Supporting Information

Non-naturally-occurring Regio Isomer of Lysophosphatidylserine Exhibits Potent Agonistic Activity Towards G Protein-coupled Receptors

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Table of Contents

Figure S1. GPR34 activation by various LysoPS analogues.

Figure S2. P2Y10 activation by various LysoPS analogues.

Figure S3. Conformational distribution of Type III

Figure S4. Distribution of conformations of Type III LysoPS derivatives with respect to the dihedral angle ω.

Figure S5. Conformational distribution of Type II-cyclic LysoPS derivatives in water

Figure S6. Docking and REST simulation of compounds 2*R* and 20

Figure S7. Core constraint applied in docking of compound 14.

Figure S8. Human and zebrafish GPR34 activation by LysoPS analogues.

Figure S9. Alignment of amino acid sequences of human, mouse, and zebrafish GPR34

Figure S1-1
GPR34

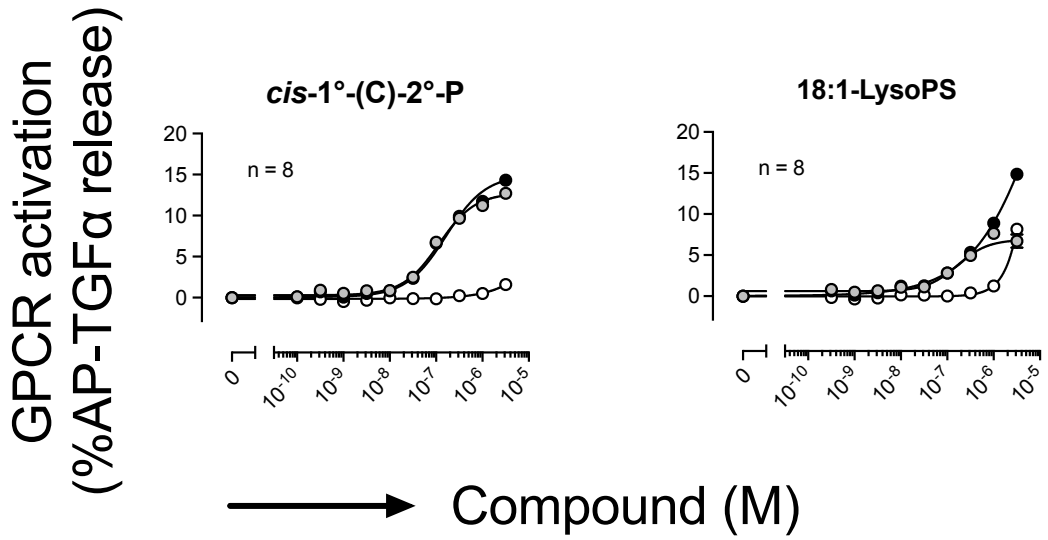
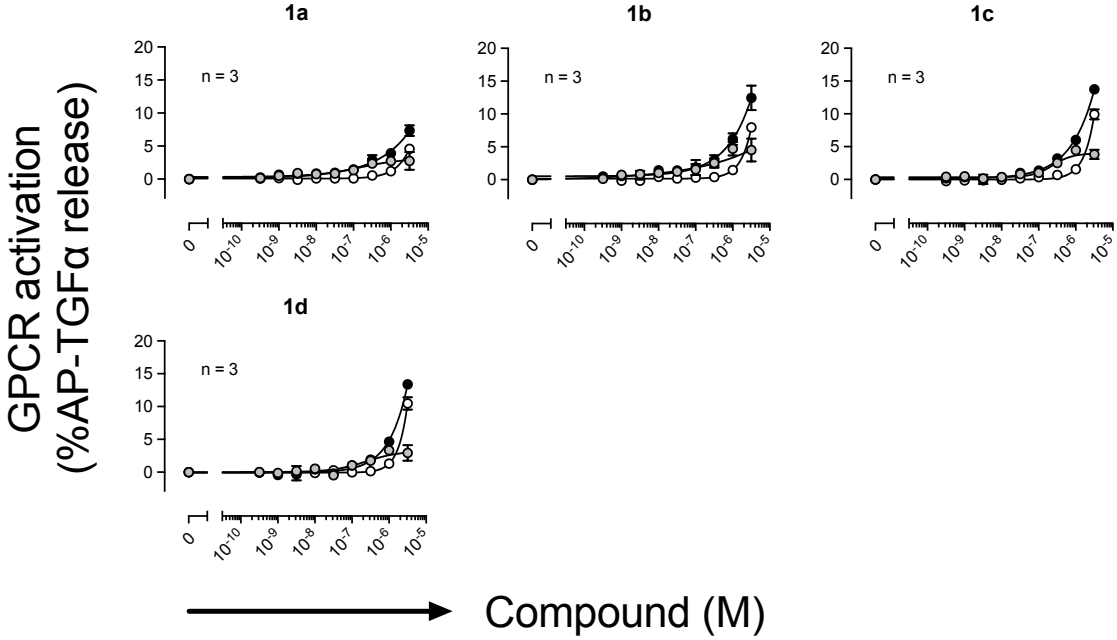


Figure S1-2
GPR34



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GPR34

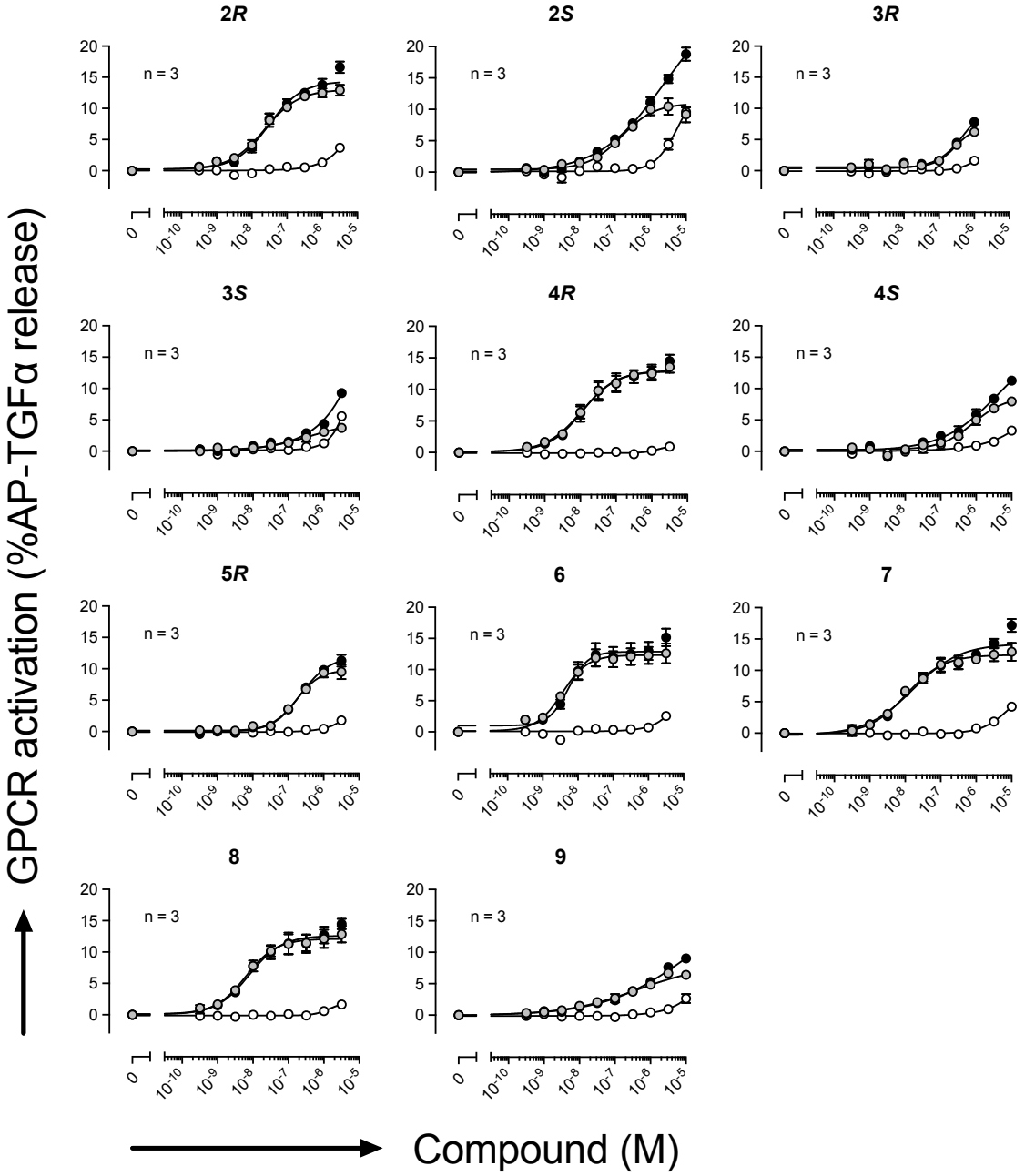


Figure S1-4
GPR34

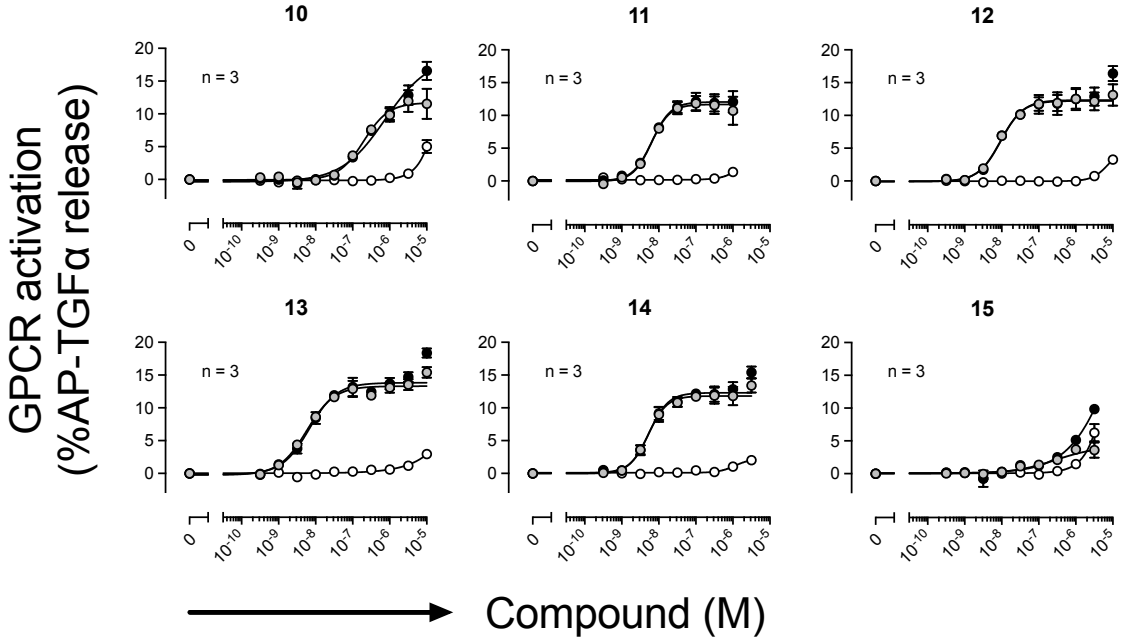


Figure S1-5
GPR34

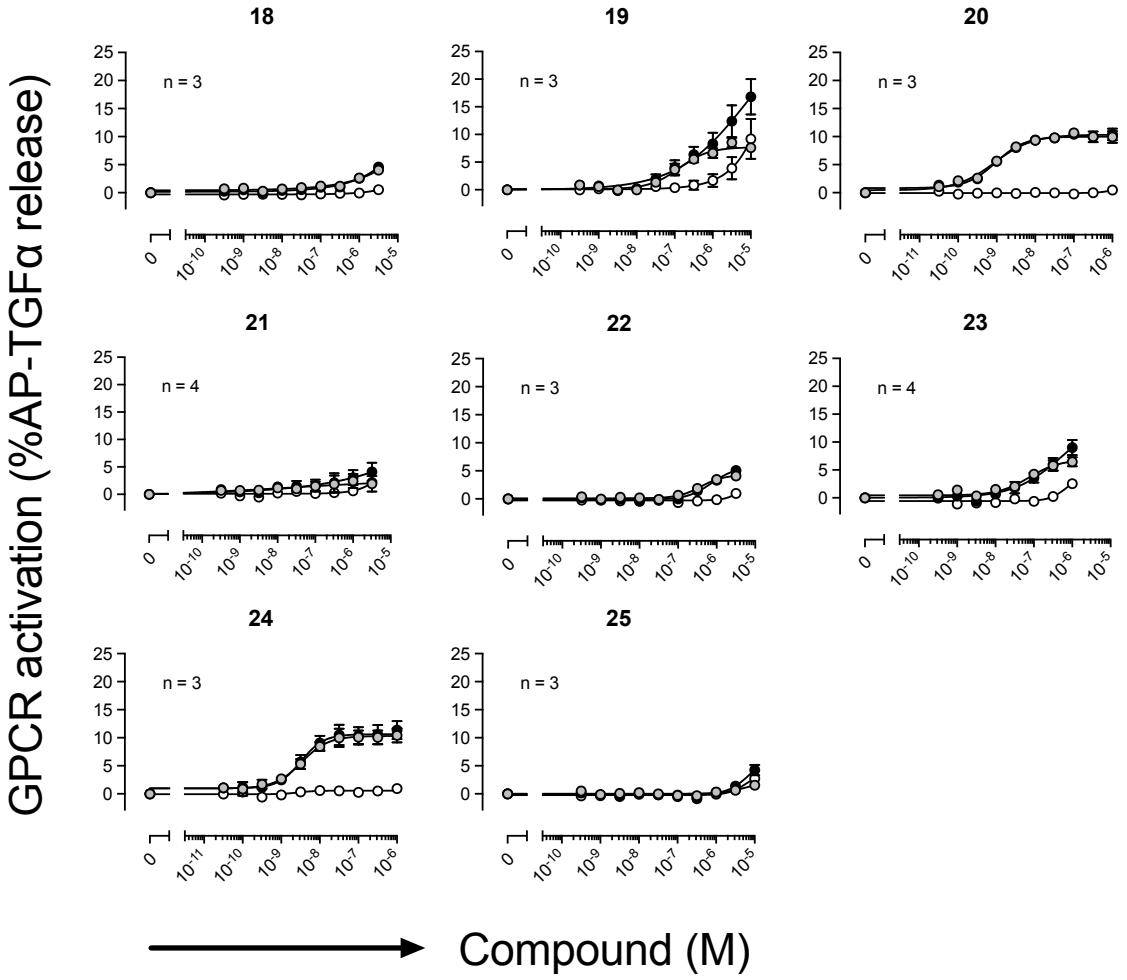
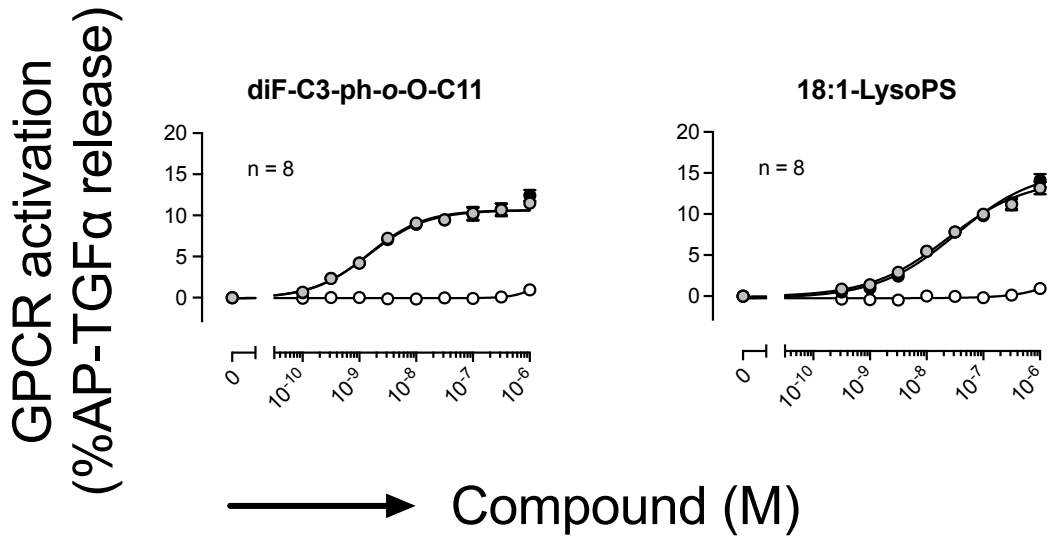


Figure S1 (S1-1 to S1-5). GPR34 activation by various LysoPS analogues.

HEK293 cells transiently transfected with mouse GPR34-encoding vector or empty vector were treated with different concentrations of each LysoPS analogue. Receptor-specific AP-TGFα releases (gray circles) were calculated by subtracting AP-TGFα releases in empty vector-transfected cells (white circles) from those in mouse GPR34-expressing cells (black circles).

Figure S2-1
P2Y10



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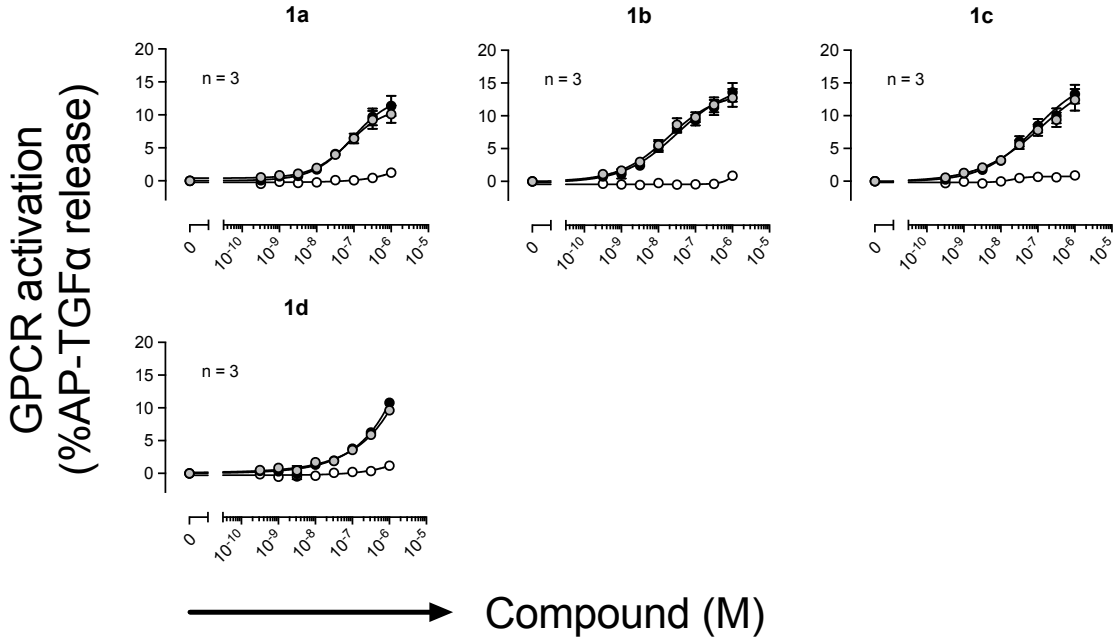
P2Y10



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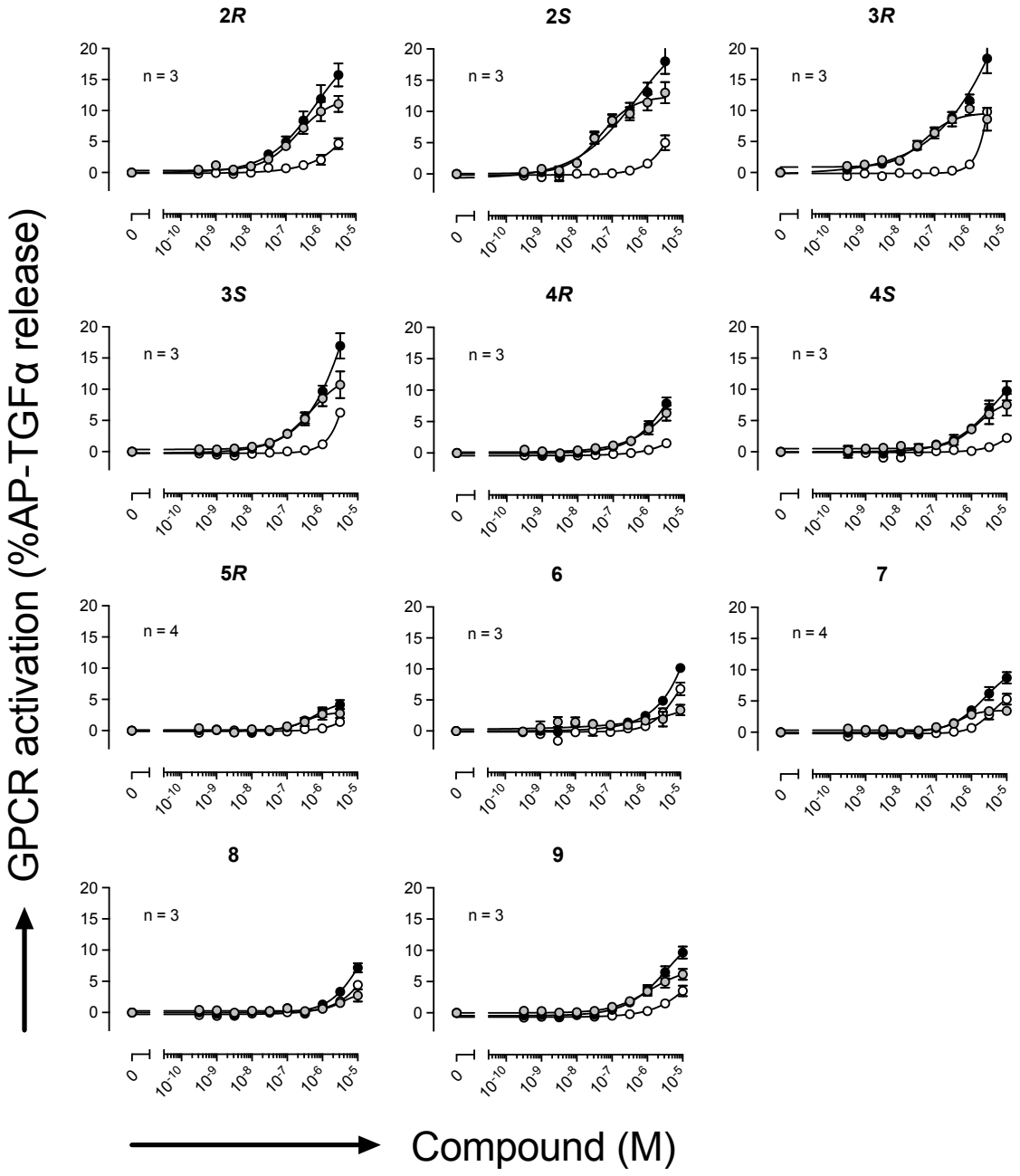
P2Y10



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P2Y10

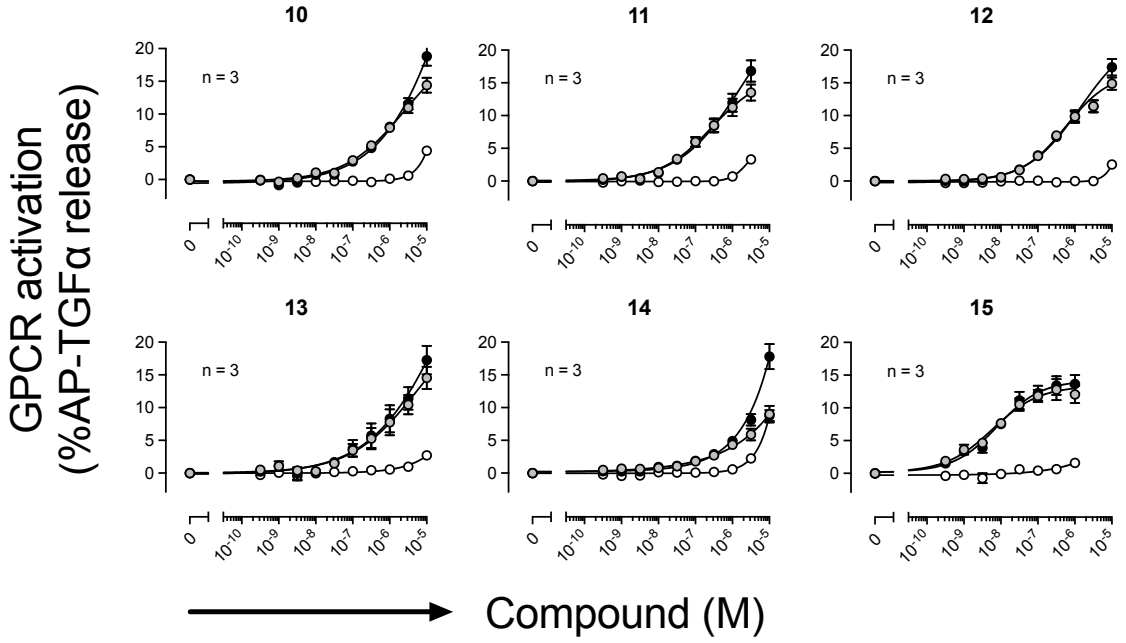


Figure S2-5
P2Y10

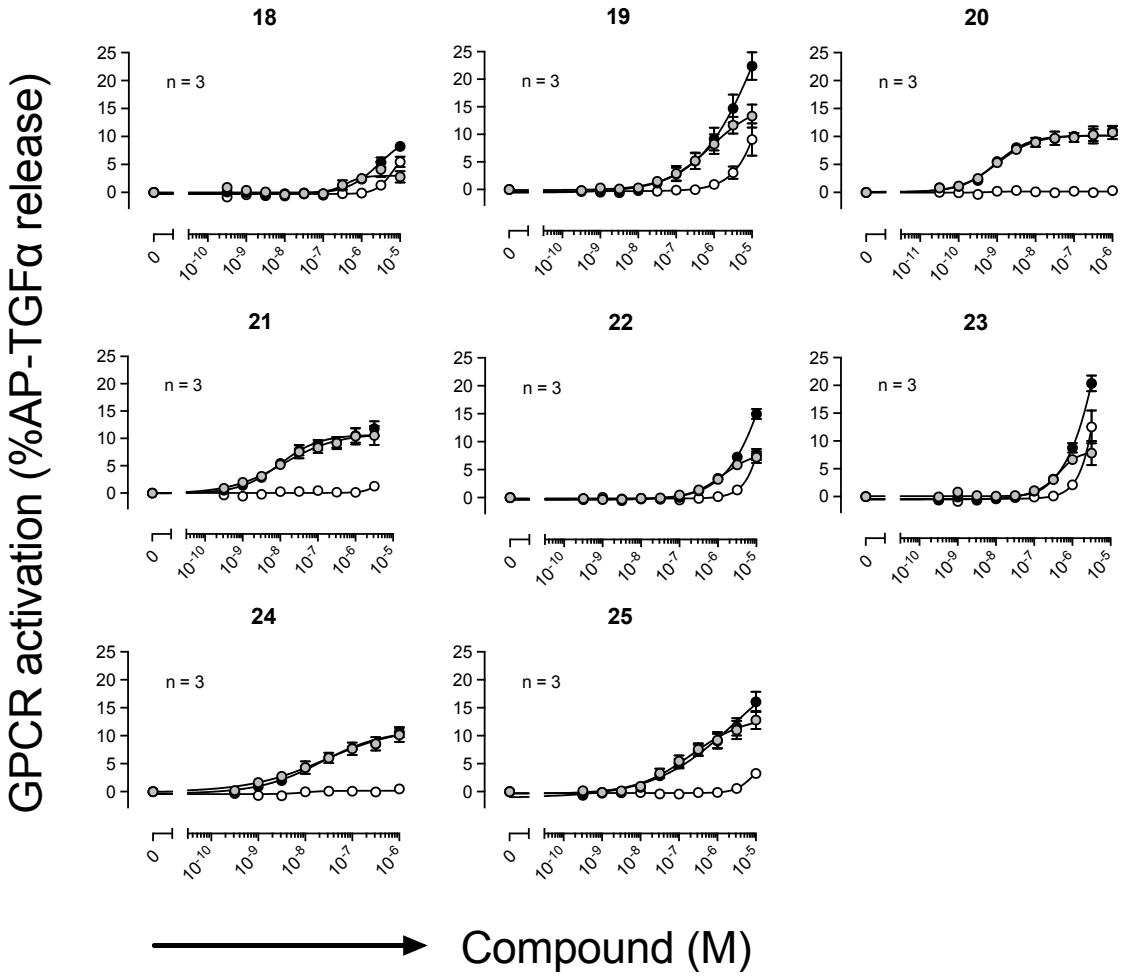
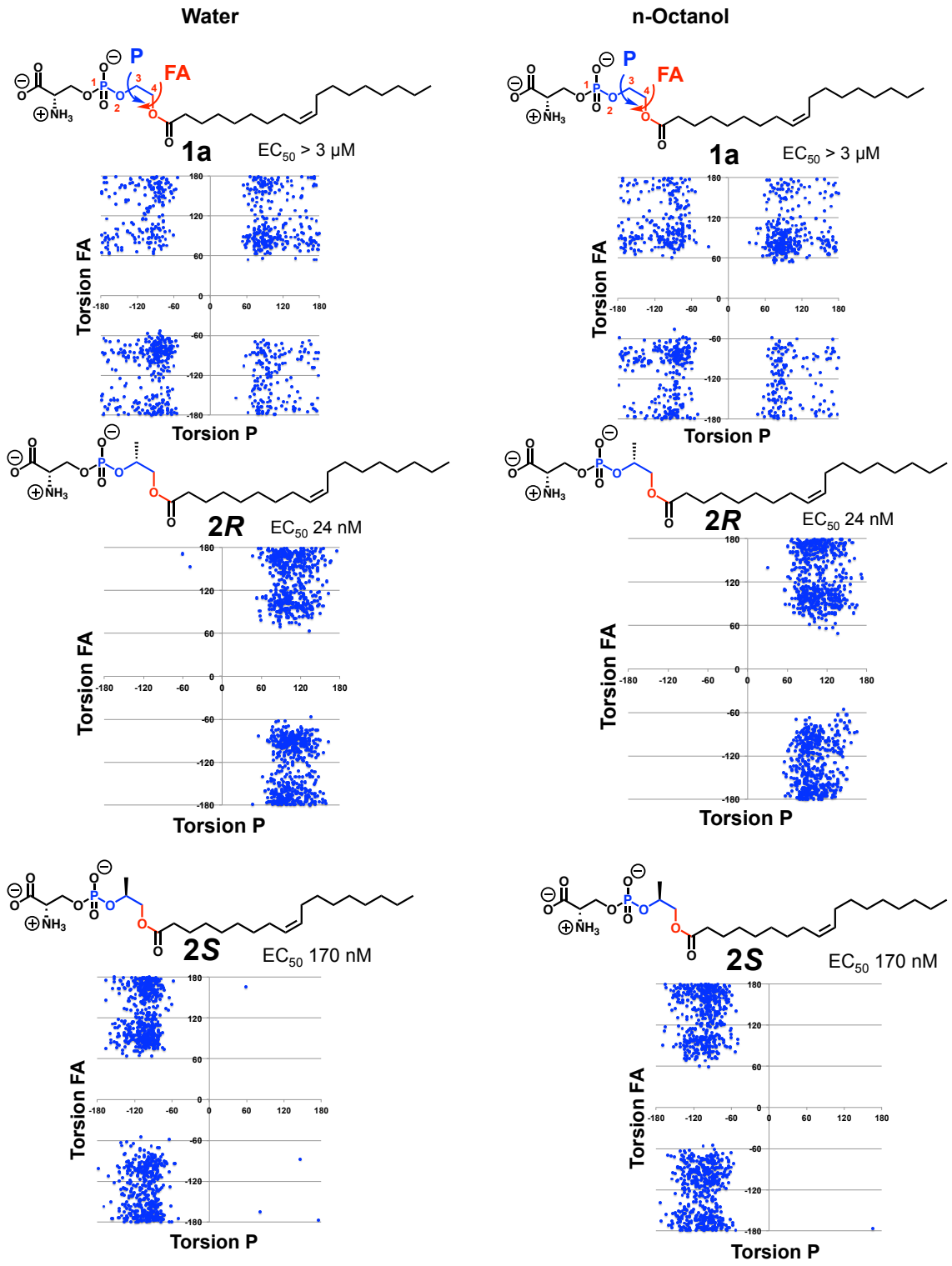
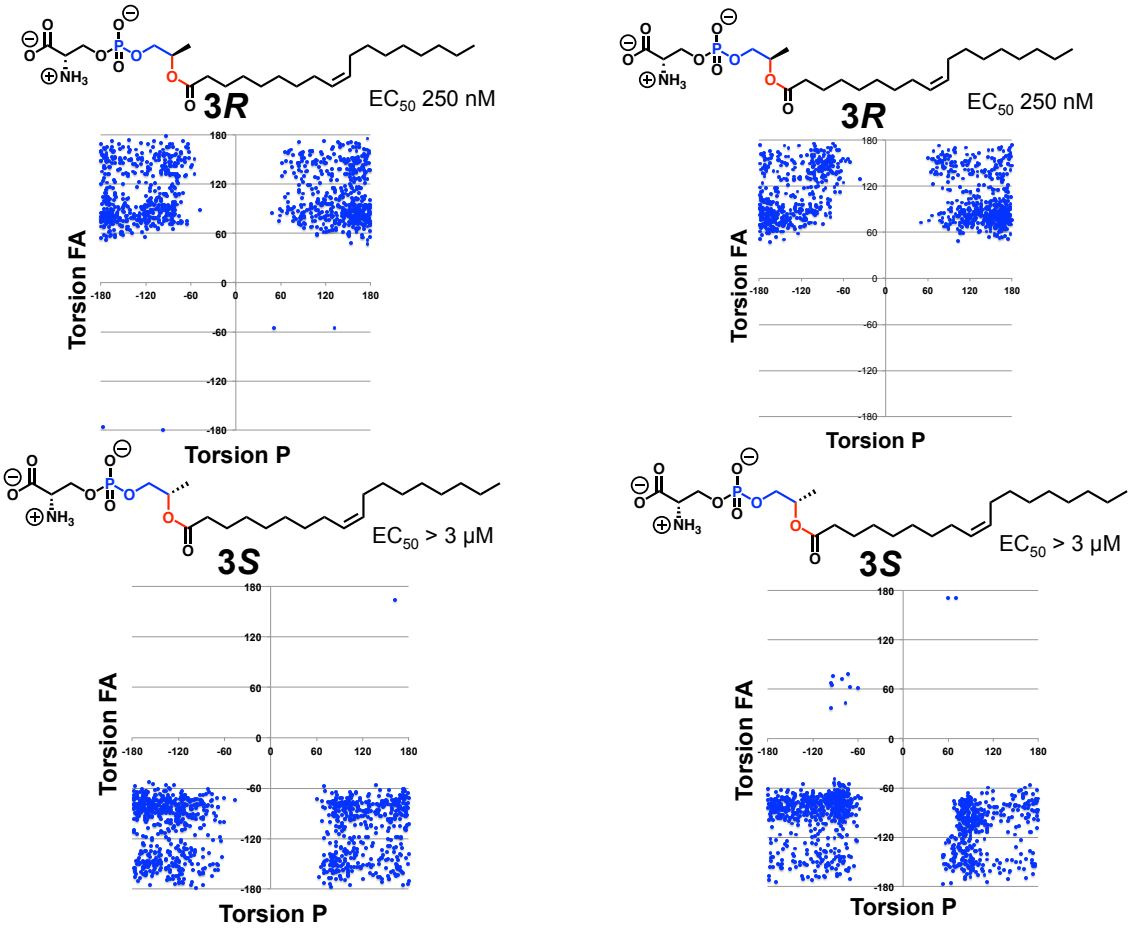


Figure S2 (S2-1 to S2-5). P2Y10 activation by various LysoPS analogues.

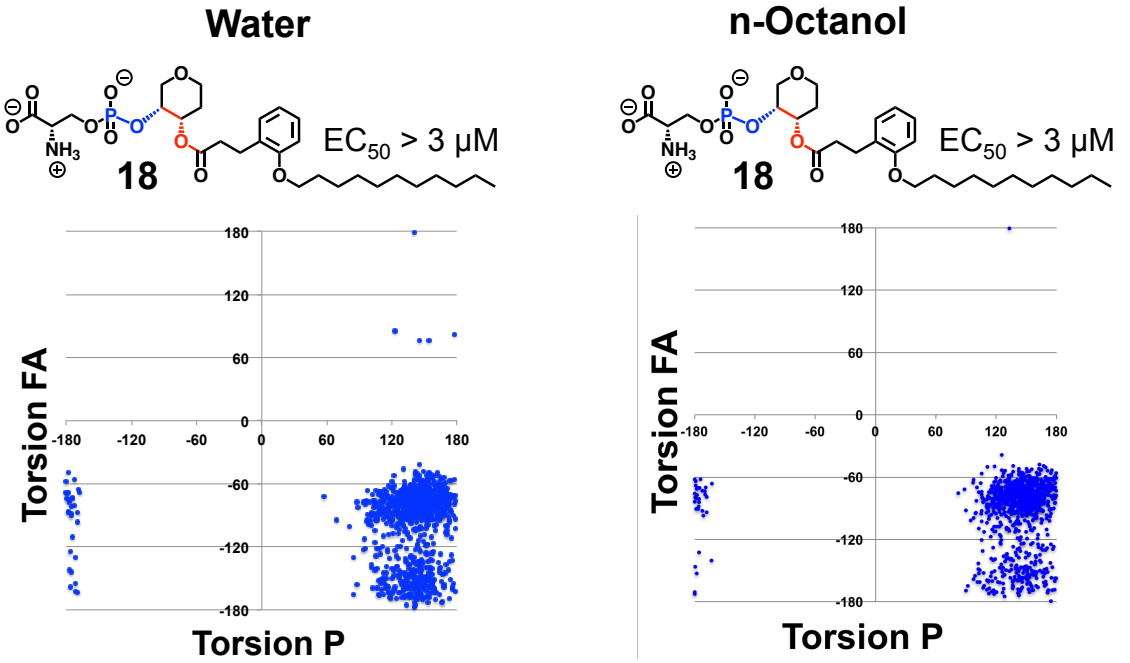
HEK293 cells transiently transfected with mouse P2Y10-encoding vector or empty vector were treated with different concentrations of each LysoPS analogue. Receptor-specific AP-TGFα releases (gray circles) were calculated by subtracting AP-TGFα releases in empty vector-transfected cells (white circles) from those in mouse P2Y10-expressing cells (black circles).

(a) non-cyclic derivatives





(b) cyclic derivatives



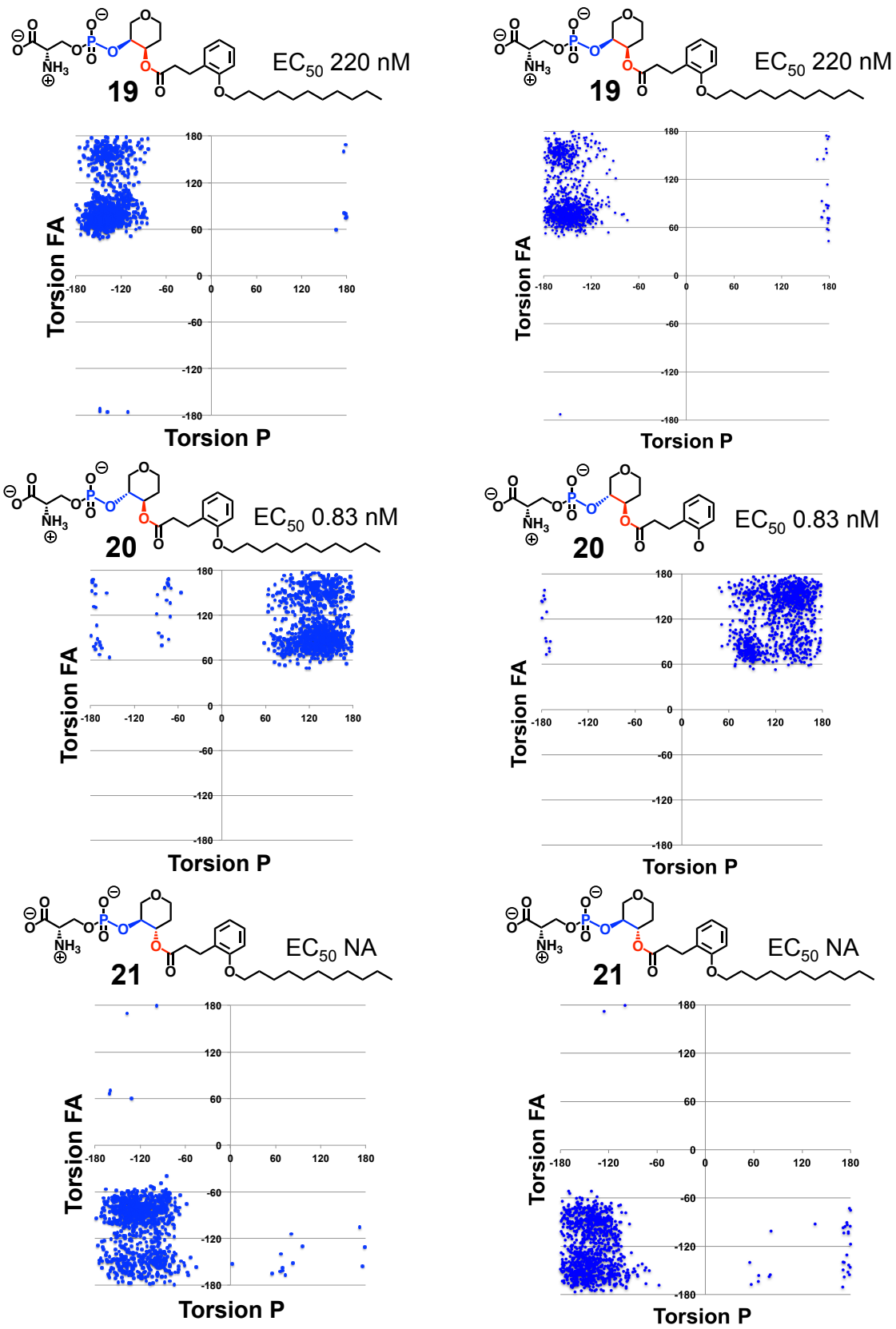
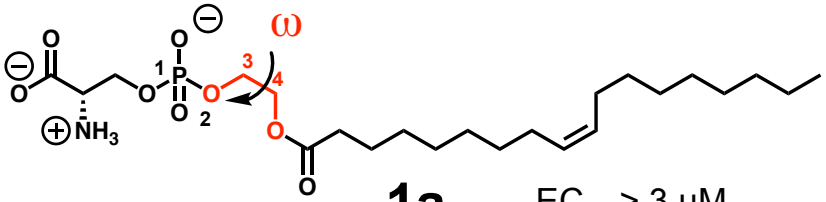
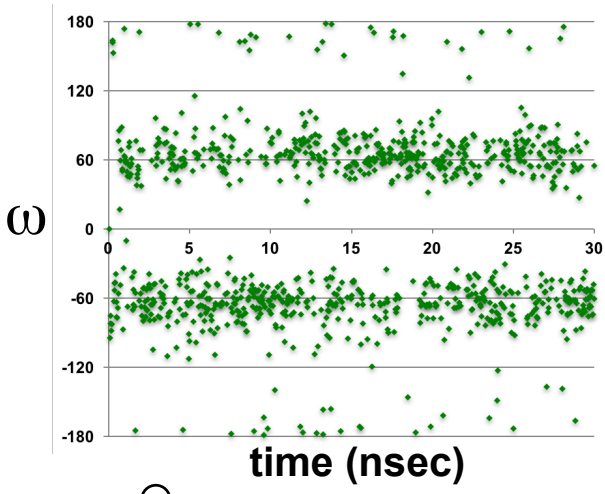
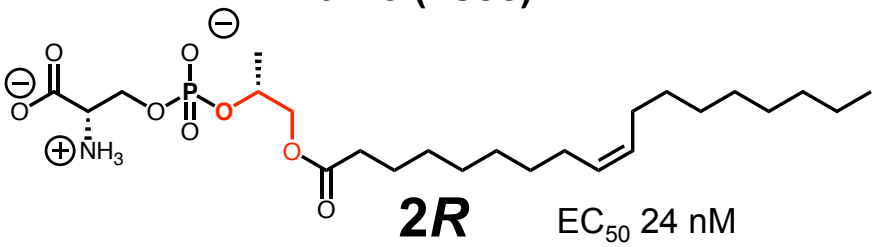


Figure S3. Conformational distribution of Type III: (a) non-cyclic LysoPS derivatives and (b) cyclic LysoPS derivatives with respect to two dihedral angles (P and FA) in n-octanol as compared with those in water: REST calculations

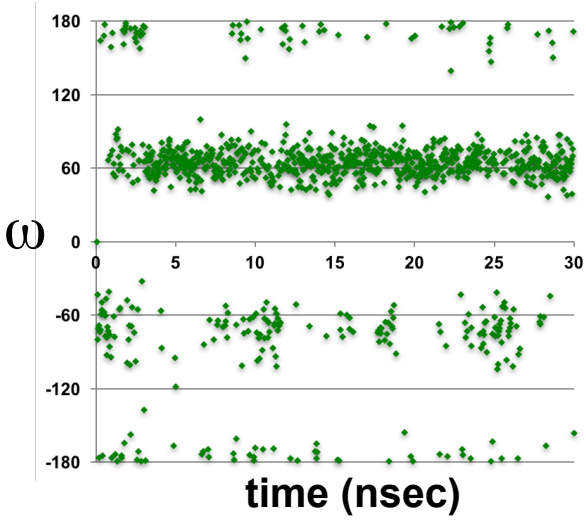


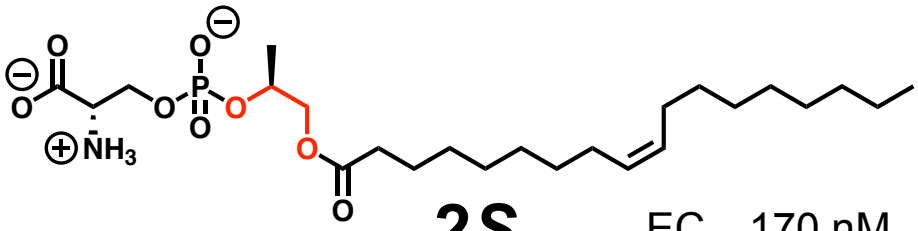
1a EC_{50} > 3 μM



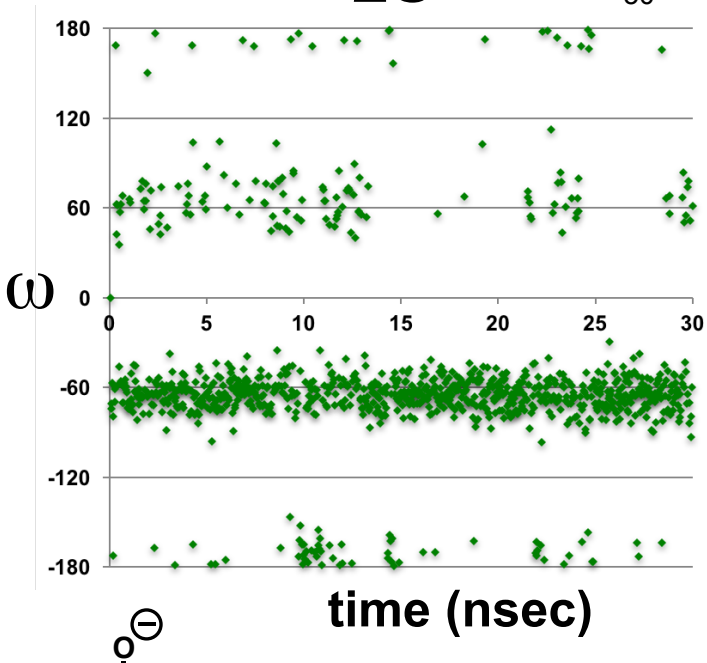


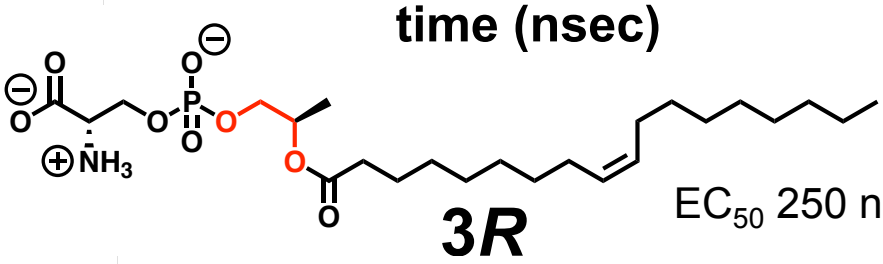
2*R* EC_{50} 24 nM



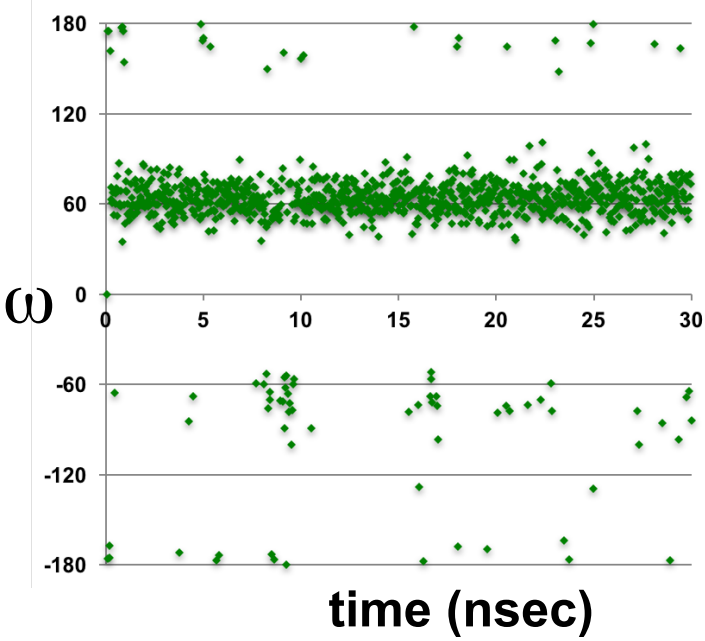


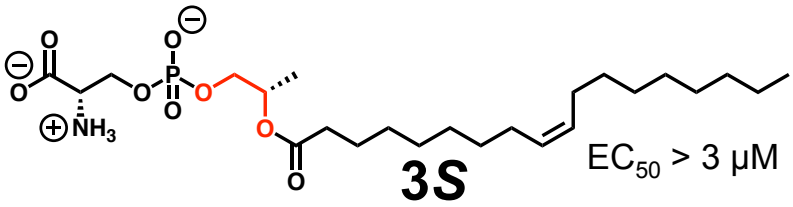
2*S* EC_{50} 170 nM

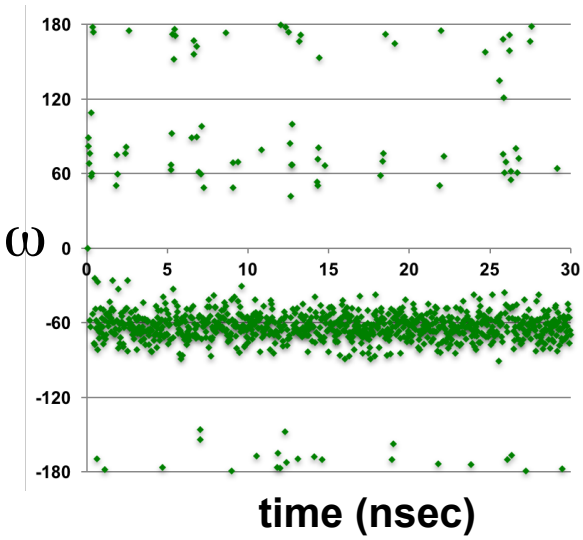


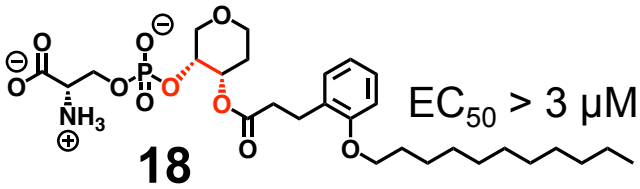


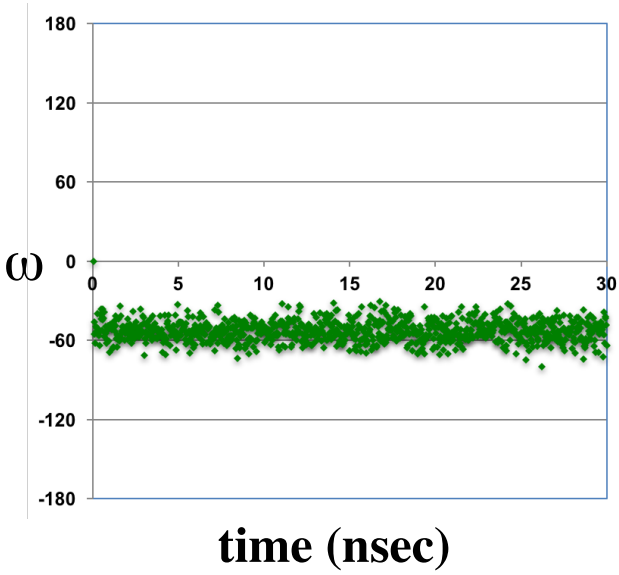
3*R* EC_{50} 250 nM

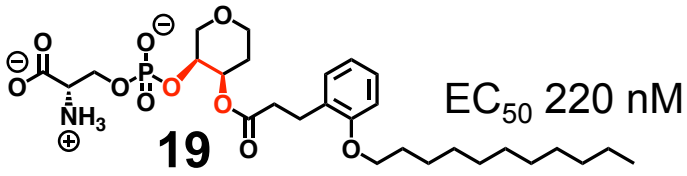


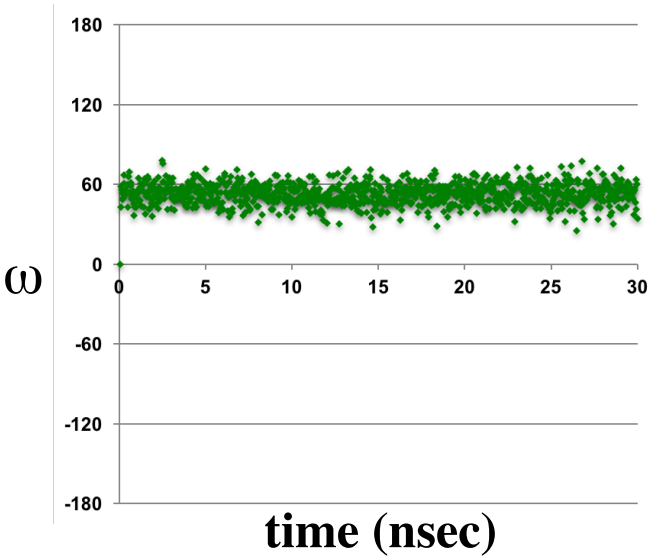

3S
EC50 > 3 μM

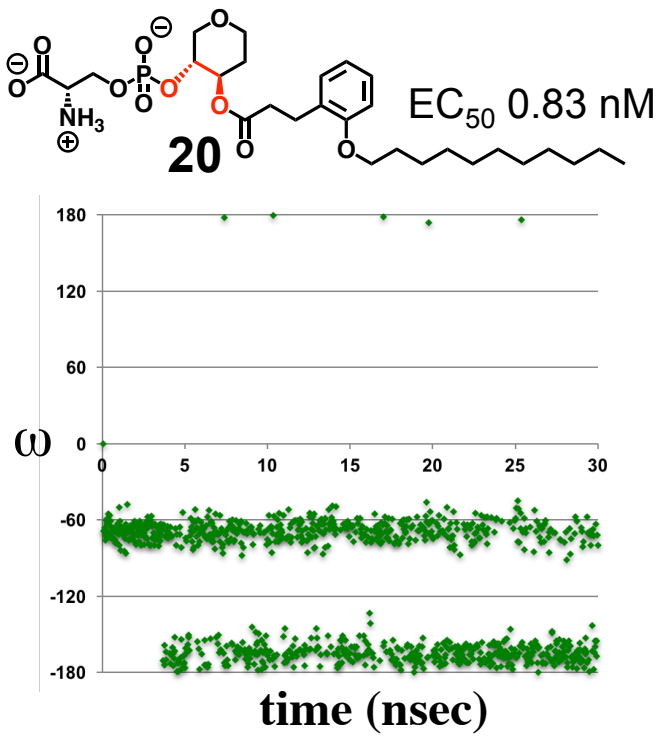

ω
180
120
60
0
-60
-120
-180
0
5
10
15
20
25
30
time (nsec)


18
EC50 > 3 μM


ω
180
120
60
0
-60
-120
-180
0
5
10
15
20
25
30
time (nsec)


19
EC50 220 nM


ω
180
120
60
0
-60
-120
-180
0
5
10
15
20
25
30
time (nsec)



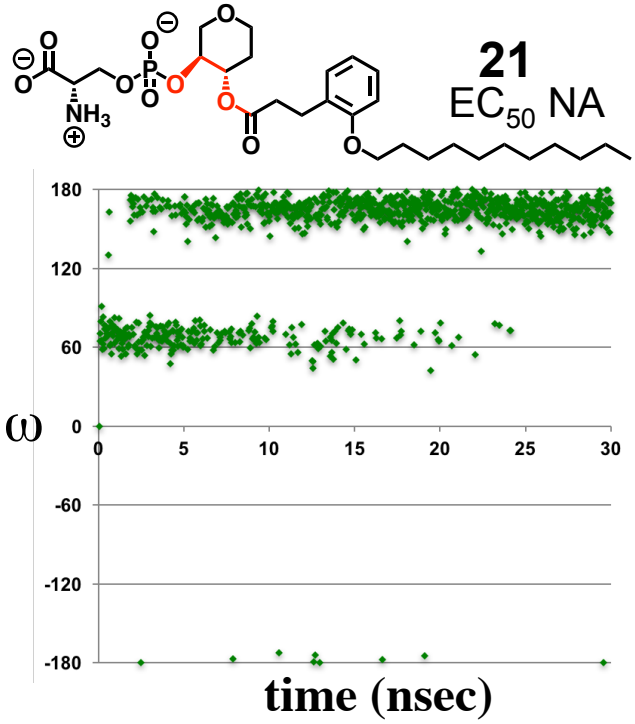


Figure S4 Distribution of conformations of Type III LysoPS derivatives with respect to the dihedral angle ω.
No correlation between bioactivity and specified conformation.
REST calculations.

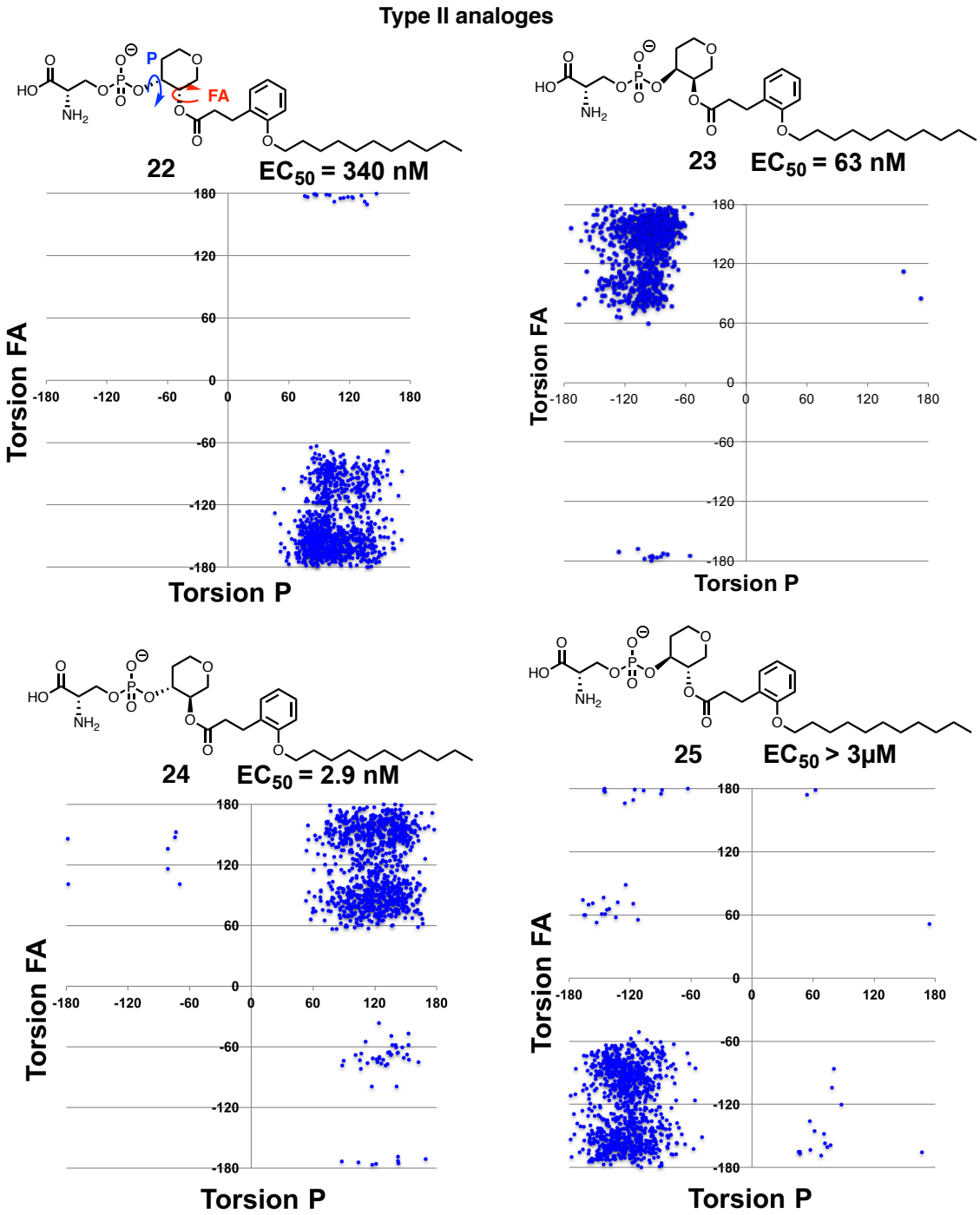


Figure S5 Conformational distribution of Type II-cyclic LysoPS derivatives in water: REST calculations.

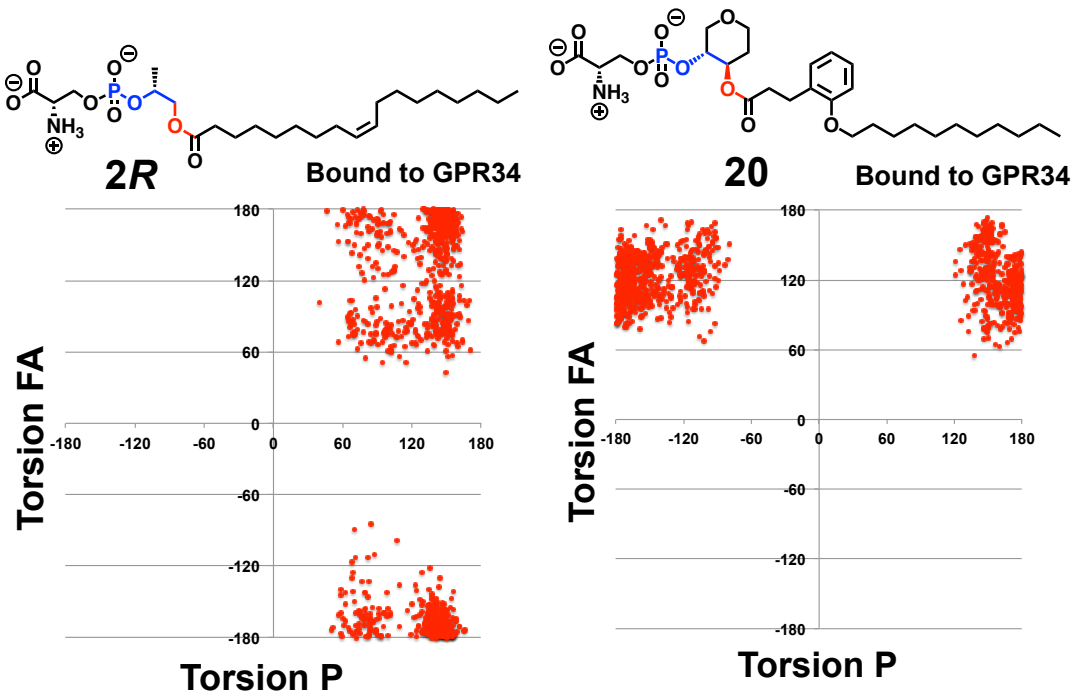


Figure S6. Docking and REST simulation of compounds 2*R* and 20

We docked compounds **2*R*** and **20** to GPR34 model structure basically following the procedure in Experimental Section of the main text that describes docking of compound **14**. However, as these compounds lack the tri-benzene fatty acid moiety (Figure S5), we did not specify the positional constraint during docking. For the docking of compound **20**, we also utilized Induced fit docking protocol (references in SI 1 and 2) which includes side-chain prediction of the residues near the ligand-binding site with an implicit membrane using Prime. For the selected poses, the (**+P**, **+FA**) conformations were found.

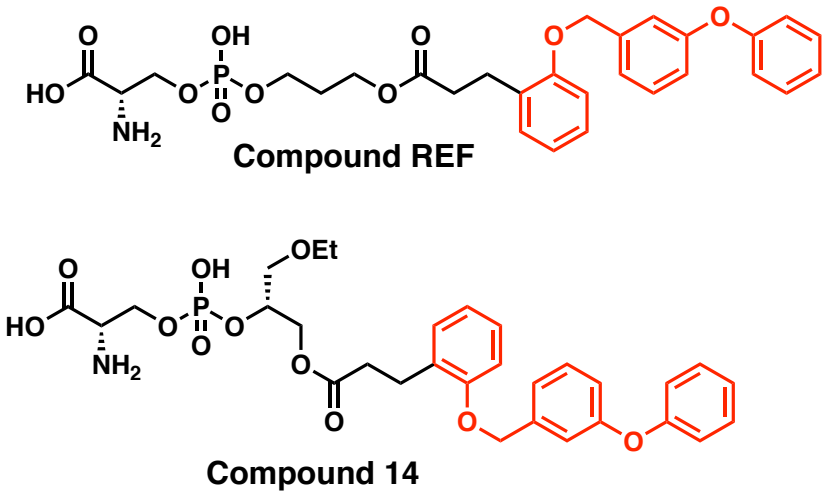


Figure S7. Core constraint applied in docking of compound 14.
The red part of compound REF was specified as the core.

Figure S8

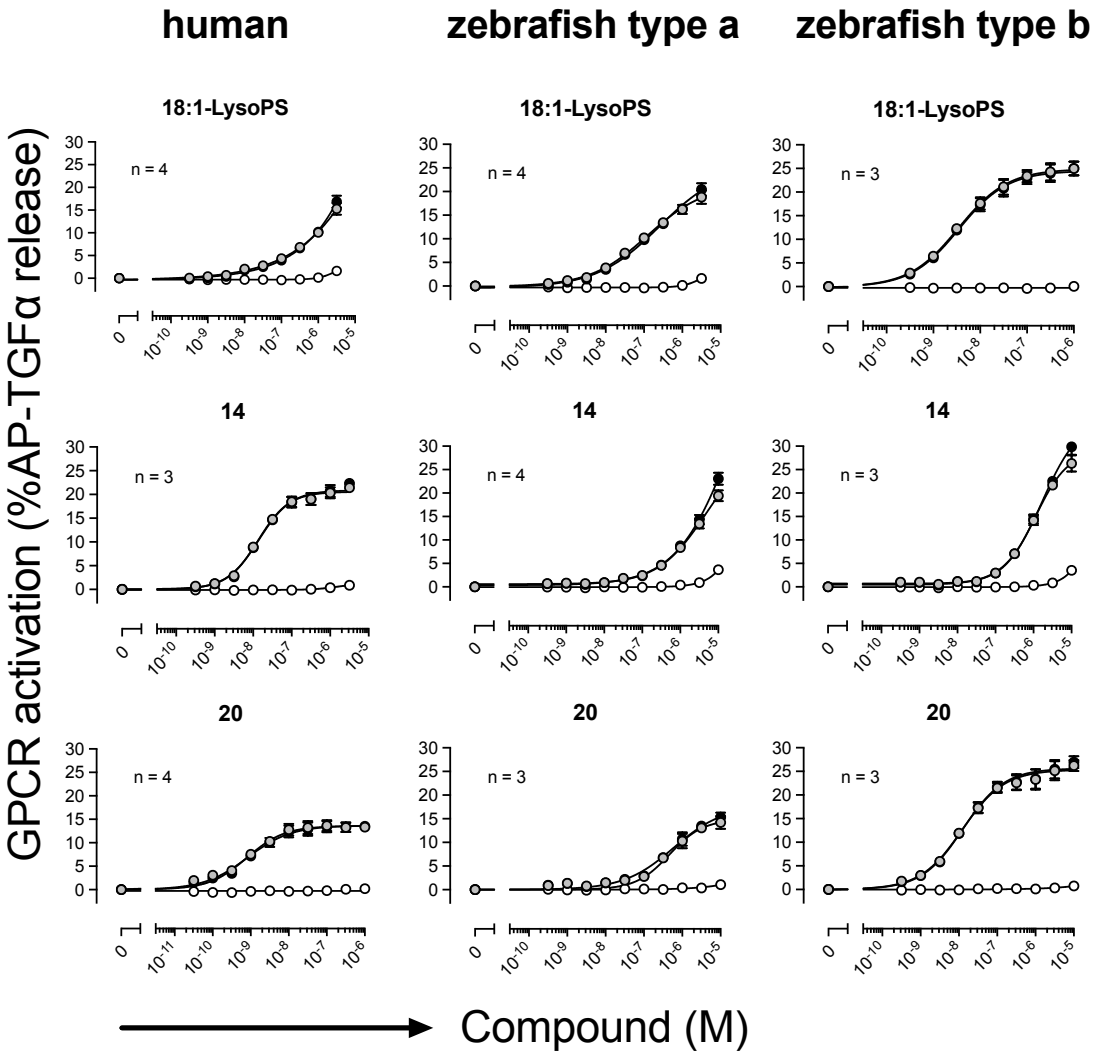


Figure S8. Human and zebrafish GPR34 activation by LysoPS analogues.

HEK293 cells transiently transfected with vectors encoding human or zebrafish GPR34 or empty vector were treated with different concentrations of each LysoPS analogue. Receptor-specific AP-TGFα releases (gray circles) were calculated by subtracting AP-TGFα releases in empty vector-transfected cells (white circles) from those in GPR34-expressing cells (black circles).

Figure S9

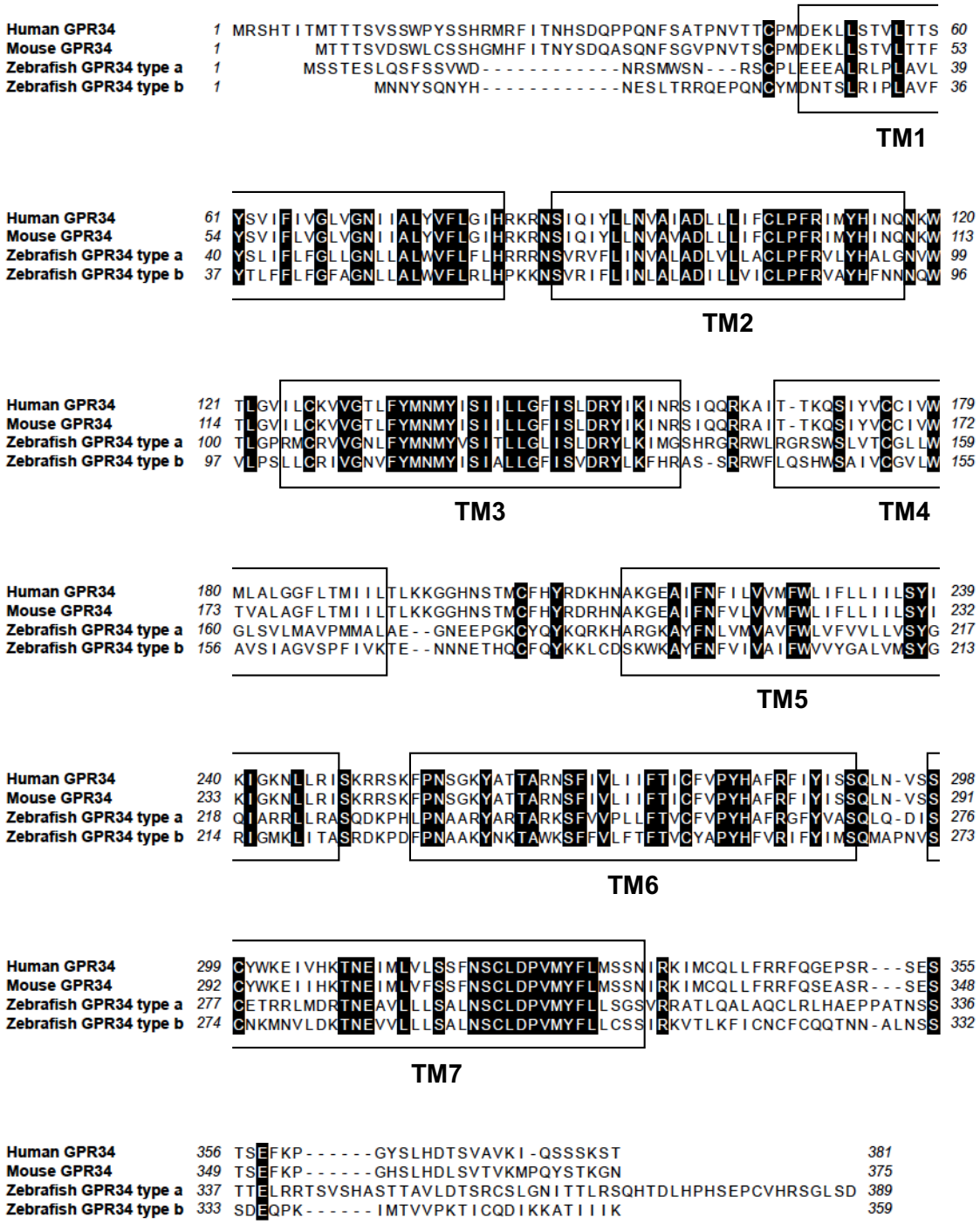


Figure S9. Alignment of amino acid sequences of human, mouse, and zebrafish GPR34

Amino acid sequences of human, mouse, and zebrafish GPR34 were aligned by using Clustal W program and are viewed with MacVector software. Predicted transmembrane domains of human GPR34 were obtained from GPCRdb (https://gpcrdb.org/) and are boxed. Amino acid residues identical to those of human GPR34 are inverted.

References

(1) Sherman, W.; Day, T.; Jacobson, M. P.; Friesner, R. A.; Farid, R. Novel Procedure for Modeling Ligand / Receptor Induced Fit Effects. *J. Med. Chem.* **2006**, *49*, 534–553.

(2) Sherman, W.; Beard, H. S.; Farid, R. Use of an Induced Fit Receptor Structure in Virtual Screening. *Chem. Biol. Drug Des.* **2006**, *67*, 83–84.