# A Survey of Structure−Property Relationships of Surfaces that Resist the Adsorption of Protein

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This paper describes the use of surface plasmon resonance (SPR) spectroscopy and self-assembled monolayers (SAMs) to determine the characteristics of functional groups that give surfaces the ability to resist the nonspecific adsorption of proteins from solution. Mixed SAMs presenting different functional groups were prepared for screening using a synthetic protocol based on the reaction of organic amines with a SAM terminated by interchain carboxylic anhydride groups. Surfaces that presented derivatives of oligo(sarcosine), *N*-acetylpiperazine, and permethylated sorbitol groups were particularly effective in resisting the adsorption of proteins. Incorporation of these groups into single-component SAMs resulted in surfaces that are comparable to (but slightly less good than) single-component SAMs that present oligo(ethylene glycol) in their ability to resist the adsorption of proteins. In the group of surfaces examined, those that resisted the adsorption of proteins had the following properties: they were hydrophilic; they contained groups that were hydrogen-bond acceptors but not hydrogen-bond donors; and they were overall electrically neutral.

## Introduction

The objective of this work was to correlate the molecular-scale structure of surfaces with their ability to resist the adsorption of proteins from buffered aqueous solutions. We combined self-assembled monolayers (SAMs) and surface plasmon resonance (SPR) spectroscopy into a system that enabled us to screen a number of functional groups rapidly for their ability to resist the adsorption of fibrinogen and lysozyme. The surfaces were prepared by the reaction of an amine HNR′**R** containing the functional group **R** of interest with a SAM presenting interchain carboxylic anhydrides on its surface (Figure 1). The ease with which these surfaces can be prepared for use in screening makes this route efficient for exploratory work.

Surfaces that do not adsorb proteins—surfaces that we refer to as "nonadsorbing" or "inert" for brevity—are part of the much broader field of biocompatible materials. Applications of nonadsorbing surfaces include prostheses, sensors, substrates for enzyme-linked immunosorbent assays (ELISAs), materials for use in contact lenses, and implanted devices.[1] Newer areas of applications include systems for patterned cell cultures,[2] tissue engineering,[3] materials used in microfluidic and analytical systems,[4] devices used for drug delivery,[5,6] and systems for high-throughput screening using proteins[7] or cells.[8]

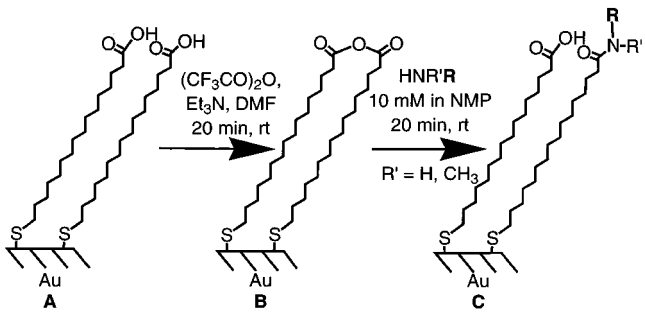


**Figure 1.** Schematic illustration of the synthesis of mixed SAMs that present a 1:1 mixture of −CONR′**R** and $-CO_2H$/$CO_2^-$ groups using the anhydride method. Dehydration of SAMs terminating in carboxylic acid groups with trifluoroacetic acid gives SAMs that present interchain carboxylic anhydride groups. Immersion of these SAMs in solutions of the appropriate amines gives the mixed SAM. The scheme suggests the composition of the mixed SAM, but not the conformation of the groups in it or the details of their distribution on the surface. The state of ionization of the carboxylic acid groups in these systems has not been established.

We find that surfaces that resist the adsorption of proteins, in the set that we studied here, incorporate groups that exhibit four molecular-level characteristics:[9] (i) They are hydrophilic. (ii) They include hydrogen-bond acceptors. (iii) They do *not* include hydrogen-bond donors. (iv) Their overall electrical charge is neutral.[10] This set of characteristics describes all of the surfaces that we examined here, but not all surfaces that are known to be inert. Mrksich has reported that SAMs presenting mannitol groups are also inert to protein adsorption, although they contain a large number of hydrogen-bond donors.[11]

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**Approaches to the Design of Inert Surfaces.** *Surfaces Presenting Poly(ethylene glycol).* The empirical observations that materials presenting poly(ethylene glycol) (PEG) moieties on their surface are biocompatible and that they resist the nonspecific adsorption of proteins have resulted in the widespread use of PEG for biomedical applications.[12] The resistance of PEG-coated surfaces increases with increasing density and length of the chains in the surface-grafted film.[13,14] PEG is useful for rendering surfaces inert to the adsorption of proteins and to the adhesion of platelets and other cells,[15,16] but it has disadvantages in some applications. PEG is a polyether that autoxidizes relatively rapidly, especially in the presence of $O_2$ and transition metal ions (most biochemically relevant solutions contain transition metal ions);[17−19] in vivo, the hydroxyl groups of PEG are oxidized enzymatically to aldehydes and acids.[20,21] Eventually, these surfaces allow cells to attach and are therefore not suitable in applications requiring patterning of cells.[2,11]

*SAMs that Resist the Adsorption of Proteins.* SAMs presenting oligo(ethylene glycol) groups [as in $HS(CH_2)_{11}-(EG)_nOH$ and $-(EG)_nOCH_3$, $n = 3-17$] on their surface also resist the adsorption of proteins, even when present at a surface in a 1:1 mixture with $HS(CH_2)_{10}CH_3$ or $HS(CH_2)_{15}COOH$.[22−24] SAMs terminated in $-(EG)_3OH$ and $-(EG)_6OH$ groups best resisted the adsorption of proteins.[23,24] SAMs presenting tripropylenesulfoxide [$-\{S(O)CH_2CH_2CH_2\}_3S(O)CH_3$] are only marginally less protein-resistant than SAMs presenting $-(EG)_6OH$ groups;[25] the $-(EG)_nOH$ group is thus not unique in its protein resistance. Prime et al. previously described a maltose-terminated SAM that resisted the adsorption of proteins.[23,24]

In 2000, Mrksich made the remarkable observation that mannitol-terminated SAMs were also inert.[11] Although mannitol-terminated surfaces have not been compared extensively with SAMs terminating in $-(EG)_nOH$ groups, Mrksich demonstrated that mannitol-terminated SAMs resist the adhesion of fibroblasts for longer periods of time than $(EG)_nOH$-terminated SAMs.[11] Because $(EG)_nOH$-terminated SAMs have been extensively characterized, we continue to use them as the standard against which to compare other systems.

**Mechanism of Protein Resistance.** *Adsorption of Proteins to Surfaces.* Adsorption probably involves a number of steps and is not mechanistically well-defined; in many cases, a combination of attractive components causes adsorption. In most cases of nonbiospecific adsorption, proteins adsorb onto surfaces irreversibly by processes that do not lend themselves to detailed kinetic treatments; individual proteins often exhibit remarkable differences in their kinetics of adsorption.[26,27] The adsorbed proteins can undergo conformational changes that increase interactions (especially hydrophobic interactions) between the proteins and the surfaces.[28] Excellent reviews of this subject have appeared elsewhere.[28−31]

*Theoretical Studies.* Many of the studies of inert surfaces have been focused on improving the biocompatibility of materials for biomedical applications; hence, the majority of this work has been performed with bulk polymers or thin polymeric films. The early discovery that grafted PEG chains made surfaces sufficiently inert for some applications generated much theoretical work to explain that observation, in the hope that a generalized understanding of inertness would emerge. Much of the early theoretical work treated the proteins as hard spheres and the polymers as random coils, without accounting for molecular structure.[13,32,33]

Andrade and de Gennes treated the protein resistance of grafted PEG chains theoretically using ideas borrowed from colloid stabilization.[13,32,33] According to the mechanism for resistance postulated by Andrade and de Gennes, the water molecules associated with the hydrated PEG chains are compressed out of the PEG layer as the protein approaches the surface. Thermodynamically, the removal of water from the PEG chains is unfavorable, and it gives rise to a steric repulsion that, according to Andrade and de Gennes, contributes to the inertness of the PEG-terminated surfaces. This theory predicts that the inertness of surfaces will increase with increasing length and density of the PEG chains.

Szleifer found that, by using single-chain mean-field (SCMF) theory for the polymer chains, it was possible to rationalize the inertness of systems with a high density of short $(EG)_nOH$ chains ($n \geq 6$), including that of SAMs; the models proposed by Andrade and de Gennes had failed to address such systems.[14,34,35]

We believe that additional inert surfaces were required to test mechanisms and that more emphasis should be placed on a *molecular-level* understanding of the surface properties that prevent adsorption of proteins. Besseling was one of the first to suggest that the chemical properties of surfaces might affect their states of hydration and the repulsive or attractive forces that result from the interactions of two such surfaces as they are allowed to interact.[36] Theoretical analysis indicated that the interaction between two surfaces that causes changes in the *orientation* of water molecules (compared to bulk water) is repulsive; such surfaces were identified as having an excess of either proton donors or acceptors.[36]

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*SAMs Presenting $(EG)_n$ Groups.* SAMs terminated with $-(EG)_{n=3-7}OH$ resist the adsorption of proteins as well as SAMs terminated with $-(EG)_{n\approx17}OH$; direct comparisons with surfaces having grafted layers of PEG have not been carried out.[23,24] Szleifer's improvements to the model of Andrade and de Gennes could rationalize the inertness of SAMs terminated with $-(EG)_{n<7}OH$, but do not provide a molecular-level explanation of resistance.[14,37]

Grunze et al. found that the chain conformation of $-(EG)_nOCH_3$ oligomers at the surface of SAMs seems to be an important determinant of resistance to protein adsorption.[38] The conformation of $-(EG)_nOCH_3$ groups in the SAMs on gold that is inert is the helical conformation (*h*-SAM); when the molecules adopt an all trans conformation on silver (*t*-SAM), the SAM is not inert.[39] Force measurements on these SAMs suggested the presence of a strong dipole field in the inert *h*-SAM.[40] Monte Carlo simulations indicated that the dipole moments of the water molecules at the interface point into the SAM and can orient 3−4 layers of water at the interface.[41,42]

Grunze and others have proposed that the interaction of water with the surface of SAMs is more important than steric stabilization of the terminal $(EG)_nOH$ chains. Theoretical and experimental work from Grunze's group indicates that the conformation and packing of the chains in SAMs affects the penetration of water in the ethylene glycol layer and the inertness of the surface.[41,43] Monte Carlo simulations suggest that *h*-SAMs interact more strongly with water than *t*-SAMs,[41,44] and sum frequency generation experiments indicate that water penetrates into the $(EG)_nOH$ layers of the SAMs and causes them to become amorphous.[43]

**Experimental Design.** It is difficult to test hypotheses for the mechanisms of protein resistance when only a few relevant data are available. Because the synthesis of alkanethiols that contain complex functional groups such as peptides and carbohydrates is not straightforward, we initially focused our efforts on developing a synthetic method that would make it possible to synthesize and screen a large number of surfaces for their ability to resist the adsorption of proteins.

We required a procedure that combined two characteristics: (i) a means of preparing model surfaces conveniently using commercially available reagents and (ii) a reliable detection system that is compatible with these model surfaces. We and others have settled on a combination of SAMs and surface plasmon resonance (SPR) to study biological phenomena that occur at interfaces.[45−49] SAMs make it possible to control the properties of surfaces at the molecular level;[50,51] SPR allows the detection of ~0.2% of a monolayer of adsorbed protein (in the experiments that we carry out) by measuring changes in the index of refraction at the surface.[52] Although several methods exist for synthesizing SAMs that present different functional groups from a common reactive intermediate,[53−55] we chose to use the reaction of amines with anhydride-terminated surfaces for its operational simplicity (we call this procedure the "anhydride method").[56] Previous work with the anhydride method established that the reaction of typical organic amines with surface anhydrides proceeds cleanly and in good yield.[56]

The use of the anhydride method in conjunction with SPR allowed us to identify the groups that were best-suited to the formation of inert surfaces. Using the results obtained using this approach, the time-consuming synthesis of alkanethiols containing complex functional groups could be undertaken with confidence.

## Results and Discussion

**Synthesis and Characterization of Surfaces.** *Anhydride Method.* Mixed SAMs were prepared using the anhydride method (Figure 1). SAMs of 16-mercaptohexadecanoic acids were dehydrated with trifluoroacetic anhydride to give SAMs terminated with interchain carboxylic anhydride groups.[56] These SAMs were allowed to react further with amines of the form HNR′**R** to give mixed SAMs that presented an approximately 1:1 mixture of $-CONR'\mathbf{R}$ and $-CO_2H/CO_2^-$ groups. The structures of the amines used in this work are given in Table 1.

*Characterization of the Synthetic Products.* Yan et al. have reported that the reaction of a structurally simple, unhindered alkylamine with a surface that presents interchain anhydride groups proceeds in essentially quantitative yield, as indicated by polarized infrared external reflectance spectroscopy (PIERS) and X-ray photoelectron spectroscopy (XPS).[56] We did not characterize the yields of the amide-forming reactions that we used to prepare the mixed SAMs summarized in Table 1. It is likely that the more sterically hindered amines that we tested (e.g., carbohydrate derivatives) did not react quantitatively with the anhydride surface. These surfaces would then present an excess of $-CO_2H/CO_2^-$ groups at the interface. We also did not exclude the possibility that some of the functional groups react more than once with the anhydride surfaces, and results described later suggest indirectly that carbohydrates might yield esters during the reactions. The potential contributions of side products to protein adsorption are the price we pay for convenience. The mixed SAMs generated in this manner are certainly useful for screening. We describe our characterization of the mixed SAMs using contact angle measurements and octan-1-ol partition coefficients (ClogP) values below.

The reaction of an amine with an anhydride-terminated surface generates a homogeneously distributed, approximately 1:1 mixture of amido groups ($-CONR'\mathbf{R}$) and $-CO_2H/CO_2^-$ groups. The longer terminal groups, such

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**Table 1. Characterization of Mixed SAMs that Present $-CONR'R/-CO_2H$ Groups**

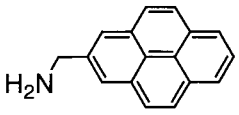
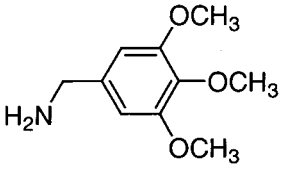
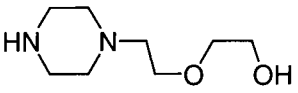
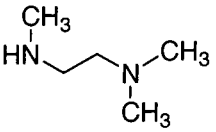
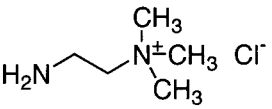
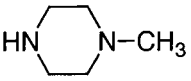
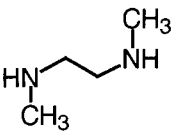
| Entry No. | HNR'**R** | Fibrinogen %ML 3 min[a] | Fibrinogen %ML 30 min[b] | Lysozyme %ML 3 min[a] | Lysozyme %ML 30 min[b] |
|---|---|---|---|---|---|
| **Hydrophobic Groups** | | | | | |
| **1** | $H_2N(CH_2)_{10}CH_3$ | 100 ± 1 | 100 ± 1 | 100 ± 1 | 100 ± 1 |
| **2** | $H_2NCH_2(CF_2)_6CF_3$ | 100 ± 2 | 96 ± 2 | 95 ± 1 | 86 ± 1 |
| **3** |  | 100 ± 4 | 106 ± 0.5 | 120 ± 0.7 | 130 ± 0.7 |
| **Ethers and derivatives of $EG_n$** | | | | | |
| **4** |  | 52 ± 0.3 | 75 ± 0.0 | 38 ± 1 | 105 ± 1.0 |
| **5** | $H_2NCH_2CH_2OCH_3$ | 25 ± 1.3 | 53 ± 0.4 | 12 ± 0.9 | 15 ± 2 |
| **6** | $H_2NCH_2CH_2OH$ | 21 ± 0.5 | 47 ± 2 | 3.8 ± 0.2 | 14 ± 0.2 |
| **7** | $HN(CH_2CH_2OCH_3)_2$ | 26 ± 1.0 | 50 ± 0.8 | 19 ± 5 | 35 ± 1.4 |
| **8** | $H_2N(CH_2CH_2O)_3CH_3$[g] | 0.8 ± 0.2 | 1.5 ± 0.2 | 1.1 ± 0.2 | 1.1 ± 0.2 |
| **9** | $H_2N(CH_2CH_2O)_3H$ | 0.9 ± 0.2 | 1.6 ± 0.5 | 1.1 ± 0.2 | 1.0 ± 0.2 |
| **10** | $H_2N(CH_2CH_2O)_6CH_3$[g] | 0 ± 0.2 | 0.3 ± 0.2 | 0 ± 0.2 | 0.5 ± 0.2 |
| **11** | $H_2N(CH_2CH_2O)_6H$ | 0.1 ± 0.2 | 0.4 ± 0.5 | 0.5 ± 0.2 | 1.0 ± 1.0 |
| **12** |  | 1.2 ± 0.2 | 3.7 ± 0.2 | 4.5 ± 0.0 | 27 ± 10 |
| **Amines/ Ammonium Salts** | | | | | |
| **13** |  | 23 ± 4 | 40 ± 2 | 1.9 ± 0.2 | 14 ± 2 |
| **14** |  | 17 ± 2 | 25 ± 4 | 3.5 ± 0.2 | 3.9 ± 0.7 |
| **15** | $H_2N(CH_2CH_2O)_2$-$CH_2CH_2NH_2$ | 14 ± 4 | 37 ± 1.6 | 8.4 ± 0.5 | 18 ± 1 |
| **16** |  | 8.2 ± 1 | 16 ± 0.2 | 1.1 ± 0.2 | 1.0 ± 0.4 |
| **17** |  | 4.4 ± 0.8 | 8.5 ± 0.2 | 4.3 ± 0.2 | 7.8 ± 0.2 |

**Table 1. (Continued)**

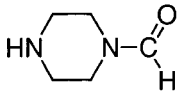
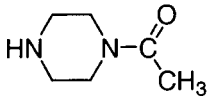
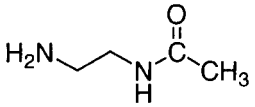
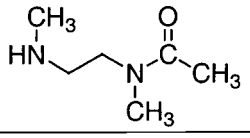
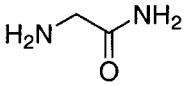
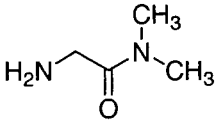
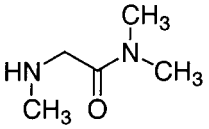
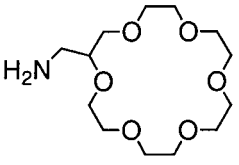
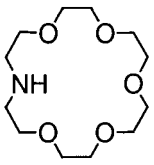
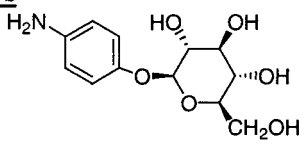
| Entry No. | HNR'**R** | Fibrinogen %ML 3 min[a] | Fibrinogen %ML 30 min[b] | Lysozyme %ML 3 min[a] | Lysozyme %ML 30 min[b] |
|---|---|---|---|---|---|
| **Simple Amides** | | | | | |
| **18** | $HN(CH_3)_2$ | 26 ± 6 | 54 ± 0.8 | 43 ± 16 | 80 ± 3 |
| **19** |  | 23 ± 1 | 39 ± 1 | 8 ± 0.3 | 12 ± 0.3 |
| **20** |  | 17 ± 1 | 38 ± 0.2 | 7.8 ± 1 | 6.5 ± 0.2 |
| **21** |  | 14 ± 1 | 40 ± 2 | 1.4 ± 0.0 | 5.4 ± 0.3 |
| **22** |  | 2.1 ± 0.3 | 12 ± 3 | 0.5 ± 0.2 | 3.8 ± 2 |
| **Amides Based on Amino Acids** | | | | | |
| **23** |  | 33 ± 3 | 58 ± 1 | 8.8 ± 1 | 30 ± 1 |
| **24** |  | 18 ± 0.5 | 33 ± 0.5 | 7.2 ± 1 | 15 ± 1 |
| | $H_2N(Gly)_1N(CH_3)_2$ | | | | |
| **25** | $H_2N(Gly)_3N(CH_3)_2$ | 8 ± 1 | 22 ± 0.4 | 2.1 ± 1.5 | 8.8 ± 0.5 |
| **26** |  | 1.0 ± 0.2 | 9.1 ± 0.3 | 0.2 ± 0.2 | 2.4 ± 0.3 |
| | $H(CH_3)N(Sar)_1N(CH_3)_2$ | | | | |
| **27** | $H(CH_3)N(Sar)_3N(CH_3)_2$ | 0.7 ± 0.2 | 2.0 ± 0.2 | 0.6 ± 0.2 | 1.1 ± 0.3 |
| **28** | $H(CH_3)N(Sar)_4N(CH_3)_2$ | 0.5 ± 0.2 | 1.7 ± 0.2 | 0.8 ± 0.2 | 1.5 ± 0.7 |
| **29** | $H(CH_3)N(Sar)_5N(CH_3)_2$ | 0.6 ± 0.3 | 1.3 ± 0.2 | 0.8 ± 0.7 | 1.0 ± 0.2 |
| **Crown Ethers** | | | | | |
| **30** |  | 44 ± 0.9 | 59 ± 2.3 | 3.9 ± 0.3 | 9.6 ± 0.5 |
| **31** |  | 3.8 ± 0.7 | 11 ± 0.3 | 2.1 ± 0.4 | 6.0 ± 0.5 |
| **Sugars** | | | | | |
| **32** |  | 51 ± 1.8 | 67 ± 0.9 | 27 ± 3.3 | 83 ± 2.3 |

**Table 1. (Continued)**

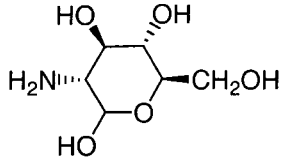
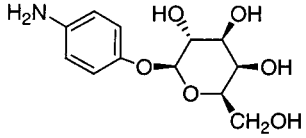
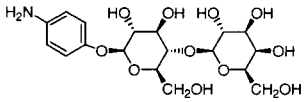
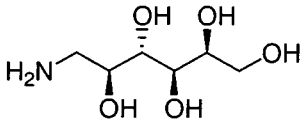
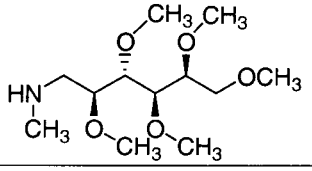
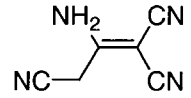
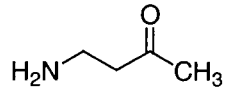
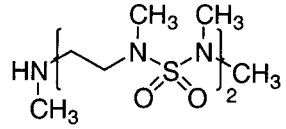
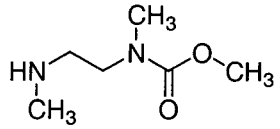
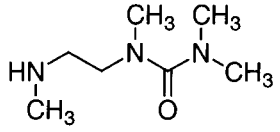
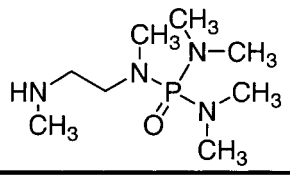
| Entry No. | HNR'**R** | Fibrinogen %ML 3 min[a] | Fibrinogen %ML 30 min[b] | Lysozyme %ML 3 min[a] | Lysozyme %ML 30 min[b] |
|---|---|---|---|---|---|
| **33** |  | 31 ± 0.7 | 55 ± 1.1 | 7.8 ± 0.2 | 25 ± 2.1 |
| **34** |  | 35 ± 5 | 49 ± 2.3 | 11 ± 1.5 | 30 ± 3.3 |
| **35** |  | 17 ± 1.4 | 32 ± 0.5 | 3.7 ± 0.2 | 9.3 ± 0.4 |
| **36** |  | 47 ± 12 | 68 ± 7 | 13 ± 7 | 20 ± 0.3 |
| **37** |  | 0.8 ± 0.5 | 2.9 ± 1.4 | 0.4 ±0.4 | 6.1 ± 4 |
| **Nitriles** | | | | | |
| **38** |  | 34 ± 1.1 | 58 ± 3 | 13 ± 0.3 | 28 ± 3.7 |
| **39** | $HN(CH_2CH_2CN)_2$ | 43 ± 1.0 | 58 ± 1.6 | 17 ± 1.0 | 40 ± 2.0 |
| **40** | $HN(CH_2CN)_2$ | 54 ± 3.8 | 73 ± 3 | 36 ± 0.5 | 92 ± 0.3 |
| **41** | $H_2NCH_2CH_2CN$ | 62 ± 1.0 | 72 ± 2 | 35 ± 0.2 | 69 ± 3 |
| **Other Groups** | | | | | |
| **42** |  | 66 ± 0.3 | 74 ± 0.3 | 31 ± 6 | 71 ± 5 |
| **43** |  | 61 ± 0.2 | 80 ± 1 | 66 ± 4 | 100 ± 2 |
| **44** | $H_2NC(CH_2CH_2CH_2OH)_3$ | 34 ± 2.2 | 57 ± 0.2 | 30 ± 0.6 | 73 ± 5 |
| **45** |  | 26 ± 0.2 | 58 ±0.3 | 4.3 ± 0.2 | 43 ± 3 |
| **46** | $H(CH_3)NCH_2CH(OCH_3)_2$ | 16 ± 2 | 36 ± 1 | 4.6 ± 2 | 19 ± 5 |
| **47** |  | 5.9 ± 0.2 | 25 ± 1 | 2.1 ± 0.2 | 11 ± 0.5 |

**Table 1. (Continued)**

| Entry No. | HNR'**R** | Fibrinogen %ML 3 min[a] | Fibrinogen %ML 30 min[b] | Lysozyme %ML 3 min[a] | Lysozyme %ML 30 min[b] |
|---|---|---|---|---|---|
| **48** |  | 0.8 ± 0.2 | 4.3 ±0.6 | 0.2 ± 0.2 | 0.3 ± 0.2 |

[a] $\%ML^3_{Fib}$ is the percent of a monolayer of fibrinogen that adsorbed onto a mixed SAM that presented $-CONH\mathbf{R}/-CO_2H$ groups. In this experiment, we assumed that a monolayer of protein adsorbed onto a mixed SAM that presented $-CONH(CH_2)_{10}CH_3/-CO_2H$ groups after a 1 mg/mL solution of fibrinogen was flowed over the surface for 3 min at 10 $\mu$L/s. $\%ML^3_{Lys}$ is the equivalent measurement using lysozyme as the protein. [b] Same as in footnote a above with the exception that the protein solution was flowed over the surface for 30 min.

as $-CON(CH_2)_{10}CH_3$, shield the $-CO_2H/CO_2^-$ groups from contact with solution more than do the shorter groups. The reaction of undecylamine with an anhydride surface generates a mixed SAM with properties similar to those of a SAM of hexadecanethiol.[56] In previous work, we tested the influence of the residual carboxylic acid groups on the properties of the surfaces by measuring the contact angles of water under cyclooctane at different values of pH on mixed SAMs formed with $H_2N(EG)_nOH$ ($n$ = 1, 3, or 6).[22] Mixed SAMs of $H_2N(EG)_nOH$ with $n$ = 3 or 6 prevented the $-CO_2H/CO_2^-$ groups from influencing the wettability for values of pH = 2 and 5−11; the values of the contact angles were independent of pH. The values of the contact angle of mixed SAMs that presented $-(EG)_1OH$ groups decreased at values of pH > 7, suggesting that the $-CO_2H/CO_2^-$ groups were at least partially exposed and ionized.

**Choice of Proteins and SPR Protocol.** We tested the adsorption of fibrinogen and lysozyme on the mixed SAMs because the properties of these two proteins (size and pI) are very different. Fibrinogen is a large protein (MW = 340 kDa for a tetrameric aggregate, pI = 5.5) that adsorbs readily onto hydrophobic and charged surfaces. Fibrinogen is structurally similar to the extracellular matrix protein fibronectin that is often used to coat surfaces to facilitate the adhesion of mammalian cells;[2,57] hence, SAMs that are inert to fibrinogen might also be useful in applications to cell patterning in which it is necessary to prevent the adhesion of mammalian cells to certain parts of the surfaces. Lysozyme is a small protein (MW = 15 kDa, pI = 10.9) that is often used as a model in studies of electrostatic adsorption and that is positively charged under the conditions of our experiment (phosphate-buffered saline, PBS, pH 7.4). The adsorption of lysozyme onto the mixed SAMs that we describe here depends at least in part on the exposure to the solution of the $-CO_2H/CO_2^-$ groups that are formed from the reaction of an amine with the anhydride groups; the positively charged protein would be attracted electrostatically to a surface with exposed $-CO_2^-$ groups.

Figure 2 displays representative SPR sensorgrams obtained during characterization of the adsorption of fibrinogen and lysozyme onto mixed SAMs prepared by the anhydride method (Figure 1). Our SPR protocol for measuring the adsorption of protein onto SAMs consisted of allowing a solution of phosphate-buffered saline (PBS, 10 mM phosphate, 138 mM NaCl, 3 mM KCl, pH 7.4) to flow over the surface for 2 min, replacing the flow of buffer with a flow of a buffered solution of protein (1 mg/mL in PBS) for 3 or 30 min, and finally allowing PBS buffer again to flow for 10 min.[58] The change in signal ($\Delta$RU = change in response units; 1 RU = $10^{-4}$°) was recorded as a function of time. The examples in Figure 2 show that the presence of different functional groups on the surface of the SAM results in the adsorption of different amounts of fibrinogen and lysozyme.[59]

**Adsorption of Fibrinogen and Lysozyme onto Mixed SAMs (Table 1 and Figure 2).** Throughout this paper, we refer to the amount of protein adsorbed onto each surface as a percentage of a monolayer of protein according to eq 1 to facilitate comparisons between surfaces. We assume that a monolayer of protein adsorbs onto mixed SAMs that present $-CONH(CH_2)_{10}CH_3$ groups [$\Delta RU_{\text{mixed SAM}(-CON(CH_2)_{10}CH_3)}$] and use that value to normalize the amounts of proteins that adsorb onto each mixed SAM [$\Delta RU_{\text{mixed SAM/COR}}$].[60] Equation 1 can be used for both single-component and mixed SAMs because $\Delta RU_{SAM(-S(CH_2)_{10}CH_3)} \approx \Delta RU_{\text{mixed SAM}(-CONH(CH_2)_{10}CH_3)}$.[56,61]

$$\%\ ML_{Protein} = \%\ \text{Monolayer} = \frac{\Delta RU_{\text{mixed SAM}(-COR)}}{\Delta RU_{\text{mixed SAM}(-CON(CH_2)_{10}CH_3)}} \times 100$$

Values of $\%ML_{Protein}$ were determined after the surfaces were exposed to solutions of protein for intervals of 30 min ($\%ML^{30}_{Protein}$) or 3 min ($\%ML^{3}_{Protein}$). On some surfaces, the amount of adsorbed protein is larger than on the $CONH(CH_2)_{10}CH_3$ mixed SAM, resulting in values of $\%ML^{30}_{Protein} > 100\%$.

*Hydrophobic Groups.* The reaction of groups such as $-H_2N(CH_2)_{10}CH_3$, $-H_2NCH_2CF_3(CF_2)_6CF_3$, and $-H_2NCH_2-$pyrene with the anhydride groups generated hydrophobic surfaces with contact angles similar to those of a SAM of hexadecanethiolate. The values of $\%ML_{Protein}$

(57) Singhvi, R.; Kumar, A.; Lopez, G. P.; Stephanopolous, G. N.; Wang, D. I. C.; Whitesides, G. M.; Ingber, D. E. *Science* **1994**, *264*, 696−698.

(58) The molar concentration of lysozyme is 20 times greater than that of fibrinogen for solutions at 1 mg/mL. We used these concentrations to simplify the comparison with previously reported data on the adsorption of these proteins onto SAMs. Fibrinogen did not dissolve in buffer to form a 20 mg/mL solution. On the other hand, a 0.05 mg/mL solution of lysozyme would make the adsorption process very slow and most likely limited by mass transport.

(59) We are uncertain about the drastic differences between the kinetics of the adsorption of fibrinogen and lysozyme displayed in Figure 2. We speculate that they are caused by electrostatic effects.

(60) Although the structure, thickness, and index of refraction of the adsorbed layer depend on the conformation of the protein on the surface, the actual quantity of protein that adsorbs onto the alkyl-terminated surface almost certainly represents a complete monolayer.

(61) Mrksich, M.; Sigal, G. B.; Whitesides, G. M. *Langmuir* **1995**, *11*, 4383−4385.

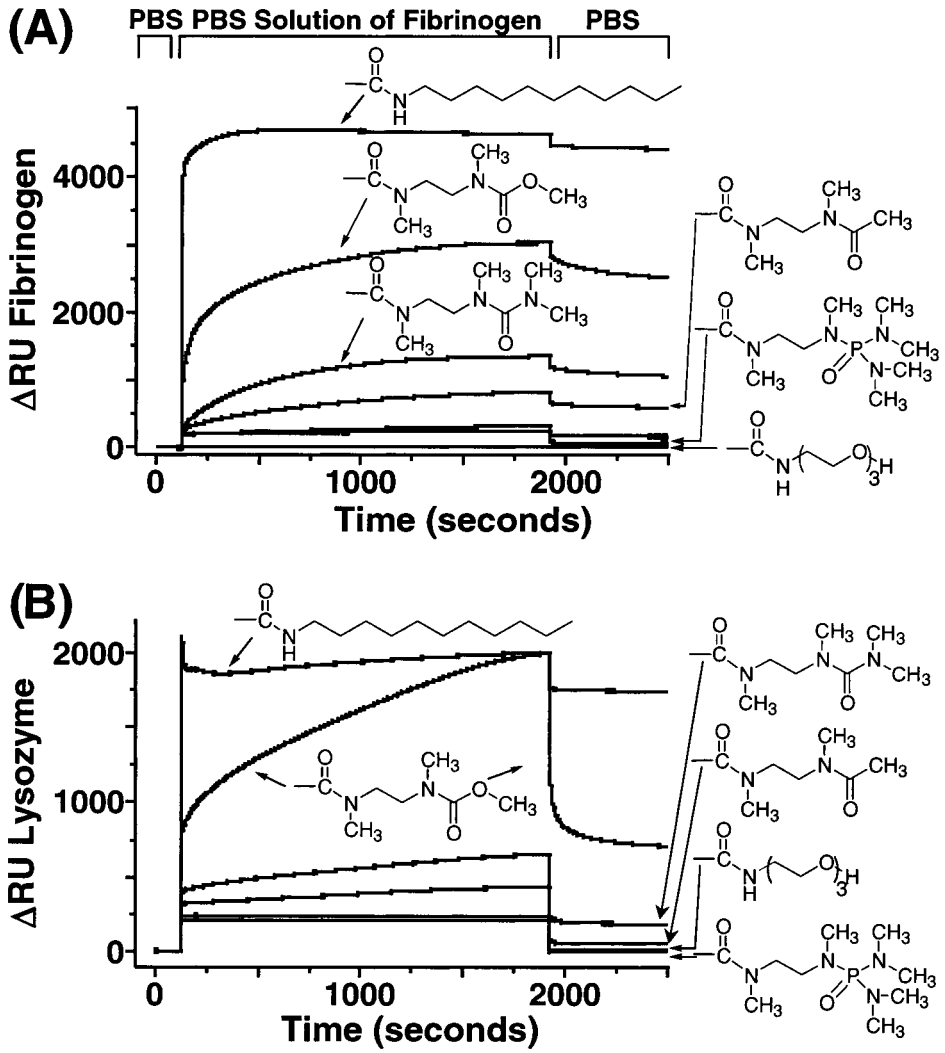



**Figure 2.** Adsorption of (A) fibrinogen and (B) lysozyme onto mixed SAMs prepared by the anhydride method. The sensorgrams illustrate the adsorption of different amounts of protein onto different SAMs.

measured on these surfaces were in the range 85−120%. These results agree with previous reports in the literature that proteins adsorb onto hydrophobic surfaces.[61,62]

*Derivatives of $(EG)_n$ and Other Ethers.* The resistance of these surfaces increased with the increasing number of oligomers of EG in the functional group, as expected from literature precedent.[14,23,63] These results are also consistent with the improved screening of the carboxylic acid groups in the mixed SAMs formed with long functional groups.[22]

*Amines/Ammonium Salts.* As a group, these surfaces were more resistant to the adsorption of lysozyme than to the adsorption of fibrinogen. We believe that one of the reasons for this behavior is the positive charge present on the amino groups of these surfaces; the positively charged lysozyme was probably more strongly repelled by the positively charged surfaces than was the negatively charged fibrinogen.

*Simple Amides and Amides Based on Amino Acids.* Functional groups with multiple amine groups made it possible to determine the influence of hydrogen-bond donors on the inertness of the surface. The derivatives of glycine and sarcosine were especially useful in this regard. The hydrogen atoms on the amino groups could be replaced with methyl groups to determine the influence of hydrogen-bond-donor groups on the adsorption of proteins onto the mixed SAMs. The inertness of the surfaces improved when amide −NH groups were replaced with $-NCH_3$ groups and when the number of oligomeric units in the chain was increased (compare also entries 21 and 22).

*Crown Ethers.* Surprisingly, the conformationally constrained aza-crown-terminated surface was more inert than a mixed SAM that presented aminomethyl-18-crown-6. The difference in the values of %ML measured for entries 30 and 31 might be due to differences in cation binding capability or in conformation. Substitution of one of the oxygen atoms of 18-crown-6 with nitrogen (entry 31) weakens complexation of hard alkali cations such as $K^+$.[64] The strong complexation of alkali cations to entry 30 might cause the surface to become more positively charged than a mixed SAM made with entry 31; this hypothesis would explain the observation that the value of $\%ML^{30}$ measured on SAMs made with entry 30 for the negatively charged fibrinogen was approximately six times larger than that measured with entry 31. The conformational differences between compounds 30 and 31, with or without a complexed cation, might also result in significant differences in the state of hydration of the corresponding

(62) Sigal, G. B.; Mrksich, M.; Whitesides, G. M. *J. Am. Chem. Soc.* **1998**, *120*, 3464−3473.
(63) Szleifer, I. *Biophys. J.* **1997**, *72*, 595−612.
(64) Frensdorf, H. K. *J. Am. Chem. Soc.* **1971**, *93*, 600−606.

mixed SAMs.[65,66] Grunze and co-workers have shown that the conformation of surface-bound groups can affect the hydration of the surface and its resistance to the adsorption of proteins.[40]

*Carbohydrates.* A mixed SAM that presented a disaccharide (entry 35) adsorbed only half as much protein as mixed SAMs that presented each of the monosaccharides that composed the disaccharide (entries 32 and 34). This result is consistent with the observation that the inertness of surfaces terminated with oligomers of amino acids increases with the number of oligomers in the chain. The replacement of hydrogen-bond donors in the hydroxyl groups of sorbitol with methyl groups improved the inertness of the surface significantly; this effect was larger than in the case of the derivatives of sarcosine. Polysaccharides are well-known to form inert coatings on synthetic surfaces and on the surfaces of cells.[67] Recently, Mrksich found that the resistance of mannitol derivatives depends on the relative stereochemistry of the hydroxyl groups;[68] inert surfaces were formed with carbohydrates that pack poorly and have low melting temperatures.[69] This observation is consistent with Grunze's inference that water causes $(EG)_nOH$ layers to become amorphous.

*Nitriles.* The inertness of these surfaces improved as the number of terminal −CN groups increased, even though this group of mixed SAMs was, overall, not very inert.[70]

*Other Groups.* The functional groups in this class that formed the most inert surfaces were those that were polar and lacked hydrogen-bond donor groups. We expected entry 43 to be resistant in light of the published report on inert surfaces generated with a SAM terminated in tri(propylene sulfoxide) groups; it is possible that this functional group is too bulky and conformationally flexible to react efficiently with the anhydride-terminated SAMs. The phosphoramide derivative is less bulky in the mixed SAMs than the sulfamide derivative, and it is effective in generating an inert surface; the functional group is polar, and it lacks hydrogen-bond donor groups.

*Removal of Adsorbed Proteins.* Adsorbed proteins could be removed, at least partially, from the mixed SAMs using solutions of sodium dodecyl sulfate (SDS, 10 mg/mL). The mixed SAMs were exposed to solutions of proteins for 30 min and PBS for 10 min, before being washed with a solution of SDS for 10 min. Figure 3A shows a typical sensorgram for such an experiment, and it illustrates our definition of $\Delta RU_{wash}$, the amount of adsorbed protein that could be removed by the 10-min SDS wash (Figure 3A). The ability to remove adsorbed proteins with SDS suggests that adsorption onto these mixed SAMs has a significant hydrophobic component.

A fraction of the proteins that adsorbed onto most SAMs was irreversibly adsorbed; the SDS wash did not completely remove the layers of adsorbed protein (Figure 3B). The SDS wash was most effective in removing the adsorbed layers of proteins from surfaces with values of %ML < 50. We have labeled several points in Figure 3 B with their corresponding entries in Table 1. The difficulty in removing

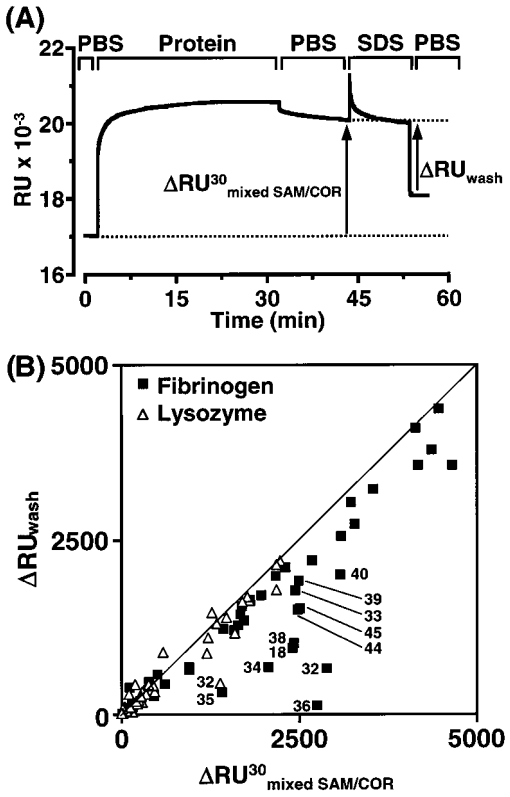



**Figure 3.** Adsorbed proteins can be removed from the mixed SAMs with solutions of SDS. (A) Sensorgram showing a typical experiment in which a solution of protein was injected over a mixed SAM for 30 min. After a 10-min rinse with PBS, the surface was exposed to a solution of SDS for 10 min. The quantities $\Delta RU_{wash}$ and $\Delta RU^{30}_{mixed\ SAM/COR}$ are labeled. (B) Plot of $\Delta RU_{wash}$ versus $\Delta RU^{30}_{mixed\ SAM/COR}$ for the removal of adsorbed fibrinogen (■) and lysozyme (△) from each mixed SAM. We have labeled some points with their corresponding entry numbers in Table 1. A straight line is drawn to illustrate that the values of $\Delta RU_{wash}$ are often smaller than those of $\Delta RU^{30}_{mixed\ SAM/COR}$. The experiments were performed once on each mixed SAM with fibrinogen and lysozyme.

adsorbed fibrinogen might be due to its large size or the possibility that it reacts covalently with the surface.

In Figure 3, most of the points that lie outside the linear range (32−36) correspond to moieties containing −OH groups that might plausibly form esters with the carboxylic anhydride groups during the initial formation of SAMs. Although the amine groups are more reactive than hydroxyl groups toward the anhydride groups, it is possible for the hydroxyl groups to also react with the surfaces to form ester bonds. This reaction might be particularly favorable as a process occurring *after* the initial formation of an amide. These ester groups, in turn, might react with lysine $\epsilon$-$NH_2$ groups on proteins to yield *covalently* immobilized proteins that cannot be washed off by solutions of SDS.

**Contact Angles.** A graph of $\%ML^{30}_{Protein}$ versus the advancing contact angle of water in cyclooctane ($\cos\theta_{adv}$) shows no correlation (Figure 4A).[71] We conclude that interfacial free energy (as measured by the contact angle)

(65) Merz, A.; Gromann, L.; Karl, A.; Parkanyi, L.; Schneider, O. *Eur. J. Org. Chem.* **1998**, *403*, 3−408.
(66) Patil, K.; Pawar, R. *J. Phys. Chem. B* **1999**, *103*, 2256−2261.
(67) Holland, N. B.; Qui, S. X.; Ruegsegger, M.; Marchant, R. E. *Nature* **1998**, *392*, 799−801.
(68) We have not tried to generate a surface terminated with mannose groups such as that used by Mrksich because we could not easily create a mixed SAM that contained an O-linked mannitol derivative.
(69) Mrksich, M. Department of Chemistry, University of Chicago. Personal communication, 2001.
(70) The reactivity of the amino group in entry 38 is probably lowered significantly by the presence of the alkene, which causes a low yield in this reaction with the anhydride surface.

(71) We chose to measure the contact angle of water in cyclooctane for several reasons. (i) Most evidence points to the crucial role played by water in the mechanism of resistance; hence, it was most appropriate to measure the interaction of water with the surfaces. (ii) Values of the contact angle of water in cyclooctane on other SAMs are available from other groups. (iii) Theoretically, provided that the solvents do not cause drastic changes in the structure of the SAMs, the contact angle of water in cyclooctane corresponds to 180° − the contact angle of cyclooctane in water.

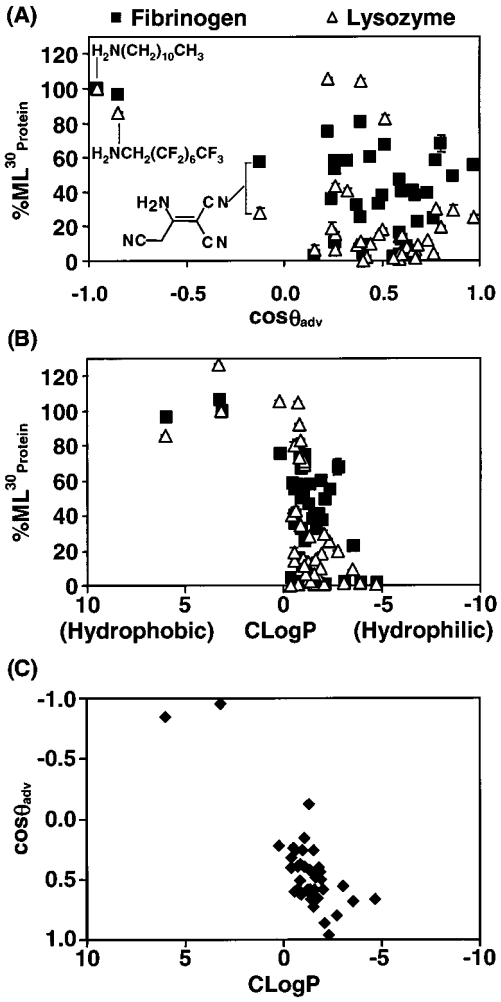



**Figure 4.** Graph of values of %ML$^{30}$Protein obtained with fibrinogen (■) and lysozyme (△) vs (A) the cosine of the advancing contact angle of water on the mixed SAM measured under cyclooctane and (B) the partition coefficient between octan-1-ol and water of the functional group that was incorporated into the mixed SAM. For graph A, the advancing contact angle could not be measured reliably on surfaces with sessile contact angles > 140° (cos $\theta$ < −0.77); in those cases, we report the sessile contact angles instead of the advancing contact angles. The contact angles were measured in triplicate on three different regions of a surface. The error bars of %ML$^{30}$Protein represent the range of the measured values. The range of the measured values of cos $\theta_{adv}$ is smaller than the height of the symbols. For graph B, we modeled the functional groups **R** with the structure $CH_3CONR'$**R**. The values of ClogP were calculated with the ChemDrawUltra software package by Cambridge Software using Viswanadhan's fragmentation method (*J. Chem. Inf. Comput. Sci.* **1989**, *29*, 163) for molecules with the structure $CH_3CON$**RR′**. (C) Plot of cos $\theta$ vs ClogP using the values plotted in graphs A and B. There is at least a crude correlation between these parameters.

does not correlate with the extent of protein adsorption. Our results confirm that hydrophobic surfaces are adsorbing and that inert surfaces are hydrophilic; because many hydrophilic surfaces are not inert, however, a surface must also have other characteristics in order for it to resist the adsorption of protein.

**Partition Coefficients, ClogP.** We used commercially available software (MaclogP) to determine the octan-1-ol/water partition coefficients (ClogP) of the amido derivatives of the functional groups that we tested. The partition coefficient is a useful indicator of the hydrophilicity of the groups used in our study. There was again no clear correlation between the values of %ML$^{30}$Protein and the values of ClogP (Figure 4B). Plots that were obtained using different methods of calculating ClogP are qualitatively similar to that in Figure 4 (see Supporting Information). Figure 4B confirms that hydrophobic compounds with values of ClogP ≈ 0−5 generate surfaces that adsorb large quantities of protein. Inert surfaces are formed with functional groups that have values of ClogP < 0; although this is a requirement for the formation of inert surfaces, it is not sufficient.

**Adsorption of Protein to Single-Component SAMs.** *SAMs from the Literature.* Table 2 summarizes values for the adsorption of fibrinogen and lysozyme onto a variety of single-component SAMs found in the literature. Typically, surfaces that adsorbed large amounts of proteins were terminated with functional groups that were (i) hydrophobic, (ii) hydrogen-bond donors, or (iii) charged.

*SAMs formed from the Successful Functional Groups.* We selected functional groups to incorporate into alkanethiols for the formation of homogeneous SAMs not strictly on the basis of low values of %ML that were measured with mixed SAMs. That criterion is reasonably good, and in fact, there are several groups in Table 1 that, if incorporated in homogeneous SAMs, might result in inert surfaces; entries 17, 22, 25, 26, 31, and 37 are such groups (Table 1). In choosing the alkanethiols to synthesize, however, we chose groups that exhibited low values of %ML with different types of polar structures. We excluded the aza-crown derivative in entry 31 because we believed that it would form a disordered SAM. The acetylpiperazine derivative was chosen because it could pack well and it would allow us to test the influence of conformational flexibility of the end group on the resistance of the SAM. The permethylated sorbitol and the sarcosine derivatives represent the broader classes of carbohydrates and amino acids that deserve to be studied further. We believe that the resistance of the phosphoramide derivative was sufficiently surprising that it warranted the synthesis of the alkanethiol.

Single-component SAMs that present tri(ethylene glycol) groups were the most resistant surfaces that we studied (Figure 5 and Table 3). Alkanethiols terminated with permethylated sorbitol, acetylpiperazine, and −(EG-)$_n$OH groups formed SAMs that were more resistant to protein adsorption than the corresponding mixed SAMs (Table 3). A homogeneous SAM that presented sarcosine trimers was approximately as resistant as the corresponding mixed SAM. We suspect that the mixed SAM formed with the phosphoramide derivatives was more resistant than the homogeneous SAM formed with the corresponding alkanethiol because the alkanethiol was bulky and caused many unfavorable steric interactions in the SAM. SAMs formed from alkanethiols 1, 2**,** and 3 (Table 3) were (i) more resistant than single-component SAMs that present tri(propylene sulfoxide) groups and (ii) comparable to single-component SAMs presenting tri(ethylene glycol). In an upcoming report, we will describe the resistance to the adhesion of cells and bacteria of these homogeneous SAMs.[72]

**Mechanism of Protein Resistance.** Grunze has discussed the importance of the interaction of water with a surface in determining its resistance to the adsorption of protein, but it is not clear whether surfaces that are inert induce a particular structure in water molecules near the surface.

Grunze and de Gennes separately suggested that the conformational flexibility of surface-bound groups is

(72) Ostuni, E.; Chapman, R. G.; Liang, M. N.; Meluleni, G.; Pier, G.; Ingber, D. E.; Whitesides, G. M. **2001**, *Langmuir*, in press.

**Table 2. Adsorption of Fibrinogen and Lysozyme onto Single-Component SAMs**

| | | % monolayer | | | |
|---|---|---|---|---|---|
| entry no. | alkanethiol | fibrinogen[a] | lysozyme[a] | detection[b] | ref |
| 1 | $HS(CH_2)_{11}CN$ | 115 | 50 | SPR | 63 |
| 2 | $HS(CH_2)_{11}OPh$ | 110 | 75 | SPR | 63 |
| 3 | $HS(CH_2)_{11}CF_3$ | 100 | 100 | SPR | 63 |
| 4 | $HS(CH_2)_{10}CH_3$ | 100 | 100 | SPR | 63 |
| 5 | $HS(CH_2)_{11}CONHCH_3$ | 80 | 10 | SPR | 63 |
| 6 | $HS(CH_2)_{11}OCH_3$ | 75 | 10 | SPR | 63 |
| 7 | $HS(CH_2)_{11}OH$ | 35−50 | 1 | E | 24, 63 |
| 8 | $HS(CH_2)_{11}EG_1OH$ | 35 | 10 | E | 23 |
| 9 | $HS(CH_2)_{11}CONH_2$ | 30 | 5 | SPR | 63 |
| 10 | $HS(CH_2)_{11}(Glc)_2OH$ | 20 | *c* | E | 24 |
| 11 | $HS(CH_2)_{11}-S(O)CH_2CH_2CH_2\}_3S(O)CH_3$ | 5 | *c* | SPR | 25 |
| 12 | $HS(CH2)_{10}O(Mal)$[d] | ∼3 | *c* | E | 24 |
| 13 | $HS(CH_2)_{11}O(Man)$[e] | ∼1 | ∼2 | SPR, E | 11 |
| 14 | $HS(CH_2)_{11}(EG)_2OH$ | ∼1 | ∼1 | E | 23 |
| 15 | $HS(CH_2)_{11}(EG)_3OH$ | ∼1 | ∼1 | SPR | 22 |
| 16 | $HS(CH_2)_{11}(EG)_3OCH_3$ | ∼1 | ∼1 | SPR | 24 |
| 17 | $HS(CH_2)_{11}(EG)_4OH$ | ∼1 | ∼1 | E | 23 |
| 18 | $HS(CH_2)_{11}(EG)_6OH$ | ∼1 | ∼1 | SPR, E | 22−24 |
| 19 | $HS(CH_2)_{11}(EG)_6OCH_3$ | ∼1 | ∼1 | SPR, E | 23−24 |
| 20 | $HS(CH_2)_{11}(EG)_{17}OCH_3$ | ∼1 | ∼1 | E | 23 |

[a] $\%ML_{Fib}$ and $\%ML_{Lys}$ were determined using eq 1. The uncertainties in these values are ±10% (relative error). Different experimental conditions were used for these experiments; each individual experiment is normalized with respect to the amount of protein that adsorbed onto a single-component SAM presenting $-S(CH_2)_{10}CH_3$ or $-(CH_2)_{15}CH_3$ groups. [b] Ellipsometry (E) and/or SPR were used for detection; if both are indicated, then both methods were used. [c] Not determined. [d] Mal = maltose [Glc-α(1,4)-Glc-β(1)-O]. [e] Man = mannitol.

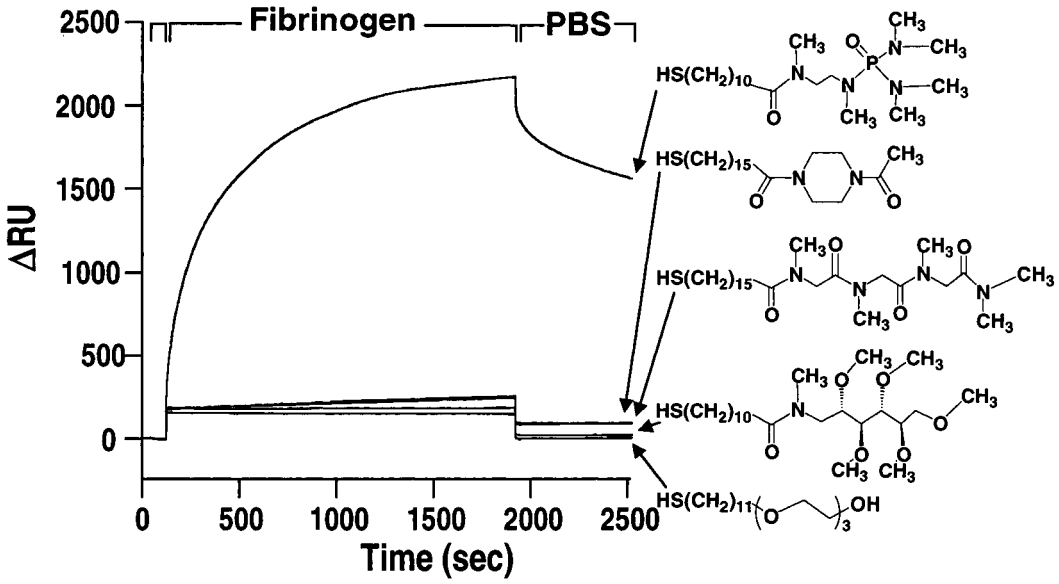



**Figure 5.** Adsorption of fibrinogen onto single-component SAMs prepared from 2 mM solutions of alkanethiols 1−5 (Table 3).

important in determining their ability to resist the adsorption of proteins. Although we found that several inert surfaces were formed with conformationally flexible groups, the piperazine derivative is conformationally very constrained (compound 2a in Table 3), and the sarcosine derivative is less flexible than the ethylene glycol derivatives (compound 5a in Table 3).

Inert surfaces that we know not to meet the design principles that we described in this paper are all carbohydrates. It is possible that the mechanism of resistance to protein adsorption of carbohydrate-based surfaces is different from that of other surfaces. Uncharged carbohydrates are claimed to orient up to three layers of water; significant repulsion can be detected when two such surfaces are brought into close proximity.[73] Parsegian and co-workers found that the repulsive interaction (attributed to the hydration layers) measured between carbohydrate surfaces with acetylated hydroxyl groups was no different than that measured between surfaces with free hydroxyl groups.[74]

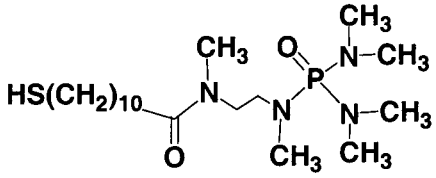
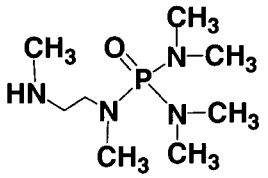
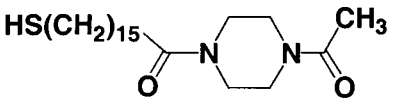
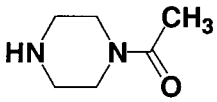
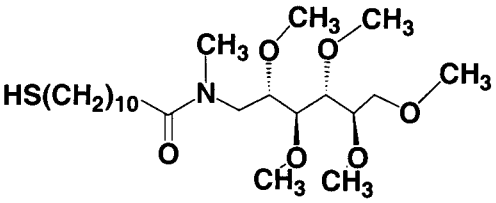
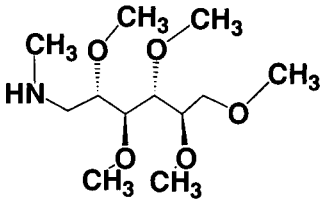
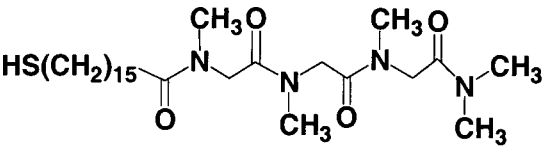
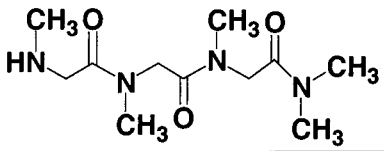
The design principles that we have described can be extended to thin polymeric films. We have previously grafted polyamines onto anhydride-terminated surfaces using procedures similar to those described in this paper.[75] The amino groups in the resulting thin films were converted to acetamido groups (−NH− to >NCOCH$_3$) to generate a hydrophilic film that did not contain any hydrogen-bond donors; thin films prepared in this manner were essentially as resistant to the adsorption of proteins and the adhesion of bacteria as SAMs terminated with $-(EG)_3OH$ groups.[75] It is possible that some of the mechanistic details of the inertness of PEG-based systems

(73) Claesson, P. M. In *Biopolymers at Interfaces*; Malmsten, M., Ed.; Marcel Dekker: New York, 1998; Vol. 75, pp 281−320.

(74) Rau, D. C.; Parsegian, V. A. *Science* **1990**, *249*, 1278−1281.

(75) Chapman, R. G.; Ostuni, E.; Liang, M. N.; Meluleni, G.; Kim, E.; Yan, L.; Pier, G.; Warren, H. S.; Whitesides, G. M. *Langmuir* **2001**, *17*, 1225−1233.

**Table 3. Characterization of Single-Component SAMs that Present Groups that Reduce the Adsorption of Protein from Solution**

| | | % Monolayer | | | |
|---|---|---|---|---|---|
| | | Fibrinogen | | Lysozyme | |
| Entry No.[i] | HNR'R or HSR | 3 min | 30 min | 3 min | 30 min |
| 1a |  | 14 ±0.2 | 39 ±2 | 4.5 ±0.2 | 13 ±0.3 |
| 1b |  | 0.8 ±0.2 | 4 ±0.6 | 4 ±0.2 | 13 ±0.2 |
| 2a |  | 0.7 ±0.2 | 2.2 ±0.2 | 0.8 ±0.3 | 0.6 ±0.2 |
| 2b |  | 17 ±1 | 38 ±0.2 | 8 ±1 | 7 ±0.2 |
| 3a | $HS(CH_2)_{11}EG_3OH$ | 0.2 ±0.2 | 0.2 ±0.2 | 0.2 ±0.2 | 0.2 ±0.2 |
| 3b | $H_2N(CH_2CH_2O)_3H$ | 1 ±0.2 | 2 ±0.5 | 1 ±0.2 | 1 ±0.2 |
| 4a |  | 0.2 ±0.2 | 0.7 ±0.2 | 0.4 ±0.2 | 0.4 ±0.2 |
| 4b |  | 0.8 ±0.5 | 2.9 ±1.4 | 0.4 ±0.4 | 6.1 ±4 |
| 5a |  | 1 ±0.2 | 3.8 ±1.2 | 2.3 ±0.2 | 1.5 ±0.7 |
| 5b |  | 0.7 ±0.2 | 2.0 ±0.2 | 0.6 ±0.2 | 1.1 ±0.3 |

[a] Results from the mixed SAMs that present the same groups are given for comparison. [b] The symbols used have been defined in Table 1.

that were proposed by Andrade and de Gennes apply to the thin films of polyamines that we have formed.

## Conclusion

We have described a range of functional groups that can make surfaces resistant to the adsorption of protein. $(EG)_nOH$-terminated surfaces are not unique in their ability to resist protein adsorption; there are doubtless other inert surfaces yet to be discovered. Our results confirm that inertness is a general property of a group of surfaces and not a specific property of $(EG)_n$OH.

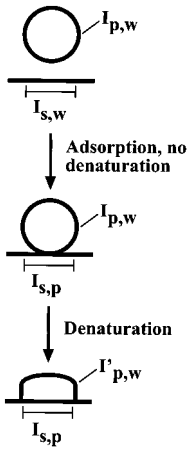



**Figure 6.** Schematic illustration of the interfaces that are involved in the process of protein adsorption onto surfaces. Legend for the labels: I = interface, p = protein, w = water, s = solid. Upon adsorption, the protein can undergo conformational changes that cause a change in its interaction with water ($I'_{p,\ w}$). Further explanation is provided in the text.

The de Gennes/Andrade approach to the rationalization of the properties of inert surfaces—based on conformational flexibility and properties of a hydrated polymer/water layer—does not provide a general description of inert surfaces. This explanation might contribute to the mechanism of inertness in some cases, but it is clearly irrelevant in others; the ability of the functional groups to interact strongly with water molecules is, however, a common attribute of the inert surfaces that we and others have described. The four properties common to *all* inert groups characterized here—polar, hydrogen-bond acceptors, no hydrogen-bond donors, electrically neutral—are compatible with the hypothesis that the interaction of the functional groups with water is an important determinant of inertness. Surface free energy is not a key determinant of inert surfaces, as the values of the advancing contact angles did not correlate with the amounts of adsorbed proteins. The inertness of the surfaces also does not correlate with hydrophilicity—as measured by ClogP—although the use of this parameter might be misleading; ClogP values were calculated for the entire amide group, when only a part of the group is exposed to the aqueous solution and available for interaction with proteins.

The mechanism of resistance to adsorption of proteins remains a problem to be solved. We and others believe that the interaction of the surface with water is a key component of the problem.[36,40,74] Adsorption of proteins, however, consists of two parts (Figure 6). The first and more important part is the formation of an interface between the surface and the protein with release of water. This interface is generated from two separate interfaces: that between the surface and water and a corresponding interface between protein and water. The second and probably less important part is reorganization of the protein on adsorption; this reorganization might cause changes in the structure of the protein/water interface (Figure 6). Detailed deconvolution of these terms is, however, just at the stage of initial hypothesis.

This paper demonstrates a strategy for the rapid evaluation of hypotheses relating molecular structures and biological properties of surfaces. The range of structures that resist the adsorption of proteins offers a range of options to those designing interfaces between nonbiological materials and solutions of biomolecules.

## Experimental Section

**Materials.** All chemicals used were reagent-grade unless stated otherwise. Fibrinogen (from bovine plasma, F8630), lysozyme (egg white, E.C. 3.2.1.17, L6876), sodium dodecyl sulfate (SDS), and phosphate-buffered saline packets were purchased from Sigma (St. Louis, MO). Anhydrous *N*-methyl-2-pyrrolidinone (NMP), 16-mercaptohexadecanoic acid, trifluoroacetic anhydride (TFAA), *N*,*N*′-dimethyl-1,2-diaminoethane [$HN(CH_3)CH_2CH_2N(CH_3)H$], *N*,*N*,*N*′-trimethyl-1,2-diaminoethane [$HN(CH_3)CH_2CH_2N(CH_3)_2$], $CH_3(CH_2)_{11}NH_2$, 1-amino-1-deoxy-D-sorbitol, di-*tert*-butyl dicarbonate, diisopropylethylamine (DIPEA), bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP−Cl), sodium hydride, methyl iodide, tetramethyl phosphorodiamidic chloride [$(CH_3)_2N)_2P(O)Cl$], and hydrazine were purchased from Aldrich (Milwaukee, WI). $H_2NCH_2CON(CH_3)_2$, ($H_2NCH_2CONH_2$), $PhCH_2OC(O)-N(CH_3)CH_2C(O)-NHS$ [*Z*-$N(CH_3)CH_2C(O)-NHS$], $HN(CH_3)CH_2C(O)OC(CH_3)_3$, and $HN(CH_3)CH_2CON(CH_3)_2$ were purchased from BACHEM (Bubendorf, Switzerland). Tri(ethylene glycol)monoamine [$H_2N(CH_2CH_2O)_3H$] was a gift from Texaco Chemical Company, which has since been bought by Huntsman Corporation (3040 Post Oak Boulevard, Houston, TX 77056 (713-235-6000). Anhydrous *N*,*N*-dimethyl formamide (DMF), triethylamine, trifluoroacetic acid (TFA), acetic anhydride and ethylene glycol were purchased from EM Science (Gibbstown, NJ). Absolute ethanol was purchased from Pharmco Products (Brookfield, CT). *tert*-Butyl-*N*-methyl-*N*-[2-(methylamino)ethyl]-carbonate [$HN(CH_3)CH_2CH_2N(CH_3)COC(CH_3)_3$][76] and *N*-carbomethoxy-*N*,*N*′-dimethyl-1,2-diaminoethane [$HN(CH_3)CH_2CH_2N(CH_3)CO_2CH_3$][77] were synthesized as described previously. Phosphate-buffered saline (PBS, 10 mM phosphate, 138 mM NaCl, and 2.7 mM KCl) was freshly prepared in deionized water and filtered through 0.22-$\mu$M filters prior to use. Brine solutions used for extractions were saturated with NaCl. The $^1$H NMR spectra were recorded at 400 MHz on a Bruker spectrometer. Chemical shifts are reported in parts per million referenced with respect to residual solvent ($CHCl_3$ = 7.26 ppm).

**Preparation of the Reactive SAM Presenting Interchain Carboxylic Anhydride.** Gold substrates for SPR spectroscopy were prepared by electron-beam evaporation of 1.5 nm of Ti, followed by 38 nm of Au, onto 50 × 18 mm$^2$ glass coverslips (Corning, no. 2). The gold-coated substrates were incubated overnight in a 2 mM solution of $HS(CH_2)_{15}CO_2H$ in ethanol/water/acetic acid (85/10/5, v/v/v), rinsed with ethanol, and dried under a stream of nitrogen.[22] The cleaned substrates were then placed in a freshly prepared solution of 0.1 M trifluoroacetic anhydride (TFAA) and 0.2 M triethylamine in anhydrous DMF without stirring for 20 min at room temperature. The substrates were removed from the TFAA solution, rinsed thoroughly with $CH_2Cl_2$, and dried in a stream of nitrogen. The resulting SAMs (which present interchain carboxylic anhydride groups) were used immediately by immersion into a 10 mM solution of the appropriate amine in NMP.[56] We added triethylamine (20 mM in NMP) to the amino compounds purchased as salts. 1-Amino-1-deoxy-D-sorbitol was not soluble in NMP; this reaction was carried out in a 25 mM phosphate buffer, pH 10, 10 mM 1-amino-1-deoxy-D-sorbitol (entry 36, Table 1). The substrates were removed from the amine solution, rinsed with ethanol, and dried under a stream of nitrogen.

**Surface Plasmon Resonance Spectroscopy.** SPR was performed on a Biacore 1000 instrument (Biacore, Uppsala, Sweden). The substrate containing the SAM to be analyzed was mounted in a SPR cartridge as described previously.[49,61] Our SPR protocol for measuring the adsorption of protein to SAMs consisted of: (i) flowing a solution of sodium dodecyl sulfate (40 mM in PBS) over the SAM surface for 3 min and then rinsing the surface with a solution of PBS buffer for 10 min; (ii) flowing PBS buffer for 2 min and then substituting the flow with a solution of protein (1 mg/mL in PBS) for 3 or 30 min; and finally, (iii) allowing PBS buffer to flow over the surface for an additional 10 min (Figure 2). The flow rate used for all experiments was 10 $\mu$L/min.

(76) Saari, W. S.; Schwering, J. E.; Lyle, P. A.; Smith, S. J.; Engelhardt, E. L. *J. Med. Chem.* **1990**, *33*, 97−101.
(77) Cravey, M. J.; Kohn, H. *J. Am. Chem. Soc.* **1980**, *102*, 3928−3939.

**Syntheses.** *Z-(N(CH$_3$)CH$_2$C(O))$_2$OC(CH$_3$)$_3$.* DIPEA (10.4 mL, 60 mmol) was added dropwise over 10 min to a mixture at 0 °C of *Z*-N(CH$_3$)CH$_2$C(O)−NHS (5.0 g, 16 mmol) and HN(CH$_3$)CH$_2$C(O)OC(CH$_3$)$_3$−HCl (2.7 g, 15 mmol) in DMF (40 mL); this solution was warmed to ambient temperature and stirred for 16 h. The solution was concentrated in vacuo; $CH_2Cl_2$ (300 mL) was added; and the organic solution was washed with water (50 mL), saturated aqueous $NaHCO_3$ (50 mL), and brine (50 mL) and dried over anhydrous $MgSO_4$. The solution was concentrated in vacuo, loaded onto a silica gel gravity column (100 g), and eluted with ethyl acetate/hexanes 1/1 (v/v) to afford *Z*-(N(CH$_3$)CH$_2$C(O))$_2$OC(CH$_3$)$_3$ as a colorless oil (4.5 g, 86%). $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 1.42 (s, 9H), 2.92−3.00 (m, 6H), 3.92−4.13 (m, 4H), 5.10 (m, 2H), 7.26−7.33 (m, 5H). HRMS-FAB: *m/z* 373.1747 ([M + Na]$^+$; calcd for $C_{18}H_{26}N_2O_5Na$, 373.1739).

*Z-(N(CH$_3$)CH$_2$C(O))$_3$OC(CH$_3$)$_3$.* *Z*-(N(CH$_3$)CH$_2$C(O))$_2$OC(CH$_3$)$_3$ (1.8 g, 5.2 mmol) was hydrogenated over Pd/C (0.1 g, 10% w/w) in EtOH (20 mL) until thin-layer chromatography (TLC) (ethyl acetate/hexanes 1/1) indicated that the *Z*-(N(CH$_3$)CH$_2$C(O))$_2$OC(CH$_3$)$_3$ had been consumed. The reaction mixture was filtered and concentrated in vacuo to give H(N(CH$_3$)CH$_2$C(O))$_2$OC(CH$_3$)$_3$, which was used in the next step without further purification. DIPEA (2.7 mL, 15 mmol) was added dropwise over 10 min to a solution at 0 °C of *Z*-N(CH$_3$)CH$_2$C(O)−NHS (1.7 g, 5.4 mmol) and H(N(CH$_3$)CH$_2$C(O))$_2$OC(CH$_3$)$_3$ (1.1 g, 5.2 mmol) in DMF (20 mL); this solution was warmed to ambient temperature and stirred for 16 h. The reaction mixture was concentrated in vacuo; $CH_2Cl_2$ (300 mL) was added; and the organic solution was washed with water (50 mL), saturated aqueous $NaHCO_3$ (50 mL) and brine (50 mL) and dried over anhydrous $MgSO_4$. The solution was concentrated in vacuo, loaded onto a silica gel gravity column (100 g), and eluted with ethyl acetate/hexanes 1/1 (v/v) to afford *Z*-(N(CH$_3$)CH$_2$C(O))$_3$OC(CH$_3$)$_3$ as a colorless oil (2.0 g, 85%). $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 1.43 (m, 9H), 2.90−3.06 (m, 9H), 3.94−4.24 (m, 6H), 5.10 (m, 2H), 7.26−7.33 (m, 5H). HRMS-FAB: *m/z* 444.2093 ([M + Na]$^+$; calcd for $C_{21}H_{31}N_3O_6Na$, 444.2111).

*Z-(N(CH$_3$)CH$_2$C(O))$_3$N(CH$_3$)$_2$, Procedure 1.* TFA (5 mL) was added to a solution of *Z*-(N(CH$_3$)CH$_2$C(O))$_2$OC(CH$_3$)$_3$ (1.7 g, 4.9 mmol) in $CH_2Cl_2$ (10 mL), and the resulting mixture was stirred for 1 h at ambient temperature. The solution was concentrated in vacuo, and the resulting oily residue was further dried for 2 days in vacuo to afford *Z*-(N(CH$_3$)CH$_2$C(O))$_2$OH as a white solid, which was used in the next step without further purification. DIPEA (2.5 mL, 14.5 mmol) was added dropwise over 5 min to a mixture at 0 °C of *Z*-(N(CH$_3$)CH$_2$C(O))$_2$OH (1.4 g, 4.9 mmol), EDAC (1.0 g, 5.2 mmol), and H$_2$N(CH$_3$)CH$_2$C(O)N(CH$_3$)$_2$ (0.65 g, 5.6 mmol) in $CH_2Cl_2$ (40 mL), and the reaction mixture was allowed to warm to ambient temperature and stirred for 16 h. $CH_2Cl_2$ (300 mL) was added, and the organic solution was washed with water (50 mL), saturated aqueous $NaHCO_3$ (20 mL), and brine (20 mL) and dried over anhydrous $MgSO_4$. The solution was concentrated in vacuo, loaded onto a silica gel gravity column (20 g), and eluted with $CH_2Cl_2$/MeOH 90/10 (v/v) to afford *Z*-(N(CH$_3$)CH$_2$C(O))$_3$N(CH$_3$)$_2$ as a white solid (1.1 g, 57%). $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 2.78−2.93 (m, 15H), 3.91−4.16 (m, 6H), 5.01 (m, 2H), 7.14−7.23 (m, 5H). HRMS-FAB: *m/z* 415.1953 ([M + Na]$^+$; calcd for $C_{19}H_{28}N_4O_5Na$, 415.1957).

*H(N(CH$_3$)CH$_2$C(O))$_3$N(CH$_3$)$_2$.* *Z*-(N(CH$_3$)CH$_2$C(O))$_3$N(CH$_3$)$_2$ (0.62 g, 1.6 mmol) was hydrogenated over Pd/C (0.1 g, 10% w/w) in EtOH/$CH_2Cl_2$ 9/1 (10 mL) until TLC ($CH_2Cl_2$/MeOH, 20/1) indicated that the *Z*-(N(CH$_3$)CH$_2$C(O))$_3$N(CH$_3$)$_2$ had been consumed. The reaction mixture was filtered to remove the Pd/C, and the filtrate was concentrated in vacuo to afford H(N(CH$_3$)CH$_2$C(O))$_3$N(CH$_3$)$_2$ as a white solid (0.42 g, 99%). $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 2.68−2.71 (m, 3H), 2.90−3.09 (m, 12H), 3.92−3.98 (m, 2H), 4.16−4.34 (m, 4H), 6.75 (bs, 1H). HRMS-FAB: *m/z* 281.1602 ([M + Na]$^+$; calcd for $C_{11}H_{22}N_4O_3Na$, 281.1590).

*Z-(N(CH$_3$)CH$_2$C(O))$_4$N(CH$_3$)$_2$.* *Z*-(N(CH$_3$)CH$_2$C(O))$_4$N(CH$_3$)$_2$ was prepared from *Z*-(N(CH$_3$)CH$_2$C(O))$_3$OC(CH$_3$)$_3$ (1.0 g, 2.4 mmol) and H$_2$N(CH$_3$)CH$_2$C(O)N(CH$_3$)$_2$ (0.52 g, 4.4 mmol) according to procedure 1 to afford *Z*-(N(CH$_3$)CH$_2$C(O))$_4$N(CH$_3$)$_2$ as a white solid (0.58 g, 52%). $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 2.87−3.00 (m, 18H), 3.95−4.21 (m, 8H), 5.07 (m, 2H), 7.23−7.29 (m, 5H). HRMS-FAB: *m/z* 486.2330 ([M + Na]$^+$; calcd for $C_{22}H_{33}N_5O_6Na$, 486.2329).

*H(N(CH$_3$)CH$_2$C(O))$_4$N(CH$_3$)$_2$.* *Z*-(N(CH$_3$)CH$_2$C(O))$_4$N(CH$_3$)$_2$ (0.50 g, 1.1 mmol) was hydrogenated over Pd/C (0.1 g, 10% w/w) in EtOH/$CH_2Cl_2$ 9/1 (10 mL) until TLC ($CH_2Cl_2$/MeOH, 9/1) indicated that *Z*-(N(CH$_3$)CH$_2$C(O))$_4$N(CH$_3$)$_2$ had been consumed. The reaction mixture was filtered to remove the Pd/C, and the filtrate was concentrated in vacuo to afford H(N(CH$_3$)CH$_2$C(O))$_4$N(CH$_3$)$_2$ as a white solid (0.35 g, 99%). $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 2.65−2.70 (m, 3H), 2.92−3.10 (m, 15H), 3.83−3.91 (m, 2H), 4.13−4.33 (m, 6H), 5.05 (bs, 1H). HRMS-FAB: *m/z* 352.1962 ([M + Na]$^+$; calcd for $C_{14}H_{27}N_5O_4Na$, 352.1961).

*Z-(N(CH$_3$)CH$_2$C(O))$_5$N(CH$_3$)$_2$.* DIPEA (0.45 mL, 2.6 mmol) was added dropwise over 10 min to a solution at 0 °C of *Z*-N(CH$_3$)CH$_2$C(O)−NHS (0.34 g, 1.1 mmol) and H(N(CH$_3$)CH$_2$C(O))$_4$N(CH$_3$)$_2$ (0.28 g, 0.85 mmol) in DMF (10 mL); this solution was warmed to ambient temperature and stirred for 16 h. The solution was concentrated in vacuo; $CH_2Cl_2$ (300 mL) was added; and the organic solution was washed with water (20 mL), saturated aqueous $NaHCO_3$ (20 mL), and brine (20 mL) and dried over anhydrous $MgSO_4$. The solution was concentrated in vacuo, loaded onto a silica gel gravity column (20 g), and eluted with $CH_2Cl_2$/MeOH 90/10 (v/v) to afford *Z*-(N(CH$_3$)CH$_2$C(O))$_5$N(CH$_3$)$_2$ (0.23 g, 51%). $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 2.86−2.99 (m, 21H), 3.92−4.22 (m, 10H), 5.07 (m, 2H), 7.23−7.29 (m, 5H). HRMS-FAB: *m/z* 557.2719 ([M + Na]$^+$; calcd for $C_{25}H_{38}N_6O_7Na$, 557.2700).

*H(N(CH$_3$)CH$_2$C(O))$_5$N(CH$_3$)$_2$.* *Z*-(N(CH$_3$)CH$_2$C(O))$_5$N(CH$_3$)$_2$ (0.23 g, 0.43 mmol) was hydrogenated over Pd/C (0.1 g, 10% w/w) in ethanol (10 mL) until TLC ($CH_2Cl_2$/MeOH, 9/1) indicated that the *Z*-(N(CH$_3$)CH$_2$C(O))$_5$N(CH$_3$)$_2$ had been consumed. The reaction mixture was filtered to remove the Pd/C, and the filtrate was concentrated in vacuo to afford H(N(CH$_3$)CH$_2$C(O))$_5$N(CH$_3$)$_2$ as a white solid (0.17 g, 99%). $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 2.56−2.61 (m, 3H), 2.77−2.97 (m, 18H), 3.83−4.24 (m, 10H), 6.50 (bs, 1H). HRMS-FAB: *m/z* 423.2332 ([M + Na]$^+$; calcd for $C_{17}H_{32}N_6O_5Na$, 423.2331).

*CH$_3$C(O)S(CH$_2$)$_{15}$C(O)(N(CH$_3$)CH$_2$C(O))$_3$N(CH$_3$)$_2$.* A mixture of CH$_3$C(O)S(CH$_2$)$_{15}$CO$_2$H (0.28 g, 0.84 mmol), BOP−Cl (0.23 g, 0.91 mmol), H(N(CH$_3$)CH$_2$C(O))$_3$N(CH$_3$) (0.18 g, 0.70 mmol), and triethylamine (0.24 mL, 1.8 mmol) in $CH_2CL_2$ (10 mL) was stirred for 30 min at 0 °C, and then the reaction mixture was warmed to ambient temperature and stirred for 16 h. Water (20 mL) was added, and the slurry was extracted with $CH_2Cl_2$ (3 × 100 mL). The combined organic solutions were washed with saturated aqueous $NaHCO_3$ (20 mL) and brine (20 mL) and dried over anhydrous $MgSO_4$. The solution was concentrated in vacuo, loaded onto a silica gel gravity column (20 g), and eluted with $CH_2Cl_2$/MeOH 97/3 (v/v) to afford CH$_3$C(O)S(CH$_2$)$_{15}$C(O)(N(CH$_3$)CH$_2$C(O))$_3$N(CH$_3$)$_2$ as a colorless oil (0.21 g, 53%). $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 1.15−1.22 (m, 22H), 1.51 (m, 4H), 2.20−2.29 (m, 2H), 2.22 (s, 3H), 2.75 (t, $J$ = 7.3 Hz, 2H), 2.80−2.99 (m, 15H), 3.97−4.20 (m, 6H). $^{13}$C NMR ($CDCl_3$, 400 MHz): $\delta$ 24.75, 24.91, 28.60, 28.92, 29.28, 29.34, 29.41, 30.43, 32.67, 33.01, 34.84, 35.07, 35.40, 35.60, 35.72, 35.90, 36.09, 36.37, 48.83, 49.17, 49.28, 49.50, 49.60, 50.83, 166.85, 167.31, 168.34, 168.68, 168.78, 169.40, 173.21, 173.58, 195.80. HRMS-FAB: *m/z* 593.3730 ([M + Na]$^+$; calcd for $C_{29}H_{54}N_4O_5SNa$, 593.3713).

*HS(CH$_2$)$_{15}$C(O)(N(CH$_3$)CH$_2$C(O))$_3$N(CH$_3$)$_2$, Procedure 2.* Nitrogen gas was bubbled through a solution of CH$_3$C(O)S(CH$_2$)$_{15}$C(O)(N(CH$_3$)CH$_2$C(O))$_3$N(CH$_3$)$_2$ (0.20 g, 0.35 mmol) in methanol (10 mL) and a solution of NaOH (1 M) for 10 min. This NaOH (3.0 mL, 3 mmol) was then added to the solution of CH$_3$C(O)S(CH$_2$)$_{15}$C(O)(N(CH$_3$)CH$_2$C(O))$_3$N(CH$_3$)$_2$ in methanol, and the reaction mixture was stirred for 3 h at ambient temperature. The solution was concentrated in vacuo, loaded onto a silica gel gravity column (20 g), and eluted with $CH_2Cl_2$/MeOH 95/5 (v/v) to afford HS(CH$_2$)$_{15}$C(O)(N(CH$_3$)CH$_2$C(O))$_3$N(CH$_3$)$_2$ as a white solid (0.17 g, 92%). $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 1.16−1.29 (m, 22H), 1.51 (m, 4H), 2.23−2.29 (m, 2H), 2.42 (q, $J$ = 7.43 Hz, 2H), 2.86−2.99 (m, 15H), 4.02−4.20 (m, 6H). $^{13}$C NMR ($CDCl_3$, 400 MHz): $\delta$ 24.48, 24.78, 24.93, 28.19, 28.89, 29.28, 29.33, 29.40, 29.45, 32.71, 32.99, 33.06, 33.88, 34.88, 35.12, 35.29, 35.44, 35.64, 35.76, 35.79, 35.94, 36.05, 36.13, 36.41, 36.47, 48.86, 49.12, 49.30, 49.41, 49.63, 50.21, 50.87, 50.98, 166.85, 166.91, 167.31, 168.26, 168.37, 168.40, 168.70, 168.99, 169.43, 173.25, 173.63. HRMS-FAB: *m/z* 551.3631 ([M + Na]$^+$; calcd for $C_{27}H_{52}N_4O_4SNa$, 551.3607).

*$CH_3C(O)S(CH_2)_{15}C(O)N(CH_3)CH_2(CH(OCH_3))_4CH_2OCH_3$.* A mixture of $CH_3C(O)S(CH_2)_{15}CO_2H$ (0.35 g, 1.1 mmol), BOP−Cl (0.32 g, 1.3 mmol), $HN(CH_3)CH_2(CH(OCH_3))_4CH_2OCH_3$ (0.28 g, 1.1 mmol), and triethylamine (0.35 mL, 2.5 mmol) in $CH_2CL_2$ (2 mL) was stirred for 30 min at 0 °C, and then the reaction mixture was warmed to ambient temperature and stirred for 16 h. Water (20 mL) was added, and the slurry was extracted with $CH_2Cl_2$ (3 × 100 mL). The combined organic solutions were washed with saturated aqueous $NaHCO_3$ (20 mL) and brine (20 mL) and dried over anhydrous $MgSO_4$. The solution was concentrated in vacuo, loaded onto a silica gel gravity column (30 g), and eluted with ethyl acetate/hexanes 67/33 (v/v) to afford $CH_3C(O)S(CH_2)_{15}C(O)N(CH_3)CH_2(CH(OCH_3))_4CH_2OCH_3$ as a colorless oil (0.39 g, 57%). $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 1.18−1.24 (m, 22H), 1.49 (m, 2H), 1.56 (m, 2H), 2.22−2.40 (m, 2H), 2.24 (s, 3H), 2.79 (t, $J$ = 7.3 Hz, 2H), [2.92 (s), 3.05 (s), 3H in total in a 1:1 ratio], 2.94−2.97 (m, 1H), 3.28−3.45 (m, 19H), 3.48−3.71 (m, 2H), 3.90 (m, 1H). $^{13}$C NMR ($CDCl_3$, 400 MHz): $\delta$ 24.99, 25.31, 28.70, 28.99, 29.03, 29.34, 29.40, 29.43, 29.52, 30.48, 32.99, 33.67, 34.13, 37.85, 49.90, 50.92, 57.23, 58.84, 58.87, 59.32, 59.43, 59.57, 60.09, 60.14, 60.21, 69.31, 69.44, 69.87, 78.25, 78.77, 79.40, 79.59, 79.73, 80.02, 80.23, 80.98, 173.19, 173.80, 196.01. HRMS-FAB: $m/z$ 600.3922 ([M + Na]$^+$; calcd for $C_{30}H_{59}NO_7SNa$, 600.3910).

*$HS(CH_2)_{15}C(O)N(CH_3)CH_2(CH(OCH_3))_4CH_2OCH_3$.* $CH_3C(O)S(CH_2)_{15}C(O)N(CH_3)CH_2(CH(OCH_3))_4CH_2OCH_3$ (0.20 g, 0.34 mmol) was deprotected according to procedure 2 to afford $HS(CH_2)_{15}C(O)N(CH_3)CH_2(CH(OCH_3))_4CH_2OCH_3$ as a colorless oil (0.11 g, 60%). $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 1.20−1.34 (m, 22H), 1.55 (m, 4H), 2.26−2.41 (m, 2H), 2.47 (q, $J$ = 7.4 Hz, 2H), 2.92−2.97 (m, 1H), [2.95 (s), 3.08 (s), 3H in total in a 1:1 ratio], 3.34−3.51 (m, 19H), 3.56 (m, 1H), 3.64−3.76 (m, 2H), 3.96 (m, 1H). $^{13}$C NMR ($CDCl_3$, 400 MHz): $\delta$ 24.60, 25.05, 25.38, 28.33, 29.03, 29.42, 29.47, 29.52, 29.58, 33.07, 33.75, 34.01, 34.20, 37.95, 49.99, 50.96, 57.30, 58.91, 58.95, 59.40, 59.56, 59.66, 60.16, 60.22, 60.40, 69.31, 69.73, 78.17, 78.67, 79.34, 79.55, 79.68, 80.03, 80.30, 80.98, 173.27, 173.89. HRMS-FAB: $m/z$ 558.3815 ([M + Na]$^+$; calcd for $C_{28}H_{57}NO_6SNa$, 558.3804).

*$CH_3C(O)S(CH_2)_{10}C(O)N(CH_3)CH_2CH_2N(CH_3)P(O)(N(CH_3)_2)_2$, Procedure 3.* A mixture of $CH_3C(O)S(CH_2)_{10}CO_2H$ (0.40 g, 1.7 mmol), EDAC (0.38 g, 2.0 mmol), and triethylamine (0.28 mL, 2.0 mmol) was stirred for 1 h at ambient temperature; $HN(CH_3)CH_2CH_2N(CH_3)P(O)(N(CH_3)_2)_2$ (0.44 g, 2.0 mmol) was added, and the reaction mixture was stirred for 16 h. The solution was concentrated in vacuo, HCl (20 mL, 0.1M) was added, and the slurry was extracted with $CH_2Cl_2$ (3 × 100 mL). The combined organic solutions were washed with saturated aqueous $NaHCO_3$ (20 mL) and brine (20 mL) and dried over anhydrous $MgSO_4$. The solution was concentrated in vacuo, loaded onto a silica gel gravity column (30 g), and eluted with $CH_2Cl_2$/MeOH 95/5 (v/v) to afford $CH_3C(O)S(CH_2)_{10}C(O)N(CH_3)CH_2CH_2N(CH_3)P(O)(N(CH_3)_2)_2$ as a colorless gummy solid (0.50 g, 54%). $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 1.12−1.19 (m, 12H), 1.37−1.47 (m, 4H), [2.13 (t, $J$ = 7.5 Hz), 2.23 (t, $J$ = 7.5 Hz), 2H total in a 3:1 ratio], 2.17 (s, 3H), [2.48 (d, $J_P$ = 9.4 Hz), 2.50 (d, $J_P$ = 9.5 Hz), 12H total in a 3:1 ratio], 2.55 (d, $J_P$ = 9.1 Hz, 3H), 2.71 (t, $J$ = 7.3 Hz, 2H), [2.81 (s), 2.90 (s), 3H total in a 1:3 ratio], 2.96 (m, 2H), [3.31 (t, $J$ = 7.1 Hz), 3.39 (t, $J$ = 6.7 Hz), 2H total in a 1:3 ratio]). $^{13}$C NMR ($CDCl_3$, 400 MHz): $\delta$ 24.59, 25.13, 28.40, 28.71, 28.75, 29.03, 29.07, 29.13, 30.28, 32.55, 33.20, 33.40, 34.00, 34.03, 35.27, 36.34, 36.38, 36.41, 36.45, 45.18, 45.20, 45.82, 45.85, 172.85, 172.91, 195.57. HRMS-ES: $m/z$ 465.3004 ([M + H]$^+$; calcd for $C_{21}H_{46}N_4O_3SP$, 465.3028).

*$HS(CH_2)_{10}C(O)N(CH_3)CH_2CH_2N(CH_3)P(O)(N(CH_3)_2)_2$.* $CH_3C(O)S(CH_2)_{10}C(O)-N(CH_3)CH_2CH_2N(CH_3)P(O)(N(CH_3)_2)_2$ (0.45 g, 1.0 mmol) was deprotected according to procedure 2 to afford $HS(CH_2)_{10}C(O)N(CH_3)CH_2CH_2N(CH_3)P(O)(N(CH_3)_2)_2$ as a gummy white solid (0.25 g, 61%). There is a slow rotation about the amide bond on the $^1$H NMR time scale, resulting in a 3:1 mixture of stereoisomers as observed by duplication of NMR signals in close proximity to the amide bond. $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 1.12−1.20 (m, 12H), 1.44 (m, 4H), [2.14 (t, $J$ = 7.5 Hz), 2.22 (t, $J$ = 7.5 Hz), 2H in total in a 3:1 ratio], 2.44 (q, $J$ = 7.3 Hz, 2H), [2.47 (d, $J_P$ = 9.5 Hz), 2.49 (d, $J_P$ = 9.5 Hz), 12H in total in a 3:1 ratio], 2.54 (d, $J_P$ = 9.1 Hz, 3H), [2.80 (s), 2.89 (s), 3H in total in a 1:3 ratio], 2.95 (m, 2H), [3.30 (t, $J$ = 7.3 Hz), 3.37 (t, $J$ = 6.6 Hz), 2H in total in a 1:3 ratio]. $^{13}$C NMR ($CDCl_3$, 400 MHz): $\delta$ 24.34, 24.67, 25.22, 28.05, 28.74, 29.11, 29.15, 29.21, 29.22, 32.63, 33.28, 33.47, 33.74, 34.09, 34.12, 34.84, 34.88, 35.36, 36.43, 36.47, 36.51, 36.54, 45.27, 45.30, 45.90, 45.94, 47.46, 47.50, 48.46, 172.92, 172.95. HRMS-ES: $m/z$ 423.2914 ([M + H]$^+$; calcd for $C_{19}H_{44}N_4O_2SP$, 423.2923).

*$CH_3C(O)S(CH_2)_{10}C(O)N(CH_2CH_2)_2NC(O)CH_3$.* $HN(CH_2CH_2)_2NC(O)CH_3$ was used as the amine in procedure 3 to afford $CH_3C(O)S(CH_2)_{10}C(O)N(CH_2CH_2)_2NC(O)CH_3$ as a white solid (0.30 g, 49%). $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 1.15−1.23 (m, 12H), 1.40−1.51 (m, 4H), 2.00 (s, 3H), 2.19 (s, 3H), 2.21 (t, $J$ = 7.6 Hz, 2H), 2.72 (t, $J$ = 7.3 Hz, 2H), 3.33−3.39 (m, 4H), 3.47−3.52 (m, 4H). HRMS-ES: $m/z$ 371.2384 ([M + H]$^+$; calcd for $C_{19}H_{35}N_2O_3S$, 371.2368).

*$HS(CH_2)_{10}C(O)N(CH_2CH_2)_2NC(O)CH_3$.* $CH_3OC(O)S(CH_2)_{10}C(O)N(CH_2CH_2)_2NC(O)CH_3$ (0.25 g, 2.0 mmol) was deprotected according to procedure 2 to afford $HS(CH_2)_{10}C(O)N(CH_2CH_2)_2NC(O)CH_3$ as a white paste (0.18 g, 72%). $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 1.13−1.21 (m, 12H), 1.44 (m, 4H), 1.98 (s, 3H), 2.19 (t, $J$ = 7.5 Hz, 2H), 2.36 (q, $J$ = 7.4 Hz, 2H), 3.31−3.36 (m, 4H), 3.44−3.49 (m, 4H). $^{13}$C NMR ($CDCl_3$, 400 MHz): $\delta$ 21.05, 24.28, 24.84, 27.96, 28.66, 29.02, 29.06, 32.88, 32.97, 33.66, 40.84, 41.08, 44.87, 45.16, 45.67, 45.96, 168.65, 168.92, 171.38, 171.62. HRMS-ES: $m/z$ 329.2269 ([M + H]$^+$; calcd for $C_{17}H_{33}N_2O_2S$, 329.2263).

*$(CH_3)_3COC(O)N(CH_3)CH_2CH_2N(CH_3)S(O)_2N(CH_3)CH_2CH_2N(CH_3)H$.* $(CH_3)_3C(O)N(CH_3)CH_2CH_2N(CH_3)H$ (2.8 g, 14 mmol) and DIPEA (2.6 mL, 15 mmol) were added to a solution of $S(O)_2Cl_2$ (1.2 mL, 14 mmol) at −78 °C, and the resulting reaction mixture was slowly warmed to ambient temperature and stirred overnight. The resulting solution was concentrated in vacuo to afford $(CH_3)_3COC(O)N(CH_3)CH_2CH_2N(CH_3)-S(O)_2Cl$ as a brown oil (5 g, 100%). This compound was used without further purification. HRMS-FAB: $m/z$ 309.0650 ([M + Na]$^+$; calcd for $C_9H_{19}N_2O_4SCl$, 309.0652). $(CH_3)_3COC(O)N(CH_3)CH_2CH_2N(CH_3)-S(O)_2Cl$ (1.5 g, 5.3 mmol) was dissolved in $CH_2Cl_2$ (50 mL) and combined with $HN(CH_3)CH_2CH_2N(CH_3)H$ (2.78 mL, 26 mmol); the resulting solution was refluxed for 4 h. The reaction mixture was cooled to ambient temperature, and $CH_2Cl_2$ (200 mL) was added. The organic solution was washed with saturated aqueous $NaHCO_3$ (50 mL) and brine (50 mL) and dried over anhydrous $MgSO_4$. The solution was concentrated in vacuo to give a yellow oil, which was loaded onto a silica gel gravity column (100 g) and eluted with $CH_2Cl_2$/MeOH 80/20 (v/v) to afford $(CH_3)_3COC(O)N(CH_3)CH_2CH_2N(CH_3)S(O)_2N(CH_3)CH_2CH_2N(CH_3)H$ as a colorless oil (1.0 g, 57%). $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 1.43 (s, 9H), 2.49 (s, 3H), 2.79 (s, 3H), 2.83 (m, 5H), 2.87 (s, 3H), 3.25 (bs, 2H), 3.30−3.38 (m, 4H). HRMS-FAB: $m/z$ 339.2055 ([M + H]$^+$; calcd for $C_{13}H_{31}N_4O_4S$, 339.2066).

*$(CH_3)_3COC(O)N(CH_3)CH_2CH_2N(CH_3)S(O)_2N(CH_3)CH_2CH_2N(CH_3)-S(O)_2N(CH_3)_2$.* $ClS(O)_2N(CH_3)_2$ (0.60 mL, 5.6 mmol) was added to a solution of $(CH_3)_3COC(O)N(CH_3)CH_2CH_2N(CH_3)S(O)_2N(CH_3)CH_2CH_2N(CH_3)H$ (0.95 g, 2.8 mmol) and triethylamine (0.78 mL, 5.6 mmol) in $CH_2Cl_2$ (50 mL), and the resulting solution was refluxed for 2 h. The reaction was cooled to ambient temperature, and $CH_2Cl_2$ (200 mL) was added. The organic solution was washed with saturated aqueous $NaHCO_3$ (50 mL) and brine (50 mL) and dried over anhydrous $MgSO_4$. The solution was concentrated in vacuo to give a brown oil, which was loaded onto a silica gel gravity column (100 g) and eluted with $CH_2Cl_2$/MeOH 95/5 (v/v) to afford $(CH_3)_3COC(O)N(CH_3)CH_2CH_2N(CH_3)S(O)_2N(CH_3)CH_2CH_2N(CH_3)S(O)_2N(CH_3)_2$ as a gummy white solid (1.1 g, 88%). $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 1.45 (s, 9H), 2.79 (s, 6H), 2.82 (s, 3H), 2.83 (s, 3H), 2.86 (s, 3H), 2.89 (s, 3H), 3.28 (bt, 2H), 3.37−3.41 (m, 6H). HRMS-FAB: $m/z$ 468.1935 ([M + Na]$^+$; calcd for $C_{15}H_{35}N_5O_6S_2Na$, 468.1926).

*$HN(CH_3)CH_2CH_2N(CH_3)S(O)_2N(CH_3)CH_2CH_2N(CH_3)S(O)_2N(CH_3)_2$.* TFA (10 mL) was added to a solution of $(CH_3)_3COC(O)N(CH_3)CH_2CH_2N(CH_3)S(O)_2N(CH_3)CH_2CH_2N(CH_3)S(O)_2N(CH_3)_2$ (0.57 g, 1.3 mmol) in $CH_2Cl_2$ (10 mL), and the resulting solution was stirred for 1 h at ambient temperature. $CH_2Cl_2$ (100 mL) was added, and the organic solution was washed with saturated aqueous $NaHCO_3$ (20 mL) and brine (20 mL) and dried over anhydrous $MgSO_4$. The solution was concentrated in vacuo to give a yellow oil, which was loaded onto a silica gel gravity column (100 g) and eluted with $CH_2Cl_2$/MeOH 80/20 (v/v) to afford $HN(CH_3)CH_2CH_2N(CH_3)S(O)_2N(CH_3)CH_2CH_2N(CH_3)S(O)_2N(CH_3)_2$ as a slightly yellow solid (0.35 g, 79%). $^1$H NMR ($CDCl_3$,

400 MHz): $\delta$ 2.36 (bs, 1H), 2.49 (s, 3H), 2.80 (s, 6H), 2.82 (m, 2H), 2.83 (s, 3H), 2.84 (s, 3H), 2.86 (s, 3H), 3.32 (t, $J$ = 6.4 Hz, 2H), 3.37 (s, 4H). $^{13}$C NMR ($CDCl_3$, 400 MHz): $\delta$ 35.47, 35.54, 35.60, 38.25, 48.67, 48.75, 49.06, 49.85. HRMS-FAB: $m/z$ 368.1407 ([M + Na]$^+$; calcd for $C_{10}H_{27}N_5O_4S_2Na$, 368.1402).

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**Supporting Information Available:** Plots of $\%ML_{Protein}$ vs values of ClogP calculated according to three different fragmentation methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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