Synthesis of a Series of Oligo(ethylene glycol)-Terminated Alkanethiol Amides Designed to Address Structure and Stability of Biosensing Interfaces

Sofia Svedhem, Carl-Åke Hollander, Jing Shi, Peter Konradsson, Bo Liedberg,† and Stefan C. T. Svensson*

Divisions of Chemistry and Applied Physics, Department of Physics and Measurement Technology, Linköpings Universitet, SE-581 83 Linköping, Sweden

sts@ifm.liu.se

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A strategy for the synthesis of a series of closely related oligo(ethylene glycol)-terminated alkanethiol amides (principally $HS(CH_2)_mCONH(CH_2CH_2O)_nH$; $m = 2, 5, 11, 15$, $n = 1, 2, 4, 6, 8, 10, 12$) and analogous esters has been developed. These compounds were made to study the structure and stability of self-assembled monolayers (SAMs) on gold in the prospect of designing new biosensing interfaces. For this purpose, monodisperse heterofunctional oligo(ethylene glycols) with up to 12 units were prepared. Selective monoacylation of the symmetrical tetra- and hexa(ethylene glycol) diols as their mesylates with the use of silver(I) oxide was performed. The synthetic approach was based on carbodiimide couplings of various oligo(ethylene glycol) derivatives to ω-(acetylthio) carboxylic acids via a terminal amino or hydroxyl function. SAM structures on gold were studied with respect to thickness, wettability (water contact angles ∼30°), and conformation. A good fit was obtained for the relation between monolayer thickness (d) and the number of units in the oligo(ethylene glycol) chain (n): $d = 2.8n + 21.8$ (Å). Interestingly, the corresponding infrared spectroscopy analysis showed a dramatic change in conformation of the oligomeric chains from all-trans ($n = 4$) to helical ($n \geq 6$) conformation. A crystalline helical structure was observed in the SAMs for $n > 6$.

Introduction

Poly- and oligo(ethylene glycols) (PEG and OEG: $H(OCH_2CH_2)_nOH$) have found widespread use in a variety of applied areas, e.g., for the preparation of crown ether type derivatives, surfactants, and ion-conducting materials, and as spacers for (bio)molecules.[1−3] Ethylene glycols provide good anchors for biological receptors and ligands, and they are known to reduce the nonspecific binding of proteins and other bioactive molecules**.** PEG derivatives are also ideal as spacer candidates because they are inexpensive, water soluble, stable, and available in a wide range of molecular weight distributions. The necessity to integrate ethylene glycol units of defined length into synthetic molecular devices makes it essential to develop approaches to general synthesis of long monodisperse chains.[4−6] Furthermore, bifunctional OEG derivatives require a difficult selective functionalization of symmetrical diols. In our research program to study self-assembled monolayers (SAMs) on gold, it was required to develop easy access to various bifunctional monodisperse OEGs with up to 12 glycol units.

Organic modifications of gold surfaces by SAMs[7] have proven to be successful in biosensor applications, e.g., in commercially available chips for biomolecular interaction analysis with surface plasmon resonance.[8] In particular, SAMs provide well-defined planar biosensing interfaces for model studies of specific aspects of biomolecular recognition such as binding mechanisms of multivalent molecules as a function of ligand density.[9,10] For example, at too close a distance, ligands will become less accessible for binding due to sterical hindrance.[11] Nonspecific binding to the sensing interface should be carefully avoided; an important criterion that is met by oligo(ethylene glycol)-terminated SAMs.[12,13] Other advantages of planar interfaces, as opposed to hydrogels, include reduction of mass transport and interaction studies involving large entities such as phages or cells.[14]

The object of this study has been to find simple methodologies for the synthesis of OEG-terminated alkanethiol amides designed to address structure and stability of the corresponding SAMs. Recently, similar

* Corresponding author. Tel: +46 13 281366; fax: +46 13 281399.
† Division of Applied Physics.

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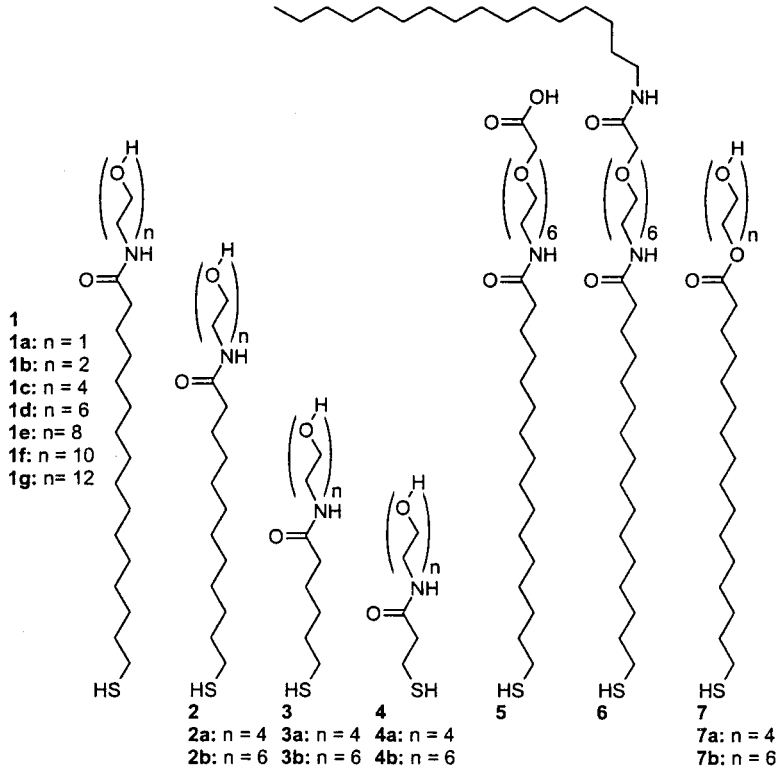
Chart 1



molecules, especially OEGs coupled as ethers to alkanethiols, have attracted interest by several researchers.[12,13,15−21] The use of amides designed in our approach[22,23] will allow greater synthetic flexibility as compared to ethers. Also, the intermediate amine derivatives can be used for direct surface coupling to carboxylate SAMs.[24] The series of closely related analogues presented here aims at a thorough understanding of the structural details of SAMs**.** In a recent work,[22] infrared reflection−absorption spectroscopy has shown that compounds **1a**−**d** ($n = 1, 2, 4, 6$) form highly crystalline SAMs irrespectively of the length of the oligo(ethylene glycol) chain. At ambient temperature, the OEGs adopt, in coexistence with amorphous structure, one of two crystalline states where the oligomeric chains take either an all-trans or a helical conformation.[18,21,22] The partitioning between the two conformers vary reversibly with temperature,[23] and the transition temperature is assumed to depend on the number of ethylene glycol units in compounds **1a**−**g**. The interplay between inter- and intramolecular interactions will be further investigated by decreasing the van der Waals interactions in the SAMs using compounds with shorter alkyl chains (**2**−**4**). It has been suggested that the helical conformation induce improved protein resistance as compared to the all-trans structure,[19] although this hypothesis may be too simplistic.[16,20,25]

The chemical resistance of the amides (**1**−**6**), in addition to the potential lateral stabilization of the SAMs, make them good candidates for use in biosensor applications. The stabilizing effect of lateral hydrogen bonding between amides will be compared to the corresponding esters (**7**).[26−29] A carboxylic acid group terminated analogue (**6**) has been included with which functionality for coupling of biomolecules of interest can be introduced into the monolayers.[17] Such synthetically flexible SAMs may also be used for immobilization of ligands that are suited for tethering of membranes to the sensing interface.[30,31] Here, a simple approach has been applied, attaching hydrophobic tails at the far end of hexa(ethylene glycol) chains.

Results and Discussion

The synthetic strategy was based on coupling of monofunctionalized oligo(ethylene glycol) amines to ω-mer-

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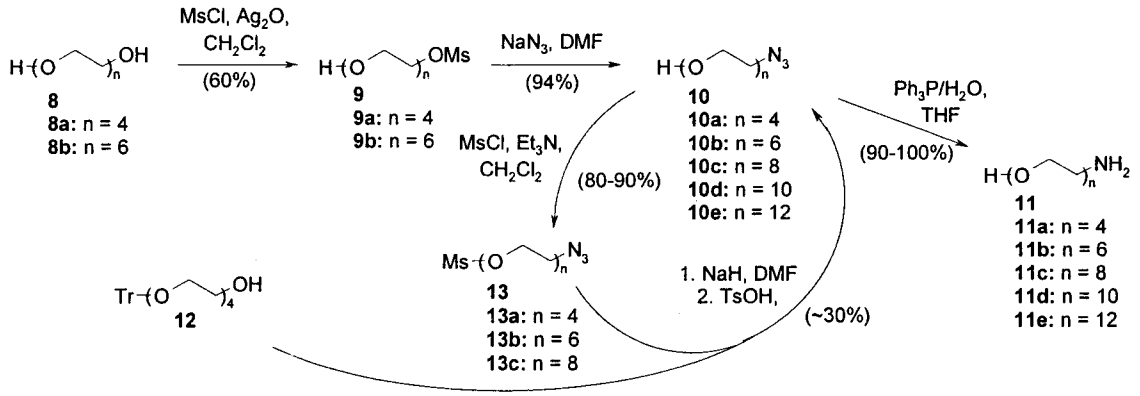
Scheme 1



captocarboxylic acids to generate the corresponding amides **1**−**5.** The shorter ethylene glycol monoamines (mono- and dimers: $n = 1, 2$) are commercially available, but longer even number homologues ($n = 4$−12) had to be prepared by selective heterofunctionalization and chain elongation. The amino function was introduced through azide displacement of monoactivated OEGs and subsequent reduction.

Synthesis of Oligo(ethylene glycol) Amines. A crucial step in the work with OEGs is to differentiate the reactivity of the two chemically equivalent terminal hydroxyl groups. In this work, for the purpose of preparing monosubstituted OEG amines, monoderivatization was subjected for further investigations. Sulfonate esters and halides have been extensively used as good leaving groups for substitution reactions at terminal ends of ethylene glycols. Especially, mesylates and tosylates have often been used as starting materials for the preparation of a variety of functionalized oligo- and poly(ethylene glycols).[1] Derivatization with a stoichiometric equivalent of protecting reagent generally yields statistical proportion of the monosubstituted product besides unreacted and disubstituted starting material. Examples are found in the literature of moderate yields up to 50%.[32−34] Disubstitution may be suppressed by the use of huge excess of glycol in proportion to the reagent. This may be accomplished, in the case of inexpensive and easily removable starting material, as ethylene glycols up to four units.[5,6,35]

The use of silver(I) oxide as a mild promotor for alkylation of hydroxyl groups is of common knowledge. The oxide acts primary as a halide acceptor in S_N2 reactions of alkyl halides, but the possibilities of complexing and chelating oxygens is also of importance. Recently it was shown that silver(I) oxide could be used for selective monoalkylation of symmetrical diols.[36] Principally monobenzylations of different symmetrical diols were reported in yields of 60−93% with reaction times of 1−15 h. Although not generally used for acylation, we found that monomesylates of tetra- and hexa(ethylene oxide) (**9a** and **9b)** could be obtained in 60% yields with nearly equivalent amounts of the glycol, mesyl chloride, and Ag_2O in methylene chloride (Scheme 1). The effect of Ag_2O was obvious as no reaction was observed when omitted in the reaction mixture. The reaction time of the concentrated heterogeneous mixture was relatively slow (2 days), but was compensated by a simplified purification by column chromatography. With 2.5 mol equiv of mesyl chloride a similar yield of the monomesylate was obtained in a reduced reaction time (12−15 h). The selectivity was on the other hand lower, and prolonged reaction from this point yielded only larger proportion of disubstitution.

The OEG azides **10a**[32,33] and **10b** were prepared in 90% yield from the mesylate by using sodium azide in DMF at 100 °C according to established methods.[37] Reduction to amines was attempted using hydrogenation (H_2/Pd),[38,39] but significant amounts of the dimeric secondary amines were formed. The dimeric structure was confirmed by ^{13}C NMR, as indicated by the nitrogen-bonded methylene at δ 49 ppm as opposed to the primary amine at δ 41 ppm. Changing catalyst[40] and solvent did not circumvent this problem. However, reduction of OEG azides with triphenylphosphine[33,41] (Staudinger reduction) in THF gave quantitative yields of the OEG amines **11** without any observed byproducts.

The observed changes in crystalline states of OEG chains in SAMs from compounds **1a**−**d**,[22] from all-trans to helical conformation with increased OEG chain length, pointed the interest to synthesis of even longer monodisperse OEG chains. As only monodisperse OEGs up to hexa(ethylene glycol) were available from commercial suppliers, chain elongation of short OEGs was required to obtain the longer OEG terminated alkanethiol amides **1e**−**g** ($n = 8$, 10, and 12).

Following the method of Chen and Baker,[6] tetra(ethylene glycol) was monotritylated in pyridine to compound **12** by using a 10-fold excess of the glycol compared to trityl chloride (Scheme 1). Compound **12** was coupled to the two mesylated tetra- and hexa(ethylene glycol) azides **13a** and **13b** by Williamson's ether synthesis using sodium hydride in DMF. Detritylation with *p*-TsOH in methanol gave the octa- and deca(ethylene glycol)azides **10c** and **10d** in low overall yields (~30%). The ether

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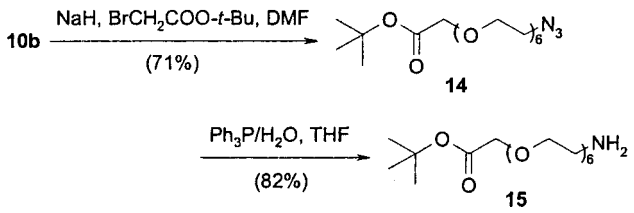
Scheme 2



couplings were sluggish and slow, and purification by preparative HPLC was necessary to obtain pure compounds. Efforts to optimize the yields by changing solvents and bases, or phase-transfer conditions,[42,43] were all unsuccessful. Earlier work on elongation of OEGs has shown that depolymerization processes may occur in basic media that suppress the outcome of the reaction.[42]

To obtain the dodeca(ethylene glycol) azide **10e**, compound **10c** was mesylated to **13c** and coupled as above with the monotritylated **12**, giving a similar yield of 34%. Staudinger reduction of the azides **10c**−**e** gave nearly quantitative yields of **11c**−**e.**

To improve the utility of the hydrophilic OEG spacers, for the purpose of linking biomolecules, a carboxyl-terminated hexa(ethylene glycol) amine was also synthesized. Compound **10b** was converted to the sodium alkoxide with NaH in DMF followed by subsequent reaction with *tert*-butyl bromoacetate to give compound **14** in 71% yield. Staudinger reduction of the azide group gave compound **15** (82%, Scheme 2).

Synthesis of Thio Alkyl OEG Amides. The synthesized OEG amine derivatives were coupled to ω-mercaptocarboxylic acids via amide linkages to give the structures **1**−**5**. Primarily 16-mercaptohexadecanoic acid was used, but ω-mercaptocarboxylic acids with shorter carbon chains (3, 6, and 12 carbons) were also prepared. Due to the high nucleophilicity of sulfur, the thiol group had to be protected during the coupling step to avoid inter- and intramolecular thioesterification. Reduction of contaminant disulfide and subsequent acetylation was carried out in a one-pot reaction with zinc and acetyl chloride in acetic acid to give the thioacetylated carboxylic acid **16** in 86% (Scheme 3). An alternative method to compound **16** was used to circumvent the contamination of disulfide. Nucleophilic displacement of the 16-bromohexadecanoic acid with potassium thioacetate in DMF gave **16** in a yield of 74%.[44] This method was also used for the preparation of the shorter ω-thioacetyl carboxylic acids **17**, **18**,[45] and **19**[44] (m = 11, 5, and 2, respectively). Starting from the corresponding ω-bromocarboxylic acids, the thioacetylated compounds were obtained in high yields (74−88%).

Couplings of the different OEG amines **11a**−**e** and to ω-thioacetyl carboxylic acids **16**−**19** were carried out via the active ester method,[46] using *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC) and 1-hydroxybenzotriazole (HOBT) in methylene chloride in good yields (60−95%) (Scheme 3). The thioacetates were subsequently deprotected to **1a**−**g** through transesterification under acidic (AcCl in MeOH) and/or basic conditions (Zemplén conditions) in generally high yields (60−90%). Basic conditions were more rapid but more sensitive for disulfide formation. Removal of air by argon atmosphere was crucial to avoid formation of disulfide during the deprotection. Under acidic conditions, the thiol formation of **1a** was competing with the transacylation of the ethanolamine group to the methyl ester. Under acidic condition, using methanol, transacylation to the thiol **1a** was competed with the ethanol amine group.

The carboxyl-terminated OEG amine **15** was coupled to the compound **16** by the aforementioned general method. Selective deprotection of the *tert*-butyl group to compound **20** was done with trifluoroacetic acid in methylene chloride, and the thioacetate was removed at neutral conditions by hydrazinium acatate in DMF[47] to give compound **5** in a total yield of 65% (three steps). With this compound in hand, access to further surface modifications and immobilizations are possible, and as an example of that, compound **6** was prepared for tethering of artificial membranes. This was done by coupling compound **20** with hexadecylamine using the same amine coupling and deprotection procedure as for the OEG amines **1**−**4** above, which gave compound **6** in a yield of 78%.

For comparison studies of the stabilizing effect of lateral hydrogen bonding between self-assembled amides, esters **7ab** were synthesized. In this case the ester coupling to compound **16** was performed with unprotected tetra- and hexa(ethylene glycol), respectively. The same carbodiimide chemistry was used as above, except that the more reactive DMAP was used instead of HOBT as base and catalyst. Using a 10-fold molar excess of the glycol suppressed the formation of diesters. The deprotection of the thioacetate went chemoselectively by hydrazinium acetate[47] and compounds **7a** and **7b** were obtained in moderate to good yields (overall yields: 74% and 50%, respectively).

To secure high quality of the monolayers, compounds **1**−**7** were carefully purified. The combination of straight-phase flash chromatography and reversed-phase preparative HPLC proved efficient to obtain high purity final products.

Physical Characterization of SAMs. SAMs from compounds **1a**−**d** have already been investigated and characterized with respect to wettability (contact angle of water), physical thickness (ellipsometry), and molecular structure (infrared reflection−absorption spectroscopy).[22,23] Here, as an illustration of this methodology, these experiments were extended to SAMs from compounds **1e**−**g** with longer ethylene glycol chains. Well-organized SAMs with this long monodisperse OEGs have not previously been reported.

Infrared reflection−absorption spectra showed a high degree of crystallinity in the SAMs, as evidenced by the strong peaks at 2917 cm^{-1} and at 2951 cm^{-1} (stretching modes from alkyl chain) and by vibrations characteristic to PEG (Figure 1). In addition to the peaks assigned previously,[22] the parallel band at 2740 cm^{-1} observed for crystalline PEG[48] was now identified in the spectra. In

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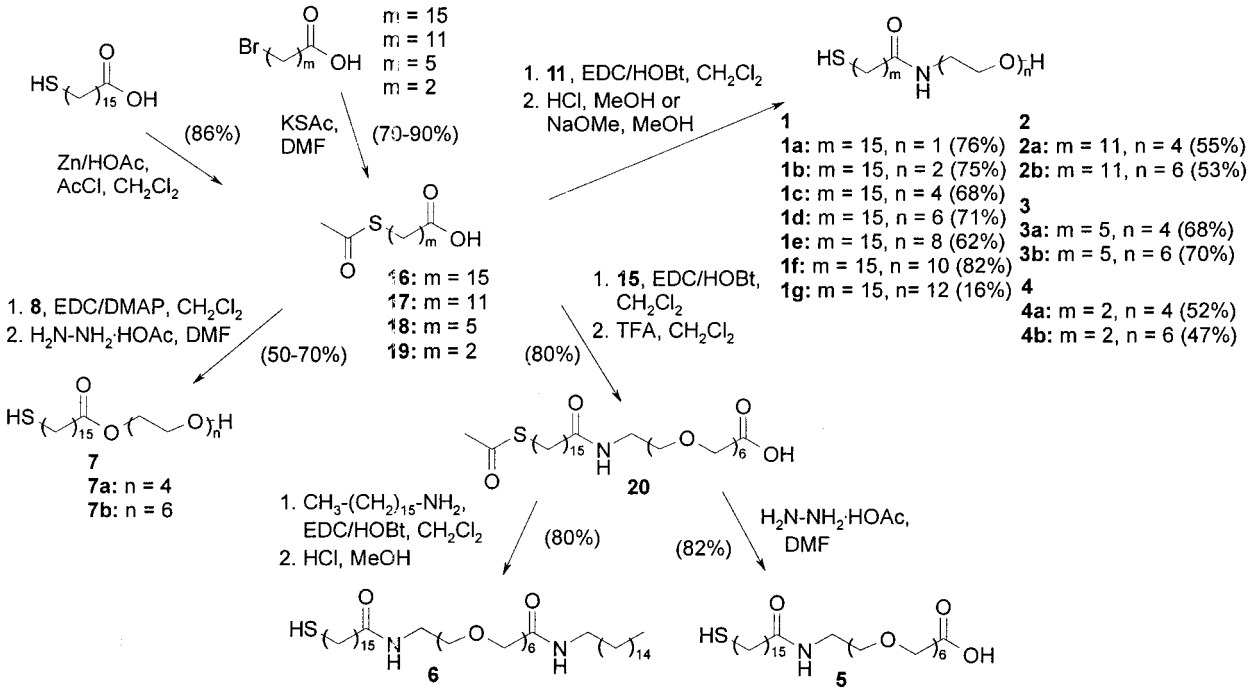
Scheme 3



the reflective mode, only vibrations having transition dipole moments oriented orthogonal to the surface were excited, and the absence of vibrations at about 1650 cm^{-1} characteristic for amide linkages (amide I) implied alignment of the carbonyl groups parallel to the surface. The apparently constant relative intensities of the alkyl stretching modes, in addition to the invariant shape and intensity of the amide II peak, suggested a firmly packed lower part of the SAMs that was independent of the OEG length.

However, a distinct change in conformation of the ethylene glycol layer, from all-trans (1146 cm^{-1}) to helical (1119 cm^{-1}), appeared for SAMs with more than four units in the OEG chain. The crystalline structures coexisted to various degrees with an amorphous phase, readily distinguished (1126 cm^{-1}) for compound **1d**.[18] The intensities of the modes assigned to the helical crystalline phase (965 cm^{-1}, 1119 cm^{-1}, 1242 cm^{-1}, 1345 cm^{-1}, 1463 cm^{-1}, 2893 cm^{-1}) increased approximately proportionally to the length of the OEG for compounds **1d**−**g**, as illustrated by the plot of the 965 and 1345 cm^{-1} peak intensities vs chain length in Figure 2.

Despite the striking conformational change of the OEGs and the high sensitivity of contact angle goniometry to surface structure, similar water contact angles (about 30°) were obtained for all SAMs. More unexpectedly, the conformational change was not either reflected in the physical thickness of the SAMs, and a remarkably good fit was obtained for the relation between monolayer thickness (d) and the number of units in the OEG chain (n): $d = 2.8n + 21.8$ (Å) (Table 1, Figure 2). The constant term corresponded well to values for SAMs of similar structure[49] ($HS(CH_2)_{16}OH$: 22 Å, $HS(CH_2)_{15}COOH$: 19 Å). Likewise, the proportionality constant was in very good agreement with the increment per repeating unit in helical ethylene glycol chains according to crystallographic[48] and theoretical[18] data, 2.78 Å. Further, the considerably larger theoretical value[18] for OEGs in all-trans conformation (3.56 Å) was not reflected in the experimentally observed thickness for SAMs from compound **1c**.

The apparently contradictory information from the different techniques suggested that a more detailed structural model would be required to fully account for the observed characteristics. For instance, there was a shift in frequency for the amide II peak indicating structural changes. The smaller cross-section area of ethylene glycol chains in the all-trans conformation probably also allowed for a larger tilt angle for the shorter OEGs resulting in a thinner film than would be expected for all-trans segments oriented perpendicular to the surface.

In conclusion, we have demonstrated a straight and simple methodology for the synthesis of a series of closely related OEG terminated alkanethiols (**1**−**7**). The compounds described here are designed to pinpoint the interplay between inherent molecular structure and interactions with adjacent molecules in well-ordered monolayer assemblies. From self-assembly experiments with analogous molecules, as illustrated above, it will be possible to extract information about how the inherent molecular structure manifests differently as a result of interactions with the environment. This information may be used to improve models for molecular interactions and thus the understanding of biomolecular function. Further progress in this area, especially in the perspective of designing new biosensing interfaces, will be reported in separate papers.

Experimental Section

General Methods. Unless otherwise stated, all starting materials were obtained from commercial suppliers and were used without further purification. TLC was performed on Merck precoated 60 F-254 plates and visualized using UV light and/or applying a solution of AMC (5 g of ammonium molybdate and 1 g of cerium(IV) sulfate in 1 L of aqueous H_2SO_4 (10%)) followed by heating. Similarly, amines were detected using ninhydrin (0.3% in ethanol). Column chromatography

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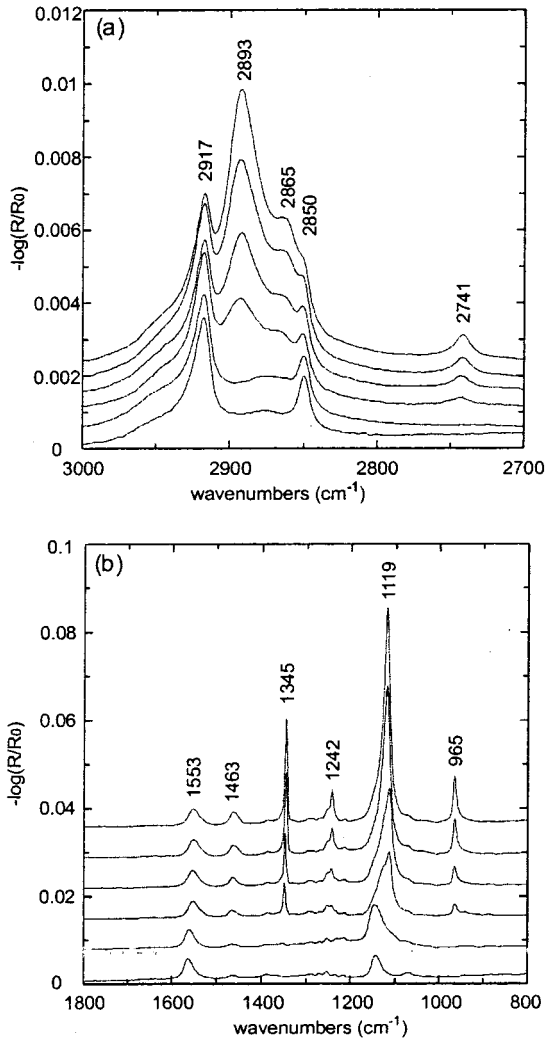

Figure 1. Infrared reflection−absorption spectra of SAMs from compounds **1b** (bottom)−**g** (top). (a) CH-stretching region, (b) fingerprint region.

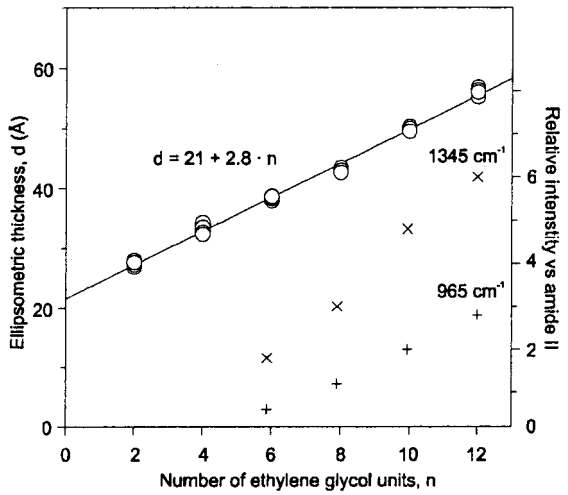

Figure 2. (○) Ellipsometric thicknesses of SAMs from compounds **1b**−**g**. The values for compounds **1b**−**d** were in good agreement with a previous study.[22] The data were fitted to a linear model: $d = 21 + 2.8n$, where d is the ellipsometric thickness of the SAM in Å and n the number of ethylene glycol units in the corresponding thiol. The standard errors of the fitted parameters were 0.2 and 0.03, respectively, and the correlation coefficient was 0.99. Also shown in the diagram are the intensities (relative to the amide II peak) of two parallel bands characteristic for crystalline poly(ethylene glycol): (×) 1345 cm^{-1} and (+) 965 cm^{-1}. Similarly to the ellipsometric thickness, the intensity of those bands increased proportionally with the number of ethylene glycol units in the SAM, although with different proportional constants.

Table 1. Ellipsometric Thicknesses and Water Contact Angles of SAMs from Compounds 1b−g

SAM from	ellipsometric thickness (Å)	contact angle of water (deg)[a]
1b	27.4 ± 0.4	28
1c	33.1 ± 0.7	31
1d	38.3 ± 0.3	29
1e	42.9 ± 0.3	31
1f	49.9 ± 0.3	26
1g	56.0 ± 0.4	28

[a] The standard deviation was <2°.

was performed using silica gel (40−63 μm, SDS) or, for reversed-phase separations, LiChroprep RP-18 (40−63 μm, Merck). Preparative HPLC was performed using a reversed-phase (C_{18}) Kromasil column at 210−230 nm. Organic extracts were dried over magnesium sulfate. Solvents were evaporated with a rotary evaporator under reduced pressure at <40° (except in the case of DMF: 50 °C), and the residue was further dried in vacuo at room temperature. NMR spectra were recorded on a Bruker AC-250 spectrometer in $CDCl_3$ using TMS (0.00 ppm) as an internal standard. Elemental analyses were carried out by Analytische Laboratorien, Lindlar, Germany or by Mikrokemi, Uppsala, Sweden.

General Procedure for Selective Mesylation of OEGs. Methanesulfonyl chloride (650 mg, 6.0 mmol) in CH_2Cl_2 (2 mL) was added to a mixture of oligo(ethylene glycol) **8** (5.0 mmol) and 1.3 g (5.5 mmol) of Ag_2O in CH_2Cl_2 (10 mL). The reaction was stopped after 48 h by filtration through Celite. Evaporation followed by flash chromatography afforded the dimesylate (25%) followed by the monomesylate **9** (60%).

11-[(Methylsulfonyl)oxy]-3,6,9-trioxaundecanol (9a)[33] [H(OCH$_2$CH$_2$)$_4$OMs]. Compound **9a** was prepared according to the above general procedure for selective mesylation of OEGs. TLC (EtOAc/MeOH 10:1) R_f 0.3; ^{1}H NMR δ 4.40−4.37 (m, 2H), 3.79−3.59 (m, 14 H), 3.09 (s, 3H); ^{13}C NMR δ 72.4, 70.6, 70.5, 70.4, 70.3, 69.3, 69.0, 61.7, 37.6.

17-(Methylsulfonyl)oxy-3,6,9,12,15-pentaoxaheptadecanol (9b) [H(OCH$_2$CH$_2$)$_6$OMs]. Compound **9b** was prepared according to the above general procedure for selective mesylation of OEGs. ^{1}H NMR δ 4.4 (m, 2H), 3.8−3.6 (m, 22H), 3.09 (s, 3H); ^{13}C NMR δ 72.7, 70.6−70.4, 70.2, 69.4, 69.0, 61.7, 37.7.

11-Azido-3,6,9-trioxaundecanol(10a)[32,33] [H(OCH$_2$CH$_2$)$_4$N$_3$]. Sodium azide (1.1 g, 16.5 mmol) was added to a solution of **9a** (3.0 g, 11 mmol) in dry DMF (12 mL). The mixture was heated at 110 °C for 2.5 h and then allowed to attain room temperature. The reaction mixture was coevaporated with toluene at 50 °C, and the residue was purified by flash chromatography giving 2.26 g (94%) of **10a** as a colorless oil. TLC (EtOAc/MeOH 10:1) R_f 0.5; ^{1}H NMR δ 3.6−3.8 (12H), 3.60 (t, 2H), 3.40 (t, 2H); ^{13}C NMR δ 72.52, 70.5−70.7, 70.35, 70.02, 61.67, 50.64.

17-Azido-3,6,9,12,15-pentaoxaheptadecanol (10b) [H(OCH$_2$CH$_2$)$_6$N$_3$]. Compound **10b** was prepared according to preparation of **10a**. Yield: 90%. TLC (EtOAc/MeOH, 10:1) R_f 0.4; ^{1}H NMR δ 3.8−3.6 (20H), 3.60 (m, 2H), 3.38 (t, 2H); ^{13}C NMR δ 72.61, 70.5−70.7, 70.31, 70.02, 61.67, 50.67.

1-Azido-23-hydroxy-3,6,9,12,15,18,21-heptaoxatricosane (10c) [H(OCH$_2$CH$_2$)$_8$N$_3$]. A suspension of NaH (0.28 g, 11.8 mmol) and monotritylated **12**[6] (2.0 g, 4.70 mmol) in dry DMF (20 mL) was stirred for 30 min at room temperature under argon. The mixture was cooled to 0 °C and **13a** (2.1 g, 7.0 mmol) dissolved in dry DMF (10 mL) was added via a syringe. The mixture was left to attain room temperature and stirred for 22 h. DMF was removed by coevaporation with toluene four times. MeOH (50 mL) was added, and the solution was acidified with *p*-TsOH (1.0 g, 5.3 mmol) and left stirring overnight. Deionized water (25 mL) was added, and MeOH

was removed by evaporation. The water phase was washed with pentane (2 × 20 mL) and concentrated. The residue was purified by chromatography (EtOAc/MeOH 10:1) followed by preparative HPLC (MeOH/H_2O 55:45) to give 0.51 g (28%) as a colorless oil. TLC (EtOAc/MeOH 3:1) R_f 0.3; ^{1}H NMR δ 3.75−3.59 (m, 30H), 3.41−3.37 (t, J = 5 Hz, 2H), 2.8 (br s, 1H); ^{13}C NMR δ 72.5, 70.7, 70.6, 70.3, 70.0, 61.7, 50.7.

1-Azido-29-hydroxy-3,6,9,12,15,18,21,24,27-nonaoxanonacosane (10d) [H(OCH$_2$CH$_2$)$_{10}$N$_3$]. A suspension of NaH (0.71 g of a 55−65% dispersion in mineral oil, 16.2−17.8 mmol) and monotritylated **12**[6] (2.0 g, 4.70 mmol) in dry DMF (10 mL) was stirred for 45 min at room temperature under argon. Compound **13b** (0.50 g, 1.3 mmol) dissolved in DMF (6 mL) was added, and the mixture was stirred for 1 h. The mixture was concentrated by coevaporation with toluene five times. MeOH (50 mL) was added, and the solution was acidified with *p*-TsOH (3.0 g, 15.8 mmol). After stirring for 2 h at room temperature, deionized water (30 mL) was added and MeOH was removed by evaporation. The water phase was washed with pentane (3 × 10 mL) and concentrated. The residue was purified by preparative HPLC to give 0.22 g (34%) of a colorless oil. TLC (EtOAc/MeOH 3:1) R_f 0.2; ^{1}H NMR δ 3.74−3.59 (m, 38H), 3.39 (t, J = 5 Hz, 2H), 2.8 (br s, 1H); ^{13}C NMR δ 72.5, 70.3, 70.0, 61.7, 50.7.

1-Azido-35-hydroxy-3,6,9,12,15,18,21,24,27,30,33-dodecaoxapentatriacontane (10e) [H(OCH$_2$CH$_2$)$_{12}$N$_3$]. A suspension of NaH (0.244 g of a 55−65% dispersion in mineral oil, 5.6−6.6 mmol) and monotritylated **12**[6] (0.611 g, 1.40 mmol) in dry DMF (15 mL) was stirred for 20 min at room temperature under argon. Compound **13c** (0.131 g, 0.276 mol) in dry DMF (10 mL) was added, and the mixture was stirred for 2.5 h. The mixture was concentrated by coevaporation with toluene three times. MeOH (50 mL) was added, and the solution was acidified with *p*-TsOH (1.2 g, 6.3 mmol) and left stirring overnight. Deionized water (25 mL) was added, and the MeOH was removed by evaporation. The water phase was washed with pentane (5 × 10 mL) and concentrated. The residue was purified by chromatography ($CHCl_3$/MeOH 11:1 → 8:1) followed by preparative HPLC (MeOH/H_2O 55:45). Yield 0.054 g (34%) as a colorless oil. TLC (EtOAc/MeOH 3:1) R_f 0.1; ^{1}H NMR δ 3.77−3.55 (m, 46 H), 3.35 (t, J = 5 Hz, 2H), 2.77 (br s, 1H); ^{13}C NMR δ 72.5, 70.5, 70.3, 70.0, 61.7, 50.7.

11-Amino-3,6,9-trioxaundecanol [H(OCH$_2$CH$_2$)$_4$NH$_2$] (11a).[33,38,39] A solution of azide **10a** (800 mg, 3.7 mmol) in dry THF (10 mL) was cooled to 0 °C. Triphenyl phosphine was added (1.1 g, 4.0 mmol) after which the mixture was allowed to attain room temperature. The reaction was monitored by TLC (*i*-PrOH/aqueous NH_3 (5%)/H_2O 6:3:1), and at completion (10 h) water was added (≥120 μL, 6.7 mmol) to hydrolyze the intermediate phosphorus adduct (<10 h). The reaction mixture was diluted with water and washed with toluene. Evaporation of the aqueous layer yielded 700 mg (99%) of compound **11a** as a pale yellow oil. TLC (CH_2Cl_2/MeOH/Et_3N 3:3:1) R_f 0.5; ^{1}H NMR δ 3.7−3.6 (12H), 3.53 (t, 2H), 2.87 (t, 2H); ^{13}C NMR δ 73.02, 70.6−70.4, 70.28, 70.11, 61.31, 41.40.

17-Amino-3,6,9,12,15-pentaoxaheptadecanol (11b)[38,39] [H(OCH$_2$CH$_2$)$_6$NH$_2$]. Compound **11b** was prepared from **10b** according to the preparation of **11a.** Yield: 98% as a pale yellow oil. TLC (CH_2Cl_2/MeOH/Et_3N 3:3:1) R_f 0.6; ^{1}H NMR δ 3.7−3.6 (20H), 3.51 (t, 2H), 2.86 (t, 2H); ^{13}C NMR δ 73.22, 72.84, 70.7−70.5, 70.34, 70.25, 61.40, 41.64.

23-Amino-3,6,9,12,15,18,21-heptaoxatricosanol (11c) [H(OCH$_2$CH$_2$)$_8$NH$_2$]. Compound **11c** was prepared from **10c** according to the preparation of **11a**, with the exception that a larger amount of triphenylphosphine (20 equiv) and longer reaction times (20 and 80 h, respectively) were used. Yield: 90% as a colorless oil. TLC (*i*-PrOH/aqueous NH_3 (5%)/H_2O 6:3:1) R_f 0.5; ^{1}H NMR δ 3.8−3.6 (m, 28H), 3.52 (t, J = 5 Hz 2H), 2.89−2.75 (m, 2H), 2.6 (m, 3H); ^{13}C NMR δ 73.1, 72.8, 71.1, 70.3, 61.5, 41.6.

29-Amino-3,6,9,12,15,18,21,24,27-nonaoxanonacosanol (11d) [H(OCH$_2$CH$_2$)$_{10}$NH$_2$]. Compound **11d** was prepared from **10d** according to the preparation of **11a**, with the exception that a larger amount of of triphenylphosphine (2 equiv) was used. After prolonged reaction time (20 h) at room temperature, more triphenylphosphine was added (2 equiv), and the temperature was raised to 50 °C for 5 h. Yield: 100% as a colorless oil. TLC (*i*-PrOH/aqueous NH_3 (5%)/H_2O 6:3:1) R_f 0.5; ^{1}H NMR δ 3.8−3.55 (m, 36H), 3.54−3.50 (m, 2H), 3.0−2.8 (m, 2H), 2.5 (m, 3H); ^{13}C NMR δ 73.2, 72.7, 70.3, 61.5, 41.7.

35-Amino-3,6,9,12,15,18,21,24,27,30,33-dodecaoxapentatriacontanol (11e) [H(OCH$_2$CH$_2$)$_{12}$NH$_2$]. Compound **11e** was prepared from **10e** according to the preparation of **11a**, with the exception that several portions of triphenyl phospine (1.5 equiv) and water (>10 equiv) were added over a period of 6 days. Yield: 98% as a colorless oil. TLC (*i*-PrOH/aqueous NH_3 (5%)/H_2O 6:3:1) R_f 0.5; ^{1}H NMR δ 3.74−3.52 (m, 48H), 3.06−2.78 (m, 3H); ^{13}C NMR δ 72.5, 72.4, 71.1, 71.0, 70.3, 70.2, 61.6, 41.4.

1-Azido-11-(methylsulfonyl)oxy-3,6,9-trioxaundecane (13a)[32] [Ms(OCH$_2$CH$_2$)$_4$N$_3$]. To a solution of **10a** (0.51 g, 2.3 mmol) and triethylamine (0.47 g, 4.7 mmol) in CH_2Cl_2 (10 mL) at 0 °C was added methanesulfonyl chloride (0.35 g, 3.0 mmol). After 10 min, the solution was allowed to attain room temperature. After further stirring for 40 min, the reaction mixture was concentrated, and the residue was purified by flash chromatography. Yield: 0.63 g (92%) as a colorless oil. TLC (EtOAc) R_f 0.4; ^{1}H NMR δ 4.40−4.36 (m, 2H), 3.79−3.75 (m, 2H), 3.70−3.66 (m, 10H), 3.41−3.37 (t, J = 5 Hz, 2H), 3.08 (s, 3H); ^{13}C NMR δ 70.6, 70.0, 69.3, 69.0, 50.7, 37.7.

1-Azido-17-(methylsulfonyl)oxy-3,6,9,12,15-pentaoxaheptadecane (13b) [Ms(OCH$_2$CH$_2$)$_6$N$_3$]. Compound **13b** was prepared from **10b** following the procedure used for compound **13a**. Yield: 76% as a yellow oil. TLC (EtOAc) R_f 0.2; ^{1}H NMR δ 4.40−4.37 (m, 2H), 3.79−3.75 (m, 2H), 3.70−3.64 (m, 18H), 3.41−3.37 (t, J = 5 Hz, 2H), 3.09 (s, 3H); ^{13}C NMR δ 70.5, 70.0, 69.3, 69.0, 50.7, 37.7.

1-Azido-23-(methylsulfonyl)oxy-3,6,9,12,15,18,21-heptaoxatricosane (13c) [Ms(OCH$_2$CH$_2$)$_8$N$_3$]. Compound **13c** was prepared from **10c** following the procedure used for compound **13a**. Yield: 92% as a yellow oil. TLC (EtOAc) R_f 0.1; ^{1}H NMR δ 4.40−4.36 (m, 2H), 3.79−3.75 (m, 2H), 3.70−3.64 (m, 26H), 3.39 (t, J = 5 Hz, 2H), 3.08 (s, 3H); ^{13}C NMR δ 70.7, 70.6, 70.5, 70.0, 69.3, 69.0, 50.7, 37.7.

***tert*-Butyl 20-azido-3,6,9,12,15,18-hexaoxaeicosanoate (14) [*t*-Bu-OOCCH$_2$(OCH$_2$CH$_2$)$_6$N$_3$].** Azide **10b** (310 mg, 1.0 mmol) was dissolved in DMF (10 mL, dried over 4 Å molecular sieves) and cooled to 0 °C. Under a flow of nitrogen, sodium hydride (88 mg, 2.0 mmol, suspension in oil) was added. When the evolution of hydrogen gas had ceased (after 5 min), *tert*-butyl bromoacetate (220 μL, 1.5 mmol) was added. The mixture was allowed to attain room temperature and was stirred for 12 h. The reaction mixture was diluted with EtOAc (20 mL), washed with water (20 mL), dried over $MgSO_4$, and concentrated. The crude product was purified by flash chromatography (toluene/EtOAc 10:1 to 0:1) to give 300 mg (71%) as a colorless oil. TLC (EtOAc) R_f: 0.3; ^{1}H NMR δ 4.02 (s, 2H), 3.7−3.6 (22H), 3.39 (t, 2H), 1.48 (s, 9H); ^{13}C NMR δ169.62, 81.40, 70.5−70.8, 70.02, 68.99, 50.64, 28.08.

***tert*-Butyl 20-Amino-3,6,9,12,15,18-hexaoxaeicosanoate (15) [*t*-Bu-OOCCH$_2$(OCH$_2$CH$_2$)$_6$NH$_2$].** Compound **15** was synthesized from **14** following the procedure used for compound **11a**. Yield: 82% as a pale yellow oil. TLC (CH_2Cl_2/MeOH/Et_3N 3:3:1) R_f 0.8;^{1}H NMR δ 4.02 (s, 2H), 3.7−3.6 (20H), 3.52 (t, 2H), 2.87 (t, 2H), 1.48 (s, 9H); ^{13}C NMR δ 169.65, 81.49, 73.19, 70.6−70.4, 70.28, 69.02, 41.70, 28.11.

16-(Acetylthio)hexadecanoic Acid (16) [AcS(CH$_2$)$_{15}$COOH]. Method A. 16-Mercaptohexadecanoic acid (Aldrich, 90%) (250 mg, 0.87 mmol) was dissolved in CH_2Cl_2 (3 mL) and acetic acid (3 mL). Zinc powder (0.5 g) was added, and after 15 min (when disulfide was no longer detected by TLC), the now clear reaction mixture was cooled to 0 °C prior to addition of acetyl chloride (1.2 mL, 17 mmol). When the evolution of hydrogen gas ceased (5 min), the reaction mixture was allowed to attain room temperature. After 10 min, zinc was removed by filtration through Celite, and the filtered organic solution was washed twice with aqueous HCl (0.1 M, 25 mL) mixed with ice. The solvent was evaporated, and the crude product

was purified by flash chromatography (toluene/EtOAc 100:1 to 10:1).

Method B. To a stirred solution of 16-bromohexadecanoic acid (200 mg, 0.60 mmol) at 0 °C in DMF (4 mL, dried over 4 Å molecular sieves) was added potassium thioacetate (200 mg, 1.8 mmol) in one portion. The deep red mixture was stirred for 30 min at room temperature, diluted with CH_2Cl_2 (10 mL), and washed three times with water. The organic solution was dried over $MgSO_4$, and the solvent was coevaporated with toluene. The yellow crude product was purified by flash chromatography (toluene/EtOAc 20:1).

Yields: Method A gave 245 mg (86%) and method B 145 mg (74%) of **16** as a white solid. TLC (toluene/EtOAc 2:1) R_f 0.5; ^{1}H NMR δ 2.86 (t, 2H), 2.34 (t, 2H), 2.32 (s, 3H), 1.7−1.4 (4H), 1.3−1.2 (14H); ^{13}C NMR δ 196.17, 180.03, 34.05, 30.67, 29.7−29.0, 28.82, 24.70.

12-(Acetylthio)dodecanoic Acid (17) [AcS(CH$_2$)$_{11}$COOH]. Compound **17** was prepared from 12-bromododecanoic acid following the procedure used for compound **16** (method B), with the exception that 1.5 equiv of potassium thioacetate was used and a reaction time of 30 min. Yield: 86% as a white solid. TLC (petroleum ether/EtOAc 1:1) R_f 0.4; ^{1}H NMR δ 2.86 (t, 2H), 2.34 (t, 2H), 2.32 (s, 3H), 1.7−1.4 (4H), 1.3−1.2 (14H); ^{13}C NMR δ 196.17, 180.03, 34.05, 30.67, 29.7−29.0, 28.82, 24.70.

6-(Acetylthio)hexanoic Acid (18)[45] [AcS(CH$_2$)$_5$COOH]. Compound **18** was prepared from 6-bromohexanoic acid following the same procedure as for compound **16** (method B). Yield: 88% as an oil. TLC (petroleum ether/EtOAc 5:1) R_f 0.4; ^{1}H NMR δ 2.87 (t, 2H), 2.36 (t, 2H), 2.33 (s, 3H), 1.8−1.5 (4H), 1.42 (m, 2H); ^{13}C NMR δ 196.11, 179.85, 33.88, 30.61, 29.20, 28.85, 28.14, 24.14.

3-(Acetylthio)propionic Acid (19)[44] [AcS(CH$_2$)$_2$COOH]. Compound **19** was prepared from 3-bromopropionic acid following the same procedure as for compound **16** (method B), except that CH_2Cl_2 extraction was replaced by several extractions with EtOAc. Yield: 1.43 g (74%) as an oil which formed crystals on standing. TLC (petroleum ether/EtOAc 1:1) R_f 0.2; ^{1}H NMR δ 3.11 (t, 2H), 2.70 (t, 2H), 2.35 (s, 3H); ^{13}C NMR δ 195.61, 177.79, 34.20, 30.55, 23.85.

General Procedure for Amine Coupling and Subsequent Deprotection of Acetylated Thiols. To a solution of thioacetylated ω-mercaptocarboxylic acids **16**−**19** (0.15 mmol) in CH_2Cl_2 (4 mL) at 0 °C were added amine (0.23 mmol), *N*-hydroxybenzotriazole (HOBt) (0.23 mmol) and finally *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC) (0.23 mmol). The reaction mixture was allowed to attain room temperature. After 12 h it was diluted with CH_2Cl_2 (10 mL) and washed with 0.1 M HCl (10 mL) and water (10 mL). The organic solution was dried over $MgSO_4$ and evaporated. The crude product was crystallized or purified by flash chromatography (EtOAc/MeOH). Occasionally, when there was a contaminant of less soluble compounds (e.g., disulfides), reversed-phase chromatography (MeOH/CH_3CN 1:5) was more suitable for purification.

Acidic Deprotection. A solution of the thioacetate in methanol was purged with argon, after which pH was decreased by addition of acetyl chloride (7.1 μL/mL, generating 0.1 M HCl). The mixture was refluxed under an atmosphere of argon for 5 h and was then concentrated. The residue was taken up in CH_2Cl_2 and washed with water until neutral. The organic solvent was dried over $MgSO_4$ and evaporated. Purification was performed by flash chromatography (EtOAc/MeOH) or crystallization.

Basic Deprotection. A solution of the thioacetate in methanol was purged with argon, after which 5 equiv of NaOMe from a fresh 1 M solution in methanol (the methanol was purged with argon prior to preparation) were added. After 1 h, the reaction mixture was neutralized with Dowex-H$^+$ and filtered. Evaporation and purification with flash chromatography (EtOAc/MeOH) yielded the final products.

***N*-(2-hydroxy-ethyl) 16-Mercaptohexadecanamide (1a) [HS(CH$_2$)$_{15}$CONHCH$_2$CH$_2$OH].** The crude product obtained following the general procedure for amine coupling of **16** and ethanolamine was crystallized in EtOAc to yield 76% of white crystals. TLC (EtOAc/MeOH 10:1) R_f 0.6; ^{1}H NMR δ 6.09 (br, 1H), 3.74 (t, 2H), 3.43 (dt, 2H), 2.86 (t, 2H), 2.32 (s, 3H), 2.21 (t, 2H), 1.7−1.5 (4H), 1.4−1.2 (22H); ^{13}C NMR δ 196.17, 174.56, 62.58, 42.46, 36.70, 30.64, 29.7−29.4, 29.35, 29.29, 29.17, 29.11, 28.82, 25.73.

Acidic conditions during deacetylation yielded 36%, whereas basic conditions gave 100% as a white solid; TLC (EtOAc) R_f 0.2; ^{1}H NMR δ 6.09 (br, 1H), 3.71 (m, 2H), 3.42 (m, 2H), 2.52 (dt, 2H), 2.20 (t, 2H), 1.7−1.5 (4H), 1.34 (t, 1H), 1.4−1.2 (22H); ^{13}C NMR δ 174.57, 62.58, 42.46, 36.70, 34.05, 29.7−29.2, 29.08, 28.38, 25.73, 24.67. Analytical data: Calcd for $C_{18}H_{37}NO_2S$: C 65.21%, H 11.25%, N 4.22%. Found: C 65.34%, H 11.12%, N 4.22%.

***N*-(5-Hydroxy-3-oxapentyl) 16-Mercaptohexadecanamide (1b) [HS(CH$_2$)$_{15}$CONH(CH$_2$CH$_2$O)$_2$H].** The general procedure for amine coupling of **16** and 2-(2-aminoethoxy)-ethanol yielded 82% of a white crystalline compound. TLC (EtOAc/MeOH 10:1) R_f 0.5; ^{1}H NMR δ 6.09 (br, 1H), 3.74 (t, 2H), 3.6−3.5 (4H), 3.46 (m, 2H), 2.86 (t, 2H), 2.32 (s, 3H), 2.18 (t, 2H), 1.7−1.5 (4H), 1.4−1.2 (22H); ^{13}C NMR δ 196.17, 173.65, 72.31, 70.02, 61.67, 39.20, 36.73, 30.64, 29.7−29.5, 29.41, 29.35, 29.17, 29.11, 28.82, 25.79.

Deprotection was performed using acid or basic conditions, both in high yields: 90% and 93%, respectively, as a white solid. TLC (EtOAc/MeOH 10:1) R_f 0.5; ^{1}H NMR δ 6.13 (br, 1H), 3.74 (m, 2H), 3.6−3.5 (4H), 3.46 (m, 2H), 2.52 (dt, 2H), 2.18 (t, 2H), 1.7−1.5 (4H), 1.33 (t, 1H), 1.4−1.2 (22H); ^{13}C NMR δ 173.56, 72.28, 70.05, 61.72, 39.20, 36.79, 34.08, 29.7−29.3, 29.08, 28.41, 25.79, 24.67. Analytical data: Calcd for $C_{20}H_{41}NO_3S$: C 63.95%, H 11.00%, N 3.73%. Found: C 63.80%, H 10.88%, N 3.76%.

***N*-11-Hydroxy-3,6,9-trioxaundecyl 16-mercaptohexadecanamide (1c) [HS(CH$_2$)$_{15}$CONH(CH$_2$CH$_2$O)$_4$H].** The general procedure for amine coupling of **16** and **11a** yielded 80%. TLC (EtOAc/MeOH 10:1) R_f 0.4; ^{1}H NMR δ 7.03 (br, 1H), 3.8−3.6 (12H), 3.53 (m, 2H), 3.42 (m, 2H), 2.86 (t, 2H), 2.32 (s, 3H), 2.17 (t, 2H), 1.7−1.5 (4H), 1.4−1.2 (22H); ^{13}C NMR δ 196.11, 173.62, 72.63, 70.66, 70.46, 70.40, 70.0−69.9, 61.55, 39.08, 36.61, 30.64, 29.8−29.4, 29.17, 29.11, 28.82, 25.85.

Deprotection under acidic conditions yielded 85% as a white solid. TLC (EtOAc/MeOH 10:1) R_f 0.3; ^{1}H NMR δ 6.97 (br, 1H), 3.6−3.8 (12H), 3.53 (m, 2H), 3.44 (m, 2H), 2.52 (dt, 2H), 2.17 (t, 2H), 1.5−1.7 (4H), 1.33 (t, 1H), 1.2−1.4 (22H); ^{13}C NMR δ 173.53, 72.60, 70.66, 70.46, 70.40, 70.1−70.0, 61.55, 39.08, 36.64, 34.05, 29.7−29.4, 29.08, 28.41, 25.84, 24.67. Analytical data: Calcd for $C_{24}H_{49}NO_5S$: C 62.16%, H 10.65%, N 3.02%. Found: C 62.30%, H 10.74%, N 2.93%.

***N*-17-Hydroxy-3,6,9,12,15-pentaoxaheptadecyl 16-Mercaptohexadecanamide (1d) [HS(CH$_2$)$_{15}$CONH(CH$_2$CH$_2$O)$_6$H].** The general procedure for amine coupling of **16** and **11b** yielded 75% as a white solid. TLC (EtOAc/MeOH 4:1) R_f 0.7; ^{1}H NMR δ 6.38 (br, 1H), 3.8−3.6 (22H), 3.55 (m, 2H), 3.44 (m, 2H), 2.86 (t, 2H), 2.32 (s, 3H), 2.17 (t, 2H), 1.7−1.5 (4H), 1.4−1.2 (22H); ^{13}C NMR δ 196.02, 173.35, 72.60, 70.63, 70.6−70.5, 70.31, 70.16, 69.99, 61.67, 39.17, 36.70, 30.64, 29.7−29.4, 29.17, 29.11, 28.82, 25.79.

Acidic deprotection yielded 95% as a white solid. TLC (EtOAc/MeOH 10:1) R_f 0.2; ^{1}H NMR δ 6.43 (br, 1H), 3.72 (m, 2H), 3.7−3.6 (18H), 3.55 (m, 2H), 3.44 (m, 2H), 2.52 (dt, 2H), 2.18 (t, 2H), 1.5−1.7 (4H), 1.33 (t, 1H), 1.2−1.4 (22H); ^{13}C NMR δ 173.41, 72.63, 70.63, 70.6−70.4, 70.28, 70.16, 69.99, 61.64, 39.14, 36.67, 34.05, 29.8−29.3, 29.08, 28.38, 25.79, 24.64. Analytical data: Calcd for $C_{28}H_{57}NO_7S$: C 60.94%, H 10.41%, N 2.54%. Found: C 60.94%, H 10.46%, N 2.38%.

***N*-(23-Hydroxy-3,6,9,12,15,18,21-heptaoxatricosyl) 16-Mercaptohexadecanamide (1e) [HS(CH$_2$)$_{15}$CONH(CH$_2$CH$_2$O)$_8$H].** The general procedure for amine coupling of **16** and **11c** yielded 67% as a white solid. TLC (EtOAc/MeOH 3:1) R_f 0.3;^{1}H NMR δ 6.22 (br s, 1H), 3.74−3.47 (m, 30H), 3.45−3.41 (m, 2H), 2.89−2.80 (m, 2H), 2.8 (br s, 1H), 2.32 (s, 3H), 2.17 (t, J = 7 Hz, 2H), 1.64−1.5 (m, 4H), 1.4−1.15 (m, 22H); ^{13}C NMR δ 196.1, 173.3, 72.6, 70.4, 70.3, 70.0, 61.7, 39.2, 36.7, 30.7, 29.7, 29.6, 29.5, 29.44, 29.38, 29.2, 28.9, 25.8.

Basic deprotection yielded 93% of a white solid. TLC (EtOAc/MeOH 3:1) R_f 0.3; ^{1}H NMR δ 6.19 (br s, 1H), 3.75−3.47 (m,

30H), 3.47−3.43 (m, 2H), 2.9 (br s, 1H), 2.57−2.48 (dt, 2H), 2.17 (t, J = 7 Hz, 2H), 1.61−1.5 (m, 4H), 1.45−1.1 (m, 22H); ^{13}C NMR δ 173.2, 72.5, 70.3, 70.2, 69.9, 61.7, 39.1, 36.7, 34.0, 29.6, 29.5, 29.4, 29.3, 29.0, 28.4, 25.7, 24.6. Analytical data: Calcd for $C_{32}H_{65}NO_9S$: C 60.06%, H 10.24%, N 2.19%. Found: C 59.8%, H 10.1%, N 2.3%.

***N*-(29-Hydroxy-3,6,9,12,15,18,21,24,27-nonaoxanonacosyl) 16-Mercaptohexadecanamide (1f) [HS(CH$_2$)$_{15}$CONH(CH$_2$CH$_2$O)$_{10}$H].** The general procedure for amine coupling of **16** and **11d** yielded 90% as a white solid. TLC (EtOAc/MeOH 2:1) R_f 0.2; ^{1}H NMR δ 6.14 (br s, 1H), 3.74−3.47 (m, 38H), 3.43 (m, 2H), 2.86 (t, J = 7 Hz, 2H), 2.6 (br s, 1H), 2.32 (s, 3H), 2.17 (t, J = 7 Hz, 2H), 1.64−1.50 (m, 4H), 1.45−1.15 (m, 22H); ^{13}C NMR δ 196.0, 173.3, 72.5, 70.4, 70.2, 70.0, 61.7, 39.1, 36.7, 30.6, 29.6, 29.5, 29.4, 29.1, 28.8, 25.8.

Basic deprotection yielded 91% of a white solid. TLC (EtOAc/MeOH 3:1) R_f 0.2. ^{1}H NMR δ 6.19 (br s, 1H), 3.74−3.54 (m, 38 H), 3.46 (m, 2H), 2.67 (br s, 1 H), 2.50(dt, 2H), 2.17 (t, J = 7 Hz, 2H), 1.7−1.5 (m, 4H), 1.4−1.15 (m, 22H); ^{13}C NMR δ 173.3, 72.5, 70.4, 70.2, 70.0, 61.7, 39.2, 36.7, 34.1, 29.64, 29.58, 29.5, 29.4, 29.1, 28.4, 25.8, 24.6. Analytical data: Calcd for $C_{36}H_{73}NO_{11}S$: C 59.39%, H 10.11%, N 1.92%. Found: C 59.5%, H 10.1%, N 2.0%.

***N*-(35-Hydroxy-3,6,9,12,15,18,21,24,27,30,33-dodecaoxapentatriacontyl) 16-mercaptohexadecanamide (1g) [HS(CH$_2$)$_{15}$CONH(CH$_2$CH$_2$O)$_{12}$H].** To a solution of **16** (23 mg, 0.067 mmol), **11e** (44 mg, 0.081 mmol), HOBt (9.0 mg, 0.10 mmol), and TEA (28 μL, 0.20 mmol) in CH_2Cl_2 (20 mL) was added EDC (19 mg, 0.010 mol) at 0 °C. After 19 h, the solution was acidified with hydrochloric acid (2 mL, 1 M HCl) and concentrated. Chromatography of the residue (CH_2Cl_2/MeOH 30:1 → 15:1) yielded 64 mg of a crude product (contaminated with TEA according to NMR). The product fraction was dissolved in CH_2Cl_2 (20 mL) and washed with water (2 × 10 mL). The water phase was separated, and the CH_2Cl_2 phase was concentrated followed by preparative HPLC (MeOH/H_2O 90:10) to provide the pure acetylated amide (16 mg, 28%). TLC (EtOAc/MeOH 3:1) R_f 0.1; ^{1}H NMR δ 6.23 (br s, 1H), 3.74−3.37 (m, 48H), 2.86 (t, J = 7 Hz, 2H), 2.32 (s, 3H), 2.3 (br s, 1H), 2.17 (t, J = 7 Hz, 2H), 1.62−1.50 (m, 4H), 1.4−1.2 (m, 22H); ^{13}C NMR δ 196.0, 173.3, 72.6, 70.6, 70.3, 70.2, 70.0, 61.7, 39.2, 36.7, 30.6, 29.64, 29.58, 29.5, 29.4, 29.2, 29.1, 28.8, 25.8.

Basic deprotection and subsequent purification by HPLC yielded 7.5 mg (56%). TLC (EtOAc/MeOH 3:1) R_f 0.1; ^{1}H NMR δ 6.13 (br s, 1H), 3.74−3.41 (m, 48H), 2.50 (dt, 2H), 2.17 (t, J = 7 Hz, 2H), 1.7−1.55 (m, 4H), 1.4−1.2 (m, 22H); ^{13}C NMR δ 173.3, 72.5, 70.6, 70.4, 70.3, 70.0, 61.7, 39.1, 36.7, 34.1, 29.6, 29.5, 29.4, 29.1, 28.4, 25.8, 24.7. Analytical data: Calcd for $C_{40}H_{81}NO_{13}S$: C 58.87%, H 10.00%, N 1.72%. Found: C 58.9%, H 10.1%, N 1.7%.

***N*-(11-Hydroxy-3,6,9-trioxaundecyl) 12-mercaptododecanamide (2a) [HS(CH$_2$)$_{11}$CONH(CH$_2$CH$_2$O)$_4$H].** The general procedure for amine coupling of **17** and **11a** yielded 70%. TLC (EtOAc/MeOH 10:1) R_f 0.2; ^{1}H NMR δ 3.8−3.6 (12H), 3.54 (m, 2H), 3.44 (m, 2H), 2.86 (t, 2H), 2.32 (s, 3H), 2.17 (t, 2H), 1.7−1.5 (4H), 1.4−1.2 (14H).

Acidic deprotection yielded 78%. TLC (EtOAc/MeOH 4:1) R_f 0.6; H NMR δ 6.98 (br, 1H), 3.8−3.6 (12H), 3.47 (m, 2H), 3.44 (m, 2H), 2.52 (dt, 2H), 2.17 (t, 2H), 1.7−1.5 (4H), 1.4−1.2 (15H); ^{13}C NMR δ 173.44, 72.60, 70.66, 70.5−70.4, 70.1−69.9, 61.55, 39.05, 36.64, 34.05, 29.6−29.4, 29.05, 28.38, 25.82, 24.64. Analytical data: Calcd for $C_{20}H_{41}NO_5S$: C 58.93%, H 10.14%, N 3.44%. Found: C 58.94%, H 10.25%, N 3.35%.

***N*-(17-Hydroxy-3,6,9,12,15-pentaoxaheptadecyl) 12-Mercaptododecanamide (2b) [HS(CH$_2$)$_{11}$CONH(CH$_2$CH$_2$O)$_6$H].** The general procedure for amine coupling of **17** and **11b** yielded 76%. TLC (EtOAc/MeOH 10:1) R_f 0.2; ^{1}H NMR δ 3.8−3.5 (22 H) 3.44 (m, 2H), 2.86 (t, 2H), 2.32 (s, 3H), 2.18 (t, 2H), 1.7−1.5 (m, 4H), 1.4−1.2 (14H).

Acidic deprotection yielded 70%. TLC (EtOAc/MeOH 4:1) R_f 0.1; ^{1}H NMR δ 6.40 (br, 1H), 3.7−3.5 (22H), 3.45 (m, 2H), 2.52 (dt, 2H), 2.18 (t, 2H), 1.7−1.5 (4H), 1.4−1.2 (15H); ^{13}C NMR δ 173.32, 72.60, 70.63, 70.6−70.5, 70.28, 70.16, 70.02, 61.69, 39.17, 36.70, 34.05, 29.3−29.6, 29.05, 28.38, 25.76, 24.64. Analytical data: Calcd for $C_{24}H_{49}NO_7S$: C 58.15%, H 9.96%, N 2.83%. Found: C 58.01%, H 10.09%, N 2.79%.

***N*-(11-Hydroxy-3,6,9-trioxaundecyl) 6-Mercaptohexanamide (3a) [HS(CH$_2$)$_5$CONH(CH$_2$CH$_2$O)$_4$H].** The general procedure for amine coupling of **18**[45] and **11a** yielded 93%. TLC (EtOAc/MeOH 10:1) R_f 0.2; ^{1}H NMR δ 3.8−3.6 (12H), 3.44 (m, 2H), 2.53 (m, 2H), 2.86 (t, 2H), 2.32 (s, 3H), 2.18 (t, 2H), 1.7−1.5 (4H), 1.35 (m, 2H).

Acidic deprotection yielded 73%. TLC (EtOAc/MeOH 4:1) R_f 0.5 ^{1}H NMR δ 7.10 (br, 1H), 3.8−3.6 (12H), 3.47 (m, 2H), 3.45 (m, 2H), 2.53 (dt, 2H), 2.19 (t, 2H), 1.75−1.55 (4H), 1.43 (m, 2H), 1.31 (t, 1H); ^{13}C NMR δ 173.09, 72.57, 70.66, 70.43, 70.37, 70.0−69.9, 61.55, 39.08, 36.29, 33.73, 28.00, 25.14, 24.44. Analytical data: Calcd for $C_{14}H_{29}NO_5S$: C 51.99%, H 9.04%, N 4.33%. Found: C 51.75%, H 8.9%, N 4.3%.

***N*-(17-Hydroxy-3,6,9,12,15-pentaoxaheptadecyl) 6-Mercaptohexanamide (3b) [HS(CH$_2$)$_5$CONH(CH$_2$CH$_2$O)$_6$H].** The general procedure for amine coupling of **18**[45] and **11b** yielded 85%. TLC (EtOAc/MeOH 10:1) R_f 0.2; ^{1}H NMR δ 3.8−3.5 (22H), 3.44 (m, 2H), 2.86 (t, 2H), 2.32 (s, 3H), 2.19 (t, 2H), 1.7−1.5 (4H), 1.35 (m, 2H).

Acidic deprotection yielded 82%. TLC (EtOAc/MeOH 4:1) R_f < 0.1. ^{1}H NMR δ 6.48 (br, 1H), 3.5−3.7 (22H), 3.45 (m, 2H), 2.53 (dt, 2H), 2.20 (t, 2H), 1.75−1.55 (4H), 1.43 (m, 2H), 1.35 (t, 1H); ^{13}C NMR δ 172.97, 72.60, 70.63, 70.6−70.5, 70.28, 70.16, 69.96, 61.69, 39.20, 36.38, 33.70, 27.97, 25.08, 24.44. Analytical data: Calcd for $C_{18}H_{37}NO_7S$: C 52.53%, H 9.06%, N 3.40%. Found: C 52.5%, H 9.3%, N 3.5%.

***N*-(11-Hydroxy-3,6,9-trioxaundecyl) 3-Mercaptopropionamide (4a) [HS(CH$_2$)$_2$CONH(CH$_2$CH$_2$O)$_4$H].** Compound **4a** was prepared following the general procedure for amine coupling of **19**[44] and **11a** with the exception that chloroform/EtOH 2:1 was used for extraction during workup. Yield 66%. TLC (EtOAc/MeOH 10:1) R_f 0.1; ^{1}H NMR δ 3.8−3.6 (12H), 3.52 (m, 2H), 3.45 (m, 2H), 3.15 (t, 2H), 2.50 (t, 2H), 2.32 (s, 3H).

Deacetylation was performed under acidic conditions as described above with the exception that extraction during workup was exchanged for coevaporation with toluene prior to purification by flash chromatography. Yield: 79%. TLC (EtOAc/MeOH 4:1) R_f 0.4; ^{1}H NMR δ 7.28 (br, 1H), 3.8−3.6 (12H), 3.48 (m, 2H), 3.45 (m, 2H), 2.82 (dt, 2H), 2.51 (t, 2H), 1.62 (t, 1H); ^{13}C NMR δ 170.91, 72.58, 70.69, 70.40, 70.22, 70.0−69.9, 61.55, 40.20, 39.17, 20.58. Analytical data: Calcd for $C_{11}H_{23}NO_5S$: C 46.96%, H 8.24%, N 4.98%. Found: C 46.8%, H 8.3%, N 4.9%.

***N*-(17-Hydroxy-3,6,9,12,15-pentaoxaheptadecyl) 3-Mercaptopropionamide (4b) [HS(CH$_2$)$_2$CONH(CH$_2$CH$_2$O)$_6$H].** Compound **4b** was prepared from compounds **19**[44] and **11b** using the same procedure as for compound **4a**. Amine coupling yielded 59%. TLC (EtOAc/MeOH 10:1) R_f 0.1; ^{1}H NMR δ 3.5−3.8 (22H), 3.44 (m, 2H), 3.15 (t, 2H), 2.52 (t, 2H), 2.32 (s, 3H).

Acidic deprotection yielded 80%. TLC (EtOAc/MeOH 4:1) R_f < 0.1; ^{1}H NMR δ 6.71 (br, 1H), 3.75−3.55 (22H), 3.45 (m, 2H), 2.82 (dt, 2H), 2.52 (t, 2H), 1.65 (t, 1H); ^{13}C NMR δ 170.85, 72.60, 70.63, 70.6−70.5, 70.28, 70.19, 69.81, 61.69, 40.29, 39.32, 20.53. Analytical data: Calcd for $C_{15}H_{31}NO_7S$: C 48.76%, H 8.46%, N 3.79%. Found: C 48.7%, H 8.6%, N 3.9%.

***N*-[18-(*N*-Hexadecylcarbamoyl)methyl-3,6,9,12,15,18-hexaoxaoctadecyl] 16-(Acetylthio)hexadecanamide (20) [AcS(CH$_2$)$_{15}$CONH(CH$_2$CH$_2$O)$_6$CH$_2$COOH].** Compounds **15** and **16** were coupled following the general procedure for amine coupling described above. The eluant for column chromatography was toluene/EtOAc (3:1 to 0:1). Yield: 80%. TLC (EtOAc/MeOH 10:1) R_f 0.5; ^{1}H NMR δ 6.23 (br, 1H), 4.02 (s, 2H), 3.7−3.6 (20H), 3.55 (t, 2H), 3.45 (m, 2H), 2.86 (t, 2H), 2.32 (s, 3H), 2.17 (t, 2H), 1.7−1.5 (4H), 1.48 (s, 9H), 1.4−1.2 (22H); ^{13}C NMR δ 196.05, 173.28, 169.66, 81.52, 70.73, 70.7−70.5, 70.02, 70.00, 69.02, 39.14, 36.73, 30.64, 29.7−29.3, 29.2−29.1, 28.82, 28.11, 25.76.

TFA (5 mL) was added to a solution of the *t*-Bu-protected compound (120 mg, 0.16 mmol) in CH_2Cl_2 (5 mL), and the mixture was stirred for 6 h. The solvent was coevaporated twice with toluene. The residue was diluted with CH_2Cl_2 (5 mL) and washed twice with water (10 mL) (until pH = 7). The organic solution was dried and evaporated to yield 109 mg

(99%) of a white solid. TLC (CH_2Cl_2 /MeOH 4:1) R_f 0.6; ^{1}H NMR δ 6.42 (s, 1H), 4.17 (s, 2H), 3.74 (m, 2H), 3.7−3.6 (18H), 3.56 (t, 2H), 3.43 (m, 2H), 2.86 (t, 2H), 2.32 (s, 3H), 2.20 (t, 2H), 1.7−1.5 (4H), 1.4−1.2 (22H); ^{13}C NMR δ 196.13, 173.99, 172.22, 71.05, 70.7−70.3, 70.20, 69.85, 68.76, 39.26, 36.61, 30.64, 29.3−29.7, 29.2−29.1, 28.82, 25.79.

***N*-(18-Carboxymethyl)-3,6,9,12,15,18-hexaoxaoctadecyl 16-Mercaptohexadecanamide (5) [HS(CH$_2$)$_{15}$CONH(CH$_2$CH$_2$O)$_6$CH$_2$COOH].** Compound **20** (105 mg, 0.16 mmol) was dissolved in DMF (10 mL), and the solution was purged with argon prior to addition of hydrazine acetate (2.4 mmol, 2.4 mL of a 1.0 M solution in DMF, purged with argon). The reaction mixture was stirred for 20 h under argon. The solvent was evaporated, and the crude product was purified by flash chromatography (CH_2Cl_2/MeOH 99:1 to 90:10) to give 80 mg (82%) of the final product as a white solid. TLC (CH_2Cl_2/MeOH 4:1); R_f 0.5; ^{1}H NMR δ 6.36 (br, 1H), 4.17 (s, 2H), 3.74 (m, 2H), 3.7−3.6 (18H), 3.55 (m, 2H), 3.44 (m, 2H), 2.53 (dt, 2H), 2.19 (t, 2H), 1.7−1.5 (4H), 1.33 (t, 1H), 1.4−1.2 (22H); ^{13}C NMR δ 173.79, 172.12, 71.02, 70.7−70.3, 70.13, 69.87, 68.87, 39.20, 36.64, 34.02, 29.7−29.4, 29.38, 29.32, 29.17, 29.05, 28.35, 25.76, 24.64. Analytical data: Calcd for $C_{30}H_{59}NO_9S$: C 59.08%, H 9.75%, N 2.30%. Found: C 58.94%, H 9.60%, N 2.24%.

***N*-[18-(*N*-Hexadecylcarbamoyl)methyl- 3,6,9,12,15,18-hexaoxaoctadecyl] 16-mercaptohexadecanamide (6) [HS(CH$_2$)$_{15}$CONH(CH$_2$CH$_2$O)$_6$CH$_2$OCONH(CH$_2$)$_{15}$CH$_3$].** Compound **20** was dissolved in CH_2Cl_2 and coupled to 1-hexadecylamine following the general procedure for amine coupling described above. Yield: 80%. TLC (EtOAc/MeOH 10:1) R_f 0.25; ^{1}H NMR δ 6.99 (s, 1H), 6.29 (s, 1H), 3.98 (s, 2H), 3.7−3.5 (m, 20H), 3.53 (tr, 2H), 3.43 (tr, 2H), 2.85 (tr, 2H), 2.32 (s, 3H), 2.17 (tr, 2H), 1.6−1.5 (m, 6H), 1.3−1.2 (50H), 0.90 (s, 3H); ^{13}C NMR δ 196.2, 173.3, 169.7, 70.7−70.0, 38.9, 36.7, 30.6, 31.9−26.9, 25.7, 22.7, 14.1.

The acetyl-protected thiol was deprotected under acidic conditions in 98% yield. TLC (EtOAc/MeOH 10:1) R_f 0.5; ^{1}H NMR δ 6.99 (s, 1H), 6.29 (s, 1H), 3.98 (s, 2H), 3.66−3.52 (m, 20H), 3.53 (tr, 2H), 3.43 (tr, 2H), 2.53 (tr, 2H), 2.17 (tr, 2H), 1.52−1.61 (m, 6H), 1.3−1.2 (50H), 0.90 (s, 3H); ^{13}C NMR δ 173.3, 169.7, 70.7−70.0, 38.9, 36.7, 34.0, 31.9−26.9, 25.7, 22.7, 14.1.

11-Hydroxy-3,6,9-trioxaundecyl 16-mercaptohexadecanoate (7a) [HS(CH$_2$)$_{15}$COO(CH$_2$CH$_2$O)$_4$H]. To a solution of **16** (100 mg, 0.30 mmol) in CH_2Cl_2 (2 mL) were added tetra(ethylene glycol) (710 mg, 3.6 mmol) and DMAP (9.2 mg, 0.076 mmol) and finally *N*-(3-(dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC) (73 mg, 0.38 mmol). The reaction was stirred overnight, diluted with CH_2Cl_2 (5 mL), and washed with 0.1 M HCl (10 mL). The aqueous layer was extracted once with CH_2Cl_2 (5 mL). The combined organic solutions were dried over $MgSO_4$, and the solvent was evaporated. The crude product was purified by flash chromatography (toluene/EtOAc 1:2 to 0:1) to give the acetylated product as a white solid (130 mg, 83%). TLC (EtOAc) R_f 0.3. ^{1}H NMR δ 4.23 (t, 2H), 3.6−3.8 (14H), 2.86 (t, 2H), 2.32 (t, 2H), 2.32 (s, 3H), 1.5−1.7 (4H), 1.2−1.4 (22H); ^{13}C NMR δ 196.1, 173.9, 72.5, 70.3−70.8, 69.3, 63.3, 61.8, 34.2, 30.7, 29.0−29.8, 28.8, 24.9.

A solution of the protected product (90 mg, 0.18 mmol) in DMF (2 mL) was purged with argon. Hydrazinium acetate (170 mg, 1.8 mmol) was added from a stock solution (1 M, DMF) that had likewise been purged with argon. After 48 h, the reaction mixture was diluted with CH_2Cl_2 (6 mL) and washed with water (10 mL). The aqueous layer was extracted once with CH_2Cl_2 (5 mL). The combined organic solutions were dried over $MgSO_4$ and coevaporated twice with toluene (5 mL). Flash chromatography yielded **7a** as a white solid (72 mg, 89%). TLC (EtOAc) R_f 0.3; ^{1}H NMR δ 4.24 (t, 2H), 3.6−3.7 (12H), 3.55 (t, 2H), 2.52 (dt, 2H), 2.33 (t, 2H), 1.5−1.7 (4H), 1.34 (t, 1H), 1.2−1.4 (22H); ^{13}C NMR δ 173.88, 72.55, 70.66, 70.5−70.6, 70.34, 69.22, 63.31, 61.75, 34.20, 34.05, 29.6−29.7, 29.52, 29.47, 29.29, 29.14, 29.08, 28.38, 24.91, 24.64. Analytical data: Calcd for $C_{24}H_{48}O_6S$: C 62.03%, H 10.41%. Found: C 61.97%, H 10.47%.

17-Hydroxy-3,6,9,12,15-pentaoxaheptadecyl 16-mercaptohexadecanoate (7b) [HS(CH$_2$)$_{15}$COO(CH$_2$CH$_2$O)$_6$H]. Compound **7b** was prepared following the same procedure as for compound **7a**. Ester coupling of **16** and hexa(ethylene glycol) gave 150 mg (81%) of the thioacetylated product. TLC (EtOAc/MeOH 10:1) R_f 0.4. ^{1}H NMR δ 4.22 (t, 2H), 3.6−3.8 (22H), 2.86 (t, 2H), 2.32 (t, 2H), 2.32 (s, 3H), 1.5−1.7 (4H), 1.2−1.4 (22H); ^{13}C NMR δ 196.1, 173.9, 72.6, 70.2−70.7, 69.2, 63.3, 61.7, 34.2, 30.6, 29.0−29.8, 28.8, 24.9.

Deprotection of the thioester yield 65 mg (62%) (in addition to >10% of disulfide). TLC (EtOAc/MeOH 20:1) R_f 0.4. ^{1}H NMR δ 4.22 (t, 2H), 3.6−3.7 (20H), 3.55 (t, 2H), 2.52 (dt, 2H), 2.32 (t, 2H), 1.5−1.7 (4H), 1.33 (t, 1H), 1.2−1.4 (22H); ^{13}C NMR δ 173.88, 72.58, 70.66, 70.6−70.5, 70.37, 69.22, 63.37, 61.75, 34.23, 34.08, 29.7−29.4, 29.29, 29.17, 29.08, 28.41, 24.94, 24.67. Analytical data: Calcd for $C_{28}H_{56}O_8S$: C 60.84%, H 10.21%. Found: C 60.95%, H 10.19%.

Contact Angle Goniometry. Static water contact angle measurements were performed at ambient with a Ramé-Hart NRL 100 goniometer using deionized water (MilliQ). Two measurements, one at each edge of the droplet, were made on two samples within a short time.

Ellipsometry. An automatic Rudolph Research AutoEL single-wavelength ellipsometer (He−Ne laser light source, λ = 632.6 nm, angle of incidence 70°) was used for the ellipsometric measurement. Three measurements were performed on different spots (2 mm apart) at each of three samples. The thickness of the SAM was determined using a three phase model (ambient − organic film − gold)[50] assuming a refractive index of 1.5 for the organic film and using the refractive index values obtained from the cleaned gold substrates as described below.

Infrared Spectroscopy. Infrared reflection−absorption spectra were recorded on a Bruker IFS 66 system equipped with a grazing angle (85°) infrared reflection accessory and a liquid nitrogen cooled MCT detector. The measurement chamber was purged with nitrogen gas during the measurements and a spectrum of a deuterated hexadecanethiol (HS(CD$_2$)$_{15}$CD$_3$) SAM was used as a reference. Two spectra were repeated twice (**1e**, **1g**), without significant variation. The acquisition time was about 10 min at 2 cm^{-1} resolution, and a three-term Blackmann−Harris apodization function was applied to the interferograms before Fourier transformation. Peak-picking was performed using the standard method supplied with the spectrometer control software (OPUS, Version 2.06 from Bruker).

Preparation of SAMs. SAMs were prepared on two different types of gold substrates. Glass slides (9 × 9 mm) coated with 2000 Å of gold (BiaCore AB, Uppsala, Sweden) were used for ellipsometric and water contact angle measurements. For the infrared spectroscopy, standard silicon (100) wafers (20 × 40 mm) were coated with 25 Å of titanium followed by 2000 Å of gold (10 Å/s) using electron beam evaporation (Balzers UMS 500 P system). The base pressure was at least 10^{-9} mbar and the evaporation pressure about 10^{-7} mbar. All gold substrates were cleaned in an aqueous solution of 4% H_2O_2 and 4% NH_3 ("TL1") for 5−10 min at close to 100 °C and rinsed in deionized water (MilliQ) prior to monolayer assembly. Ellipsometric angles Δ > 110° (average of three measurements at different spots) were taken as an indicative for a satisfactory cleaning procedure. The gold substrates were incubated for 48 h in 20 μM ethanolic solutions of compounds **1b**−**g**. Shortly before measuring, the samples were taken out of the incubation solution, ultrasonicated for 3 min in ethanol, rinsed, and blown dry by a stream of nitrogen.

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