Alkyl vs Aryl Modifications: A Comparative Study on Modular Modifications of Triphenylphosphonium Mitochondrial Vectors

How Chee Ong, João T.S. Coimbra, Maria João Ramos, Bengang Xing, Pedro Alexandrino Fernandes, Felipe Garcia

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Triphenylphosphonium (TPP+) moieties are commonly conjugated to drug molecules to confer mitochondrial selectivity due to their positive charge and high lipophilicity. Although optimisation of lipophilicity can be achieved by modifying the length of the alkyl linkers between the TPP+ moiety and the drug molecule, it is not always possible. While methylation of the TPP+ moiety is a viable alternative to increase lipophilicity and mitochondrial accumulation, there are no studies comparing these two separate modular approaches. Thus, we have systematically designed, synthesised and tested a range of TPP+ molecules with varying alkyl chain lengths and degree of aryl methylation to compare the two modular methodologies for modulating lipophilicity. The ability of aryl/alkyl modified TPP+ to deliver cargo to the mitochondria was also evaluated by confocal imaging with a TPP+-conjugated fluorescein-based fluorophore. Furthermore, we have employed molecular dynamics simulations to understand the translocation of these molecules through biological membrane model systems. These results provides further insights into the thermodynamics of this process and the effect of alkyl and aryl modular modifications.
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Abstract: Triphenylphosphonium (TPP+) moieties are commonly conjugated to drug molecules to confer mitochondrial selectivity due to their positive charge and high lipophilicity. Although optimisation of lipophilicity can be achieved by modifying the length of the alkyl linkers between the TPP+ moiety and the drug molecule, it is not always possible. While methylation of the TPP+ moiety is a viable alternative to increase lipophilicity and mitochondrial accumulation, there are no studies comparing these two separate modular approaches. Thus, we have systematically designed, synthesised and tested a range of TPP+ molecules with varying alkyl chain lengths and degree of aryl methylation to compare the two modular methodologies for modulating lipophilicity. The ability of aryl/alkyl modified TPP+ to deliver cargo to the mitochondria was also evaluated by confocal imaging with a TPP+-conjugated fluorescein-based fluorophore. Furthermore, we have employed molecular dynamics simulations to understand the translocation of these molecules through biological membrane model systems. These results provide further insights into the thermodynamics of this process and the effect of alkyl and aryl modular modifications.

Introduction

The triphenylphosphonium (TPP+) moiety has drawn widespread interest as a molecular vector for selective mitochondrial delivery owing to its high lipophilicity, cationic nature, high stability in physiological conditions and its ease of conjugation to molecular cargo. The positive charge on the TPP+ moiety allows for the accumulation within the mitochondrial matrix (100-1000x for a monocation) due to the high mitochondrial membrane potential (Δψ, ca. 180 mV) in accordance with the Nernst equation. The TPP+ platform has been demonstrated to be highly versatile, and has been employed for the delivery of a wide range of cargo, such as spin traps, antioxidants, prodrugs, protonophores, fluorophores, photodynamic therapy sensitisers as well as positron emitters into the mitochondria. With mitochondria dysfunction being inextricably linked to a myriad of diseases from neurodegenerative and cardiovascular diseases to cancer, the ability to deliver compounds to mitochondria is vital for the development of novel therapeutics. Thus, this work focuses on improving the efficacy of the TPP+ vector for enhanced mitochondrial delivery.

An effective strategy for enhancing mitochondrial uptake has been focused around tuning the molecule’s lipophilicity, which has been demonstrated to be highly relevant for TPP+-conjugated systems. The typical strategy for enhancing lipophilicity for TPP+-conjugated compounds is by selecting a molecular fragment (module) with a higher lipophilicity as the linker – usually a longer alkyl chain – between the TPP+ moiety and the cargo, which increases the rate of membrane permeation and hence the mitochondrial accumulation. However, chain length modulation has its limitations. It may be more challenging to modify linkers with a specific function, such as cleavable linkers used for drug release purposes, where modification may lead to alteration of function. Aryl methylation thus presents itself as an attractive alternative when linker modification is not synthetically viable or when it affects linker functionalities. Furthermore, it enables a modular approach for the synthesis of conjugated cargo with variable lipophilicity by varying the starting phosphine. It has also been demonstrated that aryl methylation of TPP+ can improve mitochondrial uptake and lipophilicity, in a similar manner to chain length modification. While judicious alkylation of the phenyl rings in TPP+ moieties has been shown to be an effective method to enhance lipophilicity in both mono-TPP+ and bis-TPP+, no systematic comparison between these two modular approaches has been reported in the literature. A comparative study between the two methodologies was thus needed to evaluate the mitochondrial delivery efficacies of the two series of phosphonium salts.

Herein we report the first comparative analysis of alkyl vs aryl modular modification effects on mitochondrial uptake for triphenylphosphonium moieties (Figure 1). For this purpose, we

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Results and discussion

Synthesis and characterisation of alkyltriphenylphosphonium salts.

The choice of substituted triarylphosphines was based on our previously reported work. The selected alkylated aryl groups (p-tolyl and 3,5-dimethylphenyl) as well as three alkyl groups of different lengths (ethyl, pentyl and octyl) were chosen as they exhibited the largest increase in mitochondrial uptake among mono- and dimethylated phosphonium species.\(^2^\)\(^1\),\(^2\)\(^2\)\(^1\)\(^\text{The length of alkyl chain was chosen such that any increase in aryl substitution can be matched by an identical increase in alkyl chain length based on its total atom count (i.e., structural isomerism). As such, an increase in aryl substitution can be matched by an equivalent increase in alkyl chain length (i.e., 1b/2a, 1c/2b/3a, 2c/3b are isomers one another). The synthesis and characterisation for compounds 1a – 1c was previously reported,\(^2\)\(^2\)\(^\text{whereas compounds 2a – 3c were obtained in good yields (75% - 99%) upon reacting triarylphosphines with the respective alkyl bromides in acetonitrile or toluene under reflux (Scheme 1). The compounds were characterised by \(^1\)H, \(^3\)P\(^{\text{1}}\)(H) and \(^13\)C\(^{\text{1}}\)(H) NMR spectroscopy, high-resolution mass spectrometry (HRMS), and single crystal X-ray structures were obtained for compounds 1b – 3b.}

The characterisation data was fully consistent with the proposed structures, indicating the successful synthesis of the phosphonium salts. \(^3\)\(^1\)P\(^{\text{1}}\)(H) NMR revealed a downfield shift for the chemical shift of the phosphorus atom (~+6 ppm of triarylphosphines to ~+20 ppm in the phosphonium salts), consistent with values from our previous work.\(^2\)\(^1\),\(^2\)\(^2\)\(^1\) All single-crystal structures for series a and b were obtained, with two previously unreported structures (see ESI).\(^2\)\(^2\)\(^\text{Unfortunately, compounds 1c – 3c resisted all attempts at crystallisation, presumably due to the poor packing of the long and flexible alkyl chains, forming waxy solids instead.}\(^3\)\(^1\)

In terms of structural parameters, the ranges of the average bond lengths in the six compounds were 1.794 – 1.799 Å and 1.790 – 1.798 Å for P-Aryl and P-Alkyl (i.e., P-Ar and P-Ak), respectively, while the ranges of the bond angles for Ar – P – Ar and the Ar – P – Ak bond were 108.36° – 109.71° and 109.23° – 110.57°, respectively – all of them consistent with previously reported TPP\(^+\) compounds.\(^2\)\(^1\),\(^2\)\(^2\)\(^1\)\(^\text{For the short alkyl chain modules in compounds 1a – 3a, the chains adopted a consistent anti conformation. However, for the pentyl substituted TPP\(^+\), twisting of the C-C bonds was observed for 1b and 3b further away from the phosphorus centre, while 2b had an extended hydrocarbon chain. A gauche conformation was observed in 1b for the 2\(^{\text{nd}}\) and 3\(^{\text{rd}}\) C-C bond, while the same was observed in 3b for the 3\(^{\text{rd}}\) C-C bond. The inconsistency between all three structures indicates a high degree of flexibility for the alkyl chain.}

Measurement of lipophilicity

The role of lipophilicity in drugs is well-documented and influences their biological properties greatly, and is frequently quantified by logP, where P is the partition coefficient between two immiscible phases. This is especially relevant for TPP\(^+\) compounds, as there are many studies in the literature correlating the accumulation, toxicity, or drug efficacies to lipophilicity.\(^2\)\(^3\),\(^2\)\(^4\),\(^2\)\(^5\)\(^\text{Due to the large impact that lipophilicity has on these systems, we quantified the lipophilicity of our compounds in water and 1-octanol and the values are presented in Table 1. The measurements were done using a shake-flask method as described in the literature.}\(^2\)\(^4\) The logP values obtained correlate well with the increasing atom count, with larger molecules having larger lipophilicity.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
Compound & logP in Water & logP in 1-Octanol & logP Difference \\
\hline
1a & 1.790 & 1.798 & 0.008 \\
1b & 1.794 & 1.799 & 0.005 \\
1c & 1.792 & 1.797 & 0.005 \\
2a & 1.800 & 1.805 & 0.005 \\
2b & 1.804 & 1.809 & 0.005 \\
2c & 1.806 & 1.811 & 0.005 \\
3a & 1.810 & 1.817 & 0.007 \\
3b & 1.814 & 1.820 & 0.006 \\
3c & 1.816 & 1.822 & 0.006 \\
\hline
\end{tabular}
\caption{Lipophilicity measurements of compounds 1a – 3c.}
\end{table}

The parameters (molecular volume, and solvent accessible surface area) were found to have a good correlation with lipophilicity (\(r^2 = 0.96\), in line with our previous work (see Table 1 and ESI, Figure 3)).\(^2\)\(^1\),\(^2\)\(^2\) An increase of the solvent accessible surface area (SASA) of the cations, has been linked with an increased (hydrophobic) surface area that is exposed to the solvents,
favouring the partition to the 1-octanol phase.\textsuperscript{21, 22} The molecular volume upon normalisation with respect to charge, has also been described as an important structural parameter for the prediction of mitochondrial accumulation of a series of TPP\textsuperscript{+} molecules.\textsuperscript{21} Alchemical free energy calculations focused on the van der Waals (vdW) contribution to the free energy of transfer between water and octanol, also showed a good correlation ($r^2 = 0.98$) with the experimental logP results (see ESI, Figure S3). This is also in line with our previous works.

**IC\textsubscript{50} measurements**

For a rapid screening of mitochondrial uptake, the 72-hour cell viability assay was conducted using HeLa cells by the resazurin reduction assay. The cytotoxic effects of TPP\textsuperscript{+} has been noted in the literature and has been linked to increased membrane uncoupling.\textsuperscript{24, 35} However, these bioenergetic effects induced by TPP\textsuperscript{+} moieties are unlikely to be detrimental at relevant dosages.\textsuperscript{36} Furthermore, from previously published work, mitochondrial accumulation has been found to correlate with the IC\textsubscript{50} values in TPP\textsuperscript{+} species.\textsuperscript{21} Thus, the dose-response curves of these compounds should reveal the mitochondrial accumulation of the compounds, as a higher accumulation of the TPP\textsuperscript{+} salts should result in a greater cytotoxicity. In addition to compounds 1a – 3c, methyltriphenylphosphonium (TPMP) iodide was also included as a comparison. The absolute IC\textsubscript{50} values (± 95% confidence intervals) are presented in Table 1, and the dose-response curves are plotted against the experimental logP in Figure 2.

IC\textsubscript{50} was observed to decrease with both increased alkyl chain length, as well as increasing aryl methylation. With an increase of the alkyl chain length from 1a – 1c, the IC\textsubscript{50} decreased from 16.15 to 1.64 to 0.34. On the other hand, with an increase of the aryl methylation from 1a – 3a, the IC\textsubscript{50} dropped from 16.15 to 3.83 to 0.80. The two other approaches appear to be compatible, with compound 2b having a lower IC\textsubscript{50} compared to 1b and 2a. However, the effects appear to diminish with the more lipophilic compounds. For example, 3c, with the longest alkyl chain and the most substituted aryl ring, had an IC\textsubscript{50} of 0.32 ± 0.06, which was similar to both 2c (0.30 ± 0.04) and 3b (0.32 ± 0.02). This indicates that the scope of the compounds studied approaches the upper limit for the lipophilicity-linked cytotoxicity observed in the literature, with negligible changes in IC\textsubscript{50} over an order of magnitude of [TPP\textsuperscript{+}].\textsuperscript{24}

Interestingly, while a sigmoidal model provided a good description for 1a and TPMP, a double-sigmoidal model was a more accurate model for the remaining compounds, which – to the best of our knowledge – has not been previously reported for TPP\textsuperscript{+} species. While there are multiple explanations in the literature for this phenomenon, ranging from cell population heterogeneity, differing mode of action at different concentrations, to cell-cycle phase specificity, detailed mechanistic studies are necessary to determine the cause of this phenomenon.\textsuperscript{37, 38}

**COMPUTATIONAL STUDIES**

To understand the thermodynamics of the cations transport across biological membranes, we have also performed umbrella sampling molecular dynamics simulations. The full protocol details are provided in the supporting information accompanying this paper (see ESI). We explored the thermodynamics of the cations transport using a hydrated POPC bilayer system as our model membrane. This model is much closer to biological membranes than biphasic 1-octanol:water systems, accounts for ion-membrane specific interactions, and allows for a description for the methylated TPP\textsuperscript{+} salts (1a – 3c) with TPMP. Error bars for log P are shown for standard deviation and inhibition as standard error. [TPP] is shown in μM.
and thus can be used to examine the differences between the cation-membrane interactions as a result of structural changes in the TPP$^+$ moiety (Figure 3). In our study, the membrane model is divided into four regions based on the different density compositions, namely, bulk water (region IV); most of the charged phosphate and choline density (region III), the region that contains both hydrophobic tails and a portion of the polar headgroup density, ending where the lipid tail density intercepted the choline density (region II); and a central zone that contains only the hydrophobic lipid tails (region I). The translocation free energy profile of all nine triphenylphosphonium cations ($1a$ – $3c$) as well as detailed parameters describing local free energy maxima ($\Delta G_{\text{max}}$), minima ($\Delta G_{\text{min}}$) and free energy barrier at the centre of the bilayer ($\Delta G_0$) are depicted in Figure 3 and Table S2.

The free energy profile can be qualitatively understood in 3 phases during translocation. As the TPP$^+$ ion approaches the surface of the membrane, an increase in free energy ($\Delta G_{\text{max}}$, region III) is observed. There is a subsequent decrease in free energy ($\Delta G_{\text{min}}$, region II), and finally, we observe a free energy maxima in region I ($\Delta G_0$). The potential energy profiles of TPP$^+$ ions with lipid bilayers have been previously written down as the sum of four terms, including Born, image and dipole energy contributions, and a neutral energy term.\textsuperscript{41, 42} The free energy profile diagrams obtained are highly consistent with models previously reported in the literature.\textsuperscript{43, 44}

The free energy profile diagrams obtained further emphasise the importance of lipophilicity in TPP$^+$ systems; since an increase in lipophilicity can be correlated with a respective decrease in free energy at all three critical points, $\Delta G_{\text{max}}$, $\Delta G_{\text{min}}$ and $\Delta G_0$. Notably, the maximum values of the free energy profiles relative to water (defined at 0.0 kcal-mol$^{-1}$) of isomeric compounds are comparable, with $1b/2a$ at 9.80 - 9.85 kcal-mol$^{-1}$, $1c/2b/3a$ at 7.28 - 8.61 kcal-mol$^{-1}$, and $2c/3b$ at 3.99 - 4.63 kcal-mol$^{-1}$ (see ESI, Table S2). We also observed that among the four compounds with the lowest IC$50$ (between 0.30 and 0.36 $\mu$M), $1c$, $2c$, $3c$ and $3b$, showed the smallest free energy barrier for penetrating the high-density region of the bilayer ($\Delta G_{\text{max}}$), and except for $1c$, these compounds had low $\Delta G_0$ values (see Figure 3 and ESI, Table S2).

To allow the full translocation free energy profile to be considered, the membrane partition coefficient (log$P_{\text{mem}}$) was computed from the free energy profile diagrams by calculating the standard binding free energy of the cations to the membrane (Table 1, see ESI for more details). We observed that the four compounds with the lowest IC$50$ (between 0.30 and 0.36 $\mu$M) – $2c$, $3c$, $1c$ and $3b$, were the only ones to show a positive log$P_{\text{mem}}$ (between 0.45 and 2.30). The log$P_{\text{mem}}$ obtained were also found to be well-correlated with experimental logP. ($r^2 = 0.89$, Figure S4), with a mean unsigned difference of 0.29 ± 0.23. However, these differences were to be expected as the experimental results were obtained in water and 1-octanol.

The log$P_{\text{mem}}$ for the different isomers were then compared in terms of aryl substitution vs alkyl chain length modules. For the $1b/2a$ isomers, very similar membrane partition (-0.47 ± 0.17 and -0.54 ± 0.31, respectively) were obtained. As for the other isomers, i.e., $1c/2b/3a$ and $2c/3b$, the simulations showed that $1c$ and $2c$ had a higher membrane partition when compared with their respective isomers (ranging from 0.6 to 0.8). This suggests that a longer alkyl chain length module could have an increased tendency to associate with lipid membranes despite having similar lipophilicities.

To examine the possible different tendencies to form ion-pairs for $1a$ – $3c$, which may affect translocation profiles, an ionic concentration of ca. 0.15 M of NaCl was used throughout our studies. By following the number of contacts between the cations and the Cl$^-$ ions present in the system, we did not detect the formation of ion pairs as the cations translocated the hydrated bilayer system (see Figure 3). Still, in a separate simulation, we have constrained the interatomic distance between one Cl$^-$ ion and the $1a$ cation, to investigate the effect of the formation of an ion pair in the translocation free energy profile of $1a$ (see ESI, Figure S5). We observed that ion pairing
should not be favourable for the translocation of this cation, which agrees with what has been already proposed in the literature for TPMP and using DFT-based continuum model calculations. Although we obtained the same partition with or without ion pairing, the free energy barrier for crossing the bilayer for 1a as an ion pair was higher by ca. 2 kcal/mol.

Mitochondrial uptake and localisation of TPP+-conjugated fluorescein

To quantify the difference in mitochondrial delivery between the various TPP+ vectors, a fluorescein-based dye was conjugated to the triarylphosphonium salts modules with differing chain length units. The compounds synthesised are shown in Figure 1, with modifications from previously reported molecule (MitoFluo) by Antonenko et al.46, 47 To control regioselectivity, as well as to restrict the protonophoric activity observed in mitoFluo, the methyl ester of fluorescein was used. The compounds 4a/4b/4c consist of a triphenylphosphonium linked to the fluorescent moiety with an increasing chain length, while compounds 4a/4d/4e consist of the fluorescent dye linked with a 5-carbon chain to different alkylated triarylphosphonium groups.

The synthesis of TPP+-conjugated fluorescein was achieved in three steps: esterification of fluorescein, synthesis of ω-bromoalkyltriarylphosphonium bromide and Williamson ether synthesis for the conjugation of TPP+ to the dye (Scheme 2). 4a – 4e were characterised by 1H, 13C(1H) and 31P(1H) NMR and HRMS. The experimental excitation/emission fluorescence spectra obtained for the five conjugated dyes were virtually identical in both intensities and wavelengths (see ESI, Figure S2). LogP values for the TPP+-fluorescein conjugates were also quantified experimentally and presented in Table 2. The relative increase in logP values were highly consistent with the differences in the unconjugated TPP+ salts.

To measure the mitochondrial uptake and subcellular localisation of these conjugated dyes, HeLa cells were treated with compounds 4a – 4e (100 nM) together with MitoTracker DeepRed FM(25 nM) in DMEM for 30 minutes, washed with 3 x

Table 2. Experimental data for compounds 4a – 4e

| Compound | log P (± SD) | Fluo/MT (± SD) |
|----------|-------------|----------------|
| 4a       | 0.42 ± 0.04 | 0.06 ± 0.05    |
| 4b       | 1.25 ± 0.05 | 0.521 ± 0.042 |
| 4c       | 2.72 ± 0.07 | 1.009 ± 0.091 |
| 4d       | 1.43 ± 0.01 | 0.361 ± 0.058 |
| 4e       | 2.37 ± 0.01 | 0.757 ± 0.058 |

Scheme 2: Synthesis of TPP+-fluorescein conjugates 4a-e.

Figure 1. Confocal fluorescence microscopy images of HeLa cells treated with 4a – 4e upon excitation with 488 nm and 644 nm lasers. The first row shows the fluorescence signal from MitoTracker Deep Red, the second row shows the fluorescence signal from 4a – 4e, and the third row shows the overlay of the two channels.
200 μL of PBS, and the medium was replaced with DMEM. The samples were then examined under a confocal microscope. The relative mitochondrial uptake among the cells was quantified using the ratio of fluorescence intensities between the 4a – 4e and the MitoTracker (Fluo/Mt), and the data is presented in Figure 4 and Table 2.

The images obtained revealed a colocalisation of the TPP$^+$ conjugated dyes and the MitoTracker, confirming a high selectivity of compounds 4a – 4e for the mitochondria. Evidently, the fluorescent intensity from the fluorescein dyes were correlated with lipophilicity. As the chain length was extended from 5 to 11 carbons in compounds 4a/4b/4c, the Fluo/Mt ratio increased 16 times from 0.065 to 1.009. Similarly, an increase in aryl groups methylation results in an increase in mitochondrial accumulation as the Fluo/Mt ratio for compounds 4a/4d/4e increased 12 times from 0.065 to 0.757. Both methods were found to increase mitochondrial uptake drastically, indicating the viability of both methodologies for mitochondrial delivery, consistent with other work in the literature. Fluo/Mt was found to correlate well with logP values of the TPP$^+$-fluorescein conjugate ($r^2 = 0.94$). The slightly increased performance of 4c as compared to 4e was hence attributed to the higher lipophilicity (2.72 ± 0.07 for 4c and 2.37 ± 0.01 for 4e).

Conclusions

In conclusion, we have reported the first evaluation of aryl methylation as a complementary modular strategy to the traditional methods of chain length extension for enhancing mitochondrial targeting abilities. Both strategies result in lowered membrane permeation energy and greater mitochondrial accumulation with isomeric compounds displaying analogous translocation profiles, cytotoxicity and mitochondrial accumulation. Most notably, both approaches are fully compatible, and can be simultaneously applied for the greatest increase in mitochondrial targeting. This work demonstrates that aryl methylation is both comparable and complementary with traditional methods.

Our work underscores the modular nature and complementary nature of both approaches. We hope our work will catalyse the synthesis of a wide range of fine-tuned mitochondrial delivery vectors based on a broader range of phosphonium salts and other non-conventional delivery vectors.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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Electronic Supplementary Information (ESI)

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Experimental

1. Chemicals
Triphenylphosphine, tris(3,5-dimethylphenyl)phosphine, 1-bromopentane and 1-bromooctane were purchased from Sigma Aldrich, and all other chemicals (tri-(p-tolyl)phosphine and bromoethane) were purchased from TCI Chemicals. The starting materials were used without further purification. Solvents used were dried and stored under 4 Å molecular sieves. Reactions were carried out using standard Schlenk techniques and performed under argon. Human epithelial carcinoma cell line (HeLa) was purchased from ATCC ® (ATCC no. CCL-2).

2. Instrumentation
$^1$H, $^{13}$C, $^{31}$P{${^1}$H} NMR spectra were collected using Bruker Avance III, 400 MHz and 500 MHz spectrometers with the $^1$H, $^{13}$C NMR chemical shifts internally referenced to the relevant residual solvent peaks. All NMR spectroscopic analysis were performed at room temperature (300 K). High-resolution mass spectra were obtained from Water Q-Tof Premier, with ESI mode. Reverse-phase HPLC analysis was performed on a Shimadzu Prominence I LC-2030 using a C-8/C-18 analytical column at a flow rate of 1.0 mL/min for analysis. UV absorption spectra and fluorescence emission spectra were recorded in a 10 mm path quartz cell on an Agilent Cary 300 UV-Vis spectrometer and an Agilent Cary Eclipse fluorescence spectrophotometer. Resazurin Reduction Assays were measured by Tecan’s Infinite M200 microplate reader. Confocal imaging was carried out on a Carl Zeiss LSM 800 confocal laser microscope.

3. Single crystal X-ray diffraction (SCXRD) studies
Diffraction-quality crystals were obtained by crystallization from acetonitrile/diethyl ether at room temperature. The crystals were mounted onto quartz fibers, and the X-ray diffraction intensity data were measured at 100 K with a Bruker Kappa diffractometer equipped with a CCD detector, employing Mo K$_\alpha$ radiation ($\lambda = 0.71073$ Å), with the SMART suite of programs.$^1$ All data were processed and corrected for Lorentz and polarization effects with SAINT and for absorption effects with SADABS.$^2$ Structural solution and refinement were carried out with the SHELXTL suite of programs.$^3$ The structures were solved by direct methods or Patterson maps to locate the heavy atoms, followed by difference maps for the light, non-hydrogen atoms. All non-hydrogen atoms were refined with anisotropic thermal parameters. CCDC 2078443 - 2078444 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/structures"
4. Synthesis of delocalized lipophilic cations

The synthesis for compound 1a – 1c and fluorescein methyl ester (methyl 2-(6-hydroxy-3-oxo-3H-xanthen-9-yl)benzoate) are achieved by following procedures in the literature \(^4,5\) and hence they are not described here.

4.1. Synthesis of pentyltriphenylphosphonium bromide

Triphenylphosphine (0.262 g, 1 mmol) was heated under reflux with 1-bromopentane (0.302 g, 2 mmol) in acetonitrile (5 mL) overnight. The mixture was cooled to room temperature and the product was precipitated with the addition of diethyl ether. The white precipitate was filtered and crystallized in acetonitrile and diethyl ether to obtain a white crystalline solid. Single crystals used for SCXRD were grown from acetonitrile/diethyl ether. Yield: 85 %, 0.353 g.

\(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.67 – 7.52 (m, 15H), 3.52 – 3.45 (m, 2H), 1.49 – 1.38 (m, 4H), 1.15 – 1.07 (m, 2H), 0.62 (t, \(J = 7.3\) Hz, 3H). \(^{31}\)P\(^{\{1\}H}\) NMR (162 MHz, CDCl\(_3\)) \(\delta\) 24.05.

\(^{13}\)C\(^{\{1\}H}\) NMR (101 MHz, CDCl\(_3\)) \(\delta\) 134.80 (d, \(J = 2.9\) Hz), 133.27 (d, \(J = 10.0\) Hz), 130.25 (d, \(J = 12.5\) Hz), 117.92 (d, \(J = 86.0\) Hz), 32.04 (d, \(J = 15.5\) Hz), 22.51 (d, \(J = 49.7\) Hz), 21.93 (d, \(J = 4.4\) Hz), 21.78, 13.27.

TOF-MS-ES+ for [M-Br]: Calcd. m/z 333.1772; Found 333.1771.

4.2. Synthesis of octyltriphenylphosphonium bromide

Triphenylphosphine (0.341 g, 1.3 mmol) was heated under reflux with 1-bromopentane (0.193 g, 1 mmol) in toluene (1 mL) overnight. Two liquid phases were observed, and the product solidified upon cooling to room temperature. The waxy solid was washed with toluene followed by diethyl ether. The residue was dried in vacuo to obtain a white waxy solid. Yield: 75 %, 0.342 g.

\(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.66 – 7.51 (m, 15H), 3.62 – 3.55 (m, 2H), 1.54 – 1.52 (m, 4H), 1.17 – 1.08 (m, 8H), 0.73 (t, \(J = 6.7\) Hz, 3H). \(^{31}\)P\(^{\{1\}H}\) NMR (162 MHz, CDCl\(_3\)) \(\delta\) 24.15. \(^{13}\)C\(^{\{1\}H}\) NMR (101 MHz, CDCl\(_3\)) \(\delta\) 135.02 (d, \(J = 3.1\) Hz), 133.57 (d, \(J = 10.0\) Hz), 130.49 (d, \(J = 12.4\) Hz), 118.27 (d, \(J = 85.8\) Hz), 31.54, 30.32 (d, \(J = 15.2\) Hz), 28.97, 28.70, 22.86 (d, \(J = 50.2\) Hz), 22.55 (d, \(J = 4.6\) Hz), 22.41, 13.90. TOF-MS-ES+ for [M-Br]: Calcd. m/z 375.2242; Found 375.2245.

4.3. Synthesis of pentyltri(p-tolyl)phosphonium bromide

Tri(p-tolyl)phosphine (0.396 g, 1.3 mmol) was heated under reflux with 1-bromopentane (0.302 g, 2 mmol) in toluene (1 mL) overnight. The mixture was allowed to cool to room temperature. The solution was decanted, and the solids were washed with diethyl ether. The residue was dried in vacuo to obtain a white solid. Single crystals used for SCXRD were grown from acetonitrile/diethyl ether. Yield: 75 %, 0.340 g.

\(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.64 (dd, \(J = 12.4, 8.0\) Hz, 6H), 7.46 (dd, \(J = 8.2, 3.1\) Hz, 6H), 3.56 – 3.49 (m, 2H), 2.45 (s, 9H), 1.60 – 1.55 (m, 4H), 1.31 – 1.26 (m, 2H), 0.81 (t, \(J = 7.3\) Hz, 3H). \(^{31}\)P\(^{\{1\}H}\) NMR (162 MHz, CDCl\(_3\)) \(\delta\) 23.19. \(^{13}\)C\(^{\{1\}H}\) NMR (101 MHz, CDCl\(_3\)) \(\delta\) 146.29 (d, \(J = 3.0\) Hz), 133.63
(d, J = 10.2 Hz), 131.26 (d, J = 12.9 Hz), 115.39 (d, J = 88.6 Hz), 32.54 (d, J = 15.3 Hz), 23.23 (d, J = 51.3 Hz), 22.40 (d, J = 4.5 Hz), 22.31, 21.92, 13.72. TOF-MS-ES+ for [M-Br]+: Calcd. m/z 375.2242; Found 375.2245.

4.4. Synthesis of octyltri(p-tolyl)phosphonium bromide
Tri(p-tolyl)phosphine (0.609 g, 2 mmol) was heated under reflux with 1-bromooctane (0.773 g, 4 mmol) in acetonitrile (8 mL) overnight. The mixture was cooled to room temperature, concentrated in vacuo and the product was precipitated with the addition of diethyl ether. The mixture was decanted, and the residue was washed 3 times with diethyl ether. The residue was dried in vacuo to obtain a white solid. Yield: 92 %, 0.920 g. 1H NMR (400 MHz, CDCl3) δ 7.57 (dd, J = 12.4, 8.1 Hz, 6H), 7.43 (dd, J = 8.2, 3.2 Hz, 6H), 3.42 – 3.36 (m, 2H), 2.41 (s, 9H), 1.54 – 1.52 (m, 4H), 1.20 – 1.07 (m, 8H), 0.77 (t, J = 6.8 Hz, 3H). 31P{1H} NMR (162 MHz, CDCl3) δ 22.98. 13C{1H} NMR (101 MHz, CDCl3) δ 146.27 (d, J = 3.0 Hz), 133.46 (d, J = 10.2 Hz), 131.20 (d, J = 12.8 Hz), 115.18 (d, J = 88.6 Hz), 31.63, 30.44 (d, J = 15.3 Hz), 29.04, 28.79, 23.20 (d, J = 51.3 Hz), 22.58 (d, J = 4.5 Hz), 22.50, 21.81, 13.97. TOF-MS-ES+ for [M-Br]+: Calcd. m/z 417.2711; Found 417.2709.

4.5. Synthesis of pentyltris(3,5-dimethylphenyl)phosphonium bromide
Tris(3,5-dimethylphenyl)phosphine (0.173 g, 0.5 mmol) was heated under reflux with 1-bromopentane (0.151 g, 1 mmol) in acetonitrile (5 mL) overnight. The mixture was cooled to room temperature and the product was precipitated with the addition of diethyl ether. The precipitate was filtered and washed with diethyl ether. The residue was dried in vacuo to obtain a white solid. Single crystals used for SCXRD were grown from acetonitrile/diethyl ether. Yield: 99 %, 0.246 g. 1H NMR (400 MHz, CDCl3) δ 7.35 (s, 6H), 7.31 (s, 3H), 3.55 – 3.48 (m, 2H), 2.40 (s, 18H), 1.60 – 1.56 (m, 4H), 1.33 – 1.28 (m, 2H), 0.81 (t, J = 7.3 Hz, 3H). 31P{1H} NMR (162 MHz, CDCl3) δ 23.67. 13C{1H} NMR (101 MHz, CDCl3) δ 140.60 (d, J = 13.1 Hz), 136.77 (d, J = 3.1 Hz), 130.96 (d, J = 9.9 Hz), 118.71 (d, J = 84.4 Hz), 32.42 (d, J = 15.0 Hz), 23.09 (d, J = 50.2 Hz), 22.38 (d, J = 4.4 Hz), 22.19, 21.55, 13.67. TOF-MS-ES+ for [M-Br]+: Calcd. m/z 417.2711; Found 417.2714.

4.6. Synthesis of octyltris(3,5-dimethylphenyl)phosphonium bromide
Tris(3,5-dimethylphenyl)phosphine (0.173 g, 0.5 mmol) was heated under reflux with 1-bromooctane (0.193 g, 1 mmol) in acetonitrile (5 mL) overnight. The mixture was cooled to room temperature and the product was precipitated with the addition of diethyl ether. The precipitate was filtered and washed with diethyl ether. The residue was dried in vacuo to obtain a white solid. Single crystals used for SCXRD were grown from acetonitrile/diethyl ether. Yield: 85 %, 0.229 g. 1H NMR (400 MHz, CDCl3) δ 7.36 (s, 6H), 7.33 (s, 3H), 3.59 – 3.52 (m, 2H), 2.41 (s, 18H), 1.60 – 1.57 (m, 4H), 1.25 – 1.17 (m, 8H), 0.83 (t, J = 6.8 Hz, 3H). 31P{1H} NMR (162 MHz, CDCl3) δ 23.38. 13C{1H} NMR (101
MHZ, CDCl$_3$ $\delta$ 140.50 (d, $J = 13.1$ Hz), 136.72 (d, $J = 3.2$ Hz), 130.74 (d, $J = 9.7$ Hz), 118.44 (d, $J = 84.6$ Hz), 31.61, 30.26 (d, $J = 14.9$ Hz), 28.93, 28.74, 22.99 (d, $J = 50.7$ Hz), 22.55, 22.50, 21.41, 13.93. TOF-MS-ES+ for [M-Br]$^+$: Calcd. m/z 459.3181; Found 459.3181.

4.7. Synthesis of (5-{(9-(2-(methoxycarbonyl)phenyl)-3-oxo-3H-xanthen-6-yl)oxy}pentyl)triphenylphosphonium bromide

Triphenylphosphine (0.655 g, 2.5 mmol) was dissolved in 1,5-dibromopentane (2.87 g, 12.5 mmol) and heated at 90°C for 1.5 hours. The mixture was allowed to cool to room temperature and was purified by flash column chromatography (40 : 1, DCM : MeOH) to obtain a white waxy solid (0.750 g, 1.52 mmol, 61%). Fluorescein methyl ester (0.580 g, 1.68 mmol, 1.1 equiv) and potassium carbonate (0.632 g, 4.57 mmol, 3 equiv) was added to the phosphonium salt. The mixture was suspended in DMF (10 mL) and heated at 90 degrees for 2 hours. The mixture was allowed to cool to room temperature, and 50 mL of water was added to the mixture. The suspension was extracted with 5 x 25 mL of dichloromethane and dried with anhydrous magnesium sulfate. The product was further purified by flash column chromatography (20 : 1, DCM : MeOH). The product was washed with ether and dried in vacuo to obtain an orange solid. (0.990 g, 1.31 mmol, 86%) $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.23 (dd, $J = 7.9, 1.4$ Hz, 1H), 7.89 – 7.64 (m, 17H), 7.30 (d, $J = 7.3$ Hz, 1H), 6.86 – 6.81 (m, 3H), 6.70 (dd, $J = 8.9, 2.4$ Hz, 1H), 6.52 (dd, $J = 9.7, 2.0$ Hz, 1H), 6.44 (dd, $J = 8.9, 2.4$ Hz, 1H), 6.09 – 4.01 (m, 2H), 3.99 – 3.87 (m, 2H), 3.63 (s, 3H), 1.96 – 1.82 (m, 4H). 1.78 –1.72 (m, 2H). $^{31}$P($^1$H) NMR (162 MHz, CDCl$_3$) $\delta$ 24.52. TOF-MS-ES+ for [M-Br]$^+$: Calcd. m/z 677.2457; Found 677.2457.

4.8. Synthesis of (8-{(9-(2-(methoxycarbonyl)phenyl)-3-oxo-3H-xanthen-6-yl)oxy}octyl)triphenylphosphonium bromide

Triphenylphosphine (0.655 g, 2.5 mmol) was dissolved in 1,8-dibromoocotane (3.40 g, 12.5 mmol) and heated at 90°C for 3 hours. The mixture was allowed to cool to room temperature and was purified by flash column chromatography (20 : 1, DCM : MeOH) to obtain a white waxy solid (1.03 g, 1.93 mmol, 77%). Fluorescein methyl ester (0.736 g, 2.12 mmol, 1.1 equiv) and potassium carbonate (0.801 g, 5.79 mmol, 3 equiv) was added to the phosphonium salt. The mixture was suspended in DMF (10 mL) and heated at 90 degrees for 2 hours. The mixture was allowed to cool to room temperature, and 50 mL of water was added to the mixture. The suspension was extracted with 5 x 25 mL of dichloromethane and dried with anhydrous magnesium sulfate. The product was further purified by flash column chromatography (20 : 1, DCM : MeOH). The product was washed with ether and dried in vacuo to obtain an orange solid. (1.40 g, 1.74 mmol, 90%) $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.23 (dd, $J = 7.9, 1.4$ Hz, 1H), 7.86 – 7.63 (m, 17H), 7.29 (dd, $J = 7.6, 1.4$ Hz, 1H), 6.90 (d, $J = 2.4$ Hz, 1H), 6.84 (dd, $J = 10.7, 9.3$ Hz, 2H), 6.71 (dd, $J = 8.9, 2.4$ Hz, 1H), 6.51 (dd, $J = 9.7, 2.0$ Hz, 1H), 6.42 (d, $J = 2.0$ Hz, 1H), 4.02 (t, $J = 6.5$ Hz, 2H), 3.78 – 3.66 (m, 4H).
2H), 3.62 (s, 3H), 1.78 – 1.73 (m, 2H), 1.65 – 1.59 (m, 4H), 1.43 – 1.41 (m, 2H), 1.32 – 1.30 (m, 4H). 31P{1H} NMR (162 MHz, CDCl3) δ 24.43. TOF-MS-ES+ for [M-Br]+: Calcd. m/z 719.2926; Found 719.2928.

4.9. Synthesis of (11-((9-(2-(methoxycarbonyl)phenyl)-3-oxo-3H-xanthen-6-yl)oxy)undecyl)triphenylphosphonium bromide

Triphenylphosphine (0.262 g, 1 mmol) was dissolved in 1,11-dibromoundecane (1.57 g, 5 mmol) and heated at 90°C overnight. The mixture was allowed to cool to room temperature and was purified by flash column chromatography (20 : 1, DCM : MeOH) to obtain a white waxy solid. (0.328 g, 0.569 mmol, 57 %) Fluorescein methyl ester (0.217 g, 0.626 mmol, 1.1 equiv) and potassium carbonate (0.236 g, 1.71 mmol, 3 equiv) was added to the phosphonium salt. The mixture was suspended in DMF (10 mL) and heated at 90 degrees for 1.5 hours. The mixture was allowed to cool to room temperature, and 50 mL of water was added to the mixture. The suspension was extracted with 5 x 25 mL of dichloromethane and dried with anhydrous magnesium sulfate. The product was further purified by flash column chromatography (20 : 1, DCM : MeOH). The product was washed with ether and dried in vacuo to obtain an orange solid. (0.150 g, 0.178 mmol, 31 %) 1H NMR (400 MHz, CDCl3) δ 8.23 (dd, J = 8.0, 1.4 Hz, 1H), 7.84 – 7.64 (m, 17H), 7.31 (d, J = 7.8 Hz, 1H), 6.93 (dd, J = 11.5, 9.3 Hz, 2H), 6.72 (dd, J = 8.9, 2.4 Hz, 1H), 6.52 (dd, J = 9.7, 1.9 Hz, 1H), 6.43 (d, J = 2.0 Hz, 1H), 4.04 (t, J = 6.6 Hz, 2H), 3.63 (s, 3H), 1.83 – 1.76 (m, 2H), 1.65 – 1.61 (m, 4H), 1.45 – 1.39 (m, 2H), 1.30 – 1.19 (m, 12H). 31P{1H} NMR (162 MHz, CDCl3) δ 24.41. TOF-MS-ES+ for [M-Br]+: Calcd. m/z 761.3396; Found 761.3398.

4.10. Synthesis of (5-((9-(2-(methoxycarbonyl)phenyl)-3-oxo-3H-xanthen-6-yl)oxy)pentyl)trip-tolylyphosphonium bromide

Tri(p-tolyl)phosphine (0.761 g, 2.5 mmol) was dissolved in 1,5-dibromopentane (2.87 g, 12.5 mmol) and heated at 90°C for 1.5 hours. The mixture was allowed to cool to room temperature and was purified by flash column chromatography (20 : 1, DCM : MeOH) to obtain a white waxy solid. (1.27 g, 2.37 mmol, 94 %) (5-bromopentyl)trip-tolylphosphonium bromide (0.267 g, 0.500 mmol), fluorescein methyl ester (0.190 g, 0.550 mmol, 1.1 equiv) and potassium carbonate (0.207 g, 1.50 mmol, 3 equiv) were suspended in DMF (6 mL), and heated at 90 degrees for 1.5 hours. The mixture was allowed to cool to room temperature, and 50 mL of water was added to the mixture. The suspension was extracted with 5 x 25 mL of dichloromethane and dried with anhydrous magnesium sulfate. The product was further purified by flash column chromatography (20 : 1, DCM : MeOH). The product was washed with ether and dried in vacuo to obtain an orange solid. (0.294 g, 0.368 mmol, 74 %) 1H NMR (500 MHz, CDCl3) δ 8.22 (dd, J = 7.8, 1.4 Hz, 1H), 7.74 – 7.64 (m, 8H), 7.45 (dd, J = 8.3, 3.1 Hz, 6H), 7.27 (d, J = 7.7 Hz, 1H), 6.85 – 6.81 (m, 3H), 6.69 (dd, J = 9.0, 2.4 Hz, 1H), 6.51 (dd, J = 9.7, 1.9 Hz, 1H), 6.41 (d, J = 1.9 Hz, 1H), 4.04
(s, 2H), 3.66 – 3.59 (m, 5H), 2.45 (s, 9H), 1.87 – 1.85 (m, 4H), 1.69 – 1.67 (m, 2H). $^{31}$P{$^{1}$H} NMR (202 MHz, CDCl$_3$) $\delta$ 23.24. TOF-MS-ES+ for [M-Br]$^+$: Calcd. m/z 719.2926; Found 719.2931.

4.11. Synthesis of tris(3,5-dimethylphenyl)(5-(9-(2-(methoxycarbonyl)phenyl)-3-oxo-3H-xanthene-6-yl)oxy)pentyl)phosphonium bromide

Tris(3,5-dimethylphenyl)phosphine (0.346 g, 1 mmol) was dissolved in 1,5-dibromopentane (1.15 g, 5 mmol) and heated at 90°C for 1.5 hours. The mixture was allowed to cool to room temperature and was purified by flash column chromatography (40 : 1, DCM : MeOH) to obtain a white waxy solid. (0.486 g, 0.843 mmol, 84 %) Fluorescein methyl ester (0.321 g, 0.927 mmol, 1.1 equiv) and potassium carbonate (0.350 g, 2.53 mmol, 3 equiv) was added to the phosphonium salt. The mixture was suspended in DMF (6 mL) and heated at 90 degrees for 1.5 hours. The mixture was allowed to cool to room temperature, and 50 mL of water was added to the mixture. The suspension was extracted with 5 x 25 mL of dichloromethane and dried with anhydrous magnesium sulfate. The product was further purified by flash column chromatography (20 : 1, DCM : MeOH). The product was washed with ether and dried in vacuo to obtain an orange solid. (0.290 g, 0.344 mmol, 41 %) $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.23 (dd, $J = 7.8$, 1.5 Hz, 1H), 7.69 (dtd, $J = 28.0$, 7.6, 1.4 Hz, 2H), 7.39 (s, 3H), 7.35 (s, 6H), 7.29 (d, $J = 8.7$ Hz, 1H), 6.86 – 6.81 (m, 3H), 6.70 (dtd, $J = 8.9$, 2.3 Hz, 1H), 6.51 (dd, $J = 9.8$, 1.9 Hz, 1H), 6.43 (d, $J = 1.9$ Hz, 1H), 4.07 – 4.04 (s, 2H), 3.67 – 3.60 (m, 3H), 2.40 (s, 18H), 1.93 – 1.86 (m, 2H), 1.71 – 1.60 (m, 3H). $^{31}$P{$^{1}$H} NMR (162 MHz, CDCl$_3$) $\delta$ 23.72. TOF-MS-ES+ for [M-Br]$^+$: Calcd. m/z 761.3396; Found 761.3389.
5. Measurement of lipophilicity

Lipophilicity was determined by measuring octanol–water partition coefficient using HPLC measurements as described in the literature.\textsuperscript{6} Calibration curves were obtained from standard solutions prepared (20 – 100 μM). A 100 μM sample of phosphonium salt in octanol-saturated water was stirred vigorously with water-saturated octanol in a 1.5 mL microtube and allowed to sit for 10 minutes. The two phases were separated by centrifugation, and the concentration of the phosphonium salt in the aqueous layer was quantified by HPLC using a UV detector (220 nm). The peak area in the water layer was used to calculate the partition coefficient (log P):

\[
\log P = \log \left( \frac{A_{\text{std}}}{A_{\text{w}}} - 1 \right) \left( \frac{V_{\text{w}}}{V_{\text{o}}} \right)
\]

where \(A_{\text{std}}\) and \(A_{\text{w}}\) represents the peak area for a 100 μM standard and the aqueous layer, respectively. \(V_{\text{w}}\) and \(V_{\text{o}}\) represents the volume of water and octanol used in the mixture. The measurement for each compound was repeated 3 times and the results and solvent ratios used are shown below in Table S1.

**Table S1.** Results for lipophilicity measurements

| Compound | \(V_{\text{w}}/V_{\text{o}}\) | log P    | St. Dev. |
|----------|-----------------------------|----------|----------|
| 1a       | 0.0667                      | -1.36341 | 0.091698 |
| 1b       | 0.5                         | -0.84374 | 0.005435 |
| 1c       | 2                           | 0.215889 | 0.023109 |
| 2a       | 0.5                         | -0.49856 | 0.046148 |
| 2b       | 2                           | 0.261133 | 0.021482 |
| 2c       | 1.566637                    | 0.01828  |
| 3a       | 1                           | 0.083515 | 0.016644 |
| 3b       | 0.903388                    | 0.012088 |
| 3c       | 10                          | 2.246523 | 0.050932 |
| 4a       | 10                          | 0.417092 | 0.039929 |
| 4b       | 10                          | 1.249934 | 0.046903 |
| 4c       | 100                         | 0.722392 | 0.074858 |
| 4d       | 10                          | 1.425906 | 0.011654 |
| 4e       | 100                         | 2.365476 | 0.01271  |
6. Cells culture and determination of the half maximal inhibitory concentration (IC\textsubscript{50}) test

HeLa cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) solution with 10% fetal bovine serum (FBS) and 1% Penicillin/Streptomycin under humidified atmosphere of 5% CO\textsubscript{2} at 37 °C. Resazurin sodium salt was dissolved in PBS (0.2 mg/mL) to make a stock solution, which was diluted in DMEM w/o phenol red to 0.02 mg/mL before use. Stock solutions (10 µM) for 1a – 3c was prepared by dissolving the salts in DMSO. The HeLa cells were seeded on a 96-wells containing 10000 cells per well in 100 µL DMEM media and incubated overnight before the addition of 1a – 3c. Upon incubation at 37 °C for an additional 72 h, the media was removed, and the cells were washed with PBS. Resazurin solution (100µL) was added to each well before incubation for 2 h at 37 °C. The samples were excited using a 560 nm light and the fluorescence was recorded on a Tecan’s Infinite M200 microplate reader using a 590 nm emission filter. Different concentrations of 1a – 3c were used and for each concentration and was performed in triplicate. The experiment was repeated three times and the IC\textsubscript{50} was determined from the plot of viability against concentration of samples.
7. Confocal microscopy

HeLa cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) solution with 10% fetal bovine serum (FBS) and 1% Penicillin/Streptomycin under humidified atmosphere of 5% CO₂ at 37 °C. 25 x 10⁴ HeLa cells were seeded on a microscope slide (Ibidi #80827) overnight in 200 µL of DMEM, and was subsequently treated with compounds 4a – 4e (100 nM, λ₂₅ = 488 nm) and Mitotracker DeepRed FM (50 nM, λ₂₅ = 640 nm) at 37 °C for 1 hour. The cells were washed 3 x 200 µL of PBS, and the chambers were filled with DMEM for imaging.

8. Excitation/Emission spectra

![Excitation/Emission spectra for compounds 4a – 4e.](image)

**Figure S2.** Fluorescence excitation and emission spectra for compounds 4a – 4e.
9. Theoretical methods

9.1. Molecular dynamics simulations and free energy calculations

Molecular dynamics (MD) simulations of the studied compounds were performed with the GROMACS 2018 software.\textsuperscript{7-9}

The molecules were parameterized with the Antechamber program,\textsuperscript{10} following a standard protocol: i) molecular mechanics parameters for bonded and van der Waals terms were extracted from the second-generation general amber force field (GAFF2); and ii) restrained electrostatic potential (RESP) charges\textsuperscript{11} were derived at the HF/6-31G(d)//B3LYP/6-31G(d) level of theory. The conversion from amber-generated files to GROMACS-compatible ones was achieved with parmed.\textsuperscript{12} We have confirmed that our optimized structures were true stationary points by performing a frequency calculation. All quantum mechanics calculations were performed with the Gaussian 09 software.\textsuperscript{13}

Water molecules were described with the TIP3P model,\textsuperscript{14} and 1-octanol molecules were parameterized using the previously described protocol. The amber99sb-ildn ion parameters,\textsuperscript{15} already present in GROMACS, were used to describe the chlorine counter-ions used in the simulations.

The solvated systems were assembled using either GROMACS, in the case of the hydrated systems; or packmol,\textsuperscript{16} in the case of the 1-octanol solvated systems. For the water solvated systems, we have used a cubic box of 1.5 nm, defined as the distance between any atom of the solute and the edges of the simulation box. For the 1-octanol solvated solutes, 300 1-octanol molecules were packed in a cubic box with sides of ca. 4.6 nm.

The solvated systems were then simulated using a 4-step protocol, that included: i) an optimization of the system using a steepest descent algorithm for energy minimization, until the maximum force was smaller than 100 kJ mol\textsuperscript{-1} nm\textsuperscript{-1}; ii) a 100 ps stage with the canonical (NVT) ensemble; iii) a 100 ps density equilibration using the isothermal-isobaric (NPT) ensemble; and iv) a 10 ns production run for data acquisition and for further equilibration of the systems.
A non-bonded cut-off of 1.2 nm was employed for Particle Mesh Ewald (PME) electrostatics and plain cut-off van der Waals interactions, both with a potential-shift-Verlet modifier at 0.0 nm. Long-range dispersion corrections were applied for energy and pressure. For neighbor searching we have used the Verlet scheme. Periodic boundary conditions were employed in all three directions.

The MD simulations were performed with a leap-frog stochastic integrator (2 fs time step for integration). Constraints were applied to all bonds involving hydrogen atoms, with the LINCS constraint algorithm. The same integrator was used as a thermostat, using a 2 ps time constant for temperature coupling of the system. The reference temperature was set to 298.15 K. For isotropic pressure coupling at 1 atm, we used the Berendsen barostat for the density equilibration stage, using a 2 ps time constant for pressure coupling. Then, for the production stage we have switched to the Parrinello-Rahman barostat, with the same time constant for pressure coupling.

The solvent accessible surface area (SASA) and the volume of the compounds were assessed with the gmx sasa tool also integrated in GROMACS, using a probe radius of 1.4 nm. In this analysis, we used the last 5 ns of the 10 ns production stage of the conventional MD simulations.

The alchemical solvation considering the van der Waals interactions was broken into 16 lambda states. Specifically, the Lennard-Jones interactions were scaled using \( \lambda = [0.00, 0.05, 0.10, 0.20, 0.30, 0.40, 0.50, 0.60, 0.65, 0.70, 0.75, 0.80, 0.85, 0.90, 0.95, 1.00] \). For these calculations, the charges on the solutes were set to zero. Although electrostatic interactions are necessary for obtaining the solvation/hydration free energy of the compounds, these were not considered for two reasons: i) previous studies have shown that this component showed little correlation with the experimental partition results for similar compounds; and ii) known issues have been described for compounds carrying a net charge (in particular for the solute’s charging free energy). The latter effect arises from finite-size effects due to the discrepancy between the actual simulations and the ideal bulk conditions, which results in inaccuracies on the determination of the electrostatic component of the solvation/hydration free energy for these compounds.
Each $\lambda$ state was minimized using GROMACS’ steepest descent minimization algorithm and equilibrated for a total of 150 ps. The equilibration stage included a 50 ps constant volume stage, and 100 ps constant pressure stage with the Berendsen barostat. These were followed by a 5 ns production phase at each $\lambda$, using the Parrinello-Rahman barostat. The overall simulation conditions were adapted from the work by Bannan CC et al. from 2016.\textsuperscript{23}

The contribution of the van der Waals interactions for the hydration/solvation free energy was assessed using the Multistate Bennett Acceptance Ratio (MBAR)\textsuperscript{24} through the Alchemical Analysis tool.\textsuperscript{25} For this analysis we discarded the initial 100 ps of the production stage.

The results for the vdW component of the free energy of transfer are shown as:

$$\Delta G_{aq\rightarrow oct}^{vdw} = \Delta G_{solvation}^{vdw} - \Delta G_{hydration}^{vdw}$$\textsuperscript{[1]}

The correlation plots between the experimental partition results and the SASA, Volume and $\Delta G_{aq\rightarrow oct}^{vdw}$ are shown in Figure S3.

![Correlation plots](image)

Figure S3. Correlation plots between (a) SASA and volume against the experimental logP; and (b) $\Delta G_{aq\rightarrow oct}^{vdw}$ against the experimental logP. Error bars correspond to the standard deviation and the $R^2$ values for the linear fits are provided.
9.2 Umbrella sampling simulations

System modeling. Our all-atom hydrated bilayer model system was composed of 72 1-palmitoyl-2-oleoyl-glycero-3-phosphocholine (POPC) lipids (36 lipids per leaflet), a hydration level of 60 water molecules per lipid, and an ionic concentration of ca. 0.15 M NaCl. It was assembled using the CHARMM-GUI interface using the AMBER force field. After an initial minimization and equilibration of the system, we have inserted two replicas of the studied compounds at different bilayer depths in the simulation box: one of the replicas was inserted in the water phase, and the other at the center of the lipid bilayer (see next sections for details). The cations were parameterized as described in the previous section.

Molecular dynamics simulations parameters. The all-atom simulations were performed with the GROMACS 2018 software, with the Verlet cut-off scheme. A non-bonded cut-off value of 1.0 nm was employed. The LINCS constraint algorithm was applied to all bonds involving hydrogen atoms, and for the production stage to all bonds. We have also employed a hydrogen mass repartition protocol for the production stage, which allowed for an integration time step of 4 fs. Temperature was set to 298.15 K with the v-rescale thermostat (0.5 ps time constant for coupling for the production stage and 1 ps for the equilibration stages), and a semi-isotropic pressure scaling to 1 atm was maintained with the Parrinello–Rahman barostat (5 ps time constant for coupling). During the NPT equilibration, the Berendsen barostat was employed. Dispersion corrections were applied to energy and pressure terms. Periodic boundary conditions were considered, and long-range electrostatic interactions were treated by a Particle Mesh Ewald (PME) scheme. The center of mass motion was removed in a linear fashion and individually for the upper and lower leaflets and the rest of the system (including the solvent, ions and the two solute molecules).

Umbrella sampling simulations and analysis. The hydrated bilayers coming from CHARMM-GUI were minimized and equilibrated in the NVT and NPT ensembles. Subsequently, an NPT conventional MD simulation of 300 ns was run. From the density profiles and as in previous works, we have defined a four-region model to aid in the analysis of the PMFs. Region I contained only the hydrophobic lipid
tails. Region II contained both hydrophobic tails and the initial portion of the polar headgroup density, ending where the lipid tail density intercepted the choline density. Region III contained most of the charged phosphate and choline density. Finally, region IV was composed primarily of bulk water, and a small portion of the lipid’s headgroup density.

Two replicas of each compound were inserted at different bilayer depths using the last structure of the previous run – one in the water phase and the other near the bilayer’s center. The interactions with the hydrated bilayer system were then gradually switched on during 7.5 ns, with the compounds harmonically restrained to the initial positions relative to the bilayer’s COM (with a harmonic force constant of 2000 kJ·mol⁻¹·nm⁻²). Afterwards, a constant pulling simulation of 50 ns was performed to sample the desired translocation coordinate (pulling rate of -0.000074 nm·ps⁻¹). The translocation coordinate in this case was defined by the COM distance between the solute and the lipid bilayer and was discretized into 38 sampling windows spaced by 0.1 nm. This comprised the COM distances of [-3.5; 0.2] nm and [-0.2; 3.5] nm, depending on whether the compound started in the water phase or near the center of the bilayer. Then production runs were performed for 160 ns (with a harmonic force constant of 1500 kJ·mol⁻¹·nm⁻²). The binding free energy, $\Delta G_{bind}^*$, was then derived from the energy profile using equation [2]:

$$
\Delta G_{bind}^* = -k_B T \ln \left( \frac{1}{z_b} \int_{-z_b}^{z_b} e^{-\beta w(z)} d\zeta \right) \quad [2]
$$

where $z_b = 3.4 \text{ nm}$ represents the distance at which the potential of mean force (PMF), $w(z)$, is zero and $\beta = 1/k_B T$, where $T$ is adjusted to the temperature in which the profiles were generated, and $k_B$ represents the Boltzmann constant. The partition coefficient, $P$, can then be derived using equation [3]:

$$
P = e^{-\beta \Delta G_{bind}^*} \quad [3]
$$

For the calculation of the binding free energies, we have considered the energy profiles produced from the last 100 ns of each window of the production runs. These were assessed with the weighted
histogram analysis method (WHAM) tool \(^{38,39}\) available in GROMACS 2018. A bootstrapping analysis (200 bootstraps) was also performed to assess for the error of the energy profile.

In Table S2, we provide additional parameters of the free energy diagrams for the cations’ translocation through the hydrated bilayer system.

**Table S2. Additional parameters of the energy profile diagrams.** We show the maximum at region III, \(\Delta G_{\text{max}}\); the minimum of the profile after the membrane entry and located in region II, \(\Delta G_{\text{min}}\); and the barrier at the centre of the bilayer that is in region I, \(\Delta G_B\). \(\Delta G_B\) was determined from the difference between maximum and minimum values of the energy profiles, but we also show in parenthesis the maximum value of the profiles relative to water (defined at 0.0 kcal\(\cdot\)mol\(^{-1}\)).

| compound | \(\Delta G_{\text{max}}\) / kcal\(\cdot\)mol\(^{-1}\) | \(\Delta G_{\text{min}}\) / kcal\(\cdot\)mol\(^{-1}\) | \(\Delta G_B\) / kcal\(\cdot\)mol\(^{-1}\) |
|----------|-----------------|-----------------|-----------------|
| 1a       | 3.17            | 2.14 \(^a\)     | 13.34 (13.33)   |
| 1b       | 2.58            | -0.05           | 9.86 (9.80)     |
| 1c       | 1.71            | -1.71           | 10.32 (8.61)    |
| 2a       | 2.78            | 0.13 \(^a\)     | 9.85 (9.85)     |
| 2b       | 3.02            | -0.19           | 8.17 (7.98)     |
| 2c       | 1.88            | -3.42           | 8.05 (4.63)     |
| 3a       | 2.97            | -0.61           | 7.89 (7.28)     |
| 3b       | 2.05            | -2.57           | 6.56 (3.99)     |
| 3c       | 1.73            | -4.15           | 6.40 (2.25)     |

\(^a\) The energy profile in region II did not drop below zero, but we have presented the value of the energy profile at the first minimum after the membrane entry.

We have also compared the experimental and simulated logP results. This analysis is depicted in Figure S4.
Figure S4. Correlation between logP (sim.) against logP (exptl.) (a) and comparison between experimental and simulated logP results (b). The $R^2$ value for the linear fit is provided. Error bars correspond to the standard deviation. For the logP simulation errors, we have defined the standard deviation between the partition results for the two replicate molecules in the system and they do not represent the errors from the free energy profile curves. Experimental logP results have been determined using water and 1-octanol, and simulated logP results have been measured in a hydrated bilayer system composed of POPC glycerophospholipids.

For assessing the influence of the ion pair to the translocation of 1a, we have defined two additional coordinates, representing the distance between two distinct Cl$^-$ counter-ions and the center of mass of each of the two 1a cations in the system. Using again an umbrella potential, this distance was restrained with a force constant of 1500 kJ·mol$^{-1}$·nm$^{-2}$ at 0.55 nm, for the entire translocated distance. This distance was defined from the X-ray distances between the cations and the bromide ions present in the crystals. In Figure S5 we compare the free energy profiles of 1a as a restrained ion pair and without this restrain. Figure S5 also shows the average number of contacts with Cl$^-$ ions for both situations.
Figure S5. Free energy profiles of the translocation of 1a and of 1a as an ion-pair, and average number of contacts with Cl\(^-\) ions in a POPC hydrated bilayer model system. The top two panels show a representation of the hydrated bilayer model system and the partial density profiles for the different functional groups or molecules in the system. The third panel shows the free energy profiles for the translocation of 1a and of 1a as an ion pair (black and green, respectively). The bottom panel shows the average number of contacts with Cl\(^-\) counter-ions in the simulation cell for both situations (considering a distance threshold of 0.6 nm). Vertical lines define the four-membrane regions as described in the main text.

Visual inspection of the simulations was attained with the VMD 1.9.3 software.\(^{40}\)
10. NMR Spectra

Figure S6. $^{31}$P-$^{1}$H NMR spectrum for 1b.
Figure S7. $^1$H NMR spectrum for 1b.
Figure S8. $^{13}$C$[^1]$H NMR spectrum for 1b.
Figure S9. $^{31}\text{P}^{\{1\text{H}\}}$ NMR spectrum for 1c.
Figure S10. $^1$H NMR spectrum for 1c.
Figure S11. $^{13}\text{C}^{(1)}\text{H}$ NMR spectrum for 1c.
Figure S12. $^{31}\text{P}(^{1}\text{H})$ NMR spectrum for 2b.
Figure S13. $^1$H NMR spectrum for 2b.
Figure S14. $^{13}$C($^1$H) NMR spectrum for 2b.
Figure S15. $^{31}\text{P}^{(\text{H})}$ NMR spectrum for 2c.
Figure S16. $^1$H NMR spectrum for 2c.
Figure S17. $^{13}$C($^1$H) NMR spectrum for 2c.
Figure S18. $^{31}\text{P}^{(1\text{H})}$ NMR spectrum for 3b.
Figure S19. $^1$H NMR spectrum for 3b.
Figure S20. $^{13}$C($^1$H) NMR spectrum for 3b.
Figure S21. $^{31}\text{P}^{(1\text{H})}$ NMR spectrum for 3c.
Figure S22. $^1$H NMR spectrum for 3c.
Figure S23. $^{13}$C($^1$H) NMR spectrum for 3c.
Figure S24. $^{31}$P($^1$H) NMR spectrum for 4a.
Figure S25. $^1$H NMR spectrum for 4a.
Figure S26. $^{13}$C($^1$H) NMR spectrum for 4a.
Figure S27. $^{31}$P($^1$H) NMR spectrum for 4b.
Figure S28. $^1$H NMR spectrum for 4b.
Figure S29. $^{13}$C($^1$H) NMR spectrum for 4b.
Figure S30. $^{31}$P($^1$H) NMR spectrum for 4c.
Figure S31. $^1$H NMR spectrum for 4c.
Figure S32. $^{13}$C($^1$H) NMR spectrum for 4c.
Figure S33. $^{31}$P($^1$H) NMR spectrum for 4d.
Figure S34. $^1$H NMR spectrum for 4d.
Figure S35. $^{13}$C(1H) NMR spectrum for 4d.
Figure S36. $^{31}P^{(1)}H$ NMR spectrum for 4e.
Figure S37. $^1$H NMR spectrum for 4e.
Figure S38. $^{13}$C(¹H) NMR spectrum for 4e.
11. High Resolution Mass Spectra

Figure S39. High resolution mass spectrum for 1b.
Elemental Composition Report

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
1 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0.26  H: 0.32  P: 0.1
Minimum:  80.00  -1.5
Maximum:  100.00  5.0  10.0  50.0

| Mass   | RA  | Calc. Mass | mDa | PPM | DBE | i-FIT | Norm | Conf(%) | Formula    |
|--------|-----|------------|-----|-----|-----|-------|------|---------|------------|
| 375.2245 | 100.00 | 375.2242  | 0.3 | 0.8 | 11.5 | 37.3  | n/a  | n/a     | C26 H32 P   |

Figure S40. High resolution mass spectrum for 1c.
Elemental Composition Report

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
1 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

Elements Used:
C: 0-26  H: 0-32  P: 0-1

Minimum: -1.5
Maximum:  5.0  10.0  50.0

| Mass   | Calc. Mass | mDa | PPM | DBE | i-FIT | Norm | Conf(%) | Formula |
|--------|------------|-----|-----|-----|-------|------|---------|---------|
| 375.2245 | 375.2242   | 0.3 | 0.8 | 11.5| 35.9  | n/a  | n/a     | C26 H32 P |

**Figure S41.** High resolution mass spectrum for 2b.
Figure S42. High resolution mass spectrum for 2c.
Elemental Composition Report

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

1 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

Elements Used:

C: 0-29  H: 0-38  P: 0-1

Minimum:  80.00  5.0  10.0  50.0
Maximum:  100.00

| Mass    | RA  | Calc. Mass | mDa | PPM | DBE | i-FIT | Norm | Confl(%) |
|---------|-----|------------|-----|-----|-----|-------|------|----------|
| 417.2714| 100.00 | 417.2711 | 0.3 | 0.7 | 11.5 | 199.5 | n/a | n/a | C29 H38 P |

**Figure S43.** High resolution mass spectrum for 3b.
Elemental Composition Report

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
1 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

Elements Used:
C: 0-32    H: 0-44    P: 0-1

Minimum:      -1.5
Maximum:      5.0    10.0    50.0

| Mass     | Calc. Mass | mDa | PPM | DBE | i-FIT | Norm | Conf(%) | Formula |
|----------|------------|-----|-----|-----|-------|------|---------|---------|
| 459.3181 | 459.3181   | 0.0 | 0.0 | 11.5| 33.6  | n/a  | n/a     | C32 H44 P |

**Figure S44.** High resolution mass spectrum for 3c.
**Elemental Composition Report**

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

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**Figure S45.** High resolution mass spectrum for 4a.

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| Mass  | Calc. Mass | mDa | PPM | DBE | i-FIT | Norm | Conf(%) | Formula   |
|-------|------------|-----|-----|-----|-------|------|---------|-----------|
| 677.2457 | 677.2457   | 0.0 | 0.0 | 26.5| 31.2  | n/a  | n/a     | C44 H38 O5 P |
**Elemental Composition Report**

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

### Monoisotopic Mass, Even Electron Ions

10 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

**Elements Used:**

C: 0-50  H: 0-50  O: 0-5  P: 0-1

Minimum: -1.5

Maximum: 5.0  10.0  50.0

| Mass   | Calc. Mass | mDa | PPM | DBE | i-FIT | Norm | Conf(%) | Formula |
|--------|------------|-----|-----|-----|-------|------|---------|---------|
| 719.2928 | 719.2926   | 0.2 | 0.3 | 26.5| 17.4  | n/a  | n/a     | C47 H44 O5 P |

**Figure S46.** High resolution mass spectrum for 4b.
Elemental Composition Report

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Figure S47. High resolution mass spectrum for 4c.
Elemental Composition Report

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Figure S48. High resolution mass spectrum for 4d.

| Mass   | Calc. Mass | mDa | PPM | DBE | i-FIT | Norm | Conf(%) | Formula |
|--------|------------|-----|-----|-----|-------|------|---------|---------|
| 719.2931 | 719.2926   | 0.5 | 0.7 | 26.5| 26.4  | n/a  | n/a     | C47 H44 O5 P |
**Elemental Composition Report**

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

---

**Monoisotopic Mass, Even Electron Ions**

7 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

Elements Used:

C: 0-50  H: 0-50  O: 0-5  P: 0-1

| Minimum: |  -1.5 |
| Maximum: |  5.0  |  10.0 |  50.0 |

| Mass     | Calc. Mass | mDa | PPM | DBE | i-FIT | Norm | Conf(%) | Formula |
|----------|------------|-----|-----|-----|-------|------|---------|---------|
| 761.3389 | 761.3396   | -0.7| -0.9| 26.5| 28.3  | n/a  | n/a     | C50 H50 O5 P |

**Figure S49.** High resolution mass spectrum for 4e.
### Table S3. X-ray data of compounds 2b and 3b.

|                | 2b                             | 3b                             |
|----------------|--------------------------------|--------------------------------|
| Empirical formula | C_{26}H_{32}BrP                | C_{29}H_{38}BrP                |
| Formula weight   | 455.39 g/mol                    | 497.47 g/mol                    |
| Crystal system   | monoclinic                      | monoclinic                      |
| Space group      | P2_1/c                          | C2/c                           |
| a/ Å             | 10.9878(3)                      | 17.4833(10)                    |
| b /Å             | 12.2294(3)                      | 14.5439(9)                     |
| c/ Å             | 17.2004(5)                      | 20.9956(13)                    |
| α/°              | 90                              | 90                             |
| β/°              | 90.035(2)°                      | 100.798(2)                     |
| γ/°              | 90                              | 90                             |
| Volume/ Å³       | 2311.29(11)                     | 5244.1(5)                      |
| Z                | 4                               | 8                              |
| ρ (Calc)/Mg.m⁻³  | 1.309                           | 1.260                          |
| Absorp. Coeff./ mm⁻¹ | 1.857                         | 1.643                          |
| F(000)           | 952                             | 2096                           |
| Crystal Size/ mm³ | 0.06 x 0.10 x 0.12              | 0.10 x 0.10 x 0.40             |
| Θ range/ °       | 2.49 to 30.49                   | 1.83 to 25.02                  |
| -15<=h<=15       | -19<=h<=20                      |                                |
| -17<=k<=17       | -17<=k<=17                      |                                |
| -24<=l<=24       | -24<=l<=20                      |                                |
| Refl. collected  | 27688                           | 32456                          |
| Indep. Refns. (R_{int}) | 3557 (0.061)                | 4644 (0.0649)                  |
| Completeness to Θ | 99.3%                           | 100.0%                         |
| Absorp. Corr.    | Multi-Scan                      | Multi-Scan                     |
| Max., min., transmission | 0.8970, 0.8080                | 0.8530 and 0.5590              |
| Refinement Method | Full-matrix least-squares on F² | Full-matrix least-squares on F² |
| Data/ restraint/parameters | 7004 / 0 / 257                 | 4644 / 0 / 288                 |
| Goodness-of-fit on F² | 1.031                          | 1.037                          |
| Final R indices [I>2σ(I)] | R1 = 0.0527                    | R1 = 0.0351                    |
|                  | wR2 = 0.0911                    | wR2 = 0.0721                   |
| R indices (all data) | R1 = 0.1161                    | R1 = 0.0661                    |
|                  | wR2 = 0.1110                    | wR2 = 0.0835                   |
| Largest diff. peak and hole/ e. Å⁻³ | 0.815, -0.625                | 0.369, -0.522                  |
| Temperature/ K    | 100(2)                         | 100(2)                         |
**Figure 50.** Structure of compound 2b. Hydrogen atoms are removed for clarity. The atoms were refined without using any restraints or constraints. Selected bond lengths [Å] and Angles [deg]: P1-C1 1.793(3), P1-C6 1.799(3), P1-C13 1.797(3), P1-C20 1.788(3), C1-P1-C6 112.15(14), C1-P1-C13 107.82(14), C1-P1-C20 109.20(14), C6-P1-C13 08.22(13), C6-P1-C20 108.31(14), C13-P1-C20 111.16(14).

**Figure 51.** Structure of compound 3b. Hydrogen atoms are removed for clarity. The bromide ion was disordered over 2 positions with an occupancy of 1:1. The atoms were refined without using any restraints or constraints. Selected bond lengths [Å] and Angles [deg]: P1-C1 1.802(3), P1-C9 1.792(3), P1-C17 1.804(3), P1-C25 1.798(3), C1-P1-C9 110.04(13), C1-P1-C17 107.86(13), C1-P1-C25 107.23(12), C9-P1-C17 107.19(13), C9-P1-C25 110.85(13), C17-P1-C25 113.61(13).
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