Synthesis and Characterization of BODIPY-α-Tocopherol: A New Ligand to Explore the Intracellular Transfer of Vitamin E

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ABSTRACT

Since its discovery nearly a century ago, α-tocopherol (vitamin E) research has been mainly focused on its ability to terminate the cycle of lipid peroxidation in membranes. Nitrobenzoxadiazole fluorescent analogues were made previously to study the intracellular transfer of vitamin E in cells. However, these molecules were reportedly susceptible to photobleaching while under illumination for transfer assays and microscopy.

Here is reported the synthesis of a series of fluorescent analogues of vitamin E incorporating the more robust dipyrromethene boron difluoride fluorophore (BDP-α-Tocs; λ_{ex} = 507 nm, λ_{em} = 511 nm). C8-BDP-α-Toc **42c**, having an eight-carbon chain between the chromanol and fluorophore, was shown to bind specifically to α-tocopherol transfer protein with a dissociation constant of approximately 100 nM. Another fluorescent analogue of vitamin E with a thienyl derivative of BODIPY that is excited and fluoresces at longer wavelengths (λ_{ex} = 561 nm, λ_{em} = 570 nm) is in development.

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LIST OF ABBREVIATIONS

2,2'-py_2S_2	2,2'-dipyridyl disulfide
α-toc	α-tocopherol
α-TTP	α-tocopherol transfer protein
AO	anthroyloxy
Boc_2O	di-*tert*-butyl dicarbonate
BODIPY (or **BDP**)	dipyrromethene boron difluoride
CEHC	carboxyethyl hydroxychroman
DAN	dansyl
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCE	1,2-dichloroethane
DCM	dichloromethane
DMAP	4-(dimethylamino)pyridine
DMF	*N*,*N*-dimethylformamide
DIPEA	*N*,*N*-diisopropylethylamine
DOPC	dioleoyl phosphatidylcholine
ESI	electrospray ionization
FAB	fast atom bombardment
HPLC	high performance liquid chromatography
LCMS	liquid chromatography-mass spectrometry
LHMDS	lithium bis(trimethylsilyl)amide
m/z	mass/charge ratio
NBD	nitrobenzoxadiazole
NHC	*N*-heterocyclic carbene
NMA	*N*-methylanthranilamide
NMR	nuclear magnetic resonance
nOe	nuclear Overhauser effect
PAD	potassium azodicarboxylate
PC	phosphatidylcholine
pHPLC	preparative HPLC
PUFA	polyunsaturated fatty acid
SCP-2	sterol carrier protein-2
SPhos	2-dicyclohexyphosphino-2',6'-dimethoxybiphenyl
TBAF	tetrabutylammonium fluoride
TBDP	thienylBODIPY
TEA	triethylamine
THF	tetrahydrofuran
TLC	thin layer chromatography
VLDL	very-low-density lipoprotein

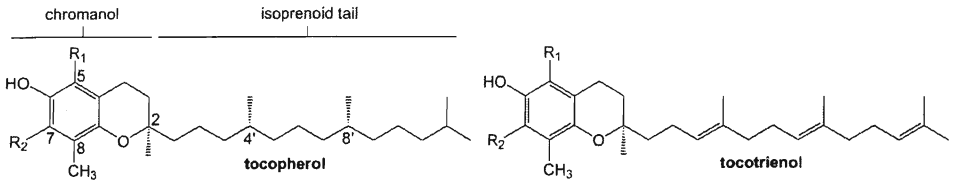
1 INTRODUCTION

1.1 Vitamin E

1.1.1 Discovery and Structure

Herbert Evans and Katherine Bishop discovered vitamin E in 1922 while studying the ability for female mice to carry to term a normal pregnancy when raised on controlled diets of casein, cornstarch, and lard.[1] They concluded that mice lacking this vitamin are able to conceive, but the fetus eventually dies and is resorbed by the mother.[1] The formal name that was given to this compound was tocopherol, which is derived from *tocos* (Greek: offspring) and *phero* (Greek: to bring forth). It is also called 'vitamin E' because of its discovery shortly after vitamin D.[2]

During its structural elucidation in 1938, it was revealed that there are a total of eight molecules belonging to the vitamin E family (**Figure 1**). Vitamin E contains a chromanol head and an isoprenoid tail that is thirteen carbons in length.[3,4] Different degrees and positions of methylation on the chromanol, as well as unsaturation along the tail, result in these different molecules (vitamers), making 'vitamin E' rather more a general term that describes a family of structurally similar molecules. Vitamin E is split into two main categories: (1) the tocopherols that contain fully saturated isoprenoid tails and (2) the tocotrienols that contain geranylgeranyl phosphate-derived tails with unsaturation at positions 3′, 7′ and 11′.[5] Both categories contain a stereocentre at position 2, but the tocopherols contain two more at positions 4′ and 8′. The form that is most readily incorporated and retained in animals is (*RRR*)-α-tocopherol.[6]



R_1	R_2	*Tocopherol*	*Tocotrienol*
CH_3	CH_3	α-tocopherol	α-tocotrienol
CH_3	H	β-tocopherol	β-tocotrienol
H	CH_3	γ-tocopherol	γ-tocotrienol
H	H	δ-tocopherol	δ-tocotrienol

Figure 1. Structures and names of the eight vitamers of vitamin E

1.1.2 Dietary Intake

Humans cannot synthesize vitamin E; instead it must be acquired by diet. The suggested daily intake of vitamin E (α-tocopherol equivalents) is 15-30 milligrams.[6] Vitamin E is synthesized exclusively by plants and can be found in leafy vegetables, cereals and oils.[3] The distribution of vitamers in these sources is not equal, however. α-Tocopherol predominates in safflower and sunflower oils whereas γ-tocopherol is mostly prominent in corn, soybean and canola oils.[6,7] It has been found that the intake of γ-tocopherol is two to four times greater than α-tocopherol in the North American diet because foods are often cooked or fried with oils containing large amounts of γ-tocopherol. This is not true for those living in Europe because these foods are cooked with oil that are richer in α-tocopherol.[7]

1.1.3 Biological Transport

After vitamin E has been consumed, tocopherols are passively absorbed across the intestinal membrane and packaged into lipid constructs or complexes, called chylomicrons, that are composed of triglycerides and phospholipids. These chylomicrons are then deposited into the intestinal lymph fluid where they eventually enter the blood stream.[8] Here, the chylomicrons are broken up into chylomicron remnants via lipoprotein

lipase and are endocytotically taken up into liver cells via remnant-receptors.[8,9] The remnants are then processed into very-low-density lipoproteins (VLDLs) within these hepatocytes (**Figure 2**).[9]

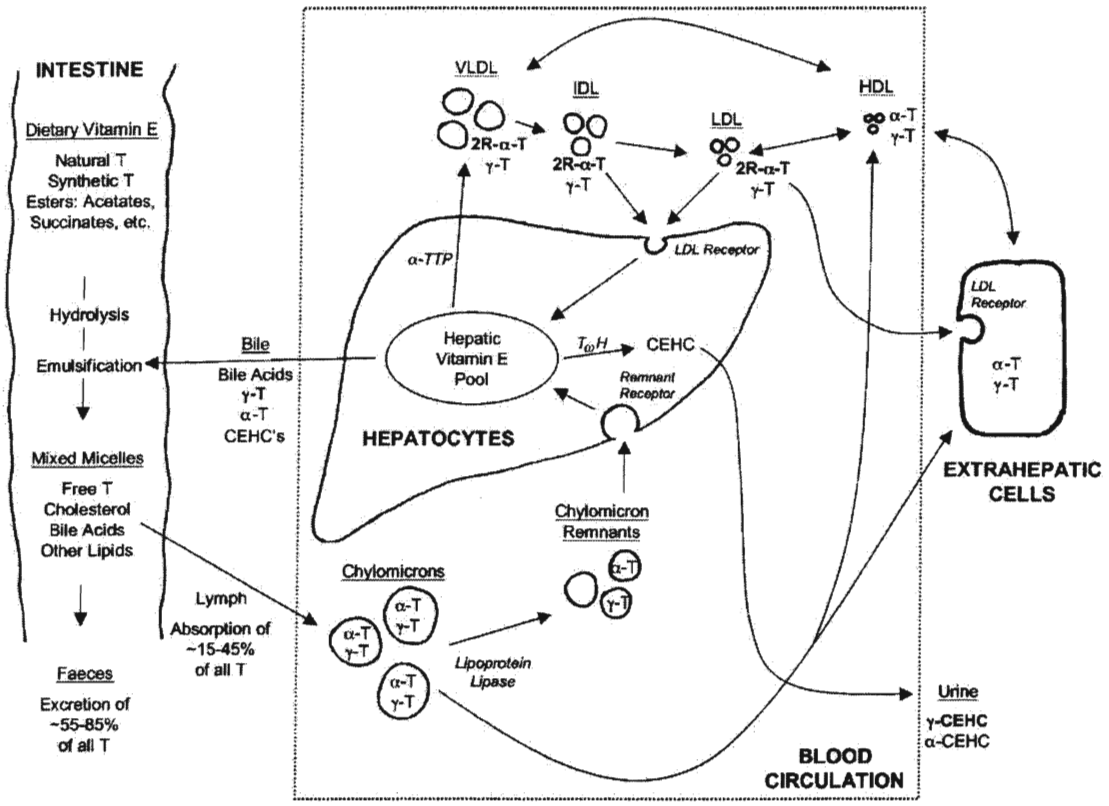


Figure 2. Molecular pathway of vitamin E *in vivo*[9]

As mentioned previously, (*RRR*)-α-tocopherol is the vitamer that is most efficiently incorporated into the body and used in tissues.[6] This evidence at first may seem confusing since the North American diet is dominated by γ-tocopherol intake. Traber and Kayden have shown that the liver differentiates the vitamers and there is selectivity for α-tocopherol deposition in tissues.[10] They demonstrated this by giving human volunteers a single dose of all-*rac*-α-tocopherol acetate or equivalent amounts of all-*rac*-α-tocopherol and (*RRR*)-γ-tocopherol.[10] Samples of bile and plasma lipoproteins were taken and analyzed for tocopherol content. High performance liquid

chromatography (HPLC) and fluorescence detection were used to quantify the relative amounts of α- and γ-tocopherol. Two days after administration of these vitamin E pills, there was a drastic decrease in the amount of γ-tocopherol in the blood plasma in comparison to α-tocopherol.[10] The opposite was observed when tocopherol quantities were determined from the bile samples retrieved from post-gall bladder surgery.[10] Samples of bile were shown to have greater amounts of γ-tocopherol in comparison to α-tocopherol.[10] This evidence suggested that although both forms of tocopherol are absorbed into the liver, here they are differentiated. α-Tocopherol is retained whereas non-α-tocopherols are excreted.

1.1.4 α-Tocopherol Transfer Protein and Substrate Specificity

Figure 2 shows the excretory pathway of β-, γ-, and δ-tocopherol after leaving the liver. They are either excreted into the bile or degraded into carboxyethyl hydroxychroman (CEHC) metabolites that are removed from the body through urine.[9] A transfer protein found in liver cells selectively retains α-tocopherol. This protein is called α-tocopherol transfer protein (α-TTP) and it assists in the secretion of α-tocopherol into VLDLs.[9]

α-TTP binds to α-tocopherol selectively over β-, γ-, and δ-tocopherol and to the 2-(*R*) isomer over the 2-(*S*) isomer.[11,12] Deuterated samples of (*RRR*)-α-tocopherol and (*SRR*)-α-tocopherol have demonstrated this stereospecificity of α-TTP.[10] Twenty-four hours after patients were administered either one of these compounds, a greater amount of the (*RRR*)-stereoisomer was present in the blood plasma, indicating a preference of having position 2 as the (*R*)-stereocentre.[13]

The substrate binding site of α-TTP is structured in such a way to accommodate a fully methylated chromanol and an (*R*)-stereocentre at position 2 (**Figure 3**).[8] These conditions make it optimal for binding and stabilization to occur with (*RRR*)-α-tocopherol.[8] Studies to determine the interactions between α-TTP and the other vitamers have shown a decrease in affinities because fewer protein-substrate interactions occur within the binding site. (*RRR*)-γ-Tocopherol has a 10-fold decrease in affinity since it lacks a methyl group at position 5 whereas (*RRR*)-δ-tocopherol binds even more poorly (50-fold less) because it lacks two methyl groups at positions 5 and 7.[8]

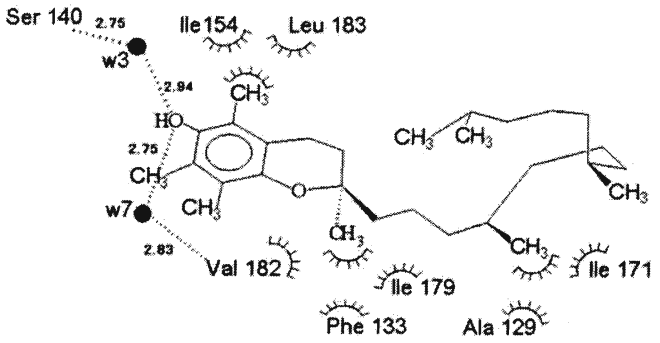


Figure 3. Atom-residue interaction between α-TTP and α-tocopherol (internal water molecules as black balls)[14]

The two remaining stereocentres of tocopherol (i.e. positions 4′ and 8′) do not play an important role in binding affinity mainly because of the great flexibility of the isoprenoid tail.[14] Crystal structure analysis shows that the phytyl tail of α-tocopherol is curved when bound to α-TTP.[14]

1.1.5 Antioxidant Properties

The main biological function of vitamin E is to inhibit the peroxidation of membrane phospholipids (**Figure 4**).[2] Although the superoxide anion ($O_2^{\bullet -}$) has the

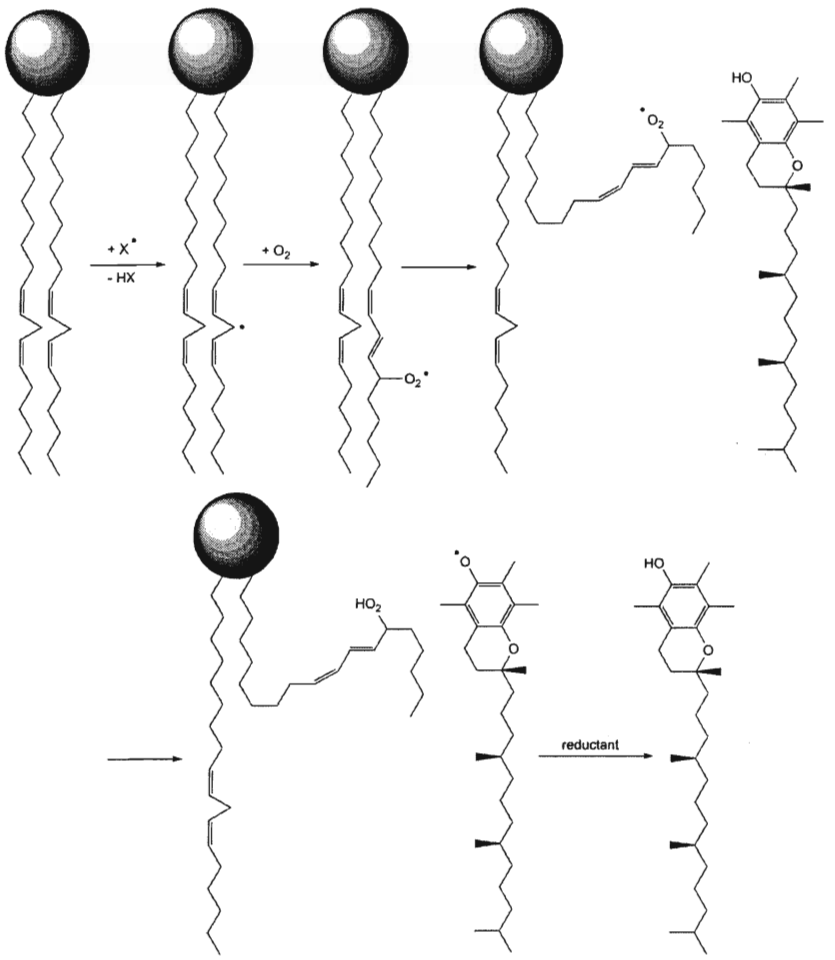


Figure 4. Peroxyl radical formation and termination in lipid membrane[15]

strength to oxidize lipids, it is too polar to enter the bilayer. Its protonated form, the perhydroxyl radical ($^{\bullet}$OOH), does have the ability to react with the lipids within the membrane, however.[16,17]

Another reactive oxygen species that causes lipid peroxidation is the hydroxyl radical ($^{\bullet}$OH).[14] Having a reduction potential of over 2300 millivolts, this species oxidizes anything that is susceptible to hydrogen abstraction. These radicals are usually generated from the homolytic cleavage of hydrogen peroxide (H_2O_2) by metal ions (e.g. Fe^{2+}).[15] Despite being considered the most important reactive oxygen species in the

initiation of lipid peroxidation, these radicals are too unstable and dimerize within nanoseconds.[16]

Peroxynitrite ($ONOO^-$) is another known oxidant that initiates lipid peroxidation.[18] It is spontaneously formed when nitric oxide (NO) and superoxide are within a few cell diameters of each other.[19] As the acid (i.e. ONOOH), it is homolytically cleaved, supplying membrane phospholipids with the hydroxyl ($^{\bullet}OH$) and nitrogen dioxide ($^{\bullet}NO_2$) radicals.[20]

The first step of lipid peroxidation, which is also the rate-limiting step, is the formation of the carbon-centred radical.[3,15] Polyunsaturated fatty acids (PUFAs) possess a characteristic 1,4-pentadiene system that is susceptible to hydrogen atom abstraction.[3,15] The pentadienyl radical rearranges to form the more stable conjugated diene and reacts readily with an oxygen molecule to form a peroxyl radical ($\Delta E^{\circ\prime}$ = 1000 mV), an oxidant that is more potent than the simple alkyl radical ($\Delta E^{\circ\prime}$ = 600 mV).[15]

In the presence of tocopherol, the peroxyl radical comes in close proximity so that the molecules' electron clouds overlap and the transfer of the phenolic hydrogen atom to the lipid is allowed, generating a lipid hydroperoxide and a tocopheroxyl radical.[3] The propagation of the radical onto α-tocopherol is favoured because the tocopheroxyl radical is delocalized on the aromatic ring (**Figure 5**).[3]

The tocopheroxyl radical can be terminated in either one of two general ways. The first way is that it can couple to another peroxyl radical ($^{\bullet}OOL$) to generate a non-radical adduct.[3] If termination occurs by this route, then each molecule of tocopherol has the capability of reducing two lipid peroxyl radicals, but is not regenerated.[3] The other way that the tocopheroxyl radical is terminated is by water-soluble reductants, such as

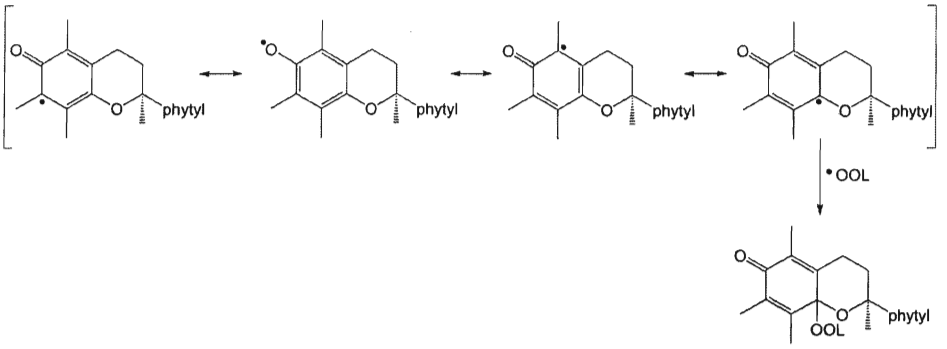


Figure 5. Resonance forms of α-tocopheroxyl radical and its termination by adduct formation with peroxyl radical[3]

ascorbate (vitamin C), glutathione, or thiols, as illustrated in **Figure 4.**[6] A molecule of tocopherol is regenerated and can continue to inhibit lipid peroxidation.[6] Without the termination of lipid peroxide species, the membrane would become damaged and a series of other biological responses soon take effect. These responses include the induction of cell apoptosis,[21] atherosclerosis,[22] and neurodegenerative diseases.[23]

1.2 The Dipyrromethene*boron* Difluoride Fluorophore

1.2.1 Discovery and Core Structure

The first reported synthesis of a *dipy*rromethene*bo*ron difluoride (BODIPY) entity was by Alfred Treibs and Franz-Heinrich Kreuzer in 1968.[24] It was not until two decades later, however, that the application of BODIPY to biology was realized. Since its discovery, the fluorophore has been introduced into probes for use in both aqueous media[25] and in cell membranes.[26] The main commercial supplier for BODIPY labels is Invitrogen/Molecular Probes, Inc. (Burlington, ON). The available quantities are not realistic for use as reagents in bioorganic synthesis, however.[27] Instead, chemists often resort to building their own fluorophores.

The conventional name for BODIPY is dipyrromethene*boron* difluoride but it has also been given the IUPAC name 4-bora-3a,4a-diaza-*s*-indacene, based on the indacene

skeleton.[28] For the purpose of this paper, it will simply be referred to as BODIPY or the further abbreviated BDP. The numbering system of BODIPY and its dipyrromethene synthetic precursor is written in **Figure 6** (Notice how BODIPY and dipyrromethene do not follow the same numbering system).

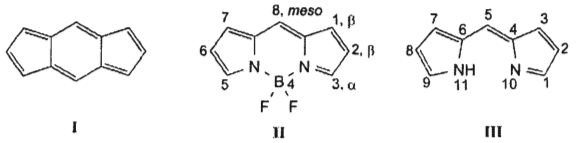


Figure 6. (**I**) Indacene; (**II**) BODIPY core; (**III**) dipyrromethene

1.2.2 Fundamental Properties

BODIPY is widely used in the research of biomolecules because of its high photostability, large extinction coefficient (90 000 $M^{-1}cm^{-1}$), high quantum yield (0.9), and negligible triplet state formation.[28,29] It is also relatively compact in size and low in polarity (electronically neutral).[30] The lipophilicity of BODIPY readily allows entry into cell membranes and other greasy environments such as plasma lipoproteins.[31,32] Another key feature of BODIPY is its large spectral overlap as a result of a small Stokes shift.[25,33] This large overlap brings forth a challenge as light scattering can interfere with the signal.[34]

At low concentrations, fatty acid analogues possessing the BODIPY unit retain key properties such as green fluorescence (~ 500 nm), high quantum yield, and insensitivity towards environment.[35] The typical green fluorescence is a result of a strong $S_0 \rightarrow S_1$ transition. This dye also appears to fluoresce at a shorter wavelength (~ 375 nm) because of a $S_0 \rightarrow S_2$ transition that is twenty times weaker in intensity.[30] At higher concentrations, BODIPY dyes have the ability to form dimers (or 'excimers') in membranes. These excimers are spectrally different from the parent dye, emitting

anywhere from green to red wavelengths.[36] For example, the phosphatidylcholine probe C_4-BDP-C_9-PC begins to show increasing fluorescence depolarization and self-quenching at equivalents 1:500 mol:mol or greater with respect to dioleoyl phosphatidylcholine (DOPC).[35] Ceramide and PC conjugates of BODIPY have both shown shifts from green to red emission, likely due to this excimer formation.[35,37]

1.2.3 Previous Applications of BODIPY to Biology

Radiolabelled and fluorescent analogues of lipids have been used extensively to study the behaviour of natural compounds. BODIPY has been incorporated into studies involving nucleic acids and proteins, as well as various lipids (e.g. fatty acids,[38] triglycerides,[39] phospholipids,[40,41] and cholesterol[42]).

Fluorescent analogues of lactosylceramide **1** (**Figure 7**) have enabled chemists to monitor how this glycosphingolipid undergoes endocytosis within different cell types by fluorescence microscopy.[29] The linker separating the glycosphingolipid from BODIPY is an all-methylene chain ($[CH_2]_n$) that does not pose any unwanted polarity, ideal for lipid probes.[29] At low temperatures, the determination of its location becomes apparent immediately after endocytosis.[29] Analogues **2** and **3** of sphingosine (**Figure 7**), a metabolite of ceramide, represent another example of BODIPY-sphingolipid analogues that are useful for biophysical and biochemical analyses.[43]

For the analysis of the distribution and transportation of cholesterol in membranes, several fluorescent analogues have been prepared but most were not highly fluorescent, were photolabile, or did bear a strong structural resemblance to cholesterol.[44] Tritiated and carbon-14-labelled sterols have been implemented in studies in the past but trends in lipid research are now moving away from radiolabelling and instead towards

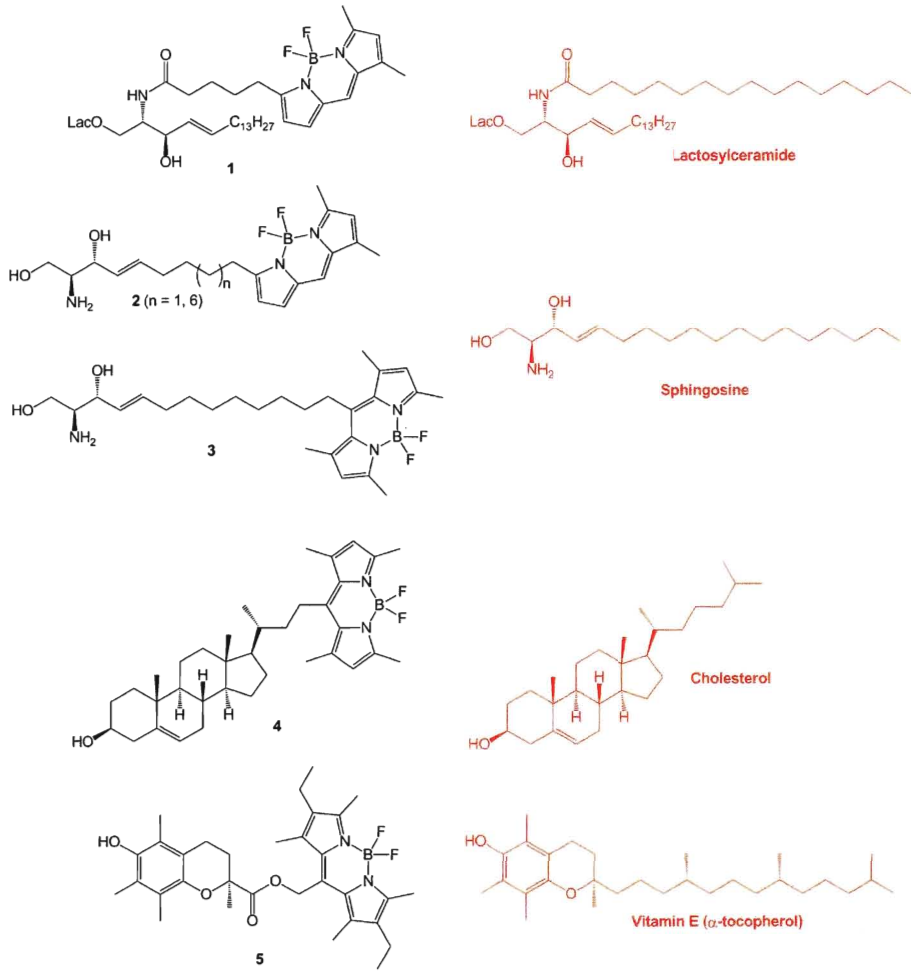


Figure 7. Recent examples of lipid analogues possessing BODIPY

fluorescence. Of the fluorescent analogues of cholesterol synthesized before a couple of years ago, none have displayed a similar behaviour to cholesterol in membranes.[45] Recently, several fluorescent analogues of cholesterol bearing the BODIPY group were synthesized.[42,46] Compound **4** (**Figure 7**) was found to mimic the properties of cholesterol whereas analogues that contained polar groups such as an ester linkage in the linker between the sterol and the fluorophore did not serve as faithful mimics of cholesterol.[44,47,48]

An emerging role of BODIPY is its use in detecting oxidative stress and identifying antioxidants that exist in membranes (e.g. vitamin E analogue **5**; **Figure 7**). As these antioxidants inhibit free-radical chain peroxidation of PUFAs, fluorescence activity may increase or decrease depending on the dye that is incorporated. BODIPY has been shown to undergo fluorescence quenching both chemically[49] and photophysically[50,51] in the presence of radicals. This property enables the use of these probes to measure lipid peroxidation both *in vitro* and *in vivo*. The ability to suppress in fluorescence was applied more recently in the study of vitamin E's antioxidant properties.[52]

1.2.4 Synthesis of the Dipyrromethene Skeleton

In the presence of acids, pyrrole goes through an Ehrlich-type condensation with pyrrole-2-carboxaldehyde to form a dipyrromethene (**Figure 8**).[43] This type of reaction may also be referred to as the MacDonald coupling, a term commonly used amongst porphyrin chemists.[53,54] Dipyrromethenes can be found as molecular subunits in nature. They are rings B and C that form biliverdin, a bile pigment, and two dipyrromethenes form one porphyrin, the large metal-chelating macrocycle that is found in chloroplasts and red blood cells.[55] Depending on the substituents on the ring, these pyrrole-2-carboxaldehydes may be available commercially or they can be easily synthesized by the Vilsmeier-Haack reaction.[56]

During this condensation, pyrrole has enough nucleophilicity to attack the aldehyde in the presence of an acid, losing a molecule of water.[55] These two-component reactions are done with equimolar amounts of each pyrrole. No step for oxidation of the aromatic system is needed since the condensation directly forms the dipyrromethene.

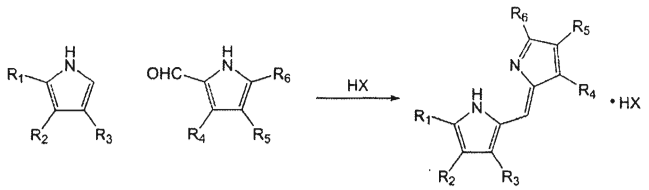


Figure 8. General two-component synthetic route to dipyrromethenes

This two-component route offers a convenient method to produce BODIPY molecules that are unsymmetrical and/or unsubstituted at the *meso*-position.[28,57]

Suitable acids for this condensation include hydrogen halides (e.g. HBr), metal salts used typically for Friedel-Crafts reactions (e.g. $ZnCl_2$), and non-metallic Lewis acids (e.g. $POCl_3$). The dipyrromethenium ion that is produced is combined with the anion of whatever acid was used (i.e. Br^-, Cl^-, $PO_2Cl_2^-$).[58] Boron trifluoride diethyl etherate can act as a Lewis acid to assist in this condensation in addition to forming the BF_2 bridge. This would be a very convenient one-pot synthesis of BODIPY directly from pyrrole.[58]

A limitation to this condensation is that the pyrrole nucleophile must not react in excess since the dipyrromethenium salt formed is much more electrophilic than the pyrrole-2-carboxaldehyde precursor.[55] Most of the time, these salts tend to precipitate out of solution so the formation of these *tri*pyrromethenes is limited.

The dipyrromethenium salts may be treated with bases (e.g. $Ca(OH)_2$ in pentane) to liberate the neutral dipyrromethene. These free-bases are less stable than their protonated forms, especially with minimal substituents (i.e. less than three alkyl groups), and may still be susceptible towards nucleophilic attack.[33,55] For example, an unsubstituted dipyrromethene molecule has been synthesized but decomposition is reported to occur at temperatures as low as –40 °C.[55] The stability of the dipyrromethenium salts increases as the number of substituents along the backbone

increases.[57] Despite this great instability, several groups have recently prepared the BODIPY core without any substituents.[59,60]

The three-component approach towards the synthesis of BODIPY produces dipyrrometh*ane* as an intermediate, as opposed to the further oxidized dipyrromethene. Dipyrromethanes are much more stable species than dipyrromethenes as long as they are in purified form. Dipyrromethanes are stored at 0 °C in the absence of light without any reported decomposition.[61] In order to convert dipyrromethanes into the required dipyrromethenes, an oxidant such as 2,3-dichloro-5,6-dicyanoquinone (DDQ) or *p*-chloranil is required, the latter of which is preferred because of its milder reactivity and easier control.[53]

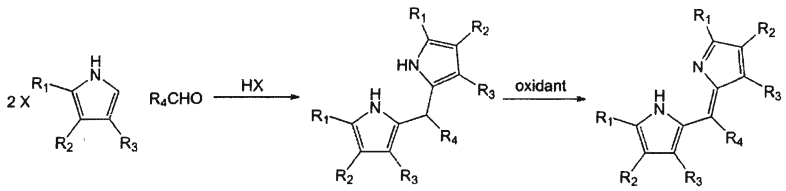


Figure 9. General three-component synthetic route to dipyrromethenes

Three separate molecules are used to form dipyrromethane by this three-component route in a one-pot reaction (**Figure 9**).[42] The ability to incorporate a wide variety of aldehydes allows a quick and simple way of synthesizing symmetrical BODIPY dyes with great diversity at the *meso*-position. Anhydrides may be used in place of aldehydes with the advantage that a carboxylic acid is generated, which can be used for further conjugation.[57,62] The yields from this three-component route are generally much lower than the two-component alternative because there is a greater chance of by-product formation.[42,63]

During the synthesis of dipyrromethanes, thin layer chromatography (TLC) often shows a small amount (less than 5%) of material tailing from the product spot and a third component that remains on the baseline. ^{1}H NMR spectra indicated that the tailing spot is the tripyrromethane formed during condensation. Tripyrromethanes appear to be less stable than dipyrromethanes. They begin as a white solid but decompose to a black substance in less than a day at room temperature.[61]

As opposed to using aldehydes or anhydrides, acyl chlorides tend to give higher yields since it produces the dipyrromethene directly without any required oxidation.[64] Electron-withdrawing groups on acyl chlorides help increase the rate of the condensation.[63] Depending on the acyl chloride chosen, this route continues to allow the synthesis of many different *meso*-substituted BODIPY derivatives.[65,66]

Oligomers and porphyrinogens may form instead of dipyrromethanes if the pyrrole reactants lack appropriate substitution at the α-position (**Figure 10**). Regioselectivity also becomes an issue since both α-positions on the pyrrole are reactive.[28] This can also be applied to inappropriately substituted dipyrromethenes following the two-component route. At these extremely reactive positions, there is

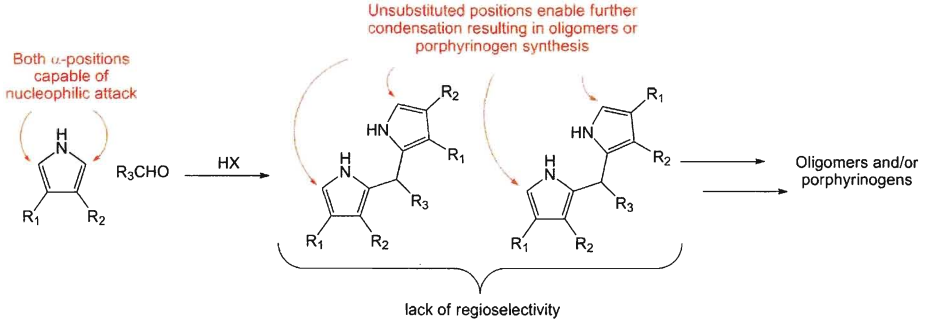


Figure 10. Oligomerization and porphyrinogen synthesis from lack of appropriate substitution of pyrrole reactants

nothing preventing an electrophilic attack.[57] In order to exclusively obtain dipyrromethanes, the pyrrole should be in excess (40 > 1) and can therefore act as solvent as well during the condensation.[61]

The extreme difficulty to form non-α-substituted BODIPY molecules has been noted in literature (**Figure 11**).[57] To maintain the molecular linearity of a probe incorporating BODIPY, substituents at positions 2 and 6 are not possible without at least methyl groups at the α-positions as placeholders. However, probes substituted at positions 3 and 5 suffer from the molecular bend that is created at the point where the fluorophore and the rest of the molecule are joined.

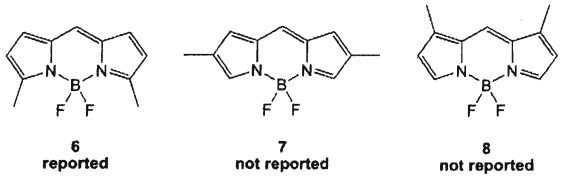


Figure 11. Synthetic limitations of BODIPY[57]

1.2.5 Conformational Analysis of Dipyrromethenes

In order to complete the formation of the BODIPY fluorophore, it is important to assess whether the dipyrromethene backbone is in the (*E*)- or (*Z*)-conformation. An earlier study of the conformation of dipyrromethenes was done in 1975.[67] They specifically looked at the possibility of both (*E*)- and (*Z*)-configurations of the exocyclic double bond on 3,4-dimethyl-5-(1*H*)-2,2′-pyrromethenone (**Figure 12**). These hydroxypyrromethenes (i.e. the tautomer of the pyrromethenone) are biologically important because they are the building blocks to many bile pigments, such as bilirubin.[68] To determine the configuration of the double bond, lanthanide shift and nuclear Overhauser effect (nOe) experiments were conducted. Falk et al. reported that both (*E*)-

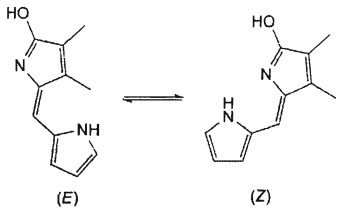


Figure 12. (*Z*)- and (*E*)-configurations of 3,4-dimethyl-5-(1*H*)-2,2′-pyrromethenone

and (*Z*)-configurations of the hydroxypyrromethenes were present.[67] Even though the hydroxypyrromethene can be viewed as a derivative of dipyrromethene, there is enough difference in electronics and structure that makes them poor candidates for comparison with other dipyrromethenes.[55]

Another study on the conformation of dipyrromethenes was published in 1977.[55] As the free-base, it was determined that dipyrromethenes must possess a hydrogen bridge in the ground state that is shared between both pyrrole nitrogens.[69] The question of whether or not this bridge directs the configuration of the exocyclic double bond was explored using *N*-CH_3, furyl, and thienyl derivatives, which would not display such an interaction.[55] The configuration of the double bond in 3′,5′-dimethylpyrromethene (*N*-H analogue) and *N*-3′,5′-trimethylpyrromethene (*N*-CH_3 analogue) was studied by nOe experiments. It was found that even without this hydrogen bridge, the *N*-CH_3 analogue preferred to form in the (*Z*)-conformation. Only under photochemical conditions did *E-Z* isomerization occur (**Figure 13**).[55] (*E*)-Dipyrromethenes make up the cyanine dyes, which have strong fluorescence with emission at 600 nm and an extinction coefficient value of 100 000 $M^{-1}cm^{-1}$ or greater.[70] The isomerization of cyanine dyes under photoexcitation would make these dyes unsuitable choices in the synthesis of fluorescent probes.[30]

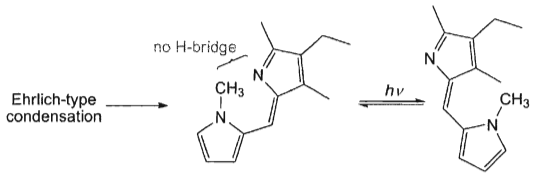


Figure 13. Photochemical *E*-*Z* isomerization of *N*-CH_3 dipyrromethenes

With the *N*-H analogue where the hydrogen bridge is present, the exocyclic double bond remained in the (*Z*)-configuration, even upon attempted photoinduction (**Figure 14**).[55] BODIPY can be considered as stabilized cyanine dyes with the BF_2 bridge locking the backbone in this *syn* conformation. Without the ability of the dipyrromethene backbone showing selectivity towards this *cis* formation, non-reactive by-products would form and complicate the synthesis of BODIPY.

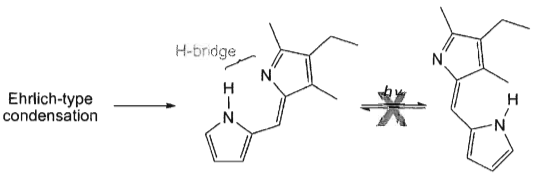


Figure 14. Hydrogen bridge stabilization of *N*-H dipyrromethenes

1.2.6 Synthesis of BODIPY: Addition of the BF_2 Bridge

Dipyrromethenes can be both protonated (e.g. during condensation) as the stable salt and deprotonated as the anionic ligand that chelates metals. Different alkali earth metal complexes have been shown to be too unstable and tend to form bis(dipyrromethene) complexes, except for the monovalent BF_2 pseudometal.[33,71,72] The BF_2 bridge can be added to the dipyrromethene backbone in the presence of an organic base such as triethylamine (TEA) or *N*,*N*-diisopropylethylamine (DIPEA) (**Figure 15**).[33]

Once the BF_2 bridge has been formed, the molecule (i.e. BODIPY) becomes much more stable than the dipyrromethene precursor, showing no observable sensitivity towards light unlike so many other fluorophores.[33] BODIPY is stable to silica gel

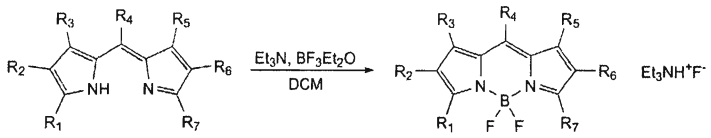


Figure 15. Last step in synthesis of BODIPY

chromatography and can be easily purified by recrystallization.[58] Another difference between BODIPY and its dipyrromethene precursor is that BODIPY has two highly delocalized resonance forms whereas the dipyrromethene has two electronic structures that are in tautomeric equilibrium (**Figure 16**).[33]

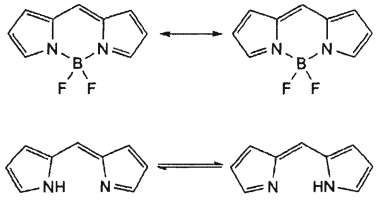


Figure 16. Different π-systems for BODIPY and dipyrromethene[33]

An interesting pattern seen by various substituted BODIPY analogues has shown that substituents at positions 2 and 6 can reduce the quantum yield (ϕ) up to half of its original value.[33] Substitution at the *meso*-position appears not to affect the wavelength for excitation or emission.[57] The quantum yield drops significantly with various aryl substituents at this position as well. In order to increase the quantum yield, BODIPY analogues should possess methyl groups at positions 1 and 7 (compound **10**; **Figure 17**),

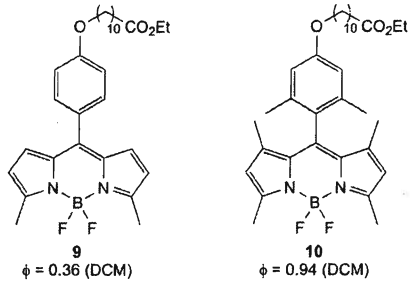


Figure 17. *meso*-Substitution and its effect on quantum yield

or the bulkiness of the *meso*-aryl group should increase, which reduces free-rotation and further locks the aryl group into an orthogonal conformation.[57,64]

1.2.7 Extending the Conjugation of BODIPY

The π-electrons are delocalized along the dipyrromethene backbone of BODIPY. Extension of this conjugation with appropriate substituents on the pyrrole rings is used in designing probes that are tailored to photoexcite at selected wavelengths.[28] As stated previously, substitution at the *meso*-position does not greatly affect the wavelength at which the fluorophore is excited or emits. A much more effective way of changing these values is by substitution at the α-positions.[73,74] In order to synthesize such analogues, pyrrole precursors with the appropriate substitutions are required since this chemistry usually cannot be done after the formation of BODIPY.

A bathochromic shift occurs when substituents are added to the BODIPY core that results in excitation and emission at longer wavelengths. Substituents that produce bathochromic shifts include alkenyl, dienyl, trienyl, and heteroaryl groups (e.g. pyrryl, thienyl, furyl, etc.). For example, when the conjugation of BODIPY is extended by a thienyl group, the excitation wavelength shifts to 558 nm and the emission wavelength shifts to 568 nm.[58]

1.3 Introduction of a BODIPY-Tocopherol Conjugate

Unlike nitrobenzoxadiazole (NBD), dansyl (DAN) and anthroyloxy (AO) fluorophores that are linked to molecules with bridges containing heteroatoms (i.e. O from esters and N from amines or amides), BODIPY analogues can possess an all-methylene linker that would maximize the lipophilicity of the probe. The addition of these heteroatoms may alter the way the probe packs into membranes because of an

added polarity in a lipophilic environment (see BODIPY-cholesterol; Section 1.2.3). The fluorescence quantum yield of BODIPY is two to three times greater than NBD; its extinction coefficient is approximately four to five times greater (**Table 1**).[35]

Table 1. Spectroscopic comparison of fluorophores BODIPY and NBD[35]

Fluorophore	*BODIPY*	*NBD*
Quantum yield[a]	0.9	0.3
$\varepsilon (M^{-1}cm^{-1})$[a]	90 000	20 000

[a]in ethanol at 25 °C

NBD (460-480 nm) has been used in the development lipid probes[75] but its sensitivity to environment, polarity, photobleaching and tendency to self-quench have made researchers seek alternatives.[76] Through ionization and fluorescent quenching measurements,[77,78] membrane probes (e.g. fatty acids) that contain NBD are reported to possess extreme curvature in the alkyl chain. This is because of a physical interaction between the fluorophore and the polar head groups of the phospholipids (**Figure 18**). In

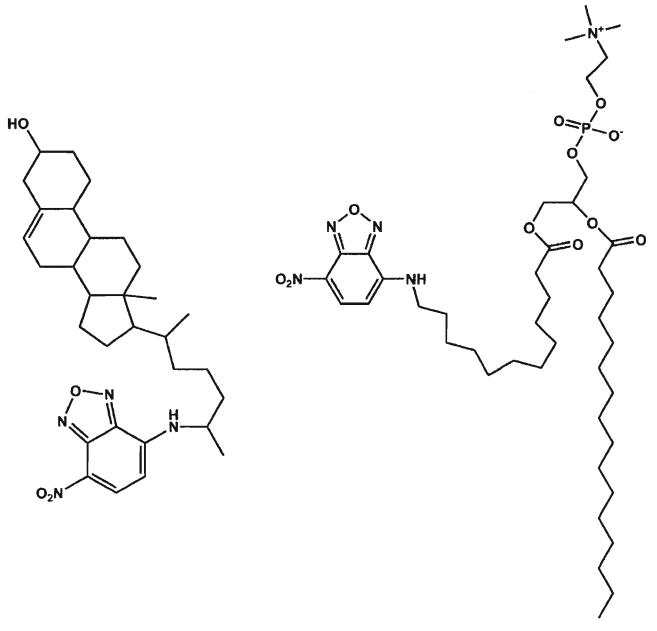


Figure 18. Location of NBD in cholesterol and PC analogues[75]

the design of synthetic probes, one seeks to prepare analogues that are of similar physical length as the original molecule and, particularly with membrane probes, that they occupy a similar volume of space. For example, the saturated fatty acyl chains of PC are relatively linear and point towards the centre of the bilayer. In contrast, NBD-PC has a curve in its methylene chain, resulting in a molecule that occupies a greater volume within the membrane (**Figure 18**).

A series of fluorescent analogues of vitamin E were prepared previously by our group with the intention of probing the localization and trafficking of vitamin E (**Figure 19**). Binding studies to α-TTP showed that only analogues from the NBD (**11d**) and AO (**12d**) series bound specifically and reversibly with dissociation constants, K_d, of 60 and 280 nM, respectively.[79] The extreme bulkiness of the AO fluorophore and its orthogonal attachment to the chain makes it a poor analogue for future use as a probe. With C9-NBD-α-Toc **11d**, significant spontaneous membrane transfer is observed. Intervesicular transfer between membranes is not uncommon amongst NBD-probes for this has been reported previously with NBD-PC.[80] Because of these similarities with the other NBD-lipid analogues, it was also postulated that C9-NBD-α- Toc **11d** must have some

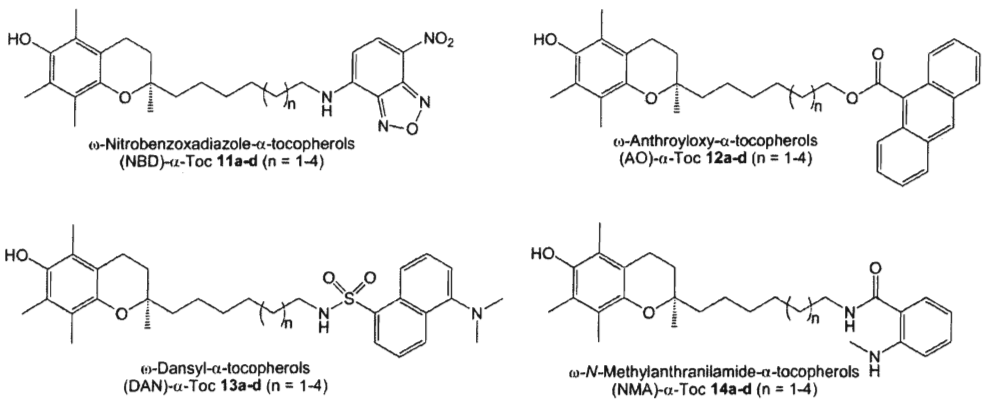


Figure 19. Fluorescent analogues of vitamin E for localization studies

curvature in the methylene chain because of an interaction of NBD with the polar phospholipid heads.

It should be expected that synthetic analogues differing structurally from intrinsic lipids bind with less affinity to target proteins and may have some degree of perturbation within membranes.[44] Recent work in our group has shown that our leading fluorescent vitamin E analogue, C9-NBD-α-Toc **11d**, is more similar to α-tocopherol than the other tocopherols in terms of the physical attributes within the membrane. This was assessed using differential scanning calorimetry.[81] Some factors to consider when designing probes include size, volume, hydrophobicity, and charge distribution. Using molecular modeling software (e.g. Spartan), it is possible to determine the approximate length of molecules following simple molecular mechanics energy minimization. A comparison of molecular length between α-tocopherol, C9-NBD-α-Toc **11d**, and a BODIPY-tocopherol conjugate (BDP-α-Toc), which is separated by a seven-carbon bridge, is shown in **Figure 20**.

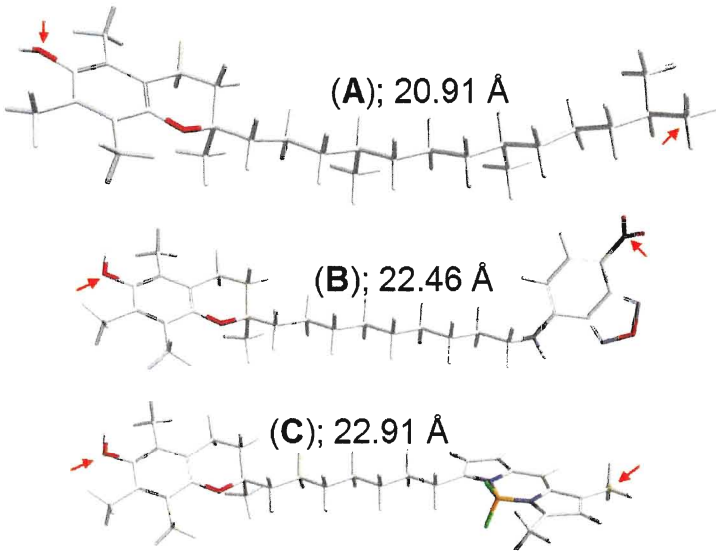


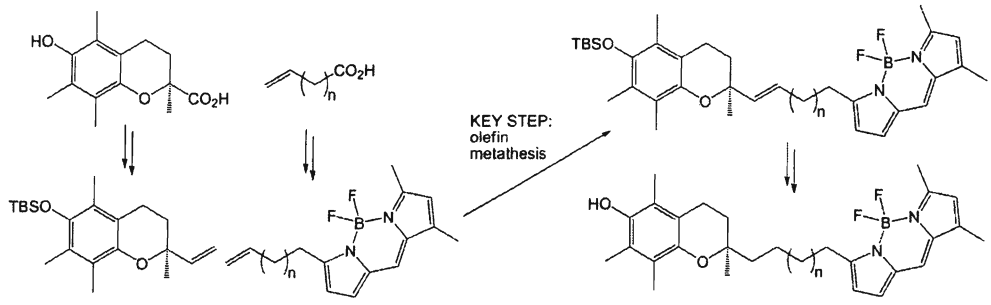
Figure 20. Molecular comparison of α-tocopherol (**A**), C9-NBD-α-Toc (**B**), and C7-BDP-α-Toc (**C**) *[Red arrows indicate atoms used for calculating molecular length]*

The high quantum yield of BDP-α-Toc allows one to use low concentrations, which may possibly reduce any negative impact on the membrane or protein receptor. Another factor to consider in the design of fluorescent probes is that there is little spectral overlap between the probe and endogenous chromophores, such as aromatic amino acids.[30] The incorporation of fluorophores that enable detection at longer wavelengths (e.g. BODIPY) reduces the interference of any cellular autofluorescence.[82]

1.4 Project Overview

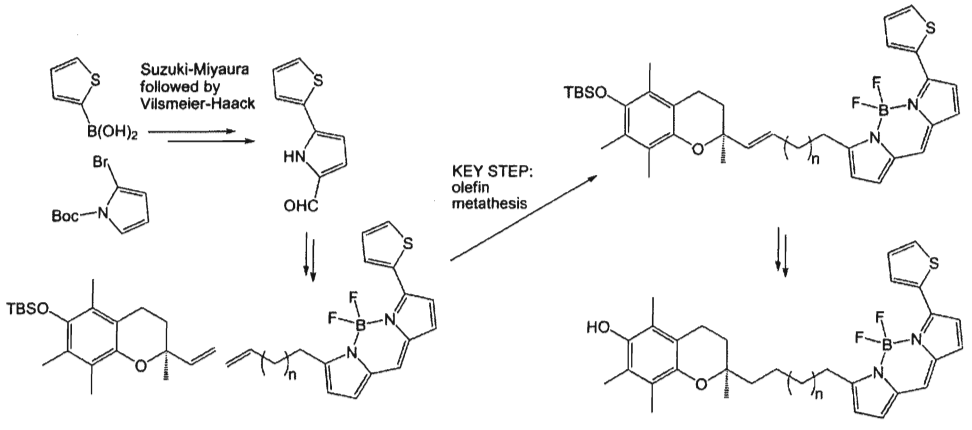
This thesis will entail the synthesis of C7-BDP-α-Toc (**Figure 20**; **C**). The choice of having seven carbons in the bridge is based on the molecular length of C7-BDP-α-Toc (22.9 Å), which is very similar to that in leading probe C9-NBP-α-Toc (22.5 Å). Once made, this analogue will be tested for its binding affinity to α-TTP. In order to examine the effect of molecular length on binding to α-TTP, C6- (21.6 Å) and C8-bridged (23.7 Å) analogues will be synthesized as well.

The key step to the synthesis of these analogues is a cross-metathesis reaction inspired by the work of Nussbaumer et al. who linked functionalized BODIPY intermediates to the sphingosine pharmacophore by this reaction.[43] Vinyl Trolox can be derived from the commercially available (*S*)-Trolox and alkenylBODIPYs can be synthesized from ω-alkenoic acids (**Scheme 1**).



Scheme 1. Synthesis of BDP-α-Toc analogues

In addition to the synthesis of these molecules, another analogue whose fluorophore conjugation is extended by a thienyl group (TBDP-α-Toc) will be made. 5-(2'-Thienyl)pyrrole-2-carboxaldehyde, which can be made by Suzuki-Miyaura cross-coupling[83] followed by formylation,[84] will be required for building the new fluorophore (**Scheme 2**). BDP-α-Toc can be paired with this thienyl derivative to observe if liver cells pool vitamin E when given in excess.

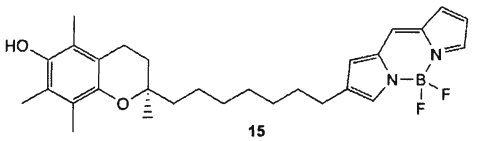


Scheme 2. Synthesis of TBDP-α-Toc analogues

2 RESULTS AND DISCUSSION

2.1 Original Design and Synthetic Attempts of BDP-α-Toc

During the planning phase of this project, the original target molecule **15** possessed a BODIPY fluorophore that was monoalkylated at position 2 (**Figure 21**). The intention behind this design was that the fluorophore should remain relatively linear with the rest of the molecule, unlike the AO-α-Tocs **12a-d** that possessed the undesirable orthogonal attachment, which made them poor binders to α-TTP.

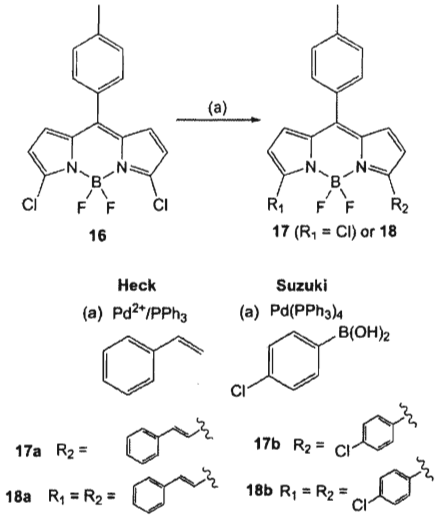


15

Figure 21. Original design of the BDP-α-Toc target molecule

This project was the first time that the synthesis of a fluorophore itself was needed. Previous syntheses of fluorescent α-tocopherol analogues in the Atkinson laboratory had used commercially available, pre-formed fluorophores that coupled to alcohols or amines.[79] The BDP-α-Tocs have been designed however to possess an all-methylene chain, which maximizes lipophilicity. Unlike the projects before, this meant having to prepare the fluorophore itself, which included extensive pyrrole chemistry. Although pyrroles are able to undergo substitution at all five positions, they are challenging molecules to make because of poor control over regiochemistry.[85]

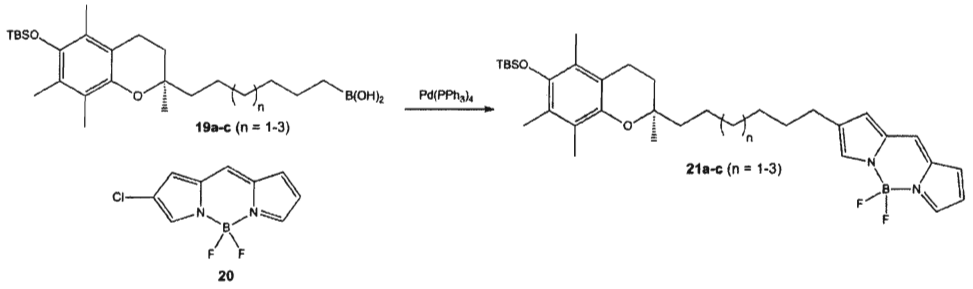
Of the very few papers that discuss alkylations or couplings to an intact BODIPY fluorophore, the one that was most attractive and seemingly most suitable for this project was the work of Wim Dehaen.[86] A novel way of functionalizing the BODIPY ring after it is assembled is exemplified in fluorophore **16** (**Scheme 3**). The chemistry of **16** has been likened to imidoyl chloride[87] and therefore cross-couplings, such as Suzuki or Heck,



Scheme 3. Reported cross-couplings using 3,5-dichloroBODIPY[86]

were done.[86] Other cross-coupling methods reportedly used on this molecule include Stille and Sonogashira.[86]

Of these coupling reactions, Suzuki would be most applicable to our target molecule because it can be done using sp^3-alkylboronic acids producing intermediate **21a-c** (**Scheme 4**), which only requires a desilylation to obtain the final compound. To perform a Suzuki cross-coupling for this reaction, the synthesis of a tocopherylboronic acid and a 2-chloroBODIPY fluorophore is needed (**Scheme 4**).



Scheme 4. Proposed Suzuki cross-coupling in synthesis of BDP-α-Toc

Unfortunately, there is no way to exclusively synthesize 4-chloro-2-formylpyrrole, which would be needed to produce a 2-chloroBODIPY fluorophore, without generating a mixture of regioisomers. However, 5-chloro-2-formylpyrrole by-product could also give a second target fluorophore, 3-chloroBODIPY. The synthesis of 2- and 3-bromoBODIPY should also be considered because they may provide a greater reactivity if the BODIPY chlorides fail.

Because pyrroles undergo electrophilic aromatic substitution with poor regioselectivity, most procedures for halogenation result in isomers that are difficult to separate. To selectively synthesize **23**, Vilsmeier-Haack formylation on 2-chloropyrrole has been done previously,[88] but 2-halopyrroles are poorly characterized because they are very unstable.[88,89] 2-Chloropyrrole decomposes to an unknown black material that remains solid up to temperatures as high as 700 °C. Sublimation of this black material yields ammonium chloride crystals, a sign that the pyrrole ring has broken apart.[88]

The selective synthesis of **23** from *N*-Boc-succinamidal **22** that avoids the 2-chloropyrrole intermediate has been reported. Under Vilsmeier-Haack conditions, succinamidals undergo dehydration to form **23** (**Figure 22**).[90] When di-*tert*-butyl dicarbonate (Boc_2O) is used to protect the succinamidal, it stabilizes the molecule during this transformation and the yields are improved. Conveniently, this protecting group is

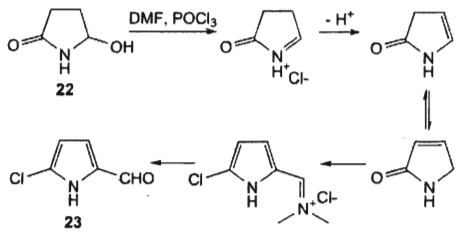
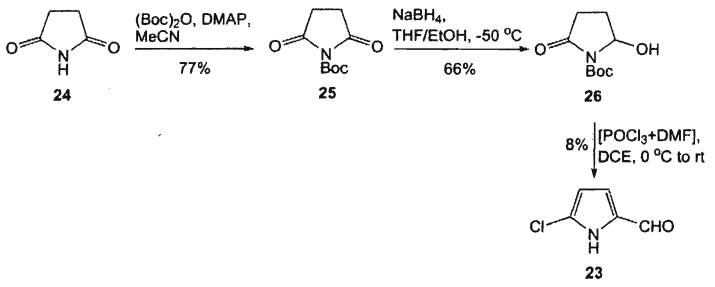


Figure 22. Suggested mechanism for the transformation of succinamidal to 5-chloro-2-formylpyrrole under Vilsmeier-Haack conditions[90]

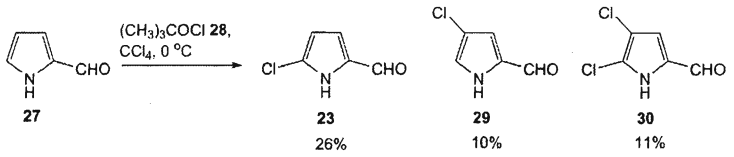
lost during the course of this reaction due to the acidic environment.[90]

Succinimide was easily protected using di-*tert*-butyl dicarbonate in good yields (**Scheme 5**). The next step required the slow addition of sodium borohydride to a stirring solution of **25** in tetrahydrofuran/ethanol 1:1. It is extremely important to add the hydride slowly and monitor the temperature of the reaction so that it does not rise above –50 °C. The amidal does not form unless these two important details are followed. Once succinamidal **26** was made, it was treated with phosphorus oxychloride and *N*,*N*-dimethylformamide to make **23**. Although this step was repeated several times, the yield (8%) remained much less than that reported in the literature (46%).[90]



Scheme 5. Synthesis of 5-chloro-2-formylpyrrole from succinimide

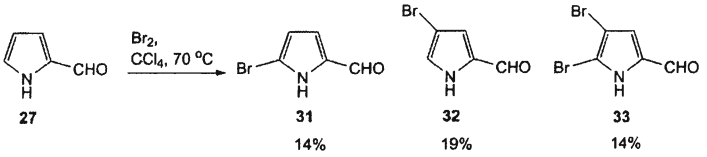
An alternative method for chlorinating pyrrole-2-carboxaldehyde uses *tert*-butyl hypochlorite prepared from bleach (i.e. NaOCl) and *tert*-butanol.[91] The preparation of *tert*-butyl hypochlorite is simple and straightforward. Bleach is cheap and readily available and the product is isolated with high purity.[92] *tert*-Butyl hypochlorite is however reportedly less stable than the inorganic hypochlorite salts and decomposes under continuous exposure to light. The compound is stable for a few months when stored in the freezer in the dark.[92]



Scheme 6. Chlorination of pyrrole-2-carboxaldehyde using *tert*-butyl hypochlorite

The slow addition of this chlorinating agent to a solution of pyrrole-2-carboxaldehyde in carbon tetrachloride on ice produced **29** (10%), **23** (26%), and 4,5-dichloro-2-formylpyrrole **30** (11%). **23** has a different R_f on TLC (hexanes/tetrahydrofuran 15:1) than **29** and **30** so it can be easily removed by silica gel chromatography. With the partially purified **29/30** fraction, **29** was purified by recrystallization from hexanes/diethyl ether 1:1 while **30** remained in solution.

A general method for the bromination of pyrrole-2-carboxaldehyde uses molecular bromine (**Scheme 7**).[93] Bromination at room temperature provided 4-bromo-2-formylpyrrole **32** and 5-bromo-2-formylpyrrole **31** in a 6:1-10:1 ratio using this method. This is because the formyl group on pyrrole-2-carboxaldehyde directs halogenation with bromine to the 4-position. At higher temperatures, the proportion of **31** increases.[93] To a solution of pyrrole-2-carboxaldehyde in carbon tetrachloride near reflux (70 °C) was slowly added a solution of bromine in the same solvent. From this reaction **32**, **31**, and 4,5-dibromo-2-formylpyrrole **33** were produced with isolated yields 19%, 14%, and 14%, respectively.

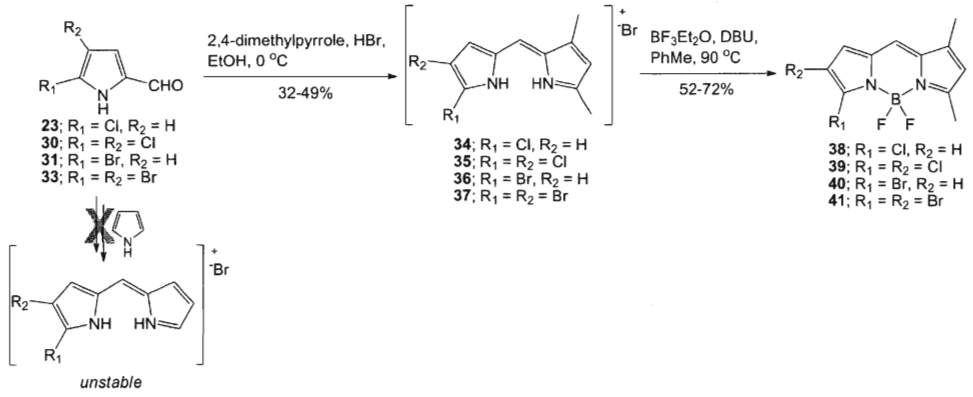


Scheme 7. Bromination of pyrrole-2-carboxaldehyde using bromine

Silica gel chromatography using the unusual hexanes/tetrahydrofuran 15:1 mixture afforded partially purified samples of **32** and pyrrole-2-carboxaldehyde in one fraction and **31/33** in another. **32** was easily isolated by recrystallization from hexanes/diethyl ether 1:1, but the separation of **31** and **33** by recrystallization was difficult. Instead, **31** was cleanly separated from **33** using sublimation (60 °C at 0.5 Torr).

With chloropyrroles **29**, **23**, **30** and their bromo counterparts in possession, they could be condensed with pyrrole to form the dipyrromethene backbone of BODIPY. When hydrobromic acid was used, the dipyrromethenium salt often precipitated.[26] This was not observed when **23** was condensed with pyrrole however (**Scheme 8**). Instead the reaction turned black and no precipitate was formed. This is understandable given that minimally substituted dipyrromethenes are unstable and only exist at very low temperatures (–40 °C).[55] Attempts to carry out this same condensation at reduced temperatures also did not yield product.

A common feature with many BODIPY fluorophores that lack *meso*-aryl groups is that they possess methyl groups at positions 1 and 3 on the same pyrrole ring. These



Scheme 8. Synthesis of BODIPY chlorides and bromides

methyl groups must be present to stabilize the dipyrromethene backbone during synthesis and therefore 2,4-dimethylpyrrole was used for the condensation. These alkyl groups also have the added advantage of increasing lipophilicity, ideal for use as membrane probes.[94]

When pyrrole was replaced with 2,4-dimethylpyrrole, bright red precipitates were rapidly formed and identified as the dipyrromethenium salts (**Scheme 8**). They were successfully prepared using **23**, **30**, **31**, and **33** as electrophiles. It should be mentioned that these salts cause irritation on inhalation (particularly to nasal passages) and must be handled with extreme caution because they are relatively volatile.[55] They were eventually transformed to the corresponding BODIPY derivatives using boron trifluoride diethyl etherate and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) without any complication. A summary of their photophysical properties is shown in **Table 2** below. It is interesting to report that their extinction coefficients (ε) are much less than the predicted value of 90 000 $M^{-1}cm^{-1}$.[35] Halogens are more electron-donating than alkyl groups and lower these values.

Table 2. Spectroscopic comparison of BODIPY halides **38-41**

BODIPY halide	λ_{max} *excitation (nm)*[a]	λ_{max} *emission (nm)*[a]	ε $(M^{-1}cm^{-1})$[a]
38	500	508	58 000
39	497	506	51 000
40	504	511	68 000
41	512	524	42 000

[a]in ethanol at 20 °C

Attempts to condense either **29** or **32** with 2,4-dimethylpyrrole did not produce the distinctly red precipitate that the other pyrroles did. Instead, these reactions turned black and no precipitate was observed. At the time, we were unaware of the problem with using non-α-substituted pyrroles to generate BODIPYs. As mentioned in Section

1.2.4, oligomers and porphyrinogens form instead of dipyrromethanes if pyrroles lack the appropriate substitution. This is also apparently true with the synthesis of dipyrromethenes from the two-component route. Oligomers and porphyrins must have formed from the overly reactive α-position on **29** or **32**. The inability to make either 2-haloBODIPY changed the original design of the target molecule from a 2-substituted fluorophore, **15**, to one that is linked at position 3 and has two methyl groups that provide stability (**Figure 23**).

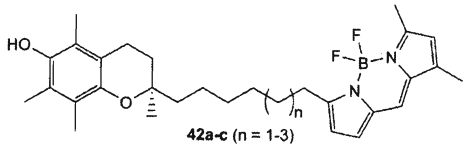
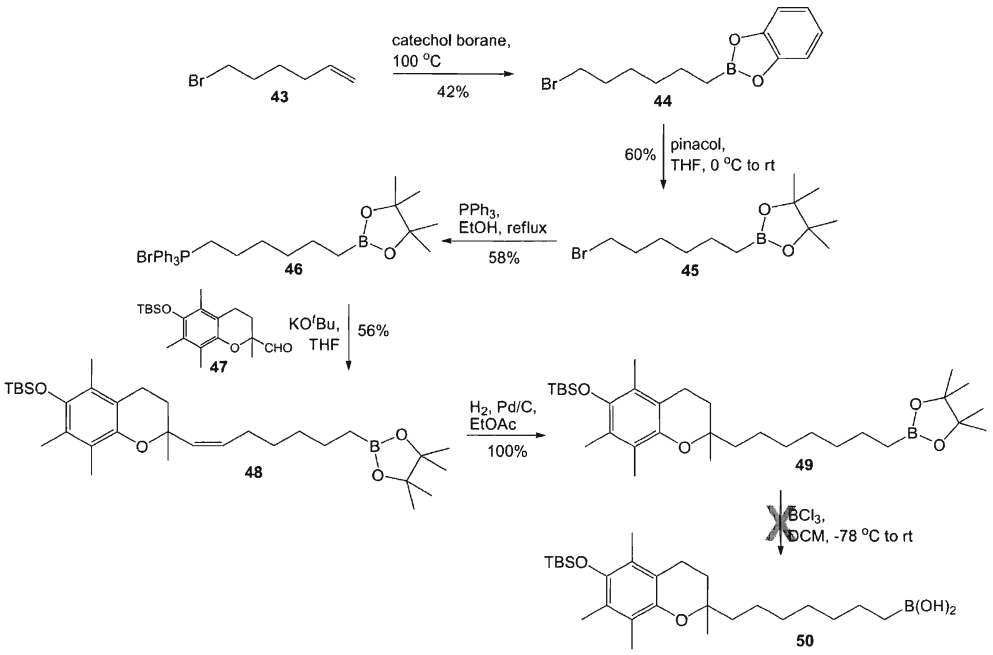


Figure 23. New design of the BDP-α-Toc target molecule

With the BODIPY halides now available for coupling, the attention was shifted towards making a tocopherylboronic acid. The proposed synthesis to obtain this intermediate is shown in **Scheme 9**. Trolox aldehyde can be generated from commercially available Trolox and has been used in the preparation of all of the other fluorescent analogues.[79] Another inspiration for the design of the synthesis came from a paper reporting the synthesis of a novel class of prostaglandins.[95] In this report, a phosphonium salt intermediate that also contained a pinacol-boronate was used.

Racemic Trolox aldehyde was originally prepared because its precursor, *rac*-Trolox, is less expensive and more easily available than optically pure (*S*)-Trolox. The synthesis of Wittig salt **46** began with the hydroboration of 6-bromo-1-hexene **43** by catecholborane. Intermediate **44** was rather unstable and blackened very easily, likely from the oxidation and polymerization of 1,2-quinone. After Kugelrohr distillation, **44** was immediately subjected to transesterification with pinacol to the much more stable

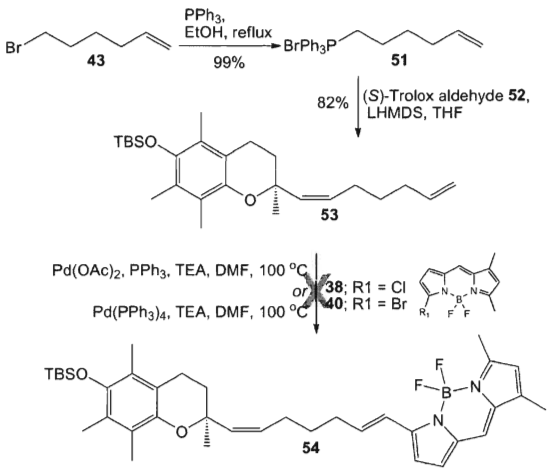


Scheme 9. Attempted synthesis of a tocopherylboronic acid pinacol-boronate, **45**. Following treatment with triphenylphosphine, Wittig salt **46** was generated. This salt is a thick sticky oil that remains stable if refrigerated.[95]

After the Wittig reaction with *rac*-Trolox aldehyde **47**, which was followed by catalytic hydrogenation, the last step to obtain tocopherylboronic acid was the removal of the pinacol ester of **49** (**Scheme 9**). There were concerns that acidic hydrolysis would also cleave the silyl ether and this prompted us to seek an alternative method. One seemingly efficient method was treating the ester with boron trichloride in dichloromethane.[96] When this reagent was used, however, neither product nor starting material was recovered from the reaction. The one spot on TLC that seemed most likely to be product, which also gave the most mass, showed multiple Ar-CH_3 signals on NMR and could not be further characterized by any other means.

Since the removal of the ester of **49** failed, the other coupling methods from Dehaen's report[86] were re-evaluated and it seemed that the Heck reaction should be the

next route to pursue (**Scheme 10**). The rationale behind this decision was that synthesizing the diene should be much easier than boronic acid **50** and it would allow us to see how the BODIPY halides reacted by cross-coupling. Heck conditions should preferentially react with the terminal alkene on **53** so there were no concerns with subjecting this diene to the coupling. Phosphonium salt **51** was easily prepared from 6-bromo-1-hexene. The Wittig reaction with (*S*)-Trolox aldehyde **52** went in good yield (82%) to generate diene **53**.

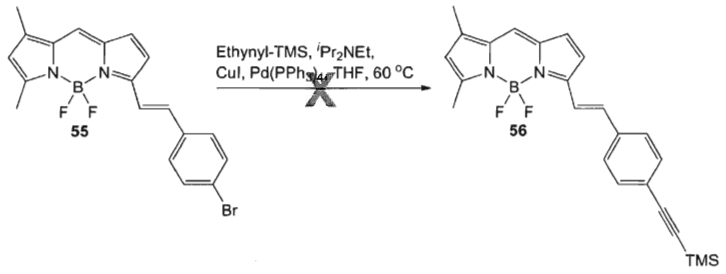


Scheme 10. Attempted Heck cross-coupling with BODIPY halides **38** and **40**

Attempts at the Heck cross-coupling of diene **53** with BODIPY chloride **38** and bromide **40** were performed using two different catalytic systems (**Scheme 10**), but with no success. After heating between 80 and 100 °C and monitoring hourly, the reactions turned purplish black. It was shown by TLC that several product spots that weakly fluoresced were produced, but none were shown to be product by NMR (i.e. neither the aromatic proton signals nor the methyl groups of the fluorophore were present). Instead, these BODIPY halides were completely consumed by the reaction. Comparing our

BODIPY halides **38-41** to Dehaen's **16** led to the speculation that their *meso*-aryl group may be providing some stability to their fluorophore under these coupling conditions.

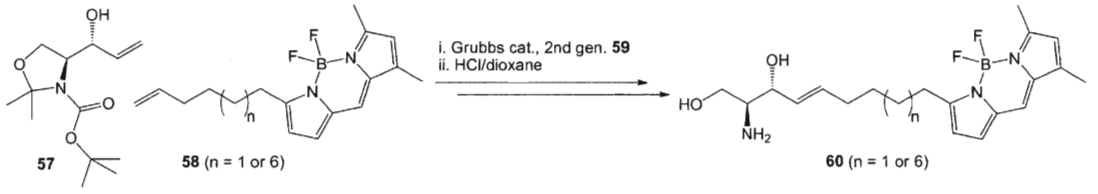
There has been one other report on the failure of a palladium-catalyzed cross-coupling in the presence of a BODIPY fluorophore, this time using the Sonogashira reaction (**Scheme 11**). Interestingly, fluorophore **55** also lacks a substituent at the *meso*-position. Attempts at this reaction reportedly produced multiple products, as was observed with our Heck reaction, that also could not be characterized.[46]



Scheme 11. Unsuccessful Sonogashira coupling of a BODIPY fluorophore lacking a *meso*-substituent[46]

2.2 Generation of BDP-α-Toc by Olefin Metathesis

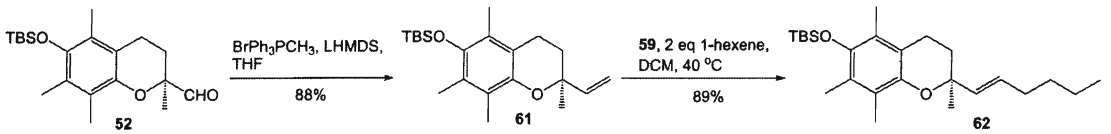
After the unsuccessful attempts at cross-coupling using the BODIPY halides, alternative methods of conjugating the fluorophore to the chromanol were sought. A successful olefin metathesis of a BODIPY intermediate functionalized with a terminal olefin was reported (**Scheme 12**).[43] The fluorophore, which lacked a *meso*-substituent and possessed two other methyl groups, is reportedly stable during this metathesis step



Scheme 12. Olefin metathesis step during the synthesis of BODIPY-sphingosine derivatives[43]

and does not interfere with the ruthenium catalyst. This key step is mild and easily controlled, requiring a gentle reflux in DCM at around 40 °C.[43]

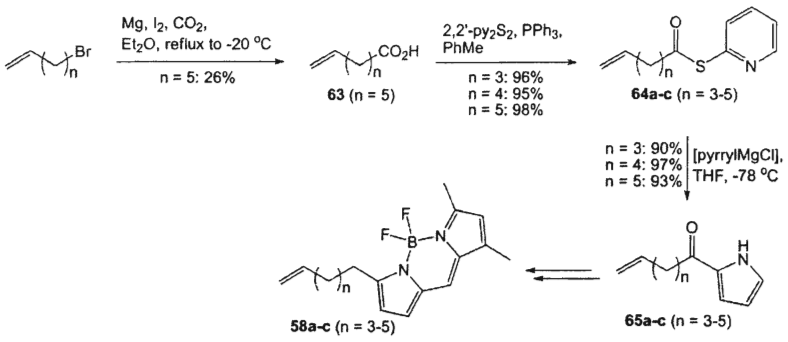
An initial concern with applying this method to our synthesis was the possibility that the chromanol would be too bulky to coordinate to Grubbs catalyst **59**. A test reaction using vinyl Trolox **61** and two equivalents of 1-hexene was successfully performed with a high yield of 89% (**Scheme 13**). The success of this test reaction prompted a new design in the synthesis of BDP-α-Toc.



Scheme 13. Test reaction for the metathesis of vinyl Trolox with 1-hexene

AlkenylBODIPYs **58a-c** can be prepared from ω-alkenoic acids. If alkenoic acids are commercially unavailable or too expensive, they can be prepared by a Grignard reaction using the corresponding alkenylbromide and carbon dioxide gas (CO_2). 7-Octenoic acid **63** was prepared from 7-bromo-1-heptene. The carbon dioxide gas that was produced from dry ice may not have fully dried as it was passed through the Drierite® drying tube, which would explain the low yield for this reaction. Other drying agents, such as calcium chloride, are used as alternatives. With the appropriate ω-alkenoic acids available, ketopyrrole intermediates **65a-c** were prepared (**Scheme 14**).

Pyrroles readily undergo electrophilic aromatic substitution due to their electron-richness. One of the ways to generate ketopyrroles is through Friedel-Craft acylation, which requires a reactive acyl halide and a catalyst (e.g. $AlCl_3$, $FeBr_3$). There is often poor regioselectivity upon this acylation however, producing mixtures of 2- and 3-



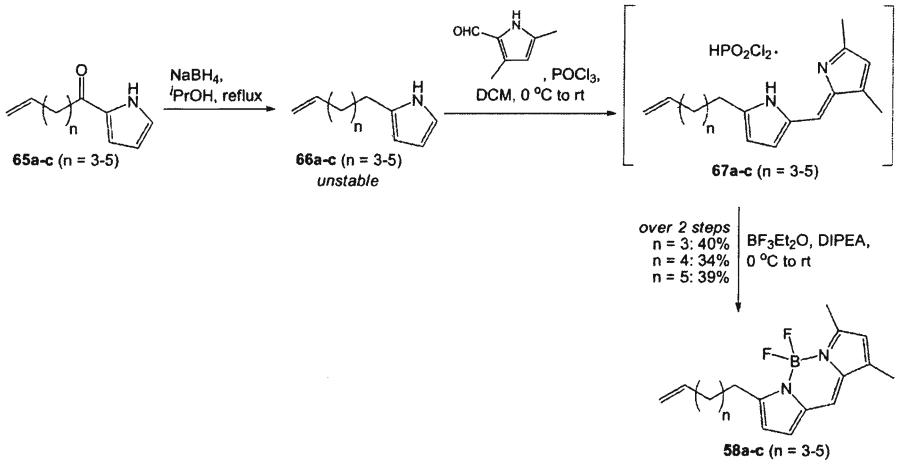
Scheme 14. Synthesis of 2-ketopyrrole intermediates

ketopyrroles.[97] Direct alkylation of pyrrylmagnesium chloride with alkyl chlorides avoids having to reduce ketopyrroles by forming alkylpyrroles directly, but regioisomers are also produced.[98] 2-Ketopyrroles can be selectively obtained from the acylation of pyrrylmagnesium chloride with either acyl halides or esters (e.g. pyridyl thioates **64a-c**; **Scheme 14**).[98] Acylations with esters are more favourable because these reactions are less vigorous and less likely to form tars because of poor control.[99]

Ketopyrroles are stable species that can be purified by chromatography. Before these intermediates can condense to form the corresponding dipyrromethenium salts, the carbonyl group must be reduced. Wolff-Kishner reduction is one method to reduce ketopyrroles. For example, acetylpyrrole is cleanly reduced to ethyl pyrrole by this method in 65% yield.[99]

In the preparation of BODIPY-sphingosine **60**, the ketopyrrole was reduced with sodium borohydride to yield the alkenylpyrrole.[43] Following this method for preparing the BDP-α-Tocs, alkenylpyrroles **66a-c** were synthesized with yields around 50% (**Scheme 15**). The purification of **66a-c** was met with difficulty because these compounds decompose on the silica gel. They are also unstable in the presence of acid, even turning dark brown in the NMR tubes when dissolved in deuterated chloroform that

contains traces of acid. The purification method for these intermediates had to be changed to neutral alumina and all NMR spectra required either the neutralization of deuterated chloroform by passing through a basic alumina column or using the less acidic deuterated dichloromethane. NMR spectra of the alkenylpyrroles after neutral alumina chromatography were still crude so it was eventually decided that after reduction with sodium borohydride, the product would be simply flushed on a short alumina plug and then taken directly to the next step.

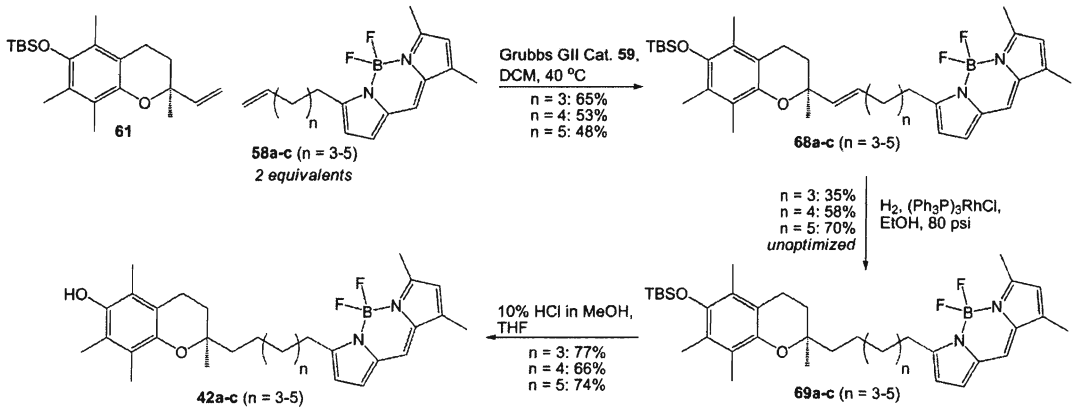


Scheme 15. Synthesis of alkenylBODIPYs **58a-c** from ketopyrrole intermediates

Initially, there were concerns about forming dipyrromethenium salts **67a-c** with hydrobromic acid because of this newfound instability of alkenylpyrroles towards acids. Despite hydrobromic acid being used to condense the alkenylpyrroles in the methods described by Peters et al. (and in a yield of 74%),[43] multiple test reactions did not yield any precipitate. An alternative reagent that is used in making dipyrromethenes is phosphorus oxychloride.[46] Replacing hydrobromic acid with phosphorus oxychloride generated **67a-c** *in situ*. The reaction went from colourless to bright orange within

minutes after the addition of phosphorus oxychloride. Treating this solution with boron trifluoride diethyl etherate and DIPEA yielded alkenylBODIPYs **58a-c**.

The olefin metathesis between one equivalent of vinyl Trolox **61** and two equivalents of alkenylBODIPY **58a-c** produced **68a-c** with the yields varied from 48-65% (**Scheme 16**). It is not too surprising to see yields that were lower than the test reaction with 1-hexene from **Scheme 13**. The fluorophore increases the steric interaction with the catalyst, even at several methylenes away from the reactive alkene. Two equivalents of alkenylBODIPY were required because there is greater ease for the metathesis between two alkenylBODIPYs. This by-product is referred to as bisBODIPY and is easily removed by silica gel chromatography. We did not detect any bisTrolox formation from this reaction.



Scheme 16. Completed synthesis of BDP-α-Tocs **42a-c**

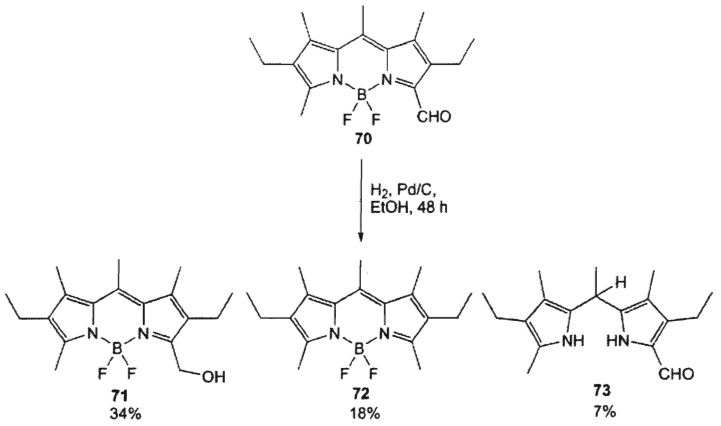
The alkenylBODIPYs proceed through the metathesis with great stereoselectivity. The new alkene that is formed is mostly in the *trans* configuration (*E*/*Z* > 95/5).[100] For the synthesis of BODIPY-sphingosine **60**, this was important because the double bond is retained in the final compound.[43] The synthesis of BDP-α-Tocs **42a-c** requires the reduction of this alkene, however.

Double bonds are easily reduced using hydrogen gas and palladium on carbon (Pd/C) as catalyst. This has been the method of choice for reducing the alkene intermediates formed during the syntheses of fluorescent vitamin E analogues **11a-d**, **12a-d**, **13a-d**, and **14a-d**.[79] There are also multiple reports that compounds possessing the BODIPY fluorophore react under these identical conditions without any reported decomposition. Such reactions include reduction of nitrophenyl groups to anilines[101-104] and hydrogenolysis of benzyl groups.[105]

When alkenes **68a-c** were treated with hydrogen gas and Pd/C, the TLC showed rapid decomposition of the starting material within minutes. None of the desired product was ever retrieved from these reactions. Instead, an unknown and uncharacterizable transparent oil that gave a fluorescent blue spot on the TLC baseline had formed everytime. These results conflicted with the published reports of high yielding reactions without decomposition. Correspondence with Professor Tetsuo Nagano of the University of Tokyo, lead author of several papers reporting such reactions,[102,103,105] provided the following unpublished information:

> *"We have also got the results similar to those shown in your e-mail. Non-substituted BODIPY derivatives at 8-position often decomposed to unknown products by reduction of H2/Pd-C immediately."*
>
> - T. Nagano, 09/19/08

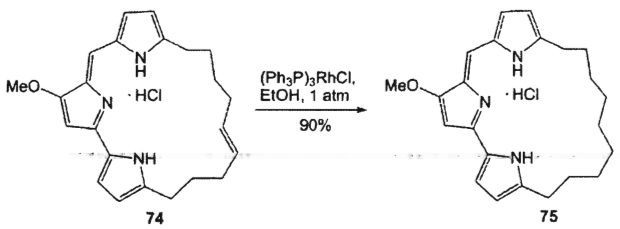
Reduction of BODIPY **70** using hydrogen gas and Pd/C has been reported to produce dipyrromethane **73** as a by-product with a yield of 7% (**Scheme 17**),[53,106,107] but this occurred after days of continuous reduction (not within minutes as seen here). Perhaps the presence of a methyl group at the *meso*-position provides some partial stability during this reaction. With regards to the other reports of nitro-group reduction[101-104] and hydrogenolysis,[105] all of these fluorophores possessed *meso*-aryl



Scheme 17. Reduction of BODIPY to dipyrromethane by catalytic hydrogenation[53,106,107]

groups, which have been shown to greatly stabilize the backbone during synthesis. Catalytic transfer hydrogenation using organic molecules, such as diimide, has been implemented in the past to reduce alkenes as an alternative method.[108] The reduction of **68a-c** using potassium azodicarboxylate (PAD)[109] however did not work, producing the same fluorescent blue spot on TLC instead. No starting material could be recovered by this method either.

The final step in the synthesis of bile pigment nonylprodigiosin **75** also reported similar difficulties in reducing a double bond using hydrogen gas and Pd/C (**Scheme 18**).[110] Attempts at this reaction led to complete destruction of the molecule. When Wilkinson's catalyst was used, the selective reduction of the alkene was achieved in good yield (i.e. 90%) without destruction of the dipyrromethene.[110] The use of Wilkinson's

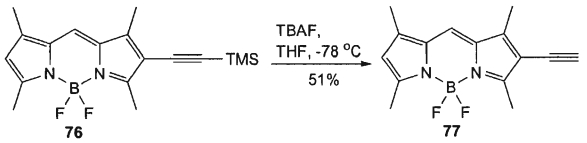


Scheme 18. Reduction of cyclic nonylprodigiosin intermediate with Wilkinson's catalyst[110]

catalyst was applied to BDP-α-Toc intermediates **68a-c** (**Scheme 16**) with the assumption that the destruction of the fluorophore in the presence of hydrogen gas and Pd/C is for the same reasons as **74**.

At 1 atm, no reaction occurred, but alkene **68a-c** is likely less accessible to Wilkinson's catalyst than **74**. Selective reduction of the alkene began at pressures of 40 psi or greater. Decomposition of the BODIPY fluorophore was still observed but there was enough chemoselectivity to obtain **69a-c** (**Scheme 16**). Increasing the concentration of the reaction in addition to further elevating the pressure provided even greater selectivity and higher yield. Currently, the best conditions for catalytic hydrogenation using Wilkinson's catalyst in ethanol is 80 psi for 28 h with an alkene concentration of 19 mM, which the product in 70% yield.

After the successful hydrogenation, the final step was the removal of the TBS group. One of the most common reagents for removing silyl protecting groups is tetrabutylammonium fluoride (TBAF). However, the BODIPY fluorophore decomposed with an obvious loss of fluorescence by TLC when **69a-c** was treated with TBAF. If BODIPY derivatives behave similarly to the dipyrromethene backbone, then dyes with fewer substituents, especially those with an open *meso*-position, are more prone to nucleophilic attack (e.g. the fluoride ion from TBAF).[55,72] The desilylation of intermediate **76** for the synthesis of an anthracene-BODIPY analogue, which also lacks a substituent at the *meso* position, was reported to occur with great difficulty (**Scheme 19**). This step was specifically stated as being the most troublesome in the entire synthesis and could only be successfully transformed if run with extreme care.[111]



Scheme 19. Desilylation of BODIPY acetylene derivative[111]

Other ways to desilylate phenols include acids such as hydrochloric acid or bases such as sodium hydroxide, 1,1,3,3-tetramethylguanidine, and DBU (e.g. DBU is reported to chemoselectively desilylate aryl silyl ethers and may be used catalytically).[112-114] Numerous reports have shown the successful hydrolysis of silyl ethers using acids that are also high yielding.[112,115-117] The final deprotection step in the preparation of BODIPY-sphingosine **60** used hydrochloric acid in dioxane to deprotect the *N*-Boc group. Although the protecting group was not a silyl ether, this reaction showed that the BODIPY moiety was stable under these conditions with only slight decomposition reported.[43] Thus the previous method of desilylation with TBAF was replaced with hydrochloric acid in tetrahydrofuran, which allowed the successful preparation of BDP-α-Tocs **42a-c** with yields of 66-77% (**Scheme 16**). These molecules are excited at 507 nm and fluoresce at 511 nm with an average extinction coefficient (ε) of 83 000 $M^{-1}cm^{-1}$ in ethanol (**Figure 24**; see experimental section for individual ε values of **42a-c**).

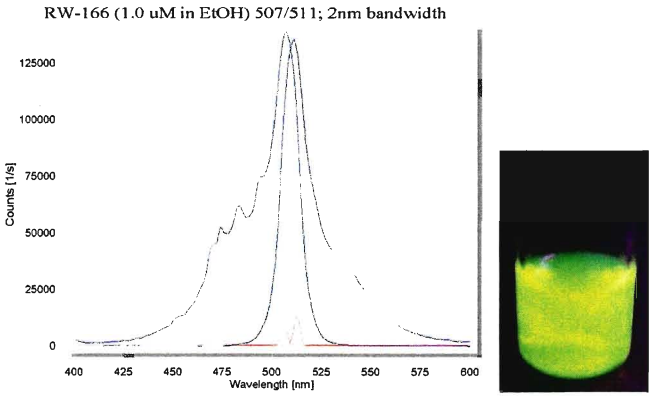


Figure 24. Excitation and emission spectrum of C7-BDP-α-Toc

2.3 Olefin isomerization and the generation of homologues

Before the binding assays can commence, the purity of these substrates had to be determined using HPLC. The NMR spectra of these substrates showed what appear to be pure samples with all of the peaks characterizable. However, the HPLC chromatograms showed multiple peaks, as exemplified for C7-BDP-α-Toc in **Figure 25**. Liquid chromatography-mass spectrometry (LCMS), with electrospray ionization (ESI) as the method for detection, showed that the main peak (R_t = 13.38 min) had a mass/charge ratio (*m/z*) value that corresponded to the molecular ion of the expected molecule. All of the peaks with shorter retention times (R_t) gave a molecular ion that decreased in *m/z* by 14 suggesting the loss of a methylene group (**Figure 25**). Contrarily, the peak with a longer retention time gave a molecular ion that increased in *m/z* by 14, suggesting the addition of a methylene group.

These findings revealed that the samples contained homologues of varying chain length. The NMR spectra appeared pure because the homologues do not have chemical

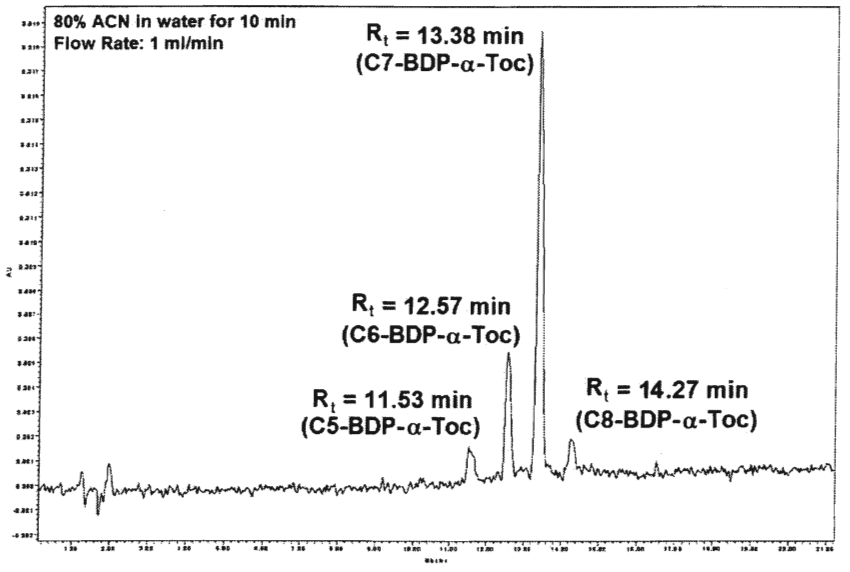


Figure 25. HPLC Chromatogram of C7-BDP-α-Toc **42b** with its homologues

shifts that are easily distinguishable. These homologues also coalesced into one spot on TLC and were eluted together during silica gel chromatography. It was initially suspected that the ω-alkenoic acids were impure, despite the supplier's claims of high purity, but the HPLC of these materials showed a single peak. In fact, all of the intermediates that led to the synthesis of the BDP-α-Tocs were pure according to HPLC until intermediates **68a-c** obtained by metathesis. The same pattern of impurities was shown in these chromatograms as **42a-c** (**Figure 25**).

A search of the literature showed that second generation olefin metathesis catalysts have developed a reputation for facilitating olefin isomerization by 1,3-hydride shifts during the intended metathesis.[118] As a result, homologues are generated. Unfortunately, these side-reactions are not always reported in the many papers that perform metatheses.[118] It is unclear if the isomerization is caused by the ruthenium catalyst itself, by some decomposition product of the catalyst, or by some impurity in the catalyst.[118] The suggested mechanism for this isomerization is shown in **Figure 26A**.[119] Another paper reports that a Grubbs catalyst decomposition product **78** possesses olefin isomerization activity (**Figure 26B**),[120] but a definitive explanation for the mechanism of isomerization remains in dispute.

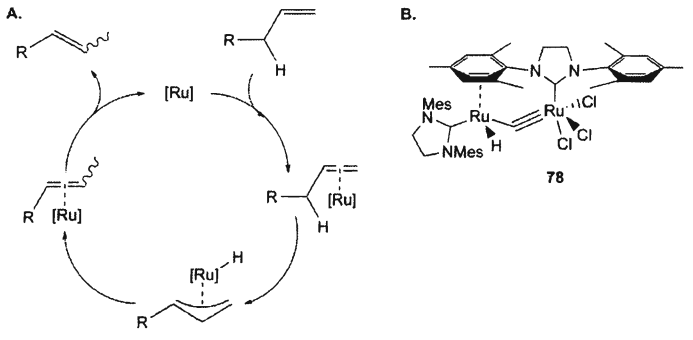


Figure 26. Suggested mechanism for olefin isomerization[119] and the decomposition product of Grubbs II catalyst that may be responsible for this event[120]

To obtain homologues that are one or two methylenes less, the isomerization of the terminal alkene must occur more than once. This mechanism however does not explain how a homologue with an extra carbon, C8-BDP-α-Toc reported in **Figure 25**, is produced. This homologue is produced by a different event (**Figure 27**) in which the alkene can isomerize after the first successful metathesis reaction and undergo a second metathesis.[118] The bisBODIPY by-products formed from these reactions are also capable of undergoing metathesis when reacted with vinyl Trolox **61**, indicating coexisting metatheses with both terminal alkenes from the reactants and internal alkenes from the products.

Homologues of any compound are often difficult to remove using simple purification methods.[121] Regular silica gel chromatography does not have the resolution

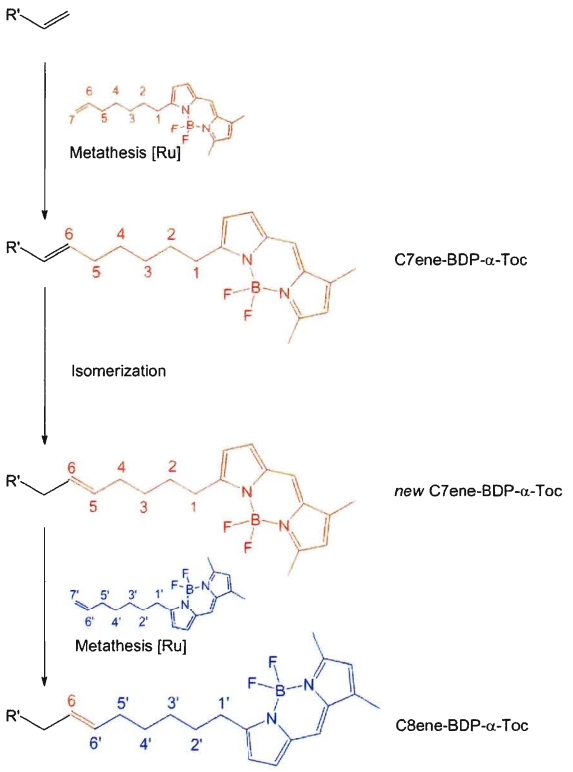


Figure 27. Suggested mechanism for homologues with additional carbons[118]

to separate these homologues. Preparative HPLC (pHPLC) can resolve these samples and allow milligrams of crude sample to be loaded onto an HPLC column with each injection (**Figure 28**). All of the crude samples were purified by this method to obtain the pure substrates. The drawback to this method is that very small amounts of crude sample can be loaded at a time. After 10-12 hours of continuous loadings and runs, an average mass of only five milligrams of pure substrate was isolated.

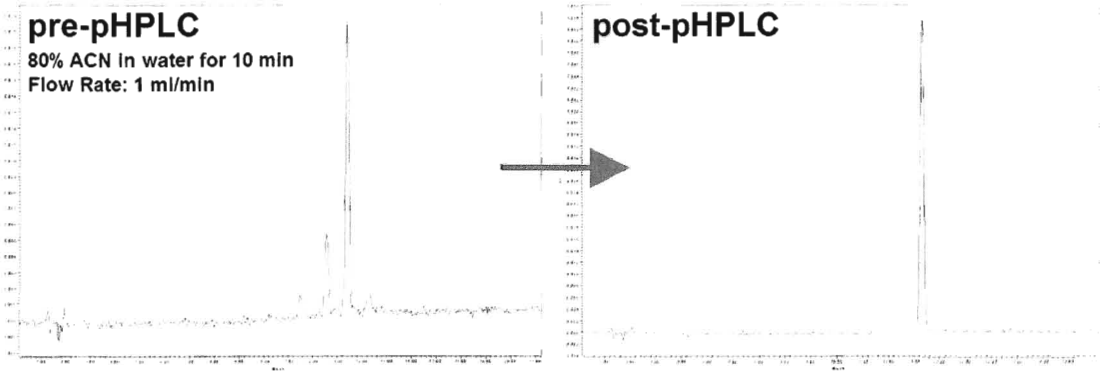


Figure 28. Purification of C7-BDP-α-Toc from its homologues by pHPLC

A much more efficient and effective way for solving the problem with olefin isomerization is to consider using an alternative catalyst, one that does not exhibit this side-reaction. A summary of metathesis reactions performed using different catalysts is provided in **Table 3**.

Bisphosphine ruthenium catalysts (e.g. Grubbs Generation I **79**) and Schrock's molybdenum catalyst reportedly do not possess this isomerization activity.[118] A study on the comparison of the potential to isomerize with Grubbs Generation I and II catalysts was performed by Fokou et al.[122] Grubbs Generation I showed very little isomerization activity that was unaffected by temperature changes or purging the system with nitrogen gas. Grubbs Generation II catalyst however showed isomerization even at temperatures

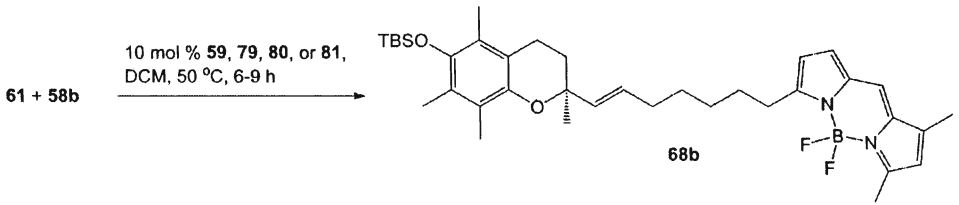
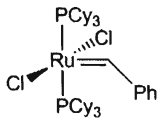
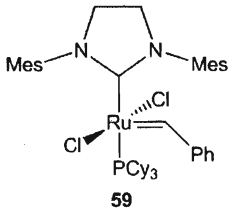
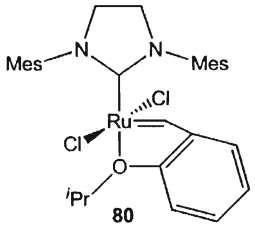
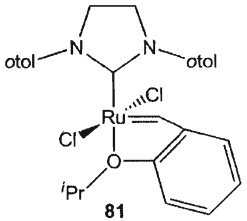


Table 3. Summary of metathesis reactions with various ruthenium catalysts

	Grubbs I		Grubbs II		Hoveyda-Grubbs II	Hoveyda-Grubbs II (*o*-tolyl derivative)
Catalyst	 79		 59		 80	 81
		from bottle	TFA	chromatographed		
Conversion	0%	53%	47%	55%	89%	50%
Composition	-	4% C8ene 69% C7ene 21% C6ene 6% C5ene	95% C7ene 5% C6ene	93% C7ene 7% C6ene	96% C7ene 4% C6ene	100% C7ene

below 60 °C. An increase in temperature or purging with nitrogen gas increased the isomerization, as monitored by HPLC.[122]

Grubbs Generation I Catalyst was used in attempts to metathesize vinyl Trolox **61** with heptenylBODIPY **58b**, but was not active enough to complex to the bulky chromanol. Instead, the corresponding bisBODIPY was exclusively produced. The HPLC chromatogram of this by-product showed only one peak, which indicates that bisphosphine catalysts do not partake in olefin isomerization.[118]

In previous attempts to eliminate this isomerization,[122] acids (e.g. trifluoroacetic acid, phenylphosphoric acid) or benzoquinones have been added during metatheses that presumably titrate the ruthenium-hydride species (e.g. **Figure 26B**). It has also been reported that removal of the impurities from the catalyst by silica gel chromatography eliminates isomerization activity.[123] Both of these methods were applied to the metathesis of vinyl Trolox **61** and heptenylBODIPY **58b** with Grubbs Generation II catalyst **59** and the reactions proceeded with average yields of 50% (**Table 3**). Even though the purities were drastically improved in comparison to the original Grubbs Generation II-catalyzed reaction, these samples would still need to undergo pHPLC for further purification.

The Hoveyda-Grubbs Generation II catalyst **80** differs from the Grubbs catalysts by the replacement of a phosphine and benzylidene ligand with an isopropoxy-phenylmethylene ligand that forms a 'boomerang complex' with the metal centre.[124] When this catalyst was used during the metathesis, an improvement in the composition was achieved (**Table 3**), but pHPLC would still need to be used for final purification. The *o*-tolyl derivative of Hoveyda-Grubbs Generation II catalyst **81** was developed to

facilitate easier interaction between catalyst and substrate (e.g. bulky alkene) because of its smaller *N*-heterocyclic carbene ligand.[125] Interestingly, the use of this catalyst for the metathesis showed only the one peak on the HPLC chromatogram corresponding to the desired homologue.

The results from **Table 3** seem to show that the *o*-tolyl derivative of Hoveyda Grubbs Generation II catalyst, **81**, has the lowest activity for olefin isomerization and should replace Grubbs Generation II catalyst in future metatheses. With BDP-α-Tocs **42a-c** in hand, binding assays to α-TTP could proceed (see Section 2.5).

2.4 Synthesis of a Thienyl Derivative of BDP-α-Toc

It was also our intention to synthesize another fluorescent analogue of vitamin E that has extended conjugation of the fluorophore by incorporating a thienyl group (thienylBODIPY or TBDP) (**Figure 29**). This new fluorophore is larger than the simpler dimethylBODIPY and further accentuates the curvature of the analogue in **82**. Molecular modeling of this analogue revealed that a methylene bridge of four carbons would afford a molecular length most similar to C9-NBD-α-Toc **11d**. In addition to C4-TBDP-α-Toc **82a**, a five methylene bridged analogue, **82b**, was made.

To synthesize this thienyl derivative, a pyrrole-2-carboxaldehyde that possesses a thienyl group instead of two methyl groups was required. Unlike 3,5-dimethylpyrrole-2-carboxaldehyde, 5-(2'-thienyl)pyrrole-2-carboxaldehyde is not commercially available

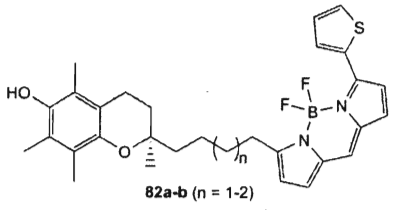
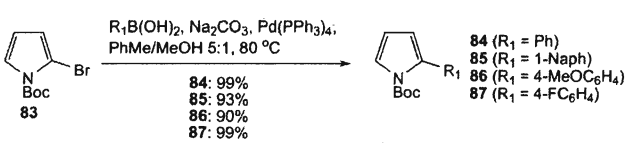


Figure 29. Structure of the TBDP-α-Toc target molecule

and must be prepared. Stille coupling using stannylpyrroles has been used to make 2-arylpyrroles, but tin is a toxic substance.[126] The Paal-Knorr cyclization of 4-oxo-4-phenylbutanal with ammonium acetate is reported to synthesize 2-arylpyrroles *de novo*.[127] This method is high yielding and can be used to prepare 2-arylpyrroles on a large scale. A methyl analogue of thienylpyrrole was reported to yield 55% of the corresponding 2-aryl derivative by this reaction.[127] Other methods for the preparation of 2-arylpyrroles include the photochemical arylation of 5-iodo-2-formylpyrrole with thiophene,[128] the condensation between methylazidoacetates and β-aryl acroleins with subsequent cyclization and decarboxylation,[84] and many more alternative methods that require *de novo* synthesis of the pyrrole ring.[129,130]

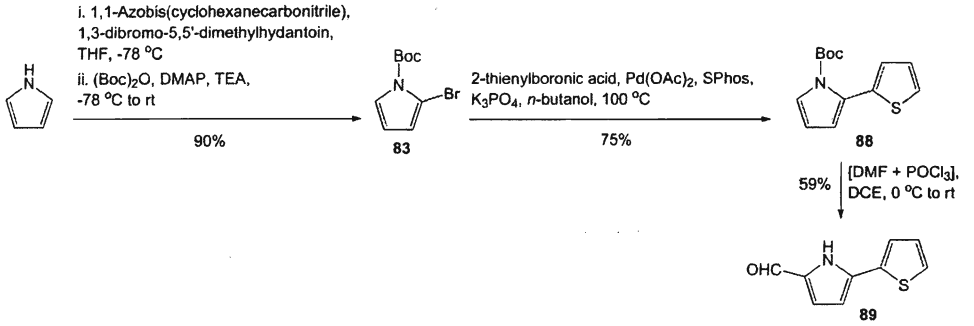
A much simpler way of making 2-arylpyrroles is through the Suzuki coupling of arylboronic acids and *N*-Boc-2-bromopyrroles **83** (**Scheme 20**). This type of cross coupling has been used before to generate other BODIPY dyes with different photophysical properties.[27] Successful coupling of **83** to various arylboronic acids, such as phenyl- and naphthylboronic acid, were reported with yields of 90% or greater.[27,131] Pyrrylboronic acids coupled to 2-arylbromides have been used as the alternative for these couplings,[132] but not often because of their inherent instabilities (e.g. deboronification of pyrrole upon heating).[133] This method was chosen to generate TBDP-α-Tocs **82a-b**.

83 can be prepared by brominating pyrrole and trapping the unstable 2-bromopyrrole intermediate *in situ* with a *N*-protecting group that withdraws electrons



Scheme 20. Suzuki coupling to generate various 2-arylpyrroles[27]

from the aromatic ring, such as Boc (**Scheme 21**). 1,3-Dibromo-5,5-dimethylhydantoin is reported to be much more regioselective than *N*-bromosuccinimide and therefore became the brominating agent of choice.[89,134] *N*-Boc-2-bromopyrrole **83** was prepared by this method with a yield of 90%.



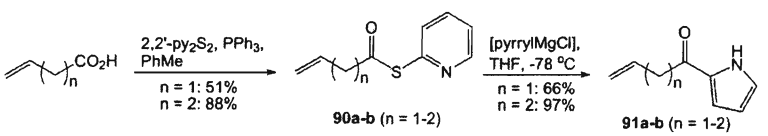
Scheme 21. Synthesis of 5-(2'-thienyl)pyrrole-2-carboxaldehyde

When the Suzuki coupling of **83** to 2-thienylboronic acid was attempted using the conditions listed in **Scheme 20**, product **88** was not produced. Instead, the reaction turned dark brown and **83** was never recovered. Unfortunately the paper that reported this method did not use heteroarylboronic acids.[27] Difficulties often arise during the coupling of thienylboronic acids. Their instability in polar solvents often results in the protodeboronation of the aromatic ring.[83]

In recent years, a higher yielding and more efficient Suzuki-Miyaura coupling reaction that uses a palladium precatalyst and a monophosphine ligand (SPhos) was reported.[83] This new approach is much more reactive than the method reported previously[27] because the catalyst generated *in situ* is able to bind to 2-thienylboronic acid.[83] When this new catalytic system was used to couple the boronic acid to **83**, 2-thienylpyrrole **88** was cleanly synthesized in 75% yield (**Scheme 21**).

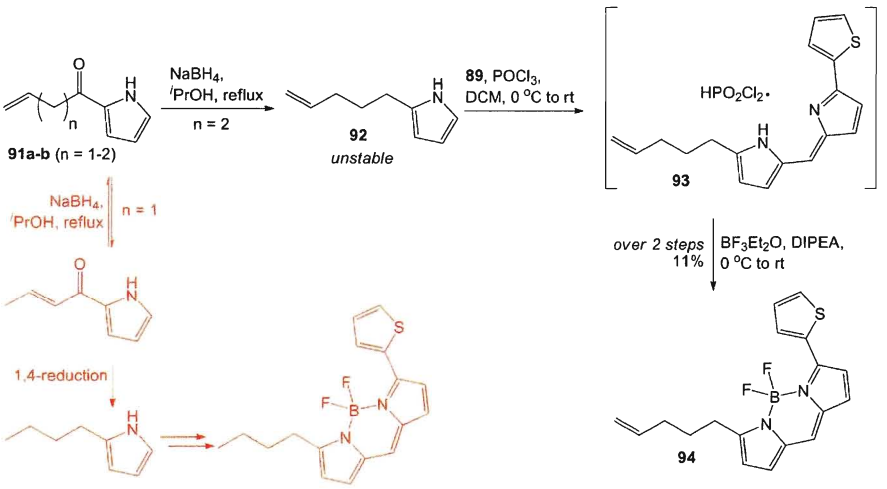
With **88** now prepared, it must be deprotected and formylated to generate **89** that is required for the condensation. The Vilsmeier-Haack reaction with thienylpyrroles has been shown to selectively formylate the pyrrole ring because of its greater electron-richness than thiophene.[84] 2-Thienylpyrroles are reported to be unstable species, even at reduced temperatures (e.g. –20 °C).[62] Stabilization is only obtained after an electron-withdrawing group (e.g. *N*-Boc, CHO) is added because the electron-richness is lowered. Based on our earlier experience with the Vilsmeier-Haack reaction on *N*-Boc-succinamidal **26**, the Boc protecting group was removed because of the acidic conditions. It was thought that if the *N*-Boc group of **88** is removed in addition to formylation of the pyrrole ring, then **89** could be made in one-pot without isolating the unstable 2-thienylpyrrole intermediate. 5-(2'-Thienyl)pyrrole-2-carboxaldehyde **89** was successfully prepared by this method in 59% yield.

Both 3-butenoic and 4-pentenoic acid underwent the same chemistry as the long-chain ω-alkenoic acids in order to prepare the alkenylpyrroles needed for condensation (**Scheme 22**). The yields from reacting 3-butenoic acid were much lower than those obtained previously, however. These reactions on short-chain ω-alkenoic acid have not been reported and the results seem to indicate that 3-butenoic acid is not amenable to these conditions. The generation of pyridyl thioate **90b** and ketopyrrole **91b** from 4-pentenoic acid gave yields that were much more similar to the long-chain ω-alkenoic acids (**Scheme 22**).



Scheme 22. Synthesis of short-chained ketopyrroles

The reduction of butenoylpyrrole **91a** with sodium borohydride followed by pyrrole condensation and treatment with boron trifluoride diethyl etherate failed to give the corresponding alkenylTBDP. Instead, a TBDP fluorophore with a fully saturated butyl tail was generated in 6% yield. The saturation of the butenyl group was likely caused by the isomerization of butenoylpyrrole **91a** to the α,β-unsaturated ketone during treatment with sodium borohydride followed by a 1,4-reduction (see **Scheme 23**). Failure to synthesize butenylTBDP by this method eliminated access to C4-TBDP-α-Toc **82a**.



Scheme 23. Synthesis of pentenylTBDP **94** from its ketopyrrole

Reduction of ketopyrrole **91b** followed by dipyrromethene formation and treatment with boron trifluoride diethyl etherate yielded **94** in 11% (over two steps) (**Scheme 23**). Unlike the red oils produced by the dimethylBODIPY intermediates, this thienyl derivative was a dark purple oil. This intermediate is excited at 561 nm and fluoresces at 570 nm with an extinction coefficient (ε) of 78 000 $M^{-1}cm^{-1}$ (**Figure 30**). The overall yield was considerably lower than that obtained from the dimethylBODIPY

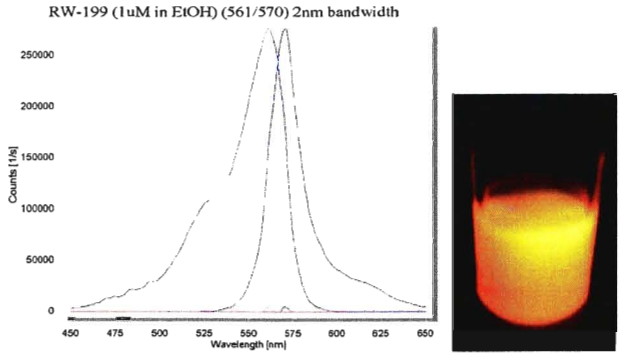
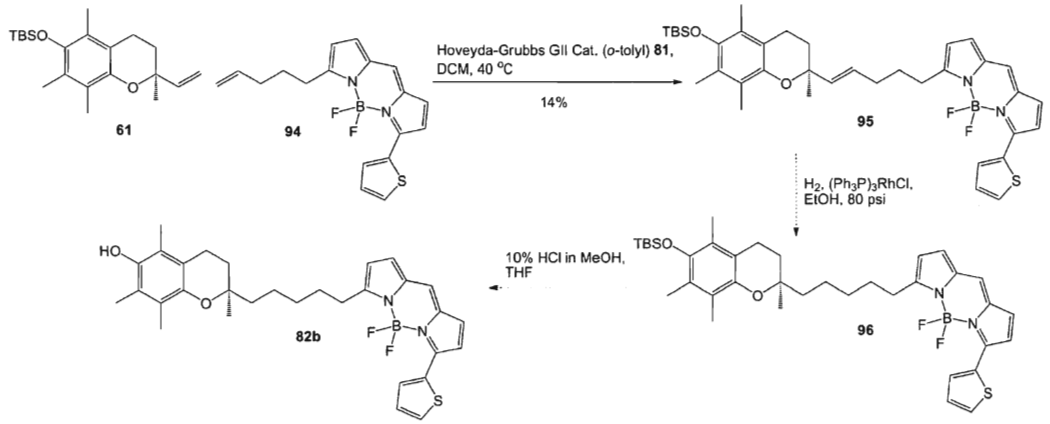


Figure 30. Excitation and emission spectrum of pentenylTBDP

series (i.e. 34-40%), likely because the fewer substituents on this dipyrromethene backbone lower the stability.

Olefin metathesis between vinyl Trolox **61** and **94** using the *o*-tolyl derivative of Hoveyda-Grubbs Generation II catalyst **81** generated product **95** with 14% yield (**Scheme 24**). This yield was much lower than those obtained from the previous metatheses. This is because the alkene on **94** is not only closer to the fluorophore, which increases steric hindrance, but also this fluorophore is much bulkier than the dimethylBODIPY fluorophore. Due to the low yields obtained from the last two reactions, only milligrams of **95** were obtained. This was not enough material to proceed through the hydrogenation so further attempts to produce **82b** were halted (**Scheme 24**).



Scheme 24. Metathesis and unperformed chemistry (dashed arrows) of C5-TBDP-α-Toc

2.5 Fluorescence Binding Studies of BDP-α-Toc

In order for BDP-α-Tocs **42a-c** to be of any utility they must possess some binding ability towards α-TTP. This binding should be both specific and reversible in order for these fluorescent probes to be suitable analogues of vitamin E. Previously, the dissociation constants (K_d) of fluorescent vitamin E analogues **11a-d**, **12a-d**, **13a-d**, and **14a-d** were determined (**Table 4**) and C9-NBD-α-Toc **11d** was shown to have the highest affinity to the protein.[79] The dissociation constant for α-tocopherol, the natural ligand for α-TTP, is 25 nM.[11] The K_d values for DAN-α-Tocs **13a-d** and NMA-α-Tocs **14a-d** could not be determined due to non-saturable binding with α-TTP. Only C9-AO-α-Toc **12d** from the anthroyloxy series was found to bind specifically.[79]

Table 4. Dissociation constants for fluorescent tocopherol analogues **11a-d** and **12d**[79]

NBD-α-Toc	K_d (nM)	AO-α-Toc	K_d (nM)
11a	299 ± 37	**12a**	-
11b	106 ± 21	**12b**	-
11c	142 ± 35	**12c**	-
11d	56 ± 15	**12d**	279 ± 124

The dissociation constants defined as [α-TTP][ligand]/[α-TTP-ligand] were assessed from fluorescence titrations of TTP with BDP-α-Tocs **42a-c** and calculated from the one-site binding model using Prism software. The average results of the multiple titrations on a range of ligand concentrations (3-640 nM) are listed in **Table 5**. C8-BDP-α-Toc **42c** appears to have the best affinity (K_d = 100 nM) for α-TTP. Literature shows that the biological function of some analogues generally increases as the linker between the pharmacophore and fluorophore is increased.[62,135] Although the results from these binding assays follow this trend, as seen with the K_d values of both NBD-α-Tocs and BDP-α-Tocs, it is just a general rule and does not apply to every case.

Table 5. Dissociation constants for BDP-α-Tocs **42a-c**

BDP-α-Toc	K_d (nM)
42a	436 ± 28
42b	146 ± 11
42c	98 ± 6

These binding assays were run with the concentration of α-TTP set to 1 μM. As the concentration of each ligand was increased upon subsequent additions, the titration curves were gradually produced (**Figures 31-33**) and were then used to determine the dissociation constants. Oddly, the maximal fluorescence appears to plateau at concentrations of ligand (~ 650 nM) that is sub-stoichiometric to the protein (1 μM). These results were not observed previously with the other fluorescent vitamin E analogues. C9-NBD-α-Toc was needed at concentrations five times in excess to protein until a plateau was reached from its binding curve.[79] This was expected since binding is in equilibrium. Radioligand binding experiments have previously established that one molecule of α-tocopherol bound per molecule of α-TTP,[136] so obtaining sub-stoichiometric plateaus (**Figure 31-33**) cannot be from non-native protein.

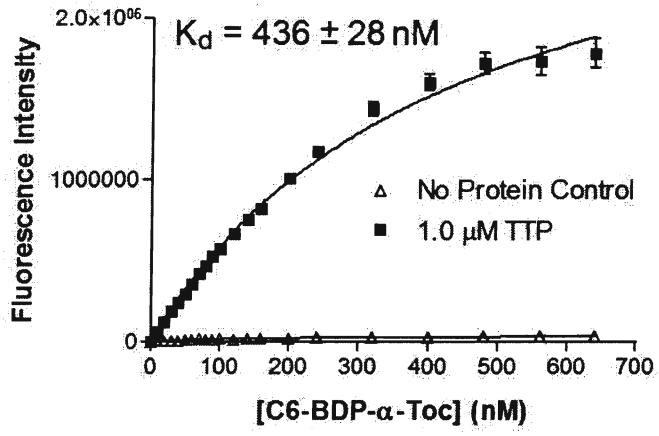


Figure 31. Titration curves showing the increase in fluorescence intensity at 514 nm during sequential additions of the C6-BDP-α-Toc, **42a** (black squares), to a 1.0 μM solution of α-TTP in SET buffer. The curves are fitted to a one-site binding model. Averages and standard errors of triplicate data sets are reported. λ_{ex} = 506 nm.

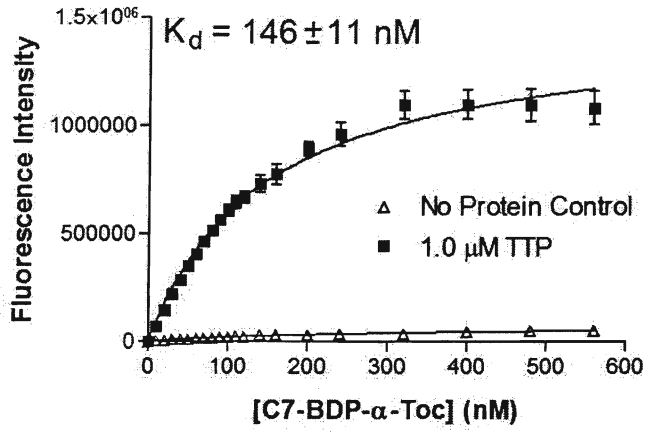


Figure 32. Titration curves showing the increase in fluorescence intensity at 514 nm during sequential additions of the C7-BDP-α-Toc, **42b** (black squares), to a 1.0 μM solution of α-TTP in SET buffer. The curves are fitted to a one-site binding model. Averages and standard errors of triplicate data sets are reported. λ_{ex} = 506 nm.

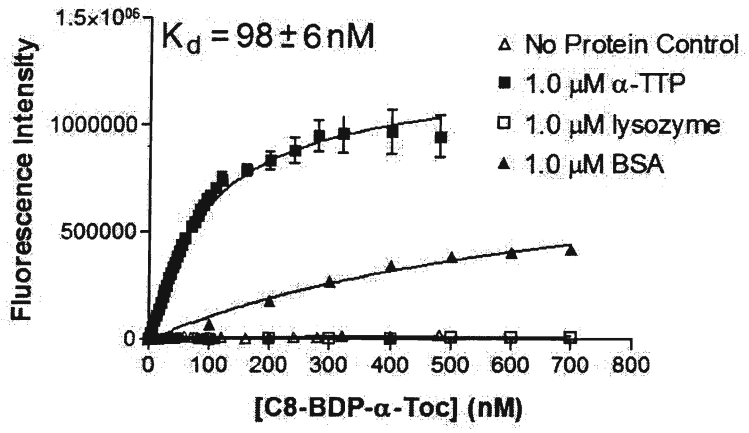


Figure 33. Titration curves showing the increase in fluorescence intensity at 514 nm during sequential additions of the C8-BDP-α-Toc, **42c** (black squares), to a 1.0 μM solution of α-TTP in SET buffer. Additional binding curves of **42c** to 1.0 μM lysozyme and 1.0 μM BSA are also reported. The curves are fitted to a one-site binding model. Averages and standard errors of triplicate data sets are reported. λ_{ex} = 506 nm.

The fluorescence signal plateaus at sub-stoichiometric concentrations of ligand-to-protein initially raised concerns with non-specific binding of the ligand to α-TTP, but that does not explain why the K_d values are low. Additional binding curves for both lysozyme and bovine serum albumin (BSA) with C8-BDP-α-Toc were run. Lysozyme is

a protein with no hydrophobic pockets that would allow binding to the ligand and therefore acted as a negative control. BSA possesses many hydrophobic pockets that does allow non-specific binding with the ligand,[137,138] as is seen in **Figure 33**.

Despite the ability of **42c** to bind non-specifically to BSA, it was important to demonstrate that we could compete the ligand from α-TTP to ensure that a specific binding event was observed. To achieve this, the protein was first equilibrated with **42c** and then titrated with α-tocopherol. This assay was then repeated using a solution of cholesterol in absolute ethanol as a non-competent control since cholesterol is known to have very low affinity to α-TTP.[11]

C8-BDP-α-Toc (**Figure 34**) exhibits a dose-dependent decrease in fluorescence upon the addition of α-tocopherol suggesting that the environmentally sensitive fluorophore is displaced from the hydrophobic-binding pocket of α-TTP by the native ligand. By 4 μM of α-tocopherol, there appears to be near total displacement of C8-BDP-α-Toc by the native ligand. There is an obvious difference in the fraction of remaining

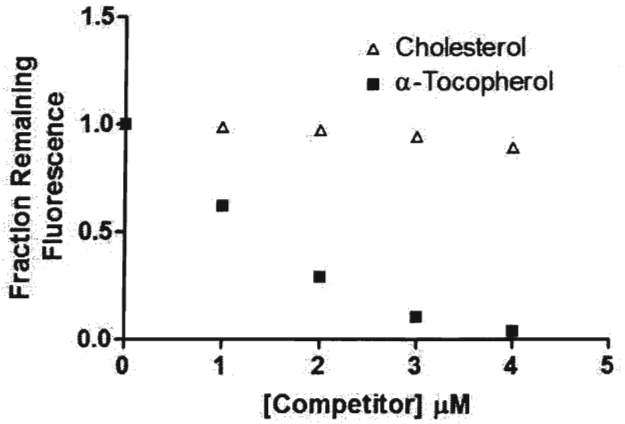


Figure 34. Competitive displacement of 1 μM C8-BDP-α-Toc, **42c**, bound to 0.2 μM α-TTP in SET buffer by addition of increasing amounts of (*RRR*)-α-tocopherol or cholesterol in ethanol. Fluorescence was monitored at 514 nm. Averages and standard errors of triplicate data sets are reported for tocopherol.

fluorescence when cholesterol is added to this assay as the control. These results indicate that there is indeed a specific binding event that occurs between C8-BDP-α-Toc and α-TTP.

It was observed that upon adding increasing amounts of cholesterol, the fluorescence began to decrease in intensity (**Figure 35**). At 40 μM cholesterol, the fraction of remaining fluorescence was reduced to near 50%. Since cholesterol does not interact with α-TTP, it was speculated that ethanol, the solvent in which all stock ligand and control solutions were made, was in fact affecting the affinity between C8-BDP-α-Toc and α-TTP. It was previously shown that ethanol affects the binding of sterol carrier protein-2 (SCP-2) with several lipids, such as cholesterol, PC, and stearic acid.[139] In the presence of 25 mM ethanol, NBD-cholesterol and NBP-PC were shown to have reduced affinity with the carrier protein. In the absence of ethanol, NBD-cholesterol and NBD-PC have association constants of $8.95 \pm 0.6 \times 10^7$ M and $9.1 \pm 1 \times 10^6$ M to SCP-2, respectively. By 25 mM of ethanol, when significant differences in binding for both ligands were first observed, the association constants were reduced to approximately 70%

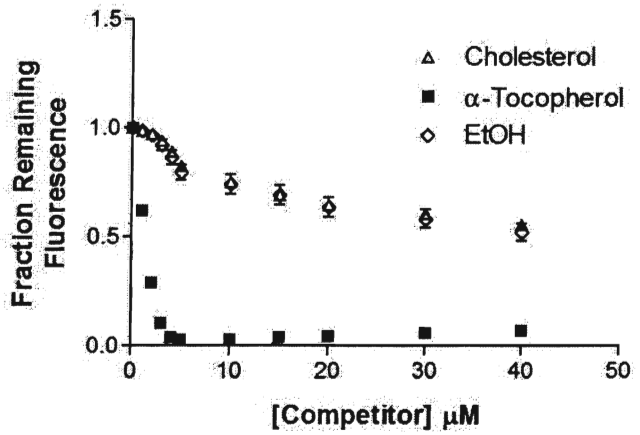


Figure 35. The effect of binding of C8-BDP-α-Toc to α-TTP in SET buffer by addition of ethanol. Fluorescence was monitored at 514 nm. Averages and standard errors of triplicate data sets are reported for ethanol.

of the original values.[139] The decrease in fluorescence of C8-BDP-α-Toc bound to α-TTP first becomes apparent at 3 μM cholesterol in **Figure 34**, which equates to 6 μl (or 34 mM) ethanol (total cuvette volume: 3 ml).

It was suggested that ethanol interacts with the hydrophobic sites within the binding pocket of SCP-2 and inhibits the interaction with the ligand.[139] Therefore, absolute ethanol was used to titrate a sample of protein equilibrated with **42c** and was shown to have a curve identical to the cholesterol control, confirming that ethanol was in fact reducing affinity of ligand to α-TTP (**Figure 35**). This would also explain how a sub-stoichiometric plateau from the binding curves (**Figures 31-33**) seems to be obtained. Ethanol removes the BDP-α-Tocs from α-TTP, appearing to 'saturate' at ligand concentrations less than the protein. This is the point at which ligand removal by ethanol was competing effectively with the binding of ligand to α-TTP. When the additions of ligand in ethanol were continued beyond the point of plateau, a decrease in fluorescence intensity was observed, suggesting that the removal of ligand from α-TTP by ethanol was occurring at a larger rate than the rate of ligand binding. There was a slight increase in fluorescence after the addition of 30-40 μM of (*RRR*)-α-tocopherol as the competitor (**Figure 35**), but this is likely because unbound C8-BDP-α-Toc is exposed to increasing concentrations of ethanol, in which the ligand fluoresces more intensely. Alternative organic solvents were sought that did not remove the nature of the binding site of α-TTP (e.g. DMF, THF) but each showed a decrease in fluorescence greater than ethanol. Despite these solvents seeming to affect the nature of the binding site, it is certain that C8-BDP-α-Toc **42c** binds to α-TTP specifically with a fairly low K_d. For now, the

binding constants obtained with ethanol as the best organic solvent (**Table 5**) can be considered as approximations.

3 CONCLUSIONS AND FUTURE PERSPECTIVES

With the successful preparation and characterization of the BDP-α-Tocs series now completed, these molecules can be used to further study the intracellular transfer of vitamin E. The synthesis of these molecules was met with challenges unprecedented to the syntheses of our previous analogues but all were eventually overcome. C8-BDP-α-Toc **42c** has been shown to bind specifically and reversibly to α-TTP, being displaced by natural ligand (*RRR*)-α-tocopherol. This ligand also has great affinity towards the protein with an apparent dissociation constant of 98 ± 6 nM.

The next step for this project is to incorporate these ligands into cells *in vitro* to learn about the transfer of tocopherols in hepatocytes. In addition, the synthesis of C5-TBDP-α-Toc shall continue so that it can one day be used alongside C8-BDP-α-Toc to study the storage mechanism of excess vitamin E in cells.

4 EXPERIMENTAL

4.1 General Methods

4.1.1 Chromatography

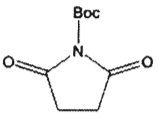
Flash chromatography was carried out on either silica gel (70-230 Å mesh, Alfa Aesar, Ward Hill, MA or Desican, Inc., Markham, ON) or alumina gel (150 Å mesh, Sigma-Aldrich, Oakville, ON) with the indicated solvent systems. Analytical thin layer chromatography (TLC) was performed on either silica gel 60 Å F_{254} plates (EMD Chemicals, Inc.) or alumina gel 60 Å F_{254} plates (EM Science).

4.1.2 Spectroscopy

Spectroscopic analysis of compounds was performed by ^{1}H, ^{13}C, ^{11}B, and ^{19}F NMR obtained using a Bruker Avance DPX-300 Digital FT-NMR spectrometer at 300 (or 600) MHz, 75 (or 151) MHz, 96 (or 193) MHz, and 282 MHz, respectively. Cambridge Isotope Laboratories, Inc. deuterated chloroform (99.8% pure) was used as the solvent with the internal reference of residual chloroform (^{1}H = 7.26 ppm, ^{13}C = 77.0 ppm). Chemical shifts are reported in ppm (δ) (multiplicity, coupling constant in Hz, number of protons, and assignment). Multiplicity is designated using the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). ^{1}H NMR (300 MHz) possesses an error of ± 0.45 Hz. Low resolution mass spectra (MS) were recorded on a Carlo Erba/ Kratos GC/ MS Concept 1S double focusing mass spectrometer interfaced to a Kratos DART acquisition system and a SUN SPARC workstation. Samples were introduced through a direct inlet system and ions were generated using electron impact (EI) at 70 eV, ESI, or fast atom bombardment (FAB) sources and are reported as *m/z* values for the parent peak and major fragments. UV/Vis spectra were

obtained using Unicam UV/Vis Spectrometer in conjunction with Vision32 software. Fluorimetric data was obtained using QuantaMaster Model QM-2001-4 cuvette-based L-format scanning spectrofluorometer from Photon Technology International (PTI) in conjunction with FeliX32 program software. All cuvettes used were made of special optical glass (10.00 mm, 3 ml) and provided by Hellma. HPLC was performed using a Synergi Hydro-RP column (C18, 4 μm, 150x4.60 mm) by Phenomenex on a Waters 626 pump and photodiode array detector in combination with Millennium 32 v.3.2 program software. pHPLC was performed using a PrimeSphere column (C18, 5 μm, 250x10.00 mm) by Phenomenex on a Hitachi L-6000 pump in combination with a Hitachi Integrator D-7500 for output.

4.2 Preparation of Compounds

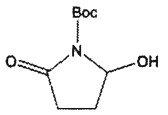


4.2.1 Synthesis of *tert*-butyl 2,5-dioxopyrrolidine-1-carboxylate (25)[90]

To a solution of succinimide (5.00 g, 50.5 mmol) in acetonitrile (100 ml) was added Boc_2O (13.9 ml, 60.6 mmol) and DMAP (617 mg, 5.05 mmol). After 4 h, the solvent was removed *in vacuo* and the crude material was subjected to flash chromatography (hexanes/ethyl acetate 5:2). The eluted material was then recrystallized from hexanes/dichloromethane to afford **25** (7.76 g, 39.0 mmol, 77%).

Pale yellow, square crystals, R_f = (0.20, hexanes/ethyl acetate 5:2, visualized by $KMnO_4$), mp 89-90 °C [lit.[90] 86 °C]. ^1H NMR (300 MHz, $CDCl_3$) δ 2.74 (s, 4H, 2x$C\underline{H}_2$), 1.54 (s, 9H, 3x$C\underline{H}_3$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.8, 146.4, 86.1, 28.4, 27.6. MS (+EI) *m/z* 184 ($[M-CH_3]^+$, 1.4%), 144 (2.0%), 126 (44.8%), 100 (31.5%), 70 (16.8%), 57

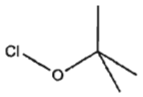
(100%), 41 (64.0%). HRMS (EI) calculated for $C_8H_{10}NO_4^{\bullet+}$: 184.06098, found 184.06064.



4.2.2 Synthesis of *tert*-butyl 2-hydroxy-5-oxopyrrolidine-1-carboxylate (26)[90]

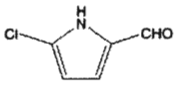
25 (513 mg, 2.58 mmol) was dissolved in a tetrahydrofuran/ethanol mixture 1:1 (7.5 ml) and cooled to –50 °C. Sodium borohydride (209 mg, 5.40 mmol) was added slowly over 0.5 h so that the temperature did not to exceed –50 °C. After 5 h, 5% hydrochloric acid (1.3 ml) was added to quench the reaction as the temperature was slowly raised to ambient. The mixture was then filtered using a Hirsch funnel and the filtrate was condensed *in vacuo*. The residue was extracted with dichloromethane and then chromatographed on silica (ethyl acetate/hexanes 8:1). Recrystallization from hexanes afforded pure amidal **26** (340 mg, 1.69 mmol, 66%).

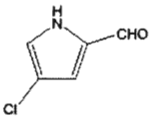
White solid, R_f = (0.45, ethyl acetate/hexanes 4:1, visualized by $KMnO_4$), mp 77-78 °C [lit.[90] 78-79 °C]. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.57-5.55 (m, 1H, C<u>H</u>OH), 4.36-4.36 (d, J = 2.1 Hz, 1H, O<u>H</u>), 2.64-2.52 (m, 1H, C<u>H</u>$_2$C=O), 2.27-2.17 (m, 1H, C<u>H</u>$_2$C=O), 2.09-1.96 (m, 1H, C<u>H</u>$_2$CHOH), 1.85-1.75 (m, 1H, C<u>H</u>$_2$CHOH), 1.35 (s, 9H, 3xC<u>H</u>$_3$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.1, 149.8, 83.1, 81.6, 30.1, 27.5, 25.4. MS (+EI) *m/z* 201 (M^+, 0.8%), 186 (1.1%), 146 (15.0%), 128 (11.9%), 85 (30.1%), 57 (100%), 41 (52.0%). HRMS (EI) calculated for $C_9H_{15}NO_4$: 201.10011, found 201.09653.

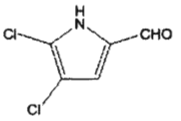


4.2.3 Synthesis of *tert*-butyl hypochlorite (28)[92]

Javex® bleach (0.5 l, 5.25% NaOCl) was cooled to 0 °C in the absence of light. Next, a solution of *tert*-butanol (37.0 ml, 390 mmol) and glacial acetic acid (24.5 ml, 428 mol) was added and stirred for 3 min. From this reaction mixture, the aqueous layer was removed and the organic layer was washed with 10% aqueous sodium carbonate (50 ml) and then water (50 ml). Product **28** (21.7 g, 0.200 mol, 51%) was a yellow transparent liquid which must be stored below 0 °C in an amber jar.







4.2.4 Synthesis of 5-chloro-1*H*-pyrrole-2-carbaldehyde (23), 4-chloro-1*H*-pyrrole-2-carbaldehyde (29), and 4,5-dichloro-1*H*-pyrrole-2-carbaldehyde (30)[91]

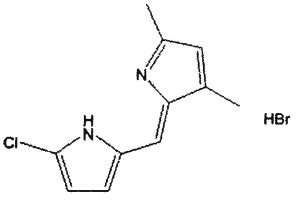
Pyrrole-2-carboxaldehyde (4.75 g, 50.0 mmol) was dissolved in carbon tetrachloride (200 ml) and cooled to 0 °C. Then a solution of *tert*-butyl hypochlorite (5.42 g, 50.0 mmol) in carbon tetrachloride (100 ml) was added dropwise using an addition funnel. After 3 h, the solvent was removed under reduced pressure and the crude material was chromatographed (hexanes/tetrahydrofuran 15:1) to **23** (1.68 g, 13.0 mmol, 26%) and a crude mixture of **29** and **30**. **29** (667 mg, 5.15 mmol, 10%) can be isolated from **30** (888 mg, 5.41 mmol, 11%) from recrystallization in hexanes/diethyl ether 1:1

23: off-white flaky powder, R_f = (0.15, hexanes/tetrahydrofuran 15:1), mp 103-105 °C [lit.[90] 110 °C]. ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.06 (br s, 1H, NH), 9.38 (s, 1H, CHO), 6.97-6.94 (m, 1H, CHCCHO), 6.23-6.21 (m, 1H, CHCCl). ^{13}C NMR (75 MHz,

$CDCl_3$) δ 178.5, 131.7, 126.6, 110.1. MS (+EI) *m/z* 129 (M^+, 100%), 100 (34.2%), 73 (35.9%), 64 (15.6%), 50 (13.4%), 41 (12.5%). HRMS (EI) calculated for C_5H_4ClNO: 128.99814, found 128.99770.

29: light brown lustrous needles, R_f = (0.25, hexanes/tetrahydrofuran 15:1), mp 128-129 °C [lit.[140] 129-129.5 °C]. ^1H NMR (300 MHz, $CDCl_3$) δ 10.35 (br s, 1H, NH), 9.44 (s, 1H, CHO), 7.11-7.10 (m, 1H, CHNH), 6.91-6.90 (m, 1H, CHCCHO). ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.2, 131.6, 124.2, 119.9, 115.1. MS (+EI) *m/z* 129 (M^+, 100%), 100 (38.3%), 73 (27.9%), 65 (17.0%), 44 (14.7%). HRMS (EI) calculated for C_5H_4ClNO: 128.99814, found 128.99829.

30: off-white needles, R_f = (0.25, hexanes/tetrahydrofuran 15:1), mp 149 °C [lit.[140] 143.5-145 °C]. ^1H NMR (300 MHz, $CDCl_3$) δ 10.46 (br s, 1H, NH), 9.55 (s, 1H, CHO), 6.21-6.20 (d, J = 2.1 Hz, 1H, CHNH). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.4, 127.3, 125.4, 125.2, 110.2. MS (+EI) *m/z* 162 ($[M-H]^+$, 100%), 134 (20.1%), 107 (21.9%), 73 (25.5%), 55 (16.5%), 45 (18.6%). HRMS (EI) calculated for $C_5H_3Cl_2NO$: 162.95917, found 162.95920.

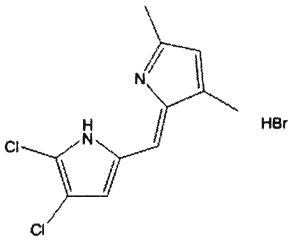


4.2.5 Synthesis of (*Z*)-2-chloro-5-((3,5-dimethyl-2*H*-pyrrol-2-ylidene)methyl)-1*H*-pyrrole hydrobromide (34)[26]

23 (102 mg, 0.790 mmol) and 2,4-dimethylpyrrole (81.3 µl, 0.790 mmol) were dissolved in absolute ethanol (3.5 ml) and cooled to 0 °C. Hydrobromic acid (133 µl, 1.22 mmol) was added dropwise and this was stirred for 1 h. The mixture was filtered

using a Hirsch funnel and the precipitate was washed with cold ethanol to yield **34** (104.5 mg, 0.360 mmol, 46%).

Bright orange powder, R_f = (0.93, dichloromethane/methanol 10:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.91 (br m, 2H, 2xNH), 7.09-7.07 (m, 2H), 6.40-6.38 (m, 1H), 6.30 (s, 1H), 2.75 (s, 3H, CH_3), 2.38 (s, 3H, CH_3).^{13}C NMR (75 MHz, $CDCl_3$) δ 162.2, 150.2, 138.4, 133.2, 129.4, 126.4, 124.4, 120.1, 114.7, 15.0, 12.3. MS (+FAB) *m/z* 207 ($[MH]^+$, 100%), 171 (6.7%). HRMS (FAB) calculated for $C_{11}H_{12}N_2Cl^+$: 207.06890, found 207.06722.

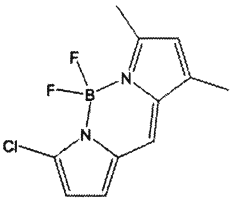


4.2.6 Synthesis of (*Z*)-2,3-dichloro-5-((3,5-dimethyl-2*H*-pyrrol-2-ylidene)methyl)-1*H*-pyrrole hydrobromide (35)[26]

30 (100 mg, 0.610 mmol) and 2,4-dimethylpyrrole (62.8 µl, 0.610 mmol) were dissolved in absolute ethanol (3.5 ml) and cooled to 0 °C. Hydrobromic acid (103 µl, 0.946 mmol) was added dropwise and this was stirred for 1 h. The mixture was filtered using a Hirsch funnel and the precipitate was washed with cold ethanol to yield **35** (95.5 mg, 0.299 mmol, 49%).

Bright reddish-orange powder, R_f = (0.85, dichloromethane/methanol 20:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 14.00-13.94 (br m, 2H, 2xNH), 7.26 (d, *J* = 2.1 Hz, 1H), 6.40-6.39 (d, *J* = 2.1 Hz, 1H), 6.34 (s, 1H), 2.75 (s, 3H, CH_3), 2.41 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.8, 151.1, 136.5, 134.1, 129.9, 123.1, 120.7, 119.7, 113.4,

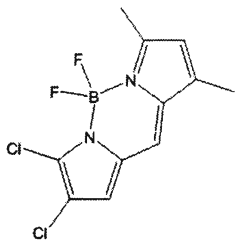
15.2, 12.3. MS (+FAB) *m/z* 241 ([MH]$^+$, 100%), 170 (8.2%), 57 (7.3%). HRMS (FAB) calculated for $C_{11}H_{11}N_2Cl_2^+$: 241.02993, found 241.02948.



4.2.7 Synthesis of 7-chloro-5,5-difluoro-1,3-dimethyl-5*H*-dipyrrolo[1,2-c:1',2'-f][1,3,2]diazaborinin-4-ium-5-uide (38)[26]

To a solution of **34** (105 mg, 0.363 mmol) in toluene (15 ml) was added boron trifluoride diethyl etherate (230 µl, 1.82 mmol) and DBU (163 µl, 1.09 mmol). The resulting mixture was heated to 90 °C for 2 h. Afterward, the solution was washed with water and the crude material was purified via chromatography (hexanes/diethyl ether 3:2) to give desired fluorophore **38** (66.8 mg, 0.261 mmol, 72%).

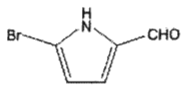
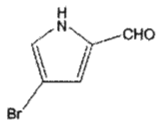
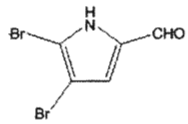
Red lustrous needles, R_f = (0.30, hexanes/diethyl ether 3:2), mp 166-167 °C. λ_{max} excitation in ethanol = 500 nm (ε_{500} = 58 000 $M^{-1}cm^{-1}$), λ_{max} emission in ethanol = 508 nm. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.03 (s, 1H), 6.83-6.82 (d, *J* = 2.4 Hz, 1H), 6.26-6.25 (d, *J* = 2.1 Hz, 1H), 6.15 (s, 1H), 2.58 (s, 3H, CH_3), 2.23 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.5, 145.8, 138.4, 136.3, 131.9, 126.7, 123.3, 121.6, 115.8. MS (+EI) *m/z* 254 (M^+, 100%), 234 ([M-HF]$^+$, 28.7%), 218 (27.4%), 204 (14.6%), 117 (20.9%), 65 (5.9%), 49 (5.8%). HRMS (EI) calculated for $C_{11}H_{10}BClN_2F_2$: 254.05936, found 254.05868.



4.2.8 Synthesis of 7,8-dichloro-5,5-difluoro-1,3-dimethyl-5*H*-dipyrrolo[1,2-c:1',2'-f][1,3,2]diazaborinin-4-ium-5-uide (39)[26]

To a solution of **35** (90.0 mg, 0.290 mmol) in toluene (15 ml) was added boron trifluoride diethyl etherate (348 µl, 2.74 mmol) and DBU (246 µl, 1.65 mmol). The resulting mixture was heated to 90 °C for 2 h. Afterward, the solution was washed with water and the crude material was purified via silica gel chromatography (hexanes/diethyl ether 3:2) to give desired fluorophore **39** (43.0 mg, 0.151 mmol, 52%).

Red lustrous needles, R_f = (0.35, hexanes/diethyl ether 3:2), decomposed when heated to 150 °C. λ_{max} excitation in ethanol = 497 nm (ε_{497} = 51 000 $M^{-1}cm^{-1}$), λ_{max} emission in ethanol = 506 nm. ^1H NMR (300 MHz, $CDCl_3$) δ 7.16 (s, 1H), 6.28 (s, 1H), 6.22 (s, 1H), 2.60 (s, 3H, CH_3), 2.30 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.3, 146.6, 136.9, 136.8, 129.0, 128.2, 122.5, 119.5, 114.3, 15.4, 11.5. MS (+EI) *m/z* 288 (M^+, 100%), 268 ([M-HF]$^+$, 35.6%), 253 (25.4%), 218 (57.8%), 169 (11.7%), 134 (25.7%), 109 (18.7%), 69 (10.7%), 57 (10.0%), 43 (8.9%). HRMS (EI) calculated for $C_{11}H_9BCl_2N_2F_2$: 288.02039, found 288.02100.

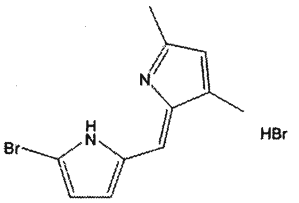
4.2.9 Synthesis of 5-bromo-1*H*-pyrrole-2-carbaldehyde (31), 4-bromo-1*H*-pyrrole-2-carbaldehyde (32), and 4,5-dibromo-1*H*-pyrrole-2-carbaldehyde (33)[93]

Pyrrole-2-carboxaldehyde (2.55 g, 26.8 mmol) was dissolved in carbon tetrachloride (200 ml) and heated to 70 °C. To this was added dropwise a solution of bromine (1.38 ml, 26.8 mmol) in carbon tetrachloride (50 ml). After 1.5 h, 20% aqueous sodium carbonate was added to neutralize evolved HBr and the reaction mixture was extracted with diethyl ether. Chromatography (hexanes/tetrahydrofuran 15:1) afforded two partially purified fractions. The first fraction contained **32** (889.3 mg, 5.11 mmol, 19%) and recovered pyrrole-2-carboxaldehyde (443.7 mg, 4.67 mmol). From this mixture, **32** can be further purified by recrystallization from hexanes/diethyl ether 1:1. The second fraction contained **31** (676.6 mg, 3.89 mmol, 14%) and **33** (983.4 mg, 3.89 mmol, 14%). From this mixture, **31** can be further purified by sublimation (60 °C at 0.5 Torr for 6 h).

31: white lustrous crystals, R_f = (0.20, hexanes/tetrahydrofuran 15:1), mp 90-92 °C [lit.[93] 93-94 °C]. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.99 (br s, 1H, NH), 9.37 (s, 1H, CHO), 6.94-6.91 (dd, J = 4.2 Hz, J' = 2.4 Hz, 1H, CHCCHO), 6.23-6.21 (dd, J = 4.2 Hz, J' = 2.4 Hz, 1H, CHCBr). ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.2, 133.7, 123.2, 113.8, 112.0. MS (+EI) *m/z* 173 (M^+, 100%), 144 (17.3%), 119 (12.2%), 93 (3.3%), 79 (2.2%), 64 (8.0%), 44 (7.2%). HRMS (EI) calculated for C_5H_4BrNO: 172.94762, found 172.94685.

32: white lustrous crystals, R_f = (0.10, hexanes/tetrahydrofuran 15:1), mp 118-121 °C [lit.[93] 123-124 °C]. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.15 (br s, 1H, NH), 9.46 (s, 1H, CHO), 7.14-7.13 (dd, J = 1.5 Hz, J' = 1.5 Hz, 1H, CHNH), 6.99-6.97 (dd, J = 1.5 Hz, J' = 2.1 Hz, 1H, CHCCHO). ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.9, 132.6, 126.3, 122.3, 98.8. MS (+EI) *m/z* 173 (M^+, 100%), 144 (18.4%), 117 (8.5%), 95 (9.4%), 65 (12.4%), 44 (5.0%). HRMS (EI) calculated for C_5H_4BrNO: 172.94762, found 172.94744.

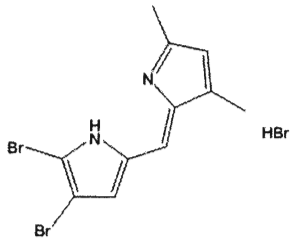
33: white powder, R_f = (0.20, hexanes/tetrahydrofuran), mp 157-159 °C [lit.[93] 155-156 °C]. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.44 (br s, 1H, NH), 9.35 (s, 1H, CHO), 6.98-6.97 (d, J = 1.2 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.7, 133.1, 123.0, 113.1, 101.9. MS (+EI) *m/z* 253 (M^+, 100%), 224 (12.9%), 197 (7.9%), 173 (4.0%), 144 (7.3%), 117 (15.7%), 93 (4.7%), 79 (3.6%), 64 (12.7%), 52 (3.1%). HRMS (EI) calculated for $C_5H_3Br_2NO$: 250.85814, found 250.85726.



4.2.10 Synthesis of (*Z*)-2-bromo-5-((3,5-dimethyl-2*H*-pyrrol-2-ylidene)methyl)-1*H*-pyrrole hydrobromide (36)[26]

31 (100 mg, 0.575 mmol) and 2,4-dimethylpyrrole (59.2 µl, 0.575 mmol) were dissolved in absolute ethanol (3.5 ml) and cooled to 0 °C. Hydrobromic acid (98.8 µl, 0.891 mmol) was added dropwise and this was stirred for 1 h. The mixture was filtered using a Hirsch funnel and the precipitate was washed with cold ethanol to yield **36** (63.0 mg, 0.185 mmol, 32%).

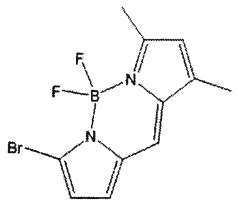
Bright orange powder, R_f = (0.50, hexanes/diethyl ether 3:2). ^{1}H NMR (600 MHz, $CDCl_3$) δ 13.89 (br m, 2H, 2xN<u>H</u>), 7.12 (s, 1H), 7.07-7.06 (dd, J = 3.0 Hz, J' = 3.0 Hz, 1H), 6.52-6.51 (d, J = 3.0 Hz, 1H), 6.32 (s, 1H), 2.76 (s, 3H, C<u>H</u>$_3$), 2.40 (s, 3H, C<u>H</u>$_3$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.5, 150.3, 133.3, 129.7, 128.2, 124.9, 123.9, 120.2, 118.4, 15.1, 12.4. MS (+FAB) *m/z* 251 ([MH]$^+$, 100%), 214 (9.9%), 171 (34.4%), 144 (7.3%), 102 (74.1%). HRMS (FAB) calculated for $C_{11}H_{12}BrN_2^+$: 251.01838, found 251.02037.



4.2.11 Synthesis of (Z)-2,3-dibromo-5-((3,5-dimethyl-2H-pyrrol-2-ylidene)methyl)-1H-pyrrole hydrobromide (37)[26]

33 (110 mg, 0.433 mmol) and 2,4-dimethylpyrrole (44.6 µl, 0.433 mmol) were dissolved in absolute ethanol (3 ml) and cooled to 0 °C. Hydrobromic acid (73.0 µl, 0.671 mmol) was added dropwise and this was stirred for 1 h. The mixture was filtered using a Hirsch funnel and the precipitate was washed with cold ethanol to yield **37** (69.3 mg, 0.169 mmol, 39%).

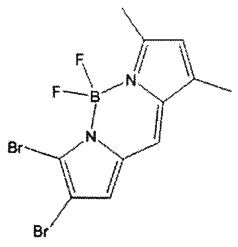
Bright reddish orange powder, R_f = (0.45, hexanes/diethyl ether 3:2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 14.12 (br m, 2H, 2xN<u>H</u>), 7.06 (s, 1H), 7.02 (s, 1H), 6.35 (s, 1H), 2.77 (s, 3H, C<u>H</u>$_3$), 2.39 (s, 3H, C<u>H</u>$_3$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.4, 151.1, 130.3, 127.3, 125.5, 120.9, 15.3, 12.4. MS (+FAB) *m/z* 331 ([MH]$^+$, 100%), 307 (13.3%), 251 (7.8%), 171 (21.7%), 136 (20.2%), 89 (10.2%), 55 (8.2%). HRMS (FAB) calculated for $C_{11}H_{11}Br_2N_2^+$: 328.92890, found 328.92960.



4.2.12 Synthesis of 7-bromo-5,5-difluoro-1,3-dimethyl-5*H*-dipyrrolo[1,2-c:1',2'-f][1,3,2]diazaborinin-4-ium-5-uide (40)[26]

To a solution of **36** (100 mg, 0.301 mmol) in toluene (15 ml) was added boron trifluoride diethyl etherate (191 µl, 1.51 mmol) and DBU (135 µl, 0.904 mmol). The resulting mixture was heated to 90 °C for 2 h. Afterward, the solution was washed with water and the crude material was purified via silica gel chromatography (hexanes/diethyl ether 3:2) to give desired fluorophore **40** (53.4 mg, 0.178 mmol, 59%).

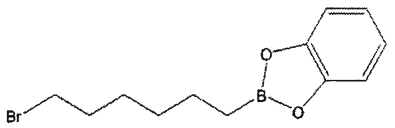
Red lustrous needles, R_f = (0.20, hexanes/diethyl ether 3:2), mp 166 °C. λ_{max} excitation in ethanol = 504 nm (ε_{504} = 68 000 $M^{-1}cm^{-1}$), λ_{max} emission in ethanol = 511 nm. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.03 (s, 1H), 6.80-6.79 (d, *J* = 3.9 Hz, 1H), 6.38-6.37 (d, *J* = 3.9 Hz, 1H), 6.16 (s, 1H), 2.58 (s, 3H, CH_3), 2.23 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.9, 145.9, 136.5, 133.6, 126.8, 125.0, 123.0, 121.9, 119.6, 15.2, 11.3. MS (+EI) *m/z* 298 (M^+, 67.2%), 278 ($[M-HF]^+$, 25.3%), 238 (38.5%), 219 (47.3%), 204 (30.7%), 192 (100%), 144 (17.8%), 119 (45.0%), 76 (32.4%), 65 (9.8%), 51 (12.4%). HRMS (EI) calculated for $C_{11}H_{10}BBrF_2N_2$: 298.00885, found 298.00928.



4.2.13 Synthesis of 7,8-dibromo-5,5-difluoro-1,3-dimethyl-5*H*-dipyrrolo[1,2-c:1',2'-f][1,3,2]diazaborinin-4-ium-5-uide (41)[26]

To a solution of **37** (65.0 mg, 0.158 mmol) in toluene (10 ml) was added boron trifluoride diethyl etherate (100 µl, 0.791 mmol) and DBU (71.0 µl, 0.475 mmol). The resulting mixture was heated to 90 °C for 2 h. Afterward, the solution was washed with water and the crude material was purified via chromatography (hexanes/diethyl ether 3:2) to give desired fluorophore **41** (33.0 mg, 0.087 mmol, 55%).

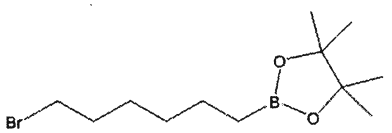
Red lustrous needles, R_f = (0.15, hexanes/diethyl ether 3:2), mp 204-206 °C. λ_{max} excitation in ethanol = 512 nm (ε_{512} = 42 000 $M^{-1}cm^{-1}$), λ_{max} emission in ethanol = 524 nm. ^1H NMR (300 MHz, $CDCl_3$) δ 6.98 (s, 1H), 6.83 (s, 1H), 6.22 (s, 1H), 2.60 (s, 3H, CH_3), 2.26 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.2, 147.2, 137.2, 132.5, 125.7, 124.9, 122.7, 122.7, 122.3, 15.4, 11.5 MS (+EI) *m/z* 378 (M^+, 62.9%), 358 ($[M-HF]^+$, 14.3%), 296 (11.9%), 256 (7.8%), 218 (43.5%), 179 (19.4%), 129 18.9%), 81 (31.8%), 69 (75.2%), 53 (100%), 43 (63.5%). HRMS (EI) calculated for $C_{11}H_9BBr_2F_2N_2$: 377.91936, found 375.91991.



4.2.14 Synthesis of 2-(6-bromohexyl)benzo[d][1,3,2]dioxaborole (44)[141]

6-Bromo-1-hexene (2.00 g, 12.3 mmol) and catecholborane (1.31 ml, 12.3 mmol) were combined and heated to 100 °C for 18 h. **44** (1.45g, 5.12 mmol, 42%) was obtained via Kugelrohr distillation (120 °C at 0.1 Torr) and subjected to transesterification immediately.

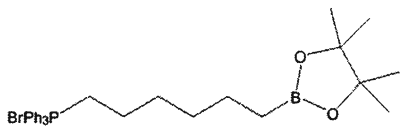
Clear and colourless liquid, R_f = (0.60, dichloromethane/methanol 7:1).



4.2.15 Synthesis of 2-(6-bromohexyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (45)[141]

To a solution of **44** (1.22 g, 4.31 mmol) in tetrahydrofuran (5 ml) at 0 °C was added pinacol (510 mg, 4.31 mmol). The mixture was stirred at 0 °C for 0.5 h and then at ambient temperature for 0.5 h. The solvent was removed *in vacuo* and then hexanes was added to the crude mixture and cooled to 4 °C for overnight to precipitate the catechol by-product. After several repetitions of dissolving in hexanes and removal of by-product catechol by filtration, pinacol-boronate **45** (750 mg, 2.57 mmol, 60%) was obtained.

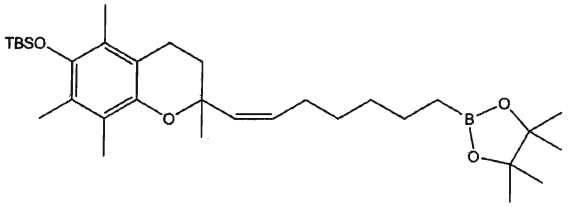
Light yellow oil, R_f = (0.80, dichloromethane/methanol, visualized by H_2SO_4/MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.26-3.22 (t, J = 6.6 Hz, 2H, C$\underline{H}_2$Br), 1.71-1.67 (m, 2H), 1.45-1.38 (m, 1H), 1.35-1.18 (m, 4H), 1.07 (m, 14H), 0.64-0.59 (t, J = 7.2 Hz, 1H, C$\underline{H}$B(OR)$_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 82.4, 82.2, 33.4, 32.4, 31.0, 27.5, 24.4, 24.2, 23.4. MS (+EI) *m/z* 291 ([MH]$^+$, 41.7%), 275 (27.5%), 227 (37.2%), 171 (18.1%), 157 (25.3%), 129 (94.9%), 83 (89.9%), 55 (88.8%), 41 (100%).



4.2.16 Synthesis of triphenyl(6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)hexyl)phosphonium bromide (46)[95]

45 (2.63 g, 9.04 mmol) and triphenylphosphine (2.37 g, 9.04 mmol) were dissolved in xylenes (25 ml) and heated to 120 °C for 72 h. Wittig salt **46** (2.88 g, 5.20 mmol, 58%) was azeotroped from diethyl ether and stored in the refrigerator.

Colourless viscous oil, R_f = (0.55, dichloromethane/methanol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74-7.58 (m, 15H, 15xArH), 3.58-3.47 (m, 2H, $CH_2P^+Ph_3$), 1.51 (m, 4H), 1.38 (m, 1H), 1.19-1.14 (m, 4H), 1.11-1.09 (m, 8H), 0.61-0.56 (t, J = 7.2 Hz, 1H, $CHB(OR)_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 134.9, 131.8, 130.4, 118.5, 117.3, 82.6, 31.4, 29.9, 29.7, 24.6, 24.5, 23.1, 22.7, 22.1. MS (+ESI) *m/z* 473 (M^+, 100%), 363 (20.1%).

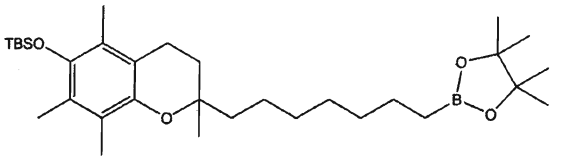


4.2.17 Synthesis of (*Z*)-*tert*-butyldimethyl(2,5,7,8-tetramethyl-2-(7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)hept-1-enyl)chroman-6-yloxy)silane (48)[95]

Wittig salt **46** (850 mg, 1.54 mmol) was dissolved in tetrahydrofuran (10 ml) under nitrogen and to this was added dropwise a 1.0 M solution of potassium *tert*-butoxide (188 mg, 1.68 mmol) in tetrahydrofuran. After stirring for 1 h, a solution of *rac*-Trolox aldehyde (486.7 mg, 1.40 mmol) in tetrahydrofuran (8.5 ml) was added and stirred for 16 h. The reaction was then quenched with saturated ammonium chloride solution and extracted with dichloromethane. The crude material was subjected to

chromatography (dichloromethane/hexanes 1:1) to afford **48** (428 mg, 0.785 mmol, 56%).

Dark brown oil, R_f = (0.60, dichloromethane/hexanes 2:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.35 (m, 1H, C=C<u>H</u>), 5.28 (m, 1H, C=C<u>H</u>), 2.58-2.57 (t, *J* = 4.8 Hz, 2H), 2.22-2.06 (m, 12H), 1.88-1.74 (m, 1H), 1.51 (m, 2H), 1.42 (s, 3H, C<u>H</u>$_3$), 1.28 (m, 10H), 1.10 (s, 9H, C(C<u>H</u>$_3$)$_3$), 0.81 (t, *J* = 7.2 Hz, 2H, C<u>H</u>B(OR)$_2$), 0.17 (s, 6H, Si(C<u>H</u>$_3$)$_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 146.0, 144.0, 133.1, 132.4, 125.6, 123.3, 122.7, 117.7, 82.6, 75.4, 33.3, 32.1, 29.7, 27.8, 27.1, 26.0, 24.7, 23.9, 21.2, 20.4, 18.5, 14.2, 13.3, 11.9, -3.4. MS (+EI) *m/z* 542 (M^+, 2.1%), 378 (1.3%), 348 (100%), 319 (63.7%), 221 33.7%), 129 (28.0%), 85 (18.0%), 73 (55.3%), 43 (30.3%).



4.2.18 Synthesis of *tert*-butyldimethyl(2,5,7,8-tetramethyl-2-(7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)heptyl)chroman-6-yloxy)silane (49)[142]

To a solution of **48** (182 mg, 0.335 mmol) in ethyl acetate (15 ml) was added 10% Pd on activated carbon (45.0 mg). The air was flushed with hydrogen gas and the reaction was stirred for 15 h at 1 atm. Filtration via Celite® followed by removal of solvent *in vacuo* yielded **49** (174 mg, 0.315 mmol, 100% completion, 94% recovered).

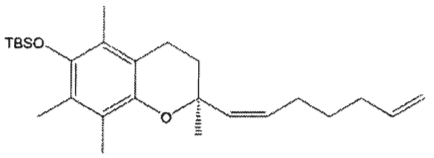
Colourless oil, R_f = (0.50, dichloromethane/hexanes 1:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.59-2.54 (t, *J* = 6.6 Hz, 2H), 2.12 (s, 3H, Ar-C<u>H</u>$_3$), 2.09 (s, 3H, Ar-C<u>H</u>$_3$), 2.07 (s, 3H, Ar-C<u>H</u>$_3$), 1.85-1.74 (m, 2H), 1.63-1.52 (m, 2H), 1.47-1.41 (m, 5H), 1.31 (m, 5H), 1.26 (m, 11H), 1.24 (s, 3H), 1.07 (s, 9H, C(C<u>H</u>$_3$)$_3$), 0.82-0.77 (t, *J* = 7.2 Hz, 2H, C<u>H</u>B(OR)$_2$), 0.14 (s, 6H, Si(C<u>H</u>$_3$)$_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 145.9, 144.0, 125.7,

123.4, 122.6, 117.4, 82.7, 74.4, 39.6, 32.3, 31.5, 30.1, 29.4, 26.1, 24.8, 24.0, 23.8, 23.6, 20.9, 18.5, 14.3, 13.4, 11.9, -3.4. MS (+EI) *m/z* 544 (M^+, 100%), 482 (5.1%), 418 (14.4%), 378 (6.6%), 348 (33.1%), 319 (28.8%), 279 (29.3%), 263 (9.2%), 221 (28.0%), 143 (9.7%), 73 (65.3%), 59 (9.7%). HRMS (EI) calculated for $C_{32}H_{57}BO_4Si$: 544.41192, found 544.41119.

4.2.19 Synthesis of hex-5-enyltriphenylphosphonium bromide (51)[143]

6-Bromo-1-hexene (2.50 g, 15.3 mmol) and triphenylphosphine (4.83 g, 18.4 mmol) were dissolved in acetonitrile (40 ml) and was refluxed for 85 h. After removal of solvent *in vacuo*, the remaining acetonitrile was removed as an azeotrope with diethyl ether, Wittig salt **51** (6.52 g, 15.3 mmol, 99%) was obtained.

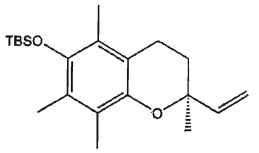
White hygroscopic powder, R_f = (0.30, dichloromethane/methanol 20:1), mp 122-125 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83-7.74 (m, 9H, 9xAr-H), 7.69-7.63 (m, 6H, 6xAr-H), 5.69-5.55 (ddt, J = 17.0 Hz, J' = 10.3 Hz, J'' = 6.7 Hz, 1H, CH=CH_2), 4.91-4.81 (m, 2H, CH=CH_2), 3.78-3.69 (m, 2H), 2.06-1.97 (m, 2H), 1.77-1.67 (m, 2H), 1.65-1.52 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.5, 134.9, 133.7, 130.5, 128.6, 118.7, 117.6, 115.2, 32.8, 29.2, 22.8, 21.7. MS (+FAB) *m/z* 345 (M^+, 100%), 262 (16.9%), 183 (16.4%), 108 (8.2%), 55 (2.9%).



4.2.20 Synthesis of (*Z*)-*tert*-butyl(2-(hepta-1,6-dienyl)-2,5,7,8-tetramethylchroman-6-yloxy)dimethylsilane (53)[143]

Wittig salt **51** (134 mg, 0.316 mmol) was dissolved in tetrahydrofuran (10 ml) under nitrogen and to this was added dropwise a 1.0 M solution of LHMDS in tetrahydrofuran (502 µl, 0.502 mmol). After stirring for 1 h, a solution of (*S*)-Trolox aldehyde (100 mg, 0.287 mmol) in tetrahydrofuran (5 ml) was added and stirred for 16 h. The reaction was then quenched with saturated ammonium chloride solution and extracted with dichloromethane. The crude material was subjected to silica gel chromatography (hexanes/tetrahydrofuran 35:1) to afford **53** (98.0 mg, 0.235 mmol, 82%).

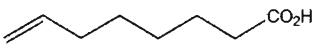
Colourless oil, R_f = (0.40, hexanes/dichloromethane 3:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.89-5.75 (ddt, J = 17.0 Hz, J' = 10.3 Hz, J'' = 6.7 Hz, 1H, C<u>H</u>=CH_2), 5.37 (m, 2H), 5.05-4.96 (m, 2H, CH=C<u>H</u>$_2$), 2.59-2.57 (t, J = 6.0 Hz, 2H), 2.42-2.25 (m, 2H), 2.16 (s, 3H, Ar-C<u>H</u>$_3$), 2.15 (s, 3H, Ar-C<u>H</u>$_3$), 2.09 (s, 3H, Ar-C<u>H</u>$_3$), 2.05-2.00 (m, 2H), 1.85-1.75 (m, 1H), 1.51 (s, 3H), 1.48-1.31 (m, 3H), 1.09 (s, 9H, C(C<u>H</u>$_3$)$_3$), 0.16 (s, 6H, Si(C<u>H</u>$_3$)$_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 146.1, 144.2, 138.7, 132.0, 125.8, 123.5, 122.4, 117.8, 114.4, 75.5, 33.5, 29.2, 27.3, 26.1, 21.3, 18.6, 14.3, 13.4, 12.2, -3.3. MS (+EI) *m/z* 414 (M^+, 100%), 278 (56.0%), 221 (37.1%), 187 (7.8%), 73 (85.5%), 59 (15.0%), 41 (12.8%). HRMS (EI) calculated for $C_{26}H_{42}O_2Si$: 414.29541, found 414.29456.



4.2.21 Synthesis of (*S*)-*tert*-butyldimethyl(2,5,7,8-tetramethyl-2-vinylchroman-6-yloxy)silane (61)[142]

Methyltriphenylphosphonium bromide (169 mg, 0.473 mmol) was dissolved in tetrahydrofuran (10 ml) under nitrogen and to this was added dropwise a 1.0 M solution of LHMDS in tetrahydrofuran (753 µl, 0.753 mmol). After stirring for 1 h, a solution of (*S*)-Trolox aldehyde (150 mg, 0.430 mmol) in tetrahydrofuran (5 ml) was added and stirred for 3 h. The reaction was then quenched with saturated ammonium chloride solution and extracted with dichloromethane. The crude material was subjected to chromatography (hexanes/dichloromethane 3:1) to afford product **61** (132 mg, 0.380 mmol, 88%).

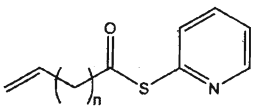
White solid, R_f = (0.30, hexanes/dichloromethane 3:1), mp 54 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.94-5.85 (dd, J = 17.1 Hz, J' = 10.8 Hz, 1H, C<u>H</u>=CH_2), 5.20-5.13 (dd, J = 17.1 Hz, J' = 1.1 Hz, 1H, CH=C<u>H</u>$_2$), 5.07-5.03 (dd, J = 10.8 Hz, J' = 1.1 Hz, 1H, CH=C<u>H</u>$_2$), 2.66-2.48 (m, 2H), 2.19 (s, 3H, Ar-C<u>H</u>$_3$), 2.17 (s, 3H, Ar-C<u>H</u>$_3$), 2.09 (s, 3H, Ar-C<u>H</u>$_3$), 2.01-1.81 (m, 2H), 1.44 (s, 3H, C<u>H</u>$_3$), 1.10 (s, 9H, C(C<u>H</u>$_3$)$_3$), 0.17 (s, 6H, Si(C<u>H</u>$_3$)$_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 146.0, 144.2, 141.9, 125.8, 123.4, 122.2, 117.5, 113.1, 75.2, 31.9, 27.0, 26.1, 21.1, 18.6, 14.3, 13.4, 12.0, -3.3. MS (+EI) *m/z* 346 (M^+, 100%), 331 (8.6%), 289 (12.4%), 278 (27.3%), 234 (10.9%), 221 (49.5%), 163 (9.0%), 129 (6.7%), 73 (53.8%), 59 (13.6%). HRMS (EI) calculated for $C_{21}H_{34}O_2Si$: 346.23281, found 346.23288.



4.2.22 Synthesis of oct-7-enoic acid (63)[110]

To a stirring solution of 7-bromo-1-heptene (2.00g, 11.3 mmol) in dry diethyl ether (10 ml) was added magnesium turnings (302 mg, 12.4 mmol) and a catalytic amount of iodine. The mixture was heated to 34 °C for 3.5 h and then cooled to –20 °C upon which carbon dioxide (dry ice subliming through a Drierite® drying tube) was bubbled into the solution. After 2 h, the reaction was quenched with 19% hydrochloric acid (10 ml) and extracted with diethyl ether. Removal of solvent *in vacuo* obtained acid **63** (452 mg, 2.94 mmol, 26%).

Clear and colourless liquid, R_f = (0.25, hexanes/ethyl acetate 5:1, visualized by H_2SO_4/MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.79 (br s, 1H, $CO_2\underline{H}$), 5.82-5.73 (ddt, J = 17.0 Hz, J' = 10.3 Hz, J'' = 6.7 Hz, 1H, $C\underline{H}$=CH_2), 5.02-4.90 (m, 2H, CH=$C\underline{H_2}$), 2.36-2.31 (t, J = 1.5 Hz, 2H, $C\underline{H_2}CO_2H$), 2.08-2.01 (m, 2H, $C\underline{H_2}CH$=CH_2), 1.66-1.61 (m, 2H), 1.43-1.28 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 180.3, 138.4, 114.3, 33.9, 33.4, 28.4, 24.4. MS (+FAB) *m/z* 143 ($[MH]^+$, 20.4%), 137 (15.2%), 125 (28.2%), 107 (9.3%), 97 (34.0%), 81 (16.1%), 67 (13.4%), 55 (100%), 41 (39.2%). HRMS (FAB) calculated for $C_8H_{15}O_2^+$: 143.10720, found 143.10647.



4.2.23a Synthesis of *S*-pyridin-2-yl but-3-enethioate (90a, n = 1)[110]

To a solution of 3-butenoic acid (902 mg, 10.5 mmol) in dry toluene (15 ml) was added 2,2′-dipyridyl disulfide (3.00 g, 13.6 mmol) and triphenylphosphine (3.57 g, 13.6 mmol). This mixture was stirred overnight. After washing the solution with water and

then washing the combined water fractions with diethyl ether, the organic phases were combined, condensed *in vacuo*, and the crude material was loaded onto a silica gel column eluting with hexanes/ethyl acetate 6:1 to afford pure thioate **90a** (967 mg, 5.36 mmol, 51%).

Light yellow syrup, R_f = (0.15, hexanes/diethyl ether 3:2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.32-8.30 (dd, J = 4.8 Hz, J' = 0.9 Hz, 1H), 7.43-7.37 (m, 1H), 7.33-7.30 (d, J = 7.8 Hz, 1H), 6.74-6.93 (m, 1H), 5.74-5.61 (ddt, J = 17.0 Hz, J' = 10.1 Hz, J'' = 6.9 Hz, 1H, C<u>H</u>=CH$_2$), 5.01-4.95 (m, 2H, CH=C<u>H$_2$</u>), 3.17-3.15 (d, J = 6.9 Hz, 2H, C<u>H$_2$</u>CO). ^{13}C NMR (75 MHz, $CDCl_3$) δ 193.0, 150.4, 149.4, 136.3, 129.2, 128.4, 122.7, 119.5, 47.5. MS (+EI) *m/z* 179 (M^+, 0.8%), 151 (16.6%), 136 (6.0%), 111 (100%), 78 (41.2%), 67 (24.1%), 51 (13.7%), 41 (45.7%). HRMS (EI) calculated for C_9H_9NOS: 179.04049, found 179.04040.

4.2.23b Synthesis of *S*-pyridin-2-yl pent-4-enethioate (90b, n = 2)

(1.70 g, 8.79 mmol, 88%). Light yellow syrup, R_f = (0.25, hexanes/diethyl ether 3:2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.42-8.40 (dd, J = 4.8 Hz, J' = 0.9 Hz, 1H), 7.54-7.49 (m, 1H), 7.43-7.41 (d, J = 7.8 Hz, 1H), 7.08-7.04 (m, 1H), 5.71-5.58 (ddt, J = 16.9 Hz, J' = 10.4 Hz, J'' = 6.6 Hz, 1H, C<u>H</u>=CH$_2$), 4.94-4.83 (m, 2H, CH=C<u>H$_2$</u>), 2.64-2.59 (t, J = 6.9 Hz, 2H, C<u>H$_2$</u>CO), 2.31-2.24 (m, 2H, C<u>H$_2$</u>CH=CH$_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 194.9, 150.8, 149.7, 136.5, 135.3, 129.5, 122.9, 115.5, 42.6, 28.5. MS (+EI) *m/z* 193 (M^+, 0.8%), 160 (4.5%), 124 (4.3%), 111 (100%), 78 (11.0%), 67 (14.7%), 55 (43.4%), 41 (4.3%). HRMS (EI) calculated for $C_{10}H_{11}NOS$: 193.05614, found 193.05645.

4.2.23c Synthesis of *S*-pyridin-2-yl hex-5-enethioate (64a, n = 3)

(1.95 g, 9.42 mmol, 96%). Light yellow syrup, R_f = (0.35, hexanes/diethyl ether 3:2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.40-8.38 (dd, J = 4.8 Hz, J' = 0.9 Hz, 1H), 7.52-7.47 (m, 1H), 7.42-7.39 (d, J = 8.1 Hz, 1H), 7.06-7.02 (m, 1H), 5.61-5.52 (ddt, J = 16.8 Hz, J' = 10.4 Hz, J'' = 6.6 Hz, 1H, C<u>H</u>=CH$_2$), 4.88-4.79 (m, 2H, CH=C<u>H</u>$_2$), 2.53-2.48 (t, J = 7.5 Hz, 2H, C<u>H</u>$_2$CO), 1.96-1.89 (m, 2H, C<u>H</u>$_2$CH=CH$_2$), 1.67-1.57 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 195.4, 150.9, 149.7, 136.6, 136.4, 129.4, 122.8, 115.1, 42.7, 32.1, 23.7. MS (+EI) *m/z* 207 (M^+, 0.4%), 179 (1.2%), 166 (3.1%), 125 (4.3%), 111 (100%), 78 (12.2%), 69 (19.4%), 55 (32.5%), 41 (38.2%). HRMS (EI) calculated for $C_{11}H_{13}NOS$: 207.07179, found 207.07210.

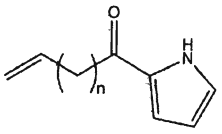
4.2.23d Synthesis of *S*-pyridin-2-yl hept-6-enethioate (64b, n = 4)

(1.42 g, 6.43 mmol, 95%). Light yellow syrup, R_f = (0.35, hexanes/diethyl ether 3:2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.29-8.26 (m, 1H), 7.37-7.30 (m, 2H), 6.93-6.89 (m, 1H), 5.48-5.42 (m, 1H, C<u>H</u>=CH$_2$), 4.74-4.64 (m, 2H, CH=C<u>H</u>$_2$), 2.42-2.37 (t, J = 7.5 Hz, 2H, C<u>H</u>$_2$CO), 1.79-1.71 (m, 2H, C<u>H</u>$_2$CH=CH$_2$), 1.47-1.37 (m, 2H), 1.20-1.10 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 194.8, 150.8, 149.3, 137.1, 136.0, 128.9, 122.4, 113.9, 42.9, 32.3, 27.0, 23.8. MS (+EI) *m/z* 221 (M^+, 0.2%), 188 (1.1%), 160 (3.1%), 125 (2.7%), 111 (100%), 78 (10.0%), 67 (13.9%), 55 (31.1%), 41 (23.8%). HRMS (EI) calculated for $C_{12}H_{15}NOS$: 221.08744, found 221.08726.

4.2.23e Synthesis of *S*-pyridin-2-yl oct-7-enethioate (64c, n = 5)

(733 mg, 3.45 mmol, 98%). Light yellow syrup, R_f = (0.35, hexanes/diethyl ether 3:2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.36-8.34 (dd, J = 4.8 Hz, J' = 1.2 Hz, 1H), 7.47-7.36 (m, 2H), 7.01-6.96 (m, 1H), 5.59-5.50 (ddt, J = 16.8 Hz, J' = 10.2 Hz, J'' = 6.6 Hz, 1H,

C<u>H</u>=C<u>H</u>$_2$), 4.80-4.69 (m, 2H, CH=C<u>H</u>$_2$), 2.47-2.43 (t, J = 7.5 Hz, 2H, C<u>H</u>$_2$CO), 1.84-1.77 (m, 2H, C<u>H</u>$_2$CH=CH$_2$), 1.53-1.43 (m, 2H), 1.22-1.09 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 195.2, 151.0, 149.5, 137.8, 136.3, 129.2, 122.7, 113.8, 43.3, 32.7, 27.7, 27.5, 24.4. MS (+EI) *m/z* 235 (M^+, 0.8%), 174 (2.2%), 125 (2.7%), 111 (100%), 78 (10.3%), 67 (13.0%), 55 (43.7%), 41 (15.1%). HRMS (EI) calculated for $C_{13}H_{17}NOS$: 235.10309, found 235.10277.



4.2.24a Synthesis of 1-(1*H*-pyrrol-2-yl)but-3-en-1-one (91a, n = 1)[110]

Pyrrole (1.49 ml, 21.4 mmol) was dissolved in tetrahydrofuran (7 ml) and cooled to 0 °C under nitrogen. To this was slowly added a 3.0 M solution of methylmagnesium chloride in tetrahydrofuran (5.36 ml, 16.1 mmol) and the resulting solution was stirred for 15 min. Next, the solution was cooled further to –78 °C and a solution of thioate **90a** (960 mg, 5.36 mmol) in tetrahydrofuran (30 ml) was added. The reaction was stirred for 1 h. Afterward, the reaction mixture was condensed *in vacuo* and then extracted with dichloromethane. This crude material was purified via column chromatography (hexanes/diethyl ether 3:2) to yield ketopyrrole **91a** (476 mg, 3.52 mmol, 66%).

Clear and colourless oil, R_f = (0.30, hexanes/diethyl ether 3:2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.65 (br s, 1H, N<u>H</u>), 7.10-7.07 (m, 1H), 7.00-6.97 (m, 1H), 6.29-6.26 (m, 1H), 6.14-6.01 (ddt, J = 17.0 Hz, J' = 10.1 Hz, J'' = 6.9 Hz, 1H, C<u>H</u>=CH$_2$), 5.27-5.26 (m, 1H, CH=C<u>H</u>$_2$), 5.23-5.18 (m, 1H, CH=C<u>H</u>$_2$), 3.60-3.57 (dt, J = 7.0 Hz, J' = 1.5 Hz, 2H, C<u>H</u>$_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 188.4, 131.5, 131.2, 125.6, 118.2, 117.1, 110.4,

42.9. MS (+EI) *m/z* 135 (M^+, 18.3%), 94 (100%), 66 (21.6%), 39 (17.6%). HRMS (EI) calculated for C_8H_9NO: 135.06841, found 135.06821.

4.2.24b Synthesis of 1-(1*H*-pyrrol-2-yl)pent-4-en-1-one (91b, n = 2)

(1.27 g, 8.53 mmol, 97%). Clear and colourless oil, R_f = (0.35, hexanes/diethyl ether 3:2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.02 (br s, 1H, NH), 7.12-7.10 (m, 1H), 7.01-6.97 (m, 1H), 6.31-6.28 (m, 1H), 6.01-5.87 (ddt, *J* = 17.0 Hz, *J'* = 10.1 Hz, *J''* = 6.9 Hz, 1H, CH=CH_2), 5.24-5.03 (m, 2H, CH=CH_2), 3.00-2.92 (t, *J* = 7.2 Hz, 2H, CH_2CO), 2.59-2.55 (m, 2H, CH_2CH=CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ 190.2, 137.1, 131.6, 125.4, 116.7, 114.9, 110.1, 36.8, 28.8. MS (+EI) *m/z* 149 (M^+, 23.1%), 134 (1.9%), 120 (1.8%), 107 (3.3%), 94 (100%), 80 (5.9%), 66 (15.3%), 53 (3.6%), 44 (4.2%). HRMS (EI) calculated for $C_9H_{11}NO$: 149.08406, found 149.08420.

4.2.24c Synthesis of 1-(1*H*-pyrrol-2-yl)hex-5-en-1-one (65a, n = 3)

(356 mg, 2.17 mmol, 90%). Clear and colourless oil, R_f = (0.40, hexanes/diethyl ether 3:2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.29 (br s, 1H, NH), 7.06 (m, 1H), 6.93 (m, 1H), 6.27-6.26 (m, 1H), 5.89-5.76 (ddt, *J* = 17.0 Hz, *J'* = 10.1 Hz, *J''* = 6.9 Hz, 1H, CH=CH_2), 5.07-4.98 (m, 2H, CH=CH_2), 2.82-2.77 (t, *J* = 7.2 Hz, 2H, CH_2CO), 2.18-2.11 (m, 2H, CH_2CH=CH_2), 1.89-1.80 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 191.0, 138.0, 131.9, 124.9, 116.4, 115.1, 110.4, 37.1, 33.2, 24.3. MS (+EI) *m/z* 163 (M^+, 25.3%), 146 (1.1%), 122 (1.6%), 109 (100%), 94 (63.0%), 80 (6.7%), 66 (14.9%), 55 (5.1%), 44 (15.4%). HRMS (EI) calculated for $C_{10}H_{13}NO$: 163.09971, found 163.09972.

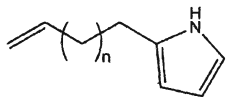
4.2.24d Synthesis of 1-(1*H*-pyrrol-2-yl)hept-6-en-1-one (65b, n = 4)

(1.11 g, 6.23 mmol, 97%). Clear and colourless oil, R_f = (0.45, hexanes/diethyl ether 3:2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.21 (br s, 1H, NH), 7.12-7.10 (m, 1H), 6.99-

6.98 (m, 1H), 6.31-6.28 (m, 1H), 5.91-5.80 (ddt, J = 17.0 Hz, J' = 10.3 Hz, J'' = 6.7 Hz, 1H, C<u>H</u>=CH$_2$), 5.12-5.01 (m, 2H, CH=C<u>H</u>$_2$), 2.87-2.82 (t, J = 7.2 Hz, 2H, C<u>H</u>$_2$CO), 2.18-2.11 (m, 2H, C<u>H</u>$_2$CH=CH$_2$), 1.88-1.77 (m, 2H), 1.58-1.48 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 191.0, 138.1, 131.6, 125.2, 116.6, 114.2, 109.9, 37.3, 33.2, 28.2, 24.5. MS (+EI) *m/z* 177 (M^+, 21.8%), 149 (2.1%), 134 (2.2%), 122 (25.9%), 109 (91.7%), 94 (100%), 80 (6.8%), 67 (36.2%), 55 (11.9%), 41 (15.4%). HRMS (EI) calculated for $C_{11}H_{15}NO$: 177.11536, found 177.11487.

4.2.24e Synthesis of 1-(1*H*-pyrrol-2-yl)oct-7-en-1-one (65c, n = 5)

(551 mg, 2.88 mmol, 93%). Clear and colourless oil, R_f = (0.45, hexanes/diethyl ether 3:2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.81 (br s, 1H, N<u>H</u>), 7.09-7.06 (m, 1H), 6.97-6.94 (m, 1H), 6.28-6.26 (m, 1H), 5.87-5.78 (ddt, J = 17.0 Hz, J' = 10.3 Hz, J'' = 6.7 Hz, 1H, C<u>H</u>=CH$_2$), 5.05-4.95 (m, 2H, CH=C<u>H</u>$_2$), 2.83-2.78 (t, J = 7.2 Hz, 2H, C<u>H</u>$_2$CO), 2.11-2.04 (m, 2H, C<u>H</u>$_2$CH=CH$_2$), 1.80-1.75 (m, 2H), 1.48-1.39 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 191.3, 138.6, 131.8, 125.2, 116.5, 114.2, 110.1, 37.7, 33.4, 28.7, 28.5, 25.1. MS (+EI) *m/z* 191 (M^+, 20.8%), 148 (2.2%), 136 (1.7%), 122 (18.5%), 109 (100%), 94 (77.5%), 80 (8.1%), 67 (19.6%), 55 (8.9%), 41 (10.1%). HRMS (EI) calculated for $C_{12}H_{17}NO$: 191.13101, found 191.13106.



4.2.25a Synthesis of 2-(pent-4-enyl)-1*H*-pyrrole (92, n = 2)[110]

Ketopyrrole **91b** (320 mg, 2.37 mmol) was dissolved in *iso*-propanol (6 ml) and heated to reflux. To this boiling solution was added a suspension of sodium borohydride (251 mg, 6.63 mmol) in *iso*-propanol (4 ml). The reflux went overnight. Afterward, the

crude material was condensed *in vacuo* and passed through a neutral alumina column (hexanes/ethyl acetate 10:1) to afford crude **92** that was reacted immediately in the next step. *Note: extreme care must be used when handling these alkenyl pyrroles for they readily decompose, especially in the presence of an acid, and must be used immediately.*

Clear and colourless liquid, fruity odour, R_f = (0.50, hexanes/ethyl acetate 6:1, alumina).

4.2.25b Synthesis of 2-(hex-5-enyl)-1*H*-pyrrole (66a, n = 3)

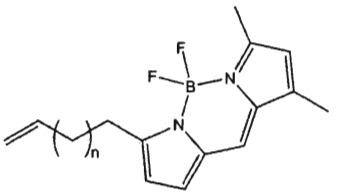
Clear and colourless liquid, fruity odour, R_f = (0.50, hexanes/ethyl acetate 6:1, alumina).

4.2.25c Synthesis of 2-(hept-6-enyl)-1*H*-pyrrole (66b, n = 4)

Clear and colourless liquid, fruity odour, R_f = (0.60, hexanes/ethyl acetate 6:1, alumina).

4.2.25d Synthesis of 2-(oct-7-enyl)-1H-pyrrole (66c, n = 5)

Clear and colourless liquid, fruity odour, R_f = (0.60, hexanes/ethyl acetate 6:1, alumina).



4.2.26a Synthesis of 7-(hex-5-enyl)-5,5-difluoro-1,3-dimethyl-5*H*-dipyrrolo[1,2-c:1',2'-f][1,3,2]diazaborinin-4-ium-5-uide (58a, n = 3)[46]

Crude **66a** and 3,5-dimethylpyrrole-2-carboxaldehyde (70.0 mg, 0.568 mmol) were dissolved in dry dichloromethane (8.5 ml) and cooled to 0 °C under nitrogen. A solution of phosphorus oxychloride (52.0 µl, 0.568 mmol) in dichloromethane (0.5 ml)

was added via syringe and the resulting solution was kept on ice for 1 h. Afterward, the reaction was brought to room temperature and stirred overnight. This solution was then cooled back to 0 °C and boron trifluoride diethyl etherate (288 µl, 2.27 mmol) and DIPEA (396 µl, 2.27 mmol) were added. After warming back to room temperature and stirring for 6 h, the crude product was extracted in dichloromethane and condensed *in vacuo*. Purification by silica gel chromatography (hexanes/diethyl ether 3:1) afforded pure **58a** (143 mg, 0.476 mmol, 40% over two steps).

Dark red oil, R_f = (0.30, hexanes/diethyl ether 3:2). λ_{max} excitation in ethanol = 507 nm (ε_{507} = 87 000 $M^{-1}cm^{-1}$), λ_{max} emission in ethanol = 511 nm. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.04 (s, 1H), 6.89-6.87 (d, *J* = 3.9 Hz, 1H), 6.28-6.26 (d, *J* = 3.9 Hz, 1H), 6.07 (s, 1H), 5.89-5.76 (ddt, *J* = 17.0 Hz, *J′* = 10.3 Hz, *J″* = 6.7 Hz, 1H, C<u>H</u>=CH_2), 5.05-4.94 (m, 2H, CH=C<u>H_2</u>), 3.01-2.96 (t, *J* = 7.8 Hz, 2H, BDP-C<u>H_2</u>), 2.55 (s, 3H, BDP-C<u>H_3</u>), 2.21 (s, 3H, BDP-C<u>H_3</u>), 2.16-2.09 (m, 2H, C<u>H_2</u>CH=CH_2), 1.81-1.70 (m, 2H), 1.58-1.48 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.5, 142.9, 138.7, 134.6, 133.3, 128.4, 123.4, 119.8, 118.9, 116.7, 114.5, 33.5, 28.7, 28.4, 28.0, 14.8, 11.2. ^{11}B NMR (193 MHz, $CDCl_3$) 1.11-0.77 (t, *J* = 32.7 Hz, <u>B</u>F_2). ^{19}F NMR (282 MHz, $CDCl_3$) -145.2 – -145.5 (overlapping q and sextet, *J* = 33.3 Hz, B<u>F_2</u>). MS (+EI) *m/z* 302 (M^+, 50.3%), 282 ([M-HF]$^+$, 3.5%), 248 (11.6%), 233 (100%), 213 (24.1%), 195 (7.5%), 149 (19.3%), 111 (12.9%), 97 (8.6%), 83 (9.6%), 71 (12.9%), 57 (22.7%), 43 (17.7%). HRMS (EI) calculated for $C_{17}H_{21}BF_2N_2$: 302.17659, found 302.17684.

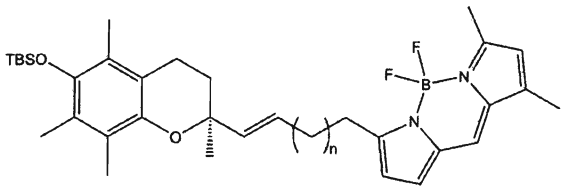
4.2.26b Synthesis of 7-(hept-6-enyl)-5,5-difluoro-1,3-dimethyl-5*H*-dipyrrolo[1,2-c:1',2'-f][1,3,2]diazaborinin-4-ium-5-uide (58b, n = 4)

(313 mg, 0.988 mmol, 34% over two steps). Dark red oil, R_f = (0.45, hexanes/diethyl ether 3:2). λ_{max} excitation in ethanol = 507 nm (ε_{507} = 86 000 $M^{-1}cm^{-1}$), λ_{max} emission in ethanol = 511 nm. ^1H NMR (300 MHz, $CDCl_3$) δ 7.00 (s, 1H), 6.84-6.83 (d, *J* = 3.9 Hz, 1H), 6.25-6.23 (d, *J* = 3.9 Hz, 1H), 6.02 (s, 1H), 5.88-5.75 (ddt, *J* = 17.0 Hz, *J'* = 10.3 Hz, *J''* = 6.7 Hz, 1H, C<u>H</u>=CH_2), 5.05-4.92 (m, 2H, CH=C<u>H_2</u>), 3.00-2.95 (t, *J* = 7.8 Hz, 2H, BDP-C<u>H_2</u>), 2.53 (s, 3H, BDP-C<u>H_3</u>), 2.15 (s, 3H, BDP-C<u>H_3</u>), 2.08-2.04 (m, 2H, C<u>H_2</u>CH=CH_2), 1.79-1.69 (m, 2H), 1.48-1.43 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.3, 158.6, 142.7, 138.7, 134.4, 133.2, 128.4, 123.3, 119.6, 116.5, 114.1, 33.5, 28.9, 28.5, 28.4, 28.3, 14.6, 10.9. ^{11}B NMR (96 MHz, $CDCl_3$) 1.29-0.60 (t, *J* = 32.7 Hz, <u>B</u>F_2). ^{19}F NMR (282 MHz, $CDCl_3$) -144.7 – -145.1 (overlapping q and sextet, *J* = 33.3 Hz, B<u>F_2</u>). MS (+EI) *m/z* 316 (M^+, 49.0%), 277 (2.1%), 248 (61.2%), 233 (100%), 213 (23.0%), 194 (4.3%), 149 (3.9%), 116 (9.9%), 81 (4.2%), 69 (7.6%), 55 (7.5%), 43 (13.2%). HRMS (EI) calculated for $C_{18}H_{23}BF_2N_2$: 316.19224, found 316.19279.

4.2.26c Synthesis of 7-(oct-7-enyl)-5,5-difluoro-1,3-dimethyl-5*H*-dipyrrolo[1,2-c:1',2'-f][1,3,2]diazaborinin-4-ium-5-uide (58c, n = 5)

(370 mg, 1.12 mmol, 39% over two steps). Dark red oil, R_f = (0.50, hexanes/diethyl ether 3:2). λ_{max} excitation in ethanol = 507 nm (ε_{507} = 85 000 $M^{-1}cm^{-1}$), λ_{max} emission in ethanol = 511 nm. ^1H NMR (300 MHz, $CDCl_3$) δ 7.02 (s, 1H), 6.87-6.85 (d, *J* = 3.9 Hz, 1H), 6.26-6.25 (d, *J* = 3.9 Hz, 1H), 6.04 (s, 1H), 5.89-5.75 (ddt, *J* = 17.0 Hz, *J'* = 10.3 Hz, *J''* = 6.7 Hz, 1H, C<u>H</u>=CH_2), 5.02-4.92 (m, 2H, CH=C<u>H_2</u>), 3.00-2.95 (t, *J* = 7.8 Hz, 2H, BDP-C<u>H_2</u>), 2.54 (s, 3H, BDP-C<u>H_3</u>), 2.18 (s, 3H, BDP-C<u>H_3</u>), 2.07-

2.02 (m, 2H, C$\underline{H}_2$CH=CH$_2$), 1.78-1.69 (m, 2H), 1.52-1.40 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.6, 158.7, 142.7, 139.0, 134.5, 133.2, 128.4, 123.4, 119.7, 116.6, 114.1, 33.7, 29.3, 28.8, 28.7, 28.6, 28.5, 14.7, 11.0. ^{11}B NMR (96 MHz, $CDCl_3$) 1.29-0.60 (t, J = 32.7 Hz, $\underline{B}F_2$). ^{19}F NMR (282 MHz, $CDCl_3$) -144.9 – -145.3 (overlapping q and sextet, J = 33.3 Hz, B$\underline{F}_2$). MS (+EI) *m/z* 330 (M^+, 10.2%), 248 (100%), 233 (40.5%), 218 (15.4%), 149 (10.6%), 116 (15.3%), 94 (10.9%), 83 (7.9%), 69 (14.3%), 57 (16.0%), 44 (40.8%). HRMS (EI) calculated for $C_{19}H_{25}BF_2N_2$: 330.20789, found 330.20818.



4.2.27a Synthesis of (*S*,*E*)-7-(6-(6-(*tert*-butyldimethylsilyloxy)-2,5,7,8-tetramethylchroman-2-yl)hex-5-enyl)-5,5-difluoro-1,3-dimethyl-5*H*-dipyrrolo[1,2-c:1',2'-f][1,3,2]diazaborinin-4-ium-5-uide (68a, n = 3)[43]

Vinyl Trolox **61** (61.9 mg, 0.179 mmol) and hexenylBODIPY **58a** (108 mg, 0.357 mmol) were dissolved in dry dichloromethane (1.5 ml). Grubbs Catalyst 2nd Generation **59** (15.2 mg, 0.018 mmol) was then added and the reaction refluxed for 6 h. The mixture was condensed *in vacuo* and the crude residue was loaded onto a silica column (hexanes/diethyl ether 6:1) to obtain **68a** (72.3 mg, 0.117 mmol, 65%).

Dark red oil, R_f = (0.50, hexanes/diethyl ether 3:2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.05 (s, 1H), 6.89-6.88 (d, J = 3.9 Hz, 1H), 6.21-6.19 (d, J = 3.9 Hz, 1H), 6.08 (s, 1H), 5.54-5.50 (m, 2H, C$\underline{H}$=C$\underline{H}$), 2.96-2.91 (t, J = 7.8 Hz, 2H, BDP-C$\underline{H}_2$), 2.56-2.50 (m, 5H, C4-C$\underline{H}_2$, BDP-C$\underline{H}_3$), 2.24 (s, 3H, Ar-C$\underline{H}_3$), 2.14 (s, 3H, BDP-C$\underline{H}_3$), 2.12 (s, 3H, Ar-C$\underline{H}_3$), 2.03 (s, 3H, Ar-C$\underline{H}_3$), 2.07-2.00 (m, 1H, C3-C$\underline{H}$), 1.94-1.71 (m, 3H, C3-C$\underline{H}$, C$\underline{H}_2$), 1.68-

1.60 (m, 2H), 1.46-1.40 (m, 2H), 1.38 (s, 3H, C$\underline{H}_3$), 1.07 (s, 9H, C(C$\underline{H}_3$)$_3$), 0.14 (s, 6H, Si(C$\underline{H}_3$)$_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.7, 158.9, 146.1, 144.1, 142.8, 134.6, 134.2, 133.3, 128.6, 128.5, 128.3, 125.7, 123.4, 122.2, 119.8, 117.7, 116.8, 74.8, 32.3, 31.9, 29.0, 28.4, 27.8, 27.3, 26.1, 21.2, 18.6, 14.8, 14.3, 13.4, 12.0, 11.2, -3.4. ^{11}B NMR (96 MHz, $CDCl_3$) 1.27-0.58 (t, J = 32.7 Hz, $\underline{B}F_2$). ^{19}F NMR (282 MHz, $CDCl_3$) -145.0 – -145.5 (overlapping q and sextet, J = 33.3 Hz, B$\underline{F}_2$). MS (+EI) *m/z* 620 (M^+, 0.6%), 378 (1.3%), 346 (13.2%), 316 (26.8%), 278 (6.1%), 233 (100%), 213 (25.4%), 149 (25.3%), 129 (6.5%), 97 (11.0%), 83 (20.2%), 71 (32.7%), 57 (54.0%), 43 (78.6%). HRMS (EI) calculated for $C_{36}H_{51}BF_2N_2O_2Si$: 620.37810, found 620.37428.

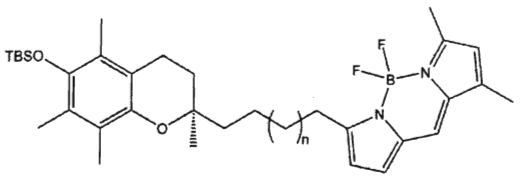
4.2.27b Synthesis of (*S*,*E*)-7-(7-(6-(*tert*-butyldimethylsilyloxy)-2,5,7,8-tetramethylchroman-2-yl)hept-6-enyl)-5,5-difluoro-1,3-dimethyl-5*H*-dipyrrolo[1,2-c:1',2'-f][1,3,2]diazaborinin-4-ium-5-uide (68b, n = 4)

(61.8 mg, 0.097 mmol, 53%). Dark red oil, R_f = (0.55, hexanes/diethyl ether 3:2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.08 (s, 1H), 6.92-6.90 (d, J = 3.9 Hz, 1H), 6.29-6.28 (d, J = 3.9 Hz, 1H), 6.10 (s, 1H), 5.54-5.51 (m, 2H, C$\underline{H}$=C$\underline{H}$), 2.99-2.94 (t, J = 7.8 Hz, 2H, BDP-C$\underline{H}_2$), 2.58 (s, 3H, BDP-C$\underline{H}_3$), 2.56-2.49 (m, 2H, C4-C$\underline{H}_2$), 2.26 (s, 3H, Ar-C$\underline{H}_3$), 2.16 (s, 3H, BDP-C$\underline{H}_3$), 2.14 (s, 3H, Ar-C$\underline{H}_3$), 2.00 (s, 3H, Ar-C$\underline{H}_3$), 1.96-1.54 (m, 6H), 1.51-1.39 (m, 4H), 1.39 (s, 3H, C$\underline{H}_3$), 1.07 (s, 9H, C(C$\underline{H}_3$)$_3$), 0.13 (s, 6H, Si(C$\underline{H}_3$)$_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.8, 158.9, 146.1, 144.1, 142.8, 134.6, 134.2, 133.3, 128.8, 128.6, 128.5, 125.7, 123.4, 122.2, 119.8, 117.7, 116.8, 74.7, 32.4, 32.0, 29.0, 28.8, 28.6, 28.4, 27.2, 26.1, 21.2, 18.6, 14.8, 14.3, 13.4, 12.0, 11.2, -3.4. ^{11}B NMR (96 MHz, $CDCl_3$) 1.30-0.61 (t, J = 32.7 Hz, $\underline{B}F_2$). ^{19}F NMR (282 MHz, $CDCl_3$) -144.9 – -145.5 (overlapping q and sextet, J = 33.3 Hz, B$\underline{F}_2$). MS (+EI) *m/z* 634 (M^+, 0.1%), 422 (1.2%),

346 (20.7%), 278 (11.6%), 233 (100%), 213 (24.6%), 195 (3.8%), 149 (11.4%), 116 (2.6%), 73 (11.5%), 41 (7.9%). HRMS (EI) calculated for $C_{37}H_{53}BF_2N_2O_2Si$: 634.39375, found 634.39555.

4.2.27c Synthesis of (*S*,*E*)-7-(8-(6-(*tert*-butyldimethylsilyloxy)-2,5,7,8-tetramethylchroman-2-yl)oct-7-enyl)-5,5-difluoro-1,3-dimethyl-5*H*-dipyrrolo[1,2-c:1',2'-f][1,3,2]diazaborinin-4-ium-5-uide (68c, n = 5)

(174 mg, 0.268 mmol, 48%). Dark red oil, R_f = (0.55, hexanes/diethyl ether 3:2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.05 (s, 1H), 6.90-6.89 (d, *J* = 3.9 Hz, 1H), 6.29-6.28 (d, *J* = 3.9 Hz, 1H), 6.08 (s, 1H), 5.53-5.50 (m, 2H, C<u>H</u>=C<u>H</u>), 3.00-2.95 (t, *J* = 7.8 Hz, 2H, BDP-C<u>H</u>$_2$), 2.57-2.49 (m, 5H, C4-C<u>H</u>$_2$, BDP-C<u>H</u>$_3$), 2.23 (s, 3H, Ar-C<u>H</u>$_3$), 2.16 (s, 3H, BDP-C<u>H</u>$_3$), 2.14 (s, 3H, Ar-C<u>H</u>$_3$), 2.06 (s, 3H, Ar-C<u>H</u>$_3$), 2.02-1.67 (m, 6H), 1.46-1.32 (m, 6H), 1.39 (s, 3H, C<u>H</u>$_3$), 1.07 (s, 9H, C(C<u>H</u>$_3$)$_3$), 0.14 (s, 6H, Si(C<u>H</u>$_3$)$_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.8, 158.8, 146.1, 144.0, 142.7, 134.5, 133.9, 133.3, 128.8, 128.5, 128.4, 125.6, 123.4, 122.1, 119.7, 117.6, 116.7, 74.7, 32.3, 32.1, 29.1, 28.9, 28.7, 28.6, 28.5, 27.2, 26.1, 21.1, 18.5, 14.7, 14.3, 13.4, 12.0, 11.1, -3.4. ^{11}B NMR (96 MHz, $CDCl_3$) 1.31-0.62 (t, *J* = 32.7 Hz, <u>B</u>F$_2$). ^{19}F NMR (282 MHz, $CDCl_3$) -145.0 – -145.4 (overlapping q and sextet, *J* = 33.0 Hz, B<u>F</u>$_2$). MS (+EI) *m/z* 648 (M^+, 0.8%), 406 (11.8%), 344 (23.8%), 233 (100%), 213 (26.2%), 194 (3.8%), 117 (2.5%), 55 (4.0%), 41 (5.4%). HRMS (EI) calculated for $C_{38}H_{55}BF_2N_2O_2Si$: 648.40940, found 648.41062.



4.2.28a Synthesis of (*R*)-7-(6-(6-(*tert*-butyldimethylsilyloxy)-2,5,7,8-tetramethylchroman-2-yl)hexyl)-5,5-difluoro-1,3-dimethyl-5*H*-dipyrrolo[1,2-c:1',2'-f][1,3,2]diazaborinin-4-ium-5-uide (69a, n = 3)[110]

A mixture of **68a** (93.6 mg, 0.151 mmol) and Wilkinson's catalyst (69.8 mg, 0.075 mmol) in absolute ethanol (25 ml) was shaken under an atmosphere of hydrogen gas (80 psi) for 52 h. Afterward, the crude product was condensed *in vacuo* and purified via column chromatography (hexanes/diethyl ether 6:1) to afford pure **69a** (32.6 mg, 0.052 mmol, 35%).

Dark red oil, R_f = (0.50, hexanes/diethyl ether 3:2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.06 (s, 1H), 6.91-6.89 (d, J = 3.9 Hz, 1H), 6.28-6.27 (d, J = 3.9 Hz, 1H), 6.08 (s, 1H), 3.00-2.94 (t, J = 7.8 Hz, 2H, BDP-C$\underline{H}_2$), 2.56-2.53 (m, 5H, C4-C$\underline{H}_2$, BDP-C$\underline{H}_3$), 2.24 (s, 3H, Ar-C$\underline{H}_3$), 2.10 (s, 3H, BDP-C$\underline{H}_3$), 2.08 (s, 3H, Ar-C$\underline{H}_3$), 2.06 (s, 3H, Ar-C$\underline{H}_3$), 1.87-1.27 (m, 12H), 1.22 (s, 3H, C$\underline{H}_3$), 1.05 (s, 9H, C(C$\underline{H}_3$)$_3$), 0.14 (s, 6H, Si(C$\underline{H}_3$)$_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.9, 158.9, 145.9, 144.0, 142.8, 134.6, 133.3, 128.5, 125.8, 123.5, 123.1, 122.7, 119.8, 117.5, 116.7, 74.4, 39.6, 31.5, 29.9, 29.7, 28.7, 28.6, 26.1, 23.8, 23.5, 20.9, 18.6, 14.8, 14.3, 13.4, 11.9, 11.2, -3.4. ^{11}B NMR (96 MHz, $CDCl_3$) 1.29-0.60 (t, J = 32.7 Hz, $\underline{B}F_2$). ^{19}F NMR (282 MHz, $CDCl_3$) -145.1 – -145.5 (overlapping q and sextet, J = 33.3 Hz, B$\underline{F}_2$). MS (+EI) *m/z* 622 (M^+, 2.6%), 433 (100%), 302 (2.8%), 233 (16.5%), 208 (7.7%), 168 (53.1%), 149 (26.8%), 69 (12.2%), 55 (18.4%), 43 (30.8%). HRMS (EI) calculated for $C_{36}H_{53}BF_2N_2O_2Si$: 622.39375, found 622.39439.

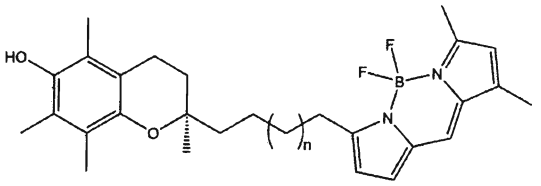
4.2.28b Synthesis of (*R*)-7-(7-(6-(*tert*-butyldimethylsilyloxy)-2,5,7,8-tetramethylchroman-2-yl)heptyl)-5,5-difluoro-1,3-dimethyl-5*H*-dipyrrolo[1,2-c:1',2'-f][1,3,2]diazaborinin-4-ium-5-uide (69b, n = 4)

(27.3 mg, 0.043 mmol, 58%). Dark red oil, R_f = (0.55, hexanes/diethyl ether 3:2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.06 (s, 1H), 6.90-6.89 (d, *J* = 3.9 Hz, 1H), 6.29-6.27 (d, *J* = 3.9 Hz, 1H), 6.08 (s, 1H), 2.99-2.94 (t, *J* = 7.8 Hz, 2H, BDP-C$\underline{H}_2$), 2.56-2.53 (m, 5H, C4-C$\underline{H}_2$, BDP-C$\underline{H}_3$), 2.24 (s, 3H, Ar-C$\underline{H}_3$), 2.10 (s, 3H, BDP-C$\underline{H}_3$), 2.08 (s, 3H, Ar-C$\underline{H}_3$), 2.06 (s, 3H, Ar-C$\underline{H}_3$), 1.83-1.68 (m, 4H), 1.46-1.42 (m, 4H), 1.41-1.27 (m, 6H), 1.22 (s, 3H, C$\underline{H}_3$), 1.05 (s, 9H, C(C$\underline{H}_3$)$_3$), 0.14 (s, 6H, Si(C$\underline{H}_3$)$_2$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 161.0, 158.9, 145.9, 144.0, 142.8, 134.6, 133.3, 128.5, 125.6, 123.5, 123.4, 122.7, 119.9, 117.5, 116.9, 74.5, 39.6, 31.5, 30.0, 29.7, 29.5, 28.7, 28.6, 26.1, 23.8, 23.5, 20.9, 18.6, 14.7, 14.2, 13.4, 12.0, 11.3, -3.3. ^{11}B NMR (96 MHz, $CDCl_3$) 1.29-0.60 (t, *J* = 32.7 Hz, $\underline{B}F_2$). ^{19}F NMR (282 MHz, $CDCl_3$) -145.1 – -145.6 (overlapping q and sextet, *J* = 33.3 Hz, B$\underline{F}_2$). MS (+EI) *m/z* 636 (M^+, 1.1%), 394 (46.0%), 380 (31.0%), 318 (5.4%), 248 (13.0%), 233 (100%), 213 (38.3%), 149 (6.1%), 115 (2.4%), 91 (14.8%), 57 (4.5%), 43 (14.5%). HRMS (EI) calculated for $C_{37}H_{55}BF_2N_2O_2Si$: 636.40940, found 636.40915.

4.2.28c Synthesis of (*R*)-7-(8-(6-(*tert*-butyldimethylsilyloxy)-2,5,7,8-tetramethylchroman-2-yl)octyl)-5,5-difluoro-1,3-dimethyl-5*H*-dipyrrolo[1,2-c:1',2'-f][1,3,2]diazaborinin-4-ium-5-uide (69c, n = 5)

(122.8 mg, 0.189 mmol, 70%). Dark red oil, R_f = (0.55, hexanes/diethyl ether 3:2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.06 (s, 1H), 6.91-6.89 (d, *J* = 3.9 Hz, 1H), 6.30-6.29 (d, *J* = 3.9 Hz, 1H), 6.08 (s, 1H), 3.02-2.97 (t, *J* = 7.8 Hz, 2H, BDP-C$\underline{H}_2$), 2.58-2.55 (m, 5H, C4-C$\underline{H}_2$, BDP-C$\underline{H}_3$), 2.24 (s, 3H, Ar-C$\underline{H}_3$), 2.13 (s, 3H, BDP-C$\underline{H}_3$), 2.11 (s, 3H, Ar-

$C\underline{H}_3$), 2.08 (s, 3H, Ar-$C\underline{H}_3$), 1.89-1.30 (m, 16H), 1.22 (s, 3H, $C\underline{H}_3$), 1.05 (s, 9H, $C(C\underline{H}_3)_3$), 0.14 (s, 6H, $Si(C\underline{H}_3)_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.9, 158.8, 145.9, 144.0, 142.7, 134.5, 133.3, 128.5, 125.7, 123.5, 123.4, 122.6, 119.8, 117.4, 116.8, 74.4, 39.6, 31.5, 29.6, 29.5, 29.4, 29.3, 28.7, 28.6, 26.1, 23.7, 23.6, 20.9, 18.6, 14.8, 14.3, 13.4, 11.9, 11.2, -3.4. ^{11}B NMR (96 MHz, $CDCl_3$) 1.32-0.63 (t, J = 32.7 Hz, $\underline{B}F_2$). ^{19}F NMR (282 MHz, $CDCl_3$) -145.0 – -145.4 (overlapping q and sextet, J = 33.0 Hz, $B\underline{F}_2$). MS (+EI) *m/z* 650 (M^+, 4.1%), 360 (14.2%), 346 (42.9%), 332 (26.2%), 233 (100%), 213 (21.7%), 195 (6.3%), 149 (3.5%), 55 (2.1%), 43 (4.2%). HRMS (EI) calculated for $C_{38}H_{57}BF_2N_2O_2Si$: 650.42505, found 650.42546.



4.2.29a Synthesis of (*R*)-5,5-difluoro-7-(6-(6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)hexyl)-1,3-dimethyl-5*H*-dipyrrolo[1,2-c:1',2'-f][1,3,2]diazaborinin-4-ium-5-uide (42a, n = 3)[117]

69a (30.0 mg, 0.048 mmol) was dissolved in tetrahydrofuran (1.2 ml) and a 10% solution of hydrochloric acid in methanol (1.2 ml) was added dropwise. The reaction stirred for 4.5 h and then was extracted in dichloromethane. After condensation, the crude residue was loaded onto a column (hexanes/diethyl ether 3:1) to afford final compound **42a** (18.8 mg, 0.039 mmol, 77%).

Dark red oil, R_f = (0.25, hexanes/diethyl ether 3:2), R_t = (25.8 min, acetonitrile, 1.00 ml/min, pHPLC). λ_{max} excitation in ethanol = 507 nm (ε_{507} = 81 000 $M^{-1}cm^{-1}$), λ_{max} emission in ethanol = 511 nm. ^1H NMR (600 MHz, $CDCl_3$) δ 7.08 (s, 1H), 6.93-6.92 (d,

J = 3.9 Hz, 1H), 6.30-6.29 (d, J = 3.9 Hz, 1H), 6.11 (s, 1H), 2.99-2.96 (t, J = 7.8 Hz, 2H, BDP-C$\underline{H}_2$), 2.63-2.61 (t, J = 7.2 Hz, 2H, C4-C$\underline{H}_2$), 2.58 (s, 3H, BDP-C$\underline{H}_3$), 2.27 (s, 3H, Ar-C$\underline{H}_3$), 2.18 (s, 3H, BDP-C$\underline{H}_3$), 2.13 (s, 6H, 2xAr-C$\underline{H}_3$), 1.84-1.71 (m, 4H), 1.63-1.50 (m, 2H), 1.47-1.41 (m, 4H), 1.39-1.35 (m, 2H), 1.22 (s, 3H, C$\underline{H}_3$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 160.9, 159.0, 145.5, 144.5, 142.8, 134.6, 133.3, 128.5, 123.5, 122.6, 121.0, 119.9, 118.5, 117.4, 116.8, 74.5, 39.4, 31.5, 29.9, 29.6, 28.7, 28.6, 23.8, 23.5, 20.8, 14.9, 12.2, 11.8, 11.3. ^{11}B NMR (96 MHz, $CDCl_3$) 1.28-0.60 (t, J = 32.7 Hz, $\underline{B}F_2$). ^{19}F NMR (282 MHz, $CDCl_3$) -145.1 – -145.5 (overlapping q and sextet, J = 33.3 Hz, B$\underline{F}_2$). MS (+EI) *m/z* 508 (M^+, 2.4%), 488 ($[M\text{-}HF]^+$, 44.4%), 368 (2.8%), 325 (16.8%), 277 (3.9%), 227 (6.9%), 213 (14.2%), 149 (10.4%), 129 (19.1%), 112 (10.7%), 83 (15.2%), 71 (16.2%), 57 (30.4%), 43 (100%). HRMS (EI) calculated for $C_{30}H_{38}BFN_2O_2$: 488.30104, found 488.30050.

4.2.29b Synthesis of (*R*)-5,5-difluoro-7-(7-(6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)heptyl)-1,3-dimethyl-5*H*-dipyrrolo[1,2-c:1',2'-f][1,3,2]diazaborinin-4-ium-5-uide (42b, n = 4)

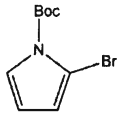
(14.8 mg, 0.029 mmol, 66%). Dark red oil, R_f = (0.10, hexanes/diethyl ether 3:1), R_t = (29.1 min, acetonitrile, 1.00 ml/min, pHPLC). λ_{max} excitation in ethanol = 507 nm (ε_{507} = 85 000 $M^{-1}cm^{-1}$), λ_{max} emission in ethanol = 511 nm. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.08 (s, 1H), 6.93-6.92 (d, J = 3.9 Hz, 1H), 6.30-6.29 (d, J = 3.9 Hz, 1H), 6.11 (s, 1H), 2.99-2.98 (t, J = 7.8 Hz, 2H, BDP-C$\underline{H}_2$), 2.63-2.61 (t, J = 7.2 Hz, 2H, C4-C$\underline{H}_2$), 2.58 (s, 3H, BDP-C$\underline{H}_3$), 2.26 (s, 3H, Ar-C$\underline{H}_3$), 2.18 (s, 3H, BDP-C$\underline{H}_3$), 2.13 (s, 6H, 2xAr-C$\underline{H}_3$), 1.82-1.72 (m, 4H), 1.62-1.53 (m, 2H), 1.45-1.28 (m, 8H), 1.22 (s, 3H, C$\underline{H}_3$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 161.0, 159.0, 145.5, 144.5, 142.8, 134.6, 133.3, 128.5, 123.5,

122.6, 121.0, 119.9, 118.5, 117.4, 116.8, 74.5, 39.5, 31.5, 30.0, 29.5, 29.4, 28.7, 28.6, 23.8, 23.6, 20.8, 14.9, 12.2, 11.8, 11.3. ^{11}B NMR (96 MHz, $CDCl_3$) 1.29-0.60 (t, J = 32.7 Hz, $\underline{B}F_2$). ^{19}F NMR (282 MHz, $CDCl_3$) -145.1 – -145.5 (overlapping q and sextet, J = 33.0 Hz, $B\underline{F}_2$). MS (+EI) *m/z* 522 (M^+, 12.1%), 502 ($[M\text{-}HF]^+$, 100%), 368 (4.8%), 302 (26.1%), 227 (14.1%), 213 (25.1%), 205 (12.5%), 165 (12.8%), 149 (22.8%), 129 (32.7%), 112 (19.5%), 86 (31.3%), 73 (49.7%), 57 (53.5%), 45 (26.1%). HRMS (EI) calculated for $C_{31}H_{40}BFN_2O_2$: 502.31669, found 502.31872.

4.2.29c Synthesis of (*R*)-5,5-difluoro-7-(8-(6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)octyl)-1,3-dimethyl-5*H*-dipyrrolo[1,2-c:1',2'-f][1,3,2]diazaborinin-4-ium-5-uide (42c, n = 5)

(17.0 mg, 0.033 mmol, 74%). Dark red oil, R_f = (0.25, hexanes/diethyl ether 3:2), R_t = (33.4 min, acetonitrile, 1.00 ml/min, pHPLC). λ_{max} excitation in ethanol = 507 nm (ε_{507} = 83 000 $M^{-1}cm^{-1}$), λ_{max} emission in ethanol = 511 nm. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.08 (s, 1H), 6.93-6.92 (d, J = 3.9 Hz, 1H), 6.31-6.30 (d, J = 3.9 Hz, 1H), 6.11 (s, 1H), 2.99-2.97 (t, J = 7.8 Hz, 2H, BDP-C$\underline{H}_2$), 2.63-2.61 (t, J = 7.2 Hz, 2H, C4-C$\underline{H}_2$), 2.58 (s, 3H, BDP-C$\underline{H}_3$), 2.26 (s, 3H, Ar-C$\underline{H}_3$), 2.18 (s, 3H, BDP-C$\underline{H}_3$), 2.13 (s, 6H, 2xAr-C$\underline{H}_3$), 1.84-1.71 (m, 4H), 1.62-1.54 (m, 2H), 1.44-1.41 (m, 4H), 1.35-1.28 (m, 6H), 1.23 (s, 3H, C$\underline{H}_3$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 161.0, 159.0, 145.5, 144.5, 142.8, 134.6, 133.3, 128.5, 123.5, 122.6, 121.0, 119.9, 118.5, 117.4, 116.9, 74.5, 39.5, 31.5, 30.1, 29.6, 29.5, 29.4, 28.7, 28.6, 23.8, 23.6, 20.8, 14.9, 12.2, 11.8, 11.3. ^{11}B NMR (96 MHz, $CDCl_3$) 1.29-0.60 (t, J = 32.7 Hz, $\underline{B}F_2$). ^{19}F NMR (282 MHz, $CDCl_3$) -145.2 – -145.5 (overlapping q and sextet, J = 33.0 Hz, $B\underline{F}_2$). MS (+EI) *m/z* 536 (M^+, 1.4%), 516 ($[M\text{-}HF]^+$, 36.8%), 408 (3.1%), 353 (10.1%), 298 (33.3%), 278 (3.6%), 233 (17.6%), 213

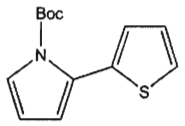
(18.4%), 191 (3.8%), 171 (100%), 129 (14.1%), 97 (17.0%), 69 (22.3%), 55 (33.3%), 43 (47.1%). HRMS (EI) calculated for $C_{32}H_{42}BFN_2O_2$: 516.33234, found 516.33308.



4.2.30 Synthesis of *tert*-butyl 2-bromo-1*H*-pyrrole-1-carboxylate (83)[134]

Pyrrole (5.00 g, 74.5 mmol) was dissolved in tetrahydrofuran (200 ml) and cooled to –78 °C under nitrogen. 1,1-Azobis(cyclohexanecarbonitrile) (182 mg, 0.745 mmol) was then added and the mixture stirred for 5 min. Over the course of 15 min, 1,3-dibromo-5,5′-dimethylhydantoin (10.7 g, 37.3 mmol) was slowly added and the solution stirred for an additional 10 min. Next, the reaction mixture stood for 2 h while maintaining a temperature below –50 °C. This mixture was then filtered via suction and TEA (4.15 ml, 29.8 mmol), Boc_2O (24.0 ml, 0.104 mol), and DMAP (91.0 mg, 0.745 mmol) were added to the stirring filtratea as it was warmed to room temperature. After stirring overnight, the crude material was condensed *in vacuo* and extracted with dichloromethane. Purification by silica gel chromatography (hexanes/ethyl acetate 20:1) afforded pure **83** (16.4 g, 67.0 mmol, 90%).

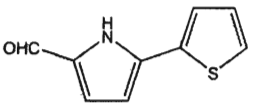
Clear and colourless oil, R_f = (0.50, hexanes/ethyl acetate 10:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30-7.25 (dd, J = 3.6 Hz, J' = 1.8 Hz, 1H, C<u>H</u>N), 6.23-6.22 (dd, J = 3.6 Hz, J' = 1.8 Hz, 1H, C<u>H</u>CBr), 6.09-6.07 (dd, J = 3.6 Hz, 1H), 1.56 (s, 9H, C(C<u>H</u>$_3$)$_3$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 147.5, 122.6, 116.8, 111.2, 99.8, 84.2, 27.5. MS (+EI) *m/z* 245 (M^+, 12.1%), 186 (4.1%), 172 (11.3%), 145 (48.6%), 119 (2.1%), 64 (5.5%), 57 (100%), 41 (45.4%). HRMS (EI) calculated for $C_9H_{12}NO_2Br$: 245.00514, found 245.00512.



4.2.31 Synthesis of *tert*-butyl 2-(thiophen-2-yl)-1*H*-pyrrole-1-carboxylate (88)[83]

To a solution of **83** (1.30 g, 5.28 mmol) and 2-thienylboronic acid (450 mg, 3.52 mmol) in *n*-butanol was added palladium (II) acetate (15.8 mg, 0.070 mmol), SPhos (57.7 mg, 0.141 mmol), and potassium phosphate tribasic (1.49 g, 7.03 mmol). The reaction was then heated to 100 °C for 5 h. Afterward, the solution was washed with water and the organic layer was collected, condensed *in vacuo*, and the crude material was purifed by silica gel chromatography (hexanes/ethyl acetate 30:1) to yield **88** (656 mg, 2.63 mmol, 75%).

Light yellow oil, R_f = (0.30, hexanes/ethyl acetate 20:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42-7.40 (dd, J = 3.3 Hz, J' = 1.8 Hz, 1H), 7.33-7.31 (dd, J = 5.1 Hz, J' = 0.9 Hz, 1H), 7.10-7.08 (dd, J = 3.6 Hz, J' = J = 0.9 Hz, 1H), 7.05-7.02 (dd, J = 5.1 Hz, J' = 3.6 Hz, 1H), 6.35-6.34 (dd, J = 3.3 Hz, J' = 1.8, 1H), 6.25-6.23 (dd, J = 3.3 Hz, J' = 3.3 Hz, 1H), 1.46 (s, 9H, $C(C\underline{H}_3)_3$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 148.9, 134.8, 127.6, 126.7, 126.3, 125.4, 123.0, 116.5, 110.4, 83.6, 27.5. MS (+EI) *m/z* 249 (M^+, 13.2%), 193 (48.7%), 176 (5.3%), 149 (90.8%), 104 (10.2%), 57 (100%), 41 (27.4%). HRMS (EI) calculated for $C_{13}H_{15}NO_2S$: 249.08235, found 249.08236.

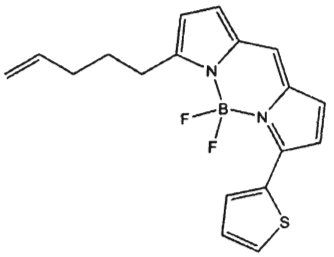


4.2.32 Synthesis of 5-(thiophen-2-yl)-1*H*-pyrrole-2-carbaldehyde (89)[144]

To a solution of *N,N*-dimethylformamide (40.8 µl, 0.529 mmol) in 1,2-dichloroethane (1 ml) at 0 °C was slowly added phosphorus oxychloride (48.5 µl, 0.529

mmol). The reaction mixture was then warmed to room temperature for 2 h. It was cooled to 0 °C again to allow the slow addition of **88** (120 mg, 0.481 mmol) in 1,2-dichloroethane (2.4 ml). The reflux went for 1 h. Then a solution of sodium acetate (360 mg) in water (1 ml) was added and boiling continued for 1 h. The organic layer was collected and condensed *in vacuo.* Flash chromatography (hexanes/ethyl acetate 8:1) was performed to obtain pure **89** (50.0 mg, 0.282 mmol, 59%).

Light yellow oil, R_f = (0.10, hexanes/ethyl acetate 10:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.01 (br s, 1H, NH), 9.50 (s, 1H, CHO), 7.41-7.40 (d, J = 3.3 Hz, 1H), 7.33-7.31 (d, J = 5.1 Hz, 1H), 7.10-7.08 (dd, J = 4.2 Hz, J' = 4.2 Hz, 1H), 7.01-6.99 (dd, J = 2.9 Hz. J' = 2.9 Hz, 1H), 6.54-6.52 (dd, J = 2.9 Hz, J' = 2.9 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.6, 134.8, 133.7, 132.9, 128.2, 125.8, 124.4, 122.9, 109.4. MS (+EI) *m/z* 177 (M^+, 100%), 148 (7.7%), 121 (30.0%), 104 (4.7%), 74 (5.6%), 63 (5.2%), 51 (4.6%), 45 (8.4%). HRMS (EI) calculated for C_9H_7NOS: 177.02484, found 177.02482.

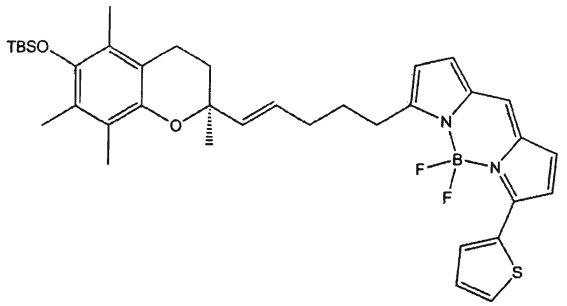


4.2.33 Synthesis of 5,5-difluoro-7-(pent-4-enyl)-3-(thiophen-2-yl)-5*H*-dipyrrolo[1,2-c:1',2'-f][1,3,2]diazaborinin-4-ium-5-uide (94)[46]

Crude **92** and **89** (190 mg, 1.70 mmol) were dissolved in dry dichloromethane (16 ml) and cooled to 0 °C under nitrogen. A solution of phosphorus oxychloride (98.1 µl, 1.70 mmol) in dichloromethane (1 ml) was added via syringe and this was kept on ice for 5 h. Afterward, boron trifluoride diethyl etherate (544 µl, 4.30 mmol) and DIPEA (748

ml, 4.30 mmol) were added and stirring was continued for overnight at room temperature. The crude product was extracted in dichloromethane and condensed *in vacuo*. The residue was purified by silica gel chromatography (hexanes/diethyl ether 3:1) to afford pure **94** (83.2 mg, 0.243 mmol, 11% over two steps).

Dark purple oil, R_f = (0.40, hexanes/diethyl ether 3:2). λ_{max} excitation in ethanol = 561 nm (ε_{561} = 78 000 $M^{-1}cm^{-1}$), λ_{max} emission in ethanol = 570 nm. ^1H NMR (300 MHz, $CDCl_3$) δ 8.16-8.15 (d, *J* = 3.6 Hz, 1H), 7.47-7.45 (d, *J* = 5.1 Hz, 1H), 7.19-7.16 (dd, *J* = 4.8 Hz, *J* = 4.2 Hz, 1H), 7.05 (s, 1H), 6.96-6.93 (dd, *J* = *J* = 4.5 Hz, 2H), 6.74-6.73 (d, *J* = 4.2 Hz, 1H), 6.38-6.37 (d, *J* = 4.2 Hz, 1H), 5.95-5.81 (ddt, *J* = 17.0 Hz, *J′* = 10.3 Hz, *J″* = 6.7 Hz, 1H, C<u>H</u>=CH_2), 5.12-5.01 (m, 2H, CH=C<u>H_2</u>), 3.11-3.05 (t, *J* = 7.8 Hz, 2H, TBDP-C<u>H_2</u>), 2.26-2.19 (m, 2H, C<u>H_2</u>CH=CH_2), 1.92-1.82 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.6, 149.7, 138.0, 136.2, 134.8, 134.1, 130.7, 130.6, 130.1, 129.7, 128.8, 126.2, 119.6, 118.9, 115.1, 33.5, 28.4, 27.8. ^{11}B NMR (96 MHz, $CDCl_3$) 1.72-1.03 (t, *J* = 24.1 Hz, <u>B</u>F_2). ^{19}F NMR (282 MHz, $CDCl_3$) -141.8 – -142.1 (overlapping q and sextet, *J* = 33.3 Hz, B<u>F_2</u>). MS (+EI) *m/z* 342 (M^+, 60.7%), 328 (4.3%), 300 (22.1%), 287 (100%), 260 (16.6%), 231 (8.8%), 218 (19.0%), 205 (49.4%), 186 (6.4%), 162 (5.7%), 149 (15.7%), 121 (5.7%), 93 (4.2%), 80 (5.7%), 57 (6.1%), 41 (4.2%). HRMS (EI) calculated for $C_{18}H_{17}BF_2N_2S$: 342.11736, found 342.11730.



4.2.34 Synthesis of (*S*,*E*)-7-(5-(6-(*tert*-butyldimethylsilyloxy)-2,5,7,8-tetramethylchroman-2-yl)pent-4-enyl)-5,5-difluoro-3-(thiophen-2-yl)-5*H*-dipyrrolo [1,2-c:1',2'-f][1,3,2]diazaborinin-4-ium-5-uide (95)[43]

Vinyl Trolox **61** (104 mg, 0.299 mmol) and pentenylTBDP **94** (205 mg, 0.598 mmol) were dissolved in dry dichloromethane (2.4 ml). Catalyst C-571 from Materia, Inc. (*o*-tolyl derivative of Hoveyda-Grubbs Catalyst 2nd Generation, **81**) (17.0 mg, 0.030 mmol) was then added and the reaction refluxed for 9 h. The mixture was condensed *in vacuo* and the crude residue was purified by silica gel chromatography (hexanes/diethyl ether 6:1) to obtain **95** (27.8 mg, 0.042 mmol, 14%).

Dark purple oil, R_f = (0.35, hexanes/diethyl ether 3:2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15-8.14 (d, *J* = 3.6 Hz, 1H), 7.48-7.47 (m, 1H), 7.21-7.15 (m, 1H), 7.09 (s, 1H), 7.02-6.99 (m, 2H), 6.78-6.77 (d, *J* = 3.6 Hz, 1H), 6.32-6.31 (d, *J* = 4.2 Hz, 1H), 5.58-5.55 (m, 2H, C$\underline{H}$=C$\underline{H}$), 3.01-2.93 (m, 2H, TBDP-C$\underline{H}_2$), 2.56-2.50 (m, 2H, C4-C$\underline{H}_2$), 2.15 (s, 3H, Ar-C$\underline{H}_3$), 2.13 (s, 3H, Ar-C$\underline{H}_3$), 2.04 (s, 3H, Ar-C$\underline{H}_3$), 1.97-1.74 (m, 6H), 1.40 (s, 3H, C$\underline{H}_3$), 1.06 (s, 9H, C(C$\underline{H}_3$)$_3$), 0.13 (s, 6H, Si(C$\underline{H}_3$)$_2$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 164.1, 149.7, 146.1, 144.2, 136.3, 134.7, 134.5, 134.2, 131.6, 130.7, 130.6, 130.2, 129.6, 128.8, 126.2, 125.8, 123.5, 122.3, 119.3, 118.8, 117.7, 77.2, 32.4, 31.6, 29.4, 28.4, 27.3, 26.1, 21.2, 18.6, 14.4, 13.4, 12.1, -3.3. ^{11}B NMR (96 MHz, $CDCl_3$) 1.68-1.00 (t, *J* = 32.7 Hz, $\underline{B}F_2$). ^{19}F NMR (282 MHz, $CDCl_3$) -141.9 – -142.4 (overlapping q

and sextet, J = 32.5 Hz, B$\underline{F}_2$). MS (+EI) *m/z* 660 (M^+, 7.3%), 640 (6.5%), 578 (6.4%), 450 (4.8%), 368 (9.1%), 342 (80.9%), 319 (100%), 294 (72.9%), 262 (28.2%), 221 (20.6%), 183 (42.5%), 149 (20.8%), 117 (13.6%), 73 (68.5%), 55 (37.5%), 43 (37.5%). HRMS (EI) calculated for $C_{37}H_{47}BF_2N_2O_2SSi$: 660.31887, found 660.31961.

4.3 Fluorescent Binding Assay Protocol

4.3.1 Preparation of Ligand Stocks

Synthetic fluorescent analogues and natural ligands were assessed for purity by NMR and HPLC prior to preparation of concentrated stocks (0.030-0.200 mM) in absolute ethanol. These stock solutions were stored at 4 °C until required and protected from direct exposure to light. Working, diluted stocks were freshly prepared in absolute ethanol at desired concentrations, mixed thoroughly by vortexing and kept on ice while performing the binding assays.

4.3.2 Fluorescence Measurements

Steady state fluorescence was measured using a spectrofluorometer employing right angle illumination with a 150 W xenon lamp. All measurements were made at 115 W with excitation and emission slit widths of 5 nm and at an excitation of 506 nm. Equilibrium fluorescence binding data was analyzed using non-linear least-squares regression analysis fitted to a one-site binding equation[145] using Graphpad Prism 4.0 software.

4.3.3 Fluorescence Titration Assay

All titration assays for BDP-α-Tocs **42a-c** were performed similarly unless otherwise noted.

Titrations were performed in a glass cuvette containing a total reaction volume of 3 ml SET buffer and protein sample. Sufficient stock of α-TTP (typically less than 50 µl) was added such that the final protein concentration was 1.0 µM. The sample was then well-mixed by repeated inversion and a baseline fluorescence measurement was recorded at 506 nm. To this solution were added aliquots (0.6-2.0 µl) of working fluorophore stock solutions (0.030-0.200 mM). Generally after 20 min of end-over-end mixing using a Roto-Torque® rotating mixer, the signal had reached a maximum and the emission spectrum was recorded. Working stock solutions were prepared such that the final concentration of ethanol did not exceed 2% v/v. No-protein controls involved titration of fluorescent tocopherols into SET buffer under identical conditions and at identical concentrations.

4.3.4 Fluorescence Competition Assay

Competition assays were performed under similar conditions as section 4.3.3. To a 0.2 µM solution of α-TTP in 3 ml SET buffer was added 2 µl of a stock solution of fluorescent analogue in absolute ethanol (1.5 mM) such that the final concentration of analogue was 1 µM. The solution was then mixed until the fluorescence signal remained the same (equilibrium was reached). To this solution were added 2 µl aliquots of α-tocopherol in absolute ethanol (1.5 mM to 15 mM) to yield final concentrations of tocopherol ranging from 1 to 40 µM. After each addition of tocopherol, the samples were mixed for 15 min to attain equilibrium, and the final fluorescence recorded. Control experiments included an identical competition substituting cholesterol as the competitor.

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