A modular approach for the construction and modification of glyco-SAMs utilizing 1,3-dipolar cycloaddition

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We report the synthesis of a broad variety of functionalized molecules for assembly on gold, allowing the formation of biologically relevant SAMs by a modular approach: either utilizing 1,3-dipolar cycloaddition of alkynes and azides in solution or by 'click on SAM'. Extensive studies into the various parameters of SAM formation and stability have been carried out, leading us to deduce reliable conditions under which glyco-decorated self-assembled monolayers can be formed and studied such as in SPR-supported binding assays.

Introduction

Eukaryotic cell surfaces are covered by a highly complex sugar coat, called the glycocalyx. It comprises of glycoproteins, glycolipids, complex oligosaccharides as well as proteoglycans and other glycoconjugates.[1] It may be considered as a cell organelle,[2] whose function is essential in cell biology, because processes like cellular recognition, cell development and differentiation, fertilisation and immune response are affiliated with molecular recognition within the glycocalyx. In addition, states of disease can depend on molecular interaction with the glycocalyx, such as in the case of cancer and metastasis.[3] Interaction of cell surface saccharides with specialized proteins called lectins and selectins[4] is of crucial biological importance. However, in spite of this, lectin–carbohydrate interactions are typically weak in *in vitro* testing, with K_D values in the millimolar or high micromolar range.[5] It has been assumed that the multivalency of carbohydrate–protein interactions[6] is fundamental to their biological effect,[7] but so far, multivalency effects occurring in carbohydrate recognition have not been conclusively understood.[8] Therefore, molecular mimetics are needed to study carbohydrate–protein interactions in detail; such designer molecules should preferably try to mimic the complexity and heterogeneity of the cell surface. There is a broad range of approaches, stretching from carbohydrate libraries to distinct multivalent glycoconjugates (such as glycodendrimers and glycopolymers, for example),[9] all of which can be regarded as 'one-dimensional' setups; however, a nano-size molecular array such as the glycocalyx rather requires a 'two-dimensional', or ideally 'three-dimensional', molecular mimicry. Consequently, surface-based glycomimetics have recently gained much interest including glyconanoparticles,[10] glycoarray technology,[11] and in particular self-assembled monolayers (SAMs).

We became interested in the synthesis of sugar-modified SAMs, the so-called 'glyco-SAMs',[12] for the study of molecular interactions of carbohydrates, because glyco-SAMs have the potential to serve as tailor-made carbohydrate arrays, allowing control over density and orientation of the carbohydrate ligands, in addition to being amenable to many spectroscopic techniques like surface plasmon resonance (SPR), ellipsometry or AFM (atomic force microscopy).

For the formation of glyco-SAMs on gold, carbohydrate-terminated long-chain thiols or thioacetates have to be prepared, as well as their non-carbohydrate analogs, in order to allow 'dilution' of the monolayer to avoid steric hindrance at the surface, which continuously increases during assembly. Furthermore, it has been our goal to include labelled molecules in SAM formation to facilitate characterization of the monolayer once it is formed. Finally, as SAM formation often is hard to control, we sought a method to allow modification of a preformed monolayer. This has led us to a modular approach for the controlled construction of glyco-functionalized SAMs involving a sequence of formation of a initial monolayer and its modification by the attachment of biological relevant headgroups 'on SAM'. We chose the copper(I)-catalysed modification of Huisgen's 1,3-dipolar cycloaddition of alkynes to azides,[13] introduced more-or-less simultaneously in 2001 by Meldal[14] and Sharpless.[15] This reaction allows modification of a preformed SAM without the requirement for classical workup or purification procedures.

This so-called 'click' chemistry[16] has recently become a popular method in organic and biological chemistry, including the field of self-assembled monolayers. Modification of alkyne-terminated SAMs with azido-functionalized aromatic molecules, nucleotides and carbohydrates has been shown,[17] as well as attachment of functionalized alkynes to azido-terminated SAMs on silicon dioxide,[18] and binding of biologically and electrochemically relevant molecules like oligonucleotides and ferrocenes to SAMs.[19] Recently, 1,3-dipolar cycloaddition has been used for construction of carbohydrate SAMs in order to measure carbohydrate–protein interactions.[20]

Here we report an extended study on the synthesis of functionalized molecules for the formation of glyco-SAMs, their modification in a modular approach utilizing 1,3-dipolar cycloaddition of alkynes and azides, and biophysical investigation of a collection of different SAMs. An essential requirement for the molecules synthesized for self-assembly is that they have to contain oligoethylene glycol (OEG) portions, to suppress non-specific adhesion of proteins to the SAM,[21] and to allow accurate

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measurement of specific ligand–receptor interactions.[22] To combine 'click' chemistry with our rather complex oligoethylene glycol spacers, first in solution and eventually 'on SAM', was one of the main challenges of this project.

Results and discussion

Our modular approach for formation of glyco-SAMs included introduction of both biorepulsive OEG moieties as well as the carbohydrate headgroups 'on SAM' (Fig. 1), after initial monolayer formation using a basic thio-functionalized type of spacer. The latter molecules were synthesized first.

Synthesis of basic spacer molecules

Two types of spacer molecules were synthesized, those with a terminal alkyne group to allow 'click on SAM' by 1,3-dipolar cycloaddition, and a second group of non-alkyne analogs, which are unreactive under cycloaddition conditions and serve to 'dilute' the alkyne-functionalized monolayer.

Thus, the commercially available 10-undecenic acid (**1**, Scheme 1) was quantitatively converted into the methyl amide **2** *via* its mixed anhydride using isobutyl chloroformate (IBCF) under basic conditions. Thioacetic acid was then added to the terminal double bond of **2** in a photoinduced radical reaction initiated with AIBN, providing **3**. An alkyne-functionalized analog of **3**, the amide **5**, was obtained from well known acetyl-protected mercaptoundecanoic acid **4**[23] by peptide coupling with propargylamine, again according to the mixed anhydride method using IBCF.

With the first two basic spacer types **3** and **5** in hand, oligoethylene glycol-containing spacers had to be prepared next, to allow the construction of monolayers resistant to non-specific binding of proteins. An oligoethylene unit can very easily be introduced using the monomethyl ether of hexaethylene glycol. Esterification of **4** with this alcohol under standard conditions employing DCC and DMAP led to the thio-functionalized

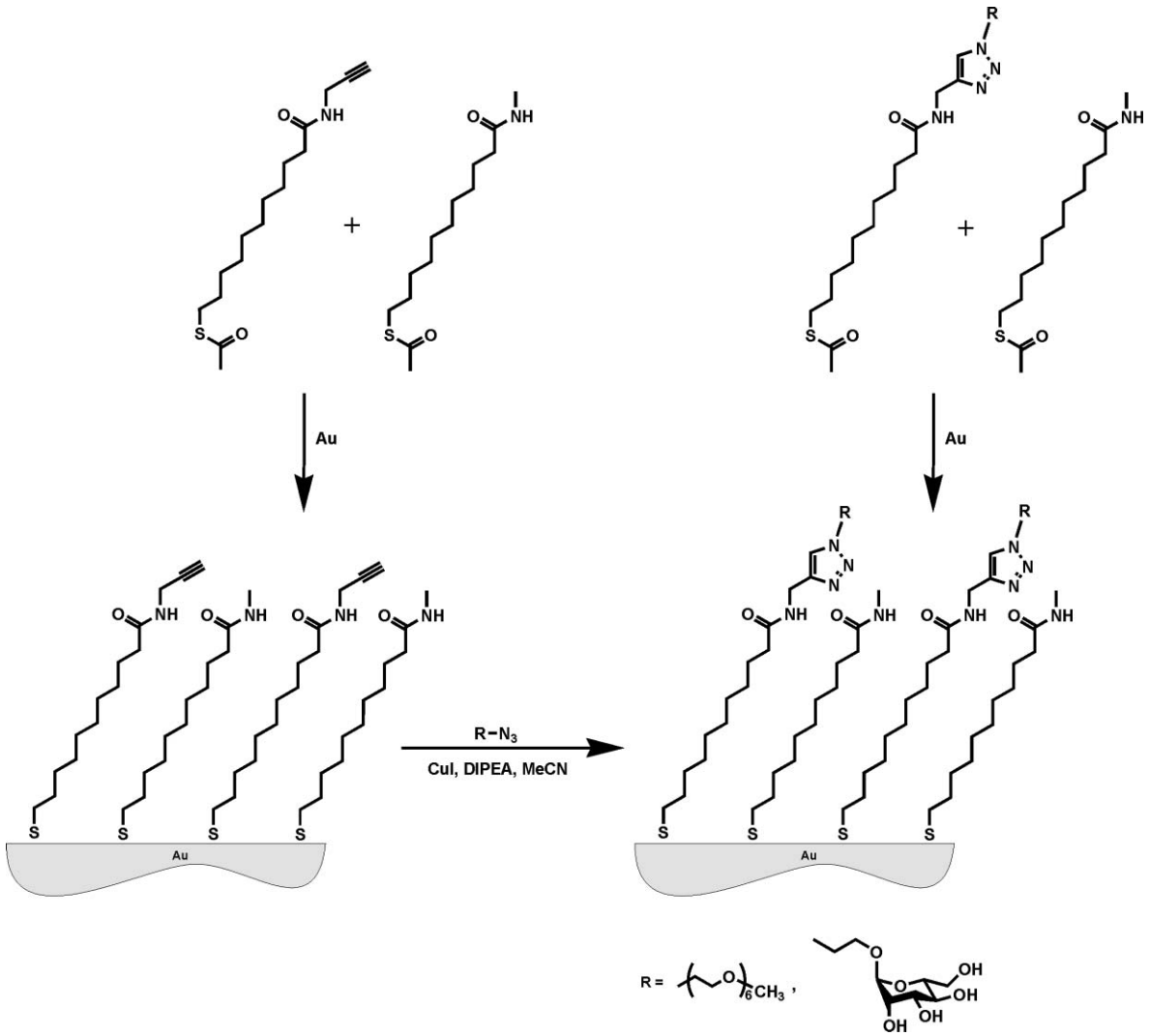
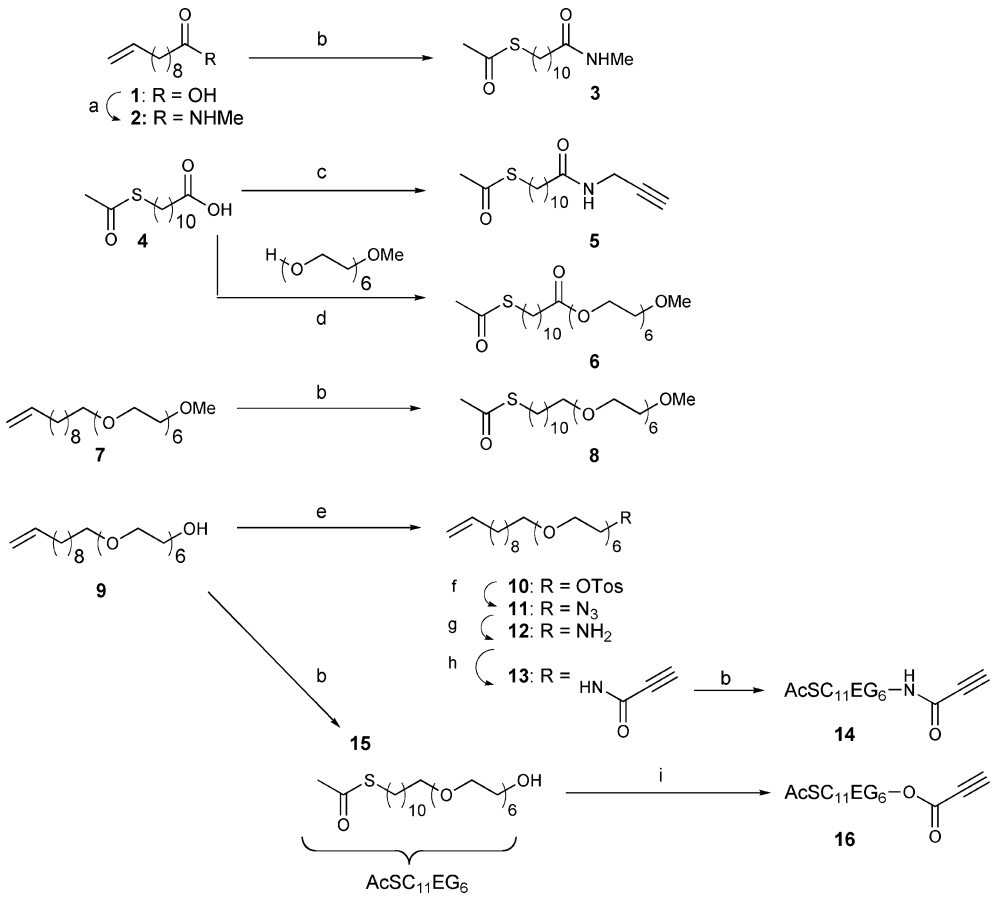


Fig. 1 Surface modification of a mixed SAM on gold by coupling of azides. The two possible concepts of generating mixed SAMs containing one functional compound generated by 'click' chemistry are shown. Right: direct assembly of the preformed molecules onto the surface. Left: SAM formation followed by 'click' reaction of the reactive groups. The latter approach permits more flexibility in the surface modification, both chemically and spatially.



Scheme 1 Synthesis of basic spacer molecules. *Reagents and conditions*: a) NH_2Me, NPr_3, IBCF, DMF, 0 °C → RT, 1.5 h, quant.; b) AcSH, AIBN, THF, *hν*, RT, 3 h, 86% for **3**, 95% for **8**, 83% for **14**, 97% for **15**; c) propargyl amine, NPr_3, IBCF, DMF, RT, 2 h, 78%; d) DCC, DMAP, DCM, −20 °C → RT, 15 h, 75%; e) TosCl, DABCO, ethyl acetate, 4 Å molecular sieves, 0 °C → RT, 1.5 h, 82%; f) NaN_3, TBAI, DMF, 70 °C, 2 h, 97%; g) PPh_3, 1 : 1 THF–H_2O, RT, 3 d, 63%; h) propiolic acid, DCC, DCM, 0 °C → RT, 3 h, 70%; i) propiolic acid, DCC, DMAP, DCM, −26 °C → RT, 15 h, 73%.

alkyloxy spacer **6** in 75% yield. On the other hand, esterification could be avoided when the literature-known alkene **7**[24] was used as the starting material, which can be obtained from bromoundec-11-en and monomethyl hexaethylene glycol under Williamson etherification conditions in over 90% yield. Radical addition of thioacetic acid under UV irradiation yielded the methoxy-terminated spacer **8** in excellent 95%. To achieve the synthesis of an alkyne-functionalized analog of **8**, the alkyl oligoethylene alcohol **9** was subjected to a sequence of OH-activation to yield the tosylate **10**, followed by nucleophilic substitution and Staudinger reduction of the resulting azide **11** to the amine **12**. It is noteworthy that the use of DABCO, according to Hünig's procedure,[25] allowed us to omit pyridine in the tosylation step.

Next, amide coupling of the amine **12** with propiolic acid had to be addressed. In this case, however, the use of IBCF led to decomposition, whereas DCC-mediated peptide coupling was successful and delivered alkyne **13** in 70%. This was in turn converted into the terminal acetyl thioate **14** in a photoaddition reaction, as described earlier. A byproduct carrying thioacetyl units at both termini of the molecule was formed in this step; it was easily removed during chromatographic purification. The amount of byproduct formation was diminished when irradiation was performed at wavelengths >295 nm using a cut-off filter. This allowed the preparation of the target alkyne **14** in 30% yield over five steps starting with **9**.

As molecules with internal amide linkages tend to form rather stable monolayers due to intermolecular hydrogen bridging,[26] an ester analog of **14** was sought for comparison reasons. This is easily accessible in two steps from **9**, through radical addition of thioacetic acid followed by DCC-mediated esterification of the resulting alcohol **15**[27] with propiolic acid, delivering **16** in 71% overall yield.

Optimization of 1,3-dipolar cycloaddition conditions for the convergent synthesis of spacer molecules

According to our synthetic plan, the convergent synthesis of more-or-less complex spacer molecules using 1,3-dipolar cycloaddition had to be accomplished next. The literature provides a vast number of different reaction conditions for the copper(I)-mediated 1,3-dipolar cycloadition, with a variety of solvents, bases and catalysts involved. Our first investigation of reaction conditions was performed with commercially available undecynic acid (**17**) and the acetylthio-functionalized alkyne **5**. Cycloaddition of the alkyne **17** to dodecyl azide delivered triazole **18** in 50% yield, whereas the reaction of **5** with the tosyl-functionalized triethylene

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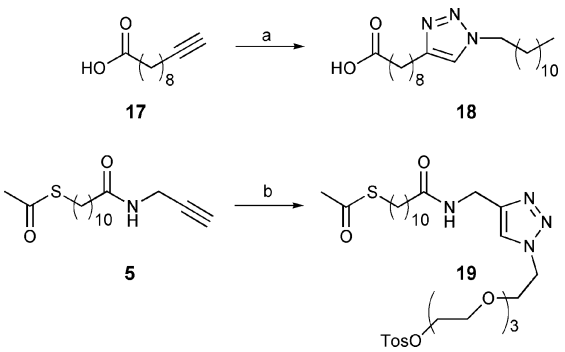


Scheme 2 Convergent synthesis of spacer molecules employing 1,3-dipolar cycloaddition. *Reagents and conditions*: a) 1-azidododecane, CuI, 2,6-lutidine, MeCN, 0 °C → RT, 15 h, 50%; b) $TosEG_4N_3$, CuI, DIPEA, MeOH, RT, 15 h, 62%.

glycolylazide $TosOEG_3N_3$ gave the cycloaddition product **19** in a reasonable 64% yield (Scheme 2).

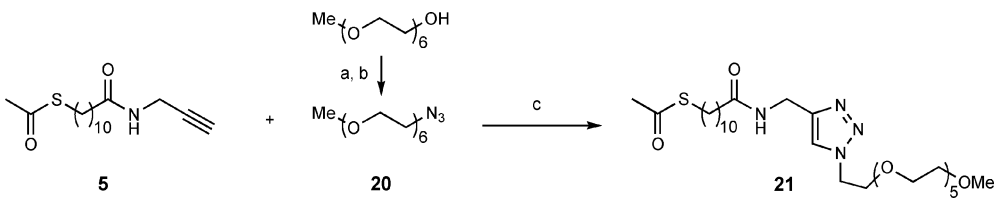
To further optimize the reaction conditions of the copper(I)-catalysed Huisgen reaction, to allow 'click on SAM' with the molecules prepared here, we performed a systematic study in a homogeneous solution employing alkyne **5** and hexaethylene glycolylazide **20** leading to triazole **21** after 1,3-dipolar cycloaddition (Scheme 3). Published reaction conditions, using water as solvent and a Cu(II) ascorbate-system,[28] were unsuccessful in our case. This might be due to the extremely polar hexaethylene glycol moiety causing problems during workup and purification in an aqueous system.

As indicated in Table 1, the solvent, base, temperature, reaction time and catalyst were varied in the synthesis of **21**. Starting materials were employed as 0.1 mM solutions. Reactions were finished after about 8 h, regardless of solvent or temperature. Extension of reaction time up to 63 h had no effect on the yields. Also, varying the temperature had little effect on conversion. As the reaction conditions were intended to be applicable 'on SAM', temperatures over 45 °C were not examined thoroughly. As ligation of the copper ion is of importance for the reaction, it can be understood that both the nitrogen base applied as well as the solvent have strong effects on the outcome of the reaction. Common nitrogen bases such as 2,6-lutidine and diisopropylethyl amine (DIPEA) were tested. Interestingly, the combination of methanol with DIPEA as the base (Table 1, entry 8) is a low yielding solvent–base system, as is lutidine in acetonitrile (entry 6). On the other hand, DIPEA in acetonitrile or lutidine in methanol provide good yields, with the desired 1,4-triazoles being the only cycloaddition products. The reaction conditions with elevated temperatures applied in entries 9 and 11 led to regioisomeric mixtures of the 1,4- and 1,5-cycloaddition products.

Finally, a 1 : 1 mixture of DMF and methanol with 0.2 equivalents of cuprous iodide and without any nitrogen base was identified to be the best system in homogeneous solution, with a 96% yield at 45 °C.

Synthesis of functionalized spacers under optimized 1,3-dipolar cycloaddition conditions

The results of our study on advantageous reaction conditions for the 1,3-dipolar cycloaddition of ethylene glycol-type spacers (*cf.* Table 1) paved the way for the synthesis of diversely functionalized spacers, suited for self-assembly of monolayers. Thus, the important dendritic building block **22**[29] was converted into the alkyne **23** using propiolic acid in a HATU-mediated peptide coupling reaction[30] (Scheme 4). Then, 1,3-dipolar cycloaddition with the azide **24**[31] proceeded in very good yield (91%). Deprotection of the resulting dendritic triester **25** using TFA in 1,2-dichloroethane

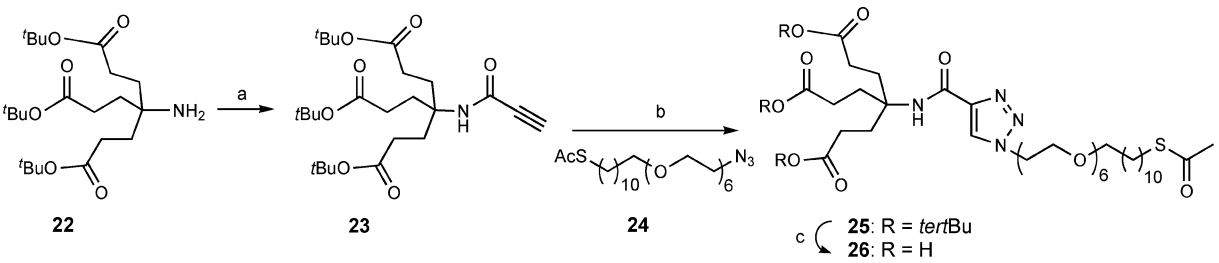


Scheme 3 Optimization of 1,3-dipolar cycloaddition conditions employing azide **20**. *Reagents and conditions*: a) TosCl, DABCO, ethyl acetate, 0 °C → RT, 1.5 h, 80%; b) NaN_3, TBABr, DMF, 90 °C, 3.5 h, 72%; c) see Table 1.

Table 1 Reaction conditions for the 1,3-dipolar cycloaddition of **5** and **20** leading to **21**

Entry	Base	Solvent	Temp./°C	Catalyst	Time/h	Yield (%)[a]
1	1.3 eq. DIPEA	MeCN	RT	1 eq. CuI	2	<10
2	1.3 eq. DIPEA	MeCN	45	1 eq. CuI	2	23
3	1.3 eq. DIPEA	MeCN	45	1 eq. CuI	8	62
4	1.3 eq. DIPEA	MeCN	45	5 eq. CuI	8	65
5	1.3 eq. DIPEA	MeCN	RT	1 eq. CuI	15	86
6	1.3 eq. 2,6-lutidine	MeCN	RT	1 eq. CuI	15	33
7	1.3 eq. 2,6-lutidine	MeOH	RT	1 eq. CuI	15	74
8	1.3 eq. DIPEA	MeOH	RT	1 eq. CuI	15	43
9	1.3 eq. DIPEA	toluene	100	1 eq. CuI	15	80[b]
10	—	H_2O–^{t}BuOH	45	$CuSO_4$ + Na ascorbate	15	50
11	—	DMF–MeOH (1 : 1)	70	0.2 eq. CuI	15	91[b]
12	—	DMF–MeOH (1 : 1)	45	0.2 eq. CuI	15	96

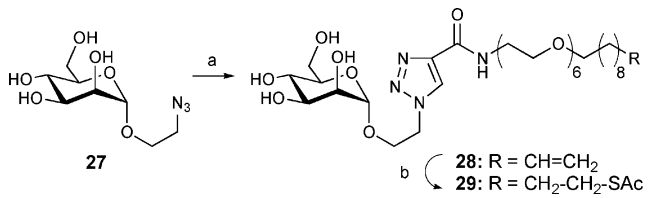
[a] Isolated yield. [b] Both regioisomeric triazoles were detected.



Scheme 4 Synthesis of dendritic triacidic spacer **26**, employing 1,3-dipolar cycloaddition. *Reagents and conditions*: a) propiolic acid, HATU, DIPEA, DMF, 0 °C → RT, 18 h, 56%; b) CuI, DIPEA, MeCN, RT, 20 h, 91%; c) TFA, DCE, RT, 1 h, 98%.

(DCE) provided the target compound **26** in almost quantitative yield. This molecule is of interest to modify polarity and acidity, respectively, of SAMs, which is of importance with regard to the high sialic acid content of many cells.

We then applied these reaction conditions to the synthesis of carbohydrate-decorated spacers. The known mannoside **27**[32] was employed as the azido component, whereas spacer **13** (Scheme 1) was used as the alkyne. Cycloaddition with CuI in DMF–MeOH (1 : 1) at 45 °C yielded 1,4-triazole **28** in 73%, which was subsequently turned into the thioacetate **29** following the usual UV irradiation protocol in THF at room temperature (Scheme 5).

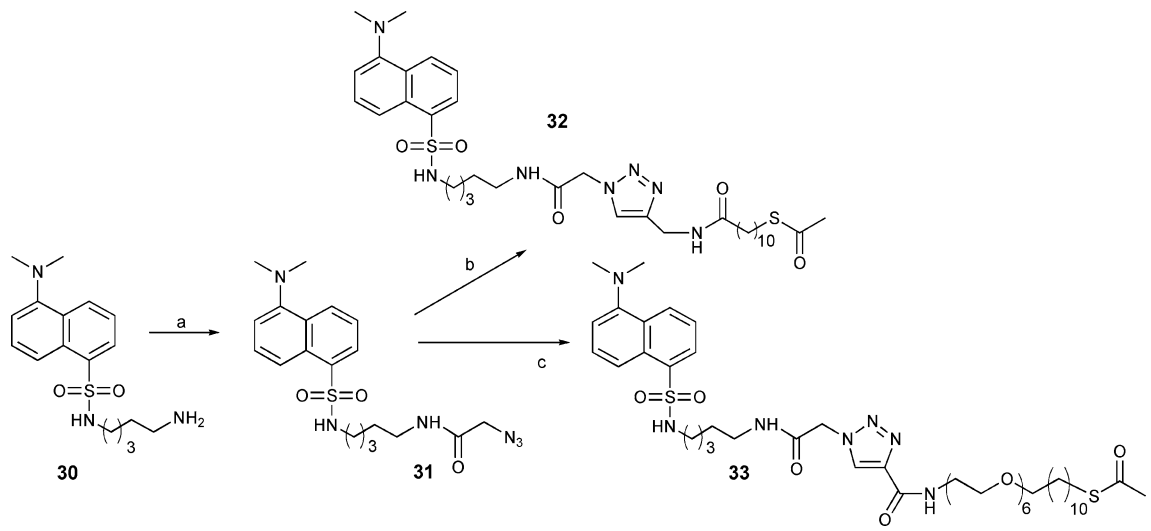


Scheme 5 Synthesis of D-mannose-decorated thioacetate spacers. *Reagents and conditions*: a) alkyne **13**, CuI, DMF–MeOH, 45 °C, 15 h, 73%; b) AcSH, AIBN, THF, *hν*, RT, 5 h, 40%.

Finally we set out to apply 1,3-dipolar cycloaddition to the synthesis of fluorescence-labeled spacers, suited for SAM formation, as labelling is an important tool in bioassays. In addition, fluorescence labels can be used to investigate the properties of an actual SAM.[33] Commercially available dansylcadaverin (**30**, Scheme 6) was used as fluorescence dye and had to be converted into an azido-functionalized derivative for 1,3-dipolar cycloaddition to a spacer alkyne. Conversion of the terminal amino group in **30** into the respective azide *via* a metal-catalysed diazo transfer with triflyl azide[34] gave the desired product in only 63% yield. Therefore, we decided to employ azidoacetic acid, which is easily accessible from chloroacetic acid in a quantitative reaction with sodium azide in an aqueous medium. DCC-mediated peptide coupling with **30** then led to the azide **31** in 92% yield. 1,3-Dipolar cycloaddition to the spacer alkynes **5** and **14** delivered triazoles **32** and **33**, respectively. Unfortunately, cycloaddition to **33** proceeded in poor yields (around 60%).

SAM formation and 'click on SAM'

With this array of thio-functionalized molecules in hand, SAM formation and 'click on SAM' chemistry were evaluated next. Ellipsometry was used to estimate the success of different reaction conditions by determination of the layer thickness *ex situ*. Initial experiments showed that the acetonitrile–CuI–DIPEA system developed for the homogeneous solutions only works well for cycloaddition of azides to alkyne-terminated monolayers, resulting in an increase of layer thickness. In contrast, azido-terminated



Scheme 6 Synthesis of fluorescence-labelled spacers. *Reagents and conditions*: a) azidoacetic acid, DCC, HOBt, DCM, 0 °C → RT, 15 h, 92%; b) **5**, CuI, DIPEA, MeCN, 45 °C, 15 h, 84%; c) **14**, CuI, DMF–MeOH, 45 °C, 15 h, 59%.

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Table 2 Thicknesses and BSA adsorptions of SAMs of triazole-bridged **21**, formed from pure **21** or from a homogeneous mixture of reaction partners (*d*: optical thickness)

Entry	Deposition conditions	Deposition time/h	d_{SAM}/Å [a]	Δd_{BSA}/Å [b]
1	Pure **21** and EtOH	15	19.9 ± 2.7	1.8 ± 3.2
2	Mixture of **5** and **20**[c]	15	10.6 ± 1.4	8.8 ± 2.1
3	Mixture of **5** and **20**[c]	72	8.8 ± 3.0	5.0 ± 3.8

[a] $d_{SAM}^{calc} = 37.4$ Å. [b] Δd_{BSA} (Au) = 19.6 ± 1.0 Å. [c] Reaction mixture: 5.0 mM thioacetate, 5.0 mM azide, 5.0 mM CuI, 6.3 mM DIPEA, MeCN.

monolayers did not react readily with alkynes. Instead of a smooth reaction, the formation of a precipitate (presumably a copper(I)–alkyne complex) was observed, whose formation removes the catalyst from the reaction solution.

Therefore, our interest focused first on the acetylenic molecule **5**, which due to its simple structure should form densely packed monolayers. In fact, deposition of this molecule from ethanolic solution reproducibly yielded monolayers with a thickness of about 19 ± 1 Å, in excellent agreement with a theoretical value of 18.8 Å for molecules that have lost their acetyl group, being packed in the monolayer with a tilt angle of 30°.

Reaction of a monolayer formed from **5** with the ethylene glycol derivative **20** is of interest since attachment of this molecule should not only increase the layer thickness, but also render the monolayer resistant to the adhesion of proteins. Two approaches were tested (Table 2); firstly, pure triazole **21**, the reaction product of **5** and **20**, was used for monolayer formation, and secondly a homogeneous reaction mixture of alkyne **5** and azide **20** was employed under 'click' conditions. The latter option is attractive, as isolation and purification of **21** could be avoided by selectively depositing it from a reaction mixture.

The layer thickness was in all cases much smaller than anticipated, hinting on incomplete monolayer formation. This is a commonly observed phenomenon for longer molecules, in particular with OEG headgroups, since unfolding of the coiled-up chain costs too much entropy.[35] Nevertheless, the resulting monolayer almost completely suppresses the adsorption of bovine serum albumin (BSA), a 'sticky' protein commonly used to test bioresistance that typically results in layers about 20 Å thick (depending on the deposition conditions). The situation becomes worse for the layers prepared from the reaction mixtures: even after prolonged immersion (entry 3) only partial monolayers could be attained, and these are not bioresistant.

If entropy is really the force suppressing the formation of denser monolayers, it would be reasonable to expect that the post-monolayer formation chemistry, such as 'click on SAM' should result in thicker layers. In fact, when the complete monolayer of **5** was treated with **20** using the above-mentioned catalyst solution at room temperature for 16 h, a layer thickness of 31.7 ± 1.8 Å (Δd = 11.4 ± 1.0 Å; Table 3, entry 1), significantly higher than attained from the preformed molecule **21**, resulted.

Nevertheless, this monolayer was still thinner than the expected (37.4 Å), showing again the influence of entropy, meaning that a number of reaction sites remain inaccessible. As a test for this hypothesis, we used so-called diluted monolayers consisting of a mixture of reactive molecule **5** with its unreactive derivative **3**. The presence of the unreactive molecule should render all the reactive sites accessible for the azide, thus permitting complete transformation. In fact, the transformation resulted not only in layers approaching the expected thickness (column 4 in Table 3) but exceeding it. Since any reactivity of the methyl derivative **3** could be excluded (as shown in entry 4 in Table 3), this excess thickness can only be interpreted as a result of a higher ratio of **5**:**3** in the SAM than in the deposition on.

Table 3 1,3-Dipolar cycloaddition of the spacer azide **20** (MeO-EG_6-N_3) to SAMs formed from a mixture of the amide **3** and alkyne-terminated **5** (*d*: optical thickness)

Entry	Ratio **5**:**3**[a]	d_{SAM}/Å	d_{SAM}^{calc}/Å	Δd_{click}/Å	Δd_{click}^{calc}/Å
1	1 : 0	20.3 ± 0.8	18.8	11.4 ± 1.0	18.6
2	1 : 3	20.3 ± 0.6	17.2	9.2 ± 0.7	4.7
3	1 : 4	18.9 ± 0.9	17.0	7.0 ± 1.1	3.7
4	0 : 1	16.0 ± 2.4	16.6	−0.6 ± 1.0	0

[a] Ratio in the deposition solution.

Since in principle all the alkyne sites should be reactive in these SAMs, we set out to use higher temperatures for the 'click' reaction. As trivial as this might sound, it should be kept in mind that most SAMs are desorbed into solution at elevated temperature.[36] We therefore tested up to which temperature diluted SAMs deposited from 1 : 1 mixtures of **3** and **5** remain stable in acetonitrile (Table 4).

Surprisingly, even up to 80 °C no complete desorption took place, although the upper limit for keeping the monolayers unaltered seemed to be 60 °C. We therefore decided to carry out 'click' reactions using azide **20** up to this temperature. For this, pure monolayers of **5** were used, (i) to learn if even in a dense monolayer all reactive groups can be approached under these conditions, and (ii) to be certain of the maximum attainable thickness after the 'click' reaction, since – as shown above – the molecular ratio in a diluted monolayer does not need to be the same as in the deposition solution. As can be seen in Table 5, the elevated temperature had only a minor influence on the completeness of the 'click' reaction: at 50 °C and 60 °C a

Table 4 Temperature stability in acetonitrile of SAMs formed from 1 : 1 mixtures of alkyne **5** and amide **3** (*d*: optical thickness)

	d_{SAM}/Å [a]	
Temperature/°C	Before treatment	After treatment
50	14.1 ± 0.3	13.1 ± 0.2
60	12.6 ± 0.3	12.9 ± 0.5
70	14.2 ± 0.1	13.1 ± 0.2
80	16.1 ± 0.4	13.7 ± 1.0

[a] $d_{SAM}^{calc} = 17.7$ Å.

Table 5 'Click' reaction of $MeOEG_6N_3$ (**20**) to SAMs of **5** at different temperatures (*d*: optical thickness)

Entry	d_{SAM}/Å [a]	Temperature/°C	Time/h	Δd_{click}/Å [b]	Δd_{BSA}/Å [c]
1	20.3 ± 0.8	25	15	11.4 ± 1.0	4.9 ± 0.8
2	19.2 ± 0.6	40	15	11.5 ± 0.9	2.0 ± 0.9
3	17.8 ± 0.7	50	16	14.9 ± 1.6	0.5 ± 2.6
4	16.3 ± 1.2	60	15	13.1 ± 1.9	−1.0 ± 2.2

[a] $d_{SAM}^{calc} = 18.8$ Å. [b] $\Delta d_{click}^{calc} = 18.6$ Å. [c] Δd_{BSA} (Au) = 20.0 ± 0.4 Å.

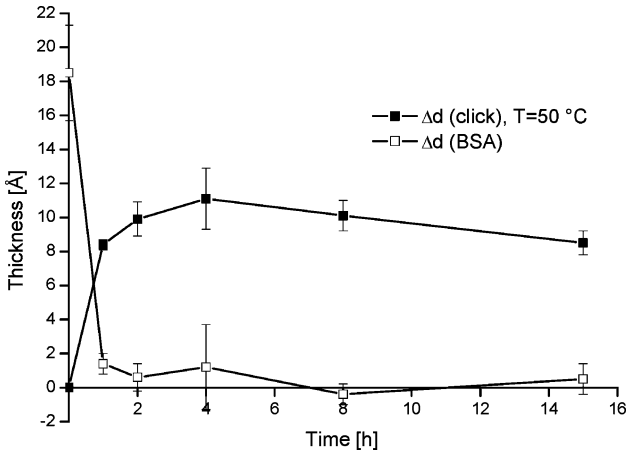

Fig. 2 Time-dependence of the added layer thickness ('click' reaction of **20** to SAM of **5** according to entry 3 in Table 5) and the BSA resistance.

couple of Å in thickness were gained (compared to Table 3, entry 1), but no complete layer was formed in either case.

Protein resistance of the monolayers on the other hand became significantly better with increasing reaction temperature (Table 5, column 5), an important criterion for future applications. In continuation of the optimization of the reaction conditions, the time dependence of the layer properties was studied after reaction at 50 °C. In a series of time-dependent experiments it was again observed that the increase in thickness never reached the theoretical value of 18.6 Å, but levelled off at about 13 Å after 4 h (Fig. 2). Despite this, complete bioresistance was not achieved before 8 h reaction time, a situation mirroring the temperature-dependent experiments. We therefore decided to perform all further 'click' reactions for 16 h at 50 °C.

Since the introduction of bioresistance was not the primary goal of this work, but to use bioresistant molecules as a background to attach recognition sites (in particular carbohydrate sites), we switched to the use of the OEG-containing alkyne **16** as SAM-forming material. The monolayers exclusively formed from **16** are not only thinner than calculated, but also are not bioresistant (Table 6, entry 1).

This comes to no surprise, since – as mentioned before – molecules this long hardly form perfect monolayers. In addition, these layers should be terminated with a significant number of hydrophobic alkyne groups, thus exposing adsorption sites for BSA. We hoped that dilution with the molecule **15** of proven bioresistance (entry 6) would render the monolayers bioresistant at some point. This expectation was more than fulfilled, since even a proportion as low as 9% of **15** (entry 3) induces bioresistance in the system.

Table 6 Bioresistance of EG_6-containing monolayers as a function of the number of alkyne groups exposed in the SAM (*d*: optical thickness)

Entry	Ratio **16**:**15**[a]	d_{SAM}/Å	d_{SAM}^{calc}/Å	Δd_{BSA}/Å
1	1 : 0	15.8 ± 1.6	36.8	14.8 ± 2.2
2	50 : 1	15.3 ± 0.9	36.7	3.1 ± 1.7
3	10 : 1	22.6 ± 1.5	36.5	1.4 ± 2.1
4	1 : 1	21.3 ± 0.2	35.3	0.9 ± 0.4
5	1 : 2	20.3 ± 0.6	34.7	−0.8 ± 0.7
6	0 : 1	24.2 ± 0.2	33.7	−2.2 ± 0.4

[a] In solution.

It should be mentioned at this point that the *de facto* proportion of **15** in the SAMs may exceed the one in solution – the reverse of the process previously observed for the **5**:**3** system. Experiments to clarify this point by determination of the number of reactive acetylenic groups by 'click on SAM' using the azide **20** again resulted in a surprise: instead of a gain in thickness, we observed a loss of matter as well as a loss of bioresistance. For example, a 1 : 1 diluted monolayer of **16** and **15**, being completely bioresistant beforehand, became thinner by almost 12 Å instead of thickening by 18.8 Å, accompanied by adherence of 3 Å of BSA compared to 0 Å before the cycloaddition. Extensive experiments (data not shown) showed that OEG-terminated thiolate SAMs in general are not compatible with Cu^+ ions in hot acetonitrile. We assume that under these conditions a Cu^+–thiolate complex is formed which becomes soluble due to the very polar OEG part of the molecule. Unfortunately no conditions could be found to achieve a 'click on SAM' reaction without desorption.

Because of this, we returned to the system based on the monolayers generated by **5** on gold to study carbohydrate–lectin interactions. Using the optimised conditions for 'click on SAM', the azidoethyl mannoside **27** was attached to a SAM of thioacetate **5** on gold. Surprisingly, the resulting increase of the layer thickness (10.8 ± 0.6 Å) is significantly larger than the expected value (6.5 Å). We attribute this either to physisorbed material or to some kind of side reaction attaching additional material. The water contact angles of the modified surfaces were 34 ± 1° (advancing) and 17 ± 2° (receding), corresponding to a hydrophilic surface. However, for a pure hydroxy-terminated surface the value of the advancing contact angle is too high. This suggests that the surface exposes not only OH groups, but also methylene groups. This assumption is supported by the relatively large contact angle hysteresis of *ca.* 17 Å, indicating an imperfectly ordered surface.

Since disorder should not hinder the recognition of the mannosyl groups (since, for example, the glycocalyx is not a laterally ordered system) we decided to perform SPR experiments to see if some specificity for concanavalin A (ConA), a strongly binding plant lectin, exists. In fact, the pure monolayer of **5** turned out not to be very adhesive for ConA, thus permitting a clear distinction from the very strong response obtained from the mannosyl-terminated monolayer (Fig. 3).

Since these data were obtained on a home-built SPR system, the quality of the data does not permit a quantitative assessment yet, but the data suggest that the specific recognition is at least five times stronger than the non-specific adhesion, thus proving our modular system suitable for further investigation.

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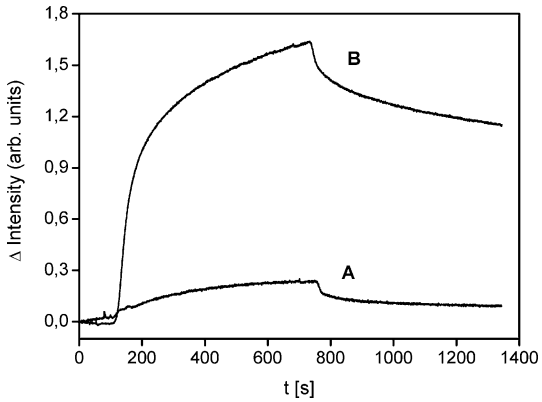

Fig. 3 The adsorption of concanavalin A onto a native monolayer of alkyne **5** (A) as well as onto a monolayer modified by 'click on SAM' chemistry using the azidoethyl mannoside **27** (B) as followed by SPR. Although some non-specific adsorption takes place in case A, the recognition of the mannosyl substituents is dominant.

Conclusion

We have reported the synthesis of a series of differently functionalized molecules for the formation of SAMs and their utilization in a modular approach employing 1,3-dipolar cycloaddition of alkynes and azides. Thus, we have provided a molecular toolbox, allowing for the preparation of biorelevant monolayers, including carbohydrate decoration. We have extensively studied the parameters of SAM formation and reported on drawbacks and limitations as well as on breakthroughs *en route* to glyco-SAMs as glycocalyx models.

We have demonstrated that 'click on SAM' chemistry permits the modification of surfaces with biologically relevant headgroups such as bioinert OEG chains or substrate groups recognized by lectins. It seems generally advisable to have the acetylenic compound for the Huisgen reaction in the SAM and the azide in solution to avoid precipitation of presumably inactive Cu acetylides rendering them unsuitable for a surface reaction. For the ligation with long OEG azides, the 'click on SAM' approach permits the synthesis of denser SAMs with a reliable bioresistance against non-specific protein adhesion as opposed to the layers obtained from the pre-formed, complete molecules. Although the optimizations of the click reactions conditions were performed on a single and stable short-chain SAM using OEG as a ligand, we have shown that the reaction conditions were also useful for the attachment of bio-entities such as mannose, resulting in surfaces recognized by lectins. Investigations are now under way to understand how surface libraries made from this building-block toolbox influence the recognition process by, for example, proteins.

Experimental

General remarks

Reactions were carried out in dried glassware under argon or nitrogen and using distilled solvents unless otherwise indicated. THF was dried by distillation from sodium/potassium ketyl, methanol by distillation from magnesium turnings, and dichloromethane by distillation from calcium hydride, each under argon. Commercially available starting materials, reagents and pure DMF were used without further purification. TLC was performed on GF254 silica gel plates (Merck), detection was effected by the use of UV light (254 nm and 366 nm) and with mixtures of either 10% sulfuric acid in ethanol or cerium(IV) sulfate and phosphomolybdate in 10% sulfuric acid followed by heat treatment. Flash chromatography was performed on silica gel 60 (230–400 mesh, particle size 0.040–0.063 mm, Merck). NMR spectra were recorded on Bruker DRX 500 (500 MHz for ^{1}H, 125.47 MHz for ^{13}C) and ARX 300 instruments (300 MHz for ^{1}H, 75.47 MHz for ^{13}C). Spectra were calibrated with respect to the solvent peak ($CDCl_3$ 7.24 ppm for ^{1}H and 77.0 ppm for ^{13}C; [D_4]methanol 3.35 ppm for ^{1}H and 49.30 ppm for ^{13}C.) Assignment of the peaks was achieved with the aid of 2D NMR techniques (^{1}H–^{1}H-COSY and ^{1}H–^{13}C-HSQC). Peak values that could not be unequivocally assigned to one atom, and may therefore be interchangeable, are marked with an asterisk. In some cases, amide isomers delivered a double set of signals, as indicated by '‡'.

Hydrogen and carbon atoms within the scaffold are indexed as follows: the sugar residue is numbered as usual from 1 to 6 with the anomeric position being number 1, and aglycon atoms are numbered in ascending order starting with the atom adjacent to the glycosidic bond being number 7, as exemplified in Fig. 4 for compound **29**. For spacer molecules without a sugar moiety, the acetyl thio-group was defined as the terminus of the molecule, and carbon and hydrogen atoms were numbered accordingly, as exemplified for **29**, **33** and the dendritic molecule **25** (Fig. 4). MALDI-TOF mass spectra were recorded on a Bruker Biflex III 19 kV instrument, with norharmane (9*H*-pyrido[3,4-*b*]indole) in

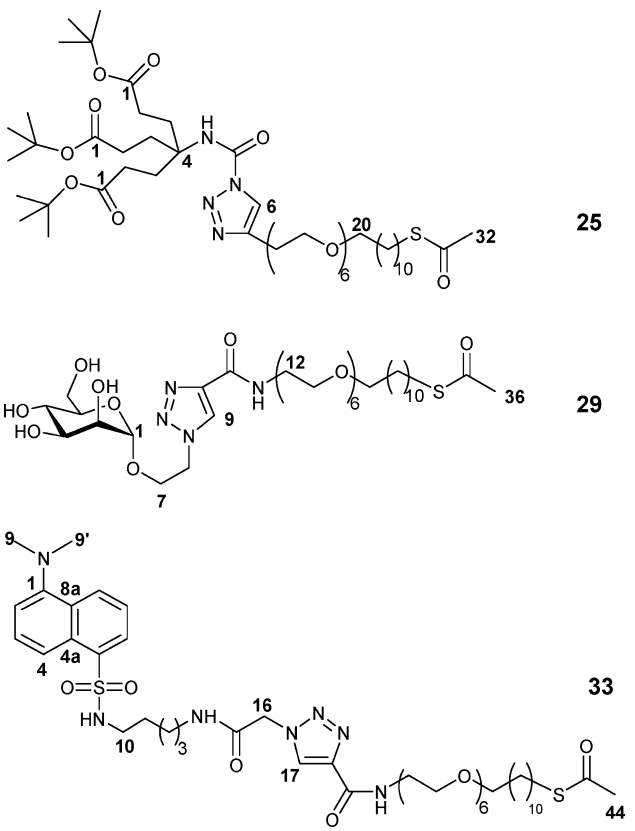

Fig. 4 Numbering of hydrogen and carbon atoms for assignment of NMR data.

THF being used as a matrix. For sample preparation, a drop of matrix solution was first placed on the target and left to evaporate. Afterwards, a solution of the sample in THF or in methanol was placed on the pre-crystallized matrix.

Even though NMR spectra showed no contamination and analytical HPLC showed no impurities, correct elemental analyses could not be obtained for most of the reported substances. ESI-MS measurements were consulted to prove purity. High-resolution mass spectra were recorded on a Mariner (Part-No. V800600) instrument. For analytical HPLC chromatography a Merck-Hitachi machine with a diode array detector L-7455 was used with either LiChrosorb® RP-8 7 μm silica or a Chromolith® performance RP-18 100 × 4.6 mm column. Preparative HPLC chromatography was carried out on a Shimadzu system with a Merck Hibar® RT250–25 mm column with LiChrosorb® RP-8 7 μm silica.

Ellipsometric contact angle measurements and protein adsorption experiments were performed under ambient conditions. Ellipsometry was performed on a SE 400 (Sentech Instruments GmbH) ellipsometer under an incident angle of 70° at a wavelength of 633 nm. Water contact angles were measured by the sessile drop method using a Multiskop (Optrel GBR, Germany) instrument.

SPR measurements were also carried out on the Multiskop instrument using the Kretschmann geometry with prism coupling (BK7), p-polarized laser radiation (785 nm) being used for the excitation of the plasmon. The prism and the gold-covered glass substrate were optically connected using a matching fluid with a refractive index of $n = 1.51$. Home-made PDMS chambers with a volume of 36 μL were used as flow cells. The solutions were pumped through the cell using syringe pumps (Braun Perfusor Secura, Germany).

Au(111)-covered substrates for the SAM formation were prepared by electron beam deposition of 1.5 nm of chromium as adhesion promotor followed by the deposition of 200 nm of gold onto Si(100) wafers (Wacker Siltronic AG). For the SPR experiments, gold-covered microscope slides (2 nm Cr, 50 nm Au) were used.

A Harrick Plasma Cleaner/Sterilizer PDG-32G was used to generate a microwave-induced hydrogen plasma for the cleaning of the gold surfaces.[37]

Monolayer formation

Before use, the gold-covered substrates were cleaned by the treatment with hydrogen plasma for 60 s. Thiolate SAMs were formed by immersion of the cleaned gold substrates in 5 mM solutions of the corresponding thioacetate in dry ethanol overnight under nitrogen. After immersion, substrates were rinsed thoroughly with ethanol and dried in a stream of nitrogen.

Monolayer modification by 'click on SAM'

Substrates covered with alkyne-terminated monolayers were treated with a solution of CuI (5.0 mM), DIPEA (6.5 mM) and the corresponding azide (5.0 mM) in oxygen-free acetonitrile overnight at a temperature of 50 °C. Then the substrates were thoroughly rinsed with acetonitrile, immersed in demineralised water for 2 h to remove physisorbed material and dried in a stream of nitrogen.

Protein adsorption

The samples were immersed for 2 h in a solution of 1 mg mL^{-1} bovine serum albumin (BSA) in aqueous KH_2PO_4/K_2HPO_4 buffer (pH = 7.0). After immersion each sample was rinsed with 100 cm^3 of demineralised water and dried in a stream of nitrogen. The amount of adsorbed BSA was determined by ellipsometric measurements.

Ellipsometry

Optical film thicknesses were determined assuming a complex refractive index $N = n - ik$ with a real part $n = 1.45$ and an imaginary coefficient $k = 0$ for both the SAMs as well as the BSA adlayers.[38] The parameters n and k of the gold substrates were obtained by ellipsometric measurements of the plasma-cleaned films before monolayer formation. For each experiment six readings were recorded to calculate an average value as well as the standard deviation.

Contact angle

Contact angles were measured for water as contact fluid. The droplets were imaged by a CCD camera and the contact angle was calculated from the shape of the recorded image using an included software. In all cases the advancing and the receding contact angle was determined. The value of each contact angle (as well as its standard deviation) was obtained by averaging six readings.

SPR measurements

The binding of concanavalin A (ConA) to mannosyl-terminated monolayers was monitored using surface plasmon resonance spectroscopy. The angle shift was calculated from the resonance angles determined before and after lectin adsorption. The measurements were carried out in HEPES-buffered saline (150 mM NaCl, 10 mM NaHEPES, 0.005% Tween 20) containing Ca^{2+} (1 mM $CaCl_2{\cdot}2H_2O$) and Mn^{2+} (1 mM $MnCl_2{\cdot}4H_2O$) ions for ConA activation. Briefly, adsorption was performed by first running buffer until a stable signal was observed, followed by 10 μM of ConA dimer in buffer solution (10 min) and then again buffer (10 min). The resonance angles were determined after the first and the second buffer purge, respectively. For all experiments a flow rate of 30 mL h^{-1} was chosen.

$H_2C{=}C_9C(O)NHMe$ (2). 10-Undecenic acid (0.91 g, 10.85 mmol) was dissolved in dried DMF (15 ml) and cooled to 0 °C. Tripropylamine (4.13 ml, 21.70 mmol) and IBCF (1.48 ml, 11.40 mmol) were added and the reaction mixture was stirred for 30 min. Then 2 M methylamine solution in THF (5.43 ml, 10.85 mmol) and more tripropylamine (2 ml, 10.85 mmol) were added, the reaction mixture was warmed to ambient temperature and kept stirring overnight. Subsequently the solvents were removed under reduced pressure and the colourless crude product was purified by column chromatography on silica using ethyl acetate and cyclohexane (1 : 1 → 2 : 1) as the solvent system. The alkene **2** was obtained as a colourless amorphous solid (2.11 g, 99%). The spectroscopic data were in accordance with the literature.[39]

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$AcSC_{10}C(O)NHMe$ (3). To a stirred solution of alkene amide **2** (1.00 g, 5.07 mmol) in abs. THF (30 cm^3) were added thioacetic acid (0.90 cm^3, 12.67 mmol) and AIBN (832.2 mg, 0.51 mmol) at room temperature and the UV irradiation ($\lambda \geq 295$ nm) was started and maintained for 2 h. For workup the solvents were removed under reduced pressure and the residual pale yellow crude oil was purified by flash chromatography on silica using an ethyl acetate–cyclohexane gradient (2 : 1 → 4 : 1). The product **3** was obtained as a colourless amorphous solid (1.19 g, 86%). ν_{max}(film/cm^{-1}) 3308s, 3088w, 2920s, 2849s, 1690s, 1643s, 1560s, 1459w, 1411m, 1237w, 1142m, 707w, 631m; δ_H (500 MHz, $CDCl_3$, Me_4Si) 5.82 (1H, br, N*H*), 2.86 (2H, m≈t, *J* 7.26, 2 × 12-H), 2.81 (3H, d, *J* 4.85, 3 × 1-H), 2.33 (3H, s, 3 × 14-H), 2.16 (m≈t, *J* 7.33, 2 × 3-H), 1.58 (4H, m, 2 × 4-H, 2 × 11-H), 1.27 (12H, m, 2 × (5–10-H)); δ_C (125 MHz, $CDCl_3$, Me_4Si) 196.18 (13-C), 173.81 (2-C), 36.71 (3-C), 30.64 (14-C), 29.45 (12-C), 29.32–28.74 (5–11-C), 26.25 (1-C), 25.75 (4-C); *m/z* (ESI-MS) 296.1302 (M^+ + Na. $C_{14}H_{27}NO_2S$ requires 296.1655).

$AcSC_{10}C(O)NHCH_2C{\equiv}CH$ (5). A solution of the carboxylic acid **4** (1.60 g, 6.14 mmol), IBCF (956.4 µl, 7.37 mmol) and tripropylamine (2.34 cm^3, 12.29 mmol) in abs. DMF (25 cm^3) was cooled to 0 °C and stirred for 50 min before a cooled solution of propargylamine (505.8 µl, 7.37 mmol) in abs. DMF (5 cm^3) and tripropylamine (1.17 cm^3, 6.15 mmol) were added dropwise. The clear reaction mixture was slowly warmed to room temperature and stirring was maintained for 2 h. Then the solvents were removed under reduced pressure and the crude product was purified by column chromatography on silica using ethyl acetate and cyclohexane (1 : 1.5) as solvent system. The product **5** was obtained as a colourless amorphous solid (1.43 g, 78%). ν_{max}(film/cm^{-1}) 3311s, 3287s, 2919s, 2849s, 1691s, 1634s, 1534s, 1534s, 1471m, 1454w, 1418m, 1357w, 1284w, 1232m, 1209w, 1141m, 959w, 643m, 579w, 549w; δ_H (500 MHz, $CDCl_3$, Me_4Si) 4.05 (2H, dd, *J* 2.56 and 5.24, 2 × 3-H), 2.86 (2H, t, *J* 7.37, 2 × 14-H), 2.32 (3H, s, 3 × 16-H), 2.23 (1H, t, *J* 2.55, 1-H), 2.20 (2H, m, 2 × 5-H), 1.63 (2H, m, 2 × 13-H), 1.56 (2H, m, 2 × 6-H), 1.27 (12H, m, 12H, 2 × (7–12-H)); δ_C (125 MHz, $CDCl_3$, Me_4Si) 196.10 (15-C), 172.78 (4-C), 79.69 (2-C), 71.38 (1-C), 36.37 (5-C), 30.59 (16-C), 29.48-28.70 (3-C, 6–13-C), 25.48 (4-C); *m/z* (ESI-MS) 298.1828 (M^+ + H. $C_{16}H_{27}NO_2S$ requires 298.1835).

$AcSC_{10}C(O)OEG_6OMe$ (6). A solution of carboxylic acid **4** (430 mg, 1.65 mmol) and hexaethylene glycol monomethyl ether (538.3mg, 1.82 mmol) in dry DCM (3 cm^3) was cooled to −20 °C and a mixture of DCC (357.8 mg, 1.73 mmol) and DMAP (20.2 mg, 0.17 mmol) in DCM (3 cm^3) were added slowly. The reaction mixture was then allowed to warm to room temperature and was stirred overnight. The solvents were removed under reduced pressure and the viscous crude product was purified by column chromatography (MeOH–DCM, 1 : 20) to yield the title compound as a pale yellow oil (667.2 mg, 75%). ν_{max}(film/cm^{-1}) 2924s, 2855s, 1735s, 1691s, 1456m, 1352m, 1298w, 1246m, 1111s, 1045w, 954m, 852m, 628m; δ_H (500 MHz, CD_3OD) 4.20 (2H, m, 2 × 13-H), 3.69 (2H, m, 2 × 12-H), 3.63 (18 H, m, 2 × (3–11-H)), 3.53 (2H, m, 2 × 2-H), 3.35 (3H, s, CH_3), 2.86 (2H, t, *J* 7.30, 2 × 24-H), 2.33 (2H, t, *J* 7.41, 15-H), 2.30 (3H, s, 3 × 26-H), 1.61 (2H, m, 2 × 16-H), 1.55 (2H, m, 23-H), 1.30 (12H, m, 2 × (17–22-H)); δ_C (125 MHz, CD_3OD) 197.50 (25-C), 175.35 (14-C), 72.96 (15-C), 71.59-71.35 (3-12-C), 70.14 (2-C), 64.55 (13-C), 59.10 (1-C), 34.96 (16-C), 32.41-29.76 (17–24-C), 30.54 (26-C), 26.00*; *m/z* (MALDI-TOF-MS) 561.5 (M^+ + Na. $C_{26}H_{50}O_9S$ + Na requires 561.5), (ESI-MS) 434.3474 (M^+ + H. $C_{23}H_{47}NO_6$ + H requires 434.3476).

$AcSC_{11}EG_6OMe$ (8). To a stirred solution of alkene **7** (2.92 g, 6.50 mmol) in abs. THF (30 cm^3), thioacetic acid (1.16 cm^3, 16.27 mmol) and AIBN (840 mg, 5.21 mmol) were added and the mixture was irradiated with UV light for approx. 3 h at room temperature. The solvents were then evaporated and the yellow crude product was purified by chromatography with a methanol–DCM gradient (1 : 20 → 1 : 18) to yield a colourless oil (3.245 g, 95%). ν_{max}(film/cm^{-1}) 2925s, 2855s, 1692s, 1461m, 1352m, 1299w, 1249w, 1116s, 952m, 884w, 627 m; δ_H (300 MHz, CD_3OD) 3.73–3.68 and 3.66–3.54 (22H, m, 2 × (2-13-H)), 3.44 (2H, t, *J* 6.81, 2 × 14-H), 3.38 (3H, s, 3 × H-1), 2.85 (2H, t, *J* 7.36, 2 × 24-H), 2.31 (3H, s, 3 × 26-H), 1.59–1.49 (4H, m, 2 × 15-H, 2 × 23-H), 1.30–1.24 [14H, m, 2 × (16–22-H)]; δ_C (75 MHz, CD_3OD) 196.12 (25-C), 71.92–70.39 (3–13-C), 59.05 (1-C), 30.66 (26-C), 29.62–28.81 (15–22-C), 26.08 (24-C); *m/z* (CI-MS) 511 (M^+ + H), 100.0%), 496 (13.5), 435 (1.3), 361 (1.4), 325 (2.5), 317 (1.7), 283 (2.8), 229 (2.0), 187 (2.6), 177 (2.8) 133 (3.9), (ESI-MS) 547.3267 (M^+ + Na. $C_{25}H_{52}O_8S$ +Na requires 547.3275).

$TosOEG_6C_9CH{=}CH_2$ (10). To a stirred solution of the alcohol **9** (4.0 g, 9.20 mmol) in ethyl acetate (13 cm^3) were added DABCO (2.07 g, 18.4 mmol) and 4 Å molecular sieves (100 mg). The reaction mixture was cooled to 0 °C and tosyl chloride (2.63 g, 13.80 mmol) was added in portions. Upon addition of TosCl a highly viscous suspension formed immediately. The mixture was warmed to room temperature and stirred for 1.5 h before the slurry was filtered through filter paper. The filtrate was acidified by dilute hydrochloric acid (5 cm^3) and washed with sat. $NaHCO_3$ (5 cm^3) in demineralised water prior to drying over Na_2SO_4. After filtration, solvents were evaporated and the crude product was purified by column chromatography using silica and an ethyl acetate–cyclohexane gradient (2 : 1 → 4 : 1). The product **10** was obtained as a pale yellow oil (4.43 g, 82%). ν_{max}(film/cm^{-1}) 3509w, 3072w, 2925s, 2856s, 1736w, 1640m, 1598m, 1454m, 1359s, 1292m, 1248m, 1189s, 1178s, 1113br, 1019m, 923s, 817m, 775m, 664s, 555s; δ_H (300 MHz, $CDCl_3$, Me_4Si) = 7.80 (2H, dt, *J* 1.88 and 8.3, 2 × Ar-*H*), 7.48 (2H, m, 2 × Ar-*H*), 5.81 (1H, ddt, *J* 6.63, 10.29, 17.1; 21-H), 5.03–5.01 and 4.97–4.90 (2H, m, 2 × 23-H), 4.16 (2H, t, *J* 4.81, 2 × 1-H), 3.70–3.56 (22H, m, 2 × (2–12-H)), 3.44 (2H, t, *J* 6.8, 2 × 15-H), 2.45 (3H, s, 3 × Ar-CH_3), 2.07–2.00 (2H, m, 2 × 21-H), 1.59–1.55 (2H, m, 2 × 14-H), 1.30-1.28 (12H, m, 2 × (15–20-H)); δ_C (75 MHz, $CDCl_3$, Me_4Si) 144.74 (*C*-Ar), 139.19 (22-C), 132.90 (*C*-Ar), 129.78 (*C*-Ar), 127.94 (*C*-Ar), 114.07 (23-C), 71.49 (2-C), 70.53 (3–11-C), 69.99 (1-C), 69.20 (12-C), 68.62 (13-C), 33.77 (21-C), 29.10 (14–19-C), 26.03*, 21.61 (*C*H_3-Ar); *m/z* (CI-MS) 598 (M^+ + H, 100%), 545 (1.5), 501 (2.6), 437 (41.4), 375 (9.6), 331 (29.6), 287 (27.7), 241 (37.5), 199 (59.8), 133 (18.9), 89 (39.5).

$N_3EG_6C_9CH{=}CH_2$ (11). The tosylate **10** (4.39 g, 7.46 mmol), sodium azide (2.43 g, 37.28 mmol) and tetrabutylammonium iodide (1.54 g, 4.20 mmol) were dissolved in abs. DMF (50 cm^3) and warmed to 70 °C for 2 h with stirring. Then the solvents were removed under reduced pressure and the crude material was purified by flash chromatography on silica using ethyl

acetate–cyclohexane (2 : 1). The title compound was obtained as a colourless oil (3.33 g, 97%). ν_{max}(film/cm^{-1}) 3590w, 3074w, 2924s, 2854s, 2103s, 1737w, 1340m, 1455s, 1348s, 1300s, 1249m, 1118s, 1039m, 994m, 910s, 853m, 722w, 644w, 556w; δ_H (500 MHz, $CDCl_3$, Me_4Si) 5.80 (1H, ddt, *J* 6.75, 10.28, 17.14; 22-H), 4.98 (1H, ddt, *J* 1.65, 2.20, 17.06; 23-H), 4.94 (1H, ddt, *J* 1.19, 2.02, 10.18; 23-H), 3.68–3.62 (20H, m, 2 × (3–12-H)), 3.58–3.56 (2H, m, 2 × 2-H), 3.43 (2H, t, *J* 6.79, 2 × 13-H), 3.38 (2H, t, *J* 5.14, 2 × 1-H), 2.05–2.00 (2H, m, 2 × 12-H), 1.56 (2H, q, *J* 14.67, 2 × 14-H), 1.36 (2H, t, *J* 7.25, 2 × 15-H), 1.32-1.28 (10H, m, 2 × (16-20-H); δ_C (125 MHz, $CDCl_3$, Me_4Si) 139.23 (22-C), 114.09 (23-C), 71.53 (13-C), 70.63 (3–12-C), 70.03 (2-C), 50.65 (1-C), 33.80 (21-C), 29.61-28.90 (14–20-C), 26.00*; *m/z* (CI-MS) 460 (M^+ + H, 9.61%), 432 (100.0), 388 (1.2), 292 (2.1), 241 (2.5), 177 (1.4), 147 (2.1), 133 (2.1), 97 (3.0), (ESI-MS) 482.3270 (M^+ + Na. $C_{23}H_{45}O_6N_3$ requires 482.3201).

$H_2NEG_6C_9CH{=}CH_2$ (12). The azide **11** (2.20 g, 4.79 mmol) was dissolved in a 1 : 1 mixture (24 cm^3) of THF and water at room temperature, triphenylphosphine (1.30 g, 4.96 mmol) was added and the solution was stirred for 3 d vigorously. Then the solution was extracted three times with a 1 : 1 mixture (200 cm^3) of diethyl ether and cyclohexane followed by three subsequent extractions with ethyl acetate, diethyl ether and cyclohexane (50 cm^3 each). The combined extracts were washed with brine and dried over Na_2SO_4 prior to column chromatography on silica using a methanol–dichloromethane gradient (1 : 20 → 1 : 10) as solvent system. Solvent evaporation provided the purified product **12** as a colourless amorphous solid (1.30 g, 63%). ν_{max}(film/cm^{-1}) 3372br, 2925s, 2856s, 1640w, 1590w, 1438s, 1350w, 1300w, 1182m, 1119s, 1037s, 996w, 750w, 722s, 696s, 542s; δ_H (500 MHz, $CDCl_3$, Me_4Si) 5.81 (1H, ddt, *J* 6.66, 10.16, 16.88; 22-H), 4.97 (2H, m, 23-H), 3.66 (18H, m, 2 × (3–11-H)), 3.58 (2H, m, 2 × 12-H), 3.51 (2H, t, *J* 5.12, 2 × 13-H), 3.43 (2H, t, *J* 6.81, 2 × 2-H), 2.86 (2H, t, *J* 5.23, 2 × 1-H), 2.03 (2H, dd, *J* 6.7, 14.36; 2 × 21-H), 1.52 (4H, m, 2 × 14-H, 2 × 20-H), 1.28 (10 H, m, 2 × (15–19-H)); δ_C (125 MHz, $CDCl_3$, Me_4Si) 139.00 (22-C), 113.96 (23-C), 71.34 (13-C), 70.45–69.88 (2–12-C), 33.62 (1-C), 29.51-28.74 (14–21-C), 25.91*; *m/z* (MALDI-TOF-MS) 456.5 (M^+ + Na. $C_{23}H_{47}NO_6$ + Na requires 456.32), (ESI-MS) 434.3474 (M^+ + H. $C_{23}H_{47}NO_6$ + H requires 434.3476).

$H_2C{=}CHC_9EG_6NHC(O)C{\equiv}CH$ (13). To a stirred solution of DCC (740 mg, 3.59 mmol) in dry DCM (19.5 cm^3), propiolic acid (0.18 cm^3, 2.92 mmol) was slowly added at 0 °C. After 10 min stirring the amine **12** (1.267 g, 2.924 mmol), dissolved in dry DCM (7 cm^3), was added dropwise. The reaction mixture was stirred for 1 h at 0 °C, warmed to room temperature and then stirred for another 2 h before the solvents were removed *in vacuo*. The crude product was purified by chromatography on silica gel (MeOH–DCM, 1 : 20) to yield the title compound as a colourless amorphous solid (1.20 g, 70%). ν_{max}(film/cm^{-1}) 3324m, 2926s, 2850s, 2108m, 1226s, 1573w, 1467w, 1436w, 1346w, 1242m, 1116s, 962w, 893w, 843w, 640m, 542m, 417w; δ_H (500 MHz, $CDCl_3$, Me_4Si) 6.99 (1H, br, N*H*), 5.81(1H, ddt, *J* 6.69, 10.18, 16.94; 25-H), 4.96 (2H, m, 2 × 26-H), 3.66 (22H, m, 2 × (4–14-H)), 3.50 (2H, m, 2 × 15-H), 3.44 (2H, t, *J* 6.82, 2 × 16-H), 2.85 (1H, s, 1-H), 2.04 (2H, m, 2 × H-24), 1.57 (2H, m, 2 × 17-H), 1.37 (2H, m, 2 × 23-H), 1.28 (10H, m, 2 × (18–22-H)); δ_C (125 MHz, $CDCl_3$, Me_4Si) 152.23 (3-C), 139.19 (25-C), 114.07 (26-C), 73.21 (2-C), 71.51 (1-C), 70.58–69.24 (5–16-C), 39.58 (4-C), 33.76 (24-C), 29.58–28.88 (17–23-C), 26.04*; *m/z* (CI-MS) 485 (M^+, 0.5%), 391 (7.4), 280 (2.5), (ESI-MS) 508.3319 (M^+ + Na. $C_{26}H_{47}NO_7$ +Na requires 508.3245).

$AcSC_{11}EG_6NHC(O)C{\equiv}CH$ (14). The alkyne **13** (200 mg, 0.42 mmol) were dissolved in abs. THF (3 cm^3), and thioacetic acid (0.06 cm^3) and AIBN (66.4 mg, 0.42 mmol) were added at room temperature. The reaction mixture was irradiated with UV light ($\lambda \geq 295$ nm) for 3 h. Then dichloroethane (2 cm^3) was added and the solvents were removed under reduced pressure to yield a brownish crude product. This was subjected to flash column chromatography (MeOH–DCM, 1 : 20) to yield a pale yellow oil (0.19 g, 83%). ν_{max}(film/cm^{-1}) 3324m, 2925s, 2852s, 2106m, 1692s, 1656m, 1573w, 1538w, 1437w, 1352m, 1245m, 1118s, 954s, 627m, 542w; δ_H (300 MHz, $CDCl_3$, Me_4Si) 3.66 (24 H, m, 2 × (4–15-H)), 3.44 (2H, t, *J* 6.83, 2 × 16-H), 2.86 (2H, t, *J* 7.29, 2 × 26-H), 2.85 (1H, s, 1-H), 2.33 (3H, s, 3H, 3 × 28-H), 1.56 (4H, m, 2 × 17-H, 2 × 25-H), 1.26 (14H, m, 2 × (18–24-H)); δ_C (75 MHz, $CDCl_3$, Me_4Si) 196.74 (27-C), 152.53 (3-C), 73.41 (2-C), 71.54 (1-C), 70.59–69.26 (4–16-C), 30.74 (28-C), 29.60-26.06 (17-26-C); *m/z* (ESI-MS) 584.3260 (M^+ + Na. $C_{28}H_{51}NO_8S$ + Na requires 584.3228).

$AcSC_{11}EG_6OC(O)C{\equiv}CH$ (16). The alcohol **15**[27] (1.29 g, 2.53 mmol) and propiolic acid (0.16 cm^3, 2.53 mmol) were dissolved in dry DCM (10 cm^3) and cooled to −26 °C. Then DCC (547.6 mg, 2.65 mmol) and DMAP (30.9 mg, 0.25 mmol) were added, the reaction mixture was warmed to room temperature and stirred overnight. For workup the solvents were removed under reduced pressure and the crude product was purified by column chromatography using an ethyl acetate–cyclohexane gradient (2 : 1 → 4 : 1) providing the title compound as a yellow oil (1.04 g, 73%). ν_{max}(film/cm^{-1}) 3217m, 2925s, 2855s, 2113s, 1716s, 1692s, 1462m, 1352m, 1228s, 1111s, 956s, 756m, 628m; δ_H (500 MHz, $CDCl_3$, Me_4Si) 4.35 (2H, m, 2 × 4-H), 3.74 (2H, m, 2 × 5-H), 3.66–3.58 (20 H, m, 2 × (6–15-H)), 3.44 (2H, t, *J* 6.81, 2 × 16-H), 2.99 (1H, s, 1-H), 2.86 (2H, t, *J* 7.26, 2 × 26-H), 2.32 (3H, s, 3 × 28-H), 1.56 (4H, m, 2 × 17-H, 2 × 25-H), 1.26 (14H, m, 2 × (18–24-H)); δ_C (125 MHz, $CDCl_3$, Me_4Si) 196.05 (27-C), 152.58 (3-C), 75.28 (2-C), 74.45 (1-C), 71.46–69.95 (5–15-C), 68.47 (16-C), 65.17 (4-C), 30.59 (28-C), 29.54 (26-C), 29.48–28.73 (17–25-C), 26.00*; *m/z* (MALDI-TOF-MS) 585.0 (M^+ + Na. $C_{28}H_{50}O_9S$ + Na requires 585.3), (ESI-MS) 585.3063 (M^+ + Na. $C_{28}H_{50}O_9S$ + Na requires 585.3068).

9-(1-Dodecyl-1*H*-[1,2,3]triazol-4-yl)nonanic acid (18). Undecynic acid (**17**, 50.0 mg, 0.27 mmol) and 1-azidododecane (60.9 mg, 0.29 mmol) were dissolved in acetonitrile (1.5 cm^3), the mixture was degassed and flushed thoroughly with nitrogen and cooled to 0 °C before CuI (104.5 mg, 0.55 mmol) and 2,6-lutidine (0.06 cm^3, (0.55 mmol) were added. The clear solution was warmed to room temperature and then stirred overnight. The reaction was quenched by addition of dilute hydrochloric acid (0.5 cm^3), the phases were separated and the aqueous phase was extracted three times with DCM (1 cm^3). The combined organic extracts were washed with brine, dried over Na_2SO_4, and then it was filtered and the filtrate evaporated under reduced pressure to provide a brownish crude product, which was purified by flash chromatography using a gradient of ethyl acetate and cyclohexane

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(2 : 1 → 4 : 1). The title triazole was obtained as a colourless amorphous solid (29.6 mg, 50%). δ_H (500 MHz, $CDCl_3$, Me_4Si) 7.25 (1H, s, 11-H), 4.30 (2H, t, *J* 7.29 Hz, 2 × 12-H), 2.70 (2H, m≈t, 2 × 9-H), 2.34 (2H, t, *J* 7.50, 2 × 2-H), 1.88 (2H, m, 2 × 13-H, 1.64 (4H, m, 2 × 3-H, 2 × 8-H), 1.38 and 1.25 (26H, m, 2 × (4–7-H, 14–22-H)), 0.88 (3H, t, *J* 6.98, 3 × 23-H); δ_H (125 MHz, $CDCl_3$, Me_4Si) 178.87 (1-C), 148.30 (10-C), 120.43 (11-C), 50.26 (12-C), 34.08 (2-C), 31.91 (21-C), 30.34-25.56 (4–9-C, 13–20-C), 24.71 (3-C), 22.69 (22-C), 14.12 (23-C); *m/z* (MALDI-TOF-MS) 394.3 (M^+ + H. $C_{23}H_{43}N_3O_2$ + H requires 394.6).

$AcSC_{10}C(O)NHCH_2$-triazole-EG_4Tos (**19**) The alkyne **5** (30 mg, 0.10 mmol) and tosylated triethylene glycol azide ($TosEG_4N_3$, 34.3 mg, 0.10 mmol) were dissolved in MeOH (1 cm^3), the solution was degassed and rinsed with nitrogen prior to addition of CuI (96.0 mg, 0.50 mmol) and DIPEA (0.09 cm^3, 0.50 mmol). The mixture was stirred at room temperature overnight, then the suspension was filtered through a 0.45 µm HPLC filter. Analytical HPLC (250/4 LiChrosorp 7 µm C8, A = H_2O, B = MeCN, 0% B → 90% B, 60 min, 1 cm^3 min^{-1}, R_t = 26.49 min) proved the high purity of product **19** (43.3 mg, 62%) without further chromatography. δ_H (500 MHz, $CDCl_3$, Me_4Si) 7.78 (2H, d, *J* 8.34, 2 × Ar-*H*), 7.73 (1H, s, 9-H), 7.35 (2H, d, *J* 8.10, 2 × Ar-*H*), 6.42 (1H, br s, N*H*), 4.51 (4H, m, 2 × 8-H, 2 × 11-H), 4.15 (2H, m, 2 × 1-H), 3.87 (2H, dd, *J* 4.63 and 9.83, 2 × 7-H), 3.69 (2H, m, 2 × 2-H), 3.60 (8H, m, 2 × (3–6-H)), 2.86 (2H, dd, *J* 7.20, 14.56; 2 × 22-H), 2.45 (3H, s, 3 × Ar-CH_3), 2.32 (3H, s, 3 × 24-H), 2.19 (2H, m, 2 × 13-H), 1.56 (4H, m, 2 × 14-H, 2 × 21-H), 1.49 (12 H, m, 2 × (15–20-H)); δ_C (125 MHz, $CDCl_3$, Me_4Si) 196.00 (23-C), 173.13 (12-C), 144.83 (Ar-*C*), 144.45 (10-C), 132.82 (Ar-*C*), 129.89 (Ar-*C*), 127.86 (Ar-*C*), 123.20 (9-C), 70.66–68.60 (1–7-C), 54.35 (Ar-*C*H_3), 50.21 (8-C), 42.67 (11-C), 36.47 (13-C), 30.57 (24-C), 29.39–28.69 (14–21-C), 25.54 (22-C); *m/z* (ESI-MS) 693.3025 (M^+ + Na. $C_{31}H_{50}N_4O_8S$ requires 693.2962).

$MeEG_6N_3$ (20). Hexaethylene glycol monomethyl ether (1.85 g, 6.24 mmol) and DABCO (1.40 g, 12.49 mmol) were dissolved in 7 cm^3 ethyl acetate, and dried 4 Å molecular sieves (100 mg) were added. The reaction mixture was cooled to 0 °C and tosyl chloride (1.79 g, 9.63 mmol) was added to the stirred reaction mixture, which was then warmed to ambient temperature. Stirring was maintained for 1.5 h, the suspension was then filtered and the filtrate washed twice with 2 M hydrochloric acid. After phase separation the aqueous layer was extracted with ethyl acetate twice and the recombined organic fractions were washed with brine and finally dried over sodium sulfate. The tosylate was obtained as a colourless oil (2.25 g, 80%) and used without further purification. The spectroscopic data were according to the literature.[40] The intermediate $MeEG_6Tos$ (1-tosy1-1,4,7,10,13,16,19-heptaoxaicosane tosylate, 2.50 g, 5.00 mmol) was dissolved in dry DMF (50 cm^3) and sodium azide (2.03 g, 31.21 mmol) and tetrabutylammonium bromide (50 mg) were added at room temperature. The stirred reaction mixture was warmed to 90 °C for 3.5 h prior to stopping the reaction by solvent removal under reduced pressure. The crude product was purified by column chromatography using a gradient of MeOH and DCM (1 : 20 → 1 : 15) providing the product **20** as a colourless oil (1.16 g, 72%). ν_{max}(film/cm^{-1}) 3441br, 2875s, 2107s, 1721w, 1642w, 1454m, 1349m, 1300m, 1250m, 1199w, 1106s, 948m, 850w; δ_H (500 MHz, $CDCl_3$, Me_4Si) 3.66 (20H, m, 2 × (3–12-H)), 3.55 (2H, m, 2 × 2-H), 3.39 (2H, m≈t, *J* 5.23, 2 × 1-H), 3.38 (3H, s, 3 × 13-H); δ_C (125 MHz, $CDCl_3$, Me_4Si) 71.87 (12-C), 70.64–70.45 (3–11-C), 69.96 (2-C), 58.96 (13-C), 50.62 (1-C); *m/z* (ESI-MS) 344.1767 (M^+ + Na. $C_{13}H_{27}N_3O_6$ + Na requires 344.1792).

$AcSC_{10}C(O)NHCH_2$-triazole-EG_6Me (21). The alkyne thioate **5** (100.0 mg, 0.54 mmol) and the azide **20** (103.1 mg, 0.32 mmol) were dissolved in acetonitrile (2 cm^3), the mixture was degassed and rinsed with nitrogen twice. CuI (61.1 mg, 0.32 mmol) was added to the stirred colourless solution before DIPEA (72.6 µl) was added slowly. The colour changed immediately to yellow. The reaction mixture was stirred overnight at room temperature prior to removal of the solvents under reduced pressure. The crude product was purified by column chromatography using a ethyl acetate–cyclohexane gradient (4 : 1 → 10 : 1). This procedure provided the title compound as a yellow amorphous solid (0.17 g, 86%). For further evaluation of the reaction conditions according to Table 1, 1 : 1 mixtures of alkyne **5** and azide **20** (20.0 mg, 0.03 mmol each) were dissolved in the solvents listed (1.5 cm^3) and the resulting solution was degassed and flushed with nitrogen twice. Catalyst was added as listed and the reaction mixture was stirred under the condition provided in Table 1; workup was performed as described above. ν_{max}(film/cm^{-1}) 3292w, 2919s, 2852m, 1694s, 1635s, 1548s, 1471s, 1421w, 1348s, 1287w, 1225w, 1110s, 962s, 848m, 775w, 714m, 664w, 631s, 419w; δ_H (500 MHz, $CDCl_3$, Me_4Si) 7.76 (1H, s, 14-H), 6.63 (1H, m≈t, *J* 5.38, N*H*), 4.54 (2H, d, *J* 4.9, 13-H), 4.52 (2H, d, *J* 5.39, 16-H), 3.87 (2H, t, *J* 5.10, 2 × 12-H), 3.63 (18H, m, 2 × (3–10-H)), 3.54 (2H, m, 2 × 2-H), 3.37 (3H, s, 3 × 1-H), 2.86 (2H, t, *J* 7.36, 2 × 27-H), 2.32 (3H, s, CH_3), 2.19 (2H, m≈t, *J* 7.50, 2 × 18-H), 1.54 (4H, m, 2 × (19-H, 26-H), 1.25 (12H, m, 2 × (20–25-H)); δ_C (125 MHz, $CDCl_3$, Me_4Si) 196.04 (28-C), 173.22 (17-C), 144.66 (15-C), 123.53 (14-C), 71.88 (2-C), 70.57–70.44 (3–12-C), 69.37 (3-C), 59.00 (1-C), 50.36 (13-C), 36.50 (16-C), 34.71 (18-C), 30.64 (29-C), 29.47–28.76 (19–27-C), 25.60 (28-C); *m/z* (MALDI-TOF-MS) 657.6 (M^+ + K. $C_{19}H_{54}N_4O_8S$ + K requires 657.4), *m/z* (ESI-MS) 641.3625 (M^+ + Na. $C_{19}H_{54}N_4O_8S$ + Na requires 641.3555).

Propiolic acid tris(2-*tert*-butoxycarbonylethyl)methylamide (23). Propargylic acid (100.0 mg, 1.42 mmol) was dissolved in abs. DMF (1 cm^3) and cooled to 0 °C. HATU (593.3 mg, 1.560 mmol) was added and a solution of the amine **22**[26] (589.5 mg, 1.42 mmol) in DMF (1.5 cm^3) was added dropwise to the stirred reaction mixture. Addition of DIPEA (0.371 cm^3, 2.128 mmol) turned the clear solution yellow. Stirring was maintained for 3 h at 0 °C, followed by stirring at room temperature overnight. For the workup the solvents were removed under reduced pressure and the crude product was purified by column chromatography with ethyl acetate–cyclohexane (1 : 1) as the eluent to yield the title alkyne as a colourless oil (373 mg, 56%). δ_H (300 MHz, $CDCl_3$, Me_4Si) 6.40 (1H, s, N*H*), 2.71 (1H, s, 7-H), 2.56 (6H, m, 6 × 2-H), 1.99 (6H, m, 6 × 3-H), 1.44 (27H, m, 9 × CH_3); δ_C (75 MHz, $CDCl_3$, Me_4Si) 172.62 (1-C), 151.16 (5-C), 80.86 (*C*CH_3), 77.80 (7-C), 71.63 (6-C), 58.79 (4-C), 29.82–29.67 (2-C, 3-C), 29.09 (*C*H_3); *m/z* (ESI-MS) 490.1831 (M^+ + Na. $C_{25}H_{41}NO_7$ + Na requires 490.2775).

4-[Tris(2-*tert*-butoxycarbonylethyl)methylcarbamoyl]-1-[ω-(11-acetylthioundecyl)hexaethylene glycol]-1*H*-[1,2,3]triazole (25). In degassed and nitrogen-saturated acetonitrile (1 cm^3) were

dissolved the azide **24** (20.0 mg, 0.037 mmol) and the alkyne **23** (26.2 mg, 0.056 mmol), and CuI (8.5 mg, 0.044 mmol) and DIPEA (8.5 × 10^{-3} cm) were added at room temperature. The reaction mixture was stirred for 20 h and was then filtered through a 0.45 μm HPLC filter before the solvent was removed *in vacuo*. Purification was performed by preparative HPLC (A = water, B = MeCN, 0% B, 10 min, 0% B → 70% B, 30 min, 70% → 100% B, 30 min, 10 cm^3 min^{-1}, R_t = 65.1 min) to yield a colourless lyophilisate (34.1 mg, 91%). δ_H (500 MHz, $CDCl_3$, Me_4Si) 8.19 (1H, s, 7-H), 6.84 (1H, s, N*H*), 4.58 (2H, m≈t, 2 × 8-H), 3.88 (2H, m≈t, 2 × 9-H), 3.65–3.63 (18H, m, 2 × (10–18-H)), 3.57 (2H, m, 2 × 19-H), 3.44 (2H, t, *J* 6.82, 2 × 20-H), 2.86 (2H, t, *J* 7.34, 2 × 30-H), 2.32 (3H, s, 3 × 32-H), 2.26 (6H, m, 6 × 2-H), 2.08 (6H, m, 6 × 3-H), 1.56 (4H, m, 2 × 21-H, 2 × 29-H), 1.43 (27H, m, 9 × CH_3), 1.26 (14H, m, 2 × (22–28-H)); δ_C (125 MHz, $CDCl_3$, Me_4Si) 196.03 (31-C), 172.33 (1-C), 159.33 (5-C), 143.55 (6-C), 126.32 (7-C), 80.51 (*C*CH_3), 71.52–70.02 (9–19-C), 69.21 (7-C), 57.68 (4-C), 57.68 (8-C), 30.62 (20-C), 30.00 (2-C), 29.64 (3-C), 29.61–28.79 (21–30-C), 28.06 (*C*H_3); *m/z* (MALDI-TOF-MS) 1026.1 [M^+ + Na. $C_{50}H_{90}N_4O_{14}S$ + Na requires 1026.3).

4-[Tris(2-carboxyethyl)methylcarbamoyl]-1-[ω-(11-acetylthioundecyl)hexaethylene glycol]-1*H*-[1,2,3]triazole (26). Into the solution of triester **25** (34.0 mg, 0.034 mmol) in DCM (0.7 cm^3) trifluoric acid (0.7 cm^3) was added at room temperature. The mixture was stirred over 1 h and then the solvent was removed under reduced pressure to yield a colourless oil (27.7 mg, 98%), which was used without further purification. ν_{max}(film/cm^{-1}) 3398br, 2926s, 2856s, 1723s, 1569m, 1511w, 1460m, 1416m, 1352m, 1301w, 1203w, 1180m, 1102s, 1027m, 952m, 628m; δ_H (300 MHz, CD_3OD) 8.46 (1H, s, 7-H), 4.67 (2H, m, 2 × 8-H), 3.95 (2H,m, 2 × 9-H), 3.66 (20H, m, 2 × (10-19-H)), 3.50 (2H, t, *J* 6.61, 2 × 20-H), 2.90 (2H, t, *J* 7.20, 2 × 30-H), 2.39 (6H, m, 6 × 2-H), 2.34 (3H, s, 3 × 32-H), 2.21 (6H, m, 6 × 3-H), 1.59 (4H, m, 2 × 21-H, 2 × 29-H), 1.34 (14H, m, 2 × (22-28)); δ_C (75 MHz, CD_3OD) 197.64 (31-C), 176.83 (1-C), 162.10 (5-C), 140.14 (6-C), 128.17 (7-C), 72.36–70.13 (9-19-C), 59.25 (4-C), 51.66 (8-C), 30.86-29.26 (2-C, 3-C, 21–30-C); *m/z* (ESI-MS) 857.4116 (M^+ + Na. $C_{38}H_{66}N_4O_{14}S$ + Na requires 857.4188).

ManC_2-triazole-C(O)NHEG$_6$C$_9$C=CH_2 (28). Alkyne **13** (70.0 mg, 0.14 mmol) and azide **27** (35.9 mg, 0.14 mmol) were dissolved in dried MeOH (0.5 cm^3) and dried DMC (0.5 cm^3), and the solution was degassed. Copper(I) iodide (5.5 mg) was then added and the reaction mixture was warmed to 45 °C and kept stirred at that temperature overnight. After that the suspension was filtered and the solvents were removed under reduced pressure. The yellowish crude product was purified by column chromatography on silica gel with methanol and dichloromethane (1 : 20) as the solvent system. The alkene **28** was obtained as a pale yellow amorphous solid (77.5 mg, 73%). δ_H (500 MHz, CD_3OD) 8.44 (1H, s, 9-H), 5.84 (ddt, 1H, *J* 6.80, 10.18, 16.98, 33-H), 4.97 (2H, m, 2 × 34-H), 4.78 (1H, m≈s, 1-H), 4.73 (2H, m, 2 × 8-H), 4.19 (1H, m, 7-H), 3.95 (2H, m, 6-H, 7-H), 3.88 (2H, m, 2 × 12-H), 3.82 (1H, dd, *J* 2.30, 11.77, 2-H), 3.78 (2H, m, 3-H, 6-H), 3.68 (20H, m, 2 × (14–23-H)), 3.63 (2H, m, 4-H, 5-H), 3.51 (2H, t, *J* 6.70, 2 × 13-H), 3.46 (2H, t, *J* 4.95, 2 × 24-H), 2.01 (2H, m≈q, 2 × 32-H), 1.61 (2H, m, 2 × 25-H), 1.35 (12H, m, 2 × (26–31-H)); δ_C (125 MHz, CD_3OD) 162.67 (11-C), 143.87 (10-C), 140.12 (33-C), 127.78 (9-C), 114.70 (34-C), [101.78 + 101.70]‡ (1-C), [75.00 + 74.84]‡ (5-C), [72.44 + 72.41]‡ (3-C), 72.37 (24-C), [72.02 + 71.84]‡ (2-C), 71.37–71.26 (–CH_2OCH_2–), [68.51 + 68.34]‡ (4-C), [62.84 + 62.77]‡ (6-C), [51.74 + 51.45]‡ (8-C), 40.03*, 34.86*, 30.66–30.09 (CH_2), 27.16*. *m/z* (MALDI-TOF-MS) 733.5 (M^+ $C_{34}H_{62}N_4O_{13}$ requires 734.9); *m/z* (ESI-MS) 735.4383 (M^+ + H. $C_{34}H_{62}N_4O_{13}$ + H requires 735.4386); *m/z* 757.4234 (M^+ + Na. $C_{34}H_{62}N_4O_{13}$ + Na requires 757.4206).

ManC_2-triazole-C(O)NH-EG$_6$C$_{11}$SAc (29). Alkene **28** (70.0 mg, 0.10 mmol) was dissolved in THF (1 cm^3) and thioacetic acid (34.0 μl, 0.48 mmol) and AIBN (20 mg, 0.12 mmol) were added. The reaction mixture was stirred at room temperature and then UV irradiation ($\lambda \geq 295$ nm) was started and maintained for 5 h. For workup the solvents were removed under reduced pressure and the residual pale yellow crude oil was purified by flash chromatography on silica using methanol–dichloromethane (1 : 18) as the solvent system. The product was obtained as a colourless oil (22.0 mg, 40%). δ_H (500 MHz, CD_3OD) 8.29 (1H, s, 9-H), 4.64 (1H, m≈s, 1-H), 4.59 (2H, m, 2 × 8-H), 4.05 (1H, m, 7-H), 3.81 (2H, m, 6-H, 7-H), 3.74 (2H, m, 2 × 12-H), 3.69 (1H, dd, *J* 2.43, 11.79, 2-H), 3.64 (2H, m, 3-H, 6-H), 3.54 (20H, m, 2 × (14–23-H)), 3.53 (2H, m, 4–5-H), 3.36 (2H, t, *J* 6.65, 2 × 13-H), 3.32 (2H, t, *J* 4.95, 2 × 24-H), 2.75 (2H, t, *J* 7.29, 2 × 34-H), 2.21 (3H, s, 2 × 36-H), 1.45 (4H, m, 2 × (24-H, 33-H), 1.20 (12H, m, 12H, 2 × (26–32-H)); δ_C (125 MHz, CD_3OD) 197.61 (35-C), 162.65 (11-C), 143.85 (10-C), 127.80 (9-C), [101.78 + 101.69]‡ (1-C), [75.03 + 74.88]‡ (5-C), [72.39 + 72.36]‡ (3-C), 71.84 (24-C), [71.44 + 71.30]‡ (2-C), 71.06–70.60 (–CH_2OCH_2–), 68.31 (4-C), 62.78 (6-C), [51.71 + 51.42]‡ (8-C), 40.05*, 30.76–29.79 (CH_2), 27.19*. *m/z* (ESI-MS) 811.4307 (M^+ + H. $C_{36}H_{66}N_4O_{14}S$ + H requires 811.4375).

***N*-Acidoacetyl-dansylcadaverine (31).** Azidoacetic acid (137 mg, 1.36 mmol) and DCC (280 mg, 1.36 mmol) HOBt (183.1 mg, 1.36 mmol) were dissolved in abs. DCM (6 cm^3) and cooled to 0 °C. To the stirred solution of dansylcadaverine (**30**, 500 mg, 1.49 mmol) in abs. DCM (4 cm^3) was slowly added. The reaction mixture was slowly warmed to room temperature over 10 min and stirring was maintained overnight. Distilled water (10 cm^3) was added and the aqueous phase was extracted with DCM (7 cm^3) four times. After drying (Na_2SO_4) the organic solvents were removed under reduced pressure and the crude material was purified by flash chromatography using a ethyl acetate–cyclohexane gradient (1 : 1 → 2 : 1). Product fractions were detected using a standard UV lamp. The fluorescent product **31** was obtained as viscous oil (523.0 mg, 92%). ν_{max}(film/cm^{-1}) 3310br, 2938s, 2863m, 2789w, 2106s, 1661s, 1575m, 1538m, 1455m, 1355w, 1314s, 1201w, 1160s, 1144s, 1074m, 912w, 792s, 732s, 683w, 626s, 571s; δ_H (500 MHz, $CDCl_3$, Me_4Si) 8.55 (1H, d, *J* 8.52, 8-H), 8.29 (1H, d, *J* 8.63, 6-H), 8.24 (1H, dd, *J* 1.15, *J* 7.27, 7-H), 7.55 (2H, m, 3–4-H), 7.45 (1H, m≈t, CON*H*), 7.20 (1H, d, *J* 7.50, 2-H), 6.27 (1H, br, SO_2N*H*), 3.97 (2H, s, 2 × 16-H), 3.15 (2H, q, *J* 7.36, 2 × 27-H), 2.90 (8H, m, 3 × 9-H, 3 × 9′-H, 2 × 10-H), 1.39 (4H, m, *J* 7.50, 2 × (11-H, 13-H)), 1.25 (2H, m, 2 × 12-H); δ_C (125 MHz, $CDCl_3$, Me_4Si) 166.65 (15-C), 152.10 (1-C), 134.74 (5-C), 130.45 (8-C), 129.93 (4a-C), 129.68 (8a-C), 129.65 (6-C), 128.43 (3-C), 123.25 (7-C), 118.70 (4-C), 115.23 (2-C), 52.74 (16-C), 45.44 (9-C), 42.93 (10-C), 38.95

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(14-C), 28.92 (11-C), 28.71 (13-C), 23.34 (12-C); *m/z* (ESI-MS) 441.1439 (M^+ + Na. $C_{19}H_{26}N_6O_3S$ + Na requires 441.1683); *m/z* (ESI-MS) 418.17871 (M^+. $C_{19}H_{26}N_6O_3S$ requires 418.17855).

Dansylcadaverine-NHC(O)CH_2-triazole-CH_2NHC(O)C_{10}SAc (32). Alkyne **5** (60.0 mg, 0.18 mmol) and azide **31** (100 mg, 0.24 mmol) were dissolved in acetonitrile (1 cm^3) and twice degassed and flushed with nitrogen. CuI (18 mg, 0.10 mmol) and DIPEA (21 µl, 0.12 mmol) were subsequently added to the stirred reaction mixture and it was heated to 45 °C overnight before the mixture was filtered and concentrated *in vacuo.* The crude product was purified by flash chromatography (ethyl acetate–cyclohexane, 4 : 1; then MeOH–DCM, 1 : 20). Product fractions were detected using a standard UV lamp. The fluorescent product **32** was obtained as viscous oil (112.8 mg, 84%). ν_{max}(film/cm^{-1}) 3297s, 3081w, 2921s, 2849s, 1692s, 1668s, 1634s, 1549s, 1446s, 1418m, 1354w, 1319m, 1261m, 1232m, 1201w, 1184w, 1143s, 1058m, 947w, 785s, 682m, 628s, 570s; δ_H (500 MHz, $CDCl_3$, Me_4Si) 8.51 (1H, d, *J* 8.47, 8-H), 8.30 (1H, d, *J* 8.63, 4-H), 8.18 (1H, d, *J* 7.26, 6-H), 7.90 (1H, s, 17-H), 7.49 (2H, m, 3-H, 7-H), 7.15 (1H, d, *J* 7.53, 2-H), 7.11 (1H, br, 15-CN*H*), 6.99 (1H, br, 20-CN*H*), 6.21 (1H, m≈t, *J* 5.64, SO_2N*H*), 5.15 (2H, s, 2 × 16-H), 4.54 (2H, m≈t, *J* 5.43, 2 × 19-H), 3.13 (2H, m, 2 × 14-H), 2.87 (6H, s, 3 × 9-H), 2.84 (4H, m≈t, *J* 7.40, 2 × (10-H, 30-H)), 2.31 (3H, s, 32-CH_3), 2.13 (2H, m≈t, *J* 7.50, 2 × 21-H), 1.51 (6H, m, 2 × (11-H, 13-H, 22-H)), 1.36 (4H, m, 2 × (12-H, 23-H)), 1.18 (12H, m, 2 × (24–29-H)); δ_C (125 MHz, $CDCl_3$, Me_4Si) 196.13 (31-C), 173.95 (20-C), 165.47 (15-C), 151.87 (1-C), 145.46 (18-C), 134.81 (5-C), 130.28 (6-C), 129.83 (4a-C), 129.56 (8a-C), 129.27 (8-C), 128.33 (3-C), 124.80 (17-C), 123.16 (7-C), 118.92 (4-C), 115.25 (2-C), 53.05 (16-C), 45.39 (9, 9′-C), 42.73 (10-C), 39.23 (14-C), 36.41 (21-C), 34.73 (19-C), 30.63 (32-C), 29.67–29.12 (13-C, 24–28-C), 29.05 (30-C), 28.76 (23-C), 28.60 (29-C), 28.19 (13-C), 25.57 (22-C), 23.23 (12-C); *m/z* (ESI-MS) 738.3398 (M^+ + Na. $C_{35}H_{53}N_7O_5S_2$ + Na requires 738.3443).

Dansylcadaverine-NHC(O)CH_2-triazole-C(O)NH-EG_6C_{11}SAc (33). Alkyne **14** (72 mg, 0.13 mmol) and azide **31** (53.6 mg, 0.13 mmol) were dissolved in a 1 : 1 mixture of dry DMF and MeOH (1.2 cm^3). After degassing and flushing with nitrogen twice, CuI (7.3 mg, 0.04 mmol) was added and the stirred suspension was warmed to 45 °C and kept stirring overnight at this temperature. The solvents were removed *in vacuo* and purification was accomplished by flash chromatography with ethyl acetate–cyclohexane (4 : 1) as the first eluent, followed by MeOH–DCM (1 : 20). The fluorescent product **33** was obtained as viscous oil (72.5 µg, 59%). δ_H (500 MHz, $CDCl_3$, Me_4Si) 8.59 (1H, d, *J* 8.52, 8-H), 8.42 (1H, s, 17-H), 8.39 (1H, d, *J* 8.68, 4-H), 8.22 (1H, dd, *J* 1.24, *J* 7.30, 6-H), 7.62 (2H, m, 3-H, 7-H), 7.30 (1H, d, *J* 7.59, 2-H), 5.19 (2H, s, 2 × 16-H), 3.65 (24H, m, 2 × (21–31-H)), 3.49 (2H, t, *J* 6.60, 2 × 32-H), 3.07 (2H, t, *J* 6.98 Hz, 2 × 14-H), 2.93 (6H, s, 6 × 9-H), 2.88 (4H, m, 2 × (10-H, 43-H)), 2.33 (3H, s, 44-CH_3), 1.58 (4H, m, 2 × (33-H, 42-H)), 1.33 (22H, m, 2 × (11-13-H, 34–41-H)); δ_C (125 MHz, $CDCl_3$, Me_4Si) 197.62 (43-C), 167.23 (15-C), 162.49 (19-C), 153.21 (1-C), 143.89 (18-C), 137.21 (5-C), 131.22 (8-C), 131.10 (4a-C), 130.99 (8a-C), 130.15 (6-C), 129.07 (3-C), 128.82 (17-C), 124.32 (7-C), 120.60 (4-C), 116.44 (2-C), 72.36-70.51 (20–32-C), 53.11 (16-C), 45.83 (9-C), 43.62 (10-C), 40.43 (14-C), 30.74 (44-C), 30.72-29.77 (33–43-C), 29.55 (11-C), 27.19 (13-C), 24.67 (12-C); *m/z* (ESI-MS) 1002.5463 (M^+ + Na. $C_{47}H_{77}N_7O_{11}S_2$ + Na requires 1002.5015).

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