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WO 2012/004008 A1

(54) Title: CONJUGATES COMPRISING HYDROXYALKYL STARCH AND A CYTOTOXIC AGENT AND PROCESS FOR THEIR PREPARATION

(57) Abstract: The present invention relates to hydroxyalkyi starch conjugates, a method for preparing the same, the hydroxyalkyi starch conjugate comprising a hydroxyalkyi starch derivative and a cytotoxic agent and the cytotoxic agent comprising at least one tertiary hydroxyl group, wherein the hydroxyalkyi starch is linked via said tertiary hydroxyl group to the cytotoxic agent. The conjugates according to the present invention have a structure according to the following formula HAS'(-L-M)„ wherein M is a residue of the cytotoxic agent, L is a linking moiety, HAS' is the residue of the hydroxyalkyi starch derivative, and n is greater than or equal to 1, and wherein the hydroxyalkyi starch derivative has a mean molecular weight (MW) above the renal threshold.

Conjugates comprising Hydroxyalkyl Starch and a cytotoxic agent and process for their Preparation

The present invention relates to hydroxyalkyl starch conjugates comprising a hydroxyalkyl starch derivative and a cytotoxic agent, the cytotoxic agent comprising at least one tertiary hydroxyl group, wherein the hydroxyalkyl starch is linked via said tertiary hydroxyl group to the cytotoxic agent. The conjugates according to the present invention have a structure according to the following formula

$$HAS'(\text{-}L\text{-}M)_n$$

wherein M is a residue of the cytotoxic agent, L is a linking moiety, HAS' is the residue of the hydroxyalkyl starch derivative, and n is greater than or equal to 1, and wherein the hydroxyalkyl starch derivative has a mean molecular weight (MW) above the renal threshold, preferably a mean molecular weight MW greater than or equal to 60 kDa, more preferably in the range of from 60 to 1500 kDa, and more preferably of from 200 to 1000 kDa, more preferably in the range of from 250 to 800 kDa, and a molar substitution (MS) in the range of from 0.6 to 1.5. Moreover, besides the conjugate, the invention relates to the method for preparing said conjugate and conjugates obtained or obtainable by said method. Further, the invention relates to the HAS cytotoxic agent conjugates for the treatment of cancer as well as to pharmaceutical compositions comprising these conjugates for the treatment of cancer.

Hydroxyalkyl starch (HAS), in particular hydroxyethyl starch (HES), is a substituted derivative of the naturally occurring carbohydrate polymer amylopectin, which is present in corn starch at a concentration of up to 95 % by weight, and is degraded by other amylases in the body. HES in particular exhibits advantageous biological properties and is used as a blood volume replacement agent and in hemodilution therapy in clinics (Sommermeyer ***et ai,*** 1987, Krankenhauspharmazie, 8(8): 271-278; Weidler ***et ai,*** 1991, Arzneimittelforschung/Drug Research, 41: 494-498).

Cytotoxic agents are natural or synthetic substances which decrease the cell growth. A major drawback of many cytotoxic agents is their extreme low water solubility which renders the *in vivo* administration of the agent extremely complicated. Thus, this poor water solubility usually has to be overcome by complex formulation techniques including various excipients, wherein these excipients usually also show toxic side effects. As an example, the emulsifier Cremophor EL and ethanol, which are used to formulate taxol-

based agents in order to deliver the required dosis of these taxol-based agents *in vivo*, shows toxic effects such as vasodilation, dispnea, and hypotension. In particular, Cremophor EL has also been shown to cause severe anaphylactoid hypersensitivity reactions, hyperlipidaemia, abnormal lipoprotein patterns, aggregation of erythrocytes and peripheral neuropathy ("Cremophor EL: the drawbacks and advantages of vehicle selection for drug formulation", European Journal of Cancer", Volume 31, Issue 13, Pages 1590-1598). In fact, the maximum dose of, for example paclitaxel, a taxol-based cytotoxic agent that can be administered to mice by injection, is dictated by the acute lethal toxicity of said Cremophor EL vehicle.

This is one reason why the potential use of soluble prodrugs, in particular macromolecular prodrugs, as a means of administering biologically effective cytotoxic agents to mammals has been proposed. Such prodrugs include chemical derivatives of the cytotoxic agents which, upon administration^ will eventually liberate the active parent compound *in vivo*. The use of such prodrugs allows the artisan to modify the onset and/or duration of action *in vivo*. In addition, the use of prodrugs was proposed to enhance the water solubility of the drug, to provide an advantageous targeting and/or an enhancement of the stability of the therapeutic agent. Further, such prodrugs were suggested to prolong the circulation lifetime, to provide an extended duration of activity, or to achieve a reduction of side effects and drug toxicity. A typical example in the preparation of prodrugs involves the conversion of alcohols or thioalcohols to either organic phosphates or esters (Remington's Pharmaceutical Science, 16th ed., A. Ozols (ed.), 1980). Numerous reviews have described the potential application of macromolecules as high molecular weight carriers for cytotoxic agents yielding in polymeric prodrugs of said agents. It was proposed that by coupling the cytotoxic agents to polymers, it is possible to increase the molecular weight and size of the prodrug so that the weight and size of the prodrugs are too high to be quickly removed by glomerular filtration in the kidney and that, as consequence, the plasma residence time can be drastically increased.

Most modifications to date have been carried out with polyethylene glycol or similar polymers with polyethylene glycol (PEG) being generally preferred as polymer because of its easy availability and the possibility to give defined products upon reaction of limited available functional groups for coupling to a cytotoxic agent being present in PEG.

For example, WO 93/24476 A1 discloses conjugates between taxane-based drugs, such as paclitaxel, to polyethylene glycol as macromolecule. In these conjugates, paclitaxel is linked to the polyethylene glycol using an ester linkage.

Similarly, US 5,977,163 B1 describes the conjugation of taxane-based drugs, such as paclitaxel or docetaxel, to similar water soluble polymers such as polyglutamic acid or polyaspartic acid.

Likewise, polyethylene glycol conjugates with cytotoxic agents, such as camptothecins, are disclosed in WO 98/07713 A1. According to WO 98/07713 A1, the polymer is linked via a linker to a hydroxyl function of the cytotoxic agent providing an ester linkage which allows for a rapid hydrolysis of the polymer drug linkage *in vivo* to generate the parent drug. This is achieved by using a linker comprising an electron-withdrawing group in close proximity to the ester bond. No polysaccharide-based conjugates were disclosed in WO 98/07713 A1.

US 6,395,266 B1 discloses branched PEG polymers linked to various cytotoxic agents. The branched polymers are considered to be advantageous compared to linear PEG conjugates since a higher loading of parent drug per unit of polymer can be achieved. The actual activity of these conjugates *in vivo* for the treatment of cancer was, however, not shown.

Similar to US 6,395,266 B1, EP 1 496 076 A1 discloses Y-shaped branched hydrophilic polymer derivatives conjugated to cytotoxic agents such as camptothecin. Again, the actual activity of these conjugates *in vivo* was not shown.

In a similar way, the following patent and non-patent literature discloses PEG conjugates: Greenwald *et al.*, J. Med. Chem., 1996, 39: 424-431 and US 5,840,900 A.

PEG, however, is known to have unpleasant or hazardous side effects such as induction of antibodies against PEG (N. J. Ganson, S.J. Kelly et al. Arthritis Research & Therapie 2006, 8:R12) and nephrotoxicity (G. A Laine, S. M. Hamid Hossain et al., The Annals of Pharmacotherapy, 1995 November, Volume 29) on use of such PEG or PEG-related conjugates. In addition, the biological activity of the active ingredients is most often greatly reduced in some cases after the PEG coupling. Moreover, the metabolism of the degradation products of PEG conjugates is still substantially unknown and possibly represents a health risk. Further, the functional groups available for coupling to cytotoxic agents are limited, so a high loading of the polymer with the respective drug is not possible.

Thus there is still a need for physiologically well tolerated alternatives to such PEG conjugates with which the solubility of poorly soluble low molecular weight substances can be improved and/or the residence time of low molecular weight substances in the

plasma can be increased and/or with which an optimized drug loading can be achieved. Further there is the need for macromolecular prodrugs which provide an advantageous targeting of the tumor and/or which, upon administration, will eventually liberate the active parent compound *in vivo* with improved pharmacodynamic properties.

It would be particularly desirable to provide prodrugs which take advantage of the so-called Enhanced Permeability and Retention (EPR) effect. This EPR effect describes the property by which certain sizes of molecules, such as macromolecules or liposomes, tend to accumulate in tumor tissue much more than they do in normal tissue (reference is made to respective passages of US 6,624,142 B2; or to Vasey P. A., Kaye S. B., Morrison R, *et al.* (January 1999) "Phase I clinical and pharmacokinetic study of PK1 [N-(2-hydroxypropyl)methacrylamide copolymer doxorubicin]: first member of a new class of chemotherapeutic agents-drug-polymer conjugates. Cancer Research Campaign Phase I/II Committee". *Clinical Cancer Research* 5 (1): 83-94). The general explanation for that effect is that tumor vessels are usually abnormal in form and architecture. This is due to the fact that, in order for tumor cells to grow quickly, they must stimulate the production of blood vessels.

Without wanting to be bound to any hypothesis, it is believed that the EPR effect allows for an enhanced or even substantially selective delivery of macromolecules to the tumor cells and as consequence, enrichment of the macromolecules in the tumor cells, when compared to the delivery of these molecules to normal tissue.

WO 03/074088 A2 describes hydroxyalkyl starch conjugates with, for example, cytotoxic agents such as daunorubicin, wherein the cytotoxic agent is usually directly coupled via an amino group to the hydroxyalkyl starch yielding in 1:1 conjugates. The hydroxyalkyl starch is described as having a substitution range preferably in the range of from 0.2 to 0.8. No use of these conjugates *in vivo* was shown. Further, in WO 03/074088 no cleavable linkage between the cytotoxic agent and hydroxyalkyl starch was described, which, upon administration, would be suitable to readily liberate the active drug *in vivo.*

Thus, there is still the need to provide new prodrugs of cytotoxic agents being bound to advantageous polymers for the treatment of cancer *in vivo.*

Thus, it is an object of the present invention to provide novel conjugates comprising a polymer linked to a cytotoxic agent. Further, it is an object of the present invention to provide a method for preparing such conjugates. Additionally, it is an object of the present invention to provide pharmaceutical compositions comprising these novel conjugates as

well as the use of the conjugates and the pharmaceutical composition, respectively, in the treatment of cancer.

Surprisingly, it was found that linking of cytotoxic agents via a tertiary hydroxyl group to a hydroxyalkyl starch derivative having a specific molecular weight MW as well as a specific molar substitution MS may lead to conjugates showing at least one of the desired beneficial properties, such as improved drug solubility, and/or optimized drug residence time *in vivo*, and/or reduced toxicity, and/or high efficiency, and/or effective targeting of tumor tissue *in vivo*. Without wanting to be bound to any theory, it is believed that the specific biodegradable hydroxyalkyl starch polymers of the invention may exhibit an optimized size, characterized by specific values of MW, which is large enough to prevent the elimination of the intact conjugate - comprised of the polymer and the cytotoxic agent - through the kidney prior to any release of the cytotoxic agent. Thus, elimination of the conjugate in the kidney by filtration through pores may be avoided. Further, the specific biodegradable hydroxyalkyl starch polymers of the invention comprised in the conjugate may exhibit an optimized molar substitution MS, and/or the conjugate as such may exhibit a preferred overall chemical constitution, so as to allow for a degradability of the hydroxyalkyl starch polymer comprised in the conjugate and release of the cytotoxic agent in a favorable time range. Further, it is believed that in contrast to most of the polymers described in the prior art, such as polyethylene glycol and derivatives thereof, the polymer fragments obtained from degradation of the conjugate of the present invention can be removed from the bloodstream by the kidneys or degraded via the lysosomal pathway without leaving any unknown degradation products of the polymer in the body.

Without wanting to be bound to any theory as to how the conjugates of the invention might operate, it is further contemplated that at least some of the conjugates of the invention might be able to deliver the respective cytotoxic agent into extracellular tissue space, such as into tissue exhibiting an EPR effect. However, it has to be understood that it is not intended to limit the scope of the invention only to such conjugates which take advantage of the EPR effect; also conjugates which show, possibly additionally, different advantageous characteristics, such as advantageous activity and/or low toxicity *in vivo* due to alternative mechanisms, are encompassed by the present invention.

Thus, the present invention relates to a hydroxyalkyl starch (HAS) conjugate comprising a hydroxyalkyl starch derivative and a cytotoxic agent, said conjugate having a structure according the following formula

$$HAS'(-L-M)_n$$

wherein M is a residue of a cytotoxic agent, wherein the cytotoxic agent comprises a tertiary hydroxyl group, L is a linking moiety (linking the HAS derivative and M), HAS' is a residue of the hydroxyalkyl starch derivative, n is greater than or equal to 1, wherein the hydroxyalkyl starch derivative has a mean molecular weight MW above the renal threshold, preferably a MW greater than or equal to 60 kDa, more preferably in the range of from 60 to 1500 kDa, and more preferably in the range of from 200 to 1000 kDa, more preferably in the range of from 250 to 800 kDa, and wherein the hydroxyalkyl starch derivative has a molar substitution MS in the range of from 0.6 to 1.5, and wherein the linking moiety L is linked to a tertiary hydroxyl group of the cytotoxic agent.

The term "linked to the tertiary hydroxyl group of the cytotoxic agent" as used in the context of the present invention is denoted to mean that the cytotoxic agent is reacted via its tertiary hydroxyl group. The resulting conjugated residue of the cytotoxic agent M is thus linked via an -O- group to the linking moiety -L- wherein the oxygen of this -O- group corresponds to the oxygen of the reacted tertiary hydroxyl group of the cytotoxic agent.

Further, the present invention relates to a method for preparing a hydroxyalkyl starch (HAS) conjugate comprising a hydroxyalkyl starch derivative and a cytotoxic agent, said conjugate having a structure according the following formula

HAS'(-L-M)n

wherein M is a residue of a cytotoxic agent, said cytotoxic agent comprising a tertiary hydroxyl group, L is a linking moiety, HAS' is a residue of the hydroxyalkyl starch derivative, and n is greater than or equal to 1, said method comprising

(a) providing a hydroxyalkyl starch (HAS) derivative having a mean molecular weight MW above the renal threshold, preferably a mean molecular weight greater than or equal to 60 kDa, more preferably in the range of from 60 to 1500 kDa, and more preferably of from 200 to 1000 kDa, more preferably in the range of from 250 to 800 kDa,, and having a molar substitution MS in the range of from 0.6 to 1.5, said HAS derivative comprising a functional group Z^1; and providing a cytotoxic agent comprising a tertiary hydroxyl group;

(b) coupling the HAS derivative to the cytotoxic agent via an at least bifunctional crosslinking compound L comprising a functional group K^1 and a functional group K^2, wherein K^2 is capable of being reacted with Z^1 comprised in the HAS derivative

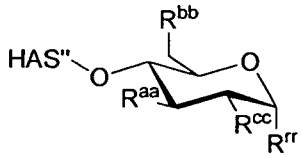
and wherein K^1 is capable of being reacted with the tertiary hydroxyl group comprised in the cytotoxic agent.

Moreover, the present invention relates to a hydroxyalkyl starch conjugate obtainable or obtained by the above-mentioned method.

Further, the present invention relates to a pharmaceutical compound or composition comprising the hydroxyalkyl starch conjugate or the hydroxyalkyl starch conjugate obtainable or obtained by the above-mentioned method. In addition, the present invention relates to the hydroxyalkyl starch conjugate as described above, or the pharmaceutical composition as described above, for the use as a medicament, in particular for the treatment of cancer. Moreover, the present invention relates to the use of the hydroxyalkyl starch conjugate as described above, or the pharmaceutical composition as described above for the manufacture of a medicament for the treatment of cancer. Moreover, the present invention relates to a method of treating a patient suffering from cancer comprising administering a therapeutically effective amount of the hydroxyalkyl starch conjugate as described above, or the pharmaceutical composition as described above.

The hydroxyalkyl starch

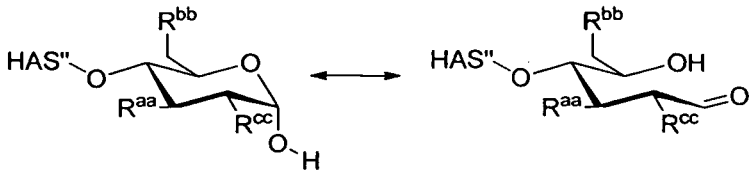
In the context of the present invention, the term "hydroxyalkyl starch" (HAS) refers to a starch derivative having a constitution according to the following formula (III)



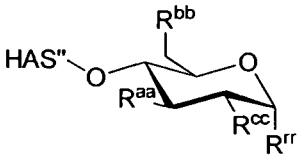
(III)

wherein the explicitly shown ring structure is either a terminal or a non-terminal saccharide unit of the HAS molecule and wherein HAS" is a remainder, i.e. a residual portion of the hydroxyalkyl starch molecule, said residual portion forming, together with the explicitly shown ring structure containing the residues R^{aa}, R^{bb} and R^{cc} and R^{rr} the overall HAS molecule. In formula (III), R^{33}, R^{bb} and R^{cc} are independently of each other hydroxyl, a linear or branched hydroxyalkyl group, or -O-HAS", in particular -O-HAS" or -[O—(CR^wR^x)—(CR^yR^z)]x-OH, wherein R^w, R^x, R^y and R^z are independently of each other selected from the group consisting of hydrogen and alkyl, x is an integer in the range of from 0 to 20, preferably in the range of from 0 to 4. Preferably, R^{a3}, R^{bb} and R^{cc} are independently of each other -0-HAS"or -[0-CH_2-CH2]$_s$-OH with s being in the range of from 0 to 4. In particular, R^{a3}, R^{bb} and R^{cc} are independently from each other -OH, -O-

CH_2-CH_2-OH (2-hydroxyethyl), or -O-HAS". Residue R^{rr} is -O-HAS" in case the explicitly shown ring structure is a non-terminal saccharide unit of the HAS molecule. In case the explicitly shown ring structure is a terminal saccharide unit of the HAS molecule, R^{rr} is -OH, and formula (III) shows this terminal saccharide unit in its hemiacetal form. This hemiacetal form, depending on e.g. the solvent, may be in equilibrium with the free aldehyde form as shown in the scheme below:



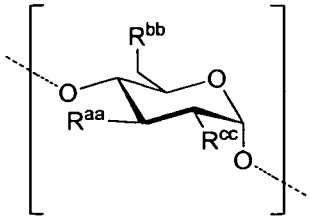
The term -O-HAS" as used in the context of the residue R" as described above is, in addition to the remainder HAS" shown at the left hand side of formula (III), a further remainder of the HAS molecule which is linked as residue R^{rr} to the explicitly shown ring structure of formula (III)



(III)

and forms, together with the residue HAS" shown at the left hand side of formula (III) and the explicitly shown ring structure the overall HAS molecule.

Each remainder HAS" discussed above comprises, preferably essentially consists of - apart from terminal saccharide units - one or more repeating units according to formula (Ilia)



(Ilia)

According to the present invention, the HAS molecule shown in formula (III) is either linear or comprises at least one branching point, depending on whether at least one of the residues R^{33}, R^{bb} and R^{cc} ofa given saccharide unit comprises yet a further remainder -O-HAS". If none of the R^{aa}, R^{bb} and R^{cc} of a given saccharide unit comprises yet a further

remainder -O-HAS", apart from the HAS" shown on the left hand side of formula (111), and optionally apart from HAS" contained in R^{π}, the HAS molecule is linear.

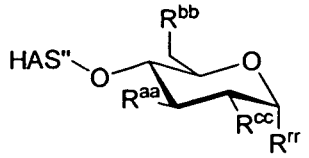
Hydroxyalkyl starch comprising two or more different hydroxyalkyl groups is also conceivable. The at least one hydroxyalkyl group comprised in the hydroxyalkyl starch may contain one or more, in particular two or more, hydroxyl groups. According to a preferred embodiment, the at least one hydroxyalkyl group contains only one hydroxyl group.

The term "hydroxyalkyl starch" as used in the present invention also includes starch derivatives wherein the alkyl group is suitably mono- or polysubstituted. Such suitable substituents are preferably halogen, especially fluorine, and/or an aryl group. Yet further, instead of alkyl groups, HAS may comprise also linear or branched substituted or unsubstituted alkenyl groups.

Hydroxyalkyl starch may be an ether derivative of starch, as described above. However, besides of said ether derivatives, also other starch derivatives are comprised by the present invention, for example derivatives which comprise esterified hydroxyl groups. These derivatives may be, for example, derivatives of unsubstituted mono- or dicarboxylic acids with preferably 2 to 12 carbon atoms or of substituted derivatives thereof. Especially useful are derivatives of unsubstituted monocarboxylic acids with 2 to 6 carbon atoms, especially derivatives of acetic acid. In this context, acetyl starch, butyryl starch and propionyl starch are preferred.

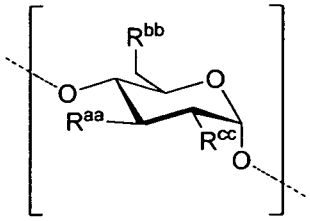
Furthermore, derivatives of unsubstituted dicarboxylic acids with 2 to 6 carbon atoms are preferred. In the case of derivatives of dicarboxylic acids, it is useful that the second carboxy group of the dicarboxylic acid is also esterified. Furthermore, derivatives of monoalkyl esters of dicarboxylic acids are also suitable in the context of the present invention. For the substituted mono- or dicarboxylic acids, the substitute group may be preferably the same as mentioned above for substituted alkyl residues. Techniques for the esterification of starch are known in the art (cf. for example Klemm, D. ***et ai,*** Comprehensive Cellulose Chemistry, vol. 2, 1998, Wiley VCH, Weinheim, New York, especially Chapter 4.4, Esterification of Cellulose (ISBN 3-527-29489-9)).

According to a preferred embodiment of the present invention, a hydroxyalkyl starch (HAS) according to the above-mentioned formula (III)



(III)

is employed. The saccharide units comprised in HAS", apart from terminal saccharaide units, may be the same or different, and preferably have the structure according to the formula (Ilia)

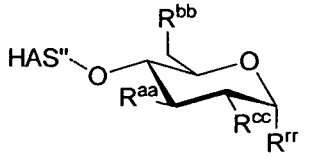


(Ilia)

as shown above.

According to the invention, the term "hydroxyalkyl starch" is preferably a hydroxyethyl starch, hydroxypropyl starch or hydroxybutyl starch, wherein hydroxyethyl starch is particularly preferred.

Thus, according to the present invention, the hydroxyalkyl starch (HAS) is preferably a hydroxyethyl starch (HES), the hydroxyethyl starch preferably having a structure according to the following formula (III)



(III)

wherein R^{aa}, R^{bb} and R^{cc} are independently of each other selected from the group consisting of -O-HES", and -[0-CH_2-CH_2]$_s$-OH, wherein s is in the range of from 0 to 4 and wherein HAS" is, in case the hydroxyalkyl starch is hydroxyethyl starch, the remainder of the hydroxyethyl starch and could be abbreviated with HES". Residue -R^{rr} is either -O-HAS" (which, in case the hydroxyalkyl starch is hydroxyethyl starch, could be abbreviated with -O-HES") or, in case the formula (III) shows the terminal saccharide unit of HES, -R^{rr} is -OH. For the sake of consistency, the abbreviation "HAS" is used

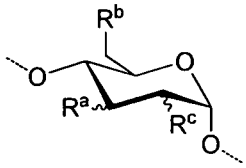
throughout all formulas in the context of the present invention, and if HAS is concretized as HES, it is explicitly mentioned in the corresponding portion of the text.

The term "Hydroxyalkyl Starch Derivative"

In the context of the present invention, the term "hydroxyalkyl starch derivative" refers to a derivative of starch being functionalized with at least one functional group Z^1, said group being a functional group capable of being linked to a further compound, in particular to the linking moiety L comprised in the structural unit-L-M which in turn is comprised in above-defined conjugate having a structure according to the following formula

$$HAS'(-L-M)_n$$

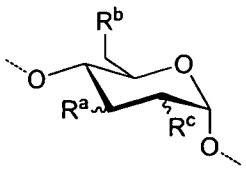
In accordance with the above-mentioned definition of HAS, the hydroxyalkyl starch derivative preferably comprises at least one structural unit according to the following formula (I)



(I)

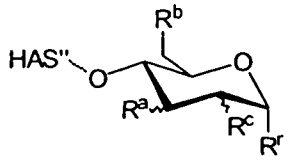
wherein at least one ofR^a, R^b or R^c comprises the functional group Z^1 and wherein -R^a, -R^b and -R^c are, independently of each other, selected from the group consisting of-O-HAS", $-[O-(CR^wR^x)-(CR^yR^z)]_x-OH$, $-[O-(CR^wR^x)-(CR^yR^z)]_y-Z^1$, $-[O-(CR^wR^x)-(CR^yR^z)]_y-[F^1]_p$-L'-$Z^1$, wherein R^w, R^x, R^y and R^z are independently of each other selected from the group consisting of hydrogen and alkyl, y is an integer in the range of from 0 to 20, preferably in the range of from 0 to 4, x is an integer in the range of from 0 to 20, preferably in the range of from 0 to 4, F^1 is a functional group, p is 0 or 1, L^1 is a linking moiety and Z^1 is a functional group which is capable of being linked to a further compound, in particular to the linking moiety L comprised in the structural unit -L-M.

In particular, a hydroxyalkyl starch derivative which comprises at least one structural unit according to the following formula (I)



(1)

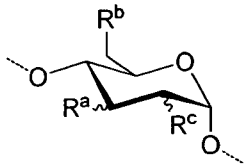
has preferably a structure according to the following formula (IV)



(IV)

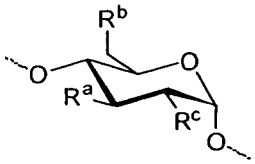
wherein -R^r is -O-HAS" or, in case the ring structure of formula (IV) shows the terminal saccharide unit of HAS, -R^r is -OH, and wherein HAS" is a remainder of the hydroxyalkyl starch derivative.

Analogously to the above-discussed definition of the term HAS" in the context of the hydroxyalkyl starch as such, the term "remainder of the hydroxyalkyl starch derivative" is denoted to mean a linear or branched chain of the hydroxyalkyl starch derivative, being linked to the oxygen groups shown in formula (IV) or being comprised in the residues R^a, R^b or R^c of formula (I), wherein said linear or branched chains comprise at least one structural unit according to formula (I)



(I)

wherein at least one ofR^a, R^b or R^c comprises the functional group Z^1 and/or one or more structural units of the formula (lb)

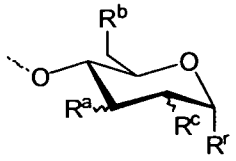


(lb)

wherein -R^a, -R^b and -R^c are, independently of each other, selected from the group consisting of -O-HAS" and -[0-{CR ^{w}R^{x}MCR ^{y}R^z)]x-OH, wherein R^w, R^x, R^y and R^z are as described above.

In case the hydroxyalkyl starch derivative has a linear starch backbone, none of R^a, R^b or R^c comprises a further group -O-HAS". In case at least one of -R^a, -R^b or -R° is -O-HAS", the hydroxyalkyl starch derivative comprises at least one branching point.

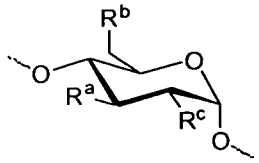
In particular, in case, the structural unit is the reducing sugar moiety of the hydroxyalkyl starch derivative, the terminal structural unit has a structure according to the following formula (la)



(Ia)

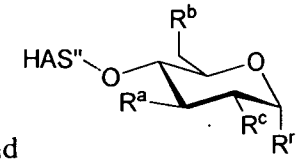
wherein -R^r is -OH or a group comprising the functional group Z^1. Residue -R^r is preferably selected from the group consisting of -OH, -Z^1 and -[F'j $_p$-L'-Z^1, most preferably -R^r is -OH, the reducing end of the hydroxyalkyl starch thus being present in unmodified form.

In the above-mentioned formula (la), the bond "~~" represents a bond with non-defined stereochemistry, i.e. this term represents a bond encompassing both possible stereochemistries. Preferably, the stereochemistry in most building blocks, preferably in all building blocks of the HAS derivative, is defined according to the formulas (lb) and (IVa)



(Ib)

and

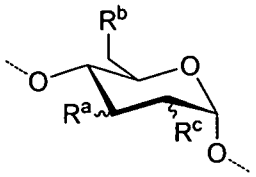


(IVa), respectively.

According to a preferred embodiment of the present invention, the hydroxyalkyl starch (HAS) derivative is a hydroxyethyl starch (HES) derivative.

Therefore, the present invention also describes a hydroxyalkyl starch derivative as described above, and a method for preparing said hydroxyalkyl starch derivative, and a conjugate comprising said hydroxyalkyl starch derivative and a cytotoxic agent, and a conjugate obtained or obtainable by the above-mentioned method wherein the conjugate comprises said hydroxyalkyl starch derivative and a cytotoxic agent, wherein the hydroxyalkyl starch derivative is a hydroxyethyl starch derivative.

Accordingly, in case the hydroxyalkyl starch (HAS) is hydroxyethyl starch (HES), the HAS derivative preferably comprises at least one structural unit according to the following formula (I)



(1)

wherein R^a, R^b and R^c are independently of each other selected from the group consisting of -O-HAS", -[0-CH_2-CH_2]s-OH, -[0-CH_2-CH_2]$_t$-Z' and - [O- CH_2- CH_2]$_t$-[F^1]$_p$-L'-Z^1, wherein at least one R^a, R^b and R^c is -[0-CH_2-CH_2]$_t$-Z^1 or –[O–CH_2–CH_2]$_t$-[F^1]$_p$-L^1-Z^1, wherein s is in the range of from 0 to 4, wherein t is in the range of from 0 to 4, and wherein p is 0 or 1.

The amount of functional groups Z^1 present in the hydroxyalkyl starch derivative

As regards the amount of functional groups Z^1 present in a given hydroxyalkyl starch derivative, preferably 0.3 % to 3 % of all residues R^a, R^b and R^c present in the hydroxyalkyl starch derivative contain the functional group Z^1.

More preferably, 0.3 % to 3 % of all residues -R^a, -R^b and -R^c present in the hydroxyalkyl starch derivative have the structure –[O–(CR^wR^x)–(CR^yR^z)]$_y$–Z^1 or -[0-(CR^wR^x)-(CR^yR^z)]$_y$–[F^1]$_p$-L^1-Z^1.

According to a particularly preferred embodiment, -R^a, -R^b and -R^c are selected from the group consisting of -O-HAS", -[0-(CR^wR^x)-(CR^yR^z)]x-OH and -[0-(CR^wR^x)-(CR^yR^z)]$_y$-Z', wherein 0.3 % to 3 % of all residues -R^a, -R^b and -R^c present in the hydroxyalkyl starch derivative have the structure -[0-(CR^wR^x)-(CR^yR^z)]$_y$- Z^1.

According to an alternative preferred embodiment, -R^a, -R^b and -R^c are selected from the group consisting of -O-HAS", -[0-(CR^wR^x)-(CR^yR^z)]x-OH and -[0-(CR^wR^x)-(CR^yR^z)]$_y$–[F^1]$_p$-L^1-Z^1, wherein 0.3 % to 3 % of all residues -R^a, -R^b and -R^c present in the hydroxyalkyl starch derivative have the structure - [O- (CR^wR^x)–(CR^yR^z)]$_y$- [F^1]$_p$-L'-Z^1.

The term "Residue of the Hydroxyalkyl Starch Derivative"

The term "residue of the hydroxyalkyl starch derivative" (HAS') refers to a hydroxyalkyl starch derivative being incorporated into a hydroxyalkyl starch conjugate. Within the meaning of the present invention the term "a conjugate comprising a hydroxyalkyl starch derivative" thus refers to a conjugate comprising a residue of a hydroxyalkyl starch derivative being incorporated into the conjugate and thus being linked to the linking moiety L comprised in the conjugate having a structure according to the following formula

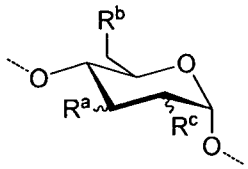
$$HAS'(-L-M)_n$$

Upon incorporation into the conjugate, the hydroxyalkyl starch derivative is coupled via at least one of its functional groups Z^1 to the crosslinking compound L (which is further reacted with M), or to the derivative of the cytotoxic agent having the structure -L-M, as described hereinabove and hereinunder, thereby forming a covalent linkage between the residue of the hydroxyalkyl starch derivative and L or -L-M, wherein the functional group X is formed upon reaction of Z^1 with L or -L-M, respectively.

Analogously to the above-discussed definition of the term "hydroxyalkyl starch derivative", the term "residue of a hydroxyalkyl starch derivative" refers to a derivative of starch being linked via at least one functional group X via a linking moiety to a further compound, in particular via the at least one linking moiety L comprised in the structural unit -L-M which in turn is comprised in above-defined conjugate having a structure according to the following formula

$$HAS'(-L-M)_n.$$

In accordance with the above-mentioned definition of the hydroxyalkyl starch derivative, the residue of the hydroxyalkyl starch derivative preferably comprises at least one structural unit according to the following formula (I)

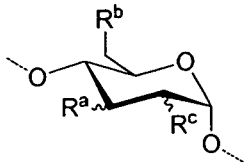


(I)

wherein $-R^a$, $-R^b$ and $-R^c$ are, independently of each other, selected from the group consisting of -O-HAS", $-[O-(CR^wR^x)-(CR^yR^z)]_x-OH$, $-[O-(CR^wR^x)-(CR^yR^z)]_y-X-$, $-[O-(CR^wR^x)-(CR^yR^z)]_y-[F^1]_p-L^1-X-$, and wherein at least one of R^a, R^b or R^c comprises the functional group $-[O-(CR^wR^x)-(CR^yR^z)]_x-X-$ or $-[O-(CR^wR^x)-(CR^yR^z)]_y-[F^1]_p-L^1-X-$,

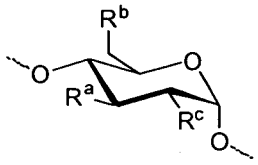
and wherein R^w, R^x, R^y and R^z are independently of each other selected from the group consisting of hydrogen and alkyl, y is an integer in the range of from 0 to 20, preferably in the range of from 0 to 4, x is an integer in the range of from 0 to 20, preferably in the range of from 0 to 4, F^1 is a functional group, p is 0 or 1, L^1 is a linking moiety and -X- is a functional group which is linked to a further compound, in particular to the linking moiety L comprised in the structural unit -L-M.

Besides the at least one structural unit according to formula (I)



(I)

wherein at least one of R^a, R^b or R^c comprises the functional group $-[O-(CR^wR^x)-(CR^yR^z)]_x-X-$ or $-[O-(CR^wR^x)-(CR^yR^z)]_y-[F^1]_p-L'-X-$, the residue of the hydroxyalkyl starch preferably comprises one or more structural units of the formula (Ib)



(Ib)

wherein $-R^a$, $-R^b$ and $-R^c$ are, independently of each other, selected from the group consisting of -O-HAS" and $-[O-(CR^wR^x)-(CR^yR^z)]_x-OH$.

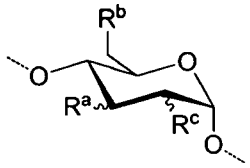
As disclosed above, preferably 0.3 % to 3 % of all residues R^a, R^b and R^c present in the hydroxyalkyl starch derivative contain the functional group Z^1. Further, preferably all functional groups Z^1 being present in a given hydroxyalkyl starch derivative are coupled according to the coupling reaction of step (b) as defined hereinabove, thereby forming the covalent linkage via functional group X. Consequently, preferably 0.3 % to 3 % of all residues R^a, R^b and R^c present in the residue of the hydroxyalkyl starch derivative contain the functional group X. Thus, preferably 0.3 % to 3 % of all residues R^a, R^b and R^c present in the residue of the conjugate of the present invention contain the functional group X.

However, in case the hydroxyalkyl starch derivative comprises at least two functional groups Z^1, it may be possible that in step (b) not all of these functional groups Z^1 are reacted with the crosslinking compound L, which in turn is reacted (either prior to or after

the reaction with the HAS derivative) with the cytotoxic agent, giving a conjugate in which the HAS derivative is linked via the linking moiety L to the residue of the cytotoxic agent M. Thus, embodiments are encompassed in which not all functional groups are reacted with the at least one crosslinking compound L, preferably the at least bifunctional crosslinking compound L, or with the derivative of the cytotoxic agent -L-M. The residue of the hydroxyalkyl starch derivative present in the conjugate of the invention may thus comprise at least one unreacted functional group Z^1. Further, in case the hydroxyalkyl starch derivative is reacted with the crosslinking compound L which comprises the functional groups K^1 and K^2 as described above, prior to the coupling reaction with the cytotoxic agent, the residue of the hydroxyalkyl starch derivative present in the conjugate of the invention may comprise at least one unreacted functional group K^2. All conjugates mentioned hereinunder and above, may comprise such unreacted groups.

To avoid possible side effects due to the presence of such unreacted functional groups Z^1 and/or unreacted functional groups K^2, the hydroxyalkyl starch conjugate may be further reacted with a suitable compound allowing for capping Z^1 and/or K^2 with a capping reagent D* in a preferably subsequent step (c) as described hereinunder in detail.

Thus, a hydroxyalkyl starch derivative comprised in a conjugate according to the invention mentioned hereinunder or above may comprise at least one structural unit according to formula (I),



(I)

wherein one or more of R^a, R^b or R^c is -[0-<CR ^{w}R^{x}HCR ^{y}R^z)]y-X-(L)$_{beta}$-D or -[O-(CR^{w}R^x)-(CR^{y}R^z)]$_y$-[F^1]$_p$-L^1-X-(L)$_{beta}$-D, wherein D is a capping group, L is the linking moiety L, as described above, beta is 0 or 1, preferably 0, and X is the functional group being formed upon reaction of at least one functional group Z^1 with a capping reagent D* thereby forming the structural unit -X-D (in this case beta is 0), or X is the functional group which is formed upon reaction of Z^1 with the crosslinking compound L, as described above, which in turn may be reacted via its functional group K^2 with a capping reagent D*, as described above, thereby forming the structural unit -L-D.

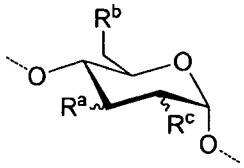
As regards the amount of functional groups X being linked to the functional moiety -L-M present in a given hydroxyalkyl starch conjugate, preferably at least 50 %, more preferably at least 75 %, more preferably at least 95 %, more preferably at least 98 % most preferably

at least 99 %, of all functional groups X present in the conjugate of the invention are linked to the functional moiety -L-M.

Alternatively, the conjugates of the present invention may also be described by the formula

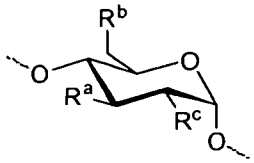
$$[D\text{-}(L)_{beta}\text{-}]_{gamma}HAS^{*}(\text{-}L\text{-}M)_{n}$$

wherein beta is 0 or 1, preferably 0, and wherein generally $0 \leq gamma < n$, preferably wherein $0 \leq gamma « < n$, especially preferably wherein gamma is 0, wherein the residue of the hydroxyalkyi starch derivative HAS* comprises at least one structural unit according to formula (I),



(I)

wherein at least one of R^a, R^b or R^c comprises the functional group X, and wherein the residue of the hydroxyalkyi starch HAS* preferably comprises one or more structural units of the formula (lb)



(lb)

wherein $-R^a$, $-R^b$ and $-R^\circ$ are, independently of each other, selected from the group consisting of-O-HAS" and $-[0\text{-}(CR^wR^xHCR^yR^z)]_x$-OH, and wherein HAS* comprises no structural units $-[0\text{-}(CR^wR^xHCR^yR^z)]_y$-X-(L)be,a-D or $-[O-(CR^wR^x)-(CR^yR^z)]_y-[F^1]_p$-$L^1$-X-$(L)_{beta}$-D.

Substitution Pattern: Molar Substitution (MS) and Degree of Substitution *CDS)*

HAS, in particular HES, is mainly characterized by the molecular weight distribution, the degree of substitution and the ratio of C_2 : C_6 substitution. There are two possibilities of describing the substitution degree.

The degree of substitution (DS) of HAS is described relatively to the portion of substituted glucose monomers with respect to all glucose moieties.

The substitution pattern of HAS can also be described as the molar substitution (MS), wherein the number of hydroxyethyl groups per glucose moiety is counted.

In the context of the present invention, the substitution pattern of the hydroxyalkyl starch (HAS), preferably HES, is referred to as MS, as described above, wherein the number of hydroxyalkyl groups present per sugar moiety is counted (see also Sommermeyer *et ai,* 1987, Krankenhauspharmazie, 8(8): 271-278, in particular page 273). The MS is determined by gaschromatography after total hydrolysis of the hydroxyalkyl starch molecule.

The MS values of the respective hydroxyalkyl starch, in particular hydroxyethyl starch starting materials, are given since it is assumed that the MS value is not affected during the derivatization procedures as well as during the coupling step of the present invention.

The MS value corresponds to the degradability of the hydroxyalkyl starch via alpha-amylase. The higher the MS value, the lower the degradability of the hydroxyalkyl starch. It was surprisingly found that the MS of the hydroxyalkyl starch derivative present in the conjugates according to the invention should preferably be in the range of from 0.6 to 1.5 to provide conjugates with advantageous properties. Without wanting to be bound to any theory, it is believed that a MS in the above mentioned range combined with the specific molecular weight range of the conjugates results in conjugates with an optimized enrichment of the cytotoxic agent in the tumor and/or residence time in the plasma allowing for a controlled release of the cytotoxic agent prior to the degradation of the polymer and the subsequent removal of polymer fragments through the kidney.

According to a preferred embodiment of the present invention, the molar substitution (MS) is in the range of from 0.70 to 1.45, more preferably in the range of from 0.80 to 1.40, more preferably in the range of from 0.85 to 1.35, more preferably in the range of from 0.95 to 1.30, such as 0.95, 1.0, 1.05, 1.1, 1.15, 1.2, 1.25 or 1.3.

Thus, the present invention also relates to a method for preparing a conjugate comprising a hydroxyalkyl starch derivative and a cytotoxic agent, as described above, and a conjugate obtained or obtainable by said method, wherein the hydroxyalkyl starch derivative has a molar substitution in the range of from 0.70 to 1.45, more preferably in the range of from 0.80 to 1.40, more preferably in the range of from 0.85 to 1.35, more preferably in the range of from 0.95 to 1.30.

Likewise, the present invention also relates to a hydroxyalkyl starch (HAS) conjugate comprising a hydroxyalkyl starch derivative and a cytotoxic agent, as described above, wherein the hydroxyalkyl starch derivative has a molar substitution MS in the range of from 0.70 to 1.45, more preferably in the range of from 0.80 to 1.40, more preferably in the range of from 0.85 to 1.35, more preferably in the range of from 0.95 to 1.30. Likewise, the present invention relates to a pharmaceutical composition comprising a hydroxyalkyl starch conjugate, as described above, or a hydroxyalkyl starch conjugate obtained or obtainable by the above described method, wherein the hydroxyalkyl starch derivative has a molar substitution MS in the range of from 0.70 to 1.45, more preferably in the range of from 0.80 to 1.40, more preferably in the range of from 0.85 to 1.35, more preferably in the range of from 0.95 to 1.30.

As far as the ratio of $C_2 : C_6$ substitution is concerned, i.e. the degree of substitution (DS) of HAS, said substitution is preferably in the range of from 2 to 20, more preferably in the range of from 2 to 15 and even more preferably in the range of from 3 to 12, with respect to the hydroxyalkyl groups.

Mean molecular weight MW (M_W)

HAS and in particular HES compounds are present as polydisperse compositions, wherein each molecule differs from the other with respect to the polymerization degree, the number and pattern of branching sites, and the substitution pattern. HAS and in particular HES is therefore a mixture of compounds with different molecular weight. Consequently, a particular HAS and in particular a HES is determined by average molecular weight with the help of statistical means.

In this context the number average molecular weight is defined by equation 1:

$$\overline{M}_n = \frac{\sum_i n_i \cdot M_i}{\sum_i n_i} \qquad (1)$$

where n_i is the number of molecules of species i of molar mass M_j.
$\overline{M}_n$ indicates that the value is an average, but the line is normally omitted by convention.

M_w is the weight average molecular weight, defined by equation 2:

$$\overline{M}_w = \frac{\sum_i n_i \cdot M_i^2}{\sum_i n_i M_i}$$

(2)

where rii is the number of molecules of species i of molar mass Mj and $\overline{M}_w$ indicates that the value is an average, but the line is normally omitted by convention.

Preferably, the hydroxyalkyl starch derivative, in particular the hydroxyethyl starch derivative comprised in the conjugate, as described above, has a mean molecular weight MW (weight mean) above the renal threshold.

The renal threshold is determined according to the method described by Waitzinger et al. (Clin. Drug Invest. 1998; 16: 151-160) and reviewed by Jungheinrich et al. (Clin. Pharmacokinet. 2006; 44(7): 681-699). Preferably, the renal threshold is denoted to mean a mean molecular weight MW above 40 kDa.

More preferably, the hydroxyalkyl starch derivative, in particular the hydroxyethyl starch derivative comprised in the conjugate, as described above, has a mean molecular weight MW above 45 kDa, more preferably above 50 kDa, more preferably above 60 kDa.

More preferably the hydroxyalkyl starch derivative, in particular the hydroxyethyl starch derivative, according to the invention, has a mean molecular weight MW (weight mean) in the range of from 60 to 1500 kDa, preferably in the range of from 200 to 1000 kDa, more preferably in the range of from 250 to 800 kDa.

The term "mean molecular weight" as used in the context of the present invention relates to the weight as determined according to MALLS (multiple angle laser light scattering) - GPC method as described in example 1.7.

According to an especially preferred embodiment, the hydroxyalkyl starch derivative has a mean molecular weight MW in the range of from 500 to 800 kDa.

Therefore, the present invention also relates to a method as described above, for preparing a hydroxyalkyl starch derivative, as well as to a method for preparing a hydroxyalkyl starch conjugate, wherein the hydroxyalkyl starch derivative has a mean molecular weight

MW above the renal threshold, preferably a mean molecular weight MW greater than or equal to 60 kDa, more preferably a mean molecular weight MW in the range of from 60 to 1500 kDa, more preferably in the range of from 200 to 1000 kDa, more preferably in the range of from 250 to 800 kDa. Likewise, the present invention relates to a hydroxyalkyl starch conjugate, as described above, comprising a hydroxyalkyl starch derivative, as well as to a hydroxyalkyl starch conjugate obtained or obtainable by the above-mentioned method, wherein the hydroxyalkyl starch derivative has a mean molecular weight above the renal threshold, preferably a MW greater than or equal to 60 kDa, more preferably a mean molecular weight MW in the range of from 60 to 1500 kDa, preferably in the range of from 200 to 1000 kDa, more preferably in the range of from 250 to 800 kDa.

According to an especially preferred embodiment, the hydroxyalkyl starch derivative has a MS in the range of from 0.8 to 1.4 and a mean molecular weight MW in the range of from 60 to 1500 kDa, more preferably a mean molecular weight MW in the range of from 200 to 1000 kDa and a molar substitution MS in the range of from 0.80 to 1.40, more preferably a mean molecular weight MW in the range of from 250 to 800 kDa and a molar substitution in the range of from 0.85 to 1.35, more preferably a mean molecular weight MW in the range of from 500 to 750 kDa and a MS in the range of from 0.95 to 1.30.

As regards integer n described above and below: According to a preferred embodiment of the present invention, n is in the range of from 1 to 400, more preferably in the range of from 2 to 300, more preferably in the range of from 3 to 200.

Drug loading

The amount of M, present in the conjugates of the invention, can further be described by the drug loading (also: drug content). The "drug loading" as used in the context of the present invention is calculated as the mean molecular weight of the cytotoxic agent measured in mg drug, i.e. cytotoxic agent, per 1 g of the conjugate.

The drug loading is determined by measuring the absorbance of M (thus the cytotoxic agent bound to HAS) at a specific wavelength in a stock solution, and calculating the content using the following equation (Lambert Beer's law):

$$c_{drug}[\mu mol\,/\,cm^3] = \frac{(A - A^0)}{\varepsilon * d}$$

where ε is the extinction coefficient of the cytotoxic agent at the specific wavelength, which is obtained from a calibration curve of the cytotoxic agent dissolved in the same

solvent which is used as in the stock solution (given in cπι²/μmo1), at the specific wavelength, A is the absorption at this specific wavelength, measured in a UV-VIS spectrometer, A^0 is the absorption of a blank sample and d the width of the cuvette (equals the slice of absorbing material in the path of the beam, usually 1 cm). The appropriate wavelength for the determination of drug loading is derived from a maximum in the UV-Vis-spectra, preferably at wavelengths above 230 nm.

With a known concentration of conjugate in the sample ($c_{conjugate}$) and the concentration of drug in the sample determined by Lambert Beer's law, the loading in μπιοϊ/g can be calculated according to the following equation:

$$Loading[\mu mol/g] = \frac{1000 * c_{drug}[\mu mol/ml]}{c_{conjugate}[mg/ml]}$$

The loading (in mg/g) can be determined taking into account the molecular weight of the drug M as shown in the following equation:

$$Loading[mg/g] = Loading[\mu mol/g] * MW_{drug}\ [\mu g/\mu mol]/1000$$

As regards the drug loading, according to a preferred embodiment of the present invention, the drug loading of the conjugates is preferably in the range of from 30 to 600 μπιοϊ drug / g conjugate, more preferably in the range of from 50 to 400 μιηοϊ drug / g conjugate, more preferably in the range of from 80 to 350 μηιοϊ drug / g conjugate, and most preferably in the range of from 100 to 250 μπιοϊ drug / g conjugate.

The cytotoxic agent

The term "cytotoxic agent" as used in the context of the present invention refers to natural or synthetic substances, which inhibit the cell growth or the cell division ***in vivo.*** The term is intended to include chemotherapeutic agents, antibiotics and toxins such as enzymatically active toxins of bacterial, fungal, plant or animal origin, or fragments thereof.

The term "residue of the cytotoxic agent" as used in the context of the present invention refers to the cytotoxic agent being linked to L via a group -0-, said group being derived from a tertiary hydroxyl group being present in the cytotoxic agent.

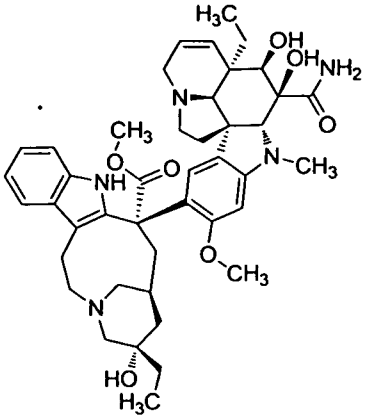
Preferably, the term "cytotoxic agent" is a natural or synthetic substance which inhibits the cell growth or the cell division of a tumor ***in vivo.*** Most preferably, the cytotoxic agent is a

chemotherapeutic agent. The therapeutic use of these preferred cytotoxic agents, most preferably of the chemotherapeutic agents, is based on this difference in the rate of cell division and cell growth of tumor cells compared to normal cells. Among others, tumor cells differ from normal cells in that tumor cells are no longer subject to physiological growth control and therefore have an increased rate of cell division. Since the toxic activity of cytotoxic agents is usually primarily directed against proliferating cells, such cytotoxic agents can be used for inhibiting a development or progression of a neoplasm *in vivo*, particularly a malignant (cancerous) lesion, such as a carcinoma, sarcoma, lymphoma, or leukemia. Inhibition of metastasis is frequently also a property of the cytotoxic agents encompassed by the present invention.

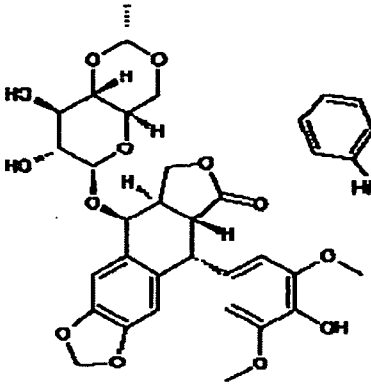
With respect to the chemistry used in the context of the present invention, any cytotoxic agent, preferably any chemotherapeutic agent, known to those skilled in the art that can be incorporated into the conjugates according to the present invention provided that this cytotoxic agent, preferably the chemotherapeutic agent, comprises a tertiary hydroxyl group. Preferably the cytotoxic agent is an agent for the treatment of cancer.

Within the meaning of the present invention the term tertiary hydroxyl group, is denoted to mean a hydroxyl group being attached to a carbon atom, this carbon atom bearing no substituents being an H. Preferably, the term tertiary hydroxyl group encompasses hydroxyl groups being attached to a tertiary carbon atom, that is a carbon atom comprising three carbon atoms as neighbours, as well as hydroxyl groups being attached to an aromatic or heteroaromatic ring.

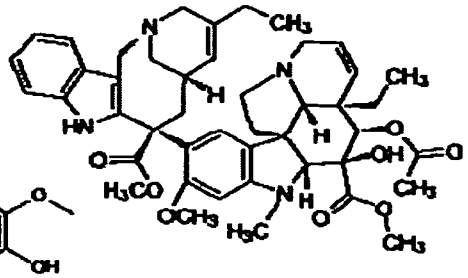
The following cytotoxic agents encompassed by the present invention are mentioned by way of example:



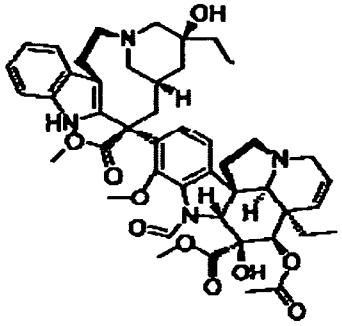
Vindesine



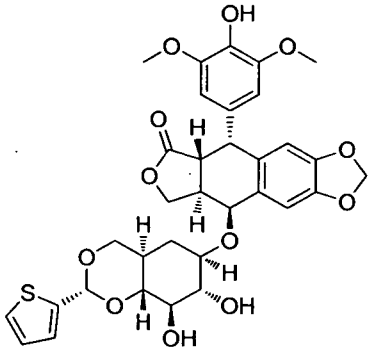
Etoposid



Vinorelbine

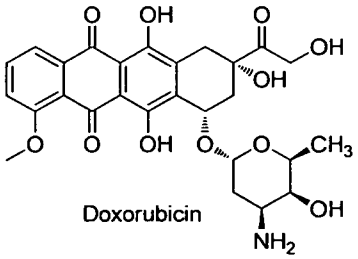


Vincristine

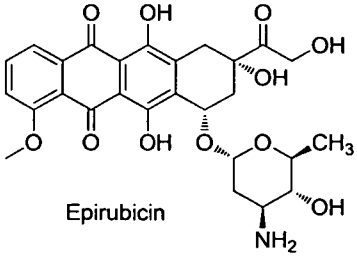


Teniposide

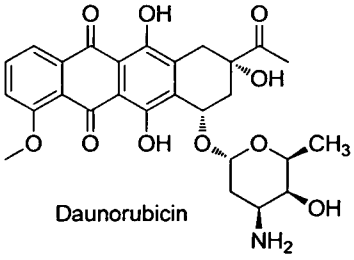
Trabectedin



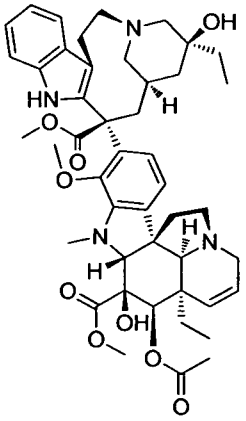
Doxorubicin



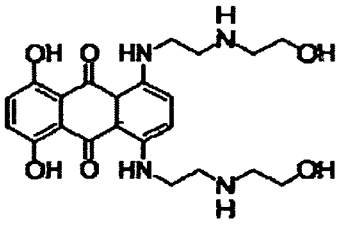
Epirubicin



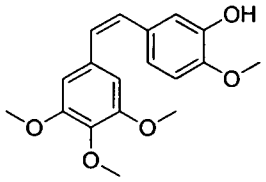
Daunorubicin



Vinblastine



Mitoxantron

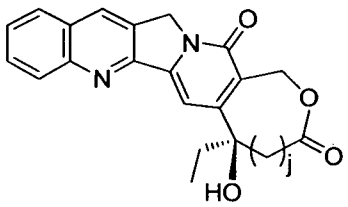


Combretastatin A4

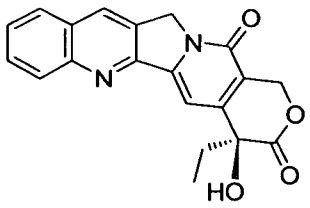
According to a preferred embodiment of the invention, the at least one tertiary hydroxyl group containing cytotoxic agent is selected from the group consisting of tubulin interacting drugs, such as tubulin inhibitors or tubulin stabilizers (such as peloruside A, the epothilone family, the taxane family, dictyostatin, discodermolide), topoisomerase (1) inhibitors (such as camptothecin, topotecan, irinotecan, silatecan (DB67), karenotecin (BNP 1350), exatecan, lurtotecan, gimatecan (ST 1481) and CKD 602), topoisomerase (II) inhibitors (such as etoposide and teniposide), DNA intercalators (such as mitoxantron), kinase inhibitors (such as rapamycin and analogues (temsirolimus, everolimus)), , antimetabolites, mitotic inhibitors, DNA damaging agents (such as trabectedin), anthracyclines (such as doxorubicin, epirubicin, daunorubicin), hormone analogues (such as fulvestrant or prednisone), vinca alkaloids (such as vindesine, vinorelbine, vincristine, vinflunine and vinblastine), vascular disrupting agents such as the combretastatin family (e.g. combretastatin A1-A4), colchinol-derivatives (e.g. N-acetyl-colchinol) and HSP-90 inhibitors (such as geldanamycin and analogues (e.g. 17-AAG)).

According to a preferred embodiment of the invention, the cytotoxic agent is a topoisomerase (I) inhibitor.

Particularly preferred cytotoxic agents according to the invention are camptothecin and camptothecin analogues. For the purpose of the present invention, the term "camptothecin analogues" refers to a class of compounds having a camptothecin ring system, shown by the core structure below



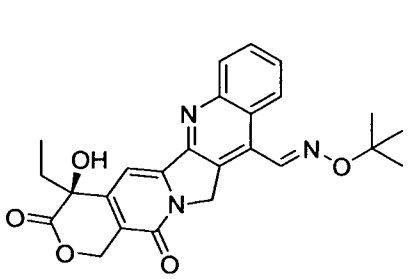
with integer j being 0 or 1, preferably 1, thus, the camptothecin analogues according to the invention preferably comprise the following core structure:

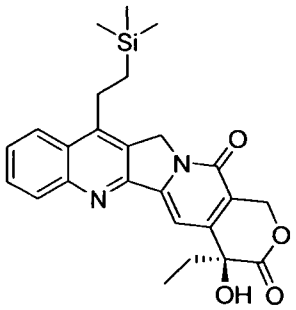


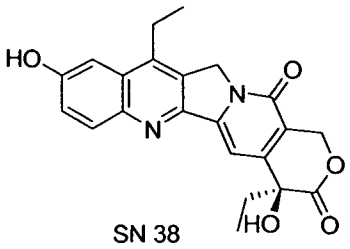
wherein these cytotoxic agents may be derived from natural sources or may have been synthesized artificially.

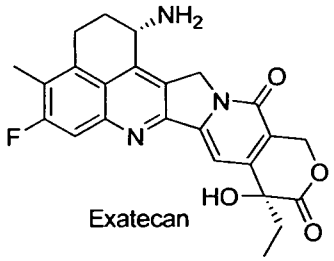
It has to be understood, that any molecule comprising this core structure is encompassed by the term "camptothecin". Apart from the hydroxyl group, the core structure may be further substituted in one or more positions.

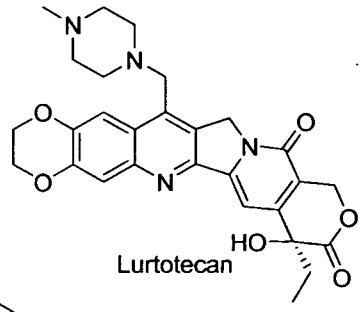
By way of example the following structures shall be mentioned:

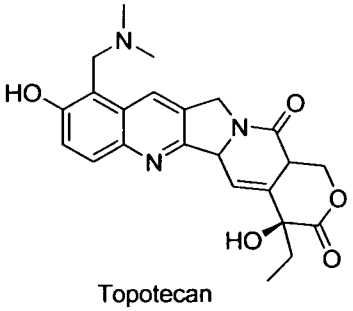

Gimatecan

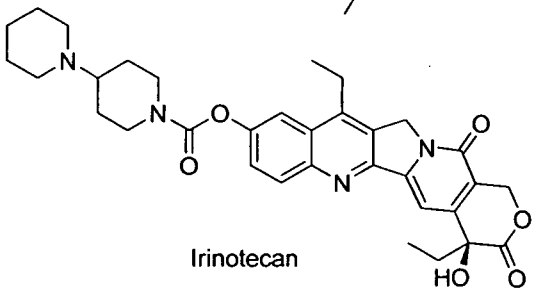

Karenitecin

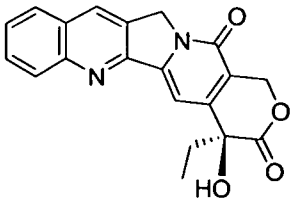

SN 38

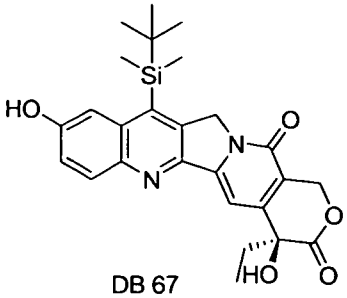

Exatecan

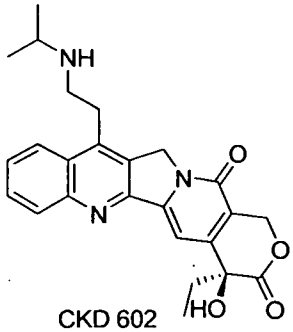

Lurtotecan


Topotecan

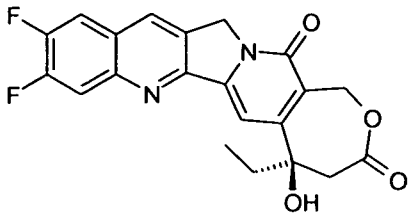

Irinotecan


camptothecin

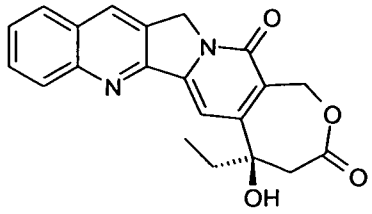

DB 67


CKD 602

Further, diflomotecan and BN80927 are mentioned by way of example.



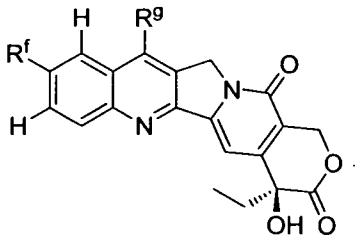
diflomotecan



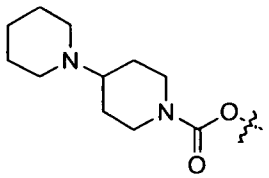
BN80927

Accordingly, the present invention preferably relates to a hydroxyalkyl starch conjugate as described above, as well as to a method for preparing a hydroxyalkyl starch conjugate and the respective conjugate obtained or obtainable by said method, the conjugate compising a cytotoxic agent selected from the group consisting of camptothecin, topotecan, irinotecan, silatecan (DB 67), BNP 1350 (cositecan), exatecan, lurtotecan, ST 1481, gimatecan, belotecan, CKD 602, karenitecin, chimmitecan, 9-aminocamptothecin, 9-nitrocamptothecin, BMS422461, diflomotecan, BN80927, BMS422461, morpholino-CPT and KOS-1584.

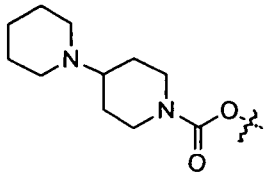
Most preferably, the cytotoxic agent according to the invention is a camptothecin or a camptothecin analogue, in particular having a structure according to the following formula



wherein - R^f is selected from the group consisting of -OH, siloxy groups, ester groups or any groups having the structure



and wherein - R^8 is -CH_2-CH_3. and wherein - R^f is preferably –OH or



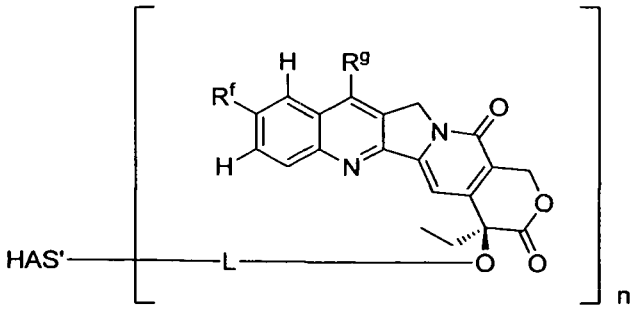
most preferably, -OH.

Accordingly, the cytotoxic agent is preferably SN-38 or irinotecan, most preferably SN-38.

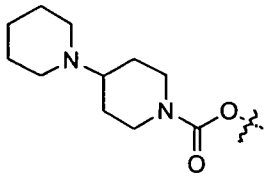
Camptothecin and SN-38 have been found to be effective anti-cancer agents. However, to date, their use is limited due to their poor water solubility and extensive toxicity. To date, only special, more soluble derivatives of camptothecin such as topotecan and prodrugs such as irinotecan can be employed in the clinic, suffering from short in vivo half lifes and still extensive adverse effects, especially severe gastro-intestinal toxicities. Common formulation techniques for such unpolar drugs normally comprise sorbitol as excipients, known to be connected to severe and dose limiting adverse effects. Such drawbacks can be overcome by the conjugates according to the present invention, wherein a hydroxyalkyl starch derivative, as described above, is linked via a linking moiety L to a tertiary hydroxyl group of the cytotoxic agent, preferably to a tertiary hydroxyl group of SN-38 or irinotecan.

In case the cytotoxic agent is SN-38 or irinotecan, the cytotoxic agent is coupled to the tertiary hydroxyl group present in the alpha-hydroxy lactone ring of the cytotoxic agent. In case the cytotoxic agent is a camptothecin analogue comprising additionally a tertiary hydroxyl group as substituent in another position of the at least pentacyclic system, coupling via such a tertiary hydroxyl group is also encompassed by the present invention.

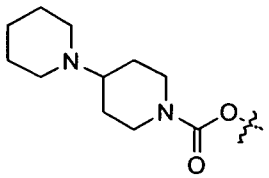
Thus, preferably, the present invention also relates to a conjugate, as described above, as well as to a conjugate obtained or obtainable by a method, as described above, the conjugate having a structure according to the following formula:



wherein - R^f is selected from the group consisting of -OH, siloxy groups, ester groups and groups having the structure

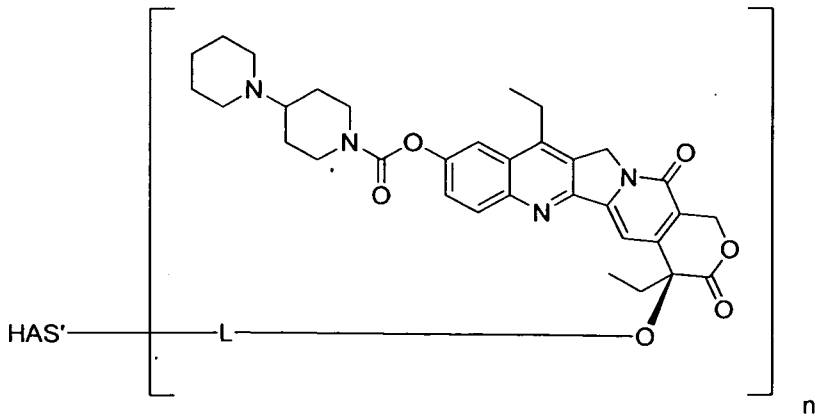


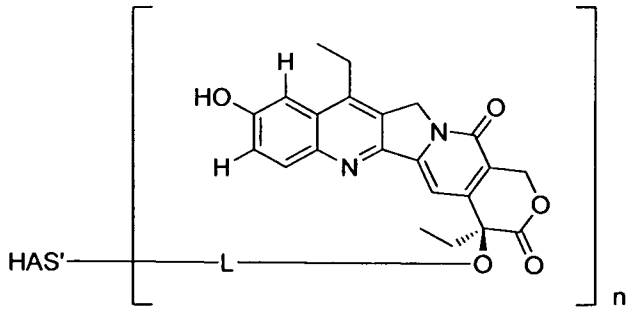
and wherein - R^8 is -CH_2-CH_3 and wherein - R^f is preferably -OH or



most preferably -OH.

The following particular preferred structures shall be mentioned:





The linking moiety L

According to the invention, the cytotoxic agent is preferably linked via a cleavable linker to the hydroxyalkyl starch derivative.

The expression "cleavable linker" refers to any linker which can be cleaved physically or chemically and preferably releases the cytotoxic agent in unmodified form. Examples for physical cleavage may be cleavage by light, radioactive emission or heat, while examples for chemical cleavage include cleavage by redox-reactions, hydrolysis, pH-dependent cleavage or cleavage by enzymes.

According to a preferred embodiment of the present invention, the cleavable linker comprises one or more cleavable bonds, preferably hydrolytically cleavable bonds, the cleavage, in particular the hydrolysis, of which releases the cytotoxic agent *in vivo.* Preferably the bond between linking moiety L and the tertiary hydroxyl group of the cytotoxic agent is a cleavable linkage.

Thus, the present invention also relates to a conjugate as described above, as well as to a conjugate obtained or obtainable by the above described method, wherein the linking moiety L and the residue of the cytotoxic agent M are linked via the tertiary hydroxyl group of the cytotoxic agent via a linkage which is cleaved, preferably which is hydrolyzed, *in vivo* and allows for the release of the cytotoxic agent, preferably in unmodified form.

Preferably, the linking moiety L has a structure -L'-F^3-, wherein F^3 is the functional group, linking L' with M, and wherein the linkage between F^3 and the group -O- derived from the tertiary hydroxyl group of the cytoxic agent, thus the structural unit —F^3-0-, is cleaved *in vivo* and releases (the residue of) the cytotoxic agent . L' is a linking moiety linking the functional group F^3 with the hydroxyalkyl starch derivative.

The functional group F^3

There are in principle no restrictions as to the nature of the functional group F^3 provided that this group forms together with the tertiary hydroxyl group of the cytotoxic agent a functional moiety capable of being cleaved *in vivo.*

Thus, the present invention also relates to a conjugate as described above, as well as to a conjugate obtained or obtainable by the above described method, wherein the bond between the functional group -F^3- and the functional group -O- of the residue of the

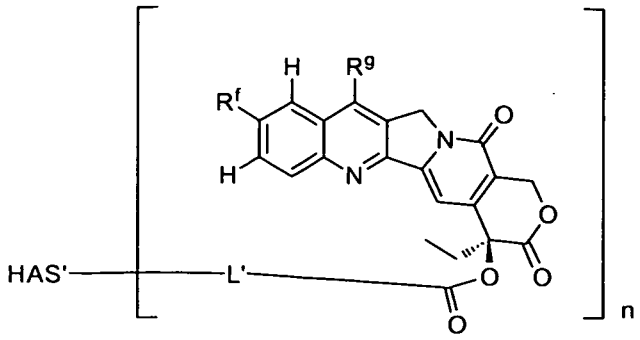
cytotoxic agent M (said group being derived from the tertiary hydroxyl group of the cytotoxic agent) is a cleavable linkage, which is cleaved *in vivo* so as to release the cytotoxic agent.

Beside the -C(=Y)- function, in particular the -C(=0)- function, this accounts, inter alia, for groups F^3 which form together with the group -O- of the residue of the cytotoxic agent M (derived from the tertiary hydroxyl group of the cytotoxic agent), the structural unit -F^3-0-, with -F^3-0- being a carbonate, thiocarbonate, xanthogenate, carbamate or thiocarbamate of the type -Y^Y-C(=Y)-0- with Y^Y being -0-, -S- or -NH- and Y being O, S or NH.

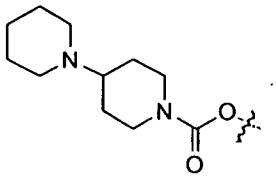
Preferably, the functional group F^3 is -C(=Y> or -Y^Y-C(=Y)-, with Y being O, NH or S and with Y^Y being -0-, -S- or -NH-. In particular, the functional group F^3 is -C(=Y)-, with Y being O, NH or S. Together with the group -O- derived from the tertiary hydroxyl group of the cytotoxic agent, the functional group F^3 therefore preferably forms a -C(=Y)-0- bond with Y being O, NH or S, in particular with Y being O or S, more preferably with Y being O, and wherein L' is a linking moiety linking the functional group F^3 with the hydroxyalkyl starch derivative.

Therefore, the present invention also relates to a hydroxyalkyl starch conjugate comprising a hydroxyalkyl starch derivative and a cytotoxic agent, said conjugate having a structure according to the following formula HAS'(-L-M)$_n$, wherein the linking moiety L has a structure -L'-F^3-, wherein F^3 is a functional group linking L' with the residue of the cytotoxic agent (M), preferably wherein F^3 is a -C(=Y)- group, with Y being O, NH or S, and wherein F^3 is linked to the group -O- derived from the tertiary hydroxyl group of the cytotoxic agent , thereby forming a -C(=Y)-0- bond with Y being O, NH or S, in particular with Y being O or S, more preferably with Y being O, and wherein L' is a linking moiety. Likewise, the present invention relates to a method for preparing a conjugate having a structure HAS'(-L-M)$_n$, wherein L has a structure -L'-F^3-, wherein F^3 is a functional group linking L' with M, preferably wherein F^3 is a -C(=Y)- group, with Y being O, NH or S, and wherein the structural unit -F^3-0- is formed upon reaction of the crosslinking compound L with the tertiary hydroxyl group of the cytotoxic agent. Likewise, the present invention relates to a conjugate obtained or obtainable by the method, as described above.

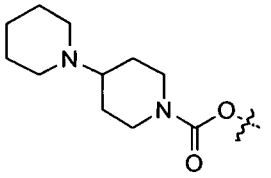
According to a particularly preferred embodiment, the present invention relates to a conjugate, as described above, as well as to a conjugate, obtained or obtainable by a method, as described above, the conjugate having a structure according to the following formula:



wherein - R^f is selected from the group consisting of -OH, siloxy groups, ester groups and groups having the structure



and wherein $-R^g$ is $-CH_2-CH_3$ and wherein $-R^f$ is preferably -OH or



most preferably -OH.

The linking moiety L'

According to a preferred embodiment of the present invention, the functional group F^3 and the hydroxyalkyi starch derivative are separated by a suitable linking moiety L', as described above. The term linking moiety L' as used in this context of the present invention relates to any suitable chemical moiety bridging F^3 and the hydroxyalkyi starch derivative.

In general, there are no particular restrictions as to the chemical nature of the linking moiety L' with the proviso that L' provides suitable chemical properties for the novel conjugates for their intended use.

Preferably, L' is a linking moiety such as an alkyl, alkenyl, alkylaryl, arylalkyl, aryl, heteroaryl, alkylheteroaryl or heteroarylalkyl group.

Within the meaning of the present invention, the term "alkyl" relates to non-branched alkyl residues, branched alkyl residues, cycloalkyl residues, as well as residues comprising one or more heteroatoms or functional groups, such as, by way of example, -0-, -S-, -NH-, -NH-C(=0)-, -C(=0)-NH-, and the like. The term also encompasses alkyl groups which are further substituted by one or more suitable substituents. The term "substituted alkyl" as used in this context of the present invention preferably refers to alkyl groups being substituted in any position by one or more substituents, preferably by 1, 2, 3, 4, 5 or 6 substituents, more preferably by 1, 2 or 3 substituents. If two or more substituents are present, each substituent may be the same or may be different from the at least one other substituent. There are in general no limitations as to the substituent. The substituents may be, for example, selected from the group consisting of aryl, alkenyl, alkynyl, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylthiocarbonyl, alkoxy, phosphate, phosphonato, phosphinato, amino, acylamino, including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido, amidino, nitro, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, alkylsulfinyl, sulfonate, sulfamoyl, sulfonamido, trifluoromethyl, cyano, azido, cycloalkyl such as e.g. cyclopentyl or cyclohexyl, heterocycloalkyl such as e.g. morpholino, piperazinyl or piperidinyl, alkylaryl, arylalkyl and heteroaryl. Preferred substituents of such organic residues are, for example, halogens, such as fluorine, chlorine, bromine or iodine, amino groups, hydroxyl groups, carbonyl groups, thiol groups and carboxyl groups.

The term "alkenyl" as used in the context of the present invention refers to unsaturated alkyl groups having at least one double bond. The term also encompasses alkenyl groups which are substituted by one or more suitable substituents.

The term "alkynyl" refers to unsaturated alkyl groups having at least one triple bond. The term also encompasses alkynyl groups which are substituted by one or more suitable substituents.

Within the meaning of the present invention, the term "aryl" refers to, but is not limited to, optionally suitably substituted 5- and 6-membered single-ring aromatic groups as well as optionally suitably substituted multicyclic groups, for example bicyclic or tricyclic aryl groups. The term "aryl" thus includes, for example, optionally substituted phenyl groups or optionally suitably substituted naphthyl groups. Aryl groups can also be fused or bridged with alicyclic or heterocycloalkyl rings which are not aromatic so as to form a polycycle, e.g., benzodioxolyl or tetraline.

The term "heteroaryi" as used within the meaning of the present invention includes optionally suitably substituted 5- and 6-membered single-ring aromatic groups as well as substituted or unsubstituted multicyclic aryl groups, for example bicyclic or tricyclic aryl groups, comprising one or more, preferably from 1 to 4 such as 1, 2, 3 or 4, heteroatoms, wherein in case the aryl residue comprises more than 1 heteroatom, the heteroatoms may be the same or different. Such heteroaryi groups including from 1 to 4 heteroatoms are, for example, benzodioxolyl, pyrrolyl, furanyl, thiophenyl, thiazolyl, isothiazolyl, imidazolyl, triazolyl, tetrazolyl, pyrazolyl, oxazolyl, isoxazolyl, pyridinyl, pyrazinyl, pyridazinyl, benzoxazolyl, benzodioxazolyl, benzothiazolyl, benzoimidazolyl, benzothiophenyl, methylenedioxyphenylyl, napthyridinyl, quinolinyl, isoquinolinyl, indolyl, benzofuranyl, purinyl, deazapurinyl, or indolizinyl.

The term "substituted aryl" and the term "substituted heteroaryi" as used in the context of the present invention describes moieties having substituents replacing a hydrogen on one or more atoms, e.g. C or N, of an aryl or heteroaryi moiety. Again, there are in general no limitiations as to the substituent. The substituents may be, for example, selected from the group consisting of alkyl, alkenyl, alkynyl, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylthiocarbonyl, alkoxy, phosphate, phosphonato, phosphinato, amino, acylamino, including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido, amidino, nitro, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, alkylsulfinyl, sulfonate, sulfamoyl, sulfonamido, trifluoromethyl, cyano, azido, cycloalkyl such as e.g. cyclopentyl or cyclohexyl, heterocycloalkyl such as e.g. morpholino, piperazinyl or piperidinyl, alkylaryl, arylalkyl and heteroaryi. Preferred substituents of such organic residues are, for example, halogens, such as fluorine, chlorine, bromine or iodine, amino groups, hydroxyl groups, carbonyl groups, thiol groups and carboxyl groups.

The term "alkylaryl" as used in the context of any linking moiety described in the present invention is denoted to mean a linking moiety having the structure -alkyl-aryl-, thus being linked on one side via the alkyl group and on the other side via the aryl group, wherein this term is meant to also encompass linking moieties such as -alkyl-aryl-alkyl- linking moieties. The term "alkylaryl group", when used in the context of any substituent described hereinunder and above, is denoted to mean a residue being linked via the alkyl portion, said alkyl portion being further substituted with an aryl moiety.

The term "arylalkyl" as used in the context of any linking moiety described in the present invention is denoted to mean a linking moiety having the structure -aryl-alkyl-, thus being linked on one side via the aryl group and on the other side via the alkyl group, wherein this

term is meant to also encompass linking moieties such as -aryl-alkyl-aryl- linking moieties. The term "arylalkyl group", when used in the context of any substituent described hereinunder and above, is denoted to mean a residue being linked via the aryl portion, said aryl portion being further substituted with an alkyl moiety.

The term "alkylheteroaryl" as used in the context of any linking moiety described in the present invention is denoted to mean a linking moiety having the structure -alkyl-heteroaryl-, thus being linked on one side via the alkyl group and on the other side via the heteroaryl group, wherein this term is meant to also encompass linking moieties such as -alkyl-heteroaryl-alkyl- linking moieties. The term "alkylheteroaryl group", when used in the context of any substituent described hereinunder and above, is denoted to mean a residue being linked via the alkyl portion, said alkyl portion being further substituted with a heteroaryl moiety.

The term "heteroarylalkyl" as used in the context of any linking moiety described in the present invention is denoted to mean a linking moiety having the structure - heteroaryl-alkyl-, thus being linked on one side via the heteroaryl group and on the other side via the alkyl group, wherein this term is meant to also encompass linking moieties such as -heteroaryl-alkyl-heteroaryl- linking moieties. The term "heteroarylalkyl group", when used in the context of any substituent described hereinunder and above, is denoted to mean a residue being linked via the heteroaryl portion, said heteroaryl portion being further substituted with an alkyl moiety.

According to a preferred embodiment of the present invention, the hydroxyalkyl starch conjugate comprises an electron-withdrawing group in close proximity to the functional group F^3. The term "electron-withdrawing group" is recognized in the art, and denotes the tendency of a functional group to attract valence electrons from neighboring atoms by means of a difference in elecronegativity with respect to the neighboring atom (inductive effect) or by withdrawal of π-electrons via conjugation (mesomeric effect)

Preferably, the electron-withdrawing group is present in alpha, beta or gamma position to the functional group F^3, more preferably in alpha or beta position. It was surprisingly found that conjugates comprising such linkages between the hydroxyalkyl starch and the cytotoxic agent show advantageous properties when used in mammals.

Without wanting to be bound to any theory, it is believed that a reason for the advantageous properties which are provided by the presence of these electron-withdrawing groups in close proximity to the functional group F^3 may be an advantageous influence on the release rate of the cytotoxic agent comprised in the conjugate in the plasma of a

mammal. The term "advantageous influence on the release rate" as used herein shall describe an influence allowing for a release rate which generates suitable amounts of the cytotoxic agent in a suitable time period so that therapeutic levels of the cytotoxic agent are delivered prior to excretion of the conjugate or conjugate fragments through the kidney or inactivation of the cytotoxic agent comprised in the conjugate by alternative mechanisms in the body. The term "suitable amounts" as used in this context of the present invention shall describe an amount with which the desired therapeutic effect of the cytotoxic agent is achieved, preferably together with a toxicity of the cytotoxic agent as low as possible. Without wanting to be bound to any theory, it is believed that the higher the tendency of the electron-withdrawing group to attract valence electrons, the faster the cytotoxic agent is released *in vivo*. Thus, it is assumed that the release rates can, inter alia, be tailored to specific needs by choosing a suitable electron-withdrawing group in alpha, beta or gamma position relative to the functional group F^3.

Therefore, the present invention also relates to a conjugate, as described above, comprising an electron-withdrawing group in alpha, beta or gamma position, preferably in alpha or beta position, to each functional group F^3. Further, the present invention also relates to a conjugate comprising an electron-withdrawing group in alpha, beta or gamma position, preferably in alpha or beta position, to each functional group F^3, obtained or obtainable by the method as described above.

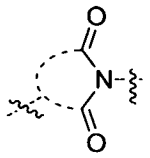
The electron-withdrawing group may be either part of the linking moiety L' or, according to an alternative embodiment, may be present in the hydroxyalkyl starch derivative, provided that the electron-withdrawing group is present in close proximity to the functional group F^3, as described above. The term "present in close proximity to", as used in the context of the present invention, is preferably denoted to mean a group which is present in alpha, beta, or gamma position to the functional group F^3.

Preferably, the electron-withdrawing group is a moiety selected from the group consisting of -O-, -S-, -SO-, **-SO2-,** $-NR^e-$, $-C(=Y^e)-$, $-NR^e-C(=Y^e)-$, $-C(=Y^e)-NR^e-$, $-NO_2$ comprising groups, -CN comprising groups, aryl groups, heteroaryl groups, cyclic imide groups and at least partially fluorinated alkyl moieties, wherein Y^e is either O, S or NR^e, and wherein R^e is one of hydrogen, alkyl, aryl, arylalkyl, heteroalkyl, alkylaryl, alkylheteroaryl or heteroarylalkyl group, and the like.

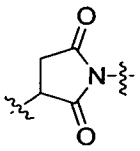
Within the meaning of the present invention, the term "at least partially fluorinated alkyl moiety" refers to, optionally substituted, alkyl groups, such as non-branched alkyl residues, branched alkyl residues, cycloalkyl residues, as well as residues comprising one or more heteroatoms or functional groups, such as, by way of example, -O-, -S-, -NH-, -NH-

C(=0), -C(=0)-NH, and the like, having at least one of the hydrogen atoms replaced with a fluorine atom. In some fluorinated alkyl groups, all the hydrogen atoms are replaced with fluorine atoms, i.e., the fluorinated alkyl group is a perfluoroalkyl group. The following groups are mentioned, by way of example: $-CH_2F$, $-CF_3$, $-CHF_2$, $-CF_2-$, -CHF-, $-CH_2-CF_3$, $-CH_2-CHF_2$ and $-CH_2-CH_2F$.

Within the context of the present invention, the term "cyclic imide groups" is denoted to mean a cyclic structural unit according to the general formula:



wherein the ring structure is preferably a 5-membered ring, 6-membered ring or 7-membered ring. Most preferably the cyclic imide is a -succinimide- having the following structure



Preferably the electron-withdrawing group is selected from the group consisting of -NH-C(=0)-, -C(=0)-NH-, -NH-, -0-, -S-, -SO-, $-SO_2-$ and -succinimide-. More preferably the electron-withdrawing group is selected from the group consisting of -C(=0)-NH-, -NH-, -0-, -S-, $-SO_2-$ and -succinimide-. According to a further embodiment, the electron-withdrawing group is selected from the group consisting of -NH-C(=0)-, -C(=0)-NH-, -NH-, -O- and -S-.

Thus, according to one preferred embodiment of the invention, the present invention also relates to a conjugate, as described above, as well as a conjugate obtained or obtainable by the above-described method, wherein the conjugate comprises an electron-withdrawing group, preferably in alpha or beta position to each functional group F^3, more particular in alpha position to each functional group F^3, wherein the electron-withdrawing group is a group selected from the group consisting of -NH-C(=0)-, -C(=0)-NH-, -NH-, -0-, -S-, -SO-, $-SO_2-$ and -succinimide-.

The nature of the electron withdrawing group has shown to influence the drug release kinetics and thus as well the activity / toxicity profile of the particular conjugates. For certain preferred linker compounds incorporated in conjugates according to the invention,

it was clearly shown by way of stability measurements in aqueous buffer (borate pH 8, 40°C) that the use of an electron-withdrawing group, has a significant influence on the release rates.

Surprisingly, in particular the groups -S- and -O- in alpha position or the group -C(=0)-NH- in alpha position or the groups -NH-C(=0)-, -C(=0)-NH- or -succinimde- in beta position allow for an advantageous influence on the release rate of the cytotoxic agent. Further, electron-withdrawing groups in beta position, allow for a minimization of non-specific toxicity, that is toxicity not directed towards a tumor (see respective Figure 6).

Accordingly, the present invention also relates to a conjugate as described above, as well as a conjugate obtained or obtainable by the above mentioned method, wherein the conjugate comprises

(i) an electron withdrawing group selected from the group consisting of -S- and -O- in alpha position to each F^3 group, or

(ii) an electron-withdrawing group selected from the group consisting of -C(=0)-NH— , -NH-C(=0)- and succinimide in beta position to each F^3 group, or

(iii) the group -C(=0)-NH in alpha position as electron-withdrawing group.

According to a particularly preferred embodiment of the present invention, the linking moiety L' has a structure according to the following formula $-[F^2]_q-[L^2]_g-[E]_e-[CR^mR^n]_f-$, wherein E is an electron-withdrawing group, L^2 is a linking moiety, F^2 is a functional group, f is 1, 2 or 3, g is 0 or 1, q is 0 or 1, e is 0 or 1, and wherein R^m and R^n are, independently of each other, H or alkyl.

Thus, the conjugate, described above, has preferably the formula

$$HAS'(-[F^2]_q-[L^2]_g-[E]_e-[CR^mR^n]_f-F^3-M)_n.$$

According to the first preferred embodiment of the invention, an electron-withdrawing group E is present in linking moiety L'. In this case, integer e is 1.

Preferably E, if present, is selected from the group consisting of -0-, -S-, -SO-, $-SO_2-$, $-NR^e-$, $-C(=Y^e)-$, $-NR^e-C(=Y^e)-$, $-C(=Y^e)-NR^e-$, $-CH(NO_2)-$, -CH(CN)-, aryl groups, heteroaryl groups, cyclic imide groups and at least partially fluorinated alkyl moieties, more preferably of the group consisting of -C(=0)-NH-, -NH-(C=0)-, -0-, -S-, -SO-, -

SO_2- and -succinimide-, more preferably **E**, if present, is selected from the group consisting of-NH-C(=O)-, -C(=O)-NH-, -succinimide-, -O- and -S-.

Accordingly, the following conjugate structures are thus particularly preferred: HAS'(-$[F^2]_q$-$[L^2]$g-C(=O)-NH-$[CR^mR^n]rF^3$-M)$_n$, HAS'(-$[F^2]_q$-$[L^2]_g$-NH-C(=O)-$[CR^mR^n]_f$-F^3-M)$_n$, HAS'(-$[F^2]_q$-$[L^2]_g$-0-$[CR^mR^n]rF^3$-M)$_n$, HAS'(-$[F^2]_q$-$[L^2]_g$-S-$[CR^mR^n]_f$-F^3-M)$_n$, HAS'(-$[F^2]_q$-$[L^2]_g$-succinimide-$[CR^mR^n]rF^3$-M)$_n$. More preferably, the electron-withdrawing group **E** is selected from the group consisting of -NH-C(=O)-, -C(=O)-NH-, -succinimide-, -O- and -S- and the functional group F^3 is a -C(=Y)- group, the hydroxyalkyl starch conjugate thus having preferably a structure selected from the group consisting of HAS'(-$[F^2]_q$-$[L^2]_g$-C(=O)-NH-$[CR^mR^n]rC(=Y)$-M)$_n$, HAS'(-$[F^2]_q$-$[L^2]_g$-NH-(C=O)-$[CR^mR^n]rC(=Y)$-M)$_n$, HAS'(-$[F^2]_q$-$[L^2]_g$-0-$[CR^mR^n]rC(=Y)$-M)$_n$, HAS'(-$[F^2]_q$-$[L^2]_g$-S-$[CR^mR^n]rC(=Y)$-M)$_n$, HAS'(-$[F^2]_q$-$[L^2]_g$-succinimide-$[CR^mR^n]rC(=Y)$-M)$_n$, wherein Y is preferably selected from O or S, in particular wherein Y is O.

According to an alternative preferred embodiment the functional group F^2 is an electron-withdrawing group present in close proximity to the functional group F^3. In this case, F^2 may for example be a group such as a -C(=0)-NH-, -NH- or -succinimide- group. In case F^2 is an electron-withdrawing group present in close proximity to the functional group F^3, that is in alpha, beta or gamma position to the functional group F^3, F^2 may be present instead of **E** or in addition to **E**.

According to this embodiment, the following conjugate structures are thus particularly preferred: HAS'(-C(=0)-NH-$[L^2]_g$-$[E]_e$-$[CR^mR^n]_f$-F^3-M)$_n$, HAS'(-NH-$[L^2]_g$-$[E]_e$-$[CR^mR^n]_f$-F^3-M)$_n$, HAS'(-S-$[L^2]_g$-$[E]_e$-$[CR^mR^n]_f$-F^3-M)$_n$ and HAS'(-succinimide-$[L^2]_g$-$[E]_e$-$[CR^mR^n]_f$-F^3-M)$_n$, more preferably a structure selected from the group consisting of HAS'(-C(=0)-NH-$[L^2]_g$-$[E]_e$-$[CR^mR^n]rC(=Y)$-M)$_n$, HAS'(-NH-$[L^2]_g$-$[E]_e$-$[CR^mR^n]rC(=Y)$-M)$_n$ HAS'(-S-$[L^2]_g$-$[E]_e$-$[CR^mR^n]_f$-C(=Y)-M)$_n$, HAS'(-0-$[L^2]_g$-$[E]_e$-$[CR^mR^n]_f$-C(=Y)-M)$_n$ and HAS'(-succinimide-$[L^2]_g$-$[E]_e$-$[CR^mR^n]rC(=Y)$-M)$_n$, wherein Y is preferably selected from O or S, in particular wherein Y is O.

According to an alternative embodiment, the electron-withdrawing group, if present in the linking moiety L', may also be present in the linking moiety L^2.

Further, the electron-withdrawing group, if present, may also be present in the structural unit $[CR^mR^n]_f$. It is recalled that integer f of the structural unit $[CR^mR^n]_f$, is preferably in the range of from 1 to 3 and R^m and R^n are, independently of each other, H or alkyl. Since the term "alkyl" as used in the context of the present invention also encompasses alkyl groups which are further substituted, the electron withdrawing group may also be present

in at least one of R^m or R^n, such as, e.g. in the form of a $-CH_2F$, $-CHF_2$ or $-CF_3$ group or the like.

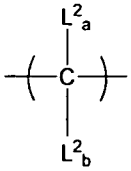
According to a further preferred embodiment of the present invention, the electron-withdrawing group, if present, is not present in the linking moiety L' but is instead part of the hydroxyalkyl starch derivative (F£AS'). In this case e is 0 and the integers q, g and fare chosen so that the electron-withdrawing group is preferably present in the hydroxyalkyl starch derivative in a position being in close proximity to the functional group F^3, as described above, preferably in alpha or beta position to the functional group F^3.

Linking moiety L^2

In general, there are no particular restrictions as to the chemical nature of the linking moiety L^2. The term "linking moiety L^2" as used in the context of the present application, relates to any suitable chemical moiety bridging F^2 and E, in case q and e are 1, or bridging F^2 and the structural unit $[CR^mR^n]_f$ in case q is 1, e is 0 and f is 1, 2 or 3, or bridging E and the hydroxyalkyl starch derivative in case q is 0 and e is 1. Thus L^2 may be an alkyl group, aryl group, heteroaryl group, alkyl aryl group, arylalkyl group and the like. The respective residues may comprise one or more substituents as described above.

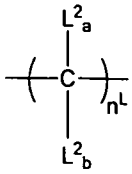
Preferably, L^2 is an alkyl group comprising 1 to 20, preferably 1 to 10, more preferably 1 to 8, more preferably 1 to 6, such as 1, 2, 3, 4, 5 or 6, more preferably 1 to 4, more preferably from 1 to 3, and most preferably from 2 to 3 carbon atoms. According to the definition of the term "alkyl", the above mentioned alkyl groups may be substituted.

In particular, L^2 comprises at least one structural unit according to the following formula

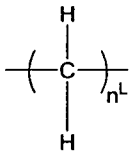


wherein L^2_a and L^2_b are independently of each other H or an organic residue selected from the group consisting of alkyl, alkenyl, alkylaryl, arylalkyl, aryl, heteroaryl, alkylheteroaryl, heteroarylalkyl, hydroxyl and halogen (such as fluorine, chlorine, bromine, or iodine).

More preferably, L^2 has a structure according to the following formula



with L^2_a and L^2_b being selected from the group consisting of H, methyl or hydroxyl, with n^L being preferably in the range of from 1 to 8, more preferably of from 1 to 6, more preferably of from 1 to 4, more preferably of from 1 to 3, and most preferably of from 2 to 3. According to an even more preferred embodiment, the spacer L^2 consists of the structural unit according to the following formula



wherein integer n^L is in the range of from 1 to 8, more preferably of from 1 to 6, more preferably of from 1 to 4, more preferably of from 1 to 3, and most preferably of from 2 to 3. Therefore, according to a preferred embodiment of the present invention, L^2 has a structure selected from the group consisting of -$\mathbf{CH_2}$-CH_2-CH_2-CH_2-CH_2-CH_2-, -CH_2-CH_2-CH_2-CH_2-CH_2-, -CH_2-CH_2-CH_2-CH_2-, -CH_2-CH_2-CH_2-, -CH_2-CH_2-, -CH_2-, more preferably L^2 is selected from the group consisting of -CH_2-, -$\mathbf{CH_2}$-$\mathbf{CH_2}$-, -CH_2-CH_2-CH_2-.

According to one preferred embodiment of the present invention, the present invention also relates to a conjugate, as described above, as well as a conjugate obtained or obtainable by the above-mentioned method, wherein the conjugate has a structure selected from the group consisting of the following formulas: HAS'(-[F^2]$_q$-[CH_2]$_g$-[E]$_e$-[CR^mR^n]$_f$-F^3-M)$_n$, HAS'(-[F^2]$_q$-[CH_2-CH_2]$_g$-[E]$_e$-[CR^mR^n]$_f$-F^3-M)$_n$, HAS'(-[F^2]$_q$-[CH_2-CH_2-CH_2]$_g$-[E]$_e$-[CR^mR^n]$_f$-F^3-M)$_n$, HAS'(-[F^2]$_q$-[CH_2-CH_2-CH_2-CH_2]$_g$-[E]$_e$-[CR^mR^n]$_f$-F^3-M)$_n$, HAS'(-[F^2]$_q$-[CH_2-CH_2-CH_2-CH_2-CH_2-CH_2]$_g$-[E]$_e$-[CR^mR^n]$_f$-F^3-M)$_n$, more preferably the conjugate is selected from the following structures: HAS'(-[F^2]$_q$-[CH_2]$_g$-[E]$_e$-[CR^mR^n]$_f$-F^3-M)$_n$, HAS'(-[F^2]$_q$-[CH_2-CH_2]$_g$-[E]$_e$-[CR^mR^n]$_f$-F^3-M)$_n$ and HAS'(-[F^2]$_q$-[CH_2-CH_2]$_g$-[E]$_e$-[CR^mR^n]$_f$-F^3-M)$_n$, more preferably from the group consisting of HAS'(-[F^2]$_q$-CH_2-[E]$_e$-[CR^mR^n]$_f$-F^3-M)$_n$, HAS'(-[F^2]$_q$-CH_2-CH_2-[E]$_e$-[CR^mR^n]$_f$-F^3-M)$_n$ and HAS'(-[F^2]$_q$-CH_2-CH_2-CH_2-[E]$_e$-[CR^mR^n]$_f$-F^3-M)$_n$.

In case g is 1, the following most preferred combinations of group L^2 with the functional unit $[E]_e$ with e = 1 are mentioned, by way of example: HAS'(-$[F^2]_q$-CH_2-C(=0)-NH-$[CR^mR^n]rF^3$-M)$_n$, HAS'(-$[F^2]_q$-CH_2-CH_2-C(=0)-NH-$[CR^mR^n]_f$-F^3-M)$_n$ and HAS'(-$[F^2]_q$-CH_2-CH_2-CH_2-C(=0)-NH-$[CR^mR^n]_f$-F^3-M)$_n$, HAS'(-$[F^2]_q$-CH_2-NH-C(=0)-$[CR^mR^n]_f$-F^3-M)$_n$, HAS'(-$[F^2]_q$-CH_2-CH_2-NH-C(=0)-$[CR^mR^n]rF^3$-M)$_n$ and HAS'(-$[F^2]_q$-CH_2-CH_2-CH_2-NH-C(=0)-$[CR^mR^n]rF^3$-M)$_n$, HAS'(-$[F^2]_q$-CH_2-S-$[CR^mR^n]_f$-F^3-M)$_n$, HAS'(-$[F^2]_q$-CH_2-CH_2-S-$[CR^mR^n]_f$-F^3-M)$_n$, HAS'(-$[F^2]_q$-CH_2-CH_2-CH_2-S-$[CR^mR^n]_f$-F^3-M)$_n$, HAS'(-$[F^2]_q$-CH_2-0-$[CR^mR^n]rF^3$-M)$_n$, HAS'(-$[F^2]_q$-CH_2-CH_2-0-$[CR^mR^n]_f$-F^3-M)$_n$, HAS'(-$[F^2]_q$-CH_2-CH_2-CH_2-0-$[CR^mR^n]_f$-F^3-M)$_n$, HAS'(-$[F^2]_q$-CH_2-succinimide-$[CR^mR^n]_f$-F^3-M)$_n$, HAS'(-$[F^2]_q$-CH_2-CH_2-succinimide-$[CR^mR^n]r$-F^3-M)$_n$, FIAS'(-$[F^2]_q$-CH_2-CH_2-CH_2-succinimide-$[CR^mR^n]_f$-F^3-M)$_n$.

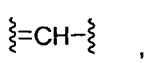
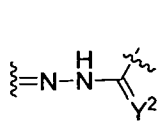
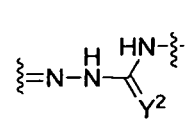
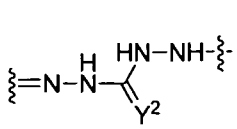
According to one preferred embodiment, L^2 is present, i.e. integer g is 1, and L^2 is -CH_2-, -CH_2-CH_2- or -CH_2-CH_2-CH_2-.

The functional group F^2

The functional group F^2 is, if present, a functional group linking the hydroxyalkyl starch derivative with the linking moiety L^2, in case g is 1, or with the electron-withdrawing group E in case g is 0 and e is 1, or with the structural unit $[CR^mR^n]f$, in case g and e are 0.

There are, in general, no particular restrictions as regards the chemical nature of the functional group F^2 provided that a stable bond is formed linking the hydroxyalkyl starch derivative with L^2, E or the structural unit $[CR^mR^n]f$, respectively. As described above, the functional group F^2 may serve as electron-withdrawing group in close proximity to the functional group F^3 to provide an optimized hydrolysis rate of the linkage between F^3 and the cytotoxic agent.

Preferably, F^2 is a group consisting of -Y^1-, -C(=Y^2)-, -C(=Y^2)-NR^{F2}-,

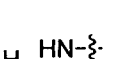
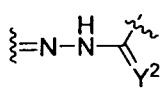
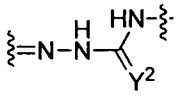
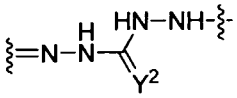





and -CH_2-CH_2-C(=Y^2)-NR^{F2}-,

wherein Y^1 is selected from the group consisting of -S-, -0-, -NH-, -NH-NH-, -CH_2-CH_2-S0$_2$-NR^{F2}-, -CH_2-CHOH-, and cyclic imides, such as succinimide, and wherein Y^2 is selected from the group consisting of NH, S and O, and wherein R^{F2} is selected from

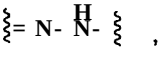
the group consisting of hydrogen, alkyl, alkylaryl, arylalkyl, aryl, heteroaryl, alkylheteroaryl or heteroarylalkyl group.

More preferably, F^2 is a group consisting of $-Y^1-$, $-C(=Y^2)-$, $-C(=Y^2)-NR^{F2}-$,

and $-CH_2-CH_2-C(=Y^2)-NR^{F2}-$.

Preferably, F^2 is selected from the group consisting of -S-, -NH-NH-,

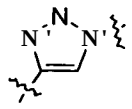
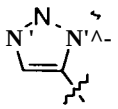


and -succinimide-, more preferably F^2 is -succinimide- or -S-, most preferably -succinimide-.

The functional group F^2 is suitably chosen depending on the functional group -X- being present in the hydroxyalkyl starch derivative.

According to one preferred embodiment of the invention, the present invention thus also relates to the conjugate as described above, wherein in the structural unit $[F^2]_q$, q is 1 and F^2 is -S- or -succinimide-, the conjugate having a structure HAS'(-succinimide-$[L^2]_g$-$[E]_e$-$[CR^mR^n]_f$-F^3-$M)_n$ or HAS'(-S-$[L^2]_g$-$[E]_e$-$[CR^mR^n]_f$-F^3-$M)_n$, more preferably HAS'(-succinimide-$[L^2]_g$-$[E]_e$-$[CR^mR^n]_f$-F^3-$M)_n$.

Furthermore, the functional group F^2 may form together with a functional group of the hydroxyalkyl starch a 1,2,3-triazole ring. In the event that the functional F^2 forms together with a functional group of the hydroxyalkyl starch derivative a 1,2,3-triazole, inter alia, the following structures are conceivable for this structural building block:

In case the conjugate comprises a triazole linking group, preferably the functional group F^2 forms together with the functional group X present in the residue of the hydroxyalkyl starch derivative a 1,2,3-triazole. Preferably such a triazole group is formed via a 1,3-dipolar cycloaddition between an azide and a terminal or internal alkynyl group to give a

1,2,3-triazole. For example in case Z^1 is an alkynyl group or azide and the linking moiety L bears a functional group K^2 being the respective azide or alkynyl, a triazole linkage may be formed when linking L to the hydroxyalkyl starch derivative.

The structural unit $[CR^mR^n]_f$

As regards the structural unit $[CR^mR^n]_f$, integer f is preferably in the range of from 1 to 3 and R^m and R" are, independently of each other, H, alkyl or aryl, more preferably H or alkyl. In case integer f is greater than 1, each repeating unit $[CR^mR^n]$ may be the same or may be different from each other.

As described above, the term "alkyl" relates to non-branched alkyl residues, branched alkyl residues, cycloalkyl residues, as well as residues comprising one or more heteroatoms or functional groups, such as, by way of example, -0-, -S-, -NH-, -NH-C(=0), -C(=0)-NH, and the like. These residues may be further substituted by one or more suitable substituents. Preferably, R^m and R" are, independently of each other, H or an unsubstituted alkyl group.

Preferably, R^m and R" are, independently of each other, selected from H or branched or linear alkyl chains, comprising 1 to 10, preferably 1 to 8, more preferably 1 to 5, most preferably 1 to 3 carbon atoms. More preferably R^m and R^n are, independently of each other, selected from the group consisting of H, methyl, ethyl, propyl, butyl, sec-butyl and tert-butyl, more preferably R^m and R^n are, independently of each other, H or methyl.

By way of example, the following preferred structures for the structural unit $[CR^mR^n]_f$ are mentioned: $-CH_2-CH_2-CH_2-$, $-CH_2-CH_2-$, $-CH_2-$, $-CH(CH_3)-$, $-C(CH_3)_2-$, $-CH(CH_2CH_3)-$, $-CH(CH(CH_3)_2)-$, $-CH(CH_3)-CH_2-$, $-CH_2-CH(CH_3)-$, $-CH(CH_3)-CH_2-CH_2-$, $-CH_2-CH(CH_3)-CH_2-$, $-CH_2-CH_2-CH(CH_3)-$, $-CH(CH_3)-CH(CH_3)-$, $CH(CH_3)-CH(CH_3)-CH_2-$, $-CH_2-CH(CH_3)-CH(CH_3)-$, $-CH(CH_3)-CH_2-CH(CH_3)$.

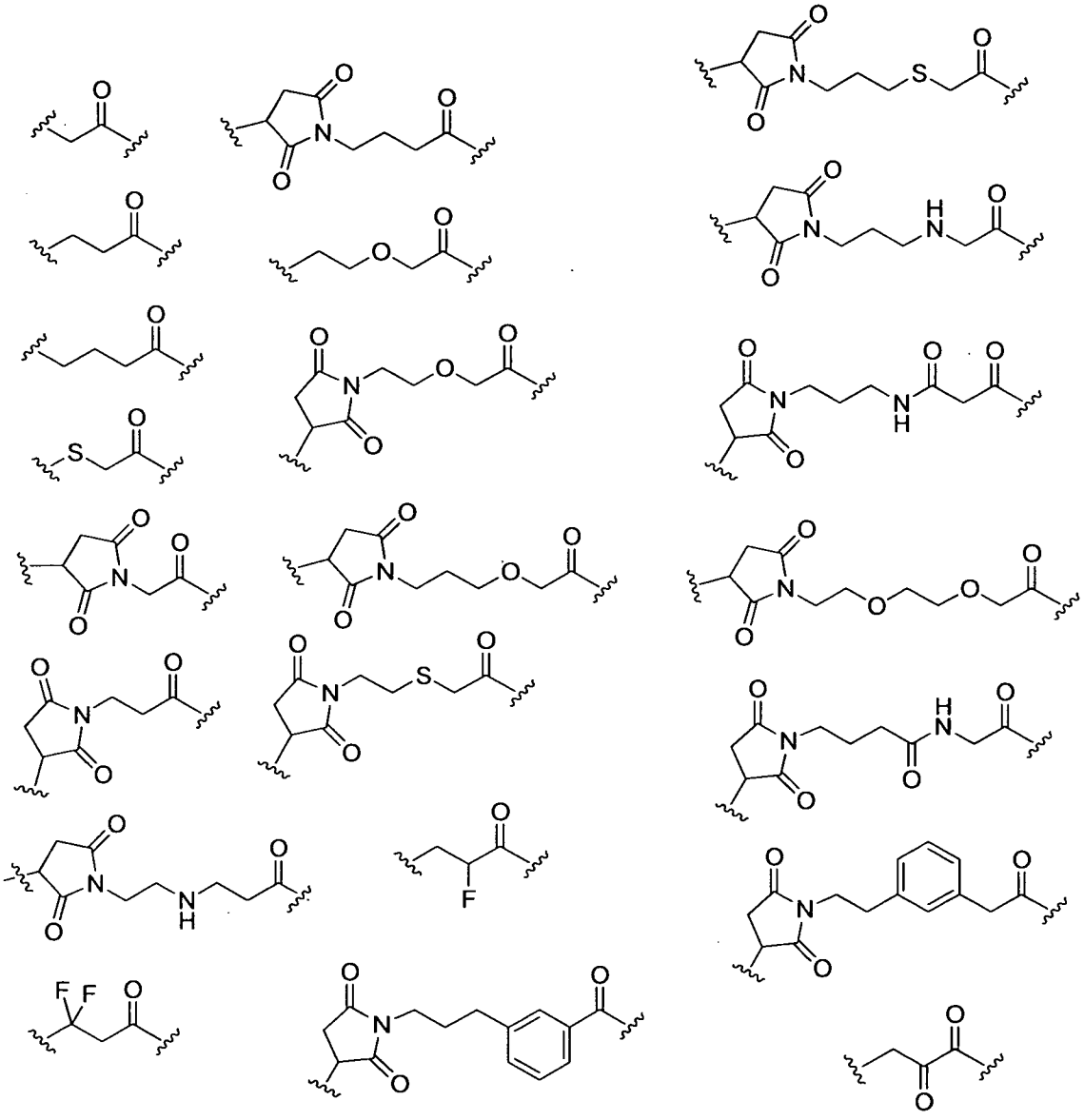
According to one particularly preferred embodiment of the present invention, R^m and R" are both H. The structural unit $[CR^mR^n]_f$ is thus preferably $-CH_2-CH_2-CH_2-$, $-CH_2-CH_2-$ or $-CH_2-$, more preferably f is 1 or 2, more preferably the structural unit $[CR^mR^n]_f$ has the structure $-CH_2-CH_2-$ or $-CH_2-$.

Thus, the present invention also relates to the conjugate as described above, the conjugate having a structure selected from the group consisting of $HAS'(-[F^2]_q-[L^2]_g-[E]_e-CH_2-CH_2-CH_2-F^3-M)_n$, $HAS'(-[F^2]_q-[L^2]_g-[E]_e-CH_2-CH_2-F^3-M)_n$ and $HAS'(-[F^2]_q-[L^2]_g-[E]_e-CH_2-F^3-$

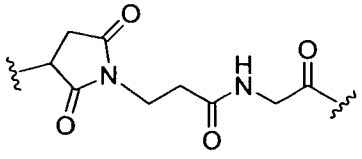
$M)_n$, more preferably HAS'(-$[F^2]_q$-$[L^2]_g$-[E]$_e$-CH_2-CH_2-F^3-$M)_n$ and HAS'(-$[F^2]_q$-$[L^2]_g$-$[E]_e$-CH_2-F^3-$M)_n$.

Examples of preferred linking moieties L

By way of example, the following preferred linking moieties L are mentioned:

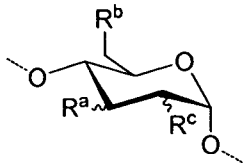


A further preferred linker is



The Residue of the Hydroxyalkyl Starch Derivative comprised in the Conjugate

In accordance with the above-mentioned definition of HAS, the residue of the hydroxyalkyl starch derivative preferably comprises at least one structural unit according to the following formula (I)

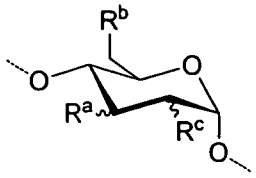


(I)

wherein at least one of R^a, R^b or R^c comprises the functional group -X- and wherein R^a, R^b and R^c are, independently of each other, selected from the group consisting of -O-HAS"; -[0-(CR^{w}R^{x}HCR^{y}R^z)]$_x$-OH, -[0-<CR^{w}R^x)-(CR^{y}R^z)]y-X-, -[0-(CR^{w}R^x)-(CR^{y}R^z)]$_y$-[F^1]$_p$-L'-X-, wherein R^w, R^x, R^y and R^z are independently of each other selected from the group consisting of hydrogen and alkyl, y is an integer in the range of from 0 to 20, preferably in the range of from 0 to 4, F^1 is a functional group, p is 0 or 1, L^1 is a linking moiety and -X- is a functional group linking the hydroxyalkyl starch derivative and the linking moiety L. Preferably X is formed upon reaction of Z^1 with the crosslinking compound L. HAS" is a remainder of the hydroxyalkyl starch derivative, as described above.

The amount of functional groups X present in the residue of the hydroxyalkyl starch derivative being incorporated into the conjugate of the invention corresponds to the amount of functional groups Z^1 present in the corresponding hydroxyalkyl starch derivative prior to the conjugation of said derivative to the crosslinking compound L or the structural unit -L-M. Thus, preferably 0.3 % to 3 % of all residues R^a, R^b and R^c present in the hydroxyalkyl starch derivative contain the functional group X. More preferably, 0.3 % to 3 % of all residues -R^a, -R^b and -R^c present in the hydroxyalkyl starch derivative have the structure -[0-(CR^{w}R^{x}MCR^{y}R^z)]$_y$-X- or -[0-<CR^{w}R^{x}HCR^{y}R^z)]$_y$-[F^1]$_p$-L^1-X-. According to a particularly preferred embodiment, -R^a, -R^b and -R^c are selected from the group consisting of-O-HAS", -[0-(CR^{w}R^{x}HCR^{y}R^z)]$_x$-OH and -[0-(CR^{w}R^{x}HCR^{y}R^z)]$_y$-X-, wherein 0.3 % to 3 % of all residues -R^a, -R^b and -R^c present in the hydroxyalkyl starch derivative have the structure -[0-(CR^{w}R^{x}HCR^{y}R^z)]$_y$-X-. According to an alternative preferred embodiment, -R^a, -R^b and -R° are selected from the group consisting of-O-HAS", -[O-(CR^{w}R^{x}HCR^{y}R^z)]$_x$-OH and -[0-(CR^{w}R^x)-(CR^{y}R^z)]$_y$-[F^1]$_p$-L^1-X-, wherein 0.3 % to 3 % of all residues -R^a, -R^b and -R^c present in the hydroxyalkyl starch derivative have the structure - [O-(CR^{w}R^x)-(CR^{y}R^z)]$_y$-[F^1]$_p$-L^1-X-.

Preferably, the present invention also describes a conjugate, comprising a residue of a hydroxyalkyl starch derivative, as described above, as well as a conjugate obtained or obtainable by the above-mentioned method, wherein the conjugate comprises a residue of a hydroxyethyl starch derivative and a cytotoxic agent, the residue of HES derivative preferably comprises at least one structural unit, according to the following formula (I)



(I)

wherein R^a, R^b and R^c are independently of each other selected from the group consisting of -O-HAS", -[0-CH_2-CH_2]s-OH, -[0-CH_2-CH_2]$_t$-X- and ***-[0-CH_2-CH_2]r-[F^1]$_p$-L^1-X-,*** wherein t is in the range of from 0 to 4, and wherein s is in the range of from 0 to 4, p being 0 or 1, and wherein at least one of R^a, R^b and R^c comprises the functional group X, and wherein -X- is linked to the linking moiety L-M comprised in the conjugate of the invention.

According to a preferred embodiment of the present invention, this linkage between –X- and L is obtained by coupling a hydroxyalkyl starch derivative being functionalized with at least one functional group Z^1, as described above, with the crosslinking compound L comprising the functional group K^2 or a derivative of a cytotoxic agent -L-M comprising the functional group K^2, thereby obtaining a covalent linkage between HAS' and L, wherein, as result, the residue of the hydroxyalkyl starch is linked via the functional group -X- to the linking moiety L. Further preferred embodiments as to this method are described below.

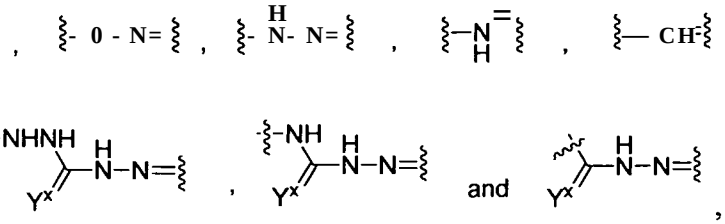
Preferably all functional groups -X- present in a given hydroxyalkyl starch derivative comprised in a conjugate according to the invention, are linked to the linking moiety L, most preferably to the structural unit -L-M.

The Functional Group X

X is a functional group linking the hydroxyalkyl starch derivative with the linking moiety L, wherein L is preferably -L'-F^3-, and wherein more preferably L' is -[F^2]$_q$-[L^2]$_g$-[E]$_e$-[CR^mR^n]r. Thus, -X- is a linking group preferably linking the hydroxyalkyl starch derivative with the functional group F^2 in case q is 1, or with the linking moiety L^2 in case

q is 0 and g is 1, or with the electron-withdrawing group E in case q and g are 0 and e is 1, or with the structural unit -[CR^mR^n]$_f$- in case q, g, e are 0 and f is 1, 2 or 3.

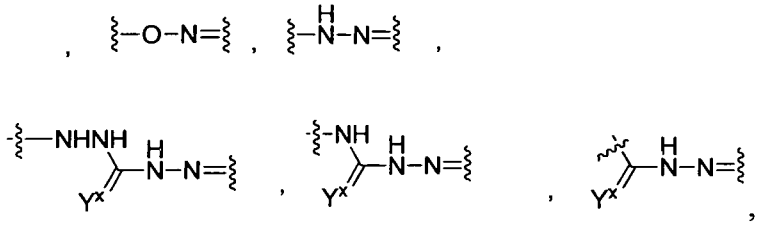
In general, there exists no limitation regarding the functional group -X- provided that the functional group -X- is able to link the hydroxyalkyl starch derivative with the linking moiety L. According to a preferred embodiment of the present invention, and depending on the respective group of the linking moiety L being linked to -X-, -X- is selected from the group consisting of -Y**-, -C(=Y^x)-, -C(=Y^x)-NR^{xx}-, -CH_2-CH_2-C(=Y^x)-NR^{xx}-,



wherein Y^{xx} is selected from the group consisting of -S-, -0-, -NH-, -NH-NH-, -CH_2-CH_2-$S0_2$-NR^{xx}-, and cyclic imides, such as -succinimide-, and wherein Y^x is selected from the group consisting of NH, S and O, and wherein R^{x*} is selected from the group consisting of hydrogen, alkyl, alkylaryl, arylalkyl, aryl, heteroaryl, alkylheteroaryl or heteroarylalkyl group.

Furthermore, the functional group X may form together with a functional group of the linking moiety L, such as with the functional group F^2 a 1,2,3-triazole ring, as described hereinabove.

More preferably -X- is selected from the group consisting of –Y^{xx}-, -C(=Y^x)-, -C(=Y^x)-NR^{xx}–,

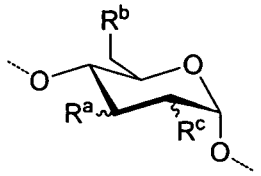


and -CH_2-CH_2-C(=Y^x)-NR^{xx}-.

Most preferably -X- is selected from the group consisting of -0-, - S–, –NH– and –NH–NH-, more preferably -0-, -S- or -NH-. Most preferably -X- is -S-.

Therefore, the present invention also describes a conjugate, comprising a residue of a hydroxyalkyl starch derivative, as described above, as well as a conjugate obtained or

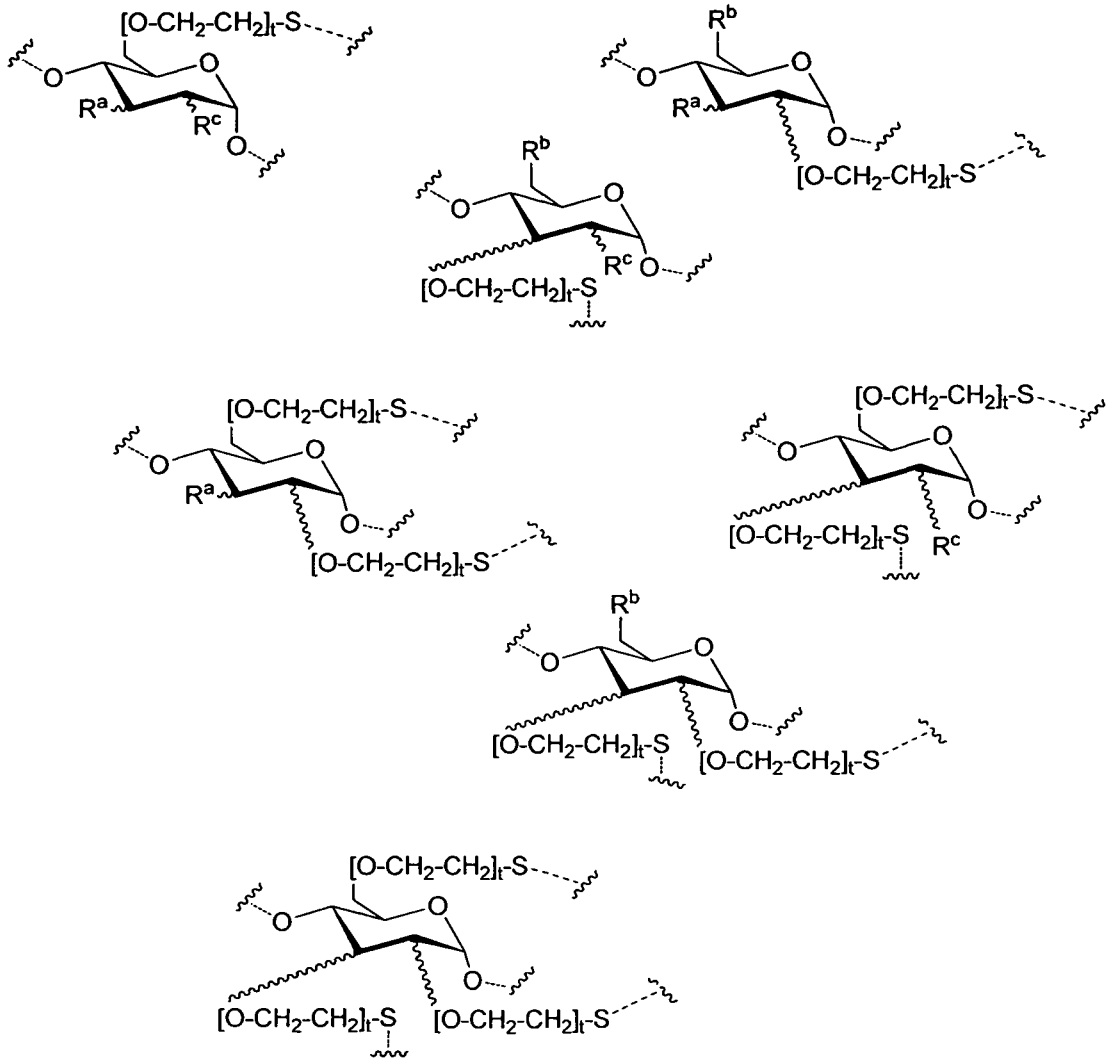
obtainable by the above-mentioned method, wherein the conjugate comprises a residue of a hydroxyalkyi starch derivative and a cytotoxic agent, the residue of the hydroxyalkyi starch derivative preferably comprises at least one structural unit according to the following formula (I)



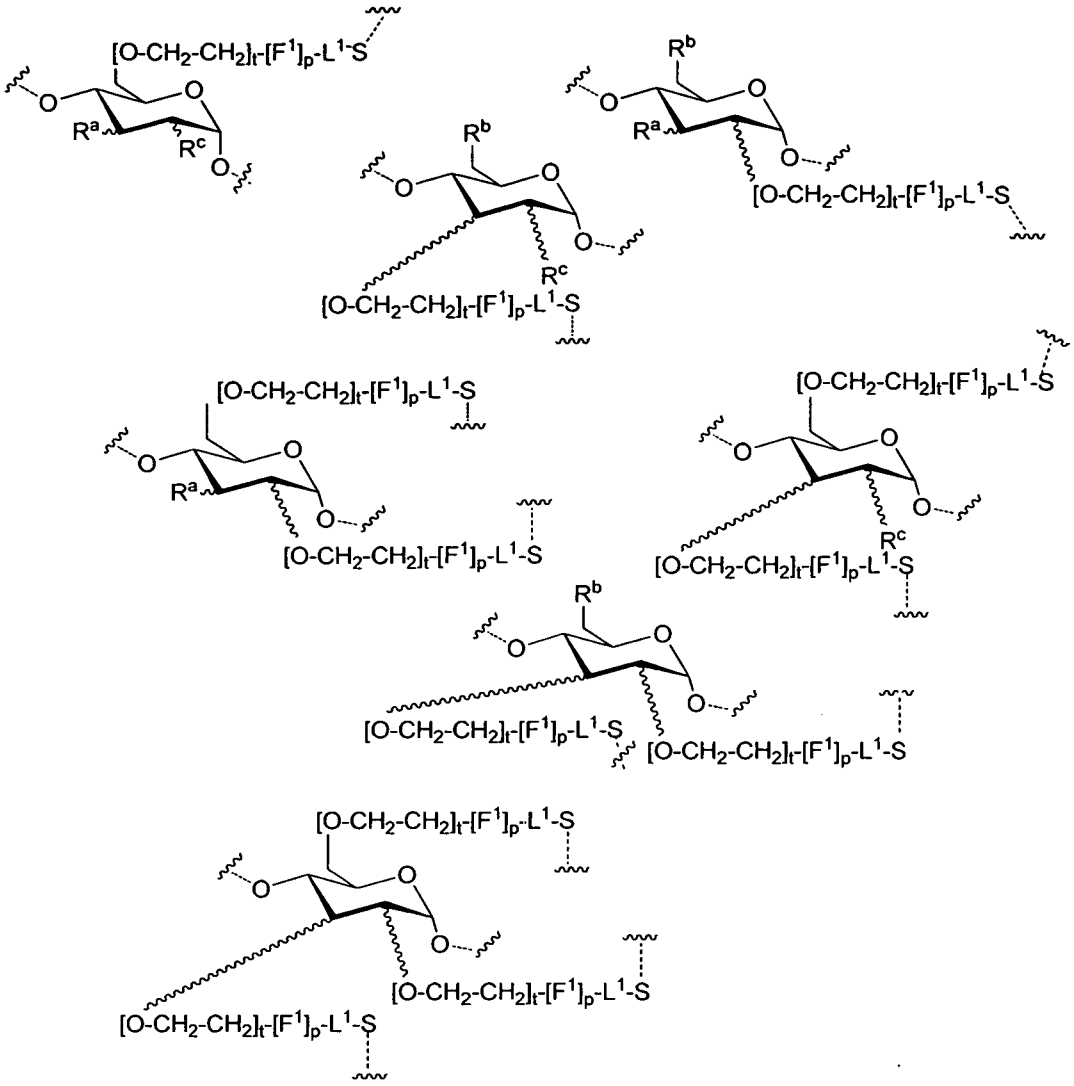
(I)

wherein at least one of R^a, R^b and R^c is -[0-(CR ^{w}R^x)-(CR^{y}R^z)]$_y$-S- $_{or}$ _[0-(CR ^{w}R^x)-(CR^{y}R^z)]$_y$-[F^1]$_p$-L^1-S- , preferably wherein at least one of R^a, R^b and R^c is -[0-CH $_2$-CH $_2$]$_t$-S- or –[O–CH$_2$–CH$_2$]$_t$-[F^1]$_p$-L^1-S-.

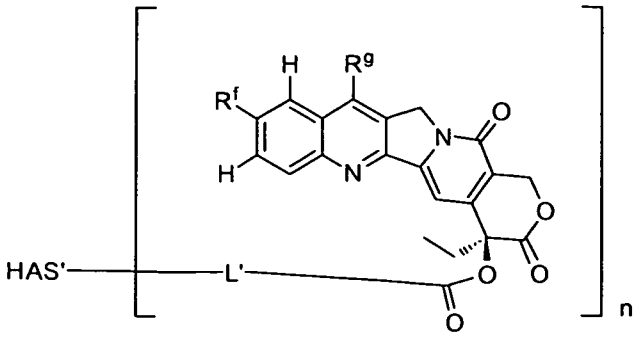
According to one preferred embodiment of the present invention, at least one ofR^a, R^b and R^c is -[0-CH $_2$-CH $_2$]$_t$-S-. Thus, the following hydroxyalkyi starch derivatives may be mentioned as preferred embodiments of the invention:



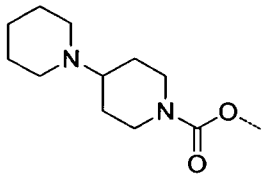
According to another preferred embodiment of the present invention, at least one of R^a, R^b and R^c is $-[O-CH_2-CH_2]_t-[F^1]_p-L^1-S-$. Thus, the following hydroxyalkyl starch derivatives may be mentioned as preferred embodiments of the invention:



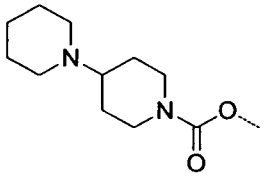
According to a preferred embodiment of the invention, the linking moiety L is directly linked to the functional group -X- of the hydroxyalkyl starch derivative and, on the other side, directly linked to a tertiary hydroxyl group of the cytotoxic agent with F^3 being -C(=0)-. According to a more preferred embodiment the conjugate of the invention, the linking moiety L is -L'-C(=0)- and the conjugate has a structure according to the following formula:



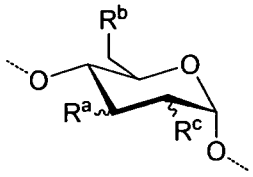
wherein - R^f is selected from the group consisting of -OH, siloxy groups, ester groups and groups having the structure



and wherein $-R^g$ is $-CH_2-CH_3$ and wherein $-R^f$ is preferably -OH or



most preferably -OH, and wherein the hydroxyalkyi starch comprises at least one structural unit according to the following formula (I)



(I)

and wherein at least one of R^a, R^b and R^c is -[0-(CR ^{w}R^x)-(CR^{y}R^z)]$_y$-S- or -[0-(CR ^{w}R^x)-(CR^{y}R^z)]$_y$-[F^1]$_p$-L'-S-, preferably wherein at least one of R^a, R^b and R^c is -[0-CH $_2$-CH $_2$]$_t$-S- or -[0 -**CH2-CH2**]r[F ^{1}]$_p$-L^1-S- and wherein L' is linked to the functional group -S-.

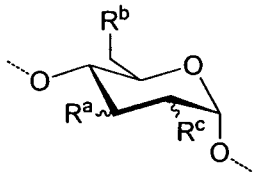
The Functional Group F^1

F^1 is a functional group, which, if present, is preferably selected from the group consisting of -Y^7-, -Y^7-C(=Y^6)-, -C(=Y^6)-, -Y^7-C(=Y^6)-Y^8-, -C(=Y^6)-Y^8-, wherein -Y^7- is selected from the group consisting of -NR^{Y7}-, -0-, -S-, -NH-NH-, -NH-0-, -CH=N-0-, -0-N=CH-,

-CH=N-, -N=CH- and cyclic imides, such as -succinimide-, $-Y^8-$ is selected from the group consisting of $-NR^{Y8}-$, -S-, -0-, -NH-NH- and Y^6 is selected from the group consisting of NR^{Y6}, O and S, wherein R^{Y6} is H or alkyl, preferably H, and wherein R^{Y7} is H or alkyl, preferably H, and wherein R^{Y8} is H or alkyl, preferably H.

According to a preferred embodiment of the present invention the functional group F^1 is, if present, selected from the group consisting of -NH-, -0-, -S-, -NH-C(=0)-, -0 -C(=0)-NH-, -NH-C(=S)-, -O-C(O)-, -C(=0)-, -NH-C(=0)-NH-, -NH-NH-C(=0)-, -C(=0)-NH-NH-, -NH-C(=0)-NH-NH-, more preferably F^1 is, if present, -O- or -0 -C(=0)-NH-.

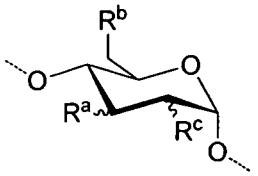
Therefore, the present invention also describes a conjugate, comprising a hydroxyalkyl starch derivative, as described above, as well as a conjugate obtained or obtainable by the above-mentioned method, the hydroxyalkyl starch derivative preferably comprising at least one structural unit according to the following formula (I)



(I)

wherein at least one of R^A, R^B and R^c is -[0-{CR ^{w}R^{x})-(CR ^{y}R^{z})]y-X- or -[0-(CR ^{w}R^{x})–(CR^{y}R^{z})]y -fFVL'-X-, preferably wherein at least one of R^A, R^b and R^C is -[0-CH $_2$-CH $_2$]$_t$-X- or –[O–CH_2–CH_2]$_t$-[F^1]$_p$-L^1-X-, more preferably wherein at least one of R^A, R^b and R^c is - [0 –CH_2–CH_2]$_t$-S- or –[O–CH_2–CH_2]$_t$-[F^1]$_p$-L^1-S-, wherein F^1, if present, is preferably -O- or -0-C(=0)-NH-.

Thus, the following preferred conjugates are described, comprising a hydroxyalkyl starch derivative, as described above, wherein the hydroxyalkyl starch derivative comprises at least one structural unit according to the following formula (I)



(I)

wherein in each unit, independently of each other unit, at least one ofR^a, R^b and R^c is

(i) -[0-CH $_2$-CH$_2$]rX- or

(ii) –[O–CH$_2$-CH$_2$]$_t$-[F^1]$_p$-L^1-X-, preferably with p being 1 and F^1 being -0-, or

(iii) -[0 -CH $_2$-CH$_2$]r[F ^{1}]$_p$-L^1-X-, preferably with p being 1 and F^1 being -0-C(=0)-NH-,

wherein -X- is -S-, and wherein t is in the range of from 0 to 4, and wherein the linking moiety L of the structural unit -L-M is directly linked to at least one functional group X, preferably wherein all groups X present in the hydroxyalkyl starch derivative are linked to the structural unit -L-M, and wherein the linking moiety L is being attached to the group -O- of M derived from the tertiary hydroxyl group of the cytotoxic agent.

The Linking Moiety L^1

The term "linking moiety L^1" as used in this context of the present invention relates to any suitable chemical moiety bridging -X- with the functional group F^1 or the building block -[0-(CR ^{w}R^x)-{CR^{y}R^z)]y- or the sugar backbone of the hydroxyalkyl starch derivative.

In general, there are no particular restrictions as to the chemical nature of the spacer L^1 with the proviso that L^1 provides for a stable linkage between the functional group -X- and the hydroxyalkyl starch building block. Preferably, L^1 is an alkyl, alkenyl, alkylaryl, arylalkyl, aryl, heteroaryl, alkylheteroaryl or heteroarylalkyl group. As described above, the terms alkenyl alkylaryl, arylalkyl, aryl, heteroaryl, alkylheteroaryl or heteroarylalkyl group also encompass groups which are substituted by one or more suitable substituent.

According to a preferred embodiment of the present invention, the linking moiety L^1 is a spacer comprising at least one structural unit according to the following formula -{[CR ^{d}R^f]$_h$-[F^4]$_U$-[CR^{dd}R^{ff}]$_z$}$_{alpha}$-, wherein F^4 is a functional group, preferably selected from the group consisting of -S-, -O- and -NH-, preferably wherein F^4 is -O- or -S-, more preferably wherein F^4 is -S-. The integer h is preferably in the range of from 1 to 20, more preferably of from 1 to 10, such as 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, more preferably of from 1 to 5, most preferably of from 1 to 3. Integer z is in the range of from 0 to 20, more preferably of from 0 to 10, such as 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, more preferably of from 0 to 3, most preferably of from 0 to 2, such as 0, 1 or 2. Integer u is 0 or 1. Integer alpha is in the range of from 1 to 10, preferably of from 1 to 5, such as 1, 2, 3, 4, 5, more preferably 1 or 2. As regards residues R^d, R^f, R^{dd} and R^{ff}, these residues are, independently of each other, preferably selected from the group consisting of halogens, alkyl groups, H or hydroxyl groups. The repeating units of -[CR^{d}R^f]$_h$- may be the same or may be different.

Likewise, the repeating units of -[$CR^{dd}R^{ff}$]$_z$- may be the same or may be different. Likewise in case integer alpha is greater than 1, the groups F^4 in each repeating unit may be the same or may be different. Further, in case alpha is greater than 1, integer h in each repeating unit may be the same or may be different, integer z in each repeating unit may be the same or may be different and integer u in each repeating unit may be the same or may be different. Thus, in case alpha is greater than 1, each repeating unit of [CR^dR^f]h-[F^4]$_u$-[$CR^{dd}R^{ff}$]$_z$ may be the same or may be different. Most preferably, R^d, R^f, R^{dd} and R^f are independently of each other H, alkyl or hydroxyl.

According to one embodiment of the present invention, u and z are 0, the linking moiety L^1 thus corresponds to the structural unit -[CR^dR^f]$_h$-.

According to an alternative embodiment of the present invention u is 1. According to this embodiment z is preferably greater than 0, preferably 1 or 2.

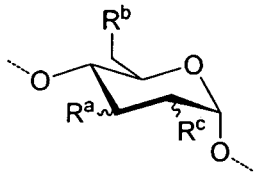
Thus, the following preferred structures for the linking moiety L^1 are mentioned by way of example: -{[CR^dR^f]$_h$-F^4-[$CR^{dd}R^{ff}$]$_z$}$_{alpha}$- and -[CR^dR^f]$_h$-.

Thus, by way of the example, the following linking moieties L^1 are mentioned:
-CH_2-,
-CH_2-CH_2-,
-CH_2-CH_2-CH_2-,
-CH_2-CH_2-CH_2-CH_2-,
-CH_2-CH_2-CH_2-CH_2-CH_2-,
-CH_2-CH_2-CH_2-S-CH_2-CH_2-,
-CH_2-CH_2-S-CH_2-CH_2-,
-CH_2-CH_2-0-CH_2-CH_2-,
-CH_2-CH_2-0-CH_2-CH_2-0-CH_2-CH_2-,
-CH_2-CHOH-CH_2-,
-CH_2-CHOH-CH_2-S-CH_2-CH_2-,
-CH_2-CHOH-CH_2-S-CH_2-CH_2-CH_2-,
-CH_2-CHOH-CH_2-NH-CH_2-CH_2-,
-CH_2-CHOH-CH_2-NH-CH_2-CH_2-CH_2- ,
-CH_2-CHOH-CH_2-0-CH_2-CHOH-CH_2-,
-CH_2-CHOH-CH_2-0-CH_2-CHOH-CH_2-S-CH_2-CH_2-,
-CH_2-CH(CH_2OH)- and
-CH_2-CH(CH_2OH)-S-CH_2-CH_2-.

According to one preferred embodiment, R^d, R^f and, if present, R^{dd} and R^{ff} are preferably H or hydroxyl, more preferably at least one of R^d and R^f of at least one repeating unit of $-[CR^dR^f]_h-$ is -OH, wherein even more preferably, in this case, both R^{dd} and R^f are H, if present. In particluar, in this case, L^1 is selected from the group consisting of $-CH_2-CHOH-CH_2-$, $-CH_2-CHOH-CH_2-S-CH_2-CH_2-$, $-CH_2-CHOH-CH_2-S-CH_2-CH_2-CH_2-$, $-CH_2-CHOH-CH_2-NH-CH_2-CH_2-$ and $-CH_2-CHOH-CH_2-NH-CH_2-CH_2-CH_2-$, more preferably from the group consisting of $-CH_2-CHOH-CH_2-$, $-CH_2-CHOH-CH_2-S-CH_2-CH_2-$ and $-CH_2-CHOH-CH_2-S-CH_2-CH_2-CH_2-$.

According to an alternative preferred embodiment, both residues R^d and R^f are H, and R^{dd} and R^{ff} are, if present, H as well. In particular, in this case, L^1 is selected from the group consisting of: $-CH_2-$, $-CH_2-CH_2-$, $-CH_2-CH_2-CH_2-$, $-CH_2-CH_2-CH_2-CH_2-$, $-CH_2-CH_2-CH_2-CH_2-CH_2-$, $-CH_2-CH_2-CH_2-S-CH_2-CH_2-$, $-CH_2-CH_2-S-CH_2-CH_2-$, $-CH_2-CH_2-CH_2-O-CH_2-CH_2-$ and $-CH_2-CH_2-O-CH_2-CH_2-$.

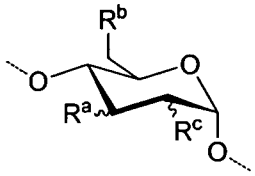
Therefore, the present invention also describes a hydroxyalkyl starch derivative, and a hydroxyalkyl starch derivative obtained or obtainable by the above-described method, the hydroxyalkyl starch derivative comprising at least one structural unit according to the following formula (I)



(I)

wherein at least one of R^a, R^b and R^c has a structure according to the following formula $-[O-CH_2-CH_2]_t-[F^1]_p-L^1-X-$, wherein L^1 is selected from the group consisting of $-CH_2-$, $-CH_2-CH_2-$, $-CH_2-CH_2-CH_2-$, $-CH_2-CH_2-CH_2-CH_2-$, $-CH_2-CH_2-CH_2-CH_2-CH_2-$, $-CH_2-CH_2-CH_2-S-CH_2-CH_2-$, $-CH_2-CH_2-S-CH_2-CH_2-$, $-CH_2-CH_2-O-CH_2-CH_2-$, $-CH_2-CH_2-O-CH_2-CH_2-O-CH_2-CH_2-$, $-CH_2-CHOH-CH_2-$, $-CH_2-CHOH-CH_2-S-CH_2-CH_2-$, $-CH_2-CHOH-CH_2-S-CH_2-CH_2-CH_2-$, $-CH_2-CHOH-CH_2-NH-CH_2-CH_2-$, $-CH_2-CHOH-CH_2-NH-CH_2-CH_2-CH_2-$, $-CH_2-CHOH-CH_2-O-CH_2-CHOH-CH_2-$, $-CH_2-CHOH-CH_2-O-CH_2-CHOH-CH_2-S-CH_2-CH_2-$, $-CH_2-CH(CH_2OH)-$ and $-CH_2-CH(CH_2OH)-S-CH_2-CH_2-$, more preferably from the group consisting of $-CH_2-CHOH-CH_2-$, $-CH_2-CHOH-CH_2-S-CH_2-CH_2-$, $-CH_2-CHOH-CH_2-S-CH_2-CH_2-CH_2-$, $-CH_2-CHOH-CH_2-NH-CH_2-CH_2-$ and $-CH_2-CHOH-CH_2-NH-CH_2-CH_2-CH_2-$, more preferably from the group consisting of $-CH_2-CHOH-CH_2-$, $-CH_2-CHOH-CH_2-S-CH_2-CH_2-$ and $-CH_2-CHOH-CH_2-S-CH_2-CH_2-CH_2-$.

Further, the present invention also relates to a conjugate, comprising a hydroxyalkyl starch derivative, as described above, as well as a conjugate obtained or obtainable by the above-mentioned method, wherein the conjugate comprises a hydroxyalkyl starch derivative and a cytotoxic agent, the hydroxyalkyl starch derivative preferably comprises at least one structural unit according to the following formula (I)



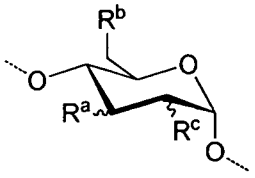
(I)

wherein at least one of R^a, R^b and R^c has a structure according to the following formula $-[O-CH_2-CH_2]_t-[F^1]_p-L^1-X-$, wherein L^1 is selected from the group consisting of $-CH_2-$, $-CH_2-CH_2-$, $-CH_2-CH_2-CH_2-$, $-CH_2-CH_2-CH_2-CH_2-$, $-CH_2-CH_2-CH_2-CH_2-CH_2-$, $-CH_2-CH_2-CH_2-S-CH_2-CH_2-$, $-CH_2-CH_2-S-CH_2-CH_2-$, $-CH_2-CH_2-0-CH_2-CH_2-$, $-CH_2-CH_2-0-CH_2-CH_2-0-CH_2-CH_2-$, $-CH_2-CHOH-CH_2-$, $-CH_2-CHOH-CH_2-S-CH_2-CH_2-$, $-CH_2-CHOH-CH_2-S-CH_2-CH_2-CH_2-$, $-CH_2-CHOH-CH_2-NH-CH_2-CH_2-$, $-CH_2-CHOH-CH_2-NH-CH_2-CH_2-CH_2-$, $-CH_2-CHOH-CH_2-0-CH_2-CHOH-CH_2-$, $-CH_2-CHOH-CH_2-0-CH_2-CHOH-CH_2-S-CH_2-CH_2-$, $-CH_2-CH(CH_2OH)-$ and $-CH_2-CH(CH_2OH)-S-CH_2-CH_2-$, more preferably from the group consisting of $-CH_2-CHOH-CH_2-$, $-CH_2-CHOH-CH_2-S-CH_2-CH_2-$, $-CH_2-CHOH-CH_2-S-CH_2-CH_2-CH_2-$, $-CH_2-CHOH-CH_2-NH-CH_2-CH_2-$ and $-CH_2-CHOH-CH_2-NH-CH_2-CH_2-CH_2-$, more preferably from the group consisting of $-CH_2-CHOH-CH_2-$, $-CH_2-CHOH-CH_2-S-CH_2-CH_2-$ and $-CH_2-CHOH-CH_2-S-CH_2-CH_2-CH_2-$.

Especially preferred conjugates according to the present invention

In the following, conjugate structures are mentioned, which comprise a particularly preferred combination of HAS' and different structural units -L-M.

According to a first especially preferred embodiment of the present invention, a residue of hydroxyalkyl starch derivative comprising at least one structural unit according to the following formula (I)



(I)

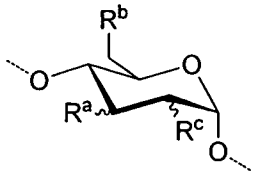
wherein in each unit, independently of each other unit, at least one of R^a, R^b and R^c is -[O-CH_2-CH_2]$_t$-X- and -X- is -S-. This hydroxyalkyl starch derivative is according to this preferred embodiment of the invention, combined with the structural unit -L-M having the structure -[F^2]$_q$-[L^2]$_g$-[E]$_e$-[CR^mR^n]$_f$-F^3-M wherein q is 0, g is 0 and e is 0.

Accordingly, in this preferred embodiment, the functional group -X- represents an electron-withdrawing group in close proximity to the functional group F^3, and -X- is directly linked to the structural unit -[CR^mR"]r- Depending on integer f, which is 1, 2 or 3, the electron-withdrawing group is either present in alpha, beta or gamma position to the functional group F^3.

Accordingly, the present invention also relates to a conjugate, comprising a hydroxyalkyl starch derivative, as described above, as well as a conjugate obtained or obtainable by the above-mentioned method, wherein the conjugate comprises a hydroxyalkyl starch derivative and a cytotoxic agent, the conjugate having a structure according to the following formula

$$HAS'(-[F^2]_q-[L^2]_g-[E]_e-[CR^mR^n]_f-F^3-M)_n$$

wherein q is 0, g is 0, e is 0, and wherein HAS' preferably comprises at least one structural unit according to the following formula (I)



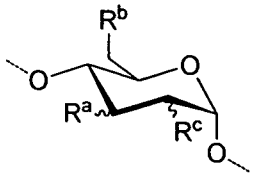
(1)

wherein at least one of R^a, R^b and R^c is -[0-CH_2-CH_2],-X- and -X- is -S- and the functional group -X- is directly linked to the -[CR^mR^n]r group.

Integer f is preferably 1, so that -X- is present in alpha position to the functional group F^3. Accordingly, the present invention also relates to a conjugate, comprising a hydroxyalkyl starch derivative, as described above, as well as a conjugate obtained or obtainable by the above-mentioned method, wherein the conjugate comprises a hydroxyalkyl starch derivative and a cytotoxic agent, the conjugate having a structure according to the following formula

$$HAS'(-[F^2]_q-[L^2]_g-[E]_e-[CR^mR^n]_f-F^3-M)_n$$

wherein q is 0, g is 0, e is 0, wherein HAS' preferably comprises at least one structural unit according to formula (1)



(I)

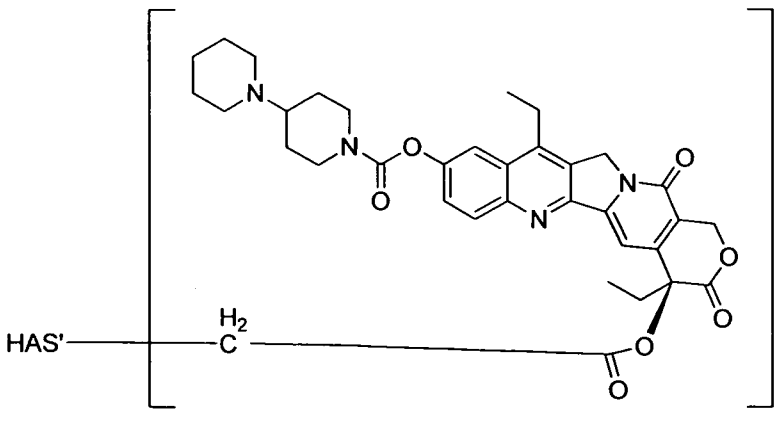
wherein in each unit, independently of each other unit, at least one of R^a, R^b and R^c is -[0-CH_2-CH_2]t-X- and -X- is -S- and the functional group -X- is directly linked to the -[CR^mR^n]r group, and wherein the hydroxyalkyl starch derivative comprises at least n functional groups X, and wherein f is 1. R^m and R^n are, independently of each other, H or alkyl. Most preferably R^m and R^n are H.

Thus, according to this embodiment, the conjugate, or the conjugate obtained or obtainable by the above-mentioned method, preferably has a structure according to the following formula

$$HAS'(-CH_2-F^3-M)_n$$

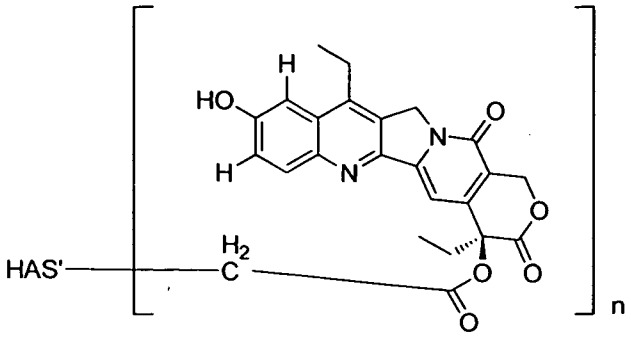
Particularly preferably F^3 in the above mentioned formula is -C(=0)-, as described above.

Most preferably the cytotoxic agent is camptothecin or a camptothecin analogue, as described above, in particular SN-38 or Irinotecan. The present invention thus also relates to a conjugate, comprising a hydroxyalkyl starch derivative, as described above, as well as a conjugate obtained or obtainable by the above-mentioned method, the conjugate having a structure according to the following formula

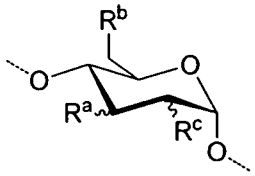


n

or the following formula



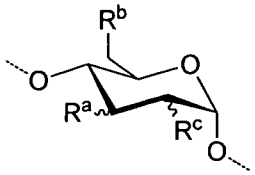
and wherein HAS' comprises at least one structural unit according to the following formula (I)



(I)

wherein in each unit, independently of each other unit, at least one of R^a, R^b and R^c is -[O-CH_2-CH_2]$_t$-X- and -X- is -S- and the functional group -X- is directly linked to the -CH_2-C(=0)-group, as shown in the formulas above.

According to a second especially preferred embodiment of the present invention, the hydroxyalkyl starch conjugate comprises a hydroxyalkyl starch derivative comprising at least one structural unit according to the following formula (I)



(1)

wherein at least one of R^a, R^b and R^c is -[0-CH_2-CH_2]$_t$-X- and -X- is -S-, thus at least one of R^a, R^b and R^c is -[0-CH_2-CH_2]$_t$-S-, and wherein the conjugate further comprises the moiety -L-M, wherein -L-M has the structure (-[F^2]$_q$-[L^2]$_g$-[E]$_e$-[CR^mR^n]$_r$$F^3$-M)$_n$, as described above, and wherein e is 1 and E is preferably -S- or -0-.

According to this embodiment, -X- is directly linked to the functional group F^2 with q and g preferably both being 1. As described above, the functional group F^2 is, if present, preferably selected from -S- and -succinimide-, preferably -succinimide-.

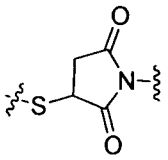
Thus, according to this embodiment, the conjugate, or the conjugate obtained or obtainable by the above-mentioned method, has in particular a structure according to the following formulas

$$HAS'(\text{-succinimide-}L^2\text{-}0\text{-}[CR^mR^n]_f\text{-}F^3\text{-}M)_n$$

or

$$rLAS'(\text{-succinimide-}L^2\text{-}S\text{-}[CR^mR^n]_f\text{-}F^3\text{-}M)_n$$

wherein HAS' comprises at least one structural unit according to formula (I), wherein in each unit, independently of each other unit, at least one of R^a, R^b and R^c is -[0-CH_2-CH_2]$_t$-X- and -X- is -S- and wherein the succinimide is directly linked to X, thereby forming a



bond.

Particularly preferably F^3 in the above mentioned formula is -C(=0)-.

As regards, the linking moiety L^2 according to this preferred embodiment, L^2 is preferably an alkyl group, as described above. More preferably L^2 is selected from the group consisting of -CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-, -CH_2-CH_2-CH_2-CH_2-CH_2-, -CH_2-CH_2-CH_2-CH_2-, -CH_2-CH_2-CH_2-, -CH_2-CH_2-, -CH_2-, more preferably L^2 is selected from the group consisting of -CH_2-, -CH_2-CH_2-, -CH_2-CH_2-CH_2-, most preferably L^2 is -CH_2-CH_2-.

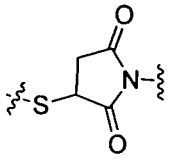
Accordingly, the present invention also relates to a conjugate, comprising a residue of a hydroxyalkyl starch derivative, as described above, as well as a conjugate obtained or obtainable by the above-mentioned method, wherein the conjugate comprises a hydroxyalkyl starch derivative and a cytotoxic agent, the conjugate having a structure according to the following formula

$$HAS'(\text{-succinimide-}CH_2\text{-}CH_2\text{-}E\text{-}[CR^mR^n]_rC(=0)\text{-}M)_n$$

more preferably a structure according to one of the following formulas

HAS'(-succinimide-CH_2-CH_2-0-[CR^mR^n]rC(=0)-M)n
and
HAS'(-succinimide-CH_2-CH_2-S-[CR^mR^n]f-C(=0)-M)n

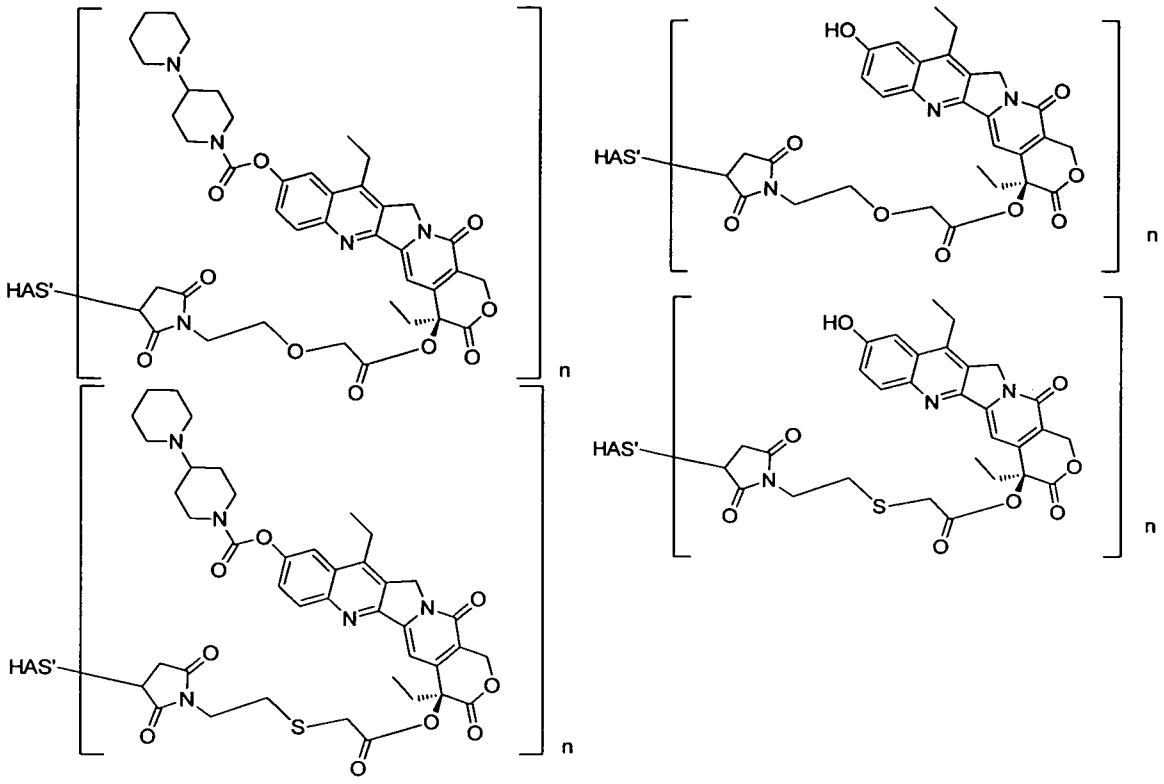
wherein HAS' preferably comprises at least one structural unit according to formula (I), wherein at least one of R^a, R^b and R^c is -[0-CH_2-CH_2],-X- and -X- is -S- and wherein the functional group -X- is directly linked to the succinimide group, thereby forming a



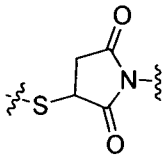
bond and wherein most preferably all functional groups -X- present in a given hydroxyalkyl starch derivative comprised in a conjugate according to the invention, are directly linked to the succinimide group.

Most preferably, according to this embodiment, R^m and R^n are both H and f is 1.

The present invention thus also relates to a conjugate, comprising a hydroxyalkyl starch derivative, as described above, as well as a conjugate obtained or obtainable by the above-mentioned method, the conjugate having a structure according to one of the following formulas:

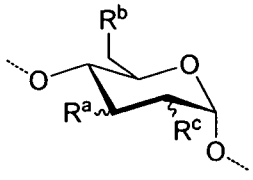


wherein HAS' comprises at least one structural unit according to the following formula (I). wherein at least one of R^a, R^b and R^c is -[0-CH $_2$-CH_2]$_t$-X- and -X- is -S-, thereby forming a



bond.

According to a third especially preferred embodiment of the present invention, the hydroxyalkyl starch conjugate comprises a hydroxyalkyl starch derivative comprising at least one structural unit according to the following formula (I)



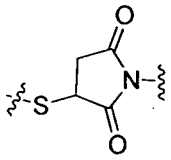
(I)

wherein at least one of R^a, R^b and R^c is -[0-CH_2-CH_2]$_t$-X- and -X- is -S-, thus at least one of R^a, R^b and R^c is -[0-CH_2-CH_2]$_t$-S-, and wherein the conjugate further comprises the moiety -L-M, wherein -L-M has the structure (-[F^2]$_q$-[L^2]$_g$-[E]$_e$-[CR^mR^n]$_r$$F^3$-M)$_n$, as described above, and wherein e is 1. In this case, E is preferably -NH-C(=0)-, -C(=0)-NH- or -succinimide-, preferably -succinimide-. In case E is a -succinimide, -X- is preferably directly linked to the functional group E with q and g thus preferably both being 0.

Thus, according to this embodiment, the conjugate, or the conjugate obtained or obtainable by the above-mentioned method, has in particular a structure according to the following formula

HAS'(-succinimide-[CR^mR^n]$_f$-F^3-M)n

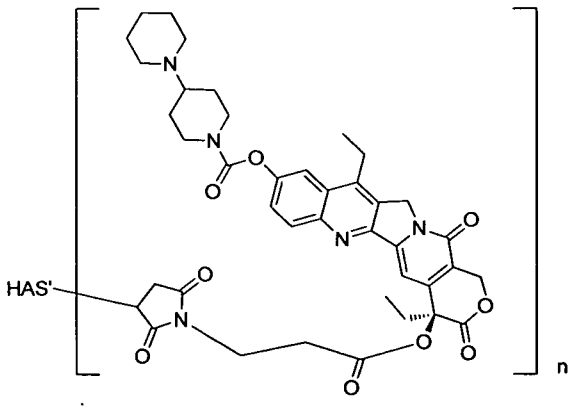
wherein HAS' comprises at least one structural unit according to formula (I), and wherein at least one of R^a, R^b and R^c is -[0-CH_2-CH_2]$_t$-X- and -X- is -S- and wherein the succinimide is directly linked to X, thereby forming a

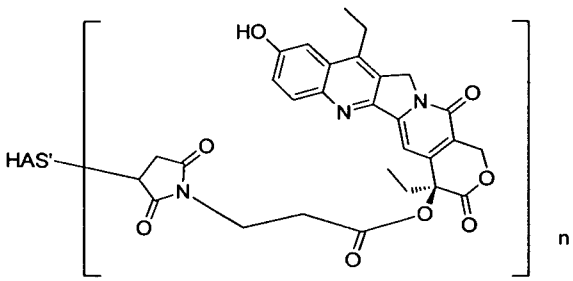


bond.

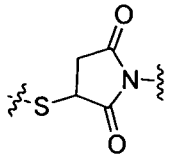
Particularly preferably F^3 in the above mentioned formula is -C(=0)-. Most preferably, according to this embodiment, R^m and R^n are both H and f is 2.

The present invention thus also relates to a conjugate, comprising a hydroxyalkyl starch derivative, as described above, as well as a conjugate obtained or obtainable by the above-mentioned method, the conjugate having a structure according to one of the following formulas:



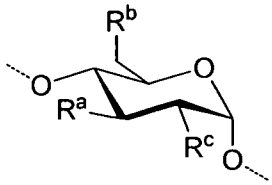


wherein HAS' comprises at least one structural unit according to formula (I), and wherein at least one of R^a, R^b and R^c is -[0-CH_2-CH_2],-X- and -X- is -S-, thereby forming a



bond.

According to a fourth especially preferred embodiment of the present invention, the hydroxyalkyl starch conjugate comprises a residue of a hydroxyalkyl starch derivative which comprises at least one structural unit according to the following formula (lb)



(Ib)

wherein at least one of R^a, R^b and R^c is $-[O-CH_2-CH_2]_t-[F^1]_p-L^1-X-$ with -X- being -S-, preferably with p being 1 and F^1 being -0-, thus at least one of R^a, R^b and R^c has preferably the structure -[0-CH_2-CH_2]$_t$-0-L^1-S-, and wherein t is in the range of from 0 to 4, and wherein L^1 is a group, as described above, preferably an alkyl group. Most preferably the linking moiety L^1 is a spacer comprising at least one structural unit according to the formula -{[CR^dR^f]$_h$-[F^4]$_u$-[$CR^{dd}R^{ff}$]$_z$}$_{alpha}$-, as described above, wherein F^4, if present, is preferably selected from the group consisting of -S-, -O- and -NH-, more preferably wherein F^4, if present, is -O- or -S-, more preferably wherein F^4 is -S-. According to this fourth especially preferred embodiment of the present invention, preferably at least one of R^d and R^f of at least one repeating unit of -[CR^dR^f]$_h$- is -OH. More preferably, R^d and R^f are either H or OH, wherein at least one of R^d and R^f of at least one repeating unit of -[CR^dR^f]$_h$- is -OH, wherein the repeating units may be the same or may be different. Most preferably R^{dd} and R^are, if present, H as well.

Particularly preferably, L^1 has a structure selected from the group consisting of -CH_2-CHOH-CH_2-, -CH_2-CHOH-CH_2-S-CH_2-CH_2-, -CH_2-CHOH-CH_2-S-CH_2-CH_2-CH_2-, -CH_2-CHOH-CH_2-NH-CH_2-CH_2-, -CH_2-CHOH-CH_2-NH-CH_2-CH_2-CH_2-, CH_2-CHOH-CH_2-0-CH_2-CHOH-CH_2-, -CH_2-CHOH-CH_2-0-CH_2-CHOH-CH_2-S-CH_2-CH_2-, more preferably from the group consisting of -CH_2-CHOH-CH_2-, -CH_2-CHOH-CH_2-S-CH_2-

CH_2- and -CH_2-CHOH-CH_2-S-CH_2-CH_2-CH_2-, most preferably L^1 is -CH_2-CHOH-CH_2-S-CH_2-CH_2-.

The hydroxyalkyl starch conjugate according to this fourth preferred embodiment, preferably further comprises the structural unit -L-M having the structure

$$-[F^2]_q-[L^2]_g-[E]_e-[CR^mR^n]_rF^3-M$$

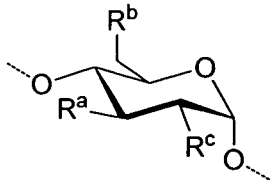
wherein q and g and e are 0.

Accordingly, in this preferred embodiment, the functional group -X- represents an electron-withdrawing group in close proximity to the functional group F^3, and -X- is directly linked to the structural unit -$[CR^mR^n]_f$. Depending on integer f, which is 1, 2 or 3, the electron-withdrawing group is either present in alpha, beta or gamma position to the functional group F^3. As regards, the position of the functional group -X- to the functional group F^3, -X- is preferably present in alpha position to the functional group F^3. Thus, according to this preferred embodiment, integer f is preferably 1, in particular with R^m and R^n being both H.

Thus, the present invention also relates to a conjugate, comprising a residue of a hydroxyalkyl starch derivative, as described above, as well as a conjugate obtained or obtainable by the above-mentioned method, wherein the conjugate comprises a residue of a hydroxyalkyl starch derivative and a cytotoxic agent, the conjugate having a structure according to the following formula

$$HAS'(-[F^2]_q-[L^2]_g-[E]_e-[CR^mR^n]_f-F^3-M)_n$$

wherein f is 1 and wherein R^m and R^n are both H, and wherein q and g and e are 0 and wherein the residue of the hydroxyalkyl starch derivative preferably comprises at least one structural unit according to the following formula (lb)



(lb)

wherein at least one of R^a, R^b and R^c is –[O–CH_2-CH_2]$_t$-[F^1]$_p$-L^1-X- with -X- being -S-, preferably with p being 1 and F^1 being -0-, thus at least one of R^a, R^b and R^c has preferably the structure -[0-CH_2-CH_2]$_t$-0-L^1-S-, wherein t is in the range of from 0 to 4, and wherein

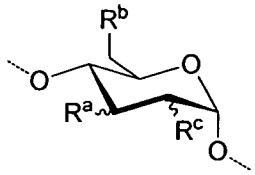
L^1 is preferably $-CH_2-CHOH-CH_2-S-CH_2-CH_2-$. Most preferably F^3 is -C(=0)- and M is preferably a residue of a camptothecin or of a camptothecin analogue, as described above, more preferably of SN-38 or of irinotecan.

According to an especially preferred embodiment, the conjugate has a structure according to the following formula

$$HAS'(-CH_2-C(=0)-M)_n$$

and wherein HAS' comprises at least one structural unit according to formula (Ib), wherein at least one of R^a, R^b and R^c is $[0-CH_2-CH_2]$,-0-$CH_2-CHOH-CH_2-S-CH_2-CH_2-S-$.

According to another preferred embodiment of the present invention, the hydroxyalkyl starch conjugate comprises a hydroxyalkyl starch derivative comprising at least one structural unit according to the following formula (I)



(I)

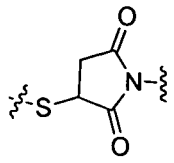
wherein at least one of R^a, R^b and R^c is $-[0-CH_2-CH_2]_t-X-$ and -X- is -S-, thus at least one of R^a, R^b and R^c is $-[0-CH_2-CH_2]_t-S-$, and wherein the conjugate further comprises the moiety -L-M, wherein -L-M has the structure $(-[F^2]_q-[L^2]_g-[E]_e-[CR^mR^n]rF^3-M)_n$, as described above, and wherein e is 1 and E is preferably -C(=0)-NH-.

According to this embodiment, -X- is directly linked to the functional group F^2 with q and g preferably both being 1. As described above, the functional group F^2 is, if present, preferably selected from -S- and -succinimide-, preferably -succinimide-.

Thus, according to this embodiment, the conjugate, or the conjugate obtained or obtainable by the above-mentioned method, has in particular a structure according to the following formula

$$HAS'(-succinimide-L^2-C(=0)-NH-[CR^mR^n]_f-M)_n$$

wherein HAS' comprises at least one structural unit according to formula (I), wherein in each unit, independently of each other unit, at least one of R^a, R^b and R^c is $-[0-CH_2-CH_2]_t-X-$ and -X- is -S- and wherein the succinimide is directly linked to X, thereby forming a



bond.

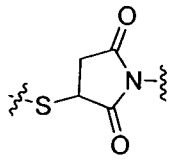
Particularly preferably F^3 in the above mentioned formula is -C(=0)-.

As regards, the linking moiety L^2 according to this preferred embodiment, L^2 is preferably an alkyl group, as described above. More preferably L^2 is $-CH_2-CH_2-CH_2-$ or $-CH_2-CH_2-$, most preferably L^2 is $-CH_2-CH_2-$.

Accordingly, the present invention also relates to a conjugate, comprising a residue of a hydroxyalkyl starch derivative, as described above, as well as a conjugate obtained or obtainable by the above-mentioned method, wherein the conjugate comprises a hydroxyalkyl starch derivative and a cytotoxic agent, the conjugate having a structure according to the following formula

$$HAS'(\text{-succinimide-}CH_2\text{-}CH_2\text{-}C(=O)\text{-}NH\text{-}[CR^mR^n]rC(=O)\text{-}M)_n$$

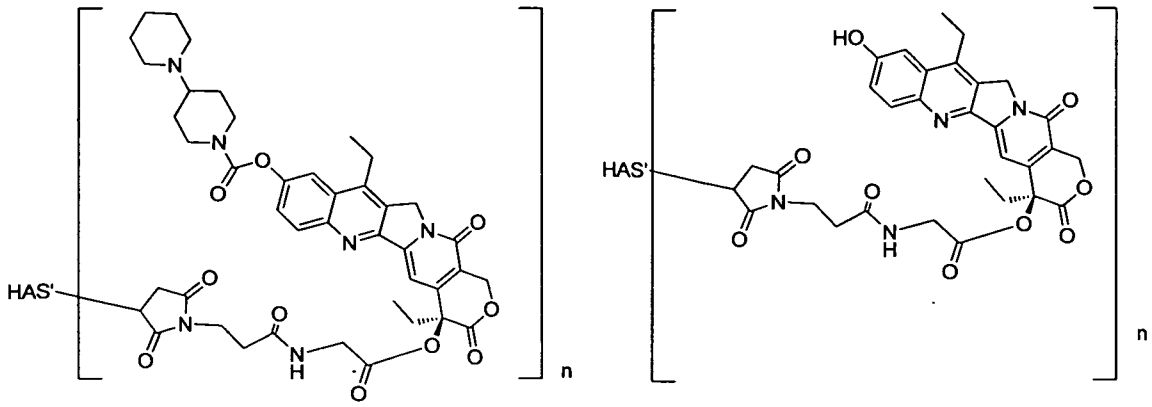
wherein HAS' preferably comprises at least one structural unit according to formula (I), wherein at least one of R^a, R^b and R^c is $-[0\text{-}CH_2\text{-}CH_2]_t$-X- and -X- is -S- and wherein the functional group -X- is directly linked to the succinimide group, thereby forming a



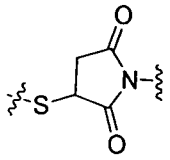
bond and wherein most preferably all functional groups -X- present in a given hydroxyalkyl starch derivative comprised in a conjugate according to the invention, are directly linked to the succinimide group.

Most preferably, according to this embodiment, R^m and R^n are both H and f is 1.

The present invention thus also relates to a conjugate, comprising a hydroxyalkyl starch derivative, as described above, as well as a conjugate obtained or obtainable by the above-mentioned method, the conjugate having a structure according to one of the following formulas:

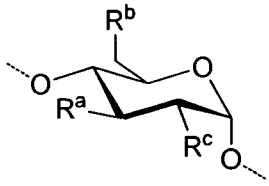


wherein HAS' comprises at least one structural unit according to the following formula (I). wherein at least one of R^a, R^b and R^c is -[0-CH_2-CH_2]$_t$-X- and -X- is -S-, thereby forming a



bond.

According to an alternative embodiment, the hydroxyalkyl starch conjugate comprises a residue of a hydroxyalkyl starch derivative which comprises at least one structural unit according to the following formula (Ib)



(Ib)

wherein at least one of R^a, R^b and R^c is -[0-CH_2-CH_2]$_t$-[F^1]$_p$-L^1-X- with -X- being -S-, preferably with p being 1 and F^1 being -0-, thus at least one of R^a, R^b and R^c has preferably the structure -[0-CH_2-CH_2]$_t$-0-L'-S-, and wherein t is in the range of from 0 to 4, and wherein L^1 is a group, as described above, preferably an alkyl group, and wherein the conjugate further comprises the moiety -L-M, as described above, with -L-M having the structure

$$-[F^2]_q-[L^2]_g-[E]_e-[CR^mR^n]_f-F^3-M$$

wherein q is 1 and F^2 is -succinimide-. More preferably F^3 is -C(=0)-. Further preferably, e is 1, and E is -O- or -S-.

Accordingly, the present invention also relates to a conjugate, comprising a residue of a hydroxyalkyl starch derivative, as described above, as well as a conjugate obtained or obtainable by the above-mentioned method, wherein the conjugate further comprises a cytotoxic agent, the conjugate having a structure according to the following formula

HAS'(-succinimide-[L ^{2}]$_{g}$-E -[CR^{m}R^{n}]rC(=0)-M)$_{n}$

more preferably a structure according to one of the following formulas

HAS'(-succinimide-[L ^{2}]$_{g}$-0-[CR ^{m}R^{n}]rC(=0)-M) $_{n}$

and

HAS'(-succinimide-[L ^{2}]$_{g}$-S-[CR^{m}R^{n}]£-C(=0)-M)$_{n}$

wherein HAS' preferably comprises at least one structural unit according to the following formula (lb), and wherein at least one of R^a, R^b and R^c is -[0-CH $_2$-CH$_2$]$_t$-[F^1]$_p$-L^1-X- with -X- being -S-, preferably with p being 1 and F^1 being -0-, thus at least one of R^a, R^b and R^c has preferably the structure -[0-CH $_2$-CH$_2$]$_t$-O-L'-S-, and wherein t is in the range of from 0 to 4, and wherein L^1 is preferably -CH$_2$-CHOH-CH$_2$-S-CH $_2$-CH$_2$-.

Depending on integer f, which is 1, 2 or 3, the electron-withdrawing group E is either present in alpha, beta or gamma position to the functional group F^3. In case E is -S- or -0-, E is preferably present in alpha position to the functional group F^3. Thus, f is preferably 1. Most preferably f is 1 and R^m and R^n are both H.

Accordingly, the present invention also relates to a conjugate, comprising a hydroxyalkyl starch derivative, as described above, as well as a conjugate obtained or obtainable by the above-mentioned method, wherein the conjugate further comprises a cytotoxic agent, the conjugate having a structure according to one of the following formulas

HAS'(-succinimide-[L ^{2}]$_{g}$-0-CH $_2$-C(=0)-M)$_{n}$

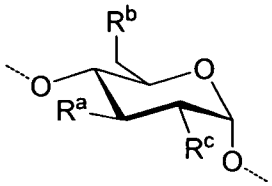
and

HAS'(-succinimide-[L ^{2}]$_{g}$-S-CH$_2$-C(=0)-M)$_{n}$

wherein HAS' preferably comprises at least one structural unit, according to formula (lb) wherein at least one of R^a, R^b and R^c is -[O-CH$_2$-CH$_2$]$_r$-[F^1]$_p$-L^1-X- with -X- being -S-, preferably with p being 1 and F^1 being -0-, thus at least one of R^a, R^b and R^c has preferably the structure -[0-CH $_2$-CH$_2$]$_t$-O-L'-S-, and wherein t is in the range of from 0 to 4, and wherein L^1 is preferably -CH$_2$-CHOH-CH$_2$-S-CH $_2$-CH$_2$-. L^2 is preferably an alkyl

group, most preferably g is 1 and L^2 has a structure selected from the group consisting of-CH_2-CH_2-, -CH_2-CH_2-CH_2- and -CH_2-CH_2-CH_2-CH_2-. Most preferably M is a residue of a camptothecin or of a camptothecin analogue, as described above, more preferably of SN-38 or of irinotecan.

According to a further preferred embodiment, the hydroxyalkyl starch conjugate comprises a residue of a hydroxyalkyl starch derivative which comprises at least one structural unit according to the following formula (lb)



(lb)

wherein at least one of R^a, R^b and R^c is -[0-CH_2-CH_2],-[F^1]$_p$-L^1-X- with -X- being -S-, preferably with p being 1 and F^1 being -0-, thus at least one of R^a, R^b and R^c has preferably the structure -[0-CH_2-CH_2]$_t$-0-L^1-S-, and wherein t is in the range of from 0 to 4, and wherein L^1 is a group, as described above, preferably an alkyl group, and wherein the conjugate further comprises the moiety -L-M, as described above, with -L-M having the structure

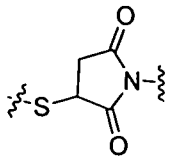
$$-[F^2]_q-[L^2]_g-[E]_e-[CR^mR^n]_f-F^3-M$$

wherein e is 1 and E is preferably -NH-C(=0)-, -C(=0)-NH- or -succinimide-, preferably -succinimide-. In case E is -succinimide-, -X- is preferably directly linked to the functional group E with q and g thus preferably both being 0.

Thus, according to this embodiment, the conjugate, or the conjugate obtained or obtainable by the above-mentioned method, has in particular a structure according to the following formula

$$HAS'(-succinimide-[CR^mR^n]rF^3-M)_n$$

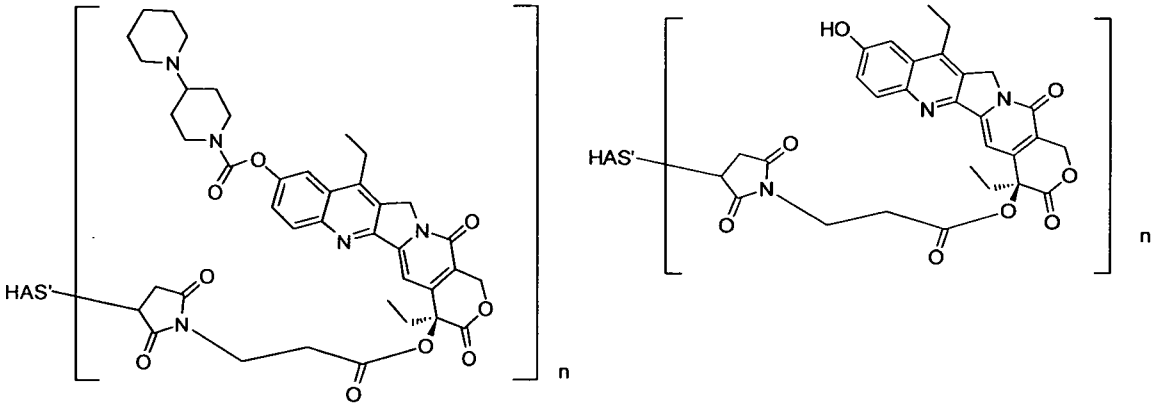
wherein HAS' comprises at least one structural unit according to formula (lb), wherein at least one of R^a, R^b and R^c is -[0-CH_2-CH_2]$_t$-[F^1]$_p$-L^1-X- with -X- being -S-, preferably with p being 1 and F^1 being -0-, and wherein the succinimide is directly linked to X, thereby forming a



bond.

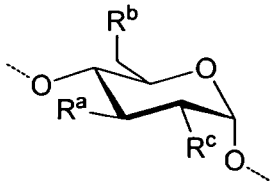
Particularly preferably F^3 in the above mentioned formula is -C(=0)-. Most preferably, according to this embodiment, R^m and R^n are both H and f is 2.

The present invention thus also relates to a conjugate, comprising a hydroxyalkyi starch derivative, as described above, as well as a conjugate obtained or obtainable by the above-mentioned method, the conjugate having a structure according to one of the following formulas:



wherein HAS' comprises at least one structural unit according to formula (lb), and wherein at least one of R^a, R^b and R^c is -[0-CH_2-CH_2]$_t$-[F^1]$_p$-L^1-X- with -X- being -S-, preferably with p being 1 and F^1 being -O, L^1 is preferably -CH_2-CHOH-CH_2-S-CH_2-CH_2-.

According to a further especially preferred embodiment of the present invention, the hydroxyalkyi starch conjugate comprises a residue of a hydroxyalkyi starch derivative which comprises at least one structural unit according to the following formula (lb)



(Ib)

wherein at least one of R^a, R^b and R^c is $-[O-CH_2-CH_2]_t-[F^1]_p-L^1-X-$ with -X- being -S-, preferably with p being 1 and with F^1 being $-Y^7-C(=Y^6)-$, $-C(=Y^6)-$, $-Y^7-C(=Y^6)-Y^8-$, wherein $-Y^7-$ is selected from the group consisting of $-NR^{Y7}-$, -O- or -S-, -succinimide, -NH-NH-, -HN-0-, -CH=N-0-, -0-N=CH-, -CH=N-, -N=CH-, $-Y^8-$ is selected from the group consisting of $-NR^{Y8}-$, -S-, -0-, -NH-NH- and Y^6 is selected from the group consisting of NR^{Y6}, O and S, wherein R^{Y6} is H or alkyl, preferably H, and wherein R^{Y7} is H or alkyl, preferably H, and wherein R^{Y8} is H or alkyl, preferably H. More preferably F^1 has the structure $-Y^7-C(=Y^6)-Y^8-$, wherein Y^6 is selected from the group consisting of NR^{Y6}, O and S, with R^{Y6} being H or alkyl, preferably H, and wherein Y^8- is selected from the group consisting of $-NR^{Y8}-$, -S-, -0-, NH-NH-, with R^{Y8} being H or alkyl, preferably H, and wherein Y^7 is -O- or -S-, preferably -0-. More preferably F^1 has the structure -0-C(=0)-NH-. As regards, the structural moiety L^1, L^1 is preferably an alkyl group, as described above. According to a preferred embodiment of the present invention, the linking moiety L^1 is a spacer comprising at least one structural unit according to the formula $-[CR^dR^f]_h-[F^4]_u-[CR^{dd}R^{ff}]_z-$, as described above, wherein F^4 is preferably selected from the group consisting of -S-, -O- and -NH-, more preferably wherein F^4, if present, is -O- or -S-, more preferably wherein F^4 is -S-. Reference is made to the discussion of linking moiety L^1 above. As described above, residues R^d, R^f, R^{dd} and R^{ff} are, independently of each other, preferably selected from the group consisting of halogens, alkyl groups, H or hydroxyl groups. More preferably, these residues, are independently of each other, H, alkyl or hydroxyl.

Preferably, in case p is 1 and F^1 has the structure $-Y^7-C(=Y^6)-Y^8-$, such as the structure -O-C(=0)-NH-, integer u and integer z (of the formula $-[CR^dR^f]_h-[F^4]_u-[CR^{dd}R^{ff}]_z-$) are 0, the linking moiety L^1 thus corresponds to the structural unit $-[CR^dR^f]_h-$.

As described above, the integer h is preferably in the range of from 1 to 20, more preferably of from 1 to 10, such as 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, more preferably of from 1 to 5, most preferably of from 1 to 3. More preferably R^d and R^f are both H. Thus, by way of example, the following preferred linking moieties L^1 are mentioned: $-CH_2-$, $-CH_2-CH_2-$, $-CH_2-CH_2-CH_2-$, $-CH_2-CH_2-CH_2-CH_2-$, $-CH_2-CH_2-CH_2-CH_2-CH_2-$, more preferably $-CH_2-CH_2-$, in the context of the fourth preferred embodiment.

The hydroxyalkyl starch conjugate according to this further preferred embodiment, preferably further comprises the structural unit -L-M having the structure

$$-[F^2]_q-[L^2]_g-[E]_e-[CR^mR^n]_f-F^3-M$$

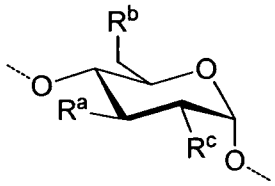
wherein q, g and e are 0.

Accordingly, in this preferred embodiment, the functional group -X- represents an electron-withdrawing group in close proximity to the functional group F^3, and -X- is directly linked to the structural unit $-[CR^mR^n]_f-$. Depending on integer f, which is 1, 2 or 3, the electron-withdrawing group is either present in alpha, beta or gamma position to the functional group F^3. As regards, the position of the functional group -X- to the functional group F^3, -X- is preferably present in alpha position to the functional group F^3. Thus, according to this preferred embodiment, the integer f is preferably 1, in particular with R^m and R^n being both H.

Thus, the present invention also relates to a conjugate, comprising a residue of a hydroxyalkyl starch derivative, as described above, as well as a conjugate obtained or obtainable by the above-mentioned method, wherein the conjugate comprises a residue of a hydroxyalkyl starch derivative and a cytotoxic agent, the conjugate having a structure according to the following formula

$$HAS'(-[F^2]_q-[L^2]_g-[E]_e-[CR^mR^n]_f-F^3-M)_n$$

wherein f is 1 and wherein R^m and R^n are both H, and wherein q and g and e are 0 and wherein the residue of the hydroxyalkyl starch derivative preferably comprises at least one structural unit according to the following formula (Ib)



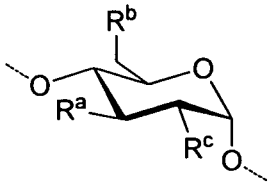
(Ib)

wherein at least one of R^a, R^b and R^c is $-[0-CH_2-CH_2]-[F^1]_p-L^1-X-$ with -X- being -S-, preferably with p being 1 and F^1 being -0-C(=0)-NH-, wherein t is in the range of from 0 to 4. Most preferably the functional group F^3 is -C(=0)-. According to an especially preferred embodiment, the conjugate has a structure according to the following formula

$$HAS'(-CH_2-C(=0)-M)_n.$$

Most preferably M is a residue of camptothecin or of a camptothecin analogue, as described above, more preferably of SN-38 or of irinotecan.

According to an alternative embodiment, the hydroxyalkyl starch conjugate comprises a residue of a hydroxyalkyl starch derivative which comprises at least one structural unit according to the following formula (lb)



(lb)

wherein at least one of R^a, R^b and R^c is -[0-CH_2-CH_2],-[F^1]$_p$-L^1-X- with -X- being -S-, preferably with p being 1 and F^1 being -0-C(=0)-NH-, wherein t is in the range of from 0 to 4, and wherein the conjugate further comprises the moiety -L-M, as described above, with -L-M having the structure

$$-[F^2]_q-[L^2]_g-[E]_e-[CR^mR^n]_f-F^3-M$$

wherein q is 1 and F^2 is -succinimide-. More preferably F^3 is -C(=0)-. Further preferably, e is 1, and E is -O- or -S-.

Accordingly, the present invention also relates to a conjugate, comprising a residue of a hydroxyalkyl starch derivative, as described above, as well as a conjugate obtained or obtainable by the above-mentioned method, wherein the conjugate further comprises a cytotoxic agent, the conjugate having a structure according to the following formula

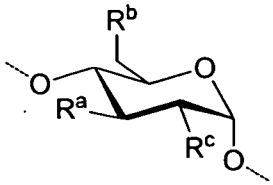
HAS'(-succinimide-[L^2]$_g$-E-[CR^mR^n]rC(=0)-M)$_n$

more preferably a structure according to one of the following formulas

HAS'(-succinimide-[L^2]$_g$-0-[CR^mR^n]rC(=0)-M)$_n$ and
FIAS'(-succinimide-[L^2]g-S-[CR^mR^n]rC(=0)-M)n

wherein HAS' preferably comprises at least one structural unit according to the formula (lb), and wherein at least one of R^a, R^b and R^c is -[0-CH_2-CH_2]r[F ']$_p$-L^1-X- with -X- being -S-, preferably with p being 1 and F^1 being -0-C(=0)-NH-, wherein t is in the range of from 0 to 4-. L^1 is preferably an alkly group, as described above. Integer f is preferably 1. Most preferably f is 1 and R^m and R" are both H.

According to another preferred embodiment, the hydroxyalkyl starch conjugate comprises a residue of a hydroxyalkyl starch derivative which comprises at least one structural unit according to the following formula (lb)



(lb)

wherein at least one of R^a, R^b and R^c is $-[O-CH_2-CH_2]_t-[F^1]_p-L^1-X-$ with -X- being -S-, preferably with p being 1 and F^1 being -0-C(=0)-NH-, wherein t is in the range of from 0 to 4, and wherein L^1 is a group, as described above, preferably an alkyl group, and wherein the conjugate further comprises the moiety -L-M, as described above, with -L-M having the structure

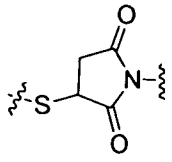
$$-[F^2]_q-[L^2]_g-[E]_e-[CR^mRYF^3-M$$

wherein e is 1 and E is preferably -NH-C(=0)-, -C(=0)-NH- or -succinimide-, preferably -succinimide-. In case E is -succinimide, -X- is preferably directly linked to the functional group E with q and g thus preferably both being 0.

Thus, according to this embodiment, the conjugate, or the conjugate obtained or obtainable by the above-mentioned method, has in particular a structure according to the following formula

$$HAS'(-succinimide-[CR^mR^n]_f-F^3-M)n$$

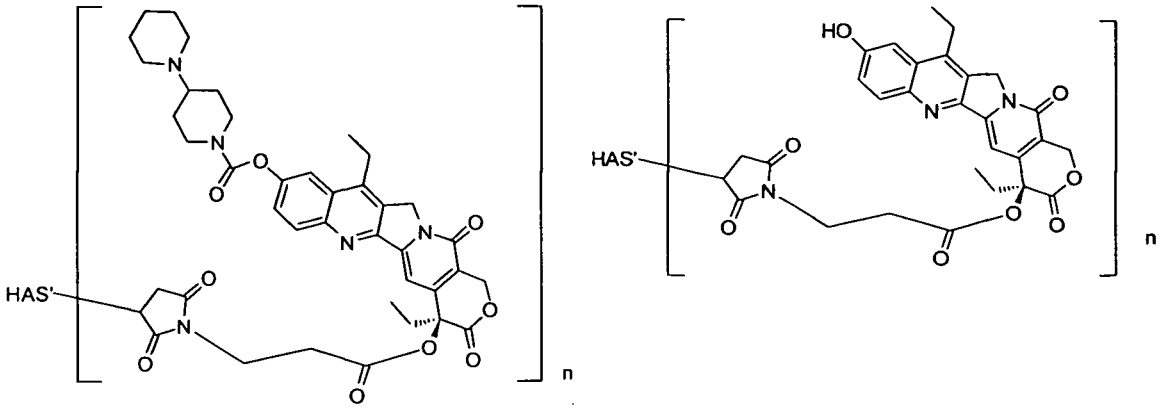
wherein HAS' comprises at least one structural unit according to formula (lb), wherein at least one of R^a, R^b and R^c is $-[0-CH_2-CH_2]_t-[F^1]_p-L^1-X-$ with -X- being -S-, preferably with p being 1 and F^1 being -0-C(=0)-NH-, wherein t is in the range of from 0 to 4, and wherein the succinimide is directly linked to X, thereby forming a



bond.

Particularly preferably F^3 in the above mentioned formula is -C(=0)-. Most preferably, according to this embodiment, R^m and R" are both H and f is 2.

The present invention thus also relates to a conjugate, comprising a hydroxyalkyl starch derivative, as described above, as well as a conjugate obtained or obtainable by the above-mentioned method, the conjugate having a structure according to one of the following formulas:



wherein HAS' comprises at least one structural unit according to formula (Ib), wherein at least one of R^a, R^b and R^c is -[O-CH$_2$-CH$_2$]$_p$-[F^1]-L^1-X- with -X- being -S-, preferably with p being 1 and F^1 being -O-C(=O)-NH-.

Synthesis of HAS Conjugates

As described above, the present invention also relates to a method for preparing a hydroxyalkyl starch conjugate comprising a hydroxyalkyl starch derivative and a cytotoxic agent, said conjugate having a structure according to the following formula HAS'(-L-M)$_n$, wherein M is a residue of a cytotoxic agent, wherein the cytotoxic agent comprises a tertiary hydroxyl group, L is a linking moiety, HAS' is a residue of the hydroxyalkyl starch derivative, and n is greater than or equal to 1,
said method comprising the steps

(a) providing a hydroxyalkyl starch derivative having a mean molecular weight MW above the renal threshold, preferably above 60 kDa, more preferably from 60 to 1500 kDa, more preferably of from 200 to 1000 kDa, more preferably of from 250 to 800 kDa, and a molar substitution MS in the range of from 0.6 to 1.5, said hydroxyalkyl starch derivative comprising a functional group Z^1; and providing a cytotoxic agent comprising a tertiary hydroxyl group,

(b) coupling the HAS derivative to the cytotoxic agent via an at least bifunctional crosslinking compound L comprising a functional group K^1 and a functional group K^2, wherein K^2 is capable of being reacted with Z^1 comprised in the HAS derivative

and wherein K^1 is capable of being reacted with the tertiary hydroxyl group comprised in the cytotoxic agent.

The at least bifunctional crosslinking compound L

The term "at least bifunctional crosslinking compound L" as used in the context of the present invention refers to an at least bifunctional compound comprising the functional groups K^1 and K^2.

Besides the functional group K^1 and the functional group K^2 the at least bifunctional crosslinking compound may optionally contain further functional groups, which may be used, for example, for the attachment of radiolabels, or the like. Hereinunder and above, the "at least bifunctional crosslinking compound L" is also referred to as "crosslinking compound L".

The crosslinking compound L is reacted via its functional group K^1 with the tertiary hydroxyl group of the cytotoxic agent, thereby forming a covalent linkage. On the other side, the at least bifunctional crosslinking compound L (in the following referred to as crosslinking compound L) is reacted via its functional group K^2 with the functional group Z^1 of the hydroxyalkyl starch derivative, thereby forming a covalent linkage as well.

The crosslinking compound L can be reacted with a cytotoxic agent either prior or subsequent to the reaction with the hydroxyalkyl starch derivative. Preferably the crosslinking compound L is coupled to the cytotoxic agent prior to the reaction with the hydroxyalkyl starch derivative.

Thus, the present invention also relates to a method for preparing a hydroxyalkyl starch conjugate comprising a hydroxyalkyl starch derivative and a cytotoxic agent, said conjugate having a structure according to the following formula $HAS'(-L-M)_n$, wherein M is a residue of a cytotoxic agent, and wherein the cytotoxic agent comprises a tertiary hydroxyl group, L is a linking moiety, HAS' is a residue of the hydroxyalkyl starch derivative, and n is greater than or equal to 1, said method comprising the steps

(a) providing a hydroxyalkyl starch derivative having a mean molecular weight MW above the renal threshold, preferably above 60 kDa, in the range of from 60 to 1500 kDa, preferably of from 200 to 1000 kDa, more preferably of from 250 to 800 kDa, and a molar substitution in the range of from 0.6 to 1.5, said hydroxyalkyl starch derivative comprising a functional group Z^1; and providing a cytotoxic agent comprising a tertiary hydroxyl group,

(b) coupling the HAS derivative to the cytotoxic agent via an at least bifunctional crosslinking compound L comprising a functional group K^1 and a functional group K^2, wherein K^2 is capable of being reacted with Z^1 comprised in the HAS derivative and wherein K^1 is capable of being reacted with the tertiary hydroxyl group comprised in the cytotoxic agent, wherein L is coupled to Z^1 via the functional group K^2 comprised in L, and wherein each cytotoxic agent is coupled via the tertiary hydroxyl group to the HAS derivative via the functional group K^1 comprised in L, and wherein the cytotoxic agent is preferably reacted with the at least one crosslinking compound L prior to the reaction with the hydroxyalkyl starch derivative, thereby forming a cytotoxic agent derivative comprising the functional group K^2, and wherein said cytotoxic agent derivative is coupled in a subsequent step to the hydroxyalkyl starch derivative according to step (a).

Further, the present invention relates to a hydroxyalkyl starch conjugate obtained or obtainable by said method.

Upon reaction of the at least bifunctional crosslinking compound L with the hydroxyalkyl starch derivative and the cytotoxic agent the hydroxyalkyl starch conjugate HAS'(-L-M)$_n$ is formed. In said conjugate, HAS' and M are linked via the linking moiety L, wherein said linking moiety L is the linking moiety derived from the at least bifunctional crosslinking compound L.

Preferably, the at least bifunctional crosslinking compound L has a structure according to the following formula,

$$K^2\text{-}L'\text{-}K^*$$

wherein L' is a linking moiety, K^2 is the functional group capable of being reacted with the functional group Z^1 of the hydroxyalkyl starch derivative and K^1 is the group capable of being reacted with the cytotoxic agent, as described above.

The functional group K^1

Accordingly, the functional group K^1 is a group capable of being coupled to a tertiary hydroxyl group of the cytotoxic agent. Upon reaction of the functional group K^1 with the tertiary hydroxyl group, preferably the linking unit -F^3-0-, as described above, is formed. Preferably, K^1 is a functional group with which (upon reaction with the hydroxyl group) a covalent linkage between L, preferably L', and M, is formed which is cleavable *in vivo* as described above.

The crosslinking compound L may be reacted with either the cytotoxic agent or the hydroxyalkyl starch in an initial step. Preferably, the crosslinking compound is reacted with the tertiary hydroxyl group of the cytotoxic agent prior to the reaction with the hydroxyalkyl starch derivative, thereby forming a derivative of the cytotoxic agent, the derivative of the cytotoxic agent preferably having the structure K^2-L'-F^3-M.

Thus, the present invention also describes a method for preparing a hydroxyalkyl starch conjugate, as described above, wherein step (b) comprises the steps

(bl) coupling the cytotoxic agent via its tertiary hydroxyl group to the crosslinking compound K^2-L'-K', thereby forming a derivative of the cytotoxic agent having the structure K^2-L'-F^3-M, wherein M is the residue of the cytotoxic agent,

(b2) coupling the derivative of the cytotoxic agent having the structure K^2-L'-F^3-M to the hydroxyalkyl starch derivative according to step (a), thereby forming the hydroxyalkyl starch conjugate.

Further, the present invention relates to a hydroxyalkyl starch conjugate obtained or obtainable by said method.

Preferably K^1 comprises the structural unit -C(=Y)-, with Y being O, NH or S. Thus, the present invention also relates to a method for preparing a hydroxyalkyl starch conjugate, as described above, wherein the cytotoxic agent is reacted with the at least one crosslinking compound L via the functional group K^1 comprised in the crosslinking compound L, wherein said functional group K^1 comprises the structural unit -C(=Y)-, with Y being O, NH or S, more preferably Y being O. Further, the present invention relates to a hydroxyalkyl starch conjugate obtained or obtainable by said method.

According to a particular preferred embodiment K^1 is a carboxylic acid group or a reactive carboxy group.

The term "reactive carboxy group" as used in this context of the present invention is intended to mean an activated carboxylic acid derivative that reacts readily with electrophilic groups, such as the -OH group of the cytotoxic agent, optionally in the presence of a suitable base, in contrast to those groups that require a further catalyst, such as a coupling reagent, in order to react. The term "activated carboxylic acid derivative" as used herein preferably refers to acid halides such as acid chlorides and also refers to activated ester derivatives including, but not limited to, formic and acetic acid derived

anhydrides, anhydrides derived from alkoxycarbonyl halides such as isobutyloxycarbonylchloride and the like, isothiocyanates or isocyanates, anhydrides derived from reaction of the carboxylic acid with N,N'-carbonyldiimidazole and the like, and esters derived from activation of the corresponding carboxylic acid with a coupling reagent. Such coupling reagents include, but are not limited to, HATU (0-(7-azabenzotriazol-l-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate); HOAt, HBTU (0-benzotriazol-l-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate); TBTU (2-(lH-benzotriazol-l-yl)-l,l,3,3-tetramethyluronium hexafluorophosphate); TFFH (N,N',N",N"-tetramethyluronium-2-fluoro-hexafluorophosphate); BOP (benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate); PyBOP (benzotriazol-l-yl-oxy-trispyrrolidino-phosphonium hexafluorophosphate); EEDQ (2-ethoxy-l-ethoxycarbonyl-l,2-dihydro-quinoline); DCC (dicyclohexylcarbodiimide); DIPCDI (diisopropylcarbodiimide); HOBt (1-hydroxybenzotriazole); NHS (N-hydroxysuccinimide); MSNT (l-(mesitylene-2-sulfonyl)-3-nitro-lH-l,2,4-triazole); aryl sulfonyl halides, e.g. triisopropylbenzenesulfonyl chloride, EDC (l-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride, CDC (l-cyclohexyl-3-(2-morpholinoethyl)carbodiimide), Pyclop, T3P, CDI, Mukayama's reagent, HODhbt, HAPyU, TAPipU, TPTU, TSTU, TNTU, TOTU, BroP, PyBroP, BOI, TOO, NEPIS, BBC, BDMP, BOMI, AOP, BDP, PyAOP, TDBTU, BOP-C1, CIP, DEPBT, Dpp-Cl, EEDQ, FDPP, HOTT, TOTT, PyCloP.

In case, K^1 is a carboxylic acid group, the coupling between the cytotoxic agent and the crosslinking compound L is preferably carried out in the presence of at least one coupling reagent, wherein the coupling reagent is preferably selected from the group of coupling reagents mentioned above. In case a coupling reagent is used, most preferably EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) is used. Additionally additives promoting the activation of the carboxylic acid, such as DMAP (4-(dimethylamino)-pyridine), may be used.

The coupling between the cytotoxic agent and the crosslinking compound L is preferably carried out in the presence of a suitable base, preferably an organic base, most preferably an amino group comprising base, most preferably a base selected from the group consisting of diisopropylamine (DIEA), triethylamine (TEA), N-methylmorpholine, N-methylimidazole, l,4-diazabicyclo[2.2.2]octane (DABCO), N-methylpiperidine, N-methylpyrrolidine, 2,6-lutidine, collidine, pyridine, 4-dimethylaminopyridine, 1,8-diaza-bicyclo[5.4.0]undec-7-ene (DBU). As regards the reaction conditions used in this coupling step, preferably, the reaction is carried out in an organic solvent, such as N-methyl pyrrolidone (NMP), dimethyl sulfoxide (DMSO), acetonitrile, acetone, dimethyl acetamide (DMA), dimethyl formamide (DMF), formamide, tetrahydrofuran (THF), 1,4-dioxane,

diethyl ether, tert.-butyl methyl ether (MTBE), dichloromethane (DCM), dimethoxyethane (DME), chloroform, tetrachloromethane, benzene, toluene, hexane and mixtures of two or more thereof. More preferably, the reaction is carried out in dichloromethane.

The temperature of the coupling reaction is preferably in the range of from 0 to 100 °C, more preferably of from 5 to 50 °C, and especially preferably of from 15 to 30 °C. During the course of the reaction, the temperature may be varied, preferably in the above given ranges, or held essentially constant.

The derivative of the cytotoxic agent, which in particular has the following structure

$$K^2\text{-}L'\text{-}F^3\text{-}M,$$

may be subjected to at least one isolation and/or purification step prior to the reaction with the hydroxyalkyl starch derivative.

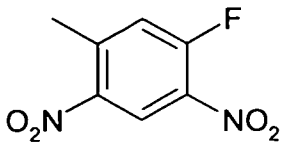
The functional group K^2 and the functional group Z^1

In the context of the present invention, K^2 is a functional group capable of being reacted with a functional group Z^1 of the hydroxyalkyl starch derivative, and Z^1 is the respective functional group capable of being reacted with the functional group K^2. Upon reaction of K^2 with Z^1 the functional unit $-X-[F^2]_q-$ is formed, with X and $-[F^2]_q-$ being as described above in the context of the conjugate structures.

Such functional groups Z^1 and K^2 may be suitably chosen. By way of example, one of the groups Z^1 and K^2, i.e. Z^1 or K^2, may be chosen from the group consisting of the functional groups according to the following list while the other group, K^2 or Z^1, is suitably selected and capable of forming a chemical linkage with Z^1 or K^2, wherein K^2 or Z^1 is also preferably selected from the above-mentioned following list:

- C-C-double bonds or C-C-triple bonds, such as alkenyl groups, alkynyl groups or aromatic C-C-bonds, in particular alkynyl groups,
  most preferably the group -C≡C-H;
- alkyl sulfonic acid hydrazides, aryl sulfonic acid hydrazides;
- the thiol group or the hydroxy group;
- thiol reactive groups such as
  -- a disulfide group comprising the structure -S-S-; such as pyridyl disulfides,
  -- maleimide group,
  -- haloacetyl groups,

-- haloacetamides,
-- vinyl sulfones,
-- vinyl pyridines,
-- haloalkanes;

- the group 
- dienes or dienophiles;
- azides;
- 1,2-aminoalcohols;
- amino groups comprising the structure -NR'R", wherein R' and R" are independently of each other selected from the group consisting of H, alkyl groups, aryl groups, arylalkyi groups and alkylaryl groups; preferably -NH_2;
- hydroxylamino groups comprising the structure -O-NR'R", wherein R' and R" are independently of each other selected from the group consisting of H, alkyl groups, aryl groups, arylalkyi groups and alkylaryl groups; preferably -0-NH_2;
- oxyamino groups comprising the structure -NR'-O-, with R' being selected from the group consisting of alkyl groups, aryl groups, arylalkyi groups and alkylaryl groups; preferably -NH-0-;
- residues having a carbonyl group, -Q-C(=G)-M', wherein G is O or S, and M' is, for example,
  -- -OH or -SH;
  -- an alkoxy group, an aryloxy group, an arylalkyloxy group, or an alkylaryloxy group;
  -- an alkylthio group, an arylthio group, an arylalkylthio group, or an alkylarylthio group;
  -- an alkylcarbonyloxy group, an arylcarbonyloxy group, an arylalkylcarbonyloxy group, an alkylarylcarbonyloxy group;
  -- activated esters such as esters of hydroxylamines having an imide structure such as N-hydroxysuccinimide,
- -NR'-NH_2, wherein R' and R" are independently of each other selected from the group consisting of H, alkyl groups, aryl groups, arylalkyi groups and alkylaryl groups; preferably wherein R' is H;
- carbonyl groups such as aldehyde groups, keto groups; hemiacetal groups or acetal groups;
- the carboxy group;
- the -N=C=0 group or the -N=C=S group;

- vinyl halide groups such as the vinyl iodide group or the vinyl bromide group or triflate;
- -(C=NH_2Cl)-OAlkyl;
- epoxide;
- residues comprising a leaving group such as e.g. halogens or sulfonates.

Preferably, Z^1 is selected from the group consisting of aldehyde, keto, hemiacetal, acetal, alkynyl, azide, carboxy groups, alkenyl, thiol reactive groups, such as maleimide, halogen acetyl, pyridyl disulfides, haloacetamides, vinyl sulfones and vinyl pyridines, -SH, -NH_2, **-O-NH_2,** -NH-O-alkyl, -(C=G)-NH-NH_2, -G-(C=G)-NH-NH_2, -NH-(C=G)-NH-NH_2, and -S0 $_2$-NH-NH_2, where G is O or S and, if G is present twice, it is independently O or S.

Thus, the present invention also relates to a method for preparing a hydroxyalkyl starch conjugate, as described above, wherein K^2 is reacted with the functional group Z^1, wherein Z^1 is selected from the group consisting of aldehyde groups, keto groups, hemiacetal groups, acetal groups, alkynyl groups, azide groups, carboxy groups, alkenyl groups, thiol reactive groups, -SH, -NH_2, -0-NH_2, -NH-O-alkyl, -(C=G)-NH-NH_2, -G-(C=G)-NH-NH_2, -NH-(C=G)-NH-NH_2, and -S0 $_2$-NH-NH_2, where G is O or S and, if G is present twice, it is independently O or S. Further, the present invention also relates to the conjugate obtained or obtainable by said method.

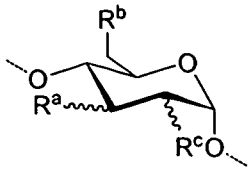
By way of example, in the following Table 1, suitable combinations of Z^1 and K^2 are mentioned:

Table 1: *Examples for K^2 and Z^1*

K^2	Z^1
-SH	thiol reactive group
-NH_2	aldehyde group, keto group, hemiacetal group, acetal group or carboxy group
-O-NH_2	aldehyde group, keto group, hemiacetal group, acetal group or carboxy group
-(C=G)-NH-NH_2	aldehyde group, keto group, hemiacetal group, acetal group or

	carboxy group
-G-(C=G)-NH-NH_2	aldehyde group, keto group, hemiacetal group, acetal group or carboxy group
-SO_2-NH-NH_2	aldehyde group, keto group, hemiacetal group, acetal group or carboxy group
alkynyl or diphenylphosphinomethylthioester	azide
azide	alkynyl or diphenylphosphinomethylthioester
aldehyde group, keto group, hemiacetal group, acetal group or carboxy group	-NH_2
aldehyde group, keto group, hemiacetal group, acetal group or carboxy group	-O-NH_2
aldehyde group, keto group, hemiacetal group, acetal group or carboxy group	-(C=G)-NH-NH_2
aldehyde group, keto group, hemiacetal group, acetal group or carboxy group	-G-(C=G)-NH-NH_2
aldehyde group, keto group, hemiacetal group, acetal group or carboxy group	-SO_2-NH-NH_2
thiol reactive group	-SH
thioester	alpha-thiol-beta-amino group
alpha-thiol-beta-amino group	thioester

It has to be understood that the groups Z^1 are statistically distributed throughout the hydroxyalkyl starch derivative. Thus, the hydroxyalkyl starch derivative formed in step (a) of the method of the present invention comprises at least one structural unit according to the following formula (I)

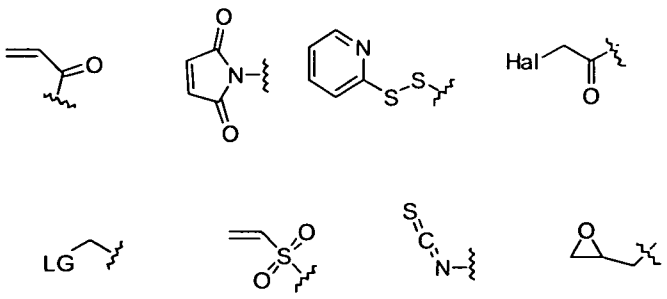


(1)

with Z^1 being comprised in at least one of R^a, R^b or R^c and are further, preferably comprised, in multiple repeating units of the structural unit according to the formula (1).

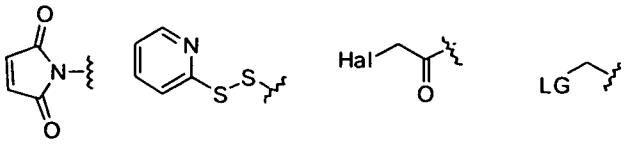
According to a preferred embodiment of the present invention, the functional group Z^1 is a thiol group. Thus, the present invention also relates to a method for preparing a hydroxyalkyl starch conjugate, as described above, wherein in step (a) a derivative is formed comprising at least one thiol group, preferably comprising multiple thiol groups, the derivative having a mean molecular weight MW above the renal threshold, preferably above 60 kDa, more preferably in the range of from 60 to 1500 kDa, more preferably of from 200 to 1000 kDa, more preferably of from 250 to 800 kDa, and a molar substitution MS in the range of from 0.6 to 1.5. The present invention further relates to the conjugate obtained or obtainable by said method.

In case Z^1 is a thiol group, K^2 is preferably a thiol reactive group, preferably a group selected from the group consisting of pyridyl disulfides, maleimide group, haloacetyl groups, haloacetamides, vinyl sulfones and vinyl pyridines. Preferably, K^2 is a thiol-reactive group selected from the group consisting of the following structures:

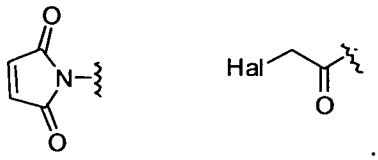


wherein Hal is a halogen, such as CI, Br, or I, and LG is a leaving group (or nucleofuge) The term "leaving group", as used in this context of the present invention, is denoted to mean a molecular fragment that departs with a pair of electrons in heterolytic bond cleavage upon reaction with the functional group Z^1. Examples are, inter alia, halogens or sulfonic esters. Examples for sulfonic esters are, inter alia, the mesyl and tosyl group.

More preferably, K^2 is a thiol-reactive group selected from the group consisting of the following structures



more preferably from the following structures



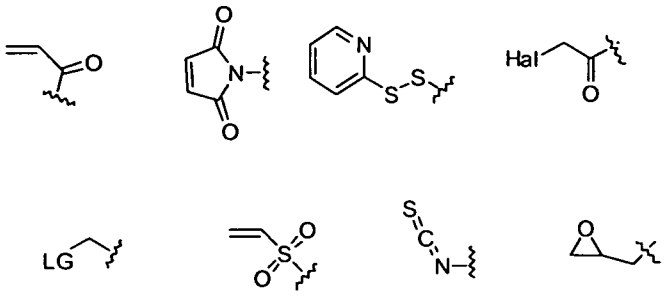
Thus, the present invention also describes a method for preparing a hydroxyalkyi starch conjugate comprising a hydroxyalkyi starch derivative and a cytotoxic agent said conjugate having a structure according to the following formula HAS'(-L-M)$_n$, wherein M is a residue of a cytotoxic agent, said cytotoxic agent comprising a tertiary hydroxyl group, L is a linking moiety, HAS' is a residue of the hydroxyalkyi starch derivative, and n is greater than or equal to 1, preferably wherein n is in the range of from 2 to 300,

said method comprising the steps

(a) providing a hydroxyalkyi starch derivative having a mean molecular weight MW above the renal threshold, preferably above 60 kDa, preferably in the range of from 60 to 1500 kDa, more preferably of from 200 to 1000 kDa, more preferably of from 250 to 800 kDa, and a molar substitution MS in the range of from 0.6 to 1.5, said hydroxyalkyi starch derivative comprising a functional group Z^1; and providing a cytotoxic agent comprising a tertiary hydroxyl group,

(b) coupling the HAS derivative to the cytotoxic agent via an at least bifunctional crosslinking compound L comprising a functional group K^1 and a functional group K^2, wherein K^2 is capable of being reacted with Z^1 comprised in the HAS derivative and wherein K^1 is capable of being reacted with the tertiary hydroxyl group comprised in the cytotoxic agent, and wherein L is coupled to Z^1 via the functional group K^2 comprised in L, and wherein each cytotoxic agent is coupled via the tertiary hydroxyl group to the hydroxyalkyi starch derivative via the functional group K^1 comprised in L,

and wherein Z^1 is -SH, and wherein K^2 is a thiol reactive group, preferably a group selected from the group consisting of the following structures:



and wherein **K^1** comprises the structural unit **-C(=Y)-,** with **Y** being **O, NH** or **S,** more preferably **Y** is **O,** preferably, wherein **K^1** is a carboxylic acid group or a reactive carboxy group. Further, the present invention also relates to the respective conjugate obtained or obtainable by said method.

Preferably, the at least bifunctional crosslinking compound L has a structure according to the following formula, **K^2-$[L^2]_g$-$[E]_e$-$[CR^mR^n]_r K^1$**, wherein **L^2** is a linking moiety, **E** is an electron-withdrawing group, and **R^m** and **R"** are, independently of each other **H** or alkyl, and g is 0 or 1, e is 0 or 1, and f is in the range of from 1 to 3, as described above.

Thus, in step (b) of the present invention, the hydroxyalkyl starch derivative according to step (a) is preferably reacted with a crosslinking compound L, with L having the structure

$$K^2\text{–}[L^2]_g\text{-}[E]_e\text{-}[CR^mR^n]_f\text{-}K^1,$$

wherein the crosslinking compound L is coupled to Z^1 comprised in the hydroxyalkyl starch derivative via the functional group K^2, and wherein each cytotoxic agent is coupled via the tertiary hydroxyl group to the hydroxyalkyl starch derivative via the functional group K^1 thereby forming a conjugate having the structure

$$\mathbf{HAS'(\text{-}[F^2]_q\text{-}[L^2]_g\text{-}[E]_e\text{-}[CR^mR^n]_f\text{-}F^3\text{-}M)n,}$$

with F^2, **L^2, E,** q, g, e and **$\text{-}[CR^mR^n]_f$** being as described hereinabove, preferably wherein **E** is an electron-withdrawing group selected from the group consisting of -0-, **-S-, -SO-,** $-SO_2-$, **$-NR^e-$,** -succinimide-, **$-C(=Y^e)-$, $-NR^e-C(=Y^e)-$, $-C(=Y^e)-NR^e-$, $-CH(NO_2)-$, -CH(CN)-,** aryl moieties or an at least partially fluorinated alkyl moiety, wherein **Y^e** is either **O, S** or **NR^e,** and **R^e** is hydrogen or alkyl, more preferably wherein **E** is selected from the group consisting of **-NH-C(=0)-, -C(=0)-NH-, -NH-,** -0-, **-S-, -SO-,** $-SO_2-$ and

-succinimide-, L^2 is a linking moiety, preferably an alkyl, alkenyl, alkylaryl, arylalkyl, heteroaryl, alkylheteroaryl, heteroarylalkyl or aryl group, f is in the range of from 1 to 3, g is 0 or 1, e is 0 or 1, and wherein R^m and R" are, independently of each other, H or alkyl, more preferably H or methyl.

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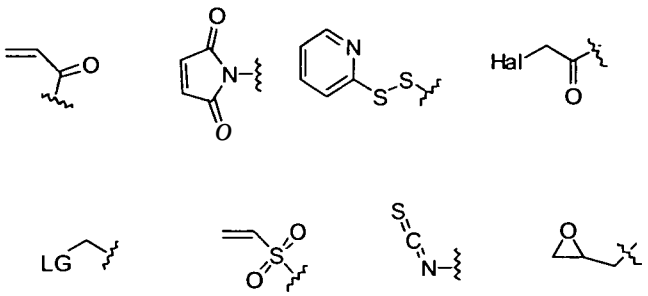
By way of example, the following preferred crosslinking compounds L are mentioned in table la:

Table la: Preferred crosslinking compounds L, by way of example

K^2-$[L^2]_g$-$[E]_e$-$[CR^mR^n]_f$-K^1					
	K^2	L^2/g	$[E]_e$	$[CR^mR^n]_f$	K^1
1	maleimide-	g is O	e is 0	$-CH_2-CH_2-$	-COOH
2	Hal-	g is O	e is 0	$-CH_2-$	-COOH
3	maleimide-	g is 1 L^2 is selected from the group: **$-CH_2-CH_2-CH_2-CH_2-$** **CH_2-CH_2-,** **$-CH_2-CH_2-CH_2-CH_2-$** CH_2-, **$-CH_2-CH_2-CH_2-CH_2-$,** **$-CH_2-CH_2-CH_2-$,** **$-CH_2-CH_2-$,** $-CH_2-$	e is 1 E is -S-	$-CH_2-$	-COOH
4	selected from group A (see entry 10)	g is 1 L^2 is -ethyl-	e is 1 E is -S-	$-CH_2-$	-COOH
5	selected from group A (see entry 10)	g is 1 L^2 is -butyl-	e is 1 E is -S-	$-CH_2-$	-COOH
6	selected from group A (see entry 10)	g is 1 L^2 is -propyl-	e is 1 E is -O-	$-CH_2-$	-COOH
7	selected from group A (see entry 10)	g is 1 L^2 is -ethyl-	e is 1 E is -O-	$-CH_2-$	-COOH
8	selected from group A (see entry 10)	g is 1 L^2 is -butyl-	e is 1 E is -O-	$-CH_2-$	-COOH

9	maleimide	g is 1 L^2 is selected from the group: $-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-$, $-CH_2-CH_2-CH_2-CH_2-CH_2-$, $-CH_2-CH_2-CH_2-CH_2-$, $-CH_2-CH_2-CH_2-$, $-CH_2-CH_2-$, $-CH_2-$	E is 1 E is -C(=0)-NH-	$-CH_2-$	-COOH
10	Group A: 				

Step (a)

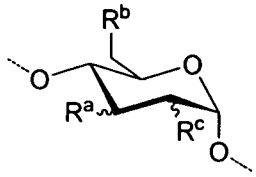
As regards, the provision of the hydroxyalkyl starch derivative according to step (a), preferably step (a) comprises the introduction of at least one functional group Z^1 into the hydroxyalkyl starch by

(i) coupling hydroxyalkyl starch via at least one hydroxyl group with at least one suitable linker comprising the functional group Z^1 or a precursor of the functional group Z^1, or

(ii) displacing a hydroxyl group present in the hydroxyalkyl starch in a substitution reaction with a precursor of the functional group Z^1 or with a bifunctional linker comprising the functional group Z^1 or a precursor of the functional group Z^1.

The term "at least one suitable linker comprising a precursor of the functional group Z^1" as used in context of the present invention is denoted to mean a linker comprising a functional group which is capable of being transformed in at least one further step to give the functional group Z^1. The term "precursor" used in the context of "displacing the hydroxyl group of hydroxyalkyl starch with a precursor", is denoted to mean a reagent which is capable of displacing the hydroxyl group, thereby forming a functional group Z^1 or a group, which can be modified in at least one further step to give the functional group Z^1.

According to a preferred embodiment of the present invention, the present invention relates to a method for preparing a hydroxyalkyl starch conjugate, as described above, wherein the hydroxyalkyl starch derivative comprises at least one structural unit according to the following formula (I)

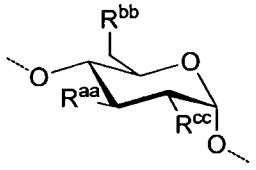


(I)

wherein at least one of R^a, R^b or R^c comprises the functional group Z^1, wherein R^a, R^b and R^c are, independently of each other, selected from the group consisting of-O-HAS", -[O-$(CR^wR^xHCR^yR^z)]_x$-OH, $-[O-(CR^wR^x)-(CR^yR^z)]_y-Z^1$, $-[O-(CR^wR^x)-(CR^yR^z)]_y-[F^1]_p-L^1-Z^1$, wherein R^w, R^x, R^y and R^z are independently of each other selected from the group consisting of hydrogen and alkyl, y is an integer in the range of from 0 to 20, preferably in the range of from 0 to 4, x is an integer in the range of from 0 to 20, preferably in the range of from 0 to 4, F^1 is a functional group, p is 1 or 0, and L^1 is a linking moiety.

and wherein step (a) comprises

(al) providing a hydroxyalkyl starch having a mean molecular weight MW above the renal threshold, preferably above 60 kDa, more preferably in the range of from 60 to 1500 kDa, more preferably of from 200 to 1000 kDa, and a molar substitution **MS** in the range of from 0.6 to 1.5, comprising the structural unit according to the following formula (II)



(II)

wherein $\mathbf{R^{aa}}$, $\mathbf{R^{bb}}$ and $\mathbf{R^{cc}}$ are independently of each other selected from the group consisting of $\mathbf{-[0-(CR^wR^x)-(CR^yR^z)]_x-OH}$ and **-O-HAS",** wherein **HAS"** is a remainder of the hydroxyalkyl starch;

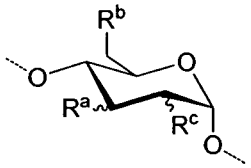
(a2) introducing at least one functional group Z^1 by

(i) coupling the hydroxyalkyl starch via at least one hydroxyl group to at least one suitable linker comprising the functional group Z^1 or a precursor of the functional group Z^1, or

(ii) displacing a hydroxyl group present in the hydroxyalkyl starch in a substitution reaction with a precursor of the functional group Z^1 or with a bifunctional linker comprising the functional group Z^1 or a precursor of the functional group Z^1.

Furthermore, the present invention relates to a conjugate obtained or obtainable by said method.

According to a preferred embodiment of the present invention, the present invention relates to a method for preparing a hydroxyalkyl starch conjugate, as described above, as well as to a conjugate obtained or obtainable by said method, wherein the hydroxyalkyl starch derivative provided in step (a2) comprises at least one structural unit according to the following formula (I)



(I)

wherein R^a, R^b and R^c are independently of each other selected from the group consisting of -O-HAS", -[0-CH $_2$-CH_2]$_s$-OH, -[0-CH $_2$-CH_2]$_t$-Z' and $-[O-CH_2-CH_2]_t-[F^1]_p-L^1-Z^1$, with p being 0 or 1, and wherein at least one of R^a, R^b and R^c comprises the functional group Z^1, and wherein t is in the range of from 0 to 4, wherein s is in the range of from 0 to 4.

Hydroxyalkyl starches having the desired properties are preferably produced from waxy maize starch or potato starch by acidic hydrolysis and reaction with ethylene oxide and purification by ultrafiltration.

Step fa2)(i)

According to a first preferred embodiment of the present invention, the functional group Z^1 is introduced by coupling the hydroxyalkyl starch via at least one hydroxyl group with at least one suitable linker comprising the functional group Z^1 or a precursor of the functional group Z^1.

Organic chemistry offers a wide range of reactions to modify hydroxyl groups with linker constructs bearing functionalities such as aldehyde, keto, hemiacetal, acetal, alkynyl, azide, carboxy, alkenyl and thiol reactive groups, such as maleimide, halogens, pyridyl disulfides, haloacetamides, vinyl sulfones, vinyl pyridines, -SH, $-NH_2$, -0-NH_2, -NH-O-alkyl, -(C=G)-NH-NH_2, -G-(C=G)-NH-NH_2, -NH-(C=G)-NH-NH_2, and -$S0_2$-NH-NH_2, wherein G is O, NH or S, preferably O or S, and if present twice may be the same or may be different from each other. However, the hydroxyalkyi starch's polymeric nature and the abundance of hydroxyl groups present in the hydroxyalkyi starch usually strongly promote the number of possible side reactions such as inter- and intramolecular crosslinking. Therefore, a method was needed to functionalize the polymer under maximum retention of its molecular characteristics such as solubility, molecular weight and polydispersity. It was surprisingly found that when using the method according to this preferred embodiment, possible side reactions such as inter- and intramolecular crosslinking can be significantly diminished.

According to a preferred embodiment of the present invention, in step (a2)(i), the hydroxyalkyi starch is coupled to a linker comprising a functional group Z^2, said functional group Z^2 being capable of being coupled to a hydroxyl group of the hydroxyalkyi starch, thereby forming a covalent linkage between the first linker and the hydroxyalkyi starch. Further, the linker preferably comprises the functional group Z^1 or a precursor thereof. According to a particularly preferred embodiment, the linker comprises a precursor of the functional group Z^1 which is transformed in at least one further step to give the functional group Z^1.

The Functional Group Z^2

The functional group Z^2 is a functional group capable of being coupled to at least one hydroxyl function of the hydroxyalkyi starch or to an activated hydroxyl function of hydroxyalkyi starch, thereby forming a covalent linkage F^1.

According to a preferred embodiment, the functional group Z^2 is a leaving group or a nucleophilic group. According to an alternative embodiment the functional group Z^2 is an epoxide.

According to a first preferred embodiment, Z^2 is a leaving group, preferably a leaving group being attached to a CH_2-group comprised in the at least one suitable linker which is reacted in step (a2)(ii) with the hydroxyalkyi starch. The term "leaving group" as used in this context of the present invention is denoted to mean a molecular fragment that departs with a pair of electrons in heterolytic bond cleavage upon reaction with the hydroxyl group

of the hydroxyalkyl starch, thereby forming a covalent bond between the oxygen atom of the hydroxyl group and the carbon atom formerly bearing the leaving group. Common leaving groups are, for example, halides such as chloride, bromide and iodide, and sulfonates such as tosylates, mesylates, fluorosulfonates, triflates and the like. According to a preferred embodiment of the present invention, the functional group Z^2 is a halide leaving group. Thus, upon reaction of the hydroxyl group with the functional group Z^2, preferably a functional group F^1 is formed, which is preferably -0-.

Alternatively, Z^2 may also be an epoxide group, which reacts with a hydroxyl group of HAS in a ring opening reaction, thereby forming a covalent bond.

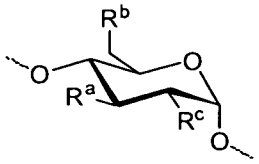
According to another embodiment, Z^2 is a nucleophile, thus a group capable of forming a covalent bond with an electrophile by donating both bonding electrons. In case Z^2 is a nucleophile, the method preferably comprises an initial step, in which at least one hydroxyl function of hydroxyalkyl starch is activated, thereby forming an electrophilic group. For example, the hydroxyl group may be activated by reacting at least one hydroxyl function with a reactive carbonyl compound, as described in detail below. Thus, the present invention also describes a method, as described above, wherein the functional group Z^2 is a nucleophile, said nucleophile being capable of being reacted with at least one activated hydroxyl function of hydroxyalkyl starch, as described above, wherein the hydroxyl group is initially activated with a reactive carbonyl compound prior to coupling the hydroxyalkyl starch in step (a2)(ii) to the at least one suitable linker comprising the functional group Z^2 and the functional group Z^1 or a precursor of the functional group Z^1.

The term "reactive carbonyl compound" as used in this context of the present invention, refers to carbonyl dication synthons having a structure R^{**}-(C=0)-R^*, wherein R^* and R^{**} may be the same or different, and wherein R^* and R^{**} are both leaving groups. As leaving groups halides, such as chloride, and/or residues derived from alcohols, may be used. The term "residue derived from alcohols", refers to R^* and/or R^{**} being a unit -0-R^f or -O-R^{88}, with -0-R^f and -O-R^{88} preferably being residues derived from alcohols such as N-hydroxy succinimide or sulfo-N-hydroxy succinimide, suitably substituted phenols such as p-nitrophenol, o,p-dinitrophenol, o,o'-dinitrophenol, trichlorophenol such as 2,4,6-trichlorophenol or 2,4,5-trichlorophenol, trifluorophenol such as 2,4,6-trifluorophenol or 2,4,5-trifluorophenol, pentachlorophenol, pentafluorophenol, heterocycles such as imidazol or hydroxyazoles such as hydroxy benzotriazole may be mentioned. Reactive carbonyl compounds containing halides are phosgene, related compounds such as diphosgene or triphosgene, chloroformic esters and other phosgene substitutes known in the art. Especially preferred are carbonyldiimidazol (CDI), N,N'-disuccinimidyl carbonate and

sulfo-N,N'-disuccinimidyl carbonate, or mixed compounds such as p-nitrophenyl chloroformate.

Preferably, the reactive carbonyl compound having the structure R^{**}-(C=0)-R^{*} is selected from the group consisting of phosgene, diphosgene, triphosgene, chloroformates and carbonic acid esters, more preferably from the group consisting of p-nitrophenylchloroformate, pentafluorophenylchloroformate, N,N'-disuccinimidyl carbonate, suIfo-N,N'-disuccinimidyl carbonate, dibenzotriazol-l-yl carbonate and carbonyldiimidazol.

Preferably upon reaction of at least one hydroxy! group with the reactive carbonyl compound R^{**}-(C=0)-R^{*} prior to the coupling step according to step (a2)(ii) an activated hydroxyalkyl starch derivative is formed, which comprises at least one structural unit, according to the following formula (lb)



(lb)

wherein R^a, R^b and R^c are independently of each other selected from the group consisting of-O-HAS", -[0-CH_2-CH_2-]$_s$-OH and -[0-CH_2-CH_2]$_t$-O-C(=0)-R^{*}, wherein t is in the range of from 0 to 4, and wherein s is in the range of from 0 to 4, and wherein at least one of R^a, R^b and R^c comprises the group $-[O-CH_2-CH_2]_t-O-C(=O)-R^{*}$, and wherein R^{*} is a leaving group, preferably a group selected from the group consisting of p-nitrophenoxy, 2,4-dichlorophenoxy, 2,4,6-trichlorophenoxy, trichloromethoxy, imidazolyl, azides and halides, such as chloride or bromide.

According to this embodiment, according to which the hydroxyalkyl starch is activated to give a hydroxyalkyl starch derivative comprising a reactive -0-C(=0)-R^{*} group, Z^2 is preferably a nucleophilic group, such as a group comprising an amino group. Possible groups are, for example, -NHR^{Z2}, -NH_2, -0-NH_2, -NH-O-alkyl, -(C=G)-NH-NH_2, -G-(C=G)-NH-NH_2, -NH-(C=G)-NH-NH_2, and -S0$_2$-NH-NH_2 wherein G is O or S, and if present twice in one structural unit, may be the same or may be different, and wherein R^{Z2} is an alkyl group, preferably methyl. More preferably Z^2 is -NH_2 or -NHR^{Z2}, most preferably -NH_2.

As described above, besides the functional group Z^2, the linker comprises either the functional group Z^1 or a precursor thereof.

Preferably, the linker further comprises the functional group W, this functional group being a group capable of being transformed in at least one further step to give the functional group Z^1. Preferably W is an epoxide or a functional group which is transformed in a further step to give an epoxide or W has the structure Z^{1*}-PG, with PG being a suitable protecting group.

Synthesis of the hydroxyalkyl starch derivative via an epoxide modified hydroxyalkyl starch derivative

According to a first preferred embodiment, in step (a2)(i), a first linker is used comprising the functional group W, wherein W is an epoxide or a functional group which is transformed in a further step to give an epoxide.

Thus, the present invention also relates to a method for preparing a hydroxyalkyl starch conjugate, as described above, and a hydroxyalkyl starch conjugate obtained or obtainable by said method, wherein step (a2)(i) comprises the step (I)

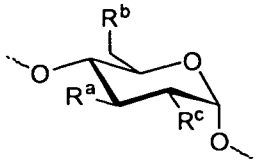
(I) coupling the hydroxyalkyl starch (HAS) via at least one hydroxyl group comprised in HAS to a first linker comprising a functional group Z^2 capable of being reacted with the at least one hydroxyl group of the hydroxyalkyl starch, thereby forming a covalent linkage between the first linker and the hydroxyalkyl starch, the first linker further comprising a functional group W, wherein the functional group W is an epoxide or a group which is transformed in a further step to give an epoxide.

Preferably, the first linker has the structure Z^2-L^w-W, wherein Z^2 is a functional group capable of being reacted with at least one hydroxyl group of hydroxyalkyl starch, as described above, and wherein L^w is a linking moiety.

Thus, the present invention also relates to a method for preparing a hydroxyalkyl starch conjugate, as described above, and a hydroxyalkyl starch conjugate obtained or obtainable by said method, wherein step (a2)(i) comprises step (I)

(I) coupling the hydroxyalkyl starch via at least one hydroxyl group comprised in HAS to a first linker having a structure according to the following formula Z^2-L^w-W, wherein Z^2 is a functional group capable of being reacted with at least one hydroxyl group of hydroxyalkyl starch, as described above, and wherein L^w is a linking

moiety, and wherein, upon reaction of the hydroxyalkyl starch, a hydroxyalkyl starch derivative is formed comprising at least one structural unit, according to the following formula (lb)



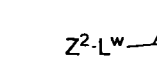
(Ib)

wherein R^a, R^b and R^c are independently of each other selected from the group consisting of -O-HAS", -[0-(CR $^{w}R^{x}$)-{CR^{y}R^{z})]x-OH and -[0-{CR $^{w}R^{x}$HCR $^{y}R^{z}$)]y-[F ^{1}]$_p$-L^{W}-W wherein R^w, R^x, R^y and R^z are independently of each other selected from the group consisting of hydrogen and alkyl, y is an integer in the range of from 0 to 20, preferably in the range of from 0 to 4, x is an integer in the range of from 0 to 20, preferably in the range of from 0 to 4 and wherein at least one of R^a, R^b and R^c comprises the group -[0-(CR $^{w}R^{x}$)-(CR $^{y}R^{z}$)]y-[F'] $_p$-L^{W}-W, and wherein [F^{1}]$_p$ is the functional group being formed upon reaction of Z^2 with the at least one hydroxyl group of the hydroxyalkyl starch, more preferably, wherein R^a, R^b and R^c are independently of each other selected from the group consisting of -O-HAS", -[0-CH $_2$-CH $_2$-]$_s$-OH and -[0-CH $_2$-CH$_2$]r[F ']p-L^{W}-W, and wherein t is in the range of from 0 to 4 and wherein s is in the range of from 0 to 4, and p is 1, and wherein at least one of R^a, R^b and R^c comprises the group -[O-CH_2-CH_2]$_T$[F ^{1}]$_p$-L^{W}-W.

According to one embodiment of the present invention, the functionalization of at least one hydroxyl group of hydroxyalkyl starch to give the epoxide comprising hydroxyalkyl starch, is carried out in a one-step procedure, wherein at least one hydroxyl group is reacted with a first linker, as described above, wherein the first linker comprises the functional group W, and wherein W is an epoxide.

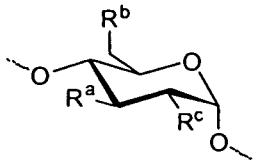
Therefore, the present invention also describes a method for preparing a hydroxyalkyl starch conjugate, as described above, as well as to a hydroxyalkyl starch conjugate obtained or obtainable by said method, wherein in step (a2) (i) (I) the hydroxyalkyl starch is reacted with a linker comprising a functional group Z^2 capable of being reacted with a hydroxyl group of the hydroxyalkyl starch, thereby forming a covalent linkage, the linker further comprising a functional group W, wherein the functional group W is an epoxide.

This linker has in this case a structure according to the following formula

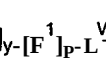


such as, for example, epichlorhydrine.

Upon reaction of this linker with at least one hydroxyl group of hydroxyalkyi starch, a hydroxyalkyi starch derivative is formed comprising at least one structural unit according to the following formula (lb)



(lb)

wherein R^a, R^b and R^c are independently of each other selected from the group consisting of -O-HAS", -[0-(CR $^{w}R^{x}$)-(CR^{y}R^{z})]$_x$-OH and -[0-(CR^{w}R^{x})-(CR^{y}R^{z})]$_y$-[F^1]$_p$-L^{w}  , an$_D$ wherein at least one of R^a, R^b and R^c comprises the group -[0-(CR ^{w}R^{x})-(CRm^{z})]$_y$-[F^1]p-L^{w}  ,

preferably wherein R^a, R^b and R^c are independently of each other selected from the group consisting of -O-HAS", -[0-CH $_2$-CH $_2$-]$_s$-OH and -P-CH$_2$-CH$_2$]^F ^{1}- L^{w}  (i.e. p is 1), and wherein t is in the range of from 0 to 4 and wherein s is in the range of from 0 to 4, and wherein at least one of R^a, R^b and R^c comprises the group -[O-CH$_2$-CH$_2$]$_t$-F^1-L^{w}  .

According to a preferred embodiment of the invention, the epoxide is generated in a two step procedure, comprising the steps (I) and (II)

(I) coupling at least one hydroxyl group of the hydroxyalkyi starch, preferably of hydroxyethyl starch, to a first linker, comprising a functional group Z^2 capable of being reacted with a hydroxyl group of the hydroxyalkyi starch, thereby forming a covalent linkage between the first linker and the hydroxyalkyi starch, the linker further comprising a functional group W, wherein the functional group W is a functional group which is capable of being transformed in a further step to give an epoxide, such as an alkenyl group,

(II) transforming the functional group W to give an epoxide.

It was surprisingly found that this two step procedure is superior to the one step procedure in that higher loadings of the hydroxyalkyi starch with epoxide groups can be achieved

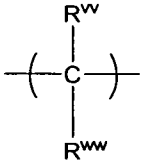
and/or undesired side reactions such as inter- and intra-molecular cross-linking can be substantially avoided.

Preferably the functional group W is an alkenyl group. In this case, step (11) preferably comprises the oxidation of the alkenyl group to give an epoxide and transforming the epoxide to give the functional group Z^1.

According to a preferred embodiment, the present invention also relates to a method for preparing a hydroxyalkyl starch conjugate, as described above, wherein the hydroxyalkyl starch, preferably the hydroxyethyl starch, is coupled in step (a2)(i) via at least one hydroxyl group to at least one suitable linker, the linker having the structure Z^2-L^w-W, wherein upon reaction of a hydroxyl group of the hydroxyalkyl starch with the linker, the leaving group Z^2 departs, thereby forming a covalent linkage between the hydroxyalkyl starch and the linking moiety L^W, and wherein the functional group F^1 which links the hydroxyalkyl starch and the linking moiety L^W, is an -O- bond. Likewise, the present invention also relates to the respective hydroxyalkyl starch conjugates obtained or obtainable by said method.

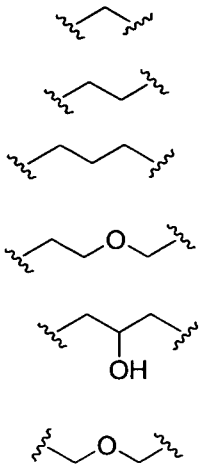
According to the present invention, the term "linking moiety L^W" as used in the context of the present invention relates to any suitable chemical moiety bridging the functional group Z^2 and the functional group W.

In general, there are no particular restrictions as to the chemical nature of the linking moiety L^w with the proviso that L^W has particular chemical properties enabling carrying out the inventive method for the preparation of the novel derivatives comprising the functional group Z^1, i.e. in particular, in case W is a functional group to be transformed to an epoxide, the linking moiety L^W has suitable chemical properties enabling the transformation of the chemical moiety W to the functional group Z^1. According to a preferred embodiment of the present invention, L^W bridging W and HAS' comprises at least one structural unit according to the following formula



wherein R" and R^{ww} are independently of each other H or an organic residue selected from the group consisting of alkyl, alkenyl, alkylaryl, arylalkyl, aryl, heteroaryl, alkylheteroaryl and heteroarylalkyl groups.

Preferably, L^W is an optionally substituted, non-branched alkyl residue such as a group selected from the following groups:



According to a first preferred embodiment of the present invention, the functional group W is an alkenyl group, wherein the first linker Z^2-L^W-W has a structure according to the following formula

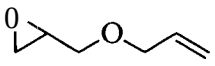
$$Z^2\text{-}L^W\text{-}CH{=}CH_2$$

preferably with Z^2 being a leaving group or an epoxide.

Thus preferred structures of the first linker are by way of example, the following structures:

Hal-CH_2-$CH{=}CH_2$, such as Cl-CH_2-$CH{=}CH_2$ or Br-CH_2-$CH{=}CH_2$ or I-CH_2-$CH{=}CH_2$,

sulfonic esters, such as TsO-CH_2-$CH{=}CH_2$ or MsO-CH_2-$CH{=}CH_2$,

epoxides such as  .

More preferably Z^2 in the first linker Z^2-L^W-W is a leaving group, most preferably the first linker Z^2-L^W-W has a structure according to the following formula

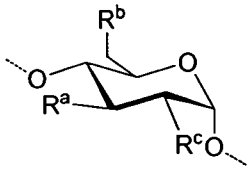
Hal-L^W-CH=CH_2.

According to an especially preferred embodiment of the present invention, the linker Z^2-L^W-W has a structure according to the following formula

$Hal\text{-}CH_2\text{-}CH{=}CH_2$

with Hal being a halogen, preferably the halogen being I, CI, or Br, more preferably Br.

Thus, the present invention also relates to a method for preparing a hydroxyalkyl starch conjugate, as described above, wherein in step (a2)(ii) the hydroxyalkyl starch, preferably the hydroxyethyl starch, is coupled via at least one hydroxyl group with at least one suitable linker having the structure $Hal\text{-}CH_2\text{-}CH{=}CH_2$, wherein upon reaction of the hydroxyalkyl starch with the linker, a hydroxyalkyl starch derivative is formed comprising at least one structural unit according to the following formula (lb)



(Ib)

wherein R^a, R^b and R^c are independently of each other selected from the group consisting of -O-HAS", -[0-(CR $^wR^xHCR\ ^yR^z)]x$-OH and -[0-(CR $^wR^x$)-(CR $^yR^z)]y$-0-CH_2-$CH{=}CH_2$, and wherein at least one of R^a, R^b and R^c comprises the group -[0-(CR $^wR^x$)-$(CR^yR^z)]_y$-0-CH_2-$CH{=}CH_2$, preferably wherein R^a, R^b and R^c are independently of each other selected form the group consisting of -OH, -O-HAS", -[0-CH_2-$CH_2]_s$-OH and -[O-CH_2-$CH_2]_t$-0-CH_2-$CH{=}CH_2$, wherein t is in the range of from 0 to 4, wherein s is in the range of from 0 to 4, and wherein at least one of R^a, R^b and R^c comprises the group -[O-CH_2-$CH_2]_t$-0-CH_2-$CH{=}CH_2$, and wherein the functional group -O- linking the $-CH_2\text{-}CH{=}CH_2$ group to the hydroxyalkyl starch is formed upon reaction of the linker $Hal\text{-}CH_2\text{-}CH{=}CH_2$ with a hydroxyl group of the hydroxyalkyl starch. Likewise, the present invention also relates to a hydroxyalkyl starch conjugate obtained or obtainable by the above-mentioned method.

As regards, the reaction conditions used in this step (1), wherein the hydroxyalkyl starch is reacted with the first linker, in particular wherein the first linker comprises the functional group W with W being an alkenyl, in principle any reaction conditions known to those skilled in the art can be used. Preferably, the reaction is carried out in an organic solvent, such as N-methyl pyrrolidone, dimethyl acetamide (DMA), dimethyl formamide (DMF), formamide, dimethyl sulfoxide (DMSO) or mixtures of two or more thereof. More preferably, the reaction is carried out in anhydrous solvents or solvent mixtures.

Preferably, the hydroxyalkyl starch is dried prior to use, by means of heating to constant weight at a temperature range from 50 to 80 °C in a drying oven or with related techniques.

The temperature of the reaction is preferably in the range of from 5 to 55 °C, more preferably in the range of from 10 to 30 °C, and especially preferably in the range of from 15 to 25 °C. During the course of the reaction, the temperature may be varied, preferably in the above given ranges, or held essentially constant.

The reaction time for the reaction of HAS with the linker Z^2-L^w-W may be adapted to the specific needs and is generally in the range of from 1 h to 7 days, preferably 2 hours to 24 hours, more preferably 3 hours to 18 hours.

More preferably, the reaction is carried out in the presence of a base. The base may be added together with the linker Z^2-L^w-W, or may be added prior to the addition of the linker, to pre-activate the hydroxyl groups of the hydroxyalkyl starch. Preferably, a base, such as alkali metal hydrides, alkali metal hydroxides, alkali metal carbonates, amine bases such as diisopropylethyl amine (D1PEA) and the like, amidine bases such as 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), amide bases such as lithium diisopropylamide (LDA) or alkali metal hexamethyldisilazyl bases (e.g. LiHMDS) may be used. Most preferably the hydroxyalkyl starch is pre-activated with sodium hydride prior to the addition of the first linker Z^2-L^w-W.

The derivative comprising the functional group W, preferably the alkenyl group, may be isolated prior to transforming this group in at least one further step to give an epoxide comprising hydroxyalkyl starch derivative. Isolation of this polymer derivative comprising the functional group W may be carried out by a suitable process which may comprise one or more steps. According to a preferred embodiment of the present invention, the polymer derivative is first separated from the reaction mixture by a suitable method such as precipitation and subsequent centrifugation or filtration. In a second step, the separated polymer derivative may be subjected to a further treatment such as an after-treatment like ultrafiltration, dialysis, centrifugal filtration or pressure filtration, ion exchange chromatography, reversed phase chromatography, HPLC, MPLC, gel filtration and/or lyophilization. According to an even more preferred embodiment, the separated polymer derivative is first precipitated, subjected to centrifugation, re-dissolved and finally subjected to ultrafiltration.

Preferably, the precipitation is carried out with an organic solvent such as ethanol, isopropanol, acetone or tetrahydrofurane (THF). The precipitated derivative is subsequently subjected to centrifugation and subsequent ultrafiltration using water or an

aqueous buffer solution having a concentration preferably from 1 to 1000 mmol/l, more preferably from 1 to 100 mmol/l, and more preferably from 10 to 50 mmol/l such as about 20 mmol/l, a pH value in the range of preferably from 3 to 10, more preferably of from 4 to 8, such as about 7. The number of exchange cycles preferably is in the range of from 5 to 50, more preferably of from 10 to 30, and even more preferably of from 15 to 25, such as about 20. Most preferably the obtained derivative comprising the functional group W is further lyophilized until the solvent content of the reaction product is sufficiently low according to the desired specifications of the product.

In case W is an alkenyl, the method preferably further comprises step (II), that is the oxidation of the alkenyl group to give an epoxide group. As to the reaction conditions used in the epoxidation (oxidation) step (II), in principle, any known method to those skilled in the art can be applied to oxidize an alkenyl group to yield an epoxide.

The following oxidizing reagents are mentioned, by way of example, metal peroxysulfates such as potassium peroxymonosulfate (Oxone®) or ammonium peroxydisulfate, peroxides such as hydrogen peroxide, tert.-butyl peroxide, acetone peroxide (dimethyldioxirane), sodium percarbonate, sodium perborate, peroxy acids such as peroxoacetic acid, meta-chloroperbenzoic acid (MCPBA) or salts like sodium hypochlorite or hypobromite.

According to a particularly preferred embodiment of the present invention, the epoxidation is carried out with potassium peroxymonosulfate (Oxone®) as oxidizing agent.

Thus, the present invention also relates to a method for preparing a hydroxyalkyl starch conjugate, as described above, wherein step (a2)(i) comprises

(I) coupling at least one hydroxyl group of the hydroxyalkyl starch, preferably of hydroxyethyl starch, to a first linker, comprising a functional group Z^2 capable of being reacted with a hydroxyl group of the hydroxyalkyl starch, thereby forming a covalent linkage between the first linker and the hydroxyalkyl starch, the linker further comprising a functional group W, wherein the functional group W is an alkenyl group,

(II) oxidizing the alkenyl group to give an epoxide, wherein as oxidizing agent, preferably potassium peroxymonosulfate is employed.

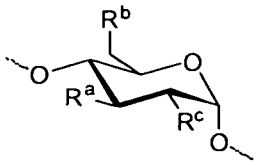
Further, the present invention also relates to a hydroxyalkyl starch conjugate obtained or obtainable by said method.

According to an even more preferred embodiment of the present invention, the reaction with Oxone® is carried out in the presence of a suitable catalyst. Catalysts may consist of

transition metals and their complexes, such as manganese (Mn-salene complexes are known as Jacobsen catalysts), vanadium, molybdenium, titanium (Ti-dialkyltartrate complexes are known as Sharpless catalysts), rare earth metals and the like. Additionally, metal free systems can be used as catalysts. Acids such as acetic acid may form peracids in situ and epoxidize alkenes. The same accounts for ketones such as acetone or tetrahydrothiopyran-4-one, which react with peroxide donors under formation of dioxiranes, which are powerful epoxidation agents. In case of non-metal catalysts, traces of transition metals from solvents may lead to unwanted side reactions, which can be excluded by metal chelation with EDTA.

Preferably, said suitable catalyst is tetrahydrothiopyran-4-one.

Upon epoxidation, in step (II) a hydroxyalkyl starch derivative is formed comprising at least one structural unit according to the following formula (lb)



(lb)

wherein R^a, R^b and R^c are independently of each other selected from the group consisting of **-O-HAS",** -[0-(CR ^{w}R^{x}HCR ^{y}R^{z})]$_x$-OH and -**[0-(CR^{w}R^{x})-(C$^{R^yR^z}$)]$_y$-[F^{1}]$_p$-L^{W}**  **, and** wherein at least one of R^a, R^b and R^c comprises the group -**[0-(CR ^{w}R^{x})-(CR^{y}R^{z})]$_y$-[F^{1}]$_p$-l-^{W}**  ,

preferably wherein R^a, R^b and R^c are independently of each other selected from the group consisting of -O-HAS", -[0-CH $_2$-CH $_2$-]$_s$-OH and -[O-CH$_2$-CHJ r- F^{1}- L^{w}  (i.e. p ig 1), and wherein t is in the range of from 0 to 4 and wherein s is in the range of from 0 to 4, and wherein at least one of R^a, R^b and R^c comprises the group -[O-CH$_2$-CH$_2$]f—F^{1}- L^{w}  .

According to a preferred embodiment, the epoxidation of the alkenyl-modified hydroxyalkyl starch derivatives is carried out in aqueous medium, preferably at a temperature in the range of from 0 to 80 °C, more preferably in the range of from 0 to 50 °C and especially preferably in the range of from 10 to 30 °C.

During the course of the epoxidation reaction, the temperature may be varied, preferably in the above-given ranges, or held essentially constant. The term "aqueous medium" as used in the context of the present invention refers to a solvent or a mixture of solvents comprising water in an amount of at least 10 % per weight, preferably at least 20 % per weight, more preferably at least 30 % per weight, more preferably at least 40 % per weight, more preferably at least 50 % per weight, more preferably at least 60 % per weight, more preferably at least 70 % per weight, more preferably at least 80 % per weight, even more preferably at least 90 % per weight or up to 100 % per weight, based on the weight of the solvents involved. The aqueous medium may comprise additional solvents like formamide, dimethylformamide (DMF), dimethylsulfoxide (DMSO), alcohols such as methanol, ethanol or isopropanol, acetonitrile, tetrahydrofurane or dioxane. Preferably, the aqueous solution contains a transition metal chelator (disodium ethylenediaminotetraacetate, EDTA, or the like) in the concentration ranging from 0.01 to 100 mM, preferably from 0.01 to 1 mM, most preferably from 0.1 to 0.5 mM, such as about 0.4 mM.

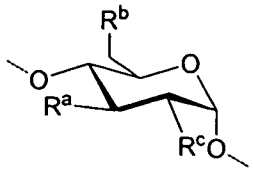
The pH value for the reaction of the HAS derivative with potassium peroxymonosulfate (Oxone®) may be adapted to the specific needs of the reactants. Preferably, the reaction is carried out in buffered solution, at a pH value in the ranges of from 3 to 10, more preferably of from 5 to 9, and even more preferably of from 7 to 8. Among the preferred buffers, carbonate, phosphate, borate and acetate buffers as well as tris(hydroxymethyl)aminomethane (TRIS) may be mentioned. Among the preferred bases, alkali metal bicarbonates may be mentioned.

According to the invention, the epoxide-modified HAS derivative may be purified or isolated in a further step prior to the transformation of the epoxide group to the functional group Z^1.

The separated derivative is optionally lyophilized.

After the purification step, the HAS derivative is preferably obtained as a solid. According to a further conceivable embodiment of the present invention, the HAS derivative solutions or frozen HAS derivative solutions may be mentioned.

The epoxide comprising HAS derivative is preferably reacted in a subsequent step (III) with at least one suitable reagent to yield the HAS derivative comprising the functional group Z^1. Preferably, the epoxide is reacted with a nucleophile comprising the functional group Z^1 or a precursor thereof. Preferably, the nucleophile reacts with the epoxide in a ring opening reaction and yields a HAS derivative comprising at least one structural unit according to the following formula (Ib)



(Ib)

wherein at least one of R^A, R^b and R^C is $-[O-(CR^wR^x)-(CR^yR^z)]_y-[F^1]_p-L^W$-CHOH-$CH_2$-Nuc, preferably wherein at least one of R^A, R^b and R^c is $-[O-CH_2-CH_2]_t-[F^1]_p-L^W$-CHOH-$CH_2$-Nuc, wherein the residue Nuc is the remaining part of the nucleophile covalently linked to the hydroxyalkyl starch after being reacted with the epoxide.

Any nucleophile capable of reacting with the epoxide thereby forming a covalent linkage and comprising the functional group Z^1 or a precursor thereof may be used. As nucleophile, for example, linker compounds comprising at least one nucleophilic functional group capable of reacting with the epoxide and at least one functional group W capable of being transformed to the functional Z^1 can be used. Alternatively, a linker such as an at least bifunctional linker comprising a nucleophilic group such as a thiol group and further comprising the functional group Z^1 may be used.

As described above, according to a particularly preferred embodiment of the present invention, Z^1 is a thiol group.

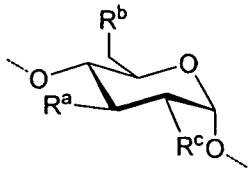
According to a further preferred embodiment of the present invention, the nucleophilic group reacting with the epoxide is a thiol group.

Thus, the present invention also relates to a method as described above, wherein step (a2)(i) comprises

(III) reacting the epoxide with a nucleophile comprising the functional group Z^1 or a precursor of the functional group Z^1, the nucleophile additionally comprising a nucleophilic group, preferably wherein Z^1 and the nucleophilic group are both -SH groups.

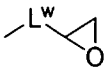
According to an especially preferred embodiment of the present invention, the present invention also relates to a method for preparing a hydroxyalkyl starch conjugate, as well as to a hydroxyalkyl starch conjugate obtained or obtainable by said method, as described above, wherein the epoxide is reacted with a nucleophile comprising the functional group Z^1, with Z^1 being a thiol group, and comprising a nucleophilic group, this group being a thiol. Thus, according to a preferred embodiment, the nucleophile is a dithiol.

The invention also relates to the respective derivative obtained or obtainable by said method, wherein said derivative is preferably transformed to the conjugate according to the invention, as described hereinunder and above, said derivative preferably comprising at least one structural unit according to the following formula (lb)

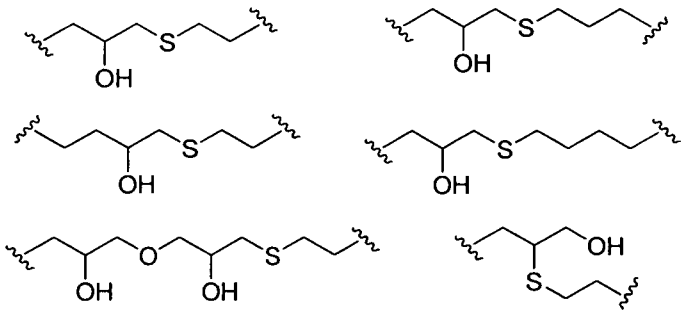


(lb)

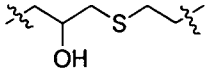
wherein at least one of R^a, R^b and R^c is $-[O-iCR^wR^x)-(CR^yR^z)]_y-[F^1]_p-L'-SH$, preferably wherein at least one of R^a, R^b and R^c is $-[0-CH_2-CH_2]_t-[F^1]_p-L^1-SH$, wherein L^1 is a linking moiety which is obtained when reacting the structural unit



with the nucleophile and which links the functional group F^1 to the functional group Z^1. According to the preferred embodiment, the linking moiety L^1 has a structure selected from the groups below:



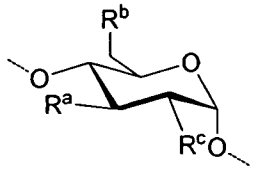
more preferably L^1 has a structure according to the following formula



According to an alternative embodiment of the present method, the epoxide is reacted with a nucleophile suitable for the introduction of thiol groups such as thiosulfate, alkyl or aryl thiosulfonates or thiourea, preferably sodium thiosulfate. Thus, the present invention also

relates to a method as described above as well as to a hydroxyalkyl starch derivative obtained or obtainable by said method, wherein the epoxide-modified hydroxyalkyl starch is reacted with a nucleophile, said nucleophile being thiosulfate, alkyl or aryl thiosulfonates or thiourea, preferably sodium thiosulfate.

Upon reaction of the thiosulfate with the epoxide in a ring opening reaction, preferably a hydroxyalkyl starch derivative is formed comprising at least one structural unit, according to the following formula (lb)



(Ib)

wherein at least one of R^a, R^b and R^c is $-[O-(CR^wR^x)-(CR^yR^z)]_x-[F^1]_p-L^w-CHOH-CH_2-SS0_3Na$, preferably wherein at least one of R^a, R^b and R^c is $-[0-CH_2-CH_2]_t-[F^1]_p-L^w-CHOH-CH_2-SS0_3Na$.

Preferably, this derivative is reduced in a subsequent step to yield the HAS derivative comprising the functional group Z^1 with Z^1 being -SH. Any suitable methods known to those skilled in the art can be used to reduce the respective intermediate shown above. Preferably, the thiosulfonate is reduced with sodium borohydride in aqueous solution.

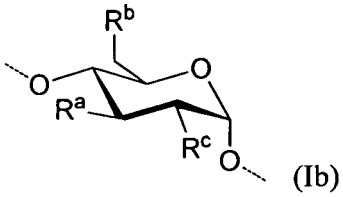
According to a preferred embodiment of the present invention, the hydroxyalkyl starch derivative comprising the functional group Z^1, obtained by the above-described method, is purified in a further step. Again, the purification of the HAS derivative from step (III) can be carried out by any suitable method such as ultrafiltration, dialysis or precipitation or a combined method using for example precipitation and afterwards ultrafiltration. Furthermore, the HAS derivative may be lyophilized, as described above, using conventional methods, prior to step (b).

Synthesis of the hydroxyalkyl starch derivative via the reaction of the carboxy activated hydroxyalkyl starch with a crosslinking compound (linker)

According to a second embodiment, in step (a2)(i), a linker is used, comprising the functional Z^1 or the functional group W, wherein W has the structure $-Z^{1*}$-PG, with PG being a suitable protecting group. Preferably, in case this linker is used, the hydroxyalkyl starch is activated prior to the reaction using a reactive carbonate as described above.

Thus, the present invention also relates to a method, as described above, wherein step (a2)(i) comprises

(aa) activating at least one hydroxyl group comprised in the hydroxyalkyl starch with a reactive carbonyl compound having the structure R^{**}-(C=0)-R^{*}, wherein R^{*} and R^{**} may be the same or different, and wherein R^{*} and R^{**} are both leaving groups, wherein upon activation a hydroxyalkyl starch derivative comprising at least one structural unit according to the following formula (lb),



(Ib)

is formed, in which R^a, R^b and R^c are independently of each other selected from the group consisting of-O-HAS", -[0-CH_2-CH_2]$_s$-OH, -[0-CH_2-CH_2]$_t$-O-C(=O)–R^*,

wherein s is in the range of from 0 to 4,

and wherein t is in the range of from 0 to 4,

and wherein at least one of R^a, R^b and R^c comprises the group -[0-CH_2-CH_2]$_t$-0-C(=0)-R*, and

(bb) reacting the activated hydroxyalkyl starch derivative according to step (aa) with the suitable linker comprising the functional group Z^1 or a precursor of the functional group Z^1.

The invention further relates to a conjugate obtained or obtainable by said method.

In particular, in step (a2)(i) the hydroxyalkyl starch is reacted with a linker comprising the functional group Z^1 or a precursor thereof and a functional group Z^2, the linker preferably having the structure Z^2-L^1-Z^1 or Z^2-L^1-Z^{1*}-PG, with Z^2 being a functional group capable of being reacted with the hydroxyalkyl starch or an activated hydroxyalkyl starch, preferably with an activated hydroxyalkyl starch, the method further comprising activating the hydroxyalkyl starch prior to the reaction with the linker using a reactive carbonate, and with Z^{1*} being the protected form of the functional group Z^1.

As described above, the linker preferably comprises a functional group Z^2, which in this case, is preferably a nucleophile, such as a group comprising an amino group, more preferably a group selected from the group consisting of -NHR^{Z2}, -NH_2, -0-NH_2, -NH-O-alkyl, -(C=G)-NH-NH_2, -G-(C=G)-NH-NH_2, -NH-(C=G)-NH-NH_2, and -$S0_2$-NH-NH_2 wherein G is O or S, and if present twice in one structural unit, may be the same or may be

different and wherein R^{Z2} is an alkyl group, preferably methyl. More preferably Z^2 is $-NH_2$ or $-NHR^{Z2}$, most preferably $-NH_2$.

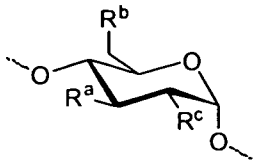
The linker has preferably a structure Z^2-L'-Z' *-PG, wherein Z^{1*} is in particular -S- (and the respective unprotected functional group Z^1 a thiol group). According to this embodiment, the linking moiety L^1 is preferably an optionally substituted alkyl group. More preferably, the linking moiety L^1 is a spacer comprising at least one structural unit according to the formula $-\{[CR^dR^f]_h-[F^4]_u-[CR^{dd}R^{ff}]_z\}_{alpha}-$, as described above, wherein integer alpha is in the range of from 1 to 10, and wherein F^4 is preferably selected from the group consisting of -S-, -O- and -NH-, more preferably wherein F^4, if present, is -O- or -S-, more preferably wherein F^4 is -S-. As described above, in the context of the preferred conjugates, residues R^d, R^f, R^{dd} and R^f are, independently of each other, preferably selected from the group consisting of halogens, alkyl groups, H or hydroxyl groups. More preferably, these residues are independently from each other H, alkyl or hydroxyl groups. Preferably, integer u and integer z of the formula $-\{[CR^dR^f]_h-[F^4]_u-[CR^{dd}R^{ff}]_z\}_{alpha}-$ are 0, and alpha is 1, the linking moiety L^1 thus corresponds to the structural unit $-[CR^dR^f]_h-$. The integer h is preferably in the range of from 1 to 20, more preferably of from 1 to 10, such as 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, more preferably of from 1 to 5, most preferably of from 1 to 3. More preferably R^d and R^f are both H. Thus, by way of example, the following preferred linker moieties L^1 are mentioned: $-CH_2-$, $-CH_2-CH_2-$, $-CH_2-CH_2-CH_2-$, $-CH_2-CH_2-CH_2-CH_2-$, $-CH_2-CH_2-CH_2-CH_2-CH_2-$, more preferably $-CH_2-CH_2-$, in the context of this embodiment.

In case Z^1 is a thiol group, and Z^{1*} is a -S- group, the group PG is preferably a thiol protecting group, more preferably a protecting group forming together with Z^{1*} a thioether (e.g. trityl, benzyl, ally]), a disulfide (e.g. S-sulfonates, S-tert.-butyl, S-(2-aminoethyl)), or a thioester (e.g. thioacetyl). In case the linker comprises a protecting group, the method further comprises a deprotection step.

In case the group Z^{1*}-PG is a disulfide, and Z^{1*} is -S-, the linker Z^2-L'-S-PG is preferably a symmetrical disulfide, with PG having the structure $-S-L^1-Z^2$. As preferred linker compound, thus cystamine and the like, may be mentioned.

In the context of this embodiment, the following linker compounds having the structure Z^2-L^1-Z^{1*}-PG are mentioned by way of example: $H_2N-CH_2-S-Trt$, $H_2N-CH_2-CH_2-S-Trt$, $H_2N-CH_2-CH_2-CH_2-S-Trt$, $H_2N-CH_2-CH_2-CH_2-CH_2-S-Trt$, $H_2N-CH_2-CH_2-CH_2-CH_2-CH_2-S-Trt$, $H_2N-CH_2-CH_2-S-S-CH_2-CH_2-NH_2$, $H_2N-CH_2-CH_2-S-S-tBu$, wherein Trt is a trityl group.

Subsequent to the activation, the hydroxyalkyl starch is preferably reacted with the linker Z^2-L^1-Z^{1*}-PG, thereby most preferably forming a derivative, comprising the functional group Z^{1*}-PG, more preferably this derivative comprises at least one structural unit according to the following formula (lb)



(Ib)

wherein at least one of R^a, R^b and R^c is $-[O-(CR^wR^x)-(CR^yR^z)]_x$-$F^1$-$L^1$-$Z^{1*}$-PG, more preferably wherein R^a, R^b and R^c are independently of each other selected from the group consisting of -O-HAS", -[0-CH_2-CH_2]$_s$-OH, and -[0-CH_2-CH_2],-F^1-L^1-Z^{1*}-PG, wherein t is in the range of from 0 to 4, and wherein s is in the range of from 0 to 4, and wherein at least one of R^a, R^b and R^c comprises the group $-[O-CH_2-CH_2]_t$-F^1-L^1-Z^{1*}-PG, and wherein F^1 is the functional group being formed upon reaction of the group -0-C(=0)-R^* with the functional group Z^2. According to a preferred embodiment, the functional group Z^2 is -NH_2, thus F^1 preferably has the structure -0-C(=0)-NH-.

The coupling reaction between the activated hydroxyalkyl starch and the linker, comprising the functional Z^1 or the functional group W, wherein W has preferably the structure -Z^{1*}PG, with PG being a suitable protecting group, in principle any reaction conditions known to those skilled in the art can be used. Preferably, the reaction is carried out in an organic solvent, such as N-methyl pyrrolidone, dimethyl acetamide (DMA), dimethyl formamide (DMF), formamide, dimethyl sulfoxide (DMSO), or mixtures of two or more thereof, preferably at a temperature in the range of from 5 to 80° C, more preferably of from 5 to 50 °C and especially preferably of from 15 to 30 °C. The temperature may be held essentially constant or may be varied during the reaction procedure.

The pH value for this reaction may be adapted to the specific needs of the reactants. Preferably, the reaction is carried out in the presence of a base. Among the preferred bases pyridine, substituted pyridines, such as 4-(dimethylamino)-pyridine, 2,6-lutidine or collidine, tertiary amine bases such as triethyl amine, diisopropyl ethyl amine (DIEA), N-methyl morpholine, amidine bases such as 1,8-diazabicyclo[5.4.0]undec-7-ene or inorganic bases such as alkali metal carbonates may be mentioned.

The reaction time for the reaction of activated hydroxyalkyl starch with the linker Z^2-L'-Z'*-PG or Z^2-L'-Z' may be adapted to the specific needs and is generally in the range of

from 1 h to 7 days, preferably of from 2 hours to 48 hours, more preferably of from 4 hours to 24 hours.

The derivative comprising the functional group Z'*-PG or Z^1, may be subjected to at least one further isolation and/or purification step. According to a preferred embodiment of the present invention, the polymer derivative is first separated from the reaction mixture by a suitable method such as precipitation and subsequent centrifugation or filtration. In a second step, the separated polymer derivative may be subjected to a further treatment such as an after-treatment like ultrafiltration, dialysis, centrifugal filtration or pressure filtration, ion exchange chromatography, reversed phase chromatography, HPLC, MPLC, gel filtration and/or lyophilization. According to an even more preferred embodiment, the separated polymer derivative is first precipitated, subjected to centrifugation, re-dissolved and finally subjected to ultrafiltration.

Preferably, the precipitation is carried out with an organic solvent such as ethanol, isopropanol, acetone or tetrahydrofurane (THF). The precipitated conjugate is subsequently subjected to centrifugation and subsequent ultrafiltration using water or an aqueous buffer solution having a concentration preferably from 1 to 1000 mmol/1, more preferably from 1 to 100 mmol/1, and more preferably from 10 to 50 mmol/1 such as about 20 mmol/1, a pH value preferably in the range of from 3 to 10, more preferably of from 4 to 8, such as about 7. The number of exchange cycles preferably is in the range of from 5 to 50, more preferably of from 10 to 30, and even more preferably of from 15 to 25, such as about 20.

Most preferably the obtained derivative is further lyophilized until the solvent content of the reaction product is sufficiently low according to the desired specifications of the product.

In case the linker comprises a protecting group (PG), the method preferably further comprises a deprotection step. The reaction conditions used are adapted to the respective protecting group used. According to a preferred embodiment of the invention, Z^1 is a thiol group, and the group Z^{1*}-PG is a disulfide, as described above. In this case, the deprotection step comprises the reduction of this disulfide bond to give the respective thiol group. This deprotection step is carried out using specific reducing agents.

As possible reducing agents, complex hydrides such as borohydrides, especially sodium borohydride, and thiols, especially dithiothreitol (DTT) and dithioerythritol (DTE) or phosphines such as tris-(2-carboxyethyl)phosphine (TCEP) are mentioned. The reduction is preferably carried out using DTT.

The deprotection step is preferably carried out at a temperature in the range of from 0 to 80 °C, more preferably of from 10 to 50 °C and especially preferably of from 20 to 40 °C. During the course of the reaction, the temperature may be varied, preferably in the above-given ranges, or held essentially constant.

Preferably, the reaction is carried out in aqueous medium. The term "aqueous medium" as used in the context of the present invention refers to a solvent or a mixture of solvents comprising water in an amount of at least 10 % per weight, preferably at least 20 % per weight, more preferably at least 30 % per weight, more preferably at least 40 % per weight, more preferably at least 50 % per weight, more preferably at least 60 % per weight, more preferably at least 70 % per weight, more preferably at least 80 % per weight, even more preferably at least 90 % per weight or up to 100 % per weight, based on the weight of the solvents involved. The aqueous medium may comprise additional solvents like formamide, dimethylformamide (DMF), dimethylsulfoxide (DMSO), alcohols such as methanol, ethanol or isopropanol, acetonitrile, tetrahydrofurane or dioxane. Preferably, the aqueous solution contains a transition metal chelator (disodium ethylenediaminetetraacetate, EDTA, or the like) in a concentration ranging from 0.01 to 100 mM, preferably from 0.01 to 1 mM, most preferably from 0.1 to 0.5 mM, such as about 0.4 mM.

The pH value in the deprotection step may be adapted to the specific needs of the reactants. Preferably, the reaction is carried out in buffered solution, at a pH value in the range of from 3 to 14, more preferably of from 5 to 11, and even more preferably of from 7.5 to 8.5. Among the preferred buffers, carbonate, phosphate, borate and acetate buffers as well as tris(hydroxymethyl)aminomethane (TRIS) may be mentioned.

Again, at least one of the isolation steps/and or purification steps, as described above, may be carried out subsequent to the deprotection step. Most preferably the obtained derivative is further lyophilized prior to step (b) until the solvent content of the reaction product is sufficiently low according to the desired specifications of the derivative.

Step (a2)(ii)

As regards step (a2)(ii) of the method according to the present invention, in this step, the functional group Z^1 is introduced by displacing a hydroxyl group present in the hydroxyalkyl starch in a substitution reaction with a precursor of the functional group Z^1 or with a bifunctional linker comprising the functional group Z^1 or a precursor thereof.

Preferably, prior to the replacement of the hydroxyl group with the functional group Z^1, the at least one hydroxyl group of the hydroxyalkyl starch is activated to generate a suitable leaving group. Preferably, a group R^L is added to the at least one hydroxyl group thereby generating a group -0-R^L, wherein the structural unit -0-R^L is the leaving group.

Thus, the present invention also relates to a method for preparing a hydroxyalkyl starch conjugate, as described above, as well as to a hydroxyalkyl starch conjugate obtained or obtainable by said method wherein in step (a2)(ii), prior to the substitution (displacement) of the hydroxyl group with the group comprising the functional group Z^1 or a precursor thereof, a group R^L is added to at least one hydroxyl group thereby generating a group -0-R^L, wherein -0-R^L is the leaving group.

The term "leaving group" as used in this context of the present invention is denoted to mean that the molecular fragment -0-R^L departs when reacting the hydroxyalkyl starch derivative with a reagent, such as a crosslinking compound, comprising the functional group Z^1 or a precursor thereof.

As regards, preferred leaving groups used in this context of the present invention, according to a preferred embodiment, the hydroxyl group is transformed to a sulfonic ester, such as a mesylic ester (-OMs), tosylic ester (-OTs), imsyl ester (imidazylsulfonyl ester) or a carboxylic ester such as trifluoracetyl ester.

Preferably, the at least one leaving group is generated by reacting at least one hydroxyl group of hydroxyalkyl starch, preferably in the presence of a base, with the respective sulfonyl chloride to give the sulfonic ester, preferably the mesylic ester.

Thus, the present invention also relates to a method for preparing a hydroxyalkyl starch conjugate as described above, as well as to a hydroxyalkyl starch conjugate obtained or obtainable by said method, wherein in step (a2)(ii), prior to the substitution (displacement) of the hydroxyl group with the group comprising the functional group Z^1 or a precursor thereof, a group R^L is added to at least one hydroxyl group, thereby generating a group -0-R^L, wherein -0-R^L is -O-Ms or -OTs, and wherein the -O-Ms group is preferably introduced by reacting at least one hydroxyl group of hydroxyalkyl starch with methanesulfonyl chloride, and -OTs is introduced by reacting at least one hydroxyl group with toluenesulfonylchloride.

The addition of the group R^L to at least one hydroxyl group of hydroxyalkyl starch, whereupon a group -0-R^L is formed, is preferably carried out in an organic solvent, such as N-methyl pyrrolidone, dimethyl acetamide (DMA), dimethyl formamide (DMF),

formamide, dimethylsulfoxide (DMSO) and mixtures of two or more thereof, preferably at a temperature in the range of from -60 to 80° C, more preferably in the range of from -30 to 50 °C and especially preferably in the range of from -30 to 30 °C. The temperature may be held essentially constant or may be varied during the reaction procedure. The pH value for this reaction may be adapted to the specific needs of the reactants. Preferably, the reaction is carried out in the presence of a base. Among the preferred bases pyridine, substituted pyridines such as collidine or 2,6-lutidine, tertiary amine bases such as triethylamine, diisopropyl ethyl amine (D1EA), N-methyl morpholine, N-methylimidazoIe or amidine bases such as l,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and inorganic bases such as metal hydrides and carbonates may be mentioned. Especially preferred are substituted pyridines (collidine) and tertiary amine bases (DIPEA, N-methylmorpholine). The reaction time for this reaction step may be adapted to the specific needs and is generally in the range of from 5 min to 24 hours, preferably of from 15 min to 10 hours, more preferably of from 30 min to 5 hours.

The derivative comprising the group -0-R^L, may be subjected to at least one further isolation and/or purification step such as precipitation and/or centrifugation and/or filtration prior to the substitution reaction according to step (a2)(ii). Likewise, instead or additionally, the derivative comprising the -0-R^L group may be subjected to an after-treatment like ultrafiltration, dialysis, centrifugal filtration or pressure filtration, ion exchange chromatography, reversed phase chromatography, HPLC, MPLC, gel filtration and/or lyophilization. According to a preferred embodiment, the derivative comprising the -0-R^L is in situ reacted with the precursor of the functional group Z^1 or with the bifunctional linker, comprising the functional group Z^1 or a precursor thereof.

As described above, the at least one hydroxyl group, preferably the at least one -0-R^L group, more preferably the O-Ms group, is displaced, in a substitution reaction, with the precursor of the functional group Z^1 or with an at least bifunctional linker comprising the functional group Z^1 or a precursor thereof.

According to a preferred embodiment of the present invention, the activated hydroxyl group, preferably the -0-R^L group, more preferably the O-Ms group, is reacted with the precursor of the functional group Z^1. The term "a precursor" as used in this context of the present invention is denoted to mean a reagent which is capable of displacing the group, thereby forming a functional group Z^1 or a group, which can be modified in at least one further step to give the functional group Z^1.

Thus, the present invention also relates to a method for preparing a hydroxyalkyl starch conjugate, as described above, as well as to a hydroxyalkyl starch conjugate, obtained or

obtainable by said method wherein in step (a2)(ii), prior to the substitution (displacement) of the hydroxyl group with the group comprising the functional group Z^1 or a precursor thereof, a group R^L is added to at least one hydroxyl group, thereby generating a group -0-R^L, wherein -0-R^L is a leaving group, and subsequently -0-R^L is replaced by a precursor of the functional group Z^1, the method further comprising converting the precursor after the substitution reaction to the functional group Z^1, and wherein Z^1 is preferably a thiol group.

In case Z^1 is an amine, reagents such as ammonia, hydrazine, acyl hydrazides, such as carbohydrazide, potassium phthalimide, azides, such as sodium azide, and the like, can be employed to introduce the functional group Z^1.

In case Z^1 is a thiol group, reagents such as thioacetic acid, alkyl- or aryl-thiosulfonates such as sodium benzenethiosulfonate, thiourea, thiosulfate or hydrogen sulfide can be employed as precursor to introduce the functional group Z^1.

According to an especially preferred embodiment of the present invention, the hydroxyl group present in the hydroxyalkyl starch is first activated and then reacted with thioacetate, thereby replacing the hydroxyl group with the structure **-S-C(=0)-CH3.** A particularly preferred reagent is potassium thioacetate. Thus, the present invention also relates to a method, as described above, wherein in step (a2)(ii) the hydroxyl group present in the hydroxyalkyl starch is reacted with thioacetate giving a functional group having the structure -S-C(=0)-CH_3.

In this substitution step, in principle any reaction conditions known to those skilled in the art can be used. Preferably, the reaction is carried out in at least one organic solvent, such as N-methyl pyrrolidone, dimethyl acetamide (DMA), dimethyl formamide (DMF), formamide, dimethyl sulfoxide (DMSO) and mixtures of two or more thereof. Preferably this step is carried out at a temperature in the range of from 0 to 80° C, more preferably of from 20 to 70 °C and especially preferably of from 40 to 60 °C. The temperature may be held essentially constant or may be varied during the reaction procedure.

The pH value for this reaction may be adapted to the specific needs of the reactants. Optionally, the reaction is carried out in the presence of a scavenger, which reacts with the leaving group -0-R^L, such as mercaptoethanol or the like.

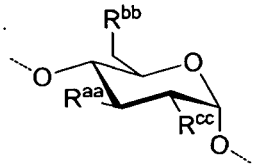
The reaction time for the substitution step is generally in the range of from 1 hour to 7 days, preferably of from 3 to 48 hours, more preferably of from 4 to 18 hours.

The derivative obtained may be subjected to at least one further isolation and/or purification step, as described above.

Preferably, the derivative is subjected to at least one further step. In particular, in case the hydroxyl group present in the hydroxyalkyl starch is reacted with thioacetate, thereby replacing the hydroxyl group with the structure -S-C(=0)-CH_3, the derivative is preferably saponified in a subsequent step to give the functional group Z^1 with Z^1 being an -SH group.

Thus, the present invention also relates to a method as described above as well as to a conjugate obtained or obtainable by said method, wherein in step (a2)(ii), the hydroxyl group present in the hydroxyalkyl starch is reacted with thioacetate giving a functional group having the structure -S-C(-0)-CH_3, wherein the method further comprises saponification of the group -S-C(=0)-CH_3 to give the functional group Z^1.

It has to be understood, that in case at least one hydroxyl group present in hydroxyalkyl starch, comprising the structural unit according to the following formula (II)

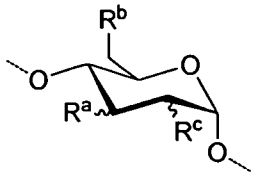


(II)

with R^{33}, R^{bb} and R^{cc} being independently of each other selected from the group consisting of - [0 –(CR^{w}R^{x}HCR ^{y}R^z)]$_x$-OH and -O-HAS", is displaced in a substitution reaction, the stereochemistry of the carbon atom which bears the respective hydroxyl function, which is displaced may be inverted.

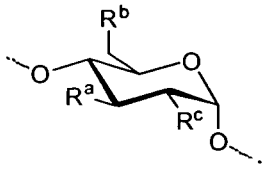
Thus, in case at least one of R^{33} and R^{bb} in the above shown structural unit is -OH, and in case, this at least one group is displaced by a precursor of the functional group Z^1, thereby yielding in a hydroxyalkyl starch derivative comprising the functional group Z^1 in this structural unit, the stereochemistry of the carbon atoms bearing this functional group Z^1 may be inverted.

Since, it cannot be excluded that such a substitution of secondary hydroxyl groups occur, in the method of the invention according to step (a2)(ii), the stereochemistry of the carbon atoms bearing the functional group R^3 and R^c is not further defined, as shown in the structural unit according to the following formula (I)



(I)

However, without wanting to be bound to any theory, it is believed that mainly primary hydroxyl groups will be displaced in the substitution reaction according to step (a2)(ii). Thus, according to this theory, the stereochemistry of most carbon atoms bearing the residues R^a or R^c will not be inverted but the respective structural unit of the hydroxyalkyl starch will comprise the stereochemistry as shown in the formula (Ib)



(Ib)

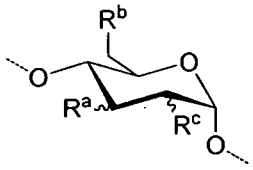
The thioacetate is preferably saponified in at least one further step to give the thiol comprising hydroxyalkyl starch derivatives. As regards the saponification of the functional group -S-C(=0)-CH_3, all methods known to those skilled in the art are encompassed by the present invention. This includes the use of at least one base (such as metal hydroxides) and strong nucleophiles (such as ammonia, amines, thiols or hydroxides) in order to saponify the present thioesters to give thiols. Preferred reagents are sodium hydroxide and ammonia.

Since thiols are well known to oxidize via the formation of disulfides, especially under basic conditions present in most saponification protocols, the molecular weight of the hydroxyalkyl starch derivative obtained may vary due to unspecific crosslinking. To prevent the formation of disulfides, preferably a reducing agent is added prior, during or after the saponification step. According to a preferred embodiment of the invention, a reducing agent is directly added to the saponification mixture in order to keep the forming thiol groups in their low oxidation state. Regarding the reduction of the thiol groups, all reduction methods known to those skilled in the art such as borohydrides, especially sodium borohydride, and thiols, especially dithiothreitol (DTT) and dithioerythritol (DTE) or phosphines such as tris-(2-carboxyethyl)phosphine (TCEP) are encompassed by the present invention. According to preferred embodiments of the present invention, dithiothreitol (DTT), dithioerythritol (DTE) or sodium borohydride are employed.

In an alternative embodiment of the reaction, aqueous sodium hydroxide is used as saponification agent together with sodium borohydride as reducing agent.

Optionally, mercaptoethanol can be used as an additive in this reaction.

Thus, the present invention also relates to a method, as described above, wherein in step (a2)(ii) the activated hydroxyl group present in the hydroxyalkyi starch is reacted with thioacetate giving a functional group having the structure -S-C(=0)-CH_3, wherein the method further comprises saponifying the group -S-C(=0)-CH_3 to give the functional group Z^1, wherein the hydroxyalkyi starch derivative comprises at least one structural unit according to the following formula (I)



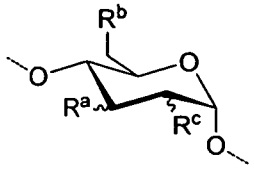
(I)

wherein R^a, R^b and R^c are independently of each other selected from the group consisting of -O-HAS", -[0-CH_2-CH_2]s-OH, -[0-CH_2-CH_2]$_t$-SH and wherein at least one R^a, R^b and R^c is -[0-CH_2-CH_2]$_t$-SH and wherein t is in the range of from 0 to 4, and wherein s is in the range of from 0 to 4.

Again, the hydroxyalkyi starch derivative, comprising the functional group SH, obtained by the above-described preferred embodiment, may be isolated/and or purified prior to step (b) in a further step. Again, the purification/isolation of the HAS derivative from step (a2)(ii) can be carried out by any suitable method such as ultrafiltration, dialysis or precipitation or a combined method using for example precipitation and afterwards ultrafiltration.

Furthermore, the hydroxyalkyi starch derivative may be lyophilized, as described above, using conventional methods.

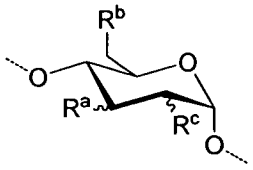
According to an especially preferred embodiment, the hydroxyalkyi starch derivative, obtained in step (a2)(ii), comprises at least one structural unit according to the following formula (I)



(I)

wherein R^a, R^b and R^c are independently of each other selected from the group consisting of-O-HAS", -[0-CH $_2$-CH $_2$]s-OH, –[O–CH_2-CH_2]$_t$-Z^1, wherein t is in the range of from 0 to 4, and wherein s is in the range of from 0 to 4, and wherein at least one of R^a, R^b and R^c is -[0-CH $_2$-CH_2]$_t$-Z', with Z^1 being -SH. This derivative is preferably reacted in step (b) with a crosslinking compound L having a structure according to the following formula K^2-[L ^{2}]$_g$-[E]$_e$-[CR^mR^n]$_f$-K' with g and e being 0, and wherein K^2 is a halogene.

According to an especially preferred embodiment the hydroxyalkyl starch derivative obtained in step (a2)(ii) comprises at least one structural unit according to the following formula (I)



(I)

wherein R^a, R^b and R^c are independently of each other selected from the group consisting of-O-HAS", -[0-CH $_2$-CH $_2$]$_s$-OH, and –[O–CH_2-CH_2]$_t$-Z^1, wherein t is in the range of from 0 to 4, and wherein s is in the range of from 0 to 4, and wherein at least one of R^a, R^b and R^c is -[0-CH $_2$-CH_2]$_t$-Z', with Z^1 being -SH. This derivative is preferably reacted in step (b) with a crosslinking compound L having a structure according to the formula K^2-[L^2]$_g$-[E]$_e$-[CR^mR^n]$_f$-K^1, wherein K^2 is maleimide, and wherein upon reaction of Z^1 with K^2, a functional group -X-F ^{2}- is formed.

<u>Step (b)</u>

As already described above, the hydroxyalkyl starch derivative obtained according to step (a) is, optionally after at least one purification and/or isolation step, further reacted in step (b).

In step (b) the HAS derivative is coupled via the functional group Z^1 to at least one cytotoxic agent via the at least bifunctional crosslinking compound L, wherein L comprises

the functional groups K^1 and K^2, wherein L is coupled to Z^1 via a functional group K^2 comprised in L, and wherein each cytotoxic agent is coupled via the tertiary hydroxyl group to the HAS derivative via the functional group K^1, comprised in L.

Thus, step (b) preferably comprises the steps (bl) and (b2)

(bl) coupling the cytotoxic agent with the crosslinking compound L, thereby forming a derivative of the cytotoxic agent having the structure -L-M; wherein M is the residue of the cytotoxic agent;

(b2) coupling the derivative of the cytotoxic agent having the structure -L-M with the hydroxyalkyl starch derivative according to step (a), thereby forming the hydroxyalkyl starch conjugate.

As to the preferred reaction conditions used in step (bl), reference is made to the details given above.

As regards to the reaction conditions used in step (b2), in principle any reaction conditions known to those skilled in the art can be used. Preferably, the reaction is carried out in an aqueous reaction medium, preferably in a mixture comprising water and at least one organic solvent, preferabaly at least one water miscible solvent, in particular a solvent selected from the group such as N-methyl pyrrolidone, dimethyl acetamide (DMA), dimethyl formamide (DMF), formamide, dimethyl sulfoxide (DMSO), acetonitrile, tetrahydrofurane (THF), dioxane, alcohols such as methanol, ethanol, isopropanol and mixtures of two or more thereof. More preferably, the reaction is carried out in DMF.

The temperature of the reaction is preferably in the range of from 5 to 55 °C, more preferably of from 10 to 30 °C, and especially preferably of from 15 to 25 °C. During the course of the reaction, the temperature may be varied, preferably in the above given ranges, or held essentially constant.

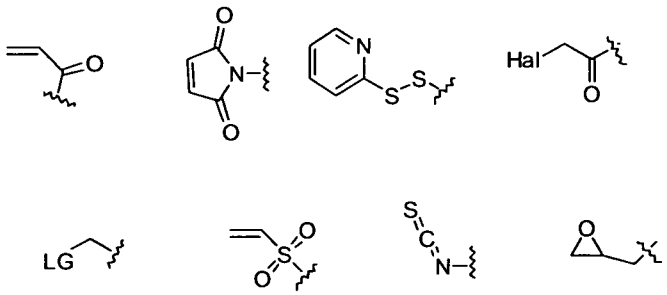
The reaction time for the reaction of step (b2) may be adapted to the specific need and is generally in the range of from 30 min to 2 days, preferably of from 1 hour to 18 hours, more preferably of from 2 hours to 6 hours.

The pH value for the reaction of step (b) may be adapted to the specific needs of the reactants. Preferably, the reaction is carried out in a buffered solution, at a pH value in the range of from 3 to 10, more preferably of from 5 to 9, and even more preferably of from 6

to 8. Among the preferred buffers, citrate buffers (pH 6.4), phosphate buffers (pH 7.5) and bicarbonate buffers (pH 8) may be mentioned.

As described above, the hydroxyalkyl starch may comprise more than one functional group Z^1, such as multiple thiol groups. Preferably, all groups Z^1 present in the hydroxyalkyl starch derivative participate in the coupling reaction in step (b2). However, it is also possible that in step (b2) not all of the functional groups Z^1 are coupled to the at least bifunctional crosslinking compound L, or preferably with the derivative of the cytotoxic agent having the structure -L-M. Thus, in this case, the hydroxyalkyl starch conjugate according to step (b2) may comprise at least one unreacted functional group Z^1.

To avoid side effects due to the presence of such unreacted functional groups Z^1, the hydroxyalkyl starch conjugate may be further reacted, as described above, in a subsequent step to step (c) with a suitable capping reagent D*. In case Z^1 is a thiol group, possible free thiol groups present in the conjugate, which may lead to unwanted side effects such as oxidative disulfide formation and consequently crosslinking, may be reacted, for example, with small molecules comprising a thiol reactive group. Examples of thiol reactive groups are given above. Preferred capping reagents D* thus in particular comprise a group selected from the group consisting of pyridyl disulfides, maleimide group, haloacetyl groups, haloacetamides, vinyl sulfones and vinyl pyridines. Preferably, the capping reagent D* comprises a thiol-reactive group selected from the group consisting of the following structures:



wherein Hal is a halogen, such as CI, Br, or I, and LG is a leaving group (or nucleofuge).

In particular D* is iodoacetic acid and/or ethylbromoacetate.

Optionally, a reducing agent such as tris-(2-carboxyethyl)phosphine (TCEP) may be added prior to the capping step in order to break existing disulfides and to keep thiols in their low oxidation state.

Thus, the present invention also decribes a method, as described above, the method further comprises

(c) reacting the hydroxyalkyl starch conjugate with a capping reagent D*.

Likewise, in case the crosslinking compound L is either reacted with the hydroxyalkyl starch derivative prior to the coupling with the cytotoxic agent, or only in a subsequent step with the cytotoxic agent, the hydroxyalkyl starch conjugate may comprise at least one unreacted functional group Z^1 and/or at least one unreacted group K^1.

In this case, the present invention may comprise a further capping step

(cl) reacting the hydroxyalkyl starch conjugate with a further capping reagent D**, wherein D** may be the same or may differ from D*, depending on the nature of functional group to be capped.

Most preferably the hydroxyalkyl starch conjugate according to step (b) comprises no unreacted functional groups Z^1 and/or no unreacted group K^1.

Preferably, the hydroxyalkyl starch conjugate obtained according to step (b), optionally according to step (c) and/or (cl), is subjected to at least one isolation and/or purification step. Isolation of the conjugate may be carried out by a suitable process which may comprise one or more steps.

According to a preferred embodiment of the present invention, the conjugate is first separated off from the reaction mixture by a suitable method such as precipitation and subsequent centrifugation or filtration. In a second step, the separated conjugate may be subjected to a further treatment such as an after-treatment like ultrafiltration, dialysis, centrifugal filtration or pressure filtration, ion exchange chromatography, reversed phase chromatography, HPLC, MPLC, gel filtration and/or lyophilization. According to an even more preferred embodiment, the separated polymer derivative is first precipitated, subjected to centrifugation, re-dissolved and finally subjected to ultrafiltration.

Preferably, the precipitation is carried out with an organic solvent such as ethanol or isopropanol. The precipitated conjugate is subsequently subjected to centrifugation and subsequent ultrafiltration using water or an aqueous buffer solution having a concentration preferably from 1 to lOOO mmol/1, more preferably from 1 to lOO mmol/1, and more preferably from 10 to 50 mmol/1 such as about 20 mmol/l, a pH value in the range of preferably of from 3 to 10, more preferably of from 4 to 8, such as about 5. The number of exchange cycles preferably is in the range of from 5 to 50, more preferably of from 10 to 30, and even more preferably of from 15 to 25, such as about 20.

Most preferably, the obtained conjugate is further lyophilized until the solvent content of the reaction product is sufficiently low according to the desired specifications of the product.

Pharmaceutical Composition

Furthermore, the present invention relates to a pharmaceutical composition comprising in a therapeutically effective amount a HAS conjugate, as described above, or a HAS conjugate, obtained or obtainable by the above described method.

As far as the pharmaceutical compositions according to the present invention comprising the hydroxyalkyl starch conjugate, as described above, are concerned, the hydroxyalkyl starch conjugate may be used in combination with a pharmaceutical excipient. Generally, the hydroxyalkyl starch conjugate will be in a solid form which can be combined with a suitable pharmaceutical excipient that can be in either solid or liquid form. As excipients, carbohydrates, inorganic salts, antimicrobial agents, antioxidants, surfactants, buffers, acids, bases, and combinations thereof may be mentioned. A carbohydrate such as a sugar, a derivatized sugar such as an alditol, aldonic acid, an esterified sugar, and/or a sugar polymer may be present as an excipient. Specific carbohydrate excipients include, for example: monosaccharides, such as fructose, maltose, galactose, glucose, D-mannose, sorbose, and the like; disaccharides, such as lactose, sucrose, trehalose, cellobiose, and the like; polysaccharides, such as raffinose, melezitose, maltodextrins, dextrans, starches, and the like; and alditols, such as mannitol, xylitol, maltitol, lactitol, xylitol, sorbitol (glucitol), pyranosyl sorbitol, myoinositol, and the like. The excipient may also include an inorganic salt or buffer such as citric acid, sodium chloride, potassium chloride, sodium sulfate, potassium nitrate, sodium phosphate monobasic, sodium phosphate dibasic, and combinations thereof. The pharmaceutical composition according to the present invention may also comprise an antimicrobial agent for preventing or determining microbial growth, such as, e.g., benzalkonium chloride, benzethonium chloride, benzyl alcohol, cetylpyridinium chloride, chlorobutanol, phenol, phenylethyl alcohol, phenylmercuric nitrate, thimersol, and combinations thereof.

The pharmaceutical composition according to the present invention may also comprise an antioxidant, such as, e.g., ascorbyl palmitate, butylated hydroxyanisole, butylated hydroxytoluene, hypophosphorous acid, monothioglycerol, propyl gallate, sodium bisulfite, sodium formaldehyde sulfoxylate, sodium metabisulfite, and combinations thereof.

The pharmaceutical composition according to the present invention may also comprise a surfactant, such as, e.g., polysorbates, or pluronics sorbitan esters; lipids, such as phospholipids and lecithin and other phosphatidylcholines, phosphatidylethanolamines, acids and fatty esters; steroids, such as cholesterol; and chelating agents, such as EDTA or zinc.

The pharmaceutical composition according to the present invention may also comprise acids or bases such as, e.g., hydrochloric acid, acetic acid, phosphoric acid, citric acid, malic acid, lactic acid, formic acid, trichloroacetic acid, nitric acid, perchloric acid, phosphoric acid, sulfuric acid, fumaric acid, and combinations thereof, and/or sodium hydroxide, sodium acetate, ammonium hydroxide, potassium hydroxide, ammonium acetate, potassium acetate, sodium phosphate, potassium phosphate, sodium citrate, sodium formate, sodium sulfate, potassium sulfate, potassium fumarate, and combinations thereof. Generally, the excipient will be present in a pharmaceutical composition according to the present invention in an amount of 0.001 to 99.999 wt.-%, preferably from 0.01 to 99.99 wt.-%, more preferably from 0.1 to 99.9 wt.-%, in each case based on the total weight of the pharmaceutical composition.

Preferably the pharmaceutical composition contains no sorbitol and/or no lactic acid.

The present invention also relates to a method of treating cancer, comprising administering to a patient suffering from cancer a therapeutically effective amount of the hydroxyalkyl starch conjugate as defined herein, or the hydroxyalkyl starch conjugate obtained or obtainable by the method according to the present invention, or the pharmaceutical composition according to the present invention.

The term "patient", as used herein, relates to animals and, preferably, to mammals. More preferably, the patient is a rodent such as a mouse or a rat. Even more preferably, the patient is a primate. Most preferably, the patient is a human. It is, however, envisaged by the method of the present invention that the patient shall suffer from cancer.

The term "cancer", as used herein, preferably refers to a proliferative disorder or disease caused or characterized by the proliferation of cells which have lost susceptibility to normal growth control. Preferably, the term encompasses tumors and any other proliferative disorders. Thus, the term is meant to include all pathological conditions involving malignant cells, irrespective of stage or of invasiveness. The term, preferably, includes solid tumors arising in solid tissues or organs as well as hematopoietic tumors (e.g. leukemias and lymphomas).

The cancer may be localized to a specific tissue or organ (e.g. in the breast, the prostate or the lung), and, thus, may not have spread beyond the tissue of origin. Furthermore the cancer may be invasive, and, thus may have spread beyond the layer of tissue in which it originated into the normal surrounding tissues (frequently also referred to as locally advanced cancer). Invasive cancers may or may not be metastatic. Thus, the cancer may be also metastatic. A cancer is metastatic, if it has spread from its original location to distant parts of the body. E.g., it is well known in the art that breast cancer cells may spread to another organ or body part, such as the lymph nodes.

Preferred cancers are breast cancer (particularly, locally advanced or metastatic breast cancer), cervical cancer, colorectal cancer, gastrointestinal cancer, leukaemia, lung cancer (particularly, locally advanced or metastatic non-small cell lung cancer), mesothelioma, non-hodgkin's lymphoma, non-small cell lung cancer, ovarian cancer, pancreatic cancer, prostate cancer (preferably, hormone-refractory prostate cancer), skin cancer, small cell lung cancer, brain tumors, uterine cancer and head and neck tumors (particularly locally advanced squamous cell carcinoma of the head and neck).

Moreover, it is also envisaged that the cancer is selected from the group consisting of Acute Lymphoblastic Leukemia (adult), Acute Lymphoblastic Leukemia (childhood), Acute Myeloid Leukemia (adult), Acute Myeloid Leukemia (childhood), Adrenocortical Carcinoma, Adrenocortical Carcinoma (childhood), AIDS-Related Cancers, AIDS-Related Lymphoma, Anal Cancer, Appendix Cancer, Astrocytomas (childhood), Atypical Teratoid/Rhabdoid Tumor (childhood), Central Nervous System Cancer, Basal Cell Carcinoma, Bile Duct Cancer (Extrahepatic), Bladder Cancer, Bladder Cancer (childhood), Bone Cancer, Osteosarcoma and Malignant Fibrous Histiocytoma, Brain Stem Glioma (childhood), Brain Tumor (adult), Brain Tumor (childhood), Brain Stem Glioma (childhood), Central Nervous System Brain Tumor, Atypical Teratoid/Rhabdoid Tumor (childhood), Brain Tumor, Central Nervous System Embryonal Tumors (childhood), Astrocytomas (childhood) Brain Tumor, Craniopharyngioma Brain Tumor (childhood), Ependymoblastoma Brain Tumor (childhood), Ependymoma Brain Tumor (childhood), Medulloblastoma Brain Tumor (childhood), Medulloepitheliom Brain Tumor (childhood), Pineal Parenchymal Tumors of Intermediate Differentiation Brain Tumor (childhood), Supratentorial Primitive Neuroectodermal Tumors and Pineoblastoma Brain Tumor, (childhood), Brain and Spinal Cord Tumors (childhood), Breast Cancer , Breast Cancer (childhood), Breast Cancer (Male), Bronchial Tumors (childhood), Burkitt Lymphoma, Carcinoid Tumor (childhood), Carcinoid Tumor,Gastrointestinal, Carcinoma of Unknown Primary, Central Nervous System Atypical Teratoid/Rhabdoid Tumor (childhood), Central Nervous System Embryonal Tumors (childhood), Central Nervous System (CNS) Lymphoma, Primary Cervical Cancer, Cervical Cancer (childhood), Childhood Cancers,

Chordoma (childhood), Chronic Lymphocytic Leukemia, Chronic Myelogenous Leukemia, Chronic Myeloproliferative Disorders, Colon Cancer, Colorectal Cancer (childhood), Craniopharyngioma (childhood), Cutaneous T-Cell Lymphoma, Embryonal Tumors, Central Nervous System (childhod), Endometrial Cancer, Ependymoblastoma (childhood), Ependymoma (childhood), Esophageal Cancer, Esophageal Cancer (childhood), Esthesioneuroblastoma (childhood), Ewing Sarcoma Family of Tumors, Extracranial Germ Cell Tumor (childhood), Extragonadal Germ Cell Tumor, Extrahepatic Bile Duct Cancer, Eye Cancer, Intraocular Melanoma, Eye Cancer, Retinoblastoma, Gallbladder Cancer, Gastric (Stomach) Cancer, Gastric (Stomach) Cancer (childhood), Gastrointestinal Carcinoid Tumor, Gastrointestinal Stromal Tumor (GIST), Gastrointestinal Stromal Cell Tumor (childhood), Germ Cell Tumor, Extracranial (childhood), Germ Cell Tumor, Extragonadal, Germ Cell Tumor, Ovarian, Gestational Trophoblastic Tumor, Glioma (adult), Glioma (childhood) Brain Stem, Hairy Cell Leukemia, Head and Neck Cancer, Heart Cancer (childhood), Hepatocellular (Liver) Cancer (adult) (Primary), Hepatocellular (Liver) Cancer (childhood) (Primary), . Histiocytosis, Langerhans Cell, Hodgkin Lymphoma (adult), Hodgkin Lymphoma (childhood), Hypopharyngeal Cancer, Intraocular Melanoma, Islet Cell Tumors (Endocrine Pancreas), Kaposi Sarcoma, Kidney (Renal Cell) Cancer, Kidney Cancer (childhood), Langerhans Cell Histiocytosis, Laryngeal Cancer, Laryngeal Cancer (childhood), Leukemia, Acute Lymphoblastic (adult), Leukemia, Acute Lymphoblastic (childhood), Leukemia, Acute Myeloid (adult), Leukemia, Acute Myeloid (childhood), Leukemia, Chronic Lymphocytic, Leukemia, Chronic Myelogenous, Leukemia, Hairy Cell, Lip and Oral Cavity Cancer, Liver Cancer (adult) (Primary), Liver Cancer (childhood) (Primary), Non-Small Cell Lung Cancer, Small Cell Lung Cancer, Non-Hodgkin Lymphoma, (adult), Non-Hodgkin Lymphoma, (childhood), Primary Central Nervous System (CNS) Lymphoma, Waldenstrom , Macroglobulinemia, Malignant Fibrous Histiocytoma of Bone and Osteosarcoma, Medulloblastoma (childhood), Medulloepithelioma (childhood), Melanoma, Intraocular (Eye)Melanoma, Merkel Cell Carcinoma, Mesothelioma (adult) Malignant, Mesothelioma (childhood), Metastatic Squamous Neck Cancer with Occult Primary, Mouth Cancer, Multiple Endocrine Neoplasia Syndromes (childhood), Multiple Myeloma/Plasma Cell Neoplasm, Mycosis Fungoides, Myelodysplastic Syndromes, Myelodysplastic/Myeloproliferative Neoplasms, Myelogenous Leukemia, Chronic, Myeloid Leukemia (adult) Acute, Myeloid Leukemia (childhood) Acute, Myeloma, Multiple, Nasal Cavity and Paranasal Sinus Cancer, Nasopharyngeal Cancer, Nasopharyngeal Cancer (childhood), Neuroblastoma, Oral Cancer (childhood), Lip and Oral Cavity Cancer, Oropharyngeal Cancer, Osteosarcoma and Malignant Fibrous, Histiocytoma of Bone, Ovarian Cancer (childhood), Ovarian Epithelial Cancer, Ovarian Germ Cell Tumor, Ovarian Low Malignant Potential Tumor, Pancreatic Cancer, Pancreatic Cancer (childhood), Pancreatic Cancer, Islet Cell Tumors, Papillomatosis (childhood), Paranasal Sinus and Nasal Cavity Cancer, Parathyroid Cancer,

Penile Cancer, Pharyngeal Cancer, Pineal Parenchymal Tumors of Intermediate Differentiation (childhood), Pineoblastoma and Supratentorial Primitive Neuroectodermal Tumors (childhood), Pituitary Tumor, Plasma Cell Neoplasm/Multiple Myeloma, Pleuropulmonary Blastoma, Pregnancy and Breast Cancer, Primary Central Nervous System (CNS) Lymphoma, Prostate Cancer, Rectal Cancer, Renal Cell (Kidney) Cancer, Renal Pelvis and Ureter Transitional Cell Cancer, Respiratory Tract Cancer with Chromosome 15 Changes, Retinoblastoma, Rhabdomyosarcoma (childhood), Salivary Gland Cancer, Salivary Gland Cancer (childhood), Sarcoma, Ewing Sarcoma Family of Tumors, Kaposi Sarcoma, Soft Tissue (adult)Sarcoma, Soft Tissue (childhood)Sarcoma, Uterine Sarcoma, Sezary Syndrome, Skin Cancer (Nonmelanoma), Skin Cancer (childhood), Skin Cancer (Melanoma), Merkel Cell Skin Carcinoma, Small Cell Lung Cancer, Small Intestine Cancer, Soft Tissue Sarcoma (adult), Soft Tissue Sarcoma (childhood), Squamous Cell Carcinoma, see Skin Cancer (Nonmelanoma), Stomach (Gastric) Cancer, Stomach (Gastric) Cancer (childhood), Supratentorial Primitive Neuroectodermal Tumors (childhood), Cutaneous T-Cell Lymphoma, Testicular Cancer, Testicular Cancer (childhood), Throat Cancer, Thymoma and Thymic Carcinoma, Thymoma and Thymic Carcinoma (childhood), Thyroid Cancer, Thyroid Cancer (childhood), Transitional Cell Cancer of the Renal Pelvis and Ureter, T Gestational rophoblastic Tumor, Unknown Primary Site, Carcinoma of adult, Unknown Primary Site, Cancer of (childhood), Unusual Cancers of hildhood, Ureter and Renal Pelvis, Transitional Cell Cancer, Urethral Cancer, Uterine Cancer, Endometrial, Uterine Sarcoma, Vaginal Cancer, Vaginal Cancer (childhood), Vulvar Cancer, Waldenstrom Macroglobulinemia, Wilms Tumor.

The terms "treating cancer" and "treatment of cancer", preferably, refer to therapeutic measures, wherein the object is to prevent or to slow down (lessen) an undesired physiological change or disorder, such as the growth, development or spread of a hyperproliferative condition, such as cancer. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. It is to be understood that a treatment can also mean prolonging survival as compared to expected survival if not receiving treatment.

The term "administering" as used herein, preferably, refers to the introduction of the hydroxyalkyl starch conjugate as defined herein, the hydroxyalkyl starch conjugate obtained or obtainable by the method according to the present invention, or the pharmaceutical composition according to the present invention into cancer patients.

Methods for administering a particular compound are well known in the art and include parenteral, intravascular, paracanceral, transmucosal, transdermal, intramuscular (i.m.), intravenous (i.v.), intradermal, subcutaneously (s.c), sublingual, intraperitoneal (i.p.), intraventricularly, intracranial, intravaginal, intratumoral, and oral administration. It is to be understood that the route of administration may depend on the cancer to be treated. Preferably, the hydroxyalkyl starch conjugate as defined herein, the hydroxyalkyl starch conjugate obtained or obtainable by the method according to the present invention, or the pharmaceutical composition according to the present invention are administered parenterally. More preferably, it is administered intravenously. Preferably, the administration of a single dose of a therapeutically effective amount of the aforementioned compounds is over a period of 5 min to 5 h.

Preferably, the conjugates are administered together with a suitable carrier, and/or a suitable diluent, such as preferably a sterile solutions for i.v., i.m., i.p. or s.c. application.

The term "therapeutically effective amount", as used herein, preferably refers to an amount of the hydroxyalkyl starch conjugate as defined herein, the hydroxyalkyl starch conjugate obtained or obtainable by the method according to the present invention, or the pharmaceutical composition according to the present invention that (a) treats the cancer (b) attenuates, ameliorates, or eliminates the cancer. More preferably, the term refers to the amount of the cytotoxic agent present in the hydroxyalkyl starch conjugate as defined herein, the hydroxyalkyl starch conjugate obtained or obtainable by the method according to the present invention, or the pharmaceutical composition according to the present invention that (a) treats the cancer (b) attenuates, ameliorates, or eliminates the cancer. How to calculate the amount of a cytotoxic agent present in the aforementioned conjugates or pharmaceutical composition is described elsewhere herein. It is particularly envisaged that the therapeutically effective amount of the aforementioned compounds shall reduce the number of cancer cells; reduce the tumor size; inhibit (i.e., slow to some extent and preferably stop) cancer cell infiltration into peripheral organs; inhibit (i.e., slow to some extent and preferably stop) tumor metastasis; inhibit, at least to some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the cancer. Whether a particular amount of the aforementioned compounds exerts these effects (and, thus is pharmaceutically effective) can be determined by well known measures. Particularly, it can be determined by assessing cancer therapy efficacy. Cancer therapy efficacy, e. g., can be assessed by determining the time to disease progression and/or by determining the response rate. Thus, the required dosage will depend on the severity of the condition being treated, the patient's individual response, the method of administration used, and the like. The skilled person is able to establish a correct dosage based on his general knowledge.

Advantageously, it has been shown in the studies carried out in the context of the present invention that

i) the cytotoxic agent is less toxic when present in the conjugates described herein as compared to an agent not being present in a conjugate and/or

ii) that the use of said conjugate, or of the pharmaceutical composition comprising said conjugate allows for a more efficient treatment of cancer in a subject (see Example 2).

Moreover, the present invention relates to the hydroxyalkyl starch conjugate as defined above, or the hydroxyalkyl starch conjugate obtained or obtainable by the method according to the present invention, or the pharmaceutical composition according to the present invention for use as a medicament.

Moreover, the present invention relates to the hydroxyalkyl starch conjugate as defined above, or the hydroxyalkyl starch conjugate obtained or obtainable by the method according to the present invention, or the pharmaceutical composition according to the present invention for the treatment of cancer.

Also envisaged by the present invention is the hydroxyalkyl starch conjugate as defined above, or the hydroxyalkyl starch conjugate obtained or obtainable by the method according to the present invention, or the pharmaceutical composition according to the present invention for the treatment of cancer selected from the group consisting of breast cancer, cervical cancer, colorectal cancer, gastrointestinal cancer, leukaemia, lung cancer, mesothelioma, non-hodgkin's lymphoma, non-small cell lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, skin cancer, small cell lung cancer, brain tumors, uterine cancer and head and neck tumors.

Finally, the present invention pertains to the use of the hydroxyalkyl starch conjugate as defined above, or the hydroxyalkyl starch conjugate obtainable or obtainable by the method according to the present invention, or the pharmaceutical composition according to the present invention for the manufacture of a medicament for the treatment of cancer. Preferably, the cancer is selected from the group consisting of breast cancer, cervical cancer, colorectal cancer, gastrointestinal cancer, leukaemia, lung cancer, mesothelioma, non-hodgkin's lymphoma, non-small cell lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, skin cancer, small cell lung cancer, brain tumors, uterine cancer and head and neck tumors.

How to administer the conjugates, compositions or medicaments has been explained elsewhere herein.

The following especially preferred embodiments are described:

1. A hydroxyalkyl starch (HAS) conjugate comprising a hydroxyalkyl starch derivative and a cytotoxic agent, said conjugate having a structure according to the following formula

$$HAS'(-L-M)_n$$

wherein
M is a residue of a cytotoxic agent, said cytotoxic agent comprising a tertiary hydroxyl group,
L is a linking moiety,
HAS' is a residue of the hydroxyalkyl starch derivative,
n is greater than or equal to 1,
wherein the hydroxyalkyl starch derivative has a mean molecular weight MW above the renal threshold, preferably a MW greater than or equal to 60 kDa,
and a molar substitution MS in the range of from 0.6 to 1.5,
and wherein the linking moiety L is linked to a tertiary hydroxyl group of the cytotoxic agent.

2. The conjugate according to embodiment 1, wherein the hydroxyalkyl starch conjugate is a hydroxyethyl starch (HES) conjugate.

3. The conjugate according to embodiment 1 or 2, wherein the hydroxyalkyl starch derivative has a mean molecular weight MW in the range of from 60 to 1500 kDa, preferably in the range of from 200 to 1000 kDa, more preferably in the range of from 250 to 800 kDa.

4. The conjugate according to any of embodiments 1 to 3, wherein the hydroxyalkyl starch derivative has a molar substitution MS in the range of from 0.70 to 1.45, more preferably in the range of from 0.80 to 1.40, more preferably in the range of from 0.85 to 1.35, more preferably in the range of from 0.95 to 1.30.

5. The conjugate according to any of embodiments 1 to 4, wherein the linking moiety L has a structure -L'-F^3-, wherein F^3 is a functional group linking L' to M via the group -O- derived from the tertiary hydroxyl group of the cytotoxic agent, thereby forming a group -F^3-O- , F^3 preferably being -C(=Y)-, with Y being O, NH or S, preferably O or S, and wherein L' is a linking moiety.

6. The conjugate according to embodiment 5, wherein the bond between the functional group F^3 and the functional group -O- of the residue of the cytotoxic agent M is a cleavable linkage, which is capable of being cleaved *in vivo* so as to release the cytotoxic agent, wherein the functional group -O- is derived from the tertiary hydroxyl group of the cytotoxic agent.

7. The conjugate according to embodiment 5 or 6, wherein the conjugate comprises an electron-withdrawing group in alpha, beta or gamma position relative to each F^3 group, wherein the electron-withdrawing group is selected from the group consisting of –0-, -S-, -SO-, -S0 $_2$-, -NR^e-, cyclic imide groups, -C(=Y^e)-, -NR^e-C(=Y^e)-, -C(=Y>NR ^{e}-, -CH(N0 $_2$)-, -CH(CN)-, aryl moieties or an at least partially fluorinated alkyl moiety, wherein Y^e is either O, S or NR^e, and R^e is hydrogen or alkyl,
preferably wherein the electron-withdrawing group is selected from the group consisting of -NH-C(=0)-, -C(=0)-NH-, -NH-, -0-, -S-, -SO-, -S0 $_2$- and -succinimide-.

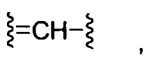
8. The conjugate according to embodiment 7, wherein the conjugate comprises
(i) an electron-withdrawing group selected from the group consisting of -S- and -O— in alpha position to each F^3 group, or
(ii) an electron-withdrawing group selected from the group consisting of -C(=0)—NH—, -NH-C(=0)- and -succinimide- in beta position to each F^3 group, or
(iii) the group -C(=0)-NH- in alpha position as electron-withdrawing group.

9. The conjugate according to any of embodiments 5 to 8, wherein L' has a structure according to the following formula

$$-[F^2]_q-[L^2]_g-[E]_e-[CR^m]RV$$

wherein E is an electron-withdrawing group, preferably selected from the group consisting of-C(=0)-NH- , -NH-C(=0)-, -NH-, -0-, -S-, -SO- , -S0 $_2$- and -succinimide-,
L^2 is a linking moiety, preferably an alkyl, alkenyl, alkylaryl, arylalkyl, aryl, heteroaryl, alkylheteroaryl or heteroarylalkyl group,

F^2 is selected from the group consisting of -Y^1- , -C(=Y ^{2})-, -C(=Y ^{2})-NR ^{F2}- ,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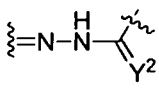
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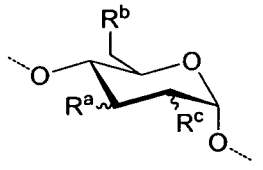
and -CH_2-CH_2-C(=Y^2)-NR^{F2}-,

wherein Y^1 is selected from the group consisting of -S-, -0-, -NH-, -NH-NH-, -CH_2-CH_2-S0 2-NR^{F2}-, -CH_2-CHOH-, and cyclic imides, and wherein Y^2 is selected from the group consisting of NH, S and O, and wherein R^{F2} is selected from the group consisting of hydrogen, alkyl, alkylaryl, arylalkyl, aryl, heteroaryl, alkylheteroaryl or heteroarylalkyl group,

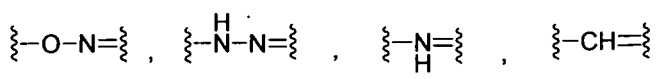
f is 1, 2 or 3, preferably 1 or 2,
g is 0 or 1,
q is 0 or 1,
e is 0 or 1,
and wherein R^m and R^n are, independently of each other, H or alkyl, preferably H or methyl, in particular H.

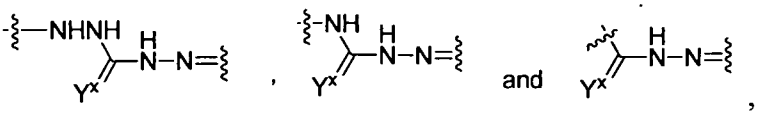
10. The conjugate according to any of embodiments 1 to 9, wherein the hydroxyalkyl starch derivative comprises at least one structural unit, preferably at least 3 structural units according to the following formula (1)



(I)

wherein R^a, R^b and R^c are, independently of each other, selected from the group consisting of-O-HAS", -[0-(CR $^wR^x$)-(CR $^yR^z$)]x-OH, -[0-(CR $^wR^x$)-(CR $^yR^z$)]y-X-, and –[O–(CR^{w}R^x)–(CR^{y}R^z)]$_y$–[F^1]$_p$-L^1-X-, wherein R^w, R^x, R^y and R^z are independently of each other selected from the group consisting of hydrogen and alkyl, y is an integer in the range of from 0 to 20, preferably in the range of from 0 to 4, and wherein x is an integer in the range of from 0 to 20, preferably in the range of from 0 to 4,
and wherein at least one of R^a, R^b and R^c is -[0-(CR $^wR^x$)-(CR $^yR^z$)]y-X- or –[O–(CR^{w}R^x)–(CR^{y}R^z)]$_y$–[F^1]$_p$-L^1-X-,
and wherein X is selected from the group consisting of - Y^{xx}- , -C(=Y^X)-, -C(=Y^X)-NR^{xx}-, -CH_2-CH_2-C(=Y^X)-NR^{xx}-,





wherein Y^{xx} is selected from the group consisting of -S-, -0-, -NH-, -NH-NH-, $-CH_2-CH2-S0_2-NR^{xx}-$, and cyclic imides, such as succinimide, and wherein Y^x is selected from the group consisting of NH, S and O, and wherein R^{xx} is selected from the group consisting of hydrogen, alkyl, alkylaryl, arylalkyl, aryl, heteroaryl, alkylheteroaryl or heteroarylalkyl group,

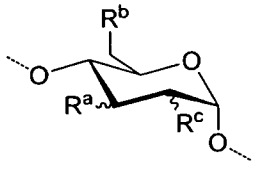
F^1 is a functional group, preferably selected from the group consisting of $-Y^7-$, $-Y^7-C(=Y^6)-$, $-C(=Y^6)-$, $-Y^7-C(=Y^6)-Y^8-$, $-C(=Y^6)-Y^8-$, wherein Y^7 is selected from the group consisting of $-NR^{Y7}-$, -0-, -S-, -succinimide, -NH-NH-, -HN-0-, -CH=N-0-, -0-N=CH-, -CH=N-, -N=CH-, Y^8 is selected from the group consisting of $-NR^{Y8}-$, -S-, -0-, -NH-NH- and Y^6 is selected from the group consisting of NR^{Y6}, O and S, wherein R^{Y6} is H or alkyl, preferably H, and wherein R^{Y7} is H or alkyl, preferably H, and wherein R^{Y8} is H or alkyl, preferably H,

p is 0 or 1,

L^1 is a linking moiety, preferably an alkyl, alkylaryl, arylalkyl, aryl, heteroaryl, alkylheteroaryl or heteroarylalkyl group,

and wherein HAS" is a remainder of HAS.

11. The conjugate according to embodiment 10, wherein the hydroxyalkyl starch derivative comprises at least one structural unit according to the following formula (I)



(I)

wherein R^a, R^b and R^c are independently of each other selected from the group consisting of –O–HAS'', $-[0-CH_2-CH_2]_s-OH$, $-[0-CH2-CH_2]$,-X- and $-[0-CH_2-CH_2]_t-[F^1]_p-L^1-X-$,

and wherein s is in the range of from 0 to 4,

and wherein t is in the range of from 0 to 4,

p is 0 or 1,

wherein at least one of R^a, R^b and R^c is -[0-CH $_2$-CH_2],-X- or-[0-CH $_2$-CH_2],-[F']p-L'-X-,

and wherein HAS" is a remainder of HAS.

12. The conjugate according to embodiment 10 or 11, wherein at least 0.3% to 3 % of all structural units present in the hydroxyalkyl starch derivative comprise the functional group X.

13. The conjugate according to embodiment 11 or 12, wherein at least one ofR^a, R^b and R^c is

(i) -[0-CH $_2$-CH_2]t-X-, or

(ii) -[0-CH $_2$-CH_2]t-[F^1]$_p$-L^1-X-, and wherein p is 1 and F^1 is -0-, or

(iii) -[0-CH $_2$-CH_2]t-[F^1]$_p$-L^1-X-, and wherein p is 1 and F^1 is -0-C(=0)-NH-,

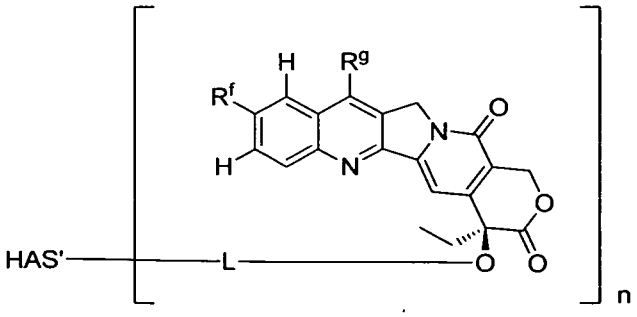
wherein X is -S-,

and wherein t is in the range of from 0 to 4.

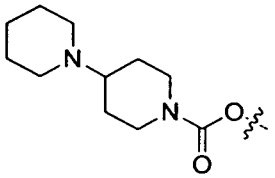
14. The conjugate according to any of embodiments 1 to 13, wherein the cytotoxic agent is a topoisomerase I inhibitor.

15. The conjugate according to any of embodiments 1 to 14, wherein the cytotoxic agent is selected from the group consisting of camptothecin, topotecan, irinotecan, DB67, BNP 1350 (cositecan), exatecan, lurtotecan, ST 1481, gimatecan, belotecan, CKD 602, karenitecin, chimmitecan, 9-aminocamptothecin, 9-nitrocamptothecin, BMS422461, diflomotecan, BN80927, BMS422461 , morpholino-CPT and, KOS-1584.

16. The conjugate according to any of embodiments 1 to 15, wherein the conjugate has a structure according to the following formula



wherein R^f is selected from the group consisting of -OH, siloxy groups, ester groups and groups having the structure



wherein R^f is preferably -OH,
and wherein R^8 is $-CH_2-CH_3$.

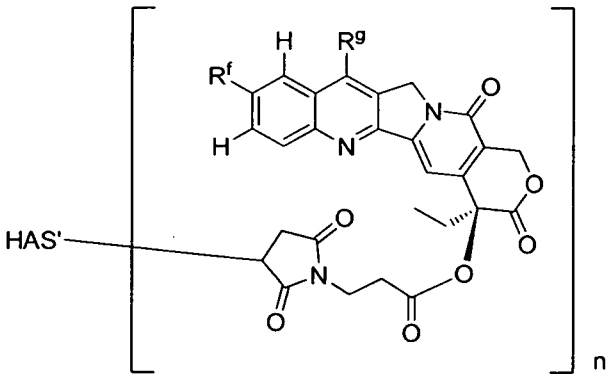
17. The conjugate according to embodiment 9, said conjugate having a structure according to the following formula

$$HAS'(-[F^2]_q-[L^2]_g-[E]_e-[CR^mR^n]_f-F^3-M)_n$$

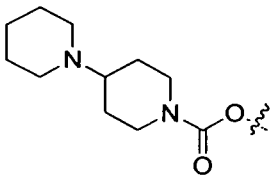
wherein q is 1, F^2 is –succinimide-, and f is 2,
wherein the structural unit $-[CR^mR^n]_f-$ is preferably $-CH_2-CH_2-$,

18. The conjugate according to embodiment 17, wherein e is 0 and g is 0.

19. The conjugate according to embodiment 17 or 18, wherein F^3 is -C(=0)-, the conjugate having a structure according to the following formula

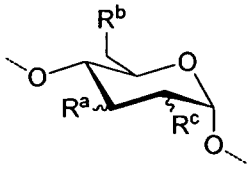


wherein R^f is selected from the group consisting of -OH, siloxy groups, ester groups or and groups having the structure



wherein R^f is preferably -OH,
and wherein R^g is $-CH_2-CH_3$.

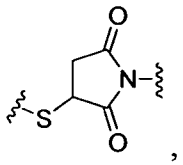
20. The conjugate according to any of embodiments 17 to 19, wherein HAS' comprises at least one structural unit, preferably 3 to 200 structural units, according to the following formula (I)



(I)

wherein R^a, R^b and R^c are independently of each other selected from the group consisting of -O-HAS", -[0-CH_2-CH_2]$_s$-OH and -[0-CH_2-CH_2],-X-,

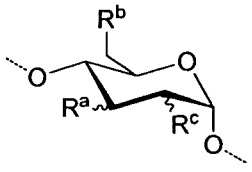
wherein s is in the range of from 0 to 4,
and wherein t is in the range of from 0 to 4,
and wherein at least one of R^a, R^b and R^c is -[0-CH_2-CH_2],-X- , wherein X is -S- and wherein X is directly bound to F^2, thereby forming a covalent linkage having the structure:



,

and wherein HAS" is a remainder of HAS.

21. The conjugate according to any of embodiments 17 to 19, wherein HAS' comprises at least one structural unit, preferably 3 to 200 structural units, according to the following formula (I)

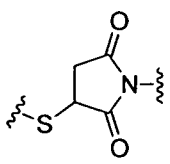


(I)

wherein R^a, R^b and R^c are independently of each other selected from the group consisting of -O-HAS", -[0-CH_2-CH_2]$_s$-OH, and -[0-CH_2-CH_2]$_t$-[F^1]p-L'-X-,

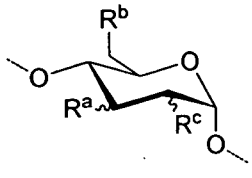
wherein s is in the range of from 0 to 4,
t is in the range of from 0 to 4,
p is 0 or 1,
and wherein at least one of R^a, R^b and R^c is -[0-CH_2-CH_2]$_t$-[F^1]$_p$-L^1-X-,

wherein F^1 is -0-,
wherein L^1 is a linking moiety having a structure according to the following formula $-\{[CR^dR^f]_h-[F^4]_{tf}-[CR^{dd}R^f]_z\}_{alpha}-$,
wherein F^4 is a functional group, preferably selected from the group consisting of-S-, -O- and -NH-, in particular -S-, wherein
z is in the range of from 0 to 20, more preferably of from 0 to 10, more preferably of from 0 to 3,
or z is in the range of from 1 to 5, preferably in the range of from 1 to 3, more preferably 2,
h is in the range of from 1 to 5, preferably in the range of from 1 to 3, more preferably 3,
u is 0 or 1,
integer alpha is in the range of from 1 to 10,
and R^d, R^f, R^{dd} and R^f are, independently of each other, selected from the group consisting of H, alkyl, hydroxyl, and halogene, preferably selected from the group consisting of H, methyl and hydroxyl, and wherein each repeating unit of $-[CR^dR^f]_h-[F^4]_u-[CR^{dd}R^{ff}]_z-$ may be the same or may be different,
wherein, more preferably, L^1 has a structure selected from the group consisting of
$-CH_2-CHOH-CH_2-$, $-CH_2-CHOH-CH_2-S-CH_2-CH_2-$,
$-CH_2-CHOH-CH_2-S-CH_2-CH_2-$, $-CH_2-CHOH-CH_2-NH-CH_2-CH_2-$,
$-CH_2-CHOH-CH_2-NH-CH_2-CH_2-CH_2-$, $-CH_2-$, $-CH_2-CH_2-$, $-CH_2-CH_2-CH_2-$,
$-CH_2-CH_2-CH_2-CH_2-$, $-CH_2-CH_2-CH_2-CH_2-CH_2-$,
$-CH_2-CH(CH_2OH)-$, $-CH_2-CH(CH_2OH)-S-CH_2-CH_2-$,
$-CH_2-CHOH-CH_2-0-CH_2-CHOH-CH_2-$,
$-CH_2-CHOH-CH_2-0-CH_2-CHOH-CH_2-S-CH_2-CH_2-$,
$-CH_2-CH_2-CH_2-S-CH_2-CH_2-$, $-CH_2-CH_2-S-CH_2-CH_2-$ and
$-CH_2-CH_2-0-CH_2-CH_2-$, more preferably from the group consisting of
$-CH_2-CHOH-CH_2-$, $-CH_2-CHOH-CH_2-S-CH_2-CH_2-$,
$-CH_2-CHOH-CH_2-S-CH_2-CH_2-CH_2-$, $-CH_2-CHOH-CH_2-NH-CH_2-CH_2-$ and
$-CH_2-CHOH-CH_2-NH-CH_2-CH_2-CH_2-$, more preferably from the group consisting of-$CH_2-CHOH-CH_2-$, $-CH_2-CHOH-CH_2-S-CH_2-CH_2-$ and
$-CH_2-CHOH-CH_2-S-CH_2-CH_2-CH_2-$,
wherein X is -S- and X is directly bound to F^2, thereby forming a covalent linkage having the structure



and wherein HAS" is a remainder of HAS.

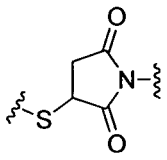
22. The conjugate according to any of embodiments 17 to 19, wherein HAS' comprises at least one structural unit according to the following formula (I)



(I)

wherein R^a, R^b and R^c are independently of each other selected from the group consisting of-O-HAS", -[0-CH $_2$-CH $_2$]$_S$-OH, and -[0-CU_2-CH_2]$_t$-[F^1]$_p$-L^1-X-,

wherein s is in the range of from 0 to 4,
t is in the range of from 0 to 4,
p is 0 or 1,
and wherein at least one of R^a, R^b and R^c is –[O–CH_2-CH_2]$_t$-[F^1]$_p$-L^1-X-,
wherein F^1 is -0-(C=0}-NH-,
wherein L^1 is an alkyl group,
wherein X is -S- and X is directly bound to F^2, thereby forming a covalent linkage having the structure



and wherein HAS" is a remainder of HAS.

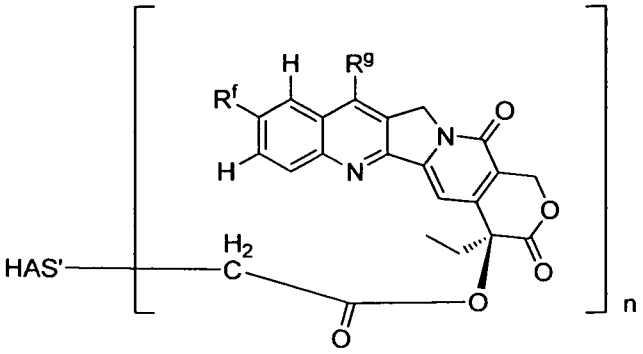
23. The conjugate according to embodiment 9, said conjugate having a structure according to the following formula

HAS'(-[F ^{2}]$_q$-[L ^{2}]$_g$-[E]$_e$-[CR ^{m}R^n]^F ^{3}-M)$_n$

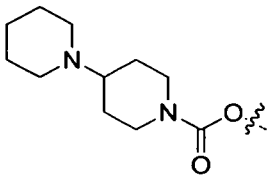
wherein e is 0,
g is 0, and
q is 0.

24. The conjugate according embodiment 23, wherein f is 1, and wherein R^m and R" are preferably H.

25. The conjugate according to embodiment 23 or 24, the conjugate having a structure according to the following formula

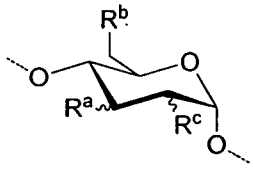


wherein R^f is selected from the group consisting of -OH, siloxy groups, ester groups and groups having the structure



and wherein R^8 is **-CH2-CH3.**

26. The conjugate according to any of embodiments 23 to 25, wherein HAS' comprises at least one structural unit according to the following formula (I)

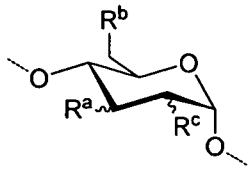


(I)

wherein R^a, R^b and R^c are independently of each other selected from the group consisting of -O-HAS", -[0-CH_2-CH_2]$_s$-OH and -[0-CH_2-CH_2],-X-,

wherein s is in the range of from 0 to 4,
and wherein t is in the range of from 0 to 4,
and wherein at least one of R^a, R^b and R^c is -[0-CH_2-CH_2],-X- , wherein X is -S- and wherein X is directly bound to -[CR $^mR^n$]$_f$-, thereby forming a covalent linkage having the structure -S-[CR $^mR^n$]$_f$-,
and wherein HAS" is a remainder of HAS.

27. The conjugate according to any of embodiments 23 to 25, wherein HAS' comprises at least one structural unit according to the following formula (I)



(I)

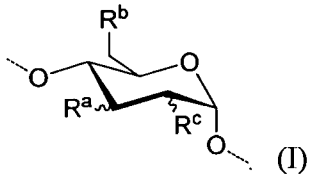
wherein R^a, R^b and R^c are independently of each other selected from the group consisting of-O-HAS", -[0-CH $_2$-CH $_2$]$_s$-OH, and - [O–CH_2-CH_2]$_t$-[F^1]$_p$-L'-X-,

wherein s is in the range of from 0 to 4,
t is in the range of from 0 to 4,
p is 0 or 1,
and wherein at least one of R^a, R^b and R^c is –[O–CH_2-CH_2]$_t$-[F^1]$_p$-L^1-X-,
wherein F^1 is -0-,
wherein L^1 is a linking moiety having a structure according to the following formula -{[CR ^{d}R^f]$_h$-[F ^{4}]$_u$-[CR ^{dd}R^f]$_z$}„- wherein F^4 is a functional group, preferably selected from the group consisting of -S-, -O- and -NH-, in particular -S-, wherein
z is in the range of from 0 to 20, more preferably of from 0 to 10, more preferably of from 0 to 3, or
z is in the range of from 1 to 5, preferably in the range of from 1 to 3, more preferably 2,
h is in the range of from 1 to 5, preferably in the range of from 1 to 3, more preferably 3,
u is 0 or 1,
a is in the range of from 1 to 10,
and wherein R^d, R^f, R^{dd} and R^f are, independently of each other, selected from the group consisting of H, alkyl, hydroxy 1, and halogen, preferably selected from the group consisting of H, methyl and hydroxyl,
and wherein each repeating unit of -[CR^dR^f]$_h$-[F^4]$_u$-[$CR^{dd}R^f$]$_z$- may be the same or may be different,
wherein, more preferably, L^1 has a structure selected from the group consisting of -CH_2-, -CH_2-CH_2-, -CH_2-CH_2-CH_2-, -CH_2-CH_2-CH_2-CH_2-, -CH_2-CH_2-CH_2-CH_2-CH_2-, -CH_2-CH_2-CH_2-S-CH_2-CH_2-, -CH_2-CH_2-S-CH_2-CH_2-, -CH_2-CH_2-0-CH $_2$-CH_2-, -CH_2-CH_2-0-CH $_2$-CH_2-0-CH $_2$-CH_2-, -CH_2-CHOH-CH_2-, -CH_2-CHOH-CH_2-S-CH_2-CH_2-, -CH_2-CHOH-CH_2-S-CH_2-CH_2-CH_2-, -CH_2-CHOH-CH_2-NH-CH_2-CH_2-, -CH_2-CHOH-CH_2-NH-CH_2-CH_2-CH_2-, -CH_2-CHOH-CH_2-0-CH $_2$-CHOH-CH_2-, -CH_2-CHOH-CH_2-0-CH $_2$-CHOH-CH_2-S-CH_2-CH_2-, -CH_2-CH(CH_2OH)- and -CH_2-CH(CH_2OFf)-S-CH_2-CH_2-, more preferably from the group consisting of -CH_2-CHOH-CH_2-, -CH_2-CHOH-CH_2-S-CH_2-CH_2-, -CH_2-CHOH-CH_2-S-CH_2-CH_2-CH_2-, -CH_2-CHOH-CH_2-NH-CH_2-CH_2- and -CH_2-CHOH-CH_2-NH-CH_2-CH_2-

CH_2-, more preferably from the group consisting of -CH_2-CHOH-CH_2-, -CH_2-CHOH-CH_2-S-CH_2-CH_2- and -CH_2-CHOH-CH_2-S-CH_2-CH_2-CH_2-,

wherein X is -S- and wherein X is directly bound to -[CR^{m}R^n]$_f$-, thereby forming a covalent linkage having the structure -S-[CR^{m}R"]$_f$-,
and wherein HAS" is a remainder of HAS.

28. The conjugate according to any of embodiments 23 to 26, wherein HAS' comprises at least one structural unit, preferably 3 to 200 structural units, according to the following formula (I)



(I)

wherein R^a, R^b and R^c are independently of each other selected from the group consisting of-O-HAS", -[O-CH_2-CH_2]$_s$-0H, and -[0-CH_2-CH_2]$_t$-[F^1]$_p$-L^1-X-,

wherein s is in the range of from 0 to 4,
t is in the range of from 0 to 4,
p is 0 or 1,
and wherein at least one of R^a, R^b and R^c is -[0-CH_2-CH_2],-[F^1]$_p$-L^1-X-,
wherein F^1 is -0-(C=0)-NH-,
wherein L^1 is an alkyl group,
wherein X is -S- and X is directly bound to -[CR^{m}R"]$_f$-, thereby forming a covalent linkage having the structure -S-[CR^{m}R^n]f-,
and wherein HAS" is a remainder of HAS.

29. A method for preparing a hydroxyalkyl starch (HAS) conjugate comprising a hydroxyalkyl starch derivative and a cytotoxic agent, said conjugate having a structure according to the following formula

HAS'(-L-M)$_n$

wherein
M is a residue of a cytotoxic agent, wherein the cytotoxic agent comprises a tertiary hydroxyl group,
L is a linking moiety,
HAS' is a residue of the hydroxyalkyl starch derivative,
and n is greater than or equal to 1,
said method comprising

(a) providing a hydroxyalkyl starch (HAS) derivative having a mean molecular weight MW above the renal threshold, preferably a mean molecular weight MW greater than or equal to 60 kDa and a molar substitution MS in the range of from 0.6 to 1.5, said HAS derivative comprising a functional group Z^1; and providing a cytotoxic agent comprising a tertiary hydroxyl group;

(b) coupling the HAS derivative to the cytotoxic agent via an at least bifunctional crosslinking compound L comprising a functional group K^1 and a functional group K^2, wherein K^2 is capable of being reacted with Z^1 comprised in the HAS derivative and wherein K^1 is capable of being reacted with the tertiary hydroxyl group comprised in the cytotoxic agent.

30. The method according to embodiment 29, wherein the functional group K^1 comprises the group -C(=Y)-, with Y being O, NH or S, wherein K^1 is preferably a carboxylic acid group or a reactive carboxy group.

31. The method according to embodiment 29 or 30, wherein the cytotoxic agent is reacted with the crosslinking compound L prior to the reaction with the HAS derivative.

32. The method according to any of embodiments 29 to 31, wherein the crosslinking compound L has a structure according to the following formula

$$K^2\text{-}L'\text{-}K'$$

wherein K^1 comprises the group -C(=Y)- and L' is a linking moiety.

33. The method according to embodiment 32, wherein K^2 is reacted with the functional group Z^1 comprised in the HAS derivative, and wherein Z'is selected from the group consisting of an aldehyde group, a keto group, a hemiacetal group, an acetal group, an alkynyl group, an azide, a carboxy group, an alkenyl group, a thiol reactive group, -SH, $-NH_2$, -0-NH_2, -NH-O-alkyl, -(C=G)-NH-NH_2, _G_{C=G)-NH-NH_2, -NH-(C=G)-NH-NH_2, and -S0$_2$-NH-NH_2, where G is O or S and, if G is present twice, it is independently O or S.

34. The method according to embodiment 32 or 33, wherein upon reaction of the tertiary hydroxyl group comprised in the cytotoxic agent with K^1, a functional group - F^3-0-

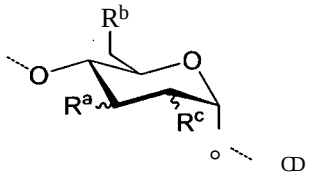
is formed, wherein F^3 is a -C(=Y)- group, with Y being O, NH or S, in particular O or S.

35. The method according to any of embodiments 29 to 34, wherein the at least one crosslinking compound L has a structure according to the following formula:

$$K^2\text{-}[L^2]_g\text{-}[E]_e\text{-}[CR^mRV\ K^1$$

wherein L^2 is a linking moiety, preferably an alkyl, alkylaryl, arylalkyl, aryl, heteroaryl, alkylheteroaryl or heteroarylalkyl group,
wherein E is an electron-wihtdrawing group,
f is 1, 2 or 3, preferably 1 or 2,
g is 0 or 1,
e is 0 or 1,
and wherein R^m and R^n are, independently of each other, H or alkyl, more preferably H or methyl, in particular H.

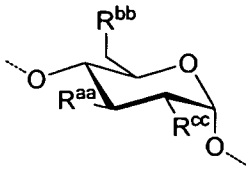
36. The method according to any of embodiments 29 to 35, wherein the derivative provided in step (a) comprises at least one structural unit, preferably 3 to 200 structural units, according to the following formula (I)



(I)

wherein R^a, R^b and R^c are, independently of each other, selected from the group consisting of-O-HAS", -[0-(CR $^wR^x$)-(CR $^yR^z$)]$_x$-OH, -[0-(CR $^wR^x$)-(CR $^yR^z$)]$_y$- Z^1, and –[O–(CR^{w}R^x)–(CR^{y}R^z)]$_y$–[F^1]$_p$-L^1-Z^1, wherein R^w, R^x, R^y and R^z are independently of each other selected from the group consisting of hydrogen and alkyl,
y is an integer in the range of from 0 to 20, preferably in the range of from 0 to 4, and
wherein x is an integer in the range of from 0 to 20, preferably in the range of from 0 to 4,
and wherein at least one of R^a, R^b and R^c is - [O–(CR^{w}R^x)–(CR^{y}R^z)]$_y$- Z^1 or - [O- (CR^{w}R^x)–(CR^{y}R^z)]$_y$- [F^1]$_p$-L^1-Z^1,
and wherein F^1 is a functional group,
p is 0 or 1,
L^1 is a linking moiety,
wherein HAS" is a remainder of HAS,
and wherein step (a) comprises

(al) providing a hydroxyalkyl starch having a mean molecular weight MW greater than or equal to 60 kDa and a molar substitution MS in the range of from 0.6 to 1.5 comprising the structural unit according to the following formula (II)



(II)

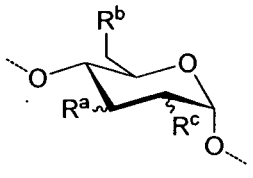
wherein R^{aa}, R^{bb} and R^{cc} are, independently of each other, selected from the group consisting of-O-HAS" and -[0-(CR $^{w}R^{x}$)-(CR $^{y}R^{z}$)]x-OH,
wherein R^{w}, R^{x}, R^{y} and R^{z} are independently of each other selected from the group consisting of hydrogen and alkyl groups, and wherein x is an integer in the range of from 0 to 20, preferably in the range of from 0 to 4,

(a2) introducing at least one functional group Z^1 into HAS by

(i) coupling the hydroxyalkyl starch via at least one hydroxyl group comprised in HAS to at least one suitable linker comprising the functional group Z^1 or a precursor of the functional group Z^1, or

(ii) displacing at least one hydroxyl group comprised in HAS in a substitution reaction with a precursor of the functional group Z^1 or with a suitable linker comprising the functional group Z^1 or a precursor thereof.

37. The method according to embodiment 36, wherein the HAS derivative formed in step (a2) comprises at least one structural unit according to the following formula (1)



(I)

wherein R^a, R^b and R^c are independently of each other selected from the group consisting of-O-HAS", -[0-CH $_2$-CH $_2$]$_S$-OH, -[0-CH $_2$-CH $_2$]$_t$-Z^1 and -[0-CH $_2$-CH_2]$_t$-[F^1]$_p$-L^1- Z^1,

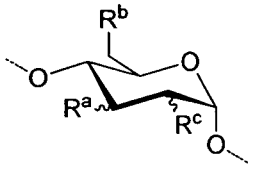
and wherein s is in the range of from 0 to 4,

and wherein t is in the range of from 0 to 4,

p is 0 or 1,

wherein at least one of R^a, R^b and R^c is -[0-CH $_2$-CH_2]$_t$- Z^1 or
- [O- CH_2-CH_2]$_t$-[F^1]$_p$-L^1- Z^1,
and wherein HAS" is a remainder of HAS.

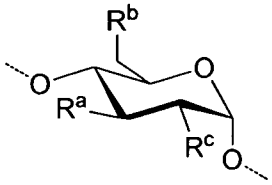
38. The method according to embodiment 36 or 37, wherein in step (a2)(i), the hydroxyalkyl starch is reacted with a suitable linker comprising the functional group Z^1 or a precursor of the functional group Z^1, and comprising a functional group Z^2, the linker preferably having the structure Z^2- L^1- Z^1 or Z^2–L^1- Z^{1*}–PG, with Z^2 being a functional group capable of being reacted with the hydroxyalkyl starch, thereby forming a hydroxyalkyl starch derivative comprising at least one structural unit, according to the following formula (I),


(I)

wherein at least one of R^A, R^B and R^C is -[0-CH2-CH_2]r [F^1]$_p$-V -Z^1 or -[0-CH_2-CH_2]r[F^1]$_p$-L^1-Z^{1*}-PG with PG being a suitable protecting group and Z^{1*} being the protected form of the functional group Z^1,
wherein Z^1 is preferably -SH, Z^{1*} is preferably -S- and PG is preferably a suitable thiol protecting group, more preferably a protecting group forming together with Z^{1*} a group selected from the group consisting of thioethers, thioesters and disulfides,
and wherein in case the linker comprises the protecting group PG, the method further comprises deprotection of Z^{1*} to give Z^1.

39. The method according to embodiment 38, wherein step (a2)(i) comprises

(aa) activating at least one hydroxyl group comprised in the hydroxyalkyl starch with a reactive carbonyl compound having the structure R^{**}–(C=O)–R^*, wherein R^* and R^{**} may be the same or different, and wherein R^* and R^* are both leaving groups, wherein upon activation a hydroxyalkyl starch derivative comprising at least one structural unit according to the following formula (I)


(I)

is formed, in which R^A, R^B and R^c are independently of each other selected from the group consisting of-O-HAS", -[0-CH_2-CH_2]$_S$-OH, and
-[0-CH_2-CH_2]$_t$-0-C(=0)-R*,
wherein at least one of R^A, R^b and R^c comprises the group
–[O–CH_2-CH_2]$_t$–O–C(=O)–R^*, and

(bb) reacting the activated hydroxyalkyl starch according to step (aa) with the suitable linker comprising the functional group Z^1 or a precursor of the functional group Z^1.

40. The method according to embodiment 39, wherein the reactive carbonyl compound having the structure R^{**}-(C=0)-R^{*} is selected from the group consisting of phosgene, diphosgene, triphosgene, chloroformates and carbonic acid esters, preferably wherein the reactive carbonyl compound is selected from the group consisting of p-nitrophenylchloroformate, pentafluorophenylchloroformate, N,N'-disuccinimidyl carbonate, sulfo-N,N'-disuccinimidyl carbonate, dibenzotriazol-l-yl carbonate and carbonyldiimidazol.

41. The method according to embodiment 39 or 40, wherein in (bb), the activated hydroxyalkyistarch derivative is reacted with a linker comprising the functional group Z^2 and the functional group Z^1 or a precursor of the functional group Z^1, the linker preferably having the structure Z^2-L^1-Z^1 or Z^2-L'-Z'*-PG, wherein
Z^2 is a functional group capable of being reacted with the -[0-CH_2-CH_2]$_t$-O-C(=0)-R^d group,
L^1 is an alkyl group,
wherein upon reaction of the -0-C(=0)-R^{*} group with the functional group Z^2, the functional group F^1 is formed, and
Z^2 is preferably -NH_2.

42. The method according to embodiment 41, wherein the linker has the structure Z^2-L^1-Z^{1*}-PG, wherein Z^{1*} is _-S- and PG is a thiol protecting group, forming together with Z^{1*} preferably a group selected from the group consisting of thioethers, thioesters and disulfides, and wherein the method further comprises deprotection of Z^{1*} to give Z^1.

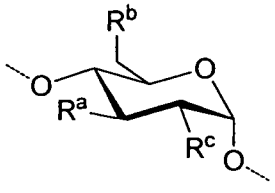
43. The method according to embodiment 36, wherein (a2)(i) comprises
(I) coupling the hydroxyalkyl starch via at least one hydroxyl group comprised in the hydroxyalkyl starch to a first linker comprising a functional group Z^2, Z^2 being capable of being reacted with a hydroxyl group of the hydroxyalkyl starch, thereby forming a covalent linkage, the first linker further comprising a functional group W, wherein the functional group W is an epoxide or a group which is transformed in a further step to give an epoxide.

44. The method according to embodiment 43, wherein the first linker has a structure according to the formula Z^2-L^W-W, wherein

Z^2 is a functional group capable of being reacted with a hydroxy 1group of the hydroxyalkyl starch,

L^w is a linking moiety,

wherein upon reaction of the hydroxyalkyl starch with the first linker, a hydroxyalkyl starch derivative is formed comprising at least one structural unit according to the following formula (lb)



(Ib)

wherein R^a, R^b and R^c are, independently of each other, selected from the group consisting of-O-HAS", -[0-CH_2-CH_2]$_s$-OH, and

-[0-CH_2-CH_2]$_t$-[F^1]$_p$- L^w-W,

wherein s is in the range of from 0 to 4,

and wherein t is in the range of from 0 to 4,

p is 0 or 1,

and wherein at least one of R^a, R^b and R^c is -[0-CH_2-CH_2]$_t$-[F^1]$_p$- L^w-W,

and wherein F^1 is the functional group being formed upon reaction of Z^2 with a hydroxyl group of the hydroxyalkyl starch, wherein F^1 is preferably -O- or -CH_2-CHOH-, preferably -0-,

and wherein HAS" is a remainder of HAS.

45. The method according to embodiment 43 or 44, wherein W is an alkenyl group and the method further comprises

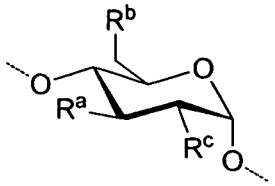
(II) oxidizing the alkenyl group W to give the epoxide, wherein as oxidizing agent, potassium peroxymonosulfate is preferably employed.

46. The method according to any of embodiments 43 to 45, wherein Z^2 is a halogene (Hal) or an epoxide, preferably a halogen, and wherein the linker Z^2- L^w- W preferably has the structure Hal-CH_2-CH=CH_2.

47. The method according to any of embodiments 44 to 46, the method comprising

(III) reacting the epoxide with a nucleophile comprising the functional group Z^1 or a precursor of the functional group Z^1, wherein the nucleophile is preferably a dithiol or a thiosulfate, thereby forming a hydroxyalkyl starch derivative

comprising at least one structural unit, preferably 3 to 200 structural units, according to the following formula (lb)



(Ib)

wherein R^a, R^b and R^c are independently of each other selected from the group consisting of-O-HAS", -[0-CH_2-CH_2]$_s$-OH, and -[O-CH_2-CH_2]$_t$-[F^1]$_p$-L'-Z^1,

wherein s is in the range of from 0 to 4,

and wherein t is in the range of from 0 to 4,

·P is 1,

at least one of R^a, R^b and R^c comprises the group -[0-CH_2-CH_2],-[F^1]$_p$-L^1-Z^1, and wherein Z^1 is -SH.

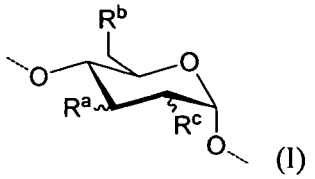
48. The method according to embodiment 47, wherein the nucleophile is ethanedithiol or sodium thiosulfate.

49. The method according to embodiment 36, wherein in (a2)(ii), prior to the displacement of the hydroxyl group, a group R^L is added to at least one hydroxyl group thereby generating a group -0-R^L, wherein -0-R^L is a leaving group, in particular a -O-Mesyl (-OMs) or -O-Tosyl (-OTs) group.

50. The method according to embodiment 36 or 49, wherein Z^1 is -SH, and wherein in step (a2)(ii) the at least one hydroxyl group comprised in the hydroxyalkyl starch is displaced by a suitable precursor of the functional group Z^1, the method further comprising converting the precursor after the substitution reaction to the functional group Z^1.

51. The method according to embodiment 50, wherein in step (a2)(ii) the at least one hydroxyl group comprised in the hydroxyalkyl starch is displaced with thioacetate giving a precursor of the functional group Z^1 having the structure -**S-C(=0)-CH_3**, wherein the method further comprises the conversion of the group -S-C(=0 **)-CH_3** to give the functional group Z^1, preferably wherein the conversion is carried out using sodium hydroxide and sodium borohydride.

52. The method according to any of embodiments 49 to 51, wherein the hydroxyalkyl starch derivative obtained according to step (a2)(ii) comprises at least one structural unit according to the following formula (1)



(I)

wherein R^a, R^b and R^c are independently of each other selected from the group consisting of-O-HAS", -[0-CH_2-CH_2]$_s$-OH, and -[0-CH_2-CH_2]$_t$-Z',

wherein s is in the range of from 0 to 4,

and wherein t is in the range of from 0 to 4,

and wherein at least one of R^a, R^b and R^c comprises the group -[0-CH_2-CH_2]$_t$-Z',

Z^1 is -SH,

and wherein HAS" is a remainder of HAS.

53. The method according to any of embodiments 29 to 52, wherein in step (b) the hydroxyalkyl starch derivative obtained according to step (a) is coupled to the derivative of the cytotoxic agent having a structure according to the formula K^2-[L^2]$_g$-[E]$_e$-[CR^mR'']$_f$-F^3-M, wherein

— g and e are 0,
f is 1, 2 or 3, preferably 1 or 2, most preferably 1,
R^m and R^n are, independently of each other, H or alkyl, preferably H or methyl, in particular H,
and K^2 is a halogene,
wherein upon reaction of Z^1 with K^2 the covalent linkage -X-[CR^mR'']$_f$- is formed;
or
— g and e are 0,
f is 1, 2 or 3, preferably 1 or 2, most preferably 2,
R^m and R" are, independently of each other, H or alkyl, preferably H or methyl, in particular H,
and K^2 is maleimide,
and wherein upon reaction of Z^1 with K^2 the covalent linkage -X-succinimide- is formed,
and wherein F^3 is preferably -C(=0)-.

54. The method according to embodiment 53, wherein Z^1 is -SH and X is -S-.

55. The method according to any embodiments 39 to 54, wherein the cytotoxic agent is selected from the group consisting of camptothecin, topotecan, irinotecan, DB67, BNP 1350 (cositecan), exatecan, lurtotecan, ST 1481, gimatecan, belotecan, CKD 602, karenitecin, chimmitecan, 9-aminocamptothecin, 9-nitrocamptothecin, BMS422461, diflomotecan, BN80927, BMS422461, morpholino-CPT and, KOS-1584.

56. A hydroxyalkyl starch conjugate obtained or obtainable by a method according to any of embodiments 29 to 55.

57. A pharmaceutical composition comprising a conjugate according to any of embodiments 1 to 28 or according to embodiment 56.

58. A hydroxyalkyl starch conjugate according to any of embodiments 1 to 28 or according to embodiment 56, or a pharmaceutical composition according to embodiment 57 for use as medicament.

59. A hydroxyalkyl starch conjugate according to any of embodiments 1 to 28 or according to claim 56, or a pharmaceutical composition according to claim 57 for the treatment of cancer.

60. A hydroxyalkyl starch conjugate according to any of embodiments 1 to 28 or according to claim 56, or a pharmaceutical composition according to claim 57 for the treatment of cancer selected from the group consisting of breast cancer, cervical cancer, colorectal cancer, gastrointestinal cancer, leukaemia, lung cancer, mesothelioma, non-hodgkin's lymphoma, non-small cell lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, skin cancer, small cell lung cancer, brain tumors, uterine cancer and head and neck tumors.

61. Use of a hydroxyalkyl starch conjugate according to any of embodiments 1 to 28 or according to embodiment 56, or of a pharmaceutical composition according to embodiment 57 for the manufacture of a medicament for the treatment of cancer.

62. The use of a hydroxyalkyl starch conjugate according to embodiment 61, wherein the cancer is selected from the group consisting of breast cancer, cervical cancer, colorectal cancer, gastrointestinal cancer, leukaemia, lung cancer, mesothelioma, non-hodgkin's lymphoma, non-small cell lung cancer, ovarian cancer, pancreatic cancer,

prostate cancer, skin cancer, small cell lung cancer, brain tumors, uterine cancer and head and neck tumors.

63. A method of treating a patient suffering from cancer comprising administering a therapeutically effective amount of a hydroxyalkyl starch conjugate according to any of embodiments 1 to 28 or according to embodiment 56, or of a pharmaceutical composition according to embodiment 57.

64. The method of embodiment 63 wherein the patient suffers from a cancer being selected from the group consisting of breast cancer, cervical cancer, colorectal cancer, gastrointestinal cancer, leukaemia, lung cancer, mesothelioma, non-hodgkin's lymphoma, non-small cell lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, skin cancer, small cell lung cancer, brain tumors, uterine cancer and head and neck tumors.

Description of the Figures

Figure 1: *Time course of the median RTV values after administering SN-38 conjugates CSN1 to CSN4 (dosage 60 mg/kg body weight; colon cancer model HT-29)*

Figure 1 shows the time course of the relative tumor volume of *human colon cancer HT-29 xenografts* growing in nude mice treated with conjugates CSN1 to CSN4 vs. mice in the control group (untreated mice (saline)) as well as vs. mice treated with Irinotecan®.

The following symbols are used:
■ = Saline, * = Irinotecan, Δ = CSN1, V = CSN2, ◇ = CSN3, O = CSN4.

The X-axis shows the time after start [d], the Y-axis shows the median relative tumor volume, RTV (median) [%].

Each measurement was carried out with a group of 8 mice. The conjugates CSN1 to CSN4 were administered once at a dosage of 60 mg/kg body weight on day 9. Irinotecan® was administered 5 times at a dosage of 15 mg/kg body weight at days 9 to 13. Median values are given. Further details are given in Table 14.

Figure 2: *Time course of the body weight change after administering SN-38 conjugates CSN1 to CSN4 (dosage 60 mg/kg body weight; colon cancer model HT-29)*

Figure 2 shows the time course of the body weight change in nude mice bearing *human colon cancer HT-29 xenografts* treated with conjugates CSN1 to CSN4 vs. mice in the control group (untreated mice (saline)) as well as vs. mice treated with Irinotecan®.

The following symbols are used:
■ = Saline, * = Irinotecan, Δ = CSN1 , V = CSN2, ◇ = CSN3, O = CSN4.

The X-axis shows the time after start [d], the Y-axis shows the body weight change, BWC [%].

Each measurement was carried out with a group of 8 mice. The conjugates CSN1 to CSN4 were administered once at a dosage of 60 mg/kg body weight on day 9. Irinotecan® was administered 5 times at a dosage of 15 mg/kg body weight at days 9 to 13. Median values are given. Further details are given in Table 14.

Figure 3: *Time course of the median RTV values after administering SN-38 conjugates CSN5, CSN7, CSN9 and CSNJ1 (dosage 60 mg/kg body weight; colon cancer model HT-29)*

Figure 3 shows the time course of the relative tumor volume of *human colon cancer HT-29 xenografts* growing in nude mice treated with conjugates CSN5, CSN7, CSN9 and CSN11 vs. mice in the control group (untreated mice (saline)) as well as vs. mice treated with lrinotecan®.

The following symbols are used:

■ = Saline, * = lrinotecan, O = CSN5, Δ = CSN7, V = CSN9, ◇ = CSN11.

The X-axis shows the time after start [d], the Y-axis shows the relative tumor volume, RTV [%].

Each measurement was carried out with a group of 7 to 8 mice. The conjugates CSN5, CSN7, CSN9 and CSN11 were administered once at a dosage of 60 mg/kg body weight on day 8. lrinotecan® was administered 5 times at a dosage of 15 mg/kg body weight at days 8 to 12. Median values are given. Further details are given in Table 15.

Figure 4: *Time course of the median RTV values after administering SN-38 conjugates CSN6, CSN8, CSNJ0 and CSN12 (dosage 60 mg/kg body weight; colon cancer model HT-29)*

Figure 4 shows the time course of the relative tumor volume of *human colon cancer HT-29 xenografts* growing in nude mice treated with conjugates CSN6, CSN8, CSN10 and CSN12 vs. mice in the control group (untreated mice (saline)) as well as vs. mice treated with lrinotecan®.

The following symbols are used:

■ = Saline, * = lrinotecan, O = CSN6, Δ = CSN8, V = CSN10, ◇ = CSN12.

The X-axis shows the time afer start [d], the Y-axis shows the relative tumor volume [%].

Each measurement was carried out with a group of 7 to 8 mice. The conjugates CSN6, CSN8, CSN10 and CSN12 were administered once at a dosage of 60 mg/kg body weight

on day 8. Irinotecan® was administered 5 times at a dosage of 15 mg/kg body weight at days 8 to 12. Median values are given. Further details are given in Table 15.

Figure 5: *Time course of the body weight change after administering SN-38 conjugates CSN5, CSN7, CSN9 and CSNl J (dosage 60 mg/kg body weight; colon cancer model HT-29)*

Figure 5 shows the time course of the body weight change in nude mice bearing *human colon cancer HT-29 xenografts* treated with conjugates CSN5, CSN7, CSN9 and CSN1 1 vs. mice in the control group (untreated mice (saline)) as well as vs. mice treated with Irinotecan®.

The following symbols are used:

■ = Saline, * = Irinotecan, O = CSN5, Δ = CSN7, V = CSN9, ◇ = CSNl 1.

The X-axis shows the time after start [d], the Y-axis shows the body weight change, BWC [%]·

Each measurement was carried out with a group of 7 to 8 mice. The conjugates CSN5, CSN7, CSN9 and CSNl 1 were administered once at a dosage of 60 mg/kg body weight on day 8. Irinotecan® was administered 5 times at a dosage of 15 mg/kg body weight at days 8 to 12. Median values are given. Further details are given in Table 15.

Figure 6: *Time course of the body weight change after administering SN-38 conjugates CSN6, CSN8, CSNIO and CSNl 2 (dosage 60 mg/kg body weight; colon cancer model HT-29)*

Figure 6 shows the time course of the body weight change in nude mice bearing *human colon cancer HT-29 xenografts* treated with conjugates CSN6, CSN8, CSNIO and CSNl 2 vs. mice in the control group (untreated mice (saline)) as well as vs. mice treated with Irinotecan®.

The following symbols are used:

■ = Saline, * = Irinotecan, O = CSN6, Δ = CSN8, V = CSNIO, ◇ = CSNl 2.

The X-axis shows the time after start [d], the Y-axis shows the body weight change, BWC [%]·

Each measurement was carried out with a group of 7 to 8 mice. The conjugates CSN6, CSN8, CSNIO and CSNl 2 were administered once at a dosage of 60 mg/kg body weight

on day 8. Irinotecan® was administered 5 times at a dosage of 15 mg/kg body weight at days 8 to 12. Median values are given. Further details are given in Table 15.

Figure 7: *Time course of the median RTV values after administering SN-38 conjugates CSN14, CSN15, CSN16, CSN17, CSN19, CSN20 (dosage 60 mg/kg body weight; colon cancer model HT-29)*

Figure 7 shows the time course of the relative tumor volume of human colon cancer HT-29 xenografts growing in nude mice treated with conjugates CSN14, CSN15, CSN16, CSN17, CSN19, CSN20 vs. mice in the control group (untreated mice (saline)) as well as vs. mice treated with Irinotecan®.

The following symbols are used:

■ = Saline, * = Irinotecan, Δ = CSN14, ⧋ = CSN15, V = CSN16, ▽ = CSN17, ◇ = CSN19, ffl = CSN20.

The X-axis shows the time after start [d], the Y-axis shows the relative tumor volume, RTV [%].

Each measurement was carried out with a group of 8 mice. The conjugates CSN14, CSN15, CSN16, CSN17, CSN19, CSN20 were administered once at a dosage of 60 mg/kg body weight on day 7. Irinotecan® was administered once at a dosage of 60 mg/kg body weight on day 7. Median values are given. Further details are given in Table 16.

Figure 8: *Time course of the body weight change after administering SN-38 conjugates CSN14, CSN15, CSN16, CSN17, CSN19, CSN20 ((dosage 60 mg/kg body weight; colon cancer model HT-29)*

Figure 8 shows the time course of the body weight change in nude mice bearing human colon cancer HT-29 xenografts treated with conjugates CSN14, CSN15, CSN16, CSN17, CSN19, CSN20 vs. mice in the control group (untreated mice (saline)) as well as vs. mice treated with Irinotecan®.

The following symbols are used:

■ = Saline, * = Irinotecan lx, Δ = CSN14, ⧋ = CSN15, V = CSN16, ▽ = CSN17, ◇ = CSN19, ffl = CSN20.

The X-axis shows the time after start [d], the Y-axis shows the relative body weight [%].

Each measurement was carried out with a group of 8 mice. The conjugates CSN14, CSN15, CSN16, CSN17, CSN19, CSN20 were administered once at a dosage of 60 mg/kg body weight on day 7. Irinotecan® was administered once at a dosage of 60 mg/kg body weight on day 7. Median values are given. Further details are given in Table 16.

Figure 9: *Time course of the median RTV values after administering SN-38 or Irinotecan conjugates CSN21, CSN23, CSN22, CIrl, CIr2, CIr3 (dosage 30 to 80 mg/kg body weight; colon cancer model HT-29)*

Figure 9 shows the time course of the relative tumor volume of human colon cancer HT-29 xenografts growing in nude mice treated with conjugates *CSN21, CSN23, CSN22, CIrl, CIr2, CIr3* vs. mice in the control group (untreated mice (saline)) as well as vs. mice treated with Irinotecan (Campto®).

The following symbols are used:

■ = saline, ★ = irinotecan (Campto®), V = **CSN21,** Δ = **CSN23,** ◇ = **CSN22,** O = **CIrl,** > = **CIr2** ◁ = **CIr3.**

The X-axis shows the time after tumor transplantation (days), the Y-axis shows the relative tumor volume, RTV [%].

Each measurement was carried out with a group of 9 to 10 mice. The conjugates *CSN22, CIrl, CIr2 and CIr3* were administered once at a dosage of 80 mg/kg body weight on day 8, the conjugate *CSN23 was* administered once at a dosage of 60 mg/kg body weight on day 8, and the conjugate *CSN21 was* administered once at a dosage of 30 mg/kg body weight on day 8. Irinotecan (Campto®) was administered once at a dosage of 60 mg/kg body weight on day 8. Median values are given. Further details are given in Table 17.

FFiigguurree 1100:: *TTiimmee ccoouurrssee ooffttthhee bbooddyy wweeiigghhtt cchhaannggee aafftteerr aaddmmiinniisstteerriinngg SSNN--3388 oorr IIrriinnootteeccaann ccoonnjjuuggaatteess CCSSNN22I1,, CCSSNN2233,, CCSSNN2222,, CCIIrrll,, CCIIrr22,, CCIIrr33 ((ddoossaaggee 3300 ttoo 8 mmgg//kkgg bbooddyy wweeiigghhtt;; ccoolloonn ccaanncceerr mmooddeell HHTT--2299))*

3355 FFiigguurree 1100 sshhoowwss tthhee ttiimmee ccoouurrssee ooff tthhee bbooddyy wweeiigghhtt cchhaannggee iinn nnuuddee mmiiccee bbeeaarriinngg hhuummaann ccoolloonn ccaanncceerr HHTT--2299 xxeennooggrraaffttss ttrreeaatteedd wwiitthh ccoonnjjuuggaatteess *CCSSNN22I1,, CCSSNN2233,, CCSSNN2222,, CCIIrrll,, CCIIrr22,, CCIIrr33* vvss.. mmiiccee iinn tthhee ccoonnttrrooll ggrroouupp ((uunnttrreeaatteedd mmiiccee ((ssaalliinnee)))) aass wweellll aass vvss.. mmiiccee treated with Irinotecan (Campto®).

The following symbols are used :

■ = saline, * = irinotecan (Campto®), V = **CSN21,** Δ = **CSN23,** ◇ = **CSN22,** O = **CIrl,** ▷ = **CIr2 < = CIr3.**

The X-axis shows the time after tumor transplantation (days), the Y-axis shows the body weight change, BWC (%).

Each measurement was carried out with a group of 9 to 10 mice. The conjugates *CSN22, CIrl, CIr2 and CIr3* were administered once at a dosage of 80 mg/kg body weight on day 8, the conjugate *CSN23 was* administered once at a dosage of 60 mg/kg body weight on day 8, and the conjugate *CSN21 was* administered once at a dosage of 30 mg/kg body weight on day 8. Irinotecan (Campto®) was administered once at a dosage of 60 mg/kg body weight on day 8. Median values are given. Further details are given in Table 17.

Figure 11: *Time course of the median RTV values after administering combretastatin conjugates CCsl and CCs2 (dosage 40 to 60 mg/kg body weight; human mammary carcinoma (MAXF-401))*

Figure 11 shows the time course of the relative tumor volume of human mammary carcinoma (MAXF-401) xenografts growing in nude mice treated with conjugates CCsl and CCs2 vs. mice in the control group (untreated mice (saline)) as well as vs. mice treated with Combretastatine A4 phosphate.

The following symbols are used:
■ = saline, · = combretastatin A4-phosphate, ▲ = **CCs2,** ♦ **= CCsl.**

The X-axis shows the days after treatment [d]; the Y-axis shows the relative tumor volume, RTV [%].

Each measurement was carried out with a group of 4 mice. The conjugate CCsl was administered once at a dosage of 60 mg/kg body weight at the beginning of the study (day 0). The conjugate CCs2 was administered once at a dosage of 60 mg/kg body weight at the beginning of the study (day 0), and once at a dosage of 40 mg/kg body weight on day 10. Combretastatine A4 phosphate was administered once at a dosage of 50 mg/kg body weight at the beginning of the study (day 0), and once at a dosage of 40 mg/kg body weight on day 10. Median values are given. Further details are given in Table 18.

Figure 12: *Time course of the body weight change after administering SN-38 or Irinotecan conjugates CCsl and CCs2 (dosage 40 to 60 mg/kg body weight; human mammary carcinoma (MAXF-401))*

Figure 12 shows the time course of the body weight change in nude mice bearing human mammary carcinoma (MAXF-401) xenografts treated with conjugates CCsl and CCs2 vs. mice in the control group (untreated mice (saline)) as well as vs. mice treated with Combretastatine A4 phosphate.

The following symbols are used:
■ = saline, · = combretastatin A4-phosphate, **A = CCs2, ♦ = CCsl.**

The X-axis shows the days after treatment [d]; the Y-axis shows the relative body weight [%]·

Each measurement was carried out with a group of 4 mice. The conjugate CCsl was administered once at a dosage of 60 mg/kg body weight at the beginning of the study (day 0). The conjugate CCs2 was administered once at a dosage of 60 mg/kg body weight at the beginning of the study (day 0), and once at a dosage of 40 mg/kg body weight on day 10. Combretastatine A4 phosphate was administered once at a dosage of 50 mg/kg body weight at the beginning of the study (day 0), and once at a dosage of 40 mg/kg body weight on day 10. Median values are given. Further details are given in Table 18.

Figure 13: *Time course of the median RTV values after administering the etoposid conjugate CEtl (dosage 20, 40 amd 50 mg/kg body weight; human lung carcinoma LXFL-529*

Figure 13 shows the time course of the relative tumor volume of *human lung carcinoma LXFL-529* xenografts growing in nude mice treated with the etoposid conjugate CEtl vs. mice in the control group (untreated mice (saline)) as well as vs. mice treated with etoposid.

The following symbols are used:
Treatment: ■ = saline, · = etoposide (V-16), ▲ = **CEtl.**

The X-axis shows the time after first treatment [days]; the Y-axis shows the relative tumor volume, RTV [%].

Each measurement was carried out with a group of 4 mice (saline with 5 mice). The etoposid conjugate CEtl was administered once at a dosage of 10 mg/kg body weight at the beginning of the study, once at a dosage of 40 mg/kg body weight on day 3, once at a dosage of 50 mg/kg body weight on day 7. Etoposid was administered once at a dosage of

20 mg/kg body weight at the beginning of the study, once at a dosage of 20 mg/kg body weight on day 3, once at a dosage of 20 mg/kg body weight on day 7. Median values are given. Further details are given in Table 19.

Figure 14: *Time course of the body weight change after administering the etoposid conjugate CEtl (dosage 20, 40 amd 50 mg/kg body weight; human lung carcinoma LXFL-529*

Figure 14 shows the time course of the body weight change in nude mice bearing of *human lung carcinoma LXFL-529* xenografts treated with conjugates with the etoposid conjugate CEtl vs. mice in the control group (untreated mice (saline)) as well as vs. mice treated with etoposid.

The following symbols are used:
■ = saline, · = etoposide (V-16), ▲ = **CEtl.**

The X-axis shows the time after first treatment [days]; the Y-axis shows the relative body weight [%].

Each measurement was carried out with a group of 4 mice. The etoposid conjugate CEtl was administered once at a dosage of 10 mg/kg body weight at the beginning of the study, once at a dosage of 40 mg/kg body weight on day 3, once at a dosage of 50 mg/kg body weight on day 7. Etoposid was administered once at a dosage of 20 mg/kg body weight at the beginning of the study, once at a dosage of 20 mg/kg body weight on day 3, once at a dosage of 20 mg/kg body weight on day 7. Median values are given. Further details are given in Table 19.

Figure 15: *Cleavage Kinetics of combretatstatin conjugates CCsl and CCs2*

Figure 15 shows the cleavage kinetics of conjugates of 5 mg/mL of CCsl and CCs2, in ACN/PBS buffer, pH 7.4 (1:1), measured at 37°C and determined by RP-HPLC.

The following symbols are used:
■ = CCs2, ♦ = CCsl .

The X-axis shows the time [h], the Y-axis shows the conjugate [%].

Figure 16: *Cleavage Kinetics of Irinotecan conjugates CIrl and CIr2*

Figure 16 shows the cleavage kinetics of conjugates of 5 mg/mL of CIrl and CIr2 in PBS buffer, pH 7.4, measured at **37°C** and determined by RP-HPLC.

The following symbols are used:
■ = CIrl, ◆ = CIr2.

The X-axis shows the time [h], the Y-axis shows the conjugate [%].

Figure 17: *Cleavage Kinetics ofSN38 conjugates CSN19, CSN22, CSN24, CSN21*

Figure 17 shows the cleavage kinetics of conjugates of 5mg/mL of CSN19, CSN22, CSN24, CSN21, in PBS buffer, pH 7.4, measured at 37°C and determined by RP-HPLC.

The following symbols are used:
■ = CSN19, ♦ = CSN22, ▲ = CSN24, · = CSN21.

The X-axis shows the time [h], the Y-axis shows the conjugate [%].

1. Examples

1.1 Materials and Methods

1.1.1 General techniques

Centrifugation was performed using a Sorvall Evolution RC centrifuge (Thermo Scientific) equipped with a SLA-3000 rotor (6x 400 ml vessels) at 9000 g and 4°C for 5-10 min.
Ultrafiltration was performed using a Sartoflow Slice 200 Benchtop (Sartorius AG) equipped with two Hydrosart Membrane cassettes (10 kD Cutoff, Sartorius). Pressure settings: p i = 2 bar, p2 = 0.5 bar.
Filtration: Solutions were filtered prior to size exclusion chromatography and HPLC using syringe filters (0.45 μπι, GHP-Acrodisc, 13 mm) or Steriflip (0.45 μm, Millipore).
Analytical HPLC spectra were measured on an Ultimate 3000 (Dionex) using a LPG-3000 pump, a DAD-3000a diode array detector and a CI8 reverse phase column (Dr. Maisch, Reprosil Gold 300A, CI8, 5μm, 150x4.6 mm). Eluents were purified water (Millipore) + 0.1% TFA (Uvasol, MERCK) and acetonitrile (HPLC grade, MERCK) + 0.1% TFA. Standard gradient was: 2% ACN to 98% ACN in 30 min.

Size exclusion chromatography was performed using an Äkta Purifier (GE-Healthcare) system equipped with a P-900 pump, a P-960 sample pump using an UV-900 UV detector and a pH/lC-900 conductivity detector. A HiPrep 26/10 desalting column (53 ml, GE-Healthcare) was used together with a HiTrap desalting column as pre-column (5 ml, GE-Healthcare). Fractions were collected using the Frac-902 fraction collector.

Freeze-drying: Samples were frozen in liquid nitrogen and lyophylized using a Christ alpha 1-2 LD plus (Martin Christ, Germany) at p = 0.2 mbar.

UV-vis absorbances were measured at a Cary 100 BIO (Varian) in either plastic cuvettes (PMMA, d = 10 mm) or quarz cuvettes (d = 10 mm, Hellma, Suprasil, 100-QS) using the Cary Win UV simple reads software.

Table 2*: Hydroxyalkyl starch used (obtainable from Presenilis Kabi Linz (Austria))*

Name	Lot	Mw	Mn	PDI	MS
HESI	073121	84.5	55.2	1.47	1.3
HES2	17090821	769.5	498.6	1.54	1.3
HES3	17091 131	694.4	441.7	1.57	1.0
HES4	17091241	700.8	375.9	1.87	0.7
HES5	17091331	985.0	500.4	1.97	0.5
HES6	1709151 1	2379.5	708.4	3.36	0.7

Table 3: *Reagents used*

Entry	Name	Quality	Supplier	Lot#
		General procedure 1		
1	4-nitrophenyl chloroformate	96%	Aldrich	02107CH-029
2	Dimethyl sulfoxide	dry, SeccoSolv	Merck	K39250731
3	Pyridine	puriss.	Merck	K37206362
4	Cystamine dihydrochloride	98%	Aldrich	MKAA1973
5	DL-Dithiothreitol	>99%	Sigma	128K1092
6	Sodium borohydride	>96%	Fluka	S3871434806003
		General procedure 2		
7	Sodium hydride	60% w/w in paraffin	Merck	S4977752
8	Allyl bromide	reagent grade 97%	Aldrich	S77053-109
9	Potassium monopersulfate Triplesalt (Oxone®)	technical grade	Aldrich	82070

10	Sodium bicarbonate	puriss.	Merck	26533223
11	Tetrahydrothiopyran-4-one	99%	Aldrich	1370210 42708159
12	Sodium thiosulfate pentahydrate	p.a.	Acros	A02049 15001
13	Ethanedithiol	99%	Fluka	01391947
		General procedure 3		
14	lodoacetic acid	synthesis grade	Merck	S06291
15	Tetrabutylammonium fluoride trihydrate	>97%	Aldrich	03513TC-516
		Analytics		
16	**5,5**'-Dithiobis **(2-**nitrobenzoic acid), Ellman's reagent	>97.5%	Fluka	1334177
		Solvents		
17	Isopropanol	puriss ACS	Fluka	
18	Methyl tert. butyl ether	99%	Acros	
19	Dimethyl formamide	pept. syn. grade	Acros	A0256931
20	Trifluoroethanol	reagent plus >99%	Aldrich	S57348-458
21	Dimethyl formamide	extra dry 99.8%	Acros	A00954967
22	Formamide	spectophotometric grade >99%	Aldrich	59096HK
23	Acetic acid	>99.8%	Fluka	91190

1.2 Synthesis of SN38-derivatives

1.2.1 Synthesis of 10-(*tert.* butyldiphenylsiloxyl)-7-ethylcamptothecin

A 250 ml three-neck flask equipped with magnetic stirring and inside thermometer and electrical heating was charged with 80 ml of DCM and 3.3 ml (22.9 mmol) of triethyl amine. 1.5 g (3.82 mmol) of SN38 (which is commercially available and sold, for example, by company Tocris Bioscience) were added and dissolved under stirring. 6.0 ml (22.9 mmol) of teri.butyldiphenylsilyl chloride was added and the reaction mixture heated to reflux for 20 h (50°C). The progress of the reaction was monitored by TLC. After cooling down, the mixture was washed twice with 50 ml of 0.2 N HCl, twice with 50 ml of saturated sodium bicarbonate solution and once with 100 ml of brine. The organic phase was dried over sodium sulphate. The solvent was removed under reduced pressure. The crude product was dissolved in a small quantity of DCM. Hexane was added until the product started to precipitate and the mixture cooled to 4°C. The precipitate was filtered

and washed with a cold mixture of hexane and DCM. This was repeated until no TBDPSC1 was detectable in the product. The colourless crystals were dried under vacuum to give 1.27 g (2.02 mmol, 53%) of the title compound.

TLC (DCM / ethyl acetate 1:1): R_f = 0.50
(hexane / ethyl acetate 1:1): R_f = 0.40

^{1}H-NMR : ($CDCl_3$, 200 MHz) δ = 8.05 (d, 9.0 Hz, 1H) ; 7.81-7.74 (m, 4H) ; 7.57-7.36 (m, 8H) ; 7.09-7.07 (m, 1H) ; 5.73 (d, 16.4 Hz, 1H) ; 5.28 (d, 16.4 Hz, 1H) ; 5.11 (s, 2H) ; 3.71 (s, 1H, OH) ; 2.73-2.56 (m, 2H) ; 1.97-1.88 (m, 2H) ; 1.17 (s, 9H) ; 1.07-0.85 (m, 6H).

1.2.2 Synthesis of 20-(bromoacetyl)-10-(feri. butyldiphenylsiloxyl)-7-ethylcamptothecin-(bromoacetyl-TBDPS-SN38, SN38-1)

A I L three-neck flask equipped with with magnetic stirring, inert gas and inside thermometer was charged with 3.0 g (4.76 mmol) of *\0-(tert.* butyldiphenylsiloxyl)-7-ethylcamptothecin and 0.99 g of bromoacetic acid (7.13 mmol). 400 ml of dichloromethane were added and the mixture cooled to 0°C by means of an ice/water bath. Then, 1.26 ml (7.13 mmol) of EDC and 260 mg of DMAP were added and the resulting mixture stirred for 30 minutes at 0°C. The reaction was allowed to warm to room temperature and the progress of the reaction monitored by TLC.
The reaction mixture was washed twice with 300 ml of a 0.5% $NaHCO_3$ solution, which was saturated with sodium chloride. The organic phase was washed with 400 ml of a 20:1 mixture of water and brine and twice with 300 ml 0.1 N hydrochloric acid. The organic phase was dried with sodium sulphate and the solvent evaporated under reduced pressure. The crude product was purified by column chromatography on silica (hexane / ethyl acetate 1:1) to give 2.6 g (3.46 mmol, 72%) of a slightly yellow solid.

TLC (Hexane/ethyl acetate **1:1):** R_f= 0.60

'H -NMR : (CDCI3, 400 MHz) : δ = 8.04 (d, *J* = 9.2 Hz, 1H); 7.79-7.74 (m, 4H); 7.51-7.36 (m, 7H); 7.17-7.11 (m, 1H); 7.09 (d, *J* = 2.6 Hz, 1H); 5.67 (d, *J* = 17.2 Hz, lH); 5.39 (d, *J* = 17.2 Hz, 1H); 5.17-5.06 (m, 2H); 4.27-4.17 (m, 2H); 2.68-2.61 (m, 2H); 2.34-2.24 (m, 1H); 2.22-2.12 (m, 1H); 1.28 (s, 9H); 1.00-0.94 (m, 3H); 0.92-0.86 (m, 3H).

^{13}C-NMR: ($CDCl_3$, 100 MHz): δ = 166.9; 166.1; 157.3; 155.0; 149.5; 147.5; 145.2; 144.8; 143.9; 135.5; 132.1; 131.7; 130.2; 128.0; 127.9; 126.7; 125.9; 119.5; 110.3; 94.9; 77.4; 67.2; 49.2; 40.4; 31.8; 26.5; 22.9; 19.5; 13.3; 7.5.

1.2.3 Synthesis of 20-(3-maleimidopropionyI)-10-(ter£ butyIdiphenylsiloxyl)-7-ethylcamptothecin (Maleimidopropionyl-TBDPS-SN38, SN38-2)

A 250 ml round bottom flask equipped with magnetic stirring was charged with 40 ml DCM and 0.13 ml (714 μπιοϊ) of EDC. The mixture was cooled down to 0°C, followed by the addition of *N*-Maleoyl-β-alanine (130 mg, 714 μmoϊ) and DMAP (26 mg, 214 μmo1). The reaction mixture was stirred for 15 minutes followed by addition of TBDPS-SN38 (300 mg, 476 μπιοϊ). The reaction mixture was allowed to warm up to room temperature and the progress of the reaction monitored by TLC. After complete conversion, the reaction mixture was washed twice with 20 ml of saturated sodium bicarbonate solution, once with 100 ml of water and twice with 20 ml of 0.1 N HC1 solution. The organic phase was dried over sodium sulphate and the solvent was removed under reduced pressure. The crude product was applied on silica and purified by column chromatography (silica, hexane/ethyl acetate 1:2) to give 230 mg (294 μmoϊ, 61%) of an off-white solid.

TLC (DCM / ethyl acetate 1:1): R_f = 0.60

^{1}H-NMR : (CDC1$_3$, 200 MHz) δ = 8.04 (d, 9.2 Hz, 1H) ; 7.82-7.70 (m, 4H) ; 7.53-7.32 (m, 7H) ; 7.13-7.07 (m, 1H) ; 7.04 (s, lH) ; 6.66 (s, 2H) ; 5.63 (d, 17.1 Hz, 1H) ; 5.33 (d, 17.2 Hz ; 1H) ; 5.10 (s, 2H) ; 3.87-3.69 (m, 2H) ; 2.92-2.79 (m, 2H) ; 2.71-2.54 (m, 2H) ; 2.33-2.05 (m, 2H) ; 1.17 (s, 9H) ; 0.92-0.74 (m, 6H).

MS: (ESI; MeOH) 836.5 [M + Na$^+$ + MeOH]; 804.5 [M + Na$^+$], 782.5 [M + H$^+$].

1.2.4 Synthesis of 20-(maleimidoacetyl)-10-(ter£. butyldiphenylsiloxyl)-7-ethylcamptothecin (MaIeimidoacetyl-TBDPS-SN38, SN38-3)

A 100 ml round bottom flask equipped with magnetic stirring was charged with 50 ml DCM and 1.1 ml (6.35 mmol) of EDC. The mixture was cooled down to 0°C, followed by the addition of *N*-Maleoyl-p-alanine (985 mg, 6.35 mmol) and DMAP (35 mg, 285 μιηο]). The reaction mixture was stirred for 15 minutes followed by addition of TBDPS-SN38 (400mg, 635 μmoϊ). The reaction mixture was allowed to warm up to room temperature and the progress of the reaction monitored by TLC. After complete conversion, the reaction mixture was washed twice with 50 ml of saturated sodium bicarbonate solution, once with 100 ml of water and twice with 50 ml of 0.1 N HC1

solution. The organic phase was dried over sodium sulphate and the solvent was removed under reduced pressure. The crude product was applied on silica and purified by column chromatography (silica, DCM/ethyl acetate 1:1) to give 340 mg (442 μπιοϊ, 69%) of an off-white solid of an off-white solid.

TLC (DCM / ethyl acetate 1:1) R_f = 0.6

'H -NMR : ($CDC1_3$, 200 MHz) δ = 8.09 (d, 9.2 Hz, 1H) ; 7.82-7.73 (m, 4H) ; 7.53-7.34 (m, 7H) ; 7.17 (s, 1H) ; 7.1 1-7.07 (m, 1H) ; 6.72 (s, 2H) ; 5.62 (d, 17.2 Hz, 1H) ; 5.35 (d, 17.2 Hz, 1H) ; 5.09 (s, 2H) ; 4.47 (m, 2H) ; 2.74-2.55 (m, 2H) ; 2.36-2.06 (m, 2H) ; 1.17 (s, 9H) ; 1.01-0.80 (m, 6H).

MS: (ESI) 790.25 [M + Na^+]; 768 [M + H^+].

1.2.5. Synthesis of (S)-9-((tert-butyldiphenyIsilyl)oxy)-4,ll-diethyl-3,14-dioxo-3,4,12,14-tetrahydro-lH-pyrano[3',4':6,7]indoIizino[l,2-b]quinolin-4-yl-2-(3-(2,5-dioxo-2,5-dihydro-lH-pyrrol-l-yl)propanamido)acetate, (TBDPS-SN38-maleimidopropyl-glycin-ester, SN38-4)

1.2.5.1 20-(N-Boc-glycinyl)-10-O-TBDPS-SN38

A 250 ml 3-neck flask was equipped with a magnetic stirring bar and an inside thermometer. An outside cooling (ice/water) was prepared. The flask was loaded with 1 g of TBDPS-SN38 and 440 mg of *N*-Boc-glycine. The mixture was dissolved in 100 ml of DCM and then cooled to 0°C. 0.45 ml of EDC and 87 mg of DMAP were added. The temperature was allowed to rise to room temperature. The finishing of the reaction was monitored by TLC after 48 h. The reaction mixture was washed twice with an aqueous sodium bicarbonate solution (0.5%, each 100 ml). Then the organic phase was washed once with 300 ml of water, twice (each 100 ml) with a HC1 solution (0.1 N) and once with brine (100 ml). The organic phase was dried over sodium sulfate. The solvent was evaporated under reduced pressure. Yield was 1.2 g (96%) of a slightly yellow solid, which was used in the following step without purification.

^{1}H-NMR ($CDCI_3$, 200 MHz): δ = 7.96 (d, *J* = 9.2 Hz, 1H), 7.70 (d, *J* = 6.7, 4H), 7.44-6.98 (m, 10H), 5.66-5.21 (m, 2H), 5.02 (s, 2H), 4.9 (br s, 1H, NH), 4.10-3.96 (m, 2H), 2.64-2.49 (m, 2H), 2.26-1.98 (m, 2H), 1.32 (s, 9H), 1.10 (s, 9H), 0.92-0.76 (m, 6H).

TLC: (ethyl acetate:hexane // 2:1), R_f = 0.50.

1.2.5.2 20-glyciny!-l 0-O-TBDPS-SN38

A 25 ml 3-neck flask was equipped with a magnetic stirring bar and an inside thermometer. An outside cooling (ice/water) was prepared. The flask was loaded with 1 g of TBDPS-SN38-*N*-Boc-glycine. The flask was evaporated and refilled with nitrogen. The substance was cooled to 0°C and 9.3 ml of TFA were added to the flask. The cooling bath was removed and the mixture was stirred for 20 minutes. Then the TFA was removed under reduced pressure. The residue was dissolved in 100 ml of DCM and washed twice with an aqueous sodium bicarbonate solution (0.5%, each 100 ml). The organic phase was dried over sodium sulfate and then the solvent was evaporated under reduced pressure. Yield was 0.7 g (80%) of a light yellow solid.

'H -NMR (CDC1$_3$, 200 MHz): δ = 8.24 (d, *J* = 9.4 Hz, 1H), 8.00 (br s, 2H, NH_2), 7.63 (d, *J* = 7.7 Hz, 6H), 7.38-7.05 (m, 8H), 5.50-5.13 (m, 4H), 4.25-3.95 (m, 2H), 2.74-2.63 (m, 2H), 1.98-1.95 (m, 2H), 1.08 (s, 9H), 0.84-0.75 (m, 6H).

TLC: (ethyl acetaterDCM // 1:1) R_f = 0.35.

1.2.5.3 Synthesis of title compound

A 100 ml 3-neck flask was equipped with a magnetic stirring bar and an inside thermometer. An outside cooling (ice/water) was prepared. The flask was loaded with 189 mg of *N*-maleoyl-/?-alanine, 171 mg of l-hydroxybenzotriazole monohydrate and 50 ml of THF. Then 700 mg of TBDPS-SN38-Gly and 0.16 ml of TEA were added. The mixture was cooled to 0°C. Afterwards 340 mg of DCC were added. The cooling bath was removed and the mixture was stirred at room temperature for 2 h. The course of the reaction was controlled by TLC. The reaction mixture was filtrated and the solvent was removed under reduced pressure. The residue was dissolved in 50 ml of DCM and the mixture was washed twice with an aqueous sodium bicarbonate solution (0.5%, each 100 ml) and once with brine (100 ml). Then the organic phase was dried over sodium sulfate and the solvent was evaporated under reduced pressure to give a solid. The residue was purified by column chromatography on silica gel using ethyl acetate:hexane // 5:1 as eluant to furnish the product as light yellow solid (0.27 g, 32%).

1H-NMR (CDCI$_3$, 200 MHz): δ = 7.96 (d, *J* = 9.2 Hz, 1H), 7.70-7.66 (m, 4H), 7.42-6.99 (m, lOH), 6.50 (s, 2H), 6.41 (br s, lH, NH), 5.56-5.21 (m, 2H), 5.01 (s, 2H), 4.35-4.22 (m,

1H), 4.08-3.93 (m, 1H), 3.74-3.64 (m, 2H), 2.57-3.33 (m, 4H), 2.22-1.98 (m, 2H), 1.09 (s, 9H), 0.88-0.77 (m, 6H).

TLC: (ethyl acetate:DCM // 1:1) R_f = 0.50.

MS (ESI) m/z: 861.29 $(M + Na)^+$ & 839.31 $(M + H)^+$.

1.2.6. Synthesis of (S)-9-((tert-butyldiphenylsilyl)oxy)-4,ll-diethyl-3,14-dioxo-3,4,12,14-tetrahydro-lH-pyrano[3',4':6,7]indolizino[l,2-b]quinolin-4-yl-6-(2,5-dioxo-2,5-dihydro-lH-pyrrol-l-yl)hexanoate (TBDPS-SN38-maleimidohexanoyl ester, SN38-5)

A 250 ml 3-neck flask was equipped with a magnetic stirring bar and an inside thermometer. An outside cooling (ice/water) was prepared. The flask was loaded with 2 g of TBDPS-SN38 and 982 mg of 6-(2,5-dioxo-2,5-dihydro-lH-pyrrol-l-yl)hexanoic acid. The mixture was dissolved in 100 ml of DCM and then cooled to 0°C. Then 0.82 ml of EDC and 170 mg of DMAP were added. The temperature was allowed to rise to room temperature. The finishing of the reaction was monitored by TLC. The reaction mixture was washed twice with an aqueous sodium bicarbonate solution (0.5%, each 100 ml). Then the organic phase was washed once with 300 ml of water, twice (each 100 ml) with a HC1 solution (0.1 N) and brine (100 ml). The organic phase was dried over sodium sulfate and then the solvent was evaporated under reduced pressure to give 2.32 g of the crude product. Column chromatography on silica gel (ethyl acetate:DCM // 1:1) gave the title compound (710 mg, 27%) as light yellow solid.

^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 7.95 (d, *J* = 9.2 Hz, 1H), 7.71-7.67 (m, 4H), 7.43-6.28 (m, 7H), 7.01 (s, 2H), 6.54 (s, 2H), 5.61-5.22 (m, 2H), 5.03 (s, 2H), 3.43-3.35 (m, 2H), 2.42-2.34 (m, 2H), 1.65-1.41 (m, 6H), 1.32-1.15 (m, 4H), 1.10 (s, 9H), 0.89-0.76 (m, 6H).

TLC: (ethyl acetate:DCM // 1:1) R_f = 0.50.

MS (ESI) m/z: 836.21 $(M+Na)^+$, 824.37 $(M+H)^+$.

1.2.7. Synthesis of 20-(bromoacetyl)-irinotecan (Irn-1)

A 500 ml 3-neck flask was equipped with a magnetic stirring bar and an inside thermometer. An outside cooling (ice/water) was prepared. The flask was loaded with 1.7 g

of irinotecan and 1.0 g of bromoacetic acid. The substances were dissolved in 340 ml DCM, and the mixture was cooled to 0°C. Then 4.9 g of 2-bromo-l-methyl-pyridinium triflate and 3.1 g of DMAP were added. The reaction mixture was stirred for 5 minutes at 0°C and then allowed to warm to room temperature. The reaction was followed by HPLC. After 105 minutes the reaction mixture was quenched with 200 ml of 0.1 N HCl solution and diluted with another 100 ml of DCM. Then the phases were separated. The organic phase was washed with brine (100 ml) and dried over sodium sulfate. Afterwards the solvent was evaporated under reduced pressure. The solid residue was purified by column chromatography on silica gel (DCMrmethanol // 10:1) to give 425 mg (21%) of the title compound as brown solid.

TLC: (DCMrmethanol // 5:1) R_f = 0.75.
(DCM:methanol // 10:1) R_f = 0.30.

MS (ESI) m/z: 709.08 [M (^{8},Br) + H^+], 707.22 [M (^{79}Br) + H^+].

1.2.8. Synthesis of 20-(3-maleimidopropionyl)-irinotecan (Irn-2)

A 500 ml 3-neck flask was equipped with a magnetic stirring bar and an inside thermometer. An outside cooling (ice/water) was prepared. The flask was loaded with 2.0 g of irinotecan and 1.44 g of *N*-maleoyl-p-alanine. The substances were dissolved in 400 ml DCM, and the mixture was cooled to 0°C. Then 4.6 g of 2-chloro-l-methylpyridinium iodide and 3.6 g of DMAP were added. The reaction mixture was stirred for 5 minutes and then allowed to warm up to room temperature. After stirring for 1.5 h, TLC showed full conversion. The reaction mixture was quenched with 400 ml of 0.1 N HCl solution and diluted with another 400. ml of DCM. Then the phases were separated. The organic phase was washed with brine (500 ml) and dried over sodium sulfate. Afterwards the solvent was evaporated under reduced pressure. Column chromatography on silica gel (DCM:methanol // 10:1) afforded 1.3 g (52%) of the title compound.

^{1}H -NMR (CDCb, 200MHz): δ = 8.15 (d, *J* = 9.2 Hz, 1H), 7.79 (d, *J* = 2.4 Hz, lH), 7,52 (dd, *J* = 9.2 Hz, *J* = 2.4 Hz, 1H), 7.06 (s, 1H), 6.60 (s, 2H), 5.58 (d, *J = 17.2* Hz, 1H), 5.32 (d, *J* = 17.2 Hz, 1H), 5.17 (s, 2H), 4.62-4.33 (m, 2H), 3.90-0.70 (m, 31H).

TLC: (DCM:methanol // 5:1) R_f = 0.80.

MS (ESI) m/z: 738.27 $(M+H)^+$.

1.2.9. Synthesis of 20-(metacryl)-irinotecan (Irn-3)

A 11 3-neck flask was equipped with a magnetic stirring bar and an inside thermometer. An outside cooling (ice/water) was prepared. The flask was loaded with 2.0 g of irinotecan and 0.72 ml of methacrylic acid. The compounds were dissolved in DCM (tech. grade, 400 ml) and the mixture was cooled to 0°C. Then 4.6 g of 2-chloro-l-methylpyridinium iodide and 3.65 g of DMAP were added. The reaction mixture was stirred for 10 minutes at 0°C and then allowed to warm up to room temperature. After stirring for 1 h, TLC and HPLC showed full conversion. The reaction mixture was quenched with 300 ml of 0.1 N HC1 solution and diluted with another 300 ml of DCM. Then the phases were separated. The organic phase was washed with brine (300 ml) and dried over sodium sulfate. Afterwards the solvent was evaporated under reduced pressure. The solid residue was purified by column chromatography on silica gel (DCM:methanol // 10:1) to give the title compound (205 mg, 9%) as brown solid.

TLC: (DCM:methanol // 5:1) R_f = 0.85.

MS (ESI) m/z: 655.30 $(M + H)^+$.

1.2.10. Synthesis of bromoacetyl combretastatin A4 (CA4-1)

A 100 ml round bottom flask was charged with combretastatin A4 (99 mg) and bromoacetic acid (335 mg) under nitrogen. The compounds were dissolved in 50 ml of dry DCM followed by addition of EDC (60 µï) and DMAP (82.6 mg). The mixture was stirred at room temperature for 90 seconds, followed by evaporation. The residue was treated twice with ethyl acetate/hexane (1:1; 25 ml) followed by centrifugation. The solvents were evaporated from the centrifugate. The residue was dissolved in ethyl acetate (40 ml) followed by washing with 6 ml of a NaHCCh-solution (5%). The organic phase was dried and evaporated to yield 140 mg of bromoacetyl combretastatin A4. The material was used in conjugation experiments without further purification.

^{1}H-NMR (DMSO-D_6): δ = 7.14 (dd, ***J*** = 8.5 Hz, ***J*** = 2.1 Hz, 1H), 7.03 (d, ***J*** = 2.1 Hz, lH), 6.86 (d, ***J*** = 8.5 Hz, IH), 6.48 (s, 2H), 6.46 (s, 2H), 3.83 (s, 3H), 3.80 (s, 3H), 3.70 (s, 3H).

1.2.11. Synthesis of maleimidopropyl combretastatin A4 (CA4-2)

A 500 ml Schlenk flask was charged with combretastatin A4 (500 mg) and 200 ml of dry DCM under nitrogen, followed by the addition of maleimidopropionic acid (542 mg). The

resulting solution was stirred at room temperature for 15 minutes. Then it was cooled in an ice/water bath, and DMAP (86.5 mg) and EDC (560 uL) were added under nitrogen. The mixture was stirred at room temperature for 75 minutes, then washed twice with each 120 ml of a $NaHCO_3$-soIution, followed by HC1 (0.1 N; 2 x 120 ml) and brine (1 x 200 ml). The organic phase was dried over sodium sulfate, filtered and evaporated. The crude product was purified by flash chromatography on silica gel using ethyl acetate as eluent. The residue was treated three times with pentane in an ultrasonic bath. It was dried under vacuum at 10^{-3} mbar to yield maleimidopropionyl combretastatin A4 (680 mg, 92%) as an off-white powder.

'H-NMR (DMSO-D_6): δ = 7.12 (dd, ***J*** = 8.5 **Hz,** ***J*** = 2.5 Hz, 1H), 7.01 (**d,** ***J*** = 2.1 Hz, 1H), 6.38 (d, ***J*** = 8.5 Hz, 1H), 6.71 (s, 2H), 6.49 (s, 1H), 6.45 (s, 1H), 3.91 (t, ***J*** = 7.2 Hz, 2H), 3.83 (s, 3H), 3.78 (s, 3H), 3.69 (s, 3H), 2.89 (t, ***J*** = 7.2 Hz, 2H).

1.2.12. Synthesis of bromoacetyl etoposide (ETO-1)

A 100 ml Schlenk flask was charged with etoposide (500 mg) and 50 ml of dry acetonitrile under nitrogen. It was warmed to about 40°C to give a solution. After cooling to room temperature, DIPEA (222 µï) and bromoacetyl chloride (81.5 µï) were added. The solution was stirred for 30 minutes followed by addition of further bromoacetyl chloride (31.8 µï). After stirring for 30 minutes the mixture was partitioned between pH 7-buffer (60 ml) and ethyl acetate (80 ml). The organic phase was washed twice with each 50 ml of brine, then dried, filtered and evaporated. The dark brown crude product was purified by repeated flash chromatography on silica gel using DCM + 5% methanol as eluent. The main fraction was filled in a brown glass bottle as a dichloromethane solution. The solvent was removed in a stream of nitrogen and the residue was dried at 10^{-3} mbar to yield 340 mg of bromoacetyl etoposide (56%).

1H-NMR (DMSO-De): δ = 6.82 (s, 1H), 6.54 (s, 1H), 6.27 (br s, 2H), 5.99 (dd, ***J*** = 11.3 Hz, ***J*** = 1.3 Hz, 2H), 5.30 (s, 1H), 4.91 (d, ***J*** = 3.5 Hz, 1H), 4.74 (q, ***J*** = 3.5 Hz, 1H), 4.62 (dd, ***J*** = 6.4 Hz, ***J*** = 3.5 Hz, 1H), 4.42 (dd, ***J*** = 10.6 Hz, ***J*** = 8.9 Hz, 1H), 4.24 (t, ***J*** = 8.3 Hz, 1H), 4.17 (dd, ***J*** = 10.7 Hz, ***J*** = 4.0 Hz, 1H), 4.09 (s, 2H), 3.73-3.68 (m, lH), 3.67 (s, 6H), 3.57 (m, 1H), 3.42 (dd, ***J*** = 8.9 Hz, ***J*** = 7.7 Hz, 1H), 3.34-3.28 (m, 2H), 2.86 (m, 1H), 1.39 (**d,** ***J*** = 5.0 Hz, 3H).

1.2.13. Synthesis of maleimidopropionyl etoposide (ETO-2)

a) Preparation of maleimidopropionyl chloride

A Schlenk tube was charged with maleimidopropionic acid (2.0 g) and thionyl chloride (4.0 ml). The mixture was heated at reflux for 20 minutes followed by evaporation. The residue was washed three times with each 10 ml of dry pentane. Then it was dried at 10^{-3} mbar to yield maleimidopropionyl chloride (2.17 g; 97.9%) as yellow solid.

b) Preparation of title compound

A Schlenk tube was charged with etoposide (251 mg) and 50 ml of dry acetonitrile. The mixture was warmed to about 40°C to yield a solution. DIPEA (108.8 µl) and maleimidopropionyl chloride (91 mg) were added at room temperature under nitrogen. The mixture was stirred for 30 minutes followed by evaporation. The residue was dissolved in 100 ml of ethyl acetate. This solution was washed with each 10 ml of $NaHCO_3$ (5%), HC1 (0.1 N) and brine. The organic phase was dried over sodium sulfate, filtered and evaporated to yield 320 mg of maleimidopropionyl etoposide. The material was used in conjugation experiments without further purification.

Synthesis of TBDPS- topotecan

Topotecan hydrochloride was neutralized by solving 1.5 g of the hydrochloride in 50 ml of distilled water. Then a diluted solution of $NaHC0_3$ was added until the pH reached 9-10. The suspension was extracted three times with 50 ml dichloromethane. The organic phase was washed once with 50 ml of brine and was dried over **Na_2S04.** Finally the solvent was evaporated under reduced pressure. The topotecan (free base) was used without further purification.

A 100 ml 3-neck flask was equipped with a magnetic stirring bar, a reflux condenser and an inside thermometer. The flask was loaded with 65 ml DCM and 2.0 ml triethyl amine and 1.1 g of topotecan. After complete dissolution, 2.4 ml of TBDPS-C1 were given to the reaction mixture. The reaction was refluxed for 12 h. After cooling to room temperature, the reaction solution was washed twice with 75 ml of 0.2 M HCl, twice with 75 ml saturated Na_2C0_3 solution and once with 150 ml of brine. Then the organic phase was dried over sodium sulfate and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica (DCMrmethanol // 10:1). The product obtained from the chromatography was purified from DCM/hexane to yield 1.0 g (1.51 mmol, 63%) of the title compound as colorless solid.

TLC: (DCM:methanol // 10:1) R_f = 0.30.

MS: (ESI) m/z = 660.13 $[M+ H]^+$; 682.10 $[M + Na]^+$; 1341.27 $[2M + Na]^+$.

Bromoacetyl Topotecan (TOP-1)

A 100 ml 3-neck flask was equipped with a magnetic stirring bar, inert atmosphere and an inside thermometer. The flask was loaded with 210 mg of bromoacetic acid and 40 ml of DCM. The solution was cooled to 0°C. Then 650 mg of DMAP and 1.034 g of 2-bromo- 1-methyl-pyridinium triflate were added. The reaction mixture was cooled to 0°C and stirred for 15 minutes. Then a solution of 400 mg of TBDPS-topotecan in 10 ml of DCM was added to the reaction mixture dropwise at 0-5°C. Stirring was continued for 3 h at 0°C. The reaction mixture was washed twice with 0.1 M HCl-solution. The organic phase was washed with 50 ml of a mixture of water and brine (20:1) and brine. Then the organic phase was dried over sodium sulfate. The solvent was evaporated under reduced pressure at room temperature. The crude product was purified by flash column chromatography on silica (DCM:methanol // 40:1) to give the title compound (60 mg, 0.076 mmol, 13%) as yellow solid.

TLC: (DCM:methanol // 10:1) R_f = 0.45.

MS: (ESI) m/z = 782.13 [M (^{81}Br) + H]$^+$, 780.15 [M (^{79}Br) + H]$^+$.

Maleimidopropyl topotecan (TOP-2)

A 100 ml 3-neck flask was equipped with a magnetic stirring bar, inert atmosphere and an inside thermometer. The flask was loaded with 3-maleimido-propionic acid (288 mg). 40 ml of DCM were given to the reaction vessel and the resulting solution was cooled to 0°C. Then 730 mg of DMAP and 922 mg of 2-chloro-l -methyl-pyridinium iodide were added and the reaction mixture was stirred for 15 minutes at 0°C. Then a solution of 450 mg TBDPS-topotecan in 10 ml DCM was added dropwise to the reaction keeping the temperature at 0-5 °C. Stirring was continued for 1 h at 0°C. After the starting material has been consumed (TLC monitoring), the reaction mixture was washed twice with a 0.1 M HC1 solution, the organic phase was washed with 40 ml of a mixture of water and brine (20:1) and with brine. The organic phase was dried over sodium sulfate. The solvent was evaporated under reduced pressure at room temperature. The crude product was purified by column chromatography on silica to give the title compound (165 mg, 0.203 mmol, 30%) as light yellow solid.

TLC: (DCM:methanol // 10:1) R_f = 0.45.

MS: (ESI) m/z = 811.24 [M+ H]$^+$, 1643.07 [2M + Na]$^+$.

1.3 Multi-thio-HES-Synthesis

1.3.1 Synthesis of multi-thio-HES (Dl) via activation with nitrophenylchloroformate

a) Activation

In a dry, three-neck round bottom flask equipped with a magnetic stirring bar, inert gas inlet and temperature probe, 15 g HES1 was dissolved in 60 ml of a 1:1 mixture of dry DMSO and pyridine under inert atmosphere. The the solution was cooled to -25°C by means of a mixture of dry ice and ethanol and maintained between -25 and -15°C. Solid 4-nitrophenyl chloroformate (9.6 g) was added in small portions under stirring (5 min). The resulting, highly viscous solution was stirred for additional 30 min in the cold and then slowly poured into 900 ml of isopropanol. The resulting precipitate was collected by filtration over a pore 4 sinter funnel and washed with 4x 100 ml of isopropanol followed by 2x 150 ml MTBE. The precipitate was used in the next step without further purification.

b) Reaction with cystamine

The activated HES from the last step was filled into a 250 ml glass bottle and dissolved in 150 ml of a 1:1 mixture of DMSO and pyridine. 28.6 g of cystamin dihydrochlorid were added and the resulting yellow suspension stirred over night in the closed bottle. After that reaction time, the solution was centrifuged. The precipitate (excess cystamin) was discarded and the clear supernatant precipitated in 770 ml isopropanol. The mixture was centrifuged and the precipitated HES collected and re-dissolved in 240 ml of water. The product was further purified by ultrafiltration (concentrated to 100 ml, 20 volume exchanges with water, concentrated to 50 ml). The retentate was freeze-dried and the lyophilisate (12.3 g) used directly in the next step.

c) Reduction with DTT

In a 250 ml round bottom flask, the lyophilized intermediate from the last step was dissolved in 70 ml of a borate buffer (pH 8.15). A solution of 949 mg of DTT in 123 ml of borate buffer was added and the resulting reaction mixture reacted at 40°C under magnetic stirring. The mixture was precipitated in 600 mL of isopropanol and the HES collected by centrifugation. The precipitate was re-dissolved in 100 mL of 20 mM acetic acid + 2 mM EDTA and subjected to ultrafiltration (15 volume exchanges with 20 mM acetic acid + 2 mM EDTA followed by 5 volume exchanges with 20 mM acetic acid. The retentate was collected and freeze-dried to give 11.2 g (75%) of a colourless solid. GPC analysis

revealed a fraction of ~5% of high molecular weight impurities (with Mw >10^7 Dalton) which were depleted by fractionate precipitation.

d) Fractionated precipitation

10.4 g of the product from the reduction step were dissolved in 100 ml of DMF (peptide syn. grade) in a 400 ml beaker. Under constant magnetic stirring, isopropanol were added until the solution became cloudy. After addition of 95 ml isopropanol, the mixture was centrifuged, the precipitate discarded and the supernatant treated with additional isopropanol. After addition of further 8 ml, the mixture was centrifuged again, resulting in a second, minor fraction of gel-like, high molecular weight HES. Further addition of isopropanol to the supernatant resulted in precipitation of the last fraction of HES, which was collected, dissolved in water and subjected to ultrafiltration (15 volume exchanges with water). Yield: 2.72 g (18% referred to starting material). Thiol loading: 148.3 nmol/mg. Mw = 71 kDa, Mn = 47 kDa.

1.4 General procedures for the synthesis of multi-EtThio-HES and Multi-MHP HES via epoxidation

1.4.1 General procedure for the synthesis of multi-allyl HES (GP1.1)

Hydroxyethyl starch used in the preparation was thoughtfully dried prior to use either on an infra-red heated balance at 80°C until the mass remained constant or by leaving in a drying oven over night at 80°C. A 10% solution of the dry HES in dry DMF or formamide (photochemical grade) was prepared in a round bottom flask equipped with a magnetic stirring bar and a rubber septum under an atmosphere of inert gas. Sodium hydride (60% w/w in paraffin) was added in one portion and the resulting cloudy solution stirred for 1 h at room temperature followed by addition of allyl bromide. The reaction mixture was stirred over night, resulting in a colourless-light brown, clear solution. The solution was then slowly poured into 7-10 times the volume of isopropanol and the precipitate collected by centrifugation. The precipitated polymer was re-dissolved in water and subjected to ultrafiltration (15-20 volume exchanges with water). Freeze-drying of the retentate yielded a colourless solid.

1.4.2 General procedure for the synthesis of multi-epoxy HES (GP1.2)

In a glass beaker, multi-allyl-HES was dissolved in a $4*10^{-4}$ M EDTA solution (10-15 ml/g HES). Tetrahydrothiopyran-4-on was added and the solution stirred on a magnetic stirring plate. Oxone® and sodium hydrogen carbonate were mixed in dry state and the mixture

added in small portions to the HES-solution resulting in formation of thick foam. The mixture was stirred at ambient temperatures for 2 h, diluted with water to a volume of 100 ml and then directly purified by ultrafiltration (15-20 volume exchanges with water). The resulting retentate was collected and directly used in the next step.

1.4.3 General procedure for the synthesis of multi-MHP HES (GP1.3)

The solution of epoxydated HES obtained from GP1.2 was filled into a round bottom flask equipped with a magnetic stirring bar and a stopper. Sodium thiosulfate was added and, in certain experiments, acetic acid (50 µL/g HES) was added to keep the pH at 7 or below (without addition of acetic acid, the pH shifted to 10-11 during the course of the reaction). The resulting clear solution was stirred for two days at ambient temperatures. The polymer was purified by ultrafiltration (15-20 volume exchanges with water), the retentate concentrated to 100 ml directly subjected to reduction according to GP1.5.

1.4.4 General procedure for the synthesis of multi-EtThio HES (GP1.4)

The solution of epoxydated HES obtained from GP1.2 was slowly poured into 7-10 times the volume of isopropanol. The precipitate was collected by centrifugation and redissolved in formamide (photochemical grade). An equal volume of DMF (peptide synthesis grade) was added and the mixture transferred into a reaction vessel equipped with a magnetic stirrer and a rubber septum. A stream of inert gas was passed through the solution by means of a cannula for ~10 min followed by addition of ethandithiol. In case of formation of an emulsion, the mixture was made homogenous by addition of additional DMF. The reaction was started by addition of a 0.1 M solution of Na_2C0_3 and stirred for two days under inert atmosphere. Finally, the mixture was slowly poured into 7-10 times the volume of cooled isopropanol (4°C). The precipitate was collected by centrifugation, the polymer redissolved in water (white emulsion due to residual ethandithiol) and purified by ultrafiltration (15-20 volume exchanges with water), resulting in a clear retentate. The retentate was concentrated to 100 ml and directly reduced according to GP1.5.

1.4.5 General procedure for the reduction of multi-EtThio HES/multi-MHP HES (GP1.5)

The HES-solution from the previous step was transferred into a round bottom flask equipped with a magnetic stirring bar and a rubber septum. A stream of inert gas was passed through the solution by means of a cannula for ~10 min, followed by addition of sodium borohydride (100 mg/g HES). The reaction was stirred 2 h or over night under an

inert atmosphere. It was quenched by acidification with acetic acid (0.5 ml/g HES) under evolution of hydrogen. The neutralized/acidified solution was purified by ultrafiltration (15-20 volume exchanges with 20 mM acetic acid). The retentate was freeze dried to yield a colourless solid.

1.4.6 General procedures for the synthesis of SH-HES by saponification of thioacetyl-HES

(a) General procedure for the synthesis of thioacetyl-HES (GP 1.6)

Hydroxyethyl starch as used in the preparation was thoughtfully dried prior to use either on an infra-red heated balance at 80°C until the mass remained constant or by leaving in a drying oven over night at 80°C. In a round bottom flask equipped with a magnetic stirring bar and a rubber septum under inert gas, HES was dissolved in formamide to give a 20% solution. After the addition of collidine, the clear solution was cooled in an ice-water bath. Then, mesyl chloride was added dropwise and the reaction mixture kept in the ice bath for ~1 h. The cooling bath was removed and the solution allowed to warm up to room temperature. After additional 1 h of stirring, potassium thioacetate was added as a solid and the resulting amber solution was allowed to stir over night at the given temperature. After cooling to room temperature, the reaction mixture was diluted 5:1 with water and subjected to ultrafiltration (concentration to a 10% w/w HES solution followed by 15-20 volume exchanges with water). The retentate was used immediately in the next step. Alternatively, the thioacetyl-HES can be lyophilized and stored without signs of degradation.

(b) General procedure for the synthesis of SH-HES by saponification of thioacetyl-HES using sodium hydroxide (GP 1.7)

A 10% (w/v) solution of thioacetyl-HES derived from GP 1.6 in water was filled in a round bottom flask equipped with a magnetic stirring bar and a rubber septum under an inert gas atmosphere. The solution was degassed by passing a stream of inert gas through the mixture while continuous stirring for ~10 minutes. A 1 M sodium hydroxide solution was added (20% of total volume), followed by addition of solid sodium borohydride (10% w/w of HES). The resulting solution was allowed to stir under inert gas for 2 h. The reaction was quenched by addition of acetic acid (-0.5 ml/gram HES, pH = 5-7). The product was purified by ultrafiltration (15-20 volume exchanges with a 20 mM solution of acetic acid in water). Freeze-drying of the retentate afforded SH-HES as a colorless solid.

1.5 General procedure for the synthesis of HES-Drug conjugates

1.5.1 Conjugation in non-aqueous conditions (GP2.1a)

A round bottom flask equipped with magnetic stirring, rubber septum and inert gas inlet was charged with the appropriate HES derivative. Under an inert atmosphere, dry DMF was added to give a 5% HES-solution. In a PP-tube, the corresponding drug derivative was dissolved in dry DMF (~1 ml/100 mg derivative). Diisopropyl ethyl amine was added to the HES solution followed by addition of the drug derivative solution. The reaction mixture was purged with inert gas for several minutes and reacted over night at ambient temperature. Then, iodoacetic acid was added as a solid and the reaction mixture was stirred for an additional hour. The polymer was precipitated by pouring the solution into cold isopropanol (60 ml/ml solution). The precipitate was collected by centrifugation.

1.5.2 Conjugation in aqueous conditions (GP2.1b)

A round bottom flask equipped with magnetic stirring, rubber septum and inert gas inlet was charged with the appropriate HES derivative. DMF (10 ml/g HES) were added and the HES derivative allowed to dissolve. In a PP-tube, the corresponding drug derivative was dissolved in dry DMF (8 ml/g HES). A 0.1 M phosphate buffer + 5mM EDTA (2 ml/g HES) was added to the HES solution followed by addition of the drug derivative solution. The reaction mixture was purged with inert gas for several minutes and reacted for 2h at ambient temperature. Then, iodoacetic acid was added as a solid and the reaction mixture was stirred for an additional hour. The polymer was precipitated by pouring the solution into cold isopropanol (40 ml/ml solution). The precipitate was collected by centrifugation.

1.5.3 Deprotection (GP2.2)

The crude conjugate was transferred into a round bottom flask and dissolved in deprotection solution (0.1 M TBAF + 1M HOAc in water, 30 ml/g HES). The mixture was allowed to react for 1 h at ambient temperature and then poured into cold isopropanol (40 ml/ml solution). The conjugate was collected by centrifugation and re-dissolved in water (-80 ml/g HES). The product was purified by size exclusion chromatography (multiple runs with 15 ml injections). The first fractions of each run, containing the polymer, were pooled and freeze dried. The product was obtained as off-white solid.

1.5.4 Determination of thiol-content of HES derivatives (GP3)

A stock solution of 4 mg/ml of 5,5'-dithio-bis(2-nitrobenzoic acid), Ellman's reagent, in 0.1 M sodium phosphate buffer + 1 mM EDTA (pH 8) buffer was freshly prepared.

A 0.2 mg/ml solution of sample in buffer was prepared and **I** ml of this solution filled into a 2 ml vial. An additional vial containing 1 ml of plain buffer was used as blank. The samples were treated with 100 µL of the reagent stock solution, placed into a mixer and mixed at 750 rpm, 21°C for 15 minutes. The sample solutions were transferred into plastic cuvettes (d = 10 mm) and measured for absorbance at 412 nm. The amount of thiols present in the vial was calculated according to following formula (A = absorbance of sample, A^0 = absorbance of blank):

$$c[\mu\text{mol ï cm}^3] = \frac{1.\text{i} * (A_{4,2} - A^0_{412})}{14.150 \frac{cm^2}{\mu\text{mol ï}} * \text{\ } cm}$$

considering the concentration of 0.2 mg/ml and 1 cm^3 = 1 ml:

$$Loading\ [nmol\ I\ mg\ \text{\ }] = \frac{1000 * c}{0.2\ \frac{mg}{ml}}$$

The final value was calculated as the average loading from the three samples.

1.6 General procedure for the determination of drug content via UV absorption (GP4)

A stock solution of the drug conjugate sample (1-3 mg per measurement) in DMF (peptide synthesis grade) was prepared (c_{st0c} k = 0.1-0.5 mg/ml). A sample of DMF (peptide synthesis grade) was used as a blank. The absorbance at 370 nm was measured and the drug content in the diluted sample calculated using following formula:

$$c_{drw}\ [\mu mol / cm^3] = \frac{(A_\lambda - A^0_\lambda)}{\varepsilon_\lambda \frac{\text{cm}^2}{\mu\text{mol ï}} * 1\,cm}$$

considering the concentration of the sample solution **(conjugate)**:

$$Loading[\mu mol / g] = \frac{1000 * c_{drug}}{c_{conjugate}}$$

Taking into account the molecular weight of the drug:

$$Loading[mg/g] = \frac{Loading[\mu mol/g]}{1000} * Mw_{drug}\ [mg/mmol]$$

The final value is calculated as an average value of 3 independent measurements.

Table of extinction coefficients (derived from a calibration curve)

Entry	Substance	Mw [g/mol]	Solvent	Wavelength [nm]	ε [cm²/µmol]
1	SN38	392.41	DMF	370	24.989
2	Irinotecan	677.19	DMF+1% HOAc	366	26.732
3	Topotecan	421.45	DMF+1% HOAc	328	12.007
4	Etoposide	588.18	TFE/H_2O 9:1	288	3.849

1.7 General procedure for the determination of the mean molecular weight MW

The mean molecular weight" as used in the context of the present invention relates to the weight as determined according to MALLS-GPC.

For the determination, 2 Tosoh BioSep GMPWXL columns connected in line (13 µm particle size, diameter 7.8 mm, length 30 cm, Art.no. 08025) were used as stationary phase. The mobile phase was prepared as follows: In a volumetric flask 3.74 g Na-Acetate*3H20, 0.344 g NaN_3 are dissolved in 800 ml Milli-Q water and 6.9 ml acetic acid anhydride are added and the flask filled up to 1 l.

Approximately 10 mg of the hydroxyalkyl starch derivative were dissolved in 1 ml of the mobile phase and particle filtrated with a syringe filter (0.22 mm, mStarll, CoStar Cambridge, MA)

The measurement was carried out at a Flow rate of 0.5 ml/min.

As detectors a multiple-angle laser light scattering detector and a refractometer maintained at a constant temperature, connected in series, were used.

Astra software (Vers. 5.3.4.14, Wyatt Technology Cooperation) was used to determine the mean M_w and the mean M_n of the sample using a dn/dc of 0.147. The value was determined at λ=690 nm (solvent NaOAc/H2O/0.02%NaN3, T=20°C) in accordance to the following literature: W.M. Kulicke, U. Kaiser, D. Schwengers, R. Lemmes, ***Starch,* Vol. 43,** Issue 10 (1991), 392-396.

1.8. General procedure for the determination of the cleaving tendency of certain tested linker compounds

The cleaving tendency of certain linker compounds were determined by incubating certain hydroxyethyl starch conjugates (see table 11a) in borate buffer at pH 8 at 40°C for 24 h. After 24 h the amount of cleaved hydroxyalkyl starch conjugate was determined using HPLC (for conditions see "Materials and Methods"). The nature of the electron withdrawing group has shown to influence the drug release kinetics and thus as well the activity / toxicity profile of the particular conjugates. Stability measurements in aqueous buffers (borate pH 8, 40°C) clearly display this correlation.

1.9 General procedure for the determination of combretastatin A content via HPLC (GP 5)

The cleavage solution was freshly prepared from 0.1 M phosphate buffer pH 7 / acetonitrile / hydrogen peroxide (35%) = 5:4:1.

A sample of HES conjugate (4-10 mg) was dissolved in the cleavage solution to give a 5 mg/ml solution. The sample was placed in a thermomixer (750 rpm, 23°C) for 1 h and then diluted with additional cleavage solution to a concentration of 1 mg/ml. The samples were measured immediately by RP-HPLC (Injection: 50 µï, for conditions see "Materials and Methods"). The peak area of combretastatin (t_R = 17.4 min) was measured at 288 nm. The combretastatin A content was calculated from the following equation (derived from a calibration curve):

$$CA4[mg/g\ conjugal = 0.1094 * Area^{2,8} - 0.0531$$

The CA4 content was determined as a mean value of two independent sample preparations.

Table 4 *Synthesis of multi-Allyl-HES intermediates (11-116) according to GPl.l*

#	HES		Solvent	NaH	AllBr	Yield
		m[g]		m[mg]	V[mL]	[%]
I1	HES1	10.0	DMF	271	0.47	91
I2	HES2	10.0	DMF	271	0.47	84
I3	HES3	10.2	DMF	380	0.63	92
I4	HES4	9.9	FA	450	0.75	88
I5a	HES5	10.2	FA	500	0.85	93
I5b	HES5	10.1	FA	491	0.85	93
I6	HES6	10.0	FA	462	0.80	94

Table 5 *Synthesis of multi-EtThio and multi-MHP-HES derivatives according to GP1.2-GP1.5*

#	Allyl HES		GP1.2			GP1.3		GP1.4			GP1.5
			Oxone®	$NaHCO_3$	THTP[a]	$Na_2S_2O_3$	HOAc	Ethanedithiol	Buffer	$V_{DMF/FA}$	$NaBH_4$
		m[g]	m[g]	m[g]	m[mg]	m[g]	V[µL]	V[mL]	V[mL]	V[mL]	m[g]
D2	I1	4.00	2.00	0.85	25	10.8[b]	-	-	-	-	0.40[b]
D3	I2	2.08	1.00	0.45	7	-	-	11.45	4	30/0	0.21
D4		4.00	2.00	0.85	25	13.5[b]	30[b]	-	-	-	0.40[b]
D5	I3	8.76	5.89	2.46	37	-	-	27.0[b]	5[b]	100/0[b]	0.50[b]
D6						31.5[b]	40[b]	-	-	-	0.50[b]
D7	I4	8.67	7.12	3.05	45	-	-	32.5[b]	5[b]	45/50[b]	0.52[b]
D8						38.4[b]	49[b]	-	-	-	0.50[b]
D9	I5a	9.34	8.33	3.65	55	-	-	76.4	10	135/175	1.02
D10	I5b	9.33	8.38	3.56	58	20.1[b]	47[b]	-	-	-	0.84[b]
D11	I6	4.76	4.15	1.79	31	-	-	19.1	5	30/25	0.26[b]
D12						22.7[b]	29[b]	-	-	-	0.25[b,c]

[a] **Tetrahydrothiopyran-4-one;**

[b] **Reaction mixture was split into two aliquots after epoxidation. Amounts refer to ½ of the starting amount of HES.**

[c] **conjugate cross-linked after work-up, reduction was repeated.**

Table 6 *Characterization of multi-EtThio and multi-MHP-HES derivatives*

#	Yield [%]	Loading[a] [nmol/mg]	Mw [kD]	Mn [kD]	#	Yield [%]	Loading[a] [nmol/mg]	Mw [kD]	Mn [kD]
D2	95	176	104	55	**D8**	69	126	1510	499
D3	71	119	688	302	**D9**	65	213	838	498
D4	83	171	1014	523	**D10**	99	164	1071	499
D5	72	182	435	372	**D12**	47	197	1365	1210
D6	75	203	675	427	**D13**	81	155	506	439
D7	64	172	815	404					

[a] Determined according to GP3

Table 6a *Synthesis and Characterization of multi-SH-HES- derivatives according to GP1.6 and GPL 7*

Derivative	HES		V (Collidine) [µl]	V (MsCl) [µl]	m (KSAc) [g]	Yield [%]	Loading [nmol/mg]	Mw [kD]	Mn [kD]
	Type	m[g]							
D14	HES7	5.0	1040	306	4.45	87	261	97	89
D15	HES10	5.0*	957	282	2.07	88	205	342	242
D16	HES3	5.0*	1196	352	4.47	81	276	864	492
D17	HES8	27.0	3102	912	6.70	n.d.	169.6	83.3	67.0
D18	HES11	606	68700	20350	304	91	172.0	94.1	67.0
D19	HES8	10.0	1928	567	4.95	89	292.5	91.6	46.9
D20	HES8	10.0	1928	567	4.95	56	260.1	85.6	67.5
D21	HES9	10.0*	2314	680	4.95	97	266.1	297.5	190.0
D22	HES8	10.0	1639	482	4.29	90	205.1	86.6	45.4
D23	HES9	10.0*	1928	567	4.95	90	192.2	294.0	176.1

* prepared from a 10% solution of HES in FA

Table 7 *Synthesis of conjugates according to GP 2.1-GP2.2*

#	Derivative		GP	Drug Derivative		DIPEA	Buffer	IAA	Deprot.[a]		Yield[b]
		m[g]			m[mg]	V[ml]	V[ml]		V[ml]	t [h]	[%]
CSN1	D1	1.00	GP2.1a	SN38-1	227	0.25	-	330	35	1	87
CSN2	D2	1.00	GP2.1b	SN38-2	206	-	2	392	40	1	82
CSN3	D3	0.50	GP2.1a	SN38-1	89	0.10	-	132	22	1	87
CSN4	D4	0.50	GP2.1b	SN38-2	100	-	1	191	20	1	76
CSN5	D5	0.99	GP2.1a	SN38-1	274	0.31	-	407	20	1.5	81
CSN6	D6	0.98	GP2.1b	SN38-2	239	-	2	454	20	1	94
CSN7	D7	0.98	GP2.1a	SN38-1	259	0.30	-	384	20	1.5	40
CSN8	D8	1.00	GP2.1b	SN38-2	147	-	2	280	20	1	89
CSN9b	D9	0.75	GP2.1a	SN38-1	240	0.275	-	357	15[c]	1.5	16
CSN9a	D9	0.75	GP2.1a	SN38-1	240	0.275	-	357	25	1.5	22
CSN10	D10	0.50	GP2.1b	SN38-2	164	-	1	183	10	1	71
CSN11	D12	0.98	GP2.1a	SN38-1	297	0.34	-	440	20	1	68
CSN12	D13	1.01	GP2.1b	SN38-2	232	-	2	345	20	1.5	66
CSN13	D5	1.00	GP2.1b	SN38-3	234	-	2	454	20	1.5	87
CSN14	D14	0.5	GP2.1a	SN38-1	147	0.223	-	291	10	1	99
CSN15	D15	0.5	GP2.1a	SN38-1	116	0.176	-	229	10	1	86
CSN16	D14	0.5	GP2.1b	SN38-2	102	-	1	186	10	1	89
CSN17	D15	0.5	GP2.1b	SN38-2	80	-	1	229	10	1	84
CSN18	D16	0.5	GP2.1b	SN38-2	108	-	1	308	10	1	69
CSN19	D14	0.51	GP2.1b	SN38-5	107	-	1	291	10	1	87
CSN20	D15	0.5	GP2.1b	SN38-5	103	-	1	229	10	1	76
CSN21	D17	1.0	GP2.1b	SN38-4	213	-	2	378	20	1	98

CSN22	D18	1.0	GP2.1b	SN38-2	202	-	2	383	20	1	87
CSN23	D19	1.0	GP2.1a	SN38-1	263	0.500	-	651	20	1	99
CSN24	D17	1.0	GP2.1a	SN38-1	128	0.290	-	378	20	1	94
CIr1	D19	1.0	GP2.1a	Irn-1	259	0.501	-	652	-	-	88
CIr2	D20	1.0	GP2.1b	Irn-2	211	-	2	536	-	-	91
CIr3	D21	1.0	GP2.1b	Irn-2	236	-	2	594	-	-	92
CCs1	D20	1.53	GP2.1a	CA4-1	240	0.203	-	426**	-	-	90
CCs2	D22	1.50	GP2.1b	CA4-2	158	-	3	178**	-	-	94
CEt1	D23	1.0	GP2.1a	ETO-1	207	0.124	-	111**	-	-	96
CEt2	D18	0.5	GP2.1b	ETO-2	77	-	0.5	51**	-	-	90
CTp1	D18	0.4	GP2.1a	TOP-1	69	0.118	-	153	10	1	83
CTp2	D18	0.75	GP2.1b	TOP-2	157	-	1.5	288	20	1	93

[a] Volume of deprotection solution (0. 1M TBAF, 1M acetic acid)

[b] Calculated as $m_{[Product]}/(m_{[Derivative]}*(1+Loading_{[mg/g]}/1000))$

[c] Addition of 13 ml DMF

Table 8: *Characterization of HES conjugates*

#	Purity[a]	Loading (GP4)		Mw	Mn
	[%]	[mg API/g]	[µmol/g]	[kD]	[kD]
CSN1	>99.9	57.9	148	323	107
CSN2	>99.9	65.7	168	302	192
CSN3	>99.9	45.1	115	2500	1027
CSN4	>99.9	43.9	112	1600	818
CSN5	>99.9	48.0	122	1800	762
CSN6	>99.9	57.0	145	1300	628
CSN7	99.6	39.6	101	5600	875
CSN8	>99.9	32.3	82	5200	767
CSN9[c]	>99.9	57.1	146	18000	9800
CSN10	99.8	37.7	96	2800	828
CSN11	>99.9	40.6	104	17600	2160
CSN12	>99.9	39.4	101	7500	1870
CSN13	>99.9	43.5	111	2900	660
CSN14	>99.9	74	189	218	135
CSN15	>99.9	62	158	476	312
CSN16	>99.9	81	207	355	165
CSN17	>99.9	72	184	462	289
CSN18	>99.9	61	156	3420	1235
CSN19	>99.9	69	176	1245	440
CSN20	>99.9	56	143	7020	1110

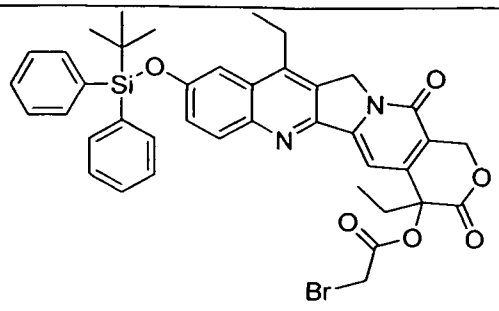
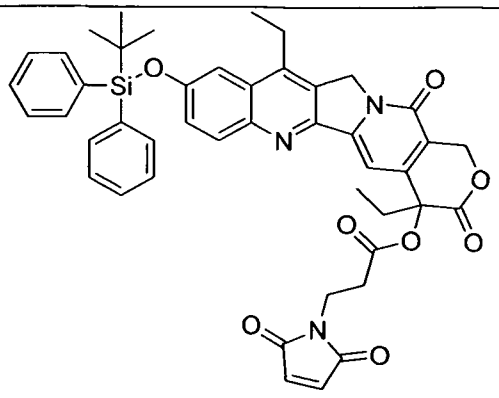
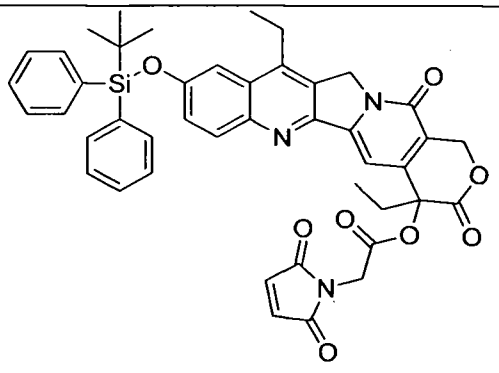
CSN21	>99.9	53.2	135	126	79
CSN22	>99.9	59.0	151	145	85
CSN23	99.5	49.4	126	222	85
CSN24	99.5	30.9	79	138	81
CIr1	>99.9	123.0	182	137	71
CIr2	99.2	77.0	114	122	85
CIr3	>99.9	89.7	132	465	262
CCs1	98.0	57.4[b]	181	139	95
CCs2	99.9	57.6[b]	182	125	60
CEt1	>99.9	104.9	178	2982	505
CEt2	>99.9	74.7	127	111	68
CTp1	>99.9	33.0	78	523	133
CTp2	>99.9	37.4	89	247	103

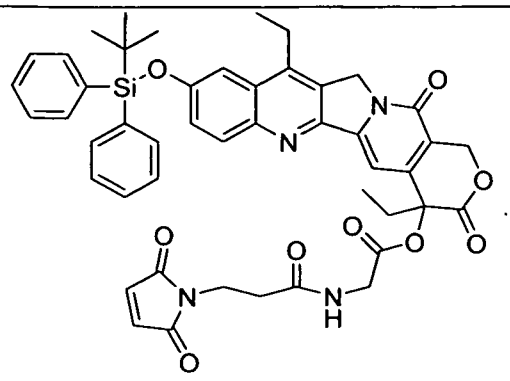
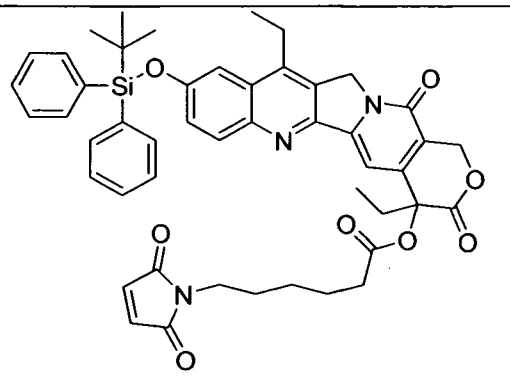
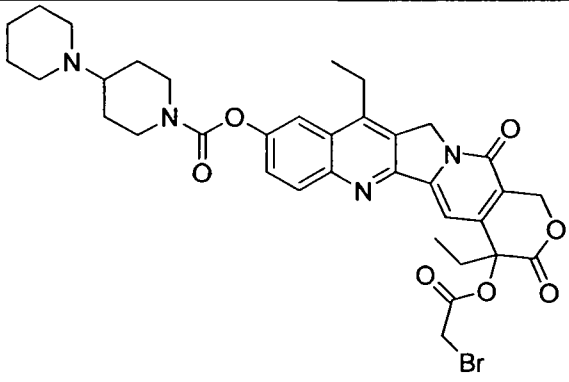
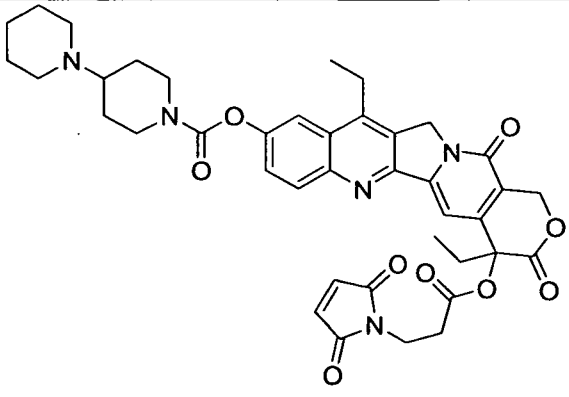
[a] Determined by HPLC

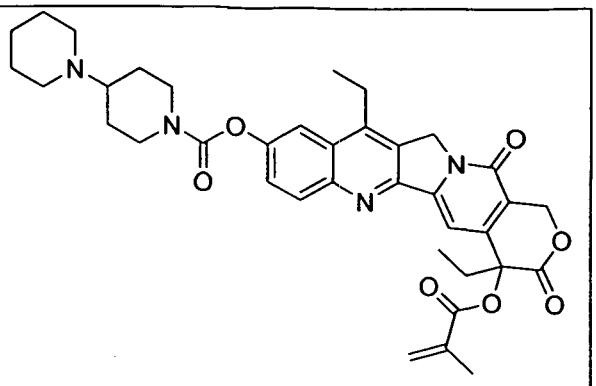
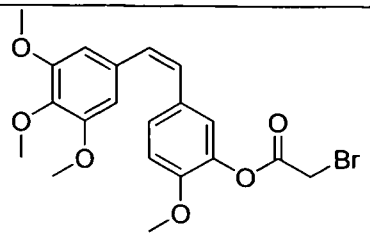
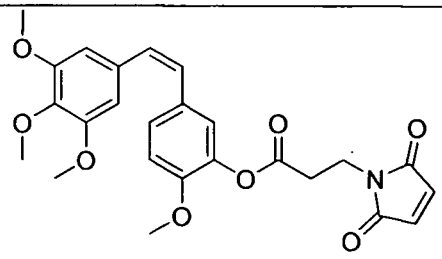
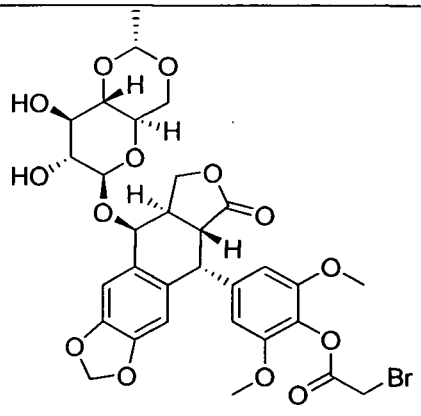
[b] According to GP5

[c] Conjugate derived from the preparations CSN9a and CSN9b

Table 9 *Overview over synthesized SN38-derivatives*

Code	Name	Formula
SN38-1	9-((tert-butyldiphenylsilyl)oxy)-4,11-diethyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl 2-bromoacetate	
SN38-2	9-((tert-butyldiphenylsilyl)oxy)-4,11-diethyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoate	
SN38-3	(*S*)-9-((tert-butyldiphenylsilyl)oxy)-4,11-diethyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl 2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetate	

SN38-4	(S)-9-((tert-butyldiphenylsilyl)oxy)-4,11-diethyl-3,14- ioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-quinolin-4-yl-2-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)acetate, (TBDPS-SN38-maleimidopropyl-glycin-ester)	
SN38-5	9-((tert-butyldiphenylsilyl)oxy)-4,11-diethyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl 2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoate	
IRN-1	[1,4']Bipiperidinyl-1'-carboxylic acid 4,11-diethyl-4-(bromoacetyloxy)-3,13-dioxo-3,4,12,13-tetrahydro-1H-2-oxa-6,12a-diaza-dibenzo[b,h]fluoren-9-yl ester	
IRN-2	[1,4']Bipiperidinyl-1'-carboxylic acid 4,11-diethyl-4-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propionyl oxy)-3,13-dioxo-3,4,12,13-tetrahydro-1H-2-oxa-6,12a-diaza-dibenzo[b,h]fluoren-9-yl ester	

IRN-3	[1,4']Bipiperidinyl-1'-carboxylic acid 4,11-diethyl-4-(2-methyl-acryloyloxy)-3,13-dioxo-3,4,12,13-tetrahydro-1H-2-oxa-6,12a-diaza-dibenzo[b,h]fluoren-9-yl ester	
CA4-1	Bromoacetyl-combretastatin A4	
CA4-2	3-Maleimidopropionyl-combretastatin A4	
ETO-1	4'-Bromoacetyl etoposide	

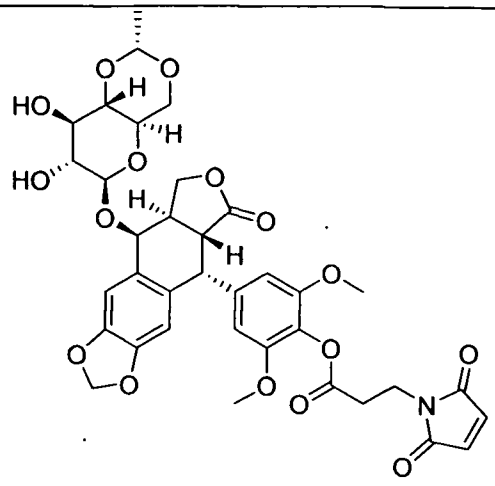
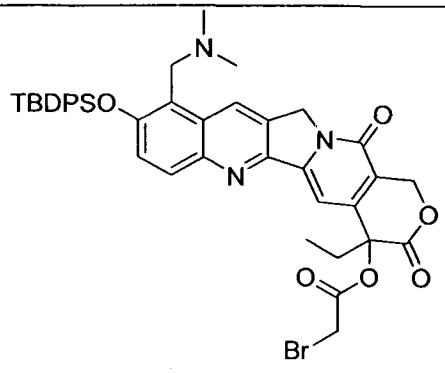
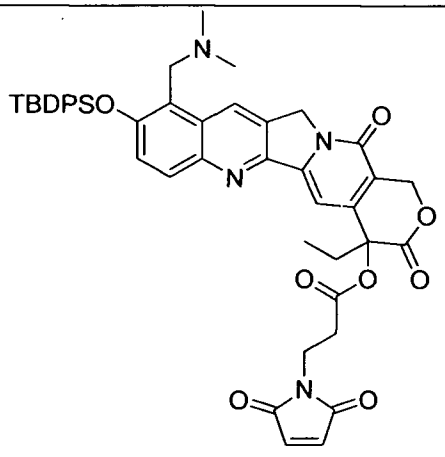
ETO-2	4'-(3-maleimidopropyl) etoposide	
TOP-1	20-(bromoacetyl)-9-dimethylaminomethyl-10-tert.-butyldiphenylsiloxy-camptothecin	
TOP2	20-(3-maleimidopropionyl)-9-dimethylaminomethyl-10-tert.-butyldiphenylsiloxy-camptothecin	

Table 10: ***Overview of synthesized hydroxyethyl starch derivatives***

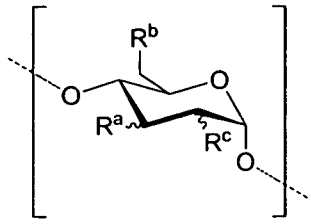
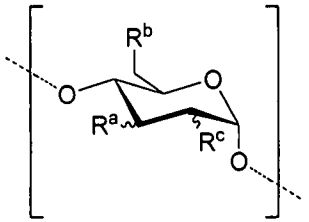
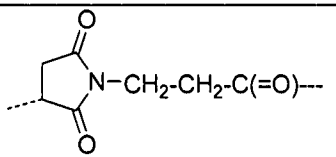
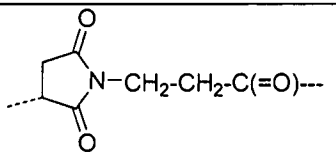
Code	Structure			
	HES used	***Structure of HES derivative***	***Linking moiety L***	***Cytotoxic agent M***
		 *with at least one of R^a, R^b or R^c of the shown structural unit being:* ···$[O–CH_2-CH_2]_t$-$[F^1]_p$-$[L^1]_{0,1}$-Z^1 wherein t is 0-4 and with -$[F^1]_p$-$[L^1]_{0,1}$-Z^1:		
D1	HES1	-O-C(=O)-NH-CH_2-CH_2-SH	---	---
D2	HES1	-O-CH_2-CHOH-CH_2-SH	---	---
D3	HES2	-O-CH_2-CHOH-CH_2-S-CH_2-CH_2-SH	---	---
D4	HES 2	-O-CH_2-CHOH-CH_2-SH	---	---
D5	HES 3	-O-CH_2-CHOH-CH_2-S-CH_2-CH_2-SH	---	---
D6	HES 3	-O-CH_2-CHOH-CH_2-SH	---	---
D7	HES4	-O-CH_2-CHOH-CH_2-S-CH_2-CH_2-SH	---	---
D8	HES4	-O-CH_2-CHOH-CH_2-SH	---	---
D9	HES5	-O-CH_2-CHOH-CH_2-S-CH_2-CH_2-SH	---	---
D10	HES5	-O-CH_2-CHOH-CH_2-SH	---	--
D12	HES6	-O-CH_2-CHOH-CH_2-S-CH_2-CH_2-SH	---	---
D13	HES6	-O-CH_2-CHOH-CH_2-SH	---	---
D14	HES7	-S-	---	---
D15	HES 10	-S-	---	---
D16	HES 3	-S-	---	---
D17	HES 8	-S-	---	---
D18	HES 9	-S-	---	---
D19	HES 8	-S-	---	---
D20	HES 8	-S-	---	---
D21	HES 9	-S-	---	---
D22	HES 8	-S-	---	---
D23	HES 9	-S-	---	---

Table 11: *Overview of synthesized hydroxyethyl starch conjugates*

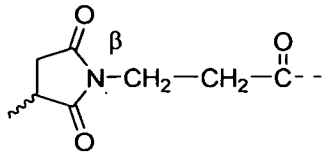
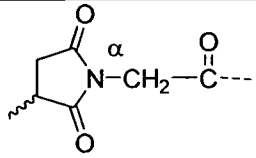
Code	Structure			
	HES used	*Structure of HES derivative*	*Linking moiety L*	*Cytotoxic agent M*
		 *with at least one of R^a, R^b or R^c of the shown structural unit being:* $\cdots[O]_v-[CH_2-CH_2]_t-[F^1]_p-[L^1]_{0,1}-X\cdots$ wherein t is 0-4 and with **$-[F^1]_p-[L^1]_{0,1}-X-$:**		
CSN1	HES1	-O-C(=O)-NH-CH_2-CH_2-S-	-CH_2-C(=O)-	-SN-38
CSN2	HES1	-O-CH_2-CHOH-CH_2-S-		-SN-38
CSN3	HES2	-O-CH_2-CHOH-CH_2-S-CH_2-CH_2-S-	-CH_2-C(=O)-	-SN-38
CSN4	HES 2	-O-CH_2-CHOH-CH_2-S-		-SN-38
CSN5	HES 3	-O-CH_2-CHOH-CH_2-S-CH_2-CH_2-S-	-CH_2-C(=O)-	-SN-38

CSN6	HES 3	-O-CH_2-CHOH-CH_2-S-		-SN-38
CSN7	HES4	-O-CH_2-CHOH-CH_2-S-CH_2-CH_2-S-	-CH_2-C(=O)-	-SN-38
CSN8	HES 4	-O-CH_2-CHOH-CH_2-S-		-SN-38
CSN9	HES 5	-O-CH_2-CHOH-CH_2-S-CH_2-CH_2-S-	-CH_2-C(=O)-	-SN-38
CSN10	HES 5	-O-CH_2-CHOH-CH_2-S-		-SN-38
CSN11	HES 6	-O-CH_2-CHOH-CH_2-S-CH_2-CH_2-S-	-CH_2-C(=O)-	-SN-38
CSN12	HES6	-O-CH_2-CHOH-CH_2-S-		-SN-38
CSN13	HES 3	-O-C(=O)-NH-CH_2-CH_2-S-		-SN-38
CSN14	HES 7	-S-	-CH_2-C(=O)-	-SN-38
CSN15	HES 10	-S-	-CH_2-C(=O)-	-SN-38

CSN16	HES 7	-S-		-SN-38
CSN17	HES 10	-S-		-SN-38
CSN18	HES 3	-S-		-SN-38
CSN19	HES 7	-S-		-SN-38
CSN20	HES 10	-S-		-SN-38
CSN21	HES 8	-S-		-SN-38
CSN22	HES 9	-S-		-SN-38

CSN23	HES 8	-S-	-CH_2-C(=O)-	-SN-38
CSN24	HES 8	-S-	-CH_2-C(=O)-	-SN-38
Clr1	HES 8	-S-	-CH_2-C(=O)-	-IRN
Clr2	HES 8	-S-		-IRN
Clr3	HES 9	-S-	-S-CH_2-CH(CH_3)-C(=O)-	-IRN
CCs1	HES 8	-S-	-CH_2-C(=O)-	-CA4
CCs2	HES 8	-S-		-CA4
CEt1	HES 9	-S-	-CH_2-C(=O)-	-ETO
CEt2	HES 9	-S-		-ETO
CTp1	HES9	-S-	-CH_2-C(=O)-	-TOP
CTp2	HES9	-S-		-TOP

Table 11a *Overview of the amount of cleaved hydroxyethyl starch conjugates in borate buffer, at 40 °C, pH 8 after 24 h*

Entry	Linker	SN38 released*
1	-S-CH_2-(C=O)-	54 %
2		25 %
3		68 %

*40°C, pH 8, amount SN38 determined by HPLC

2. In vivo testing SN38 and Irinotecan

2.1 Test animals

Adult female NMRI:nu/nu mice (TACONIC Europe, Lille Skensved, Denmark) bred in the own (EPO) colony were used throughout the study. At the start of experiment they were 6-8 weeks of age and had a median body weight of 19.0 to 32.6 g.

All mice were maintained under strictly controlled and standardized barrier conditions. They were housed - maximum five mice/cage - in individually ventilated cages (Macrolon Typ-ll, system Techniplast, Italy). The mice were held under standardized environmental conditions: 22 ± 1°C room temperature, 50 ± 10% relative humidity, 12 hour-light-dark-rhythm. They received autoclaved food and bedding (Ssniff, Soest, Germany) and acidified (pH 4.0) drinking water ad libitum.

Animals were randomly assigned to 12 experimental groups with 8 mice each. At treatment initiation the ears of the animals were marked and each cage was labeled with the cage number, study number and animal number per cage.

Table 12 provides an overview of the animal conditions.

Table 12 Summary of animal conditions

Subject	Conditions
Animals, gender and strain	female NMRI:nu/nu mice
Age	6-8 weeks
Body weight	19.0 to 32.6 g at the start of treatment
Supplier	EPO, Berlin
Environmental Conditions	Strictly controlled and standardised barrier conditions, IVC System Techniplast DCC (TECNIPLAST DEUTSCHLAND GMBH, HohenpeiBenberg)
Caging	Macrolon Type-II wire-mesh bottom
Feed type	Ssniff NM, Soest, Germany
Drinking water	autoclaved tap water in water bottles (acidified to pH 4 with HC1)
Feeding and	ad libitum 24 hours per day

drinking time	
Room temperature	22±1°C
Relative humidity	50±10%
Light period	artificial; 12-hours dark/12 hours light rhythm (light 06.00 to 18.00 hours)
Health control	The health of the mice was examined at the start of the experiment and twice per day during the experiment.
Identification	Ear mark and cage labels
Tumor model	HT-29, human colon carcinoma, ATCC-Nr. HTB-38 Human tumor cells in vitro. New York: Plenum Press; 1975

2.2 Tumor model

The human colon carcinoma HT-29 was used as s.c. xenotransplantation model in immunodeficient female NMRI:nu/nu mice.

The cells were obtained from ATCC and are cryo-preserved within the EPO tumor bank. They were thawed, expanded *in vitro* and transplanted as cell suspension subcutaneously (s.c.) in female NMRI:nu/nu mice. The tumor line HT-29 is used for testing new anticancer drugs or novel therapeutic strategies. It was therefore selected for this study. HT-29 xenografts are growing relatively fast and uniform.

Experimental procedure

For experimental use 10^7 tumor cells/mouse from the in vitro passage were transplanted s.c. into the flank of each of 10 mice/group at day 0.

Treatment

At palpable tumor size (30-100 mm^3) treatment started (day 8). The application volume was 0.2 ml/20g mouse body weight. The test compounds, the vehicle controls and the reference compounds were all given intravenously (i.v.).

2.3 Therapeutic evaluation

Tumor growth inhibition was used as therapeutic parameter. Additionally, body weight change was determined as signs for toxicity (particularly, potential hematological or gastrointestinal side effects).

Tumor measurement

Tumor diameters were measured twice weekly with a caliper. Tumor volumes were calculated according to V = (length x (width)^2)/2- For calculation of the relative tumor volume (RTV) the tumor volumes at each measurement day were related to the day of first treatment. At each measurement day the median and mean tumor volumes per group and also the treated to control (T/C) values in percent were calculated.

Body weight

Individual body weights of mice were determined twice weekly and mean body weight per group was related to the initial value in percent (body weight change, BWC).

End of experiment

On the day of necropsy mice were sacrificed by cervical dislocation and inspected for gross organ changes.

Statistics

Descriptive statistics were performed on the data of body weight and tumor volume. These data are reported in tables as median values, means and standard derivations, see Tables 14-17 in appendix. Statistical evaluation was performed with the U-test of Mann and Whitney with a significance level of $p \leq 0.05$, using the Windows program STATISTICA 6.

2.4 Analysis of the effects of Irinotecan conjugates on tumor growth and body weight

2.4.1 Tested substances

All HES-drug-conjugates were stored in a freeze-dried form at -20°C until use. Solutions were prepared immediately before injection by solving the conjugates in saline solution by vortexing in combination with centrifugation until a clear solution of the necessary concentration of the drug was obtained.

All solutions were prepared and injected under sterile conditions.

The reference compound Irinotecan® (Pfizer Pharma GmbH, Berlin; Nr. 43209.00.00, Lot. 7ZL030-A, 5% Glucose), was stored at 4°C in the dark and diluted in saline before administration.

As a further control, saline solution was intravenously administered.

The following table provides an overview on the dosage scheme for the various tested substances. Usually, the SN38-conjugates were administered only once at a dosage of 60 mg/kg body weight. Usually, the reference compound Irinotecan® was administered 5 times at a dosage of 15 mg/kg on 5 consecutive days each. A more comprehensive overview on the dosage scheme can be found in tables 14-17. For the analogue irinotecan-HES conjugates reference is made to table 17.

2.4.2 **Test results**

Tables 14 to 15 summarize the results for the tested SN38-conjugates and the reference compound Irinotecan®. The tables show, inter alia,

i) the tested compounds,
ii) the relative tumor volume in mice at the day the control group was sacrificed (in cm^3),
iii) the lowest value of the relative tumor volume vs. the relative tumor volume of the control group (RTV T/C) together with the day, when this optimum was reached,
iv) the maximum body weight loss in mice together with the day, when this minimum was reached.

The loss of body weight is known to be an indicator of gastrointestinal and hepatotoxicity of the tested compound.

The time course of the body weight change as well as the relative tumor volume for the tested compounds and the reference compound Irinotecan® is shown in Figures 1 to 6.

As it can be seen from the tables 14 to 15 and the Figures 1 to 6 as well as tables 16 to 17 and figures 7 to 10, the administration of a SN38-conjugate i) allows for a more efficient reduction of tumor size and/or ii) is less toxic (as indicated by the body weight change) than the administration of non-conjugated drug. The same accounts for the analogue irinotecan-HES conjugates (table 17 and figure 16).

3. In vivo experiments Combretastatin and Etoposide

3.1 Test animals

The athymic nude mouse is immunodeficient, thus enabling the xenotransplantation and growth of human tumors. Subcutaneous tumor implantation is a well-described methodology allowing visualization and quantification of tumor growth.

Specific Information:
Mouse strain: NMRI nu/nu, female
Animals supplied by: Charles River, Germany

Age of mice at implantation: 5-7 weeks

Animal Health and Monitoring:
All experiments were conducted according to the guidelines of the German Animal Welfare Act (Tierschutzgesetz). Animal health was examined prior to tumor implantation and randomization to ensure that only animals without any symptoms of disease were selected to enter testing procedures. During the experiments, animals were monitored daily regarding tumor burden, general condition, feed and water supply.

Animal Identification:
Animals were arbitrarily numbered during tumor implantation using ear clips. At the beginning of the experiments, each cage was labelled with a record card indicating the experiment number, date of tumor implantation, date of randomization, tumor type, tumor number and passage, mouse strain, gender, and individual mouse numbers. After randomization, the group identity, test compound, dosage, schedule, and route of administration were added.

Housing Conditions
The animals were housed in autoclaved individually ventilated cages (TECNIPLAST Sealsafe™-IVC, TECNIPLAST, Hohenpeissenberg, Germany). Depending on group size, they were housed in either type III cages or type II long cages. Dust-free bedding Lignocel® PS 14 was used (ssniff Spezialdiaten GmbH, Soest, Germany). The cages including the bedding were changed weekly. The temperature inside the cages was maintained at 25 ± 1°C with a relative humidity at 60 ± 10%. The animals were kept under a natural daylight cycle.

Diet and Water Supply
The animals were fed autoclaved ssniff NM complete feed for nude mice (ssniff Spezialdiaten GmbH, Soest, Germany) and had access to sterile filtrated and acidified (pH 2.5) tap water.

Bottles were autoclaved prior to use; they were changed twice a week. Food and water were provided ad libitum.

3.2 Tumor models

The tumor xenografts LXFL-529 and MAXF-401 (Fiebig HH, Berger DP, Dengler WA, Wallbrecher E, Winterhalter BR: Combined In Vitro/In Vivo Test Procedure with Human Tumor Xenografts for New Drug Development. Contrib. Oncol., Basel, Karger, 1992, Vol. 42, pp 321-351) used in this study were derived from surgical specimen from patients treated at the University Hospital in Freiburg, Germany, and directly implanted into nude mice. Prior to surgery, most of the patients had not received any chemotherapy.

Following their primary implantation into nude mice (passage 1), the tumor xenografts were passaged until establishment of stable growth patterns. Master stocks of early passage xenografts were then frozen in liquid nitrogen. Usually, a particular master stock batch itself is only used for maximally 30 passages. Therefore, the xenografts closely reflect the initial primary histology..

Tumor fragments were obtained from xenografts in serial passage in nude mice. After removal from donor mice, tumors were cut into fragments (4-5 mm diameter) and placed in PBS until subcutaneous implantation. Recipient mice were anaesthetized by inhalation of isoflurane. A small incision was made in the back and one tumor fragment per animal was transplanted with tweezers. The mice were monitored daily.

At randomization, tumor-bearing animals were stratified according to tumor volume into treatment and vehicle (control) groups. Only animals carrying one tumor of appropriate size (approximately 50-250 mm^3) were considered for randomization. Mice were randomized when the required number of mice qualified for randomization. The day of randomization was designated as day 0, which was also the first day of dosing.

3.3 Sample preparations

All test items were formulated in 0.9% NaCl solution and given as i.v. bolus injection.

Combretastatin A4 phosphate disodium salt was purchased from Toroma Organics (Saarbriicken), Lot. TB422. Etoposide was purchased from Sequoia Research Products, Lot. 1101012600e. Individual treatment schedules and results can be extracted from tables 18 and 19.

3.4 Therapeutic evaluation

Measurement of tumor volume and body weight as well as calculations of relative tumor volumes were carries out analgue to the procedure described in **2.3.**

3.5 Test results

3.5.1 Combretastatin

The data (table 18, figures 11, 12 and 15) illustrate that HES-Combretastatin A4 conjugates show a significant inhibition of tumor growth in a tumor model in which the reference drug combretastatin A4 phosphate (CA4P) shows only modest anti-tumor activity. The slower releasing conjugate **CCs2** showed advantages over **CCsl** in that only temporary toxic effects were observed.

3.5.2 Etoposide

In this experimental setting (table 19, figure 13-14), HES-etoposide **(CEtl)** showed a clear inhibition of tumor growth. Further, during the course of the experiment, the dose of etoposide equivalents in **CEtl** could be more than doubled without any detectable toxic effects. Compared to native etoposide, the tumor shrinkage after day 7 was more pronounced for the group treated with **CEtl** (40% loss of tumor volume between d7 and dl6 compared to 26% for the etoposide group). At the last day of the experiment, 4/5 tumors of the HES-conjugate group were still shrinking or at least stabile, which accounted only for 2/5 tumors of the etoposide group.

Table 14 Summary of the results for the tested SN-38 conjugates

		Treatment	Dose	BWC	Toxic	Group	Tumor	RTV T/C (%)
Group	**Mice**	(d)	(mg/kg/inj.)	[%]	death	**sacrif.**	**volume**	Optimum
	n			(at day)	day	(at day)	cm^3/d54	(at day)
Saline	8	9		-1		54	0,764+/-0,350	
				(13)				
Irinotecan	8	9-13	15	-9	1	54	0,419+/-0,271	48,2
				(13-16)	(34)			(54)
CSN1	8	9	60	-3		54	0,211+/-0,166**	23,5
				(13)				(54)
CSN2	8	9	60	-4		54	0,514+/-0,523	35,2
				(13)				(54)
CSN4	8	9	60	-2		54	0,139+/-0,113**	12,4
				(13-16)				(54)
CSN3	8	9	60	-8	1	54	0,186/-0,113**	23,5
				(13)	(14)			(54)

* Significantly different to saline ($p<0.01$)

Table 15 Summary of the results for the tested SN-38 conjugates

		Treatment	Dose	Toxic	Group	BWC	Tumor	RTV T/C (%)
Group	**Mice**	(d)	(mg/kg/inj.)	**death**	**sacrif.**	[%]	volume	Optimum
	n			(at day)	(at day)	(at day)	cm^3/d54	(at day)
Saline	8	8			34	-2	1,369+/-0,519	
						(13)	(d34)	
Irinotecan	8	8-12	15		54	-2	1,238+/-0,664	27,7*
						(13)		-30
CSN5	8	8	60	2	57	-18	0,662+/-0,202	11,1**
				(14+16)		(13)		-30
CSN6	8	8	60	1	57	-2	0,740+/-0,395	13,0**
				(15)		(13)		-30
CSN7	8	8	60	1	57	-11	0,725+/-0,195	15,8**
				(16)		(13)		-30
CSN8	7	8	60		57	0	0,852+/-0,258	19,6*
						(13-16)		-30
CSN9	8	8	60		54	0	0,970+/-0,428	29,1*
						(13)		-30
CSN10	7	8	60		54	-1	1,289+/-0,514	38,2*
						(13)		-34
CSN11	8	8	60		57	-11	0,831+/-0,367	14,2*
						(13)		-30
CSN12	7	8	60		54	-1	1,149+/-0,378	21,1*
						(13)		-30
CSN13	8	8	60	8		1	0,044+/-0,004	31,9
				(13)		(13)	(d13)	-13

* Significantly different to saline ($p<0.05$); ** Significantly different to saline and irinotecan ($p<0.05$)

Table 16: Summary of the results for the tested SN-38 conjugates

Group	Mice	Treatment	Dose	Toxic	Group	BWC	Tumor	RTV T/C (%)
	n	(d)	(mg/kg/inj.)	death	sacrif.	[%]	volume	Optimum
				(at day)	(at day)	(at day)	cm^3/d42	(at day)
Saline	8	7	60		42	>0	1.25+/-0.43	
Irinotecan	8	7	60		42	-4.8	0.92+/-0.5	66.3
						(10)		(21)
CSN14	8	7	60		42	-7.5	0.31+/-0.22	22.9**
						(14)		(28)
CSN15	8	7	60	1	42	-16.1	0.22+/-11	17.1**
				(17)		(14)		(35)
CSN16	8	7	60		42	-1.8	0.72+/-0.37	51.9*
						(14)		(21)
CSN17	8	7	60		42	-1.4	0.56+/-0.12	33.4*
						(14)		(21)
CSN19	8	7	60		42	-3.5	0.75+/-0.24	60.0*
						(10)		(42)
CSN20	8	7	60		42	>0	1.04+/-0.45	83.5
								(42)

* Significantly different to saline ($p<0.05$); ** Significantly different to saline and irinotecan ($p<0.05$).

Table 17: Summary of the results for the tested SN-38 and Irinotecan conjugates

Substance		Treatment	Dose	Toxic	Group	BWC	Tumor	RTV T/C (%)
	Mice	(d)	(mg/kg/inj.)	death	sacrif.	[%]	volume	Optimum
	n			(at day)	(at day)	(at day)	cm^3/d42	(at day)
Saline	9	8		-	24		1.84+/-0.86	
Irinotecan	9	8	60	1 (20)	24	-0.8	1.06+/-0.51	45.3 (21)
(Campto®)						(10)		
CSN21	9	8	30	-	24	-8.7	0.13+/-0.05**	6.6 (17)
						(14)		
CSN23	10	8	60	-	24	-6.9	0.14+/-0.04**	7.2 (21)
						(14)		
CSN22	9	8	80	-	24	-0.7	0.21+/-0.16**	10.6 (17)
						(10)		
CIr1	9	8	80	-	24	-12.9	0.47+/-0.28**	21.9 (17)
						(10)		
CIr2	9	8	80	1 (11)	24	-11.8	0.49+/-0.20**	23.5 (17)
						(10)		
CIr3	9	8	80	1 (13)	24	-7.1	0.63+/-0.31*	31.3 (17)
						(10)		

* Significantly different to saline $p<0.01$

** Significantly different to saline (pO.01) and to irinotecan (Gr. F:$p<0.05$, all others pO.01)

Table 18: Summary of the results for the tested Combretastatin-conjugates

Substance	Mice n	Treatment (d)	Dose (mg/kg/inj.)	Mortality (at day)	BWC [%] (at day)	RTV T/C (%) optimum (at day)
Saline	4	0, 10	-	1 (14)	-	-
Combretastatin A4 Phosphate	4	0, 10	50, 40	0	-1.8 (7)	59.8 (13)
CCsl	4	0	60	3 (3,7,7)	-23.7 (7)	49.7 (3)
CCs2	4	0, 10	60, 40	1 (6)	-7.5 (3)	24.7 (13)

Table 19: Summary of the results for the tested Etoposid conjugates

Substance	**Mice** n	**Treatment** (d)	**Dose** (mg/kg/inj.)	**Mortality** (at day)	**BWC** [%] (at day)	**Tumor** volume $cm^3/dl6$	**RTV T/C (%)** optimum (at day)	**RTV T/C (%)** re-normalized on day 7
Saline	5	0, 3, 7	-	1(13)	-	2.037 +/-0.549	-	-
V-16 (Etoposid)	4	0, 3, 7	20	-	-	0.293 +/-0.137	15.5 (16)	22.6
CEtl	4	0, 3, 7	20, 40, 50	-	-	0.476 +/-0.210	17.7 (16)	19.7

Claims

1. A hydroxyalkyl starch (HAS) conjugate comprising a hydroxyalkyl starch derivative and a cytotoxic agent, said conjugate having a structure according to the following formula

$$HAS'(-L-M)_n$$

wherein
M is a residue of a cytotoxic agent, wherein the cytotoxic agent comprises a tertiary hydroxyl group,
L is a linking moiety,
HAS' is a residue of the hydroxyalkyl starch derivative,
n is greater than or equal to 1,
wherein the hydroxyalkyl starch derivative has a mean molecular weight MW above the renal threshold, preferably a MW greater than or equal to 60 kDa,
and a molar substitution MS in the range of from 0.6 to 1.5,
and wherein the linking moiety L is linked to a tertiary hydroxyl group of the cytotoxic agent.

2. The conjugate according to claim 1, wherein the hydroxyalkyl starch conjugate is a hydroxyethyl starch (HES) conjugate.

3. The conjugate according to claim 1 or 2, wherein the hydroxyalkyl starch derivative has a mean molecular weight MW in the range of from 60 to 1500 kDa, preferably in the range of from 200 to 1000 kDa, more preferably in the range of from 250 to 800 kDa.

4. The conjugate according to any of claims 1 to 3, wherein the hydroxyalkyl starch derivative has a molar substitution MS in the range of from 0.70 to 1.45, more preferably in the range of from 0.80 to 1.40, more preferably in the range of from 0.85 to 1.35, more preferably in the range of from 0.95 to 1.30.

5. The conjugate according to any of claims 1 to 4, wherein the linking moiety L has a structure -L'-F^3-, wherein F^3 is a functional group linking L' to M via the group -O- derived from the tertiary hydroxyl group of the cytotoxic agent, thereby forming a

group - F^3-0-, F^3 preferably being -C(=Y)-, with Y being O, NH or S, preferably O or S, and wherein L' is a linking moiety.

6. The conjugate according to claim 5, wherein the bond between the functional group F^3 and the functional group -O- of M is a cleavable linkage, which is capable of being cleaved *in vivo* so as to release the cytotoxic agent, wherein the functional group -CD- is derived from the tertiary hydroxyl group of the cytotoxic agent.

7. The conjugate of claim 5 or 6, wherein the conjugate comprises an electron-withdrawing group in alpha, beta or gamma position relative to each F^3 group, wherein the electron-withdrawing group is selected from the group consisting of -0-, -S-, -SO-, -S0 $_2$-, -NR^e-, cyclic imide groups, -C(=Y^e)-, -NR^e-C(=Y^e)-, -C(=Y^e)-NR^e-, -CH(N0 $_2$)-, -CH(CN)-, aryl moieties or an at least partially fluorinated alkyl moiety, wherein Y* is either O, S or NR^e, and R^e is hydrogen or alkyl,
preferably wherein the electron-withdrawing group is selected from the group consisting of -NH-C(=0)-, -C(=0}-NH-, -NH-, -0-, -S-, -SO-, -S0 $_2$- and -succinimide-.

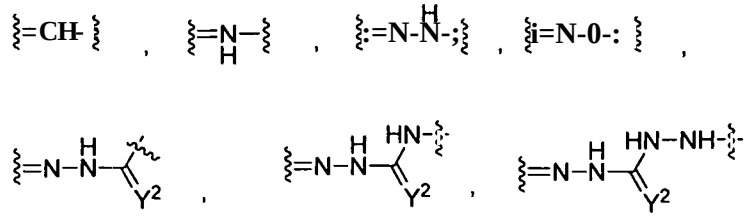
8. The conjugate according to claim 7, wherein the conjugate comprises
   (i) an electron-withdrawing group selected from the group consisting of-S- and -O- in alpha position to each F^3 group, or
   (ii) an electron-withdrawing group selected from the group consisting of -C(=0)-NH-, -NH-C(=0)- and -succinimide- in beta position to each F^3 group.

9. The conjugate according to any of claims 5 to 8, wherein L' has a structure according to the following formula

$$-[F^2]_q-[L^2]g-[E]_e-[CR^mRV$$

wherein E is an electron-withdrawing group, preferably selected from the group consisting of-C(=0)-NH-, -NH-C(=0)-, -NH-, -0-, -S-, -SO- , -S0 $_2$- and -succinimide-,
L^2 is a linking moiety, preferably an alkyl, alkenyl, alkylaryl, arylalkyl, aryl, heteroaryl, alkylheteroaryl or heteroarylalkyl group,

F^2 is selected from the group consisting of -Y^1- , -C(=Y^2)-, -C(=Y^2)-NR^{F2}- ,

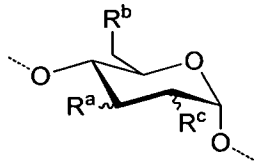


and $-CH_2-CH_2-C(=Y^2)-NR^{F2}-$,

wherein Y^1 is selected from the group consisting of -S-, -0-, -NH-, -NH-NH-, $-CH_2-CH_2-S0_2-NR^{F2}-$, $-CH_2-CHOH-$, and cyclic imides, and wherein Y^2 is selected from the group consisting of NH, S and O, and wherein R^{F2} is selected from the group consisting of hydrogen, alkyl, alkylaryl, arylalkyl, aryl, heteroaryl, alkylheteroaryl or heteroarylalkyl group,

f is 1, 2 or 3, preferably 1 or 2,
g is 0 or 1,
q is 0 or 1,
e is 0 or 1,
and wherein R^m and R^n are, independently of each other, H or alkyl, preferably H or methyl, in particular H.

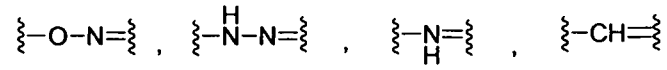
10. The conjugate according to any of claims 1 to 9, wherein the hydroxyalkyl starch derivative comprises at least one structural unit, according to the following formula (I)

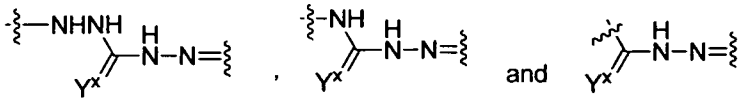


(I)

wherein R^a, R^b and R^c are, independently of each other, selected from the group consisting of-O-HAS", $-[0-<CR^wR^x)-(CR^yR^z)]_x-OH$, $-[0-(CR^wR^x)-(CR^yR^z)]y-X-$, and $-[O-(CR^wR^x)-(CR^yR^z)]_y-[F^1]_p-L^1-X-$, wherein R^w, R^x, R^y and R^z are independently of each other selected from the group consisting of hydrogen and alkyl, y is an integer in the range of from 0 to 20, preferably in the range of from 0 to 4, and wherein x is an integer in the range of from 0 to 20, preferably in the range of from 0 to 4,
and wherein at least one of R^a, R^b and R^c is $-[0-(CR^wR^x)-(CR^yR^z)]y-X-$ or $-[()-(CR^wR^x)-(CR^yR^z)]_y-p^1]_p-L'-X-$,

and wherein X is selected from the group consisting of - Y^{xx}- , -C(=Y^{x})-, -C(-Y^{x})-NR**-, -CH_2-CH_2-C(=Y^{x})-NR^{x_x}-,





wherein Y** is selected from the group consisting of -S-, -0-, -NH-, -NH-NH-, -CH_2-CH_2-S0 $_2$-NR^{xx}-, and cyclic imides, such as succinimide, and wherein Y^{x} is selected from the group consisting of NH, S and O, and wherein R^{x_x}* is selected from the group consisting of hydrogen, alkyl, alkylaryl, arylalkyl, aryl, heteroaryl, alkylheteroaryl or heteroarylalkyl group,

F^1 is a functional group, preferably selected from the group consisting of -Y^7-, -Y^7-C(=Y^6)- , -C(=Y^6)-, -Y^7-C(=Y^6)-Y^8-, -C(=Y^6)-Y^8-, wherein Y^7 is selected from the group consisting of -NR^{Y_7}-, -0-, -S-, -succinimide, -NH-NH-, -HN-0-, -CH=N-0-, -0-N=CH-, -CH=N-, -N=CH-, Y^8 is selected from the group consisting of -NR^{Y8}-, -S-, -0-, -NH-NH- and Y^6 is selected from the group consisting of NR^{Y6}, O and S, wherein R^{Y_6} is H or alkyl, preferably H, and wherein R^{Y_7} is H or alkyl, preferably H, and wherein R^{Y8} is H or alkyl, preferably H,

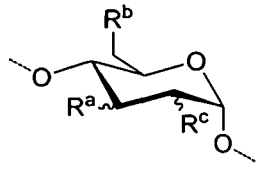
p is 0 or 1,

L^1 is a linking moiety, preferably an alkyl, alkylaryl, arylalkyl, aryl, heteroaryl, alkylheteroaryl or heteroarylalkyl group,

and wherein HAS" is a remainder of HAS,

preferably wherein at least 0.3% to 3 % of all structural units present in the hydroxyalkyi starch derivative comprise the functional group X.

1 1. The conjugate according to claim 10, wherein the hydroxyalkyi starch derivative comprises at least one structural unit according to the following formula (I)



(I)

wherein R^a, R^b and R^c are independently of each other selected from the group consisting of-O-HAS", -[0-CH $_2$-CH $_2$]$_s$-OH, -[0-CH $_2$-CH$_2$],-X- and -[0-CH $_2$-CH$_2$]$_t$-[F^1]p-L^1-X-,

and wherein s is in the range of from 0 to 4,

and wherein t is in the range of from 0 to 4,

p is 0 or 1,

wherein at least one of R^a, R^b and R^c is -[0-CH $_2$-CH$_2$]$_t$-X- or -[0-CH $_2$-CH$_2$]$_t$-[F'] $_p$-L'-X-,

and wherein HAS" is a remainder of HAS.

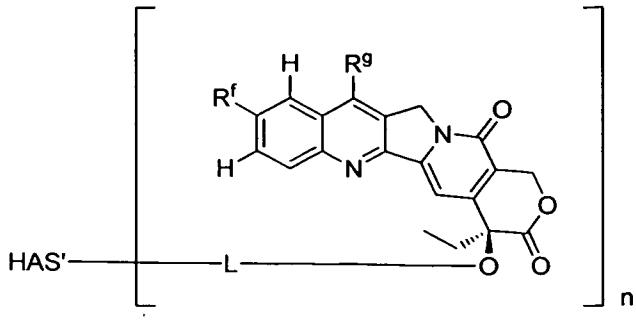
12. The conjugate according to claim 11, wherein at least one ofR^a, R^b and R^c is
(i) -[0-CH $_2$-CH$_2$]$_t$-X-, or
(ii) -[0-CH $_2$-CH$_2$]r[F ^{1}]$_p$-L^1-X-, and wherein p is 1 and F^1 is -0-, or
(iii) -[0-CH $_2$-CH$_2$]$_t$-[F ^{1}]$_p$-L ^{1}-X-, and wherein p is 1 and F^1 is -0-C(=0)-NH-,

wherein X is -S-,

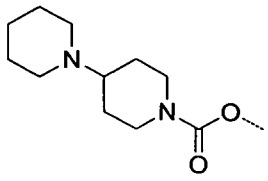
and wherein t is in the range of from 0 to 4.

13. The conjugate according to any of claims 1 to 12, wherein the cytotoxic agent is a topoisomerase I inhibitor,
more preferably wherein the cytotoxic agent is selected from the group consisting of camptothecin, topotecan, irinotecan, DB67, BNP 1350 (cositecan), exatecan, lurtotecan, ST 1481, gimatecan, belotecan, CKD 602, karenitecin, chimmitecan, 9-aminocamptothecin, 9-nitrocamptothecin, BMS422461, diflomotecan, BN80927, BMS422461, morpholino-CPT and KOS-1584.

14. The conjugate according to any of claims 1 to 13, wherein the conjugate has a structure according to the following formula



wherein R^f is selected from the group consisting of -OH, siloxy groups, ester groups and groups having the structure

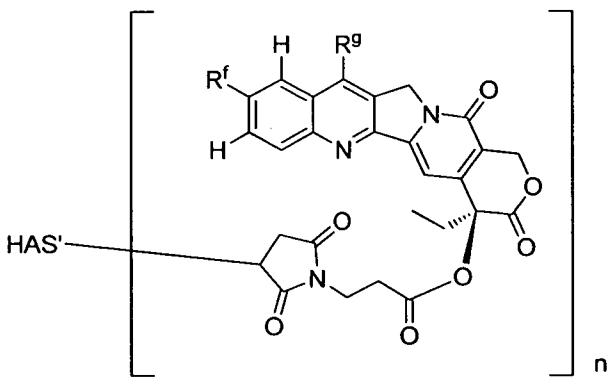


wherein R^f is preferably -OH,
and wherein R^8 is **$-CH_2-CH3$.**

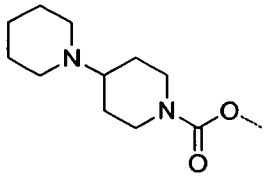
15. The conjugate according to claim 9, said conjugate having a structure according to the following formula

$$HAS'(-[F^2]_q-[L^2]_g-[E]_e-[CR^mR^n]r-F^3-M)_n$$

wherein q is 1, F^2 is -succinimide-, and f is 2,
wherein F^3 is -C(=0)-,
wherein the structural unit $-[CR^mR^n]_f-$ is preferably $-CH_2-CH_2-$, more preferably wherein e is 0 and g is 0, more preferably wherein the conjugate has a structure according to the following formula

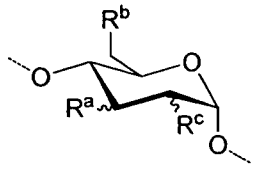


wherein R^f is selected from the group consisting of -OH, siloxy groups, ester groups or groups having the structure



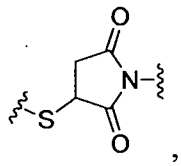
wherein R^f is preferably -OH,
and wherein R^8 is $-CH_2-CH_3$.

16. The conjugate according claim 15, wherein HAS' comprises at least one structural unit, preferably 3 to 200 structural units, according to the following formula (I)


(I)

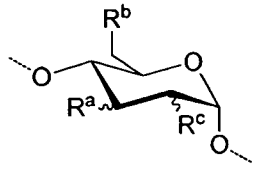
wherein R^a, R^b and R^c are independently of each other selected from the group consisting of -O-HAS", $-[0-CH_2-CH_2]_s-OH$ and $-[0-CH_2-CH_2]_t-X-$,

wherein s is in the range of from 0 to 4,
wherein t is in the range of from 0 to 4,
and wherein at least one of R^a, R^b and R^c is $-[0-CH_2-CH_2]_t-X-$, wherein X is -S- and wherein X is directly bound to F^2, thereby forming a covalent linkage having the structure:


,

and wherein HAS" is a remainder of HAS.

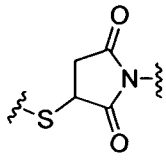
17. The conjugate according to claim 15 or 16, wherein HAS' comprises at least one structural unit, preferably 3 to 200 structural units, according to the following formula (I)


(I)

wherein R^a, R^b and R^c are independently of each other selected from the group consisting of-O-HAS", -[0-CH 2-CH 2]s-OH, and -[0-CH 2-CH2]r[F 1]p-L 1-X-,

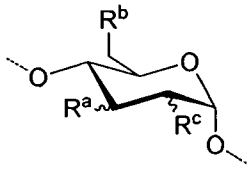
wherein s is in the range of from 0 to 4,
t is in the range of from 0 to 4,
p is 0 or 1,
and wherein at least one of R^a, R^b and R^c is $-[O-CH_2-CH_2]_t-[F^1]_p-L^1-X-$,
wherein F1 is -0-,
wherein L1 is a linking moiety having a structure according to the following formula
$-\{[CR^dR^f]_h-[F^4]_u-[CR^{dd}R^{ff}]_z\}_{alpha}-$,
wherein F4 is a functional group, preferably selected from the group consisting of-S-, -O- and -NH-, in particular -S-, wherein
z is in the range of from 0 to 20, more preferably of from 0 to 10, more preferably of from 0 to 3 h is in the range of from 1 to 5, preferably in the range of from 1 to 3, more preferably 3,
u is 0 or 1,
integer alpha is in the range of from 1 to 10,
and R^d, R^f, R^{dd} and R^f are, independently of each other, selected from the group consisting of H, alkyl, hydroxyl, and halogene, preferably selected from the group consisting of H, methyl and hydroxyl, and wherein each repeating unit of-[CR dRf]h-[F4]u-[CR ddRff]z- may be the same or may be different,
wherein, more preferably, L1 has a structure selected from the group consisting of
-CH 2-CHOH-CH 2-, -CH 2-CHOH-CH 2-S-CH 2-CH 2-,
-CH 2-CHOH-CH 2-S-CH 2-CH 2-CH 2-, -CH 2-CHOH-CH 2-NH-CH 2-CH 2-,
-CH 2-CHOH-CH 2-NH-CH 2-CH 2-CH 2-, -CH 2-, -CH 2-CH 2-, -CH 2-CH 2-CH 2-,
-CH 2-CH 2-CH 2-CH 2-, -CH 2-CH 2-CH 2-CH 2-CH2-,
-CH2-CH(CH2OH)-, -CH2-CH(CH2OH)-S-CH2-CH 2-,
-CH2-CHOH-CH2-0-CH 2-CHOH-CH 2-,
-CH2-CHOH-CH2-0-CH 2-CHOH-CH 2-S-CH2-CH2-,
-CH 2-CH 2-CH 2-S-CH 2-CH 2-, -CH 2-CH 2-S-CH 2-CH 2- and
-CH 2-CH 2-0-CH 2-CH 2-, more preferably from the group consisting of
-CH 2-CHOH-CH 2-, -CH 2-CHOH-CH 2-S-CH 2-CH 2-,
-CH 2-CHOH-CH 2-S-CH 2-CH 2-CH 2-, -CH 2-CHOH-CH 2-NH-CH 2-CH 2- and
-CH 2-CHOH-CH 2-NH-CH 2-CH 2-CH 2-, more preferably from the group consisting of-CH 2-CHOH-CH 2-, -CH 2-CHOH-CH 2-S-CH 2-CH 2- and
-CH 2-CHOH-CH 2-S-CH 2-CH 2-CH 2-,

wherein X is -S- and X is directly bound to F^2, thereby forming a covalent linkage having the structure



and wherein HAS" is a remainder of HAS.

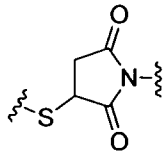
18. The conjugate according to any of claims 15 to 17, wherein HAS' comprises at least one structural unit according to the following formula (1)



(I)

wherein R^a, R^b and R^c are independently of each other selected from the group consisting of-O-HAS", -[0-CH_2-CH_2]$_s$-OH, and $-[O-CH_2-CH_2]_t-[F^1]_p-L^1-X-$,

wherein s is in the range of from 0 to 4,
t is in the range of from 0 to 4,
p is 0 or 1,
and wherein at least one of R^a, R^b and R^c is -[0-CH_2-CH_2]$_t$-[F']$_p$-L^1-X-,
wherein F^1 is -0-(C=0)-NH-,
wherein the linking moiety L^1 is an alkyl group,
wherein X is -S- and X is directly bound to F^2, thereby forming a covalent linkage having the structure



and wherein HAS" is a remainder of HAS.

19. The conjugate according to claim **9**, said conjugate having a structure according to the following formula

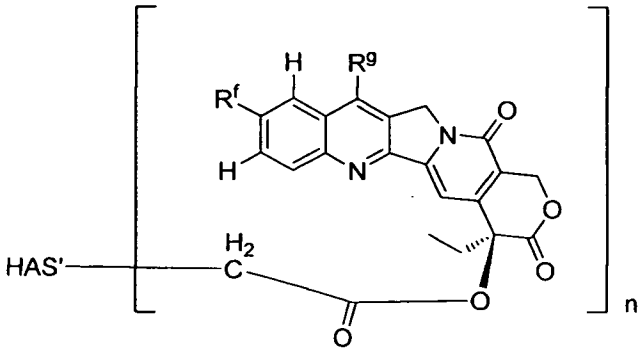
$$HAS'(-[F^2]_q-[L^2]_g-[E]_e-[CR^mR^n]_f-F^3-M)_n$$

wherein e is 0,

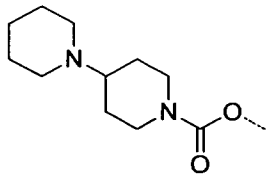
g is 0, and

q is 0, preferably

wherein f is 1, and wherein R^m and R" are preferably H, most preferably wherein the conjugate has a structure according to the following formula

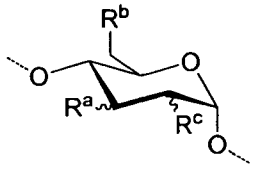


wherein R^f is selected from the group consisting of -OH, siloxy groups, ester groups and groups having the structure



and wherein R^8 is $-CH_2-CH_3$.

20. The conjugate according to claim 19, wherein HAS' comprises at least one structural unit according to the following formula (I)



(I)

wherein R^a, R^b and R^c are independently of each other selected from the group consisting of -O-HAS", $-[0-CH_2-CH_2]_s-OH$ and $-[0-CH_2-CH_2]_t-X-$,

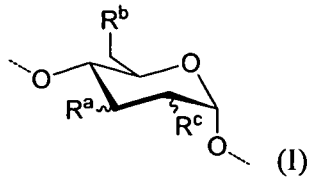
wherein s is in the range of from 0 to 4,

and wherein t is in the range of from 0 to 4,

and wherein at least one of R^a, R^b and R^c is $-[0-CH_2-CH_2]-X-$, wherein X is -S- and wherein X is directly bound to $-[CR^mR^n]_f-$, thereby forming a covalent linkage having the structure $-S-[CR^mR^n]_f-$,

and wherein HAS" is a remainder of HAS.

21. The conjugate according to claim 19, wherein HAS' comprises at least one structural unit according to the following formula (I)



(I)

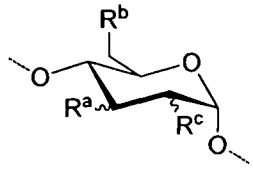
wherein R^A, R^b and R^c are independently of each other selected from the group consisting of-O-HAS", -[0-CH $_2$-CH $_2$]$_S$-OH, and –[O–CH$_2$-CH$_2$]$_t$-[F^1]$_p$-L^1-X-,

wherein s is in the range of from 0 to 4,
t is in the range of from 0 to 4,
p is 0 or 1,
and wherein at least one of R^A, R^b and R^C is -[O-CH $_2$-CH $_2$]$_t$-[F^1]$_p$-L^1-X-,
wherein F^1 is -0-,
wherein L^1 is a linking moiety having a structure according to the following formula - {[CR^{D}R^F]$_H$[F^4]$_u$-[CR^{DD}R^f]$_z$}aip$_h$a- wherein F4 is a functional group, preferably selected from the group consisting of-S-, -O- and - NH -, in particular -S-, wherein z is in the range of from 0 to 20, more preferably of from 0 to 10, more preferably of from 0 to 3, h is in the range of from 1 to 5, preferably in the range of from 1 to 3, more preferably 3,
u is 0 or 1,
alpha is in the range of from 1 to 10,
and wherein R^D, R^f, R^{DD} and R^F are, independently of each other, selected from the group consisting of H, alkyl, hydroxy!, and halogen, preferably selected from the group consisting of H, methyl and hydroxyl,
and wherein each repeating unit of -[CR^{d}R^f]$_h$-[F^4]$_U$-[CR^d$_D$R^{ff}]$_Z$- may be the same or may be different,
wherein, more preferably, L^1 has a structure selected from the group consisting of -CH $_2$-, -CH $_2$-CH $_2$-, -CH $_2$-CH $_2$-CH $_2$-, -CH $_2$-CH $_2$-CH $_2$-CH $_2$-, -CH $_2$-CH $_2$-CH $_2$-CH $_2$-CH $_2$-, -CH $_2$-CH $_2$-CH $_2$-S-CH$_2$-CH $_2$-, -CH $_2$-CH $_2$-S-CH$_2$-CH $_2$-, -CH $_2$-CH $_2$-0-CH $_2$-CH $_2$-, -CH $_2$-CH$_2$-0-CH $_2$-CH $_2$-0-CH $_2$-CH $_2$-, -CH $_2$-CHOH-CH $_2$-, -CH $_2$-CHOH-CH $_2$-S-CH$_2$-CH $_2$-, -CH $_2$-CHOH-CH $_2$-S-CH$_2$-CH $_2$-CH $_2$-, -CH $_2$-CHOH-CH $_2$-NH-CH $_2$-CH $_2$-, -CH $_2$-CHOH-CH $_2$-NH-CH $_2$-CH $_2$-CH $_2$-, -CH $_2$-CHOH-CH $_2$-0-CH $_2$-CHOH-CH $_2$-, -CH $_2$-CHOH-CH $_2$-0-CH $_2$-CHOH-CH $_2$-S-CH $_2$-CH $_2$-, -CH $_2$-CH(CH $_2$OH)- and -CH $_2$-CH(CH $_2$OH)-S-CH $_2$-CH $_2$-, more preferably from the group consisting of -CH $_2$-CHOH-CH $_2$-, -CH $_2$-CHOH-CH $_2$-S-CH$_2$-CH $_2$-, -CH $_2$-CHOH-CH $_2$-S-CH$_2$-CH$_2$-CH $_2$-, -CH $_2$-CHOH-CH $_2$-NH-CH $_2$-CH $_2$- and -CH $_2$-CHOH-CH $_2$-NH-CH $_2$-CH $_2$-

CH_2-, more preferably from the group consisting of -CH_2-CHOH-CH_2-, -CH_2-CHOH-CH_2-S-CH_2-CH_2- and -CH_2-CHOH-CH_2-S-CH_2-CH_2-CH_2-,

wherein X is -S- and wherein X is directly bound to -[CR ^{m}R^n]f-, thereby forming a covalent linkage having the structure -S-[CR ^{m}R^n]f-,
and wherein HAS" is a remainder of HAS.

22. The conjugate according to claim 19, wherein HAS' comprises at least one structural unit, preferably 3 to 200 structural units, according to the following formula (I)



(I)

wherein R^a, R^b and R^c are independently of each other selected from the group consisting of-O-HAS", -[0-CH $_2$-CH $_2$]$_s$-OH, and - [O- CH_2-CH_2]$_t$-[F^{1}j$_p$-L'-X-,

wherein s is in the range of from 0 to 4,
t is in the range of from 0 to 4,
p is 0 or 1,
and wherein at least one ofR ^{a}, R^b and R^c is -[O-CHz-CH^-tF'j $_p$-L'-X-,
wherein F^1 is -0-(C=0)-NH-,
wherein L^1 is an alkyl group,
wherein X is -S- and X is directly bound to -[CR ^{m}R^n]$_f$-, thereby forming a covalent linkage having the structure -S-[CR ^{m}R^n]$_f$-,
and wherein HAS" is a remainder of HAS.

23. A method for preparing a hydroxyalkyi starch (HAS) conjugate comprising a hydroxyalkyi starch derivative and a cytotoxic agent, said conjugate having a structure according to the following formula

HAS'(-L-M)$_n$

wherein
M is a residue of the cytotoxic agent, wherein the cytotoxic agent comprises a tertiary hydroxyl group,
L is a linking moiety,
HAS' is a residue of the hydroxyalkyi starch derivative,
and n is greater than or equal to 1,
said method comprising

(a) providing a hydroxyalkyl starch (HAS) derivative having a mean molecular weight MW above the renal threshold, preferably a mean molecular weight MW greater than or equal to 60 kDa and a molar substitution MS in the range of from 0.6 to 1.5, said HAS derivative comprising a functional group Z^1; and providing a cytotoxic agent comprising a tertiary hydroxyl group;

(b) coupling the HAS derivative to the cytotoxic agent via an at least bifunctional crosslinking compound L comprising a functional group K^1 and a functional group K^2, wherein K^2 is capable of being reacted with Z^1 comprised in the HAS derivative and wherein K^1 is capable of being reacted with the tertiary hydroxyl group comprised in the cytotoxic agent, preferably

wherein the functional group K^1 comprises the group -C(=Y)-, with Y being O, NH or S, wherein K^1 is preferably a carboxylic acid group or a reactive carboxy group, more preferably wherein the crosslinking compound L has a structure according to the following formula

$$K^2\text{-}L'\text{-}K^1$$

wherein L' is a linking moiety.

24. The method according to claim 23, wherein the cytotoxic agent is reacted with the crosslinking compound L prior to the reaction with the HAS derivative.

25. The method according to claim 23 or 24, wherein K^2 is reacted with the functional group Z^1 comprised in the HAS derivative, wherein Z^1 is selected from the group consisting of an aldehyde group, a keto group, a hemiacetal group, an acetal group, an alkynyl group, an azide, a carboxy group, an alkenyl group, a thiol reactive group, -SH, $-NH_2$, -0-NH_2, -NH-O-alkyl, -(C=G)-NH-NH_2, -G-(C=G)-NH-NH_2, -NH-(C=G)-NH-NH_2, and -S0$_2$-NH-NH_2, where G is O or S and, if G is present twice, it is independently O or S.

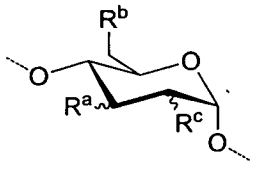
26. The method according to claim 25, wherein upon reaction of the tertiary hydroxyl group comprised in the cytotoxic agent with K^1, a functional group -F^3-0- is formed, wherein F^3 is a -C(=Y)- group, with Y being O, NH or S, in particular O or S.

27. The method according to any of claims 23 to 26, wherein the at least one crosslinking compound L has a structure according to the following formula:

$$K^2\text{-}[L^2]_g\text{-}[E]_e\text{-}[CR^mR^n]_f\text{-}K^1$$

wherein L^2 is a linking moiety, preferably an alkyl, alkylaryl, arylalkyl, aryl, heteroaryl, alkylheteroaryl or heteroarylalkyl group,
wherein E is an electron-withdrawing group,
f is 1, 2 or 3, preferably 1 or 2,
g is 0 or 1,
e is 0 or 1,
and wherein R^m and R" are, independently of each other, H or alkyl, more preferably H or methyl, in particular H.

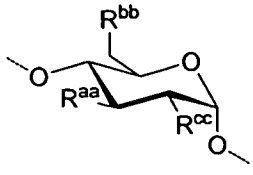
28. The method according to any of claims 23 to 27, wherein the HAS derivative provided in step (a) comprises at least one structural unit, preferably 3 to 200 structural units, according to the following formula (1)



(I)

wherein R^a, R^b and R^c are, independently of each other, selected from the group consisting of-O-HAS", -[0-(CR $^wR^x$)-(CR $^yR^z$)]$_x$-OH, $-[O-(CR^wR^x)-(CR^yR^z)]_y-Z^1$, and $-[O-(CR^wR^x)-(CR^yR^z)]_y-[F^1]_p-L^1-Z^1$, wherein R^w, R^x, R^y and R^z are independently of each other selected from the group consisting of hydrogen and alkyl,
y is an integer in the range of from 0 to 20, preferably in the range of from 0 to 4, and wherein x is an integer in the range of from 0 to 20, preferably in the range of from 0 to 4,
and wherein at least one of R^a, R^b and R^c is $-[O-(CR^wR^x)-(CR^yR^z)]_y-Z^1$ or
- [0 -(CR^{w}R^x)-(CR ^{y}R^z)]y-[F ^{1}]$_p$-L^1-Z ^{1},
and wherein F^1 is a functional group,
p is 0 or 1,
L^1 is a linking moiety,
wherein HAS" is a remainder of HAS,
and wherein step (a) comprises

(al) providing a hydroxyalkyl starch having a mean molecular weight MW greater than or equal to 60 kDa and a molar substitution MS in the range of from 0.6 to 1.5 comprising the structural unit according to the following formula (II)



(II)

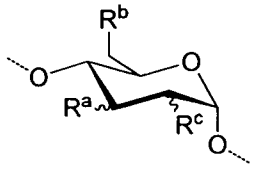
wherein R^{aa}, R^{bb} and R^{cc} are, independently of each other, selected from the group consisting of-O-HAS" and - [0-(CRwRx)-(CRyRz)]x- OH,
wherein R^{w}, R^{x}, R^{y} and R^{z} are independently of each other selected from the group consisting of hydrogen and alkyl groups, and wherein x is an integer in the range of from 0 to 20, preferably in the range of from 0 to 4,

(a2) introducing at least one functional group Z^1 into HAS by

(i) coupling the hydroxyalkyl starch via at least one hydroxyl group comprised in HAS to at least one suitable linker comprising the functional group Z^1 or a precursor of the functional group Z^1, or

(ii) displacing at least one hydroxyl group comprised in HAS in a substitution reaction with a precursor of the functional group Z^1 or with a suitable linker comprising the functional group Z^1 or a precursor thereof.

29. The method according to claim 28, wherein the HAS derivative formed in step (a2) comprises at least one structural unit according to the following formula (I)



(I)

wherein R^a, R^b and R^c are independently of each other selected from the group consisting of-O-HAS", -[0-CH 2-CH 2]s-OH, -[0-CH 2-CH2]»-Z' and -[0-CH 2-CH2]t-[F1]p-L1- Z1,

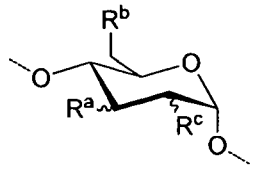
and wherein s is in the range of from 0 to 4,

and wherein t is in the range of from 0 to 4,

p is 0 or 1,

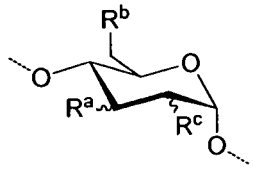
wherein at least one of R^a, R^b and R^c is -[0-CH 2-CH 2]t- Z1 or
-[O-CH 2-CH 2]t-[F1]p-L1- Z1,
and wherein HAS" is a remainder of HAS.

30. The method according to claim 28 or 29, wherein in step (a2)(i), the hydroxyalkyl starch is reacted with a suitable linker comprising the functional group Z^1 or a precursor of the functional group Z^1, and comprising a functional group Z^2, the linker preferably having the structure Z^2-L^1-Z^1 or Z^L'-Z^-PG, with Z^2 being a functional group capable of being reacted with the hydroxyalkyl starch, thereby forming a hydroxyalkyl starch derivative comprising at least one structural unit, according to the following formula (1),

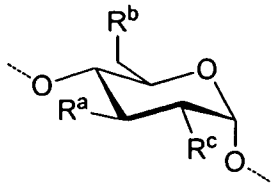

(I)

wherein at least one of R^a, R^b and R^c is -[0-CH_2-CH_2]$_t$-[F^1]$_p$-L^1-Z^1 or -[0-CH_2-CH_2]$_t$-[F^1]$_p$-L^1-Z^{1*}-PG with PG being a suitable protecting group and Z^{1*} being the protected form of the functional group Z^{1*},
wherein Z^1 is preferably -SH, Z^{1*} is preferably -S- and PG is preferably a suitable thiol protecting group, more preferably a protecting group forming together with Z^{1*} a group selected from the group consisting of thioethers, thioesters and disulfides,
and wherein in case the linker comprises the protecting group PG, the method further comprises deprotection of Z^{1*} to give Z^1.

31. The method according to claim 30, wherein step (a2)(i) comprises
(aa) activating at least one hydroxyl group comprised in the hydroxyalkyl starch with a reactive carbonyl compound having the structure R^{**}-(C=0)-R*
wherein R^* and R^{**} may be the same or different, and wherein R* and R** are both leaving groups, wherein upon activation a hydroxyalkyl starch derivative comprising at least one structural unit according to the following formula (1)


(I)

preferably (lb)



(Ib)

is formed, in which R^a, R^b and R^c are independently of each other selected from the group consisting of-O-HAS", -[0-CH_2-CH_2]$_s$-OH, and -[0-CH_2-CH_2]$_t$-0-C(=0)-R *,
wherein at least one of R^a, R^b and R^c comprises the group -[0-CH_2-CH_2]$_t$-0-C(=0)-R *, and

(bb) reacting the activated hydroxyalkyl starch according to step (aa) with the suitable linker comprising the functional group Z^1 or a precursor of the functional group Z^1.

32. The method according to claim 31, wherein the reactive carbonyl compound having the structure R**-(C=0)-R * is selected from the group consisting of phosgene, diphosgene, triphosgene, chloroformates and carbonic acid esters, preferably wherein the reactive carbonyl compound is selected from the group consisting of p-nitrophenylchloroformate, pentafluorophenylchloroformate, N,N'-disuccinimidyl carbonate, sulfo-N,N'-disuccinimidyl carbonate, dibenzotriazol-l-yl carbonate and carbonyldiimidazol.

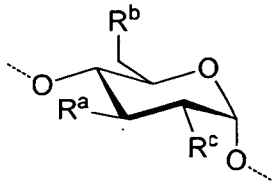
33. The method according to claim 31 to 32, wherein in (bb), the activated hydroxyalkylstarch derivative is reacted with a linker comprising the functional group Z^2 and the functional group Z^1 or a precursor of the functional group Z^1, the linker preferably having the structure Z^2- L^1- Z^1 or Z^L'-Z^-PG, wherein
Z^2 is a functional group capable of being reacted with the -[0-CH_2-CH_2],-0-C(=0)-R * group,
Z^{1*} is the protected form of the functional group Z^1
L^1 is an alkyl group,
wherein upon reaction of the -0-C(=0)-R * group with the functional group Z^2, the functional group F^1 is formed, and
Z^2 is preferably -NH_2.

34. The method according to claim 33, wherein the linker has the structure Z^2-L'-Z' *-PG, wherein Z^{1*} is _-S- and PG is a thiol protecting group, forming together with Z^{1*}

preferably a group selected from the group consisting of thioethers, thioesters and disulfides, and wherein the method further comprises deprotection of Z^{1*} to give Z^1.

35. The method according to claim 28, wherein (a2)(i) comprises
(I) coupling the hydroxyalkyl starch via at least one hydroxyl group comprised in the hydroxyalkyl starch to a first linker comprising a functional group Z^2, Z^2 being capable of being reacted with a hydroxyl group of the hydroxyalkyl starch, thereby forming a covalent linkage, the first linker further comprising a functional group W, wherein the functional group W is an epoxide or a group which is transformed in a further step to give an epoxide.

36. The method according to claim 35, wherein the first linker has a structure according to the formula Z^2-L^w-W, wherein
Z^2 is a functional group capable of being reacted with a hydroxyl group of the hydroxyalkyl starch,
L^w is a linking moiety,
wherein upon reaction of the hydroxyalkyl starch with the first linker, a hydroxyalkyl starch derivative is formed comprising at least one structural unit according to the following formula (lb)



(Ib)

wherein R^a, R^b and R^c are, independently of each other, selected from the group consisting of-O-HAS", -[0-CH_2-CH_2]$_s$-OH, and
-[0-CH_2-CH_2]r-[FVL^w-W,

wherein s is in the range of from 0 to 4,

and wherein t is in the range of from 0 to 4,

p is 0 or 1,
and wherein at least one of R^a, R^b and R^c is -[0-CH_2-CH_2],-[F']$_p$-L^w-W,
and wherein F^1 is the functional group being formed upon reaction of Z^2 with a hydroxyl group of the hydroxyalkyl starch, wherein F^1 is preferably -O- or -CH_2-CHOH-, preferably -0-,
and wherein HAS" is a remainder of HAS.

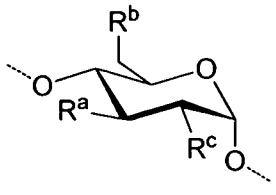
37. The method according to claim 35 or 36, wherein W is an alkenyl group and the method further comprises

(11) oxidizing the alkenyl group W to give the epoxide, wherein as oxidizing agent, potassium peroxymonosulfate is preferably employed.

38. The method according to any of claims 35 to 37, wherein Z^2 is a halogene (Hal) or an eeppooxxiiddee,, pprreeffeerraabbllyy a hhaalloogeenn, and wherein the linker Z^2-L^w-W preferably has the structure Hal-CH_2-CH=CH_2.

39. The method according to any of claims 36 to 38, the method comprising

(III) reacting the epoxide with a nucleophile comprising the functional group Z^1 or a precursor of the functional group Z^1, wherein the nucleophile is preferably a dithiol or a thiosulfate, thereby forming a hydroxyalkyl starch derivative comprising at least one structural unit, preferably 3 to 200 structural units, according to the following formula (lb)



(Ib)

wherein R^a, R^b and R^c are independently of each other selected from the group consisting of-O-HAS", -[0-CH_2-CH_2]$_s$-OH, and -[0-CH_2-CH_2],-[F ^{1}]$_p$- L ^{1}- Z ^{1},

wherein s is in the range of from 0 to 4,

and wherein t is in the range of from 0 to 4,

P is 1,

at least one of R^a, R^b and R^c comprises the group -[0-CH_2-CH_2]$_t$-[F ^{1}]$_p$- L ^{1}- Z ^{1},

and wherein Z^1 is -SH,

preferably wherein the nucleophile is ethanedithiol or sodium thiosulfate.

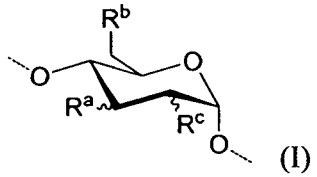
40. The method according to claim 28, wherein in (a2)(ii), prior to the displacement of the hydroxyl group, a group R^L is added to at least one hydroxyl group thereby generating a group -0-R^L, wherein -0-R^L is a leaving group, in particular a -O-Mesyl (-OMs) or -O-Tosyl (-OTs) group.

4 1. The method according to claim 28 or 40, wherein Z^1 is -SH, and wherein in step (a2)(ii) at least one hydroxyl group comprised in the hydroxyalkyl starch is displaced

by a suitable precursor of the functional group Z^1, the method further comprising converting the precursor after the substitution reaction to the functional group Z^1.

42. The method according to claim 41, wherein in step (a2)(ii) the at least one hydroxyl group comprised in the hydroxyalkyl starch is displaced with thioacetate giving a precursor of the functional group Z^1 having the structure - **S- C(=0)-CH3,** wherein the method further comprises the conversion of the group -S-C(=0 **)-CH_3** to give the functional group Z^1, preferably wherein the conversion is carried out using sodium hydroxide and sodium borohydride.

43. The method according to any of claims 40 to 42, wherein the hydroxyalkyl starch derivative obtained according to step (a2)(ii) comprises at least one structural unit according to the following formula (I)



(I)

wherein R^a, R^b and R^c are independently of each other selected from the group consisting of-O-HAS", -[0-CH_2-CH_2]$_s$-OH, and -[0-CH_2-CH_2],-Z^1,

wherein s is in the range of from 0 to 4,

and wherein t is in the range of from 0 to 4,

and wherein at least one of R^a, R^b and R^c comprises the group -[0 **-CH2-**CH_2]$_t$-Z',

Z^1 is -SH,

and wherein HAS" is a remainder of HAS.

44. The method according to any of claims 23 to 43, wherein in step (b) the hydroxyalkyl starch derivative obtained according to step (a) is coupled to the derivative of the cytotoxic agent having a structure according to the formula K^2-[L^2]$_g$-[E]$_e$-[CR^{m}R^n]r-F^3-M, wherein

— g and e are 0,

f is 1, 2 or 3, preferably 1 or 2, most preferably 1,

R^m and R" are, independently of each other, H or alkyl, preferably H or methyl, in particular H,

and K^2 is a halogene,

wherein upon reaction of Z^1 with K^2 the covalent linkage -X-[CR ^{m}R^n]$_f$- is formed;

or

— g and e are 0,

f is 1, 2 or 3, preferably 1 or 2, most preferably 2,

R^m and R" are, independently of each other, H or alkyl, preferably H or methyl, in particular H,

and K^2 is maleimide,

and wherein upon reaction of Z^1 with K^2 the covalent linkage -X-succinimide- is formed,

and wherein F^3 is preferably -C(=0)-,

preferably wherein Z^1 is -SH and X is -S-.

45. The method according to any claims 23 to 44, wherein the cytotoxic agent is selected from the group consisting of camptothecin, topotecan, irinotecari, DB67, BNP 1350 (cositecan), exatecan, lurtotecan, ST 1481, gimatecan, belotecan, CKD 602, karenitecin, chimmitecan, 9-aminocamptothecin, 9-nitrocamptothecin, BMS422461, diflomotecan, BN80927, BMS422461, morpholino-CPT and KOS-1584.

46. A hydroxyalkyl starch conjugate obtained or obtainable by a method according to any of claims 23 to 45.

47. A pharmaceutical composition comprising a conjugate according to any of claims 1 to 22 or according to claim 46.

48. A hydroxyalkyl starch conjugate according to any of claims 1 to 22 or according to claim 46, or a pharmaceutical composition according to claim 47 for use as medicament.

49. A hydroxyalkyl starch conjugate according to any of claims 1 to 22 or according to claim 46, or a pharmaceutical composition according to claim 47 for the treatment of cancer,

preferably for the treatment of cancer selected from the group consisting of breast cancer, cervical cancer, colorectal cancer, gastrointestinal cancer, leukaemia, lung cancer, mesothelioma, non-hodgkin's lymphoma, non-small cell lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, skin cancer, small cell lung cancer, brain tumors, uterine cancer and head and neck tumors.

50. Use of a hydroxyalkyl starch conjugate according to any of claims 1 to 22 or according to claim 46, or of a pharmaceutical composition according to claim 47 for the manufacture of a medicament for the treatment of cancer,
preferably wherein the cancer is selected from the group consisting of breast cancer, cervical cancer, colorectal cancer, gastrointestinal cancer, leukaemia, lung cancer, mesothelioma, non-hodgkin's lymphoma, non-small cell lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, skin cancer, small cell lung cancer, brain tumors, uterine cancer and head and neck tumors.

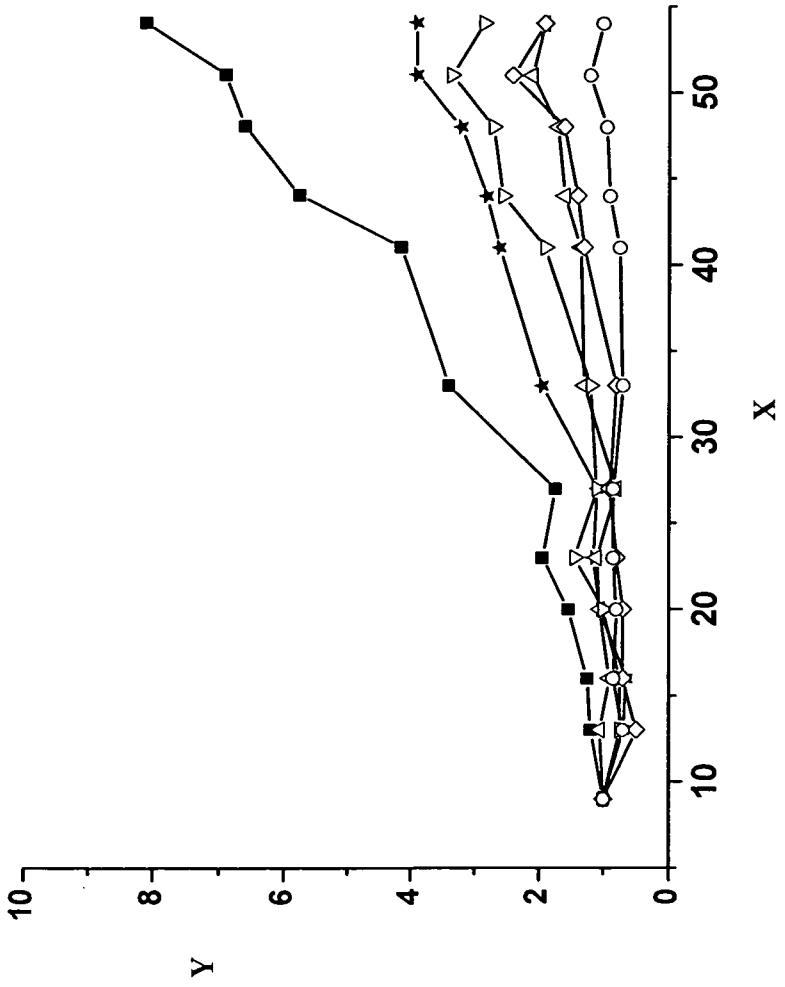


Fig. 1:

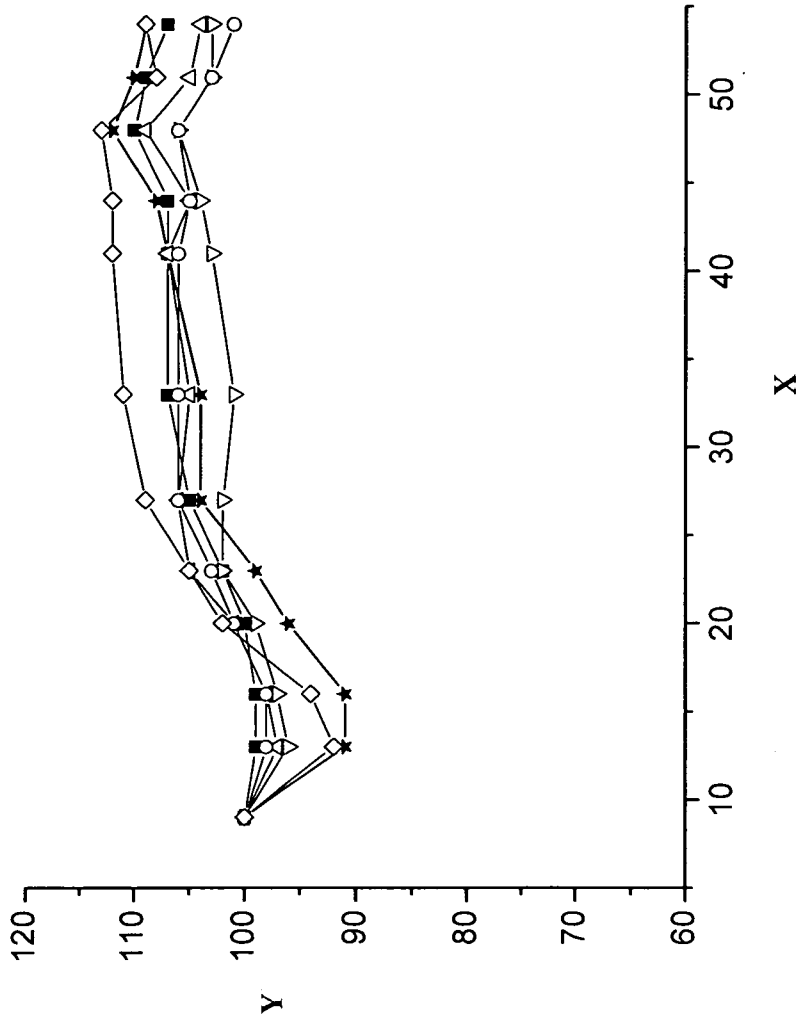


Figure 2:

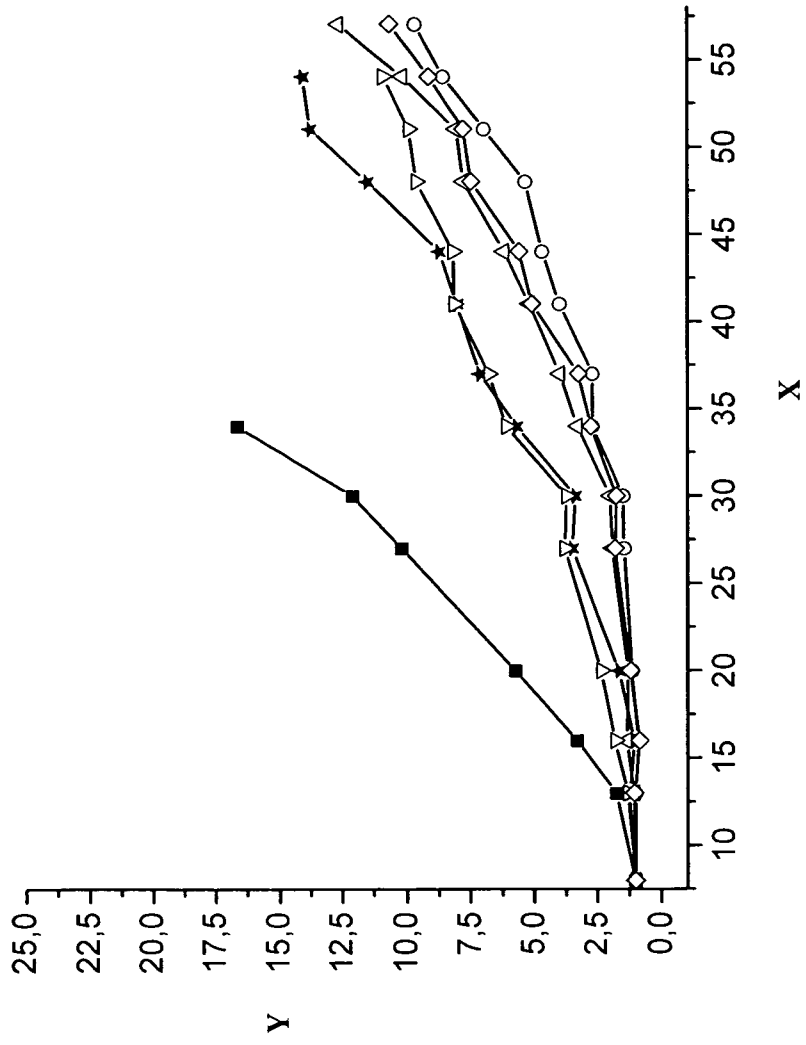

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Fig. 3:

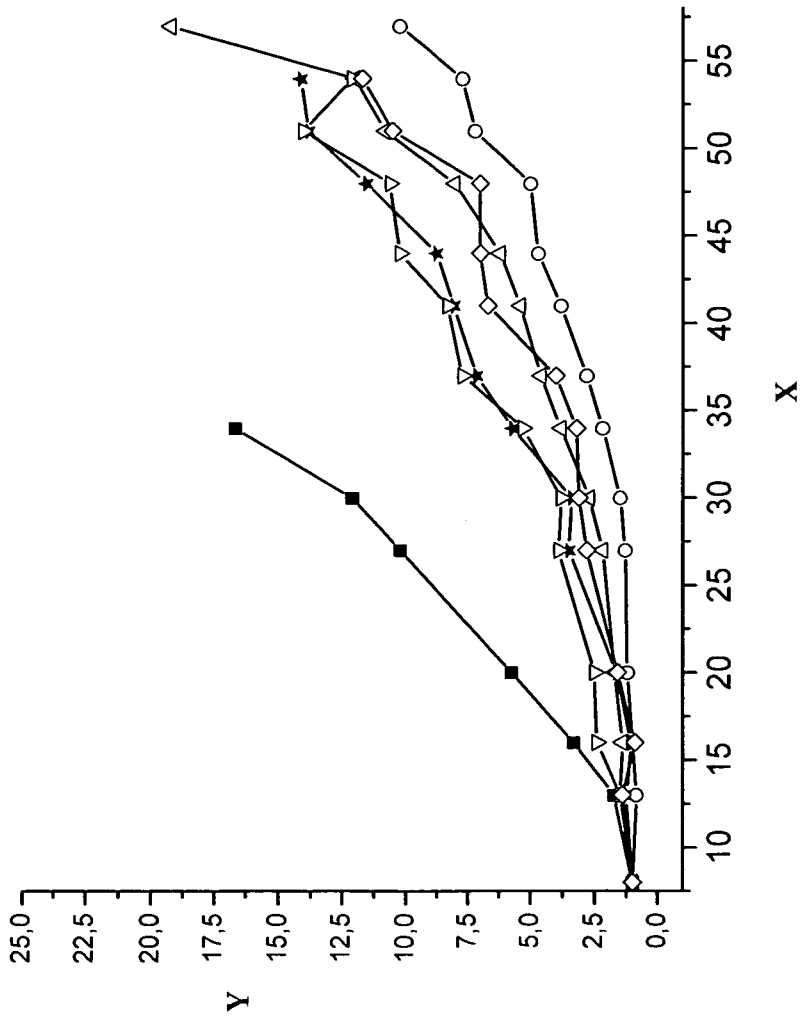
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Fig. 4:

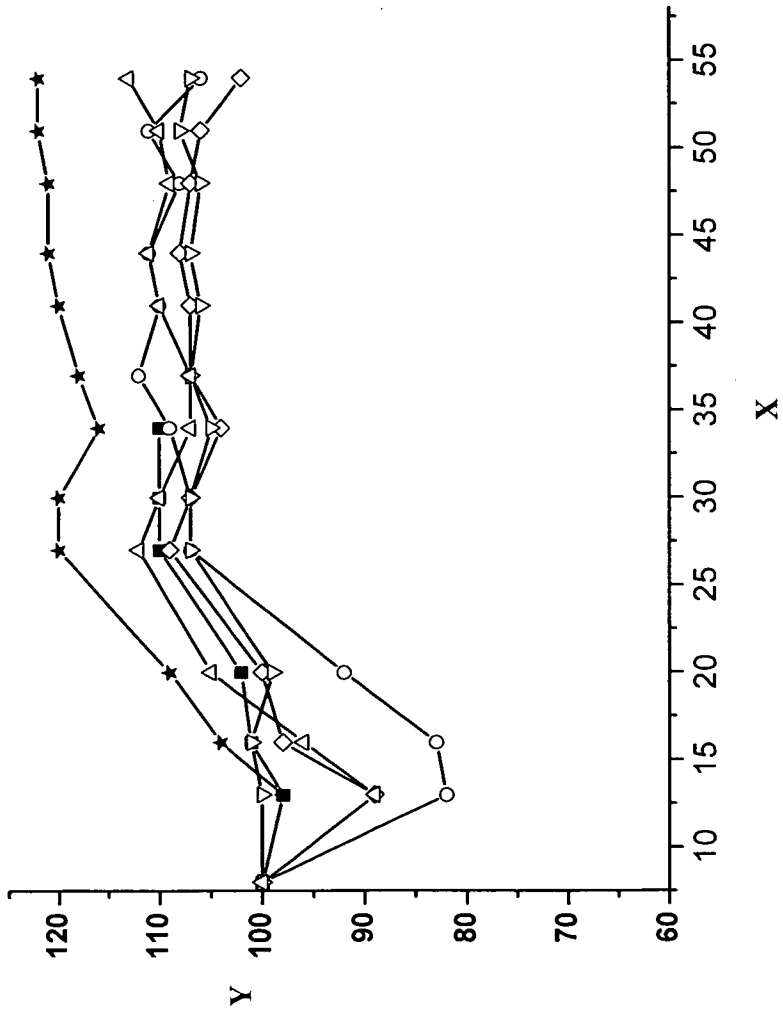

Fig. 5:

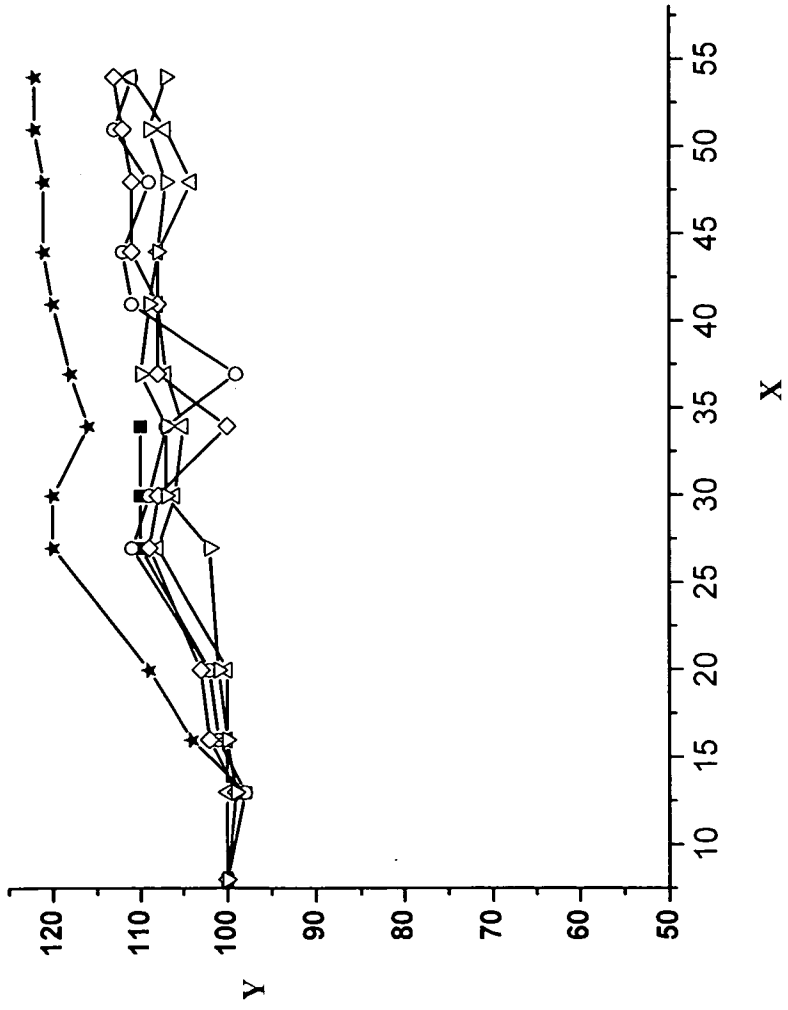

Fig. 6:

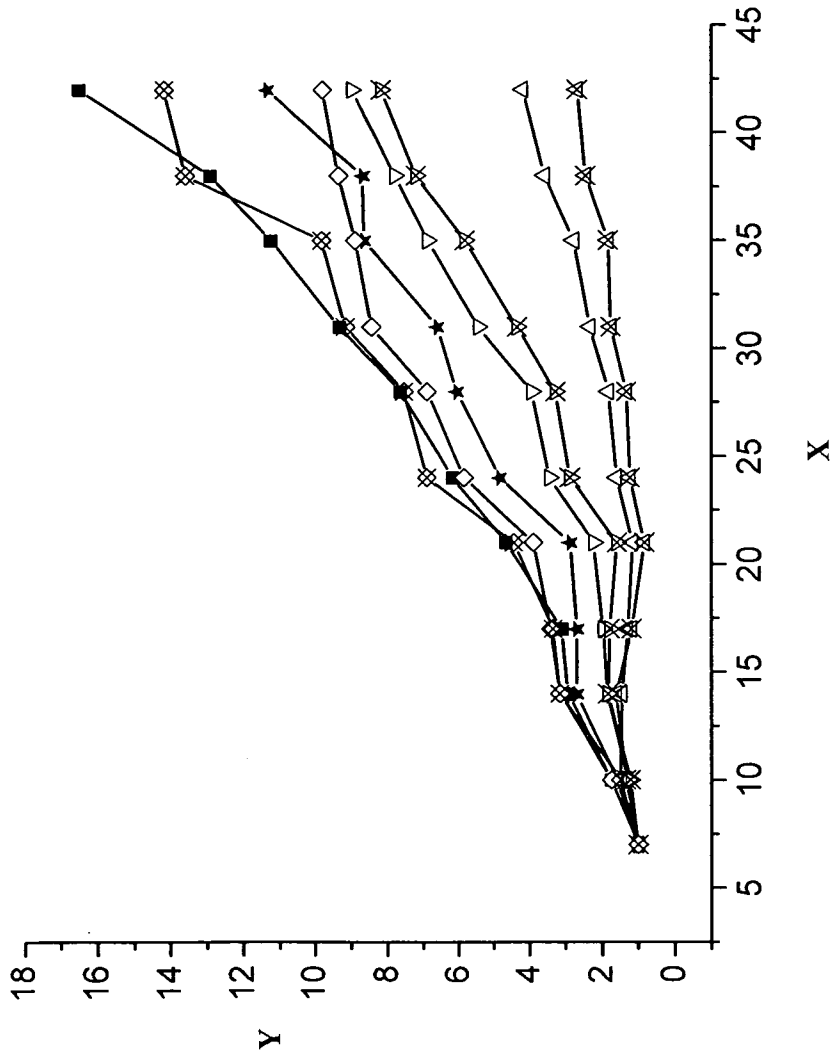

Fig. 7:

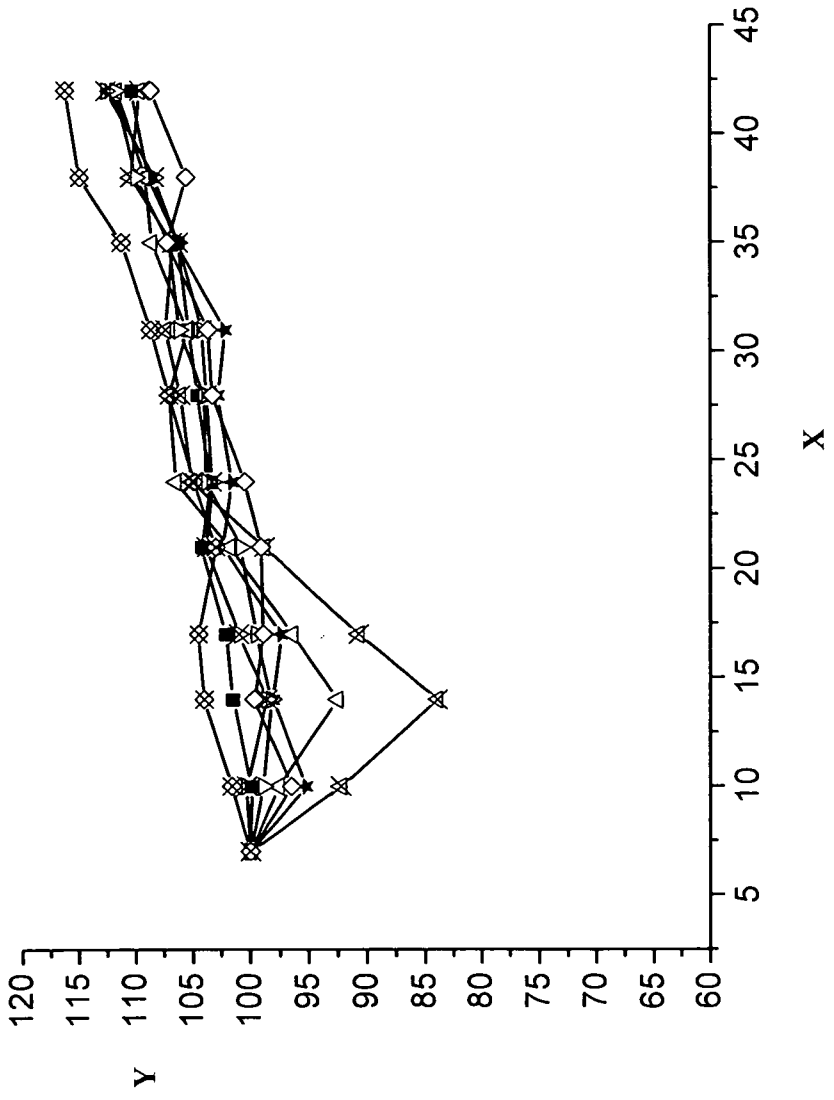
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Fig. 8:

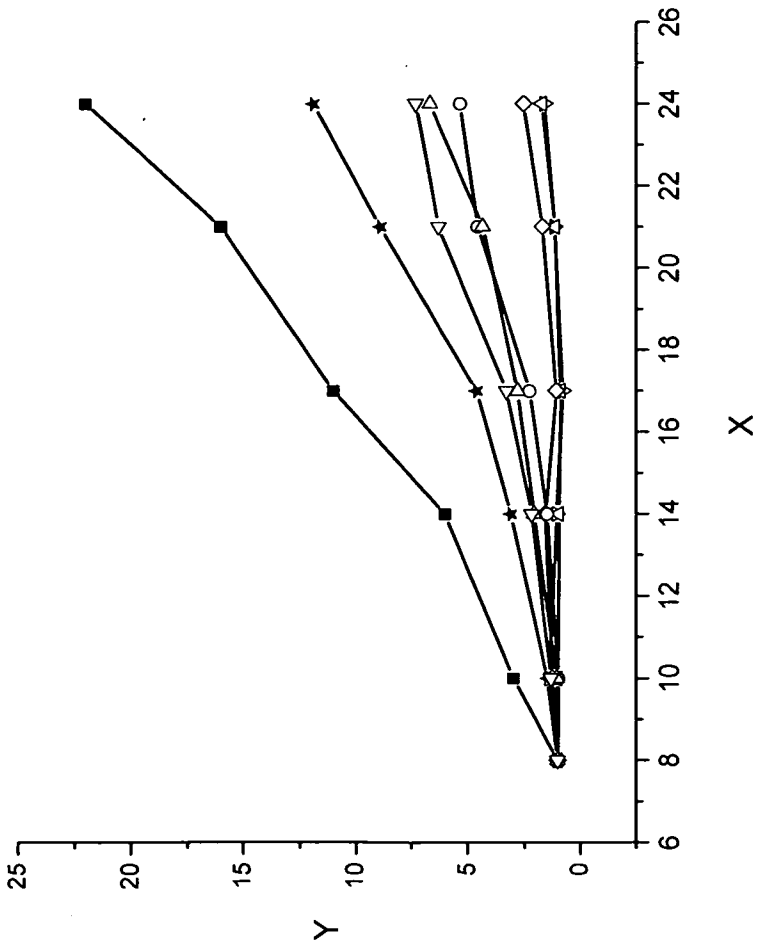


Figure 9:

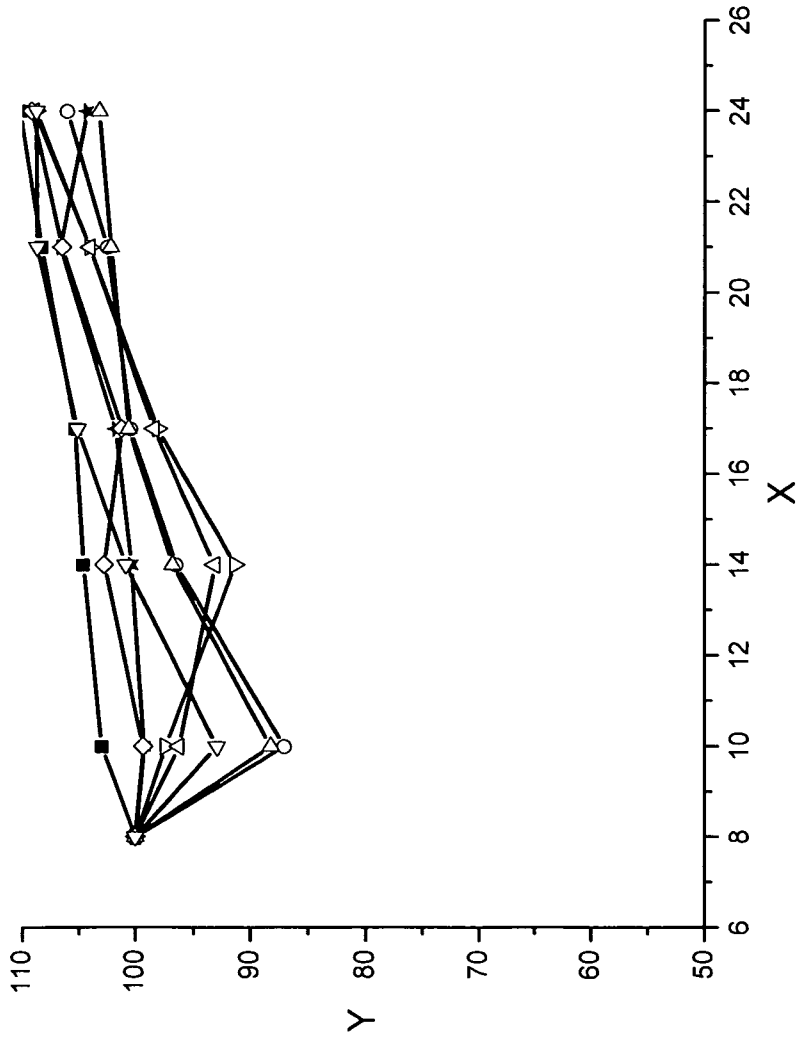
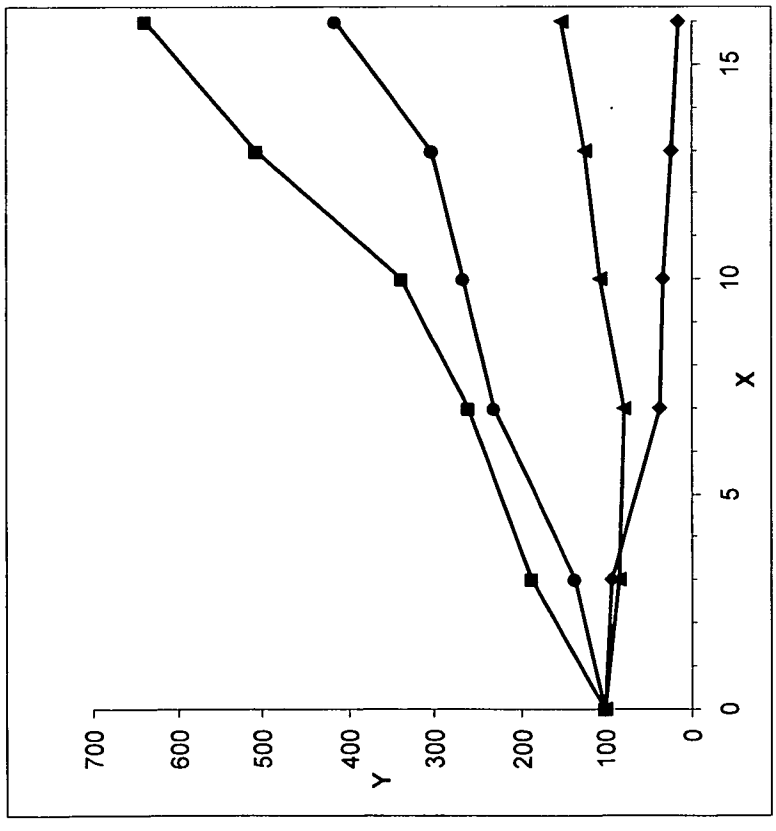


Figure 10:

Figure 11:



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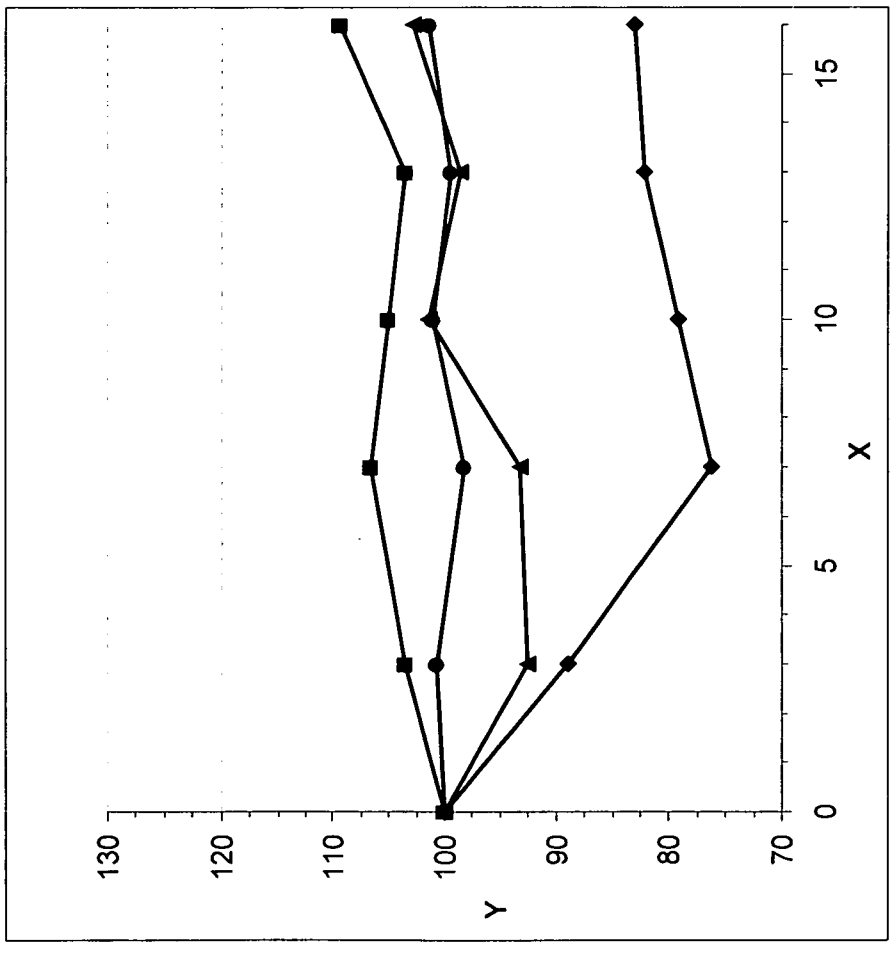

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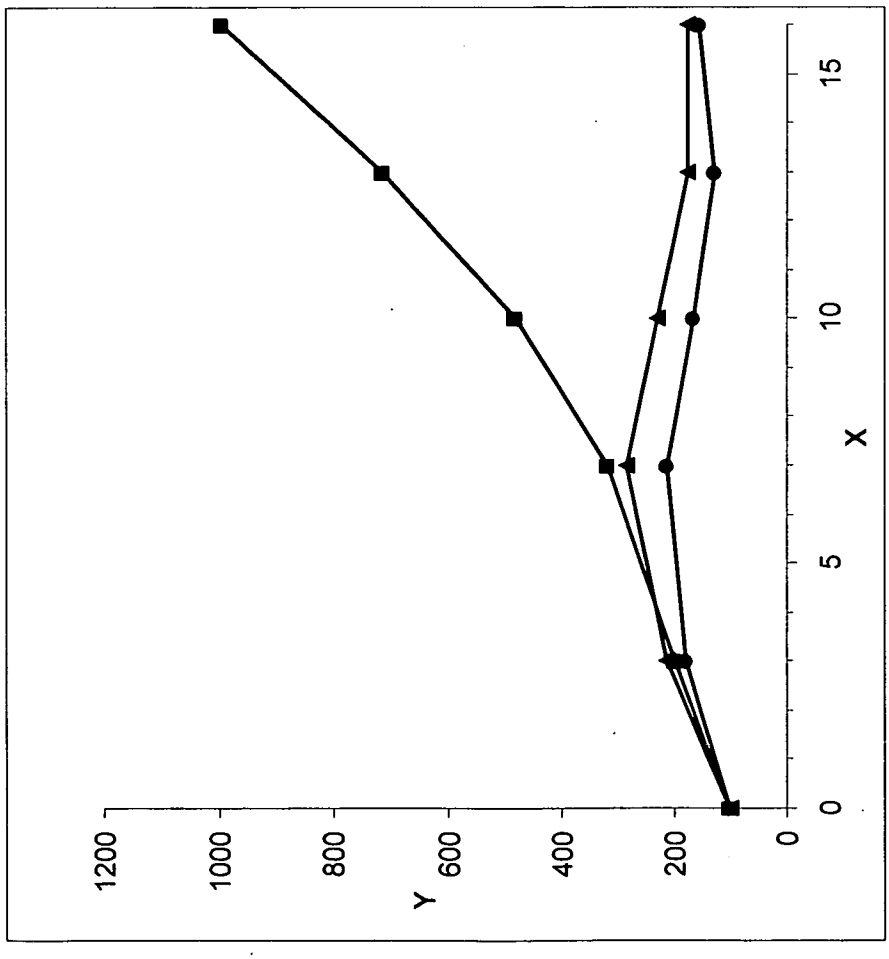

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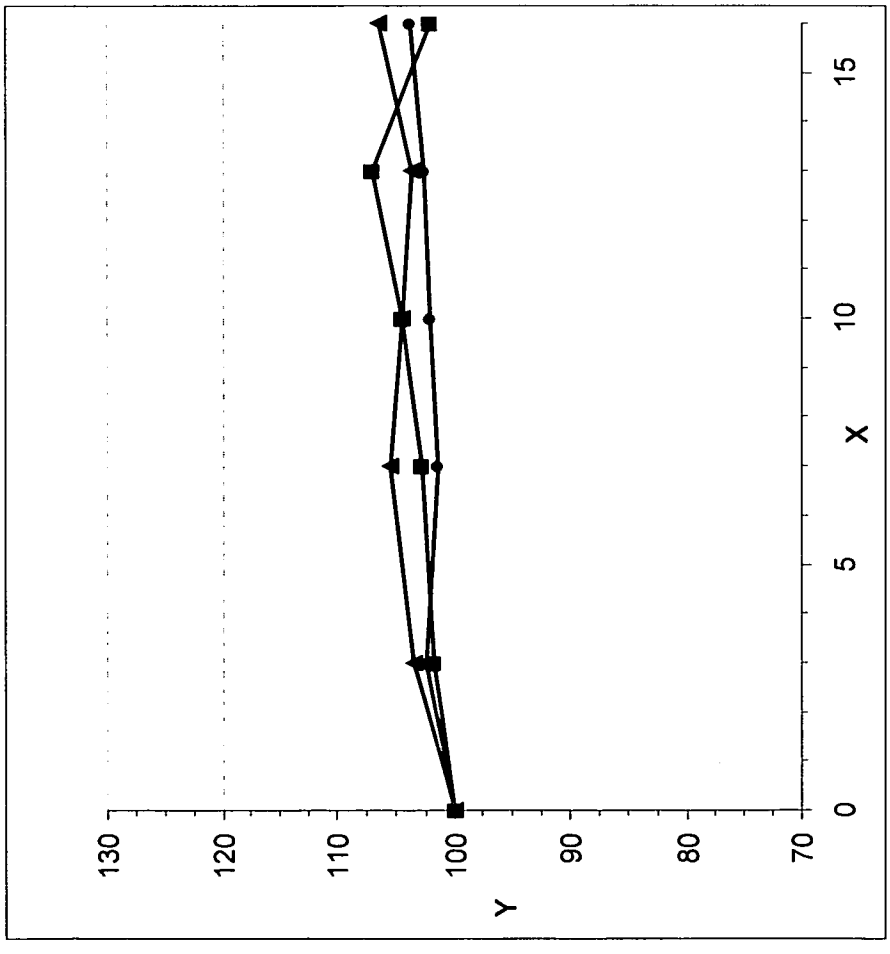

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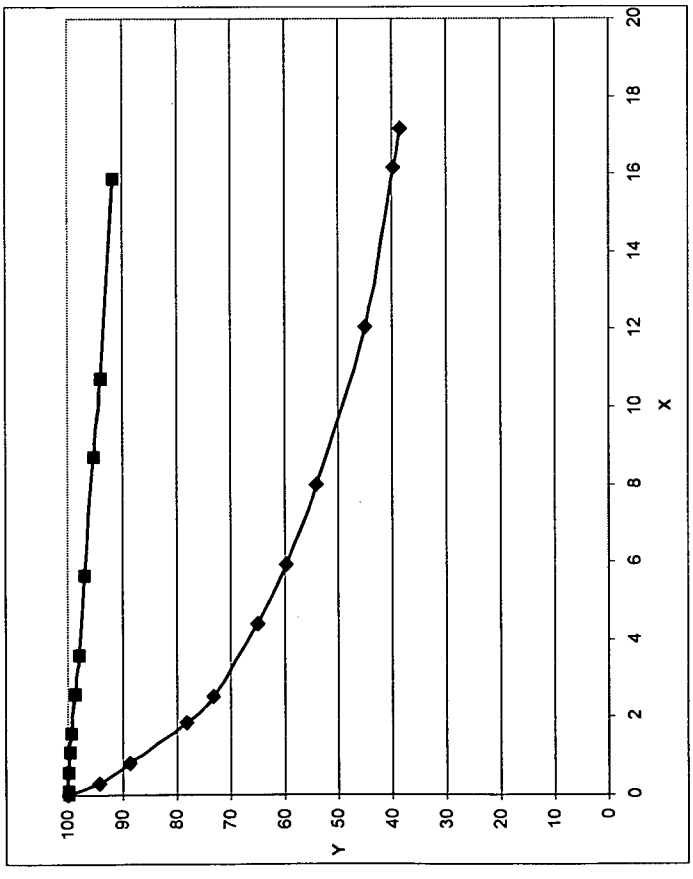


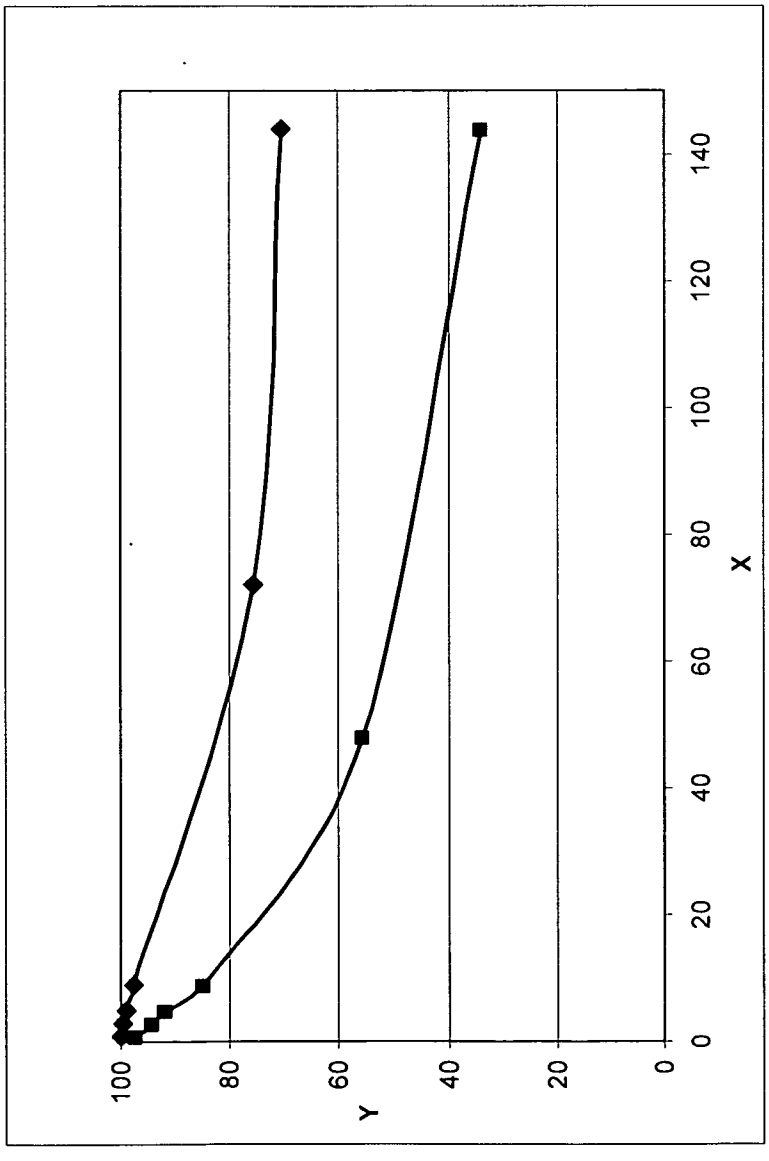
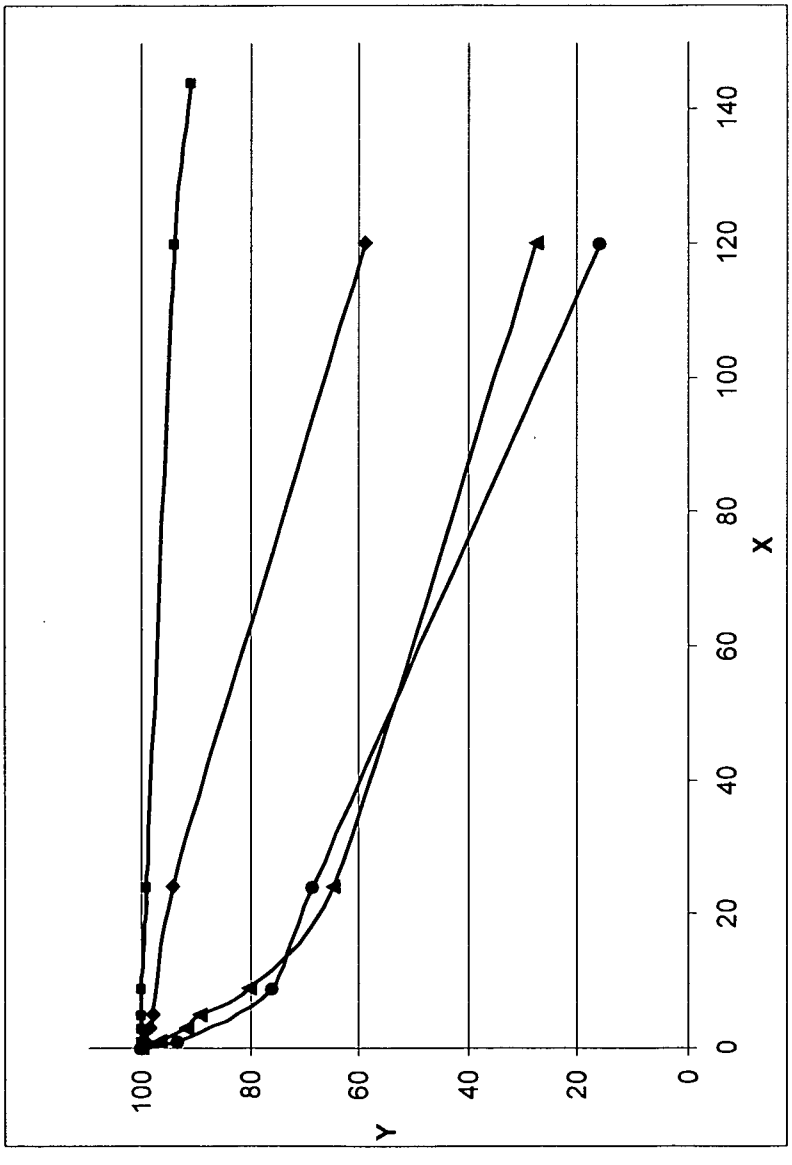


Figure 16:

Figure 17:



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2011/003461

A. CLASSIFICATION OF SUBJECT MATTER
INV. C08B31/12 C08L3/08 A61K47/48
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)
C08B C08L A61K

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 practical, search terms used)
EPO-Internal , BIOSIS, COMPENDEX, INSPEC, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X	WO 03/074088 A2 (BIOTECHNOLOGIE GES MITTELHESSE [DE] ; ORLANDO MICHELE [DE] ; HEMBERGER J) 12 September 2003 (2003-09-12) cited in the application claims ; examples	1-50
X	EP 2 070 950 Al (FRESENIUS KABI DE GMBH [DE]) 17 June 2009 (2009-06-17) examples page 117	1-50

-/- ·

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

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
30 November 2011	06/12/2011

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Vaccaro, El eonora

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2011/003461

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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