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(54) Title: PEPTIDE BINDER

(57) Abstract: The present invention relates to a sulfonamide of formula (A); a conjugate comprising a sulfonamide of formula (I) and an active pharmaceutical ingredient; a process for preparing a conjugate comprising a sulfonamide of formula (I) and an active pharmaceutical ingredient; a pharmaceutical composition comprising the conjugate comprising a sulfonamide of formula (I) and an active pharmaceutical ingredient; and to the use of the conjugate as a medicament.

Peptide Binder

5 Description

Provided herein are sulfonamides of general formula (A); a conjugate comprising a sulfonamide of formula (I) and an active pharmaceutical ingredient or a diagnostic compound; a process for preparing a conjugate comprising a sulfonamide of formula
10 (I) and an active pharmaceutical ingredient; a pharmaceutical composition comprising the conjugate comprising a sulfonamide of formula (I) and an active pharmaceutical ingredient or a diagnostic compound; and to the use of the conjugate as a medicament.

The release of insulin by the pancreas is strictly coupled to the concentration of the
15 blood glucose. Elevated blood glucose levels, such as occur after meals, are rapidly compensated by a corresponding increase in insulin (a blood glucose lowering hormone) secretion. In the fasting state, the plasma insulin level falls to a basal value which is adequate to guarantee a continuous supply of insulin-sensitive organs and tissue with glucose and to keep hepatic glucose production low in the night. Diabetes
20 mellitus is a metabolic disorder in which this tight regulation of blood glucose is disturbed.

Diabetes mellitus is characterized by either a reduced/missing production of insulin by the pancreas and/or the incapability to use insulin. As a consequence, blood
25 glucose levels are inadequately controlled and therefore elevated. Blood glucose levels which are increased for years without initial symptoms are a considerable health risk. The large-scale DCCT study in the USA (The Diabetes Control and Complications Trial Research Group (1993) N. Engl. J. Med. 329, 977-986) demonstrated clearly that chronically elevated blood glucose levels are essentially
30 responsible for the development of diabetic late complications, such as microvascular and macrovascular damage which is manifested, under certain circumstances, as retinopathy, nephropathy or neuropathy and leads to loss of sight, kidney failure and the loss of extremities. Moreover diabetes is accompanied by an increased risk of cardiovascular diseases.

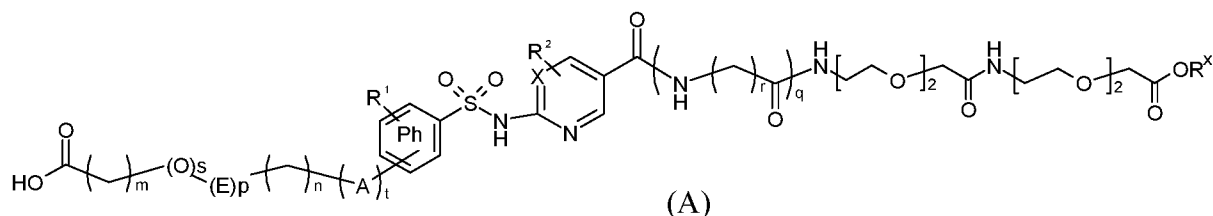
Worldwide, in 2016, approximately 422 million people suffer from type 1 and type 2 diabetes mellitus. Diabetes mellitus is classified in type 1 and type 2 diabetes. In type 1 diabetes patients, no insulin produced by the body itself is available. Therefore, since no cure is available, for type 1 diabetics the substitution of the lacking endocrine insulin secretion is the only currently possible therapy. The affected persons are dependent lifelong on insulin injections, as a rule a number of times daily. In contrast to type 1 diabetes, there is not basically a deficiency of insulin in type 2 diabetes, but in a large number of cases, especially in the advanced stage, treatment with insulin, optionally in combination with an oral antidiabetic, is regarded as the most favourable form of therapy.

The goal of current diabetes mellitus therapy is primarily to keep the blood glucose as closely as possible in the physiological range. Current therapy recommendations include treatment with oral anti-diabetic drugs, GLP-1 receptor agonists and finally treatment with insulin.

Human insulin is a polypeptide of 51 amino acids, which is comprised of 2 amino acid chains: the A chain having 21 amino acids and the B chain having 30 amino acids. The chains are connected to one another by means of 2 disulfide bridges (interchain disulfide bridges are between Cys(A7) and Cys(B7) and between Cys(A20) and Cys(B19)). Additionally, an intrachain disulfide bridge is present between Cys(A6) and Cys(A11). Insulin preparations have been employed for diabetes mellitus therapy for many years. Not only human insulins are used here, but recently also insulin derivatives and analogs.

In view of the problems and discomforts associated with a daily or repeated daily injection(s), there are ongoing efforts to find insulin preparations with a prolonged profile of action – the aim is a once weekly dosage regimen.

Provided herein are sulfonamides of formula (A)



As used herein, the terms "analog of insulin" and "insulin analog" refer to a polypeptide which has a molecular structure which formally can be derived from the structure of a naturally occurring insulin, for example that of human insulin, by deleting and/or exchanging at least one amino acid residue occurring in the naturally occurring insulin and/or adding at least one amino acid residue. The added and/or exchanged amino acid residue can either be codable amino acid residues or other naturally occurring residues or purely synthetic amino acid residues. Examples of analogs of insulin include, but are not limited to, the following:

- (i). 'Insulin aspart' is human insulin where the amino acid B28 (i.e. the amino acid no. 28 in the B chain of human insulin), which is proline, is replaced by aspartic acid. Insulin aspart is a short-acting insulin.
- (ii). 'Insulin lispro' is human insulin where the penultimate lysine and proline residues on the C-terminal end of the B-chain of are reversed (human insulin: ProB28LysB29; insulin lispro: LysB28ProB29). Insulin lispro is a short-acting insulin.
- (iii). 'Insulin glulisine' differs from human insulin in that the amino acid asparagine at position B3 is replaced by lysine and the lysine in position B29 is replaced by glutamic acid. Insulin glulisine is a short-acting insulin.
- (iv). "Insulin glargine" differs from human insulin in that the asparagine at position A21 is replaced by glycine and the B chain is extended at the carboxy terminal by two arginines. Insulin glargine a long-acting insulin.

As used herein, the term "insulin conjugate" is synonymous with „derivative of insulin" and "insulin derivative" – the term refers to a polypeptide which has a molecular structure which formally can be derived from the structure of a naturally occurring insulin, for example that of human insulin, in which one or more organic substituents (e.g. a fatty acid) is bound to one or more of the amino acids. The one or more organic substituents are designed to interact with serum proteins like albumin and are called herein "binder(s)" or "binder molecule(s)". Optionally, one or more amino acids occurring in the naturally occurring insulin may have been deleted and/or replaced by

other amino acids, including non-codeable amino acids, or amino acids, including non-codeable, have been added to the naturally occurring insulin. Examples of conjugates of insulin include, but are not limited to, the following:

- (i). 'Insulin detemir' which differs from human insulin in that amino acid threonine at position B30 is deleted and a fatty acid residue (myristic acid) is attached to the epsilon-amino function of the lysine in position B29. Insulin detemir is a long-acting insulin.
- (ii). 'Insulin degludec' which differs from human insulin in that the amino acid threonine at position B30 is deleted and that a hexadecanedioic acid is conjugated to the amino acid lysine B29 via a gamma-L-glutamyl-linker. Insulin degludec is a long-acting insulin.

As used herein, the term "fast acting insulin" refers to insulin analogs and/or insulin derivatives, wherein the insulin-mediated effect begins within 5 to 15 minutes and continues to be active for 3 to 4 hours. Examples of fast acting insulins include, but are not limited to, the following: (i). insulin aspart; (ii). insulin lispro and (iii). insulin glulisine.

As used herein, the term "long acting insulin" refers to insulin analogs and/or insulin derivatives, wherein the insulin-mediated effect begins within 0.5 to 2 hours and continues to be active, for about or more than 24 hours. Examples of fast acting insulins include, but are not limited to, the following: (i). insulin glargin; (ii). insuline detemir and (iii). insulin degludec.

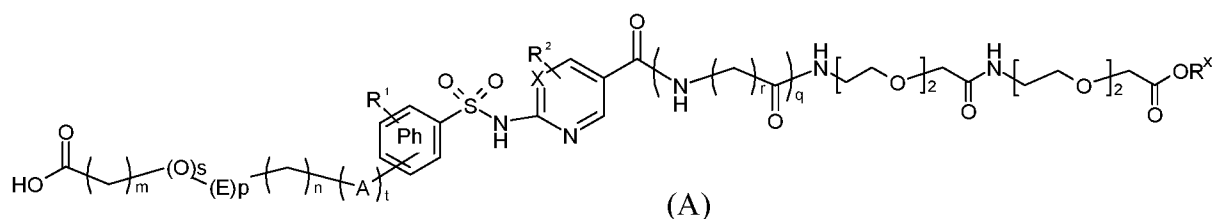
Provided herein are serum albumin binding moieties, which when coupled to a peptide lead to improved pharmacodynamics and/or pharmacokinetic properties of the peptide for example, an extended pharmacokinetic half life in blood and/or blood plasma and/or a prolonged profile of action, i.e. a prolonged reduction of blood glucose level.

Surprisingly, it was found that such peptide conjugates can be provided using specific sulfonamides, which can be used for peptide conjugates. The resulting peptide conjugates exhibit favorable half-life in blood and/or blood plasma and a prolonged profile of action. It could be shown that the resulting peptide conjugates have an increased pharmacokinetic half-life ($t_{1/2}$) and also an increased Mean Residence Time (MRT) compared to the unconjugated peptides. Moreover, the

peptide conjugates have a significant prolongation of the duration of action in vivo in relation to the unconjugated peptides.

Thus, provided herein are sulfonamides of formula (A)

5



wherein:

- A is selected from the group consisting of oxygen atom, -CH₂CH₂- group, -OCH₂- group and -CH₂O- group;
- E represents a -C₆H₃R- group with R being a hydrogen atom or a halogen atom, wherein the halogen atom is selected from the group consisting of fluorine, chlorine, bromine and iodine atom;
- X represents a nitrogen atom or a -CH- group;
- m is an integer in the range from 5 to 17;
- n is zero or an integer in the range from 1 to 3;
- p is zero or 1;
- q is zero or 1;
- r is an integer in the range from 1 to 6;
- s is zero or 1;
- t is zero or 1;
- R¹ represents at least one residue selected from the group of hydrogen atom, halogen atom, C1 to C3 alkyl group and halogenated C1 to C3 alkyl group;
- R² represents at least one residue selected from the group of hydrogen atom, halogen atom, C1 to C3 alkyl group and halogenated C1 to C3 alkyl group;
- R^x represents a hydrogen atom or an activation group, optionally an activation group selected from the group consisting of 7-azabenzotriazole (optionally derived from HATU [1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate] or HBTU [3-[bis(dimethylamino)methylumyl]-3H-benzotriazol-1-oxide hexafluorophosphate]),

4-nitro benzene and N-succinimidyl-group, wherein Rx is optionally a N-succinimidyl -group.

In some embodiments, the combination of s being 1, p being zero, n being zero, A being an oxygen atom and t being 1 is excluded. In some embodiments, s is zero, wherein the remaining residues and indices have the meaning as indicated above for formula (A).

For example, the halogenated C1 to C3 alkyl group of R¹ and/or the halogenated C1 to C3 alkyl group of R² is/are partially halogenated or per halogenated. In some embodiments, the halogenated C1 to C3 alkyl group of R¹ and/or the halogenated C1 to C3 alkyl group of R² is/are per halogenated.

As used herein, the term "sulfonamides of formula (A)" comprises the sulfonamides of formula (A), pharmaceutically acceptable salts thereof and all pharmaceutically acceptable isotopically-labeled sulfonamides of formula (A), wherein one or more atoms are replaced by atoms having the same atomic number, but an atomic mass or mass number different from the atomic mass or mass number which predominates in nature. The same applies to all subtypes of the sulfonamides of formula (A), i.e. to the sulfonamides of formula (A-1) to (A-5) as detailed below and also to their substructures respectively, for example, the sulfonamides of formula (A-1-1). That is, the term "sulfonamides of formula (A-...)", wherein (A-...) represents the number of the sulfonamides of formula (A-1) to (A-5) as detailed below and also their substructures, comprises the compounds themselves, pharmaceutically acceptable salts and all pharmaceutically acceptable isotopically-labeled compounds thereof.

Pharmaceutically acceptable salts of the sulfonamides of formula (A) are base salts. Suitable base salts are formed from bases which form non-toxic salts. Examples include the aluminium, arginine, benzathine, calcium, choline, diethylamine, bis(2-hydroxyethyl)amine (diolamine), glycine, lysine, magnesium, meglumine, 2-aminoethanol (olamine), potassium, sodium, 2-amino-2-(hydroxymethyl)propane-1,3-diol (tris or tromethamine) and zinc salts. For a review on suitable salts, see Handbook of Pharmaceutical Salts: Properties, Selection, and Use by Stahl and Wermuth (Wiley-VCH, 2002).

The sulfonamides of formula (A), and pharmaceutically acceptable salts thereof, may exist in unsolvated and solvated forms. The term `solvate` is used herein to describe a molecular complex comprising the sulfonamides of formula (A), or a
5 pharmaceutically acceptable salt thereof, and one or more pharmaceutically acceptable solvent molecules, for example, ethanol. The term `hydrate` is employed when said solvent is water.

10 Examples of isotopes suitable for inclusion in the sulfonamides of formula (A) include isotopes of hydrogen, such as ^2H and ^3H , carbon, such as ^{11}C , ^{13}C and ^{14}C , chlorine, such as ^{36}Cl , fluorine, such as ^{18}F , iodine, such as ^{123}I and ^{125}I , nitrogen, such as ^{13}N and ^{15}N , oxygen, such as ^{15}O , ^{17}O and ^{18}O , and sulfur, such as ^{35}S .

15 Certain isotopically-labelled sulfonamides of formula (A), for example those incorporating a radioactive isotope, are useful in drug and/or substrate tissue distribution studies. The radioactive isotopes tritium, i.e. ^3H , and carbon-14, i.e. ^{14}C , are particularly useful for this purpose in view of their ease of incorporation and ready means of detection.

20 Substitution with heavier isotopes such as deuterium, i.e. ^2H , may afford certain therapeutic advantages resulting from greater metabolic stability, for example, increased in vivo half-life or reduced dosage requirements.

25 Substitution with positron emitting isotopes, such as ^{11}C , ^{18}F , ^{15}O and ^{13}N , can be useful in Positron Emission Topography (PET) studies for examining substrate receptor occupancy.

Isotopically-labeled sulfonamides of formula (A) can generally be prepared by conventional techniques known to those skilled in the art.

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Pharmaceutically acceptable solvates in accordance with the invention include those wherein the solvent of crystallization may be isotopically substituted, e.g. D_2O , d_6 -acetone, d_6 -DMSO.

In order to identify suitable binder molecules, which when bound to a peptide, such as an insulin, are able to improve the half-life in plasma and to prolong the profile of action, a system was established based on affinity chromatography with serum albumin columns, i.e. columns with immobilized serum albumin.

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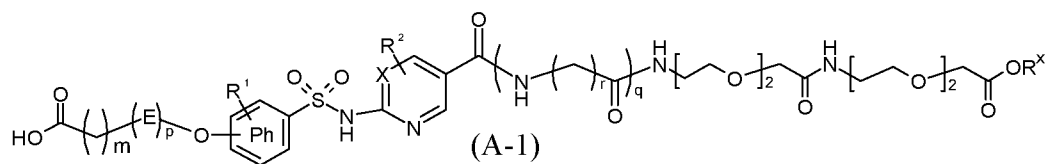
The net retention time of the binders (samples) was calculated according to the following formula:

$$\text{Net retention time} = \text{RetTime}_{\text{Sample}} - \text{RetTime}_{\text{t0 marker}}$$

10

Sulfonamides of formula (A) have a net retention in the range of from 9 to 19, for example in the range of from 9.5 to 18, and were consequently considered to be useful binders for peptide conjugates, such as insulin conjugates.

15 According to one embodiment, the sulfonamide has the formula (A-1)



wherein:

- E represents a $-C_6H_3R-$ group with R being a hydrogen atom or a halogen atom, wherein the halogen atom is selected from the group consisting of fluorine, chlorine, bromine and iodine atom and is for example a fluorine atom;
- X represents a nitrogen atom or a $-CH-$ group;
- p is zero or 1;
- q is zero or 1;
- 25 r is an integer in the range from 1 to 6;
- R¹ represents at least one residue selected from the group of hydrogen atom and halogen atom, wherein the halogen atom is for example a fluorine or chlorine atom;
- R² represents at least one residue selected from the group of hydrogen atom, C1 to C3 alkyl group and halogenated C1 to C3 alkyl group, wherein the C1 to C3 alkyl group is for example a methyl group and the halogenated C1 to C3 alkyl group is for example perhalogenated such as a trifluoromethyl group;
- 30

R^x represents a hydrogen atom or an activation group, optionally an activation group selected from the group consisting of 7-azabenzotriazole (optionally derived from HATU or HBTU), 4-nitro benzene and N-succinimidyl-group, wherein R^x is optionally a N-succinimidyl-group;

- 5 with m being an integer in the range from 5 to 15 if p is zero, or m being an integer in the range from 7 to 15 if p is 1.

In one embodiment of the sulfonamide, R^1 and R^2 are hydrogen atoms.

- 10 In one embodiment of the sulfonamide, X represents a nitrogen atom.

According to another embodiment of the sulfonamide, the $\text{HOOC}-(\text{CH}_2)_m-(\text{O})_s-(\text{E})_p-(\text{CH}_2)_n-(\text{A})_t$ group of formula (A) or the $\text{HOOC}-(\text{CH}_2)_m-(\text{E})_p-\text{O}-$ group of formula (A-1) is situated in meta or para position on phenyl ring Ph with respect to the $-\text{S}(\text{O})_2-$ group.

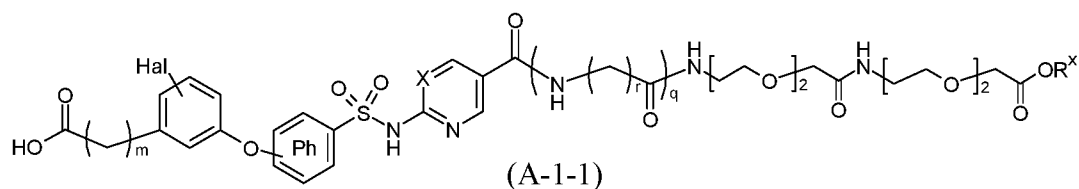
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According to another embodiment of the sulfonamide, if p is 1, the $\text{HOOC}-(\text{CH}_2)_m-(\text{O})_s$ -group and the $-(\text{CH}_2)_n-(\text{A})_t$ -group are situated in meta or para position on $(\text{E})_p$ of formula (A) or the $\text{HOOC}-(\text{CH}_2)_m$ -group and the $-\text{O}-$ are situated in meta or para position on $(\text{E})_p$ of formula (A-1).

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According to another embodiment of the sulfonamide, q is zero.

According to another embodiment, the sulfonamide has the formula (A-1-1)



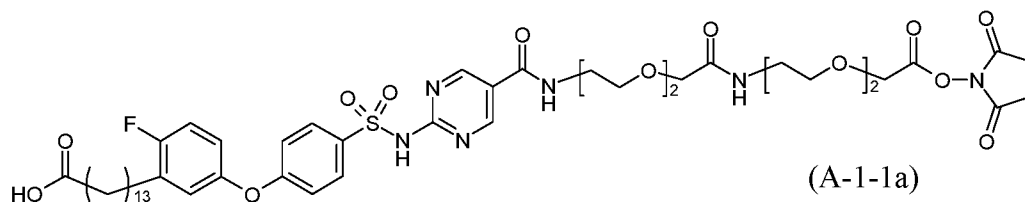
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wherein X is a nitrogen atom or a $-\text{CH}-$ group, for example a nitrogen atom; m is an integer in the range from 7 to 15; r is an integer in the range from 1 to 6; q is zero or 1, for example zero; Hal is a halogen atom selected from the group consisting of fluorine, chlorine, bromine and iodine atom, for example a fluorine atom; R^x is a hydrogen atom or an activation group, optionally an activation group selected from the group consisting of 7-azabenzotriazole (optionally derived from HATU or HBTU), 4-nitro benzene and N-succinimidyl-group, wherein R^x is optionally a N-succinimidyl-group;

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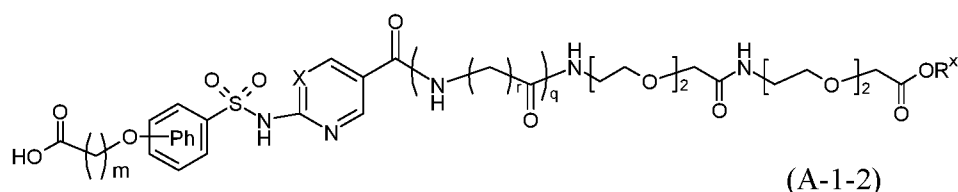
and the $\text{HOOC}-(\text{CH}_2)_m-\text{C}_6\text{H}_3\text{Hal}-\text{O}-$ group is situated in meta or para position on phenyl ring Ph with respect to the $-\text{S}(\text{O})_2-$ group.

According to one embodiment, the sulfonamide has the formula (A-1-1a)



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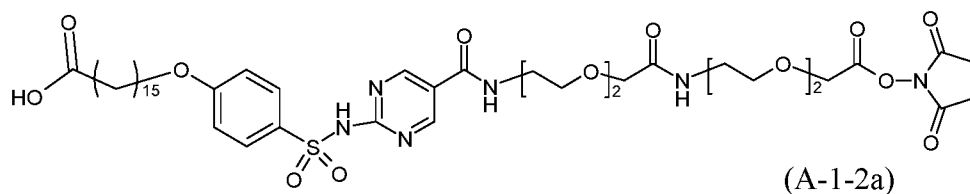
According to another embodiment, the sulfonamide has the formula (A-1-2)



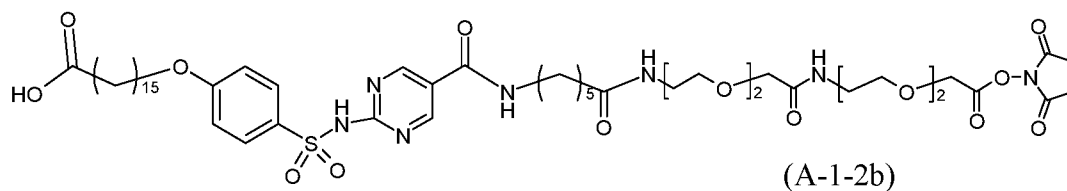
wherein X is a nitrogen atom or a $-\text{CH}-$ group, for example a nitrogen atom; m is an integer in the range from 5 to 15; r is an integer in the range from 1 to 6; q is zero or 1, for example zero; R^x is a hydrogen atom or an activation group, optionally an activation group selected from the group consisting of 7-azabenzotriazole (optionally derived from HATU or HBTU), 4-nitro benzene and N-succinimidyl-group, wherein R^x is optionally a N-succinimidyl-group; and the $\text{HOOC}-(\text{CH}_2)_m-\text{O}-$ group is situated in meta or para position on phenyl ring Ph with respect to the $-\text{S}(\text{O})_2-$ group.

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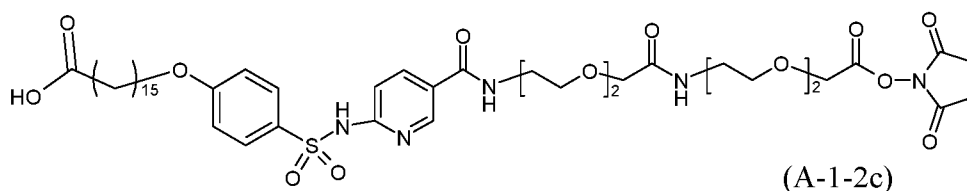
According to one embodiment, the sulfonamide has the formula (A-1-2a)



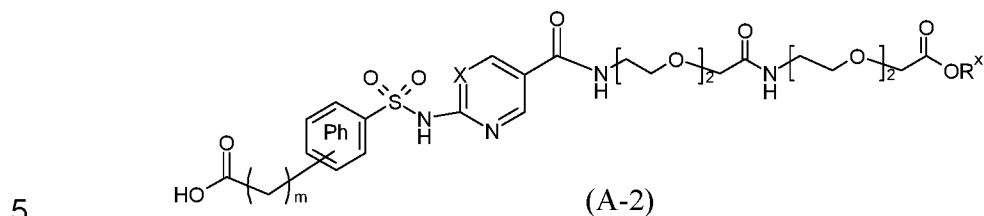
or the formula (A-1-2b)



20 or the formula (A-1-2c)



According to another embodiment, the sulfonamide has the formula (A-2)



wherein

X represents a nitrogen atom or a -CH- group;

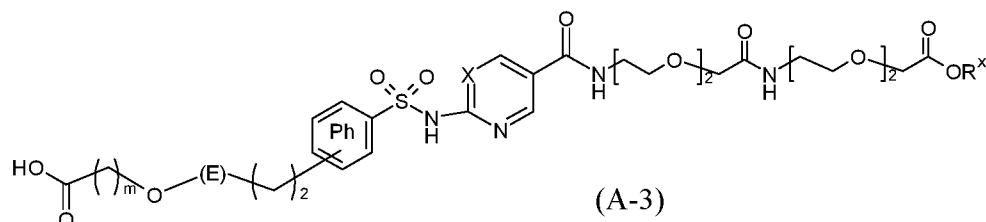
10 R^x represents a hydrogen atom or an activation group, optionally an activation group selected from the group consisting of 7-azabenzotriazole (optionally derived from HATU or HBTU), 4-nitro benzene and N-succinimidyl-group, wherein R^x is optionally a N-succinimidyl-group; and

m is an integer in the range from 5 to 17, for example in the range from 11 to 17.

15 According to one embodiment of the sulfonamide of formula (A-2), the HOOC-(CH₂)_m-group is situated in meta or para position on phenyl ring Ph with respect to the -S(O)₂-group.

According to another embodiment, the sulfonamide has the formula (A-3)

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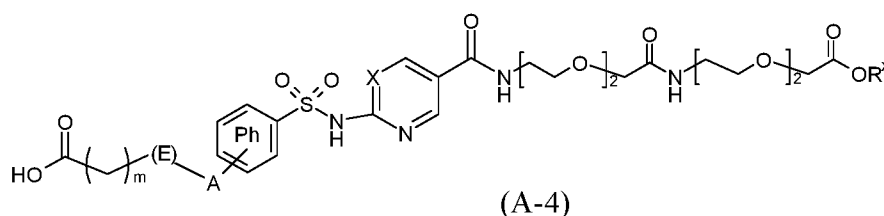
wherein

25 E represents a -C₆H₃R- group with R being a hydrogen atom or a halogen atom, wherein the halogen atom is selected from the group consisting of fluorine, chlorine, bromine and iodine atom;

- X represents a nitrogen atom or a -CH- group;
 R^x represents a hydrogen atom or an activation group, optionally an activation group selected from the group consisting of 7-azabenzotriazole (optionally derived from HATU or HBTU), 4-nitro benzene and N-succinimidyl-group,
 5 wherein R^x is optionally a N-succinimidyl-group; and
 m is an integer in the range from 5 to 17, for example 11.

According to one embodiment of the sulfonamide of formula (A-3), the HOOC-(CH₂)_m-O- group and the -(CH₂)₂- group are situated in para position on (E) of formula (A-3)
 10 and the HOOC-(CH₂)_m-O-(E)-(CH₂)₂- group is situated in para position on phenyl ring Ph with respect to the -S(O)₂- group.

According to another embodiment, the sulfonamide has the formula (A-4)

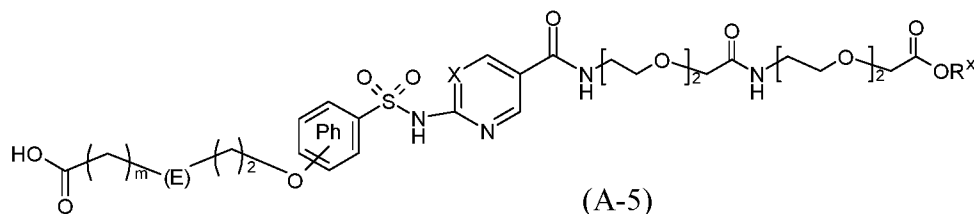


wherein

- A is a -OCH₂- group or a -CH₂O- group;
 E represents a -C₆H₃R- group with R being a hydrogen atom or a halogen atom, wherein the halogen atom is selected from the group consisting of fluorine,
 20 chlorine, bromine and iodine atom;
 X represents a nitrogen atom or a -CH- group;
 R^x represents a hydrogen atom or an activation group, optionally an activation group selected from the group consisting of 7-azabenzotriazole (optionally derived from HATU or HBTU), 4-nitro benzene and N-succinimidyl-group,
 25 wherein R^x is optionally a N-succinimidyl-group; and
 m is an integer in the range of from 5 to 17, for example in the range of from 9 to 13.

According to one embodiment of the sulfonamide of formula (A-4), the HOOC-(CH₂)_m- group and the -A- group are situated in para position on (E) of formula (A-4) and the -A- group is situated in para position on phenyl ring Ph with respect to the -S(O)₂- group.
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According to another embodiment, the sulfonamide has the formula (A-5)



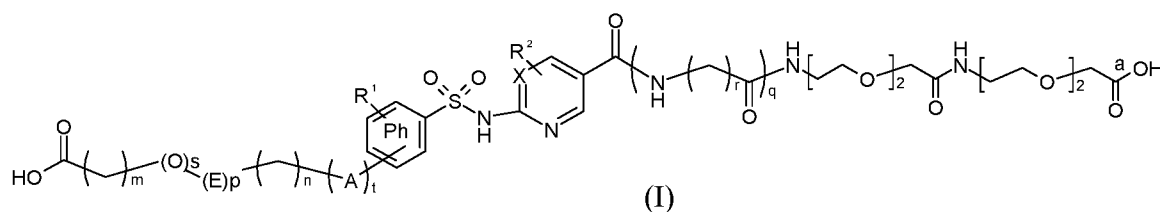
wherein

- 5 E represents a $-C_6H_3R-$ group with R being a hydrogen atom or a halogen atom, wherein the halogen atom is selected from the group consisting of fluorine, chlorine, bromine and iodine atom;
- X represents a nitrogen atom or a $-CH-$ group;
- 10 R^x represents a hydrogen atom or an activation group, optionally an activation group selected from the group consisting of 7-azabenzotriazole (optionally derived from HATU or HBTU), 4-nitro benzene and N-succinimidyl-group, wherein R^x is optionally a N-succinimidyl-group; and
- m is an integer in the range of from 5 to 17, for example in the range of from 7 to 9.

15 According to one embodiment of the sulfonamide of formula (A-5), the $HOOC-(CH_2)_m-$ group and the $-(CH_2)_2-$ group are situated in para position on (E) of formula (A-5) and the $HOOC-(CH_2)_mE-(CH_2)_2-O-$ group is situated in para position on phenyl ring Ph with respect to the $-S(O)_2-$ group.

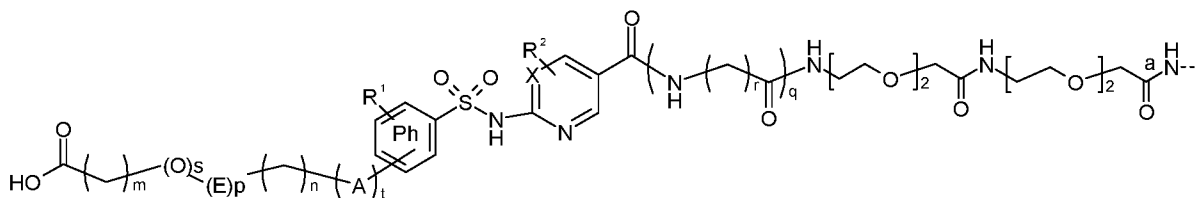
20 Conjugate

Provided herein are conjugates comprising a sulfonamide of formula (I) and an active pharmaceutical ingredient or a diagnostic compound



wherein in the sulfonamide of formula (I):

- A is selected from the group consisting of oxygen atom, -CH₂CH₂- group, -OCH₂- group and -CH₂O- group;
- E represents a -C₆H₃R- group with R being a hydrogen atom or a halogen atom, wherein the halogen atom is selected from the group consisting of fluorine, chlorine, bromine and iodine atom;
- 5 X represents a nitrogen atom or a -CH- group;
- m is an integer in the range from 5 to 17;
- n is zero or an integer in the range from 1 to 3;
- p is zero or 1;
- 10 q is zero or 1;
- r is an integer in the range from 1 to 6;
- s is zero or 1;
- t is zero or 1;
- R¹ represents at least one residue selected from the group of hydrogen atom, halogen atom, C1 to C3 alkyl group and halogenated C1 to C3 alkyl group;
- 15 R² represents at least one residue selected from the group of hydrogen atom, halogen atom, C1 to C3 alkyl group and halogenated C1 to C3 alkyl group;
- wherein the sulfonamide of formula (I) is covalently bound to the active pharmaceutical ingredient or the diagnostic compound in that the terminal carboxy group "a" of the sulfonamide of formula (I) is covalently bound to a suitable functional group of the active pharmaceutical ingredient or of the diagnostic compound, for example to an amino group or a hydroxyl group of the active pharmaceutical ingredient or of the diagnostic compound.
- 20
- 25 For example, the active pharmaceutical ingredient is a peptide, wherein the peptide and the sulfonamide of formula (I) are for example connected by an amide bond, for example formed between the terminal carboxy group "a" of the sulfonamide of formula (I) and an amino group of the peptide. It goes without saying that in case of an amide bond, the carboxyl group "a" is present in the conjugate as carbonyl group -C(=O)- , as shown below, wherein all residues E, A, R¹, R², X, as well as the indices m, s, p, n, t, r and q have the meaning as indicated above for formula (I) and the NH---- group is already the part remaining from the peptide's amino group :
- 30



In some embodiments, the combination of s being 1, p being zero, n being zero, A being an oxygen atom and t being 1 is excluded for the sulfonamide of formula (I). In some embodiments, s is zero, wherein the remaining residues and indices have the meaning as indicated above for formula (I).

In some embodiments, the halogenated C1 to C3 alkyl group of R¹ and/or the halogenated C1 to C3 alkyl group of R² of the sulfonamide of formula (I) is/are partially halogenated or per halogenated. In some embodiments, the halogenated C1 to C3 alkyl group of R¹ and/or the halogenated C1 to C3 alkyl group of R² of the sulfonamide of formula (I) is/are per halogenated.

As already discussed above, it was surprisingly found that said conjugates exhibit favourable half life in blood and/or blood plasma and a prolonged profile of action, which has, for example, been proven in pre-clinical animal models.

As used herein, the term “conjugates comprising a sulfonamide of formula (I) and an active pharmaceutical ingredient or a diagnostic compound” comprises the conjugates themselves, pharmaceutically acceptable salts thereof and all pharmaceutically acceptable isotopically-labeled conjugates, wherein one or more atoms are replaced by atoms having the same atomic number, but an atomic mass or mass number different from the atomic mass or mass number which predominates in nature. The same applies to all subtypes of the conjugates, i.e. to the conjugates comprising sulfonamides of formula (I-1) to (I-5) as detailed below and also to their substructures, for example, conjugates comprising the sulfonamides of formula (I-1-1). The same applies to all subtypes of the sulfonamides of formula (I), i.e. to the sulfonamides of formula (I-1) to (I-5) as detailed below and also to their substructures respectively, for example, the sulfonamides of formula (I-1-1). That is, the term “conjugate comprising a sulfonamide of formula (I-...)”, wherein (I-...) represents the number of the sulfonamides of formula (I-1) to (I-5) as detailed below and also their

substructures, comprises the conjugates themselves, pharmaceutically acceptable salts and all pharmaceutically acceptable isotopically-labeled compounds thereof.

Pharmaceutically acceptable salts of the conjugates include acid addition and base salts. Suitable acid addition salts are formed from acids which form non-toxic salts. Examples include the acetate, adipate, aspartate, benzoate, besylate, bicarbonate/carbonate, bisulfate/sulfate, borate, camsylate, citrate, cyclamate, edisylate, esylate, formate, fumarate, gluceptate, gluconate, glucuronate, hexafluorophosphate, hibenzate, hydrochloride/chloride, hydrobromide/bromide, hydroiodide/iodide, isethionate, lactate, malate, maleate, malonate, mesylate, methylsulfate, naphthylate, 2-napsylate, nicotinate, nitrate, orotate, oxalate, palmitate, pamoate, phosphate/hydrogen phosphate/dihydrogen phosphate, pyroglutamate, saccharate, stearate, succinate, tannate, tartrate, tosylate, trifluoroacetate, 1,5-naphthalenedisulfonic acid and xinafoate salts. Suitable base salts are formed from bases which form non-toxic salts. Examples include the aluminium, arginine, benzathine, calcium, choline, diethylamine, bis(2-hydroxyethyl)amine (diolamine), glycine, lysine, magnesium, meglumine, 2-aminoethanol (olamine), potassium, sodium, 2-amino-2-(hydroxymethyl)propane-1,3-diol (tris or tromethamine) and zinc salts. Hemisalts of acids and bases may also be formed, for example, hemisulfate and hemicalcium salts. For a review on suitable salts, see Handbook of Pharmaceutical Salts: Properties, Selection, and Use by Stahl and Wermuth (Wiley-VCH, 2002).

The conjugates, and pharmaceutically acceptable salts thereof, may exist in unsolvated and solvated forms. The term `solvate` is used herein to describe a molecular complex comprising the compound of Formula I, or a pharmaceutically acceptable salt thereof, and one or more pharmaceutically acceptable solvent molecules, for example, ethanol. The term `hydrate` is employed when said solvent is water.

30

Examples of isotopes suitable for inclusion in the conjugates include isotopes of hydrogen, such as ^2H and ^3H , carbon, such as ^{11}C , ^{13}C and ^{14}C , chlorine, such as ^{36}Cl , fluorine, such as ^{18}F , iodine, such as ^{123}I and ^{125}I , nitrogen, such as ^{13}N and ^{15}N , oxygen, such as ^{15}O , ^{17}O and ^{18}O , and sulfur, such as ^{35}S .

Certain isotopically-labelled conjugates, for example those incorporating a radioactive isotope, are useful in drug and/or substrate tissue distribution studies. The radioactive isotopes tritium, i.e. ^3H , and carbon-14, i.e. ^{14}C , are particularly
5 useful for this purpose in view of their ease of incorporation and ready means of detection.

Substitution with heavier isotopes such as deuterium, i.e. ^2H , may afford certain therapeutic advantages resulting from greater metabolic stability, for example,
10 increased in vivo half-life or reduced dosage requirements.

Substitution with positron emitting isotopes, such as ^{11}C , ^{18}F , ^{15}O and ^{13}N , can be useful in Positron Emission Topography (PET) studies for examining substrate receptor occupancy.

15 Isotopically-labeled conjugates can generally be prepared by conventional techniques known to those skilled in the art.

Pharmaceutically acceptable solvates in accordance with the invention include those
20 wherein the solvent of crystallization may be isotopically substituted, e.g. D_2O , d_6 -acetone, d_6 -DMSO.

As used herein, the term "active pharmaceutical ingredient" (API) includes any pharmaceutically active chemical or biological compound and any pharmaceutically
25 acceptable salt thereof and any mixture thereof, that provides some pharmacologic effect and is used for treating or preventing a condition. As used herein, the terms "active pharmaceutical ingredient", "active agent", "active ingredient", "active substance" and "drug" are meant to be synonyms, i.e., have identical meaning.

30 In one embodiment, the active pharmaceutical ingredient is selected from the group comprising antidiabetic agent, antiobesity agent, appetite regulating agent, antihypertensive agent, agent for the treatment and/or prevention of complications resulting from or associated with diabetes and agents for the treatment and/or prevention of complications and disorders resulting from or associated with obesity.

Examples of these active pharmaceutical ingredient are: insulin, sulphonylureas, biguanides, meglitinides, glucosidase inhibitors, glucagon antagonists, DPP-IV (dipeptidyl peptidase-IV) inhibitors, inhibitors of hepatic enzymes involved in stimulation of gluconeogenesis and/or glycogenolysis, glucose uptake

- 5 modulators, compounds modifying the lipid metabolism such as antihyperlipidemic agents as HMG CoA inhibitors (statins), Gastric Inhibitory Polypeptides (GIP analogs), compounds lowering food intake, RXR agonists and agents acting on the ATP-dependent potassium channel of the β -cells; cholestyramine, colestipol, clofibrate, gemfibrozil, lovastatin, pravastatin, simvastatin, probucol,
- 10 dextrothyroxine, neteglinide, repaglinide; α -blockers such as alprenolol, atenolol, timolol, pindolol, propranolol and metoprolol, ACE (angiotensin converting enzyme) inhibitors such as benazepril, captopril, enalapril, fosinopril, lisinopril, alatriopril, quinapril and ramipril, calcium channel blockers such as nifedipine, felodipine, nicardipine, isradipine, nimodipine, diltiazem and verapamil, and α -
- 15 blockers such as doxazosin, urapidil, prazosin and terazosin; CART (cocaine amphetamine regulated transcript) agonists, NPY (neuropeptide Y) antagonists, PYY agonist, PYY2 agonists, PYY4 agonists, mixed PYY2/PYY4 agonists, MC4 (melanocortin 4) agonists, orexin antagonists, TNF (tumor necrosis factor) agonists, CRF (corticotropin releasing factor) agonists, CRF BP (corticotropin
- 20 releasing factor binding protein) antagonists, urocortin agonists, α agonists, MSH (melanocyte-stimulating hormone) agonists, MCH (melanocyte-concentrating hormone) antagonists, CCK (cholecystokinin) agonists, serotonin re-uptake inhibitors, serotonin and noradrenaline re-uptake inhibitors, mixed serotonin and noradrenergic compounds, 5HT (serotonin) agonists, bombesin agonists, galanin
- 25 antagonists, growth hormone, growth hormone releasing compounds, TRH (thyrotropin releasing hormone) agonists, UCP 2 or 3 (uncoupling protein 2 or 3) modulators, leptin agonists, DA agonists (bromocriptin, doprexin), lipase/amylase inhibitors, RXR (retinoid X receptor) modulators, TR agonists; histamine H3 antagonists, Gastric Inhibitory Polypeptide to agonists or antagonists (GIP
- 30 analogs), gastrin and gastrin analogs. In one embodiment, the active pharmaceutical ingredient is selected from the group consisting of antidiabetic agent, antiobesity agent, appetite regulating agent, antihypertensive agent, agent for the treatment and/or prevention of complications resulting from or associated with diabetes and agents for the treatment and/or prevention of complications and

disorders resulting from or associated with obesity. Examples of these active pharmaceutical ingredient are: Insulin, sulphonylureas, biguanides, meglitinides, glucosidase inhibitors, glucagon antagonists, DPP-IV (dipeptidyl peptidase-IV) inhibitors, inhibitors of hepatic enzymes involved in stimulation of gluconeogenesis and/or glycogenolysis, glucose uptake modulators, compounds modifying the lipid metabolism such as antihyperlipidemic agents as HMG CoA inhibitors (statins), Gastric Inhibitory Polypeptides (GIP analogs), compounds lowering food intake, RXR agonists and agents acting on the ATP-dependent potassium channel of the - cells; cholestyramine, colestipol, clofibrate, gemfibrozil, lovastatin, pravastatin, simvastatin, probucol, dextrothyroxine, neteglinide, repaglinide; -blockers such as alprenolol, atenolol, timolol, pindolol, propranolol and metoprolol, ACE (angiotensin converting enzyme) inhibitors such as benazepril, captopril, enalapril, fosinopril, lisinopril, alatriopril, quinapril and ramipril, calcium channel blockers such as nifedipine, felodipine, nicardipine, isradipine, nimodipine, diltiazem and verapamil, and α -blockers such as doxazosin, urapidil, prazosin and terazosin; CART (cocaine amphetamine regulated transcript) agonists, NPY (neuropeptide Y) antagonists, PYY agonist, PYY2 agonists, PYY4 agonists, mixed PYY2/PYY4 agonists, MC4 (melanocortin 4) agonists, orexin antagonists, TNF (tumor necrosis factor) agonists, CRF (corticotropin releasing factor) agonists, CRF BP (corticotropin releasing factor binding protein) antagonists, urocortin agonists, 3 agonists, MSH (melanocyte-stimulating hormone) agonists, MCH (melanocyte-concentrating hormone) antagonists, CCK (cholecystokinin) agonists, serotonin re-uptake inhibitors, serotonin and noradrenaline re-uptake inhibitors, mixed serotonin and noradrenergic compounds, 5HT (serotonin) agonists, bombesin agonists, galanin antagonists, growth hormone, growth hormone releasing compounds, TRH (thyreotropin releasing hormone) agonists, UCP 2 or 3 (uncoupling protein 2 or 3) modulators, leptin agonists, DA agonists (bromocriptin, doprexin), lipase/amylase inhibitors, RXR (retinoid X receptor) modulators, TR agonists; histamine H3 antagonists, Gastric Inhibitory Polypeptide to agonists or antagonists (GIP analogs), gastrin and gastrin analogs.

In one embodiment, the active pharmaceutical ingredient is a therapeutically active peptide, wherein the peptide comprises at least 2 amino acids. In some embodiments, the peptide comprises at least 10 amino acids, or at least 20 amino acids. In some

embodiments, the peptide comprises not more than 1000 amino acids, such as not more than 500 amino acids, for example not more than 100 amino acids.

In one embodiment of the conjugate, the active pharmaceutical ingredient is an antidiabetic agent, such as a peptide. In some embodiments, the peptide is GLP-1, GLP-1 analog, GLP-1 agonist; dual GLP-1 receptor/glucagon receptor agonist; human FGF21, FGF21 analog, FGF21 derivative; insulin (for example human insulin), insulin analog, or insulin derivative.

According to one embodiment of the conjugate, the active pharmaceutical ingredient is selected from the group comprising insulin, insulin analog, GLP-1, and GLP-1 analog (for example GLP(-1) agonist). In one embodiment of the conjugate, the active pharmaceutical ingredient is selected from the group consisting of insulin, insulin analog, GLP-1, and GLP-1 analog (for example GLP(-1) agonist).

15

As used herein, the terms "GLP-1 analog" refer to a polypeptide which has a molecular structure which formally can be derived from the structure of a naturally occurring glucagon-like-peptide-1 (GLP-1), for example that of human GLP-1, by deleting and/or exchanging at least one amino acid residue occurring in the naturally occurring GLP-1 and/or adding at least one amino acid residue. The added and/or exchanged amino acid residue can either be codable amino acid residues or other naturally occurring residues or purely synthetic amino acid residues.

20

As used herein, the term "GLP(-1) agonist" refers to analogs of GLP(-1), which activate the glucagon-like-peptide-1-rezeptor (GLP-1-rezeptor). Examples of GLP(-1) agonists include, but are not limited to, the following: lixisenatide, exenatide / exendin-4, semaglutide, taspoglutide, albiglutide, dulaglutide.

25

Lixisenatide has the following amino acid sequence (SEQ ID NO: 1): His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Ser-Lys-Lys-Lys-Lys-Lys-Lys-NH₂

30

Exenatide has the following amino acid sequence (SEQ ID NO: 2):

H-His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser-NH₂

- 5 Semaglutide – Albuminbinder coupled to Lys(20) has the following amino acid sequence (SEQ ID NO: 3):

H-His-Aib-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys(AEEAc-AEEAc-γ-Glu-17-carboxyheptadecanoyl)-Glu-Phe-Ile-Ala-Trp-Leu-Val-Arg-Gly-Arg-Gly-OH

10

Dulaglutide (GLP1 (7-37) coupled via peptidic linker to an fc-fragment) has the following amino acid sequence (SEQ ID NO: 4):

H-His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly

15

As used herein, the term “FGF-21” means “fibroblast growth factor 21”. FGF-21 compounds may be human FGF-21, an analog of FGF-21 (referred to “FGF-21 analog”) or a derivative of FGF-21 (referred to “FGF-21 derivative”).

- 20 According to one embodiment of the conjugate, the active pharmaceutical ingredient is insulin or an insulin analog, for example human insulin analog, wherein the amino group of the peptide, to which the sulfonamide of formula (I) is covalently bound, is an epsilon amino group of a lysine present in the insulin or insulin analog or is the N-terminal amino group of the B chain of the insulin or insulin analog. For example, the
- 25 insulin or insulin analog has one lysine in the A chain and/or B chain. In some embodiments, the insulin or insulin analog has one lysine in the A and in the B chain.

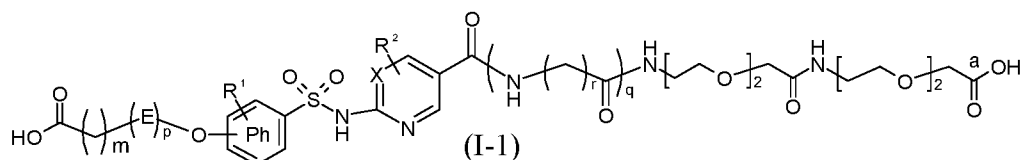
- According to one embodiment of the conjugate, the amino group of the peptide, to which the sulfonamide of formula (I) is covalently bound is an epsilon amino group of
- 30 a lysine present at position B26 to B29, for example B29, of the B chain of human insulin or human insulin analog, for example of human insulin analog.

According to another embodiment of the conjugate, the diagnostic compound is a contrast agent, such as a radio contrast agent. In some embodiments, the contrast

agent is a gadolinium or iodine based magnetic resonance imaging (MRI) contrast agent. In some embodiments, the contrast agent is gadopentetate dimeglumine, gadoterate meglumine, gadobenate dimeglumine, gadoteridol, gadodiamide, gadoversetamide, gadoxetate disodium, amidotrizoate or a salt of amidotrizoate, for example a meglumine, sodium and/or lysine salt of amidotrizoate, iohexol (5-[acetyl(2,3-dihydroxypropyl)amino]-1-N,3-N-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-benzene-1,3-dicarboxamide), iopamidol (1-N,3-N-bis(1,3-dihydroxypropan-2-yl)-5-[(2S)-2-hydroxypropanoyl]amino]-2,4,6-triiodobenzene-1,3-dicarboxamide), iopromide (1-N,3-N-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-5-[(2-methoxyacetyl)amino]-3-N-methylbenzene-1,3-dicarboxamide) or ioxidanol (5-[acetyl-[3-[acetyl-[3,5-bis(2,3-dihydroxypropyl)carbonyl]-2,4,6-triiodo-phenyl]amino]-2-hydroxy-propyl]amino]-N,N'-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-benzene-1,3-dicarboxamide). In some embodiments, the contrast agent is selected from the group consisting of gadopentetate dimeglumine, gadoterate meglumine, gadobenate dimeglumine, gadoteridol, gadodiamide, gadoversetamide, gadoxetate disodium, amidotrizoate or a salt of amidotrizoate, for example a meglumine, sodium and/or lysine salt of amidotrizoate, iohexol (5-[acetyl(2,3-dihydroxypropyl)amino]-1-N,3-N-bis(2,3-dihydroxypropyl)-2,4,6-triiodobenzene-1,3-dicarboxamide), iopamidol (1-N,3-N-bis(1,3-dihydroxypropan-2-yl)-5-[(2S)-2-hydroxypropanoyl]amino]-2,4,6-triiodobenzene-1,3-dicarboxamide), iopromide (1-N,3-N-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-5-[(2-methoxyacetyl)amino]-3-N-methylbenzene-1,3-dicarboxamide) or ioxidanol (5-[acetyl-[3-[acetyl-[3,5-bis(2,3-dihydroxypropyl)carbonyl]-2,4,6-triiodo-phenyl]amino]-2-hydroxy-propyl]amino]-N,N'-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-benzene-1,3-dicarboxamide).

As discussed above, the sulfonamide of formula (I) is covalently bound to the diagnostic compound in that the terminal carboxy group "a" of the sulfonamide of formula (I) is covalently bound to a suitable functional group of the diagnostic compound. The suitable functional group can be, for example, an amino group (primary or secondary) or a hydroxyl group of the diagnostic compound.

According to one embodiment of the conjugate, the sulfonamide has the formula (I-1)



wherein:

E represents a $-C_6H_3R-$ group with R being a hydrogen atom or a halogen atom, wherein the halogen atom is selected from the group consisting of fluorine, chlorine, bromine and iodine atom and is for example a fluorine atom;

X represents a nitrogen atom or a $-CH-$ group;

p is zero or 1;

q is zero or 1;

r is an integer in the range from 1 to 6;

R^1 represents at least one residue selected from the group of hydrogen atom and halogen atom, wherein the halogen atom is for example a fluorine or chlorine atom;

R^2 represents at least one residue selected from the group of hydrogen atom, C1 to C3 alkyl group and halogenated C1 to C3 alkyl group, wherein the C1 to C3 alkyl group is for example a methyl group and the halogenated C1 to C3 alkyl group is for example perhalogenated such as a trifluoromethyl group;

with m being an integer in the range from 5 to 15 if p is zero, or m being an integer in the range from 7 to 15 if p is 1.

In one embodiment of the conjugate, the residues R^1 and R^2 of the sulfonamide are hydrogen atoms.

In one embodiment of the conjugate, the residue X of the sulfonamide represents a nitrogen atom.

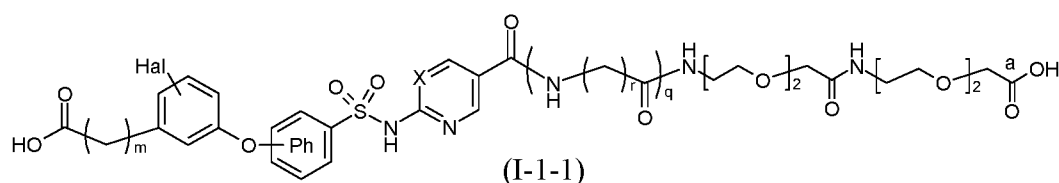
According to another embodiment of the conjugate, the $HOOC-(CH_2)_m-(O)_s-(E)_p-(CH_2)_n-(A)_t$ group of formula (I) or the $HOOC-(CH_2)_m-(E)_p-O-$ group of formula (I-1) of the sulfonamide is situated in meta or para position on phenyl ring Ph with respect to the $-S(O)_2-$ group.

According to another embodiment of the conjugate, if p is 1, the $HOOC-(CH_2)_m-(O)_s-$ group and the $-(CH_2)_n-(A)_t$ group are situated in meta or para position on $(E)_p$ of

formula (I) of the sulfonamide or the $\text{HOOC}-(\text{CH}_2)_m$ - group and the -O- are situated in meta or para position on (E)_p of formula (I-1).

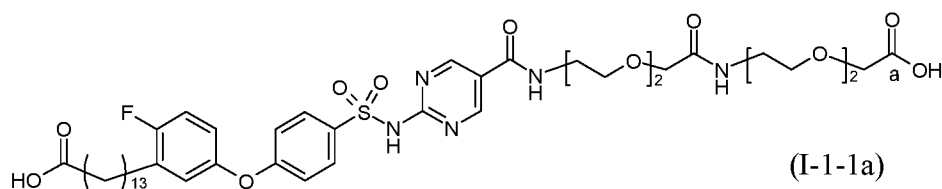
According to another embodiment of the conjugate, the index q of the sulfonamide is zero.

According to another embodiment of the conjugate, the sulfonamide has the formula (I-1-1)

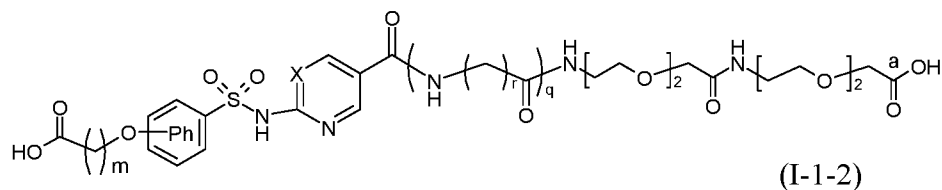


wherein X is a nitrogen atom or a -CH- group, for example a nitrogen atom; m is an integer in the range from 7 to 15; r is an integer in the range from 1 to 6; q is zero or 1, for example zero; Hal is a halogen atom selected from the group consisting of fluorine, chlorine, bromine and iodine atom, for example a fluorine atom; and the $\text{HOOC}-(\text{CH}_2)_m$ -C₆H₃Hal-O- group is situated in meta or para position on phenyl ring Ph with respect to the -S(O)₂- group.

According to one embodiment of the conjugate, the sulfonamide has the formula (I-1-1a)



According to another embodiment of the conjugate, the sulfonamide has the formula (I-1-2)

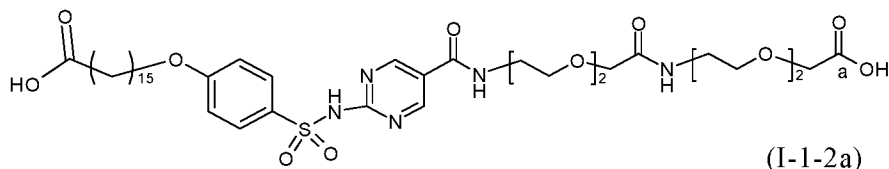


wherein X is a nitrogen atom or a -CH- group, for example a nitrogen atom; m is an integer in the range from 5 to 15; r is an integer in the range from 1 to 6; q is zero or 1,

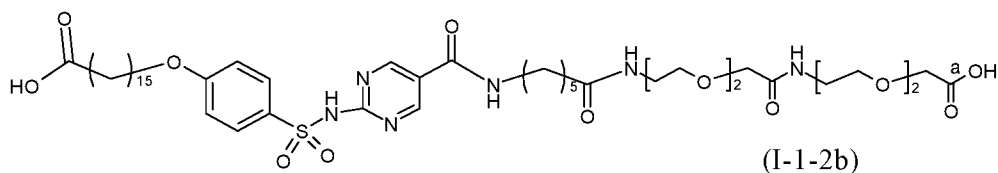
for example zero; and the $\text{HOOC}-(\text{CH}_2)_m\text{-O-}$ group is situated in meta or para position on phenyl ring Ph with respect to the $-\text{S}(\text{O})_2-$ group.

According to one embodiment of the conjugate, the sulfonamide has the formula (I-1-

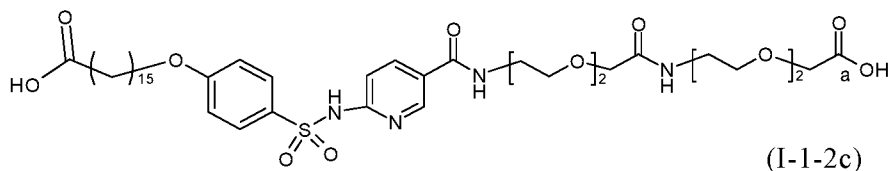
5 2a)



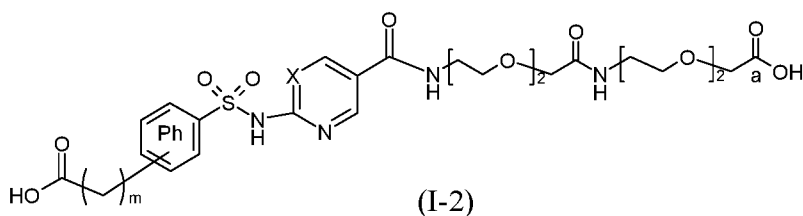
or the formula (I-1-2b)



or the formula (I-1-2c)



According to another embodiment of the conjugate, the sulfonamide has the formula (I-2)



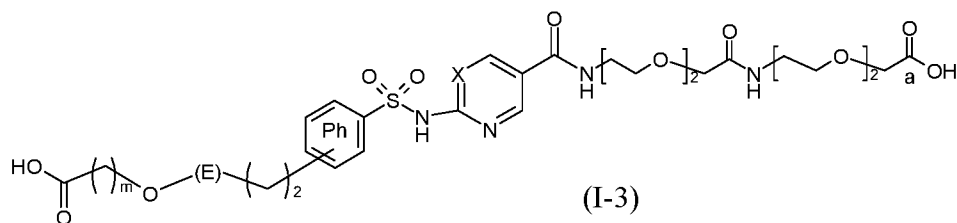
15 wherein

X represents a nitrogen atom or a $-\text{CH}-$ group; and

m is an integer in the range from 5 to 17, for example in the range from 11 to 17.

20 According to one embodiment of the conjugate, the $\text{HOOC}-(\text{CH}_2)_m-$ group of the sulfonamide of formula (I-2) is situated in meta or para position on phenyl ring Ph with respect to the $-\text{S}(\text{O})_2-$ group.

According to another embodiment of the conjugate, the sulfonamide has the formula (I-3)



wherein

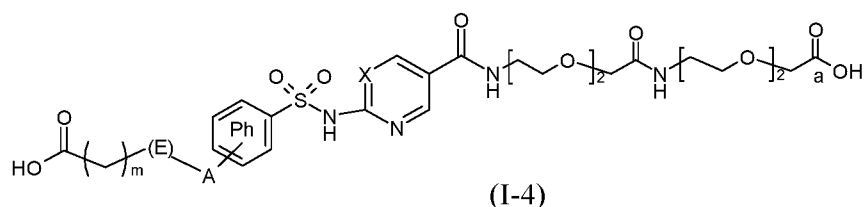
E represents a $-C_6H_3R-$ group with R being a hydrogen atom or a halogen atom, wherein the halogen atom is selected from the group consisting of fluorine, chlorine, bromine and iodine atom;

X represents a nitrogen atom or a $-CH-$ group;

m is an integer in the range from 5 to 17, for example 11.

According to one embodiment of the conjugate, the $HOOC-(CH_2)_m-O-$ group and the $-(CH_2)_2-$ group of the sulfonamide of formula (I-3) are situated in para position on (E) of formula (I-3) and the $HOOC-(CH_2)_m-O-(E)-(CH_2)_2-$ group is situated in para position on phenyl ring Ph with respect to the $-S(O)_2-$ group.

According to another embodiment of the conjugate, the sulfonamide has the formula (I-4)



wherein

A is a OCH_2- group or a $-CH_2O-$ group;

E represents a $-C_6H_3R-$ group with R being a hydrogen atom or a halogen atom, wherein the halogen atom is selected from the group consisting of fluorine, chlorine, bromine and iodine atom;

X represents a nitrogen atom or a $-CH-$ group;

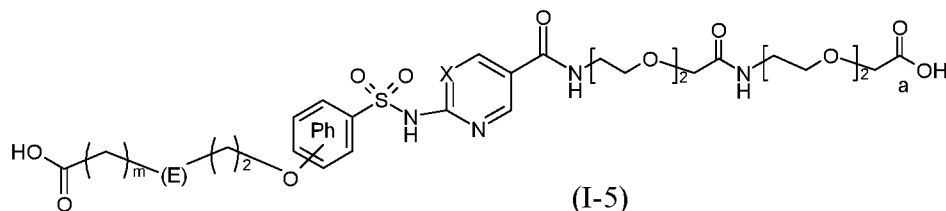
m is an integer in the range of from 5 to 17, for example in the range of from 9 to 13.

According to one embodiment of the conjugate, the $HOOC-(CH_2)_m-$ group and the $-A-$ group of the sulfonamide of formula (I-4) are situated in para position on (E) of formula

(I-4) and the -A- group is situated in para position on phenyl ring Ph with respect to the -S(O)₂- group.

According to another embodiment of the conjugate, the sulfonamide has the formula

5 (I-5)



wherein

E represents a -C₆H₃R- group with R being a hydrogen atom or a halogen atom, wherein the halogen atom is selected from the group consisting of fluorine, chlorine, bromine and iodine atom;

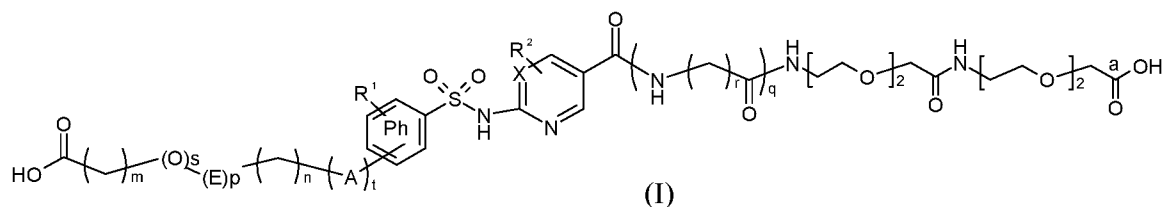
X represents a nitrogen atom or a -CH- group;

m is an integer in the range of from 5 to 17, for example in the range of from 7 to 9.

15 According to one embodiment of the conjugate, the HOOC-(CH₂)_m group and the -(CH₂)₂- group, of the sulfonamide of formula (I-5) are situated in para position on (E) of formula (I-5) and the HOOC-(CH₂)_m (E)-(CH₂)₂-O- group is situated in para position on phenyl ring Ph with respect to the -S(O)₂- group.

20 Process for preparing a conjugate

Provided herein are processes for preparing a conjugate comprising a sulfonamide of formula (I) and an active pharmaceutical ingredient



wherein in the sulfonamide of formula (I):

A is selected from the group consisting of oxygen atom, -CH₂CH₂- group, -OCH₂- group and -CH₂O- group;

E represents a $-C_6H_3R-$ group with R being a hydrogen atom or a halogen atom, wherein the halogen atom is selected from the group consisting of fluorine, chlorine, bromine and iodine atom;

X represents a nitrogen atom or a $-CH-$ group;

5 m is an integer in the range from 5 to 17;

n is zero or an integer in the range from 1 to 3;

p is zero or 1;

q is zero or 1;

r is an integer in the range from 1 to 6;

10 s is zero or 1;

t is zero or 1;

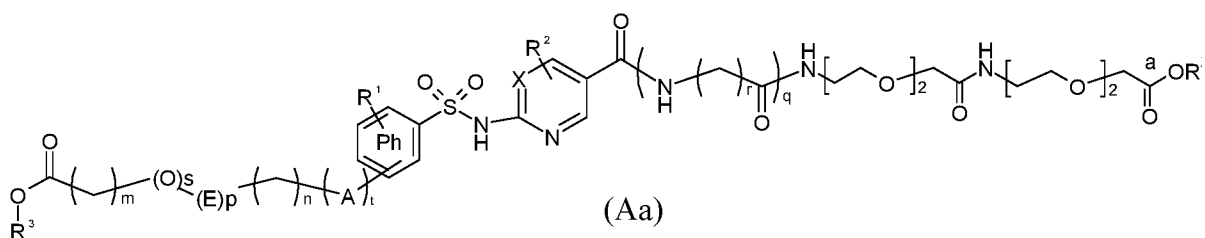
R^1 represents at least one residue selected from the group of hydrogen atom, halogen atom, C1 to C3 alkyl group and halogenated C1 to C3 alkyl group;

R^2 represents at least one residue selected from the group of hydrogen atom, halogen atom, C1 to C3 alkyl group and halogenated C1 to C3 alkyl group;

wherein the sulfonamide of formula (I) is covalently bound to the active pharmaceutical ingredient in that the terminal carboxy group "a" of the sulfonamide of formula (I) is covalently bound to an amino group of the active pharmaceutical ingredient;

(a) providing a sulfonamide of formula (Aa)

20



wherein X, Y, A, E, R^1 , R^2 and the indices m, n, p, q, r, s, t have the meaning as defined above with respect to formula (I), R^x is a hydrogen atom or an activation group, optionally an activation group selected from the group consisting of 7-azabenzotriazole (optionally derived from HATU or HBTU), 4-nitro benzene and N-succinimidyl-group, wherein R^x is optionally a N-succinimidyl-group, and R^3 is a protective group or a hydrogen atom, optionally a hydrogen atom; and a active pharmaceutical ingredient having a protected or unprotected C terminus;

30 (b) reacting the sulfonamide of formula (Aa) and the active pharmaceutical ingredient having a protected or unprotected C terminus under conditions

suitable to form an amide bond between the free or activated, optionally activated, carboxy group "a" of the sulfonamide of formula (Aa) and an amino group of the active pharmaceutical ingredient having a protected or unprotected C terminus;

- 5 (c) optionally removing one or both protection groups, for example removing both protective groups.

10 In some embodiments of the process, the combination of s being 1, p being zero, n being zero, A being an oxygen atom and t being 1 is excluded for the sulfonamide of formula (I) as well as for the sulfonamide of formula (Aa). In some embodiments, s is zero for the sulfonamide of formula (I) as well as for the sulfonamide of formula (Aa), wherein the remaining residues and indices have the meaning as indicated above for formula (I) and (Aa) respectively.

15 Provided herein are processes for preparing a conjugate comprising a sulfonamide of formula (I) and a diagnostic compound, wherein the diagnostic compound is covalently bound with a suitable functional group to a free or activated, optionally activated, carboxy group "a" of the sulfonamide of formula (Aa) in accordance with the method described above for the bonding with the active pharmaceutical ingredient.

20

It is also possible to prepare a conjugate as described herein above by a process comprising:

- a) providing a sulfonamide of formula (Aa) wherein R^x represents an activation group (R^x = activation group);
- 25 b) Providing an aqueous solution of an active pharmaceutical ingredient, wherein the aqueous solution optionally comprises an alcohol;
- c) Contacting the aqueous solution of b) with the sulfonamide of formula (Aa) (R^x = activation group) of a); and
- d) Reacting the sulfonamide of formula (Aa) with the active pharmaceutical
- 30 ingredient, obtaining a solution comprising the conjugate of the sulfonamide and the active pharmaceutical ingredient, wherein the sulfonamide is covalently bound to the active pharmaceutical ingredient.

In this process, the active pharmaceutical ingredient is optionally an insulin polypeptide having a free amino group, optionally an insulin analog as described herein above or a precursor thereof, each having a free amino group, wherein the precursor of the insulin analog comprises an additional linker peptide which has a
5 length of at least two amino acids, or a length in the range from 2 to 30 amino acids, or a length in the range from 4 to 9 amino acids. In this process, the aqueous solution provided in a) has a pH value in the range of from 9 to 12, or in the range of from 9.5 to 11.5, or in the range of from 10 to 11, wherein the pH value is determined with a pH sensitive glass electrode according to ASTM E 70:2007; wherein the pH value is
10 optionally adjusted in the respective range by addition of a base, optionally a base selected from the group consisting of alkali hydroxides (lithium hydroxide, sodium hydroxide, potassium hydroxide), alkyl amines and mixtures of two or more thereof ; optionally selected from the group of tertiary alkyl amines $N(C1-C5 \text{ alkyl})_3$, primary alkyl amines $H_2N-C(C1-C5 \text{ alkyl})_3$ and mixtures of two or more thereof, wherein the
15 C1-C5 alkyl groups of the tertiary amines and of the primary amines are each independently selected from branched or straight C1-C5 alkyl groups and wherein each C1-C5 alkyl group has at least one substituent selected from the group of hydrogen atom, hydroxyl group and carboxyl group; optionally selected from the group of tertiary alkyl amines $N(C1-C3 \text{ alkyl})_3$, primary alkyl amines $H_2N-C(C1-C3 \text{ alkyl})_3$ and mixtures of two or more thereof, wherein the C1-C3 alkyl groups of the
20 tertiary amines and of the primary amines are each independently selected from branched or straight C1-C3 alkyl groups and wherein each C1-C3 alkyl group has at least one substituent selected from the group of hydrogen atom, hydroxyl group and carboxyl group; optionally selected from the group of bicine, trimethylamine, tris(hydroxymethyl)aminomethane and mixtures of two or more thereof; wherein the
25 base optionally comprises at least triethylamine.

In one variant of this process, contacting the aqueous solution of b) with the sulfonamide of formula (Aa) (R^x = activation group) of a) according to step c) is done in that the sulfonamide of formula (Aa) (R^x = activation group) of a) is added as a
30 solution of the sulfonamide of formula (Aa) (R^x = activation group) to the aqueous solution of b), wherein the solution of the sulfonamide of formula (Aa) (R^x = activation group) is optionally an organic solution, optionally a solution comprising the sulfonamide of formula (Aa) (R^x = activation group) and a polar aprotic organic solvent, optionally a polar aprotic organic solvent having an octanol-water-partition

coefficient (K_{ow}) in the range of from 1 to 5, or in the range of from 2 to 4 at standard conditions (T: 20-25 °C, p: 1013 mbar); optionally selected from the group consisting of tetrahydrofuran, acetonitrile, dimethylformamide, and mixtures of two or more thereof; or selected from the group of tetrahydrofuran, acetonitrile and mixtures of tetrahydrofuran and acetonitrile.

In one variant of this process, contacting the aqueous solution of b) with the sulfonamide of formula (Aa) (R^x = activation group) of a) according to step c) is done in that the sulfonamide of formula (Aa) (R^x = activation group) of a) is added in solid form to the aqueous solution of b), or at least partially in crystalline form, or at least 90 weight-% in crystalline form.

In this process, step d) optionally comprises: d.1) Reacting the sulfonamide of formula (Aa) (R^x = activation group) with a precursor of the insulin analog at a pH in the range from 9 to 12, or in the range from 9.5 to 11.5, or in the range from 10 to 11, obtaining a pre-conjugate comprising the sulfonamide of formula (I) and the precursor of the insulin analog, wherein the sulfonamide of formula (I) is covalently bound to the precursor of the insulin analog by an amide bond $C(=O)-NH-$ formed between the $-C(=O)-O(R)$ of the sulfonamide of Formula (I) and the amino group of the precursor of the insulin analog; d.2) Enzymatic digestion, optionally at a pH in the range below 9, or at a pH in the range of 7 to 9, of the precursor of the insulin analog of the pre-conjugate obtained according to d.1), obtaining a solution comprising the conjugate of the sulfonamide of formula (I) and the insulin analog. The process optionally comprises: e) Isolating the conjugate of the sulfonamide of formula (I) and the insulin analog from the solution obtained in d) or d.2).

In this process, the activation group R^x of the sulfonamide of formula (Aa) is optionally selected from the group consisting of 7-azabenzotriazole (optionally derived from HATU or HBTU), 4-nitro benzene and N-succinimidyl-group, wherein R^x optionally a N-succinimidyl-group.

In one variant of this process, the aqueous solution of the precursor of the insulin analog according to b) comprises an alcohol which is selected from the group consisting of C1-C4 monoalcohols and mixtures of two or more thereof, or from the group consisting of methanol, ethanol, propan-2-ol, propan-1-ol, butan-1-ol and

mixtures of two or more thereof, or from the group consisting of ethanol, propan-2-ol, propan-1-ol, and mixtures of two or more thereof. Optionally, the alcohol is present in the aqueous solution in an amount in the range from 0.0001 to 35 volume-%, or in the range from 0.001 to 30 volume-%, or in the range from 0.01 to 25 volume-%, or in the range from 0.1 to 20 volume-%, each based on the total volume of water and alcohol. In this process, the enzymatic digestion according to d.2) comprises use of at least one enzyme selected from the group consisting of trypsin, a TEV protease (Tobacco Etch Virus protease) and mixtures of two or more thereof. In this process, the insulin analog is an insulin analog as described herein above. In this process, the sulfonamide of formula (I) is covalently bound to the insulin analog and the precursor thereof respectively by an amide bond $C(=O)-NH-$ formed between the $-C(=O)-O(R^3)$ of the sulfonamide of formula (I) and the free amino group of the insulin analog and the precursor thereof respectively, wherein the free amino group of the insulin analog and the precursor thereof respectively is optionally the amino group of a lysine comprised in the insulin analog and the precursor thereof respectively, or a terminal lysine, or a lysine present at a C terminus of the insulin analog and the precursor thereof respectively, or a lysine present at the C terminus of the B-chain.

Provided herein are conjugates comprising a sulfonamide of formula (I) and an active pharmaceutical ingredient or a diagnostic compound obtained or obtainable from the processes as described above.

Provided herein are pharmaceutical compositions comprising in a pharmaceutically or diagnostically effective amount, the conjugate comprising a sulfonamide of formula (I) and an active pharmaceutical ingredient or a diagnostic compound as described above.

Provided herein are conjugates comprising a sulfonamide of formula (I) and an active pharmaceutical ingredient as described above for use as a medicament.

One embodiment relates to the conjugate comprising a sulfonamide of formula (I) and an active pharmaceutical ingredient as described above for use as a medicament for treatment of a disease selected from the group consisting of gestational diabetes, diabetes mellitus type 1, diabetes mellitus type 2 and hyperglycemia and/or for lowering blood glucose levels.

Provided herein are methods of treating a patient suffering from a disease selected from the group consisting of gestational diabetes, diabetes mellitus type 1, diabetes mellitus type 2, and hyperglycemia and/or being in need of lowering blood glucose levels; comprising administering a therapeutically effective amount of the conjugate comprising a sulfonamide of formula (I) and an active pharmaceutical ingredient as described above.

Provided herein are uses of the conjugate comprising a sulfonamide of formula (I) and an active pharmaceutical ingredient as described above for the manufacture of a medicament for treatment of a disease selected from the group consisting of gestational diabetes, diabetes mellitus type 1, diabetes mellitus type 2 and hyperglycemia and/or for lowering blood glucose levels.

Provided herein are conjugates comprising a sulfonamide of formula (I) and a diagnostic compound as described above for use as a diagnostic agent.

Provided herein are methods of diagnosing a disease, for example a disease selected from the group of cardiovascular diseases and cancers, in a patient or for determining the risk of a patient to develop a diseases, for example a disease selected from the group of cardiovascular diseases and cancers, comprising administering a diagnostically effective amount of the conjugate comprising a sulfonamide of formula (I) and a diagnostic compound as described above.

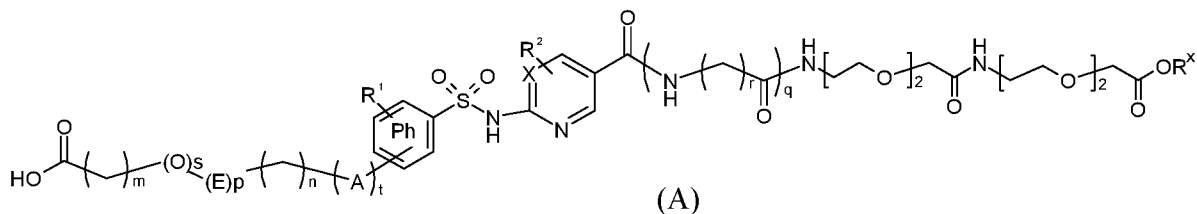
Provided herein are uses of the conjugate comprising a sulfonamide of formula (I) and a diagnostic compound as described above for the manufacture of a diagnostic agent for diagnosis of a disease, for example a disease selected from the group of cardiovascular diseases and cancers.

The present invention is further illustrated by the following embodiments and combinations of embodiments as indicated by the respective dependencies and back-references. In particular, it is noted that in each instance where a range of embodiments is mentioned, for example in the context of a term such as "The ... of any of embodiments 1 to 4", every embodiment in this range is meant to be explicitly

disclosed for the skilled person, i.e. the wording of this term is to be understood by the skilled person as being synonymous to "The ... of any of embodiments 1, 2, 3, and 4".

1. A sulfonamide of formula (A)

5



wherein:

A is selected from the group consisting of oxygen atom, -CH₂CH₂- group, -OCH₂- group and -CH₂O- group;

10 E represents a -C₆H₃R- group with R being a hydrogen atom or a halogen atom, wherein the halogen atom is selected from the group consisting of fluorine, chlorine, bromine and iodine atom;

X represents a nitrogen atom or a -CH- group;

m is an integer in the range from 5 to 17;

15 n is zero or an integer in the range from 1 to 3;

p is zero or 1;

q is zero or 1;

r is an integer in the range from 1 to 6;

s is zero or 1;

20 t is zero or 1;

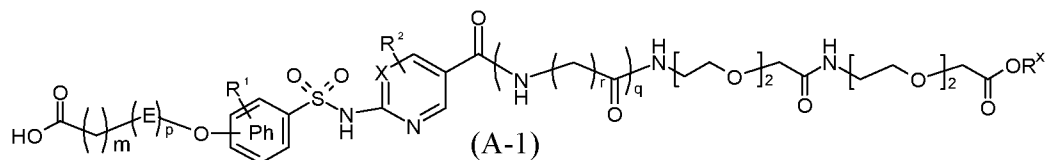
R¹ represents at least one residue selected from the group of hydrogen atom, halogen atom, C1 to C3 alkyl group and halogenated C1 to C3 alkyl group;

25 R² represents at least one residue selected from the group of hydrogen atom, halogen atom, C1 to C3 alkyl group and halogenated C1 to C3 alkyl group;

R^x represents a hydrogen atom or an activation group, optionally an activation group selected from the group consisting of 7-azabenzotriazole (optionally derived from HATU or HBTU), 4-nitro benzene and N-succinimidyl-group, wherein R^x is optionally a N-succinimidyl-group.

30

2. The sulfonamide according to embodiment 1 having the formula (A-1)



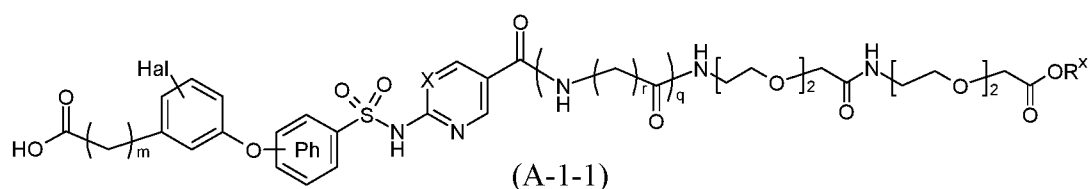
wherein:

- 5 E represents a -C₆H₃R- group with R being a hydrogen atom or a halogen atom, wherein the halogen atom is selected from the group consisting of fluorine, chlorine, bromine and iodine atom;
- X represents a nitrogen atom or a -CH- group;
- p is zero or 1;
- 10 q is zero or 1;
- r is an integer in the range from 1 to 6;
- R¹ represents at least one residue selected from the group of hydrogen atom and halogen atom;
- R² represents at least one residue selected from the group of hydrogen atom, C1 to C3 alkyl group and halogenated C1 to C3 alkyl group;
- 15 R^x represents a hydrogen atom or an activation group, optionally an activation group selected from the group consisting of 7-azabenzotriazole (optionally derived from HATU or HBTU), 4-nitro benzene and N-succinimidyl-group, wherein R^x is optionally a N-succinimidyl-group; and
- 20 with m being an integer in the range from 5 to 15 if p is zero, or m being an integer in the range from 7 to 15 if p is 1.
3. The sulfonamide according to embodiment 1 or 2, wherein R¹ and R² are hydrogen atoms.
- 25 4. The sulfonamide according to any of embodiments 1 to 3, wherein X represents a nitrogen atom.
5. The sulfonamide according to any of embodiments 1 to 4, wherein the HOOC-(CH₂)_m-(O)_s-(E)_p-(CH₂)_n-(A)_t group of formula (A) or the HOOC-(CH₂)_m-(E)_p-O-group of formula (A-1) is situated in meta or para position on phenyl ring Ph with respect to the -S(O)₂- group.
- 30

6. The sulfonamide according to any of embodiments 1 to 5, wherein, if p is 1, the $\text{HOOC}-(\text{CH}_2)_m-\text{O}-$ group and the $-(\text{CH}_2)_n-(\text{A})_t-$ group are situated in meta or para position on $(\text{E})_p$ of formula (A) or the $\text{HOOC}-(\text{CH}_2)_m-$ group and the $-\text{O}-$ are situated in meta or para position on $(\text{E})_p$ of formula (A-1).

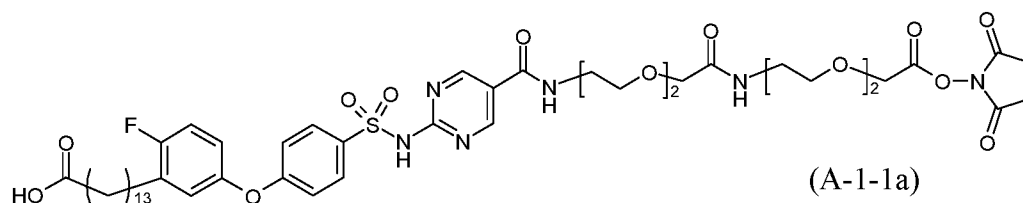
7. The sulfonamide according to any of embodiments 1 to 6, wherein q is zero.

8. The sulfonamide according to any of embodiments 1 to 7, wherein the sulfonamide has the formula (A-1-1)

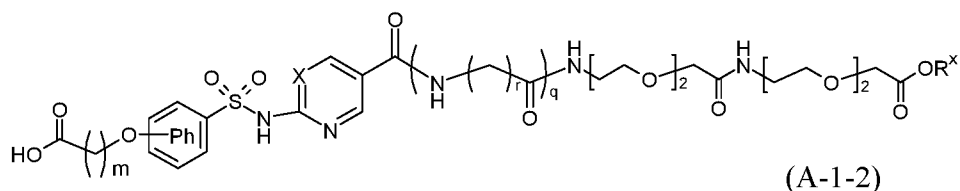


wherein X is a nitrogen atom or a $-\text{CH}-$ group; m is an integer in the range from 7 to 15; r is an integer in the range from 1 to 6; q is zero or 1; Hal is a halogen atom selected from the group consisting of fluorine, chlorine, bromine and iodine atom; R^x represents a hydrogen atom or an activation group, optionally an activation group selected from the group consisting of 7-azabenzotriazole (optionally derived from HATU or HBTU), 4-nitro benzene and N-succinimidyl-group, wherein R^x is optionally a N-succinimidyl-group; and the $\text{HOOC}-(\text{CH}_2)_m-\text{C}_6\text{H}_3\text{Hal}-\text{O}-$ group is situated in meta or para position on phenyl ring Ph with respect to the $-\text{S}(\text{O})_2-$ group.

9. The sulfonamide according to any of embodiments 1 to 8, wherein the sulfonamide has the formula (A-1-1a)

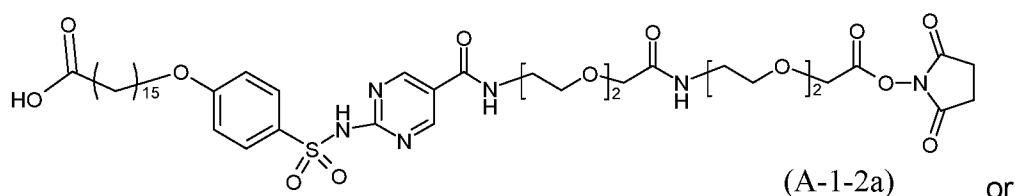


10. The sulfonamide according to any of embodiments 1 to 7, wherein the sulfonamide has the formula (A-1-2)

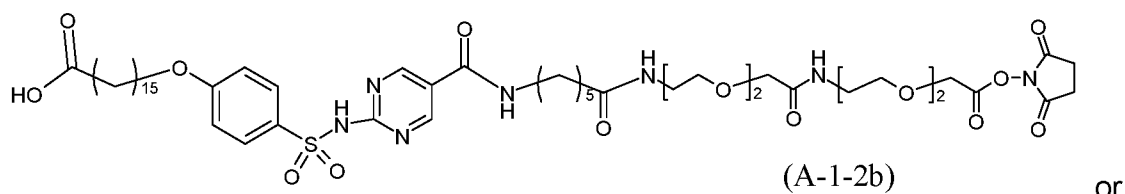


wherein X is a nitrogen atom or a -CH- group; m is an integer in the range from 5 to 15; r is an integer in the range from 1 to 6; q is zero or 1; and the HOOC-(CH₂)_m-O- group is situated in meta or para position on phenyl ring Ph with respect to the -S(O)₂- group.

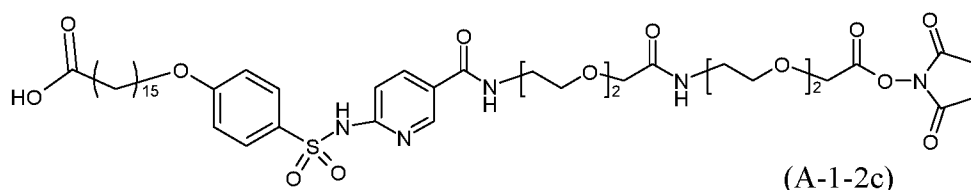
11. The sulfonamide according to any of embodiments 1 to 7 or 10, wherein the sulfonamide has the formula (A-1-2a)



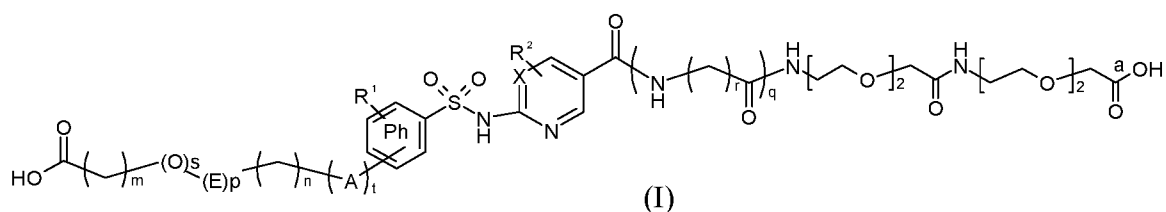
- 10 the formula (A-1-2b)



the formula (A-1-2c)



12. A conjugate comprising a sulfonamide of formula (I) and an active pharmaceutical ingredient or a diagnostic compound



wherein in the sulfonamide of formula (I):

A is selected from the group consisting of oxygen atom, -CH₂CH₂- group, -OCH₂-group and -CH₂O- group;

E represents a -C₆H₃R- group with R being a hydrogen atom or a halogen atom, wherein the halogen atom is selected from the group consisting of fluorine, chlorine, bromine and iodine atom, preferably a fluorine atom;

X represents a nitrogen atom or a -CH- group;

m is an integer in the range from 5 to 17;

n is zero or an integer in the range from 1 to 3;

p is zero or 1;

q is zero or 1;

r is an integer in the range from 1 to 6;

s is zero or 1;

t is zero or 1;

R¹ represents at least one residue selected from the group of hydrogen atom, halogen atom, C1 to C3 alkyl group and halogenated C1 to C3 alkyl group;

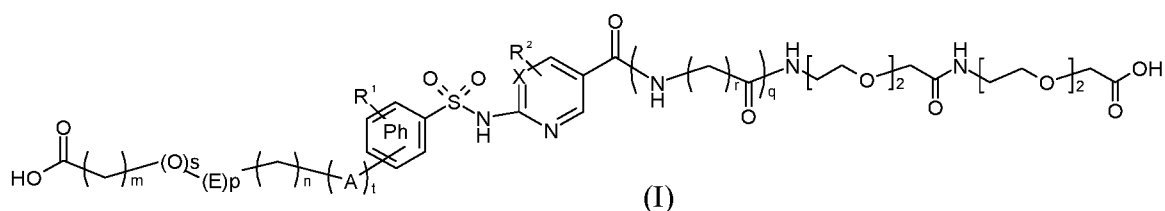
R² represents at least one residue selected from the group of hydrogen atom, halogen atom, C1 to C3 alkyl group and halogenated C1 to C3 alkyl group;

wherein the sulfonamide of formula (I) is covalently bound to the active pharmaceutical ingredient or the diagnostic compound in that the terminal carboxy group "a" of the sulfonamide of formula (I) is covalently bound to a suitable functional group of the pharmaceutically active agent or of the diagnostic compound.

13. The conjugate according to embodiment 12, wherein the active pharmaceutical ingredient is selected from the group consisting of insulin, insulin analog, GLP-1, and GLP-1 analog.

14. The conjugate according to embodiment 12 or 13, wherein the active pharmaceutical ingredient is insulin or an insulin analog, wherein the amino group of the peptide, to which the sulfonamide of formula (I) is covalently bound, is an epsilon amino group of a Lysine present in the insulin or insulin analog or is the N-terminal amino group of the B chain of the insulin or insulin analog.

15. The conjugate according to embodiment 14, wherein the amino group of the peptide, to which the sulfonamide of formula (I) is covalently bound is an epsilon amino group of a Lysine present at position B26 to B29 of the B chain of human insulin or human insulin analog.
16. A process for preparing a conjugate comprising a sulfonamide of formula (I) and an active pharmaceutical ingredient



wherein in the sulfonamide of formula (I):

A is selected from the group consisting of oxygen atom, -CH₂CH₂- group, -OCH₂- group and -CH₂O- group;

E represents a -C₆H₃R- group with R being a hydrogen atom or a halogen atom, wherein the halogen atom is selected from the group consisting of fluorine, chlorine, bromine and iodine atom, preferably a fluorine atom;

X represents a nitrogen atom or a -CH- group;

m is an integer in the range from 5 to 17;

n is zero or an integer in the range from 1 to 3;

p is zero or 1;

q is zero or 1;

r is an integer in the range from 1 to 6;

s is zero or 1;

t is zero or 1;

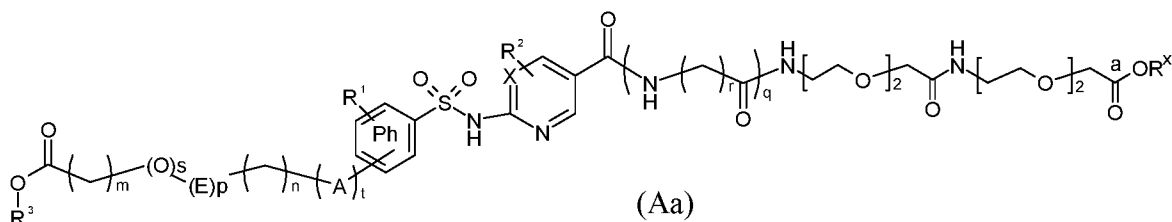
R¹ represents at least one residue selected from the group of hydrogen atom, halogen atom, C1 to C3 alkyl group and halogenated C1 to C3 alkyl group;

R² represents at least one residue selected from the group of hydrogen atom, halogen atom, C1 to C3 alkyl group and halogenated C1 to C3 alkyl group;

wherein the sulfonamide of formula (I) is covalently bound to the active pharmaceutical ingredient in that the terminal carboxy group "a" of the sulfonamide of formula (I) is covalently bound to an amino group of the active pharmaceutical ingredient;

comprising:

(a) providing a sulfonamide of formula (Aa)



wherein X, Y, A, E, R¹, R² and the indices m, n, p, q, r, s, t have the meaning as defined in embodiment 1, R^x is a hydrogen atom or an activation group, preferably an activation group selected from the group consisting of 7-azabenzotriazole (preferably derived from HATU or HBTU), 4-nitro benzene and N-succinimidyl-group, wherein R^x is preferably a N-succinimidyl -group; and R³ is a protective group or a hydrogen atom, preferably a hydrogen atom; and a active pharmaceutical ingredient having a protected or unprotected C terminus;

(b) reacting the sulfonamide of formula (Aa) and the active pharmaceutical ingredient having a protected or unprotected C terminus under conditions suitable to form an amide bond between the free or activated, preferably activated, carboxy group "a" of the sulfonamide of formula (Aa) and an amino group of the active pharmaceutical ingredient having a protected or unprotected C terminus;

(c) optionally removing one or both protection groups.

17. A conjugate comprising a sulfonamide of formula (I) and an active pharmaceutical ingredient obtained or obtainable from the process according to embodiment 16.

18. Pharmaceutical composition comprising in a pharmaceutically or diagnostically effective amount, the conjugate comprising a sulfonamide of formula (I) and an

active pharmaceutical ingredient or a diagnostic compound according to any of embodiments 12 to 15 or according to embodiment 17.

19. The conjugate comprising a sulfonamide of formula (I) and an active pharmaceutical ingredient according to any of embodiments 12 to 15 or according to embodiment 17 for use as a medicament.
20. The conjugate comprising a sulfonamide of formula (I) and an active pharmaceutical ingredient according to any of embodiments 12 to 15 or according to embodiment 17 for use as a medicament for treatment of a disease selected from the group consisting of gestational diabetes, diabetes mellitus type 1, diabetes mellitus type 2 and hyperglycemia and/or for lowering blood glucose levels.
21. The conjugate comprising a sulfonamide of formula (I) and a diagnostic compound according to any of embodiments 12 to 15 for use as a diagnostic agent.

The present invention is further illustrated by the following examples.

Examples

1. List of used abbreviations:

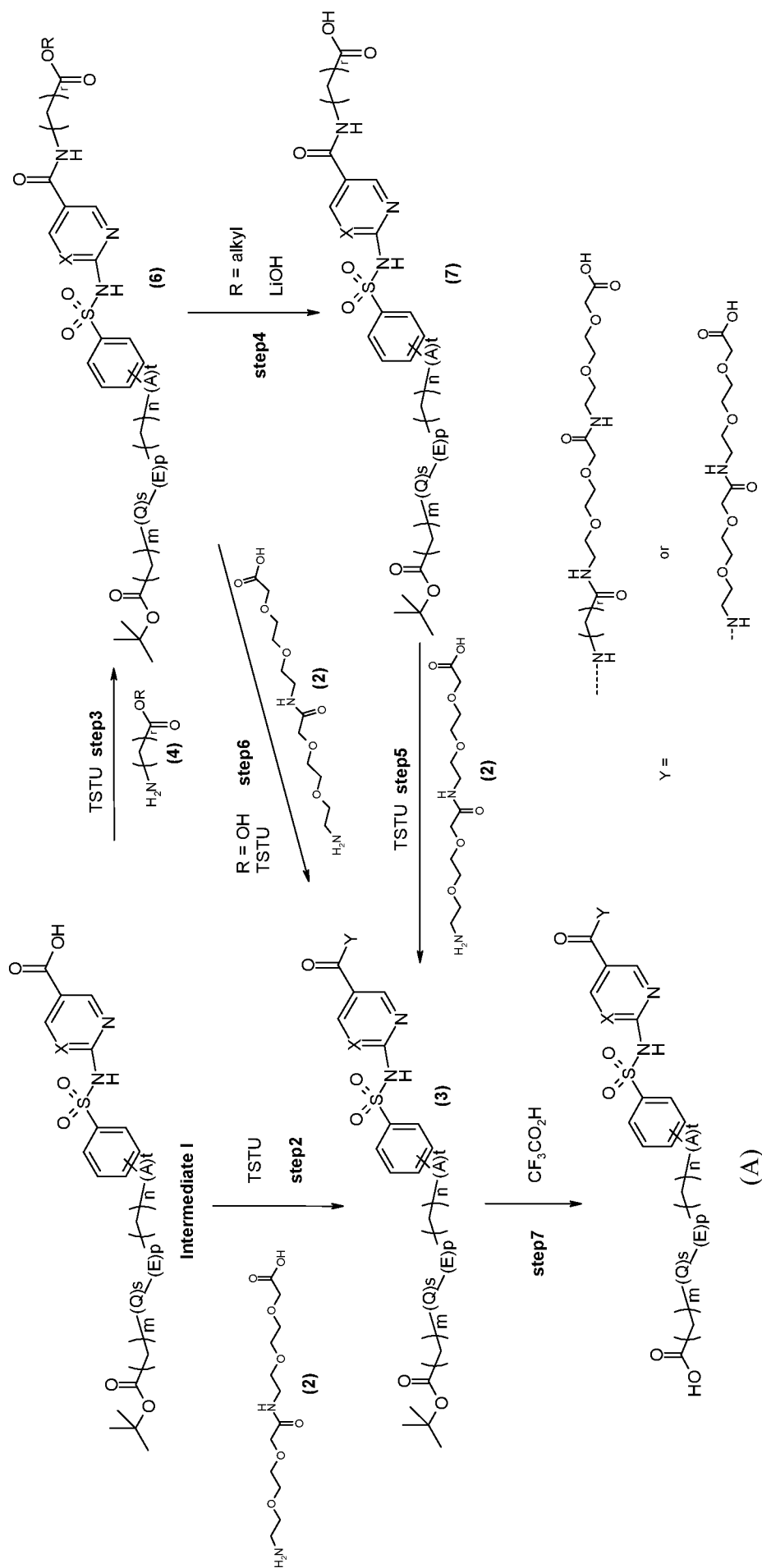
Ac	Acetyl
Boc	<i>tert</i> -Butyloxycarbonyl
DCM	Dichlormethane
DIAD	Diisopropyl azodicarboxylate
DIPEA	N,N-diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
EA	Ethyl acetate

HMPA	Hexamethylphosphoramide
HPLC	high performance liquid chromatography
LC	liquid chromatography
LCMS	liquid chromatography / mass spectrometry
MeCN	MeCN
NMM	N-methylmorpholine
NMP	N-methyl-2-pyrrolidone
PE	Petroleum ether
RP	reversed phase
RT	room temperature (25 °C)
TEA	Triethylamine
TEMPO	2,2,6,6-Tetramethylpiperidine-N-oxide
TFA	Trifluoroacetic acid
TFAA	Trifluoroacetic anhydride
TMS	Trimethyl silyl
Ts	Tosyl
TSTU	O-(N-Succinimidyl)-N,N,N,N-tetramethyluronium tetrafluoroborate

General processes suitable for preparing compounds of the formula (A) are described below. The compounds of the formula I were prepared by different chemical processes. The groups and indices mentioned in the following methods, especially in the schemes, have the abovementioned meaning indicated for formula (I) unless they are explicitly defined otherwise.

2. General synthesis of compounds of formula (A)

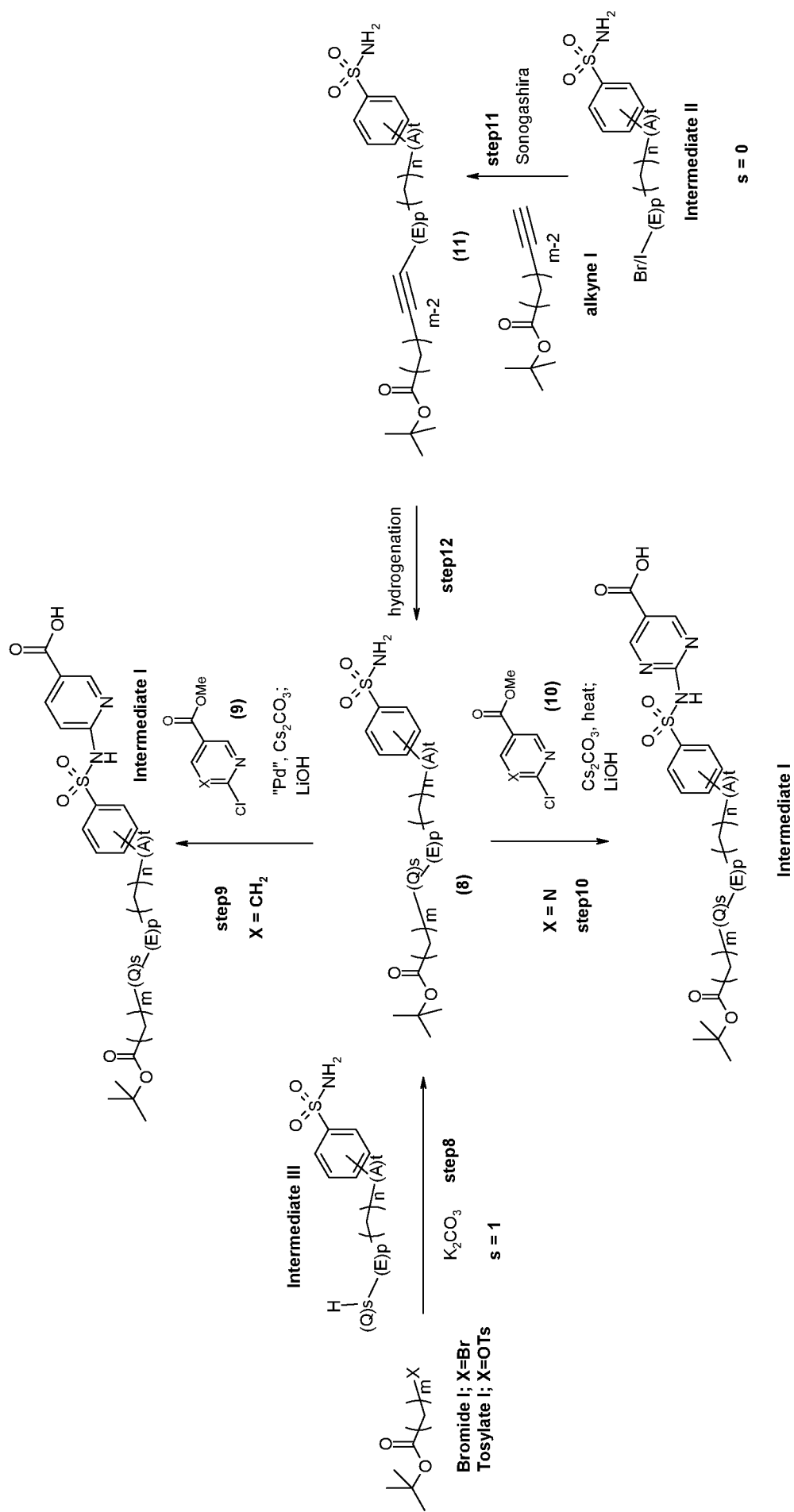
- Compounds of the formula (A) were synthesized starting from the corresponding intermediate I (scheme 1). After activation with TSTU the intermediate I was coupled either with amino acid (4) (step3) or compound (2) (step2) to give (3) and (6), respectively. In case in step3 an alkyl ester (R = alkyl) was utilized, saponification with LiOH was achieved. Both carboxylic acid (6) and (7) were activated with TSTU and coupled with (2) to yield in (3). To finish the synthesis of compounds of the formula (I), *tert*-Butyl ester of (3) was cleaved in the final step7 by treatment with CF₃CO₂H. The synthesis of intermediate I is shown in scheme 2.



Scheme 1

2.1 General synthesis of intermediate I

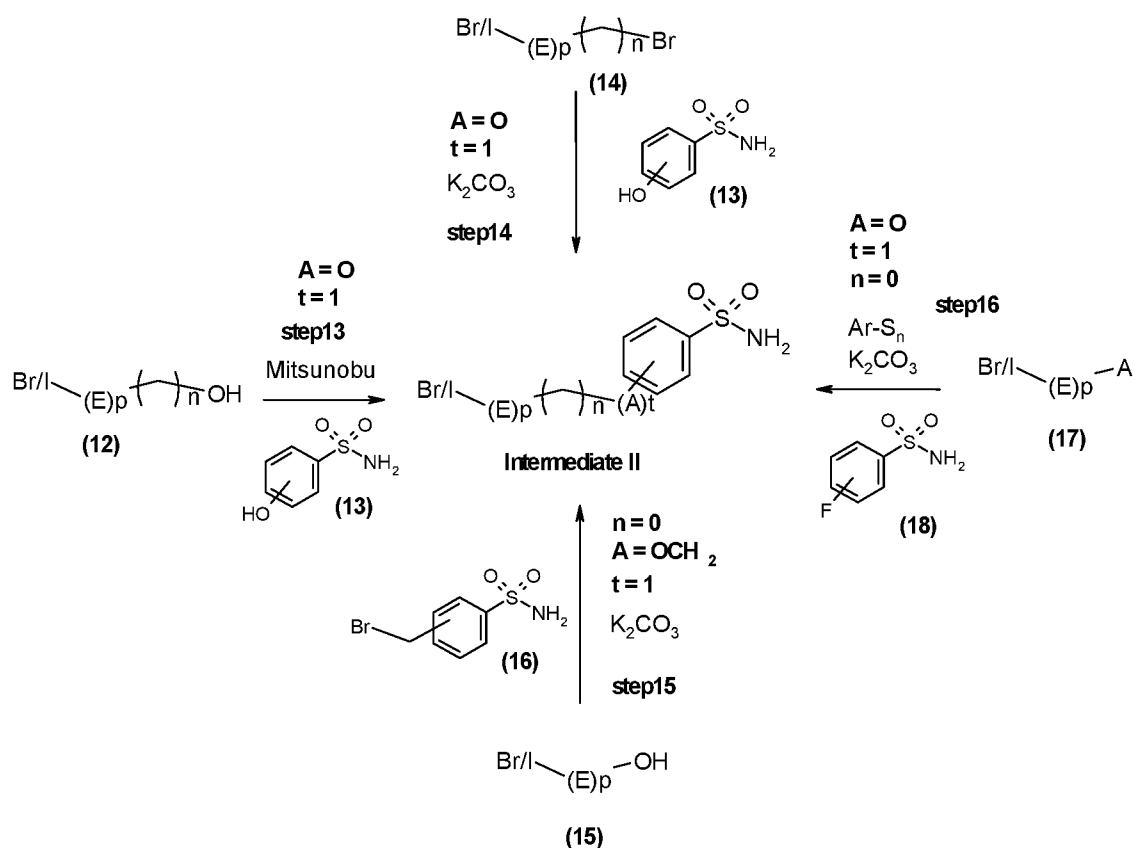
Intermediate I was synthesized as shown in scheme 2. Starting from Bromide I or
5 Tosylate I alkylation of intermediat III was achieved in the presence of K_2CO_3 (step
8). Alternatively (8) was isolated after a sequence of reactions starting with a
Sonogashira reaction of alkyne I and intermediate II (step 11) followed by a
hydrogenation of the resulting (11) under a hydrogen atmosphere catalyzed by
palladium and platinum, respectively (step 12). (8) was then condensed either with 2-
10 chloro pyridine (9) (step 9) in a palladium catalyzed reaction or or thermically
condensed with 2-chloro pyrimidine (10) (step 10). In both cases the alkyl ester was
subsequently hydrolyzed with LiOH to obtain the desired intermediate I.



Scheme 2

2.2 General synthesis of intermediate II

As shown in scheme 3, intermediate II was isolated after a Mitsunobu reaction of phenol (13) and alcohol (12) (step 13). Alternatively, intermediate II was synthesized via alkylation of either phenol (13) (step14) or phenol (15) (step 15) in the presence of K_2CO_3 . Suitable alkylating agents were (14) and (16), respectively. Nucleophilic aromatic substitution of fluorid (18) with phenol (17) also yielded in intermediate II (step16).

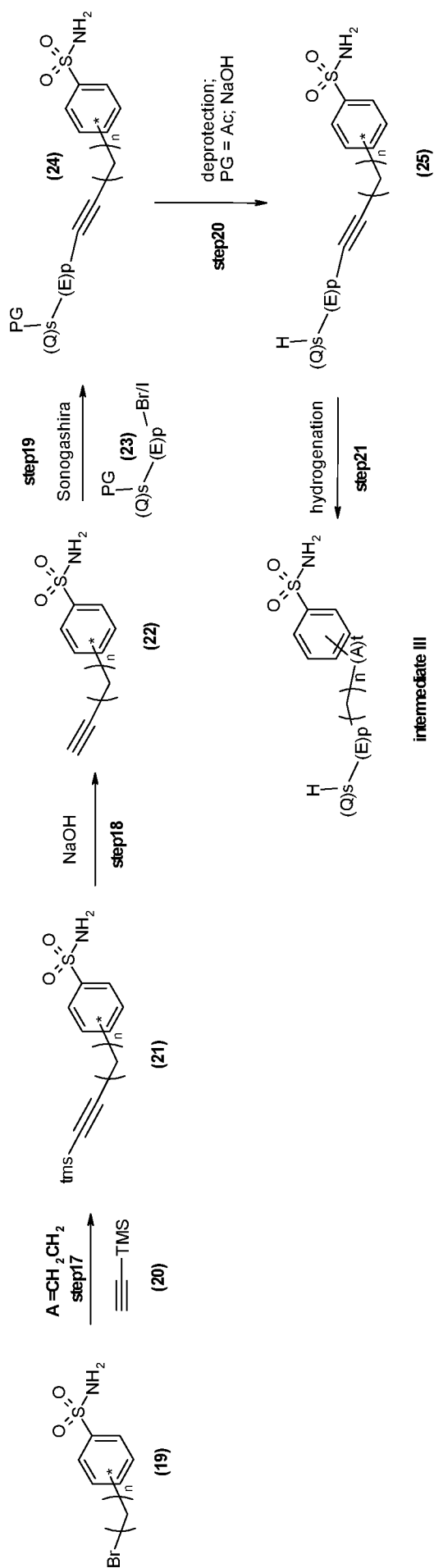


Scheme 3

2.3 General synthesis of intermediate III

Intermediate III was obtained after a linear reaction sequence as described in scheme 4. Starting with an alkylation of alkyne (20) with bromide (19) TMS protected alkyne (21) was isolated. (21) was deprotected under basic conditions using NaOH. Subsequent Sonogashira reaction of the isolated alkyne (22) with a corresponding aromatic halide (23) (step19) yielded in (24). A suitable protecting group for (24) was

for example acetyl (PG = Ac), which was cleaved upon treatment with NaOH (step20). The final hydrogenation step 21 was catalyzed by palladium or platinum under a H₂ atmosphere to provide the desired intermediate III.



Scheme 4

2.4 General synthesis of alkyne I and bromide I

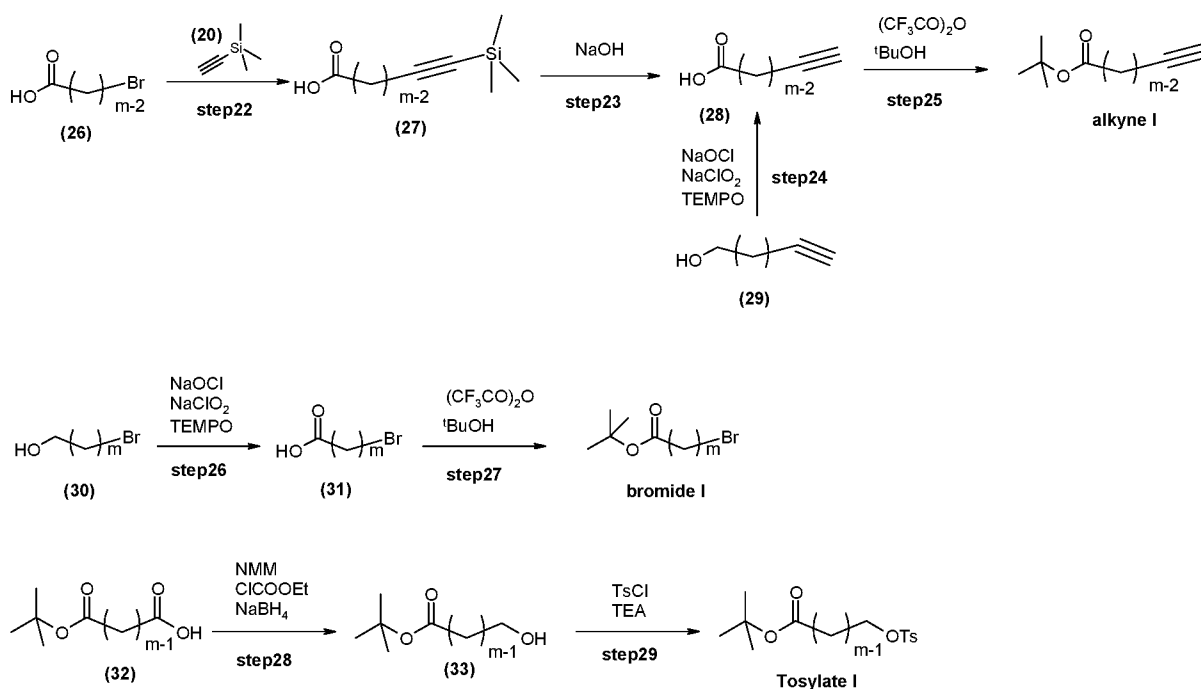
Starting materials bromide I and alkyne I were synthesized as shown in scheme 5.

For alkyne I two different synthetic routes were utilized. Carboxylic acid (28) was

either isolated after oxidation of alcohol (29) - the mentioned oxidation was achieved through a mixture of NaOCl and NaClO₂ in the presence of a catalytic amount of TEMPO (step24) – or by an alkylation / deprotection sequence of bromide (26). For the alkylation reagent (20) was used. The isolated product (27) was then treated with NaOH to cleave the TMS protecting group. The necessary protection of carboxylic acid (28) as a *tert*-Butyl ester to obtain desired alkyne I was achieved after activation with (CF₃CO)₂O and reaction with *tert*-ButylOH.

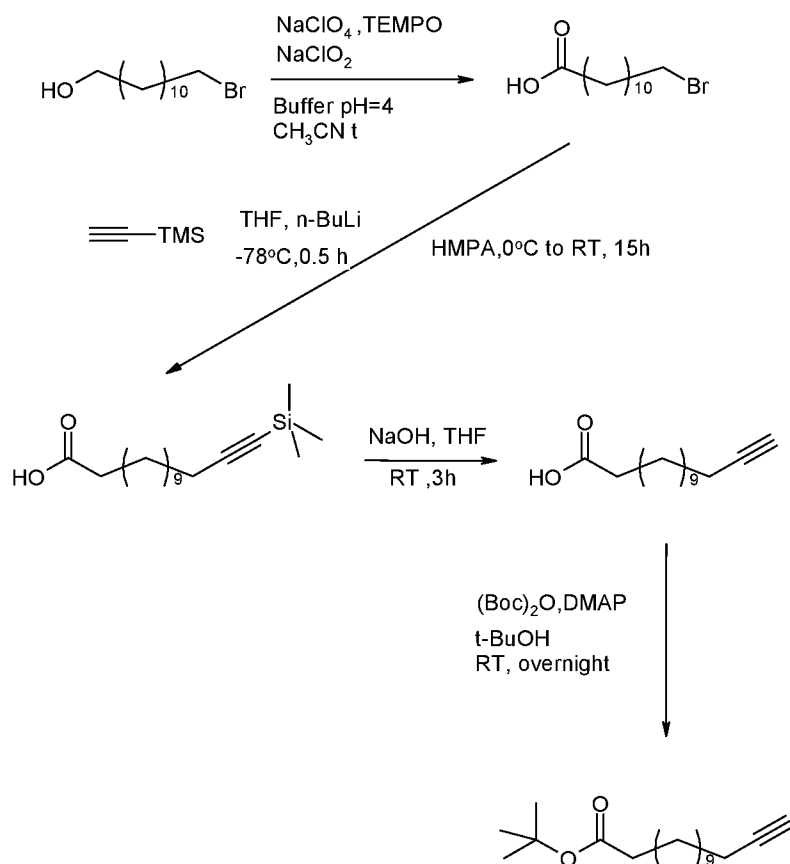
For the synthesis of bromide I a similar sequence as described for the conversion of (29) to alkyne I was used (step24 and 25). Oxidation of alcohol (30) and subsequent protection of the resulting carboxylic acid (31) yielded in the desired bromide I.

Tosylate I can be synthesized by a tosylation of the alcohol (33) (step29). (33) was isolated after a reduction of the carboxylic acid (32), which was in situ transferred into the mixed anhydride and subsequently reduced with NaBH₄ (step28).



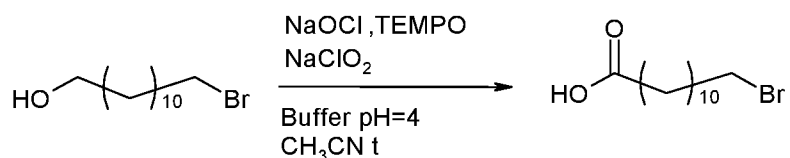
Scheme 5

2.5 Examples for the synthesis of alkynes I and bromides I according to scheme 5



5

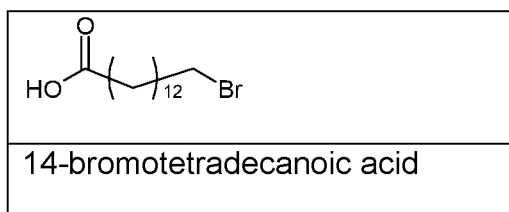
2.5.1 Synthesis of 12-bromododecanoic acid



To a solution of 12-Bromo-dodecan-1-ol (20g, 75.4 mmol) and TEMPO (5.9g, 37.7 mmol) in CH₃CN (400 ml) and pH 4-buffer solution (60 ml) was added a solution of NaClO₂ (37.5g, 414.8 mmol) in H₂O (60 ml) and a 10% solution of NaOCl (28g, 37.7mmol) simultaneously. The reaction mixture was stirred at RT overnight. The mixture was diluted with EA (1200 ml), washed with water (1000 ml) and brine, dried over Na₂SO₄, and concentrated under vacuum to afford the desired product 12-bromododecanoic acid (20 g, 71.6 mmol, yield, 95%) as a yellow solid.

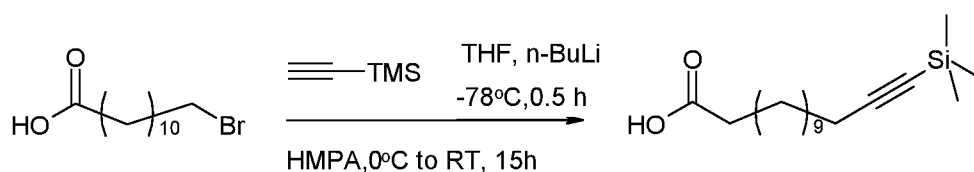
^1H NMR (400 MHz, DMSO) δ 11.96 (s, 1H), 3.52 (t, J = 6.6 Hz, 2H), 2.18 (t, J = 7.2 Hz, 2H), 1.85 – 1.72 (m, 2H), 1.55 – 1.43 (m, 2H), 1.37 (s, 2H), 1.21 (d, J = 32.6 Hz, 12H).

5 Following compounds were synthesized accordingly:



10

2.5.2 Synthesis of 14-(trimethylsilyl)tetradec-13-ynoic acid

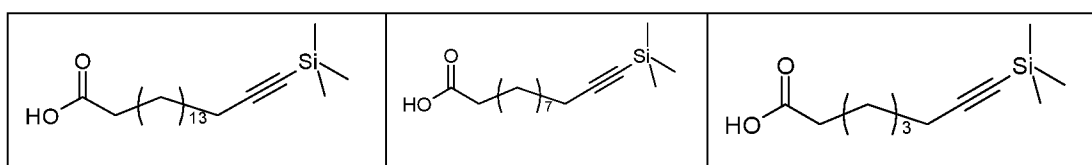


15

To a mixture of Ethynyl-trimethyl-silane (63.3g, 644.7 mmol) in THF (300 ml) was added n-BuLi (2.5M in hexane) (258 ml, 644.7mmol) at -78°C under N_2 , after 10 min , HMPA (115.5g, 644.7 mmol) was added and the mixture was warmed to 0°C for 30 min. Then 12-bromododecanoic acid (30g, 107.45 mmol) in THF (300 ml) was added. Then the mixture was stirred at RT overnight. Water (1200 ml) was added into the mixture slowly at 0°C , then pH value was adjusted to 3 with aqueous HCl solution, extracted with EA (800 ml). The organic phase was washed with brine, dried over Na_2SO_4 , concentrated under vacuum to afford the crude product 14-(trimethylsilyl)tetradec-13-ynoic acid (35g) as a brown oil and used for next step.

25

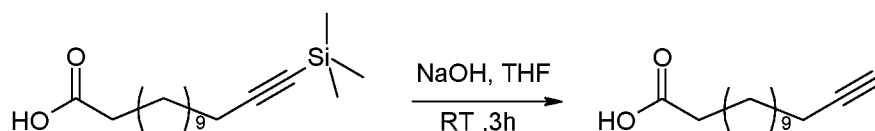
Following compounds were synthesized accordingly:



18-trimethylsilyloctadec-17-ynoic acid	12-trimethylsilyldodec-11-ynoic acid	8-trimethylsilyloct-7-ynoic acid
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5

2.5.3 Synthesis of tetradec-13-ynoic acid



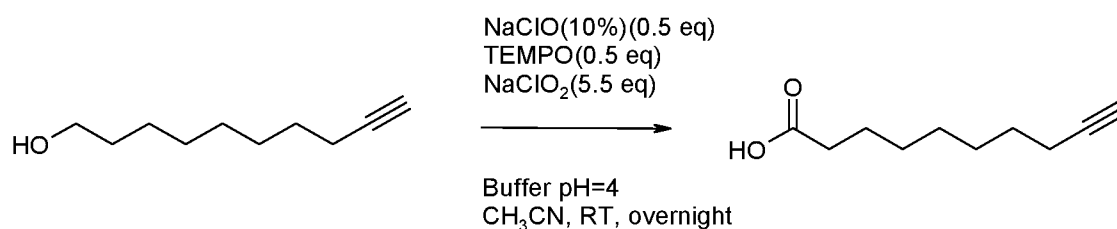
To a mixture of 14-(trimethylsilyl)tetradec-13-ynoic acid (35g, 107.45 mmol) in H₂O (150 ml) and THF (150 ml) was added NaOH (8.6g, 214.9 mmol). Then the mixture was stirred at RT for 3h. Then pH value was adjusted to 4 with aqueous HCl solution, extracted with EA (300 ml*2). The organic phases were washed with brine, dried over Na₂SO₄, concentrated under vacuum. The crude was purified by silica gel chromatography (PE:EA=4:1) to afford the desired product tetradec-13-ynoic acid (23 g, 102.5 mmol, 2 step yield: 95%) as a yellow solid.

¹H NMR (400 MHz, DMSO) δ 11.96 (s, 1H), 2.73 (s, 1H), 2.17 (dd, J = 16.3, 8.9 Hz, 4H), 1.51 – 1.21 (m, 18H).

20 Following compounds were synthesized accordingly:

octadec-17-ynoic acid	dodec-11-ynoic acid	oct-7-ynoic acid

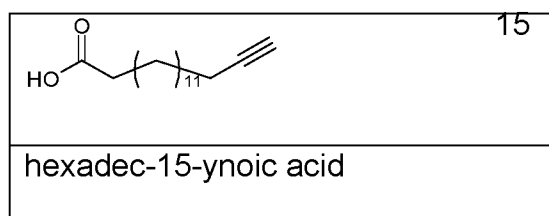
2.5.4 Synthesis of dec-9-ynoic acid



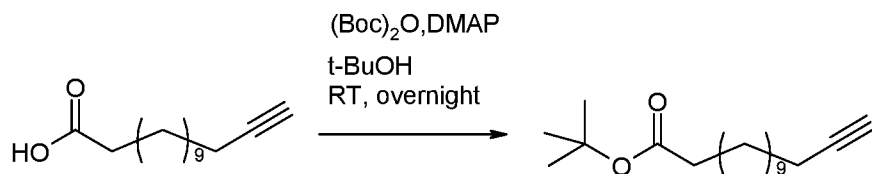
To a solution of dec-9-yn-1-ol (15 g, 97.4 mmol) and TEMPO (7.6 g, 48.7 mmol) in CH₃CN (300 ml) and pH 4-buffer solution (75 ml) was added a solution of NaClO₂ (48.2 g, 536 mmol) and NaOCl (36.0 g, 48.7 mmol) simultaneously. The reaction mixture was stirred at RT overnight, diluted with EA (900 ml), washed with water (900 ml) and brine, dried over Na₂SO₄, concentrated under vacuum. The crude was purified by silica gel chromatography (PE/EA = 1/1) to afford the desired dec-9-ynoic acid (20 g, crude) as a colourless oil.

¹H NMR (400 MHz, CDCl₃) δ 2.36 (t, J = 7.3 Hz, 2H), 2.18 (td, J = 6.9, 2.3 Hz, 2H), 1.93 (t, J = 2.3 Hz, 1H), 1.72 – 1.59 (m, 2H), 1.54 (td, J = 14.1, 7.2 Hz, 2H), 1.48 – 1.30 (m, 6H) ppm.

Following compounds were synthesized accordingly:



2.5.5 Synthesis of *tert*-butyl tetradec-13-ynoate



To a mixture of tetradec-13-ynoic acid (23g, 102.5 mmol) in t-BuOH (200 ml) was added (Boc)₂O (33.6g, 153.8 mmol) and DMAP (3.7g, 30.7 mmol). Then the mixture was stirred at RT overnight. The solvent was removed under vacuum. Water (400 ml) was added into the mixture, and extracted with EA (400 ml). The organic phase was washed with brine, dried over Na₂SO₄, filtered and concentrated. The residue was purified by silica gel chromatography (PE:EA=30:1) to give the desired product *tert*-butyl tetradec-13-ynoate (23.5g, 83.8 mmol, 82% yield) as a yellow liquid.

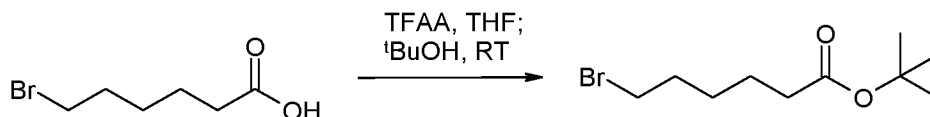
^1H NMR (400 MHz, DMSO) δ 2.72 (s, 1H), 2.15 (d, J = 8.4 Hz, 4H), 1.49 – 1.21 (m, 27H).

Following compounds were synthesized accordingly:

5

<i>tert</i> -butyl octadec-17-ynoate	<i>tert</i> -butyl dodec-11-ynoate	<i>tert</i> -butyl hexadec-15-ynoate
<i>tert</i> -butyl oct-7-ynoate	<i>tert</i> -butyl dec-9-ynoate	

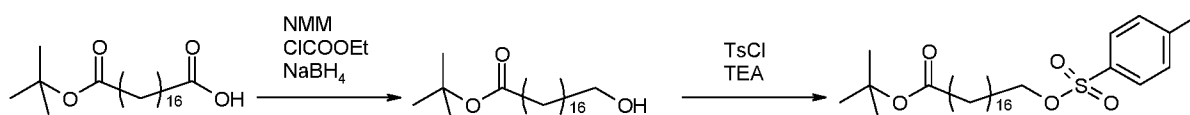
2.5.6 Synthesis of *tert*-butyl 6-bromohexanoate



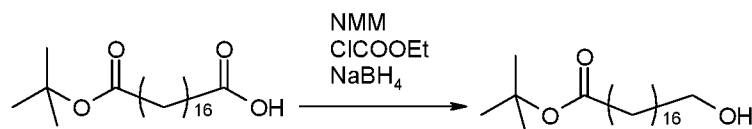
- 10 6-bromohexanoic acid (6.0 g, 31 mmol), TFAA (26.0 g, 124 mmol) was added to THF (60 ml), the mixture reacted at RT for 1 h. Then *tert*-ButylOH (30 ml) was added to the mixture, and stirred for 16 h at RT. Then the pH of reaction mixture was adapted to pH=8 with NaHCO_3 solution, the mixture was extracted with EA (150 ml*3), dried over Na_2SO_4 , concentrated to afford the target compound *tert*-butyl 6-bromohexanoate (7.6 g, 30.4 mmol, 98% yield).
- 15

^1H NMR (400 MHz, DMSO) δ 3.52 (t, J = 6.6 Hz, 2H), 2.20 (dd, J = 15.0, 7.8 Hz, 2H), 1.85 – 1.74 (m, 2H), 1.52 (ddd, J = 19.3, 10.9, 5.7 Hz, 2H), 1.44 – 1.32 (m, 9H).

20 2.5.6 Synthesis of tosylates I



2.5.7 Synthesis of tert-butyl 18-hydroxyoctadecanoate



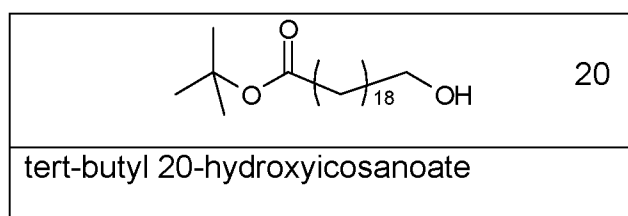
To a solution of 18-tert-butoxy-18-oxooctadecanoic acid (5 g, 13.5 mmol) in THF (150 ml) was added N-methylmorpholine (1638 mg, 16.5 mmol). The mixture was cooled to -25°C before adding ethyl chloroformate (1277 mg, 13.5 mmol) dropwise.

The mixture was stirred at -25°C for 20 minutes and the solid was removed by filtration. The solution was carefully added to a solution of NaBH₄ (770 mg, 20.25 mmol) in water (15 mL) at 0°C. The mixture was stirred for 1 hour at room

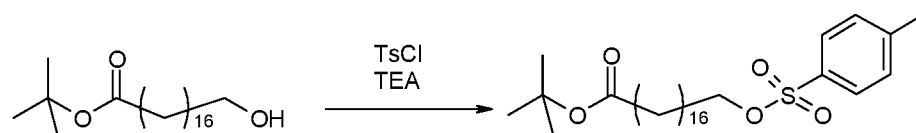
temperature. THF was removed under vacuum and the aqueous phase was extracted with EA (3 x 50 mL). The combined organic phases were dried over MgSO₄ and concentrated under vacuum to give tert-butyl 18-hydroxyoctadecanoate as a white solid (4.7g, 99.8% yield).

¹H NMR (400 MHz, CDCl₃) δ 3.63 (t, J = 6.6 Hz, 2H), 2.19 (t, J = 7.5 Hz, 2H), 1.57 (dd, J = 13.0, 6.5 Hz, 4H), 1.43 (d, J = 3.9 Hz, 9H), 1.38 – 1.20 (m, 27H).

Following compounds were synthesized accordingly:



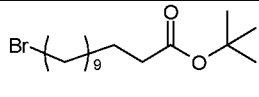
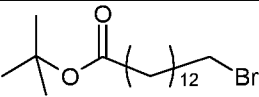
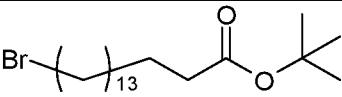
2.5.8 Synthesis of tert-butyl 18-(p-tolylsulfonyloxy)octadecanoate



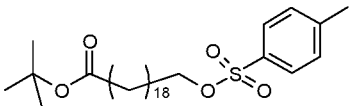
To a solution of tert-butyl 18-hydroxyoctadecanoate (4700 mg, 13.2 mmol) and TsCl (2508 mg, 13.2 mmol) in DCM (100 mL) was added TEA (400 mg, 39.6 mmol). The reaction mixture was stirred at room temperature overnight. Water (50 mL) was

added, and extracted with DCM (50 mL *2). The combined organic phase was washed with brine (100 mL), dried over Na₂SO₄, filtrated and concentrated. The crude was purified by silica gel column (EA/n-hexane = 1:20) to afford tert-butyl 18-(p-tolylsulfonyloxy)octadecanoate (4.5g, 67% yield).

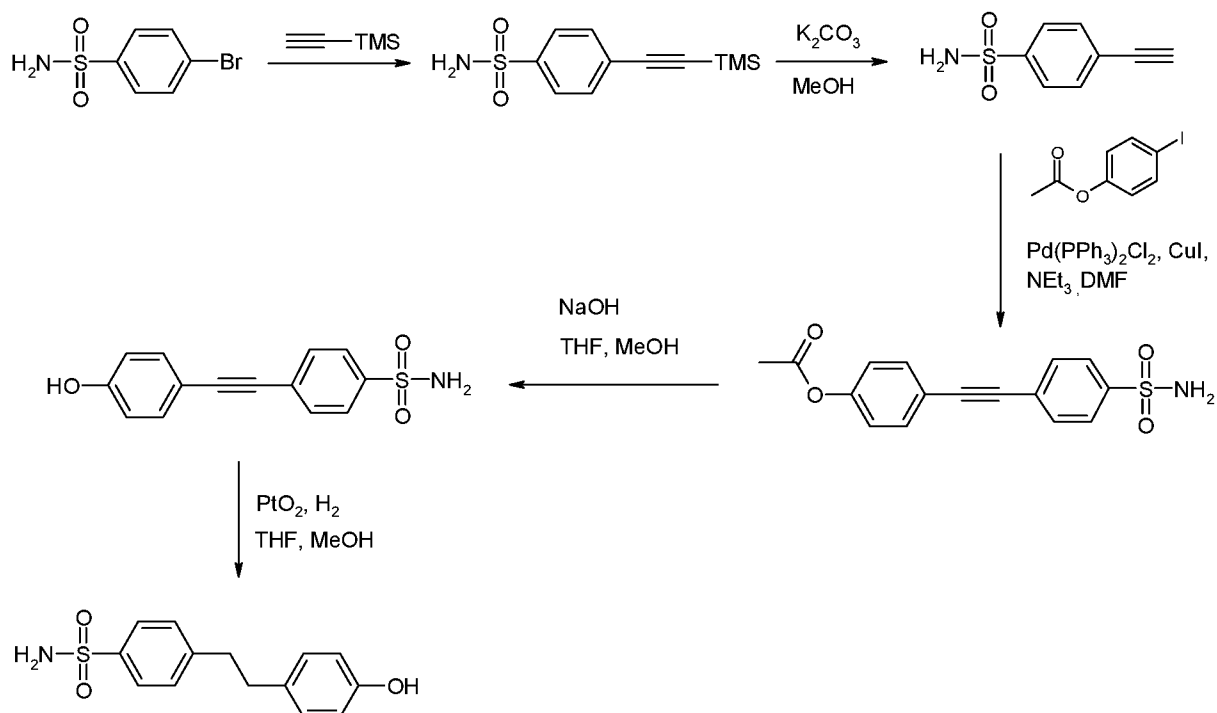
- 5 ¹H NMR (400 MHz, CDCl₃) δ 7.79 (d, J = 8.2 Hz, 2H), 7.34 (d, J = 8.1 Hz, 2H), 4.02 (t, J = 6.5 Hz, 2H), 2.45 (s, 3H), 2.20 (t, J = 7.5 Hz, 2H), 1.69 – 1.57 (m, 4H), 1.44 (s, 9H), 1.25 (t, J = 12.1 Hz, 24H).

		
<i>tert</i> -butyl 12-bromododecanoate	<i>tert</i> -butyl 14-bromotetradecanoate	<i>tert</i> -butyl 16-bromohexadecanoate

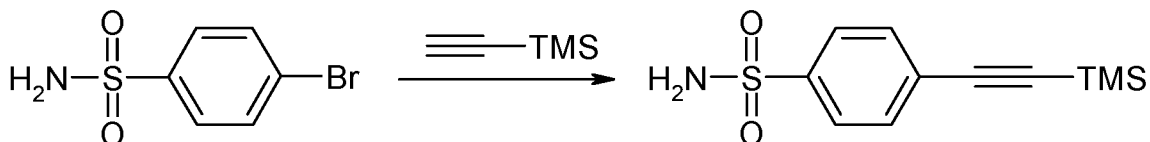
- 10 Following compounds were synthesized accordingly:


tert-butyl 20-(p-tolylsulfonyloxy)icosanoate
15

2.6 Examples for the synthesis of intermediates III according to scheme 4



2.6.1 Synthesis of 4-((trimethylsilyl)ethynyl)benzenesulfonamide



5

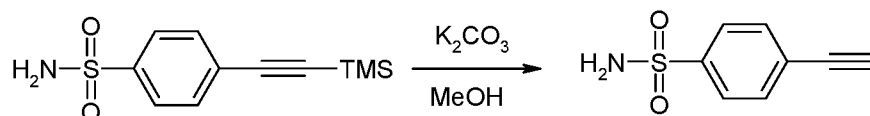
A mixture of 4-bromobenzenesulfonamide (61 g, 260 mmol), trimethylsilylacetylene (38.2 g, 0.09 mol), tetrakis(triphenylphosphine) palladium (7.5 g, 6.5 mmol) and copper iodide (2.5 g, 13 mmol) in triethylamine (500 ml) was heated to 80 °C under a nitrogen atmosphere for 8 h. The mixture was concentrated *in vacuo* and extracted with EA (300 ml). The combined organic layers were dried (Na₂SO₄) and concentrated under reduced pressure. The crude was purified by silica gel chromatography (eluting with 70% DCM in PE) to afford 4-((trimethylsilyl)ethynyl)benzenesulfonamide (50 g, 75 %).

10

15 LC-Mass Method: Mobile phase: A = 10 mM TFA/H₂O, B = MeCN; Gradient: B = 5 % - 95 % in 1.5 min; Flow rate: 2.0 ml/min; Column: Xbridge-C₁₈, 50 × 4.6mm, 3.5 μm. LC purity: 90 % (214 nm); Mass: find peak 254.0 (M + H)⁺ at 1.98 min.

2.6.2 Synthesis of 4-ethynylbenzenesulfonamide

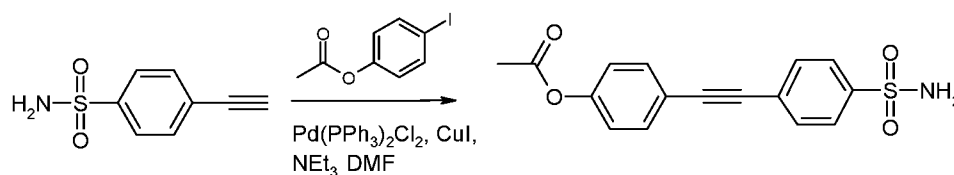
20



4-((trimethylsilyl)ethynyl)benzenesulfonamide (40 g, 158 mmol), K_2CO_3 (2.2 g, 15.8 mmol), and methanol (400 ml) were stirred at rt for 12h. After the reaction was completed (monitored by LCMS), diluted with water (200 ml), and extracted with EA (2x200 ml). The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. The crude was purified by silica gel chromatography (eluting with 100% DCM in PE) to afford 4-ethynylbenzenesulfonamide (22 g, 77 %).

LC-Mass Method: Mobile phase: A = 10 mM TFA/ H_2O , B = MeCN; Gradient: B = 5 % - 95 % in 1.5 min; Flow rate: 2.0 ml/min; Column: Xbridge- C_{18} , 50×4.6 mm, 3.5 μ m. LC purity: 90 % (214 nm); Mass: find peak 182.1 ($M + H$)⁺ at 1.65 min.

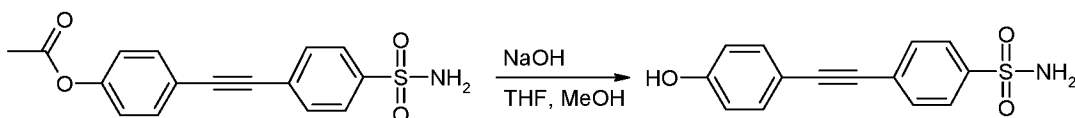
2.6.3 Synthesis of 4-((4-sulfamoylphenyl)ethynyl)phenyl acetate



To a mixture of 4-ethynylbenzenesulfonamide (15 g, 83 mmol) in DMF (150 ml) was added $Pd(PPh_3)_2Cl_2$ (5.8 g, 8.3 mmol), CuI (1.6 g, 8.3 mmol), Et_3N (25 g, 249 mmol) and (4-iodophenyl) acetate (27 g, 103 mmol). The flask was evacuated and backfilled with N_2 . Then the mixture was stirred at RT overnight. Water (200 ml) was added into the mixture, suction filtration and drying in air provides 4-((4-sulfamoylphenyl)ethynyl)phenyl acetate as brown solid (18 g, 70 %).

LC-Mass Method: Mobile phase: A = 10 mM TFA/ H_2O , B = MeCN; Gradient: B = 5 % - 95 % in 1.5 min; Flow rate: 2.0 ml/min; Column: Xbridge- C_{18} , 50×4.6 mm, 3.5 μ m. LC purity: 90 % (214 nm); Mass: find peak 338 ($M + Na$)⁺ at 1.88 min.

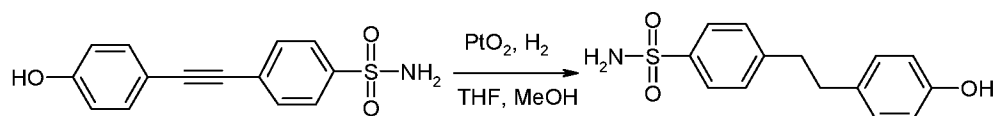
2.6.4 Synthesis of 4-((4-hydroxyphenyl)ethynyl)benzenesulfonamide



To a solution of 4-((4-sulfamoylphenyl)ethynyl)phenyl acetate (18 g, 57 mmol) in THF (60 ml), MeOH (60 ml) and H₂O (30 ml) was added NaOH (4.5 g, 114 mmol) at 0 °C. The mixture was stirred at RT for 2 h. After the reaction was completed (monitored by LCMS), upon the solution was diluted with EA (50 ml) and washed with water (20 ml), and saturated aqueous NaCl, dried over MgSO₄. The filtrate was concentrated in vacuo to provide crude product. The crude product was slurried with DCM. Suction filtration and drying in air provides 4-((4-hydroxyphenyl) ethynyl)benzenesulfonamide as brown solid (10.9 g, 70 %).

LC-Mass Method: Mobile phase: A = 10 mM TFA/H₂O, B = MeCN; Gradient: B = 5 % - 95 % in 1.5 min; Flow rate: 2.0 ml/min; Column: Xbridge-C₁₈, 50 × 4.6mm, 3.5 um. LC purity: 95 % (214 nm); Mass: find peak 296.1 (M + Na)⁺ at 1.75 min.

2.6.5 Synthesis of 4-(4-hydroxyphenethyl)benzenesulfonamide



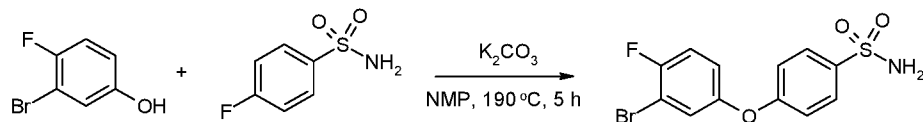
To a solution of 4-((4-hydroxyphenyl)ethynyl)benzenesulfonamide (10.9 g, 40 mmol) in 40 ml of THF and 40 ml of MeOH was added PtO₂ (1 g). The reaction mixture was stirred at RT under H₂ for 24h. After the reaction was completed (monitored by LCMS), the mixture was then filtered. The filtrate was concentrated in vacuo to provide 4-(4-hydroxyphenethyl)benzenesulfonamide (9.5 g, 86 %).

LC-Mass Method: Mobile phase: A = 10 mM TFA/H₂O, B = MeCN; Gradient: B = 5 % - 95 % in 1.5 min; Flow rate: 2.0 ml/min; Column: Xbridge-C₁₈, 50 × 4.6mm, 3.5 um. LC purity: 100 % (214 nm); Mass: find peak 278.1 (M + H)⁺ at 1.67 min.

¹H NMR (400 MHz, DMSO) δ 9.14 (s, 1H), 7.71 (d, *J* = 8.3 Hz, 2H), 7.38 (d, *J* = 8.3 Hz, 2H), 7.26 (s, 2H), 7.00 (d, *J* = 8.4 Hz, 2H), 6.72 – 6.60 (m, 2H), 2.96 – 2.84 (dd, *J* = 9.2, 6.2 Hz, 2H), 2.77 (dd, *J* = 9.2, 6.3 Hz, 2H).

2.7 Examples for the synthesis of intermediates II according to scheme 3

2.7.1 Synthesis of 4-(3-bromo-4-fluorophenoxy)benzenesulfonamide

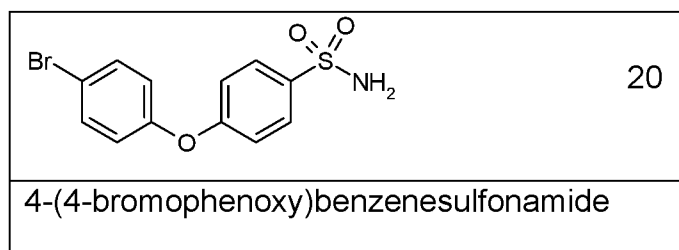


- 5 A mixture of 3-bromo-4-fluoro-phenol (12.8 g, 66.8 mmol), 4-fluorobenzene sulfonamide (9.00 g, 51.4 mmol) and K_2CO_3 (14.2 g, 103 mmol) in NMP (50 ml) was stirred at 190 °C for 5 h. The reaction mixture was diluted with EA (500 ml), washed with water (50 ml), brine (50 ml*3), dried over Na_2SO_4 , filtered and concentrated. The residue was purified by flash chromatography on silica gel (eluting with PE/EA = 3/1)
- 10 to afford 4-(3-bromo-4-fluorophenoxy)benzenesulfonamide as a white solid (10.8 g, 31.3 mmol, 61% yield).

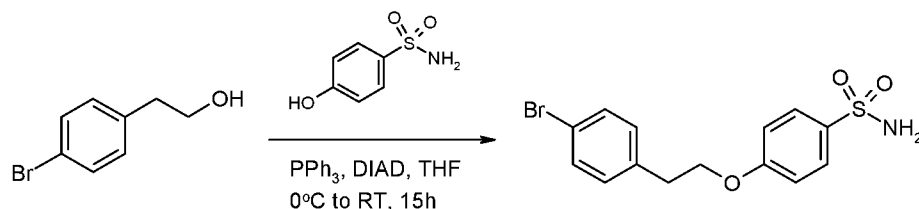
LC-Mass Method: Mobile phase: A = 2.5mM TFA/ H_2O , B = 2.5mM TFA/MeCN;

- Gradient: B = 10%-95% in 1.0 min; Flow rate: 1.5 ml/min; Column: Xbridge- C_{18} , 30 × 4.6mm, 2.5 μm . LC (desired product) purity: 88% (214 nm); Mass: find peak 368.0 (M + Na) $^+$ at 1.74 min.
- 15

Following compounds were synthesized accordingly:



25 2.7.2 Synthesis of 4-(4-bromophenethoxy)benzenesulfonamide

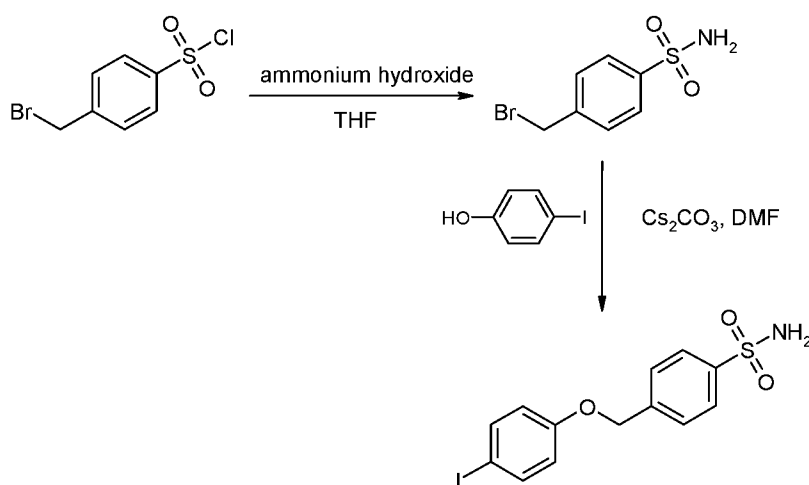


- To a solution of 2-(4-bromophenyl)ethanol (10 g, 49.8 mmol), 4-hydroxybenzenesulfonamide (8.6 g, 49.8 mmol) and PPh_3 (14.3 g, 54.795 mmol) in dry THF (200 ml) was added DIAD (11.1 g, 54.7 mmol) at 0°C dropwise. The reaction was allowed to
- 30 warm to RT with stirring for 20h. The solvent was removed under reduced pressure

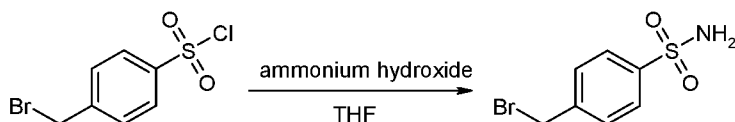
and the residue was dissolved in EA (200 ml) and then washed with water (50 ml) and brine (50 ml). The organic phase dried over Na₂SO₄. After filtration, the solvent was removed under reduced pressure and the residue was purified by column chromatography (silica gel, eluting with EA in PE from 0 to 40%) to obtain 4-(4-bromophenethoxy)benzenesulfonamide (6.8 g as white solid) in 39% yield.

LC-Mass Method: Mobile phase: H₂O (0.01%TFA (A) / MeCN (0.01%TFA), (B); Gradient: 5 % B for 0.2 min, increase to 95%B within 1.3 min; Flow rate: 1.8 ml/min; Column: SunFire, 50 × 4.6mm, 3.5 μm. LC purity: 95% (214 nm); Mass: find peak 356 (M + H)⁺ at 2.08 min

2.7.3 Synthesis scheme 4-((4-iodophenoxy)methyl)benzenesulfonamide



2.7.4 Synthesis of 4-(bromomethyl)benzenesulfonamide

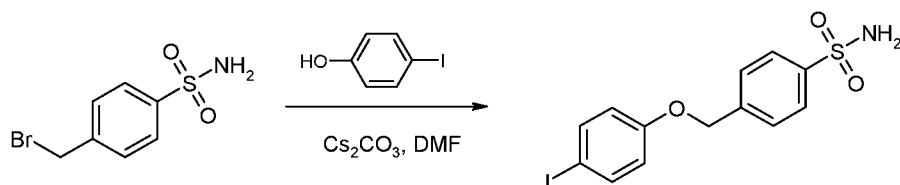


A solution of 4-(bromomethyl)benzenesulfonyl chloride (7 g, 26 mmol) in THF (80 ml) was cooled to 0°C, 28% aqueous ammonia (6.5 ml) was added thereto and the mixture was stirred at RT for 2 h. The reaction solution was concentrated and ethyl acetate (200 ml) was added. The organic layer was separated, dried and concentrated. The crude 4-(bromomethyl)benzenesulfonamide was used directly without further purification. (5.5 g, 86 %)

LC-Mass Method: Mobile phase: A = 10 mM TFA/H₂O, B = MeCN; Gradient: B = 5 % - 95 % in 1.5 min; Flow rate: 2.0 ml/min; Column: Xbridge-C₁₈, 50 × 4.6mm, 3.5 μm. LC purity: 90 % (214 nm); Mass: find peak 250.1 (M + H)⁺ at 1.64 min.

5

2.7.5 Synthesis of 4-((4-iodophenoxy)methyl)benzenesulfonamide

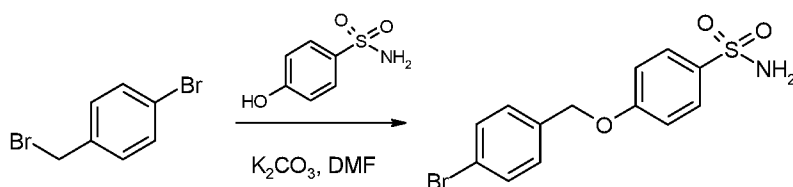


To a mixture of 4-(bromomethyl)benzenesulfonamide (5.5 g, 22 mmol) in DMF (50 ml) was added Cs₂CO₃ (10.7 g, 33 mmol) and 4-iodophenol (6 g, 27.5 mmol). Then the mixture was stirred at RT for 12h. Water (200 ml) was added into the mixture, the resulting solid filtered, and then slurried with Et₂O (50 ml); suction filtration and drying in air provides the desired product as a white solid (5.5 g, 65 %).

LC-Mass Method: Method: Mobile phase: A = 10 mM TFA/H₂O, B = MeCN; Gradient: B = 5 % - 95 % in 1.5 min; Flow rate: 1.8 ml/min; Column: Xbridge-C₁₈, 50 x 4.6mm, 3.5 μm. LC purity: 80% (214 nm); Mass: find peak 389.7 (M+H)⁺ at 1.98 min.

2.7.6 Synthesis of 4-(4-bromobenzyloxy)benzenesulfonamide

20

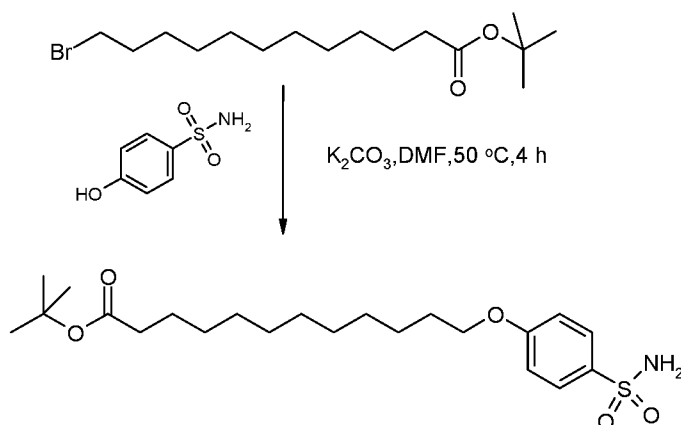


To a mixture of 1-bromo-4-(bromomethyl)benzene (6.5 g, 26 mmol) in DMF (50 ml) was added K₂CO₃ (5.5 g, 40 mmol), and 4-hydroxybenzenesulfonamide (4.5 g, 26 mmol). Then the mixture was stirred at 50°C for 2h. Water (200 ml) was added into the mixture, the solid was filtered. Then the solid was slurried with PE:EA=1:2 (50 ml), suction filtration and drying in air provides the desired product as a white solid. (5.3 g, 60 %).

LC-Mass Method: Method: Mobile phase: A = 10 mM TFA/H₂O, B = MeCN; Gradient: B = 5 % - 95 % in 1.5 min; Flow rate: 1.8 ml/min; Column: Xbridge-C₁₈, 50 × 4.6mm, 3.5 um. LC purity: 80% (214 nm); Mass: find peak 364 (M+Na)⁺ at 1.81min.

5 2.8 Examples for the synthesis of intermediates I according to scheme 2

2.8.1 Synthesis of *tert*-butyl 12-(4-sulfamoylphenoxy)dodecanoate

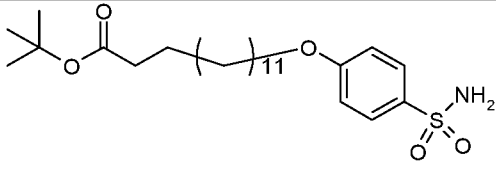
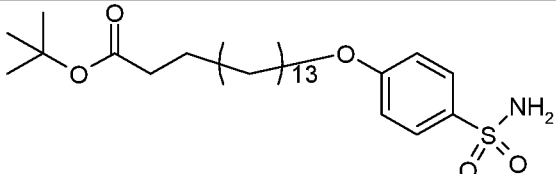
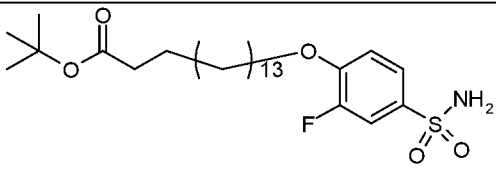
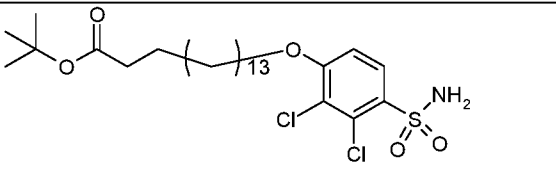
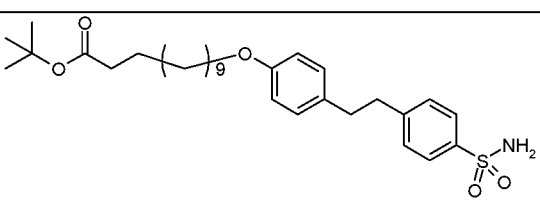
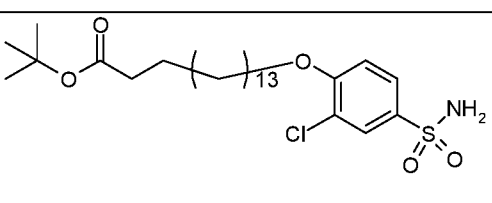
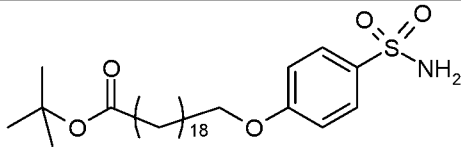
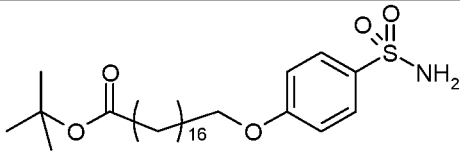
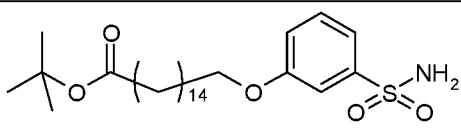
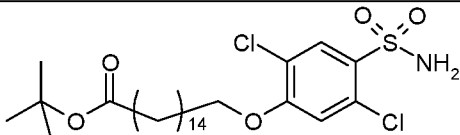


- 10 A mixture of *tert*-butyl 12-bromododecanoate (6 g, 18 mmol), 4-hydroxybenzene sulfonamide (3g, 18 mmol) and K₂CO₃ (5 g, 36 mmol) in DMF (50 ml) was heated to 50 °C and stirred for 4 h. Then water (300 ml) was added. The resulting precipitate was collected and dried to give the crude *tert*-butyl 12-(4-sulfamoylphenoxy)dodecanoate, which was slurried with EA/PE (1/5, 100 ml) to yield
- 15 7 g (93%) of 12-(4-sulfamoylphenoxy) dodecanoate:

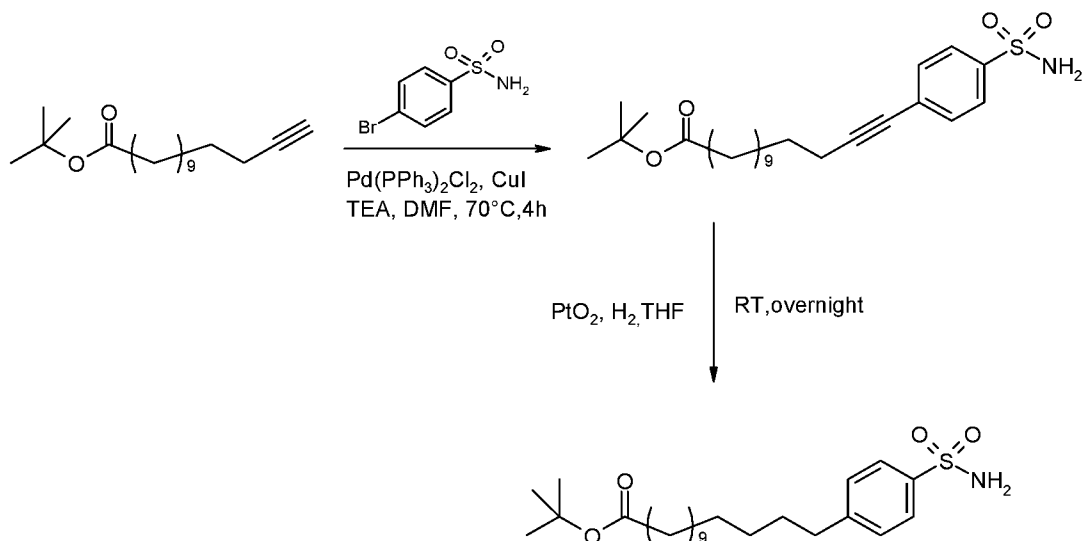
LC-Mass Method: Mobile phase: A: water (0.01%TFA) B: MeCN (0.01%TFA). Gradient: 5%B for 0.2min,increase to 95%B within 1.3min,95%B for 1.5min,back to 5%B within 0.01min; Flow Rate :1.8ml/min; Column :Sunfire, 50*4.6mm,3.5um Column Temperature:50 °C. LC-MS purity: 100% (214 nm); Mass: find peak 450.2 (M +Na)⁺ at 2.23 min.

¹H NMR (400 MHz, CDCl₃) δ 7.83 (t, J = 14.8 Hz, 2H), 6.96 (d, J = 8.8 Hz, 2H), 4.89 (s, 2H), 4.03 (dt, J = 13.0, 6.6 Hz, 2H), 2.20 (t, J = 7.5 Hz, 2H), 1.73-1.80 (m, 2H), 1.50-1.57 (m, 2H), 1.40-1.48 (m, 11H), 1.37 – 1.19 (m, 12H).

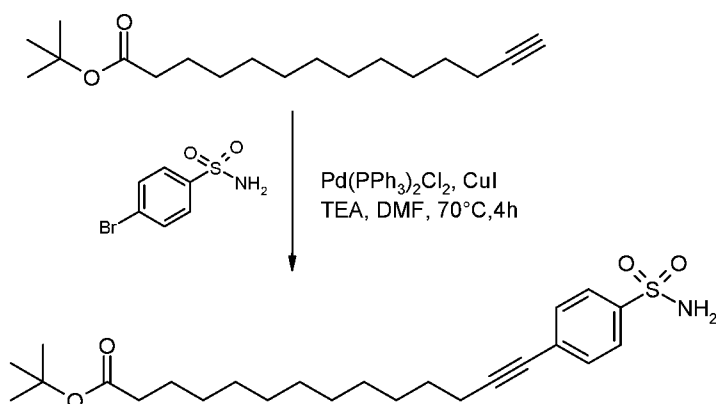
- 25 Following compounds were synthesized accordingly:

	
<i>tert</i> -butyl 14-(4-sulfamoylphenoxy)tetradecanoate	<i>tert</i> -butyl 16-(4-sulfamoylphenoxy)hexadecanoate
	
<i>tert</i> -butyl 16-(2-fluoro-4-sulfamoylphenoxy) hexadecanoate	<i>tert</i> -butyl 16-(2,3-dichloro-4-sulfamoylphenoxy) hexadecanoate
	
<i>tert</i> -butyl 12-[4-[2-(4-sulfamoylphenyl)ethyl]phenoxy]dodecanoate	<i>tert</i> -butyl 16-(2-chloro-4-sulfamoylphenoxy) hexadecanoate
	
<i>tert</i> -butyl 20-(4-sulfamoylphenoxy)icosanoate	<i>tert</i> -butyl 18-(4-sulfamoylphenoxy)octadecanoate
	
<i>tert</i> -butyl 16-(3-sulfamoylphenoxy)hexadecanoate	<i>tert</i> -butyl 16-(2,5-dichloro-4-sulfamoylphenoxy)hexadecanoate

Synthetic scheme: Synthesis of 14-(4-sulfamoylphenyl)tetradecanoate



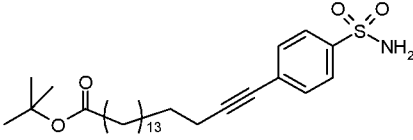
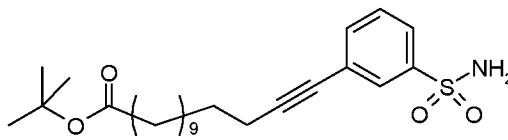
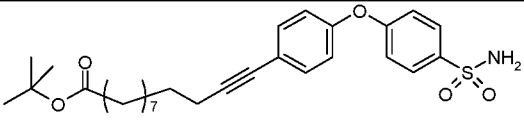
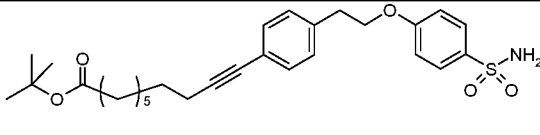
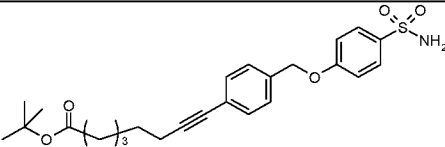
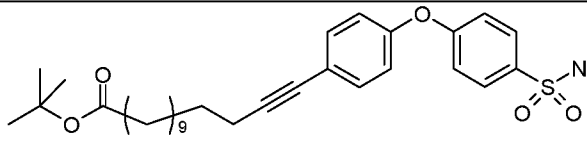
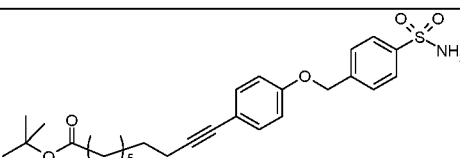
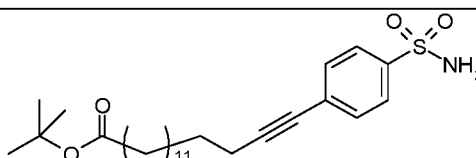
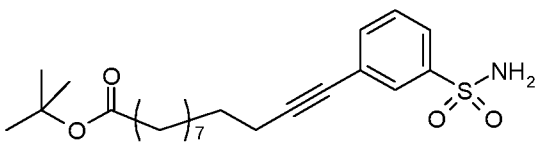
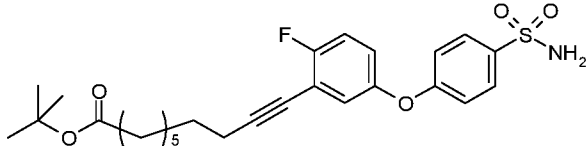
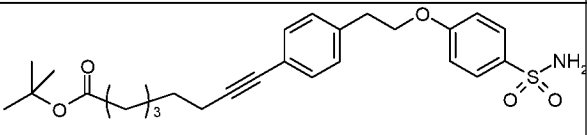
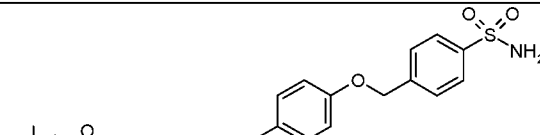
2.8.2 Synthesis of *tert*-butyl 14-(4-sulfamoylphenyl)tetradec-13-ynoate

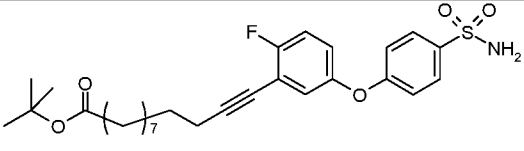
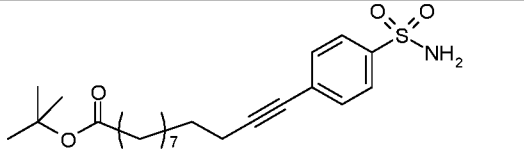
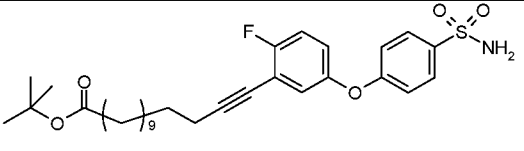
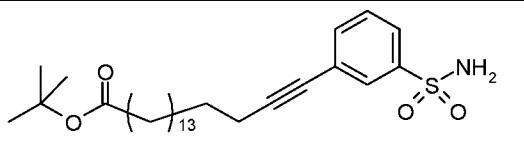
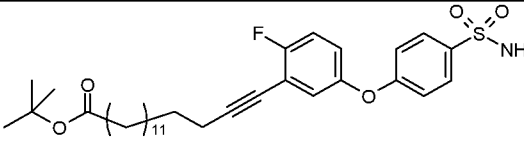
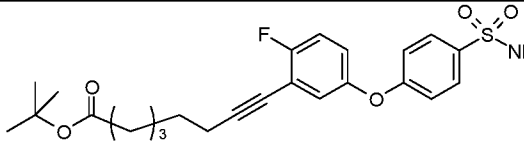
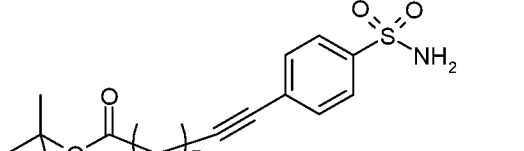


To a mixture of 4-bromobenzenesulfonamide (1.6g, 6.8 mmol) in DMF (20 ml) was added $\text{Pd(PPh}_3)_2\text{Cl}_2$ (0.47g, 0.68mmol), CuI (0.13g, 0.68 mmol), Et_3N (2g, 20.33mmol) and *tert*-butyl tetradec-13-ynoate (2.2g, 7.8 mmol). The flask was evacuated and backfilled with N_2 . Then the mixture was stirred at 70°C for 4h. Water (80ml) was added into the mixture, extracted by EA (80 ml*2). The combined organic phase was washed with brine, dried over Na_2SO_4 , concentrated under the vacuum. The crude was purified by silica gel chromatography (PE:EA=4:1) to give *tert*-butyl 14-(4-sulfamoylphenyl)tetradec-13-ynoate (2.2g, 5.05 mmol, yield:76%) as a yellow solid.

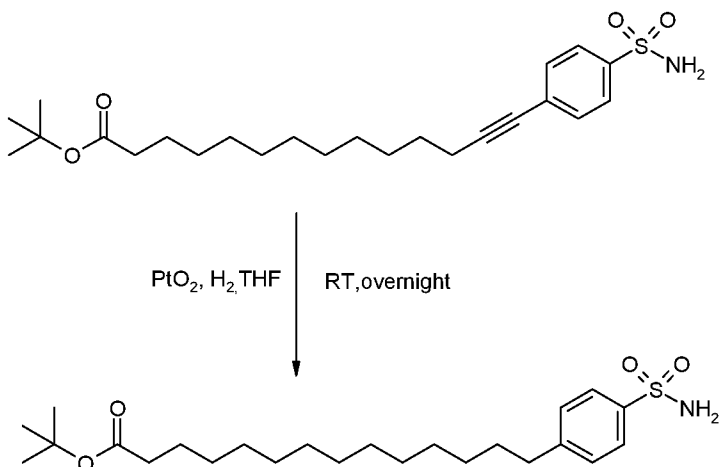
LC-Mass Method: Method: Mobile phase: A = 10 mM TFA/ H_2O , B = MeCN; Gradient: B = 5 % - 95 % in 1.5 min; Flow rate: 1.8 ml/min; Column: Xbridge- C_{18} , 50 x 4.6mm, 3.5 μm . LC purity: 98% (214 nm); Mass: find peak 458 ($\text{M}+\text{H}$)⁺ at 2.37min.

Following compounds were synthesized accordingly:

	
<i>tert</i> -butyl 18-(4-sulfamoylphenyl) octadec-17-ynoate	<i>tert</i> -butyl 14-(3-sulfamoylphenyl) tetradec-13-ynoate
	
<i>tert</i> -butyl 12-[4-(4-sulfamoylphenoxy) phenyl]dodec-11-ynoate	<i>tert</i> -butyl 10-[4-[2-(4-sulfamoyl-phenoxy) ethyl]phenyl]dec-9-ynoate
	
<i>tert</i> -butyl 8-[4-(4-sulfamoyl phenoxy) methyl]phenyl]oct-7-ynoate	<i>tert</i> -butyl 14-[4-(4-sulfamoylphenoxy) phenyl]tetradec-13-ynoate
	
<i>tert</i> -butyl 10-[4-(4-sulfamoylphenyl) methoxy]phenyl]dec-9-ynoate	<i>tert</i> -butyl 16-(4-sulfamoylphenyl)hexadec-15-ynoate
	
<i>tert</i> -butyl 12-(3-sulfamoylphenyl)dodec- 11-ynoate	<i>tert</i> -butyl 10-[2-fluoro-5-(4-sulfamoylphenoxy)phenyl]dec-9-ynoate
	
<i>tert</i> -butyl 8-[4-[2-(4-sulfamoylphenoxy) ethyl]phenyl]oct-7-ynoate	<i>tert</i> -butyl 14-[4-(4-sulfamoylphenyl) methoxy]phenyl]tetradec-13-ynoate

	
<i>tert</i> -butyl 12-[2-fluoro-5-(4-sulfamoylphenoxy) phenyl]dodec-11-ynoate	<i>tert</i> -butyl 12-(4-sulfamoylphenyl) dodec-11-ynoate
	
<i>tert</i> -butyl 14-[2-fluoro-5-(4-sulfamoylphenoxy)phenyl]tetradec-13-ynoate	<i>tert</i> -butyl 18-(3-sulfamoylphenyl) octadec-17-ynoate
	
<i>tert</i> -butyl 16-[2-fluoro-5-(4-sulfamoylphenoxy) phenyl]hexadec-15-ynoate	<i>tert</i> -butyl 8-[2-fluoro-5-(4-sulfamoylphenoxy)phenyl]oct-7-ynoate
	
<i>tert</i> -butyl 10-(4-sulfamoylphenyl)dec-9-ynoate	

2.8.3 Synthesis of *tert*-butyl 14-(4-sulfamoylphenyl)tetradecanoate



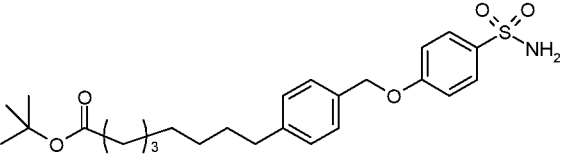
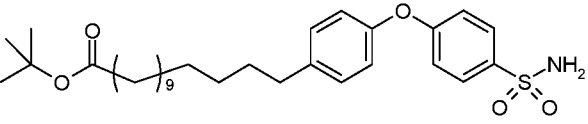
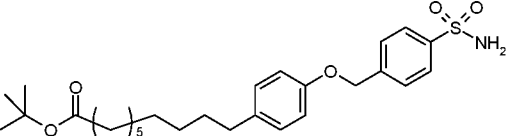
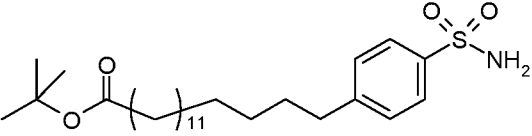
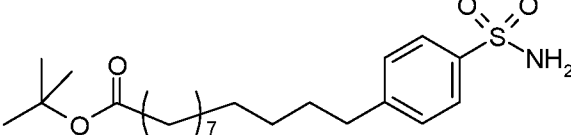
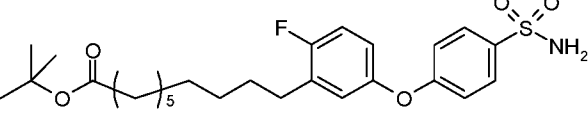
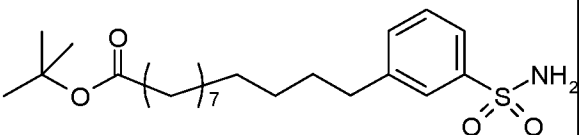
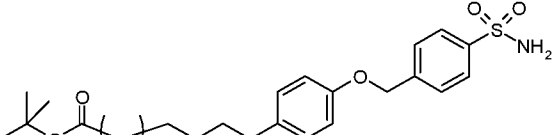
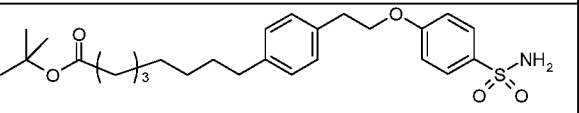
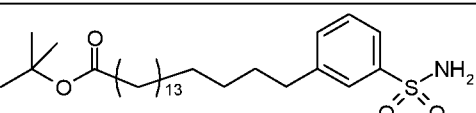
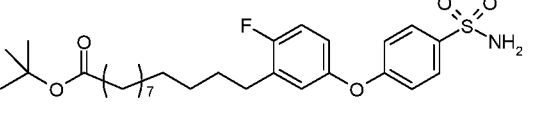
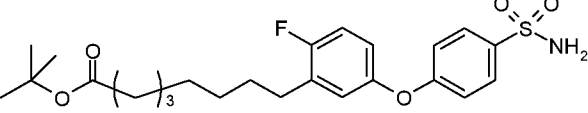
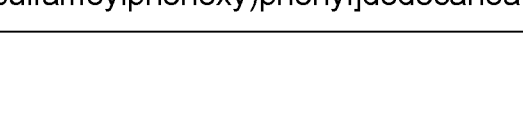
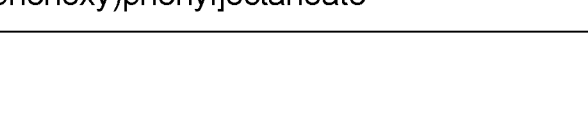
To a mixture of *tert*-butyl 14-(4-sulfamoylphenyl)tetradec-13-ynoate (2.2g, 5.05mmol) in THF (30 ml) was added PtO_2 (0.23g, 1.01mmol). The flask was evacuated and backfilled with H_2 . Then the mixture was stirred at RT overnight. Filtered, concentrated under the vacuum to afford 14-(4-sulfamoylphenyl)tetradecanoate (2g, 4.55mmol, yield: 90%) as a gray solid.

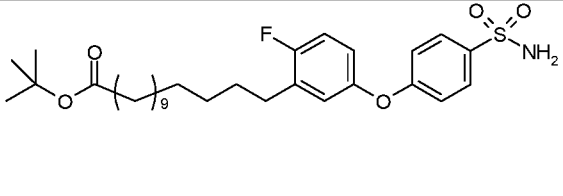
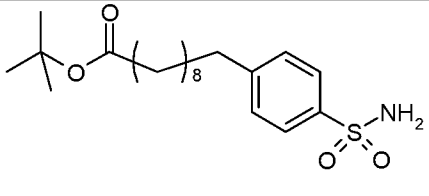
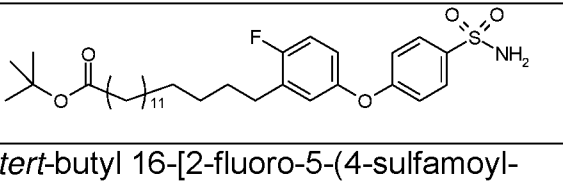
LC-Mass Method: Mobile phase: A = 10 mM TFA/ H_2O , B = MeCN; Gradient: B = 5 % - 95 % in 1.5 min; Flow rate: 1.8 ml/min; Column: Xbridge- C_{18} , $50 \times 4.6\text{mm}$, 3.5 μm . LC purity: 93% (214 nm); Mass: find peak 462 ($\text{M}+\text{H}$)⁺ at 2.44min.

^1H NMR (400 MHz, DMSO) δ 7.72 (d, J = 8.1 Hz, 2H), 7.37 (d, J = 8.1 Hz, 2H), 7.26 (s, 2H), 2.63 (t, J = 7.6 Hz, 2H), 2.16 (t, J = 7.3 Hz, 2H), 1.57 (s, 2H), 1.51 – 1.43 (m, 2H), 1.38 (s, 9H), 1.25 (d, J = 14.5 Hz, 18H).

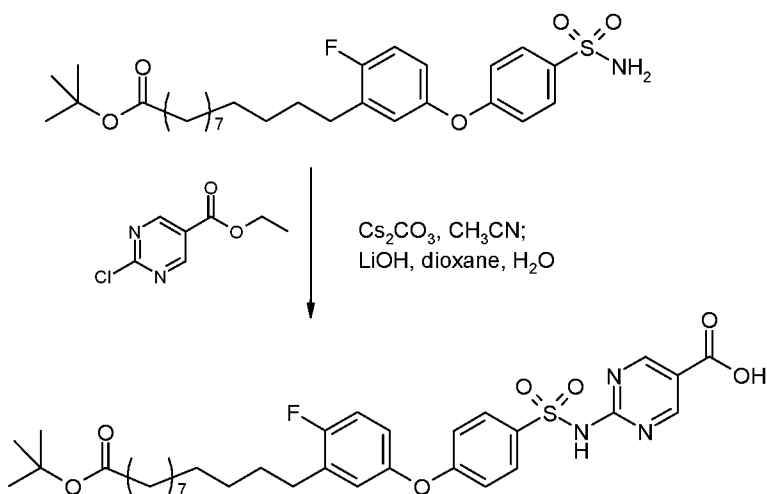
Following compounds were synthesized accordingly:

<i>tert</i> -butyl 18-(4-sulfamoylphenyl)octadecanoate	<i>tert</i> -butyl 14-(3-sulfamoylphenyl)tetradecanoate

<i>tert</i> -butyl 12-[4-(4-sulfamoylphenoxy)phenyl] dodecanoate	<i>tert</i> -butyl 10-[4-[2-(4-sulfamoylphenoxy)ethyl] phenyl]decanoate
	
<i>tert</i> -butyl 8-[4-(4-sulfamoylphenoxy)methyl]phenyl]octanoate	<i>tert</i> -butyl 14-[4-(4-sulfamoylphenoxy)phenyl]tetradecanoate
	
<i>tert</i> -butyl 10-[4-[(4-sulfamoylphenyl)methoxy]phenyl]decanoate	<i>tert</i> -butyl 16-(4-sulfamoylphenyl)hexadecanoate
	
<i>tert</i> -butyl 12-(4-sulfamoylphenyl)dodecanoate	<i>tert</i> -butyl 10-[2-fluoro-5-(4-sulfamoylphenoxy) phenyl]decanoate
	
<i>tert</i> -butyl 12-(3-sulfamoylphenyl) dodecanoate	<i>tert</i> -butyl 14-[4-(4-sulfamoylphenyl)methoxy]phenyl]tetradecanoate
	
<i>tert</i> -butyl 8-[4-[2-(4-sulfamoylphenoxy)ethyl]phenyl]octanoate	<i>tert</i> -butyl 18-(3-sulfamoylphenyl) octadecanoate
	
<i>tert</i> -butyl 12-[2-fluoro-5-(4-sulfamoylphenoxy)phenyl]dodecanoate	<i>tert</i> -butyl 8-[2-fluoro-5-(4-sulfamoylphenoxy)phenyl]octanoate
	

	
<i>tert</i> -butyl 14-[2-fluoro-5-(4-sulfamoylphenoxy) phenyl]tetradecanoate	<i>tert</i> -butyl 10-(4-sulfamoylphenyl)decanoate
	
<i>tert</i> -butyl 16-[2-fluoro-5-(4-sulfamoylphenoxy)phenyl]hexadecanoate	

2.8.4 Synthesis of 2-[[4-[3-(12-*tert*-butoxy-12-oxo-dodecyl)-4-fluorophenoxy]phenyl]sulfonyl amino] pyrimidine-5-carboxylic acid



5

A mixture of *tert*-butyl 12-[2-fluoro-5-(4-sulfamoylphenyl)dodecanoate (300 mg, 575 μmol), ethyl 2-chloropyrimidine-5-carboxylate (112 mg, 603 μmol) and Cs_2CO_3 (656 mg, 2.01 mmol) in MeCN (6 ml) was heated to 60 °C and stirred for 3 h (TLC control). The reaction mixture was used in the next saponification step without

10

The suspension was diluted with dioxane (6 ml) and a solution of LiOH (37 mg, 1.56 mmol) in water (6 ml) was added. The mixture was stirred at RT for 16h and additional LiOH (37 mg, 1.56 mmol) was added. Overall the mixture was stirred at RT for 36h. The suspension was poured on a aqueous solution of citric acid (10percent,

15

50ml). The suspension was filtered and the filter cake washed with water and dried in vacuum. The title compound 2-[[4-[3-(12-*tert*-butoxy-12-oxo-dodecyl)-4-fluoro-

phenoxy]phenyl]sulfonylamino] pyrimidine -5-carboxylic acid was obtained as white solid (350 mg, quan.).

¹H NMR (400.23 MHz, DMSO-d₆) δ ppm 12.2 (bs, 2 H), 8.89 (s, 2 H), 7.99 (d, J=8.93 Hz, 2 H), 7.21 (t, J=9.17 Hz, 1 H), 7.05 (m, 4 H), 2.58 (br t, J= 7.46 Hz, 2 H), 2.15 (t, J= 7.27 Hz, 2 H), 1.53 (m, 2 H), 1.47 (m, 2 H), 1.38 (s, 9 H), 1.26-1.22 (m, 14 H).

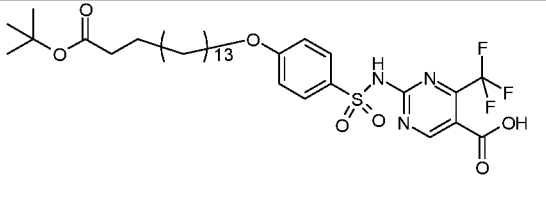
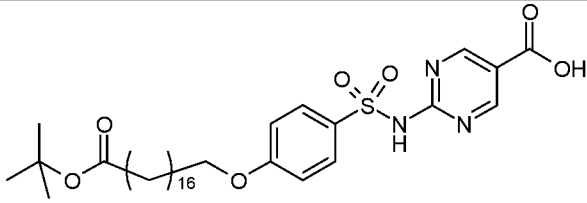
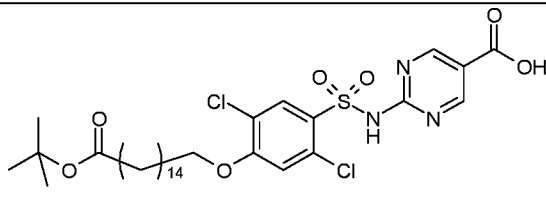
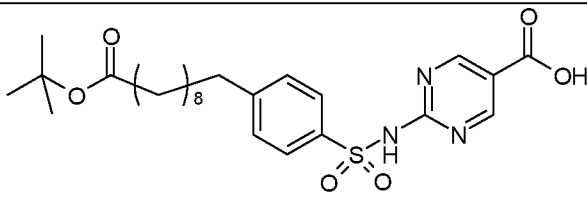
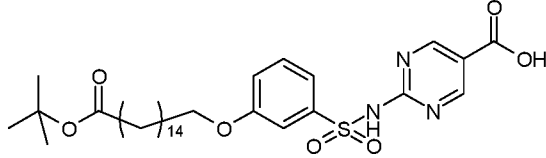
In case the desired product did not precipitate upon pouring on aqueous citric acid, the aqueous layer was extracted with ethyl acetate, the combined organic layers dried with Na₂SO₄, filtered and concentrated in vacuo. The crude products were subjected to column chromatography using MeOH / CH₂Cl₂ as eluent.

Following compounds were synthesized accordingly:

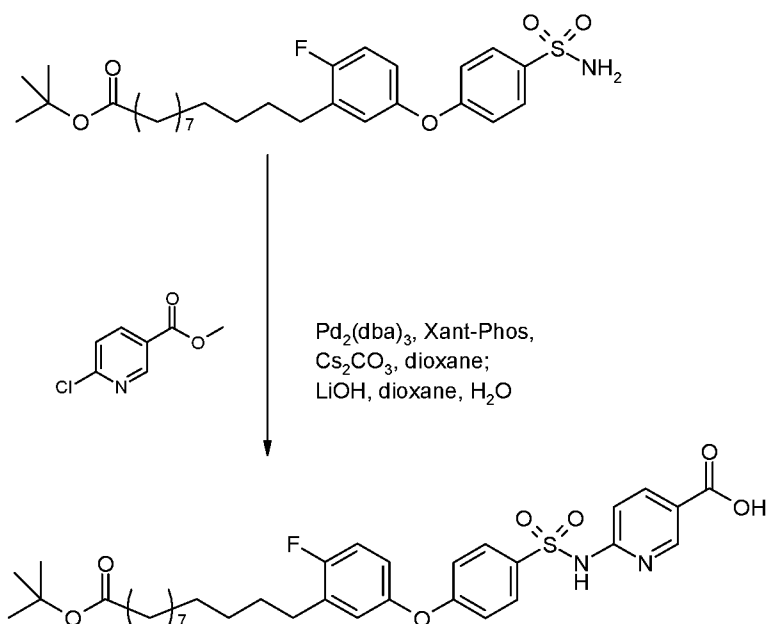
5-[[4-(12- <i>tert</i> -butoxy-12-oxododecoxy)phenyl]sulfonylamino]pyrimidine-2-carboxylic acid	5-[[4-[[4-(10- <i>tert</i> -butoxy-10-oxodecyl)phenoxy]methyl]phenyl]sulfonylamino]pyrimidine-2-carboxylic acid
5-[[4-(14- <i>tert</i> -butoxy-14-oxotetradecyl)phenyl]sulfonylamino]pyrimidine-2-carboxylic acid	5-[[4-[[4-(14- <i>tert</i> -butoxy-14-oxotetradecyl)phenoxy]phenyl]sulfonylamino]pyrimidine-2-carboxylic acid
5-[[4-[3-(16- <i>tert</i> -butoxy-16-oxohexadecyl)-4-fluorophenoxy]phenyl]sulfonylamino]pyrimidine-2-carboxylic acid	5-[[3-(14- <i>tert</i> -butoxy-14-oxotetradecyl)phenyl]sulfonylamino]pyrimidine-2-carboxylic acid

5-[[4-[2-[4-(12- <i>tert</i> -butoxy-12-oxododecoxy)phenyl]ethyl]phenyl]sulfonylamino]pyrimidine-2-carboxylic acid	5-[[4-(18- <i>tert</i> -butoxy-18-oxooctadecyl)phenyl]sulfonylamino]pyrimidine-2-carboxylic acid
2-[[4-(16- <i>tert</i> -butoxy-16-oxohexadecoxy)phenyl]sulfonylamino]-4-methyl-pyrimidine-5-carboxylic acid	5-[[4-(14- <i>tert</i> -butoxy-14-oxotetradecoxy)phenyl]sulfonylamino]pyrimidine-2-carboxylic acid
2-[[4-(16- <i>tert</i> -butoxy-16-oxohexadecoxy)-3-fluoro-phenyl]sulfonylamino]pyrimidine-5-carboxylic acid	2-[[4-(16- <i>tert</i> -butoxy-16-oxohexadecoxy)-3-chloro-phenyl]sulfonylamino]pyrimidine-5-carboxylic acid
2-[[4-(16- <i>tert</i> -butoxy-16-oxohexadecyl)phenyl]sulfonylamino]pyrimidine-5-carboxylic acid	2-[[4-(12- <i>tert</i> -butoxy-12-oxododecyl)phenyl]sulfonylamino]pyrimidine-5-carboxylic acid

2-[[3-(12- <i>tert</i> -butoxy-12-oxo-dodecyl)phenyl]sulfonylamino]pyrimidine-5-carboxylic acid	2-[[3-(18- <i>tert</i> -butoxy-18-oxo-octadecyl)phenyl]sulfonylamino]pyrimidine-5-carboxylic acid
5-[[4-[2-[4-(10- <i>tert</i> -butoxy-10-oxo-decyl)phenyl]ethoxy]phenyl]sulfonylamino]pyrimidine-2-carboxylic acid	2-[[4-[3-(8- <i>tert</i> -butoxy-8-oxo-octyl)-4-fluorophenoxy]phenyl]sulfonylamino]pyrimidine-5-carboxylic acid
5-[[4-[3-(14- <i>tert</i> -butoxy-14-oxotetradecyl)-4-fluorophenoxy]phenyl]sulfonylamino]pyrimidine-2-carboxylic acid	2-[[4-[[4-(14- <i>tert</i> -butoxy-14-oxotetradecyl)phenoxy]methyl]phenyl]sulfonylamino]pyrimidine-5-carboxylic acid
5-[[4-(16- <i>tert</i> -butoxy-16-oxohexadecoxy)phenyl]sulfonylamino]pyrimidine-2-carboxylic acid	2-[[4-[3-(10- <i>tert</i> -butoxy-10-oxo-decyl)-4-fluorophenoxy]phenyl]sulfonylamino]pyrimidine-5-carboxylic acid
5-[[4-[[4-(8- <i>tert</i> -butoxy-8-oxooctyl)phenyl]methoxy]phenyl]sulfonylamino]pyrimidine-2-carboxylic acid	2-[[4-[2-[4-(8- <i>tert</i> -butoxy-8-oxooctyl)phenyl]ethoxy]phenyl]sulfonylamino]pyrimidine-5-carboxylic acid

	
2-[[4-(16- <i>tert</i> -butoxy-16-oxo-hexadecoxy) phenyl]sulfonylamino]-4-(trifluoromethyl) pyrimidine-5-carboxylic acid	2-[[4-(18- <i>tert</i> -butoxy-18-oxo-octadecoxy)phenyl]sulfonylamino]pyrimidine-5-carboxylic acid
	
2-[[4-(16- <i>tert</i> -butoxy-16-oxo-hexadecoxy)-2,5-dichloro-phenyl]sulfonylamino]pyrimidine-5-carboxylic acid	2-[[4-(10- <i>tert</i> -butoxy-10-oxo-decyl)phenyl]sulfonylamino]pyrimidine-5-carboxylic acid
	
2-[[3-(16- <i>tert</i> -butoxy-16-oxo-hexadecoxy)phenyl]sulfonylamino]pyrimidine-5-carboxylic acid	

2.8.5 Synthesis of 6-[[4-[3-(12-*tert*-butoxy-12-oxo-dodecyl)-4-fluorophenoxy]phenyl]sulfonyl-amino] pyridine-3-carboxylic acid



A mixture of *tert*-butyl 12-[2-fluoro-5-(4-sulfamoylphenoxy)phenyl]dodecanoate (300 mg, 575 μmol), methyl 6-chloronicotinoate (102 mg, 603 μmol), Cs_2CO_3 (468 mg, 1.44 mmol), tris(dibenzylideneacetone)dipalladium (26 mg, 29 μmol) and 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene ("xantphos", 17 mg, 29 μmol) in dioxane (6 ml) was heated to 80 °C under an argon atmosphere for 3 h (TLC control). The reaction mixture was used in the next saponification step without further purification.

- 10 The suspension was diluted with dioxane (6 ml) and a solution of LiOH (37 mg, 1.56 mmol) in water (6 ml) was added. The mixture was stirred at RT for 16h and additional LiOH (37 mg, 1.56 mmol) was added. Overall the mixture was stirred at RT for 36h. The suspension was poured on a aqueous solution of citric acid (10percent, 50ml). The suspension was filtered and the filter cake washed with water and dried in
- 15 vacuum. The title compound 6-[[4-[3-(12-*tert*-butoxy-12-oxo-dodecyl)-4-fluorophenoxy]phenyl]sulfonyl-amino]pyridine-3-carboxylic acid was obtained as white solid (350 mg, quan.).

- 20 ^1H NMR (400.23 MHz, DMSO-d_6) δ ppm 12.5 (br s, 1H), 8.54 (br s, 1 H), 8.11 (dd, $J=8.93, 2.20$ Hz, 1 H), 7.91 (br d, $J=8.68$ Hz, 2 H), 7.80 (m, 1 H), 7.19 (m, 2 H), 7.04 (m, 4 H), 2.58 (br t, $J=7.46$ Hz, 2 H), 2.15 (t, $J=7.27$ Hz, 2 H), 1.48 (m, 4 H), 1.38 (s, 9 H), 1.26-1.22 (m, 14 H).

In case the desired product did not precipitate upon pouring on aqueous citric acid, the aqueous layer was extracted with ethyl acetate, the combined organic layers dried with Na₂SO₄, filtered and concentrated in vacuo. The crude products were subjected to column chromatography using MeOH / CH₂Cl₂ as eluent.

5

Following compounds were synthesized accordingly:

6-[[4-[4-(12- <i>tert</i> -butoxy-12-oxo-dodecyl)phenoxy]phenyl]sulfonylamino]pyridine-3-carboxylic acid	6-[[4-(12- <i>tert</i> -butoxy-12-oxo-dodecoxy)phenyl]sulfonylamino]pyridine-3-carboxylic acid
6-[[4-[4-(10- <i>tert</i> -butoxy-10-oxo-decyl)phenoxy]methyl]phenyl]sulfonylamino]pyridine-3-carboxylic acid	6-[[4-[2-[4-(10- <i>tert</i> -butoxy-10-oxo-decyl)phenyl]ethoxy]phenyl]sulfonylamino]pyridine-3-carboxylic acid
6-[[4-(14- <i>tert</i> -butoxy-14-oxo-tetradecyl)phenyl]sulfonyl amino]pyridine-3-carboxylic acid	6-[[4-[3-(14- <i>tert</i> -butoxy-14-oxo-tetradecyl)-4-fluoro-phenoxy]phenyl]sulfonyl amino]pyridine-3-carboxylic acid
6-[[4-[3-(16- <i>tert</i> -butoxy-16-oxo-hexadecyl)-4-fluoro-phenoxy]phenyl]sulfonyl amino]pyridine-3-carboxylic acid	6-[[4-(18- <i>tert</i> -butoxy-18-oxo-octadecyl)phenyl]sulfonyl amino]pyridine-3-carboxylic acid

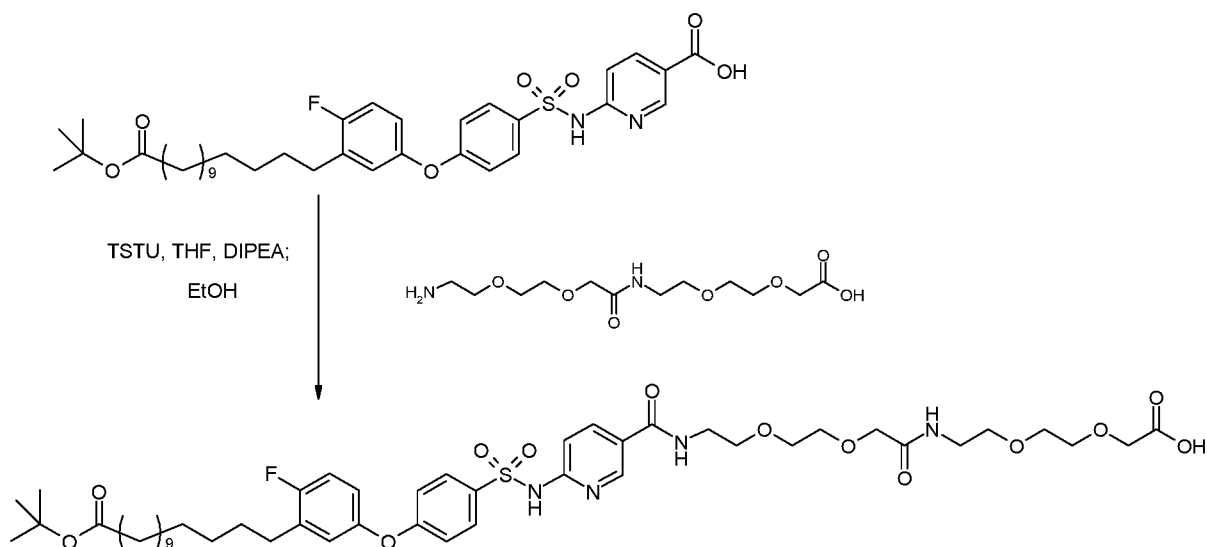
phenyl]sulfonylamino] pyridine-3-carboxylic acid	sulfonylamino]pyridine-3-carboxylic acid
6-[[4-(14- <i>tert</i> -butoxy-14-oxo-tetradecoxy)phenyl]sulfonylamino]pyridine-3-carboxylic acid	6-[[4-(16- <i>tert</i> -butoxy-16-oxo-hexadecoxy)-2,3-dichloro-phenyl]sulfonylamino] pyridine-3-carboxylic acid
6-[[4-(16- <i>tert</i> -butoxy-16-oxo-hexadecoxy)-3-fluoro-phenyl]sulfonylamino]pyridine-3-carboxylic acid	6-[[4-[[4-(14- <i>tert</i> -butoxy-14-oxo-tetradecyl)phenoxy]methyl]phenyl]sulfonylamino]pyridine-3-carboxylic acid
6-[[4-(16- <i>tert</i> -butoxy-16-oxo-hexadecyl)phenyl] sulfonylamino]pyridine-3-carboxylic acid	6-[[4-2-[4-(8- <i>tert</i> -butoxy-8-oxo-octyl)phenyl]ethoxy]phenyl]sulfonylamino]pyridine-3-carboxylic acid
6-[[3-(18- <i>tert</i> -butoxy-18-oxo-octadecyl)phenyl]sulfonylamino]pyridine-3-carboxylic acid	6-[[4-(16- <i>tert</i> -butoxy-16-oxo-hexadecoxy)-3-chloro-phenyl]sulfonylamino]pyridine-3-carboxylic acid

6-[[4-[4-(14- <i>tert</i> -butoxy-14-oxo-tetradecyl)phenoxy]phenyl]sulfonylamino]pyridine-3-carboxylic acid	6-[[4-(16- <i>tert</i> -butoxy-16-oxo-hexadecoxy)phenyl]sulfonylamino]pyridine-3-carboxylic acid
6-[[4-(10- <i>tert</i> -butoxy-10-oxo-decoxy)phenyl]sulfonylamino]pyridine-3-carboxylic acid	6-[[4-(18- <i>tert</i> -butoxy-18-oxo-octadecoxy)phenyl]sulfonylamino]pyridine-3-carboxylic acid
6-[[3-(14- <i>tert</i> -butoxy-14-oxo-tetradecyl)phenyl]sulfonylamino]pyridine-3-carboxylic acid	6-[[4-[2-[4-(8- <i>tert</i> -butoxy-8-oxo-octyl)phenyl]ethoxy]phenyl]sulfonylamino]pyridine-3-carboxylic acid
6-[[4-(10- <i>tert</i> -butoxy-10-oxo-decoxy)phenyl]sulfonylamino]pyridine-3-carboxylic acid	6-[[4-(10- <i>tert</i> -butoxy-10-oxo-decoxy)phenyl]sulfonylamino]pyridine-3-carboxylic acid
6-[[3-(14- <i>tert</i> -butoxy-14-oxo-tetradecyl)phenyl]sulfonylamino]pyridine-3-carboxylic acid	6-[[4-(20- <i>tert</i> -butoxy-20-oxo-icosoxy)phenyl]sulfonylamino]pyridine-3-carboxylic acid

6-[[4-(16- <i>tert</i> -butoxy-16-oxohexadecoxy)phenyl]sulfonylamino]pyridine-3-carboxylic acid	6-[[3-(12- <i>tert</i> -butoxy-12-oxododecyl)phenyl]sulfonylamino]pyridine-3-carboxylic acid
6-[[4-[2-[4-(12- <i>tert</i> -butoxy-12-oxododecoxy)phenyl]ethyl]phenyl]sulfonylamino]pyridine-3-carboxylic acid	

2.9 Examples for the synthesis of compounds with formula I according to scheme 1

- 5 2.9.1 Synthesis of 2-[2-[2-[2-[2-[2-[6-[[4-[3-(14-*tert*-butoxy-14-oxotetradecyl)-4-fluorophenoxy]phenyl]sulfonylamino]pyridine-3-carboxyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid



- 10 A mixture of 6-[[4-[3-(14-*tert*-butoxy-14-oxotetradecyl)-4-fluorophenoxy]phenyl]sulfonyl amino]pyridine-3-carboxylic acid (169 mg, 251 μ mol), TSTU (80 mg, 264 μ mol) and DIPEA (132 μ l, 97 mg, 1.25 mmol) in 6 ml of THF were stirred

at RT for 16h. After 16h the solvent was removed under reduced pressure and a solution of [2-(2-{2-[2-(2-Amino-ethoxy)-ethoxy] -acetylamino}-ethoxy)-ethoxy]-acetic acid (85 mg, 277 μ mol) in 6 ml abs. EtOH was added and the mixture was stirred at RT for 16h. Volatile components were removed under reduced pressure, the

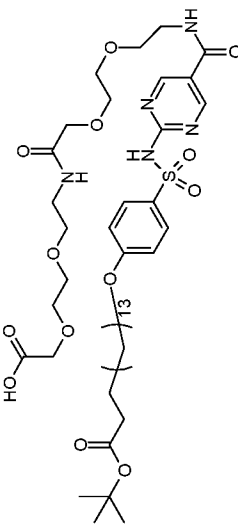
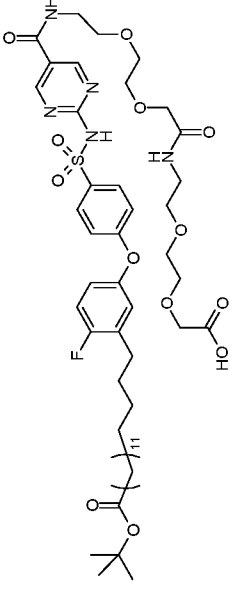
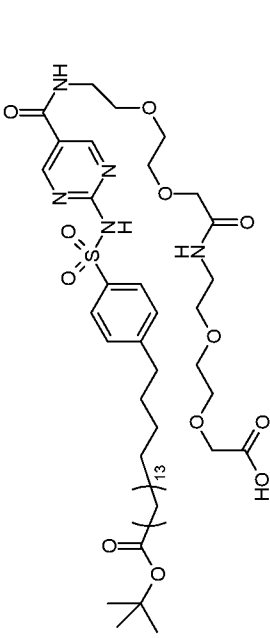
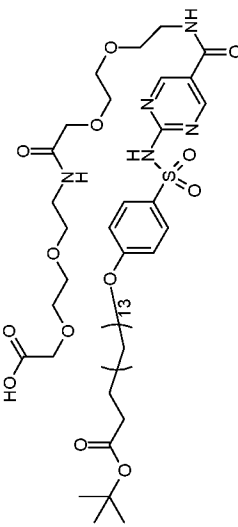
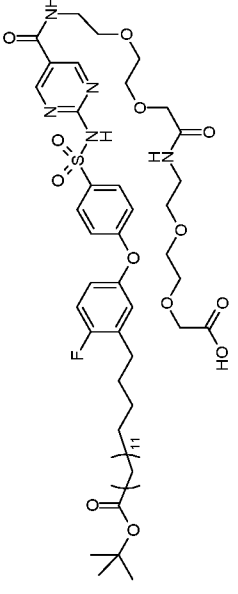
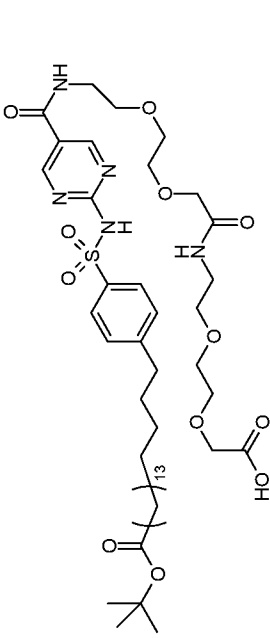
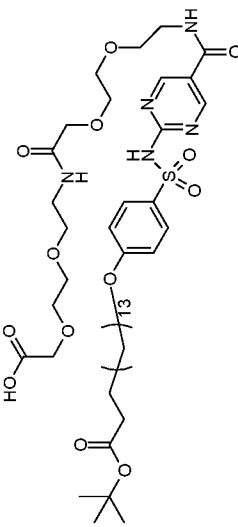
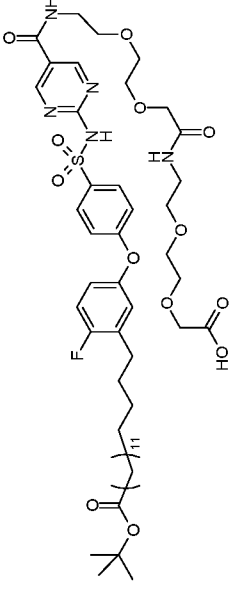
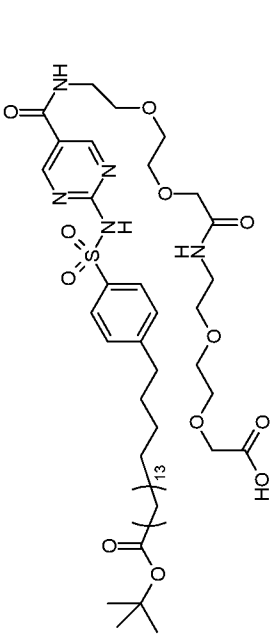
5 resulting residue dissolved in CH₂Cl₂ and washed with aq. 10% KHSO₄ solution. The organic layer was washed with water and brine, dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. The crude product was purified by RP HPLC to afford 2-[2-[2-[[2-[2-[2-[[6-[[4-[3-(14-*tert*-butoxy-14-oxo-tetradecyl)-4-fluoro-phenoxy]phenyl] sulfonylamino]pyridine-3-carbonyl]amino]ethoxy]ethoxy]acetyl] amino]ethoxy] ethoxy]acetic acid (106 mg, 44 %).

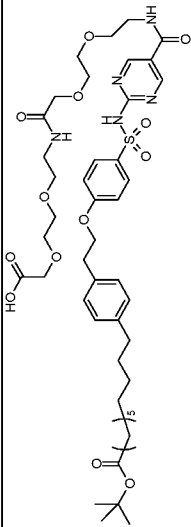
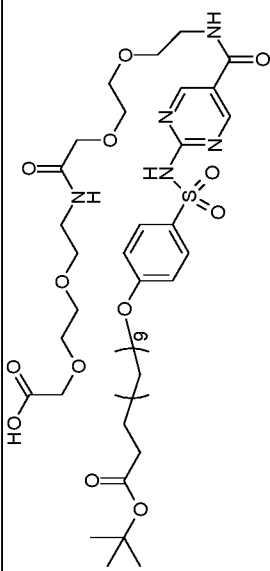
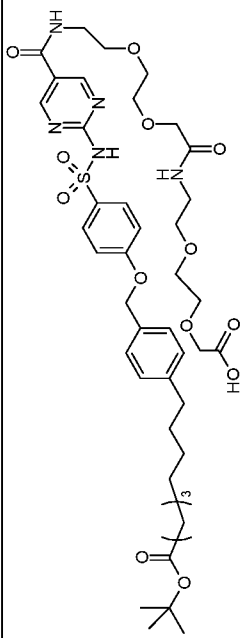
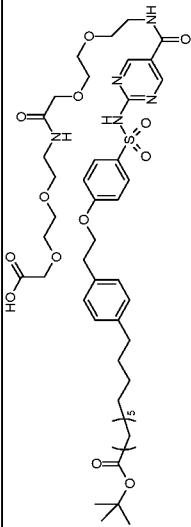
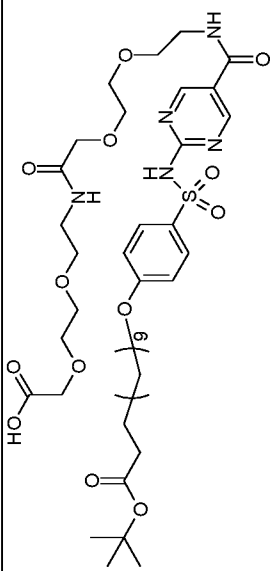
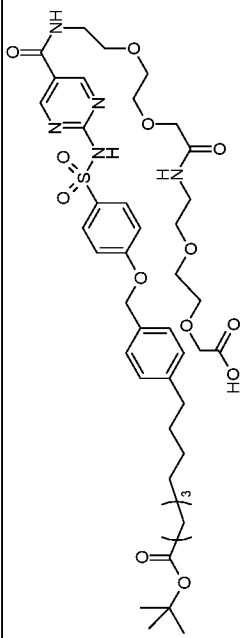
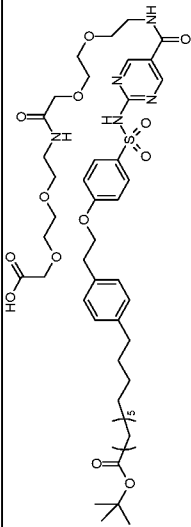
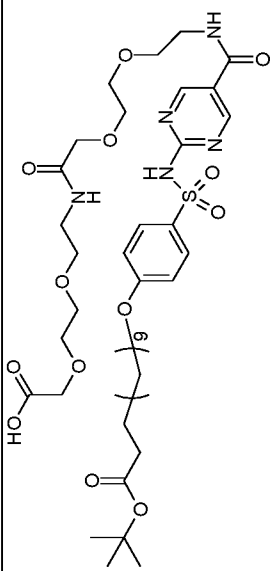
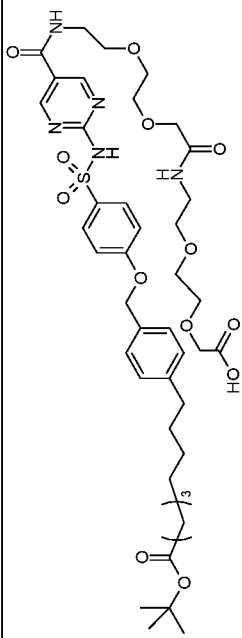
¹H NMR (400.23 MHz, DMSO-d₆) δ ppm 12.29 (br s, 1 H), 8.52 (m, 2 H), 8.09 (dd, J=8.93, 2.32 Hz, 1 H), 7.89 (d, J=8.80 Hz, 2 H), 7.61 (br t, J=5.69 Hz, 1 H), 7.18 (m, 15 2 H), 7.03 (m, 4 H), 4.01 (s, 2 H), 3.86 (s, 2 H), 3.20 – 3.68 (m, 16 H), 2.58 (br t, J=7.52 Hz, 2 H), 2.15 (t, J=7.27 Hz, 2 H), 1.49 (m, 4 H), 1.38 (s, 9 H), 1.25 (m, 18 H).

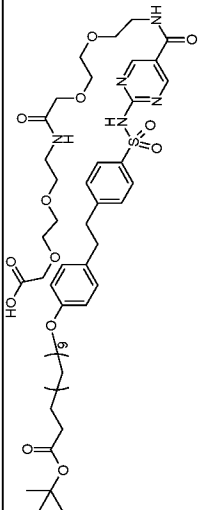
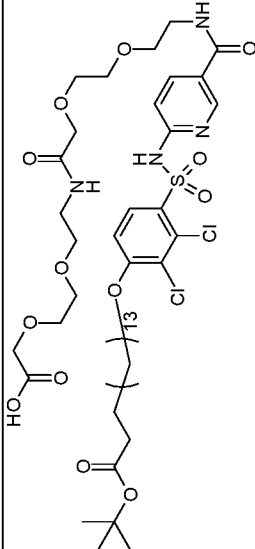
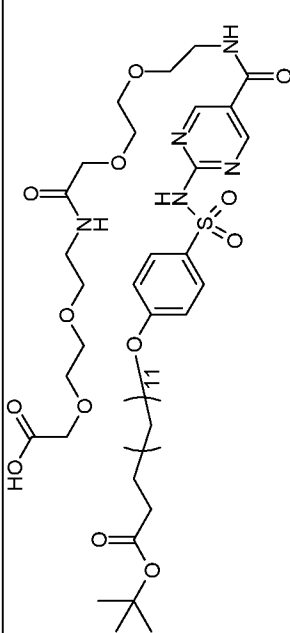
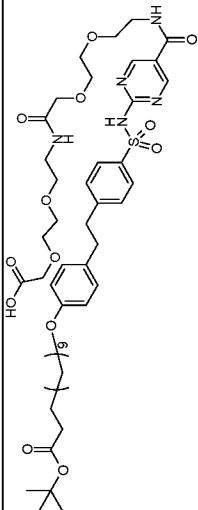
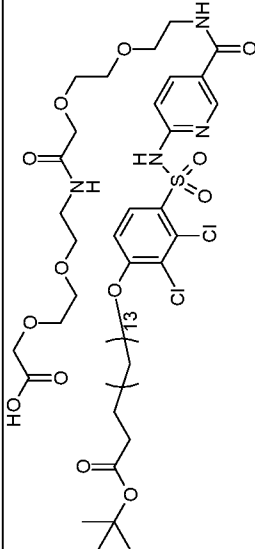
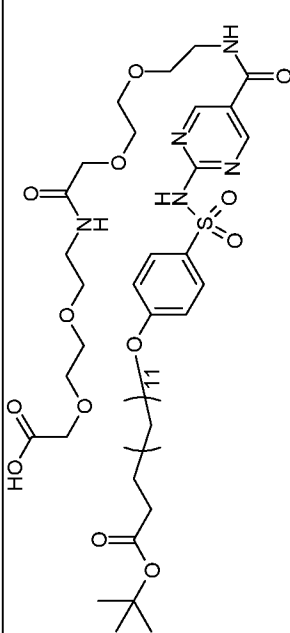
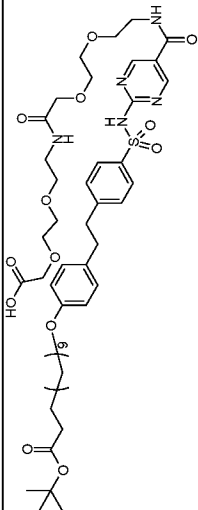
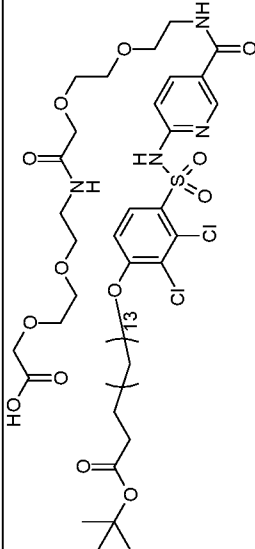
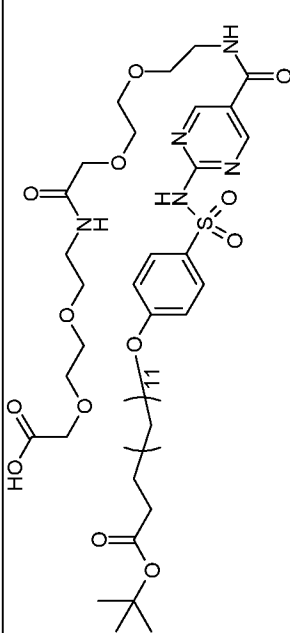
Following compounds were synthesized accordingly:

	2-[2-[2-[2-[2-[6-[4-(14- <i>tert</i> -butoxy-14-oxo-tetradecyl)]phenyl]sulfonyl]amino]pyridine-3-carbonyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid
	2-[2-[2-[2-[2-[6-[4-(16- <i>tert</i> -butoxy-16-oxo-hexadecyl)]-4-fluorophenoxy]phenyl]sulfonyl]amino]pyridine-3-carbonyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid
	2-[2-[2-[2-[2-[6-[4-(12- <i>tert</i> -butoxy-12-oxo-dodecoxy)]phenyl]sulfonyl]amino]pyridine-3-carbonyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid

mino]ethoxy]ethoxy]acetic acid	
acetyl]amino]ethoxy]ethoxy]acetic acid	
carbonyl]amino]ethoxy]ethoxy]acetic acid	
2-[2-[2-[2-[2-[6-[3-(14-tert-butoxy-14-oxo-tetradecyl)]phenyl]sulfonylamino]pyridine-3-carbonyl]amino]ethoxy]ethoxy]acetic acid	
2-[2-[2-[2-[2-[6-[4-[4-(12-tert-butoxy-12-oxo-dodecyl)]phenoxy]phenyl]sulfonylamino]pyridine-3-carbonyl]amino]ethoxy]ethoxy]acetic acid	
2-[2-[2-[2-[2-[6-[4-[4-(10-tert-butoxy-10-oxo-decyl)]phenoxy]phenyl]sulfonylamino]pyridine-3-carbonyl]amino]ethoxy]ethoxy]acetic acid	

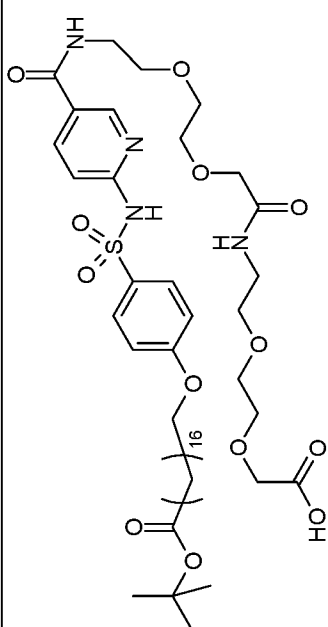
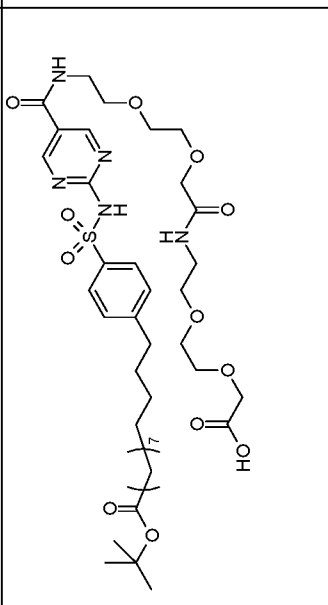
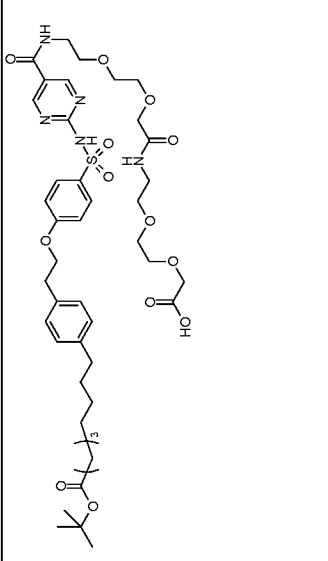
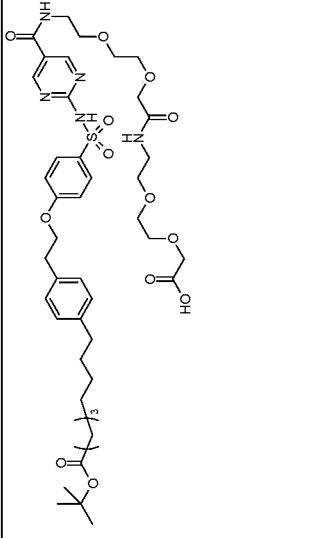
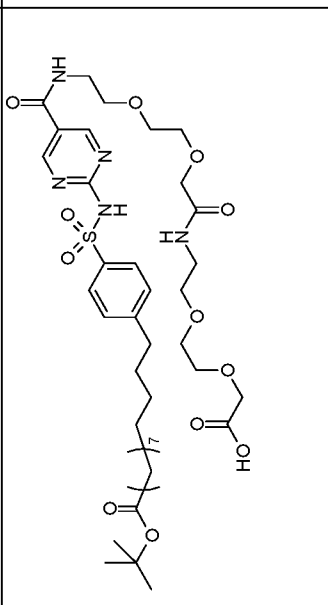
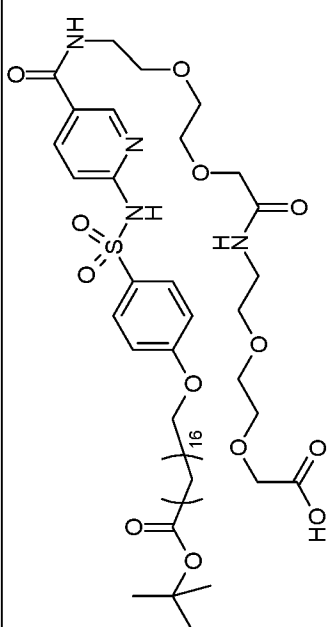
carbonyl]lamino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid		carbonyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid		amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid	
2-[2-[2-[2-[2-[5-[4-(16- <i>tert</i> -butoxy-16-oxo-hexadecoxy)phenyl]sulfonylamino]pyrimidine-2-carbonyl]amino]ethoxy]ethoxy]acetyl]lamino]ethoxy]ethoxy]acetic acid		2-[2-[2-[2-[2-[5-[4-[3-(16- <i>tert</i> -butoxy-16-oxo-hexadecyl)-4-fluorophenoxy]phenyl]sulfonylamino]pyrimidine-2-carbonyl]amino]ethoxy]ethoxy]acetyl]lamino]ethoxy]ethoxy]acetic acid		2-[2-[2-[2-[2-[5-[4-[4-(18- <i>tert</i> -butoxy-18-oxo-octadecyl)phenyl]sulfonylamino]pyrimidine-2-carbonyl]amino]ethoxy]ethoxy]acetyl]lamino]ethoxy]ethoxy]acetic acid	
2-[2-[2-[2-[2-[5-[4-[3-(14- <i>tert</i> -butoxy-14-oxo-tetradecyl)-4-fluorophenoxy]phenyl]sulfonylamino]pyrimidine-2-carbonyl]amino]ethoxy]ethoxy]acetyl]lamino]ethoxy]ethoxy]acetic acid		2-[2-[2-[2-[2-[5-[4-[3-(14- <i>tert</i> -butoxy-14-oxo-tetradecyl)phenoxy]phenyl]sulfonylamino]pyrimidine-2-carbonyl]amino]ethoxy]ethoxy]acetyl]lamino]ethoxy]ethoxy]acetic acid		2-[2-[2-[2-[2-[5-[4-[4-(10- <i>tert</i> -butoxy-10-oxo-decyl)phenoxy]methyl]phenyl]sulfonylamino]pyrimidine-2-carbonyl]amino]ethoxy]ethoxy]acetyl]lamino]ethoxy]ethoxy]acetic acid	

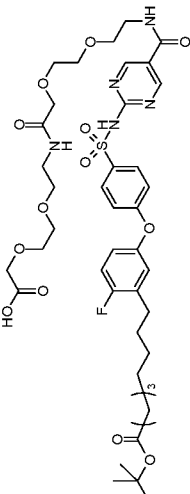
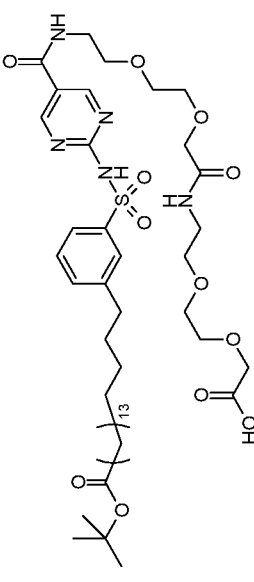
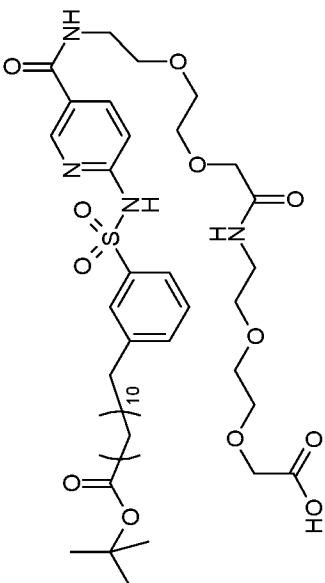
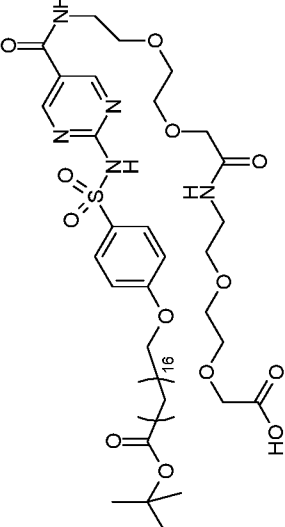
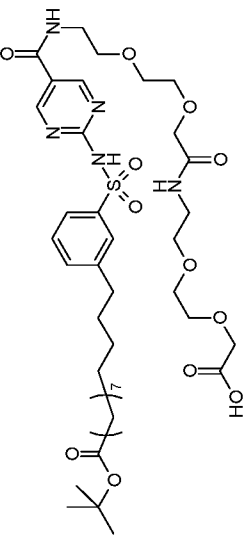
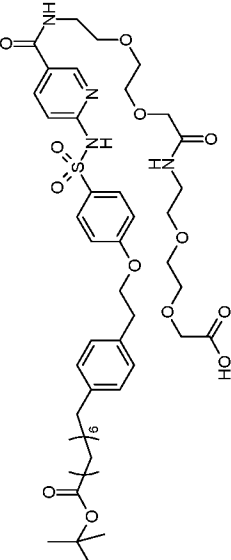
pyrimidine-2-carbonyl]amino] ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]ethoxy]acetic acid		ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid		carbonyl]amino] ethoxy]ethoxy]acetyl] amino]ethoxy]ethoxy]acetic acid	
2-[2-[2-[2-[2-[5-[4-[2-[4-(10- <i>tert</i> -butoxy-10-oxo-decyl)]phenyl]]sulfonamino]]pyrimidine-2-carbonyl]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid		2-[2-[2-[2-[2-[5-[4-[12- <i>tert</i> -butoxy-12-oxo-dodecoxy)]phenyl]]sulfonamino]]pyrimidine-2-carbonyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid		2-[2-[2-[2-[2-[5-[4-[4-(8- <i>tert</i> -butoxy-8-oxo-octyl)]phenyl]]sulfonamino]]pyrimidine-2-carbonyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid	
2-[2-[2-[2-[2-[5-[4-[2-[4-(14- <i>tert</i> -butoxy-14-oxo-tetradecyl)]phenyl]]sulfonamino]]pyrimidine-2-carbonyl]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid		2-[2-[2-[2-[2-[5-[4-[3-(12- <i>tert</i> -butoxy-12-oxo-dodecyl)]-4-fluoro-phenyl]]sulfonamino]]pyrimidine-2-carbonyl]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid		2-[2-[2-[2-[2-[5-[4-[4-(14- <i>tert</i> -butoxy-14-oxo-tetradecyl)]phenyl]]sulfonamino]]pyrimidine-2-carbonyl]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid	

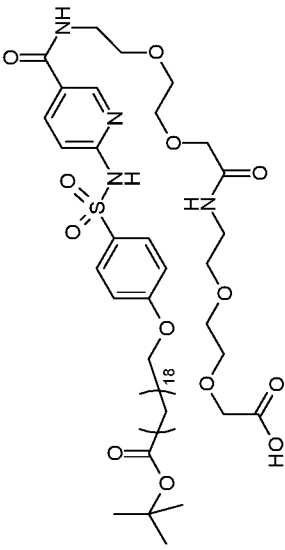
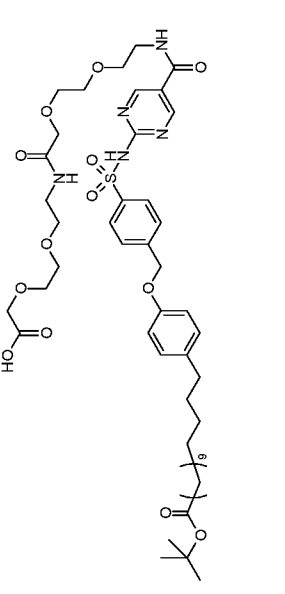
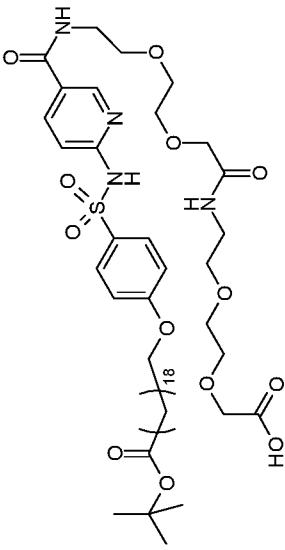
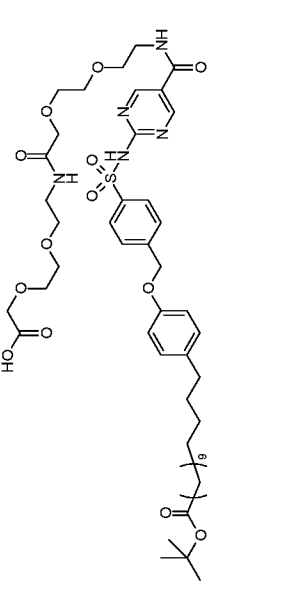
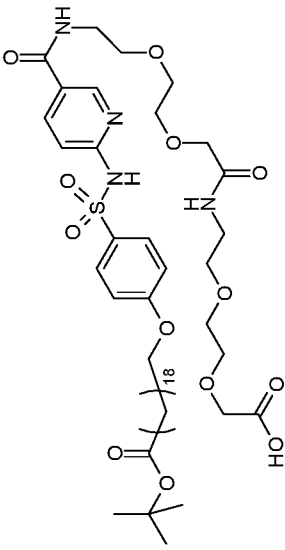
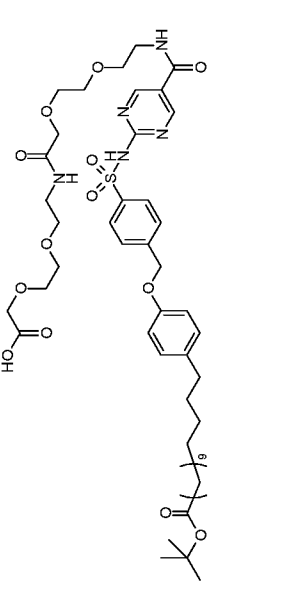
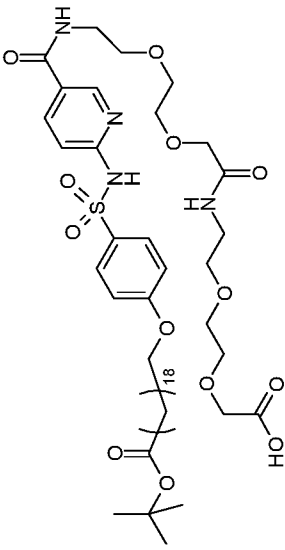
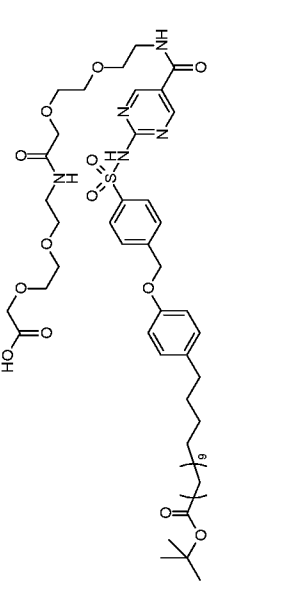
sulfonyl]amino]pyrimidine-2-carbonyl]amino] ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid 	phenoxy]phenyl] sulfonyl amino]pyrimidine-2-carbonyl]amino] ethoxy]ethoxy]acetyl] amino]ethoxy]ethoxy]acetic acid 	phenyl]sulfonyl]amino] pyrimidine-2-carbonyl]amino]ethoxy] ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid 
2-[2-[2-[2-[2-[5-[4-[2-[4-(12-tert-butoxy-12-oxo-dodecoxy)phenyl] ethyl]phenyl]sulfonyl]amino]pyrimidine-2-carbonyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid 	2-[2-[2-[2-[2-[6-[4-(16-tert-butoxy-16-oxo-hexadecoxy)-2,3-dichlorophenyl]sulfonyl]amino]pyridine-3-carbonyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid 	2-[2-[2-[2-[2-[5-[4-(14-tert-butoxy-14-oxo-tetradecoxy)phenyl]sulfonyl]amino]pyrimidine-2-carbonyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid 
2-[2-[2-[2-[2-[5-[4-[2-[4-(12-tert-butoxy-12-oxo-dodecoxy)phenyl] ethyl]phenyl]sulfonyl]amino]pyrimidine-2-carbonyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid 	2-[2-[2-[2-[2-[6-[4-(16-tert-butoxy-16-oxo-hexadecoxy)-2,3-dichlorophenyl]sulfonyl]amino]pyridine-3-carbonyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid 	2-[2-[2-[2-[2-[5-[4-(14-tert-butoxy-14-oxo-tetradecoxy)phenyl]sulfonyl]amino]pyrimidine-2-carbonyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid 

2-[2-[2-[2-[2-[2-[6-[4-(14- <i>tert</i> -butoxy-14-oxo-tetradecoxy)phenyl]sulfonylamino]pyridine-3-carbonyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid	
2-[2-[2-[2-[2-[2-[4-(16- <i>tert</i> -butoxy-16-oxo-hexadecoxy)-3-fluorophenyl]sulfonylamino]pyridine-3-carbonyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid	
2-[2-[2-[2-[2-[2-[4-(16- <i>tert</i> -butoxy-16-oxo-hexadecoxy)-3-fluorophenyl]sulfonylamino]pyrimidine-5-carbonyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid	
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2-[2-[2-[2-[2-[2-[4-(16- <i>tert</i> -butoxy-16-oxo-hexadecoxy)-3-chlorophenyl]sulfonylamino]pyridine-3-carbonyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid	
2-[2-[2-[2-[2-[2-[4-(16- <i>tert</i> -butoxy-16-oxo-hexadecoxy)-3-fluorophenyl]sulfonylamino]pyrimidine-5-carbonyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid	

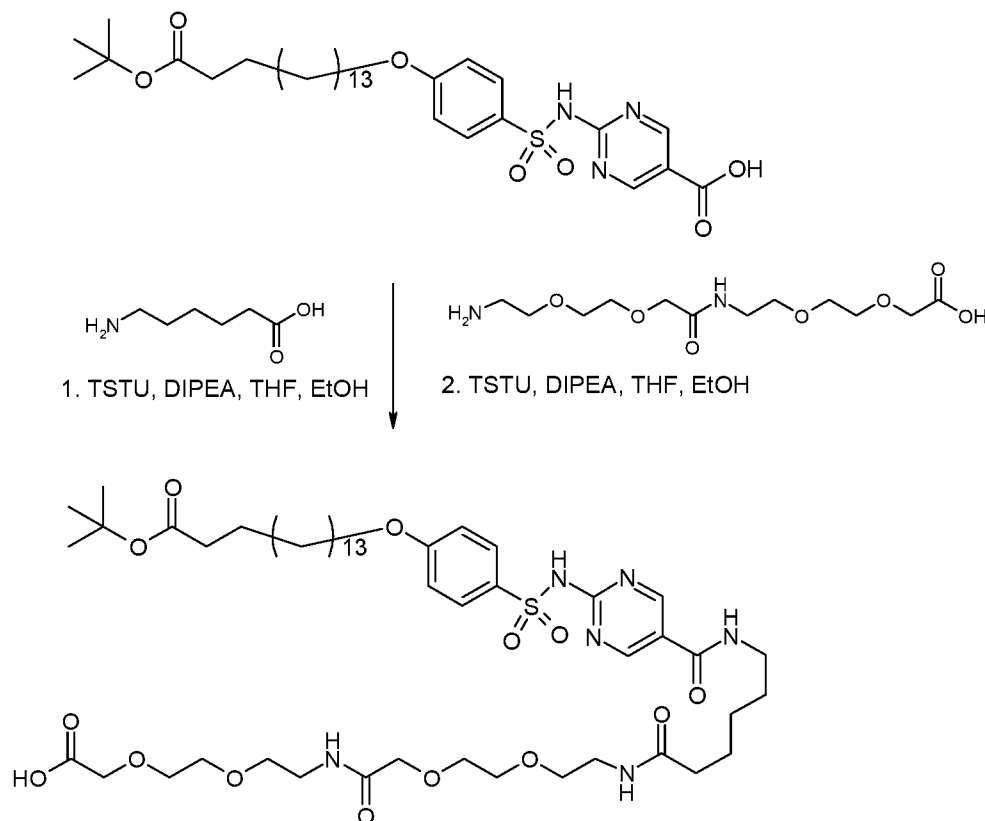
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2-[2-[2-[2-[2-[2-[4-(16- <i>tert</i> -butoxy-16-oxo-hexadecyl)]phenyl]]sulfonylamino]pyrimidine-5-carbonyl]amino]ethoxy]ethoxy]acetic acid	2-[2-[2-[2-[2-[2-[4-(14- <i>tert</i> -butoxy-14-oxo-tetradecyl)]phenoxy]]phenyl]]sulfonylamino]pyridine-3-carbonyl]amino]ethoxy]ethoxy]acetic acid	2-[2-[2-[2-[2-[2-[4-(16- <i>tert</i> -butoxy-16-oxo-hexadecyl)]phenyl]]sulfonylamino]pyrimidine-5-carbonyl]amino]ethoxy]ethoxy]acetic acid
		
2-[2-[2-[2-[2-[4-(8- <i>tert</i> -butoxy-8-oxo-octyl)]phenyl]]sulfonylamino]pyrimidine-5-carbonyl]amino]ethoxy]ethoxy]acetic acid	2-[2-[2-[2-[2-[4-(12- <i>tert</i> -butoxy-12-oxo-dodecyl)]phenyl]]sulfonylamino]pyrimidine-5-carbonyl]amino]ethoxy]ethoxy]acetic acid	2-[2-[2-[2-[2-[6-[4-(18- <i>tert</i> -butoxy-18-oxo-octadecyl)]phenyl]]sulfonylamino]pyridine-3-carbonyl]amino]ethoxy]ethoxy]acetic acid
		

	2-[2-[2-[2-[2-[4-[3-(8-<i>tert</i>-butoxy-8-oxo-octyl)-4-fluorophenoxy]pyrimidine-5-sulfonylamino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid		2-[2-[2-[2-[2-[2-[3-(18-<i>tert</i>-butoxy-18-oxo-octadecyl)]phenyl]sulfonylamino]pyrimidine-5-carbonyl]amino]ethoxy]ethoxy]acetic acid		2-[2-[2-[2-[2-[6-[3-(12-<i>tert</i>-butoxy-12-oxo-dodecyl)]phenyl]sulfonylamino]pyridine-3-carbonyl]amino]ethoxy]ethoxy]acetic acid
	2-[2-[2-[2-[2-[4-[3-(8-<i>tert</i>-butoxy-8-oxo-octyl)-4-fluorophenoxy]pyrimidine-5-sulfonylamino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid		2-[2-[2-[2-[2-[2-[3-(12-<i>tert</i>-butoxy-12-oxo-dodecyl)]phenyl]sulfonylamino]pyrimidine-5-carbonyl]amino]ethoxy]ethoxy]acetic acid		2-[2-[2-[2-[2-[6-[3-(12-<i>tert</i>-butoxy-12-oxo-dodecyl)]phenyl]sulfonylamino]pyridine-3-carbonyl]amino]ethoxy]ethoxy]acetic acid

pyrimidine-5-carbonyl]amino]ethoxy]ethoxy]acetic acid		carbonyl]amino]ethoxy]ethoxy]acetic acid		carbonyl]amino]ethoxy]ethoxy]acetic acid
2-[2-[2-[2-[2-[6-[4-(20-tert-butoxy-20-oxo-icosoxy)phenyl]sulfonyl]amino]ethoxy]pyridine-3-carbonyl]amino]ethoxy]ethoxy]acetic acid		2-[2-[2-[2-[2-[6-[4-(14-tert-butoxy-14-oxo-tetradecyl)phenoxy]methyl]phenyl]sulfonyl]amino]pyrimidine-5-carbonyl]amino]ethoxy]ethoxy]acetic acid		2-[2-[2-[2-[2-[6-[4-(10-tert-butoxy-10-oxo-decyl)phenyl]sulfonyl]amino]pyridine-3-carbonyl]amino]ethoxy]ethoxy]acetic acid
				

2.9.2 Synthesis of 2-[2-[2-[2-[2-[2-[6-[[5-[[4-(16-*tert*-butoxy-16-oxo-hexadecoxy)phenyl] sulfonylamino] pyrimidine-2-carbonyl]amino]-hexanoylamino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy] acetic acid



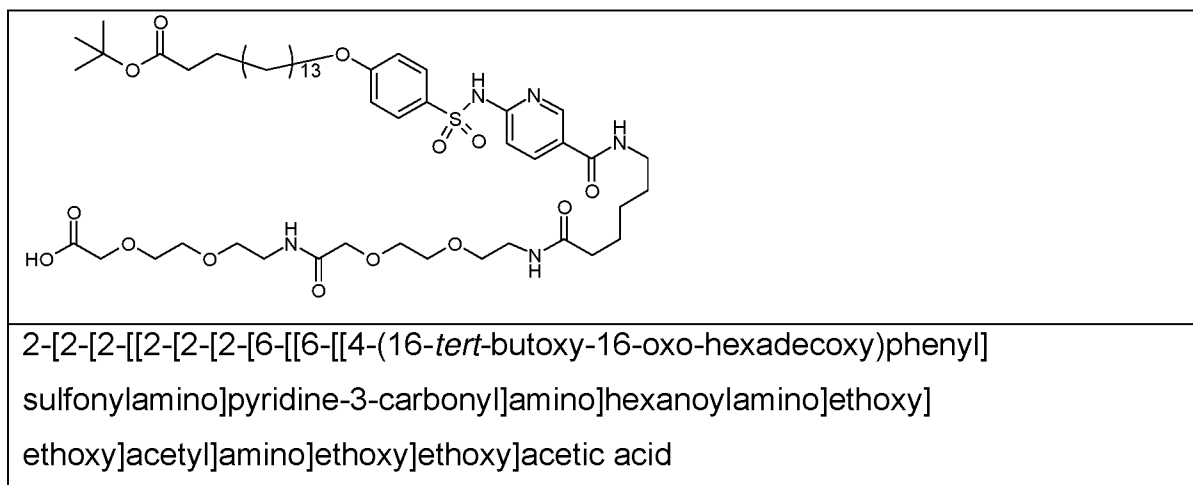
- 5 A mixture of 5-[[4-(16-*tert*-butoxy-16-oxo-hexadecoxy)phenyl]sulfonylamino]pyrimidine-2- carboxylic acid (500 mg, 825 μ mol), TSTU (310 mg, 1.0 mmol) and DIPEA (360 μ l, 266 mg, 2.06 mmol) in 6 ml of THF were stirred at RT for 16h. After 16h the solvent was removed under reduced pressure and a solution of 6-aminohexanoic acid (130 mg, 990 μ mol) and DIPEA
- 10 (360 μ l, 266 mg, 2.06 mmol) in 6 ml abs. EtOH was added and the mixture was stirred at RT for 16h. Volatile components were removed under reduced pressure, the resulting residue dissolved in CH_2Cl_2 and washed with aq. 10% KHSO_4 solution. The organic layer was washed with water and brine, dried over anhydrous Na_2SO_4 , filtered and concentrated under reduced pressure. 900mg of obtained crude 6-[[5-[[4-
- 15 (16-*tert*-butoxy-16-oxo-hexadecoxy)phenyl] sulfonylamino] pyrimidine-2-carbonyl]amino]hexanoic acid were used in the next step without further purification.

- A mixture of 6-[[5-[[4-(16-*tert*-butoxy-16-oxo-hexadecoxy)phenyl]sulfonylamino]pyrimidine-2-carbonyl]amino]hexanoic acid (900 mg crude, 65% purity, 814 μ mol),
- 20 TSTU (306 mg, 1.02 mmol) and DIPEA (355 μ l, 262 mg, 2.03 mmol) in 6 ml of THF

were stirred at RT for 16h. After 16h the solvent was removed under reduced pressure and a solution of 2-[2-[2-[2-[2-(2-aminoethoxy)ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid (301 mg, 976 μ mol) and DIPEA (355 μ l, 262 mg, 2.03 mmol) in 6 ml abs. EtOH was added and the mixture was stirred at RT for 16h. Volatile components were removed under reduced pressure, the resulting residue dissolved in CH₂Cl₂ and washed with aq. 10% KHSO₄ solution. The organic layer was washed with water and brine, dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. The crude product was purified by RP HPLC to afford 2-[2-[2-[2-[2-[2-[6-[[5-[[4-(16-*tert*-butoxy-16-oxo-hexadecoxy)phenyl]sulfonylamino]pyrimidine-2-carbonyl]amino]hexanoylamino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid (78 mg, 10 %).

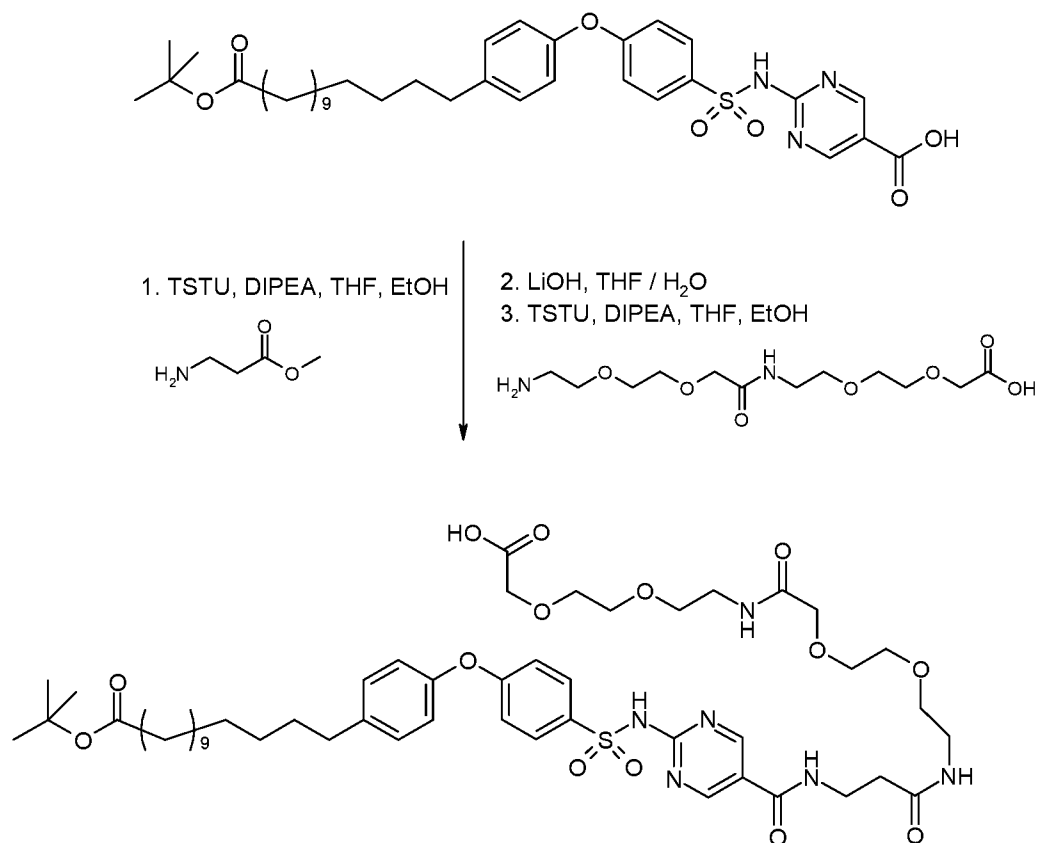
¹H NMR (400.23 MHz, DMSO-d₆) δ ppm 12.29 (br s, 1 H), 8.82 (s, 2 H), 8.47 (br s, 1 H), 7.90 (d, J=8.93 Hz, 2 H), 7.79 (t, J=5.50 Hz, 1 H), 7.63 (t, J=5.75 Hz, 1 H), 7.07 (d, J=8.93 Hz, 2 H), 4.01 (m, 4 H), 3.87 (s, 2 H), 3.20 – 3.68 (m, 18 H), 2.15 (t, J=7.27 Hz, 2 H), 2.05 (t, J=7.34 Hz, 2 H), 1.70 (m, 2 H), 1.48 (m, 6 H), 1.38 (s, 9 H), 1.25 (m, 24 H).

Following compound was synthesized accordingly:

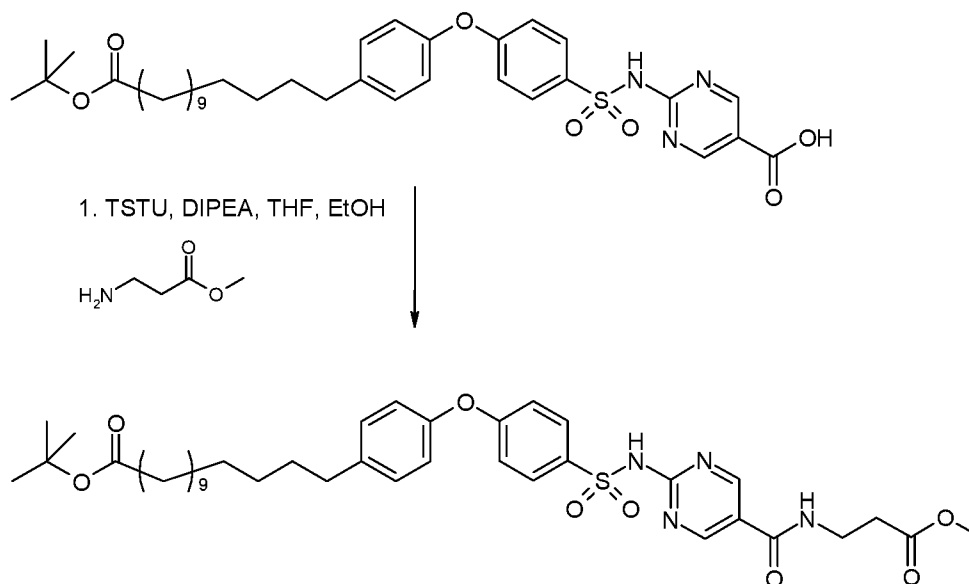


2.10 Incorporation of (2)

2.10.1 Synthesis of 2-[2-[2-[2-[2-[2-[3-[5-[4-[4-(14-*tert*-butoxy-14-oxo-tetradecyl)phenoxy] phenyl]sulfonyl amino]pyrimidine-2-carbonyl]-amino]propanoylamino]ethoxy]ethoxy]acetyl] amino]ethoxy] ethoxy]acetic acid



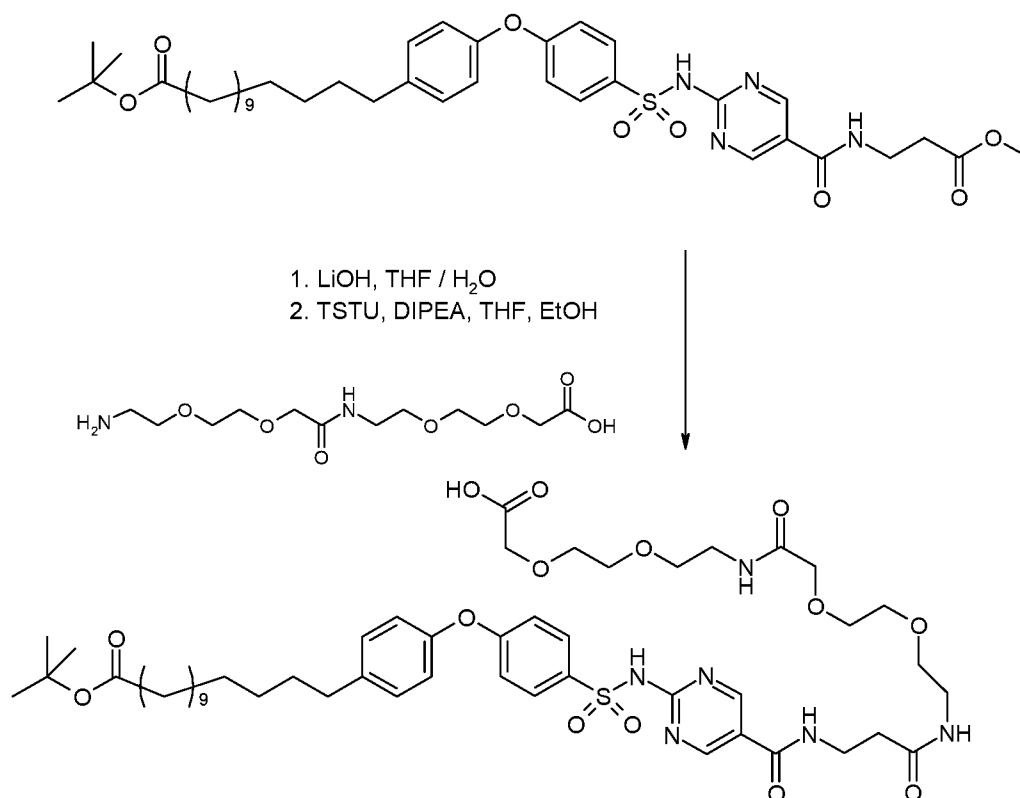
2.10.2 Synthesis of *tert*-butyl 14-[4-[4-[5-[[(3-methoxy-3-oxo-propyl)carbamoyl] pyrimidin-2-yl] sulfamoyl] phenoxy]phenyl]tetradecanoate



A mixture of 5-[[4-[4-(14-*tert*-butoxy-14-oxo-tetradecyl)phenoxy]phenyl]sulfonyl amino]pyrimidine-2-carboxylic acid (1.0 g, 764 μ mol), TSTU (241 mg, 803 μ mol) and DIPEA (494 mg, 3.82 mmol) in 10 ml of THF were stirred at RT for 16h. Additional TSTU was added (80 mg, 267 μ mol) and stirring at RT was continued for 2h. Methyl 3-aminopropanoate hydrochloride (117 mg, 841 μ mol) was added and stirring at RT was continued for 16h. Volatile components were removed under reduced pressure, the resulting residue dissolved in CH₂Cl₂ and washed with aq. 10% KHSO₄ solution. The organic layer was washed with water and brine, dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. The crude product was purified by RP preparative HPLC to afford 14-[4-[4-[[5-[(3-methoxy-3-oxo-propyl) carbamoyl] pyrimidin-2-yl]sulfamoyl]phenoxy]phenyl] tetradecanoate (235mg, 42%).

¹H NMR (400.23 MHz, DMSO-d₆) δ ppm 12.11 (br s, 1 H), 8.84 (s, 2 H), 8.66 (t, J=5.44 Hz, 1 H), 7.98 (d, J=8.93 Hz, 2 H), 7.26 (d, J=8.44 Hz, 2 H), 7.04 (m, 4 H), 3.60 (s, 3 H), 3.46 (m, 2H), 2.57 (m, 4 H), 2.15 (t, J=7.27 Hz, 2 H), 1.56 (m, 2 H), 1.46 (m, 2 H), 1.38 (s, 9 H), 1.29 (m, 18 H).

2.10.3 Synthesis of 2-[2-[2-[[2-[2-[2-[3-[[5-[[4-[4-(14-*tert*-butoxy-14-oxo-tetradecyl)phenoxy] phenyl]sulfonyl amino]pyrimidine-2-carbonyl]amino]propanoylamino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid



A mixture of 14-[4-[4-[[5-[(3-methoxy-3-oxo-propyl) carbamoyl] pyrimidin-2-yl]sulfamoyl] phenoxy]phenyl] tetradecanoate (235 mg, 318 μ mol), LiOH (38 mg, 1.59 mmol), THF (5 ml) and H₂O (5 ml) was stirred at RT for 2h. The reaction mixture was acidified to approx. pH = 1.0 with HCl (2.0 M) and extracted with CH₂Cl₂. The organic layer was washed with brine dried with Na₂SO₄, filtered and concentrated to afford 3-[[5-[[4-[4-(14-*tert*-butoxy -14-oxo-tetradecyl)phenoxy]phenyl]sulfonylamino]pyrimidine-2-carbonyl]amino]propanoic acid (207mg, 89% yield) as a white solid, which was used in the next reaction without further purification.

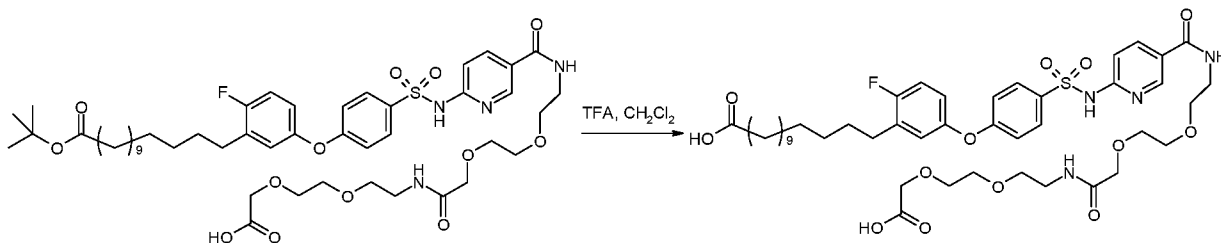
A mixture of 3-[[5-[[4-[4-(14-*tert*-butoxy -14-oxo-tetradecyl)phenoxy]phenyl]sulfonylamino]pyrimidine-2-carbonyl]amino]propanoic acid (207mg, 285 μ mol), TSTU (90 mg, 300 μ mol) and DIPEA (150 μ l, 110 mg, 850 μ mol) in 6 ml of THF were stirred at RT for 1h. After 1h the solvent was removed under reduced pressure and a solution of 2-[2-[2-[[2-[2-(2-aminoethoxy)ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid (97 mg, 314 μ mol) and DIPEA (150 μ l, 110 mg, 850 μ mol) in 6 ml abs. EtOH was added and the mixture was stirred at RT for 16h. Volatile components were removed under reduced pressure, the resulting residue dissolved in CH₂Cl₂ and washed with aq. 10% KHSO₄ solution. The organic layer was washed with water and brine, dried over

anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. The crude product was purified by RP HPLC to afford 2-[2-[2-[[2-[2-[2-[3-[[5-[[4-[4-(14-*tert*-butoxy-14-oxo-tetradecyl)phenoxy]phenyl]sulfonylamino]pyrimidine-2-carbonyl]amino]propanoylamino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid (163mg, 56%).

¹H NMR (400.23 MHz, DMSO-d₆) δ ppm 12.11 (br s, 2 H), 8.84 (s, 2 H), 8.61 (t, J=5.62 Hz, 1 H), 7.97 (m, 3 H), 7.62 (t, J=5.56 Hz, 1 H), 7.26 (d, J=8.44 Hz, 2 H), 7.04 (m, 4 H), 4.01 (s, 2 H), 3.86 (s, 2 H), 3.20 – 3.60 (m, 18 H), 2.58 (m, 2 H), 2.34 (t, J=7.03 Hz, 2 H), 2.15 (t, J=7.27 Hz, 2 H), 1.56 (m, 2 H), 1.46 (m, 2 H), 1.38 (s, 9 H), 1.29 (m, 18 H).

2.11 Deprotection

Synthesis of 14-[5-[4-[[5-[2-[2-[2-[2-[2-(carboxymethyloxy)ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy] ethylcarbamoyl]-2-pyridyl]sulfamoyl]phenoxy]-2-fluorophenyl]tetradecanoic acid

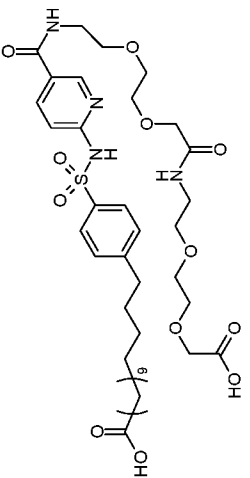
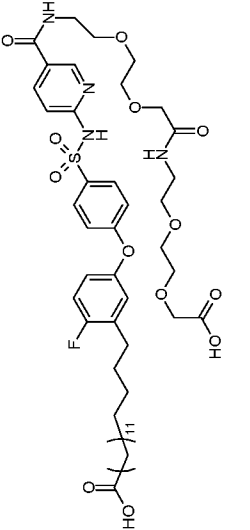
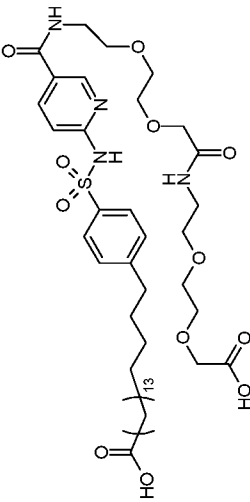
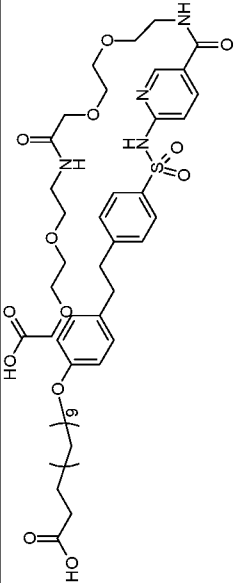
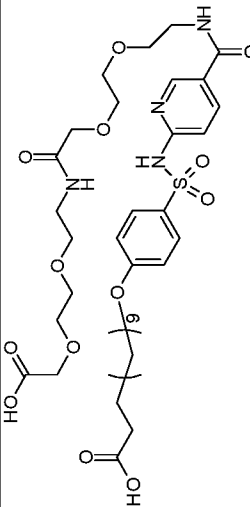
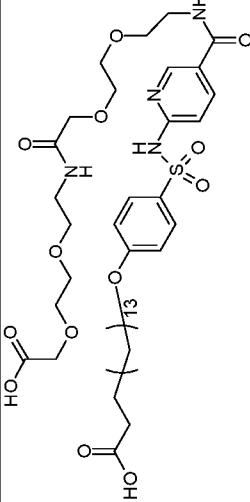


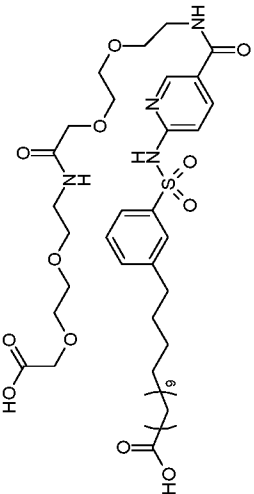
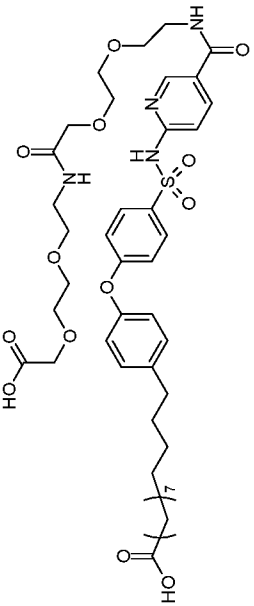
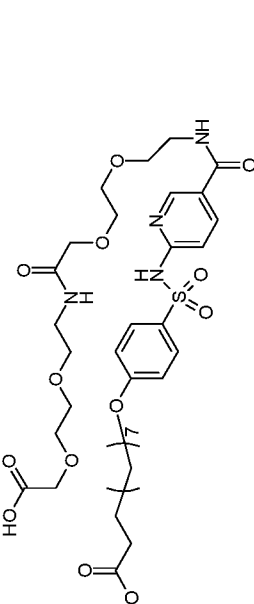
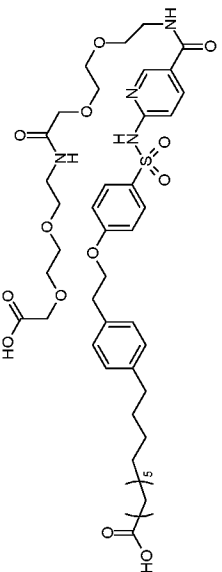
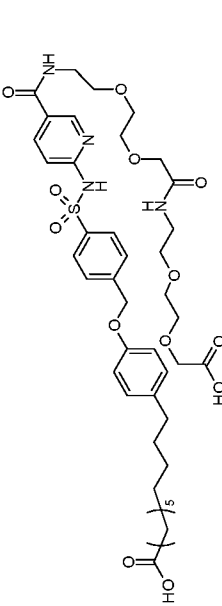
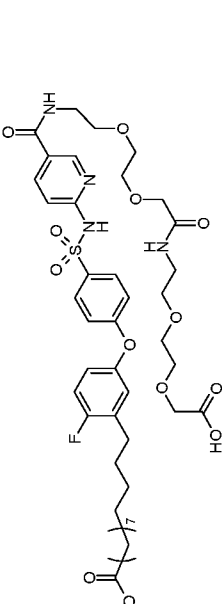
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¹H NMR (400.23 MHz, DMSO-d₆) δ ppm 12.19 (br s, 1 H), 8.51 (m, 2 H), 8.09 (dd, J=8.93, 2.32 Hz, 1 H), 7.89 (d, J=8.93 Hz, 2 H), 7.61 (br t, J=5.56 Hz, 1 H), 7.20 (t, J=8.93 Hz, 1 H), 7.15 (d, J=8.19 Hz, 1 H), 7.03 (m, 4 H), 4.01 (s, 2 H), 3.86 (s, 2 H),

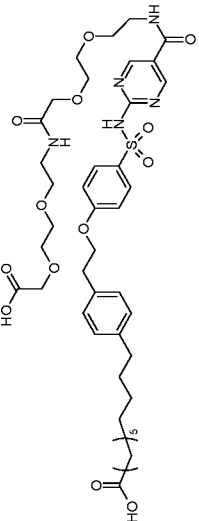
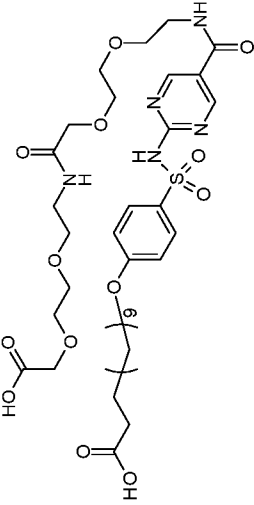
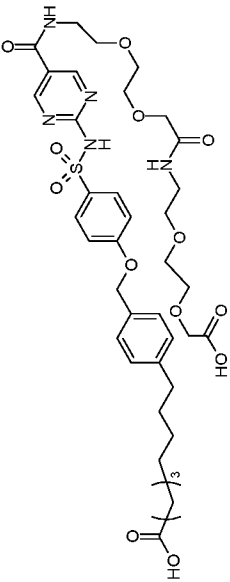
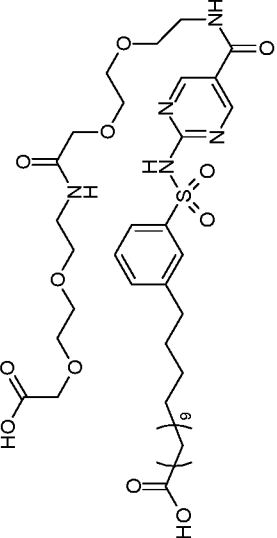
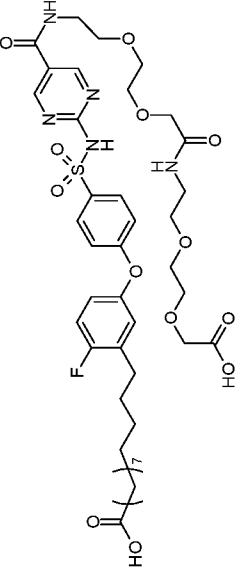
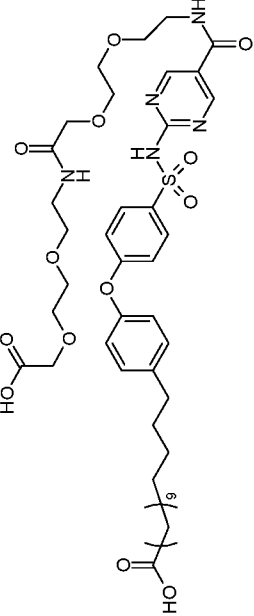
3.20 – 3.68 (m, 16 H), 2.58 (br t, J=7.52 Hz, 2 H), 2.17 (t, J=7.34 Hz, 2 H), 1.49 (m, 4 H), 1.25 (m, 18 H).

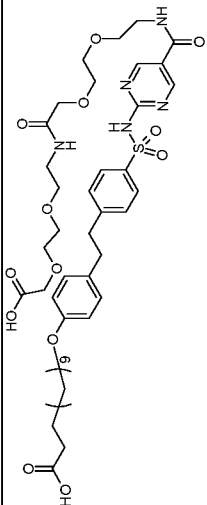
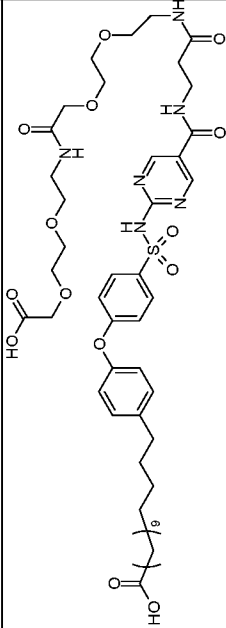
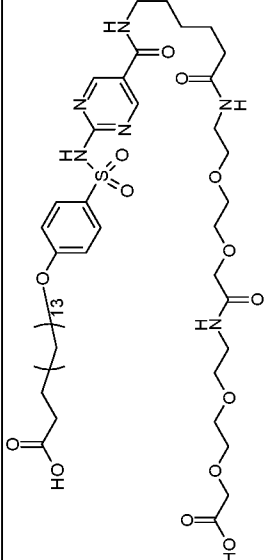
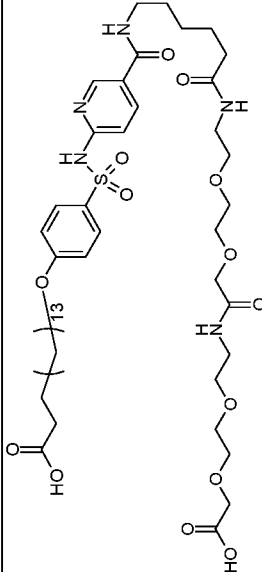
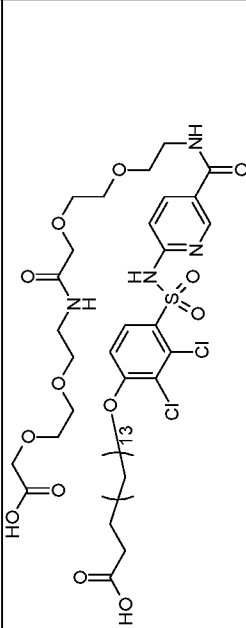
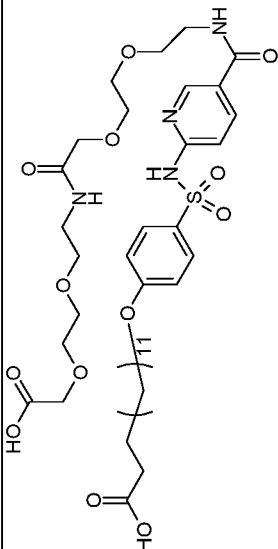
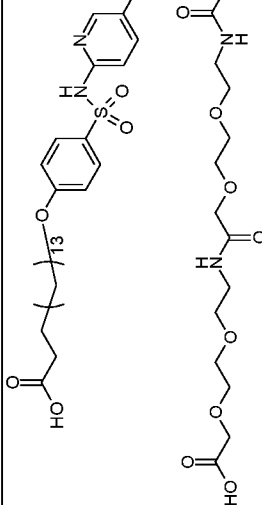
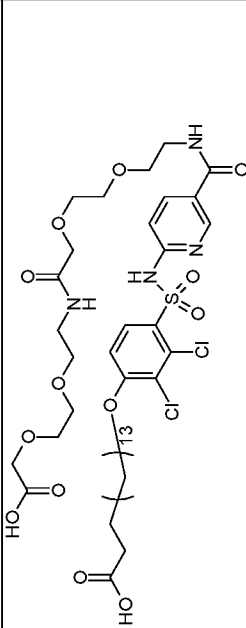
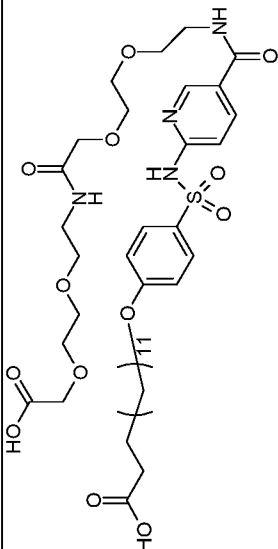
Following compounds were synthesized accordingly:

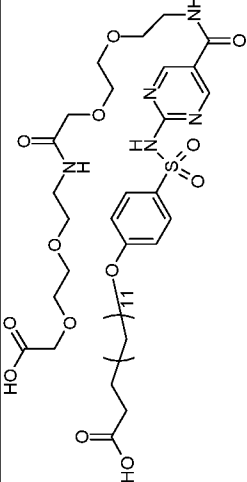
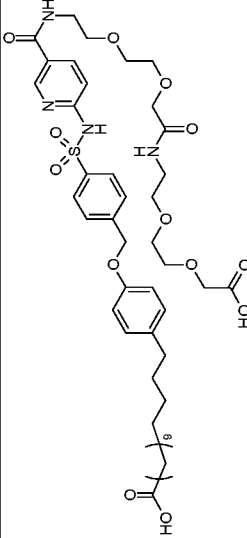
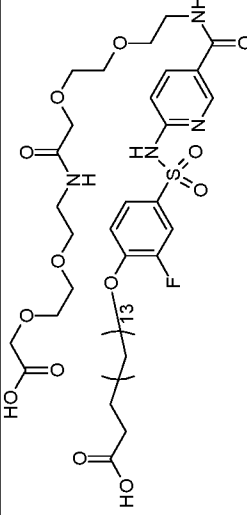
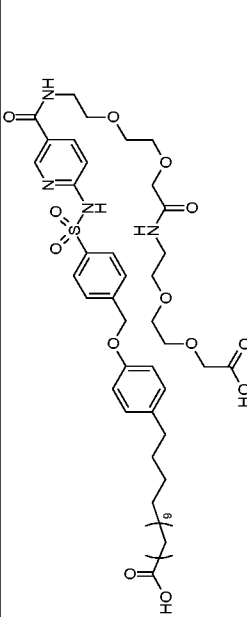
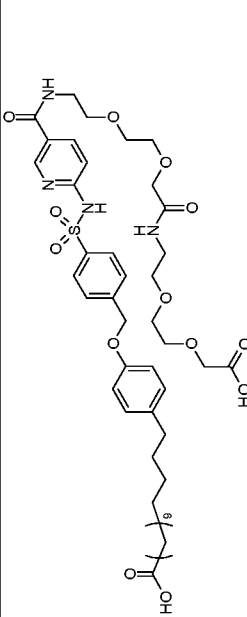
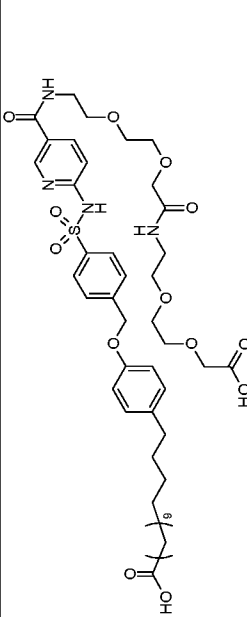
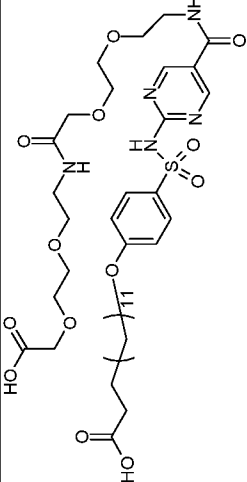
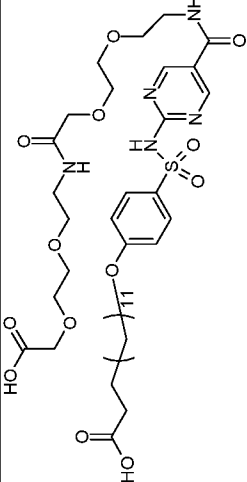
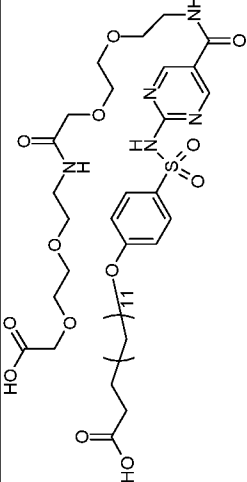
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	14-[3-[[5-[2-[2-[2-[2-[(carboxymethyloxy)ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy] ethylcarbamoyl]-2-pyridyl] sulfamoyl]phenyl]tetradecanoic acid		12-[4-[4-[[5-[2-[2-[2-[2-[(carboxymethyloxy)ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy] ethylcarbamoyl]-2-pyridyl] sulfamoyl]phenoxy]phenyl]dodecanoic acid		10-[4-[[5-[2-[2-[2-[2-[(carboxymethyloxy)ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy] ethylcarbamoyl]-2-pyridyl] sulfamoyl]phenoxy]phenyl]dodecanoic acid
	10-[4-[2-[4-[[5-[2-[2-[2-[2-[(carboxymethyloxy)ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy] ethylcarbamoyl]-2-pyridyl] sulfamoyl]phenyl]decanoic acid		10-[4-[4-[[5-[2-[2-[2-[2-[(carboxymethyloxy)ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy] ethylcarbamoyl]-2-pyridyl] sulfamoyl]phenoxy]phenyl]methoxy]phenyl]decanoic acid		12-[5-[4-[[5-[2-[2-[2-[2-[(carboxymethyloxy)ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy] ethylcarbamoyl]-2-pyridyl] sulfamoyl]phenoxy]phenyl]dodecanoic acid

	16-[4-[5-[2-[2-[2-[2-[2-(carboxymethyloxy)ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy]ethylcarbamoylethylpyrimidin-2-yl)sulfamoyl]phenoxy]hexadecanoic acid
	16-[5-[4-[5-[2-[2-[2-[2-(carboxymethyloxy)ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy]ethylcarbamoylethylpyrimidin-2-yl)sulfamoyl]phenoxy]-2-fluorophenyl]hexadecanoic acid
	14-[4-[5-[2-[2-[2-[2-(carboxymethyloxy)ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy]ethylcarbamoylethylpyrimidin-2-yl)sulfamoyl]phenyl]tetradecanoic acid
	14-[5-[4-[5-[2-[2-[2-[2-(carboxymethyloxy)ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy]ethylcarbamoylethylpyrimidin-2-yl)sulfamoyl]phenoxy]-2-fluorophenyl]hexadecanoic acid
	18-[4-[5-[2-[2-[2-[2-(carboxymethyloxy)ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy]ethylcarbamoylethylpyrimidin-2-yl)sulfamoyl]phenyl]octadecanoic acid
	10-[4-[4-[5-[2-[2-[2-[2-(carboxymethyloxy)ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy]ethylcarbamoylethylpyrimidin-2-yl)sulfamoyl]phenyl]methoxy]phenyl]decanoic acid

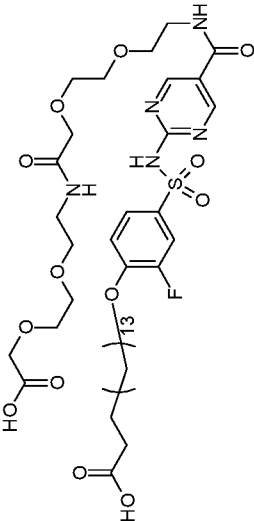
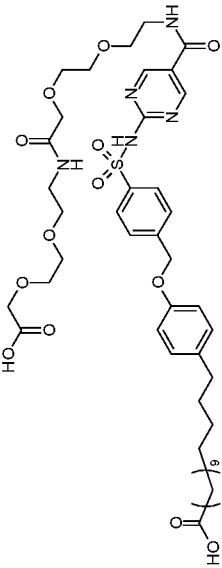
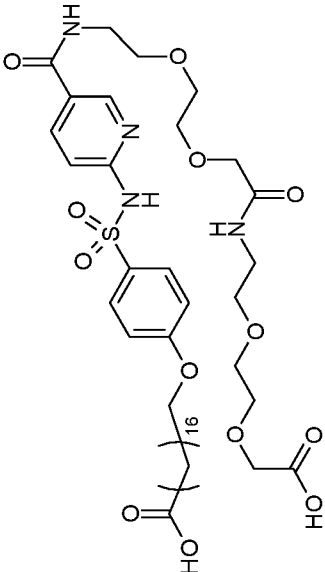
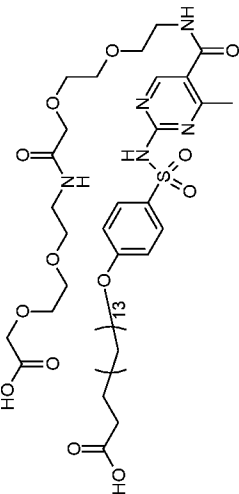
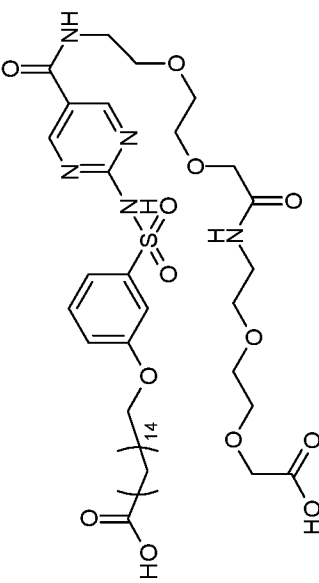
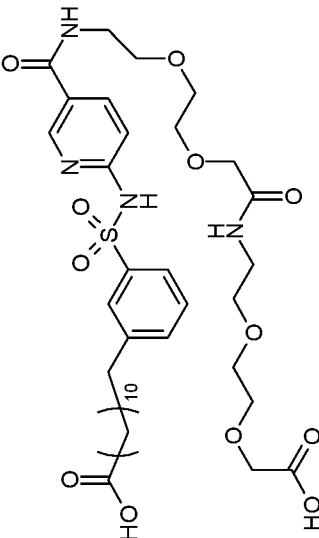
phenyl]tetradecanoic acid		oic acid
		
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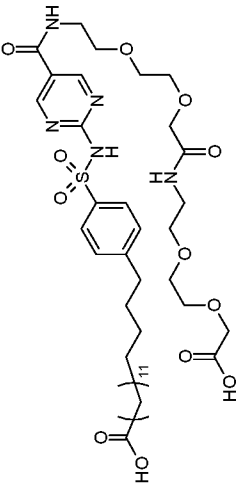
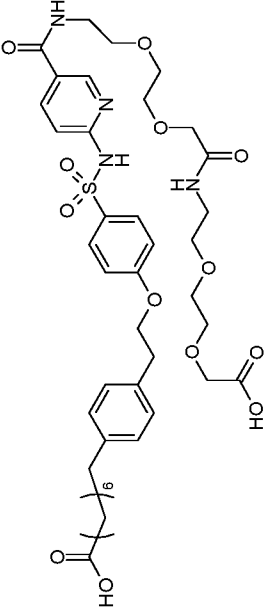
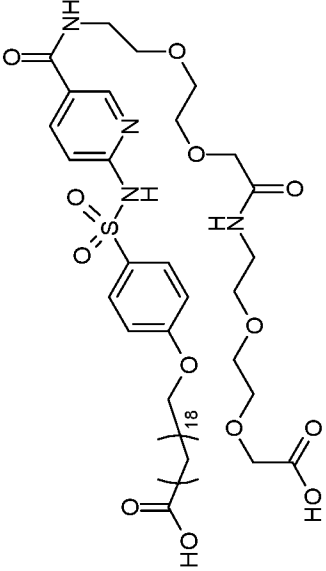
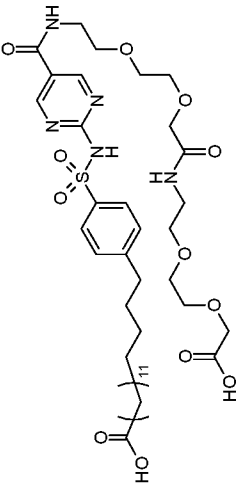
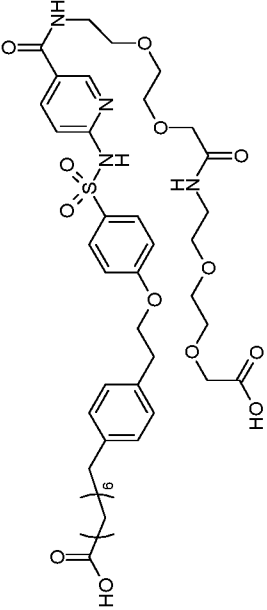
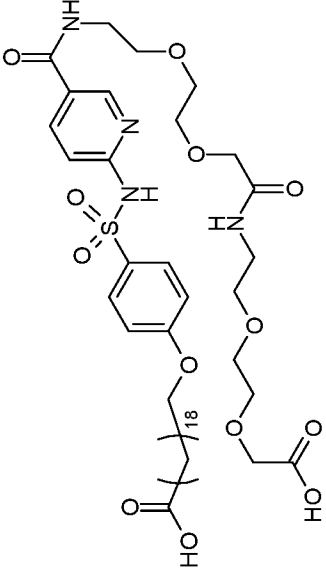
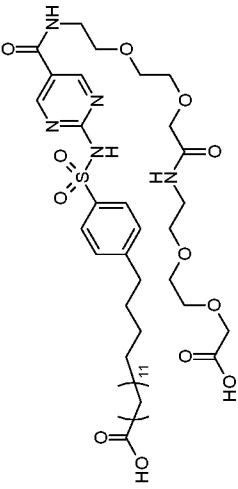
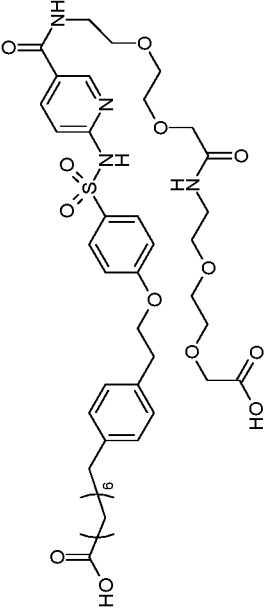
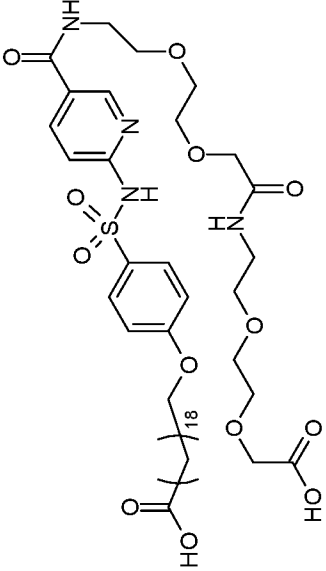
ethylcarbamoyl]pyrimidin-2-yl] sulfamoyl]phenyl[tetradecanoic acid	ethylcarbamoyl]pyrimidin-2-yl] sulfamoyl]phenoxy]phenyl[tetradecanoic acid	ethylcarbamoyl]pyrimidin-2-yl] sulfamoyl]phenyl[dodecanoic acid
		
12-[4-[2-[4-[5-[2-[2-[2-[2- (carboxymethoxy]ethoxy]ethylamino]-2- oxo-ethoxy]ethoxy] ethylcarbamoyl]pyrimidin-2- yl]sulfamoyl]phenyl]ethyl]phenoxy] dodecanoic acid	14-[4-[4-[5-[3-[2-[2-[2-[2- (carboxymethoxy]ethoxy]ethylamino]-2- oxo-ethoxy]ethoxy]ethylamino]-3-oxo- propyl]carbamoyl]pyrimidin-2- yl]sulfamoyl]phenoxy]phenyl[tetradecanoic acid	16-[4-[4-[5-[6-[2-[2-[2-[2-[2- (carboxymethoxy]ethoxy]ethylamino]-2- oxo-ethoxy]ethoxy] ethylamino]-6-oxo- hexyl] carbamoyl]pyrimidin-2-yl] sulfamoyl]phenoxy]hexadecanoic acid
		
16-[4-[4-[5-[6-[2-[2-[2-[2- (carboxymethoxy]ethoxy]ethylamino]-2- oxo-ethoxy]ethoxy]ethylamino]-2- yl] sulfamoyl]phenyl]ethyl]phenoxy] dodecanoic acid	16-[4-[4-[5-[5-[2-[2-[2-[2- (carboxymethoxy]ethoxy]ethylamino]-2- oxo-ethoxy]ethoxy]ethylamino]-2- yl] sulfamoyl]phenyl]ethyl]phenoxy] dodecanoic acid	14-[4-[4-[5-[2-[2-[2-[2- (carboxymethoxy]ethoxy]ethylamino]-2- oxo-ethoxy]ethoxy]ethylamino]-2- yl] sulfamoyl]phenyl]ethyl]phenoxy] dodecanoic acid
		

oxo-ethoxy[ethoxy]ethylamino]-6-oxo-hexyl]carbamoyle]-2-pyridyl]sulfamoyl]phenoxy]hexadecanoic acid		oxo-ethoxy[ethoxy]ethylamino]-2-pyridyl]sulfamoyl]-2,3-dichlorophenoxy]hexadecanoic acid		oxo-ethoxy[ethoxy]ethylamino]-2-pyridyl]sulfamoyl]phenoxy]tetradecanoic acid	
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16-[4-[5-[2-[2-[2-[2-(carboxymethoxy)ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy]ethylcarbamoyle]-2-pyridyl]sulfamoyl]phenyl]methoxy]phenyl]tetradecanoic acid		16-[4-[5-[2-[2-[2-[2-(carboxymethoxy)ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy]ethylcarbamoyle]-2-pyridyl]sulfamoyl]phenyl]methoxy]phenyl]tetradecanoic acid		16-[4-[5-[2-[2-[2-[2-(carboxymethoxy)ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy]ethylcarbamoyle]-2-pyridyl]sulfamoyl]phenyl]methoxy]phenyl]tetradecanoic acid	

oxo-ethoxy[ethoxy]ethyl[carbamoyl]-2-pyridyl[sulfamoyl]-2-chloro-phenoxy]hexadecanoic acid		oxo-ethoxy[ethoxy]ethyl[carbamoyl]-2-pyridyl[sulfamoyl]phenoxy]ethyl]phenyl]octanoic acid	
oxo-ethoxy[ethoxy]ethyl[carbamoyl]-4-(trifluoromethyl)pyrimidin-2-yl[sulfamoyl]phenoxy]hexadecanoic acid		oxo-ethoxy[ethoxy]ethyl[carbamoyl]-4-(trifluoromethyl)pyrimidin-2-yl[sulfamoyl]phenoxy]hexadecanoic acid	
16-[4-[[5-[2-[2-[2-(2-(carboxymethoxy)ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy]ethyl]carbamoyl]pyrimidin-2-yl[sulfamoyl]phenyl]dodecanoic acid		12-[4-[[5-[2-[2-[2-(2-(carboxymethoxy)ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy]ethyl]carbamoyl]pyrimidin-2-yl[sulfamoyl]phenyl]dodecanoic acid	
18-[3-[[5-[2-[2-[2-[2-(2-(carboxymethoxy)ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy]ethyl]carbamoyl]pyrimidin-2-yl[sulfamoyl]phenyl]hexadecanoic acid		18-[3-[[5-[2-[2-[2-[2-(2-(carboxymethoxy)ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy]ethyl]carbamoyl]pyrimidin-2-yl[sulfamoyl]phenyl]hexadecanoic acid	

(carboxymethyloxy)ethoxy[ethylamino]-2-oxo-ethoxy[ethoxy]ethylcarbamoyl]pyrimidin-2-yl[sulfamoyl]phenoxy]-2-fluorophenyl]decanoic acid		(carboxymethyloxy)ethoxy[ethylamino]-2-oxo-ethoxy[ethoxy]ethylcarbamoyl]pyrimidin-2-yl[sulfamoyl]phenoxy]-2-fluorophenyl]decanoic acid	
(carboxymethyloxy)ethoxy[ethylamino]-2-oxo-ethoxy[ethoxy]ethylcarbamoyl]pyrimidin-2-yl[sulfamoyl]phenyl]octadecanoic acid		(carboxymethyloxy)ethoxy[ethylamino]-2-oxo-ethoxy[ethoxy]ethylcarbamoyl]pyrimidin-2-yl[sulfamoyl]phenyl]octadecanoic acid	
(carboxymethyloxy)ethoxy[ethylamino]-2-oxo-ethoxy[ethoxy]ethylcarbamoyl]pyrimidin-2-yl[sulfamoyl]phenyl]octadecanoic acid		(carboxymethyloxy)ethoxy[ethylamino]-2-oxo-ethoxy[ethoxy]ethylcarbamoyl]pyrimidin-2-yl[sulfamoyl]phenyl]octadecanoic acid	
14-[4-[4-[5-[2-[2-[2-[2-(carboxymethyloxy)ethoxy]ethylamino]-2-oxo-ethoxy]ethylcarbamoyl]-2-pyridyl]sulfamoyl]phenoxy]phenyl]tetradecanoic acid		14-[4-[4-[5-[2-[2-[2-[2-(carboxymethyloxy)ethoxy]ethylamino]-2-oxo-ethoxy]ethylcarbamoyl]-2-pyridyl]sulfamoyl]phenyl]tetradecanoic acid	

	16-[4-[[5-[2-[2-[2-[2-(carboxymethoxy)ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy]ethylcarbamoyl]-pyrimidin-2-yl]sulfamoyl]-2-fluorophenoxy]hexadecanoic acid		14-[4-[[5-[2-[2-[2-(carboxymethoxy)ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy]ethylcarbamoyl]pyrimidin-2-yl]sulfamoyl]phenyl]methoxy]phenyl]tetradecanoic acid		18-[4-[[5-[2-[2-[2-(carboxymethoxy)ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy]ethylcarbamoyl]-2-pyridyl]sulfamoyl]phenoxy]octadecanoic acid
	16-[4-[[5-[2-[2-[2-(carboxymethoxy)ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy]ethylcarbamoyl]-4-methylpyrimidin-2-yl]sulfamoyl]phenyl]dodecanoic acid		16-[3-[[5-[2-[2-[2-(carboxymethoxy)ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy]ethylcarbamoyl]pyrimidin-2-yl]sulfamoyl]phenoxy]hexadecanoic acid		12-[3-[[5-[2-[2-[2-(carboxymethoxy)ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy]ethylcarbamoyl]-2-pyridyl]sulfamoyl]phenyl]dodecanoic acid

y][sulfamoyl]phenoxy]hexadecanoic acid			
16-[4-[5-[2-[2-[2-(carboxymethyloxy)ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy]ethyl]carbamoylethyl]pyrimidin-2-yl][sulfamoyl]phenyl]hexadecanoic acid			
			

8-[4-[2-[4-[5-[2-[2-[2-[2-(carboxymethoxy)ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy]ethylcarbamoyl]pyrimidin-2-yl]sulfamoyl]phenoxy]ethyl]-phenyl]octanoic acid		10-[4-[[5-[2-[2-[2-[2-(2-(carboxymethoxyloxy)ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy]ethylcarbamoyl]pyrimidin-2-yl]sulfamoyl]phenyl]decanoic acid
10-[4-[5-[2-[2-[2-[2-(2-(carboxymethoxyloxy)ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy]ethylcarbamoyl]-2-pyridyl]sulfamoyl]phenyl]decanoic acid		18-[4-[[5-[2-[2-[2-[2-(2-(carboxymethoxyloxy)ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy]ethylcarbamoyl]pyrimidin-2-yl]sulfamoyl]phenoxy]octadecanoic acid
8-[5-[4-[5-[2-[2-[2-[2-(carboxymethoxyloxy)ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy]ethylcarbamoyl]pyrimidin-2-yl]sulfamoyl]phenoxy]-2-fluorophenyl]octanoic acid		

3. Insulins and Conjugate synthesis

3.1 human insulin

5 The amino acid sequences of the A and B chain of human insulin are:

A-chain: GIVEQCCTSICSLYQLENYCN (SEQ ID NO: 5)

B-chain: FVNQHLCGSHLVEALYLVCGERGFFYTPKT (SEQ ID NO:6)

10 An intrachenar disulfide bridge is present between *Cys(A6)* and *Cys(A11)*, two interchenar disulfide brigdes are present between *Cys(A7)* and *Cys(B7)* and between *Cys(A20)* and (*Cys(B19)*).

3.2 Insulin analog 1

15

Insulin analog 1 is based on human insulin with mutations in positions A14, B16, B25 and a removal of the amino acid at position B30:

20 *Glu(A14)*: The amino acid at position 14 of the A-chain of human insulin (Y, tyrosine, Tyr) is substituted by glutamic acid (E, Glu),

His(B16): The amino acid at position 16 of the B-chain of human insulin (Y, tyrosine, Tyr) is substituted by histidine (H, His),

His(B25):The amino acid at position 25 of the B-chain of human insulin (F, phenylalanine, Phe) is substituted by histidine (H, His),

25 *Des(B30)*:The amino acid at position 30 of the B-chain of human insulin is deleted.

The complete amino acid sequence of insulin analog 1 in view of A and B chain is:

A-chain: GIVEQCCTSICSLQLENYCN (SEQ ID NO: 7)

30 *B-chain*: FVNQHLCGSHLVEALHLVCGERGFFHYTPK- (SEQ ID NO: 8)

The one intrachenar and the two interchenar disulfide bridges are in accordance with human insulin.

3.3 Conjugate with human insulin / Synthesis of [16-[4-[[5-[2-[2-[2-[2-
(carboxymethyloxy)ethoxy]ethylamino]-2-oxo-
ethoxy]ethoxy]ethylcarbamoyl]pyrimidin-2-yl]sulfamoyl]phenoxy]hexadecanoic
acid]Lys(B29)-insulin

5

A conjugate was prepared from human insulin according to 3.1 and 2-[2-[2-[[2-[2-[2-
[[2-[[4-(16-tert-butoxy-16-oxo-hexadecoxy) phenyl]sulfonylamino]pyrimidine-5-
carbonyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid from Example
2.9:

10

Synthesis of 16-[4-[[5-[2-[2-[2-[2-[2-(2,5-dioxopyrrolidin-1-yl) oxy-2-oxo-ethoxy]
ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy]ethylcarbamoyl]pyrimidin-2-
yl]sulfamoyl]phenoxy]hexadecanoate:

15 To a solution of 296 mg 2-[2-[2-[[2-[2-[2-[[2-[[4-(16-tert-butoxy-16-oxo-hexadecoxy)
phenyl]sulfonylamino]pyrimidine-5-carbonyl]amino]ethoxy]ethoxy]acetyl]amino]-
ethoxy]ethoxy]acetic acid in 9 ml DMF, 92.7 µl triethylamine, 106 mg TSTU and a
trace of DMAP were added. The solution was stirred for one hour.

100 ml methylene chloride were added and the resulting solution was washed three
20 times with 50 ml brine. The organic layer was separated, dried with sodium sulfate,
filtered and concentrated in vacuo. The crude product was taken up in 11 ml
methylene chloride and 5.5 ml trifluoro acetic acid and stored overnight in at 5 °C:

The solution was concentrated. Then, the crude product was three times dissolved in
25 30 ml methylene chloride and evaporated. The solid material was suspended in 5 ml
methyl tert-butyl ether, the ether decanted. The residue was dried in vacuo and used
without further purification.

A solution of 480 mg insulin was suspended in 25 ml water and then 0.45 ml
30 triethylamine was added. To the clear solution 25 ml MeCN and then 0.9ml (45.89
mM in DMF) 16-[4-[[5-[2-[2-[2-[2-[2-(2,5-dioxopyrrolidin-1-yl)oxy-2-oxo-
ethoxy]ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy]ethylcarbamoyl]pyrimidin-2-
yl]sulfamoyl]phenoxy]hexadecanoate were added. The solution was stirred for 3
hours at roomtemperature. The reaction was analyzed with waters UPLC H-class at

214 nm in a sodium chloride phosphate buffer. Waters BEH300 10 cm. Retention time insulin: 3.85 min. Retention time insulin conjugate 6.46 min. The product was purified by HPLC with ÄKTA avant 25. Kinetex 5 µm C18 100 Å 250 x 21.2 mm. Column volume (CV) 88 ml.

5 Column volume (CV) 88 ml.

Solvent A: 0.5% acetic acid in water

Solvent B: 0.5 acetic acid in water / MeCN 2 : 8

Gradient: 95 % A 5 % B to 40 % A 60 % B in 14 CV

The reaction was analyzed with Waters UPLC H-class at 214 nm in a sodium
10 chloride phosphate buffer. Waters BEH300 10 cm. Retention time insulin conjugate:
6.419 min. The solution was lyophilized and gave the desired product. 93 mg 34 %
yield. Mass spec.: 6629.6 g / mol.

3.4 Conjugates with insulin analog 1

15

Conjugates of insulin analog 1 according to 3.2 were prepared with binder molecules from Example 2.9:

Binder 5: 16-[4-[[5-[2-[2-[2-[2-[2-(carboxymethyloxy)ethoxy]ethylamino]-2-oxo-
ethoxy]ethoxy] ethylcarbamoyl]pyrimidin-2-yl]

20

sulfamoyl]phenoxy]hexadecanoic acid;

tert-butyl ester:

2-[2-[2-[[2-[2-[2-[2-[4-(16-tert-butoxy-16-oxo-hexadecoxy)
phenyl]sulfonylamino]pyrimidine-5-

carbonyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic

25

Binder 8: 14-[5-[4-[[5-[2-[2-[2-[2-[2-(carboxymethyloxy)ethoxy]ethylamino]-2-oxo-
ethoxy]ethoxy] ethylcarbamoyl]pyrimidin-2-yl]sulfamoyl]phenoxy]-2-
fluoro-phenyl]tetradecanoic acid; tert-butyl ester:

2-[2-[2-[[2-[2-[2-[2-[5-[4-[3-(14-tert-butoxy-14-oxo-tetradecyl)-4-fluoro-
phenoxy]phenyl] sulfonylamino] pyrimidine-2-carbonyl]amino]

30

ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid

Binder 50: 16-[4-[[5-[2-[2-[2-[2-[2-(carboxymethyloxy)ethoxy]ethylamino]-2-oxo-
ethoxy]ethoxy]ethylcarbamoyl]pyrimidin-2-yl]sulfamoyl]-2-chloro-
phenoxy]hexadecanoic acid; tert-butyl ester:

2-[2-[2-[[2-[2-[2-[[2-[4-(16-*tert*-butoxy-16-oxo-hexadecoxy)-3-chloro-phenyl]sulfonylamino]pyrimidine-5-carbonyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid

Binder 54: 16-[4-[[5-[2-[2-[2-[2-[2-(carboxymethyloxy)ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy]ethylcarbamoyl]pyrimidin-2-yl]sulfamoyl]phenyl]hexadecanoic acid; and *tert*-butyl ester:

2-[2-[2-[[2-[2-[2-[[2-[4-(16-*tert*-butoxy-16-oxo-hexadecyl)phenyl]sulfonylamino]pyrimidine-5-carbonyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic acid

3.4.1 Synthesis of Glu(A14)His(B16)His(B25)[16-[4-[[5-[2-[2-[2-[2-(carboxymethyloxy)ethoxy]ethylamino]-2-oxoethoxy]ethoxy]-ethylcarbamoyl]pyrimidin-2-yl]sulfamoyl]phenoxy]hexadecanoic acid]Lys(B29)Des(B30)-insulin

An amide bond was formed between the ϵ -amino group of lysine B29 and the activated acetic acid residue of the binder in its *tert*-butyl ester form 2-[2-[2-[[2-[2-[2-[[2-[4-(16-*tert*-butoxy-16-oxo-hexadecoxy) phenyl]sulfonylamino]pyrimidine-5-carbonyl]amino]ethoxy]ethoxy]acetyl]amino]ethoxy]ethoxy]acetic as follows:

A solution of 400 mg of insulin analog 1 (Glu(A14)His(B16)His(B25)Des(B30)-insulin according to Example 3.2) was suspended in 20 ml water and then 0.4 ml triethylamine was added. To the clear solution 20 ml DMF and then 5 ml (17.04 mM in DMF) *tert*-butyl 16-[4-[[5-[2-[2-[2-[2-[2-(2,5-dioxopyrrolidin-1-yl)oxy-2-oxo-ethoxy]ethoxy]ethylamino]-2-oxo-ethoxy]ethoxy]ethylcarbamoyl]pyrimidin-2-yl]sulfamoyl]phenoxy]hexadecanoate) were added. The solution was stirred for 2 hours at room temperature. The reaction was analyzed with Waters UPLC H-class at 214 nm in a sodium chloride phosphate buffer.

Waters BEH300 10 cm.

Retention time insulin: 2.643 min.

Retention time insulin conjugate 6.224 min.

The product was purified by HPLC with ÄKTA avant 25.

Kinetex 5 μ m C18 100 A 250 x 21.2 mm. Column volume (CV) 88 ml.

Solvent A: 0.5% acetic acid in water

Solvent B: 0.5% acetic acid in water / MeCN 4 : 6

Gradient: 80 % A 20 % B to 20 % A 80 % B in 10 CV

After lyophilisation of the product, the powder was dissolved in 2 ml trifluor acetic acid. After one hour, the solution was neutralized with diluted sodium bicarbonate.

- 5 The product was purified by HPLC with ÄKTA avant 25. Kinetex 5 µm C18 100 A 250 x 21.2 mm. Column volume (CV) 88 ml.

Solvent A: 0.5% acetic acid in water

Solvent B: 0.5% acetic acid in water / MeCN 4 : 6

Gradient: 70 % A 30 % B to 30 % A 70 % B in 8 CV

- 10 The reaction was analyzed with waters UPLC H-class at 214 nm in a sodium chloride phosphate buffer.

Waters BEH300 10 cm.

Retention time insulin conjugate: 5.121 min.

The solution was lyophilized and gave the desired product.

- 15 63 mg 14 % yield.

Mass spec.: 6453.9 g / mol.

Conjugates of Binders 8, 50 and 54 and insulin analog 1 were prepared accordingly.

- 20 4. Analytical data

4.1 Liquid chromatography mass spectrometry (LCMS) analysis

Mass spectrometry Method	Method description	Mass spectrometry type
a	Waters SQD Single Quadrupol, 0.13s scantime for mass 100-1400	UPLC esi
b	Waters SQD Single Quadrupol, 0.5s scantime for mass 100-1400	UPLC esi

Liquid chromatography Method	Method description
---------------------------------	--------------------

A	Waters ACQUITY UPLC BEH C18 1.7 μ m 2.1x50mm MV Kit; H ₂ O+0.05% TFA:MeCN+0.035% TFA 98:2(0min) to 98:2(0.2min) to 2:98(3.8min) to 2:98(4.3min) to 98:2(4.5min), 1 ml/min 55°C
B	Waters ACQUITY UPLC BEH C18 1.7 μ m 2.1x50mm; H ₂ O+0.05% TFA:MeCN+0.035% TFA 95:5(0min) to 5:95(2min) to 5:95(2.6min) to 95:5(2.7min) to 95:5(3min), 0.9 ml/min 55°C
C	Waters ACQUITY UPLC BEH C18 1.7 μ m 2.1x50mm; H ₂ O+0.05% TFA:MeCN+0.035% TFA 98:2(0min) to 98:2(0.2min) to 2:98(3.8min) to 2:98(4.3min) to 98:2(4.5min), 1 ml/min 55°C

Table 1 in section 4.2 shows the LCMS analysis results of the isolated binders.

4.2 Analysis of albumin binding

5

Instrument: Waters Alliance 2795 / Waters PDA 2996 or or Waters H-Class UPLC equipped with a Waters Acquity photodiode-array detector

Software: Waters Empower 3

Column: CHIRALPAK® HSA 50 x 4 mm; 5 μ m Particle size
Chiraltech Order Numbers: HSA: 34712

Eluent A: Phosphate Buffer saline (PBS) at pH=7.4
Gibco PBS pH7.4 (10x) Phosphate Buffered Saline 500 ml;
Order Number: 70011-036 (500 ml)

Eluent B: iso-propanol
Fisher Order Number: A461-1 (1L)

Gradient:

Time [min]	%A	%B
------------	----	----

0	98	2
2	98	2
17	60	40
22	60	40
22.2	98	2
30	98	2
31	95	5

Column temperature:	25 °C
Flow rate:	1.0 ml/min
Detection:	$\lambda = 220 \text{ nm}$
Injection volume:	20 μL
Sample Conc.:	• 1mg/ ml insulin solution in PBS for insulin samples • 5μL of 10mM DMSO stock solution (DMSO evaporated and re-dissolved in 200 μL i-propanol/water 1:1 v/v) for isolated binder samples (250μM, 0.2mg/ ml at 800Da MolWeight)
t ₀ marker	Sodium Nitrate (NaNO_3) solution in water, 0.05mg/ ml Diluted from aqueous 1mg/ ml stock solution (Fluka Order Number: 74246-100ML)
Reported Value	Net retention time of sample: RetTime Sample – RetTime t ₀ marker

Affinity chromatography was carried out i) for insulin conjugates according to Examples 3.3 and 3.4 on a Waters Alliance Separation Module 2695 equipped with a Waters photodiode-array detector 2996 or Waters H-Class UPLC equipped with a Waters Acquity photodiode-array detector and ii) for isolated binders according to Example 2.11 on a Waters Alliance Separation Module 2795 equipped with a Waters photodiode-array detector 2996 or Waters H-Class UPLC equipped with a Waters Acquity photodiode-array detector.

10

Waters Empower 3 was used for all measurements as data processing software.

Columns with immobilized human serumalbumin (50 x 4mm; 5µm particle size) were purchased from Chiralpak and used for separations.

Phosphate Buffer Saline (PBS) was purchased from Gibco and used as Eluent A,
5 isopropanol was purchased from Fisher and used as Eluent B.

The applied gradient with a flow of 1.0 ml/min is shown below:

Time [min]	%Eluent A	%Eluent B
0	98	2
2	98	2
17	60	40
22	60	40
22.2	98	2
30	98	2
31	95	5

The columns with immobilized serum albumin were kept at 25°C during the LC run,
10 UV detection was carried out at 220 nm and injection volume was 20 µL.

The net retention time of the samples was reported according to

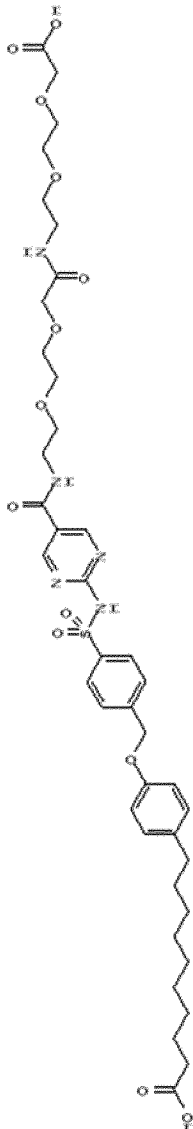
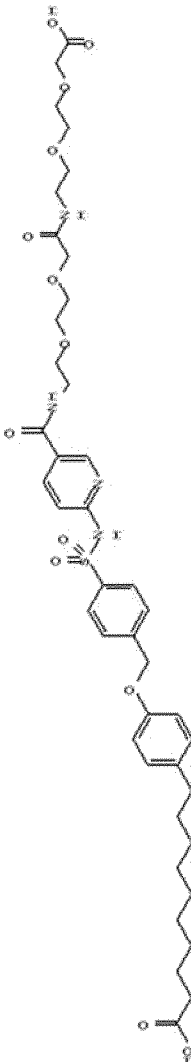
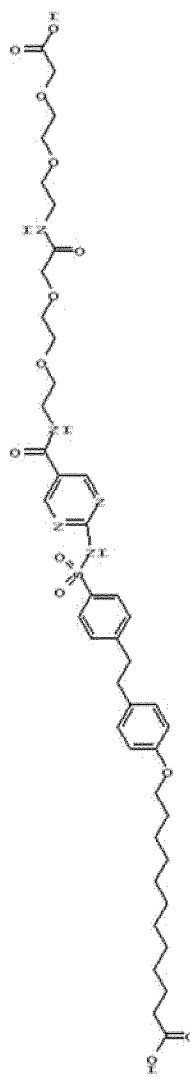
Net retention time = RetTime Sample – RetTime t0 marker

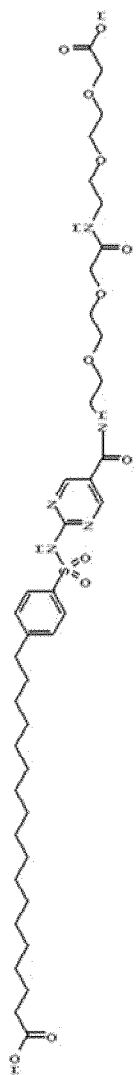
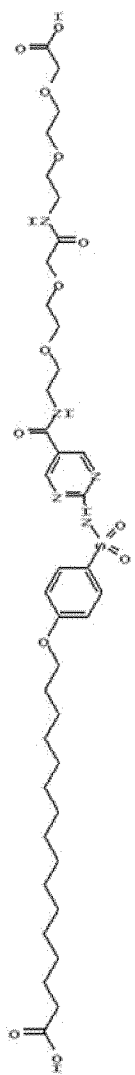
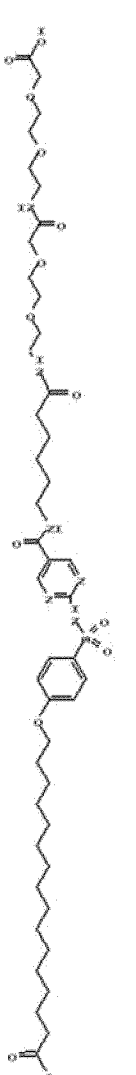
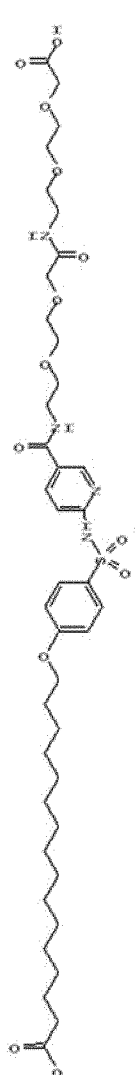
15 Table 1 shows the albumin binding results of the isolated binders, together with the LCMS data from section 4.1.

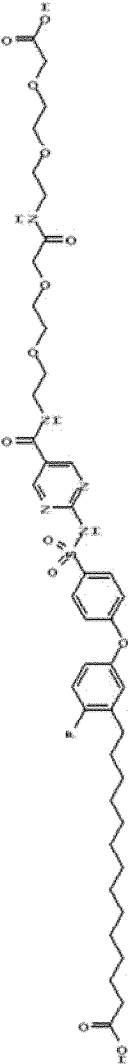
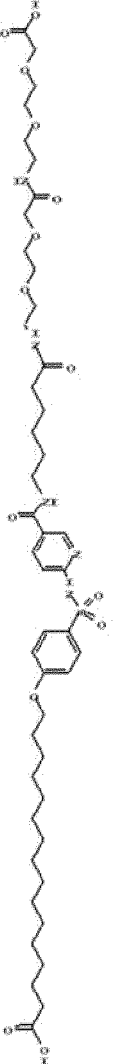
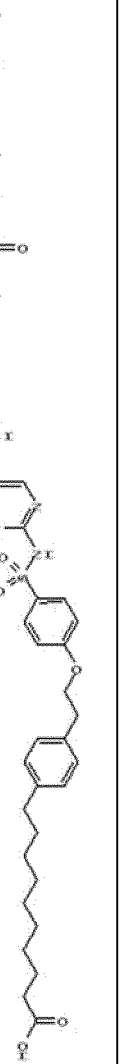
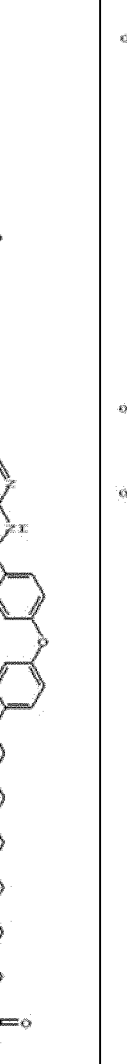
The abbreviations used in Table 1 are defined as follows:

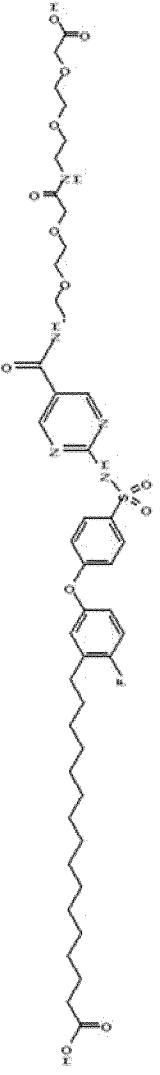
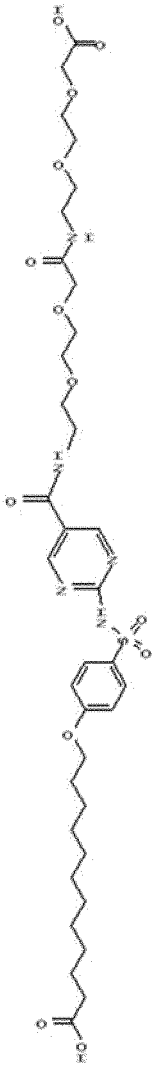
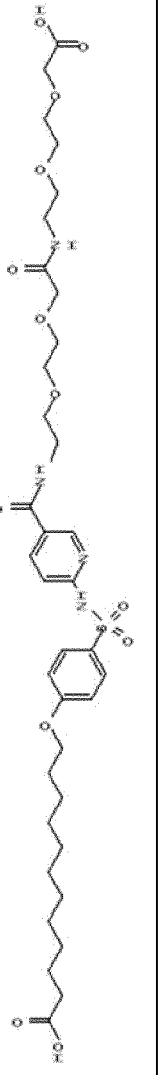
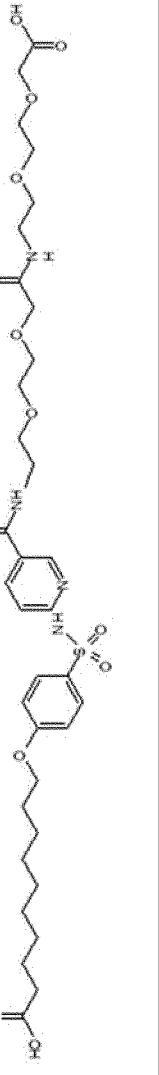
- NRT: Netto retention time on columns with immobilized human serumalbumin
- 20 LCMS: Liquid chromatography mass spectrometry
- MSM: Mass spectrometry method
- OIM: Observed ion mass
- OIT: Observed ion type
- IM: Ionization method
- 25 LCRT: Liquid chromatography Retention time
- LCM: Liquid chromatography Method

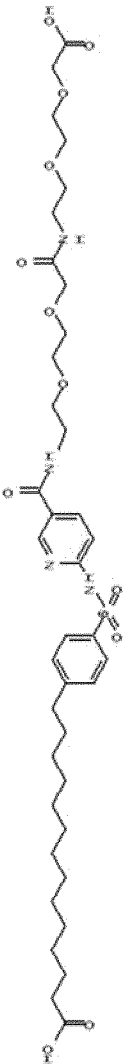
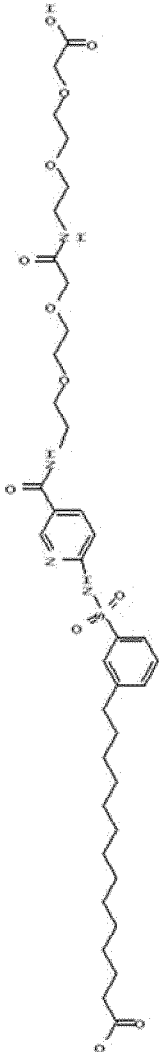
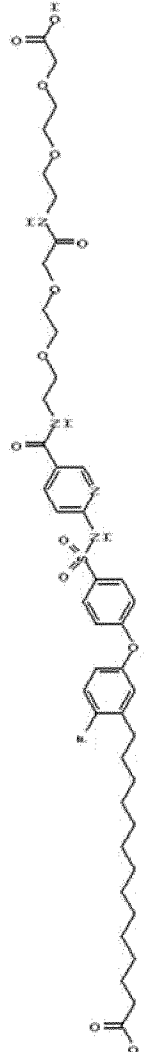
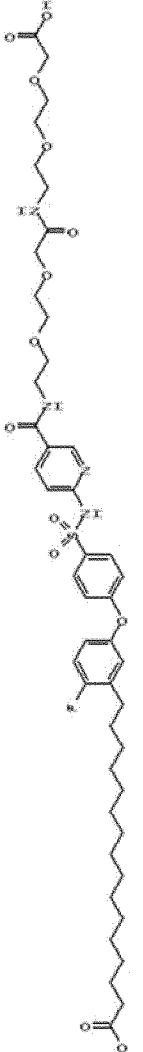
Table 1
Results from Columns with immobilized serumalbumin and LCMS data

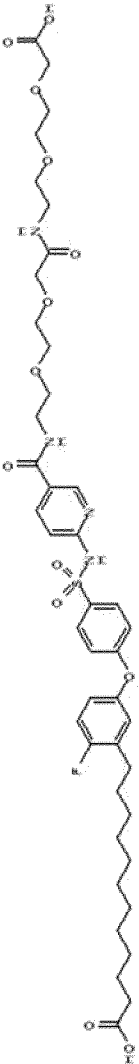
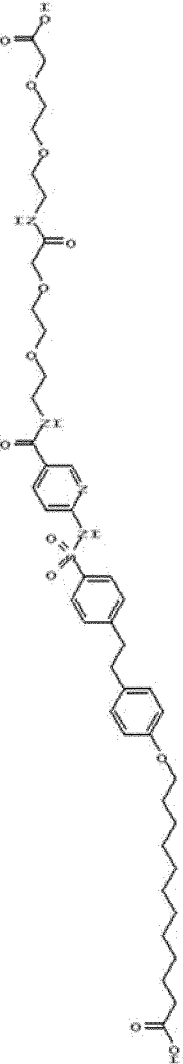
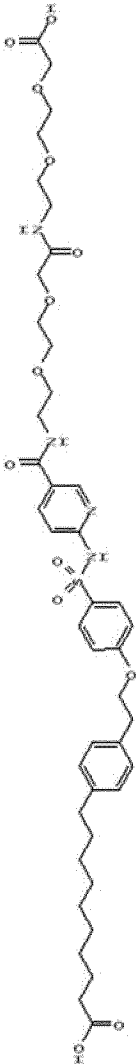
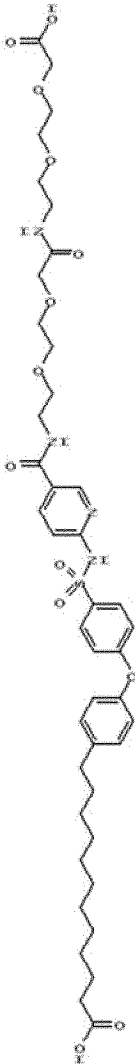
LCMS							
	LCM	B	C	C			
	LCRT [min]	1.78	2.28	2.52			
	IM	ES+	ES+	ES+			
	OIT	[M+H] ⁺	[M+H] ⁺	[M+H] ⁺			
	OIM	846.37	845.37	888.35			
MSM	b	b	b				
	NRT [min]	16.76	17.88	17.51			
	Structure						
No.	1	2	3				

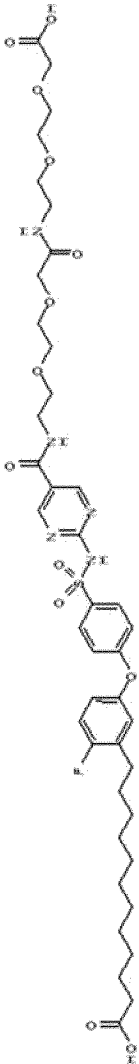
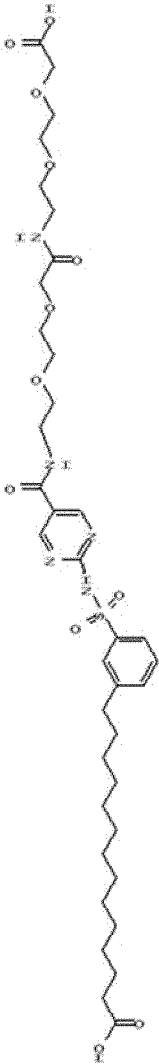
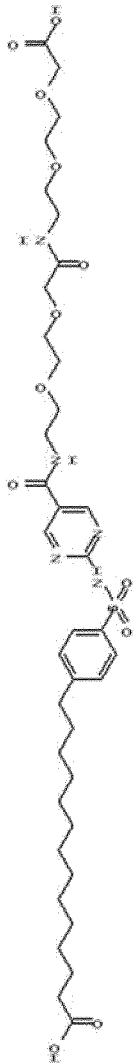
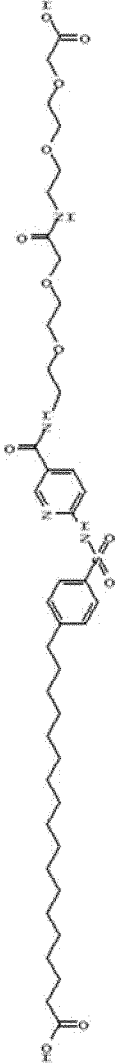
	No.	Structure	LCMS					
			LCM	LCRT	IM	OIT	OIM	MSM
				[min]				
			NRT					
			[min]					
	4		15.75	2.88	ES+	[M+H] ⁺	852.7	b
	5		14.32	2.59	ES+	[M+H] ⁺	840.35	b
	6		14.31	2.58	ES+	[M+H] ⁺	953.46	b
	7		14.65	2.58	ES+	[M+H] ⁺	839.4	b

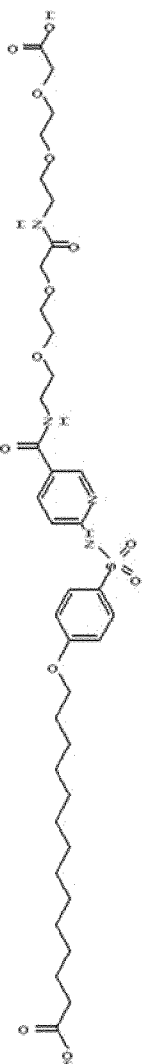
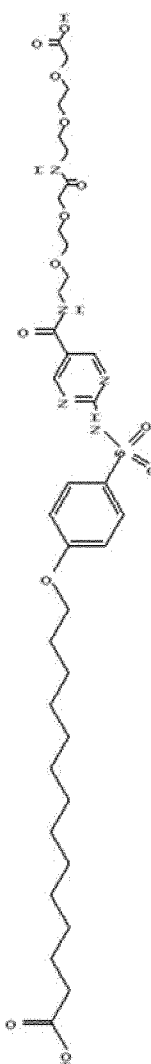
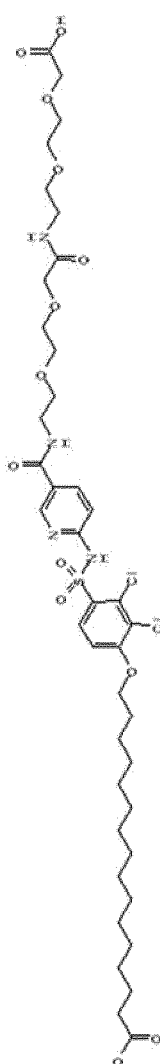
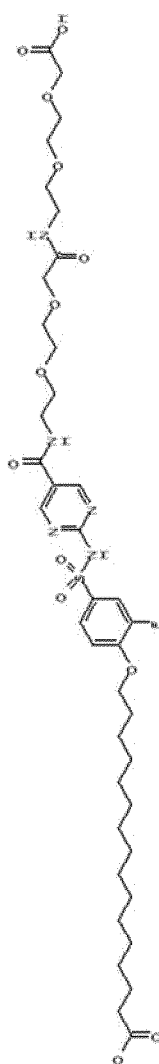
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		IM	ES+	ES+	ES+	ES+	ES-
		OIT	[M+H] ⁺	[M+H] ⁺	[M+H] ⁺	[M+H] ⁺	[M-H] ⁻
		OIM	906.52	952.4	860.32	888.7	957.7
		MSM	a	b	b	b	b
		NRT [min]	15.29	14.01	15.65	14.52	14.95
No.	Structure						
8							
9							
10							
11							
12							

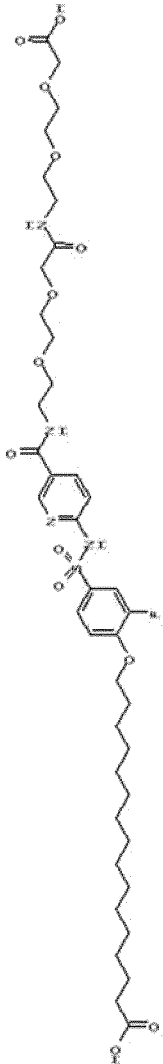
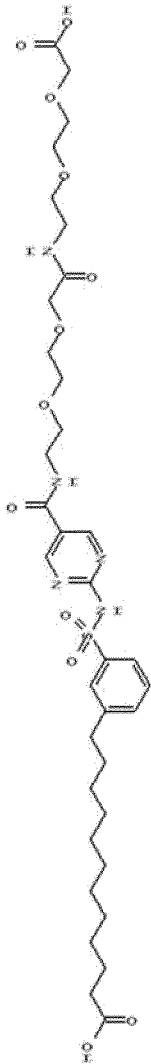
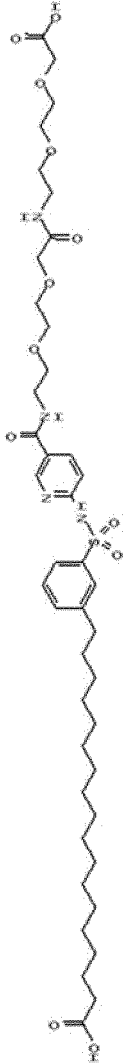
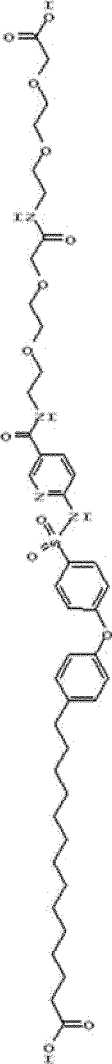
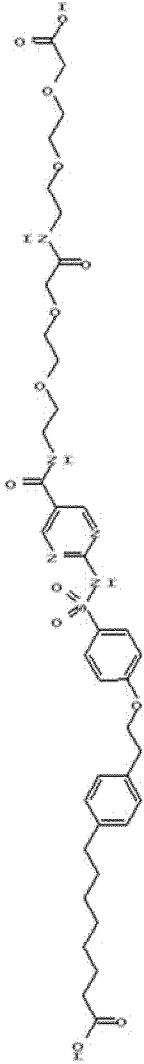
	No.	Structure	LCMS					
			LCM	A	C	C	C	
			LCRT [min]	2.99	2.14	2.13	1.87	
			IM	ES+	ES+	ES+	ES+	
			OIT	[M+H] ⁺	[M+H] ⁺	[M+H] ⁺	[M+H] ⁺	
			OIM	934.78	784.28	783.34	755.28	
			MSM	b	a	a	a	
			NRT [min]	15.29	11.88	12.37	10.82	
								
	13							
	16							
	18							
	19							

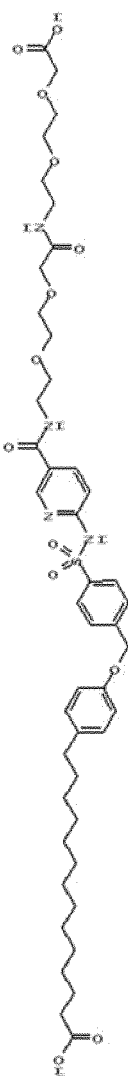
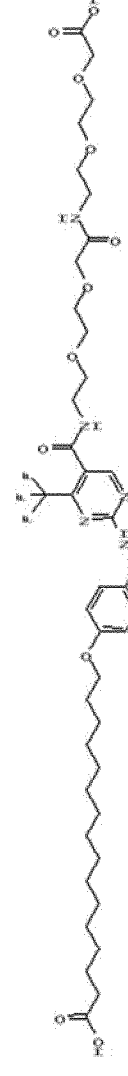
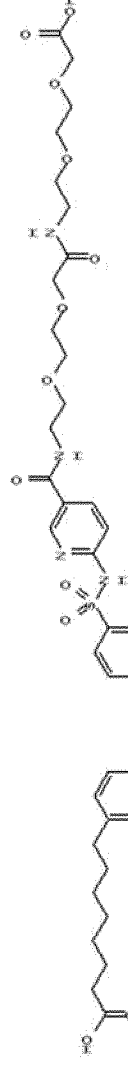
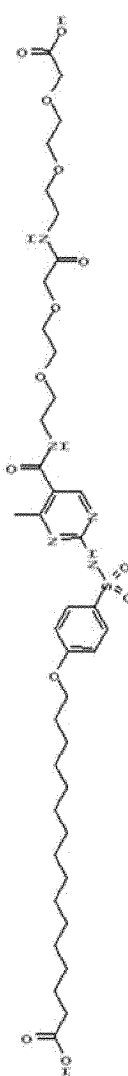
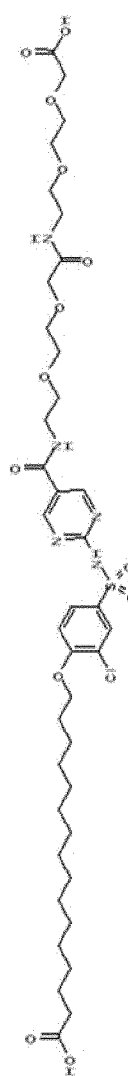
	No.	Structure	LCMS					
			LCM	C	C	C	C	C
			LCRT [min]	2.4	2.38	2.77	2.99	
			IM	ES+	ES+	ES+	ES+	
			OIT	[M+H] ⁺	[M+H] ⁺	[M+H] ⁺	[M+H] ⁺	
			OIM	795.37	795.37	905.47	933.54	
			MSM	a	a	a	a	
			NRT [min]	14.8	16.26	15.55	15.61	
								
	20							
	21							
	22							
	23							

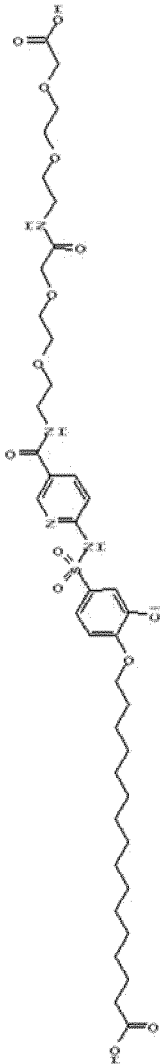
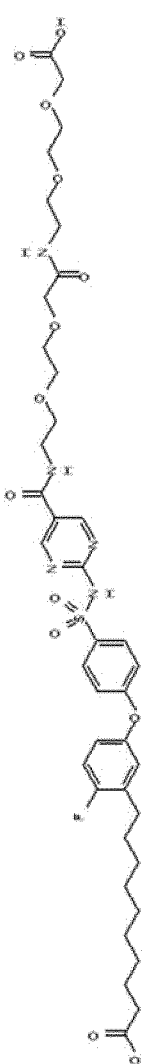
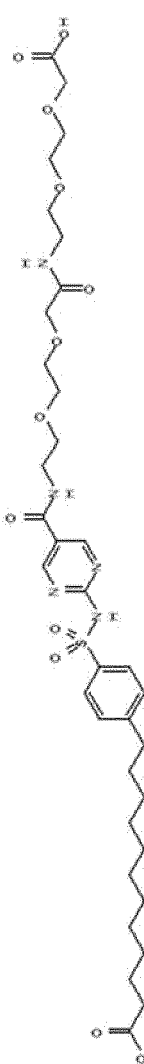
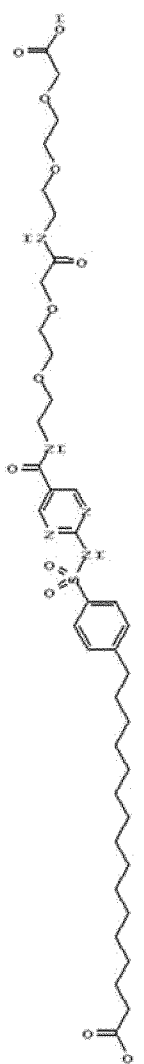
	No.	Structure	LCMS					
			LCM	C	C	C	C	C
			LCRT [min]	2.55	2.54	2.41	2.55	2.55
			IM	ES+	ES+	ES+	ES+	ES+
			OIT	[M+H] ⁺	[M+H] ⁺	[M+H] ⁺	[M+H] ⁺	[M+H] ⁺
			OIM	877.44	887.49	859.44	859.45	859.45
			MSM	a	a	a	a	a
			NRT [min]	15.76	17.48	14.98	15.01	15.01
	24							
	25							
	26							
	27							

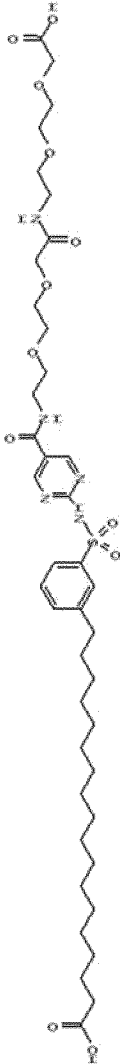
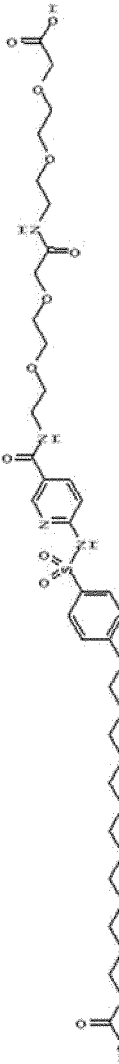
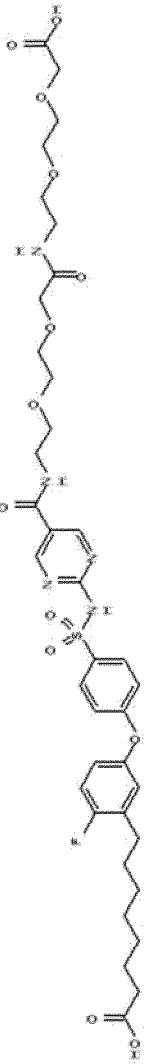
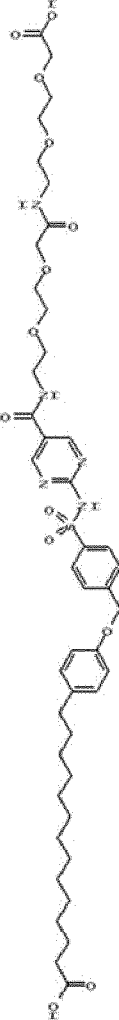
	No.	Structure	LCMS					
			LCM	C	C	C	C	C
			LCRT [min]	2.57	2.39	2.41	2.88	
			IM	ES+	ES+	ES+	ES+	
			OIT	[M+H] ⁺	[M+H] ⁺	[M+H] ⁺	[M+H] ⁺	
			OIM	878.4	796.4	796.44	851.51	
			MSM	a	a	a	a	
			NRT [min]	15.39	15.66	14.36	15.77	
								
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	29							
	30							
	31							

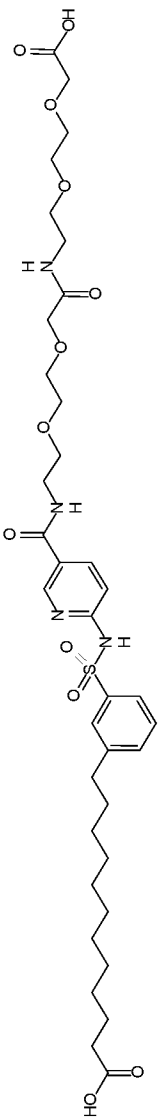
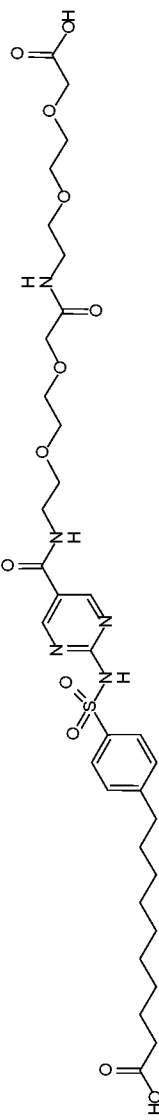
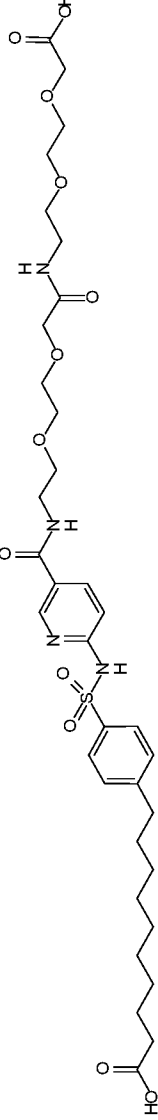
	No.	Structure	LCMS					
			LCM	C	C	C	C	C
			LCRT [min]	2.36	2.37	2.74	2.62	
			IM	ES+	ES+	ES+	ES+	
			OIT	[M+H] ⁺	[M+H] ⁺	[M+H] ⁺	[M+H] ⁺	
			OIM	811.29	812.23	907.59	858.6	
			MSM	a	a	a	a	
			NRT [min]	13.9	13.57	13.89	13.53	
								
	32							
	33							
	36							
	38							

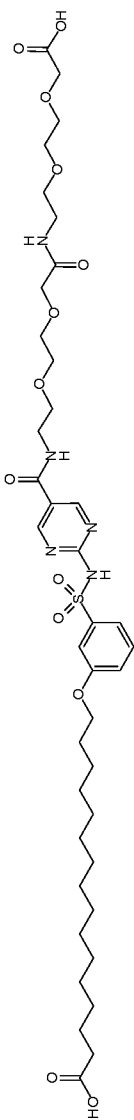
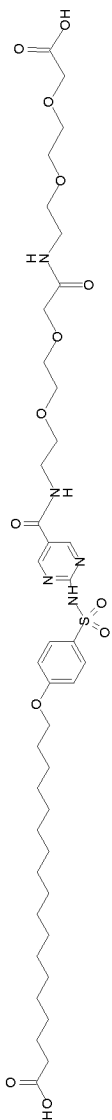
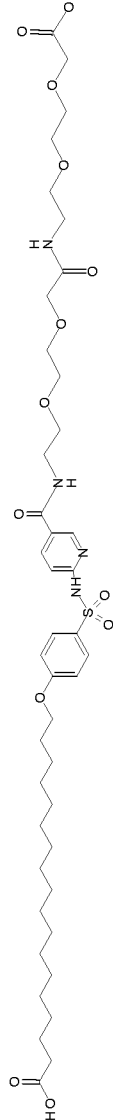
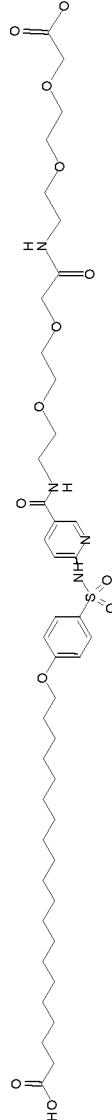
	Structure	LCMS					
		LCM	C	C	C	C	C
		LCRT [min]	2.6	2.14	2.82	2.76	2.18
		IM	ES+	ES+	ES+	ES+	ES+
		OIT	[M+H] ⁺	[M+H] ⁺	[M+H] ⁺	[M+H] ⁺	[M+H] ⁺
		OIM	857.62	768.6	851.7	887.65	832.6
		MSM	a	a	a	a	a
		NRT [min]	13.68	11.44	14.98	14.35	11.36
No.							
39							
40							
41							
42							
43							

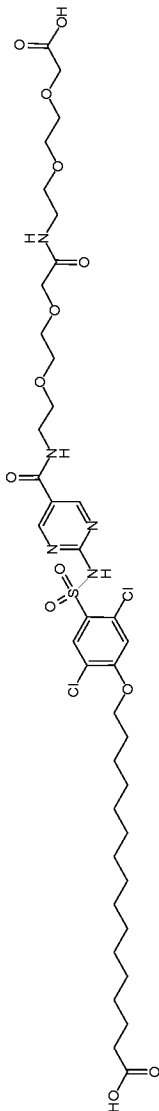
	Structure	LCMS					
		LCM	C	C	C	C	C
		LCRT [min]	2.76	2.77	2.16	2.63	2.7
		IM	ES+	ES+	ES+	ES+	ES+
		OIT	[M+H] ⁺	[M+H] ⁺	[M+H] ⁺	[M+H] ⁺	[M+H] ⁺
		OIM	901.72	908.66	831.6	854.7	874.6
		MSM	a	a	a	a	a
		NRT [min]	15.04	13.8	14.48	13.73	13.74
							
No.			44	45	46	47	50

	LCMS		LCM	C	C	C	C
			LCRT [min]	2.68	2.33	2.16	2.64
			IM	ES-	ES+	ES+	ES+
			OIT	[M-H]-	[M+H]+	[M+H]+	[M+H]+
			OIM	871.6	850.54	768.5	824.61
			MSM	a	a	a	a
		NRT [min]	13.59	14.56	12.29	15.19	
	Structure						
No.	51	52	53	54			

	Structure	LCMS					
		LCM	C	C	C	C	C
		LCRT [min]	2.84	2.62	2.11	2.78	
		IM	ES+	ES-	ES+	ES+	
		OIT	[M+H] ⁺	[M-H] ⁻	[M+H] ⁺	[M+H] ⁺	
		OIM	852.58	821.62	822.52	902.65	
		MSM	a	a	a	a	
		NRT [min]	15.01	15.47	13.42	14.78	
No.	Structure						
55							
59							
63							
64							

	LCMS					
	LCM	C	C	C	C	
	LCRT [min]	2.13	1.92	1.91		
	IM	ES+	ES+	ES+		
	OIT	[M+H] ⁺	[M+H] ⁺	[M+H] ⁺		
	OIM	767.61	740.57	739.57		
	MSM	a	a	a		
	NRT [min]	12.21	9.87	10.99		
	Structure					
No.	65	66	67			

	No.	Structure	LCMS					
			LCM	C	C	C	C	C
			LCRT [min]	2.60	2.84	2.83	3.06	
			IM	ES+	ES+	ES+	ES+	
			OIT	[M+H] ⁺	[M+H] ⁺	[M+H] ⁺	[M+H] ⁺	
			OIM	840.57	868.82	867.83	895.81	
			MSM	a	a	a	a	
			NRT [min]	14.74	14.24	14.26	14.42	
								
			68	69	70	71		

LCMS	LCM	C
	LCRT [min]	2.77
	IM	ES+
	OIT	[M+H] ⁺
	OIM	908.79
	MSM	a
	NRT [min]	13.96
	Structure	
No.	72	

5 Insuline receptor binding affinity

Insulin receptor binding affinity for the insulin, insulin analog 1 and the respective conjugates listed in Table 2 was determined as described in Hartmann et al. (Effect of the long-acting insulin analogues glargine and degludec on cardiomyocyte cell signaling and function. *Cardiovasc Diabetol.* 2016;15:96). Isolation of insulin receptor embedded plasma membranes (M-IR) and competition binding experiments were performed as previously described (Sommerfeld et al., *PLoS One.* 2010; 5(3): e9540). Briefly, CHO-cells overexpressing the IR were collected and re-suspended in ice-cold 2.25 STM buffer (2.25 M sucrose, 5 mM Tris-HCl pH 7.4, 5 mM MgCl₂, complete protease inhibitor) and disrupted using a Dounce homogenizer followed by sonication. The homogenate was overlaid with 0.8 STM buffer (0.8 M sucrose, 5 mM Tris-HCl pH 7.4, 5 mM MgCl₂, complete protease inhibitor) and ultra-centrifuged for 90 min at 100,000g. Plasma membranes at the interface were collected and washed twice with phosphate buffered saline (PBS). The final pellet was re-suspended in dilution buffer (50 mM Tris-HCl pH 7.4, 5 mM MgCl₂, complete protease inhibitor) and again homogenised with a Dounce homogenizer. Competition binding experiments were performed in a binding buffer (50 mM Tris-HCl, 150 mM NaCl, 0.1 % BSA, complete protease inhibitor, adjusted to pH 7.8) in 96-well microplates. In each well 2 µg isolated membrane was incubated with 0.25 mg wheat germ agglutinin polyvinyltoluene polyethylenimine scintillation proximity assay (SPA) beads. Constant concentrations of [125I]-labelled human Ins (100 pM) and various concentrations of respective unlabelled Ins (0.001–1000 nM) were added for 12 h at room temperature (23 °C). The radioactivity was measured at equilibrium in a microplate scintillation counter (Wallac Microbeta, Freiburg, Germany)."

The insulin receptor binding affinity relative to human insuline for the analoga depicted in Table 2 comprises the following ranges: A (≥ 40%); B (< 20%). Conjugate human insulin + binder no. 5 belongs to category A whereas all other conjugates and insulin analog 1 were classified under category B.

Table 2

Insulin receptor B binding affinity relative to human insuline

No. of binder	Backbone	<i>In vitro</i> insulin receptor B activation
5	Human insulin	A
5	Insulin analog 1	B
8	Insulin analog 1	B
50	Insulin analog 1	B
54	Insulin analog 1	B
---	Insulin analog 1	B

6. *In vivo* testing - Evaluation of pharmacokinetic effects

Healthy, normoglycemic Göttingen minipigs (aged 8-11 months, body weight ~12-18 kg) were used to evaluate the pharmacodynamic and pharmacokinetic effects of very long-acting insulin analogs in animals. The pigs were kept under standard animal house conditions and were fed once daily with access to tap water *ad libitum*. After overnight fasting, the pigs were treated with a single subcutaneous injection of a solution that contained either a placebo formulation, insulin or an insulin analog or the respective conjugate. Pure human insulin and pure insulin analog 1 as well as the conjugate of binder no. 5 with human insulin and the conjugates of binders 5, 50 and 54 with insulin analog 1 were tested.

Blood collection was performed via pre-implanted central venous catheters for determination of blood glucose, pharmacokinetics and additional biomarkers from K-EDTA plasma. Blood sampling started before the administration of the test item (baseline) and was repeated 1-4 times per day until study end. During the study, the animals were fed after the last blood sampling of the day. All animals were handled regularly and clinical signs were recorded at least twice on the day of treatment and once daily for the remaining duration of the study. The animals were monitored carefully for any clinical signs of hypoglycemia, including behavior, coat, urine and fecal excretion, condition of body orifices and any signs of illness. In case of severe

hypoglycemia, the investigator was allowed to offer food or infuse glucose solution intravenous (i.v.) in case food intake was not possible. After the last blood sampling, the animals were transported back to the non-GLP animal keeping facility.

For determination of the pharmacokinetic parameters following experimental conditions were used.

6.1 Materials and Chemicals

MeCN (hyperSolv chromanorm), dimethyl sulfoxide (uvasol), 2-propanol, methanol (hyperSolv chromanorm), water (hyperSolv chromanorm), formic acid (98-100%) were purchased from Merck (Darmstadt, Germany). Analyte and suitable internal reference were obtained from Sanofi. Blank plasma (K2-EDTA as anticoagulant) was obtained from Seralab (West Sussex, UK).

6.2 Stock and working solutions of test compound and internal standard

Stock solutions of the test compound and its internal standard were prepared in MeCN /water/formic acid (50:50:1, v/v/v) at a concentration of 1 mg/ml. The working solutions of the test compound and the corresponding internal standard were prepared in the same solvent at a concentration of respectively 100 µg/ml and 1250 ng/ml.

6.3 Plasma sample preparation

A 25 µl portion of plasma was spiked with 10 µl of internal standard working solution (1250 ng/ml) into a 1.5 ml Eppendorf tube. After sealing and mixing 75 µl of MeCN/methanol (80:20, v/v) was added and the samples were vortexed for 5 s and vortexed for 10 min at approximately 5 °C and 3000g. Then, 75 µl of supernatant was transferred into an autosampler vial containing 75 µl of water. The vial were sealed, mixed and analyzed.

6.4 LC-MS/MS Analysis

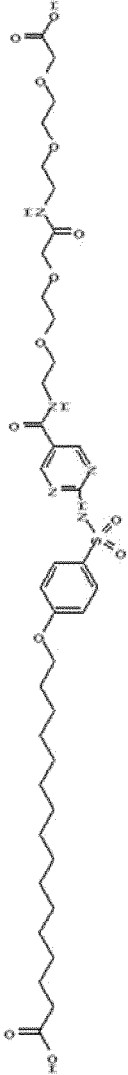
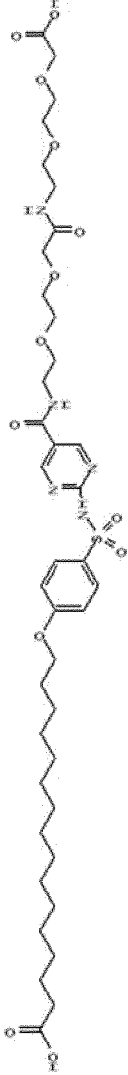
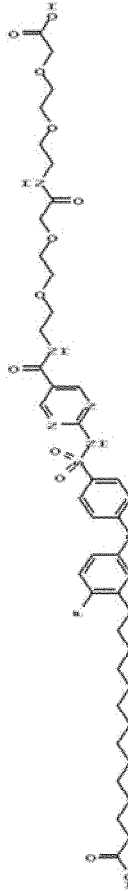
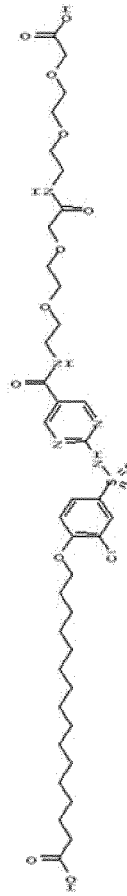
The LC-MS/MS analysis of the intact insulin was performed on an Agilent 1290 Series HPLC (Waldbronn, Germany), coupled to an ABSciex QqQ API 4000 mass spectrometer (Darmstadt, Germany). The LC was equipped with an Aeris PEPTIDE XB-C18 analytical column (100 x 2.1 mm, particle size 3.6 μ m, Phenomenex) operated at 40°C. The mobile phase A consisted in water/formic acid/DMSO (100:0.1:1, v/v/v) and mobile phase B in MeCN/formic acid/DMSO (100:0.1:1, v/v/v). The HPLC program started by keeping the initial conditions of 2% B for 0.5 min, then a gradient of 2% B to 90%B within 7.5 minutes was applied and the column was reequilibrated for 2 minutes. The flow rate was 600 μ l/min and a volume of 40 μ l was injected into the system. The mass spectrometer was operated in the positive mode at an ion spray voltage of 5500 V, and the declustering potential was optimized for efficient isolation of the 5-fold protonated molecules. The mass spectrometer was operating in positive mode and the MS compound specific parameters were optimized for best sensitivity. Nitrogen was used as collision gas.

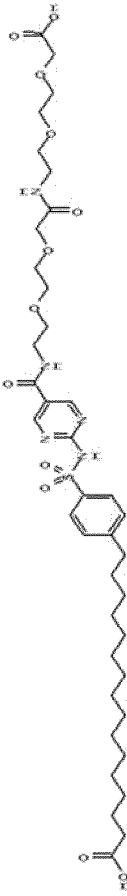
The pharmacokinetic (PK) parameters half-life time ($t_{1/2}$) and Mean Residence Time (MRT) are shown in Table 3.

For human insuline, the literatue MRT value obtained in chronic diabetic Yucatan minipigs is given (Lin, S.; Chen, L.-L. H.; Chien, Y.W. The journal of pharmacology and experimental therapeutics, 1998, 286, 959-966). The listed $t_{1/2}$ has been calculated as an approximation using the formula $t_{1/2} * 1.44$ according to the text book Clinical Pharmacokinetics Concepts and applications by Tozer and Rowland, 3rd edition (Publisher Lippincott Williams & Wilkins), 1995- Section II-6).

As can be seen, conjugation of insuline derivatives, here human insulin or insulin analog 1, with the binders of the invention had a significant impact in the PK (pharmacokinetics) properties of the resulting conjugates, leading in all cases to increased $t_{1/2}$ and MRTs.

Table 3
Pharmacokinetic results of pure insulins vs. conjugates

No. of binder	Structure of binder	Backbone	PK t _{1/2}	MRT
---	No binder	Human insulin	~2h**	2.8 h*
5		Human insulin	30 h	47.2 h
5		Insulin analog 1	37h	57.7 h
8		Insulin analog 1	38h	63.3 h
50		Insulin analog 1	52h	83 h

No. of binder	Structure of binder	Backbone	PK t _{1/2}	MRT
54		Insulin analog 1	33h	56.5 h

* From: Lin, S.; Chen, L.-L. H.; Chien, Y.W. The journal of pharmacology and experimental therapeutics, 1998, 286, 959-966.

** Calculated from $t_{1/2} = MRT / 1.44$ according to Clinical Pharmacokinetics Concepts and applications by Tozer and Rowland, 3rd edition (Publisher Lippincott Williams & Wilkins), 1995- Section II-6).

The pharmacodynamic effects of several insulines and conjugates are shown in Figures 1 and 2, i.e., the effect on blood glucose after s.c. administration is depicted. The data demonstrated a significant prolongation of the duration of action for all the insuline-
5 binder conjugates tested (> 48 h), in relation to insulin analog 1 and to human insulin reselectively, for which a duration of action at the tested doses lower than 24 hr was observed. For the test insuline conjugates with a reduced insuling receptor binding affinity, the chosen *in vivo* dose was higher as for the corresponding parent insulins, which were not tested at higher dosis to avoid hypoglychemic effects.

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Short description of the Figures

Fig. 1 shows the blood glucose lowering effect after s.c. application of the conjugates of insulin analog 1 with binder no. 50 and binder no. 54 respectively in
15 (Göttingen) minipigs (12-18 kg, n = 3). Both compounds were tested at a dose of (18 nmol/kg).

Fig. 2 shows blood glucose lowering effect after s.c. application of the insulins and insuline conjugates respectively in (Göttingen) minipigs (19-20 kg, n = 3):
20 Human insulin + binder no. 5 (18 nmol/kg), human insulin (3 nmol/kg), insulin analog 1 + binder no. 5 (18 nmol/kg), insulin analog 1 (3 nmol/kg).

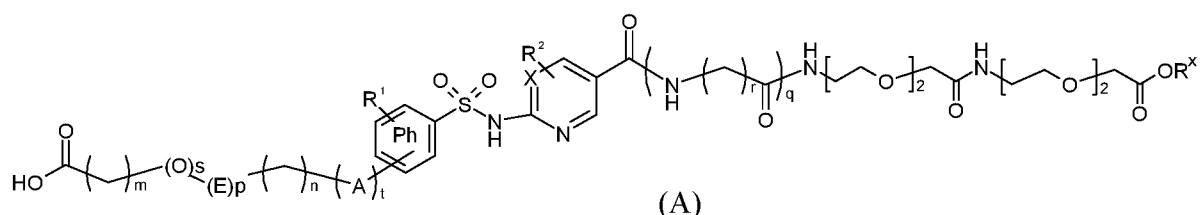
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- 25
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 - Handbook of Pharmaceutical Salts: Properties, Selection, and Use by Stahl and Wermuth (Wiley-VCH, 2002).

35

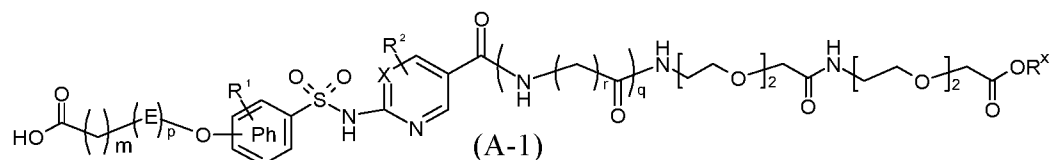
Claims

- 5 1. A sulfonamide of formula (A)



wherein:

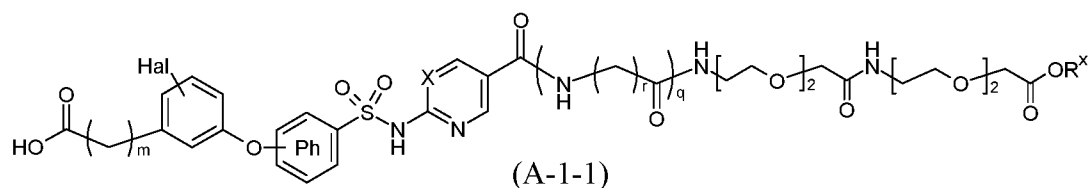
- 10 A is selected from the group consisting of oxygen atom, -CH₂CH₂- group, -OCH₂- group and -CH₂O- group;
- E represents a -C₆H₃R- group with R being a hydrogen atom or a halogen atom, wherein the halogen atom is selected from the group consisting of fluorine, chlorine, bromine and iodine atom;
- X represents a nitrogen atom or a -CH- group;
- 15 m is an integer in the range from 5 to 17;
- n is zero or an integer in the range from 1 to 3;
- p is zero or 1;
- q is zero or 1;
- r is an integer in the range from 1 to 6;
- 20 s is zero or 1;
- t is zero or 1;
- R¹ represents at least one residue selected from the group of hydrogen atom, halogen atom, C1 to C3 alkyl group and halogenated C1 to C3 alkyl group;
- R² represents at least one residue selected from the group of hydrogen atom, halogen atom, C1 to C3 alkyl group and halogenated C1 to C3 alkyl group;
- 25 R^x represents a hydrogen atom or an activation group.
2. The sulfonamide according to claim 1 having the formula (A-1)



wherein:

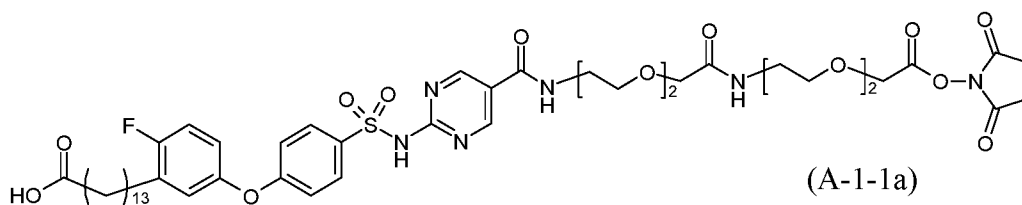
- E represents a $-C_6H_3R-$ group with R being a hydrogen atom or a halogen atom, wherein the halogen atom is selected from the group consisting of fluorine, chlorine, bromine and iodine atom;
- X represents a nitrogen atom or a $-CH-$ group;
- p is zero or 1;
- q is zero or 1;
- r is an integer in the range from 1 to 6;
- R^1 represents at least one residue selected from the group of hydrogen atom and halogen atom;
- R^2 represents at least one residue selected from the group of hydrogen atom, C1 to C3 alkyl group and halogenated C1 to C3 alkyl group;
- R^x represents a hydrogen atom or an activation group; and
- with m being an integer in the range from 5 to 15 if p is zero, or m being an integer in the range from 7 to 15 if p is 1.

3. The sulfonamide according to claim 1 or 2, wherein the sulfonamide has the formula (A-1-1)

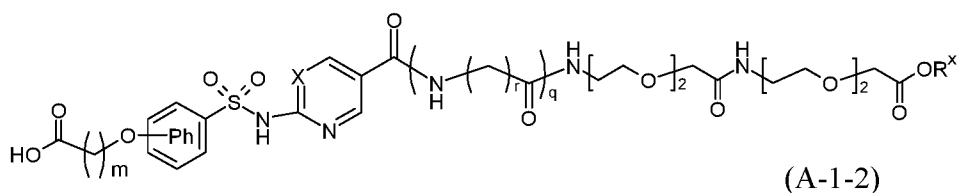


wherein X is a nitrogen atom or a -CH- group; m is an integer in the range from 7 to 15; r is an integer in the range from 1 to 6; q is zero or 1; Hal is a halogen atom selected from the group consisting of fluorine, chlorine, bromine and iodine atom; R^x represents a hydrogen atom or an activation group; and the HOOC-(CH₂)_m-C₆H₃Hal-O- group is situated in meta or para position on phenyl ring Ph with respect to the -S(O)₂- group.

4. The sulfonamide according to any of claims 1 to 3, wherein the sulfonamide has the formula (A-1-1a)

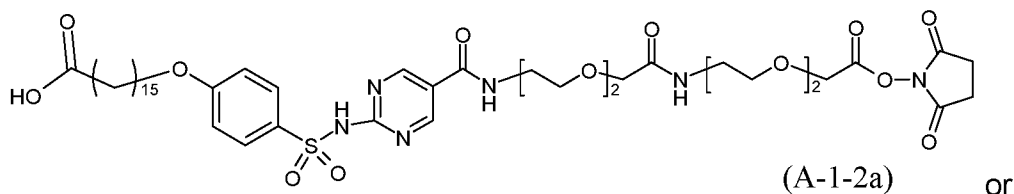


5. The sulfonamide according to claim 1 or 2, wherein the sulfonamide has the formula (A-1-2)

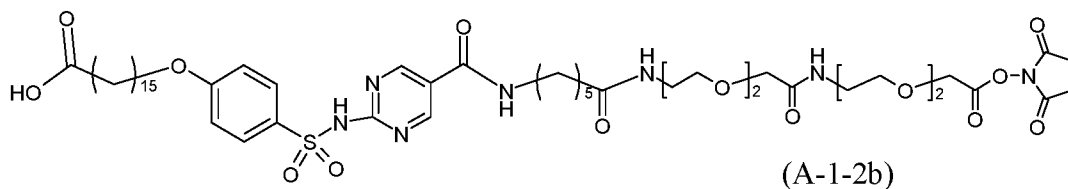


wherein X is a nitrogen atom or a -CH- group; m is an integer in the range from 5 to 15; r is an integer in the range from 1 to 6; q is zero or 1; and the HOOC-(CH₂)_m-O- group is situated in meta or para position on phenyl ring Ph with respect to the -S(O)₂- group.

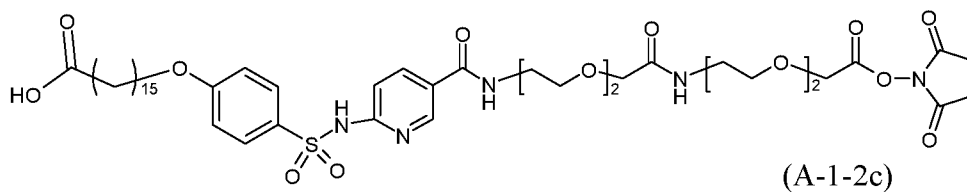
6. The sulfonamide according to any of claims 1 to 2 or 5, wherein the sulfonamide has the formula (A-1-2a)



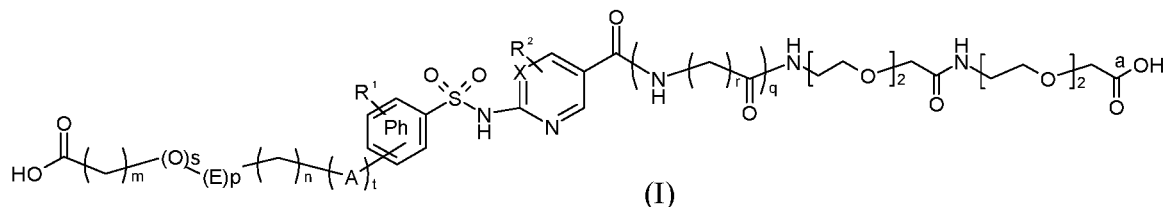
the formula (A-1-2b)



the formula (A-1-2c)



7. A conjugate comprising a sulfonamide of formula (I) and an active pharmaceutical ingredient

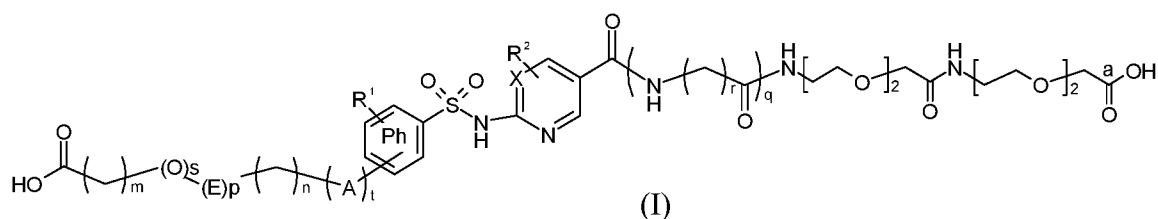


wherein in the sulfonamide of formula (I):

- 5 A is selected from the group consisting of oxygen atom, -CH₂CH₂- group, -OCH₂-group and -CH₂O- group;
- E represents a -C₆H₃R- group with R being a hydrogen atom or a halogen atom, wherein the halogen atom is selected from the group consisting of fluorine, chlorine, bromine and iodine atom;
- 10 X represents a nitrogen atom or a -CH- group;
- m is an integer in the range from 5 to 17;
- n is zero or an integer in the range from 1 to 3;
- p is zero or 1;
- q is zero or 1;
- 15 r is an integer in the range from 1 to 6;
- s is zero or 1;
- t is zero or 1;
- R¹ represents at least one residue selected from the group of hydrogen atom, halogen atom, C1 to C3 alkyl group and halogenated C1 to C3 alkyl group;
- 20 R² represents at least one residue selected from the group of hydrogen atom, halogen atom, C1 to C3 alkyl group and halogenated C1 to C3 alkyl group;
- wherein the sulfonamide of formula (I) is covalently bound to the active pharmaceutical ingredient in that the terminal carboxy group "a" of the sulfonamide of formula (I) is covalently bound to an amino group of the pharmaceutically active agent.
- 25
8. The conjugate according to claim 7, wherein the active pharmaceutical ingredient is selected from the group consisting of insulin, insulin analog, GLP-1, and GLP-1 analog.

8. The conjugate according to claim 7, wherein the active pharmaceutical ingredient is selected from the group consisting of insulin, insulin analog, GLP-1, and GLP-1 analog.

9. The conjugate according to claim 7 or 8, wherein the active pharmaceutical ingredient is insulin or an insulin analog, wherein the amino group of the peptide, to which the sulfonamide of formula (I) is covalently bound, is an epsilon amino group of a lysine present in the insulin or insulin analog or is the N-terminal amino group of the B chain of the insulin or insulin analog.
10. A process for preparing a conjugate comprising a sulfonamide of formula (I) and an active pharmaceutical ingredient



wherein in the sulfonamide of formula (I):

A is selected from the group consisting of oxygen atom, -CH₂CH₂- group, -OCH₂- group and -CH₂O- group;

E represents a -C₆H₃R- group with R being a hydrogen atom or a halogen atom, wherein the halogen atom is selected from the group consisting of fluorine, chlorine, bromine and iodine atom;

X represents a nitrogen atom or a -CH- group;

m is an integer in the range from 5 to 17;

n is zero or an integer in the range from 1 to 3;

p is zero or 1;

q is zero or 1;

r is an integer in the range from 1 to 6;

s is zero or 1;

t is zero or 1;

R¹ represents at least one residue selected from the group of hydrogen atom, halogen atom, C1 to C3 alkyl group and halogenated C1 to C3 alkyl group;

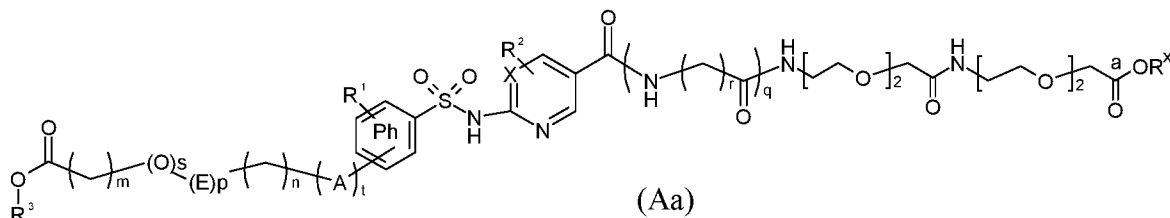
R² represents at least one residue selected from the group of hydrogen atom, halogen atom, C1 to C3 alkyl group and halogenated C1 to C3 alkyl group;

wherein the sulfonamide of formula (I) is covalently bound to the active pharmaceutical ingredient in that the terminal carboxy group "a" of the sulfonamide

of formula (I) is covalently bound to an amino group of the active pharmaceutical ingredient;

comprising:

(a) providing a sulfonamide of formula (Aa)



wherein X, Y, A, E, R¹, R² and the indices m, n, p, q, r, s, t have the meaning as defined in claim 1, R^x is a hydrogen atom or an activation group; and R³ is a protective group or a hydrogen atom; and an active pharmaceutical ingredient having a protected or unprotected C terminus;

(b) reacting the sulfonamide of formula (Aa) and the active pharmaceutical ingredient having a protected or unprotected C terminus under conditions suitable to form an amide bond between the free or activated carboxy group "a" of the sulfonamide of formula (Aa) and an amino group of the active pharmaceutical ingredient having a protected or unprotected C terminus;

(c) optionally removing one or both protection groups.

11. A conjugate comprising a sulfonamide of formula (I) and an active pharmaceutical ingredient obtained or obtainable from the process according to claim 10.

12. Pharmaceutical composition comprising in a pharmaceutically effective amount the conjugate comprising a sulfonamide of formula (I) and an active pharmaceutical ingredient according to any of claims 7 to 9 or according to claim 11.

13. The conjugate comprising a sulfonamide of formula (I) and an active pharmaceutical ingredient according to any of claims 7 to 9 or according to claim 11 for use as a medicament.

14. The conjugate comprising a sulfonamide of formula (I) and an active pharmaceutical ingredient according to any of claims 7 to 9 or according to claim 11 for use as a medicament for treatment of a disease selected from the group

consisting of gestational diabetes, diabetes mellitus type 1, diabetes mellitus type 2 and hyperglycemia and/or for lowering blood glucose levels.

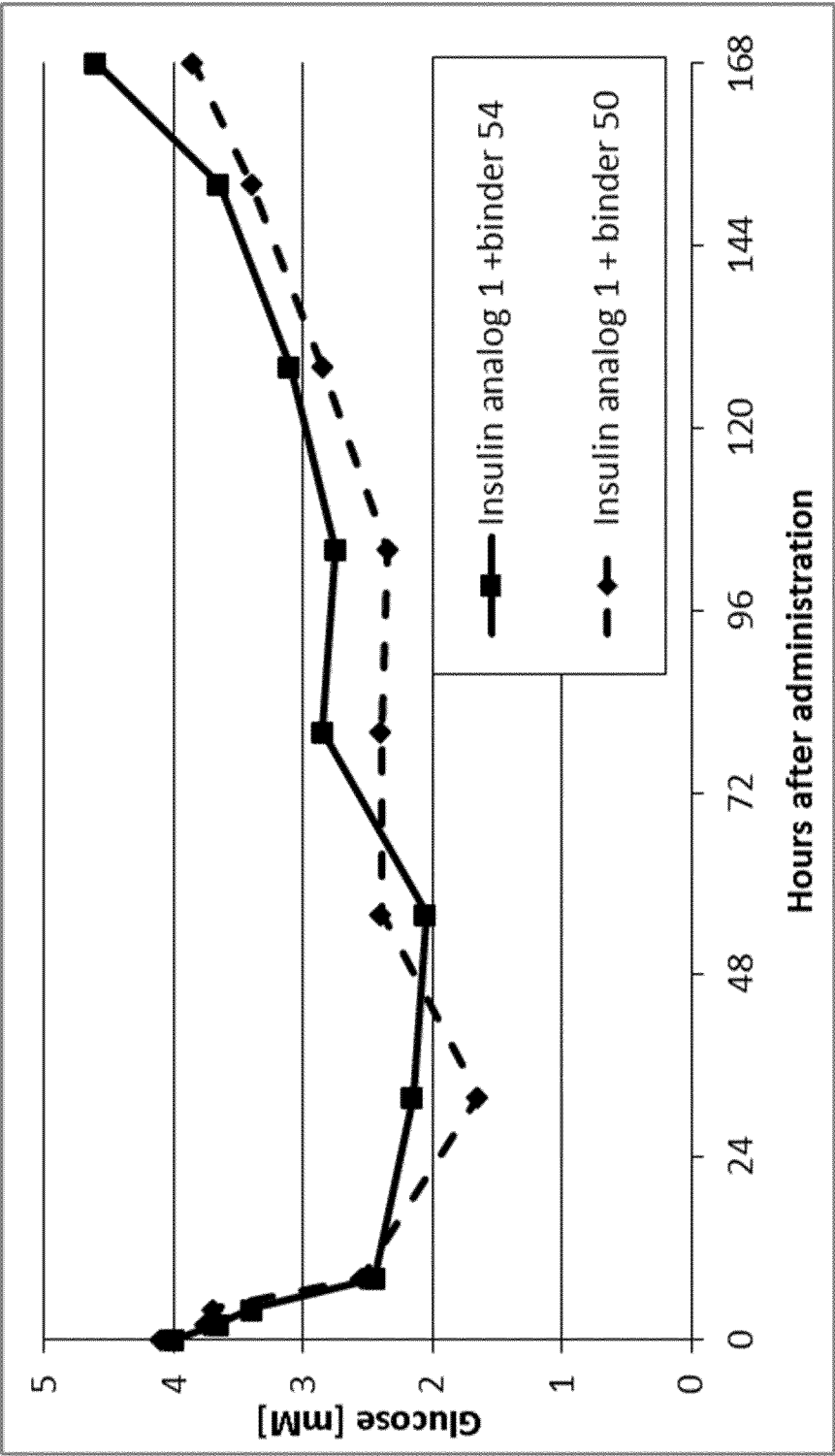


Fig. 1

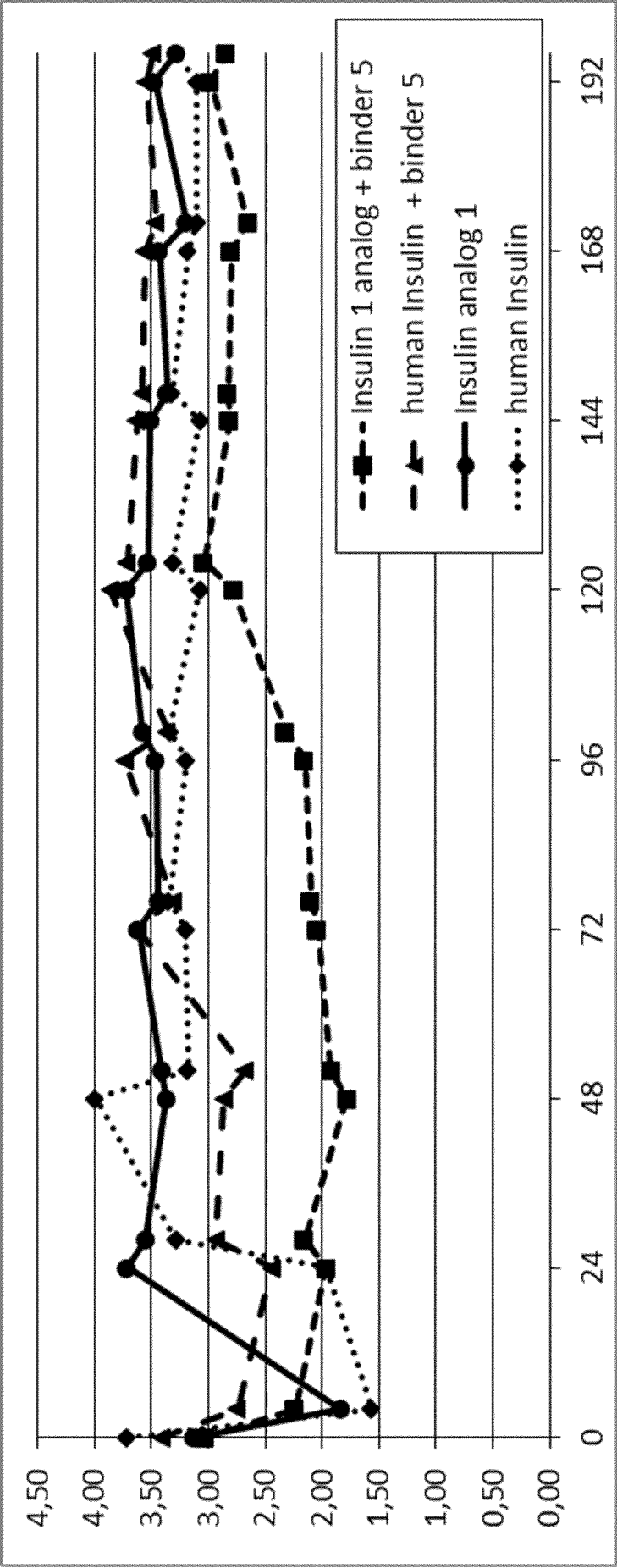


Fig. 2

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