Poly(ethylene glycol)-conjugated Alkylamines as Novel Blocking Reagents for Immunoassays

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Für Henning und für meine Familie

最愛の家族に捧ぐ

Zusammenfassung

Blockierungsreagenzien stellen zwar eine unentbehrliche Komponente eines Festphasenassays dar, sind dafür jedoch vergleichsweise schlecht erforscht. Die Entwicklung neuer Blockierungsreagenzien war daher das zentrale Anliegen dieser Arbeit. Daher wurden Poly(ethylenglycol)-konjugierte Alkylamine, eine Klasse von Tensiden, die bislang hauptsächlich in der Industrie Verwendung findet, erstmals bezüglich ihrer Eignung als Blockingreagenzien für Immunoassays studiert. Im ersten Schritt wurde eine Reihe von Poly(ethylenglycol)-konjugierten Alkylaminen dargestellt, und im zweiten Schritt wurden die synthetisierten Tenside mit Hilfe von Festphasenassays auf ihre Blockierungseigenschaften untersucht.

Es wurde eine modulare Baukasten-Synthese entworfen, mit Hilfe deren Poly(ethylenglycol)-konjugierte Alkylamine verschiedenster Struktur effizient dargestellt werden konnten. Diese Synthese ermöglichte die Herstellung von Tensiden mit jeweils einer oder zwei Poly(ethylenglycol)-Gruppe(n) und Alkylkette(n) unterschiedlicher Länge, die über eine kurze Brücke miteinander verbunden sind. Die Synthesestrategie erlaubte weiterhin den Einbau von einer oder zwei Aminogruppe(n) zwischen der Poly(ethylenglycol)-Gruppe und der Brücke und/oder am Ende der Alkylkette. Die synthetisierten Substanzen wurden erfolgreich charakterisiert, dabei wurden unter anderem Löslichkeit und kritische mizellare Konzentration bestimmt.

Die synthetisierten kationischen Tenside wurden mit Hilfe unterschiedlicher Festphasenassays untersucht. In einer Reihe von Voruntersuchungen wurde gezeigt, dass kationische Tenside optimal unter üblichen Assaybedingungen, wie z.B. neutralem pH-Wert oder physiologischen Ionenkonzentrationen, arbeiten. Drei unterschiedliche ELISA-Formate wurden angewandt, um die Blockierungseigenschaften der kationischen Tenside mit denen kommerziell erhältlicher Blockierungsreagenzien zu vergleichen. Dabei stellte sich heraus, dass keines der kommerziell erhältlichen Reagenzien sowohl gute Spezifität als auch gute Sensitivität bieten konnte. Hingegen waren viele der synthetisierten Reagenzien in der Lage, angemessene Blockierungseigenschaften sowohl in Hinsicht der Sensitivität als auch der Spezifität zu gewährleisten. Einige der kationischen Tenside konnten in dieser Hinsicht sehr gute Ergebnisse in allen drei ELISA-Formaten erzielen.

Zusammenfassend lässt sich sagen, dass die besten Vertreter der synthetisierten Tenside kommerziell erhältliche Blockierungsreagenzien in ihren Blockierungsfähigkeiten übertreffen. Synthetische Blockierungsreagenzien auf Basis Poly(ethylenglycol)-konjugierter Alkylamine schließen des Weiteren viele Nachteile aus, die bei proteinhaltigen Blockierungsreagenzien auftreten, wie z.B. Kreuzreaktivität, Heterogenität, Verderblichkeit, Chargenunterschiede, etc. Die vielversprechenden Ergebnisse dieser Arbeit lassen eine baldige Kommerzialisierung der neuartigen Blockierungsreagenzien erhoffen. Auch wenn weitergehende Untersuchungen sicherlich nötig sind, sollte eine industrielle Produktion der kationischen Tenside kostengünstig möglich sein.

Abstract

Blocking reagents represent an essential albeit not widely studied part of solid phase supported immunoassays. The development of novel blocking reagents was the main purpose of this thesis. In this regard, poly(ethylene glycol)-conjugated alkyl amines, which are typical surfactants with up to now mainly industrial applications, were studied as candidates for novel blocking reagents in immunoassays for the first time. First, a variety of poly(ethylene glycol)-conjugated alkyl amines was synthesised. Second, the blocking performance of the synthesised surfactants was investigated in assay experiments.

A modular synthesis was designed that permitted the efficient preparation of poly(ethylene glycol)-conjugated alkyl amines of different structures. Notably, one or two poly(ethylene glycol)-groups could be linked to one or two alkyl chains, both of variable length, via a short and simple bridge. Moreover, one or two amine groups could be inserted between the bridge and the poly(ethylene glycol)-group and/or terminally appended on the hydrophobic side. The synthesised substances were successfully characterised, which included the determination of the solubility and the critical micelle concentration.

The synthesised surfactants were subjected to a number of different assay experiments. Preliminary assays showed that cationic surfactants perform best under standard assay conditions such as neutral pH value and physiological ion concentrations. In three distinct ELISA formats the blocking performance of the synthesised surfactants was compared to that of commercially available blocking reagents. It was found that no single commercially available blocking reagent was able to achieve both good specificity and sensitivity. On the other hand, many of the PEG-conjugated alkylamines were able to combine acceptable specificity and sensitivity and some yielded very good results in both respects in all assay experiments.

In conclusion, the best synthesised surfactants outperform the blocking reagents that are commercially available to date. In addition, they do not exhibit any of the drawbacks of proteinaceous blocking reagents, such as cross-reactivity, heterogeneity and lot-to-lot variability. Since these novel blocking reagents achieved very promising results, a commercialisation is hoped for in the near future. Though further research is obviously needed, industrial preparation of the compounds should be feasible and enable a cost-efficient production.

Contents

1. Introduction

1.1 Immunoassays

The immune system of an organism uses antibodies to target foreign objects. The recognition of an antigen by its antibody must be highly specific. This specificity is provided by an exact three-dimensional match of the epitope (the structural part of the antigen interacting with the antibody) to its counterpart, the paratope. The interaction between epitope and paratope involves a variety of synergistic forces. Hence, the binding of an antibody to its antigen is very strong (affinity constants of about 10^5 - 10^9 M^{-1})[1] despite being non-covalent. Immunoassays are analytical tests which employ these unique properties of antibody-antigen pairs to investigate a sample. The analyte usually is an antigen and is detected by a labelled antibody. A signal is produced by the label and can be used to interpret the test result, since the signal strength correlates with the analyte concentration. This basic principle of an immunoassay is implemented in a variety of setups, which differ in assay design and type of label and can be used for qualitative as well as quantitative determinations.

Solid Phases are Needed for Purification Steps

Immunoassays usually are supported by a so-called solid phase. It is prepared in such a way that biomolecules of interest, such as an antigen or an antibody, adsorb and bind firmly to its surface. Once the antibody or antigen is immobilised, all subsequently added reagents which specifically bind to the immobilised component become attached to the solid phase surface as part of the antibody-antigen complex. The advantage of this procedure is that all non-specifically bound molecules can be removed by washing the solid phase after each step. Thus, a solid phase facilitates purification of the reacting partners. Many different materials and formats are used as solid phases, but polystyrene- and polypropylene-based microtitre plates as well as nitrocellulose-, poly(vinylidene fluoride)- (PVDF) and nylon-based membranes are the most common.

Pros and Cons of Different Assay Designs

The simplest design of an immunoassay is a direct assay. In this setup, an antigen is adsorbed to the surface of the solid phase and is detected in a second incubation step by a labelled antibody (Fig. 1A). The direct assay is a time-saving and robust system, but it is not very sensitive. In an indirect assay, the antigen is detected by an unlabelled primary antibody, which in turn is recognised by a labelled secondary antibody (Fig. 1B). The indirect system is highly versatile, because labelled species-specific antibodies can be used to target any primary antibody created in members of the respective species. The time-consuming and expensive labelling process of antibodies can be omitted because almost any anti-species antibody conjugate is commercially available nowadays. Furthermore, primary antibodies with multiple epitopes for a secondary antibody can lead to signal amplification in an indirect setup and increase the sensitivity of the assay this way. Additional enhancement in sensitivity can be achieved with a triple-step indirect assay, e.g. by including a biotin-streptavidin pair (Fig. 1C).

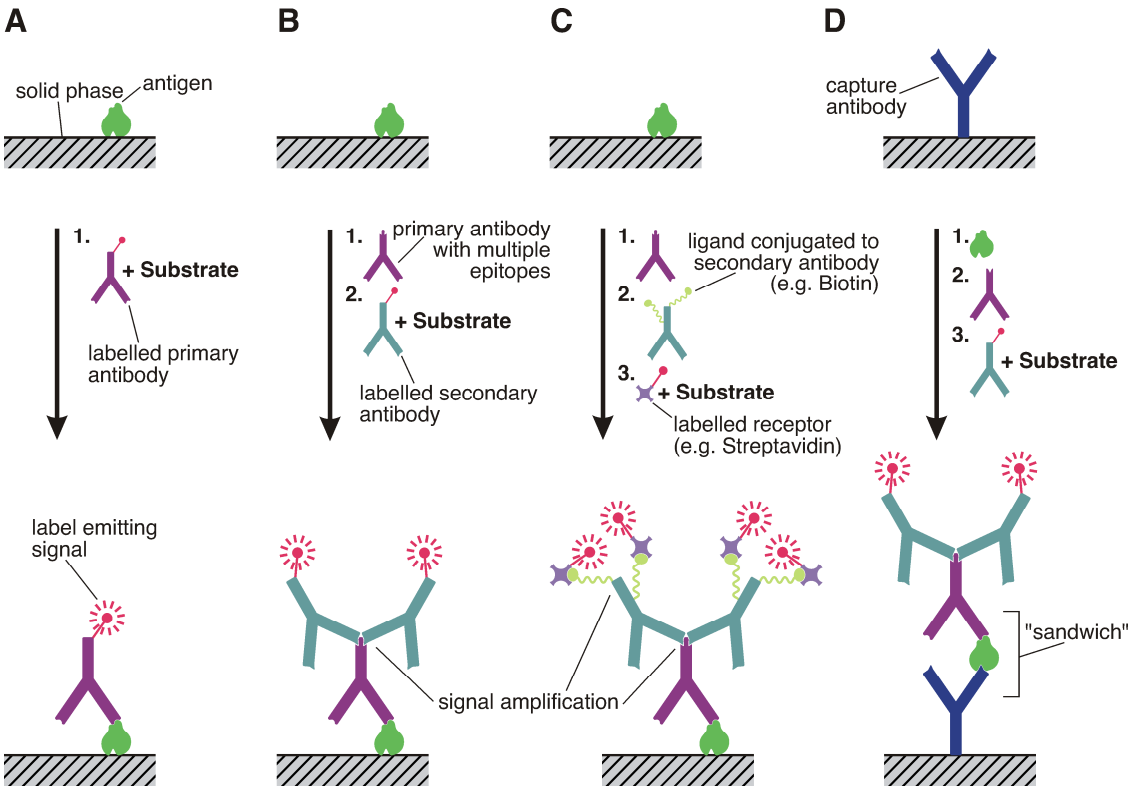


Fig. 1: Different setups for immunoassays. **A**: direct assay, **B**: indirect assay, **C**: three-step indirect assay, **D**: indirect sandwich assay.

A third assay setup with good sensitivity is the so-called sandwich assay. A capture antibody is immobilised to the solid phase instead of the antigen and binds the antigen of interest out of a solution. The captured antigen then can be detected either directly or indirectly as described before (Fig. 1D). This double recognition of the antigen by both, the capture and the detection antibody, enhances the specificity of this assay. The sandwich format is limited to antigens with more than one epitope, because both capture and detection antibodies must bind to the antigen.

Finally, the competitive or inhibition assay is an assay type that is often applied for quantitative analysis if the antigen possesses only one epitope or if the antigen is very small, so that the epitopes overlap. This method requires a calibrated assay system with known quantitative outcomes. The analyte is inhibiting or competing with one of the reagents of the calibrated system so that the signal is reduced correlative to the amount of analyte contained in the sample (Fig. 2)[2]

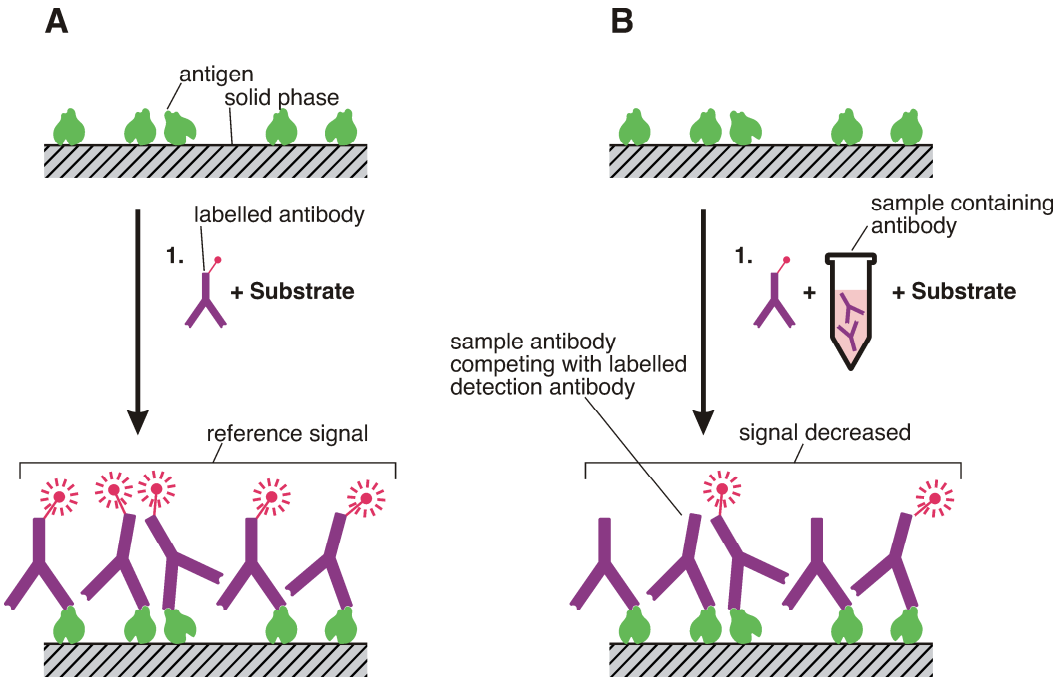


Fig. 2: Direct competitive assay for the determination of an antibody. **A**: Pre-titrated system with known quantities of reagents. **B**: Antibody in the sample is competing with the labelled detection antibodies and causes a decrease in signal.

Various Labels and Assay Technologies are Used for Immunoassays

The signal in an immunoassay is produced by a label linked to one of the detection reagents. Numerous labels are available. Radioactive labels applied in the radioimmunoassay (RIA) used to be very popular due to their high sensitivity, but they implicate health risks and disposal problems inherent to radionuclides. Nowadays, optically detectable labels, such as fluorescent, luminescent or phosphorescent dyes, are more common. The most important class of labels, however, are enzymes that are able to catalyse a reaction of a chromogenic substrate. They are used in an enzyme-linked assay (EIA) or enzyme-linked immunosorbent assay (ELISA, to emphasise the use of a solid phase). The ELISA is probably the most frequently used assay next to immunoblots, which are carried out on different types of membranes and apply mainly dye-labelled detection molecules. Automated high-throughput assay techniques are often based on the ELISA or on some other type of immunoassay concept, but utilise special kinds of solid phases (e.g. particles). Such automated systems, as well as more elaborately designed assays e.g. ELISPOT (a method for direct analysis of cells), are predominantly used in clinical diagnostics. A lateral flow assay is a qualitative immunoassay with an additional chromatographic separation step which is often used in over-the counter products, e.g. pregnancy tests.

Reagents and Analytes Used in an Immunoassay: a Terminological Question

Although immunoassays were originally developed on the basis of antigen-antibody pairs, many other combinations of specifically and strongly binding biomolecules are known today. Therefore, assays can be conducted analogous to immunoassays without using any antibody-antigen pairs. Referring to such assays as an immunoassay often is considered inappropriate

because the word “immuno” indicates the presence of an antibody and an antigen. A terminologically correct solution, e.g. “solid phase assay” or a “bioassay” is used instead of the term “immunoassay” in such a case, but the difference is not essential for this thesis.

1.2 Blocking Reagents

While in principle an immunoassay can be conducted as described above, each assay needs to be optimised with regard to its sensitivity and specificity. In a quantitative immunoassay, the sensitivity is determined by the proportion of the signal strength in relation to the amount of signal-inducing analyte. E.g., a highly sensitive assay will produce sufficient signal strength even for low analyte concentrations. The specificity of an assay on the other hand states how much signal is produced by unintended reactions, e.g. a highly specific assay will only produce signals derived from the intended antigen-antibody interaction. In terms of statistical analysis, the sensitivity equals the ability to detect true positives (exclusion of type II error) and the specificity equals the ability to detect true negatives (exclusion of type I error). Both abilities are essential requirements for a good signal-to-noise ratio (S/N) of an assay.

The Specificity of an Assay Is Decreased by Non-Specific Binding

In a first step of an immunoassay, an assay component is adsorbed onto the solid phase surface. This adsorption process is referred to as the coating step. Since an excess of coating reagents leads to instabilities in the binding of the coated material to the surface, the solid phase cannot be fully covered by the coating component. As a consequence, all components present in subsequent assay steps may encounter unoccupied spots of the solid phase surface and adhere in an unintended manner. This non-specific binding (NSB)[3] is a major cause of false-positive detection (observable as background) and thus decreases specificity and signal-to-noise ratio. In most cases, the overall signal-to-noise ratio cannot be enhanced by using higher amounts of sample and detection reagents, as this will usually increase both signal and background. Besides that, the amount of sample is limited in many situations. As a consequence, the reduction of NSB is of utmost importance in order to improve an assay.

How Blocking Reagents Work

A very common procedure for the prevention of NSB is the saturation of the solid phase surface with a blocking reagent. For this purpose, coating is followed by a blocking step, where remaining free spots on the solid phase surface are covered with blocking reagent molecules. Requirements for a good blocking reagent are very strict. An ideal blocking reagent must saturate the solid phase surface fully and has to bind tightly to the solid phase surface. The blocking reagent is furthermore required to repel any subsequently added assay components and must not interfere with the intended antibody-antigen recognition process in any way (Fig. **3**A). Any shortcoming regarding these requirements will end up in either lower sensitivity or lower specificity. If blocking reagent molecules cover or replace coating material, the outcome in signal is reduced and the sensitivity is decreased (Fig. **3**B). On the other hand, prevention of NSB will be insufficient if the blocking reagent is not covering all remaining unoccupied spots on the solid phase surface, or if the blocking reagent is only loosely bound and is easily replaced by subsequently added reagents. (Fig. **3**C). If blocking

reagents are not acting repulsively or if they exhibit cross-reactive properties, NSB still takes place but the location of NSB events is shifted (from solid phase surface to blocking reagents, Fig. **3**C).

From a physicochemical point of view, the treatment of a solid phase surface with a blocking reagent is an adsorption process which can be described by the equation:

$$[BR]_{aq} + [S^*] \rightleftharpoons [S\text{-}BR] \qquad \text{(Eq. 1-1)}$$

Here, $[BR]_{aq}$ is the concentration of the dissolved blocking reagent, [S*] represents free spots and [S-BR] represents spots occupied by a blocking reagent on the solid phase surface. Both sides of the equation are in equilibrium as indicated by the right- over leftward arrows. This means that the adsorption is a reversible process (which we assume here since it is true for many adsorption processes). Although this simplified equation does not accurately reflect a real adsorption process of heterogeneous blocking reagents on a solid phase with a (on molecular scale) very irregular surface,[4] it still provides useful information. The equilibrium constant K_{Ads} for the adsorption, a ratio of the concentrations, is described as follows:

$$K_{Ads} = \frac{[S-BR]}{[BR]_{aq}[S^*]} \qquad \text{(Eq. 1-2)}$$

Good blocking reagents exhibit high values for K_{Ads}, and the blocking reagent concentration $[BR]_{aq}$ needs to be high enough in order to keep the concentration of free spots on the surface [S*] near zero. The reversibility of the adsorption process also demands that all subsequent solutions applied to the saturated surface must contain the blocking reagent, because otherwise desorption will occur to reach equilibrium once again.

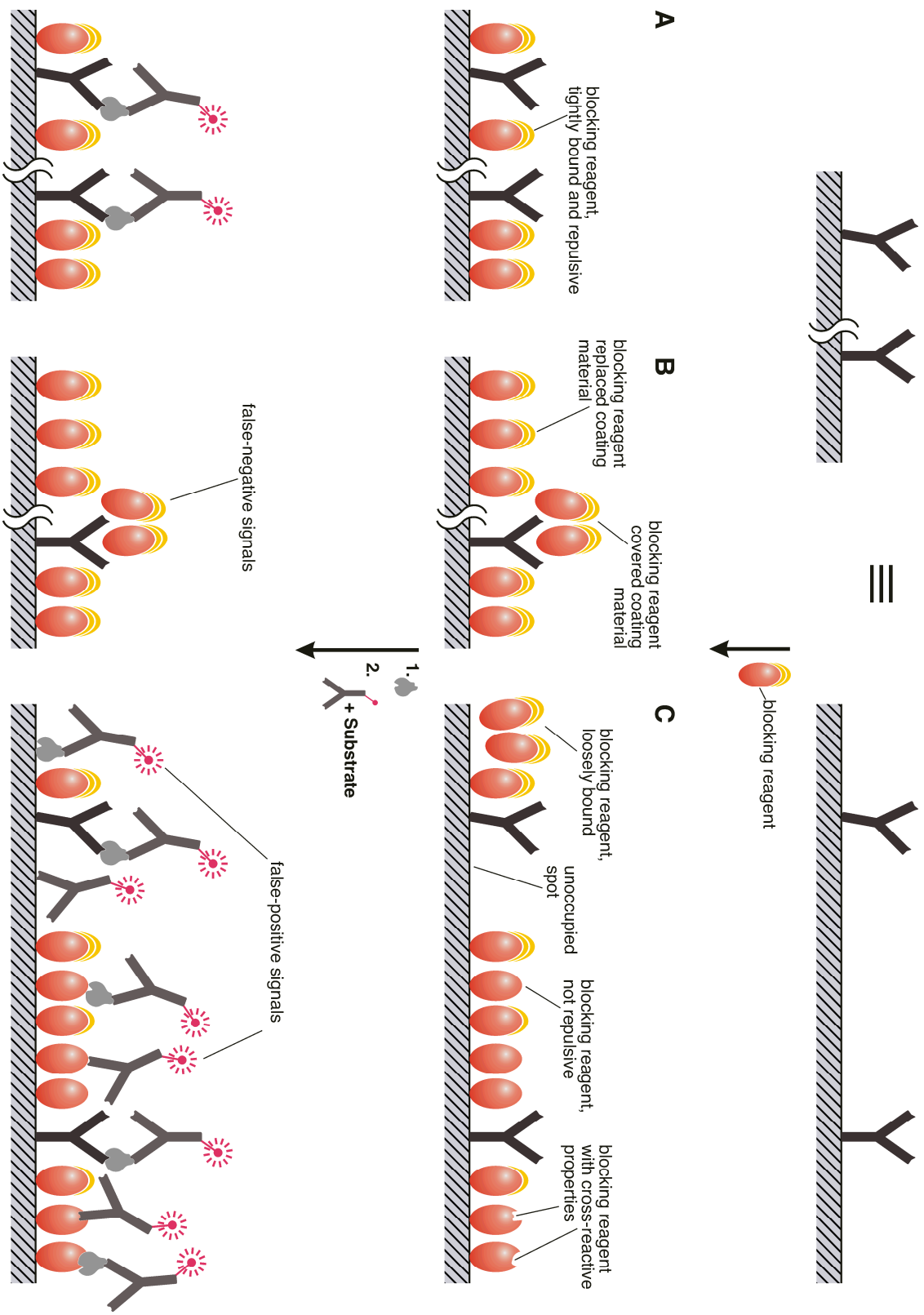


Fig. 3: Possible flaws of a blocking reagent. **A**: ideal situation. The blocking reagent prevents NSB completely without disturbing the detection. **B**: The blocking reagent is covering or replacing coating material and the sensitivity is therefore decreased. **C**: NSB occurs if the blocking reagent 1. does not saturate the solid phase completely, 2. is only weakly adsorbed, 3. has insufficient repulsion properties, or 4. is cross-reactive. As a consequence, false-positive signals and/or high background are induced and the specificity (and thus, the signal-to-noise ratio, too) is decreased.

State of the Art Blocking Reagents and their Drawbacks

The majority of commercially available blocking reagents is derived from biological sources and includes materials such as animal sera, gelatine, skimmed milk, treated or non-treated proteins and protein fractions like bovine serum albumin (BSA),[5] casein or casein hydrolysate. Detergents and polymers like Tween20 or Poly (vinyl pyrrolidone) (PVP)[6] are commonly used protein-free blocking reagents.

Existing blocking reagents provide a sufficient solution for many NSB problems, but they exhibit serious disadvantages. Due to their biological origin, proteinaceous blocking reagents are not only heterogeneous, lot-to-lot variable and decomposable but may also be subject to import and export restrictions (e.g. materials of bovine origin). These reagents furthermore tend to cross react [7, 8] and may even interfere with important recognition processes like streptavidin-biotin binding.[9] Problems with cross reactivity have led to the development of reagents like fish sera,[10] which show less cross reactivity with reagents of mammalian origin. Some biologically derived blocking reagents e.g. skimmed milk are known to possess very good NSB reducing abilities, but they may also decrease the sensitivity of the assay by covering or replacing the coating material. [11, 12] Synthetic blocking reagents do not share these disadvantages, but they often are insufficient in reducing NSB and could therefore not establish a solid market share.[13]

In conclusion, blocking reagents are normally obligatory in a solid phase based immunoassay, but determination of the most appropriate reagent for a specific application is a difficult task. Many experimental assays are still not used in routine applications, because of the above mentioned issues. Despite that, investigations on blocking reagent performance or even developments of new reagents are rare. A commercial supplier of blocking reagents describes this problem as follows: "*For true optimization of the blocking step for a particular immunoassay, empirical testing is essential. …[]… No single blocking agent is ideal for every occasion because each antibody-antigen pair has unique characteristics.*".[14] This statement reflects a demand for an optimised, versatile blocking reagent. Because of the above mentioned intrinsic shortcomings of reagents from biological sources, the development of novel blocking reagents derived from a class of synthetic molecules such as surfactants is highly desirable.

1.3 Poly(ethylene glycol)-Based Surfactants as Prospective Blocking Reagents

Surfactants, sometimes also termed tensides, are amphiphilic substances which are able to lower the surface tension of a liquid. The etymology of the word already expresses this property: surfactant is a contraction of "surface active agent". The word is also used commonly as a name for the pulmonary surfactant in a medical context, but this meaning is not of interest for this thesis. Like all amphiphilic molecules, surfactants possess a hydrophilic and a hydrophobic part, and they often show a match-like structure. This is why the hydrophilic and hydrophobic groups sometimes are referred to as head and tail group. While the hydrophobic part of the molecule is always composed of mostly linear alkyl, or more generally, hydrocarbon groups, the hydrophilic moiety can be made of many different

functional groups. Based on the net charge of the hydrophilic head group, surfactants are classified into neutral, anionic, cationic or amphoteric (Fig. 4).

Term	Hydrophilic group	Head Hydrophobic tail
neutral	-OH, ether, carbohydrates	
anionic	$-COO^-$, $-SO_3^-$, $-OSO_3^-$	
cationic	ammonium derivatives	
amphoteric	often $-COO^-$ and ammonium group	

Fig. 4: Surfactant classification based on the charge of the head group.

Surfactants are found in a wide range of different applications. They can be used as detergents, in personal care products and cosmetics, as well as emulsifiers, foaming agents, defoamers, adhesives and as additives in paints and other products. The amphiphilic structure of the molecules facilitates a self-organising process that leads to aggregations of various stages. A similar process permits emulsification of immiscible phases (Fig. 5A). The lowest concentration of the surfactant at which the molecules start to form micelles is an important characteristic and is called critical micelle concentration (CMC) (Fig. 5B).

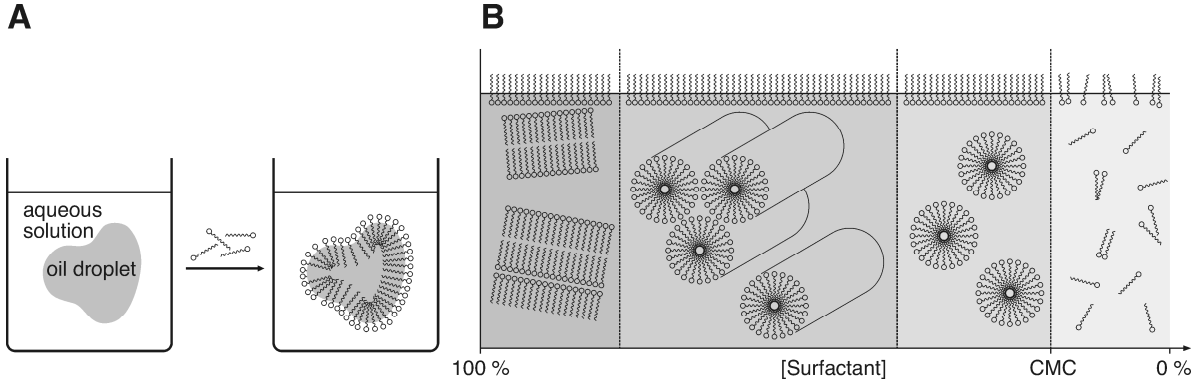


Fig. 5: Schematic illustrations of **A**: surfactant molecules emulsifying an oil droplet in aqueous solution, **B**: aggregation behaviour of an aqueous surfactant solution at different concentration.[15]

Poly(ethylene glycol) as a Surfactant Head Group

Being a poly-ether, poly(ethylene glycol) (PEG), sometimes also named poly(ethylene oxide) (PEO) or poly(oxyethylene) (POE), can be used as a head group for neutral surfactants (if no further ionic groups are added). PEGs contain the repeating unit $-CH_2CH_2O-$ and are produced in large-scale by acidic or basic polymerisation of ethylene oxide. Thus, they normally are polydisperse, i.e. they show a distribution of molecular masses. The masses of the oligomers are separated by a factor of approximately 44 Da, the mass of the repeating unit. Exceptions, i.e. monodisperse PEGs, are known for shorter chains. The synthesis of monodisperse PEGs is possible on lab-scale, but the procedure is very complex.[16] The chain length

furthermore is very limited so that many would refer to these molecules as to oligo(ethylene glycol)s rather than PEGs. The polydispersity, i.e. the range of the distribution, depends on the polymerisation method and can be described by the polydispersity index PI:

$$PI = \frac{M_W}{M_N} \qquad \text{(Eq. 1-3)}$$

with $$M_W = \frac{1}{m}\sum_i m_i M_i \qquad \text{(Eq. 1-4)}$$

and $$M_N = \frac{1}{N}\sum_i N_i M_i \qquad \text{(Eq. 1-5)}$$

Here, m is the total mass of the sample, m_i is the mass of a particular molecule with the index *i* and M_i is the corresponding molecular mass so that M_W is the weight average molecular mass (Eq. 1-4). Similarly, N is the total number of molecules present and N_i is the number of molecules with the index *i*, thus M_N is the number average molecular mass (Eq. 1-5). The parameter PI is always greater than 1 for polydisperse materials, but the smaller PI, the narrower the distribution. Although physical properties of PEGs vary greatly with the average molecular mass, PEGs do not differ too much in chemical aspects. They are quite inert, tend to react only at the end groups, and a slight decrease in reactivity can be seen for PEGs with higher M_W. Short PEGs are liquid at room temperature and slightly better soluble in less polar solvents, but most PEGs are solid at room temperature and soluble in several different solvents, notably in water, dichloromethane, methanol, THF and DMF. PEGs are extensively investigated due to their utility in research and also clinical applications, where they are valued for their low toxicity and the good solubility in water. Many applications are known for PEGs in pure form (use as laxative, lubricant, additive in cosmetics etc.) but also for PEG-bound molecules, which can be prepared by a conjugation reaction. Quite often, this PEGylation reaction is used to improve the solubility of a substance in water. It is moreover known that modification of a substance surface with a PEG layer generates a bio-repulsive structure. The modified surface repels biological substances from adsorption onto the surface more or less effectively.[17,18] So far, this characteristic of PEGs has mostly been studied with the intention to create anti-fouling coatings or devices for medical applications. However, this bio-repulsive property of PEG-modified surfaces appears highly useful for blocking reagents, because it matches the main purpose of a blocking reagent closely: a blocked solid phase surface must act bio-repulsively in order to prevent adsorption of further (mostly biological) material.

Why PEG-Based Surfactants Are Well Suited as Blocking Reagents

Given that PEGs are bio-repulsive, why then is Tween20, a PEGylated sorbitan, able to serve as a blocking reagent, while pure PEG is not? The reason lies in the fact that most solid phase materials, for instance PVDF, polypropylene, polystyrene etc., are hydrophobic. PEG, as a very hydrophilic group, is highly unlikely to bind tightly to such hydrophobic surfaces. In contrast, Tween20 is not only PEGylated but also esterified with a fatty acid (Fig. 6), so that

the hydrocarbon part can interact with the solid phase surface via hydrophobic forces. This suggests a possible mechanism how surfactants with a PEG head group are able to serve as a blocking reagent. The hydrophobic tail of a surfactant interacts with the solid phase, while the hydrophilic PEG group is responsible for the bio-repulsive property. This way, the basic requirements of a blocking reagent, namely stable adsorption and repulsion of biological material, are met by this class of surfactants.

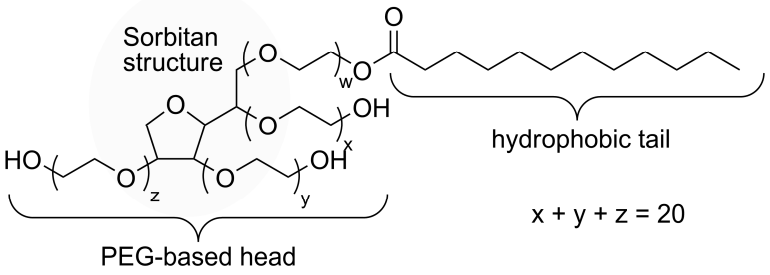


Fig. 6: Surfactant structure of Tween20.

1.4 Purpose of the Thesis

The blocking performance of Tween20 is known to be surpassed by many proteinaceous blocking reagents. It is essential for the development of prospective novel reagents to consider how this performance can be improved. As the bio-repulsive properties of PEGs are quite pronounced and well investigated,[19,20,21] it may be assumed that in particular the surfactant tail group, which is responsible for a tight binding to the solid phase surface, has room for improvement. It was observed some time ago that cation-rich peptides seemed to bind much stronger to polystyrene-based solid phases than other peptides.[22] This is believed to be caused by an electrostatic interaction between cationic parts of the peptides with electronegative groups present on the solid phase surface. Carboxylate groups are known to exist on commercially available polystyrene-based solid phases and may serve as a counterpart for electrostatic interactions. Another possible explanation is the so-called cation-π-interaction.[23] This phenomenon is very characteristic for a binding of a cation to aromatic molecules, which are exposed on polystyrene surfaces. Therefore it appears reasonable to assume that the blocking ability of PEG containing surfactants can be enhanced by equipping the molecules with cationic groups at their hydrophobic part.

The outline so far has clarified the existing problem of a lack of optimal blocking reagents for immunoassays and implicated a possible solution. The main aim of this thesis therefore lies in the development of novel synthetic blocking reagents with improved blocking performance. This will be achieved in the course of the thesis by

1. synthesis of PEG-conjugated surfactants containing cationic groups,
2. investigation of the synthesised surfactants regarding their blocking behaviour, and
3. interpretation of the results and final evaluation of the novel blocking reagents.

The synthesis is designed in a way that the structural properties of the surfactants, e.g. length and number of hydrophobic / hydrophilic chains and location of number of cationic groups, can be set systematically.

The blocking performance of the novel blocking reagents shall be investigated in relation to state of the art blocking reagents. The influence of different assay conditions on the blocking behaviour as well as the effect of the structural properties of the novel blocking reagents shall be determined in particular. The results shall help to clarify which structural aspects are needed for a surfactant with ideal blocking abilities.

A final assessment of the surfactants shall explain if and why the novel blocking reagents outperform conventional blocking reagents.

2. Synthesis

2.1 Known Substances and Synthesis Procedures

PEG containing surfactants are well studied substances[24] with a broad range of applications. They are used commonly in research as well as in consumer products and in industry. Cationic derivatives almost always contain amines and are usually synthesised in industry by reacting fatty amines with ethylene oxide, thus providing ethoxylated alkyl amines (Fig. 7).[25]

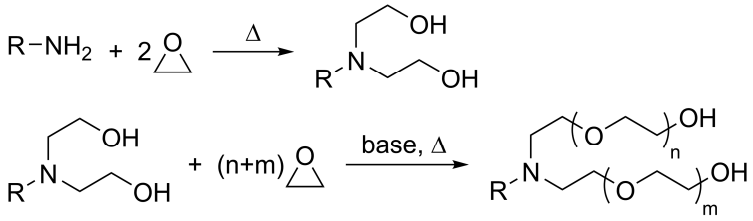


Fig. 7: Two-step ethoxylation process of an alkyl amine.

Comparable to pure PEGs, PEG-conjugated alkyl amines consist of a polydisperse mixture of oligomers. Ethoxylated derivatives of coco-, lauryl-, tallow-, oleyl- and stearyl amines are commercially produced and typically contain 2-50 equivalents of ethylene oxide per alkyl amine on average.[26] Amine ethoxylates with structures derived from alkyl propane diamines,[27] diamidoamines[28] and dialkylamines are also available (Fig. 8).

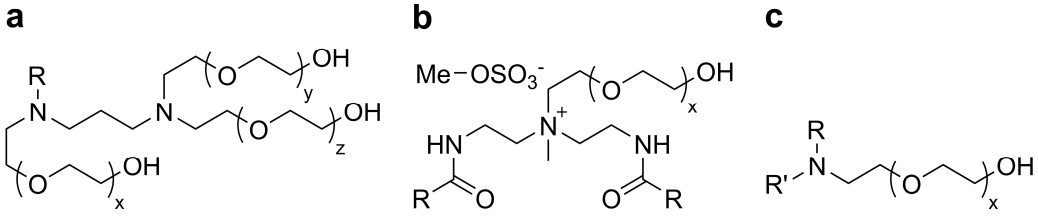


Fig. 8: **a**: an ethoxylated alkyl propane diamine; **b**: an ethoxylated diamidoamine quaternary ammonium methosulfate, **c**: an ethoxylated dialkylamine

These substances are used in many different areas, which range from industrial applications like use as emulsifying agents, aids in road construction,[27] additives for plastic,[29] corrosion inhibitors and defoamers,[26] to consumer products such as fabric softeners[28] or personal care products.

Synthesis in Research Relies on End Group Reactions Instead of Ethoxylation

The synthesis of PEG conjugates on laboratory scale normally is completely different from industrial production, as the polymerisation with ethylene oxide demands technically sophisticated equipment. End group functionalisation of commercially available PEGs, which requires less technical effort, is favoured over down scaling the ethoxylation process and is a widely accepted method in scientific research.[30] Synthetic pathways for substituted PEG-amines are also known. Such procedures are e.g. reductive amination of PEG-amines with aldehydes or of PEG-aldehydes with amines (Fig. 9):[31]

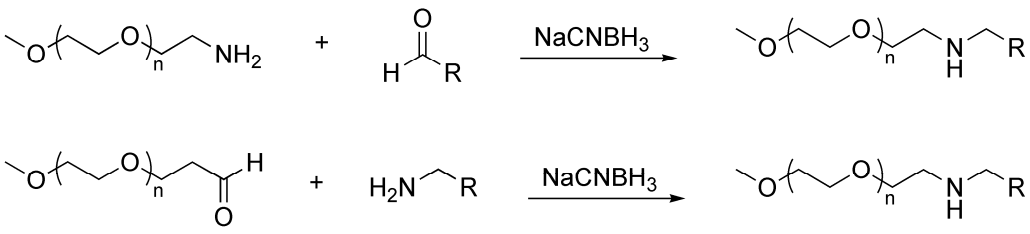


Fig. 9: Synthesis of substituted PEG-amines by reductive amination with $NaCNBH_3$.

Another suitable method is nucleophilic substitution of modified PEGs that bear a suitable leaving group with amines (Fig. 10):[31,32]

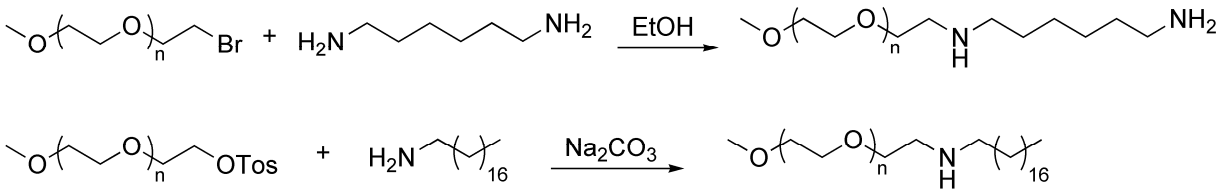


Fig. 10: Nucleophilic substitution of PEG-Bromide and PEG-tosylate with amines.

PEG-conjugated cationic surfactants are a special type of substituted PEG-amines. The reactions presented above are thus useful methods for the synthetic work of this thesis.

2.2 Building Block Synthesis

The synthesis of individual PEG-conjugated alkylamines seems to be easily realisable with the literature procedures at hand. However, as already briefly pointed out in the previous chapter, the synthesis needs to fulfil further requirements. It is, for instance, important to understand how certain structural features affect possible blocking abilities of the synthesised surfactants. Detailed knowledge about this relationship will not only help to clarify what kind of interactions and forces are involved on the molecular scale during blocking but will also facilitate the identification of preferred structures of the surfactants necessary for ideal blocking performance.

For these reasons, it was essential to design a synthesis that allows the preparation of a series of PEG-conjugated alkylamines which differ from each other systematically in only distinct structural variables. The variables were carefully chosen with respect to a potential effect on the blocking behaviour of the surfactants.

Basic Structural Variables of the Surfactants Relate to PEG, Alkyl or Amine Group

The overall assembly of the PEG, the alkyl and the amine groups will be discussed first. To simplify synthesis, a bridge is used to link the hydrophilic with the hydrophobic part. The bridge will also allow attaching of up to two chains of each group. The number of chains was restricted because molecules with complex structures are more difficult to synthesise, and they unnecessarily complicate evaluation of the structural variables in regard to the blocking behaviour. Amines were linked to the alkyl group terminally or inserted between PEG chain and bridge. With this positioning the amine was hoped to be close enough to the hydrophobic group to create a synergistic effect as mentioned above, without affecting hydrophobic inter-

actions considerably. Candidate surfactants with a modular structural design were devised with these settings (Fig. 11).

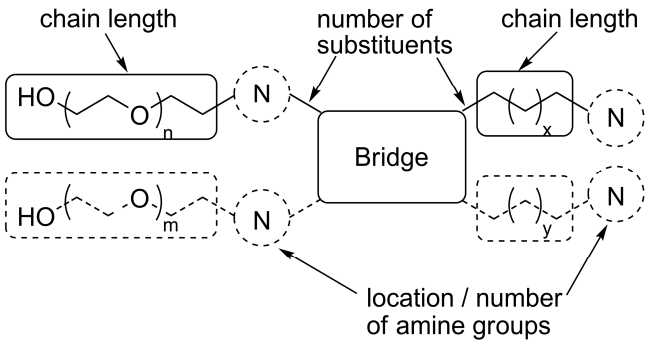


Fig. 11: Basic configuration and variables of the target structure.

The chain length of the hydrophilic, and also of the hydrophobic group is a crucial parameter. Both variables are directly associated with the solubility of the molecule and the hydrophilic-lipophilic balance (HLB), an important characteristic of a surfactant. The alkyl chain enhances the adsorption to a hydrophobic solid phase surface, but the solubility of the surfactant (in buffers) will be insufficient for blocking if the alkyl chain is too long. PEG chains on the other hand increase buffer-solubility of the molecule and they are responsible for the repulsive action of the blocked surface. Longer PEG chains may exhibit steric disadvantages and disturb hydrophobic interactions of the adsorption process due to their highly hydrophilic proportions. Amines were already mentioned to be introduced because cationic groups are assumed to enhance the adsorption process of the surfactants on polystyrene surfaces. Although it seems beneficial to add as many amines as possible, their high hydrophilicity should be taken into consideration. Since the amines ought to be placed close to the alkyl group to create a synergistic effect for adsorption improvement, they will partly counteract hydrophobic forces between the alkyl chain and the solid phase surface. Considering the above adjustments, the target structure is well defined and the variables are limited to structural aspects essential for an investigation of the surfactants as novel blocking reagents (Fig. 11).

The Range of Structural Variables is Devised on the Basis of Reference Structures

The presence of both PEG and alkyl group in sufficient amounts is necessary for effective blocking, but either group is expected to show disadvantages if employed excessively. For this reason, it is expected that the amount of both hydrophilic and hydrophobic group have an optimum range with respect to a good blocking performance of the surfactant. Obviously, it is desirable to set the chain length of PEG and alkyl group in a way so that this optimum range is covered.

Two different commercially available surfactants **1** and **2** (Fig. 12) were thoroughly investigated in the course of preliminary assay studies. Since the surfactants showed promising results, their structures were used as a reference point for the chain length. Surfactant **1** is an ethoxylated oleyl amine with approximately 20 equivalents of ethylene oxide. Surfactant **2** is an ethoxylated di-coco amine with about 30 equivalents of ethylene oxide. The alkyl groups of **2** show a distribution of the alkyl chain length, with the average chain length being roughly 14 C atoms.

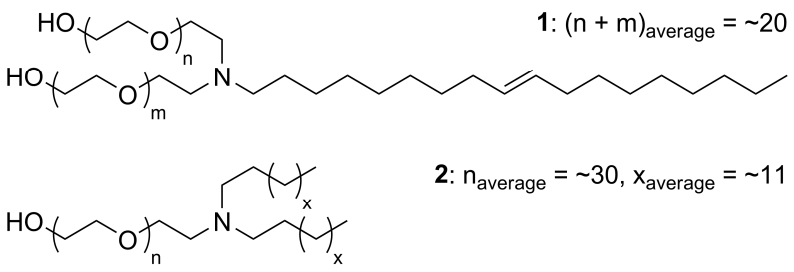


Fig. 12: Commercially available surfactants **1** (ethoxylated oleyl amine) and **2** (ethoxylated di-coco amine)

I.e. **1** has one alkyl group with a chain length of 18 C atoms, while **2** possesses two alkyl groups with a chain length of roughly 2×14 C atoms. Moreover, surfactant **2** has only one PEG group with an average molecular mass around 1300 g/mol, but **1** possesses two PEG groups with a total average molecular mass of about 900 g/mol. The chain lengths of prospective novel blocking reagents were chosen in such a way as to cover the range of chain lengths of the reference surfactants. Precursors for the alkyl group were assigned to bear either 12 or 20 C atoms, so that surfactants which possess one or two of either short or long chains, i.e. alkyl groups with 12, 20, 2×12 or 2×20 C atoms, could be investigated. Similarly, PEG precursors with an average molecular mass of approximately 550 g/mol or 2000 g/mol were used. Investigation of surfactants which exhibit PEG groups with average molecular masses of about 550, 1100, 2000 or 4000 g/mol is possible this way.

Efficient Synthesis of the Target Structures Is Achieved by a Building Block Approach

At this point, almost all parameters of the candidate surfactants are defined and a synthesis design can be prepared. In what follows, the structure of the bridge is described. The surfactants are intended to be model compounds that allow the investigation of specific structure-activity relationships. Hence, further unpredictable structural influences on the blocking behaviour should be avoided. The bridging unit was therefore kept as short and simple as possible and no additional functional groups are used.

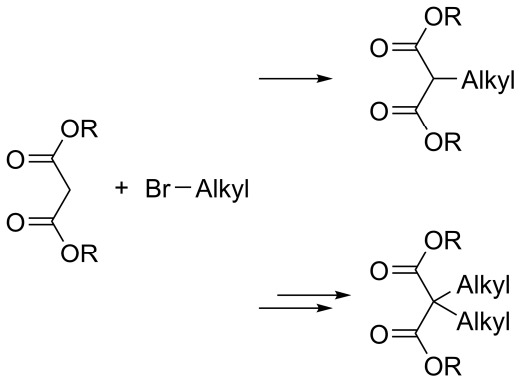


Fig. 13: Malonic ester synthesis with mono- (top) and di- (bottom) substitution shown.

Malonic ester synthesis is a highly versatile and reliable C-C bond forming reaction with usually good yields, which are obtained under rather mild conditions. This reaction type was used to connect the bridging unit with the alkyl precursors. Evidently, monosubstituted malonic esters were prepared to introduce only one alkyl group while incorporation of two alkyl groups was achieved by disubstitution (Fig. 13).

The 1,3-propane dicarboxylic function was used to connect the alkylated bridge to PEG precursors. To insert an amine between the bridge and the PEG group, the malonic ester derivative was hydrolysed to the corresponding malonic acid, coupled with a PEG-amine to an amide and reduced to a secondary amine. The malonic acid was decarboxylated prior to the linkage to PEG moieties if only one PEG group was intended to be attached. The carboxylic

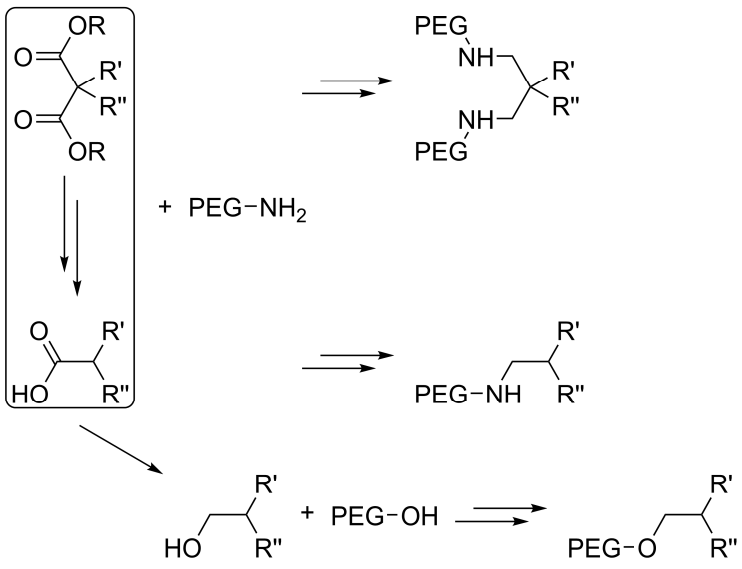


Fig. 14: Connecting options of PEG precursors to alkylated malonic acid derivatives. R = -Alkyl or -H, R' = -Alkyl, R'' = -Alkyl or -H.

acids prepared by decarboxylation of the malonic acid derivatives were also reduced to a hydroxyl group and directly linked to a PEG via Williamson ether synthesis. (Fig. 14).

The development of an ideal synthesis route for each molecule is very time-consuming and ineffective if a large group of similar compounds is synthesised. In contrast, the building block approach as outlined so far uses a very limited set of reaction types. The synthesis design is convergent for the most part to improve the yield. Many intermediate compounds are used for several different target structures and the overall amount of reaction steps is thus kept small. These reasons strongly suggest that the building block synthesis is well suited and very efficient for the preparation of the series of target structures.

Detailed Structure of Building Blocks

The alkyl building blocks are linear alkyl bromides (**3a**) or ω-phthalimido alkyl bromides (**3c**). A bromide is an appropriate leaving group for the nucleophilic substitution of the malonic ester synthesis. The phthalimido group is a protecting group for amines that is stable to many reaction conditions.[33] It was used here to enable an optional introduction of a terminal amino group in the target structure. These alkyl precursors then were converted to a group of alkyl-bridge building blocks. Mono- and di (alkyl- or ω-phthalimidoalkyl-) substituted malonic acids (**6a**, **6b** and **6c**), 2-substituted alkanoic acids (**6e**) as well as ω-phthalimidoalkanoic acids (**6f**) and ω-phthalimidoalkanols (**6g**) are alkyl-bridge building blocks. A regioselective reaction at only one end of a linear PEG is desirable for the connection of PEG building blocks to alkyl-bridge building blocks. Hence, methoxy PEGs (**4a**) and methoxy PEG amines (**4d**) were used as mono-functional PEG building blocks because methoxy groups are inert for most reaction conditions (Fig. 15).

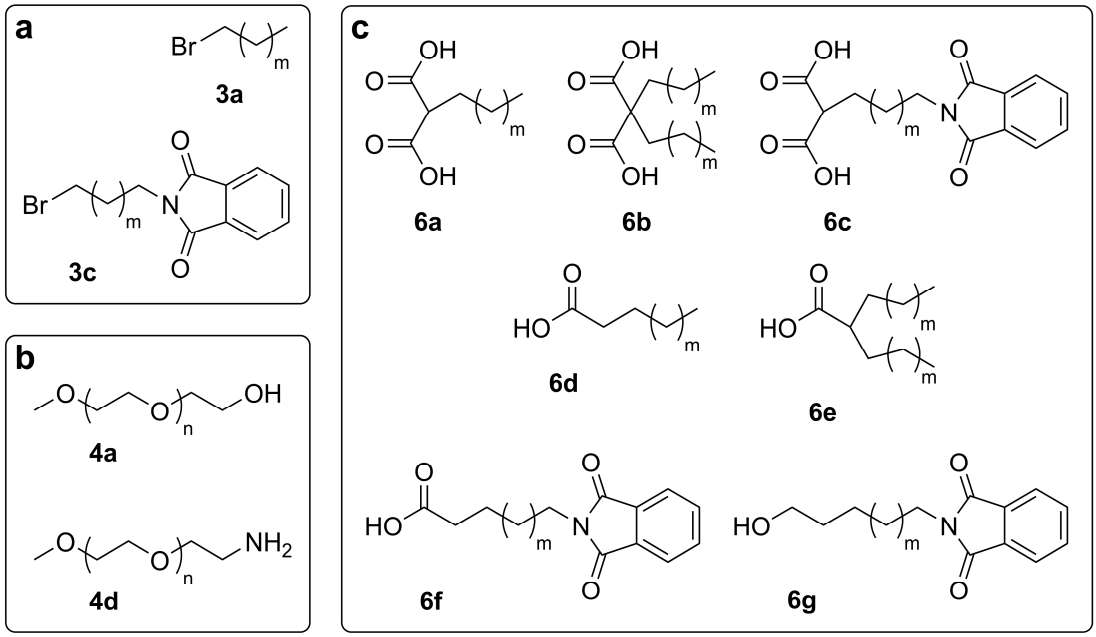


Fig. 15: alkyl- (**a**), PEG- (**b**) and alkyl-bridge (**c**) building blocks

2.3 Preparational Details

For a simplified identification, all compounds with the same core structure but different alkyl or PEG chain length were tagged with a unique combination of a number and a letter. The chain length of alkyl and PEG moieties is indicated by numbers in parenthesis, separated by a comma if both PEG and alkyl chain are present. E.g. **3a**(12) represents dodecylbromide, **3a**(20) eicosylbromide and **4d**(550) PEG amine with an average molecular mass of the PEG chain of approximately 550 g/mol.

Alkyl Building Blocks

The alkyl bromides **3a**(12) and **3a**(20) as well as dodecan-1,12-diol were purchased and used without further purification. Eicosane-1,20-diol was prepared by conversion of the eicosane dioic acid into the diacid chloride with neat thionyl chloride, followed by a reduction with $LiAlH_4$ with quantitative yields. A direct reduction of the dioic acid was avoided due to low solubility of eicosane dioic acid in conventional solvents. The α,ω-diols were mono-brominated to ω-bromoalcanols **3b** with good yields by a modified procedure of a literature protocol.[34] The ω-phthalimido alkyl bromides **3c**(12) and **3c**(20) were prepared by a Mitsunobu reaction of ω-bromoalcanols **3b**(12) and **3b**(20) with diisopropyl azodicarboxylate (DIAD), PPh_3 and phthalimide with very good yields (Fig. 16).

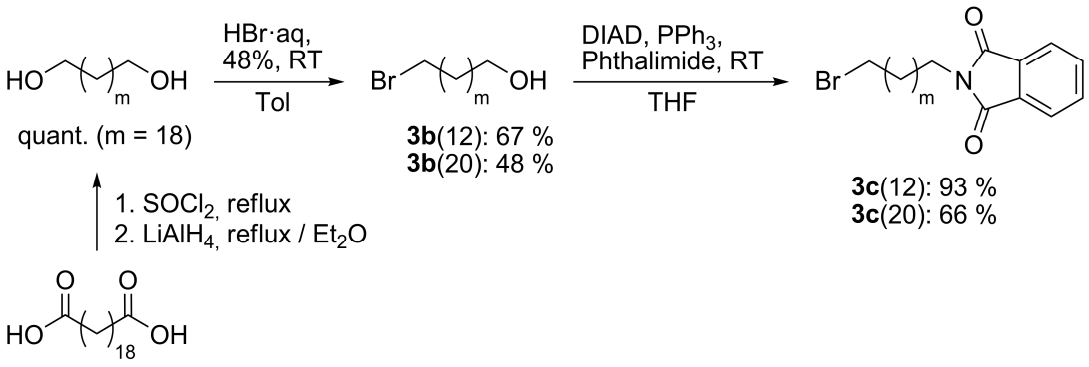


Fig. 16: Synthesis of Alkyl Building Blocks. m = 10 for (12) derivatives, m = 18 for (20) derivatives.

PEG Building Blocks

Mono-methoxylated PEGs **4a**(550) and **4a**(2000) (mPEG) were purchased and used without further purification. Numerous possible pathways to PEG amines are known to date,[35, 32] but many of them exhibit drawbacks, such as the use of very inconvenient reagents e.g. gaseous ammonia,[36] low purity of the product due to incomplete conversion[37] or formation of secondary amine by-products.[30] The product purity and the yields of the procedure used by Menger et al.[38] and Zych et al.[39] appeared very promising and the method was used with slight modifications. First methoxy PEG tosylates **4b**(550) and **4b**(2000) were prepared by reacting mPEG **4a** with tosylchloride. Nucleophilic substitution of **4b** with sodium azide gave mPEG azides **4c**(550) and **4c**(2000). The crude **4c** was then converted to the mPEG amine **4d**(550) or **4d**(2000) via Staudinger reduction. This synthetic approach resulted in excellent overall yields (Fig. 17).

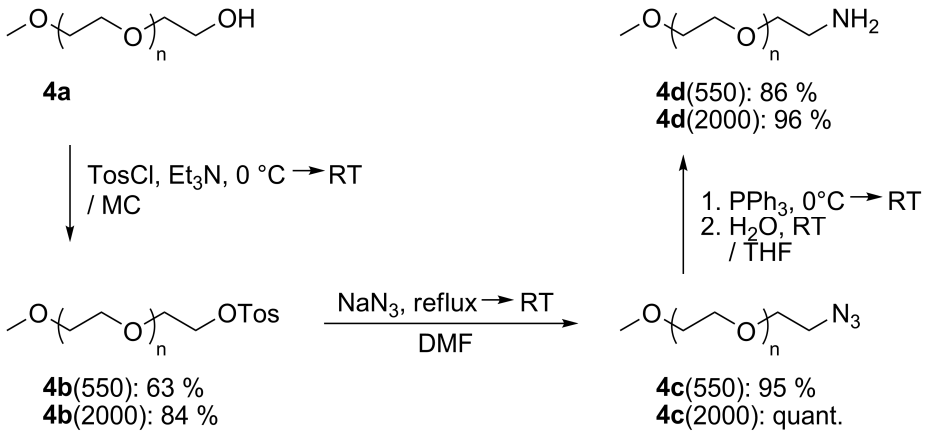


Fig. 17: Synthesis of PEG Building Blocks. $n_{average}$ = approximately 11 for (550) derivatives, $n_{average}$ = approximately 44 for (2000) derivatives.

Alkyl-Bridge Building Blocks

The alkyl building blocks **3a** and **3c** were linked to the bridge by malonic ester synthesis. The disubstituted malonic ester **5b** was synthesised by separating the reaction mixture of **5a** in two equal portions and repeating the substitution *in situ* for one half of the portions. The malonic ester syntheses gave good total yields for all substitution reactions. Basic hydrolysis of the diethyl malonic esters **5a** and **5b** was uncomplicated and gave excellent yields except for **6b**(20). The extremely low solubility of this molecule led to a loss of product upon workup.

Decarboxylation of malonic acids **6b**(12) and **6b**(20) led to the carboxylic acids **6e**(12) and **6e**(20) with quantitative yield as no workup was required (Fig. 18). Linear alkanoic acids **6d** were obtainable the same way by decarboxylation of malonic acids **6a**, but commercially available material was used instead to reduce reaction steps and to save on reagents.

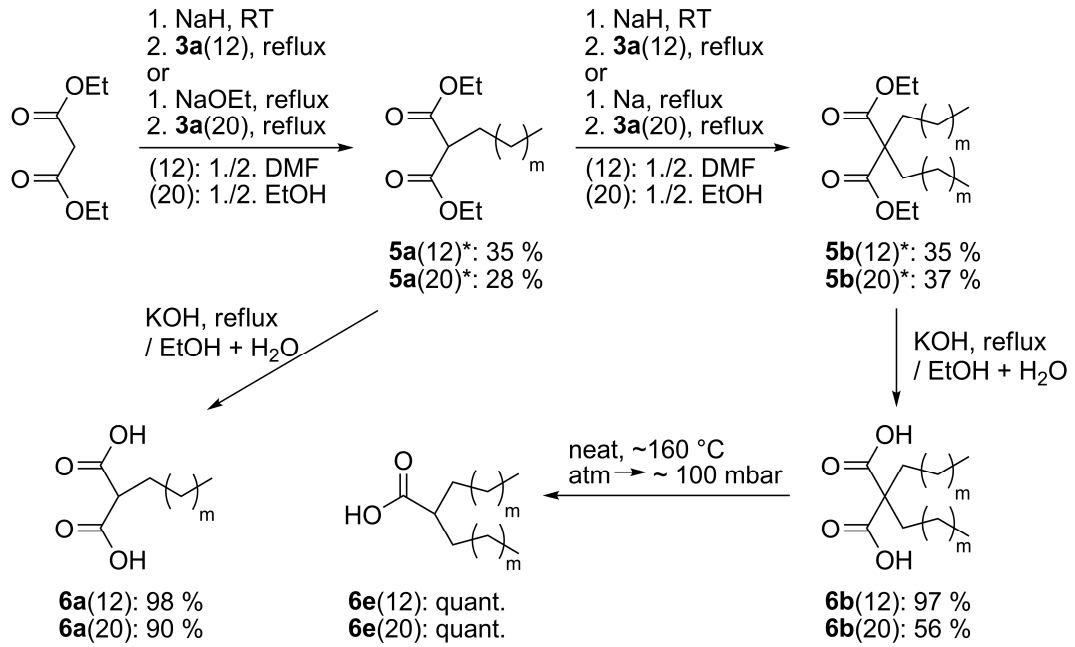


Fig. 18: Synthesis of alkyl-bridge building blocks based on alkyl group **3a**. m = 10 for (12) derivatives, m = 18 for (20) derivatives. * Yields with respect to 100 % of the unmodified malonic ester. The reaction mixture of the monosubstitution reaction was separated in two equal portions, which were subjected to either workup or to a disubstitution reaction.

The monosubstituted malonic esters **5c** were prepared similarly to **5a** but a di-*tert*-butyl malonate was employed because *tert*-butyl esters can be safely hydrolysed without harming a phthalimide group. It should be noted that the diethyl ester analogues of **5c** were also prepared and that attempts were made to selectively hydrolyse these esters without affecting the amine protecting group. Some evidence is found in the literature that a basic[40] or harsh acidic hydrolysis[41, 42] or a modification of the phthalimide[43] group is promising for this reaction, but several experiments remained unsuccessful and gave only crude material with a complex mixture of by–products. In contrast, acidic hydrolysis of the di-*t*-butyl malonic esters **5c** could be performed without difficulty, leading to pure product with quantitative yields after removal of all by–products and excess reagent. Prolonged reaction times (up to three days) were needed for a full conversion of the ester to malonic acids **6c**. Carboxylic acids **6f** were obtained by decarboxylation of **6c** with again quantitative yields (Fig. 19).

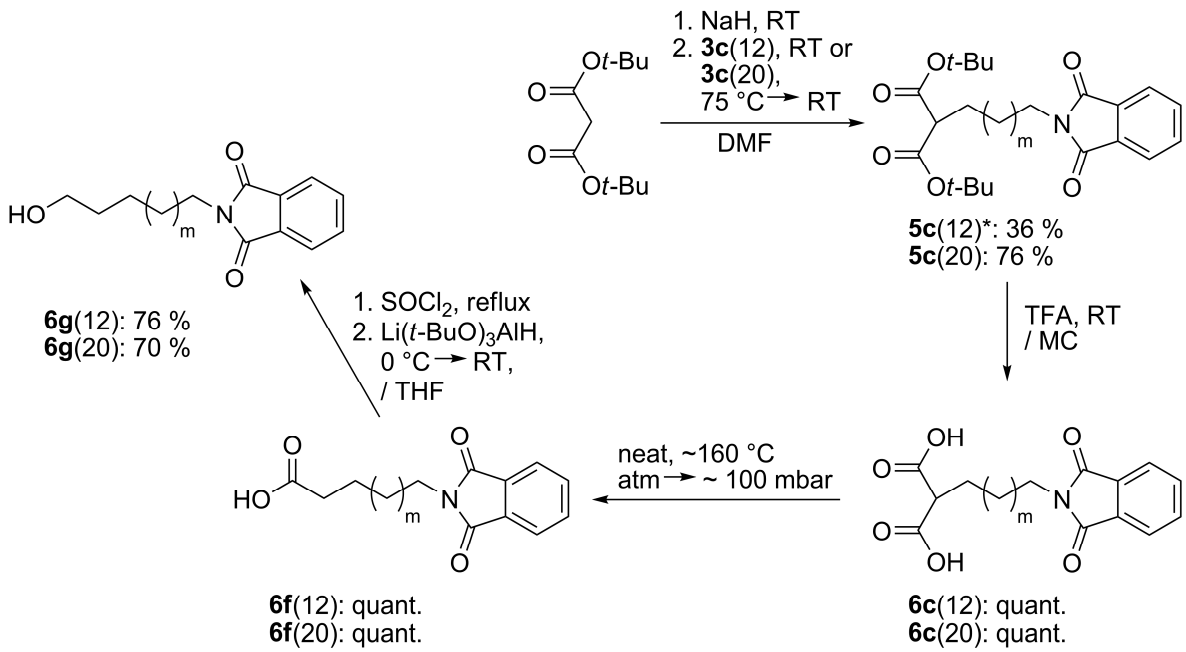


Fig. 19: Synthesis of alkyl-bridge building blocks based on alkyl group **3c**. m = 10 for (12) derivatives, m = 18 for (20) derivatives. * Yields with respect to 100 % of the unmodified malonic ester. The reaction mixture of the monosubstitution reaction was separated in two equal portions, which were subjected to either workup or to a disubstitution reaction.

The ω-phthalimidoalcanols **6g** were obtained by reduction of ω-phthalimidoacids **6f** (Fig. 19). A direct and selective reduction of the acids using either the $NaBH_4/I_2$[44] or $DCC/LiBH_4$[45] system failed, although the former method was reported not to harm methyl esters and the latter was observed to retain amides. Both attempts led to a complex mixture of products and a decomposition of the phthalimide group was observed by thin layer chromatography (TLC). It appears that an acyloxyborohydride was intermediately formed (as proposed for $NaBH_4/I_2$[44]), which was more reactive than the original reducing agent[46] and interfered with the phthalimide group. The reduction of the acid chloride of acids **6f** (the acid chlorides were initially prepared for coupling with PEG amine **4d** later on) on the other hand was successfully performed with the sterically very demanding reducing agent $Li(t\text{-}BuO)_3AlH$[47] with very good yields.

Assembly of Building Blocks and Preparation of Target Structures

The preparation of the target structures that display a direct link between PEG and bridge without an amine is presented first. The Williamson ether synthesis of phthalimidoalcanol **6g** with the PEG tosylate **4b** was performed under different conditions (e.g. temperature, reaction time, deprotonating base etc.), but the preparation was not successful. Nucleophile/ electrophile roles were therefore switched and the trifluoromethane sulfonates of **6g** were synthesised. The triflates of **6g** were then reacted *in situ* with a deprotonated mPEG **4a**.[48] Hydrazinolyis of the phthalimide group provided amine surfactants **7g** with moderate yields (Fig. 20). The yields were almost the same for **7g**(550,12) and for **7g**(2000,12). This is striking, because conversion rates for reactions involving PEGs tend to be higher the shorter and the more reactive the participating PEGs are. An acceleration effect of PEGs for

nucleophilic substitution reactions involving organo alkali metal compounds is discussed in the literature.[49] In terms of the present case, a self-catalytic activity of the PEG is well imaginable to be stronger for the longer PEG chain and this might have compensated the loss of general reactivity caused by the larger size of **7g**(2000,12).

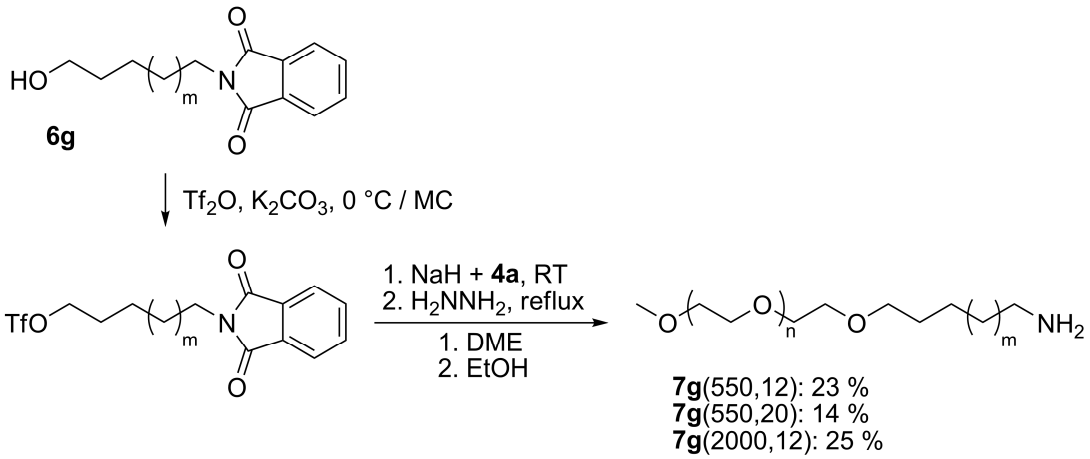


Fig. 20: Williamson ether synthesis of **7g**. m = 10 for (12) derivatives, m = 18 for (20) derivatives. $n_{average}$ = approximately 11 for (550) derivatives, $n_{average}$ = approximately 44 for (2000) derivatives.

Target structures which possess an amine between PEG and bridge are prepared as described in the following part. The malonic acid and carboxylic acid derivatives **6a**, **6b**, **6d** and **6e** were converted to the corresponding (malonic) acid chlorides using thionyl chloride. The acid chlorides were then coupled with mPEG amines **4d**, and amide derivatives **7a**, **7b**, **7d** and **7e** were obtained with good to excellent yields. The phthalimides **6f** were coupled to **4d** in the same way and the resulting product was deprotected with hydrazine to afford amines **7f** (Fig. 21). Peptide coupling reagents e.g. HCTU[50] were also used for this conversion, but tedious purification steps were necessary for the removal of by-products, thereby decreasing the yields dramatically. Coupling yields via acid chlorides in contrast were satisfying to excellent.

In general, yields in the synthesis of PEG based surfactants seem to be mainly controlled by difficulties in purification and workup. Moreover, PEGs are rather inreactive compared to analogous smaller molecules with the same functional group, and they are quite robust. This may be a reason why simple reactions with very reactive substances (e.g. coupling with acid chlorides) work better than sophisticated and complex reactions.

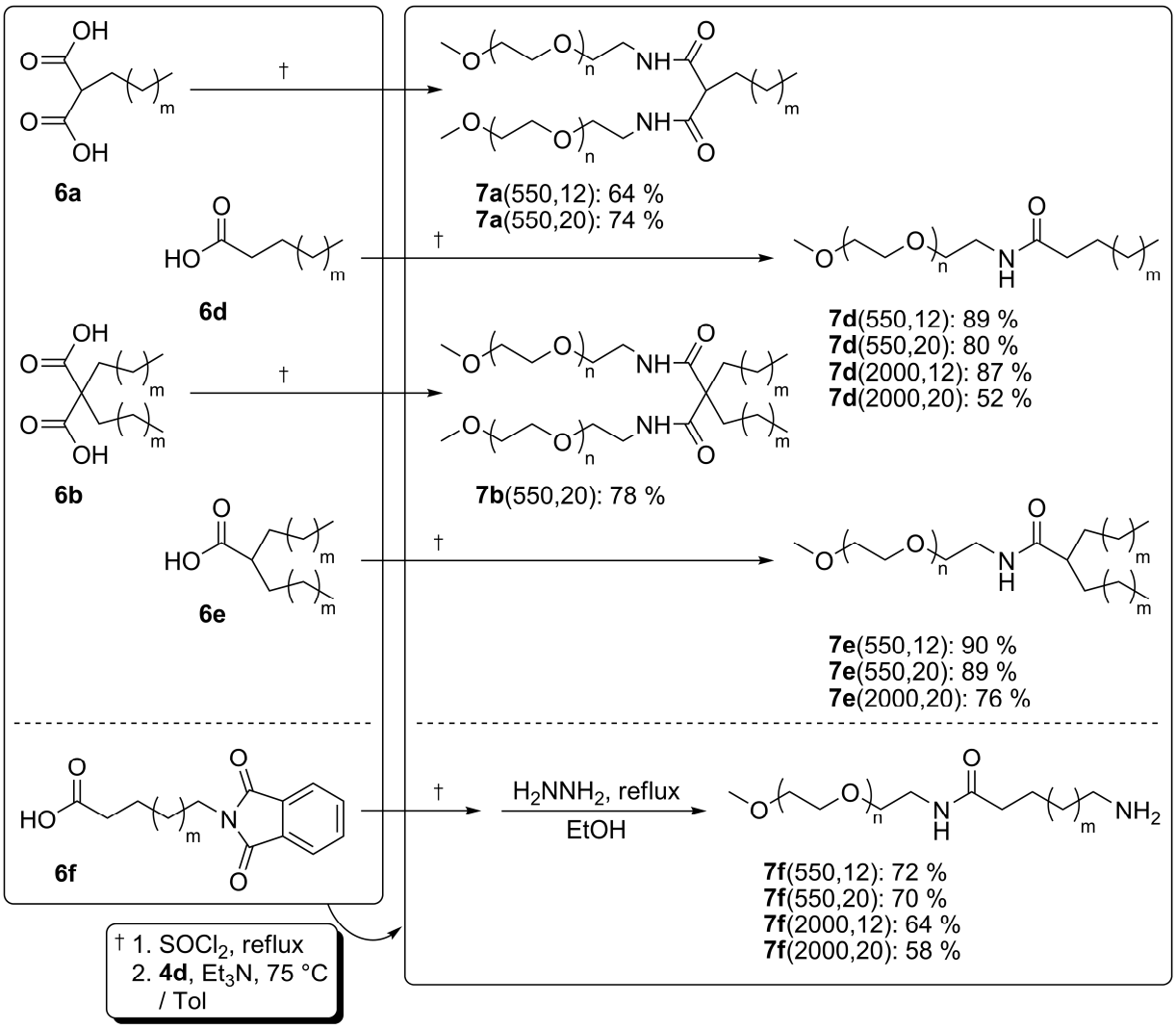


Fig. 21: Coupling of PEG building blocks with alkyl-bridge building blocks. m = 10 for (12) derivatives, m = 18 for (20) derivatives. $n_{average}$ = approximately 11 for (550) derivatives, $n_{average}$ = approximately 44 for (2000) derivatives.

Several attempts to reduce the PEG–amides with $LiAlH_4$ showed that this reduction agent leads to unreliable conversion rates and side reactions, e.g. amide fracturing to primary amines. An alternative reduction method using 1M BH_3 in THF supplied the secondary amines more reliably and with fewer by–products. The reduction rate itself seemed to be quite efficient as indicated by TLC, but complications in the workup procedure were decreasing the yield. BH_3 was also preferable as an alternative reduction agent because it normally generates only by-products that can easily be separated by an aqueous workup. But even an aqueous workup procedure caused trouble in this case due to the amphiphilic behaviour of the molecules. Most often phase separation only occurred on saturating the aqueous phase with salt, on changing the pH or on gentle heating. Even then the phase separation frequently took a lot of time to be completed. A stable emulsion of dichloromethane and water was formed by the surfactants during workup although dichloromethane is known to be highly preferred to water as a solvent for PEGs.[35] Further purification of the surfactants was similarly difficult. In some cases, i.e. if no side–products with similar molecular mass as the product itself were

present, it was possible to purify crude products via column chromatography using Sephadex LH20 as the stationary phase but aggregate formation in the (organic) solvent that was used as eluent sometimes prevented successful purification. Conventional column chromatography on silica as stationary phase was used for most of the synthesised substances. Purification of the crude products was successful with this method, but loss of product material to impure fractions was inevitable due to tailing issues typical for materials with a molecular mass distribution. Under the prevailing circumstances, yields for target surfactants **8a**, **8d**, **8e** and **8f** were good to very good while **8b** was obtained quantitatively as the purification step could be omitted (Fig. 22).

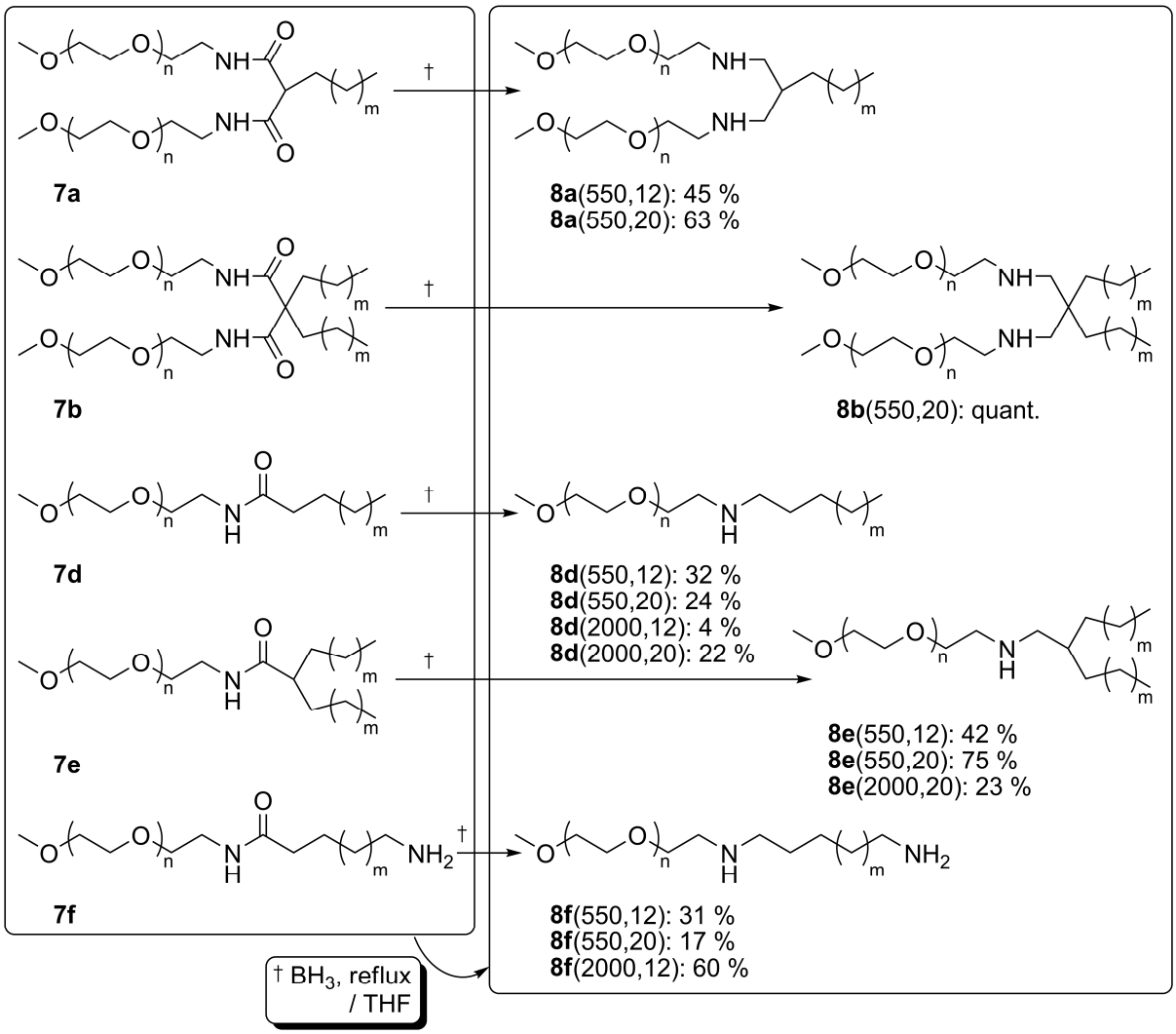


Fig. 22: Reduction of amides with borane. m = 10 for (12) derivatives, m = 18 for (20) derivatives. $n_{average}$ = approximately 11 for (550) derivatives, $n_{average}$ = approximately 44 for (2000) derivatives.

2.4 Characterisation

Analysis of the synthesised surfactants needed careful selection of the characterisation methods due to the unique features of polydispersity. E.g., elemental analysis is often routinely performed to provide proofs of the identity of organic compounds. However,

elemental analysis is not a suitable characterisation technique in this case, because an exact determination of the molecular mass distribution or the average molecular mass is necessary to calculate a precise CHN composition. Polydisperse macromolecules are also not easily analysed by X-ray diffraction techniques. Typically, NMR measurements were performed to clarify the identity of the products, but the signals of the PEG- and alkyl chains were disturbing the interpretation by covering other signals and also by distorting integral relations. Detection of most end groups still was possible with NMR techniques, and especially ^{13}C measurements proved to be valuable for the identification of certain functional groups.[30]

The most important tool next to NMR spectroscopy for confirmation of the molecular structure of the surfactants was mass spectrometry (MS). First experiments with the ESI-MS technique were unsuccessful and because MALDI-TOF MS was reported by other groups to be an important tool for the characterisation of polymers[51] and even ethoxylated fatty amines, the MALDI-TOF MS method was used instead.[52] Spectra obtained with the MALDI-TOF MS technique showed peaks referring to $[M+H]^+$, $[M+Na]^+$ or sometimes $[M+K]^+$ for each oligomer, resulting in a typically bell-shaped distribution of oligomers (Fig. 23).

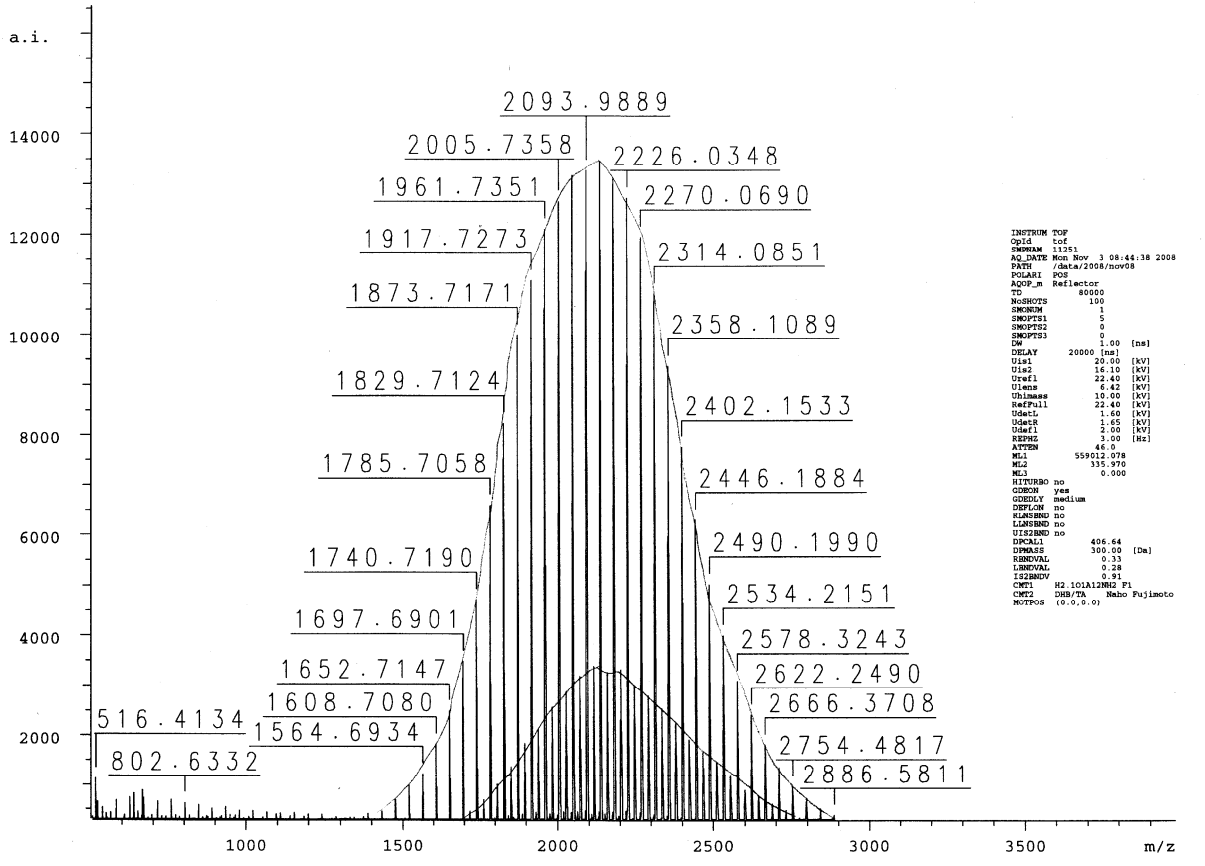


Fig. 23: A MALDI-TOF spectrum typical for the PEG-based surfactants synthesised in this thesis. The spectrum of the substance **7g**(2000,12) is shown. A bell-shaped distribution of oligomer masses for $[M+H]^+$ and $[M+Na]^+$ is distinguishable.

Solubility in Buffer

The solubility of the surfactants is most likely an important parameter for the blocking performance. As the blocking abilities of the surfactants were investigated using a solution of the substances in Dulbecco's phosphate buffered saline (D-PBS) by default, the solubility in D-PBS was roughly determined. Saturated D-PBS solutions of the surfactants were prepared if enough material was available, and a defined volume of these solutions was extracted repeatedly with dichloromethane. The solvent was removed from the extracts and the residues were dried thoroughly. The solubility of a compound was calculated as the ratio of the mass of the extract residue to the volume of the extracted, saturated solution (Table 1). Although the method is rather simple, the results reflect a relationship between structural aspects and solubility as expected. E.g. an increase in the alkyl group is decreasing solubility (**8e**(550,12) against **8e**(550,20) or **8a**(550,12) against **8a**(550,20) etc.) but the longer the PEG chains, the better the solubility becomes (e.g. **8a**(550,20) against **8d**(550,20) or **8d**(2000,20) against **8d**(550,20) etc.).

Critical Micelle Concentration (CMC)

As briefly discussed in the introduction, the CMC is an important characteristic of amphiphilic molecules like surfactants. CMC values of the target structures were determined via tensiometry. This method is based on the fact that surfactant molecules are able to decrease the surface tension of an aqueous solution. As long as the surfactant concentration $c_{Surfactant}$ is in the range below the CMC, the surface tension is decreasing with the concentration, because surfactant molecules are able to occupy positions at the liquid surface. The correlation of the surface tension for this concentration range is linear to log ($c_{Surfactant}$). Once $c_{Surfactant}$ reaches the CMC value, the surfactant molecules in the solution start to form micelles and other aggregates, and thereby indicate that the liquid surface is saturated with surfactant molecules. The surface tension therefore should not change above the CMC in an ideal system. In reality, the surface tension often continues to decrease logarithmically with $c_{Surfactant}$, but not as rapidly as below the CMC. The CMC value can therefore be determined as the intersection point between two linear correlations of surface tension against log ($c_{Surfactant}$) (Fig. 24).

The Du Noüy ring method[53,54] was used to experimentally investigate surface tensions of surfactant solutions for a series of different concentrations. A platinum ring was dipped into a surfactant solution and the solution surface was lowered slowly. A liquid film is then formed and stretched between the ring and the surface of the solution. The pull on the ring was recorded at the moment the liquid film teared off the ring. This force is approximately the same as the maximum pull on the ring (F_{max}) applied during the measuring process. With the assumption that the weight of the liquid volume beneath the ring (F_V) is constant for all concentration ranges, F_{max} is correlated linearly to the surface tension (Eq. 2-1):

$$\sigma = \frac{F_{max} - F_V}{L * \cos\theta} \qquad L: \text{wetted length}, \quad \theta: \text{contact angle, } 0° \text{ for max. pull} \qquad \text{(Eq. 2-1)}$$

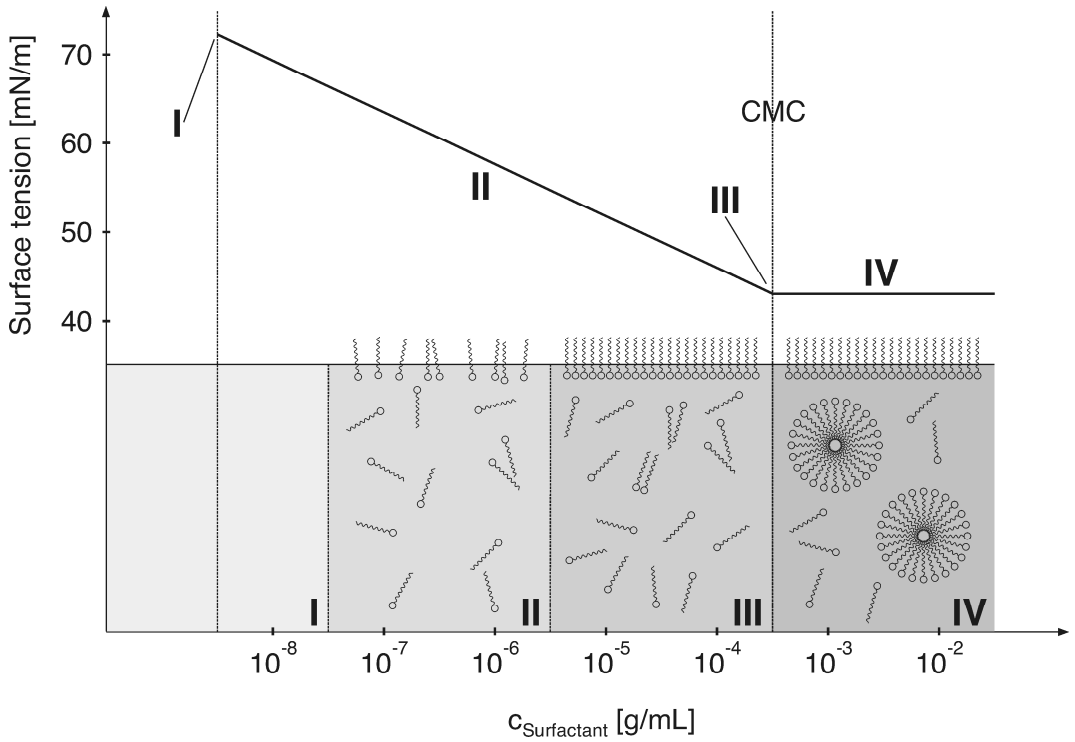


Fig. 24: Schematic illustration of the relationship between surface tension and concentration of surfactant molecules. Concentration numbers are arbitrary, only exemplary specifications to represent the logarithmic notation of the x-axis. **I**: No surfactant molecules present, **II**: Low surfactant concentration, no aggregation, **III**: surfactant molecules form a monolayer at the liquid surface, **IV**: Surfactant molecules additionally aggregate as micelles at concentrations above the CMC.

Correction factors are known for the precise determination of surface tensions. However, they cannot be used here since the density of the investigated liquids (which is unknown) is needed to apply the adjustment. Errors regarding the surface tension are estimated to be negligible in this case. Furthermore, the intersection point of the surface tension functions would be only slightly affected by the corrections, because the correction factor relates quite evenly and is relatively independent from the surfactant concentration. Separate linear regressions were carried out for both of the two visually distinguishable concentration regions below and above the CMC for the function of the recorded pull force (approximately F_{max}) against log $c_{Surfactant}$. As surface tension and F_{max} are linearly correlated, the concentration at the intersection points remains unchanged. CMC values were then obtained by calculating the intersection point of the two fit functions (Table 1).

Table 1: CMC and solubility data of cationic surfactants

Substance	**CMC**[1] [g/mL]	**CMC** [mM]	**Solubility** [g/mL]	**Solubility** [mM]
Tween20	0.0000767	0.0625	n.d.	n.d.
7f(550,12)	0.0013077	1.6983	0.158	204.8
7f(550,20)	0.0000716	0.0804	0.184	206.6
7f(2000,12)	>0.0033000[2]	n.d.[2]	0.173	78.1

7f(2000,20)	0.0001010	0.0432	0.151	64.4
7g(550,12)	0.0002903	0.3976	0.456	624.7
7g(550,20)	n.d.[3]	n.d.[3]	0.171	196.6
7g(2000,12)	>0.0033000[2]	n.d.[2]	0.240	110.1
8a(550,12)	0.0003447	0.2632	0.253	193.1
8a(550,20)	0.0001808	0.1273	0.171	120.4
8b(550,20)	0.0001091	0.0642	0.044	25.8
8d(550,12)	0.0003812	0.5083	n.d.	n.d.
8d(550,20)	0.0000698	0.0831	0.036	43.3
8d(2000,12)	0.0014124	0.6420	n.d.	n.d.
8d(2000,20)	0.0000994	0.0430	0.213	92.4
8e(550,12)	0.0000971	0.1067	0.071	78.6
8e(550,20)	0.0000421	0.0369	0.028	24.6
8e(2000,20)	0.0007274	0.2809	0.092	35.5
8f(550,12)	0.0022099	2.9077	0.128	168.4
8f(550,20)	0.0001020	0.1159	0.229	260.2
8f(2000,12)	>0.0033000[2]	n.d.[2]	0.255	115.2
1	0.0000275	0.0239	0.173	150.6
2	0.0001115	0.0664	0.196	116.7

[1] CMC values are uncorrected, i.e., correction factors for the surface tension due to temperature, geometry and/or density influences have not been used for calculation. n.d.: [2] CMC not determined in the concentration range observed, assumably higher than highest measured dilution (0.0033 g/mL); [3] not determined

Although some of the surfactants exhibit a quite high CMC, many others show CMC values in the usual range of surfactant molecules (Table 1). For micelles in an aqueous solution, the hydrophilic group of a surfactant forms an outer layer, which shields the hydrophobic core of the micelles from the surrounding solution. The micellar structure suggests that surfactants with small hydrophobic fractions start to form micelles at low concentrations, because only few molecules are needed to form a micelle. In practice, the relationship seems to be the other way round, i.e. surfactants with long alkyl chains exhibit particularly low CMC values (e.g. the CMC of **8e**(550,20) is 0.0369 mM while the CMC of **8e**(550,12) is 0.1067 mM, etc.). It appears that the low solubility of compounds with particularly high hydrophobic proportions is the reason for this aggregation behaviour at low concentrations, and that this solubility effect is more pronounced than the structural influence on micelle formation.

3. Immunoassays

3.1 Preliminary Studies

As discussed in the previous chapter, the commercially available surfactants **1** and **2**, both of which belong to the class of PEG-conjugated alkyl amines, serve as a reference against which the performance of all other surfactants is measured. In contrast to the synthesised surfactants, substances **1** and **2** were available in sufficient amounts so that the number of immunoassay experiments with these substances was not limited in practical terms. Therefore many preliminary investigations were conducted only with surfactants **1** and **2**.

NSB Prevention Is Studied with Foetal Bovine Serum as Reference Material

A model-assay for the determination of NSB as an indicator for the blocking performance of a substance was developed and used for most preliminary experiments. The ability of blocking reagents to prevent NSB is basically independent from the coating material, so the assay was started by blocking an uncoated polystyrene microtitre plate. Biological samples usually contain a variety of biological components beside the analyte and provoke the main part of NSB. Samples may be e.g. sputum, faeces, urine or blood, among a range of other examples, but sera are routinely analysed with ELISAs. Foetal bovine serum (FBS) was obtainable in large quantities of one specific lot and used as a reference sample. The influence of the amount of potentially non-specifically binding matter on the blocking behaviour was studied by varying the concentration of FBS, which was applied as a serial dilution (unless otherwise noted). Non-specifically bound serum components were determined by use of Concanavalin A (ConA). This lectin was thought to be able to detect as many serum components as possible, as most proteins, including immunoglobulins and serum albumin, are at least partly glycosylated and ConA recognises a very common glycosylation pattern[55] (internal non-reducing terminal mannose groups). The ConA was Biotin-conjugated and allowed the indirect detection with a horseradish peroxidase-conjugated streptavidin (SA-HRP). 3,3'-5,5'-tetramethylbenzidine (TMB) is a sensitive chromogenic substrate for horseradish peroxidase (HRP) and was used to visualise the detection provided by the Biotin-ConA / SA-HRP system. Spectrophotometrical intensities were measured and expressed as optical density (OD). With this general setup, the signal strength of OD indicates the degree of NSB. Negative controls were conducted by stepwise replacement of each incubation solution by pure blocking solution. I.e. blocking solution was used instead of incubation solution for (**a**) FBS incubation, (**b**) both FBS and ConA incubation steps and (**c**) FBS, ConA and SA-HRP incubations.

If the concentration of the variable parameter (e.g. FBS in the model assay) was applied in a serial dilution, a four parameter logistic function (Eq 3-1) was fitted to the raw data to describe the sigmoidal correlation between OD and the logarithm of the concentration.

$$f(x) = \frac{A - D}{1 + \left(\frac{x}{C}\right)^B} + D \qquad \begin{array}{l} A: \text{Bottom}, B: \text{Slope} \\ C: \text{Inflexion point}, D: \text{Top} \\ x: \text{concentration}, f(x): \text{signal (OD)} \end{array} \qquad \text{(Eq. 3-1)}$$

An analytical software (Prism 4) was used for the fit and initial values were set to A = 0.1, B = 1.0, C = 10.0 and D = 4.0. The results were summarised by plotting both OD as well as fit function against the concentration. A logarithmic scale was used for the concentration.

Neutral pH Values Are Ideal for the Blocking Performance of a Reference Surfactant

The pH value of blocking solutions is mainly determined by the buffer in which the blocking reagent is dissolved, because the pH range in which buffering agents are able to keep a pH value relatively constant is narrow ($pK_a \pm 1$; pK_a is the negative common logarithm of the acid dissociation constant of the buffering agent). Most immunoassays are conducted at neutral pH values, but some reagents demand basic or acidic conditions due to stability aspects. Moreover, amine-containing surfactants can be present in an aqueous solution as an amine derivative or as the protonated form of an amine, an ammonium compound. The concentration ratio of the two possible forms is determined by the pH value and will be 1 at a pH value of the pK_a of the ammonium derivative:

$$pH = pK_a + \log_{10}\frac{[\text{Amine form}]}{[\text{Ammonium form}]} \quad \text{Henderson – Hasselbalch equation applied for amines} \qquad \text{(Eq. 3-2)}$$

The pK_a of ammonium compounds is typically about 10, so the protonated form prevails at neutral or acidic conditions, while a considerable amount of the amine form is found in basic solutions. Assuming that the two possible forms have different blocking abilities, then different results for the blocking performance should be obtained for one and the same surfactant at different pH values.

To investigate the influence of different pH values, blocking solutions with distinct pH values were tested with the model-assay as described before. 0.5% (w/v) blocking solutions of surfactant **1** were prepared with (**a**) a piperazine-1,4-bis-2-ethanesulfonic acid (PIPES) buffer with pH values of 5.8, 6.3, 6.8 or 7.3, (**b**) a phosphate-buffer with pH values of 6.3, 6.8, 7.3, 7.8 or 8.3 and (**c**) a tris(hydroxymethyl)aminomethane (Tris) buffer with pH values of 7.3, 7.8, 8.3 or 8.8. The selected pH values are within the ideal buffer range of each buffering agent as stated above (pK_a values are 6.76 for PIPES, 7.20 for $H_2PO_4^-$, and 8.06 for Tris[56]).

The results for PIPES-buffers do not differ substantially for the four pH values investigated (Fig. 25). Signal strength deviations of the fitted curves seem to be statistical irregularities rather than tendencies for worse or better blocking behaviour at certain pH values. Contrary to these findings, the fitted curves of the data obtained with the phosphate-buffers clearly show a preference for neutral pH values. Signal increase of the fit functions indicative for higher NSB starts at lower FBS concentrations for slightly lower or slightly higher pH values than 7.3. A similar yet much more explicit result is obtained with the Tris-buffers. A huge shift in the slope of the fitted curve to lower FBS concentrations, i.e. higher NSB, is seen from pH 7.8 to 8.3, and especially to 8.8 (Fig. 25).

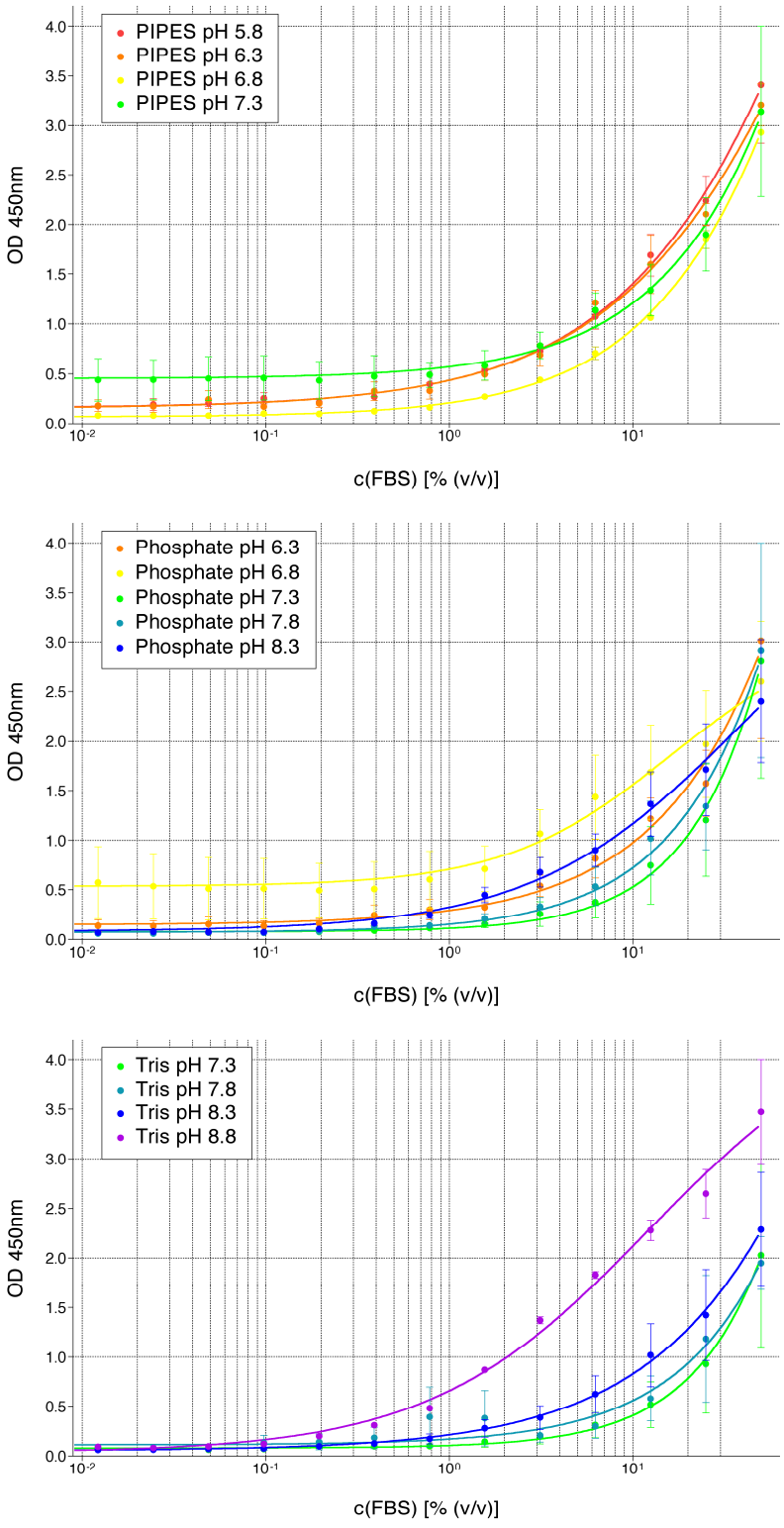


Fig. 25: Influence of pH values on the blocking behaviour of surfactant **1**. OD data points are given as mean ± range (N=2). The signals are false-positive and reflect the degree of NSB caused by FBS.

To sum up, basic conditions led to a very poor blocking performance of surfactant **1** as seen by Tris- and Phosphate-based blocking solutions. On the other hand, an acidic pH value either resulted in a moderate decrease of the blocking abilities of surfactant **1** (phosphate-buffer results) or showed insignificant overall influence (PIPES results). However, it should be noted

that a difference in the buffering agent may also affect the blocking performance, so that any conclusion that depends only on the pH value needs to be weighed carefully. Nevertheless, the striking agreement of the logistic curves of phosphate- and Tris-based blocking solutions at the pH values that were common to both buffer systems (7.3, 7.8 and 8.3) suggest that the influence of different buffering agents on the blocking performance is negligible at least for these two buffers. This, in turn, indicates that the best blocking performance of surfactant **1** is achieved at neutral pH values.

Ionic Strength of Common Buffers is Adequate for Blocking with a Reference Surfactant

Ions play an important role in the simulation of physiological conditions in buffers. Normal saline is a solution of 0.9% (w/v) or 154 mM sodium chloride. Although the physiological nature is questioned in literature,[57] normal saline is routinely used in medicine (e.g. for intravenous infusions) and the sodium chloride concentration of normal saline provides a basis for many buffer compositions used in immunoassay experiments (e.g. D-PBS). The aggregation behaviour of surfactants, however, is likely to be related to the presence and concentration of ions like sodium or chloride, because ions may affect the hydrophilic forces of the surfactant molecules. Hydrophilic interactions of the amines and the polystyrene surface (e.g. cation-π or electrostatic interactions) might be affected similarly, e.g. cations could compete with the amine/ammonium groups.

The blocking behaviour of surfactant **1** was investigated with the model-assay for blocking solutions with specific amounts of ions. In a first simple approach, 0.5 % (w/v) blocking solutions of surfactant **1** were prepared with 2- to 0.2-fold concentrated D-PBS solution and with 1- or 0.5-fold concentrated lite-PBS (L-PBS) solution (Fig. 26). The ion concentrations of these solutions were as follows (Table 2):

Table 2: Concentration of ions in different blocking solutions

Solution	$c(H_2PO_4^-) + c(HPO_4^{2-})$ [mM]	$c(Na^+)$ [mM]	$c(K^+)$ [mM]	$c(Cl^-)$ [mM]
D-PBS 2x	19.2	306.4	8.4	139.7
D-PBS 1x	9.6	153.2	4.2	139.7
D-PBS 0.5x	4.8	76.6	2.1	69.9
D-PBS 0.2x	1.9	30.6	0.84	27.9
L-PBS 1x	10.0	20.0	0	10.0
L-PBS 0.5x	5.0	10.0	0	5.0

The blocking solutions contain 0.5% (w/v) surfactant **1.** The ionic strength is indicated as nx for an n-fold concentrated solution. The factor n refers to ion concentrations of buffers (D-PBS and L-PBS) used elsewhere in this thesis.

The results of this study revealed a very obvious correlation between the ionic strength and the blocking performance. At high ion concentrations, a relatively normal blocking behaviour is achieved. Decreasing the concentration from 1-fold to 0.5-fold and to 0.2-fold concentrated

D-PBS improved the blocking behaviour strongly. But at even lower ion concentrations, i.e. 1-fold and 0.5-fold L-PBS the blocking ability was lost completely (Fig. 26). L-PBS does not contain any potassium ions in contrast to D-PBS and the proportion of the other ions also differs between L-PBS and D-PBS, so the loss of blocking ability might have been caused by a different ion composition instead of the ionic strength too.

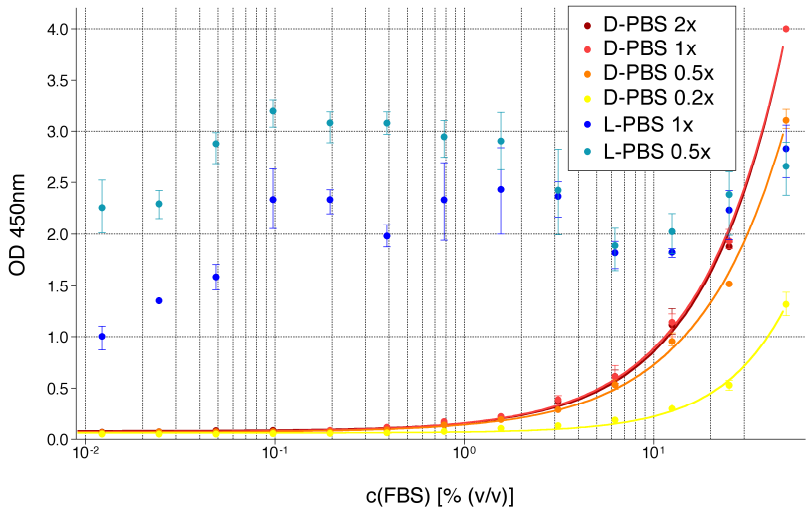


Fig. 26: Influence of ionic strength on the blocking behaviour of surfactant **1**. OD data points are given as mean ± range (N=2). The four parameter logistic function was not applied for data without a recognisable logistic relationship between OD and c(FBS) (L-PBS 1x and 0.5x).

A second experiment was designed in order to substantiate the findings that low ionic strength lead to a breakdown of the blocking performance. Furthermore, it was investigated whether specific ions have an influence on the blocking behaviour. Three series of blocking solutions (0.5% (w/v) surfactant **1**) were prepared, which varied in only two ion concentrations for each solution series and contained only one cation type (besides protons/hydronium ions which are inherent to aqueous solutions). The concentrations of (a) sodium and chloride, (b) potassium and chloride or (c) ammonium and chloride ions were modified in each series, while the concentration of other ions was kept constant. The blocking solutions were designated according to the cation present in the buffer and by a number indicating the ionic strength. E.g. Na^+ 2x refers to a blocking solution with a 2-fold sodium concentration. A cation concentration of 150 mM was defined to be 1-fold for the blocking solutions and the concentration of the buffering agent (H_2PO_4/HPO_4^{2-}) was kept constant at 10 mM, in reference to concentrations used in 1-fold D-PBS (Table 3).

Table 3: Concentration of ions in different blocking solutions

Solution	c(Na^+) [mM]	c(K^+) [mM]	c(NH_4^+) [mM]	c(Cl^-) [mM]
Na^+ 5x	750	0	0	734.5
Na^+ 2x	300	0	0	284.5
Na^+ 1x	150	0	0	134.5

Na^+ 0.5x	75	0	0	59.5
Na^+ 0.2x	30	0	0	14.5
Na^+ 0.1x	15.5	0	0	0
K^+ 5x	0	750	0	734.5
K^+ 2x	0	300	0	284.5
K^+ 1x	0	150	0	134.5
K^+ 0.5x	0	75	0	59.5
K^+ 0.2x	0	30	0	14.5
K^+ 0.1x	0	15.5	0	0
NH_4^+ 5x	0	0	750	734.5
NH_4^+ 2x	0	0	300	284.5
NH_4^+ 1x	0	0	150	134.5
NH_4^+ 0.5x	0	0	75	59.5
NH_4^+ 0.2x	0	0	30	14.5
NH_4^+ 0.1x	0	0	15.5	0
D-PBS	153.2	4.2	0	139.7

The blocking solutions contain 0.5% (w/v) surfactant **1.** For comparison the last entry shows the ion concentrations of D-PBS.

The inability of surfactant **1** to block at very low ion strength was confirmed by all three series of blocking solutions. Even the limit for reliable blocking was the same. For the 0.1-fold blocking solution the blocking occurred, if at all, in a sporadic manner and random background signals showed up already at very low FBS concentrations. This was consistently the case in all three series (Fig. 27). For the 0.2x ion concentration a certain threshold appears to be exceeded and a moderate, even background signal along with a good blocking behaviour is observed. A further increase in ionic strength, however, did not improve blocking. On the contrary, the logistic curves shift substantially and stepwise from 0.2- to 5-fold ion concentration, showing a decline in the blocking performance for each step. Most interestingly, the series reveal different effects of specific ions on the blocking behaviour of **1** for higher ion concentrations. The Na^+ 0.2x blocking solution for instance is superior to Na^+ 5x by roughly one order of magnitude of FBS concentration, whereas the blocking solutions containing ammonium ions do not differ considerably in the blocking performance for all concentrations, with the exception of the 0.1-fold solution (Fig. 27). Blocking solutions containing potassium ions range in between. K^+ 0.2x shows much better blocking abilities than K^+ 5x, but the effect is not as strong as in solutions containing sodium ions (Fig. 27). If results of the three blocking solution series are related to each other, it can be suspected that the influence of ion concentrations on the blocking behaviour depends on the size of the cations, as the radius of

the ions decreases in the order of ammonium, potassium, sodium. But since buffers seldom contain large quantities of cations other than those investigated in this experiment, this discussion is of rather theoretical interest.

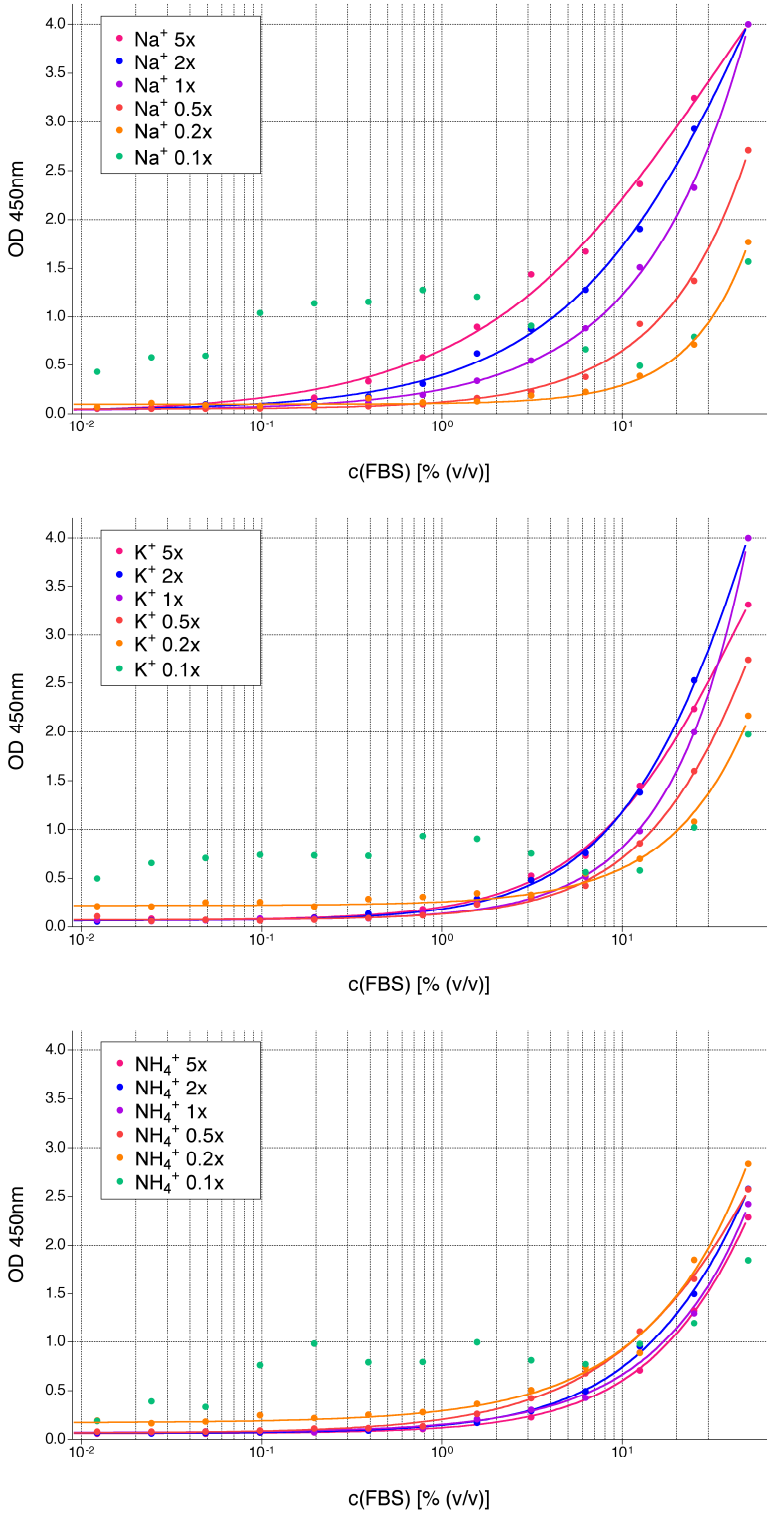


Fig. 27: Influence of specific ion concentrations on the blocking behaviour of surfactant **1**. OD data points are given for a single measurement. The four parameter logistic function was not applied for data without a recognisable logistic relationship between OD and c(FBS) (Na^+ 0.1x, K^+ 0.1x and NH_4^+ 0.1x).

In conclusion, it was shown that the ionic strength of the blocking solution has a pronounced impact on the blocking behaviour of surfactant **1**. An ion concentration of roughly the same as a 0.2-fold D-PBS concentration was determined to be ideal for the blocking behaviour. Since an ionic strength below 30 mM cation and/or 14.5 mM anion is quickly leading to a complete loss of the blocking ability, concentrations of 75-150 mM monovalent cation and 59.5-134.5 mM monovalent anion, which is equivalent to about 0.5- to 1-fold D-PBS, can be considered safe for assay purpose.

The Presence of Blocking Reagent in All Incubation Steps Is Essential

Once the solid phase is blocked, all subsequent incubation steps needs to be conducted with reagents dissolved in blocking solution. Otherwise, partial desorption of the blocking reagent will occur as the adsorption process of the blocking reagent to the solid phase appears to be at least partially reversible. As a consequence, unoccupied spots are created, which can be affected by NSB of the incubated reagent. The importance of using blocking solutions for all incubation steps is demonstrated by the following experiment, in which the blocking reagents were omitted from the incubation solutions.

The model-assay was used and the incubation solutions for each of the four incubations, i.e. blocking, FBS incubation, ConA incubation, and SA-HRP incubation, were prepared with either a blocking solution of 0.5% (w/v) surfactant **1** in D-PBS or D-PBS without blocking reagent. This setup led to 16 (two possible settings for four incubations, i.e. 2^4) experiments with a unique combination of different incubation solutions. The OD was recorded for a dilution series of FBS concentration as usual. However, only 4 experiments showed data analysable with a logistic function, and the difference of these experiments was well reflected by the heights of the lower plateau of the respective fit functions. To summarise the results concisely, instead of the fitted curves, only the OD of the negative controls as well as the OD at a low FBS concentration of about 0.1% (w/v) (indicating either high background signals due to insufficient blocking for the whole FBS concentration range or, differences in the height of the lower plateau of the fit functions, where applicable) is shown (Fig. 28).

Omitting the blocking reagent in the blocking step (experiments 9-16) obviously leads to elevated background signals, but this can only be seen if most of the other incubation steps contain the blocking reagent and as long as FBS or ConA were present in their corresponding incubation step (red and blue bars, see experiment 9 and 10 compared to 1 and 2). The signals increase similarly if the FBS incubation step does not contain the blocking reagent. This behaviour can be observed as long as the ConA incubation step contains the blocking reagent and FBS was incubated (red bars, see experiments 5, 6, 13 and 14 compared to 1, 2, 9 and 10). This shows that the presence of blocking reagent in the incubation solution of ConA is crucial. If we compare the experiments in which both ConA and blocking reagent were present in the ConA incubation step (blue bars, experiments 1, 2, 5, 6, 9, 10, 13 and 14) with the corresponding experiments without blocking reagent in the incubation solution (blue bars, experiments 3, 4, 7, 8, 11, 12, 15 and 16), the increase in false-positive signals is dramatic. A similar trend but less dramatic differences were observed for the SA-HRP incubation step

(green bars, experiments with odd numbers were compared to experiments with even numbers), from which it can be deduced that SA-HRP does not generate as much NSB as ConA or FBS.

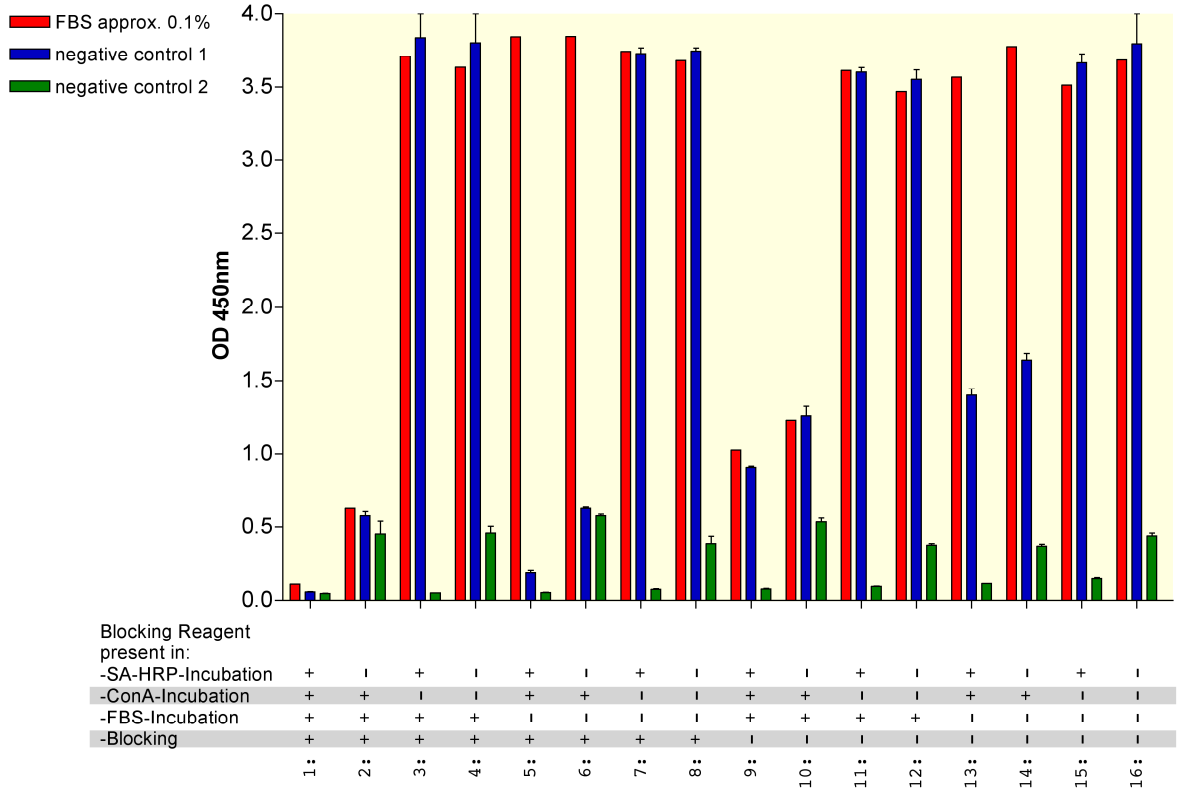


Fig. 28: Results of the model-assay for different combinations of two possible incubation solutions ("+": 0.5% (w/v) surfactant **1** in D-PBS; "-": D-PBS without blocking reagent). Negative controls contained (1) no FBS or (2) neither FBS nor ConA in the corresponding incubation solution. Bars refer to a single measurement for an approximately 0.1% FBS concentration, and the OD data are given as mean ± standard error for negative controls (N=2).

To sum up, it was shown that omitting the blocking reagent in any of the incubation steps leads to a considerable increase of false-positive signals. Results obtained by studying the negative controls indicate that different reagents cause different degrees of NSB, i.e. in general SA-HRP generates less background signals than ConA.

Blocking Reagents Are Able to Enhance TMB Oxidation Catalysed by HRP

Literature data[58,59] suggest that certain tenside molecules can inhibit the activity of important enzymes such as horseradish peroxidase (HRP). The signal strength of all signals, regardless whether true- or false-positive, would be decreased by such inhibition. In regard to false-positive signals, this means that detection of NSB with the model-assay setup would be disturbed. A decrease of true-positive signals would simulate a lower sensitivity of the assay in general.

A new assay setup was used to investigate the influence of different blocking solutions on the kinetic behaviour of horseradish peroxidase. For this purpose, polystyrene microtitre plates

were coated with an HRP-conjugated anti-mouse antibody and incubated with different blocking solutions, i.e. D-PBS solutions of surfactants **1** (0.5 % (v/v)) and **2** (1 % (v/v)), as well as Tween20 (0.5 % (v/v)), skimmed milk (5 % (v/v)), casein (1 % (v/v)), and with pure D-PBS as a negative control. TMB substrate solution was added subsequently and the OD was measured immediately thereafter every 5 s for 3 min without addition of a stopping solution, since a kinetic observation of the signal was intended. The adsorption spectrum of tetramethylbenzidine is different before and after addition of a stopping solution.[60] The intermediate reaction product, a charge-transfer complex of the educt diamine and the final oxidation product diimine, prevails before addition of the stopping solution and has two different adsorption maxima at 370 nm and 655 nm.[61] On the other hand, the final oxidation product generated by stopping the substrate development shows only one adsorption maximum at 450 nm. This is why the OD is usually measured at 450 nm if an ELISA uses a TMB substrate. Since no stopping reagent was added in this experiment, the OD was recorded at 370 nm and at 655 nm (Fig. 29).

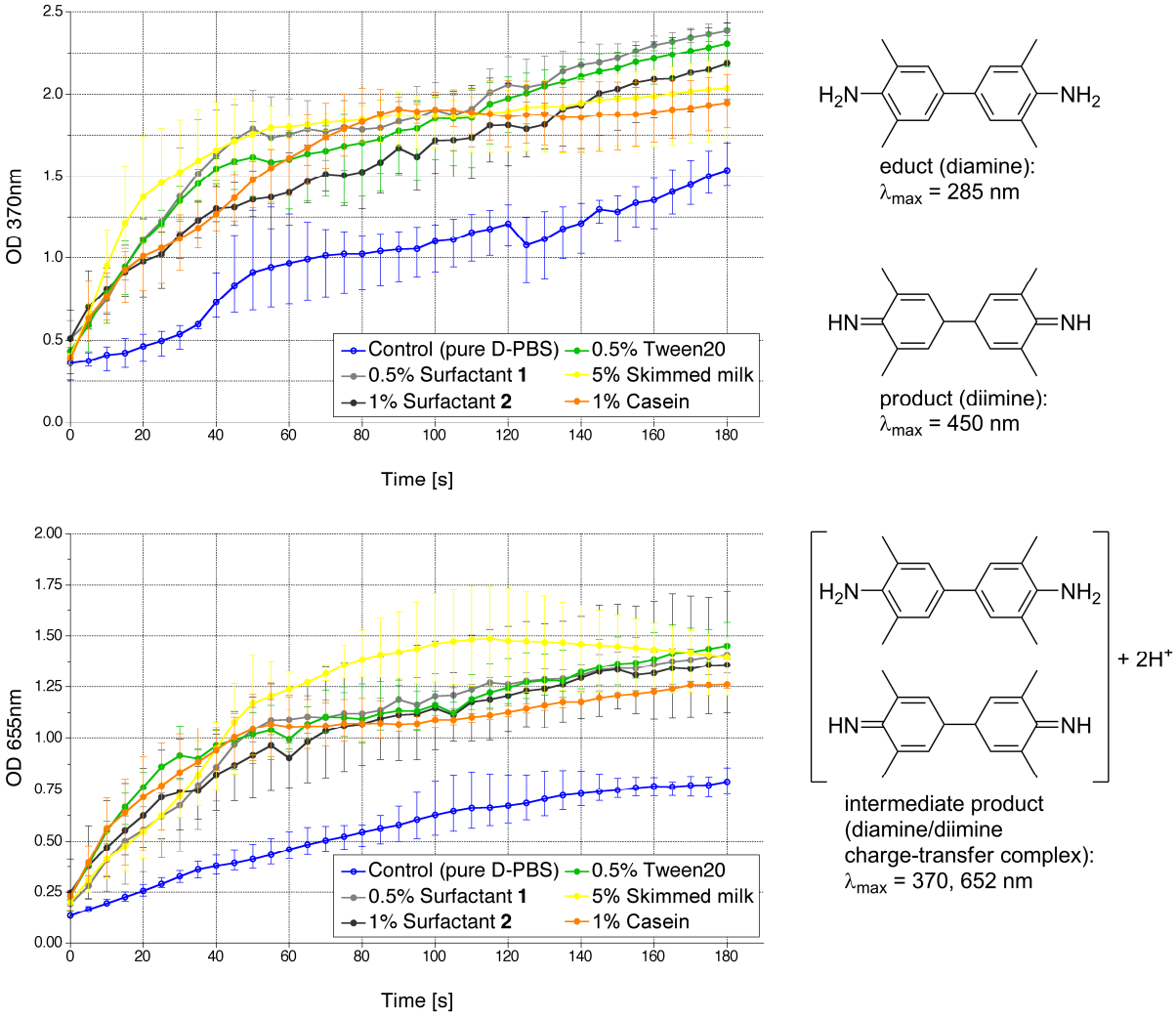


Fig. 29: Left: Influence of different blocking solutions on the time-dependent OD development of TMB oxidation by HRP at 370 nm and 655 nm. OD data is given as mean ± range (N=3). Right: Structure of molecules generated by TMB oxidation.[61]

No inhibition effects were observable with this experimental setup. On the contrary, all investigated blocking solutions increased the kinetics of the TMB oxidation reaction compared to the negative control.

This rather short-time determination was supplemented with a further experiment of the same design, but this time the OD was recorded after addition of a stopping solution. The experiment was repeated for several different periods of TMB development time, ranging from 0-20 min (Fig. 30).

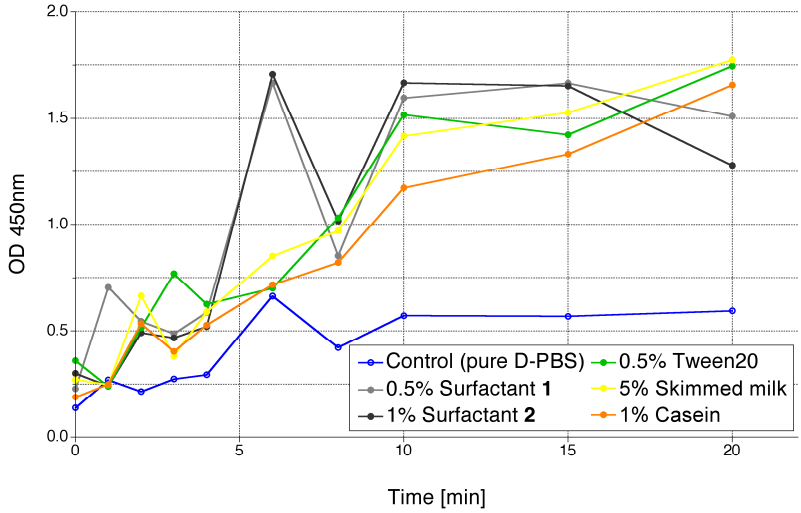


Fig. 30: Influence of different blocking solutions on the TMB oxidation reaction by HRP after different periods of development time. OD is measured after addition of stopping solution and is shown for single measurements.

This experiment again clearly showed an enhancement of the oxidation reaction rather than inhibition compared to the negative control. The rate of enhancement was surprisingly similar for all blocking reagents tested, both in short- and long-time determination. While it cannot be concluded with certainty that this improvement of the TMB reaction kinetics by all investigated blocking solutions is due to an enhancement of the catalytic activity of HRP, the effect for the assay setups used in this thesis is positive nonetheless. The assay sensitivity is increased as a consequence of the TMB reaction enhancement, regardless of the cause.

3.2 Specificity-ELISA

The quantification of antibody titres in human serum (HS) is a standard application of solid phase based immunoassays. It is therefore essential for a blocking reagent to be able to prevent NSB of antibodies from a serum sample. The model-assay was modified to an ELISA setup that allowed the determination of the degree of NSB arising from IgG antibodies in human serum. The conditions for each assay were chosen carefully to keep sample consumption low as the amount of human serum from one specific lot was limited. In general, polystyrene microtitre plates were incubated with different concentrations of human serum instead of FBS after blocking. For a negative control, the human serum incubation solution was replaced by blocking solution without serum. IgG adsorbed on the plate then was detected with a combination of Biotin-labelled anti-human IgG antibody and SA-HRP. TMB

substrate was used again to visualise the detection, and false-positive signals caused by non-specifically bound human IgG were recorded as OD (Fig. 31).

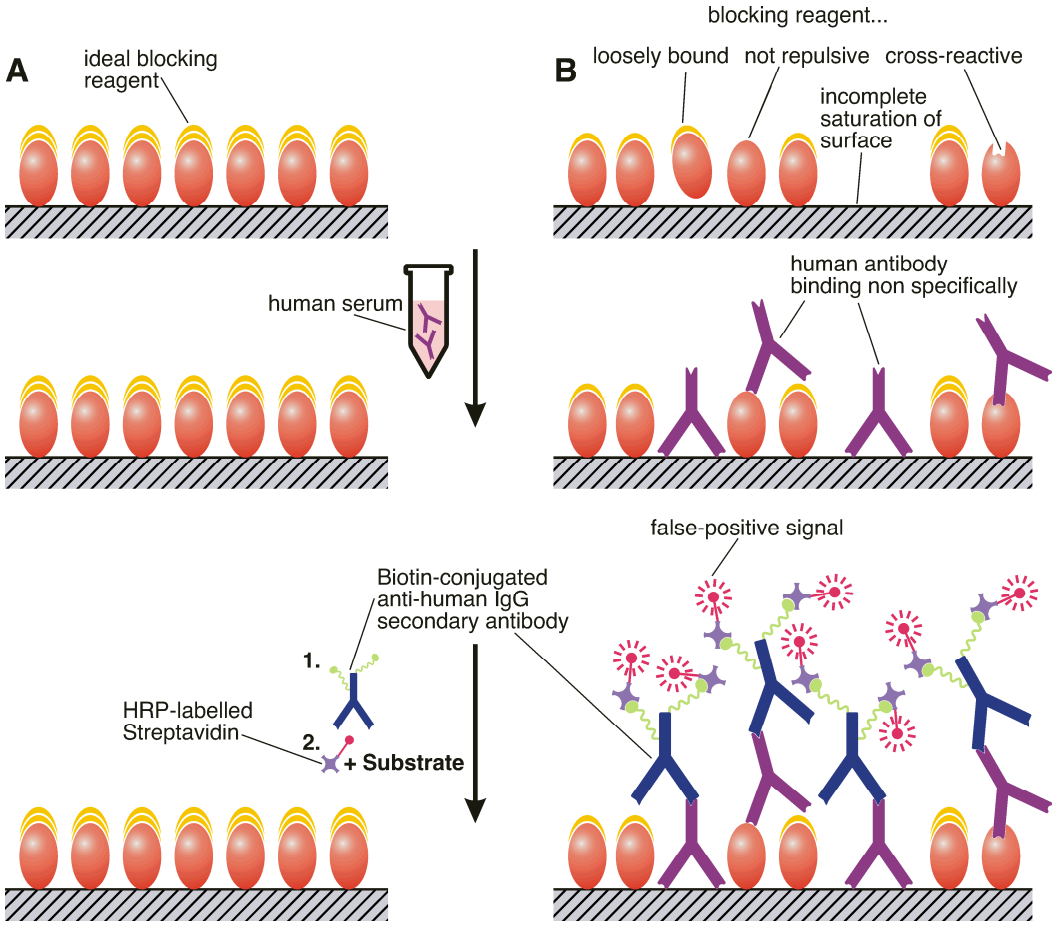


Fig. 31: Schematic illustration of the Specificity-ELISA. **A**: an ideal blocking reagent prevents NSB completely. **B**: different flaws of a blocking reagent lead to false-positive signals.

In what follows, the assay setup is referred to as Specificity-ELISA, because the main purpose of this design is the determination of NSB which decreases the specificity of an assay.

The Blocking Behaviour of a Surfactant Strongly Depends on its Concentration

The concentration of a blocking solution is a basic parameter that needs to be adjusted appropriately. Although it is an obvious assumption that the adsorption rate of a blocking reagent increases with its concentration in the blocking solution, concentrations higher than a value sufficient to induce saturation of the solid phase surface are not only unnecessary, but were also found to counteract an optimal blocking behaviour. Blocking solutions of surfactant **2** in D-PBS with concentrations ranging from 5% to 0.1% (w/v) were tested with the Specificity-ELISA. The incubation with human serum was performed as a serial dilution.

In the experiment, an optimum range of blocking reagent concentration was observed (Fig. 32). The blocking ability of surfactant **2** is very good at a concentration of 1% (w/v) as indicated by a slope of the fitted curve at high human serum concentrations, but the blocking declines for lower concentrations, i.e. 0.5%, 0.2% and 0.1% (w/v), and in this order. Strikingly, a similar decrease in blocking performance is seen for increasing surfactant

concentrations, and the effect is dramatic for a concentration of 5% (w/v). An explanation for this result, although rather hypothetical, may be that the adsorption process of added reagents is somehow enhanced by high surfactant concentrations. This could be the case if e.g. a detection molecule forms an aggregate with excess surfactant molecules and the aggregate then adsorbs onto a blocked surface or exchanges with adsorbed surfactant molecules.

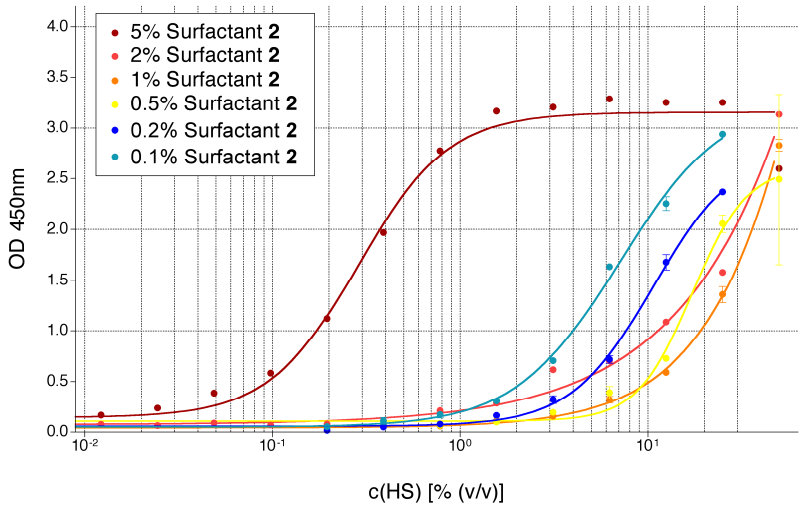


Fig. 32: Blocking performance of surfactant **2** at different blocking solution concentrations. OD data are shown for single measurements and serve as a measure for NSB arising from human IgG.

For the time being, an important conclusion of this experiment is that surfactant **2** possesses the best blocking ability if used in a concentration of roughly 1% (w/v).

Synthesised Surfactants Perform as Well as Reference Reagents in the Specificity-ELISA

All synthesised PEGylated alkylamines, and also the non-reduced amides **7f** were tested in various assay setups. The blocking solutions prepared with these compounds, as well as blocking solutions of commercially available blocking reagents and of surfactants **1** and **2** were given an entry number (BS1-30), to be able to easily refer to one specific blocking solution (Table 4). On account of the results of the previous experiment, a 1% (v/v) concentration was chosen for most of the cationic surfactants.

Table 4: Overview and concentrations of investigated blocking solutions

Commercially available blocking reagents			Synthetic surfactants as novel blocking reagents[3]					
Entry No	Reagent	Conc. [g/mL]	Entry No	Reagent	Conc. [g/mL]	Entry No	Reagent	Conc. [g/mL]
BS1	BSA	1%	BS9	**7f**(550,12)	1%	BS20	**8d**(550,20)	1%
BS2	Fish gelatine	1%	BS10	**7f**(550,20)	1%	BS21	**8d**(2000,12)	1%
BS3	Tween20	0.5 %	BS11	**7f**(2000,12)	1%	BS22	**8d**(2000,20)	1%
BS4	Casein	1%	BS12	**7f**(2000,20)	1%	BS23	**8e**(550,12)	1%

BS5	Casein hydrolysate	1%	BS13	**7g**(550,12)	1%	BS24	**8e**(550,20)	0.2 %
			BS14	**7g**(550,20)	1%	BS25	**8e**(2000,20)	1%
BS6	Skimmed milk	5%	BS15	**7g**(2000,12)	1%	BS26	**8f**(550,12)	1%
			BS16	**8a**(550,12)	1%	BS27	**8f**(550,20)	1%
BS7	Aqua Block	Neat[1]	BS17	**8a**(550,20)	1%	BS28	**8f**(2000,12)	1%
BS8	Roti Block	10x [2]	BS18	**8b**(550,20)	0.5 %	BS29	**1**	0.5 %
			BS19	**8d**(550,12)	1%	BS30	**2**	1%

The solutions were prepared in D-PBS if not otherwise stated. [1] ready to use solution. [2] 10 fold diluted in doubly distilled water (ddH_2O). [3] The pH of the blocking solutions was adjusted to approximately 7.2 with 32% (w/v) hydrochloric acid before diluting to the final concentration in case of the novel reagents.

The basic assay design of the Specificity-ELISA was kept but half-area polystyrene microtitre plates were used to save on materials. The NSB rates were determined at 20% and 2% human serum concentration, as well as with the corresponding blocking solution without human serum for a negative control. Results were recorded as ODs for NSB-induced signals (Fig. 33).

High signals correlate to high NSB, resulting from poor blocking ability of the blocking reagent, and low signals indicate low NSB, due to efficient blocking. Because of a hook effect[62] signals at 20% (v/v) human serum are mostly lower than respective signals at 2% (v/v) human serum (Fig. 33). The commercially available blocking reagents, which served as a reference for blocking performance, differ dramatically in their ability to prevent NSB. BSA, although widely used and approved as a blocking reagent, has the worst blocking performance (Fig. 33). Fish gelatine and Tween20 also show a very poor blocking performance as indicated by an OD > 2 for 2% (v/v) human serum. A much better blocking performance is observed for casein and casein hydrolysate with 1.5 > OD > 0.5 for 2% (v/v) human serum. Skimmed milk prevents NSB very efficiently, and AquaBlock (a fish plasma-based blocking solution) and RotiBlock (a commercially available blocking reagent containing PVP) show NSB-induced signals which are only slightly higher than the background signal as given by the negative control. The blocking performance of the synthesised surfactants varies highly, too (Fig. 33). Subjected to 2% (v/v) human serum, some of the surfactant-based blocking solutions (BS10, 14, 24, 25 and 27) block as poorly as fish gelatine or Tween20 with an OD > 2, while others show good blocking performances similar to casein or casein hydrolysate with 1.5 > OD > 0.5 (BS12, 15, 18, 19, 21 and 29). Most of the surfactants (BS9, 11, 13, 16, 17, 20, 22, 23, 26 and 30) prevent NSB very effectively (OD < 0.5), comparable to skimmed milk, AquaBlock or RotiBlock. BS17 (compound **8a**(550,20)) has the lowest NSB-derived signal of all tested blocking solutions.

All in all, the majority of surfactants which were used as novel blocking reagents shows a blocking performance that is comparable to the best commercially available reference blocking reagents. Many of the surfactants outperform routinely used reference reagents such as BSA or casein significantly as novel blocking reagents (Fig. 33).

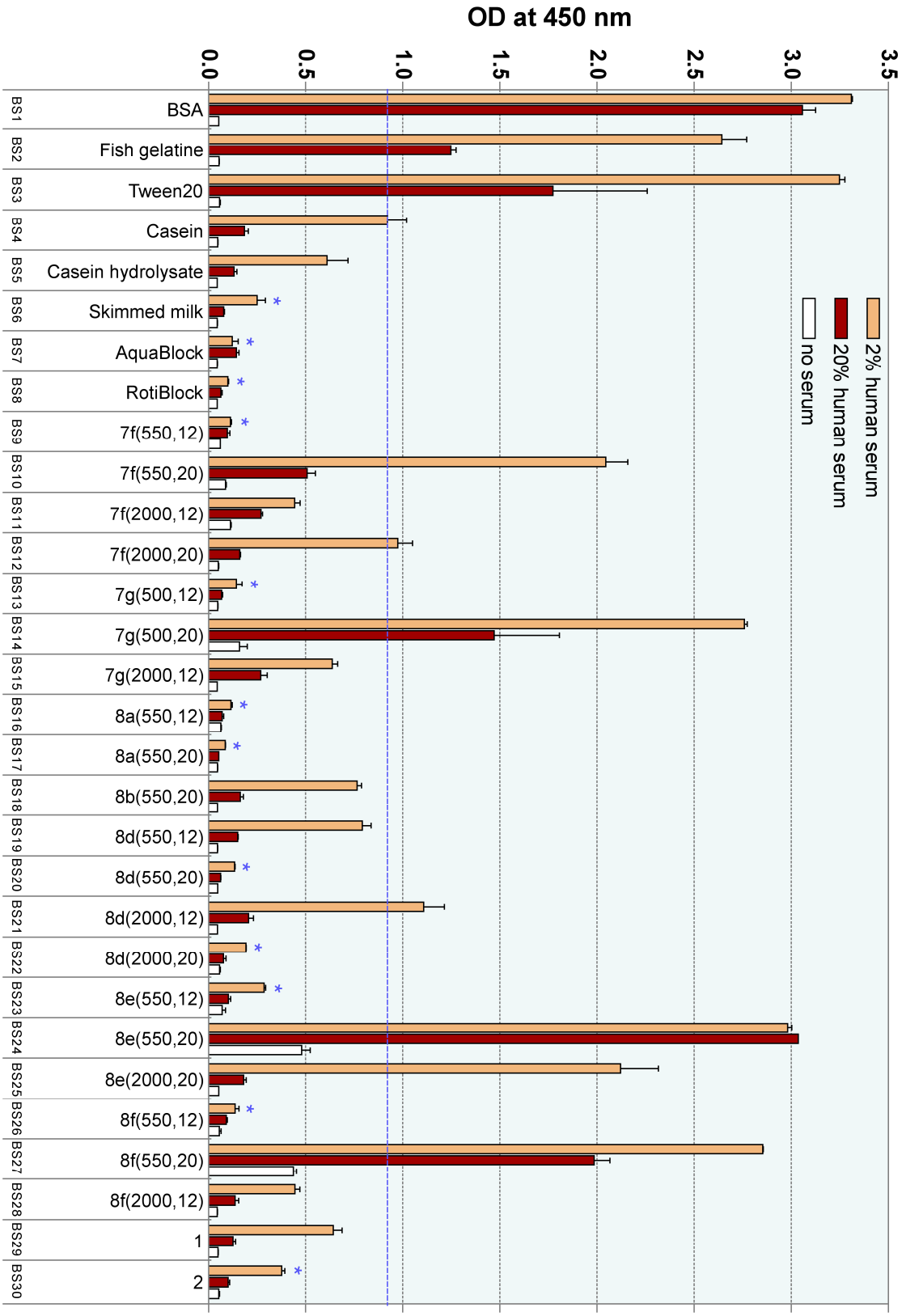


Fig. 33: Test results of synthesised surfactants compared to commercially available blocking reagents in the Specificity-ELISA. OD is given for 20% (v/v) and 2% (v/v) human serum. Bars indicate mean +/- standard error. Signals at 2% human serum marked with a blue asterisk are significantly lower (Oneway ANOVA, Bonferroni post hoc test, $p<0.05$, N=4 for BS1-8 and BS29-30, N=2 for BS9-28) than the signal of casein at 2 % human serum (blue dotted line).

3.3 Prion-ELISA

A good NSB reduction ability is a necessary yet not sufficient criterion for a prospective blocking reagent. To achieve high sensitivity, a blocking reagent also must not disturb the antigen-antibody interaction necessary for detection. This happens, for instance, if a blocking reagent covers or replaces the coating material, both of which will lead to false-negative signals (Fig. 34)

To investigate the effect of different blocking reagents on the sensitivity, an ELISA for the detection of prion protein (PrP) in reference samples was designed. The reference samples contained 50 and 3 ng/mL of a recombinant prion protein fragment (PrP90-231),[63] and were prepared in lite-PBS (L-PBS). Polystyrene microtitre plates were coated with these reference samples as well as with L-PBS as a negative control, and blocked with different blocking solutions. Detection was carried out with a monoclonal anti-PrP antibody and an HRP-labelled secondary antibody (Fig. 34).

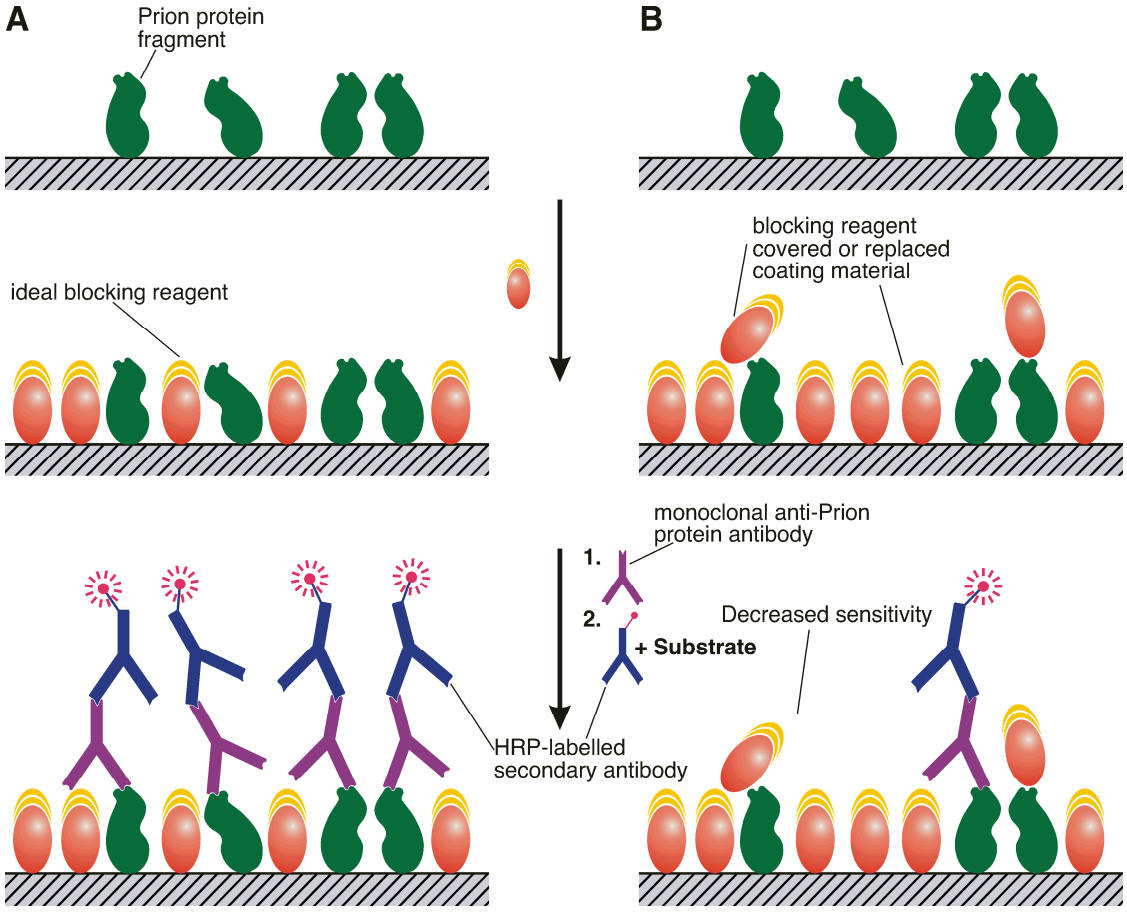


Fig. 34: Schematic illustration of the Prion-ELISA. **A**: ideal blocking reagent. **B**: different flaws of a blocking reagent lead to false-negative signals.

The results of the experiment show clearly that all blocking reagents except BSA are able to sufficiently prevent NSB in this setup as seen by very low signals of the negative control (Fig. 35). For BSA, the OD of the negative control is almost as high as the signal generated by the

reference sample containing 50 ng/mL PrP90-231. So the relatively high signals of the reference samples caused by blocking with BSA should not be mistaken for high sensitivity.

For all remaining blocking solutions the signal strength of the reference samples correlates with the sensitivity of the assay. A very poor to mediocre sensitivity is observed for the commercially available blocking reagents (except BSA, as stated above). Only Tween20 is able to produce a slightly elevated signal compared to the negative control at a concentration of 3 ng/mL PrP90-231 in the reference sample, whereas the other reference reagents show ODs very close to the negative control at this concentration (Fig. 35). At a PrP90-231 concentration of 50 ng/mL, blocking with Tween20 results in an OD of about 0.5, while blocking with fish gelatine, casein and skimmed milk lead to ODs of roughly 0.2 to 0.3. The assay failed to detect PrP90-231 in the reference samples completely even with a concentration of 50 ng/mL if casein hydrolysate, AquaBlock or RotiBlock was used as blocking reagent (Fig. 35).

The blocking solutions based on the surfactants on the other hand, perform very well also in regard to the assay sensitivity. At a reference sample concentration of 50 ng/mL PrP90-231, all of the tested surfactants led to elevated signals. A very high signal, significantly higher than the signal generated by Tween20, the best-performing reference reagent, is achieved with many of the blocking solutions prepared with the synthesised surfactants (BS14, 16-21 and 23-25). Even at a PrP90-231 concentration of 3 ng/mL considerably increased signals can be seen for many blocking solutions based on surfactants (BS14, 16-20, 23) (Fig. 35).

To conclude, this experiment proved that with regard to the assay sensitivity in the Prion-ELISA, almost all of the cationic surfactants are equal or better than the best-performing reference blocking reagent, Tween20. Many reference reagents, especially AquaBlock and RotiBlock, showed a very poor sensitivity in this experimental setup.

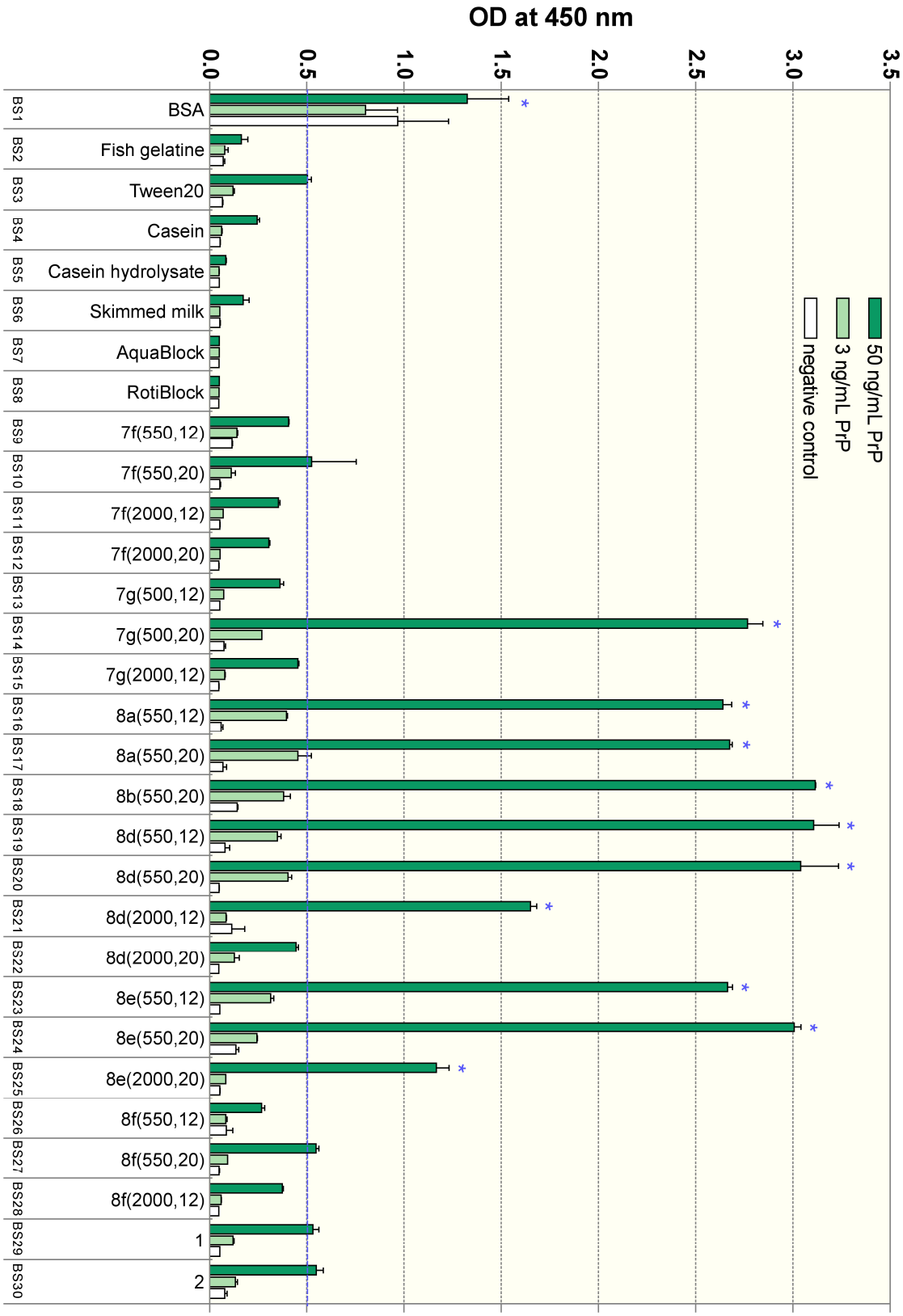


Fig. 35: Test results of synthesised surfactants compared to commercially available blocking reagents in the Prion-ELISA. OD for 50 and 3 ng/ml PrP90-231 is given. Signals marked with a blue asterisk are significantly higher (Oneway ANOVA, Bonferroni post hoc test, $p<0.05$, N=4 for BS1-8 and BS29-30, N=2 for BS9-28) than the signal of Tween20 at 50 ng/mL PrP90-231 (blue dotted line).

3.4 Hepatitis B-ELISA

In some cases it is important that a test yields a high sensitivity not only at certain analyte concentrations but over a whole concentration range. It was intended to investigate such a relationship, preferably with clinical relevance. For this purpose, an assay for the determination of hepatitis B surface antigen (HBsAg) as used in diagnostic test kits was chosen. As most diagnostic kits are supplied with a pre-coated and -blocked solid phase, the assay was developed in-house. Following a standard setup for direct sandwich ELISAs, polystyrene microtitre plates were first coated with an anti-HBsAg antibody and then subjected to different blocking solutions. In a next step, HBsAg reference samples were applied in a serial dilution and detected with an HRP-labelled anti-HBsAg antibody (Fig. 36). Negative controls were conducted by replacing either the HBsAg-sample incubation or both, the coating and the sample incubation, with pure blocking solution. After visualisation with a TMB substrate solution the OD was determined. Four parameter logistic functions were fitted to the raw data, and both OD as well as the fit function were plotted against the (logarithmic) HBsAg concentration, as usual.

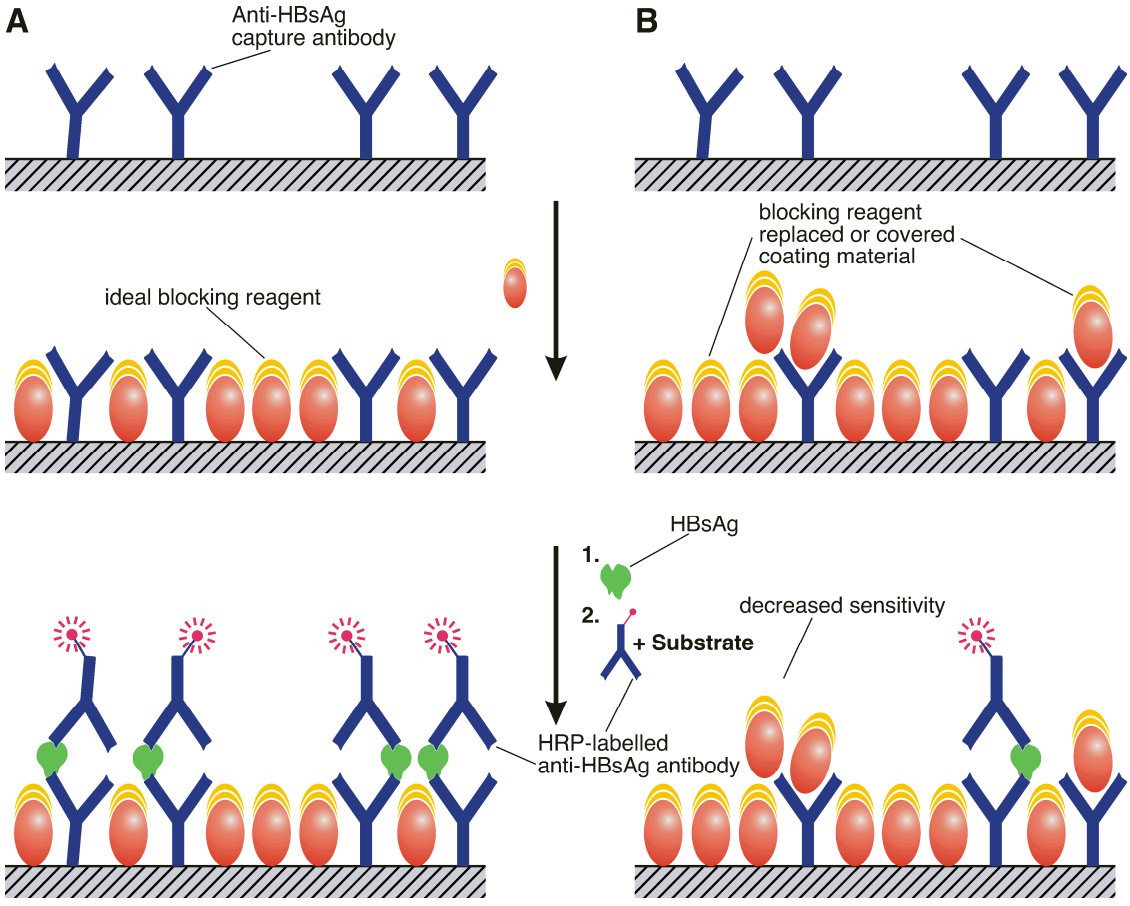


Fig. 36: Schematic illustration of the Hepatitis B-ELISA. **A**: ideal blocking reagent. **B**: different flaws of a blocking reagent lead to false-negative signals.

The findings of the previous experiment could be confirmed with respect to the reference reagents. Overall, the reference reagents led to a relatively poor sensitivity of the assay. Indeed, for blocking solutions based on casein, casein hydrolysate, skimmed milk, AquaBlock

or RotiBlock no signals above background could be achieved with this assay. A promising signal increase at high HBsAg concentrations was generated if BSA or fish gelatine were used as blocking reagent, but a complete sigmoidal curve with lower and upper plateau could only be obtained for Tween20 (Fig. 37).

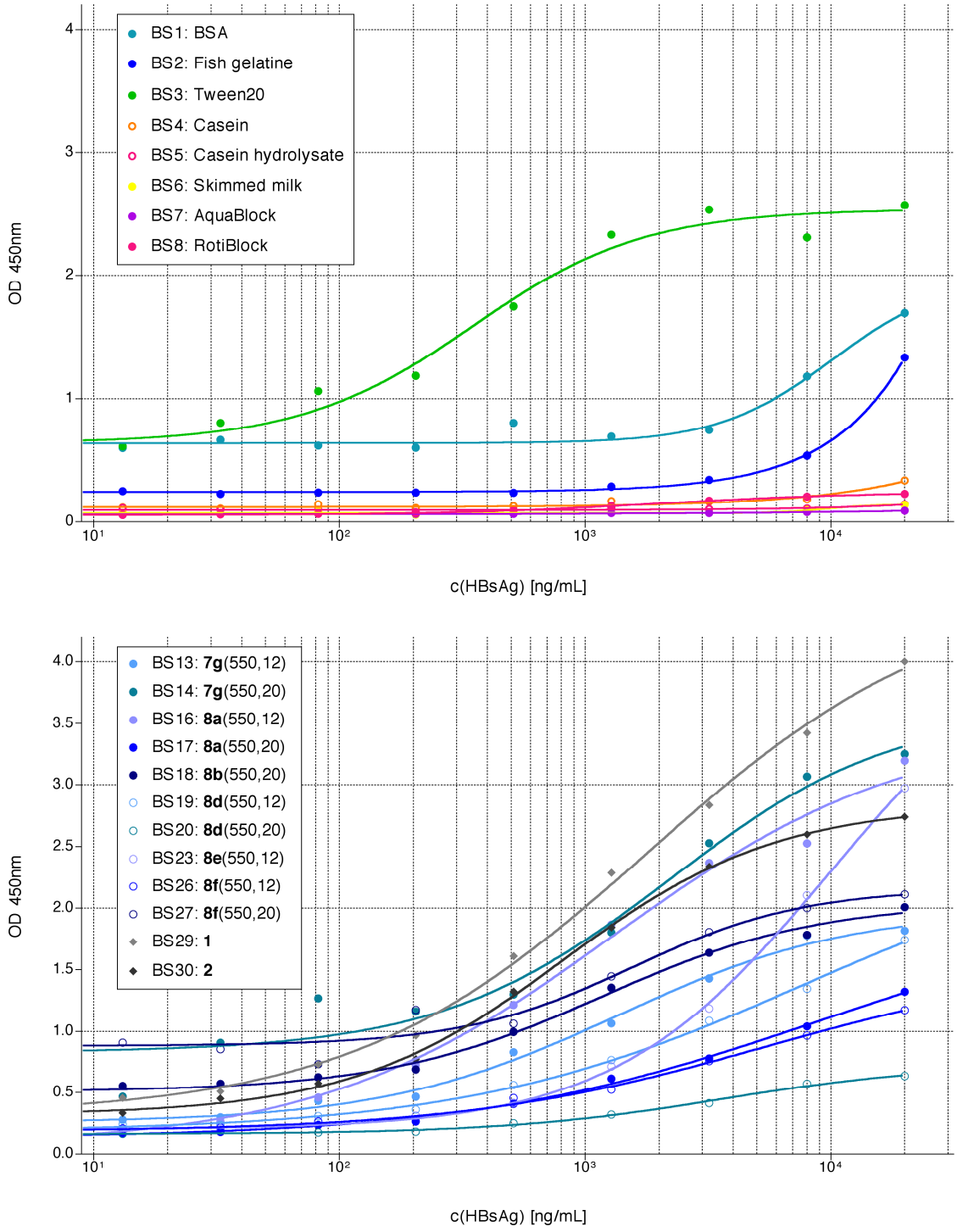


Fig. 37: Test results of synthesised surfactants compared to commercially available blocking reagents in the Hepatitis B-ELISA. Top: reference blocking reagents, Bottom: synthesised surfactants and surfactants **1** and **2**. Results of synthesised surfactants with no significant signal increase are not shown. OD data of single measurements are given.

In contrast to the majority of the commercially available blocking reagents, the application of surfactants as a blocking reagent resulted for more than half of the cases in increased signals, i.e. almost complete sigmoidal curves were achieved. Interestingly, in some cases the labelled anti-HBsAg antibody recognised the coating antibody, which can be deduced from an elevated lower plateau of the corresponding fitted curves. This behaviour was confirmed by negative controls without coating antibody (Fig. 37). In conclusion, most of the cationic surfactants proved to generate better sensitivity if used as a blocking reagent than all reference reagents except Twen20 in this assay setup.

Moreover, the fit functions were analysed to yield quantitative data. The limit of detection (LOD) was determined by t-statistics (95% confidence level, calculations executed with Prism 4) using the lower plateau of the logistic fit functions as background level[64]. The inflexion point and the signal-to-noise ratio S/N (defined here as the ratio of the maximum OD to the minimum OD of the four parameter curve) were also calculated. All results are summarised in Table 5.

Table 5: Quantitative analysis of the Hepatitis B ELISA fit functions

Blocking solution / reagent		Limit of detection [µg/mL HBsAg]	Inflexion point [µg/mL HBsAg]	Signal-to-noise ratio at maximum OD
BS1	BSA	n.d. [2]	n.d. [2]	n.d. [2]
BS2	Fish gelatine	0.569	n.d. [3]	n.d. [3]
BS3	Tween20	0.041	0.210	4.329
BS4	Casein	n.d. [1]	n.d. [1]	n.d. [1]
BS5	Casein hydrolysate	n.d. [1]	n.d. [1]	n.d. [1]
BS6	Skimmed milk	n.d. [1]	n.d. [1]	n.d. [1]
BS7	AquaBlock	n.d. [1]	n.d. [1]	n.d. [1]
BS8	RotiBlock	n.d. [1]	n.d. [1]	n.d. [1]
BS9	**7f**(550,12)	n.d. [2]	n.d. [2]	n.d. [2]
BS10	**7f**(550,20)	n.d. [2]	n.d. [2]	n.d. [2]
BS11	**7f**(2000,12)	n.d. [2]	n.d. [2]	n.d. [2]
BS12	**7f**(2000,20)	n.d. [1]	n.d. [1]	n.d. [1]
BS13	**7g**(550,12)	0.063	1.332	7.796
BS14	**7g**(550,20)	0.282	2.191	4.394
BS15	**7g**(2000,12)	0.271	n.d. [3]	n.d. [3]
BS16	**8a**(550,12)	0.052	1.299	53.094

BS17	**8a**(550,20)	0.032	8.408	15.603
BS18	**8b**(550,20)	0.061	1.159	4.043
BS19	**8d**(550,12)	0.032	7.378	14.119
BS20	**8d**(550,20)	0.162	3.612	4.525
BS21	**8d**(2000,12)	n.d. [1]	n.d. [1]	n.d. [1]
BS22	**8d**(2000,20)	n.d. [1]	n.d. [1]	n.d. [1]
BS23	**8e**(550,12)	0.092	9.878	22.119
BS24	**8e**(550,20)	n.d. [1]	n.d. [1]	n.d. [1]
BS25	**8e**(2000,20)	n.d. [1]	n.d. [1]	n.d. [1]
BS26	**8f**(550,12)	0.070	5.433	8.615
BS27	**8f**(550,20)	0.329	1.568	2.450
BS28	**8f**(2000,12)	n.d. [1]	n.d. [1]	n.d. [1]
BS29	**1**	0.035	1.270	11.738
BS30	**2**	0.018	0.771	9.164

n.d.: [1] No significant sigmoidal curve distinguishable; [2] Background problems (negative control gave signals), fit curve accuracy too low (R^2<0.95); [3] inflexion point > 20 µg/mL HBsAg

Mostly due to lack of a distinguishable sigmoidal curve, but also due to background signals or due to low curve accuracy, the above mentioned values were not determined (n.d.) for many reagents, including almost all of the commercially available reagents. If the calculated inflexion point of the fit function exceeded the maximum HBsAg concentration employed in the assay (20 µg/mL), both inflexion point and signal-to-noise ratio were listed as n.d. While among the commercially available reagents only Tween20 showed a satisfactory sensitivity as indicated by a completely evaluable fitted curve, more than half of the surfactants used as novel blocking reagents led to an analysable fit function. Although the LOD is convincingly low for Tween20, some of the cationic surfactants show similar (BS13, 16, 18 and 26) or even lower (BS17, 19, 29 and 30) LOD values. The signal-to-noise ratio furthermore is equal (BS14, 18, and 20) or 2-12 times higher (BS13, 16, 17, 18, 19, 26, 29, and 30) for the cationic surfactants. The low inflexion point for Tween20 seems to point to a dose-response at low concentrations, but may also be a result of the relatively low maximum OD of the fit curve.

To summarise the results, many of the novel blocking reagents led to equal or better sensitivity in a Hepatitis B-ELISA over a wide range of analyte concentrations compared to Tween20, the best-performing commercially available reagent.

3.5 Immunoblots

A polystyrene-based solid phase appears advantageous for the blocking performance of cationic surfactants, since they feature aromatic and electronegative groups, which might lead to an enhanced adsorption of the surfactants as illustrated in the introduction. For this reason, in the experiments described so far only polystyrene-made microtitre plates were used. Polystyrene plates are wide-spread and used very commonly, but on the other hand, many immunoassays use other solid phase formats and other materials and require a blocking reagent as well. Therefore, the performance of the cationic surfactants was also investigated with typical immunoblot membranes as solid phases, where enough synthesised material was available. Nitrocellulose (NC) and Poly(vinylidene fluoride) (PVDF) membranes were blocked either with different blocking solutions or with pure D-PBS as negative control and incubated with an anti-mouse antibody labelled with a fluorescing dye (AlexaFluor680). The amount of fluorescence caused by the non-specifically binding labelled antibody was determined on a fluorescence imager. The fluorescence intensity was normalised linearly to the intensity of the negative control containing no blocking reagent defined as 0% and to the lowest measured intensity defined as 100% blocking effect.

Almost all of the commercially available reference blocking reagents blocked non-specific adsorption of the labelled antibody very effectively, with a blocking effect of near 100% achieved for both the PVDF and the nitrocellulose membrane. Exceptions were BSA and fish gelatine, which showed blocking effects of about 70% and 50% for the PVDF membrane and both about 90% for the nitrocellulose membrane, as well as RotiBlock with a blocking effect of about 70% for the PVDF membrane. The blocking behaviour of the synthesised surfactants varied as expected. While some of them exhibited a quite poor blocking performance, some (BS11, 12, 22, 30) were able to block PVDF membranes as good as reference blocking reagents, i.e. a blocking effect of > 80%, and even more of them showed a blocking effect near 100 % for NC membranes (BS18, 22, 23, 25, 28, 29, 30).

In conclusion, some of the surfactants are able to prevent NSB on a PVDF and on a nitrocellulose membrane in an immunoblot experiment as good as reference reagents.

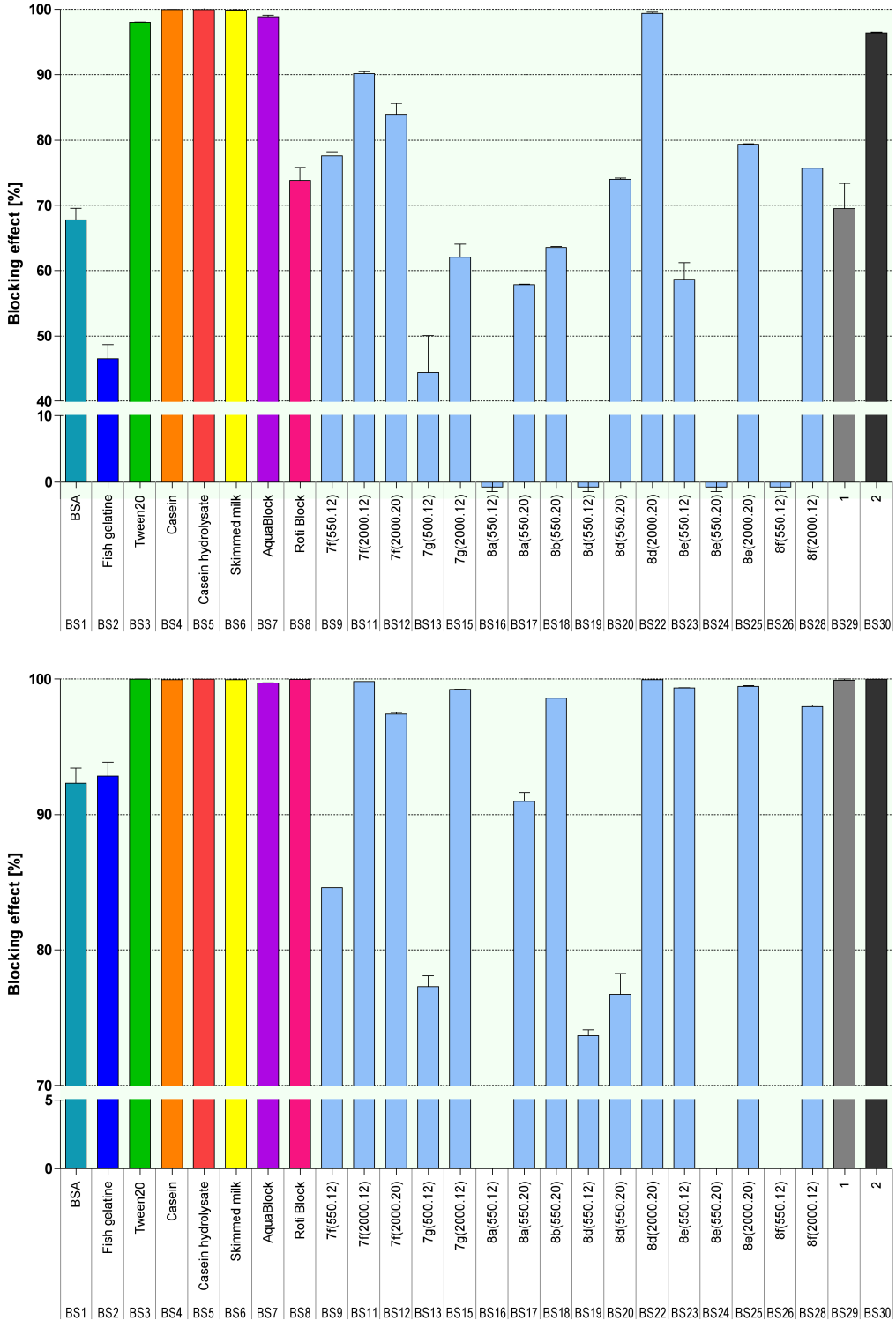


Fig. 38: Results of an Immunoblot. The fluorescence indicative for non-specific antibody binding was normalised linearly by defining the fluorescence of the negative control (no blocking reagent used) as 0% blocking effect and the lowest measured fluorescence as 100% blocking effect. Bars indicate mean ± standard error (N=2 for all blocking solutions). Top: Blocking effect of different blocking solutions on PVDF membranes. Bottom: Blocking effect of different blocking solutions on nitrocellulose membranes.

4. Discussion and Conclusion

The two main achievements of this thesis are as follows. First, a large number of different PEG-conjugated alkylamines were synthesised. These were deemed to be promising candidates for novel blocking reagents. Second, in a series of assay experiments the blocking performance of the cationic surfactants was investigated and compared with commercially available blocking reagents.

A modular so-called building block synthesis was designed and it was shown in theoretical and practical terms that this approach allowed a successful preparation of a series of PEG-conjugated alkylamines. These cationic surfactants possessed one or two PEG- and one or two Alkyl-chains of variable length, which were connected by a simple and short bridge. The bridge had no further functional groups except an amide as a precursor in some cases and amine groups were possibly present between the PEG group and the bridge or terminally on the hydrophobic side, or at both locations. In the practical work several synthetic challenges had to be overcome, which, in some cases, led to the discovery of interesting properties of PEG-containing molecules. For instance, it was found that simple, more traditional reactions are better suited than elaborate complex ones. Moreover, a solubility and CMC characterisation was successfully conducted for the cationic surfactants (both, the synthesised ones and the reference surfactants **1** and **2**), and the obtained data provide a useful basis for the interpretation of the assay experiments.

In order to determine the degree of NSB that arises from serum components of FBS, a model-assay was designed. The model-assay permitted, in a series of preliminary studies, to investigate the influence of various assay conditions on the blocking behaviour of reference surfactants. These experiments clearly showed that both pH value and ionic strength of the blocking solution are important factors which affect the blocking performance of the surfactant. A neutral pH value and an ion concentration equal to or somewhat lower than concentrations of commonly used buffers were found to be ideal. It was demonstrated that all incubation steps have to be conducted with the blocking reagents present in the incubation solution in order to prevent NSB. Furthermore, the kinetics of the TMB oxidation catalysed by HRP were found to be enhanced, rather than inhibited, by many blocking reagents, including the reference surfactants and some commercially available blocking reagents. Too high or too low concentrations of the blocking reagent in a blocking solution were found to decrease the blocking ability of a reference surfactant. The blocking performance of the synthesised cationic surfactants was investigated with a Specificity-ELISA, which determined the degree of NSB caused by human IgG, and with a Prion- and a Hepatitis B-ELISA that revealed the influence of the blocking reagents on the sensitivity of the assay at certain concentrations (PrP90-231) or for a whole dilution series (HBsAg) of reference samples. While the majority of cationic surfactants performs about as good as the best commercial blocking reagents in the Specificity-ELISA, both Prion- and Hepatitis B-ELISA demonstrated clearly that most of the surfactants are superior in terms of sensitivity. Finally, an immunoblot experiment was conducted wherein some of the surfactants were shown to be able to prevent

NSB on solid phases other than a polystyrene-based material, i.e. a PVDF and a nitrocellulose membrane, as good as the reference reagents.

4.1 Discussion of Experimental Results

Size Matters: Why Sensitivity Is Low for Many Commercial Blocking Reagents

The most salient result of the assay experiments is that, with respect to sensitivity, most of the cationic surfactants are clearly superior to many of the commercially available blocking reagents. A probable reason lies in the fact that size does matter, only sometimes large is simply too large.

Most of the molecules involved in an assay, i.e. the analytes, the detection molecules etc., are proteins, which, while highly variable in size, are almost never smaller than several ten thousands of Daltons. Now, most of the commercially available blocking reagents are either proteins, too (casein, fish gelatine, BSA), or at least protein-based (casein hydrolysate, skimmed milk, AquaBlock). Consequently, these blocking reagents are at least of similar size and possibly even larger than the analyte and the detection reagents. IgGs for instance are relatively large with a mass of about 150 kDa corresponding to a hydrodynamic diameter of about 10 nm.[65] Yet, casein micelles as present in skimmed milk exceed even this size by far with their diameters ranging from about 20 nm to more than 100 nm.[66] Even if the blocking reagents only develop a uniform monolayer on the surface, a sterical effect of adsorbed blocking reagents on adjacent coating reagents is highly likely. Such a sterical influence might affect the specific binding between an antigen/antibody offered in the solution and the coated material (Fig. 39). In practical use, blocking solutions contain a huge excess of blocking reagent, several thousand times more than what is needed to saturate the solid phase surface with a monolayer. In such high concentrations, a protein-protein based secondary adsorption on the protein monolayer is probable and has been proposed to explain experimental results in the literature.[67] In this respect, too, protein-based blocking reagents are likely to cover coated reagents partly or in whole, which may lead to the diminished sensitivity that was observed in this thesis for assays utilising proteinaceous blocking reagents (Fig. 39).

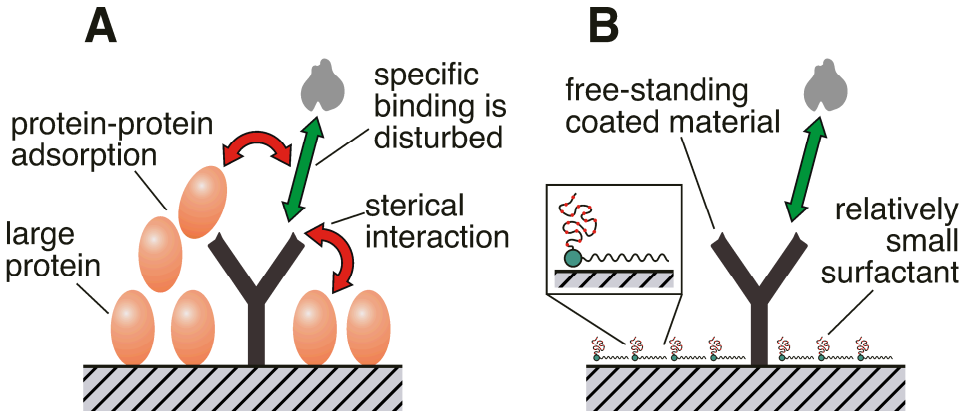


Fig. 39: Schematic illustration of how the blocking reagent size might affect the sensitivity of an assay. **A**: Large proteins as blocking reagents may disturb the specific antigen-antibody recognition process by sterical interaction and protein-protein adsorption. B: Surfactants are far smaller and do not exhibit pronounced influences on the specific antigen-antibody binding.

Tween20 was the only commercially available blocking reagent that led to a sensitivity that was as good as some of the novel blocking reagents in both, the Prion- and the Hepatitis B-ELISA. Strikingly, Tween20 is, with a molecular mass of about 1.2 kDa, by far the smallest of the commercially available blocking reagents. Indeed, its size is in the same order of magnitude as the cationic surfactants, which have molecular masses of about 0.7 to 3 kDa. It should be noted that there was no data available about the molecular mass of PVP used in RotiBlock. Nevertheless, this polymer seems to be widely used with an average molecular mass of about 40 kDa[68,69] and more,[6] which is close to the size of proteins. To sum up, the fact that Tween20 and the cationic surfactants share small size and good blocking performance in terms of sensitivity should be seen as evidence for the discussion above.

Also within the tested cationic surfactants small size of the blocking reagent seems to be correlated with high sensitivity of the assay. In fact, while cationic surfactants based on PEG groups with a molecular mass of 550 Da are able to produce elevated signals for a reference sample concentration of 3 ng/mL in the Prion-ELISA, those surfactants with a 2 kDa PEG group only lead to mediocre signal strengths. The same is true for the Hepatitis B-ELISA: evaluation of the fitted curves was only possible with surfactants with a 550 Da PEG-head group.

In conclusion, all results obtained within this thesis indicate that smaller molecules of about < 3 kDa weight are much better suited as blocking reagents for improved sensitivity of an assay than large molecules such as proteins.

Finally, suggestions were made[70] that too large blocking reagent molecules such as BSA would, because of sterical effects, not be able to cover a solid phase surface completely without leaving gaps. This could explain the poor specificity obtained with BSA as a blocking reagent. On the other hand, other smaller proteins, e.g. casein hydrolysate, are able to sufficiently prevent NSB in our experiments. Therefore, it seems reasonable that an influence of the blocking reagent size on the specificity of an assay only exists for large molecules (> 50 kDa) and that other features of the molecule such as shape and chemical properties may play a more important role for the specificity.

NSB Prevention Requires Unexpectedly High Blocking Reagent Concentrations

It was mentioned briefly in the last section that the blocking reagents are used in a large excess in blocking solutions. Plate dimensions of a typical microtitre plate are a liquid volume of 200 µL and a covered area of 1.54 cm^2 per well (Fig. 40). Now, if a Stokes radius of 3.5 nm is assumed for BSA, the area covered by one BSA molecule is $\pi \times r^2 = \pi \times (3.5 \times 10^{-7}$ $cm)^2 = \sim 3.85 \times 10^{-13}$ cm^2, and the mass for one molecule is about 66,000 g/mol / 6.022×10^{23} $mol^{-1} = 1.10 \times 10^{-19}$ g. This means that a mass per area of 1.10×10^{-19} g / 3.85×10^{-13} $cm^2 = 286$ ng/cm^2 is needed to cover the solid phase with a monolayer, which is about 440 ng for the well described above. Related to the volume of 200 µL, the concentration needed for a monolayer of BSA on the solid phase surface lies at only 2.2×10^{-6} g/mL. Experiments described in literature have suggested that protein monolayer formation via

protein-to-polystyrene adsorption occurs down to a concentration calculated above, and that a protein-to-protein adsorption on such a layer is probable for higher concentrations.[67]

A similar calculation for surfactant **1** (PEGylated oleylamine) is conducted. For a rough estimate, the somewhat unrealistic assumption is made that the alkyl part is lying flatly spread on the surface, so that all atoms of the alkyl group are touching the surface. The area covered by the molecule then can be calculated by multiplying a length of 18 sp^3-sp^3 C-C bonds of 154 pm each (assuming the double bond is negligible) with a width of 2 C-H bonds of 110 pm each, which leads to an area of 6.10×10^{-15} cm^2 (Fig. 40). With a molecular mass of about 1.15 kDa, the amount per area needed for a monolayer of **1** on a solid phase surface is 313 ng/cm^2, or a concentration of 2.41×10^{-6} g/mL for a well with dimensions as stated above. The CMC of the cationic surfactants in contrast lies in the range of 10^{-5} to 10^{-3} g/mL as shown in chapter 2. Although the CMC therefore is in a concentration range one to two order of magnitudes higher than what is needed for a dense saturation of the surface, the adsorption rate of surfactants to hydrophobic surfaces seems to increase with the surfactant concentration until it is stabilised around the CMC.[71]

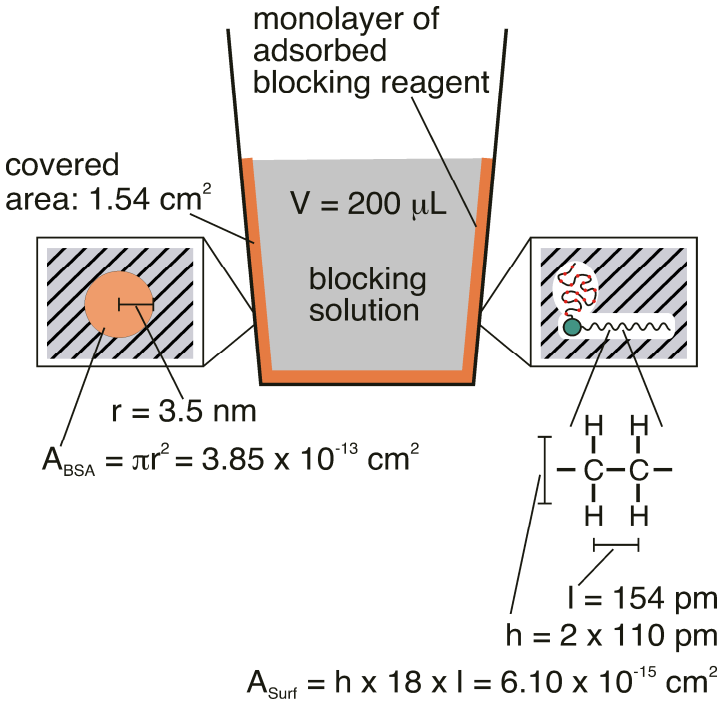


Fig. 40: Dimensions of a typical well of a microtitre plate. Left side: Calculation of the area covered by one BSA molecule. Right side: Calculation of the area covered by one molecule of surfactant **1**. Illustration is not true to scale.

In the experiments conducted in this thesis, it was proved that blocking solution concentrations far beyond the CMC, about 0.01 g/mL are needed for an effective NSB prevention if cationic surfactants are used as a blocking reagent. The same is true for commercially available proteinaceous blocking reagents, which need far more concentrated blocking solutions (0.01 – 0.05 g/mL) than a complete saturation of the surface with a protein monolayer actually requires. This observation clearly shows that the blocking reagent must serve another purpose besides the saturation of the solid phase surface.

A possible explanation is the non-specific interaction of the blocking reagent with incubated materials, which might compete with the NSB of incubated reagents to the solid phase surface. The far stronger specific antibody-antigen recognition would not be disturbed by such a competition, but the weaker NSB can be hindered in two ways, because blocking reagent

molecules are attracted non-specifically both to the solid phase surface as well as to components in the incubation solution (Fig. 41). This explanation is substantiated by the finding that the presence of blocking reagent is needed in all incubation steps.

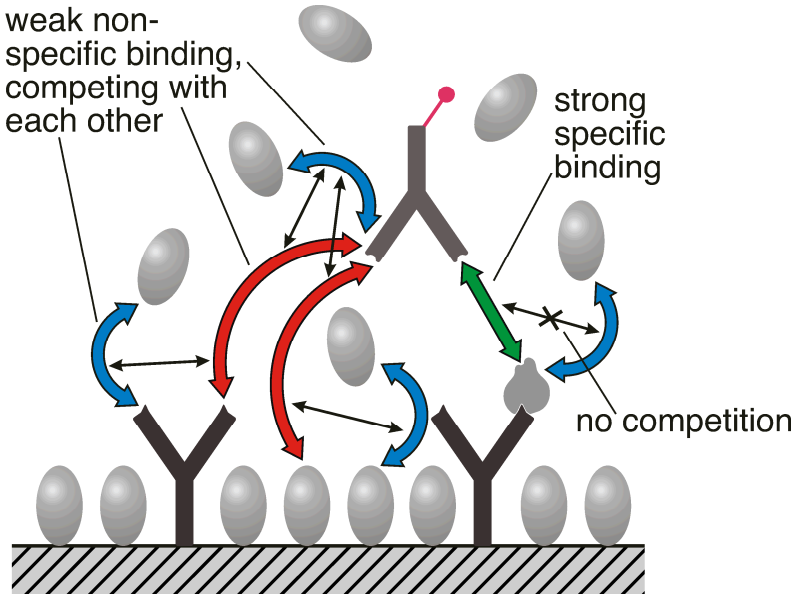


Fig. 41: How blocking reagent molecules present in the liquid phase might reduce NSB. A detection antibody is non-specifically interacting (red arrows) with both surface and coated material. Blocking reagent molecules interact in the same way, but also with the molecules of the detection system (blue arrows), and thus generate competition for the NSB-inducing interactions. Since the antigen-antibody binding is specific and strong (green arrow), it is not disturbed by the non-specific forces of the blocking reagent.

Such a support in the discrimination between strong specific and weak non-specific interaction in an assay can be especially effective for the cationic surfactants because of their aggregation behaviour. As already mentioned, the ideal concentrations of blocking solutions are far beyond the CMC, so micelles and other aggregation forms are found in surfactant based blocking solutions, which might lead to mixed aggregation forms with components of the incubation solutions.

The Aggregation and Biorepulsivity of Surfactants on the Solid Phase Surface

It is hypothesised in this thesis that the biorepulsivity of the cationic surfactants is mainly due to the PEG head group. For this mechanism to work, the surfactant molecules need to aggregate in a way so that the surface is densely covered by PEG groups but whether or not this might be the case is not yet clear. The aggregation state of surfactants on a surface is still controversially discussed and appears to depend on the type of the surfactant, as well as the surface characteristics.[71]

Adsorption studies of ionic surfactants onto non-crystalline hydrophobic surfaces are rare. In the few studies available monolayer type structures like hemi-cylindrical aggregates are assumed.[72, 73] In a monolayer structure the surface would be covered by PEG groups as required for a good repulsive action. The actual aggregation state of cationic surfactants on polystyrene microtitre plates, however, is difficult to determine. Solid phase surfaces that are

intended for immunoassays are far from being smooth, let alone homogeneous. Scanning force microscopy studies have even shown volatile hydrocarbon contaminants to be present on polystyrene microtitre wells.[4] Many analytical techniques, which are usually used in surface chemistry, such as light scattering,[74] or reflectometry,[75] require the use of artificial model surfaces such as particles, coated phases or very smooth materials. Even if the model surfaces are of the same material as the solid phase, it is far from clear whether conclusions drawn from studies on such surfaces may be applicable to solid phase surfaces used for immunoassays. On the other hand, it is very unlikely that any hydrophobic part of the adsorbed surfactant is not oriented towards the surface, but sticks out into an aqueous solution. Therefore, it appears reasonable that the solid phase surface is mainly covered by a PEG-layer, which provides the repulsive behaviour of the blocked surface towards NSB.

In chapter 3, it was seen that the best blocking performance is achieved for a certain range of ionic strength of the surfactant solution. Both lower and higher ionic strength on the other hand, are correlated with worse blocking performance. A possible explanation of this phenomenon is based on the relationship between the ionic strength of a surfactant solution and the aggregation state on a hydrophobic surface as discussed in the literature.[72] Two effects are mentioned, of which the first is an increase in aggregate curvature and the second is a decrease in aggregate separation, both of which are a consequence of an interaction of the ionic components in the solution with the hydrophilic headgroup of the surfactant. While the first effect, an increase in aggregate curvature, would inhibit a flat, lamellar arrangement of the molecules and prefer curved aggregates such as hemi-cylinders or hemi-micelles, the converse is true for a decrease in aggregate separation (second effect). Since curved aggregates are likely to leave gaps on the surface, the first effect is likely to inhibit effective NSB prevention, while the second effect is likely to enhance it. Moreover, a decrease in aggregate separation induces a higher surface density of the PEG groups, which is preferred for a good biorepulsivity of PEGs.[20] Possibly, the second effect might prevail at lower ionic strength, whereas the first effect might outbalance the second at higher ion concentrations. This seems a probable explanation of why an ideal ionic strength is found for the blocking behaviour of a surfactant. This explanation also suggests that the surface is mainly covered by a PEG-layer, as such an aggregation state is required for the above mentioned effects to take place.

Adsorptive Forces of Cationic Surfactants on Polystyrene Surfaces

So far, the adsorption of the novel blocking reagents was reviewed and compared to commercial blocking reagents in terms of concentration of the blocking solution, as well as the aggregation behaviour of surfactants and the biorepulsivity of PEGs. One further reason for the better performance of cationic surfactants might be the particularly strong interaction between these surfactants and a polystyrene surface. While the proteinaceous blocking reagents mainly bind through non-specific hydrophobic interactions, three adsorptive forces are thought to exist for cationic surfactants.

The long, hydrophobic alkyl chain interacts via a van-der-Waals force with the likewise hydrophobic polystyrene surface. The linear chain is believed to support a higher ordered, and

therefore denser packing of the adsorbed molecules, as additional hydrophobic interaction may occur between two flatly adsorbed alkyl chains lying side by side. Now, the surfactants bear an amine group at either end of the alkyl chain (ignoring the short, hydrocarbon-based bridge), or at both ends. High-binding polystyrene surfaces, which are most commonly used for immunoassays, exhibit carboxylate groups on the surface due to a mild oxidative treatment in the production process. These negatively charged groups are highly attractive to the amines as a counterion for electrostatic interactions. Furthermore, cationic functional groups, especially ammonium derivatives have been shown to be attracted to electron-rich groups with π-orbitals. This phenomenon is called cation-π-interaction and the binding strength is comparably high as hydrogen bonds under certain circumstances.[76, 77] A cation-π-interaction is very characteristic for aromatic molecules with a doughnut-shaped π-electron ring (where the cationic group fits very well), so the cationic groups of the surfactants are thought to interact with aromatic rings of the polystyrene via strong cation-π-bonds.

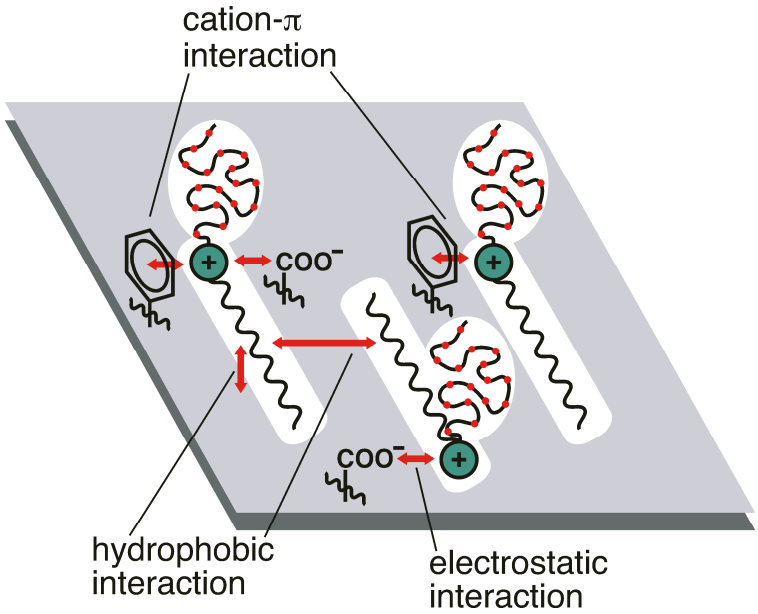


Fig. 42: Schematic illustration of the three main adsorptive forces proposed for a cationic surfactant adsorbed on a polystyrene surface. The alkyl chain is lying flatly on the surface and interacts hydrophobically with the polystyrene surface, as well as with other alkyl chains from neighbouring surfactant molecules. The cationic groups are attracted either electrostatically to a carboxylate group or via a cation-p-interaction to a benzene ring.

In total, these three forces, a weak hydrophobic interaction, as well as the somewhat more pronounced electrostatic and cation-π-interaction, are thought to sum up synergistically to a tight bond of cationic surfactants to polystyrene surfaces.

4.2 Conclusion and Outlook

In this chapter a number of arguments and considerations were reviewed in order to explain why cationic surfactants are so well-suited as novel blocking reagents in an immunoassay. For a final evaluation of the assay experiments, the relevant data is presented in a concise way in Table 6.

Table 6: Summarised results of Specificity-, Prion-, and Hepatitis B-ELISA

Blocking solution / reagent		**1** Spec. 2% HS	**2** Prion S/N 3 ng/mL	**3** Prion S/N 50 ng/mL	**4** HepB LOD	**5** HepB Inflex.P.	**6** HepB S/N
BS1	BSA	3,31	0,83	1,37	n.d.	n.d.	n.d.
BS2	Fish gelatine	2,64	1,12	2,32	0,57	n.d.	n.d.
BS3	Tween20	3,25	1,85	7,66	0,04	0,21	4,33
BS4	Casein	0,92	1,14	4,48	n.d.	n.d.	n.d.
BS5	Casein hydrolysate	0,61	0,99	1,69	n.d.	n.d.	n.d.
BS6	Skimmed milk	0,25	0,97	3,22	n.d.	n.d.	n.d.
BS7	AquaBlock	0,12	1,03	1,02	n.d.	n.d.	n.d.
BS8	RotiBlock	0,10	1,02	1,04	n.d.	n.d.	n.d.
BS9	**7f**(550,12)	0,11	1,22	3,51	n.d.	n.d.	n.d.
BS10	**7f**(550,20)	2,05	2,08	9,80	n.d.	n.d.	n.d.
BS11	**7f**(2000,12)	0,44	1,31	6,61	n.d.	n.d.	n.d.
BS12	**7f**(2000,20)	0,98	1,13	6,33	n.d.	n.d.	n.d.
BS13	**7g**(550,12)	0,14	1,40	6,96	0,06	1,33	7,80
BS14	**7g**(550,20)	2,76	3,62	37,29	0,28	2,19	4,39
BS15	**7g**(2000,12)	0,64	1,61	9,47	0,27	n.d.	n.d.
BS16	**8a**(550,12)	0,12	6,73	44,80	0,05	1,30	53,09
BS17	**8a**(550,20)	0,09	6,69	39,40	0,03	8,41	15,60
BS18	**8b**(550,20)	0,77	2,68	21,88	0,06	1,16	4,04
BS19	**8d**(550,12)	0,79	4,37	38,95	0,03	7,38	14,12
BS20	**8d**(550,20)	0,13	8,30	62,51	0,16	3,61	4,53
BS21	**8d**(2000,12)	1,11	0,73	14,40	n.d.	n.d.	n.d.
BS22	**8d**(2000,20)	0,19	2,69	9,38	n.d.	n.d.	n.d.
BS23	**8e**(550,12)	0,29	5,94	50,25	0,09	9,88	22,12
BS24	**8e**(550,20)	2,98	1,80	22,27	n.d.	n.d.	n.d.
BS25	**8e**(2000,20)	2,12	1,59	22,42	n.d.	n.d.	n.d.

BS26	**8f**(550,12)	0,14	0,97	3,11	0,07	5,43	8,62
BS27	**8f**(550,20)	2,85	1,90	11,20	0,33	1,57	2,45
BS28	**8f**(2000,12)	0,44	1,25	7,86	n.d.	n.d.	n.d.
BS29	**1**	0,64	2,33	10,22	0,04	1,27	11,74
BS30	**2**	0,38	1,69	7,01	0,02	0,77	9,16

Columns indicate the following: **1** Mean of OD values obtained at 2% human serum in the Specificity-ELISA is given. The OD values are either significantly better (green), better but not significantly (orange), or worse than OD values of Casein (One-Way ANOVA, Bonferroni post hoc test, $p<0.05$, N=4 for BS1-8 and BS29-30, N=2 for BS9-28). **2** and **3**: Signal-to-noise ratio of OD values obtained at 3 and 50 ng/mL PrP90-231 are given. The OD values of the signal are either significantly better than the noise (green), better but not significantly (orange), or the S/N values are worse than the S/N value of Tween20 (One-Way ANOVA, Bonferroni post hoc test, $p<0.05$, N=4 for BS1-8 and BS29-30, N=2 for BS9-28). **4**, **5** and **6**: quantitative analysis of fit functions for the Hepatitis B-ELISA as presented in Chapter 3. The values are either better (green) or worse (orange) than the value obtained for Tween20, or were not determined (red).

The table shows very clearly that no single commercially available blocking reagent was able to achieve both good specificity and sensitivity. Tween20 leads to reasonable sensitivity for both, the Prion- and the Hepatitis B-ELISA, but the specificity is very low. Casein, casein hydrolysate, skimmed milk, AquaBlock and RotiBlock are able to efficiently prevent NSB, but the sensitivity is inacceptable both in the Prion- and in the Hepatitis B-ELISA. Moreover, AquaBlock and RotiBlock show an extremely good blocking behaviour in terms of specificity, but a signal-to-noise ratio of about 1 in the Prion-ELISA suggests that these blocking reagents are simply preventing any signal, regardless whether false or true.

On the other hand, many of the PEG-conjugated alkylamines are able to combine acceptable specificity and sensitivity (i.e. BS13, 15, 18, 19, 22, 29 and 30) and some perform very good in all assay experiments (i.e. BS16, 17, 20 and 23).

In summary, PEG-conjugated alkylamine were successfully synthesised and tested as novel blocking reagents. These cationic surfactants are able to outperform commercial blocking reagents, they are appropriate for the usual assay conditions such as neutral pH and ionic strength of common buffers, and they do not exhibit any of the drawbacks of proteinaceous blocking reagents mentioned in the introduction, such as cross-reactivity, heterogeneity and lot-to-lot differences.

Since these novel blocking reagents achieved very promising results, a commercialisation is hoped for in the near future.[78] Though further research is obviously needed, industrial preparation of the compounds should be feasible and enable a cost-efficient production.

5. Experimental Part

5.1 Materials

Equipment and Consumables

Educational tensiometer K6	Krüss, Hamburg
Handheld UV lamp (254 / 366 nm)	Carl Roth, Karlsruhe
Microplate reader VersaMax	Molecular Devices, Ismaning
Microplate washer Columbus	Tecan, Crailsheim
Odyssey Infrared Imaging System	LI-COR Biosciences, Bad Homburg
SpeedVac-Concentrator SPD121P	ThermoElectron, Frankfurt
Water purification system Purelab Ultra	ELGA Labwater, High Wycombe, UK
96-well polystyrene microtitre plates	
high-binding, flat bottom	Corning, Wiesbaden
high-binding, flat bottom, half area	Corning
Microcentrifuge tubes, 2.0 and 0.5 mL	Sarstedt, Nümbrecht
Microcentrifuge tubes, 1.5 mL	Eppendorf, Hamburg
Nitrocellulose transfer membrane	Schleicher & Schuell BioScience, Dassel
PVDF transfer membrane	Whatman, Sanford, ME, USA
Silica gel 60, 0.04 – 0.063 mm	Carl Roth
Sephadex LH-20	GE Healthcare Europe, Freiburg
TLC plates, Alugram, UV_{254}	Carl Roth

Software

Adobe Photoshop 7	Adobe Systems, San Jose, CA, USA
CorelDraw 10	Corel Corporation, Ottawa, Canada
CS ChemDraw Std 9	CambridgeSoft, Cambridge, MA, USA
Odyssey Application Software 2.1	LI-COR Biosciences
Prism 4	GraphPad Software, San Diego, CA, USA
iNMR 0.7	Mestrelab Research, Santiago de Compostela, Spain
Microsoft Office 2004	Microsoft, Red Wood City, OR, USA
SciFinder Scholar 2007	CAS, Columbus, OH, USA
SoftMax Pro 4.8	Molecular Devices

Chemicals

All standard stockroom reagents were purchased from Sigma-Aldrich, Taufkirchen (including the brands Fluka, Aldrich, Sigma-Aldrich and Riedel-de Haën) or Carl Roth, and are not explicitly listed. All reagents were of at least p.a. grade purity, unless otherwise noted.

Reagents

Borane, 1M in tetrahydrofuran	Sigma-Aldrich
1-Bromoeicosane	Sigma-Aldrich
1-Bromododecane	Sigma-Aldrich
Diisopropyl azodicarboxylate	Merck, Darmstadt
Docosanoic acid	TCI Europe, Zwijndrecht, Belgium
1,12-Dodecanediol	Sigma-Aldrich
Eicosanedioic acid	TCI Europe
Hydrazine monohydrate	Sigma-Aldrich
Hydrobromic acid, 48% (w/v) aqueous solution	Sigma-Aldrich
Lithium aluminium hydride	Sigma-Aldrich
Lithium tri-*tert*-butoxyaluminium hydride, 97%	Sigma-Aldrich
Malonic acid diethyl ester	Sigma-Aldrich
Malonic acid di-*tert*-butyl ester	Merck
2-(α-methoxy[poly(ethylene oxy)]) ethanol, 550 Da	Sigma-Aldrich
2-(α-methoxy[poly(ethylene oxy)]) ethanol, 2 kDa	Sigma-Aldrich
Molecular sieves, 4 Å, beads	Sigma-Aldrich
Ninhydrin	Sigma-Aldrich
Phosphomolybdic acid hydrate	Sigma-Aldrich
Phthalimide	Sigma-Aldrich
Piperazine-*N*,*N*'-bis(2-ethanesulfonic acid) (PIPES)	Sigma-Aldrich
Potassium carbonate, anhydrous	Sigma-Aldrich
Sodium, pieces stored in heavy mineral oil	Sigma-Aldrich
Sodium azide	Sigma-Aldrich
Sodium hydride, 60 % dispersion in mineral oil	Sigma-Aldrich
Tetrabutyl ammonium borohydride (TBABH)	Sigma-Aldrich
Tetradecanoic acid	TCI Europe

3,3',5,5'-tetramethylbenzidine	Sigma-Aldrich
Thionyl chloride	Merck
para-Toluenesulfonyl chloride	Merck
Triethylamine	Sigma-Aldrich
Trifluoroacetic acid	Sigma-Aldrich
Trifluoromethane sulfonic acid anhydride	Alfa Aesar, Karlsruhe
Triphenylphosphine	Acros Organics, Geel, Belgium
Tris(hydroxymethyl)aminomethane (Tris)	Sigma-Aldrich
Tween20	Sigma-Aldrich

Solvents

All anhydrous solvents were purchased from Sigma-Aldrich. All other solvents were of at least p.a. grade purity.

Cyclohexane	Carl Roth
Dichloromethane (MC)	Carl Roth
Diethyl ether	Merck
N,*N*-Dimethylformamide (DMF)	Sigma-Aldrich
Ethanol	Carl Roth
Ethyl acetate	Carl Roth
Methanol	Merck
Toluene	Carl Roth

Stains for thin layer chromatography (TLC)

Phosphomolybdic acid dip-solution:

20 g Phosphomolybdic acid hydrate in 100 mL ethanol, stored in the dark. This stain produced blue-black spots on yellow-green background on heating for almost all compounds.

Ninhydrin spray-solution:

0.2 g Ninhydrin and 5 mL acetic acid in 100 mL ethanol. This stain produced red spots for amines on heating.

Iodine:

Development was conducted by storing the TLC plate for several minutes in a jar with a few crystals of iodine. This stain produced non-permanent brown spots of sublimated and adsorbed iodine for almost all compounds.

UV-light:

UV-active spots were visualised with a handheld UV lamp.

Conditions and Commonly Used Abbreviations

Room temperature	25 °C (± 5 °C)
o/n: over night	(approximately 17 h)
ddH_2O: double distilled water	ASTM Grade 1, obtained with Purelab Ultra

Reagents for Assay Experiments

Sera

Human serum (HS):

Human serum was prepared from a freshly collected blood sample drawn from a healthy adult blood donor (the sampling of blood for this purpose was approved by the Ethics Committee of the Medical University of Lübeck). 250 mL blood was drawn and incubated o/n at room temperature. The clotted blood was then incubated on ice for 1 h and centrifuged for 10 min at an rcf of 1,800 × g. The supernatant was divided sterilely in 1.5 mL portions. The aliquots were snap-frozen in liquid nitrogen and stored at -80 °C.

Pooled normal human serum:

Pooled normal human (mixed blood types) serum was purchased from Innovative Research, Novi, MI, USA.

Foetal Bovine serum (FBS):

FBS was purchased from Allgäu Biotech, Kempten.

Antibodies and detection reagents

Concanavalin A (ConA), biotin-labelled	Vector Laboratories, Burlingame, CA, USA
Goat anti-Hepatitis B surface Antigen antibody	AbD Serotec, Kidlington, Oxford, UK
Goat anti-Hepatitis B surface Antigen antibody horseradish peroxidase (HRP)-labelled	AbD Serotec
Goat anti-human IgG antibody, biotin-labelled	Southern Biotech, Birmingham, AL, USA
Goat anti-mouse IgG antibody, AlexaFluor680-labelled	Invitrogen, Carlsbad, CA, US
Goat anti-mouse IgG antibody, HRP-labelled	Southern Biotech
Hepatitis B surface Antigen (HBsAg), recombinant	AbD Serotec
Mouse anti-Prion Protein antibody clone 6H4	Prionics, Schlieren, Switzerland
Prion protein fragment consisting of amino acids 90-231 of murine prion protein, his6-tagged (PrP90-231)[63]	provided by Dr. Steffen Bade, Forschungszentrum Borstel
Streptavidin, HRP-labelled	Vector Laboratories

Other proteins and protein-containing materials

Bovine serum albumine (BSA), fraction V	MP Biomedicals, Solon, OH, USA
Casein, Hammarsten grade	VWR International, Darmstadt
Fish gelatine, HiPure Liquid Gelatine	Norland Products, Cranbury, NJ, USA
Skimmed milk, powder	Lactoland, Dülmen

TMB Substrate solutions

Solution A:

205 mM citric acid, pH adjusted with KOH to 4.0, 3.075 mM H_2O_2. Solution A was stored at 4 °C.

Solution B:

41 mM TMB and 8.1 mM TBABH, dissolved in anhydrous, neat dimethylacetamide. Solution B was stored in microcentrifuge tubes at 4 °C in the dark.

Buffers

The volume of the following buffers was adjusted with double distilled water.

D-PBS (10x) Dulbecco's phosphate buffered saline:

Per litre: 80 g NaCl, 11.5 g Na_2HPO_4, 2 g KCl, 2 g KH_2PO_4.

D-PBS (1x, 1.47 mM KH_2PO_4, 8.10 mM Na_2HPO_4, 137 mM NaCl, 2.68 mM KCl, pH 7.4):

Per litre: 100 mL D-PBS (10x).

PBST (D-PBS with 0.05% (w/v) Tween20):

1 L D-PBS (1x), 5 mL 10% (w/v) Tween20.

L-PBS (Lite PBS, 10.0 mM NaH_2PO_4 and 10.0 mM NaCl, pH 7.0):

Per litre: 0.584 g NaCl, 1.38 g $NaH_2PO_4 \times H_2O$, pH adjusted with 1 M NaOH to 7.0.

Phosphate-Buffer (4x)

Per 50 mL: 107.2 mg NaH_2PO_4, 157.1 mg Na_2HPO_4, 420 µL 2 M KCl, 6 mL 5 M NaCl.

Phosphate-Buffer (1x, pH 6.3, 6.8, 7.3, 7.8, 8.3)

Per 40 mL: 10 mL Phosphate-Buffer (4x), pH adjusted with 1 M HCl or 1 M NaOH to each of the above mentioned values.

PIPES-Buffer (2x)

Per 100 mL: 604.7 mg PIPES, 420 µL 2 M KCl, 6 mL 5 M NaCl, pH adjusted with 1 M HCl to 5.8.

PIPES-Buffer (1x, pH 5.8, 6.3, 6.8, 7.3)

Per 40 mL: 20 mL PIPES-Buffer (2x), pH adjusted with 1 M HCl or 1 M NaOH to each of the above mentioned values.

Tris-Buffer (4x)

Per 50 mL: 242.3 mg Tris, 420 µL 2 M KCl, 6 mL 5 M NaCl.

Tris-Buffer (1x, pH 7.3, 7.8, 8.3, 8.8)

Per 40 mL: 10 mL Tris-Buffer (4x), pH adjusted with 1 M HCl or 1 M NaOH to each of the above mentioned values.

Na^+-Buffer (0.1x to 5x)

Per 200 mL: 107.2 mg NaH_2PO_4, 157.1 mg Na_2HPO_4, and one of the following amounts of NaCl: 0 mg for 0.1x, 169.1 mg for 0.2x, 695.1 mg for 0.5x, 1.572 g for 1x, 3.325 g for 2x and 8.585 g for 5x, pH adjusted with 1 M NaOH to 7.2.

K^+-Buffer (0.1x to 5x)

Per 200 mL: 121.6 mg KH_2PO_4, 192.7 mg K_2HPO_4, and one of the following amounts of KCl: 0 mg for 0.1x, 215.7 mg for 0.2x, 886.7 mg for 0.5x, 2.005 g for 1x, 4.242 g for 2x and 10.95 g for 5x, pH adjusted with 1 M KOH to 7.2.

NH_4^+-Buffer (0.1x to 5x)

Per 200 mL: 2 mL 1 M H_3PO_4, 3.04 mL 1 M NH_4OH, and one of the following amounts of NH_4Cl: 0 mg for 0.1x, 155.1 mg for 0.2x, 636.5 mg for 0.5x, 1.439 g for 1x, 3.044 g for 2x and 7.858 g for 5x, pH adjusted with 1 M NH_4OH or 1 M HCl to 7.2.

Blocking Solutions

Blocking solutions BS1-8 were prepared with commercially available blocking reagents:

BS1: BSA, 1% (w/v) in D-PBS

BS2: Fish gelatine, 1% (w/v) in D-PBS

BS3: Tween20, 0.5% (v/v) in D-PBS

BS4: Casein, 1% (w/v) in D-PBS

BS5: Casein hydrolysate, 1% (w/v) in D-PBS

1 g casein was dissolved in 80 mL of 0.3 M aqueous NaOH at 37 °C and allowed to hydrolyse for several hours. Then 10 mL of D-PBS (10x) were added, the pH was set to 8 by addition of diluted HCl and the volume was adjusted with double distilled water to 100 mL.

BS6: Skimmed milk, 5% (w/v) in D-PBS

BS7: AquaBlock (fish plasma-based blocking reagent)

Ready to use solution, purchased from EastCoastBio, North Berwick, ME, USA.

BS8: Rotiblock (PVP containing blocking reagent)

Purchased from Carl Roth, 10 fold diluted in double distilled water.

Blocking solutions BS9-30 were prepared with cationic surfactants as novel blocking reagents:

BS9: ***7f****(550,12), 1% (w/v) in D-PBS, pH adjusted with 1 M HCl or 1 M NaOH to 7.2*

BS10: ***7f****(550,20), 1% (w/v) in D-PBS, pH adjusted with 1 M HCl or 1 M NaOH to 7.2*

BS11: ***7f****(2000,12), 1% (w/v) in D-PBS, pH adjusted with 1 M HCl or 1 M NaOH to 7.2*

BS12: ***7f****(2000,20), 1% (w/v) in D-PBS, pH adjusted with 1 M HCl or 1 M NaOH to 7.2*

BS13: ***7g****(550,12), 1% (w/v) in D-PBS, pH adjusted with 1 M HCl or 1 M NaOH to 7.2*

BS14: ***7g****(550,20), 1% (w/v) in D-PBS, pH adjusted with 1 M HCl or 1 M NaOH to 7.2*

BS15: ***7g****(2000,12), 1% (w/v) in D-PBS, pH adjusted with 1 M HCl or 1 M NaOH to 7.2*

BS16: ***8a****(550,12), 1% (w/v) in D-PBS, pH adjusted with 1 M HCl or 1 M NaOH to 7.2*

BS17: ***8a****(550,20), 1% (w/v) in D-PBS, pH adjusted with 1 M HCl or 1 M NaOH to 7.2*

BS18: ***8b****(550,20), 0.5% (w/v) in D-PBS, pH adjusted with 1 M HCl or 1 M NaOH to 7.2*

BS19: ***8d****(550,12), 1% (w/v) in D-PBS, pH adjusted with 1 M HCl or 1 M NaOH to 7.2*

BS20: ***8d****(550,20), 1% (w/v) in D-PBS, pH adjusted with 1 M HCl or 1 M NaOH to 7.2*

BS21: ***8d****(2000,12), 1% (w/v) in D-PBS, pH adjusted with 1 M HCl or 1 M NaOH to 7.2*

BS22: ***8d****(2000,20), 1% (w/v) in D-PBS, pH adjusted with 1 M HCl or 1 M NaOH to 7.2*

BS23: ***8e****(550,12), 1% (w/v) in D-PBS, pH adjusted with 1 M HCl or 1 M NaOH to 7.2*

BS24: ***8e****(550,20), 0.2% (w/v) in D-PBS, pH adjusted with 1 M HCl or 1 M NaOH to 7.2*

BS25: ***8e****(2000,20), 1% (w/v) in D-PBS, pH adjusted with 1 M HCl or 1 M NaOH to 7.2*

BS26: ***8f****(550,12), 1% (w/v) in D-PBS, pH adjusted with 1 M HCl or 1 M NaOH to 7.2*

BS27: ***8f****(550,20), 1% (w/v) in D-PBS, pH adjusted with 1 M HCl or 1 M NaOH to 7.2*

BS28: ***8f****(2000,12), 1% (w/v) in D-PBS, pH adjusted with 1 M HCl or 1 M NaOH to 7.2*

BS29: ***1****, 0.5% (w/v) in D-PBS*

BS30: ***2****, 1% (w/v) in D-PBS*

5.2 Syntheses

Column chromatography was performed on silica gel 60 if not stated otherwise. MALDI–TOF spectrometry was used to characterise derivatives containing polydisperse polymer units. As the molecular mass of the repetitive unit of poly(ethylene glycol) is approximately 44 Da, signals separated by a multiple of 44 Da belong to the same distribution. For each distinguishable distribution in one spectrum the central signal of the top three most abundant signals is given and the range of the (approximately bell-shaped) distribution is indicated (considering signals with at least an 0.1-fold count of the maximum signal) in parenthesis. ^{1}H and ^{13}C NMR spectra were measured in chloroform-d_1 if not otherwise indicated. Spectra of amine containing compounds were described by the chemical shifts of either the amine- or the protonated form, whichever prevailed. A remark was added to the spectrum if the shifts given belonged to the protonated form.

General Procedures

Mitsunobu reaction of alcohols to phthalimides (general procedure A):
The respective alcohol and a 1.1-fold molar amount each of triphenylphosphine and phthalimide were dissolved in anhydrous THF. A 1.1-fold molar amount of diisopropyl azo dicarboxylate was added slowly while stirring and keeping the temperature at room temperature. The cooling was removed and the reaction mixture stirred for 2.5 h - 1 d at room temperature. The reaction process was monitored via thin layer chromatography. The solvent was removed and the crude product was purified by column chromatography.

Malonic ester synthesis (general procedure B):
The respective malonic ester was deprotonated by either dissolving it in ethanol and dropping the solution into a carefully prepared sodium ethanolate solution containing an equimolar amount of sodium in ethanol (**B1**) or dissolving it in an appropriate solvent (THF or DMF) and adding the solution to an equimolar amount of sodium hydride in the respective solvent (**B2**) and subsequently stirring at room temperature – under reflux for 10 min - 5 h until hydrogen formation ceased. Then an equimolar amount of alkylbromide was added and the reaction mixture was stirred at room temperature – under reflux for 3 h - 1 d. The reaction progress was monitored by thin layer chromatography. The solvent was removed *in vacuo* and the residue was taken up in diethyl ether or ethyl acetate. The organic fractions were washed once with ddH_2O, dried over $MgSO_4$ and concentrated. The crude product was purified by column chromatography.

Hydrolysis of substituted malonic acid diethyl esters (general procedure C):
The respective malonic acid diethyl ester was stirred at reflux for 4.5 - 6 h with a freshly prepared solution of an approximately four-fold excess of potassium hydroxide dissolved in a 1:2 mixture of ddH_2O and ethanol. The solvent was evaporated and the residue was taken up in ddH_2O. Concentrated hydrochloric acid (32% (w/v)) was added until the pH reached approximately 1. The aqueous phase was extracted four times with diethyl ether. The organic fractions were combined, dried over $MgSO_4$ and concentrated.

Hydrolysis of substituted malonic acid di-tert-butyl esters (general procedure D):
The respective malonic acid di-*tert*-butyl ester was dissolved in dichloromethane and an approximately ten fold molar excess of trifluoroacetic acid was added. The solution was stirred at room temperature for 3 d and the reaction progress was monitored by thin layer chromatography. The solvent and any remaining trifluoroacetic acid and ester were removed *in vacuo*.

Decarboxylation of substituted malonic acids (general procedure E):
The respective malonic acid was stirred at 150 - 160 °C and at atmospheric pressure for 70 min - 5 h and for further 1 - 5 h at reduced pressure (approximately 100 mbar) until gas formation ceased.

Preparation of carboxylic or malonic acid chlorides (general procedure F):
The respective carboxylic acid or malonic acid was stirred at reflux with an eight- to twenty-fold molar excess of thionyl chloride for 1.5 - 7.5 h. The volatiles were removed *in vacuo* and the residue was co-distilled with toluene. The crude product was used for the next step without further purification.

Reduction of carboxylic acid chlorides to alcohols (general procedure G):
The respective carboxylic acid chlorides were dissolved in anhydrous THF and cooled to 0 °C. An approximately 2.4 fold molar amount of lithium tri(*tert*-butoxy) aluminium hydride was added under argon atmosphere and vigorous stirring and the reaction mixture was stirred at 0 °C for 1 h and for further 80 min - 2 h at room temperature. The reaction mixture then was poured into an excess of 0.1 M hydrochloric acid and the aqueous phase was extracted four times with ethyl acetate. The organic fractions were combined, dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography.

Condensation of carboxylic or malonic acid chlorides with mPEG-amine to the respective amide (general procedure H):
The respective carboxylic acid chloride and an equimolar amount of **3 / 3'** or the respective malonic acid chlorides and a 2.0-fold molar amount of **3 / 3'** were dissolved in toluene and stirred at room temperature for 5 - 30 min. An equimolar amount of triethylamine was added and the reaction mixture was stirred at 75 °C bath temperature for o/n - 4 d. The reaction mixture was cooled to room temperature and the precipitate was filtered off and washed with toluene. The filtrate and the washing solutions were combined and washed once with 0.2 M HCl and once with saturated sodium bicarbonate solution. The organic fractions were combined, dried over $MgSO_4$ and concentrated.

Williamson ether synthesis of ω-phthalimido alkan-1-ols with mPEGs (general procedure I):
The respective ω-phthalimido alkan-1-ol was dissolved in anhydrous dichloromethane and cooled to 0 °C. A 1.2-fold molar amount of trifluoromethane sulfonic acid anhydride was added dropwise under an argon atmosphere and the reaction mixture was stirred at 0 °C for 35 - 90 min. Concurrently, a 1.1-fold molar amount of 2-(α-methoxy[poly(ethylene

oxy)])ethanol was dissolved in anhydrous dimethoxyethane and freshly dried molecular sieves 4 Å and a 1.05-fold molar amount of sodium hydride were added. The dimethoxyethane solution was stirred at room temperature for 50 - 90 min. Meanwhile the dichloromethane solution was filtered and the precipitate was washed with ice cold dichloromethane. The filtrate and the washing solution were combined and washed once with ice cold saturated sodium bicarbonate solution. The organic fractions were combined, dried over $MgSO_4$ and the solvent was removed *in vacuo* at a temperature not exceeding 30 °C. The residue was dissolved in anhydrous dimethoxyethane and added to the 2-(α-methoxy[poly(ethylene oxy)]) ethanol solution. The reaction mixture was stirred at room temperature o/n - 5 d, then it was filtered and the filtrate was concentrated. The residue was purified by column chromatography.

Hydrazinolysis of phthalimides (general procedure J):
The respective phthalimide was dissolved in ethanol with an approximately eight-fold molar excess of hydrazine monohydrate. The solution was stirred at reflux o/n - 1 d. The solvent was evaporated and the residue was dissolved in 0.1 M hydrochloric acid. The aqueous phase was washed twice with ethyl acetate and then treated with neat sodium hydroxide until the pH reached 14. The aqueous phase was then extracted four times with dichloromethane. The organic fractions were combined, dried over $MgSO_4$ and concentrated.

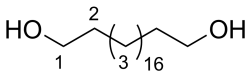
Reduction of carboxylic or malonic acid amides to amines (general procedure K):
The respective carboxylic acid amide or malonic acid amide was dissolved in anhydrous THF and added slowly under an argon atmosphere at 0 °C to an 2.4- to 5.1-fold molar amount of 1 M borane in THF. The solution was allowed to warm to room temperature and then stirred at reflux o/n - 4 d. The reaction was stopped by adding a 0.9-fold molar amount of 6 M hydrochloric acid. The solvent was removed and the residue was dissolved in 1 M hydrochloric acid. The pH was adjusted to 14 using neat sodium hydroxide. The aqueous phase was extracted four times with dichloromethane. The organic fractions were combined, dried over $MgSO_4$ and concentrated.

Synthesis of Alkyl Building Blocks

Eicosane-1,20-diol:
Eicosanedioic acid (50.1 g, 146 mmol) was stirred at reflux with thionyl chloride (85.5 mL, 1.17 mol) for 2.5 h. The volatiles were removed *in vacuo* and the residue was co-distilled with toluene twice. The crude diacid chloride was dissolved in anhydrous diethyl ether (100 mL) and the solution was added slowly to a suspension of LiAlH4 (6.66 g, 175 mmol) in anhydrous diethyl ether (250 mL) while stirring and keeping the temperature at 0 °C. The reaction mixture was stirred at room temperature o/n and at reflux for 90 min. The mixture was cooled to 0 °C and ddH_2O (50 mL), as well as 10% (v/v) H_2SO_4 were added slowly while keeping the temperature at 0 °C. The mixture was allowed to warm to room temperature and stirred o/n. The resulting emulsion was repeatedly extracted with hot toluene. The toluene

phases were combined, washed once with saturated sodium bicarbonate solution and once with ddH_2O, dried over $MgSO_4$ and were concentrated.

Yield: 45.5 g (145 mmol), 99% of a colourless crystalline solid.

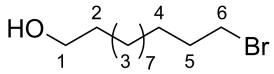


^{1}H NMR (360 MHz, $CDCl_3$+DMSO-d_6, 25 °C): δ 1.05 (m, 32H, H-3), 1.33 (m, 4H, H-2), 3.36 (t, 4H, $^3J_{H-H}$ = 6.7 Hz, H-1).

12-Bromododecan-1-ol [3b(12)]:

Dodecane-1,12-diol (20.2 g, 100 mmol) was suspended in toluene (200 mL) and hydrobromic acid (48% (w/v), 12.5 mL, 110 mmol) was added to the suspension. The mixture was stirred at reflux for 1 d at a Dean-Stark apparatus, and allowed to cool to room temperature. The resulting mixture was decanted and the residue was washed three times with toluene. The decanted supernatant and the washing solutions were combined, and washed once with saturated bicarbonate solution and once with saturated sodium chloride solution. The organic fractions were combined, dried over $MgSO_4$ and were concentrated. The crude product was purified by column chromatography (cyclohexane / ethyl acetate 9:1 → cyclohexane / acetone 9:1).

Yield: 17.7 g (66.6 mmol), 67% of a colourless crystalline solid.

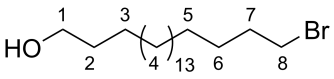


^{1}H NMR (360 MHz, $CDCl_3$, 25 °C): δ 1.28 (m, 14H, H-3), 1.42 (m, 2H, H-4), 1.57 (m, 2H, H-2), 1.85 (tt, 2H, $^3J_{H-H}$ = 7.1 Hz, $^3J_{H-H}$ = 7.1 Hz, H-5), 3.41 (t, 2H, $^3J_{H-H}$ = 6.8 Hz, H-6), 3.64 (t, 2H, $^3J_{H-H}$ = 6.7 Hz, H-1).

20-Bromoeicosan-1-ol [3b(20)]:

Eicosane-1,20-diol (45.5 g, 145 mmol) was suspended in toluene (270 mL) and hydrobromic acid (48% (w/v), 19.7 mL, 174 mmol) was added to the suspension. The mixture was stirred at reflux for 1 d at a Dean-Stark apparatus, and allowed to cool to room temperature. Cyclohexane (300 mL) was added and the resulting mixture was washed once with saturated bicarbonate solution and once with ddH_2O. The organic fractions were combined, dried over $MgSO_4$ and were concentrated. The crude product was purified by column chromatography (cyclohexane → cyclohexane / acetone 9:1).

Yield: 26.6 g (69.4 mmol), 48% of a colourless crystalline solid.



^{1}H NMR (360 MHz, $CDCl_3$, 25 °C): δ 1.26 (m, 30H, H-3, H-4, H-5), 1.42 (m, 2H, H-6), 1.57 (m, 2H, H-2), 1.85 (tt, 2H, $^3J_{H-H}$ = 7.1 Hz, $^3J_{H-H}$ = 7.1 Hz, H-7), 3.40 (t, 2H, $^3J_{H-H}$ = 6.8 Hz, H-8), 3.64 (t, 2H, $^3J_{H-H}$ = 6.7 Hz, H-1).

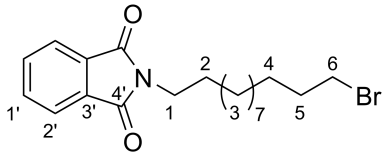
^{13}C NMR (90 MHz, $CDCl_3$, 25 °C): δ 25.9 (C-3), 28.3 (C-6), 28.9 (C-5), 29.5–29.8 (C-4), 33.0 (C-7), 34.2 (C-8), 63.3 (C-1).

***N*-(12-Bromododecyl) phthalimide [3c(12)]:**

Following general procedure A, **3b(12)** (18.6 g, 70.0 mmol), triphenylphosphine (20.2 g, 77.0 mmol), phthalimide (11.3 g, 77.0 mmol) and diisopropyl azo dicarboxylate (16.1 mL, 77.0 mmol) were reacted in THF (350 mL) for 2.5 h and the mixture was processed accordingly. The crude product was purified by column chromatography (cyclohexane / ethyl acetate 9:1).

Yield: 25.7 g (65.2 mmol), 93 % of a colourless crystalline solid.

^{1}H NMR (360 MHz, $CDCl_3$, 25 °C): δ 1.26 (m, 14H, H-3), 1.41 (m, 2H, H-4), 1.67 (m, 2H, H-2), 1.85 (m, 2H, H-5), 3.40 (t, 2H, $^{3}J_{H\text{-}H}$ = 6.8 Hz, H-6), 3.67 (t, 2H, $^{3}J_{H\text{-}H}$ = 7.4 Hz, H-1), 7.70 (m, 2H, H-1'), 7.84 (m, 2H, H-2').



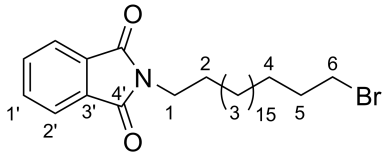
^{13}C NMR (90 MHz, $CDCl_3$, 25 °C): δ 27.0 (C-3), 28.3 (C-4), 28.8 (C-2), 28.9–29.6 (C-3), 33.0 (C-5), 34.2 (C-6), 38.2 (C-1), 123.3 (C-2'), 132.4 (C-3'), 134.0 (C-1'), 168.6 (C-4').

***N*-(20-Bromoeicosyl) phthalimide [3c(20)]:**

Following general procedure A, **3b(20)** (26.2 g, 69.4 mmol), triphenylphosphine (20.0 g, 76.3 mmol), phthalimide (11.2 g, 76.3 mmol) and diisopropyl azo dicarboxylate (15.5 mL, 76.3 mmol) were reacted in THF (300 mL) for 1 d and the mixture was processed accordingly. The crude product was purified by column chromatography (toluene).

Yield: 11.5 g (22.7 mmol), 66 % of a colourless crystalline solid.

^{1}H NMR (360 MHz, $CDCl_3$, 25 °C): δ 1.24 (m, 14H, H-3), 1.42 (m, 2H, H-4), 1.67 (m, 2H, H-2), 1.85 (m, 2H, H-5), 3.40 (t, 2H, $^{3}J_{H\text{-}H}$ = 7.0 Hz, H-6), 3.67 (t, 2H, $^{3}J_{H\text{-}H}$ = 7.2 Hz, H-1), 7.70 (m, 2H, H-1'), 7.84 (m, 2H, H-2').



^{13}C NMR (90 MHz, $CDCl_3$, 25 °C): δ 27.0 (C-3), 28.3 (C-4), 28.8 (C-2), 28.9–29.8 (C-3), 33.0 (C-5), 34.2 (C-6), 38.3 (C-1), 123.3 (C-2'), 132.4 (C-3'), 133.9 (C-1'), 168.6 (C-4').

Synthesis of PEG Building Blocks

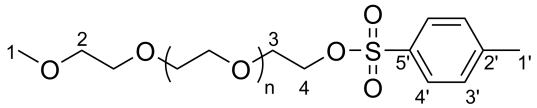
α-methoxy[poly(ethylene oxy)] *para*-toluene sulfonate (average molecular mass ~0.70 kDa) [4b(550)]:

2-(α-methoxy[poly(ethylene oxy)]) ethanol (average molecular mass ~550 Da, 55.0 g, 100 mmol) was dissolved in anhydrous dichloromethane (400 mL). Freshly dried molecular sieves 4Å and triethylamine (35.0 mL, 250 mmol) were added and the solution was cooled to 0 °C. *Para*-toluene sulfonylchloride (47.7 g, 250 mmol) was added under exclusion of humidity. The mixture was stirred at 0 °C for 60 min, warmed to room temperature and stirred for another 80 min. The solvent was removed and the residue was taken up in toluene. The mixture was filtered and the residue was washed with toluene. The filtrate and the washing solution were combined and concentrated. The residue was dissolved in toluene and purified

twice by column chromatography (1. toluene → dichloromethane → dichloromethane / triethylamine 19:1; 2. toluene → acetonitril).

Yield: 44.4 g (63.1 mmol), 63% of a light-brown oil.

^{1}H NMR (360 MHz, $CDCl_3$, 25 °C): δ 2.44 (s, 3H, H-1'), 3.37 (s, 3H, H-1), 3.62 (m, polymer backbone), 4.15 (m, 2H, H-4), 7.33 (d, 2H, $^3J_{H-H}$ = 7.9 Hz, H-4'), 7.79 (d, 2H, $^3J_{H-H}$ = 8.3 Hz, H-3').



^{13}C NMR (90 MHz, $CDCl_3$, 25 °C): δ 21.8 (C-1'), 59.2 (C-1), 68.8 (C-4), 69.4 (C-3), 70.0–71.0 (polymer backbone), 72.1 (C-2), 128.1 (C-3'), 129.9 (C-4'), 133.2 (C-2'), 144.9 (C-5').

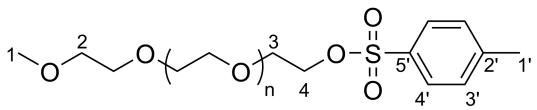
MALDI–TOF: 671.3 (451.3–891.7) $[M+H]^+$, 693.4 (473.2–913.6) $[M+Na]^+$, 709.4 (489.2–929.6) $[M+K]^+$

α-methoxy[poly(ethylene oxy)] *para*-toluene sulfonate (average molecular mass ~2.15 kDa) [4b(2000)]:

2-(α-methoxy[poly(ethylene oxy)]) ethanol (average molecular mass ~2 kDa, 100 g, 50.0 mmol) was dissolved in anhydrous dichloromethane (300 mL). Freshly dried molecular sieves 4 Å and triethylamine (17.4 mL, 125 mmol) were added and the solution was cooled to 0 °C. *Para*-toluene sulfonylchloride (23.8 g, 125 mmol) was added under exclusion of humidity. The mixture was stirred at 0 °C for 40 min, warmed to room temperature and further stirred o/n. The solvent was removed and the residue was taken up in toluene. The precipitate was filtered off and washed with toluene. The filtrate and the washing solution were combined and concentrated. The residue was taken up in dichloromethane and filtered over a small amount (approximately 50 g) of silica gel. The silica gel was washed with dichloromethane. The filtrate and the washing solution were again combined and concentrated. The residue was precipitated by addition of diethyl ether. The precipitate was filtered, washed with diethyl ether and dissolved in dichloromethane. The precipitation and washing steps were repeated, and the precipitate was dried *in vacuo*.

Yield: 90.5 g (42.0 mmol), 84 % of a colourless solid.

^{1}H NMR (360 MHz, $CDCl_3$, 25 °C): δ 2.44 (s, 3H, H-1'), 3.37 (s, 3H, H-1), 3.63 (m, polymer backbone), 4.15 (m, 2H, H-4), 7.33 (d, 2H, $^3J_{H-H}$ = 8.0 Hz, H-4'), 7.79 (d, 2H, $^3J_{H-H}$ = 8.3 Hz, H-3').

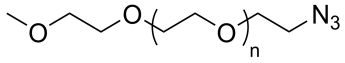


^{13}C NMR (90 MHz, $CDCl_3$, 25 °C): δ 21.8 (C-1'), 59.1 (C-1), 68.8 (C-4), 69.3 (C-3), 70.0–71.5 (polymer backbone), 72.1 (C-2), 128.1 (C-3'), 129.9 (C-4'), 133.2 (C-2'), 144.9 (C-5').

2-(α-methoxy[poly(ethylene oxy)]) ethyl azide (average molecular mass ~575 Da) [4c(550)]:

4b(550) (29.5 g, 41.9 mmol) and sodium azide (4.09 g, 62.9 mmol) were suspended in anhydrous DMF (80mL) and stirred at reflux for 3 h and at room temperature for 1 d. The reaction mixture was cooled and the solvent was removed *in vacuo*. The residue was co-

distilled twice with toluene and then taken up in a 1:1 solution of toluene and diethyl ether. The precipitate was filtered off and rinsed with toluene. The filtrate and the washing solutions were combined, concentrated and taken up in diethyl ether. The precipitate was filtered off and rinsed with diethyl ether. The filtrate and the washing solutions were combined, concentrated and dried *in vacuo*.



Yield: 23.0 g (39.8 mmol), 95% of a light-brown oil.

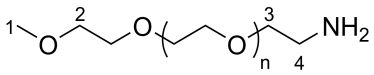
The crude product was used in the following step without further purification.

2-(α-methoxy[poly(ethylene oxy)]) ethyl amine (average molecular mass ~550 Da) [4d(550)]:

A solution of **4c(550)** (23.0 g, 40.0 mmol) in anhydrous THF (100 mL) was cooled to 0 °C. Triphenylphosphine (22.0 g, 83.0 mmol) was added and the mixture was stirred at 0 °C for 45 min. The mixture was allowed to warm to room temperature and stirred o/n. The reaction progress was followed by thin layer chromatography. ddH_2O (2.27 mL, 126 mmol) was added and the reaction mixture was further stirred for 3 h. The reaction mixture was concentrated *in vacuo* and the residue was diluted with ddH2O. The aqueous phase was acidified to pH 1 using 2 M HCl and washed once with toluene. The aqueous phase was adjusted to pH 13 using neat sodium hydroxide and extracted three times with dichloromethane. The dichloromethane fractions were combined, dried over $MgSO_4$ and concentrated *in vacuo*.

Yield: 18.9 g (34.4 mmol), 86% of a light-yellow oil.

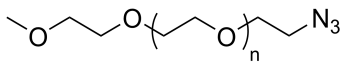
^{1}H NMR (360 MHz, $CDCl_3$, 25 °C): δ 2.89 (m, 2H, H-4), 3.37 (s, 3H, H-1), 3.63 (m, polymer backbone).



^{13}C NMR (90 MHz, $CDCl_3$, 25 °C): δ 41.8 (C-4), 59.1 (C-1), 70.0–71.5 (polymer backbone), 72.1 (C-2), 72.7 (C-3).

2-(α-methoxy[poly(ethylene oxy)]) ethyl azide (average molecular mass ~2.03 kDa) [4c(2000)]:

4b(2000) (43.1 g, 20.0 mmol) and sodium azide (1.95 g, 30.0 mmol) were suspended in anhydrous DMF (80 mL) and stirred at reflux for 60 min. The reaction mixture was cooled to room temperature and stirred o/n. The solvent was removed *in vacuo* and the residue was taken up in toluene. Insoluble components were removed by centrifugation (15 - 30 min; 1,700 rcf) and the supernatant was carefully transferred into a flask. The centrifugation pellet was resuspended in toluene and the above mentioned steps were repeated 3 times. The combined supernatants were concentrated and dried *in vacuo*.



Yield: 40.5 g (20.0 mmol), 100% of a light-brown solid.

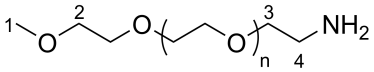
The crude product was used in the following step without further purification.

2-(α-methoxy[poly(ethylene oxy)]) ethyl amine (average molecular mass ~2.00 kDa) [4d(2000)]:

A solution of **4c(2000)** (40.5 g, 20.0 mmol) in anhydrous THF (85 mL) was cooled to 0 °C. Triphenylphosphine (10.5 g, 40.0 mmol) was added and the mixture was stirred at 0 °C for 20 min. The mixture was allowed to warm to room temperature and stirred for 3 d. The reaction progress was monitored by thin layer chromatography. ddH_2O (1.08 mL, 60.0 mmol) was added and the reaction mixture was stirred o/n. ddH_2O was added and the aqueous phase was washed with toluene once and extracted with dichloromethane three times. The dichloromethane fractions were combined, dried over $MgSO_4$ and concentrated *in vacuo*.

Yield: 38.4 g (19.2 mmol) 96% of a light yellow solid.

^{1}H NMR (360 MHz, $CDCl_3$, 25 °C): δ 3.17 (m, 2H, H-4), 3.37 (s, 3H, H-1), 3.63 (m, polymer backbone).



^{13}C NMR (90 MHz, $CDCl_3$, 25 °C): δ 41.0 (C-4), 59.2 (C-1), 70.0–71.5 (polymer backbone), 72.1 (C-2).

MALDI–TOF: 2189.6 (1792.6–2717.7) $[M+H]^+$, 2211.6 (1814.6–2739.7) $[M+Na]^+$

Synthesis of Alkyl-Bridge Building Blocks

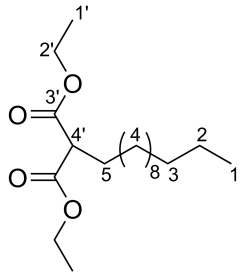
2-dodecyl propane dioic acid diethyl ester [5a(12)] and 2,2'-bisdodecyl propane dioic acid diethyl ester [5b(12)]:

Sodium hydride in mineral oil (60% (w/w), 1.60 g, 40.0 mmol) was washed with anhydrous hexane and reacted with malonic acid diethyl ester (6.07 mL, 40.0 mmol) in anhydrous THF (50 mL) for 10 min at room temperature according to general procedure B2. The mixture then was reacted with dodecylbromide (9.6 mL, 40 mmol) in anhydrous THF (50 mL) for 7 h at reflux and o/n at room temperature according to general procedure B. The reaction mixture was separated into two equal portions.

One portion was worked up with ethyl acetate as described (general procedure B). Column chromatography (cyclohexane / ethyl acetate 19:1) gave **5a(12)**.

Sodium hydride in mineral oil (60% w/w, 1.10 g, 27.5 mmol) was washed with anhydrous hexane and reacted with the other portion for 10 min at room temperature according to general procedure B2 (in deviation from this procedure, the mixture was used as provided without further dilution). The mixture then was reacted with dodecylbromide (4.5 mL, 19 mmol) for 6 h at reflux and o/n at room temperature. The work-up was performed as described (general procedure B) with ethyl acetate. Column chromatography (cyclohexane → cyclohexane / ethyl acetate 19:1) gave **5b(12)**.

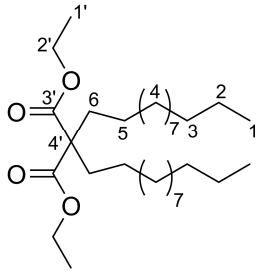
5a(12):
Yield: 4.60 g (14.0 mmol), 35% of a colourless oil.



^{1}H NMR (360 MHz, $CDCl_3$, 25 °C): δ 0.88 (t, 3H, $^{3}J_{H\text{-}H}$ = 6.8 Hz, H-1), 1.25 (m, 26H, H-2, H-3, H-4, H-1'), 1.88 (m, 2H, H-5), 3.30 (t, 1H, $^{3}J_{H\text{-}H}$ = 7.5 Hz, H-4'), 4.19 (q, 4H, $^{3}J_{H\text{-}H}$ = 7.2 Hz, H-2').

^{13}C NMR (90 MHz, $CDCl_3$, 25 °C): δ 14.2 (C-1, C-1'), 22.8 (C-2), 27.5 (C-5), 28.9–29.8 (C-4), 32.0 (C-3), 52.3 (C-4'), 61.4 (C-2'), 169.8 (C-3').

5b(12):
Yield: 6.96 g (14.0 mmol), 35% of a colourless oil.



^{1}H NMR (360 MHz, $CDCl_3$, 25 °C): δ 0.88 (t, 6H, $^{3}J_{H\text{-}H}$ = 6.8 Hz, H-1), 1.14 (m, 4H, H-2), 1.25 (m, 42H, H-3, H-4, H-5, H-1'), 1.85 (m, 4H, H-6), 4.17 (q, 4H, $^{3}J_{H\text{-}H}$ = 7.1 Hz, H-2').

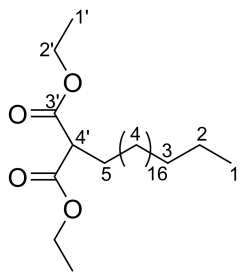
^{13}C NMR (90 MHz, $CDCl_3$, 25 °C): δ 14.3 (C-1, C-1'), 22.8 (C-2), 24.1 (C-6), 29.5–29.9 (C-4), 32.1 (C-3), 32.3 (C-5), 57.7 (C-4'), 61.0 (C-2'), 172.2 (C-3').

2-eicosyl propane dioic acid diethyl ester [5a(20)] and 2,2'-biseicosyl propane dioic acid diethyl ester [5b(20)]:
A solution of malonic acid diethyl ester (10.6 mL, 70.0 mmol) in ethanol (10 mL) and a solution of sodium ethanolate (1.63 g sodium, 70.9 mmol) in ethanol (60 mL) were reacted for 30 min at reflux according to general procedure B1. The mixture then was reacted with eicosylbromide (25.4 g, 70.3 mmol) for 3 h at reflux according to general procedure B. The reaction mixture was separated into two equal portions.

One portion was worked up with diethyl ether as described (general procedure B). Column chromatography (cyclohexane → cyclohexane / ethyl acetate 19:1) gave **5a(20)**.

Sodium (810 mg, 35.2 mmol) was added to the other portion and the reaction mixture was stirred at reflux for 20 min. Eicosylbromide (12.7 g, 35.0 mmol) was added and the reaction mixture was stirred at reflux o/n. The work-up was performed as described (general procedure B) with diethyl ether. Column chromatography (cyclohexane / ethyl acetate 19:1) gave **5b(20)**.

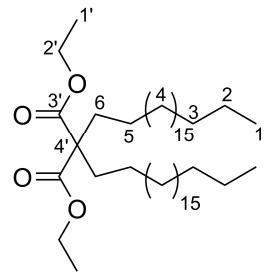
5a(20):
Yield: 8.56 g (19.4 mmol), 28% of a colourless oil.



^{1}H NMR (360 MHz, $CDCl_3$, 25 °C): δ 0.88 (t, 3H, $^{3}J_{H\text{-}H}$ = 6.8 Hz, H-1), 1.25 (m, 42H, H-2, H-3, H-4, H-1'), 1.88 (m, 2H, H-5), 3.31 (t, 1H, $^{3}J_{H\text{-}H}$ = 7.5 Hz, H-4'), 4.19 (q, 4H, $^{3}J_{H\text{-}H}$ = 7.2 Hz, H-2').

^{13}C NMR (90 MHz, $CDCl_3$, 25 °C): δ 14.3 (C-1, C-1'), 22.9 (C-2), 27.5 (C-5), 28.9–29.8 (C-4), 32.1 (C-3), 52.3 (C-4'), 61.4 (C-2'), 169.8 (C-3').

5b(20):
Yield: 18.7 g (25.9 mmol), 37% of a colourless oil.



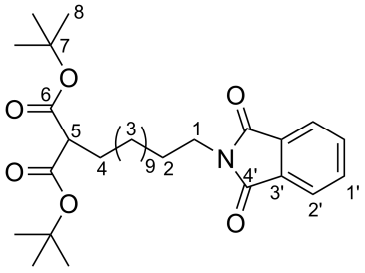
^{1}H NMR (360 MHz, $CDCl_3$, 25 °C): δ 0.88 (t, 6H, $^3J_{H-H}$ = 6.8 Hz, H-1), 1.25 (m, 78H, H-2, H-3, H-4, H-5, H-1'), 1.85 (m, 4H, H-6), 4.17 (q, 4H, $^3J_{H-H}$ = 7.1 Hz, H-2').

^{13}C NMR (90 MHz, $CDCl_3$, 25 °C): δ 14.3 (C-1, C-1'), 22.8 (C-2), 24.1 (C-6), 29.5–29.9 (C-4), 32.1 (C-3), 32.3 (C-5), 57.7 (C-4'), 61.0 (C-2'), 172.2 (C-3').

2-(12-phthalimido dodecyl) propane dioic acid di-*tert*-butyl ester [5c(12)]:
Malonic acid di-*tert*-butyl ester (10.0 mL, 43.8 mmol) and sodium hydride in mineral oil (55 % w/w) (1.91 g, 43.8 mmol) were reacted in anhydrous DMF (70 mL) for 5 h at room temperature according to general procedure B2. **3c(12)** (17.3 g, 43.8 mmol) was added to the mixture and the reaction was stirred at room temperature o/n according to procedure B. The reaction mixture was separated into two equal portions.

One portion was worked up with diethyl ether as described (general procedure B). Column chromatography (cyclohexane / acetone 19:1 + 0.1% (v/v) triethylamine) gave **5c(12)**.

Yield: 8.45 g (16.0 mmol), 36% of a colourless oil.

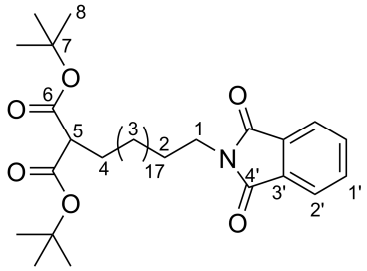


^{1}H NMR (360 MHz, $CDCl_3$, 25 °C): δ 1.24 (m, 18H, H-3), 1.45 (s, 18 H, H-8), 1.65 (m, 2H, H-2), 1.78 (m, 2H, H-4), 3.10 (t, 1H, $^3J_{H-H}$ = 7.6 Hz, H-5), 3.67 (t, 2H, $^3J_{H-H}$ = 7.4 Hz, H-1), 7.70 (m, 2H, H-1'), 7.83 (m, 2H, H-2').

^{13}C NMR (90 MHz, $CDCl_3$, 25 °C): δ 27.0 (C-2), 27.4 (C-4), 28.1 (C-8), 28.8–29.7 (C-3), 38.2 (C-1), 54.2 (C-5), 81.2 (C-7), 123.3 (C-2'), 132.4 (C-3'), 133.9 (C-1'), 168.6 (C-4'), 169.2 (C-6).

2-(20-phthalimido eicosyl) propane dioic acid di-*tert*-butyl ester [5c(20)]:
Malonic acid di-*tert*-butyl ester (6.56 mL, 29.3 mmol) and sodium hydride in mineral oil (55 % w/w) (1.28 g, 29.3 mmol) were reacted in anhydrous DMF (120 mL) for 50 min at room temperature according to general procedure B2. **3c(20)** (23.0 g, 45.4 mmol) was added to the mixture and the reaction mixture was stirred for 1 h at 75 °C bath temperature and at room temperature o/n. Additional sodium hydride in mineral oil (55 % (w/w), 708 mg, 16.2 mmol) was added and the reaction mixture was further stirred at room temperature for 7 h. The work-up was performed with diethyl ether as described (general procedure B). Column chromatography (cyclohexane → cyclohexane / acetone 9:1) gave **5c(20)**.

Yield: 12.5 g (19.5 mmol), 67% of a colourless oil.



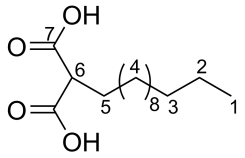
^{1}H NMR (360 MHz, $CDCl_3$, 25 °C): δ 1.24 (m, 34H, H-3), 1.45 (s, 18 H, H-8), 1.65 (m, 2H, H-2), 1.78 (m, 2H, H-4), 3.10 (t, 1H, $^{3}J_{H-H}$ = 7.6 Hz, H-5), 3.67 (t, 2H, $^{3}J_{H-H}$ = 7.2 Hz, H-1), 7.70 (m, 2H, H-1'), 7.84 (m, 2H, H-2').

^{13}C NMR (90 MHz, $CDCl_3$, 25 °C): δ 27.0 (C-2), 27.4 (C-4), 28.1 (C-8), 28.8–29.9 (C-3), 38.3 (C-1), 54.2 (C-5), 81.3 (C-7), 123.3 (C-2'), 132.3 (C-3'), 134.0 (C-1'), 168.6 (C-4'), 169.2 (C-6).

2-dodecyl propane dioic acid [6a(12)]:

Following general procedure C, a solution of potassium hydroxide (4.93 g, 87.8 mmol) in ddH_2O (6 mL) and ethanol (12 mL) was reacted with **5a(12)** (4.12 g, 12.6 mmol) for 6 h and the mixture was processed accordingly.

Yield: 3.35 g (12.3 mmol), 98 % of a colourless crystalline solid.



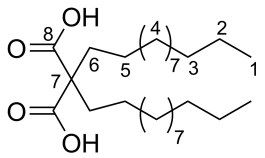
^{1}H NMR (360 MHz, $CDCl_3$+DMSO-d_6, 25 °C): δ 0.83 (t, 3H, $^{3}J_{H-H}$ = 6.7 Hz, H-1), 1.20 (m, 20H, H-2, H-3, H-4), 1.86 (m, 2H, H-5), 3.25 (t, 1H, $^{3}J_{H-H}$ = 7.2 Hz, H-6).

^{13}C NMR (90 MHz, $CDCl_3$+DMSO-d_6, 25 °C): δ 14.1 (C-1), 22.7 (C-2), 27.4 (C-5), 29.4–29.7 (C-4), 31.9 (C-3), 51.7 (C-6), 172.3 (C-7).

2,2'-bisdodecyl propane dioic acid [6b(12)]:

Following general procedure C, a solution of potassium hydroxide (0.82 g, 15 mmol) in ddH_2O (1 mL) and ethanol (2 mL) was reacted with **5b(12)** (1.81 g, 3.64 mmol) for 5.5 h and the mixture was processed accordingly.

Yield: 1.56 g (3.54 mmol), 97 % of a colourless crystalline solid.



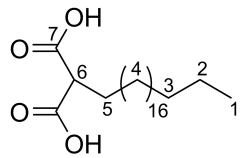
^{1}H NMR (360 MHz, $CDCl_3$, 25 °C): δ 0.88 (t, 6H, $^{3}J_{H-H}$ = 7.0 Hz, H-1), 1.25 (m, 40H, H-2, H-3, H-4, H-5), 1.93 (m, 4H, H-6).

^{13}C NMR (90 MHz, $CDCl_3$, 25 °C): δ 14.3 (C-1), 22.8 (C-2), 24.9 (C-6), 29.4–29.8 (C-4), 32.1 (C-3), 35.6 (C-5), 57.9 (C-7), 177.3 (C-8).

2-eicosyl propane dioic acid [6a(20)]:

Following general procedure C, a solution of potassium hydroxide (7.62 g, 136 mmol) in ddH_2O (10 mL) and ethanol (20 mL) was reacted with **5a(20)** (8.56 g, 19.4 mmol) for 5.5 h and the mixture was processed accordingly.

Yield: 6.68 g (17.4 mmol), 90 % of a colourless crystalline solid.



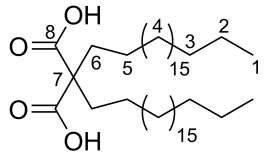
^{1}H NMR (360 MHz, $CDCl_3$+DMSO-d_6, 25 °C): δ 0.76 (t, 3H, $^{3}J_{H-H}$ = 6.8 Hz, H-1), 1.14 (m, 36H, H-2, H-3, H-4), 1.76 (m, 2H, H-5), 3.15 (t, 1H, $^{3}J_{H-H}$ = 7.4 Hz, H-6).

^{13}C NMR (90 MHz, $CDCl_3$+DMSO-d_6, 25 °C): δ 14.0 (C-1), 22.5 (C-2), 27.3 (C-5), 28.9–29.5 (C-4), 31.7 (C-3), 51.8 (C-6), 171.9 (C-7).

2,2'-biseicosyl propane dioic acid [6b(20)]:

Following general procedure C, a solution of potassium hydroxide (10.2 g, 182 mmol) in ddH_2O (13 mL) and ethanol (26 mL) was reacted with **5b(20)** (18.7 g, 26.0 mmol) for 4.5 h and the mixture was processed accordingly.

Yield: 9.66 g (14.5 mmol), 56 % of a colourless crystalline solid.



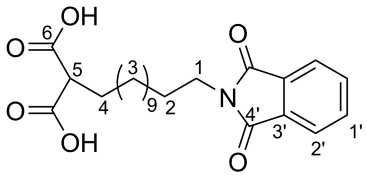
^{1}H NMR (360 MHz, $CDCl_3$+DMSO-d_6, 25 °C): δ 0.79 (t, 6H, $^3J_{H-H}$ = 6.7 Hz, H-1), 1.17 (m, 72H, H-2, H-3, H-4, H-5), 1.80 (m, 4H, H-6).

^{13}C NMR (90 MHz, $CDCl_3$+DMSO-d_6, 25 °C): δ 14.1 (C-1), 22.6 (C-2), 24.9 (C-6), 29.3–29.8 (C-4), 31.9 (C-3), 35.8 (C-5), 57.3 (C-7), 176.2 (C-8).

2-(12-phthalimido dodecyl) propane dioic acid [6c(12)]:

Following general procedure D, **5c(12)** (8.45 g, 16.0 mmol) and trifluoroacetic acid (11.9 mL, 160 mmol) were reacted in dichloromethane (12 mL) at room temperature and the mixture was processed accordingly.

Yield: 6.66 g (15.9 mmol), 100% of a colourless solid.



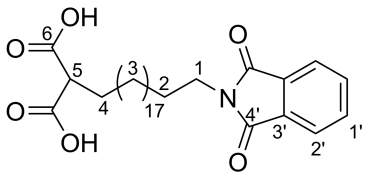
^{1}H NMR (360 MHz, $CDCl_3$, 25 °C): δ 1.25 (m, 18H, H-3), 1.66 (m, 2H, H-2), 1.96 (m, 2H, H-4), 3.45 (t, 1H, $^3J_{H-H}$ = 7.4 Hz, H-5), 3.68 (t, 2H, $^3J_{H-H}$ = 7.4 Hz, H-1), 7.70 (m, 2H, H-1'), 7.85 (m, 2H, H-2').

^{13}C NMR (90 MHz, $CDCl_3$, 25 °C): δ 26.9 (C-4), 27.2 (C-2), 28.7–29.6 (C-3), 38.3 (C-1), 51.3 (C-5), 123.4 (C-2'), 132.3 (C-3'), 134.1 (C-1'), 168.9 (C-4'), 174.1 (C-6).

2-(20-phthalimido eicosyl) propane dioic acid [6c(20)]:

Following general procedure D, **5c(20)** (12.5 g, 19.5 mmol) and trifluoroacetic acid (14.5 mL, 195 mmol) were reacted in dichloromethane (15 mL) at room temperature and the mixture was processed accordingly.

Yield: 10.3 g (19.5 mmol), 100% of a colourless amorphous solid.



^{1}H NMR (360 MHz, $CDCl_3$, 25 °C): δ 1.25 (m, 18H, H-3), 1.67 (m, 2H, H-2), 1.96 (m, 2H, H-4), 3.45 (t, 1H, $^3J_{H-H}$ = 7.4 Hz, H-5), 3.68 (t, 2H, $^3J_{H-H}$ = 7.2 Hz, H-1), 7.70 (m, 2H, H-1'), 7.85 (m, 2H, H-2').

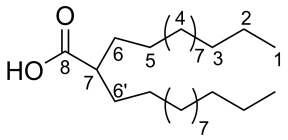
^{13}C NMR (90 MHz, $CDCl_3$, 25 °C): δ 27.0 (C-4), 27.4 (C-2), 28.8–29.8 (C-3), 38.3 (C-1), 51.2 (C-5), 123.4 (C-2'), 132.3 (C-3'), 134.1 (C-1'), 168.8 (C-4'), 173.9 (C-6).

2-dodecyl tetradecanoic acid [6e(12)]:

6b(12) (3.09 g, 7.01 mmol) was allowed to decarboxylate for 5 h at atmospheric pressure and 2.5 h at reduced pressure according to general procedure E.

Yield: 2.75 g (6.94 mmol), 99% of a colourless crystalline solid.

^{1}H NMR (360 MHz, $CDCl_3$, 25 °C): δ 0.88 (t, 6H, $^{3}J_{H\text{-}H}$ = 6.8 Hz, H-1), 1.26 (m, 40H, H-2, H-3, H-4, H-5), 1.46 (m, 2H, H-6), 1.61 (m, 2H, H-6'), 2.35 (m, 1H, H-7).



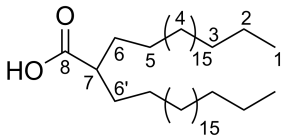
^{13}C NMR (90 MHz, $CDCl_3$, 25 °C): δ 14.3 (C-1), 22.8 (C-2), 27.5 (C-5), 29.4–29.8 (C-4), 32.1 (C-3), 32.3 (C-6/6'), 45.5 (C-7), 181.8 (C-8).

2-eicosyl docosanoic acid [6e(20)]:

6b(20) (3.99 g, 6.00 mmol) was allowed to decarboxylate for 2 h at atmospheric pressure and 2 h at reduced pressure according to general procedure E.

Yield: 3.73 g (6.00 mmol), 100% of a colourless crystalline solid.

^{1}H NMR (360 MHz, $CDCl_3$+DMSO-d_6, 25 °C): δ 0.70 (t, 6H, $^{3}J_{H\text{-}H}$ = 6.8 Hz, H-1), 1.08 (m, 40H, H-2, H-3, H-4, H-5), 1.24 (m, 2H, H-6), 1.40 (m, 2H, H-6'), 2.08 (m, 1H, H-7).



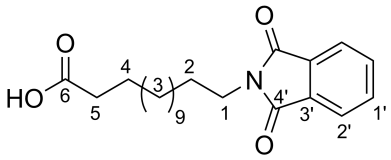
^{13}C NMR (90 MHz, $CDCl_3$+DMSO-d_6, 25 °C): δ 13.8 (C-1), 22.3 (C-2), 27.1 (C-5), 29.0–29.3 (C-4), 31.6 (C-3), 32.1 (C-6/6'), 45.3 (C-7), 178.3 (C-8).

14-phthalimido tetradecanoic acid [6f(12)]:

6c(12) (4.18 g, 10.0 mmol) was allowed to decarboxylate for 3 h at atmospheric pressure and 5 h at reduced pressure according to general procedure E.

Yield: 3.66 g (9.79 mmol), 98% of a colourless solid.

^{1}H NMR (360 MHz, $CDCl_3$+DMSO-d_6, 25 °C): δ 1.05 (m, 18H, H-3), 1.40 (m, 2H, H-4), 1.47 (m, 2H, H-2), 2.06 (t, 2H, $^{3}J_{H\text{-}H}$ = 7.6 Hz, H-5), 3.47 (t, 2H, $^{3}J_{H\text{-}H}$ = 7.2 Hz, H-1), 7.53 (m, 2H, H-1'), 7.64 (m, 2H, H-2').



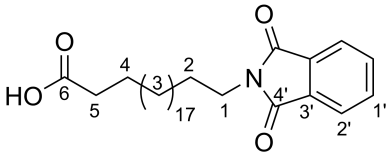
^{13}C NMR (90 MHz, $CDCl_3$+DMSO-d_6, 25 °C): δ 24.6 (C-4), 26.5 (C-2), 28.2–29.2 (C-3), 33.9 (C-5), 37.7 (C-1), 122.8 (C-2'), 131.8 (C-3'), 133.6 (C-1'), 168.0 (C-4'), 175.6 (C-6).

22-phthalimido docosanoic acid [6f(20)]:

6c(20) (4.77 g, 9.00 mmol) was allowed to decarboxylate for 70 min at atmospheric pressure and 1 h at reduced pressure and processed according to general procedure E.

Yield: 4.32 g (8.89 mmol), 99% of a colourless solid.

^{1}H NMR (360 MHz, $CDCl_3$, 25 °C): δ 1.24 (m, 34H, H-3), 1.67 (m, 4H, H-2, H-4), 2.35 (t, 2H, $^{3}J_{H\text{-}H}$ = 7.4 Hz, H-5), 3.67 (t, 2H, $^{3}J_{H\text{-}H}$ = 7.4 Hz, H-1), 7.70 (m, 2H, H-1'), 7.84 (m, 2H, H-2').



^{13}C NMR (90 MHz, $CDCl_3$, 25 °C): δ 24.8 (C-4), 27.0 (C-2), 28.8–29.8 (C-3), 34.0 (C-5), 38.3 (C-1), 123.3 (C-2'), 132.4 (C-3'), 134.0 (C-1'), 168.6 (C-4'), 178.9 (C-6).

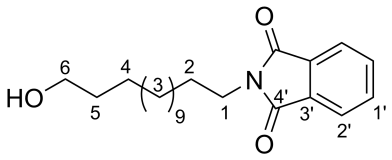
***N*-(14-hydroxy tetradecyl) phthalimide [6g(12)]:**

Following general procedure F, **6f(12)** (2.90 g, 7.76 mmol) and thionyl chloride (11.3 mL, 155 mmol) were reacted for 5 h and the mixture was processed accordingly.

Following general procedure G, a solution of the crude acid chloride (2.26 g, 5.76 mmol) in anhydrous THF (50 mL) and lithium tri(*tert*-butoxy) aluminium hydride (3.46 g, 13.6 mmol) were reacted at 0 °C and for 80 min at room temperature, and the mixture was processed accordingly. Column chromatography (cyclohexane / acetone 3:1) gave **6g(12)**.

Yield: 1.46 g (4.40 mmol), 76% (over two steps) of a colourless crystalline solid.

^{1}H NMR (360 MHz, $CDCl_3$, 25 °C): δ 1.25 (m, 20H, H-3, H-4), 1.56 (m, 2H, H-5), 1.67 (m, 2H, H-2), 3.64 (t, 2H, $^{3}J_{H\text{-}H}$ = 6.7 Hz, H-6), 3.67 (t, 2H, $^{3}J_{H\text{-}H}$ = 7.2 Hz, H-1), 7.70 (m, 2H, H-1'), 7.84 (m, 2H, H-2').



^{13}C NMR (90 MHz, $CDCl_3$, 25 °C): δ 25.9 (C-4), 27.0 (C-2), 28.8–29.7 (C-3), 33.0 (C-5), 38.3 (C-1), 63.3 (C-6), 123.3 (C-2'), 132.4 (C-3'), 134.0 (C-1'), 168.6 (C-4').

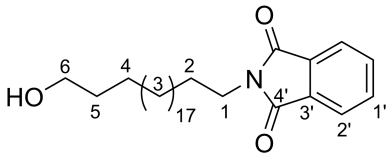
***N*-(22-hydroxy docosyl) phthalimide [6g(20)]:**

Following general procedure F, **6f(20)** (4.32 g, 8.89 mmol) and thionyl chloride (13.2 mL, 180 mmol) were reacted for 7 h and the mixture was processed accordingly.

Following general procedure G, a solution of the crude acid chloride (2.46 g, 4.88 mmol) in anhydrous THF (50 mL) and lithium tri(*tert*-butoxy) aluminium hydride (2.93 g, 11.5 mmol) were reacted at 0 °C and for 2 h at room temperature and the mixture was processed accordingly. Column chromatography (dichloromethane / methanol 95:5) gave **6g(20)**.

Yield: 1.62 g (3.43 mmol), 70% (over two steps) of a colourless crystalline solid.

^{1}H NMR (360 MHz, $CDCl_3$, 25 °C): δ 1.25 (m, 20H, H-3, H-4), 1.56 (m, 2H, H-5), 1.67 (m, 2H, H-2), 3.64 (t, 2H, $^{3}J_{H\text{-}H}$ = 6.5 Hz, H-6), 3.67 (t, 2H, $^{3}J_{H\text{-}H}$ = 7.2 Hz, H-1), 7.70 (m, 2H, H-1'), 7.84 (m, 2H, H-2').



Assembly of Building Blocks and Synthesis of Target Structures

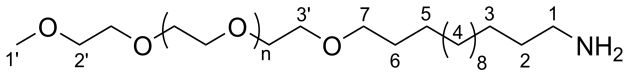
14-(α-methoxy[poly(ethylene oxy)] tetradecane-1-amine (average molecular mass ~760 Da) [7g(550,12)]:

According to general procedure I, **6g(12)** (663 mg, 2.00 mmol) was reacted with trifluoromethane sulfonic acid anhydride (0.45 mL, 2.7 mmol) in dichloromethane (10 mL) for 35 min. At the same time 2-(α-methoxy[poly(ethylene oxy)]) ethanol (average molecular mass ~550 Da, 1.21 g, 2.20 mmol) was reacted with anhydrous potassium carbonate (335 mg, 2.42 mmol) and with sodium hydride in mineral oil (55 % w/w, 94 mg, 2.2 mmol) in anhydrous dimethoxyethane (10 mL) for 50 min. The dichloromethane solution was worked up, the resulting residue was dissolved in anhydrous dimethoxyethane (10 mL), added to the 2-(α-methoxy[poly(ethylene oxy)]) ethanolate solution, and the mixture was reacted o/n and

processed, all according to general procedure I. The crude product was purified by column chromatography (dichloromethane/methanol 95:5).

The resulting product was treated with hydrazine monohydrate (0.23 mL, 4.7 mmol) for 1 d, and the reaction mixture was processed, both according to general procedure J.

Yield: 354 mg (0.465 mmol), 23% of a light-yellow oil.



^{1}H-NMR (360 MHz, $CDCl_3$): δ 1.25 (m, 20H, H-3, H-4, H-5), 1.56 (m, 4H, H-2, H-6), 2.79 (m, 2H, H-1), 3.37 (s, 3H, H-1'), 3.44 (m, 2H, H-7), 3.64 (m, polymer backbone).

^{13}C-NMR (90 MHz, $CDCl_3$): δ 26.2 (C-5), 26.9 (C-3), 28.8 - 29.7 (C-4, C-6), 30.9 (C-2), 41.3 (C-1), 59.2 (C-1'), 70.2 (C-3'), 70.4 - 70.8 (polymer backbone), 71.6 (C-7), 72.1 (C-2').

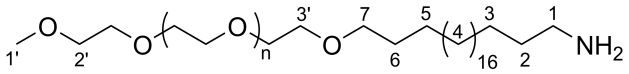
MALDI-TOF: 772.3 (596.3 - 948.4) $[M+H]^+$

22-(α-methoxy[poly(ethylene oxy)] docosane-1-amine (average molecular mass ~870 Da) [7g(550,20)]:

According to general procedure I, **6g(20)** (943 mg, 2.00 mmol) was reacted with trifluoromethane sulfonic acid anhydride (0.40 mL, 2.4 mmol) in dichloromethane (25 mL) for 1.5 h. At the same time 2-(α-methoxy[poly(ethylene oxy)]) ethanol (average molecular mass ~550 Da, 1.16 g, 2.10 mmol) was reacted with anhydrous potassium carbonate (498 mg, 3.60 mmol) and with sodium hydride in mineral oil (55 % w/w, 60 mg, 1.4 mmol) in anhydrous dimethoxyethane (15 mL) for 1.5 h. The dichloromethane solution was worked up, the resulting residue was dissolved in anhydrous dimethoxyethane (10 mL), added to the 2-(α-methoxy[poly(ethylene oxy)]) ethanolate solution, and the mixture was reacted for 4 d and processed, all according to general procedure I. The crude product was purified by column chromatography (dichloromethane/methanol 9:1).

The resulting product was treated with hydrazine monohydrate (0.49 mL, 9.9 mmol) o/n, and the reaction mixture was processed, both according to general procedure J.

Yield: 249 mg (0.285 mmol), 14% of a light-yellow oil.



^{1}H-NMR (360 MHz, $CDCl_3$, protonated form): δ 1.25 (m, 36H, H-3, H-4, H-5), 1.57 (m, 2H, H-6), 1.70 (m, 2H, H-2), 2.94 (m, 2H, H-1), 3.38 (s, 3H, H-1'), 3.45 (m, 2H, H-7), 3.65 (m, polymer backbone).

^{13}C-NMR (90 MHz, $CDCl_3$, protonated form): δ 26.2 (C-5), 26.8 (C-3), 27.7 (C-2), 28.8 - 29.7 (C-4, C-6), 40.3 (C-1), 59.1 (C-1'), 70.1 (C-3'), 70.6 - 70.7 (polymer backbone), 71.7 (C-7), 72.0 (C-2').

MALDI-TOF: 884.9 (620.8 - 1149.0) $[M+H]^+$, 906.9 (686.8 - 1171.0) $[M+Na]^+$

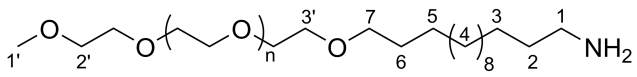
14-(α-methoxy[poly(ethylene oxy)] tetradecane-1-amine (average molecular mass ~2.21 kDa) [7g(2000,12)]:

According to general procedure I, **6g(12)** (663 mg, 2.00 mmol) was reacted with trifluoromethane sulfonic acid anhydride (0.42 mL, 2.5 mmol) in dichloromethane (10 mL) for 70 min. At the same time 2-(α-methoxy[poly(ethylene oxy)]) ethanol (average molecular mass ~2.0 kDa, 4.40 g, 2.20 mmol) was reacted with anhydrous potassium carbonate (338 mg, 2.45 mmol) and with sodium hydride in mineral oil (55 % w/w, 94 mg, 2.2 mmol) in dimethoxyethane (15 mL) for 50 min. The dichloromethane solution was worked up, the resulting residue was dissolved in anhydrous dimethoxyethane (10 mL), added to the 2-(α-methoxy[poly(ethylene oxy)]) ethanolate solution, and the mixture was reacted for 5 d and processed, all according to general procedure I. The crude product was purified by column chromatography (dichloromethane/methanol 9:1).

The resulting product was treated with hydrazine monohydrate (0.79 mL, 16 mmol) o/n, and the reaction mixture was processed, both according to general procedure J.

Yield: 1.10 g (0.497 mmol), 25% of a light-yellow crystalline solid.

^{1}H-NMR (360 MHz, $CDCl_3$, protonated form): δ 1.26 (m, 20H, H-3, H-4, H-5), 1.56 (m, 2H, H-6), 1.67 (m, 2H, H-2), 2.91 (m, 2H, H-1), 3.37 (s, 3H, H-1'), 3.45 (m, 2H, H-7), 3.63 (m, polymer backbone).



^{13}C-NMR (90 MHz, $CDCl_3$, protonated form): δ 26.1 (C-5), 26.8 (C-3), 27.7 (C-2), 29.1 - 29.7 (C-4, C-6), 40.3 (C-1), 59.2 (C-1'), 70.2 (C-3'), 70.4 - 70.7 (polymer backbone), 71.6 (C-7), 72.1 (C-2').

MALDI-TOF: 2138.0 (1608.7 - 2666.4) $[M+H]^+$, 2160.0 (1762.7 - 2688.4) $[M+Na]^+$

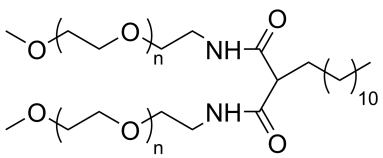
***N,N'*-di(α-methoxy[poly(ethylene oxy)] ethyl)-2-dodecyl propane-1,3-diamide (average molecular mass ~1.33 kDa) [7a(550,12)]:**

Following general procedure F, **6a(12)** (1.09 g, 4.00 mmol) and thionyl chloride (11.7 mL, 160 mmol) were reacted for 5 h and the mixture was processed accordingly.

Following general procedure H, the crude malonic acid chloride was reacted with **4d(550)** (4.39 g, 8.01 mmol) in toluene (20 mL) for 30 min and with triethylamine (1.1 mL, 7.9 mmol) for 1 d and the mixture was processed accordingly.

Yield: 3.42 g (2.56 mmol) 64% of a light-yellow amorphous solid.

The crude product was used in the following step without further purification.

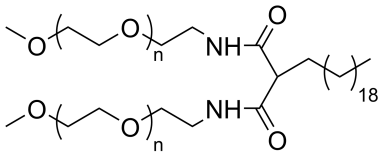


***N,N'*-di(α-methoxy[poly(ethylene oxy)] ethyl)-2-eicosyl propane-1,3-diamide (average molecular mass ~1.45 kDa) [7a(550,20)]:**

Following general procedure F, **6a(20)** (1.15 g, 2.99 mmol) and thionyl chloride (8.75 mL, 120 mmol) were reacted for 5 h and the mixture was processed accordingly.

Following general procedure H, the crude malonic acid chloride was reacted with **4d(550)** (3.30 g, 6.0 mmol) in toluene (15 mL) for 30 min and with triethylamine (0.84 mL, 6.0 mmol) o/n and the mixture was processed accordingly.

Yield: 3.19 g (2.21 mmol), 74% of a light-yellow amorphous solid.



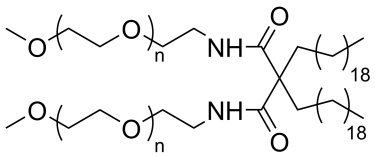
The crude product was used in the following step without further purification.

***N,N'*-di(α-methoxy[poly(ethylene oxy)] ethyl)-2,2'-dieicosyl propane-1,3-diamide (average molecular mass ~1.73 kDa) [7b(550,20)]:**

Following general procedure F, **6b(20)** (1.33 g, 2.03 mmol) and thionyl chloride (5.8 mL, 80 mmol) were reacted for 5 h and the mixture was processed accordingly.

Following general procedure H, the crude malonic acid chloride was reacted with **4d(550)** (2.20 g, 4.01 mmol) in toluene (20 mL) for 5 min and with triethylamine (0.56 mL, 4.0 mmol) o/n and the mixture was processed accordingly.

Yield: 2.71 g (1.57 mol), 78% of a colourless amorphous solid.



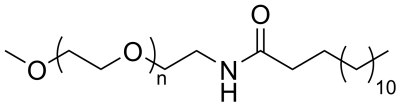
The crude product was used in the following step without further purification.

***N*-(α-methoxy[poly(ethylene oxy)] ethyl) tetradecanamide (average molecular mass ~760 Da) [7d(550,12)]:**

Following general procedure F, myrisitic acid (942 mg, 4.00 mmol) and thionyl chloride (5.8 mL, 80 mmol) were reacted for 3.5 h and the mixture was processed accordingly.

Following general procedure H, the crude acid chloride (0.49 g, 2.0 mmol) was reacted with **4d(550)** (1.10 g, 2.00 mmol) in toluene (15 mL) for 40 min and with triethylamine (0.28 mL, 2.0 mmol) for 2 d and the mixture was processed accordingly.

Yield: 1.36 g (1.79 mmol), 89% (over two steps) of a colourless solid.



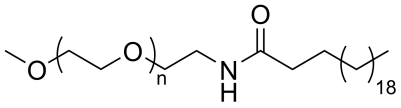
The crude product was used in the following step without further purification.

***N*-(α-methoxy[poly(ethylene oxy)] ethyl) docosanamide (average molecular mass ~870 Da) [7d(550,20)]:**

Following general procedure F, behenic acid (1.08 g, 3.01 mmol) and thionyl chloride (4.4 mL, 60 mmol) were reacted for 5 h and the mixture was processed accordingly.

Following general procedure H, the crude acid chloride was reacted with 4**d(550)** (1.6 g, 3.0 mmol) in toluene (5 mL) for 30 min and with triethylamine (0.42 mL, 3.0 mmol) for 4 d and the mixture was processed accordingly.

Yield: 2.05 g (2.40 mmol), 80% of a colourless solid.



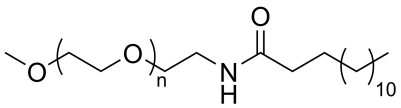
The crude product was used in the following step without further purification.

***N*-(α-methoxy[poly(ethylene oxy)] ethyl) tetradecanamide (average molecular mass ~2.21 kDa) [7d(2000,12)]:**

Following general procedure F, myrisitic acid (942 mg, 4.00 mmol) and thionyl chloride (5.8 mL, 80 mmol) were reacted for 3.5 h and the mixture was processed accordingly.

Following general procedure H, the crude acid chloride (0.49 g, 2.0 mmol) was reacted with **4d(2000)** (4.00 g, 2.00 mmol) in toluene (15 mL) for 40 min and with triethylamine (0.28 mL, 2.0 mmol) for 2 d and the mixture was processed accordingly.

Yield: 3.828 g (1.73 mmol), 87% (over two steps) of a colourless solid.



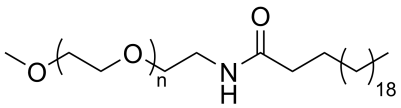
The crude product was used in the following step without further purification.

***N*-(α-methoxy[poly(ethylene oxy)] ethyl) docosanamide (average molecular mass ~2.32 kDa) [7d(2000,20)]:**

Following general procedure F, behenic acid (1.44 g, 4.01 mmol) and thionyl chloride (5.8 mL, 80 mmol) were reacted for 1.5 h and the mixture was processed accordingly.

Following general procedure H, the crude acid chloride (0.72 g, 2.0 mmol) was reacted with **4d(2000)** (4.00 g, 2.00 mmol) in toluene (10 mL) for 40 min and with triethylamine (0.28 mL, 2.0 mmol) for 1 d and the mixture was processed accordingly.

Yield: 2.45 g (1.05 mmol), 52% (over two steps) of a colourless solid.



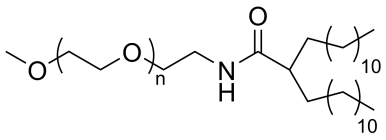
The crude product was used in the following step without further purification.

***N*-(α-methoxy[poly(ethylene oxy)] ethyl)-2-dodecyl tetradecanamide (average molecular mass ~930 Da) [7e(550,12)]:**

Following general procedure F, **6e(12)** (793 mg, 2.00 mmol) and thionyl chloride (2.95 mL, 40.4 mmol) were reacted for 5 h and the mixture was processed accordingly.

Following general procedure H, the crude acid chloride was reacted with **4d(550)** (1.10 g, 2.00 mmol) in toluene (7 mL) for 20 min and with triethylamine (0.28 mL, 2.0 mmol) for 1 d and the mixture was processed accordingly.

Yield: 1.66 g (1.79 mmol), 90% of a colourless amorphous solid.



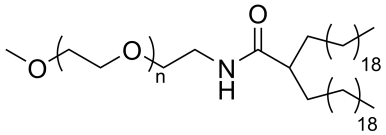
The crude product was used in the following step without further purification.

***N*-(α-methoxy[poly(ethylene oxy)] ethyl)-2-eicosyl docosanamide (average molecular mass ~1.15 kDa) [7e(550,20)]:**

Following general procedure F, **6e(20)** (1.25 g, 2.01 mmol) and thionyl chloride (2.95 mL, 40.4 mmol) were reacted for 5 h and the mixture was processed accordingly.

Following general procedure H, the crude acid chloride was reacted with **4d(550)** (1.10 g, 2.00 mmol) in toluene (5 mL) for 1 h and with triethylamine (0.28 mL, 2.0 mmol) for 1 d and the mixture was processed accordingly.

Yield: 2.06 g (1.79 mmol), 89% of a colourless amorphous solid.



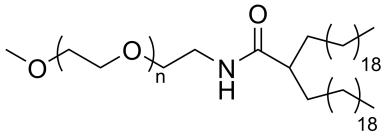
The crude product was used in the following step without further purification.

***N*-(α-methoxy[poly(ethylene oxy)] ethyl)-2-eicosyl docosanamide (average molecular mass ~2.60 kDa) [7e(2000,20)]:**

Following general procedure F, **6e(20)** (1.25 g, 2.00 mmol) and thionyl chloride (2.92 mL, 40.0 mmol) were reacted for 6.5 h and the mixture was processed accordingly.

Following general procedure H, the crude acid chloride was reacted with **4d(2000)** (4.00 g, 2.00 mmol) in toluene (10 mL) for 30 min and with triethylamine (0.28 mL, 2.0 mmol) for 2 d and the mixture was processed accordingly.

Yield: 3.97 g (1.53 mmol), 76% of a colourless amorphous solid.



The crude product was used in the following step without further purification.

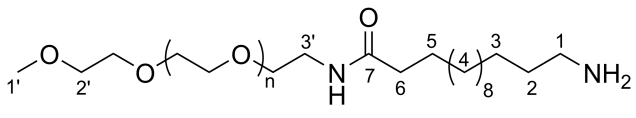
***N*-(α-methoxy[poly(ethylene oxy)] ethyl)-14-amino tetradecanamide (average molecular mass ~770 Da) [7f(550,12)]:**

Following general procedure F, **6f(12)** (750 mg, 2.01 mmol) and thionyl chloride (2.95 mL, 40.4 mmol) were reacted for 7.5 h the mixture was and processed accordingly.

Following general procedure H, the crude acid chloride was reacted with **4d(550)** (1.10 g, 2.00 mmol) in toluene (10 mL) for 20 min and with triethylamine (0.28 mL, 2.0 mmol) for 2 d and the mixture was processed accordingly. The resulting product was treated with hydrazine monohydrate (0.72 mL, 15 mmol) for 1 d, and the reaction mixture was processed, both according to general procedure J.

Yield: 1.12 g (1.45 mmol), 72% of a light-yellow amorphous solid.

1H-NMR (360 MHz, $CDCl_3$): δ 1.26 (m, 18H, H-3, H-4), 1.54 (m, 2H, H-2), 1.60



(m, 2H, H-5), 2.16 (t, 2H, $^3J_{H-H}$ = 7.6 Hz, H-6), 2.77 (t, 2H, $^3J_{H-H}$ = 7.6 Hz, H-1), 3.36 (s, 3H), 3.43 (m, 2H, H-3'), 3.64 (m, polymer backbone).

13C-NMR (90 MHz, $CDCl_3$): δ 25.8 (C-5), 26.9 (C-3), 29.4 - 29.5 (C-4), 36.8 (C-6), 39.2 (C-3'), 41.3 (C-1), 59.1 (C-1'), 70.1 - 70.6 (polymer backbone), 72.0 (C-2'), 173.5 (C-7).

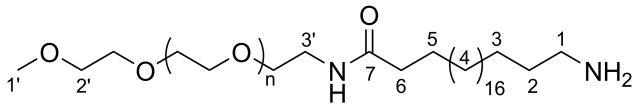
MALDI-TOF: 785.6 (564.8 - 961.7) $[M+H]^+$

***N*-(α-methoxy[poly(ethylene oxy)] ethyl)-22-amino docosanamide (average molecular mass ~890 Da) [7f(550,20)]:**

Following general procedure F, **6f(20)** (4.32 g, 8.89 mmol) and thionyl chloride (13.2 mL, 180 mmol) were reacted for 7 h and the mixture was processed accordingly.

Following general procedure H, the crude acid chloride (1.01 g, 2.00 mmol) was reacted with **4d(550)** (1.10 g, 2.00 mmol) in toluene (10 mL) for 10 min and with triethylamine (0.28 mL, 2.0 mmol) for 4 d and the mixture was processed accordingly. The resulting product was treated with hydrazine monohydrate (0.71 mL, 15 mmol) for 1 d, and the reaction mixture was processed, both according to general procedure J.

Yield: 1.25 g (1.40 mmol), 70% (over two steps) of a light-yellow amorphous solid.



1H-NMR (360 MHz, $CDCl_3$, protonated form): δ 1.24 (m, 34H, H-3, H-4), 1.61 (m, 2H, H-5), 1.68 (m, 2H, H-2), 2.17 (m, 2H, H-6), 2.89 (m, 2H, H-1), 3.37 (s, 3H), 3.44 (m, 2H, H-3'), 3.65 (m, polymer backbone).

13C-NMR (90 MHz, $CDCl_3$, protonated form): δ 25.9 (C-5), 26.8 (C-3), 27.1 (C-2), 29.3 – 30.4 (C-4), 36.8 (C-6), 39.3 (C-3'), 40.3 (C-1), 59.2 (C-1'), 70.1 - 70.7 (polymer backbone), 72.1 (C-2'), 173.6 (C-7).

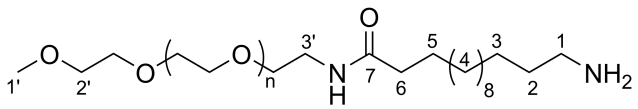
MALDI-TOF: 897.7 (677.7 - 1073.8) $[M+H]^+$

***N*-(α-methoxy[poly(ethylene oxy)] ethyl)-14-amino tetradecanamide (average molecular mass ~2.22 kDa) [7f(2000,12)]:**

Following general procedure F, **6f(12)** (2.90 g, 7.76 mmol) and thionyl chloride (11.3 mL, 155 mmol) were reacted for 5 h and the mixture was processed accordingly.

Following general procedure H, the crude acid chloride (785 mg, 2.00 mmol) was reacted with **4d(2000)** (4.00 g, 2.00 mmol) in toluene (10 mL) for 40 min and with triethylamine (0.28 mL, 2.0 mmol) for 4 d and the mixture was processed accordingly. The resulting product was treated with hydrazine monohydrate (0.63 mL, 13 mmol) for 1 d, and the reaction mixture was processed, both according to general procedure J.

Yield: 2.87 g (1.29 mmol), 64% (over two steps) of a light-yellow amorphous solid.



1H-NMR (360 MHz, $CDCl_3$): δ 1.24 (m, 18H, H-3, H-4), 1.48 (m, 2H, H-2), 1.60 (m, 2H, H-5), 2.16 (t, 2H, $^3J_{H-H}$ = 7.6 Hz,

H-6), 2.72 (t, 2H, $^{3}J_{H-H}$ = 7.4 Hz, H-1), 3.36 (s, 3H), 3.43 (m, 2H, H-3'), 3.63 (m, polymer backbone).

13C-NMR (90 MHz, $CDCl_3$): δ 25.8 (C-5), 27.0 (C-3), 29.3 - 29.6 (C-4), 36.8 (C-6), 39.3 (C-3'), 41.8 (C-1), 59.1 (C-1'), 69.7 - 70.7 (polymer backbone), 72.1 (C-2'), 173.3 (C-7).

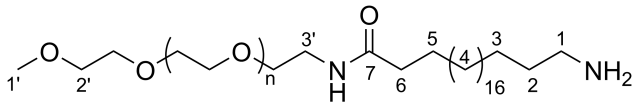
MALDI-TOF: 2371.4 (2063.2 - 2856.1) $[M+H]^+$

***N*-(α-methoxy[poly(ethylene oxy)] ethyl)-22-amino docosanamide (average molecular mass ~2.34 kDa) [7f(2000,20)]:**

Following general procedure F, **6f(20)** (4.32 g, 8.89 mmol) and thionyl chloride (13.2 mL, 180 mmol) were reacted for 7 h and the mixture was processed accordingly.

Following general procedure H, the crude acid chloride (1.01 g, 2.00 mmol) was reacted with **4d(2000)** (4.00 g, 2.00 mmol) in toluene (10 mL) for 10 min and with triethylamine (0.28 mL, 2.0 mmol) for 4 d and the mixture was processed accordingly. The resulting product was treated with hydrazine monohydrate (0.67 mL, 14 mmol) for 1 d, and the reaction mixture was processed, both according to general procedure J.

Yield: 2.69 g (1.15 mmol), 58% (over two steps) of a light-yellow amorphous solid.



1H-NMR (360 MHz, $CDCl_3$, protonated form): δ 1.24 (m, 34H, H-3, H-4), 1.60 (m, 2H, H-5), 1.70 (m, 2H, H-2), 2.16 (m, 2H, H-6), 2.90 (m, 2H, H-1), 3.36 (s, 3H), 3.43 (m, 2H, H-3'), 3.63 (m, polymer backbone).

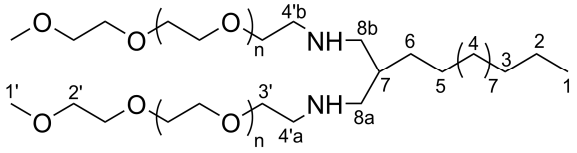
13C-NMR (90 MHz, $CDCl_3$, protonated form): δ 25.9 (C-5), 26.6 (C-3), 27.7 (C-2), 29.1 – 29.8 (C-4), 36.8 (C-6), 39.3 (C-3'), 40.3 (C-1), 59.1 (C-1'), 70.1 - 70.7 (polymer backbone), 72.1 (C-2'), 173.5 (C-7).

MALDI-TOF: 2439.2 (2219.2 - 2880.4) $[M+H]^+$

***N,N'*-di(α-methoxy[poly(ethylene oxy)] ethyl)-2-dodecyl propane-1,3-diamine (average molecular mass ~1.31 kDa) [8a(550,12)]:**

A solution of **7a(550,12)** (3.42g, 2.56 mmol) in anhydrous THF **(**25 mL) and a 1 M solution of borane in THF (12.0 mL, 12.0 mmol) were reacted o/n and the mixture was processed, both according to general procedure K. The crude product was purified by column chromatography (Sephadex LH20, ethanol).

Yield: 1.50 g (1.15 mmol), 45% of a light-yellow amorphous solid.



^{1}H-NMR (360 MHz, $CDCl_3$, protonated form): δ 0.87 (t, 3H, H-1), 1.25 (m, 22H, H-2, H-3, H-4, H-5, H-6), 2.63 (m, 2H, H-8a), 2.85 (m, 2H, H-8b), 3.02 (m, 2H, H-4'a), 3.10 (m, 2H, H-4'b), 3.37 (s, 6H, H-1'), 3.64 (m, polymer backbone).

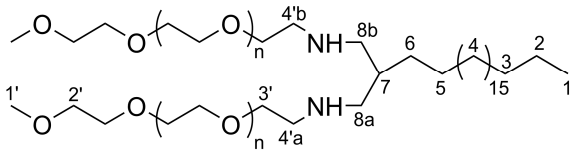
^{13}C-NMR (90 MHz, $CDCl_3$, protonated form): δ 14.3 (C-1), 22.8 (C-2), 26.9 (C-5), 29.5 - 29.9 (C-4), 31.5 (C-6), 32.1 (C-3), 33.8 (C-7), 48.1 (C-4'), 55.1 (C-8), 59.2 (C-1'), 68.0 (C-3'), 70.4 - 70.7 (polymer backbone), 72.1 (C-2').

MALDI-TOF: 1371.8 (1195.7-1591.9) $[M+H]^+$

***N,N'*-di(α-methoxy[poly(ethylene oxy)] ethyl)-2-eicosyl propane-1,3-diamine (average molecular mass ~1.42 kDa) [8a(550,20)]:**

A solution of **7a(550,20)** (3.19 g, 2.20 mmol) in anhydrous THF **(**20 mL) and a 1 M solution of borane in THF (10.3 mL, 10.3 mmol) were reacted o/n and the mixture was processed, both according to general procedure K. The crude product was purified by column chromatography (Sephadex LH20, ethanol).

Yield: 1.98 g (1.40 mmol), 63% of a light-yellow amorphous solid.



^{1}H-NMR (360 MHz, $CDCl_3$, protonated form): δ 0.88 (t, 3H, H-1), 1.25 (m, 38H, H-2, H-3, H-4, H-5, H-6), 2.64 (m, 2H, H-8a), 2.86 (m, 2H, H-8b), 3.02 (m, 2H, H-4'a), 3.12 (m, 2H, H-4'b), 3.37 (s, 6H, H-1'), 3.63 (m, polymer backbone).

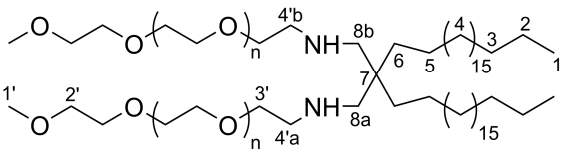
^{13}C-NMR (90 MHz, $CDCl_3$, protonated form): δ 14.3 (C-1), 22.8 (C-2), 26.9 (C-5), 29.4 – 30.0 (C-4), 31.5 (C-6), 32.1 (C-3), 33.8 (C-7), 48.1 (C-4'), 55.1 (C-8), 59.2 (C-1'), 68.0 (C-3'), 70.4 - 70.7 (polymer backbone), 72.1 (C-2').

MALDI-TOF: 1351.8 (1087.8 - 1660.0) $[M+H]^+$

***N,N'*-di(α-methoxy[poly(ethylene oxy)] ethyl)-2,2'-dieicosyl propane-1,3-diamine (average molecular mass ~1.70 kDa) [8b(550,20)]:**

A solution of **7b(550,20)** (2.71 g, 1.57 mmol) in anhydrous THF **(**20 mL) and a 1 M solution of borane in THF (8.0 mL, 8.0 mmol) were reacted for 1 d and the mixture was processed, both according to general procedure K. The product was obtained in sufficient purity.

Yield: 2.63 g (1.55 mmol), 99% of a colourless amorphous solid.



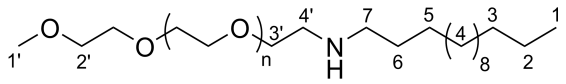
^{1}H-NMR (360 MHz, $CDCl_3$, protonated form): δ 0.87 (t, 3H, H-1), 1.25 (m, 76H, H-2, H-3, H-4, H-5, H-6), 2.83 (m, 2H, H-8a), 2.93 (m, 2H, H-8b), 3.05 (m, 2H, H-4'a), 3.22 (m, 2H, H-4'b), 3.37 (s, 6H, H-1'), 3.64 (m, polymer backbone).

^{13}C-NMR (90 MHz, $CDCl_3$, protonated form): δ 14.3 (C-1), 22.7 (C-2), 29.5 – 30.1 (C-4), 32.1 (C-3), 33.4 (C-5), 37.8 (C-6), 39.4 (C-7), 48.3 (C-4'), 56.6 (C-8), 59.2 (C-1'), 68.0 (C-3'), 70.4 - 70.7 (polymer backbone), 72.1 (C-2').

***N*-(α-methoxy[poly(ethylene oxy)] ethyl) tetradecane-1-amine (average molecular mass ~750 Da) [8d(550,12)]:**

A solution of **7d(550,12)** (1.36 g, 1.79 mmol) in anhydrous THF **(**15 mL) and a 1 M solution of borane in THF (5.1 mL, 5.1 mmol) were reacted for 2 d and the mixture was processed, both according to general procedure K. The crude product was purified by column chromatography (dichloromethane → dichloromethane/methanol 9:1).

Yield: 427 mg (0.573 mmol), 32% of a colourless solid.



^{1}H-NMR (360 MHz, $CDCl_3$): δ 0.88 (t, 3H, $^{3}J_{H\text{-}H}$ = 6.8 Hz, H-1), 1.25 (m, 22H, H-2, H-3, H-4, H-5), 1.67 (m, 2H, H-6), 2.83 (m, 2H, H-7), 3.02 (m, 2H, H-4'), 3.37 (s, 3H, H-1'), 3.64 (m, polymer backbone), 3.79 (m, 2H, H-3').

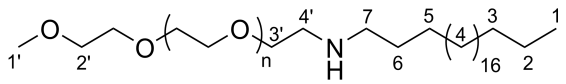
^{13}C-NMR (90 MHz, $CDCl_3$): δ 14.3 (C-1), 22.8 (C-2), 27.2 (C-5), 29.4 - 29.8 (C-4, C-6), 32.1 (C-3), 48.3 (C-7), 49.0 (C-4'), 59.2 (C-1'), 67.8 (C-3'), 70.3 - 70.7 (polymer backbone), 72.1 (C-2').

MALDI-TOF: 756.8 (536.8 - 932.8) $[M+H]^+$, 778.8 (602.8 - 954.7) $[M+Na]^+$

***N*-(α-methoxy[poly(ethylene oxy)] ethyl) docosane-1-amine (average molecular mass ~860 Da) [8d(550,20)]:**

A solution of **7d(550,20)** (1.96 g, 2.25 mmol) in anhydrous THF **(**25 mL) and a 1 M solution of borane in THF (5.4 mL, 5.4 mmol) were reacted for 3 d and the mixture was processed, both according to general procedure K. The crude product was purified by column chromatography (dichloromethane → dichloromethane/methanol 9:1).

Yield: 468 mg (0.546 mmol), 24% of a colourless solid.



^{1}H-NMR (360 MHz, $CDCl_3$, protonated form): δ 0.87 (t, 3H, $^{3}J_{H\text{-}H}$ = 6.7 Hz, H-1), 1.25 (m, 38H, H-2, H-3, H-4, H-5), 1.81 (m, 2H, H-6), 2.94 (m, 2H, H-7), 3.12 (m, 2H, H-4'), 3.37 (s, 3H, H-1'), 3.64 (m, polymer backbone), 3.89 (m, 2H, H-3').

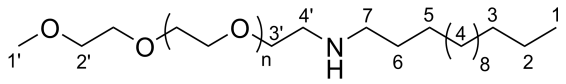
^{13}C-NMR (90 MHz, $CDCl_3$, protonated form): δ 14.3 (C-1), 22.8 (C-2), 26.5 (C-6), 27.0 (C-5), 29.3 - 29.9 (C-4, C-6), 32.1 (C-3), 47.6 (C-7), 48.5 (C-4'), 59.2 (C-1'), 66.5 (C-3'), 70.3 - 70.7 (polymer backbone), 72.1 (C-2').

MALDI-TOF: 868.7 (648.6 - 1044.8) $[M+H]^+$

***N*-(α-methoxy[poly(ethylene oxy)] ethyl) tetradecane-1-amine (average molecular mass ~2.20 kDa) [8d(2000,12)]:**

A solution of **7d(2000,12)** (3.83 g, 1.73 mmol) in anhydrous THF **(**20 mL) and a 1 M solution of borane in THF (4.9 mL, 4.9 mmol) were reacted for 2 d and the mixture was processed, both according to general procedure K. The crude product was purified by column chromatography (dichloromethane → dichloromethane/methanol 9:1).

Yield: 135 mg (0.0615 mmol), 4% of a colourless solid.



^{1}H-NMR (360 MHz, $CDCl_3$, protonated form): δ 0.88 (t, 3H, $^3J_{H-H}$ = 6.8 Hz, H-1), 1.26 (m, 22H, H-2, H-3, H-4, H-5), 1.84 (m, 2H, H-6), 2.96 (m, 2H, H-7), 3.15 (m, 2H, H-4'), 3.38 (s, 3H, H-1'), 3.64 (m, polymer backbone), 3.93 (m, 2H, H-3').

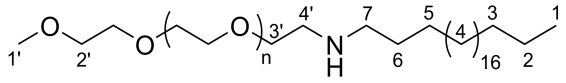
^{13}C-NMR (90 MHz, $CDCl_3$, protonated form): δ 14.3 (C-1), 22.8 (C-2), 26.1 (C-6), 26.9 (C-5), 29.2 - 29.8 (C-4, C-6), 32.1 (C-3), 47.7 (C-7), 48.3 (C-4'), 59.2 (C-1'), 65.9 (C-3'), 70.1 - 70.7 (polymer backbone), 72.1 (C-2').

MALDI-TOF: 2342.7 (1990.2 - 2783.1) $[M+H]^+$, 2364.8 (2100.2 - 2761.1) $[M+Na]^+$

***N*-(α-methoxy[poly(ethylene oxy)] ethyl) docosane-1-amine (average molecular mass ~2.31 kDa) [8d(2000,20)]:**

A solution of **7d(2000,20)** (2.32 g, 1.00 mmol) in anhydrous THF **(**20 mL) and a 1 M solution of borane in THF (2.8 mL, 2.8 mmol) were reacted for 4 d and the mixture was processed, both according to general procedure K. The crude product was purified by column chromatography (dichloromethane → dichloromethane/methanol 9:1).

Yield: 519 mg (0.225 mmol), 22% of a colourless solid.



^{1}H-NMR (360 MHz, $CDCl_3$, protonated form): δ 0.87 (t, 3H, $^3J_{H-H}$ = 6.8 Hz, H-1), 1.25 (m, 38H, H-2, H-3, H-4, H-5), 1.84 (m, 2H, H-6), 2.97 (m, 2H, H-7), 3.16 (m, 2H, H-4'), 3.37 (s, 3H, H-1'), 3.64 (m, polymer backbone), 3.92 (m, 2H, H-3').

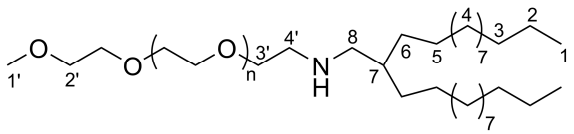
^{13}C-NMR (90 MHz, $CDCl_3$, protonated form): δ 14.3 (C-1), 22.8 (C-2), 26.1 (C-6), 26.9 (C-5), 29.3 - 29.8 (C-4, C-6), 32.1 (C-3), 47.6 (C-7), 48.3 (C-4'), 59.2 (C-1'), 65.9 (C-3'), 70.1 - 70.7 (polymer backbone), 72.1 (C-2').

MALDI-TOF: 2278.2 (1750.1 - 2807.6) $[M+H]^+$, 2364.8 (2100.2 - 2761.1) $[M+Na]^+$

***N*-(α-methoxy[poly(ethylene oxy)] ethyl)-2-dodecyl tetradecane-1-amine (average molecular mass ~910 Da) [8e(550,12)]:**

A solution of **7e(550,12)** (1.66 g, 1.79 mmol) in anhydrous THF **(**17 mL) and a 1 M solution of borane in THF (5.0 mL, 5.0 mmol) were reacted for 1 d and the mixture was processed, both according to general procedure K. The crude product was purified by column chromatography (dichloromethane → dichloromethane/methanol 9:1).

Yield: 687 mg (0.752 mmol), 42% of a colourless oil.



^{1}H-NMR (360 MHz, $CDCl_3$): δ 0.88 (t, 6H, $^3J_{H-H}$ = 6.8 Hz, H-1), 1.26 (m, 44H, H-2, H-3, H-4, H-5, H-6), 1.70 (m, 1H, H-7), 2.71 (m, 2H, H-8), 2.99 (m, 2H, H-4'), 3.38 (s, 3H,

H-1'), 3.64 (m, polymer backbone), 3.77 (H-3').

^{13}C-NMR (90 MHz, $CDCl_3$): δ 14.3 (C-1), 22.8 (C-2), 26.5 (C-5), 29.5 - 30.1 (C-4), 31.8 (C-6), 32.1 (C-3), 36.6 (C-5), 48.8 (C-4'), 52.8 (C-8), 59.2 (C-1'), 70.4 - 70.8 (polymer backbone), 72.1 (C-2').

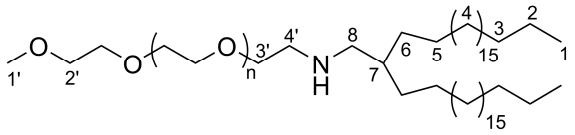
MALDI-TOF: 924.9 (792.8 - 1056.9) $[M+H]^+$, 946.9 (858.9 - 1078.9) $[M+Na]^+$

***N*-(α-methoxy[poly(ethylene oxy)] ethyl)-2-eicosyl docosane-1-amine (average molecular mass ~1.14 kDa) [8e(550,20)]:**

A solution of **7e(550,20)** (2.06 g, 1.79 mmol) in anhydrous THF **(**15 mL) and a 1 M solution of borane in THF (5.0 mL, 5.0 mmol) were reacted o/n and the mixture was processed, both according to general procedure K. The crude product was purified by column chromatography (dichloromethane → dichloromethane/methanol 9:1).

Yield: 1.53 g (1.35 mmol), 75% of a colourless solid.

^{1}H-NMR (360 MHz, $CDCl_3$): δ 0.88 (t, 6H, $^3J_{H-H}$ = 6.8 Hz, H-1), 1.25 (m, 76H, H-2, H-3, H-4, H-5, H-6), 1.92 (m, 1H, H-7), 2.90 (m, 2H, H-8), 3.19 (m, 2H, H-4'), 3.38 (s, 3H, H-1'), 3.64 (m, polymer backbone), 3.94 (H-3').



^{13}C-NMR (90 MHz, $CDCl_3$): δ 14.3 (C-1), 22.8 (C-2), 26.2 (C-5), 29.5 - 30.2 (C-4), 31.3 (C-6), 32.1 (C-3), 35.1 (C-5), 48.0 (C-4'), 51.8 (C-8), 59.2 (C-1'), 65.6 (C-3'), 70.3 - 70.7 (polymer backbone), 72.1 (C-2').

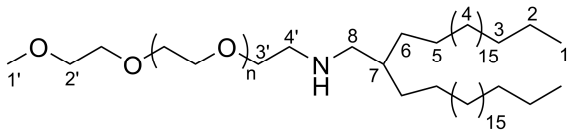
MALDI-TOF: 1148.6 (972.6 - 1324.6) $[M+H]^+$, 1214.6 (1038.6 - 1390.6) $[M+Na]^+$

***N*-(α-methoxy[poly(ethylene oxy)] ethyl)-2-eicosyl docosane-1-amine (average molecular mass ~2.59 kDa) [8e(2000,20)]:**

A solution of **7e(2000,20)** (3.77 g, 1.45 mmol) in anhydrous THF **(**20 mL) and a 1 M solution of borane in THF (5.5 mL, 5.5 mmol) were reacted for 3 d and the mixture was processed, both according to general procedure K. The crude product was purified by column chromatography (dichloromethane → dichloromethane/methanol 9:1).

Yield: 873 mg (0.337 mmol), 23% of a colourless solid.

^{1}H-NMR (360 MHz, $CDCl_3$): δ 0.88 (t, 6H, $^3J_{H-H}$ = 6.8 Hz, H-1), 1.25 (m, 76H, H-2, H-3, H-4, H-5, H-6), 1.92 (m, 1H, H-7), 2.90 (m, 2H, H-8), 3.19 (m, 2H, H-4'), 3.38 (s, 3H, H-1'), 3.64 (m, polymer backbone), 3.94 (H-3').



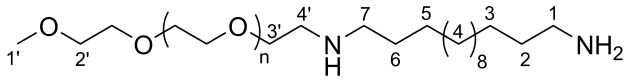
^{13}C-NMR (90 MHz, $CDCl_3$): δ 14.3 (C-1), 22.8 (C-2), 26.2 (C-5), 29.5 - 30.1 (C-4), 31.3 (C-6), 32.1 (C-3), 35.1 (C-5), 48.1 (C-4'), 51.8 (C-8), 59.2 (C-1'), 65.6 (C-3'), 70.2 - 70.7 (polymer backbone), 72.1 (C-2').

MALDI-TOF: 2690.6 (2426.5 - 3131.4) $[M+H]^+$, 2756.6 (2492.5 - 3197.9) $[M+Na]^+$

N-(α-methoxy[poly(ethylene oxy)] ethyl)-14-amino tetradecane-1-amine (average molecular mass ~760 Da) [8f(550,12)]:

A solution of **7f(550,12)** (1.00 g, 1.30 mmol) in anhydrous THF **(**13 mL) and a 1 M solution of borane in THF (4.8 mL, 4.8 mmol) were reacted for 3 d and the mixture was processed, both according to general procedure K. The crude product was purified by column chromatography (dichloromethane/methanol 9:1 → dichloromethane/methanol 9:1 + 0.1% (v/v) triethylamine).

Yield: 305 mg, (0.401 mol), 31% of a light-yellow oil.



^{1}H-NMR (360 MHz, $CDCl_3$): δ 1.26 (m, 20H, H-3, H-4, H-5), 1.54 (m, 2H, H-2), 1.59 (m, 2H, H-6), 2.69 (t, 2H, $^3J_{H-H}$ = 7.6 Hz, H-1), 2.77 (t, 2H, $^3J_{H-H}$ = 7.4 Hz, H-7), 2.87 (t, 2H, $^3J_{H-H}$ = 5.2 Hz, H-4'), 3.38 (s, 3H, H-1'), 3.64 (m, polymer backbone), 3.84 (H-3').

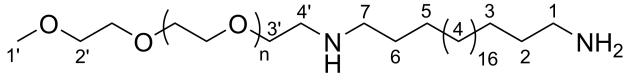
^{13}C-NMR (90 MHz, $CDCl_3$): δ 26.9 (C-3), 27.2 (C-5), 28.8 - 29.7 (C-4, C-6), 31.8 (C-2), 41.6 (C-1), 48.8 (C-7), 49.7 (C-4'), 59.2 (C-1'), 69.3 (C-3'), 70.4 - 70.8 (polymer backbone), 72.1 (C-2').

MALDI-TOF: 771.6 (551.5 - 996.7) $[M+H]^+$

***N*-(α-methoxy[poly(ethylene oxy)] ethyl)-22-amino docosane-1-amine (average molecular mass ~870 Da) [8f(550,20)]:**

A solution of **7f(550,20)** (1.11 g, 1.25 mmol) in anhydrous THF **(**15 mL) and a 1 M solution of borane in THF (3.75 mL, 3.75 mmol) were reacted for 4 d and the mixture was processed, both according to general procedure K. The crude product was purified by column chromatography (dichloromethane/methanol 9:1 → dichloromethane/methanol 9:1 + 0.1% (v/v) triethylamine).

Yield: 184 mg (0.211 mmol), 17% of a colourless amorphous solid.



^{1}H-NMR (360 MHz, $CDCl_3$): δ 1.25 (m, 36H, H-3, H-4, H-5), 1.80 (m, 4H, H-2, H-6), 3.00 (m, 4H, H-1, H-7), 3.14 (m, 2H, H-4'), 3.38 (s, 3H, H-1'), 3.64 (m, polymer backbone), 3.95 (H-3').

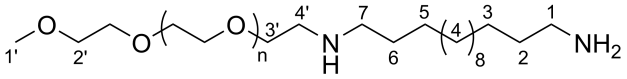
^{13}C-NMR (90 MHz, $CDCl_3$): δ 26.1 (C-6), 26.9 (C-5), 27.0 (C-3), 27.7 (C-2), 28.7 - 29.8 (C-4), 40.5 (C-1), 47.3 (C-7), 48.9 (C-4'), 59.2 (C-1'), 66.3 (C-3'), 70.2 - 70.8 (polymer backbone), 72.1 (C-2').

MALDI-TOF: 839.6 (663.5 - 1015.7) $[M+H]^+$

***N*-(α-methoxy[poly(ethylene oxy)] ethyl)-14-amino tetradecane-1-amine (average molecular mass ~2.21 kDa) [8f(2000,12)]:**

A solution of **7f(2000,12)** (2.67 g, 1.20 mmol) in anhydrous THF **(**13 mL) and a 1 M solution of borane in THF (4.5 mL, 4.5 mmol) were reacted for 3 d and the mixture was processed, both according to general procedure K. The crude product was purified by column chromatography (Sephadex LH20, ethanol).

Yield: 1.59 g (0.719 mmol), 60% of a light-yellow amorphous solid.



^{1}H-NMR (360 MHz, $CDCl_3$): δ 1.27 (m, 20H, H-3, H-4, H-5), 1.70 (m, 2H, H-2), 1.77 (m, 2H, H-6), 2.82 (m, 2H, H-1), 2.88 (m, 2H, H-7), 3.00 (m, 2H, H-4'), 3.37 (s, 3H, H-1'), 3.64 (m, polymer backbone), 3.83 (H-3').

^{13}C-NMR (90 MHz, $CDCl_3$): δ 26.5 (C-3), 26.7 (C-5), 28.3 - 29.6 (C-4, C-6), 40.7 (C-1), 48.1 (C-7), 49.2 (C-4'), 59.2 (C-1'), 70.3 - 70.8 (polymer backbone), 72.1 (C-2').

^{1}H-NMR (360 MHz, $CDCl_3$): δ 1.27 (m, 16H), 1.70 (m, 2H), 1.77 (m, 2H), 2.82 (m, 2H), 2.88 (m, 2H), 3.00 (m, 2H), 3.37 (s, 3H), 3.64 (m, polymer backbone).

^{13}C-NMR (90 MHz, $CDCl_3$): δ 26.5, 26.7, 28.3 - 29.6, 40.7, 48.1, 49.2, 59.2, 70.3 - 70.8, 72.1.

MALDI-TOF: 2400.4 (2136.5 - 2841.2) $[M+H]^+$

5.3 CMC- and Solubility Measurements

CMC Determination

The surface tension of 3 mL of a specific blocking solution (BS3 and BS9-30) was determined four times each for different concentrations. The highest concentration was 0.0033 g/mL and second highest 0.0025 g/mL blocking reagent in D-PBS. The latter solution (0.0025 g/mL) was 2 fold serially diluted (e.g. concentrations measured were 0.0033 g/mL, 0.0025 g/mL, 0.00125 g/mL, 0.000625 g/mL, 0.0003125 g/mL and so on), resulting in 8-13 different concentrations. The (uncorrected) surface tension was measured with a manual tensiometer and plotted against the logarithmic concentration. CMC was determined (via linear regression) as the intersection of the two linear parts on the surface tension versus log-transformed concentration curve. In some cases, no change was seen in the rate of surface tension decrease although the solutions were highly diluted and the surface tension already approached the value for D-PBS. In these cases it is assumed that the CMC is higher than 0.0033 g/mL.

Solubility Experiment

To prepare saturated solutions, an excess amount of a specific blocking reagent (all cationic surfactants except **8d**(550,12) and **8d**(2000,12)) was dissolved in D-PBS (150 µL – 1.5 mL) at 40 °C. The mixture was allowed to cool to room temperature, and the pH of the mixture was adjusted with concentrated hydrochloric acid (32% (w/v)) to 7.2. The mixture was

centrifuged at an rcf of 16,100 × g until the excess amount of blocking reagent formed a pellet (5 to 20 min). A defined volume of the supernatant was transferred to a microcentrifuge tube and the volume was recorded. The solvent of the transferred supernatant was removed *in vacuo* with a SpeedVac concentrator and the residue was resuspended in 100 µL of ddH_2O. This suspension was extracted three times with 500 µL of dichloromethane. The organic fractions were combined and the dichloromethane was removed *in vacuo* with a SpeedVac concentrator. The weight of the residue was recorded, and the solubility was calculated as the ratio of extract weight to the volume of the extracted D-PBS solution.

The procedure was carried out once for all blocking reagents.

5.4 Assay Experiments

Model-Assay for the Determination of NSB as an Indicator of Blocking Performance

High-binding polystyrene microtitre plates were blocked with 250 µL/well of a specific blocking solution (0.5% (v/v) of Surfactant **1** in different buffers, see below) for 7 h at room temperature. After washing the plates four times with 300 µL/well of D-PBS, 75 µL/well of a FBS solution (two-fold serially diluted over 16 wells, i.e. 50%, 25%, 12.5%, …, 0.0015%, 0.0008% (v/v) FBS, in the corresponding blocking solution) were added to each well. 75 µl/well of the blocking solution alone were added to two wells for each blocking reagent as a negative control, and the plates were incubated o/n at 4 °C. The plates were washed four times with 300 µL/well of D-PBS and incubated for 3 h at room temperature with 75 µl/well of a solution of biotin-labelled ConA (1.0 µg/mL in the corresponding blocking solution). One of the two wells reserved for negative controls was incubated with 75 µl/well of the blocking solution alone (also for 3 h at room temperature). The plates were washed six times with 300 µL/well of PBST, and 75 µL/well of a solution of HRP-labelled streptavidin (1 µg/mL in the corresponding blocking solution) were added. The plates were incubated for 90 min at room temperature. After washing the plates six times with 300 µL/well of PBST, 75 µL/well of a freshly prepared TMB substrate solution (8 mL solution A + 200 µL solution B) were added and allowed to develop colour in the dark for 10 min at room temperature. The reaction was stopped by adding 125 µL of 1 M sulphuric acid to each well. Optical densities at 450 nm and 405 nm were measured with a microplate reader.

The procedure was carried out for blocking solutions prepared with the following buffers:

- D-PBS 2x, 1x, 0.5x and 0.2x, L-PBS 1x and 0.5x, each in duplicate
- Na^+ 5x to 0.1x, K^+ 5x to 0.1x, NH_4^+ 5x to 0.1x, as single measurement each
- PIPES-buffer (1x, pH 5.8 to 7.3), Phosphate-buffer (1x, pH 6.3 to 8.3), Tris-buffer (1x, pH 7.3 to 8.8), each in duplicate

The procedure was also carried out for a blocking solution of 0.5% (v/v) of Surfactant **1** in D-PBS (1x) with the difference that all incubation steps were conducted either in blocking solution (as described above) or in D-PBS. This set of 16 (two possible settings for four

incubations, i.e. 2^4) experiments was conducted once for each unique combination of different incubation solutions.

Assay for the Investigation of the Kinetics of HRP-Catalysed TMB Oxidation

High-binding polystyrene microtitre plates were coated with 75 µL/well of a solution of a HRP-labelled goat anti-mouse IgG antibody (1:10,000 diluted in D-PBS) o/n at 4 °C. After washing the plates six times with 300 µL/well of PBS, the plates were incubated with 75 µL/well of D-PBS or of a specific blocking solution (BS3, 4, 6, 29 and 30) for 90 min at room temperature. After washing the plates three times with 300 µL/well of PBS, 75 µL/well of a freshly prepared TMB substrate solution (8 mL solution A + 200 µL solution B) were added to each well.

In a first experiment, no stopping solution was added. Optical densities at 655 nm and 370 nm were measured immediately after addition of the TMB substrate solution with a microplate reader. The measurement was repeated every 5 sec for a time span of 3 min. The experiment was conducted three times for each combination of blocking solution and wave length.

In a second experiment, the TMB substrate solution was allowed to develop colour in the dark at room temperature but the reaction was stopped by adding 125 µL of 1 M sulphuric acid to each well immediately after TMB addition, or after 1, 2, 3, 4, 6, 8, 10, 15, or 20 min. Optical densities at 450 nm and 405 nm were measured with a microplate reader. The procedure was carried out once for each blocking solution.

ELISA for the Determination of NSB for Different Blocking Solution Concentrations

High-binding polystyrene microtitre plates were blocked with 250 µL/well of a specific blocking solution of surfactant **2** in D-PBS (5%, 2%, 1%, 0.5%, 0.2%, 0.1% (w/v) surfactant **2**) for 7 h at room temperature. After washing the plates four times with 300 µL/well of D-PBS, 75 µL/well of a pooled normal human serum solution (two-fold serially diluted over 16 wells, i.e. 50%, 25%, 12.5%, …, 0.0015%, 0.0008% (v/v) pooled human serum, in the corresponding blocking solution) were added to each well. 75 µl/well of the blocking solution alone were added to two wells for each blocking solution as a negative control, and the plates were incubated o/n at 4 °C. The plates were washed four times with 300 µL/well of D-PBS and incubated for 3 h at room temperature with 75 µl/well of a solution of a goat anti-human IgG antibody (0.25 µg/mL in the corresponding blocking solution). One of the two wells reserved for negative controls was incubated with 75 µl/well of the blocking solution alone (also for 3 h at room temperature). The plates were washed six times with 300 µL/well of PBST, and 75 µL/well of a solution of HRP-labelled streptavidin (1 µg/mL in the corresponding blocking solution) were added. The plates were incubated for 90 min at room temperature. After washing the plates six times with 300 µL/well of PBST, 75 µL/well of a freshly prepared TMB substrate solution (8 mL solution A + 200 µL solution B) were added and allowed to develop colour in the dark for 10 min at room temperature. The reaction was stopped by adding 125 µL of 1 M sulphuric acid to each well. Optical densities at 450 nm and 405 nm were measured with a microplate reader.

The assay was carried out once for each blocking solution.

Specificity-ELISA

High-binding half-area polystyrene microtitre plates were blocked with 150 µL/well of a specific blocking solution (BS1-30) for 7 h at room temperature. After washing the plates four times with 150 µL/well of D-PBS, 45 µL/well of a human serum solution (either 20% (v/v) or 2% (v/v) of a human serum sample in the corresponding blocking solution) or the blocking solution alone as a negative control were added to each well and the plates were incubated o/n at 4 °C. The plates were washed four times with 150 µL/well of D-PBS and incubated for 3 h at room temperature with 45 µl/well of a solution of a biotin-labelled goat anti-human IgG antibody (0.25 µg/mL in the corresponding blocking solution). The plates were washed six times with 150 µL/well of PBST and 45 µL/well of a solution of HRP-labelled streptavidin (1 µg/mL in the corresponding blocking solution) were added. The plates were incubated for 90 min at room temperature. After washing the plates six times with 150 µL/well of PBST, 45 µL/well of a freshly prepared TMB substrate solution (5 mL solution A + 125 µL solution B) were added and allowed to develop colour in the dark for 10 min at room temperature. The reaction was stopped by adding 75 µL of 1 M sulphuric acid to each well. Optical densities at 450 nm and 405 nm were measured with a microplate reader.

The procedure was carried out independently in duplicate for BS9-28 and in quadruplicate for BS1-8 and BS29-30.

Prion-ELISA

For each blocking reagent six wells of high-binding half-area polystyrene microtitre plates were coated with 45 µL/well of a solution of a recombinant prion protein fragment (PrP90-231, 50 ng/mL or 3 ng/mL, in L-PBS), or with L-PBS alone as a negative control o/n at 4 °C. After washing the plates three times with 150 µL/well of PBST, the plates were blocked with 150 µL/well of a specific blocking solution (BS1-30) for 7 h at room temperature. The plates were washed four times with 150 µL/well of PBST and incubated o/n at 4 °C with 45 µL/well of a solution of a monoclonal anti-PrP antibody (0.25 ng/mL in the corresponding blocking solution containing additional 0.1% (v/v) Tween20). The plates were washed four times with 150 µL/well of PBST and 45 µL/well of a solution of a HRP-labelled goat anti-mouse IgG antibody (1:2,000 diluted in the corresponding blocking solution) were added. The plates were incubated for 90 min at room temperature. After washing the plates six times with 150 µL/well of PBST, 45 µL/well of a freshly prepared TMB substrate solution (5 mL solution A + 125 µL solution B) were added to each well and allowed to develop colour in the dark for 10 min at room temperature. The reaction was stopped by adding 75 µL/well of 1 M sulphuric acid. Optical densities at 450 nm and 405 nm were measured with a microplate reader.

The procedure was carried out independently in duplicate for BS9-28 and in quadruplicate for BS1-8 and BS29-30.

Hepatitis B-ELISA

High-binding half-area polystyrene microtitre plates were coated with 45 µL/well of a solution of a goat anti-HBsAg antibody (0.5 µg/mL in D-PBS) o/n at 4 °C. After washing the plates four times with 150 µL/well of D-PBS, plates were blocked for 3 h at room temperature with 150 µL/well of a specific blocking solution (BS1-30). The plates were washed four times with 150 µL/well of D-PBS and incubated for 2 h at room temperature with 45 µL/well of a solution of a recombinant Hepatitis B surface Antigen (serial dilution, 20,000, 8,000, 3,200, 1,280, 512, 205, 82, 33, 13 and 5 ng/mL, in the corresponding blocking solution). The plates were washed four times with 150 µL/well of D-PBS followed by addition of 45 µL/well of a solution of a HRP-labelled goat anti-HBsAg antibody (4 µg/mL in the corresponding blocking solution). The plates were incubated for 60 min at room temperature. After washing the plates six times with 150 µL/well of PBST, 45 µL/well of a freshly prepared TMB substrate solution (5 mL solution A + 125 µL solution B) were added and allowed to develop colour in the dark for 30 min at room temperature. The reaction was stopped by adding 75 µL/well of 1 M sulphuric acid. Optical densities at 450 nm and 405 nm were measured with a microplate reader.

The assay was carried out once for BS9-28 and in duplicate for BS1-8 and BS29-30.

Immunoblots

In the following experiment, the nitrocellulose membranes were used without any pre-treatment. The PVDF membranes were wetted with 750 µL of ethanol (1.63 mL/cm^2) for 1 min at room temperature before blocking.

Membranes (11.5 mm × 4 mm) were blocked in 500 µL (1.1 mL/cm^2) of a specific blocking solution (BS1-30) or of D-PBS as negative control for 60 min at room temperature. The membranes were subsequently incubated for 60 min at room temperature with 500 µL (1.1 mL/cm^2) of a solution of an AlexaFluor680-labelled goat anti-mouse IgG antibody (0.8 µg/mL, in the corresponding blocking solution or in D-PBS in case of the negative control). After incubation the membranes were washed six times with 750 µL (1.6 mL /cm^2) of D-PBS for 10 min at room temperature. Fluorescence was quantitated on a Odyssey infrared imager using appropriate software.

The procedure was carried out in duplicate for each membrane and blocking solution.

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7. Appendix

7.1 Abbreviations

BSA	Bovine serum albumin
CMC	Critical micelle concentration
ConA	Concanavalin A
D-PBS	Dulbecco's phosphate buffered saline
DCC	Dicyclohexyl carbodiimide
DIAD	Diisopropyl azodicarboxylate
DME	Dimethoxyethane
DMF	*N*,*N*-Dimethylformamide
EIA	Enzyme-linked immunoassay
ELISA	Enzyme-linked immunosorbent assay
ESI-MS	Electrospray ionisation mass spectrometry
FBS	Foetal bovine serum
HBsAg	Hepatitis B surface antigen
HCTU	2-(6-Chloro-1-H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HRP	Horseradish peroxidase
HS	Human serum
IgG	Immunoglobulin G
L-PBS	Lite-PBS
LOD	Limit of detection
MALDI-TOF MS	Matrix-assisted laser desorption/ionisation time of flight mass spectrometry
MC	Methylene chloride
NC	Nitrocellulose
NMR	Nuclear magnetic resonance
NSB	Non-specific binding
OD	Optical density
PEG	Poly(ethylene glycol)

PIPES	Piperazine-1,4-bis-2-ethanesulfonic acid
POE	Poly(oxyethylene)
PrP	Prion protein
PVDF	Poly(vinylidene fluoride)
PVP	Poly(vinyl pyrrolidone)
RIA	Radioimmunoassay
RT	Room temperature
S/N	Signal-to-noise ratio
SA	Streptavidin
TBABH	Tetrabutyl ammonium borohydride
Tf_2O	Trifluoromethane sulfonic acid anhydride
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMB	3,3',5,5'-tetramethylbenzidine
TosCl	*Para*-Toluenesulfonyl chloride
Tol	Toluene
Tris	Tris(hydroxymethyl)aminomethane

7.2 Hazardous Reagents

Listed below are all reagents and chemicals used within this work along with the respective hazard code(s) and the risk (R-) and safety (S-) phrase(s).

Table 7: List of hazardous reagents

Name	Hazard code(s)	R-phrase(s)	S-phrase(s)
Borane 1.0 M in THF	F, Xn	14/15-19-22-36/37/38	16-33-36/37/39-7/9
1-Bromoeicosane	-	-	22-24/25
1-Bromododecane	Xi	36/37/38	26-36
Cyclohexane	F, Xn, N	11-38-50/53-65-67	9-16-25-33-60-61-62
Dichloromethane	Xn	40	23-24/25-36/37
Diethyl ether	F+, Xn	12-19-22-66-67	9-16-29-33
Dimethoxyethane	F, T	60-61-11-19-20	53-45
N,*N*-Dimethylformamide	T	61-20/21-36	53-45
Diisopropyl azodicarboxylate	Xn	36/37/38-40-48/20/22	36
Docosanoic acid	-	-	-
1,12-Dodecanediol	-	-	-
Eicosanedioic acid	Xi	36/37/38	26-37/39
Ethanol	F	11	7-16
Ethyl acetate			
Hexane	F, Xn, N	11-38-48/20-51/53-62-65-67	9-16-29-33-36/37-61-62
Hydrazine monohydrate	T, N	45-10-23/24/25-34-43-50/53	53-45-60-61
Hydrobromic acid, 48% (w/v)	C	35-37	26-45-7/9
Lithium aluminium hydride	F	15	24/25-43-7/8
Lithium tri-*tert*-butoxyaluminium hydride	F, C	11-14-34	16-26-36/37/39-45
Malonic acid ditheyl ester	-	-	-
Malonic acid di-*tert*-butyl ester	-	-	23-24/25
Methanol	F, Xi	11-36-66-67	16-26-33

2-(α-methoxy[poly(ethylene oxy)]) ethanol, 550 Da	-	-	-
2-(α-methoxy[poly(ethylene oxy)]) ethanol, 2kDa	-	-	-
Molecular Sieves, 4 Å	Xi	36/37	26
Ninhydrin	Xn	22-36/37/38	26
Phosphomolybdic acid hydrate	O, C	8-34	17-26-36/37/39-45
Sodium, pieces stored in heavy mineral oil	F, C	14/15-34	8-43-45
Sodium azide	T+, N	28-32-50/53	28-45-60-61
Sodium hydride, 60% dispersion in mineral oil	F	15	7-24/25-43
Tetrabutyl ammonium boro-hydride	Xi	36/37/38	26-36
Tetradecanoic acid	-	-	-
Tetrahydrofuran	F, Xi	11-19-36/37	16-29-33
3,3',5,5'-tetramethylbenzidine	Xi	36/37/38	26-36
Thionyl chloride	C	14-20/22-29-35	26-36/37/39-45
Toluene	F, Xn	11-38-48/20-63-65-67	36/37-46-62
Para-Toluenesulfonyl chloride	C	34	26-36/37/39-45
Triethylamine	F,C	11-20/21/22-35	3-16-26-29-36/37/39-45
Trifluoroacetic acid	C	20-35-52/53	9-26-27-28-45-61
Trifluoromethane sulfonic acid anhydride	C	20/21/22-34	26-27-28-36/37/39-45
Triphenylphosphine	Xn	22-43-53	36/37-60
Tris(hydroxymethyl)methylamine	Xi	36/37/38	26-36
Tween20	-	-	-

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Curriculum Vitae

Education

2005

July — Enrolled in PhD programme at the University of Hamburg. Experimental work is carried out externally at the Research Center Borstel, supervised by PD Dr Andreas Frey.

May — Chemistry Diploma awarded (overall grade: very good)
Thesis title: „Glycosylceramides: Synthesis and biophysical properties“

2004

June-July — Diploma examinations (oral): Organic Chemistry - very good
Biochemistry - good
Inorganic Chemistry - very good
Physical Chemistry - very good

2001

July — Pre-Diploma examinations (oral, overall grade: very good)

1999

Oct — Enrolled in Chemistry at the University of Hamburg

June — Abitur

Publications

Peer reviewed publication

Ferrocene-based ligands in ruthenium alkylidene chemistry; Butler, I. R.; Coles, S. J.;Hursthouse, M. B.; Roberts, D. J.; Fujimoto, N.; *Inorg. Chem. Commun*. **2003**, *6*, 760-762.

Conference proceedings and presentations

Peptide-based optical contrast agents for targeting of intestinal malignancies; Roeckendorf, N.; Helfmann, J.; Fujimoto, N.; Wehry, K.; Buerger, M.; Frey, A.; *Proceedings of SPIE-The International Society for Optical Engineering* **2007**, *6633*, 66332A/1-66332A/9.

Glycosylceramides: Synthesis and biophysical properties; Fujimoto, N.; Howe, J.; Brandenburg, K.; *Abstracts of Papers, 234th ACS National Meeting, Boston*, **2007**, ORGN-155.

Chiral Self-Assembled Structures of Columnar Oligopeptides; Kamikawa, Y.; Fujimoto, N.; Kato, T.; *Proceedings of Japanese Liquid Crystal Society annual meeting* **2005**, 2B02.

International Experiences

2002

Mar-June — Experimental work at Bangor University, Wales, UK, supervised by Dr Ian Butler.

2003

Feb-May — Experimental work at the University of Tokyo, Tokyo, Japan, supervised by Prof Takashi Kato.

Awards / Grants

2007 — Participant of the NESACS-NSYCC/GDCh-JCF Exchange Program in Boston, USA with attendance of the ACS National Meeting

1999-2005 — Scholarship of the German National Academic Foundation (Studienstiftung des deutschen Volkes)

1994-1999 — Participant of different contests, eg Mathematical Olympiad, International Mathematics Tournament of Towns, Federal Contest in Mathematics (Germany), Chemistry Olympiad, and “Jugend musiziert”; awards at federal and state levels.

Erklärungen im Sinne der Promotionsordnung

Eidesstattliche Versicherung

Hamburg, den 11. Juni 2009

Hiermit erkläre ich an Eides Statt, dass die vorliegende Dissertationsschrift selbständig und allein von mir unter den angegebenen Hilfsmitteln angefertigt wurde.

Naho Fujimoto

Erklärung über frühere Promotionsversuche

Hamburg, den 11. Juni 2009

Hiermit erkläre ich, Naho Fujimoto, dass vorher keine weiteren Promotionsversuche unternommen worden sind, oder an einer anderen Stelle vorgelegt wurden.

Naho Fujimoto