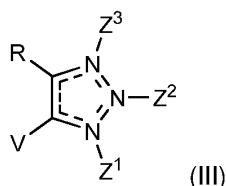


CLAIMS

1. 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 (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:

- **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 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 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⁺;

5 with the provisos that:

- at least one of **Z**¹, **Z**² 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⁺.

10 2. The composition according to claim 1, further comprising at least one nucleic acid molecule to be transfected in a cell, preferably a nucleic acid molecule 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
15 a plasmid encoding said nucleic acid molecule or a plasmid expressing said nucleic acid molecule.

3. The composition according to claim 2, wherein the at least one nucleic acid molecule is a DNA.

4. The composition according to any one of claims 1 to 3, wherein **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.
20

25

5. The composition according to any one of claims 1 to 4, 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⁺, wherein **X**₁, **X**₂, **R**₃ and **P**⁺ are as defined in claim 1; 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
30
- (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.

6. The composition according to any one of claims 1 to 5, wherein:

- 5 if (i) **Z**¹ 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**¹ 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
- 10 (ii) **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
- (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
- 15 (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.

20

7. The composition according to any one of claims 1 to 6, wherein:

- 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
- 25 (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
- 30 (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.

8. The composition according to any one of claims 1 to 7, wherein:

if (i) **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 CH₂, **X**₂ represents CO, and **R**₃ represents (CH₂)_m, with m

5 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

10 (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.

15

9. The composition according to any one of claims 1 to 8, wherein:

if (i) **R** or **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 herein; more preferably **Z**³ represents $X_1-R_3-X_2-P^+$, wherein **X**₁ represents CH₂, **X**₂ represents CO, and **R**₃ represents (CH₂)_m, with m

20 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

25 (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.

10. The composition according to any one of claims 1 to 9, wherein:

30 if (i) **R** or **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 herein; more preferably **Z**³ represents $X_1-R_3-X_2-P^+$, 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.

11. The composition according to any one of claims 1 to 10, 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.

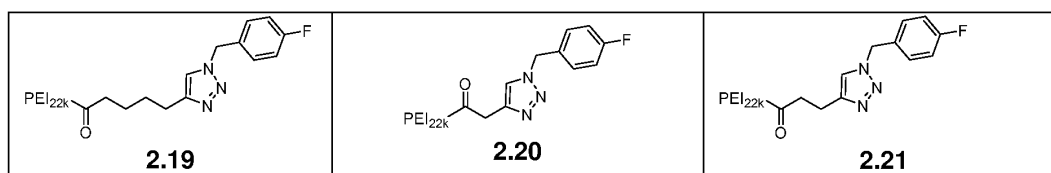
12. The composition according to claim 11, wherein the graft cationic polymer is a linear or branched PEI, more preferably a linear PEI.

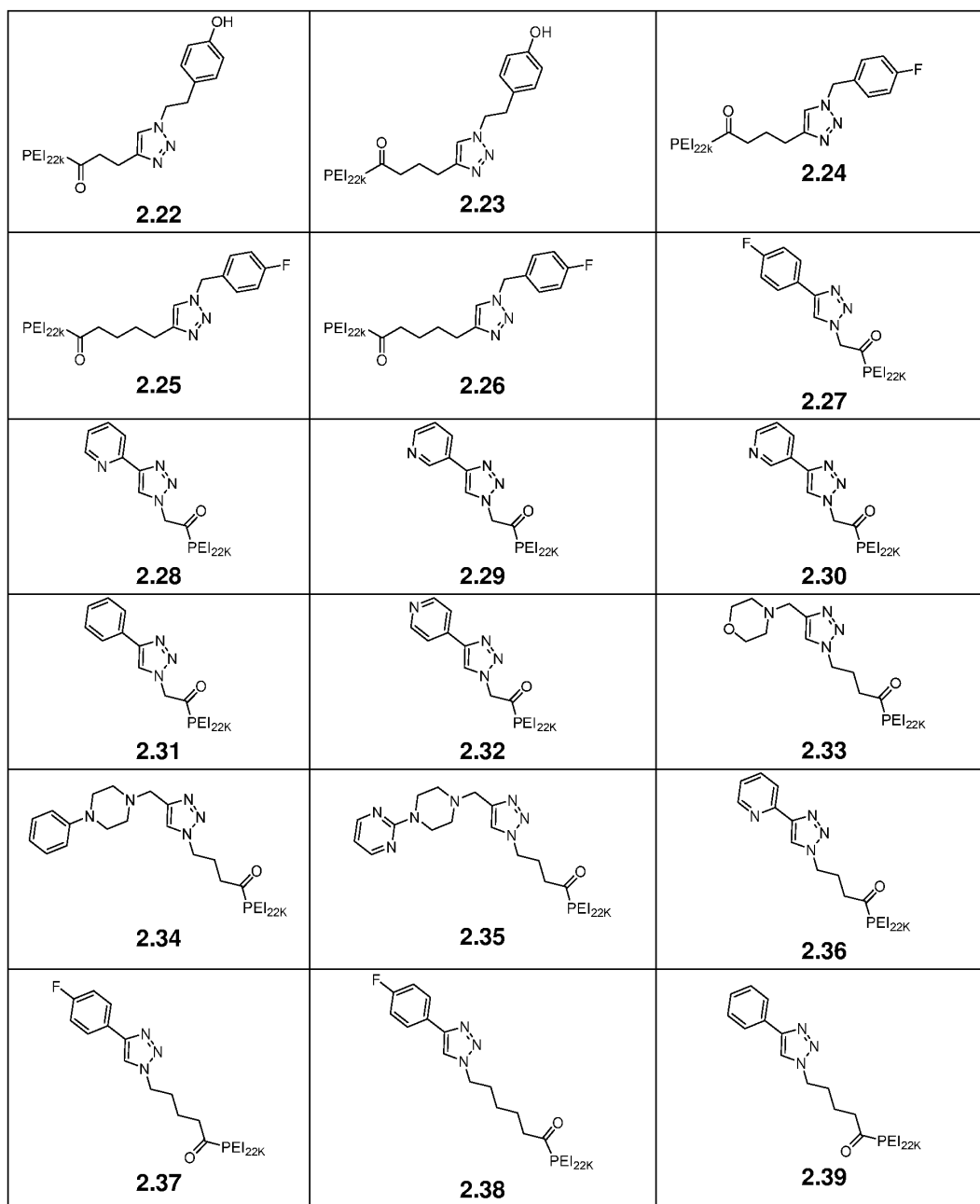
13. The composition according to any one of claims 1 to 12, wherein the graft cationic polymer has a grafting ratio ranging from 1 to 50%, preferably from 5 to 30%, more preferably is 20%.

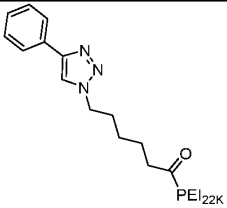
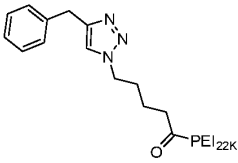
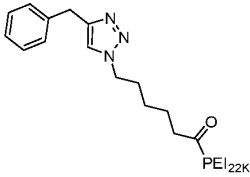
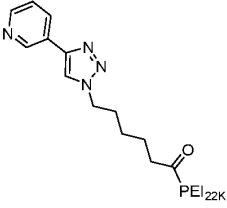
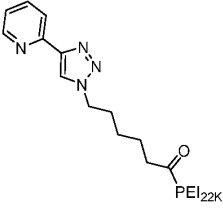
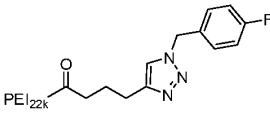
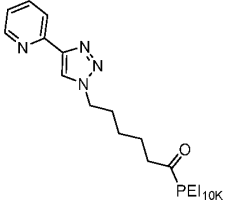
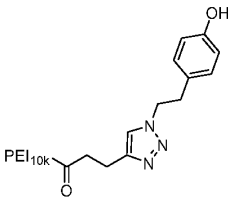
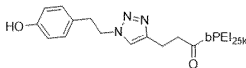
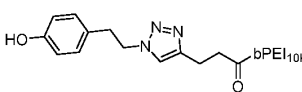
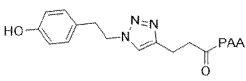
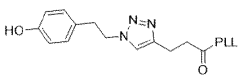
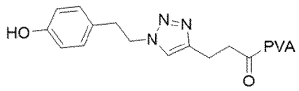
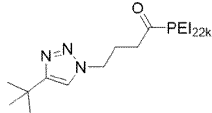
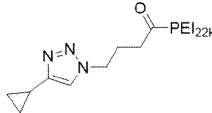
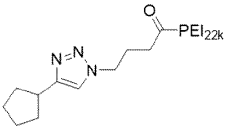
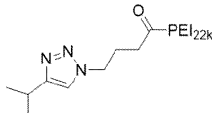
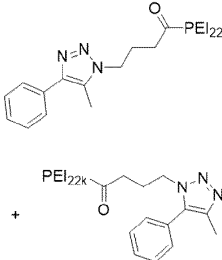
14. The composition according to any one of claims 1 to 13, wherein the graft cationic polymer has an average molecular weight (Mw) ranging from 1 kDa to 500 kDa, preferably from 1 kDa to 50 kDa, more preferably from 5 kDa to 50 kDa or from 1 kDa to 15 kDa.

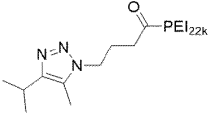
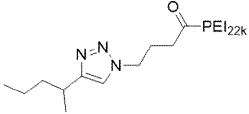
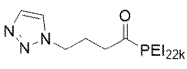
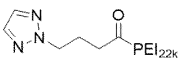
15. The composition according to claim 14, wherein the graft cationic polymer has an average molecular weight (Mw) of 6, 8, 10, 15, 22 or 30 kDa, preferably of 6, 8, 10, 15 or 30 kDa.

16. The composition according to any one of claims 1 to 15, wherein the at least one compound of general formula (III) is selected from the group consisting of the following compounds:

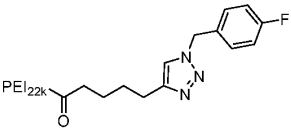
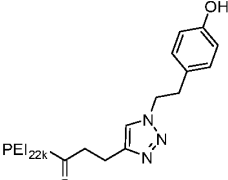
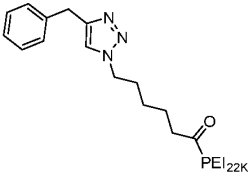
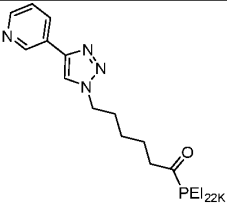
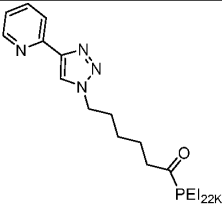
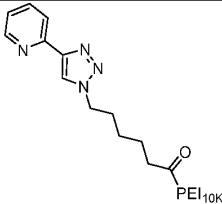
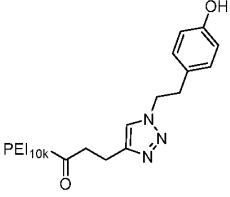
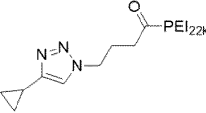
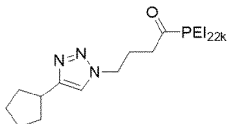


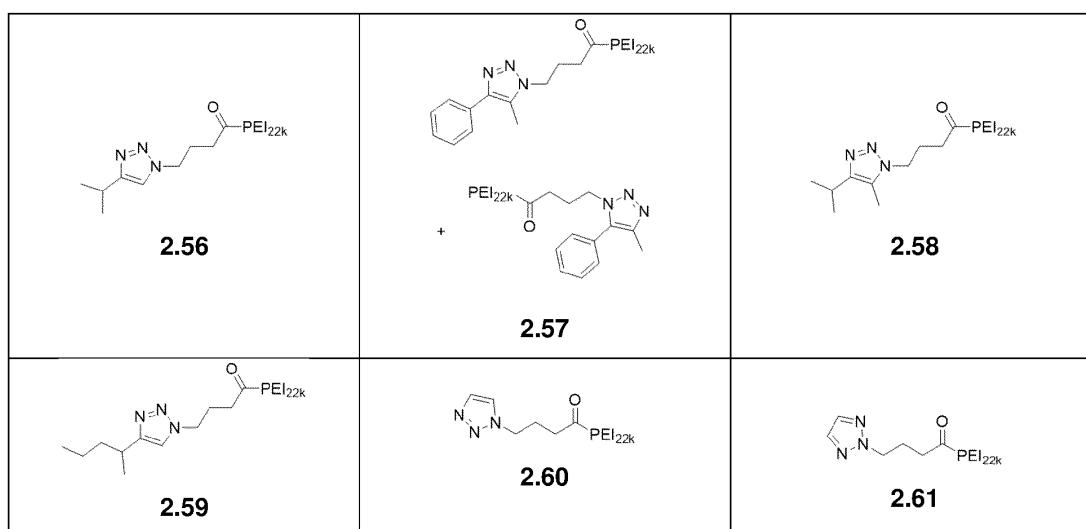


 2.40	 2.41	 2.42
 2.43	 2.44	 2.45
 2.46	 2.47	 2.48
 2.49	 2.50	 2.51
 2.52	 2.53	 2.54
 2.55	 2.56	 2.57

 2.58	 2.59	 2.60
 2.61		

17. The composition according to claim 16, wherein 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



18. The composition according to claim 17, wherein the at least one compound of general formula (III) is compound **2.22**.

5 19. A method for *in vitro* or *ex vivo* transfection of live cells comprising introducing in the cells the composition according to any one of claims 2 to 18.

20. *In vitro* or *ex vivo* use of the composition according to any one of claims 2 to 18 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.

15 21. *In vitro* or *ex vivo* use of the composition according to any one of claims 2 to 18 for genome engineering, for cell reprogramming, for differentiating cells, or for gene-editing.

22. 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 any one of claims 2 to 18 comprises multiple nucleic acid molecules for co-transfection; or (iii) viral

or virus-like particles, wherein the composition according to any one of claims 2 to 18 comprises multiple nucleic acid molecules for co-transfection.

23. The method according to claim 22, for the production of AAV, said composition comprising (i)
5 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.

24. The method according to claim 22, for the production of LV, said composition comprising (i) at
10 least the compound **2.22**, and (ii) an acceptable excipient, buffering agent, cell culture medium, or
transfection medium.

25. The method according to claim 22, for the production of recombinant virus, said composition
comprising a plurality of expression vectors such as plasmid vectors to transfect in an adherent or
15 suspension cell, such as HEK293, 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.

20 26. The method according to claim 25, wherein said recombinant virus is for use in *in vivo*
applications for cell therapy or for gene therapy.