and compounds of **Formula B** can exist and can be used as salts, solvates and hydrates, preferably as salts with alkaline metal cations including Na^+, Li^+, K^+ and organic ammonium or organic phosphonium cations

In another aspect of the present disclosure, there is provided a method for an automated carbohydrate mixture composition pattern profiling comprising the steps of
a) providing a first sample containing a first carbohydrate mixture composition;
b) labelling of said carbohydrate mixture composition with a first fluorescent label;
c) providing a second sample containing a second carbohydrate mixture composition labelled with a second fluorescent label which ~~may be~~is added ~~optionally~~ to said first sample;
d) generating electropherograms/chromatograms of the carbohydrate mixture composition of said sample using electrokinetic/chromatographic separation techniques combined with fluorescence or laser induced fluorescence detection;
e) analyzing the identity and/or differences between the carbohydrate mixture composition pattern profiles of the first and the second sample,
wherein the first fluorescent label of the first sample is different to the second fluorescent label of the second sample and wherein at least one of the first fluorescent label and the second fluorescent label is a fluorescent dye disclosed herein.

In a further aspect of the present disclosure, there is provided a method for calibration of a multi wavelength fluorescence detection system, in particular, a capillary-gel electrophoresis system, with acridone and/or pyrene based fluorescent dyes which ~~may optionally be~~are present as conjugates with a substrate moiety including carbohydrates,
whereby the method includes the detection of at least one of the compounds according to **Formula A** or **C~~B~~** as ~~defined in claim 1~~disclosed herein together with additional fluorescent dyes and their carbohydrate conjugates emitting at different wavelengths, preferably including at least one of the compounds: ***APTS***, ***6-R***, ~~***8-H***,~~ ***15***, ***19***, ***20***, ***23*** or ***23b***, as shown in the following scheme:

combined with fluorescence or laser induced fluorescence detection, like capillary gel electrophoresis-laser induced fluorescence;

e) analyzing the identity and/or differences between the carbohydrate mixture composition pattern profiles of the first and the second sample,

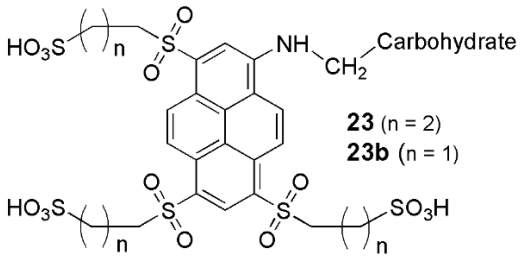
wherein the first fluorescent label of the first sample is different to the second fluorescent label of the second sample and wherein at least one of the first fluorescent label and the second fluorescent label is a fluorescent dye disclosed herein; and a fluorescent dye disclosed herein.

In another aspect of the present disclosure, there is provided the use of a carbohydrate dye conjugate comprising fluorescent dyes disclosed herein in a method disclosed herein.

In a further aspect of the present disclosure, there is provided a calibration standard, like an oligosaccharide standard, including a fluorescence dye according to **Formula A**~~, **B**,~~ or **C** ~~or **D**~~ ~~which may~~ being conjugated with a carbohydrate, optionally further comprising at least one of compounds ***19***, ***20***.

In another aspect of the present disclosure, there is provided a kit containing a calibration standard disclosed herein and, optionally, instructions for use.

In a further aspect of the present disclosure, there is provided a compound having Formula 23 or 23b



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

In another aspect of the present disclosure, there is provided a standard composition composed of compounds labelled with a fluorescence dye according to **Formula A** or ~~**B, in particular, of**~~ **Formula C** ~~**or D**~~ or different dyes of **Formulae A** ~~**to**~~ and ~~**C**~~**D**~~.

In a further aspect of the present disclosure, there is provided a kit containing a standard composition disclosed herein, in particular, for use in a disclosed herein and, optionally, instructions for use.

Description

A wide range of strategies and analytical techniques for analyzing glycoconjugates including glycoproteins, glycopeptides and released *N*-glycans or *O*-glycans have been established. For example, complex samples containing a variety of different oligosaccharides can be separated by chromatographic or electrokinetic techniques. These techniques include chromatographic techniques like size exclusion chromatography (SEC), hydrophilic interaction chromatography (HILIC), reversed phase liquid chromatography (RPLC) and reversed phase ion pairing chromatography (RPIPC), as well as porous graphitized carbon chromatography (PGC). Further, structural data of complex molecules including carbohydrates derived from glycoconjugates are either analyzed by mass-spectrometry (MS) or nuclear magnetic resonance spectroscopy (NMR) which are generally laborious and time-consuming techniques regarding sample preparation and data interpretation. For example, a

Claims

1. A method for an automated determination and/or identification of carbohydrates and/or carbohydrate mixture composition pattern profiling comprising the steps of:

a) obtaining a sample containing at least one carbohydrate;

b) labelling said carbohydrate(s) with a first fluorescent label;

c) providing a standard of known composition labelled with a second fluorescent label;

d) determining the migration/retention time(s) of said carbohydrate(s) and the standard of known composition using electrokinetic/chromatographic separation techniques combined with fluorescence or laser induced fluorescence detection;

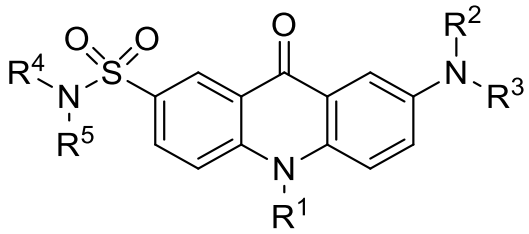
e) aligning the migration/retention time(s) to migration/retention time indice(s) based on given standard migration/retention time indice(s) of the standard;

f) comparing these migration/retention time indice(s) of the carbohydrate(s) with standard migration/retention time indice(s) from a database;

g) identifying or determining the carbohydrate(s) and/or the carbohydrate mixture composition pattern based on step f),

wherein the standard composition is added to the sample containing the unknown carbohydrate and/or carbohydrate mixture composition, the first fluorescent label and the second fluorescent label are different and wherein the first fluorescent label or the second fluorescent label is a fluorescent dye, preferably having multiple ionizable and/or negatively charged groups

which is selected from the group consisting of compounds of the following general **Formula A** and **B**:



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 of $(CH_2)_mO$-$COOR^7$ or $COOR^7$, where m = 1-12 and R^7 = methyl, ethyl, tert-butyl, benzyl, fluoren-9-yl, CH_2CN, *N*-succinimidyl, sulfo-*N*-succininmidyl, 1-oxybenzotriazolyl, phenyl, substituted phenyl group, e.g., 2- or 4-nitrophenyl, pentachlorophenyl, penta-fluorophenyl, 2,3,5,6-tetrafluorophenyl, 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;

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;

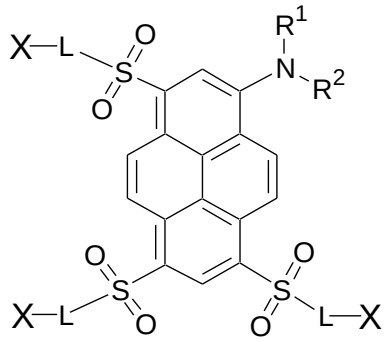
optionally R^2---R^3 (R^4---R^5) may form a four-, five, six-, or seven-membered heterocycle with an additional 1-3 heteroatoms such as O, N or S included into this heterocycle;

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;

and compounds of **Formula A** can exist and can be used as salts, solvates and hydrates, preferably as salts with alkaline metal cations including Na^+, Li^+, K^+ and organic ammonium or organic phosphonium cations;



Formula B

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

H, CH_3, C_2H_5, a linear or branched C_3-C_{12} alkyl or perfluoroalkyl group, or a substituted C_2–C_{612} 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 $COOR^4$, 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-tetrafluoro-phenyl, 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 contain a terminal alkyloxyamino group $(CH_2)_nONH_2$ with n = 1–12, which can be linked via one or multiple alkylamino $(CH_2)_mNH$, alkylamido $(CH_2)_mCONH$, alkyl ether or ester group(s) in all possible combinations with m = 0–12;

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, pentafluoro-phenyl, *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 comprises at least one carbon atom and may comprise alkyl, heteroalkyl, in particular 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 $N(CH_2CH_2O-)_2$, perfluoroalkyl, like single or multiple difluoromethyl (CF_2), alkene or alkyne 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 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 analogon 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;
Compounds of **Formula B** can exist and can be used as salts, solvates and hydrates, preferably as salts with alkaline metal cations including Na^+, Li^+, K^+ and organic ammonium.
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, $OP(O)(OH)_2$, $OP(O)(OH)R^a$, 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 is provided;
and compounds of **Formula B** can exist and can be used as salts, solvates and hydrates, preferably as salts with alkaline metal cations including Na^+, Li^+, K^+ and organic ammonium or organic phosphonium cations;

2. The method according to claim 1 wherein the standard of known composition is a standard base pair ladder and/or a known carbohydrate mixture composition.

3. A method for an automated carbohydrate mixture composition pattern profiling comprising the steps of
a) providing a first sample containing a first carbohydrate mixture composition;
b) labelling of said carbohydrate mixture composition with a first fluorescent label;
c) providing a second sample containing a second carbohydrate mixture composition labelled with a second fluorescent label which ~~may be~~is added ~~optionally~~ to said first sample;

d) generating electropherograms/chromatograms of the carbohydrate mixture composition of said sample using electrokinetic/chromatographic separation techniques combined with fluorescence or laser induced fluorescence detection;

e) analyzing the identity and/or differences between the carbohydrate mixture composition pattern profiles of the first and the second sample,

wherein the first fluorescent label of the first sample is different to the second fluorescent label of the second sample and wherein at least one of the first fluorescent label and the second fluorescent label is a fluorescent dye as defined in claim 1.

4. A method for an automated carbohydrate mixture composition pattern profiling according to claim 3 comprising the steps of

a) providing a sample containing a first carbohydrate mixture composition;

b) labelling of said carbohydrate mixture composition with a first fluorescent label;

c) providing a second sample labelled with a second fluorescent label containing a second carbohydrate mixture composition to be compared with;

d) generating electropherograms/chromatograms of the carbohydrate mixture composition of the first and second sample using electrokinetic/chromatographic separation techniques combined with fluorescence or laser induced fluorescence detection;

e) comparing the standard migration/retention time indices calculated from the obtained electropherogram/chromatogram of the first sample and the second sample;

f) analyzing the identify and/or differences between the carbohydrate mixture composition pattern profiles of the first and second sample, wherein standard migration/retention time indices of the carbohydrates present in the sample are calculated based on internal standards of known composition labelled with a third fluorescent label and wherein one of the first or the second fluorescent label is a fluorescent dye as defined in claim 1.

5. The method according to any one of the preceding claims whereby at least two orthogonal standards are added to the sample and orthogonal cross-alignment is performed based on the given standard migration/retention time indices of the at least two orthogonal standards.

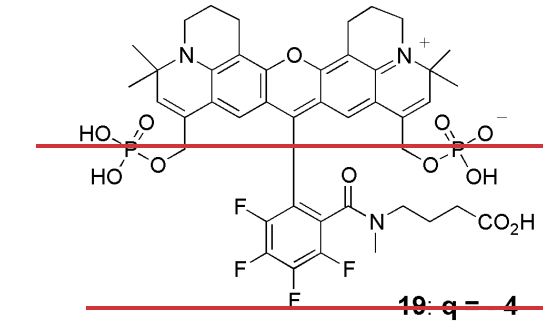
6. The method according to any one of the preceding claims wherein the sample contains a mixture of carbohydrates.

7. The method according to any one of the preceding claims wherein the sample is an extraction of glycans and the method allows for the identification of a glycosylation pattern profile.

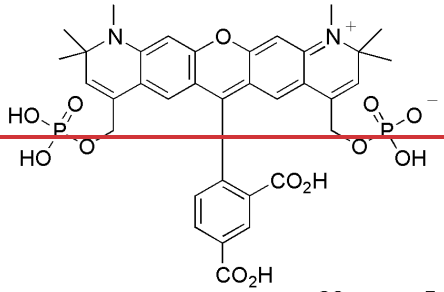
8. The method according to any one of the preceding claims wherein the glycosylation pattern of a glycoprotein is identified.

9. The method according to any one of the preceding claims wherein the components of the carbohydrate mixture are determined quantitatively.

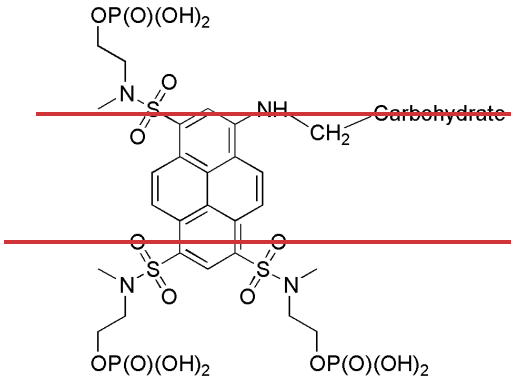
~~10. A method for calibration of a multi wavelength fluorescence detection system, in particular, a capillary-gel electrophoresis system, with acridone and/or pyrene based fluorescent dyes which may optionally be present as conjugates with a substrate moiety including carbohydrates,~~
~~whereby the method includes the detection of at least one of the compounds according to **Formula A** or **B** as defined in claim 1 together with additional fluorescent dyes and their carbohydrate conjugates emitting at different wavelengths, preferably including at least one of the compounds: ***APTS***, ***6-R***, ***8-H***, ***15***, ***19***, ***20***, ***23*** or ***23b***, as shown in the following scheme:~~



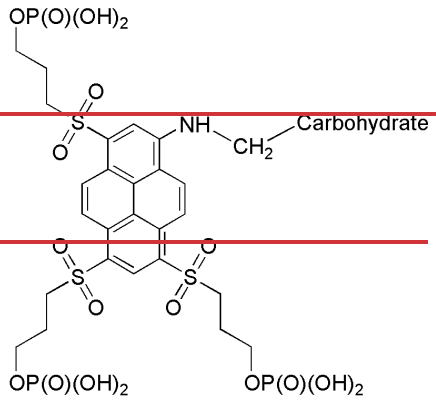
~~19~~: **~~q = 4~~**



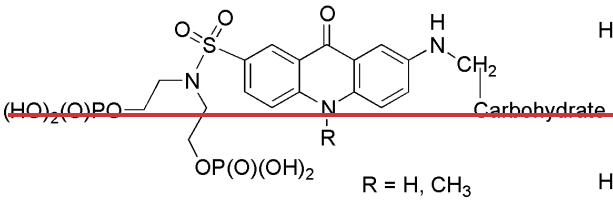
~~20~~: **~~q = 5~~**



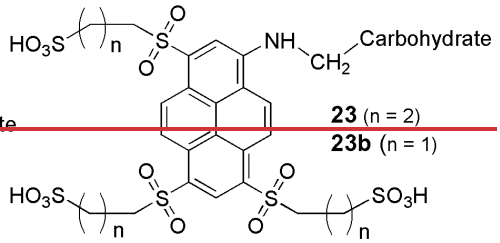
~~8-H~~ carbohydrate: **~~q = 6~~**



~~15~~ carbohydrate: **~~q = 6~~**



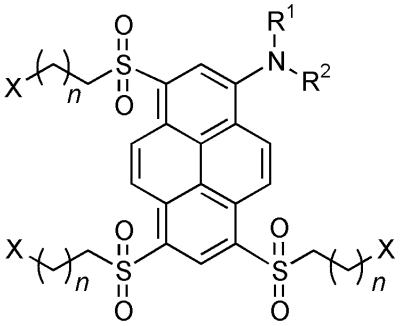
~~6~~ R carbohydrate: **~~q = 4~~**



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

~~11. The method according to claim 10 wherein the acridone and/or pyrene based dyes, which may optionally be present as conjugates with a substrate moiety including carbohydrates, include the combination of ***APTS (8-Amino-pyrene-1,3,6-trisulfonic acid)***, ***6-H***, ***19*** and ***20***, or ***APTS***, ***6-Me***, ***19*** and ***20***, or ***15***, ***6-Me***, ***19*** and ***20***, or ***APTS***, ***15***, ***19*** and ***20***, or ***APTS***, ***15***, ***6-Me*** and ***20***, or ***APTS***, ***8-H***, ***6-Me*** and ***19***, or ***APTS***, ***8-H***, ***6-Me*** and ***20***, or ***APTS***, ***8-H***, ***19*** and ***20***, or ***APTS***, ***23***, ***19*** and ***20***, or ***APTS***, ***15***, ***6-Me*** and ***19***, or ***APTS***, ***23***, ***6-Me*** and ***19***, or ***APTS***, ***23***, ***6-Me*** and ***20***, or ***23***, ***6-Me***, ***19*** and ***20***, or ***APTS***, ***8-H***, ***6-Me***, ***20*** and ***19***, or ***APTS***, ***15***, ***6-Me***, ***20*** and ***19***, or ***APTS***, ***23***, ***6-Me***, ***20*** and ***19***, or ***APTS***, ***8-H***, ***6-H***, ***20*** and ***19***, or ***APTS***, ***15***, ***6-H***, ***20*** and ***19***, or ***APTS***, ***23***, ***6-H***, ***20*** and ***19, as defined in claim 10***.~~

102. The method according to any one of the preceding claims wherein the fluorescent dye of **Formula B** is a dye having the following **Formula C** with n = 0-12



Formula C

wherein

R^1 and/or R^2 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 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 = 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 $COOR^4$, 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-tetrafluoro-phenyl, 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 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, pentafluoro-phenyl, sulfo-*N*-succinimidyl, *N*-succinimidyl or 1-oxybenzotriazolyl;

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;

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 C** and R^7 = H or alkyl;

one of R^1 or R^2 groups may be a hydroxy group to form aryl hydroxylamines Ar-NR^8OH where Ar is the entire pyrene residue in **Formula C** and R^7 = H or alkyl;

one of R^1 or R^2 groups may contain a terminal alkyloxyamino group $(CH_2)_nONH_2$ with n = 1–12, which can be linked via one or multiple alkylamino $(CH_2)_mNH$, alkylamido $(CH_2)_mCONH$, alkyl ether or alkyl ester group(s) in all possible combinations with m = 0–12;

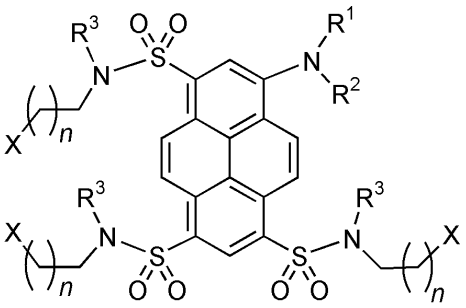
the (CH_2)n-CH_2 linker, with n = 1-5, between the SO_2 fragment and the residue X in **Formula B** may represent a straight-chain, branched or cyclic group having 2–6 carbon atoms;

X = SH, COOH, SO_3H, $OP(O)(OH)_2$, $OP(O)(OH)R^a$, 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, $OP(O)(OH)_2$, $OP(O)(OH)R^a$, 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;

and compounds of **Formula C** can exist and can be used as salts, solvates and hydrates, preferably as salts with alkaline metal cations including Na^+, Li^+, K^+ and organic ammonium or organic phosphonium cations;

11~~3~~. The method according to any one of the preceding claims wherein the fluorescent dye of **Formula B** is a dye having the following **Formula D**



Formula D

wherein

R^1 and/or R^2 are independent from each other and may represent H, 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 $COOR^5$, 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, pentafluoro-phenyl, 2,3,5,6-tetrafluorophenyl, 2-pyridyl, 4-pyridyl;

$(CH_2)_mN_3$, 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 = 1-12, preferably 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 Ar-NR^7NH_2 where Ar is the entire pyrene residue in **Formula D** and R^7 = H or alkyl;

one of R^1 or R^2 groups may be a hydroxy group to form aryl hydroxylamines Ar-NR^8OH where Ar is the entire pyrene residue in **Formula D** and R^8 = H or alkyl;

one of R^1 or R^2 groups may contain a terminal alkyloxyamino group $(CH_2)_nONH_2$ with n = 1–12, which can be linked via one or multiple alkylamino $(CH_2)_mNH$, alkylamido $(CH_2)_mCONH$, alkyl ether or alkyl ester group(s) in all possible combinations with m = 0–12;

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 = H, $(CH_2)_qCH_2X$, C_2H_5, a straight or branched C_3-C_6 alkyl group, $C_mH_{2m}OR$, where m = 2–6, with a straight or branched alkan-diyl chain C_mH_{2m}, and R = 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;

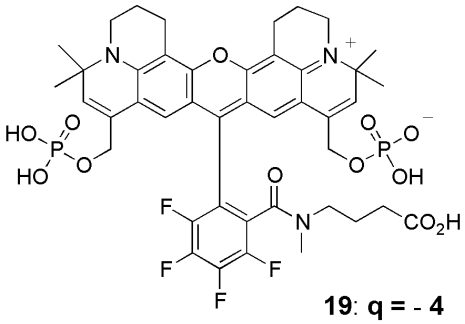
in **Formula D**, the $(CH_2)_n$-CH_2 linker, with n = 1-12, preferably 1-5, between the sulfonamide fragment SO_2N and the residue X may represent a straight-chain, branched or cyclic group having 2–6 carbon atoms;

X = SH, COOH, SO_3H, $OP(O)(OH)_2$, $OP(O)(OH)R^a$, 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;

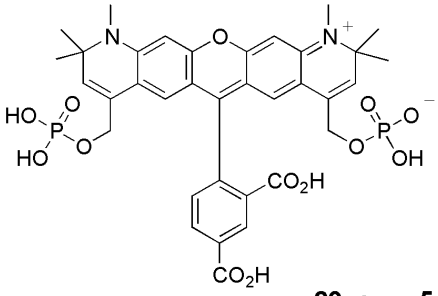
with the proviso that in all compounds represented by **Formula D** three, six, nine or twelve negatively charged groups are present in the residues X of **Formula C** under basic conditions, i.e. 7 < pH < 14, and these negatively charged groups represent at least partially deprotonated residues of ionizable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

and compounds of **Formula D** can exist and can be used as salts, solvates and hydrates, preferably as salts with alkaline metal cations including Na^+, Li^+, K^+ and organic ammonium or organic phosphonium cations.

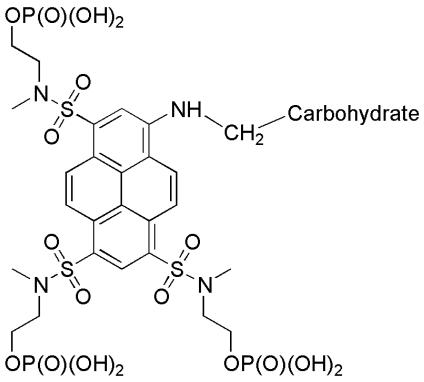
12. A method for calibration of a multi wavelength fluorescence detection system, in particular, a capillary-gel electrophoresis system, with acridone and/or pyrene based fluorescent dyes which are present as conjugates with a substrate moiety including carbohydrates,
whereby the method includes the detection of at least one of the compounds according to **Formula A** as defined in claim 1 or Formula C as defined in claim 10 together with additional fluorescent dyes and their carbohydrate conjugates emitting at different wavelengths, preferably including at least one of the compounds: ***APTS***, ***6-R***, ***15***, ***19***, ***20***, ***23*** or ***23b***, as shown in the following scheme:



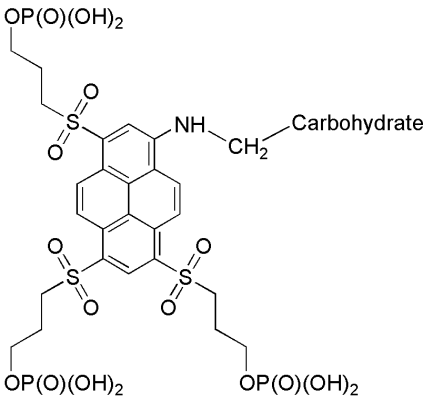
19: **q = - 4**



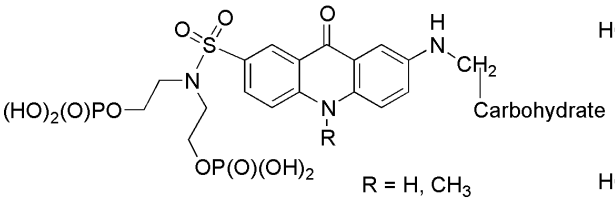
20: **q = - 5**



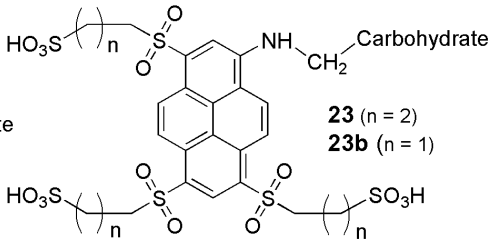
8-H-carbohydrate: **q = - 6**



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



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

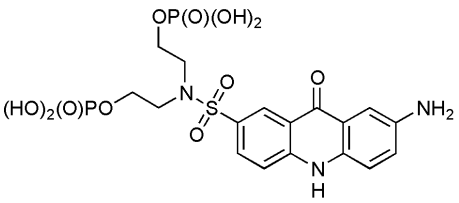


23, **23b**-carbohydrate: **q = - 3**

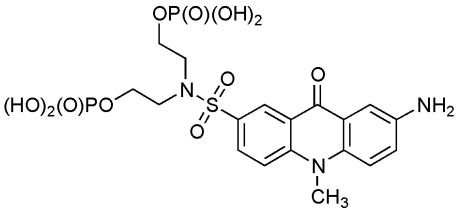
13. The method according to claim 12 wherein the acridone and/or pyrene based dyes, which is present as conjugates with a substrate moiety including carbohydrates, include the combination of ***APTS (8 Amino-pyrene-1,3,6-trisulfonic acid)***, ***6-H***, ***19*** and ***20***, or ***APTS***, ***6-Me***, ***19*** and ***20***, or ***15***, ***6-Me***, ***19*** and ***20***, or ***APTS***, ***15***, ***19*** and ***20***, or ***APTS***, ***15***, ***6-Me*** and ***20***, or ***APTS***, ***23***, ***19*** and ***20***, or ***APTS***, ***15***, ***6-Me*** and ***19***, or ***APTS***, ***23***, ***6-Me*** and ***19***, or ***APTS***, ***23***, ***6-Me*** and ***20***, or ***23***, ***6-Me***, ***19*** and ***20***, or ***APTS***, ***8-H***, ***6-Me***, ***20*** and ***19***, or ***APTS***, ***15***, ***6-Me***, ***20*** and ***19***, or ***APTS***, ***23***, ***6-Me***, ***20*** and ***19***, or ***APTS***, ***15***, ***6-H***, ***20*** and ***19***, or ***APTS***, ***23***, ***6-H***, ***20*** and ***19, as defined in claim 12***.

14. The method according to any one of the preceding claims wherein R^1 and/or R^2 in formula B, or D represent: H, deuterium, alkyl or deutero-substituted substituted alkyl, wherein one, several or all H atoms of the alkyl group may be replaced by deuterium atoms, in particular alkyl or deutero-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 .

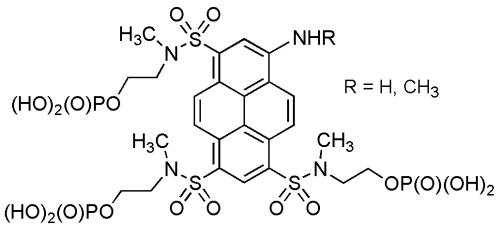
15. The method according to any one of the preceding claims wherein the compound of **Formulae A** to **D** is selected from:



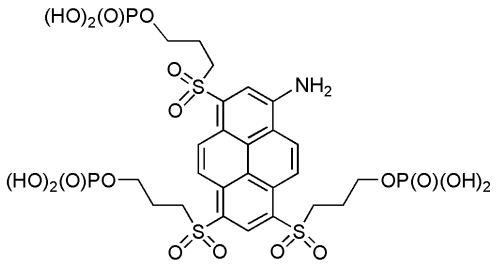
6-H



6-Me

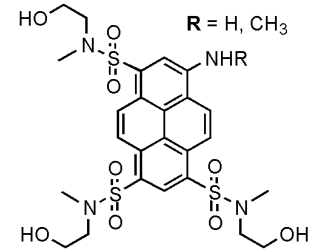


8-R

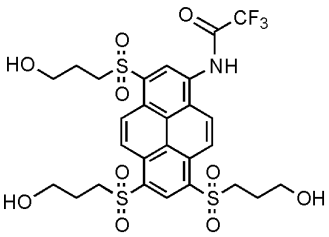


15

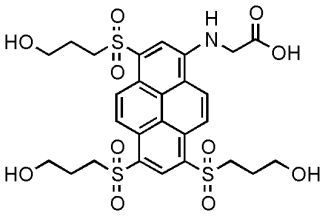
or a compound of ***7-R*** (R =H, Me), ***13a***, ***13b***, ***16***, ***18***, **23** and ***23b***



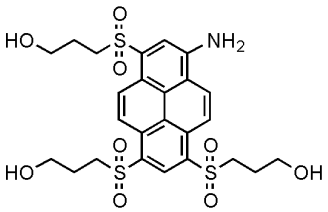
7-R



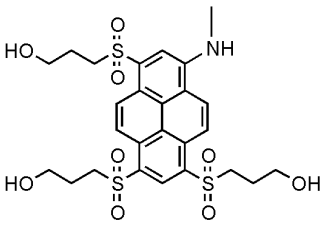
13a



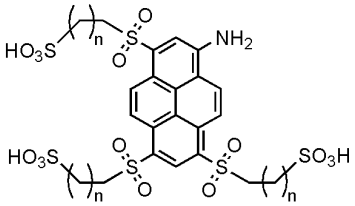
16



13b



18



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

or salts thereof.

16. A kit or system for determining and/or identifying carbohydrate mixture composition patterns comprising a data processing unit having a non-transient memory, said memory containing a database, said database containing aligned migration/retention times and/or aligned migration/retention time indices of carbohydrates, said migration/retention times and/or migration/retention time indices are obtained by an automated determination and/or identification of carbohydrates and/or identification of carbohydrates and/or carbohydrate mixture composition pattern profiling comprising the steps of:

a) obtaining a sample containing at least one carbohydrate;

b) labelling said carbohydrate(s) with a first fluorescent label;

c) providing a standard of known composition labelled with a second fluorescent label;

d) determining the migration/retention time(s) of said carbohydrate(s) and the standard of known composition using electrokinetic/chromatographic separation techniques combined with fluorescence or laser induced fluorescence detection;

e) aligning the migration/retention time(s) to migration/retention time indice(s) based on given standard migration/retention time indice(s) of the standard;

f) comparing these migration/retention time indice(s) of the carbohydrate(s) with standard migration/retention time indice(s) from a database;

g) identifying or determining the carbohydrate(s) and/or the carbohydrate mixture composition pattern, based on step f),

wherein the standard composition is added to the sample containing the unknown carbohydrate mixture composition, the first fluorescent label and the second fluorescent label are different and wherein the first fluorescent label or the second fluorescent label is a fluorescent dye, preferably having multiple ionizable and/or negatively charged groups which is selected from the group consisting of compounds of the general **Formulae A** to **D** as defined in any one of claims 1, 10, 11, 14 and ~~12 to~~ 15; and a fluorescent dye as defined in any one of claims 1, 10, 11, 14 and ~~12 to~~ 15.

17. A kit or system for an automated carbohydrate mixture composition pattern profiling comprising a data processing unit having a non-transient memory, said memory containing a database, said database containing aligned migration/retention times and/or aligned migration/retention time indices of carbohydrates, said migration/retention times and/or migration/retention time indices are obtained by an automated determination and/or identification of carbohydrates and/or identification of carbohydrates and/or carbohydrate mixture composition pattern profiling comprising the steps of

a) providing a first sample containing an unknown carbohydrate mixture composition;

b) labelling of said carbohydrate mixture composition with a first fluorescent label;

c) adding a second sample having a known carbohydrate mixture composition pattern labelled with a second fluorescent label to said first sample;

d) generating electropherograms/chromatograms of the carbohydrate mixture composition of said sample using electrokinetic/chromatographic separation techniques combined with fluorescence or laser induced fluorescence detection, like capillary gel electrophoresis-laser induced fluorescence;

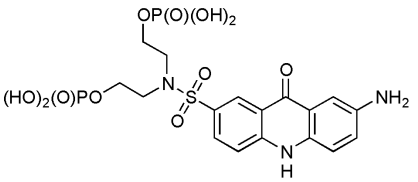
e) analyzing the identity and/or differences between the carbohydrate mixture composition pattern profiles of the first and the second sample,

wherein the first fluorescent label of the first sample is different to the second fluorescent label of the second sample and wherein at least one of the first fluorescent label and the second fluorescent label is a fluorescent dye as defined in any one of claims 1, 10, 11, 14 and ~~12 to~~ 15; and a fluorescent dye as defined in any one of claims 1, 10, 11, 14 and ~~12 to~~ 15.

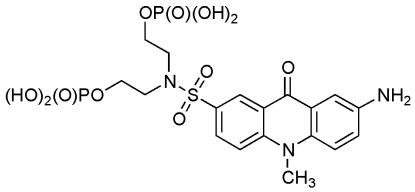
18. A kit or system according to any one of claims 16 or 17 further comprising a capillary gel electrophoresis-laser induced fluorescence apparatus, in particular, wherein the capillary gel electrophoresis-laser induced fluorescence apparatus is a capillary DNA-sequencer.

19. The use of a carbohydrate dye conjugate comprising fluorescent dyes as defined in any one of claims 1, 10, 11, 14~~12 to~~ and 15 in a method according to any one of claims 1, 10, 11, 14 and ~~to~~ 15.

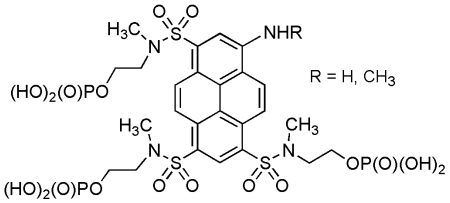
20. The use of a carbohydrate dye conjugate according to claim 19 wherein the dye is selected from the compounds of the formula below



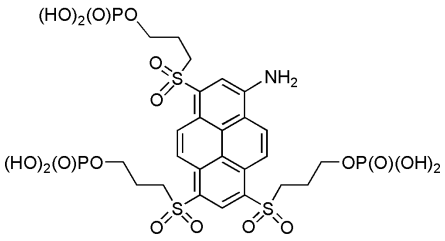
6-H



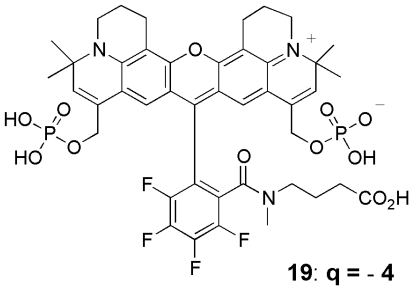
6-Me



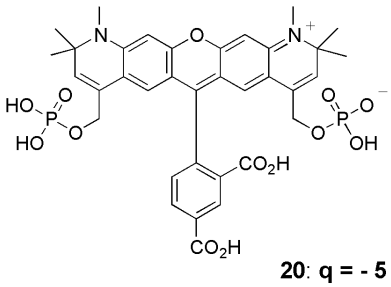
8-R



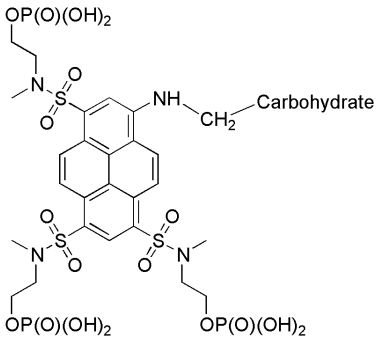
15



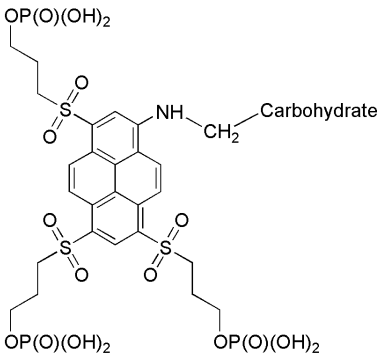
19: **q = - 4**



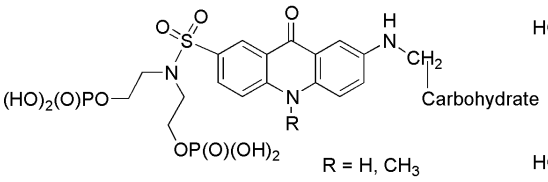
20: **q = - 5**



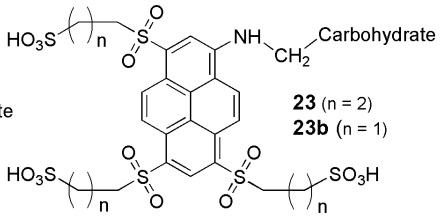
8-H-carbohydrate**: q = - 6**



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

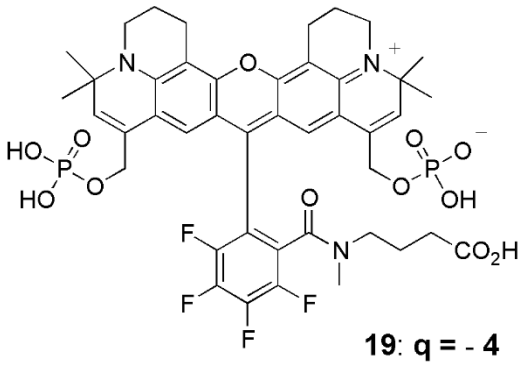


6-R-carbohydrate: **q = - 4**

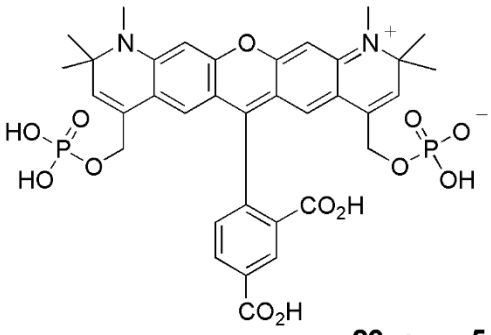


23, **23b**-carbohydrate: **q = - 3**

21. The use of a kit or composition in a method according to any of claims 1 to 15, said kit or composition comprising one or more of the dyes as defined in claims 1, 10, 11, 14 and ~~12 to~~ 15 or one or more of the carbohydrate dye conjugates of claims 19 or 20, or compound 19 or 20



19: **q = - 4**

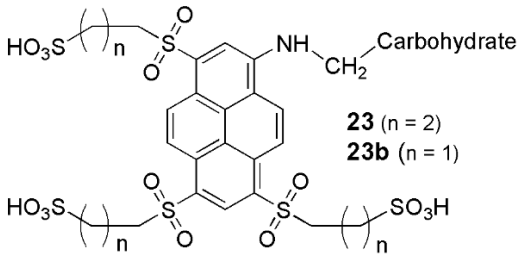


20: **q = - 5**

22. A calibration standard, like an oligosaccharide standard, including a fluorescence dye according to **Formula A**~~, **B**,~~ or **C** ~~or **D**~~ being conjugated with a carbohydrate, optionally further comprising at least one of compounds ***19***, ***20***.

23. A kit containing a calibration standard according to claim 22 and, optionally, instructions for use.

24. A compound having Formula 23 or 23b



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

25. A standard composition composed of carbohydrates or DNA base pair ladder or nucleic acid base standards labelled with a fluorescence dye according to **Formula A** or ~~**B**, in particular, of~~ **Formula C** ~~or **D**~~ or different dyes of **Formulae A** ~~to~~ and **C**~~**D**~~.

26. The standard composition according to claim 25 wherein the fluorescence dye is at least one dye selected from ***6-H***, ***6-Me***, ~~***8-R***,~~ ***15***, ***13a***, ***13b***, ***16***, ***18***, ***23*** and ***23b***, as defined in claim 20***.***

27. A kit containing a standard composition according to any one of claims 25 to 26, in particular, for use in a method according to any one of claims 1 to 15 and, optionally, instructions for use.