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Preparation of fluorescent tocopherols for use in protein binding and localization with the α-tocopherol transfer protein

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Abstract—Sixteen fluorescent analogues of the lipid-soluble antioxidant vitamin α-tocopherol were prepared incorporating fluorophores at the terminus of ω-functionalized 2-*n*-alkyl-substituted chromanols (**1a**–**d** and **4a**–**d**) that match the methylation pattern of α-tocopherol, the most biologically active form of vitamin E. The fluorophores used include 9-anthroyloxy (AO), 7-nitrobenz-2-oxa-1,3-diazole (NBD), *N*-methyl anthranilamide (NMA), and dansyl (DAN). The compounds were designed to function as fluorescent reporter ligands for protein-binding and lipid transfer assays. The fluorophores were chosen to maximize the fluorescence changes observed upon moving from an aqueous environment (low fluorescence intensity) to an hydrophobic environment such as a protein's binding site (high fluorescence intensity). Compounds **9d** (anthroyloxy) and **10d** (nitrobenzoxadiazole), having a C9-carbon chain between the chromanol and the fluorophore, were shown to bind specifically and reversibly to recombinant human tocopherol transfer protein (α-TTP) with dissociation constants of approximately 280 and 60 nM, respectively, as compared to 25 nM for the natural ligand 2*R*,4′*R*,8′*R*-α-tocopherol. Thus, compounds have been prepared that allow the investigation of the rate of α-TTP-mediated inter-membrane transfer of α-tocopherol and to investigate the mechanism of α-TTP function at membranes of different composition.

1. Introduction

α-Tocopherol is now accepted to be the dominant lipid-soluble antioxidant in biological membranes.[1] The bioavailability and biokinetics of α-tocopherol[2,3] and γ-tocopherol[4,5] have been well studied in humans and it is clear that the selective retention of the natural stereoisomer (*RRR*) of α-tocopherol[6] is due to the presence of the α-tocopherol transfer protein (α-TTP) expressed predominantly in liver.[7–9] Of all the ligands tested so far, α-TTP binds α-tocopherol with the highest affinity, approximately 25 nM.[10] However, the mechanisms by which α-TTP delivers α-tocopherol from hepatocytes to carrier lipoproteins in the plasma are poorly understood. Particularly opaque are the mechanisms by which very-low density lipoprotein (VLDL) particles are enriched with vitamin E prior to or during their secretion from the liver[6,11] and the molecular basis of transport of tocopherol between different intracellular sites. A significant barrier to studies with this vitamin is that current techniques for determining tissue or plasma levels require extraction in organic solvents followed by chromatographic analysis and often mass spectrometry. These analytical barriers especially complicate determinations of where the tocopherol resides in a cell and of the dynamics of α-TTP-mediated tocopherol transfer between membranes. While tocopherol is weakly fluorescent ($\lambda_{abs} \sim 290$ nm, $\lambda_{em} \sim 330$ nm) in EtOH,[12,13] its absorption spectrum overlaps with those of many other cell components and does not allow for selective monitoring techniques such as fluorescence resonance energy transfer (FRET) or fluorescence microscopy.

Similar difficulties were encountered in the study of other hydrophobic ligands and their transfer proteins, and these were overcome by the preparation of novel fluorescently labeled reporters such as the anthroyloxy stearic acids[14,15] and more recently the BODIPY fatty acids.[16,17] Fluorescently-labeled ligands have been particularly useful probes for quantitative binding assays and for the elucidation of the mechanism underlying protein-mediated inter-membrane transfer of the ligand

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in the case of fatty acid-binding protein (FABP),[18–20] sterol transfer protein,[21–24] and cholesterol transfer protein.[25–28] Based on the documented success of fluorescent hydrophobic ligands, we designed and prepared fluorescent analogues of α-tocopherol that enable quantitative studies of ligand binding and transfer by α-TTP.

2. Results and discussion

As we have noted previously in the design of photoaffinity ligands of tocopherol,[29] any manipulation of the tocopherol skeleton must not interfere with the chromanol head group as this will lessen or abolish the affinity of such ligands to α-TTP.[10,30] As a consequence, the phytyl tail remains the only place available for modification with a fluorescent group. To simplify this task the side-chain methyl groups can be omitted, as their presence is not necessary for the biological activity of the tocopherols.[31] Thus, the sole remaining possible variation in structure is the choice of fluorophore and the length of the alkyl chain connecting the fluorophore to the chromanol. The recent X-ray structure determinations of human α-TTP[32,33] offer some guidance in this regard. We chose to prepare structures with varying chain lengths that 'bracket' the approximate side-chain length of α-tocopherol. The general synthetic approach follows very closely that reported in our earlier work with tocopherol photoaffinity labels[29] and is outlined in Figure 1.

The terminal alcohols **1a**–**d** are prepared from the commercially available (*S*)-Trolox® after conversion to the methyl ester, protection of phenol as the *tert*-butyldimethylsilyl ether, reduction to the aldehyde, and reaction with the ω-functionalized phosphonium salts of varying chain lengths.[29] Compounds **1a**–**d** can easily be esterified to 9-anthracenecarboxylic acid (oxalyl chloride, cat. DMF, CH_2Cl_2 then Et_3N, and **1a**–**d**) giving the protected ω-anthroyloxy tocopherols **5a**–**d** that are readily deprotected (TBAF, THF) to give the free phenols **9a**–**d**. Despite the fact that these compounds lack the normal phytyl side-chain of the tocopherols, for simplicity we call these 'anthroyloxy-α-tocopherols' or AO-α-Tocs.

In order to prepare conjugates of other fluorophores, it was necessary to transform the alcohol functional group in compounds **1a**–**d** to the terminal amines in **4a**–**d**. This was accomplished in a straightforward manner by mesylation, substitution with azide, and hydride reduction. The amines **4a**–**d** were coupled to fluorophores following standard procedures.[34] It is wisest to store the silyl ether-protected compounds rather than the free phenols as the easily cleavable silyl ethers (TBAF, THF) are less prone to oxidative decomposition—a problem common to the tocopherols. In the end, 16 compounds (four homologous series) were prepared having four chain lengths and four different fluorophores attached to a 2-alkyl chromanol that mimics natural source α-tocopherol with respect to the chromanol methyl groups at C-5, C-7 and C-8, and the C-2 *R* stereochemistry. The final compounds are shown in Figure 2.

We were primarily interested in developing a fluorescent form of tocopherol that would be useful in FRET assays to monitor inter-membrane tocopherol transfer. We focused our attention on the AO-α-Tocs, as they are analogous to the anthroyloxy fatty acids that have been well characterized as FRET partners with NBD-labeled phospholipids and used for monitoring the fatty acid transfer activity of FABP.[18] The NBD absorption spectrum ($\lambda_{abs} \sim 465$ nm) overlaps the emission spectrum of the AO-α-Tocs ($\lambda_{em} \sim 460$ nm). Thus, when a donor membrane or α-TTP containing AO-Toc is mixed with an acceptor membrane containing an NBD-phospholipid, one can monitor the decay in fluorescence of the AO-α-Toc as it arrives in the acceptor vesicle. A similar transfer assay system can be constructed using an

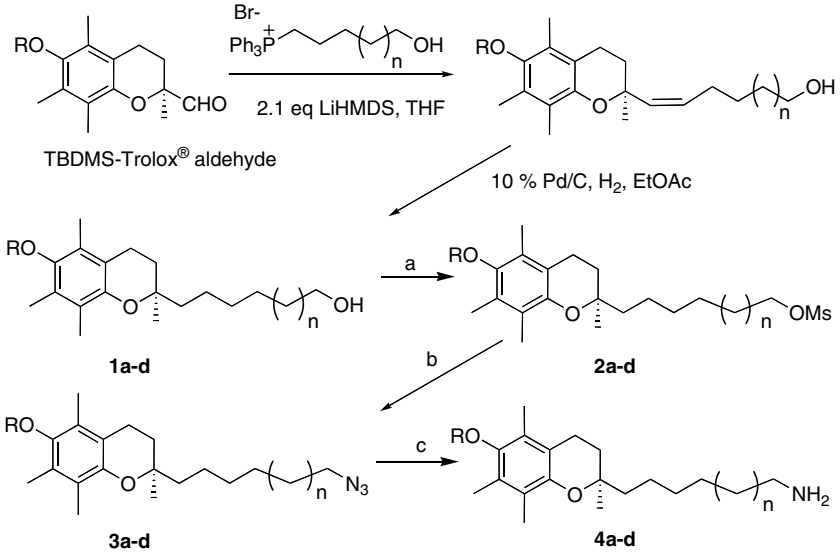


Figure 1. R = TBDMS, *n* = 1–4. Reagents and conditions: (a) MsCl, Et_3N, DMAP, CH_2Cl_2, 0 °C, rt, 80–85%; (b) NaN_3, DMF, 55 °C, 85–89% from **1a**–**d**; (c) $LiAlH_4$, THF, 0 °C, 83–90%.

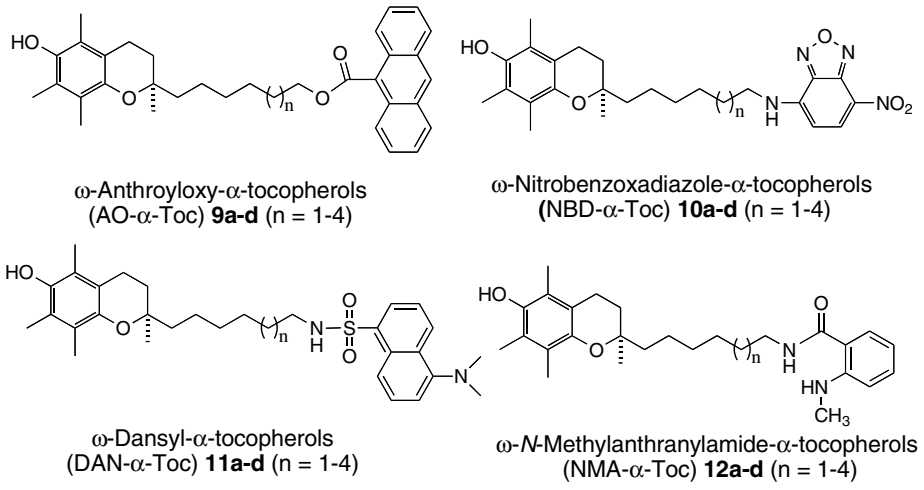


Figure 2. Structures of the 16 ω-functionalized fluorescent analogues of α-tocopherol prepared in this work.

NBD-α-Toc as FRET donor and a tetramethylrhodamine phospholipid such as TRITC-PE as a FRET acceptor ($\lambda_{abs} \sim 540$ nm), or a Marina Blue-labeled phospholipid as a FRET donor ($\lambda_{abs} \sim 362$ nm, $\lambda_{em} \sim 459$ nm) and the NBD-α-Toc as the acceptor.[35]

Transfer of α-tocopherol between membranes in vivo is understood to be mediated by the α-tocopherol transfer protein (α-TTP).[7,8,36] Thus, if the fluorescent analogues are to be of any utility, it is required that they bind specifically and reversibly to this protein so that they may act as competent probes of the unmodified, naturally occurring vitamin. Figure 3 shows fluorescent titrations of recombinant human α-TTP with C9-NBD-α-Toc, **10d**. Figures 4 and 5 show that both the C9-NBD-α-Toc and C9-AO-α-TOH analogues exhibit a dose-dependent decrease in fluorescence upon the addition of tocopherol. This suggests that the environmentally sensitive fluorophores are displaced from the hydrophobic-binding pocket of α-TTP by the native ligand.

Competitive assays with these hydrophobic ligands are not straightforward to interpret since both the

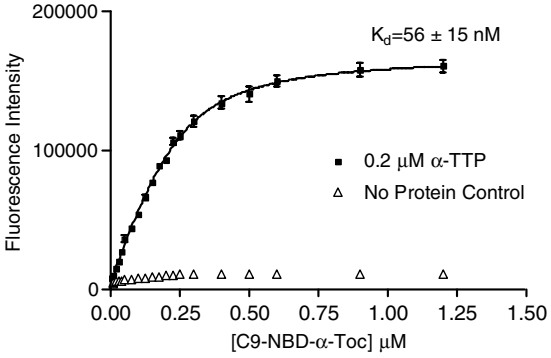


Figure 3. Titration curves showing the increase in fluorescence intensity at 535 nm during sequential additions of the C9-NBD-α-Toc, **10d** (black squares), to a 0.2 μM solution of α-TTP in SET buffer (see Section 4). The curves are fitted to a one-site bimolecular association model.[37] Averages and standard errors of triplicate data sets are reported. $\lambda_{ex} = 469$ nm.

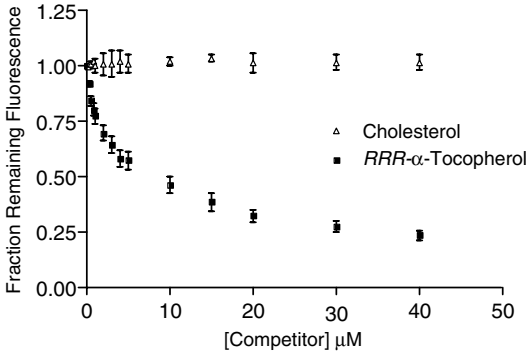


Figure 4. Competitive displacement of C9-NBD-α-Toc (**10d**) bound to α-TTP by addition of increasing amounts of *RRR*-α-tocopherol or cholesterol. Fluorescence was monitored at 535 nm. The starting protein–ligand complex included 0.2 μM α-TTP and 1.0 μM C9-NBD-α-Toc. Competitions were performed in the same buffer as reported in Figure 3 plus 100 μM Triton X-100.

fluorophore and the competitor are near their solubility limits. By way of comparison, the water solubility of retinol[38] and cholesterol[39] in water is 60 and 37 nM, respectively. The solubility of α-tocopherol has been reported to be 49 μM,[40] but in our experience, the preparation and use of pure tocopherol solutions in aqueous media does not approach this value. The provision of organic co-solvents (generally 1% or less of EtOH, acetonitrile, or DMSO) is useful for preparing low concentration 'solutions' (generally below ~20 μM), but higher concentrations of ligand with similar content of organic co-solvent do not exist in solution, but rather as distinct populations of ligand aggregates in suspension, and/or adhered to the surfaces of glass or plasticware. As a result, attempting a competitive displacement of bound C9-NBD-α-Toc (**10d**) with α-tocopherol is not possible in buffer lacking detergent. This is the result of the inability to supply sufficient dissolved tocopherol in a solution lacking detergent to achieve competitive equilibrium in a reasonable length of time. The ability of the ligand to dissociate from the binding site is predicated on having a thermodynamically favorable place to move to, and generally an aqueous buffer did not

provide this requirement. This was a more pronounced problem for the anthroyloxy compounds than for the NBD analogues. In effect, the ligand prefers to stay in the binding site than dissociate into solution where its solubility is very low. Complicating this is the observation that adding detergent to the assay buffer raises the background fluorescence as the fluorophore is removed from the protein. The displaced fluorophore then exists in a hydrophobic complex with detergent and generates a higher fluorescence signal than in buffer alone. The result is what looks like an incomplete competition; down to only about 30% of the original fluorescence. It is worth noting that detergent alone does not remove any of the fluorophores once bound to α-TTP. This is exemplified by the constant fluorescence signal of the ligand in Figures 4 and 5. Competitions with cholesterol, which has a very low affinity to TTP,[10] showed an apparent increase in fluorescence for the C9-AO-α-Toc (Figure 5). This is an artifact due to the very long times required for equilibration of α-TTP with fluorophore in detergent-containing buffers. During fluorescence titrations in buffers lacking detergent, equilibration (i.e., a stable fluorescence intensity) was achieved in 5–10 min with all ligands, but in detergent-containing buffers the time to a similar stable fluorescence signal required 1 h for NBD-α-Toc and >4 h for AO-α-Toc. As a result the cholesterol competition in Figure 5 was not yet at equilibrium (~95%) and consequently normalization of data at higher concentrations appeared to give >100% relative fluorescence.

The dissociation constants K_d, defined as [TTP]/[TTP-ligand][ligand], have also been assessed by fluorescence titrations. The anthroyloxy tocopherol analogues display higher K_d values (lower affinity) for α-TTP than their similar chain length NBD-α-Tocs. The C9-AO-α-Toc (**9d)** is the only compound of **9a**–**d** for which a K_d for binding with α-TTP could be reliably determined (Figure 6). Compounds **9a**–**c**, although able to bind to α-TTP, all showed significant non-specific binding (data not shown) which could not be corrected for by non-specific controls and thus K_d-values could not be determined. One advantage of the AO-α-Tocs is their very weak fluorescence in aqueous solution. Contrarily, while the C9-NBD-α-Toc **10d** had less than 2% of the fluorescence in buffer than an equimolar amount in ethanol, the shorter chain lengths had greater fluorescence in aqueous buffers: **10a** (~20%), **10b** (~10%) and **10c** (~6%). Dissociation constants for the NBD- and AO-α-Tocs are listed in Table 1.

Unfortunately, the dansyl (DAN) and *N*-methylanthranilamide (NMA) compounds did not behave as expected in fluorescence titrations. While they may show specific binding to α-TTP, these analogues retained substantial fluorescence intensity in aqueous solution thus complicating distinction between free and bound ligands. This is illustrated for C9-DAN-α-Toc in Figure 7. Titration of α-TTP with sequential additions of any of the DAN or NMA fluorophores resulted in a linear, non-saturable increase in fluorescence that did not differ greatly from controls lacking α-TTP. The minor increase in fluorescence in the protein-containing samples (versus no protein control) likely reflects a combination of specific and non-specific binding to the protein. It is possible that the DAN and NMA fluorophores may be too polar to be accepted into the hydrophobic α-TTP-binding site.

The binding specificity of C9-AO-α-Toc (**9d**) was further supported by the results of modeling calculations. The goal of these calculations was to determine whether

Table 1. Dissociation constants, K_d, for fluorescent tocopherol analogues **9a**–**d** and **10d** binding to recombinant α-TTP

NBD-α-Toc	K_d (nM)	AO-α-Toc	K_d (nM)
10a	299 ± 37	**9a**	nd
10b	106 ± 21	**9b**	nd
10c	142 ± 35	**9c**	nd
10d	56 ± 15	**9d**	279 ± 124

nd, accurate value could not be determined due to non-specific binding.

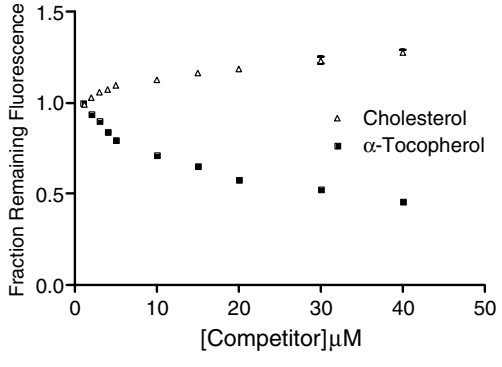


Figure 5. Competitive displacement of C9-AO-α-Toc, **9d**, bound to α-TTP by addition of increasing amounts of *RRR*-α-tocopherol or cholesterol. Fluorescence was monitored at 460 nm. Averages and standard errors of triplicate data sets are reported for tocopherol, duplicates for cholesterol. The starting protein–ligand complex included 0.2 μM α-TTP and 1.0 μM C9-AO-α-Toc. Competitions were performed in the same buffer as reported in Figure 3 plus 100 μM Triton X-100.

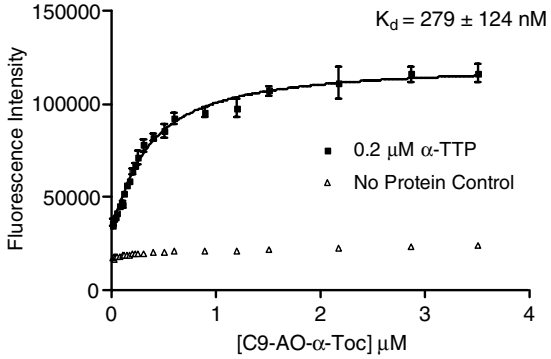


Figure 6. Titration curves showing the increase in fluorescence intensity at 460 nm during sequential additions of the C9-AO-α-Toc, **9d** (black squares), to a 0.2 μM solution of α-TTP in SET buffer (see Section 4). The curves are fitted to a one-site bimolecular association model.[37] Averages and standard errors of triplicate data sets are reported. λ_{ex} = 363 nm.

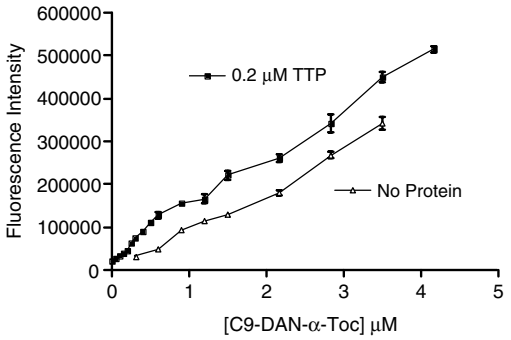


Figure 7. Titration curve showing the increase in fluorescence intensity at 510 nm during sequential additions of the fluorophore C9-DAN-α-Toc, **11d** (black squares) to a 0.2 μM solution of α-TTP in SET buffer (see Section 4). No saturable binding was observed.

the known binding site of α-TTP could accommodate a low-energy conformation of the fluorescent ligand, without requiring significant distortion to accommodate the bulky fluorophore. Using the X-ray coordinates of human α-TTP in complexation with α-Toc,[32] the initial conformation of C9-AO-α-Toc (**9d**) was manually docked into the binding site, imposing the same atomic coordinates for the chromanol head group as those observed for the native ligand. The dihedral angles of the alkyl tail with its pendant anthroyloxy substituent were manually adjusted to fit within the confines of the binding site, without regard to energetic considerations. Subsequently, two investigations were carried out—one with all atoms of the protein fixed in their crystallographic positions, and in the second, the protein backbone atoms were subjected to harmonic constraints, whereas all atoms of the side-chains were unconstrained. In both studies, short (15 ps) high-temperature (500 K) molecular dynamics (MD) simulations were performed to enable the C9-AO-α-Toc to locate lower-energy conformations accessible from the one initially assigned. Thirty conformations of the protein–ligand complex, taken at 0.5 ps intervals from each of the two resultant MD trajectories, were subjected to energy minimization, under constraint conditions reflecting those applied during the simulations. Only four distinct conformations of the C9-AO-α-Toc were found in this manner from the simulation where none of the protein atoms were allowed to relax around the initial conformation of the anthroyloxy ligand. The thirty C9-AO-α-Toc conformations selected from the simulation boasting less-constrained conditions for the protein environment were divisible into three conformational clusters having somewhat different orientations of the alkyl tail. All of the low-energy C9-AO-α-Toc conformations generated from this second, less-constrained protein simulation possessed considerably lower intramolecular energies than those obtained when all atoms of the protein were fixed to the X-ray coordinates of the α-Toc/α-TTP complex. Allowing the side-chains to be conformationally mobile facilitated the movement of the anthroyloxy moiety away from the linking alkyl chain and to assume conformations with less steric crowding. The first method yielded conformers in which the anthroyloxy moiety was wedged between immobile side-chains into a bent position. A comparison of the putative ligand position produced by these simulations and that of α-tocopherol in the X-ray crystal structure of α-TTP is shown in Figure 8. The lowest-energy conformation of C9-AO-α-Toc in α-TTP obtained here is depicted. We do not claim that this ligand/protein conformation is optimal, given that the above investigations do not constitute comprehensive conformational searches. Nevertheless, it is possible to state that the known binding site of

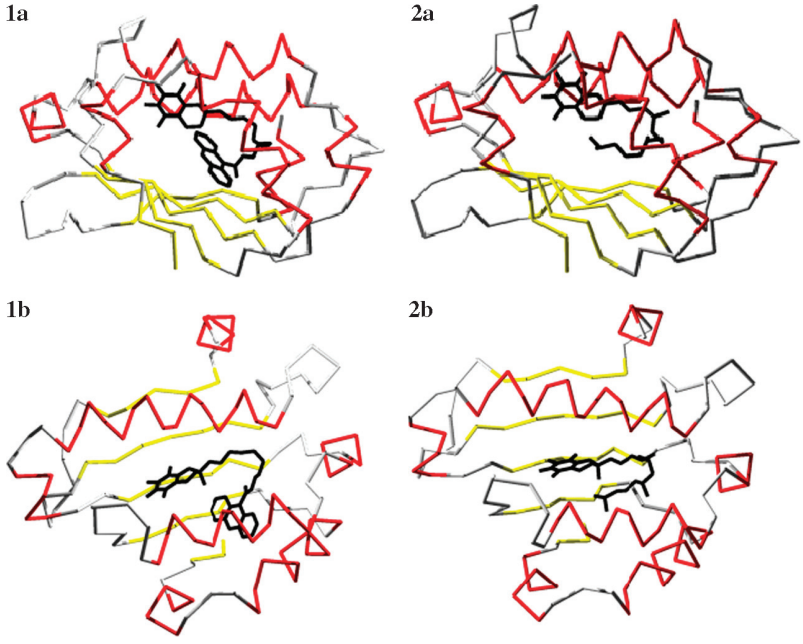


Figure 8. Comparison of the calculated structures of C9-AO-α-Toc, **9d** bound to α-TTP (panels 1a and 1b) with the α-Toc/α-TTP complex observed by X-ray diffraction[32] (panels 2a and 2b). Perspectives 1a and 2a are looking down the long axis of the binding site; 1b and 2b from 'above'. Only amino acid residues 92–225, comprising the bulk of the ligand-binding site, are shown for clarity.

α-TTP could accommodate C9-AO-α-Toc without significant rearrangement of the protein backbone.

3. Summary

The synthetic procedures employed in the preparation of these fluorescent analogues of α-tocopherol were all relatively straightforward and were routinely performed on a 10–45 mg scale of the final silyl ether-protected compounds. We have since used the C9-NBD-α-Toc (**10d**) in a study of the intracellular location and transport of tocopherol in hepatocytes[41] and have begun work on the nature of the mechanism of α-TTP transfer of tocopherol from one lipid environment (liposome and vesicles) to another. This will explore the effect of vesicle phospholipid composition (i.e., acyl chain unsaturation, head group charge) and curvature as well as the effect of mutant forms of α-TTP on rates by which tocopherol is transferred between vesicles.

4. Experimental

4.1. Materials

4.1.1. Reagents. Reagent-grade chemicals were from Aldrich Chemical Co., Oakville, Ontario. Solvents were from Caledon Labs, Ontario. Activated forms of the fluorophores (*N*-methylisatoic anhydride, 4-chloro-7-nitrobenz-2-oxa-1,3-diazole, and 5-dimethylaminonaphthalene-1-sulfonylchlroide) were from Molecular Probes, Eugene, Oregon.

4.2. Analytical methods

4.2.1. Spectroscopy. Optical rotations were registered on a Rudolph Autopol III polarimeter. Infrared (IR) spectra were recorded on a Mattson Research Series FT-IR spectrophotometer and the resonance frequencies are reported in cm^{-1}. ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker Advance DPX-300 Digital FT-NMR spectrometer (300 and 75 MHz, respectively) in $CDCl_3$ with residual chloroform as internal reference ($^{1}H = 7.26$ ppm, $^{13}C = 77.0$ ppm) unless otherwise noted. Chemical shifts are reported δ values (ppm), and coupling constants (*J*) are reported in Hertz (Hz). The following NMR abbreviations are used: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), ar (aryl), and br (broad). 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. Fluorescence experiments were performed on a Photon Technologies International (London, Ontario) QuantaMaster Model QM-2001 L-format, equipped with double-grating monochromators, a 150 W xenon lamp, running Felix 32 software.

4.2.2. Chromatography. Analytical thin-layer chromatography (TLC) was performed on 0.25 mm pre-coated silica gel 60 Å F-254 plates. Column chromatography was carried out on silica gel (200–300 Å mesh) with the indicated solvent systems.

4.3. Fluorescence titrations

Recombinant human α-TTP was expressed and purified as described previously[10] and was stored in the elution buffer from metal affinity columns used for purification (250 mM NaCl, 25 mM phosphate at pH 7.4). Fractions rich in α-TTP were usually in the first 400 mM imidazole elutions. The imidazole had only minor effects on background fluorescence and did not affect binding. Titrations were performed in quartz cuvettes containing a total volume of 3 mL SET buffer (250 mM sucrose, 1 mM EDTA, 50 mM Tris–HCl, and 100 mM KCl, pH 7.5) and protein sample. Sufficient stock of recombinant α-TTP (typically less than 50 μL) from metal affinity column fractions was added such that the final concentration was 0.2 μM α-TTP. The cuvette was then mixed by repeated inversion, equilibrated at room temperature (19–20 °C), and a baseline fluorescence measurement was recorded at the wavelength of the fluorophore being used. To this solution were added 2 μL aliquots of stock solutions of fluorophores (~0.2 mM in absolute ethanol). After each addition, samples were mixed on a Rototorque rotating mixer, and the fluorescence was monitored until a maximum signal was obtained, which generally required 3–4 min for the NBD-α-Tocs and 6–8 min for the AO-α-Tocs. The final concentration of ethanol did not exceed 2% (v/v) and was usually less than 1% v/v. Fluorescence data were fitted to a one-site binding equation[37] using Prism 4.0 software.

The AO-α-tocopherols **9a**–**d** displayed a degree of non-specific binding to α-TTP (likely due to their greater hydrophobicity as compared to the other fluorophores) that often led to failure of the non-linear regression analysis. In order to assess the extent of non-specific binding of the AO-α-tocopherols, the raw fluorescence data were corrected by fluorescence recorded during a titration of α-TTP with **9a**–**d** in the presence of 40 μM α-tocopherol in SET buffer.

Competitive assays were performed under similar conditions. To a 0.2 μM solution of α-TTP in 3 mL SET buffer plus 100 μM Triton X-100 was added 2 μL of a stock solution of fluorophore in absolute EtOH such that the final concentration of fluorophore was 1 μM. The solution was then mixed until the fluorescence signal had reached about 95% of its final value. This took about 15 min for **10d**, **11d**, and **12d**, but four hours for **9d** (AO-α-Tocs). To this solution were added 2 μL aliquots of *RRR*-α-tocopherol in absolute ethanol 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.

4.4. Computational methods

The structural model is based on the crystal structure of bound α-tocopherol transfer protein (TTP) with

α-tocopherol in the binding site (pdb code: 1OIP).[42,32] The conformation of residues 9–24 was taken from the crystal structure of unbound TTP (pdb code: 1OIZ).[42,32] Using Quanta 4.1,[43] α-tocopherol was modified in isolation from α-TTP to yield a preliminary structure for C9-AO-α-tocopherol. Since the chromanol head group of C9-AO-α-tocopherol was superimposed on α-tocopherol, C9-AO-α-tocopherol was positioned similarly to α-tocopherol in the binding site of α-TTP. To yield the initial conformation of the tail of C9-AO-α-tocopherol, dihedral angles were modified manually to assume as favorable positions as possible. Partial charges were assigned to atoms using charge templates of Quanta and the charge was smoothed over all atoms. The hydrogen atoms on the protein were generated using the H-build algorithm of CHARMm 27.1.[44]

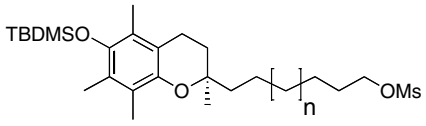
CHARMm was also used for all computer simulations. All non-bonded interactions were smoothly turned off by the atom shift function with a start distance of 8.0 Å and a cut-off radius of 12.0 Å. The radius of the non-bonded neighbor list was set to 14.0 Å, and the list was updated using heuristic methods.[45,46] Covalent bonds to hydrogen atoms were constrained using the SHAKE algorithm.[47] During molecular dynamics simulations, a time-step of 1 fs was used.

As the goal of these experiments was to find a low-energy conformation of the ligand in the binding site of α-TTP, all the protein atoms were held fixed to their original positions. The initial conformation of C9-AO-α-tocopherol in the binding site was manually implemented, thus it is unlikely that the potential energy of the system was at a minimum. Post-fixing the atoms of the protein, possible strain was released via Adopted Basis Newton-Raphson (ABNR) energy minimization for 50,000 steps; however, the minimization converged in 825 steps with a root-mean-square gradient of 0.001 kcal/mol/Å satisfied. During the initial 5 ps of computer simulation, the system was heated to 500 K by rescaling the velocities of each atom and increasing the temperature by 5 K every 0.05 ps. In preliminary experiments, the system was heated to 300 K, but this temperature did not provide sufficient energy to move the ligand out of unfavorable conformations in which the anthroyloxy moiety was deformed from its desired planar conformation. Subsequently, the system was allowed to equilibrate for 15 ps, during which time period the velocity of each atom was rescaled only if the average temperature fell outside the window of (500 ± 10) K. Every tenth conformer in the 300-conformer trajectory was energy-minimized using the ABNR algorithm for 50,000 steps. In all cases, the minimization converged with a root-mean-square gradient of 0.001 kcal/mol/Å satisfied. This resulted in 30 low-energy conformations; however, there were approximately 4 distinct conformers. The energy terms used to evaluate the conformations represent intra-ligand interactions and ligand–protein interaction; they do not represent intra-protein interactions.

In order to allow the ligand more flexibility, another set of simulations was performed. The minimized conformation of TTP with bound C9-AO-α-tocopherol from the previous experiment was used as the starting conformation, and the system was relaxed by ABNR energy minimization for 300 steps without any constraints. Subsequently, harmonic constraints were applied to all the atoms of the protein backbone about their current positions with a force constant of 500.0 kcal/mol/Å^2 used in conjunction with mass weighting. The system was heated to 500 K and equilibrated in the same manner as described formerly. After dynamics, the harmonic constraints were released and the protein backbone was fixed in position. Every tenth conformer in the 300-conformer trajectory was energy-minimized using the ABNR algorithm for 50,000 steps or until the root-mean-square gradient of 0.001 kcal/mol/Å was satisfied. This resulted in 30 low-energy conformations; however, this set was not as easily categorized into distinct conformers as the above set. The lowest-energy conformation we found is shown in Fig. 8. The same procedure was repeated for C6-, C7-, and C8-AO-α-tocopherol with similar results.

4.5. Synthesis

4.5.1. General procedure for the synthesis of compounds 2a–d.



2a-d, n=1-4

The synthesis of compounds **2a**–**d** was conducted under identical conditions and gave similar product yields (80–85%).

4.5.1.1. Methanesulfonic acid 6-[(R)-6-(*tert*-butyl-dimethyl-silanyloxy)-2,5,7,8-tetramethyl-chroman-2-yl]-hexyl ester (2a, *n* = 1). To a stirred solution of the alcohol **1a** (50.5 mg, 0.120 mmol) in dry CH_2Cl_2 (5 ml) were added dry triethylamine (25 μl, 0.180 mmol), methanesulfonyl chloride (14 μl, 0.180 mmol), and a catalytic amount of DMAP (1.9 mg, 0.015 mmol) at 0 °C under argon. After 15 min, the ice bath was removed and the reaction mixture was allowed to stir at room temperature for an additional 1/2 h. The mixture was then diluted with ether, washed with saturated aqueous $NaHCO_3$ (15 ml) and water (3× 10 ml). The organic layer was dried with Na_2SO_4, decanted and concentrated to give the crude mesylate as a cloudy yellow oil. This intermediate was used in the next reaction without further purification. (50.3 mg, 84%), clear colorless oil, $R_f = 0.30$ (CH_2Cl_2/hexane 2:1), $[\alpha]_D^{20}$ +1.9° (*c* 0.041, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 4.22 (t, 2H, *J* = 7 Hz), 3.02 (s, 3H), 2.54 (t, 2H, *J* = 7 Hz), 2.09 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 1.76 (m, 4H), 1.53 (m, 2H), 1.44 (m, 6H), 1.21 (s, 3H), 1.04 (s, 9H), 0.11 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 145.7, 144.0, 125.8, 123.4, 122.5, 117.3, 74.2, 70.1, 39.3, 37.2, 31.5, 29.4, 29.0, 26.0, 25.3, 23.7, 23.3, 20.8, 18.5, 14.2, 13.3, 11.9, −3.4; MS (EI) *m*/*z* 498 (M^+,

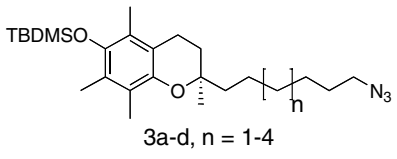
100%), 402 (16.2%), 279 (33.3%), 153 (32.5%), 73 (99.2%). HRMS (EI): calcd for $C_{26}H_{46}O_5SSi$: 498.28352. Found: 498.28360.

4.5.1.2. Methansulfonic acid 7-(*R*)-6-[*tert*-butyl(dimethyl)silanyloxy-2,5,7,8-tetramethyl-chroman-2-yl]-heptyl ester (2b, *n* = 2). (61.2 mg, 85%), clear colorless oil, $R_f = 0.30$ (CH_2Cl_2/hexane 2:1), $[\alpha]_D^{20}$ +1.8° (*c* 0.084, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 4.22 (t, 2H, $J = 6$ Hz), 2.98 (s, 3H), 2.53 (t, 2H, $J = 6$ Hz), 2.10 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 1.77 (m, 4H), 1.54 (m, 2H), 1.42 (m, 8H), 1.22 (s, 3H), 1.05 (s, 9H), 0.12 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 145.7, 144.0, 125.7, 123.4, 122.5, 117.3, 74.2, 70.0, 39.3, 37.1, 31.5, 29.8, 29.0, 28.9, 26.0, 25.2, 23.6, 23.3, 20.7, 18.4, 14.2, 13.3, 11.8, −3.4; MS (EI) *m*/*z* 512 (M^+, 99.3%), 416 (23.1%), 279 (30.3%), 153 (39.1%), 73 (100%). HRMS (EI): calcd for $C_{27}H_{48}O_5SSi$: 512.29917. Found: 512.29835.

4.5.1.3. Methansulfonic acid 8-(*R*)-6-[*tert*-butyl(dimethyl)silanyloxy-2,5,7,8-tetramethyl-chroman-2-yl]-octyl ester (2c, *n* = 3). (38.4 mg, 80%), clear colorless oil, $R_f = 0.32$ (CH_2Cl_2/hexane 2:1), $[\alpha]_D^{20}$ +1.7° (*c* 0.070, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 4.22 (t, 2H, $J = 7$ Hz), 2.99 (s, 3H), 2.56 (t, 2H, $J = 7$ Hz), 2.11 (s, 3H), 2.09 (s, 3H), 2.07 (s, 3H), 1.75 (m, 4H), 1.54 (m, 2H), 1.40 (m, 4H), 1.31 (m, 6H), 1.23 (s, 3H), 1.05 (s, 9H), 0.12 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 145.8, 144.0, 125.8, 123.5, 122.6, 117.4, 74.3, 70.2, 39.5, 37.2, 31.5, 30.0, 29.4, 29.1, 29.0, 26.1, 25.4, 23.8, 23.5, 20.8, 18.5, 14.3, 13.4, 11.9, −3.3; MS (EI) *m*/*z* 526 (M^+, 9.8%), 430 (91.7%), 307 (41.0%), 149 (100%), 57 (63.2%). HRMS (EI): calcd for $C_{28}H_{50}O_5SSi$: 526.31482. Found: 526.31549.

4.5.1.4. Methansulfonic acid 9-(*R*)-6-[*tert*-butyl(dimethyl)silanyloxy-2,5,7,8-tetramethyl-chroman-2-yl]-nonyl ester (2d, *n* = 4). (41.1 mg, 81%), clear colorless oil, $R_f = 0.32$ (CH_2Cl_2/hexane 2:1), $[\alpha]_D^{20}$ +1.6° (*c* 0.063, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 4.21 (t, 2H, $J = 7$ Hz), 2.98 (s, 3H), 2.54 (t, 2H, $J = 7$ Hz), 2.09 (s, 3H), 2.07 (s, 3H), 2.05 (s, 3H), 1.76 (m, 4H), 1.54 (m, 2H), 1.39 (m, 4H), 1.28 (m, 8H), 1.21 (s, 3H), 1.04 (s, 9H), 0.11 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 145.8, 143.9, 125.7, 123.6, 122.5, 117.4, 74.3, 70.1, 39.4, 37.2, 31.4, 30.0, 29.4, 29.2, 29.0, 28.9, 26.0, 25.3, 23.7, 23.5, 20.8, 18.5, 14.2, 13.3, 11.8, −3.4; MS (EI) *m*/*z* 540 (M^+, 42.6%), 428 (80.1%), 279 (17.1%), 153 (56.8%), 73 (100%). HRMS (EI): calcd for $C_{29}H_{52}O_5SSi$: 540.33047. Found: 540.32960.

4.5.2. General procedure for the preparation of compounds 3a–d.



3a-d, n = 1-4

The synthesis of compounds **3a**–**d** were conducted under identical conditions and gave similar product yields (85–88%).

4.5.2.1. [(*R*)-2-(6-Azido-hexyl)-2,5,7,8-tetramethyl-chromen-6-yloxy]-(*tert*-butyl)dimethylsilane (3a, *n* = 1). To a solution of mesylate **2a** (0.268 mmol) in dry DMF (3 ml) at room temperature was added sodium azide (86.9 mg, 1.34 mmol). The mixture was then warmed to and stirred at 55 °C for 1 h. The reaction was then diluted with ether (15 ml), washed with water (3× 25 ml), dried with Na_2SO_4, decanted, and concentrated. The resulting oily residue was purified by column chromatography (CH_2Cl_2/hexane 2:1) to afford the desired product **3a**.

(105 mg, 88% over two steps), clear colorless oil, $R_f = 0.41$ (CH_2Cl_2/hexane 1:1), $[\alpha]_D^{20}$ +2.5° (*c* 0.013, $CHCl_3$), IR (neat): 2098 (–N_3); ^1H NMR ($CDCl_3$): δ 3.24 (t, 2H, $J = 7$ Hz), 2.55 (t, 2H, $J = 7$ Hz), 2.09 (s, 3H), 2.07 (s, 3H), 2.05 (s, 3H), 1.76 (m, 2H), 1.59 (m, 4H), 1.33 (m, 6H), 1.22 (s, 3H), 1.04 (s, 9H), 0.11 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 145.8, 144.0, 125.8, 123.5, 122.6, 117.4, 74.3, 51.4, 39.4, 31.5, 29.6, 28.7, 26.7, 26.0, 23.7, 23.4, 20.8, 18.5, 14.3, 13.4, 11.9, −3.3; MS (EI) *m*/*z* 445 (M^+, 15.9%), 415 (59.8%), 358 (20.7%), 279 (27.0%), 73 (100%). HRMS (EI): calcd for $C_{25}H_{43}N_3O_2Si$: 445.31245. Found: 445.31246.

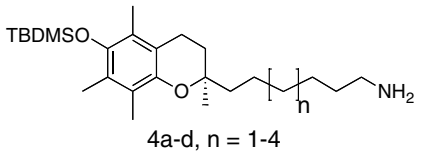
4.5.2.2. [(*R*)-2-(7-Azido-heptyl)-2,5,7,8-tetramethyl-chromen-6-yloxy]-(*tert*-butyl)dimethylsilane (3b, *n* = 2). (182 mg, 88% over two steps), clear colorless oil, $R_f = 0.42$ (CH_2Cl_2/hexane 1:1), $[\alpha]_D^{20}$ +2.4° (*c* 0.040, $CHCl_3$), IR (neat): 2059 (–N_3); ^1H NMR ($CDCl_3$): δ 3.25 (t, 2H, $J = 7$ Hz), 2.56 (t, 2H, $J = 7$ Hz), 2.10 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H), 1.77 (m, 2H), 1.59 (m, 4H), 1.33 (m, 8H), 1.22 (s, 3H), 1.05 (s, 9H), 0.12 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 145.8, 144.0, 125.8, 123.5, 122.6, 117.4, 74.3, 51.4, 39.4, 31.5, 29.9, 29.1, 28.8, 26.6, 26.0, 23.7, 23.4, 20.8, 18.5, 14.3, 13.4, 11.9, −3.3; MS (EI) *m*/*z* 459 (M^+, 18.6%), 429 (54.3%), 372 (18.2%), 279 (26.4%), 73 (100%). HRMS (EI): calcd for $C_{26}H_{45}N_3O_2Si$: 459.32810. Found: 459.32887.

4.5.2.3. [(*R*)-2-(8-Azido-octyl)-2,5,7,8-tetramethyl-chromen-6-yloxy]-(*tert*-butyl)dimethylsilane (3c, *n* = 3). (180 mg, 88.5% over two steps), clear colorless oil, $R_f = 0.43$ (CH_2Cl_2/hexane 1:1), $[\alpha]_D^{20}$ +2.5° (*c* 0.035, $CHCl_3$), IR (neat): 2098 (–N_3); ^1H NMR ($CDCl_3$): δ 3.25 (t, 2H, $J = 7$ Hz), 2.55 (t, 2H, $J = 7$ Hz), 2.10 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H), 1.81 (m, 2H), 1.57 (m, 4H), 1.31 (m, 10H), 1.22 (s, 3H), 1.05 (s, 9H), 0.12 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 145.8, 143.9, 125.8, 123.4, 122.6, 117.4, 74.3, 51.4, 39.4, 31.4, 29.9, 29.4, 29.0, 28.7, 26.6, 26.0, 23.7, 23.5, 20.8, 18.5, 14.2, 13.3, 11.9, −3.3; MS (EI) *m*/*z* 473 (M^+, 39.7%), 445 (93.4%), 279 (45.2%), 221 (32.4%), 73 (100%). HRMS (EI): calcd for $C_{27}H_{47}N_3O_2Si$: 473.34375. Found: 473.34235.

4.5.2.4. [(*R*)-2-(9-Azido-nonyl)-2,5,7,8-tetramethyl-chromen-6-yloxy]-(*tert*-butyl)dimethylsilane (3d, *n* = 4). (154 mg, 85% over two steps), clear colorless oil, $R_f = 0.45$ (CH_2Cl_2/hexane 1:1), $[\alpha]_D^{20}$ +1.9° (*c* 0.065, $CHCl_3$), IR (neat): 2098 (–N_3); ^1H NMR ($CDCl_3$): δ 3.25 (t, 2H, $J = 7$ Hz), 2.55 (t, 2H, $J = 7$ Hz), 2.10 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H), 1.77 (m, 2H), 1.57 (m, 4H), 1.29 (m, 12H), 1.22 (s, 3H), 1.05 (s, 9H), 0.12 (s,

6H); ^{13}C NMR ($CDCl_3$): δ 145.8, 143.9, 125.8, 123.4, 122.6, 117.4, 74.3, 51.4, 39.5, 31.4, 30.0, 29.4, 29.3, 29.1, 28.8, 26.6, 26.0, 23.7, 23.5, 20.8, 18.5, 14.2, 13.3, 11.9, −3.3; MS (EI) *m*/*z* 487 (M^+, 22.0%), 457 (75.8%), 279 (27.8%), 221 (19.3%), 73 (100%). HRMS (EI): calcd for $C_{28}H_{49}N_3O_2Si$: 487.35940. Found: 487.35939.

4.5.3. General procedure for the preparation of compounds 4a–d.



4a-d, n = 1-4

The synthesis of compounds **4a**–**d** was conducted under identical conditions to give similar product yields (83–90%).

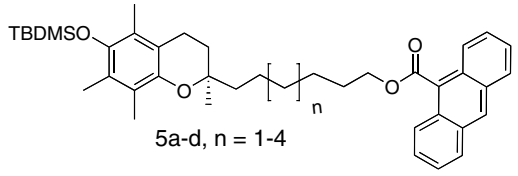
4.5.3.1. 6-((2*R*)-6-{[*tert*-Butyl(dimethyl)silyl]oxy}-2,5,7,8-tetramethyl-3,4,-dihydro-2*H*-chromene-2-yl)-hexylamine. To a stirred solution of azide **3a** (167.0 mg, 0.37 mmol) in dry THF (3 ml) cooled to 0 °C was added $LiAlH_4$ (0.48 ml, 0.48 mmol, 1 M in THF) dropwise. This was then stirred for an additional 5 min at 0 °C and subsequently for 10 min at room temperature. The reaction was then acidified using acetic acid (2 M), neutralized with saturated $NaHCO_3$, and extracted with ether (3× 25 ml). The organic layers were combined, dried with Na_2SO_4, and concentrated under reduced pressure. The resulting crude product was then further purified by column chromatography by application to 4 ml silica and passing through 100% ethyl acetate (25 ml) followed by EtAOc/MeOH (8:2) to afford the desired product. (131.5 mg, 83%), clear colorless oil, R_f = 0.31 (EtOAc/MeOH + 1% HCl 7:2), $[\alpha]_D^{20}$ +1.9° (*c* 0.020, $CHCl_3$), IR (neat): 3368 (N–H), 3300 (N–H); ^{1}H NMR ($CDCl_3$): δ 2.88 (br, 2H), 2.68 (br, 2H), 2.52 (t, 2H, *J* = 6 Hz), 2.07 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 1.74 (m, 2H), 1.48 (m, 6H), 1.28 (m, 4H), 1.19 (s, 3H), 1.02 (s, 9H), 0.09 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 145.8, 144.0, 125.8, 123.4, 122.6, 117.4, 74.3, 41.6, 39.5, 32.8, 31.5, 29.9, 26.8, 26.0, 23.7, 23.5, 20.8, 18.5, 14.3, 13.3, 11.9, −3.4; MS (EI) *m*/*z* 419 (M^+, 100%), 358 (15.1%), 279 (33.3%), 221 (24.6%), 73 (71.1%). HRMS (EI): calcd for $C_{25}H_{45}NO_2Si$: 419.32195. Found: 419.32160.

4.5.3.2. 7-((2*R*)-6-{[*tert*-Butyl(dimethyl)silyl]oxy}-2,5,7,8-tetramethyl-3,4,-dihydro-2*H*-chromene-2-yl)-heptylamine (4b, *n* = 2). (219.4 mg, 87%), clear colorless oil, R_f = 0.31 (EtOAc/MeOH + 1% HCl 7:2), $[\alpha]_D^{20}$ +1.9° (*c* 0.034, $CHCl_3$), IR (neat): 3368 (N–H), 3275 (N–H); ^{1}H NMR ($CDCl_3$): δ 2.64 (t, 2H, *J* = 7 Hz), 2.52 (t, 2H, *J* = 7 Hz), 2.16 (br, 2H), 2.07 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 1.76 (m, 2H), 1.50 (m, 2H), 1.48 (br, 4H), 1.26 (br, 6H), 1.19 (s, 3H), 1.02 (s, 9H), 0.09 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 145.7, 143.8, 125.6, 123.3, 122.5, 117.3, 74.5, 41.8, 39.4, 33.2, 31.3, 29.9, 29.3, 26.7, 25.9, 23.6, 23.4, 20.7, 18.4, 14.0, 13.2, 11.8, −3.5; MS (EI) *m*/*z* 433 (M^+, 100%), 372 (19.2%), 279 (35.1%), 221 (24.1%), 73 (76.1%). HRMS (EI): calcd for $C_{26}H_{47}NO_2Si$: 433.33760. Found: 433.33801.

4.5.3.3. 8-((2*R*)-6-{[*tert*-Butyl(dimethyl)silyl]oxy}-2,5,7,8-tetramethyl-3,4,-dihydro-2*H*-chromene-2-yl)-octylamine (4c, *n* = 3). (72.6 mg, 85%), clear colorless oil, R_f = 0.31 (EtOAc/MeOH+1% HCl 7:2), $[\alpha]_D^{20}$ +1.9° (*c* 0.043, $CHCl_3$), IR (neat): 3368 (N–H), 3291 (N–H); ^{1}H NMR ($CDCl_3$): δ 2.67 (t, 2H, *J* = 7 Hz), 2.52 (t, 2H, *J* = 7 Hz), 2.32 (br, 2H), 2.07 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 1.77 (m, 2H), 1.48 (m, 6H), 1.27 (br, 8H), 1.19 (s, 3H), 1.02 (s, 9H), 0.09 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 145.6, 143.8, 125.6, 123.3, 122.4, 117.2, 74.2, 41.7, 39.3, 33.0, 31.3, 29.8, 29.3, 29.2, 26.6, 25.8, 23.6, 23.3, 20.6, 18.3, 14.1, 13.1, 11.7, −3.5; MS (EI) *m*/*z* 447 (M^+, 77.7%), 386 (24.8%), 279 (42.2%), 221 (28.7%), 73 (100%). HRMS (EI): calcd for $C_{27}H_{49}NO_2Si$: 447.35325. Found: 447.35557.

4.5.3.4. 9-((2*R*)-6-{[*tert*-Butyl(dimethyl)silyl]oxy}-2,5,7,8-tetramethyl-3,4,-dihydro-2*H*-chromene-2-yl)-nonylamine (4d, *n* = 4). (161.2 mg, 90%), clear colorless oil, R_f = 0.31 (EtOAc/MeOH+1% HCl 7:2), $[\alpha]_D^{20}$ +2.5° (*c* 0.032, $CHCl_3$), IR (neat): 3384 (N–H), 3308 (N–H); ^{1}H NMR ($CDCl_3$): δ 2.53 (t, 2H, *J* = 7 Hz), 2.45 (br, 2H), 2.08 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 1.75 (m, 2H), 1.48 (m, 6H), 1.26 (br, 12H), 1.20 (s, 3H), 1.03 (s, 9H), 0.09 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 145.8, 144.0, 125.8, 123.5, 122.6, 117.4, 74.4, 39.6, 33.2, 31.5, 30.9, 30.1, 29.6, 29.4, 26.8, 26.1, 23.8, 23.6, 20.9, 18.6, 14.3, 13.4, 11.9, −3.3; MS (EI) *m*/*z* 461 (M^+, 46.9%), 457 (100%), 400 (23.6%), 279 (32.8%), 221 (23.0%), 73 (63.1%). HRMS (EI): calcd for $C_{28}H_{51}NO_2Si$: 461.36890. Found: 461.36459.

4.5.4. General procedure for the preparation of compounds 5a–d.



5a-d, n = 1-4

The synthesis of compounds **5a**–**d** was conducted under identical conditions and gave product yields ranging from 60% to 89%.

4.5.4.1. Anthracene-9-carboxylic acid 6-[(*R*)-6-(*tert*-butyl-dimethyl-silanyloxy)-2,5,7,8-tetramethyl-chroman-2-yl-hexyl ester (5a, *n* = 1). To a stirred solution of 9-anthracene carboxylic acid (71.4 mg, 0.321 mmol) in dry DCM (3 ml) under argon were added oxalyl chloride (41 μl, 0.321 mmol) and a catalytic amount of DMF (1 μl, 0.016 mmol). It was then allowed to stir for 2 h, thereby generating the acid chloride in situ. Reaction progress could be monitored by the poor initial solubility of 9-anthracene carboxylic acid as a cloudy yellow mixture in DCM to a resulting clear transparent yellow solution and by the evolution of CO_2 and CO bubbles. To this

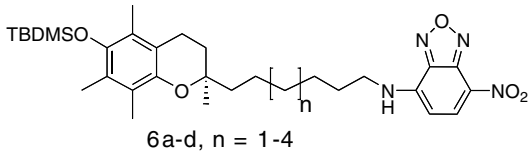
was added dropwise via syringe a solution of appropriate alcohol **1a** (104.1 mg, 0.247 mmol) in dry DCM (2 ml) that was stirred for 15 min in the presence of TEA (38 μl, 0.272 mmol). The combined reaction components were then mixed for a further 3 h, at which point alcohol **1a** could no longer be detected by TLC. The reaction was quenched with dH_2O (15 ml) and extracted with DCM (3× 25 ml). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (15 ml) and then dH_2O (15 ml) prior to drying with anhydrous Na_2SO_4, decanting, and concentration under reduced pressure. Purification was achieved by column chromatography (Et_2O/hexane 1:2). (104.9 mg, 68%), clear light yellow oil, $R_f = 0.53$ (CH_2Cl_2/hexane 10:1), $[\alpha]_D^{20}$ +2.4° (*c* 0.010, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.55 (s, 1H), 8.02 (m, 4H), 7.5 (m, 4H), 4.59 (t, 2H, $J = 6$ Hz), 2.49 (t, 2H, $J = 6$ Hz), 2.07 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 1.86 (m, 2H), 1.76 (m, 2H), 1.46 (m, 4H), 1.40 (m, 4H), 1.19 (s, 3H), 1.02 (s, 9H), 0.09 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 169.7 (C═O), 145.8, 144.0, 130.9, 129.1, 128.5, 128.3, 128.1, 126.8, 125.8, 125.4, 125.0, 123.5, 122.6, 117.4, 74.3, 65.9, 39.5, 31.5, 29.7, 29.7, 28.7, 26.0 (3 CH_3), 23.7, 23.5, 20.8, 18.5, 14.3, 13.4, 11.9, −3.3 (2 CH_3); MS (EI) *m*/*z* 624 (M^+, 1.4%), 208 (100%), 180 (66.6%), 152 (46.0%), 76 (35.2%). HRMS (EI): calcd for $C_{40}H_{52}O_4Si$: 624.36348. Found: 624.37719.

4.5.4.2. Anthracene-9-carboxylic acid 7-[(*R*)-6-(*tert*-butyl-dimethyl-silanyloxy)-2,5,7,8-tetramethyl-chroman-2-yl-heptyl ester (5b, *n* = 2). (165.2 mg, 60%), clear light yellow oil, $R_f = 0.54$ (CH_2Cl_2/hexane 10:1), $[\alpha]_D^{20}$ +1.7° (*c* 0.006, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.52 (s, 1H), 8.01 (m, 4H), 7.51 (m, 4H), 4.58 (t, 2H, $J = 6$ Hz), 2.48 (t, 2H, $J = 6$ Hz), 2.07 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 1.86 (m, 2H), 1.75 (m, 2H), 1.45 (m, 6H), 1.39 (m, 4H), 1.19 (s, 3H), 1.02 (s, 9H), 0.08 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 169.8 (C═O), 145.9, 144.1, 130.7, 129.2, 128.6, 128.4, 128.2, 126.7, 125.7, 125.3, 125.0, 123.5, 122.6, 117.2, 74.2, 65.8, 39.5, 31.5, 30.1, 29.6, 29.5, 28.6, 26.0 (3 CH_3), 23.6, 23.5, 20.7, 18.6, 14.4, 13.4, 11.8, −3.4 (2 CH_3); MS (EI) *m*/*z* 638 (M^+, 21.4%), 279 (19.4%), 205 (48.3%), 73 (36.5%), 43 (100%). HRMS (EI): calcd for $C_{41}H_{54}O_4Si$: 638.37913. Found: 638.37231.

4.5.4.3. Anthracene-9-carboxylic acid 8-[(*R*)-6-(*tert*-butyl-dimethyl-silanyloxy)-2,5,7,8-tetramethyl-chroman-2-yl-octyl ester (5c, *n* = 3). (89.5 mg, 87%), clear light yellow oil, $R_f = 0.54$ (CH_2Cl_2/hexane 10:1), $[\alpha]_D^{20}$ +1.5° (*c* 0.007 $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.50 (s, 1H), 8.01 (m, 4H), 7.48 (m, 4H), 4.59 (t, 2H, $J = 6$ Hz), 2.51 (t, 2H, $J = 6$ Hz), 2.06 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 1.83 (m, 2H), 1.73 (m, 2H), 1.47 (m, 6H), 1.37 (m, 6H), 1.18 (s, 3H), 1.02 (s, 9H), 0.08 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 169.8 (C═O), 145.9, 144.1, 131.0, 129.2, 128.6, 128.4, 128.2, 126.9, 125.9, 125.5, 125.1, 123.6, 122.7, 117.5, 74.5, 66.0, 39.6, 31.5, 30.1, 29.7, 29.6, 29.2, 28.8, 26.1 (3 CH_3), 23.8, 23.6, 20.9, 18.6, 14.4, 13.4, 12.0, −3.2 (2 CH_3); MS (EI) *m*/*z* 652 (M^+, 100%), 446 (8.8%), 279 (25.8%), 205 (52.0%), 73 (38.1%). HRMS (EI): calcd for $C_{42}H_{56}O_4Si$: 652.39478. Found: 652.39563.

4.5.4.4. Anthracene-9-carboxylic acid 9-[(*R*)-6-(*tert*-butyl-dimethyl-silanyloxy)-2,5,7,8-tetramethyl-chroman-2-yl-nonyl ester (5d, *n* = 4). (159.2 mg, 89%), clear light yellow oil, $R_f = 0.55$ (CH_2Cl_2/hexane 10:1), $[\alpha]_D^{20}$ +1.2° (*c* 0.008, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.50 (s, 1H), 8.01 (m, 4H), 7.49 (m, 4H), 4.59 (t, 2H, $J = 6$ Hz), 2.51 (t, 2H, $J = 6$ Hz), 2.06 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3 H), 1.83 (m, 2H), 1.73 (m, 2H), 1.47 (m, 6H), 1.37 (m, 8H), 1.19 (s, 3H), 1.02 (s, 9H), 0.09 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 169.7 (C═O), 145.8, 144.0, 130.9, 129.1, 128.5, 128.3, 128.1, 126.8, 126.0, 125.4, 125.0, 123.5, 122.6, 117.4, 74.4, 65.9, 39.5, 31.9, 30.1, 29.6, 29.5, 29.4, 29.2, 28.7, 26.0 (3 CH_3), 23.7, 23.5, 20.8, 18.5, 14.3, 13.3, 11.9, −3.3 (2 CH_3); MS (EI) *m*/*z* 666 (M^+, 18.5%), 472 (8.7%), 205 (55.1%), 73 (45.0%), 43 (100%). HRMS (EI): calcd for $C_{43}H_{58}O_4Si$: 666.41043. Found: 666.41082.

4.5.5. General procedure for the preparation of compounds 6a–d.



6a-d, n = 1-4

The synthesis of compounds **6a**–**d** was conducted under identical conditions and gave similar product yields (73–76%).

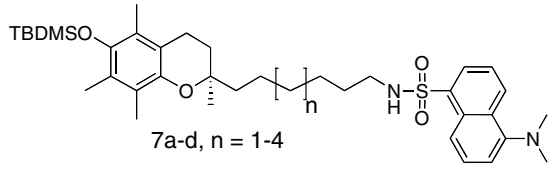
4.5.5.1. {9-[(R)-6-(*tert*-Butyl-dimethyl-silanyloxy)-2,5,7,8-tetramethyl-chroman-2-yl]-hexyl}-(7-nitro-benzo[1,2,5]oxadiazol-4-yl)-amine (6a, *n* = 1). The amine **4a** (44.8 mg, 0.106 mmol) was dissolved in dry THF (1 ml) and then dry TEA (30 μl, 0.212 mmol) was added under argon. This was stirred at room temperature for 15 min prior to dropwise addition via cannula of a solution of NBD-Cl (23.2 mg, 0.116 mmol) in dry THF (1 ml). After stirring for a further 1 h, the reaction mixture was diluted with dH_2O (10 ml), extracted with EtOAc (3× 30 ml), dried over Na_2SO_4, and concentrated under reduced pressure to give the crude product, which was purified by column chromatography (Et_2O/hexane 1:2) to afford the pure compound **6a**. (47.3 mg, 76%), dark browny orange oil, $R_f = 0.28$ (Et_2O/hexane 1:1), $[\alpha]_D^{20}$ +2.1° (*c* 0.005, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.44 (d, 1H, $J = 9$ Hz), 6.24 (br, 1H), 6.12 (d, 1H, $J = 9$ Hz), 3.45 (q, 2H, $J = 7$ Hz), 2.53 (t, 2H, $J = 7$ Hz), 2.06 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 1.77 (m, 4H), 1.41 (m, 8H), 1.20 (s, 3H), 1.01 (s, 9H), 0.08 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 145.7, 144.2, 144.0, 143.8, 143.8, 136.5, 125.8, 123.8, 123.5, 122.5, 117.4, 98.5, 74.2, 43.9, 39.3, 31.5, 29.6, 28.4, 26.8, 26.0 (3 CH_3), 23.7, 23.3, 20.8, 18.5, 14.3, 13.3, 11.9, −3.4 (2 CH_3); MS (EI) *m*/*z* 582 (M^+, 50.3%), 548 (31.6%), 418 (21.5%), 279 (24.5%), 73 (100%). HRMS (EI): calcd for $C_{31}H_{46}N_4O_5Si$: 582.32374. Found: 582.32418.

4.5.5.2. {9-[(R)-6-(*tert*-Butyl-dimethyl-silanyloxy)-2,5,7,8-tetramethyl-chroman-2-yl]-heptyl}-(7-nitro-benzo[1,2,5]oxadiazol-4-yl)-amine (6b, n = 2). (52.5 mg, 76%), dark browny orange oil, $R_f = 0.30$ (Et_2O/hexane 1:1), $[\alpha]_D^{20}$ +2.0° (*c* 0.007, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.45 (d, 1H, $J = 9$ Hz), 6.20 (br, 1H), 6.13 (d, 1H, $J = 9$ Hz), 3.44 (q, 2H, $J = 7$ Hz), 2.52 (t, 2H, $J = 7$ Hz), 2.06 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 1.76 (m, 4H), 1.41 (m, 10H), 1.19 (s, 3H), 1.01 (s, 9H), 0.08 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 145.7, 144.2, 144.0, 143.8, 143.8, 136.5, 125.8, 123.9, 123.5, 122.5, 117.4, 98.5, 74.3, 43.9, 39.8, 31.5, 29.9, 29.1, 28.5, 26.8, 26.0 (3 CH_3), 23.7, 23.4, 20.8, 18.5, 14.3, 13.4, 11.9, −3.3 (2 CH_3); MS (EI) *m*/*z* 596 (M^+, 32.4%), 562 (17.9%), 432 (69.2%), 279 (28.3%), 73 (100%). HRMS (EI): calcd for $C_{32}H_{48}N_4O_5$ Si: 596.33939. Found: 596.34291.

4.5.5.3. {9-[(*R*)-6-(*tert*-Butyl-dimethyl-silanyloxy)-2,5,7,8-tetramethyl-chroman-2-yl]-octyl}-(7-nitro-benzo[1,2,5]oxadiazol-4-yl)-amine (6c, *n* = 3). (39.2 mg, 74%), dark browny orange oil, $R_f = 0.33$ (Et_2O/hexane 1:1), $[\alpha]_D^{20}$ +2.4° (*c* 0.009, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.47 (d, 1H, $J = 9$ Hz), 6.21 (br, 1H), 6.15 (d, 1H, $J = 9$ Hz), 3.45 (q, 2H, $J = 7$ Hz), 2.53 (t, 2H, $J = 7$ Hz), 2.07 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 1.77 (m, 4H), 1.41 (m, 4H), 1.30 (m, 8H), 1.19 (s, 3H), 1.01 (s, 9H), 0.08 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 145.8, 144.2, 144.0, 143.8, 143.8, 136.5, 125.8, 123.9, 123.5, 122.6, 117.4, 98.5, 74.3, 43.9, 39.4, 31.5, 29.9, 29.3, 29.1, 28.5, 26.9, 26.0 (3 CH_3), 23.8, 23.4, 20.8, 18.5, 14.3, 13.4, 11.9, −3.3 (2 CH_3); MS (EI) *m*/*z* 610 (M^+, 20.2%), 576 (21.0%), 446 (48.2%), 279 (55.2%), 73 (100%). HRMS (EI): calcd for $C_{33}H_{50}N_4O_5$ Si: 610.35504. Found: 610.35132.

4.5.5.4. {9-[(*R*)-6-(*tert*-Butyl-dimethyl-silanyloxy)-2,5,7,8-tetramethyl-chroman-2-yl]-nonyl}-(7-nitro-benzo[1,2,5]oxadiazol-4-yl)-amine (6d, *n* = 4). (29.5 mg, 73%), dark browny orange oil, $R_f = 0.36$ (Et_2O/hexane 1:1), $[\alpha]_D^{20}$ +1.7° (*c* 0.007, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.46 (d, 1H, $J = 9$ Hz), 6.18 (br, 1H), 6.14 (d, 1H, $J = 9$ Hz), 3.45 (q, 2H, $J = 6$ Hz), 2.53 (t, 2H, $J = 7$ Hz), 2.07 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 1.77 (m, 4H), 1.39 (m, 6H), 1.28 (br, 8H), 1.20 (s, 3H), 1.02 (s, 9H), 0.09 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 145.8, 144.2, 144.0, 143.8, 143.8, 136.5, 125.8, 124.0, 123.5, 122.6, 117.4, 98.5, 74.4, 43.9, 39.5, 31.5, 30.0, 29.4, 29.3, 29.1, 28.5, 26.9, 26.0 (3 CH_3), 23.8, 23.5, 20.8, 18.5, 14.3, 13.4, 11.9, −3.3 (2 CH_3); MS (EI) *m*/*z* 624 (M^+, 30.3%), 590 (20.3%), 460 (45.6%), 279 (54.2%), 73 (100%). HRMS (EI): calcd for $C_{34}H_{52}N_4O_5$Si: 624.37069. Found: 624.36959.

4.5.6. General procedure for the preparation of compounds 7a–d.



The synthesis of compounds **7a**–**d** was conducted under identical conditions and gave similar product yields (81–82%).

4.5.6.1. 5-Dimethylamino-naphthalene-1-sulfonic acid {9-[(*R*)-6-(*tert*-butyl-dimethyl-silanyloxy)-2,5,7,8-tetramethyl-chroman-2-yl]hexyl}-amide (7a, *n* = 1). A solution of amine **4a** (47.8 mg, 0.113 mmol) and dry TEA (47 μl, 0.339 mmol) in dry DCM (2 ml) was stirred at 0 °C for 15 min prior to dropwise addition of a solution of DAN-Cl (33.5 mg, 0.124 mmol) in dry DCM (1 ml). The reaction was maintained at this temperature for another five minutes at which point it was allowed to proceed at room temperature for a further one hour. The reaction was then quenched with dH_2O (15 ml) and extracted with CH_2Cl_2 (3× 20 ml), dried over Na_2SO_4, decanted, and concentrated in vacuo to give the crude product. Further purification by column chromatography (Et_2O/hexane 2:1) afforded the desired product. (60.1 mg, 81%), lime green solid, mp 46–47 °C, R_f= 0.42 (Et_2O/hexane 2:1), $[\alpha]_D^{20}$ +2.7° (*c* 0.006, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.51 (d, 1H, $J = 8$ Hz), 8.26 (d, 1H, $J = 8$ Hz), 8.22 (d, 1H, $J = 7$ Hz), 7.51 (q, 2H, $J = 8$ Hz), 7.14 (d, 1H, $J = 7$ Hz), 4.62 (t, 1H, $J = 6$ Hz), 2.84 (s, 8H), 2.50 (t, 2H, $J = 6$ Hz), 2.07 (s, 3H), 2.07 (s, 6H), 1.70 (m, 2H), 1.39 (m, 2H), 1.35 (m, 4H), 1.31 (m, 4H), 1.15 (s, 3H), 1.02 (s, 9H), 0.09 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 151.9, 145.7, 144.0, 134.7, 130.3, 129.8, 129.6, 129.6, 128.3, 125.8, 123.5, 123.2, 122.6, 118.7, 117.4, 115.1, 74.2, 45.4 (2 CH_3), 43.2, 39.3, 31.4, 29.4 (2 CH_2), 26.3, 26.0 (3 CH_3), 23.7, 23.3, 20.8, 18.5, 14.3, 13.3, 11.9, −3.3 (2 CH_3); MS (EI) *m*/*z* 652 (M^+, 90.5%), 543 (15.6%), 445 (51.6%), 279 (28.8%), 73 (100%). HRMS (EI): calcd for $C_{37}H_{56}N_2O_4SSi$: 652.37300. Found: 652.36645.

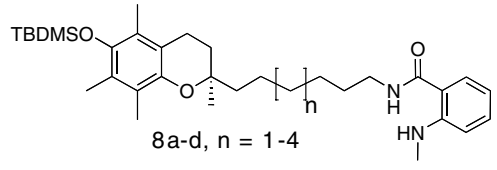
4.5.6.2. 5-Dimethylamino-naphthalene-1-sulfonic acid {9-[(*R*)-6-(*tert*-butyl-dimethyl-silanyloxy)-2,5,7,8-tetramethyl-chroman-2-yl]heptyl}-amide (7b, *n* = 2). (48.3 mg, 82%), lime green solid, mp 59–61 °C, $R_f = 0.42$ (Et_2O/hexane 2:1), $[\alpha]_D^{20}$ +2.9° (*c* 0.007, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.51 (d, 1H, $J = 9$ Hz), 8.29 (d, 1H, $J = 9$ Hz), 8.23 (d, 1H, $J = 7$ Hz), 7.54 (t, 1H, $J = 8$ Hz), 7.50 (t, 1H, $J = 8$ Hz), 7.15 (d, 1H, $J = 7$ Hz), 4.77 (t, 1H, $J = 6$ Hz), 2.87 (s, 8H), 2.52 (t, 2H, $J = 6$ Hz), 2.08 (s, 3H), 2.04 (s, 6H), 1.73 (m, 2H), 1.41 (m, 2H), 1.32 (m, 4H), 1.18 (s, 3H), 1.07 (s, 6H), 1.03 (s, 9H), 0.10 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 151.9, 145.7, 143.9, 134.7, 130.3, 129.8, 129.5 (2 Ar-C), 128.3, 125.7, 123.4, 123.1, 122.5, 118.7, 117.4, 115.1, 74.3, 45.3 (2 CH_3), 43.2, 39.4, 31.4, 29.6, 29.4, 28.8, 26.3, 26.0 (3 CH_3), 23.7, 23.4, 20.8, 18.5, 14.3, 13.3, 11.9, −3.4 (2 CH_3); MS (EI) *m*/*z* 666 (M^+, 22.7%), 307 (26.1%), 279 (6.7%), 171 (15.8%), 149 (100%). HRMS (EI): calcd for $C_{38}H_{58}N_2O_4SSi$: 666.38865. Found: 666.38290.

4.5.6.3. 5-Dimethylamino-naphthalene-1-sulfonic acid {9-[(*R*)-6-(*tert*-butyl-dimethyl-silanyloxy)-2,5,7,8-tetramethyl-chroman-2-yl]octyl}-amide (7c, *n* = 3). (28.4 mg, 82%), lime green sticky residue, $R_f = 0.44$ (Et_2O/hexane 2:1), $[\alpha]_D^{20}$ +2.6° (*c* 0.008, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.51 (d, 1H, $J = 8$ Hz), 8.26 (d, 1H, $J = 9$ Hz), 8.22 (d, 1H,

J = 7 Hz), 7.54 (t, 1H, J = 8 Hz), 7.50 (t, 1H, J = 8 Hz), 7.15 (d, 1H, J = 7 Hz), 4.57 (t, 1H, J = 6 Hz), 2.87 (s, 8H), 2.51 (t, 2H, J = 7 Hz), 2.07 (s, 3H), 2.03 (s, 3H), 2.03 (s, 3H), 1.73 (m, 2H), 1.48 (m, 2H), 1.30 (m, 4H), 1.18 (s, 3H), 1.07 (s, 8H), 1.02 (s, 9H), 0.09 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 151.8, 145.8, 144.0, 134.7, 130.3, 129.7, 129.6, 128.3, 125.8, 123.4, 123.5, 123.2, 122.6, 118.8, 117.4, 115.1, 74.3, 45.4 (2 CH_3), 43.3, 39.5, 31.4, 29.9, 29.6, 29.4, 29.3, 26.3, 26.0 (3 CH_3), 23.7, 23.5, 20.8, 18.5, 14.3, 13.4, 11.9, −3.3 (2 CH_3); MS (EI) m/z 680 (M^+, 6.4%), 473 (10.3%), 279 (6.5%), 73 (43.9%), 43 (100%). HRMS (EI): calcd for $C_{39}H_{60}N_2O_4SSi$: 680.40430. Found: 680.40426.

4.5.6.4. 5-Dimethylamino-naphthalene-1-sulfonic acid {9-[(*R*)-6-(*tert*-butyl-dimethyl-silanyloxy)-2,5,7,8-tetramethyl-chroman-2-yl]nonyl}-amide (7d, *n* = 4). (34.3 mg, 81%), lime green oil, R_f = 0.44 (Et_2O/hexane 2:1), $[\alpha]_D^{20}$ +2.2° (c 0.009, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.52 (d, 1H, J = 9 Hz), 8.28 (d, 1H, J = 9 Hz), 8.22 (d, 1H, J = 7 Hz), 7.54 (t, 1H, J = 8 Hz), 7.51 (t, 1H, J = 8 Hz), 7.16 (d, 1H, J = 7 Hz), 4.67 (t, 1H, J = 6 Hz), 2.85 (s, 8H), 2.52 (t, 2H, J = 7 Hz), 2.07 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 1.74 (m, 2H), 1.48 (m, 2H), 1.30 (m, 4H), 1.19 (s, 3H), 1.15 (s, 4H), 1.06 (s, 6H), 1.03 (s, 9H), 0.09 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 151.8, 145.8, 143.9, 134.7, 130.2, 129.7, 129.6, 129.5, 128.3, 125.8, 123.4, 123.2, 122.6, 118.8, 117.4, 115.1, 74.3, 45.4 (2 CH_3), 43.2, 39.5, 31.4, 30.0, 29.4, 29.3, 29.2, 28.9, 26.3, 26.0 (3 CH_3), 23.7, 23.5, 20.8, 18.5, 14.3, 13.3, 11.9, −3.3 (2 CH_3); MS (EI) m/z 694 (M^+, 9.1%), 460 (12.2%), 279 (12.3%), 149 (41.2%), 69 (100%). HRMS (EI): calcd for $C_{40}H_{62}N_2O_4SSi$: 694.41995. Found: 694.42075.

4.5.7. General procedure for the preparation of compounds 8a–d.



The synthesis of compounds **8a**–**d** was conducted under identical conditions and gave similar product yields (90–93%).

4.5.7.1. *N*-{9-[(*R*)-6-(*tert*-Butyl-dimethyl-silanyloxy)-2,5,7,8-tetramethyl-chroma-2-yl]-hexyl}-2-methylamino-benzamide (8a, *n* = 1). To solid *N*-methylisatoic anhydride[34] (20.0 mg, 0.112 mmol) and 4-dimethylaminopyridine (1.2 mg, 0.010 mmol) under argon is added dry DMF (1 ml) via syringe. After stirring for 1 min, a solution of amine **4a** (43.0 mg, 0.102 mmol) in dry DMF (1 ml) was added via cannula. The reaction mixture was stirred for an additional 30 min., after which is diluted with dH_2O and extracted with ethyl acetate (3× 30 ml). The combined organic layers are then dried with Na_2SO_4, decanted, and evaporated to give the crude product which is purified by column chromatography (EtOAc/hexane 2:1) to yield the desired product in excellent yield. (51.9 mg, 92%), clear light yellow sticky residue, R_f = 0.62 (EtOAc/hexane 2:1), $[\alpha]_D^{20}$ 2.8° (c 0.003, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.56 (br, 1H), 7.30 (m, 2H), 6.65 (d, 1H, J = 9 Hz), 6.57 (t, 1H, J = 7 Hz), 6.06 (br, 1H), 3.35 (q, 2H, J = 6 Hz), 2.83 (s, 3H), 2.51 (t, 2H, J = 6 Hz), 2.08 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H), 1.75 (m, 2H), 1.54 (m, 4H), 1.34 (m, 6H), 1.20 (s, 3H), 1.03 (s, 9H), 0.10 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 169.7 (C═O), 150.2, 145.7, 143.9, 132.6, 126.9, 125.8, 123.4, 122.6, 117.4, 115.5, 114.6, 111.2, 74.3, 39.6, 39.4, 31.4, 29.8, 29.7, 29.6, 26.9, 26.0 (3 CH_3), 23.7, 23.5, 20.8, 18.5, 14.2, 13.3, 11.9, −3.4 (2 CH_3); MS (EI) m/z 552 (M^+, 76.0%), 445 (5.5%), 279 (11.8%), 134 (89.6%), 73 (100%). HRMS (EI): calcd for $C_{33}H_{52}N_2O_3Si$: 552.37472. Found: 552.37384.

4.5.7.2. *N*-{9-[(*R*)-6-(*tert*-Butyl-dimethyl-silanyloxy)-2,5,7,8-tetramethyl-chroma-2-yl]-heptyl}-2-methylamino-benzamide (8b, *n* = 2). (86.3 mg, 91%), clear light yellow sticky residue, R_f = 0.64 (EtOAc/hexane 2:1), $[\alpha]_D^{20}$ +2.2° (c 0.004, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.57 (br, 1H), 7.30 (m, 2H), 6.63 (d, 1H, J = 9 Hz), 6.56 (t, 1H, J = 7 Hz), 6.08 (br, 1H), 3.34 (q, 2H, J = 7 Hz), 2.83 (s, 3H), 2.53 (t, 2H, J = 7 Hz), 2.08 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H), 1.75 (m, 2H), 1.54 (m, 4H), 1.31 (m, 8H), 1.20 (s, 3H), 1.03 (s, 9H), 0.10 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 169.7 (C═O), 150.2, 145.7, 143.9, 132.6, 126.9, 125.7, 123.4, 122.5, 117.4, 115.5, 114.5, 111.0, 74.3, 39.6, 39.4, 31.4, 29.9, 29.7, 29.6, 29.2, 26.9, 26.0 (3 CH_3), 23.7, 23.5, 20.8, 18.5, 14.2, 13.3, 11.7, −3.4 (2 CH_3); MS (EI) m/z 566 (M^+, 100%), 458 (12.9%), 279 (10.1%), 221 (12.2%), 134 (65.6%). HRMS (EI): calcd for $C_{34}H_{54}N_2O_3Si$: 566.39037. Found: 566.39190.

4.5.7.3. *N*-{9-[(*R*)-6-(*tert*-Butyl-dimethyl-silanyloxy)-2,5,7,8-tetramethyl-chroma-2-yl]-octyl}-2-methylamino-benzamide (8c, *n* = 3). (42.3 mg, 93%), clear faint yellow oil, R_f = 0.67 (EtOAc/hexane 2:1), $[\alpha]_D^{20}$ +2.1° (c 0.004, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.56 (br, 1H), 7.30 (m, 2H), 6.64 (d, 1H, J = 9 Hz), 6.57 (t, 1H, J = 7 Hz), 6.05 (br, 1H), 3.35 (q, 2H, J = 7 Hz), 2.83 (s, 3H), 2.53 (t, 2H, J = 7 Hz), 2.08 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 1.75 (m, 2H), 1.55 (m, 4H), 1.28 (m, 10H), 1.20 (s, 3H), 1.03 (s, 9H), 0.09 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 169.7 (C═O), 150.2, 145.8, 143.9, 132.6, 126.9, 125.8, 123.4, 122.6, 117.4, 115.5, 114.6, 111.2, 74.4, 39.6, 39.5, 31.4, 30.0, 29.7, 29.6, 29.4, 29.2, 26.9, 26.0 (3 CH_3), 23.7, 23.5, 20.8, 18.5, 14.2, 13.3, 11.9, −3.4 (2 CH_3); (EI) m/z 580 (M^+, 100%), 446 (32.7%), 279 (24.5%), 134 (70.4%), 73 (95.0%). HRMS (EI): calcd for $C_{35}H_{56}N_2O_3Si$: 580.40602. Found: 580.41120.

4.5.7.4. *N*-{9-[(*R*)-6-(*tert*-Butyl-dimethyl-silanyloxy)-2,5,7,8-tetramethyl-chroma-2-yl]-nonyl}-2-methylamino-benzamide (8d, *n* = 4). (60.2 mg, 90%), clear faint yellow oil, R_f = 0.68 (EtOAc/hexane 2:1), $[\alpha]_D^{20}$ +1.6° (c 0.007, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.55 (br, 1H), 7.30 (m, 2H), 6.64 (d, 1H, J = 8 Hz), 6.57 (t, 1H, J = 6 Hz), 6.06 (br, 1H), 3.35 (q, 2H, J = 7 Hz), 2.83 (s, 3H), 2.53 (t, 2H, J = 7 Hz), 2.08 (s, 3H), 2.06 (s, 3H), 2.03 (s, 3H), 1.75 (m, 2H), 1.51 (m, 4H), 1.27 (m, 12H), 1.20 (s, 3H), 1.03 (s, 9H), 0.10 (s, 6H); ^{13}C NMR ($CDCl_3$):

δ 169.7 (C=O), 150.2, 145.8, 143.9, 132.6, 126.9, 125.7, 123.4, 122.6, 117.4, 115.5, 114.6, 111.2, 74.4, 39.6, 39.5, 31.4, 30.0, 29.7, 29.6, 29.5, 29.4, 29.2, 26.9, 26.0 (3 CH_3), 23.7, 23.5, 20.8, 18.5, 14.8, 13.3, 11.9, −3.4 (2 CH_3); MS (EI) *m/z* 594 (M^+, 100%), 322 (23.7%), 279 (16.7%), 134 (78.2%), 73 (61.2%). HRMS (EI): calcd for $C_{36}H_{58}N_2O_3Si$: 594.42167. Found: 594.42157.

4.5.8. General procedure for the preparation of compounds 9a–d, 10a–d, 11a–d, and 12a–d. Syntheses providing the deprotected fluorescent tocopherols **9a**–**d**, **10a**–**d**, **11a**–**d**, and **12a**–**d** were conducted under identical conditions, except where noted, to give similar product yields (83–89%).

4.5.8.1. ω-Anthroyloxy-α-tocopherols (AO-α-Toc) anthracene-9-carboxylic acid 6-(*R*)-6-hydroxy-2,5,7,8-tetramethyl-chroman-2-yl-hexyl ester (9a, *n* = 1). A solution of tetrabutylammonium fluoride (TBAF) (1 M in THF, 61 μl, 0.212 mmol) was added dropwise via syringe to a stirred solution of TBSO-C6-AO-α-Toc **5a** (26.5 mg, 0.042 mmol) in dry THF (1 ml). The mixture was stirred at room temperature for 30 min until no starting material **5a** was detected by TLC. The reaction was then quenched with 1 ml of 1 N HCl and diluted with 15 ml ether, to which an additional 10 ml dH_2O was added. The water phase was extracted again with ether (2× 15 ml), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was then chromatographed on silica gel (Et_2O/hexane 1:2) to give the pure product **9a**. (19.7 mg, 84%), light yellow oil, R_f = 0.54 (Et_2O/hexane 4:1), $[\alpha]_D^{20}$ +0.8° (*c* 0.003, EtOH); UV (EtOH) λ_{abs} 362 nm (ε = 6731); fluorescence λ_{em} 470 nm; ^{1}H NMR ($CDCl_3$): δ 8.50 (s, 1H), 8.01 (t, 4H, *J* = 7 Hz), 7.49 (m, 4H), 4.59 (t, 2H, *J* = 7 Hz), 2.57 (t, 2H, *J* = 7 Hz), 2.13 (s, 3H), 2.08 (s, 6H), 1.84 (m, 2H), 1.74 (m, 2H), 1.50 (m, 8H), 1.21 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 169.8, 145.5, 144.6, 131.0, 129.2, 128.6, 128.4, 128.2, 126.9, 125.4, 125.0, 122.6, 121.0, 118.5, 117.3, 74.4, 65.9, 39.5, 31.5, 29.7, 28.7, 26.1, 23.7, 23.5, 20.7, 12.2, 11.8, 11.3; MS (EI) *m/z* 510 (M^+, 4.9%), 205 (16.1%), 165 (18.8%), 138 (54.0%), 43 (100%). HRMS (EI): calcd for $C_{34}H_{38}O_4$: 510.27701. Found: 510.27581.

4.5.8.2. Anthracene-9-carboxylic acid 7-(*R*)-6-hydroxy-2,5,7,8-tetramethyl-chroman-2-yl-heptyl ester (9b, *n* = 2). (33.9 mg, 83%), light yellow oil, R_f = 0.56 (Et_2O/hexane 4:1), $[\alpha]_D^{20}$ +0.4° (*c* 0.007, EtOH); UV (EtOH) λ_{abs} 362 nm (ε = 6718); fluorescence λ_{em} 470 nm; ^{1}H NMR ($CDCl_3$): δ 8.54 (s, 1H), 8.06 (t, 4H, *J* = 9 Hz), 7.53 (m, 4H), 4.64 (t, 2H, *J* = 7 Hz), 4.26 (br, 1H), 2.61 (t, 2H, *J* = 7 Hz), 2.13 (s, 3H), 2.09 (s, 6H), 1.91 (m, 2H), 1.79 (m, 2H), 1.28 (m, 10H), 1.24 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 169.8, 145.4, 144.5, 130.9, 129.2, 128.6, 128.3, 128.2, 126.8, 125.4, 124.9, 122.5, 121.0, 118.5, 117.3, 74.4, 65.9, 39.4, 31.5, 30.0, 29.1, 28.7, 26.0, 23.7, 23.5, 20.7, 12.2, 11.7, 11.2; MS (EI) *m/z* 524 (M^+, 3.4%), 348 (20.7%), 205 (24.1%), 165 (62.0%), 43 (100%). HRMS (EI): calcd for $C_{35}H_{40}O_4$: 524.29266. Found: 524.29270.

4.5.8.3. Anthracene-9-carboxylic acid 8-(*R*)-6-hydroxy-2,5,7,8-tetramethyl-chroman-2-yl-octyl ester (9c, *n* = 3). (20.8 mg, 84%), light yellow oil, R_f = 0.58 (Et_2O/hexane 4:1), $[\alpha]_D^{20}$ +0.6° (*c* 0.007, EtOH); UV (EtOH) λ_{abs} 362 nm (ε = 6835); fluorescence λ_{em} 470 nm; ^{1}H NMR ($CDCl_3$): δ 8.51 (s, 1H), 7.99 (t, 4H, *J* = 8 Hz), 7.50 (m, 4H), 4.60 (t, 2H, *J* = 6 Hz), 4.18 (br, 1H), 2.57 (t, 2H, *J* = 7 Hz), 2.14 (s, 3H), 2.09 (s, 6H), 1.86 (m, 2H), 1.76 (m, 2H), 1.38 (m, 12H), 1.19 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 169.7, 145.5, 144.5, 130.9, 129.1, 128.5, 128.3, 128.1, 126.8, 125.4, 125.0, 122.5, 121.0, 118.4, 117.3, 74.4, 65.9, 39.4, 31.5, 30.0, 29.4, 29.1, 28.7, 26.0, 23.7, 23.5, 20.7, 12.2, 11.7, 11.2; MS (EI) *m/z* 538 (M^+, 3.0%), 376 (19.3%), 262 (49.5%), 165 (62.2%), 43 (100%). HRMS (EI): calcd for $C_{36}H_{42}O_4$: 538.30831. Found: 538.30037.

4.5.8.4. Anthracene-9-carboxylic acid 9-(*R*)-6-hydroxy-2,5,7,8-tetramethyl-chroman-2-yl-nonyl ester (9d, *n* = 4). (29.5 mg, 85%), light yellow oil, R_f = 0.61 (Et_2O/hexane 4:1), $[\alpha]_D^{20}$ +0.4° (*c* 0.008, EtOH); UV (EtOH) λ_{abs} 363 nm (ε = 6933); fluorescence λ_{em} 470 nm; ^{1}H NMR ($CDCl_3$): δ 8.50 (s, 1H), 8.01 (t, 4H, *J* = 8 Hz), 7.49 (m, 4H), 4.60 (t, 2H *J* = 7 Hz), 4.24 (br, 1H), 2.57 (t, 2H, *J* = 7 Hz), 2.13 (s, 3H), 2.09 (s, 6H), 1.84 (m, 2H), 1.74 (m, 2H), 1.48 (m, 4H), 1.36 (m, 2H), 1.28 (m, 8H), 1.20 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 169.7, 145.4, 144.4, 130.9, 129.1, 128.5, 128.3, 128.1, 126.8, 125.4, 124.9, 122.5, 121.0, 118.4, 117.3, 74.4, 65.9, 39.4, 31.4, 30.0, 29.5, 29.4, 29.2, 28.7, 26.0, 23.8, 23.5, 20.7, 12.2, 11.7, 11.2; MS (EI) *m/z* 552 (M^+, 16.4%), 222 (14.4%), 205 (100%), 165 (30.2), 83 (31.1%). HRMS (EI): calcd for $C_{37}H_{44}O_4$: 552.32395. Found: 552.32126.

4.5.8.5. ω-Nitrobenzoxadiazole-α-tocopherols (NBD-α-Toc). The following change was made to the above protocol: (i) column chromatography was conducted using an ethyl acetate/hexane (1:2) solvent system.

4.5.8.6. (*R*)-2,5,7,8-tetramethyl-chroman-2-[9-(7-nitro-benzo[1,2,5]oxadiazol-4-ylamino)-hexyl]-chroman-6-ol (10a, *n* = 1). (7.6 mg, 85 %), dark browny orange oil, R_f = 0.43 (EtOAc/hexane 1:1), $[\alpha]_D^{20}$ +4.5° (*c* 0.005, EtOH); UV (EtOH) λ_{abs} 468 nm (ε = 18,750); fluorescence λ_{em} 527 nm; ^{1}H NMR ($CDCl_3$): δ 8.49 (d, 1H, *J* = 9 Hz), 6.17 (br, 1H), 6.16 (d, 1H, *J* = 9 Hz), 4.20 (s, 1H), 3.46 (q, 2H, *J* = 6 Hz), 2.60 (t, 2H, *J* = 6 Hz), 2.15 (s, 3H), 2.11 (s, 3H), 2.10 (s, 3H), 1.77 (m, 4H), 1.48 (m, 8H), 1.22 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 145.3, 144.6, 144.2, 143.8, 143.7, 136.4, 124.0, 122.5, 121.0, 118.5, 117.3, 98.5, 74.2, 43.9, 39.2, 31.6, 29.6, 28.4, 26.9, 23.7, 23.3, 20.7, 12.2, 11.8, 11.3; MS (EI) *m/z* 468 (M^+, 8.3%), 304 (20.2%), 203 (20.9%), 165 (100%), 43 (84.7%). HRMS (EI): calcd for $C_{25}H_{32}N_4O_5$: 468.23727. Found: 468.22557.

4.5.8.7. (*R*)-2,5,7,8-tetramethyl-chroman-2-[9-(7-nitro-benzo[1,2,5]oxadiazol-4-ylamino)-heptyl]-chroman-6-ol (10b, *n* = 2). (9.2 mg, 84%), dark browny orange oil, R_f = 0.45 (EtOAc/hexane 1:1), $[\alpha]_D^{20}$ +3.9° (*c* 0.005, EtOH); UV (EtOH) λ_{abs} 468 nm (ε = 18,415); fluorescence λ_{em} 529 nm; ^{1}H NMR ($CDCl_3$): δ 8.48 (d, 1H, *J* = 9 Hz), 6.19 (br, 1H), 6.16 (d, 1H, *J* = 9 Hz), 4.18

(s, 1H), 3.44 (q, 2H, $J = 6$ Hz), 2.58 (t, 2H, $J = 7$ Hz), 2.13 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H), 1.76 (m, 4H), 1.56 (m, 2H), 1.48 (m, 8H), 1.19 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 145.4, 144.5, 144.2, 143.8, 143.7, 136.4, 123.9, 122.5, 121.0, 118.5, 117.3, 98.4, 74.3, 43.9, 39.3, 31.5, 29.8, 29.1, 28.5, 26.8, 23.7, 23.4, 20.7, 12.2, 11.8, 11.3; MS (EI) *m/z* 482 (M^+, 7.6%), 318 (31.0%), 203 (16.1%), 165 (100%), 43 (92.8%). HRMS (EI): calcd for $C_{26}H_{34}N_4O_5$: 482.25292. Found: 482.25347.

4.5.8.8. (*R*)-2,5,7,8-tetramethyl-chroman-2-[9-(7-nitro-benzo[1,2,5]oxadiazol-4-ylamino)-octyl]-chroman-6-ol (10c, *n* = 3). (8.2 mg, 84%), dark browny orange oil, $R_f = 0.46$ (EtOAc/hexane 1:1), $[\alpha]_D^{20}$ +4.1° (*c* 0.004, EtOH); UV (EtOH) λ_{abs} 469 nm ($\varepsilon = 5809$); fluorescence λ_{em} 528 nm; ^{1}H NMR ($CDCl_3$): δ 8.47 (d, 1H, $J = 9$ Hz), 6.24 (br, 1H), 6.14 (d, 1H, $J = 9$ Hz), 4.23 (br, 1H), 3.46 (q, 2H, $J = 6$ Hz), 2.58 (t, 2H, $J = 7$ Hz), 2.13 (s, 3H), 2.08 (s, 3H), 2.03 (s, 3H), 1.73 (m, 4H), 1.57 (m, 2H), 1.42 (m, 10H), 1.20 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 145.4, 144.5, 144.2, 143.9, 143.8, 136.5, 123.9, 122.5, 121.0, 118.5, 117.3, 98.4, 74.4, 43.9, 39.1, 31.6, 29.8, 29.2, 29.0, 28.5, 26.9, 23.8, 23.4, 20.7, 12.2, 11.8, 11.3; MS (EI) *m/z* 496 (M^+, 11.8%), 332 (22.2%), 203 (17.1%), 165 (100%), 43 (43.0%). HRMS (EI): calcd for $C_{27}H_{36}N_4O_5$: 496.26857. Found: 496.26402.

4.5.8.9. (*R*)-2,5,7,8-tetramethyl-chroman-2-[9-(7-nitro-benzo[1,2,5]oxadiazol-4-ylamino)-nonyl]-chroman-6-ol (10d, *n* = 4). (14.9 mg, 86%), dark browny orange oil, $R_f = 0.49$ (EtOAc/hexane 1:1), $[\alpha]_D^{20}$ +4.7° (*c* 0.004, EtOH); UV (EtOH) λ_{abs} 469 nm ($\varepsilon = 17{,}602$); fluorescence λ_{em} 527 nm; ^{1}H NMR ($CDCl_3$): δ 8.49 (d, 1H, $J = 9$ Hz), 6.26 (br, 1H), 6.17 (d, 1H, $J = 9$ Hz), 4.24 (s, 1H), 3.47 (q, 2H, $J = 6$ Hz), 2.58 (t, 2H, $J = 7$ Hz), 2.15 (s, 3H), 2.10 (s, 3H), 2.05 (s, 3H), 1.77 (m, 4H), 1.55 (m, 2H), 1.43 (m, 12H), 1.22 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 145.5, 144.5, 144.2, 143.9, 143.8, 136.5, 123.9, 122.5, 121.0, 118.5, 117.3, 98.5, 74.4, 43.9, 39.3, 31.5, 29.9, 29.4, 29.3, 29.2, 28.5, 26.9, 23.8, 23.5, 20.7, 12.2, 11.8, 11.3; MS (EI) *m/z* 510 (M^+, 13.5%), 346 (21.4%), 203 (17.6%), 165 (100%), 43 (18.5%). HRMS (EI): calcd for $C_{28}H_{38}N_4O_5$: 510.28422. Found: 510.28290.

4.5.8.10. ω-Dansyl-α-tocopherols (DAN-α-Toc). The following changes were made to the above protocol: (i) extraction of the crude product was done using dichloromethane, (ii) column chromatography was conducted using an ether/hexane (2:1) solvent system.

4.5.8.11. 5-Dimethylamino-naphthalene-1-sulfonic acid [9-((*R*)-6-hydroxyl-2,5,7,8-tetramethyl-chroman-2-yl)-hexyl-amine (11a, *n* = 1). (20.8 mg, 88%), light lime green solid, mp 60–63 °C, $R_f = 0.56$ (Et_2O/hexane 5:1), $[\alpha]_D^{20}$ +0.8° (*c* 0.010, EtOH); UV (EtOH) λ_{abs} 340 nm ($\varepsilon = 3797$); fluorescence λ_{em} 510 nm; ^{1}H NMR ($CDCl_3$): δ 8.51 (d, 1H, $J = 9$ Hz), 8.25 (d, 1H, $J = 8$ Hz), 8.23 (d, 1H, $J = 8$ Hz), 7.53 (t, 1H, $J = 8$ Hz), 7.50 (t, 1H, $J = 8$ Hz), 7.14 (d, 1H, $J = 7$ Hz), 4.57 (t, 1H, $J = 6$ Hz), 4.26 (s, 1H), 2.87 (s, 6H), 2.82 (m, 2H), 2.56 (t, 2H, $J = 7$ Hz), 2.13 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H), 1.71 (m, 2H), 1.41 (m, 2H), 1.33 (m, 6H), 1.15 (s, 3H), 1.08 (br, 2H); ^{13}C NMR ($CDCl_3$): δ 151.4, 145.4, 144.5, 134.7, 130.2, 129.7, 129.6, 129.5, 128.2, 123.2, 122.5, 121.1, 118.9, 118.6, 117.2, 115.1, 74.2, 45.4 (2 CH_3), 43.2, 38.9, 31.5, 29.3, 29.3, 26.2, 23.7, 23.2, 20.6, 12.2, 11.7, 11.2; MS (EI) *m/z* 538 (M^+, 100%), 374 (6.7%), 205 (12.5%), 165 (74.6%), 43 (58.1%). HRMS (EI): calcd for $C_{31}H_{42}N_2O_4S$: 538.28653. Found: 538.28642.

4.5.8.12. 5-Dimethylamino-naphthalene-1-sulfonic acid [9-((*R*)-6-hydroxyl-2,5,7,8-tetramethyl-chroman-2-yl)-heptyl-amine (11b, *n* = 2). (18.1 mg, 87%), light lime green sticky solid, mp 80–82 °C, $R_f = 0.57$ (Et_2O/hexane 5:1), $[\alpha]_D^{20}$ +1.0° (*c* 0.009, EtOH); UV (EtOH) λ_{abs} 341 nm ($\varepsilon = 3865$); fluorescence λ_{em} 507 nm; ^{1}H NMR ($CDCl_3$): δ 8.51 (d, 1H, $J = 8$ Hz), 8.26 (d, 1H, $J = 9$ Hz), 8.21 (d, 1H, $J = 8$ Hz), 7.54 (t, 1H, $J = 8$ Hz), 7.50 (t, 1H, $J = 8$ Hz), 7.15 (d, 1H, $J = 7$ Hz), 4.61 (t, 1H, $J = 6$ Hz), 4.24 (s, 1H), 2.87 (s, 6H), 2.83 (m, 2H), 2.56 (t, 2H, $J = 6$ Hz), 2.13 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H), 1.72 (m, 2H), 1.42 (m, 2H), 1.35 (m, 6H), 1.17 (s, 3H), 1.07 (br, 4H); ^{13}C NMR ($CDCl_3$): δ 151.4, 145.4, 144.5, 134.7, 130.2, 129.7, 129.6, 129.6, 128.2, 123.2, 122.5, 121.0, 118.9, 118.5, 117.2, 115.1, 74.3, 45.4 (2 CH_3), 43.2, 39.2, 31.4, 29.7, 29.4, 28.8, 26.2, 23.7, 23.3, 20.6, 12.2, 11.7, 11.2; MS (EI) *m/z* 552 (M^+, 76.4%), 388 (5.8%), 203 (15.6%), 165 (60.6%), 69 (100%). HRMS (EI): calcd for $C_{32}H_{44}N_2O_4S$: 552.30218. Found: 552.30216.

4.5.8.13. 5-Dimethylamino-naphthalene-1-sulfonic acid [9-((*R*)-6-hydroxyl-2,5,7,8-tetramethyl-chroman-2-yl)-octyl-amine (11c, *n* = 3). (8.0 mg, 87%), light lime green sticky residue, $R_f = 0.58$ (Et_2O/hexane 5:1), $[\alpha]_D^{20}$ +1.5° (*c* 0.004, EtOH); UV (EtOH) λ_{abs} 342 nm ($\varepsilon = 3655$); fluorescence λ_{em} 511 nm; ^{1}H NMR ($CDCl_3$): δ 8.51 (d, 1H, $J = 9$ Hz), 8.25 (d, 1H, $J = 8$ Hz), 8.22 (d, 1H, $J = 8$ Hz), 7.53 (t, 1H, $J = 7$ Hz), 7.49 (t, 1H, $J = 7$ Hz), 7.15 (d, 1H, $J = 8$ Hz), 4.60 (t, 1H, $J = 6$ Hz), 4.24 (s, 1H), 2.86 (s, 6H), 2.84 (t, 2H, $J = 7$ Hz), 2.56 (t, 2H, $J = 7$ Hz), 2.13 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H), 1.73 (m, 2H), 1.45 (m, 2H), 1.31 (m, 6H), 1.17 (s, 3H), 1.14 (br, 6H); ^{13}C NMR ($CDCl_3$): δ 151.4, 145.4, 144.4, 134.6, 130.2, 129.7, 129.6, 129.5, 128.2, 123.2, 122.5, 121.1, 118.9, 118.6, 117.2, 115.1, 74.3, 45.4 (2 CH_3), 43.2, 39.2, 31.4, 29.7, 29.5, 29.4, 28.8, 26.2, 23.7, 23.3, 20.6, 12.1, 11.6, 11.1; MS (EI) *m/z* 566 (M^+, 24.9%), 205 (20.1%) 165 (37.3%), 57 (62.5%), 43 (100%). HRMS (EI): calcd for $C_{33}H_{46}N_2O_4S$: 566.31783. Found: 566.31688.

4.5.8.14. 5-Dimethylamino-naphthalene-1-sulfonic acid [9-((*R*)-6-hydroxyl-2,5,7,8-tetramethyl-chroman-2-yl)-nonyl-amine (11d, *n* = 4). (20.8 mg, 88%), lime green sticky residue, $R_f = 0.59$ (Et_2O/hexane 5:1), $[\alpha]_D^{20}$ +0.3° (*c* 0.010, EtOH); UV (EtOH) λ_{abs} 340 nm ($\varepsilon = 4009$); fluorescence λ_{em} 507 nm; ^{1}H NMR ($CDCl_3$): δ 8.52 (d, 1H, $J = 9$ Hz), 8.26 (d, 1H, $J = 8$ Hz), 8.22 (d, 1H, $J = 8$ Hz), 7.54 (t, 1H, $J = 7$ Hz), 7.50 (t, 1H, $J = 7$ Hz), 7.16 (d, 1H, $J = 8$ Hz), 4.62 (t, 1H, $J = 6$ Hz), 4.25 (s, 1H), 2.87 (s, 6H), 2.84 (t, 2H, $J = 7$ Hz), 2.57 (t, 2H, $J = 7$ Hz), 2.13 (s, 3H), 2.08 (s, 6H), 1.74 (m, 2H), 1.47 (m, 2H), 1.34 (m, 6H), 1.19 (s, 3H), 1.15 (br, 8H); ^{13}C NMR ($CDCl_3$) δ 151.5, 145.5, 144.5, 134.7, 130.3, 129.8,

129.7, 129.6, 128.3, 123.3, 122.5, 121.0, 119.0, 118.5, 117.3, 115.2, 74.4, 45.4 (2 CH_3), 43.3, 39.3, 31.5, 30.0, 29.5, 29.3, 29.2, 28.9, 26.3, 23.8, 23.5, 20.7, 12.2, 11.8, 11.3; MS (EI) *m*/*z* 580 (M^+, 21.5%), 205 (22.7%), 149 (30.8%), 57 (100%), 43 (97.6%). HRMS (EI): calcd for $C_{34}H_{48}N_2O_4S$: 580.33348. Found: 580.33409.

4.5.8.15. ω-*N*-Methylanthranylamide-α-tocopherols (NMA-α-Toc). The following change was made to the above protocol: (i) column chromatography was conducted using an ethyl acetate/hexane (1:1) solvent system.

4.5.8.16. *N*-[9-((*R*)-6-hydroxy-2,5,7,8-tetramethylchroma-2-yl)-hexyl]-2-methylamino-benzamide (12a, *n* = 1). (12.3 mg, 88%), light yellow solid, mp 48–49 °C, $R_f = 0.61$ (EtOAc/hexane 2:1), $[\alpha]_D^{20}$ +3.0° (*c* 0.005, EtOH); UV (EtOH) λ_{abs} 340 nm ($\varepsilon = 2729$); fluorescence λ_{em} 423 nm; ^{1}H NMR ($CDCl_3$): δ 7.27 (q, 2H, $J = 8$ Hz), 6.73 (d, 1H, $J = 7$ Hz), 6.62 (t, 1H, $J = 7$ Hz), 6.19 (br, 1H), 4.31 (br, 1H), 3.28 (q, 2H, $J = 7$ Hz), 2.80 (s, 3H), 2.52 (t, 2H, $J = 7$ Hz), 2.08 (s, 3H), 2.03 (s, 6H), 1.71 (m, 2H), 1.51 (m, 4H), 1.28 (m, 6H), 1.14 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 169.4, 150.0, 145.4, 144.5, 132.8, 127.1, 122.5, 121.1, 118.6, 117.3, 116.5, 115.8, 112.4, 74.4, 39.8, 39.3, 31.5, 29.8, 29.5, 27.0, 23.7, 23.5, 20.7, 12.2, 11.8, 11.3; MS (EI) *m*/*z* 438 (M^+, 23.8%), 305 (6.9%), 165 (22.2%), 134 (100%), 69 (53.2%). HRMS (EI): calcd for $C_{27}H_{38}N_2O_3$: 438.28824. Found: 438.28643.

4.5.8.17. *N*-[9-((*R*)-6-hydroxy-2,5,7,8-tetramethylchroma-2-yl)-heptyl]-2-methylamino-benzamide (12b, *n* = 2). (15.2 mg, 89%), light yellow solid, mp 53–55 °C, $R_f = 0.63$ (EtOAc/hexane 2:1), $[\alpha]_D^{20}$ +5.3° (*c* 0.007, EtOH); UV (EtOH) λ_{abs} 346 nm ($\varepsilon = 3898$); fluorescence λ_{em} 423 nm; ^{1}H NMR ($CDCl_3$): δ 7.29 (m, 2H), 6.66 (d, 1H, $J = 8$ Hz), 6.58 (t, 1H, $J = 7$ Hz), 6.07 (br, 1H), 4.33 (br, 1H), 3.32 (q, 2H, $J = 7$ Hz), 2.83 (s, 3H), 2.57 (t, 2H, $J = 7$ Hz), 2.13 (s, 3H), 2.08 (s, 6H), 1.74 (m, 2H), 1.55 (m, 4H), 1.30 (m, 8H), 1.19 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 169.7, 150.0, 145.4, 144.5, 132.6, 127.0, 122.5, 121.1, 118.6, 117.3, 115.6, 114.8, 111.4, 74.3, 39.6, 39.2, 31.5, 29.9, 29.6, 29.2, 26.8, 23.7, 23.4, 20.7, 12.2, 11.7, 11.2; MS (EI) *m*/*z* 452 (M^+, 11.3%), 319 (4.2%), 165 (16.7%), 134 (53.9%), 69 (100%). HRMS (EI): calcd for $C_{28}H_{40}N_2O_3$: 452.30389. Found: 452.30454.

4.5.8.18. *N*-[9-((*R*)-6-hydroxy-2,5,7,8-tetramethylchroma-2-yl)-octyl]-2-methylamino-benzamide (12c, *n* = 3). (11.0 mg, 88%), light yellow oil, $R_f = 0.66$ (EtOAc/hexane 2:1), $[\alpha]_D^{20}$ +1.5° (*c* 0.005, EtOH); UV (EtOH) λ_{abs} 347 nm ($\varepsilon = 3213$); fluorescence λ_{em} 423 nm; ^{1}H NMR ($CDCl_3$): δ 7.33 (t, 2H, $J = 7$ Hz), 6.72 (d, 1H, $J = 7$ Hz), 6.60 (t, 1H, $J = 7$ Hz), 6.09 (br, 1H), 4.33 (br, 1H), 3.32 (q, 2H, $J = 7$ Hz), 2.84 (s, 3H), 2.57 (t, 2H, $J = 7$ Hz), 2.13 (s, 3H), 2.08 (s, 6H), 1.74 (m, 2H), 1.54 (m, 4H), 1.26 (m, 10H), 1.19 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 169.6, 149.8, 145.4, 144.5, 132.7, 127.0, 122.5, 121.0, 118.5, 117.3, 115.9, 115.3, 111.9, 74.4, 39.7, 39.2, 31.5, 29.9, 29.6, 29.4, 29.1, 27.0, 23.8, 23.4, 20.7, 12.2, 11.7, 11.2; MS (EI) *m*/*z* 466 (M^+, 5.6%), 329 (3.6%), 165 (16.4%), 134 (23.4%), 69 (100%). HRMS (EI): calcd for $C_{29}H_{42}N_2O_3$: 466.31954. Found: 466.31689.

4.5.8.19. *N*-[9-((*R*)-6-hydroxy-2,5,7,8-tetramethylchroma-2-yl)-nonyl]-2-methylamino-benzamide (12d, *n* = 4). (19.2 mg, 89%), light yellow oil, $R_f = 0.67$ (EtOAc/hexane 2:1), $[\alpha]_D^{20}$ +1.3° (*c* 0.006, EtOH); UV (EtOH) λ_{abs} 346 nm ($\varepsilon = 3969$); fluorescence λ_{em} 424 nm; ^{1}H NMR ($CDCl_3$): δ 7.30 (t, 2H, $J = 7$ Hz), 6.68 (d, 1H, $J = 8$ Hz), 6.60 (t, 1H, $J = 7$ Hz), 6.10 (br, 1H), 4.31 (br, 1H), 3.34 (q, 2H, $J = 7$ Hz), 2.83 (s, 3H), 2.57 (t, 2H, $J = 7$ Hz), 2.13 (s, 3H), 2.08 (s, 6H), 1.74 (m, 2H), 1.55 (m, 4H), 1.25 (m, 12H), 1.20 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 169.7, 149.8, 145.4, 144.5, 132.7, 127.0, 122.5, 121.1, 118.6, 117.3, 115.9, 115.2, 111.7, 74.4, 39.7, 39.3, 31.5, 30.0, 29.6, 29.5, 29.4, 29.3, 27.0, 23.8, 23.5, 20.7, 12.2, 11.8, 11.3; MS (EI) *m*/*z* 480 (M^+, 6.4%), 163 (39.9%), 149 (32.3%), 105 (100%), 69 (38.5%). HRMS (EI): calcd for $C_{30}H_{44}N_2O_3$: 480.33519. Found: 480.33222.

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References and notes

1. Burton, G. W.; Ingold, K. U. *Acc. Chem. Res.* **1986**, *19*, 194–201.
2. Burton, G. W.; Foster, D. O.; Hughes, L.; Traber, M. G.; Kayden, H. J.; Ingold, K. U. In *Medical, Biochemical and Chemical Aspects of Free Radicals*; Hayaishi, O., Niki, E., Kondo, M., Yoshikawa, T., Eds.; Elsevier: Amsterdam, 1989; pp 241–250.
3. Burton, G. W.; Traber, M. G. *Annu. Rev. Nutr.* **1990**, *10*, 357–382.
4. Galli, F.; Lee, R.; Atkinson, J.; Floridi, A.; Kelly, F. J. *Free Radic. Res.* **2003**, *37*, 1225–1233.
5. Galli, F.; Lee, R.; Dunster, C.; Atkinson, J.; Floridi, A.; Kelly, F. *J. Biofactors* **2001**, *15*, 65–69.
6. Traber, M. G.; Burton, G. W.; Ingold, K. U.; Kayden, H. J. *J. Lipid Res.* **1990**, *31*, 675–685.
7. Kaempf-Rotzoll, D. E.; Traber, M. G.; Arai, H. *Curr. Opin. Lipidol.* **2003**, *14*, 249–254.
8. Blatt, D. H.; Leonard, S. W.; Traber, M. G. *Nutrition* **2001**, *17*, 799–805.
9. Traber, M.; Sies, H. *Annu. Rev. Nutr.* **1996**, *16*, 321–347.
10. Panagabko, C.; Morley, S.; Hernandez, M.; Cassolato, P.; Gordon, H.; Parsons, R.; Manor, D.; Atkinson, J. *Biochemistry* **2003**, *42*, 6467–6474.
11. Cohn, W.; Loechleiter, F.; Weber, F. *J. Lipid Res.* **1988**, *29*, 1359–1366.
12. Kagan, V. E.; Bakalova, R. A.; Serbinova, E. E.; Stoytchev, T. S. *Methods Enzymol.* **1990**, *186*, 355–367.
13. Jemiola-Rzemimska, M.; Kruk, J.; Strzalka, K. *Chem. Phys. Lipids* **2003**, *123*, 233–243.

14. Thulborn, K. R.; Sawyer, W. H. *Biochim. Biophys. Acta* **1978**, *511*, 125–140.
15. Vandermeulen, D. L. *Govindjee. Biochim. Biophys. Acta* **1976**, *449*, 340–356.
16. Johnson, I. D.; Kang, H. C.; Haugland, R. P. *Anal. Biochem.* **1991**, *198*, 228–237.
17. Kang, H. C.; Haugland, R. P.; Molecular Probes, U.S. Patent 5,338,854, 1994.
18. Storch, J.; Thumser, A. E. *Biochim. Biophys. Acta* **2000**, *1486*, 28–44.
19. Huang, H.; Starodub, O.; McIntosh, A.; Kier, A. B.; Schroeder, F. *J. Biol. Chem.* **2002**, *277*, 29139–29151.
20. Faergeman, N. J.; DiRusso, C. C.; Elberger, A.; Knudsen, J.; Black, P. N. *J. Biol. Chem.* **1997**, *272*, 8531–8538.
21. Petrescu, A. D.; Gallegos, A. M.; Okamura, Y.; Strauss, J. F., 3rd; Schroeder, F. *J. Biol. Chem.* **2001**, *276*, 36970–36982.
22. Atshaves, B. P.; Starodub, O.; McIntosh, A.; Petrescu, A.; Roths, J. B.; Kier, A. B.; Schroeder, F. *J. Biol. Chem.* **2000**, *275*, 36852–36861.
23. Frolov, A.; Petrescu, A.; Atshaves, B. P.; So, P. T.; Gratton, E.; Serrero, G.; Schroeder, F. *J. Biol. Chem.* **2000**, *275*, 12769–12780.
24. Avdulov, N. A.; Chochina, S. V.; Igbavboa, U.; Warden, C. S.; Schroeder, F.; Wood, W. G. *Biochim. Biophys. Acta* **1999**, *1437*, 37–45.
25. Dagher, G.; Donne, N.; Klein, C.; Ferre, P.; Dugail, I. *J. Lipid Res.* **2003**, *44*, 1811–1820.
26. Wiegand, V.; Chang, T. Y.; Strauss, J. F., 3rd; Fahrenholz, F.; Gimpl, G. *FASEB J.* **2003**, *17*, 782–784.
27. Mukherjee, S.; Zha, X.; Tabas, I.; Maxfield, F. R. *Biophys. J.* **1998**, *75*, 1915–1925.
28. Bisgaier, C. L.; Essenburg, A. D.; Minton, L. L.; Homan, R.; Blankley, C. J.; White, A. *Lipids* **1994**, *29*, 811–818.
29. Lei, H. S.; Atkinson, J. *J. Org. Chem.* **2000**, *65*, 2560–2567.
30. Hosomi, A.; Arita, M.; Sato, Y.; Kiyose, C.; Ueda, T.; Igarashi, O.; Arai, H.; Inoue, K. *FEBS Lett.* **1997**, *409*, 105–108.
31. Ingold, K. U.; Burton, G. W.; Foster, D. O.; Hughes, L. *Free Radic. Biol. Med.* **1990**, *9*, 205–210.
32. Meier, R.; Tomizaki, T.; Schulze-Briese, C.; Baumann, U.; Stocker, A. *J. Mol. Biol.* **2003**, *331*, 725–734.
33. Min, K. C.; Kovall, R. A.; Hendrickson, W. A. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 14713–14718.
34. Venuti, M. C. *Synthesis* **1982**, *4*, 266–268.
35. Morley, S.; Cross, V.; Cecchini, M.; Nava, P.; Atkinson, J.; Manor, D. *Biochemistry* **2006**, *45*, 1075–1081.
36. Brigelius, F.-R.; Traber, M. G. *FASEB J.* **1999**, *13*, 1145–1155.
37. Norris, A. W.; Cheng, L.; Giguere, V.; Rosenberger, M.; Li, E. *Biochim. Biophys. Acta* **1994**, *1209*, 10–18.
38. Szuts, E. Z.; Harosi, F. I. *Arch. Biochem. Biophys.* **1991**, *287*, 297–304.
39. Matsuoka, K.; Kuranaga, Y.; Moroi, Y. *Biochim. Biophys. Acta* **2002**, *1580*, 200–214.
40. Dubbs, M. D.; Gupta, R. B. *J. Chem. Eng. Data* **1998**, *43*, 590–591.
41. Qian, J.; Morley, S.; Wilson, K.; Nava, P.; Atkinson, J.; Manor, D. *J. Lipid Res.* **2005**, *46*, 2072–2082.
42. Berman, H. M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T. N.; Weissig, H.; Shindyalov, I. N.; Bourne, P. E. *Nucleic Acids Res.* **2000**, *28*, 235–242.
43. Quanta, version 4.1, Accelrys, Inc., San Diego, CA, 1998.
44. Brooks, B. R.; Bruccoler, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. *J. Comput. Chem.* **1983**, *4*, 187–217.
45. Tompson, S. M. *In CCP5 Quarterly* **1983**, 20–28.
46. Fincham, D.; Ralston, B. *J. Int. Comput. Phys. Commun.* **1981**, 127–134.
47. Ryckaert, J. P.; Ciccotti, G.; Berendsen, H. J. C. *Int. J. Comput. Phys.* **1977**, 327–341.