Organic & Biomolecular Chemistry

Dynamic Article Links

Cite this: *Org. Biomol. Chem.*, 2011, **9**, 7468

www.rsc.org/obc

PAPER

Synthesis and ion transport activity of oligoesters containing an environment-sensitive fluorophore†

Joanne M. Moszynski and Thomas M. Fyles*

Received 29th June 2011, Accepted 5th August 2011
DOI: 10.1039/c1ob06047c

A series of oligoesters based on a rigid triphenyl-diyne core is described. The molecules were readily synthesized from key intermediates, and retained good solubility properties. One of the compounds displayed modest ion transport activity in vesicles, was capable of forming highly conducting single channels in planar bilayers and exhibited an irregular non-linear current–voltage response. All the reported molecules had minimal aqueous fluorescence while being highly fluorescent in less-polar media including lipid vesicles; their partitioning into the membrane could be monitored by a significant blue-shift and increase in fluorescence intensity, as well as a decreased extent of quenching in vesicles over that in water. The combined data indicated that the compounds are highly aggregated in aqueous solution, which limits their membrane partitioning and ion transport activity, in agreement with mechanisms proposed for other 'simple' oligoester channels.

Introduction

Synthetic ion channel research has been extensively pursued for the past several decades, and entire classes of transport-active molecules have been made over this time.[1–6] A number of these compounds exhibit interesting properties in addition to ion transport; they have been used as antibiotic agents[7–10] and sensors for analytes such as sugars.[11] Synthetic systems are also becoming increasingly sophisticated; ion transport activity can be regulated by voltage,[12–14] light[15] and ligand-binding[16] and additional examples continue to be reported in the literature.

One ongoing focus has been the design and synthesis of structurally 'simple' compounds which are believed to act as aggregate channels in the membrane.[1] While these structures are easily accessible by relatively facile syntheses and can be highly transport-active,[17–19] in most cases the in-membrane structures of these species remain unknown. Accordingly, increasing attention is being paid to characterizing the fate of these compounds as they interact with lipid bilayers. Fluorescently-labelled synthetic ion channels offer one such possibility of monitoring the bilayer partitioning and dynamics of putative channel-forming compounds,[20,21] which could lead to a better understanding of how these 'simple' channels act.

Recent efforts from our own lab have involved a series of oligoester ion channels containing both fully-saturated alkyl chains,[22–24] as well as a modified diphenylacetylene or '**Dip**' moiety.[25] The **Dip** molecules exhibit environment-sensitive fluorescence due to the diphenylacetylene chromophore,[26–28] this allowed direct detection of partitioning dynamics which in turn lent support to a proposed mechanism based on rate-limiting aqueous phase aggregation.[25] The **Dip** oligoesters were also found to be highly transport-active, with rates enhanced by up to 5-fold over the saturated analogs, due to more efficient partitioning of the rigid **Dip** moiety and/or increased stabilization of the channels formed within the membrane.[18,29]

While the **Dip** fluorescence is a valuable tool, its intensity is fairly low; a more efficient fluorophore would be more useful as a 'light up' membrane probe,[30] as well as simplifying detection of low concentrations of compound. An extended rigid fluorophore derived from diphenylacetylene would therefore be promising in this regard. In addition, as the **Dip** studies indicated a positive correlation existed between compound rigidity and ion transport activity, the potential to enhance this further by a longer rigid aromatic moiety was another motivation. A number of structures were considered but a majority were rejected based on the known poor solubilities of oligo phenylacetylenes.[31] We reasoned that this was in part due to the high symmetry of these compounds and as we were interested in end-differentiated molecules, we focussed our attention on lower symmetry fluorophores.

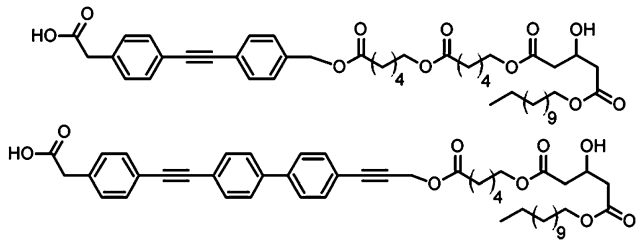
This paper reports the design, synthesis and functional characterization of a series of potential channel formers built upon an extended, tri-aromatic '**Trip**' scaffold. An example of such a target structure; HO_2C-**Trip-Hex-G(12)**-OH‡ is shown below, in

Department of Chemistry, University of Victoria, PO Box 3065, Victoria, BC, Canada. E-mail: tmf@uvic.ca; Fax: +1 250 721 7147; Tel: +1 250 7217192

† Electronic supplementary information (ESI) available: synthetic experimental details and characterization (^{1}H and ^{13}C NMR, HPLC, MS) of new compounds; transport and fluorescence assay details; bilayer clamp summary data, as discussed in text. See DOI: 10.1039/c1ob06047c

‡ The trivial naming of the compounds follows from the linear structures with the carboxyl and hydroxyl termini explicitly specified; named subunits are linked as esters. The ***G(12)*** unit indicates a diester of 3-hydroxy glutaric

comparison to the previously-developed **Dip** oligomer HO_2C-**Dip-Hex-Hex-G(12)**-OH. The **Trip**-containing molecules retain similar structural features as the **Dip** oligomers such as a membrane-spanning length of approximately 3.5 nm from carboxyl to hydroxyl termini, and an overall dissymmetry to potentially access voltage-gated ion transport behaviours.



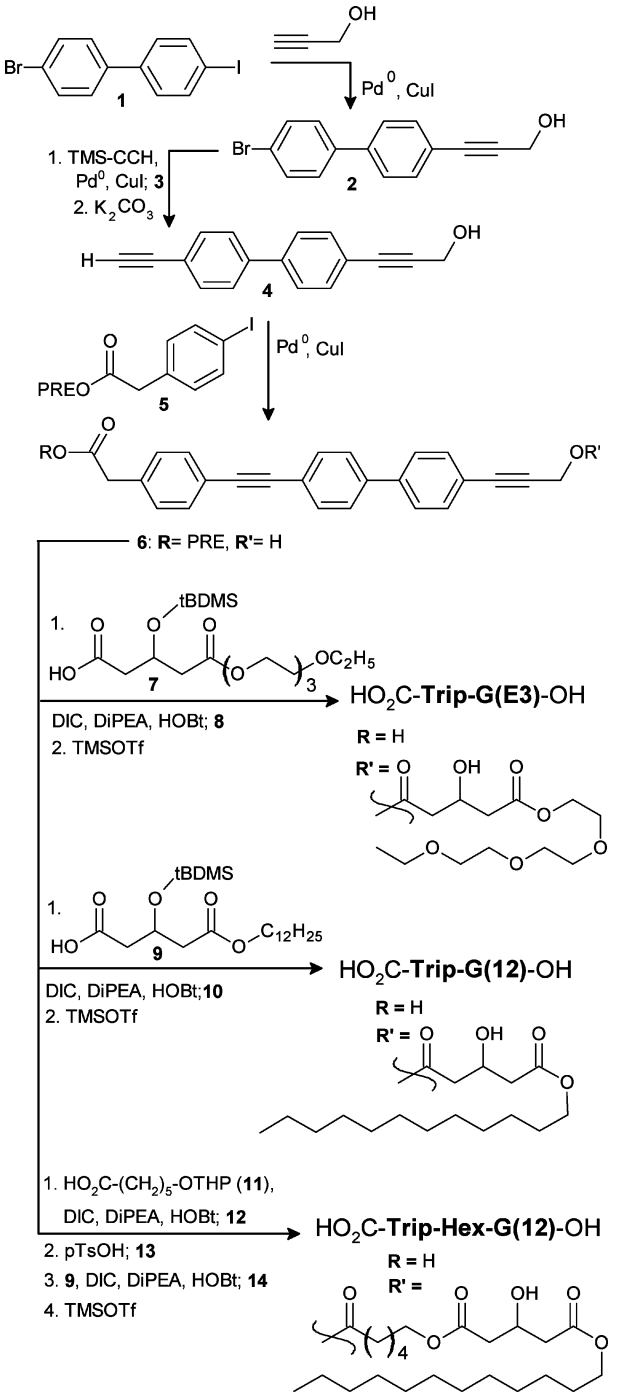
Results & discussion

Synthesis

The synthesis of the **Trip** scaffold-containing key intermediate **6** began with the facile Sonogashira[32,33] coupling of propargyl alcohol to the known[34] 4-bromo-4′-iodobiphenyl compound **1** to produce **2** in 89% yield (Scheme 1). Under the utilized conditions, only the iodo substituent underwent the coupling reaction. The next step required a higher-temperature Sonogashira reaction with TMS acetylene to effect cross coupling of the bromo substituent of **2**, to produce **3** in a fairly good yield of 71%. After a simple TMS deprotection with K_2CO_3, the terminal alkyne **4** underwent another cross-coupling reaction with the prenyl-protected aromatic iodide **5**, to furnish the protected **Trip** scaffold **6**. Compound **5** was made simply by the ester coupling of prenyl alcohol to 4-iodophenylacetic acid.†

Once in hand, the **Trip** scaffold was extended into dimeric or trimeric oligoesters by utilizing the ester coupling and deprotection reactions previously developed for the **Dip** compounds.[25] The alkyl 'tails' appended to the hydroxyl terminus of **6** under the standard conditions were either known from previous work (compounds **9** and **11**),[23] or easily synthesized from commercially-available starting materials (compound **7**).†In all three cases, the ester coupling reactions between the protected alkyl tails and the **Trip** scaffold **6** were fairly good yielding; 71% for the reaction yielding **8**, 73% for **10**, and ~60% for each of the 2 steps leading to **14**, *via* **12** and **13**. The final step in all three syntheses was the simultaneous removal of the prenyl and tBDMS protecting groups present on the carboxyl and hydroxyl termini, respectively. This was accomplished in one step using TMS triflate,[25,35] leading to the final products HO_2C-**Trip-G(E3)**-OH, HO_2C-**Trip-G(12)**-OH and HO_2C-**Trip-Hex-G(12)**-OH in overall acceptable yields.

The final compounds were easily purified by HPLC to produce high purity samples for further analysis, and they retained favourable properties throughout the syntheses, with the final, deprotected oligomers being soluble in solvents such as methanol and chloroform at millimolar concentrations. The fact that the key intermediate **6** could be easily diversified into the desired final compounds using known and highly-optimized reactions



Scheme 1 Synthesis of Trip-containing molecules. Abbreviations standard except PRE is prenyl ($-CH_2CHC(CH_3)_2$).

in a maximum of 9 steps from commercially-available starting materials is also significant.

Ion transport activity

Lipid vesicle assays

The vesicle-based HPTS assay was used to assess ion transport activity.[36] In this experiment, the pH-sensitive dye (HPTS) is entrapped in vesicles to which a putative channel-forming molecule

acid with a 12-carbon alkyl ester. The a,ω-hydroxy acids of 6 and 10 carbons are indicated as ***Hex*** and ***Dec***, respectively, while the ester of 4-carboxymethyl-4′-(hydroxymethyl)diphenylacetylene is referred to as ***Dip***. Only the final structures are named in this way.

is introduced. An aliquot of NaOH is then added to initiate a pH gradient. By monitoring the differential emission from the acid and conjugate-base forms of the dye as ions are transported across the membrane and the pH gradient is collapsed, the extent of transport by the channel forming-molecule can be obtained by procedures reported previously.[22] Apparent rate constants can then be obtained by plotting the extent of transport as a function of concentration of the assayed compound. The results of this assay for the polyether-tailed HO_2C-**Trip-G(E3)**-OH are shown in Fig. 1A and B; the compound is active at low concentrations, as above-baseline activity was observed down to 3 μM of added transporter. At concentrations above 20 μM, however, the rate appears to level off, with only minor rate increases from 20 μM to the maximum tested concentration of approximately 150 μM being observed (Fig. 1B). Fitting the concentration-rate profile to a saturation model gave a V_{max} of 0.0155 s^{-1} and a concentration at half maximal activity of 9.8 μM ($r^2 = 0.94$).

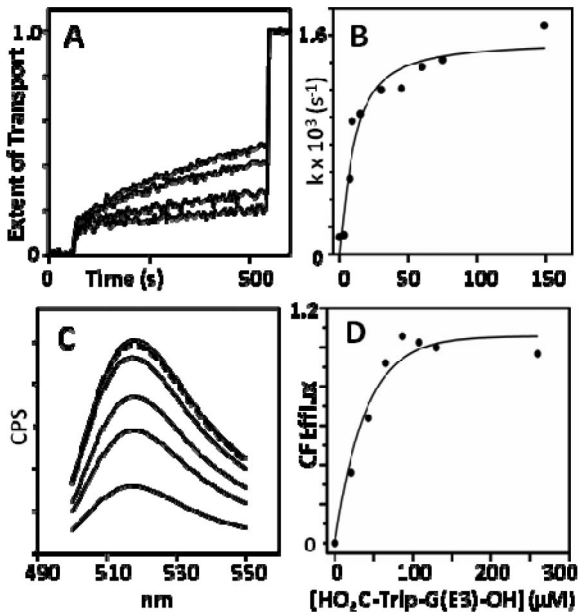


Fig. 1 Vesicle-based transport activity. HPTS (**A**, **B**) and CF (**C**, **D**) data for HO_2C-**Trip-G(E3)**-OH. Raw data from both assays is shown in panels **A** and **C**. The data were then analysed as discussed in text and SI to provide transport rate constants (HPTS assay, **B**) or extent of CF efflux (CF assay, **D**).

Other **Trip**-containing molecules were completely inactive, with rates no higher than that of the THF or MeOH controls. The activity of HO_2C-**Trip-G(E3)**-OH is modest in comparison to the previously-reported **Dip** oligomers; the maximum rate achieved is only 40% of that exhibited by HO_2C-**Dip-Hex-Hex-(G12)**-OH, the most active in the series. The supposition that rigidity leads to improved transport activity is therefore in question. The relatively low activity of the only compound uncovered among the first few examples prepared limited our enthusiasm for preparing the full range of sequence isomers.

Saturation behaviour, as shown by HO_2C-**Trip-G(E3)**-OH, has been associated with membrane-disruption by surfactants.[36] Structurally, the amphiphilic HO_2C-**Trip-G(E3)**-OH resembles a classic surfactant such as Triton X100, which solubilises membranes at or below its critical micelle concentration (cmc) of 200 μM. To test for surfactant-like activity, the carboxyfluorescein (CF) assay was therefore carried out on HO_2C-**Trip-G(E3)**-OH. This assay detects large defects in the membrane of diameters greater than 10 Å, the approximate size of CF, by measuring the extent of fluorescence enhancement as the self-quenched encapsulated dye is released and diluted in the exterior aqueous medium.[2,36] As shown in Fig. 1C and D, extensive CF efflux equal to that of Triton X100 was observed for HO_2C-**Trip-G(E3)**-OH at relatively low concentrations (~80 μM, Fig. 1D). Above 80 μM, further increases in compound concentration up to 260 μM actually reduced the extent of CF efflux, although this is most likely due to poor solubility of the **Trip** compound in aqueous solution at these high concentrations. This 'saturation' of CF efflux occurred at a similar concentration range as did the HPTS ion-transport activity, suggesting that the saturation is simply a solubility issue. Note however that CF release requires a significantly higher concentration of compound at the half-maximum (50 μM) that was required in the HPTS assay.

Planar bilayer assay

The vesicle-based transport results suggest that HO_2C-**Trip-G(E3)**-OH behaves as a membrane disruptor or surfactant in a manner that is closely akin to Triton X100. This was an intriguing observation in light of the long history of single-channel observations of Triton itself, and of other structurally-derived ion channels.[37,38,19,36] The original observation of Triton single channels reported regular, long-lived (up to a few minutes), predominantly 'square-top' type conductance behaviour with conductances ranging from 100 to 400 pS, although shorter lifetime openings and multiple-step openings were also observed;[39] other common surfactants failed to produce single channel events. Triton channels were found to be cation-selective, and had voltage-dependent conductance under certain conditions. Despite reports from other groups that support the existence of Triton single-channels,[40,41] they remain elusive due to their poor reproducibility and the unconventional methods used to introduce Triton to the bilayer membrane.[18,29]

Examples of bilayer activity observed for HO_2C-**Trip-G(E3)**-OH are presented in Fig. 2; other **Trip**-containing molecules were completely inactive by this transport assay as well. HO_2C-**Trip-G(E3)**-OH exhibits a variety of behaviours ranging from short-lived, highly-conducting spikes through irregular yet longer-lived openings to almost idealized square-tops of fairly low conductance (~30 pS for the trace in Fig. 2A). As shown in Fig. 2B, multiple behaviours were usually observed in a single trace over a short period of time, indicating a variety of active structures. Openings varied greatly in both lifetime and conductance, and occurred in approximately equivalent proportions in each tested electrolyte (KCl, CsCl, NMe_4Cl). The bilayer results thus reinforce the similarity to Triton, and that HO_2C-**Trip-G(E3)**-OH can act in a dual role as both a surfactant and a discrete ion channel at low concentrations.

The observed behaviours were further analysed by a procedure recently developed in our lab,[42] the results of which are shown in the bottom panel of Fig. 2. Each type of conductance activity is assigned a corresponding colour (green for square-top, yellow for flickers, blue for multiple opening, red for spikes, purple for erratic), which is plotted on an activity grid. The horizontal axis

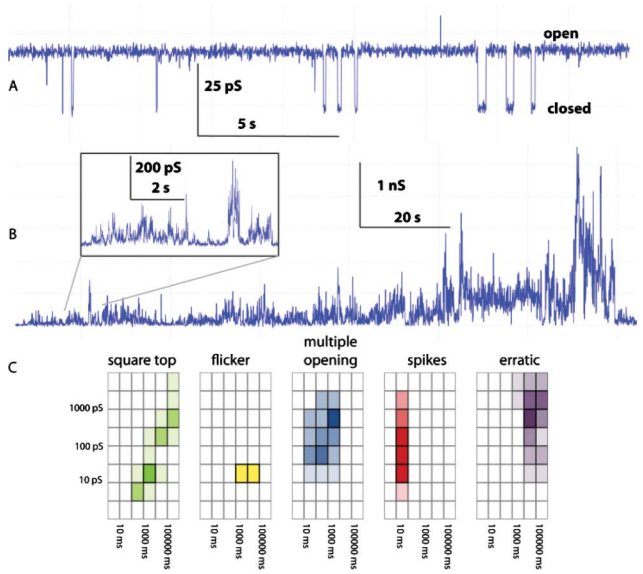


Fig. 2 Voltage clamp activity of HO_2C-**Trip-G(E3)**-OH. *Conditions*: **A**: diPhyPC, 1 M CsCl, MeOH solution added to a bulk concentration of 0.5 μM, + 150 mV, 5 min following addition. **B**: diPhyPC, 1 M KCl, concentration as **A**, +100 mV, 1 h following addition. **C**: cumulative activity grids, see text.

represents the event lifetime (on a log scale from 10 ms to 100 s), while conductance (in half log unit steps from 3 to 3000 pS) is on the vertical axis. Every experimental record was analysed and the activities were summed to produce the grids shown. The color intensity reflects the frequency of a particular conductance-duration within a type of conductance behavior. The dominant activity is of the multiple-opening type (40% of all records contain this activity). Other prevalent activities are spikes and erratic behaviours (25% each). Only 10% of the observations were of the square-top type shown in Fig. 2A and flicker activity was only observed on a single occasion. Although statistically unfounded, the summary grids further confirm the strong similarity of HO_2C-**Trip-G(E3)**-OH and Triton.[42]

Consistently high conductances are produced by HO_2C-**Trip-G(E3)**-OH. In 75% of collected traces, conductances of over 100 pS were predominant, regardless of the type of activity observed, and in over 20% of total experiments, the predominant behaviour showed conductances greater than 1 nS. Occasionally, truly giant pores corresponding to Hille diameters[43] greater than 10 Å were observed. These frequent large openings are due to channel formation, and not membrane rupture, as direct detergent action on the bilayer was rarely observed during the bilayer experiments. These types of very large pores are rare in the synthetic ion channel literature; some Triton openings are of high conductance,[42,44] but these do not occur with the **Dip** oligoesters,[25] suggesting that the increased rigidity of the **Trip** scaffold may be able to better stabilize these larger channels.

Dissymmetric oligoesters potentially could give rise to voltage-dependent conductance, but this has not been observed for any previous compound of this class.[45,25] As noted above, large long-lived openings, sometimes of the square-top type, are formed by HO_2C-**Trip-G(E3)**-OH and it was therefore possible to alter the applied potential during the period of a single opening. A non-rectifying channel would show a constant conductance; Fig. 3

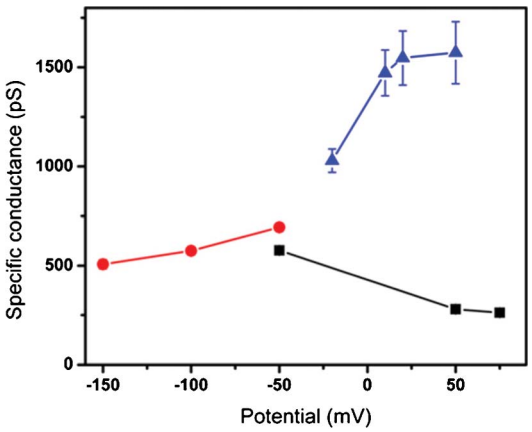


Fig. 3 Conductance *versus* potential for HO_2C-**Trip-G(E3)**-OH (1 M NMe_4Cl, diPhyPC) determined within the lifetime of a single opening event. The three segments occurred in three separate experiments over a span of 10 min.

shows three sections of the same experiment separated by about 10 min in which the conductance of these openings is clearly a function of applied potential. Equally clearly there are dynamic processes that alter the pore during the time of the opening such that the magnitude and sign of the voltage dependence varies. The Hille diameters in these cases range from 0.4 to 1.2 nm. The asymmetry of the conductance behaviour implies overall asymmetry of the conducting pore. The same must be true of the channels formed by Triton X100, which are sometimes reported to exhibit a voltage-dependent response, although this was not conclusively observed by all authors.[42]

Fluorescence studies

Fluorescence in solution

Previous studies on the **Dip** oligoesters exploited their environment-sensitive excimer fluorescence to track the partitioning of the molecules from an aqueous solution into the vesicle membrane. In water the compounds emit at 380 nm from an excimer state; the introduction of lipid vesicles shifts this to the 'monomer' emission at 320 nm. Over time, the recovery of the 380 nm emission suggests the formation of in-membrane aggregates.[25]

The low fluorescence intensity of the **Dip** moiety and its small (~15 nm in MeOH) Stokes shift prompted the development of the **Trip** fluorophore; it was therefore satisfying to observe that in organic solutions such as THF or methanol, the **Trip** compounds exhibit fluorescence intensity enhanced by at least an order of magnitude over that seen for the **Dip** oligoesters (Fig. 4). Significant fluorescence signal can still be observed at sub-micromolar concentrations, below the detectable limit for the **Dip** molecules. The absorption, excitation and emission spectra of the **Trip**-containing compounds are all significantly red-shifted in comparison to the **Dip** moiety as well. Quenching studies such as those reported for the **Dip** molecules were also carried out, and the **Trip** fluorophore can be quenched by $CuSO_4$ in methanolic solution, yielding derived Stern–Volmer constants of approximately 230 M^{-1} (Table 1). The **Trip** molecules are also

Table 1 Comparison of representative photophysical parameters of the Dip and Trip fluorophores in methanolic solution

Property	Dip[a]	Trip[b]
ε ($M^{-1}cm^{-1}$)	20 000	90 000
Absorption (λ_{max}, nm)	289	313
Excitation (λ_{max}, nm)	305	325
Emission (λ_{max}, nm)	320	365
$CuSO_4$ quenched/K_{SV}	Yes/1000 M^{-1}	Yes/230 M^{-1}

[a] 16 μM HO_2C-**Dip-Hex-Hex-(G12)**-OH[25] [b] 16 μM HO_2C-**Trip-G(E3)**-OH

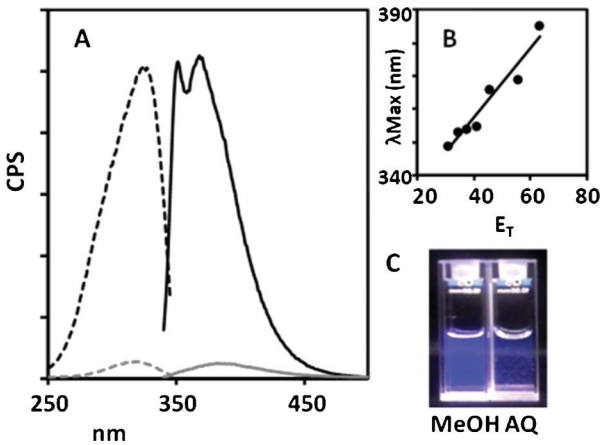


Fig. 4 **A:** Excitation (dashed lines) or emission (solid lines) spectra of 14 μM HO_2C-**Trip-G(E3)**-OH in CH_3OH (black line) or aqueous buffer (10 mM Na_3PO_4, 100 mM NaCl, pH = 6.4) (grey line). **B**: Emission wavelength maximum as a function of solvent polarity in a variety of tested solvents. **C**: photograph under UV light of solutions shown in **A**.

visibly fluorescent at low micromolar concentrations in MeOH under UV illumination (Fig. 4C).

While the Trip fluorophore exhibited an intense emission in organic solution, this was much reduced in water, as clearly demonstrated in Fig. 4A and C. The fluorescence observed in aqueous solution is also red-shifted in comparison to that present in MeOH, shifting by 15 nm for HO_2C-**Trip-G(E3)**-OH, and nearly 40 nm for the longer trimer HO_2C-**Trip-Hex-G(12)**-OH. These characteristics are immediately suggestive of excimer emission, analogous to that observed for the **Dip** isomers; however, the extremely low intensity of this emission makes it of little practical utility. More interestingly, the **Trip** compounds exhibit solvatochromism that can be roughly correlated ($r^2 = 0.94$) to the polarity of the solvent, based on the E_T scale[46] (Fig. 4B).

The low fluorescence intensity of the **Trip** molecules in aqueous solution was not completely unexpected as they were discovered to visibly aggregate at fairly low concentrations; aggregation-induced self-quenching[47] is therefore a reasonable possibility. The extent of aqueous aggregation is commonly assessed by the pyrene assay.[48,49] This was carried out for both the fully-saturated and **Dip** oligoesters, yielding similar apparent cmc values of approximately 20 μM. The **Trip** molecules cannot be assayed in this manner due to their fluorescence overlapping that of pyrene, however, an apparent cmc of approximately 5 μM was obtained for HO_2C-**Trip-G(E3)**-OH from the point at which a concentration-intensity plot deviated from linearity (data in the SI).[50] The extent of aggregation of the more hydrophobic **Trip** compounds was yet more difficult to assess due to their extremely low aqueous fluorescence. However, even at these very low intensities, the fluorescence response was clearly not linear with concentration, and the maximum intensity was reached at concentrations as low as 3 μM; they are evidently far less soluble than the less-rigid precursors.

Besides aggregation-induced self-quenching, another possible explanation for these molecules' very low fluorescence in aqueous solution is that water actually quenches the **Trip** fluorophore by some specific solvent effect[47] as has been postulated for a number of known membrane probes.[51–53] To determine whether this was indeed a factor, a Stern–Volmer type experiment in which a solution of compound **6** in MeOH was titrated against increasing concentrations of aqueous buffer was carried out. Selected scans are shown in Fig. 5A, while 5B illustrates a plot of the fluorescence intensity as a function of water concentration. It is evident from the data that any water quenching does not follow a linear relationship. Rather, the observed behaviours are much more consistent with an aggregation process as the fluorescence intensity remains unchanged up to 40% water (22 M), and then suddenly decreases by half at 45% water (24.8 M). Therefore, it appears that above some critical concentration of water (24.5 M, as determined from a logistic fit of the data) the **Trip**-containing compound collapses into an aggregate, resulting in a dramatic fluorescence decrease. The nature of the aggregate is not known but it must have significant stability to form in the presence of a significant concentration of methanol.

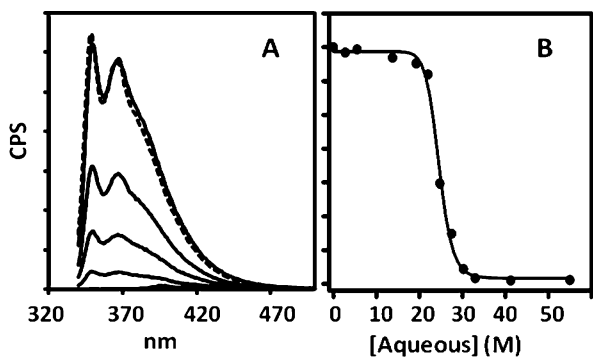


Fig. 5 **A**: Fluorescence emission spectra (Ex~325 nm) of 14 μM **6** in aqueous buffer/MeOH mixtures; from top to bottom, solid lines = [buffer] of 22, 24.8, 27.5, 30.3 and 41.3 M. Dashed line = 100% MeOH. **B**: fluorescence intensity as a function of water concentration, fit to a logistic function, $r^2 > 0.99$.

A linear Stern–Volmer relationship was observed when $CuSO_4$ was used as quencher. In analogy to the **Dip** molecules, the quenching observed for the **Trip** fluorophore in aqueous solution was significantly more efficient than in MeOH, with K_{SV} values in water approximately four-fold higher (1000 M^{-1}, see the ESI for spectra†). The fact that quenching by copper ion was observed in both media for the **Trip** fluorophore indicated that analogous experiments as carried out for the **Dip** compounds, in particular quenching in the presence of vesicles, could be conducted on these molecules as well.

Membrane localization

When a suspension of lipid vesicles was introduced into an aqueous solution of HO_2C-**Trip-G(E3)**-OH, the fluorescence

intensity rapidly increased by approximately 2-fold, as shown in Fig. 6A. This rapid increase in intensity is analogous to that seen for the **Dip** compounds when interacting with vesicles, and was previously used to monitor bilayer partitioning.[25] As the fluorescence intensity of the **Trip** molecules is much higher in less-polar media, this suggests that bilayer partitioning is also being observed by the experiment in Fig. 6A. Significantly, the response displayed by the **Trip** isomers, which levels off after a few minutes, is much faster than that observed for the **Dip** oligomers, which continued to exhibit intensity changes for periods of time up to 1 h. Further indication that the **Trip** molecule is associated with the bilayer comes from the fact that the quenching seen with externally-added aqueous copper is decreased by over 50% when the compound is incubated with vesicles (see ESI†).

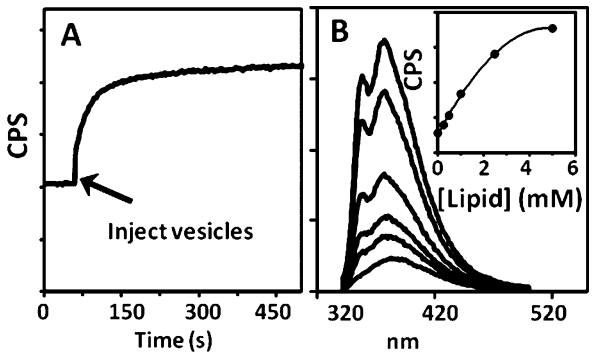


Fig. 6 **A**: Emission over time of an aqueous solution of 16 μM HO_2C-**Trip-G(E3)**-OH to which 0.5 mM lipid (as vesicles) has been added at the time indicated. **B**: Static emission spectra of 16 μM HO_2C-**Trip-G(E3)**-OH at varying lipid concentrations. From bottom to top, lipid concentrations = 0, 0.25, 0.5, 1, 2.5, 5 mM. Inset: Plot of CPS *versus* lipid concentration, saturation not reached, K_p not determined. The line is to guide the eye. Ex = 325 nm.

Attempts to quantify the extent of partitioning are shown in Fig. 6B; in this experiment, a constant concentration of HO_2C-**Trip-G(E3)**-OH was titrated against increasing concentrations of lipid vesicles (0–5 mM). This procedure has been used by others to obtain a partitioning constant (K_p)[51,54,55] however, in order for this to occur, the maximal response upon complete partitioning must be known. A K_p could not be obtained for HO_2C-**Trip-G(E3)**-OH (inset to Fig. 6B), as saturation was not reached at the assayed lipid concentrations, and further increases were precluded by the high turbidity of the suspension above these concentrations. Despite this, the results are significant as they give at least a rough indication of the affinity of this compound for the membrane. It is clearly modest in comparison to that of known membrane probes such as DPH (diphenylhexatriene), which exhibits lipid EC_{50} values (indicating the lipid concentration needed to incorporate 50% of the probe molecule) of 43 μM.[54] HO_2C-**Trip-G(E3)**-OH is not fully partitioned even at 5 mM of lipid. Therefore, despite its transport activity, the efficiency of partitioning for the **Trip** molecule is poor.

This poor partitioning has consequences when considering the vesicle-based activity assays discussed above (Fig. 1). In these assays, the concentration of lipid as vesicles is kept constant at 0.5 mM, a concentration at which the compound is incompletely partitioned. Similar incomplete partitioning has been recently observed for a series of fluorescently-labelled peptidic ion channels reported by You and Gokel; these authors suggest that the ion transport activity observed for these compounds is only a fraction of what could be possible.[21]

Polarity in vesicles

The titration results in Fig. 6B reveal a shift of the emission maxima from that initially seen in water (385 nm) to 375 nm, and the growth of a new band at 356 nm. A blue-shifting of emission maximum upon vesicle introduction is a common occurrence for many polarity-sensitive fluorophores, and has been used to probe the polarity of the bilayer environment,[47,56] and the localization of synthetic ion channels.

The membrane localization behaviours of the **Trip** molecules were determined for the polyether-tailed HO_2C-**Trip-G(E3)**-OH as well as the longer trimer HO_2C-**Trip-Hex-G(12)**-OH, as both compounds exhibited a linear E_T-wavelength dependence (Fig. 4 and 7).

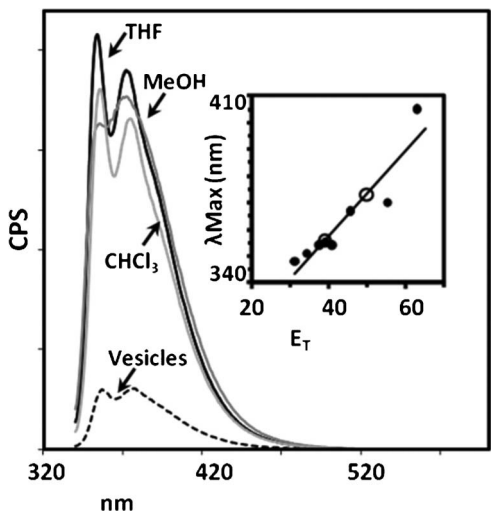


Fig. 7 Solvent effects for HO_2C-**Trip-Hex-G(12)**-OH. Fluorescence emission spectra (Ex~325 nm) of 17 μM compound in selected solvents (solid lines), and pre-loaded into vesicles at *ca.* 3.5 μM (dashed line). INSET: λMax as a function of solvent polarity. Black circles = tested solvents, open circles = fit of vesicle wavelengths onto linear relationship.

While HO_2C-**Trip-G(E3)**-OH was simply incubated with vesicles for a short period of time, the more hydrophobic trimer was pre-incorporated into the lipid, as it does not freely partition from solution. Once the two maxima were compared to the linear emission-polarity relationships, they corresponded to derived in-vesicle E_T values of 39 and 50 for HO_2C-**Trip-Hex-G(12)**-OH (Fig. 7). This compound showed two equivalent maxima, possibly indicating that the **Trip** moiety is acting as two separate fluorophores that probe different membrane environments. This is plausible as the length of the **Trip** scaffold from carboxyl terminus to hydroxyl terminus is approximately 20 Å, so it can clearly extend through different regions of the bilayer.

The E_T derived polarity for HO_2C-**Trip-G(E3)**-OH is very high (E_T = 57), comparable to or more polar than MeOH (E_T = 55.4). A number of conformations could account for this high value; for example this could indicate that the compound is protruding into the phosphocholine head group region. Another possibility open to HO_2C-**Trip-G(E3)**-OH is that the high polarity E_T value

is reporting an aqueous aggregate, as the fluorescence intensity of this molecule in aqueous solution is relatively high in comparison to the other, more hydrophobic analogs, and the blue-shift from aqueous to in-vesicle of 10 nm is not as significant as that seen for HO_2C-**Trip-Hex-G(12)**-OH (up to 49 nm). The increasing intensity of the band at 356 nm, which only appears in the presence of vesicles and corresponds to a low-polarity E_T value of 39, must be tracking the actual membrane-inserted species (Fig. 6B). Significantly, the presence and intensity of this band appears to be *inversely correlated* with transport activity.

Conclusions

A series of triphenyldiynes based on a highly fluorescent **Trip** chromophore was synthesized and characterized. While their ion transport activity in vesicle assays was modest, the active compound, HO_2C-**Trip-G(E3)**-OH, exhibited a 'dual role' as both a membrane-disrupting agent as well as a discrete ion channel, similar to the reported activity of other 'Triton-like' molecules.

Based on the functional similarities between HO_2C-**Trip-G(E3)**-OH and the Triton channels, it is tempting to extrapolate to a common mechanism based on the additional environmental information provided by the compounds prepared. The following structural constraints appear to be established: the **Trip** unit of HO_2C-**Trip-G(E3)**-OH is in a relatively polar but membrane-associated environment, possibly as a surface-exposed structure, while the **Trip** unit of HO_2C-**Trip-Hex-G(12)**-OH resides in a significantly less polar environment. The inactive compounds are thus inserted into the membrane but are unable to form productive channels. The partitioning of HO_2C-**Trip-G(E3)-OH** is relatively poor, yet the channels form easily and reliably. The channels once formed are highly conductive in most cases, and can show asymmetry. Taken together, these results suggest the insertion of the **Trip** occurs from one side of the bilayer. Since it is unlikely that the glycol portion directs entry, and it is also unlikely that the carboxylate leads the insertion, a U-shaped insertion seems most probable and most consistent with the data. The aggregation of these U-insertions in one leaflet, followed by flip-flop to the other would build a structure of the right size. It could also lead to the observed, but irregular asymmetry of the conductance-potential relationship as different numbers of molecules would be engaged at different times. Triton is more flexible than HO_2C-**Trip-G(E3)**-OH, but the same sequence of events could lead to similar conducting structures.

Experimental

General procedures

Most chemicals and solvents were used as received from known suppliers, except THF which was dried and distilled before use. NMR spectra were collected on at 300 MHz or 500 MHz (protons). UV spectra were run on a Cary 5 UV-VIS spectrometer in a 10 × 10 mm quartz cell. ESI Mass spectra were recorded on a Waters MicroMass Q-TOF instrument running in negative ion mode; **Trip**-containing compounds aggregate in solution so spectra frequently contain intermolecular fragment-transfer (M+**Trip**) and fragment (M–**Trip**) ions in addition to the molecular ion. HPLC was performed using an HP Series 1100 instrument, with either a Macherey–Nagel "Nucleosil" RP C18 analytical (4 mm × 250 mm) or a Grace Davison "Alltima" RP C18 semi-prep (10 mm × 150 mm) column. Solvents used (ACN, CH_3OH; HPLC-grade) were filtered through a Millipore sub-micrometre filter before use. HPLC elution was monitored at various UV wavelengths (typically 254, 280 and 220 nm) and fluorometrically. Fluorescence spectra were run on a PTI QM-2 instrument at T = 20 °C in either 10 × 10 mm quartz cells equipped with a micro stir rod, or 1 × 10 mm quartz cells (pyrene, CF assay). The syntheses followed directly from previously-reported procedures.[25]

Sonogashira coupling:[32] To a round bottomed flask equipped with a septum, 1.2–1.5 equivalents (in relation to the alkyne starting material) of the halogen-containing reactant and 6–10% CuI were dissolved in dry solvent (typically DMF or THF), which was then deoxygenated under vacuum. The alkyne reactant, 3–5% $Pd(PPh_3)_4$, and 2–5 equivalents of NEt_3 were then sequentially added. The reaction was then stirred in the dark under N_2 at temperatures ranging from rt to 70 °C. Reactions were monitored by TLC (silica gel, EtOAc/hexanes as eluent, visualized by UV, *p*-anisaldehyde and/or vanillin stain). Once complete, reactions were cooled if necessary, diluted with EtOAc and washed with saturated EDTA (2–3 times), H_2O (once), saturated NaCl (once), dried over sodium sulfate, and concentrated under vacuum. Unless noted otherwise, the crude products were purified by column chromatography on silica gel, typically using EtOAc/hexanes as eluent.

Ester coupling:[25] To a solution of 1.3–2 equivalents of either the alcohol or acid building block in relation to 1 equivalent of the other (excess reagent choice determined by ease of synthesis or availability) in dry solvent (typically THF) were added 1.3–2 equivalents of DIC, HOBt and 2.6–4 equivalents of DIPEA. The reaction was sealed under an atmosphere of N_2, and stirred in the dark at temperatures varying between rt and 50 °C. Reaction completion was monitored by TLC. Once complete, the reaction was cooled (if necessary), filtered to remove DIU, and diluted with DCM or EtOAc. The organic phase was extracted with H_2O (twice), saturated $NaHCO_3$ (twice), rinsed with sat. NaCl (once), dried with anhydrous sodium sulfate, and concentrated under vacuum. Unless noted otherwise, the crude product was purified by column chromatography on silica gel, typically using EtOAc/hexanes as eluent.

THP removal: pTsOH (5–25%) was added to a solution of compound in ~10–30% CH_3OH: DCM, which was stirred at rt for 1–3 h, as monitored by TLC. Once complete, the reaction was diluted with DCM, washed with H_2O (once), saturated $NaHCO_3$ (twice), and saturated NaCl (once), then dried over sodium sulfate and concentrated under vacuum. If necessary, further purification was carried out as noted.

Prenyl deprotection: Adapted:[35] 0.025–0.1 equivalents TMSOTf were added to the compound dissolved in DCM, which was stirred at rt. Once complete (as monitored by TLC, generally < 1 h), the reaction mixture was diluted further into DCM, washed with H_2O, saturated $NaHCO_3$ and saturated NaCl, then dried over sodium sulfate and concentrated under vacuum. Further purification was carried out as noted. Full spectroscopic and characterization data for all new compounds is available in the ESI.

Transport assays: the bilayer clamp,[57,58] HPTS,[22] CF,[59] pyrene, quenching and partitioning assays, as well as vesicle preparation[25] have all been reported previously, details of the minor

modifications utilized are available in the ESI. Bilayer clamp experiments conducted: diPhyPC, 1 M electrolyte (KCl, CsCl, NMe_4Cl), 5 μL of a 1 mM MeOH solution introduced to 5 mL of electrolyte (0.5 μM bulk aqueous concentration), potentials between 150 and −150 mV, 2–5 h of observations in replicate for each electrolyte. Full trace summary information is available in the ESI.†

Acknowledgements

The ongoing support of the Natural Science and Engineering Research Council of Canada and that of the Nora and Mark DeGoutiere Memorial foundation (JMM) is gratefully acknowledged.

References

1 T. M. Fyles, *Chem. Soc. Rev.*, 2007, **36**, 335–347.
2 J. T. Davis, O. Okunolaa and R. Quesada, *Chem. Soc. Rev.*, 2010, **39**, 3843–3862.
3 G. W. Gokel and A. Mukhopadhyay, *Chem. Soc. Rev.*, 2001, **30**, 274–286.
4 S. Matile, A. V. Jentzsch, J. Montenegro and A. Fin, *Chem. Soc. Rev.*, 2011, **40**, 2453–2474.
5 S. Matile, S. A. Abhigyan and N. Sorde, *Tetrahedron*, 2004, **60**, 6405–6435.
6 B. A. Mcnally, M. W. Leevy and B. D. Smith, *Supramol. Chem.*, 2007, **19**(1–2), 29–37.
7 G. Gokel and I. A. Carasel, *Chem. Soc. Rev.*, 2007, **36**, 378–389.
8 W. M. Leevy, M. E. Weber, P. H. Schlesinger and G. W. Gokel, *Chem. Commun.*, 2005, 89–91.
9 T. Renkes, H. J. Schafer, P. M. Siemens and E. Neumann, *Mater. Sci. Eng., C*, 2002, **22**, 275–275.
10 X. Li, B. Shen, X.-Q. Yao and D. Yang, *J. Am. Chem. Soc.*, 2009, **131**, 13676–13680.
11 S. Matile, H. Tanaka and S. Litvinchuk, *Top. Curr. Chem.*, 2007, **277**, 219–250.
12 T. M. Fyles, D. Loock and X. Zhou, *J. Am. Chem. Soc.*, 1998, **120**(13), 2997–3003.
13 C. Goto, M. Yamamura, A. Satake and Y. Kobuke, *J. Am. Chem. Soc.*, 2001, **123**, 12152–12159.
14 Y. Kobuke and T. J. Nagatani, *J. Org. Chem.*, 2001, **66**, 5094–5101.
15 P. V. Jog and M. S. Gin, *Org. Lett.*, 2008, **10**(17), 3693–3696.
16 S. Litvinchuk, H. Tanaka, T. Miyatake, D. Pasini, T. Tanaka, G. Bollot, J. Mareda and S. Matile, *Nat. Mater.*, 2007, **6**, 576–580.
17 T. M. Fyles, R. Knoy, K. Mullen and M. Sieffert, *Langmuir*, 2001, **17**, 6669–6674.
18 W. Wang, R. Li and G. W. Gokel, *Chem. Commun.*, 2009, 911–913.
19 T. Renkes, H. J. Schafer, P. M. Siemens and E. Neumann, *Angew. Chem., Int. Ed.*, 2000, **39**(14), 2512–2515.
20 E. Abel, G. E. Maguire, O. Murillo, I. Suzuki, S. L. De Wall and G. W. Gokel, *J. Am. Chem. Soc.*, 1999, **121**(39), 9043–9052.
21 L. You and G. W. Gokel, *Chem.–Eur. J.*, 2008, **14**, 5861–5870.
22 T. M. Fyles and H. Luong, *Org. Biomol. Chem.*, 2009, **7**, 733–738.
23 T. M. Fyles and H. Luong, *Org. Biomol. Chem.*, 2009, **7**, 725–732.
24 T. M. Fyles, C.-W. Hu and H. Luong, *J. Org. Chem.*, 2006, **71**, 8545–8551.
25 J. M. Moszynski and T. M. Fyles, *Org. Biomol. Chem.*, 2010, **8**, 5139–5149.
26 B. Brocklehurst, D. C. Bull, M. Evans, P. M. Scott and G. Stanney, *J. Am. Chem. Soc.*, 1975, **97**(11), 2977–2978.
27 Y. Hirata, *Bull. Chem. Soc. Jpn.*, 1999, **72**, 1647–1664.
28 R. L. Letsinger, T. Wu, J.-S. Yang and F. D. Lewis, *Photochem. Photobiol. Sci.*, 2008, **7**, 854–859.
29 W. Wang, L. Ruiqiong and G. W. Gokel, *Chem.–Eur. J.*, 2009, **15**, 10543–10553.
30 A. P. Demchenko, *Introduction to Fluorescence Sensing*; Springer: Berlin, 2009.
31 P. F. Schwab, M. D. Levin and J. Michl, *Chem. Rev.*, 1999, **99**(7), 1863–1934.
32 K. Sonogashira, Y. Tohda and N. Hagihara, *Tetrahedron Lett.*, 1975, **16**, 4467–4470.
33 R. Chinchilla and C. Najera, *Chem. Rev.*, 2007, **107**, 874–922.
34 A. Moroda and H. Togo, *Tetrahedron*, 2006, **62**, 12408–12414.
35 M. Nishizawa, K. Yamamoto, H. Seo, H. Imagawa and T. Sugihara, *Org. Lett.*, 2002, **4**, 1947–1949.
36 S. Matile and N. Sakai, The Characterization of Synthetic Ion Channels and Pores. In *Analytical Methods in Supramolecular Chemistry*; C. A. Schalley, Ed.; Wiley-VCH: Weinheim, 2007; pp 391–418.
37 K. S. Iqbal, M. C. Allen, F. Fucassi and P. J. Cragg, *Chem. Commun.*, 2007, 3951–3953.
38 O. Lawal, K. Iqbal, A. Mohamadi, P. Razavi, H. T. Dodd, M. C. Allen, S. Siddiqui, F. Fucassi and P. J. Cragg, *Supramol. Chem.*, 2009, **21**(1–2), 55–60.
39 P. Schlieper and E. De Robertis, *Arch. Biochem. Biophys.*, 1977, **184**, 204–208.
40 T. K. Rostovtseva, C. L. Bashford, A. A. Lev and C. A. Pasternak, *Journal of Membrane Biology*, 1994, **141**, 83–90.
41 G. M. Alder, W. M. Arnold, C. L. Bashford, A. F. Drake, C. A. Pasternak and U. Zimmerman, *Biochim. Biophys. Acta, Biomembr.*, 1991, **1061**, 111–120.
42 J. K. Chui and T. M. Fyles, *Chem. Soc. Rev.*, 2011, DOI: 10.1039/c1cs15099e.
43 B. Hille, *Ion Channels of Excitable Membranes*, 3rd ed.; Sinauer Associates: USA, 2001.
44 L. Ma, W. A. Harrell and J. T. Davis, *Org. Lett.*, 2009, **11**(7), 1599–1602.
45 H. Luong, *Towards Voltage-Gated Ion Channels Synthesized by Solid Phase Organic Synthesis*; Doctoral Dissertation; University of Victoria: Victoria, 2008.
46 C. Reichardt, *Chem. Rev.*, 1994, **94**, 2319–2358.
47 J. R. Lakowicz, Ed. *Principles of Fluorescence Spectroscopy*, 3rd ed.; Springer: NY USA, 2006.
48 K. Kalyanasundaram and J. K. Thomas, *J. Am. Chem. Soc.*, 1977, **99**, 2039–2044.
49 C. Yihwa, M. Kellermann, M. Becherer, A. Hirsch and C. Bohne, *Photochem. Photobiol. Sci.*, 2007, **6**, 525–531.
50 R. M. Cardoso, H. A. Filipe, F. Gomes, N. D. Moriera, W. L. Vaz and M. J. Moreno, *J. Phys. Chem. B*, 2010, **114**, 16337–16346.
51 A. Kyrychenko, F. Wu, R. P. Thummel, J. Waluk and A. S. Ladokhin, *J. Phys. Chem. B*, 2010, **114**, 13574–13584.
52 V. Y. Postupalenko, V. V. Shvadchak, G. Duportail, V. G. Pivovarenko, A. S. Klymchenko and Y. Mely, *Biochim. Biophys. Acta, Biomembr.*, 2011, **1808**, 424–432.
53 E. Perochon, A. Lopez and J. F. Tocanne, *Biochemistry*, 1992, **31**, 7672–7682.
54 Z. Huang and R. P. Haugland, *Biochem. Biophys. Res. Commun.*, 1991, **181**(1), 166–171.
55 S. H. White, W. C. Wimley, A. S. Ladokhin and K. Hristova, *Methods Enzymol.*, 1998, **295**, 62–87.
56 L. Davenport, *Methods Enzymol.*, 1997, **278**, 487–512.
57 M. B. Buchmann, T. M. Fyles and T. Sutherland, *Bioorg. Med. Chem.*, 2004, **12**, 1315–1324.
58 P. K. Eggers, T. M. Fyles, K. D. Mitchell and T. Sutherland, *J. Org. Chem.*, 2003, **68**, 1050–1058.
59 T. M. Fyles and C. Hu, *J. Supramol. Chem.*, 2001, **1**, 207–215.