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Electronic Supplementary Information.

Effect of Methyl and Halogen Substituents on the Transmembrane Movement of the Lipophilic Ions

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Synthesis.

General procedure of synthesis of dodecyl(triphenyl)phosphonium bromides with different substitutions in the phenyl ring (compound 1-6).

Substituted triphenylphosphine (0.1-0.16 mmol) in a mixture with excess of 12-bromdecane (0.15-0.6 mmol) in the presence of ethanol (100 μl) and two-three drops of acetic acid heated in a hermetically closed flask at 80°C for 48 hours. Upon completion of the reaction the product was precipitated with excess of ether or hexane, decanted if necessary several times, and sediment after centrifugation dried dryness. The yealds of the compounds **1-6** were 50-70% of the theoretical values. The target substances were identified using TLC in the dichloromethane - methanol – trifluoracetic acid (100:10:0.1) (DMT) as eluent by UV- Brumberg hemiscope and Dragendorf reagent. If necessary, compounds were purified on chromatographic column of silica gel in suitable system. Chromatographic mobility and molecular mass were determined for purified substances by using TLC and LCMS-method (Liquid Chromatography Mass Spectrometry), respectively.

The following compounds **1-6** (Figure 1A) were synthesized according to described method:

(1) *n*-Dodecyl[tri(*p*-chlorophenyl)]phosphonium bromide

The yield 57.6%, R_f (DMT) 0.58. Calculated mass for $C_{30}H_{37}Cl_3P$ M=534.95; found mass M=535.1

(2) *n*-Dodecyl[tri(*p*-fluorophenyl)]phosphonium bromide

The yield 63%, R_f (DMT) 0.79. Calculated mass for $C_{30}H_{37}F_3P$ M=485.58; found mass M=485.4

(3) *n*-Dodecyl[tri(*p*-tolyl)]phosphonium bromide

The yield 69%, R_f (DMT) 0.55. Calculated mass for $C_{33}H_{46}P$ M=473.69; found mass M=473.8

(4) *n*-Dodecyl[tri(*p*-methoxyphenyl)]phosphonium bromide

The yield 69%, R_f (DMT) 0.52. Calculated mass for $C_{33}H_{46}O_3P$ M=521.69; found mass M=521.3

(5) *n*-Dodecyl[tris(2,4,6-trimethylphenyl)]phosphonium bromide

The yield 52%, R_f (DMT) 0.71. Calculated mass for $C_{39}H_{58}P$ M=557.85; found mass M=557.9

(6) *n*-Dodecyl[tri(3,5-dimethylphenyl)]phosphonium bromide

The yield 50%, R_f (DMT) 0.65. Calculated mass for $C_{36}H_{52}P$ M=515.79; found mass M=515.64.

Synthesis of *n*-dodecyl(trinaphthyl)phosphonium bromide (7) was performed analogously to described earlier for compounds (**1-6**) with some modifications.

The mixture of 59 mg (0.14 mmol) of trinaphthylphosphine, 250 mg (1 mmol) of 12-bromododecane was heated with 100 μl of ethanol, 200 μl of dimethylformamide, toluene and three drops of acetic acid in a tightly closed flask at 80°C for 48 hours. Then the reaction mixture was evaporated in vacuum, the residue was diluted in minimal quantity of dichloromethane and precipitated by excess of hexane. After decantation the precipitate was dried in vacuum desiccator. The yield 51 mg (55%), R_f (DMT) 0.65, calculated mass for $C_{42}H_{48}P$ M=581.80; found mass M=581.7.

Octanol–water partition coefficient

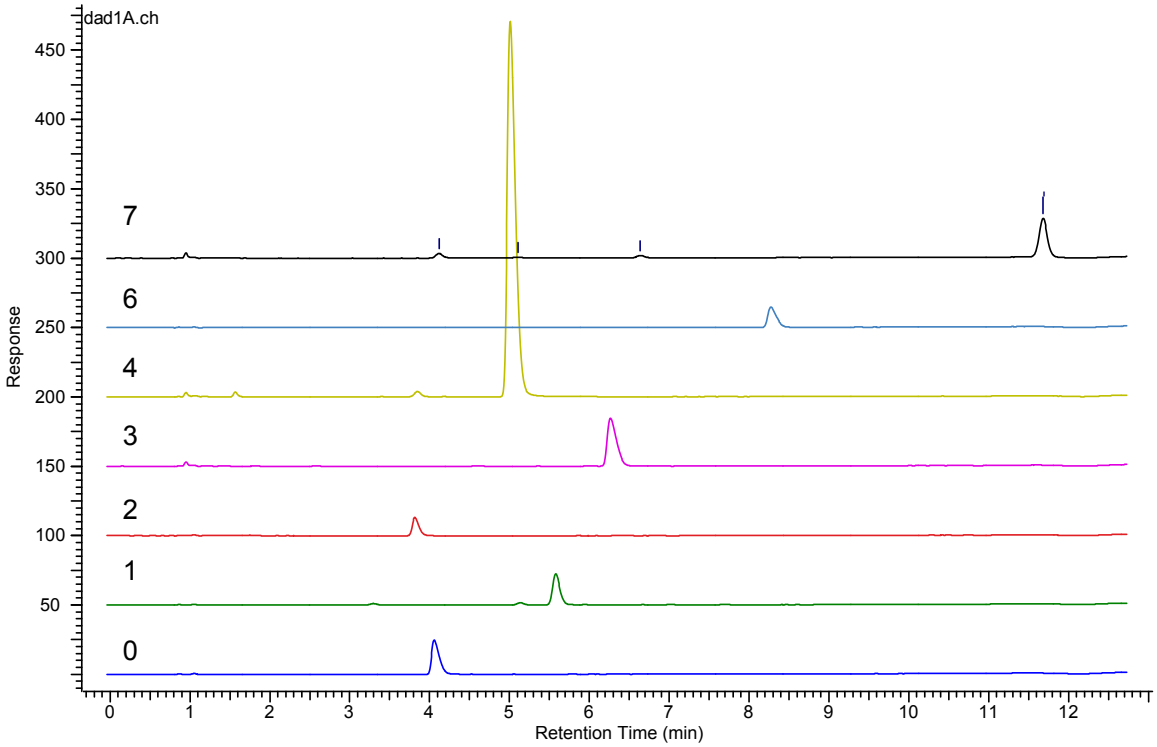
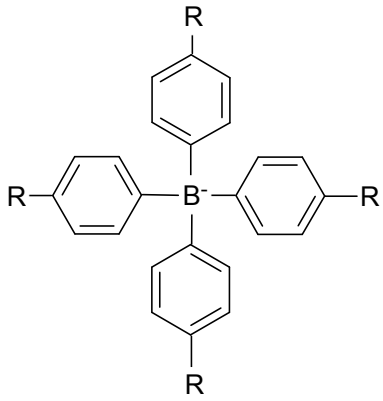


Figure S1. The chromatograms of compounds **0-7** in a reverse-phase high-performance liquid chromatography (HPLC) column.

Table S1. Experimental results from a reverse-phase high-performance liquid chromatography (HPLC) column and calculated octanol-water partition coefficient for compound **0-4**, **6**, **7**.

Name	t_R, min	log(t_R-t_0)	logP_{ow}	P_{ow}
0	4.061	2.264015	3.56	3.6E+03
1	5.578	2.438827	3.81	6.5E+03
2	3.815	2.22763	3.50	3.2E+03
3	6.266	2.499632	3.90	7.9E+03
4	5.014	2.381729	3.73	5.3E+03
6	8.273	2.639865	4.10	1.3E+04
7	11.678	2.806641	4.35	2.2E+04

Current relaxation measurements on the bilayer lipid membrane in the presence of tetraphenylborate and its analogues. Estimation of the flip-flop constant.



Scheme S1. Chemical structure of tetraphenylborate (R=H, TPB) and its analogues: tetrakis-(4-fluorophenylborate) (R=F, TPFB), tetrakis-(4-chlorophenylborate) (R=Cl, TPCB) and tetrakis-(4-methylphenylborate) (R=CH_3, TPMB).

Planar bilayer lipid membranes (BLM) were formed from a 2% solution of 1,2-di-O-phytanoyl-sn-glycero-3-phosphocholine (DPhPC) in n-decane (Avanti Polar Lipids, Alabaster, AL) as previously described.

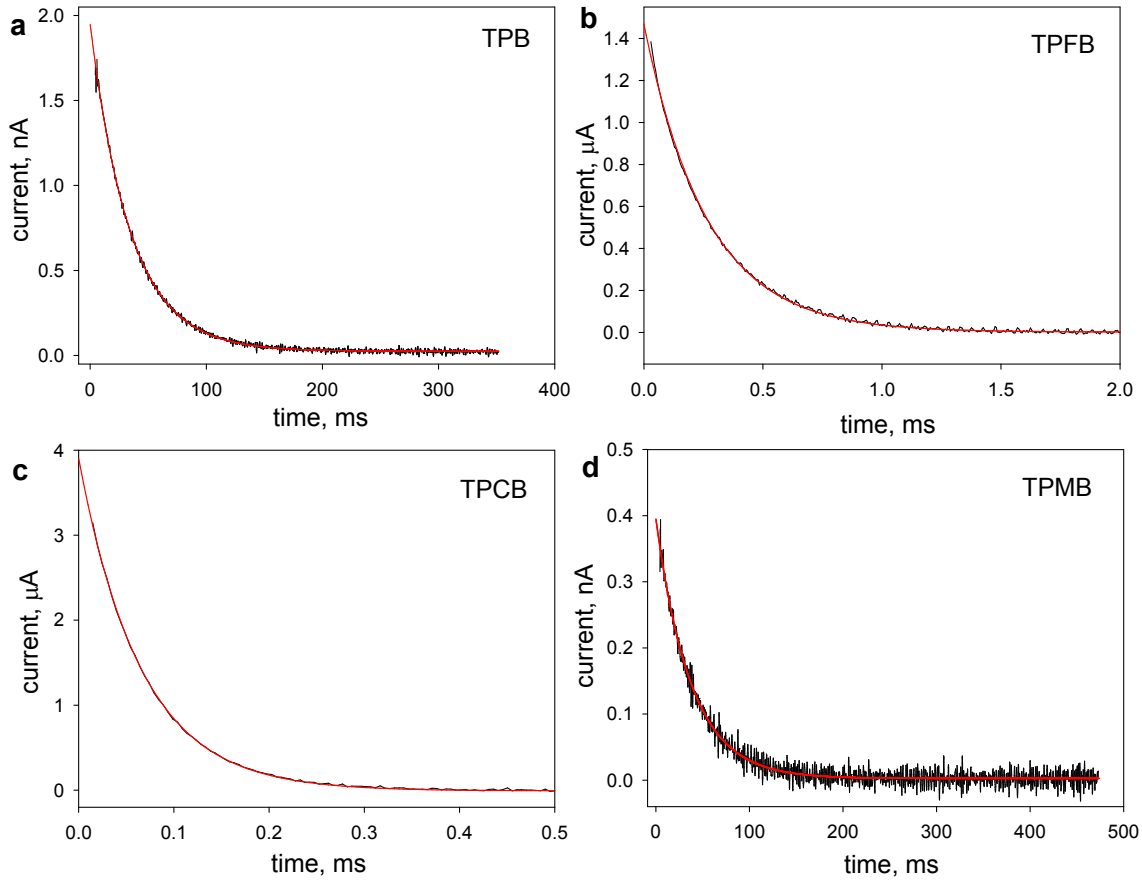


Figure S2. Time courses of electrical current through planar BLMs after application of a voltage jump from 0 mV to 25 mV (black curves) and their best fit by a monoexponential function (red curves) in the presence of 0.1 µM TPB (a, τ=34.5 ms), 0.1 µM TPFB (b, τ=265 µs), 0.1 µM TPCB (c, τ=65.5 µs) and 0.2 µM TPMB (d, τ=37.5 ms).

Free energy differences for the transfer of the C_{12}TPP, TPB and their analogues across membranes calculated from experimental data.

In order to compare the experimental data with the results of the theoretical calculations of the free energies of solvation, the binding constant, *K*, and the free energy of adsorption, ΔG, were calculated by using the partition coefficient γ between aqueous and lipid phases, according to

$$K = \gamma / l_a \quad (3)$$

$$\Delta G = -RT \ln K \quad (4),$$

where l_a is equal to the thickness of the adsorption plane of charge groups in the lipid membrane. It was assumed that l_a approximately equal to 0.5 nm (corresponding to the thickness of the polar headgroup layer). It is evident from a comparison of the data on Table 1 that analogues with larger rate constant of transfer have smaller partition coefficients. For the most hydrophobic derivatives the value of γ are reduced by two orders of magnitude. This could be caused by a small shifts of the adsorption planes as a results of higher hydrophobicity of certain derivatives or could be related to the aggregation of the lipophilic ions in the aqueous solution which should increase upon the increase of the hydrophobicity. The change of the free energy difference, ΔG^0, between the aqueous phase and the center of the membrane may be calculated as proposed by Benz, 1988, Biophys. J. 54:25-33.

$$\Delta G^0 = -RT \ln(RT \times G_{0,0} \times d / (F^2 \times D \times c)) \quad (5),$$

where $G_{0,0}$ is the initial ohmic conductance at zero voltage, *d* is the membrane thickness and equal to 5 nm, and D=10^{-6} cm^2/s is the diffusion coefficient.[1,2] Introducing the expression for $G_{0,0}$ [3] the equation becomes

$$\Delta G^0 = -RT \ln(\gamma \times k_i \times d / D) \quad (6),$$

$$\Delta G^0 - \Delta G = -RT \ln(k_i \times d \times l_a / D) \quad (7).$$

It should be noted that according (7) a value of the central barrier (ΔG^0 - ΔG) for lipophilic ions is independent of the partition coefficient. Table S2 shows the values for ΔG, ΔG^0 and for absolute height of the central barrier of some analogues with similar partition coefficients (**0**, **3** and **4**) for membrane formed from DPhytanylPC. Table S3 represents the same parameters of C_{12}TPP analogues (**1**, **2**, **3** and **4**) for membrane formed from DPhytanylPC and modified by 5 μM of phloretin.

Table S2. Calculated values of the adsorption constant and free energy differences of the C_{12}TPP analogues on BLM from diphytanylphosphotidylcholine.

compound	$\gamma/10^{-2}$, cm	K/10^4	ΔG, kJ/mol	k_i, s^{-1}	ΔG^0, kJ/mol	ΔG^0-ΔG, kJ/mol
0	1.7	34	-31.4	0.075	18.1	49.6
3	0.8	16	-29.6	2.1	11.8	41.4
4	0.64	12.8	-29	0.19	18.3	47.3

Table S3. Calculated values of the adsorption constant and free energy differences of the C_{12}TPP analogues on BLM from diphytanylphosphotidylcholine, modified by 5 μM of phloretin.

compound	$\gamma/10^{-3}$, cm	K/10^4	ΔG, kJ/mol	k_i, s^{-1}	ΔG^0, kJ/mol	ΔG^0-ΔG, kJ/mol
0	4.7	9.4	-28.3	1.7	13.6	41.9
1	2.5	5	-26.7	0.1	22.2	48.9
2	4.0	8	-27.9	0.02	25.0	52.9
3	3.7±0.4	7.3	-27.7	44.2	6.2	33.85
4	3.6±0.5	7.2	-27.6	5.3	11.5	39.1

Parameters of current relaxation after voltage jump, thermodynamic parameters of the adsorption and free energy differences for the movement of the TPB analogues across membranes made from 1,2-di-O-phytanoyl-sn-glycero-3-phosphocholine are summarized in Table S4.

Table S4

compound	$\gamma/10^{-2}$, cm	$K/10^4$	ΔG, kJ/mol	k_i, s^{-1}	ΔG^0, kJ/mol	$\Delta G^0-\Delta G$, kJ/mol
TPB	1.3	26	-30.8	10.2	6.7	37.5
TPFB	2.3	46	-32.2	1780	-7.5	24.7
TPCB	1.7	34	-31.4	7600	-10.3	21.1
TPMB	0.12	2.4	-24.9	10.9	12.4	37.3

1 R. Benz, P. Lauger and K. Janko, *Biochim. Biophys. Acta*, 1976**, 455***,* 701-720.

2 R. Benz, *Biophys. J*, 1988**, 54***,* 25-33.

3 B. Ketterer, B. Neumcke and P. Lauger, *J Membrane Biol.*, 1971**, 5***,* 225-245.

Theoretical calculations of the free energies of solvation for TPB and analogues.

Table S5. Free energy barrier values (ΔG^0-ΔG) estimated from experimental data on BLM formed from DPhytanoylPC for TPB and analogues and difference between the free energies of solvation in octane and water (ΔG_s^{oct} -ΔG_s^{wat}) calculated using DFT/PCM.

Name	ΔG^0-ΔG, kJ/mol BLM experiments	Name	ΔG_s^{oct}, kJ/mol	ΔG_s^{wat}, kJ/mol	ΔG_s^{oct} -ΔG_s^{wat}, kJ/mol
TPB	37.5	Me-TPB$^-$-H	-119.7	-176.6	56.9
TPFB	24.7	Me-TPB$^-$-F	-105.3	-145.1	39.9
TPCB	21.1	Me-TPB$^-$-Cl	-112.2	-139.0	26.8
TPMB	37.3	Me-TPB$^-$-CH_3	-123.4	-176.0	52.6