Molecular Recognition on Functionalized Self-Assembled Monolayers of Alkanethiols on Gold

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Abstract: A system for probing molecular recognition events at organic interfaces using fluorescent receptors is described. Receptors formed from the bis(2,6-diaminopyridine) amide of isophthalic acid are incorporated in mixed self-assembled monolayers (SAMs) of alkanethiols on gold and shown to interact with barbituric acid derivatives from solution. Individual parameters that affect the ability of receptors on surfaces to recognize ligands from solution along with varieties of solvents for ligand solutions have been examined.

Introduction

The understanding of interactions at the molecular level at the solid−liquid or solid−vapor interface is an important focus of research in surface and interface science.[1] Interfacial interactions play an essential role in lubrication,[2] inhibition of corrosion,[3] adhesion,[4] coatings, wetting,[5] electrochemistry,[6] chromatography,[7] formation of detection devices and sensors,[8] formation of biocompatible material,[9] biofouling,[10] catalysis,[11] and biological membranes[12] including cellular interactions[13] and cell−surface biochemistry.[14] The complexity of these systems and their frequent lack of defined structure make the preparation of well-ordered and easily characterized model systems essential to gain insight into fundamental interactions involved in such systems.

The single layers of molecules on each side of an interface in large part define the physical and chemical interactions of the interface. Well-defined systems consisting of a single layer of molecules (monolayers) can be used as model systems for many interfacial interactions. Organic monolayer films, forming well-defined surface environments, form versatile model systems in which to study interfacial phenomena. Two methods for preparing such organic monolayer films have been employed. The Langmuir−Blodgett technique[15] offers the advantage of constructing multilayers in addition to monolayers and also allows for the incorporation of a broad range of lipophilic materials in the mono- or multilayer. However, because the components of the layers are only physically (rather than covalently) linked to the surface, these materials suffer from poor stability. Thermal shock, dust, and even solvent can easily destroy the monolayer. A more robust alternative to the Langmuir−Blodgett technique involves the chemisorption of the film components to the substrate from solution.[16] Such self-assembled monolayer films afford more chemically resistant monolayers but are only obtained with very specific pairs of substrates and adsorbates. A number of self-assembling systems

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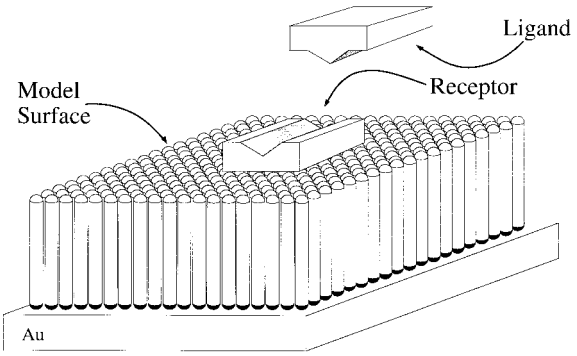

Figure 1. Schematic representation of a system for the study of molecular recognition events between a ligand from solution and a receptor on a functionalized alkanethiolate monolayer on gold film.

have been investigated[17] including of silanes on silicon,[18] carboxylic acids on metal oxides,[19] and organosulfur compounds (including dialkyl sulfides,[20] dialkyl disulfides,[21] and thiols[22]) on gold. Organosulfur self-assembled monolayers on gold offer several advantages over other self-assembled monolayer systems. They provide the highest structural order by densely packing long alkanethiolate chains on the surface and forming crystalline or quasi-crystalline monolayers. In addition, their ease of preparation and characterization, their flexibility in functionalization of terminal group at the monolayer surface, and the inertness of gold toward most organic functional groups make these substrates ideal systems in which to model many important interfacial phenomena.

Molecular recognition on a functionalized monolayer surface, in which the model surface is constructed from a mixed monolayer of alkanethiolates and receptor-terminated alkanethiolates on gold films, is shown schematically in Figure 1. This system permits the examination of the individual parameters that impact recognition, such as solvent/ligand and solvent/receptor interactions,[23] and the impact that the surface environment has on the ability of the receptor on the surface to recognize ligands from solutions. Prior to our initial report in this field,[24] there were only two similar sets of studies, by Ringsdorf and co-workers and Knoll and co-workers, on molecular recognition of biotin receptors on self-assembled monolayers with streptavidin,[25] and by Niwa and co-workers, on specific binding of glycolipid monolayers with Concanavalin A.[26] Since our disclosure, several other studies have appeared in the literature describing nonspecific molecular recognition of cavitand-functionalized self-assembled monolayers by small organic molecules,[27] molecular recognition of ferrocenyl functionalized self-assembled monolayers with calixarene hosts,[28] application of functionalized self-assembled monolayers as immunosensors using molecular recognition,[29] and molecular recognition of carceplexe monolayers.[30] Among other studies,[31] self-assembled monolayer supported membranes have also been exploited in the design of biosensors based on proteins, peptides, and ionophores.[32]

We chose to study the interaction of barbituric acid derivatives with mixed monolayers of alkanethiols and the bis(2,6-diaminopyridine) amide of isophthalic acid-functionalized decanethiol on thin gold films (Figure 2). Similar systems have been studied in solution by Hamilton and co-workers and found to form 1 to 1 complexes in hydrophobic solvents.[33,34] By changing the composition and ratio of thiols from which the mixed monolayers are formed, the environment surrounding the receptor can be easily altered. By manipulating the terminal functional

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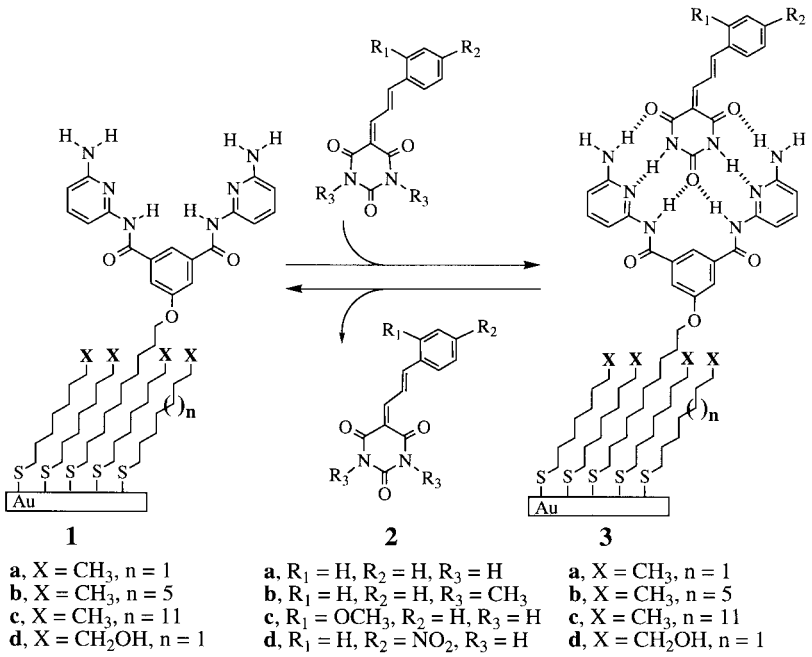
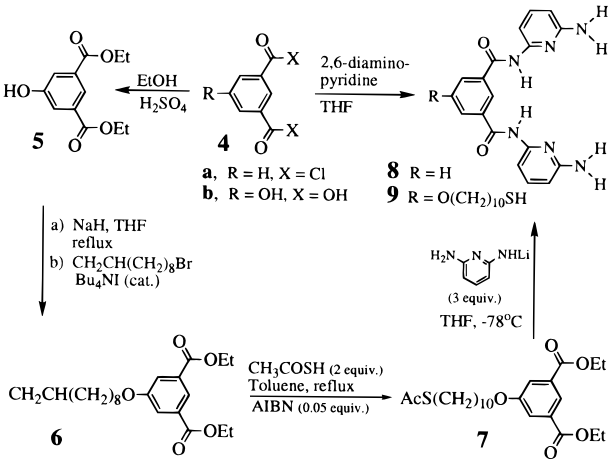
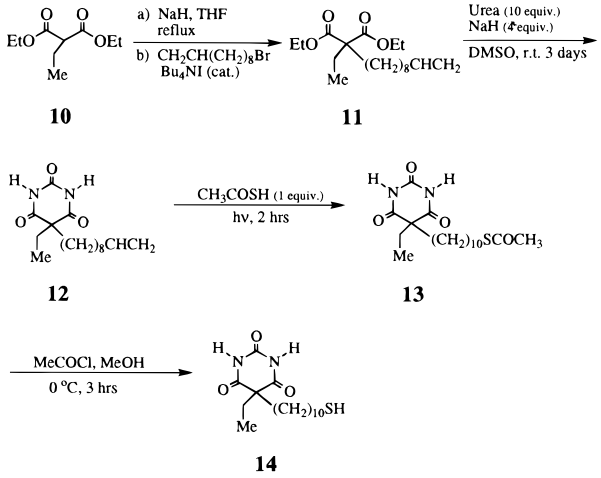


Figure 2. Molecular recognition events between barbituric acid derivatives and the bis(2,6-diaminopyridine) amide of isophthalic acid receptor functionalized alkanethiol on the surface of self-assembled thiolate monolayers on gold film.

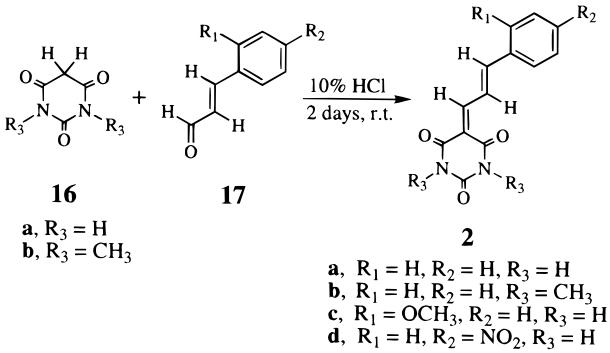
Scheme 1



Scheme 2



Scheme 3



group X and the chain length *n*, the polarity and charge of the surface surrounding the receptor and the extent to which the receptor is buried in the membrane can be regulated (Figure 2). We expected changes in each of these factors to impact the interaction of **2** with **1**. In the context of our experiments the alkanethiol tether appended to the receptor serves two functions. First, the thiol groups serve as the surface active groups for covalently bonding to the gold surface, and second, the long alkyl chain serves as an insulating medium, separating the receptor from the metallic surface and minimizing quenching of the fluorescence of the receptor by gold.[35]

Results and Discussions

Molecular Recognition in Solution. The receptor, receptor-functionalized thiol, and barbiturates used for these experiments were easily synthesized from readily available starting materials as described in Schemes 1, 2, and 3 (see the Experimental Section). Before the examination of the recognition events between the ligand and the receptor in the context of the monolayer membrane, interactions between the host and the guest molecules were studied in solution. Like Hamilton we determined, using ^{1}H NMR spectroscopy, that receptors and ligands formed 1 to 1 complexes in chloroform.[36]

Having confirmed the stoichiometry of the complex using NMR, we considered methods for characterization of the binding

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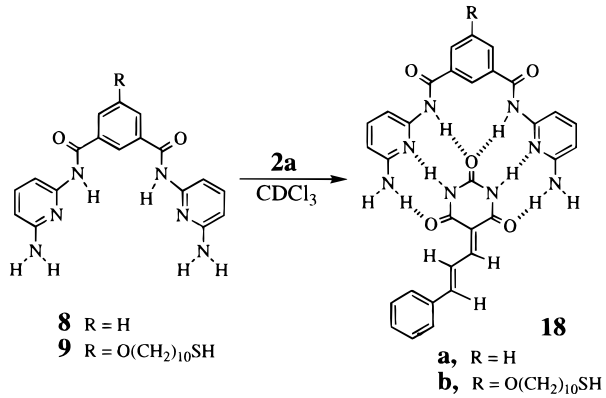

Figure 3. Molecular recognition between the bis(diaminopyridine) amide of isophthalic acid host and the barbituric acid guest in solution.

event in the context of the monolayer. We had noted that bisamide receptors **8** and **9** were fluorescent and reasoned that the wavelength of the fluorescence emission might be altered by the presence of a barbiturate ligand in the binding site. Both guest molecule **2** and host molecules **8** and **9** were shown to fluoresce in several different solvents, and so a series of experiments were conducted to observe the changes in fluorescence during host/guest complexation by incremental addition of ligand to the receptor. Since both host and guest molecules fluoresce, either fluorescence could have been used to monitor the changes during the experiments. However, the fluorescence of the host molecules (**8** and **9**) was chosen as the monitoring probe, since, when these host molecules are used as the receptors on monolayer surfaces, the same fluorescence changes would be more useful as a probe for detection of ligands binding to the receptor. This fluorescent receptor and this detection technique allow for screening of an array of different ligands without the requirement of having a fluorescent ligand.

Solution Fluorescence Experiments. The interaction through hydrogen bonding of barbiturate **2a** and receptor **8** or **9** (Figure 3) was expected to show a strong solvent effect, being favored by nonpolar, aprotic solvents and disfavored in polar protic media. This effect should be manifested by a modest change in the wavelength ($\Delta\lambda$) of fluorescence of receptors **8** and **9** upon treatment with ligand **2a** in a polar solvent. A large $\Delta\lambda$ would be expected in a nonpolar or aprotic solvent, where hydrogen bonding between the ligand and receptor would be strong. The association of the ligand with the receptor would be expected to result in a red shift (positive $\Delta\lambda$) in the fluorescence emission, due to preferential stabilization of the more polarized excited state by the highly polarized ligand complexing with the chromophore.[37] Fluorescence spectra (excitation at 330−335 nm) of **8** in ethanol, acetonitrile, and dichloromethane were obtained (Table 1). The solutions were then treated with 1 equiv of barbiturate **2a**, and the fluorescence spectra were recorded again. As expected, the $\Delta\lambda$ in ethanol was small, showing only a 4-nm bathochromic shift. In contrast, the dichloromethane and acetonitrile solutions containing equimolar amounts of **8** and **2a** showed relatively large bathochromic shifts of 11 and 9 nm, respectively. Thus, the $\Delta\lambda$ behaved as expected both in direction and magnitude.

Table 1. Solution Fluorometric Analysis of **8** and **2a**

	emission wavelength (nm)[a]		
solvent[b]	**8**	**18**	$\Delta\lambda$[c]
CH_3CH_2OH	489	493	4
CH_3CN	473	482	9
CH_2Cl_2	499	510	11

[a] Excitation wavelength = 333 ± 2 nm for all cases. [b] All solvents were of spectrophotometric grade. [c] Shifts ($\Delta\lambda$) to longer wavelength are reported as positive numbers.

Functionalized Self-Assembled Monolayers. Our objective was to study the binding of the ligand to the receptor without interference from receptor−receptor interactions. The receptor-functionalized thiols were diluted with simple octanethiol in the monolayer to minimize interaction of receptor molecules with each other on the surface. In their most extended conformations, receptor **9** measures ca. 20 Å in width. Assuming a hexagonal close-packed array of receptors (radius, ca. 10 Å) over hexagonal close-packed thiolate on gold (radius ca. 5.0 Å), approximately 12 unfunctionalized thiols would be obscured under the umbrella of each receptor-functionalized thiol.

Self-assembled monolayers containing receptors and octanethiols were formed by immersion of thin films of evaporated gold into an ethanolic solution of receptor-functionalized thiol (**9**) (1 mM) and simple alkanethiol diluent (18 mM) for 3−12 h. The exact ratio of alkanethiolates to receptors on the monolayer surfaces was not determined. To remove any physisorbed materials, the samples were then rinsed with ca. 10 mL of anhydrous ethanol and dried with a stream of dry nitrogen. The monolayers formed in this manner are stable to a variety of solvents.[38] To minimize air oxidation of the monolayer, monolayers were stored in degassed solvent under inert atmosphere.[39] In later studies, mixed monolayers were formed from solution mixtures of receptor and longer-chain alkanethiol (C_{12}, and C_{18}) or hydroxyl-terminated alkanethiol chains to study the surface environment effects on the recognition process (see below).

Surface Fluorescence Experiments. We have found that fluorescence spectroscopy is a highly effective technique for the study of thin films. Several studies of fluorescent probes on self-assembled monolayers have been reported.[40] In addition to the work presented here, other examples of host−guest interactions on monolayer surfaces has been shown using fluorescence spectroscopy.[41]

(36) To establish the 1:1 stoichiometry in the complex, we performed two sets of titration experiments using both the nonalkylated (**8**) and alkylated (**9**) hosts with the barbiturate guest **2a** (Figure 3). In each set of experiments, aliquots of the barbiturate (0.33 equiv) were added to 1 mM solutions of the host in $CDCl_3$ solvent. The changes in the chemical shift of amide and amine protons for host **8** and imide protons for guest **2a** were followed carefully. For the host **8,** in $CDCl_3$, the amide protons of the isophthalate appear at 8.40 ppm and the amine protons at 4.36 ppm. In the same solvent, the imide protons of the barbiturate **2a** resonate at 7.79 ppm. A stoichiometry-dependent deshielding of all of the indicated signals was observed during the titration. Upon addition of 1 equiv of **2a** to a solution of **8** at room temperature, a downfield shift for both sets of protons in **8** is observed, to 9.97 and 5.49 ppm, respectively. However, the imide protons of **2a** showed a very broad signal at 12−13 ppm, which was difficult to distinguish from the baseline. Addition of excess of barbiturate **2a** did not result in further deshielding of the indicated signals, thus confirming the 1:1 stoichiometry.

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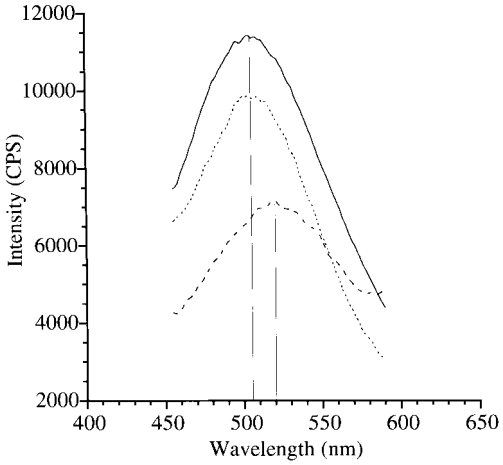

Figure 4. Fluorescence emission spectra of monolayer surfaces with free receptors (**1a**) (—), after complexation with barbiturate **2a** from dichloromethane (**3a**) (- - -), and after decomplexation using ethanol (•••).

Table 2. Surface Fluorometric Analysis for Complex Formation of Monolayer **1a** with Ligand **2a** from Different Solvents

solvent[a] used for complexation	emission wavelength (nm)[b]		
	1a	**3a**	$\Delta\lambda$[c]
CH_2Cl_2	506	520	14
CH_3CN	506	518	12
20% H_2O in CH_3CN	506	516	10
CH_3CH_2OH	506	506	0

[a] All solvents were of spectrophotometric grade. [b] Excitation wavelength = 335 ± 1 nm for all cases. [c] Shifts ($\Delta\lambda$) to longer wavelength are reported as positive numbers.

Fluorometric experiments on the monolayer surfaces reported here were conducted in the front-face mode, with samples held in the sample compartment in air and positioned normal to the incident beam. This geometry proved to be very useful due to the ease of alignment of the sample and greater reproducibility in the fluorescence spectra. The detector was set at 10−15° off of normal to the plane of the monolayer. The fluorescence emission spectra of monolayer **1a** containing receptor **9** were surprisingly similar to those of **8** or **9** in solution. Excitation at 335 nm gave rise to a fluorescence emission at 506 nm for monolayer **1a** (Figure 4) compared to 499 nm for **9** in dichloromethane. As expected, the 10 carbon atom chain that linked the fluorophore to the gold surface served to insulate the fluorophore from the metal and to minimize quenching of the excited state by the gold.

Following examination of the fluorescence of monolayer **1a**, the interactions of this monolayer with solutions of **2** were examined. Formation of complexed monolayer **3a** was achieved by immersing **1a** into 1 mM solutions of **2a** in dichloromethane, acetonitrile, or ethanol for a minimum of 5 min (Table 2). The self-assembled monolayer was then removed from solution and treated in one of several ways. Direct air-drying of the sample without any rinsing led to the formation of a physisorbed multilayer of **2a** on the monolayer surface. This film exhibited spectroscopic properties similar to those of a thin film of **2a** on gold (emission at 485 nm with excitation at 370 nm). Gentle rinsing of capped monolayer with a stream of ca. 5 mL of dichloromethane effectively removed the noncomplexed ligand. The fluorescence spectrum of the monolayer was again obtained. The capped monolayer **3a** showed an emission that had been shifted substantially to longer wavelength from 506 nm for **1a** to 520 nm to **3a** (Figure 4). This 14-nm bathochromic shift is consistent with the $\Delta\lambda$ observed in the solution experiments and is indicative of the formation of **3a**. Similar behavior was observed when complexation experiments were carried out using anhydrous acetonitrile as the solvent. In analogy to the solution complexation experiments, treatment of **1a** with ethanolic solutions of **2a** gave monolayers that did not show any shift in the wavelength of maximum fluorescence emission. Interestingly, when monolayer **1a** was capped from a 1 mM solution of **2a** in 20% water in acetonitrile, the capped monolayer **3a** showed an emission band at 516 nm.

The small $\Delta\lambda$ of 4 nm (Table 1) exhibited in solution titration of **8** after addition of **2a** in ethanol suggested that the receptor−ligand interaction in this solvent is relatively weak. This result further suggests that treatment of capped monolayer **3a** with ethanol might reverse the complexation, leading to the formation of **1a**. Thus, after treatment of capped monolayer **3a** with ethanol, the fluorescence maximum of the monolayer returned to 506 nm (Figure 4), the wavelength of emission of **1a**. Extensive rinsing (>30 mL of ethanol) was required to achieve complete removal of the ligand as evidenced by a restoration of the fluorescence emission to the 506 nm of monolayer **1a**. Short rinse cycles lead to peak broadening and a blue shift in the emission spectra, which was attributed to the partial removal of ligand **2a**. Two derivatives of the cinnamaldehyde barbiturates were also examined. Complexation with both electron-rich (**2c**) and electron-poor (**2d**) ligand derivatives gave 15- and 14-nm red shifts, respectively, in the fluorescence emission of the receptor on the monolayer surface.

To confirm that barbiturate ligand **2a** interacts with monolayer **1a** through hydrogen bonding, the *N*-methylated barbiturate derivative **2b** was examined. Treatment of monolayer **1a** with solutions of *N*,*N*-dimethylbarbiturate **2b** in several solvents resulted in no $\Delta\lambda$, suggesting that the interaction of monolayer **1a** with barbiturate ligand **2a** occurs via hydrogen bonding. Furthermore, identical capping procedures with gold substrate and octanethiol monolayer followed by a dichloromethane rinse resulted in materials that gave no fluorescence emissions, confirming that there was little or no interaction between the barbituric ligand with the simple alkanethiol monolayer.

The fluorescence emission of monolayer **1a** showed a modest dependence on the treatment of the monolayer prior to the fluorescence experiment. Typically, the monolayers were rinsed with ethanol after adsorption and prior to fluorescence examinations. However, rinsing the monolayer with dichloromethane instead of ethanol, followed by air-drying (ca. 3 min) gave a fluorescence emission at 501 nm. This 5-nm blue shift, relative to emission after ethanol treatment, is attributed to the displacement of ethanol by dichloromethane in the binding pocket of the receptor and hence disruption of ethanol/receptor hydrogen bonding. This effect is even more pronounced when the monolayers were exposed to high vacuum for 2 h, at which they showed fluorescence emission at 496 nm.

To examine the selectivity of receptor functionalized monolayer **1a**, a series of competitive complexation experiments were conducted. Treatment of monolayer **1a** with solutions of nonbarbiturate ligands, such as 2-imidazolidone, resulted only in a minor change in the fluorescence emission of the receptor (2−3-nm red shift), suggesting weak interaction between the **1a** and 2-imidazolidone. Competitive capping experiments from a solution of **2a** and 2-imidazolidone each at 1 mM concentration in dichloromethane showed preferential binding of the barbiturate ligand over the cyclic urea, based on the large $\Delta\lambda$ values (14 nm) observed for the capped monolayer. Similar results were also observed when the complexation was attempted from 1:10 solution of **2a** and 2-imidazolidone in dichloro-

methane at concentrations of 1 and 10 mM for the two ligands, respectively.

Binding Constant Measurements. The binding constant K_b for the complexation of the barbiturate ligands (**2**) to the receptor on a monolayer surface is defined according to eq **1** as

$$K_b = \frac{[\text{capped}]}{[\text{free}][\text{ligand}]} \quad (1)$$

where the concentrations of capped receptor and free receptor are defined as the numbers of complexed receptors and free receptors on the monolayer surface per unit area and the concentration of ligand is the concentration of the ligand solution used for complexation. Thus, when the populations of capped and uncapped receptors are equal, the binding constant for the receptor is reduced to the reciprocal of the concentration of ligand in solution used for complexation. We hoped to determine the fraction of bound receptors in the monolayer following treatment with ligand solution by examination of the fluorescence spectra. However, due to the broadness of the emission bands for both capped and uncapped receptors and the relatively small $\Delta\lambda$, direct integration of the fluorescence signals for the capped and uncapped receptors is not a viable method for determination of the fraction of receptors that are occupied. We could, however, obtain the desired information indirectly by comparing fluorescence intensities at two points above and below the average of the emission of the capped and uncapped monolayers (513 nm). Assuming the receptors on monolayer surface are fully occupied when the monolayer is capped from a 1 mM solution of **2a** in dichloromethane (since higher concentrations and longer complexation times did not cause any further changes), lower concentrations of **2a** should result in a partial occupation of the receptors on the monolayer surface. Therefore, it was envisioned that, through a series of capping experiments with solutions of ligand at lower concentrations, the partial capping of receptors could be achieved. We further observed, to a first approximation, that band shapes of the fluorescence emissions of capped and uncapped receptors were similar. Thus, on the basis of these observations, we were able to conclude that the reciprocal of the concentration of the ligand used for complexation at which the ratio of intensities at 471 and 555 nm is equal to 1 is the binding constant of the receptor on the monolayer surface.

Monolayers capped from solutions containing ligand **2a** at concentrations from 10^{-6} to 10^{-3} M were examined. The samples of monolayer **1a** were immersed in the solutions of **2a** over the indicated range of concentrations for at least 5 min. The samples were then removed from the solutions and rinsed rapidly with ca. 1 mL of dichloromethane. We had shown previously that even prolonged rinsing of capped monolayers with dichloromethane did not result in significant changes in the fluorescence spectra of the samples. Thus we were confident that brief rinsing of the samples capped from solutions of **2a** at varying concentrations would allow us to take a "snapshot" of the monolayer. Prior to complexation, monolayers showed a fluorescence intensity ratio of 1.17 for the signal at 471 nm over 555 nm. For the fully capped monolayer **3a** the same ratio is 0.78. Monolayers capped from ligand solutions of 10^{-4} M concentrations showed a fluorescence intensity ratio of about 1.0 for the signal at 471 nm over 555 nm. Consequently, the binding constant of ligand **2a** to the receptor on the monolayer surface from dichloromethane was estimated to be 10^4 M^{-1}. This value is in agreement with Hamilton's reported value for the acyclic receptor of this type in chloroform.[33a] Capping experiments with more dilute solutions of ligand led to very minor changes in the fluorescence emission of the receptor as no significant binding took place.

Table 3. Surface Fluorometric Analysis of Monolayer **1a**−**d** Interactions with Ligand **2a**

monolayers used for complexation	emission wavelength (nm)[a]		
	before capping	after capping	$\Delta\lambda$[b]
1a	506	520	14
1b	507	517	10
1c	509	513	04
1d	523	523	00

[a] Excitation wavelength = 335 ± 1 nm for all cases. [b] Shifts ($\Delta\lambda$) to longer wavelength are reported as positive numbers.

Receptor Environment and Recognition of Ligand. To examine the way in which the surface environment affects the recognition processes, mixed monolayers were formed from solution mixtures of **9** and simple alkanethiols of different chain lengths: octanethiol monolayer (**1a**), dodecanethiol monolayer (**1b**), and octadecanethiol monolayer (**1c**). The deformation of the binding pocket of the receptor on the monolayer surface, presumably due to steric crowding by the longer alkyl chains, appears to prevent the complete binding of the ligand to the receptors as evidenced by the decrease in $\Delta\lambda$ as a function of increasing chain length (Table 3).

To prove how the surface hydrophilicity affects the recognition process, mixed monolayers of receptor-functionalized alkanethiols and hydroxy-terminated thiols were formed. Monolayer **1d** was prepared by adsorption from ethanol containing **9** (1 mM) and 8-hydroxy-1-octanethiol (18 mM) (see the Experimental Section).

The initial effect of the hydrophilic surface was a dramatic change in the fluorescence emission spectra of the receptor on the monolayer surface. Due to interaction of the receptor with the hydroxylic surface, perhaps through hydrogen bonding, excitation of monolayer **1d** at 335 nm gave rise to emission at 523 nm, a 17-nm red shift relative to monolayer **1a**. The corruption of the binding site by interactions with the surface through hydrogen bonding resulted in very small changes in the fluorescence emission of the monolayer **1d** after monolayer capping attempts. Again, presumably due to hydrogen bonding with the surface and the collapse of the binding cavity, no appreciable binding was expected and practically no $\Delta\lambda$ was observed.

Complexation of Barbiturate Receptor on Monolayer Surface. We next examined the recognition of fluorescent diaminopyridine ligands by monolayers functionalized with barbiturates (Figure 5). These experiments served two purposes. First, they allowed us to show that the molecular recognition event we were studying would occur when the barbiturate component was covalently linked to the monolayer surface. Second, and perhaps more importantly, these experiments would allow us to establish that the fluorescence we were observing was due to the presence of fluorescent diaminopyridine on the monolayer and not due to the presence of some other fluorescing species or the gold itself. Formation of mixed monolayers of **14** and octanethiol on gold was achieved by immersing gold substrates in 1:18 solution mixtures of **14** and octanethiol in ethanol. As expected, no fluorescence was observed for these monolayers when they were excited at 335 nm. However, after treating monolayer **19** with a 1 mM solution of **8** in dichloromethane, the monolayers showed a fluorescence emission band corresponding to that of **8** on surface (510 nm). The reversible removal of the cap was achieved by rinsing the monolayer sample with ethanol. This cycle could be repeated at least four times with almost identical results. When treated

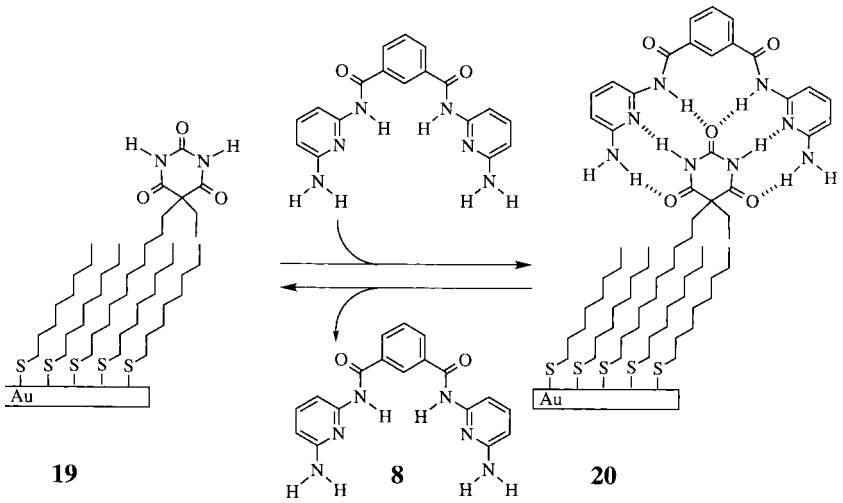

Figure 5. Molecular recognition of nonfluorescent barbiturate receptor-functionalized alkanethiol with the fluorescent ligand bis(diaminopyridine) amide of isophthalic acid on the surface of a self-assembled thiolate monolayer.

the same way, neither gold samples nor octanethiol monolayers resulted in any fluorescence signal. With this set of experiments we were able to demonstrate, beyond a reasonable doubt, that a reversible binding occurs between these pairs of ligands and receptors. Presence and absence of the fluorescent signal on monolayer **20** is the key evidence for such complexation.

Conclusions

Molecular recognition of a series of barbiturate ligands, from solution, by a receptor functionalized self-assembled monolayer of alkanethiolates on gold is shown. As demonstrated here, well-organized self-assembled monolayers of alkanethiols on gold films can be used as model systems for the study of interfacial recognition events in the context of membrane mimics. Such systems afford models which permit for control and variation of the surface environment properties. In addition, such functionalized surfaces can be used for selective and specific detection of compounds in drug-screening processes. We are currently developing new functionalized monolayers for a range of applications.

Experimental Section

General Methods. All reagents were purchased from Aldrich Chemical Co., Inc., and were used without further purification unless otherwise noted. Diethyl ether and tetrahydrofuran were freshly distilled from deep blue solutions of sodium benzophenone ketyl. Dichloromethane, toluene, and triethylamine were freshly distilled from calcium hydride. Methanol was distilled from magnesium/magnesium oxide.

All reactions were carried under a positive pressure of argon. Chromatography was carried out according to the method of Still[42] using Fisher silica gel (230−425 mesh). Analytical thin-layer chromatography was performed using 0.25-mm silica gel plates (Baker, F_{254}). For organic reactions, a standard workup procedure of quenching with 1 M $NaHCO_3$, extracting three times with ethyl acetate, followed by sequential treatment of the organic extracts with brine and anhydrous $MgSO_4$ was used. After filtration, all samples were concentrated in vacuo. All melting points are uncorrected.

NMR spectra were recorded using a AF 200 or AM 360 Bruker spectrometer. All spectra were acquired in $CDCl_3$ (99.8% D, Cambridge Isotope Laboratories, Woburn, MA) unless otherwise indicated. Chemical shifts (δ) are in parts per million (ppm) relative to $SiMe_4$, and coupling constants (J values) are in hertz.

Fluorescence Spectroscopy. Fluorescence measurements were performed with a SPEX Fluorolog 212 fluorometer equipped with dM3000 software. Spectra were collected at room temperature using excitation and emission slit widths of 4 and 8 mm, respectively. The data were collected over a 1 s/nm scan time in the single photon counting mode. The spectra were recorded in the right-angle mode for solution fluorometric experiments and in the front-face mode for the surface fluorometric experiments. Spectra are not corrected.

Preparation of Gold Surface. Silicon wafers (100, Silicon Sense, Inc., Nashua, NH) were precoated with 100 Å of chromium to promote adhesion of gold, followed by 1000 Å of gold. Silicon wafers were used as supplied by the manufacturer. Both chromium and gold were evaporated at 4×10^{-6} Torr from a resistively heated tungsten rod for Cr and resistively heated tungsten boat for Au. The thicknesses of the deposited metallic films were measured by the change in frequency of oscillation of a quartz crystal.

Preparation of Monolayers. Gold-coated silicon samples were immersed in anhydrous ethanolic solutions of (**9** or **14**) (1 mM) and alkanethiols (18 mM) for at least 3 h. To remove any solution-deposited material, the slides were then rinsed with ca. 10 mL of anhydrous ethanol and dried with a stream of dry nitrogen. Some samples were dried under high vacuum (0.05 Torr) for 2 h to remove solvent.

Capping and Uncapping the Monolayer. Capping the mixed monolayer receptor (**1** or **19**) was achieved by immersion of the prepared monolayer slide in a solution of the ligand (**2** or **8**) in either dichloromethane, acetonitrile, or a mixture of water and acetonitrile for 5 min. To remove weakly bound material, the capped monolayers were rinsed with dichloromethane (ca. 20 mL) and dried with a stream of dry nitrogen. Uncapping the capped monolayer was only possible by an extensive ethanol rinse (ca. 30 mL).

5-Cinnamylidenepyrimidine-2,4,6-(1*H*,3*H*,5*H*)-trione (2a). This compound was synthesized from barbituric acid according to the procedure by Wood and co-workers:[43] mp >250 °C; UV−vis (CH_2Cl_2) λ_{max} 383 nm (ϵ 4.7 × 10^7); ^{1}H NMR (360 MHz, acetone-d_6) δ 7.49 (m, 3 H), 7.66 (d, 1 H, J = 15.5 Hz), 7.73 (dd, 2 H); HRMS (EI) calcd for M^+ $C_{13}H_{10}N_2O_3$ 242.069 071, found 242.069 142.

5-Cinnamylidenepyrimidine-2,4,6-(1*CH*$_3$,3*CH*$_3$,5*H*)-trione (2b). To a solution of 1,3-dimethylbarbituric acid (2.0 g, 12.8 mmol) in 70 mL of 10% HCl at room temperature was added *trans*-cinnamaldehyde (1.86 g, 14.1 mmol). After 2 h of stirring at room temperature, the reaction was extracted with ethyl acetate (ca. 500 mL). The organic extracts were washed with 1 M $NaHCO_3$ followed by brine and dried over anhydrous $MgSO_4$. After filtration, the solvent was removed in vacuo to leave 3.26 g (94.5%, 12.1 mmol) of crude product containing a small amount of cinnamaldehyde. Analytical samples of this material could be prepared by flash chromatography (silica gel, 1:3 ethyl

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acetate/hexanes): mp 183.0−188.5 °C; ^{1}H NMR (360 MHz, acetone-d_6) δ 3.28 (s, 3 H), 3.29 (s, 3 H), 7.49 (m, 3 H), 7.67 (d, 1 H, J = 15.44 Hz), 7.75 (m, 2 H), 8.13 (dd, 1 H, J = 11.95, 0.80 Hz), 8.63 (dd, 1 H, J = 15.51, 11.92 Hz).

5-(*o*-Methoxycinnamylidene)pyrimidine-2,4,6-(1*H*,3*H*,5*H*)-trione (2c). Barbituric acid (0.20 g, 1.56 mmol) was dissolved in 50 mL of 10% HCl. After 10 min of stirring at room temperature, 2-methoxycinnamaldehyde (0.24 g, 1.48 mmol) was added to the clear solution. After 20 min, an orange precipitate started to form. The reaction was then stirred at room temperature for 2 days. The reaction was stopped by extracting with ethyl acetate (ca. 500 mL). The organic extracts were washed with brine and dried over anhydrous $MgSO_4$. After filtration, the solvent was removed in vacuo and the residue was stirred in a hexanes/ethyl acetate (95:5) mixture overnight. After filtration, the orange residue was rinsed with ethanol and dried under high vacuum to give 350 mg (86.9%, 1.28 mmol) of pure product: mp > 250 °C; ^{1}H NMR (360 MHz, acetone-d_6) δ 3.96 (s, 3 H), 7.06 (dd, 1 H, J = 7.3, 7.3 Hz), 7.12 (d, 1 H, J = 8.4 Hz), 7.47 (ddd, 1 H, J = 7.4, 7.4, 1.7 Hz), 7.74 (dd, 1 H, J = 7.8, 1.6 Hz), 7.86 (d, 1 H, J = 15.6 Hz), 8.09 (d, 1 H, J = 12.2 Hz), 8.65 (dd, 1 H, J = 15.7, 12.1 Hz), 9.97 (br s, 1 H), 10.03 (br s, 1 H).

5-(*p*-Nitrocinnamylidene)pyrimidine-2,4,6-(1*H*,3*H*,5*H*)-trione (2d). Barbituric acid (0.20 g, 1.56 mmol) was dissolved in 50 mL of 10% HCl. After 10 min of stirring at room temperature, 4-nitrocinnamaldehyde (0.26 g, 1.48 mmol) was added to the clear solution. After 5 min, a yellowish precipitate started to form. The reaction was stirred at room temperature overnight. The reaction mixture was filtered, and the yellowish residue was rinsed with ethanol (ca. 30 mL), followed by hexanes (ca. 50 mL). The residue was then stirred in 100 mL of hexanes overnight and filtered again. After drying under high vacuum, 250 mg (58.9%, 0.87 mmol) of pure product was isolated: mp > 250 °C; ^{1}H NMR (360 MHz, DMSO-d_6) δ 7.79 (d, 1 H, J = 15.6 Hz), 7.85 (d, 2 H, J = 8.9 Hz), 7.99 (d, 1 H, J = 11.7 Hz), 8.29 (d, 2 H, J = 8.7 Hz), 8.53 (dd, 1 H, J = 15.6, 11.8 Hz), 11.30 (br s, 1 H), 11.34 (br s, 1 H).

1-Bromo-9-decene. 9-Decen-1-ol (13.14 g, 84 mmol) was dissolved in 100 mL of dry diethyl ether, and the solution was cooled to 0 °C in an ice bath. To this solution was then added dropwise PBr_3 (11.38 g, 42 mmol). The solution was stirred at 0 °C for 2 h and then at room temperature overnight. The reaction was quenched by the slow addition of methanol (ca. 50 mL). The mixture was then washed with a 1 M solution of $NaHCO_3$ (ca. 100 mL) and brine, dried over anhydrous $MgSO_4$, and then filtered. The solvent was removed in vacuo to leave a light yellow oil. Flash chromatography (silica gel, hexane) yielded 9.64 g (52%, 44 mmol) of pure product. A second impure fraction was also obtained. This fraction was chromatographed as before to afford an additional 7.67 g (40%, 35 mmol) of product. The combined yield of 17.31 g (92%, 79 mmol) of product was obtained: ^{1}H NMR (360 MHz) δ 1.29 (m, 6 H), 1.39 (m, 4 H), 1.83 (m, 2 H), 2.03 (m, 2 H), 3.39 (t, 2 H, J = 6.1 Hz), 4.95 (m, 2 H), 5.78 (ddt, 1 H, J = 16.9, 13.3, 6.6 Hz); ^{13}C NMR (90 MHz) δ 28.78, 29.34, 29.49, 29.62, 29.90, 33.45, 34.39, 34.51, 114.80, 139.67.0.

Diethyl 5-Hydroxyisophthalate (5). 5-Hydroxyisophthalic acid (**4b**) (10.00 g, 55 mmol) was dissolved in 150 mL of anhydrous ethanol. To that solution was added 3 mL of 18 M H_2SO_4, and the solution was heated at reflux for 48 h. The reaction was quenched with a 1 M solution of $NaHCO_3$ (ca. 50 mL) and extracted with ethyl acetate. The extracts were washed with brine and dried over anhydrous $MgSO_4$ and then filtered. The solvent was then removed in vacuo to afford **5** as a slightly yellow crystalline solid. This solid was washed with cold hexanes to remove any colored impurities and dried under high vacuum to afford 12.50 g (95.65%, 52.5 mmol) of pure product: mp 104.7−105.1 °C; ^{1}H NMR (200 MHz) δ 1.40 (t, 6 H, J = 7.1 Hz), 4.40 (q, 4 H, J = 7.2 Hz), 7.05 (br s, 1 H), 7.81 (s, 2 H), 8.22 (s, 1 H); ^{13}C NMR (90 MHz) δ 14.81, 62.38, 121.68, 123.06, 132.52, 157.36, 167.00.

Diethyl 5-(9-Decenoxy)isophthalate (6). Diethyl 5-hydroxyisophthalate (**5**) (5.00 g, 21 mmol) was dissolved in 30 mL of dry THF. To that solution was added 0.798 g of 60% NaH in mineral oil (20 mmol). The mixture was stirred for 10 min. To the above solution were added 1-bromo-9-decene (3.15 g, 14.8 mmol) and tetrabutylammonium iodide (0.27 g, 0.74 mmol), and the mixture was heated at reflux overnight. The reaction was quenched by addition of 10% HCl (ca. 20 mL) and then washed with a 1 M solution of $NaHCO_3$ (ca. 50 mL) and extracted with ethyl acetate. The organic extracts were washed with brine and dried over anhydrous $MgSO_4$ and then filtered and concentrated in vacuo. The residue was purified by flash chromatography (silica gel, 1:3 ethyl acetate/hexanes) to give 4.91 g (91.6%, 13.2 mmol) of pure **6**: UV−vis (CH_2Cl_2) λ_{max} 230 nm (e 4.0 × 10^8); ^{1}H NMR (200 MHz) δ 1.32 (m, 10 H), 1.40 (t, 6 H, J = 7.2 Hz), 1.78 (m, 2 H), 2.01 (m, 2 H), 4.01 (t, 2 H, J = 6.4 Hz), 4.37 (q, 4 H, J = 7.2 Hz), 4.93 (m, 2 H), 5.80 (ddt, 1 H, J = 16.8, 10.2, and 3.2 Hz), 7.71 (s, 2 H), 8.23 (s, 1 H); ^{13}C NMR (50 MHz) δ 14.28, 25.93, 28.87, 29.01, 29.08, 29.26, 29.35, 33.75, 61.29, 68.53, 114.14, 119.64, 122.63, 131.99, 139.07, 159.13, 165.73.

Diethyl 5-[10-(Thioacetyl)decanoxy]isophthalate (7). Diethyl 5-(9-decenoxy)isophthalate (**6**) (1.0 g, 2.66 mmol) was dissolved in 10 mL of degassed toluene. To this solution was added thioacetic acid (0.404 g, 5.32 mmol) followed by a catalytic amount of azobisisobutyronitrile (AIBN) (0.022 g, 0.133 mmol, Alfa Research Chemicals and Accessories, Ward Hill, MA). The solution was then heated to reflux. After 20 h at reflux, the reaction was quenched by the addition of 1 M $NaHCO_3$ (ca. 20 mL) and extracted with ethyl acetate. The organic extracts were treated sequentially with 1 M NaOH, brine, and anhydrous $MgSO_4$. After filtration, the solvent was removed in vacuo. The residue was purified by flash chromatography (silica gel, 1:10 ethyl acetate/hexanes) to give 0.80 g (66.5%, 1.77 mmol) of pure **7**: mp 46.2−46.8 °C; ^{1}H NMR (360 MHz) δ 1.29 (m, 12 H), 1.40 (t, 6 H, J = 7.2 Hz), 1.58 (m, 2 H), 1.80 (m, 2 H), 2.31 (s, 3 H), 2.86 (t, 2 H, J = 7.3 Hz), 4.03 (t, 2 H, J = 6.5 Hz), 4.39 (q, 4 H, J = 7.2 Hz), 7.73 (s, 2 H), and 8.25 (s, 1 H); ^{13}C NMR (90 MHz) δ 14.29, 25.93, 28.75, 29.04, 29.10, 29.26, 29.34, 29.41, 29.45, 30.61, 61.32, 68.53, 119.65, 122.63, 131.98, 159.12, 165.78, 196.03.

***N*,*N*-Bis(6-aminopyridyl)-5-(10-thiodecanoxy)isophthaloyl Diamide (9).** To a solution of 2,6-diaminopyridine (recrystallized from hot ethyl acetate) (0.506 g, 4.65 mmol) in 10 mL of THF at −78 °C was added a 2.0 M solution of *n*-BuLi (2.28 mL, 4.57 mmol) in hexane. After 10 min at −78 °C, a solution of diethyl 5-(10-thioacetyldecanoxy)-isophthalate (**7**) (0.30 g, 0.66 mmol) in 5 mL of THF was added. The reaction mixture was stirred for 7 h at −78 °C and then gradually warmed to room temperature and stirred overnight. The reaction was then quenched with a 1 M solution of $NaHCO_3$ (ca. 50 mL) and extracted with ethyl acetate. The organic extracts were washed with water and then brine and dried over anhydrous $MgSO_4$. After filtration, the solvent was removed in vacuo and the residue was purified using flash chromatography (silica gel, 2:1 ethyl acetate/hexane) to give 0.25 g (70.3%, 0.47 mmol) of pure **8**: mp 116.5−117.3 °C; UV−vis (CH_2Cl_2) λ_{max} 231 nm (ϵ 3.5 × 10^7); ^{1}H NMR (360 MHz, acetone-d_6) δ 1.36 (m, 11 H), 1.57 (m, 4 H), 1.80 (m, 2 H), 2.48 (dt, 2 H, J = 7.4, 7.4 Hz), 4.11 (t, 2 H, J = 6.4 Hz), 5.38 (s, 4 H), 6.32 (d, 2 H, J = 7.4 Hz), 7.43 (dd, 2 H, J = 7.9, 7.9 Hz), 7.59 (d, 2 H, J = 7.4 Hz), 7.70 (s, 2 H), 8.22 (s, 1 H), 9.38 (s, 2 H); ^{13}C NMR (90 MHz, acetone-d_6) δ 24.53, 26.39, 28.74, 29.53, 29.98, 34.58, 68.85, 102.76, 104.67, 117.65, 118.61, 137.08, 139.75, 151.20, 159.20, 160.19, 165.05; HRMS (FAB) calcd for M^+ $C_{28}H_{36}N_6O_3S$ 537.2656, found 537.2648.

Diethyl (9-Decenyl)ethylmalonate (11). Diethyl malonate (**10**) (2.05 g, 10.96 mmol) was dissolved in 10 mL of dry THF. To this solution was added 0.40 g of 60% NaH in mineral oil (10.0 mmol). The mixture was stirred for 30 min. To the above solution were added 1-bromo-9-decene (2.00 g, 9.13 mmol) and tetrabutylammonium iodide (30 mg, 0.08 mmol), and the mixture was heated at reflux for 2 days. The reaction was quenched by the addition of $NaHCO_3$ (ca. 50 mL) and extracted with ethyl acetate. The organic extracts were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (silica gel, 1:10 ethyl acetate/hexanes) to give 2.71 g (91.0%, 8.31 mmol) of pure **11**: ^{1}H NMR (360 MHz) δ 0.78 (t, 3 H, J = 7.5 Hz), 1.21 (t, 6 H, J = 7.1 Hz), 1.24 (m, 12 H), 1.82 (m, 2 H), 1.89 (q, 2 H, J = 7.6 Hz), 1.99 (m, 2 H), 4.14 (q, 4 H, J = 7.1 Hz), 4.93 (m, 2 H), 5.77 (ddt, 1 H, J = 16.9, 13.3, 6.6 Hz); ^{13}C NMR (90 MHz) δ 9.02, 14.72, 24.44, 25.75, 29.52, 29.69, 29.90, 29.98, 30.45, 32.17, 34.40, 58.54, 61.49, 114.74, 139.76, 172.54.

5-Ethyl-5-decenylpyrimidine-2,4,6-(1*H*,3*H*,5*H*)-trione (12). Finely ground urea (2.76 g, 46 mmol) was dissolved in 10 mL of dimethyl sulfoxide. To this solution was added sodium hydride (0.44 g, 18.3 mmol), at which time the solution became turbid. After 10 min, the solution became clear again and diethyl (9-decenyl)ethylmalonate (**11**) (1.50 g, 4.6 mmol) was added. The mixture was stirred at room temperature for 3 days. The reaction was quenched by pouring over an ice−water slurry (ca. 50 mL) which was then acidified with a 10% HCl (ca. 10 mL) mixture and was extracted with ethyl acetate. The organic extracts were washed with brine and dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (silica gel, 1:3 ethyl acetate/hexanes) to give 0.42 g (31.1%, 1.43 mmol) of pure **12**: mp 74.5−77.0 °C; ^{1}H NMR (360 MHz) δ 0.88 (t, 3 H, J = 7.2 Hz), 1.23 (m, 10 H), 1.33 (m, 2 H), 1.99 (m, 4 H), 2.04 (q, 2 H, J = 7.5 Hz), 4.94 (m, 2 H), 5.78 (ddt, 1 H, J = 16.9, 13.3, 6.6 Hz), 8.79 (br s, 2 H); ^{13}C NMR (90 MHz) δ 10.12, 25.85, 29.51, 29.66, 29.78, 29.93, 30.10, 33.19, 34.43, 39.49, 58.13, 114.87, 139.81, 150.25, 173.37.

5-Ethyl-5-[10-(thioacetyl)decyl]pyrimidine-2,4,6-(1*H*,3*H*,5*H*)-trione (13). 5-Ethyl-5-decenylpyrimidine-2,4,6-(1*H*,3*H*,5*H*)-trione (**12**) (0.23 g, 0.80 mmol) was dissolved in 1 mL of $CDCl_3$ in an NMR tube. To this solution was added thioacetic acid (57 mL, 0.80 mmol). This reaction mixture was irradiated for 2 h with a 450-W medium-pressure mercury lamp at a distance of 2 in. The reaction mixture was then concentrated in vacuo and kept under high vacuum for several hours to give 0.29 g (97.%, 0.78 mmol) of pure product: mp 108.5−110.2 °C; ^{1}H NMR (360 MHz) δ 0.88 (t, 3 H, J = 7.4 Hz), 1.22 (m, 14 H), 1.54 (m, 2 H), 1.97 (m, 2 H), 2.04 (q, 2 H, J = 7.5 Hz), 2.32 (s, 3 H), 2.85 (t, 2 H, J = 7.4 Hz), 8.75 (br s, 2 H); ^{13}C NMR (90 MHz) δ 10.19, 25.77, 29.38, 29.68, 29.81, 29.91, 30.00, 30.12, 31.31, 33.16, 39.44, 58.08, 149.82, 173.51, 197.05.

5-Ethyl-5-(10-mercaptodecyl)pyrimidine-2,4,6-(1*H*,3*H*,5*H*)-trione (14). To 10 mL of methanol at 0 °C was added acetyl chloride (1.0 mL). After 10 min at 0 °C, 5-ethyl-5-(10-thioacetyldecyl)-pyrimidine-2,4,6-(1*H*,3*H*,5*H*)-trione (**13**) (0.10 g, 0.28 mmol) was added to this solution. The resulting mixture was stirred at 0 °C for 3 h, gradually warmed to room temperature, and stirred overnight. The reaction was stopped by addition of 50 mL of degassed distilled water. The reaction mixture was then extracted with ethyl acetate. The organic extracts were washed with brine, dried over anhydrous $MgSO_4$, filtered, and finally concentrated in vacuo. The residue was purified by flash chromatography (silica gel, 1:3 ethyl acetate/hexanes) to give 80 mg (88.8%, 0.25 mmol) of pure **14**: mp 98.6−101.0 °C; UV−vis (CH_2Cl_2) λ_{max} 232 nm (e 1.2 × 10^6); ^{1}H NMR (360 MHz) δ 0.88 (t, 3 H, J = 7.4 Hz), 1.22 (m, 12 H), 1.31 (t, 1 H, J = 7.8 Hz), 1.37 (m, 2 H), 1.59 (m, 2 H), 1.92 (m, 2 H), 2.04 (q, 2 H, J = 7.4 Hz), 2.50 (dt, 2 H, J = 7.4, 7.4 Hz), 8.66 (br s, 2 H); ^{13}C NMR (90 MHz) δ 10.11, 25.28, 25.82, 28.96, 29.62, 29.76, 29.99, 30.07, 33.14, 34.63, 39.42, 58.09, 150.09, 173.66; HRMS (FAB) calcd for M$^+$ $C_{16}H_{28}N_2O_3S$ 329.1899, found 329.1899.

8-(Acetylthio)-1-octanol. Potassium thioacetate (2.18 g, 19.14 mmol) was suspended in 35 mL of THF at room temperature for 15 min. To this suspension was added 8-bromo-1-octanol (2.0 g, 9.57 mmol). The reaction mixture was stirred at room temperature for 15 h. The reaction was then quenched by addition of 1 M $NaHCO_3$ (ca. 35 mL) and extracted with ethyl acetate. The organic extracts were washed with water, followed by a brine wash, and then dried over anhydrous $MgSO_4$. After filtration, the solvent was removed in vacuo and the residue was purified using flash chromatography (silica gel, 5% ethyl acetate in hexanes) to give 1.44 g (73.8%, 7.06 mmol) of pure product.

8-Hydroxy-1-octanethiol. 8-(Acetylthio)-1-octanol (1.0 g, 4.90 mmol) was added to 40 mL of dry HCl/methanol (2 mL of acetyl chloride in 38 mL of methanol) solution at 0 °C and was stirred for 3 h. After this time, the reaction was warmed to room temperature and was stirred overnight. The reaction was quenched with 1 M $NaHCO_3$ (ca. 25 mL) and extracted with ethyl acetate. The organic extracts were washed with water and then brine and dried over anhydrous $MgSO_4$. After filtration, the solvent was removed in vacuo and the residue was purified using flash chromatography (silica gel, 2:1 hexanes/ethyl acetate) to give 0.794 g (100%, 4.90 mmol) of pure products: ^{1}H NMR (360 MHz) δ 1.21 (m, 9 H), 1.48 (m, 4 H), 2.42 (dt, 2 H, J = 7.5, 7.2 Hz), 2.98 (br s, 1 H), 3.49 (t, 2 H, J = 6.6 Hz); ^{13}C NMR (90 MHz) δ 24.32, 25.39, 28.00, 28.74, 28.97, 32.32, 33.73, 62.24.

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