Tether Lipid Molecules For High Precision Metrology Of Integral Proteins

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SURF
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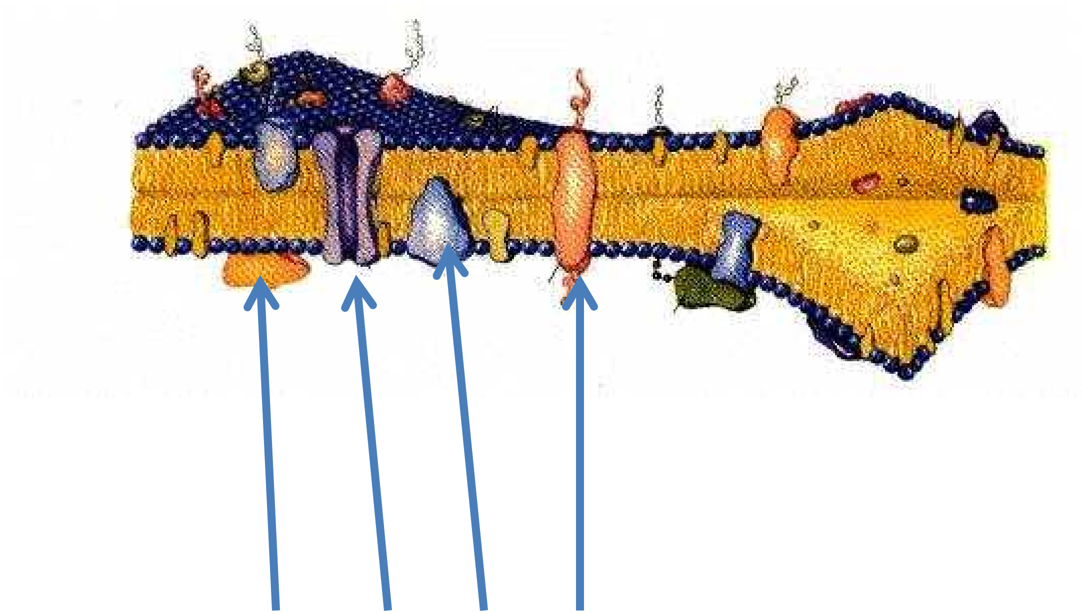
Natural Lipid Bilayers

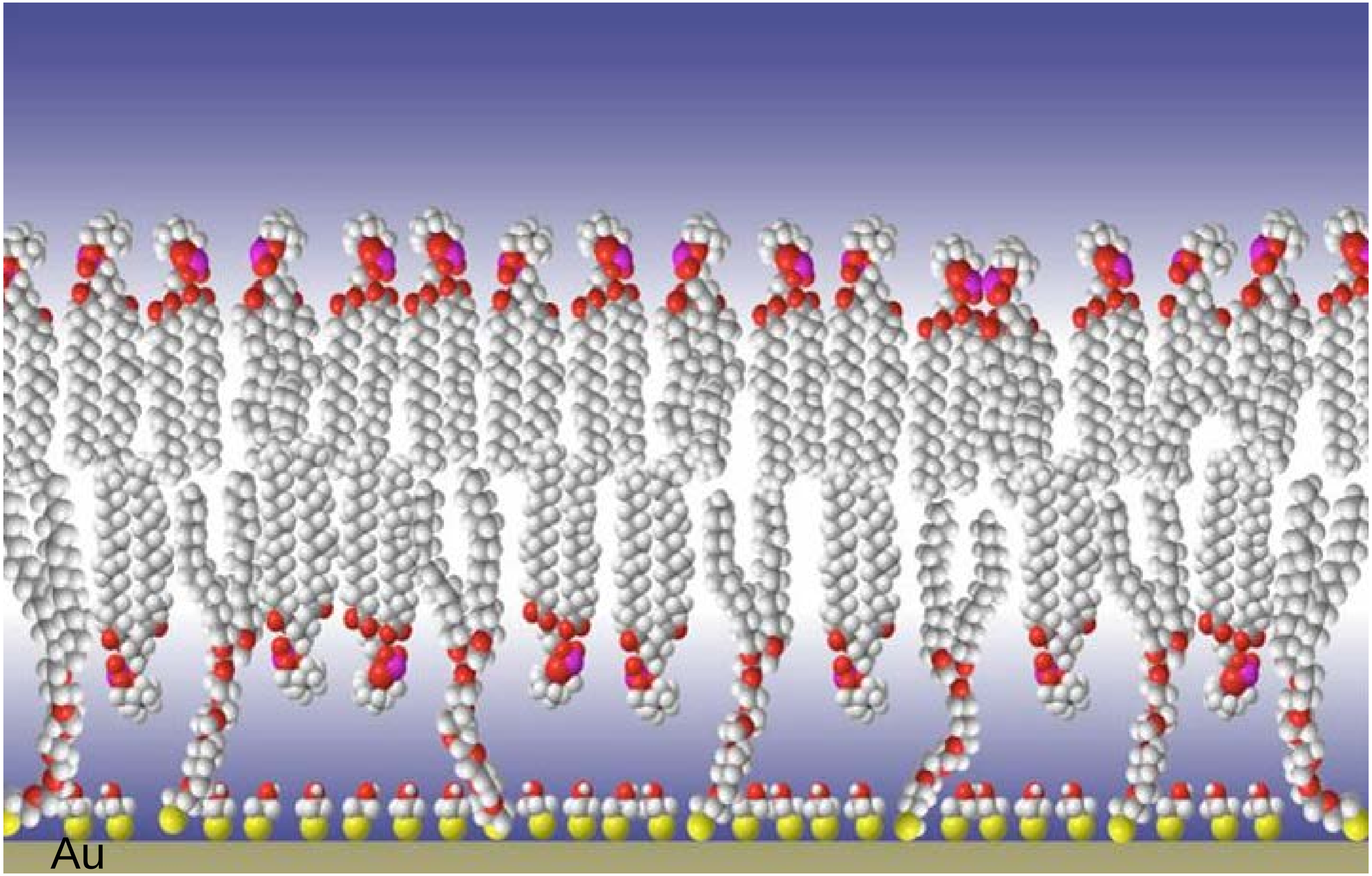


Integral membrane proteins (IMPs)

Objective: High precision metrology – structure-function studies of IMPs

Tethered Bilayer Lipid Membrane (tBLM) System

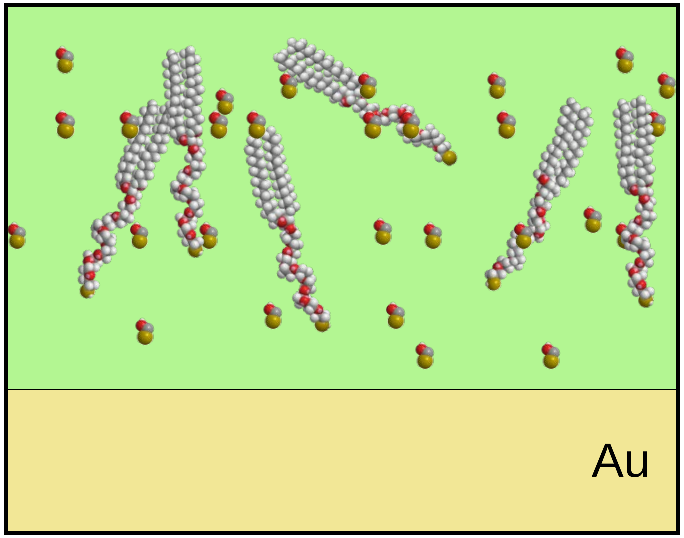
Synthetic (tethering) Lipid/β-mercaptoethanol (βME)/lipids



tBLM Preparation

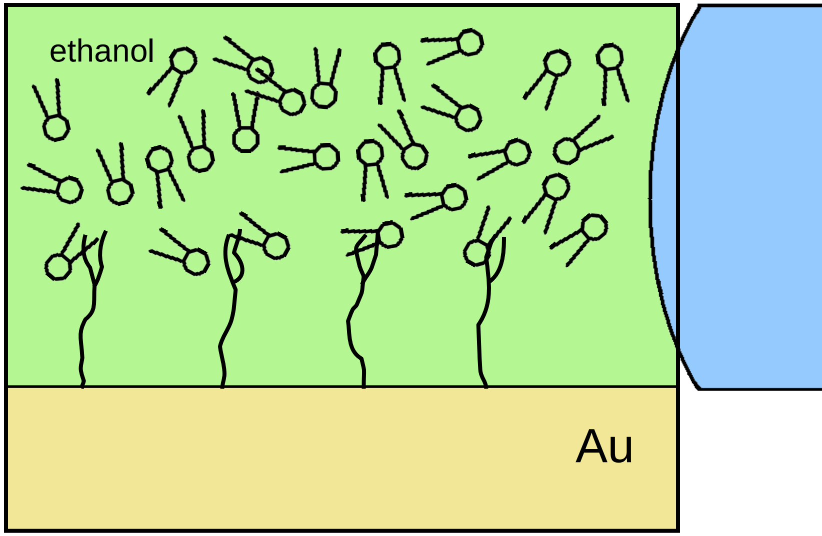
Step 1: Mixed Self-Assembled Monolayer (SAM)

Incubation with lipidic anchor molecule and β-mercaptoethanol



Step 2: Create tBLM

Rapid Solvent Exchange

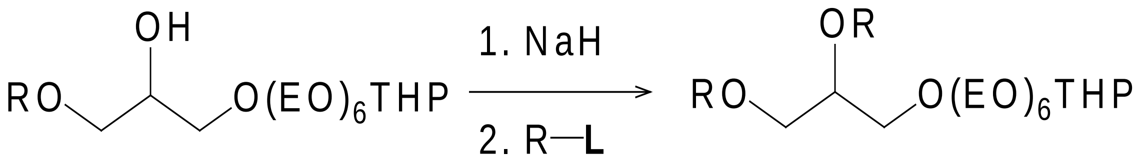


tBLMs are stable for days, highly insulating ($C_{tBLM} \leq 0.8\ \mu F$) and provide an aqueous-filled sub-membrane space

Ng 2008 SURF talk

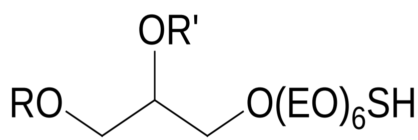
Summer Goals

1. **Synthesis optimization of dialkylation products**

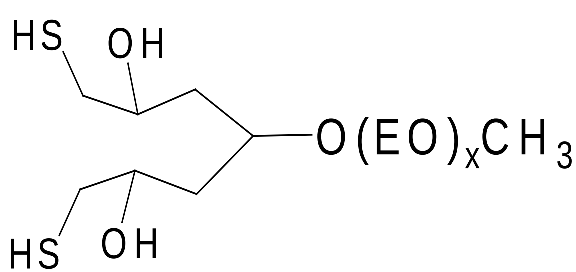


L= leaving group

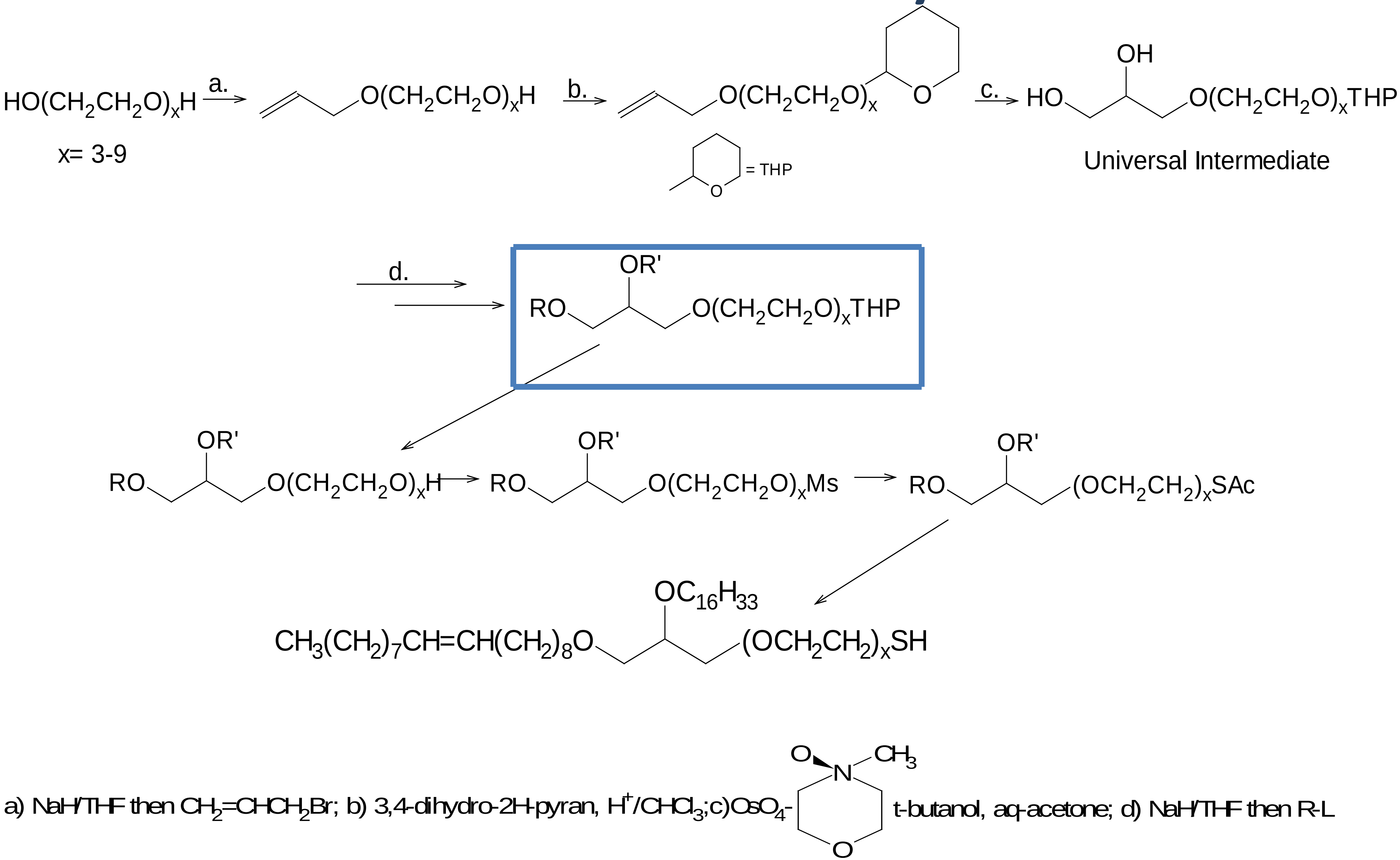
2. **Synthesis of a new compound:**



3. **Synthetic strategy of double anchor molecules:**

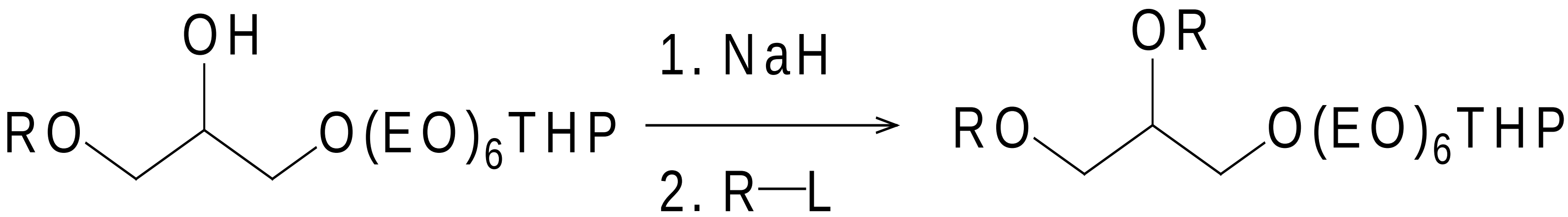


Outline Of Tether Synthesis



a) NaH/THF then CH_2=$CHCH_2Br$; b) 3,4-dihydro-2H-pyran, H^+/$CHCl_3$;c)OsO_4- [N-methylmorpholine N-oxide] t-butanol, aq-acetone; d) NaH/THF then R-L

Goal 1: Synthesis Optimization

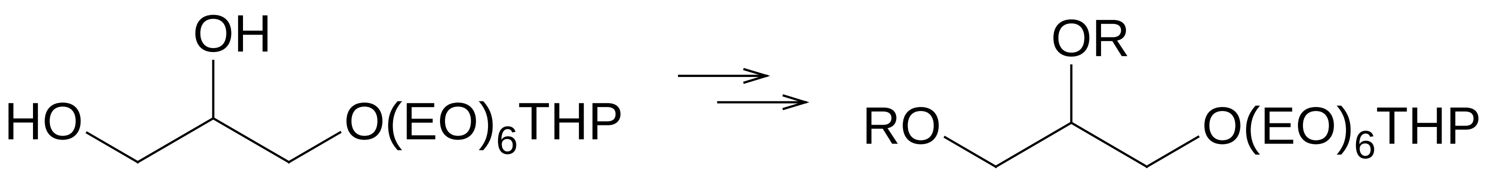


R= oleyl = $CH_3(CH_2)_7CH{=}CH(CH_2)_8$

L= Br ~30% yield

L= Ms ~80% yield

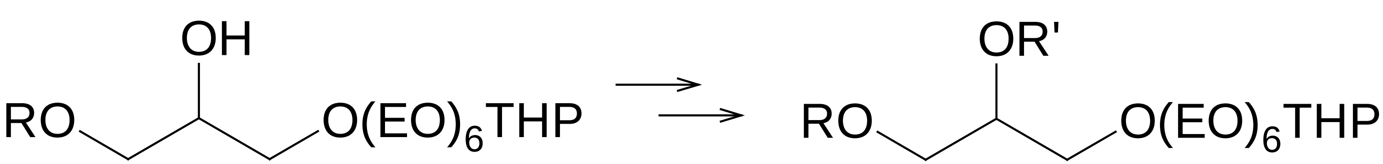
Goal 2: Synthesis Of New Compound



Key intermediate

60-70% yield with monoalkyl as other product

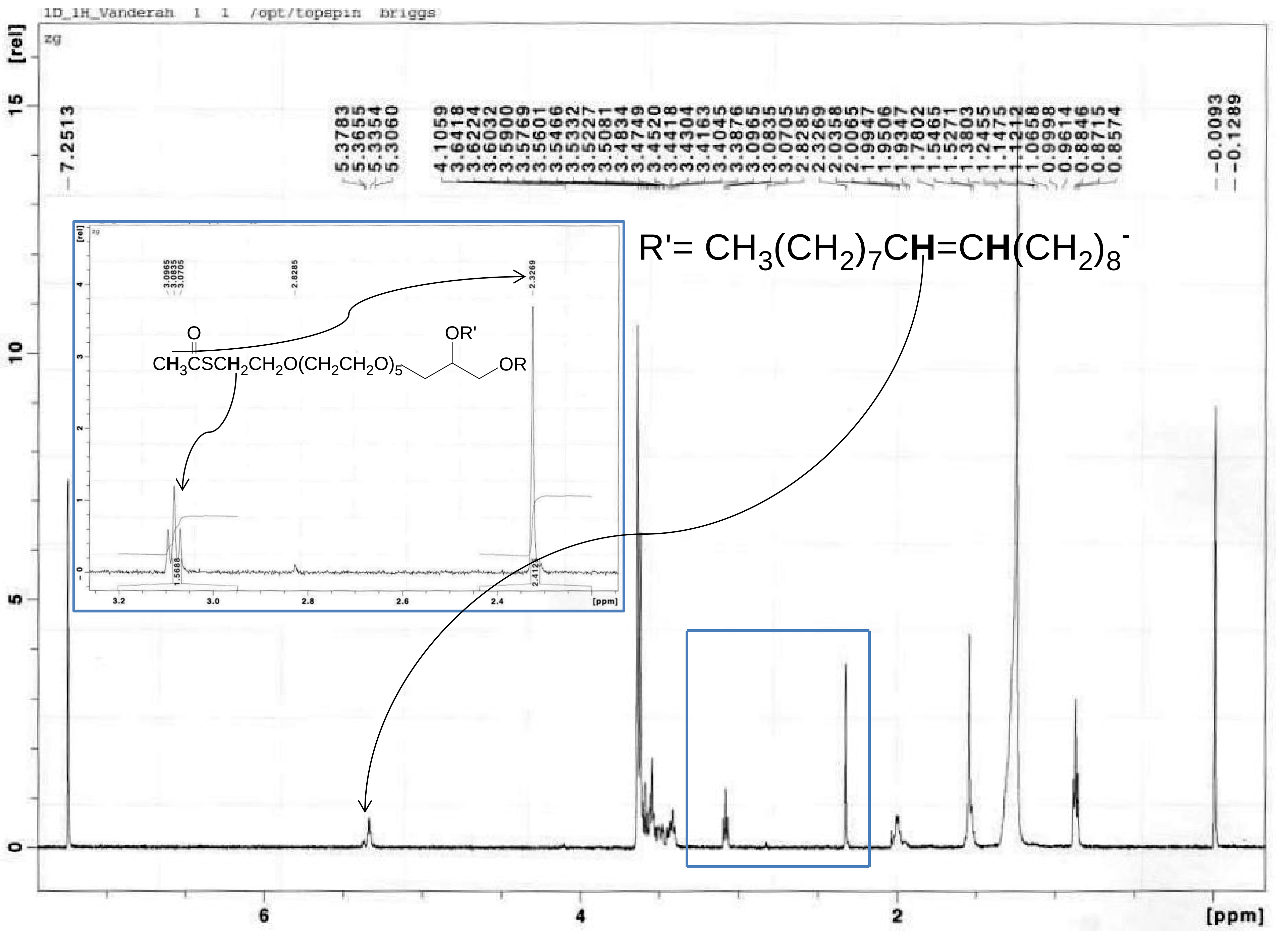
Monoalkylated material, purified through chromatography, is then the source for next reaction:



30-35% yield with bromine as leaving group

R= oleyl

R'= $C_{16}H_{33}$



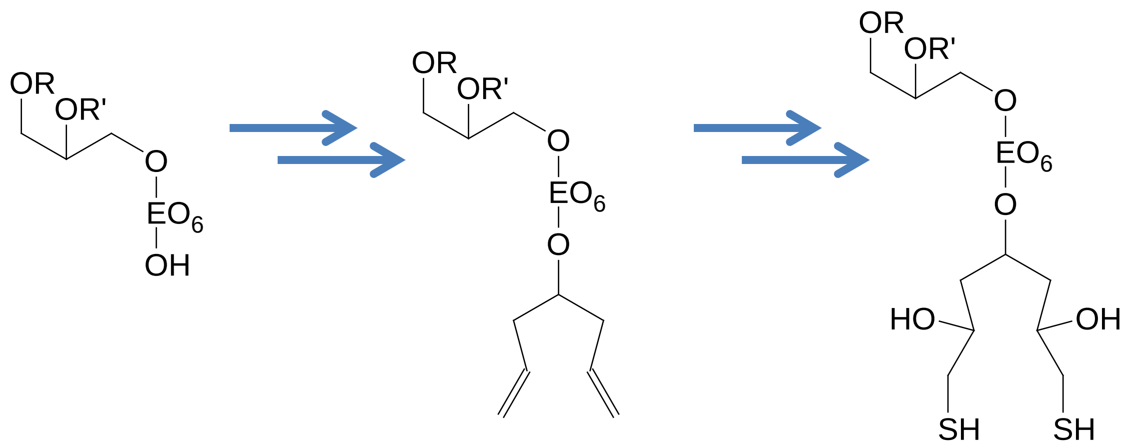
1D_1H_Vanderah 1 1 /opt/topspin briggs
zg
[rel]
R'= CH3(CH2)7CH=CH(CH2)8-
CH3CSCH2CH2O(CH2CH2O)5
O
OR'
OR
3.0965
3.0835
3.0705
2.8285
2.3269
1.5688
2.412
7.2513
-0.0093
-0.1289
[ppm]

Goal 3: Lipidic Anchor Molecules with Two Anchoring Groups

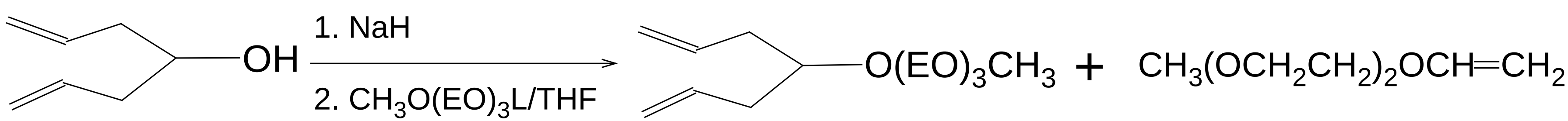
1. Increases stability in the binding of the tBLM to the substrate (Au)
2. Incorporation of alcohol (-OH) groups should increase the hydrophilicity in the submembrane space and the potential of hydrogen bonding between anchor molecules may add stability to the tBLM [may be able to eliminate the "spacer" molecule β-mercaptoethanol (βME)]

More water between the Au and the tBLM makes the bilayer environment more likely to reconstitute IMPs

Synthetic Strategy



Model System to Evaluate Leaving Groups



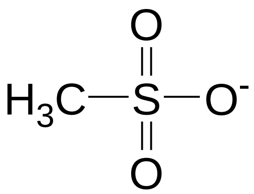
L = leaving group

Leaving group	Substitution	Elimination
Bromine	30%	70%
Mesylate	96%	4%
Tosylate	100%	0%

With the tosylate as leaving group, no elimination product was observed (NMR data).

Conclusions

- A higher yield can result from a mesylate leaving group. Likely because of stability of the mesyl anion ($CH_3SO_3^-$):



- A new lipidic anchor molecule with two different alkyl groups was prepared this summer.

- A tosylate was shown to be superior to other leaving groups in the synthesis of lipidic molecules with two anchoring group.

Acknowledgement

IBBR: Dave Vanderah, John Marino

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