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Photocontrôle d'évènements de reconnaissance moléculaire au sein de récepteurs greffés sur des surfaces: vers des ardoises supramoléculaires

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Photocontrôle d'évènements de reconnaissance moléculaire au sein de récepteurs greffés sur des surfaces: vers des ardoises supramoléculaires

Des récepteurs de barbiturates greffés avec des groupements anthracène photoactifs possédant différentes fonctions d'ancrage ont été synthétisés et caractérisés, en vue de transférer leurs propriétés de reconnaissance photocontrôlable à des substrats par diverses techniques de modification, comme la formation de liaisons amide, de liaisons thioacétate, ou par réaction click. Les propriétés photophysiques et photochimiques de ces récepteurs ont été étudiées en solution, et la fluorescence, la durée de vie et le rendement quantique ont été mesurés à différentes températures. Des surfaces d'or modifiées ont été fabriquées et caractérisées par ellipsométrie, mesure d'angle de contact, AFM et PM-IRRAS. Les résultats montrent qu'il est possible de moduler les propriétés de reconnaissance moléculaire des récepteurs de manière réversible via une combinaison d'irradiation lumineuse (365 nm) et de chaleur (80 °C).

Photocontrol of recognition events in surface-bound anthracene gated receptors

Anthracene-appended photoactive barbiturate receptors possessing various anchoring groups are synthesized and characterized in view of transferring their photocontrolled binding properties onto substrates through various surface grafting techniques, such as amide bond formation, direct thioacetate linkage, or post click reaction modification. The photophysical and photochemical properties of the synthesized receptors were investigated in solution using variable temperature fluorescence, lifetime measurement and reaction quantum yield measurements. Receptor-modified gold substrates were characterized using ellipsometry, contact angle, AFM and PM-IRRAS experiments. The results showed that it is possible to reversibly modulate the binding properties of the anthracene-appended receptors through a combination of the irradiation with light (365 nm) and heat (80°C).

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Abbreviation

Chemical reagents

C ₆₀	fullerene
DMF	dimethylformamide
EDC	3-(dimethylamino)-1-ethylpropylcarbodiimide
NaAsc	sodium ascorbate
NHS	<i>N</i> -Hydroxysuccinimide
TBTA	(tris[(1-benzyl-1H-1,2,3-triazol-4-yl) methyl]amine
TMS	trimethylsilyl

Physical and chemical characteristics

AFM	atomic force spectroscopy
ATR	attenuated total reflection
CAM	contact angle measurements
CV	cyclic voltammetry
HPLC	high performance liquid chromatography
PM-IRRAS	polarization modulation infrared reflection-absorption spectroscopy
SAM	self-assembled monolayer
SECM	scanning electrochemical microscopy
TRES	time-resolved emission spectra
UV	ultra violet
XPS	X-ray photoelectron spectroscopy
E _a	activation energy
HH	head-to-head
HT	head-to-tail
K _{SV}	Stern-Volmer constant
Rxn	reaction
rt	room temperature
S _N 2	bimolecular nucleophilic substitution
T	temperature
Φ	quantum yield
Φ _F	fluorescence quantum yield
λ	wavelength
λ _{em}	emission wavelength
λ _{ex}	excitation wavelength
<i>k</i>	rate constant
<i>k_F</i>	rate constant of the fluorescence

k_{NR}	rate constant of non-radiative deactivation process
k_{IC}	rate constant of the internal conversion
k_{ISC}	rate constant of the intersystem crossing
k_{ex}	rate constant of the excimer formation
k_D	rate constant of the photodimer formation
τ	lifetime
θ_C	contact angle

Units

$\text{\AA}$	angstrom (10^{-10} m)
<i>cal</i>	calorie
d	day
g	gram
h	hour
L	liter
s	second
m	meter
mol	mole
M	molar
mM	10^{-3} M
nm	nanometer (10^{-9} m)
ns	nanosecond
μ M	10^{-6} M

Chapter 1. Introduction

1.1 Current studies of molecular recognition on substrates

Nanotechnology deals with natural and artificial structures on the nanometer scale, i.e. in the range from *ca.* 100 nm down to the nanometer. The properties of such small objects can be dramatically different when compared to their properties in the bulk.¹ One important characteristic of nanotechnology is the frequent need to precisely position molecules and/or nanoparticles on surfaces, so that they may be addressed and manipulated for the bottom-up construction of nanoscale devices. The interface chemistry of the nanostructures and substrates plays a very important role in the positioning and immobilization of these structures, and it is attractive to separate the interface design from the nanostructure fabrication in order to gain better control over the interfacial properties. This requires the development of versatile and generally applicable interface chemistry to manipulate the nanostructures and control their position.

There are two principal mechanisms for the adsorption of molecules on solid surfaces. If accumulation of adsorbates on the surface occurs on account of weak van der Waals forces, the adsorption is termed physisorption.² When the molecules are held on the solid surface by chemical bonds, the adsorption is termed chemisorption.² In physisorption, a surface does not necessarily show preference for particular adsorbates, and the process is generally reversible, whereas chemisorption is generally highly specific and irreversible. Furthermore, while physisorption may result in the formation of ordered or disordered single- or multi-molecular layers, chemisorption generally leads to a uni-molecular layer on the surface.

Ideally, parameters such as stoichiometry, binding strength and dynamics, packing density and order, and reversibility, should be controllable. Covalent immobilization does not generally fulfill these criteria due to the irreversible nature of covalent bonds. On the other hand, physisorption processes do not present the mechanical strength that is required for many applications (*e.g.* sensors) or in processes (*e.g.* electrode deposition and adherence), and may not be generally patternable. An alternative solution is to interpose a covalently-bound monolayer possessing specific interactions for a pre-determined constituent that would then serve as an adhesion promoter for a wide variety of active materials. Furthermore, in the event that such an adhesion layer can be externally addressed, then the resulting hierarchically organized architecture will be likewise addressable. More specifically, the adhesion layer could be composed of molecular host-guest pairs. Self-assembled monolayers (SAMs) on solid surfaces could then be used to covalently attach such receptors.

The fixing of a receptor to a substrate can lead to a multivalent arrangement of interaction sites. The density of these sites is an important parameter in the binding of complementary multivalent guests. Multivalency,^{3,4} which describes the interaction between multiple interacting sites on one entity with multiple interacting sites on another, is therefore a fundamental principle governing the stability and dynamics of such systems and offers the means to control the binding properties of an entity binding to a substrate. This control can be exerted by systematic variation and optimization of the number of interacting sites, the

intrinsic binding strength of an individual interacting pair, and the geometry of the multivalent building blocks.

Molecular recognition by synthetic receptors in solution has reached a high degree of complexity.^{5,6} However, for possible applications at the device level, such receptors need to be confined in space. Self-assembled monolayers of molecules on gold are relatively easy to obtain and generally have a high degree of molecular organization.^{7,8} The most-studied monolayers are those based on thiols as anchoring groups, although stable monolayers of dialkyl sulfides have also been reported.⁹⁻¹² To obtain devices for the transduction of molecular recognition into macroscopic properties, the self-assembly of receptor molecules, such as resorcin[4]arene,¹³⁻¹⁵ calix[4]arene,^{16,17} and carceplex derivatives¹⁶ on gold has been reported (Figure 1.1). Binding studies carried out with a quartz crystal microbalance and surface plasmon resonance proved that these monolayers are able to complex guest molecules.^{18,19} These were the early attempts to functionalize a surface with synthetic receptors able to display molecular recognition.

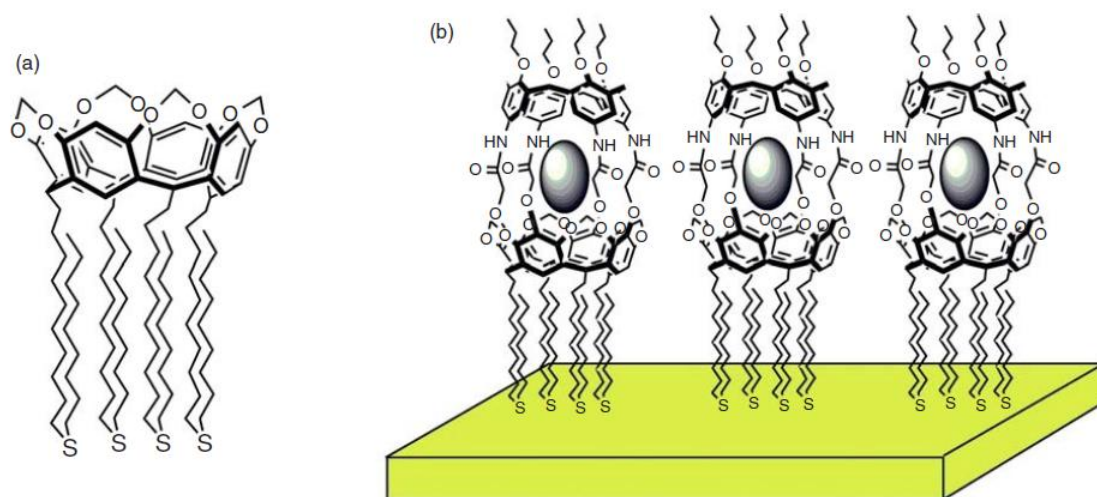


Figure 1.1 (a) Example of a cavitand adsorbate based on a resorcin[4]arene scaffold.¹⁶ (b) Schematic representation of a monolayer of carceplex molecules trapping a guest in their cavity.¹⁷ Adapted with permission from ref 41. Copyright 2011 Elsevier.

The Reinhoudt group (University of Twente) has extended this concept through the use of β -cyclodextrin as a receptor molecule for host-guest chemistry on surfaces as a form of molecular printing.²⁰⁻²⁵ The Dutch system is based on the interactions between calixarenes and hydrophobic guests to induce the assembly of functionalized dendrimers containing redox-active units, gold nanoparticles, or oligosaccharides (Figure 1.2, (a)).²⁵ Patterning is principally achieved by the micro-contact printing technique, though their work has also involved the use of scanning electrochemical microscopy (SECM) to direct desorption through electrostatic interactions (Figure 1.2, (b)).²⁶

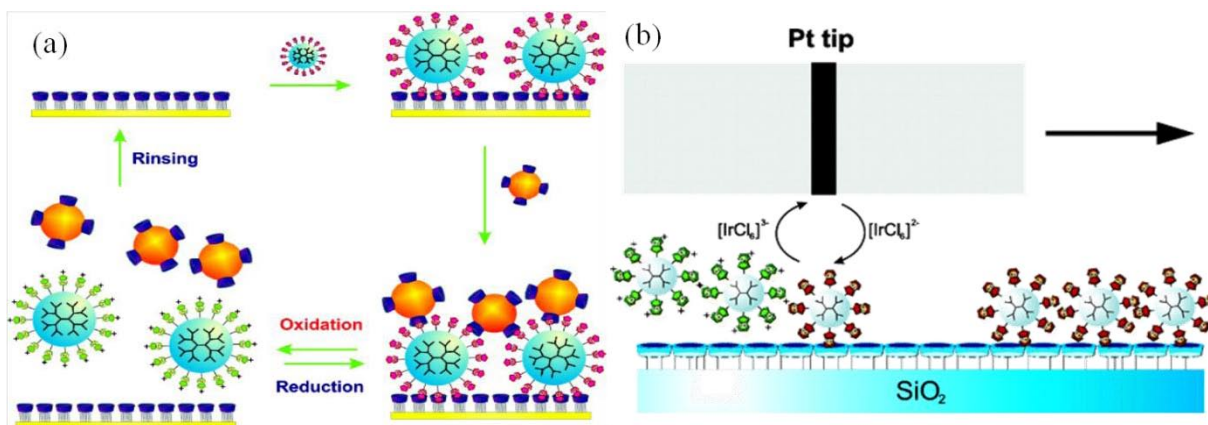


Figure 1.2 (a) Reversible attachment of nanostructures to a molecular printboard²⁵
 (b) SECM surface modification.²⁶ Adapted with permission from ref 25,26. Copyright
 2005,2008 American Chemical Society.

In general, a molecular printboard is understood to be a solid substrate or a monolayer of host molecules onto which guest molecules can be attached with control over position, binding strength, and binding dynamics.²⁷ In principle, a unit that can be reversibly switched on and off could be used to reversibly control the formation and properties of the assembled structure. However, the design of a molecular receptor whose affinity can be reversibly switched from very strong to very weak is a major challenge. Many examples to control the binding site of calixarenes using the well-known dithienylethylene “Irie switch”, or the use of azobenzenes to modulate the accessibility of a binding site, show that this remains a difficult task.²⁸

The Mattay group in Bielefeld University (Germany) has recently demonstrated a bistable supramolecular host-guest system where the supramolecular receptor cavity of a resorc[4]arene is combined with two photoactive anthracene moieties whose photodimerization can be externally switched by UV light and temperature.^{29,30} The receptor was also modified with four didecylsulfide linkers for surface immobilization on gold substrates via molecular self-assembly (Figure 1.3). This photochemical macrocyclic receptor has been investigated using AFM to elucidate its functional properties to act as a photochemical single-molecule switch, which showed that the reversible affinity switching of the receptor molecule by means of UV (open → close cavity) and heat (close → open cavity) is possible and can be monitored by AFM.

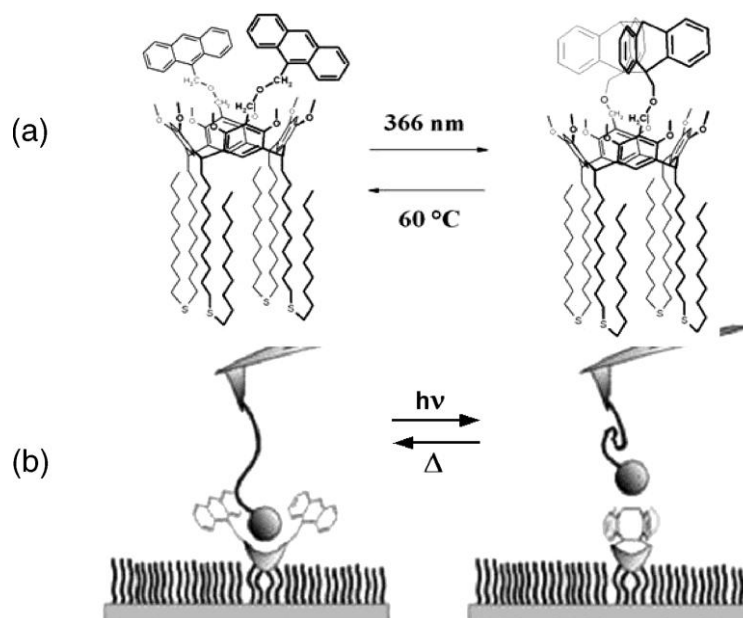


Figure 1.3 Resorc[4]arene photoswitch for self-assembly (a) and a pictogram visualizing the self-assembled monolayer of this photoswitch for single-molecule affinity using AFM (b).³⁰ Adapted with permission from ref 30. Copyright 2007 American Chemical Society.

1.2 Previous studies in our group

In 1988, Andrew Hamilton's group in Princeton University designed and synthesized a receptor with the ability to strongly bind barbiturate derivatives.^{31,32} Their approach exploits the complementarity between a 2,6-diaminopyridine group³³ and one of the two imide sites on barbituric acid through the formation of three (3) complementary hydrogen bonds. The integration of two such 2,6-diaminopyridine units into a macrocyclic structure allows the complexation of the six accessible hydrogen bonding sites in 5,5-disubstituted barbiturates to afford a 1:1 assemblies possessing a high association constant ($K=1.37 \times 10^6$ in CDCl_3) (Figure 1.4).³¹

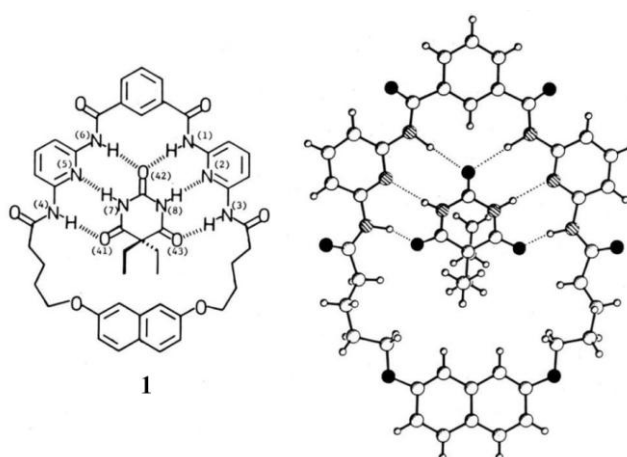


Figure 1.4 The X-ray structure of a Hamilton receptor binding 5,5'-diethylbarbituric acid through six complementary hydrogen bonds.³² Adapted with permission from ref 32.

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In the course of our previous studies on the control of supramolecular interactions by light, a receptor for barbituric acid whose binding affinity could be modulated by light through the photodimerization of appended anthracene units was investigated (compound **2** in Figure 1.5).^{34,35} In this system, the binding cleft is again based on the highly selective barbiturate receptor developed by Hamilton and co-workers (Figure 1.5). However, the macrocyclic nature of the original receptor was replaced by two anthracene-terminated propyloxy chains. In solution, irradiation with 365-nm light leads to efficient photodimerization of the two pendant anthracene moieties to afford an intramolecular photodimer. (Figure 1.5).

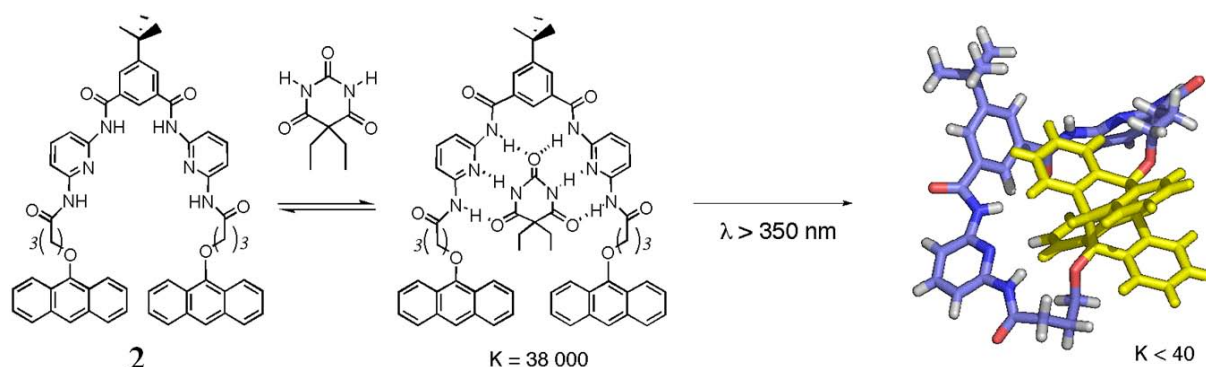


Figure 1.5 The open receptor **2** shows a strong binding to barbituric acid, which is considerably lowered upon photodimerization. The X-ray structure of the closed receptor after photodimerization is shown on the right.^{34,35}

Most importantly, the binding affinity of the photodimerized (closed) receptor drops from 38000 to $< 40\text{ M}^{-1}$ in CDCl_3 , which represents a *ca.* 1000-fold decrease in binding strength when compared to the uncyclized (open) receptor. To fully appreciate this achievement, one may compare it to the much smaller 35-fold modulation of the association constant obtained using a cyclodextrin receptor incorporating a photoisomerizable switch.²⁸ The reason for such a control on binding affinity by the anthracene system is easily seen from the X-ray structure of the photodimerized receptor (Figure. 1.5, right), which shows how the entire binding cleft is occupied and thus blocked by the voluminous anthracene photodimer. In the case of this photodimer, heating to 80°C for a few hours induces slow retro-cyclization to afford the open receptor.

Although head-to-tail anthracene photodimers are thermally stable, it is also known that they can be cleanly cleaved through a one-electron redox process.^{36,37} Chloranil was previously shown by de Mayo to photosensitize the cleavage of anthracene photodimers in solution through an oxidative PET mechanism.³⁶ Thus, electrochemical or photoinduced (via sensitized electron transfer) generation of the radical cation of the anthracene photodimer of **2** may be expected to result in its cleavage to afford two anthracene molecules after reduction of the stable intermediate anthracene radical cation (Figure 1.6).

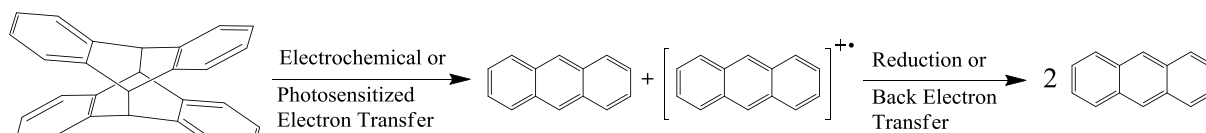


Figure 1.6 Anthracene photodimers are rapidly cleaved by one-electron redox process^{36,37}

1.3 The goal of this thesis

The use of self-assembled monolayers of molecular receptors for molecular sensors or device applications requires a high degree of order and packing of the monolayer.^{7,8} Order is necessary to ensure that the binding clefts of the receptors share a similar local environment, whereas close packing of the monolayer minimizes nonspecific adsorption or reaction with the underlying substrate. The capability of modifying regions of a chemisorbed molecular layer can further enable the fabrication of materials in which specific properties, such as pH, conductivity, or binding, are spatially controlled. When the modification alters the binding properties of the surface, it is possible to conceive architectures in which separate molecular recognition events are hierarchically organized to promote the self-assembly of a chosen architecture. This concept was first introduced by Ringsdorf using biotin and streptavidine recognition,³⁸ and is now widely applied to numerous biological and abiotic systems, including the binding of semiconducting nanoparticles, dendrimers, DNA, enzymes, or viruses.³⁹ Compared to covalent linkers, the use of supramolecular interactions to connect molecules to a surface allows rearrangement, self-correction, and the possibility of tuning the association constant to induce adsorption or desorption of species. From the above, it emerges that the anthracene appended barbituric acid receptor **2** is likely to be a good candidate for the fabrication of a light driven reversible molecular printboard, as illustrated schematically in Figure 1.7.

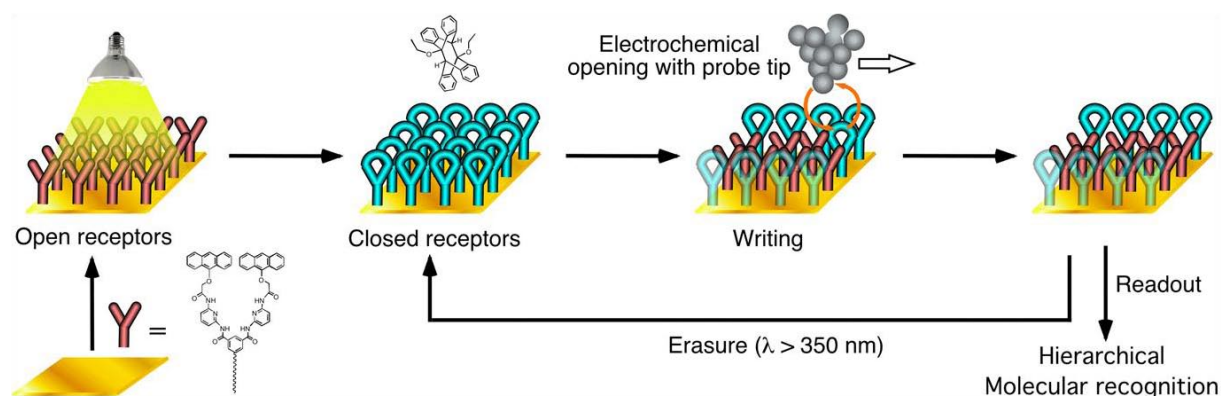


Figure 1.7 A write-read-erase molecular print board designed based on the previously-developed photochromic barbiturate receptor **2**.

In the system proposed, the host-guest interactions that are at the basis of the molecular binding event are based on the recognition between barbituric acid and a complementary receptor analogous to that reported by Andy Hamilton in 1988. The immobilization of a similar receptor in SAMs was demonstrated by Myles and co-workers (Figure 1.8),⁴⁰ who showed that a well-organized SAM of this receptor on gold can be used as a model system for the study of interfacial recognition. By using surface immobilized receptors based on **2**, photo-patterning will be possible, and the open receptor on the surface can serve as a support for the assembly of a variety of photo- or electroactive barbiturate-appended substrates. The facile substitution of barbituric acid in the C-5 position, located distal to its binding site, should allow the easy introduction of fluorescent or electroactive substituents. For example, previous work in the group reported the straightforward synthesis of fullerene C₆₀, oligothiophenes, CdS and gold nanoparticles appended with barbituric acid or melamine derivatives and demonstrated their use in the construction of self-assembled electroactive architectures (Figure 1.9).^{38,39}

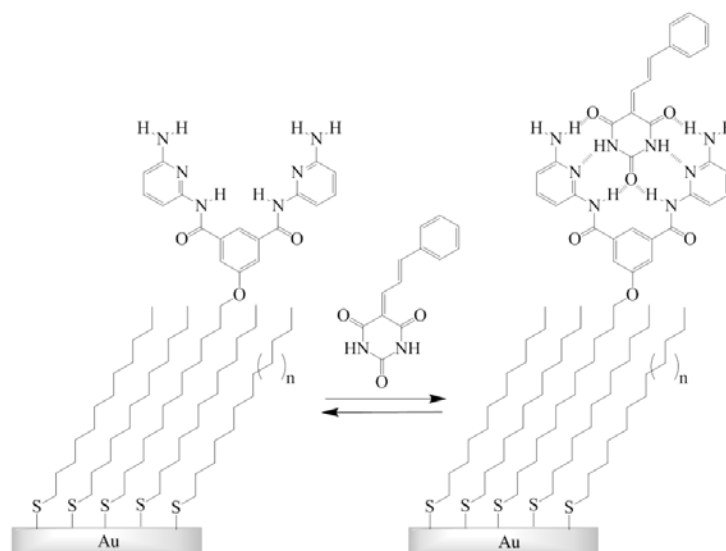


Figure 1.8 Molecular recognition between barbituric acid and a Hamilton receptor immobilized on a gold surface.⁴⁰ Adapted with permission from ref 40. Copyright 1998 American Chemical Society.

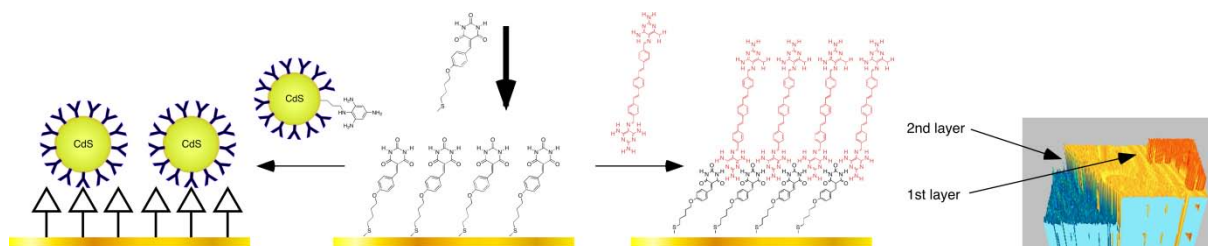


Figure 1.9 Example using barbituric acid as a basis for hierarchical molecular recognition on surfaces, leading to the selective deposition of Au or CdS nanoparticles³⁸ or oligophenylenevinyls appended with complementary melamine units.³⁹ Adapted with permission from ref 39. Copyright 2006 Elsevier.

1.4 The outline of this thesis

This thesis is divided into three main chapters according to the principal tasks involved in this work. The first task is to synthesize the anthracene-appended barbiturate receptors with suitable spacers and linkers which can allow their grafting onto various types of substrates such as gold, silicon or silicon oxide (Figure 1.10). The synthesis and the different grafting strategies for various substrates will be described in detail in chapter 2, as well as the synthesis of barbiturate guest derivatives with different substituents.

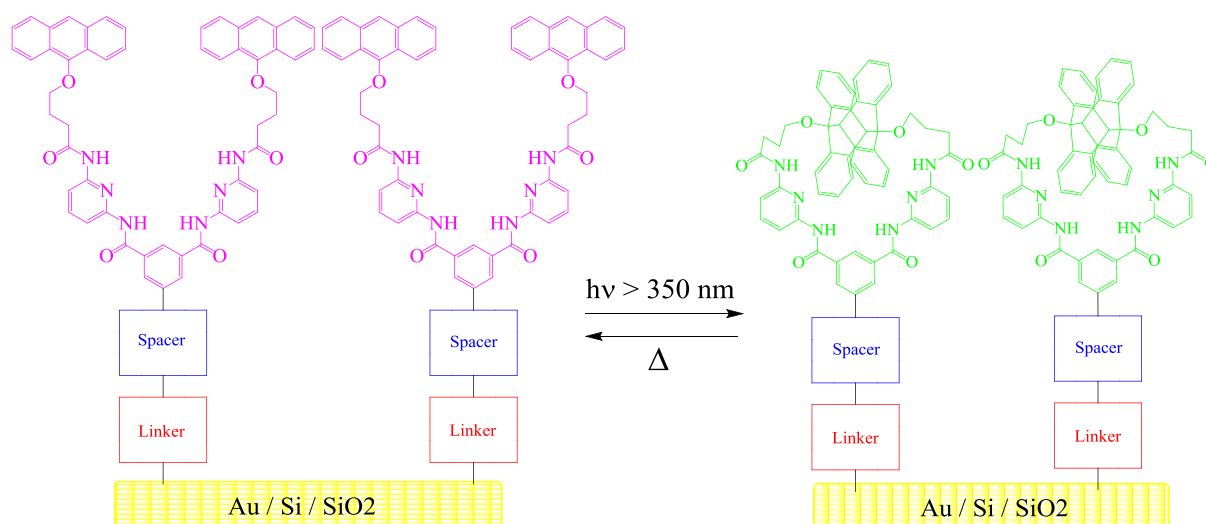


Figure 1.10 Molecular design of receptor molecule on the substrates

Because the photodimerization of the anthracene moieties on the photochromic receptor molecules is used to write the information on the monolayer, it is important to understand the photophysical and photochemical properties of the receptors to better optimize the photodimerization process. The investigation of these properties was undertaken in solution for the convenience of experiments, and these results are described in Chapter 3.

A large part of this work involved the surface modification of substrates, and substrate characterization using different instrumental analysis such as ellipsometry, contact angle and AFM measurements. The direct photodimerization on monolayer of receptor molecules was also examined and the results are shown in Chapter 4.

References

1. J. Charles P. Poole and F. J. Owens, *Introduction to Nanotechnology*, John Wiley & Sons, **2003**.
2. K. Oura, V. G. Lifshits, A. A. Saranin and A. V. Zotov, *Surface Science: An Introduction*, Springer, **2010**.
3. M. Mammen, S.-K. Choi and G. M. Whitesides, *Angew. Chem., Int. Ed.*, **1998**, *37*, 2754-2794.
4. A. Mulder, J. Huskens and D. N. Reinhoudt, *Org. Biomol. Chem.*, **2004**, *2*, 3409-3424.
5. J.-M. Lehn, *Angew. Chem., Int. Ed.*, **1988**, *27*, 89-112.
6. D. M. Rudkevich, Z. Brzozka, M. Palys, H. C. Visser, W. Verboom and D. N. Reinhoudt, *Angew. Chem., Int. Ed.*, **1994**, *33*, 467-468.
7. A. Ulman, *An Introduction to Ultrathin Organic Films: From Langmuir--Blodgett to Self--Assembly* Academic Press, San Diego, CA, **1991**.
8. A. J. Bard and I. Rubinstein, *Electroanalytical Chemistry*, Marcel Dekker, New York, **1996**.
9. E. B. Troughton, C. D. Bain, G. M. Whitesides, R. G. Nuzzo, D. L. Allara and M. D. Porter, *Langmuir*, **1988**, *4*, 365-385.
10. S. Steinberg and I. Rubinstein, *Langmuir*, **1992**, *8*, 1183-1187.
11. B. Hagenhoff, A. Benninghoven, J. Spinke, M. Liley and W. Knoll, *Langmuir*, **1993**, *9*, 1622-1624.
12. M. Zhang and M. R. Anderson, *Langmuir*, **1994**, *10*, 2807-2813.
13. E. U. Thoden van Velzen, J. F. J. Engbersen and D. N. Reinhoudt, *J. Am. Chem. Soc.*, **1994**, *116*, 3597-3598.
14. E. U. Thoden van Velzen, J. F. J. Engbersen and D. N. Reinhoudt, *Synthesis*, **1995**, 989-997.
15. H. Schönherr, G. J. Vancso, B.-H. Huisman, F. C. J. M. van Veggel and D. N. Reinhoudt, *Langmuir*, **1997**, *13*, 1567-1570.
16. B.-H. Huisman, E. U. T. van Velzen, F. C. J. M. van Veggel, J. F. J. Engbersen and D. N. Reinhoudt, *Tetrahedron Lett.*, **1995**, *36*, 3273-3276.
17. B.-H. Huisman, D. M. Rudkevich, F. C. J. M. van Veggel and D. N. Reinhoudt, *J. Am. Chem. Soc.*, **1996**, *118*, 3523-3524.
18. K. D. Schierbaum, T. Weiss, E. U. T. van Veizen, J. F. J. Engbersen, D. N. Reinhoudt and W. Göpel, *Science*, **1994**, *265*, 1413-1415.
19. B.-H. Huisman, R. P. H. Kooyman, F. C. J. M. van Veggel and D. N. Reinhoudt, *Adv. Mater.*, **1996**, *8*, 561-564.
20. J. Huskens, M. A. Deij and D. N. Reinhoudt, *Angew. Chem., Int. Ed.*, **2002**, *41*, 4467-4471.
21. F. Corbellini, A. Mulder, A. Sartori, M. J. W. Ludden, A. Casnati, R. Ungaro, J. Huskens, M. Crego-Calama and D. N. Reinhoudt, *J. Am. Chem. Soc.*, **2004**, *126*,

- 17050-17058.
22. C. A. Nijhuis, J. Huskens and D. N. Reinhoudt, *J. Am. Chem. Soc.*, **2004**, *126*, 12266-12267.
 23. S. Onclin, J. Huskens, B. J. Ravoo and D. N. Reinhoudt, *Small*, **2005**, *1*, 852-857.
 24. C. A. Nijhuis, B. A. Boukamp, B. J. Ravoo, J. Huskens and D. N. Reinhoudt, *J. Phys. Chem. C*, **2007**, *111*, 9799-9810.
 25. X. Y. Ling, D. N. Reinhoudt and J. Huskens, *Chem. Mater.*, **2008**, *20*, 3574-3578.
 26. C. A. Nijhuis, F. Yu, W. Knoll, J. Huskens and D. N. Reinhoudt, *Langmuir*, **2005**, *21*, 7866-7876.
 27. R. Salvio, J. Huskens and D. N. Reinhout, in *Handbook of Nanofabrication*, Elsevier B.V., **2010**.
 28. A. Mulder, A. Jukovic, L. N. Lucas, J. van Esch, B. L. Feringa, J. Huskens and D. N. Reinhoudt, *Chem. Commun.*, **2002**, 2734-2735.
 29. V. Walhorn, C. Schaefer, T. Schroeder, J. Mattay and D. Anselmetti, *Nanoscale*, **2011**, *3*, 4859-4865.
 30. C. Schaefer, R. Eckel, R. Ros, J. Mattay and D. Anselmetti, *J. Am. Chem. Soc.*, **2007**, *129*, 1488-1489.
 31. S. K. Chang and A. D. Hamilton, *J. Am. Chem. Soc.*, **1988**, *110*, 1318-1319.
 32. S. K. Chang, D. Van Engen, E. Fan and A. D. Hamilton, *J. Am. Chem. Soc.*, **1991**, *113*, 7640-7645.
 33. B. Feibush, A. Figueroa, R. Charles, K. D. Onan, P. Feibush and B. L. Karger, *J. Am. Chem. Soc.*, **1986**, *108*, 3310-3318.
 34. Y. Molard, D. M. Bassani, J.-P. Desvergne, P. N. Horton, M. B. Hursthouse and J. H. R. Tucker, *Angew. Chem., Int. Ed.*, **2005**, *44*, 1072-1075.
 35. Y. Molard, D. M. Bassani, J.-P. Desvergne, N. Moran and J. H. R. Tucker, *J. Org. Chem.*, **2006**, *71*, 8523-8531.
 36. R. A. Barber, P. De Mayo, K. Okada and S. K. Wong, *J. Am. Chem. Soc.*, **1982**, *104*, 4995-4997.
 37. J. M. Masnovi and J. K. Kochi, *J. Am. Chem. Soc.*, **1985**, *107*, 6781-6788.
 38. R. Baron, C.-H. Huang, D. M. Bassani, A. Onopriyenko, M. Zayats and I. Willner, *Angew. Chem., Int. Ed.*, **2005**, *44*, 4010-4015.
 39. C.-H. Huang, N. D. McClenaghan, A. Kuhn, G. Bravic and D. M. Bassani, *Tetrahedron*, **2006**, *62*, 2050-2059.
 40. K. Motesharei and D. C. Myles, *J. Am. Chem. Soc.*, **1998**, *120*, 7328-7336.
 41. R. Salvio, J. Huskens, D.N. Reinhoudt, *4.06 - Molecular Printboards: From Supramolecular Chemistry to Nanofabrication, Comprehensive Nanoscience and Technology*, Academic Press, Amsterdam, 2011, Pages 145-181

Chapter 2. Synthesis

2.1 Introduction to surface modification on solid substrates

Self-assembled monolayers (SAMs) have attracted extensive interest because of their potential applications related to the control of wettability, biocompatibility and corrosion resistance of the surface of a wide range of materials.¹ Self-assembled monolayers provide an opportunity to define the chemical functionality with molecular precision. Functional group transformations on SAMs can be studied and may provide new insights as well as routes to modified surface properties. These transformations allow, for example, the tethering of biological molecules to surfaces at precisely controlled positions, which can be important in a wide range of studies in chemical biology and microarray technology.²

Self-assembled monolayers are highly ordered molecular assemblies, which form spontaneously by chemisorption of functionalized, surfactant-like molecules.³ When the molecules adsorb to a surface, they may organize laterally through van der Waals interactions between long aliphatic chains (Figure 2.1). The choice of the terminal group thus determines the properties of the surface, as the underlying substrate becomes completely inaccessible to molecules in solution. For example, clean gold is naturally hydrophilic, but the formation of SAMs makes it possible to control the contact angle of water on the surface to any value between 0° ($-\text{OH}$ and $-\text{COOH}$ terminal functional group) and 118° ($-\text{CF}_3$ groups) depending on the functional group on the surface.⁴

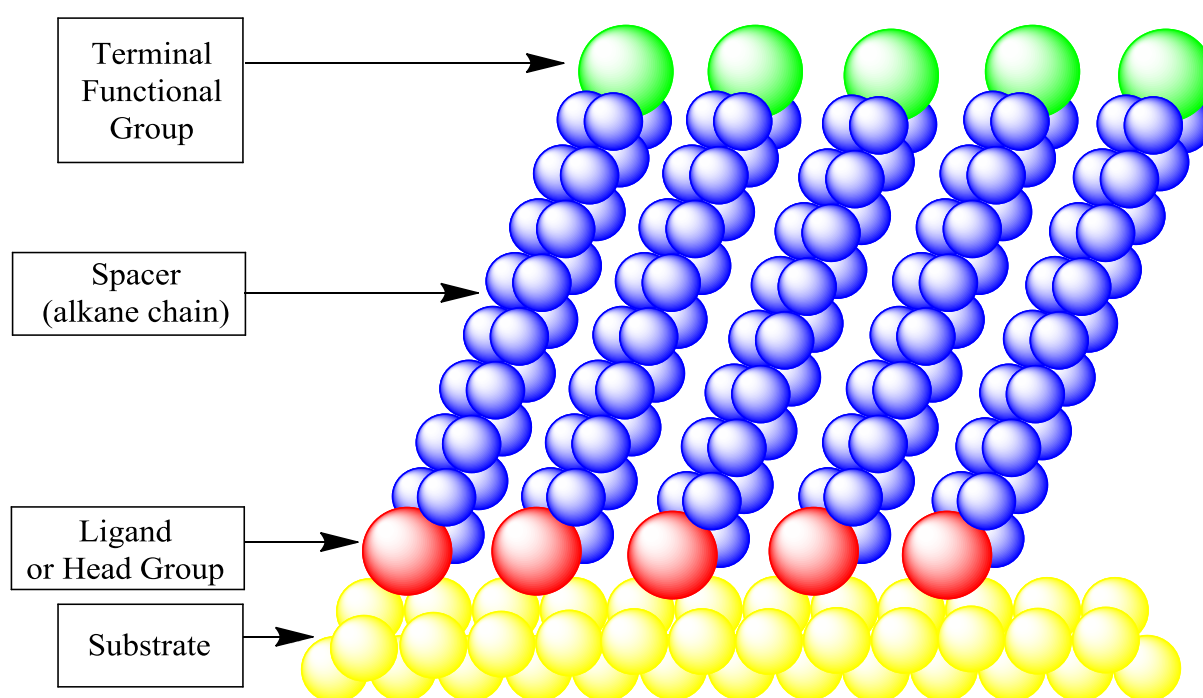


Figure 2.1 Schematic diagram of an ideal, single crystalline SAM of a functionalized alkanethiol supported on a gold surface.⁵ Adapted with permission from ref 5. Copyright 2005 American Chemical Society.

It is not only possible to control a surface's physical properties through variation of the terminal functional group of a monolayer, but also possible to introduce more chemically interesting properties such as specific binding of proteins to surfaces. It is often more convenient to use a number of standard SAMs to modify a surface's chemistry instead of synthesizing different thiols/silanes with different functional groups. Performing reactions on SAMs allows one to adjust the properties of surfaces, but due to the nature of the SAMs (tightly packed, restricted movement of molecules within the monolayer) the choice of reaction is important. One has to consider that the steric effects are likely to be exacerbated for certain surface reactions, leading to an energy barrier higher than would be expected in solution chemistry. To successfully functionalize a SAM, the reaction conditions must not cause destruction of the monolayer or damage the underlying substrate. This is a special consideration with monolayers on substrates such as gold, where desorption of the monolayer can occur more readily than in systems based on the covalent attachment of trichlorosilanes on silicon oxides. Further information can be found in several reviews which highlight the use of different modification sequences to introduce chemical functionalities into monolayer system.⁶⁻⁹ A list of selected reactions performed on SAMs is given in Table 2.1.

Table 2.1 Selected transformation reactions on SAMs

SAM functionalization	Reaction Type	Reagent	Product	Refs.
Alcohol	Acylation	Acid chlorides	Esters	10
		Anhydrides	Esters	
	Nucleophilic Substitution	Alkyltrichlorosilane	Double layer	SAM 11
Alkyl Halide	S _N 2	Strong anionic nucleophiles	Substituted Product	12,13
Amine	Nucleophilic Substitution	Carboxylic acid	Imine	14
		Chlorodimethylsilane (S _N 2)	Amides	15
	Acylation	Acid chloride	Amides	16
		Active esters		17
Aldehyde	Nucleophilic Substitution	Amines	Imines	18
Carboxylic Acid	Esterification	Trifluoroethanol/di- <i>tert</i> -butylcarbodiimide	Ester	19
	Nucleophilic Substitution	Amines	Amides	20
Azide	Click chemistry	Alkyne	Cycloaddition product	21
Alkyne	Click chemistry	Azide		22,23

2.2 General strategy for the synthesis of photoactive receptors

The design of an anthracene-appended Hamilton receptor suitable for immobilization onto a substrate while retaining both its photoreactivity and its molecular recognition properties requires some considerations. Based on the work of Myles,²⁴ we may consider that the C-3 position of the central phenyl ring is a suitable point of attachment (Figure 2.2):

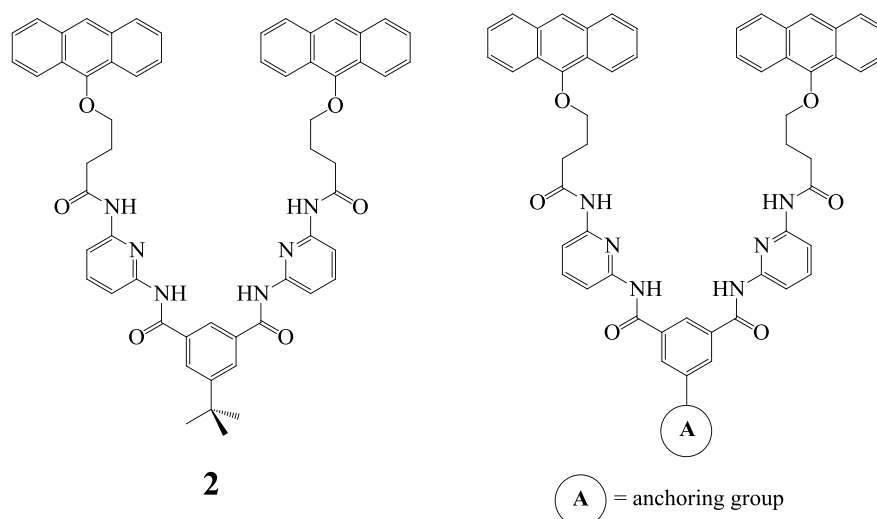
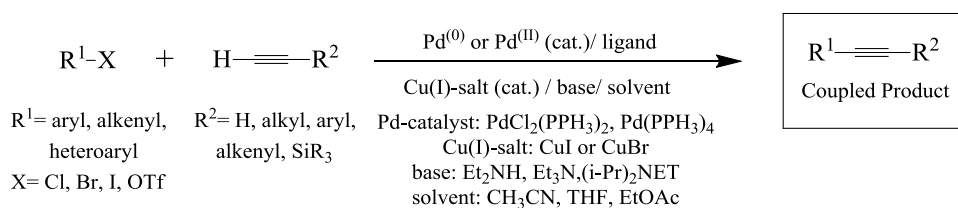


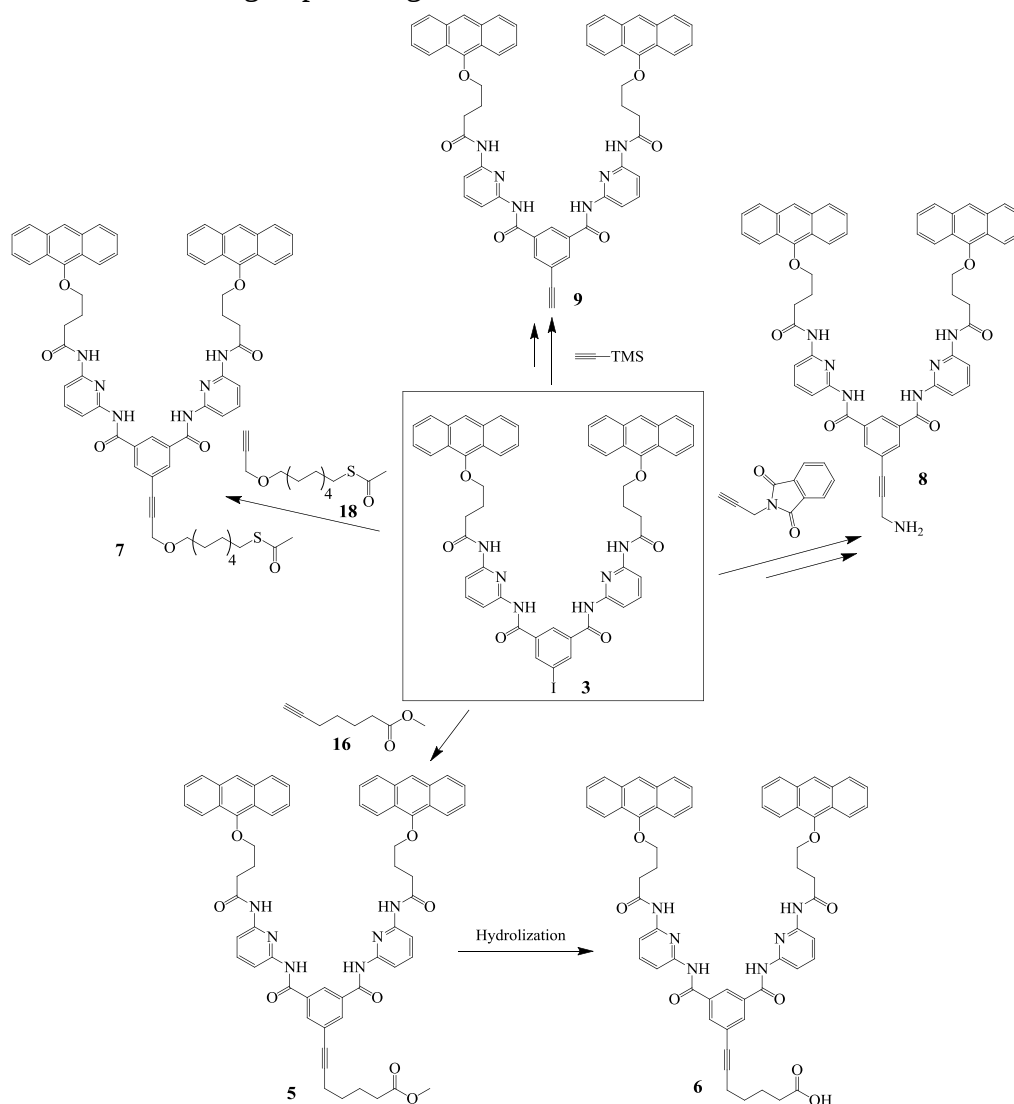
Figure 2.2 Rational design of an anthracene-appended Hamilton receptor

Because the multi-steps synthesis of **2** is long, a convergent strategy for the introduction of various anchoring groups would be advantageous. Possible candidates involve transition-metal catalyzed coupling reactions such as Suzuki, Sonogashira, or Stille coupling. The palladium catalyzed C-C bond formation process which is able to couple a terminal sp hybridized carbon of an alkyne with a sp^2 carbon of an aryl or vinyl halide is commonly termed as a Sonogashira coupling. The name arises from the discovery in 1975 by Sonogashira, Tohda, and Hagihara that this process could be performed easily at room temperature using a palladium source such as $\text{PdCl}_2(\text{PPh}_3)_2$ as catalyst, combined with a co-catalyst (CuI) and an amine as base (Scheme 2.1).²⁵⁻²⁷ It is one of the most important and widely used sp^2 — sp carbon-carbon bond formation reactions in organic synthesis, frequently employed in the synthesis of natural products, biologically active molecules, heterocycles, molecular electronics, etc.²⁵⁻²⁷ In our system, it can be employed to combine the photochromic receptor with different linkers to afford various molecules suited for different surface grafting techniques.



Scheme 2.1 General reaction conditions for the Sonogashira cross coupling reaction.²⁸

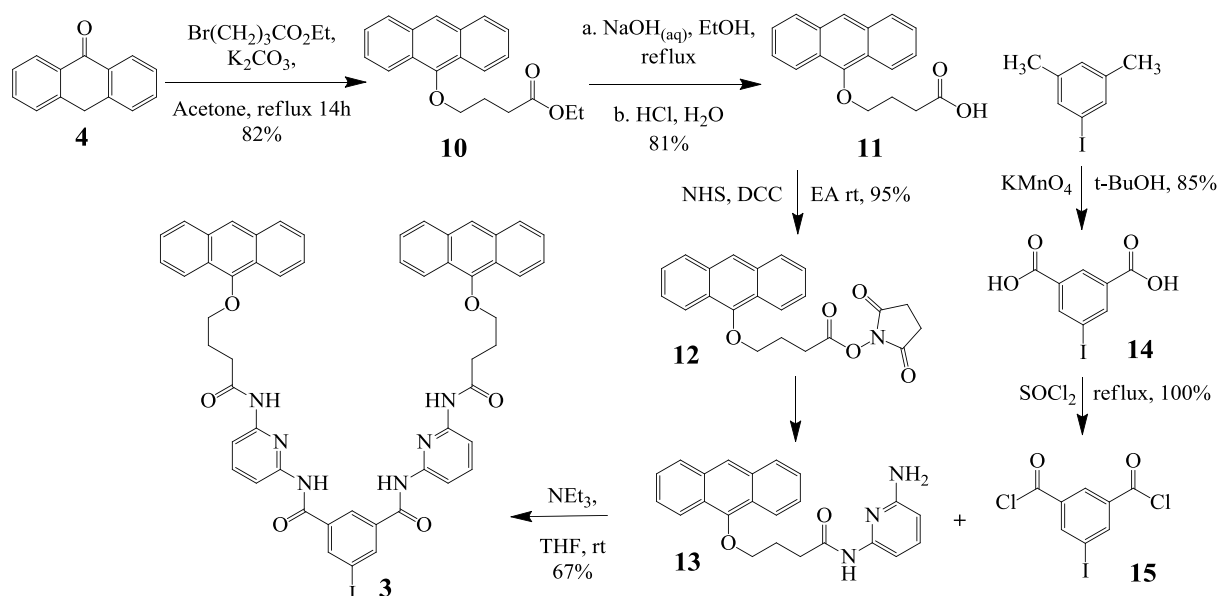
Five molecules containing a Hamilton receptor motif appended with different end functional groups for surface coupling were synthesized (Scheme 2.2). These include the thioacetate group for direct anchoring to gold substrates, as well as a primary amine or carboxylic acid group for amide-coupling methodology, or a terminal alkyne used for click reactions onto suitably derivatized substrates. Their synthesis is highly convergent and begins from a common receptor intermediate containing an aryl iodide moiety for coupling to different functional groups using Sonogashira coupling methodology. The major advantage is that we can readily synthesize receptors with different functional end-groups and apply them to various aspects of our studies. For example, the receptor with a methyl ester terminated group (**5**) was used in investigating the photophysical and photochemical properties in solution, while receptors with thioacetates (**7**) were used in surface modification on gold. Receptors with acid groups (**6**) can be grafted onto a SAM with an amine terminal group whereas receptors with amino groups (**8**) can be grafted onto a SAM with a carboxylic acid group on the surface. Finally, receptors with terminal alkynes (**9**) were grafted on a SAM functionalized with azide groups through a click reaction.



Scheme 2.2 General convergent strategy for the synthesis of various functionalized open receptors

2.3 Synthesis of receptor intermediate 3

The synthetic route of receptor intermediate **3** is illustrated in Scheme 2.3. The heart of receptor was obtained starting from 5-iodo-xylene, which could be easily oxidized to the diacid **14**, and then transformed to acid chloride **15** by refluxing in thionyl chloride. The synthesis of the anthracene side arms **13** is based on the previously reported synthesis of **2**,^{29,30} and begins from commercially available anthrone **4**, to prepare the corresponding ester via *O*-alkylation with ethyl 4-bromobutyrate. Then, hydrolysis of the ester group affords the desired carboxylic acid **11**. Coupling between **11** and 2,6-diaminopyridine was performed in two steps via the corresponding *N*-succinimidoyl ester intermediate (**12**, prepared by reaction with *N*-hydroxysuccinimide and *N,N'*-dicyclohexylcarbodiimide in ethyl acetate at 25°C) by reaction with a large excess of 2, 6-diaminopyridine in dry CH₂Cl₂ and diisopropylethylamine. Finally, the receptor intermediate **3** was obtained in good yield (68%) by amide bond formation between the acyl chloride **15** and compound **13** in THF in the presence of triethylamine. It is worth noting that the separation between receptor **3** and residual anthracene side arm **13** is very difficult due to the similar polarity of the two compounds. To minimize this complication, excess acid chloride **15** was added to completely consume any remaining compound **13** after the coupling reaction and prior to purification by column chromatography to afford pure **3**.

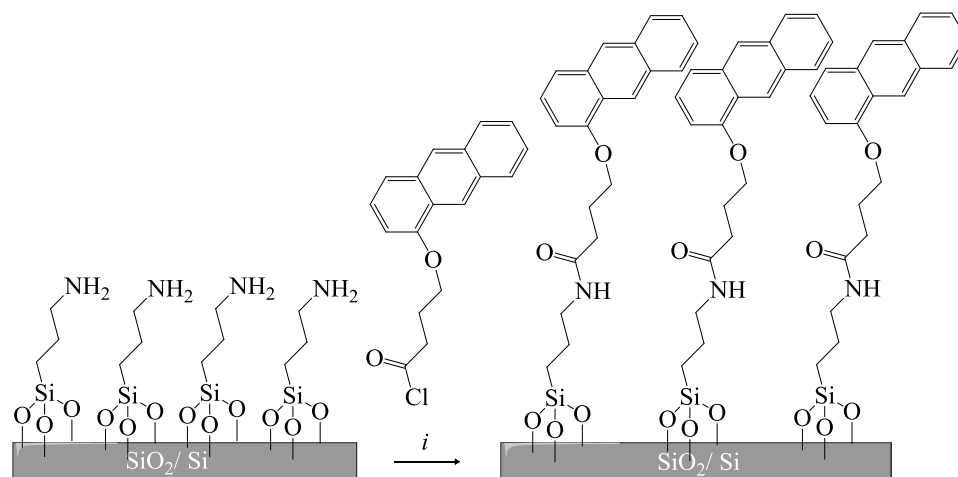


Scheme 2.3 Synthesis of receptor intermediate **3**

2.4 Synthesis of receptors with methyl ester and acid group

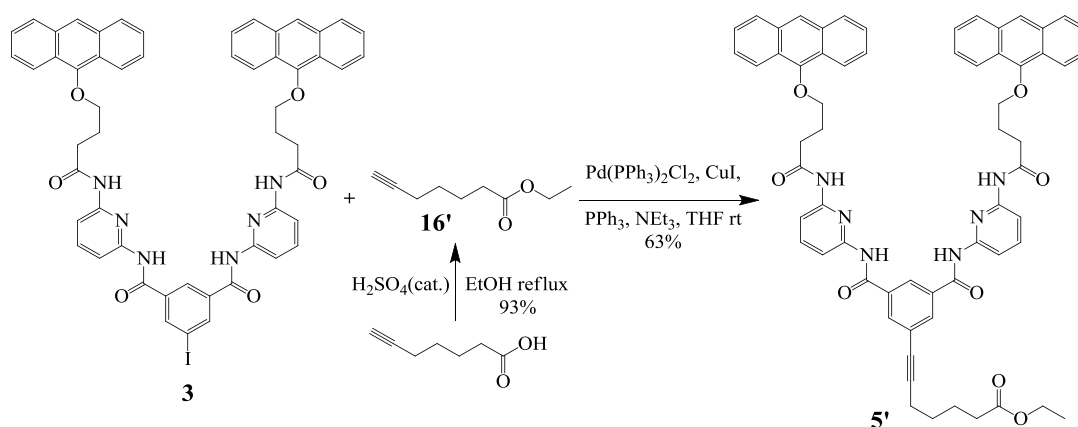
The formation of aminopropylsilane SAMs on glass or Si/SiO₂ is a standard surface modification frequently used for the covalent coupling of DNA fragment to microchip arrays,³¹⁻³³ or the base-by-base synthesis of oligonucleotides.^{34,35} Because of their use in DNA

chips, reactions on amine-terminated monolayers have received considerable attention and are well documented. Amine SAMs react readily with acylating agents such as acid chlorides,^{16,36} active esters^{17,37} and quinones (Scheme 2.4).³⁸ Therefore, we began by investigating grafting the receptor molecule onto SiO₂ surfaces using amide formation between an amine terminated SAM and the carboxylic acid linker group in compound **6**. Additionally, the methyl ester intermediate **5** is useful for the solution-based studies of the receptors' photochromic properties as esters generally provide improved solubility.



Scheme 2.4 Example of surface modification on amino SAMs with an acid chloride
(i) CH₃CN, rt, Ar, 24 hr.³⁶

To begin, the corresponding ethyl ester compound **5'** was chosen as the end group for the receptor. The Sonogashira reaction went quite smoothly, although the yield was not quantitative (63%, Scheme 2.5). However, although various hydrolysis conditions for cleaving the ethyl ester group were tested, none of them proved successful (Table 2.2). Sodium hydroxide was too strong a reagent and induced degradation of the receptor. Lithium hydroxide is a much milder reagent for the hydrolysis of ester groups, but could not hydrolyze **5'** at room temperature nor under reflux conditions. The last trial was performed using trimethylsilyl chloride to deprotect the ester group, but no reaction was obtained.

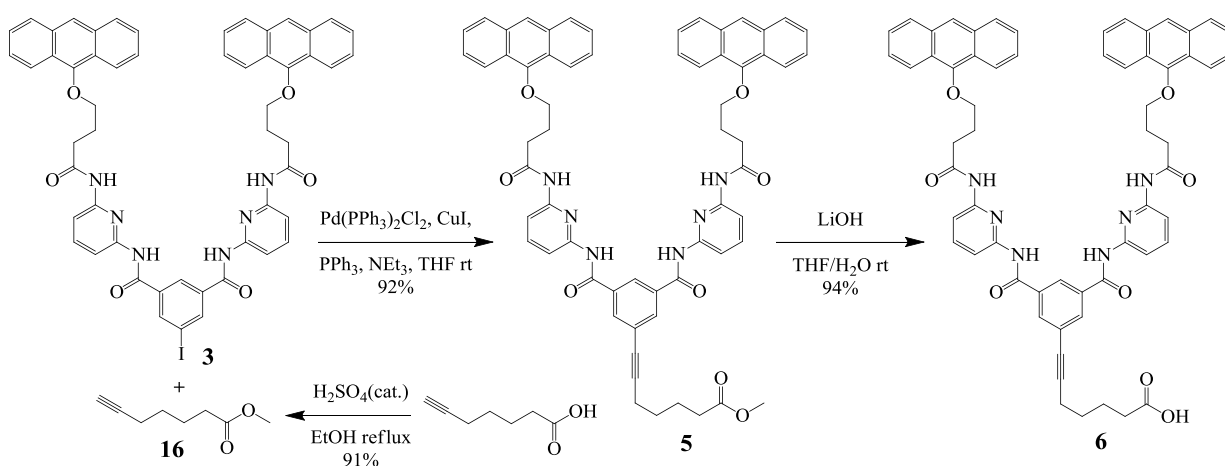


Scheme 2.5 Synthesis of ethyl ester receptor **5'**

Table 2.2 Reaction conditions for hydrolyzation of ethyl ester receptor

Conditions	NaOH	LiOH	LiOH	TMSCl/NaI
Solvent	EtOH _(aq)	THF/H ₂ O	THF/H ₂ O	THF
Temperature	rt	rt	reflux	rt
Results	Degradation	No Rxn	No Rxn	No Rxn

Interestingly, it was not as easy as expected to deprotect the ethyl ester without damaging the receptor **5'**. Therefore, the ethyl ester in **16'** was replaced by methyl ester **16** to reduce the difficulty of deprotection. The Sonogashira coupling reaction worked well and afforded the methyl ester receptor **5** in near quantitative yield (92%). Finally, the ensuing hydrolysis using lithium hydroxide at room temperature went smoothly to afford the desired carboxylic acid functionalized receptor **6** in 95% yield (Scheme 2.6).

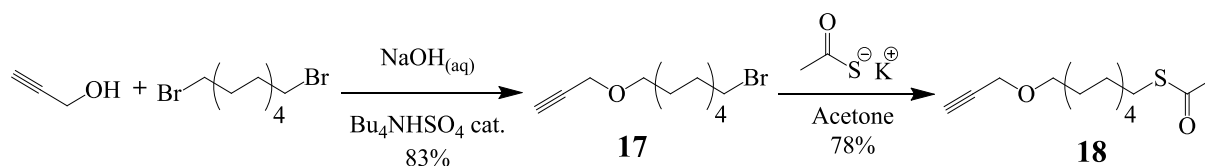
Scheme 2.6 Synthesis of methyl ester **5** and acidic receptor **6**

2.5 Synthesis of receptors with a thioacetate group

To chemically assemble a monolayer on a gold surface, a molecule should have an anchoring group such as a thiol, disulfide or thiolate group. Generally, thioacetate-terminated conjugated molecules are used for SAMs fabrication because they are more inert than thiols, which can easily undergo auto-oxidation to form disulfides or other oxidized products in the presence of oxygen. The thioacetate group can be deprotected *in situ* to the corresponding thiolate group by reacting with small amount of acid or base, and then forms a self-assembled monolayer on the Au surface.^{39,40} It is also known that the thioacetate group can in some cases be used directly to self-assemble onto a gold surface without removing the acetyl group, although higher concentration and longer adsorption time may be required to achieve similar surface coverage relative to those of a free thiol group.³⁹ In later studies, we found that the use of unprotected thiols or thiolates sometimes led to the occasional deposition of aggregates onto the gold surface. These nano-size aggregates were difficult to remove by washing and

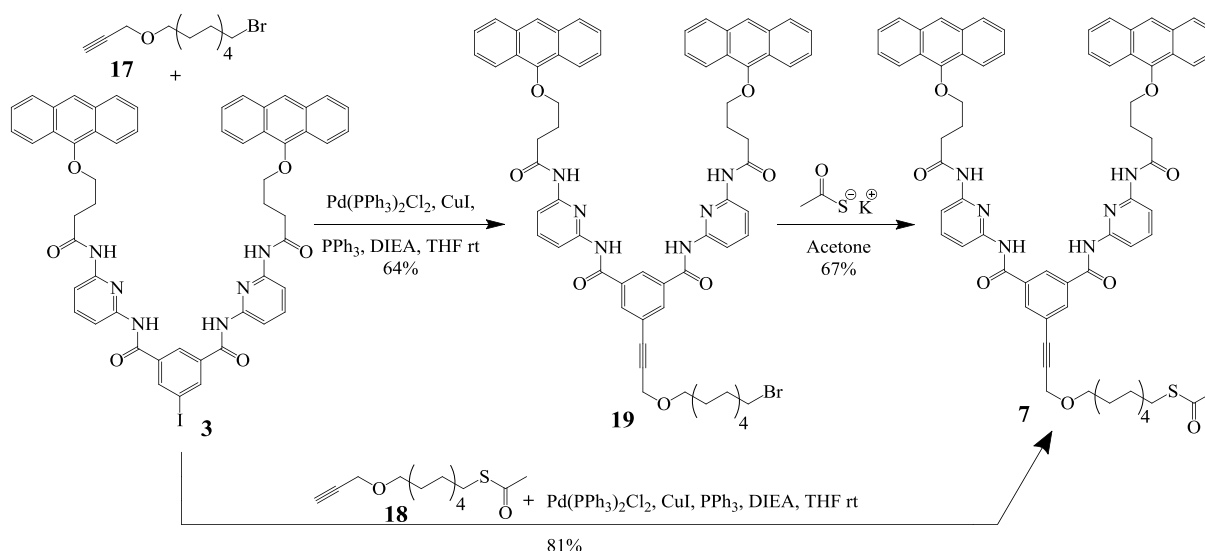
resulted in device failure. Thus, it would be very interesting to directly use the thioacetate-terminated molecules without converting them to the corresponding thiolates for the monolayer assembly.

Based on the advantages of thioacetate approach described above, a receptor with a thioacetate end group was designed and synthesized. Propargyl alcohol was chosen to provide the required terminal alkyne functional group, and reacted with 1,10-dibromodecane under biphasic conditions in a Williamson ether synthesis to give the bromide precursor **17**. Then the bromine atom was easily transformed to the thioacetate linker **18** using potassium acetate in acetone (Scheme 2.7).



Scheme 2.7 Synthesis of thioacetate linker

Initially, the Sonogashira reaction was used to first couple with the bromo linker **17** to **3**. The reason for this was to avoid possible complex poisoning of the Pd catalyst by the thioacetate group, which would lead to reduction of its catalytic ability. In a second step, the terminal bromine was substituted with potassium thioacetate to afford the desired thioacetate receptor **18** (Scheme 2.8). Nevertheless, the direct coupling between receptor intermediate **3** and the thioacetate spacer **18** was also tested, and the result showed that the Sonogashira coupling reaction was not affected by the thioacetate group, leading to an overall yield of **7** which was considerably improved.

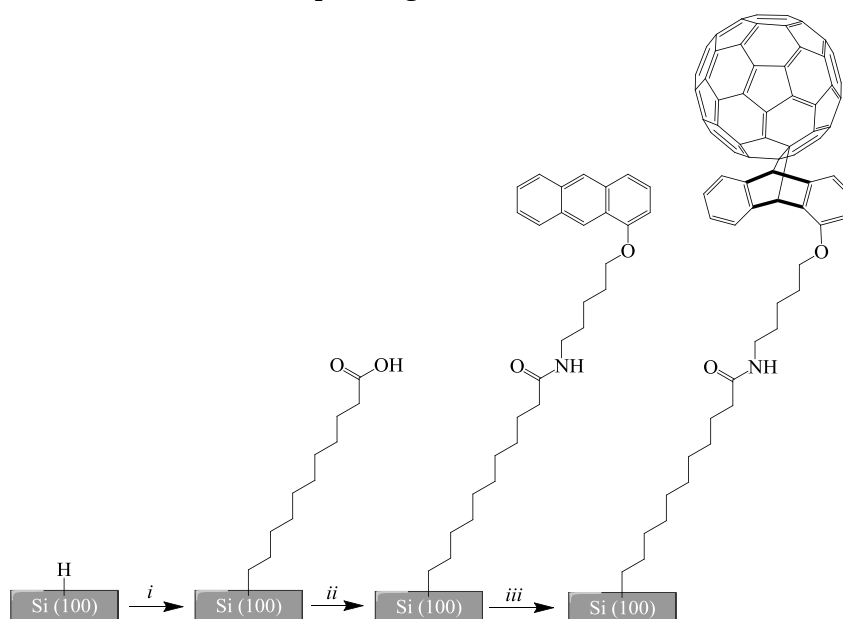


Scheme 2.8 Synthesis of receptor **7** with a thioacetate-terminated linker

2.6 Synthesis of receptor with a terminal amino group

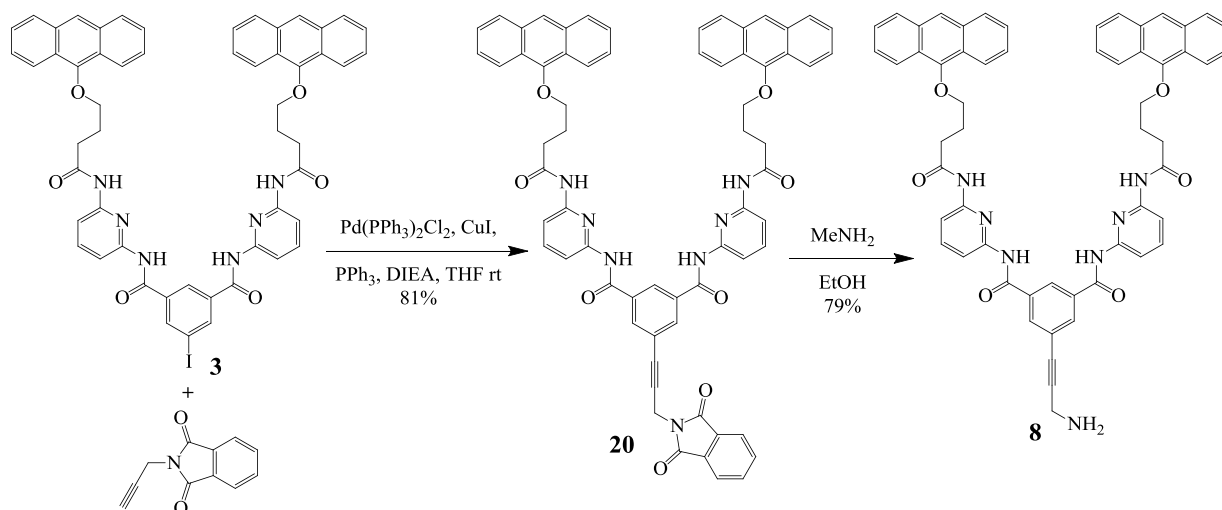
The “activated ester” method is widely used for the introduction onto carboxylic acid terminated SAMs of various functional groups that can be used for the binding of biological molecules to the surface.⁴¹ *N*-Hydroxysuccinimide (NHS) activation is often applied for this purpose to introduce a variety of amine-terminated molecules to such SAMs under mild conditions. The reaction proceeds quickly in high yield and is compatible with a wide range of functional groups (Scheme 2.9).^{31,42}

Alternatively, carboxylic acid terminated SAMs can be converted into a reactive ester by immersing the surface into a solution of 3-(dimethylamino)-1-ethylpropylcarbodiimide (EDC) and pentafluorophenol in DMF. The reaction proceeded quickly (*ca.* 20 mins) under mild conditions. The carboxylic acid groups are activated to pentafluorophenyl esters, which are *ca.* 10 times more reactive than the corresponding NHS esters.



Scheme 2.9 Example of surface modification on carboxylic acid SAMs.⁴³ (i) Neat undecylenic acid, 300 nm, 3.5 h; (ii) 0.1 M NHS + 0.2 M EDC in DMF, rt, 3h; then 1-(5-aminopentyl)anthracene (1.5×10^{-2} M) in CH_2Cl_2 , rt, overnight; (iii) C_{60} (1 mM) in toluene, 45°C, 3.5 days

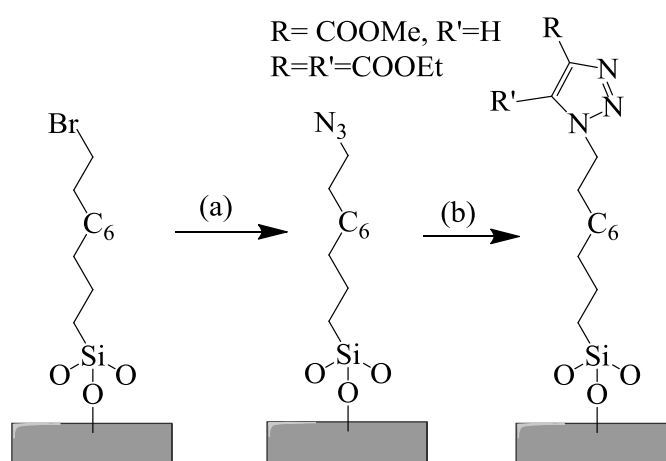
Based on the grafting technique described above, we designed and synthesized a receptor with an aminoalkane end group. The synthesis began with an alkyne possessing a protected amine (*N*-propargylphthalimide) in view of coupling with the receptor intermediate **3** through a Sonogashira coupling reaction, followed by subsequent deprotection of the amine with methyl amine to afford the desired amino functionalized receptor (Scheme 2.10). The final product **8** was extremely prone to autooxidation and therefore needed great care in its purification. All the solvents and silica gel were degassed before column chromatography, and the pure compound had to be sealed in a glass tube following freeze-pump-thaw degassing for storage.



Scheme 2.10 Synthesis of amine-functionalized receptor **8**

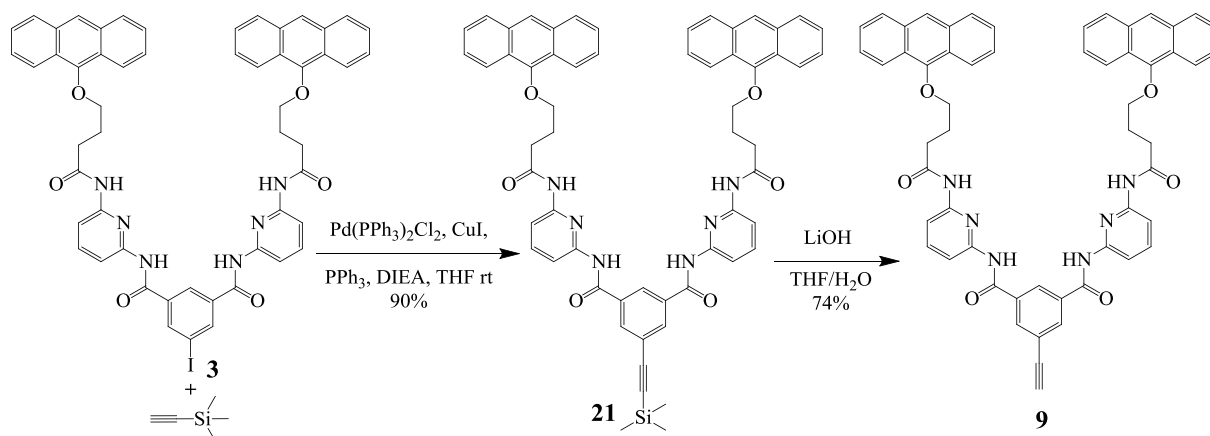
2.7 Synthesis of receptors with alkynyl or alkane thiol groups

Since the approach was made popular by Sharpless in 2001, click chemistry transformations have attracted enormous attention in various fields of chemistry, especially in polymer synthesis, material science, and biology.⁴⁴⁻⁴⁶ The definition of click-chemistry fulfills several criteria that are also advantageous for reactions performed on a surface: high yields, absence of byproducts, stereo specificity, and the ability to work under mild conditions. To date, the most used click reaction is the Huisgen 1,3-dipolar cycloaddition of an organic azide and an acetylene mediated by Cu.^{1,47} The first reported Huisgen 1,3-dipolar cycloaddition performed on silicon substrates was reported by Lummerstorfer and Hoffman.⁴⁸ 11-Bromoundecylsiloxane monolayers were reacted with sodium azide to obtain the corresponding azide-terminated monolayers, which were subsequently reacted with different acetylenes (Scheme 2.11).



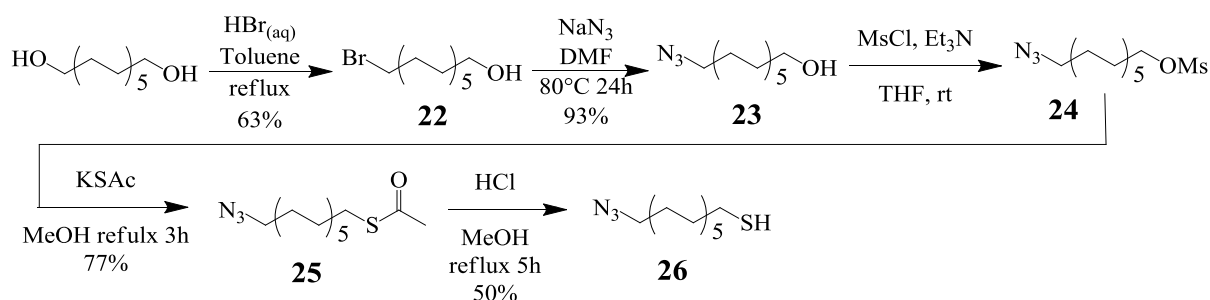
Scheme 2.11 Schematic representation of the 1,3-dipolar cycloaddition on Br-terminated monolayers on SiO₂ substrates. (a) S_N2 with NaN₃ and (b) cycloaddition with methoxycarbonyl or bis(ethoxycarbonyl) acetylene.

In our synthetic strategy to prepare surface-bound receptors, the Sonogashira coupling between a terminal alkyne linker and an aryl iodide is employed to synthesize different receptors suited for surface grafting techniques. However, a terminal aryl alkyne is in itself a useful intermediate for click modification of a surface. The design of the required receptor started with a protected alkyne, trimethylsilylethyne, to couple with compound **3**, followed by deprotection with lithium hydroxide to afford the receptor with terminal alkyne group **9** in 74% yield.



Scheme 2.12 Synthesis of the alkyne-terminated receptor **9**

To prepare a gold surface modified with azido end groups, 12-azidododecan-1-thiol **26** was synthesized starting from 1,12-dihydroxydodecane. One of the hydroxyl groups was replaced by a bromine atom (**22**), followed by substitution with sodium azide to provide the azido group (**23**). The remaining hydroxyl group was then activated using methanesulfonyl chloride (**24**), followed by substitution with potassium thioacetate to afford **25**. Finally the thioacetate group was hydrolyzed by refluxing in methanol with hydrochloric acid to afford the desired thiol molecule **26** (Scheme 2.13).



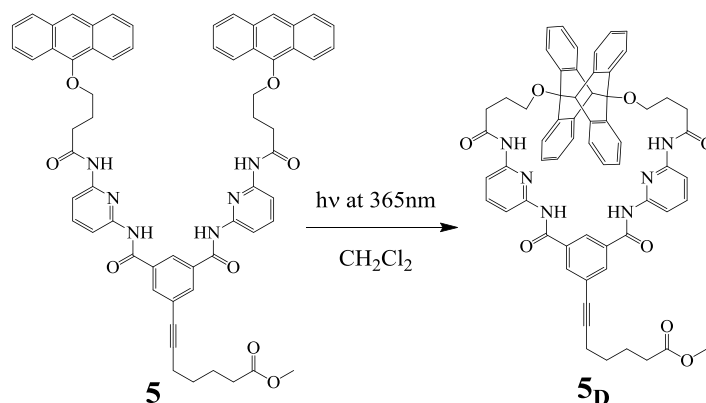
Scheme 2.13 Synthesis of 12-azido-dodecanyl thiol **26**

2.8 Synthesis of photodimerized receptors

The bisanthracene receptors **5-9** are designed to undergo photoinduced intramolecular

photodimerization as a means of controlling access to the binding cleft. For this reason, it is interesting to have access to substrates grafted with either the open or closed form of the receptors. The latter can be obtained by preparative scale irradiation of the open receptor in solution, and then isolated and characterized.

Upon photo-irradiation at 365 nm in CH_2Cl_2 , the two anthracene moieties in compound **5** undergo photodimerization via a $[4\pi + 4\pi]$ cyclization.^{49,50} The reaction can be monitored using absorption spectroscopy by observing the disappearance of the characteristic absorption bands of anthracene at 350, 371 and 390 nm.



Scheme 2.14 Photodimerization of receptor-ester **5**

During irradiation, it is important to exclude oxygen to avoid photo-oxidation of the anthracenes. Therefore, successive freeze-pump-thaw cycles were used for small-scale irradiation, while continuous bubbling of nitrogen or argon was used for larger-scale preparations. The effect of incomplete oxygen removal can be seen in Fig 2.3, in which the formation of anthraquinones may be inferred on the basis of their characteristic absorption tail at 340 nm. The photodimer **5D**, after isolation by column chromatography, was used for calibration of the HPLC detector in view of quantitative analyses for the determination of quantum yields.

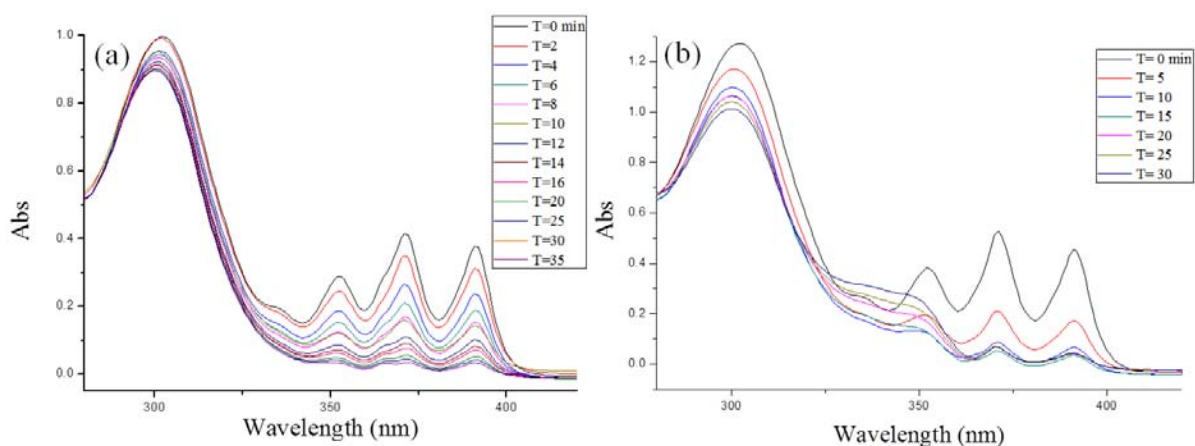
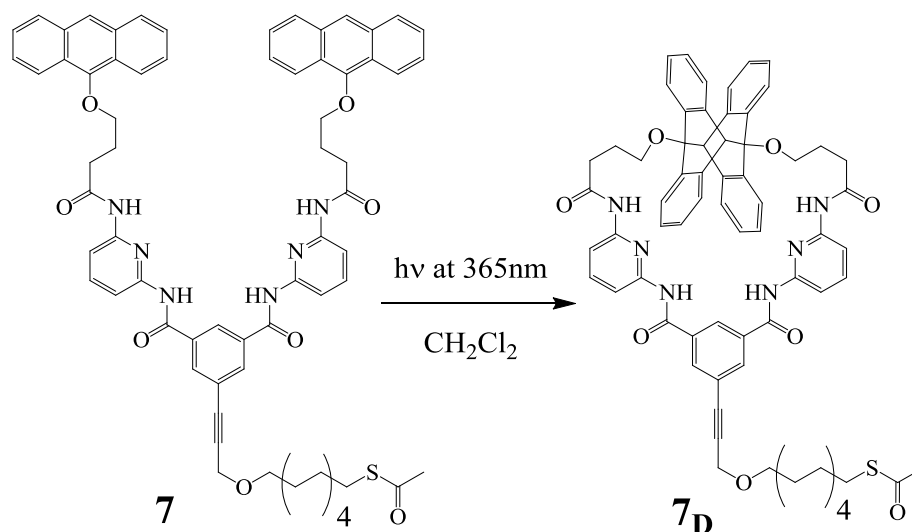


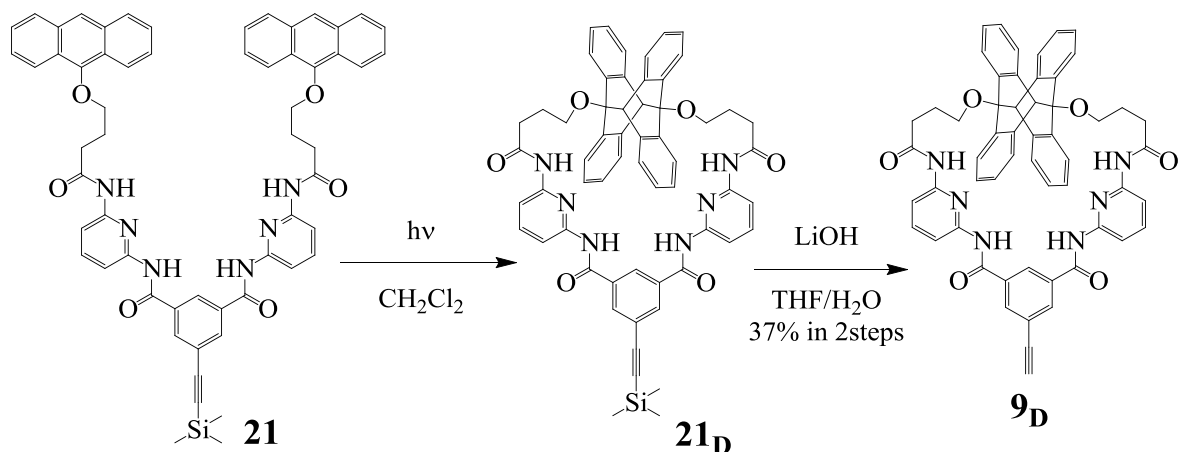
Figure 2.3 Evolution of the absorption of **5** upon irradiation using 365-nm light under fully degassed (a) and partially degassed (b) conditions.

To obtain samples of photodimerized receptor for the preparation of control substrates to compare the properties between open and closed receptor surfaces, compound **7** possessing a thioacetate group was irradiated at 365 nm. However, some residual open receptor remained and proved difficult to purify by conventional column chromatography due to their similar R_f of **7** and **7_D**. Therefore, the photolyzed receptor was further purified using a semi-preparative HPLC (kromasil silica column, gradient ethyl acetate/cyclohexane as mobile phase) to afford pure closed photodimer (Scheme 2.15). Both HPLC and mass spectroscopy confirmed that only the intramolecular photodimer was formed, and no intermolecular photodimer formation during the photoirradiation could be detected.



Scheme 2.15 Photodimerization of receptor-SAc **7_D**

The receptor possessing a terminal alkyne (**9**) was used for surface modification via a click reaction approach. Therefore the corresponding closed receptor with a terminal alkyne was also necessary. The preparation of **9_D**, began with the photodimerization of the TMS-protected receptor **21**. Subsequent deprotection of the TMS group was performed directly without further purification of the photodimer, and therefore only one purification step is required (Scheme 2.16). It is also worth mentioning that the solubility of **9_D** in CH_2Cl_2 is poor, as is often the case for anthracene photodimers. After irradiation and deprotection of the TMS group, the solid obtained was washed with CH_2Cl_2 to remove any remaining open receptor, affording the pure photodimer **9_D** without the need for HPLC separation. Again, mass spectrum analysis confirmed that only the intramolecular photodimerization was obtained.



Scheme 2.16 Synthesis of the photodimer **9_D**

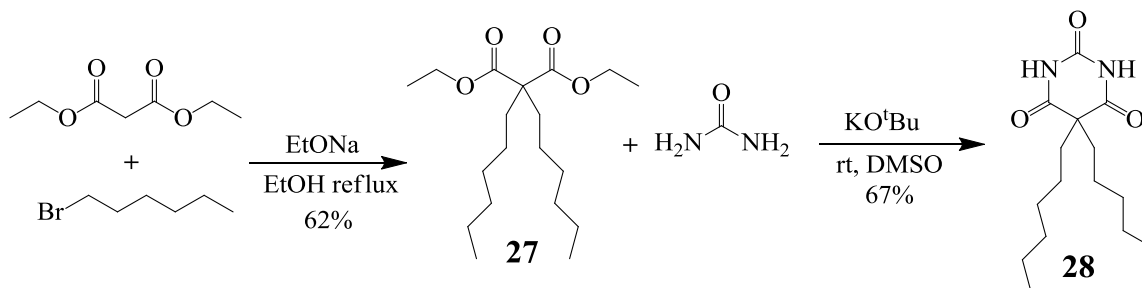
2.9 Synthesis of barbiturate guest molecules

To exploit the binding properties of the receptor molecules, a series of functionalized barbituric acid derivatives was gathered. These include functionalities based on photo- or electro-active substituents, such as pyrene, ferrocene or fullerene, or substituents capable of interacting with DNA (9-aminoacridine), as presented in Figure 2.4.

Compound Structure					
Targeted process	Binding ability	Energy transfer	Electrochemistry	Electron transfer	DNA binding
Source	Synthesized	Nathan McClellan	Gift from Prof. JHR Tucker	Synthesized	Synthesized

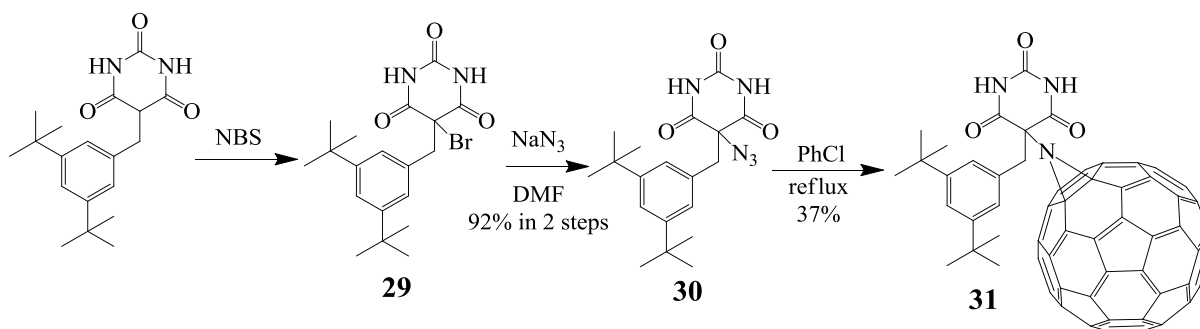
Figure. 2.4 Guest molecules used in this thesis

All of the guest molecules possess six hydrogen bonding sites that are fully complementary in number and spatial organization to those of the receptor binding site. Dihexyl barbituric acid is prepared from the di-substitution reaction of diethyl malonate with 5-bromohexane, followed by condensation with urea using potassium *tert*-butoxide as a base in DMSO. The introduction of the hexyl chains is used to increase the solubility of barbituric acid in less-polar solvents such as dichloromethane (Scheme 2.17).



Scheme 2.17 Synthesis of dihexyl barbituric acid **28**

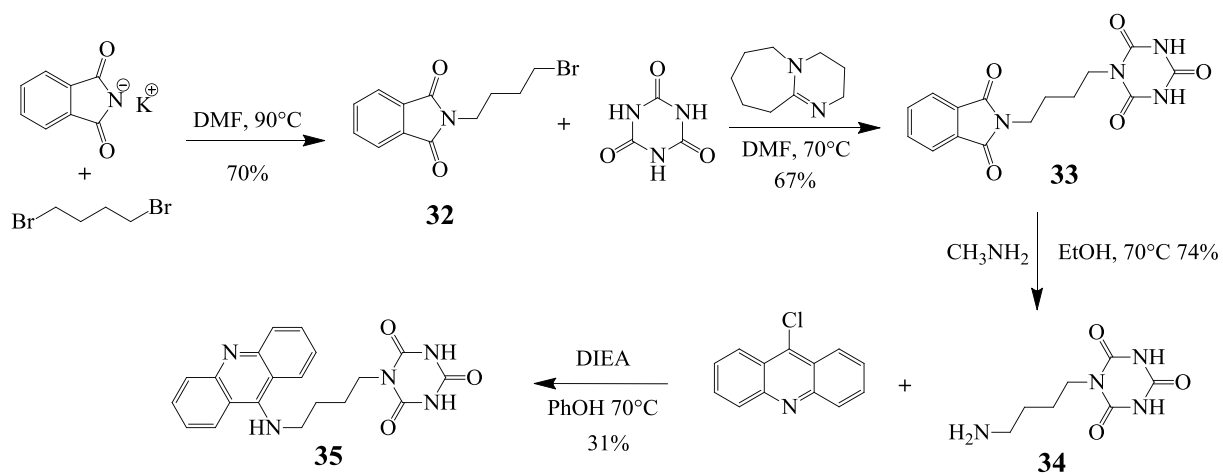
A fullerene-barbituric acid analogue, compound **31**, can be used to investigate the effect of supramolecular interaction on the electronic properties of supramolecular architectures. The synthetic route was previously developed in our group.⁵¹ An aromatic ring with two *tert*-butyl groups was introduced in order to enhance the solubility of the fullerene. Bromination and subsequent introduction of the azide provided precursor **30**. The [3+2] cyclization onto fullerene C₆₀, followed by nitrogen elimination, proceeded smoothly in chlorobenzene to afford the desired compound (Scheme 2.18).



Scheme 2.18 Synthesis of barbiturate-C₆₀ **31**

For more elaborate biological systems such as DNA or enzymes, direct covalent modification with a barbituric acid moiety is not desirable. In these systems, an attractive option may be to design a tether joining barbituric acid and a functional unit capable of binding the biological component. For example, substrates or co-factors are routinely employed to bind proteins to surfaces, whereas intercalators (*e.g.* 9-aminoacridines) are used to bind DNA. Therefore, an acridine-cyanuric acid molecule was synthesized as shown in Scheme 2.19. The cyanuric acid part can provide H-binding ability to the receptor molecule while the aminoacridine part can bind to double stranded-DNA or RNA due to its ability to intercalate.⁵² The synthesis of compound **35** begins with the reaction of potassium phthalimide with 1,4-dibromobutane to provide a protected amine possessing an alkyl bromide. Subsequent substitution of the bromide with cyanuric acid in the presence of DBU as a base afforded compound **33**. The phthalimide group was removed using methylamine to provide a terminal primary amino group **34** for the final step. The last reaction was to react the amino group with 9-chloroacridine in the presence of diisopropylethyl amine in phenol to

afford the desired guest molecule **35**.



Scheme 2.19 Synthesis of acridine-cyanuric acid dyad **35**

Reference

1. M. Mrksich, *Chem. Soc. Rev.*, **2000**, 29, 267-273.
2. M. C. Pirrung, *Angew. Chem., Int. Ed.*, **2002**, 41, 1276-1289.
3. A. Ulman, *Chem. Rev.*, **1996**, 96, 1533-1554.
4. C. D. Bain, J. Evall and G. M. Whitesides, *J. Am. Chem. Soc.*, **1989**, 111, 7155-7164.
5. J. C. Love, L. A. Estroff, J. K. Kriebel, R. G. Nuzzo and G. M. Whitesides, *Chem. Rev.*, **2005**, 105, 1103-1169.
6. V. Chechik, R. M. Crooks and C. J. M. Stirling, *Adv. Mater.*, **2000**, 12, 1161-1171.
7. S. Onclin, B. J. Ravoo and D. N. Reinhoudt, *Angew. Chem., Int. Ed.*, **2005**, 44, 6282-6304.
8. T. P. Sullivan and W. T. S. Huck, *Eur. J. Org. Chem.*, **2003**, 17-29.
9. C. Haensch, S. Hoeppener and U. S. Schubert, *Chem. Soc. Rev.*, **2010**, 39, 2323-2334.
10. I. Engquist, M. Lestelius and B. Liedberg, *Langmuir*, **1997**, 13, 4003-4012.
11. A. Ulman and N. Tillman, *Langmuir*, **1989**, 5, 1418-1420.
12. N. Balachander and C. N. Sukenik, *Langmuir*, **1990**, 6, 1621-1627.
13. G. E. Fryxell, P. C. Rieke, L. L. Wood, M. H. Engelhard, R. E. Williford, G. L. Graff, A. A. Campbell, R. J. Wiacek, L. Lee and A. Halverson, *Langmuir*, **1996**, 12, 5064-5075.
14. J. H. Moon, J. W. Shin, S. Y. Kim and J. W. Park, *Langmuir*, **1996**, 12, 4621-4624.
15. D. G. Kurth and T. Bein, *Langmuir*, **1993**, 9, 2965-2973.
16. M. A. Fox, J. K. Whitesell and A. J. McKerrow, *Langmuir*, **1998**, 14, 816-820.
17. F. Mirkhalaf and D. J. Schiffrin, *J. Chem. Soc., Faraday Trans.*, **1998**, 94, 1321-1327.
18. R. C. Horton, T. M. Herne and D. C. Myles, *J. Am. Chem. Soc.*, **1997**, 119, 12980-12981.
19. D. A. Hutt and G. J. Leggett, *Langmuir*, **1997**, 13, 2740-2748.
20. H. C. Yang, D. L. Dermody, C. Xu, A. J. Ricco and R. M. Crooks, *Langmuir*, **1996**, 12, 726-735.
21. N. K. Devaraj, P. H. Dinolfo, C. E. D. Chidsey and J. P. Collman, *J. Am. Chem. Soc.*, **2006**, 128, 1794-1795.
22. J. K. Lee, Y. S. Chi and I. S. Choi, *Langmuir*, **2004**, 20, 3844-3847.
23. Y. Zhang, S. Luo, Y. Tang, L. Yu, K.-Y. Hou, J.-P. Cheng, X. Zeng and P. G. Wang, *Anal. Chem.*, **2006**, 78, 2001-2008.
24. K. Motesharei and D. C. Myles, *J. Am. Chem. Soc.*, **1998**, 120, 7328-7336.
25. R. Chinchilla and C. Najera, *Chem. Rev.*, **2007**, 107, 874-922.
26. R. Chinchilla and C. Najera, *Chem. Soc. Rev.*, **2011**, 40, 5084-5121.
27. M. M. Heravi and S. Sadjadi, *Tetrahedron*, **2009**, 65, 7761-7775.
28. L. Kürti and B. Czako, *Strategic applications of named reactions in organic synthesis: background and detailed mechanisms*, Elsevier Academic Press, **2005**.

29. Y. Molard, D. M. Bassani, J.-P. Desvergne, P. N. Horton, M. B. Hursthouse and J. H. R. Tucker, *Angew. Chem., Int. Ed.*, **2005**, *44*, 1072-1075.
30. Y. Molard, D. M. Bassani, J.-P. Desvergne, N. Moran and J. H. R. Tucker, *J. Org. Chem.*, **2006**, *71*, 8523-8531.
31. J. M. Brockman, A. G. Frutos and R. M. Corn, *J. Am. Chem. Soc.*, **1999**, *121*, 8044-8051.
32. L. A. Chrisey, G. U. Lee and C. E. O'Ferrall, *Nucleic Acids Res.*, **1996**, *24*, 3031-3039.
33. Z. Guo, R. A. Guilfoyle, A. J. Thiel, R. Wang and L. M. Smith, *Nucleic Acids Res.*, **1994**, *22*, 5456-5465.
34. S. Fodor, J. Read, M. Pirrung, L. Stryer, A. Lu and D. Solas, *Science*, **1991**, *251*, 767-773.
35. G. H. McGall, A. D. Barone, M. Diggelmann, S. P. A. Fodor, E. Gentalen and N. Ngo, *J. Am. Chem. Soc.*, **1997**, *119*, 5081-5090.
36. D. Ray, C. Belin, F. Hui, B. Fabre, P. Hapiot and D. M. Bassani, *Chem. Commun.*, **2011**, *47*, 2547-2549.
37. P. Wagner, M. Hegner, H.-J. Guentherodt and G. Semenza, *Langmuir*, **1995**, *11*, 3867-3875.
38. Z. He, S. Bhattacharyya, M. C. Leavy, W. E. Cleland Jr, R. C. Sabapathy and C. L. Hussey, *J. Electroanal. Chem.*, **1998**, *458*, 7-16.
39. L. Cai, Y. Yao, J. Yang, D. W. Price and J. M. Tour, *Chem. Mater.*, **2002**, *14*, 2905-2909.
40. J. M. Tour, L. Jones, D. L. Pearson, J. J. S. Lamba, T. P. Burgin, G. M. Whitesides, D. L. Allara, A. N. Parikh and S. Atre, *J. Am. Chem. Soc.*, **1995**, *117*, 9529-9534.
41. J. Lahiri, E. Ostuni and G. M. Whitesides, *Langmuir*, **1999**, *15*, 2055-2060.
42. E. A. Smith, M. J. Wanat, Y. Cheng, S. V. P. Barreira, A. G. Frutos and R. M. Corn, *Langmuir*, **2001**, *17*, 2502-2507.
43. B. Fabre, D. M. Bassani, C.-K. Liang, D. Ray, F. Hui and P. Hapiot, *J. Phys. Chem. C*, **2011**, *115*, 14786-14796.
44. C. R. Becer, R. Hoogenboom and U. S. Schubert, *Angew. Chem., Int. Ed.*, **2009**, *48*, 4900-4908.
45. H. C. Kolb, M. G. Finn and K. B. Sharpless, *Angew. Chem., Int. Ed.*, **2001**, *40*, 2004-2021.
46. J.-F. Lutz, *Angew. Chem., Int. Ed.*, **2007**, *46*, 1018-1025.
47. R. Huisgen, *1,3-Dipolar Cycloaddition Chemistry*, Wiley, New York, **1984**.
48. T. Lummerstorfer and H. Hoffmann, *J. Phys. Chem. B*, **2004**, *108*, 3963-3966.
49. H. Bouas-Laurent, A. Castellan, J.-P. Desvergne and R. Lapouyade, *Chem. Soc. Rev.*, **2001**, *30*, 248-263.
50. H. Bouas-Laurent, A. Castellan, J.-P. Desvergne and R. Lapouyade, *Chem. Soc. Rev.*, **2000**, *29*, 43-55.
51. C.-C. Chu, *Hydrogen-bonded supramolecular materials for organic photovoltaic*

applications, UNIVERSITÉ BORDEAUX 1, **2009**.

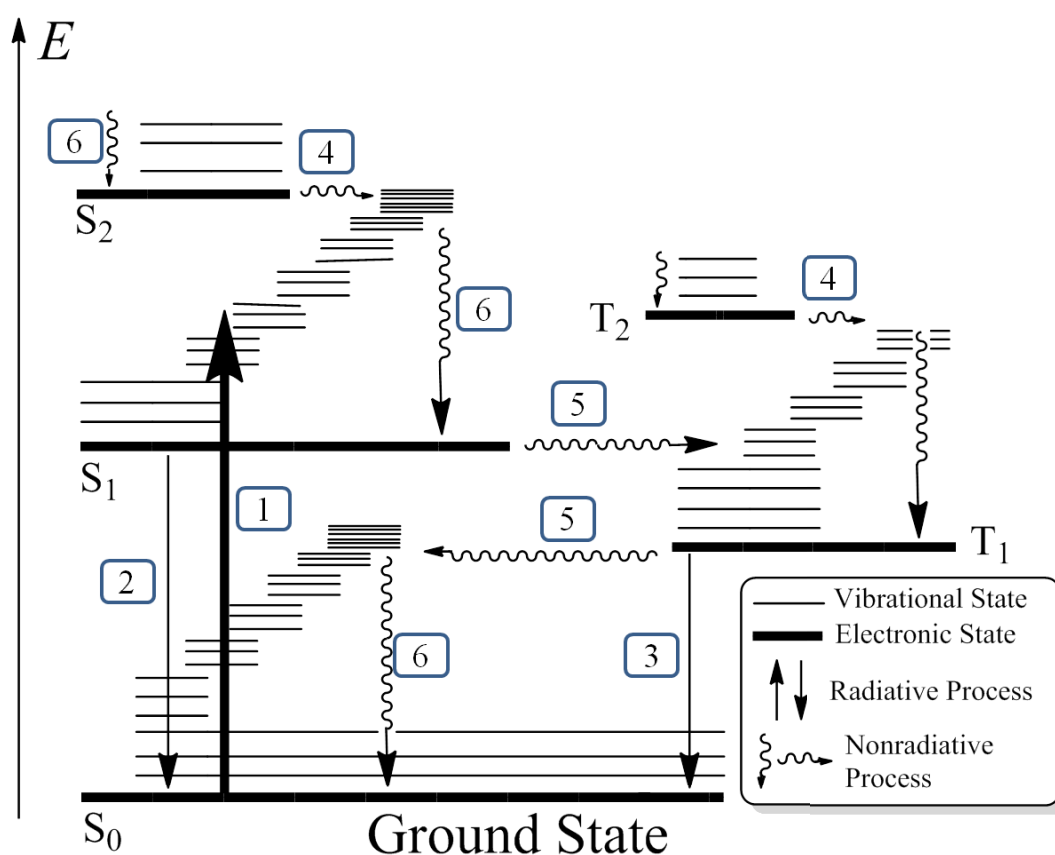
52. D. M. Bassani, J. Wirz, R. Hochstrasser and W. Leupin, *J. Photochem. Photobiol., A*, **1996**, *100*, 65-76.

Chapter 3. Photochemical and Photophysical Studies

3.1 Introduction to photochemistry

3.1.1 Jabłoński-Perrin diagram

The energy levels and excited-state processes of a molecule in its different electronic states are generally represented in a Jablonski-Perrin diagram.¹ The lowest horizontal line represents the ground-state electronic energy of the molecule which is generally a singlet state (S_0). At room temperature, the majority of the molecules in solution are in this state. The upper lines represent the energy levels of the electronically excited states: S_1 and S_2 represent the first and second electronic singlet states, respectively, whereas T_1 and T_2 represent the first and second electronically excited triplet states, respectively. The energy of the triplet state is lower than the energy of the corresponding singlet states by the electronic pairing energy, as expected from the Pauli principle.²



Scheme 3.1. Jabłoński Diagram: 1. absorption, 2. fluorescence, 3. phosphorescence, 4. internal conversion, 5. intersystem crossing, 6. vibrational relaxation

When a fluorophore absorbs light, it is usually excited to a higher vibrational energy level in the first or second excited state S_1 and S_2 , and then rapidly relaxes to the lowest energy level S_1 (Kasha's postulate).² This process is a combination of vibrational relaxation and internal conversion ($S_1 \leftarrow S_2$) and occurs in about a picosecond or less. Fluorescence rate

constants are typically four orders of magnitude slower than vibrational relaxation, giving the molecules ample time to achieve a thermally equilibrated lowest-energy excited state prior to fluorescence emission.

Photochemical processes are initiated from an electronically excited state and yield a primary photoproduct that is chemically different from the original reactant. Photochemical processes are in competition with photophysical processes that eventually return the reactant to the ground state by radiative or non-radiative dissipation of energy. Photoreactions can be efficient only if they are faster than the competing photophysical processes. Therefore, it is essential to have a feeling for the typical time scales of common photophysical processes (Table 3.1).¹

Table 3.1 Summary of Photophysical Process Time Scale¹

Process	Name	Time Scale (s)
1	Absorption	10^{-15}
2	Fluorescence	$10^{-9} - 10^{-7}$
3	Phosphorescence	$10^{-6} - 10^{-3}$
4	Internal Conversion	$10^{-12} - 10^{-6}$
5	Intersystem crossing (S→T)	$10^{-12} - 10^{-6}$
5	Intersystem crossing (T→S)	$10^{-9} - 10^{-1}$
6	Vibration Relaxation	$10^{-13} - 10^{-12}$

3.1.2 Fluorescence quantum yield

The quantum yield $\Phi_x(\lambda)$ is defined as equal to the number n_x of a photochemical or a photophysical event x that occurred divided by the number n_p of photons that were absorbed by the reactant. Both n_x and n_p are measured in moles or einsteins (1 einstein = 1 mole of photons) and the maximum value of Φ_x is unity (eq 3.1).

$$\Phi_x = \frac{n_x}{n_p} \quad \text{eq. 3.1}$$

A general expression for the quantum yield of a photochemical processes p is given by:

$$\Phi_p = \Phi^* \frac{k_p}{k_p + \sum k_i} = \Phi^* k_p \tau \quad \text{eq. 3.2}$$

where Φ^* is the formation efficiency of the excited state, k_p is the rate constant for the process p , $\sum k_i$ is the sum of all rate constants that deactivate the excited state (*e.g.* internal conversion or intersystem crossing) except k_p , and τ is the lifetime of the excited state; i.e., $\tau \equiv (k_p + \sum k_i)^{-1}$.² The experimental lifetime τ , and therefore the experimental quantum

yield of the process Φ_F , depend crucially on the magnitude $\sum k_i$, which can be dependent of the experimental conditions.

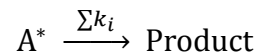
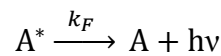
Data derived from fluorescence are best interpreted in terms of eq. 3.3 which is a specific form of eq. 3.2 ($\Phi^* = 1.0$, since the emitting state is the absorbing state).

$$\Phi_F = \frac{k_F}{k_F + \sum k_i} = k_F \tau \quad \text{eq. 3.3}$$

where $\tau = (k_F + \sum k_i)^{-1}$. The equation above has two limiting situations: (a) $k_F \gg \sum k_i$, in which case $\Phi_F \sim 1.0$, and (b) $\sum k_i \gg k_F$ in which case $\Phi_F \equiv k_F / \sum k_i$. In terms of these limits we note that Φ_F will approach unity when $\sum k_i$ is very small. Also Φ_F tends towards zero when $\sum k_i$ is very large.

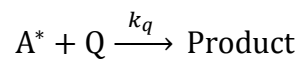
3.1.3 Stern-Volmer quenching relation

The Stern-Volmer relationship, named after Otto Stern and Max Volmer, describes the relative efficiency of a photophysical intermolecular deactivation process based on the rate constants of the intervening competitive processes.² An excited molecule (A^*) can be deactivated by emission of light, a chemical reaction, or other photophysical process (*e.g.* internal conversion or intersystem crossing). The reaction efficiency of emissive relaxation (Φ_F) can be expressed in the following manner:



$$\Phi_F = \frac{k_F}{k_F + \sum k_i} = k_F \tau \quad (\tau \equiv \frac{1}{k_F + \sum k_i}) \quad \text{eq. 3.4}$$

where k_F and k_i represent the rate constant of the radiative and non-radiative pathways, respectively. In the presence of an added quencher, an additional deactivation pathway is introduced. The bimolecular rate constant, k_q describes the quenching process by quencher Q according to



In the presence of Q, Φ_F becomes:

$$\Phi_F = \frac{k_F}{k_F + \sum k_i + k_q [Q]} \quad \text{eq. 3.5}$$

By recording the relative fluorescence intensity or lifetime in the absence and presence of added quencher, a straight line described by Equation 3.6 is obtained, where the slope $k_q \tau$ is termed as the Stern-Volmer constant K_{sv} , which indicates the quenching efficiency and is equal to the product $k_q \tau$.

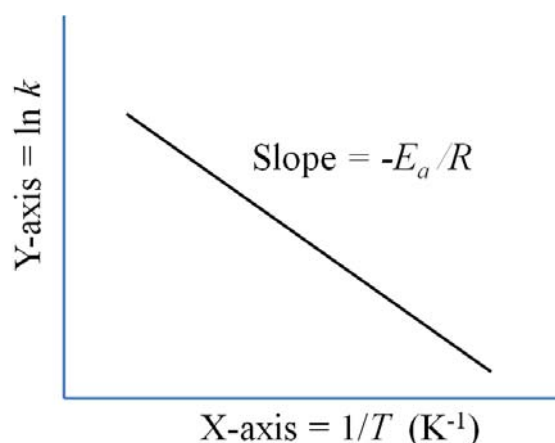
$$\frac{\Phi_R^0}{\Phi_R} = \left(\frac{k_R}{k_R + k_{nR}} \right) \left(\frac{k_R + k_{nR} + k_q[Q]}{k_R} \right) = 1 + k_q \tau [Q] = 1 + K_{sv} [Q] \quad \text{eq. 3.6}$$

3.1.4 Arrhenius plot

In 1889 the Swedish chemist Svante Arrhenius proposed the Arrhenius equation from his direct observations of the plots of the variation of chemical rate constants with temperature. In short, the Arrhenius equation gives the dependence of the rate constant k of chemical reactions on the temperature T (in absolute temperature Kelvins) and activation energy E_a .^{3,4}

$$k = Ae^{-\frac{E_a}{RT}} \quad \text{or} \quad \ln k = \ln A - \frac{E_a}{R} \cdot \frac{1}{T} \quad \text{eq. 3.7}$$

The units of the pre-exponential factor A are identical to those of the rate constant and will vary depending on the order of the reaction. Simply put, k is the number of collisions that result in a reaction per second, A is the total number of collisions (leading to a reaction or not) per second and $e^{-E_a/RT}$ is the probability that any given collision will result in a reaction. Thus it can be seen that either increasing the temperature or decreasing the activation energy (ex. through the use of catalysts) will result in an increase in the rate of reaction.



Scheme 3.2 Example of Arrhenius plot

3.2 Introduction to anthracene photodimerization

Owing to their versatile photophysical and photochemical properties, anthracene and its derivatives are employed in many systems, for instance as triplet sensitizers,⁵⁻⁸ molecular fluorosensors,⁹⁻¹⁵ electron acceptor or donor chromophores in artificial photosynthesis,¹⁶⁻¹⁸ photochromic substrates in 3D memory materials,^{19,20} *etc.* One remarkable feature is their ability to undergo $[4\pi + 4\pi]$ photodimerization under UV irradiation which induces

considerable changes in their physical properties, and this has been applied in molecular or ionic receptors, to modulate their binding ability.²¹⁻²⁵ Two examples in which anthracene photodimerization is used to modulate the binding ability for receptors are shown in Figure 3.1.

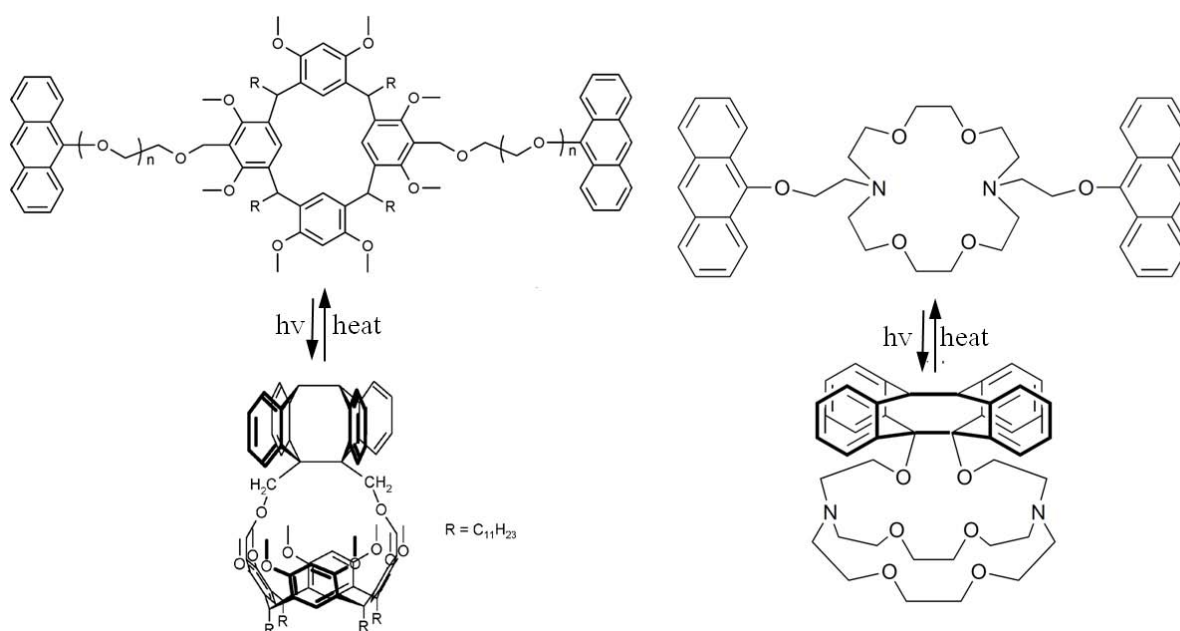
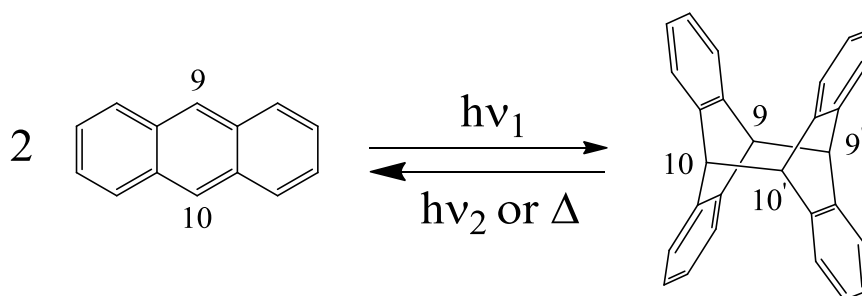


Figure 3.1 Two examples in which anthracene photodimerization is used to modulate the binding ability for receptors.^{23,25} Adapted with permission from ref 23,25. Copyright 1969 and 2004 Royal Society of Chemistry.

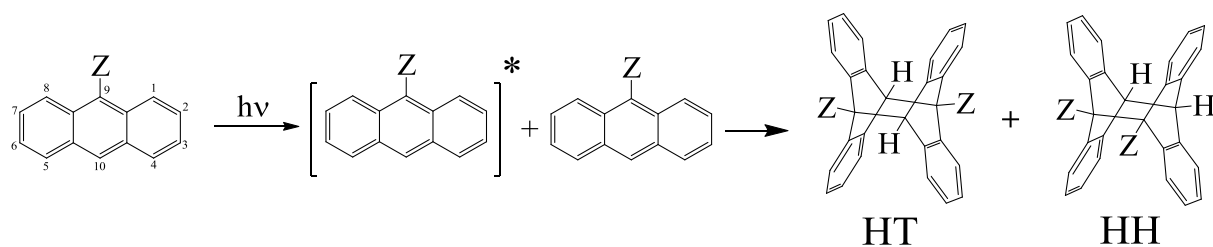
3.2.1 Structural aspects of the photodimerization of anthracenes

Photodimerization reactions are of special interest in synthesis and have been the object of extensive studies.²⁶⁻³⁰ One of the oldest known photodimerizations is that of anthracene outlined in Scheme 3.3: Upon photoirradiation in a degassed solution, two anthracenes form a photodimer, which can revert back to anthracene thermally or under UV irradiation below 300 nm.³¹⁻³⁷



Scheme 3.3 Photodimerization of anthracenes

In the case of two anthracene monomers substituted in C-9 position, photodimerization with a head-to-tail (HT, also termed *trans*) or a head-to-head (HH, also termed *cis*) orientation to form the HT and HH photodimers (Scheme 3.4), can take place.



Scheme 3.4 Head-to-tail (HT) and head-to-head (HH) anthracene photodimers

Because the photodimerization process results in the reduction of the conjugated system (from anthracene to *ortho*-disubstituted benzene) the photodimers do not absorb light > 300 nm, as shown in their UV spectra (Figure 3.2). Such a shift between the absorption spectra is an advantage for kinetic studies and instills photochromic properties to the system, allowing direct monitoring of the transformation to the photoproducts using absorption spectroscopy.

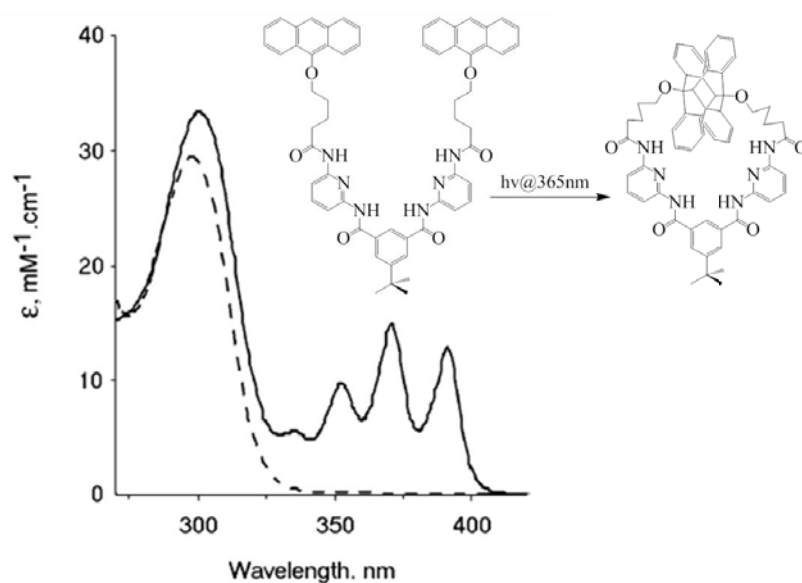


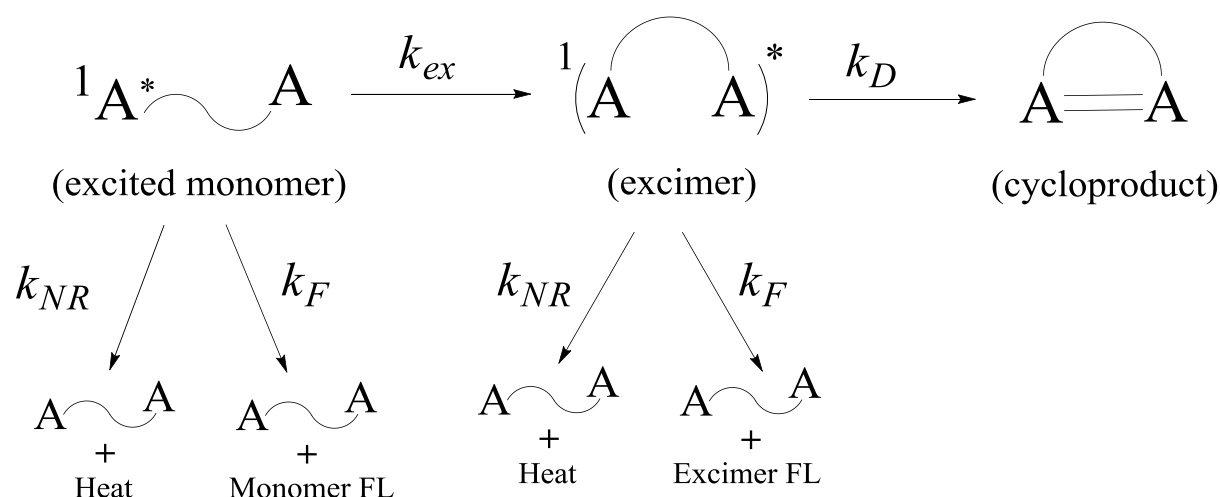
Figure 3.2 Absorption spectra of anthracene receptor **2** (solid line) and its photodimer (dashed line) in CH_2Cl_2 ^{38,39} Adapted with permission from ref 39. Copyright 2006 American Chemical Society.

3.2.2 Mechanistic aspects of intramolecular photodimerization of anthracenes

Based on fluorescence quenching, photodimerization quantum yields measurements, and the influence of dioxygen and solvents, a singlet state pathway has been proposed for the

photodimerization of anthracene.²⁹ The photodimerization rate constants are found to be generally high, unless they are slowed down or inhibited by steric strain. In several cases, emissive excimers have been demonstrated to be intermediates and in such cases it was shown that excimer fluorescence and photocyclization are competitive processes.^{34,35,40,41}

In bis-anthracene systems, both intramolecular and intermolecular photodimerization are possible. For unsubstituted anthracene, and most of its derivatives, it has been shown that intermolecular photodimerization via the excited singlet state is competitive at concentrations $\geq 10^{-3}$ M.⁴² In our anthracene-appended receptor system, we would like to avoid the possibility of intermolecular photodimerization, and therefore the sample concentrations were always kept at or below 10^{-5} M. In the following, we will discuss the intramolecular photodimerization mechanism. In the classical reaction mechanism, a fluorescent excimer is proposed as an intermediate as described in Scheme 3.5. Upon absorption of light by one of the anthracene chromophores, there are several possibilities: Fluorescence (k_F), non-radiative deactivation processes (k_{NR}) such as internal conversion (k_{IC}) or intersystem crossing (k_{ISC}), or formation of an excimer (k_{ex}). After excimer formation, the latter can emit excimer fluorescence, undergo non-radiative decay to the ground state or form cycloproducts (k_D).



Scheme 3.5 Kinetic scheme for intramolecular photocycloaddition via a fluorescent excimer.⁴³

3.3 UV absorption, fluorescence and photodimerization of receptor 5

As mentioned in the previous chapter, the receptor appended with a methyl ester chain (compound 5) was used to investigate the photophysical and photochemical behavior in solution. The UV absorption and fluorescence spectra of 5 are showed in Figure 3.3. The UV absorption reveals the characteristic absorption bands of anthracene at 350, 371 and 390 nm, which are attributed to vibronic transition as the 0-2, 0-1, and 0-0 vibrational bands of the $S_1 \leftarrow S_0$ 1L_b electronic transition. Similarly, the fluorescence also shows a vibronic progression at 400, 420, 440 nm corresponding to anthracene emission as the 0-0, 0-1, 0-2 bands. From

the similarity of the absorption and fluorescence spectra of **5** to that of 9-alkoxyanthracene **10**, it is concluded that the photophysical properties are mainly those of the anthracene chromophore which are not considerably affected by the functional groups of the receptor molecule.

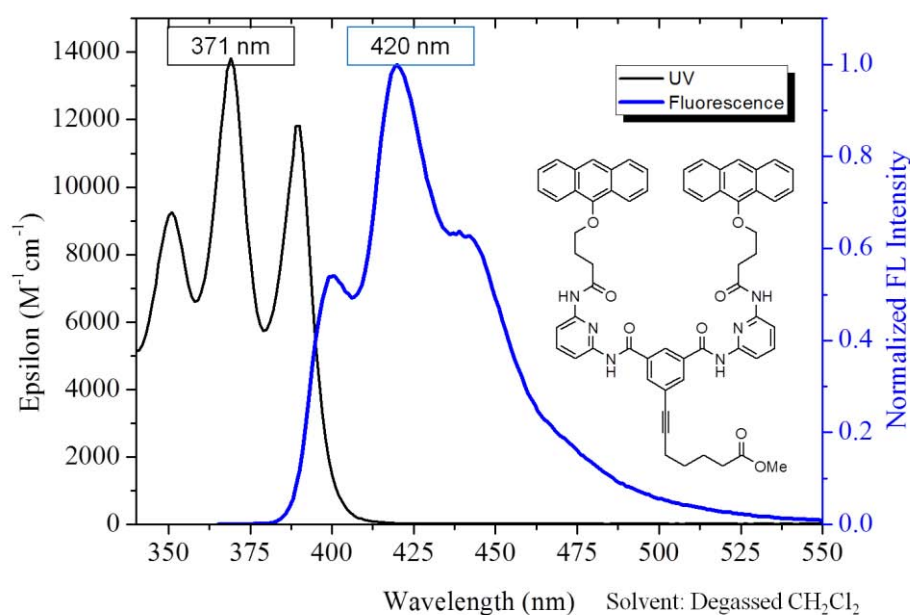


Figure 3.3 UV absorption and fluorescence emission ($\lambda_{\text{ex}} = 370 \text{ nm}$) of receptor **5** in degassed CH_2Cl_2

The two anthracene moieties on a receptor molecule such as **5** will undergo intramolecular photodimerization in dilute dichloromethane solution upon irradiation at 365 nm. According to previously described methodology,⁴⁴ the intensity of the irradiation was determined by chemical actinometry using potassium ferrioxalate as standard ($\Phi_{\text{Fe}^{2+}} \lambda_{\text{ex}}(365 \text{ nm}) = 1.21$). When exposed to light, 85% of **5** ($4.7 \times 10^{-5} \text{ M}$, 3.5 mL) is photodimerized within 2 hours and the photodimerization quantum yield is calculated to be 0.025 (Figure 3.4). The receptor is designed to bind barbiturates by utilizing multiple hydrogen bonding interactions, and therefore it is expected that the quantum yield of photodimerization is affected by adding barbiturate derivative into the receptor solution (Figure 3.5). The quantum yield for photodimerization of receptor **5** ($4.7 \times 10^{-5} \text{ M}$) in the presence of an excess of dihexylbarbiturate **28** ($4.8 \times 10^{-2} \text{ M}$, to ensure the complete consumption of **5**) is significantly decreased: Less than 15% is photodimerized upon > 6 hours of irradiation, giving a quantum yield of only 0.001. This effect is due to two anthracene moieties requiring close proximity to form an excimer and undergo photodimerization, which is reduced due to the binding to **28**. This therefore leads to a significant reduction of the photodimerization efficiency.

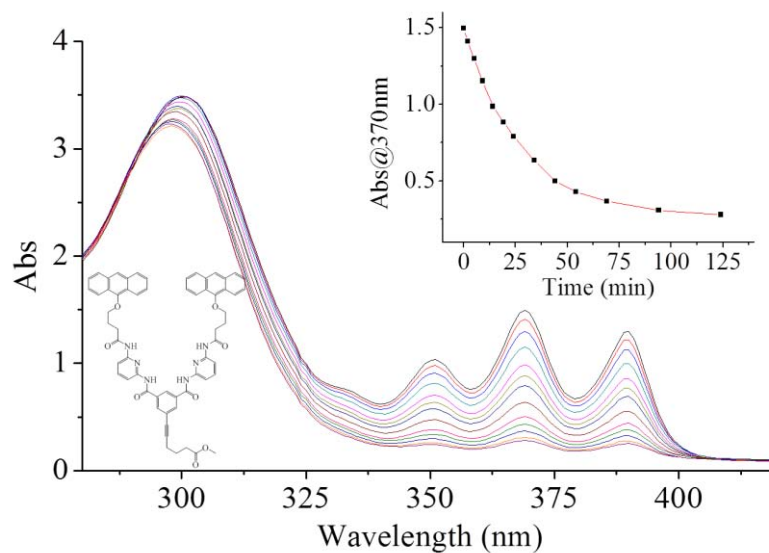


Figure 3.4 Photodimerization of receptor **5** ($4.7 \times 10^{-5}\text{M}$) in degassed CH_2Cl_2 at rt.

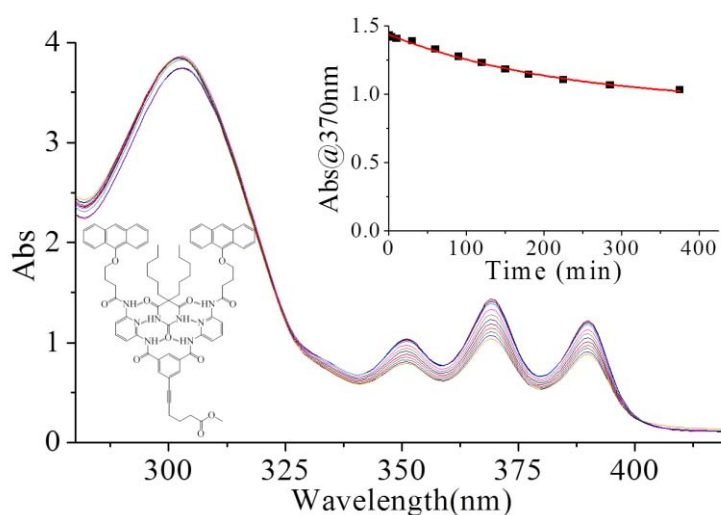
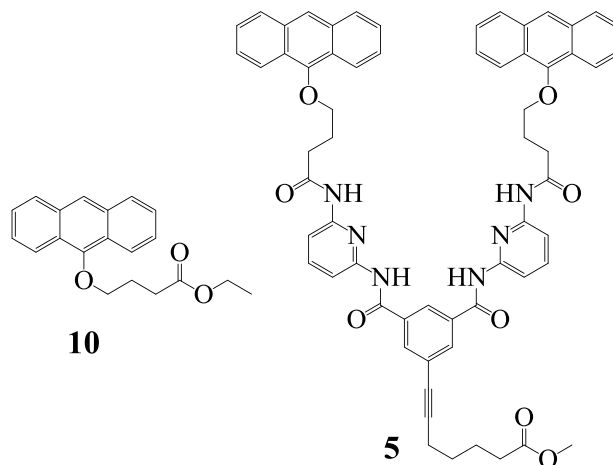


Figure 3.5 Photodimerization of receptor **5** ($4.7 \times 10^{-5}\text{M}$) with **28** ($4.8 \times 10^{-2}\text{M}$) in degassed CH_2Cl_2 at rt

3.4 Photophysical and photochemical properties of model and receptor molecules

Upon photo excitation, compound **5** can emit fluorescence, undergo uni-molecular processes such as internal conversion or intersystem crossing, or undergo intramolecular photodimerization. Therefore, to simplify such a complex system, the anthracene derivative **10** was studied as a sub-unit of **5**.



Scheme 3.6 Compound **10** is a single-chromophore model for the photophysical and photochemical properties of **5**.

3.4.1 Photophysical properties of anthracene compound **10**

The temperature dependence of the fluorescence quantum yield of **10** was determined in CH_2Cl_2 solution degassed by repeated freeze-pump-thaw cycles. According to a previously described methodology, quinine sulfate in 1 N sulfuric acid was used as a secondary standard for fluorescence emission intensity upon excitation at 366 nm ($\Phi_f = 0.54$).⁴⁴ The temperature variation was achieved using an Oxford Instrument variable-temperature liquid nitrogen cryostat (Optistat^{DN}) and an ITC⁵⁰² controller, which is designed to control the temperature between 77 K and 320 K with a precision of $\pm 0.1\text{K}$.

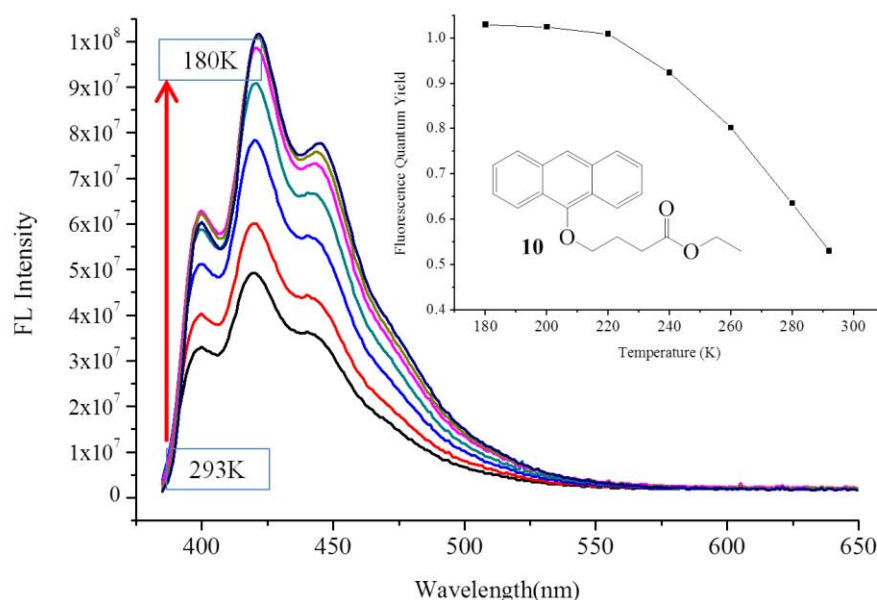
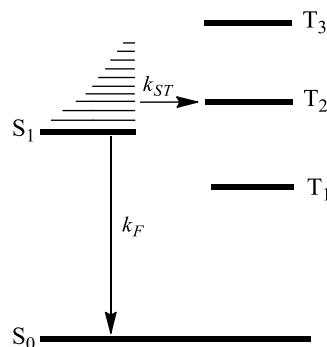


Figure. 3.6 Temperature dependence of the fluorescence emission and quantum yield of **10** ($2.6 \times 10^{-5}\text{M}$) in degassed CH_2Cl_2 .

The fluorescence quantum yield is increased when the temperature is lowered, and compound **10** becomes fully fluorescent below 220 K (Figure 3.6). It is known that in

anthracenes, the excited singlet state can undergo intersystem crossing to the triplet state via a temperature-activated process from a higher S_1 vibrational state (Scheme 3.7).⁴⁵⁻⁴⁷



Scheme 3.7 Kinetic scheme of competitive process between intersystem crossing and fluorescence in anthracene.⁵⁰

Fluorescence decay profiles of model compound **10** were also obtained at different temperatures in CH_2Cl_2 solutions that were degassed by three freeze-pump-thaw cycles. The data is collected in Table 3.2 and fitted to a one-exponential decay and the goodness-of-fit judged by the χ^2 values (1.04-1.19) and the residual distribution. The lifetime data were processed according to an Arrhenius plot to extract the temperature dependence. The plot of $\ln(1/\tau) = \ln(\sum k_i)$ vs T^{-1} is non-linear, which means that more than one temperature-dependent processes is present. It can be deduced that one of them is temperature independent (from 220K to 180K) while the other is a temperature dependent processes (from 300K to 220K). The temperature-independent process is attributed to fluorescence with a pre-exponential factor of $6.6 \times 10^7 \text{ s}^{-1}$ and nearly zero activation energy. The temperature-dependent process is attributed to a non-radiative process and the activation energy is calculated to be 7.72 kcal mol^{-1} with pre-exponential factor as $3.8 \times 10^{13} \text{ s}^{-1}$ (Figure 3.7).

Table 3.2 Lifetime of anthracene model **10**

Temp (K)	$\tau(\text{ns})$	χ^2	$\text{Ln}(1/\tau)$	Φ_F	$k_F(\text{s}^{-1})$
300	6.93	1.04	18.78	0.516	7.45×10^7
280	9.65	1.07	18.45	0.604	6.26×10^7
260	12.2	1.12	18.22	0.738	6.05×10^7
240	13.7	1.16	18.10	0.840	6.13×10^7
220	14.4	1.11	18.06	0.921	6.40×10^7
200	14.5	1.19	18.05	0.923	6.37×10^7
180	14.4	1.14	18.06	0.919	6.38×10^7

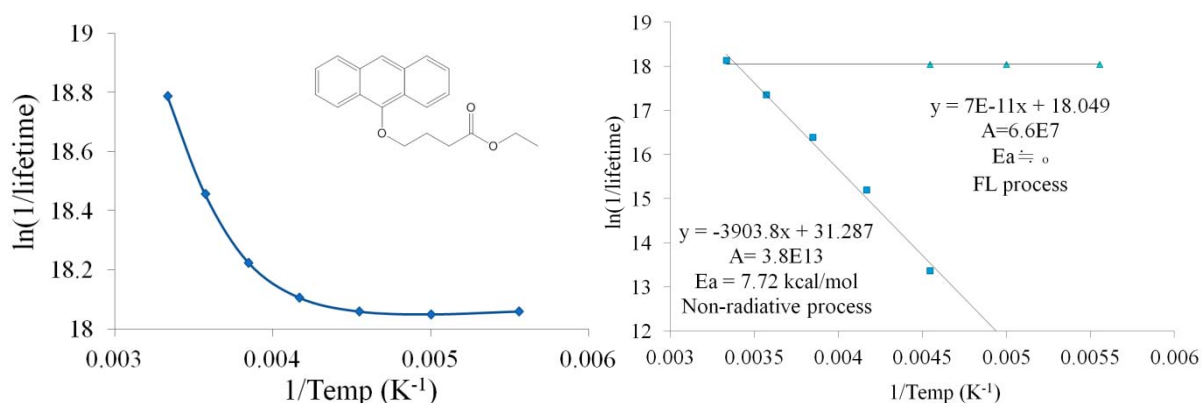


Figure 3.7 Arrhenius plot of anthracene **10** as obtained from experimental data (left) and as deduced from kinetic Scheme 3.7 (right)

3.4.2 Photophysical properties of **5**

The temperature dependence of the fluorescence quantum yield of compound **5** was determined in CH_2Cl_2 solution degassed by repeated freeze-pump-thaw cycles as described for compound **10**. A similar trend was observed, with increased fluorescence intensity at lower temperatures. The quantum yield reached a maximum of 0.93 when the temperature is lowered to 200 K.

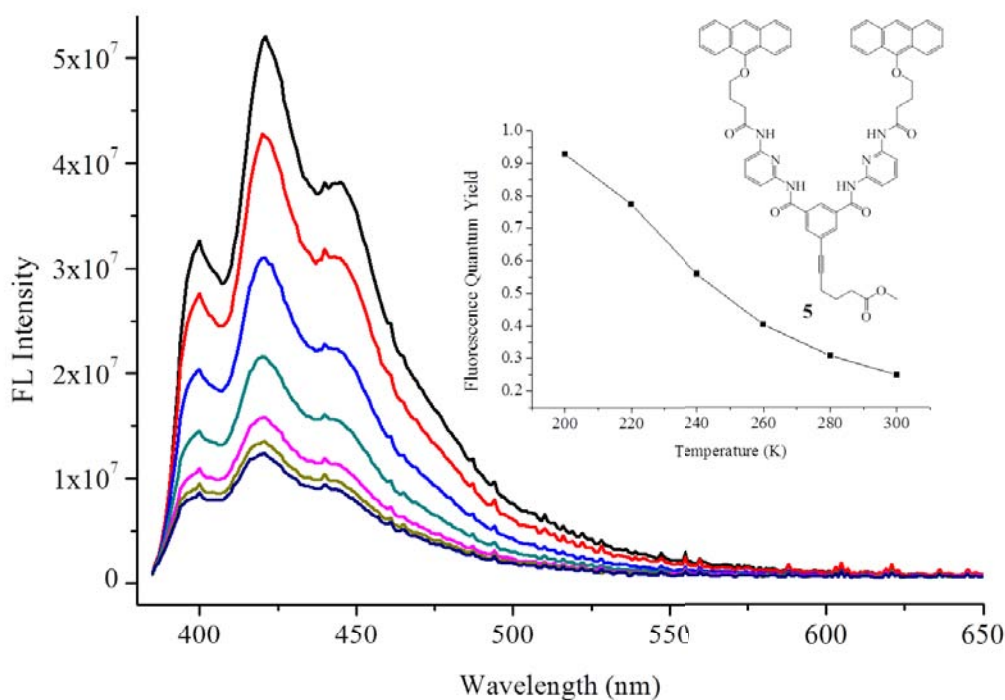


Figure 3.8 Temperature dependence of the fluorescence emission and quantum yield of **5** ($1.37 \times 10^{-5} \text{ M}$) in degassed CH_2Cl_2

Photoreaction quantum yields were determined upon excitation at 365 nm on an optical bench equipped with a 200 W Hg-Xe lamp and a monochromator using Aberchrome 540 as a chemical actinometer. Temperature variation was achieved as described for fluorescence measurements of compound **10**. Compound **5** (5×10^{-5} M in CH_2Cl_2) was stirred during the irradiation, and the amount of converted photoproduct was determined by HPLC using synthesized compound **5_D** as a reference. The schematic experimental setup is shown in Figure 3.9. A stream of argon was first passed through a dichloromethane solution to provide a saturated gas flow to degas the sample for 20 minutes without changing the sample concentration, and then the sample solution was transferred to the optical Dewar for irradiation for 5 minutes. After irradiation, the sample solution was withdrawn and immediately analyzed by HPLC using a kromasil silica column (ethyl acetate/cyclohexane, 10% EA to 100% EA in 50 minutes).

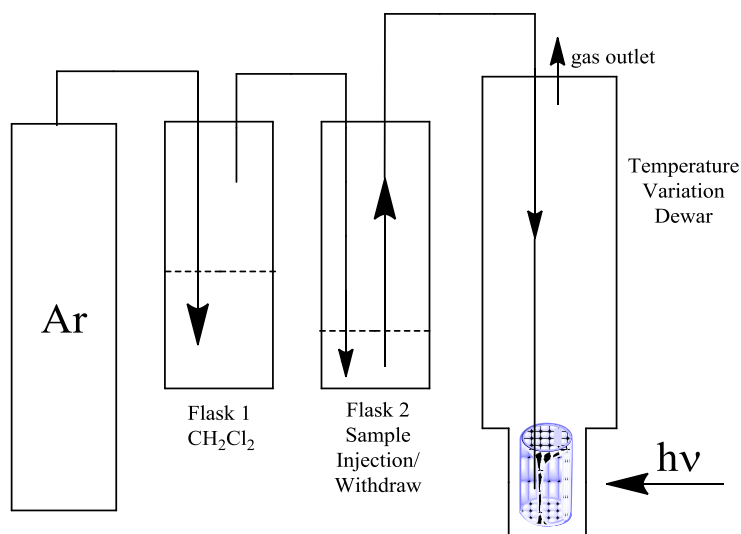
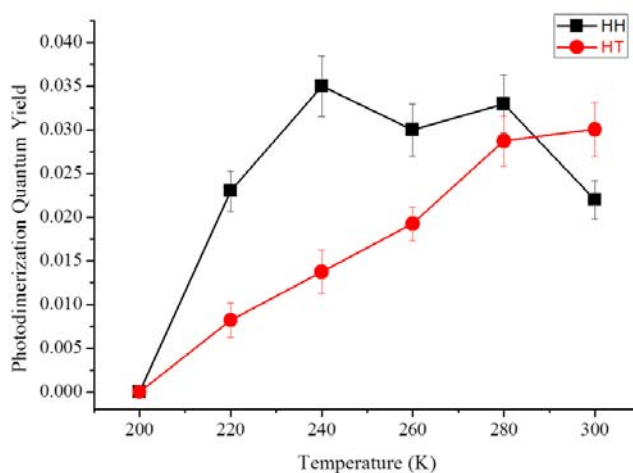


Figure 3.9 Experimental setup for the determination of photodimerization quantum yields at low temperature

It is known that upon photoirradiation, the 9-substituted anthracenes can form two different photodimers characterized by a head-to-tail (HT) or head-to-head (HH) geometry. It was previously shown that the HT photodimer is thermally stable, whereas the HH photodimer is thermally reversible at room temperature. Upon irradiation of compound **5**, HPLC analysis of the photolized solution shows the presence of two photoproducts, which are assigned to the HH and HT photodimers. To attribute the photoproducts, a sample was reanalyzed by HPLC after 48 hours. Only one photoproduct remained, which was assigned to the HT geometry. The loss of the HH photodimer was accompanied by an increase in the integral of **5** (Figure 3.10). At room temperature, both HT and HH photodimers are formed with similar quantum yields. When the temperature is decreased, the formation of the HT photodimer is found to be more temperature dependent compared to the HH photodimer. When the temperature is further lowered to 200 K, formation of both HH and HT photodimers is suppressed.



Scheme 3.8 Quantum yields for the formation of HH and HT photodimers of **5** as a function of temperature.

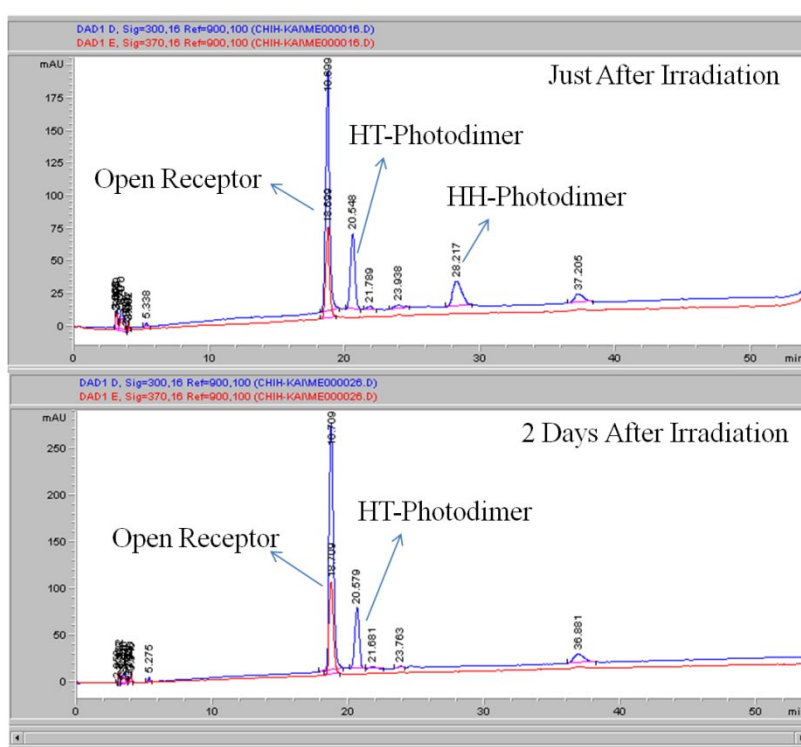


Figure 3.10 HPLC analysis of irradiated solution of **5**. Red trace is obtained at 370 nm, whereas blue trace is obtained at 300 nm

Fluorescence decay profiles of **5** were collected as function of temperature at three different emission wavelengths λ_{em} = 420, 500 and 550 nm in CH_2Cl_2 solutions that were degassed by three freeze-pump-thaw cycles. The data, collected in Tables 3.3 to 3.5, was obtained from fitting to a three-exponential decay function, and the goodness-of-fit judged by the χ^2 values (0.98-1.33) and the residual distributions. An example of typical decay curve is shown in Figure 3.11.

Table 3.3 Fluorescence decay of 5^a

420nm	τ_1	A_1	τ_2	A_2	τ_3	A_3	χ^2
300K	1.67	0.038 (13%)	4.77	0.023 (21%)	6.27E-5 s	5.36E-6 (66%)	1.12
280K	2.36	0.024(29%)	3.90	0.027(54%)	6.73	0.005(17%)	0.99
260K	1.02	0.004 (2%)	4.41	0.047 (90%)	7.93	0.002 (8%)	1.07
240K	3.04	0.001 (1%)	6.19	0.046 (97%)	0.7	0.0067 (2%)	0.97
220K	2.92	0.0016 (1%)	8.57	0.042 (96%)	0.57	0.015 (2%)	1.027
200K	4.7	0.0042 (5%)	11.1	0.035 (92%)	0.87	0.016 (3%)	1.05
180K	3.43	0.029 (14%)	12.6	0.046 (80%)	0.73	0.066 (6%)	1.15

^a In ns, λ_{ex} = 370 nm, λ_{em} = 420 nm in degassed CH₂Cl₂Table 3.4 Fluorescence decay of 5^a

500nm	τ_1	A_1	τ_2	A_2	τ_3	A_3	χ^2
300K	1.82	0.042 (40%)	0.22	-0.07 (8%)	5.33	0.025 (68%)	1.19
280K	2.6	0.028 (36%)	3.66	0.015 (27%)	7.06	0.0106 (37%)	1.209
260K	4.4	0.048 (84%)	11.9	0.0025 (12%)	25.9	0.0004 (4%)	1.18
240K	5.7	0.031 (55%)	7.05	0.016 (35%)	33.5	0.00096 (10%)	1.12
220K	5.42	0.004 (5%)	8.76	0.042 (85%)	48.7	8.36E-4 (10%)	1.1
200K	9.52	0.026 (46%)	12.95	0.019 (45%)	74.3	0.0007 (9%)	1.12
180K	0.29	-0.07 (-2%)	12	0.087 (82%)	49.4	0.0051 (20%)	1.33

^a In ns, λ_{ex} = 370 nm, λ_{em} = 500 nm in degassed CH₂Cl₂

Table 3.5 Fluorescence decay of **5**^a

550nm	τ_1	A_1	τ_2	A_2	τ_3	A_3	χ^2
300K	1.27	0.045 (23%)	0.51	-0.085 (-17%)	6.00	0.04 (94%)	1.22
280K	2.6	0.011 (18%)	7.2	0.014 (66%)	0.43	0.055 (16%)	0.96
260K	4.93	0.028 (49%)	18.2	0.0067 (43%)	0.65	0.035 (8%)	1.03
240K	6.42	0.034 (51%)	32.7	0.006 (46%)	0.76	0.017 (3%)	0.98
220K	0.743	0.013 (2%)	8.64	0.035 (50%)	52.2	0.005 (48%)	0.944
200K	12.16	0.034 (50%)	3.66	0.006 (3%)	79.5	0.005 (47%)	1.02
180K	13	0.065 (34%)	30.6	0.022 (28%)	96.9	0.001 (40%)	1.09

^a In ns, $\lambda_{\text{ex}} = 370$ nm, $\lambda_{\text{em}} = 550$ nm in degassed CH_2Cl_2

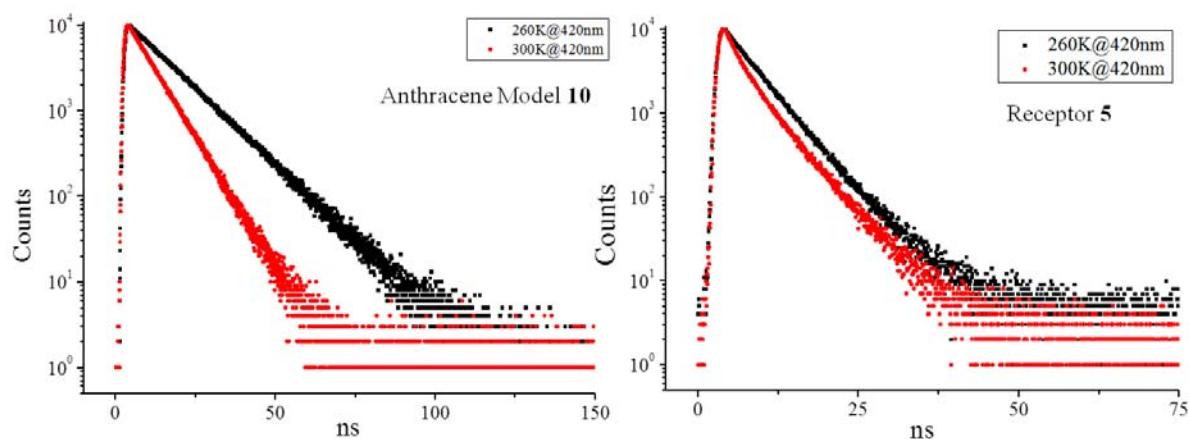


Figure 3.11 Fluorescence decay profile of **10** and **5** at 260K and 300K
($\lambda_{\text{ex}} = 370$ nm, $\lambda_{\text{em}} = 420$ nm)

Time-resolved emission spectra (TRES, Figure 3.12) were obtained at low temperature (200 K) to better understand the emission of **5**. After absorption of light, the initial emission profile of **5** is similar to the anthracene monomer fluorescence, in which the maximum is located at 420 nm. As time goes by, this anthracene monomer emission intensity continuously decreases, while a weaker red-shifted emission located at 530 nm remains. This broad red-shift emission is typical of excited-state dimers and is therefore assigned to excimer emission.

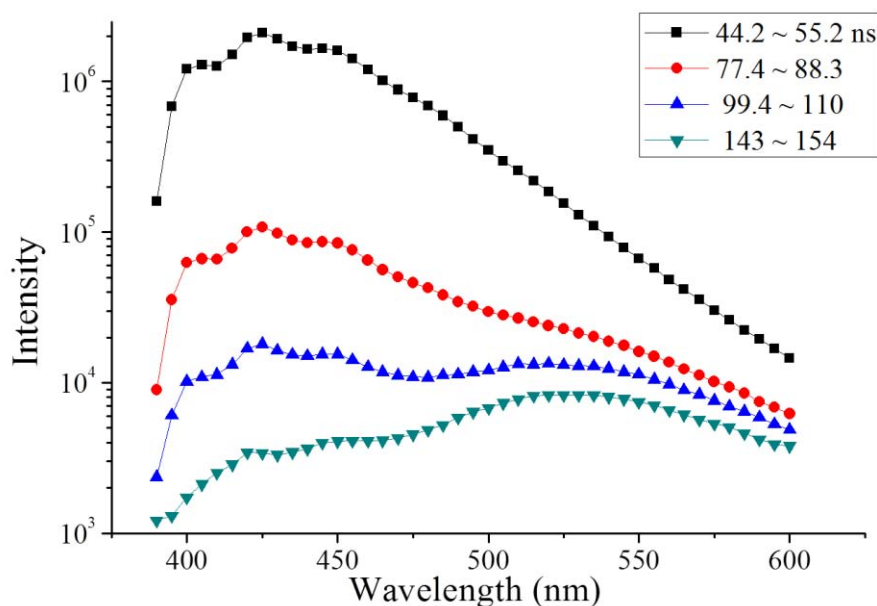


Figure 3.12 TRES of receptor **5** at 200 K (λ_{ex} = 370 nm in degassed CH_2Cl_2)

The parameters in the various decay functions in Table 3.3-3.5 are correlated and difficult to resolve. The resolution of correlated parameters can be improved by the use of global analysis in which the decay parameters are reduced to a single set that is fitted to all of the decay data.⁴⁸ Global analysis of the variable temperature decay profiles of **5** obtained at different emission wavelength can be fitted to a three-component model to yield a short decay component (τ_{LE}) and two longer decay components (τ_{ex1} and τ_{ex2} , Table 3.6). In Figure 3.13, one can observe that the parameters form three distinct series of decay constants, of which two are temperature dependent and one is temperature independent. The temperature independent decay is attributed to the locally excited anthracene state (LE) because it is very short-lived and more important in the shorter wavelength region, where anthracene emission is strongest. The other two decay components are attributed to excimer emission. The increased excimer lifetime at low temperature could be due to a lowered probability of intersystem crossing or photodimerization. The observation of structured emission, characteristic of the locally excited anthracene state, at long delays (> 100 ns) in the TRES (Fig 3.12) indicate that, at that temperature, excimer formation is reversible.

Table 3.6 Global analysis of decay parameters of **5** (ns)

Temp (K)	τ_{LE}	τ_{EX1}	τ_{EX2}
300	1.10	3.67	6.48
280	0.92	3.42	7.08
260	0.43	4.58	16.95
240	0.42	6.18	31.11
220	0.41	8.52	52.42
200	0.74	10.90	63.83

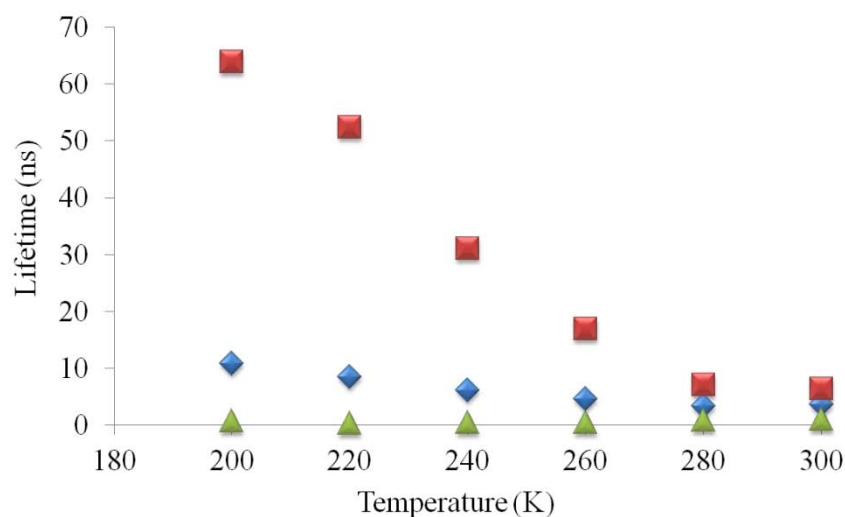
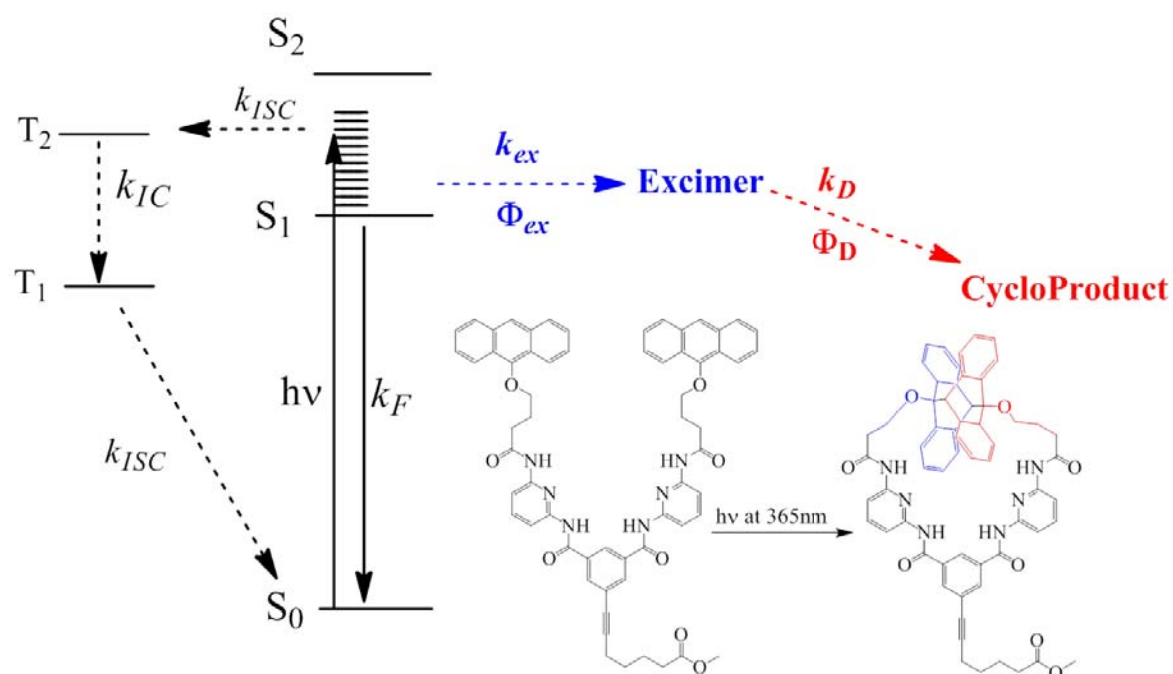
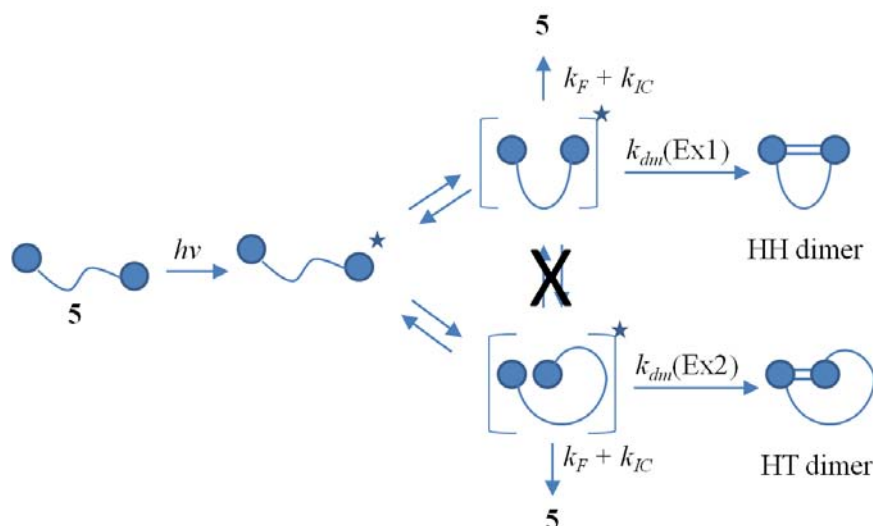


Figure 3.13 Temperature-dependence of the fluorescence decay data of **5**

A plausible kinetic scheme for the photodimerization of receptor **5** is shown in Scheme 3.10. In this model, the anthracenes in **5** are mobile and can explore various conformations in the ground state. After the absorption of light, one of the anthracene moieties is excited to its singlet excited state and reversibly forms one of the excimers with either a pseudo-HH or HT conformation that are not directly interconverting on a short timescale. The excimers can either undergo non-radiative or radiative deactivation to return to the ground state, or undergo photodimerization to form either the HH or HT photodimer.



Scheme 3.9 Reaction scheme of the photodimerization of **5**



Scheme 3.10 Photodimerization scheme *via* intramolecular excimer formation for receptor **5** in solution

The lifetime data of anthracene monomer **10** can be used to obtain the activation energy of the radiative and non-radiative process for the anthracene locally excited state. The shortest lifetime (less than 1 ns) is attributed to the residual emission from the anthracene. It is significantly shorter than that of compound **10** due to intramolecular deactivation processes. The two longer lifetimes are assigned to intramolecular excimers possessing different geometries and whose interconversion is slow with respect to their lifetime. Assuming that excimer dissociation to populate the locally excited state is negligible compared to the other deactivation pathways of the excimer, the data can then be used to extract the rate constants of excimer formation in receptor **5** by subtracting the reciprocal of the monomer lifetime from the reciprocal of the locally excited states lifetime in **5** (eq. 3.8).

$$k_{ex} = \frac{1}{\tau_{LE}} - \frac{1}{\tau_{anthracene\ 10}} \quad \text{eq. 3.8}$$

The overall quantum yield of excimer formation (i.e. $\Phi_{ex1} + \Phi_{ex2}$) can then be deduced from the excimer rate constant and the monomer lifetime (eq. 3.9).

$$\Phi_{ex} = \frac{k_{ex}}{k_{ex} + k_{monomer}} \left(\frac{1}{\tau_{monomer}} = k_{monomer} \right) \quad \text{eq. 3.9}$$

Table 3.7 $k_{excimer}$ calculation from $\tau_{monomer}$ and τ_{LE}				
Temp(K)	$\tau_{Anthracene10}(ns)$	$\tau_{LE}(ns)$	k_{ex}	Φ_{Ex}
300	6.9	1.1	7.61E+08	0.840
280	9.7	0.9	9.77E+08	0.904
260	12.2	0.4	2.22E+09	0.964
240	13.7	0.4	2.30E+09	0.969
220	14.4	0.4	2.36E+09	0.971
200	14.5	0.7	1.27E+09	0.948
180	14.4	1.2	7.52E+08	0.915

Since the HPLC-measured photodimerization quantum yield is the product of the quantum yield for excimer formation and the photodimerization quantum yield of the excimer (eq. 3.10),

$$\Phi_{HPLC} = \Phi_{ex} \times \Phi_{photodimerization} \quad \text{eq. 3.10}$$

the quantum yield of photodimerization can be therefore extracted by dividing the HPLC-measured quantum yields with the quantum yield of excimer formation:

$$\Phi_{photodimerization} = \frac{\Phi_{HPLC}}{\Phi_{ex}} \quad \text{eq. 3.11}$$

Table 3.8 Calculation of $\Phi_{photodimerization}$				
Temp(K)	Φ_{HT}	Φ_{HH}	$\Phi_D(HT)^a$	$\Phi_D(HH)^a$
300	0.030	0.022	3.6×10^{-2}	2.6×10^{-2}
280	0.029	0.034	3.2×10^{-2}	3.7×10^{-2}
260	0.019	0.031	2.0×10^{-2}	3.2×10^{-2}
240	0.014	0.036	1.4×10^{-2}	3.7×10^{-2}
220	0.008	0.023	0.8×10^{-2}	2.4×10^{-2}

$$^a\Phi_D = \Phi_{photodimerization}$$

The photodimerization rate constant can then be deduced by dividing the photodimerization quantum yield by excimer decay data. Because two excimer decays are observed, two possible answer sets are obtained.

$$k_{dimerization} = \frac{\Phi_{dimerization}}{\tau_{ex}} \quad \text{eq. 3.12}$$

Table 3.9 Calculation of $k_{Dimerization}$

Temp(K)	$k_D(\text{HT}-\tau_{\text{EX1}})$	$k_D(\text{HH}-\tau_{\text{EX2}})$	$k_D(\text{HT}-\tau_{\text{EX2}})$	$k_D(\text{HH}-\tau_{\text{EX1}})$
300	5.5×10^6	7.1×10^6	9.7×10^6	4.0×10^6
280	4.5×10^6	1.1×10^7	9.3×10^6	5.3×10^6
260	1.2×10^6	6.9×10^6	4.3×10^6	1.9×10^6
240	4.5×10^5	6.0×10^6	2.3×10^6	1.2×10^6
220	1.6×10^5	2.8×10^6	0.9×10^6	0.4×10^6

Finally, the activation energy of the photodimerization rates can be obtained through an Arrhenius plot.

$$\ln(k_{dimerization}) \text{ v.s } \frac{1}{T} \rightarrow E_a \quad \text{eq 3.13}$$

Combining all the experimental data, we arrive at two possible answer sets: The first originates from the attribution of the shorter lived excimer 1 to the formation of the HT photodimer, and the longer lived excimer 2 is assigned to the HH photodimer. This answer set gives similar activation energies for both HH and HT photodimer formation to be *ca.* 4 kcal mol⁻¹, as determined from the Arrhenius plots (Figure 3.14, left). The second answer set assigns the longer lived excimer 2 to the formation of the HT photodimer, with an activation energy of *ca.* 6.0 kcal mol⁻¹, and the shorter lived excimer 1 to the HH photodimer with a *ca.* 1.7 kcal mol⁻¹ activation energy (Figure 3.14, right) (Table 3.10). However, this reasoning assumes that the conformational equilibrium between the pseudo-HH and pseudo-HT geometries (Scheme 3.10) is relatively insensitive to temperature. Further experiments, such as competitive quenching experiments, are necessary to distinguish between the two possible answer sets.

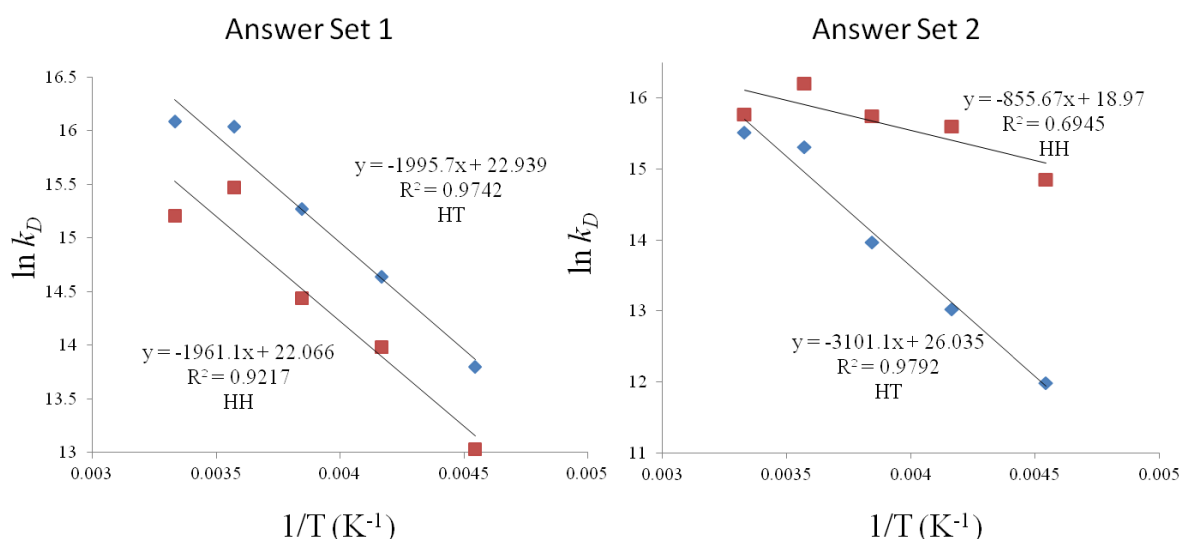
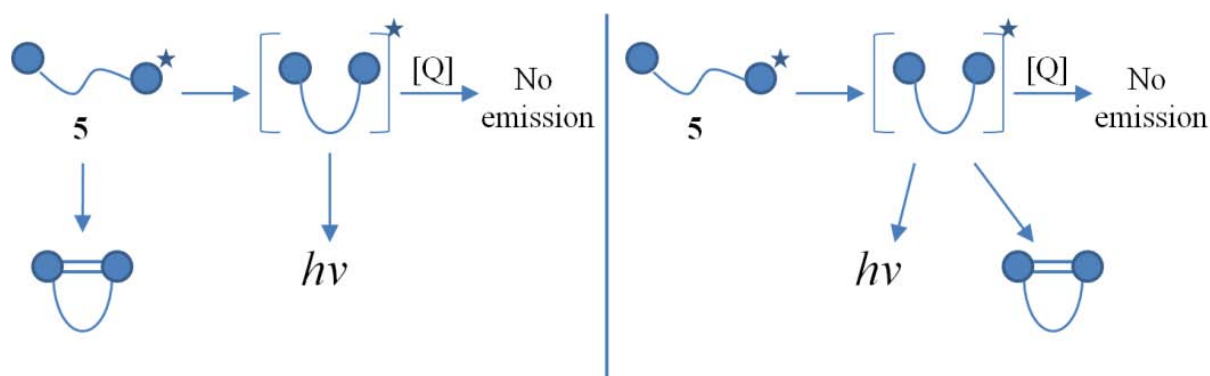
Figure 3.14 Arrhenius plots for the photodimerization reaction of **5**

Table 3.10 Calculated activation energies for photodimerization

Answer set 1			Answer set 2	
Cycloproduct	HT	HH	HT	HH
Excimer lifetime	Excimer 1	Excimer 2	Excimer 2	Excimer 1
E_a (kcal mol ⁻¹)	3.96	3.89	6.15	1.69

3.4.3 Effect of oxygen on the photophysical and photochemical properties of **5**

The formation of a fluorescent excimer has been shown to lie on the pathway for intramolecular photodimerization of a number of bis-anthracene systems. However, the observation of two distinct excimer-like emissions from **5** cannot be taken as proof that such intermediates are indeed precursors to the photodimers. While direct evidence can be difficult to obtain, the observation of identical quenching rates for a postulated intermediate and its photoproduct is generally considered a strong argument. The reaction schemes below show the effect of an exterior quencher for the case where a fluorescent excimer is formed in competition (Scheme 3.11, left), or on the way to photodimers (Scheme 3.11, right). In the first case, only quenching of the excimer fluorescence will be observed, whereas in the latter case both excimer fluorescence and product formation will be quenched by the same amount.



Scheme 3.11 Effect of the exterior quencher in the photodimerization

Oxygen was selected as an intermolecular quencher, because its quenching rate is generally diffusion limited ($k_{diff} = 1.6 \times 10^{10} \text{ L mol}^{-1} \text{ s}^{-1}$ at 25°C in CH_2Cl_2).⁴⁹ Furthermore, because the residual lifetime of the anthracene component is very short, we can neglect quenching of the locally excited anthracene. Two concentrations of oxygen were experimentally investigated, air-saturated and oxygen-saturated (bubbled with oxygen for 5 minutes). The aerated and oxygen-saturated fluorescence quantum yields and decays were measured at room temperature. The results show that the fluorescence for both **10** and **5** is moderately affected by oxygen. The fluorescence quantum yields and decay rates are reduced in the oxygen-saturated condition (Table 3.11). From a Stern-Volmer analysis, the oxygen quenching constants can be calculated (Table 3.12). From the fluorescence quantum yield or decay rates,

these are found to be quite similar.

Table 3.11 Lifetime data in different conditions

		τ (ns)			Φ_F	
	[O ₂](M)	10	5^a	5^b	10	5
Air	2.2×10^{-3}	6.34	5.09	1.88	0.420	0.250
O ₂	10.7×10^{-3}	3.41	3.3	1.46	0.280	0.212

^a λ_{ex} = 370 nm, λ_{em} = 550 nm ^b λ_{ex} = 370 nm, λ_{em} = 420 nm

Table 3.12 Calculated quenching rate for **10** and **5** by oxygen

k_q (L mol ⁻¹ s ⁻¹)	10	5^a
Fluorescence	0.97×10^{10}	0.9×10^{10}
Lifetime	1.45×10^{10}	1.75×10^{10}

^a λ_{ex} = 370 nm, λ_{em} = 420 nm

The aerated and oxygen-saturated photodimerization quantum yields were measured at room temperature. Photodimerization is also reduced by oxygen when compared to the degassed conditions, although from the results the formation of the HT photodimer is found to be less sensitive to oxygen when compared to the HH photodimer. Both HT and HH photodimerization are further suppressed when the solution is fully oxygenated.

Table 3.13 Photodimerization results in different content of oxygen

	HT ^a	HH ^a
Aerated	0.011	0.004
Oxygenated	0.005	0.003

^a at room temperature in CH₂Cl₂

From these results, it can be observed that the degree of oxygen quenching of the excimer fluorescence (or decay) and product formation are very similar (Table 3.14). This leads us to conclude that the fluorescent excimers are indeed intermediates in the kinetic scheme leading to photodimer formation. In this respect, intramolecular anthracene photodimerization is similar to the previously investigated intermolecular variant. Furthermore, two possible answer sets were obtained in the previous section, which can now be distinguished based on the relative propensity of excimer quenching by oxygen. We may safely assume that the intrinsic rate of quenching of the excimers by oxygen is similar for both excimers, and that differences in the observed K_{SV} are due to differences in excimer lifetime. When comparing the emission quenching and photodimerization data in aerated and oxygenated media, it is found that the extent of quenching by oxygen is within experimental error in one case. For the longer excimer lifetime, K_{SV} = 1.5 while HT quantum yield gives K_{SV} = 2.2. For the HH photodimer, and shorter lifetime, both give K_{SV} = 1.3. Thus, the longer excimer lifetime can

be attributed to the excimer leading to the HT photodimer, while the shorter lived excimer leads to the formation of the HH photodimer.

Table 3.14 Comparison between lifetime and photodimerization quantum yield
in aerated and oxygenated data

	τ_{550}	HT QY	τ_{420}	HH QY
Air	5.09 ns	0.011	1.88 ns	0.004
O ₂	3.3 ns	0.005	1.46 ns	0.003
K _{sv}	1.5	2.2	1.3	1.3

Reference

1. P. Klán and J. Wirz, *Photochemistry of Organic Compounds: From Concepts to Practice*, John Wiley, **2009**.
2. N. J. Turro, *Modern Molecular Photochemistry*, University Science Books, **1991**.
3. IUPAC. Compendium of Chemical Terminology, 2nd ed. (the "Gold Book"). Compiled by A. D. McNaught and A. Wilkinson. Blackwell Scientific Publications, Oxford (1997).
4. R. A. a. R. J. S. Alberty, *Physical chemistry*, Wiley.
5. R. R. Islangulov, D. V. Kozlov and F. N. Castellano, *Chem. Commun.*, **2005**, 3776-3778.
6. R. R. Islangulov and F. N. Castellano, *Angew. Chem., Int. Ed.*, **2006**, 45, 5957-5959.
7. M. Kuki, Y. Koyama and H. Nagae, *Journal of Physical Chemistry*, **1991**, 95, 7171-7180.
8. B. R. Nielsen, K. Jorgensen and L. H. Skibsted, *J. Photochem. Photobiol., A*, **1998**, 112, 127-133.
9. T. Gunnlaugsson, A. P. Davis, G. M. Hussey, J. Tierney and M. Glynn, *Org. Biomol. Chem.*, **2004**, 2, 1856-1863.
10. A. Ojida, Y. Mitooka, M. Inoue and I. Hamachi, *J. Am. Chem. Soc.*, **2002**, 124, 6256-6258.
11. J. Y. Kwon, N. J. Singh, H. N. Kim, S. K. Kim, K. S. Kim and J. Yoon, *J. Am. Chem. Soc.*, **2004**, 126, 8892-8893.
12. T. Gunnlaugsson, A. P. Davis and M. Glynn, *Chem. Commun.*, **2001**, 2556-2557.
13. T. Gunnlaugsson, A. P. Davis, J. E. O'Brien and M. Glynn, *Organic Letters*, **2002**, 4, 2449-2452.
14. L. Fabbrizzi, M. Licchelli, P. Pallavicini, A. Perotti, A. Taglietti and D. Sacchi, *Chem. Eur. J.*, **1996**, 2, 75-82.
15. T. Gunnlaugsson, A. P. Davis, J. E. O'Brien and M. Glynn, *Org. Biomol. Chem.*, **2005**, 3, 48-56.
16. Y. Terazono, P. A. Liddell, V. Garg, G. Kodis, A. Brune, M. Hamburger, A. L. Moore, T. A. Moore and D. Gust, *J. Porphyrins Phthalocyanines*, **2005**, 9, 706-723.
17. G. Kodis, Y. Terazono, P. A. Liddell, J. Andreasson, V. Garg, M. Hamburger, T. A. Moore, A. L. Moore and D. Gust, *J. Am. Chem. Soc.*, **2006**, 128, 1818-1827.
18. B. Jing and D. Zhu, *Tetrahedron Lett.*, **2004**, 45, 221-224.
19. A. S. Dvornikov and P. M. Rentzepis, *Res. Chem. Intermed.*, **1996**, 22, 115-128.
20. A. S. Dvornikov, I. Cokgor, F. McCormick, R. Piyaket, S. Esener and P. M. Rentzepis, *Optics Communications*, **1996**, 128, 205-210.
21. V. Walhorn, C. Schäfer, T. Schroeder, J. Mattay and D. Anselmetti, *Nanoscale*, **2011**, 3, 4859-4865.
22. C. Schäfer, R. Eckel, R. Ros, J. Mattay and D. Anselmetti, *J. Am. Chem. Soc.*, **2007**,

- 129, 1488-1489.
23. J. H. R. Tucker, H. Bouas-Laurent, P. Marsau, S. W. Riley and J.-P. Desvergne, *Chem. Commun.*, **1997**, 1165-1166.
 24. G. McSkimming, J. H. R. Tucker, H. Bouas-Laurent and J.-P. Desvergne, *Angew. Chem., Int. Ed.*, **2000**, 39, 2167-2169.
 25. C. Schäfer and J. Mattay, *Photochemical & Photobiological Sciences*, **2004**, 3, 331-333.
 26. A. Gilbert and J. Baggott, *Essentials of Molecular Photochemistry*, Blackwell, Oxford, **1991**.
 27. H. Bouas-Laurent and J. P. Desvergne, *Studies in Organic Chemistry (Amsterdam)*, **1990**, 40, 561-630.
 28. D. O. Cowan and R. L. Drisko, *Elements of Photochemistry*, Plenum Press, New York, **1976**.
 29. H. Bouas-Laurent, A. Castellan, J.-P. Desvergne and R. Lapouyade, *Chem. Soc. Rev.*, **2001**, 30, 248-263.
 30. H. Bouas-Laurent, A. Castellan, J.-P. Desvergne and R. Lapouyade, *Chem. Soc. Rev.*, **2000**, 29, 43-55.
 31. H. D. Becker, *Chem. Rev.*, **1993**, 145-172.
 32. H. D. Becker, *Advances in Photochemistry*, **1990**, 15, 139-227.
 33. J. Ferguson, *Chem. Rev.*, **1986**, 86, 957-982.
 34. H. Bouas-Laurent, A. Castellan and J. P. Desvergne, *Pure and Applied Chemistry*, **1980**, 52, 2633-2648.
 35. A. Castellan, R. Lapouyade and H. Bouas-Laurent, *Bulletin de la Societe Chimique de France*, **1976**, 210-216.
 36. B. Stevens, *Advances in Photochemistry*, **1971**, 8, 161-226.
 37. E. J. Bowen, *Advances in Photochemistry*, **1963**, 1, 23-42.
 38. Y. Molard, D. M. Bassani, J.-P. Desvergne, P. N. Horton, M. B. Hursthouse and J. H. R. Tucker, *Angew. Chem., Int. Ed.*, **2005**, 44, 1072-1075.
 39. Y. Molard, D. M. Bassani, J.-P. Desvergne, N. Moran and J. H. R. Tucker, *J. Org. Chem.*, **2006**, 71, 8523-8531.
 40. H. Dürr and H. Bouas-Laurent, *Photochromism: Molecules and Systems: Molecules and Systems*. Elsevier Science, **2003**.
 41. A. Castellan, R. Lapouyade and H. Bouas-Laurent, *Bulletin de la Societe Chimique de France*, **1976**, 201-209.
 42. A. S. Dvornikov, J.-P. Desvergne, D. A. Oulianov, H. Bouas-Laurent and P. M. Rentzepis, *Helvetica Chimica Acta*, **2001**, 84, 2520-2532.
 43. J. B. Birks, *Photophysics of Aromatic Molecules*, Wiley Intersciences, New York, **1970**.
 44. M. Montalti, A. Credi, L. Prodi and M. T. Gandolfi, *Handbook of Photochemistry*, CRC Press, **2006**.

45. L. Sun and H. Goerner, *J. Phys. Chem.*, **1993**, 97, 11186-11193.
46. R. G. Bennett and P. J. McCartin, *J. Chem. Phys.*, **1966**, 44, 1969-1972.
47. T. Brotin, J. P. Desvergne, F. Fages, R. Utermöhlen, R. Bonneau and H. Bouas-Laurent, *Photochem. Photobiol.*, **1992**, 55, 349-358.
48. J. R. Lakowicz, in *Principles of Fluorescence Spectroscopy*, Springer, New York, 3rd edn., **2010**, p. 144.
49. M. Montalti, A. Credi, L. Prodi and T. Gandolfi, *Handbook of Photochemistry, Third Edition*, Taylor & Francis, **2006**.
50. S. Schoof, H. Güsten and C. Von Sonntag, *Berichte der Bunsengesellschaft für physikalische Chemie*, **1977**, 81, 305-309.

Chapter 4. Surface Characterization

4.1 Characterization techniques of SAMs

When working with reactions on monolayers, the problems of monitoring the progress of the reaction, determining the products and estimating the yield of conversion can become quite significant. The extremely small quantities involved render many analytical tools impractical, and, very often, a combination of techniques is necessary to adequately characterize the structure of a modified substrate.

In optimal cases, such as SAMs on smooth, reflective surfaces, transformation can be studied by a wide range of techniques including infrared spectroscopy, scanning electron microscopy, contact angle measurements, atomic force microscopy (AFM), surface plasmon resonance, ellipsometry, X-ray photoelectron spectroscopy (XPS), quartz crystal microbalance, electrochemical methods, confocal and optical microscopes. In practice, IR spectroscopy, ellipsometry and XPS are the techniques most widely used to study chemical transformations, whereas AFM is particularly useful for image-patterned surfaces. Here, a change inside the pattern can be easily compared with the nonchanging background that acts as an internal reference. Various techniques for the characterization of SAMs are reviewed elsewhere.^{1,2} In the following introductory sections we will describe the techniques that we used to characterize the surface modification, mainly ellipsometry, contact angle, and AFM techniques.

4.1.1 Introduction to ellipsometry

Ellipsometry is an optical measurement which has been used for about one hundred years to characterize sample surfaces. The name originates from the fact that the most common case of polarized light is elliptical. A remarkable feature of spectroscopic ellipsometry is the high precision of the measurement and sensitivity. With resolution of 0.1 nm or better, it is therefore suitable for studies of single layers of molecular dimensions on solid substrates.³ Ellipsometry refers to a class of optical experiments which is self-referenced and measures the polarization states before and after reflection from a reflecting sample surface. The simultaneous measurement of the ellipsometry parameters, ψ and Δ provides information about the sample under investigation, and can allow quantitative analysis of the optical index (n) and thickness (d) of a thin layer based on fitting theoretical models to the experimental results.

4.1.1.1 Instrumental setup of an ellipsometer

Figure 4.1 shows a schematic diagram of a nulling ellipsometer with a quarter-wave plate positioned before the sample surface. In most cases the light source is a single wavelength laser or a white light source with a monochromator to select a specific wavelength. A polarizer is generally placed in front of the light source and is used to extract linearly

polarized light from the unpolarized light. On the detection side, an analyzer is placed in front of a light detector and the state of the polarization of the reflected light beam is determined from the intensity of the light transmitted through the analyzer. Although a polarizer and an analyzer are the same optical element, they are named separately due to the difference in their roles. A compensator (or retarder) is generally placed behind a polarizer or in front of an analyzer and is employed to convert linear polarization to circular polarization and vice versa. The light detector is generally a photomultiplier tube to provide maximum sensitivity.

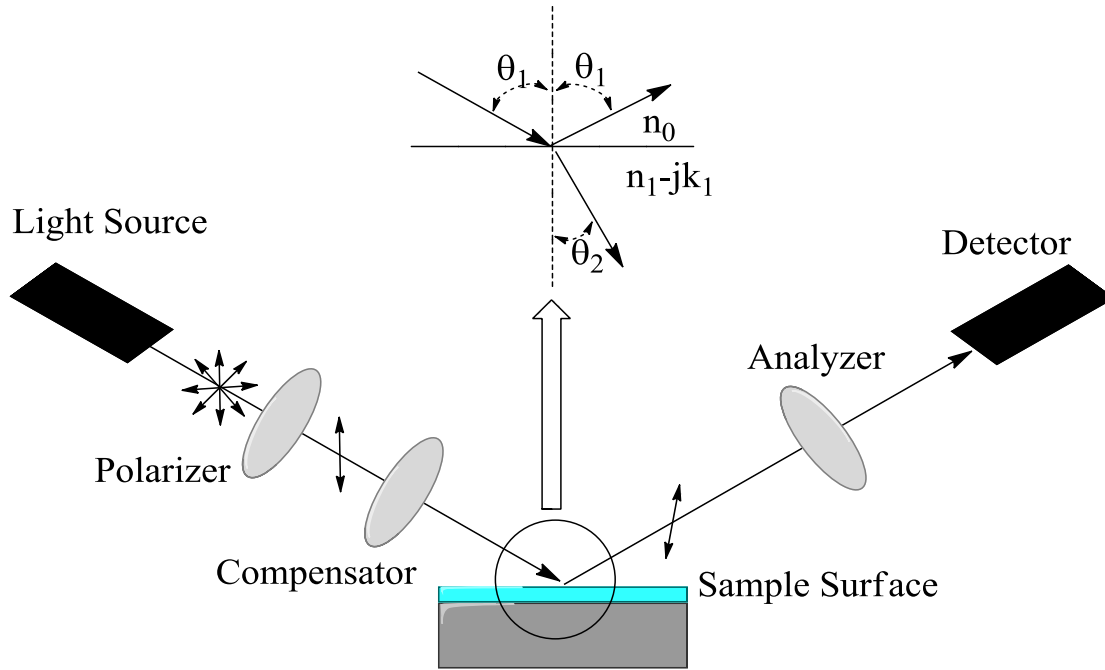


Figure 4.1 Instrument setup of an ellipsometer

When a linearly polarized light beam is incident on the sample surface, the reflected light will in general exhibit an elliptical state of polarization. The other way around, the same elliptical state of polarization (but with a reversed sense of rotation) incident on a surface will generate a linearly polarized reflection. More generally, by using the polarizer and compensator combination mentioned above, one can always find an elliptical polarization that produces a linearly polarized reflection (if the sample surface is not depolarizing). The relative orientation of the polarizer and compensator plate give the ellipsometric parameters Ψ and Δ .

4.1.1.2 Principle of ellipsometry

In ellipsometry, p - and s -polarized light waves are irradiated onto a sample near the Brewster angle, and the optical constants and film thickness of the sample are determined from the change in the polarization state of light that is reflected or transmitted. Figure 4.2 illustrates the measurement principle of ellipsometry. The state of polarization is expressed by superimposing waves propagating along two orthogonal axes. The polarization states of the

incident and reflected light waves are described by the coordinates of p - and s -polarizations. The vectors on the incident and reflection sides overlap completely when $\theta = 90^\circ$ (straight-through configuration). In Figure 4.2, the incident light is linearly polarized and oriented at $+45^\circ$ relative to the E_{ip} axis. In particular, $E_{ip} = E_{is}$ is true for this polarization since the amplitudes of p - and s -polarizations are the same and the phase difference between the polarizations is zero.

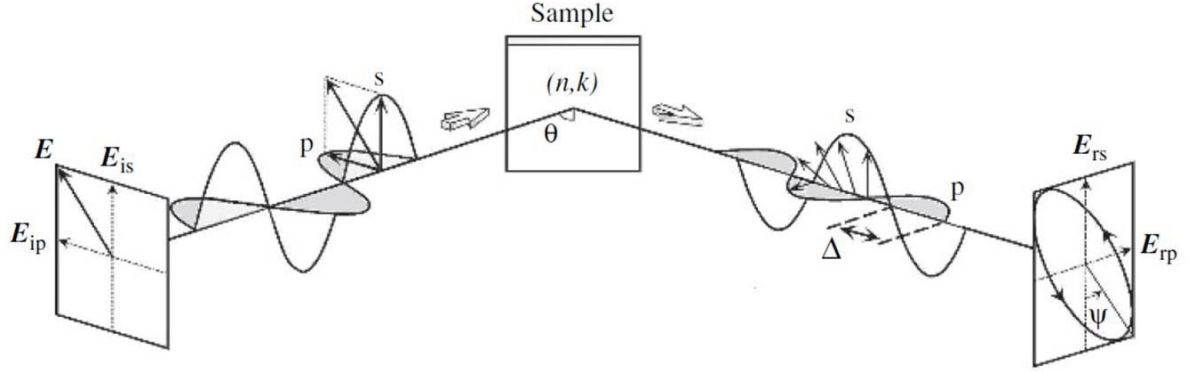


Figure 4.2 Measurement principle of ellipsometry⁴ Reprinted with permission from ref 4.
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The amplitude reflection coefficients for p - and s -polarizations differ significantly due to the difference in the electric dipole of the radiation. Thus, upon reflection from the sample, the p - and s -polarizations will show different changes in amplitude and phase. As shown in Figure 4.2, ellipsometry measures the two values (Ψ, Δ) that express the amplitude ratio and phase difference between p - and s -polarizations, respectively. In ellipsometry, therefore, the variation of light reflection with p - and s -polarizations is measured as the change in polarization state. In particular, when a sample structure is composed of homogeneous layers, the amplitude ratio is related to the refractive index n , while Δ represents light absorption described by the extinction coefficient k . In this case, the two values (n, k) can be determined directly from the two ellipsometry parameters (Ψ, Δ) experimentally obtained from a sample by applying the Fresnel equations. The (Ψ, Δ) measured from ellipsometry are defined from the ratio of the amplitude reflection coefficients for p - and s -polarizations:

$$\rho \equiv \tan \psi \cdot \exp(i\Delta) = \frac{r_p}{r_s} \quad \text{eq. 4.1}$$

If we apply the definitions of the amplitude reflection coefficients $r_p \equiv E_{rp}/E_{ip}$ and $r_s \equiv E_{rs}/E_{is}$, we can rewrite eq. 4.1 as follows:

$$\rho \equiv \tan \psi \cdot \exp(i\Delta) = \frac{r_p}{r_s} \equiv \left(\frac{E_{rp}}{E_{ip}} \right) / \left(\frac{E_{rs}}{E_{is}} \right) \quad \text{eq. 4.2}$$

As confirmed from eq. 4.2, r_p and r_s are originally defined by the ratio of the reflected electric field to the incident electric field, and $\tan \psi \cdot \exp(i\Delta)$ is further defined by the ratio

of r_p to r_s . In the case of Figure 4.2, eq. 4.2 can be simplified to $\tan\psi \exp(i\Delta) = E_{rp}/E_{rs}$ since $E_{ip} = E_{is}$. In Figure 4.2, therefore, ψ represents the angle determined from the amplitude ratio between reflected p - and s -polarizations, while Δ expresses the phase difference between reflected p - and s -polarizations.

If we use polar coordinates to represent the amplitude reflection coefficients, it follows from eq. 4.1 that

$$\tan\psi = \left(\frac{|r_p|}{|r_s|}\right) \text{ and } \Delta = (\delta_{rp} - \delta_{rs}) \quad \text{eq. 4.3}$$

These are the ellipsometry signals obtained from the measurement by applying the Fresnel equation, and combined with the theoretical fitting to the experimental data, various optical properties can be determined for the sample under investigation. In spectroscopic ellipsometry, ψ and Δ spectra are measured over an ensemble of wavelengths of the incident light. This is particularly useful for samples whose optical parameters (n, k) and thickness (d) are unknown. In such cases, if n and k are wavelength dependent, it is possible to fit the ensemble of the data to a single value of the sample thickness (d). Further details can be found in the literature.⁴

4.1.2 Principle of contact angle measurements

Contact angle measurement is a simplified method for characterizing the interfacial tension present between a solid, a liquid, and a vapor. If a liquid with well known properties is used for the measurement, then the resulting interfacial tension can be used to identify the nature of the solid used in the measurement. The technique is extremely surface-sensitive, with the ability to detect monolayers.⁵

When a drop of liquid rests on the surface of a solid, its shape is determined by the balance of the interfacial liquid/vapor/solid forces. The contact angle is linked to the surface energy and one can calculate the surface energy and discriminate between polar and nonpolar interactions. These interactions are described by the cohesion and adhesion forces which are intermolecular forces. The balance between the cohesive forces of similar molecules such as between liquid molecules (i.e. hydrogen bonds and Van der Waals forces) and the adhesive forces between dissimilar molecules (such as between the liquid and solid molecules i.e. mechanical and electrostatic forces) will determine the contact angle created at the solid and liquid interface. The traditional definition of a contact angle θ_c is the angle a liquid creates with the solid or liquid when it is deposited on it (Figure 4.3).

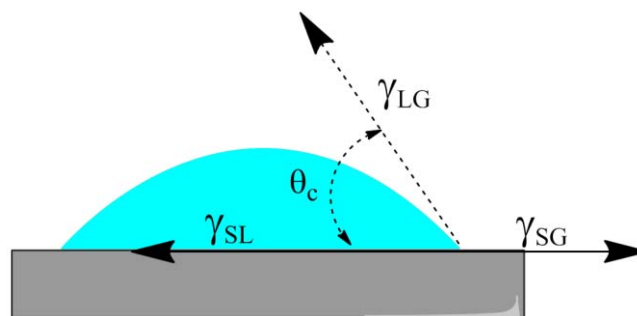


Figure 4.3 Common definition of contact angle

When the contact angle is small, the cohesive forces are weaker than the adhesive forces and the molecules of the liquid tend to interact more with the solid rather than the liquid molecules. When the contact angle is large, the cohesive forces are stronger than the adhesive forces and the molecules of the liquid tend to interact more with each other than with the solid. Contact angle measurement can be therefore used to detect the presence of films, coatings, or contamination which have a surface energy that is different than the underlying substrates. It provides a versatile tool to examine the properties of self-assembled monolayers on the substrates.

4.1.3 Introduction to AFM

The basic idea behind AFM is that three-dimensional images of surfaces can be obtained by sensing the force between a sample and a sharp tip mounted to the end of a soft cantilever. To this end, the specimen is mounted on a piezoelectric scanner, which allows three dimensional positioning with sub-nanometer accuracy while the force on the tip-cantilever assembly is monitored with picoNewton sensitivity by measuring the deflection of the cantilever. The deflection, or vertical bending of the cantilever, is usually detected using a laser beam reflected from the free end of the cantilever and focused onto a photodiode array. AFM cantilevers and tips are typically made of silicon or silicon nitride using micro-fabrication techniques.

A number of different AFM imaging modes are available, which differ mainly in the way the tip is moving over the sample. In contact mode, the AFM tip is in contact with the sample surface and is raster-scanned over the sample while the cantilever deflection, thus the force applied to the tip, is kept constant using feedback control. In tapping mode, also called dynamic or intermittent mode, the tip is set into vibration by a separate piezo electronic element and oscillates near its resonant frequency. As the vibrating tip is brought close to the sample, the substrate-tip interactions affect the tip's resonant frequency and are detected as a phase-shift in the frequency of the vibration. Because lateral forces during imaging are greatly reduced, this mode may be advantageous for imaging soft biological samples. Further information on the different imaging modes can be found in the literature.⁶⁻⁹ In force

spectroscopy, the cantilever deflection is recorded as a function of the vertical displacement of the piezoelectric scanner, i.e. as the sample is pushed towards the tip and retracted. This results in a cantilever deflection (d) vs. scanner displacement (z) curve, which can be transformed into a force–distance curve by converting the cantilever deflection into a force (F) using Hooke’s law ($F = -k \times d$, where k is the cantilever spring constant) and subtracting the deflection from the scanner displacement to obtain the distance ($z - d$). The point of contact (zero separation distance) is determined as the position of the linear vertical part of the force–distance curve in the contact region. Because AFM does not provide an independent measurement of the tip–sample separation distance, determining the point of contact can sometimes be difficult, especially when long-range surface forces or sample deformation effects dominate. Force–distance curves can be recorded either at single, well-defined locations of the (x , y) plane or at multiple locations to yield a so-called ‘force–volume image’. In doing so, spatially resolved maps of sample properties and molecular interaction forces can be produced. For quantitative force measurements, the actual spring constants of the cantilevers must be independently calibrated by using geometric or thermal methods.

A typical force–distance curve is shown in Figure 4.4. As the cantilever approaches the surface, the forces are initially too small to give a measurable deflection of the cantilever, and the cantilever remains in its undisturbed position. At a specific tip–sample distance, the attractive forces (usually Van der Waals, or capillary forces in air) overcome the cantilever spring constant and the tip jumps into contact with the surface. Once the tip is in contact with the sample, it remains on the surface as the separation between the base of the cantilever and the sample decreases further, causing a deflection of the cantilever and an increase in the repulsive contact force. As the cantilever is retracted from the surface, the tip may remain in contact with the surface due to some adhesion and the cantilever is deflected downwards. This can be used to estimate the surface energy of solids or the binding forces between a substrate and the tip, if the tip is coated with a compound exhibiting specific interactions with the surface. Further details on the principles of force spectroscopy can be found in the excellent review by Butt and co-workers.¹⁰

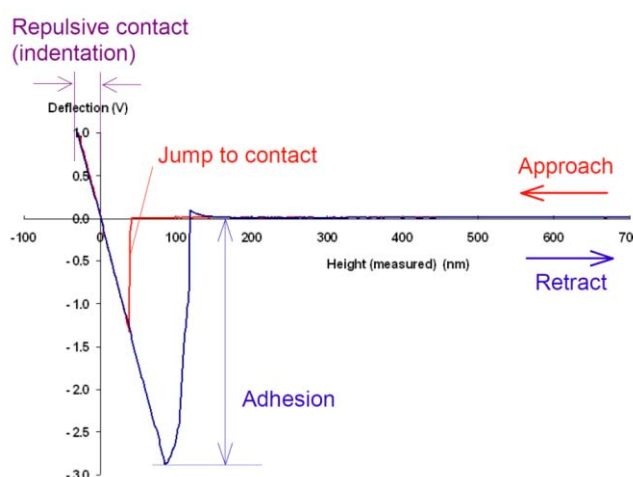


Figure 4.4 Typical force–distance curve of approach(red) and retract(blue) curves for a contact mode cantilever and clean mica in air¹¹

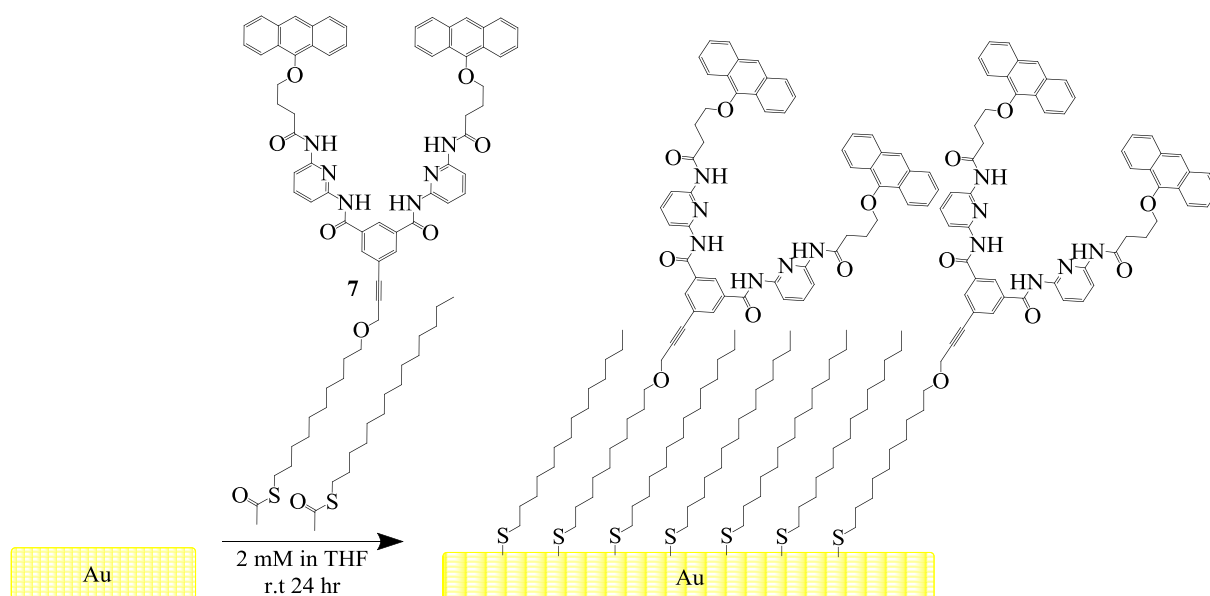
4.2 Surface modification on gold surface through thioacetate anchoring groups

4.2.1 Preparation of 7-modified substrates

The synthesis of receptor with thioacetate **7** was shown in chapter 2. To modify the gold surface with a self-assembled monolayer, the gold surface was first treated with freshly prepared piranha solution to clean the surface, and then incubated with **7** in THF (2 mM) at room temperature for 24 hours (Scheme 4.1). The receptor solutions were prepared with a mixture of **7** and a long chain alkane thioacetate ($n\text{-C}_{14}\text{H}_{29}\text{SAC}$) to dilute the bulky compound **7** in a more tightly-packed C_{14} monolayer. The ratios of **7**/ C_{14}SAC investigated in view of affording better packing are shown in Table 4.1. After the incubation period, the surface was cleaned with copious THF and dichloromethane to remove physically adsorbed molecules. Finally, the substrate was dried with a stream of nitrogen gas and its thickness measured by ellipsometry (Table 4.1).

Table 4.1 Thickness results after surface modification

Sample	7	C_{14}SAC	Thickness (nm)
1	100%	0%	1.2 ($\pm$ 0.3)
2	90%	10%	1.3 ($\pm$ 0.2)
3	75%	25%	1.2 ($\pm$ 0.1)
4	50%	50%	1.1 ($\pm$ 0.2)
5	20%	80%	1.0 ($\pm$ 0.1)
6	10%	90%	1.0 ($\pm$ 0.1)
7	0%	100%	0.4 ($\pm$ 0.0)



Scheme 4.1 Surface modification using a receptor with a thioacetate anchoring group

4.2.2 Characterization of 7-modified substrates: Fluorescence

From the observed thickness one may conclude that the ratio of 7/C₁₄-thioacetate has a minor effect on the density of the monolayer. All of the modified substrates have a similar thickness, which ranges from 1.0 to 1.3 nm. To further prove that the receptor molecules are on the gold surface, the substrate fluorescence was measured. The fluorescence signal is weak due to only one monolayer of fluorescent molecules covering the substrate, and its intensity was not proportional to the increased ratio of receptor (Figure 4.5, left). It should be noted that the experimental setup involving placing the substrate in exactly the same orientation every time is difficult, and therefore quantitative interpretation of the fluorescence signal is not straightforward.

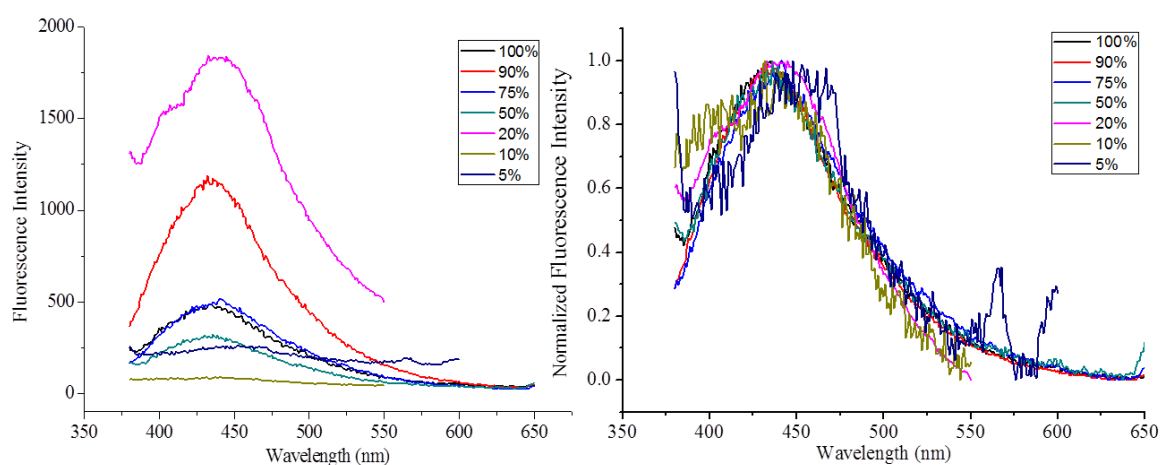


Figure 4.5 Fluorescence spectra from modified gold surface (λ_{ex} 370 nm);
left: original spectra, right: normalized spectra

To further analyze the substrate fluorescence, the spectra were normalized to see whether there is any shift in emission for different ratios. The normalized spectra showed that all of the modified substrates possessed similar fluorescence spectra whose maximum is located at around 450 nm (Figure 4.5, right). When comparing the fluorescence emission between the receptor immobilized on a substrate and in solution, one notices that there is a 30-nm red shift in the emission profile. This is mainly due to a difference in the chromophores' environment and local polarity. It is also possible that the close-packing of the monolayer encourages overlap between the anthracenes, which would favor excimer formation (Scheme 4.2). In solution, the movement of two anthracenes on 7 is not restricted, and therefore its emission behavior is monomer-like. However, when the receptor molecule is immobilized on the gold surface, the two anthracenes may be forced into proximities due to π - π stacking between the anthracenes, and therefore give rise to excimer emission. It may also explain why substrates with the different ratios of 7/C₁₄-thioacetate delivered similar spectra. Since the two anthracene moieties are on the same receptor molecule, they are always in close proximity to

each other when immobilized on the substrate. One may therefore expect little difference when changing the proportion of receptor/ C_{14} -thioacetate. In other words, there appear to be little intermolecular interactions between two proximate molecules of **7** on the substrate.

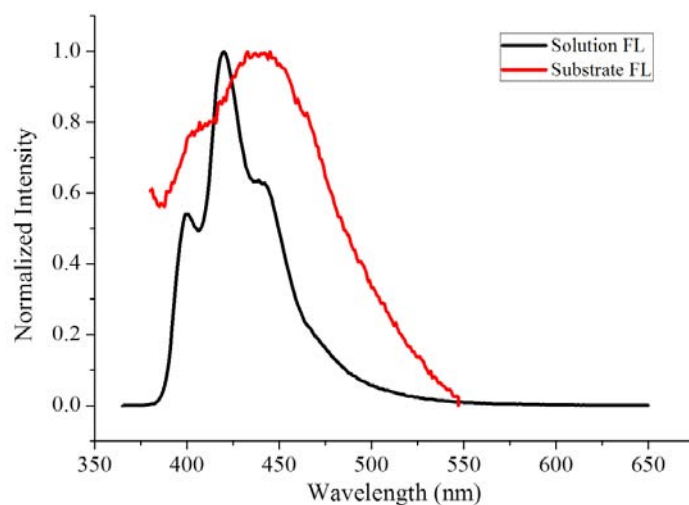
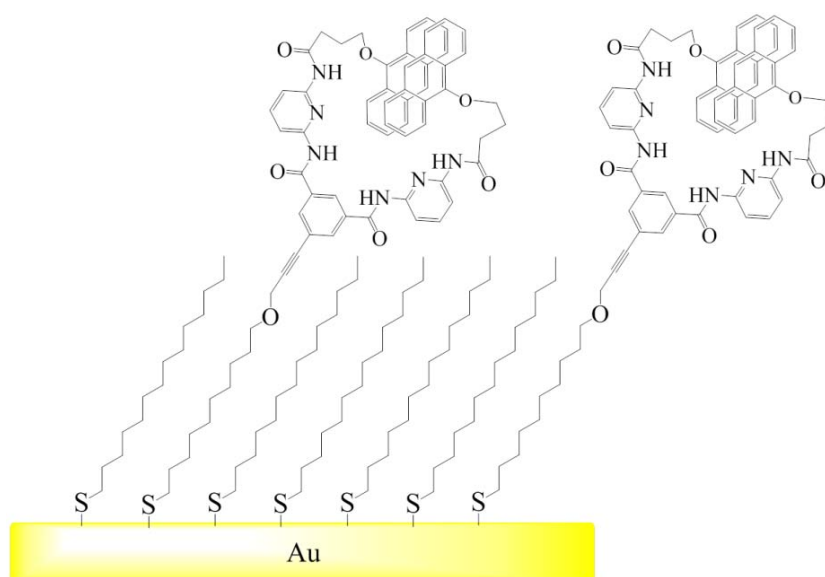


Figure 4.6 Comparison of the fluorescence comparison between **7** in solution (CH_2Cl_2 , black curve) and on a gold substrate (20% **7**/ $C_{14}SAc$, red curve). $\lambda_{ex} = 370$ nm



Scheme 4.2 Possible conformation of **7** on gold substrate that may give rise to excimer fluorescence

4.2.3 Characterization of **7**-modified substrates: electrochemical methods

To further investigate the structure of the SAMs and their molecular organization, the samples were characterized using electrochemistry. This work was done in collaboration with Dr. Galina Dubacheva (ISM) and the MaCSE electrochemistry group of the Rennes Chemistry Institute (ISCR). To begin, a 40% $AnOC_{12}SAc/C_{14}SAc$ modified gold substrate was chosen as a reference monolayer (Figure 4.7). The surface coverage of the self-assembled monolayer can be readily evaluated by electrochemistry when the chemisorbed molecules

serve as a barrier to block a redox process involving a species in solution.¹² In this case, we investigated the CV response of modified gold electrodes using TCNQ as an electrochemically reversible redox probe diffusing in solution. Figure 4.8 shows the comparison between the voltammetric profiles obtained on gold substrates covered with 40% AnOC₁₂Sac/C₁₄Sac and that of a bare gold electrode. There is not much difference between the current potential (I-E) responses for the electroactive substance of the SAM covered electrodes and a bare gold surface. This confirms the weak blocking properties of the modified substrates, and that the diffusion through pinholes and defects in the monolayer is efficient.

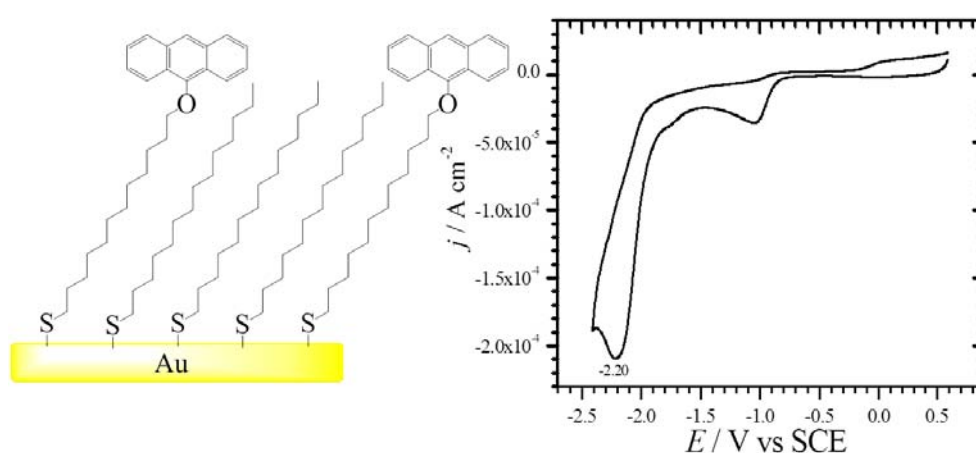


Figure 4.7 CV at 0.1 V/s of 40% AnOC₁₂Sac / C₁₄Sac modified gold surface in CH₃CN + 0.1 M Bu₄NPF₆

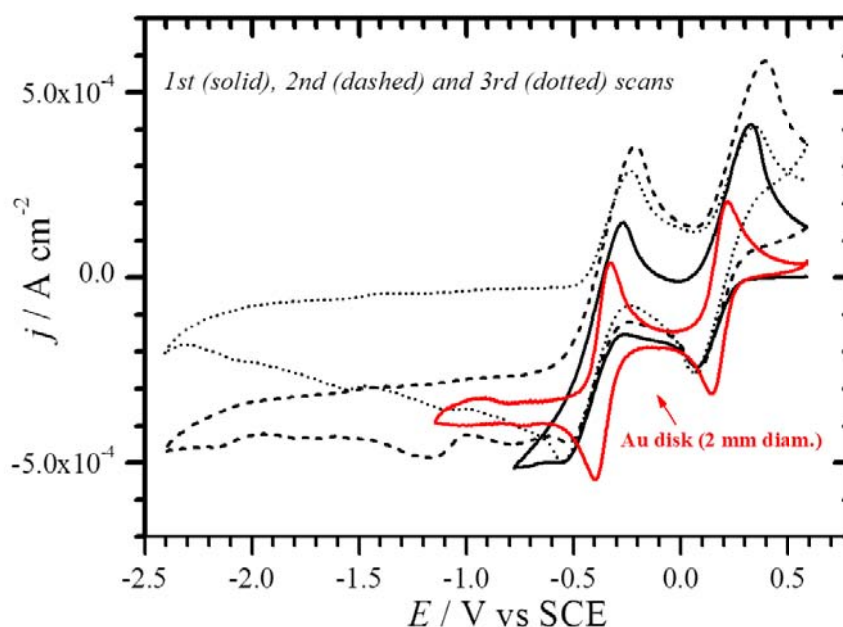


Figure 4.8 CV at 0.1 V/s of anthracene-modified gold surface 40% AnOSac with C₁₄Sac + 1 mM TCNQ in CH₃CN + 0.1 M Bu₄NPF₆.

Cyclic voltammetry measurements were also performed with gold substrates modified with a monolayer composed of 100% open- or closed-receptor-SAc (Figures 4.9 and 4.11). Both CV curves are much more complicated when compared with those of the AnOC₁₂SAc-modified substrates. The CV responses of modified gold electrodes using TCNQ as an electrochemically reversible redox probe diffusing in solution were also investigated. Figure 4.10 shows the comparison between the voltammetric profiles obtained on gold substrates covered with 100% **7**-modified gold substrate and those from a bare gold electrode. Again, there is not much difference between the current potential (I-E) responses for the electroactive substance at SAM covered electrodes and a bare gold surface. It also confirms the weak blocking properties on the modified substrates, and that the diffusion through pinholes and defects in the monolayer is possible. Figure 4.12 shows the comparison between the voltammetric profiles obtained on gold substrates covered with 100% **7_D**-modified gold substrate and that on a bare gold electrode. The current potential (I-E) shows better blocking properties compared with open receptor, but still not good enough for molecular electronics applications.

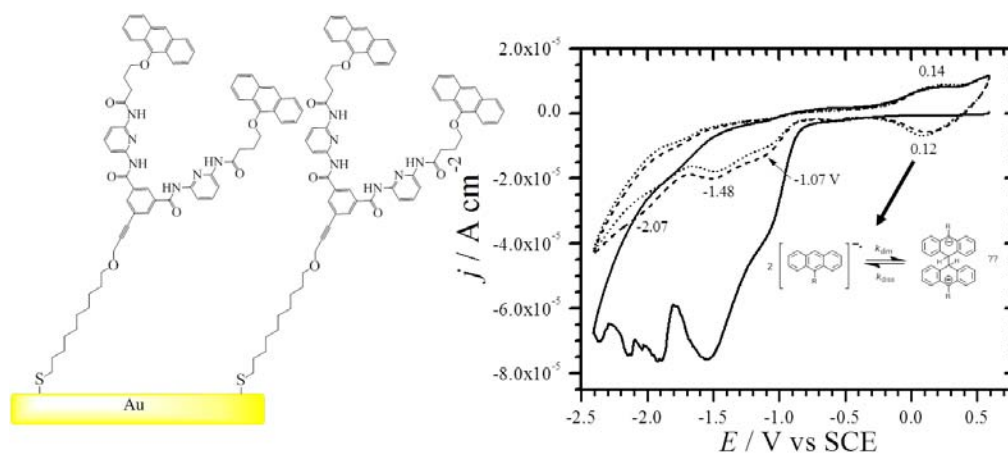


Figure 4.9 CV at 0.1 V/s of a **7**-modified gold surface in CH₃CN + 0.1 M Bu₄NPF₆. 1st (solid), 2nd (dashed) and 3rd (dotted) scans.

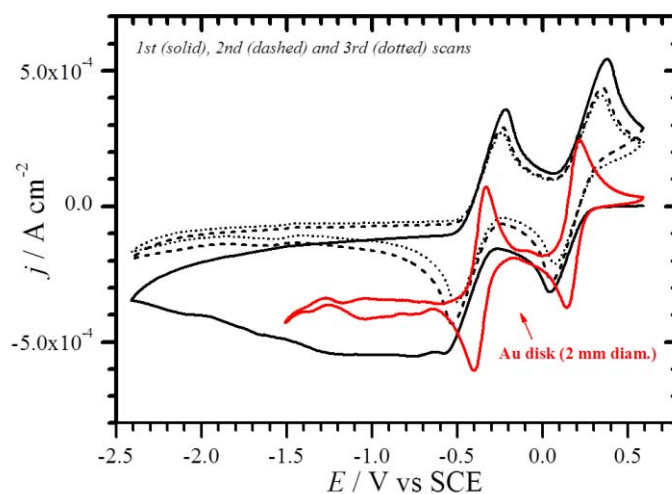


Figure 4.10 CV at 0.1 V/s of a **7**-modified gold surface + 1 mM TCNQ in CH₃CN + 0.1 M Bu₄NPF₆.

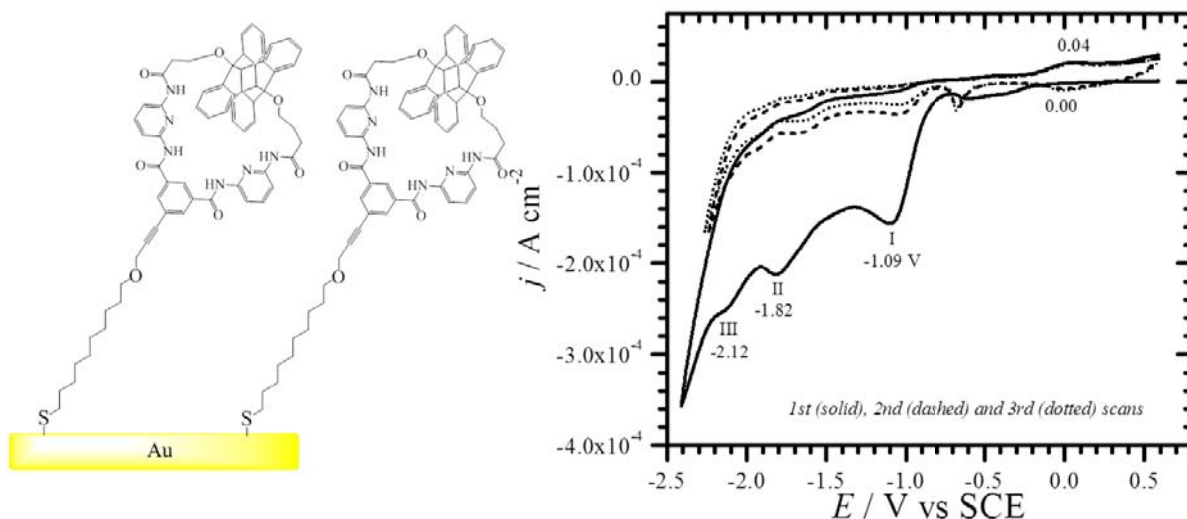


Figure 4.11 CV at 0.1 V/s of a 7_D-modified monolayer deposited on a gold surface in CH₃CN + 0.1 M Bu₄NPF₆

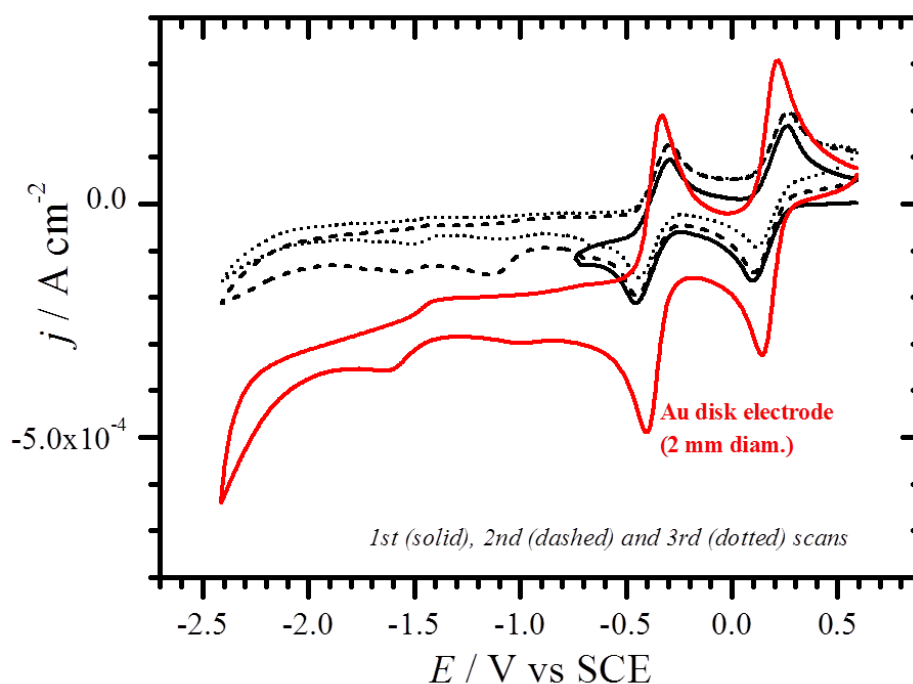


Figure 4.12 CV at 0.1 V/s of a 7_D-modified monolayer deposited on a gold surface in CH₃CN + 0.1 M Bu₄NPF₆, in the presence of 1 mM TCNQ

In summary, although deposition of the closed and open anthracene receptor molecules onto gold substrates using a thioacetate anchoring group was successful, as is confirmed by ellipsometry and fluorescence measurements, the electrochemical data shows that the quality of a SAM on the substrate is not as good as expected, and therefore further improvement is necessary. Further electrochemical experiments involving the use of a 7-modified gold electrode and barbiturates featuring electroactive ferrocene or fullerene groups revealed that there is little electronic communication between bound analytes and the gold surface,

presumably due to the intervening alkane layer acting as a dielectric.

4.3 Comparison of surface modification on gold surface between thiol and thioacetate anchoring group

Although the thioacetate anchoring group has the advantage of being insensitive towards oxidation, the performance of the modified substrate was not as good as expected. The thioacetate group is presumably deprotected *in situ* to the corresponding thiol group by reacting with small amount of acid or base, and then formed a self-assembled monolayer (SAM) on the Au surface.¹³ We therefore chose to directly deprotect the thioacetate group to thiol *in situ* to improve monolayer formation. In the literature, one finds three common conditions for deprotection which are listed in Table 4.2. C₁₄SAC was chosen to form a SAM to test these deprotection conditions, and then the thickness measured by ellipsometry. To simplify the experimental tests, only NH₄OH and Et₃N were used as base; the thioacetate-only condition was also examined as a control experiment (Table 4.2).

Table 4.2 Experimental conditions to cleave thioacetate to thiol

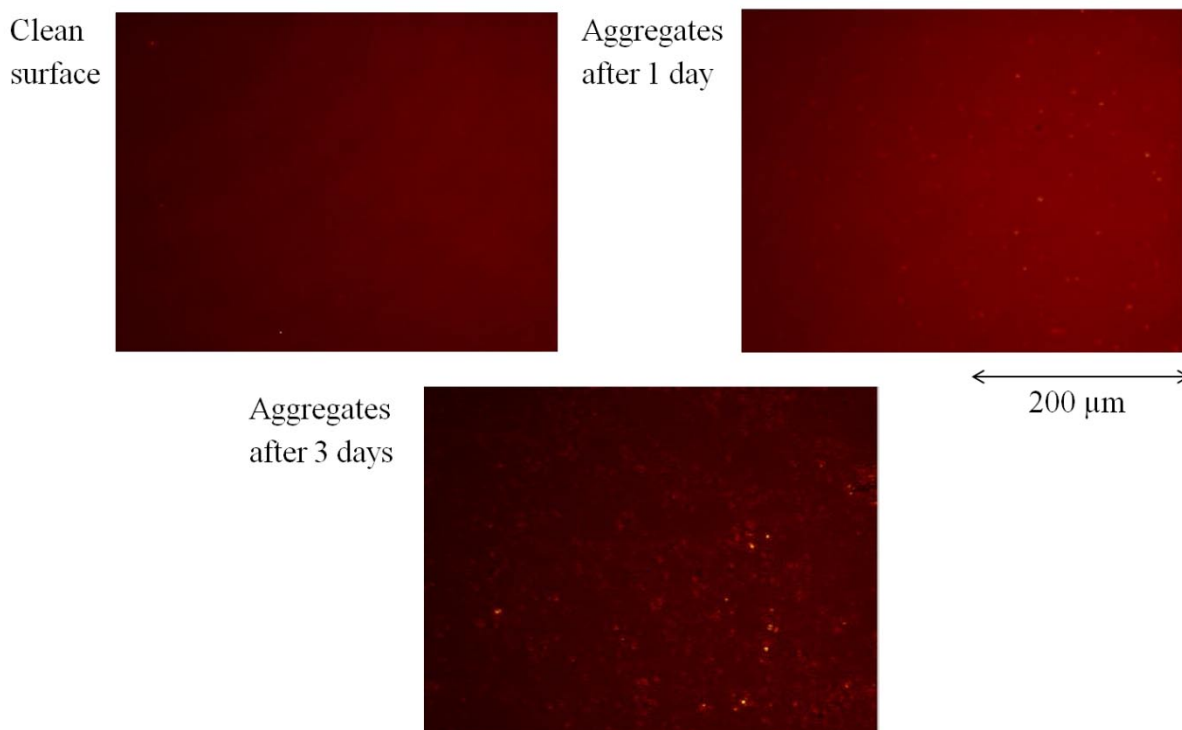
Procedure	Molecular Type	Ref.
3-5 μ L 30% NH ₄ OH per mg in THF	rigid-rod conjugated SAc	14
15% Et ₃ N in THF, bad effect of >2 eq. Bu ₄ NOH	rigid-rod conjugated SAc	15
0.5 eq. TBACN in MeOH, incubation 3h at 50°C before immersion	alkane-SAc	16

The test results are shown in Table 4.3. To observe the impact of the incubation time on the SAMs' growth, the incubation duration was varied from one day to three days. After one day incubation, non-protected thioacetate, 10 μ L NH₄OH with 1mM C₁₄-SAc and Et₃N delivered SAM growth (0.5-1.0 nm) with clean surface. Higher concentration of C₁₄-SAc (3mM) with NH₄OH or low concentration C₁₄-SAc with higher concentration NH₄OH (2 eq.) gave a SAM with aggregates on the surface, and after two minutes sonication in THF the substrate surface was clean but the thickness was then reduced (from 1.0 nm to 0.6 nm). The receptor-SAc 7 was also examined using the NH₄OH deprotection conditions. However, after one day incubation it formed a SAM with aggregates on the surface which was not better than in the previous case without deprotection. With prolonged incubation time (three days) for C₁₄SAC SAM, only Et₃N gave a SAM with clean surface, although there was no further thickness growth. Non-protected thioacetate and lower-concentrated NH₄OH (1mM) formed a thicker SAM (2.5 nm) with aggregates on the surface, and the aggregates remained on the surface even after 2 minutes sonication in different solvents (Figure 4.13).

Table 4.3 Experimental results of C₁₄-SAM growth by thioacetate deprotection *in situ*

Deprotection	Concentration	Time ^a	Thickness	Surface ^b	Thickness ^c
-	1 mM	1	0.6 nm	C	-
			0.7 nm		
		3	2.5 nm	A	2.2 nm, aggregates
+ 10 μ L NH ₄ OH	1 mM	1	0.7 nm	C	-
			0.5 nm		
		3	2.5 nm	A	2.0 nm, aggregates
	3 mM		1.0 nm		
	3 mM + 20% of 7	1	1.0 nm	A	-
+ 2 eqv NH ₄ OH	1 mM	1	1.0 nm	A	0.6 nm, clean
+ 15% Et ₃ N	1 mM	1	1.0 nm	C	-
		3	1.0 nm	C	

^a unit: day; ^b C: clean, A: aggregates; ^c thickness growth after 3 days incubation

Figure 4.13 Ellipsometry images of Au surface after SAM preparation with C₁₄SAC.

To get a better idea of how a well-packed SAM should respond, the C₁₄-SH thiol was used to modify the gold surface (Table 4.4). After one day incubation in 1 mM EtOH solution, it delivered a clean surface with 1.5 nm thickness growth. With prolonged incubation time to three days, the result remained the same. In summary, comparing the results between various thioacetate deprotected conditions and pure thiols, one may conclude that the pure thiol has the advantage of easier preparation and better results over *in situ* deprotected thioacetate.

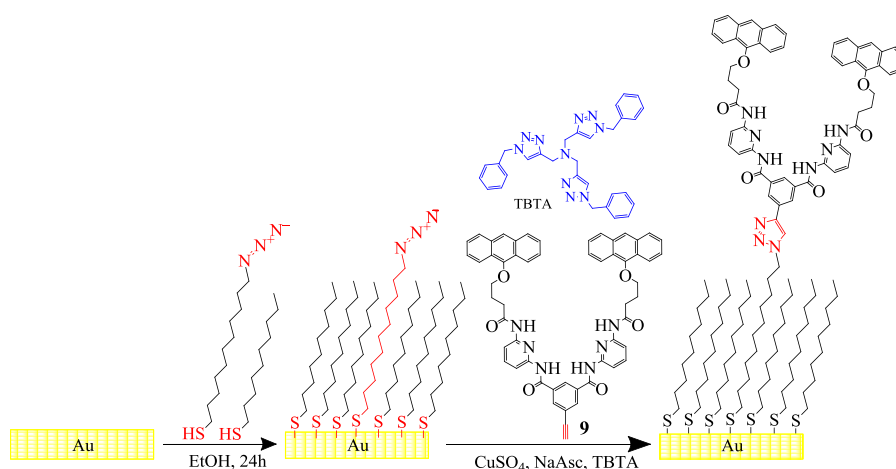
Table 4.4 Experimental results of C₁₄-SH SAM growth

Procedure	Concentration	Rinsing	Time	Thickness	Surface
2 mL EtOH, RT	1 mM	EtOH	1 day	1.5 nm	clean
			3 days	1.5 nm	clean

4.4 Post modification via click reaction and characterizations on SAM

4.4.1 Preparation of 9-modified gold substrates

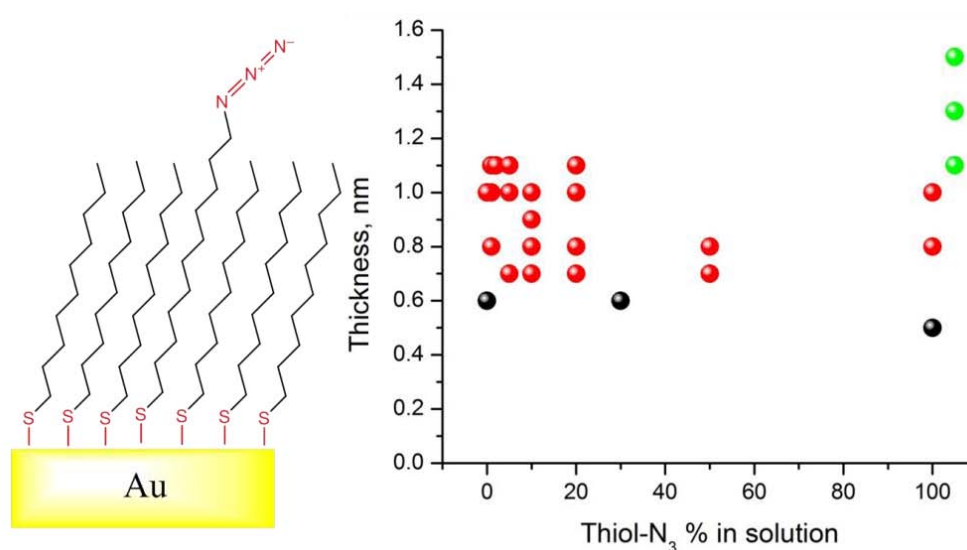
An alternative strategy was explored for the immobilization of the receptor molecules on substrates. It is based on the selective grafting of receptor **9** on a preformed SAM via a Huisgen cycloaddition reaction, allowing control of the amount and accessibility of the immobilized receptors (Scheme 4.3).¹⁷⁻¹⁹ Alkyne functional groups and azide-containing binary SAMs have been previously reported in the literature.¹⁷⁻¹⁹ The click reaction is efficiently catalyzed by copper (I), resulting exclusively in the formation of 1,4-bisubstituted 1,2,3-triazole rings. Because triazole formation is irreversible and usually quantitative, this method has been advantageously used for surface modification.²⁰⁻²⁴ Decan-1-thiol was chosen as a diluting component in the SAMs to minimize non-specific adsorption phenomena, which is important to reduce interactions between receptor-modified SAMs and guest molecules. The surface modification is described in detail in the following sections.



Scheme 4.3 Immobilization through azide-alkyne-click reaction on a gold surface

SAM-coated substrates were prepared by dipping a clean gold substrate into a solution of mixed thiols (3-5 mM total concentration in absolute ethanol) at room temperature for 24 hours. Two thiols were used for SAM preparation: $C_{10}SH$ and $N_3C_{12}SH$. The gold surface was cleaned by UV-ozone treatment for 20 minutes, followed by immersion in stirring absolute ethanol for 20 minutes before dipping into thiol solution. The results obtained from ellipsometry characterizing at the SAMs are listed in Scheme 4.4 and Table 4.5.

For control experiments, newly purchased $C_{14}SH$ with high purity ($\geq 98\%$) was used to provide good SAMs on gold substrates with an average thickness of 1.35 nm as a reference. In the first experiment, $C_{10}SH$ (reagent grade, 96% purity) was newly purchased and used without further purification. The azide derivative, $N_3C_{12}SH$, was synthesized in the lab and purified with column chromatography. However, the results of the SAMs obtained on the substrates were not as good as expected, and the average thickness of the SAMs was only 0.57 nm. The reason for the reduced thickness of the SAMs is attributed to the presence of impurity in the thiols, and therefore further purification of the thiols was undertaken. Both $N_3C_{12}SH$ and $C_{10}SH$ were purified by repeated column chromatography ($\sim 90\% \rightarrow 98\%$ by NMR spectroscopy). Following purification, the thickness of the SAMs on gold substrates was increased to 0.92 nm, which is close to the expected value (1.1 nm thickness for $C_{10}SH$ on gold substrate²⁵). Therefore the next step of click reaction on the substrates was applied.



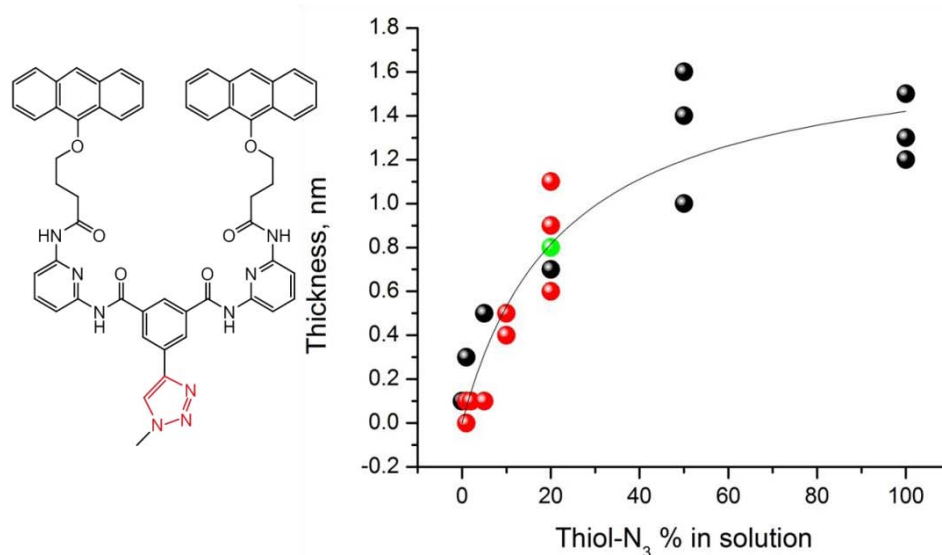
Scheme 4.4 Thickness results from SAM modification
($C_{10}SH/N_3C_{10}SH$ purity: black: 96%, red: 98%. $C_{14}SH$ purity: green: $\geq 98\%$)

Table 4.5 Experimental conditions and results of SAM preparation on gold substrates

Procedure	Thiols	Average thickness ^b
Abs. EtOH, 24h, RT, 3-5 mM, Rinsing with EtOH	C ₁₀ SH (purchased, 96%)	0.57±0.06 nm
	N ₃ C ₁₂ SH (Synthesized, 90%) ^a	
	C ₁₀ SH (purified, 98%) ^a	0.92±0.15 nm
	N ₃ C ₁₂ SH (purified, 98%) ^a	
	C ₁₄ SH (purchased, ≥ 98%)	1.35±0.19 nm

^aPurity is estimated by NMR; ^bthickness is measured by ellipsometry

To graft receptor **9** on the modified gold substrates, the substrates were rinsed with ethanol and dried with a stream of nitrogen, and then immersed in a water/DMSO (1:2) solution at room temperature containing 0.5 mM CuSO₄, 0.5 mM TBTA (tris[(1-benzyl-1H-1,2,3-triazol-4-yl) methyl]amine, which is a commonly used ligand for stabilizing the Cu(I) catalyst), 2.8 mM sodium ascorbate (which is necessary for generation of the Cu(I) catalyst) and 0.5 mM receptor **9**. Exposure to light was kept to a minimum in order to prevent photo-oxidation and photodimerization of the SAMs. Various incubation times were tested (3, 4 and 5 hours), and the thickness results obtained listed in Scheme 4.5. Further detail can be found in the experimental section. In general, incubation time did not have much influence on thickness growth when examined by ellipsometry or AFM, and all condition tested delivered a clean surface (Figure 4.14 and 4.15).



Scheme 4.5 Thickness results of click reaction on SAMs
(Incubation time: green: 3h, red: 4hr, black: 5 hr)

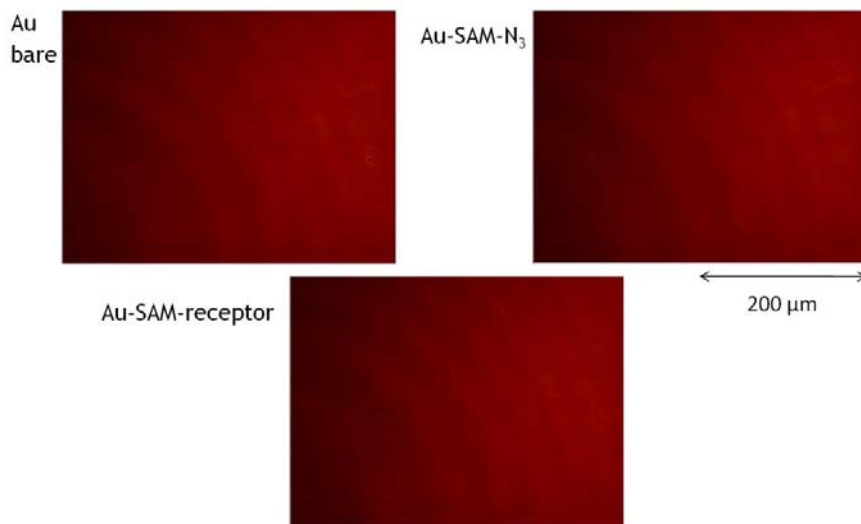


Figure 4.14 Ellipsometry images of Au surface during receptor-SAM preparation.

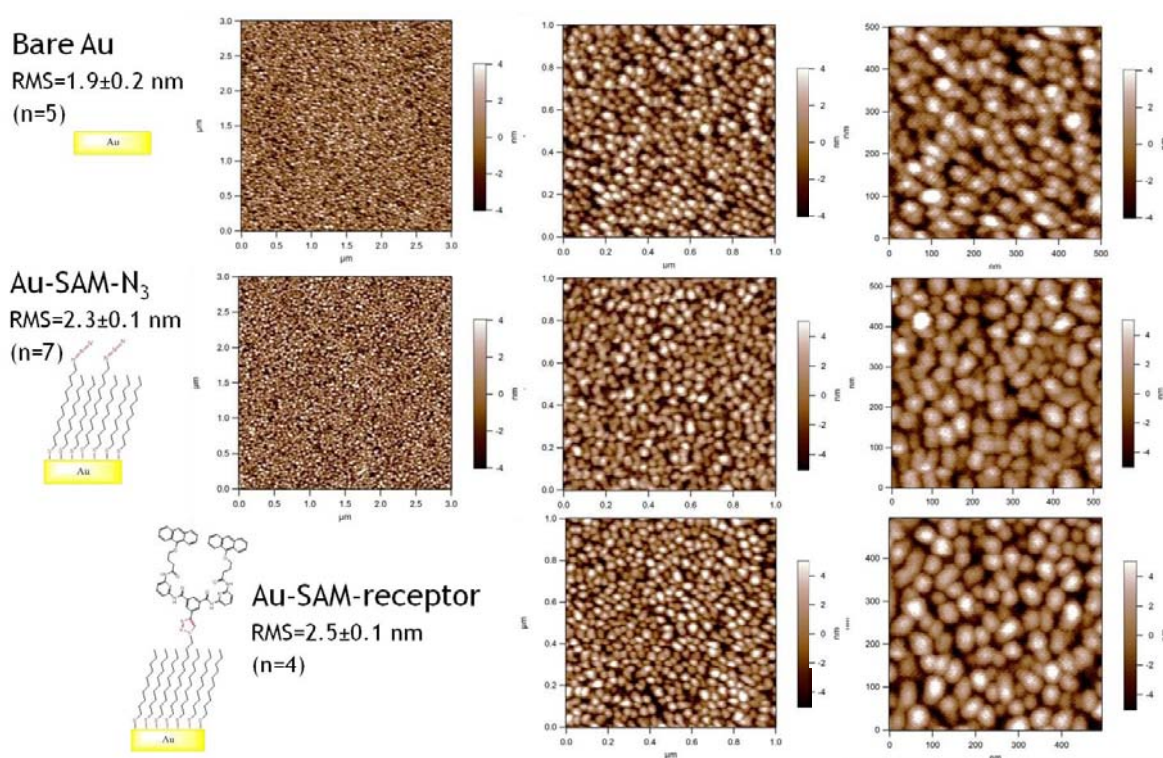
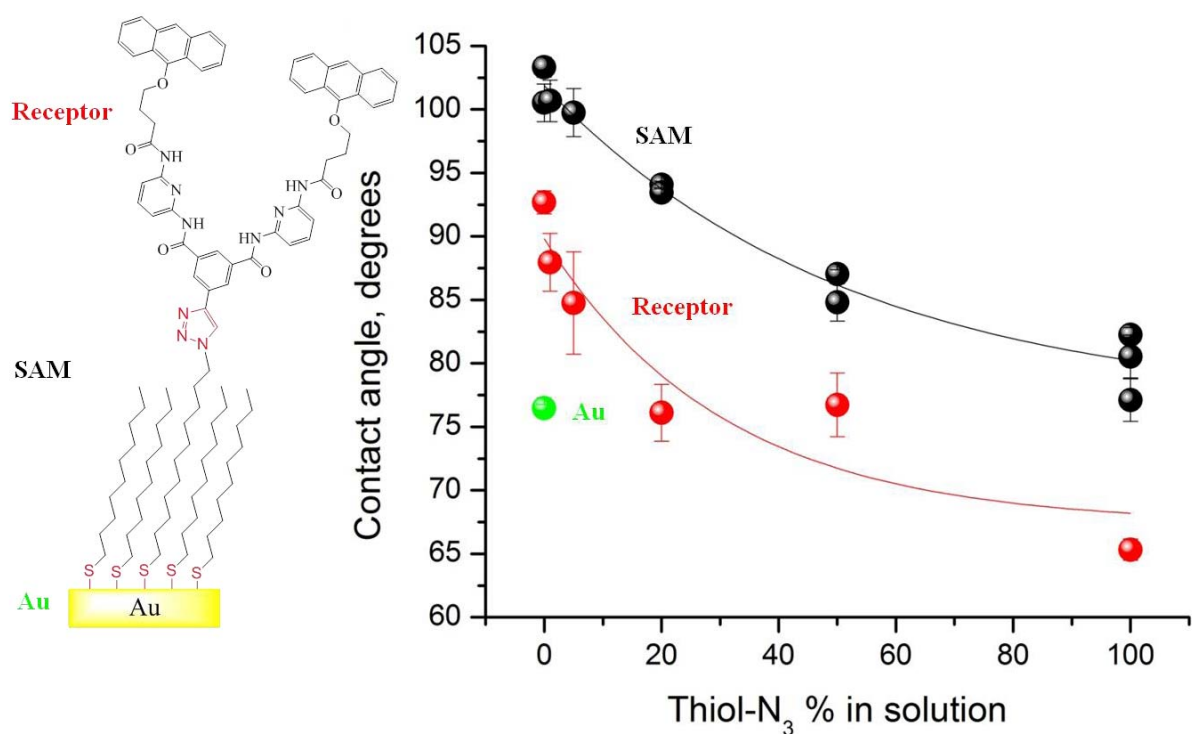


Figure 4.15 AFM Images of bare gold, SAM-Au and 9-SAM-Au substrates. In all cases, Au grains are visible.

4.4.2 Characterization of 9-modified substrates using contact angle measurements

Contact angle measurements (CAM) using water were used to monitor the hydrophobic properties of the surface. The contact angles were determined at each stage of surface modification, and the results are shown in Scheme 4.6 and Figure 4.16. The bare gold surface was quite hydrophilic due to the ozone plasma treatment, and gave a contact angle of 77 degrees. After grafting $C_{10}SH/N_3C_{12}SH$ SAM, the surface became more hydrophobic but the extent of hydrophobicity mainly depended on the composition of $N_3C_{12}SH$ and $C_{10}SH$.

Because azido groups were more hydrophilic compared to alkyl groups, higher ratios of $\text{N}_3\text{C}_{12}\text{SH}$ lead to a more hydrophilic surface and lower contact angles. The receptor-modified substrates after click reaction always gave a lower contact angle when compared to the SAMs before modification. This may be due to the interactions between water and hydrogen binding sites inside the receptor. The pyridine and amide groups can hydrogen bond with water, and therefore the surface would be more hydrophilic and lead to lower contact angles. In the extreme case (full coverage of the receptor on substrate), the contact angle is lower than that of bare gold surface (Figure 4.16).



Scheme 4.6 Contact angle results for surface modification
(Green: Bare Au, Red: Au-SAM-9, Black: Au-SAM)

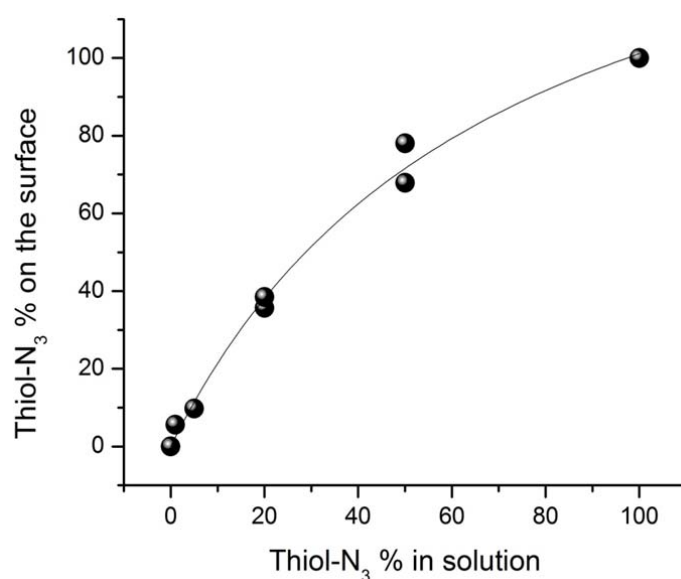


Figure 4.16 Contact angle images for surface modification

It has been shown that for binary SAMs, the relationship between the alkanethiol mole fraction in the solution and on the surface can be directly related to contact angle of the SAM using the CAM.²⁶ Thus, the percentage of $\text{N}_3\text{C}_{12}\text{SH}$ on a gold surface after the formation of mixed SAMs was determined using Cassie's law:³⁵

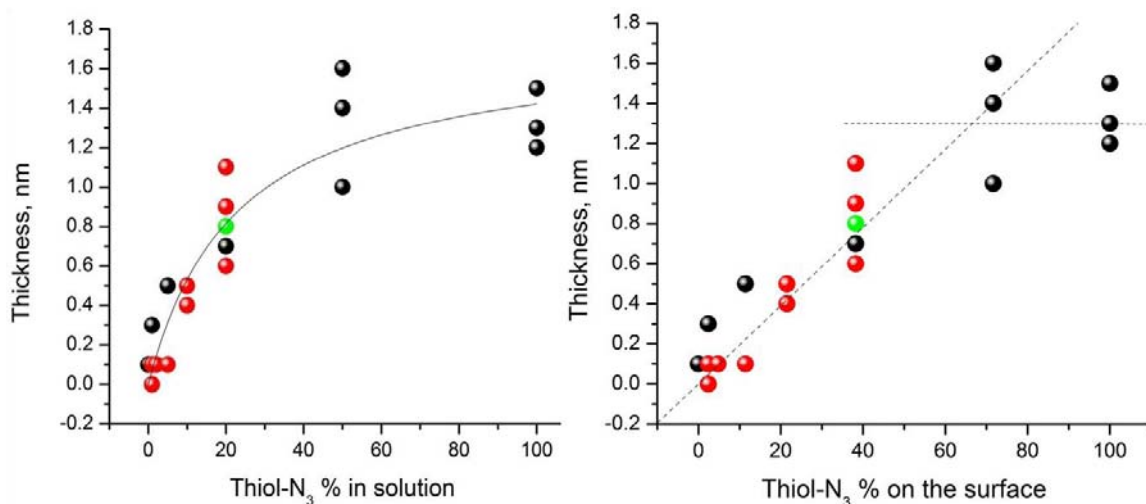
$$\cos \theta = f_1 \cos \theta_1 + f_2 \cos \theta_2$$

where θ , θ_1 , θ_2 are the contact angles on the mixed SAM and the two single-component SAMs, respectively, and f_1 and f_2 are the fractions of each component present in the mixed SAM.^{27,35} Scheme 4.7 shows the percentage of $\text{N}_3\text{C}_{12}\text{SH}$ on the gold surface as obtained from CAM using Cassie's law vs. the molar fraction of $\text{N}_3\text{C}_{12}\text{SH}$ in the solution used to prepare the mixed SAMs. The angles measured in this experiment varied from $\theta_1 = 102.5$ (100% $\text{N}_3\text{C}_{12}\text{SH}$) to $\theta_2 = 82.5$ (100% C_{10}SH). It is known that depending on the nature of the thiols, their ratio in solution differs from that in the resulting SAM.^{19,26} As shown in Scheme 4.7, one finds that the mole fraction of $\text{N}_3\text{C}_{12}\text{SH}$ in the SAM is higher than that in the solution. Therefore, this result suggests that the rate of adsorption of $\text{N}_3\text{C}_{12}\text{SH}$ onto gold is faster than the rate of adsorption of C_{10}SH .



Scheme 4.7 Relation between $\text{N}_3\text{C}_{12}\text{SH}$ mole fractions in the solution used to prepare binary SAM and the $\text{N}_3\text{C}_{12}\text{SH}$ mole fraction on a gold surface after SAM formation as determined by contact angle measurements

Since the relation between $\text{N}_3\text{C}_{12}\text{SH}$ mole fraction in the solution used to prepare binary SAM and the $\text{N}_3\text{C}_{12}\text{SH}$ mole fraction on a gold surface after SAM formation is known, one can plot the thickness of the receptor layer as a function of $\text{N}_3\text{C}_{12}\text{SH}$ mole fraction on the surface (Scheme 4.8). This plot shows linear increase of the receptor amount with $\text{N}_3\text{C}_{12}\text{SH}$ mole fraction until saturation regime is achieved. The linear increase shows that the amount of receptor on the substrate can be easily varied by changing the mole fraction of $\text{N}_3\text{C}_{12}\text{SH}/\text{C}_{10}\text{SH}$ in solution during assembly. These findings prove the efficiency and selectivity of the click modification. The presence of a saturation regime is not surprising with regards to the steric bulk of **9**.



Scheme 4.8 Thickness growth after click modification on SAMs

4.4.3 Characterization of **9**-modified substrates: Fluorescence

The fluorescence and excitation spectra of an open-receptor modified substrate were measured. The fluorescence emission maximum was located at 440 nm, corresponding to a 20-nm red shift when compared to the emission of **5** in solution. This shift is attributed to a difference in the local environment of the anthracene chromophore. The excitation spectrum showed a maximum absorption located at 310 nm that is blue-shifted when compared to the long wavelength absorption band of anthracene in solution. This blue-shifted absorption should be from the anthracene $S_0 \rightarrow S_2$ $\pi-\pi^*$ transition, which is 3 or 4 times more intense than the lower energy absorption band.

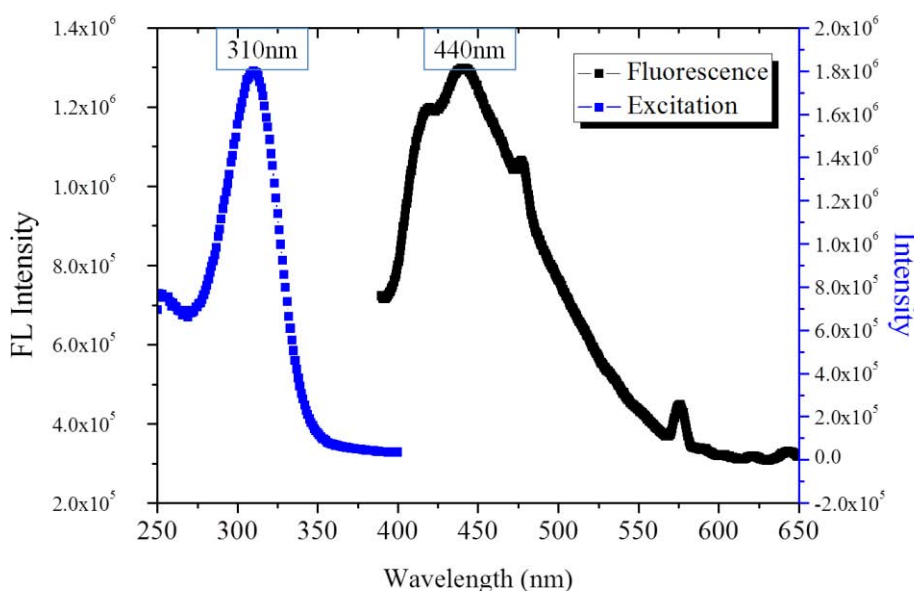


Figure 4.17 Fluorescence and excitation spectra of **9**-modified substrate
(Fluorescence: λ_{ex} =370 nm, excitation: λ_{em} = 440 nm)

4.4.4 Characterization of 9-modified substrates using AFM

Atomic force microscopy (AFM) is a powerful tool that can be used to probe the physical and electronic properties of a surface with high resolution. In collaboration with Dr. Galina Dubacheva (ISM), AFM was applied towards the characterization of the surface properties of the modified gold substrates. Substrates modified with the open and closed forms of **5** were used to examine the surface binding properties (Table 4.6). In order to directly probe the specific binding interactions between the surface-bound receptor and its guest, barbituric acid, a gold-coated AFM tip was modified with a barbiturate derivative. Additionally, an unmodified gold tip was used as control experiment. The force-distance curves of open- and closed-receptor substrates are shown in Figure 4.18 and 4.20.

Table 4.6 Substrates that were used under AFM investigation

Substrate	Material	Thickness
10% Au-SAM-N ₃	Open receptor 9	0.5 nm
	Closed Receptor 9_D	0.7 nm

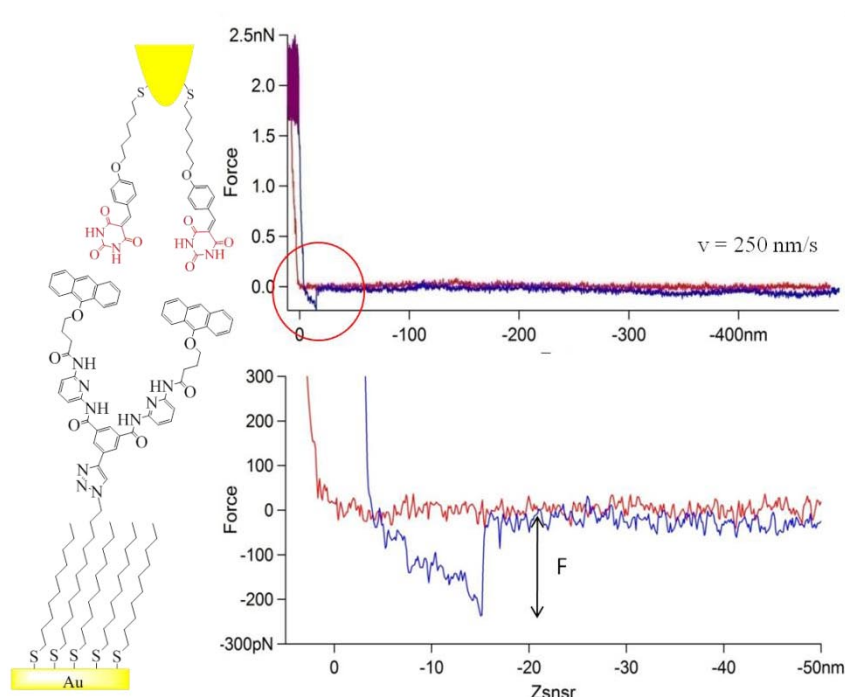


Figure 4.18 AFM force-distance curve between **9**-modified substrate and barbiturate modified AFM tip (red curve: Approach, blue curve: Retract)

Force-distance curves were first recorded between the open-receptor modified substrate and barbiturate molecules at the apex of an AFM tip (Figure 4.18 and 4.19(a)). According to the force-distance histogram obtained, most retraction curves displayed single binding events yielding a binding force histogram with a maximum force centered at 172 pN with an overall binding probability of 50.6% (n=468; Figure 4.19(a)). It is also worth noting that the

probability distribution profile at *ca.* 350 pN corresponding to twice the force of the single event at 172 pN, with a smaller probability. It is likely due to simultaneous binding of two barbiturates and two receptors on the surface. The interaction between an unmodified control tip and open-receptor substrate showed a much lower binding profile with overall probability of only 4.9%. Furthermore, no clear adhesion peak could be distinguished on the histogram, thus suggesting that no specific recognition event occurred between the control tip and the open-receptor modified substrate.

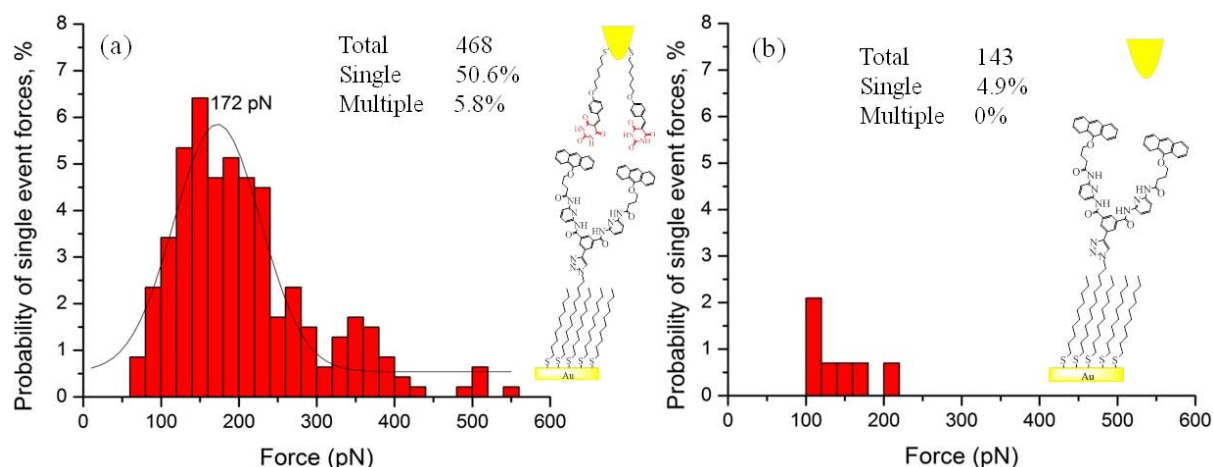


Figure 4.19 Adhesion histogram of **9**-modified substrates:
(a) with barbiturate modified gold tip, (b) with unmodified gold tip

Another set of measurements were performed on a substrate modified with a closed-receptor (**9_D**) and a barbiturate modified tip. In the force-distance histograms obtained, the retracting curve also displayed a single binding event yielding a binding force histogram with a maximum force centered at 169 pN with overall binding probability of 12.9% (*n*=412; Figure 4.21(a)). The similar binding force between the open- and closed- receptor substrates suggests that it is the same type of binding. It was expected that there would be no specific binding between the modified tip and closed-receptor substrate, because the previous studies it showed that the binding ability between the receptor and barbiturates would be significantly reduced due to the binding site being occupied by the steric hindered anthracene-photodimer.

After the re-examination by HPLC of the closed-receptor molecules used in the click reaction for the substrate, it was found that there was *ca.* 2% of open receptor molecules. It is possible that a small amount of open receptor may contribute to the specific binding observed. When corrected for the background events (*ca.* 5%), this represents *ca.* 8% binding probability. The interaction between the control tip and closed-receptor substrate showed that no specific recognition event occurred, just as the case in open-receptor substrate (Figure 4.21(b)).

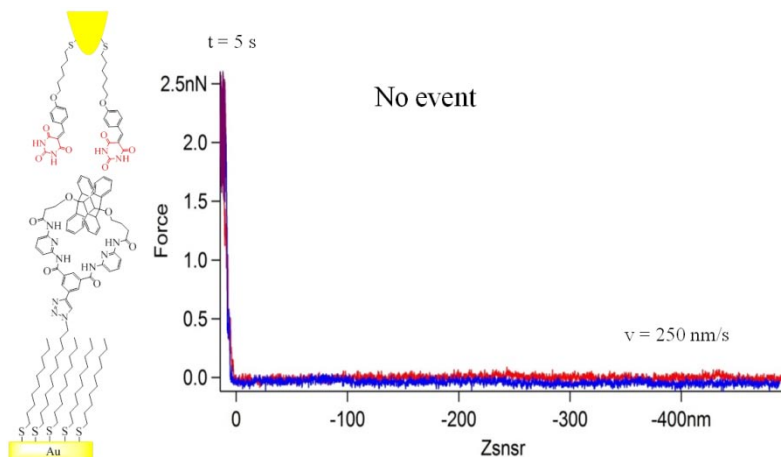


Figure 4.20 AFM force curve of closed receptor substrate

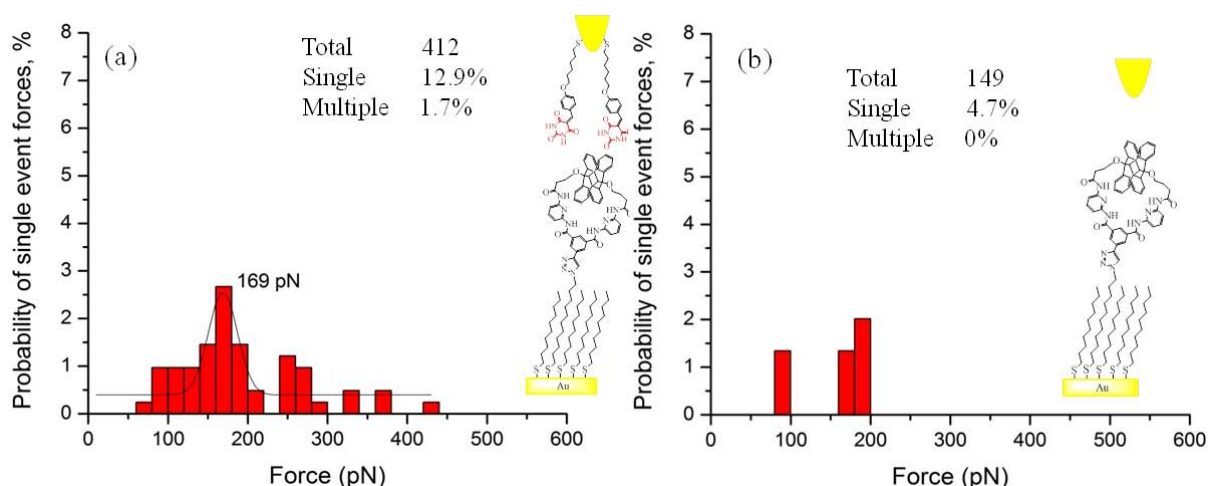


Figure 4.21 Adhesion histogram of **9_D**-modified substrates:

(a) with barbiturate modified gold tip, (b) with unmodified gold tip

From the AFM data we know that once the receptor is photodimerized, the binding ability decreases significantly. The different binding probabilities between the open- and closed-receptor substrates can be used to monitor the photodimerization process of the open-receptor modified substrate. The open-receptor modified substrate was photo-irradiated at 350-390 nm. During the photoirradiation the substrate was immersed in a degassed toluene solution to provide local solvation of the anthracene moieties. After photoirradiation, the substrate was again probed by AFM using the barbiturate-modified tip to record the binding probabilities. The results are shown in Figure 4.22. At 15 minutes of irradiation, the binding probability was decreased from 54% to 41%. However, when the irradiation time was extended to 60 minutes, the binding probability was only further decreased to 40%. The binding probability distribution profile remained the same during the photo-irradiation, which suggests that a portion of the receptor molecules on the substrate remains active towards binding, although their photo-reactivity is lost.

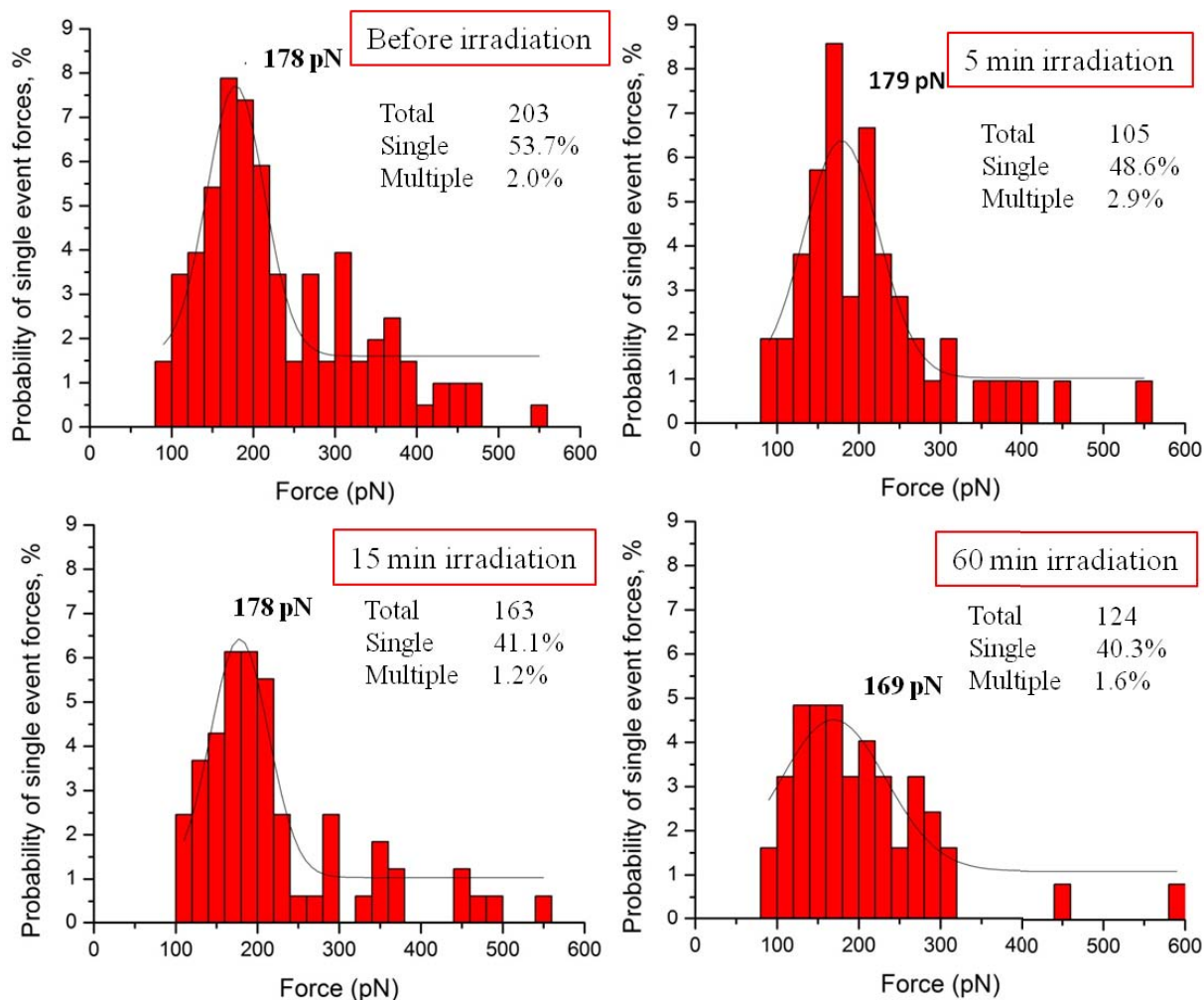


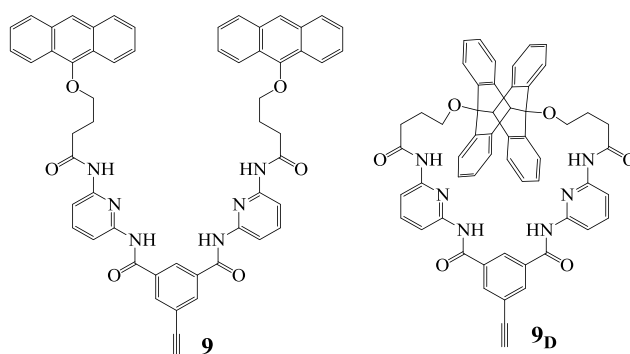
Figure 4.22 Adhesion histogram of **9**-modified substrates during photodimerization

4.4.5 Characterization of **9**-modified substrates using PM-IRRAS

Polarization modulation infrared reflection-absorption spectroscopy (PM-IRRAS) is a direct and nondestructive method to characterize various functional groups of SAMs deposited onto metallic substrates and to acquire molecular information on these bi-dimensional systems, such as the conformation of the alkyl chains and the orientation of the functional groups.³⁶ The PM-IRRAS method exploits the IRRAS advantages of the electric field enhancement and of the surface selection rule. Furthermore, it also demonstrates the abilities of high sensitivity in surface absorption detection and the ability to do *in situ* experiments even in infrared absorbing isotropic media.³⁷ PM-IRRAS has been used successfully to obtain vibrational spectra of Langmuir-Blodgett (LB) monolayers, lipid bilayers and SAMs deposited metallic substrates. We therefore applied it to investigate the surface properties of the modified gold substrates. These experiments were performed in collaboration with Dr. T. Buffeteau and D. Cavagnat (ISM).

4.4.5.1 ATR experiments

To begin, the *p*- and *s*-polarized Ge-ATR spectra of **9** and **9_D** (Scheme 4.9) were recorded in order to check whether a possible orientation of the open and closed receptor molecules would occur after deposition onto the Ge crystal, leading to anisotropic optical constants. As shown in Figure 4.23, the *p*-polarized ATR spectra of the open and close receptors have twice the intensity of the *s*-polarized ones over the entire spectral range, in agreement with an isotropic arrangement of the molecules at the crystal surface. As a consequence, the optical constants determined from the polarized ATR spectra are similar in the plane (*xy*) and out of the plane (*z*) of the layers. The variation of the isotropic refractive indexes $n(\bar{\nu})$ and extinction coefficients $k(\bar{\nu})$ are reported in Figure 4.23 in the 4000-600 cm^{-1} spectral range for the two molecules.



Scheme 4.9 Receptors **9** and **9_D** were investigated by ATR measurements

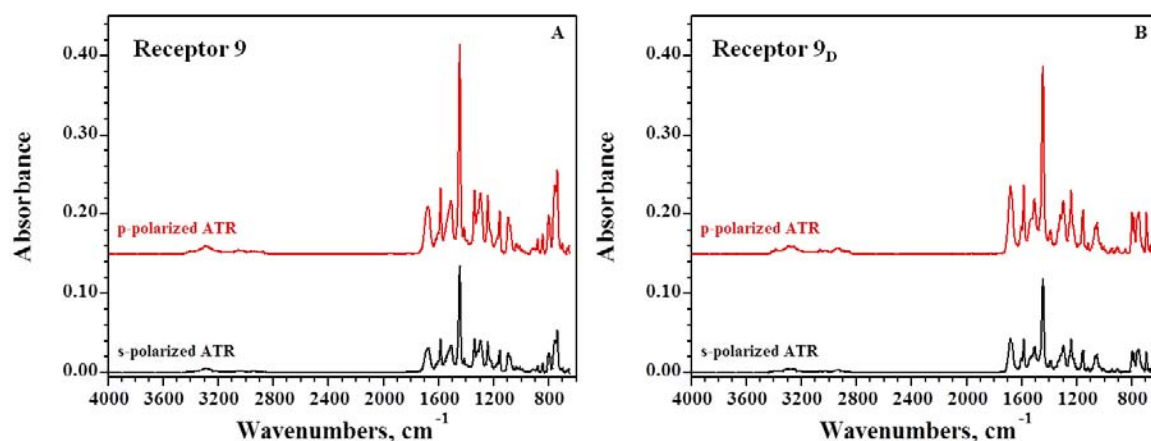


Figure 4.23 *p*- and *s*-polarized ATR spectra of **9** (A) and **9_D** (B)

The isotropic extinction coefficients $k(\bar{\nu})$ of the open and closed receptors are shown in Figure 4.24 in the 1800-1000 cm^{-1} spectral range. The $k(\bar{\nu})$ spectra can be used to assign the major infrared band of the receptor. This assignment has been performed according to the data in the literature²⁸⁻³⁰ and calculations of the optimized geometries, vibrational frequencies and

infrared intensities of the molecules using Gaussian 09 package. These calculations have been performed at the density functional theory (DFT) level using the B3PW91 functional and 6-31G* basis set. The wavenumbers, assignment and origin of the different infrared bands are given in Table 4.7. The three bands at 1415, 1390 and 1340 cm^{-1} are chosen to monitor the open and close forms of the receptor because they are easily distinguished from the other bands of the receptor. The bands at 1415 and 1340 cm^{-1} can be assigned to the $\nu\text{C}=\text{C}$ stretching vibration of the central ring of anthracene. The band at 1415 cm^{-1} is characteristic to the $\text{C}=\text{C}$ bonds common with the external rings whereas the band at 1340 cm^{-1} is coupled with the $\delta\text{C}=\text{C}-\text{H}$ bending vibration of the central ring. The band at 1390 cm^{-1} can be assigned to the ωCH_2 wagging vibrations of the three methylene linked to the anthracene and to the photodimer. This band exhibits a medium intensity for the closed receptor (**9_D**) whereas its intensity is very low for the open receptor (**9**).

Table 4.7 Assignments of the major bands in ATR spectra of **9** and **9_D**

Position, cm^{-1}	Assignment ^a	Origin
1680	$\nu\text{C}=\text{O}$	amide
1621	$\nu\text{C}=\text{C}$, $\delta\text{C}=\text{C}-\text{H}$ (external ring)	anthracene
1605, 1585	$\nu\text{C}=\text{C}$	pyridine ring
1511	δNH	amide
1469	$\nu\text{C}=\text{C}$, $\delta\text{C}=\text{C}-\text{H}$ (external ring)	photodimer
1449	$\nu\text{C}=\text{C}$	pyridine ring
1415	$\nu\text{C}=\text{C}$ (common bond)	anthracene
1390	ωCH_2 (3 CH_2 linked to photodimer)	photodimer
1340	$\nu\text{C}=\text{C}$ (central ring), $\nu\text{C}-\text{O}$	anthracene
1296	$\nu\text{N}=\text{C}$, $\nu\text{C}=\text{C}$	pyridine ring
1242	$\nu\text{N}=\text{C}$, $\nu\text{C}=\text{C}$	pyridine ring
1155	$\delta\text{C}=\text{C}-\text{H}$	pyridine ring
1118	$\delta\text{C}-\text{H}$, $\nu\text{C}-\text{O}$	photodimer
1095	$\delta\text{C}=\text{C}-\text{H}$	anthracene

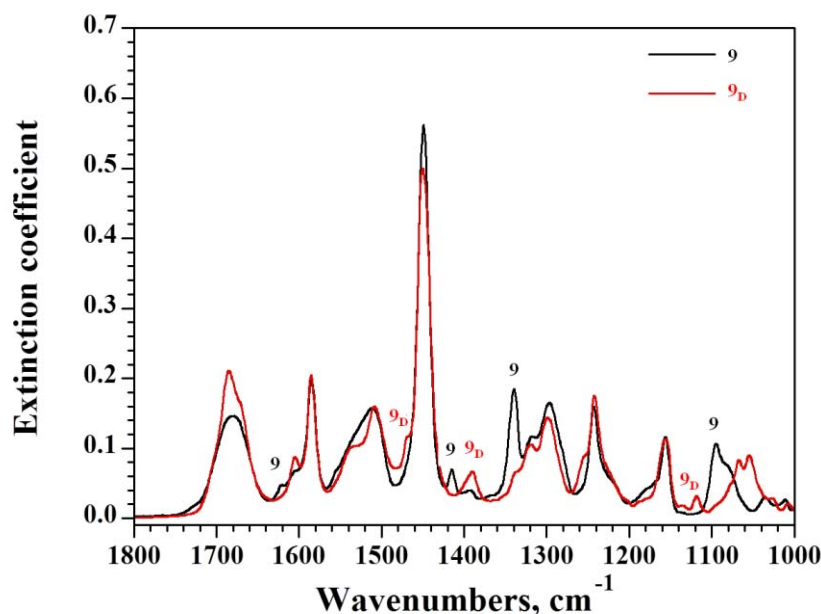


Figure 4.24 Isotropic extinction coefficients of **9** (black) and **9_D** (red). The bands assigned to mono-substituted anthracene and to photodimer are labeled **9** and **9_D**, respectively.

4.4.5.2 PM-IRRAS spectra of SAMs containing **9**, **9_D** and mixture

To characterize the surface properties, three modified substrates were prepared as references, which were **9**-, **9_D**-modified gold substrate and a 1:1 mixture of **9** and **9_D** substrate (Table 4.8). The PM-IRRAS spectra of SAMs containing open, close and mixed (1:1 mixture of **9** and **9_D**) receptors are shown in Figure 4.25. These spectra exhibit bands at 2920 and 2850 cm^{-1} , assigned to the asymmetric ($\nu_{\text{a}}\text{CH}_2$) and symmetric ($\nu_{\text{s}}\text{CH}_2$) stretching vibrations of the methylene groups, at 2105 cm^{-1} , assigned to asymmetric stretching vibration of the azide groups, and at wavenumbers lower than 1800 cm^{-1} , assigned to the vibrations of the receptor (Table 4.7). First, it is noteworthy that PM-IRRAS spectra were obtained with a very good signal-to-noise ratio, allowing observation of the chemical modification of the receptor onto a SAM. The zoom-in of the 1440-1260 cm^{-1} region (Figure 4.25 (B)) reveals clearly the presence of mono-substituted anthracene (bands at 1415 and 1340 cm^{-1}) for the open form of the receptor and the presence of photodimer (band at 1390 cm^{-1} associated with the disappearance of the bands at 1415 and 1340 cm^{-1}) for the closed form of the receptor. The PM-IRRAS spectrum of the SAM containing a mixture of **9** and **9_D** exhibits bands at 1415, 1390 and 1340 cm^{-1} with intermediate intensities, which indicates that it is possible to track the percentage of photodimerization directly on SAMs. Secondly, the wavenumbers observed for the $\nu_{\text{a}}\text{CH}_2$ (2920 cm^{-1}) and $\nu_{\text{s}}\text{CH}_2$ (2850 cm^{-1}) vibrations of methylene groups suggest that the monolayer is composed of an ordered arrangement of the alkyl chains with a preferential all-*trans* conformation. In the literature, these two modes are typically in the range 2915-2918 and 2846-2850 cm^{-1} for all-*trans* extended chains³¹ and at ~ 2928 and ~ 2856 cm^{-1} for liquid-like disordered chains.³² The presence of the $\nu_{\text{a}}\text{N}=\text{N}=\text{N}$ band at 2105 cm^{-1} , with a

significant intensity, reveals that only a portion of the SAM- $C_{12}N_3$ is reacted with the receptor.

Table 4.8 Substrate used in the investigation of PM-IRRAS

Sample ^a	Thickness (nm) ^b
9	1.3
9_D	0.6
9:9_D = 1:1	0.9

^a SAMs containing 100% $N_3C_{12}SH$; ^b thickness growth after click reaction determined by ellipsometry

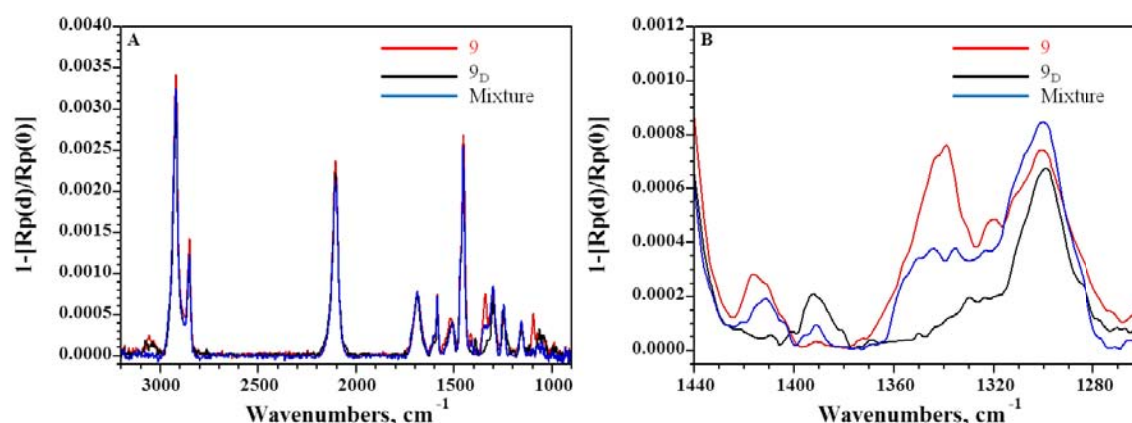


Figure 4.25 (A) PM-IRRAS spectra of SAMs containing **9** (red), **9_D** (black), and mixture (blue). (B) Zoom in the 1440-1260 cm^{-1} region.

The proportion of azide groups reacted with the receptor can be determined by comparing the PM-IRRAS spectra of a SAM- $C_{12}N_3$ and the SAM containing receptors, as shown in Figure 4.26. The decrease of the intensity of the $\nu_a N=N=N$ band at 2105 cm^{-1} is directly associated with the conversion of azide groups. The percentages of azide conversion have been calculated for the three investigated samples and listed in Table 4.9. An average value of azide conversion of 41.3 ± 1.9 % is found.

Table 4.9 Percentage of conversion of azido group on SAMs

Samples	% of azide conversion
9	41.5
Mixture	43.7
9_D	38.6

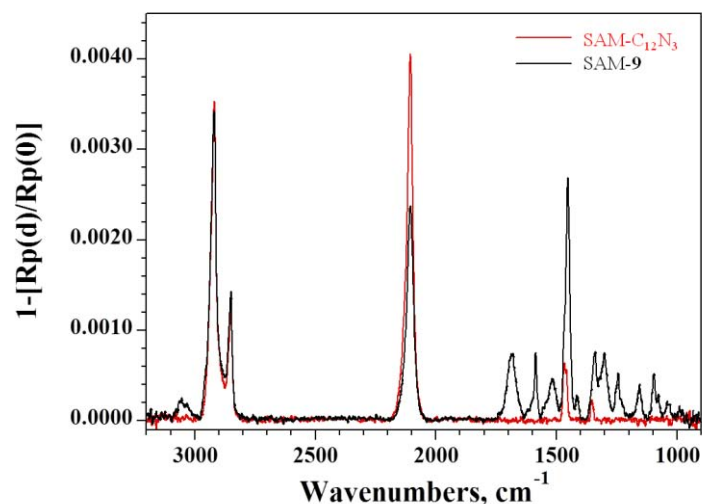


Figure 4.26 PM-IRRAS spectra comparison between SAM-C₁₂N₃ (red) and SAM-**9** (black)

The coverage of the receptor on the surface can be estimated by comparing the experimental PM-IRRAS spectrum of the **9**-modified SAM and the IRRAS spectrum calculated for a compact isotropic layer of **9**. We have estimated the thickness of the receptor layer at 10 Å (value measured by ellipsometry). As shown in Figure 4.27, the calculated IRRAS spectrum from the isotropic optical constants of **9** reproduces the relative intensities of the bands observed in the experimental spectrum. This feature suggests that the receptor molecules have no preferential orientation on the surface. However, the intensities calculated for the overall bands are 1.6 times higher than those measured in the experimental spectrum. This result indicates a surface coverage of **9** at the surface of about 62%.

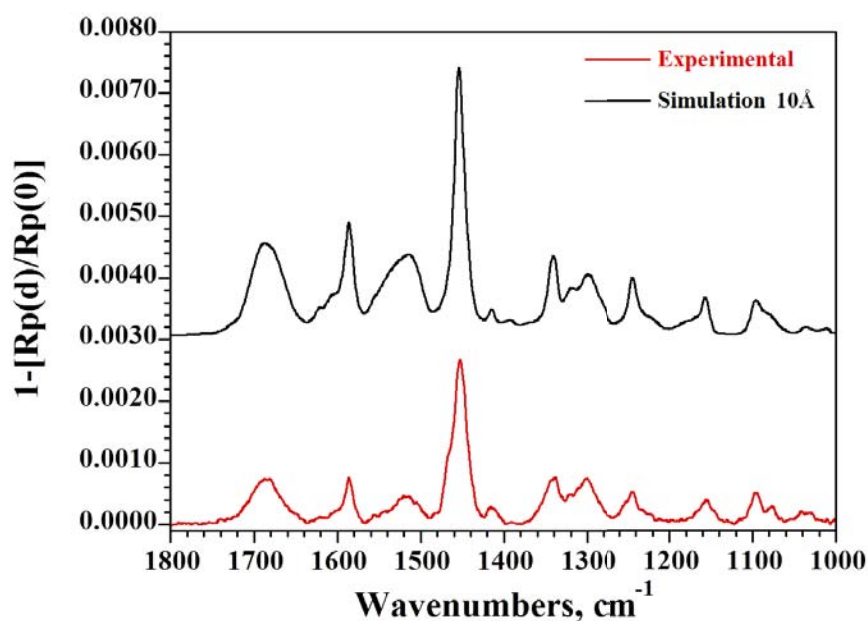


Figure 4.27 Experimental PM-IRRAS spectrum of **9**-modified SAM (black) and calculated IRRAS spectrum for a compact isotropic layer of **9** (thickness: 10 Å)

4.4.5.3 Reversible photodimerization and thermal retrocyclization on 9-modified substrate

From the solution experiments and the AFM data, we know that once the receptor is photodimerized, the binding ability decreases significantly. The different binding probabilities between the open- and closed-receptor substrates can be used to monitor the photodimerization process of the open-receptor modified substrate. The open-receptor modified substrate was photo-irradiated at 350-390 nm. From the experimental data above, we know that it is possible to monitor the photodimerization by observing the change of intensity of bands in $1440\text{--}1260\text{ cm}^{-1}$. Therefore we irradiated a 9-modified substrate in degassed CH_2Cl_2 using a lead nitrate solution filter (350-390 nm) for 20 minutes. The light source was an EXFO E3000-01 UV source lamp equipped with a 50W (HgXe) lamp and a 320-390 nm filter (Figure 4.28).

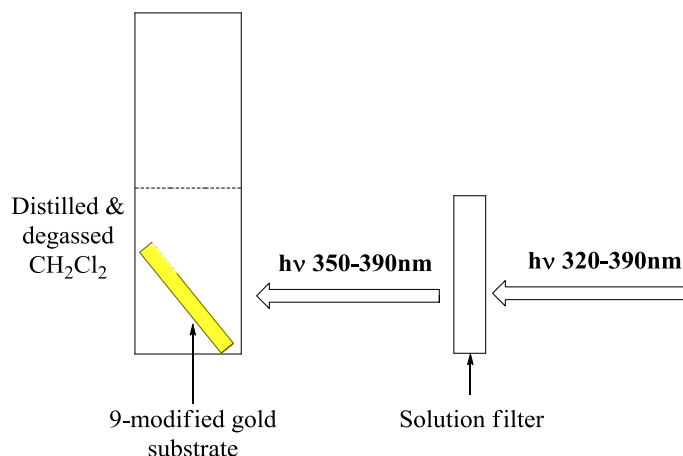


Figure 4.28 Experimental set up for photoirradiation on a 9-modified substrate

The open $\rightarrow$ closed conversion of the receptor was followed by PM-IRRAS experiments. The comparison of PM-IRRAS spectra of 9-modified substrate before and after irradiation is shown in Figure 4.29. It shows an overall decrease of PM-IRRAS intensities of about 20% after the irradiation, and therefore we can affirm that a small amount of molecules were lost during the irradiation. The zoom-in of the $1440\text{--}1260\text{ cm}^{-1}$ region (Figure 4.29 (B)) shows that the bands at 1415 and 1340 cm^{-1} , related to monosubstituted anthracene, decrease whereas the band at 1390 cm^{-1} , related to photodimer, appears. This result clearly indicates that the open $\rightarrow$ close conversion of the receptor occurs during irradiation. Using the intensity of the 1340 cm^{-1} band, we can estimate a conversion of about 70%.

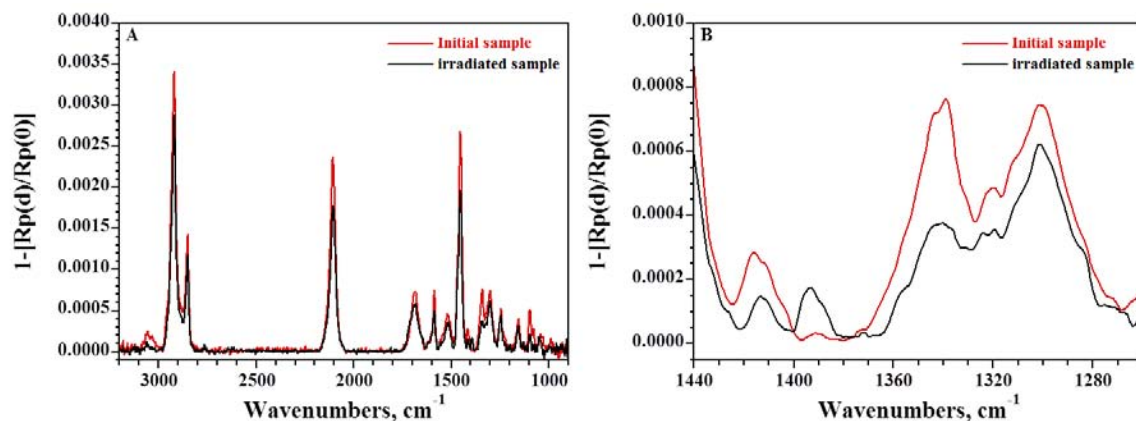


Figure 4.29 (A) PM-IRRAS spectra comparison of SAM-9 between before (red) and after (black) photoirradiation in degassed CH_2Cl_2 . (B) Zoom in the $1440\text{--}1260\text{ cm}^{-1}$ region.

The PM-IRRAS spectra confirms that it is possible to photodimerize the receptor directly on the substrate. The next step is to see whether the photodimer can be opened thermally on the substrate. The same irradiated substrate following irradiation (containing 70% **9_D**) was heated at 80°C under high vacuum for 20 minutes, and then directly analyze by PM-IRRAS without further treatment (Figure 4.30).

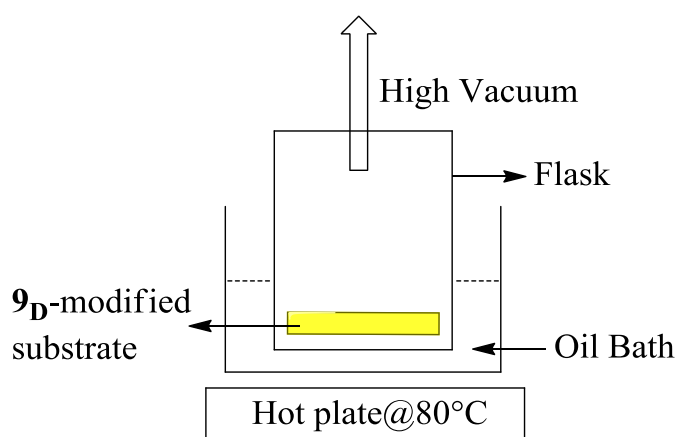


Figure 4.30 Experimental set up for thermal retro-cyclization on a **9_D**-modified substrate

The comparison of PM-IRRAS spectra of **9_D**-modified substrate before and after heating is shown in Figure 4.31. The intensities of the major bands remain unchanged, indicating that no degradation of the SAM occurs during the heating treatment. A very small disorder of the alkyl chains is observed for the heated sample, as demonstrated in Figure 4.31(B) by an increase of the width and the intensity of the $\nu_a(\text{CH}_2)$ and $\nu_s(\text{CH}_2)$ bands. The increase of the width is due to the presence of alkyl chains with *gauche* defects, giving rise to absorptions at higher wavenumbers (~ 2928 and $\sim 2856\text{ cm}^{-1}$) whereas the increase in the intensities is due to a variation in the conformational orientation of the methylene chains, resulting in an increase of the projection of their transition moment. Nevertheless, the disorder of the alkyl chains is very low with respect to those published in the literature for heated LB monolayers.^{33,34} An

expansion of the spectrum in the range of 1440-1260 cm^{-1} (Figure 4.31C) shows that the bands at 1415 and 1340 cm^{-1} increase in intensity whereas the band at 1390 cm^{-1} vanishes. This result clearly indicates that the close $\rightarrow$ open conversion of the receptor occurs during the heating.

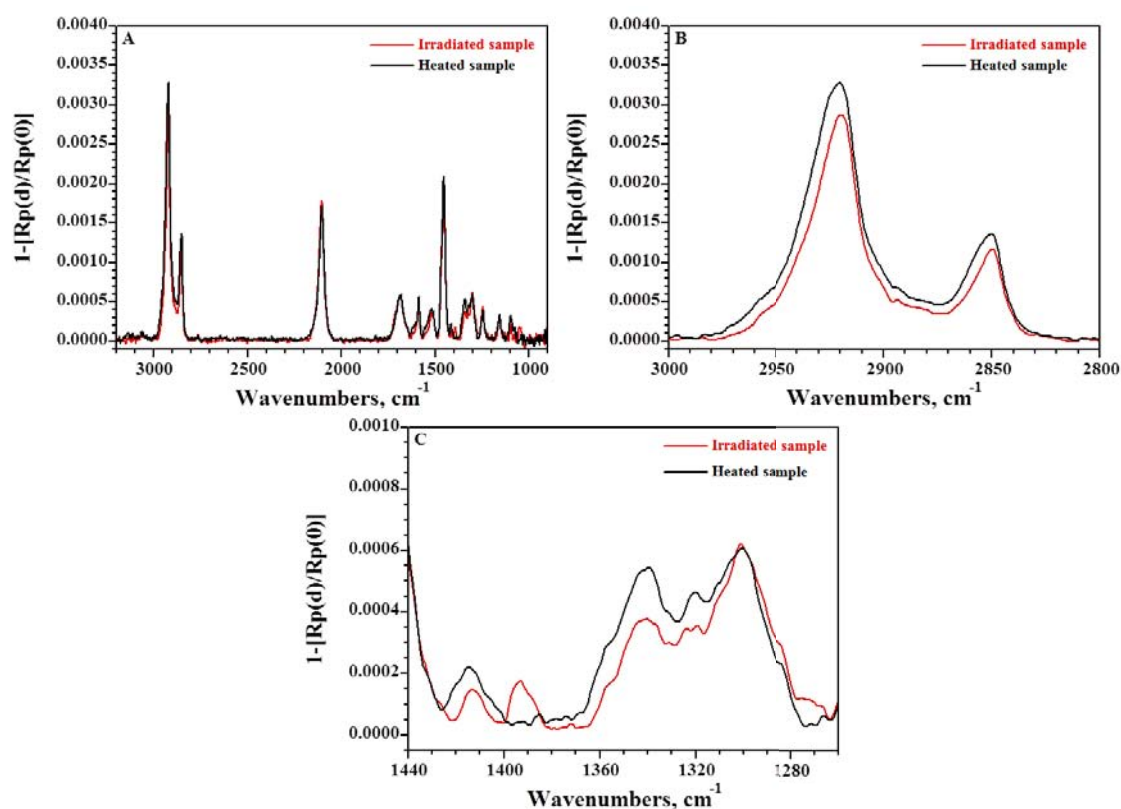


Figure 4.31 (A) PM-IRRAS spectra comparison of SAM-**9_D** between before (red) and after (black) heating at 80°C. Expansion of the 3000-2800 (B) and 1440-1260 cm^{-1} (C) regions.

A comparison of the PM-IRRAS spectra of **9**-modified substrate between before and after the irradiation-heating cycle is shown in Figure 4.32. The intensities of the 1415 and 1340 cm^{-1} bands are almost the same, which is evidence that the receptor on SAM remained intact.

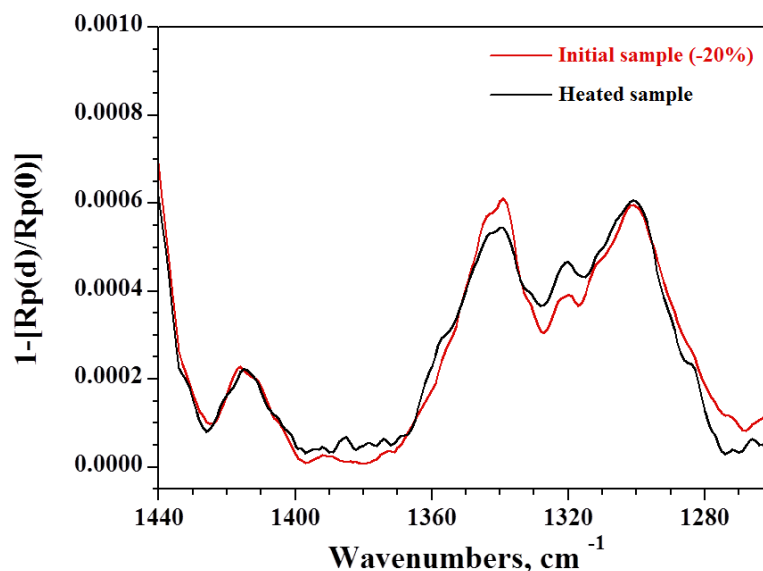


Figure 4.32 PM-IRRAS spectra comparison of **9**-modified substrate between before and after irradiation-heating cycle in the 1440-1260 cm^{-1} .

In summary, the grafting of the receptor molecules onto the gold substrate using a click reaction was successful. The substrate's properties were characterized using multiple techniques such as ellipsometry, contact angle, electrochemistry, AFM and PM-IRRAS. The results showed that it is possible to reversibly modulate the binding properties of the anthracene-appended receptors through a combination of irradiation with light and heat.

References

1. F. L. Carter, R. E. Siatkowski and H. Wohltjen, *Molecular Electronic Devices: Proceedings of the 3rd International Symposium on Molecular Electronic Devices*, North-Holland, 1988.
2. G. Roberts, *Langmuir-Blodgett Films*, Springer, **1990**.
3. H. Arwin, *Thin Solid Films*, **1998**, 313–314, 764-774.
4. H. Fujiwara, *Spectroscopic Ellipsometry: Principles and Applications*, John Wiley, **2007**.
5. P. E. Laibinis, G. M. Whitesides, D. L. Allara, Y. T. Tao, A. N. Parikh and R. G. Nuzzo, *J. Am. Chem. Soc.*, **1991**, 113, 7152-7167.
6. B. Bhushan, *Springer Handbook of Nanotechnology*, Springer, **2004**.
7. R. J. Colton, *Procedures in scanning probe microscopies*, John Wiley & Sons, **1998**.
8. B. P. Jena and J. K. H. Hörber, *Atomic Force Microscopy in Cell Biology*, Academic Press, **2002**.
9. V. J. Morris, A. R. Kirby and A. P. Gunning, *Atomic force microscopy for biologists*, Imperial College Press, **2009**.
10. H.-J. Butt, B. Cappella and M. Kappl, *Surface Science Reports*, **2005**, 59, 1-152.
11. *A practical guide to AFM force spectroscopy and data analysis*.
12. M. D. Porter, T. B. Bright, D. L. Allara and C. E. D. Chidsey, *J. Am. Chem. Soc.*, **1987**, 109, 3559-3568.
13. M. I. Béthencourt, L.-o. Srisombat, P. Chinwangso and T. R. Lee, *Langmuir*, **2009**, 25, 1265-1271.
14. J. M. Tour, L. Jones, D. L. Pearson, J. J. S. Lamba, T. P. Burgin, G. M. Whitesides, D. L. Allara, A. N. Parikh and S. Atre, *J. Am. Chem. Soc.*, **1995**, 117, 9529-9534.
15. H. Valkenier, E. H. Huisman, P. A. van Hal, D. M. de Leeuw, R. C. Chiechi and J. C. Hummelen, *J. Am. Chem. Soc.*, **2011**, 133, 4930-4939.
16. T. Park, H. Kang, I. Choi, H. Chung, E. Ito, M. Hara and J. Noh, *Bull. Korean Chem. Soc.*, **2009**, 30, 441-444.
17. J. P. Collman, N. K. Devaraj and C. E. D. Chidsey, *Langmuir*, **2004**, 20, 1051-1053.
18. G. V. Dubacheva, A. I. Van Der Heyden, P. Dumy, O. Kaftan, R. Auzély-Velty, L. Coche-Guerente and P. Labbé, *Langmuir*, **2010**, 26, 13976-13986.
19. G. A. Hudalla and W. L. Murphy, *Langmuir*, **2009**, 25, 5737-5746.
20. R. Chelmowski, D. Kaefer, S. D. Koester, T. Klasen, T. Winkler, A. Terfort, N. Metzler-Nolte and C. Woell, *Langmuir*, **2009**, 25, 11480-11485.
21. I. F. Gallardo and L. J. Webb, *Langmuir*, **2010**, 26, 18959-18966.
22. J. K. Lee, Y. S. Chi and I. S. Choi, *Langmuir*, **2004**, 20, 3844-3847.
23. R.-V. Ostaci, D. Damiron, S. Capponi, G. Vignaud, L. Leger, Y. Grohens and E. Drockenmuller, *Langmuir*, **2008**, 24, 2732-2739.
24. Y. Zhang, S. Luo, Y. Tang, L. Yu, K.-Y. Hou, J.-P. Cheng, X. Zeng and P. G. Wang,

- Anal. Chem.*, **2006**, 78, 2001-2008.
25. J.-S. Park, A. C. Smith and T. R. Lee, *Langmuir*, **2004**, 20, 5829-5836.
 26. J. P. Collman, N. K. Devaraj, T. P. A. Eberspacher and C. E. D. Chidsey, *Langmuir*, **2006**, 22, 2457-2464.
 27. L. He, J. W. F. Robertson, J. Li, I. Kärcher, S. M. Schiller, W. Knoll and R. Naumann, *Langmuir*, **2005**, 21, 11666-11672.
 28. A. Bree and R. A. Kydd, *J. Chem. Phys.*, **1968**, 48, 5319-5325.
 29. J. G. Radziszewski and J. Michl, *J. Chem. Phys.*, **1985**, 82, 3527-3533.
 30. G. Socrates, *Infrared Characteristic Group Frequencies: Tables and Charts*, John Wiley & Sons, **1994**.
 31. R. A. MacPhail, H. L. Strauss, R. G. Snyder and C. A. Elliger, *J. Phys. Chem.*, **1984**, 88, 334-341.
 32. R. G. Snyder, H. L. Strauss and C. A. Elliger, *J. Phys. Chem.*, **1982**, 86, 5145-5150.
 33. C. Naselli, J. F. Rabolt and J. D. Swalen, *J. Chem. Phys.*, **1985**, 82, 2136-2140.
 34. T. Hasegawa, S. Takeda, A. Kawaguchi and J. Umemura, *Langmuir*, **1995**, 11, 1236-1243.
 35. A. B. D. Cassie and S. Baxter, *Trans. Faraday Soc.*, **1944**, 40, 546-551.
 36. A.E. Dowrey, C. Marcott, *Appl. Spectrosc.*, **1982**, 36, 414
 37. J.C. Riviere, *Surface Analytical Techniques*, Clarendon Press, Oxford, **1990**.

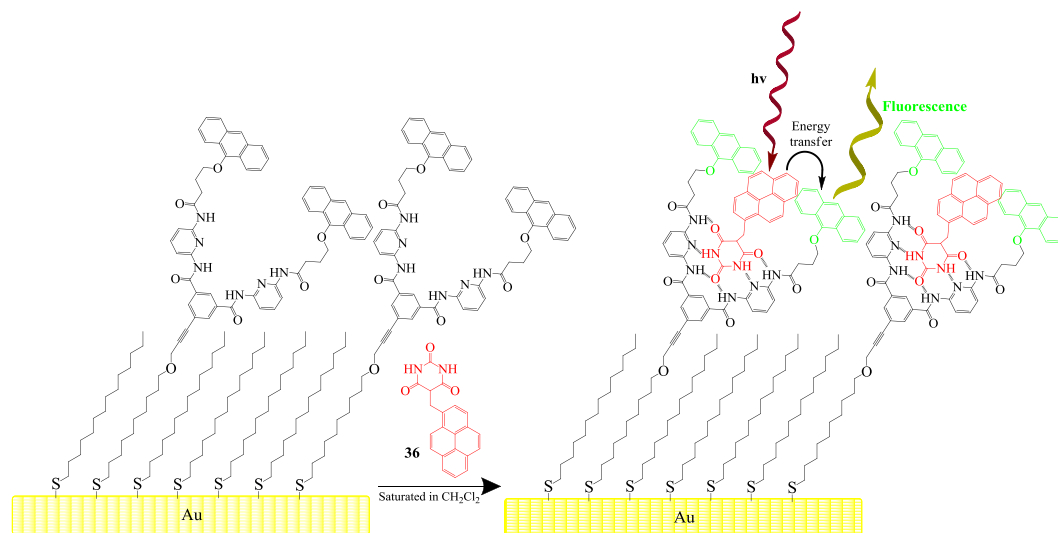
Chapter 5. Conclusion and Future Perspectives

5.1 Future perspective

The assembly of the anthracene-appended receptor onto gold substrates via a click reaction is quite successful, and we showed that it is possible to control the binding ability of the **9**-modified substrate using light. Clearly, the next step will be to use the specific binding between the open receptors and barbituric acid derivatives to build hierarchical supramolecular architecture at desired locations on a substrate. In the following sub-sections, the results obtained from the preliminary investigation of the binding abilities of the surface-bound receptors are presented. The first example makes use of a pyrene-functionalized barbituric acid to investigate Förster energy transfer in surface-bound supramolecular assemblies. In the second example, an acridine derivative is used to promote binding of double-stranded DNA to the substrate surface.

5.1.1 Preliminary binding ability test on receptor 7-modified substrate

The binding ability of the **7**-modified substrate was examined using pyrene-barbituric acid **36** as an energy donor molecule. The latter is appended with a barbituric acid molecular recognition motif that is complementary to **7**. A **7**/C₁₄-thioacetate (20:80) substrate was incubated with a saturated solution of **36** in dichloromethane at room temperature for 24 hours (Scheme 5.1). After incubation, the substrate was washed with dichloromethane to remove residual **36**, and then dried with a stream of nitrogen. The measured fluorescence emission is shown in Figure 5.1, and the corresponding excitation spectra are shown in Figure 5.2.



Scheme 5.1 Incubation of Modified Substrate with Pyrene-Barbiturate

The emission intensity of the fluorescence from the substrate modified with the pyrene-barbiturate **36** was increased about five times compared to that of the receptor-only substrate (Figure 5.1). Even though, as noted previously, the intensity can be difficult to quantify, such a large increase is likely to be beyond the variability of the experimental setup

and may be an indication of resonant energy transfer (FRET) between the pyrene, which acts as an antenna, to the anthracenes. Pyrene monomer possess a higher energy S_1 state than anthracene ($E_s = 322$ vs 296 kJmol^{-1} for pyrene and anthracene¹, respectively), and the process is thus favored energetically. To further confirm this, the excitation spectra were measured. The result is shown in Figure 5.2, where it can be seen that the strong pyrene $\pi-\pi^*$ absorption band at 306 nm can be easily identified. The characteristic vibronic progression of the pyrene $S_0 \rightarrow S_2$ absorption is absent, perhaps lost due to the large slit width used (5 nm) and broadening. The receptor-only substrate showed the excitation maximum located at around 370 nm, assigned to the $\pi-\pi^*$ $S_0 \rightarrow S_1$ of the anthracene. However, the results should to be further confirmed as the anthracene $S_0 \rightarrow S_2$ $\pi-\pi^*$ transition is also located near the observed pyrene absorption band (at 308 nm in CH_2Cl_2). We therefore tentatively conclude that the open receptor molecule binds the pyrene-functionalized barbiturates and that energy transfer between the two can take place.

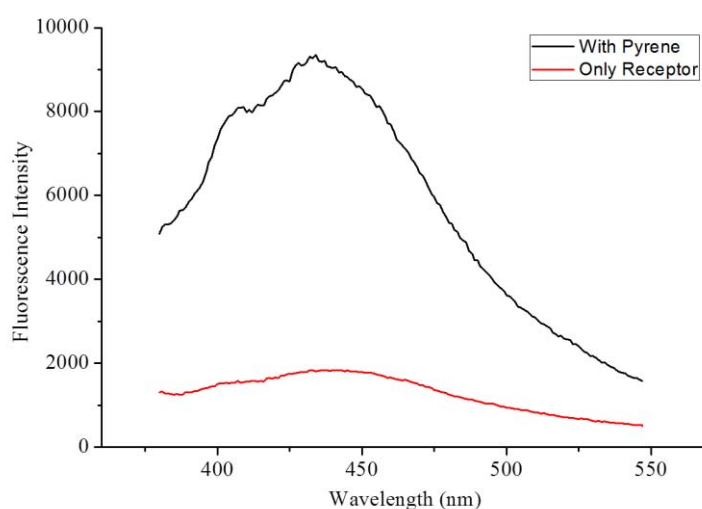


Figure 5.1 Fluorescence emission ($\lambda_{\text{ex}}=370 \text{ nm}$) from 7/ C_{14} -thioacetate (20:80) substrate before (red curve) and after (black curve) incubation with **36**.

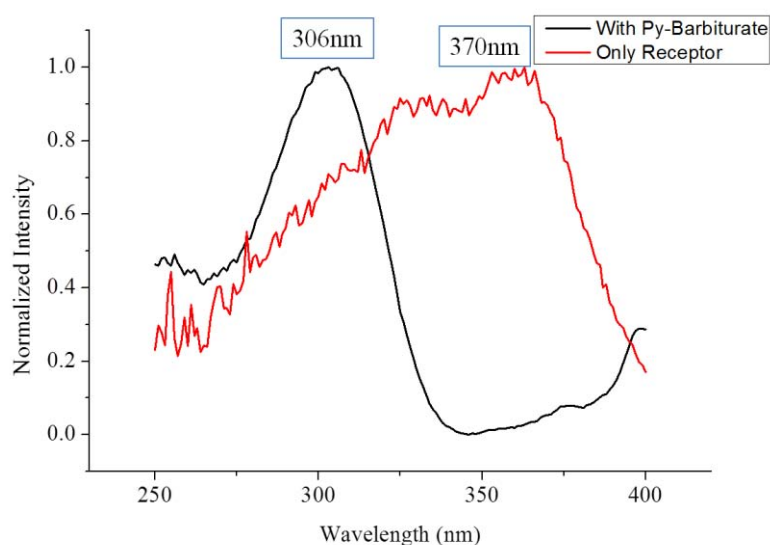


Figure 5.2 Fluorescence excitation spectra ($\lambda_{\text{em}}=440 \text{ nm}$) from 7/ C_{14} -thioacetate (20:80) substrate before (red curve) and after (black curve) incubation with **36**.

5.1.2 Preliminary binding ability test on receptor 9-modified substrate

The binding ability of receptor **9** immobilized on a modified gold substrate towards an acridine-cyanuric acid derivative (compound **35**) was examined. The acridine heterocycle was selected as it possess the ability to intercalate in DNA and RNA double strands (ds), and thus possibly construct multi-layer structures on the gold substrate. A substrate covalently modified with **9** (20% N₃C₁₂SH/C₁₀SH) was used and results are listed in Table 5.1. In the first trial, the modified substrate was immersed into an acridine-cyanuric acid solution (50μM of **35** in water) for 30 minutes, and then rinsed with water to remove residual physisorbed **35** and characterized using ellipsometry. The thickness of the modified substrate was slightly increased (by 0.3 nm). The second trial involved dipping the modified substrate into a mixed solution of acridine-cyanuric acid **35** (12μM) and DNA (62.5μM in base pairs) with phosphate buffer for 30 minutes. The increased thickness of substrate was significant (1.8 nm), which indicates that the DNA is adsorbed onto the substrate (Figure 5.3, left). As a control experiment, the binding between a substrate bearing the immobilized receptor **9** on the surface and DNA in phosphate buffer (without the acridine derivative **35**) was tested. Interestingly, the thickness of this substrate was also increased by 1.7 nm, which is nearly identical to that observed for the Au-**9**/**35**/DNA. Because double-strand DNA is known not to physisorb to hydrophobic substrates, it is possible that the receptor itself is capable of specific DNA binding interactions, as anthracene derivatives are known to also bind to double-stranded DNA by intercalation (Figure 5.3, right).²⁻⁵ This result is particularly interesting because the photodimerized receptor is not expected to bind DNA, which may lead to substrates whose DNA-binding ability can be reversibly gated using light.

Table 5.1 Results of binding test of receptor-modified gold substrate

Molecules	Conditions	Rinsing	Thickness
Acridine-Cyanuric Acid 35 (50μM in H ₂ O)	30min in H ₂ O	H ₂ O	+0.3 nm
Acridine-Cyanuric Acid 35 (12.5μM)	30 min in 0.04M phosphate buffer	Buffer / H ₂ O	+1.8 nm
DNA (62.5μM)	0.1M NaCl, pH 7.5		
	30 min in 0.04M phosphate buffer	Buffer / H ₂ O	+1.7 nm
DNA (62.5μM)	0.1M NaCl, pH 7.5		

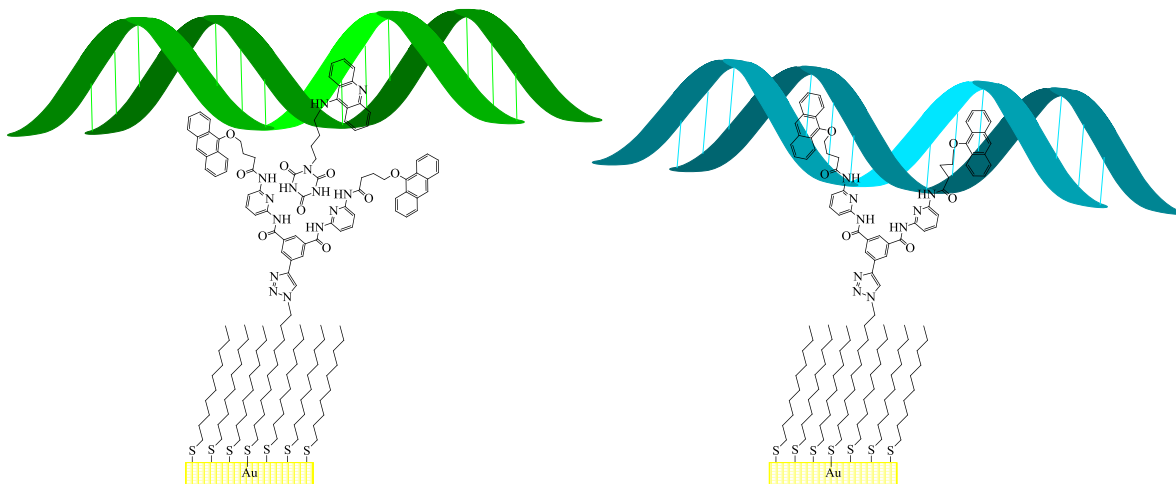


Figure 5.3 Schematic representation of receptor immobilized substrates bound with DNA via the assistance of acridine derivative **22** (left) or anthracene moiety (right).

5.2 Conclusion

A series of anthracene-appended photoactive barbiturate receptors with different end groups **5**, **6**, **7**, **8**, **9** and their corresponding photodimers **5_D**, **7_D**, **9_D** were synthesized. These receptor molecules can be used in combination with various substrate grafting techniques, such as amide bond formation using amine- or carboxylic-acid-terminated SAMs, direct thioacetate anchoring on gold substrates, and a post-click reaction modification on azide-terminated SAMs. A series of functionalized barbituric acid derivatives suited to binding the receptor was also prepared, and used to obtain preliminary information on the suitability of this system to construct hierarchically organized supramolecular devices.

The photophysical and photochemical properties of receptor **5** were investigated in solution. These were compared to the anthracene monomer **10** to differentiate the behaviors of the bis-anthracene system from that of the isolated anthracene chromophore. Several techniques were applied to characterize the photophysical and photochemical properties, including variable temperature lifetime, fluorescence and photodimerization quantum yield measurements. Upon photoirradiation of **5** in CH₂Cl₂ at 365 nm, we found that two photodimers are formed, namely the HT and HH photodimers. In the fluorescence decay data we can also observe two excimer lifetimes for **5**, which we attribute to the existence of two non-interconverting excimer geometries. Based on the variable temperature data and relative rate of intermolecular quenching of the emissive excimer states and photodimer formation, we further propose that the intramolecular excimers are intermediates in the photodimerization reaction of **5**.

To graft the photochromic receptors onto a surface, the first approach involved using receptor **7** possessing a thioacetate group to directly anchor it onto gold substrates. Although both ellipsometry and fluorescence measurements confirmed that **7** is grafted onto the gold substrate, cyclic voltammetry measurements showed that the packing of SAMs on the surface was not as good as expected. Therefore, to obtain a closed packed SAM, we turned to a

post-modification approach via a Huisgen 1,3-dipolar cycloaddition (click) reaction. The grafting of receptor **9** onto the gold substrates was again confirmed by ellipsometry measurements, and the SAMs thus obtained were confirmed to be densely packed on the surface by cyclic voltammetry measurements. The surface binding properties were examined by AFM measurements using **9**- and **9_D**-modified substrates using force measurements. With a barbiturate modified gold tip, strong binding interactions between the tip and **9**-modified substrate were detected, whereas the **9_D**-modified substrate did not show specific interactions. The control of photodimerization and thermal retro-cyclization on the gold substrates was examined by PM-IRRAS measurements. By irradiating the **9**-modified substrate in CH₂Cl₂ at 350-390 nm, we can photodimerize *ca.* 70% of **9** into **9_D** directly on the substrate. The same substrate can undergo a thermal retro-cyclization by heating the substrate to 80°C. Thus it is possible to reversibly modulate the binding properties of the anthracene-appended receptors though a combination of the irradiation with light and heat. Further work on such systems will involve exploring the selective binding of functionalized barbituric acid derivative, which is to fulfill the purpose of building a space-resolved architecture on devices.

References

1. M. Montalti, A. Credi, L. Prodi and T. Gandolfi, *Handbook of Photochemistry, Third Edition*, Taylor & Francis, **2006**.
2. C. V. Kumar and E. H. Asuncion, *J. Am. Chem. Soc.*, **1993**, *115*, 8547-8553.
3. C. V. Kumar, E. H. A. Punzalan and W. B. Tan, *Tetrahedron*, **2000**, *56*, 7027-7040.
4. N. K. Modukuru, K. J. Snow, B. S. Perrin, Jr., J. Thota and C. V. Kumar, *J. Phys. Chem. B*, **2005**, *109*, 11810-11818.
5. W. D. Wilson, Y. H. Wang, S. Kusuma, S. Chandrasekaran, N. C. Yang and D. W. Boykin, *J. Am. Chem. Soc.*, **1985**, *107*, 4989-4995.

Chapter 6. Experimental Section

6.1 Materials

Commercially-available starting materials were obtained from Acros, Sigma-Aldrich, Avocado, Lancaster or TCI unless other mentioned. Anhydrous solvents, such as absolute ethanol, chlorobenzene, DMSO and DMF were used as received. Dichloromethane, toluene and acetonitrile were distilled over calcium hydride (CaH_2). Tetrahydrofuran (THF) and diethyl ether were dried over sodium/benzophenone and distilled immediately before use.

6.2 Thin-layer chromatography and column

Thin-layer chromatography was performed on Merck 60 silica plate F254. The plates are visualized under UV at 254 nm and 365 nm. Column chromatography was performed on silica gel (silica gel Merck, particle size of 63-200 microns, 230-400 mesh or size from 40 to 63 microns).

6.3 Nuclear magnetic resonance (NMR)

The ^1H and ^{13}C NMR spectra were recorded on Bruker DPX200 (^1H : 200 MHz), AC-250 (^1H : 250 MHz, ^{13}C : 62.9 MHz), Avance 300 (^1H : 300 MHz, ^{13}C : 75 MHz) spectrometer. Chemical shifts δ are expressed relative to tetramethylsilane (TMS) using the residual signals of deuterated solvents (CDCl_3 , CD_2Cl_2 , D_2O , d_6 -DMSO, CD_3OD) as internal reference. The coupling constants are calculated in Hertz (Hz). For the assignment of signals, the following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublet, m = multiplet.

6.4 Mass spectrometry

Mass spectra were obtained by Centre d'Etude Stucturale et d'Analyse des Molécules Organiques (CESAMO) at the University Bordeaux I. The impact spectra (EI) ($E = 70$ eV) and LSIMS⁺ ($E = 35$ keV) were performed on a spectrometer Micromass VG Autospec-Q. Electrospray spectra were performed on a spectrometer Varian Saturn-4D. MALDI-TOF mass spectra were recorded on an Applied Biosystem Voyager. The high resolution spectra were recorded using the method of LSIMS⁺ with a resolution of 10000.

6.5 UV-visible absorption spectroscopy

The electronic absorption spectra were recorded on a double beam Varian Cary 5000 spectrophotometer whose wavelength range extends from 170 to 3300 nm.

6.6 Fluorescence quantum yields

Fluorescence quantum yields were determined by comparison with a known standard: quinine bisulfate in 0.05 M H₂SO₄ solution, $\Phi_F = 0.546$.¹ The quantum yield was determined using the equation:

$$\Phi_F = \frac{A_{ST}}{A_F} \times \frac{F_F}{F_{ST}} \times \left(\frac{n_F}{n_{ST}} \right)^2 \times \Phi_{ST}$$

where Φ_F : fluorescence quantum yield

A_{ST} : absorbance of standard at the excitation wavelength

A_F : absorbance at the excitation wavelength

F_F : Emission intensity (integrated)

F_{ST} : Emission intensity of standard (integrated)

n_F : refractive index of the solvent

n_{ST} : refractive index of the standard's solvent ($n_{DCM}=1.424$ $n_{water}=1.34$)

6.7 Photodimerization quantum yields

The photodimerization quantum yield is defined as:

$$\Phi = \frac{\text{number of photodimerized molecule per time unit}}{\text{number of photons absorbed per time unit}}$$

Actinometry allows determination of the photon flux for a system.¹ In our case we used potassium ferrioxalate as chemical actinometer. The quantity of ferrous ions formed during the irradiation period is monitored by conversion to the colored tris-phenanthroline complex ($\epsilon=11100$ L mol⁻¹ cm⁻¹ at $\lambda_{max}=510$ nm). Ferric ions are not complexed and thus do not contribute to the absorption at 510 nm. After irradiation, the formation of ferrous ions can be measured by UV absorption at 510 nm, and therefore the photon influx can be deduced by the following equation:

$$\text{Photon influx} = \frac{\text{Abs at 510 nm}}{\epsilon} \times V \div \Phi_{\lambda} \times t$$

where V equals the volume in UV cell, ϵ equals extinction coefficient of actinometer reference, Φ_{λ} equals the quantum yield of ferrous ion at wavelength λ , and t equals irradiation time.

The quantitative determination of the photodimerization product can be measured by two methods. The first one is to measure the difference of absorption spectrum at 371 nm between

before and after photoirradiation, and then divide this by the extinction coefficient of the chromophore to deduce the concentration of photodimer. The second one is measure the integration area of the product by HPLC. The moles of photodimer is deduced by calibration with a solution of 5 (1.069 mg/ 10 mL CH₂Cl₂ (1.04 x 10⁻⁴ M), injection volume 15 µL, UV detector set at 300 nm, integration area = 2283) and 5_D as standards and comparing the integration area of the photolyzed sample.

6.8 Preparative irradiation procedure

For the preparation of photodimers on the anthracene-appended receptors, preparative photoirradiation is necessary. For a large scale preparation, a solution filter is used (lead nitrate/ potassium bromide, Pb(NO₃)₂ 7 g L⁻¹/KBr 540 g L⁻¹), which cut off the UV light below 350 nm.² Light source is a Hanovia 450W HgXe lamp. During the irradiation, a continuous bubbling of argon or nitrogen is necessary to avoid photo-oxidation. The reaction can be monitored by UV-absorption spectrum using the reduction of the absorption band at 370 nm.

6.9 Ellipsometry measurement

The thickness of the films formed was determined using an Accurion nanofilm EP³-SE ellipsometer equipped with a 20 mW 658 nm laser and Xe Arc lamp with 46 filters. It operates on the principle of classical null ellipsometry. Optical parameters were obtained from the ellipsometric angles (ψ and Δ) measured with an incidence angle of 70 degrees (for Au substrates), which give the null conditions. The spatial resolution was about 2 µm, and the width of images was 400 µm with a x10 magnification lens. The angles of the polarizer, compensator, and analyzer that obtained the null condition allow one to obtain the (Δ , Ψ) angles, which are related to the optical properties of the sample. For ultrathin films, Δ is proportional to the film thickness. Analysis of the measured data with standard optical models (n,k) included in the ellipsometric software leads to a deduction of film thickness for a given refractive index. The refractive index value for the self-assembled monolayer is determined to be 1.5 for the fitting of the thickness.

6.10 Contact angle

Contact angles were measured using a *KRÜ SS DSA100* instrument with milliQ water was used as the testing liquid. Once the droplet was deposited on the surface, the droplet image was taken and then fitted by the models provided in the software.

6.11 Preparation of surface modification on gold via a click reaction

1. Piranha solution ($\text{H}_2\text{O}_2 / \text{H}_2\text{SO}_4 = 1:3$) was used to clean all the glass bottles overnight, and then were washed with copious MilliQ water and absolute ethanol to remove residual acid and dried in the oven overnight.

WARNING: Piranha solution should be handled with caution; in some circumstances (most probably when it had been mixed with significant quantities of an oxidizable organic material), it has detonated unexpectedly)

2. Gold substrates were cleaned by UV-ozone treatment for 20 minutes, followed by immersion in stirring absolute ethanol for another 20 minutes.³

(UV-ozone was generated by Novascan PSDP-UV8T with OES-1000D as ozone neutralizer)

3. Ellipsometry was measured in Brewster's angle of 70 degree with wavelength variation from 370 to 720.5 nm and a blank substrate recorded as the reference.

4. For SAM preparation, 1~3 mM thiol solution in absolute ethanol was prepared. The clean substrates were incubated in the thiol solution at 30°C for 48 hours. After incubation, the substrates were washed with copious absolute ethanol to remove residual thiol molecules and dried by a stream of N_2 .

5. Ellipsometry was measured at Brewster's angle (70°) with wavelength variation from 370 to 720.5 nm. At least three measurements were recorded at different locations and converted to thickness for each substrate.

6. Click modification on SAM:

For 600 μL : 1) 30 μL 10 mM CuSO_4 in H_2O

2) 200 μL 1.5 mM TBTA (Tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine) in DMSO

3) 170 μL 10mM NaAsc in H_2O

4) 200 μL 1.5mM Receptor-alkyne in DMSO (must be freshly prepared)

After mixing CuSO_4 and TBTA, the solution was slightly blue, and after addition of NaAsc the solution became colorless (evidence that Cu(II) has been reduced to Cu(I)). After addition of the alkyne solution, the gold substrate with an azide-terminated SAMs were put into the solution and kept from light at 30°C for 4 hours.

9. Washing procedure:

1) Fresh DMSO incubation for 2 mins, then washed with 10 mL DMSO washing

2) Fresh milliQ H_2O incubation for 2 mins, then washed with 10 mL H_2O washing.

3) Dry the substrate by a stream of N_2 .

10. Samples were characterized using ellipsometry (angle: 70°) with wavelength variation from 370 to 720.5 nm. At least three measurements were recorded at different locations and converted to thickness for every substrate.

6.12 Photodimerization on 9-modified gold substrate for PM-IRRAS measurements

The experimental setup is illustrated in Figure 6.1. A gold substrate modified with **9** was placed in a clean test tube, sealed by a septum and then put under vacuum to remove oxygen. Another flask was injected with distilled CH_2Cl_2 and degassed by bubbling Ar for 30 minutes, and then the degassed CH_2Cl_2 was transferred into the test tube by cannula. The solution filter is as described in section 6.8. The solution filter was put in front of the substrate to make sure all the light irradiated on the substrate was filtered. The light source was an EXFO E3000-01 UV lamp equipped with a 50W lamp and a 320-390 nm filter. The light intensity was adjusted to 5 and the irradiation time was 20 minutes. After irradiation, the substrate was washed with distilled CH_2Cl_2 and dried by a stream of N_2 . The characterization was done by PM-IRRAS.

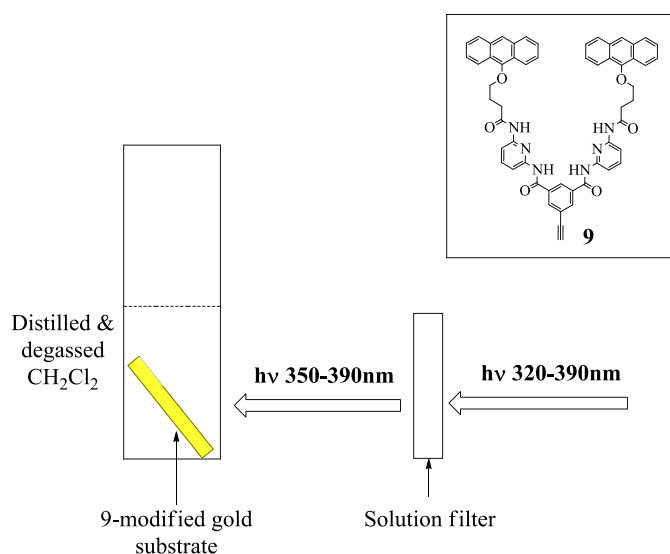


Figure 6.1 Schematic setup for photodimerization of **9**-modified gold substrate in CH_2Cl_2

6.13 Retro-cyclization of **9_D**-modified gold substrate

The **9_D**-modified substrate was placed in a clean flask connected to high vacuum and heated in oil bath for 20 minutes (Figure 6.2). After heating, the flask was back-filled with nitrogen and directly analyzed by PM-IRRAS characterization without further treatment.

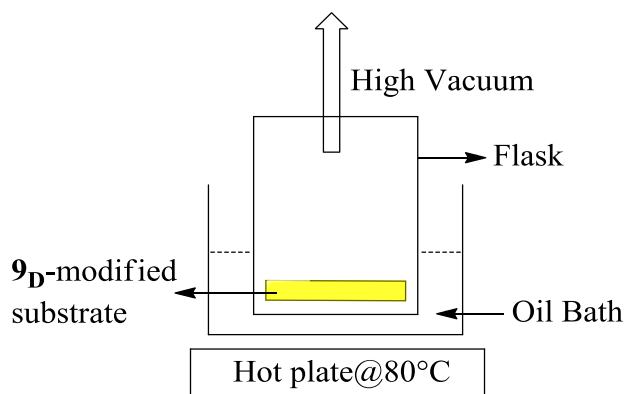


Figure. 6.2 Schematic set up of retro-cyclization on **9_D**-modified substrate

6.14 ATR experiments

ATR spectra were recorded on a ThermoNicolet Nexus 670 FTIR spectrometer at a resolution of 4 cm^{-1} , by coadding 500 scans. ATR experiments were performed using a single-reflection ATR accessory (Specac) equipped with a germanium (Ge) crystal and a liquid nitrogen cooled narrow-band mercury cadmium telluride (MCT) detector. A BaF_2 wire grid polarizer was added to record the spectra in the p- and s-polarizations.

6.15 PM-IRRAS experiments

PM-IRRAS spectra were recorded on a ThermoNicolet Nexus 670 FTIR spectrometer at a resolution of 4 cm^{-1} , by integration of several blocks of 1500 scans (30 minutes acquisition time). Generally, eight blocks (4 hours acquisition time) are necessary to obtain PM-IRRAS spectra of SAMs with good signal-to-noise ratio. Experiments were performed at an incidence angle of 75° using an external homemade goniometer reflection attachment.⁴ The infrared parallel beam (modulated in intensity at frequency ω_i lower than 5 KHz) was directed out of the spectrometer with an optional flipper mirror and made slightly convergent with a first BaF_2 lens (191 mm focal length). The IR beam passed through a BaF_2 wire grid polarizer (Specac) to select the *p*-polarized radiation and a ZnSe photoelastic modulator (PEM, Hinds Instruments, type III). The PEM modulated the polarization of the beam at a fixed high frequency, $\omega_m=74$ KHz, between the parallel and perpendicular linear states. After reflection on the sample, the double modulated (in intensity and in polarization) infrared beam was focused with a second ZnSe lens (38.1 mm focal length) onto a photovoltaic MCT detector (Kolmar Technologies, Model KV104) cooled at 77 K. The polarization modulated signal I_{AC} was separated from the low frequency signal I_{DC} (ω_i between 500 and 5000 Hz) with a 40 KHz high pass filter and then demodulated with a lock-in amplifier (Stanford Model SR 830). The output time constant was set to 1 s. The two interferograms were high-pass and low-pass filtered (Stanford Model SR 650) and simultaneously sampled in the dual channel electronics of the spectrometer. In all experiments, the PEM was adjusted for a maximum efficiency at 2500 cm^{-1} to cover the mid-IR range in only one spectrum. For calibration measurements, a second linear polarizer (oriented parallel or perpendicular to the first preceding the PEM) was inserted between the sample and the second ZnSe lens. This procedure was used to calibrate and convert the PM-IRRAS signal

in terms of the IRRAS signal (i.e. $1 - \frac{R_p(d)}{R_p(0)}$, where $R_p(d)$ and $R_p(0)$ stand for the *p*-polarized reflectance of the film/substrate and bare substrate systems, respectively).^{5,6}

6.16 Spectral simulations and determination of the optical constants

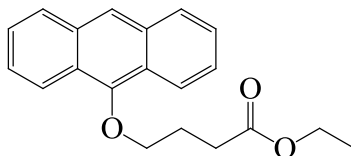
The computer program used to calculate the IRRAS spectra of SAMs deposited onto gold substrates is based on the Abeles' matrix formalism,⁷ which has been generalized for anisotropic layers.⁸ Several parameters must be fixed in the program such as the number of layers, the angle of incidence (set to 75°) and the polarization of the infrared beam. The anisotropic optical constants (refractive index $n(\bar{\nu})$ and extinction coefficient $k(\bar{\nu})$ in the three space directions) of SAMs have to be determined beforehand (vide infra). Since the deposition of SAMs does not induce orientation in the xy plane (substrate surface), an uniaxial symmetry of molecular orientation can be assumed for the calculations ($n_x = n_y = n_{xy}$ and $k_x = k_y = k_{xy}$).

The optical constants of SAMs have been determined from polarized attenuated total reflectance (ATR) spectra, using the interdependence of $n(\bar{\nu})$ and $k(\bar{\nu})$ by the Kramers-Kronig relations. Dignam et al. have shown how the Kramers-Kronig relations can be applied to polarized ATR spectra. The in-plane optical constants (n_{xy} and k_{xy}) were calculated from the s-polarized ATR spectrum, whereas the out-of-plane optical constants (n_z and k_z) were obtained from the p-polarized ATR spectrum and the before determined n_{xy} and k_{xy} . Then, the isotropic optical constants of SAMs have been calculated from the in-plane and out-of-plane optical constants using the relations :

$$n_{\text{iso}} = (2n_{xy} + n_z)/3 \quad \text{and} \quad k_{\text{iso}} = (2k_{xy} + k_z)/3.$$

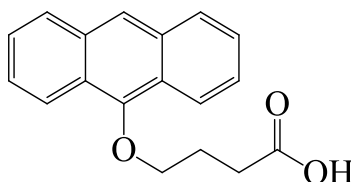
6.17 Synthetic procedures

Ethyl 4-(anthracen-9-yloxy)butanoate (Compound 10, C₂₀H₂₀O₃, MW: 308.37)⁹



Anthrone (3.0 g, 15.4 mmol), K₂CO₃ (2.35 g, 17 mmol), and acetone (150 mL) were placed in a 250 mL round-bottomed flask equipped with a reflux condenser. After the addition of ethyl 4-bromobutyrate (23 mmol, 1.5 equiv), the mixture was heated under reflux overnight. The mixture was then allowed to cool to room temperature and filtered, and the solvent was removed under reduced pressure. The residue was then dissolved in 50 mL of CH₂Cl₂, washed with H₂O (3 x 20 mL), and dried over MgSO₄. After removal of the solvent, the crude product was purified by column chromatography (dichloromethane/ethyl acetate) to afford light yellow solid 3.85 g (82%). ¹H NMR (300MHz, CDCl₃): δ 1.34 (t, *J* = 7.1Hz, 3H); 2.42 (quint, *J* = 6.7Hz, 2H); 2.82 (t, *J* = 7.4Hz, 2H); 4.21-4.28 (s, 4H); 7.47-7.53 (br s, 4H); 8.01 (d, *J* = 9.7Hz, 2H); 8.24 (s, 1H); 8.31 (d, *J* = 9.1Hz, 2H). ¹³C NMR (400 MHz, CDCl₃): δ 134.1, 133.5, 132.4, 128.5, 127.2, 125.6, 125.2, 124.6, 122.2, 74.5, 60.6, 31.2, 26.0, 14.3. HRMS: [C₂₀H₂₀O₃]⁺: calcd:308.1412; obtained: 308.1414.

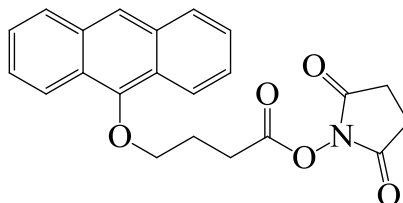
4-(Anthracen-9-yloxy)butanoic acid (Compound 11, C₁₈H₁₆O₃, MW: 280.32)⁹



Compound 2 (3.17 g, 10 mmol) was dissolved in 80 mL of ethanol in a round-bottomed flask equipped with a reflux condenser. After the addition of an aqueous solution of NaOH (1 g, 25 mmol in 10 mL H₂O), the mixture was heated under reflux for 14 h and then allowed to cool to room temperature. The solvent was then removed under vacuum, giving a solid that was then dissolved in 250 mL of distilled water. Addition of 1 mL of concentrated hydrochloric acid under stirring led to the precipitation of the compound that was collected by filtration and dried under vacuum at 60 °C for 14 h to remove residual water and afford **11** as a yellow solid 2.3 g (81%). ¹H NMR (300 MHz, CDCl₃): δ 2.44 (quint, *J* = 6.4Hz, 2H); 2.90 (t, *J* = 7.4Hz); 4.27 (t, *J* = 6.3Hz, 2H); 7.47-7.52 (br s, 4H); 8.01 (d, *J* = 9.5Hz); 8.25 (s, 1H); 8.29 (d, *J* = 9.9Hz). ¹³C NMR (400 MHz, CDCl₃/CD₃CN): δ 134.3, 132.4, 128.4, 126.9, 125.6, 125.4, 124.5, 122.2, 117.1, 74.6, 30.2, 25.7. HRMS: [C₁₈H₁₆O₃+H]⁺: calcd: 281.1177; obtained:

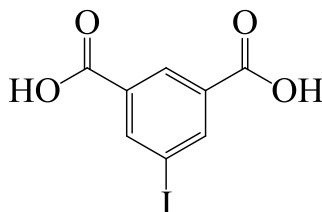
281.1166

2,5-Dioxopyrrolidin-1-yl 4-(anthracen-9-yloxy)butanoate (Compound 12, C₂₂H₁₉NO₅, MW: 377.39)⁹



Compound **3** (8.2g, 29 mmol) and *N*-hydroxysuccinimide (3.7g, 32 mmol, 1.1 equiv) were dissolved in dry ethyl acetate (200 mL). A solution containing *N,N'*-dicyclohexylcarbodiimide (6.64g, 32 mmol, 1.1 eq.) in ethyl acetate (100 mL) was then added under stirring, which was maintained for 24h. The mixture was then filtered, and the filtrate was concentrated under reduced pressure and used without further purification. ¹H NMR (300MHz, CDCl₃): δ 2.48 (quint, *J* = 6.4Hz, 2H); 2.86 (s, 4H); 3.18 (t, *J* = 7.4Hz, 2H); 4.29 (t, *J* = 6.1Hz, 2H); 7.45-7.54 (br s, 4H); 8.01 (d, *J* = 9.5Hz, 2H); 8.27 (m, 3H). HRMS: [M+H]⁺ calcd: 378.1341; obtained: 378.1337

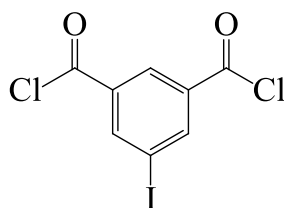
5-Iodo-isophthalic acid (Compound 14, C₈H₅IO₄, MW: 292.03)¹⁰



KMnO₄ (21.67 g, 0.137 mol) was added to a suspension of 5-iodo-xylene (12 g, 0.0648 mol, 8.82 mL) in *tert*-butanol/H₂O (120 mL, 1:1). After heating the suspension for 1 h at 100°C another batch of KMnO₄ (21.67 g, 0.137 mol) was added. The suspension was heated at 100°C for another 20 h and then cooled to rt. After filtration over Celite and washing with water the filtrate was reduced to one-third and acidified with conc. HCl. The white precipitate that appeared was dissolved in conc. NaHCO₃ solution and washed three times with diisopropylether (100 mL). After further acidification with concd HCl the white precipitate was collected and dried at 80°C overnight. Yield: 12.8 g (85%).

¹H NMR (300 MHz, DMSO) δ 13.53 (br s, 2H), 8.40 (s, 3H). ¹³C NMR (75 MHz, DMSO) δ 165.24, 141.42, 133.12, 129.11, 94.84. HRMS: [C₈H₅IO₄+H]⁺: calcd: 292.9311, obtained: 292.9307

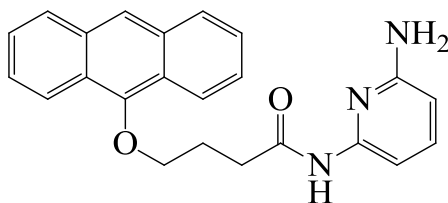
5-Iodo-isophthaloyl dichloride (Compound 15, C₈H₃Cl₂IO₂, MW: 328.92)¹⁰



A solution of 5-iodo-isophthalic acid (4.44 g, 15 mmol) in thionyl chloride (80 mL) and DMF (five drops as catalyst) was refluxed overnight under dry conditions with subsequent removal of the excess of the thionyl chloride under high vacuum to afford a light yellow solid. Yield: 5.00 g (100%).

¹H NMR (DMSO-*d*₆, 400 MHz): δ 8.38 (s, 3H). ¹³C NMR (DMSO-*d*₆, 100 MHz): δ 165.2, 141.4, 133.0, 129.1, 94.8.

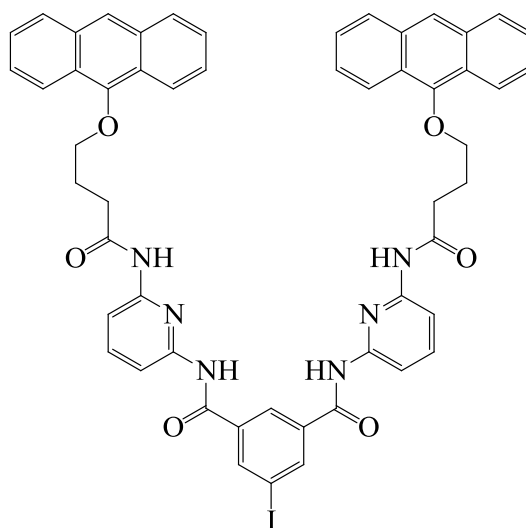
***N*-(6-Aminopyridin-2-yl)-4-(anthracen-9-yloxy)butanamide (Compound 13, C₂₃H₂₁N₃O₂, MW: 371.43)⁹**



A large excess of 1,5-diaminopyridine (25.53g, 0.234 mol, 8 equiv) was suspended in dry CH₂Cl₂ (250 mL) along with *N,N'*-diisopropylethylamine (7.6 mL, 44 mmol, 1.5 equiv). A solution of 4 (29 mmol, 1 equiv) in CH₂Cl₂ (100 mL) was then added dropwise with stirring at 25 °C. The mixture was then brought to reflux, which was maintained for 3 days. The mixture was allowed to cool and then filtered. The filtrate was washed with water (3 x 100 mL), and the organic phase was collected and dried over MgSO₄, and the solvent was removed under reduced pressure. The crude product was then purified by column chromatography on silica (eluent: CH₂Cl₂/ethyl acetate 90:10) to afford light yellow solid 6.61 g (61 %)

¹H NMR (300 MHz, CDCl₃): δ 2.48 (quint, *J* = 6.7Hz, 2H); 2.87 (t, *J* = 7.6Hz, 2H); 4.29 (t, *J* = 6.2Hz, 2H); 4.37 (s, 2H, NH₂); 6.29 (d, *J* = 7.9Hz, 1H); 7.45 - 7.53 (m, 5H); 7.61 (d, *J* = 7.7Hz, 1H); 7.99 - 8.03 (m, 2H); 8.24-8.29 (m, 3H). ¹³C NMR (300 MHz, CDCl₃): δ 170.77, 157.08, 150.92, 149.82, 140.12, 132.23, 128.40, 125.42, 125.21, 124.55, 122.19, 122.15, 104.26, 103.32, 74.49, 33.97, 26.17. HRMS: [C₂₃H₂₁N₃O₂+H]⁺: calcd: 372.1712, obtained: 372.1698.

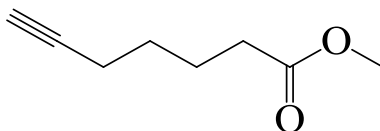
***N*¹,*N*³-Bis(6-(4-(anthracen-9-yloxy)butanamido)pyridin-2-yl)-5-iodoisophthalamide
(Compound 3, C₅₄H₄₃IN₆O₆, MW: 998.86)**



A solution of compound **15** (5.00 g, 15 mmol) dissolved in dry THF (60 mL) was added dropwise to a solution of compound **13** (11g, 29.6 mmol) and triethylamine (5 mL, 29.6 mmol) in dry THF (150 mL) at room temperature. The solution was stirred at room temperature for 12 h. Another solution of compound **15** (1.00 g, 3 mmol) in THF (12 mL) was added to the reaction mixture, and stirred for 2h. The residue was filtered off and the solvent removed under reduced pressure. Purification by column chromatography on silica gel (CH₂Cl₂/ethyl acetate 90: 10 as eluent) gave a yellowish solid. Yield: 9.72 g (67%).

¹H NMR (300 MHz, CDCl₃) δ 8.60 (br s, 2H), 8.46 (br s, 2H), 8.08 (d, *J* = 7.4 Hz, 9H), 7.85 (d, *J* = 7.4 Hz, 8H), 7.57 (t, *J* = 8.0 Hz, 2H), 7.37 – 7.25 (8, 4H), 4.10 (t, *J* = 5.8 Hz, 4H), 2.75 (d, *J* = 6.7 Hz, 4H), 2.44 – 2.23 (m, 4H). ¹³C NMR (75 MHz, CDCl₃) δ 173.45, 171.46, 163.19, 150.51, 149.65, 149.05, 140.54, 139.47, 132.11, 128.36, 125.28, 125.13, 122.37, 121.92, 110.40, 109.92, 94.46, 74.33, 33.71, 25.88. HRMS: [C₅₄H₄₃IN₆O₆+Na]⁺: calcd: 1021.2186, obtained: 1021.2188.

Methyl hept-6-ynoate (Compound 16, C₈H₁₂O₂, MW: 140.18)

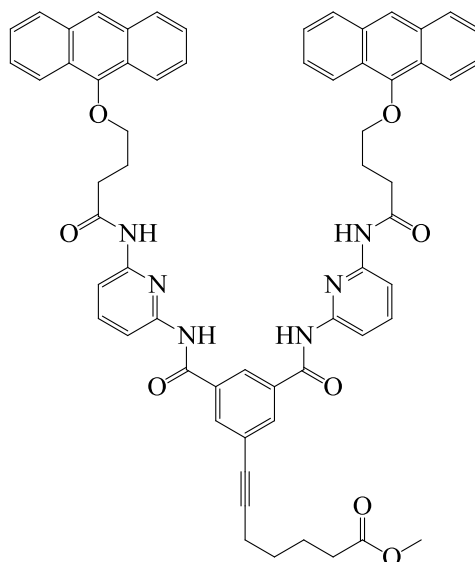


A solution of 6-heptynoic acid (2g, 15.8 mmol) was dissolved in methanol (20 mL) and one drop of concentrated H₂SO₄ was added as catalyst. The solution was brought to reflux overnight. The solvent was removed in vacuum and then extracted with CH₂Cl₂/H₂O, and then dried with MgSO₄. After removal of the solvent, a light yellow liquid was obtained (2.01g, 91%) and used directly without further purification.

^1H NMR (300 MHz, CDCl_3): δ 3.64 (s, 3H), 2.31 (t, J = 7.4 Hz, 2H), 2.17 (dd, J = 13.0, 6.1 Hz, 2H), 1.93 (s, 1H), 1.81 – 1.66 (m, 2H), 1.55 (m, 2H).

^{13}C NMR (75 MHz, CDCl_3): δ 173.81, 83.89, 68.56, 51.51, 33.49, 27.81, 23.96, 18.10.

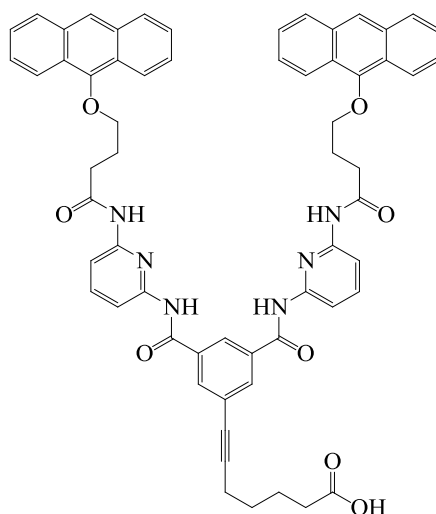
Methyl 7-(3,5-bis((6-(4-(anthracen-9-yloxy)butanamido)pyridin-2-yl)carbamoyl)phenyl)hept 6-ynoate (Compound 5, $\text{C}_{62}\text{H}_{54}\text{N}_6\text{O}_8$, MW: 1011.13)



In a 2-necked flask was added compound **3** 0.92g (0.92 mmol), $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$ 0.065g (0.09 mmol, 0.1 equiv), CuI 0.053g (0.27 mmol, 0.3 equiv) and PPh_3 0.012g (0.046 mmol, 0.05 equiv). The flask was placed under vacuum and back-filled with Ar 3 times. Another flask was filled with 20 mL dried THF, compound **16** 0.018g (1.38 mmol, 1.5 equiv) and triethylamine (11 mL) and then bubbled with Ar to degas. After 20 minutes, the solution was transferred by cannula into the 2-necked flask and stirred at room temperature overnight. The solvent was removed under vacuum and the solid partition between $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O}$, the organic phase was concentrated and purified by column chromatography (CH_2Cl_2 /ethyl acetate 90:10 as eluent) to afford **5** as a white solid 0.85g (91%).

^1H NMR (400 MHz, CDCl_3) δ 8.51 (s, 2H), 8.21 – 8.18 (m, 6H), 8.16 (s, 2H), 8.04 – 7.97 (m, 4H), 7.97 – 7.90 (m, 5H), 7.71 (t, J = 8.1 Hz, 2H), 7.44 – 7.35 (m, 8H), 4.23 (t, J = 6.1 Hz, 4H), 3.66 (s, 3H), 2.86 (t, J = 7.2 Hz, 4H), 2.40 (m, 8H), 1.84 – 1.74 (m, 2H), 1.61 (dd, J = 9.1, 6.3 Hz, 2H). ^{13}C NMR (100 MHz, CDCl_3) δ 173.91, 171.20, 163.92, 150.82, 149.69, 149.29, 140.98, 134.78, 133.63, 132.35, 128.50, 125.45, 125.29, 124.54, 122.35, 122.07, 110.14, 109.80, 92.91, 79.05, 74.42, 51.59, 34.15, 33.49, 27.80, 26.08, 24.11, 19.09. HRMS: $[\text{C}_{62}\text{H}_{54}\text{N}_6\text{O}_8 + \text{Ag}]^+$: calcd: 1117.3048, obtained: 1117.3034

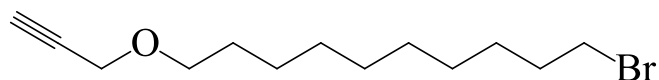
7-(3,5-Bis((6-(4-(anthracen-9-yloxy)butanamido)pyridin-2-yl)carbamoyl)phenyl)hept-6-ynoic acid (Compound 6, C₆₁H₅₂N₆O₈, MW: 997.10)



To a solution of THF and water (1:1) compound 5 0.565g (0.56 mmol) and LiOH • H₂O 0.54g (13 mmol, 23 eq) were mixed and stirred at room temperature overnight. NH₄Cl_(aq) was then added to adjust the pH to neutral. The THF was removed under vacuum, and the residual washed with water (to remove salts) to afford 6 as a light yellow powder 0.528g (94%).

¹H NMR (300 MHz, DMSO) δ 10.63 (s, 1H), 10.37 (s, 1H), 8.43 (s, 1H), 8.34 (s, 1H), 8.24 (dd, *J* = 6.4, 3.5 Hz, 4H), 8.12 (d, *J* = 1.4 Hz, 2H), 8.09 – 8.00 (m, 4H), 7.92 – 7.74 (m, 6H), 7.48 (dq, *J* = 6.5, 3.2 Hz, 8H), 4.18 (t, *J* = 6.3 Hz, 4H), 2.84 (t, *J* = 7.2 Hz, 4H), 2.33-2.25 (m, 8H), 1.78 – 1.68 (m, 4H). ¹³C NMR (75 MHz, DMSO) δ 174.40, 171.80, 164.72, 150.65, 150.13, 140.13, 134.85, 133.61, 131.97, 128.46, 125.74, 125.57, 124.12, 123.81, 122.02, 110.59, 110.05, 92.41, 79.42, 74.97, 33.21, 32.75, 27.55, 25.79, 23.86, 18.44 . HRMS: [C₆₁H₅₂N₆O₈+Na]⁺: calcd: 1119.3744, obtained:1119.3766

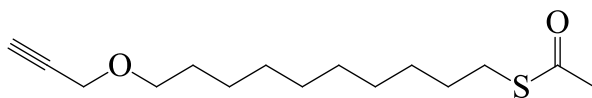
1-Bromo-10-(prop-2-yn-1-yloxy)decane (Compound 17, C₁₃H₂₃BrO, MW: 275.23)



To a solution of propargyl alcohol (0.34mL,) in 10mL H₂O was added 1,10-dibromodecane 5.3g (3eq), tetrabutylammonium bisulfate(Bu₄NHSO₄) 0.024g (1.25%), and NaOH 2g. The solution was refluxed at 120°C for 3 hours. After cooling to room temperature, the reaction solution was extracted with CH₂Cl₂/H₂O; then dried with MgSO₄. Column chromatography with 5% diethyl ether/petroleum ether afforded **17** as a colorless liquid 1.341g (83%)

¹H NMR (300 MHz, CDCl₃) δ 4.13 (d, *J* = 2.4 Hz, 2H), 3.50 (t, *J* = 6.6 Hz, 2H), 3.40 (t, *J* = 6.9 Hz, 2H), 2.41 (t, *J* = 2.4 Hz, 1H), 1.92 – 1.77 (m, 2H), 1.66 – 1.51 (m, 2H), 1.48 – 1.22 (m, 12H).

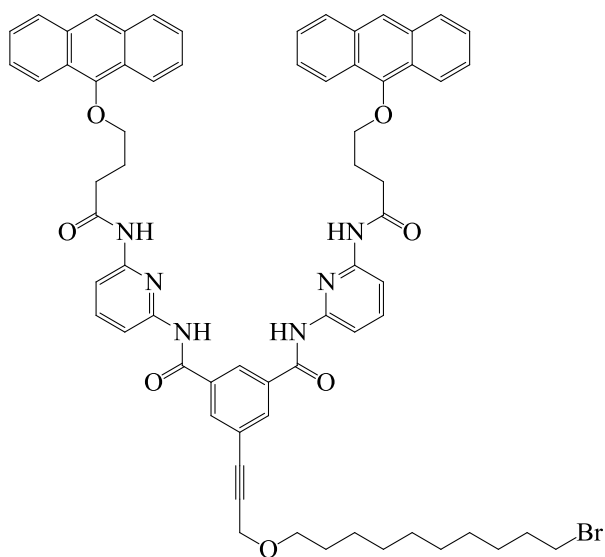
10-(Prop-2-yn-1-yloxy)decyl ethanethioate (Compound 18, C₁₅H₂₆O₂S, MW: 270.43)



To a solution of 100 mL acetone was added potassium thioacetate 1.36g (12 mmol, 1.6 eq.), followed by adding compound **17** 2.03g (7.3 mmol, 1eq.) and stirred at 30°C in the dark. After 50 hr, the reaction mixture was extracted by CH₂Cl₂/H₂O, and the organic phase was dried over MgSO₄, purified by column chromatography with petroleum ether/ diethyl ether (100:5) to afford **18** a yellow liquid 1.556g (78%).

¹H NMR (300 MHz, CDCl₃) δ 4.12 (d, *J* = 2.4 Hz, 2H), 3.50 (t, *J* = 6.6 Hz, 2H), 2.85 (t, *J* = 7.3 Hz, 2H), 2.41 (t, *J* = 2.4 Hz, 1H), 2.31 (s, 3H), 1.57 (m, 4H), 1.42 – 1.20 (m, 12H). ¹³C NMR (75 MHz, CDCl₃) δ 195.99, 80.05, 73.99, 70.26, 57.98, 30.62, 29.47, 29.35, 29.13, 29.05, 28.77, 26.04.

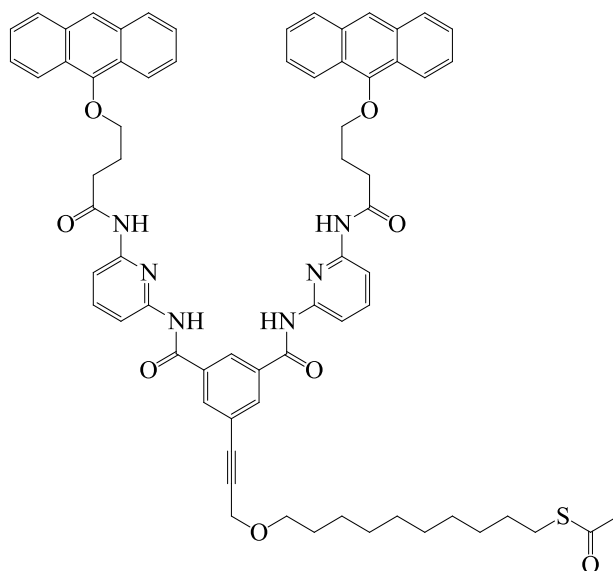
***N*¹,*N*³-Bis(6-(4-(anthracen-9-yloxy)butanamido)pyridin-2-yl)-5-(3-((10-bromodecyl)oxy)prop-1-yn-1-yl)isophthalamide (Compound 19, C₆₇H₆₅BrN₆O₇, MW: 1146.17)**



In a 2-necked flask was added compound **3** 0.68g (0.68 mmol), Pd(PPh₃)₂Cl₂ 0.050g (0.07 mmol, 0.1 equiv), CuI 0.040g (0.21 mmol, 0.3 equiv) and PPh₃ 0.002g (0.006 mmol, 0.05 equiv), and then the flask was placed under vacuum and back-filled with Ar 3 times. A different flask was filled with 20 mL dried THF, compound **17** 0.26g (0.94 mmol, 1.3 equiv) and DIEA 6.5 mL and then degassed by bubbling Ar. After 20 minutes, the solution was transferred by cannula into the 2-necked flask and the solution was stirred in room temperature overnight. The solvent was removed by vacuum and extracted with CH₂Cl₂/H₂O, and then purified with column (CH₂Cl₂/ethyl acetate 90:10 as eluent) to afford **19** as a white solid product 0.503g (64%).

^1H NMR (300 MHz, CDCl_3) δ 8.33 – 8.22 (m, 6H), 8.20 (s, 2H), 8.07 (d, J = 8.3 Hz, 4H), 8.01 – 7.91 (m, 7H), 7.81 (t, J = 8.1 Hz, 2H), 7.48 – 7.38 (m, 8H), 4.38 (s, 2H), 4.29 (t, J = 6.1 Hz, 4H), 3.58 (t, J = 6.6 Hz, 2H), 3.37 (t, J = 6.9 Hz, 2H), 2.89 (m, 4H), 2.54 – 2.40 (m, 4H), 1.90 – 1.73 (m, 2H), 1.66 – 1.61 (m, 2H), 1.42 – 1.19 (m, 12H).

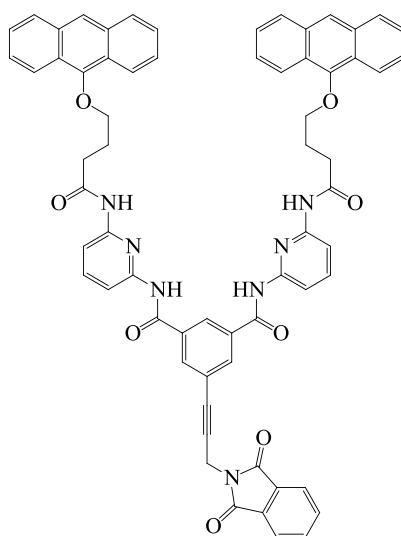
***S*-(10-((3-(3,5-Bis((6-(4-(anthracen-9-yloxy)butanamido)pyridin-2-yl)carbamoyl)phenyl)prop-2-yn-1-yl)oxy)decyl) ethanethioate (Compound 7, $\text{C}_{69}\text{H}_{68}\text{N}_6\text{O}_8\text{S}$, MW: 1141.3786)**



To a solution of 20 mL acetone was added compound **19** 0.503g (0.43 mmol) and potassium thioacetate 0.10g (0.87 mmol, 2 eq.). After stirring at room temperature overnight, the reaction mixture was extracted with $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O}$ and the organic phase was dried over MgSO_4 , and purified by column chromatography with CH_2Cl_2 /Ethyl acetate (10:1) to afford **7** as a yellow solid 0.331g (67%).

^1H NMR (300 MHz, CDCl_3) δ 8.48 (s, 2H), 8.31 – 8.11 (m, 9H), 8.09 – 7.85 (m, 9H), 7.72 (t, J = 7.9 Hz, 2H), 7.49 – 7.32 (m, 8H), 4.32 (s, 2H), 4.22 (t, J = 5.9 Hz, 4H), 3.54 (t, J = 6.6 Hz, 2H), 2.93 – 2.77 (m, 6H), 2.48 – 2.39 (m, 4H), 2.29 (s, 3H), 1.66 – 1.46 (m, 4H), 1.41 – 1.18 (m, 12H). ^{13}C NMR (75 MHz, CDCl_3): δ 194.97, 171.32, 163.85, 150.68, 149.71, 149.22, 140.75, 132.23, 128.41, 125.36, 125.17, 124.41, 122.27, 121.95, 110.27, 109.92, 74.38, 70.55, 58.48, 33.71, 29.48, 29.34, 29.09, 29.06, 28.72, 26.03, 25.94. HRMS: $[\text{C}_{69}\text{H}_{68}\text{N}_6\text{O}_8\text{S}+\text{Na}]^+$: calcd: 1163.4711; obtained: 1163.4703

***N*¹,*N*³-Bis(6-(4-(anthracen-9-yloxy)butanamido)pyridin-2-yl)-5-(3-(1,3-dioxisoindolin-2-yl)prop-1-yn-1-yl)isophthalamide (Compound 20, C₆₅H₄₉N₇O₈, MW: 1056.13)**

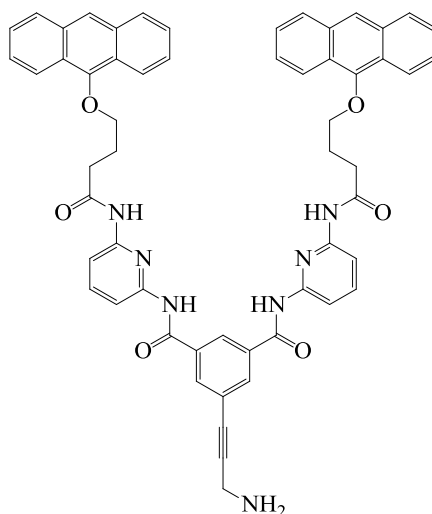


In a 2-necked flask was added compound **3** 0.314g (0.31 mmol), *N*-propargylphthalimide 0.069g (0.37 mmol, 1.2 eq.) Pd(PPh₃)₂Cl₂ 0.022g (0.03 mmol, 0.1 equiv), CuI 0.017g (0.12 mmol, 0.3 equiv) and PPh₃ 0.0012g (0.015 mmol, 0.05 equiv). The flask was placed under vacuum and back-filled with Ar 3 times. A different flask was filled with 10 mL dried THF and Et₃N 3 mL and then bubbled with Ar to degas. After 20 minutes, the solution was transferred by cannula into the 2-necked flask and the solution stirred at room temperature overnight. The solvent was removed under vacuum and extracted with CH₂Cl₂/H₂O, and purified by column chromatography (CH₂Cl₂/ethyl acetate 80:20 as eluent) to afford **20** as a white solid product 0.269g (81%).

¹H NMR (300 MHz, CDCl₃) δ 8.79 (s, 3H), 8.25 (s, 1H), 8.10 (d, *J* = 8.3 Hz, 4H), 8.05 (s, 2H), 7.84 (d, *J* = 9.1 Hz, 6H), 7.78 (s, 2H), 7.69 – 7.59 (m, 3H), 7.57 – 7.39 (m, 4H), 7.39 – 7.28 (m, 8H), 4.52 (s, 2H), 4.12 (t, *J* = 5.5 Hz, 4H), 2.82 (s, 4H), 2.37 (s, 4H).

¹³C NMR (75 MHz, CDCl₃) δ 171.56, 169.77, 166.98, 163.76, 150.64, 149.75, 149.14, 134.02, 132.15, 131.54, 128.35, 125.32, 125.13, 124.36, 123.34, 122.19, 121.93, 110.15, 109.71, 83.35, 80.76, 74.41, 33.80, 27.49, 25.96.

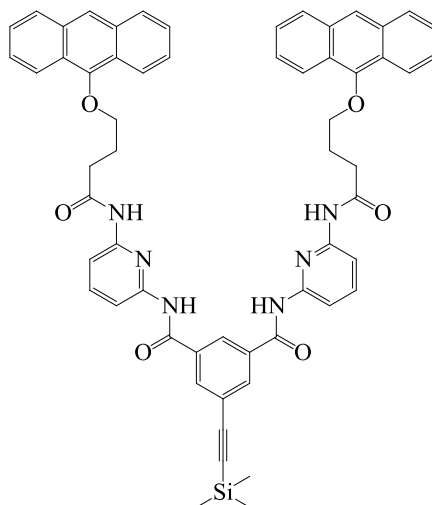
5-(3-Aminoprop-1-yn-1-yl)-*N*¹,*N*³-bis(6-(4-(anthracen-9-yloxy)butanamido)pyridin-2-yl)isophthalamide (Compound **8, C₅₇H₄₇N₇O₆, MW: 926.03)**



A solution of ethanol (5mL) and THF (5mL) containing compound **20** 0.221g (0.2 mmol) was degassed by bubbling Ar for 30 minutes. Methylamine 1 mL (33% in abs. ethanol) was added into the reaction mixture and stirred in room temperature for 3 hours. The solvent was removed under vacuum and purified by column chromatography using silica gel that was deactivated by 2% Et₃N/CH₂Cl₂. After packing, the column was washed with 200 mL CH₂Cl₂ to remove residual Et₃N. All the solvents used for purification were degassed. Elution with CH₂Cl₂/MeOH (100/2) afforded **8** as a light yellow solid 0.153g (79%)

¹H NMR (300 MHz, CDCl₃) δ 8.88 (br s, 2H), 8.23 – 8.00 (m, 8H), 7.96 – 7.77 (m, 9H), 7.77 – 7.64 (m, 2 1H), 7.52 (s, 2H), 7.38 – 7.27 (m, 8H), 4.13 (t, *J* = 6.1 Hz, 4H), 3.38 (s, 2H), 2.81 (t, *J* = 6.8 Hz, 4H), 2.35 (m, 4H). ¹³C NMR (75 MHz, CDCl₃) δ 171.49, 164.06, 150.73, 149.93, 149.23, 140.65, 134.63, 134.08, 132.39, 132.25, 128.43, 127.19, 125.38, 125.20, 124.45, 122.28, 121.96, 110.33, 109.80, 73, 74.47, 53.40, 33.90, 31.65, 26.05. HRMS: [C₅₇H₄₇N₇O₆+H]⁺: calcd: 926.3660, obtained: 926.3636

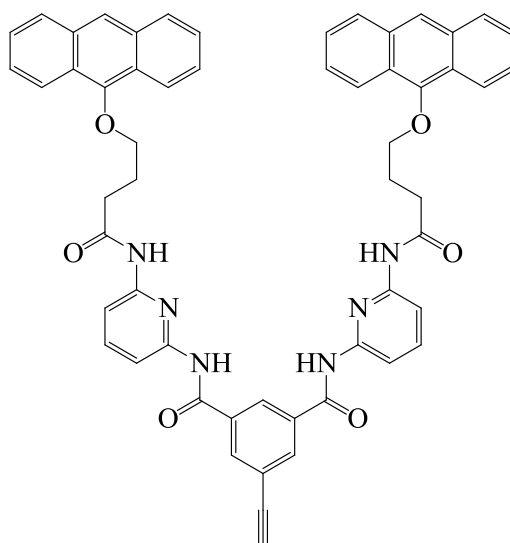
***N*¹,*N*³-Bis(6-(4-(anthracen-9-yloxy)butanamido)pyridin-2-yl)-5-((trimethylsilyl)ethynyl)-isophthalamide (Compound 21, C₅₉H₅₂N₆O₆Si, MW: 969.17)**



In a 2-necked flask was added compound **3** 1.5g (1.5 mmol), CuI 0.0935g (0.45 mmol, 0.3 eq), Pd(PPh₃)₂Cl₂ 0.112g (0.15 mmol, 0.1 eq), PPh₃ 0.014g (0.075 mmol, 0.05eq) and the flask was placed under vacuum and sealed with a septum. To a solution of distilled THF 15 mL was added with Ethynyltrimethylsilane 0.25 mL (1.8 mmol, 1.2 eq) and diisopropylethylamine 6 mL (30 mmol, 20 eq) and degassed by bubbling argon for 30 minutes. The solution was transferred by cannula into the 2-necked flask and stirred at room temperature overnight. The solvent was removed under vacuum and extracted with CH₂Cl₂/H₂O. The residual was purified by column chromatography (CH₂Cl₂/ethyl acetate 90:10 as eluent) to afford **21** as a light yellow product 1.36 g (90%).

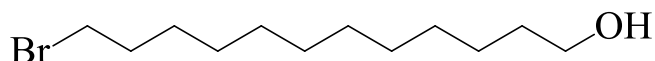
¹H NMR (300 MHz, CDCl₃) δ 8.49 (s, 2H), 8.28 (s, 2H), 8.24 – 8.09 (m, 8H), 8.01 – 7.86 (m, 10H), 7.65 (t, *J* = 8.1 Hz, 2H), 7.41 – 7.32 (m, 8H), 4.19 (t, *J* = 6.1 Hz, 4H), 2.81 (t, *J* = 6.7 Hz, 4H), 2.46 – 2.34 (m, 4H), 0.27 – 0.23 (m, 27H). ¹H NMR (75 MHz, CDCl₃) δ 171.10, 163.67, 150.73, 149.20, 132.28, 128.44, 125.39, 125.21, 124.47, 122.30, 121.99, 110.22, 109.81, 102.34, 97.79, 74.38, 60.35, 34.04, 26.04, 14.16, -0.25. MALDI: [C₅₉H₅₂N₆O₆Si+ Na]⁺: calcd: 991.4, obtained: 991.0.

***N*¹,*N*³-Bis(6-(4-(anthracen-9-yloxy)butanamido)pyridin-2-yl)-5-ethynylisophthalamide (Compound 9, C₅₆H₄₄N₆O₆, MW: 896.99)**



To a solution of 20 mL THF/H₂O (7:3) was added into compound **21** 1.36g (1.4 mmol) and LiOH · H₂O 0.62g (14 mmol, 10eq.), and then stirred under N₂ at room temperature overnight. The solvent was removed under vacuum, extracted by CH₂Cl₂/H₂O, and dried by MgSO₄. Column chromatography with CH₂Cl₂/ethyl acetate (100:10) to afford **9** as a yellow solid 0.93 g(74%). ¹H NMR (400 MHz, CDCl₃) δ 8.39 (s, 2H), 8.26 (s, 1H), 8.22 – 8.19 (m, 4H), 8.19 (s, 2H), 8.10 – 7.93 (m, 11H), 7.74 (t, *J* = 8.0 Hz, 2H), 7.41 (m, 8H), 4.24 (t, *J* = 6.0 Hz, 4H), 3.18 (s, 1H), 2.87 (t, *J* = 6.8 Hz, 4H), 2.45 (dd, *J* = 13.0, 6.4 Hz, 4H). ¹³C NMR (100 MHz, CDCl₃) δ 171.16, 163.56, 150.78, 149.68, 149.18, 141.00, 134.96, 134.08, 132.35, 128.52, 125.46, 125.30, 124.53, 122.40, 122.04, 110.27, 109.82, 81.23, 80.11, 74.42, 34.21, 26.08. HRMS: [C₅₆H₄₄N₆O₆+Na]⁺: calcd: 919.3214, obtained: 919.3236.

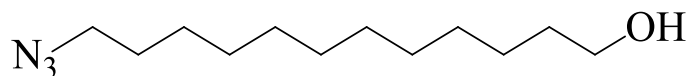
12-Bromododecan-1-ol (Compound 22, C₁₂H₂₅BrO, MW: 265.23)¹¹



To a solution of 1,12-dodecanediol (8.2 g, 40.5 mmol) in toluene (200 mL), was added an aqueous solution of HBr (5.2 mL, 48 wt% in H₂O, 60 mmol). The flask was equipped with a Dean-Stark apparatus and the mixture heated at reflux (water removed every 30 mins) for 6 hours. The reaction mixture was washed with NaOH(aq) (1 M, 30 mL), brine (30 mL), and the organic phase separated was dried over anhydrous Na₂SO₄, and the solvents removed in vacuo. The crude was purified using flash column chromatography (Et₂O/petroleum ether 2:3) to give the desired bromoalcohol as a colorless oil (6.8 g, 63 %), which slowly solidified upon standing.

^1H NMR (300 MHz, CDCl_3) δ 3.64 (t, J = 6.6 Hz, 2H), 3.41 (t, J = 6.9 Hz, 2H), 1.94 – 1.78 (m, 2H), 1.65 – 1.49 (m, 2H), 1.48 – 1.21 (m, 16H).

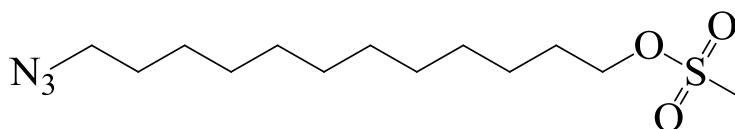
12-Azidododecan-1-ol (Compound 23, $\text{C}_{12}\text{H}_{25}\text{N}_3\text{O}$, MW: 227.35)¹²



To 150mL of DMF, 10g (38 mmol) of $\text{Br}(\text{CH}_2)_{12}\text{OH}$ and 2.95g (45 mmol, 1.2 eq) of NaN_3 were added and stirred at 80 °C for 24 hours. After cooling to room temperature, 150mL of H_2O was added and the mixture was extracted three times with diethyl ether. The organic phase was washed with water three times and dried over MgSO_4 . Rotary Evaporation yielded a pale yellow oil 8.05g (93%).

^1H NMR (300 MHz, CDCl_3) δ 3.64 (dd, J = 11.4, 6.4 Hz, 2H), 3.25 (t, J = 6.9 Hz, 2H), 1.67 – 1.49 (m, 4H), 1.43 – 1.18 (m, 16H).

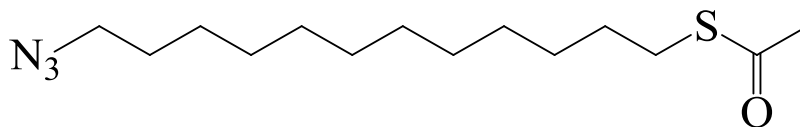
1-Azidoundecan-12-methylsulfonate (Compound 24, $\text{C}_{13}\text{H}_{27}\text{N}_3\text{O}_3\text{S}$, MW: 305.44)¹²



To 8g (35 mmol) of **23** in distilled THF 100 mL, 8mL (103 mmol) of methanesulfonyl chloride were added. In a separate vial, 14mL of triethylamine was dissolved in 40mL THF. The triethylamine solution was slowly added to the stirring solution of **23** over 5 minutes. The reaction continued at room temperature for 2 hours. Then, 100mL of ice-cold water were added, and the organic phase was separated from the aqueous phase. The aqueous phase was extracted twice with diethyl ether (2x35mL). The combined organic phases were washed with 1M HCl, H_2O , NaHCO_3 , and H_2O . After drying over MgSO_4 , the solvent was removed under vacuum yielding **24** as a pale yellow oil 10.02g (93%).

^1H NMR (300 MHz, CDCl_3) δ 4.22 (t, J = 6.6 Hz, 2H), 3.25 (t, J = 6.9 Hz, 2H), 3.00 (s, 3H), 1.81 – 1.67 (m, 2H), 1.66 – 1.52 (m, 2H), 1.46 – 1.21 (m, 8H).

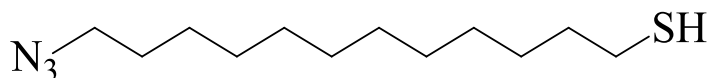
1-Azidoundecan-12-thioacetate (Compound 25, C₁₄H₂₇N₃OS, MW: 285.45)¹²



Compound **24** 3.05g (10 mmol) and potassium thioacetate 2.42g (21 mmol) were dissolved in 100mL of methanol. The solution was degassed and refluxed under N₂ for three hours. After cooling to room temperature, excess methanol was removed via rotary evaporation and ice cold water (30mL) was added. The mixture was extracted 3 times with diethyl ether (30mL) and washed three times with water (3x30mL). The solution was dried over MgSO₄ and the solvent removed by rotary evaporation yielding **25** as a yellow oil 2.21g (77%).

¹H NMR (300 MHz, CDCl₃) δ 3.25 (t, *J* = 7.0 Hz, 2H), 2.32 (s, 3H), 1.67 – 1.49 (m, 4H), 1.4-1.26 (m, 8H).

12-Azidododecane-1-thiol (Compound 26, C₁₂H₂₅N₃S, MW: 243.41)¹²



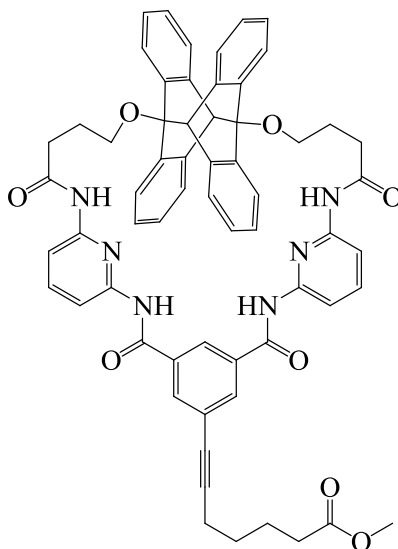
To a solution of 120 mL methanol, compound **25** 2.102g (7.3 mmol) was added. The solution was degassed thoroughly and filled with nitrogen. Then 7 mL concentrated HCl was added into the solution and the entire mixture was refluxed for 5 hrs. The reaction mixture was quenched with water and extracted twice with diethyl ether. The organic phase was washed twice with water (2x20mL) and dried over MgSO₄. After removal of the solvent, a yellow oil was obtained, and purified by column chromatography with 3% CH₂Cl₂/petroleum ether to afford **26** as a colorless oil 0.90g (50%).

¹H NMR (300 MHz, CDCl₃) δ 3.25 (t, *J* = 6.9 Hz, 2H), 2.52 (dd, *J* = 14.7, 7.5 Hz, 2H), 1.68 – 1.51 (m, 4H), 1.44 – 1.22 (m, 16H). ¹³C NMR (75 MHz, CDCl₃) δ 51.47, 34.03, 29.51, 29.47, 29.44, 29.13, 29.05, 28.82, 28.36, 26.69, 24.65.

General procedure for the synthesis of macrocyclic compounds 5_D and 7_D

A solution of **5** or **7** in 10 mL of distilled CH₂Cl₂ at a concentration of 5 x10⁻⁴ M was degassed by freeze-pump-thaw cycles and irradiated for 14 h with a Hanovia 450W HgXe lamp using a solution filter (lead nitrate/ potassium bromide, Pb(NO₃)₂ 7 g L⁻¹/KBr 540 g L⁻¹). After removal of the solvent, the solid was purified by conventional column chromatography (ethyl acetate/dichloromethane=10/90) or semi-preparative HPLC (mobile phase: gradient 10% ethyl acetate/cyclohexane to 100% ethyl acetate in 50 minutes) to afford the pure photodimers.

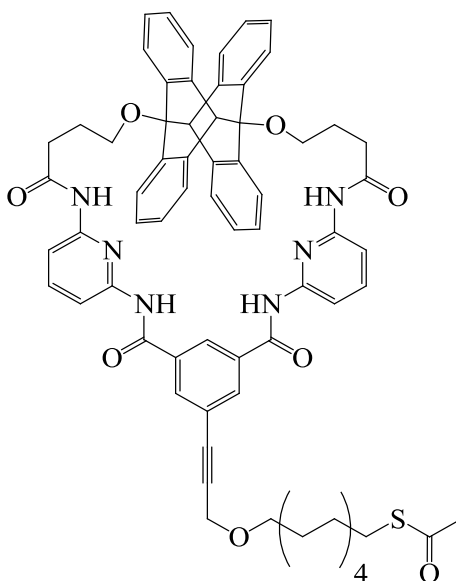
Compound 5_D (C₆₂H₅₄N₆O₈, MW: 1011.13)



¹H NMR (300 MHz, CDCl₃) δ 8.63 (s, 1H), 8.13 (s, 1H), 8.03 (d, *J* = 8.2 Hz, 2H), 7.91 (t, *J* = 8.0 Hz, 2H), 7.82 (s, 1H), 7.34 (s, 1H), 7.13 (d, *J* = 6.8 Hz, 3H), 6.89 (d, *J* = 7.0 Hz, 3H), 6.74 (dd, *J* = 13.7, 7.1 Hz, 6H), 4.46 (s, 2H), 3.80 (s, 4H), 3.72 (s, 3H), 2.79 (s, 4H), 2.51 (t, *J* = 6.9 Hz, 2H), 2.43 (t, *J* = 7.3 Hz, 2H), 2.29 (s, 4H), 1.96 – 1.78 (m, 2H), 1.78 – 1.63 (m, 2H).

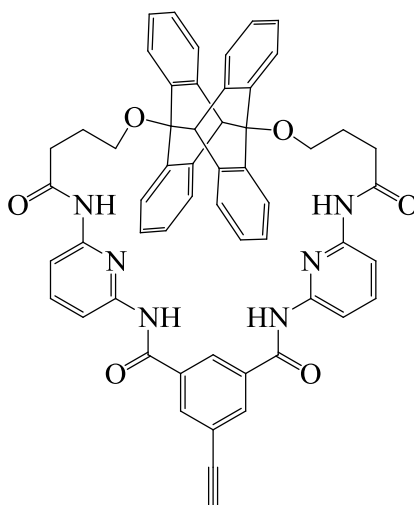
¹³C NMR (75 MHz, CDCl₃) δ 171.99, 165.11, 149.66, 149.23, 141.52, 140.93, 140.59, 136.01, 127.61, 126.09, 125.31, 110.90, 110.40, 90.02, 79.05, 63.43, 51.58, 33.54, 27.85, 26.32, 24.15, 19.15.

Compound 7_D (Compound 7, C₆₉H₆₈N₆O₈S, MW: 1141.3786)



^1H NMR (300 MHz, CDCl_3) δ 8.60 (s, 1H), 8.15 (s, 1H), 8.06 (s, 1H), 8.00 (d, J = 8.2 Hz, 2H), 7.87 (t, J = 8.0 Hz, 2H), 7.79 (s, 2H), 7.35 (s, 1H), 7.09 (d, J = 7.1 Hz, 3H), 6.85 (d, J = 6.7 Hz, 3H), 6.79 – 6.58 (m, 6H), 4.40 (d, J = 12.1 Hz, 2H), 3.76 (t, J = 5.4 Hz, 3H), 3.58 (t, J = 6.6 Hz, 2H), 2.87 – 2.80 (m, 2H), 2.75 (s, 2H), 2.33 – 2.20 (m, 5H), 1.90 – 1.80 (m, 1H), 1.76 – 1.47 (m, 7H), 1.42 – 1.15 (m, 11H). ^{13}C NMR (75 MHz, CDCl_3) δ 172.10, 164.89, 150.34, 149.68, 149.17, 141.52, 140.95, 140.59, 136.13, 127.60, 126.08, 125.32, 125.25, 110.95, 110.40, 89.96, 88.85, 83.63, 70.55, 63.58, 63.43, 43.44, 35.36, 30.61, 30.15, 29.53, 29.44, 29.37, 29.15, 29.06, 28.77. LRMS: $[\text{C}_{69}\text{H}_{68}\text{N}_6\text{O}_8\text{S}+\text{H}]^+$ calcd: 1141.5, obtained: 1141.5

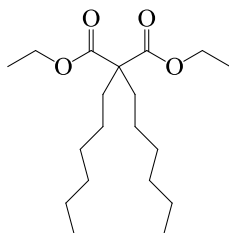
Compound 9_D ($\text{C}_{56}\text{H}_{44}\text{N}_6\text{O}_6$, MW: 896.98)



A solution of compound **21** 0.612g in 1100 mL CH_2Cl_2 (0.55 mM) was degassed by continuous bubbling argon for 40 minutes, and then irradiated by a Hg lamp with solution filter as described above for 2 hr. After the removal of the solvent, the solid was dissolved in 25 mL $\text{THF}/\text{H}_2\text{O}$ (7/3) and $\text{LiOH} \cdot \text{H}_2\text{O}$ 0.304 g (7 mmol, 10 eq.) was added. After stirring at room temperature overnight, the solvent was removed under vacuum and the residual was collected on a PTFE filter, and purified by washing with CH_2Cl_2 to afford **9** as a white solid 0.212g (37%, combined two steps yield).

^1H NMR (400 MHz, CDCl_3) δ 8.60 (s, 1H), 8.20 (s, 1H), 8.06 (s, 1H), 8.00 (d, J = 7.9 Hz, 2H), 7.88 (t, J = 8.0 Hz, 2H), 7.74 (s, 2H), 7.36 (s, 1H), 7.10 (d, J = 7.3 Hz, 3H), 6.86 (d, J = 7.0 Hz, 3H), 6.71 (dd, J = 16.0, 8.4 Hz, 6H), 4.44 (s, 2H), 3.78 (t, J = 5.9 Hz, 4H), 3.25 (s, 1H), 2.76 (br s, 4H), 2.26 (br s, 4H). ^{13}C NMR (75 MHz, DMSO) δ 172.88, 165.83, 150.62, 150.32, 141.24, 140.33, 139.99, 135.61, 133.87, 127.22, 125.58, 125.09, 124.90, 122.43, 109.98, 109.26, 88.64, 81.84, 63.52, 62.70, 33.63, 26.06. HRMS: $[\text{C}_{56}\text{H}_{44}\text{N}_6\text{O}_6+\text{Na}]^+$ calcd: 919.3220, obtained: 919.3233.

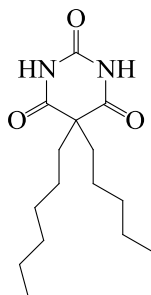
Diethyl 2,2-dihexylmalonate (Compound 27, C₁₉H₃₆O₄, MW: 328.49)



In a 2-necked flask sodium metal 3.8g (165 mmol) (cut into small pieces) was placed and absolute ethanol (60 mL) was added. After the sodium was fully dissolved to produce sodium ethoxide, a syringe was used to transfer 24 mL of ethoxide solution to a solution of diethyl malonate 4.75 mL (31.3 mmol) in absolute ethanol. After addition the reaction mixture formed a white precipitate, and a heat gun was used to dissolve the precipitate and the stirring was continued for 10 minutes. Then bromohexane 4.8 mL (34.2 mmol, 1.1 eq.) was added into the homogeneous reaction solution and stirred at room temperature for 15 minutes. Another 24 mL of EtONa was added into the reaction solution, and stirred for 15 minutes. Then, another aliquot of bromohexane (4.8 mL, 34.2 mmol, 1.1 eq.) was added and stirred at room temperature for 15 minutes. The reaction mixture was further refluxed overnight. The solvent was removed under vacuum and the residual extracted with diethyl ether/H₂O. The organic phase was dried over MgSO₄. The product was purified by column chromatography with petroleum ether/diethyl ether (100:3) to afford **27** as a white solid 6.36g (62%).

¹H NMR (300 MHz, CDCl₃) δ 4.17 (q, *J* = 7.1 Hz, 4H), 1.92 – 1.79 (m, 6H), 1.38 – 1.03 (m, 20H), 0.87 (dd, *J* = 8.7, 4.5 Hz, 6H).

Dihexyl barbituric acid (DHB, Compound 28, C₁₆H₂₈N₂O₃, MW: 296.41)¹³

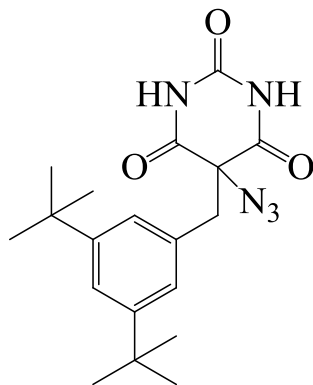


To a 2-necked flask was added diethyl 2,2-dihexylmalonate (**27**) 2.01g (6.12 mmol), urea 1.84g (30.4 mmol, 5 eq.) and dry DMSO 15 mL, and this mixture was stirred at room temperature until all the materials dissolved. Potassium *tert*-butoxide 1.38 g (12.3 mmol, 2 eq.) was dissolved in 20 mL dried DMSO and then added into the reaction mixture, and the reaction was stirred at room temperature for overnight. The reaction mixture was hydrolyzed with 10% HCl_(aq) 10 mL, extracted with diethyl ether/H₂O and the organic phase was dried with MgSO₄. After removal of the solvent under vacuum, the residual obtained was collected

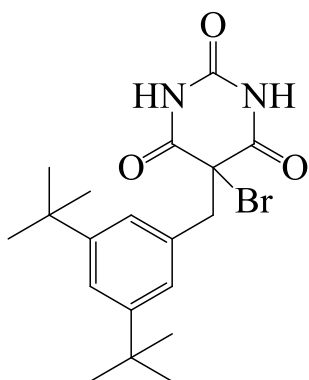
and washed with petroleum ether to remove residual diethyl 2,2-dihexylmalonate to afford **28** as a white solid 1.21g (67%).

^1H NMR (300 MHz, CDCl_3) δ 8.27 (s, 2H), 1.97 (dd, J = 9.5, 6.7 Hz, 4H), 1.34 – 1.10 (m, 16H), 0.86 (m, 6H). ^{13}C NMR (75 MHz, CDCl_3) δ 173.00, 149.23, 56.78, 39.27, 31.34, 29.10, 25.08, 22.47, 13.99.

5-Azido-5-(3,5-di-*tert*-butylbenzyl)pyrimidine-2,4,6(1H,3H,5H)-trione (Compound 30, $\text{C}_{19}\text{H}_{25}\text{N}_5\text{O}_3$, MW: 371.43)¹⁴

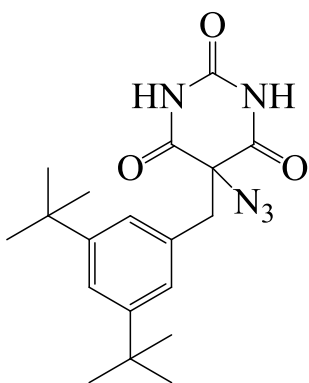


To a DMF solution (20 mL) containing 5-(3,5-di-*tert*-butylbenzyl)-pyrimidine-2,4,6(1H,3H,5H)-trione (2.0 g, 6.1 mmol), NBS (1.19 g, 6.6 mmol) was added dropwise and the solution stirred at rt for 4 h. Sodium azide (786 mg, 12.1 mmol) in DMF (4 mL) was then added and stirring continued at 60°C overnight. Water was added and the mixture was extracted with ethyl acetate. The organic layer was washed with water and dried. The solvent was removed under reduced pressure to afford **30** as a pale brown oil. (2.08 g, yield = 92 %).



Intermediate, Compound 29:

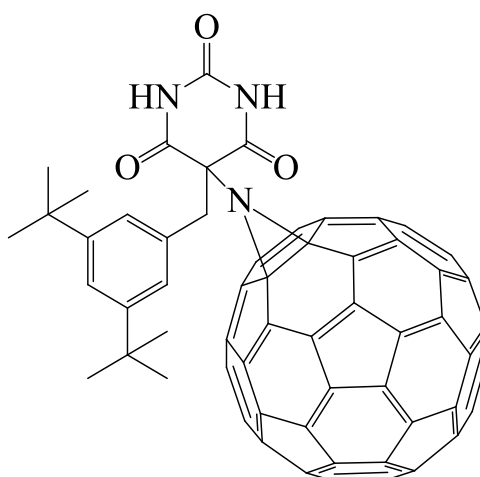
^1H NMR (300 MHz, acetone- d_6) δ 10.59 (s, 1H), 7.35 (d, J = 1.5 Hz, 1H), 7.05 (d, J = 1.5 Hz, 1H), 3.79 (s, 2H), 1.25 (s, 18H); ^{13}C NMR (75MHz, acetone- d_6) 167.6, 151.8, 148.4, 133.8, 125.5, 122.2, 51.0, 43.6, 35.3, 31.6 ESI- m/z 431.09 ($[\text{C}_{19}\text{H}_{25}\text{BrN}_2\text{O}_3 + \text{Na}]^+$).



Product, Compound 30:

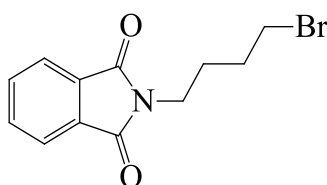
^1H NMR (300 MHz, CDCl_3) δ 7.30 (d, J = 1.5 Hz, 1H), 6.94 (d, J = 1.5 Hz, 1H), 3.57 (s, 2H), 1.25 (s, 18H); ^{13}C NMR (75MHz, CDCl_3) 167.5, 151.6, 147.5, 129.4, 124.0, 122.1, 67.1, 43.7, 34.7, 31.2; IR (KBr) 2216 cm^{-1} ; ESI- m/z (%) 394.18 ($[\text{C}_{19}\text{H}_{25}\text{N}_5\text{O}_3 + \text{Na}]^+$, 100), 765.37 ($[\text{C}_{38}\text{H}_{50}\text{N}_{10}\text{O}_6 + \text{Na}]^+$, 27).

N-[5''-(3'',5''-Di-*tert*-butylbenzyl)-2'',4'',6''-trioxo-hexahydropyrimidin-5''-yl] aziridino[2',3':1,2][60]fullerene24 (Compound 31, C₇₉H₂₅N₃O₃, MW: 1064.06)¹⁴



A chlorobenzene solution (200 mL) containing C₆₀ (1.223 g, 1.62 mmol) was degassed by three cycles of freeze-pump-thaw. The solution was brought to reflux and azido di-*tert*-butylbenzyl barbiturate (276 mg, 0.81 mmol) in chlorobenzene (this solution was also degassed by three cycles of freeze-pump-thaw) was added dropwise. After refluxing for 2 days, the solvent was removed under reduced pressure. The crude product was dry-loaded onto a column and washed with toluene to remove C₆₀ prior to eluting with mixture of ethyl acetate in toluene (5%) to afford the desired product. The solvent was dried under vacuum to afford brown powder product. (315 mg, yield = 37%) MALDI-ms m/z (%) 1063.95 ([C₇₉H₂₅N₃O₃ + H]⁺, 83), 1085.98 ([C₇₉H₂₅N₃O₃ + Na]⁺, 100), 1101.93 ([C₇₉H₂₅N₃O₃ + K]⁺, 30).

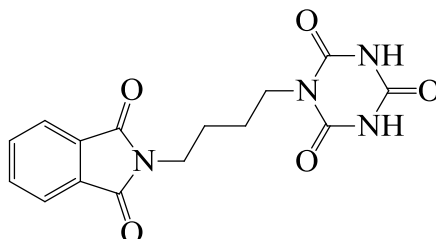
N-(4-Bromobutyl) phthalimide (Compound 32, C₁₂H₁₂BrNO₂, MW: 282.13)¹⁵



A mixture of potassium phthalimide (7.56 g, 40.8 mmol) and 1,4-dibromobutane 14.3 mL (120 mmol, 3 eq.) was stirred in dry DMF 200ml at 90°C overnight. The DMF was removed under high vacuum at 50°C and the residual liquid was dissolved in diethyl ether and then filtered to remove insoluble impurities. The solvent was removed under vacuum and the residual reprecipitated with petroleum ether to afford **32** as a white solid 8.05g (70%).

¹H NMR (300 MHz, CDCl₃) δ 7.85 (dd, *J* = 5.4, 3.1 Hz, 2H), 7.72 (dd, *J* = 5.5, 3.0 Hz, 2H), 3.73 (t, *J* = 6.7 Hz, 2H), 3.45 (t, *J* = 6.4 Hz, 2H), 1.98 – 1.79 (m, 4H).

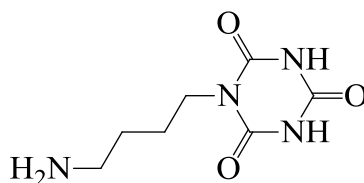
1-(4-(1,3-Dioxisoindolin-2-yl)butyl)-1,3,5-triazinane-2,4,6-trione
(Compound 33, C₁₅H₁₄N₄O₅, MW: 330.30)¹⁵



A solution of cyanuric acid 2.503g (1.0 eq.), *N*-(4-bromobutyl) phthalimide (5.008g, 1.0 eq.), and DBU (2.8 mL, 1.0 eq.) in dry DMF (40 mL) was heated to 70 °C under Ar overnight. After cooling, the yellow solution was poured into cold H₂O (300 mL), and the resulting white precipitate was filtered off and dried under vacuum. The white solid obtained was sonicated in diethyl ether/ ethyl acetate (8/1) solution to remove impurities to afford **33** as a white solid 3.94g (67%).

¹H NMR (300 MHz, DMSO) δ 11.38 (s, 2H), 7.85 – 7.81 (m, 4H), 3.75 – 3.60 (m, 2H), 3.56 (dd, *J* = 8.4, 4.6 Hz, 2H), 1.55 (s, 4H).

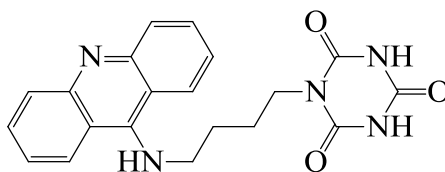
1-(4-Aminobutyl)-1,3,5-triazinane-2,4,6-trione (Compound 34, C₇H₁₂N₄O₃, MW: 200.20)¹⁵



A solution of methylamine (33% in EtOH, 6 mL, 40 equiv.) was added to a solution of phthalimide (0.3g, 1.0 eq.) in EtOH (10 mL), and the mixture was heated at 70°C under Ar for 150 min. After the mixture had cooled, the resulting white precipitate was filtered off, washed with EtOH three times, and dried under vacuum. Amino cyanuric acid **34** was obtained as white solids 0.135g (74%).

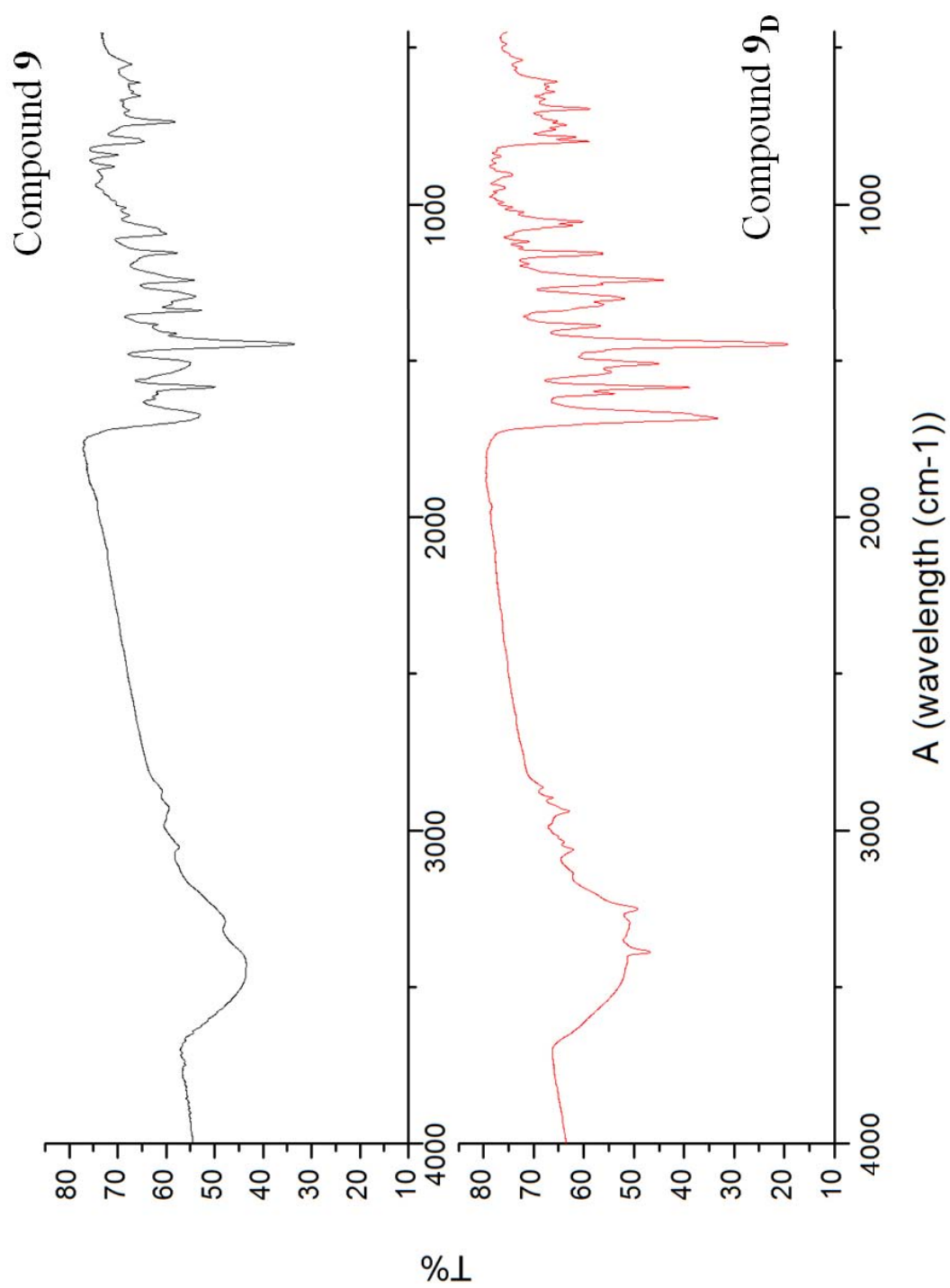
¹H NMR (300 MHz, D₂O) δ 3.74 (t, *J* = 5.7 Hz, 2H), 2.98 – 2.87 (m, 2H), 1.61 (dd, *J* = 6.8, 3.4 Hz, 4H).

1-(4-(Acridin-9-ylamino)butyl)-1,3,5-triazinane-2,4,6-trione (Compound 35, C₂₀H₁₉N₅O₃, MW: 377.40)



To a 2-necked flask was added compound **34** 0.10g (0.5 mmol, 1.25 eq), 9-chloroacridine 0.08g (0.4 mmol, 1 eq.), diisopropylethylamine 0.1 mL (0.5 mmol, 1.25 eq.) and phenol 1.14g (1.2 mmol, 3 eq.), and placed under vacuum and then back-filled with Ar three times. The reaction mixture was then heated to 70°C for 30 minutes, and then cooled to room temperature. Methanol (7 mL) was added into the flask and then the mixture triturated with diethyl ether to precipitate crude **35** as a yellowish solid, which was purified using column chromatography (CH₂Cl₂/MeOH (9:1)) to afford **35** as a yellow solid 0.045g (31%).

¹H NMR (300 MHz, MeOD) δ 8.40 (d, *J* = 8.5 Hz, 2H), 7.92 – 7.82 (m, 2H), 7.76 – 7.69 (m, 2H), 7.53 – 7.45 (m, 2H), 4.92 (s, 3H), 4.13 (t, *J* = 7.2 Hz, 2H), 3.83 (t, *J* = 6.9 Hz, 2H), 2.07 – 1.92 (m, 2H), 1.87 – 1.72 (m, 2H). ¹³C NMR (50 MHz, MeOD) δ 159.59 (s), 151.52 (s), 150.13 (s), 141.28 (s), 136.41 (s), 126.72 (s), 124.93 (s), 119.69 (s), 113.88 (s), 41.43 (s), 27.61 (s), 26.14 (s). HRMS: [C₂₀H₁₉N₅O₃+H]⁺ calcd: 378.1560, obtained: 378.1551.



References

1. M. Montalti, A. Credi, L. Prodi and M. T. Gandolfi, *Handbook of Photochemistry*, CRC Press, **2006**.
2. A. Schönberg, G. O. Schenck and O. A. Neumüller, *Preparative organic photochemistry*, Springer-Verlag, **1968**.
3. H. Ron, S. Matlis and I. Rubinstein, *Langmuir*, **1998**, *14*, 1116-1121.
4. T. Buffeteau, B. Desbat and J. M. Turlet, *Applied Spectroscopy*, **1991**, *45*, 380-389.
5. T. Buffeteau, B. Desbat, D. Blaudez and J. M. Turlet, *Applied Spectroscopy*, **2000**, *54*, 1646-1650.
6. M. A. Ramin, G. Le Bourdon, N. Daugey, B. Bennetau, L. Vellutini and T. Buffeteau, *Langmuir*, **2011**, *27*, 6076-6084.
7. T. Buffeteau and B. Desbat, *Applied Spectroscopy*, **1989**, *43*, 1027-1032.
8. K. Yamamoto and H. Ishida, *Applied Spectroscopy*, **1994**, *48*, 775-787.
9. Y. Molard, D. M. Bassani, J.-P. Desvergne, N. Moran and J. H. R. Tucker, *J. Org. Chem.*, **2006**, *71*, 8523-8531.
10. F. Wessendorf and A. Hirsch, *Tetrahedron*, **2008**, *64*, 11480–11489
11. A. Grube, C. Timm, and M. Köck, *Eur. J. Org. Chem.* **2006**, 1285–1295
12. J. P. Collman, N. K. Devaraj and C. E. D. Chidsey, *Langmuir*, **2004**, *20*, 1051-1053.
13. W. Fraser, C. J. Suckling and H. C. S. Wood, *Journal of the Chemical Society, Perkin Transactions 1*, **1990**, 3137-3144.
14. C.-C. Chu, *Hydrogen-bonded supramolecular materials for organic photovoltaic applications*, UNIVERSITÉ BORDEAUX 1, **2009**.
15. X. Kong, Z. He, Y. Zhang, L. Mu, C. Liang, B. Chen, X. Jing and A. N. Cammidge, *Organic Letters*, **2011**, *13*, 764-767.
16. O. Félix, M. Crego-Calama, I. Luyten, P. Timmerman and David N. Reinhoudt, *European Journal of Organic Chemistry*, **2003**, 1463-1474.

List of compounds

