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(54) Title: METHODS AND REAGENTS FOR CROSS-LINKING CELLULAR DNA AND RNA VIA STRAIN-PROMOTED DOUBLE-CLICK REACTIONS

(57) Abstract: The present invention relates to new reagents and methods for modifying nucleic acids where an azide-containing nucleoside or nucleotide derivative is applied to living cells followed by a nitrogenous derivative of dibenzo-1,5-cyclooctadiene (CODY). The azide-containing derivative is metabolically incorporated into cellular DNA and/or RNA, and the CODY derivative undergoes a strain-promoted double-click reaction to give nucleic acid – nucleic acid cross links, and/or optionally, in the presence of an exogenously added azide (X- N₃); to give nucleic acid – "X" cross links.

Methods and Reagents for Cross-Linking Cellular DNA and RNA via Strain-Promoted Double-Click Reactions

The present invention relates to new reagents and methods for modifying cellular DNA and/or RNA where an azide-containing nucleoside or nucleotide derivative is applied to living cells followed by a nitrogenous derivative of dibenzo-1,5-cyclooctadiyne (CODY). The azide-containing derivative is metabolically incorporated into cellular DNA and/or RNA, and the CODY derivative undergoes a strain-promoted double-click reaction to give nucleic acid – nucleic acid cross links, and/or optionally, in the presence of an exogenously added azide (X-N₃); to give nucleic acid – “X” cross links.

Background

Crosslinking of cellular DNA and/or RNA is required for numerous therapeutic and bioanalytical procedures. Bioorthogonal chemical reactions, such as strain-promoted azide-alkyne “click” (SPAAC) reactions (*J. Am. Chem. Soc.* **2004**, 126, 15046–15047), hold promise for crosslinking, but are limited by their inability to modify duplex nucleic acids in live cells. Due to the high stringency of cellular kinases and polymerases, the metabolic incorporation and cellular labeling of only a small handful of azide-containing nucleoside/nucleotide analogs compatible with SPAAC reactions are known (*ChemBioChem* **2014**, 15, 789–793; *ChemBioChem* **2016**, 17, 2149–2152; *Proc. Natl. Acad. Sci.* **2018**, 115, E1366-E1373.) Despite their promise for catalyst-free reactions on native duplexes, these studies utilized copper-catalyzed reactions, and/or chemical denaturation of the cellular nucleic acids prior to a copper-free click reaction. In natively folded, duplex nucleic acids, steric hinderance of the bioorthogonal functional groups inhibited catalyst-free reactions when standard reagents were used. For example, duplex DNA containing a 5-vinyl-pyrimidine residue gave no detectable product formation upon addition of an activated tetrazine or triazolindinedione (*Angew. Chemie. Int. Ed.* **2014**, 53, 9168–9172; *Angew. Chemie Int. Ed.* **2017**, 56, 10850–10853) and the reaction of duplex DNA containing 5-methylazido-pyrimidine residues with the strained cyclooctyne “BCN” gave little or no detectable product formation in cells unless the cellular DNA was first denatured into single strands (*ChemBioChem* **2014**, 15, 789–793). In the absence of the desired reactions, side-reactions became dominant, leading to high background signals in cells.

Proximity effects resulting from non-covalent binding interactions can accelerate reaction rates thereby facilitating selective covalent modification of nucleic acids. In the context of metabolic labeling strategies, a new approach was needed for accessing sterically hindered bioorthogonal functional groups in natively folded DNA/RNA molecules. By integrating strained alkynes directly into the scaffold of a planar, polycyclic core that also serves as an

intercalating agent, steric access to azide groups in folded nucleic acids can be gained via low-affinity, non-covalent intercalation reactions that effectively “scan” the duplex for azide-containing sites. The inventors choose dibenzocycloocta-1,5-diyne (“CODY”) also known as the Sondheimer diyne to evaluate this concept, due to the high importance of cross linking reactions in medicine and bioanalytics (*Org. Biomol. Chem.* **2010**, 8, 4051; WO2011118394A1, **2010**; *Angew. Chemie Int. Ed.* **2015**, 54, 15410). Since two alkyne groups are present in the CODY scaffold, tandem SPAAC reactions can generate a crosslink between an azido nucleic acid and azide-modified tag, or alternatively, interstrand cross links between opposing strands of nucleic acids if azide groups are present in both strands.

10 Terms and definitions

“Nucleotides” in the context of the present invention are nucleic acid or nucleic acid analogue building blocks, oligomers of which are capable of forming selective hybrids with RNA or DNA oligomers on the basis of base pairing. The hybridizing sequence may be composed of any of the nucleotides below, or mixtures thereof. The term nucleotides in this context includes the classic ribonucleotide building blocks adenosine, guanosine, uridine (and ribosylthymine), cytidine, the classic deoxyribonucleotides including deoxyadenosine, deoxyguanosine, thymidine, deoxyuridine and deoxycytidine. Nucleotides comprise nucleoside monophosphate, nucleoside diphosphate and nucleoside triphosphate. Nucleotide analogues comprise modifications of the base and/or ribose or deoxyribose moiety such as azide or -alkyl-N₃ moieties alone or in combination with modifications of the phosphate moieties, in particular phosphate triester.

“Nucleosides” in the context of the present invention are nucleic acid or nucleic acid analogue building blocks as defined above without a phosphate group.

The term “non-toxic” in the context of the present specification relates to the property of a compound of being non-lethal to mammalian cells. Lethality is determined using HeLa or U2OS cell cultures and the Alamar Blue assay that measures mitochondrial activity (*Bull. Environ. Contam. Tox.* **1981**, 26, 145). Non-toxic compounds are characterized by EC₅₀ > 100 μM.

The term “non-inhibiting substrate for a polymerase” relates to nucleotides and nucleotide analogues that do not significantly impact on the activity of a eukaryotic or prokaryotic DNA polymerase, in particular eukaryotic Family B polymerases, or a eukaryotic or prokaryotic DNA-directed RNA polymerase such as RNA polymerase II. The enzymatic activity of the polymerase with regard to the non-inhibiting substrate ranges from 80 % to 100 %, in particular 90 % to 100 %, compared to the enzymatic activity of the polymerase with regard to its natural substrate, i.e. nucleoside triphosphates selected from adenosine triphosphate

(ATP), guanosine triphosphate (GTP), cytidine triphosphate (CTP) and uridine triphosphate (UTP) for RNA polymerases and 2'-deoxyadenosine triphosphate (dATP), 2'-deoxyguanosine triphosphate (dGTP), 2'-deoxycytidine triphosphate (dCTP) and 2'-deoxythymidine triphosphate (dTTP) for DNA polymerases.

- 5 The term "physiological conditions" relates to the naturally occurring internal milieu of an organism or cell, in particular a mammalian cell. Typical conditions are a temperature of 20 °C to 40 °C, in particular 37 °C and pH 6 to 8.

The term "affinity label" relates to tags that are useful for affinity purification and/or imaging techniques. Non-limiting examples for affinity labels are streptavidin tag, biotin tag, FLAG-
10 tag, HaloTag ligand, poly-histidine tag, antibody, antibody fragment.

The term "scaffold moiety" relates to a non-toxic, planar, water soluble molecule that intercalates in duplex DNA and/or RNA strands.

The term "ex vivo" is to be understood as it is commonly used in the field of biology, biochemistry or medicine. "Ex vivo" relates to methods outside the human or animal body.

- 15 Cells, tissues or organs used in ex vivo methods may be in particular obtained from mammals, particularly humans. Cells can also include immortalized cell lines.

The term "phosphoester" relates to phosphodiester, phosphotriester, phosphoramidate or phosphorodiamidate moieties. The term "phosphoester" is not directed towards polyphosphate moieties such as a di- or triphosphate moiety.

20 Description

Based on the above mentioned state of the art, the objective of the present invention is to provide substances and methods to crosslink or to label cellular DNA and/or RNA. This objective is attained by the claims of the present specification.

- According to a first aspect of the invention, a kit of parts for crosslinking or labelling nucleic
25 acid comprising two separate components is provided. The first component comprises one or more non-toxic nucleoside analogues or nucleotide analogues, in particular one or more non-toxic nucleotide analogues, wherein each of the non-toxic nucleoside analogues or each of the non-toxic nucleotide analogues comprises one or two fusion moieties X which are covalently linkable to a counter fusion moiety Y, and the second component comprises one
30 or more non-toxic compounds BY₂, wherein B is a scaffold moiety, and Y is said counter fusion moiety which is covalently linkable to said fusion moiety X.

In certain embodiments, the kit of parts for crosslinking or labelling nucleic acid comprises two separate components, wherein the first component comprises one non-toxic nucleoside analogue or nucleotide analogue, in particular one non-toxic nucleotide analogue, wherein

the non-toxic nucleoside analogue or the non-toxic nucleotide analogue comprises one or two fusion moieties X which are covalently linkable to a counter fusion moiety Y, and the second component comprises one non-toxic compound BY₂, wherein B is a scaffold moiety, and Y is said counter fusion moiety which is covalently linkable to said fusion moiety X.

- 5 The first component of the kit of parts may comprise more than one nucleotide analogue, e.g. a deoxyadenosine phosphoester and a deoxycytidine nucleotide phosphoester. Alternatively, the first component may comprise one nucleotide analogue, e.g. a deoxyadenosine phosphoester. The wording "one or more" relates to types of nucleotide analogues. It does not relate to the number of discrete molecules. As shown in the examples, a plurality of
10 nucleotide analogue molecules may be added to the culture medium of cells (e.g. 1 to 100 µM nucleotide analogue per 40 000 cells in a cavity of a 24 well plate).

The same applies to the compound BY₂: Either one type of compounds BY₂ or a mix of two or more different compounds BY₂ may be used.

- 15 In certain embodiments of all aspects of the invention, the first component comprises at least one fusion moiety X.

In certain embodiments of all aspects of the invention, the first component comprises two fusion moieties X.

- 20 Crosslinking of nucleic acids is achieved by cellular uptake of the first component and subsequent incorporation of said component into DNA and/or RNA. For this, the first component might undergo intracellular modification such as esterase activity, nucleoside kinase activity, nucleotide kinase activity, nucleosidase kinase activity or spontaneous chemical cleavage of phosphate moieties to yield a nucleoside triphosphate that comprises one or two fusion moieties X. Incorporation occurs by a DNA and/or RNA polymerase. Upon cellular uptake of the second component, the counter fusion moieties Y of the second
25 component bind covalently to the fusion moieties X resulting in crosslinked DNA and/or RNA (see also Scheme 1 in Fig. 16).

The nucleoside analogue or nucleotide analogue comprising one or two fusion moieties X may be an azido-nucleoside or azido-nucleotide-phosphoester. The one or two azido moieties (-N₃) may be present at the ribose/deoxyribose and/or base moiety.

- 30 The fusion moiety and counter fusion moiety are covalently linkable by "click" chemistry. For example, the fusion moiety X may be an azide and the counter fusion moiety may be an alkyne. The azide and the alkyne react under physiological conditions by strain-promoted alkyne-azide cyclization.

- 35 An advantageous effect of the present invention is the absence of toxic initiator molecules such as a Cu(I) catalyst. Furthermore, the reaction of the fusion moiety and counter fusion

moiety does not require elevated temperatures and is performed under physiological conditions, in particular between 20 °C and 40 °C and pH 6-8.

The general crosslinking mechanism is depicted in Scheme 1 as shown in Fig. 16.

5 In certain embodiments, the kit of parts does not comprise a catalytically active molecule or means or substances for inducing an activating stimulus, in particular for inducing a change in temperature or pH. In certain embodiments, the kit of parts does not comprise a Cu(I) catalyst.

In certain embodiments of all aspects of the invention, the fusion moiety X reacts with the counter fusion moiety Y by strain-promoted alkyne-azide cyclization.

10 The nucleoside triphosphate analogue is a non-inhibiting substrate for a polymerase, in particular for a DNA-directed DNA-polymerase such as eukaryotic Family B polymerases, or a DNA-directed RNA polymerase such as RNA polymerase II.

The fusion moiety X is covalently linkable to the counter fusion moiety Y under physiological conditions. Typically, there is no need for an additional catalytic molecule or an activating
15 stimulus such as temperature increase or change of pH. The fusion moiety X binds primarily to the counter fusion moiety Y and not to components of a cell such as proteins, carbohydrates, signaling molecules or cellular membranes. Also the counter fusion moiety Y binds primarily to the fusion moiety X and not to components of a cell such as proteins, carbohydrates, signaling molecules or cellular membranes.

20 In certain embodiments of all aspects of the invention, the scaffold moiety B is water soluble. The scaffold moiety B is characterized by a water solubility of 0.1 nmol/l to 100 mmol/l, in particular 1 mmol/l to 10 mmol/l.

In certain embodiments of all aspects of the invention, the scaffold moiety B is planar. The atoms of the scaffold moiety B are mainly arranged in one plane. A deviation up to 20% of
25 this plane may occur.

In certain embodiments of all aspects of the invention, the scaffold moiety B is a nucleic acid intercalating moiety, in particular a duplex intercalating moiety.

The scaffold moiety B is not covalently linkable to components of a cell such as unmodified proteins, carbohydrates, signaling molecules or cellular membranes under physiological
30 conditions.

In certain embodiments, said kit of parts further comprises a third component, wherein the third component is a molecule comprising one or more fusion moieties X and optionally an affinity label, wherein particularly the third component is a molecule selected from a dye, a metal complex, a radioisotope, a spin probe, an RNA molecule, a DNA molecule, a peptide,

a protein and a carbohydrate each comprising one or more fusion moieties X and optionally an affinity label.

In certain embodiments of all aspects of the invention, said third component is a molecule comprising one or more fusion moieties X.

- 5 In certain embodiments of all aspects of the invention, said third component is a molecule comprising one or more fusion moieties X and an affinity label.

In certain embodiments of all aspects of the invention, said third component is a dye, a metal complex, a radioisotope, a spin probe, an RNA molecule, a DNA molecule, a peptide, a protein or a carbohydrate comprising one or more fusion moieties X.

- 10 In certain embodiments of all aspects of the invention, said third component is a dye, a metal complex, a radioisotope, a spin probe, an RNA molecule, a DNA molecule, a peptide, a protein or a carbohydrate comprising one or more fusion moieties X and an affinity label.

In certain embodiments of all aspects of the invention, said third component is a dye, a metal complex, a radioisotope, a spin probe comprising one or more fusion moieties X.

- 15 In certain embodiments of all aspects of the invention, said third component is a dye, a metal complex, a radioisotope, a spin probe comprising one or more fusion moieties X and an affinity label.

Particularly, the second component is administered 24 hours to 72 hours after administration of the first component. An incubation time of 24 hours to 72 hours for the first component is particularly performed if nucleoside analogues or nucleotide analogues for crosslinking and/or labeling of DNA are used.

- 20

Particularly, the second component is administered 2 hours to 24 hours, in particular 2 hours to 12 hours, more particularly 8 hours, after administration of the first component. An incubation time of 2 hours to 24 hours for the first component is particularly performed if nucleoside analogues or nucleotide analogues for crosslinking and/or labeling of RNA are used.

- 25

Preferably, the second component is administered 48 hours after administering the first component.

Particularly, the second component is administered 48 hours to 72 hours after administering the first component.

- 30

The time between administering the first and the second component may be adjusted according to the replication time. For interstrand crosslinking, the time chosen should be sufficient for at least two rounds of replication.

If the time between administration of the first and second component is 48 hours to 72 hours, the X-containing nucleoside analogues are incorporated in both strands of duplex DNA of a proliferating cell. Upon cellular uptake of the second component, one counter fusion moiety of the second component may react with one fusion moiety incorporated in one DNA strand and the other counter fusion moiety of the second component may react with another fusion moiety that is incorporated in the complementary DNA strand yielding interstrand crosslinked DNA.

Particularly, the second component is administered 24 hours after administering the first component.

Particularly, the third component is administered 24 hours after administering the second component.

The time between administering the first and the second component may be adjusted according to the replication time. For nucleic acid labeling using the three component kit, the time chosen should allow only one round of replication.

If the time between administration of the first and second component is 24 hours, the X-containing nucleoside analogues are mainly incorporated only in one strand of duplex DNA of a proliferating cell. Upon cellular uptake of the second component, only one counter fusion moiety may react with a fusion moiety incorporated in the DNA strand. The other counter fusion moiety may react with the third component leading to a tagged but not crosslinked DNA.

The third component may be used for monitoring the incorporation of the first component into nucleic acids. For this, a nucleotide modified by a fusion moiety X is first incorporated into nucleic acid as described above. Upon incubation with the second component, one counter fusion moiety Y of the second component binds covalently to X. The other counter fusion moiety may then react to a further fusion moiety X of the third component, e.g. an azide-modified fluorescent dye. To prevent binding of two third components to one second component, the second and third components are not administered simultaneously but separately, e.g. the third component is administered 24 hours after administration of the second component.

In certain embodiments of all aspects of the invention, the third component is non-toxic and/or cell permeable.

In certain embodiments of all aspects of the invention, the third component is a dye, in particular a fluorescent dye that comprises a fusion moiety X, wherein in particular the fusion moiety X comprises an azide moiety.

The general use of the kit of parts with three components is depicted in Scheme 2 as shown in Fig. 17.

According to a second aspect of the invention, a method for crosslinking nucleic acid *ex vivo* is provided. The method comprises the steps of

- 5 a. providing one or more non-toxic nucleoside analogues or nucleotide analogues, in particular one or more non-toxic nucleotide analogues, wherein each nucleoside analogue or nucleotide analogue comprises one or two fusion moieties X which are covalently linkable to a counter fusion moiety Y,
- 10 b. in a pulse step, contacting a proliferating cell with said non-toxic nucleoside analogues or nucleotide analogues yielding at least two nucleic acid strands that are each tagged with at least one fusion moiety X or yielding one nucleic acid strand that is tagged with at least two fusion moieties X,
- c. providing one or more non-toxic compounds BY_2 , wherein B is a scaffold moiety, and Y is a counter fusion moiety which is covalently linkable to said fusion moiety X,
- 15 d. in a crosslinking step, contacting, particularly incubating, said cell with said compound BY_2 yielding interstrand crosslinked nucleic acid strands and/or an intrastrand crosslinked nucleic acid strand, in particular interstrand crosslinked nucleic acid strands.

20 According to a third aspect of the invention, a method for labeling nucleic acid *ex vivo* is provided. The method for labeling nucleic acid comprising the steps of

- a. providing one or more non-toxic nucleoside analogues or nucleotide analogues, in particular one or more non-toxic nucleotide analogues, wherein said non-toxic nucleoside analogue or said non-toxic nucleotide analogue comprises one or two fusion moieties X which are covalently linkable to a counter fusion moiety Y,
- 25 b. in a pulse step, contacting a proliferating cell with said non-toxic nucleoside analogue or nucleotide analogue yielding a nucleic acid strand which is tagged by at least one fusion moiety X,
- c. providing one or more non-toxic compounds BY_2 , wherein B is a scaffold moiety, and Y is a counter fusion moiety which is covalently linkable to said fusion moiety X,
- 30 d. in a crosslinking step, contacting, particularly incubating, said cell with said compound BY_2 yielding a double-tagged nucleic acid strand which comprises said

compound BY_2 , wherein one counter fusion moiety Y is covalently bound to the fusion moiety X,

- 5 e. in a labeling step, contacting, particularly incubating, said cell with a third component, wherein the third component is a molecule comprising one or more fusion moieties X, in particular a molecule selected from a dye, a metal complex, a radioisotope, a spin probe, an RNA molecule, a DNA molecule, a peptide, a protein, a carbohydrate each comprising one or more fusion moieties X and optionally an affinity label, yielding a triple-tagged nucleic acid strand that comprises said double-tagged nucleic acid strand covalently bound to the fusion moiety X of the third component.
- 10

In certain embodiments of the second or third aspect of the invention, one non-toxic nucleoside analogue or nucleotide analogue, in particular one non-toxic nucleotide analogue is provided in step a, wherein the nucleoside analogue or nucleotide analogue comprises one or two fusion moieties X which are covalently linkable to a counter fusion moiety Y.

- 15 In certain embodiments of the second or third aspect of the invention, one non-toxic compound BY_2 is provided in step c.

In step a, more than one nucleotide analogue, e.g. a deoxyadenosine phosphoester and a deoxycytidine nucleotide phosphoester, may be provided. Alternatively, only one nucleotide analogue, e.g. a deoxyadenosine phosphoester, may be provided. The wording "one or more" relates to types of nucleotide analogues. It does not relate to the number of discrete molecules. As shown in the examples, a plurality of nucleotide analogue molecules may be added to the culture medium of cells (e.g. 1 to 100 μ M nucleotide analogue per 40 000 cells in a cavity of a 24 well plate).

20

The same applies to the compound BY_2 : Either one type of compounds BY_2 or a mix of two or more different compounds BY_2 may be used.

25

The method for crosslinking nucleic acid is performed outside a human or animal body. The method may be performed in vitro, e.g. in cells of a cell line, or ex vivo, e.g. in cells obtained from an animal or patient.

In certain embodiments of the second or third aspect of the invention, the method does not comprise a catalytically active molecule or means or substances for inducing an activating stimulus, in particular for inducing a change in temperature or pH.

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In certain embodiments of the second or third aspect of the invention, the method does not comprise a Cu(I) catalyst.

The same advantageous effects of a catalyst free reaction under physiological conditions apply also to the method for crosslinking nucleic acid.

In certain embodiments of the second or third aspect of the invention, the proliferating cell is a mammal cell.

- 5 In certain embodiments of the second or third aspect of the invention, the proliferating cell is a human cell.

In certain embodiments of the second or third aspect of the invention, the proliferating cell is obtained from a mammal.

- 10 In certain embodiment of the second or third aspect of the invention, the proliferating cell is obtained from a human.

In certain embodiments of the second or third aspect of the invention, the crosslinking step is performed 24 hours to 72 hours after the pulse step. An incubation time of 24 hours to 72 hours after the pulse step is particularly performed if nucleoside analogues or nucleotide analogues for crosslinking and/or labeling of DNA are used.

- 15 In certain embodiments of the first aspect of the invention, the crosslinking step is performed 2 hours to 24 hours, in particular 2 hours to 12 hours, more particularly 8 hours after the pulse step. An incubation time of 2 hours to 24 hours after the pulse step is particularly performed if nucleoside analogues or nucleotide analogues for crosslinking and/or labeling of RNA are used.

- 20 In certain embodiments of the second aspect of the invention, the crosslinking step is performed 48 hours after the pulse step.

In certain embodiments of the second aspect of the invention, the crosslinking step is performed 48 hours to 72 hours after the pulse step.

- 25 If the time between the pulse and the crosslinking step is 48 hours, the X-containing nucleoside analogues are incorporated in both strands of duplex DNA of a proliferating cell. Upon cellular uptake of the second component, one counter fusion moiety of the second component may react with one fusion moiety incorporated in one DNA strand and the other counter fusion moiety of the second component may react with another fusion moiety that is incorporated in the complementary DNA strand yielding interstrand crosslinked DNA.

- 30 In case two nucleoside analogues are incorporated in close proximity in one strand, also intrastrand crosslinking may occur.

In certain embodiments of the third aspect of the invention, the crosslinking step is performed 24 hours after the pulse step.

In certain embodiments of the third aspect of the invention, the labeling step is performed 24 hours after the crosslinking step.

If the time between the pulse and the crosslinking step is 24 hours, the X-containing nucleoside analogues are mainly incorporated only in one strand of duplex DNA of a proliferating cell. Upon cellular uptake of the second component, only one counter fusion moiety may react with a fusion moiety incorporated in the DNA strand. The other counter fusion moiety may react with the third component leading to a tagged but not crosslinked DNA.

In certain embodiments of the first, second or third aspect of the invention, said first component comprises a compound A-X_v with X being said fusion moiety X and v being 1 or 2, wherein the moiety A is a moiety of formula (I) and/or (II) and/or (III) and/or (IIIa),

D-M- (I), D'-M- (II) or -D''-M- (III), -D'''-M (IIIa) wherein

- D is a phosphoester moiety,
- D' is a triphosphate moiety,
- D'' is a monophosphate moiety,
- D''' is a hydroxyl group, and
- M is a nucleoside or an analogue or a derivative thereof.

In certain embodiments of the first, second or third aspect of the invention, D is a phosphodiester or phosphotriester moiety, in particular a phosphotriester moiety.

In certain embodiments of the first, second or third aspect of the invention, said nucleoside analogue or nucleotide analogue is a compound A-X_v with X being said fusion moiety X and v being 1 or 2, wherein the moiety A is a moiety of formula (I) or (II) or (III) or (IIIa).

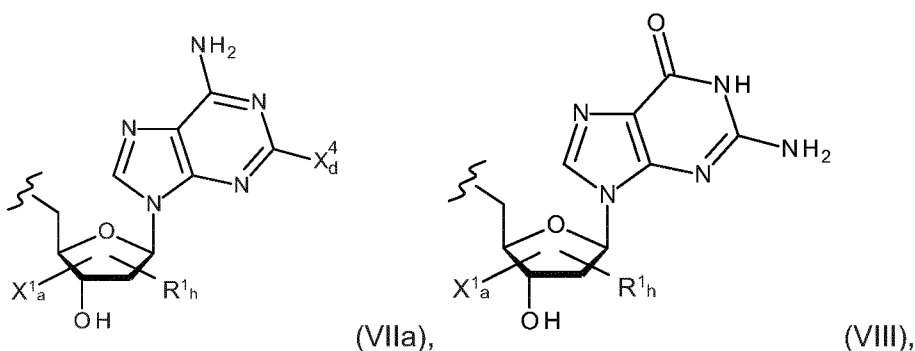
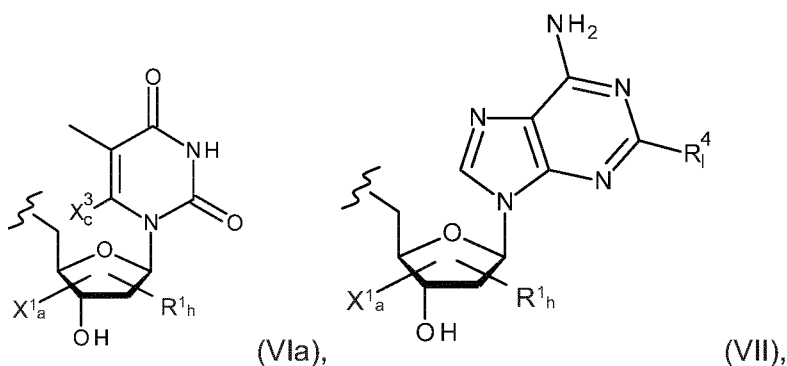
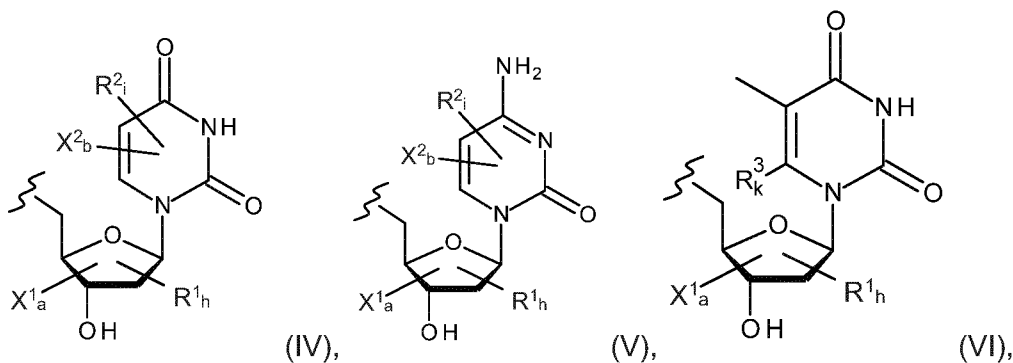
In certain embodiments of the first, second or third aspect of the invention, said nucleoside analogue or nucleotide analogue is a compound A-X_v with X being said fusion moiety X and v being 1 or 2, wherein the moiety A is a moiety of formula (I) or (IIIa), in particular of formula (I).

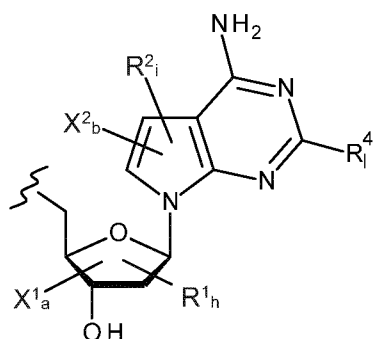
Nucleoside analogues such as 5-(azidomethyl)-2'-deoxyuridine (AmdU) are known to require phosphorylation by cellular kinases prior to incorporation into DNA or RNA. Some cells do not express kinases that are able to phosphorylate nucleoside analogues. To circumvent the lack of suitable kinases, an efficient pro-monophosphate strategy is the use of nucleotide analogues that comprise a phosphoester moiety such as compounds of formula (I). The phosphoester moiety may be cleaved by cellular esterases to generate a monophosphate moiety which is subsequently phosphorylated to generate nucleoside triphosphate analogue that is suitable as substrate for a polymerase.

Nucleotide analogues that comprise a mono-, di- or triphosphate moiety are negatively charged under physiological conditions and thus very stable against non-enzymatic hydrolysis by nucleophiles. Furthermore, cellular uptake of such nucleotide analogues occurs mainly by active transport or endo-/exocytosis. In contrast to this, nucleotide analogues that

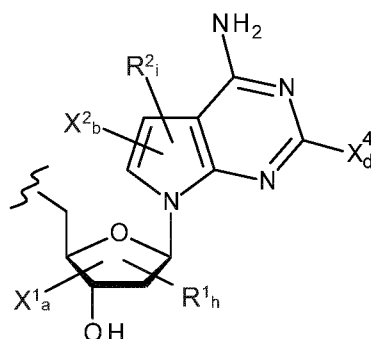
5 comprise a phosphoester moiety are usually non-charged and able to diffuse across the plasma membrane. Once inside the cell, the esters are cleaved by chemical or enzymatic hydrolysis and charged phosphate moieties are generated. Thus, nucleotide analogues comprising a phosphoester enter easily the cell. Due to the intracellular generation of phosphate moieties, diffusion out of the cell is prevented.

- 10 In certain embodiments of the first, second or third aspect of the invention, $-M(X)_v$ of D-M- $(X)_v$, D'-M- $(X)_v$, D''-M- $(X)_v$ or D'''-M- $(X)_v$, in particular of D-M- $(X)_v$, is a moiety of formula (IV), (V), (VI), (VIa), (VII), (VIIa), (VIII), (IX), (IXa) or (X), in particular of formula (IV), (V), (VI), (VII), (VIII), (IX) or (X)

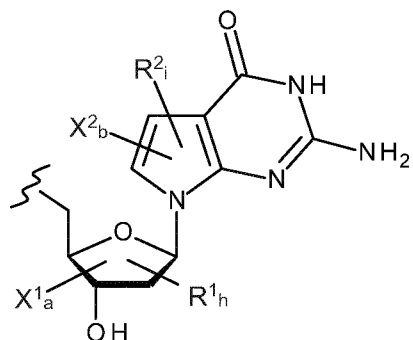




(IX),



(IXa),



(X), with

each X^1 , each X^2 , X^3 and X^4 being independently from each other selected from $-N_3$ and $-C_{1-4}$ -alkyl- N_3 ,

5 a and b being independently from each other an integer between 0 and 2,

c being 0 or 1, wherein the sum of a and c is equal to v,

d being 0 or 1, wherein the sum of a, b and d is equal to v,

R^1 , R^2 , R^3 and R^4 are independently from each other selected from $-F$, $-Cl$, $-Br$, $-I$, $-C_{1-4}$ -alkyl, $-C_{2-4}$ -alkenyl, $-O-C_{1-4}$ -alkyl, $-O-C(=O)-C_{1-4}$ -alkyl, $-S-C_{1-4}$ -alkyl, $-S-C(=O)-C_{1-4}$ -alkyl, $-OH$, $-NH_2$, $-N-HR^{20}$ and $-N-R^{20}_2$,

10

with R^{20} being $-C_{1-4}$ -alkyl

h is an integer between 0 and 5, wherein the sum of a and h is equal to or smaller than 5,

i is an integer between 0 and 2, wherein the sum of b and i is equal to or smaller than 2,

15

k and l are independently from each other 0 or 1.

In certain embodiments of the first, second or third aspect of the invention, $-M(X)_v$ of D-M- $(X)_v$, D'-M- $(X)_v$, D''-M- $(X)_v$ or D'''-M- $(X)_v$, in particular of D-M- $(X)_v$, is a moiety of formula (IV), (V), (VI), (VIa), (VII), (VIIa), (VIII), (IX), (IXa) or (X), in particular of formula (IV), (V), (VII), (VIII), (IX) or (X), with

20

each X^1 , each X^2 , X^3 and X^4 being independently from each other selected from $-N_3$ and $-C_{1-2}$ -alkyl- N_3 ,

a and b being independently from each other an integer between 0 and 1, wherein the sum of a and b is equal to v,

5 c being 0, wherein the sum of a and c is equal to v,

d being 0 or 1, in particular 0, wherein the sum of a, b and d is equal to v,

R^1 , R^2 , R^3 and R^4 are independently from each other selected from -F, -Cl, -Br, -I, $-C_{1-2}$ -alkyl, $-C_2$ -alkenyl, $-O-C_{1-2}$ -alkyl, $-O-C(=O)-C_{1-42}$ -alkyl, $-S-C_{1-2}$ -alkyl, $-S-C(=O)-C_{1-2}$ -alkyl, -OH, $-NH_2$, $-N-HR^{20}$ and $-N-R^{20}_2$,

10 with R^{20} being $-C_{1-4}$ -alkyl

h is an integer between 0 and 2, wherein the sum of a and h is equal to or smaller than 4,

i is an integer between 0 and 1, wherein the sum of b and i is equal to or smaller than 2,

15 k and l are independently from each other 0 or 1.

In certain embodiments of the first, second or third aspect of the invention, $-M-(X)_v$ or $D-M-(X)_v$, $D'-M-(X)_v$, $D''-M-(X)_v$ or $D'''-M-(X)_v$, in particular of $D-M-(X)_v$, is a moiety of formula (IV), (V), (VI), (VIa), (VII), (VIIa), (VIII), (IX), (IXa) or (X), in particular of formula (IV), (V), (VII), (VIII), (IX) or (X), with

20 each X^1 , each X^2 , X^3 and X^4 being independently from each other selected from $-N_3$ and $-CH_2-N_3$,

a and b being independently from each other an integer between 0 and 1, wherein the sum of a and b is equal to v,

c being 0, wherein the sum of a and c is equal to v,

25 d being 0, wherein the sum of a, b and d is equal to v,

R^1 , R^2 , R^3 and R^4 are independently from each other selected from -F, -Cl, $-CH_3$, $-O-CH_3$, $-O-C(=O)-CH_3$, $-S-CH_3$, -OH, particularly from -OH, -F and -Cl

with R^{20} being $-C_{1-4}$ -alkyl,

h is an integer between 0 and 1, particularly h is 0,

30 i is 0,

k and l are independently from each other 0 or 1.

In certain embodiments of the first, second or third aspect of the invention, the 2' position of the ribose moiety is not substituted (2' deoxy ribose moiety) or substituted with one substituent X^1 or R^1 in 2'S configuration or with two substituents independently selected from X^1 and R^1 .

- 5 In certain embodiments of the first, second or third aspect of the invention, the 2' position of the ribose moiety is not substituted (2' deoxy ribose moiety).

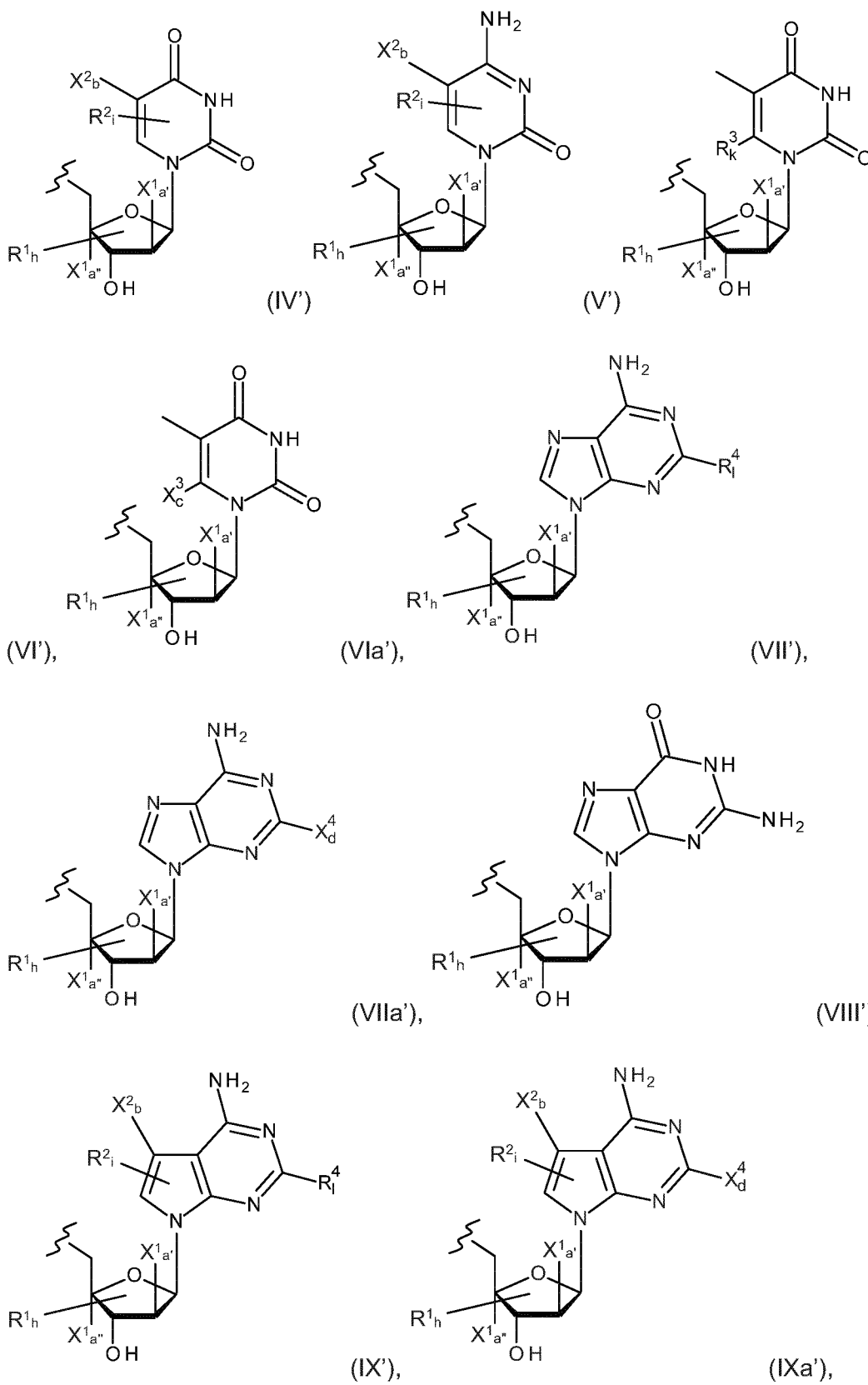
In certain embodiments of the first, second or third aspect of the invention, the 2' position of the ribose moiety is substituted with one substituent X^1 or R^1 in 2'R configuration or with two substituents independently selected from X^1 and R^1 .

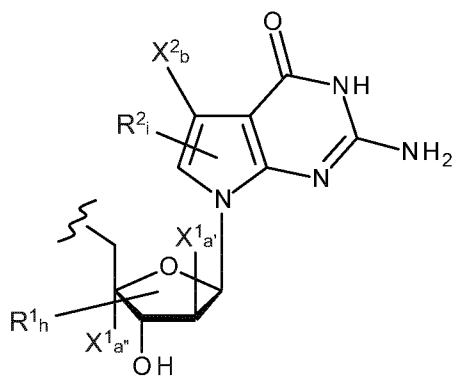
- 10 In certain embodiments of the first, second or third aspect of the invention, the 2' position of the ribose moiety is substituted with one substituent X^1 or R^1 in 2'R configuration.

For the incorporation of nucleoside analogues into nucleic acids, the substituents of the 2' position of the ribose moiety determine if the nucleoside analogue is a substrate of a DNA polymerase or a DNA-directed RNA polymerase. If the ribose moiety is not substituted at the

- 15 2' position (2' deoxy ribose moiety), the nucleoside analogue is a suitable substrate for a DNA polymerase and incorporated into DNA. If the ribose moiety is substituted in *R*-configuration at the 2' position by one substituent, e.g. by -OH or -N₃, the nucleoside analogue is a suitable substrate for a DNA-directed RNA polymerase and incorporated into RNA (2'S = H). If the ribose moiety is substituted in *S*-configuration at the 2' position by one
 20 substituent, e.g. -OH or -N₃, the nucleoside analogue is a suitable substrate for a DNA polymerase and/or DNA-directed RNA polymerase and incorporated in DNA and/or RNA (2'R = H). If the ribose moiety is substituted in both *R*- and *S*-configuration at the 2' position by two substituents, e.g. -OH or -N₃, the nucleoside analogue is a suitable substrate for a
 25 DNA polymerase and/or DNA-directed RNA polymerase and incorporated in DNA and/or RNA (2'R = H). If a 2'S-substituted or 2'S-2'R-substituted nucleoside analogue is primarily incorporated into DNA or RNA or into a mixture of DNA and RNA depends on the cell type.

- In certain embodiments of the first, second or third aspect of the invention, -M-(X)_v of D-M-(X)_v, D'-M-(X)_v, D''-M-(X)_v or D'''-M-(X)_v, in particular of D-M-(X)_v, is a moiety of formula (IV'), (V'), (VI'), (VIa'), (VII'), (VIIa'), (VIII'), (IX'), (IXa') or (X'), in particular of formula (IV'), (V'),
 30 (VI'), (VII'), (VIII'), (IX'), or (X'),





(X'), with

each X^1 , each X^2 , X^3 and X^4 being independently from each other selected from $-N_3$ and $-C_{1-4}$ -alkyl- N_3 ,

a' , a'' , b , c and d being independently from each other 0 or 1, wherein the sum of a' , a'' , b , c and d is equal or larger than 1,

R^1 being defined as described above,

R^2 , R^3 and R^4 are defined as described above,

h is an integer between 0 and 4, wherein the sum of a' , a'' and h is equal to or smaller than 4,

i is 0 or 1,

k and l are independently from each other 0 or 1.

In certain embodiments of the first, second or third aspect of the invention, $-M-(X)_v$ of D-M- $(X)_v$, D'-M- $(X)_v$, D''-M- $(X)_v$ or D'''-M- $(X)_v$, in particular of D-M- $(X)_v$, is a moiety of formula (IV'), (V'), (VI'), (VIa'), (VII'), (VIIa'), (VIII'), (IX'), (IXa') or (X'), in particular of formula (IV'), (V'), (VI'), (VII'), (VIII'), (IX'), or (X'), with

each X^1 , each X^2 , X^3 and X^4 being independently from each other selected from $-N_3$ and C_{1-2} -alkyl- N_3 ,

a' , a'' , b , c and d being independently from each other 0 or 1, wherein the sum of a' , a'' , b , c and d is equal or larger than 1,

R^1 being $-OH$,

R^2 , R^3 and R^4 $-Cl$ or $-F$,

h is an integer between 0 and 2, wherein the sum of a' , a'' and h is equal to or smaller than 4,

i is an 0 or 1,

k and l are independently from each other 0 or 1.

In certain embodiments of the first, second or third aspect of the invention, $-M(X)_v$ of $D-M(X)_v$, $D'-M(X)_v$, $D''-M(X)_v$ or $D'''-M(X)_v$, in particular of $D-M(X)_v$, is a moiety of formula (IV'), (V'), (VI'), (VIa'), (VII'), (VIIa'), (VIII'), (IX'), (IXa') or (X'), in particular of formula (IV'), (V'), (VI'), (VII'), (VIII'), (IX'), or (X'), with

5 each X^1 , each X^2 , X^3 and X^4 being independently from each other selected from $-N_3$ and $-CH_2N_3$,

- a' is 0, a'' is 1 and b is 0, or
- a' is 1, a'' is 0 and b is 0, or
- a' is 1, a'' is 0 and b is 1, and

10 c being 0 or 1,

d being 0 or 1,

R^1 being $-OH$,

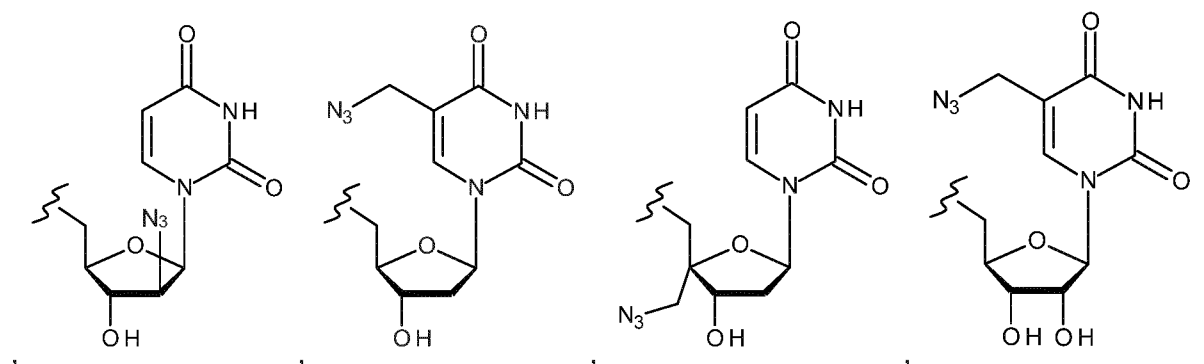
R^2 , R^3 and R^4 being $-Cl$ or $-F$,

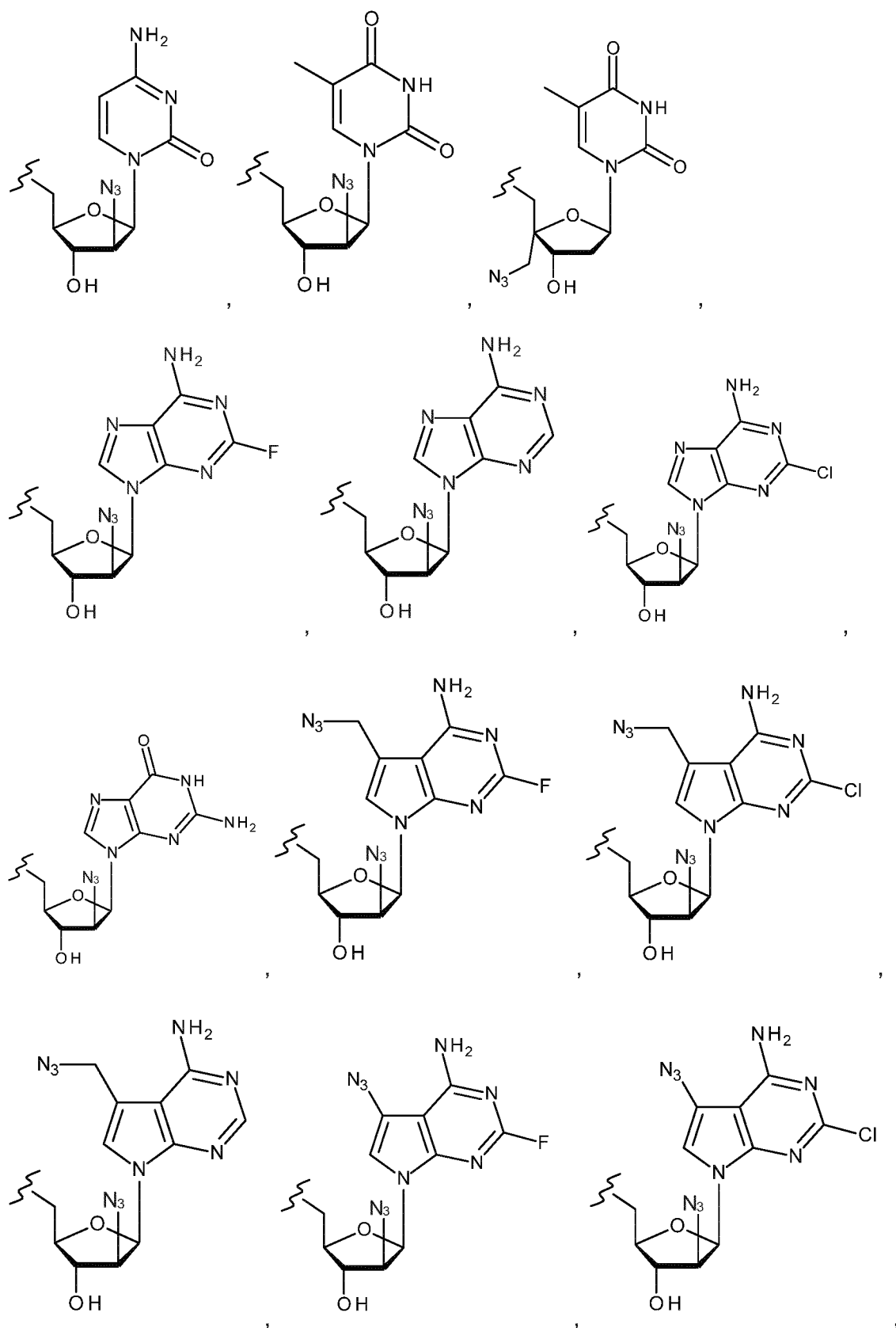
h is 0 or 1, wherein the sum of a' , a'' and h is equal to or smaller than 4,

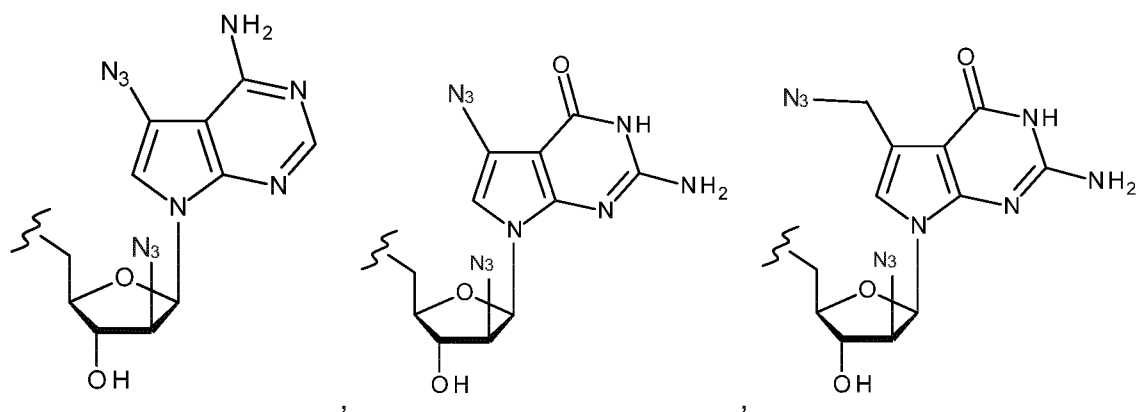
15 i is 0 or 1,

k and l are independently from each other 0 or 1.

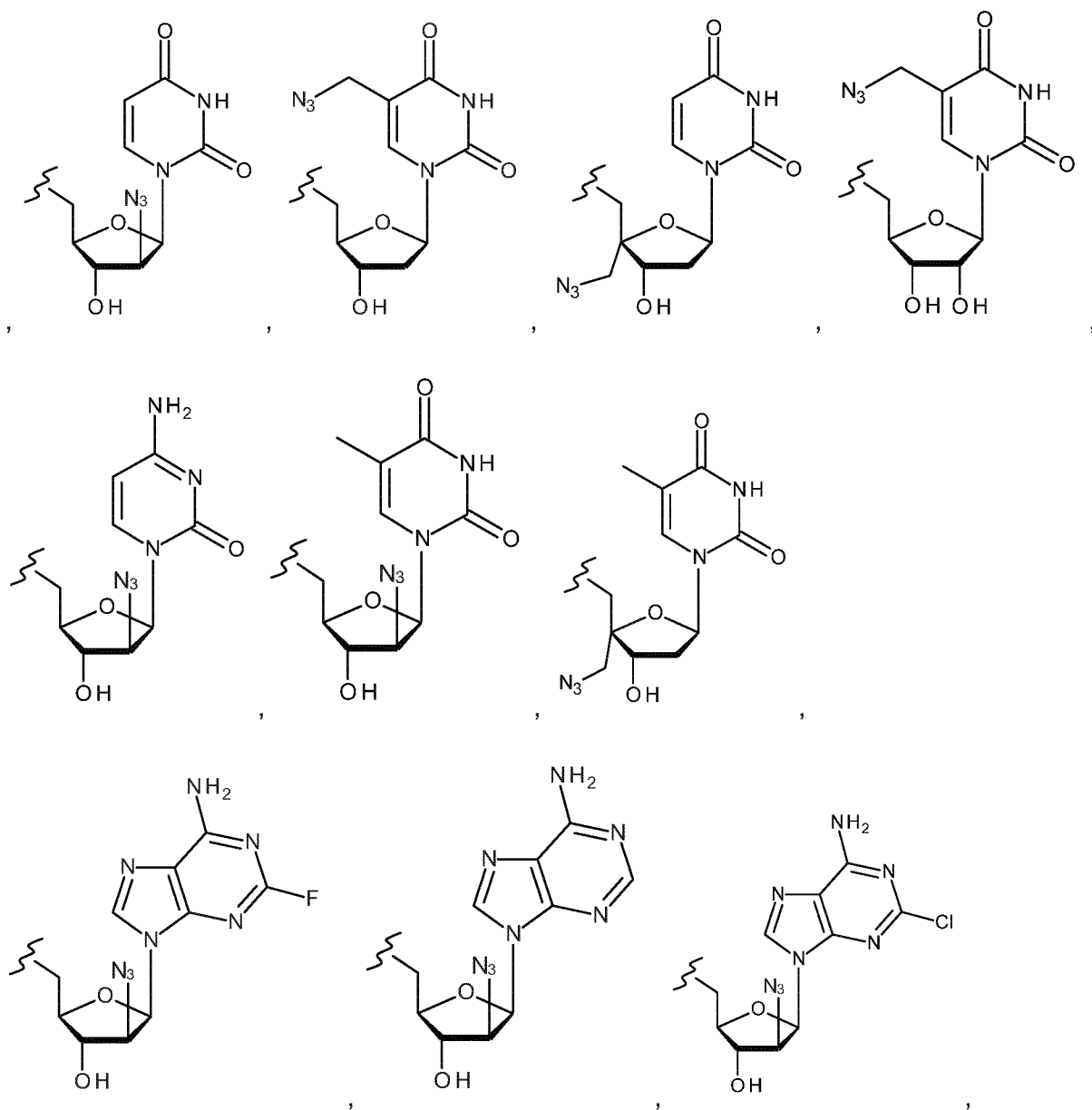
In certain embodiments of the first, second or third aspect of the invention, $-M(X)_v$ of $D-M(X)_v$, $D'-M(X)_v$, $D''-M(X)_v$ or $D'''-M(X)_v$, in particular of $D-M(X)_v$, is a moiety selected from

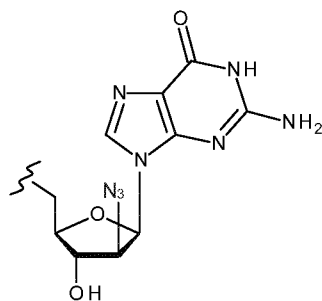




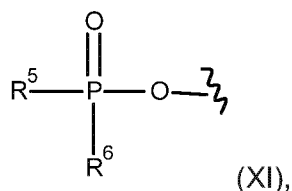


In certain embodiments of the first, second or third aspect of the invention, $-M-(X)_v$ of $D-M-(X)_v$, $D'-M-(X)_v$, $D''-M-(X)_v$ or $D'''-M-(X)_v$, in particular of $D-M-(X)_v$, is a moiety selected from





In certain embodiments of the first, second or third aspect of the invention, D is a phosphoester of formula (XI),



5 with R⁵ and R⁶ being independently selected from a moiety of formula (XII), (XIII) or (XIV),

L-G-E (XII), with

L being selected from -O-, -NH-, -NR⁷- and -S-,

10 G being selected from -C₁₋₆-alkyl-, -C₆₋₁₄-aryl-, -R⁸-C₆₋₁₄-aryl-, -C₆₋₁₄-aryl-R⁹-, -R⁸-C₆₋₁₄-aryl-R⁹-, -C₆₋₁₄-cycloalkyl-, -R⁸-C₆₋₁₄-cycloalkyl-, -C₆₋₁₄-cycloalkyl-R⁹-, -R⁸-C₆₋₁₄-cycloalkyl-R⁹-, -R⁸-O-C(=O)-R⁹-C₆₋₁₄-aryl-, -R⁸-C(=O)-O-R⁹-C₆₋₁₄-aryl-, -furyl- and -thiofuryl-,

R⁸ and R⁹ being independently from each other C₁₋₄-alkyl,

15 E being selected from -O-C(=O)-O-R¹⁰, -O-C(=O)-R¹⁰, -C(=O)-O-R¹⁰, -S-S-R¹⁰, -S-C(=O)-O-R¹⁰, -O-C(=O)-S-R¹⁰, -S-C(=O)-R¹⁰, -C(=O)-S-R¹⁰, and -NO₂,

R¹⁰ is independently selected from H or -C₁₋₆-alkyl, or

L-G (XIII), with

L being selected from -O-, -NH-, -NR⁷- and -S-, and

20 G being selected from -C₁₋₆-alkyl-, -C₆₋₁₄-aryl-, -R⁸-C₆₋₁₄-aryl-, -C₆₋₁₄-aryl-R⁹-, -R⁸-C₆₋₁₄-aryl-R⁹-, -C₆₋₁₄-cycloalkyl-, -R⁸-C₆₋₁₄-cycloalkyl-, -C₆₋₁₄-cycloalkyl-R⁹ and -R⁸-C₆₋₁₄-cycloalkyl-R⁹,

R⁸ and R⁹ being independently from each other C₁₋₄-alkyl, or

L-E (XIV), with

L being selected from -O-, -NH-, -NR⁷- and -S-,

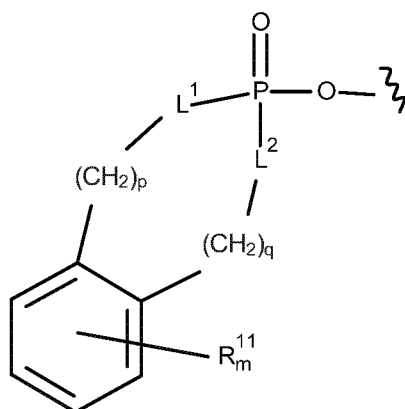
E being selected from $-C(=O)-O-R^{10}$ and $-C(=O)-S-R^{10}$,

R^{10} is independently selected from H or $-C_{1-6}$ -alkyl,

G of formula (XII) or (XIII) and/or R^{10} of formula (XII) or (XIV) may be optionally substituted by one or more substituents independently selected from -F, -Cl, -Br, -I, -
 5 C_{1-4} -alkyl, $-O-C_{1-4}$ -alkyl, $-CH_2-C(=O)-O-(CH_2)_{1-4}$ and $-CH_2-O-C(=O)-(CH_2)_{1-4}$, and/or

R^7 of formula (XII), (XIII) or (XIV) is independently selected from -G-E, -G or -E with G and E as defined above, or

D is a phosphoester of formula (XV),



(XV), with

10 L^1 and L^2 being independently from each other selected from -O-, -NH-, $-NR^7$ - and -S-,

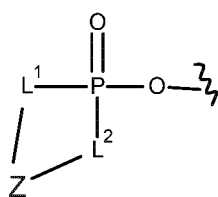
R^7 being independently selected from -G-E, -G or -E with G, E and n as defined above,

p and q being independently from each other an integer between 0 and 4,

15 R^{11} being independently selected from -F, -Cl, -Br, -I, $-C_{1-4}$ -alkyl, $-O-C_{1-4}$ -alkyl,

m being an integer between 0 and 4, or

D is a phosphoester of formula (XVI),



(XVI), with

20 L^1 and L^2 being independently from each other selected from -O-, -NH-, $-NR^7$ - and -S-,

R^7 being independently selected from -G-E, -G or -E with G, E and n as defined above,

Z being a C_r -alkyl, optionally substituted with R^{12}_s ,

r being an integer between 3 and 5,

5 s being an integer between 0 and 5, wherein s is equal or smaller than r,

R^{12} being -G or -E as defined for formula (XIII) or (XIV).

Upon cellular uptake of nucleotide analogues that comprise a phosphoester group as described above, the moiety L-G-E is cleaved. The moiety E is an enzyme sensitive group that may be cleaved by a carboxyesterase or reductase. Subsequently, the leaving group G
10 is released. L represents a linker.

In certain embodiments of the first, second or third aspect of the invention, D is a phosphoester of formula (XI).

In certain embodiments of the first, second or third aspect of the invention, D is a phosphoester of formula (XI) with R^5 and R^6 being independently selected from a moiety of
15 formula (XII) or (XIII).

In certain embodiments of the first, second or third aspect of the invention, D is a phosphoester of formula (XI), with R^5 and R^6 being independently selected from a moiety of formula (XII), (XIII) or (XIV),

- L-G-E (XII) with

20 L being selected from -O-, -NH- and -NR⁷-,

G being selected from -C₁₋₆-alkyl-, -C₆-aryl-, -R⁸-C₆-aryl-, -C₆-aryl-R⁹-, -R⁸-C₆-aryl-R⁹-, -C₆-cycloalkyl-, -R⁸-C₆-cycloalkyl-, -C₆-cycloalkyl-R⁹-, -R⁸-C₆-cycloalkyl-R⁹-, -R⁸-O-C(=O)-R⁹-C₆-aryl-, -R⁸-C(=O)-O-R⁹-C₆-aryl-, -furyl- and -thiofuryl-,

25 R^8 and R^9 being independently from each other C₁₋₄-alkyl, in particular C₁₋₂-alkyl,

E being selected from -O-C(=O)-O-R¹⁰, -O-C(=O)-R¹⁰, -C(=O)-O-R¹⁰, -S-S-R¹⁰, -S-C(=O)-O-R¹⁰, -O-C(=O)-S-R¹⁰, -S-C(=O)-R¹⁰, -C(=O)-S-R¹⁰, and -NO₂,

R^{10} is independently selected from H or -C₁₋₆-alkyl, or

30 - L-G (XIII) with

L being selected from -O-, -NH- and -NR⁷-,

G being selected from -C₁₋₆-alkyl, -C₆-aryl, -R⁸-C₆-aryl, -C₆-aryl-R⁹, -R⁸-C₆-aryl-R⁹, -C₆-cycloalkyl, -R⁸-C₆-cycloalkyl, -C₆-cycloalkyl-R⁹ and -R⁸-C₆-cycloalkyl-R⁹,

R⁸ and R⁹ being independently from each other C₁₋₄-alkyl, or

5 - L-E (XIV) with

L being selected from -O-, -NH- and -NR⁷,

E being selected from -C(=O)-O-R¹⁰ and -C(=O)-S-R¹⁰,

R¹⁰ is independently selected from H or -C₁₋₄-alkyl,

10 G of formula (XII) or (XIII) and/or R¹⁰ of formula (XII) or (XIV) may be optionally substituted by one or more substituents independently selected from -F, -Cl, -Br, -I, -C₁₋₄-alkyl, -O-C₁₋₄-alkyl, -CH₂-C(=O)-O-(CH₂)₁₋₂ and -CH₂-O-C(=O)-(CH₂)₁₋₂, and/or R⁷ of formula (XII), (XIII) or (XIV) is independently selected from -G-E, -G or -E with G and E as defined above, or

D is a phosphoester of formula (XV), with

15 L¹ and L² being independently from each other selected from -O-, -NH- and -NR⁷,

R⁷ being independently selected from -G-E, -G or -E with G and E as defined above,

p and q being independently from each other an integer between 0 and 2,

20 R¹¹ being independently selected from -F, -Cl, -Br, -I, -C₁₋₄-alkyl, -O-C₁₋₄-alkyl, m being an integer between 0 and 4, or

D is a phosphoester of formula (XVI) with

L¹ and L² being independently from each other selected from -O-, -NH- and -NR⁷-,

25 R⁷ being independently selected from -G-E, -G or -E with G, E and n as defined above,

Z being a C_r-alkyl, optionally substituted with R¹²_s,

r being an integer between 3 and 5,

s being an integer between 0 and 3, wherein s is equal or smaller than r,

30 R¹² being -G or -E as defined for formula (XIII) or (XIV),

in particular D is a phosphoester of formula (XI) with R^5 and R^6 being independently selected from a moiety of formula (XII) (XIII) or (XIV), more particularly, D is a phosphoester of formula (XI) with R^5 and R^6 being independently selected from a moiety of formula (XII) or (XIII).

5 In certain embodiments of the first, second or third aspect of the invention, D is a phosphoester of formula (XI), with R^5 and R^6 being independently selected from a moiety of formula (XII), (XIII) or (XIV),

- L-G-E (XII) with

L being selected from -O-, -NH- and -NR⁷-,

10 G being selected from -C₁₋₄-alkyl-, particularly -C₁₋₂-alkyl-, -C₆-aryl-, -R⁸-C₆-aryl-, -C₆-cycloalkyl-, -R⁸-O-C(=O)-R⁹-C₆-aryl-, -furyl- and -thiofuryl-,

R^8 and R^9 being independently from each other C₁₋₄-alkyl, in particular C₁₋₂-alkyl,

15 E being selected from -O-C(=O)-O-R¹⁰, -O-C(=O)-R¹⁰, -C(=O)-O-R¹⁰, -S-S-R¹⁰, -S-C(=O)-O-R¹⁰ and -NO₂,

R^{10} is independently selected from H or -C₁₋₆-alkyl, or

- L-G (XIII) with

L being selected from -O-, -NH- and -NR⁷-,

20 G being selected from -C₁₋₆-alkyl, in particular -C₁₋₂-alkyl, -C₆-aryl, -C₆-cycloalkyl and -R⁸-C₆-cycloalkyl,

R^8 and R^9 being independently from each other C₁₋₄-alkyl, or

- L-E (XIV) with

L being selected from -O-, -NH- and -NR⁷-,

E being selected from -C(=O)-O-R¹⁰,

25 R^{10} is independently selected from H or -C₁₋₄-alkyl,

G of formula (XII) or (XIII) and/or R^{10} of formula (XII) or (XIV) may be optionally substituted by one or more substituents independently selected from -F, -Cl, -Br, -I, -C₁₋₂-alkyl, -O-C₁₋₂-alkyl, -CH₂-C(=O)-O-(CH₂)₁₋₂ and -CH₂-O-C(=O)-(CH₂)₁₋₂, and/or R^7 of formula (XII), (XIII) or (XIV) is independently selected from -G-E, -G or -E with G and E as defined above, or

30 D is a phosphoester of formula (XV), with

L^1 and L^2 being independently from each other selected from -O-, -NH- and -NR⁷,

R^7 being independently selected from -G-E, -G or -E with G and E as defined above,

5 p being 0 and q being,

R^{11} being independently selected from -F, -Cl, -Br, -I, -C₁₋₄-alkyl, -O-C₁₋₄-alkyl, in particular selected from -F, -Cl, -C₁₋₂-alkyl, -O-C₁₋₂-alkyl,

m being an integer between 0 and 4, in particular 0 and 2, or

D is a phosphoester of formula (XVI) with

10 L^1 and L^2 being independently from each other selected from -O-, -NH- and -NR⁷-,

R^7 being independently selected from -G-E, -G or -E with G and E as defined above,

Z being a C_r-alkyl, optionally substituted with R¹²_s,

15 r being 3,

s being 0 or 1,

R^{12} being -G or -E as defined for formula (XIII) or (XIV) ,

in particular D is a phosphoester of formula (XI) with R^5 and R^6 being independently selected from a moiety of formula (XII) (XIII) or (XIV), more particularly, D is a phosphoester of formula (XI) with R^5 and R^6 being independently selected from a moiety of formula (XII) or (XIII).

20

In certain embodiments of the first, second or third aspect of the invention, D is a phosphoester of formula (XI) with R^5 and R^6 being independently selected from a moiety of formula (XII) or (XIII),

25 - L-G-E (XII) with

L being -O- or -NH-,

G being -C₁₋₂-alkyl-,

E being selected from -O-C(=O)-O-R¹⁰, -O-C(=O)-R¹⁰, -C(=O)-O-R¹⁰,

R^{10} is independently selected from H or -C₁₋₆-alkyl, in particular -C₁₋₆-alkyl, or

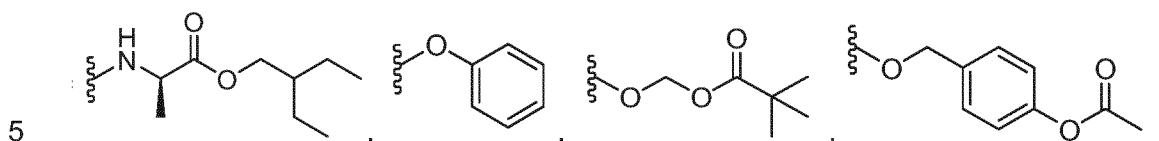
30

- L-G (XIII) with

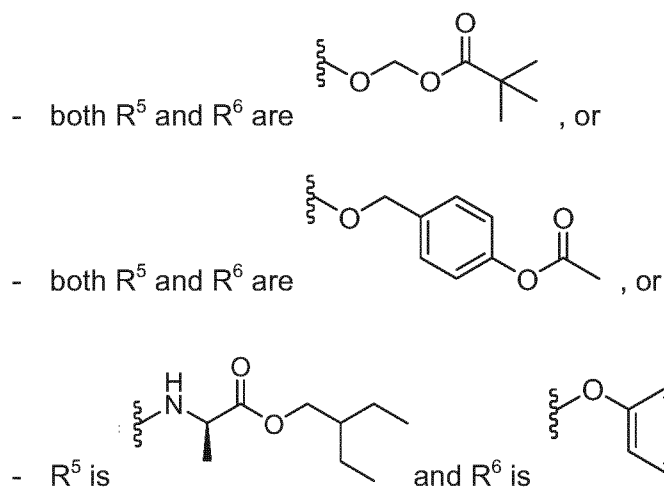
L being -O-,

G being -C₆-aryl or -C₆-cycloalkyl, in particular -C₆-aryl.

In certain embodiments of the first, second or third aspect of the invention, R⁵ and R⁶ are independently selected from



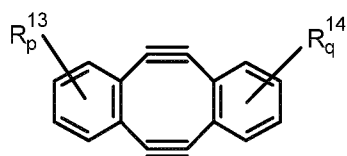
In certain embodiments,



- 10 In certain embodiments of the first, second or third aspect of the invention, said counter fusion moiety Y is a carbon-carbon triple bond, wherein in particular the carbon-carbon triple bond is part of a cyclic system of said scaffold moiety B.

As described above, the carbon-carbon triple bond may react with an -N₃ moiety in a strain-promoted alkyne-azide cyclization.

- 15 In certain embodiments of the first, second or third aspect of the invention, said compound BY₂ is a compound of formula (XVII),



(XVII), wherein

R¹³ and R¹⁴ are polar moieties that increase the solubility of dibenzocyclooctadiyne (CAS No. 53397-65-2) in water, and

- 20 p and q are integers between 0 and 4, in particular between 0 and 2, more particularly p and q are independently 1 or 2.

The polar moieties R^{13} and R^{14} are hydrophilic moieties having a net dipole.

Upon cellular uptake, the compound BY_2 intercalates into cellular duplex DNA or RNA. If the cell was treated with a first compound $A-X_v$ prior to the treatment with compound BY_2 , the DNA and/or RNA is modified by azide-containing nucleosides. If an azide moiety is in close proximity of the intercalated BY_2 compound, strain-promoted alkyne-azide cyclization occurs.

In certain embodiments of the first, second or third aspect of the invention, R^{13} and R^{14} are independently selected from

$-O-R^{15}$, $-NH-R^{16}$ or $-N(R^{16})_2$, wherein

R^{15} and R^{16} are independently from each other selected from

$-C_{1-4}$ -alkyl, $-C_{3-6}$ -cycloalkyl, C_6 -aryl, 7 to 10, in particular 7 to 8, more particularly 8 membered bicycle, 7 to 10, in particular 7 to 8, more particularly 8 membered heterobicycle, in particular $-C_{1-3}$ -alkyl or 7 to 10 membered heterobicycle,

$-C_{1-4}$ -alkyl- R^{17} , in particular C_{1-3} -alkyl- R^{17} ,

$-C_{1-4}$ -alkyl- R^{18} - C_{1-4} -alkyl, in particular $-C_{1-3}$ -alkyl- R^{18} - C_{1-3} -alkyl, and

$-C_{1-4}$ -alkyl- R^{18} - C_{1-4} -alkyl- R^{17} , in particular $-C_{1-4}$ -alkyl- R^{18} - C_{1-4} -alkyl- R^{17} , wherein

the C_{1-4} -alkyl- C_{3-6} -cycloalkyl or C_6 -aryl may optionally be substituted by one or more substituents independently selected from $-F$, $-Cl$, $-Br$, $-I$, $-OH$, $-O-C_{1-2}$ -alkyl, $-NH_2$,

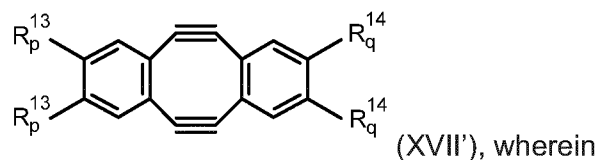
R^{17} is selected from $-NH_2$, $-NR^{19}H$, $-N(R^{19})_2$, $-OH$, $-O-C_{1-2}$ -alkyl, 3 to 6 membered heterocycle, 7 to 10, in particular 7 to 8, more particularly 8 membered bicycle, 7 to 10, in particular 7 to 8, more particularly 8 membered heterobicycle, 5 or 6 membered heteroaryl, C_6 -aryl, C_{3-6} -cycloalkyl, in particular $-NH_2$, $-NR^{19}H$, $-N(R^{19})_2$, $-OH$, $-O-C_{1-2}$ -alkyl, 3 to 6 membered heterocycle, $-O$ -*tert*-butyldimethylsilyl,

R^{18} is selected from $-O-$, $-C(=O)-$, $-C(=O)-O-$, $-O-C(=O)-$, $-O-C(=O)-O-$, $-NH-$, $-C(=O)-NH-$, $-NH-C(=O)-$, $-O-C(=O)-NH-$, $-NH-C(=O)-O-$,

R^{19} being independently selected from $-C_{1-3}$ -alkyl, $-C_{1-3}$ -alcohol.

In case of R^{13} and/or R^{14} being $-N(R^{16})_2$, both residues R^{16} may be identical or differ from each other.

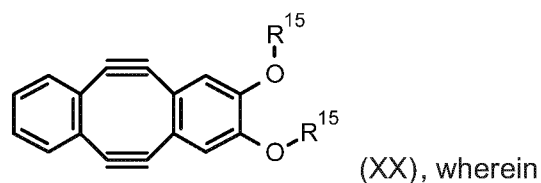
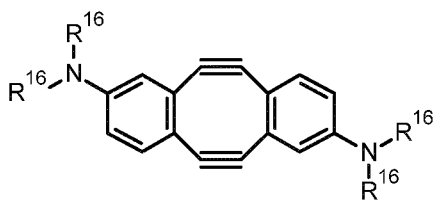
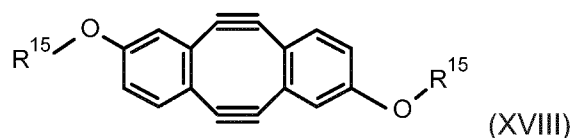
In certain embodiments of the first, second or third aspect of the invention, said compound BY_2 is a compound of formula (XVII'),



each R^{13} and each R^{14} are independently from each other defined as above,
and

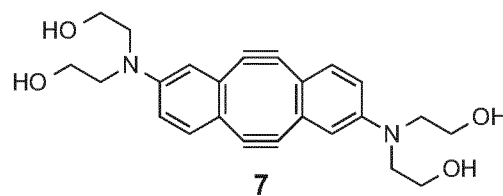
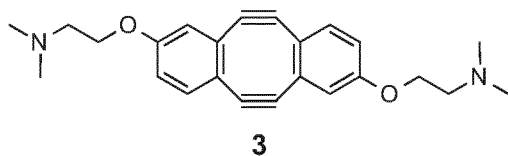
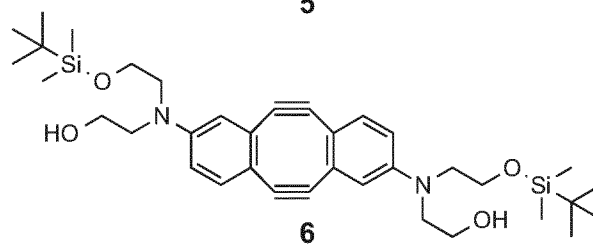
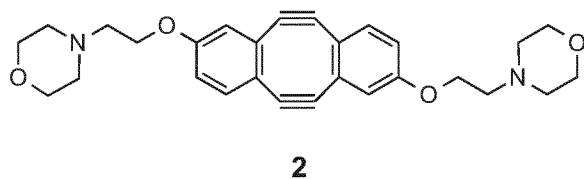
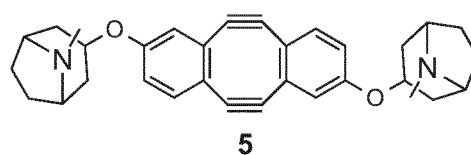
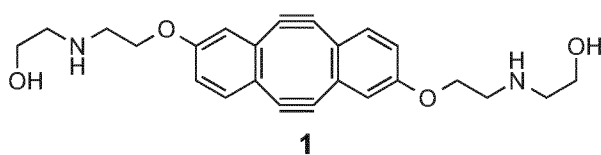
each p and each q are independently from each other 0 or 1, wherein in
particular the sum of p and q is larger than 1, more particularly the sum of p
and q is 2.

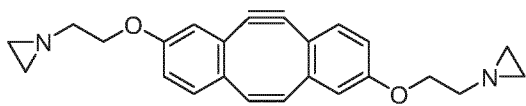
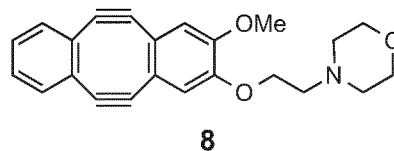
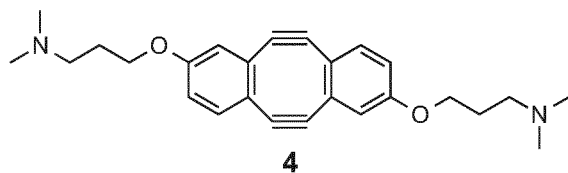
In certain embodiments of the first, second or third aspect of the invention, said compound
 BY_2 is a compound of formula (XVIII), (XIX) or (XX),



each R^{15} and each R^{16} are independently from each other defined as described above.

In certain embodiments of the first, second or third aspect of the invention, compound BY_2 is
selected from





According to a fourth aspect of the invention, a combination medicament comprising the kit of parts according to the first aspect of the invention for use in a method in the treatment of a disease is provided.

Particularly, the combination medicament for use in a method for treatment of a disease comprises the first and the second component according to the first aspect or any embodiment thereof.

Particularly, the combination medicament for use in a method in the treatment of a disease does not comprise the third component according to any embodiment of the first aspect.

According to a fifth aspect of the invention, a medicament comprising the kit of parts according to the first aspect of the invention for use in a method in the treatment of cancer is provided.

Particularly, the combination medicament for use in a method for treatment of cancer comprises the first and the second component according to the first aspect or any embodiment thereof.

Particularly, the combination medicament for use in a method in the treatment of cancer does not comprise the third component according to any embodiment of the first aspect.

In certain embodiments of the fourth or fifth aspect of the invention, the combination medicament is administered according to the following dosage regimen:

First, administration of the first component.

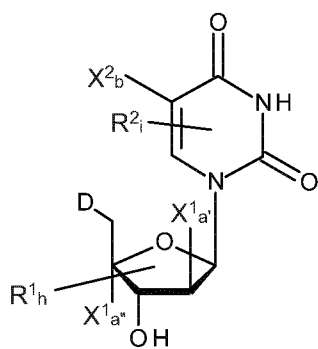
Second, administration of the second component separately from administration of the first component.

In certain embodiments of the fourth or fifth aspect of the invention, the first component of the combination medicament comprises a 2' deoxy ribose moiety that

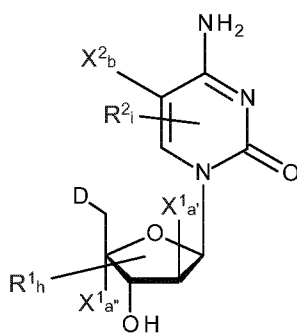
- is not substituted at the 2' position, or
- is substituted with one substituent at the 2' position in *S* configuration, or
- is substituted with two substituents at the 2' position.

In certain embodiments of the fourth or fifth aspect of the invention, the first component of the combination medicament comprises a 2' deoxy ribose moiety that is not substituted at the 2' position.

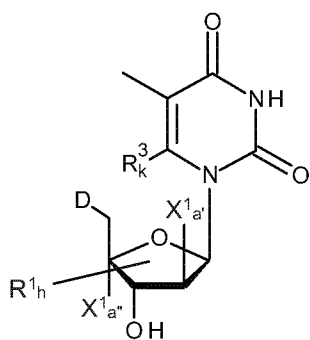
According to a sixth aspect of the invention, a compound of formula (XXI), (XXII), (XXIII), (XXIV), (XXV), (XXVI) or (XXVII), in particular (XXI), (XXII), (XXIV), (XXV), (XXVI) or (XXVII) is provided,



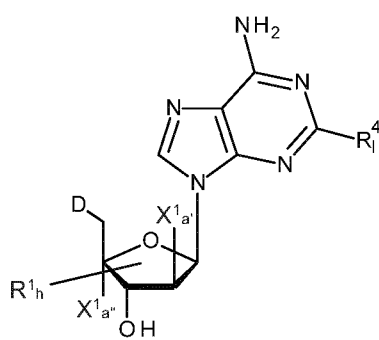
(XXI),



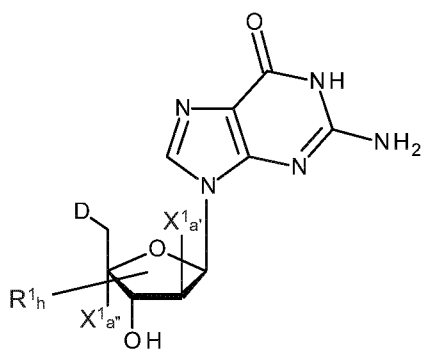
(XXII),



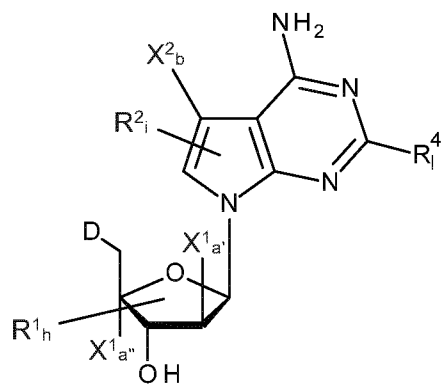
(XXIII),



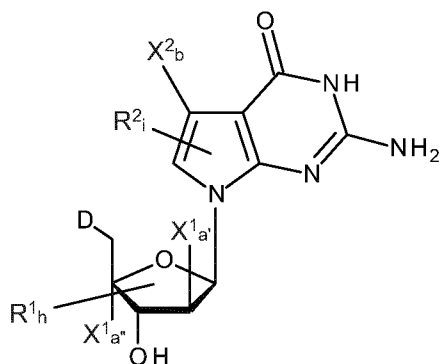
(XXIV),



(XXV),



(XXVI),



(XXVII), with

X^1 and X^2 being independently from each other selected from $-N_3$ and $-CH_2-N_3$,

a' , a'' and b being independently from each other 0 or 1, wherein the sum of a' , a'' and b is 1 or 2, in particular a' is 0, a'' is 1, and b is 0 or a' is 1, a'' is 0, and b is 0 or a' is 1, a'' is 0, and b is 1,

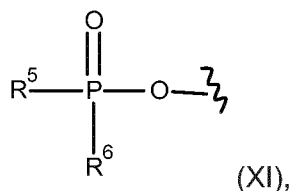
R^1 , R^2 , R^3 and R^4 are independently from each other selected from $-OH$, $-F$ and $-Cl$, wherein in particular R^1 is $-OH$ and R^2 , R^3 and R^4 are independently from each other selected from $-F$ and $-Cl$,

h is an integer between 0 and 1, in particular 0,

i is an integer between 0 and 1, in particular 0,

k and l are independently from each other 0 or 1,

D being a phosphoester of formula (XI),



(XI),

with R^5 and R^6 being independently selected from a moiety of formula (XII) or (XIII),

$L-G-E$ (XII), with

L being selected from $-O-$, $-NH-$ and $-NR^7-$,

G being selected from $-C_{1-4}-alkyl-$, particularly $-C_{1-2}-alkyl-$, $-C_6-aryl-$, $-R^8-C_6-aryl-$, $-C_6-cycloalkyl$ and $-R^8-C_6-cycloalkyl$,

R^8 being $C_{1-4}-alkyl$, in particular $C_{1-2}-alkyl$,

E being selected from $-O-C(=O)-O-R^{10}$, $-O-C(=O)-R^{10}$, $-C(=O)-O-R^{10}$, $-S-S-R^{10}$, $-S-C(=O)-O-R^{10}$ and $-NO_2$,

R^{10} is independently selected from H or $-C_{1-6}-alkyl$, or

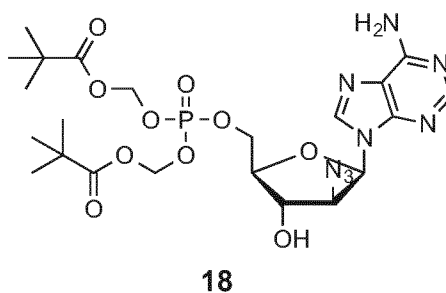
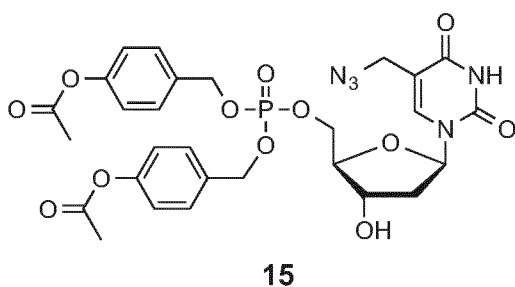
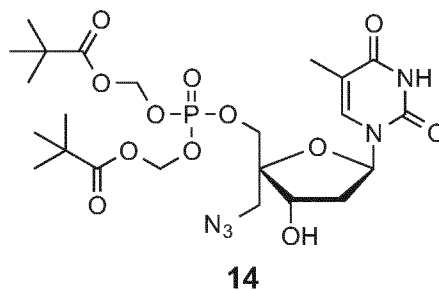
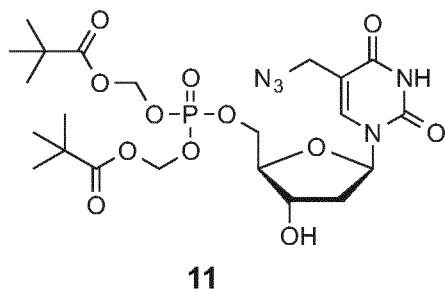
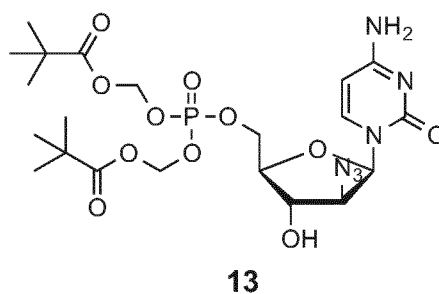
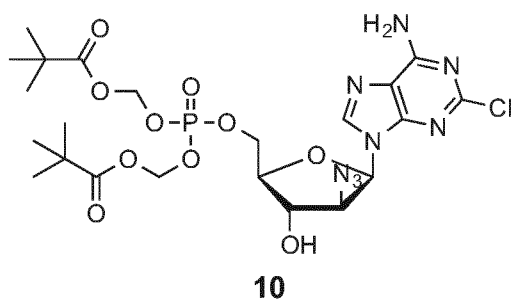
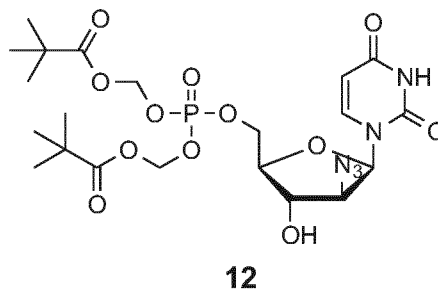
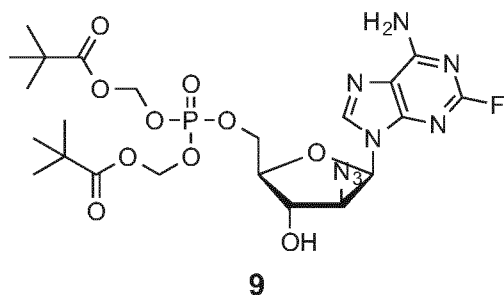
L-G (XIII), with

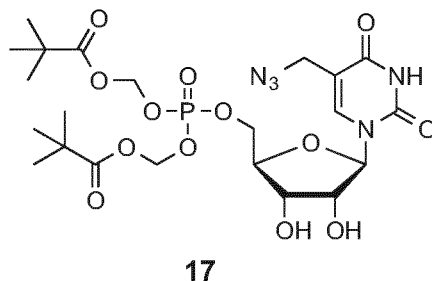
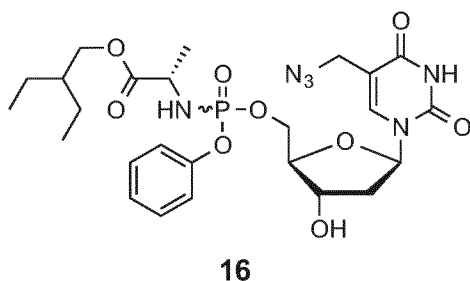
L being selected from -O-, -NH- and -NR⁷-,

G being selected from -C₁₋₆-alkyl, in particular -C₁₋₂-alkyl, -C₆-aryl, -R⁸-C₆-aryl, -C₆-cycloalkyl and -R⁸-C₆-cycloalkyl,

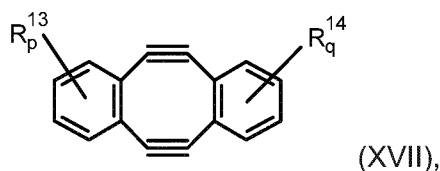
5 R⁸ being C₁₋₄-alkyl.

In certain embodiments of the sixth aspect of the invention, the compound is selected from





According to a seventh aspect of the invention, a compound of formula (XVII) is provided,



wherein

5 R^{13} and R^{14} are independently selected from

-O- R^{15} , -NH- R^{16} or -N(R^{16})₂, wherein

R^{15} and R^{16} are independently from each other selected from

-C₁₋₄-alkyl, -C₃₋₆-cycloalkyl, C₆-aryl, 7 to 10, in particular 7 to 8, more particularly 8 membered bicycle, 7 to 10, in particular 7 to 8, more particularly 8 membered heterobicycle, in particular -C₁₋₃-alkyl or 7 to 10 membered heterobicycle,

-C₁₋₄-alkyl- R^{17} , in particular -C₁₋₃-alkyl- R^{17} ,

-C₁₋₄-alkyl- R^{18} -C₁₋₄-alkyl, in particular -C₁₋₃-alkyl- R^{18} -C₁₋₃-alkyl, and

-C₁₋₄-alkyl- R^{18} -C₁₋₄-alkyl- R^{17} , in particular -C₁₋₃-alkyl- R^{18} -C₁₋₃-alkyl- R^{17} , wherein

15 the C₁₋₄-alkyl-C₃₋₆-cycloalkyl or C₆-aryl may optionally be substituted by one or more substituents independently selected from -F, -Cl, -Br, -I, -OH, -O-C₁₋₂-alkyl, -NH₂,

20 R^{17} is selected from -NH₂, -NR¹⁹H, -N(R^{19})₂, -OH, -O-C₁₋₂-alkyl, 3 to 6 membered heterocycle, 7 to 10, in particular 7 to 8, more particularly 8 membered bicycle, 7 to 10, in particular 7 to 8, more particularly 8 membered heterobicycle, 5 or 6 membered heteroaryl, C₆-aryl, C₃₋₆-cycloalkyl, in particular -NH₂, -NR¹⁹H, -N(R^{19})₂, -OH, -O-C₁₋₂-alkyl, 3 to 6 membered heterocycle, -O-*tert*-butyldimethylsilyl,

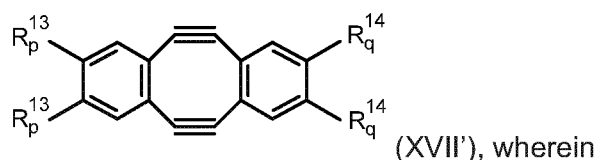
25 R^{18} is selected from -O-, -C(=O)-, -C(=O)-O-, -O-C(=O)-, -O-C(=O)-O-, -NH-, -C(=O)-NH-, -NH-C(=O)-, -O-C(=O)-NH-, -NH-C(=O)-O-,

R^{19} being independently selected from $-C_{1-3}$ -alkyl, $-C_{1-3}$ -alcohol

wherein at least one residue R^{13} and/or R^{14} , in particular all residues R^{13} and/or R^{14} , comprises a residue R^{17} selected from $-NH_2$, $-NR^{19}H$, $-N(R^{19})_2$ and/or R^{18} selected from $-NH-$, $-C(=O)-NH-$, $-NH-C(=O)-$, $-O-C(=O)-NH-$, $-NH-C(=O)-O-$.

- 5 In case of R^{13} and/or R^{14} being $-N(R^{16})_2$, both residues R^{16} may be identical or differ from each other.

In certain embodiments of the seventh aspect of the invention, the compound is a compound of formula (XVII'),

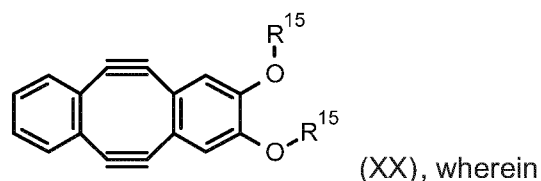
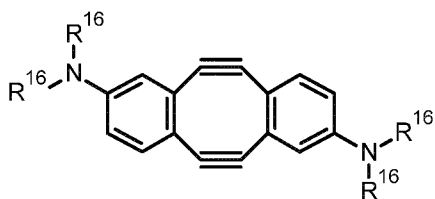
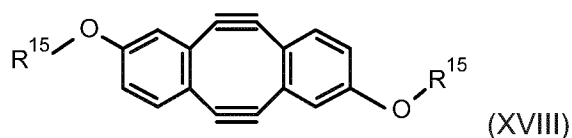


- 10 each R^{13} and each R^{14} are independently from each other defined as described above, and

each p and each q are independently from each other 0 or 1

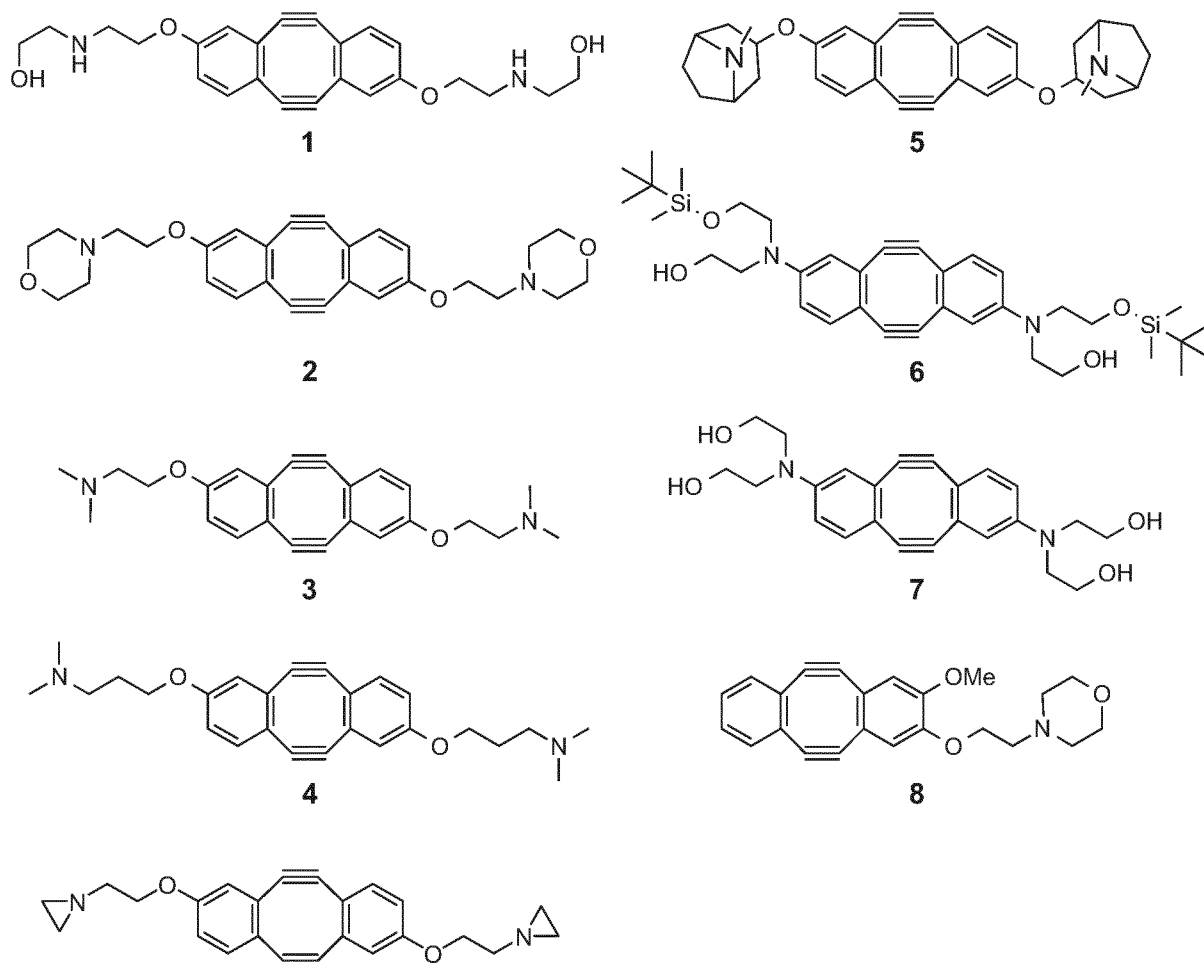
- 15 wherein at least one residue R^{13} and/or R^{14} , in particular all residues R^{13} and/or R^{14} , comprises a residue R^{17} selected from $-NH_2$, $-NR^{19}H$, $-N(R^{19})_2$ and/or R^{18} selected from $-NH-$, $-C(=O)-NH-$, $-NH-C(=O)-$, $-O-C(=O)-NH-$, $-NH-C(=O)-O-$.

In certain embodiments of the seventh aspect of the invention, the compound is a compound of formula (XVIII), (XIX) or (XX),



- 20 each R^{13} and each R^{14} are independently from each other defined as described above.

In certain embodiments of the seventh aspect of the invention, the compound is selected from



5 Brief description of the figures

- Fig. 1 shows the synthesis of 5-(azidomethyl)-2'-deoxyuridine (AmdU) monophosphates containing bisacetoxymethyl phosphoester (AB), bispivaloyloxymethyl phosphoester (POM), or phenyloxalanylphosphoramidate (Protide). BOPCI = bis(2-oxo-3-oxazolidinyl)phosphinic chloride, DIPEA = *N,N*-diisopropylethylamine, THF = tetrahydrofuran, DCM = dichloromethane.
- Fig. 2 shows HeLa cell viability according to the Alamar Blue assay and relative incorporation efficiencies by CuAAC staining (A). Metabolic activities of HeLa or U2OS cell cultures relative to untreated controls after 72 hour incubations (B): Cells were incubated with variable concentrations of AmdU, POM-AmdU, AB-AmdU and Prot-AmdU in DMEM with 10% FBS and 0.1% DMSO for 24h or 72 h prior to addition of Resasurin; cells receiving only DMSO were used to

define 100 % activity. (C). Visualization of azide-modified DNA in HeLa cells treated with 10 μ M AmdU, POM-AmdU, AB-AmdU, or Prot-AmdU for 24 h, followed by fixation and CuAAC staining with Alexa-Fluor 594 alkyne (magenta) in the presence of copper(I) and THPTA, and finally DAPI (cyan). Negative controls received no nucleoside/nucleotide, but were otherwise treated identically.

Fig. 3 shows staining of azide-containing DNA in zebrafish (72 hpf) that had been injected with 5.8 pmol of POM-AmdU (**8**) or AmdU at the zygote (one-cell) stage. Whole mounts were fixed and stained with SiR alkyne using CuAAC reactions. Nuclei were visualized using DAPI. Negative controls received no nucleoside/nucleotide, but were otherwise treated identically. Lateral views of the tail region are shown.

Fig. 4 shows staining of azide-containing DNA in zebrafish (5 hpf and 24 hpf) that had been injected with 5.8 pmol of POM-AmdU (**8**) at the zygote (one-cell) stage, followed by fixation and CuAAC staining with SiR alkyne and DAPI. The animal view of a 5 hpf embryo, and a lateral view of the tail region of a 24 hpf embryo are shown.

Fig. 5 shows staining of azide-containing DNA in zebrafish (96 hpf) that had been soaked in 0.5 mM AmdU or POM-AmdU prior to fixation. Tissues were sectioned and stained with SiR alkyne using CuAAC reactions, followed by DAPI. Negative controls received no nucleoside/nucleotide, but were otherwise treated identically. Transverse sections of the intestine are shown.

Fig. 6 shows the HPLC chromatograph of the SPDC reaction between AmdU and CODY showing two products: isomer 1 (24.3 min.), isomer 2 (27.0 min.), and unreacted AmdU (28.8 min.). The structures of products were determined by NMR and MS spectrometry.

Fig. 7 shows the synthesis of cyclooctadiynes containing polar side chains and predicted LogP values (*J. Chem. Inf. Model.*, **2014**, 54, 3284). LHMSD = lithium bis(trimethylsilyl)amide, THF = tetrahydrofuran, LDA = lithium diisopropylamide, MOM-Cl = chloromethyl methyl ether, DIPEA = *N,N*-diisopropylethylamine, DCM = dichloromethane.

Fig. 8 shows examples of new amine-containing CODY derivatives.

Fig. 9 Toxicity enhancement by the sequential treatment of POM-AmdU followed by a CODY derivative. (A) shows HeLa, and (B) U2OS cell viability after treatment with cyclooctadiynes (CODY or **8**) for 72 hours alone, or following a

pre-incubation of the cells with 100 μ M of POM-AmdU (**8**) for 72 hours. (C) shows toxicity results of HeLa, (D) shows AML-2, and (E) shows AML-3 cell cultures treated with POM-AmdU (0.3–30 μ M) for 72 hours followed by incubation with an amino CODY derivative “**2**” (0.3–30 μ M for HeLa, 0.1–10 μ M for AML-2, 3) following the 72 hour pre-treatment by POM-AmdU according to a trypan blue cell counting assay. Abbreviations: POM-AmdU = 5-(azidomethyl)-2'-deoxyuridine-5'-bispivaloyloxymethylphosphotriester; CODY = dibenzo-1,5-cyclooctadiyne.

Fig. 10 shows an example of chemoselective DNA-DNA cross-linking of azide-modified DNA by an amino CODY derivative according to scheme 1. (A) shows chemical structures of the azide-modified DNA and the amino CODY derivative used in these examples. (B) shows sequences of duplex DNA containing azide-modifications in each strand but in different positions (bold-underlined). (C) shows denaturing gel analysis after the treatment of duplexes 1–4 with an amino CODY derivative “**2**” (lanes 1, 2, 5–10). For comparison, duplex-1' contains only a single azide group (lanes 3, 4). (D) shows MALDI-MS analyses for the DNA-DNA cross-linked adducts of duplex-1 (calcd.: 17612.3), (E) shows products for duplex-2 (calcd.: 17621.3) and (F) shows products of duplex-3 (calcd.: 17621.3). Abbreviations: FAM = fluorescein amidite; ICL = interstrand cross-linking; Mono-adduct = FAM-oligonucleotides conjugated with only **2**; MALDI-MS = matrix-assisted laser desorption-ionization mass spectrometry; CODY = dibenzo-1,5-cyclooctadiyne.

Fig. 11 shows an example of cross-linking reaction according to scheme 1. Detection of DNA-DNA interstrand cross links in HeLa cells treated with POM-AmdU followed by an amino CODY derivative “**2**”. (A) shows chemical structures of compounds used in these examples. (B) shows denaturing polyacrylamide gel analysis of isolated, S1-digested genomic DNA from cells treated with POM-AmdU (**8**) (100 μ M for 72 h; lanes 3, 4), **2** (30 μ M for 24 h; lanes 5, 6) or cisplatin (20, 100 μ M for 24 h; lanes 7–10), or the combination of POM-AmdU (**8**) and amino CODY derivative “**2**” (100 μ M for 72 h and 30 μ M for 24h, sequentially; lanes 11, 12). Abbreviations: POM-AmdU = 5-(azidomethyl)-2'-deoxyuridine- 5'-bispivaloyloxymethylphosphotriester; CODY = dibenzo-1,5-cyclooctadiyne; Con. = conditions; S1 = nuclease that digests single strand nucleic acids; neg. = negative control.

Fig. 12 shows an example of a three-component cross-linking reaction according to scheme 2. Chemoselective labeling of azide-modified duplex DNA by addition

of an amino CODY derivative "DIMOC" and an azide-dye (N_3 -TAMRA). (A) shows chemical structures of compounds used in this example. (B) shows structure of duplex DNA containing an azide-modification "I" and terminal FAM fluorescent tag. (C) shows gel electrophoresis analysis of native duplex reactivity, and (D) shows gel electrophoresis analysis of the denatured, single-stranded DNA reactivity towards tandem DIMOC + N_3 -TAMRA treatment versus treatment with BCN-TAMRA. (E) shows HeLa cells treated with 30 μ M of POM-AmdU for 24 h, followed by fixation and SPAAC reaction for 20 min with TAMRA-BCN (10 μ M) (magenta) or DiMOC (10 μ M for 10 min) and N_3 -TAMRA (10 μ M for 10 min). Nuclei were non-covalently stained by DAPI. Negative controls received no POM-AmdU, but were otherwise treated identically. Scale bars show 20 μ m.

Fig. 13 shows example of cross-linking reactions according to scheme 2. Metabolic incorporation of AzC and AzA into DNA of HeLa cells followed by chemoselective labeling by combination of CODY derivative "DIMOC" and azido-dye N_3 -TAMRA. HeLa cells were treated with 50 μ M of AzC or AzA for 24 hours prior to fixation with paraformaldehyde, and tandem treatment with 10 μ M of DIMOC for 10 min. followed by 10 μ M of N_3 -TAMRA for 10 min.

Fig. 14 shows examples of new azide-containing nucleotide monophosphate triesters.

Fig. 15 shows examples of three-component RNA cross-linking reactions according to scheme 2. HeLa cells were incubated with 200 μ M of each nucleoside or nucleotide for 8 hours prior to fixation with paraformaldehyde, and tandem treatment with 10 μ M of DIMOC for 10 min. followed by 10 μ M of N_3 -TAMRA for 10 min.

Fig. 16 shows Scheme 1. Metabolic incorporation of azido-nucleoside/nucleotide analogs into DNA or RNA followed by chemoselective cross-linking between either DNA-DNA, DNA-RNA or RNA-RNA by a CODY derivative. Where: a) nucleotidase activity or spontaneous chemical cleavage; b) esterase activity or spontaneous chemical cleavage; c) nucleoside kinase activity; d) nucleotide kinase activity; e) DNA polymerase activity; f) RNA and DNA polymerase activity; g) RNA polymerase activity h) SPAAC reaction. Where: R^1 and R^2 are independently chosen from carbon-containing functional groups; R^3 - R^6 are independently chosen from one or more azide-containing functional groups; R^7 - R^{10} are independently chosen from hydrogen and at least one nitrogen-containing functional group. Abbreviations: CODY = dibenzo-1,5-cyclooctadiyne; SPAAC = strain-promoted alkyne-azide cyclization.

Fig. 17 shows Scheme 2. Metabolic incorporation of azido-nucleoside/nucleotide analogs into DNA or RNA followed by chemoselective ligation by the combination of a CODY derivative and another azide-modified molecule "R¹¹". Where: a) nucleotidase activity or spontaneous chemical cleavage; b) 5 esterase activity or spontaneous chemical cleavage; c) nucleoside kinase activity; d) nucleotide kinase activity; e) DNA polymerase activity; f) RNA polymerase activity; g) 1st SPAAC reaction; h) 2nd SPAAC reaction. Where: R¹ and R² are independently chosen from carbon-containing functional groups; R³–R⁶ are independently chosen from one or more azide-containing functional groups; R⁷–R¹⁰ are independently chosen from hydrogen and at least one nitrogen-containing functional group; R¹¹ represents an azide-containing target for cross-linking such as a fluorescent or affinity tag, protein, peptide, carbohydrate, lipid, or nucleic acid. Abbreviations: CODY = dibenzo-1,5-cyclooctadiyne; SPAAC = strain-promoted alkyne-azide cyclization.

15 Examples

Example 1: DNA-DNA interstrand crosslinking according to Scheme 1 (Fig. 16)

5-(azidomethyl)-2'-deoxyuridine (AmdU) is a non-toxic azido nucleoside which can be metabolically incorporated into whole cells and animals and subsequently modified by copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reactions. Being a nucleoside derivative, AmdU requires phosphorylation by cellular kinases prior to its incorporation into DNA. Some cells do not express kinases that are able to phosphorylate AmdU. The inventors therefore introduced a phosphoester group at the 5'-OH of AmdU that is cleaved by cellular esterases to generate the corresponding nucleotide monophosphate inside of cells. Using modified versions of published synthetic procedures (*Bioorg. Chem.*, **1984**, 12, 118; *J. Pharm. Sci.*, **1983**, 72, 324; *Antiviral Res.*, **1992**, 17, 311), the inventors synthesized 5'-bisacetoxybenzylphosphoester- (AB-AmdU), 5'-bispivaloyloxymethylphosphoester- (POM-AmdU), and 5'-phenyloxalanylphosphoramidate- 5-(azidomethyl)-2'-deoxyuridine (Protide-AmdU, Figure 1). These AmdU "pro-monophosphates" were evaluated for toxicity in HeLa (cervical cancer) and U2OS (osteosarcoma) cell cultures using the Alamar Blue assay that measures mitochondrial activity (*Bull. Environ. Contam. Tox.* **1981**, 26, 145). The parent compound AmdU and its pro-phosphates exhibited lower toxicity (EC₅₀ > 100 μM) than the well-known metabolic label EdU (EC₅₀ = 1–10 μM). Among the three pro-phosphates, POM-AmdU exhibited the lowest toxicity.

To evaluate the AmdU pro-monophosphates in terms of their impact on cellular proliferation and respiration, Resazurin-reduction (Alamar Blue) assays were used (*Br. J. Radiol.* **2005**,

78, 945–947). Consistent with a previous report (*ChemBioChem* **2014**, 15, 789–793), AmdU itself exhibited little or no toxicity towards HeLa cell cultures, even when applied at 100 μ M for 72 h. “POM-AmdU” and “Prot-AmdU” (Protide-AmdU) also exhibited very little or no toxicity ($IC_{50} \geq 100$ μ M) after 72 hour incubations. In comparison, the common metabolic probe for DNA synthesis, 5-ethynyl-2-deoxyuridine (EdU) exhibited potent toxicity ($IC_{50} = 1 - 10$ μ M) after 72 hours (Figure 2A), but no apparent toxicity after 24 hours according to this assay. “AB-AmdU” exhibited an intermediate potency ($IC_{50} = 10 - 30$ μ M) in both HeLa and U2OS cells, with the same apparent activities after both 24 and 72 hour incubation times (Figure 2B), suggesting a mode of toxicity for AB-AmdU that is independent of DNA synthesis and damage response pathways.

To compare their DNA metabolic incorporation efficiencies, HeLa cells were treated with 10 μ M of AmdU, Prot-AmdU, POM-AmdU and AB-AmdU for 24 h. After fixation, the cells were stained with Alexa-Fluor 594 alkyne in the presence of copper(I). According to confocal microscopy, HeLa cells treated with AmdU or Prot-AmdU exhibited little or no specific staining even when applied at 100 μ M. Cells treated with 10 μ M of AB-AmdU exhibited weak staining that co-localized with the non-covalent stain DAPI (Figure 2C). Cells treated with POM-AmdU exhibited superior staining at all concentrations and time points evaluated. Selective incorporation of POM-AmdU into DNA was reflected by specific staining of metaphase and telophase chromosomes (data not shown). POM-AmdU could be detected at a concentration as low as 3 μ M, and after incubation times of only 30 – 60 minutes. Similar results were obtained in both HeLa (cervical cancer) and U2OS (osteosarcoma) cells. Little or no incorporation of Prod-AmdU was apparent in these cells because its metabolic conversion into AmdU triphosphate requires the presence of certain enzymes (ex. histidine triad nucleotide binding protein-1) that are expressed only in certain cell types (ex. hepatocytes). Differences in enzyme expression in different cell types therefore provide a means to target specific cell types for crosslinking reactions.

Both POM-AmdU and AB-AmdU exhibited rapid incorporation into the DNA of HeLa and U2OS cells, yet, AB-AmdU exhibited a lower incorporation efficiency and higher toxicity than POM-AmdU. To evaluate this apparent contradiction, we evaluated the chemical stabilities of AB-AmdU and POM-AmdU in the same cellular media (DMEM + 10% FCS) used for all toxicity and cell labeling experiments. Surprisingly, AB-AmdU exhibited a very short half life ($T_{1/2} \approx 60$ minutes) under these conditions in the absence of cells, whereas little or no loss in POM-AmdU was observed after 120 minutes (data not shown). Taken together with the rapid onset of AB-AmdU's toxic effects and its limited incorporation efficiency (Figure 2), these results suggest that non-enzymatic, extracellular degradation of AB-AmdU limits its cellular uptake and incorporation into DNA. At the same time, AB-AmdU releases an active agent,

most likely quinone methide, that causes toxicity independent of AmdU anabolism. Taken together, these results demonstrate that POM-AmdU exhibits enhanced chemical stability, lower toxicity and greater metabolic incorporation into DNA as compared to AB-AmdU.

To evaluate relative incorporation efficiencies in vivo, variable amounts (0.4 pmol, 2.1 pmol or 5.8 pmol) of AmdU or POM-AmdU were microinjected into the cytosol of zebrafish at the 1-cell-stage (zygote). After 24 – 72 hours of growth, the fish were sacrificed, fixed, and stained using Cu(I) catalysis and the silicon rhodamine dye “SiR alkyne” (*Nat Methods*, 2014, 7, 731–733). At all concentrations and time points tested, animals treated with POM-AmdU exhibited superior staining as compared to AmdU. (Figure 3). Since the compounds were directly injected into the cytosol, these results suggest that the enhanced incorporation of POM-AmdU into DNA is due to its improved metabolism into nucleotide triphosphate, rather than improved cellular uptake. The staining patterns of POM-AmdU at 5 hours post fertilization (hpf) exhibited extensive overlap with DAPI (Figure 4). This staining pattern became “spotty” within the nuclei at 24 – 72 hpf, consistent with a rapid consumption of POM-AmdU (< 24 hpf) and continued division of the labeled cells in the absence of POM-AmdU to give individual chromosomes containing azide groups (Figures 3 and 4). Taken together with the normal developmental morphologies and viability of the POM-AmdU-treated fish, these results demonstrate that azide-modified zebrafish genomes are functional and that the azide groups remained stable in the DNA over days and multiple rounds of cellular division.

Next the inventors investigated whether metabolic incorporation of azide groups into zebrafish DNA can be achieved by simply soaking the fish in water containing POM-AmdU. Live zebrafish (72 hpf) were incubated in solutions containing 0.5 mM of AmdU or POM-AmdU for 24 hours, sacrificed, fixed, sectioned, and stained using Cu(I) catalysis and SiR (*Nat Methods*, 2014, 7, 731–733). Fish soaked in POM-AmdU, but not AmdU, exhibited staining in regions known to contain proliferating cells throughout the body, including the intestine (Figure 5), liver, retina and forebrain (data not shown). Together these results demonstrate the POM-AmdU is able to penetrate into deep tissues of living animals and deliver azide groups into the DNA of dividing cells. POM-AmdU therefore provides the first generally-applicable metabolic label for the biosynthesis of azide-modified DNA in cells and animals. Remarkably, azide-modified genomes continued to function and divide in living zebrafish and mammalian cell cultures, with no evidence for any DNA repair or degradation over a period of three or more days. These studies have therefore opened the door to producing functional, azide-containing genomes in wild-type cells and animals.

The Sondheimer diyne “CODY” (Figure 6) is known to react with two azide groups via a strain-promoted double-click (SPDC) reaction in the absence of Cu(I). To evaluate the

suitability of AmdU in this reaction, two equivalents of AmdU were reacted with CODY in methanol for 5 hours to give a 1:1 mixture of the *cis* and *trans* bis triazoles (Figure 6). CODY is poorly soluble in aqueous solutions due to its lipophilicity (predicted LogP: 3.96) (*J. Chem. Inf. Model.*, **2014**, 54, 3284). CODY also reacts with naturally abundant nucleophiles in cells such as thiols. Increasing the electron density of the system by introducing ether groups can potentially prevent such side reactions involving nucleophilic addition. The inventors therefore designed and synthesized tetramethoxymethoxy (tetra-MOM; TMC)-, monomorpholinoethoxy (mono-Mor; MMOC)- and dimorpholinoethoxy (di-Mor; DIMOC)-substituted CODY analogs (Figure 7). The new CODY derivatives (Figure 8) exhibited similar reactivities towards AmdU as the Sonheimer diyne while their solubility in aqueous solution was dramatically increased.

Non-covalent binding of unmodified, duplex DNA by the CODY derivative "DIMOC" was assessed by measuring changes in the UV-visible absorbance spectrum of DIMOC upon addition of calf thymus (CT) DNA, giving changes that were consistent with the presence of a weak binding interaction ($K_d \approx 122 \pm 12 \mu\text{M}$). To determine the binding mode of DIMOC to duplex DNA, the viscosity of CT DNA solutions were monitored upon addition of DIMOC (not shown). DIMOC caused increased viscosities of CT DNA solutions, consistent with its intercalation into the double helix. Consistent with reversible, non-covalent intercalation into duplex DNA, DiMOC exhibited no reactivity towards unmodified duplex DNA, yet CT DNA did not inhibit the chemical reaction between DiMOC and AmdU (not shown).

According to the Alamar Blue assay (*Bull. Environ. Contam. Tox.* **1981**, 26, 145), the CODY derivatives exhibited little or no toxicity towards HeLa cells. However, pre-incubation of POM-AmdU with HeLa cells for 72 hours, which is enough time for incorporation of AmdU in both DNA strands, made the CODY derivatives much more toxic (Figure 9). This toxicity is potentially the result of DNA-DNA interstrand cross-linking (ICL) reactions on the genomic DNA.

To evaluate the feasibility of ICL reactions on genomic DNA, oligonucleotides containing AmdU were evaluated in vitro. AmdU is not compatible with typical solid-phase DNA synthesis due to the phosphoramidite reacting with the azide moiety via the Staudinger reaction. The oligonucleotides containing an AmdU modification in the center of a sequence were therefore prepared by a primer extension reaction with AmdU triphosphate, which was synthesized in 6 steps from thymidine (*Analyst*, **2015**, 140, 2671). The ICL reactions were carried out using 1 equivalent of DIMOC + duplex DNA at 37° C in PBS buffer. After 4 hours, ICL products were clearly observed by denaturing polyacrylamide gel electrophoresis and MALDI-MS analyses (Figure 10). The duplex 1' containing a single AmdU modification did not give an ICL-adduct, but only a DIMOC "mono-adduct" (Figure 10C, lane 4). Duplex 2

containing AmdU residues spaced by one unmodified base pair gave the highest yield (27%) for the double-click reaction to form a DNA-DNA ICL. This yield is much higher than conventional crosslinking agents such as the nitrogen mustards, that normally give yields of only 1 – 5 % of the ICL product (*CancerRes.* 1999, 59, 4363).

- 5 To evaluate the formation of DNA-DNA ICL in living cells using this approach, HeLa cells were treated with POM-AmdU (100 μ M) for 72 hours followed by DiMOC for 24 hours. The total genomic DNA was isolated from the treated cells and mechanically sheared by sonication to obtain 200-400 bp DNA fragments. The DNA fragments were denatured by NaOH (pH 13.0) and renatured by neutralization with HCl then the fragments were digested
- 10 by S1 nuclease which can selectively hydrolyze single-strand DNA. The digested samples were subjected to super-denaturing PAGE (7M-urea, 20%-formamide, 8%-PAGE) at 60°C (Fig. 11). The cisplatin treated HeLa cells (positive control) gave undigested DNA fragments as a smear band with lower mobility region (Fig. 11B lanes 8, 10) and the same gel pattern was obtained by the combination treatment of POM-AmdU and DiMOC (Fig. 11g lane 12).
- 15 Taken together, these results demonstrate that the administration of two relatively non-toxic compounds POM-AmdU and DiMOC caused the formation of highly toxic DNA-DNA ICLs in living cells.

Example 2: DNA-“R¹¹” crosslinking according to Scheme 2 (Fig. 17)

- This approach can also be used in a three-component modality. When live cells are treated
- 20 for a relatively short period of time, one round of DNA replication in the presence of POM-AmdU results in AmdU residues in only one of the two strands of DNA in each chromosome. Subsequent addition of an amino CODY derivative such as DIMOC gives “mono-adducts” on the DNA (similar to Figure 10C, lane 4) containing a single cyclooctyne group that can be reacted with a third added component containing an azide group. This third component can
- 25 be, for example, a fluorescent azide such as N₃-TAMRA (Figure 12A). To evaluate the feasibility of this approach, azide-containing duplex DNA was prepared using enzymatic extension of a 5'-fluorescein (FAM) modified primer in the presence of 5-(azidomethyl)-2'-deoxyuridine (AmdU) triphosphate. The template was designed such that a single AmdU unit was incorporated in the center of the duplex (Figure 12B). The AmdU-containing duplex
- 30 exhibited little or no SPAAC product formation upon its incubation with 50 equivalents of the “standard” cyclooctyne derivative “BCN-TAMRA” (Figure 12C). In contrast, consecutive treatment of the azide-containing duplex with 10 eq. of the intercalating reagent DIMOC for 10 min, followed by 10 eq. of N₃-TAMRA for 10 minutes resulted in quantitative formation of a DNA-DIMOC-TAMRA cross link (Figure 12C). Denaturation of the duplex into single-
- 35 stranded DNA prior to addition of 50 eq. of the standard cyclooctyne probe “BCN-TAMRA” facilitated partial conversion (46%) of the single-stranded DNA into the SPAAC product

(Figure 12D). Tandem treatment of this single-stranded DNA with DiMOC and N₃-TAMRA furnished quantitative (~99%) formation of the cross-linked product (Figure 12D). These results demonstrate that standard cyclooctynes such as BCN preferentially react with single-stranded DNA, whereas CODY derivatives such as DIMOC rapidly react with both duplex and single-stranded azide-containing DNA. The resulting “monoadducts” of these reactions can be quantitatively crosslinked to a third component, such as N₃-TAMRA.

To evaluate the feasibility of this approach in cells, the nucleotide monophosphate triester “POM-AmdU” was added to the growth media of dividing HeLa cells, where it was incorporated into the cellular DNA with a much higher efficiency than AmdU itself (Example 1). Subsequent fixation with formaldehyde and incubation with the standard cyclooctyne probe “BCN-TAMRA” yielded little or no SPAAC staining of cellular DNA (Figure 12E), unless the DNA was first denatured into single stands (not shown). In contrast, tandem treatment of POM-AmdU-treated cells with DiMOC and N₃-TAMRA yielded strong staining of native, duplex DNA (Figure 12E). These results mirrored those obtained using oligonucleotides in vitro, where DiMOC but not BCN, was capable of reacting with duplex DNA containing azide (Figure 12 C).

A wide variety of nucleosides and nucleotides are compatible with this approach. For example, the cytarabine drug analog “AzC” (*Proc. Natl. Acad. Sci.* **2018**, 115, E1366-E1373.) is metabolically incorporated into the DNA of living HeLa and U2OS cell cultures, where it causes stalling of replication forks and reversible cell cycle arrest. To evaluate the suitability of this approach for AzC modification, living HeLa cells were treated with 50 μM of AzC for 24 hours fixed, and stained with tandem treatments with the CODY derivative “DIMOC” and N₃-TAMRA (Fig. 13). Highly punctuated staining of DNA was observed, indicative of stalled replication foci. Similar results were obtained using an analogous adenosine derivative “Aza” (Fig. 13). Unexpectedly, both AzC and Aza were incorporated mostly into the RNA of leukemia cell cultures OCI-AML2 and OCI-AML3 (not shown).

Example 3: RNA-“R¹¹” crosslinking according to Scheme 2 (Fig. 17)

The incorporation of any particular nucleoside/nucleotide analog into DNA versus RNA is often difficult to predict *a priori*. For example, the cytarabine drug analog “AzC” (*Proc. Natl. Acad. Sci.* **2018**, 115, E1366-E1373.) is metabolically incorporated into the DNA of HeLa and U2OS cell cultures, yet this same nucleoside is primarily incorporated into the RNA of leukemia cell lines OCI-AML2 and OCI-AML3 (data not shown). Widely-used nucleoside and nucleotide-based drugs such as gemcitabine (*Cancer Res.* **1990**, 50, 4417) and fludarabine (*Blood* **1989**, 74, 19.) are incorporated primarily into DNA, into RNA, or into a mixture of DNA and RNA, depending on the exact cell type (*Biochem Pharmacol.* **1993**, 46, 762.). The pharmacological activities of these compounds can be associated with their incorporation

into RNA (*Cancer Res.* **1991**, 51, 1829). To generate compounds with the potential for enhanced RNA incorporation, we synthesized a collection of new substances based on azido nucleotide monophosphate triesters (Fig. 14). To assess the ability of these compounds to modify RNA in a three-component modality, selected derivatives and the corresponding nucleosides were added to living HeLa cells to a final concentration of 200 μ M and incubated for 8 hours. This represents a higher concentration and shorter incubation time as compared to experiments aimed at DNA labeling (Examples 2 and 3). The cells were then fixed, and stained with tandem treatments with the CODY derivative “DIMOC” and N₃-TAMRA (Fig. 15). Under these conditions, the initial application of nucleoside “AzA” and the corresponding POM derivative caused staining of both RNA and DNA. In contrast, the fluorinated and chlorinated AzA derivatives exhibited relatively weak labelling intensities as compared to their corresponding POM derivatives that exhibited highly intense and selective RNA labelling (Fig. 15). These results demonstrate that the presence of a halogen substituent at the 2-position of adenine and a POM monophosphate triester at the 5' position can greatly enhance the incorporation of POM azido nucleotides into RNA.

Experimental section

Cell cultures: For all experiments, HeLa and U2OS cells were cultivated at 37 °C under 5% CO₂ in DMEM containing FBS (10 %), penicillin (100 unit), and streptomycin (0.1 mg/mL).

Metabolic labeling: All experiments were carried out as previously reported (*ChemBioChem* **2014**, 15, 789–793). Cells were seeded in 24-well plates with glass cover slips at 40,000 cells per well, and incubated overnight. The medium was replaced with the medium containing various concentrations of AmdU or its prodrugs.

Click staining in fixed cells: The cells were fixed with paraformaldehyde (4%) for 15 min at room temperature, quenched by glycine-NH₄Cl solution (50 mM each) for 5 min, and washed with PBS buffer twice. The cells were permeabilized with 0.2% Triton-100X in PBS and 0.1% Tween-20 in PBS, respectively. After denaturation of the cells by HCl (2 M) for 30 min, neutralized with Borax (0.1 M) for 10 min, and washed with PBS, cells on coverslips were incubated upside-down with 25 μ L of freshly prepared Click-staining mix (AlexaFluor 594 alkyne (10 μ M), CuSO₄ (1 mM), THPTA (2 mM), aminoguanidine (1 mM) and sodium ascorbate (1 mM) in PBS buffer) for 2h at room temperature in the dark. Cells were washed with PBS, 1% Triton-100X in PBS, and 0.1% Tween-20 in PBS. The cells were stained by DAPI (2 μ M in PBS buffer) for 15 min at room temperature and washed by PBS buffer twice. Preheated glycerol was mounted on the coverslips and they were glued upside-down on microscope slides.

Zebrafish maintenance and breeding: Zebrafish were kept at 26°C and bred as previously described (*Curr. Biol.* **1994**, 4, 189-202). Embryos were raised in E3 medium (5 mM NaCl,

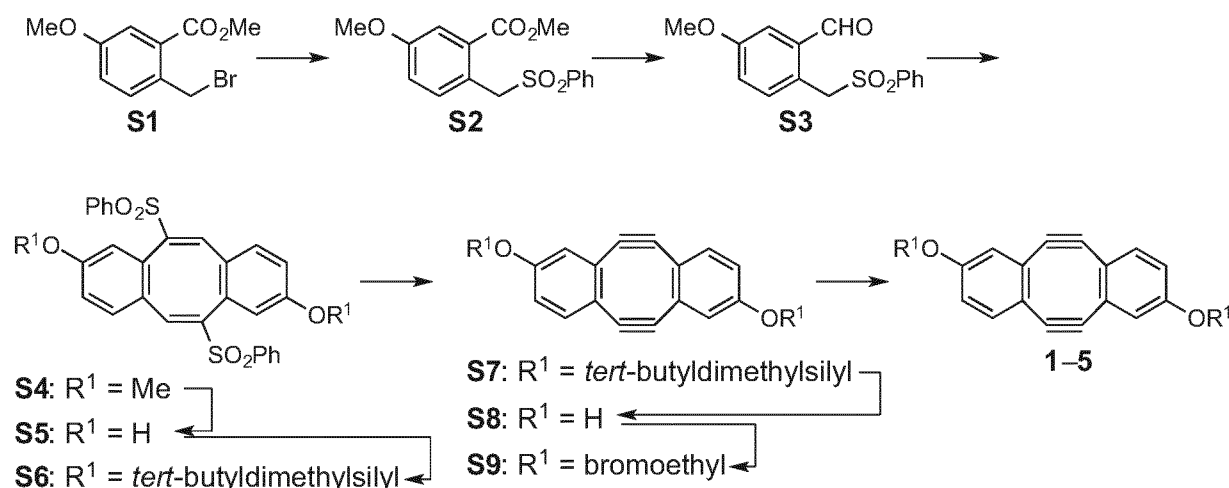
0.17 mM KCl, 0.33 mM CaCl₂, 0.33 mM MgSO₄) at 28°C and staged according to development in hours postfertilization (dpf) (*Dev. Dyn.* **1995**, 203, 253-310). All experiments were performed in accordance with internationally recognized guidelines for the use of fish in biomedical research.

- 5 **Metabolic labelling of zebrafish embryos:** For microinjection experiments, 0.4 pmol, 2.1 pmol, or 5.8 pmol POM-AmdU or AmdU were microinjected into the cytosol of one-cell-stage embryos, and embryos were raised at 28°C in E3 medium until the desired stage. For soaking experiments, zebrafish were incubated in E3 medium containing 0.5 mM POM-AmdU or AmdU and 1% DMSO at 28°C.

10 Chemical synthesis

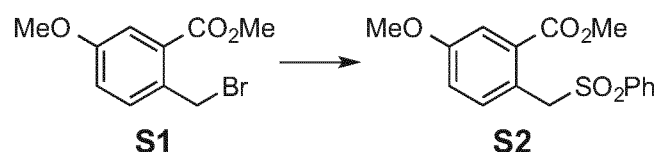
Dibenzo-1,5-cyclooctadiyne derivatives (Compounds 1 to 5)

Compounds **1** to **5** (Fig. 10) were synthesized according to Scheme 3.



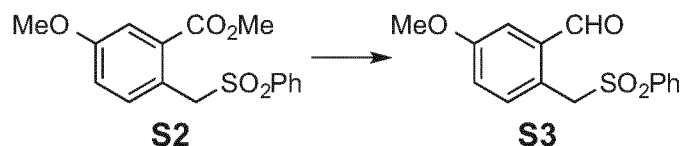
Scheme 3. Synthetic pathway A for dibenzo-1,5-cyclooctadiyne derivatives.

15 Compound 1

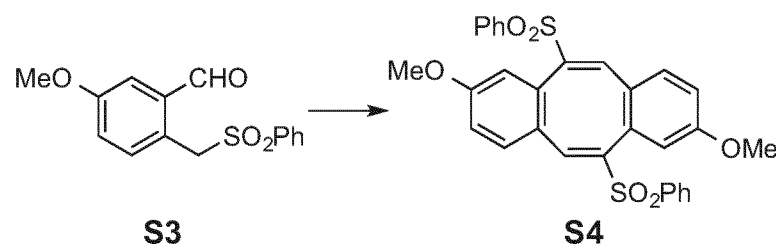


- A mixture of methyl 2-(bromomethyl)-5-methoxybenzoate (**S1**) (51.1 mmol), benzenesulfinic acid sodium salt (61.3 mmol), and DMF (85mL) was stirred for 2 h at 80 °C. The reaction mixture was cooled to rt, EtOAc was added, washed with water, dried over MgSO₄. The solvent was removed in vacuo, the residue was subjected to recrystallization from EtOAc to give **S2** (30.6 mmol; 50%) as a white powder. ¹H NMR (400 MHz, CDCl₃) δ 7.64-7.55 (m, 3H), 7.46-7.42 (m, 2H), 7.39 (d, *J* = 2.8 Hz, 1H), 7.23 (d, *J* = 8.5 Hz, 1H), 7.00 (dd, *J* = 2.8, 8.5 Hz, 1H), 4.97, (s, 2H), 3.84 (s, 3H), 3.73 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 166.9,

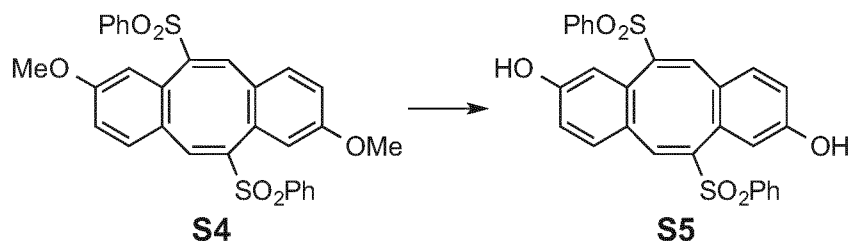
159.7, 138.4, 134.7, 133.4, 131.9, 128.8, 120.9, 117.9, 116.1, 58.8, 55.5, 52.2; HRMS (ESI-TOF) 343.0610 ($M + Na$)⁺ calcd for C₁₆H₁₆O₅SNa 343.0611.



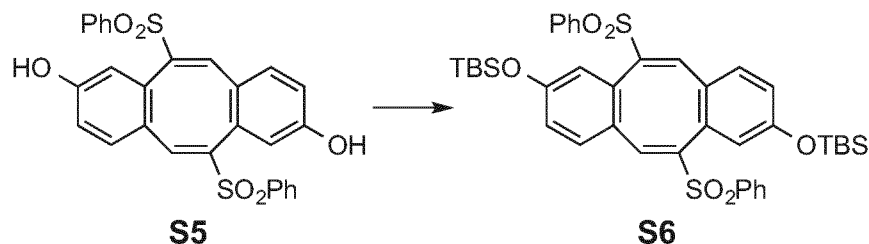
To a solution of methyl 5-methoxy-2-(phenylsulfonylmethyl)benzoate (**S2**) (12.0 mmol) in THF (45 mL), DIBAL-H (30 mmol; 1 mol/L in heptane) was added at 0 °C and stirred for 2 h at rt. To the reaction mixture, saturated NH₄Cl solution and HCl (2 mol/L) was added and extracted with EtOAc, dried over MgSO₄. To the crude product in dichloroethane (45 mL), MnO₂ (60.0 mmol) was added and stirred at 80 °C for 2 h. The reaction mixture was cooled to rt and subjected to filtration through a pad of silica gel and the residue was subjected to recrystallization from EtOAc to give **S3** (6.4 mmol, 53%) as a white powder. ¹H NMR (400 MHz, CDCl₃) δ 9.80 (s, 1H), 7.69-7.67 (m, 2H), 7.62-7.58 (m, 1H), 7.48-7.44 (m, 2H), 7.31 (d, *J* = 8.4 Hz, 1H), 7.26 (d, *J* = 2.8 Hz, 1H), 7.09 (dd, *J* = 2.8, 8.4 Hz, 1H), 4.90 (s, 2H), 3.88 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 191.4, 160.6, 138.3, 135.9, 135.3, 133.9, 129.0, 128.8, 120.9, 119.4, 118.5, 57.3, 55.8; HRMS (ESI-TOF) 313.0506 ($M + Na$)⁺ calcd for C₁₅H₁₄O₄SNa 313.0505.



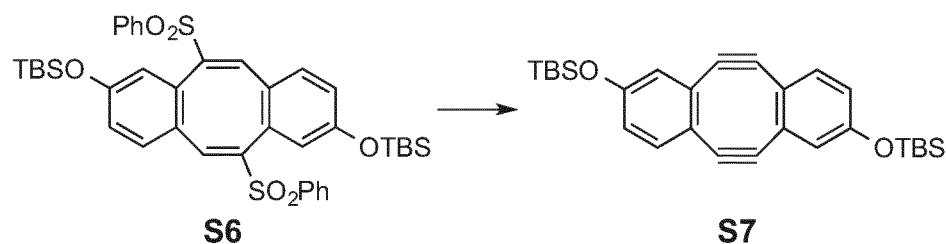
To a solution of 5-methoxy-2-(phenylsulfonylmethyl) benzaldehyde (**S3**) (24.4 mmol) and diethyl chlorophosphate (29.3 mmol) in THF (50 mL), lithium bis(trimethylsilyl)amide (49 mmol, 1 mol/L in THF) was added at -78 °C and stirred for 30 min and 1 h at rt. To the reaction mixture, saturated NH₄Cl solution was added and extracted with EtOAc, dried over MgSO₄. The residue was subjected to recrystallization from EtOAc to give **S4** (2.8 mmol; 22%) as a yellowish white powder. ¹H NMR (400 MHz, CDCl₃) δ 7.57 (t, *J* = 7.2 Hz, 2H), 7.43-7.33 (m, 10H), 7.20 (s, 2H), 6.73 (dd, *J* = 2.5, 8.8 Hz, 2H), 6.41 (d, *J* = 2.5 Hz, 2H), 3.67 (s, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 160.3, 144.8, 139.24, 138.7, 137.4, 133.9, 132.4, 129.1, 128.2, 120.9, 114.6, 111.9, 55.5; HRMS (ESI-TOF) 567.0908 ($M + Na$)⁺ calcd for C₃₀H₂₄O₆S₂Na 567.0907.



To a solution of **S4** (0.44 mmol) in dichloromethane (8 mL), $\text{BF}_3 \cdot \text{OEt}_2$ solution in dichloromethane (2.6 mmol; 1 mol/L in dichloromethane) was added at 0 °C, stirred for 30 min and 2 h at rt. To the reaction mixture, methanol was added at 0 °C and saturated NaHCO₃ solution was added at rt. The mixture was extracted with dichloromethane and dried over MgSO₄. The residue was subjected to recrystallization from dichloromethane to give **S5** (0.44 mmol; 99%) as a white powder. ¹H NMR (400 MHz, CDCl₃) δ 7.75-7.70 (m, 2H) 7.59-7.55 (m, 4H), 7.30-7.38 (m, 4H), 7.27 (d, *J* = 8.6 Hz, 2H), 7.17 (s, 2H), 6.76 (dd, *J* = 2.6, 8.6 Hz, 2H), 6.51 (d, *J* = 2.6 Hz, 2H).

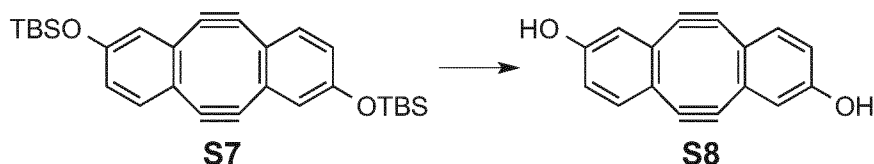


To the solution of **S5** (1.09 mmol), imidazole (8.72 mmol), and DMF (2.5 mL), TBS-Cl (5.99 mmol) was added and stirred for 2 h at rt. To the reaction mixture, EtOAc was added, washed with water, dried over MgSO₄. The residue was subjected to recrystallized from EtOAc to give **S6** (0.85 mmol, 78%) as a white solid. ¹H NMR (400 MHz, CDCl₃) δ 7.63 (t, *J* = 7.4 Hz, 2H), 7.47-7.36 (m, 10H), 7.19 (s, 2H), 6.73 (dd, *J* = 2.4, 8.6 Hz, 2H), 6.72 (d, *J* = 2.4 Hz, 2H), 0.93 (s, 18H), 0.15 (s, 12H); ¹³C NMR (100 MHz, CDCl₃) δ 156.7, 144.7, 139.2, 138.3, 137.5, 133.9, 132.4, 129.0, 128.2, 121.6, 120.5, 118.4, 25.7, 18.4, -4.3; HRMS (ESI-TOF) 767.2327 (*M* + Na)⁺ calcd for C₄₀H₄₈O₆S₂Si₂Na 767.2323.

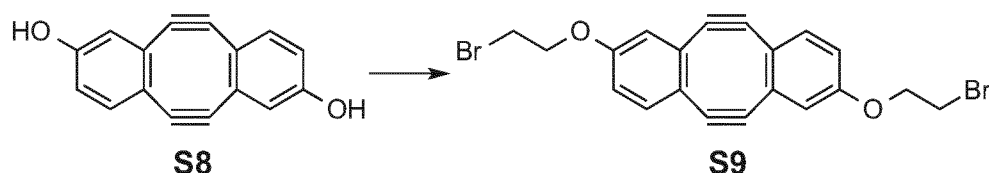


To a solution of **S6** (0.85 mmol) in THF (10 mL), lithium diisopropylamide (4.2 mL, 1 mol/L in THF) was added at -78 °C and stirred for 2h. To the reaction mixture, saturated NH₄Cl aqueous was added and extracted by EtOAc, dried over MgSO₄. The residue was subject to silica gel column chromatography (hexane:EtOAc = 10:1) to give **S6** (0.46 mmol, 54%) as a

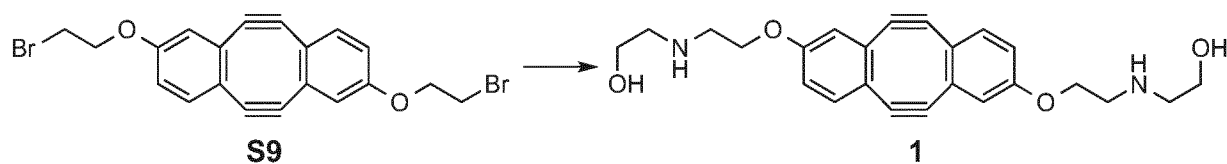
dark orange solid. ^1H NMR (400 MHz, CDCl_3) δ 6.58 (d, J = 8.2 Hz, 2H), 6.45 (dd, J = 2.4, 8.2 Hz, 2H), 6.25 (d, J = 2.4 Hz, 2H), 0.94 (s, 18H), 0.17 (s, 12H); ^{13}C NMR (100 MHz, CDCl_3) δ 156.6, 134.9, 127.8, 124.8, 119.8, 119.1, 110.1, 107.5, 25.6, 18.2, -4.5.



- 5 To a solution of **S7** (0.23 mmol) in MeOH (3 mL) and THF (2 mL), KHF_2 (0.91 mmol) was added at rt and stirred for 1h. To the reaction mixture, EtOAc was added and washed with saturated NH_4Cl solution, water, dried over MgSO_4 . The residue was purified on silica gel column chromatography (EtOAc) to give **S7** (0.22 mmol, 96%) as a dark orange solid. ^1H NMR (400 MHz, CD_3CN) δ 7.25 (s, 2H), 6.65, (d, J = 8.3Hz, 2H), 6.39 (dd, J = 2.5, 8.3 Hz, 2H), 6.30 (d, J = 2.5 Hz, 2H); ^{13}C NMR (100 MHz, CD_3CN) δ 159.2, 135.8, 129.4, 123.6, 116.4, 115.4, 111.1, 107.9.
- 10



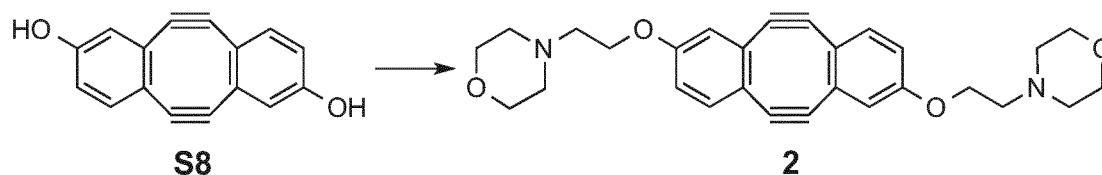
- To a solution of **S8** (0.43 mmol), K_2CO_3 (2.17 mmol), and tetrabutylammonium iodide (catalytic amount) in DMF (5 mL), dibromoethane (6.51 mmol) was added and stirred for 12 h at 65 °C. The reaction mixture was cooled and filtered through a pad of celite. To the crude mixture, EtOAc was added and the precipitation was collected by filter paper and washed with water to give **S9** as a dark grey powder (0.22 mmol, 52%). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 6.81 (d, J = 8.4 Hz, 2H), 6.61 (dd, J = 2.5, 8.4 Hz, 2H), 6.60 (d, J = 8.4 Hz, 2H), 4.30 (t, J = 5.3 Hz, 4H), 3.73, (t, J = 5.4 Hz, 4H); ^{13}C NMR (100 MHz, CDCl_3) δ 158.7, 133.8, 127.9, 123.1, 114.9, 113.6, 109.7, 107.0, 67.9, 30.5.
- 15
- 20



- A mixture of **S9** (0.034 mmol) and ethanolamine (0.15 mL) was stirred for 2 h at 90 °C. The mixture was subjected to C18 reverse-phase column chromatography (0.2% AcOH, 5% water, 95% MeCN) to give **1** (0.012 mmol, 35%) as a yellow solid. ^1H NMR (400 MHz, D_2O) δ 6.73 (d, J = 8.4 Hz, 2H), 6.57 (dd, J = 2.5, 8.4 Hz, 2H), 6.47 (d, J = 2.5 Hz, 2H), 4.25 (t, J = 4.7 Hz, 4H), 3.91, (t, J = 5.2 Hz, 4H), 3.52 (t, J = 4.7 Hz, 4H), 3.31 (t, J = 5.2 Hz, 4H); ^{13}C
- 25

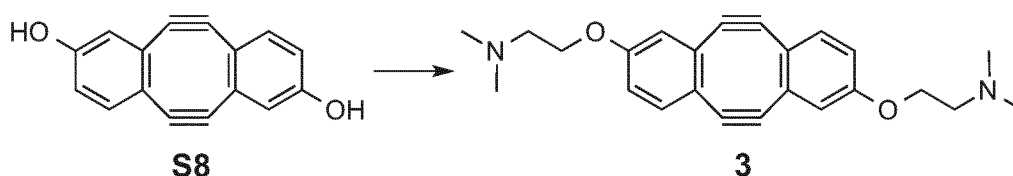
NMR (100 MHz, D₂O) δ 159.0, 134.6, 129.0, 124.6, 115.6, 113.9, 110.7, 108.1, 63.6, 57.0, 49.7, 46.8; HRMS (ESI-TOF) 407.1961 (M + Na)⁺ calcd for C₂₄H₂₇O₄N₂ 407.1965.

Compound 2



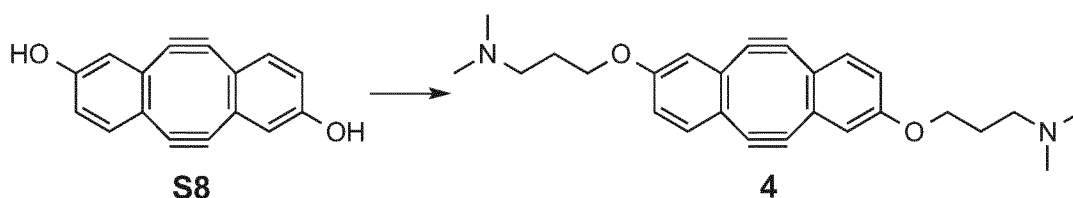
- 5 To a solution of **S8** (0.11 mmol), 4-(2-hydroxyethyl)morpholine (0.43 mmol), and triphenylphosphine (0.43 mmol) in THF (1 mL), diisopropyl azodicarboxylate (0.43 mmol) was added at rt and stirred for 2 h at 60 °C. The precipitation was collected by filter paper and washed with EtOAc three times to give **2** as a yellow powder (0.08 mmol, 73%). ¹H NMR (400 MHz, D₂O, 350K) δ 7.40 (d, *J* = 8.5 Hz, 2H), 7.19 (dd, *J* = 2.6, 8.5 Hz, 2H), 7.10 (d, *J* = 2.6 Hz, 2H), 4.90 (t, *J* = 4.8 Hz, 4H), 4.53 (br, 8H), 4.16, (t, *J* = 4.9 Hz, 4H), 3.98 (br, 8H); ¹³C
- 10 NMR (100 MHz, D₂O, MeCN as an internal standard) δ 159.5, 135.2, 129.6, 125.2, 116.3, 114.5, 111.3, 108.7, 64.2, 57.6, 50.3, 47.4; HRMS (ESI-TOF) 459.2279 (M + H)⁺ calcd for C₂₈H₃₁O₄N₂ 459.2278.

Compound 3



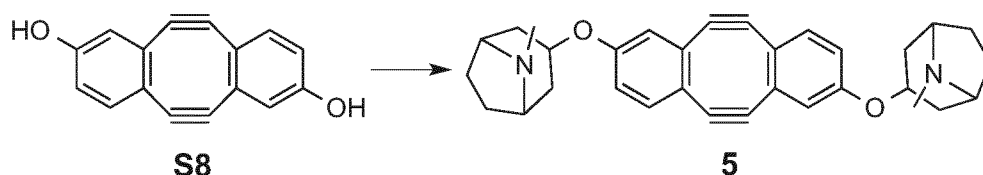
- 15 To a solution of **S8** (0.11 mmol), 3-dimethylamino-1-ethanol (0.44 mmol), and triphenylphosphine (0.44 mmol) in THF (1 mL), diisopropyl azodicarboxylate (0.44 mmol) was added at rt and stirred for 1 h at 60 °C. To the reaction mixture, HCl (0.2 mol/L, 1 mL) was added and washed with EtOAc twice. The aqueous phase was subjected to C18 reverse-
- 20 phase column chromatography (0.2% AcOH, 15% water, 85% MeCN) to give **3** (0.072 mmol, 64%) as a yellow solid. ¹H NMR (400 MHz, CD₃CN-D₂O) δ 6.73 (d, *J* = 8.6 Hz, 2H), 6.53 (dd, *J* = 2.6, 8.6 Hz, 2H), 6.44 (d, *J* = 2.6 Hz, 2H), 4.16 (t, *J* = 4.9 Hz, 4H), 3.41, (t, *J* = 4.9 Hz, 4H), 2.81 (s, 12H); ¹³C NMR (100 MHz, CD₃CN-D₂O) δ 159.3, 135.0, 129.3, 125.0, 116.2, 114.3, 110.8, 108.1, 62.7, 56.8, 43.8.

25 Compound 4



To a solution of **S8** (0.11 mmol), 3-dimethylamino-1-propanol (0.43 mmol), and triphenylphosphine (0.43 mmol) in THF (1 mL), diisopropyl azodicarboxylate (0.43 mmol) was added at rt and stirred for 2 h. The precipitation was collected by centrifuge. The residue was suspended in HCl (0.2 mol/L, 0.3 mL), washed with EtOAc three times. The precipitation was collected by filter paper to give **4** (0.082 mmol, 76%) as a yellow powder. ¹H NMR (400 MHz, D₂O) δ 6.81 (d, *J* = 8.5 Hz, 2H), 6.60 (dd, *J* = 2.6, 8.5 Hz, 2H), 6.59 (d, *J* = 2.6 Hz, 2H), 4.09 (t, *J* = 5.7 Hz, 4H), 3.33 (t, *J* = 7.8 Hz, 4H), 2.92, (s, 6H), 2.22-2.16 (m, 4H).

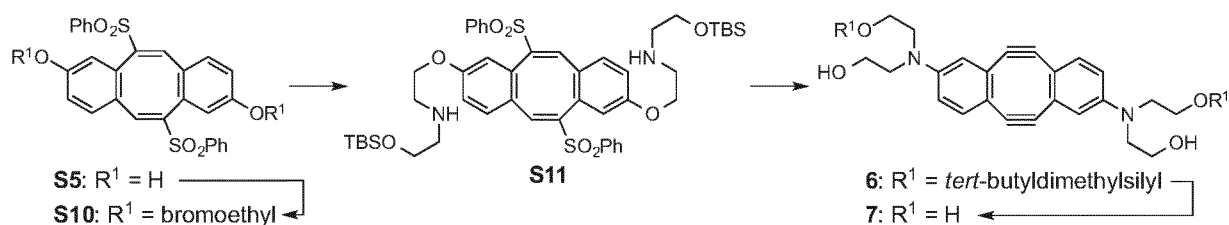
Compound 5



To a solution of **S8** (0.11 mmol), tropine (0.43 mmol), and triphenylphosphine (0.43 mmol) in THF (1 mL), diisopropyl azodicarboxylate (0.43 mmol) was added at rt and stirred for 2 h. The precipitation was collected by filter paper and washed with EtOAc three times to give **5** (0.031 mmol, 29%) as a yellow powder. ¹H NMR (400 MHz, D₂O-CD₃CO₂D) δ 6.74 (d, *J* = 8.5 Hz, 2H), 6.60 (dd, *J* = 2.6, 8.5 Hz, 2H), 6.46 (d, *J* = 2.6 Hz, 2H), 4.70 (m, 2H), 3.98 (br, 4H), 2.77, (s, 6H), 2.40-2.32 (br-m, 8H), 2.11-2.06 (br-m, 4H), 1.96-1.91 (br-m, 4H).

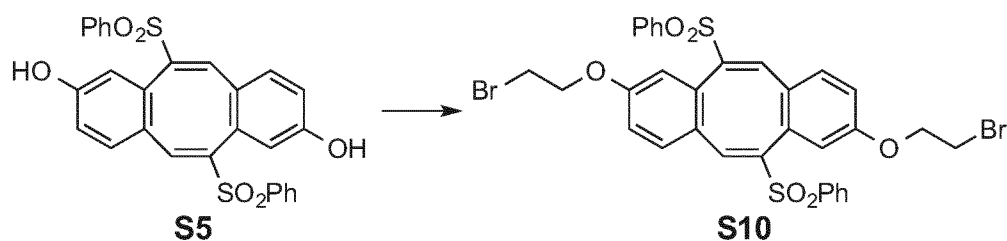
Dibenzo-1,5-cyclooctadiyne derivatives (Compounds 6 to 7)

Compounds **6** to **7** (Fig. 10) were synthesized according to Scheme 4.

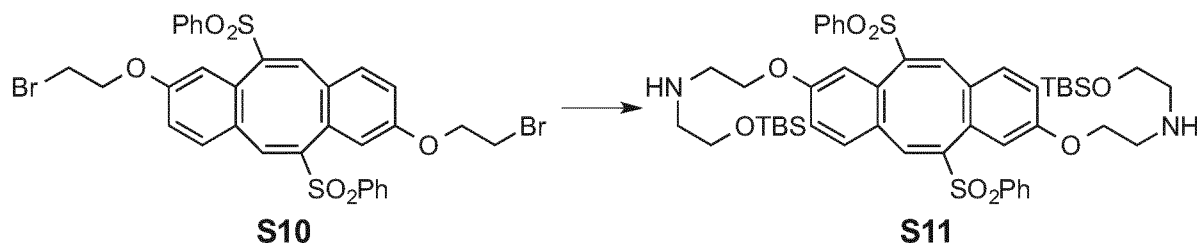


Scheme 4. Synthetic pathway B for dibenzo-1,5-cyclooctadiyne derivatives.

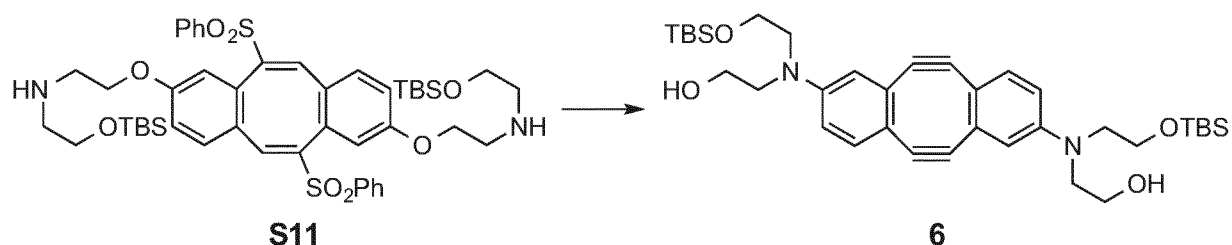
Compound 6



A mixture of **S5** (0.19 mmol), K₂CO₃ (5.68 mmol), dibromoethane (2.89 mmol), and DMF (2 mL) was stirred for 12 h at 75 °C. To the reaction mixture EtOAc was added and washed with water, dried over MgSO₄. The residue was subjected to silica gel column chromatography (hexane-EtOAc = 5:1) to give **S10** (0.08 mmol, 42%) as a white solid. ¹H NMR (400 MHz, CDCl₃) δ 7.64 (t, *J* = 7.28 Hz, 2H), 7.50-7.41 (m, 10H), 7.27 (s, 2H), 6.80 (dd, *J* = 2.6, 8.8 Hz, 2H), 6.50 (d, *J* = 2.6 Hz, 2H), 4.21 (m, 4H), 3.59 (t, *J* = 6.1 Hz, 4H); ¹³C NMR (100 MHz, CDCl₃) δ 158.8, 144.9, 139.1, 138.6, 137.5, 134.0, 132.5, 129.2, 128.2, 121.7, 115.1, 112.7, 67.9, 28.8; HRMS (ESI-TOF) 750.9433 (*M* + Na)⁺ calcd for C₃₂H₂₆O₆S₂Br₂Na 750.9430.

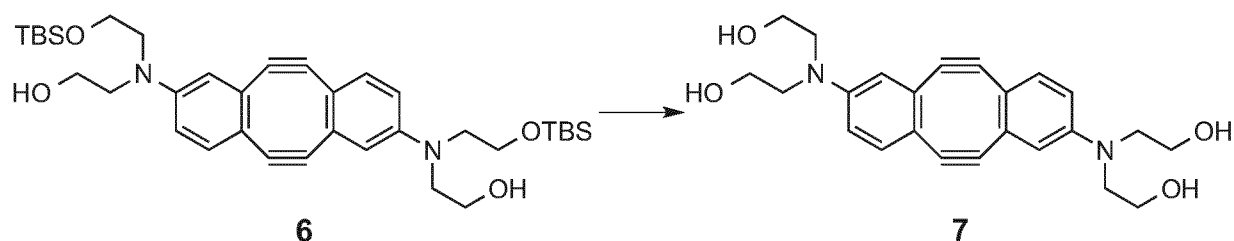


A mixture of **S10** (0.058 mmol) and 2-((*tert*-butyldimethylsilyl)oxy)ethan-1-amine (0.68 mmol) was stirred for 3 h at 100 °C. The crude mixture was subjected to silica gel column chromatography (dichloromethane:EtOAc:MeOH = 3:2:0.1) to give **S11** (0.058 mmol, 80%) as a yellowish syrup. ¹H NMR (400 MHz, CDCl₃) δ 7.64-7.61 (m, 2H), 7.49-7.45 (m, 4H), 7.42-7.39 (m, 6H), 7.24 (s, 2H), 6.78 (dd, *J* = 2.4, 8.8 Hz, 2H), 6.46 (d, *J* = 2.4 Hz, 2H), 4.00-3.98 (m, 4H), 3.73 (t, *J* = 5.2 Hz, 4H), 2.99 (t, *J* = 5.0 Hz, 4H), 2.77 (t, *J* = 5.2 Hz, 4H), 0.88 (s, 18H), 0.05 (s, 12H); ¹³C NMR (100 MHz, CDCl₃) δ 159.4, 144.6, 139.0, 138.5, 137.2, 133.8, 132.2, 128.9, 128.0, 120.8, 114.8, 112.3, 67.6, 62.1, 51.5, 48.3, 25.9, 18.3, -5.3.



To a solution of **S11** (0.077 mmol) in THF (2 mL), lithium diisopropylamide (0.39 mmol, 1 mol/L in THF) was added at -78 °C and stirred for 1 h. To the reaction mixture, saturated NH₄Cl aqueous was added and extracted by EtOAc, dried over MgSO₄. The residue was subject to silica gel column chromatography (dichloromethane:EtOAc:MeOH = 3:2:0.0.5) to give **6** (0.028 mmol, 36%) as a yellow solid. ¹H NMR (400 MHz, CD₃Cl) δ 6.58 (d, *J* = 8.5 Hz, 2H), 6.16 (d, *J* = 2.6 Hz), 6.12 (dd, *J* = 2.6, 8.5 Hz, 2H), 3.78 (t, *J* = 5.2 Hz, 4H), 3.74 (m, 4H), 3.51 (t, *J* = 5.2 Hz, 4H), 3.47 (t, *J* = 5.0 Hz, 4H), 3.18 (br, 2H), 0.87 (s, 18H), 0.04 (s, 12H); ¹³C NMR (100 MHz, CDCl₃) δ 148.3, 135.12, 127.6, 118.1, 111.9, 110.9, 109.9, 107.3, 60.9, 60.8, 55.4, 53.8, 25.9, 18.4, -5.5.

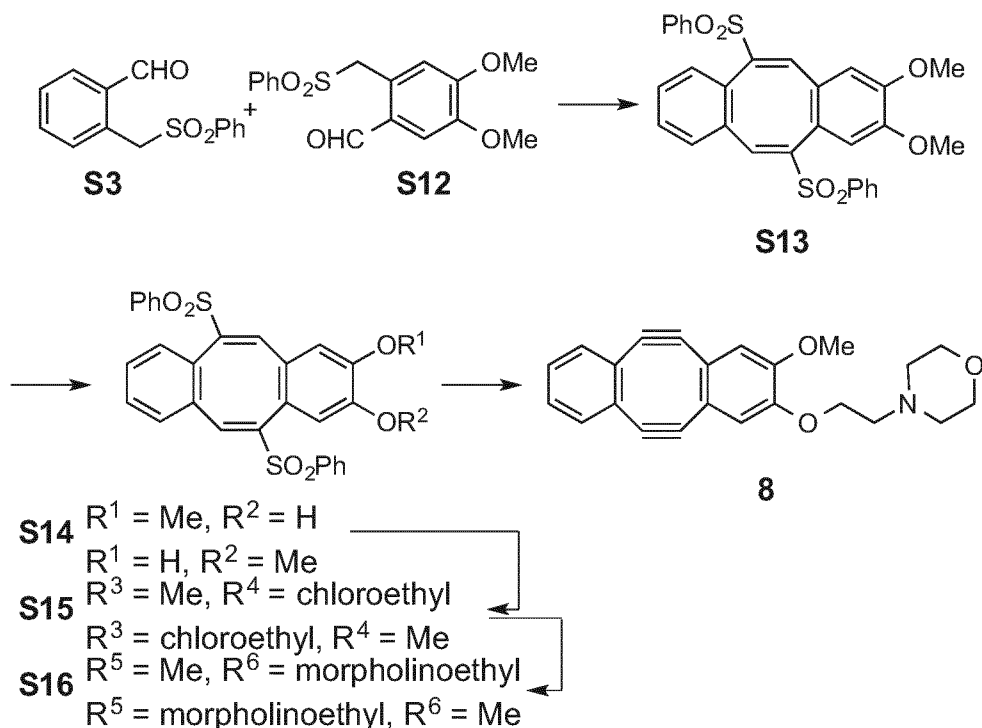
Compound 7



A mixture of **S12** (0.025 mmol), tetrabutylammonium fluoride (0.13 mmol) and THF (2 mL) was stirred for 1h at rt. To the reaction mixture, EtOAc was added, washed with water, dried over MgSO₄. The crude product was subjected to silica gel column chromatography (dichloromethane:EtOAc:MeOH = 3:2:0.3) to give **6** (0.0098 mmol, 40%) as a pale orange solid. ¹H NMR (400 MHz, CD₃CO₂D) δ 6.57 (d, *J* = 8.0 Hz, 2H), 6.24 (s, 2H), 6.23 (d, *J* = 8.0 Hz, 2H), 3.80 (t, *J* = 5.6 Hz, 8H), 3.53 (t, *J* = 5.6 Hz, 8H).

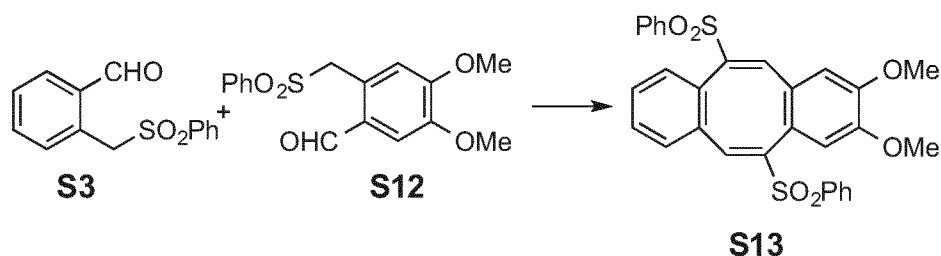
Dibenzo-1,5-cyclooctadiyne derivatives (Compound 8)

Compound **8** (Fig. 10) was synthesized according to Scheme 5.

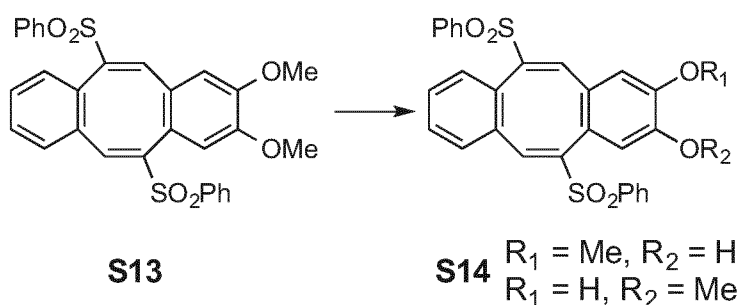


Scheme 5. Synthetic pathway C for dibenzo-1,5-cyclooctadiyne derivatives.

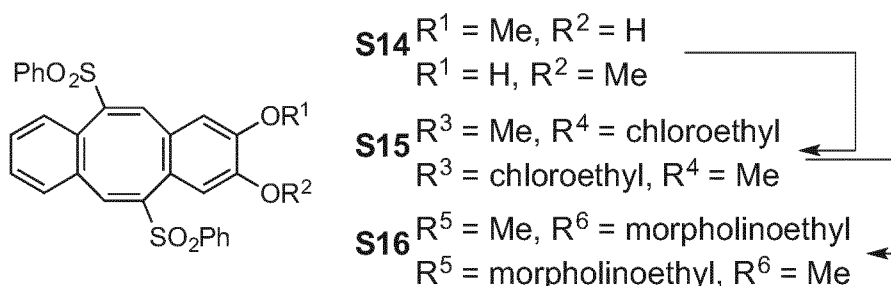
Compound 8



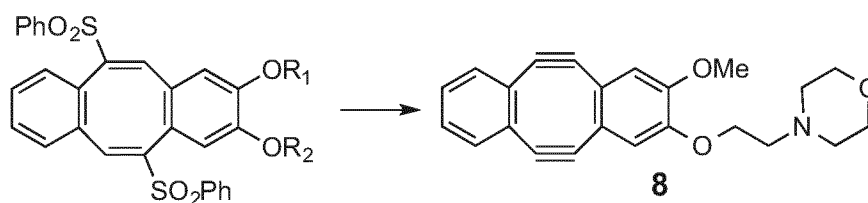
To a solution of **S3** (1.0 mmol), **S12** (1.0 mmol), and diethyl chlorophosphate (2.35 mmol) in THF (37 mL), lithium bis(trimethylsilyl)amide (4.0 mmol, 1 mol/L in THF) was added at -78 °C and stirred for 30 min and for 2 h at rt. To the mixture, saturated NH₄Cl solution was added and extracted with EtOAc, dried over MgSO₄. The residue was subjected to silica gel column chromatography (hexane:dichloromethane:EtOAc = 4:2:1) to give **S13** (0.22 mmol, 22%) as a yellowish solid. ¹H NMR (400 MHz, CDCl₃) δ 7.65-7.62 (m, 2H), 7.54-7.40 (m, 9H), 7.35 (s, 1H), 7.32 (s, 1H), 7.30-7.28 (m, 2H), 6.98 (s, 2H), 6.44 (s, 1H), 3.80 (s, 3H), 3.79 (s, 3H).



To a solution of **S13** (0.44 mmol) in dichloromethane (5 mL), BF₃•OEt₂ solution in dichloromethane (1.3 mmol; 1 mol/L in dichloromethane) was added at 0 °C, stirred for 2 h. To the reaction mixture, methanol was added at 0 °C and saturated NaHCO₃ solution was added at rt. The mixture was extracted with dichloromethane and dried over MgSO₄. The residue was subjected to silica gel column chromatography (hexane:dichloromethane:EtOAc = 2:1:1) to give **S14** (0.22 mmol, 22% as a mixture of regioisomer) as a white solid. ¹H NMR (400 MHz, CDCl₃) δ 7.57-7.54 (m, 2H), 7.40-7.19 (m, 12H), 6.94-6.92 (m, 2H), 6.45 (s, for major product)-6.36 (s, for minor product) (1H), 5.64 (s, for major product)-5.51 (for minor product) (1H), 3.76 (s, for major product)-3.74 (for minor product) (3H).



To a solution of **S14** (0.096 mmol) and K_2CO_3 (0.48 mmol) in DMF (0.5 mL), 1-bromo-2-chloroethane (0.29 mmol) was added and stirred for 1 h at 65 °C. To the reaction mixture, K_2CO_3 (0.48 mmol) and 1-bromo-2-chloroethane (0.29 mmol) were added and stirred for further 2 h at 70 °C. To the reaction mixture, EtOAc was added, washed with water, dried over $MgSO_4$ to give **S15** as a crude product and was used without further purification. A crude mixture of **S15** and morpholine (0.4 mL) was stirred for 1 h at 110 °C. The residue was subjected to silica gel column chromatography (dichloromethane:EtOAc:MeOH = 3:2:0.5) to give **S16** (0.063 mmol, 66% as a mixture of regioisomer) as a white syrup. 1H NMR (400 MHz, $CDCl_3$) δ 7.77-7.73 (m, 2H), 7.65-7.39 (m, 12H), 7.15-7.09 (m, 2H), 7.15 (s, for minor product)-7.09 (s, for major product) (1H), 6.59 (s, for major product)-6.56 (for minor product) (1H), 3.89 (s, 3H), 3.85-3.80 (br, 4H), 3.73-3.69 (br, 2H), 2.94-2.91 (br, 2H), 2.70 (br, 4H).

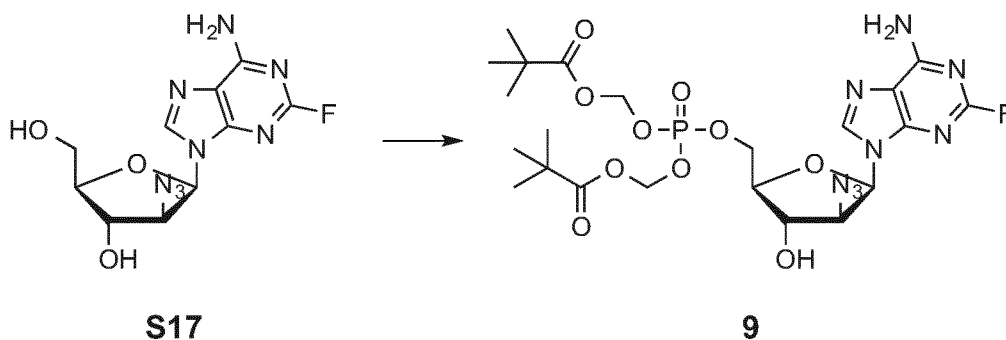


S16 $R_1 = Me$, $R_2 =$ morpholinoethyl
 $R_1 =$ morpholinoethyl, $R_2 = Me$

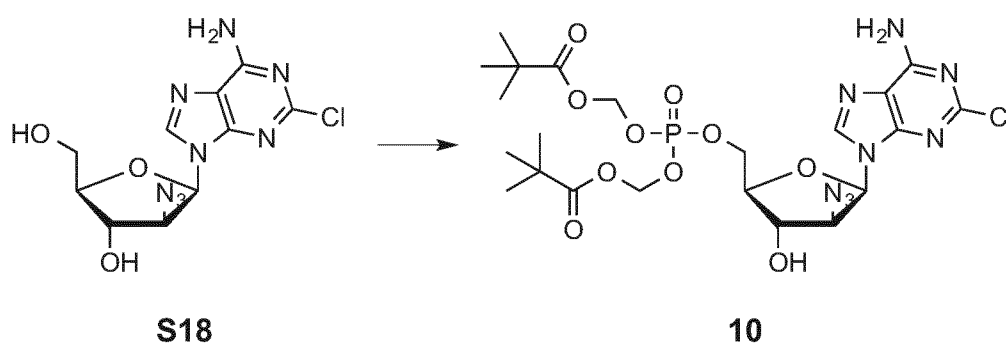
To a solution of **S16** (0.070 mmol) in THF (2 mL), lithium diisopropylamide (0.35 mL, 1 mol/L in THF) was added at -78 °C and stirred for 2h. To the reaction mixture, saturated NH_4Cl aqueous was added and extracted by EtOAc, dried over $MgSO_4$. The residue was subject to silica gel column chromatography (dichloromethane:EtOAc:MeOH = 3:2:0.5). The eluted mixture was further purified on preparative TLC (toluene:MeOH = 4:1) and the silica gel was extracted with dichloromethane and filtrated by a pad of celite to give **8** (0.034 mmol, 54%) as a dark brown solid. 1H NMR (400 MHz, $CDCl_3$) δ 6.88-6.83 (m, 2H), 6.67-6.62 (m, 2H), 6.35 (s, 1H), 6.32 (s, 1H), 4.05 (t, $J = 5.8$ Hz, 2H), 3.75 (s, 3H), 3.74 (t, $J = 4.5$ Hz, 4H), 2.80 (t, $J = 5.8$ Hz), 2.59 (br, 4H).

Azide-containing nucleotide monophosphate triesters (Compounds 9 to 18)

Compounds **9** to **18** (Fig. 11) were synthesized as follows.

Compound **9**

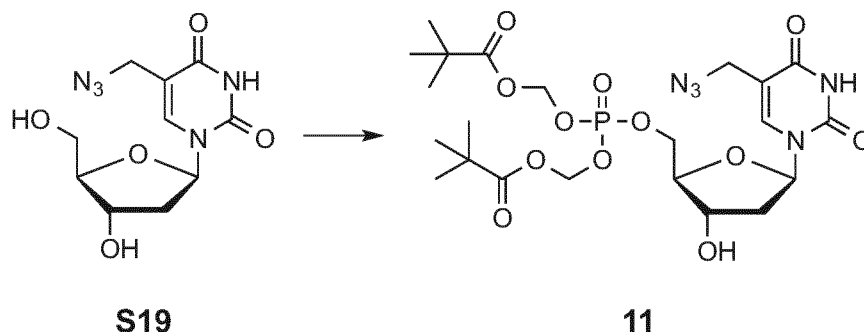
Compound **S17** (48 mg, 0.155 mmol) was added to triethylammonium bis(isopropoxyloxycarbonyloxymethyl)phosphate (0.23 mmol) and the mixture was co-
 5 evaporated with pyridine (3 x 1 mL). The residue was dissolved in anhydrous THF (2.5 mL) and at 4°C, diisopropylethylamine (81.6 μ L, 0.46 mmol) was added, followed by BOP-Cl (79 mg, 0.31 mmol) and 3-nitro-1,2,4-triazole (35 mg, 0.31 mmol). The mixture was stirred at rt for overnight, diluted with EtOAc, washed with water and dried with MgSO_4 . The residue was purified on Silica gel column (DCM: CH_3OH ; 10:0.2) to yield 17mg (17%) of **9**. ^1H NMR (300 MHz, DMSO-d_6) δ : 8.16 (s, 1H), 7.9 (br. s, NH_2), 6.33 (d, $J = 9$ Hz, 1H), 6.21 (d, $J = 6$ Hz, OH), 5.61 (s, 2H), 5.56 (s, 2H), 4.66 (t, $J = 9$ Hz, 1H), 4.48 (m, 1H), 4.34 (m, 2H), 4.01 (m, 1H), 1.14 (s, 9H), 1.12 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ : 177.19, 160.75, 157.97, 139.35, 125.03, 83.04, 83.00, 81.53, 81.42, 73.90, 67.42, 66.83, 38.73, 26.77; ^{31}P NMR (121 MHz, DMSO-d_6) δ : -3.97; HR-ESI MS (m/z): $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{22}\text{H}_{32}\text{FN}_8\text{O}_{10}\text{P}$ 619.1963,
 10 found 619.2036

Compound **10**

Compound **S18** (54 mg, 0.16 mmol) was added to triethylammonium bis(isopropoxyloxycarbonyloxymethyl)phosphate (0.248 mmol) and the mixture was co-
 20 evaporated with pyridine (3 x 1 mL). The residue was dissolved in anhydrous THF (4 mL) and at 4°C, diisopropylethylamine (86.7 μ L, 0.49 mmol) was added, followed by BOP-Cl (83.6 mg, 0.33 mmol) and 0.45 M tetrazole solution in acetonitrile (0.75 ml, 0.33 mmol). The mixture was stirred at rt for overnight, diluted with EtOAc, washed with water and dried with MgSO_4 . The residue was purified on Silica gel column (DCM: CH_3OH ; 10:0.2) to yield 45 mg

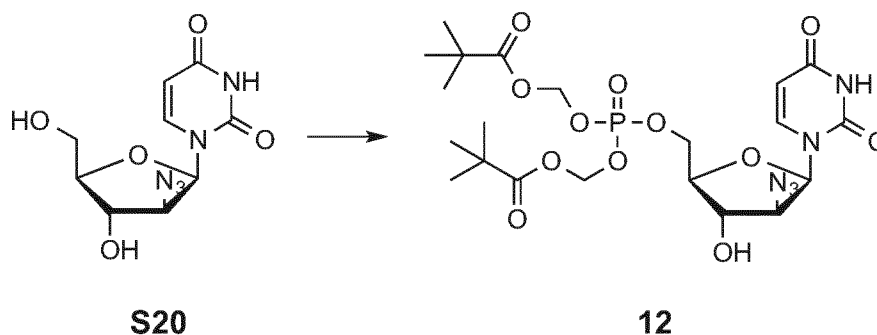
(44%) of **10**. ^1H NMR (300 MHz, DMSO- d_6) δ : 8.19 (s, 1H), 7.88 (br. s, NH_2), 6.38 (d, J = 6 Hz, 1H), 6.24 (d, J = 6 Hz, OH), 5.60 (s, 2H), 5.56 (s, 2H), 4.67 (t, J = 9 Hz, 1H), 4.37 (m, 3H), 4.00 (m, 1H), 1.13 (s, 9H), 1.12 (s, 9H); ^{13}C NMR (75 MHz, DMSO- d_6) δ : 175.88, 156.77, 153.08, 150.04, 140.14, 117.73, 82.74, 82.67, 81.87, 80.71, 72.70, 66.68, 38.10, , 26.35; ^{31}P NMR (121 MHz, DMSO- d_6) δ : -3.98; HR-ESI MS (m/z): $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{22}\text{H}_{32}\text{ClN}_8\text{O}_{10}\text{P}$ 657.1565, found 657.1559

Compound 11



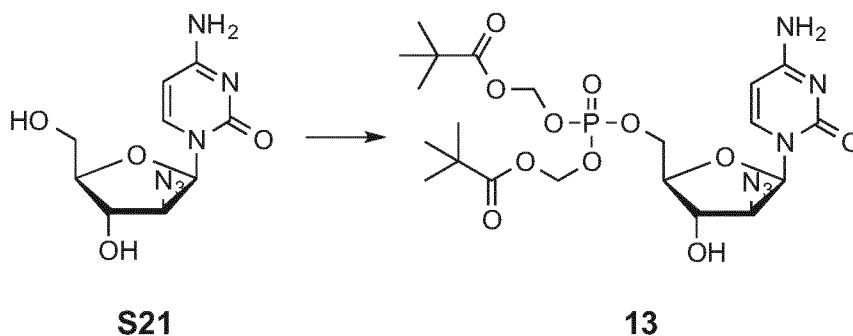
To a solution of triethylammonium bis(isopropoxyloxycarbonyloxymethyl)phosphate (0.31 mmol), **S19** (0.38 mmol), diisopropylethylamine (1.50 mmol), and tetrazole (0.75 mmol), bis(2-oxo-3-oxazolidinyl)phosphinic chloride (0.75 mmol) was added at 0 °C and stirred for 12 h. To the reaction mixture, EtOAc was added and washed with water, saturated NaHCO_3 solution, and dried over MgSO_4 . The residue was subject to silica gel column chromatography (dichloromethane : methanol = 97:3) to give **11** (0.11 mmol, 29%) as clear viscous oil. ^1H NMR (400 MHz, CD_3CN) δ 9.20 (br, 1H), 7.69 (s, 1H), 6.20 (dd, J = 6.6, 6.7 Hz, 1H), 5.63 (s, 2H), 5.60 (s, 12H), 4.35 (m, 1H), 4.25 (m, 2H), 4.08 (s, 2H), 4.02 (m, 1H), 3.55 (br, 1H), 2.27 (m, 1H), 2.15 (b, 1H), 1.22 (s, 9H), 1.21 (s, 9H); ^{13}C NMR (100 MHz, CD_3CN) δ 177.6, 163.7, 151.0, 140.3, 110.2, 86.1, 85.5 (d, J = 7.8 Hz), 84.1 (d, J = 1.8 Hz), 84.0 (d, J = 1.8 Hz), 71.2, 68.6 (d, J = 5.9 Hz), 48.0, 40.5, 39.3, 27.1; ^{31}P NMR (162 MHz, CD_3CN) δ -3.97; HRMS (ESI-TOF) calcd for $\text{C}_{22}\text{H}_{35}\text{O}_{12}\text{N}_5\text{P}$: 592.2014; observed: 592.2020 ($\text{M} + \text{H}$) $^+$.

Compound 12

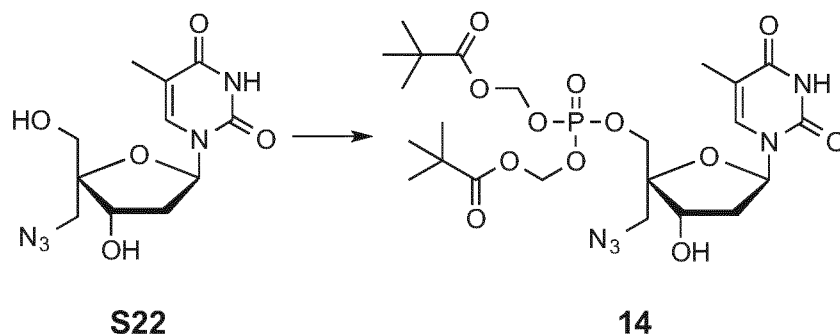


Compound **S20** (33 mg, 0.11 mmol) was added to triethylammonium bis(isopropoxyloxycarbonyloxymethyl)phosphate (0.16 mmol) and the mixture was co-evaporated with pyridine (3 x 1 mL). The residue was dissolved in anhydrous THF (2 mL) and at 4°C, diisopropylethylamine (58 μ L, 0.33 mmol) was added, followed by BOP-Cl (56.6 mg, 0.22 mmol) and 0.45 M tetrazole solution in acetonitrile (0.49 ml, 0.22 mmol). The mixture was stirred at rt for overnight, diluted with EtOAc, washed with water and dried with MgSO₄. The residue was purified on Silica gel column (DCM: CH₃OH; 10:0.25) to yield 28 mg (44%) of **12**. ¹H NMR (300 MHz, DMSO-d₆) δ : 11.48 (s, NH), 7.55 (d, J= 9 Hz, 1H), 6.38 (d, J= 6 Hz, 1H), 6.21 (d, J= 6 Hz, 1H), 6.15 (d, J= 3 Hz, 1H), 5.64 (s, 2H), 5.59 (s, 2H), 4.48 (t, J= 6 Hz, 1H), 4.29 (m, 2H), 4.03 (m, 1H), 3.94 (m, 1H), 1.18 (s, 9H), 1.17 (s, 9H); ¹³C NMR (75 MHz, DMSO-d₆) δ : 175.94, 162.87, 150.19, 140.65, 101.39, 82.78, 82.71, 80.72, 80.61, 72.90, 66.84, 66.73, 38.15, 26.38; ³¹P NMR (121 MHz, DMSO-d₆) δ : -3.85; HR-ESI MS (m/z): [M + H]⁺ calcd for C₂₁H₃₂N₅O₁₂P 577.1785, found 578.1862.

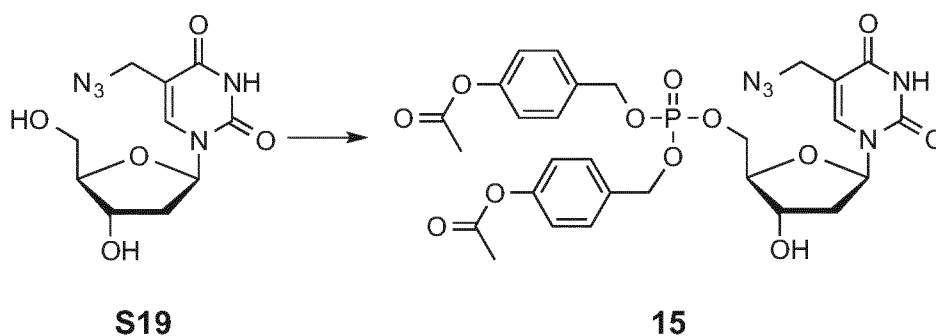
Compound **13**



Compound **S21** (35 mg, 0.11 mmol) was added to triethylammonium bis(isopropoxyloxycarbonyloxymethyl)phosphate (0.17 mmol) and the mixture was co-evaporated with pyridine (3 x 1 mL). The residue was dissolved in anhydrous THF (2 mL) and at 4°C, diisopropylethylamine (61.7 μ L, 0.35 mmol) was added, followed by BOP-Cl (59.9 mg, 0.23 mmol) and 0.45 M tetrazole solution in acetonitrile (0.49 ml, 0.22 mmol). The mixture was stirred at rt for overnight, diluted with EtOAc, washed with water and dried with MgSO₄. The residue was purified on Silica gel column (DCM: CH₃OH; 10:0.3) to yield 14 mg (22%) of **13**. ¹H NMR (300 MHz, DMSO-d₆) δ : 8.01 (d, J= 9 Hz, 1H), 6.33 (d, J= 9 Hz, 1H), 6.16 (d, J= 6 Hz, 1H), 5.58 (s, 2H), 5.53 (s, 2H), 4.49 (t, J= 6 Hz, 1H), 4.02 (m, 1H), 3.74 (m, 2H), 3.70 (m, 1H), 1.14 (s, 9H), 1.13 (s, 9H); ¹³C NMR (75 MHz, DMSO-d₆) δ : 176.20, 155.04, 133.53, 113.29, 90.84, 83.06, 82.22, 82.21, 71.29, 67.44, 58.75, 38.10, 26.39; ³¹P NMR (121 MHz, DMSO-d₆) δ : -4.75; HR-ESI MS (m/z): [M + H]⁺ calcd for C₂₁H₃₃N₆O₁₁P 577.1945, found 577.2023

Compound **14**

Compound **S22** (33 mg, 0.11 mmol) was added to triethylammonium bis(isopropoxyxycarbonyloxymethyl)phosphate (0.16 mmol) and the mixture was co-
 5 evaporated with pyridine (3 x 1 mL). The residue was dissolved in anhydrous THF (2 mL) and at 4°C, diisopropylethylamine (58 μ L, 0.33 mmol) was added, followed by BOP-Cl (56.6 mg, 0.22 mmol) and 3-nitro-1,2,4-triazole (26.8 mg, 0.23 mmol). The mixture was stirred at rt for overnight, diluted with EtOAc, washed with water and dried with MgSO_4 . The residue was purified on Silica gel column (DCM: CH_3CN ; 1:1) to yield 14 mg (20%) of **14**. ^1H NMR (300
 10 MHz, MeOD) δ : 7.45 (s, 1H), 6.35 (t, J = 6 Hz, 1H), 5.70 (s, 2H), 5.65 (s, 2H), 4.47 (t, J = 6 Hz, 1H), 4.22 (d, 2H), 3.65 (d, J = 12 Hz, 1H), 3.38 (d, J = 12 Hz, 1H), 2.35 (m, 2H), 1.90 (s, 3H), 1.23 (s, 9H), 1.22 (s, 9H); ^{13}C NMR (75 MHz, MeOD) δ : 177.82, 165.85, 151.79, 146.10, 137.07, 112.05, 87.74, 85.88, 84.00, 72.29, 69.89, 52.37, 40.94, 39.52, 27.52; ^{31}P NMR (121 MHz, MeOD) δ : -4.58; HR-ESI MS (m/z): $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{23}\text{H}_{36}\text{N}_5\text{O}_{12}\text{P}$ 628.1996, found
 15 628.1990

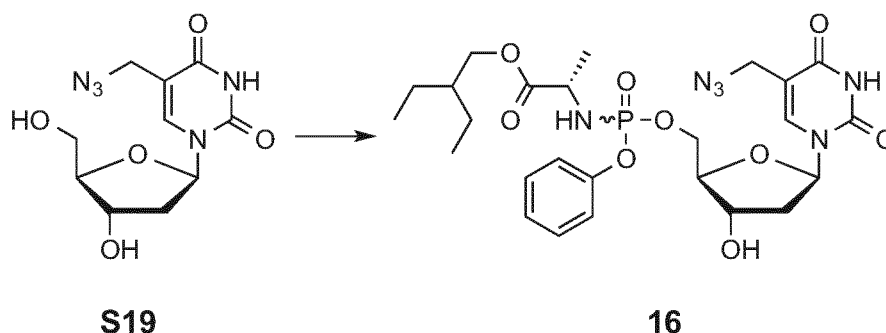
Compound **15**

To a solution of bis(4-acetoxybenzyl) phosphate (0.12 mmol) in THF (1.2 mL), **S19** (0.10 mmol), diisopropylethylamine (0.40 mmol), and 3-nitro-1H-1,2,4-triazole (0.20 mmol), bis(2-oxo-3-oxazolidinyl)-phosphinic chloride (0.20 mmol) was added at 0 °C stirred for 15 h at rt.
 20

To the reaction mixture, dichloromethane was added and washed with water, saturated NaHCO_3 solution, and dried over MgSO_4 . The residue was subject to silica gel column chromatography (dichloromethane : methanol = 50:1–30:1) to give **15** as clear viscous oil

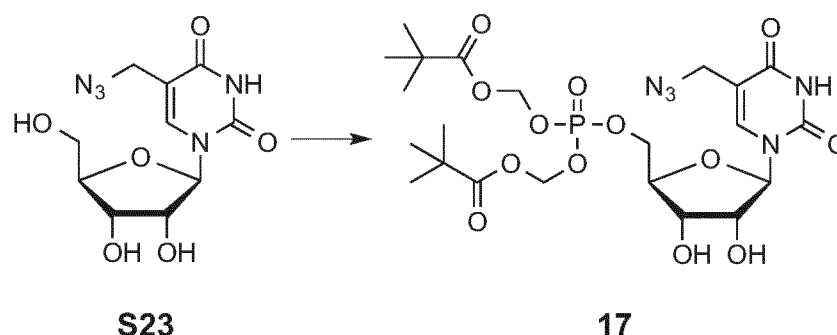
(0.031 mmol, 31%). ^1H NMR (400 MHz, CD_3CN) δ 9.11 (br, 1H), 7.70 (s, 1H), 7.41 (m, 4H), 7.11 (m, 4H), 6.17 (dd, J = 6.4, 6.6 Hz, 1H), 5.06 (d, J = 8.4 Hz, 4H), 4.26 (m, 1H), 4.17 (m, 2H), 4.01 (s, 2H), 3.99 (m, 1H), 3.54 (br, 1H), 2.26 (s, 6H), 2.24 (m, 1H), 2.06 (m, 1H); ^{13}C NMR (100 MHz, CD_3CN) δ 170.6, 163.6, 152.1, 151.0, 140.3, 134.7 (dd, J = 1.5, 6.3 Hz), 130.3 (d, J = 1.9 Hz), 123.1 (d, J = 1.4 Hz), 110.1, 86.1, 85.8 (d, J = 7.3 Hz), 71.3, 69.7 (dd, J = 2.0, 5.5 Hz), 68.0 (d, J = 5.8 Hz), 48.1, 40.6, 21.3; ^{31}P NMR (162 MHz, CDCl_3) δ -0.50; HRMS (ESI-TOF) calcd for $\text{C}_{28}\text{H}_{31}\text{O}_{12}\text{N}_5\text{P}$: 660.1701; observed: 660.1699 ($\text{M} + \text{H}$) $^+$

Compound 16



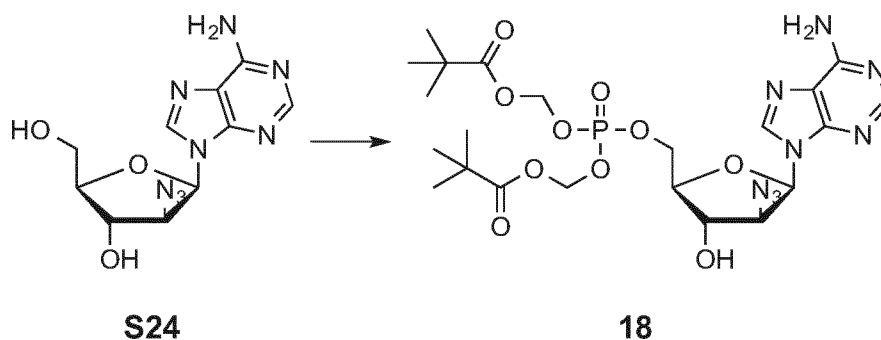
To a solution of **S19** (0.10 mmol), 2-ethylbutyl ((4-nitrophenoxy)(phenoxy)phosphoryl)-L-alaninate (0.16 mmol), and MgCl_2 (0.16 mmol) in dry MeCN (2.0 mL), diisopropylethylamine (0.34 mmol) was added and stirred for 15 hours at rt. To the reaction mixture, EtOAc was added, washed with water and brine. The residue was subjected to silica gel column chromatography (dichloromethane : methanol = 50:1–30:1) to give **16** (0.037 mmol, 37% as a mixture of diastereomers) as clear viscous oil. ^1H NMR (400 MHz, CDCl_3) δ 9.26 (br, 1H), 7.73 (s, 1H), 7.33 (m, 2H), 7.22 (m, 3H), 6.23 (m, 1H), 4.48 (m, 1H), 4.11 (m, 7H), 2.40 (m, 1H), 2.07 (m, 1H), 1.50 (m, 1H), 1.37 (m, 7H), 0.87 (m, 6H); ^{13}C NMR (100 MHz, CDCl_3) δ 173.7, 162.5, 150.3, 150.0, 138.6, 129.9, 125.3, 120.1, 109.7, 85.3, 70.6, 67.8, 65.9, 50.4, 47.0, 40.3, 40.2, 23.1, 21.0, 10.9; ^{31}P NMR (162 MHz, CDCl_3) δ 3.22; HRMS (ESI-TOF) calcd for $\text{C}_{25}\text{H}_{35}\text{O}_9\text{N}_6\text{PNa}$: 617.2095; observed: 617.2097 ($\text{M} + \text{Na}$) $^+$.

Compound 17



Compound **S23** (34 mg, 0.11 mmol) was added to triethylammonium bis(isopropoxyloxycarbonyloxymethyl)phosphate (0.124 mmol) and the mixture was co-evaporated with pyridine (3 x 1 mL). The residue was dissolved in anhydrous THF (3 mL) and at 4°C, diisopropylethylamine (59.5 μ L, 0.34 mmol) was added, followed by BOP-Cl (57.8 mg, 0.22 mmol) and 0.45 M tetrazole solution in acetonitrile (0.49 ml, 0.22 mmol). The mixture was stirred at rt for overnight, diluted with EtOAc, washed with water and dried with MgSO₄. The residue was purified on Silica gel column (DCM: CH₃OH; 10:0.4) to yield 16 mg (24%) of **17**. ¹H NMR (300 MHz, DMSO-d₆) δ : 11.57 (s, 1H), 7.72 (s, 1H), 5.71 (d, J= 6 Hz, 1H), 5.55 (s, 2H), 5.52 (s, 2H), 5.50 (d, J= 6 Hz, OH), 5.29 (d, J= 6 Hz, OH), 4.17 (m, 2H), 3.91 (m, 5H), 1.10 (s, 9H), 1.09 (s, 9H); ¹³C NMR (75 MHz, DMSO-d₆) δ : 175.94, 162.78, 150.33, 140.03, 108.66, 88.64, 82.79, 82.72, 81.59, 72.58, 69.30, 46.61, 38.15, 28.37; ³¹P NMR (121 MHz, DMSO-d₆) δ : -3.87; HR-ESI MS (m/z): [M + Na]⁺ calcd for C₂₂H₃₄N₅O₁₃P 630.1788, found 630.1784

Compound **18**



Compound **S24** (56 mg, 0.19 mmol) was added to triethylammonium bis(isopropoxyloxycarbonyloxymethyl)phosphate (0.28 mmol) and the mixture was co-evaporated with pyridine (3 x 1 mL). The residue was dissolved in anhydrous THF (4 mL) and at 4°C, diisopropylethylamine (100 μ L, 0.57 mmol) was added, followed by BOP-Cl (97 mg, 0.38 mmol) and 0.45 M tetrazole solution in acetonitrile (0.85 ml, 0.38 mmol). The mixture was stirred at rt for overnight, diluted with EtOAc, washed with water and dried with MgSO₄. The residue was purified on Silica gel column (DCM: CH₃OH; 100:5) to yield 53 mg (46%) of **18**. ¹H NMR (300 MHz, DMSO-d₆) δ : 8.18 (s, 1H), 8.16 (s, 1H), 7.33 (br. s, NH₂), 6.45 (d, J= 6 Hz, 1H), 6.24 (br. s, OH), 5.60 (s, 2H), 5.56 (s, 2H), 4.68 (t, J= 9 Hz, 1H), 4.54 (t, J= 9 Hz, 1H), 4.37 (m, 3H), 4.02 (m, 1H), 1.13 (s, 9H), 1.11 (s, 9H). ³¹P NMR (121 MHz, DMSO-d₆) δ : -3.96.

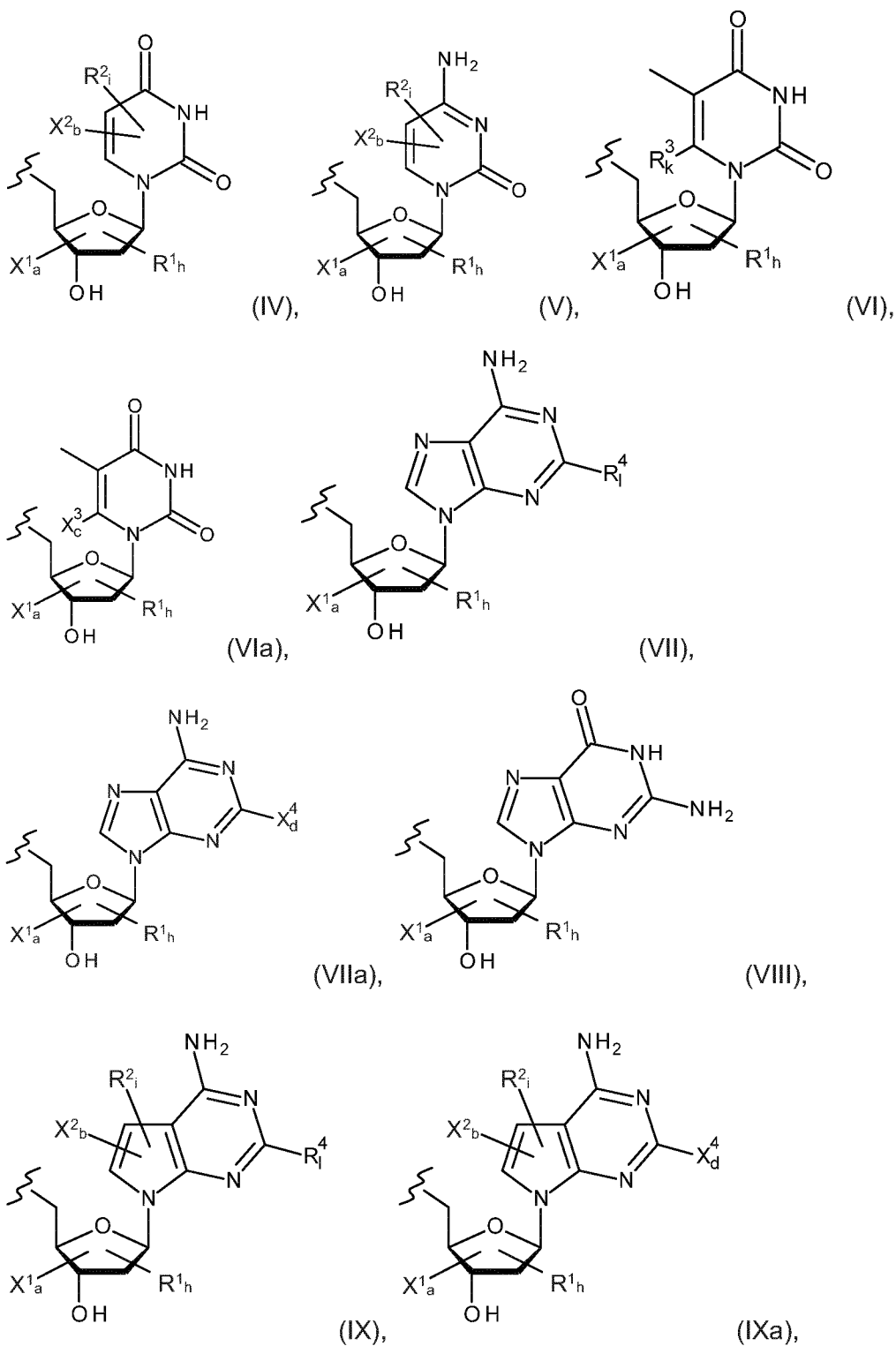
Claims

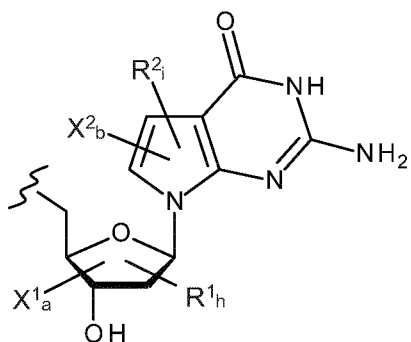
1. A kit of parts for crosslinking nucleic acid comprising two separate components, wherein
 - the first component comprises one or more non-toxic nucleoside analogues or nucleotide analogues, in particular one or more non-toxic nucleotide analogues, wherein each of said non-toxic nucleoside analogues or each of said non-toxic nucleotide analogues comprises one or two fusion moieties X which are covalently linkable to a counter fusion moiety Y, and
 - the second component comprises one or more non-toxic compounds BY₂, wherein B is a scaffold moiety, and Y is said counter fusion moiety which is covalently linkable to said fusion moiety X, wherein in particular

said kit of parts further comprises a third component, wherein said third component is a molecule comprising one or more fusion moieties X and optionally an affinity label, wherein particularly the third component is a molecule selected from a dye, a metal complex, a radioisotope, a spin probe, an RNA molecule, a DNA molecule, a peptide, a protein and a carbohydrate each comprising one or more fusion moieties X and optionally an affinity label.
2. A method for crosslinking nucleic acid ex vivo comprising the steps of
 - a. providing one or more non-toxic nucleoside analogues or nucleotide analogues, in particular one or more non-toxic nucleotide analogues, wherein each nucleoside analogue or nucleotide analogue comprises one or two fusion moieties X which are covalently linkable to a counter fusion moiety Y,
 - b. in a pulse step, contacting a proliferating cell with said non-toxic nucleoside analogues or nucleotide analogues yielding at least two nucleic acid strands that are each tagged with at least one fusion moiety X or yielding one nucleic acid strand that is tagged with at least two fusion moieties X,
 - c. providing one or more non-toxic compounds BY₂, wherein B is a scaffold moiety, and Y is a counter fusion moiety which is covalently linkable to said fusion moiety X,
 - d. in a crosslinking step, contacting (particularly incubating) said cell with said compound BY₂ yielding interstrand crosslinked nucleic acid strands and/or an intrastrand crosslinked nucleic acid strand, in particular interstrand crosslinked nucleic acid strands.
3. A method for labeling nucleic acid ex vivo comprising the steps of

- a. providing one or more non-toxic nucleoside analogues or nucleotide analogues, in particular one or more non-toxic nucleotide analogues, wherein said non-toxic nucleoside analogue or said non-toxic nucleotide analogue comprises one or two fusion moieties X which are covalently linkable to a counter fusion moiety Y,
 - b. in a pulse step, contacting a proliferating cell, in particular a mammal cell, more particularly a human cell, obtained from a mammal, particularly a human, with said non-toxic nucleoside analogue or nucleotide analogue yielding a nucleic acid strand which is tagged by at least one fusion moiety X,
 - c. providing one or more non-toxic compounds BY_2 , wherein B is a scaffold moiety, and Y is a counter fusion moiety which is covalently linkable to said fusion moiety X,
 - d. in a crosslinking step, contacting (particularly incubating) said cell with said compound BY_2 yielding a double-tagged nucleic acid strand which comprises said compound BY_2 , wherein one counter fusion moiety Y is covalently bound to the fusion moiety X,
 - e. in a labeling step, contacting (particularly incubating) said cell with a third component, wherein the third component is a molecule comprising one or more fusion moieties X, in particular a molecule selected from a dye, a metal complex, a radioisotope, a spin probe, an RNA molecule, a DNA molecule, a peptide, a protein, a carbohydrate, each comprising one or more fusion moieties X and optionally an affinity label, yielding a triple-tagged nucleic acid strand that comprises of said double-tagged nucleic acid strand covalently bound to the fusion moiety X of the third component.
4. The kit of parts according to claim 1 or the method according to claim 2 or 3, wherein said first component comprises a compound $A-X_v$ with X being said fusion moiety X and v being 1 or 2, wherein the moiety A is a moiety of formula (I) and/or (II) and/or (III) and/or (IIIa),
- D-M- (I), D'-M- (II) and/or -D''-M- (III), and/or D'''-M (IIIa) wherein
- D is a phosphoester moiety,
 - D' is a triphosphate moiety,
 - D'' is a monophosphate moiety,
 - D''' is a hydroxy group, and
 - M is a nucleoside or an analogue or a derivative thereof.

5. The kit of parts or the method according to claim 4, wherein $-M(X)_v$ of $D-M(X)_v$, $D'-M(X)_v$, $D''-M(X)_v$ or $D'''-M(X)_v$ is a moiety of formula (IV), (V), (VI), (VIa), (VII), (VIIa), (VIII), (IX), (IXa) or (X), in particular of formula (IV), (V), (VI), (VII), (VIII), (IX) or (X),





(X), with

each X^1 , X^2 , X^3 and X^4 being independently from each other selected from $-N_3$ and $-C_{1-4}$ -alkyl- N_3 , in particular $-N_3$ and $-C_{1-2}$ -alkyl, more particularly $-N_3$ and $-CH_2-N_3$,

a and b being independently from each other an integer between 0 and 2, in particular 0 and 1, wherein the sum of a and b is equal to v,

c being 0 or 1, in particular 0, wherein the sum of a and c is equal to v,

d being 0 or 1, in particular 0, wherein the sum of a, b and d is equal to v,

R^1 , R^2 , R^3 and R^4 are independently from each other selected from $-F$, $-Cl$, $-Br$, $-I$, $-C_{1-4}$ -alkyl, $-C_{2-4}$ -alkenyl, $-O-C_{1-4}$ -alkyl, $-O-C(=O)-C_{1-4}$ -alkyl, $-S-C_{1-4}$ -alkyl, $-S-C(=O)-C_{1-4}$ -alkyl, $-OH$, $-NH_2$, $-N-HR^{20}$ and $-N-R^{20}_2$, in particular from $-F$, $-Cl$, $-Br$, $-I$, $-C_{1-2}$ -alkyl, $-C_{2-4}$ -alkenyl, $-O-C_{1-2}$ -alkyl, $-O-C(=O)-C_{1-4-2}$ -alkyl, $-S-C_{1-2}$ -alkyl, $-S-C(=O)-C_{1-2}$ -alkyl, $-OH$, $-NH_2$, $-N-HR^{20}$ and $-N-R^{20}_2$, more particularly from $-F$, $-Cl$, $-CH_3$, $-O-CH_3$, $-O-C(=O)-CH_3$, $-S-CH_3$, $-OH$, even more particularly from $-OH$, $-F$ and $-Cl$

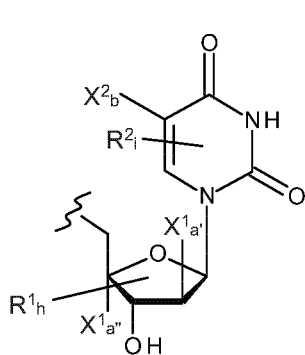
with R^{20} being $-C_{1-4}$ -alkyl

h is an integer between 0 and 5, in particular 0 and 2, more particularly 0 and 1, even more particularly 0, wherein the sum of a and h is equal to or smaller than 5,

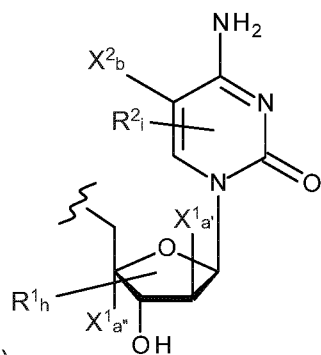
i is an integer between 0 and 2, in particular 0 and 1, more particularly 0, wherein the sum of b and i is equal to or smaller than 2,

k and l are independently from each other 0 or 1, wherein in particular

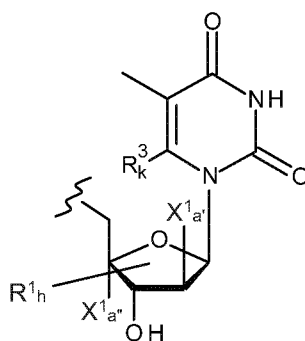
$-M-(X)_v$ of $D-M-(X)_v$, $D'-M-(X)_v$, $D''-M-(X)_v$ or $D'''-M-(X)_v$ is a moiety of formula (IV'), (V'), (VI'), (VIIa'), (VII'), (VIII'), (IX'), (IXa') or (X'), in particular of formula (IV'), (V'), (VI'), (VII'), (VIII'), (IX'), or (X'),



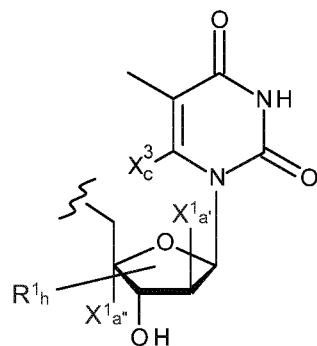
(IV')



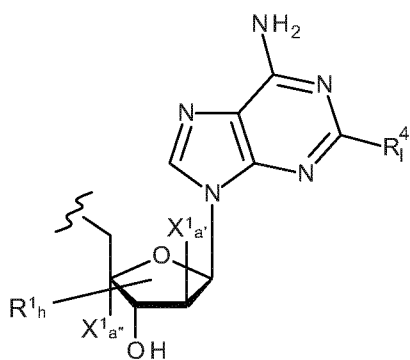
(V')



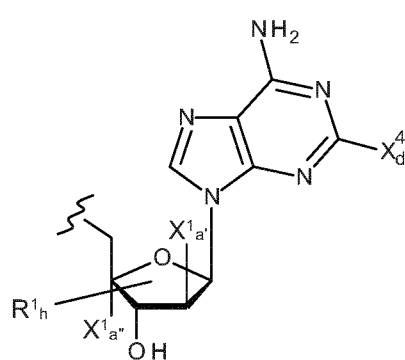
(VI'),



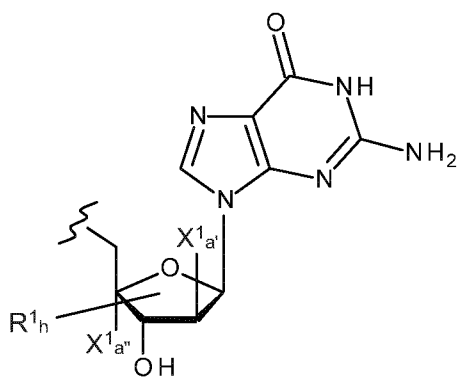
(VIa'),



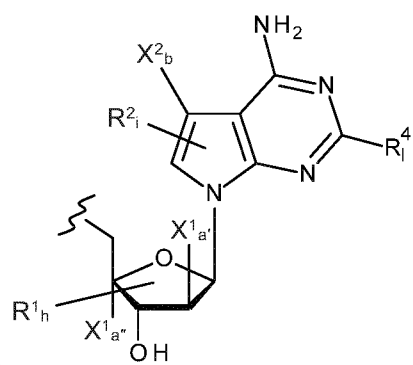
(VII'),



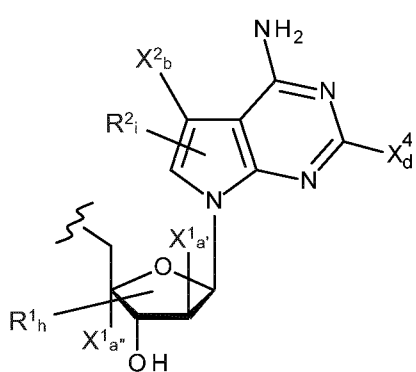
(VIIa'),



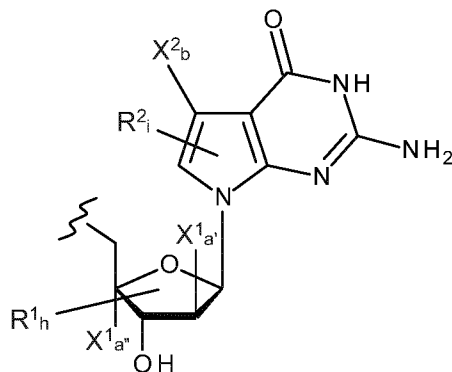
(VIII'),



(IX'),



(IXa'),



(X'), with

each X^1 , each X^2 , X^3 and X^4 being independently from each other selected from $-N_3$ and $-C_{1-4}$ -alkyl- N_3 , in particular $-N_3$ and C_{1-2} -alkyl- N_3 , more particularly $-N_3$ and $-CH_2-N_3$,

a' , a'' , b , c and d being independently from each other 0 or 1, wherein the sum of a' , a'' , b and c and d is equal or larger than 1, in particular the sum of a' , a'' and b is 1 or 2, more particularly

a' is 0, a'' is 1 and b is 0, or

a' is 1, a'' is 0 and b is 0, or

a' is 1, a'' is 0, and b is 1, and

R^1 being in particular being $-OH$,

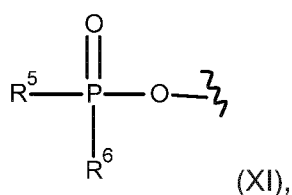
R^2 , R^3 and R^4 are in particular $-Cl$ or $-F$,

h is an integer between 0 and 4, in particular 0 and 2, more particularly 0 and 1, wherein the sum of a' , a'' and h is equal to or smaller than 4,

i is 0 or 1,

k and l are independently from each other 0 or 1.

6. The kit of parts or the method according to any one of claims 4 to 5, wherein D is a phosphoester of formula (XI),



(XI),

with R^5 and R^6 being independently selected from a moiety of formula (XII), (XIII) or (XIV),

- L-G-E (XII), with

L being selected from $-O-$, $-NH-$, $-NR^7-$ and $-S-$,

G being selected from $-C_{1-6}$ -alkyl-, $-C_{6-14}$ -aryl-, $-R^8-C_{6-14}$ -aryl-, $-C_{6-14}$ -aryl- R^9 -, $-R^8-C_{6-14}$ -aryl- R^9 -, $-C_{6-14}$ -cycloalkyl-, $-R^8-C_{6-14}$ -cycloalkyl-, $-C_{6-14}$ -cycloalkyl- R^9 -, $-R^8-C_{6-14}$ -cycloalkyl- R^9 -, $-R^8-O-C(=O)-R^9-C_{6-14}$ -aryl-, $-R^8-C(=O)-O-R^9-C_{6-14}$ -aryl-, -furyl- and -thiofuryl-,

R^8 and R^9 being independently from each other C_{1-4} -alkyl,

E being selected from $-O-C(=O)-O-R^{10}$, $-O-C(=O)-R^{10}$, $-C(=O)-O-R^{10}$, $-S-S-R^{10}$, $-S-C(=O)-O-R^{10}$, $-O-C(=O)-S-R^{10}$, $-S-C(=O)-R^{10}$, $-C(=O)-S-R^{10}$, and $-NO_2$,

R^{10} is independently selected from H or $-C_{1-6}$ -alkyl, or

- L-G (XIII), with

L being selected from -O-, -NH-, $-NR^7$ - and -S-, and

G being selected from $-C_{1-6}$ -alkyl-, $-C_{6-14}$ -aryl-, $-R^8-C_{6-14}$ -aryl-, $-C_{6-14}$ -aryl- R^9 -, $-R^8-C_{6-14}$ -aryl- R^9 -, $-C_{6-14}$ -cycloalkyl-, $-R^8-C_{6-14}$ -cycloalkyl-, $-C_{6-14}$ -cycloalkyl- R^9 and $-R^8-C_{6-14}$ -cycloalkyl- R^9 ,

R^8 and R^9 being independently from each other C_{1-4} -alkyl, or

- L-E (XIV), with

L being selected from -O-, -NH-, $-NR^7$ - and -S-,

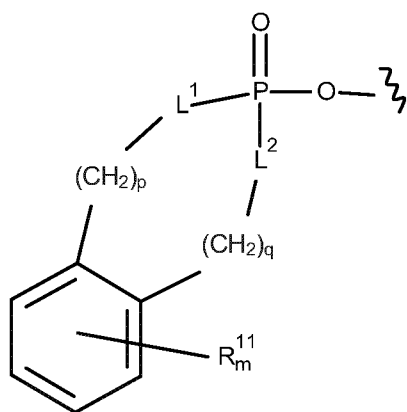
E being selected from $-C(=O)-O-R^{10}$ and $-C(=O)-S-R^{10}$,

R^{10} is independently selected from H or $-C_{1-6}$ -alkyl,

G of formula (XII) or (XIII) and/or R^{10} of formula (XII) or (XIV) may be optionally substituted by one or more substituents independently selected from -F, -Cl, -Br, -I, $-C_{1-4}$ -alkyl, $-O-C_{1-4}$ -alkyl, $-CH_2-C(=O)-O-(CH_2)_{1-4}$ and $-CH_2-O-C(=O)-(CH_2)_{1-4}$, and/or

R^7 of formula (XII), (XIII) or (XIV) is independently selected from -G-E, -G or -E with G and E as defined above, or

D is a phosphoester of formula (XV),



(XV), with

L^1 and L^2 being independently from each other selected from -O-, -NH-, -NR⁷- and -S-,

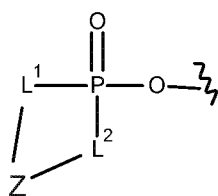
R^7 being independently selected from -G-E, -G or -E with G, E and n as defined above,

p and q being independently from each other an integer between 0 and 4,

R^{11} being independently selected from -F, -Cl, -Br, -I, -C₁₋₄-alkyl, -O-C₁₋₄-alkyl,

m being an integer between 0 and 4, or

D is a phosphoester of formula (XVI),



(XVI), with

L^1 and L^2 being independently from each other selected from -O-, -NH-, -NR⁷- and -S-,

R^7 being independently selected from -G-E, -G or -E with G, E and n as defined above,

Z being a C_r-alkyl, optionally substituted with R¹²_s,

r being an integer between 3 and 5,

s being an integer between 0 and 5, wherein s is equal or smaller than r,

R^{12} being -G or -E as defined for formula (XIII) or (XIV),

in particular D is a phosphoester of formula (XI) with R⁵ and R⁶ being independently selected from a moiety of formula (XII) (XIII) or (XIV), more particularly D is a

phosphoester of formula (XI) with R^5 and R^6 being independently selected from a moiety of formula (XII) or (XIII).

7. The kit of parts or the method according to claim 6, wherein D is a phosphoester of formula (XI), with R^5 and R^6 being independently selected from a moiety of formula (XII), (XIII) or (XIV),

- L-G-E (XII) with

L being selected from -O-, -NH- and -NR⁷-,

G being selected from -C₁₋₆-alkyl-, -C₆-aryl-, -R⁸-C₆-aryl-, -C₆-aryl-R⁹-, -R⁸-C₆-aryl-R⁹-, -C₆-cycloalkyl-, -R⁸-C₆-cycloalkyl-, -C₆-cycloalkyl-R⁹-, -R⁸-C₆-cycloalkyl-R⁹-, -R⁸-O-C(=O)-R⁹-C₆-aryl-, -R⁸-C(=O)-O-R⁹-C₆-aryl-, -furyl- and -thiofuryl-,

R^8 and R^9 being independently from each other C₁₋₄-alkyl, in particular C₁₋₂-alkyl,

E being selected from -O-C(=O)-O-R¹⁰-, -O-C(=O)-R¹⁰-, -C(=O)-O-R¹⁰-, -S-S-R¹⁰-, -S-C(=O)-O-R¹⁰-, -O-C(=O)-S-R¹⁰-, -S-C(=O)-R¹⁰-, -C(=O)-S-R¹⁰-, and -NO₂,

R^{10} is independently selected from H or -C₁₋₆-alkyl, or

- L-G (XIII) with

L being selected from -O-, -NH- and -NR⁷-,

G being selected from -C₁₋₆-alkyl-, -C₆-aryl-, -R⁸-C₆-aryl-, -C₆-aryl-R⁹-, -R⁸-C₆-aryl-R⁹-, -C₆-cycloalkyl-, -R⁸-C₆-cycloalkyl-, -C₆-cycloalkyl-R⁹ and -R⁸-C₆-cycloalkyl-R⁹-,

R^8 and R^9 being independently from each other C₁₋₄-alkyl, or

- L-E (XIV) with

L being selected from -O-, -NH- and -NR⁷-,

E being selected from -C(=O)-O-R¹⁰ and -C(=O)-S-R¹⁰,

R^{10} is independently selected from H or -C₁₋₄-alkyl,

G of formula (XII) or (XIII) and/or R^{10} of formula (XII) or (XIV) may be optionally substituted by one or more substituents independently selected from -F, -Cl, -Br, -I, -C₁₋₄-alkyl, -O-C₁₋₄-alkyl, -CH₂-C(=O)-O-(CH₂)₁₋₂ and -CH₂-O-C(=O)-(CH₂)₁₋₂, and/or R^7 of formula (XII), (XIII) or (XIV) is independently selected from -G-E, -G or -E with G and E as defined above, or

D is a phosphoester of formula (XV), with

L^1 and L^2 being independently from each other selected from -O-, -NH- and -NR⁷,

R^7 being independently selected from -G-E, -G or -E with G and E as defined above,

p and q being independently from each other an integer between 0 and 2,

R^{11} being independently selected from -F, -Cl, -Br, -I, -C₁₋₄-alkyl, -O-C₁₋₄-alkyl,

m being an integer between 0 and 4, or

D is a phosphoester of formula (XVI) with

L^1 and L^2 being independently from each other selected from -O-, -NH- and -NR⁷-,

R^7 being independently selected from -G-E, -G or -E with G, E and n as defined above,

Z being a C_r-alkyl, optionally substituted with R¹²_s,

r being an integer between 3 and 5,

s being an integer between 0 and 3, wherein s is equal or smaller than r,

R^{12} being -G or -E as defined for formula (XIII) or (XIV) ,

in particular D is a phosphoester of formula (XI) with R^5 and R^6 being independently selected from a moiety of formula (XII) (XIII) or (XIV), more particularly D is a phosphoester of formula (X) with R^5 and R^6 being independently selected from a moiety of formula (XII) or (XIII).

8. The kit of parts or the method according to any one of claims 6 or 7, wherein D is a phosphoester of formula (XI), with R^5 and R^6 being independently selected from a moiety of formula (XII), (XIII) or (XIV),

- L-G-E (XII) with

L being selected from -O-, -NH- and -NR⁷-,

G being selected from -C₁₋₄-alkyl-, particularly -C₁₋₂-alkyl-, -C₆-aryl-, -R⁸-C₆-aryl-, -C₆-cycloalkyl-, -R⁸-O-C(=O)-R⁹-C₆-aryl-, -furyl- and -thiofuryl-,

R^8 and R^9 being independently from each other C₁₋₄-alkyl, in particular C₁₋₂-alkyl,

E being selected from -O-C(=O)-O-R¹⁰, -O-C(=O)-R¹⁰, -C(=O)-O-R¹⁰, -S-S-R¹⁰, -S-C(=O)-O-R¹⁰ and -NO₂,

R^{10} is independently selected from H or $-C_{1-6}$ -alkyl, or

- L-G (XIII) with

L being selected from $-O-$, $-NH-$ and $-NR^7-$,

G being selected from $-C_{1-6}$ -alkyl, in particular $-C_{1-2}$ -alkyl, $-C_6$ -aryl, $-C_6$ -cycloalkyl and $-R^8-C_6$ -cycloalkyl,

R^8 and R^9 being independently from each other C_{1-4} -alkyl, or

- L-E (XIV) with

L being selected from $-O-$, $-NH-$ and $-NR^7-$,

E being selected from $-C(=O)-O-R^{10}$,

R^{10} is independently selected from H or $-C_{1-4}$ -alkyl,

G of formula (XII) or (XIII) and/or R^{10} of formula (XII) or (XIV) may be optionally substituted by one or more substituents independently selected from $-F$, $-Cl$, $-Br$, $-I$, $-C_{1-2}$ -alkyl, $-O-C_{1-2}$ -alkyl, $-CH_2-C(=O)-O-(CH_2)_{1-2}$ and $-CH_2-O-C(=O)-(CH_2)_{1-2}$, and/or

R^7 of formula (XII), (XIII) or (XIV) is independently selected from $-G-E$, $-G$ or $-E$ with G and E as defined above, or

D is a phosphoester of formula (XV), with

L^1 and L^2 being independently from each other selected from $-O-$, $-NH-$ and $-NR^7-$,

R^7 being independently selected from $-G-E$, $-G$ or $-E$ with G and E as defined above,

p being 0 and q being,

R^{11} being independently selected from $-F$, $-Cl$, $-Br$, $-I$, $-C_{1-4}$ -alkyl, $-O-C_{1-4}$ -alkyl, in particular selected from $-F$, $-Cl$, C_{1-2} -alkyl, $O-C_{1-2}$ -alkyl,

m being an integer between 0 and 4, in particular 0 and 2, or

D is a phosphoester of formula (XVI) with

L^1 and L^2 being independently from each other selected from $-O-$, $-NH-$ and $-NR^7-$,

R^7 being independently selected from $-G-E$, $-G$ or $-E$ with G and E as defined above,

Z being a C_r -alkyl, optionally substituted with R^{12}_s ,

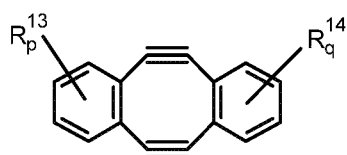
r being 3,

s being 0 or 1,

R^{12} being -G or -E as defined for formula (XIII) or (XIV) ,

in particular D is a phosphoester of formula (XI) with R^5 and R^6 being independently selected from a moiety of formula (XII) (XIII) or (XIV), more particularly D is a phosphoester of formula (XI) with R^5 and R^6 being independently selected from a moiety of formula (XII) or (XIII).

9. The kit of parts or the method according to any one of the preceding claims, wherein said counter fusion moiety Y is a carbon-carbon triple bond, wherein in particular the carbon-carbon triple bond is part of a cyclic system of said scaffold moiety B.
10. The kit of parts or the method according to any one of the preceding claims, wherein said compound BY_2 is a compound of formula (XVII),



(XVII), wherein

R^{13} and R^{14} are polar moieties that increase the solubility of dibenzocyclooctadiene in water, in particular

R^{13} and R^{14} are independently selected from

-O- R^{15} , -NH- R^{16} or -N(R^{16})₂, wherein

R^{15} and R^{16} are independently from each other selected from

-C₁₋₄-alkyl, -C₃₋₆-cycloalkyl, C₆-aryl, 7 to 10 membered bicycle, 7 to 10 membered heterobicycle, in particular -C₁₋₃-alkyl or 7 to 10 membered heterobicycle,

-C₁₋₄-alkyl- R^{17} , in particular C₁₋₃-alkyl- R^{17} ,

-C₁₋₄-alkyl- R^{18} -C₁₋₄-alkyl, in particular -C₁₋₃-alkyl- R^{18} -C₁₋₃-alkyl, and

-C₁₋₄-alkyl- R^{18} -C₁₋₄-alkyl- R^{17} , in particular -C₁₋₄-alkyl- R^{18} -C₁₋₄-alkyl- R^{17} , wherein

the C₁₋₄-alkyl-C₃₋₆-cycloalkyl or C₆-aryl may optionally be substituted by one or more substituents independently selected from -F, -Cl, -Br, -I, -OH, -O-C₁₋₂-alkyl, -NH₂,

R^{17} is selected from -NH₂, -NR¹⁹H, -N(R^{19})₂, -OH, -O-C₁₋₂-alkyl, 3 to 6 membered heterocycle, 7 to 10 membered bicycle, 7 to 10 membered heterobicycle, 5 or 6 membered heteroaryl, C₆-aryl, C₃₋₆-cycloalkyl, in

particular -NH₂, -NR¹⁹H, -N(R¹⁹)₂-OH, -O-C₁₋₂-alkyl, 3 to 6 membered heterocycle, -O-*tert*-butyldimethylsilyl,

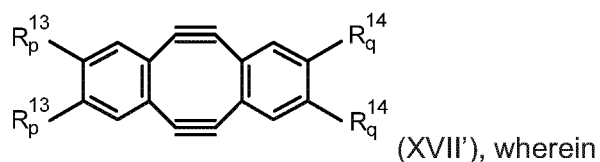
R¹⁸ is selected from -O-, -C(=O)-, -C(=O)-O-, -O-C(=O)-, -O-C(=O)-O-, -NH-, -C(=O)-NH-, -NH-C(=O)-, -O-C(=O)-NH-, -NH-C(=O)-O-,

R¹⁹ being independently selected from -C₁₋₃-alkyl, -C₁₋₃-alcohol,

and

p and q are integers between 0 and 4, in particular between 0 and 2.

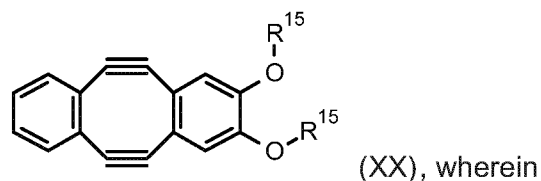
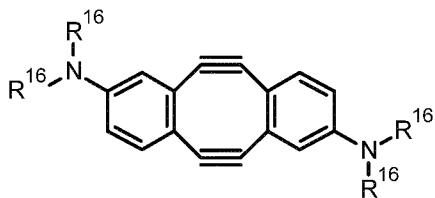
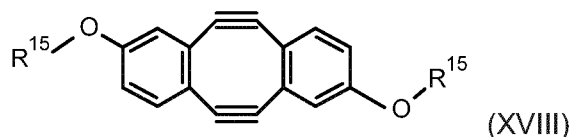
11. The kit or parts or the method according to any one of claims 9 to 10, wherein said compound BY₂ is a compound of formula (XVII'),



each R¹³ and each R¹⁴ are independently from each other defined according to any one of claims 12 to 13, and

each p and each q are independently from each other 0 or 1, in particular

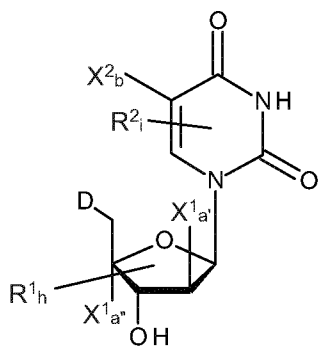
said compound BY₂ is a compound of formula (XVIII), (XIX) or (XX),



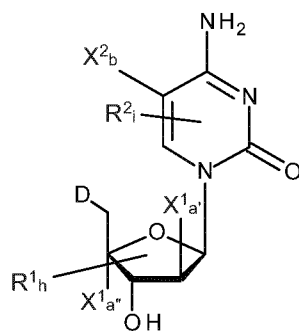
each R¹⁵ and each R¹⁶ are independently from each other defined according to any one of claims 13 to 14.

12. A combination medicament comprising the kit of parts according to claims 1 or 4 to 11 for use in a method in the treatment of a disease, wherein in particular said nucleotide analogue or said nucleoside analogue comprises a 2'-deoxyribose moiety.
13. A combination medicament comprising the kit of parts according to claims 1 or 4 to 11 for use in a method in the treatment of cancer, wherein in particular said nucleotide analogue or said nucleoside analogue comprises a 2'-deoxyribose moiety.

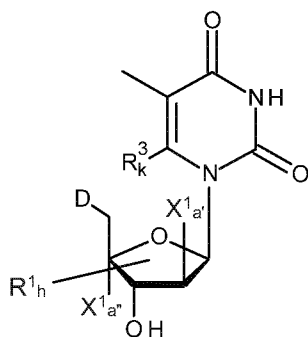
14. A compound of formula (XXI), (XXII), (XXIII), (XXIV), (XXV), (XXVI) or (XXVII), in particular (XXI), (XXII), (XXIV), (XXV), (XXVI) or (XXVII),



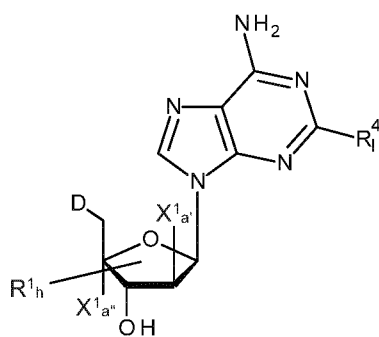
(XXI),



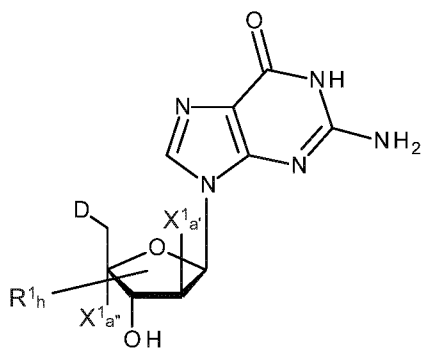
(XXII),



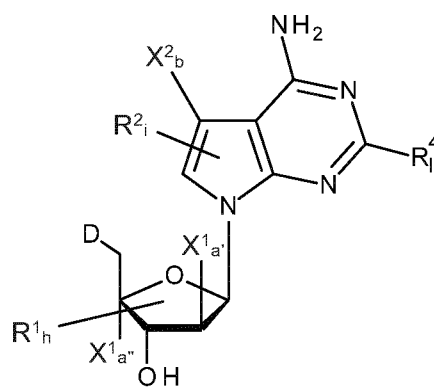
(XXIII),



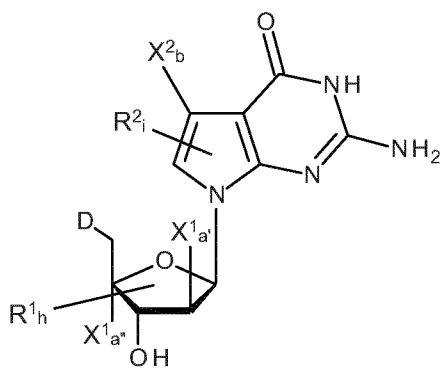
(XXIV),



(XXV),



(XXVI),



(XXVII), with

X^1 and X^2 being independently from each other selected from $-N_3$ and $-CH_2-N_3$,

a' , a'' and b being independently from each other 0 or 1, wherein the sum of a' , a'' and b is 1 or 2, in particular a' is 0, a'' is 1, and b is 0 or a' is 1, a'' is 0, and b is 0 or a' is 1, a'' is 0, and b is 1,

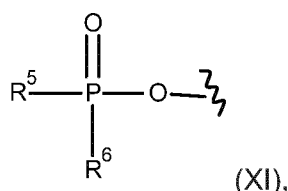
R^1 , R^2 , R^3 and R^4 are independently from each other selected from -OH, -F and -Cl, wherein in particular R^1 is -OH and R^2 , R^3 and R^4 are independently from each other selected from -F and -Cl,

h is an integer between 0 and 1, in particular 0,

i is an integer between 0 and 1, in particular 0,

k and l are independently from each other 0 or 1,

D being a phosphoester of formula (XI),



with R^5 and R^6 being independently selected from a moiety of formula (XII) or (XIII),

L-G-E (XII), with

L being selected from -O-, -NH- and -NR⁷-,

G being selected from -C₁₋₄-alkyl-, particularly -C₁₋₂-alkyl-, -C₆-aryl-, -R⁸-C₆-aryl-, -C₆-cycloalkyl and -R⁸-C₆-cycloalkyl,

R⁸ being C₁₋₄-alkyl, in particular C₁₋₂-alkyl,

E being selected from -O-C(=O)-O-R¹⁰, -O-C(=O)-R¹⁰, -C(=O)-O-R¹⁰, -S-S-R¹⁰, -S-C(=O)-O-R¹⁰ and -NO₂,

R¹⁰ is independently selected from H or -C₁₋₆-alkyl, or

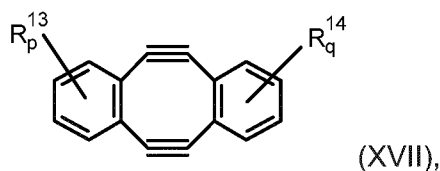
L-G (XIII), with

L being selected from -O-, -NH- and -NR⁷-,

G being selected from -C₁₋₆-alkyl, in particular -C₁₋₂-alkyl, -C₆-aryl-, -R⁸-C₆-aryl-, -C₆-cycloalkyl and -R⁸-C₆-cycloalkyl,

R⁸ being C₁₋₄-alkyl.

15. A compound of formula (XVII),



wherein

R^{13} and R^{14} are independently selected from

$-O-R^{15}$, $-NH-R^{16}$ or $-N(R^{16})_2$, wherein

R^{15} and R^{16} are independently from each other selected from

$-C_{1-4}$ -alkyl, $-C_{3-6}$ -cycloalkyl, C_6 -aryl, 7 to 10 membered bicycle, 7 to 10 membered heterobicycle, in particular $-C_{1-3}$ -alkyl or 7 to 10 membered heterobicycle,

$-C_{1-4}$ -alkyl- R^{17} , in particular $-C_{1-3}$ -alkyl- R^{17} ,

$-C_{1-4}$ -alkyl- R^{18} - C_{1-4} -alkyl, in particular $-C_{1-3}$ -alkyl- R^{18} - C_{1-3} -alkyl, and

$-C_{1-4}$ -alkyl- R^{18} - C_{1-4} -alkyl- R^{17} , in particular $-C_{1-3}$ -alkyl- R^{18} - C_{1-3} -alkyl- R^{17} , wherein

the C_{1-4} -alkyl- C_{3-6} -cycloalkyl or C_6 -aryl may optionally be substituted by one or more substituents independently selected from $-F$, $-Cl$, $-Br$, $-I$, $-OH$, $-O-C_{1-2}$ -alkyl, $-NH_2$,

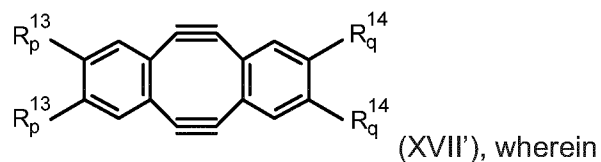
R^{17} is selected from $-NH_2$, $-NR^{19}H$, $-N(R^{19})_2$, $-OH$, $-O-C_{1-2}$ -alkyl, 3 to 6 membered heterocycle, 7 to 10 membered bicycle, 7 to 10 membered heterobicycle, 5 or 6 membered heteroaryl, C_6 -aryl, C_{3-6} -cycloalkyl, in particular $-NH_2$, $-NR^{19}H$, $-N(R^{19})_2$, $-OH$, $-O-C_{1-2}$ -alkyl, 3 to 6 membered heterocycle,

R^{18} is selected from $-O-$, $-C(=O)-$, $-C(=O)-O-$, $-O-C(=O)-$, $-O-C(=O)-O-$, $-NH-$, $-C(=O)-NH-$, $-NH-C(=O)-$, $-O-C(=O)-NH-$, $-NH-C(=O)-O-$,

R^{19} being independently selected from $-C_{1-3}$ -alkyl, $-C_{1-3}$ -alcohol

wherein at least one residue R^{13} and/or R^{14} , in particular all residues R^{13} and/or R^{14} , comprises a residue R^{17} selected from $-NH_2$, $-NR^{19}H$, $-N(R^{19})_2$ and/or R^{18} selected from $-NH-$, $-C(=O)-NH-$, $-NH-C(=O)-$, $-O-C(=O)-NH-$, $-NH-C(=O)-O-$, in particular

a compound of formula (XVII'),

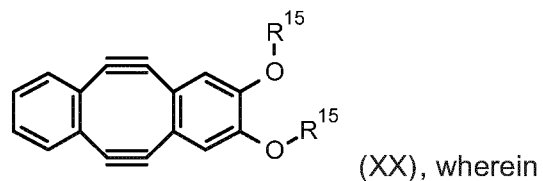
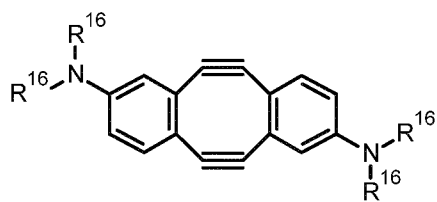
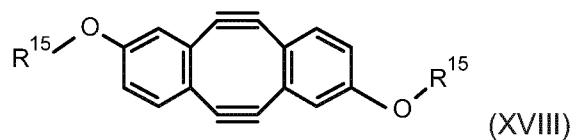


each R^{13} and each R^{14} are independently from each other defined as described above, and

each p and each q are independently from each other 0 or 1

wherein at least one residue R^{13} and/or R^{14} , in particular all residues R^{13} and/or R^{14} , comprises a residue R^{17} selected from $-NH_2$, $-NR^{19}H$, $-N(R^{19})_2$ and/or R^{18} selected from $-NH-$, $-C(=O)-NH-$, $-NH-C(=O)-$, $-O-C(=O)-NH-$, $-NH-C(=O)-O-$, more particularly

a compound of formula (XVIII), (XIX) or (XX),



each R^{13} and each R^{14} are independently from each other defined as described above.

Fig. 1

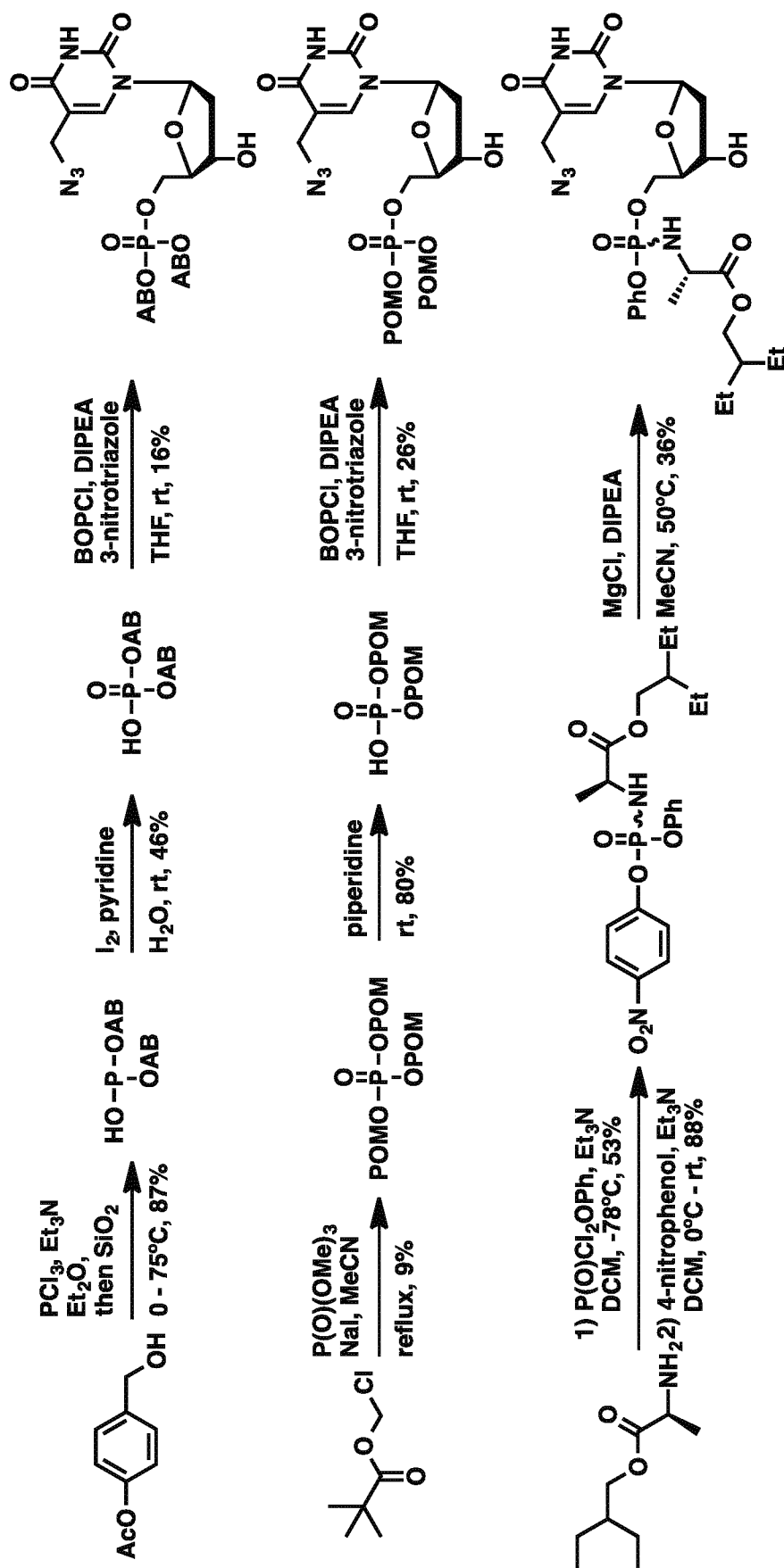
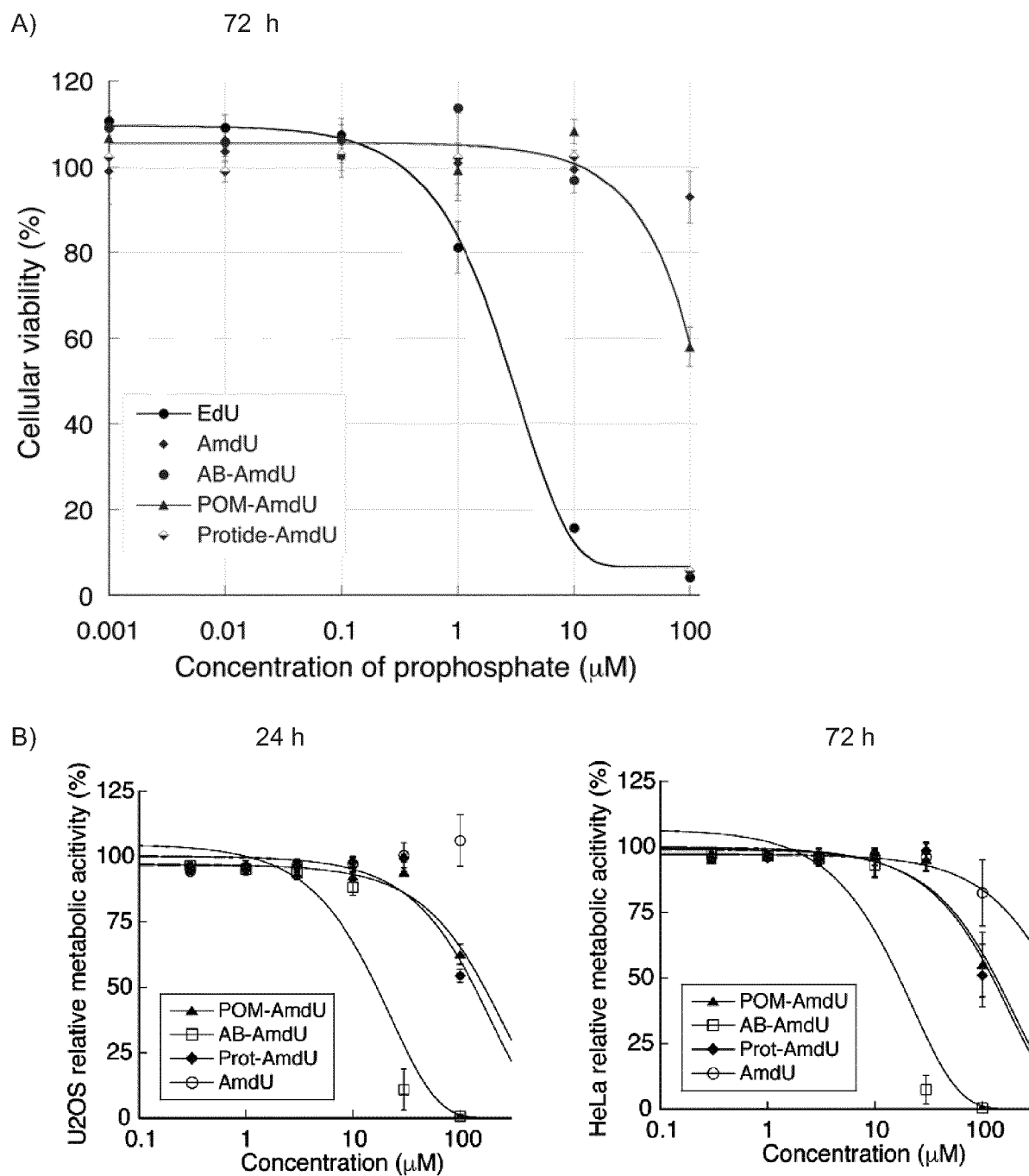


Fig. 2



C)

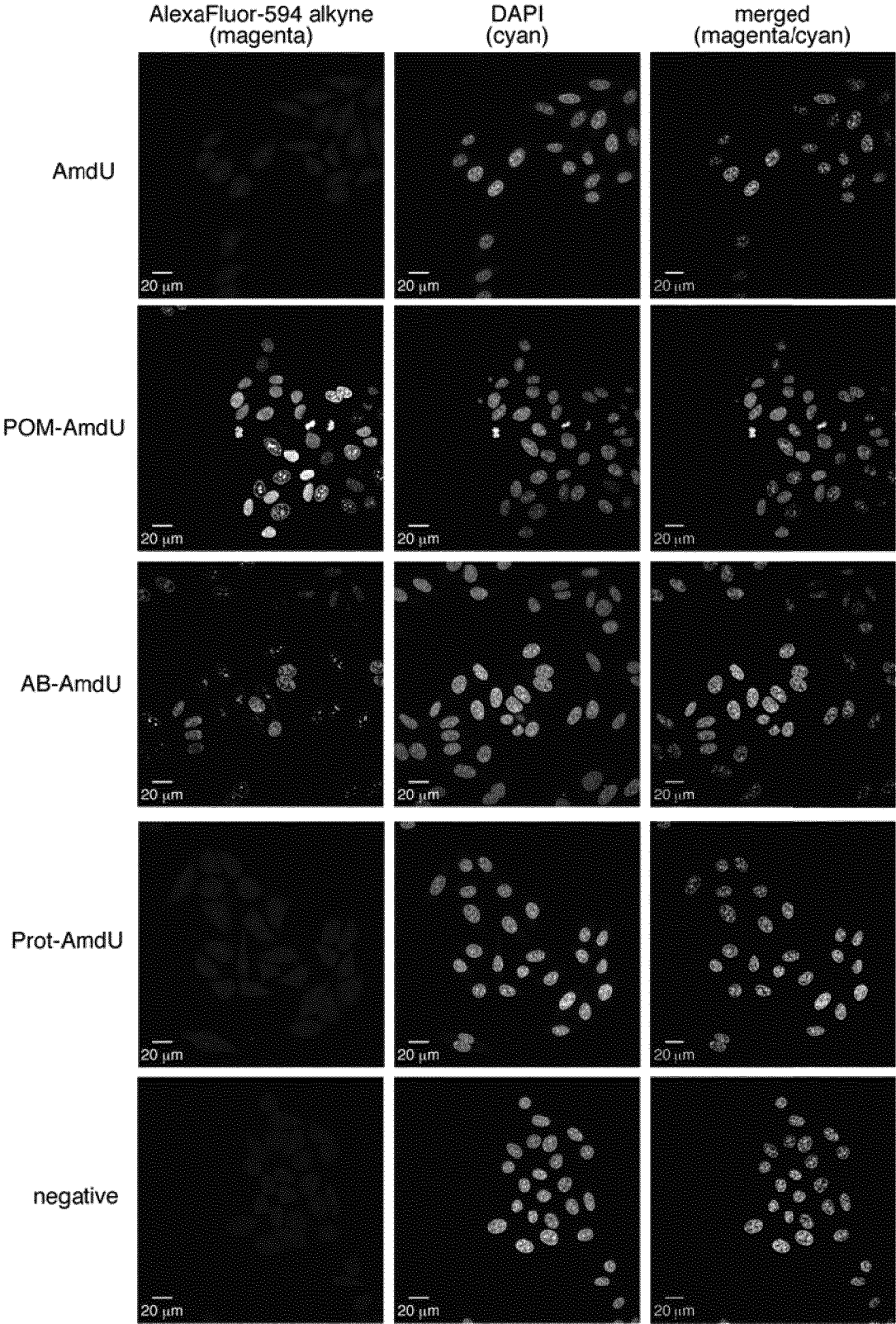


Fig. 3

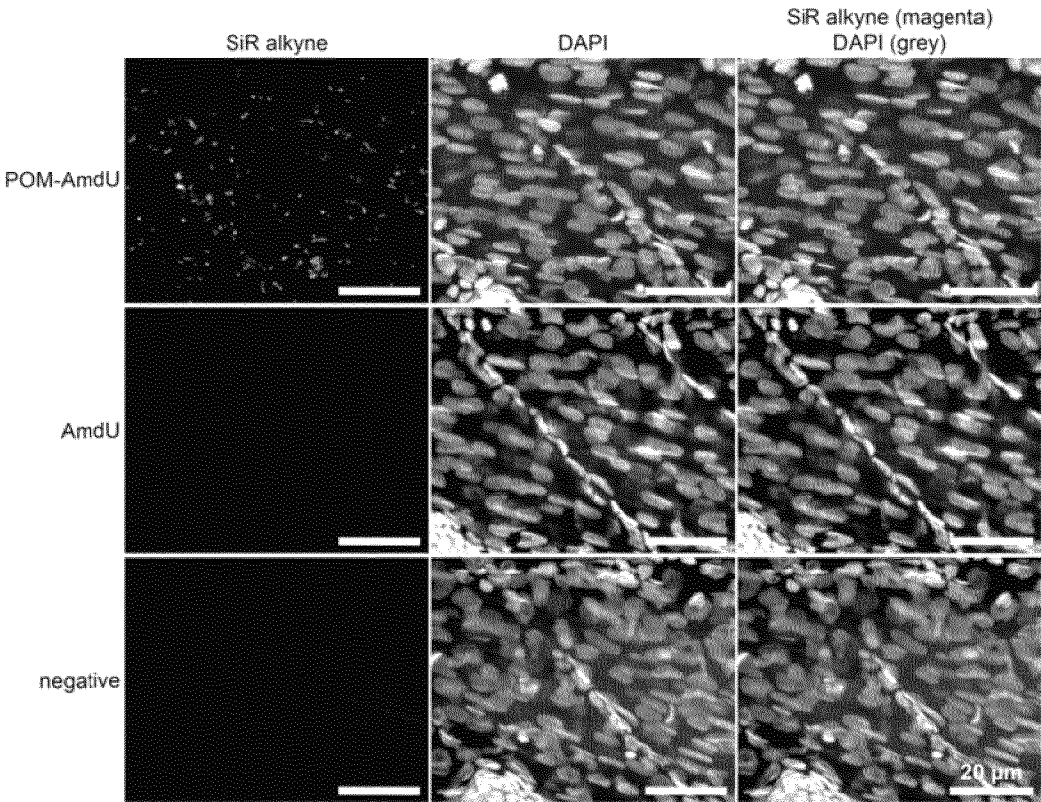


Fig. 4

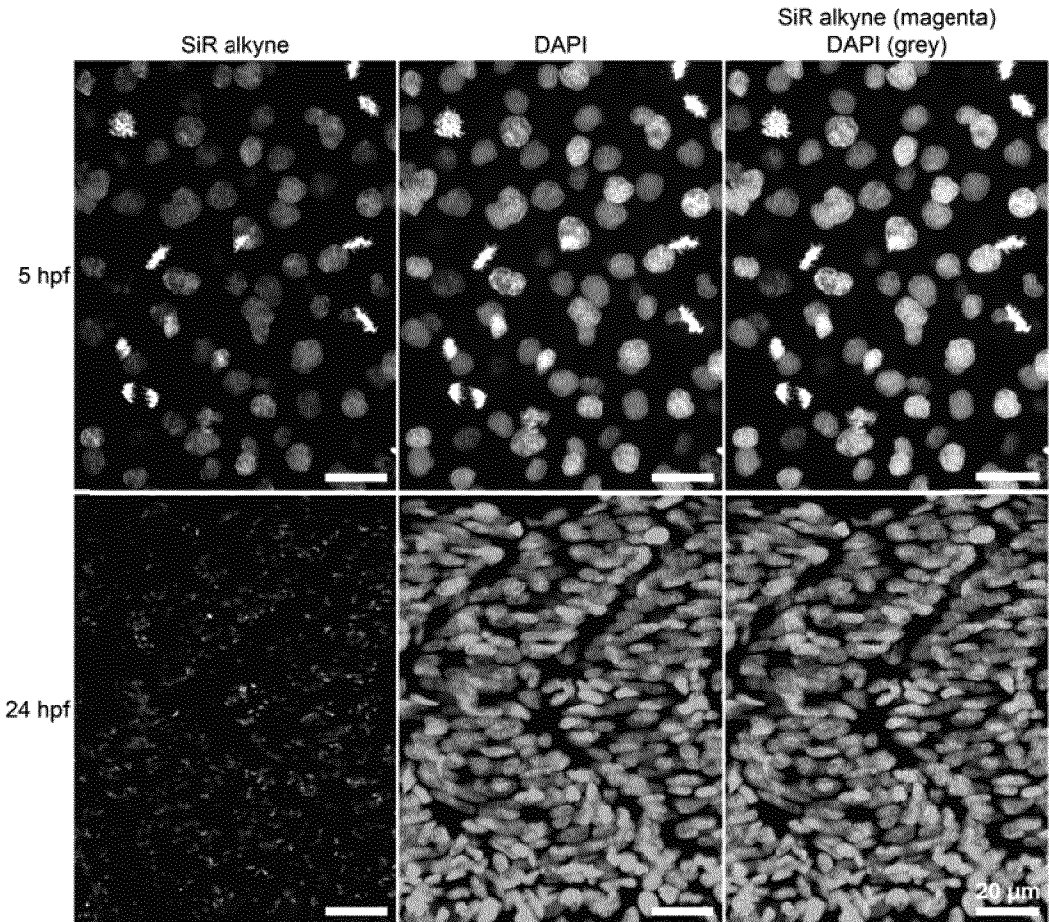
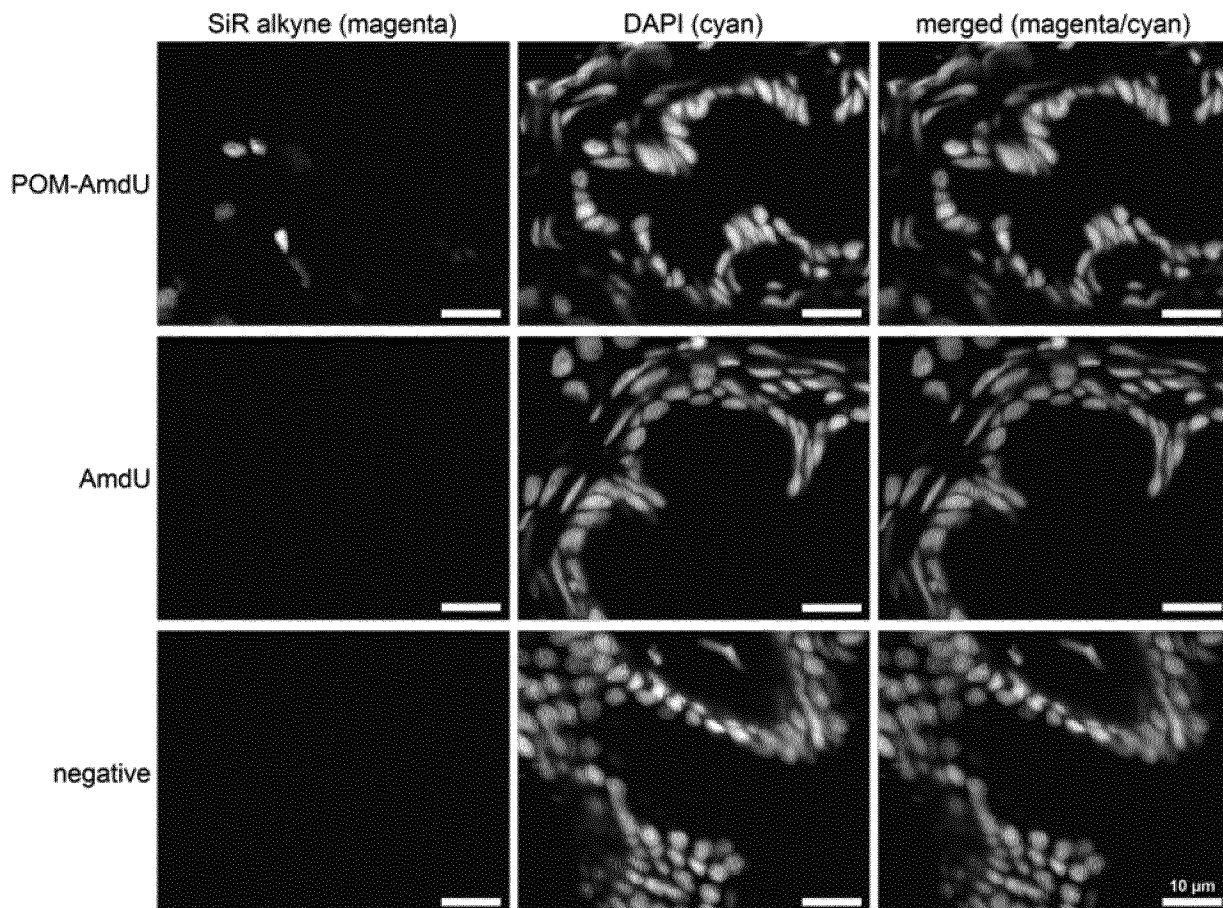


Fig. 5



AmdU

CODY

trans-product

MeOH, rt

AU

Minutes

COc1cc(C=O)cc(C=O)c1 $\xrightarrow{\text{MeI, K}_2\text{CO}_3, \text{NBS, AIBN, PhSO}_2\text{Na, DIBAL-H}}$ COc1cc(C=O)cc(C=O)c1 $\xrightarrow{\text{LHMDS, THF, -78}^\circ\text{C, 20\% each product}}$ COc1cc(C=O)cc(C=O)c1 $\xrightarrow{\text{1) BBr}_3, \text{DCM; 2) MOM-Cl, DIPEA, DCM; 3) LDA, THF, -78}^\circ\text{C}}$ COc1cc(C=O)cc(C=O)c1 $\xrightarrow{\text{1) morpholine; 2) LDA, -78}^\circ\text{C}}$ COc1cc(C=O)cc(C=O)c1

Reagents and conditions for the synthesis of the macrocyclic compound:

- 1) $\text{MeI, K}_2\text{CO}_3$ 2) NBS, AIBN 3) PhSO_2Na 4) DIBAL-H
- LHMDS, THF, -78°C , 20% each product
- 1) BBr_3, DCM 2) $\text{MOM-Cl, DIPEA, DCM}$ 3) $\text{LDA, THF, -78}^\circ\text{C}$
- 1) morpholine 2) $\text{LDA, -78}^\circ\text{C}$

The final product is a macrocyclic compound, DI-Mor-CODY (DIMOC), with a LogP of 1.34.

Fig. 8

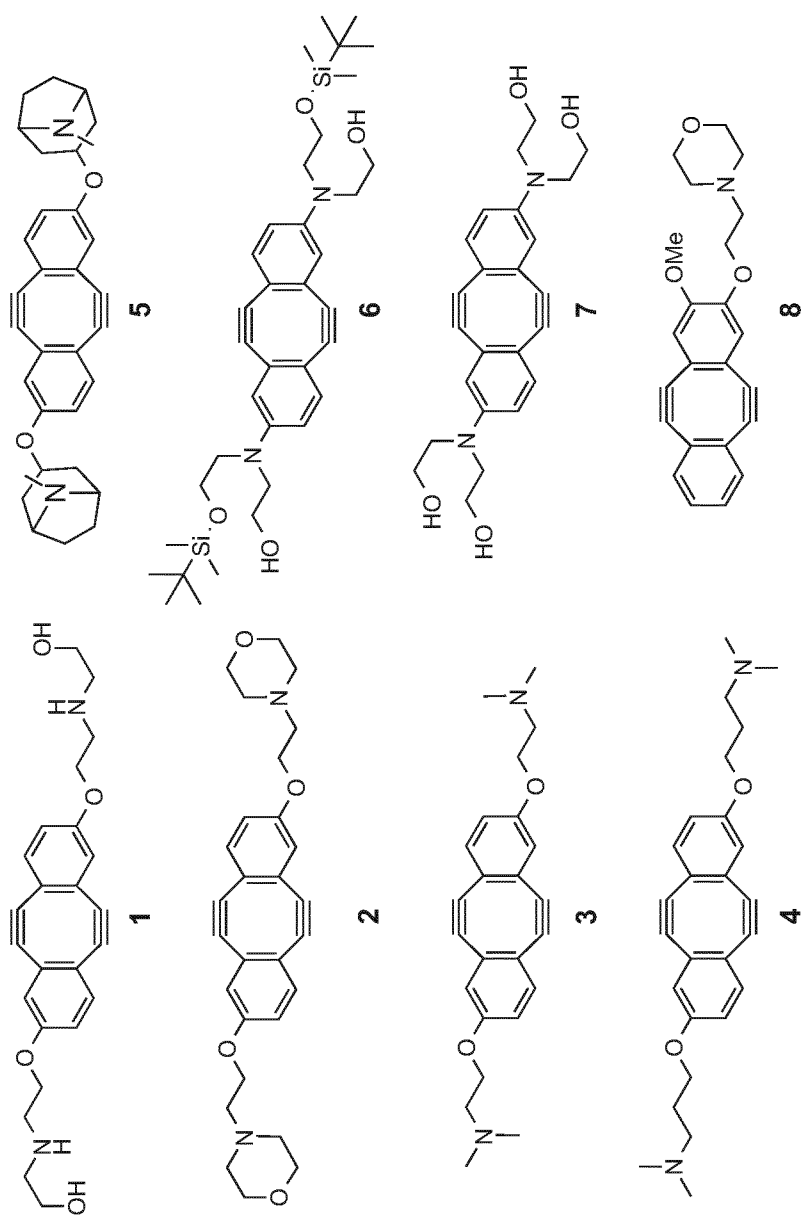


Fig. 9

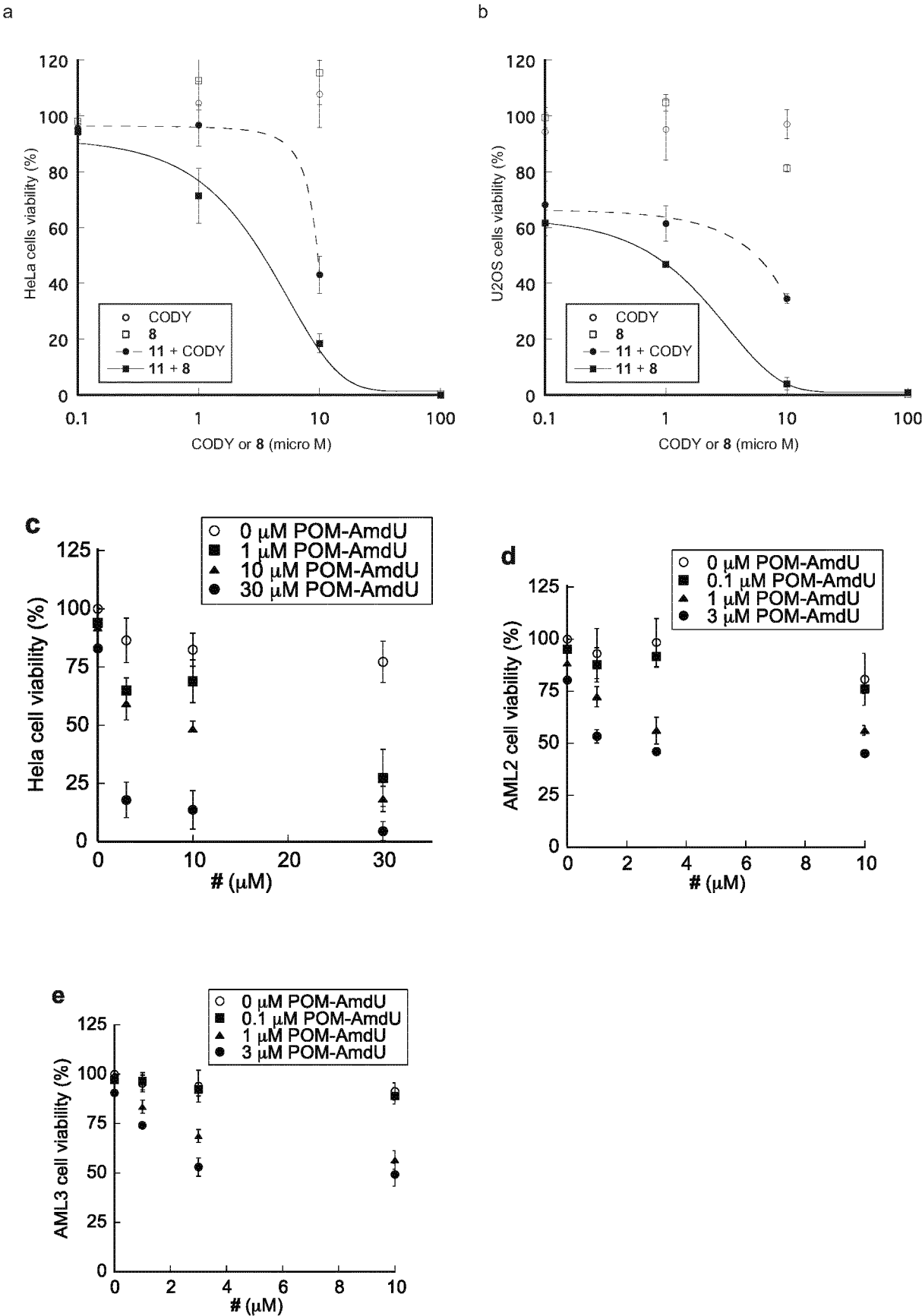


Fig. 10

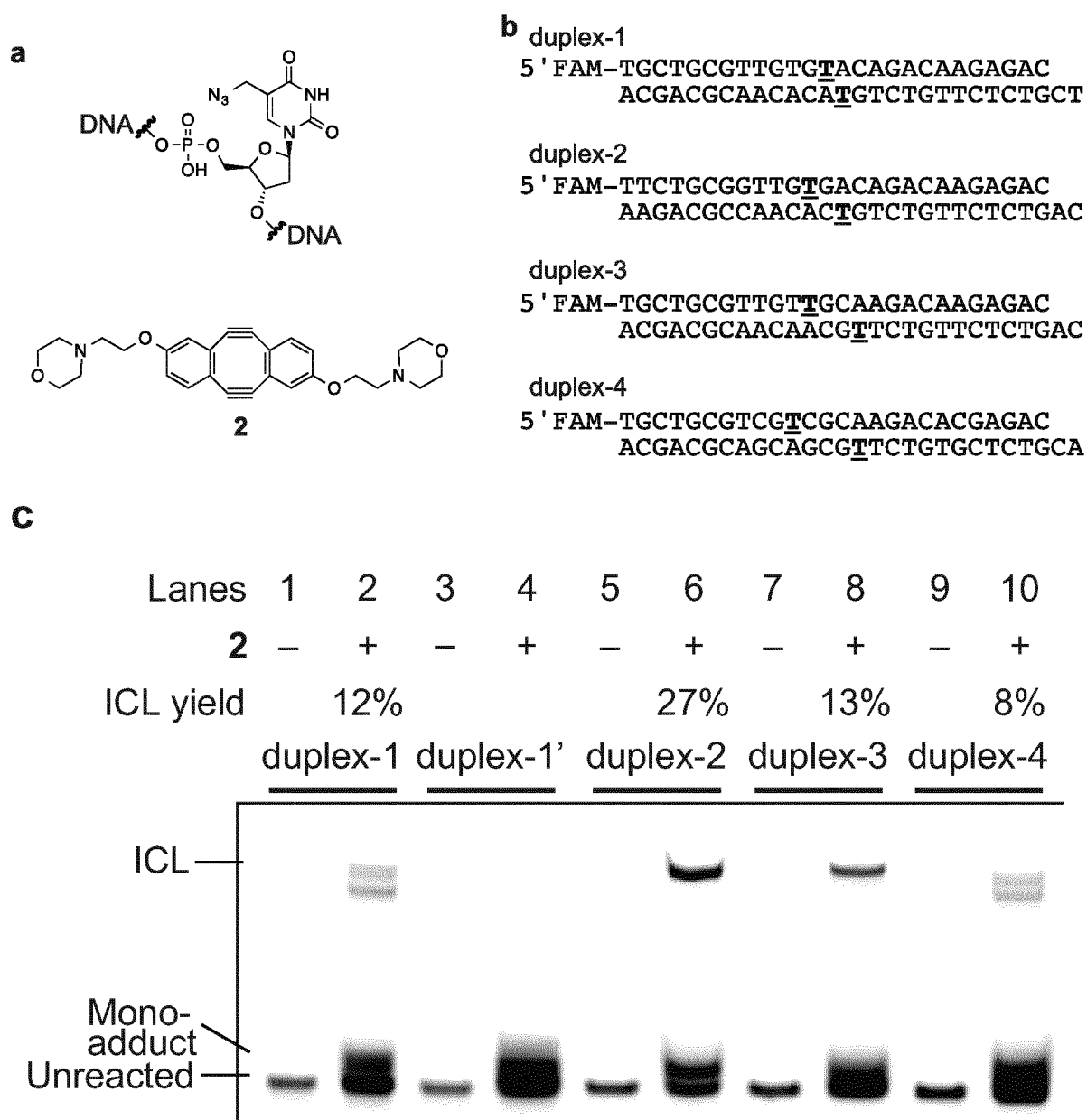


Fig. 10d, e, f

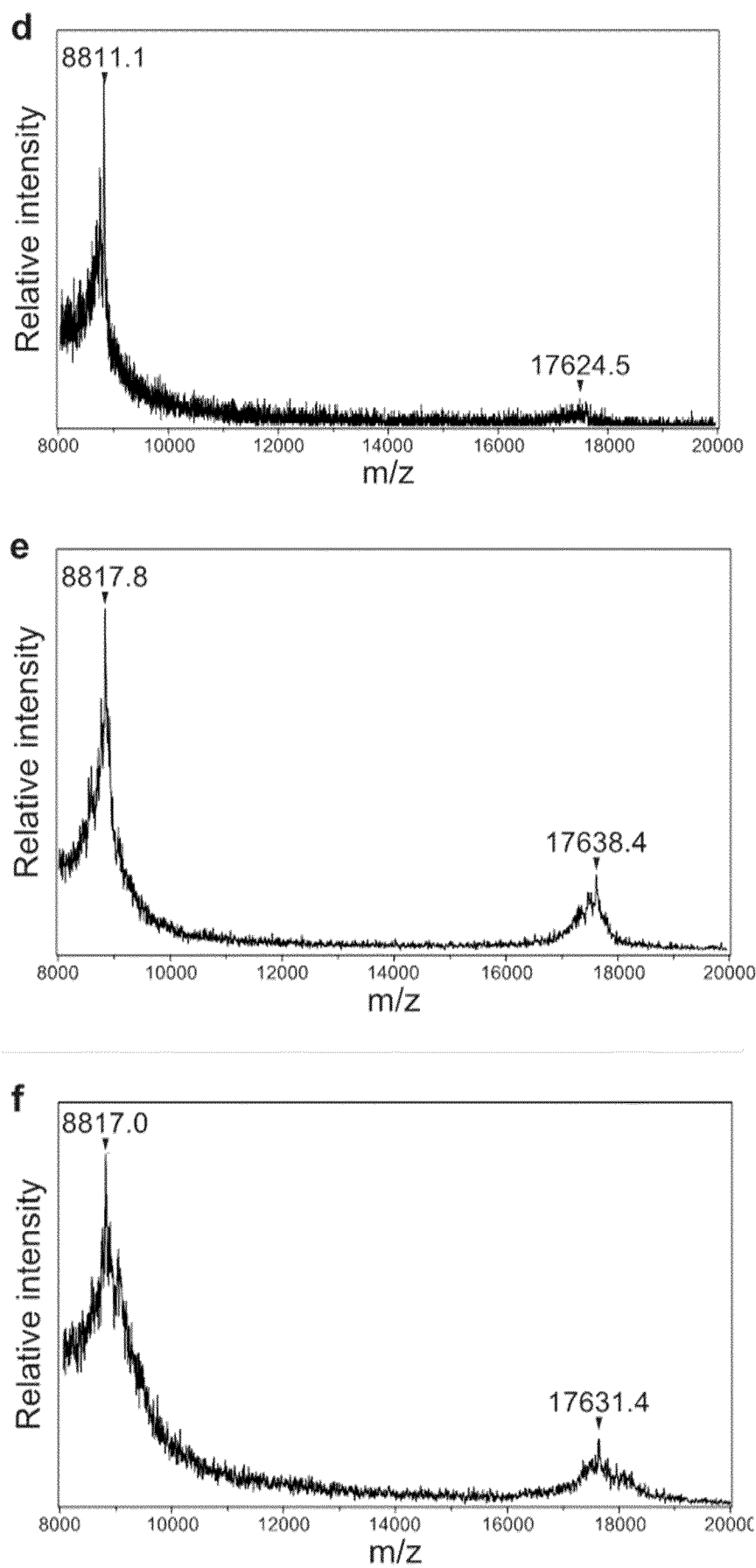
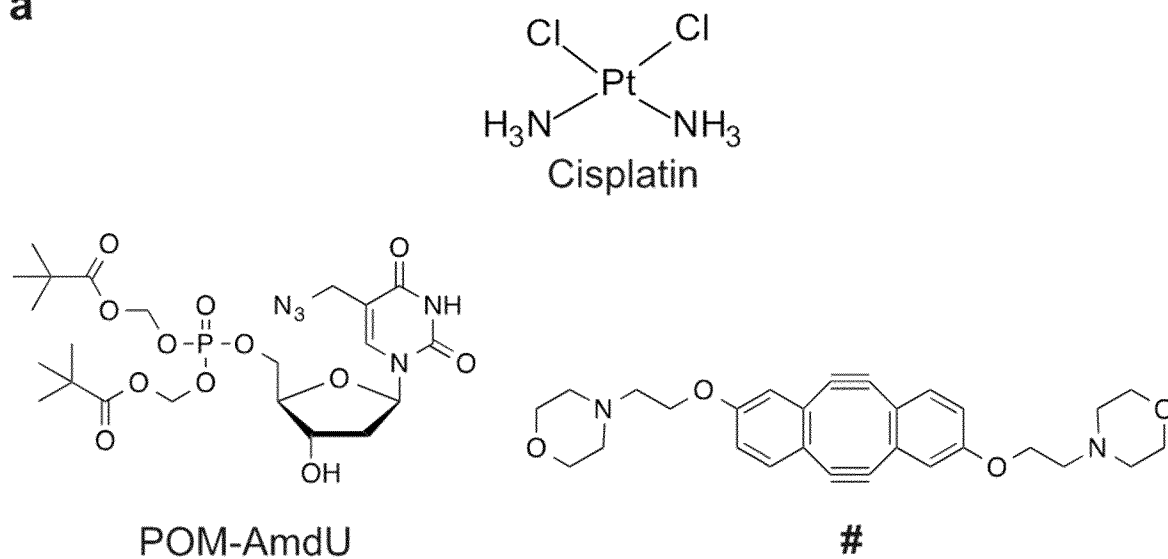


Fig. 11a, b

a**b**

	1	2	3	4	5	6	7	8	9	10	11	12
Con.:	neg.		POM-AmdU		#		Cisplatin 20 μ M		Cisplatin 100 μ M		POM-AmdU then #	
S1:	—	+	—	+	—	+	—	+	—	+	—	+

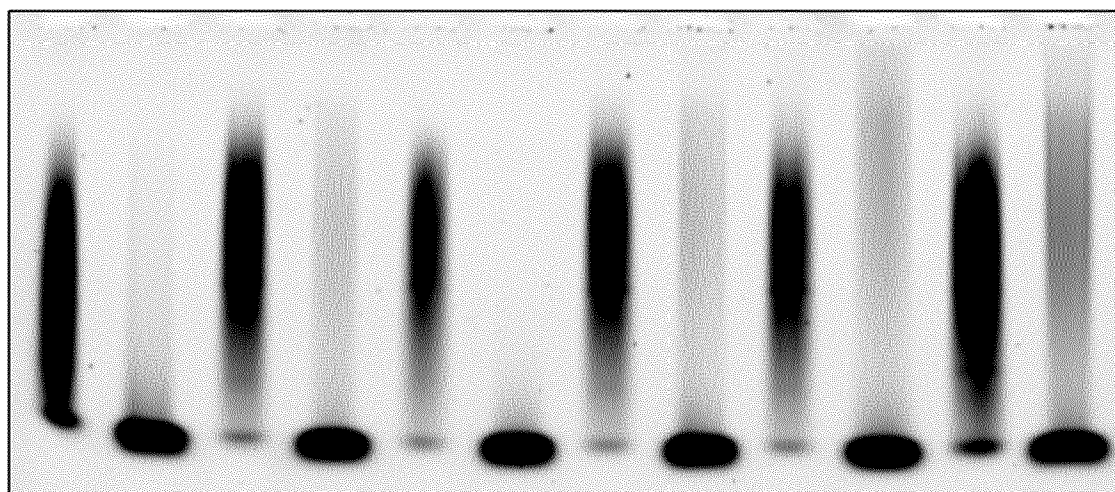


Fig. 12

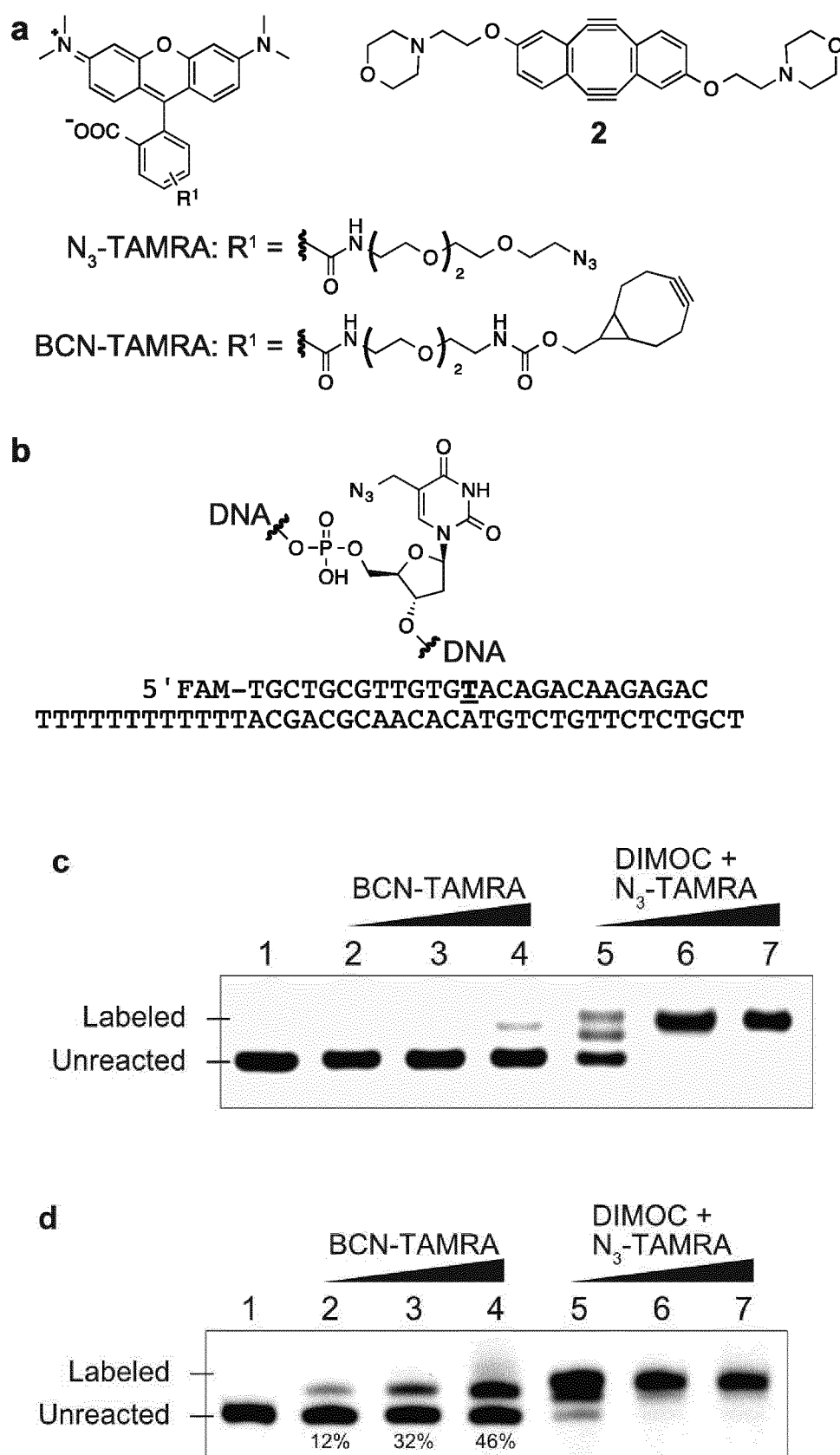


Fig. 12

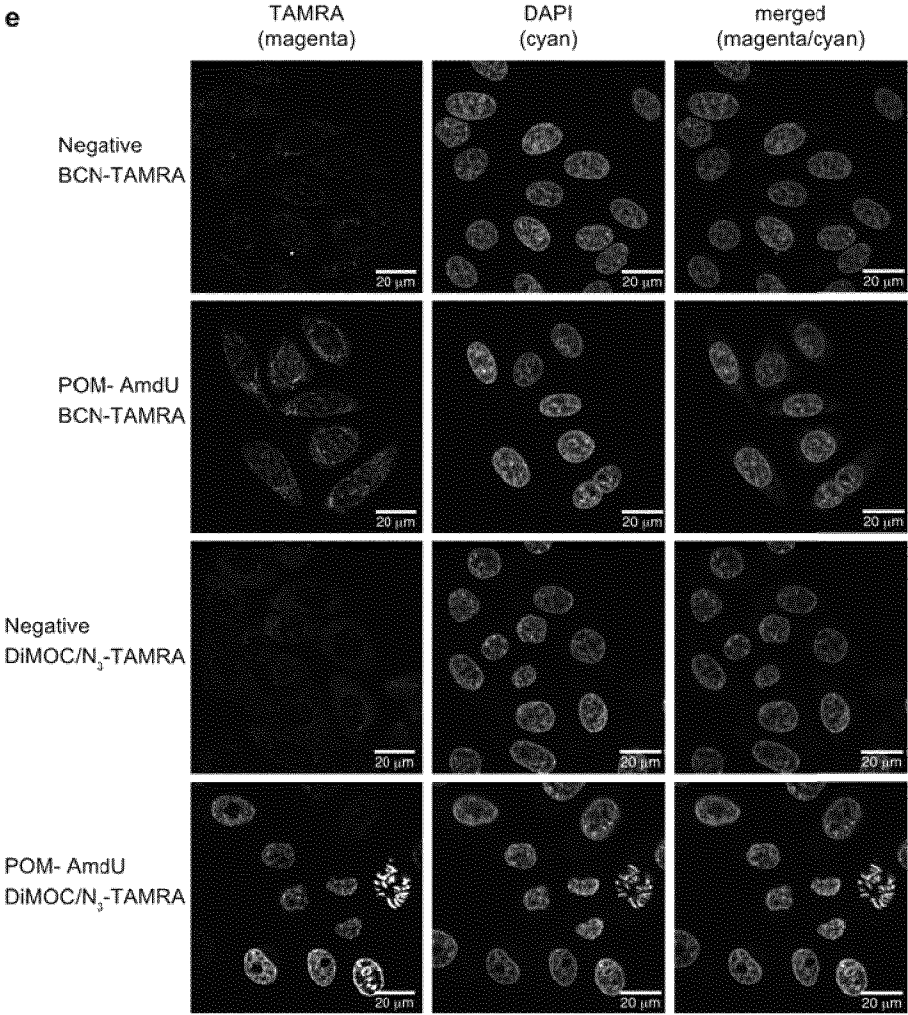


Fig. 13

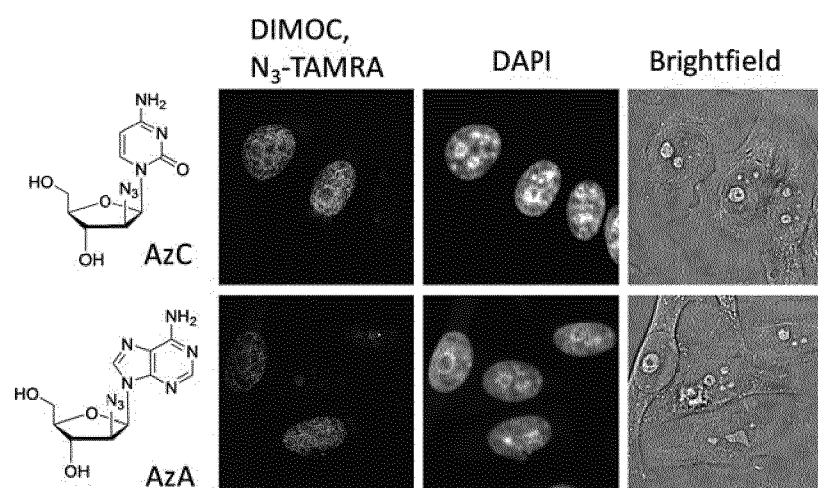


Fig. 14

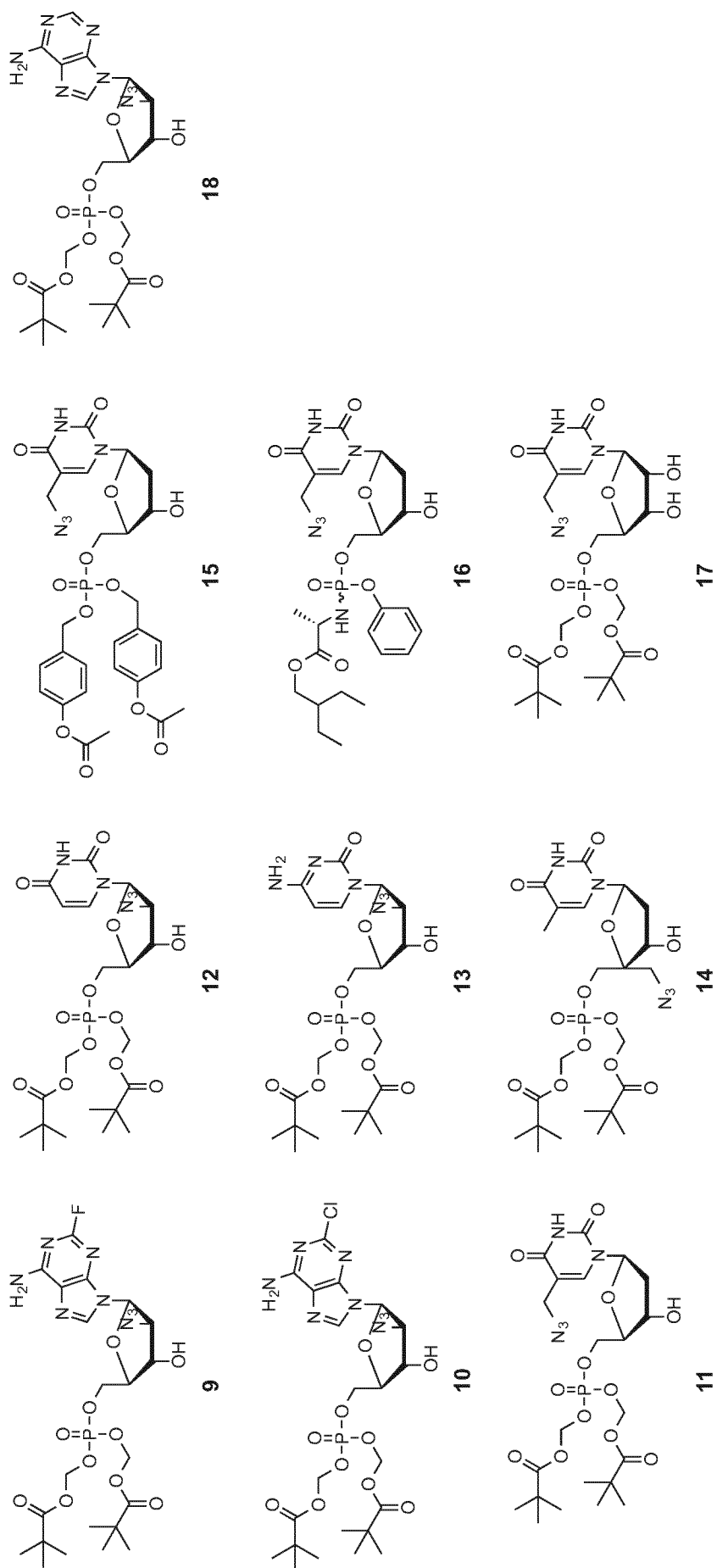


Fig. 15

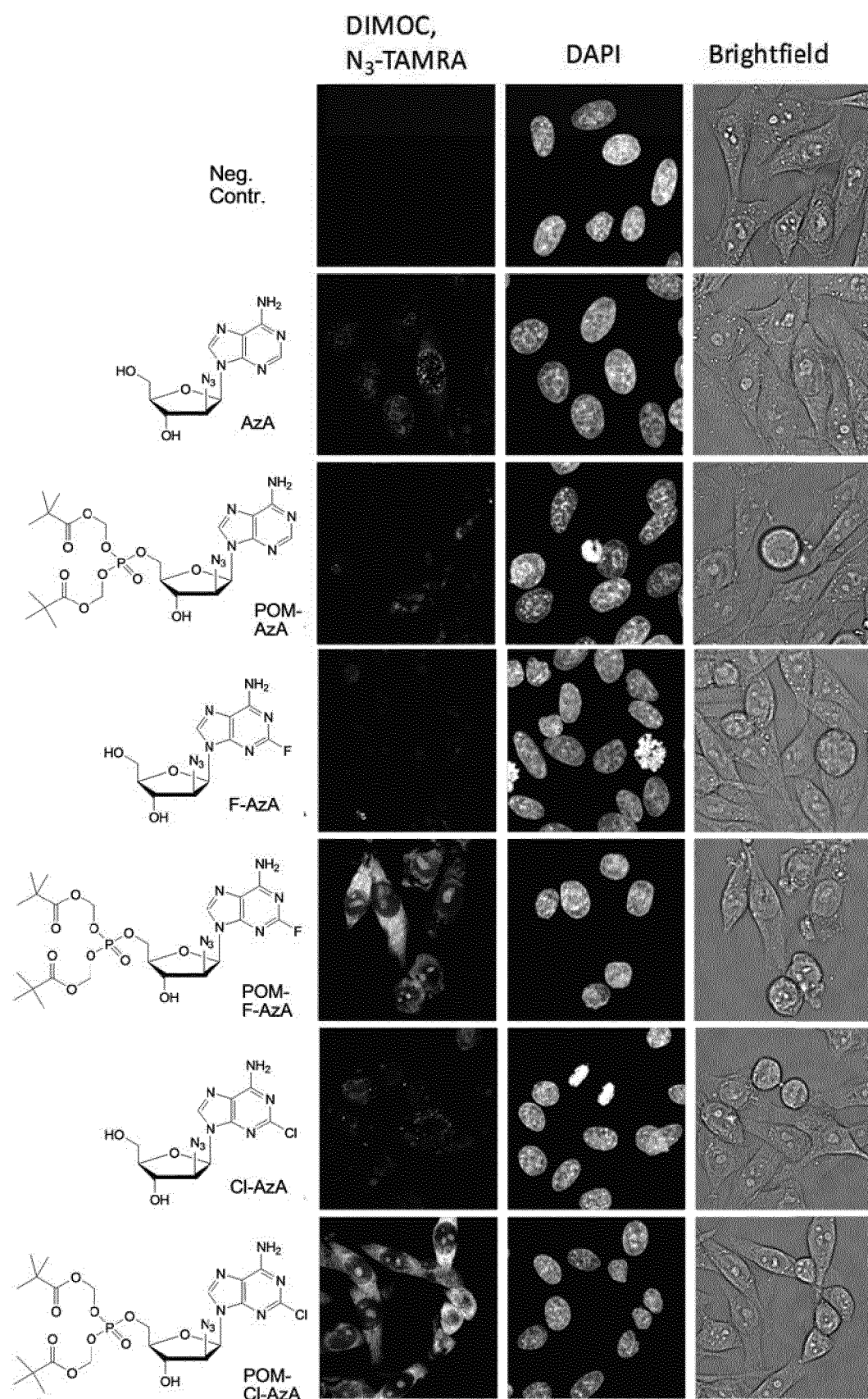


Fig. 16

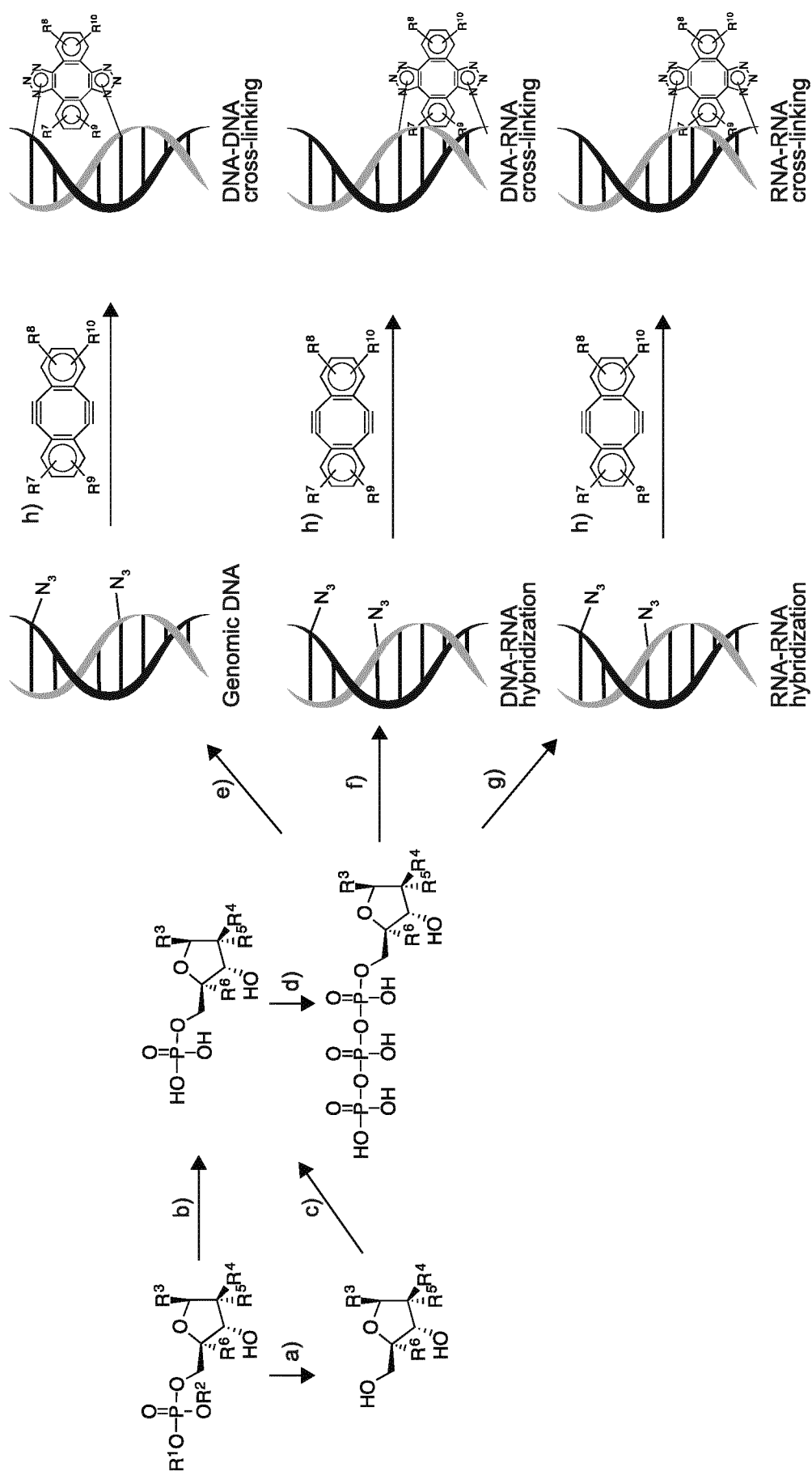
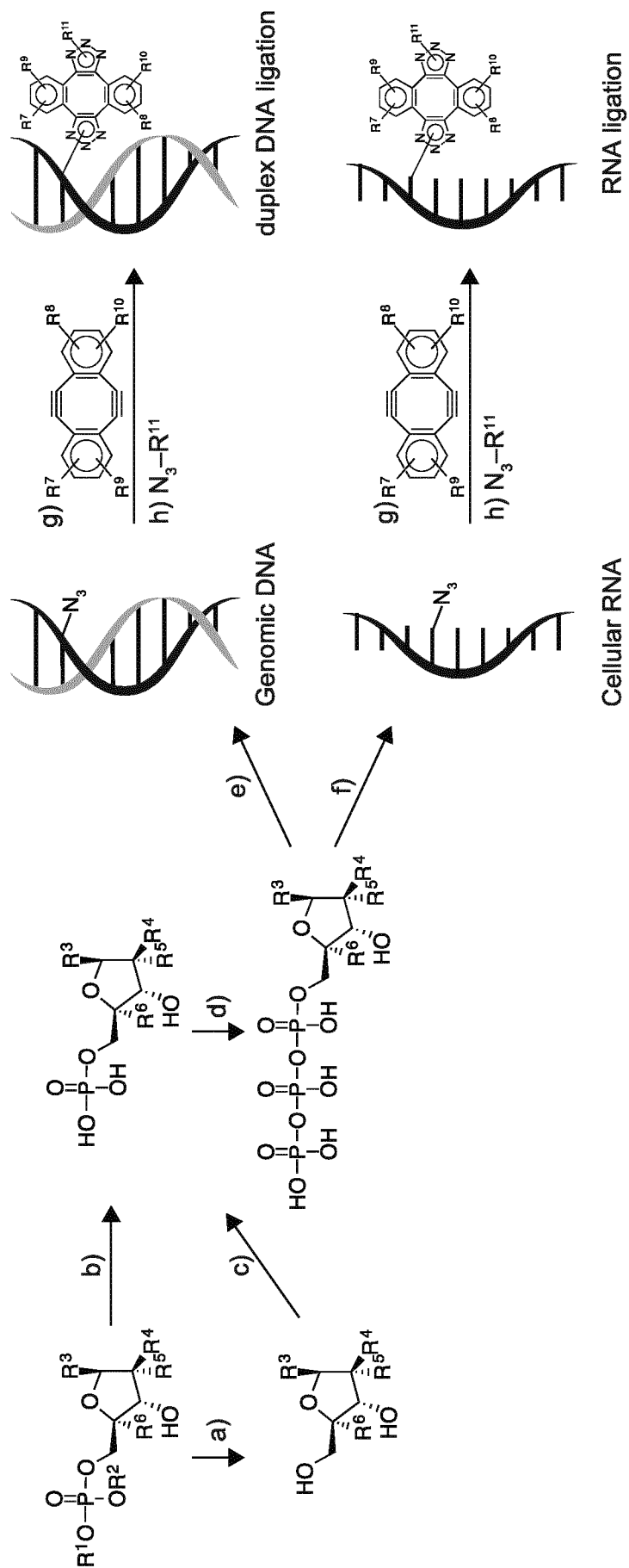


Fig. 17



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/067074

A. CLASSIFICATION OF SUBJECT MATTER		
INV.	C07H1/00 C07H21/04 C07H21/02 C07H19/067 C07H19/073	
	C07H19/167 C07H19/173 A61K31/7052 A61P35/00	
ADD.		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols)		
C07H A61K A61P		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
EPO-Internal, CHEM ABS Data, WPI Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	MASAYUKI TERA ET AL: "In Vivo Incorporation of Azide Groups into DNA by Using Membrane-Permeable Nucleotide Triesters", CHEMBIOCHEM, vol. 19, no. 18, 28 June 2018 (2018-06-28) , pages 1939-1943, XP055589828, ISSN: 1439-4227, DOI: 10.1002/cbic.201800351 scheme 1 ----- -/--	14
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search		Date of mailing of the international search report
25 July 2019		08/08/2019
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Klein, Didier

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2019/067074

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DEWEY A. SUTTON ET AL: "Cyclopropanone-caged Sondheimer diyne (dibenzo[a,e]cyclooctadiyne): a photoactivatable linchpin for efficient SPAAC crosslinking", CHEMICAL COMMUNICATIONS, vol. 52, no. 3, 1 January 2016 (2016-01-01), pages 553-556, XP055589528, ISSN: 1359-7345, DOI: 10.1039/C5CC08106H scheme 1 -----	15
X	SURESH S. PUJARI ET AL: "Cross-Linked DNA Generated by "Bis-click" Reactions with Bis-functional Azides: Site Independent Ligation of Oligonucleotides via Nucleobase Alkynyl Chains", THE JOURNAL OF ORGANIC CHEMISTRY, vol. 75, no. 24, 11 November 2010 (2010-11-11), pages 8693-8696, XP055031965, ISSN: 0022-3263, DOI: 10.1021/jo101809w the whole document -----	1-4
X	Michael E. Harris ET AL: "RNA Crosslinking Methods" In: "METHODS IN ENZYMOLOGY", 1 January 2009 (2009-01-01), ACADEMIC PRESS, US, XP055590325, ISSN: 0076-6879 vol. 468, pages 127-146, DOI: 10.1016/S0076-6879(09)68007-1, the whole document -----	1-4
A	ANNE B. NEEF ET AL: "An Azide-Modified Nucleoside for Metabolic Labeling of DNA", CHEMBIOCHEM, vol. 15, no. 6, 14 April 2014 (2014-04-14) , pages 789-793, XP055451708, ISSN: 1439-4227, DOI: 10.1002/cbic.201400037 cited in the application Scheme 1 -----	1-15
A	US 2013/011901 A1 (HOSOYA TAKAMITSU [JP] ET AL) 10 January 2013 (2013-01-10) cited in the application the whole document in particular page 7 right-hand column lines 1-6 ----- -/--	1-15

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2019/067074

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SARAH NAINAR ET AL: "Metabolic Incorporation of Azide Functionality into Cellular RNA", CHEMBIOCHEM, vol. 17, no. 22, 17 November 2016 (2016-11-17), pages 2149-2152, XP055589868, ISSN: 1439-4227, DOI: 10.1002/cbic.201600300 the whole document -----	1-15
T	MASAYUKI TERA ET AL: "Intercalation-enhanced "Click" Crosslinking of DNA", ANGEWANDTE CHEMIE, INTERNATIONAL EDITION, vol. 57, no. 47, 19 November 2018 (2018-11-19), pages 15405-15409, XP055589511, DE ISSN: 1433-7851, DOI: 10.1002/anie.201808054 the whole document -----	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2019/067074

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2013011901 A1	10-01-2013	JP 5741572 B2	01-07-2015
		JP W02011118394 A1	04-07-2013
		US 2013011901 A1	10-01-2013
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