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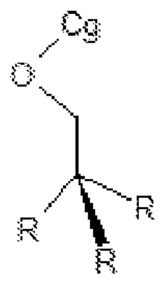
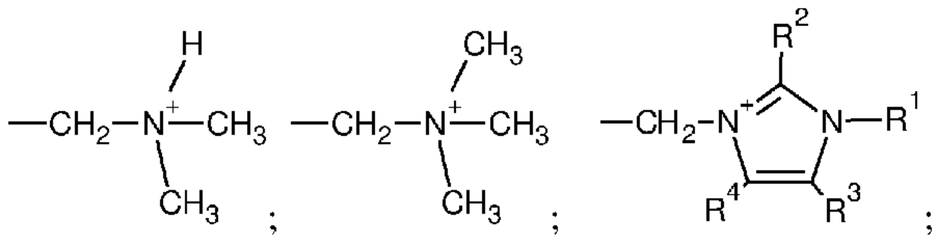
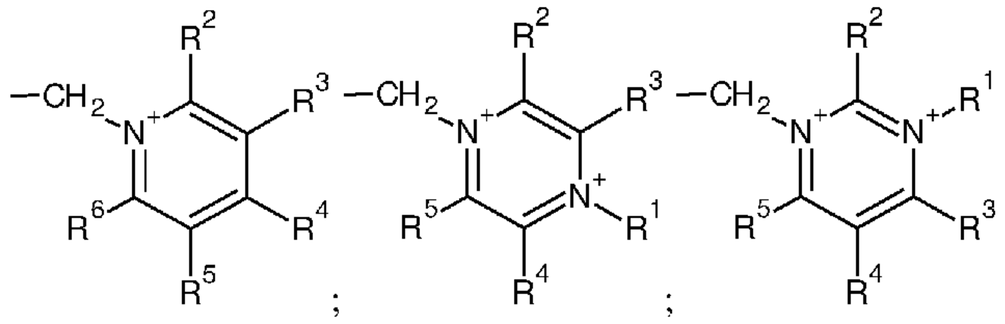
(54) Title: COMPOUNDS FOR MODIFICATION OF NEGATIVELY CHARGED CARRIER SURFACE, METHOD OF THEIR PREPARATION AND USE THEREOF



(I)



(II) ; (III) ; (IV) ;



(V) ; (VI) ; (VII)

(57) **Abstract:** The present invention relates to compounds of general formula (I) (I) wherein Cg is $-CH_2-C\equiv CH$ or $-CH_2-CH=CH_2$; R is independently selected from the group consisting of (Cl-C6)alkyl, which may be linear or branched; (C5-C6)cykloalkyl; R^1 is H, (C1-C6)alkyl, which may be linear or branched; or (C5-C6)cykloalkyl; R^2 to R^6 are independently selected from the group consisting of -H, (Cl-C6)alkyl, (Cl- C6)alkoxyl, $-N(R^7)2$, wherein R^7 is independently (Cl-C6)alkyl; wherein the compound of general formula (I) bears at least one positive charge. The invention further relates to the methods of synthesis thereof and their use for modification of negatively charged solid surfaces.

[Continued on next page]

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Compounds for modification of negatively charged carrier surface, method of their preparation and use thereof

Field of Art

The present invention relates to multiply positively charged substances – *anchors*, to which it is possible to easily covalently bind almost any *substance*, a method for their preparation and the use of such prepared *modifiers* for the modification of negatively charged surfaces. Negatively charged solid surfaces can then be modified by simply immersing the solid in an aqueous solution of the modifier. In this way, many materials used in practice can be prepared in a much easier manner. This is demonstrated by the preparation of a modified silica gel phase suitable for the chromatographic separation of enantiomers.

Background Art

When it is necessary to modify the surface of a solid so that the modifier adheres firmly to the surface, then covalent bonding of the surface with the modifier is usually used, i.e., a chemical reaction, which is a costly process requiring special equipment, chemicals and solvents, which must then be disposed of in an environmentally friendly manner.

Another modification method already uses ionic interactions, which utilise attractive electrostatic forces between the positive and negative charges present in the modifier and on the solid surface. For example, in some Pirkle-type chromatography columns for chiral separations (Pirkle, W. H. et al., A Useful and Conveniently Accessible Chiral Stationary Phase for the Liquid Chromatographic Separation of Enantiomers. In E. L. Eliel et al. (Eds.), Asymmetric Reactions and Processes in Chemistry (Vol. 185, pp. 245–260), 1982), the active substance is ionically bound by one negative charge on the active substance and one positive charge on a solid carrier (Pirkle, W. H. et al. J. Org. Chem., 1981, 46, 2935–2938), thus allowing easy preparation of columns that can also be used for preparative separations of enantiomers (Pirkle, W. H. et al. J. Org. Chem., 1982, 47, 4037–4040). Their main disadvantage, due to which this method of bonding is no longer preferred, is that the ionic bonding of the active substance to the solid phase is weak due to only one ionic bond. Thus the active substance elutes in more polar solvents. Besides, a carboxyl group of the active substance and an amino group covalently bonded to silica gel were used to form the ionic bond, i.e. it was necessary to use already chemically modified silica gel for the preparation of the column, which is significantly more expensive than unmodified silica gel.

Commonly used solids, to which oppositely charged substances are bound by ionic bonding, are ion exchangers (ionexes) whether for ion chromatography (Fritz, J. S. et al. Ion Chromatography (4 edition). 2009. Weinheim: Wiley-VCH), water treatment or other applications (Zagorodni, A. A. Ion exchange materials: properties and applications (1st ed.). 2007. Amsterdam ; Boston: Elsevier). In all these applications, equilibrium is used, where an excess of some ions replaces the ions still bound to the ionex. But the use of ionexes as carriers of active substances, which bind to the ionex by a strong ionic bond, is not common. One of the most widely used methods for modifying the surface of materials using electrostatic bonding is the layer-by-layer technique (Ariga, K. et al. Chem. Lett., 2013, 43(1), 36–68), which uses polyelectrolytes to form thin polymer films on the surface of the carrier. However, films prepared in this way usually have poor mechanical properties, unless they are subsequently covalently crosslinked.

The prior art lacks a universal and economically advantageous arrangement which would ensure a strong ionic bond of a non-polymeric substance (compound) to a solid carrier without eluting this substance in polar solvents.

Summary of the Invention

The present invention relates to the development of a synthesis of multiply positively charged anchors, which could be easily covalently attached to almost any substance containing at least one functional group selected from the group consisting of an azide group (-N_3), a hydroxyl group (-OH), a thiol group (-SH), an amine group (primary or secondary), a carbonyl group, a carboxyl group or a reactive functional derivative thereof, for example, a halo carbonyl, an anhydride, an active ester; an isocyanate group (-N=C=O) and an isothiocyanate group (-N=C=S), and the resulting modifier is bound by a strong ionic bond to the surface of a solid carrier which contains (even weak) negative charges. One of the practical applications of this method is, for example, the preparation of stationary phases based on modified silica gel and their use for chromatographic separations.

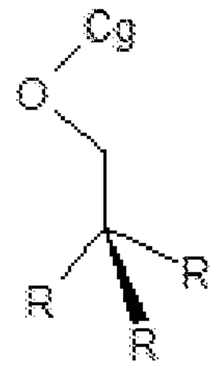
Figure 1 shows the general structure of the resulting system of the present invention, which comprises a positively charged anchor (with one to three positive charges), an optional (oligo ethylene glycol) linker and substances A (compounds A) attached thereto. The multiply positively charged anchor provides strong ionic bonding of substance A to the surface of the solid carrier; the linker allows to regulate the distance of substance A from the surface (according to the purpose of use); and the optional multiplier of the charged anchor allows up to seven multiple charged anchors to be bound to a single molecule, increasing the total charge from one, two or three up to

7, 14 or 21 positive charges, thus ensuring an even stronger ionic bond of the anchor to the solid carrier surface.

Quaternary ammonium groups were chosen as the positively charged groups, but only those that do not easily undergo Hofmann elimination (Lethesh, K. C. et al. RSC Adv., 2013, 4, 4472-4477) so that they can be used over a wide pH range.

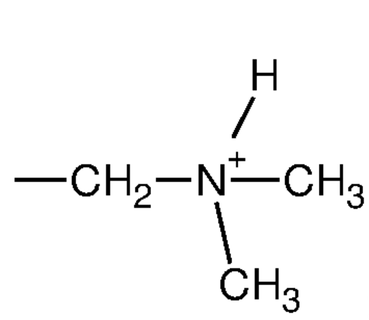
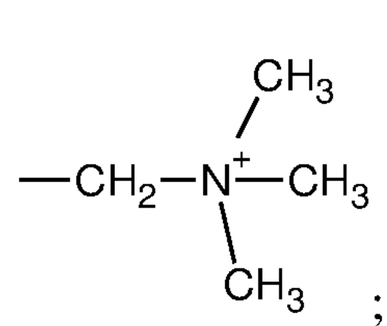
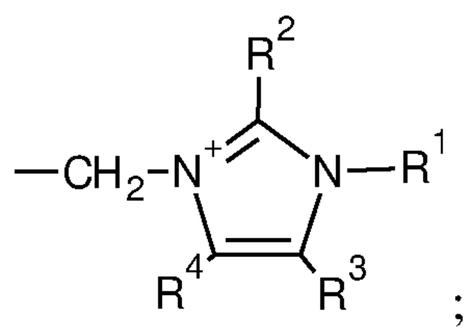
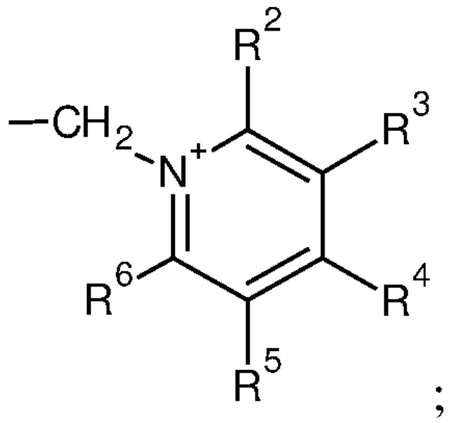
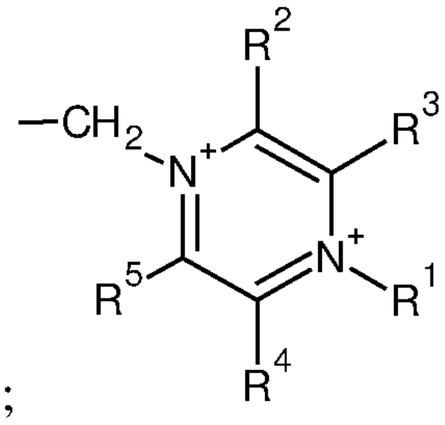
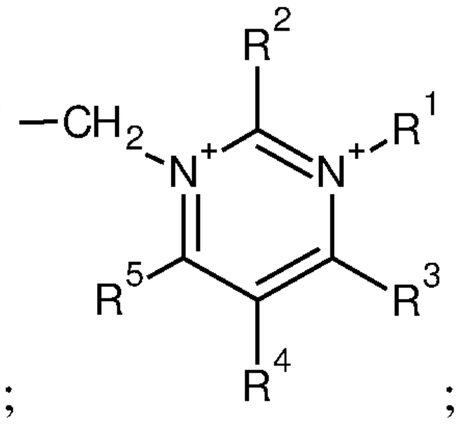
For the binding of substance A to the anchor, groups (Cg – 'clickable group') were chosen, which allow the formation of covalent bonds by means of so-called 'click' reactions. (Kolb, H. C. et al. Angew. Chem. Int. Ed., 2001, 40, 2004–2021). It is mainly a propargyl group, which in recent years has proved very useful in the reaction with azide compounds, with which CuAAC (copper-catalysed azide-alkyne cycloaddition) (Hein, J. E. et al. Chem. Soc. Rev., 2010, 39, 1302–1315) reaction forms stable triazole derivatives. Alternatively, it is possible to use an allyl group, which can be introduced into the substances described below under very similar conditions as the propargyl group. The allyl group allows easy attachment of other types of compounds, most easily those containing a thiol group, via a thiol-ene reaction (Hoyle, C. E. et al. Angew. Chem. Int. Ed., 2010, 49, 1540–1573). The propargyl and allyl groups can be further oxidatively cleaved to the corresponding carboxyl or aldehyde derivatives, which can also be used for covalent binding of substances by amide or imine bond formation, or for reductive amination to form amine, carbamate or thiocarbamate bonds. The amino group obtained by reductive amination can also be used to bind a carboxyl group-containing substance A via an amide bond.

The object of the present invention is a compound (anchor) of the general formula (I),



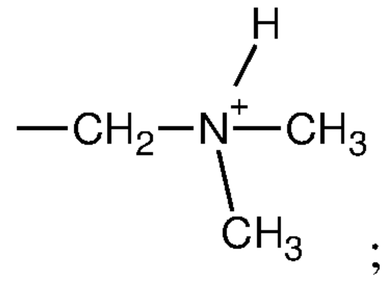
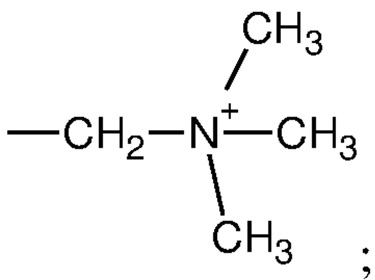
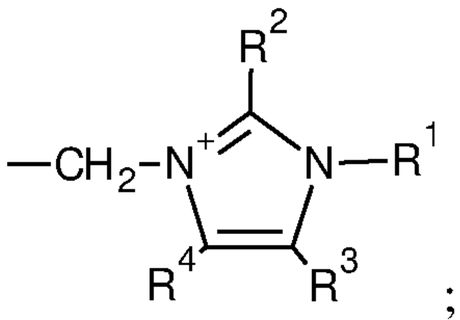
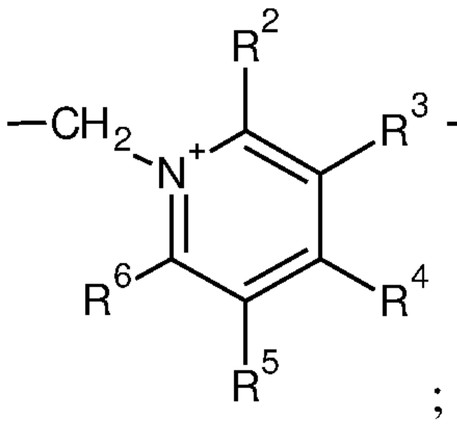
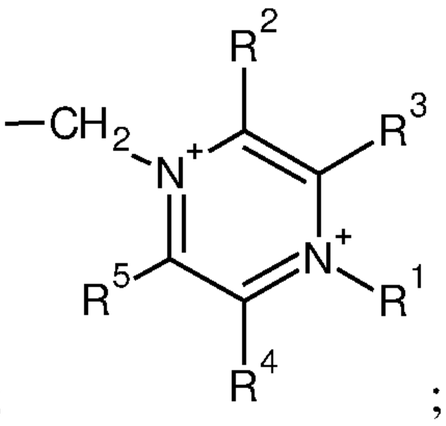
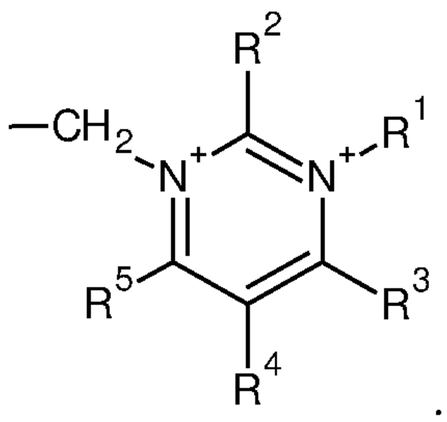
(I),

wherein Cg is propargyl (-CH_2-C≡CH) or allyl (-CH_2-CH=CH_2);

R is independently selected from the group consisting of (C1-C6)alkyl, which may be linear or branched; (C5-C6)cykloalkyl;  ;  ;  ;  ;  ;  ;

R^1 is H, (C1-C6)alkyl, which may be linear or branched; or (C5-C6)cykloalkyl;

R^2 to R^6 are independently selected from the group consisting of –H, (C1-C6)alkyl, (C1-C6)alkoxyl, $–N(R^7)_2$, wherein R^7 is independently (C1-C6)alkyl;

wherein at least one R is selected from the group consisting of  ;  ;  ;  ;  ;  .

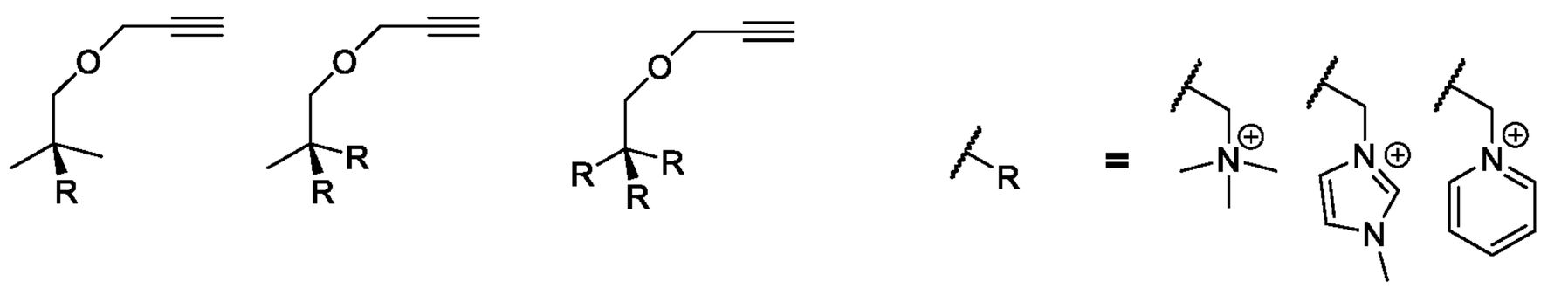
In one embodiment, R^1 is H, methyl, ethyl, cyclopentyl or cyclohexyl.

In one embodiment, R^2 to R^6 are independently selected from the group consisting of –H, methyl, ethyl, methoxyl, ethoxyl and $–N(R^7)_2$, wherein R^7 is independently methyl or ethyl.

As the number of positive charges in the molecule increases, the strength of the ionic bond with the negatively charged surface increases, but also the proximity of the positive charges increases

the instability of the molecule. The positive charges of the anchors of general formula (I) are compensated by anions, preferably selected from the group consisting of iodides, triflates (trifluoromethanesulphonates), carbonates and chlorides.

In one embodiment, the compounds (anchors) of general formula (I) are compounds, the structures thereof with one, two or three positive charges are shown in Scheme 1.



Scheme 1: Prepared positively charged anchors with one (K1), two (K2) or three (K3) permanent charges contained in the trimethylammoniomethyl (TMAM), methylimidazoliomethyl (MIMM) or pyridiniomethyl (PYRM) group.

Any substance A containing an azide functional group can be attached to the propargyl group (Cg) of the compound of general formula (I) using Huisgen cycloaddition of azide to form a triazole bridge. In this way, essentially any azide group-containing substance can be attached via the anchor of the general formula (I) to a solid carrier with negatively charged surface. Preferably, this substance A can be attached to the propargyl group by means of a CuAAC reaction, which is regioselective.

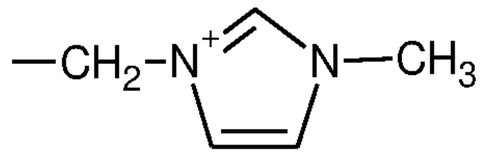
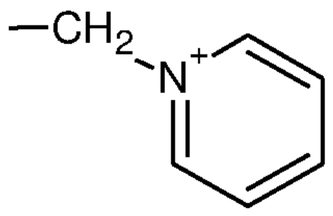
Essentially any thiol group-containing substance A can be attached to the allyl group (Cg) of the compound of general formula (I) by the above-mentioned thiol-ene reaction to form a sulphide bridge. It can also be readily converted, e.g. by ozonolysis, to a formylmethyl group to which amino group-containing substances A can be attached either to form an imine bond or by reductive amination to form an amine bond. The formylmethyl group can also be converted to an aminoethyl group by reductive amination and use it either directly to react with substance A containing a carboxyl (Hartman, T. et al. Tetrahedron Asymmetry, 2012, 23, 1571-1583), isocyanate or isothiocyanate functional group, or to conversion to an isocyanate or isothiocyanate reactive group to which substances A containing an amino or hydroxyl group can be attached. Both types of Cg groups can then be converted to a carboxymethyl derivative by standard oxidation reactions, to

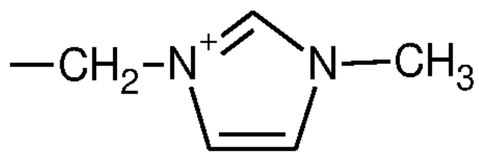
which amino group-containing substances can be attached to form an amide bond, e.g., using methods used in peptide chemistry.

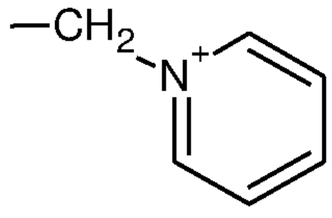
The solid support is preferably selected from the group comprising cation-exchange materials, most of which are commercially available:

- catex resins and zeolites (aluminosilicates) (Wang, S. et al. Chem. Eng. J., 2010, 156, 11–24) also used for water treatment
- stationary phase for ion-exchange chromatography (Haddad, P. R. et al. (Eds.). Chapter 3 Ion-Exchange Stationary Phases for Ion Chromatography. In Journal of Chromatography Library (pp. 29–77) 1990)
- Nafion (Kusoglu, A. et al. Chem. Rev., 2017, 117, 987–1104), copolymer of perfluoro-3,6-dioxa-4-methyl-7-octene sulphonic acid with tetrafluorethylen used commercially mainly in the form of membranes, e.g., in fuel cells and electrolysers
- silica gel (Ciriminna, R. et al. Chem. Rev., 2013, 113, 6592–6620.) – a very widespread sorbent used for chromatographic separations, which is very little ionised, but when using anchors with a larger number of charges, this material can also be used
- materials containing hydrated silica, for example a glass surface
- metal oxides, e.g. Al_2O_3, ZrO_2, TiO_2 (Nawrocki, J. et al. J. Chromatogr. A, 2004, 1028(1), 1–30), which are also used as chromatographic sorbents
- surfaces of uncharged materials modified to obtain a negative charge, e.g., by sulphonation (Koivula, R. et al. React. Funct. Polym., 2012, 72, 92–97) or plasma treatment (Jacobs, T. et al. Plasma Chem. Plasma Process., 2012, 32, 1039–1073)

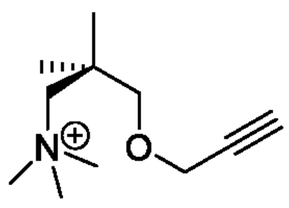
In one embodiment, the two R groups are methyl and the resulting molecule has one or two positive charges; in another embodiment one R group is methyl and the resulting molecule has two, three or four positive charges; in yet another embodiment no R group is methyl and the resulting molecule has at least three positive charges.

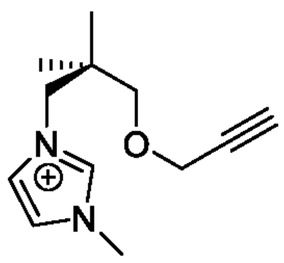
In one embodiment, R is selected from $-CH_3$, $-CH_2-N^+(CH_3)_3$,  and  .

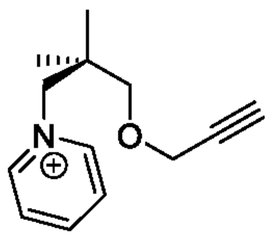
In one preferred embodiment, R is selected from $-CH_3$, $-CH_2-N^+(CH_3)_3$, 

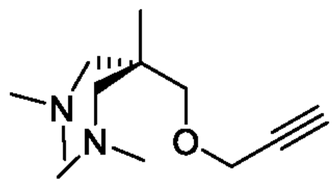
and  and Cg is propargyl.

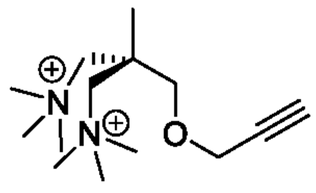
In a preferred embodiment, the compound of general formula (I) is selected from the group comprising:

 N,N,N,2,2-Pentamethyl-3-(prop-2-yn-1-yloxy)propan-1-aminium;

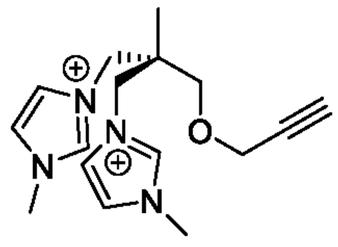
 3-(2,2-Dimethyl-3-(prop-2-yn-1-yloxy)propyl)-1-methyl-1H-imidazol-3-ium;

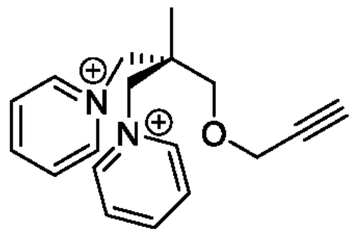
 1-(2,2-Dimethyl-3-(prop-2-yn-1-yloxy)propyl)pyridin-1-ium;

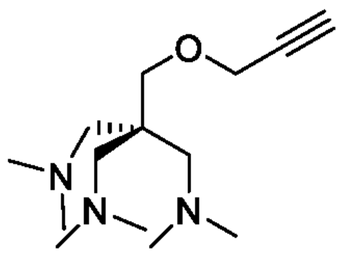
 N^1,N^1,N^3,N^3,2-Pentamethyl-2-((prop-2-yn-1-yloxy)methyl)propan-1,3-diamin;



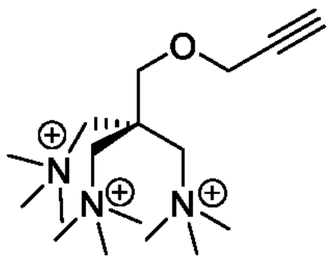
N^1,N^1,N^1,N^3,N^3,N^3,2-Heptamethyl-2-((prop-2-yn-1-yloxy)methyl)propan-1,3-diaminium;

 3,3'-(2-Methyl-2-((prop-2-yn-1-yloxy)methyl)propan-1,3-diyl)bis(1-methyl-1H-imidazol-3-ium);

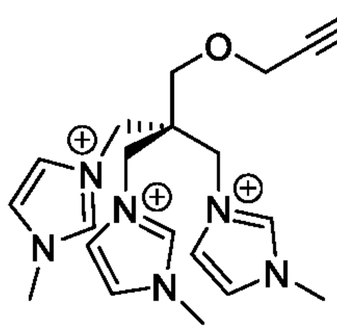
 1,1'-(2-Methyl-2-((prop-2-yn-1-yloxy)methyl)propan-1,3-diyl)bis(pyridin-1-ium);



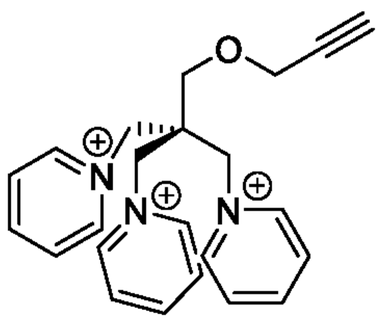
2-((Dimethylamino)methyl)-N^1,N^1,N^3,N^3-tetramethyl-2-((prop-2-yn-1-yloxy)methyl)propan-1,3-diamin;



N^1,N^1,N^1,N^3,N^3,N^3-Hexamethyl-2-((prop-2-yn-1-yloxy)methyl)-2-((trimethylamonio)methyl)propane-1,3-diaminium;



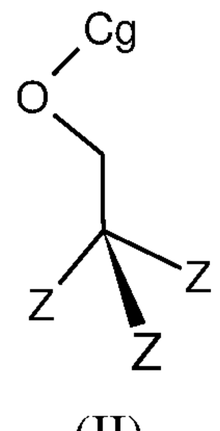
3,3'-(2-((1-Methyl-1H-imidazol-3-ium-3-yl)methyl)-2-((prop-2-yn-1-yloxy)methyl)propan-1,3-diyl)bis(1-methyl-1H-imidazol-3-ium);



1,1'-(2-((Prop-2-yn-1-yloxy)methyl)-2-(pyridin-1-ium-1-ylmethyl)propane-1,3-diyl)bis(pyridin-1-ium).

The present invention further relates to a method of preparation of a compound of general formula (I) which comprises the following steps:

(i) providing a compound of formula (II),

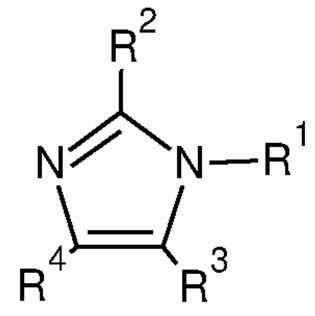
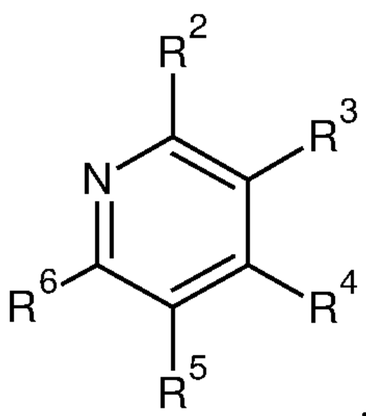
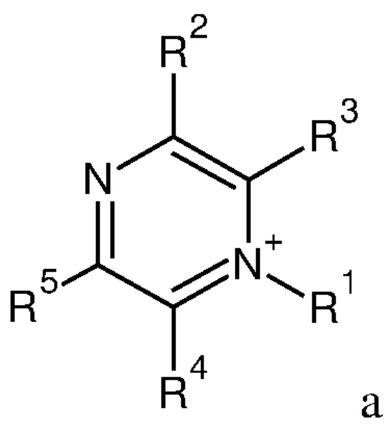
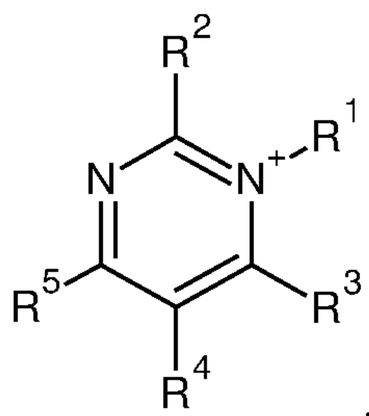


(II),

wherein Cg is propargyl (-CH_2-C≡CH) or allyl (-CH_2-CH=CH_2);

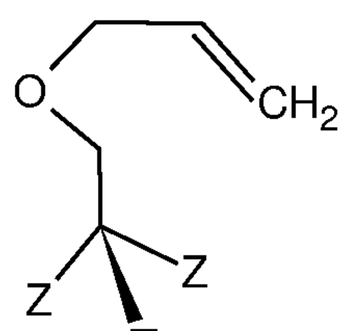
and Z is independently –CH_2OH, (C1-C6)alkyl, which may be linear or branched, or (C5-C6)cykloalkyl; wherein at least one Z is –CH_2OH; preferably Z is independently –CH_2OH or methyl;

(ii) at least one hydroxyl group of the compound of formula (II) reacts with anhydride or halide of fluorinated alkylsulphonic acid (especially selected from trifluoromethanesulphonic, tetrafluoroethanesulphonic, nonafluorobutanesulphonic, heptadecafluorobutanesulphonic acid), preferably with trifluoromethanesulphonic acid anhydride (Tf_2O), to form fluorinated alkylsulphonic acid ester;

(iii) the fluorinated alkylsulphonic acid ester from step (ii) reacts with at least one compound selected from the group consisting of trimethylamine, dimethylamine,  ,  ,  a  , wherein R^1 to R^6 are as defined above, preferably the compound is selected from the group consisting of trimethylamine, dimethylamine, 1-methylimidazole and pyridine, to give a compound of general formula (I);

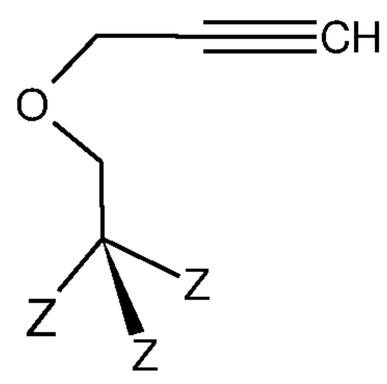
(iv) if dimethylamine is used in step (iii), the last step is the quaternisation of the dimethylamine group to a trimethylammonium group by methylating agent, preferably selected from the group comprising methyl halides, dimethyl sulphate and methyl esters of alkyl or arylsulphonic acids.

Step (i) of providing a compound of formula (II) comprises providing compounds of general formula (IIa)



(IIa)

and compounds of general formula (IIp)

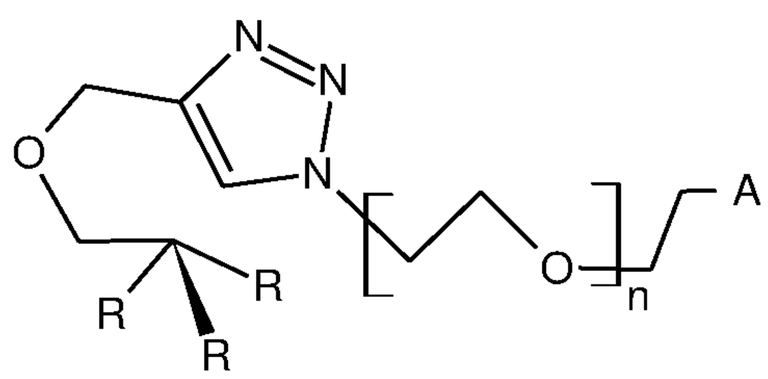


(IIp),

wherein Z is as defined above.

Compounds of general formula (IIa) and (IIp) are commercially available or can be prepared according to known procedures – IIa (Z=1×CH_2OH,2×CH_3) (Effenberger, F. et al. Tetrahedron Asymmetry, 1995, 6(1), 271–282), IIp (Z=2×CH_2OH,1×CH_3) (Dyke, J. C. et al. J. Mater. Chem., 2012, 22(43), 22888), IIp (Z=3×CH_2OH) (Feng, Y. et al. Nucl. Med. Biol., 2018, 61, 1–10).

A further object of the present invention is a modifier of a surface of a negatively charged carrier, the modifier having the general formula (III),



(III),

which comprises a substance A linked via an oligoethylene glycol linker,

wherein R is as defined above and *n* is an integer in the range of from 0 to 20, preferably from 2 to 10, more preferably from 3 to 7.

Substance A is preferably selected from the group comprising cyclodextrin (CD) derivatives (Crini, G. Chem. Rev., 2014, 114(21), 10940–10975) used for chiral separations due to their ability of inclusion complexation or also the ability to serve as a skeleton to which a large number of other groups can be covalently bound, e.g., phenylcarbamoyl groups (Yang, B. et al. Electrophoresis, 2017, 38, 1939–1947.) and thereby multiply the efficiency; amino acid derivatives that can be used as chiral selectors, such as N-(3,5-dinitrophenyl)amino acid derivatives used in Pirkle-type columns (Pirkle, WH et al. J. Chromatogr. A, 1986, 369, 175-177); or other modifiers hitherto used for the preparation of chiral solid phases using a covalent bond (Teixeira, J. et al. Molecules, 2019, 24, 865) based on macrocyclic compounds, proteins, carbohydrates, crown ethers or cinchonine derivatives.

In a preferred embodiment, the chiral modifier is amylose or cellulose, with free hydroxyl groups or modified by reaction with phenylisocyanates (Ali, I. et al. Sep. Purif. Rev., 2009, 38, 97–147),

peptides with an amino acid number of 2 to 200 or proteins used hitherto in covalently bonded form (Bocian, S. et al. J. Sep. Sci., 2016, 39, 83–92).

The oligoethylene glycol linker serves to regulate the distance of the substance A binding site from the surface of the negatively charged carrier. Substance A is linked via the oligoethylene glycol linker to the compound of general formula (I) by means of the triazole bridge formed by the addition of azide (oligoethylene glycol linker end group) to the propargyl group of the compound of general formula (I) (the modifier is derived from the anchor of general formula (I), wherein Cg is propargyl).

For substance A to be attached to the oligoethylene glycol linker, it must contain at least one functional group selected from the group comprising an azide group ($-N_3$), a hydroxyl group (-OH), a thiol group (-SH), an amine group (primary or secondary), a carbonyl group, a carboxyl group or a reactive functional derivative thereof, for example a halo carbonyl, an anhydride, an active ester; an isocyanate group (-N=C=O), an isothiocyanate group (-N=C=S), a leaving group allowing nucleophilic substitution on an adjacent carbon (halogen, alkyl- or arylsulphonyloxy group). This reactive functional group then reacts with the oligoethylene glycol derivative containing, in addition to the end azide group, for example an amino group (amino-azido-oligoethylene glycol), a carboxyl group (carboxyl-azido-oligoethylene glycol), a carbonyl group (carbonyl-azido-oligoethylene glycol) or hydroxyl group (azido-oligoethylene glycol) at the other end, to form a covalent bond between the above functional group of substance A and the amino, carboxyl, carbonyl or hydroxyl group of the oligoethylene glycol linker, which is subsequently attached via an azide group to the propargyl group of the compound of general formula (I) by Huisgen cycloaddition to form a triazole bridge.

In one preferred embodiment, substance A is α-, β-, or γ-cyclodextrin. The oligoethylene glycol linker is attached to the cyclodextrin via position 6 of the glucose unit of cyclodextrin by converting the OH group to an amino group, to which the oligoethylene glycol linker, attached to the anchor of general formula (I) via the triazole bridge, is bound.

Another object of the invention is a method of synthesis of modifiers of general formula (III), wherein the bound substance A is a covalently attached α-, β-, or γ-cyclodextrin (hereinafter CD modifier), which comprises the following steps:

(i) reaction of monotosyl of α-, β-, or γ-cyclodextrin (Popr, M. et al. Beilstein J. Org. Chem., 2014, 10, 1390–1396) with an amino-azido-oligoethylene glycol linker with the number of ethylene glycol units from 1 to 20, preferably from 2 to 10, more preferably from 4 to 8 (Tran, F. et al.

Molecules, 2013, 18, 11639-11657), optionally followed by acetylation of the free amino group, to give a cyclodextrin derivative with attached oligoethylene glycol linker terminated with a reactive azide group;

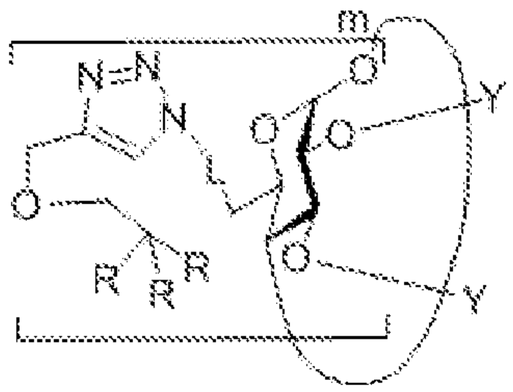
(ii) binding of the charged anchor of general formula (I), wherein Cg is propargyl, by azido-alkyne Huisgen cycloaddition (CuAAC) to the CD derivatives prepared in step (i) to give a modifier of general formula (III), wherein the bound substance A is α-, β-, or γ-cyclodextrin.

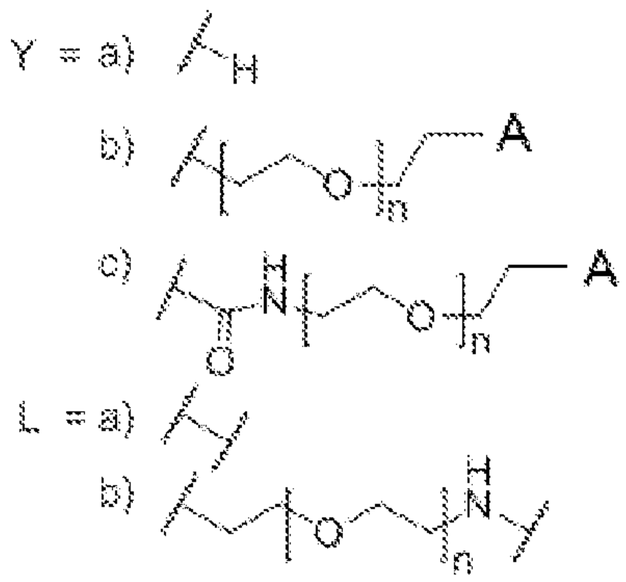
Alternatively, substance A, if it contains an $-N_3$ group, can be attached directly to the anchor of general formula (I), wherein Cg is propargyl, by azido-alkyne Huisgen cycloaddition (without the use of the oligoethylene glycol linker). This gives a surface modifier of the negatively charged carrier of general formula (III), where *n* is 0. An example of such substance A is a 6-azido-CD derivative (Boffa, L. et al. New J. Chem., 2010, 34, 2013.).

In one embodiment, between steps (i) and (ii), a phenylcarbamoyl group is introduced, which may be further independently substituted with -Cl or methyl group, preferably in position 3 and 5, to all free hydroxyl groups and amino group of the CD intermediate prepared in step (i). The cyclodextrin modifier thus obtained contains phenylcarbamoyloxy groups instead of OH groups; the bound substance A is thus in this embodiment the phenylcarbamoyl derivative of cyclodextrin.

In one embodiment, the CD modifier may contain, in addition to the CD unit, a fluorescent group that can be used to easily monitor the binding strength of the modifier to solids. It can be prepared, for example, by introducing a naphthalimide group (Dian, J. et al. Monatshefte Für Chem. - Chem. Mon., 2017, 148, 1929–1935) onto the amino group of the CD intermediate prepared in step (i) instead of acetylating the free amino group, followed by CuAAC reaction with the charged anchor as in step (ii).

In one embodiment, the surface modifier of the negatively charged carrier further comprises a multiplier located between the bound substance A and the anchor of general formula (I). The multiplier is preferably the most common and cheapest of the above-mentioned cyclodextrins - β-cyclodextrin (cyclic oligosaccharide composed of 7 glucose units), or its other commercially available analogues α- and γ-cyclodextrin (containing 6 and 8 glucose units). The result is a substance of general formula (IV),





(IV),

wherein *m* is an integer in the range of from 6 to 8, the value of which depends on the number of anchors that the given type of multiplier is able to bind to itself (in the case of β-CD modified on primary hydroxyls it is at most 7) and the anchor is the compound of general formula (I). The bound substance A is defined above. L is a bond or oligoethylene glycol linker $–(CH_2)_2–(O–(CH_2)_2)_n–NH–$, covalently attached to the CD multiplier via position 6 of the cyclodextrin glucose unit whose OH group has been converted to an amino group; wherein *n* is an integer from 1 to 20; Y represents possible substituents on the secondary hydroxyls of CD. Y is selected from the group consisting of H, an oligoethylene glycol linker with attached substance A, wherein the oligoethylene glycol linker may be attached to the hydroxyl group of CD via ether or carbamate bond, and wherein at least one Y of the modifier of formula (IV) is not H. In the case of β-CD, it is possible to covalently bind from one to 14 molecules of substance A, optionally using oligoethylene glycol linkers, preferably using an ether (b) or carbamate (c) bond.

The resulting substance of general formula (IV) contains a high number of positive charges concentrated in a small volume and is thus capable of very strong ionic interaction with the surface of a negatively charged solid.

The object of the present invention is also a method of preparation of the modifier of general formula (IV), wherein the multiplier is the cyclodextrin derivative per-(6-azido-6-deoxy)-β-cyclodextrin, which comprises the following steps:

(i) The compound of general formula (I) according to the present invention is prepared, wherein Cg is a propargyl group;

(ii) Substance A as defined above, preferably selected from the group consisting of phenylcarbamoyl, N-(3,5-dinitrophenyl) amino acid derivatives, macrocyclic compounds, peptides and proteins, carbohydrates, crown ethers, cinchonine derivatives, is attached to free hydroxyl groups of per-6-azido-β-cyclodextrin (Jicsinszky, L. et al. Beilstein J. Org. Chem., 2016, 12, 2364–2371);

(iii) The compound of general formula (I) from step (i) reacts with the multiplier comprising azide groups and the attached substance A from step (ii) to form the compound of general formula (IV).

Another object of the present invention is the use of the compound of general formula (I), (III) or (IV) for binding of substance A to the surface of a negatively charged solid (carrier), which may be selected from the group comprising catex resins, zeolites, stationary phases for ion-exchange chromatography, copolymer of perfluoro-3,6-dioxa-4-methyl-7-octenesulphonic acid with tetrafluoroethylene, silica gel, glass, metal oxides, and the use of the compound of general formula (I), (III) or (IV) for chromatographic separation and purification of substances, or as a sorbent for solid-phase extraction or as a heterogeneous catalyst.

Type (III) and (IV) modifiers with optionally attached chromophore can be attached to negatively charged carriers selected from the group comprising catex resins and zeolites, stationary phases for ion-exchange chromatography, silica gel, glass, metal oxides, surfaces of uncharged materials modified to obtain a negative charge, e.g. by sulphonation or plasma treatment, by mixing the carrier with an aqueous solution of the modifier, preferably with a 0.1 to 1% aqueous solution of the modifier. If the modifier contains a bound chromophore, the course of the reaction can be monitored spectrophotometrically, for example until the decrease in UV absorption is stopped. Under these conditions (depending on the carrier), the modifier binding half-life is in the order of hours at most. Furthermore, in the case of such modified carriers, the binding strength of the modifier to the carrier was determined by eluting with eluents of different polarity and ionic strength.

Brief description of drawings

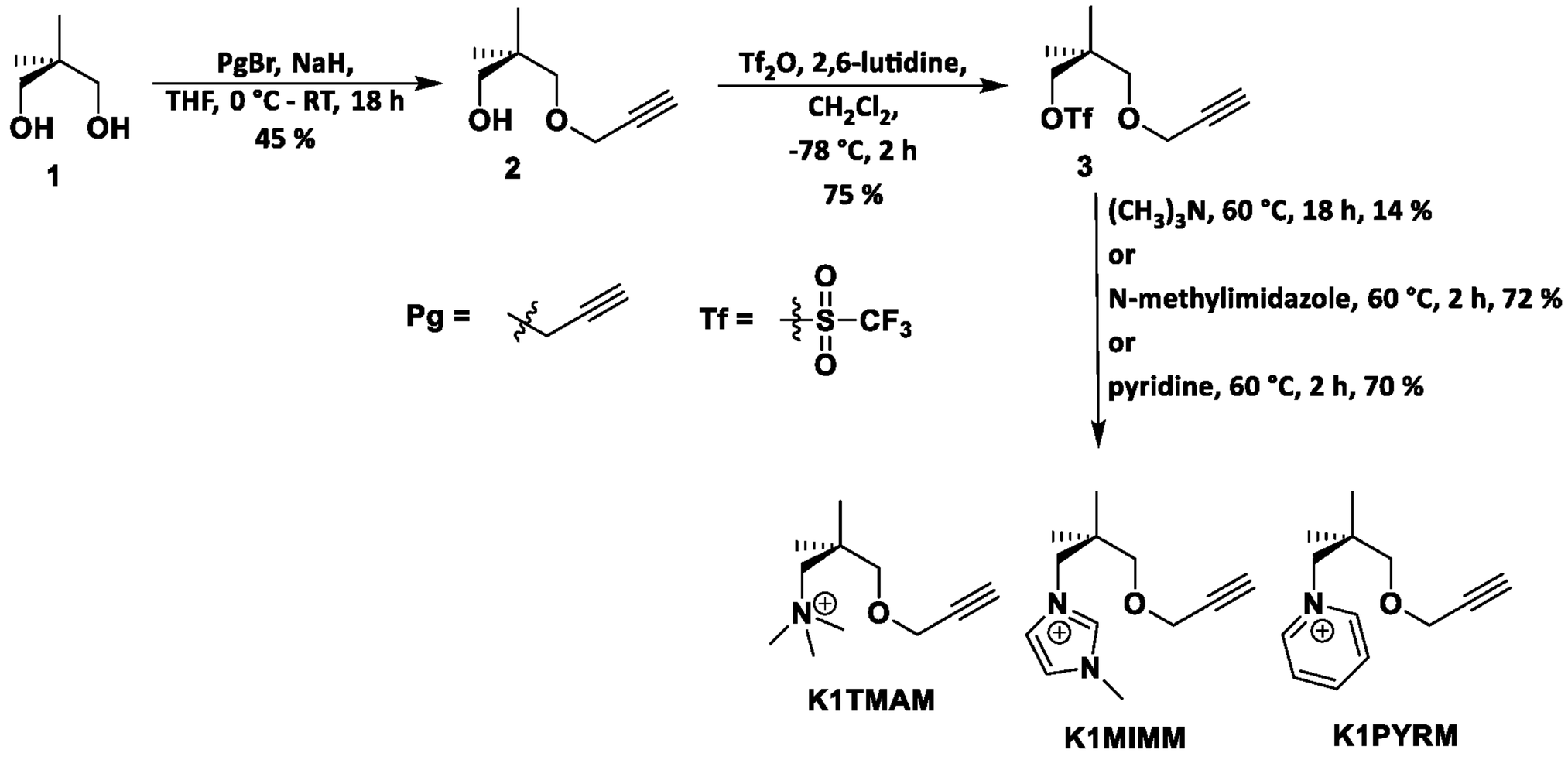
Figure 1: General scheme of modifying the surface of a negatively charged solid using a multiply positively charged modifier, which is the subject-matter of the patent.

Examples

Example 1: Syntheses of compounds of formula (I), wherein Cg is a propargyl group

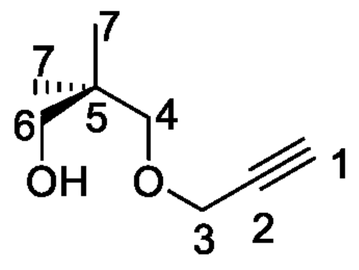
1.1 Synthesis of K1 anchors

The synthesis of K1 anchors of formula I with one charged TMAM, MIMM or PYRM group is described in Scheme 2, followed by detailed procedures for the preparation of intermediates and final products shown in the scheme.



Scheme 2: Synthesis of K1 anchors

2,2-Dimethyl-3-(prop-2-yn-1-yloxy)propan-1-ol (2).



2,2-Dimethylpropan-1,3-diol **1** (26.3 g, 0.252 mol) was dissolved in dry THF (40 mL). The mixture was cooled to 0 °C and sodium hydride (1.0 g, 25.2 mmol) was carefully added portion-wise. The reaction mixture was stirred at 0 °C for 1 hour. Propargyl bromide (2.80 mL, 25.2 mmol) was added dropwise and the reaction mixture was allowed to stir overnight at laboratory temperature. The reaction mixture was monitored by TLC ($CHCl_3$/MeOH 30/1 - detection with aqueous basic potassium permanganate solution). The reaction mixture was filtered through celite and extracted between toluene (100 mL) and water (100 mL). The organic phase was washed with water (3 × 100 mL), dried over $MgSO_4$ (0.7 g), filtered and evaporated on a rotary vacuum evaporator at 50 °C. The product was dried using an oil rotary pump at 50 °C and obtained as a yellowish oil in a yield of 45 % (1.64 g). IR(KBr): 3433 ν(O-H), 3287 ν(C-H alkyne), 2963 ν(C-H), 2921 ν(C-H), 2866 ν(C-H), 2120 ν(C-C alkyne), 1476 ν(C-H), 1455 ν(CH), 1434 ν(C-H), 1357 ν(C-H), 1270 ν(C-H), 1099 ν(C-O), 1044 ν(C-O) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 4.14 (d, *J* = 2.4 Hz, 2H, H-3), 3.44 (d, *J* = 6.1 Hz, 2H, H-6), 3.37 (s, 2H, H-4), 2.43 (t, *J* = 2.4 Hz, 1H, H-1), 2.20 (t, *J* = 6.1 Hz, 1H),

0.93 (s, 3H, H-7) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 78.56 (C-2), 78.34 (C-4), 74.46 (C-1), 71.02 (C-6), 58.66 (C-3), 38.16 (C-5), 21.78 (C-7) ppm. ESI MS: for $C_8H_{14}O_2$ calcd.: *m/z* 142.1, found 143.0 $[M+H]^+$. HRMS: for $C_8H_{14}O_2$ calcd.: *m/z* 142.0994, found 143.1064 $[M+H]^+$, Δ 2.1 ppm.

2,2-Dimethyl-3-(prop-2-yn-1-yloxy)propyl trifluormethansulphonate (3).

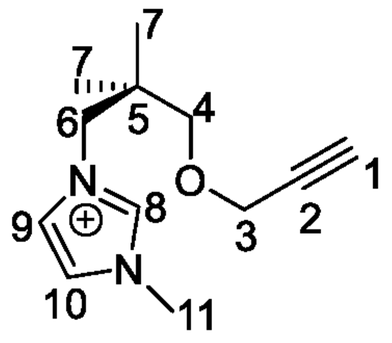
2,2-Dimethyl-3-(prop-2-yn-1-yloxy)propan-1-ol **2** (1.40 g, 9.85 mmol) was dissolved in dry dichloromethane (45 mL) and 2,6-lutidine (1.06 g, 9.85 mmol) was added. The reaction mixture was cooled to -78 °C, Tf_2O (2.78 g, 9.85 mmol) was added dropwise to the reaction mixture, and the reaction mixture was stirred at this temperature for 2 hours. The reaction mixture was monitored by TLC (hexane/EtOAc 10/1 – detection with aqueous basic potassium permanganate solution or 1% ethanolic solution of 4-(4-nitrobenzyl)pyridine and then by concentrated aqueous ammonia solution). The reaction mixture was extracted between Et_2O (120 mL) and 1M HCl (80 mL). The organic phase was then extracted with saturated aqueous solutions of $NaHCO_3$ (80 mL) and NaCl (80 mL). The organic phase was dried over $MgSO_4$ (0.7 g), filtered and evaporated on a rotary vacuum evaporator at laboratory temperature. The product was dried at laboratory temperature using an oil rotary pump and obtained as a light brown oil in a yield of 75 % (2.04 g). IR(KBr): 3300 ν(C-H alkyne), 2968 ν(C-H), 2926 ν(C-H), 2854 ν(C-H), 2122 ν(C-C alkyne), 1482 ν(C-H), 1422 ν(C-H), 1248 ν(C-H), 1204 ν(C-H), 1150 ν(C-O), 1102 ν(C-O) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.33 (s, 2H, H-6), 4.14 (d, *J* = 2.4 Hz, 2H, H-3), 3.31 (s, 2H, H-4), 2.43 (t, *J* = 2.4 Hz, 1H, H-1), 1.02 (s, 6H, H-7) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 118.83 (q, *J* = 319.7 Hz, C-8), 81.84 (C-6), 79.46 (C-2), 74.77 (C-1), 74.18 (C-4), 58.63 (C-3), 36.15 (C-5), 21.44 (C-7) ppm.

N,N,N,2,2-Pentamethyl-3-(prop-2-yn-1-yloxy)propan-1-aminium (K1TMAM).

2,2-Dimethyl-3-(prop-2-yn-1-yloxy)propyl trifluoromethanesulphonate **3** (2.29 g, 8.36 mmol) was mixed with freshly distilled and dried trimethylamine (60 ml) at -78 °C. The reaction vessel was carefully sealed, heated to 60 °C and stirred overnight. The reaction mixture was monitored by TLC (MeOH/HOAc/1% aqueous solution of NH_4OAc 10/1/9 – detection with aqueous basic solution of potassium permanganate). After cooling the reaction vessel to 0 °C and opening it, the trimethylamine was evaporated at room temperature by standing in a fume hood. The crude product was extracted between water (60 mL)

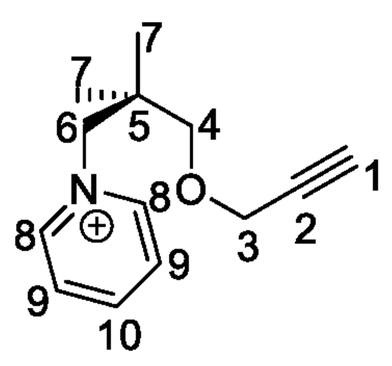
and toluene (60 mL). The aqueous phase was washed with toluene (60 mL) and evaporated on a rotary vacuum evaporator at 50 °C. The residue (1.77 g) was dissolved in water (40 mL) and purified on strong Dowex 50W catex resin (17 mL). The elution solutions were water, 5% aqueous solution of NH_3, concentrated aqueous solution of NH_3, 5% aqueous solution of NH_4HCO_3 and 20% aqueous solution of NH_4HCO_3. The latter elution solution containing the product was evaporated on a rotary vacuum evaporator at 50 °C and the product (0.74 g) was extracted into MeOH (10 mL). The solution was filtered and evaporated on a rotary vacuum evaporator at 50 °C. The product was dried at 60 °C using an oil rotary pump and obtained as a light brown solid in a yield of 14 % (0.28 g). IR(KBr): 3437 ν(O-H), 3396 ν(O-H), 3168 ν(C-H alkyne), 3113 ν(C-H), 2977 ν(C-H), 2899 ν(C-H), 2857 ν(C-H), 2612 ν(C=O), 2104 ν(C-C alkyne), 1658 ν(C=O), 1422 ν(C-H), 1431 ν(C-H), 1356 ν(C-H), 1266 ν(C-H), 1248 ν(C-H), 1093 ν(C-O) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ = 4.19 (d, *J* = 2.4 Hz, 2H, H-3), 4.06 (s, 2H, H-6), 3.87 (s, 3H, H-11), 3.54 (t, *J* = 2.4 Hz, 1H, H-1), 3.35 (s, 4H, H-4, H-6), 3.17 (s, 9H, H-8), 1.50 (s, 1H, H-10), 1.10 (s, 6H, H-7) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 157.64 (C-9), 79.81 (C-2), 77.78 (C-1), 76.18 (C-4), 72.21 (C-6), 57.91 (C-3), 54.46 (C-8), 36.67 (C-5), 24.86 (C-7) ppm. ESI MS: for $C_{11}H_{22}NO^+$ calcd.: *m/z* 184.2, found 184.2 $[M]^+$. HRMS: for $C_{11}H_{22}NO^+$ calcd.: *m/z* 184.1696, found 184.1693 $[M]^+$, Δ 1.6 ppm.

3-(2,2-Dimethyl-3-(prop-2-yn-1-yloxy)propyl)-1-methyl-1H-imidazol-3-ium (K1MIMM). 2,2-Dimethyl-3-(prop-2-yn-1-yloxy)propyl trifluoromethanesulphonate **3** (2.04 g, 7.44 mmol) was dissolved in N-methylimidazole (38 mL), the reaction mixture was heated to 60 °C and stirred at this temperature for 2 hours. The reaction mixture was monitored by TLC (MeOH/HOAc/1% aqueous solution NH_4OAc 10/1/9 – detection with aqueous basic solution of potassium permanganate). N-methylimidazole was distilled off under reduced pressure (1-10 mbar) at 80 °C. The crude product was extracted between water (60 mL) and toluene (160 mL). The aqueous phase was evaporated on a rotary vacuum evaporator at 55 °C. The product was dried at 85 ° C using an oil rotary pump and obtained as a light brown oil in a yield of 72 % (1.93 g). IR(KBr): 3150 ν(C-H alkyne), 3117 ν(C-H), 2968 ν(C-H), 2875 ν(C-H), 2113 ν(C-C alkyne), 1628 ν(imidazole), 1580 ν(imidazole), 1482 ν(C-H), 1431 ν(C-H), 1356 ν(C-H), 1254 ν(C-H), 1225 ν(C-H), 1159 ν(C-O), 1099 ν(C-O) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ = 8.98 (s, 1H, H-8), 7.71 (s, 1H, H-10), 7.61 (s, 1H, H-9), 4.18 (d, *J* = 2.4 Hz, 2H, H-3), 4.06 (s, 2H, H-6), 3.87 (s, 3H, H-11), 3.49 (t, *J* = 2.4 Hz, 1H, H-1), 3.16 (s, 2H, H-4), 0.90 (s, 6H, H-7) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 137.32 (C-8), 123.71 (C-9), 123.17 (C-10), 80.04 (C-2), 77.54 (C-1), 74.90



(C-4), 57.94 (C-3), 55.62 (C-6), 35.83 (C-11), 35.68 (C-5), 22.17 (C-7) ppm. UV-VIS (acetone), λ_{max1}, nm: 216.0, λ_{max2}, nm: 320.0, 3×10^{-2} M. ESI MS: for $C_{12}H_{19}N_2O^+$ calcd.: *m/z* 207.1, found 207.2 $[M]^+$. HRMS: for $C_{12}H_{19}N_2O^+$ calcd.: *m/z* 207.1492, found 207.1464 $[M]^+$, Δ 13.5 ppm.

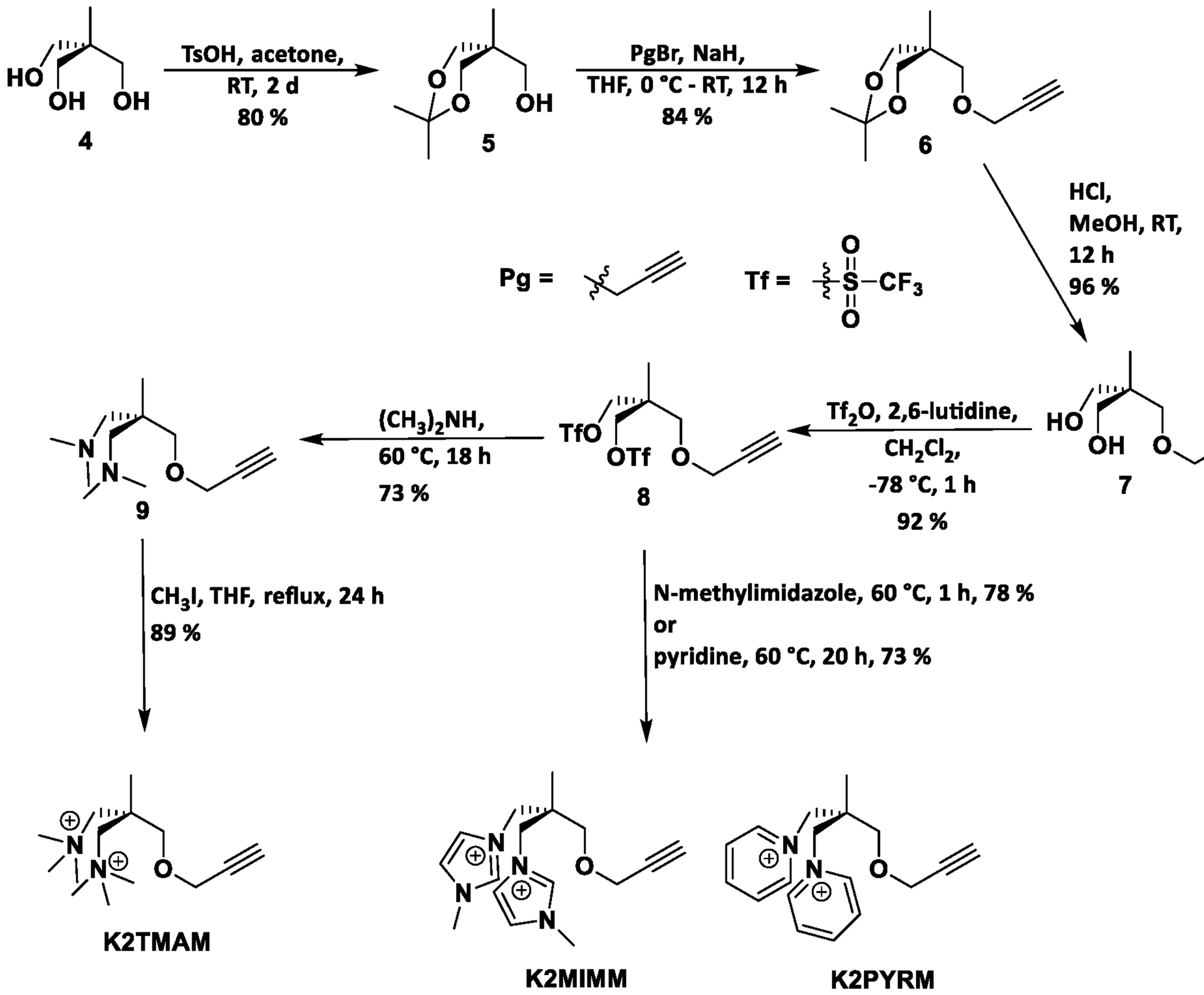
1-(2,2-Dimethyl-3-(prop-2-yn-1-yloxy)propyl)pyridin-1-ium (K1PYRM).



2,2-Dimethyl-3-(prop-2-yn-1-yloxy)propyl trifluoromethanesulphonate **3** (0.31 g, 1.13 mmol) was dissolved in dry pyridine (6 mL), the reaction mixture was heated to 60 °C and stirred at this temperature for 2 hours. The reaction mixture was monitored by TLC (MeOH/HOAc/1% aqueous solution of NH_4OAc 10/1/9 – detection with aqueous basic solution of potassium permanganate). The pyridine was distilled off under reduced pressure (1-10 mbar) at 60 °C. The crude product was extracted between water (20 mL) and toluene (20 mL). The aqueous phase was evaporated on a rotary vacuum evaporator at 50 °C. The product was dried at 50 °C using an oil rotary pump and obtained as a light brown oil in a yield of 70 % (0.28 g). IR(KBr): 3258 ν(pyridine), 3141 ν(C-H alkyne), 3091 ν(C-H), 2971 ν(C-H), 2878 ν(C-H) 2116 ν(C-C alkyne), 1634 ν(pyridine), 1494 ν(C-H), 1257 ν(pyridine), 1228 ν(C-H), 1162 ν(pyridine), 1096 ν(C-O), 1033 ν(C-O) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ = 8.90 (m, 2H, H-8), 8.66 (tt, *J* = 7.8, 1.4 Hz, 1H, H-10), 8.16 (dd, *J* = 7.9, 6.5 Hz, 2H, H-9), 4.54 (s, 2H, H-6), 4.16 (d, *J* = 2.4 Hz, 2H, H-3), 3.51 (t, *J* = 2.4 Hz, 1H, H-1), 3.21 (s, 2H, H-4), 0.94 (s, 6H, H-7) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 145.92 (C-10), 145.73 (C-8), 127.67 (C-9), 79.85 (C-2), 77.77 (C-1), 74.59 (C-4), 66.93 (C-6), 57.87 (C-3), 36.52 (C-5), 21.98 (C-7) ppm. UV-VIS (acetone), λ_{max1}, nm: 218.5, λ_{max2}, nm: 320.0, 3×10^{-2} M. ESI MS: for $C_{13}H_{18}NO^+$ calcd.: *m/z* 204.1, found 204.2 $[M]^+$. HRMS: for $C_{13}H_{18}NO^+$ calcd.: *m/z* 204.1383, found 204.1378 $[M]^+$, Δ 2.4 ppm.

1.2 Synthesis of K2 anchors

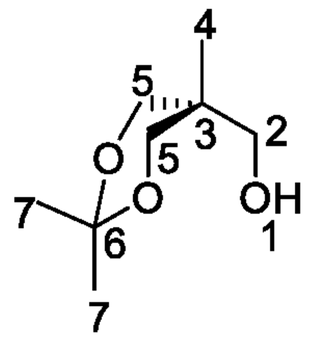
The synthesis of K2 anchors of formula I with two charged groups TMAM, MIMM or PYRM is described in Scheme 3. The following are detailed procedures for the preparation of compounds shown in the scheme.



Scheme 3: Synthesis of K2 anchors

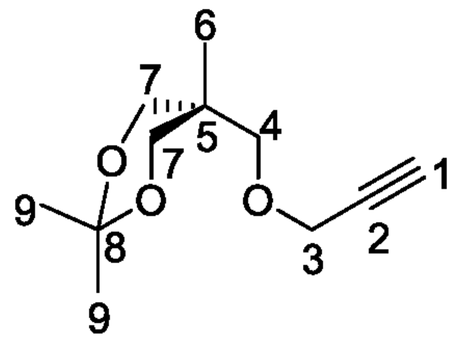
5-(Hydroxymethyl)-2,2,5-trimethyl-1,3-dioxane (5).

1,1,1-Tris(hydroxymethyl)ethane 4 (60 g, 0.5 mol) and *p*-toluenesulphonic acid monohydrate (60 mg, 315 μmol) was dissolved in dry acetone (600 mL). The reaction mixture was stirred at laboratory temperature for 2 days. The reaction mixture was monitored by TLC using the mixture $CHCl_3$/MeOH 20/1. Substances were detected by immersing the TLC plate in a mixture of ammonium sulphate tetrahydrate (0.5 g), ammonium molybdate tetrahydrate (2.5 g), sulphuric acid (5 mL) and water (45 mL) followed by heating. The reaction mixture was neutralized with potassium carbonate (1.5 g, 11 mmol), filtered and evaporated on a rotary vacuum evaporator at 40 °C. The product was purified by distillation under reduced pressure (130 °C, 1.5 mbar). The product was obtained as a colourless oil in a yield of 80 % (64.5 g). IR(KBr): 3518 ν(O-H), 3452 ν(O-H), 3381 ν(O-H), 2995 ν(C-H), 2947 ν(C-H), 2872 ν(C-H), 1658 ν(C-H), 1455 ν(C-H), 1374 ν(C-H), 1263 ν(C-H), 1210 ν(C-H), 1156 ν(C-O) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 3.61 (m, 6H, H-2, H-5), 2.50



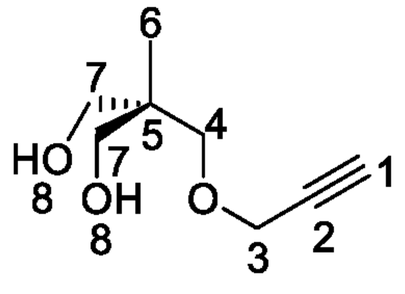
(t, J = 5.6 Hz, 1H, H-1), 1.41 (s, 3H, H-7), 1.36 (s, 3H, H-7), 0.80 (s, 3H, H-4) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 98.07 (C-6), 66.38 (C-5), 65.77 (C-2), 34.84 (C-3), 27.38 (C-7), 20.23 (C-7), 17.67 (C-4) ppm. ESI MS: for $C_8H_{16}O_3$ calcd.: *m/z* 160.1, found 198.2 $[M+K]^+$. ^{1}H and ^{13}C NMR spectra agree with the literature (Sun, J. et al. *Biomacromolecules*, **2018**, *19*, 4677–4690).

2,2,5-Trimethyl-5-((prop-2-yn-1-yloxy)methyl)-1,3-dioxane (6).



5-(Hydroxymethyl)-2,2,5-trimethyl-1,3-dioxane **5** (30.6 g, 191 mmol) was dissolved in dry THF (270 mL) and cooled to 0 °C. Sodium hydride (11.5 g, 287 mmol, 60% dispersion in oil) was added to the solution over 30 minutes. The suspension was stirred at 0 °C for 2 hours. It was then cooled to -78 °C and propargyl bromide (32 mL, 287 mmol, 80% solution in toluene) was added dropwise over 30 minutes. The reaction mixture was warmed to laboratory temperature and allowed to stir overnight. The reaction mixture was monitored by TLC using hexane/EtOAc 5/1 mixture. Substances were detected by immersing the TLC plate in a mixture of ammonium sulphate tetrahydrate (0.5 g), ammonium molybdate tetrahydrate (2.5 g), sulphuric acid (5 mL) and water (45 mL) followed by heating. The reaction mixture was filtered through celite and evaporated on a rotary vacuum evaporator at 40 °C. The product was purified by distillation under reduced pressure (115 °C, 1.5 mbar). The product was obtained as a colourless oil in a yield of 84 % (32,0 g). IR(KBr): 3285 ν(C-H alkyne), 2992 ν(C-H), 2953 ν(C-H), 2860 ν(C-H), 2113 (C-C alkyne), 1658 ν(C-H), 1449 ν(C-H), 1374 ν(C-H), 1263 ν(C-H), 1210 ν(C-H), 1090 ν(C-O) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.16 (d, J = 2.4 Hz, 2H, H-3), 3.70 (d, J = 12.0 Hz, 2H, H-7), 3.55 (d, J = 12.0 Hz, 2H, H-7), 3.52 (s, 2H, H-4), 2.41 (t, J = 2.4 Hz, 1H, H-1), 1.43 (s, 3H, H-9), 1.40 (s, 3H, H-9), 0.88 (s, 3H, H-6) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 98.01 (C-8), 80.06 (C-2), 74.30 (C-1), 73.07 (C-4), 66.63 (C-7), 58.85 (C-3), 34.34 (C-5), 26.58 (C-9), 21.20 (C-9), 18.32 (C-6) ppm. ESI MS: for $C_{11}H_{18}O_3$ calcd.: *m/z* 198.1, found 221.1 $[M+Na]^{1+}$. ^{1}H and ^{13}C NMR spectra agree with the literature (Jia, Z. et al. *ACS Macro Lett.*, **2012**, *1*, 780–783).

2-Methyl-2-((prop-2-yn-1-yloxy)methyl)propan-1,3-diol (7).



2,2,5-Trimethyl-5-((prop-2-yn-1-yloxy)methyl)-1,3-dioxane **6** (32 g, 162 mmol) was dissolved in MeOH (190 mL) and concentrated HCl (13 mL, 162 mmol) was added. The reaction mixture was allowed to stir overnight at laboratory temperature. The reaction mixture was monitored by TLC using hexane/EtOAc 1/2 mixture. Substances were detected by immersing the TLC plate in a mixture of

ammonium sulphate tetrahydrate (0.5 g), ammonium molybdate tetrahydrate (2.5 g), sulphuric acid (5 mL) and water (45 mL) followed by heating. The reaction mixture was neutralized with 50% aqueous NaOH solution. The resulting NaCl precipitate was filtered off. The filtrate was evaporated on a rotary vacuum evaporator at 40 °C. The residue was dissolved in as little $CHCl_3$ as possible and purified by column chromatography (600 g of silica gel) with elution $CHCl_3$/MeOH 30/1 mixture. The product was obtained as a yellowish oil in a yield of 96 % (24.5 g). IR(KBr): 3485 ν(O-H), 3351 ν(O-H), 3094 ν(C-H), 2986 ν(C-H), 2965 ν(C-H), 2938 ν(C-H), 2875 ν(C-H), 2860 ν(C-H), 2122 ν(C-C alkyne), 1718 ν(C-H), 1706 ν(C-H), 1658 ν(C-H), 1622 ν(C-H), 1353 ν(C-H), 1344 ν(C-H), 1248 ν(C-H), 1204 ν(C-H), 1099 ν(C-O), 1048 ν(C-O) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 4.13 (d, *J* = 2.4 Hz, 2H, H-3), 3.64 (d, *J* = 11.0 Hz, 2H, H-7), 3.55 (d, *J* = 11.0 Hz, 2H, H-7), 3.50 (s, 2H, H-4), 2.98 (bs, 2H, H-8), 2.45 (t, *J* = 2.4 Hz, 1H, H-1), 0.82 (s, 3H, H-6) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 79.65 (C-2), 74.87 (C-1), 74.72 (C-4), 67.66 (C-7), 58.86 (C-3), 40.86 (C-5), 17.17 (C-6) ppm. ESI MS: for $C_8H_{14}O_3$ calcd.: *m/z* 158.1, found 181.0 $[M+Na]^{1+}$. HRMS: for $C_8H_{14}O_3$ calcd.: *m/z* 158.0943, found 159.0984 $[M+H]^+$, Δ 20.1 ppm. ^1H NMR spectrum agrees with the literature *(Dyke, J. C. et al. J. Mater. Chem.,* ***2012****, 22, 22888).*

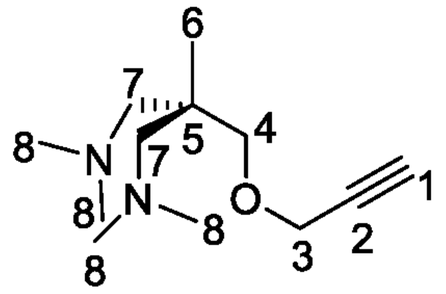
2-Methyl-2-((prop-2-yn-1-yloxy)methyl)propan-1,3-diyl bis(trifluormethanesulphonate) (8).

2-Methyl-2-((prop-2-yn-1-yloxy)methyl)propan-1,3-diol **7** (1.04 g, 6.58 mmol) was dissolved in dry dichloromethane (30 mL) and 2,6-lutidine (1.41 g, 13 mmol) was added. The reaction mixture was cooled to -78 °C and Tf_2O (3.71 g, 13 mmol) was added dropwise to the reaction mixture. The reaction mixture was stirred at this temperature for 1 hour. The reaction mixture was monitored by TLC using hexane/EtOAc 10/1 mixture. Substances were detected by immersing the TLC plate in a 1% ethanolic solution of 4-(4-nitrobenzyl)pyridine, followed by heating the plate and immersing it in concentrated aqueous ammonia solution. The reaction mixture was extracted between Et_2O (60 ml) and 1M HCl (40 mL). The organic phase was then extracted with saturated aqueous solutions of $NaHCO_3$ (40 mL) and NaCl (40 mL). The organic phase was dried over $MgSO_4$ (1 g), filtered and evaporated on a rotary vacuum evaporator at laboratory temperature. The product was dried on an oil rotary pump at laboratory temperature and obtained as a light brown oil in a yield of 92 % (2.55 g). IR(KBr): 3303 ν(C-H alkyne), 2983 ν(C-H), 2911 ν(C-H), 2869 ν(C-H), 2122 ν(C-C alkyne), 1422 ν(C-H), 1248 ν(C-H), 1207 ν(C-H), 1144 ν(C-O), 1108 ν(C-O) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 4.45 (s, 4H, H-7), 4.17 (d, *J* = 2.4 Hz, 2H, H-3), 3.48 (s, 2H, H-4), 2.48 (t, *J* = 2.4 Hz, 1H, H-1),

1.17 (s, 3H, H-6) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 118.72 (q, *J* = 319.7 Hz, C-8), 78.44 (C-2), 76.68 (C-7), 75.76 (C-1), 69.74 (C-4), 58.80 (C-3), 40.63 (C-5), 16.34 (C-6) ppm. ESI MS: for $C_{10}H_{12}F_6O_7S_2$ calcd.: *m/z* 422.0, found 445.0 $[M+Na]^{1+}$. HRMS: for $C_{10}H_{12}F_6O_7S_2$ calcd.: *m/z* 421.9929, found 444.9816 $[M+Na]^{1+}$, Δ 1.1 ppm.

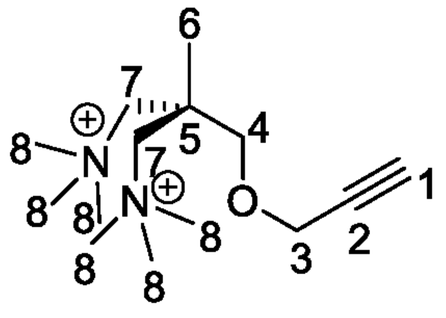
N^1,N^1,N^3,N^3,2-Pentamethyl-2-((prop-2-yn-1-yloxy)methyl)propan-1,3-diamine (9).

2-Methyl-2-((prop-2-yn-1-yloxy)methyl)propane-1,3-diyl bis(trifluormethanesulphonate) **8** (0.30 g, 0.71 mmol) was mixed with freshly distilled and dried dimethylamine (3 mL) at -78 °C. The reaction vessel was tightly sealed and the mixture was heated to 60 °C and allowed to stir overnight. The reaction vessel was cooled to -78 °C and opened. The reaction mixture was monitored by TLC ($CHCl_3$/MeOH/concentrated aqueous solution NH_3 90/10/0.5 – detection with aqueous basic solution of potassium permanganate). The reaction mixture was extracted between dichloromethane (10 mL) and 5% aqueous solution of NaOH (10 mL). The organic phase was evaporated on a rotary vacuum evaporator at laboratory temperature. The residue was suspended in water (20 mL) and co-distilled at 110 °C. The distillate was extracted with dichloromethane (20 mL). The organic phase was dried over $MgSO_4$ (0.1 g), filtered and evaporated on a rotary vacuum evaporator at laboratory. The product was obtained as a colourless oil in a yield of 73 % (0.113 g). IR(KBr): 3303 ν(C-H alkyne), 2968 ν(C-H), 2938 ν(C-H), 2851 ν(C-H), 2815 ν(C-H), 2764 ν(C-H), 2119 ν(C-C alkyne), 1467 ν(C-H), 1452 ν(C-H), 1359 ν(C-H), 1266 ν(C-H), 1093 ν(C-O), 1045 ν(C-O) cm^{-1}. ^{1}H NMR (400 MHz, D_2O): δ = 4.34 (d, *J* = 2.4 Hz, 2H, H-3), 3.77 (s, 2H, H-4), 3.51 (d, *J* = 14.1 Hz, 2H, H-7), 3.36 (d, *J* = 14.1 Hz, 2H, H-7), 3.01 (d, *J* = 4.1 Hz, 12H, H-8), 2.99 (t, *J* = 2.4 Hz, 1H, H-1), 1.28 (s, 3H, H-6) ppm. ^{13}C NMR (100 MHz, D_2O, tBuOH): δ = 79.21 (C-2), 76.68 (C-7), 77.73 (C-1), 72.26 (C-4), 70.51 (tBuOH), 64.88 (C-7), 59.03 (C-3), 47.92 (C-8), 47.52 (C-8), 38.75 (C-5), 30.29 (tBuOH), 17.97 (C-6) ppm. The sample for NMR measurement was converted to the hydrochloric acid salt to obtain better spectra. ESI MS: for $C_{12}H_{24}N_2O$ calcd.: *m/z* 213.0, found 212.2 $[M+H]^{1+}$. HRMS: for $C_{12}H_{24}N_2O$ calcd.: *m/z* 212.1889, found 213.1968 $[M+H]^{1+}$, Δ 3.3 ppm.



N^1,N^1,N^1,N^3,N^3,N^3,2-Heptamethyl-2-((prop-2-yn-1-yloxy)methyl)propan-1,3-diaminium (K2TMAM).

N^1,N^1,N^3,N^3,2-Pentamethyl-2-((prop-2-yn-1-yloxy)methyl)propan-1,3-diamine **9** (0.38 g, 1.79 mmol) was dissolved in dry THF (10 mL) and methyl iodide (5.08 g, 35.8 mmol) was added slowly. The reaction mixture



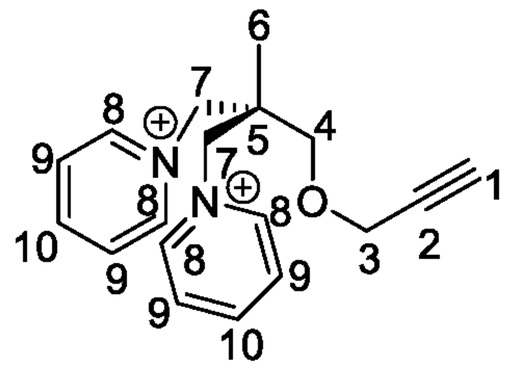
was brought to a boil and stirred for 24 hours. The reaction mixture was monitored by TLC ($CHCl_3$/MeOH/concentrated aqueous solution of NH_3 90/10/0.5 – for substance **9**, detection with aqueous basic solution of potassium permanganate, MeOH/HOAc/1% aqueous solution of NH_4OAc 10/1/9 – for product, detection with aqueous basic solution of potassium permanganate). A precipitate formed. The THF was filtered off and the precipitate was washed with THF (3 × 8 mL). The precipitate was dissolved in water and evaporated on a rotary vacuum evaporator at 50 °C. The product was dried at 50 °C on an oil rotary pump and obtained as a yellowish oil in a yield of 89 % (0.79 g). IR(KBr): 3219 ν(C-H alkyne), 3010 ν(C-H), 2971 ν(C-H), 2878 ν(C-H), 2116 ν(C-C alkyne), 1482 ν(C-H), 1416 ν(C-H), 1368 ν(C-H), 1269 ν(C-H), 1099 ν(C-O) cm^{-1}. ^1H NMR (400 MHz, D_2O): δ = 4.36 (d, *J* = 2.4 Hz, 2H, H-3), 3.86 (s, 2H, H-4), 3.84 (d, *J* = 14.2 Hz, 2H, H-7), 3.63 (d, *J* = 14.2 Hz, 2H, H-7), 3.34 (s, 18H, H-8), 2.98 (t, *J* = 2.4 Hz, 1H, H-1), 1.54 (s, 3H, H-6) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 78.97 (C-2), 78.13 (C-1), 73.37 (C-7), 72.78 (C-4), 59.19 (C-3), 56.89 (C-8), 44.98 (C-5), 22.14 (C-6) ppm. ESI MS: for $C_{14}H_{30}N_2O^{2+}$ calcd.: *m/z* 121.1 and for $C_{14}H_{30}IN_2O^{1+}$ calcd.: *m/z* 369.1, found 121.0 $[M]^{2+}$ and 370.0 $[M+I^-]^{1+}$. HRMS: for $C_{14}H_{30}IN_2O^{1+}$ calcd.: *m/z* 369.1397, found 369.1404 $[M+I^-]^{1+}$, Δ 1.9 ppm.

3,3'-(2-Methyl-2-((prop-2-yn-1-yloxy)methyl)propan-1,3-diyl)bis(1-methyl-1H-imidazol-3-ium) (K2MIMM).

2-Methyl-2-((prop-2-yn-1-yloxy)methyl)propan-1,3-diyl bis(trifluormethanesulphonate) **8** (0.21 g, 0.49 mmol) was dissolved in N-methylimidazole (2 ml), the mixture was heated to 60 °C and stirred at this temperature for 1 hour. The reaction mixture was monitored by TLC (MeOH/HOAc/1% aqueous solution of NH_4OAc 10/1/9 – detection with aqueous basic solution of potassium permanganate). N-Methylimidazole was distilled off from the reaction mixture under reduced pressure (1-10 mbar) at 100 °C. Residual N-methylimidazole was extracted with EtOAc (2 × 5 mL). The crude product was extracted between water (5 mL) and $CHCl_3$ (5 mL). The aqueous phase was extracted with additional $CHCl_3$ (8 × 5 mL) and evaporated on a rotary vacuum evaporator at 50 °C. The product was dried at 70 °C on an oil rotary pump and obtained as a light brown oil in a yield of 78 % (0.23 g). IR(KBr): 3144 ν(C-H alkyne), 3088 ν(C-H), 2881 ν(C-H), 2110 ν(C-C alkyne), 1616 ν(imidazole), 1574 ν(imidazole), 1562 ν(imidazole), 1449 ν(C-H), 1425 ν(C-H), 1359 ν(C-H), 1278 ν(C-H), 1168 ν(C-O), 1093 ν(C-O) cm^{-1}. ^1H NMR (400 MHz, D_2O): δ = 8.84 (s, 2H, H-8), 7.52 (s, 4H, H-9), 4.48 (d, *J* = 14.3 Hz, 2H, H-7), 4.28 – 4.25 (m, 4H, H-3, H-7), 3.95 (s, 6H, H-10), 3.23 (s, 2H, H-4), 3.02 (t, *J* = 2.4 Hz, 1H, H-1), 1.03 (s, 3H, H-6) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 139.19

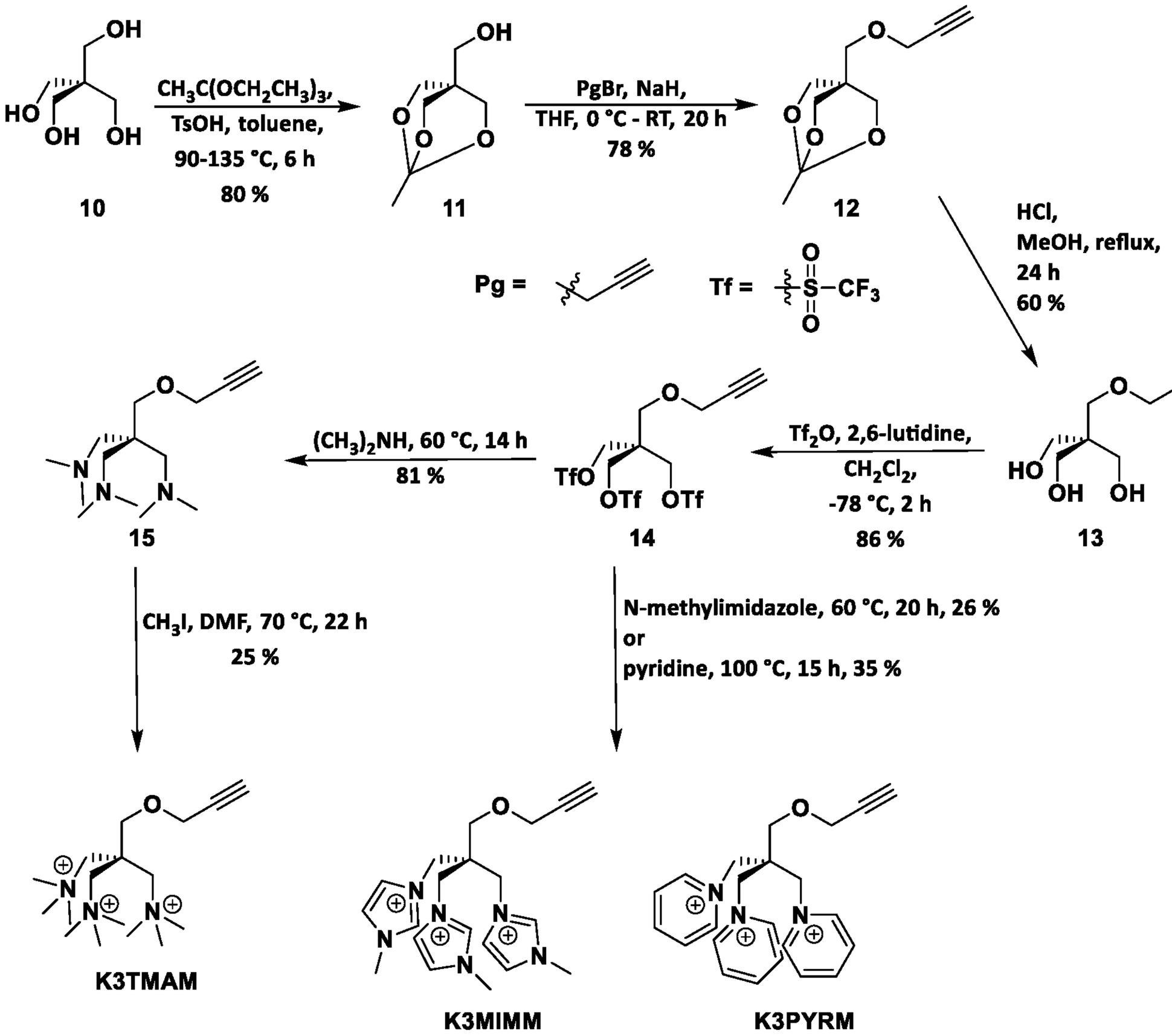
(C-8), 125.40 – 125.03 (C-9), 79.91 (C-2), 77.61 (C-1), 71.06 (C-4), 59.28 (C-3), 54.39 (C-7), 41.46 (C-5), 37.02 (C-10), 18.37 (C-6) ppm. UV-VIS (H_2O), λ_{max1}, nm: 224.5, 7×10^{-3} M. ESI MS: for $C_{16}H_{24}N_4O^{2+}$ calcd.: *m/z* 144.1, found 144.0 $[M]^{2+}$. HRMS: for $C_{16}H_{24}N_4O^{2+}$ calcd.: *m/z* 144.0970, found 144.0581 $[M]^{2+}$, Δ 270.0 ppm.

1,1'-(2-Methyl-2-((prop-2-yn-1-yloxy)methyl)propan-1,3-diyl)bis(pyridin-1-ium) (K2PYRM). 2-Methyl-2-((prop-2-yn-1-yloxy)methyl)propan-1,3-diyl bis(trifluormethanesulphonate) **8** (1.9 g, 4.57 mmol) was dissolved in dry pyridine (35 mL), the mixture was heated to 60 °C and stirred overnight. The reaction mixture was monitored by TLC (MeOH/HOAc/1% aqueous solution of NH_4OAc 10/1/9 – detection with aqueous basic solution of potassium permanganate). Pyridine was distilled off from the reaction mixture under reduced pressure (1-10 mbar) at 60 °C. The crude product was extracted between water (50 mL) and $CHCl_3$ (50 mL). The aqueous phase was extracted once more with $CHCl_3$ (50 mL) and evaporated on a rotary vacuum evaporator at 40 °C. The product was dried at 60 °C on an oil rotary pump and obtained as a brownish oil in a yield of 73 % (1.9 g). IR(KBr): 3261 ν(pyridine), 3141 ν(C-H alkyne), 3094 ν(C-H), 2974 ν(C-H), 2105 ν(C-C alkyne), 1637 ν(pyridine), 1613 ν(pyridine), 1491 ν(C-H), 1267 ν(pyridine), 1228 ν(C-H), 1171 ν(pyridine), 1096 ν(C-O), 1030 ν(C-O) cm^{-1}. ^1H NMR (400 MHz, D_2O): δ = 8.83 (m, 4H, H-8), 8.62 (tt, *J* = 7.9, 1.4 Hz, 2H, H-10), 8.12 (dd, *J* = 8.0, 6.6 Hz, 4H, H-9), 4.95 (d, *J* = 13.6 Hz, 2H, H-7), 4.68 (d, *J* = 13.6 Hz, 2H, H-7), 4.23 (d, *J* = 2.4 Hz, 2H, H-3), 3.18 (s, 2H, H-4), 2.94 (t, *J* = 2.4 Hz, 1H, H-1), 1.01 (s, 3H, H-6) ppm. ^{13}C NMR (100 MHz, D_2O, tBuOH): δ = 147.55 (C-10), 146.53 (C-8), 129.11 (C-9), 79.27 (C-2), 77.76 (C-1), 70.48 (tBuOH), 67.99 (C-4), 64.65 (C-7), 58.52 (C-3), 42.20 (C-5), 30.29 (tBuOH), 17.01 (C-6) ppm. UV-VIS (H_2O), λ_{max1}, nm: 216.0, λ_{max2}, nm: 259.0, 16×10^{-3} M. ESI MS: for $C_{18}H_{22}N_2O^{2+}$ calcd.: *m/z* 141.1, found 141.2 $[M]^{2+}$. HRMS: for $C_{18}H_{22}N_2O^{2+}$ calcd.: *m/z* 141.0861, found 141.0849 $[M]^{2+}$, Δ 8.5 ppm.



1.3 Synthesis of K3 anchors

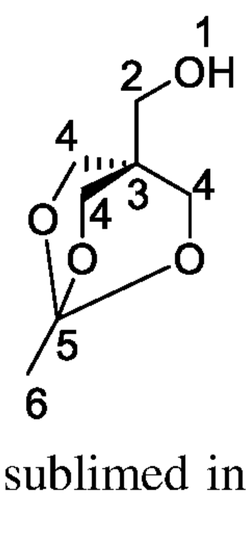
The synthesis of K3 anchors of formula I with three charged groups TMAM, MIMM or PYRM is described in Scheme 4. The following are detailed procedures for the preparation of compounds shown in the scheme.



Scheme 4: Synthesis of K3 anchors

(1-Methyl-2,6,7-trioxabicyklo[2.2.2]oktan-4-yl)methanol (11).

Pentaerythritol **10** (15 g, 0.11 mol) was suspended in toluene (11 mL). Triethyl orthoacetate (17.9 g, 0.11 mol) and *p*-toluenesulphonic acid monohydrate (55 mg, 0.28 mmol) were added to the mixture. The mixture was heated to 90 °C, and the resulting ethanol was distilled off from the reaction mixture. After distilling off the ethanol, the temperature was raised to 135 °C, and toluene was distilled off. The gel



residue was transferred to a special elongated flask with $CHCl_3$ and the product was sublimed in the Kugelrohr apparatus (180 °C, 5 mbar). The product is obtained as a white solid in a yield of 80 % (14.9 g). IR(KBr): 3452 ν(O-H), 3351 ν(O-H), 2956 ν(C-H), 2935 ν(C-H), 2887 ν(C-H), 1718 ν(C-H), 1655 ν(C-H), 1365 ν(C-H), 1245 ν(C-H), 1153 ν(C-O), 1036 ν(C-O) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 4.02 (s, 6H, H-4), 3.47 (d, *J* = 4.7 Hz, 2H, H-2), 1.51 (t, *J* = 4.7 Hz, 1H, H-1), 1.46 (s, 3H, H-6) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 108.65 (C-5), 69.41 (C-4), 61.40

(C-2), 35.71 (C-3), 23.51 (C-6) ppm. ESI MS: for $C_7H_{12}O_4$ calcd.: *m/z* 160.1, found 161.1 $[M+H]^{1+}$. HRMS: for $C_7H_{12}O_4$ calcd.: *m/z* 160.0736, found 161.0802 $[M+H]^+$, Δ 3.7 ppm. ^1H and ^{13}C NMR spectrum agrees with the literature (Feng, Y. et al. *Nucl. Med. Biol.*, **2018**, *61*, 1–10).

1-Methyl-4-((prop-2-yn-1-yloxy)methyl)-2,6,7-trioxabicyklo[2.2.2]octane (12).

(1-Methyl-2,6,7-trioxabicyklo[2.2.2]octan-4-yl)methanol **11** (1.0 g, 6.25 mmol) was dissolved in dry THF (9 mL) and the solution was cooled to 0 °C. Sodium hydride (0.37 g, 9.37 mmol, 60% dispersion in mineral oil) was carefully added and the mixture was stirred at 0 °C for 2 hours. Propargyl bromide (1.3 g, 9.37 mmol, 80% dispersion in toluene) was slowly added dropwise and the mixture was allowed to stir overnight at laboratory temperature. The reaction mixture was monitored by TLC (hexane/EtOAc 5/1 – detection with aqueous basic solution of potassium permanganate). The mixture was filtered through celite and the filtrate was evaporated on a rotary evaporator at 50 °C. The residue was transferred to a special elongated flask with $CHCl_3$ and the product was sublimed in the Kugelrohr apparatus (170 °C, 5 mbar). The product is obtained in the form of a white solid in a yield of 78 % (0.95 g). IR(KBr): 3261 ν(C-H alkyne), 3007 ν(C-H), 2932 ν(C-H), 2881 ν(C-H), 2851 ν(C-H), 2122 ν(C-C alkyne), 1476 ν(C-H), 1410 ν(C-H), 1356 ν(C-H), 1299 ν(C-H), 1269 ν(C-H), 1132 ν(C-O), 1102 ν(C-O), 1048 ν(C-O) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 4.09 (s, 2H, H-3), 4.00 (s, 2H, H-6), 3.29 (s, 2H, H-4), 2.44 (s, 1H, H-1), 1.45 (s, 3H, H-8) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 108.70 (C-7), 78.96 (C-2), 75.34 (C-1), 69.54 (C-6), 68.11 (C-4), 58.87 (C-3), 34.84 (C-5), 23.56 (C-8) ppm. ESI MS: for $C_{10}H_{14}O_4$ calcd.: *m/z* 198.1, found 199.0 $[M+H]^{1+}$. HRMS: for $C_{10}H_{14}O_4$ calcd.: *m/z* 198.0892, found 199.0959 $[M+H]^+$, Δ 3.0 ppm. ^1H and ^{13}C NMR spectra agree with the literature (Feng, Y. et al. *Nucl. Med. Biol.*, **2018**, *61*, 1–10).

2-(Hydroxymethyl)-2-((prop-2-yn-1-yloxy)methyl)propan-1,3-diol (13).

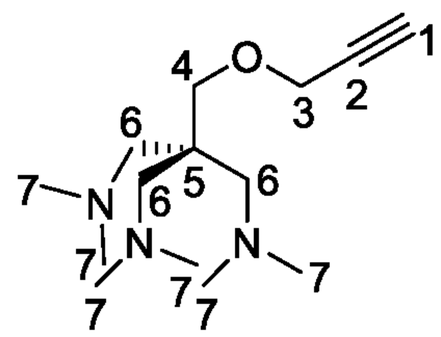
1-Methyl-4-((prop-2-yn-1-yloxy)methyl)-2,6,7-trioxabicyklo[2.2.2]octane **12** (12.0 g, 60 mmol) was dissolved in MeOH (240 mL) and concentrated HCl (3.2 g, 30.0 mmol) was added. The mixture was brought to a boil and stirred overnight. The reaction mixture was monitored by TLC ($CHCl_3$/MeOH 15/1 – detection with aqueous basic solution of potassium permanganate). The reaction mixture was cooled to laboratory temperature, neutralized with 5% aqueous solution of NaOH and evaporated on a rotary vacuum evaporator at 50 °C. The residue was extracted between water (500 mL) and $CHCl_3$ (500 mL). The aqueous phase was further

extracted with $CHCl_3$ (2 × 400 mL), evaporated on a rotary vacuum evaporator at 50 °C and the residue was suspended in acetone (200 mL). The mixture was filtered and the filtrate was evaporated on a rotary vacuum evaporator at 40 °C. The product was dried at 60 °C on an oil rotary pump and obtained as a yellowish oil in a yield of 60 % (6.4 g). IR(KBr): 3357 ν(O-H), 2935 ν(C-H), 2881 ν(C-H), 2116 ν(C-C alkyne), 1721 ν(C-H), 1649 ν(C-H), 1365 ν(C-H), 1245 ν(C-H), 1093 ν(C-O), 1042 ν(C-O) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 4.15 (d, *J* = 2.4 Hz, 2H, H-3), 3.72 (s, 6H, H-6), 3.57 (s, 2H, H-4), 2.47 (t, *J* = 2.4 Hz, 1H, H-1) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 79.41 (C-2), 75.16 (C-1), 71.59 (C-4), 64.57 (C-6), 59.04 (C-3), 45.15 (C-5) ppm. ESI MS: for $C_8H_{14}O_4$ calcd.: *m/z* 174.1, found 175.0 $[M+H]^{1+}$. ^1H and ^{13}C NMR spectra agree with the literature (Feng, Y. et al. *Nucl. Med. Biol.*, **2018**, *61*, 1–10).

2-((Prop-2-yn-1-yloxy)methyl)-2-((((trifluormethyl)sulfonyl)oxy)methyl)propan-1,3-diyl bis(trifluormethanesulphonate) (14).

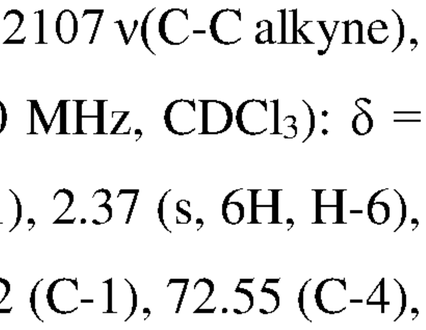
2-(Hydroxymethyl)-2-((prop-2-yn-1-yloxy)methyl)propan-1,3-diol **13** (1 g, 5.5 mmol) was dissolved in dry dichloromethane (30 mL) and 2,6-lutidine (1.84 g, 17.2 mmol) was added. The reaction mixture was cooled to -78 °C and Tf_2O (4.86 g, 17.2 mmol) was added dropwise to the reaction mixture. The reaction mixture was stirred at this temperature for 2 hours. The reaction mixture was monitored by TLC ($CHCl_3$/MeOH 15/1 – for the starting triol, detection with aqueous basic potassium permanganate solution, hexane/EtOAc 10/1 – for product, detection by immersing the TLC plate in a 1% ethanolic solution of 4-(4-nitrobenzyl) pyridine, followed by heating the plate and immersion in a concentrated aqueous ammonia solution). The reaction mixture was extracted between Et_2O (60 mL) and 1M HCl (40 mL). The organic phase was washed with saturated aqueous solutions of $NaHCO_3$ (40 mL) and NaCl (40 mL), dried over $MgSO_4$ (1 g), filtered and evaporated on a rotary vacuum evaporator at laboratory temperature. The product was dried at laboratory temperature on an oil rotary pump and obtained as a brown solid in a yield of 86 % (2.84 g). IR(KBr): 3303 ν(C-H alkyne), 2980 ν(C-H), 2905 ν(C-H), 2122 ν(C-C alkyne), 1428 ν(C-H), 1407 ν(C-H), 1245 ν(C-H), 1210 ν(C-H), 1147 ν(C-O), 1108 ν(C-O) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 4.57 (s, 6H, H-6), 4.22 (d, *J* = 2.4 Hz, 2H, H-3), 3.64 (s, 2H, H-4), 2.54 (t, *J* = 2.4 Hz, 1H, H-1) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 118.65 (q, *J* = 319.9 Hz, C-7), 77.36 (C-2), 76.75 (C-1), 71.43 (C-6), 64.61 (C-4), 59.05 (C-3), 44.84 (C-5) ppm. ESI MS: for $C_{11}H_{11}F_9O_{10}S_3$ calcd.: *m/z* 569.9, found 587.9 $[M+NH_4]^{1+}$. HRMS: for $C_{11}H_{11}F_9O_{10}S_3$ calcd.: *m/z* 569.9371, found 592.9265 $[M+Na]^{1+}$, Δ 0.3 ppm.

2-((Dimethylamino)methyl)-*N*^{1},*N*^{1},*N*^{3},*N*^{3}-tetramethyl-2-((prop-2-yn-1-yloxy)methyl)propan-1,3-diamine (15).



2-((Prop-2-yn-1-yloxy)methyl)-2-((((trifluormethyl)sulfonyl)oxy)methyl)propan-1,3-diyl bis(trifluormethanesulphonate) **14** (0.36 g, 0.62 mmol) was mixed with freshly distilled and dried dimethylamine (3.6 ml) at -78 °C. The reaction vessel was tightly sealed and the mixture was heated to 60 °C and allowed to stir overnight. The reaction vessel was cooled to -78 ° C and opened. The reaction mixture was monitored by TLC ($CHCl_3$/MeOH/concentrated aqueous solution of NH_3 90/10/0.5 – detection with aqueous basic solution of potassium permanganate). The reaction mixture was extracted between dichloromethane (12 mL) and 5% aqueous solution of NaOH (12 mL). The organic phase was evaporated on a rotary vacuum evaporator at laboratory temperature. The residue was suspended in water (30 mL) and co-distilled at 120 °C. The distillate was extracted with $CHCl_3$ (30 mL). The organic phase was dried over $MgSO_4$ (0.7 g), filtered and evaporated on a rotary vacuum evaporator at laboratory temperature. The product was dried at laboratory temperature on an oil rotary pump and obtained as a colourless oil in a yield of 81 % (0.13 g). IR(KBr): 3309 ν(C-H alkyne), 2971 ν(C-H), 2941 ν(C-H), 2860 ν(C-H), 2815 ν(C-H), 2768 ν(C-H), 2107 ν(C-C alkyne), 1458 ν(C-H), 1263 ν(C-H), 1096 ν(C-O), 1036 ν(C-O) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.10 (d, *J* = 2.4 Hz, 2H, H-3), 3.46 (s, 2H, H-4), 2.38 (d, *J* = 2.4 Hz, 1H, H-1), 2.37 (s, 6H, H-6), 2.27 (s, 18H, H-7) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 80.12 (C-2), 77.32 (C-1), 72.55 (C-4), 61.86 (C-6), 58.42 (C-3), 49.11 (C-7), 46.98 (C-5) ppm. ESI MS: for $C_{14}H_{29}N_3O$ calcd.: *m/z* 255.2, found 256.3 $[M+H]^{1+}$. HRMS: for $C_{14}H_{29}N_3O$ calcd.: *m/z* 255.2311, found 256.2389 $[M+H]^{1+}$, Δ 2.3 ppm.

N^1,N^1,N^1,N^3,N^3,N^3-Hexamethyl-2-((prop-2-yn-1-yloxy)methyl)-2-((trimethylamonio)methyl)propan-1,3-diaminium (K3TMAM).

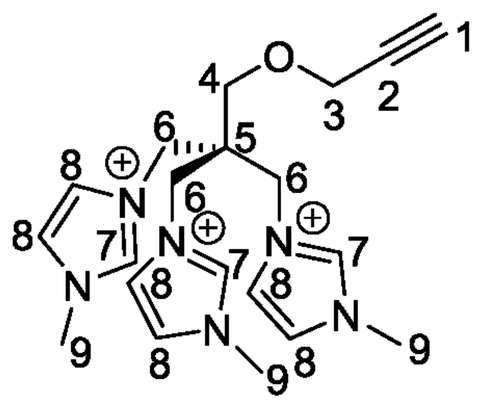


2-((Dimethylamino)methyl)-*N*^{1},*N*^{1},*N*^{3},*N*^{3}-tetramethyl-2-((prop-2-yn-1-yloxy)methyl)propan-1,3-diamine **15** (0.51 g, 1.99 mmol) was dissolved in DMF (13 mL) and CH_3I (8.5 g, 59.9 mmol) was added dropwise. The mixture was heated to 70 °C and allowed to stir for 22 hours. The reaction mixture was monitored by TLC (MeOH/HOAc/1% aqueous solution of NH_4OAc 10/10/9 – detection with aqueous basic solution of potassium permanganate). The reaction mixture was filtered and DMF was distilled off from the reaction mixture under reduced

pressure (1-10 mbar) at 60 °C. The crude product was then dissolved in as little water as possible and purified using weak cation exchange resin Amberlite (11 mL, NH_4^+ form). The elution solutions were successively water, 1% aqueous solution of NH_4HCO_3, 5% aqueous solution of NH_4HCO_3 and 10% aqueous solution of NH_4HCO_3. Fractions containing pure product were evaporated on a rotary vacuum evaporator at 50 °C. The residue was suspended in MeOH (10 mL); the solution was filtered, neutralised with 1M HCl and re-evaporated on the rotary vacuum evaporator at 50 °C. The product was dried at 50 °C on an oil rotary pump and obtained as a brownish glassy material in a yield of 25 % (0.203 g). ^{1}H NMR (400 MHz, D_2O, tBuOH): δ = 4.49 (d, *J* = 2.4 Hz, 2H, H-3), 4.42 (s, 2H, H-4), 4.12 (s, 6H, H-6), 3.47 (s, 27H, H-7), 3.05 (t, *J* = 2.4 Hz, 1H, H-1), 1.25 (s, tBuOH) ppm. ^{13}C NMR (100 MHz, D_2O, tBuOH): δ = 78.86 (C-1), 77.52 (C-2), 70.40 (tBuOH), 70.24 (C-6), 68.65 (C-4), 58.81 (C-3), 57.54 (C-7), 52.67 (C-5) ppm. ESI MS: for $C_{17}H_{38}N_3O^{3+}$ calcd.: *m/z* 100.1 and for $C_{17}H_{38}IN_3O^{2+}$ calcd.: *m/z* 213.6 and for $C_{17}H_{38}I_2N_3O^{1+}$ calcd.: 554.1, found 213.7 $[M+I^-]^{2+}$ and 554.1 $[M+2I^-]^{1+}$.

3,3'-(2-((1-Methyl-1H-imidazol-3-ium-3-yl)methyl)-2-((prop-2-yn-1-yloxy)methyl)propan-1,3-diyl)bis(1-methyl-1H-imidazol-3-ium) (K3MIMM).

2-((Prop-2-yn-1-yloxy)methyl)-2-((((trifluormethyl)sulphonyl)oxy)methyl)propan-1,3-diyl bis(trifluormethanesulphonate) **14** (2.75 g, 4.82 mmol) was dissolved in

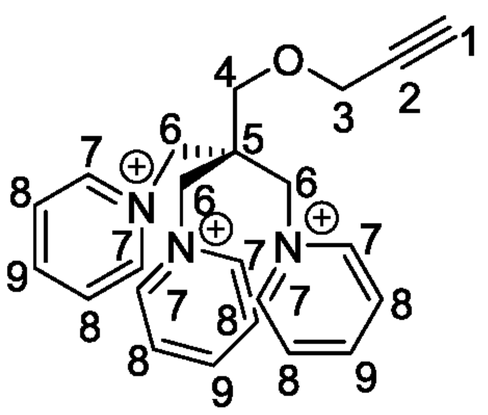


N-methylimidazole (35 mL). The reaction mixture was heated to 60 °C and allowed to stir for 20 hours. The reaction mixture was monitored by TLC (MeOH/HOAc/1% aqueous solution of NH_4OAc 10/10/9 – detection with aqueous basic solution of potassium permanganate). N-methylimidazole was distilled off under reduced pressure (1-10 mbar) at 80 °C. The crude product was dissolved in water (100 mL) and extracted with $CHCl_3$ (4 × 100 mL). The crude product in aqueous solution was purified using weak Amberlite cation exchange resin (60 mL, NH_4^+ form). The elution solutions were successively water, 5% aqueous solution of NH_3, 1% aqueous solution of NH_4HCO_3, 5% aqueous solution of NH_4HCO_3 and 10% aqueous solution of NH_4HCO_3. Fractions containing pure product were evaporated on a rotary vacuum evaporator at 50 °C. The residue was suspended in MeOH (40 mL); the solution was filtered, neutralised with 1M HCl and re-evaporated on the rotary vacuum evaporator at 50 °C. The product was dried at 50 °C on an oil rotary pump and obtained as a brownish glassy material in a yield of 26 % (0.594 g). IR(KBr): 3153 ν(C-H alkyne), 3082 ν(C-H), 2863 ν(C-H), 2113 ν(C-C alkyne), 1637 ν(imidazole), 1577 ν(imidazole), 1559 ν(imidazole), 1431 ν(C-H), 1344 ν(C-H), 1177 ν(C-O), 1096 ν(C-O) cm^{-1}. ^{1}H

NMR (400 MHz, DMSO-d6): δ = 9.52 (s, 3H, H-7), 7.80 (s, 6H, H-8), 4.61 (s, 6H, H-6), 4.13 (d, *J* = 2.4 Hz, 2H, H-3), 3.90 (s, 9H, H-9), 3.64 (t, *J* = 2.3 Hz, 1H, H-1), 3.61 (s, 2H, H-4) ppm. ^{13}C NMR (100 MHz, DMSO-d6): δ = 138.47 (C-7), 123.91 – 123.49 (C-8), 79.21 (C-2), 78.38 (C-1), 68.36 (C-4), 57.95 (C-3), 49.87 (C-6), 42.26 (C-5), 36.03 (C-9) ppm. UV-VIS (H_2O), λ_{max1}, nm: 222.0, λ_{max2}, nm: 271.5, 6×10^{-3} M. ESI MS: for $C_{20}H_{29}N_6O^{3+}$ calcd.: *m/z* 123.1 for $C_{20}H_{28}ClN_6O^{1+}$ calcd.: *m/z* 403.2, found 403.2 $[M\text{-}H^+\text{+}Cl^-]^{1+}$.

1,1'-(2-((Prop-2-yn-1-yloxy)methyl)-2-(pyridin-1-ium-1-ylmethyl)propane-1,3-diyl)bis(pyridin-1-ium) (K3PYRM).

2-((Prop-2-yn-1-yloxy)methyl)-2-((((trifluormethyl)sulphonyl)oxy)methyl)propan-1,3-diyl bis(trifluormethanesulphonate) **14** (1.22 g, 2.14 mmol) was dissolved in dry pyridine (23 mL) and activated 4Å molecular sieves were added. The reaction mixture was heated to 100 °C and allowed to stir for 17 hours.



The reaction mixture was monitored by TLC (MeOH/HOAc/1% aqueous solution of NH_4OAc 10/10/9 – detection with aqueous basic solution of potassium permanganate). The pyridine was distilled off under reduced pressure (1-10 mbar) at 50 °C. The crude product was purified using weak Amberlite cation exchange resin (32 mL, NH_4^+ form). The elution solutions were successively water, 1% aqueous solution of NH_4HCO_3 and 10% aqueous solution of NH_4HCO_3. Fractions containing pure product were evaporated on a rotary vacuum evaporator at 40 °C. The residue was suspended in MeOH (20 mL); the solution was filtered, neutralised with 1M HCl and re-evaporated on the rotary vacuum evaporator at 40 °C. The product was dried at 50 °C on an oil rotary pump and obtained as a dark purple glassy material in a yield of 35 % (0.413 g). IR(KBr): 3452 ν(pyridine), 3064 ν(C-H), 2113 ν(C-C alkyne), 1640 ν(pyridine), 1491 ν(C-H), 1275 ν(pyridine), 1180 ν(pyridine), 1093 ν(C-O), 1015 ν(C-O) cm^{-1}. ^{1}H NMR (400 MHz, D_2O): δ = 8.94 (d, *J* = 6.2 Hz, 6H, H-7), 8.74 (t, *J* = 7.9 Hz, 3H, H-9), 8.22 (t, *J* = 7.0 Hz, 1H, H-8), 5.20 (s, 6H, H-6), 4.11 (s, 2H, H-3), 3.95 (s, 2H, H-4) ppm. UV-VIS (H_2O), λ_{max1}, nm: 259.5, λ_{max2}, nm: 404.0, 1×10^{-3} M. ESI MS: for $C_{23}H_{26}N_3O^{3+}$ calcd.: *m/z* 120.1 and for $C_{24}H_{26}F_3N_3O_4S^{2+}$ calcd.: *m/z* 254.6, found 254.7 $[M+TfO^-]^{2+}$. HRMS: for $C_{23}H_{26}Cl_2N_3O^+$ calcd.: *m/z* 430.1447, found 430.1420 $[M+2Cl^-]^+$, Δ 6.3 ppm.

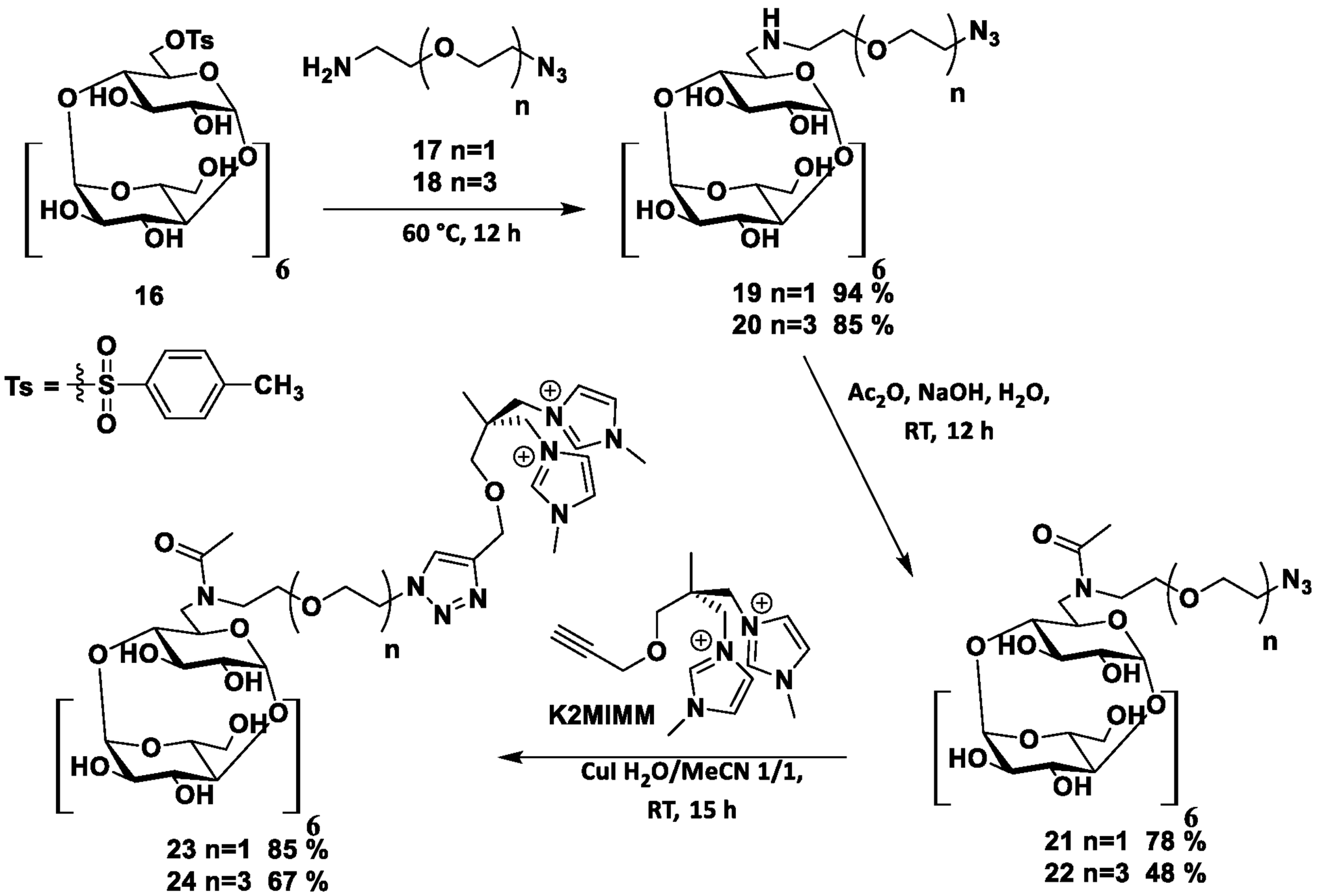
Example 2: Chemical stability of anchors

It has been verified that the substances of general formula (I) are stable even in a basic environment, e.g., K2TMAM withstanding heating with 5% aqueous hydroxide up to 90 °C, K2MIMM and K2PYRM up to 50 °C.

Example 3: *Synthesis of modifiers of general formula (III)*

3.1 Cyclodextrin-containing modifiers

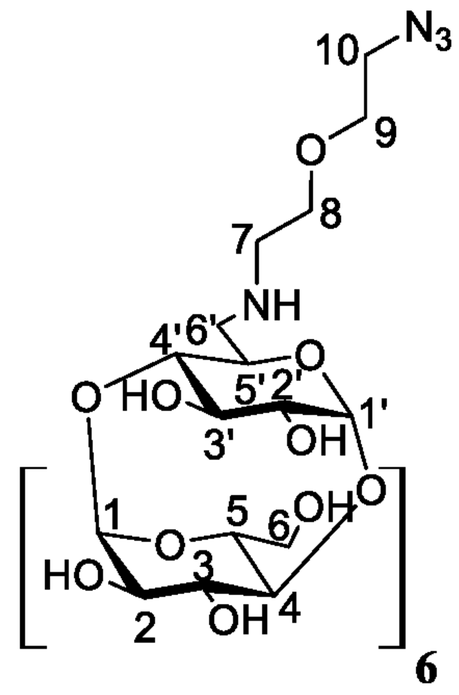
The procedure for preparing modifiers of general formula III containing cyclodextrin as substance A attached via an oligoethylene glycol linker to the charged anchor, shown in Scheme 5, is based on the reaction of 6^A-*O*-*p*-toluensulphonate of cyclodextrin with an amino-azido-oligoethylene glycol linker followed by acetylation of the secondary amine and CuAAC by reaction with a propargyl anchor.



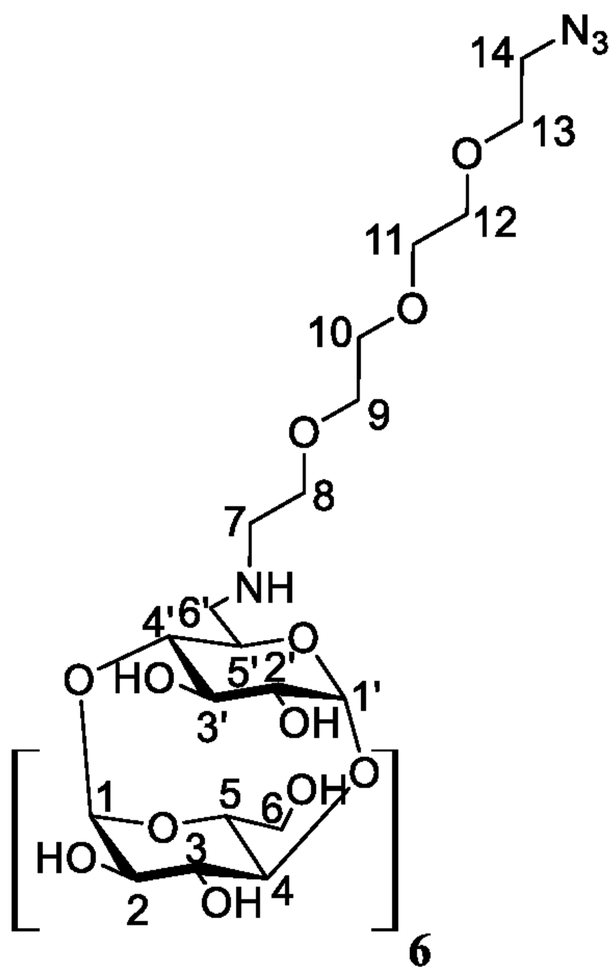
Scheme 5: Preparation of the modifier of general formula (III) containing cyclodextrin linked via an oligoethylene glycol linker to an anchor.

N-(2-(2-Azidoethoxy)eth-1-yl)-6^{A}-amino-6^{A}-deoxy-β-cyclodextrin (19).

6^{A}-*O*-*p*-Toluensulphonyl-β-cyclodextrin **16** (1.00 g, 0.78 mmol) was mixed with 2-(2-azidoethoxy)ethan-1-amine **17** (2.0 ml) and the heterogeneous mixture was heated to 60 °C. Homogenisation took place and the solution was stirred at this temperature overnight. The reaction mixture was monitored by TLC using the mixture PrOH/H_2O/EtOAc/concentrated aqueous solution of NH_3 6/3/1/1 and the substances were detected by carbonisation in 50% aqueous sulphuric acid solution. The solution was diluted with water (2 mL) and the reaction mixture was poured into acetone (200 mL). The resulting precipitate was separated by filtration, washed with acetone and dried for 3 hours at laboratory temperature using an oil rotary pump. The crude product (1.02 g) was dissolved in water (14 mL) and poured again into acetone (200 mL). The resulting precipitate was separated by centrifugation, dissolved in as little water as possible and purified by ion exchange column chromatography. The strong cation exchange resin Amberlite IR 120 (160 mL) was used in the H^+ cycle. The by-products were removed with water and the product was eluted with 5% aqueous solution of NH_3. The product-containing solution was evaporated at 50 °C using a rotary vacuum evaporator. The residue (0.93 g) was dissolved in water (18 mL) and lyophilised. The product was obtained as a white solid in a yield of 94 % (0.92 g). $[\alpha]^{25}_{D}$ +121.4° (α +0.085, 7.0 mg, H_2O). IR(KBr): 3324 ν(O-H), 2932 ν(C-H), 2113 ν(azide), 1651 ν(C-H), 1455 ν(C-H), 1300 ν(C-H), 1155 ν(C-H), 1079 ν(C-O), 1032 ν(C-O) cm^{-1}. ^{1}H NMR (600 MHz, D_2O): δ = 5.07 – 5.02 (m, 7H, H-1, H-1‘), 3.92 – 3.56 (m, 44H, H-2, H-2‘, H-3, H-3‘, H-4, H-4‘, H-5, H-5‘, H-6, H-8, H-9), 3.42 (t, *J* = 4.6 Hz, 2H, H-10), 3.10 – 2.74 (m, 4H, H-6‘, H-7) ppm. ^{13}C NMR (150 MHz, D_2O, tBuOH): δ = 102.62 – 101.98 (C-1, C-1‘), 84.46 – 81.12 (C-4, C-4‘), 73.71 –73.40 (C-3, C-3‘), 72.58 – 72.32 (C-2, C-2‘, C-5), 70.28 (C-5‘), 70.10 – 69.72 (C-8, C-9), 60.62 – 60.45 (C-6), 50.65 (C-10), 49.63 (C-6‘), 47.77 (C-7) ppm. ESI MS: for $C_{46}H_{78}N_4O_{35}$ calcd.: *m/z* 1246.4, found 1248.0 $[M+H]^{1+}$. HRMS: for $C_{46}H_{78}N_4O_{35}$ calcd.: *m/z* 1246.4447, found 1247.4518 $[M+H]^{1+}$, Δ 0.1 ppm.



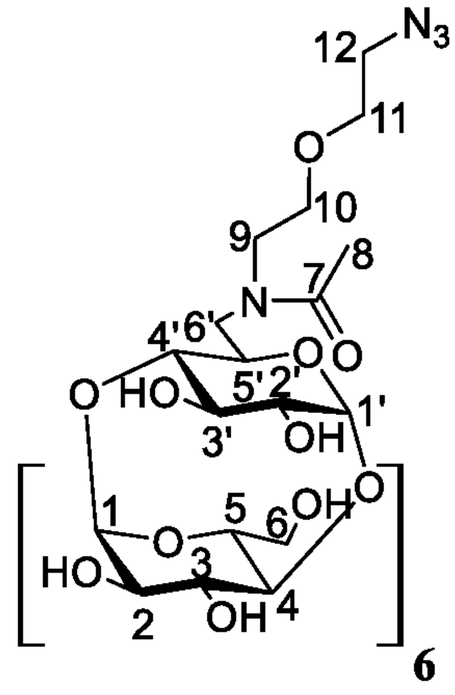
N-(2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)eth-1-yl)-6^{A}-amino-6^{A}-deoxy-β-cyclodextrin (20).



6^{A}-O-*p*-Toluensulphonyl-β-cyclodextrin **16** (1.00 g, 0.78 mmol) was mixed with 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethan-1-amin **27** (2,0 ml) and the heterogeneous mixture was heated to 60 °C. Homogenisation took place and the solution was stirred at this temperature overnight. The reaction mixture was monitored by TLC using the mixture PrOH/H_2O/EtOAc/concentrated aqueous solution of NH_3 6/3/1/1 and the substances were detected by carbonisation in 50% aqueous sulphuric acid solution. The solution was diluted with water (2 mL) and the reaction mixture was poured into acetone (200 mL). The resulting precipitate was separated by filtration, washed with acetone and dried for 2 hours at laboratory temperature using an oil rotary pump. The crude product (1.10 g) was dissolved in water (15 mL) and poured again into acetone (200 mL). The resulting precipitate was separated by centrifugation, dissolved in as little water as possible and purified by ion exchange column chromatography. The strong cation exchange resin Amberlite IR 120 (160 mL) was used in H^+ cycle. The by-products were removed with water and the product was eluted with 5% aqueous solution of NH_3. The product-containing solution was evaporated at 50 °C using a rotary vacuum evaporator. The residue (0.94 g) was dissolved in water (18 mL) and lyophilised. The product was obtained as a white solid in a yield of 85 % (0.88 g). $[\alpha]^{25}_{D}$ +89.4° (α +0.059, 6.6 mg, H_2O). IR(KBr): 3361 ν(O-H), 2925 ν(C-H), 2113 ν(azide), 1645 ν(C-H), 1301 ν(C-H), 1154 ν(C-H), 1080 ν(C-O), 1031 ν(C-O) cm^{-1}. ^1H NMR (600 MHz, D_2O): δ = 5.07 – 5.03 (m, 7H, H-1, H-1‘), 3.92 – 3.56 (m, 52H, H-2, H-2‘, H-3, H-3‘, H-4, H-4‘, H-5, H-5‘, H-6, H-8, H-9, H-10, H-11, H-12, H-13), 3.48 (m, 2H, H-14), 3.11 – 2.74 (m, 4H, H-6‘, H-7), ppm. ^{13}C NMR (150 MHz, D_2O, tBuOH): δ = 102.46 – 101.78 (C-1, C-1‘), 84.35 – 80.99 (C-4, C-4‘), 73.60 – 73.33 (C-3, C-3‘), 72.43 – 72.19 (C-2, C-2‘, C-5), 70.72 (C-5‘), 70.32 – 69.39 (C-8, C-9, C-10, C-11, C-12, C-13), 60.52 – 60.39 (C-6), 50.51 (C-14), 49.43 (C-6‘), 47.74 (C-7) ppm. ESI MS: for $C_{50}H_{86}N_4O_{37}$ calcd.: *m/z* 1334.5, found 1336.0 $[M+H]^{1+}$. HRMS: for $C_{50}H_{86}N_4O_{37}$ calcd.: *m/z* 1334.4971, found 1335.4979 $[M+H]^{1+}$, Δ 4.8 ppm.

N-Acetyl-N-(2-(2-azidoethoxy)eth-1-yl)-6^{A}-amino-6^{A}-deoxy-β-cyclodextrin (21).

N-(2-(2-azidoethoxy)eth-1-yl)-6^{A}-amino-6^{A}-deoxy-β-cyclodextrin **19** (0.40 g, 0.32 mmol) was dissolved in deionised water (9 mL) and NaOH solution (51 mg in 1 mL water) was added. Acetic anhydride (0.12 mL, 1.28 mmol) was then added and the solution was stirred at laboratory temperature for 4 hours. The reaction mixture was monitored by TLC using $CHCl_3$/MeOH/H_2O 5/4/1 and the substances were detected by carbonisation in 50% aqueous sulphuric acid solution. No starting material was observed, only the product and overreacted by-products. Additional NaOH solution (100 mg in 1.8 mL of water) was added to the reaction mixture and the reaction mixture was stirred overnight at laboratory temperature. The reaction mixture was again monitored by TLC using the mixture $CHCl_3$/MeOH/H_2O 5/4/1 and the substances were detected by carbonisation in 50% aqueous sulphuric acid solution. No overreacted by-products were observed. The reaction mixture was neutralised with 1M HCl, and silica gel (2 g) was added to the solution. The suspension was evaporated on a rotary vacuum evaporator at 55 °C. The crude product adsorbed on silica gel was purified by column chromatography (20 g of silica gel) with elution mixture $CHCl_3$/MeOH/H_2O 5/4/1. The product-containing fractions were evaporated on a rotary vacuum evaporator at 50 °C. The product was dried at 60 °C using an oil rotary pump and obtained as a white free-flowing material in a yield of 78 % (320 mg). $[\alpha]^{25}_{D}$ +137.3° (α +0.070, 5.1 mg, DMSO). IR(KBr): 3378 ν(O-H), 2926 ν(C-H), 2113 ν(azide), 1622 ν(C=O), 1419 ν(C-H), 1251 ν(C-H), 1156 ν(C-H), 1078 ν(C-O), 1039 ν(C-O) cm^{-1}. ^{1}H NMR (600 MHz, DMSO-d_6): δ = 5.97 – 5.66 (m, 14H, 2,3-OH), 4.88 – 4.80 (m, 7H, H-1, H-1‘), 4.53 – 4.20 (m, 7H, 6-OH, H-5‘), 3.83 – 2.98 (m, 48H, H-2, H-2‘, H-3, H-3‘, H-4, H-4‘, H-5, H-5‘, H-6, H-6‘, H-9, H-10, H-11, H-12), 2.02 (s, 1.8H, H-8), 1.97 (s, 1.2H, H-8) ppm. ^{13}C NMR (150 MHz, DMSO-d_6): δ = 170.35 (C-7), 170.01 (C-7), 102.42 – 101.55 (C-1, C-1‘), 84.68 – 81.17 (C-4, C-4‘), 72.97 – 68.04 (C-2, C-2‘, C-3, C-3‘, C-5, C-5‘, C-9, C-10, C-11), 63.26 – 59.56 (C-6), 50.45 (C-12), 50.22 (C-12), 49.32 – 46.17 (C-5‘, C-6‘), 21.51 (C-8), 21.28 (C-8) ppm. ESI MS: for $C_{48}H_{80}N_4O_{36}$ calcd.: *m/z* 1288.5, found 1289.0 $[M+H]^{1+}$, 1311.0 $[M+Na]^{1+}$. HRMS: for $C_{48}H_{80}N_4O_{36}$ calcd.: *m/z* 1246.4552, found 1249.4636 $[M+H]^{1+}$, Δ 0.9 ppm.



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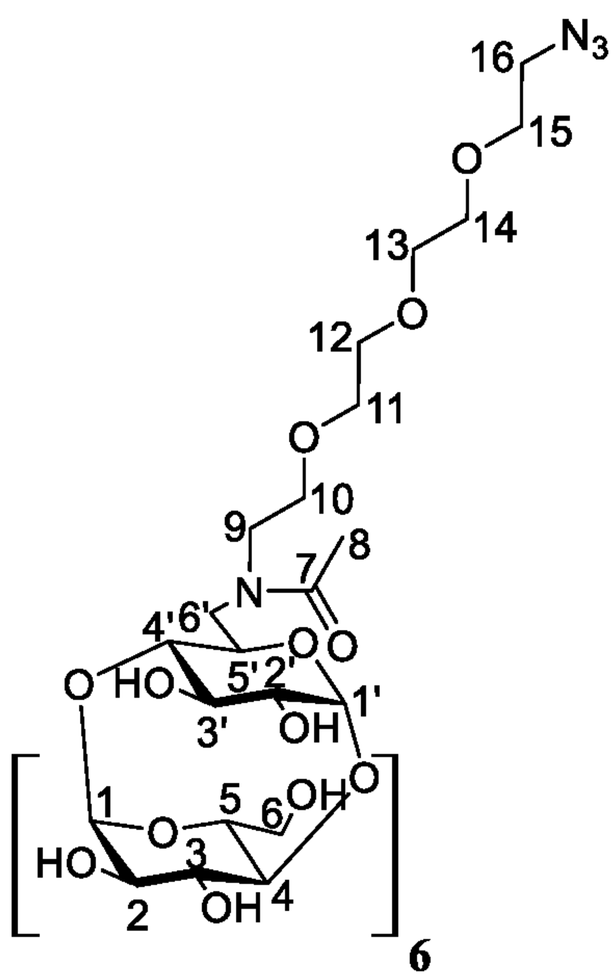
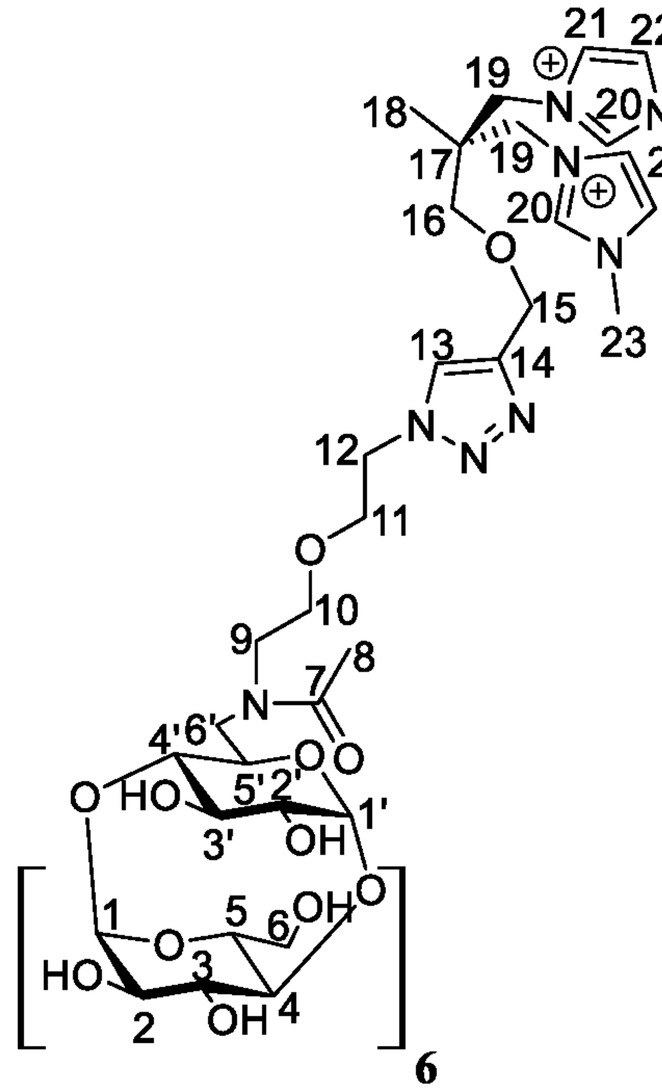
N-Acetyl-N-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)eth-1-yl)-6^{A}-amino-6^{A}-deoxy-β-cyclodextrin (22).



N-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)eth-1-yl)-6^{A}-amino-6^{A}-deoxy-β-cyclodextrin **20** (0.40 g, 0.30 mmol) was dissolved in deionised water (9 mL) and NaOH solution (48 mg in 0.9 mL water) was added. The acetic anhydride (0.11 mL, 1.20 mmol) was then added and the solution was stirred at laboratory temperature for 3 hours. The reaction mixture was monitored by TLC using the mixture $CHCl_3$/MeOH/H_2O 5/4/1 and the substances were detected by carbonisation in 50% aqueous sulfuric acid. No starting material was observed, only the product and overreacted by-products. Additional NaOH solution (100 mg in 1.8 mL of water) was added to the mixture and the reaction mixture was stirred overnight at laboratory temperature. The reaction mixture was again monitored by TLC using the mixture $CHCl_3$/MeOH/H_2O 5/4/1 and the substances were detected by carbonisation in 50% aqueous sulphuric acid solution. No overreacted by-products were observed. The reaction mixture was neutralised with 1M HCl, and silica gel (2 g) was added to the solution. The suspension was evaporated on a rotary vacuum evaporator at 55 °C. The crude product adsorbed on silica gel was purified by column chromatography (20 g of silica gel) with elution mixture $CHCl_3$/MeOH/H_2O 5/4/1. The product was not obtained sufficiently pure. The product-containing fractions were evaporated on a rotary vacuum evaporator at 50 °C. The residue (0.49 g) was dissolved in water and purified by C18-reversed phase column chromatography (10 g of silica gel). The product was eluted with 10% aqueous solution of MeOH. The product-containing fractions were evaporated on a rotary vacuum evaporator at 50 °C. The product was dried at 60 °C using an oil rotary pump and obtained as a white free-flowing material in a yield of 48 % (190 mg). $[\alpha]^{25}_{D}$ +133.3° (α +0.096, 7.2 mg, H_2O). IR(KBr): 3351 ν(O-H), 2920 ν(C-H), 2116 ν(azide), 1622 ν(C=O), 1413 ν(C-H), 1251 ν(C-H), 1159 ν(C-H), 1081 ν(C-O), 1036 ν(C-O) cm^{-1}. ^{1}H NMR (600 MHz, DMSO-d_6): δ = 5.95 – 5.68 (m, 14H, 2,3-OH), 4.89 – 4.80 (m, 7H, H-1, H-1‘), 4.51 – 4.23 (m, 7H, 6-OH, H-5‘), 3.80 – 2.93 (m, 51H, H-2, H-2‘, H-3, H-3‘, H-4, H-4‘, H-5, H-5‘, H-6, H-6‘, H-9, H-10, H-11, H-12, H-13, H-14, H-15, H-16), 2.03 (s, 2H, H-8), 1.98 (s, 1H, H-8) ppm. ^{13}C NMR (150 MHz, DMSO-d_6): δ = 170.24 (C-7), 170.10 (C-7), 102.40 – 101.64 (C-1, C-1‘), 84.63 – 81.17 (C-4, C-4‘), 73.02 – 67.70 (C-2, C-2‘, C-3, C-3‘, C-5, C-9, C-10, C-11, C-12, C-13, C-14, C-15), 60.01 – 59.14 (C-6), 49.99 (C-16), 49.00 – 45.95 (C-5‘, C-6‘), 21.48 (C-8), 21.28 (C-8) ppm. ESI MS: for $C_{52}H_{88}N_4O_{38}$ calcd.: *m/z* 1376.5, found 1415.0 $[M+K]^{1+}$

3,3'-(2-(((1-(2-(3-(*N*-(6^A-Deoxy-β-cyclodextrin-6^A-yl)acetamido)propoxy)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)-2-methylpropan-1,3-diyl)bis(1-methyl-1H-imidazol-3-ium) (23)

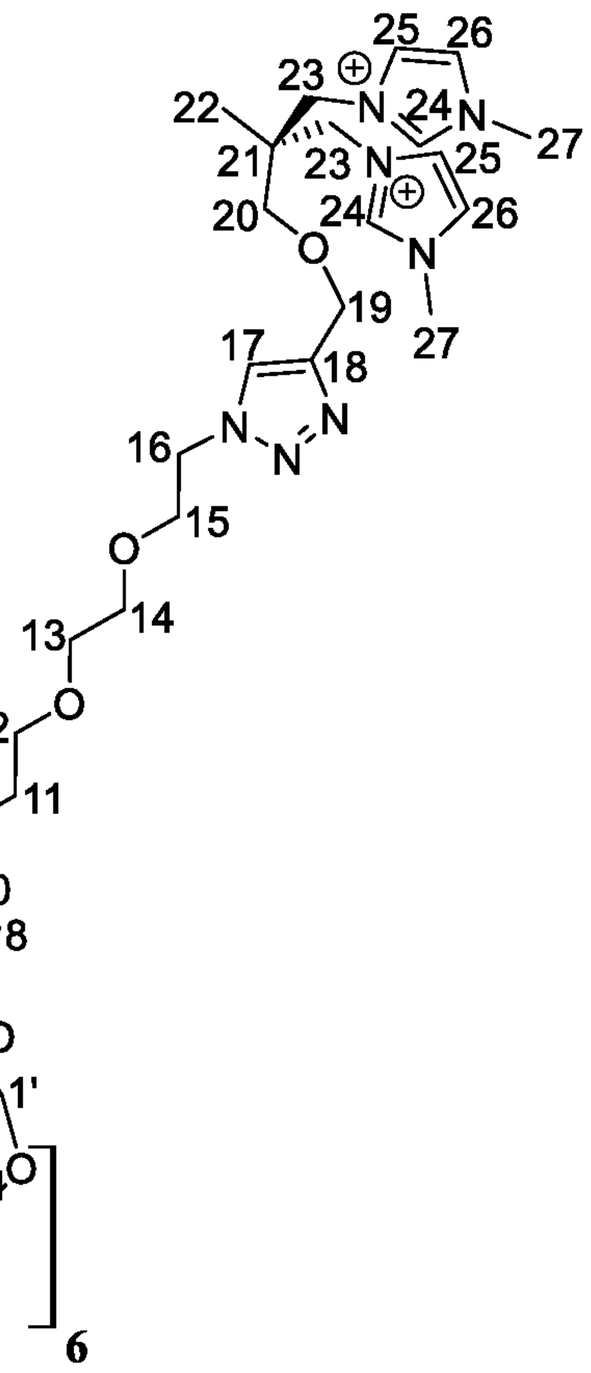


N-acetyl-*N*-(2-(2-azidoethoxy)eth-1-yl)-6^A-amino-6^A-deoxy-β-cyclodextrin **21** (0.139 g, 0.108 mmol) and 3,3'-(2-methyl-2-((prop-2-yn-1-yloxy)methyl)propan-1,3-diyl)bis(1-methyl-1H-imidazol-3-ium) **K2MIMM** (51 mg, 86.2 μmol) were dissolved in the mixture of deionised water/MeCN 1/1 (10 mL) and the solution was bubbled with argon for 10 minutes. Subsequently, CuI (16 mg, 86.2 μmol) was added and the reaction mixture was stirred at laboratory temperature for 12 hours. The reaction mixture was monitored by TLC using the mixture MeOH/concentrated HOAc/1% aqueous solution of NH_4HCO_3 10/1/9 and the substances were detected by UV lamp and by carbonisation in 50% aqueous sulphuric acid solution. The reaction mixture was concentrated on a rotary vacuum evaporator at 50 °C. The residue (0.246 g) was suspended in water (20 mL) and the insoluble CuI was filtered off through a layer of celite. The product in aqueous solution was purified by C18-reversed phase column chromatography (5 g of silica gel). The product was eluted with 5% aqueous solution of MeOH. The product-containing fractions were evaporated on a rotary vacuum evaporator at 50 °C and propanol was added during evaporation to give a free-flowing product. The product was dried at 60 °C using an oil rotary pump and obtained as a white free-flowing material in a yield of 85 % (138 mg). $[\alpha]^{25}_D$ +90.0° (α +0.072, 8.0 mg, H_2O). IR(KBr): 3306 ν(O-H), 2929 ν(C-H), 1619 ν(C=O), 1422 ν(C-H), 1281 ν(C-H), 1153 ν(C-H), 1081 ν(C-O), 1036 ν(C-O) cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6): δ = 9.04 – 9.03 (m, 2H, H-20), 8.09 (s, 0,4H, H-13), 8.06 (s, 0,6H, H-13), 7.74 (s, 2H, H-21), 7.60 (s, 2H, H-22), 6.12 – 5.76 (m, 14H, 2,3-OH), 4.91 – 4.78 (m, 7H, H-1, H-1‘), 4.58 – 4.49 (m, 11H, 6-OH, H-5‘, H-12, H-15), 4.31 (d, *J* = 14.1 Hz, 1H, H-19), 4.18 (d, *J* = 14.1 Hz, 1H, H-19), 3.88 (s, 6H, H-23), 3.82 – 2.95 (m, 45H, H-2, H-2‘, H-3, H-3‘, H-4, H-4‘, H-5, H-6, H-6‘, H-9, H-10, H-11), 3.11 (s, 2H, H-16), 1.96 (s, 1.2H, H-8), 1.91 (s, 1.8H, H-8), 0.85 (m, 3H, H-18) ppm. ^{13}C NMR (150 MHz, DMSO-d_6): δ = 170.42 (C-7), 170.17 (C-7), 142.96 (C-14), 137.74 (C-20), 124.64 (C-13), 124.56 (C-13), 123.72 (C-22), 123.50 (C-21), 102.03 – 101.67 (C-1, C-1‘), 84.54 – 80.95 (C-4, C-4‘), 73.04 – 67.31 (C-2, C-2‘, C-3, C-3‘, C-5, C-5‘, C-9, C-10, C-11), 63.46 (C-15), 63.42

(C-15), 60.10 – 59.29 (C-6), 52.57 (C-19), 49.46 (C-12), 48.63 – 45.37 (C-5‘, C-6‘), 39.52 (C-17, solvent overlay), 35.96 (C-23), 21.53 (C-8), 21.26 (C-8), 17.35 (C-18), 17.32 (C-18) ppm. UV-VIS (H_2O), λ_{max}, nm: 209.0, 6.7×10^{-5} M. ESI MS: for $C_{64}H_{104}N_8O_{37}^{2+}$ calcd.: *m/z* 788.3, found 788.2 $[M]^{2+}$.

3,3'-(2-(((1-(3-(6^A-Deoxy-β-cyclodextrin-6^A-yl)-2-oxo-6,9,12-trioxa-3-azatetradekan-14-yl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)-2-methylpropan-1,3-diyl)bis(1-methyl-1H-imidazol-3-ium) (24)

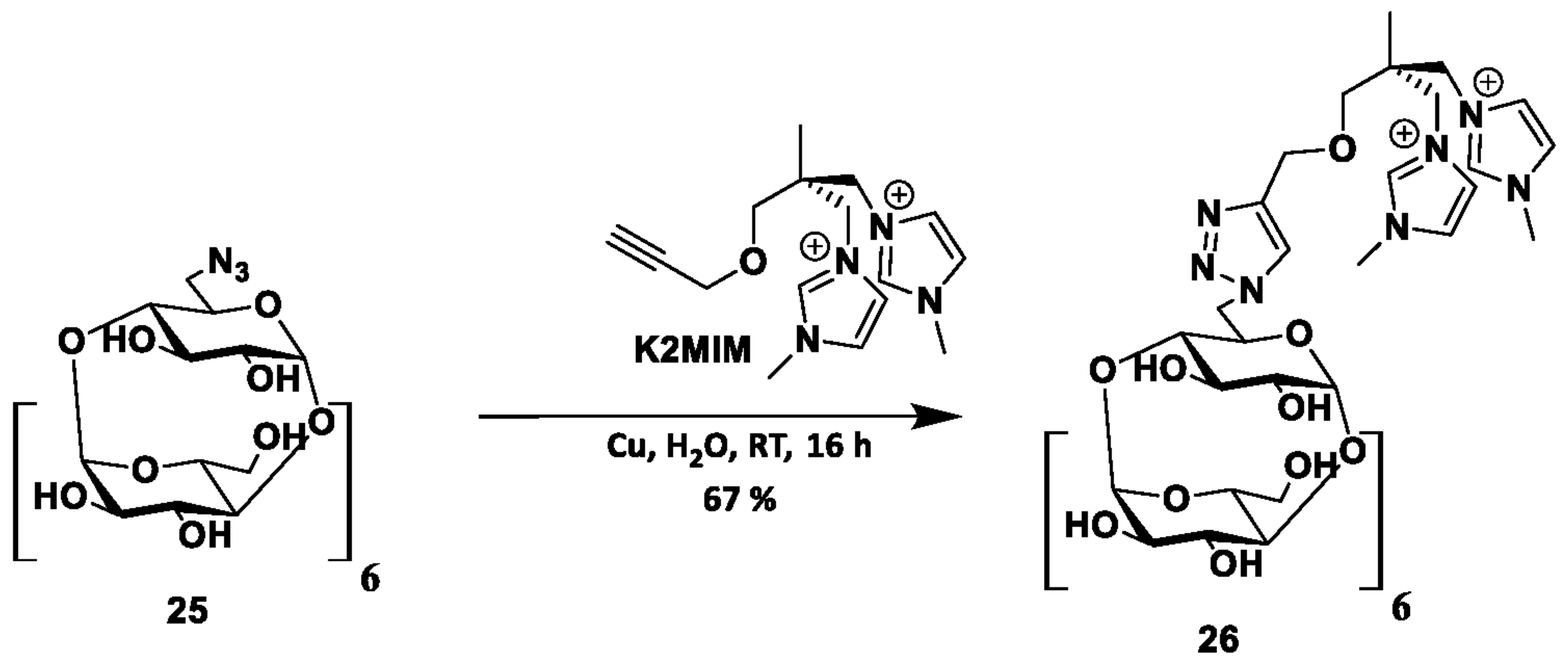
N-Acetyl-*N*-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)eth-1-yl)-6^A-amino-6^A-deoxy-β-cyclodextrin **22** (0.091 g, 65.9 μmol) and 3,3'-(2-methyl-2-((prop-2-yn-1-yloxy)methyl) propan-1,3-diyl)bis(1-methyl-1H-imidazol-3-ium) **K2MIMM** (31 mg, 52.8 μmol) were dissolved in the mixture of deionised water/MeCN 1/1 (6 ml) and the solution was bubbled with argon for 10 minutes. Subsequently, CuI (10 mg, 52.8 μmol) was added and the reaction mixture was stirred at laboratory temperature for 15 hours. The reaction mixture was monitored by TLC using the mixture of MeOH/concentrated HOAc/1% aqueous solution of NH_4HCO_3 10/1/9 and the substances were detected by UV lamp and carbonisation in 50% aqueous sulphuric acid solution. The reaction mixture was evaporated on a rotary vacuum evaporator at 50 °C. The residue (0.104 g) was suspended in water (15 mL) and the insoluble CuI was filtered through a layer of celite. The product in aqueous solution was purified by C18-reversed phase column chromatography (3.2 g of silica gel). The product was eluted with 5% aqueous solution of MeOH. The product-containing fractions were evaporated on a rotary vacuum evaporator at 50 °C and propanol was added during evaporation to give the free-flowing product. The product was dried at 60 °C using an oil rotary pump and obtained as a white free-flowing material in a yield of 67 % (69 mg). $[\alpha]^{25}_D$ +95.9° (α +0.070, 7.3 mg, H_2O). IR(KBr): 3357 ν(O-H), 2935 ν(C-H), 1622 ν(C=O), 1425 ν(C-H), 1254 ν(C-H), 1159 ν(C-H), 1081 ν(C-O), 1030 ν(C-O) cm^{-1}. ^{1}H NMR (600 MHz, DMSO-d_6): δ = 9.04 – 9.03 (m, 2H, H-24), 8.13 (s, 1H, H-17), 7.74 (s, 2H, H-25), 7.59 (s, 2H, H-26), 5.99 – 5.69 (m,



14H, 2,3-OH), 4.85 – 4.80 (m, 7H, H-1, H-1‘), 4.58 – 4.20 (m, 11H, 6-OH, H-16, H-19, H-5‘), 4.31 (d, *J* = 13.9 Hz, 1H, H-23), 4.17 (d, *J* = 14.0 Hz, 1H, H-23), 3.87 (s, 6H, H-27), 3.85 (t, *J* = 5.2 Hz, 1H, H-15), 3.79 – 2.96 (m, 51H, H-2, H-2‘, H-3, H-3‘, H-4, H-4‘, H-5, H-6, H-6‘, H-9, H-10, H-11, H-12, H-13, H-14), 3.10 (s, 2H, H-20), 2.02 (s, 1.8H, H-8), 1.97 (s, 1.2H, H-8), 0.85 (s, 3H, H-22) ppm. ^{13}C NMR (150 MHz, DMSO-d_6): δ = 170.30 (C-7), 170.14 (C-7), 142.89 (C-18), 137.71 (C-24), 124.67 (C-17), 124.63 (C-17), 123.70 (C-26), 123.48 (C-25), 102.38 – 101.65 (C-1, C-1‘), 84.58 – 81.15 (C-4, C-4‘), 73.04 – 67.35 (C-2, C-2‘, C-3, C-3‘, C-5, C-5‘, C-9, C-10, C-11, C-12, C-13, C-14, C-15), 63.39 (C-19), 60.11 – 59.24 (C-6), 52.56 (C-23), 49.43 (C-16), 48.97 – 45.73 (C-5‘, C-6‘), 39.52 (C-21, solvent overlay), 35.95 (C-27), 21.50 (C-8), 21.30 (C-8), 17.33 (C-22) ppm. UV-VIS (H_2O), λ_{max}, nm: 209.0, 7.6×10^{-5} M. ESI MS: for $C_{68}H_{112}N_8O_{39}^{2+}$ calcd.: *m/z* 832.4, found 832.5 $[M]^{2+}$.

3.2 Modifier with cyclodextrin directly attached to the charged anchor

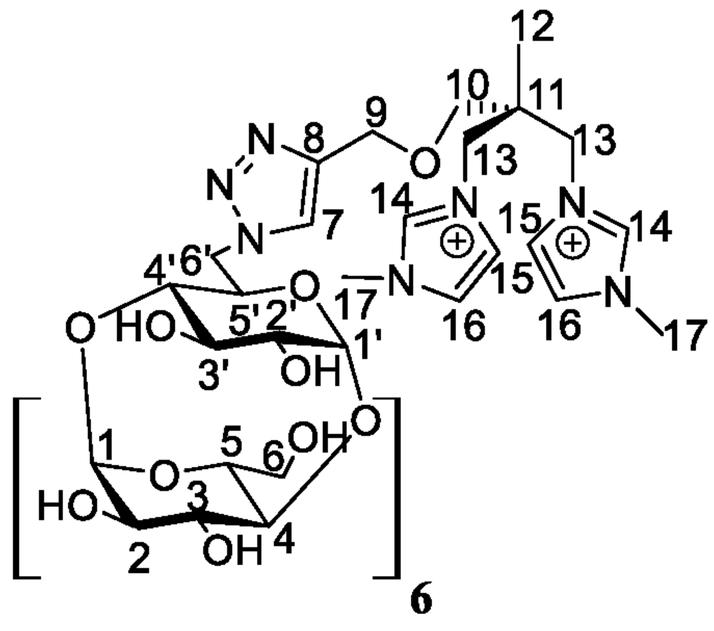
The procedure for preparing the modifier of general formula (III) which contains cyclodextrin directly attached to a charged anchor is shown in Scheme 6. The preparation is based on the commercially available 6^A-azido-6^A-deoxy-β-cyclodextrin, which gives the desired product by CuAAC reaction with the propargyl anchor.



Scheme 6: Preparation of CD modifier without linker

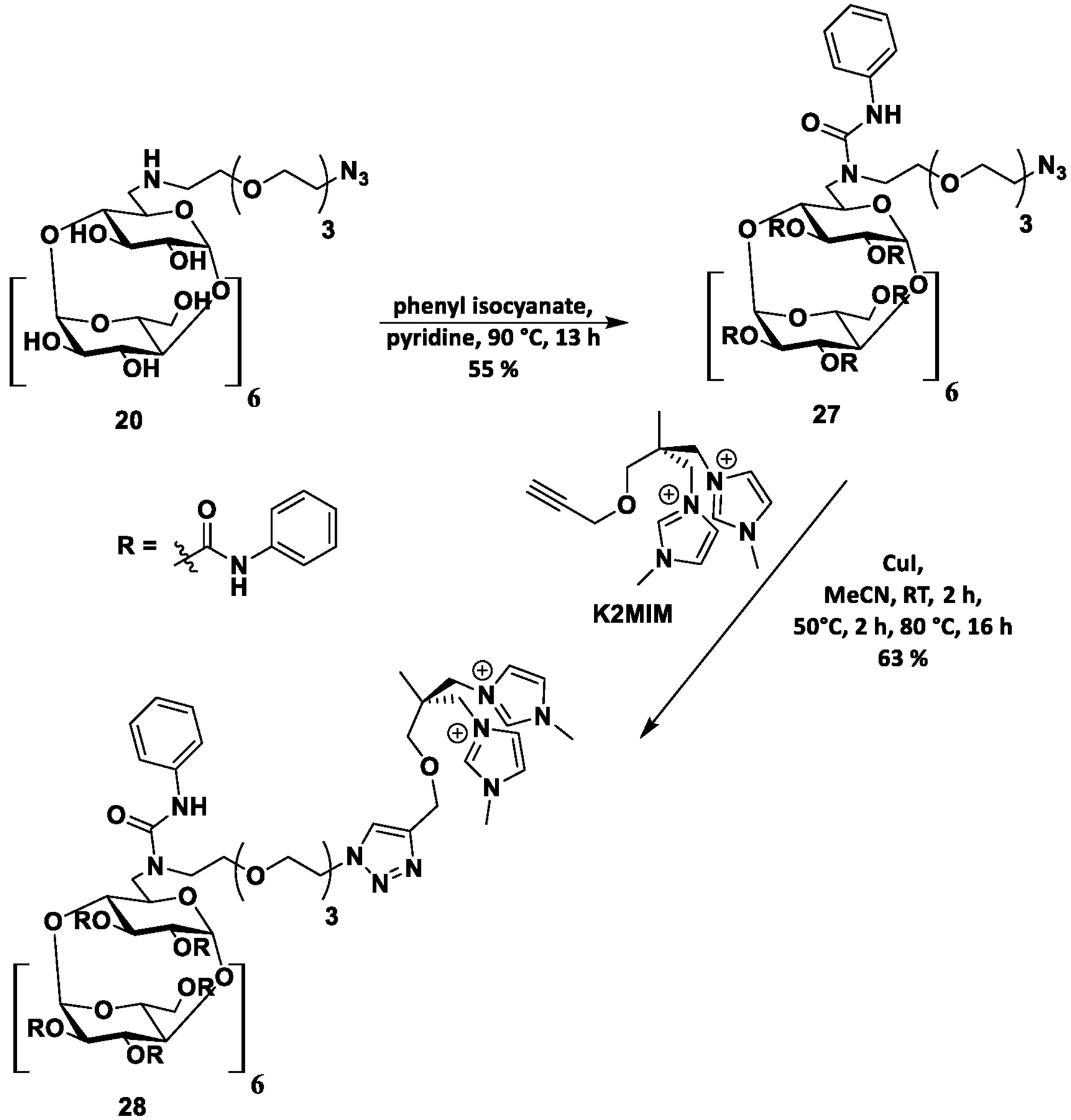
3,3'-(2-(((1-(6^{A}-Deoxy-β-cyclodextrin-6^{A}-yl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)-2-methylpropan-1,3-diyl)bis(1-methyl-1H-imidazol-3-ium) (26)

3,3'-(2-methyl-2-((prop-2-yn-1-yloxy)methyl)propan-1,3-diyl)bis(1-methyl-1H-imidazol-3-ium) **K2MIMM** (0.390 g, 0.720 mmol) and 6^{A}-azido-6^{A}-deoxy-β-cyclodextrin **18** (1.0 g, 0.863 mmol) were dissolved in deionised water (20 mL). The metallic copper (1.37 g, 21.5 mmol) was added and the reaction mixture was stirred at laboratory temperature for 16 hours. The reaction mixture was monitored by TLC using the mixture MeOH/concentrated HOAc/1% aqueous solution of NH_4HCO_3 10/1/9 and the substances were detected by carbonisation in 50% aqueous sulphuric acid solution. The reaction mixture was filtered through a layer of celite. The green filtrate was mixed with weak cation exchange resin (Amberlite CG50, 14 g of wet polymer) in H^+ cycle. After 15 minutes, a colourless solution was obtained. The mixture was filtered and the aqueous product solution was purified by C18-reversed phase column chromatography (10 g of silica gel). The product was eluted with water and the solution was evaporated on a rotary vacuum evaporator at 50 °C and dried at 60 °C using an oil rotary pump. The product was obtained as a light orange glassy material in a yield of 67 % (817 mg). $[\alpha]^{25}_{D}$ +71.4° (α +0.045, 6.3 mg, H_2O). IR(KBr): 3327 ν(O-H), 2923 ν(C-H), 1619 ν(C=O), 1625 ν(C-H), 1425 ν(C-H), 1293 ν(C-H), 1156 ν(C-H), 1078 ν(C-O), 1030 ν(C-O) cm^{-1}. ^{1}H NMR (600 MHz, D_2O): δ = 8.80 – 8.78 (m, 2H, H-14), 8.23 (s, 1H, H-7), 7.51 (s, 2H, H-15), 7.45 (m, 2H, H-16), 5.22 – 4.69 (m, 9H, H-1, H-1‘, H-9), 4.47 – 4.20 (m, 5H, H-13, H-5‘), 4.07 – 3.53 (m, 45H, H-2, H-2‘, H-3, H-3‘, H-4, H-4‘, H-5, H-6, H-17), 3.23 (s, 2H, H-10), 3.20 – 2.83 (m, 2H, H-6‘), 1.00 (s, 3H, H-12) ppm. ^{13}C NMR (150 MHz, D_2O, tBuOH): δ = 143.90 (C-8), 137.48 (C-14), 127.23 (C-7), 124.20 – 124.08 (C-15, C-16), 102.51 – 101.88 (C-1, C-1‘), 83.54 – 81.10 (C-4, C-4‘), 73.46 – 70.04 (C-2, C-2‘, C-3, C-3‘, C-5, C-5‘,C-10), 63.32 – 59.56 (C-6, C-6‘), 53.33 – 53.25 (C-13), 51.66 (C-9) 40.40 (C-11), 36.35 (C-17), 17.43 (C-12) ppm. UV-VIS (H_2O), λ_{max1}, nm: 241.0, λ_{max2}, nm: 279.0, 4.7×10^{-4} M. ESI MS: for $C_{58}H_{93}N_7O_{35}^{2+}$ calcd.: *m/z* 723.8, found 724.0 $[M]^{2+}$. HRMS: for $C_{58}H_{93}N_7O_{35}^{2+}$ calcd.: *m/z* 723.7851, found 723.7860 $[M]^{2+}$, Δ 1.2 ppm.



3.3 Modifiers containing phenylcarbamoylated cyclodextrin

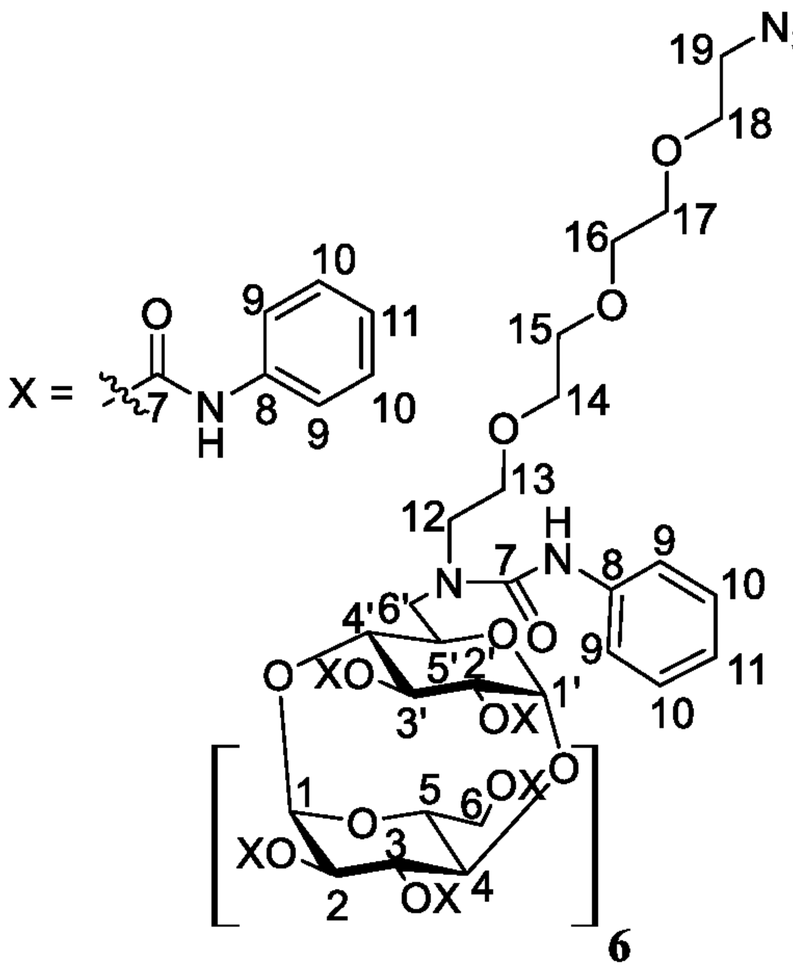
The procedure for preparing the modifier of general formula (III) which contains phenylcarbamoylated cyclodextrin as substance A is described in Scheme 6.



Scheme 6: Synthesis of a type (III) modifier containing per(phenylcarbamate)cyclodextrin attached via a tetraethylene glycol linker to a charged anchor.

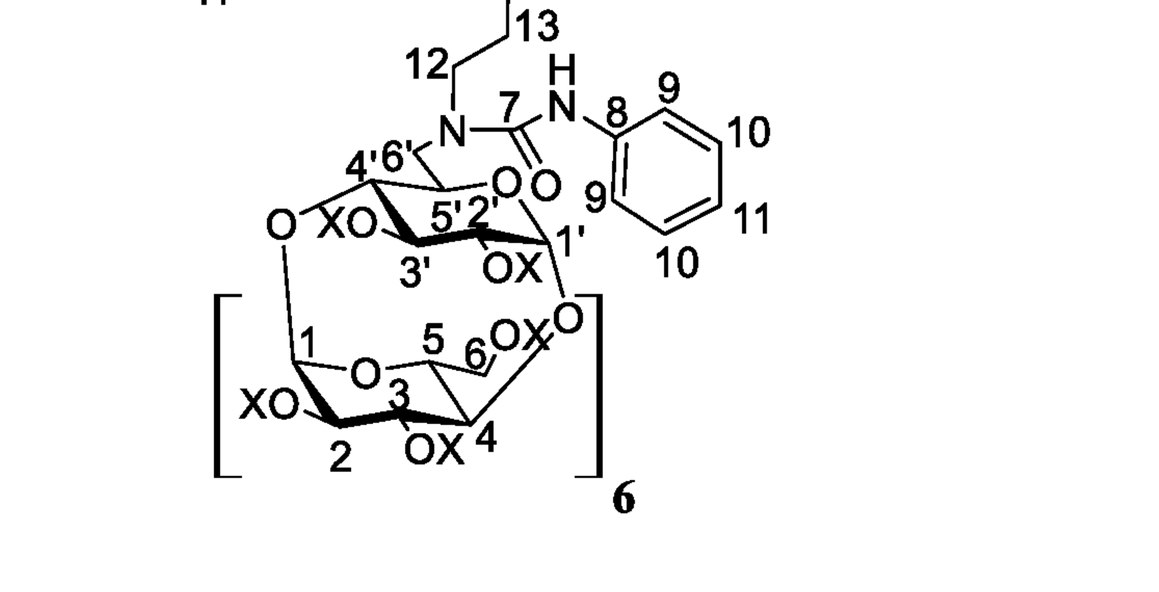
Perphenylcarbamoyl-*N*-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)eth-1-yl)-6^A-amino-6^A-deoxy-β-cyclodextrin (27).

N-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)eth-1-yl)-6^A-amino-6^A-deoxy-β-cyclodextrin **20** (0.1 g, 74.9 µmol) was dissolved in dry pyridine (1.4 mL) followed by dropwise addition of phenyl isocyanate (0.25 mL, 2.25 mmol). The reaction mixture was heated to 90 °C and stirred at this temperature for 13 hours. The reaction mixture was monitored by TLC using the mixture toluene/MeOH 6/1 and the substances were detected by UV lamp and carbonisation in 50% aqueous sulphuric acid solution. The pyridine was then distilled off from the reaction mixture at 90 °C using an oil rotary pump. The residue (0.31 g) was dissolved in the mixture $CHCl_3$/MeOH 1/1, silica gel (1.5 g) was added and the mixture was evaporated on a rotary evaporator at 50 °C. The crude product adsorbed on silica gel was purified by column chromatography (15 g silica gel) with elution mixture toluene/MeOH 10/1. The product was not obtained in pure form. The product-containing fractions were evaporated on a rotary vacuum evaporator at 50 °C. The residue (0.23 g) was dissolved in $CHCl_3$, silica gel (1.1 g) was added and the mixture was evaporated on a rotary vacuum evaporator at 50 °C. The second time adsorbed product was again purified by column chromatography (5 g of silica gel) with eluting mixtures hexane/EtOAc 7/1 and hexane/EtOAc 1/1. The product-containing fractions were evaporated on a rotary vacuum evaporator at 40 °C. The product was dried at 50 °C using an oil rotary pump and obtained as a white free-flowing material in a yield of 55 % (0.150 g). ^{1}H NMR (600 MHz, DMSO-d_6): δ = 9.79 – 8.38 (m, 21H, NH), 7.54 – 6.72 (m, 105H, H-9, H-10, H-11), 5.51 – 3.55 (m, 28H, H-2, H-2‘, H-3, H-3‘, H-4, H-4‘, H-5, H-5‘), 5.34 (bs, 7H, H-1, H-1‘), 4.64 (m, 2H, H-6‘), 3.45 – 3.29 (s, 26H, H-6, H-12, H-13, H-14, H-15, H-16, H-17, H-18), 3.26 (t, *J* = 4.9 Hz, 2H, H-19) ppm. ^{13}C NMR (150 MHz, DMSO-d_6): δ = 157.09 – 152.47 (C-7), 140.66 – 138.00 (C-8), 129.19 – 118.26 (C-9, C-10, C-11), 98.51 – 97.57 (C-1, C-1‘), 71.73 – 70.11 (C-2, C-2‘, C-3, C-3‘, C-4, C-4‘, C-5, C-5‘), 69.98 – 68.86 (C-6, C-12, C-13, C-14, C-15, C-16, C-17, C-18), 62.94 – 62.47 (C-6‘), 49.62 (C-19) ppm. ESI MS: for $C_{197}H_{191}N_{25}O_{58}$ calcd.: *m/z* 3834.28, found 1941.2 $[M+2Na]^{2+}$.



Compound 28.

Compound **27** (0.30 g, 78.2 µmol) and 3,3'-(2-methyl-2-((prop-2-yn-1-yloxy)methyl)propan-1,3-diyl)bis(1-methyl-1H-imidazol-3-ium) **K2MIMM** (69 mg, 117.4 µmol) were dissolved in acetonitrile (7 ml) and the solution was bubbled with argon for 30 minutes. Subsequently, CuI (15 mg, 78.2 µmol) was added and the reaction mixture was stirred at laboratory temperature for 2 hours. The reaction mixture was monitored by TLC using the mixture MeOH/concentrated HOAc/1% aqueous solution of NH_4HCO_3 10/1/9 and the substances were detected by UV lamp and carbonisation in 50% aqueous sulphuric acid solution. The reaction mixture was virtually unreacted. The temperature was raised to 50 °C and the reaction was stirred for 2 hours. Additional CuI (15 mg, 78.2 µmol) was added, the temperature was raised to 80 °C, and the reaction was stirred for another 4 hours. Additional CuI (15 mg, 78.2 µmol) was then added and the reaction was allowed to react for an additional 12 hours. The C18-reversed phase silica gel (1.8 g) was added to the reaction mixture and the mixture was evaporated using a rotary vacuum evaporator at 50 °C. The crude product adsorbed was purified by column chromatography with C18-reversed phase silica gel (7 g). The product was eluted with 80% aqueous solution of MeOH. The product-containing fractions were evaporated on a rotary vacuum evaporator at 50 °C. The product was dried at 65 °C using an oil rotary pump and obtained as a white free-flowing material in a yield of 63% (0.219 g). IR(KBr): 3309 ν(N-H), 3070 ν(N-H), 2926 ν(C-H) 2854 ν(C-H), 1736 ν(C=O), 1601 ν(C=O), 1539 ν(C=O), 1443 ν(C-H), 1314 ν(C-H), 1222 ν(C-H), 1084 ν(C-O), 1030 ν(C-O) cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6): δ = 9.76 – 8.34 (m, 21H, NH), 8.99 (bs, 2H, H-27), 8.03 (s, 1H, H-20), 7.71 (s, 2H, H-28), 7.56 (s, 2H, H-29), 7.52 – 6.73 (m, 105H, H-9, H-10, H-11), 5.52 – 3.05 (m, 64H, H-2, H-2‘, H-3, H-3‘, H-4, H-4‘, H-5, H-5‘, H-6‘, H-6, H-12, H-13, H-14, H-15, H-16, H-17, H-18, H-19, H-22, H-23, H-26), 5.34 (bs, 7H, H-1, H-1‘), 0.81 (s, 3H, H-25) ppm.


X =
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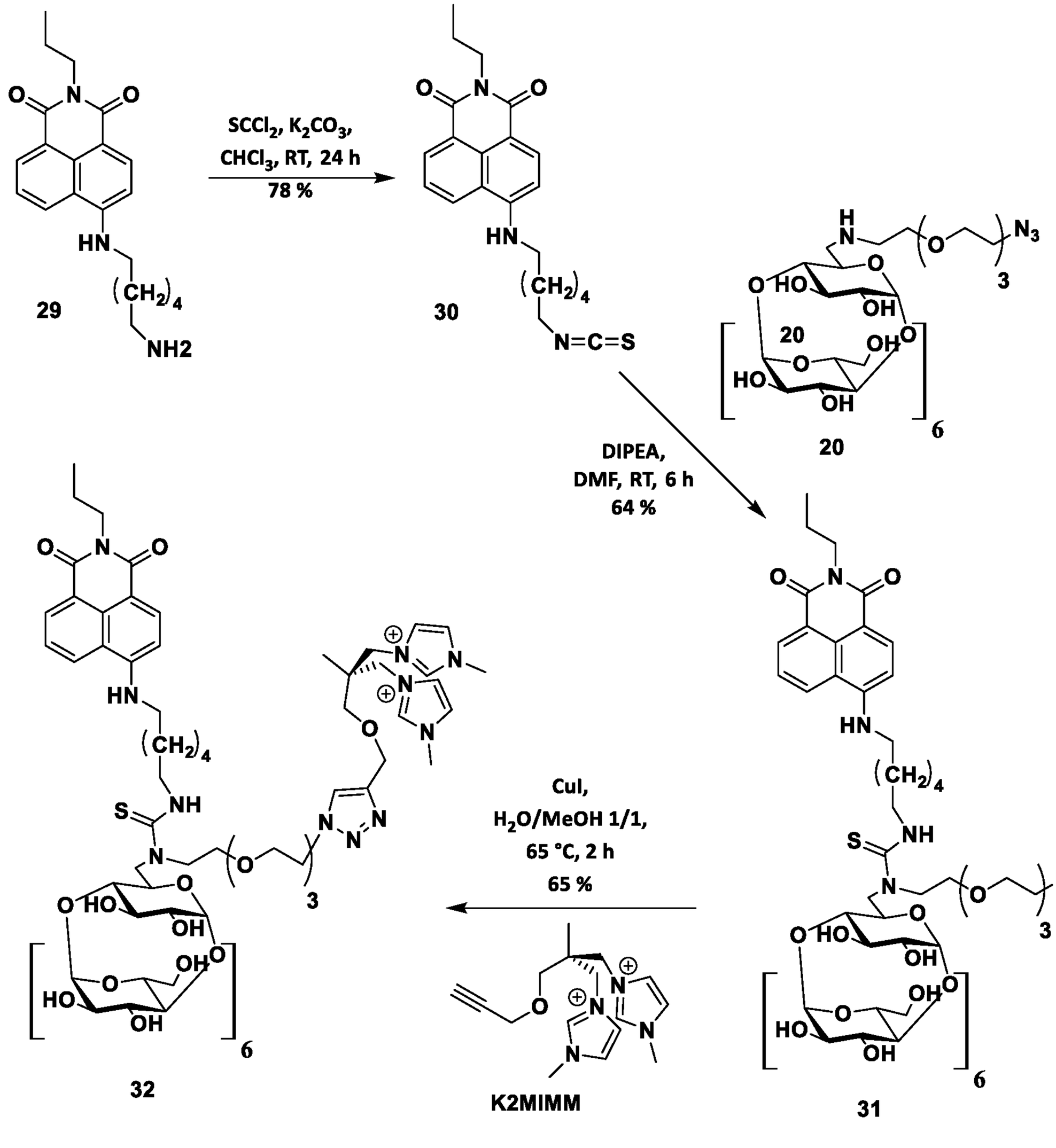
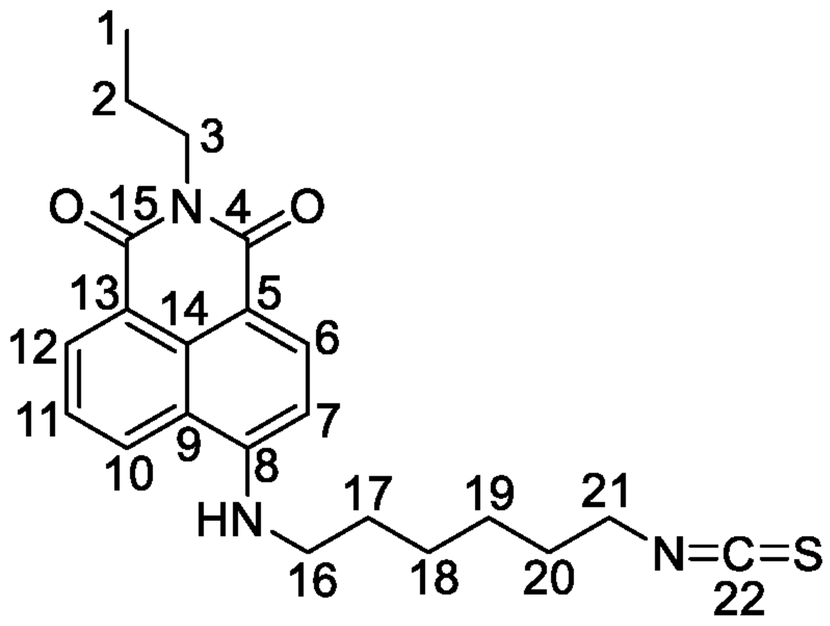
3.4 Modifier containing cyclodextrin and a fluorescent group

The procedure for preparing the modifier of general formula (III) which contains both cyclodextrin and a fluorescent group is described in Scheme 7.



Scheme 7: Synthesis of a type (III) modifier containing a cyclodextrin and a fluorophore group attached via a tetraethylene glycol linker to a charged anchor.

6-((6-Isothiocyanatohexyl)amino)-2-propyl-1H-benzo[de]isochinolin-1,3(2H)-dion (30).

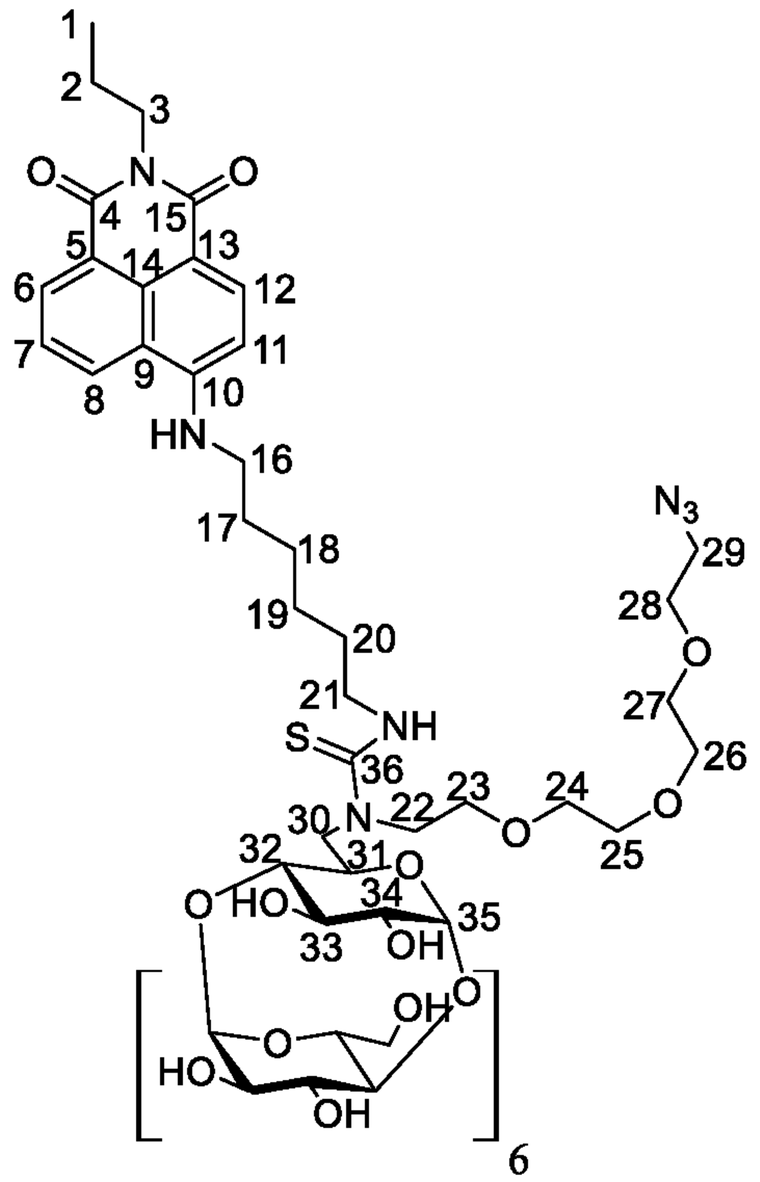


6-((6-Aminohexyl)amino)-2-propyl-1H-benzo[de]isochinolin-1,3(2H)-dion **29** (0.57 g, 1.62 mmol) was dissolved in $CHCl_3$ (100 mL) and K_2CO_3 (0.67 g, 4.84 mmol) was added. Thiophosgene (0.28 g, 2.42 mmol) was added dropwise and the reaction mixture was stirred at laboratory temperature for 3 hours. Additional thiophosgene (0.28 g, 2.42 mmol) was added dropwise and the mixture was stirred for an additional 16 hours. Subsequently, K_2CO_3 (0.67 g, 4.84 mmol) and thiophosgene (0.28 g, 2.42 mmol) were added and the reaction mixture was stirred for another 4 hours. The reaction mixture was monitored by TLC using hexane/EtOAc 2/1 to monitor the product and $CHCl_3$/MeOH 1/1 to monitor the starting substance and the substances were detected by UV lamp. The reaction mixture was extracted between water (50 mL) and Et_2O (200 mL). The organic phase was diluted with Et_2O (150 mL), washed with water (2 × 100 mL) and dried over $MgSO_4$ (3 g). The drying agent was removed by filtration and the filtrate was evaporated at 40 °C using a rotary vacuum evaporator. The residue (0.9 g) was dissolved in $CHCl_3$, silica gel (4.5 g) was added and the suspension was evaporated again at 40 °C using the rotary vacuum evaporator. The adsorbed product was purified by column chromatography (40 g of silica gel) with eluting mixture hexane/EtOAc 3/1. The product-containing fractions were evaporated at 40 °C using a rotary vacuum evaporator. The product was dried at laboratory temperature with an oil vacuum pump to give an orange solid in a yield of 78 % (0.49 g). IR(KBr): 3387 ν(naphthalimide), 2962 ν(C-H), 2938 ν(C-H), 2872 ν(C-H), 2857 ν(C-H), 2187 ν(isothiocyanate), 2125 ν(isothiocyanate), 1676 ν(naphthalimide), 1634 ν(naphthalimide), 1619 ν(naphthalimide), 1589 ν(naphthalimide), 1574 ν(naphthalimide), 1551 ν(naphthalimide), 1395 ν(C-H), 1374 ν(C-H), 1365 ν(C-H), 1350 ν(C-H), 1245 ν(naphthalimide) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 8.58 (dd, *J* = 7.3, 1.1 Hz, 1H, H-12), 8.46 (d, *J* = 8.3 Hz, 1H, H-6), 8.09 (dd, *J* = 8.5, 1.1 Hz, 1H, H-10), 7.62 (dd, *J* = 8.4, 7.3 Hz, 1H, H-11), 6.72 (d, *J* = 8.4 Hz, 1H, H-7), 5.25 (t, *J* = 5.2 Hz, 1H, NH), 4.17 – 4.07 (m, 2H, H-3), 3.55 (t, *J* = 6.4 Hz, 2H, H-21), 3.43 (td, *J* = 7.1, 5.1 Hz, 2H, H-16), 1.89 – 1.81 (m, 2H, H-17), 1.80 – 1.70 (m, 4H, H-2, H-20), 1.56 – 1.53 (m, 4H, H-18, H-19), 1.00 (t, *J* = 7.4 Hz, 3H, H-1) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 164.82 (C-15), 164.29 (C-4), 149.34 (C-8), 134.53 (C-6), 131.24 (C-12), 129.92 (C-13, C22), 125.82 (C-10), 124.89 (C-11), 123.39 (C-14), 120.30 (C-9), 110.65 (C-5), 104.48 (C-7), 45.09 (C-21), 43.64 (C-16), 41.82 (C-3), 29.95 (C-20), 28.97 (C-17),

26.55 (C-18, C-19), 21.60 (C-2), 11.72 (C-1) ppm. UV-VIS ($CHCl_3$), λ_{max1}, nm: 261, λ_{max2}, nm: 278, λ_{max3}, nm: 426, 12×10^{-5} M. ESI MS: for $C_{22}H_{25}N_3O_2S$ calcd.: *m/z* 395.2, found 396.2 $[M+H]^{1+}$. HRMS: for $C_{22}H_{25}N_3O_2S$ calcd.: *m/z* 395.1667, found 396.1737 $[M+H]^{1+}$, Δ 0.8 ppm.

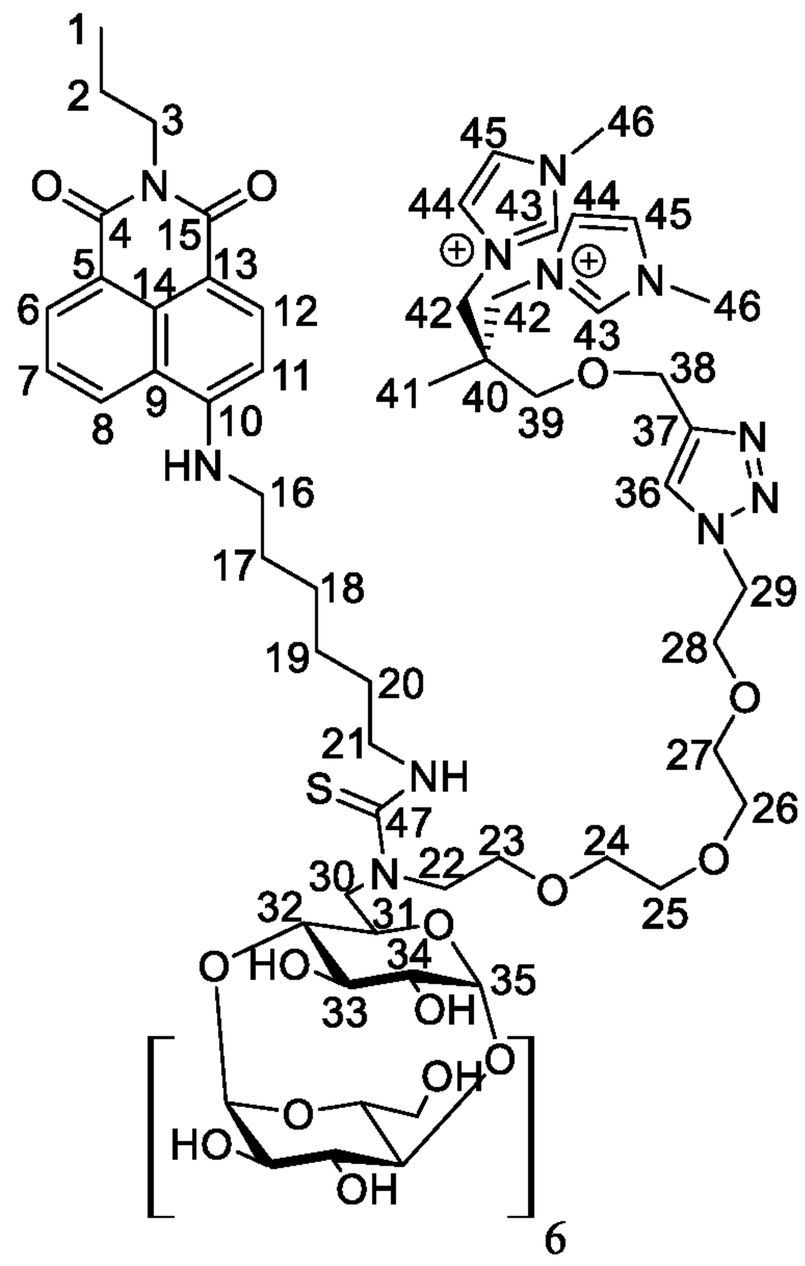
1-(2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)ethyl)-3-(6-((1,3-dioxo-2-propyl-2,3-dihydro-1H-benzo[de]isochinolin-6-yl)amino)hexyl)-1-(6^{A}-deoxy-β-cyclodextrin-6^{A}-yl)thiourea (31).

N-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)eth-1-yl)-6^{A}-amino-6^{A}-deoxy-β-cyclodextrin 20 (0.47 g, 0.35 mmol) was dissolved in DMF (18 mL), 6-((6-isothiocyanatohexyl)amino)-2-propyl-1H-benzo[de]isochinolin-1,3(2H)-dion **30** (0.21 g, 0.53 mmol) and DIPEA (70 µl, 0,40 mmol) were added and the reaction the mixture was stirred at laboratory temperature for 6 hours. The reaction mixture was monitored by TLC using the mixture n-propanol/water/EtOAc/concentrated aqueous solution of NH_3 6/3/1/1 and the substances were detected by UV lamp and carbonisation in 50% aqueous sulphuric acid solution. DMF was distilled off at 80 °C using an oil vacuum pump. The residue (0.87 g) was dissolved in MeOH, C18-reversed phase silica gel (4.5 g) was added and the suspension was evaporated again at 40 °C using the rotary vacuum evaporator. The adsorbed product was purified by column chromatography with C18-reversed phase silica gel (18 g). The product was eluted with 50% aqueous solution of MeOH. The product-containing fractions were evaporated at 55 °C using a rotary vacuum evaporator. The product was dried at 60 °C with an oil vacuum pump and obtained as a yellow free-flowing substance in a yield of 64 % (0.396 g). [α]25 *D* +98.1° (α +0.058, 6.1 mg, DMSO), IR(KBr): 3411 ν(OH), 2923 ν(C-H), 2851 ν(C-H), 2113 ν(azide), 1643 ν(naphthalimide), 1616 ν(naphthalimide), 1577 ν(naphthalimide), 1362 ν(C-H), 1326 ν(C-H), 1248 ν(C-H), 1159 ν(C-O), 1030 ν(C-O) cm^{-1}. ^{1}H NMR (600 MHz, DMSO-d_6): δ = 8.71 (d, *J* = 8.4 Hz, 1H, H-6), 8.43 (d, *J* = 7.2 Hz, 1H, H-12), 8.27 (d, *J* = 8.5 Hz, 1H, H-8), 7.74 (t, *J* = 5.5 Hz, 1H, NH1), 7.67 (dd, *J* = 8.4, 7.3 Hz, 1H, H-7), 7.24 (d, *J* = 37.7 Hz, 1H, NH2), 6.79 (d, *J* = 8.6 Hz, 1H, H-11), 6.00 – 5.63 (m, 14H, sec. OH), 4.89 – 4.80 (m, 7H, H-35), 4.49 – 4.13 (m, 6H, prim. OH), 3.97 (t, *J* = 7.4 Hz, 2H, H-3), 3.88 – 3.13 (m, 62H, H-16, H-21, H-22, H-23, H-24, H-25, H-26, H-27, H-28, H-29, H-30, H-31, H-32, H-33, H-34), 1.79 – 1.30 (m, 8H, H-17, H-18, H-19, H-20), 1.62 (h, *J* = 7.4 Hz, 2H, H-2), 0.90 (t,



J = 7.4 Hz, 3H, H-1) ppm. ^{13}C NMR (150 MHz, DMSO-d_6): δ = 181.81 (C-36), 163.79 (C-4), 162.95 (C-15), 150.68 (C-10), 134.37 (C-8), 130.65 (C-12), 129.46 (C-5), 128.61 (C-6), 124.21 (C-7), 121.85 (C-14), 120.11 (C-9), 107.45 (C-13), 103.81 (C-11), 102.55 – 101.55 (C-35), 84.68 – 80.84 (C-32), 73.09 – 71.79 (C-31, C-33, C-34), 69.96 – 68.32 (C-23, C-24, C-25, C-26, C-27, C-28), 59.97 – 59.20 (C-30), 49.96 (C-29), 45.96 – 40.06 (C-16, C-21, C-22), 40.73 (C-3), 28.57 – 26.34 (C-17, C-18, C-19, C-20), 20.98 (C-2), 11.40 (C-1) ppm. UV-VIS (H_2O), λ_{max1}, nm: 204.0, λ_{max2}, nm: 256.5, λ_{max3}, nm: 283.5, λ_{max4}, nm: 450.0, 1.5×10^{-5} M. ESI MS: for $C_{72}H_{111}N_7O_{39}S$ calcd.: *m/z* 1729.7, found 1753.2 $[M + Na]^+$. HRMS: pro $C_{72}H_{111}N_7O_{39}S$ calcd.: *m/z* 1729.6638, found 1730.6549 $[M + H]^+$, Δ 9.4 ppm.

3,3'-(2-(((1-(20-((1,3-Dioxo-2-propyl-2,3-dihydro-1H-benzo[de]isochinolin-6-yl)amino)-12-(6^A-deoxy-β-cyclodextrin-6^A-yl)-13-thioxo-3,6,9-trioxa-12,14-diazaikosyl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)-2-methylpropan-1,3-diyl)bis(1-methyl-1H-imidazol-3-ium) (32).

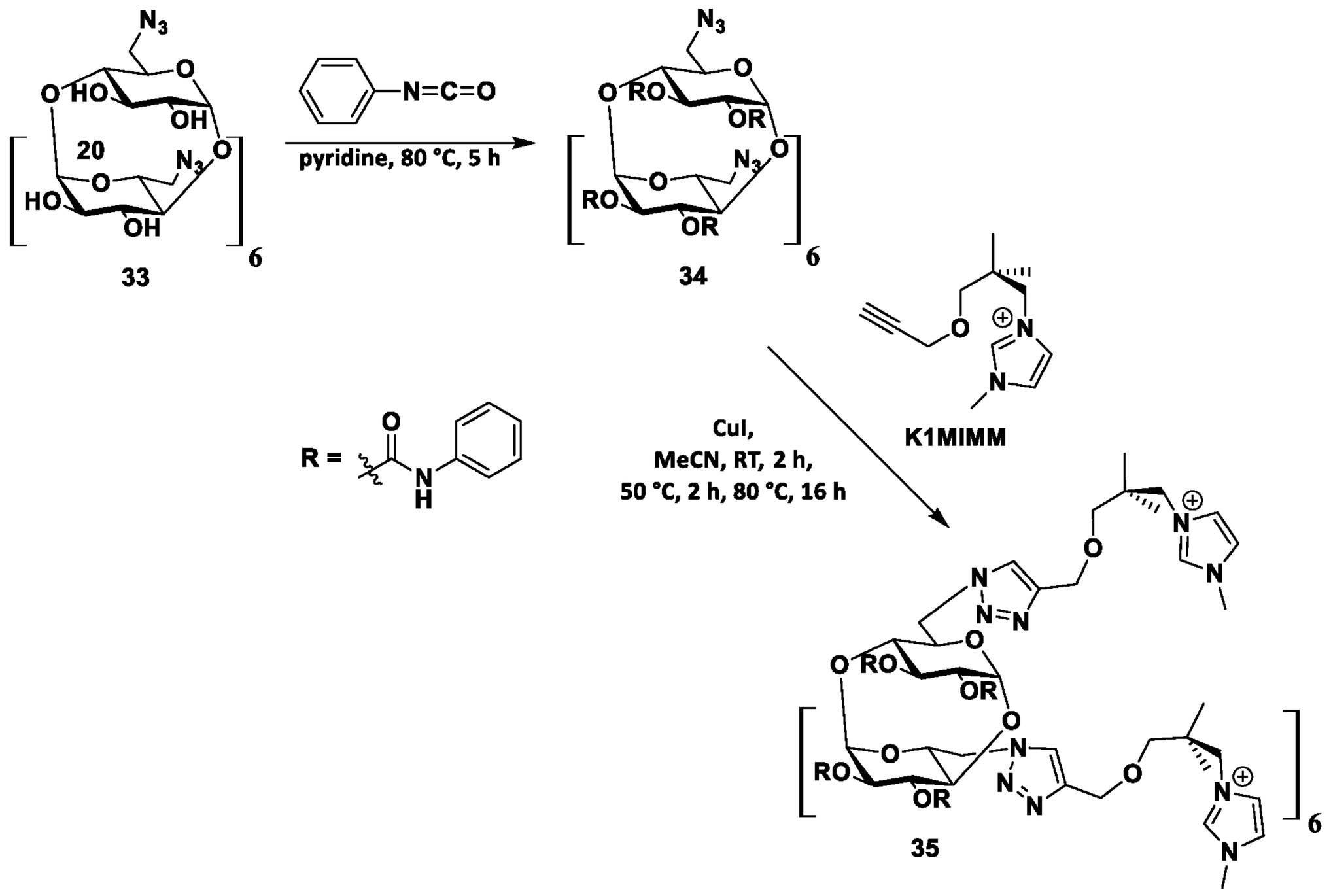


1-(2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)ethyl)-3-(6-((1,3-dioxo-2-propyl-2,3-dihydro-1H-benzo[de]isochinolin-6-yl)amino)hexyl)-1-(6^A-deoxy-β-cyclodextrin-6^A-yl)thiourea **31** (0.35 g, 0.21 mmol) and 3,3'-(2-methyl-2-((prop-2-yn-1-yloxy)methyl)propan-1,3-diyl)bis(1-methyl -1H-imidazol-3-ium) **K2MIMM** (97 mg, 0.17 mmol) were dissolved in the mixture deionised water/MeOH 1/1 (10 ml) and the solution was bubbled with argon for 30 minutes. Subsequently, CuI (32 mg, 0.17 mmol) was added and the reaction mixture was stirred at laboratory temperature for 12 hours. The reaction mixture was monitored by TLC using the mixture MeOH/concentrated HOAc/1% aqueous solution of NH_4HCO_3 10/1/9 and the substances were detected by UV lamp and carbonisation in 50% aqueous sulphuric acid solution. The reaction mixture had almost no reaction and so the temperature was raised to 65 °C. The reaction was complete in 2 hours. The reaction mixture was evaporated at 55 °C using a rotary vacuum evaporator. The residue (0.75 g) was dissolved in water and purified by column chromatography with C18-reversed phase silica gel (8 g). The product was eluted with 20% aqueous solution of MeOH. The product-containing fractions were evaporated at 50 °C using the rotary vacuum evaporator; n-propanol was added to the product

during evaporation for the substance to be free-flowing. The product was dried at 70 °C with an oil vacuum pump and obtained as a yellow free-flowing material in a yield of 65 % (0.25 g). [α]25 *D* +67.3° (α +0.035, 5.2 mg, DMSO). IR(KBr): 3336 ν(OH), 2932 ν(C-H), 2869 ν(C-H), 1679 ν(naphthalimide), 1634 ν(naphthalimide), 1580 ν(imidazole), 1553 ν(naphthalimide), 1281 ν(C-H), 1251 ν(C-H), 1159 ν(C-O), 1033 ν(C-O) cm^{-1}. ^{1}H NMR (600 MHz, DMSO-d_6): δ = 9.00 (s, 2H, H-43), 8.70 (dd, *J* = 8.8, 2.3 Hz, 1H, H-6), 8.43 (d, *J* = 7.3 Hz, 1H, H-12), 8.27 (d, *J* = 8.5 Hz, 1H, H-8), 8.11 (s, 1H, H-36), 7.73 (s, 3H, NH1, H-45), 7.68 (t, *J* = 7.8 Hz, 1H, H-7), 7.58 (s, 2H, H-44), 7.22 (d, *J* = 64.1 Hz, 1H, NH2), 6.78 (dd, *J* = 8.7, 3.4 Hz, 1H, H-11), 6.89 – 5.65 (m, 14H, sec. OH), 4.84 – 4.82 (m, 7H, H-35), 4.58 – 4.38 (m, 10H, prim. OH, H-29, H-38), 4.29 (d, *J* = 14.0 Hz, 2H, H-42), 4.16 (d, *J* = 14.0 Hz, 2H, H-42), 3.97 (t, *J* = 7.6 Hz, 2H, H-3), 3.86 (s, 6H, H-46), 3.81 (d, *J* = 5.2 Hz, 2H, H-28), 3.74 – 3.17 (m, 58H, H-16, H-21, H-22, H-23, H-24, H-25, H-26, H-27, H-30, H-31, H-32, H-33, H-34), 3.09 (s, 2H, H-39), 1.73 – 1.28 (m, 8H, H-17, H-18, H-19, H-20), 1.63 (h, *J* = 7.3 Hz, 2H, H-2), 0.89 (t, *J* = 6.7 Hz, 3H, H-1), 0.83 (s, 3H, H-41) ppm. ^{13}C NMR (150 MHz, DMSO-d_6): δ = 181.71 (C-47), 163.76 (C-4), 162.94 (C-15), 150.64 (C-10), 142.90 (C-37), 137.37 (C-43), 134.07 (C-8), 130.39 (C-12), 129.43 (C-5), 128.29 (C-6), 124.33 (C-36), 123.95 (C-7), 123.40 (C-44), 123.19 (C-45), 121.83 (C-14), 120.08 (C-9), 107.44 (C-13), 103.51 (C-11), 102.23 – 101.21 (C-35), 84.31 – 80.54 (C-32), 72.79 – 71.54 (C-31, C-33, C-34), 70.09 – 68.37 (C-23, C-24, C-25, C-26, C-27, C-28, C-39), 63.08 (C-38), 59.72 – 59.00 (C-30), 52.25 (C-42), 49.11 (C-29), 45.10 – 42.61 (C-16, C-21, C-22), 40.45 (C-3), 39.52 (C-40, solvent overlay), 35.66 (C-46), 28.26 – 26.02 (C-17, C-18, C-19, C-20), 20.70 (C-2), 17.02 (C-41), 11.12 (C-1) ppm. UV-VIS (H_2O), λ_{max1}, nm: 204.5, λ_{max2}, nm: 256.5, λ_{max3}, nm: 283.5, λ_{max4}, nm: 450.0, 1.0×10^{-5} M. ESI MS: for $C_{88}H_{135}N_{11}O_{40}S^{2+}$ calcd.: *m/z* 1008.9, found 1009.3 $[M]^{2+}$. HRMS: for $C_{88}H_{135}N_{11}O_{40}S^{2+}$ calcd.: *m/z* 1008.9289, found 1008.9153 $[M]^{2+}$, Δ 13.5 ppm.

3.5 Modifier of formula IV with a multiplier carrying phenylcarbamate groups

Scheme 8 describes the synthesis of a type IV modifier in which per(6-azido-6-deoxy)-β-cyclodextrin appears as a multiplier to which seven type I anchors – K1MIMM bind and the active groups are phenylcarbamate groups that are attached to all free hydroxyl groups of cyclodextrin.



Scheme 8: Synthesis of a type IV modifier containing phenylcarbamoyl groups attached to the multiplier with seven bound charged anchors.

Heptakis(6-azido-2,3-*O*-bisphenylcarbamoyl-6-deoxy)-β-cyclodextrin (34). Heptakis(6-azido-6-deoxy)-β-cyclodextrin **33** (0.05 g, 38.2 μmol) was dissolved in dry pyridine (1 mL), phenyl isocyanate (90 μL, 0.802 mmol) was added, and the reaction mixture was heated to 80 °C and stirred for 5 hours. The reaction mixture was monitored by TLC using the mixtures n-propanol/water/EtOAc/concentrated aqueous solution of NH_3 6/3/1/1 and hexane/EtOAC 5/1 and the substances were detected by UV lamp and carbonisation in 50% aqueous sulphuric acid solution. Pyridine was distilled off from the reaction mixture at 80 °C using an oil vacuum pump. The distillation residue was dissolved in $CHCl_3$ and silica gel (0.6 g) was added. The mixture was evaporated at 50 °C using a rotary vacuum evaporator. The adsorbed crude product was purified by column chromatography using elution mixtures hexane/EtOAc 5/1 and 1/1. The product-

containing fractions were evaporated at 50 °C using the rotary vacuum evaporator and the product obtained (0.070 g) was used for the next reaction.

Heptakis(6-(4-((2,2-dimethyl-3-(1-methyl-1H-imidazol-3-ium-3-yl)propoxy)methyl)-1H-1,2,3-triazol-1-yl)-2,3-O-bisphenylcarbamoyl)-β-cyclodextrin (35). Substance **34** (0.07 g, 23.1 µmol) and 3-(2,2-dimethyl-3-(prop-2-yn-1-yloxy)propyl)-1-methyl-1H-imidazol-3-ium **K1MIMM** (0.115 g, 0.32 mmol) were dissolved in DMF (1.5 ml) and the solution was bubbled with argon for 10 minutes. Subsequently, copper sulphate (6 mg, 23.1 µmol) and sodium ascorbate (9 mg, 46.1 µmol) were added. The reaction mixture was heated to 60 °C and stirred for 2 hours. The reaction mixture was monitored by TLC using the mixtures hexane/EtOAc 2/1 and MeOH/HOAc/1% aqueous solution of NH_4OAc 10/1/9 and the substances were detected by UV lamp and carbonisation in 50% aqueous sulphuric acid solution. DMF was distilled off at 60 °C using an oil vacuum pump. The distillation residue was dissolved in n-propanol, C18-reversed phase silica gel (0.6 g) was added and the mixture was evaporated at 50 °C using a rotary vacuum evaporator. The crude product was purified by column chromatography with C18-reversed phase silica gel (2.5 g). The product was eluted with 70% aqueous solution of MeOH. However, a large amount of product was retained on the sorbent. The product-containing fractions were evaporated at 50 °C using a rotary vacuum evaporator. Small amount of product (15 mg) was obtained as a white free-flowing material.

Example 4: Binding of modifiers to a solid carrier

The modifiers of general formula (III) K1MIMM, K2MIMM, K3MIMM and K3TMAM with bound chromophore according to Example 3.4 were bound to negatively charged carriers selected from the group consisting of cation exchange resins and zeolites, stationary phases for ion exchange chromatography, silica gel, surfaces of uncharged materials modified to obtain a negative charge, e.g., by sulphonation or plasma treatment, by mixing the carrier with a 0.1 to 1% aqueous solution of the modifier of general formula (III) until the decrease in UV absorption is stopped. Under these conditions (depending on the carrier), the modifier binding half-life is in the order of hours at most. Furthermore, in the case of such modified carriers, the binding strength of the modifier to the carrier was determined by eluting with eluents of different polarity and ionic strength.

The results of the elution experiment for silica gel and modifiers with different numbers of charges are summarized in Table 1. It follows from it that when using a basic buffer in the mobile phase, a two or three times charged anchor can be used for stable binding of the modifier even for very

polar elution mixtures. For even less polar solvents (butanol, acetonitrile, acetone, ethyl acetate, chloroform, dichloromethane, tetrahydrofuran, 1,4-dioxane, diethyl ether, toluene, benzene, hexane) the elution of the modifier was not detected either.

Table 1: Comparison of stability of bonds of modifiers with different number of charges on silica gel in basic environment

Carrier:	**Silica gel**		
Anchor:	**K1MIMM**	**K2MIMM**	**K3MIMM**
Elution solution:			
0.01M NH_4HCO_3 H_2O	o	o	o
0.01M NH_4HCO_3 MeOH/H_2O 1/1	+	o	o
0.01M NH_4HCO_3 MeOH/H_2O 3/1	+	o	o
0.01M NH_4HCO_3 MeOH	+	o	o
0.01M NH_4OAc H_2O	o	o	o
Methanol	o	o	o
Ethanol	o	o	o
Propanol	o	o	o
Isopropanol	o	o	o

The modifier (3 μmol) containing a naphthalimide group attached to the corresponding anchor bound in 0.5 ml of 0.1M NH_4HCO_3 on 50 mg of Merck silica gel for column chromatography. Continuous elution of the modifier: **o** - not detected, **+** - was detected.

In an acidic environment, a significant binding strength of the K3TMAM anchor to the strongly acidic cation exchange resin Amberlite IR 120 was found, as evidenced in Table 2, where the binding strength of this anchor to silica gel and sulphonated silica gel is also shown for comparison.

Table 2: Comparison of the stability of the modifier bond with the K3TMAM anchor to different carriers in an acidic environment

Anchor:	**K3TMAM**		
Carrier:	**Silica gel**	**Silica gel-SO_3H**	**Amberlite IR 120**
Elution solution (H_2O):			
H_2O	+	o	o
0.01M NH_4Cl, pH 2	+	o	o
0.10M NH_4Cl, pH 2	+	o	o
1.00M NH_4Cl, pH 2	+	+	o
0.01M $CaCl_2$, pH 2	+	o	o
0.10M $CaCl_2$, pH 2	+	o	o
1.00M $CaCl_2$, pH 2	+	+	o
0.01M enCit, pH 2	+	o	o
0.10M enCit, pH 2	+	+	o
1.00M enCit, pH 2	+	+	o

The modifier (1.5 mg) containing a naphthalimide group attached to the K3TMAM anchor was dissolved in water and applied to a column (250 µl) of the carrier in a glass column and subsequently eluted with elution solutions having their pH adjusted to 2 with HCl. enCit - ethylenediamine citrate. Continuous elution of the modifier: **o** - not detected, **+** - was detected.

Example 5: Binding of multiplier-containing modifiers to a solid carrier

From per-6-azido-6-deoxy-β-CD (**33**), modifiers containing 7, 14 and 21 positive charges were prepared by reaction with K1MIMM, K2MIMM and K3MIMM anchors as described above for the preparation of substance **35**. Furthermore, the strength of their bond to silica gel and aluminium oxide, i.e. the substances to which the anchors of general formula I, with one to three charges, are bound relatively weakly, was studied. The strength of the bond was determined using TLC chromatography, when it was monitored whether the modifier, applied in a 1% aqueous solution (1-2 µl), moves from the place on the TLC plate to which it was applied, using different eluents. The sensitivity of the determination of CD derivatives on TLC using carbonisation as the method of detection is very high and approaches the sensitivity of a mass spectrometer. The results are

shown in Table 3 and Table 4 and show that the elution of these modifiers in the commonly used eluents for HPLC in the case of silica gel is almost non-existent. In the case of aluminium oxide, the 7-charge modifier is eluted with an acidic pH eluent. From silica gel, all types of modifiers can be eluted with 50% formic acid (but not acetic acid or concentrated salt solutions). In the case of aluminium oxide, the use of a concentrated solution of ethylenediamine citrate destroyed the sorbent layer on the TLC plate.

Table 3: Elution of modifiers with 7, 14 and 21 positive charges on a silica gel TLC plate

Carrier	**Silica gel**		
Number of charges in the modifier	**7**	**14**	**21**
Eluents:			
MeCN, EtOAc, toluene, benzene, CH_2Cl_2, $CHCl_3$, Et_2O, THF, 1,4-dioxan, acetone	o	o	o
butanol, propanol, ethanol, methanol	o	o	o
0.01M, 0.1M, 1M NH_4OAc	o	o	o
MeCN/H_2O/AcOH/TFA 93/7/1/0.025	o	o	o
MeCN/H2O/HCOOH 95/5/0.1	o	o	o
MeOH/H_2O/TEA/NH_4OAc 95/5/0.6/1	o	o	o
0.01M, 0.1M, 1M $CaCl_2$ (pH=2; HCl)	o	o	o
0.01M, 0.1M, 1M enCit	o	o	o
AcOH/H_2O 1/1	o	o	o
HCOOH/H_2O 1/1	+	+	+

Elution of the modifier: **o** – not detected, **+** – was detected. enCit – ethylenediamine citrate.

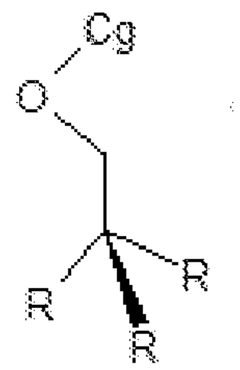
Table 4: Elution of modifiers with 7, 14 and 21 positive charges on a TLC plate with aluminium oxide

Carrier	**Aluminium oxide**		
Number of charges in the modifier	**7**	**14**	**21**
Eluents:			
0.01M, 0.1M, 1M NH_4OAc	o	o	o
MeCN/H_2O/AcOH/TFA 93/7/1/0.025	+	o	o
MeCN/H2O/HCOOH 95/5/0.1	o	o	o
MeOH/H_2O/TEA/NH_4OAc 95/5/0.6/1	o	o	o
0.01M, 0.1M, 1M $CaCl_2$ (pH=2; HCl)	o	o	o
0.01M, 0.1M enCit	o	o	o
1M enCit	x	x	x

Elution of the modifier: **o** – not detected, **+** – was detected, x – the sorbent layer on the TLC plate was destroyed. enCit – ethylenediamine citrate.

CLAIMS

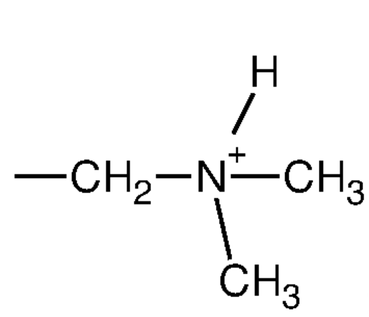
1. A compound of general formula (I)



(I),

wherein Cg is -CH_2-C≡CH or -CH_2-CH=CH_2;

R is independently selected from the group consisting of (C1-C6)alkyl, which may be linear or branched; (C5-C6)cykloalkyl;



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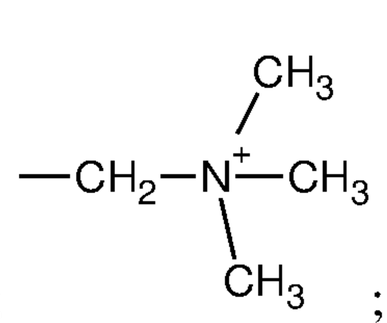

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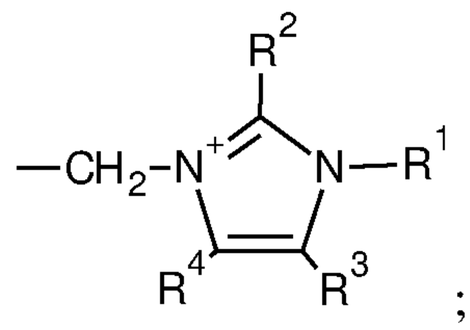

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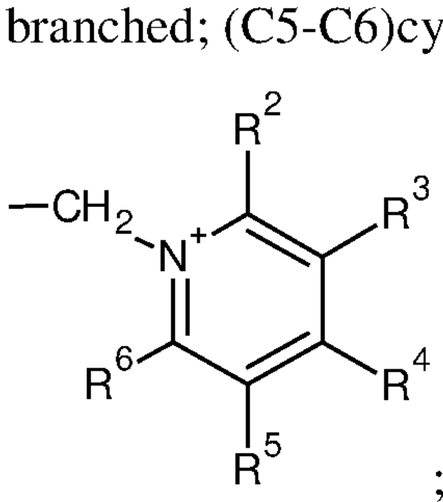

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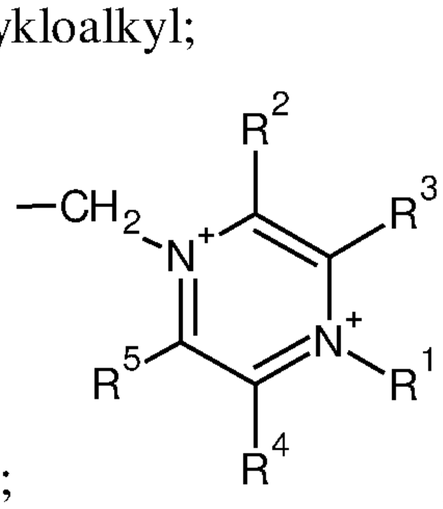

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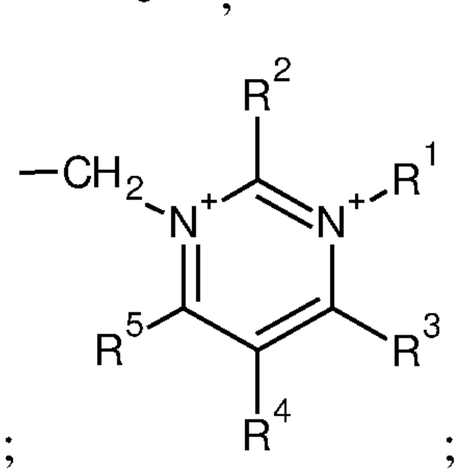

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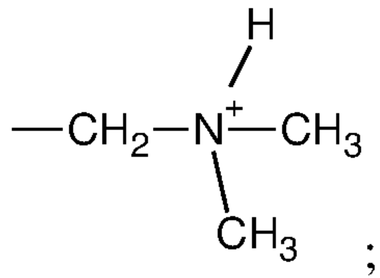
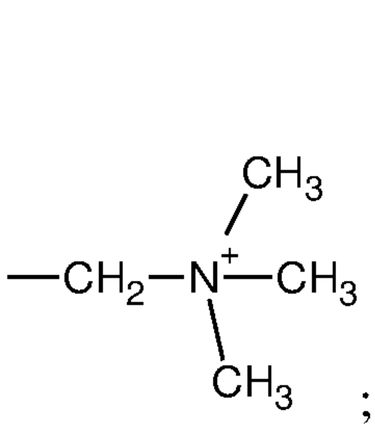
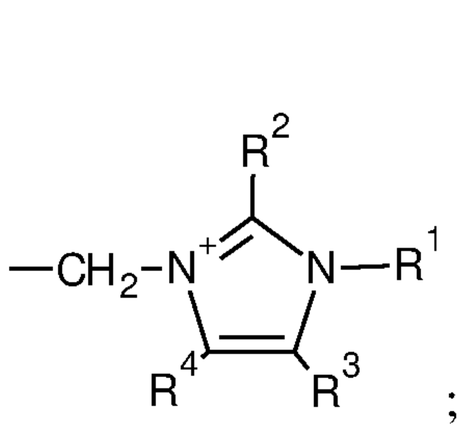
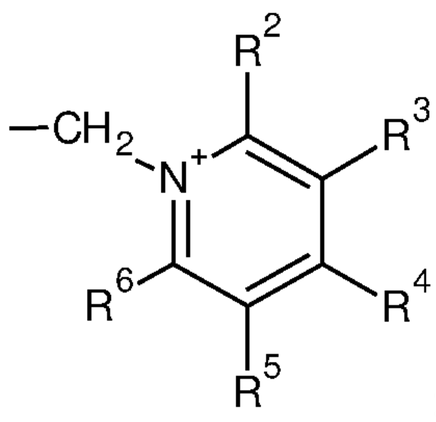
R^1 is H, (C1-C6)alkyl, which may be linear or branched; or (C5-C6)cykloalkyl;

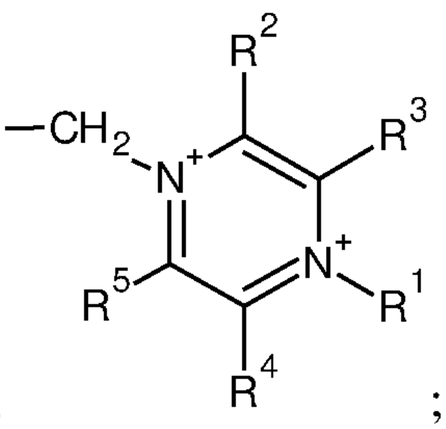
R^2 to R^6 are independently selected from the group consisting of –H, (C1-C6)alkyl, (C1-C6)alkoxyl, –N(R^7)$_2$, wherein R^7 is independently (C1-C6)alkyl;

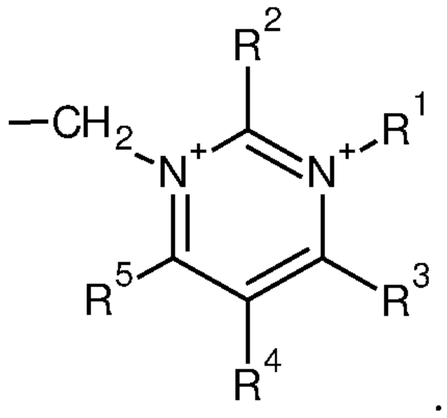
wherein at least one R is selected from the group consisting of  ;

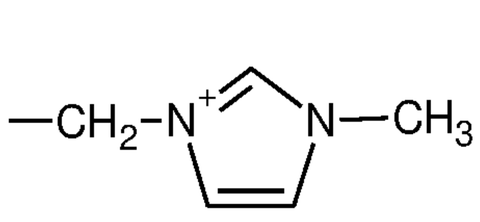
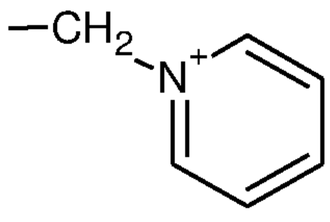
 ;

 ;

 ;

 ;

 .

2. The compound of general formula (I) according to claim 1, wherein Cg is -CH_2-C≡CH, and R is independently selected from the group consisting of –CH_3, -CH_2-$N^+(CH_3)_3$,  and  ;

wherein at least one R is other than methyl.

3. The compound of general formula (I) according to claim 1 or 2, which is selected from the group consisting of:

N,N,N,2,2-Pentamethyl-3-(prop-2-yn-1-yloxy)propan-1-aminium;

3-(2,2-Dimethyl-3-(prop-2-yn-1-yloxy)propyl)-1-methyl-1H-imidazol-3-ium;

1-(2,2-Dimethyl-3-(prop-2-yn-1-yloxy)propyl)pyridin-1-ium;

N^1,N^1,N^3,N^3,2-Pentamethyl-2-((prop-2-yn-1-yloxy)methyl)propan-1,3-diamin;

N^1,N^1,N^1,N^3,N^3,N^3,2-Heptamethyl-2-((prop-2-yn-1-yloxy)methyl)propan-1,3-diaminium;

3,3'-(2-Methyl-2-((prop-2-yn-1-yloxy)methyl)propan-1,3-diyl)bis(1-methyl-1H-imidazol-3-ium);

1,1'-(2-Methyl-2-((prop-2-yn-1-yloxy)methyl)propan-1,3-diyl)bis(pyridin-1-ium);

2-((Dimethylamino)methyl)-N^1,N^1,N^3,N^3-tetramethyl-2-((prop-2-yn-1-yloxy)methyl)propan-1,3-diamin;

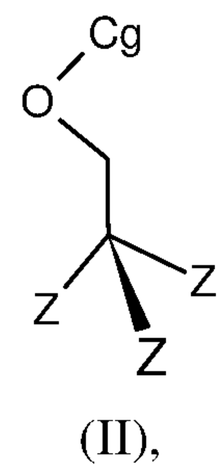
N^1,N^1,N^1,N^3,N^3,N^3-Hexamethyl-2-((prop-2-yn-1-yloxy)methyl)-2-((trimethylamonio)methyl)propane-1,3-diaminium;

3,3'-(2-((1-Methyl-1H-imidazol-3-ium-3-yl)methyl)-2-((prop-2-yn-1-yloxy)methyl)propan-1,3-diyl)bis(1-methyl-1H-imidazol-3-ium);

1,1'-(2-((Prop-2-yn-1-yloxy)methyl)-2-(pyridin-1-ium-1-ylmethyl)propane-1,3-diyl)bis(pyridin-1-ium).

4. A method of preparation of a compound of general formula (I), **characterized in that** it comprises the following steps:

(i) providing a compound of general formula (II),

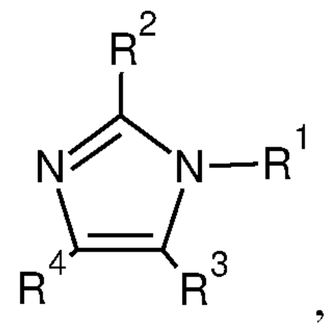


(II),

wherein Cg is -CH_2-C≡CH or -CH_2-CH=CH_2;

and Z is independently –CH_2OH, (C1-C6)alkyl, which may be linear or branched, or (C5-C6)cykloalkyl; wherein at least one Z is –CH_2OH; preferably Z is independently –CH_2OH or methyl;

(ii) at least one hydroxyl group of the compound of general formula (II) reacts with anhydride or halide of fluorinated alkylsulphonic acid to form fluorinated alkylsulphonic acid ester; preferably the fluorinated alkylsulphonic acid is selected from the group comprising trifluoromethanesulphonic acid, tetrafluoroethanesulphonic acid, nonafluorobutanesulphonic acid, heptadecafluorobutanesulphonic acid; more preferably at least one hydroxyl group of the compound of general formula (II) reacts with trifluoromethanesulphonic acid anhydride;

(iii) the fluorinated alkylsulphonic acid ester from step (ii) reacts with at least one compound selected from the group consisting of trimethylamine, dimethylamine, 

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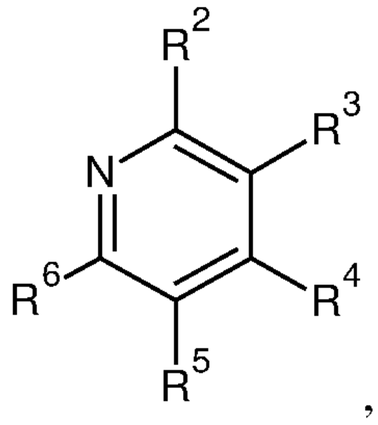
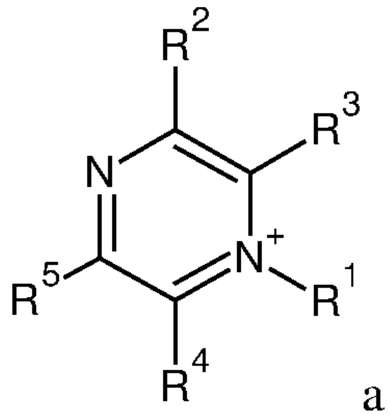
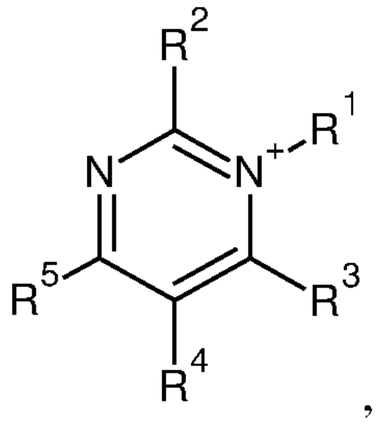
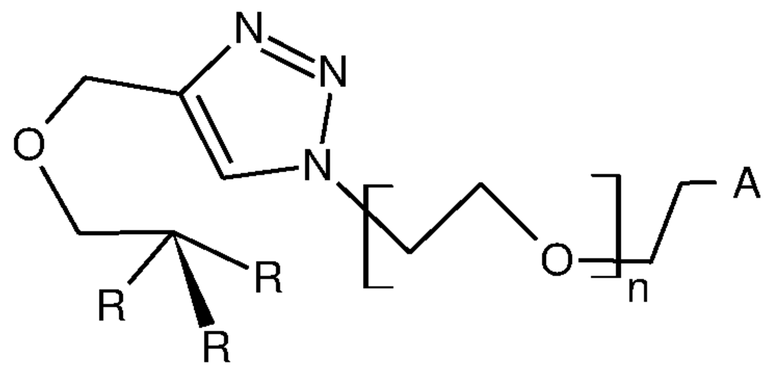
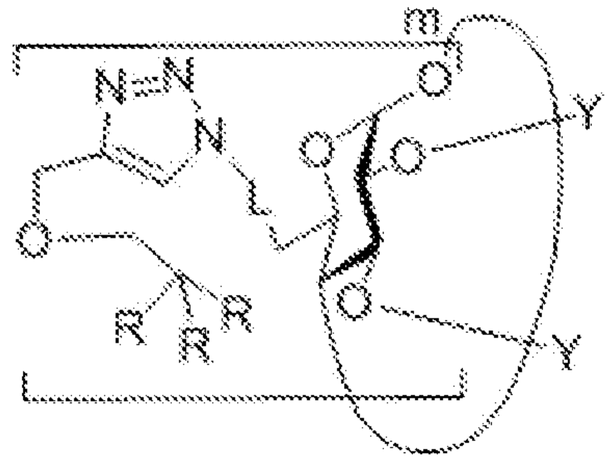

, 
a 
, preferably the compound is selected from the group consisting of trimethylamine, dimethylamine, 1-methylimidazole and pyridine, to give the compound of general formula (I);

(iv) if dimethylamine is used in step (iii), the last step is the quaternisation of the dimethylamine group to a trimethylammonium group using methylating agent, preferably selected from the group comprising methyl halides, dimethyl sulphate and methyl esters of alkyl- or arylsulphonic acids.

5. A modifier of a surface of a negatively charged carrier, the modifier having the general formula (III) or (IV),



(III),



(IV),

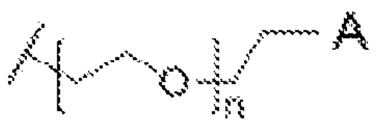
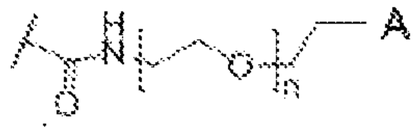
wherein

R is as defined in claim 1;

n is an integer in the range of from 0 to 20;

m is an integer in the range of from 6 to 8;

L is a bond or oligoethylene glycol linker $–(CH_2)_2–(O–(CH_2)_2)_n–NH–$, covalently attached to the cyclodextrin via position 6 of the cyclodextrin glucose unit;

Y is selected from the group consisting of H,  and  ;

wherein the oligoethylene glycol linker is bound to the hydroxyl group of cyclodextrin via ether or carbamate bond;

and wherein at least one Y is other than H;

A is selected from the group comprising α-, β-, or γ-cyclodextrin, optionally substituted with phenylcarbamoyl groups or fluorescent groups; amino acids, optionally N-(3,5-dinitrophenyl)-substituted amino acids; macrocyclic compounds; peptides and proteins; saccharides; crown-ethers; cinchonine derivatives.

6. The modifier of a surface of a negatively charged carrier according to claim 5, wherein the substance A is α-, β-, or γ-cyclodextrin, optionally substituted with at least one phenylcarbamoyl group, which may be further independently substituted with –Cl or methyl group, preferably in position 3 and 5, and/or with at least one fluorescent group, preferably the fluorescent group is naphthalimide group.

7. A method of synthesis of the modifier of general formula (III) according to claim 6, **characterized in that** it comprises the following steps:

(i) providing of α-, β-, or γ-cyclodextrin bound to an oligoethylene glycol linker with the number of ethylene glycol units from 1 to 20 and terminated with an azide end group; or

providing of α-, β-, or γ-cyclodextrin containing an azide group;

(ii) optionally, incorporation of a phenylcarbamoyl group, which may be further independently substituted with -Cl or methyl group, preferably in position 3 and 5, to a hydroxyl group and/or amino group of the α-, β-, or γ-cyclodextrin prepared in step (i); optionally, incorporation of at least one fluorescent group to a hydroxyl group and/or amino group of the α-, β-, or γ-cyclodextrin prepared in step (i);

(iii) azido-alkyne Huisgen cycloaddition binding of the cyclodextrin derivative prepared in step (i) or (ii) to the compound of general formula (I), wherein Cg is propargyl, to give the modifier of general formula (III), wherein the substance A is cyclodextrin.

8. The method of synthesis according to claim 7, **characterized in that** step (i) comprises reaction of monotosyl cyclodextrin with an amino-azido-oligoethylene glycol linker with the number of ethylene glycol units from 1 to 20, optionally followed by acetylation of the free amino

group, to give a cyclodextrin derivative with a bound oligoethylene glycol linker terminated with a reactive azide group.

9. Use of the compound of general formula (I), (III) or (IV) according to claim 1, 2, 3, 5 or 6, for binding of substance A to the surface of a negatively charged solid, preferably selected from the group comprising catex resins, zeolites, stationary phases for ion-exchange chromatography, copolymer of perfluoro-3,6-dioxa-4-methyl-7-octenesulphonic acid with tetrafluoroethylene, silica gel, glass, metal oxides.

10. Use of the compound of general formula (I) according to any one of claims 1, 2 or 3, or use of the modifier of the general formula (III) or (IV) according to claim 5 or 6, for preparing a material usable for chromatographic separation and purification of substances, or as a sorbent for solid-phase extraction or as a heterogeneous catalyst.

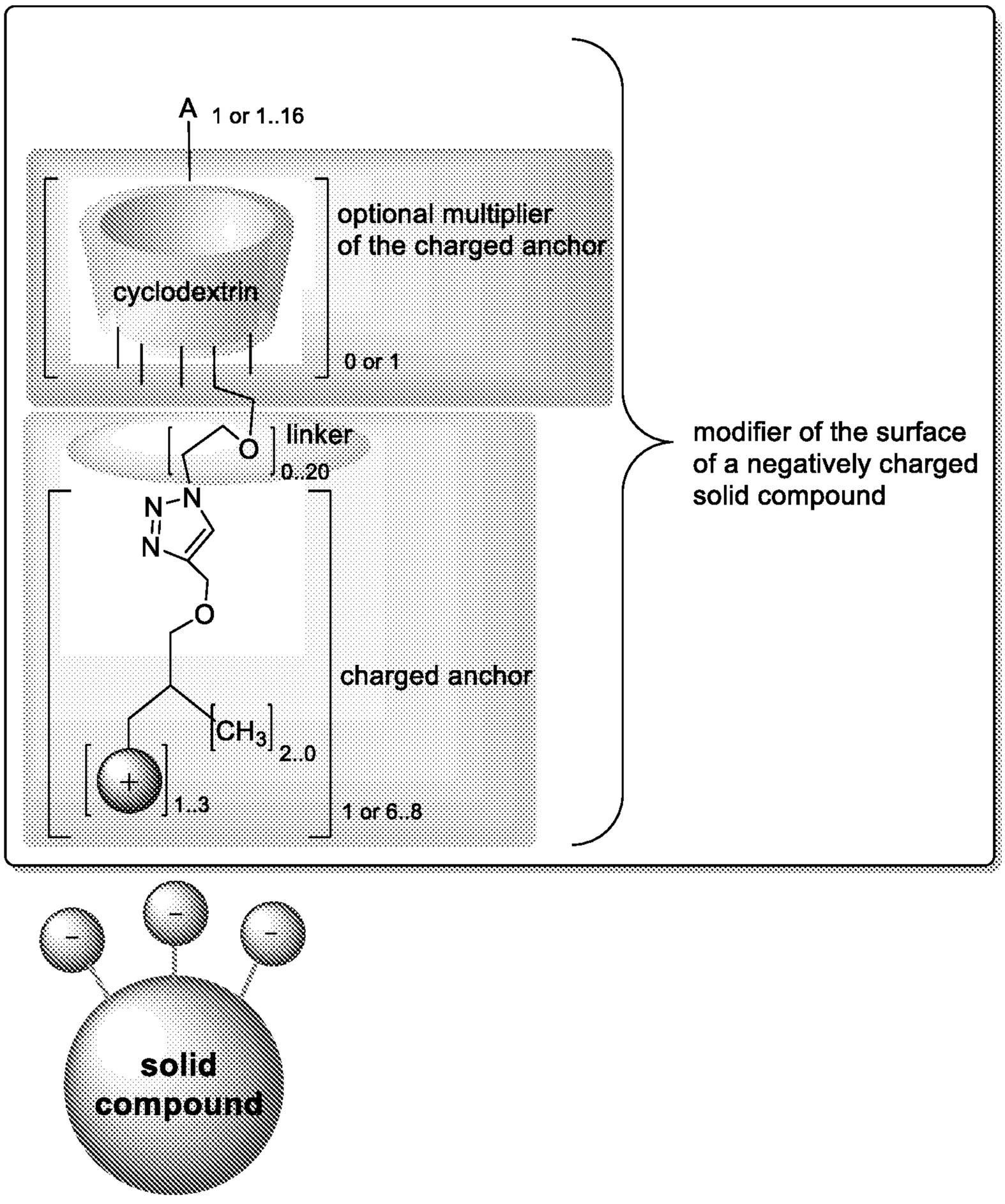

A
1 or 1..16
optional multiplier
of the charged anchor
cyclodextrin
0 or 1
linker
0..20
charged anchor
1 or 6..8
modifier of the surface
of a negatively charged
solid compound
solid
compound

Figure 1

INTERNATIONAL SEARCH REPORT

International application No
PCT/CZ2020/050088

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D233/60 C07D213/20 C07C217/08 C08L5/16 B01J20/32
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07D C07C C09J C08L G01N B01J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	FERNANDES CARLA ET AL: "Small Molecules as Chromatographic Tools for HPLC Enantiomeric Resolution: Pirkle-Type Chiral Stationary Phases Evolution", CHROMATOGRAPHIA, vol. 76, no. 15-16, 9 May 2013 (2013-05-09), pages 871-897, XP055776329, DE ISSN: 0009-5893, DOI: 10.1007/s10337-013-2469-8 Retrieved from the Internet: URL:http://link.springer.com/article/10.1007/s10337-013-2469-8/fulltext.html> whole document, especially chiral stationary phases in Table 2 on page 875 ----- -/--	1-10

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 February 2021	25/02/2021
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Guspanová, Jana

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INTERNATIONAL SEARCH REPORT

International application No
PCT/CZ2020/050088

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 912 563 A1 (LINDNER WOLFGANG PROF DR [AT]) 6 May 1999 (1999-05-06) claims 1,5; figure 5	1-10
A	LI XIA ET AL: "Enantioseparation performance of novel benzimido-[beta]-cyclodextrins derivatized by ionic liquids as chiral stationary ph", ANALYTICA CHIMICA ACTA, ELSEVIER, AMSTERDAM, NL, vol. 819, 19 February 2014 (2014-02-19), pages 122-129, XP028631234, ISSN: 0003-2670, DOI: 10.1016/J.ACA.2014.02.020 abstract; figure 2	1-10
A	WO 2016/176461 A1 (WATERS TECHNOLOGIES CORP [US]; BROUSMICHE DARRYL W [US] ET AL.) 3 November 2016 (2016-11-03) claim 1; figures 1A,1B	1-10

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/CZ2020/050088

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0912563 A1	06-05-1999	AT 259807 T	15-03-2004
		DE 69727679 T2	22-07-2004
		EP 0912563 A1	06-05-1999
		US 6313247 B1	06-11-2001
		WO 9746557 A1	11-12-1997
WO 2016176461 A1	03-11-2016	CN 108136275 A	08-06-2018
		EP 3288658 A1	07-03-2018
		JP 6768790 B2	14-10-2020
		JP 2018522729 A	16-08-2018
		US 2018154332 A1	07-06-2018
		WO 2016176461 A1	03-11-2016