide) δ 7.71 – 7.19 (s, 1H), 7.15 – 6.34 (m, 4H), 4.65 – 4.09 (m, 2H), 4.06 – 0.57 (m, 26H).

15

- Synthesis of Product 2.51

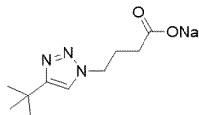
Product **2.51** was prepared analogously to the general procedure, step 1. Yield= 36% ; m= 13 mg ; ^1H NMR (400 MHz, Deuterium Oxide) δ 7.68 – 7.45 (m, 1H), 7.14 – 6.62 (m, 4H), 4.67 – 4.42 (m, 2H), 4.39 – 4.17 (m, 5H), 3.38 – 2.73 (m, 16H), 2.58 – 2.33 (m, 2H), 1.99 – 1.01 (m, 35H).

20

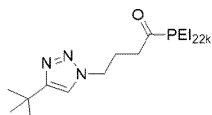
- Synthesis of Product 2.52

Product **2.52** was prepared analogously to the general procedure, step 1. Yield= 43% ; m= 63 mg ; ^1H NMR (400 MHz, Deuterium Oxide) δ 7.84 – 7.31 (s, 1H), 7.28 – 6.48 (m, 4H), 4.69 – 4.33 (m, 2H), 4.30 – 1.04 (m, 25H).

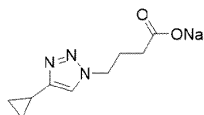
25

- Synthesis of Product 2.53

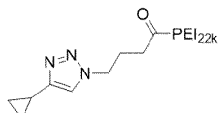
Intermediate **2.53a** was prepared analogously to the general procedure, steps 3 & 4. Yield= 34% ; m= 151 mg ; ¹H NMR (400 MHz, MeOD) δ 6.69 (s, 1H), 3.39 – 3.21 (m, 2H), 1.20 – 0.94 (m, 4H), 0.26 (s, 9H).



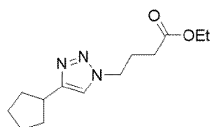
5 Product **2.53** was prepared analogously to the general procedure, step 1. Yield= 37% ; m= 25 mg ; ¹H NMR (400MHz, Deuterium Oxide) δ 8.07 – 7.88 (m, 1H), 4.60 – 3.35 (m, 2H), 4.08 – 3.05 (m, 24H), 2.90 – 1.97 (m, 4H), 1.55 – 1.05 (m, 9H)

- Synthesis of Product 2.54

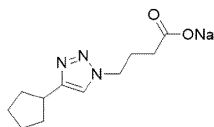
10 Intermediate **2.54a** was prepared analogously to the general procedure, step 3 & 4. Yield= 69% ; m= 287 mg ; ¹H NMR (400 MHz, MeOD) δ 6.64 (s, 1H), 3.32 (d, *J* = 6.9 Hz, 2H), 1.17 – 1.00 (m, 4H), 0.90 (tt, *J* = 8.4, 5.0 Hz, 1H), -0.02 – -0.18 (m, 2H), -0.22 – -0.35 (m, 2H).



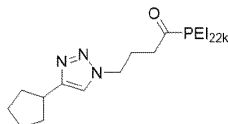
15 Product **2.54** was prepared analogously to the general procedure, step 1. Yield= 34% ; m= 24 mg ; ¹H NMR (400MHz, Deuterium Oxide) δ 7.99 – 7.78 (m, 1H), 4.61 – 4.38 (m, 2H), 4.06 – 3.26 (m, 21H), 2.66 – 1.95 (m, 5H), 1.15 – 1.01 (m, 2H), 0.88 – 0.70 (m, 2H).

- Synthesis of Product 2.55

20 Intermediate **2.55a** was prepared analogously to the general procedure, step 3. Yield= 52% ; m= 251 mg ; ¹H NMR (500 MHz, CDCl₃) δ 7.28 (s, 1H), 4.39 (t, *J* = 6.9 Hz, 2H), 4.14 (q, *J* = 7.1 Hz, 2H), 3.28 – 3.09 (m, 1H), 2.34 (t, *J* = 6.9 Hz, 2H), 2.22 (p, *J* = 7.0 Hz, 2H), 2.11 (s, 2H), 1.88 – 1.56 (m, 6H), 1.26 (t, *J* = 7.1 Hz, 3H).

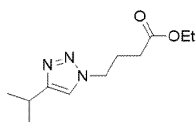


Intermediate **2.55b** was prepared analogously to the general procedure, step 4. Yield= quantitative ; m= 223 mg ; ^1H NMR (400 MHz, MeOD) δ 7.65 (s, 1H), 4.35 – 4.21 (m, 2H), 3.12 – 2.96 (m, 1H), 2.15 – 1.91 (m, 6H), 1.77 – 1.49 (m, 6H).

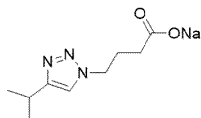


- Product **2.55** was prepared analogously to the general procedure, step 1.
- 5 Yield= 40% ; m= 22 mg ; ^1H NMR (400MHz, Deuterium Oxide) δ 8.04 – 7.83 (m, 1H), 4.59 – 4.34 (m, 2H), 4.07 – 3.04 (m, 25H), 2.60 – 1.97 (m, 6H), 1.80 – 1.47 (m, 6H)

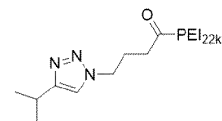
- Synthesis of Product 2.56



- Intermediate **2.56a** was prepared analogously to the general procedure, step 3.
- 10 Yield= 80% ; m= 350 mg ; ^1H NMR (400 MHz, CDCl_3) δ 7.26 (s, 1H), 4.38 (t, J = 6.9 Hz, 2H), 4.14 (q, J = 7.1 Hz, 2H), 3.09 (hept, J = 6.9 Hz, 1H), 2.34 (dd, J = 7.4, 6.4 Hz, 2H), 2.29 – 2.14 (m, 2H), 1.31 (s, 3H), 1.29 (s, 3H), 1.26 (td, J = 7.1, 0.6 Hz, 3H).

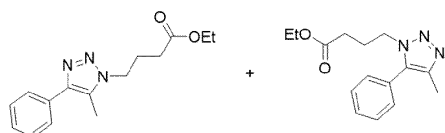


- Intermediate **2.56b** was prepared analogously to the general procedure, step 4.
- 15 Yield= quantitative ; m= 340 mg ; ^1H NMR (400 MHz, MeOD) δ 7.75 (d, J = 0.8 Hz, 1H), 4.39 (td, J = 6.4, 5.8, 2.8 Hz, 2H), 3.03 (pd, J = 6.9, 0.7 Hz, 1H), 2.25 – 2.05 (m, 4H), 1.30 (s, 3H), 1.29 (s, 3H).

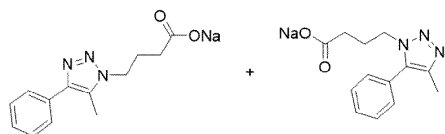


Product **2.56** was prepared analogously to the general procedure, step 1.

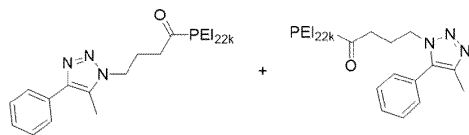
Yield= 45% ; m= 32 mg ; ^1H NMR (400MHz, Deuterium Oxide) δ 8.09 – 7.90 (m, 1H), 4.61 – 4.36 (m, 2H), 4.09 – 3.20 (m, 21H), 3.17 – 2.97 (m, 1H), 2.62 – 2.03 (m, 4H), 1.45 – 1.16 (m, 6H).

- Synthesis of Product 2.57

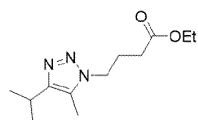
Intermediate **2.57a** was prepared analogously to the general procedure, step 5. Yield= 55% ; m= 287 mg ; ^1H NMR (400 MHz, CDCl_3) δ 7.73 – 7.66 (m, 2H), 7.56 – 7.40 (m, 4H), 7.38 – 7.27 (m, 2H), 4.39 (t, J = 7.1 Hz, 2H), 4.31 (t, J = 6.9 Hz, 1H), 4.14 (q, J = 7.1 Hz, 2H), 4.04 (q, J = 7.1 Hz, 1H), 2.48 (s, 3H), 2.43 (t, J = 7.0 Hz, 2H), 2.30 (s, 2H), 2.31 – 2.23 (m, 1H), 2.27 – 2.19 (m, 2H), 2.15 – 2.03 (m, 1H), 1.26 (t, J = 7.1 Hz, 3H), 1.19 (t, J = 7.1 Hz, 2H).



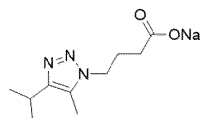
Intermediate **2.57b** was prepared analogously to the general procedure, step 4. Yield= quantitative ; m= 280 mg ; ^1H NMR (400 MHz, MeOD) δ 7.79 – 7.10 (m, 5H), 4.56 – 4.17 (m, 2H), 2.57 – 1.89 (m, 7H).



Product **2.57** was prepared analogously to the general procedure, step 1. Yield= 55% ; m= 44 mg ; ^1H NMR (400MHz, Deuterium Oxide) δ 7.68 – 6.86 (m, 5H), 4.53 – 3.03 (m, 20H), 2.62 – 1.58 (m, 7H).

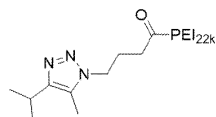
- Synthesis of Product 2.58

Intermediate **2.58a** was prepared analogously to the general procedure, step 5. Yield= 49% ; m= 224 mg ; ^1H NMR (400 MHz, CDCl_3) δ 4.35 – 4.25 (m, 2H), 4.19 – 4.08 (m, 2H), 3.23 – 2.96 (m, 1H), 2.42 – 2.34 (m, 4H), 2.22 – 2.09 (m, 2H), 1.36 – 1.30 (m, 6H), 1.29 – 1.18 (m, 3H).



Intermediate **2.58b** was prepared analogously to the general procedure, step 4.

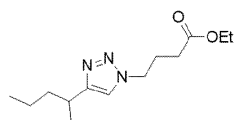
Yield= quantitative ; m= 219 mg ; ^1H NMR (400 MHz, MeOD) δ 4.37 – 4.27 (m, 2H), 3.31 – 3.18 (m, 1H), 2.33 (s, 3H), 2.27 – 2.13 (m, 2H), 2.15 – 2.03 (m, 2H), 1.34 (s, 3H), 1.32 (s, 3H).



Product **2.58** was prepared analogously to the general procedure, step 1.

- 5 Yield= 64% ; m= 48 mg ; ^1H NMR (400MHz, Deuterium Oxide) δ 4.49 – 4.22 (m, 2H), 3.99 – 3.03 (m, 20H), 2.76 – 1.87 (m, 7H), 1.34 – 1.04 (m, 6H).

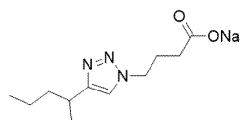
- Synthesis of Product 2.59



10

Intermediate **2.59a** was prepared analogously to the general procedure, step

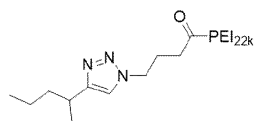
3. Yield= 41% ; m= 199 mg ; ^1H NMR (400 MHz, CDCl_3) δ 7.25 (s, 1H), 4.39 (t, J = 6.9 Hz, 2H), 4.14 (q, J = 7.1 Hz, 2H), 2.96 (h, J = 7.0 Hz, 1H), 2.34 (td, J = 7.1, 1.0 Hz, 2H), 2.21 (p, J = 7.0 Hz, 2H), 1.75 – 1.62 (m, 1H), 1.60 – 1.46 (m, 1H), 1.41 – 1.16 (m, 8H), 0.90 (t, J = 7.3 Hz, 3H).



Intermediate **2.59b** was prepared analogously to the general procedure,

15

step 4. Yield= quantitative ; m= 194 mg ; ^1H NMR (400 MHz, MeOD) δ 7.75 (s, 1H), 4.44 – 4.35 (m, 2H), 2.92 (h, J = 7.0 Hz, 1H), 2.19 – 2.08 (m, 4H), 1.74 – 1.49 (m, 2H), 1.47 – 1.10 (m, 6H), 0.91 (t, J = 7.4 Hz, 3H).



Product **2.59** was prepared analogously to the general procedure, step 1.

- 20 Yield= 58% ; m= 44 mg ; ^1H NMR (400MHz, Deuterium Oxide) δ 7.98 – 7.77 (m, 1H), 4.58 – 4.31 (m, 2H), 4.07 – 3.16 (m, 20H), 3.05 – 2.77 (m, 1H), 2.60 – 1.98 (m, 4H), 1.66 – 1.42 (m, 2H), 1.34 – 1.04 (m, 5H), 0.92 – 0.64 (m, 3H).

- Synthesis of Product 2.60

Intermediate **2.60a** was prepared analogously to the general procedure, step 6.
Yield= 44% ; m= 586 mg ; ¹H NMR (400 MHz, CDCl₃) δ 7.75 – 7.68 (m, 1H), 7.58 (s, 1H), 4.48 (td, *J* = 6.8, 1.9 Hz, 2H), 4.21 – 4.04 (m, 2H), 2.43 – 2.29 (m, 2H), 2.29 – 2.19 (m, 2H), 1.33 – 1.18 (m, 3H).



Intermediate **2.60b** was prepared analogously to the general procedure, step 4.
Yield= quantitative ; m= 567 mg ; ¹H NMR (400 MHz, MeOD) δ 8.01 (d, *J* = 1.3 Hz, 1H), 7.71 (d, *J* = 1.2 Hz, 1H), 4.52 – 4.43 (m, 2H), 2.23 – 2.10 (m, 4H).



Product **2.60** was prepared analogously to the general procedure, step 1. Yield= 46% ; m= 33 mg ; ¹H NMR (400MHz, Deuterium Oxide) δ 8.09 – 7.95 (m, 1H), 7.89 – 7.76 (m, 1H) 4.59 – 4.41 (m, 2H), 4.04 – 3.12 (m, 16H), 2.55 – 1.99 (m, 4H).

- Synthesis of Product 2.61

Intermediate **2.61a** was prepared analogously to the general procedure, step 6.
Yield= 27% ; m= 362 mg ; ¹H NMR (400 MHz, CDCl₃) δ 7.59 (s, 2H), 4.51 (td, *J* = 6.6, 1.1 Hz, 2H), 4.13 (qd, *J* = 7.1, 1.2 Hz, 2H), 2.36 – 2.26 (m, 4H), 1.24 (td, *J* = 7.1, 1.1 Hz, 3H).



Intermediate **2.61b** was prepared analogously to the general procedure, step 4.
Yield= quantitative ; m= 350 mg ; ¹H NMR (400 MHz, MeOD) δ 7.54 (s, 2H), 4.42 – 4.34 (m, 2H), 2.16 – 2.00 (m, 4H).



Product **2.61** was prepared analogously to the general procedure, step 1. Yield= 41% ; m= 29 mg ; ¹H NMR (400MHz, Deuterium Oxide) δ 7.84 – 7.67 (m, 2H), 4.60 – 4.39 (m, 2H), 4.08 – 3.12 (m, 16H), 2.57 – 1.99 (m, 4H).

Example 3. Compounds 2.19 to 2.26**25 Screening of transfection activity**

Compounds **2.19 to 2.26** were evaluated for their ability to transfect DNA in four different cell lines, Caco-2 (human colon epithelial cells), Hep G2 (human hepatocarcinoma cells), MDCK (Madin-Darby canine kidney epithelial cells) and MCF-10A (human mammary epithelial cells). The screening of compounds (**Figure 2**) was performed in 96-well plate by transfecting 200 ng of pCMV-EGFP_{Luc} DNA (Clontech) complexed with 0.6 or 0.8 μL of one compound of the invention,

i.e. one compound selected from the group consisting of compounds **2.19** to **2.26** (at 7.5 mM nitrogen concentration), defining a ratio of 1 µg DNA/ 3 µL of compound (ratio 1:3) or a ratio of 1 µg DNA/ 4 µL of compound (ratio 1:4), respectively. The percentage of cells expressing the GFP (%GFP) was determined by cytometry assay one day post-transfection. A transfection was performed with jetPEI® as a control which is a linear polyethylenimine of 22KDa and represents the parental cationic polymer backbone of the tested compounds.

The compounds **2.19** to **2.26** represent polymers wherein the triazole ring was used to graft fluorobenzyl or hydroxyphenol (or 4-hydroxyphenethyl) moiety and wherein the cationic polymer is grafted to **R** or **V** of the formula (III). All the compounds showed significant transfection activity whereas the best compound was dependant to the cell line used.

Example 4. Bioproduction of recombinant virus with compounds 2.22, 2.23, 2.41, 2.42, 2.43, 2.46 and 2.47.

DNA transfection is one of the mainly used technologies in the bioproduction of recombinant proteins and viruses by a process of transient gene expression (TGE). Concerning the production of AAV and lentivirus the most commonly used method is the transfection to deliver the viral and therapeutic genes in the producer cell lines, HEK293 adherent or suspension cells. In most systems, the co-transfection of many plasmids is performed by a chemical method, such as the co-precipitation with the calcium phosphate or the transfection mediated with the cationic polymer polyethylenimine (PEI), such as PEIpro® (Polyplus-transfection) commercially recommended for such a bioproduction of recombinant virus.

AAV and lentivirus particles were produced from HEK-293T cells through transient co-transfection of several plasmids containing the gene of interest and necessary viral components to produce full recombinant virions. AAV-2 and lentivirus vectors expressing the GFP reporter gene were produced with various compounds and the virus productivity was determined by assessing the transducing unit (TU/mL) 3 days post-transfection. The levels of productivity were compared to those obtained with the PEIpro® transfection reagent extensively used in adherent and suspension virus production systems.

Many compounds of Example 3 were tested for the production of AAV-2 as well as other compounds wherein the triazole ring was grafted by benzyl (**2.41** or **2.42**) or pyridinyl (**2.43** to **2.46**) moiety and wherein the cationic polymer was linked to the triazole ring in position Z¹ of the formula (III). **Figure 3** presents some of the results obtained. At a ratio of 1:2 (1 µg total DNA per µL of compound) used for the transfection, some compounds performed similarly in virus productivity than PEIpro® but most of them increased significantly by 3- to 8-fold the viral titer. This

improvement was confirmed for most of the compounds and enhanced by using a ratio of 1:3 with the highest increase of viral titer superior to 10-fold for compound **2.43**.

Similarly, lentiviruses were produced in suspension HEK-293T cells after co-transfection of 4 plasmids (pRSV-REV packaging vector, pCgpV Packaging Vector, pCMV-VSV-G Envelop
5 Vector and pLenti6.3/V5-GW/EmGFP Expression Control Vector). Lentivirus titers (TU/mL) were determined 72 hours post-transfection (**Figure 4**). An improvement of the LV production yield of about 10-fold was obtained when compared to the productivity with PEIpro® by using the compound **2.22** at a ratio of 1:3.

10 **Example 5. Compounds 2.53 to 2.61**

Screening of transfection activity

Compounds **2.53 to 2.61** were screened in transfection (**Figure 6**) similarly as previously described for compounds of Example 3, in 96-well plate by transfecting 200 ng of pCMV-EGFPLuc DNA (Clontech) complexed with 0.6 or 0.8 µL of one compound of the invention (at 7.5 mM
15 nitrogen concentration), defining a ratio of 1 µg DNA/ 3 µL of compound or ratio of 1 µg DNA/ 4 µL of compound, respectively.

Compounds **2.53 to 2.61** represent compounds having a triazole ring wherein the cationic polymer is linked at **Z¹** of the formula (III) and wherein various alkyl or cycle moiety were added on position **R** or **V** of the formula (III). **Figure 6** shows that grafting of alkyl or cycloalkyl moiety at
20 the position **R** or **V** on the triazole ring provides efficient compounds in transfection as exemplified by the compounds **2.54**, **2.56**, **2.58** or **2.57**. Surprisingly, compounds **2.60** and **2.61** with unsubstituted triazole ring on position R and V of the formula (III) were not able to transfect efficiently the Hep G2 cells.

Bioproduction of recombinant virus

25 Compounds **2.53 to 2.61** were tested for the production of AAV-2 and **Figure 7** presents the results obtained of compounds at ratio 1:2 µg DNA/µL reagent. AAV titers (transducing unit, TU/mL) were determined 72 hours post-transfection. The results are expressed as relative AAV-2 transducing Units/mL (TU/mL).

The compound **2.22** was used as a positive control. Compounds **2.54** and **2.57** showed
30 promising results and in correlation with the transfection activity presented in **Figure 6**. Contrary to the experiments of transfection in Hep G2 cells, compounds **2.60** and **2.61** wherein R and V=H showed high levels of AAV-2 productivity in HEK-293T cells

Example 6. Key parameters for the production of AAV-2 from suspension HEK-293T cells.

The production of recombinant virus is achieved by co-transfection of many plasmids in HEK293 cells. The virus productivity is greatly influenced by the total amount of plasmids and the volume of transfection reagent. **Figure 8** illustrates the AAV-2 production using the compound **2.22** (formulated at 15 mM nitrogen concentration). Different amounts of plasmids were used to transfect the HEK293-T cells in suspension. Many ratios of transfection reagent were also tested and expressed as $\mu\text{g DNA}/\mu\text{L reagent}$ per millions of cells the day of transfection. The results show that the virus productivity depends on the amount of plasmids transfected. In addition, for each amount of DNA transfected, the optimal productivity depends on the ratio of $\mu\text{g DNA}/\mu\text{L reagent}$. This example illustrates the transfection conditions with the compounds of formula (III) can be adapted easily to obtain an optimal virus productivity. **Figure 9** presents the influence of time of DNA complexation with the compound **2.22** on the production of AAV-2 from suspension HEK-293T cells. A minimal time of DNA complexation of 15 minutes before adding the transfection complexes into the cell culture is required to obtain high yield of virus production. A longer time of DNA complexation above 15 minutes can be used without affecting the virus yield, indicating a good stability of the transfection complexes in virus production activity. This property indicates that the compound **2.22** is particularly suitable for large scale applications in bioreactors where the time window during the transfer of the transfection complexes mixture needs to be adapted according to the cell culture volume.

Conclusion

Many compounds based on grafting of polyamine with heterocycles of formula (I), preferably of formula (III) showed improved performances to induce gene expression in "hard to transfect" cells such as cancer cells, or to increase the productivity of biologics such as viruses, AAV or LV.

Many compounds of Example 3, 4 or 5, particularly polyamine grafted with benzyl, fluorobenzyl, hydroxyphenyl, 4-hydroxyphenethyl, pyridine or phenyl triazole derivative showed high transfection efficiencies.

Selected compounds of Example 3, 4 or 5 also showed improved productivity of biologics such as AAV or LV, indicating a combined effect of high transfection efficiency and gene expression in cells resulting in high virus titers expressed as transducing units. Improved virus productivity was observed whatever the type of transfected cells, *e.g.* adherent or in suspension.

The results obtained indicated that such compounds might be also of interest to produce other biologics such as recombinant proteins, peptides or antibodies.

Taken together, the compounds of formula (I), preferably of formula (III) of the invention represent novel reagents for transfection and bioproduction purposes wherein a fine optimisation
5 of the chemical structure may be adapted for each application, cell types or transfection conditions.

The person skilled in the art can adapt the transfection method with the compounds of general formula (I), preferably of general formula (III) of the invention for *in vivo* applications with an acceptable excipient or buffering agent. The compounds of general formula (I), preferably of general formula (III) can be mixed with DNA to generate DNA complexes suitable for direct
10 injection into animals or humans. Particularly low salt buffering agents such as TRIS, phosphate, or citrate buffer or excipient such as glucose, dextrose, or maltose are known to provide acceptable formulation for direct injection into animals and humans. Many mixture methods between the DNA and the compounds of general formula (I), preferably of general formula (III) are suitable as they are able to generate formulation containing small size particles (non aggregated
15 DNA complexes) that can be injected through various routes of administration.

References

- Kaestner L, Schol A, Lipp P. Conceptual and technical aspects of transfection and gene delivery. *Bioorganic & Medicinal Chemistry Letters*, 25(6), 1171-1176 (2015).
- 20 Merten OW, Hebben M, Bovolenta C. Production of lentiviral vectors. *Mol Ther Methods Clin Dev*. 3:16017 (2016).
- Van der Loo JC, Wright JF. Progress and challenges in viral vector manufacturing. *Hum Mol Genet*. 25(R1):R42-52 (2015).
- Labat-Moleur F, Steffan AM, Brisson C, Perron H, Feugeas O, Furstenberger P, Oberling F,
25 Brambilla E, Behr JP. An electron microscopy study into the mechanism of gene transfer with lipopolyamines. *Gene Ther.*, 3(11):1010-7 (1996).
- Mislick KA, Baldeschwieler JD. Evidence for the role of proteoglycans in cation-mediated gene transfer. *Proc Natl Acad Sci U S A*, 93(22):12349-54 (1996).
- Felgner PL, Gadek TR, Holm M, Roman R, Chan HW, Wenz M, Northrop JP, Ringold GM,
30 Danielsen M. Lipofection: a highly efficient, lipid-mediated DNA-transfection procedure. *Proc Natl Acad Sci U S A*, 84(21):7413-7 (1987).
- Behr JP, Demeneix B, Loeffler JP, Perez-Mutul J. Efficient gene transfer into mammalian primary endocrine cells with lipopolyamine-coated DNA. *Proc Natl Acad Sci USA*, 86: 6982-6986 (1989).

- Gao X, Huang L. A novel cationic liposome reagent for efficient transfection of mammalian cells. *Biochem Biophys Res Commun.*, 179(1):280-5 (1991).
- Wu GY, Wu CH. Receptor-mediated in vitro gene transformation by a soluble DNA carrier system. *The Journal of Biological Chemistry*, 262, 4429-4432 (1987).
- 5 Zenke M, Steinlein P, Wagner E, Cotton M, Beug H, Birnstiel ML. Receptor-mediated endocytosis of transferrin-polycation conjugates: an efficient way to introduce DNA into hematopoietic cells. *Proc Natl Acad Sci USA*, 87(10) 3655-3659 (1990).
- Erbacher P, Roche AC, Monsigny M, Midoux P. Putative role of chloroquine in gene transfer into a human hepatoma cell line by DNA/lactosylated polylysine complexes. *Exp Cell Res.*, 225(1):186-10 94 (1996).
- Plank C, Oberhauser B, Mechtler K, Koch C, Wagner E. The influence of endosome-disruptive peptides on gene transfer using synthetic virus-like gene transfer systems. *J Biol Chem.*, 269(17):12918-24 (1994).
- Boussif O, Lezoualc'h F, Zanta MA, Mergny MD, Scherman D, Demeneix B, Behr JP. A versatile 15 vector for gene and oligonucleotide transfer into cells in culture and in vivo: polyethylenimine. *Proc Natl Acad Sci U S A.*, 92(16): 7297–7301 (1995).
- Sonawane ND, Szoka FC Jr, Verkman AS. Chloride accumulation and swelling in endosomes enhances DNA transfer by polyamine-DNA polyplexes. *J Biol Chem.*, 278(45):44826-31 (2003).
- Itaka K, Harada A, Yamasaki Y, et al. In situ single cell observation by fluorescence resonance 20 energy transfer reveals fast intracytoplasmic delivery and easy release of plasmid DNA complexed with linear polyethylenimine. *J Gene Med*, 6: 76-84 (2004).
- Chandrashekhar C, Pons B, Muller CD, Tounsi N, Mulherkar R, Zuber G. Oligobenzylethylenimine enriches linear polyethylenimine with a pH-sensitive membrane-disruptive property and leads to enhanced gene delivery activity. *Acta Biomaterialia*, 9(2):4985-4993 (2012).
- 25 Erbacher P, Zou S, Bettinger T, Steffan AM, Remy JS. Chitosan-based vector/DNA complexes for gene delivery: biophysical characteristics and transfection ability. *Pharm Res.*, 15(9):1332-9 (1998).
- Tomalia DA, Baker D, Dewald J, Hall M, Kallos G, Martin S, Roeck J, Ryder, Smith J. A New Class of Polymers: Starburst-Dendritic Macromolecules *Polymer Journal* 17, 117–132 (1985).
- 30 Haensler J, Szoka FC. Polyamidoamine cascade polymers mediate efficient transfection of cells in culture. *Bioconjugate Chem.*, 45, 372-379 (1993).
- Tang MX, Redemann CT, Szoka FC Jr. In vitro gene delivery by degraded polyamidoamine dendrimers. *Bioconjug Chem.*, 7(6):703-14 (1996).

- Liu X, Wu J, Yammine M, Zhou J, Posocco P, Viel S, Liu C, Ziarelli F, Fermeiglia M, Priol S, Victorero G, Nguyen C, Erbacher P, Behr JP, Peng L. Structurally flexible triethanolamine core PAMAM dendrimers are effective nanovectors for DNA transfection in vitro and in vivo to the mouse thymus. *Bioconjug Chem.*, 22(12):2461-73 (2011).
- 5 Little SR, Lynn D, Ge Q, Anderson DG, Puram SV, Chen J, Eisen H, Langer R. From The Cover: Poly- amino ester-containing microparticles enhance the activity of non-viral genetic vaccines. *Proc Natl Acad Sci USA*, 101(26), 9534-9539 (2004).
- Akinc A, Anderson DG, Lynn DM, Langer R. Synthesis of poly(beta-amino ester)s optimized for highly effective gene delivery. *Bioconjug Chem.*, 14:979–988 (2003).
- 10 Cryan S, Donohue R, Ravoo BJ, Darcy R, O'Driscoll CM. Cationic cyclodextrin amphiphiles as gene delivery vectors. *Journal of Drug Delivery Science and Technology*, 14(1), Pages 57-62 (2004).
- Dréan M, Debuigne A, Jérôme C, Goncalves C, Midoux P, Rieger J, Guégan P. Poly(N-methylvinylamine)-Based Copolymers for Improved Gene Transfection. *Macromol Biosci.*, 18(4): e1700353 (2018).
- 15 Boussif O, Delair T, Brua C, Veron L, Pavirani A, Kolbe HV. Synthesis of polyallylamine derivatives and their use as gene transfer vectors in vitro. *Bioconjug Chem.* 10(5):877-83 (1999).
- Dong Y, Skoultchi AI, Pollard JW. Efficient DNA transfection of quiescent mammalian cells using poly-L-ornithine. *Nucleic Acids Res.*, 21(3): 771–772 (1993).
- 20 Alhakamy NA, Berkland CJ. Polyarginine molecular weight determines transfection efficiency of calcium condensed complexes. *Mol Pharm.*, 10(5):1940-8 (2013).
- Putnam D, Zelikinb AN, Izumrudovc VA, Langer R. Polyhistidine–PEG:DNA nanocomposites for gene delivery *Biomaterials* 24:4425–4433 (2003).
- Gupta B, Levchenko TS, Torchilin VP. Intracellular delivery of large molecules and small particles by cell-penetrating proteins and peptides. *Advanced Drug Delivery Reviews*, 57(4), 637-651
- 25 (2005).
- Brunner S, Fürtbauer E, Sauer T, Kursa M, Wagner E. Overcoming the Nuclear Barrier: Cell Cycle Independent Nonviral Gene Transfer with Linear Polyethylenimine or Electroporation. *Mol. Ther.*, 5(1), 80-86 (2002).
- 30 Lukacs GL, Haggie P, Seksek O, Lechardeur D, Freedman N, Verkman AS. Size-dependent DNA mobility in cytoplasm and nucleus. *J Biol Chem.* 275(3):1625-9 (2000).
- Lechardeur D, Sohn KJ, Haardt M, Joshi PB, Monck M, Graham RW, Beatty B, Squire J, O'Brodovich H, Lukacs GL. Metabolic instability of plasmid DNA in the cytosol: a potential barrier to gene transfer. *Gene Ther.* 6(4):482-97 (1999).

Bai H, Schiralli Lester GM, Petishnok LC, Dean DA. Cytoplasmic transport and nuclear import of plasmid DNA. Bioscience Reports 37, BSR20160616 (2017).