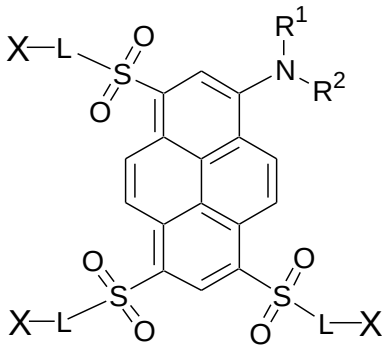
functionally substituted 1,6,8-trisulfonamido-3-aminopyrenes (APTS derivatives), an analyte-reactive group, a cleavable anchor as well as a porous solid phase. WO 2010/116142 A2 describes a large variety of fluorophores and fluorescent sensors compounds which also encompass aminopyrene-based dyes. However, none of these dyes has been shown or suggested to have superior spectral and electrophoretic properties, in particular as conjugates with carbohydrates, in comparison with APTS.

In view of the drawbacks of the fluorescent dyes of the prior art, the main objective of the present invention was to provide novel fluorescent dyes with improved properties, such as even higher electrophoretic mobility and/or higher brightness or other favorable spectroscopic characteristics, as compared to APTS. These properties are highly demanded from fluorescent tags for carbohydrate analysis based on electrokinetic separation with fluorescence detection and thus would provide a superior performance, here.

This objective has been achieved by providing novel fluorescent acridone and pyrene dyes with multiple negatively charged groups according to claims 1-14, as well as the use of the disclosed novel fluorescent dyes according to, e.g., claim 18. Further aspects and more specific embodiments of the invention are the subject of further claims.

Summary

In an aspect of the present disclosure, there is provided a fluorescent dye having multiple ionizable and/or negatively charged groups which is selected from the group consisting of compounds of the following general Formulae B and A or of salts thereof:



Formula B

wherein R^1 and/or R^2 are independent from each other and may represent:

H, deuterium, alkyl or deutero-substituted alkyl, wherein one, several or all H atoms in the alkyl group may be replaced by deuterium atoms, in particular alkyl or deutero-substituted alkyl with 1-12 C atoms, preferably 1-6 C atoms, e.g. CH_3, C_2H_5, a linear or branched C_3-C_{12} alkyl or perfluoroalkyl group, or a substituted C_2–C_{12} alkyl group; in particular, $(CH_2)_nCOOR^3$, where n = 1-12, preferably 1-5, R^3 may be H, alkyl, in particular C_1-C_6, CH_2CN, benzyl, fluorene-9-yl, polyhalogenoalkyl, polyhalogenophenyl, *e.g.* tetra- or pentafluorophenyl, pentachlorophenyl, 2- and 4-nitrophenyl, *N*-succinimidyl, sulfo-*N*-succinimidyl, 1-oxybenzotriazolyl, or other potentially nucleophile-reactive leaving groups, and the alkyl chain in $(CH_2)_n$ may be straight or branched; and

R^1---R^2 may form a four-, five, six-, or seven-membered non-aromatic carbocycle with an additional primary amino group NH_2, secondary amino group NHR^a, where R^a = C_1–C_6 alkyl, or hydroxyl group OH attached to one of the carbon atoms in this cycle; optionally R^1---R^2 may form a four-, five, six-, or seven-membered non-aromatic heterocycle with an additional heteroatom such as O, N or S included into this heterocycle;

a hydroxyalkyl group $(CH_2)_mOH$, where m = 1-12, preferably 2–6, with a straight or branched alkyl chain;

one of R^1 or R^2 groups may be a carbonate or carbamate derivative $(CH_2)_mOCOOR^4$ or $(CH_2)_mNHCOOR^4$, where m = 1-12 and R^4 = methyl,

ethyl, 2-chloroethyl, *N*-succinimidyl, sulfo-*N*-succinimidyl, 1-oxybenzotriazolyl, a phenyl group or substituted phenyl group, e.g., 2- and 4-nitrophenyl, pentachlorophenyl, pentafluorophenyl, 2,3,5,6-tetrafluorophenyl, 2-pyridyl, or 4-pyridyl;

$(CH_2)_mNR^aR^b$, where m = 1-12, preferably 2-6, with a straight or branched alkyl chain; R^a, R^b are independent from each other and may be H, or optionally substituted C_1–C_4 alkyl group(s), in particular, one of R^1 or R^2 groups may be an alkyl azide group $(CH_2)_mN_3$ with m = 2-6 and a straight or branched alkyl chain; one of R^1 or R^2 may be $(CH_2)_nSO_2NR^5NH_2$ with n = 1-12, while the substituent R^5 can be represented by H, alkyl, hydroxyalkyl or perfluoroalkyl groups C_1-C_{12};

one of R^1 or R^2 groups may be a primary amino group to form aryl hydrazines Ar-NR^6NH_2 where Ar is the entire pyrene residue in Formula B and R^6 = H or alkyl;

one of R^1 or R^2 groups may be a hydroxy group to form aryl hydroxylamines Ar-NR^7OH where Ar is the entire pyrene residue in Formula B and R^7 = H or alkyl;

one of R^1 or R^2 groups may be an alkyloxyamino group $(CH_2)_nONH_2$ with n = 1–12;

one of R^1 or R^2 groups may be $CO(CH_2)_nCOOR^8$, with n = 1-5 and a straight or branched alkyl chain $(CH_2)_n$ and with R^8 selected from H, straight or branched C_1-C_6 alkyl, CH_2CN, 2- and 4-nitrophenyl, 2,3,5,6-tetrafluorophenyl, pentachlorophenyl, pentafluorophenyl, *N*-succinimidyl, sulfo-*N*-succinimidyl, 1-oxybenzotriazolyl;

further, one of R^1 or R^2 may be $(CH_2)_nCONHR^9$, with n = 1–5 and R^9 = H, C_1-C_6 alkyl, $(CH_2)_mN_3$, $(CH_2)_m$-*N*-maleimido, $(CH_2)_m$-*N*$HCOCH_2X$ (X = Br or I), where m = 2-6 and with straight or branched alkyl chains in (CH_2)n and R^9;

or one of R^1 or R^2 may represent CH_2-C_6H_4-NH_2, COC_6H_4-NH_2, $CONHC_6H_4$-NH_2 or $CSNHC_6H_4$-NH_2 with C_6H_4 being a 1,2-, 1,3- or 1,4-phenylene, COC_5H_3N-NH_2 or CH_2-C_5H_3N-NH_2 with C_5H_3N being pyridin-2,4-diyl, pyridin-2,5-diyl, pyridin-2,6-diyl, or pyridin-3,5-diyl; or one

of R^1 or R^2 may be an alkyl azide $(CH)N_3$ or alkine, in particular propargyl;

The linker L may be divalent or polyvalent and comprises at least one carbon atom and may comprise alkyl, heteroalkyl, perfluoroalkyl, alkyloxy, such as CH_2OCH_2, CH_2CH_2O, $CH_2CH_2OCH_2$, alkylamino or dialkylamino, particularly diethanolamine or *N*-methyl (alkyl) monoethanolamine moieties such as $N(CH_3)CH_2CH_2O$- and $N(CH_2CH_2O-)_2$, one or multiple amide, esther or urethane moieties, single or multiple difluoromethyl (CF_2), alkene or alkine moieties in any combinations, at any occurrence, linear or branched, with the length ranging from C_1 to C_{12};

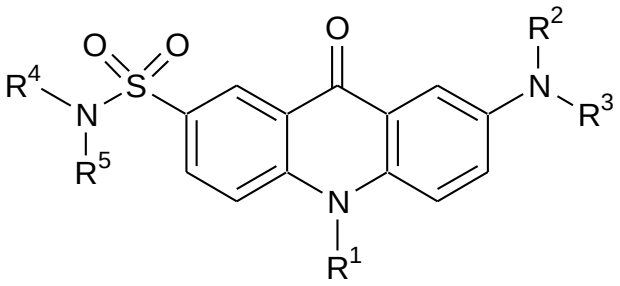
The linker L may also include a carbonyl (CH_2CO, CF_2CO) moiety, also as a part of an amide group;

The linker L may also comprise or contain a residue of 1,3,5-triazine, thus providing two attachment points for group X;

X denotes a solubilizing and/or ionizable anion-providing moiety, in particular consisting of or including a moiety selected from the group comprising hydroxyalkyl $(CH_2)_nOH$, thioalkyl $((CH_2)_nSH)$, carboxy alkyl $((CH_2)_nCO_2H)$, alkyl sulfonate $((CH_2)_nSO_3H)$, alkyl sulfate $((CH_2)_nOSO_3H)$, alkyl phosphate $((CH_2)_nOP(O)(OH)_2)$ or phosphonate $((CH_2)_nP(O)(OH)_2)$, wherein n is an integer ranging from 0 to 12, or an analog thereof wherein one or more of the CH_2 groups are replaced by CF_2,
further, the anion-providing moieties may be linked by means of non-aromatic O, N and S-containing heterocycles, e. g., piperazines, pipecolines, or, alternatively, one of the groups X may bear any of the moieties listed above for groups R^1 and R^2, also with any type of linkage listed for group L, and independently from other substituents;

with the proviso that in all compounds represented by Formula B three or six negatively charged groups are present in the residues X of Formula B under basic conditions, i.e. $7 < pH < 14$, and these negatively charged groups represent at least

partially deprotonated residues of ionizable groups selected from the following: SH, COOH, SO_3H, OSO_3H, $OP(O)(OH)_2$, $OP(O)(OH)R^a$, where R^a = C_1–C_4 alkyl or substituted C_1–C_4 alkyl, $P(O)(OH)_2$, $P(O)(OH)R^a$, where R^a = C_1–C_4 alkyl or substituted C_1–C_4 alkyl;



Formula A

wherein

R^1, R^2, R^3, R^4, R^5 are independent from each other and may represent:

H, CH_3, C_2H_5, a straight or branched C_3-C_{12}, preferably C_3-C_6, alkyl or perfluoroalkyl group, a phosphonylated alkyl group $(CH_2)_mP(O)(OH)_2$, where m = 1-12, preferably 2–6, with a straight or branched alkyl chain, $(CH_2)_nCOOH$, where n = 1-12, preferably 1-5, or $(CH_2)_nCOOR^6$, where n = 1–12, preferably 1-5, and R^6 may be alkyl, in particular C_1-C_6, CH_2CN, benzyl, fluorene-9-yl, polyhalogenoalkyl, polyhalogenophenyl, *e.g.* tetra- or pentafluorophenyl, pentachlorophenyl, 2- and 4-nitrophenyl, *N*-succinimidyl, sulfo-*N*-succinimidyl, 1-oxybenzotriazolyl or other potentially nucleophile-reactive leaving groups, alkyl sulfonate ($(CH_2)_nSO_3H$) or alkyl sulfate ($(CH_2)_nOSO_3H$) where n = 1–12, preferably 1-5, and the alkyl chain in any $(CH_2)_n$ may be straight or branched;

a hydroxyalkyl group $(CH_2)_mOH$ or thioalkyl group $(CH_2)_mSH$, where m = 1-12, preferably 2–6, with a straight or branched alkyl chain, a phosphorylated hydroxyalkyl group $(CH_2)_mOP(O)(OH)_2$, where m = 1-12, preferably 2–6, with a straight or branched alkyl chain; one of R^1 or R^2 groups may be a carbonate or carbamate derivative $(CH_2)_mOCOOR^7$ or $(CH_2)_mNHCOOR^7$, where m = 1-12 and R^7 =

methyl, ethyl, tert-butyl, benzyl, fluoren-9-yl, CH_2CN, *N*-succinimidyl, sulfo-*N*-succinimidyl, 1-oxybenzotriazolyl, phenyl, substituted phenyl group, e.g., 2- or 4-nitrophenyl, pentachlorophenyl, pentafluorophenyl, 2,3,5,6-tetrafluoro-phenyl, 2-pyridyl, 4-pyridyl, pyrimid-4-yl;

$(CH_2)_mNR^aR^b$, where m = 1-12, preferably 2–6, with a straight or branched alkyl chain; R^a, R^b are independent from each other and represent hydrogen and/or C_1–C_4 alkyl groups, a hydroxyalkyl group $(CH_2)_mOH$, where m = 2–6, with a straight or branched alkyl chain, a phosphorylated hydroxyalkyl group $(CH_2)_mOP(O)(OH)_2$, where m = 1-12, preferably 2–6, with a straight or branched alkyl chain;

an alkyl azide $(CH_2)_mN_3$, where m = m = 1-12, preferably 2–6, with a straight or branched alkyl chain;

R^1, R^2, R^3, R^4, R^5 may contain a terminal alkyloxyamino group $(CH_2)_mONH_2$, where m = 1-12, preferably 2–6, with a straight or branched alkyl chain, that can include one or multiple alkylamino $(CH_2)_mNH$ or alkylamido $(CH_2)_mCONH$ groups in all possible combinations with m = 0–12;

$(CH_2)_nCONHR^8$, with n = 1-12, preferably 1–5; R^8 = H, C_1-C_6 alkyl, $(CH_2)_mN_3$, or $(CH_2)_m$-N-maleimido, $(CH_2)_m$-NH-$COCH_2X$ (X = Br or I), with m = 1–12, preferably 2-6, and with straight or branched alkyl chains in (CH_2)n, $(CH_2)_m$ and R^8;

a primary amino group, preferably as R^1, R^2, or R^3, which forms aryl hydrazines;

a hydroxy group, preferably as R^2 or R^3, which forms aryl hydroxylamines;

an alkyloxyamino group $(CH_2)_nONH_2$ with n = 1–12;

further, one of the residues R^1, R^2, R^3, R^4, R^5 may represent CH_2-C_6H_4-NH_2, COC_6H_4-NH_2, $CONHC_6H_4$-NH_2 or $CSNHC_6H_4$-NH_2 with C_6H_4 being a 1,2-, 1,3- or 1,4-phenylene, COC_5H_3N-NH_2 or CH_2-C_5H_3N-NH_2 with C_5H_3N being pyridin-2,4-diyl, pyridin-2,5-diyl, pyridin-2,6-diyl, or pyridin-3,5-diyl;

additionally, R^2---R^3 (R^4---R^5) may form a four-, five, six-, or seven-membered cycle, or a four-, five, six-, or seven-membered

cycle with or without a primary amino group NH_2, secondary amino group NHR^a, where R^a = C_1–C_6 alkyl, a hydroxyl group OH, or a phosphorylated hydroxyl group $-OP(O)(OH)_2$ attached to one of the carbon atoms in this cycle;
optionally R^2---R^3 (R^4---R^5) may form a four-, five, six-, or seven-membered heterocycle with an additional 1-3 heteroatoms such as O, N or S included into this heterocycle;
further, R^1 may represent an unsubstituted phenyl group, a phenyl group with one or several electron-donor substituents chosen from the set of OH, SH, NH_2, NHR^a, NR^aR^b, R^aO, R^aS, where R^a and R^b are independent from each other and may be C_1-C_6 alkyl groups with straight or branched carbon chains, a phenyl group with one or several electron-acceptors chosen from the set of NO_2, CN, COH, COOH, CH=CHCN, $CH=C(CN)_2$, SO_2R^a, COR^a, $COOR^a$, $CH=CHCOR^a$, $CH=CHCOOR^a$, $CONHR^a$, $SO_2NR^aR^b$, $CONR^aR^b$, where R^a and R^b are independent from each other and may be H, or C_1-C_6 alkyl group(s) with straight or branched carbon chains;
or R^1 may represent a heteroaromatic group;
with the proviso that in all compounds of Formula A above at least two, preferably at least 3, 4, 5 or 6 negatively charged groups are present under basic conditions, i.e. 7 < pH < 14, and these negatively charged groups represent at least partially deprotonated residues of ionizable groups selected from the following: SH, COOH, a sulfonic acid residue SO_3H, a primary phosphate group $OP(O)(OH)_2$, a secondary phosphate group $OP(O)(OH)R^a$, where R^a = C_1–C_4 alkyl or substituted C_1–C_4 alkyl, a primary phosphonate group $P(O)(OH)_2$, a secondary phosphonate group $P(O)(OH)R^a$, where R^a = C_1–C_4 alkyl or substituted C_1–C_4 alkyl.

In another aspect of the present disclosure, there are provided fluorescent dyes of Formulae A and B that have a hydroxylamine or a hydrazine moiety as an analyte-reactive group, which is connected directly the dye core or where the hydroxylamine,

hydrazine or sulfonyl hydrazide moieties are connected via a linker group, in particular alkyl, heteroalkyl or a linker L, as defined herein for Formula B.

In a further aspect of the present disclosure, there is provided a compound according to Formula A above which has a net charge q of 0, -2, -4 or higher, preferably -4, or a compound of Formulae B, C and D above which has a net charge z of 0, -1, -3, -6 or -12, preferably -6 or -12, in particular in an aqueous medium at a pH range of 7-13.

In another aspect of the present disclosure, there is provided a compound as disclosed herein and salts thereof for use in the reductive amination and in the condensation with reducing sugars, i.e. monomeric, oligomeric or polymeric carbohydrates possessing an aldehyde group in a free form or as semiacetal, including glycans.

In a further aspect of the present disclosure, there is provided a compound as disclosed herein and salts thereof for use as a fluorescent label for amino acids, peptides, proteins, including primary and secondary antibodies, single-domain antibodies, docetaxel, avidin, streptavidin and their modifications, aptamers, nucleotides, nucleic acids, toxins, lipids, carbohydrates, including 2-deoxy-2-amino glucose and other 2-deoxy-2-aminoaminopyranosides, glycans, biotin, and other small molecules, i.e. having molecular masses of less than 1500 Da, e.g., jasplakinolide and its modifications.

In another aspect of the present disclosure, there is provided a use of the compounds as disclosed herein and salts thereof as fluorescent reagents for conjugation to analytes, wherein the conjugation comprises formation of at least one covalent chemical bond or at least one molecular complex with a chemical

entity or substance, such as amine, carboxylic acid, aldehyde, alcohol, aromatic compound, heterocycle, dye, amino acid, amino acid residue coupled to any chemical entity, peptide, protein, carbohydrate, nucleic acid, toxin and lipid.

In a further aspect of the present disclosure, there are provided carbohydrate-dye conjugates comprising fluorescent dyes as disclosed herein.

In another aspect of the present disclosure, there is provided a kit or composition comprising one or more of the dyes as disclosed herein or one or more of the carbohydrate conjugates as disclosed herein.

In a further aspect of the present disclosure, there is provided a method for synthesizing fluorescent aminopyrene dyes with absorbance maxima of 477 – 510 nm, net charge of 0 or down to -6, emission maxima of 535 – 560 nm in water, having the general formulae B and C, particularly compounds **7-R**, **13a**, **13b**, **15**, **16**, **18**, **23a** and **23b**, which involves an exchange reaction of the aryl halogen to a substituted thioalkyl function, followed by oxidation to the sulfone and, optionally, by phosphorylation at the hydroxyl site.

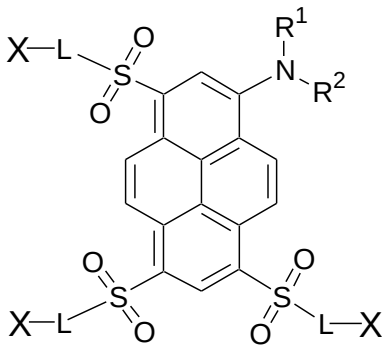
In another aspect of the present disclosure, there is provided a method for synthesizing phosphorylated fluorescent aminopyrene or aminoacridone dyes with net charges of -4, -6 and larger, that have the general formulae A and D, particularly compounds **6-R** and **8-R**, which involves sulfamidation of amino alcohols, followed by phosphorylation at the hydroxyl site.

Description of the invention

In their ionized (deprotonated) form, the novel fluorescent dyes of the invention feature multiple negatively charged residues and an aromatic amino or hydrazine group attached to the fluorophore, which is excitable e.g. with an argon ion laser. Importantly, the novel dyes and their carbohydrate conjugates emit light with the maximum that is considerably shifted from that of APTS-labeled analogs. Multiple negative net charges, which are especially high in the phosphorylated derivatives

Claims

1. A fluorescent dye having multiple ionizable and/or negatively charged groups which is selected from the group consisting of compounds of the following general Formulae B and A or of salts thereof:



Formula B

wherein R^1 and/or R^2 are independent from each other and may represent:

H, deuterium, alkyl or deutero-substituted alkyl, wherein one, several or all H atoms in the alkyl group may be replaced by deuterium atoms, in particular alkyl or deutero-substituted alkyl with 1-12 C atoms, preferably 1-6 C atoms, e.g. CH_3, C_2H_5, a linear or branched C_3-C_{12} alkyl or perfluoroalkyl group, or a substituted C_2–C_{12} alkyl group; in particular, $(CH_2)_nCOOR^3$, where n = 1-12, preferably 1-5, R^3 may be H, alkyl, in particular C_1-C_6, CH_2CN, benzyl, fluorene-9-yl, polyhalogenoalkyl, polyhalogenophenyl, *e.g.* tetra- or pentafluorophenyl, pentachlorophenyl, 2- and 4-nitrophenyl, *N*-succinimidyl, sulfo-*N*-succinimidyl, 1-oxybenzotriazolyl, or other potentially nucleophile-reactive leaving groups, and the alkyl chain in $(CH_2)_n$ may be straight or branched; and

R^1---R^2 may form a four-, five, six-, or seven-membered non-aromatic carbocycle with an additional primary amino group NH_2, secondary amino group NHR^a, where R^a = C_1–C_6 alkyl, or hydroxyl group OH attached to one of the carbon atoms in this cycle;

optionally R^1---R^2 may form a four-, five, six-, or seven-membered non-aromatic heterocycle with an additional heteroatom such as O, N or S included into this heterocycle;
a hydroxyalkyl group $(CH_2)_mOH$, where m = 1-12, preferably 2–6, with a straight or branched alkyl chain;
one of R^1 or R^2 groups may be a carbonate or carbamate derivative $(CH_2)_mOCOOR^4$ or $(CH_2)_mNHCOOR^4$, where m = 1-12 and R^4 = methyl, ethyl, 2-chloroethyl, *N*-succinimidyl, sulfo-*N*-succinimidyl, 1-oxybenzotriazolyl, a phenyl group or substituted phenyl group, e.g., 2- and 4-nitrophenyl, pentachlorophenyl, pentafluorophenyl, 2,3,5,6-tetrafluorophenyl, 2-pyridyl, or 4-pyridyl;
$(CH_2)_mNR^aR^b$, where m = 1-12, preferably 2–6, with a straight or branched alkyl chain; R^a, R^b are independent from each other and may be H, or optionally substituted C_1–C_4 alkyl group(s), in particular, one of R^1 or R^2 groups may be an alkyl azide group $(CH_2)_mN_3$ with m = 2-6 and a straight or branched alkyl chain; one of R^1 or R^2 may be $(CH_2)_nSO_2NR^5NH_2$ with n = 1-12, while the substituent R^5 can be represented by H, alkyl, hydroxyalkyl or perfluoroalkyl groups C_1-C_{12};
one of R^1 or R^2 groups may be a primary amino group to form aryl hydrazines Ar-NR^6NH_2 where Ar is the entire pyrene residue in Formula B and R^6 = H or alkyl;
one of R^1 or R^2 groups may be a hydroxy group to form aryl hydroxylamines Ar-NR^7OH where Ar is the entire pyrene residue in Formula B and R^7 = H or alkyl;
one of R^1 or R^2 groups may be an alkyloxyamino group $(CH_2)_nONH_2$ with n = 1–12;
one of R^1 or R^2 groups may be $CO(CH_2)_nCOOR^8$, with n = 1-5 and a straight or branched alkyl chain $(CH_2)_n$ and with R^8 selected from H, straight or branched C_1-C_6 alkyl, CH_2CN, 2- and 4-nitrophenyl, 2,3,5,6-tetrafluorophenyl, pentachlorophenyl, pentafluorophenyl, *N*-succinimidyl, sulfo-*N*-succinimidyl, 1-oxybenzotriazolyl;

further, one of R^1 or R^2 may be $(CH_2)_nCONHR^9$, with n = 1–5 and R^9 = H, C_1-C_6 alkyl, $(CH_2)_mN_3$, $(CH_2)_m$-*N*-maleimido, $(CH_2)_m$-*N*$HCOCH_2X$ (X = Br or I), where m = 2-6 and with straight or branched alkyl chains in (CH_2)n and R^9;
or one of R^1 or R^2 may represent CH_2-C_6H_4-NH_2, COC_6H_4-NH_2, $CONHC_6H_4$-NH_2 or $CSNHC_6H_4$-NH_2 with C_6H_4 being a 1,2-, 1,3- or 1,4-phenylene, COC_5H_3N-NH_2 or CH_2-C_5H_3N-NH_2 with C_5H_3N being pyridin-2,4-diyl, pyridin-2,5-diyl, pyridin-2,6-diyl, or pyridin-3,5-diyl; or one of R^1 or R^2 may be an alkyl azide $(CH)N_3$ or alkine, in particular propargyl;

The linker L may be divalent or polyvalent and comprises at least one carbon atom and may comprise alkyl, heteroalkyl, perfluoroalkyl, alkyloxy, such as CH_2OCH_2, CH_2CH_2O, $CH_2CH_2OCH_2$, alkylamino or dialkylamino, particularly diethanolamine or *N*-methyl (alkyl) monoethanolamine moieties such as $N(CH_3)CH_2CH_2O$- and $N(CH_2CH_2O-)_2$, one or multiple amide, esther or urethane moieties, single or multiple difluoromethyl (CF_2), alkene or alkine moieties in any combinations, at any occurrence, linear or branched, with the length ranging from C_1 to C_{12};

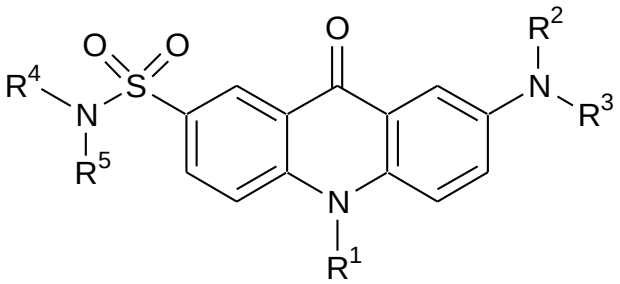
The linker L may also include a carbonyl (CH_2CO, CF_2CO) moiety, also as a part of an amide group;

The linker L may also comprise or contain a residue of 1,3,5-triazine, thus providing two attachment points for group X;

X denotes a solubilizing and/or ionizable anion-providing moiety, in particular consisting of or including a moiety selected from the group comprising hydroxyalkyl $(CH_2)_nOH$, thioalkyl ($(CH_2)_nSH$), carboxy alkyl ($(CH_2)_nCO_2H$), alkyl sulfonate ($(CH_2)_nSO_3H$), alkyl sulfate ($(CH_2)_nOSO_3H$), alkyl phosphate ($(CH_2)_nOP(O)(OH)_2$) or phosphonate ($(CH_2)_nP(O)(OH)_2$), wherein n is an integer ranging from 0 to 12, or an analog thereof wherein one or more of the CH_2 groups are replaced by CF_2,
further, the anion-providing moieties may be linked by means of non-aromatic O, N and S-containing heterocycles, e. g.,

piperazines, pipecolines, or, alternatively, one of the groups X may bear any of the moieties listed above for groups R^1 and R^2, also with any type of linkage listed for group L, and independently from other substituents;

with the proviso that in all compounds represented by Formula B three or six negatively charged groups are present in the residues X of Formula B under basic conditions, i.e. 7 < pH < 14, and these negatively charged groups represent at least partially deprotonated residues of ionizable groups selected from the following: SH, COOH, SO_3H, OSO_3H, $OP(O)(OH)_2$, $OP(O)(OH)R^a$, where R^a = C_1–C_4 alkyl or substituted C_1–C_4 alkyl, $P(O)(OH)_2$, $P(O)(OH)R^a$, where R^a = C_1–C_4 alkyl or substituted C_1–C_4 alkyl;



Formula A

wherein

R^1, R^2, R^3, R^4, R^5 are independent from each other and may represent:
H, CH_3, C_2H_5, a straight or branched C_3-C_{12}, preferably C_3-C_6, alkyl or perfluoroalkyl group, a phosphonylated alkyl group $(CH_2)_mP(O)(OH)_2$, where m = 1-12, preferably 2-6, with a straight or branched alkyl chain, $(CH_2)_nCOOH$, where n = 1-12, preferably 1-5, or $(CH_2)_nCOOR^6$, where n = 1-12, preferably 1-5, and R^6 may be alkyl, in particular C_1-C_6, CH_2CN, benzyl, fluorene-9-yl, polyhalogenoalkyl, polyhalogenophenyl, *e.g.* tetra- or pentafluorophenyl, pentachlorophenyl, 2- and 4-nitrophenyl, *N*-succinimidyl, sulfo-*N*-succinimidyl, 1-oxybenzotriazolyl or other potentially nucleophile-reactive leaving groups, alkyl

sulfonate ($(CH_2)_nSO_3H$) or alkyl sulfate ($(CH_2)_nOSO_3H$) where n = 1-12, preferably 1-5, and the alkyl chain in any $(CH_2)_n$ may be straight or branched;

a hydroxyalkyl group $(CH_2)_mOH$ or thioalkyl group $(CH_2)_mSH$, where m = 1-12, preferably 2-6, with a straight or branched alkyl chain, a phosphorylated hydroxyalkyl group $(CH_2)_mOP(O)(OH)_2$, where m = 1-12, preferably 2-6, with a straight or branched alkyl chain; one of R^1 or R^2 groups may be a carbonate or carbamate derivative $(CH_2)_mOCOOR^7$ or $(CH_2)_mNHCOOR^7$, where m = 1-12 and R^7 = methyl, ethyl, tert-butyl, benzyl, fluoren-9-yl, CH_2CN, *N*-succinimidyl, sulfo-*N*-succinimidyl, 1-oxybenzotriazolyl, phenyl, substituted phenyl group, e.g., 2- or 4-nitrophenyl, pentachlorophenyl, pentafluorophenyl, 2,3,5,6-tetrafluoro-phenyl, 2-pyridyl, 4-pyridyl, pyrimid-4-yl;

$(CH_2)_mNR^aR^b$, where m = 1-12, preferably 2-6, with a straight or branched alkyl chain; R^a, R^b are independent from each other and represent hydrogen and/or C_1-C_4 alkyl groups, a hydroxyalkyl group $(CH_2)_mOH$, where m = 2-6, with a straight or branched alkyl chain, a phosphorylated hydroxyalkyl group $(CH_2)_mOP(O)(OH)_2$, where m = 1-12, preferably 2-6, with a straight or branched alkyl chain;

an alkyl azide $(CH_2)_mN_3$, where m = m = 1-12, preferably 2-6, with a straight or branched alkyl chain;

R^1, R^2, R^3, R^4, R^5 may contain a terminal alkyloxyamino group $(CH_2)_mONH_2$, where m = 1-12, preferably 2-6, with a straight or branched alkyl chain, that can include one or multiple alkylamino $(CH_2)_mNH$ or alkylamido $(CH_2)_mCONH$ groups in all possible combinations with m = 0-12;

$(CH_2)_nCONHR^8$, with n = 1-12, preferably 1-5; R^8 = H, C_1-C_6 alkyl, $(CH_2)_mN_3$, or $(CH_2)_m$-N-maleimido, $(CH_2)_m$-NH-$COCH_2X$ (X = Br or I), with m = 1-12, preferably 2-6, and with straight or branched alkyl chains in $(CH_2)n$, $(CH_2)_m$ and R^8;

a primary amino group, preferably as R^1, R^2, or R^3, which forms aryl hydrazines;

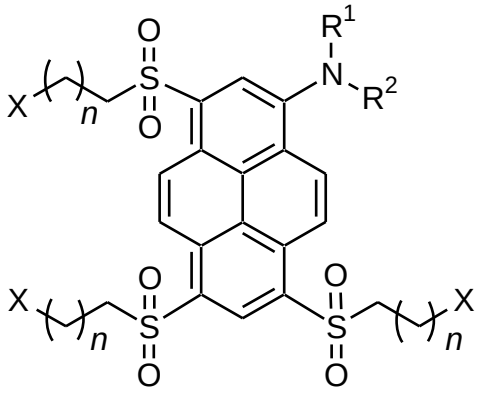
a hydroxy group, preferably as R^2 or R^3, which forms aryl hydroxylamines;
an alkyloxyamino group $(CH_2)_nONH_2$ with n = 1–12;

further, one of the residues R^1, R^2, R^3, R^4, R^5 may represent CH_2-C_6H_4-NH_2, COC_6H_4-NH_2, $CONHC_6H_4$-NH_2 or $CSNHC_6H_4$-NH_2 with C_6H_4 being a 1,2-, 1,3- or 1,4-phenylene, COC_5H_3N-NH_2 or CH_2-C_5H_3N-NH_2 with C_5H_3N being pyridin-2,4-diyl, pyridin-2,5-diyl, pyridin-2,6-diyl, or pyridin-3,5-diyl;
additionally, R^2---R^3 (R^4---R^5) may form a four-, five, six-, or seven-membered cycle, or a four-, five, six-, or seven-membered cycle with or without a primary amino group NH_2, secondary amino group NHR^a, where R^a = C_1–C_6 alkyl, a hydroxyl group OH, or a phosphorylated hydroxyl group $-OP(O)(OH)_2$ attached to one of the carbon atoms in this cycle;
optionally R^2---R^3 (R^4---R^5) may form a four-, five, six-, or seven-membered heterocycle with an additional 1-3 heteroatoms such as O, N or S included into this heterocycle;
further, R^1 may represent an unsubstituted phenyl group, a phenyl group with one or several electron-donor substituents chosen from the set of OH, SH, NH_2, NHR^a, NR^aR^b, R^aO, R^aS, where R^a and R^b are independent from each other and may be C_1-C_6 alkyl groups with straight or branched carbon chains, a phenyl group with one or several electron-acceptors chosen from the set of NO_2, CN, COH, COOH, CH=CHCN, $CH=C(CN)_2$, SO_2R^a, COR^a, $COOR^a$, $CH=CHCOR^a$, $CH=CHCOOR^a$, $CONHR^a$, $SO_2NR^aR^b$, $CONR^aR^b$, where R^a and R^b are independent from each other and may be H, or C_1-C_6 alkyl group(s) with straight or branched carbon chains;
or R^1 may represent a heteroaromatic group;
with the proviso that in all compounds of Formula A above at least two, preferably at least 3, 4, 5 or 6 negatively charged groups are present under basic conditions, i.e. 7 < pH < 14, and these negatively charged groups represent at least partially deprotonated residues of ionizable groups selected from the following: SH, COOH, a sulfonic acid residue SO_3H, a primary

phosphate group $OP(O)(OH)_2$, a secondary phosphate group $OP(O)(OH)R^a$, where R^a = C_1–C_4 alkyl or substituted C_1–C_4 alkyl, a primary phosphonate group $P(O)(OH)_2$, a secondary phosphonate group $P(O)(OH)R^a$, where R^a = C_1–C_4 alkyl or substituted C_1–C_4 alkyl.

2. A fluorescent dye according to claim 1 having Formula B wherein X at each occurrence may represent 1 to 4 groups SO_3H attached to the respective linker group L at one or more sites.

3. A fluorescent dye according to claim 1 having Formula C and at least one alkyl sulfonyl group



Formula C

or a salt thereof,
wherein
n = 1-12, preferably 1-6;
R^1 and/or R^2 are independent from each other and may represent: H, deuterium, alkyl or deutero-substituted alkyl, wherein one, several or all H atoms in the alkyl group may be replaced by deuterium atoms, in particular alkyl or deutero-substituted alkyl with 1-12 C atoms, preferably 1-6 C atoms, e.g. CH_3, C_2H_5, a straight or branched C_3-C_{12}, preferably C_3-C_6, alkyl group, or a substituted C_2–C_{12}, preferably C_2-C_6, alkyl group; in particular, $(CH_2)_nCOOR^3$, where n = 1-12, preferably 1-5, R^3 may be H, CH_2CN,

2- and 4-nitrophenyl, 2,3,5,6-tetrafluorophenyl, pentachlorophenyl, pentafluorophenyl, *N*-succinimidyl, sulfo-*N*-succinimidyl, 1-oxybenzotriazolyl, and the alkyl chain in $(CH_2)_n$ may be straight or branched; and

R^1---R^2 may form a four-, five, six-, or seven-membered non-aromatic carbocycle with an additional primary amino group NH_2, secondary amino group NHR^a, where R^a = C_1–C_6 alkyl, or hydroxyl group OH attached to one of the carbon atoms in this cycle; optionally R^1---R^2 may form a four-, five, six-, or seven-membered non-aromatic heterocycle with an additional heteroatom such as O, N or S included into this heterocycle;

a hydroxyalkyl group $(CH_2)_mOH$, where m = 2-12, preferably 2–6, with a straight or branched alkyl chain; one of R^1 or R^2 groups may be a carbonate or carbamate derivative $(CH_2)_mOCOOR^4$ or $(CH_2)_mNHCOOR^4$, where m = 1-12 and R^4 = methyl, ethyl, 2-chloroethyl, *N*-succinimidyl, sulfo-*N*-succinimidyl, 1-oxybenzotriazolyl, a phenyl group or substituted phenyl group, e.g., 2- and 4-nitrophenyl, pentachlorophenyl, pentafluorophenyl, 2,3,5,6-tetrafluorophenyl, 2-pyridyl, or 4-pyridyl;

$(CH_2)_mNR^aR^b$, where m = 2-12, preferably 2–6, with a straight or branched alkyl chain; R^a, R^b are independent from each other and may be H, or optionally substituted C_1–C_4 alkyl group(s), in particular, one of R^1 or R^2 groups may be an alkyl azide group $(CH_2)_mN_3$ with m = 2-6 and a straight or branched alkyl chain; one of R^1 or R^2 groups may be $(CH_2)_nCOOR^5$, with n = 1-5 and a straight or branched alkyl chain $(CH_2)_n$ and with R^5 selected from H, straight or branched C_1-C_6 alkyl, CH_2CN, 2- and 4-nitrophenyl, 2,3,5,6-tetrafluorophenyl, pentachlorophenyl, pentafluorophenyl, *N*-succinimidyl, sulfo-*N*-succinimidyl, 1-oxybenzotriazolyl;

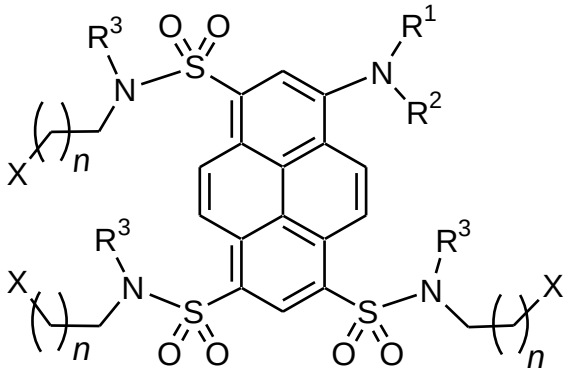
one of R^1 or R^2 groups may be a primary amino group to form aryl hydrazines, as described for Formula B;

one of R^1 or R^2 groups may be a hydroxy group to form aryl hydroxylamines, as described for Formula B;

one of R^1 or R^2 groups may be an alkyloxyamino group $(CH_2)_nONH_2$ with n = 1–12;
further, one of R^1 or R^2 may be $(CH_2)_nCONHR^6$, with n = 1-12, preferably 1–5, and R^6 = H, C_1-C_6 alkyl, $(CH_2)_mN_3$, $(CH_2)_m$-*N*-maleimido, $(CH_2)_m$-*N*$HCOCH_2X$ (X = Br or I), where m = 2-6 and with straight or branched alkyl chains in (CH_2)n and R^6; or one of R^1 or R^2 may represent CH_2-C_6H_4-NH_2, COC_6H_4-NH_2, $CONHC_6H_4$-NH_2 or $CSNHC_6H_4$-NH_2 with C_6H_4 being a 1,2-, 1,3- or 1,4-phenylene, COC_5H_3N-NH_2 or CH_2-C_5H_3N-NH_2 with C_5H_3N being pyridin-2,4-diyl, pyridin-2,5-diyl, pyridin-2,6-diyl, or pyridin-3,5-diyl;
the (CH_2)n-CH_2 linker, with n = 1-12, preferably 1-5, between the SO_2 fragment and the residue X in Formula B may represent a straight-chain, branched or cyclic group having 2–6 carbon atoms;
X = SH, COOH, SO_3H, OSO_3H, $OP(O)(OH)_2$, $OP(O)(OH)R^a$, where R^a = optionally substituted C_1–C_4 alkyl, $P(O)(OH)_2$, $P(O)(OH)R^a$, where R^a = optionally substituted C_1–C_4 alkyl;
with the proviso that in all compounds represented by Formula C three or six negatively charged groups are present in the residues X of Formula B under basic conditions, i.e. 7 < pH < 14, and these negatively charged groups represent at least partially deprotonated residues of ionizable groups selected from the following: SH, COOH, SO_3H, OSO_3H, $OP(O)(OH)_2$, $OP(O)(OH)R^a$, where R^a = C_1–C_4 alkyl or substituted C_1–C_4 alkyl, $P(O)(OH)_2$, $P(O)(OH)R^a$, where R^a = C_1–C_4 alkyl or substituted C_1–C_4 alkyl.

4. A fluorescent dye having Formula C according to claim 2 wherein X at each occurrence is SO_3H and n is 1-12, preferably 1-6.

5. A fluorescent dye according to claim 1 having the following Formula D and at least one sulfonamide group



Formula D

or a salt thereof,

wherein

R^1 and/or R^2 are independent from each other and may represent H, deuterium, alkyl or deutero-substituted alkyl, wherein one, several or all H atoms in the alkyl group may be replaced by deuterium atoms, in particular alkyl or deutero-substituted alkyl with 1-12 C atoms, preferably 1-6 C atoms, e.g. CH_3, C_2H_5, or a straight or branched, optionally substituted, C_3-C_{12}, preferably C_3-C_6, alkyl group; in particular, $(CH_2)_nCOOR^4$, where n = 1-12, preferably 1-5,

R^4 may be H, CH_2CN, 2- and 4-nitrophenyl, 2,3,5,6-tetrafluorophenyl, pentachlorophenyl, pentafluorophenyl, *N*-succinimidyl, sulfo-*N*-succinimidyl, 1-oxybenzotriazolyl, and the alkyl chain in $(CH_2)_n$ may be straight or branched; and

R^1---R^2 may form a four-, five, six-, or seven-membered non-aromatic carbocycle with an additional primary amino group NH_2, secondary amino group NHR^a, where R^a = optionally substituted C_1-C_6 alkyl, or hydroxyl group OH attached to one of the carbon atoms in this cycle; or optionally R^1---R^2 may form a four-, five, six-, or seven-membered non-aromatic heterocycle with a heteroatom such as O, N or S included into this heterocycle;

R^1 and/or R^2 may further represent:

a hydroxyalkyl group $(CH_2)_mOH$, where m = 1-12, preferably 2-6, with a straight or branched, optionally substituted alkyl chain;

one of R^1 or R^2 groups may be a carbonate or carbamate derivative $(CH_2)_mOCOOR^5$ or $(CH_2)_mNHCOOR^5$, where m = 1-12 and R^5 = methyl, ethyl, 2-chloroethyl, CH_2CN, *N*-succinimidyl, sulfo-*N*-

succinimidyl, 1-oxybenzotriazolyl, a phenyl group or substituted phenyl group, such as 2- and 4-nitrophenyl, pentachlorophenyl, pentafluorophenyl, 2,3,5,6-tetrafluorophenyl, 2-pyridyl, 4-pyridyl;

$(CH_2)_mNR^aR^b$, where m = 1-12, preferably 2–6, with a straight or branched alkyl chain; R^a, R^b are independent from each other and represent hydrogen and/or optionally substituted C_1–C_4 alkyl groups;

$(CH_2)_mN_3$, m = 1-12, preferably 2-6, with a straight or branched alkyl chain;

$CH_2)_nCONHR^6$, where n = 1-12, preferably 1–5 and R^6 = H, substituted or unsubstituted C_1-C_6 alkyl, $(CH_2)_mN_3$, (CH_2)m-N-maleimido, (CH_2)m-$NHCOCH_2Y$ (Y = Br, I) where m = 2-6, with straight or branched alkyl chains in (CH_2)n and R^6;

one of R^1 or R^2 groups may be a primary amino group to form aryl hydrazines, as described for Formula B;

one of R^1 or R^2 groups may be a hydroxy group to form aryl hydroxylamines, as described for Formula B,

one of R^1 or R^2 groups may be an alkyloxyamino group $(CH_2)_nONH_2$ with n = 1–12;

further, R^1 or R^2 may represent CH_2-C_6H_4-NH_2, COC_6H_4-NH_2, $CONHC_6H_4$-NH_2 or $CSNHC_6H_4$-NH_2 with C_6H_4 being a 1,2-, 1,3- or 1,4-phenylene, COC_5H_3N-NH_2 or CH_2-C_5H_3N-NH_2 with C_5H_3N being pyridin-2,4-diyl, pyridin-2,5-diyl, pyridin-2,6-diyl, or pyridin-3,5-diyl;

R^3 may represent H, $(CH_2)_qCH_2X$, C_2H_5, a straight or branched C_3-C_6 alkyl group, $C_mH_{2m}OR^7$, where m = 2–6, with a straight or branched alkan-diyl chain C_mH_{2m}, and R^7 = H, CH_3, C_2H_5, C_3H_7, $CH_3(CH_2CH_2O)_kCH_2CH_2$; with k = 1–12; while the $(CH_2)_qCH_2$ linker may represent a straight-chain, branched or cyclic group having 2–6 carbon atoms;

in Formula D, the $(CH_2)_n$-CH_2 linker, with n = 1-12, preferably 1-5, between the sulfonamide fragment SO_2N and the residue X may represent a straight-chain, branched or cyclic group having 2–6 carbon atoms;

X = SH, COOH, SO_3H, OSO_3H, $OP(O)(OH)_2$, $OP(O)(OH)R^a$, where R^a = substituted or unsubstituted C_1–C_4 alkyl, $P(O)(OH)_2$, $P(O)(OH)R^a$, where R^a = substituted or unsubstituted C_1–C_4 alkyl;

with the proviso that in all compounds represented by Formula D three, six, nine or twelve negatively charged groups are present in the residues X of Formula C under basic conditions, i.e. 7 < pH < 14, and these negatively charged groups represent at least partially deprotonated residues of ionizable groups selected from the following: SH, COOH, SO_3H, OSO_3H, $OP(O)(OH)_2$, $OP(O)(OH)R^a$, where R^a = C_1–C_4 alkyl or substituted C_1–C_4 alkyl, $P(O)(OH)_2$, $P(O)(OH)R^a$, where R^a = C_1–C_4 alkyl or substituted C_1–C_4 alkyl.

6. A fluorescent dye according to claims 1-5 wherein R^1 and/or R^2 in Formula B, C or D represents H, deuterium, alkyl or deutero-substituted alkyl, wherein one, several or all H atoms in the alkyl group may be replaced by deuterium atoms, in particular alkyl or deutero-substituted alkyl with 1-12 C atoms, preferably 1-6 C atoms, 4,6-dihalo-1,3,5-triazinyl ($C_3N_3X_2$) where halogen X is preferably chlorine, 2-, 3- or 4-aminobenzoyl ($COC_6H_4NH_2$), N-[(2-, N-[(3- or N-[(4-aminophenyl)ureido group ($NHCONHC_6H_4NH_2$), N-[(2-, N-[(3- or N-[(4-aminophenyl)thioureido group ($NHCSNHC_6H_4NH_2$) or linked carboxylic acid residues and their reactive esters of the general formulae $(CH_2)_{m1}COOR^3$, $(CH_2)_{m1}OCOOR^3$, $(CH_2)_{n1}COOR^3$ or $(CO)_{m1}(CH_2)_{m2}(CO)_{n1}(NH)_{n2}(CO)_{n3}(CH_2)_{n4}COOR^3$ where the integers m1,m2 and n1,n2,n3,n4 independently range from 1 to 12 and from 0 to 12, respectively, with the chain $(CH_2)_{m/n}$ being straight, branched, saturated, unsaturated, partially or completely deuterated, and/or or included into a carbo- or heterocylcle containing N, O or S, whereas R^3 is H, D or a nucleophile-reactive leaving group, preferably including but not limited to *N*-succinimidyl, sulfo-*N*-succinimidyl, 1-oxybenzotriazolyl, cyanomethyl, polyhalogenoalkyl, polyhalogenophenyl, *e.g.* tetra- or pentafluorophenyl, 2- or 4-nitrophenyl.

7. Fluorescent dyes of Formulae A and B that have a hydroxylamine or a hydrazine moiety as an analyte-reactive group, which is connected directly the dye core or where the hydroxylamine, hydrazine or sulfonyl hydrazide moieties are connected via a linker group, in particular alkyl, heteroalkyl or a linker L, as defined in claim 1 for Formula B.

8. A fluorescent dye according to claim 1, wherein R^1 in Formula A represents an unsubstituted phenyl group, a phenyl group with one or several electron-donor substituents chosen from the set of OH, SH, NH_2, NHR^a, NR^aR^b, R^aO, R^aS, where R^a and R^b are independent from each other and may be C_1-C_6 alkyl groups with straight or branched carbon chains, a phenyl group with one or several electron-acceptors chosen from the set of NO_2, CN, CF_3, COH, COOH, CH=CHCN, $CH=C(CN)_2$, SO_2R^a, SO_3R, COR^a, $COOR^a$, $CH=CHCOR^a$, $CH=CHCOOR^a$, $CONHR^a$, $SO_2NR^aR^b$, $CONR^aR^b$, $P(O)(OR^a)(OR^b)$ where R^a and R^b are independent from each other and may be H, or C_1-C_6 alkyl group(s) with straight or branched carbon chains; or wherein R^1 represents an aromatic heterocyclic group, in particular, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-thienyl, 3-thienyl, pyrimidin-4-yl, pyrimidin-2-yl, pyrimidin-5-yl, or other electron acceptor groups derived from aromatic heterocycles, such as 4-pyridyl-*N*-oxides, *N*-alkylpyridinium salts, or betaines, in particular, *N*-(ω-sulfoalkyl)-4-pyridinium, *N*-(ω-sulfoalkyl)-2-pyridinium, *N*-(1-hydroxy-4,4,5,5-tetrafluoro-cyclopent-1-en-3-on-2-yl)-4-pyridinium, *N*-(1-hydroxy-4,4,5,5-tetrafluorocyclopent-1-en-3-on-2-yl)-2-pyridinium.

9. A fluorescent dye according to claim 1 or 7, wherein the substituents R^1, R^2 and R^3 in Formula A represent one or more carbonyl-reactive aryl hydrazine moieties, e.g. R^2 = H, R^1 or R^3= NH_2 or R^1 = NH_2, R^2,R^3 = alkyl, perfluoroalkyl or alkyl, and which

are further conjugated or substituted with solubilizing and/or anion-providing moieties, listed as possible candidates for R^4 and R^5, particularly: hydroxyalkyl $(CH_2)_nOH$, thioalkyl $((CH_2)_nSH)$, carboxyalkyl $((CH_2)_nCO_2H)$, alkyl sulfonate $((CH_2)_nSO_3H)$, alkyl sulfate $((CH_2)_nOSO_3H)$, alkyl phosphate $((CH_2)_nOP(O)(OH)_2)$ or phosphonate $((CH_2)_nP(O)(OH)_2)$, wherein n is an integer ranging from 0 or 1 to 12;

or, alternatively, the hydrazine derivatives might be represented by sulfonyl hydrazide moieties, where $R^4 = NH_2$, while R^5 are alkyl, perfluoroalkyl or alkyl groups conjugated or substituted with solubilizing and/or anion-providing groups of the types mentioned above.

10. The fluorescent dye according to claim 1, wherein the aryl amino groups NR^1 and NR^2R^3 in Formula A are connected to an analyte-reactive group, in particular selected from the group comprising an aromatic or heterocyclic amine, carboxylic acid, ester of the carboxylic acid, in particular *N*-hydroxysuccinimidyl, sulfo-*N*-succinimidyl, 1-oxybenzotriazolyl or another amino reactive ester, alkyl azide, alkine, $(CH_2)_nONH_2$, maleimido, $COCH_2X$ with X = Cl, Br and I, $NRCOCH_2X$, with R = H, C_1-C_6-alkyl, X = Cl, Br, I, either directly or indirectly via a (poly)methylene, carbonyl, amido, nitrogen, oxygen or sulfur-containing linker, particularly $(CH_2)_mCON(R^9)$, CO(O)NR', $CO(CH_2)_mN(R^9)$, $CO(CH_2)_mS(CH_2)_n$, $(CH_2)_mS(CH_2)_nCO$, $CO(CH_2)_mSO_2(CH_2)_n$, $(CH_2)_mSO_2(CH_2)_nCO$, or combinations thereof; with m and n being integers in the range from 0 or 1 to 12, and substituent R^9 may be a residue as defined for any of R^1, R^2, R^3, R^4 and R^5 in claim 1 above;
or, alternatively, wherein the aryl amino groups NR^1 and/or NR^2R^3 in Formula A are connected to an acyl hydrazine or alkyl hydrazine moiety indirectly, via linkers, in particular linkers as defined above, thus representing hydrazides $(ZCONHNH_2)$ or hydrazines $(ZNHNH_2)$, respectively;

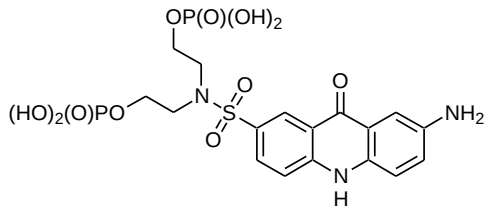
wherein Z denotes the dye residue of Formula A that includes aryl amino groups and linkers;

in particular, R^1 and R^2 may be represented by: $(CH_2)_mCON(R^{10})$, $CO(CH_2)_mN(R^{10})$, $CO(CH_2)_mS(CH_2)_n$, $(CH_2)_mS(CH_2)_nCO$, $CO(CH_2)_mSO_2(CH_2)_n$, $(CH_2)_mSO_2(CH_2)_nCO$ and their combinations; with m and n being integers ranging from 0 or 1 to 12;

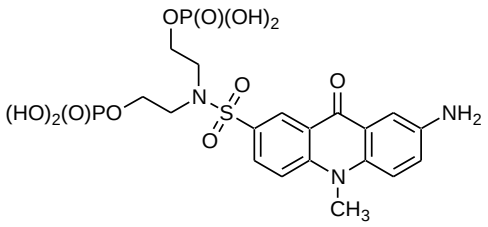
R^{10} is defined as any of the functional groups R^1, $R,^2$ R^3, R^4 and R^5 in claim 1 above; in particular hydroxyalkyl $(CH_2)_nOH$, thioalkyl $((CH_2)_nSH)$, carboxyalkyl $((CH_2)_nCO_2H)$, alkyl sulfonate $((CH_2)_nSO_3H)$, alkyl sulfate $((CH_2)_nOSO_3H)$, alkyl phosphate $((CH_2)_nOP(O)(OH)_2)$ or phosphonate $((CH_2)_nP(O)(OH)_2)$, wherein n is an integer ranging from 0 or 1 to 12;

linkers may also be represented by non-aromatic O, N and S-containing heterocycles, e.g., piperazines, pipecolines.

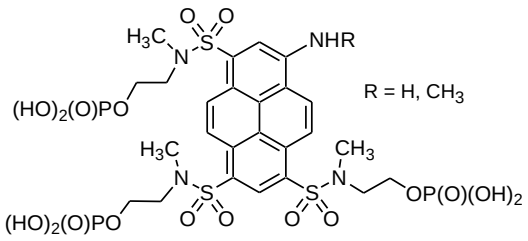
11. A compound according to one of the general formulae A-D of claims 1-6 having one of the following formulae:



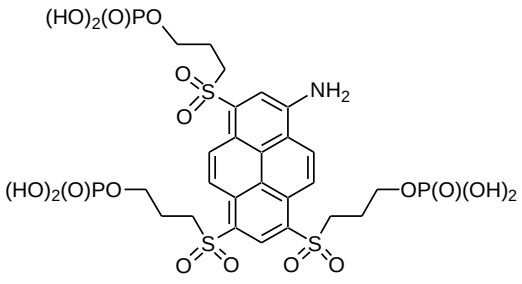
6-H



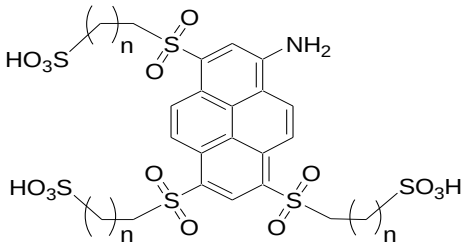
6-Me



8-R



15



23a (n = 2)
23b (n = 1)

or a salt thereof.

12. A compound according to Formula A above which has a net charge q of 0, -2, -4 or higher, preferably -4, or a compound of Formulae B, C and D above which has a net charge z of 0, -1, -3, -6 or -12, preferably -6 or -12, in particular in an aqueous medium at a pH range of 7-13.

13. A fluorescent dye salt according to any one of claims 1-12, comprising negatively charged sulfonate and/or phosphate groups and counterions selected from inorganic or organic cations, preferably alkaline metal cations, ammonium cations or cations of organic ammonium and phosphonium compounds, and/or comprising a positively charged group or a charge-transfer complex formed at the nitrogen site $N(R^1)R^2$ in the dye of formulae A-D with a counterion selected from anions of a strong mineral, organic or a Lewis acid.

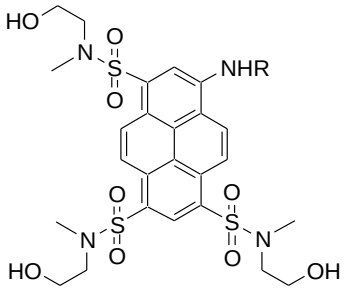
14. A fluorescent dye according to any one of claims 1-13, wherein carbohydrate-reactive groups, particularly hydrazine $N(R)NH_2$, hydroxylamine N(R)OH or aminooxy ONH_2 groups with R being H, alkyl, heteroalkyl, alkenyl or perfluoroalkyl are connected to the nitrogen site $N(R^1)R^2$ in the dye of formulae A or B via all types of linkers listed for group L in formula B.

15. A compound according to any one of claims 1-14 and salts thereof for use in the reductive amination and in the condensation with reducing sugars, i.e. monomeric, oligomeric or

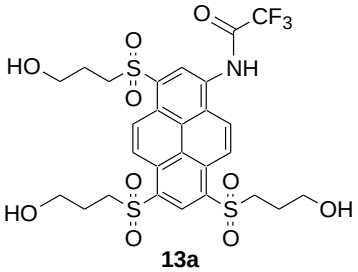
polymeric carbohydrates possessing an aldehyde group in a free form or as semiacetal, including glycans.

16. A compound according to any one of claims 1–14 and salts thereof for use as a fluorescent label for amino acids, peptides, proteins, including primary and secondary antibodies, single-domain antibodies, docetaxel, avidin, streptavidin and their modifications, aptamers, nucleotides, nucleic acids, toxins, lipids, carbohydrates, including 2-deoxy-2-amino glucose and other 2-deoxy-2-aminoaminopyranosides, glycans, biotin, and other small molecules, i.e. having molecular masses of less than 1500 Da, e.g., jasplakinolide and its modifications.

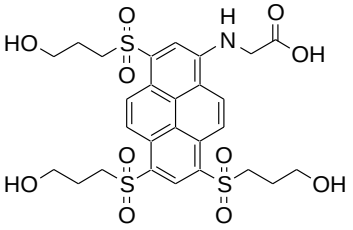
17. Compounds according to claim 16 which belong to the following compound set: **7-R** (R = H, Me), **13a**, **13b**, **16** and **18**.



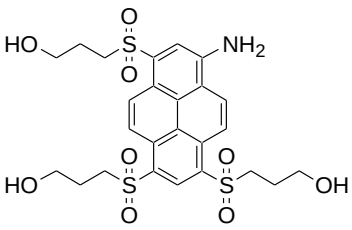
7-R
R = H, Me



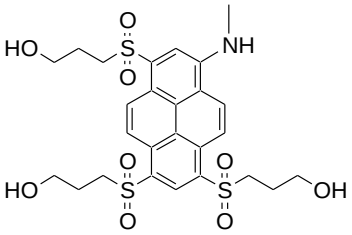
13a



16



13b

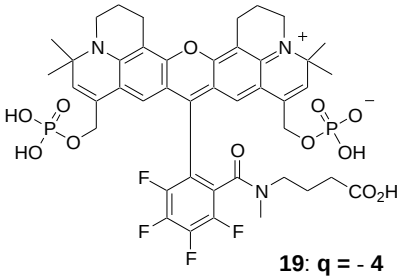


18

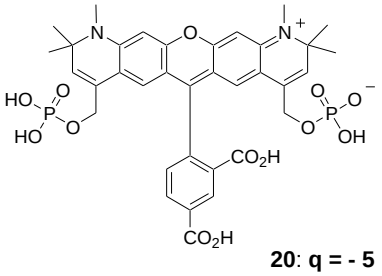
18. Use of the compounds according to any one of claims 1-14 and salts thereof as fluorescent reagents for conjugation to analytes, wherein the conjugation comprises formation of at least one covalent chemical bond or at least one molecular complex with a chemical entity or substance, such as amine, carboxylic acid, aldehyde, alcohol, aromatic compound, heterocycle, dye, amino acid, amino acid residue coupled to any chemical entity, peptide, protein, carbohydrate, nucleic acid, toxin and lipid.

19. Carbohydrate-dye conjugates comprising fluorescent dyes according to any one of claims 1-14.

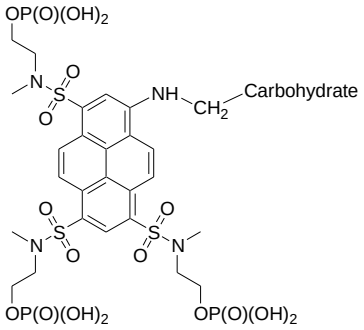
20. The carbohydrate-dye conjugates according to claim 19 wherein the dye is selected from the compounds of the formulae **6**–H, **6**–Me, **8**–H, **15**, **23a** and **23b**, as shown in claim 11 and below



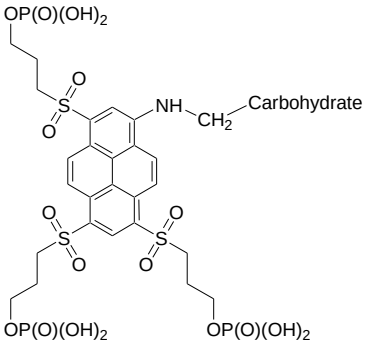
19: **q = - 4**



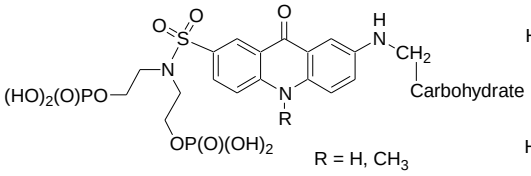
20: **q = - 5**



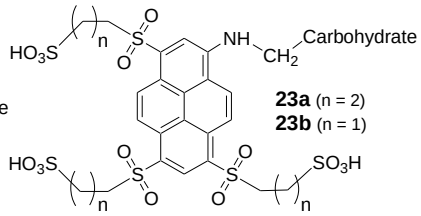
8-H-carbohydrate**: q = - 6**



15-carbohydrate: **q = - 6**



6-R-carbohydrate: **q = - 4**



23a,b-carbohydrate: **q = - 3**

21. A kit or composition comprising one or more of the dyes of claims 1-14 or one or more of the carbohydrate conjugates of claims 19 and 20.

22. A method for synthesizing fluorescent aminopyrene dyes with absorbance maxima of 477 – 510 nm, net charge of 0 or down to -6, emission maxima of 535 – 560 nm in water, having the general formulae B and C, particularly compounds **7-R, 13a, 13b, 15, 16, 18, 23a** and **23b,** which involves an exchange reaction of the aryl halogen to a substituted thioalkyl function, followed by oxidation to the sulfone and, optionally, by phosphorylation at the hydroxyl site.

23. A method for synthesizing phosphorylated fluorescent aminopyrene or aminoacridone dyes with net charges of -4, -6 and larger, that have the general formulae A and D, particularly compounds **6-R** and **8-R**, which involves sulfamidation of amino alcohols, followed by phosphorylation at the hydroxyl site.