Supporting Information

Elucidating the Interaction of γ-Hydroxymethyl-γ-Butyrolactone Substituents with Model Membranes and Protein Kinase C-C1 domains

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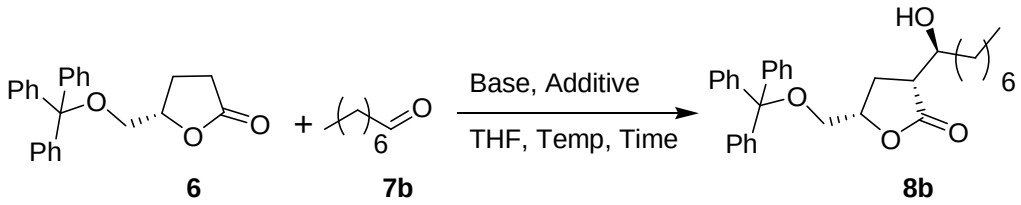
Table S1. Optimization of the aldol reaction conditions[a]

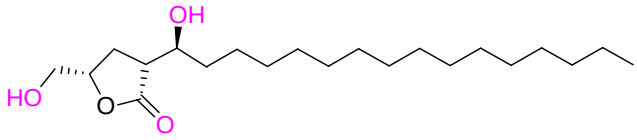


6 **7b** **8b**

entry	base	Additive (equiv)	solvent	Temp (°C)	Time (h)	Yield (%)[b]
1	NaH	LiCl (2.0)	THF	rt-60	24	n.r
2	TEA	LiCl (2.0)	THF	rt-60	24	-
3	DIPEA	LiCl (2.0)	THF	rt-60	24	-
4	DBU	LiCl (2.0)	THF	rt-60	24	-
5	DABCO	LiCl (2.0)	THF	rt-60	24	-
6	LDA	CuCN/LiCl (1.0)/(2.0)	THF	-78	0.5	70
7	n-BuLi	CuCN/LiCl (1.0)/(2.0)	THF	-78	0.2	80
8	n-BuLi	CuI (2.0)	THF	-78	1	56
9	n-BuLi	$ZnCl_2$ (2.0)	THF	-78	1	50

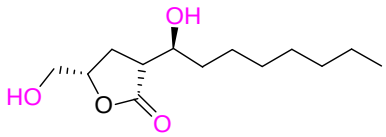
[a]Performed with protected γ-lactone (1.0 equiv), n-BuLi (1.5 equiv), CuCN/LiCl (1.0 equiv)/(2.0 equiv) and aldehyde (1.5 equiv) in dry THF. [b]Isolated yields.

Characterization of the synthesized compounds:

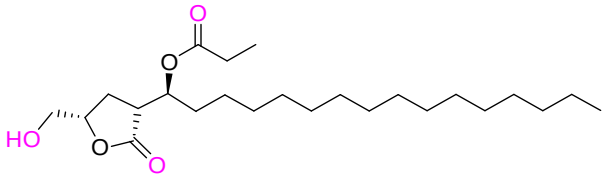


(3*R*,5*S*)-dihydro-5-(hydroxymethyl)-3-((*S*)-1-hydroxypropyl)furan-2(3*H*)-one (1a):

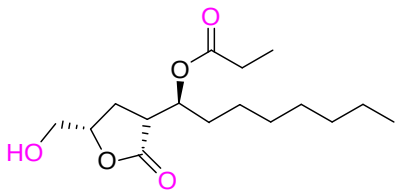
Yellowish semisolid, ^{1}H NMR (400 MHz, $CDCl_3$): δppm 5.01-5.00 (m, 1H), 4.65-4.63 (m, 1H), 3.97-3.94 (m, 1H), 3.90-3.88 (m, 1H), 3.69-3.64 (m, 2H), 1.53-1.47 (m, 1H), 1.33-1.20 (m, 28H), 0.89-0.85 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δppm 179.2, 79.3, 72.0, 63.6, 46.1, 37.3, 35.0, 34.6, 34.0, 32.2, 29.2, 28.2, 27.6, 27.5, 27.4, 27.3, 26.9, 25.2, 22.9, 20.0, 14.3.



(3*R*,5*S*)-dihydro-5-(hydroxymethyl)-3-((*S*)-1-hydroxypropyl)furan-2(3*H*)-one (1b): Yellowish semisolid, ^{1}H NMR(400 MHz, $CDCl_3$): δ_{ppm} 4.78-4.73 (m, 1H), 3.65-3.60 (m, 2H), 3.50-3.43 (m, 1H), 1.59-1.50 (m, 1H), 1.49-1.46 (m, 2H), 1.43-1.40 (m, 2H), 1.36-1.14 (m, 10H), 0.99-0.86 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ_{ppm} 74.6, 72.8, 66.7, 39.1, 38.5, 35.5, 29.9, 29.6, 26.1, 26.0, 23.5, 14.3.

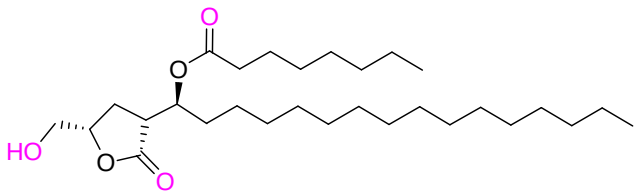


(*S*)-1-((3*R*, 5*S*)-tetrahydro-5-(hydroxymethyl)-2-oxofuran-3-yl) propyl propionate. (**2a**): Yellowish semisolid, ^{1}H NMR(400 MHz, $CDCl_3$): δ_{ppm} 4.88-4.82 (m, 1H), 4.10-4.08 (m, 1H), 4.00-3.95 (m, 1H), 3.79 (bs, 1H), 3.68-3.60 (m, 1H), 2.32-2.29 (m, 2H), 1.59-1.52 (m, 1H), 1.44-1.34 (m, 2H), 1.32-1.14 (m, 28H), 0.89-0.83 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ_{ppm} 174.5, 174.3, 74.4, 70.5, 68.5, 39.2, 38.8, 34.8, 34.6, 34.4, 32.1, 29.9, 29.8, 29.7, 29.5, 29.5, 29.4, 27.5, 25.9, 25.3, 24.6, 23.5, 14.3.

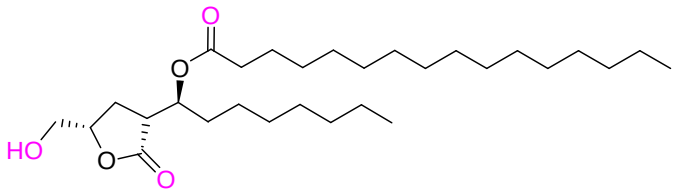


(S)-1-((3R, 5S)-tetrahydro-5-((hydroxymethyl)-2-oxofuran-3-yl)propyl propionate (2b): Yellowish semisolid, ^{1}H NMR(400 MHz, $CDCl_3$): δ_{ppm} 5.15-5.07 (m, 1H), 4.12-

4.06 (m, 1H), 4.00-3.95 (m, 1H), 3.84-3.78 (m, 1H), 2.37-2.30 (m, 2H), 1.61-1.47 (m, 1H), 1.43-1.36 (m, 2H), 1.33-1.18 (m, 12H), 1.14-1.09 (m, 2H), 0.90-0.83 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ_{ppm} 174.9, 74.4, 70.5, 68.6, 39.2, 38.9, 35.2, 34.1, 29.9, 27.7, 27.5, 26.0, 25.9, 25.1, 23.5, 14.3.



(*S*)-1-((3*R*, 5*S*)-tetrahydro-5-(hydroxymethyl)-2-oxofuran-3-yl) propyl propionate (3a):Yellowish semisolid, ^{1}H NMR(400 MHz, $CDCl_3$): δ_{ppm} 5.01-4.92 (m, 1H), 3.66-3.64 (m, 1H), 2.96-2.95 (m, 1H), 2.89-2.88 (m, 1H), 1.89-1.86 (m, 2H), 1.60-1.57 (m, 1H), 1.33-1.20 (m, 40H), 0.89-0.83 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$, DMSO): δppm 176.4, 69.8, 59.6, 31.5, 29.9, 29.3, 29.0, 22.3, 19.6, 13.8.



(*S*)-1-((3*R*, 5*S*)-tetrahydro-5-(hydroxymethyl)-2-oxofuran-3-yl) propyl propionate (3b):Yellowish semisolid, ^{1}H NMR(400 MHz, $CDCl_3$): δ_{ppm} 4.77-4.74 (m, 1H), 3.90-3.84 (m, 1H), 3.69-3.67 (m, 1H), 2.41-2.39 (m, 1H), 1.96 (bs, 1H), 1.72-1.70 (m, 1H), 1.64-1.61 (m, 2H), 1.38-1.14 (m, 40H), 0.89-0.87 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ_{ppm} 174.5, 154.4, 56.5, 49.9, 36.2, 33.0, 32.1, 31.1, 29.9, 29.9, 29.8, 29.7, 29.6, 29.6, 29.5, 26.6, 25.7, 25.7, 25.6, 24.9,22.9, 14.3

NMR spectra of the synthesized compounds

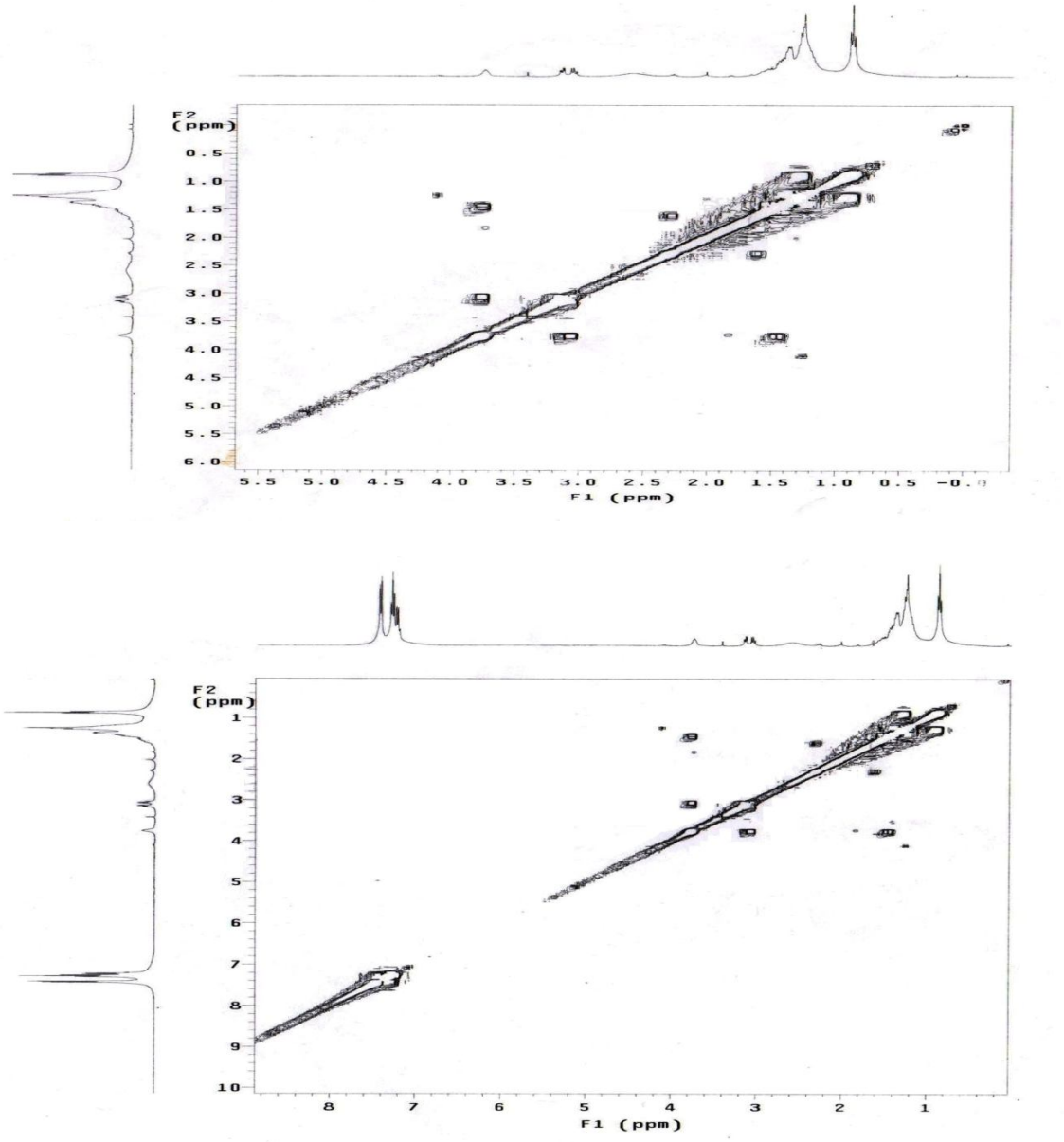


Figure S1: COSY NMR of compound **8b**.

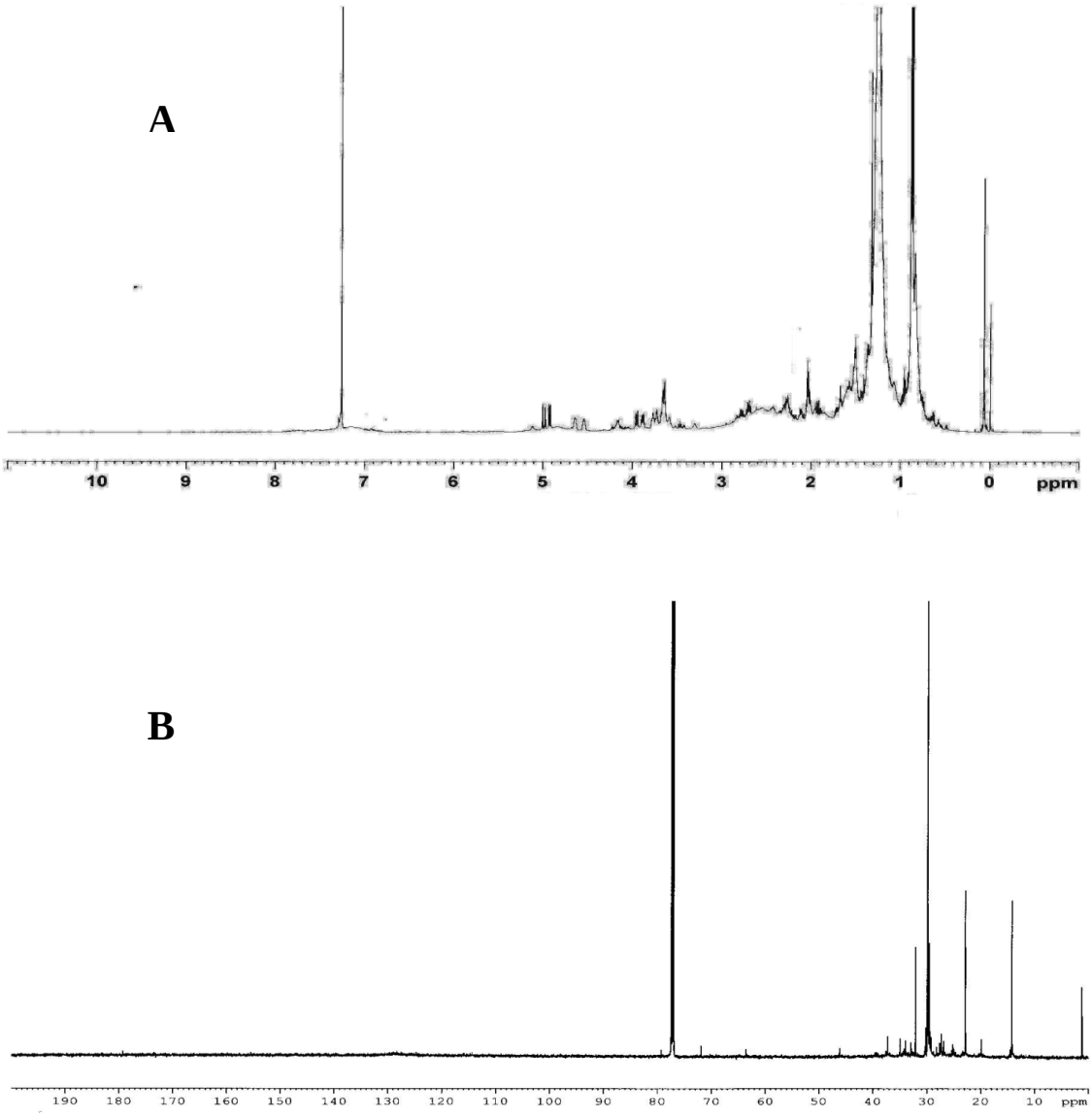


Figure S2: ^{1}H NMR (A) and ^{13}C NMR (B) of compound **1a**.

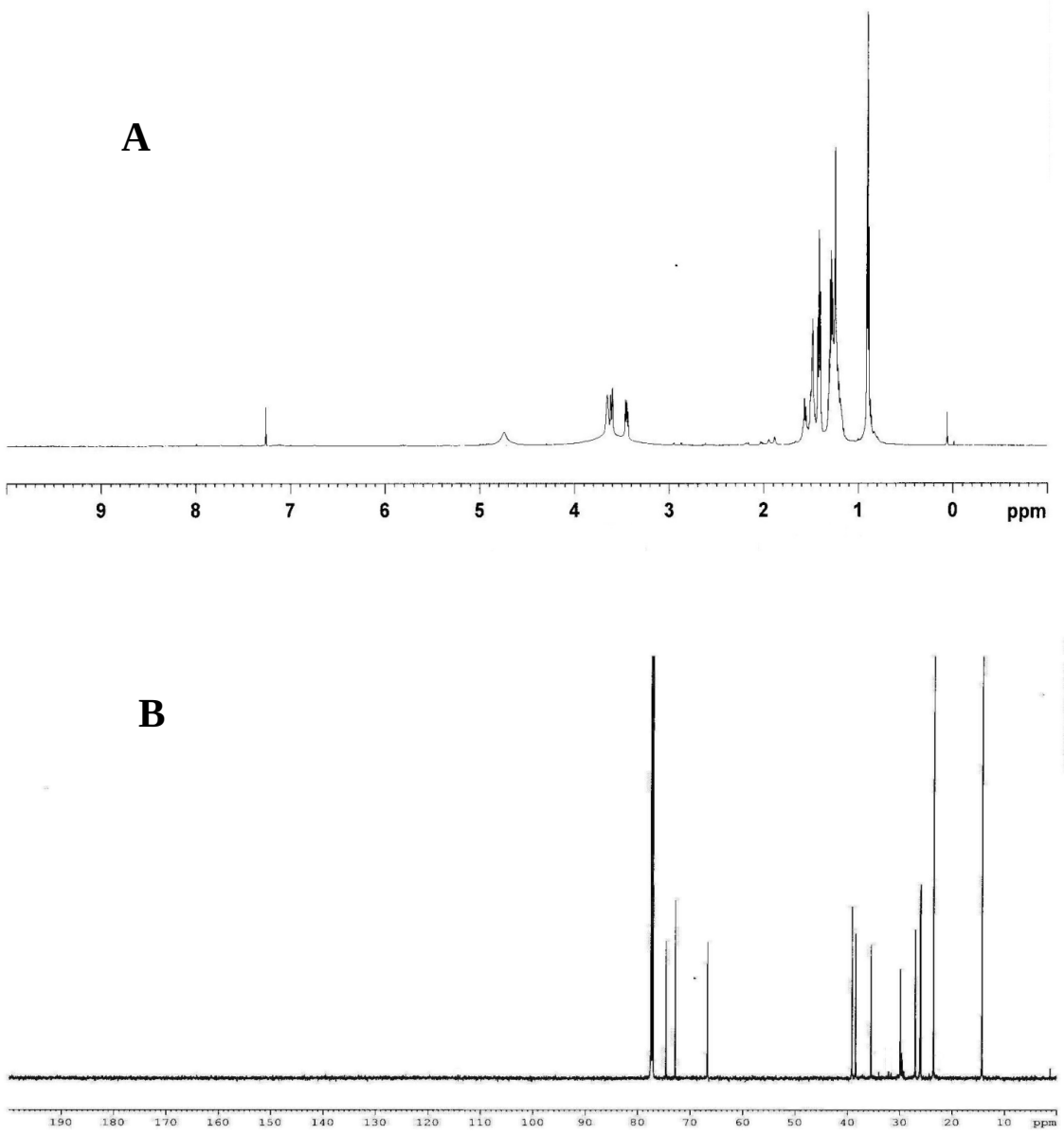


Figure S3: ^{1}H NMR (A) and ^{13}C NMR (B) of compound **1b**.

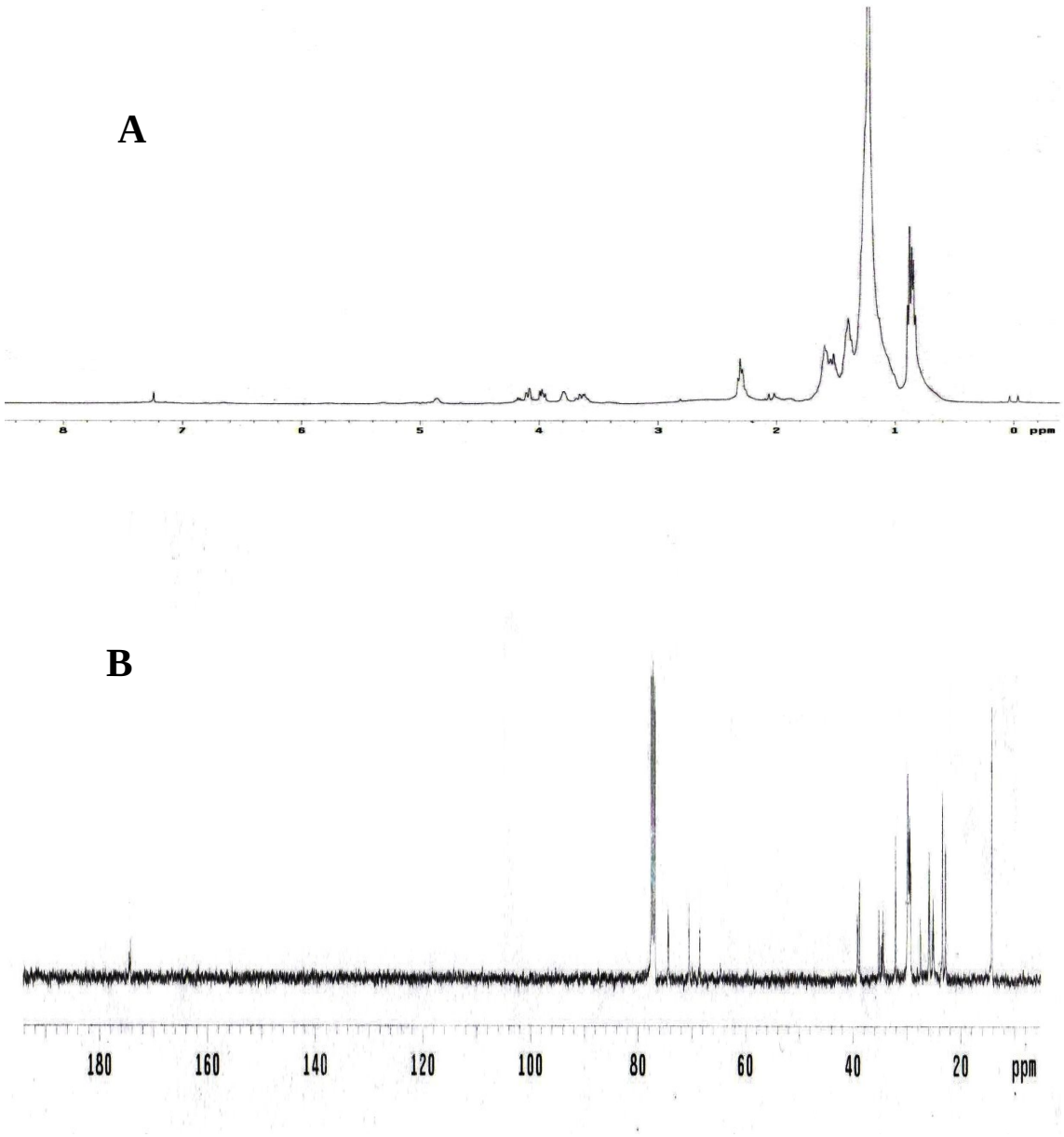


Figure S4: ^{1}H NMR (A) and ^{13}C NMR (B) of compound **2a.**

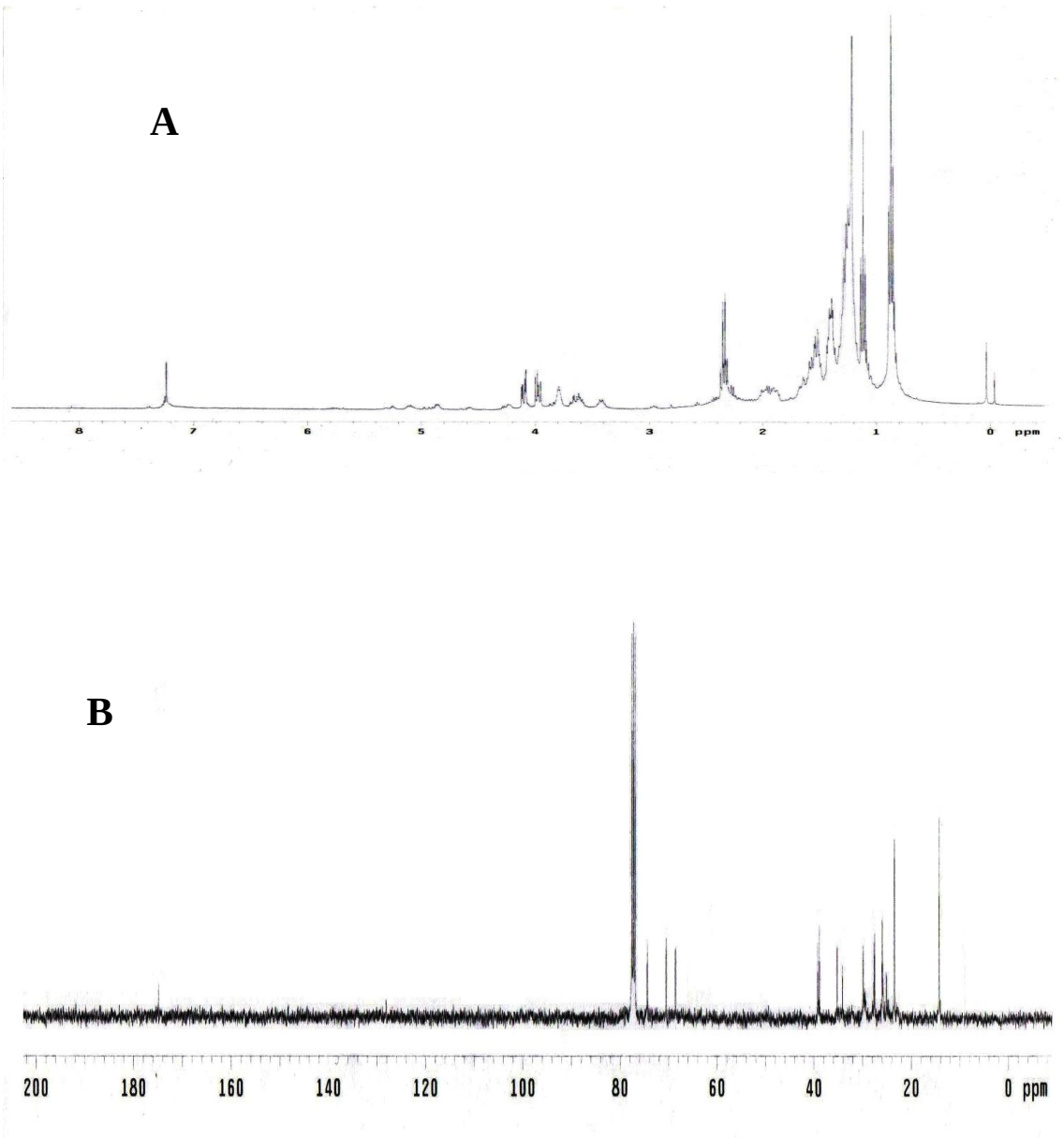


Figure S5: ^{1}H NMR (A) and ^{13}C NMR (B) of compound **2b.**

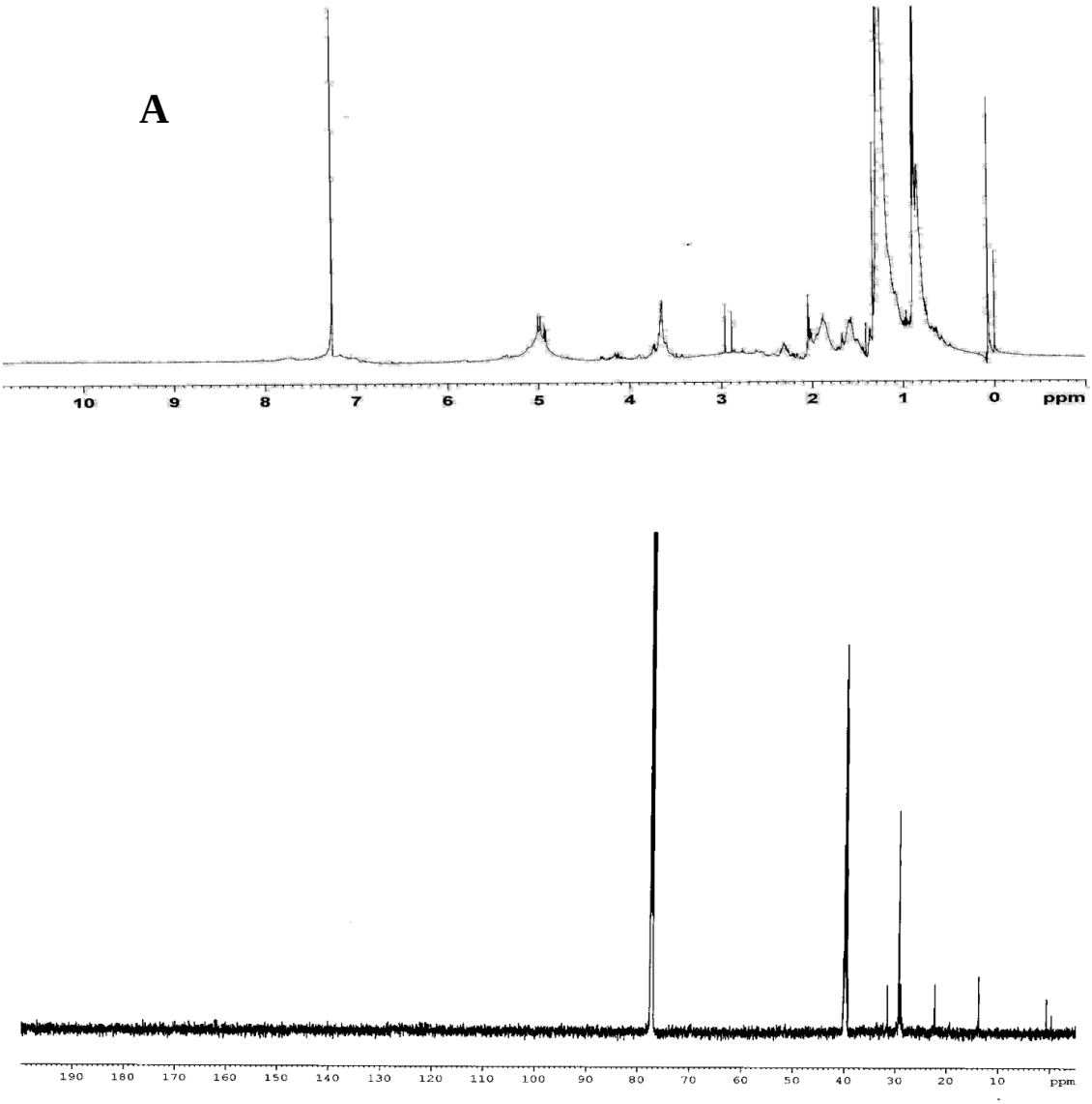


Figure S6: ^{1}H NMR (A) and ^{13}C NMR (B) of compound **3a**.

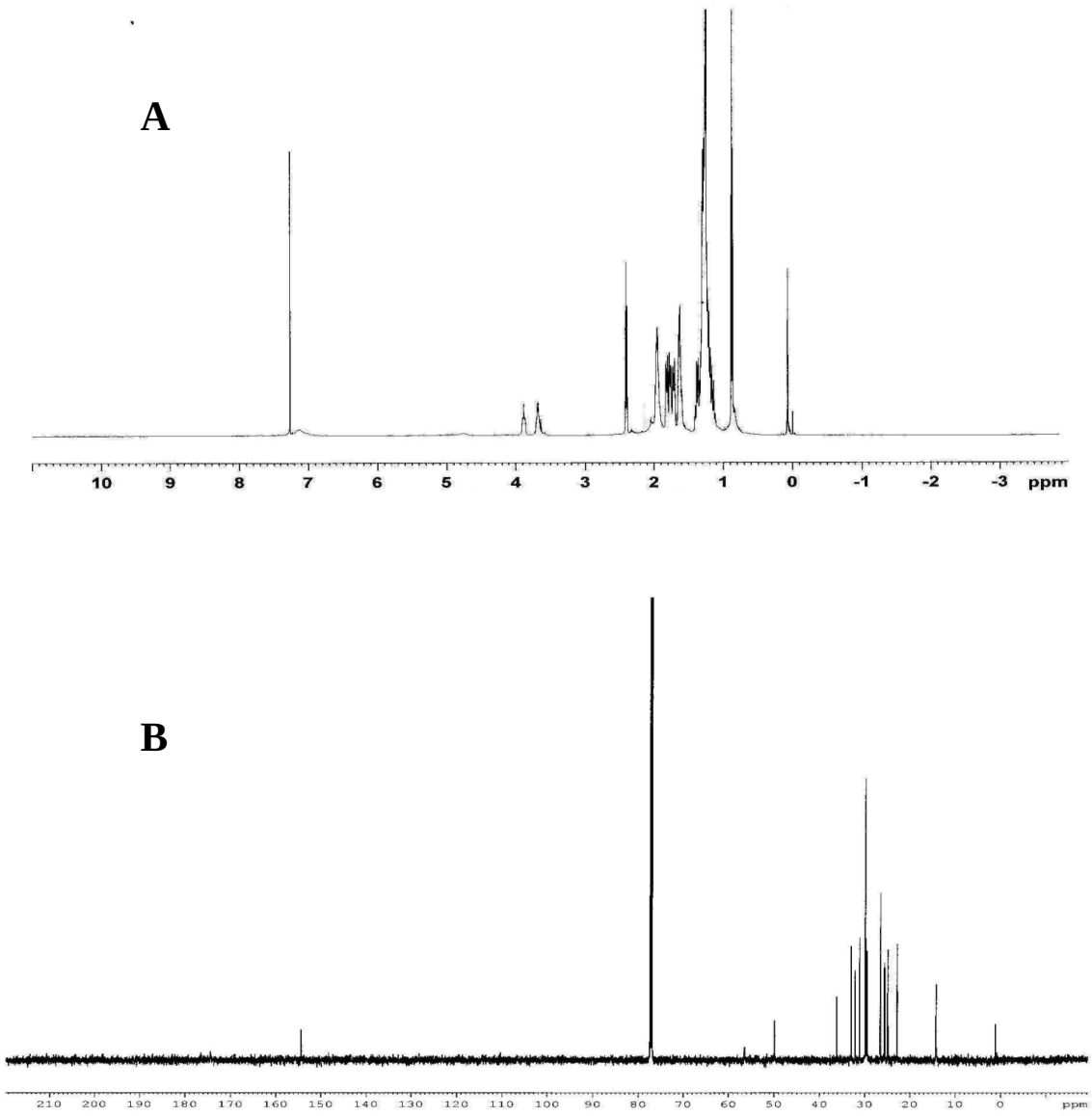


Figure S7: ^{1}H NMR (A) and ^{13}C NMR (B) of compound **3b.**

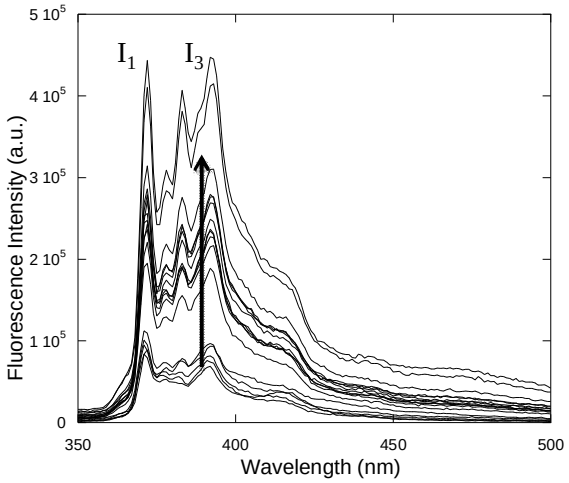


Figure S8: Fluorescence intensity of pyrene probe (2 μM) in the presence of increasing concentration (0-160 μM) of compound **2a** in aqueous solution.

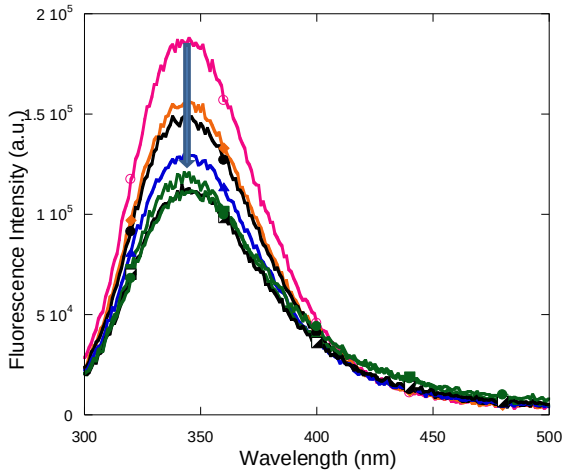


Figure S9: Representative quenching plot of PKCδ-C1b fluorescence by ligand. Addition of increased concentration of compound **2b** (0-12 μM**)** to PKCδ-C1b (1 μM) quenched the intrinsic fluorescence intensity.

Table S2. Anisotropy[a] values of the compounds in the presence and absence of the PKCδ and PKCθ C1b proteins at room temperature.

Compound	PKCδ C1b	PKCθ C1b	Compound	PKCδ C1b	PKCθ C1b
Buffer[b]	0.0439 (0.0045)	0.0667 (0.0053)	DAG_8[c]	0.0978 (0.0063)	0.1069 (0.0051)
DAG_{16}[c]	0.1028 (0.0061)	0.0727 (0.0077)	**1b**[c]	0.1821 (0.0037)	0.1332 (0.0087)
1a[c]	0.1759 (0.0045)	0.1767 (0.0067)	**2b**[c]	0.1925 (0.0062)	0.1409 (0.0029)
2a[c]	0.1882 (0.0035)	0.1733 (0.0043)	**3b**[c]	0.1630 (0.0111)	0.1123 (0.0036)
3a[c]	0.1530 (0.0035)	0.1460 (0.0116)			

[a)]Values in the parenthesis indicate standard deviations.
[b)]Protein, 1 μM in buffer (20 mM Tris, 160 mM NaCl, 50 μM $ZnSO_4$, pH 7.4).
[c)]**DAG, 1-3**, 10 μM; protein, 1 μM in buffer (20 mM Tris, 160 mM NaCl, 50 μM $ZnSO_4$, pH 7.4).

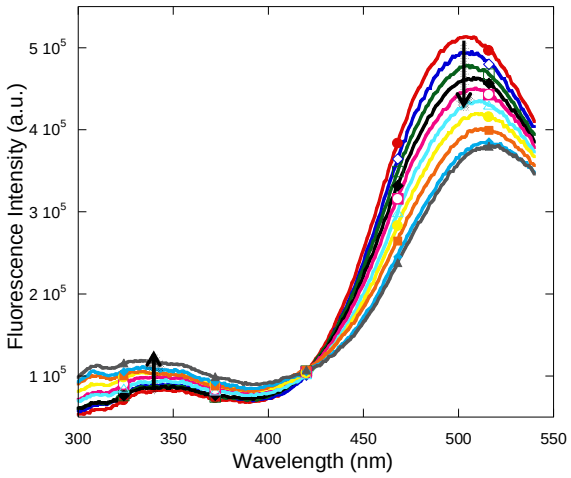


Figure S10: Representative protein-to-membrane FRET assessment under liposomal environment. Addition of increased concentration of DAG_8 (0 - 80 μM) to PKCδ-C1b sundomain (1 μM) bound to the active liposome (PC/PE/dPE/ligand (75/15/5/5)) of compound **2a** decreases the FRET signal at 500 nm. All the measurements were performed in 20 mM Tris, pH 7.4 containing 160 mM NaCl and 50 μM $ZnSO_4$.

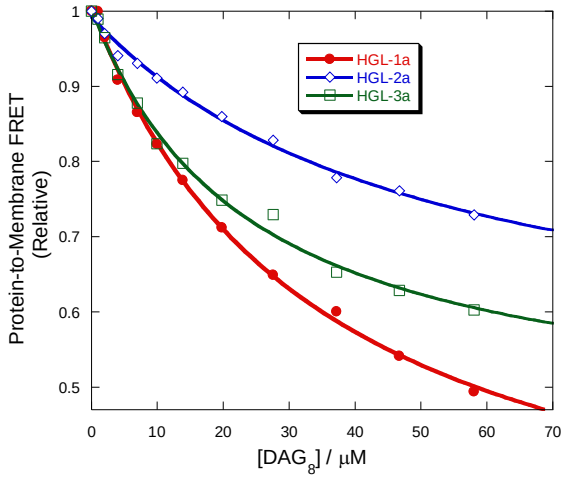


Figure S11: Competitive displacement assay for the PKCθ-C1b (A) subdomains (1 μM) bound to liposome containing ligands **1a, 2a** and **3a**. The bound complex was titrated with the DAG_8.

Table S3. Binding parameters of compound **1a** and **2a** containing liposome[a] with the PKCδ-C1b protein[b] at room temperature.

Compound	**1a** (nM)	**2a** (nM)
PKCδ-C1b	147 ± 14	127 ± 17

[a]Liposome composition, PC/PE/PS /$Ligand_{16}$ (55/20/20/5).
[b]Protein, 0.25 μM in buffer (20 mM Tris, 150 mM NaCl, 50 μM $ZnSO_4$, pH 7.4).
Values represent the mean ± SD from triplicate measurements.

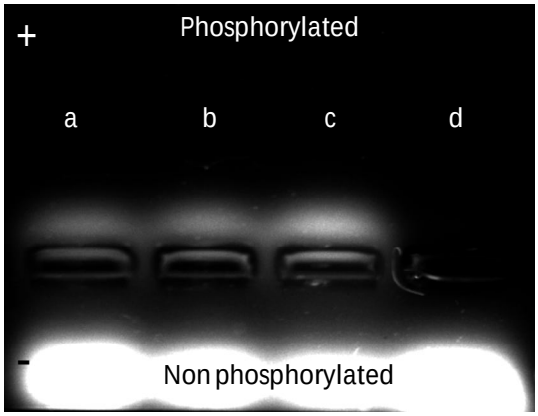


Figure S12: The PKC activity in the absence and presence of compound **2a**. Representative UV-illuminated agarose gel image of the product of reactions run with PKC full-length enzyme. Control with no PS containing PKC activator solution and activator (lane d). PKC activity assay in the presence of compound **2a** with 1μM (lane a), 2μM (lane b) and 4μM (lane c) concentrations.