# TOWARDS SYNTHETIC CATCH BONDS: SYNTHESIS AND BINDING PROPERTIES OF A HOOK-SHAPED ELECTRON-RICH HOST

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Abstract

A hook-shaped molecule was synthesised in an attempt to act as a π-stacking host with electron deficient guests, before incorporating this system into synthetic catch bonds. The synthesised hook-shaped host consists in two methoxy-substituted anthracene molecules coupled together and bridged to make the structure more rigid upon pulling. Three electron deficient guests were used in this attempt and changes in absorption and steady-state fluorescence were monitored. For all three combinations the absorbance increased significantly while the changes in emission spectra were different for each guest. The dioctyl-bipyridinium guest increased the fluorescence, while the 4,4'-dinitrobiphenyl guest quenched it. For the third guest (perfluorinated biphenyl) no notable change in fluorescence was observed. The changes in absorbance and fluorescence were attributed to the predicted host-guest interaction. However, more insight into the characterisation, interactions and behaviour are required for a better understanding of this host-guest system.
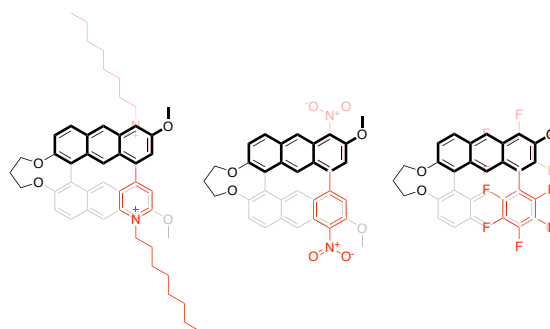
Introduction

Catch bonds are non-covalent bonds that, upon application of tensile force, become stronger and of which the lifetime increases [1,2]. This behaviour is counterintuitive and opposite to the common bonds, that weaken with force [1]. In nature, catch bonds occur between receptors and ligands where the bond strengthens by an applied tensile mechanical force [1]. Several examples of catch bonds have been reported, of which the most studied are the blood cell adhesion protein selectins and the bacterial adhesive protein FimH [2]. Despite the counterintuitive character of this behaviour, no synthetic catch bonds have yet been reported to the best of our knowledge [1,2]. One possibility to synthesise catch bonds is with the principle of a hook and thread to imitate the host-guest interactions, as shown schematically in **scheme 1**. When the hook and thread are in the right arrangement, the pulling force makes their unbinding less likely. For the host and guest to dissociate in the absence of pulling, a certain amount of thermal energy is required. Upon pulling apart, considerably larger thermal energy is required thus the lifetime is longer, and the host and guest undergo catch bond behaviour. An important milestone before
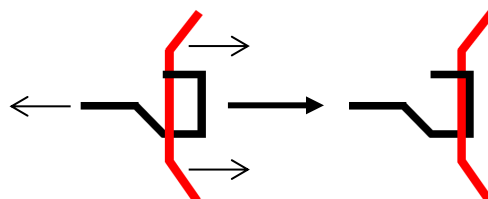
Scheme 1 – schematic representation of a hook (black) and thread (red) catch bond and their predicted behaviour.

reaching this catch bond behaviour, is the demonstration of non-covalent host-guest interactions.

The design of a possible hook-shaped host is a polyaromatic molecule with a rigid backbone. Here, two substituted anthracenes are coupled together to create the 'hook' shape. Bridging the two leftover hydroxy groups, prevents the molecule from planarizing upon pulling. The predicted structure of the host-guest complex is the guest sandwiched between the two anthracene of the host. In this structure, π-stacking is the main supramolecular interaction predicted to occur. This is maximised if the two molecules have opposing electron densities [5]. The electron-rich host is anticipated to bind with electron-poor aromatic guests. **Scheme 2** shows the predicted hook-thread structure and interaction between the hook-shaped host and three intended guests.
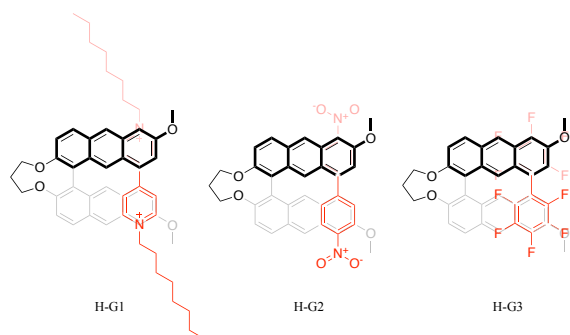
Scheme 2 – Predicted structures of the host-guest (H-G) complexes with the three different intended guests.

The structures of these intended guests feature two aromatic rings with electron-withdrawing groups. Guest 1 (**G1**) is dioctyl-bipyridinium, an analogue of viologen. The pyridiniums are substituted by octyl chains to increase their solubility in organic solvents. The positive formal charge on both nitrogen atoms pulls on the electrons of the aromatic rings thus decreasing the electron density [3]. Guests 2 (**G2**) and 3 (**G3**) are symmetrical biphenyl

compounds with substituents on the benzene rings. **G2** is 4,4'-dinitrobiphynl which has nitro functional groups on the *para* positions. This is a strong electron-withdrawing group thus decreasing the electron density of the rings [4]. **G3** is perfluorinated biphenyl. The high electronegativity of fluorine strongly decreases the electron density of the benzene rings.

In the attempt to eventually reach synthetic catch bonds, first validation of host-guest interactions is required. This is predicted to be with electron poor guests sandwiched in between a 'hook'-shaped electron rich host via π-stacking interaction. Many π-stacking based host-guest complexes have been reported in chloroform, making it an interesting option to study these kinds of interactions while avoiding the harsher synthesis of large water-soluble aromatic molecules [6].

Experimental section

Synthesis

The procedures were adapted from the methods from Zhu, Zhang, and Wongma [7,8,9].

6-methoxyanthracen-2-ol (2). In 25 ml dimethylformamide, 2,6-anthracenediol (3.31 g, 14.9 mmol, 1.0 eq) and K_2CO_3 (2.22 g, 16.0 mmol, 1.1 eq) were dissolved. To this solution, methyl iodide (0.44 ml, 7.14 mmol, 0.5 eq) was added dropwise. The mixture was stirred at 25°C and monitored by TLC (SiO_2/Al_2O_3, toluene/methanol 40:1). At the start, the colour changed quickly from yellow to dark brown as well as the formation of precipitate. After 48 hours, the mixture was diluted with water (140 ml) and 12M hydrochloric acid (1 ml). With the addition

of dichloromethane (70 ml) and brine (13 ml), the precipitate was clearly visible between the layers. This was filtered off and washed with DCM to extract the product. The product was extracted from the liquid solution with DCM (6x 70 ml). The combined organic layer was concentrated under reduced pressure and then dissolved in ethyl acetate (600 ml). The insoluble solids were filtered off and washed with ethyl acetate (200 ml). The combined ethyl acetate layers were washed with water (6x 100 ml) and dried over $MgSO_4$. Evaporating off the ethyl acetate resulted in a dark brown solid. This was purified with column chromatography (silica gel; toluene/methanol 50:1) to afford a red-brown solid (12.5 mg, 55.7 µmol, 0.8%).

^{1}H-NMR (400MHz, $CDCl_3$): 8.22 (s, 1H, H-10), 8.15 (s, 1H, H-9) 7.88 (d, J=9Hz, 1H, H-8), 7.83 (d, J=9Hz, 1H, H-4), 7.22 (d, J=2Hz, 1H, H-5), 7.14 (m, 3H), 4.92 (s, 1H, O-H), 3.95 (s, 3H, O-CH_3)

MALDI $[M+H]^+$ *m/z*: 224.05 (100%), 225.05 (22%), 226.06 (8%)

6,6'-dimethoxy-[1,1'-bianthracene]-2,2'-diol (3). In 6.3 ml chloroform, 6-methoxyanthracen-2-ol* (0.355 g, 1.58 mmol, 1eq) and manganese diiodide (25.0 mg, 0.081 mmol, 5 mol%) were dissolved. This solution was heated to 50˚C and the reaction was monitored by TLC (SiO_2/Al_2O_3, toluene/methanol 5:1). After 24 hours no reaction had occurred, thus manganese diiodide (10.4 mg, 0.034 mmol, 2 mol%) in 2.0 ml chloroform was added. After refluxing for 3 days, the chloroform was removed. A dark red solution was obtained after dissolving the acquired solid in dichloromethane (50 ml). This was washed with water (6x40 ml) and dried over $MgSO_4$. The dichloromethane was removed

under reduced pressure and the obtained dark red solid was purified by column chromatography (silica gel; toluene/methanol 10:1). The obtained dark red solid (16.6 mg) was characterised by ^{1}H-NMR ($CDCl_3$) to prove no product was formed.

* multiple samples were added together that was synthesised using the same procedure as described

12,19-dimethoxy-5,6-dihydro-4H-dianthra[2,1-f:1',2'-h][1,5]dioxonine (4). 6,6'-dimethoxy-[1,1'-bianthracene]-2,2'-diol* (18 mg, 42.2 µmol, 1eq) and potassium carbonate (57 mg, 415 µmol, 10 eq) were dissolved in 1.5 ml acetone. To this solution 1,3-dibromopropane (4.3 µl, 42.2 µmol, 1eq) was added, whereafter the solution was refluxed (~23 hours) the reaction was monitored by TLC (SiO_2/Al_2O_3, toluene/ethyl acetate 50:1). The acetone was removed under reduced pressure and then the obtained red crystals were dissolved in 100 ml ethyl acetate. This was washed with the combination of water (7x30 ml) and brine (7x5 ml). The organic layer was dried over $MgSO_4$ thereafter the ethyl acetate was removed under reduced pressure. The obtained red crystals were further purified by column chromatography (silica gel, toluene/ethyl acetate 75:1). As final product, a brown solid (0.64 mg, 1.3 µmol, 3%) was obtained.

MALDI $[M+H]^+$ *m/z*: 286.10 (100%), 287.10 (52%), 288.11 (25%)

UV-vis (λ_{max} nm, $CHCl_3$): 267, 330, 406

*6,6'-dimethoxy-[1,1'-bianthracene]-2,2'-diol used, was previously synthesised by B.M.A. Vial using the same procedure discussed with a reaction time of 23 hours.

Analysis host-guest interactions

First, 20µM stock solutions were prepared of the host (**4**) and each guest (**G1**, **G2**, **G3**) were prepared in chloroform.

UV-vis spectroscopy

A reference absorption spectrum of the host and guests were performed at 10µM concentration (1 ml stock solution + 1 ml chloroform). Next, the absorption spectra of the host with each guest separately, at a 1:1 ratio, were taken, as shown in **table 1**.

Table 1 – Volumes of host and each guest 20µM stock solution used for 1:1 solutions.

	20µM **4**	20µM **G1**	20µM **G2**	20µM **G3**
4 + G1	1 ml	1 ml		
4 + G2	1 ml		1 ml	
4 + G3	1 ml			1 ml

Steady-state fluorescence spectroscopy

From the reference absorption spectra, the absorption wavelengths of the host and guests were used as excitation wavelength for the emission spectra. Fluorescence spectroscopy was done on a diluted solution (10µM) of the host and guests stock solutions before mixed the host and each guest at a 1:1 ratio (**table 1**). **Table 2** shows
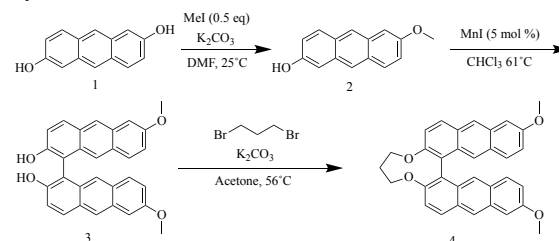
the corresponding wavelength to the solution.

Results and discussion

Synthesis

Scheme 3 – Synthesis route to hook-shaped host **4**. The final product was synthesised via three steps (**scheme 3**). During the methylation of the anthracene-2,6-diol (step 1), large quantities of by-products were formed. The main by-products were a black powder and the dimethylated compound. The black powder was a precipitate formed during the reaction thus filtered off. On the other hand, the dimethylated compound was soluble and therefore removed during the column chromatography. The overall yield was 12%.

For the aromatic coupling (step 2), the reaction time lasted four times longer than intended. This can have caused possible further reaction of the intended coupling product. Small quantity in the mixture of large amount of by-products made it challenging to purify the product. No

Table 2 – Excitation wavelength used for corresponding solution.

	241nm	267nm	269nm	309nm	330nm	406nm
4		X			X	X
G1			X			
G2				X		
G3	X	X				
4 + G1		X	X		X	X
4 + G2		X		X	X	X
4 + G3	X	X			X	X

desired product could be isolated in this trial. However, the product has been already available as an intermediate in another synthesis and therefore it could be spectroscopically characterised.
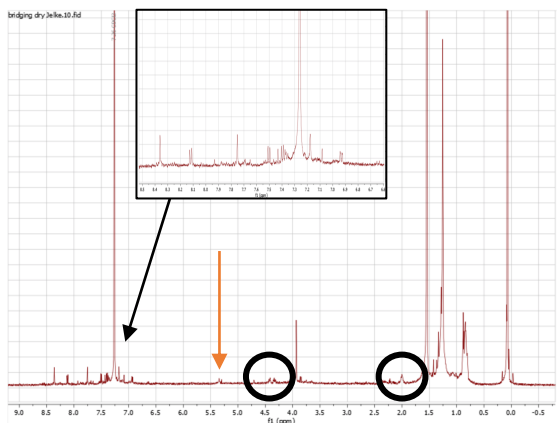

Figure 1 – ^{1}H-NMR spectra of final product in CDCl$_3$.

Due to low solubility, only diluted NMR spectra were acquired of the final product. **Figure 1** shows a diluted ^{1}H-NMR spectrum, the impurities seen in this spectrum are solvent (CDCl$_3$ 7.26 ppm) and grease (0.07 ppm, 0.86 ppm, 1.26 ppm). On the spectrum, it is visible that the signal for the hydroxy proton has disappeared (5.2 ppm) and two new signals (4.3 ppm and 2.0 ppm) have appeared. The new signals correspond to the signals reported by Wongma for the alkyl bridge protons [9]. In addition to the MALDI TOF spectrum obtained of the final product, it can be assumed that the desired compound was formed.

Table 3 – Molar absorption coefficient of each compound.

Compound	Molar absorption coefficient (μM^{-1} cm^{-1})
4	27419
G1	25951
G2	22215
G3	1991

To improve the solubility, a modification to the host structure might be required. One suggestion for a modification is to increase the carbon chain on the methoxy to a branched alkyl chain. Another solution is dissolving the product at higher temperatures such as in toluene at 70˚C, which seemed to be suitable conditions to dissolve **4**.

Spectroscopic analysis

To investigate the possible host-guest interactions, change to absorption and emission were monitored when mixing host and guests. First, the molar absorption coefficients of the host and three guests were determined using the Beer-Lambert law. The coefficients are shown in **table 3**. This shows that the host absorbs light the strongest.

As a reference, the absorption of only host and guests were measured (**figure 2**) at 10μM concentrations. The host absorbs slightly more than **G1** and **G2**, while **G3** absorbs much less. Only **G2** absorbs at a different wavelength. The emission spectra were based on the resulting absorption wavelengths (**figure 3**). The host has two fluorescence bands of which the excitation
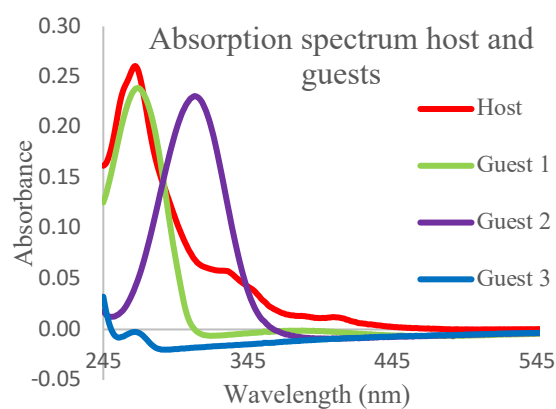

Figure 2 – Absorption spectra of each compound at 10μM concentration. All spectra were recorded in chloroform.
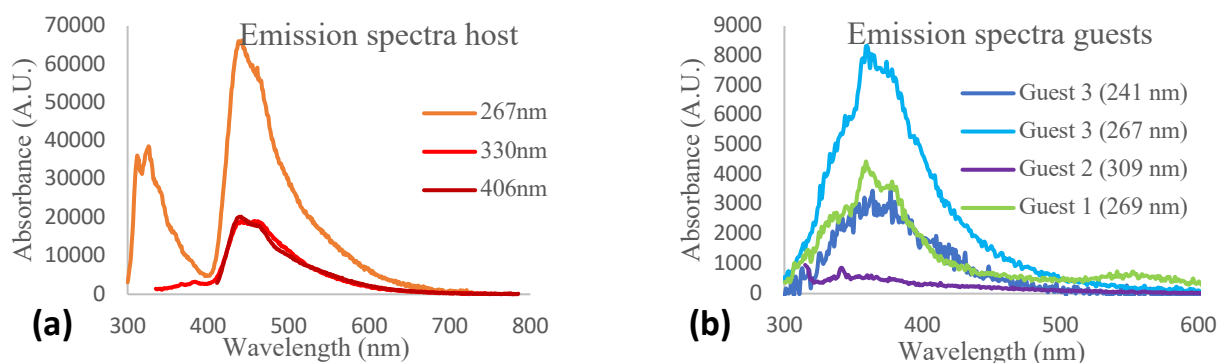
Figure 3 – (a) emission spectra of 10μM host (b) emission spectra of each guest at 10μM. All spectra were recorded in chloroform.

at 267nm gives highest emission intensity at 450nm. On the other hand, the guests have a lower fluorescence intensity, of which **G3** has the highest. Coincidently, this is at the same excitation wavelength (267nm) as the host. Due to the highest emission intensity by the excitation at 267nm, this is the focus for the host-guest combinations.

When the host and guests are combined, both absorption and emission spectra change (**figure 4**). The UV-vis and fluorescence changes were monitored directly after the addition of the 20μM

solutions of host and guest, resulting in 10μM solutions. The absorption increases significantly for all three host-guest combinations while the emission change is different for each guest. For each absorption spectrum, the absorption spectra of individual host and guest were mathematical summed up to give a theoretical reference spectrum, which would be obtained in the absence of any host-guest interactions.

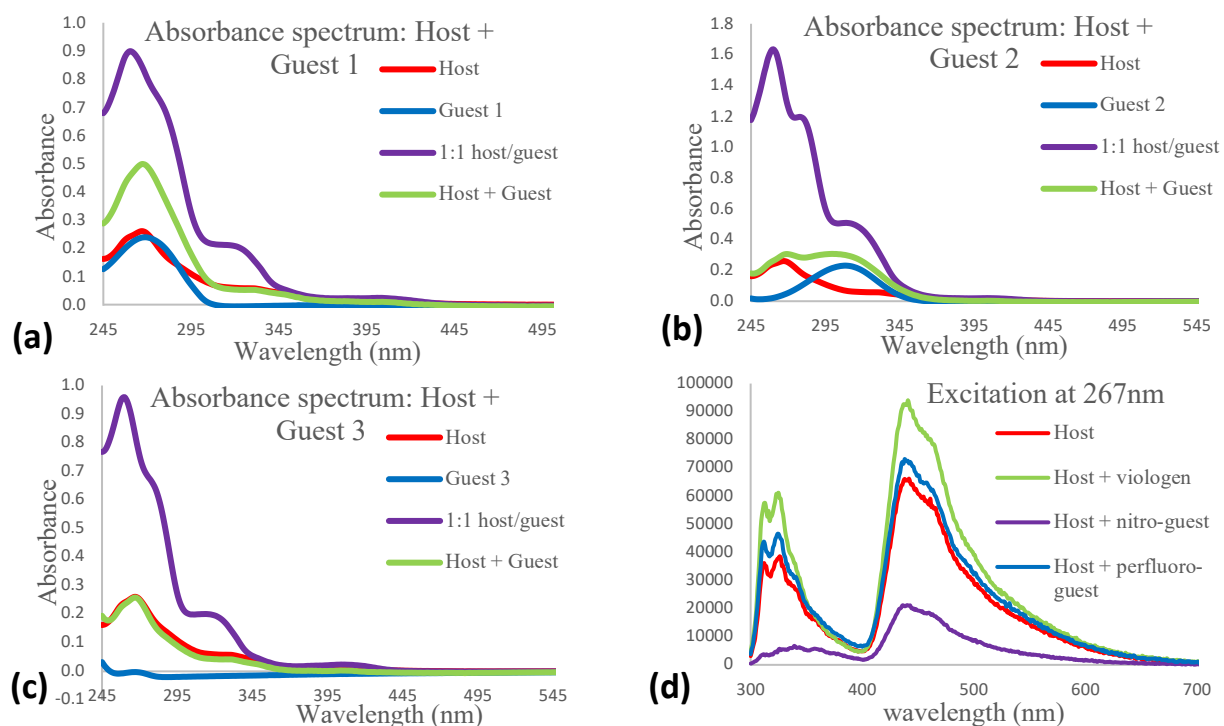In the case of **G1** (viologen analogue guest), the absorption of the **4** + **G1** solution is

Figure 4 – (a) absorption spectra of 10μM host and 10μM **G1** (b) absorption spectra of 10μM host and 10μM **G2** (c) absorption spectra of 10μM host and 10μM **G3** (d) emission spectra of 10μM host with 10μM each guest with 267nm excitation wavelength. All spectra were recorded in chloroform.

almost double that of the mathematical spectrum, this is the lowest of all guests. On the other hand, the fluorescence increased the most with the addition of the viologen guest. The increase in emission is possibility induced by the hindrance in motion of the complexation [10].

G2 (4,4'-dinitrobiphenyl guest) showed opposite changes. This combination increased the absorption by the highest factor (5.3-fold) although the fluorescence seemed to be quenched by the supramolecular interactions. It is possible that the resulting increase reflects a charge transfer process during the formation of supramolecular interactions [10]. Here, inner filter effects were neglected because this guest only absorbs weakly at 267nm.

For **G3** (perflourinated biphenyl guest), the absorption increased by a factor of almost four compared to the mathematical spectrum but only slightly higher than that of the host-viologen combination. This difference is partially caused by absorption of the guest itself. Viologen absorbs at a similar wavelength and intensity as the host while **G3** with the fluorine substituents barely absorbs at all. The emission intensity changes insignificantly for the **4** + **G3** combination, even though this guest is the most fluorescent and lowest absorption.

Conclusion

A hook-shaped molecule was synthesised with the assumption of identity based on MALDI TOF spectrum and low concentration ^{1}H-NMR. The final product has low solubility in organic solvents. Therefore, next steps to be taken is in a precise characterisation by obtaining ^{1}H and ^{13}C-NMR spectra at higher concentrations as well as IR spectrum. Spectroscopic

studies were carried out to study the binding properties of the host with three electron-deficient guests: dioctyl biyridinium; 4,4'-dinitrobiphenyl; and perfluorinated biphenyl. All three guests changed the absorption and emission spectra of the host. This gives a strong indication that the host-guest complex is formed and non-covalent interactions between the host and guest take place. Each guest has a different effect on the fluorescence, but the absorption increases consistently for each host-guest solution compared to individual host and guests. Addition of viologen to the host has the lowest increase in absorbance but highest increase in fluorescence. On the other hand, the nitro-substituted guest has the highest increase in absorbance but quenches the fluorescence. The fluorine-substituted guest showed an intermediate absorption increase with respect to these two guests and had a small effect on the resulting fluorescence.

To better understand the nature of the binding, further research is necessary into the effects of the host/guest ratios. Both bonding behaviour as well as the confirmation of the predicted sandwich structure could offer interesting avenues for research in the future. Techniques such as X-ray spectroscopy might be employed for further insight into the nature of this host-guest complexation. If such sandwich-structure were to be confirmed, its incorporation into a gel or an AFM-force measurement setup could lead to the formation of a synthetic catch bond.

with supramolecular chemistry. I would also like to thank B.M.A. Vial for the daily supervision. His enthusiasm for my research made the project very enjoyable, allowing me to learn new skills which will be useful for the rest of my career in chemistry.

Reference

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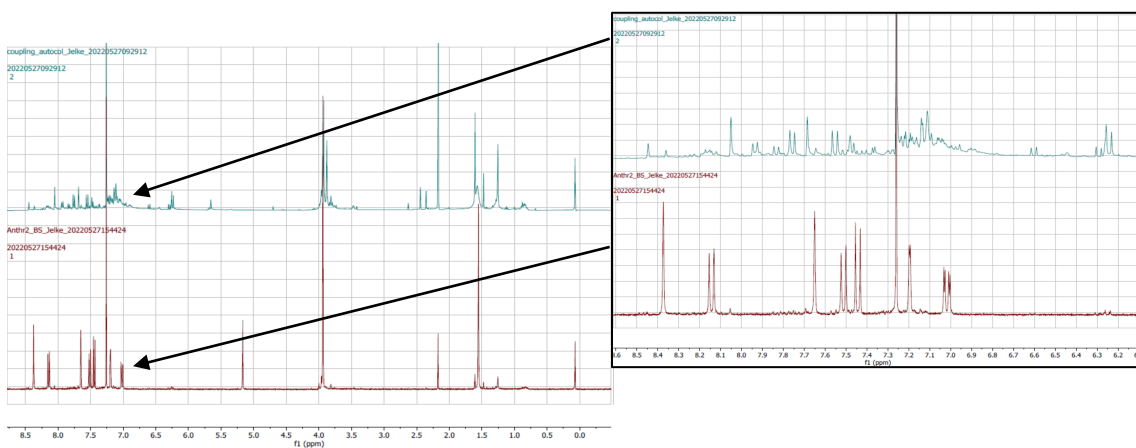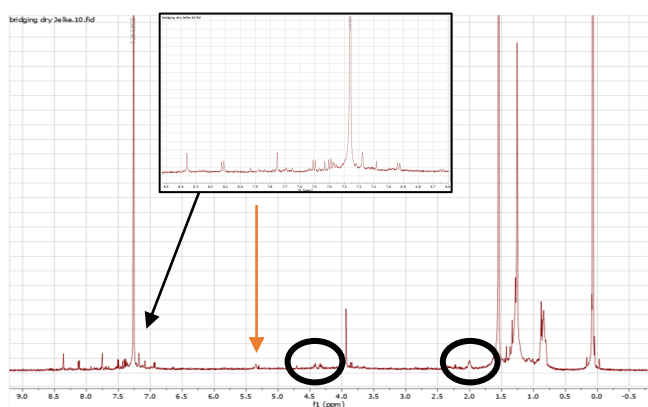
Supplementary information
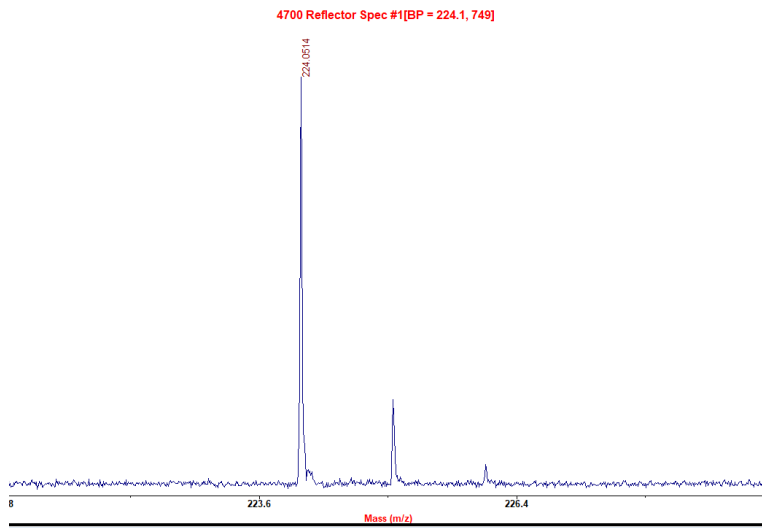
NMR spectra

Step 1:
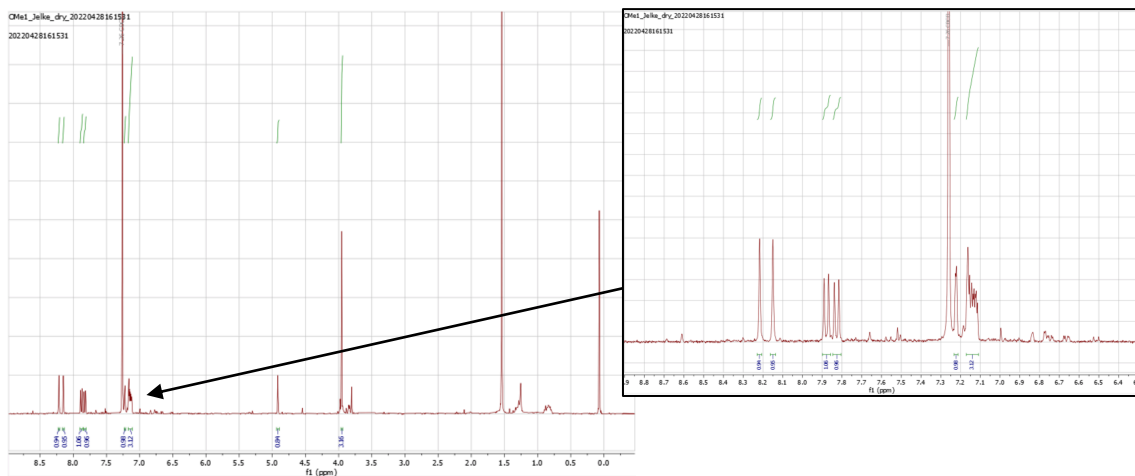
Step 2:

Obtain product in green, desired product in red

Step 3:

MALDI TOF

Step 1 product:
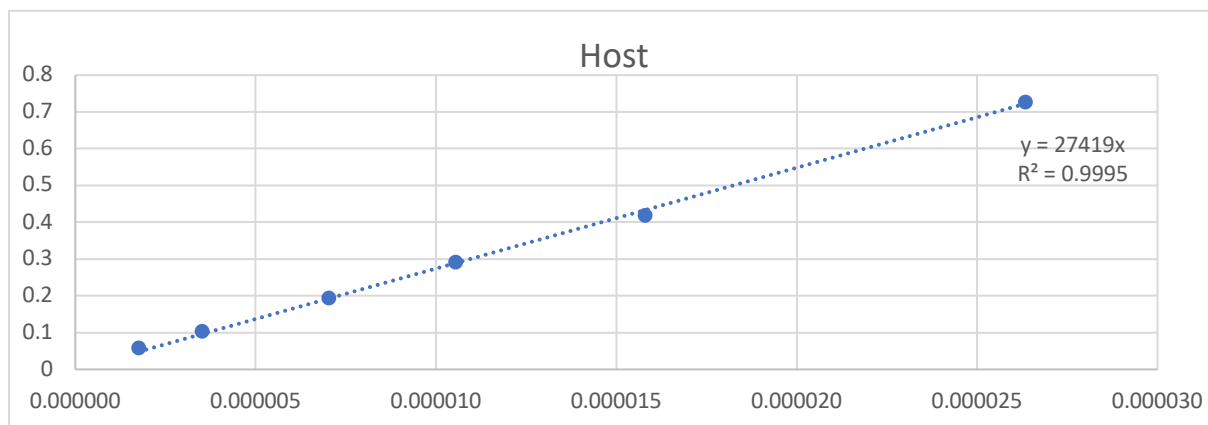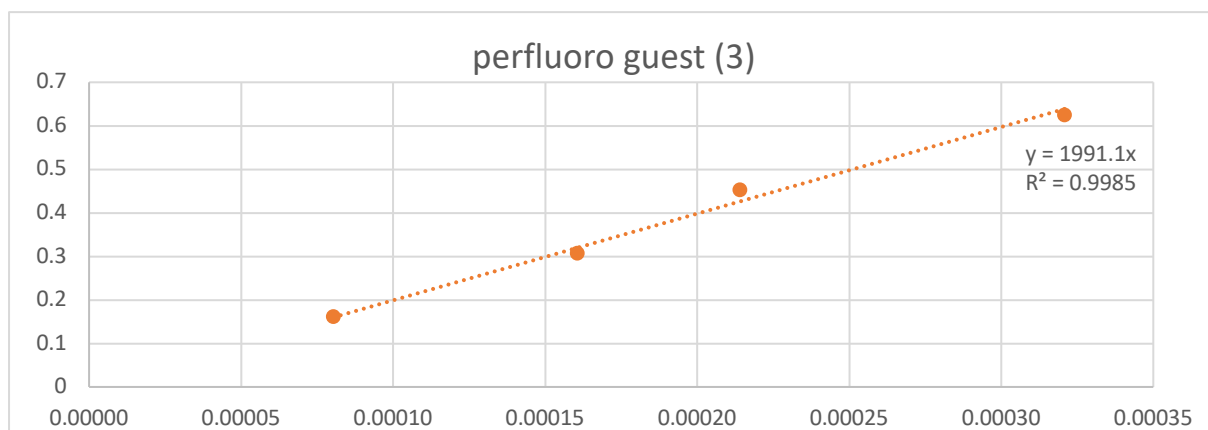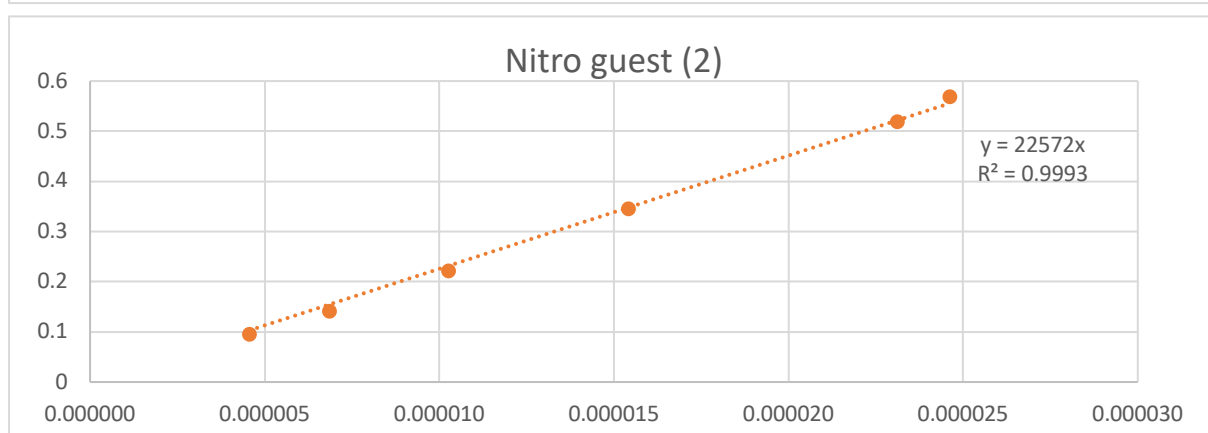
4700 Reflector Spec #1[BP = 224.1, 749]

224.0514

223.6   226.4

Mass (m/z)

Step 3 product:

4700 Reflector Spec #1[BP = 312.3, 602]

486.1024

488.1140

486.4   489.6

Mass (m/z)
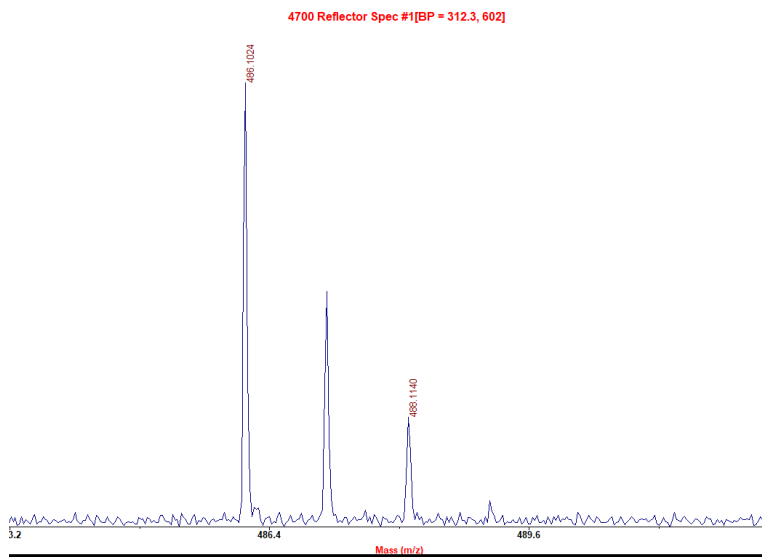
Molar absorption coefficient determination

Viologen guest (1)

$y = 25951x$
$R^2 = 0.9992$

Nitro guest (2)

$y = 22572x$
$R^2 = 0.9993$

perfluoro guest (3)

$y = 1991.1x$
$R^2 = 0.9985$

Host

$y = 27419x$
$R^2 = 0.9995$
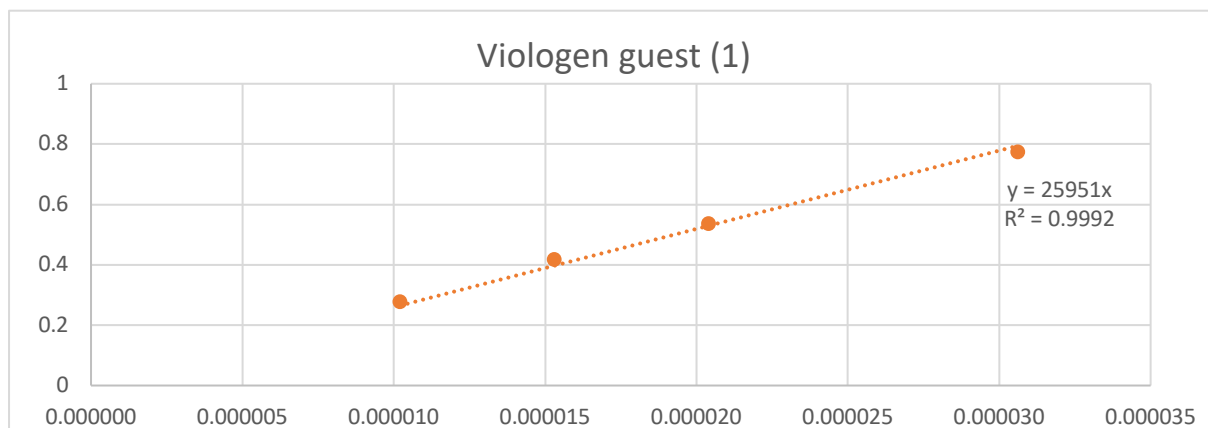
Fluorescence spectra 330nm and 406nm