Phospholipid Research Center

Synthetic alternatives to natural tetraether lipids – chance or illusion?

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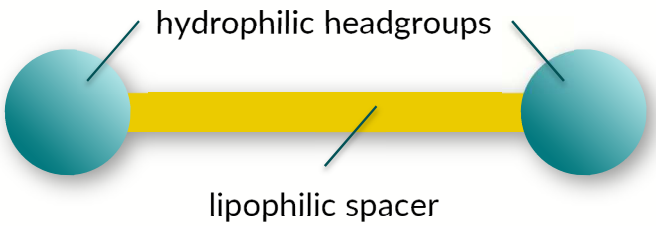
OUTLINE

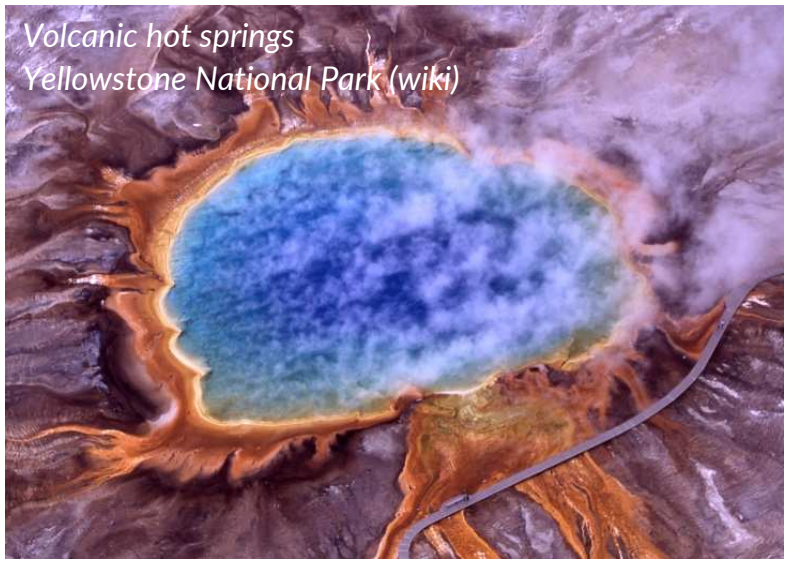
- Short introduction … what are bolalipids?
- The synthesis of natural occurring TELs
- Simplifications of the chemical structure
- How simple can you go?
- Summary

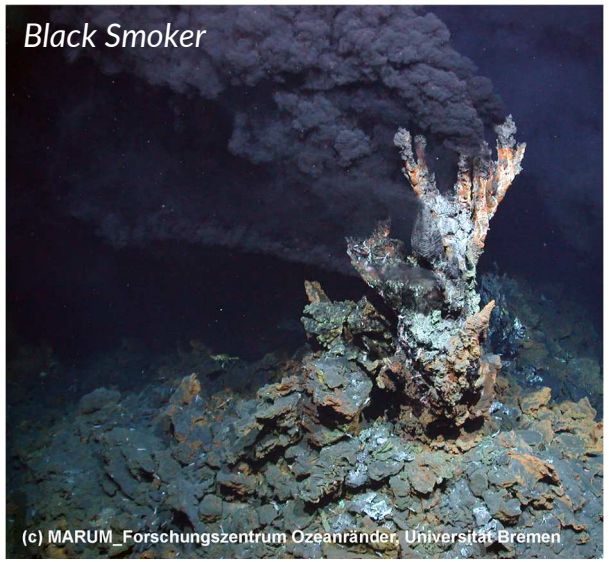
What are bolalipids?

- Bipolar (phospho)lipids – including TELs
- Originate in membrane lipids of Archaea



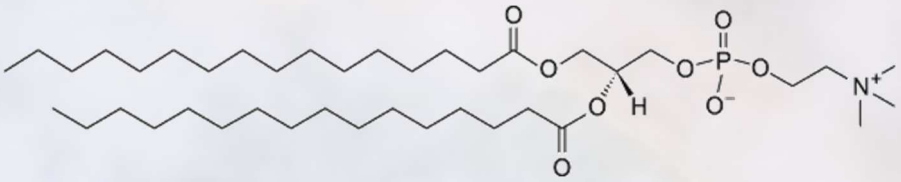
- Archaea thrive under extreme living conditions:
  - *Methanogenic* - anaerobic milieu
  - *Thermoacidophiles* - high temperatures and low pH values



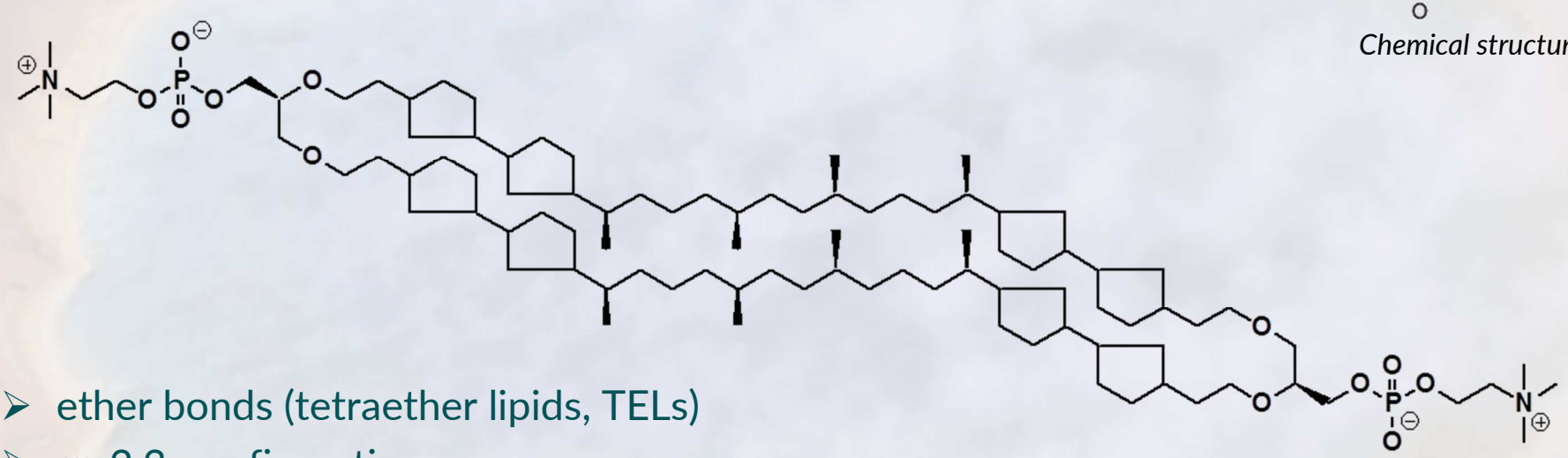


What are bolalipids?

- Reasons for the **extraordinary stability**



Chemical structure of DPPC



Chemical structure of a natural bolalipid

  - ether bonds (tetraether lipids, TELs)
  - *sn*-2,3-configuration
  - membrane-spanning
  - methyl branches and 5-membered rings

- several groups use **natural** as well as **artificial** bolalipids (TELs):
  Bakowsky, Benvegnu, Fischer, Fricker, Treusch, Uhl, Xu, Yang; NovoArc

The synthesis of natural occurring TELs

- First by **Katsumi Kakinuma:** Total Synthesis of Archaeal 72-Membered Macrocyclic Tetraether Lipids. ***J. Org. Chem.* 1998**, 63, 2689-2698.

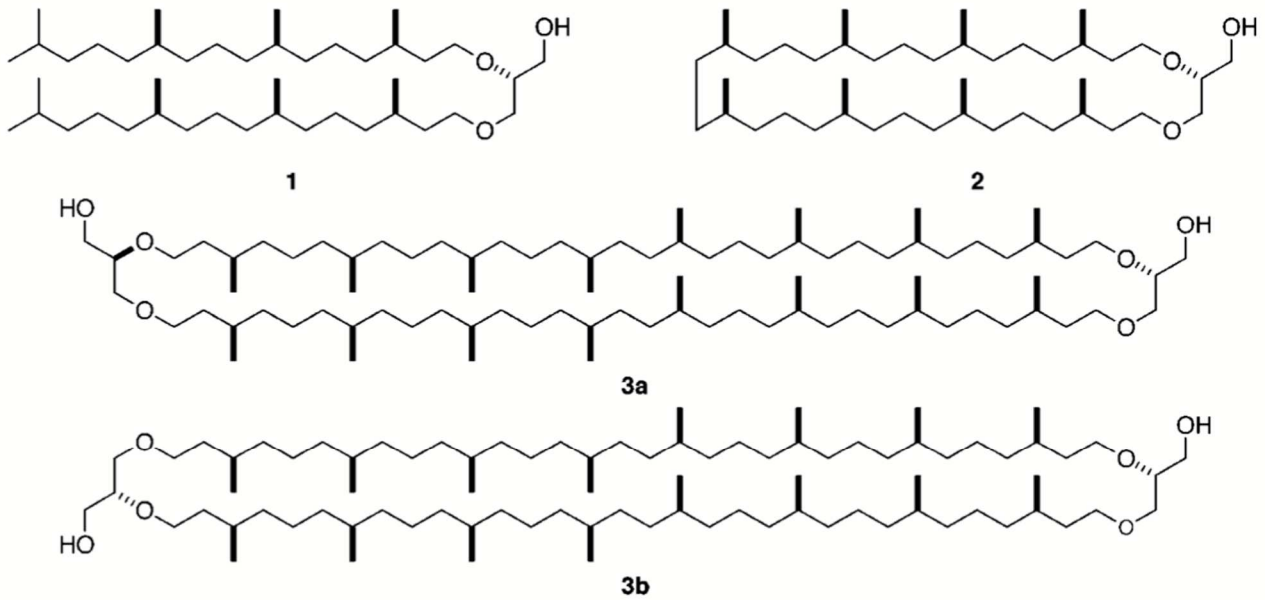


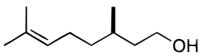
Figure 1. Typical structures of archaeal membrane lipids **1**, **2**, and **3ab**.

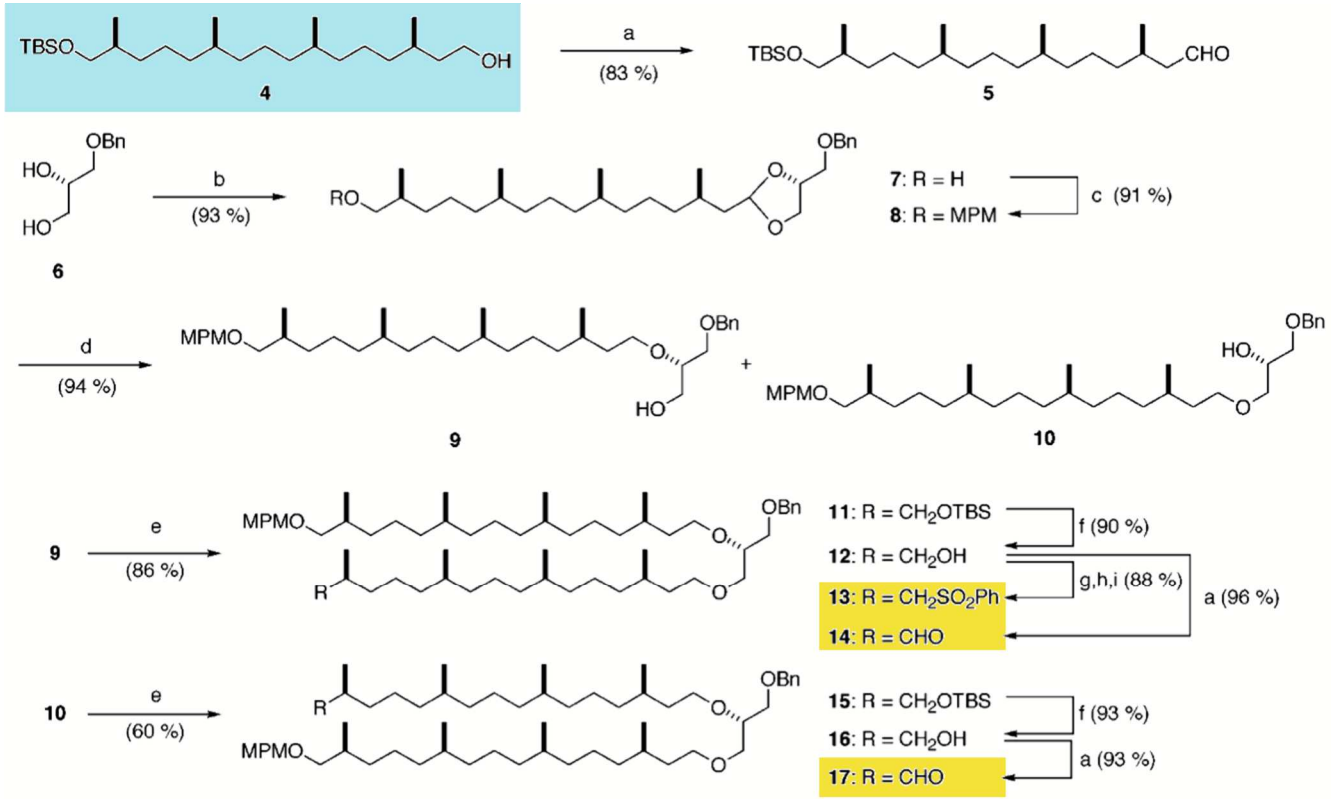
The synthesis of natural occurring TELs

- **First by Katsumi Kakinuma:** Total Synthesis of Archaeal 72-Membered Macrocyclic Tetraether Lipids. *J. Org. Chem.* **1998**, 63, 2689-2698.

Starting material (**4**) from: Total Synthesis of Archaeal 36-Membered Macrocyclic Diether Lipid. *J. Org. Chem.* **1997**, 62, 1924-1933.

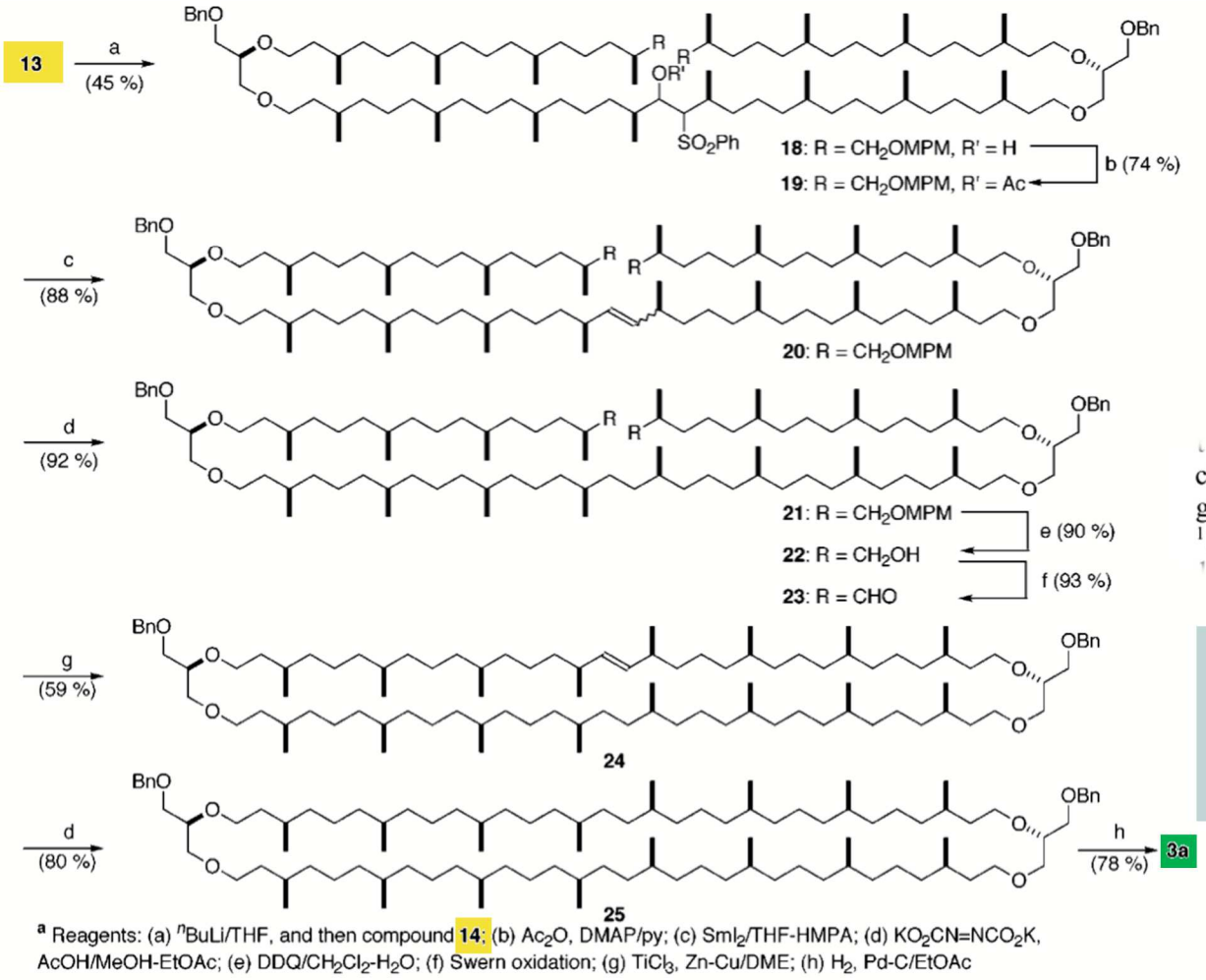
18 steps (!) starting from (*R*)-citronellol





[a]Reagents: (a) Swern oxidation; (b) (1) compound **5**, *p*-TsOH, $MgSO_4/CH_2Cl_2$, (2) TBAF/THF; (c) NaH, MPMCl/DMF; (d) DIBAH/toluene; (e) NaH, mesylate of **4**/DMSO; (f) TBAF/THF; (g) MsCl, Et_3N/CH_2Cl_2; (h) PhSH, K_2CO_3/DMF; (i) *m*-CPBA/CH_2Cl_2

The synthesis of natural occurring TELs



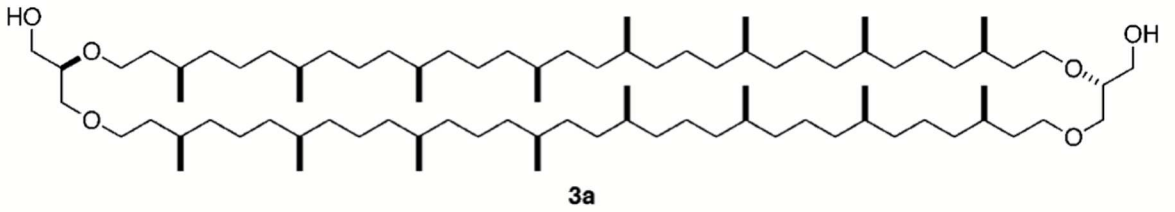
[a] Reagents: (a) ^{n}BuLi/THF, and then compound **14**; (b) Ac_2O, DMAP/py; (c) SmI_2/THF-HMPA; (d) $KO_2CN{=}NCO_2K$, AcOH/MeOH-EtOAc; (e) DDQ/CH_2Cl_2-H_2O; (f) Swern oxidation; (g) $TiCl_3$, Zn-Cu/DME; (h) H_2, Pd-C/EtOAc

chromatographed over silica gel with hexanes−EtOAc (5:1) to give **3a** (50 mg, 78%) as an oil: $[\alpha]^{25}_D$ +8.68 (*c* 1.68, $CHCl_3$); ^{1}H NMR (300 MHz) δ 0.80−0.92 (m, 48H), 0.97−1.68 (m,

17 steps (!) starting compound **4** with an overall yield of **3.7%**.

The synthesis of natural occurring TELs

- First by **Katsumi Kakinuma:** Total Synthesis of Archaeal 72-Membered Macrocyclic Tetraether Lipids. ***J. Org. Chem.* 1998**, 63, 2689-2698.

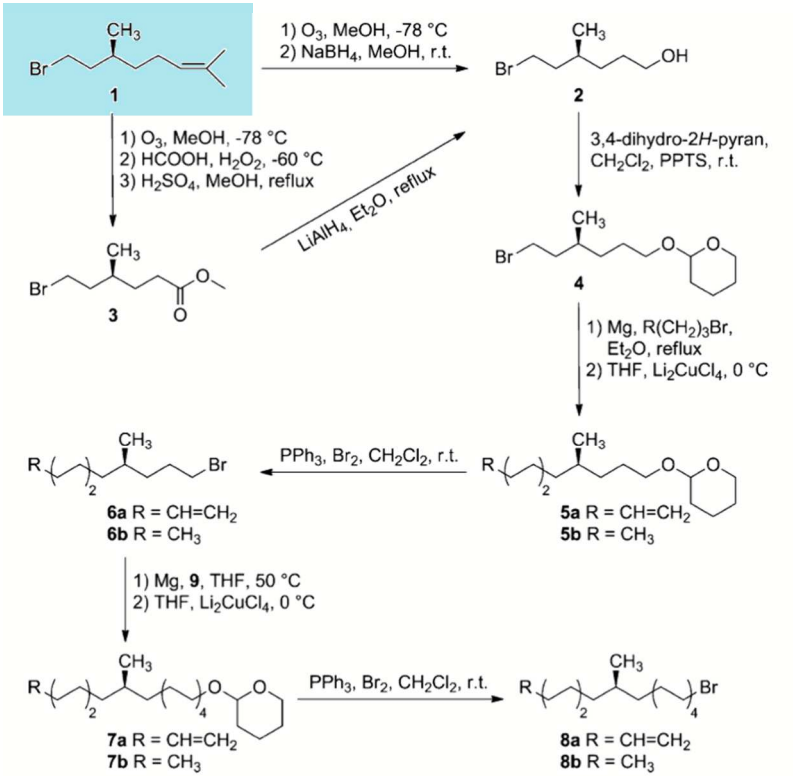


3a

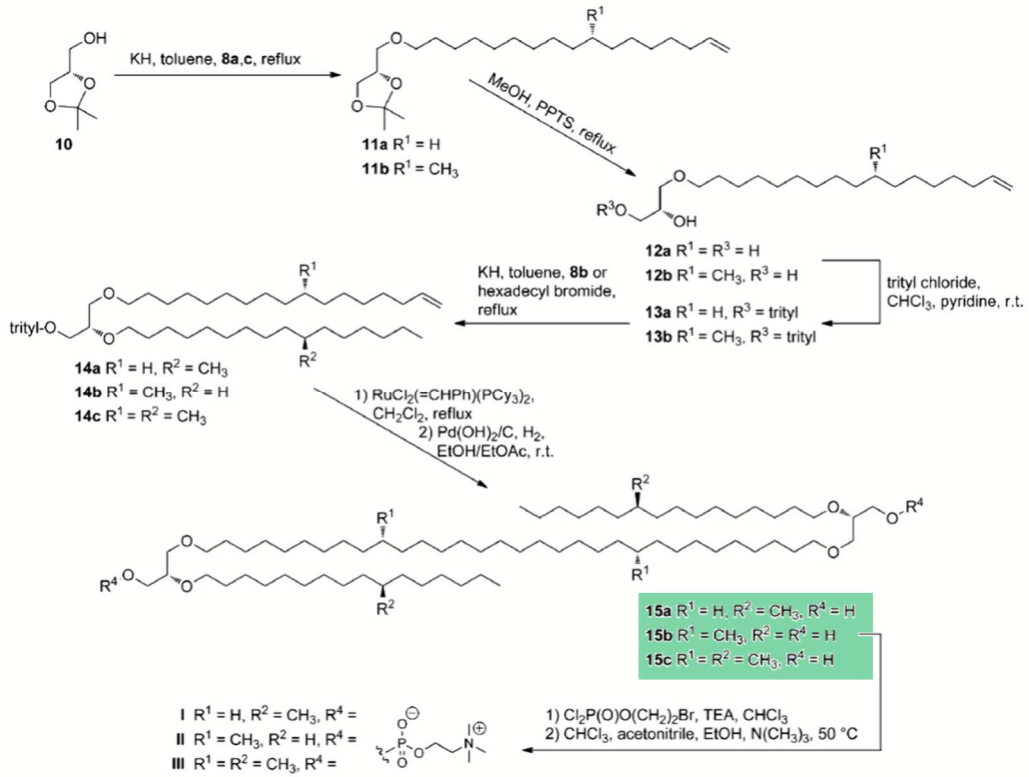
- Are **two membrane-spanning** alkyl chain necessary?
- Are **16** chiral methyl groups necessary?
- Are **chiral** methyl groups necessary at all? Or are **racemic** groups just as possible?

Simplifications of the chemical structure

- Synthesis by **Thomas Markowski**



Scheme 1. Synthesis of optically pure methyl-branched alkyl bromides **8a**,**b** (PPTS: pyridinium *p*-toluenesulfonate).



12 steps starting compound **1** with an overall yield of **1.5-5,7%**.

Markowski, Drescher et al. *Eur. J. Org. Chem.* **2011**, 5894.

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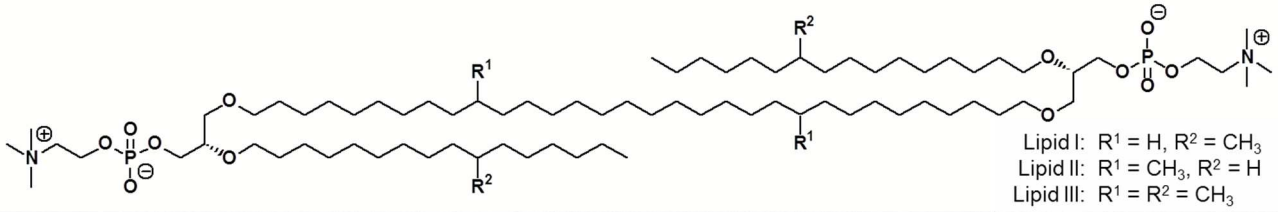
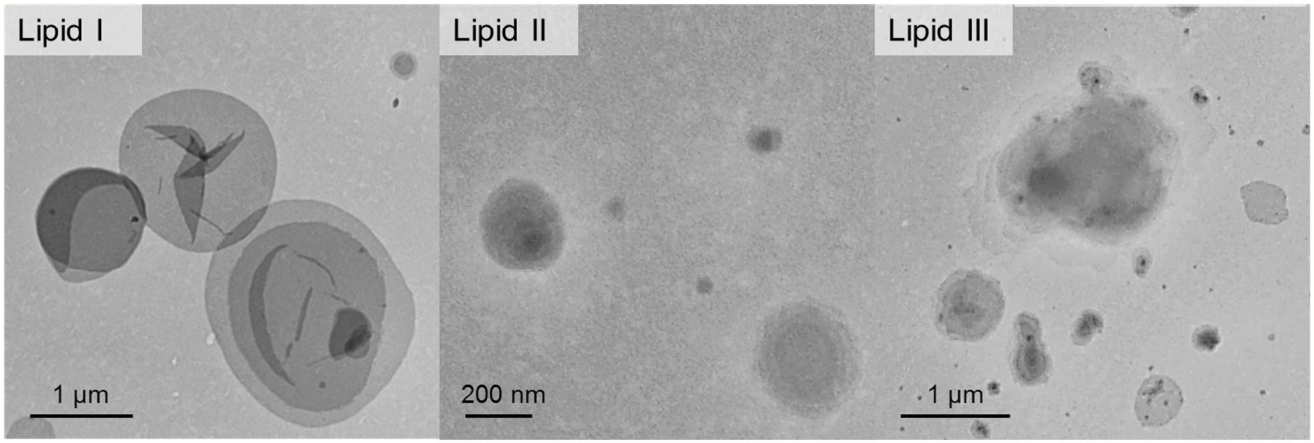
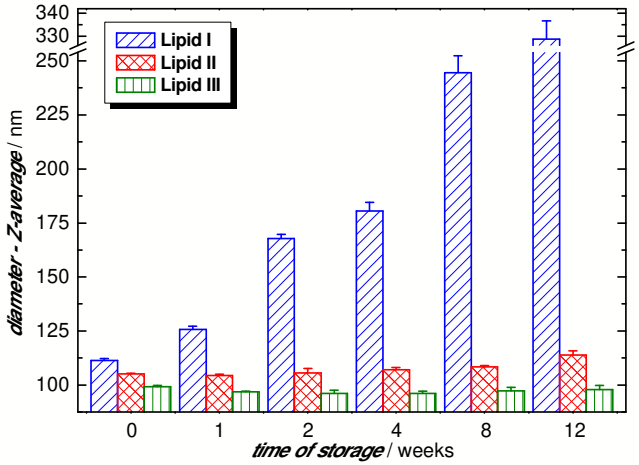
Simplifications of the chemical structure – does it work?

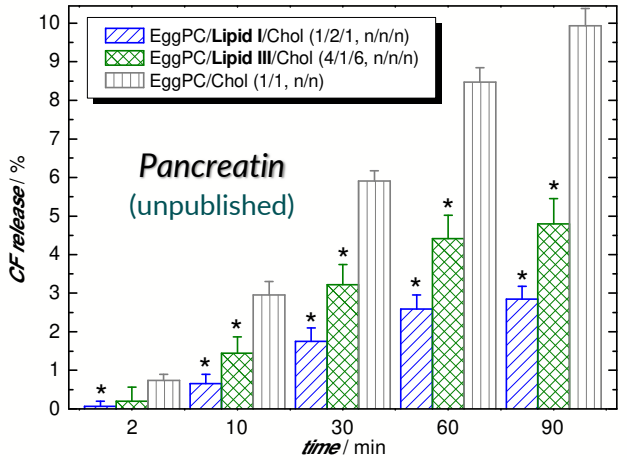
- Synthesis by **Thomas Markowski**





***TEM**: c = 0.03-0.10 mg/ml samples stained with UA (not extruded)*

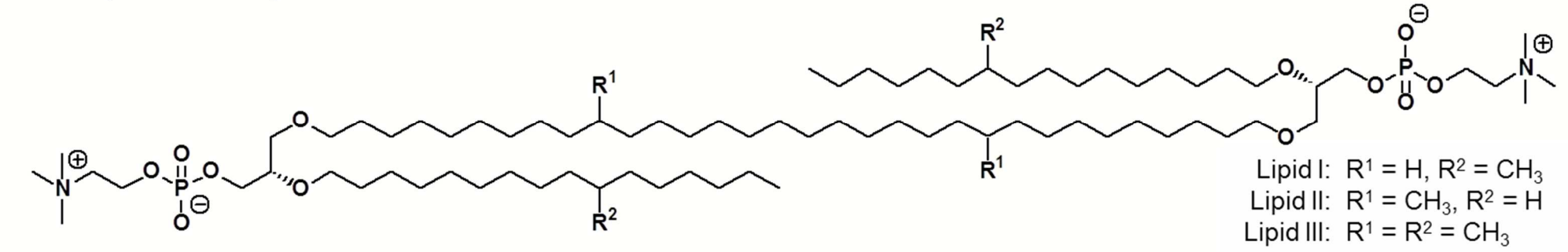




Markowski, Drescher et al. *Org. Biomol. Chem.* **2014**, 12, 3649.

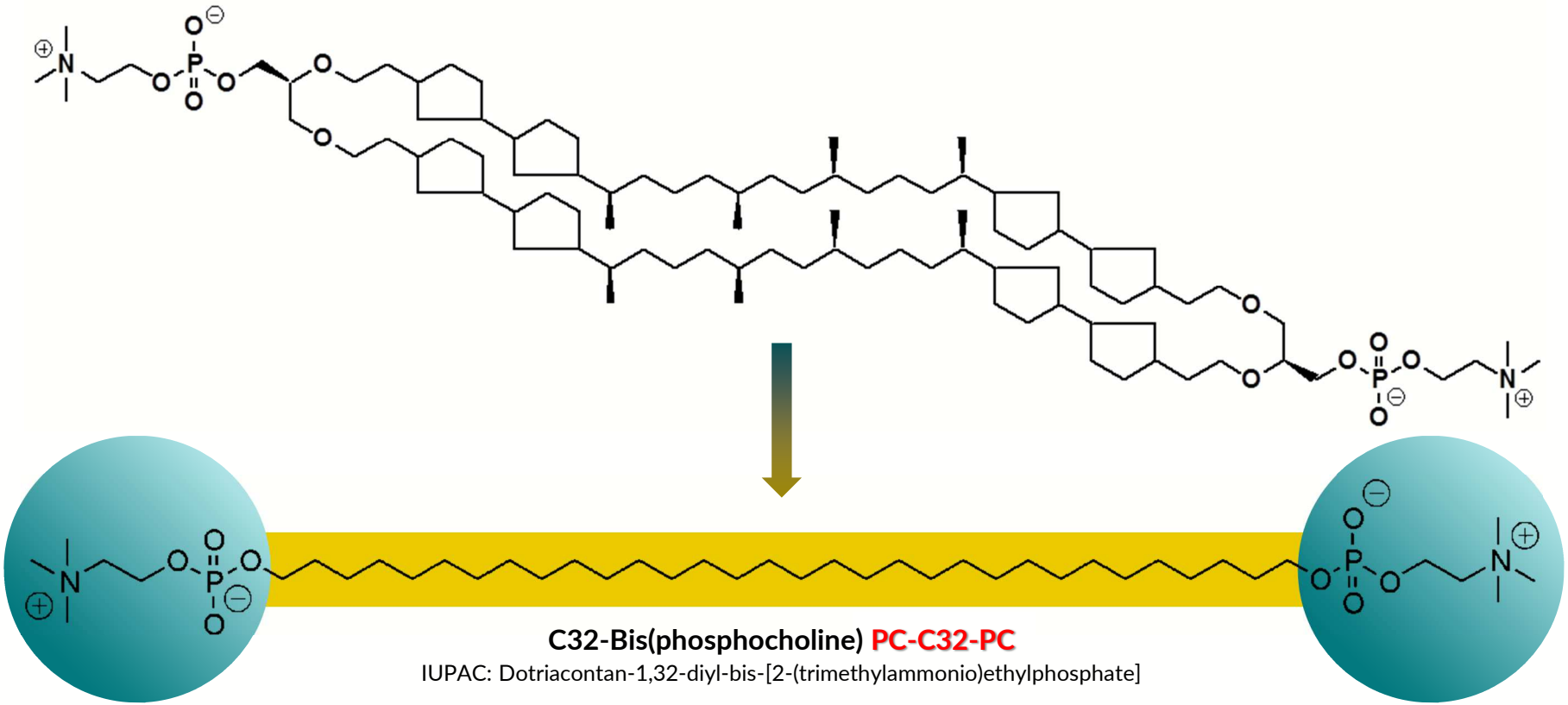
Simplifications of the chemical structure

- Synthesis by **Thomas Markowski**



- One **membrane-spanning** alkyl chain still works.
- **Two or four** chiral methyl groups are necessary.
- **Racemic** groups do the job as well.

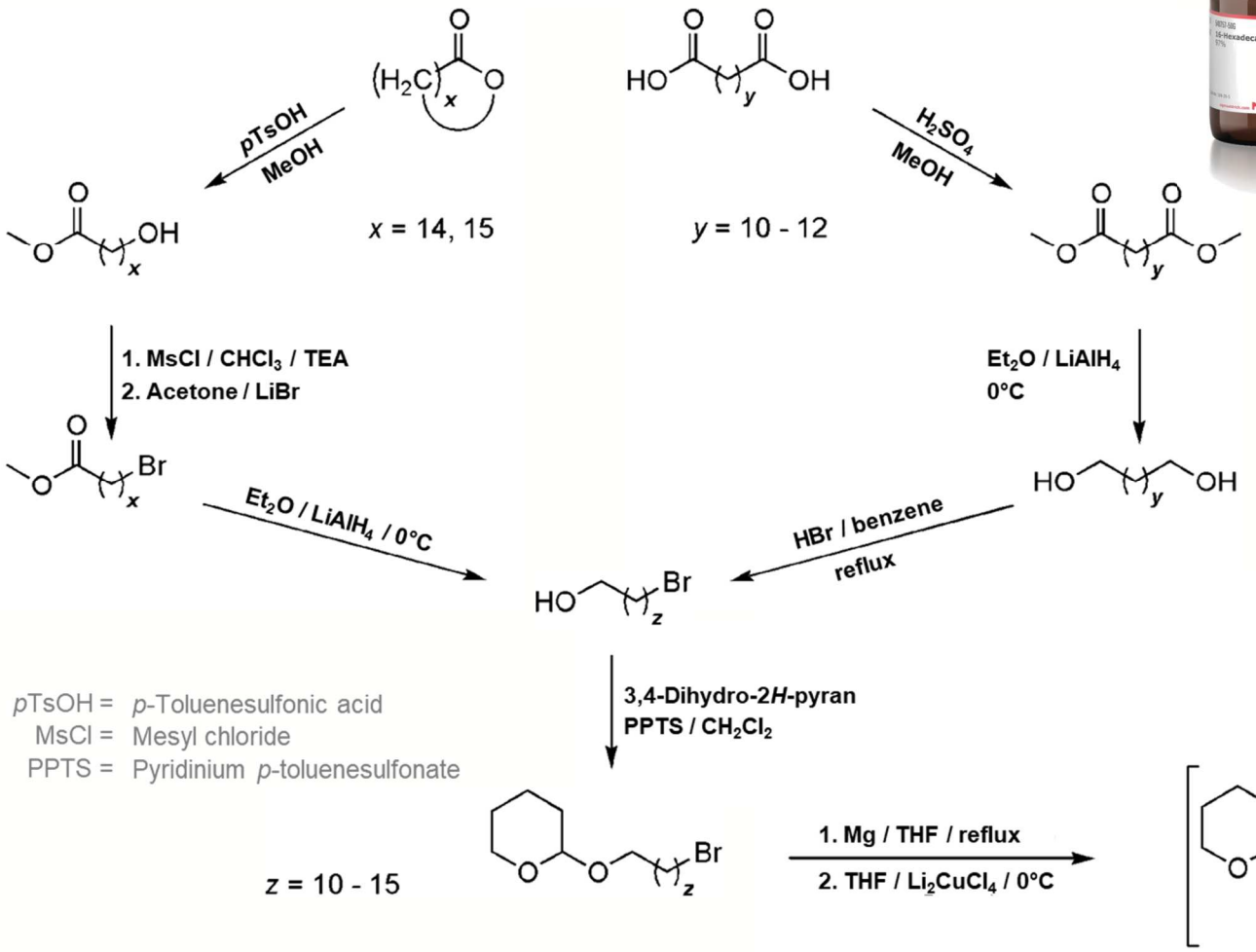
Simplifications of the chemical structure – How simple can you go?



C32-Bis(phosphocholine) PC-C32-PC

IUPAC: Dotriacontan-1,32-diyl-bis-[2-(trimethylammonio)ethylphosphate]

How simple can you go? Synthesis of PC-C32-PC



Overall yield ≈ **40–45 %**
Starting from 50 g educt
→ ca. 20 g HO-C32-OH

60–70 % yield after purification

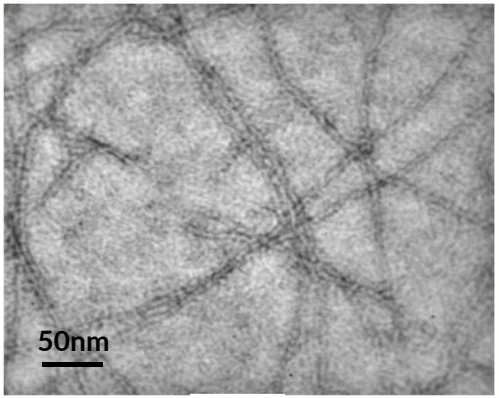
Drescher et al. *Chem. Eur. J.* **2007**, 13, 5300.

The model bolalipid PC-C32-PC

- Aggregation behavior
  - self-assembles into long, flexible *nanofibers* due to hydrophobic interactions
  - built up a dense network, which gels water very efficiently

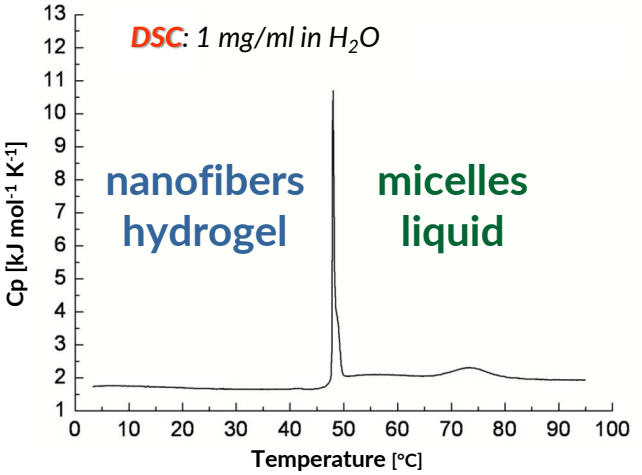
Köhler et al. *Angew. Chem. Int. Ed.* **2004**, 43, 245 | *J. Am. Chem. Soc.* **2004**, 126, 16804.
Drescher et al. *Chem. Eur. J.* **2007**, 13, 5300.

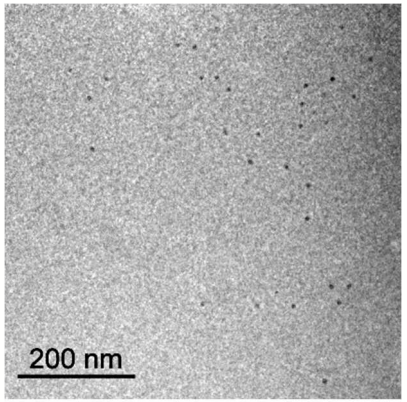
c = 1 mg/ml



***TEM**: c = 0.3 mg/ml, r.t.*

- Temperature dependency
  - heating leads to transformation into *small micelles*
  - after heating, gel character is lost
  - completely reversible

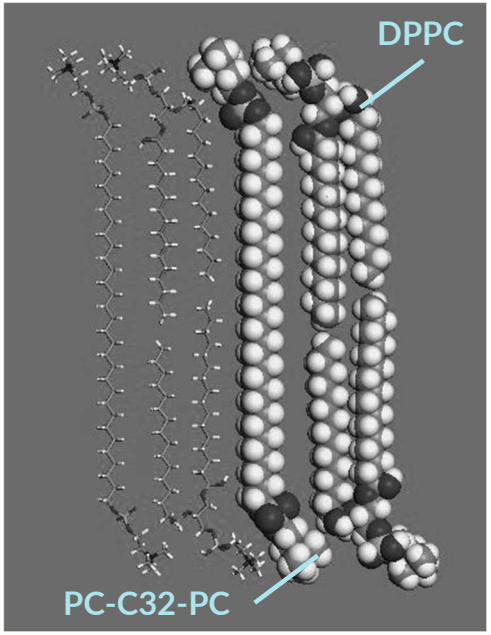


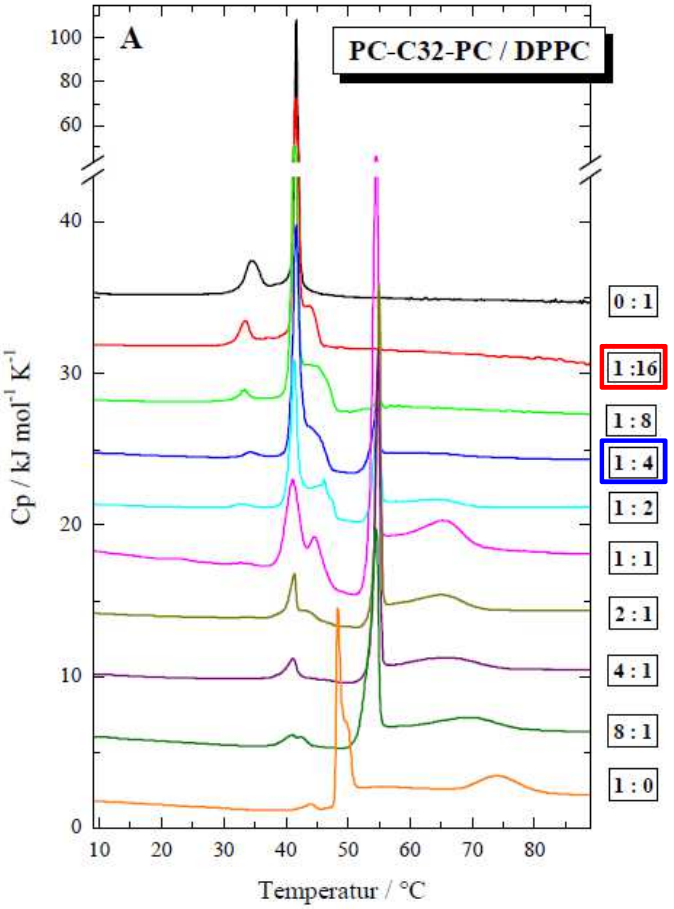


***cryo-TEM**: 1 mg/ml, 55°C*
(by Göran Karlsson, Uppsala)

The model bolalipid PC-C32-PC – the miscibility issue

- What about the miscibility with classical phospholipids?



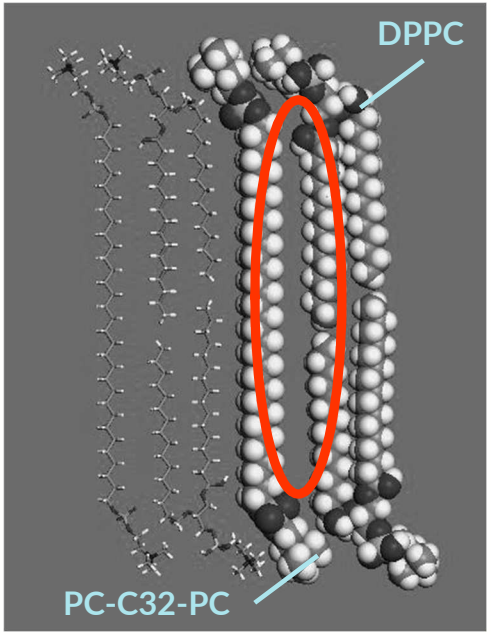


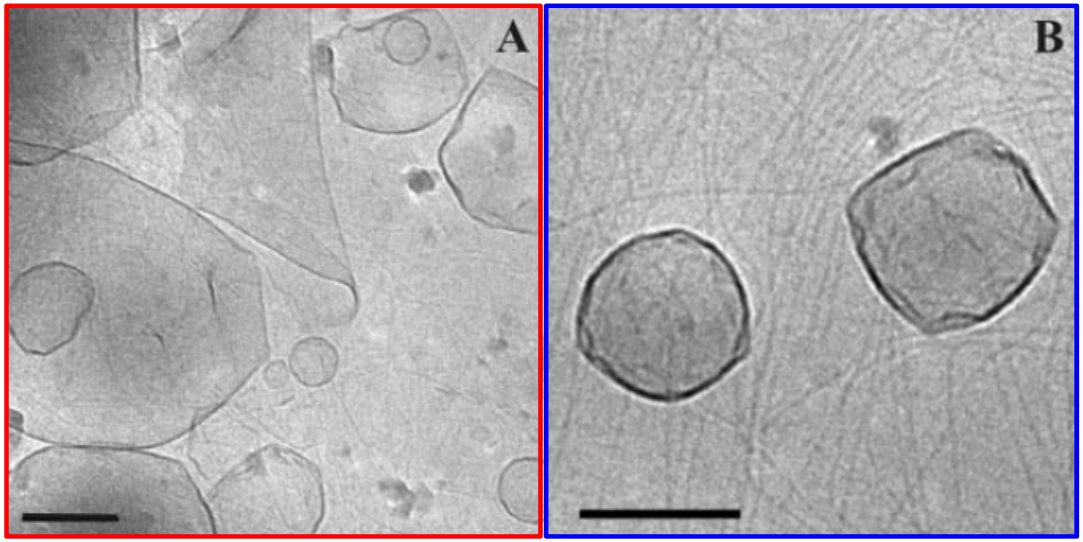
Meister, Köhler, Drescher et al. *Soft Matter* **2007**, 3, 1025.

***DSC**, 3mM in phosphate buffer pH 7.4*

The model bolalipid PC-C32-PC – the miscibility issue

- What about the miscibility with classical phospholipids?





***Cryo-TEM**: PC-C32-PC:DPPC mixtures (c = 3 mM, r.t.)*
(A) 1:16, (B) 1:4. The bar corresponds to 100 nm.

→ Only marginal incorporation of bolalipids into phospholipid (DPPC) bilayers – and *vice versa*.

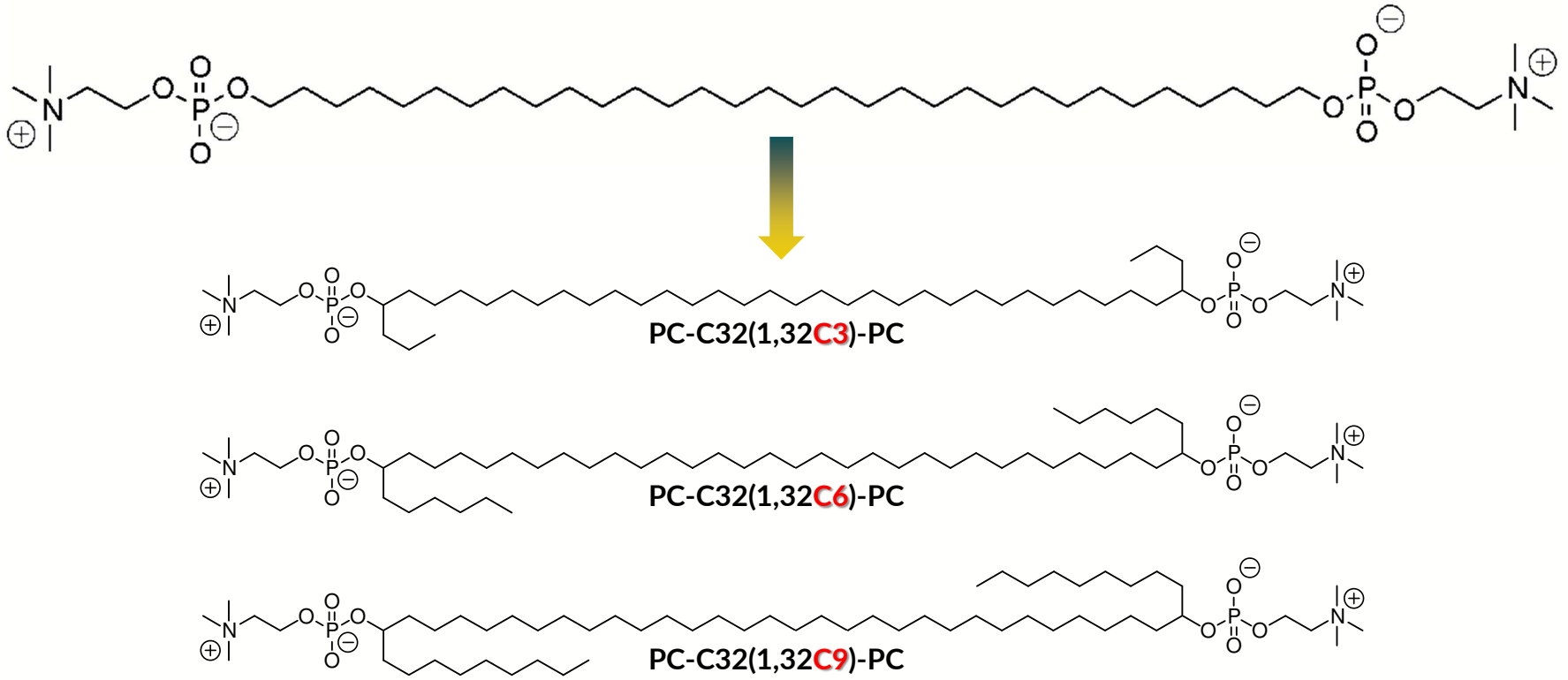
→ **No** liposome stabilization.

→ Reason: packing problems.

Meister, Köhler, Drescher et al. *Soft Matter* **2007**, 3, 1025.

Simplifications of the chemical structure – not that simple!

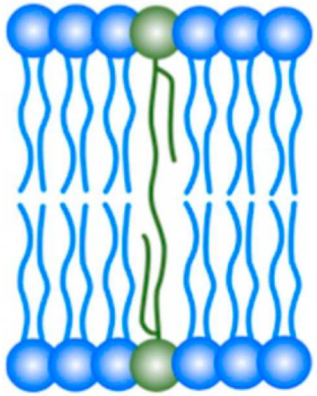
- Synthesis by **Kai Gruhle**



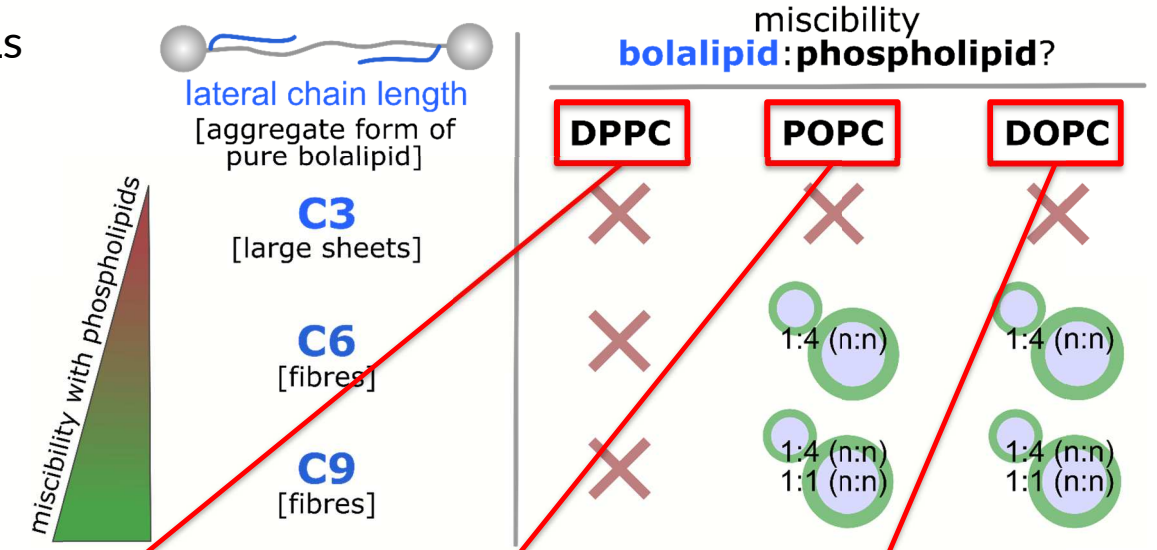
Gruhle, … Drescher *Org. Biomol. Chem.* **2018**, 16, 2711.

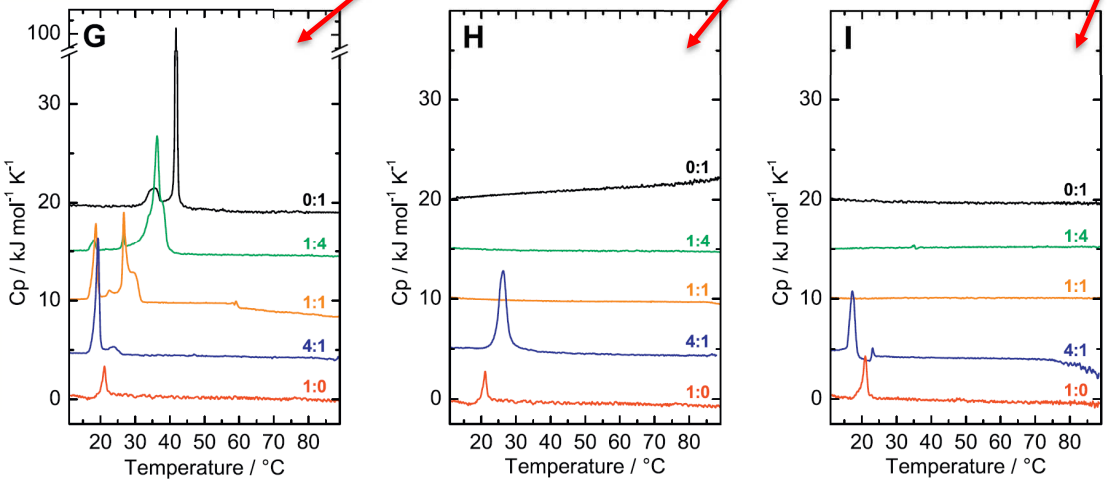
Bolalipids bearing lateral alkyl chains – the solution regarding miscibility?

- Mixing behavior with ‘classical’ PLs



Müller, … Drescher *Biophys. Chem.* **2019**, 244, 1.





DSC

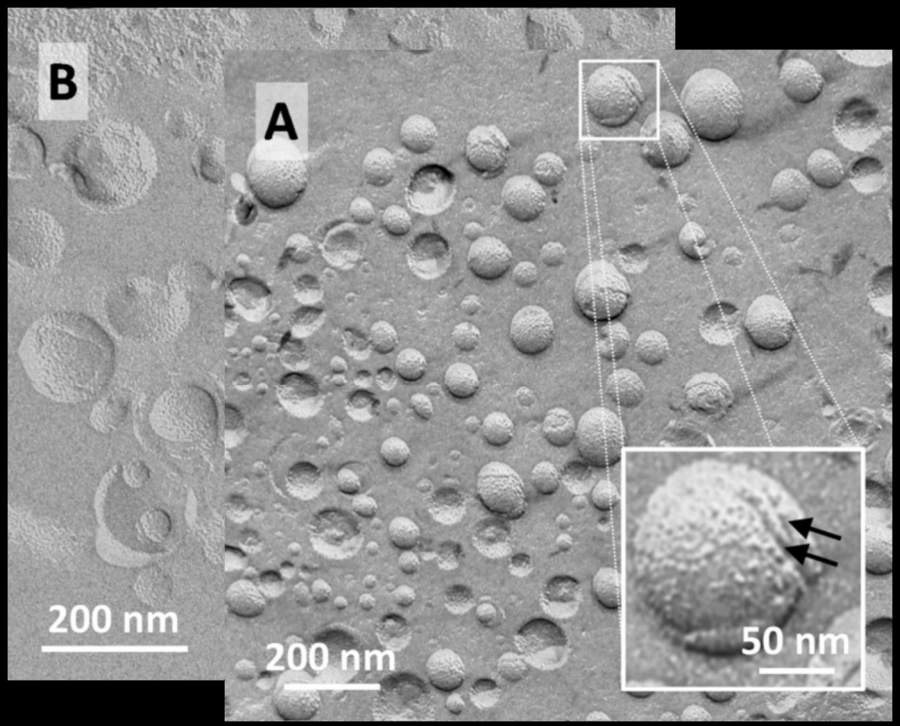
C9*-bolalipid:phospholipid,*
c = 3 mM in PBS pH = 7.4

Bolalipids bearing lateral alkyl chains – the solution regarding miscibility?

FFEM

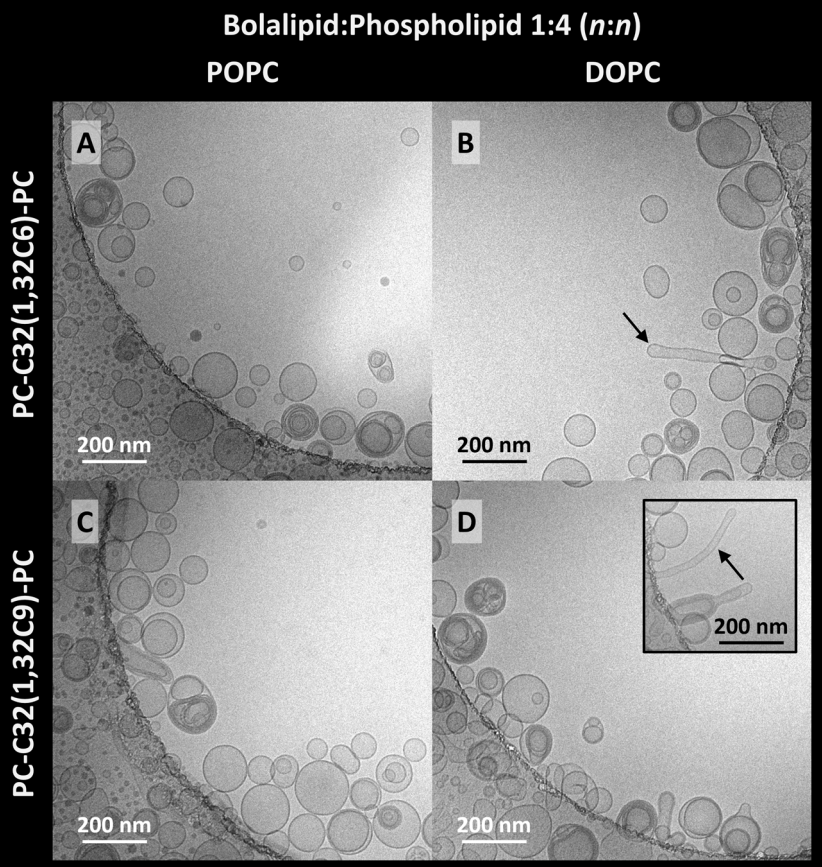
PC-C32(1,32C9)-PC : DOPC (1:4)

in PBS pH 7.4, c = 6 mM



Cryo-EM

c = 3 mM, in PBS pH 7.4



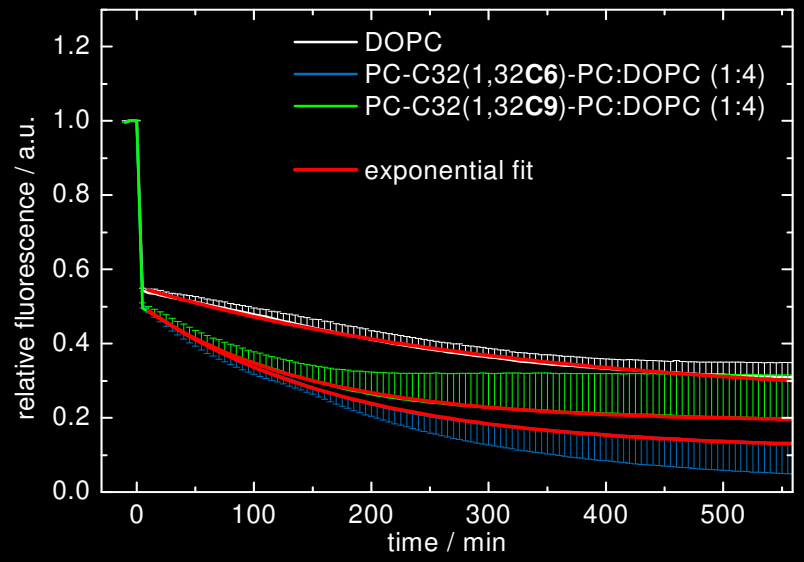
Müller, … Drescher *Biophys. Chem.* **2019**, 244, 1.

Bolalipids bearing lateral alkyl chains – the solution regarding miscibility?

Dithionite assay

c(lipid) = 3 mM, c(NBD-lipid) = 1 mol%,

T = 20 °C

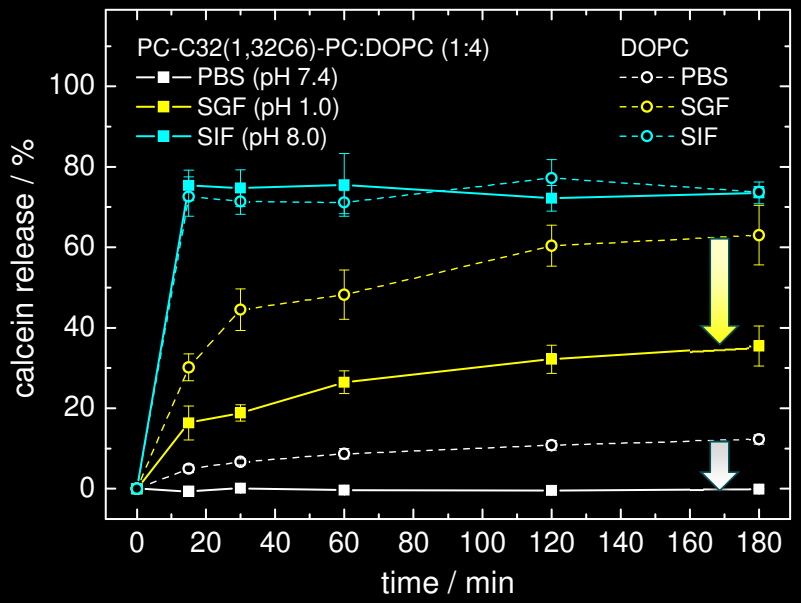


Liposomes	Rate constant *k*
DOPC	$3{,}0 \times 10^{-3}$ min^{-1}
PC-C32(1,32C6)-PC:DOPC	$5{,}8 \times 10^{-3}$ min^{-1}
PC-C32(1,32C9)-PC:DOPC	$7{,}1 \times 10^{-3}$ min^{-1}

Müller, … Drescher *Pharmaceutics* **2019**, 11, 646.

Calcein release assay

c = 3 mM, T = 37 °C



SGF: simulated gastric fluid (HCl, NaCl, KCl, pH ≈ 2; pepsin 1 mg/ml)

SIF: simulated intestinal fluid (phosphate buffer pH ≈ 8; sodium taurocholate 6 mg/ml, trypsin 1 mg/ml)

Take home messages

Type of TELs/bolalipid	The Synthesis	Applicability
Natural TEL	Perfect	+++
Synthetic: *Kakinuma*	Possible but very time-consuming and costly	?
Synthetic: *Markowski*	Possible but still laborious	++
Synthetic: *Drescher*	Feasible	–
Synthetic: *Gruhle*	Feasible but low yields	+

Thanks!

- Sindy Müller, Kai Gruhle (my former group, Halle, Germany)
- Alfred Blume, Christian Schwieger, Annette Meister, Gesche Graf (Physicochemistry, Halle, Germany)
- Bodo Dobner, Thomas Markowski, Susan Becker, Katrin Helmis, Stefan Sonnenberger (Halle, Germany)
- Vasil Garamus (Helmholtz Hereon, Geesthacht, Germany)
- Gerd Hause (Biocenter Halle, Germany)
- Lea-Ann Dailey and her group (Vienna, Austria)
- Andrea Sinz and her group (Halle, Germany)
- Sandro Keller and his group (Graz, Austria)
- …

Phospholipid Research Center

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Fraunhofer IZI

Lipoid
We Invest in Quality.

DFG Deutsche Forschungsgemeinschaft

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Synthetic alternatives to natural tetraether lipids – chance or illusion?

Thank you for your attention! Happy to take questions …

PD DR. HABIL. SIMON DRESCHER
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Phospholipid Symposium 2024 | Heidelberg | September 09, 2024