'Q-wires': Synthesis, electrochemical properties and their application in electro-enzymology

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CHAPTER 2

Exploring the chemistry of quinone-terminated oligo(phenylenevinylene) molecular wires

2.1 Introduction

The ‘Q-wires’ introduced in chapter 1 have three different functionalities: a quinone moiety that can interact with enzymes; a conjugated oligo(phenylenevinylene) (OPV) molecular wire that enables non-rate-limiting electron transport between electrode and enzyme [1, 2]; and a terminal methyl thiol that allows the wire to bind gold surfaces, forming a ‘self-assembled monolayer’ or ‘SAM’ [3]. As can be observed in figure 1, the quinol moiety (here: ubiquinone and menaquinone) and the OPV system are connected through a methylene bridge. This *sp*^{3} carbon separates the quinone moiety from the molecular wire, preserving its natural redox properties, and, in addition, providing flexibility suspected to be essential for interactions with enzymes.

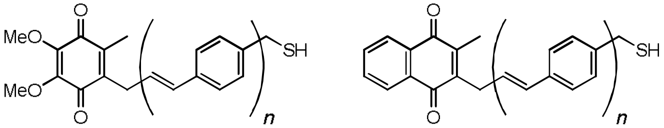


Figure 1 General structure of the ubiquinone- (left) and menaquinone-terminated (right) ‘Q-wires’

As will be described below, it was decided to synthesize the quinone moieties and OPV wires (of different lengths) separately, and to join both parts in a later stage using a Grubbs olefin metathesis reaction [4, 5, 6]. This would allow for many possible quinone-OPV wire combinations. However, complications forced the envisioned strategy to be considerably modified.

One alternative synthesis method removes the necessity of the Grubbs metathesis reaction altogether, and features a Wittig coupling instead. Another strategy, which proved the most successful, changes the location of the Grubbs metathesis reaction in the molecule, allowing for a cross metathesis reaction between two vinyl groups, instead of an allyl and vinyl group.

As mentioned previously, oligo(phenylenevinylene) (OPV) molecular wires are capable of fast electron transport [1, 2]. To verify whether the use of an OPV section in the Q-wires indeed improves electron transport rates, a fully saturated ‘wire’ (i.e. a quinone moiety attached to an alkanethiol) was synthesized as well. In addition, it was attempted to synthesize a fully conjugated wire, linking the quinone unit directly to the OPV system, in order to assess the influence of the aforementioned methylene bridge on electron transfer rates. The (unsuccessful) synthesis of the latter Q-wire is described below. The electron transfer characteristics of the Q-wires will be elaborated on in chapter 4.

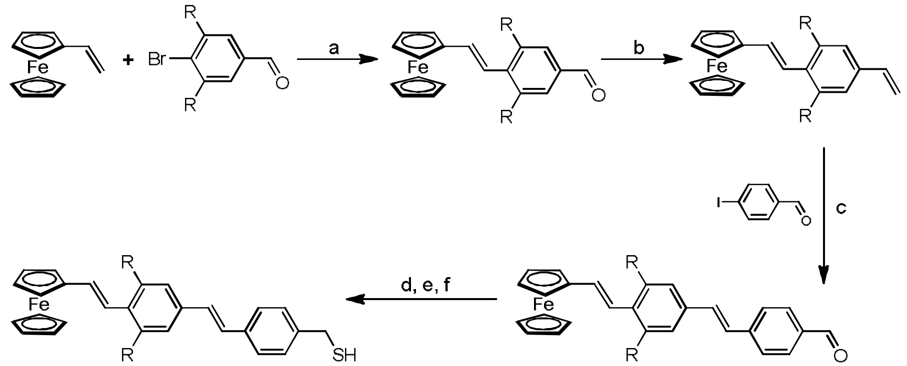
The ultimate goal of this study was to achieve a fully immobilized (sub)monolayer of a redox enzyme on an electrode surface, where a Q-wire was to provide non-rate-limiting electron transport between enzyme and electrode. Because it was suspected that the Q-wires may not achieve enzyme immobilization by themselves, some effort was put into the optimization of the SAM composition. Trimethyl-ammonium-terminated alkanethiols, for instance, could aid in the anchoring of enzymes that carry negatively charged surface patches. This approach was inspired by quatenary ammonium-modified anion-exchange materials widely used for protein purification. As will be explained below, these efforts have not yet led to a clear enhancement in binding/activity of the enzymes that were tested.

This chapter aims to reflect the exploration of the chemistry of the Q-wires that was required to arrive at a functional and practical method of synthesis, while chapter 3 provides a detailed description of the method that was developed here, which eventually allowed for the synthesis of the desired Q-wires.

2.2.1 Background

The synthesis of oligo(phenylenevinylene) can be achieved by alternating Heck coupling [7] and Horner-Wadsworth-Emmons coupling [8] or Wittig reactions [9], in which (*p*-halogen) benzaldehydes often play a crucial role [1, 10, 11, 12]. Scheme 1 exemplifies this recurring strategy: a Heck coupling between vinylferrocene and a *p*-bromobenzaldehyde is followed by a Wittig conversion of the terminal aldehyde to a vinyl, to allow for a second Heck coupling with *p*-iodobenzaldehyde. The introduction of the terminal methyl thiol is achieved by reduction of the terminal aldehyde, followed by the conversion of the resulting benzyl alcohol to benzyl thioacetate by means of a Mitsunobu reaction [13]. Deacetylation provides the desired benzyl thiol.

Longer OPV chains suffer from poor solubility in most solvents and thus require solubilization by addition of solubilizing substituents to the phenyl rings (ethoxy substituents in scheme 1, for example). It may be argued that such modifications have undesired interactions with enzymes or affect the redox properties of the molecule, and are therefore to be avoided. Furthermore, such modifications may hinder (desired) dense packing in a self-assembled monolayer on a gold surface. However, the effects on proper SAM formation were previously found to be of minimal concern [1]. Nevertheless, in the synthesis strategy outlined below, it was decided to avoid adding substituents to the phenyl rings if possible.

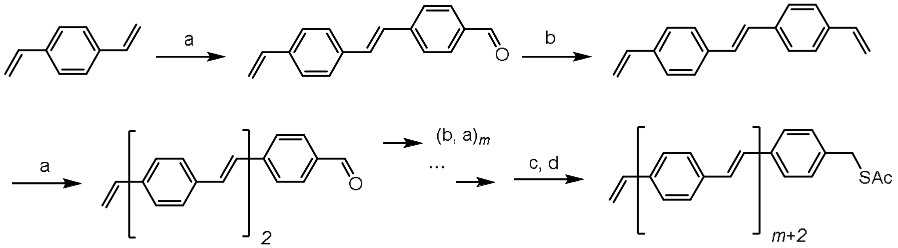


Scheme 1 Synthesis of a ferrocene-OPV-methylthiol wire (adapted from reference [1]): (a) $Pd(OAc)_2$, P(*o*-tolyl)$_3$, NBu_3, DMA; (b) *n*-BuLi, PPh_3CH_3Br, THF; (c) palladacycle, NaOAc, DMA; (d) $NaBH_4$, THF; (e) DEAD, PPh_3, AcSH, THF; (f) $LiAlH_4$, THF

2.2.2 Original synthesis strategy

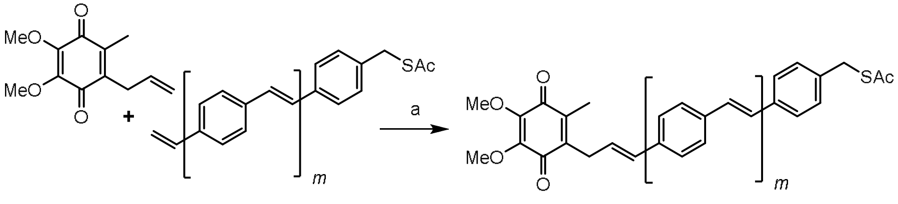
Although scheme 1 served as a starting point in designing a synthesis strategy, it was decided to introduce the quinone moiety – the redox-active part of the wire – during a late stage in the synthesis, thereby allowing for the attachment of different types of quinones to the same OPV wire. Furthermore, in the final product, the quinone moiety is uncoupled from the conjugated wire by a methylene bridge, which prohibits a reaction comparable to the initial Heck coupling reaction encountered in scheme 1. As a final step (scheme 3), a Grubbs olefin metathesis reaction between the allylic compound **1** and the vinyl of an OPV wire was therefore selected (comparable to, e.g. [4]). Additionally, by attaching the quinone moieties during a late stage in the synthesis, concerns about the stability of these groups were largely circumvented.

The synthesis of the methyl-thioacetate-terminated OPV wires, starting from *p*-divinyl benzene, is summarized in scheme 2.



Scheme 2 Schematic overview of the synthesis of methyl thioacetate-terminated OPV wires of arbitrary length; wire elongation is achieved by alternating the depicted Heck (a) and Wittig (b) reactions: (a) *p*-Br-benzaldehyde, Et_3N, $Pd(OAc)_2$, $P(o\text{-tolyl})_3$, DMF, 80°C, 24 h; (b) PPh_3CH_3Br, *t*-BuOK, THF, 0°C 1.5 h, then RT 1 h; (c) reducing agent; (d) DCAD, PPh_3, AcSH, THF, RT, 18 h

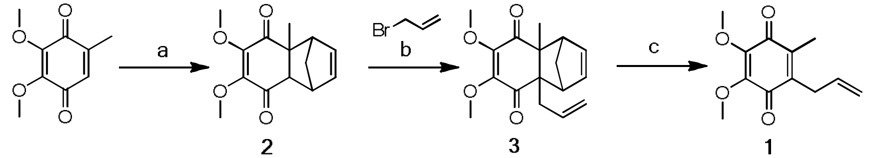
The attachment of 'allyl ubiquinone' (**1**) to the OPV wires by means of a Grubbs olefin metathesis reaction is depicted in scheme 3.



Scheme 3 Synthesis of a ubiquinone-terminated 'Q-wire' of arbitrary length, by means of a Grubbs cross metathesis reaction: (a) 2nd generation Grubbs catalyst, DCM, RT, ≥ 18 h

Several different methods for obtaining 'allyl ubiquinone' (**1**) can be found in literature [14, 15]. Perhaps the most straightforward strategy has been outlined in scheme 4. Here, commercially available 2,3-dimethoxy-5-

methyl-1,4-benzoquinone is reacted with cyclopentadiene, resulting in the Diels-Alder cycloadduct **2** [16], which is subsequently treated with potassium *tert*-butoxide. After addition of allyl bromide, compound **3** is obtained. This compound undergoes a retro-Diels-Alder reaction by heating, which gives the desired 'allyl ubiquinone' (**1**).

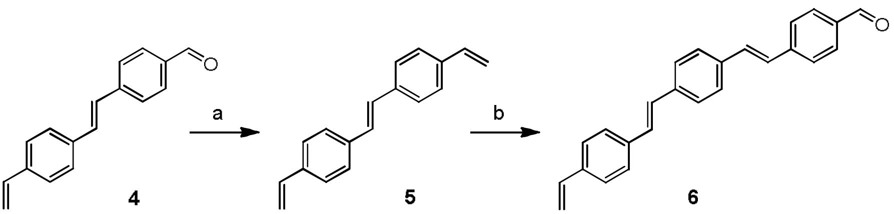


2 3 1

Scheme 4 Synthesis of 'allyl ubiquinone' **1** (adapted from [14]): (a) cyclopentadiene, glacial AcOH, RT, 94%; (b) *t*-BuOK, THF, 0°C, 80%; (c) toluene, 110°C, 98%

2.2.3 Experimental challenges

During synthesis of the OPV wires (scheme 5), solubility issues were encountered already after the first Heck coupling reaction. Compound **4** proved to be poorly soluble in common solvents, causing complications during purification (e.g. broad elution peaks) and analysis (precipitation). A second Heck coupling between *p*-bromobenzaldehyde and **5** resulted in compound **6**, exhibiting even worse solubility properties, obstructing further reactions with this compound. An attempt to address this problem was made by reacting **5** (and p-divinylbenzene) with 4-bromo-2,5-dimethoxy-benzaldehyde instead. However, this did not lead to a detectable amount of product.

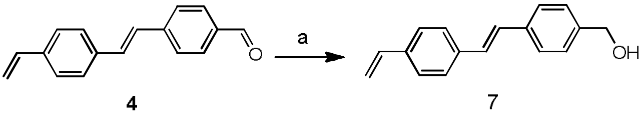


4 5 6

Scheme 5 Synthesis of **6**: (a) PPh_3CH_3Br, *t*-BuOK, THF, 0°C 1.5 h, then RT, 1 h; (b) *p*-Br-benzaldehyde, Et_3N, $Pd(OAc)_2$, $P(o\text{-tolyl})_3$, DMF, 80°C, 170 h

Further complications were encountered during the reduction of compound **4**. Reduction using sodium borohydride to afford compound **7** was unsuccessful. Subsequent efforts to obtain **7** included the use of milder reducing agents, such as sodium cyanoborohydride and sodium triacetoxyborohydride, and changing solvent composition (THF, MeOH, $CHCl_3$, DCM, *i*-PrOH, or mixtures thereof). However, none of these efforts resulted in the desired product.

During a later stage of the synthesis, when the strategy outlined here was already abandoned, it was discovered that diisobutylaluminium hydride (DIBAL-H) is very suitable for the reduction of compound **4** to **7**, achieving yields up to 80%.

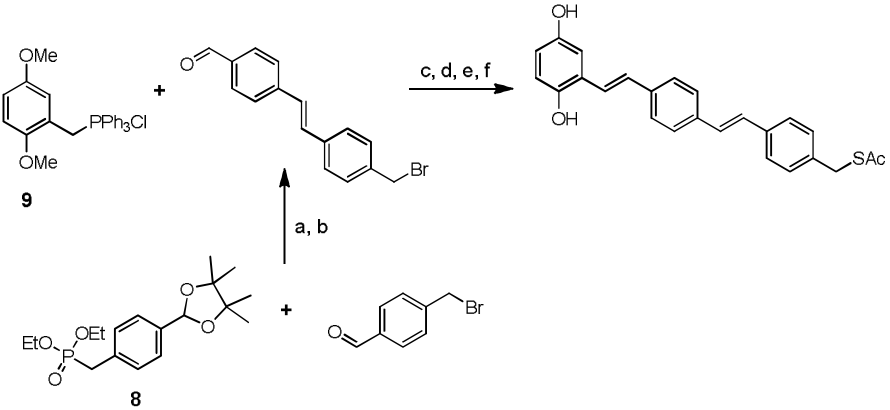


Scheme 6 Reduction of **4**: (a) THF, 0°C, dropwise addition of 1 M DIBAL-H in THF, then RT, 15 min

In addition to the aforementioned obstacles, the discovery that the Grubbs olefin metathesis depicted in scheme 3 would not be successful for *m > 0*, presented a more insurmountable problem and ultimately led to the abandonment of the strategy presented in the above sections. Several alternatives were considered, two of which are elaborated on below.

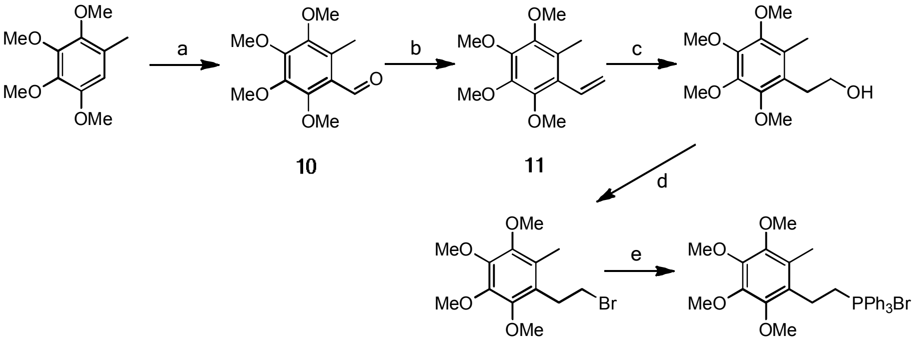
2.3.1 Wittig reagent alternative

Scheme 7 depicts the synthesis of a fully conjugated hydroquinone OPV wire [12], which does not rely on a Grubbs olefin metathesis. A Horner-Wadsworth-Emmons coupling between 4-(bromomethyl)benzaldehyde and compound **8**, results in an OPV wire featuring a terminal aldehyde. This aldehyde can then be used to couple the (protected) quinone moiety to the wire, by means of a Wittig reaction. The Wittig reagent **9**, a triphenyl phosphonium salt, is pivotal in this reaction.



Scheme 7 Synthesis of a hydroquinone-terminated OPV wire (adapted from [12]): (a) *t*-BuOK, THF; (b) HCl, THF, 65°C; (c) NaH, THF; (d) KSAc, DMF, 80°C; (e) I_2 (cat.), toluene, reflux; (f) BBr_3, DCM, -78°C

The synthesis of a comparable Wittig reagent, consisting of a (protected) ubiquinone moiety, can be envisioned. Once obtained, this reagent can be used to couple a ubiquinone unit to any aldehyde-terminated OPV wire, abolishing the need for a Grubbs metathesis reaction. A possible synthesis method can be found in scheme 8. After the introduction of an aldehyde group to commercially available 1,2,3,4-tetramethoxy-5-methylbenzene by means of a Rieche formylation reaction [17, 18] and its subsequent conversion to a vinyl group (Wittig), 9-borabicyclo(3.3.1)nonaan (9-BBN) and hydrogen peroxide are used to specifically convert the vinyl to a terminal, primary alcohol [19]. Phosphorus tribromide is then used to convert the alcohol to an alkyl bromide [17, 20], which, after reacting with triphenylphosphine, yields the desired Wittig reagent.

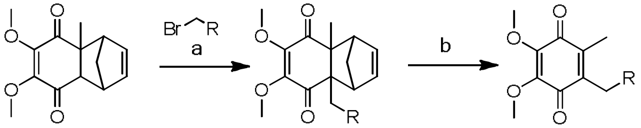


Scheme 8 Synthesis of a Wittig reagent carrying a protected ubiquinone unit: (a) HCl_2COCH_3, 1 M $TiCl_4$ dropwise, DCM, 0°C, then RT, 5 h; (b) PPh_3CH_3Br, *t*-BuOK, THF, 0°C, then RT, 18 h; (c) 9-BBN, THF, then NaOH, H_2O_2, H_2O; (d) PBr_3, Et_2O, 0°C, 20 min; (e) PPh_3, toluene, reflux

Although compound **11** was successfully obtained from **10** and this synthesis method may probably still be feasible, it was suspended in favor of the alternative method described below.

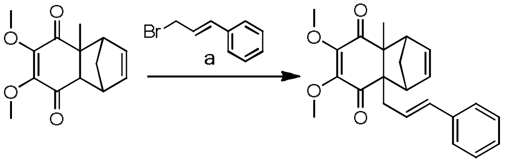
2.3.2 Diels-Alder cycloadduct alternative

Another method to circumvent the problems associated with the Grubbs metathesis reaction encountered in the original synthesis strategy, could utilize the approach outlined in scheme 4 and generalized in scheme 9. Here – in principle – any convenient *R* moiety could be introduced, allowing for many different types of subsequent reactions.



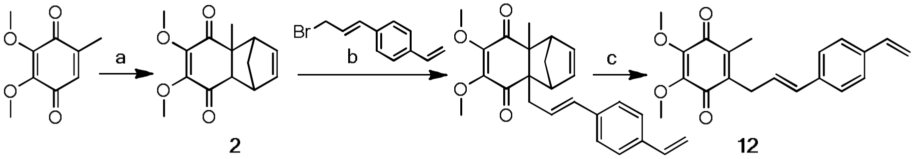
Scheme 9 Generalized approach to obtain a ubiquinone moiety carrying any methylene-R group: (a) *t*-BuOK, THF, 0°C (b) toluene, reflux

In reality, however, only allylic bromides could be attached successfully, perhaps due to the increased stability of the allylic cation. The successful attachment of cinnamyl bromide (scheme 10) inspired a new strategy (scheme 11 and 12). Although still dependent on a Grubbs cross metathesis, this reaction would now essentially couple two substituted styrenes, which had previously led to relatively high yields (e.g. see scheme 16).



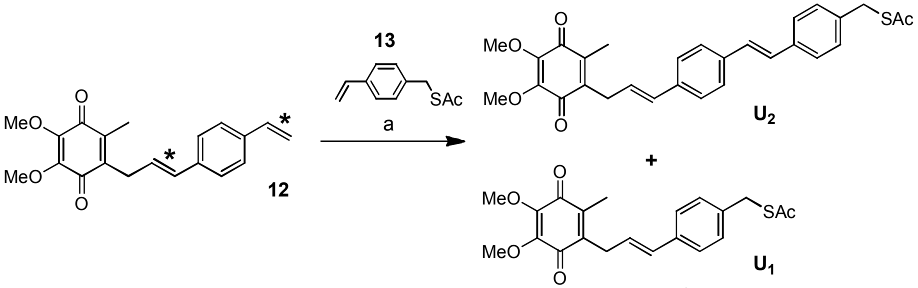
Scheme 10 Cinnamyl-modified compound **2**: (a) *t*-BuOK, THF, 0°C

As mentioned above, the attachment of *p*-vinyl cinnamyl bromide – the synthesis of which can be found in chapter 3 – to compound **2**, and subsequent removal of the cyclopentadiene ring (scheme 11), yielded a styryl-terminated compound **12**, which was suspected to successfully couple to *S*-4-vinylbenzyl thioacetate (**13**) by means of a Grubbs cross metathesis reaction (scheme 12).



Scheme 11 Synthesis of **12**: (a) cyclopentadiene, glacial AcOH, RT; (b) *t*-BuOK, THF, 0°C; (c) toluene, reflux

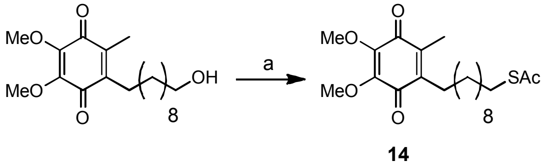
Besides the formation of dimers of especially compound **13**, an additional complication was observed. Not only the terminal vinyl proved to participate in the cross metathesis, but an internal double bond as well (indicated with an asterisk in scheme 12), leading to the inadvertent synthesis of Q-wires of different length (i.e. **U$_1$** and **U$_2$**; for naming convention, see chapter 1). Separation of the desired product from the numerous byproducts and starting materials proved challenging and required several rounds of careful purification, leading to relatively low, yet – for our purposes – satisfactory yields. The synthesis strategy outlined here, together with the fully analogous synthesis of menaquinone-terminated Q-wires, will be discussed in greater detail in chapter 3.



Scheme 12 Synthesis of **U$_2$** (and **U$_1$** as a byproduct): (a) 2nd generation Grubbs catalyst, DCM, RT, ≥ 18 h. The asterisks indicate reactive double bonds

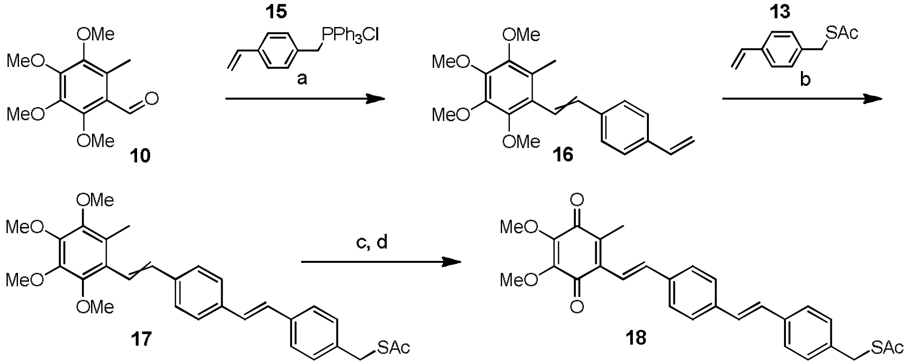
2.4 Verification of fast electron transport by Q-wires

To verify whether the OPV sections in the Q-wires indeed improve electron transfer rates, in comparison with equally long ubiquinone-terminated alkanethiol wires, it was decided to synthesize compound **14**, which is similar in length to compound **U$_2$**. Starting from commercially available idebenone, a single Mitsunobu reaction [13] sufficed to afford the desired product (scheme 13).



Scheme 13 Synthesis of **14**: (a) DCAD, PPh$_3$, AcSH, THF, 0°C, then RT, 18 h

In addition, to assess the influence of the aforementioned *sp^3* carbon on electron transfer rates, it was attempted to synthesize a fully conjugated Q-wire (**18** - scheme 14). Here, the quinone moiety is not uncoupled from the conjugated OPV wire, which is anticipated to influence the redox properties of the quinone unit.



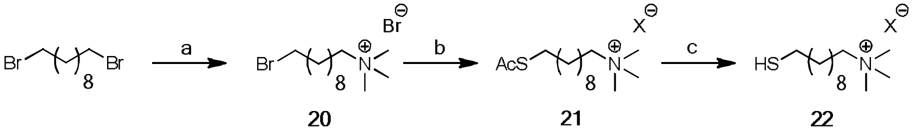
Scheme 14 Synthesis of **18**: (a) *t*-BuOK, THF, 0°C, then RT, 40 h; (b) 2nd generation Grubbs catalyst, DCM, RT, 40 h; (c) I_2 (cat.), toluene, reflux, 3h; (d) several unsuccessful attempts

Reacting the aldehyde **10** with the phosphonium salt **15** in a Wittig coupling reaction, results in an *E*/*Z* mixture of compound **16**. Similar to the cross metathesis encountered in scheme 12, coupling **13** and **16** yielded compound **17**, after which isomerization was performed to obtain the all-trans Q-wire. Although extensively described in the literature [14, 15, 17], the oxidative demethylation of (specifically) the *para*-methyl ethers by ceric ammonium nitrate (CAN) proved unsuccessful to afford **18**. In addition, oxidation by silver (II) oxide [15, 21] yielded no product. Deprotection attempts using (bis(trifluoroacetoxy)iodo)benzene (PIFA) [22, 23] or boron tribromide [12] were not successful either. Efforts to deprotect compound **16** instead, prior to the Grubbs metathesis, were ineffective as well.

2.5 Additional electrode surface modifications

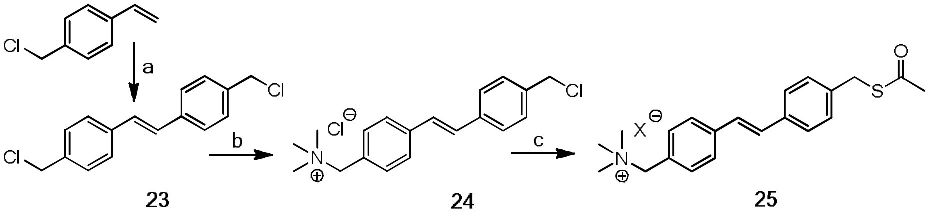
The ultimate goal of this study was to achieve a fully immobilized (sub)monolayer of a redox enzyme of interest on an electrode surface, where an appropriate Q-wire was to provide non-rate-limiting electron transport between enzyme and electrode. Because it was considered to be unlikely that Q-wires could achieve enzyme immobilization by themselves, some effort was put into the optimization of the SAM composition. Trimethyl-ammonium-terminated alkanethiols, for instance, could aid in the anchoring of enzymes that carry negatively charged surface patches.

The synthesis of one such trimethyl-ammonium-terminated alkanethiol, compound **22**, is outlined in scheme 15. Starting from 1,10-dibromodecane, two subsequent substitution reactions and a thioester hydrolysis yielded the desired compound **22** [24, 25].



Scheme 15 Synthesis of **22**: (a) NMe_3, toluene, RT, 120 h; (b) KSAc, H_2O, 60°C, 16 h; (c) 4 M HCl, 85°C, 1 h

In addition, instead of a fully saturated linker, a fully conjugated linker was considered as well. The synthesis of compound **25**, outlined in scheme 16, is fully analogous to the previous method.



Scheme 16 Synthesis of **25**: (a) 2nd generation Grubbs catalyst, DCM, RT, 60 h; (b) NMe_3, toluene, RT, 48 h; (c) KSAc, aceton/MeOH, RT, 18 h

Preliminary experiments revealed that compound **22** does not form a full SAM. However, when supplemented with an additional, uncharged co-SAM, such as heptanethiol, a denser SAM was formed. Electrodes decorated with Q-wire, compound **22** and heptanethiol (comparable to the standard electrode surface modification in chapter 5) did not yet show enhanced activity or immobilization of the cenzymes that were studied (*data not shown*). Further attempts at optimization of the aforementioned system were therefore abandoned. However, some possible applications of the wires introduced here will be discussed in the following section.

2.6 Conclusion & outlook

As will be discussed in chapter 3, the synthetic strategy developed in this chapter allowed for the synthesis of a series of ubiquinone- and menaquinone-terminated OPV molecular wires. Without solubilizing substituents on the phenyl rings, a maximum length of three ‘OPV units’ was achieved ($n = 3$ in figure 1). An even longer wire, however, may be required to reach a particularly deeply buried active site of an enzyme, or to keep an enzyme at a secure distance from the electrode. Recently, an alternative to the OPV synthesis strategy outlined in scheme 1 was described [26]; Horner-Wadsworth-Emmons reagents similar to reagent **8** depicted in scheme 7, carrying solubilizing substituents and a *p*-nitrile instead of a *p*-acetal, were used to elongate an aldehyde-terminated OPV chain. Subsequent conversion of the now terminal nitrile to an aldehyde then allowed for further elongation, resulting in chains up to seven ‘OPV units’ in length in good yield. The Wittig reagent depicted in scheme 8 could then be reacted with the terminal aldehyde of such an OPV wire, resulting in a Q-wire of superior length. In this scenario, however, similar issues regarding the deprotection of compound **17** may be encountered. It may therefore be necessary to consider the use of alternative protecting groups, capable of withstanding the reactions summarized in scheme 8, in addition to the Wittig reaction described above. This strategy may additionally lead to the completion of the synthesis of compound **18**. A comparison of the electrochemical behavior of this Q-wire to that of $\mathbf{U_2}$ and $\mathbf{U_{SAT}}$, which are of similar length, would shed more light on the rather puzzling results described in chapter 4.

It may be of interest to subject the trimethyl-ammonium-terminated wires, introduced in the previous section, to further study. As described for the wires depicted in figure 3, chapter 1, a – possibly genetically engineered – region near the active site of an enzyme of interest could be used to tether the enzyme to an electrode using the wires discussed here, establishing a direct electron pathway. A patch of negatively charged amino acids, for instance, could provide the interactions required for anchoring the enzyme. Once immobilized, protein film voltammetry could then be employed to assess the differences between compounds **22** and (deprotected) **25** in terms of their electron transfer characteristics. In an alternative scenario, said wires may aid in the prevention of undesired interactions between positively charged proteins and the electrode surface. In the study of Complex III – cytochrome *bc*$_1$ – for example, interactions between the electrode and the positively charged substrate cytochrome *c*, present in the electrolyte, may be undesired. The aforementioned wires could then repel cytochrome *c* from the electrode, preventing interfering interactions.

As mentioned before, the Q-wires may bind enzymes only transiently, and may therefore not be able to fully immobilize them. Stronger enzyme-wire interactions may be achieved by utilizing alternative head groups, consisting of, for example, (parts of) enzyme inhibitors or non-natural quinone derivatives. The respiratory chain inhibitor HQNO (N-oxo-2-heptyl-4-hydroxyquinoline), for instance, binds tightly to quinone binding sites [27 28, 29]. Therefore, an HQNO-terminated OPV wire (figure 2) could be a viable candidate for further research.

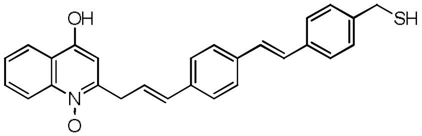
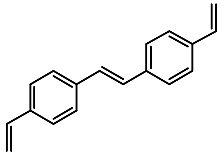


Figure 2 HQNO-terminated Q-wire

2.7 Experimental section

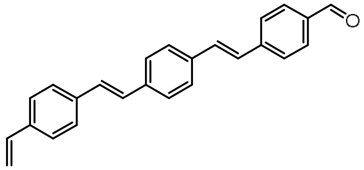
This section describes the synthesis of the compounds that do not feature in chapter 3. If the synthesis of a compound is not listed here, the compound can either be: described in chapter 3; obtained from a commercial source; or taken from literature. THF and diethyl ether were dried over 60% sodium hydride in mineral oil for several hours, prior to distillation under reduced pressure. Dry DCM and DMF were purchased. Flash chromatography was performed on Screening Devices B.V. silica gel 60 (0.040-0.063 mm). NMR spectra were recorded on a Bruker DPX-300 spectrometer (300/75 MHz).

(*E*)-1,2-bis(4-vinylphenyl)ethane (5)



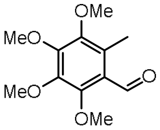
To a stirring suspension of methyltriphenylphosphonium bromide (3.57 g, 10 mmol) in 100 mL dry THF under an argon atmosphere at 0°C, potassium *t*-butoxide (1.12 g, 10 mmol) was added in portions. A separate solution of (*E*)-4-(4-vinylstyryl)benzaldehyde (**4**) (2.35 g, 10 mmol) in 50 mL dry THF was added dropwise. The mixture was stirred for 1.5 hrs at 0°C and an additional hour at room temperature, after which TLC indicated completion. The solvent was evaporated and the resulting slurry was redissolved in diethyl ether, to which silica powder was added. After drying to the air, the powder-like substance was poured on top of a silica gel column (PET), and the product was eluted with PET, resulting in a broad elution peak. This provided approximately 500 mg (20% - further purification of impure fractions may improve the yield) of a light yellow solid. ^{1}H NMR (300 MHz, CDCl$_3$): δ = 7.48 (d, *J* = 8.4 Hz, 4H), 7.41 (d, *J* = 8.4 Hz, 4H), 7.10 (s, 2H), 6.72 (dd, J_1 = 17.6 Hz, J_2 = 10.9 Hz, 2H), 5.77 (d, *J* = 17.7 Hz, 2H), 5.26 (d, *J* = 10.8 Hz, 2H). ^{13}C NMR (75 MHz, CDCl$_3$): δ = 137.0, 136.5, 128.3, 126.8, 126.7, 113.9.

4-((*E*)-4-((*E*)-4-vinylstyryl)styryl)benzaldehyde (6)



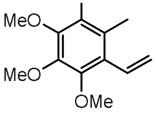
p-bromobenzaldehyde (285 mg, 1.5 mmol), (*E*)-1,2-bis(4-vinylphenyl)ethene (**5**) (200 mg, 0.9 mmol) and triethylamine (233 mg, 2.3 mmol) were added to a round-bottom flask containing 10 mL of dry DMF. After purging with argon, palladium (II) acetate (23 mg, 0.1 mmol) and tri(*o*-tolyl)phosphine (47 mg, 0.15 mmol) were added to the stirring solution. The mixture was allowed to react for approximately 170 hrs at 80°C under an argon atmosphere. During this period, palladium (II) acetate, tri(*o*-tolyl)phosphine and triethylamine were replenished twice. The solvent was subsequently removed under vacuum. The residue was redissolved in diethyl ether and silica powder was added. After evaporation of the ether, the resulting powder was poured on top of a silica gel column, after which a crude purification was performed, using DCM as eluent. The fractions containing the crude product were combined and after the eluent was removed under vacuum, a second round of silica gel column chromatography was performed, using a PET/DCM gradient (1:0 to 0:1). This purification was repeated once more, resulting in 30 mg (10%) of a virtually insoluble light yellow solid. ^{1}H NMR (300 MHz, CD_2Cl_2): δ = 9.75 (s, 1H), 7.88 (d, *J* = 9.0 Hz, 2H), 7.71 (d, *J* = 9.0 Hz, 2H), 7.58 (s, 4H), 7.53 (d, *J* = 9.0 Hz, 2H), 7.46 (d, *J* = 6.0 Hz, 2H), 7.27 (d, *J* = 15.0 Hz, 2H), 7.16 (s, 2H), 6.71 (dd, J_1 = 18.0 Hz, J_2 = 6.0 Hz, 1H), 5.79 (d, *J* = 21.0 Hz, 1H), overlaps with DCM peak (d, 1H).

2,3,4,5-tetramethoxy-6-methylbenzaldehyde (10)



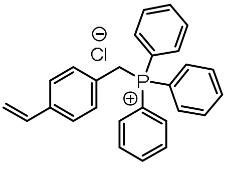
The following procedure was derived from a previously described protocol [18]. To a stirring solution of 2 g (9.5 mmol) 1,2,3,4-tetramethoxy-5-methylbenzene and 5 mL (35 mmol) dichloromethyl methyl ether in 50 mL dry DCM under an argon atmosphere at 0°C, 3 mL of a 1M solution of $TiCl_4$ in DCM (3 mmol) was added dropwise over a period of several minutes. The reaction was continued at room temperature for 5 hrs under argon, after which the reaction mixture was poured into cold water. The aqueous phase was extracted with DCM twice (250 mL total). The organic phase was then dried over $MgSO_4$ and, after filtration, the solvent was evaporated to afford 2 g (88%) of product that required no further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 10.43 (s, 1H), 4.03 (s, 3H), 3.95 (s, 3H), 3.91 (s, 3H), 3.77 (s, 3H), 2.46 (s, 3H).

1,2,3,4-tetramethoxy-5-methyl-6-vinylbenzene (11)



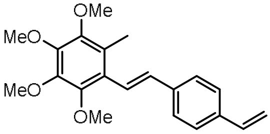
Methyltriphenylphosphonium bromide (223 mg, 0.6 mmol) and 2,3,4,5-tetramethoxy-6-methylbenzaldehyde (**10**) (100 mg, 0.4 mmol) were added to 5 mL of dry THF at 0°C. While stirring under an argon atmosphere, potassium *t*-butoxide (70 mg, 0.6 mmol) was added in portions. The reaction mixture was stirred overnight at room temperature, after which chloroform was added. The mixture was filtered and the solvents were evaporated, after which the residue was purified by means of silica gel column chromatography, utilizing a PET/ethyl acetate gradient (1:0 to 4:1). This provided 30 mg (30%) of the desired compound. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.67 (dd, J_1 = 17.9 Hz, J_2 = 11.6 Hz, 1H), 5.58 (dd, J_1 = 17.9 Hz, J_2 = 1.9 Hz, 1H), 5.50 (dd, J_1 = 11.7 Hz, J_2 = 2.1 Hz, 1H), 3.93 (s, 3H), 3.91 (s, 3H), 3.79 (s, 3H), 3.77 (s, 3H), 2.22 (s, 3H).

Triphenyl(4-vinylbenzyl)phosphonium chloride (15)



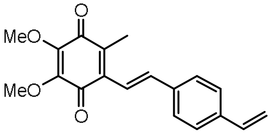
To 50 mL of toluene, 4 mL of 4-vinylbenzyl chloride (21 mmol) and 5 g of triphenylphosphine (19 mmol) were added. The mixture was refluxed for 8 hrs, after which it was cooled to room temperature and filtered. The residue was dried to provide 2 g (25%) of a white powder, which required no further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.59-7.83 (m, 15H), 7.15 (d, J = 8.1 Hz, 2H), 7.07 (d, J = 8.4 Hz, 2H), 6.60 (dd, J_1 = 17.6 Hz, J_2 = 10.9 Hz, 1H), 5.67 (d, J = 17.4 Hz, 1H), 5.52 (d, J = 14.7 Hz, 2H), 5.23 (d, J = 10.8 Hz, 1H).

(*E*)-1,2,3,4-tetramethoxy-5-methyl-6-(4-vinylstyryl)benzene (16)



Triphenyl(4-vinylbenzyl)phosphonium chloride (**15**) (2.8 g, 6.7 mmol) and 2,3,4,5-tetramethoxy-6-methylbenzaldehyde (**10**) (1.2 g, 5 mmol) were added to 50 mL dry THF, after which the stirred suspension was purged with argon and cooled to 0°C. Potassium *t*-butoxide (850 mg, 7.6 mmol) was then added in portions. The mixture was then stirred for 40 hrs under an argon atmosphere at room temperature, after which it was filtered. The solvent was removed and the resulting crude product was subjected to silica gel column chromatography, utilizing a PET/DCM gradient (1:0 to 1:1), to afford approximately 1.1 g (65%) of a yellow oil, composed of a 63% *E* and 37% *Z* isomer mixture. To obtain the desired *E* isomer, 150 mg of this mixture was dissolved in 10 mL toluene, to which a catalytic amount of I_2 was added. After refluxing for 3 hrs, the reaction mixture was cooled to room temperature and treated with a 10% aqueous potassium thiosulfate solution. The aqueous phase was extracted with DCM and the solvents were evaporated. The crude product was purified as described above, affording 46 mg (31%) of the title compound as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.47 (d, *J* = 8.3 Hz, 2H), 7.40 (d, *J* = 8.3 Hz, 2H), 7.11 (d, *J* = 16.5 Hz, 1H), 7.03 (d, *J* = 16.8 Hz, 1H), 6.72 (dd, J_1 = 17.6 Hz, J_2 = 10.9 Hz, 1H), 5.76 (d, *J* = 17.6 Hz, 1H), 5.24 (d, *J* = 10.9 Hz, 1H), 3.95 (s, 3H), 3.93 (s, 3H), 3.81 (s, 3H), 3.78 (s, 3H), 2.30 (s, 3H).

Towards (*E*)-2,3-dimethoxy-5-methyl-6-(4-vinylstyryl)cyclohexa-2,5-diene-1,4-dione (19)

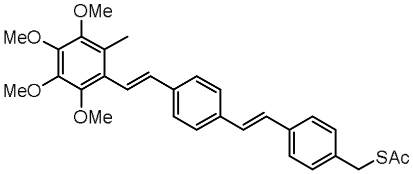


Several attempts were made to obtain the title compound from compound **16** by demethylation of its 1- and 4-methoxy, none of which proved successful. The methods listed below were derived from literature ([17], [21] and [23], respectively):

1. Compound **16** (40 mg, 0.12 mmol) was dissolved in a 5 mL mixture of 2:1 THF:water, to which 1.5 mL of a solution of 186 mg (0.34 mmol) ceric ammonium nitrate (CAN) was added dropwise at 0°C under an argon atmosphere. The reaction mixture was warmed to room temperature and reacted for 2 hrs. After addition of water and extraction of the aqueous phase with DCM, evaporation of the organic phase resulted in a residue containing no detectable product.
2. To a suspension of 147 mg of compound **16** (0.43 mmol) and 525 mg of silver (II) oxide (4.24 mmol) in 10 mL THF, stirring at 0°C, 3.5 mL of 6 N nitric acid was added, after which the reaction mixture turned clear. After 15 mins of reacting at room temperature, 50 mL of water was added. The aqueous phase was extracted with chloroform. The organic phase was washed with brine and subsequently dried over $MgSO_4$. After filtration and removal of the solvent, the resulting thick orange oil was subjected to NMR-analysis, revealing no appreciable amount of the title compound.
3. A suspension of 100 mg of compound **16** (0.3 mmol) and 260 mg of (bis(trifluoroacetoxy)iodo)benzene (PIFA) (0.6 mmol) in 1.5 mL water, containing 0.05 mL methanol, was stirred for 45 mins at room temperature, after which the mixture was extracted with

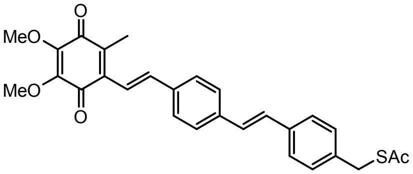
ethyl acetate. The residue after evaporation of the solvents was subjected to silica gel column chromatography (using a DCM:ethyl acetate gradient 1:0 to 20:1). None of the fractions contained the title product.

***S*-4-((*E*)-4-((*E*)-2,3,4,5-tetramethoxy-6-methylstyryl)styryl)benzyl thioacetate (17)**



S-4-vinylbenzyl thioacetate (**13**) (750 mg, 4 mmol) and 1,2,3,4-tetramethoxy -5-methyl-6-(4-vinylstyryl) benzene (**16**, mixture of isomers) (950 mg, 2.8 mmol) were dissolved in 25 mL dry, argon purged DCM. After the addition of 10 mg of Grubbs catalyst, 2nd generation, the mixture was stirred for 24 hrs under an argon atmosphere. After removal of the solvent, the crude mixture was applied to a silica gel column (PET), eluting with a gradient of PET/ethyl acetate (1:0 to 3:1) to afford 280 mg (20% - further purification of impure fractions may improve the yield) of the desired product as a mixture of *E* and *Z* isomers. Isomerization was performed as described for compound (**16**), however no further purification was required. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.49 (s, 4H), 7.08 (s, 2H), 7.03-7.46 (m, 6H), 4.10 (s, 2H), 3.95 (s, 3H), 3.93 (s, 3H), 3.81 (s, 3H), 3.79 (s, 3H), 2.34 (s, 3H), 2.31 (s, 3H).

Towards *S*-4-((*E*)-4-((*E*)-2-(4,5-dimethoxy-2-methyl-3,6-dioxocyclohexa-1,4-dien-1-yl)vinyl)styryl)benzyl thioacetate (18)



Several attempts were made to obtain the title compound from compound **17** by demethylation of its 1- and 4-methoxy, none of which proved successful. The methods listed below were derived from literature ([17], [23], [21] and [12], respectively):

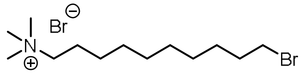
1. In a 5 mL 2:2:1 mixture of THF:acetonitrile:water stirring at 0°C, 140 mg (0.28 mmol) of compound **17** was dissolved. A solution of 325

mg (0.6 mmol) ceric ammonium nitrate (CAN) in a 1:1 mixture of acetonitrile and water was added dropwise to the former solution, over a period of 15 mins. After stirring at 0°C for 15 mins, the reaction mixture was allowed to warm to room temperature and allowed to react for an additional 15 mins, after which 10 mL of water was added. The mixture was extracted with four portions of 25 mL DCM. The organic phases were combined and the solvent was evaporated. The residue was subjected to silica column chromatography, but no appreciable amount of product was found.

2. Compound **17** (40 mg, 0.08 mmol) was dissolved in 2.5 mL of a 4:1 acetonitrile:water mixture. While being stirred at 0°C under an argon atmosphere, 68 mg (0.16 mmol) (bis(trifluoroacetoxy)iodo)benzene (PIFA) was added. The reaction was allowed to proceed for 2 hrs at room temperature, after which 5 mL of a dilute bicarbonate solution was added. The mixture was extracted with chloroform. The organic phase was treated with brine and dried over $MgSO_4$, after which the solvents were removed. NMR analysis revealed relatively intact starting material, but no desired product.
3. To a suspension of 40 mg of compound **17** (0.08 mmol) and 60 mg of silver (II) oxide (0.48 mmol) in 3 mL THF, stirring at 0°C, 0.4 mL of 6 N nitric acid was added. After 20 mins of reacting at room temperature, water was added. The aqueous phase was extracted with chloroform. The organic phase was washed with a 1 M bicarbonate solution and brine, after which the solvent was evaporated. The resulting residue was subjected to NMR-analysis, revealing destruction of the starting material and no appreciable amount of the title compound.
4. To 5 mL of dry DCM under argon, compound **17** was added (50 mg, 0.1 mmol). The stirring solution was cooled to -78°C, after which 0.3 mL of a 1 M boron tribromide (0.3 mmol) solution was added dropwise. The reaction mixture was allowed to warm to room temperature and react for 30 mins. Water was added to the mixture and stirring was continued for another 30 mins, after which the mixture was extracted with ethyl acetate. The organic phase

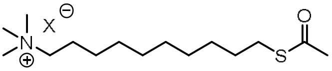
was treated with brine and dried over $MgSO_4$, after which the solvents were evaporated. NMR analysis of the residue suggested that no reaction had occurred, and therefore no product had been formed.

10-bromo-*N*,*N*,*N*-trimethyldecan-1-aminium bromide (20)



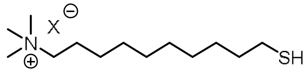
The following protocol was based on a method described earlier [25]. To a stirred solution of 6 g of 1,10-dibromodecane (20 mmol) in 30 mL toluene, 4 mL of a 33% solution of trimethylamine in ethanol (~18 mmol) was added dropwise. The reaction mixture was stirred for 5 days, after which it was treated as described in the aforementioned protocol. This afforded 1.5 g (22%) of the title compound. ^{1}H NMR (300 MHz, D_2O): δ = 3.53 (t, *J* = 6.9 Hz, 2H), 3.28-3.34 (m, 2H), 3.10 (s, 9H), 1.83-1.93 (m, 2H), 1.72-1.83 (m, 2H), 1.3-1.5 (m, 12H).

10-(acetylthio)-*N*,*N*,*N*-trimethyldecan-1-aminium (21)



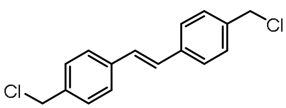
A solution of 1.5 g (4 mmol) of 10-bromo-*N*,*N*,*N*-trimethyldecan-1-aminium bromide (**20**) and 0.7 g (6 mmol) potassium thioacetate in 15 mL water was stirred for 16 hrs at 60°C, after which work-up was performed as described previously [24]. An additional purification by means of silica gel column chromatography, utilizing DCM/methanol gradient (1:0 to 4:1), was required to obtain 420 mg (30%) of the title product as a brown solid. ^{1}H NMR (300 MHz, D_2O): δ = 3.35-3.40 (m, 2H), 3.16 (s, 9H), 2.89 (t, *J* = 7.3 Hz, 2H), 2.38 (s, 3H), 1.75-1.86 (m, 2H), 1.52-1.64 (m, 2H), 1.32-1.43 (m, 12H).

10-mercapto-*N*,*N*,*N*-trimethyldecan-1-aminium (22)



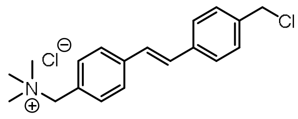
To achieve deacetylation, compound **21** was dissolved in 10 mL 4M HCl and stirred at 85°C for 1 hr, as described previously [24]. The aqueous phase was then extracted with DCM. After removal of the organic phase, 40 mg (10%) of the title compound was obtained as a brown liquid. ^{1}H NMR (300 MHz, D_2O): δ = 3.26-3.32 (m, 2H), 3.09 (s, 9H), 2.53 (t, *J* = 7.1 Hz, 2H), 1.70-1.82 (m, 2H), 1.53-1.64 (m, 2H), 1.24-1.40 (m, 12H).

(*E*)-1,2-bis(4-(chloromethyl)phenyl)ethane (23)



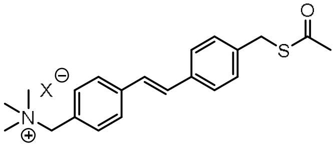
To 40 mL of dry, argon-purged DCM, 4-vinylbenzyl chloride (5 mL, 26 mmol) and 10 mg of Grubbs catalyst, 2nd generation, were added. The solution was stirred for 60 hrs under an argon atmosphere, during which the catalyst was replenished once. The solvent was removed under vacuum and PET was added to the crude mixture to form a suspension, which was subsequently filtered. The residue was washed with PET and dried to afford 1.6 g (46%) of the target compound as an off-white solid, which required no further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.51 (d, *J* = 8.1 Hz, 4H), 7.38 (d, *J* = 8.4 Hz, 4H), 7.11 (s, 2H), 4.60 (s, 4H).

(*E*)-1-(4-(4-(chloromethyl)styryl)phenyl)-*N*,*N*,*N*-trimethylmethanaminium chloride (24)



(*E*)-1,2-bis(4-(chloromethyl)phenyl)ethane (1 g, 3.6 mmol) (**23**) and 0.86 mL of a 33% trimethylamine solution in ethanol (3.6 mmol) were added to 50 mL toluene. The mixture was stirred for 2 days at room temperature, after which it was diluted with diethyl ether and filtered. The residue was washed with additional ether and allowed to dry, affording approximately 1 g (~85%) of the desired product. ^{1}H NMR (300 MHz, MeOD): δ = 7.61 (d, *J* = 7.8 Hz, 2H), 7.49 (d, *J* = 8.7 Hz, 4H), 7.33 (d, *J* = 8.1 Hz, 2H), 7.21 (d, *J* = 16.8 Hz, 1H), 7.13 (d, *J* = 16.5 Hz, 1H), 4.57 (s, 2H), 4.50 (s, 2H), 3.06 (s, 9H).

(*E*)-1-(4-(4-((acetylthio)methyl)styryl)phenyl)-*N*,*N*,*N*-trimethyl-methanaminium (25)



To 7 mL of a 3:1 aceton:methanol mixture, 300 mg potassium thioacetate (2.6 mmol) and 0.85 g (2.5 mmol) of (*E*)-1-(4-(4-(chloromethyl) styryl)phenyl)-*N*,*N*,*N*-trimethyl-methanaminium chloride (**24**) were added, after which the mixture was stirred overnight at room temperature. The mixture was then diluted with methanol and filtered. The solvent was removed from the filtrate and the resulting crude product was applied to a silica gel column (DCM) and purified using an eluent gradient (DCM/methanol 1:0 to 0:1), yielding 410 mg (44%) of the title compound. ^{1}H NMR (300 MHz, MeOD): δ = 7.63 (d, *J* = 8.4 Hz, 2H), 7.49 (d (overlapping), *J* = 8.4 Hz, 2H), 7.44 (d (overlapping), *J* = 8.4 Hz, 2H), 7.22 (d (overlapping), *J* = 8.1 Hz, 2H), 7.21 (d (overlapping), *J* = 15.9 Hz, 1H), 7.13 (d (overlapping), *J* = 16.5 Hz, 1H), 4.49 (s, 2H), 4.05 (s, 2H), 3.06 (s, 9H), 2.23 (s, 3H).

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