Supplementary Information 2

Design and synthesis of lipid-coupled inositol 1,2,3,4,5,6-hexakisphosphate derivatives exhibiting high-affinity binding for HIV-1 MA domain

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Table S1. The dissociation constants (*K*d) for IP_3-MA and **1***-MA obtained in the dimethysulfoxide-containing buffer (the present research) and buffer without containing dimethylsulfoxide (the previous report**).

compounds	*K*d (μM)	
	The present research (with dimethylsulfoxide)	The previous report** (without dimethylsulfoxide)
IP_3	272±48	568±52
1*	16.9±1.3	5.64±1.43

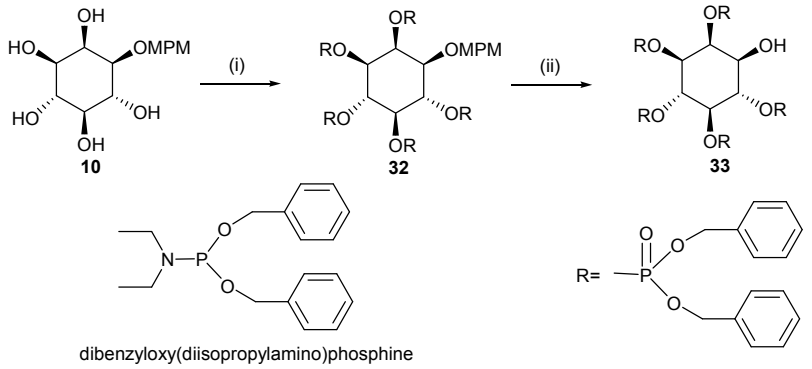
* named di-C_8-PIP_2 in ref 7

** ref 7

Table S1 shows the *K*d values of IP_3-MA and **1***-MA complex in a buffer with or without dimethylsulfoxide. Whereas the *K*d value measured in the buffer used in the previous report** showed some dispersion, the *K*d value obtained in the improved dimethylsulfoxide-containing buffer did not disperse.

Structure confirmation of regioisomers of 2 and 2'

As shown in **Scheme S1**, to confirm the structure of **2** and **2'**, the 2,3,4,5,6-pentakishydroxyl compound **10** was converted to pentakisphosphonate **32** by treatment with phosphoramitdite considered not to be caused phosphate migration, dibenzyl *N*, *N*-diethylphosphine, and 1*H*-tetrazole and subsequent oxidation with MCPBA in 81% yield. Oxidative cleavage of *p*-methoxybenzyl group with CAN [16] gave the $InsP_6$ fragment **33** having 1-hydroxy free of *myo*-inositol as a one spot over the thin layer silica gel chromatography in 62% yield. As the same procedure in **Scheme 5**, the glycerol moiety **18** was reacted with Benzyl-*N, N, N', N'*-tetraisopropylphosphoramidite and 1*H*-tetrazole, subsequently condensed with compound **33**, and oxidation with tBuOOH gave compound **34** in 61% yield. Finally, protecting groups were removed by hydrogenolysis with palladium carbon to give compound **35** in 80% yield.



Scheme S1 *Reagents and conditions*: (i) (a) dibenzyl *N*,*N*-diethylphosphoramidite, *1H*-tetrazole, CH_2Cl_2, rt, overnight; (b) MCPBA, CH_2Cl_2, rt, 1 hr, 81%; (ii) CAN, CH_3CN-H_2O, rt, 1 hr, 62% yield.

DL-1-*O*-(*p*-methoxybenzyl)-2,3,4,5,6-penta-*O*-(dibenzoyloxyphosphoryl)-*myo*-inositol (32)

To a suspension of **10** (0.10 g, 0.333 mmol) in CH_2Cl_2 (5 ml) was added MS4A, and the resulting suspension was stirred at room temperature under argon for 15 min. To the mixture was added dibenzyl *N*,*N*-diethylphosphoramidite (1.0 g, 3.15 mmol) followed by 1*H*-tetrazole (0.232 g, 3.32 mmol), the resulting mixture was stirred at room temperature under argon for overnight. To the mixture was added *m*-chloroperbenzoic acid (0.569 g, 3.30 mmol) in small portions, and the resulting mixture was stirred at –78 °C to room temperature for 1hr. The mixture was purified by

silica gel column chromatography (AcOEt:Hexane=2:1) to afford **32** (0.430 g, 81%) as a colorless oil.

^{1}H NMR ($CDCl_3$) δ: 3.54-3.58 (1H, d, *J*=9.9Hz, C<u>H</u>), 3.67 (3H, s, C<u>H</u>$_3$), 4.35-4.44 (1H, d, *J*=10.3Hz, C<u>H</u>, 1H, t, *J*=9.9, C<u>H</u>), 4.52-4.61 (1H, dd, *J*=9.5, 19.4Hz, C<u>H</u>), 4.64-4.67 (1H, d, *J*=10.6Hz, C<u>H</u>), 4.73-5.23 (22H, m, C<u>H</u>$_2$C$_6$H$_5$ x 10, C<u>H</u> x 2), 5.51-5.53 (1H, d, *J*=9.5Hz, C<u>H</u>$_2$), 6.66-6.69 (1H, d, *J*=8.8, C<u>H</u>$_2$), 7.06-7.34(52H, m, C$_6$<u>H</u>$_5$). ^{13}C NMR (CDCl3) δ: 55.4, 69.7, 69.8, 70.1, 70.2, 70.3, 70.4, 72.2, 74.0, 75.2, 76.6, 76.8, 113.9, 127.9, 128.0, 128.2, 128.3, 128.5, 128.6, 128.7, 130.1, 130.7, 159.6. IR (KBr) 3035, 2970, 1610, 1515, 1280, 1215, 880, 740, 700 cm^{-1}. HRMS(FAB) *m/z* calcd for $C_{84}H_{85}O_{22}P_5$ $(M+Na)^+$ 1623.4118 Found:1623.4149. Anal. Calcd for $C_{84}H_{85}O_{22}P_5$: C, 63.00; H, 5.35. Found: C, 62.76; H, 5.38.TLC; R*f* 0.40 (Hexane:AcOEt=1:2).

DL-2,3,4,5,6-penta-*O*-(dibenzoyloxy phosphoryl)-*myo*-inositol (33)

To a solution of **32** (0.30 g, 0.187 mmol) in CH_3CN-H_2O (9:1, 5 ml) was added diammonium cerium(IV) nitrate (0.256 g, 0.469 mmol) and the resulting mixture was stirred at room temperature for 1 hr. The mixture was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (CH_2Cl_2:AcOEt=1:5) to afford **33** (0.17 g, 62%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 3.72-3.75 (1H, d, *J*=8.8Hz, C<u>H</u>), 4.26-4.32 (1H, t, *J*=9.5, C<u>H</u>), 4.41-4.58 (2H, m, C<u>H</u> x 2), 4.75-5.17 (21H, m, C<u>H</u>$_2$C$_6$H$_5$ x 10, <u>CH</u>), 5.24-5.27 (1H, d, *J*=8.8Hz, C<u>H</u>), 5.41-5.42 (1H, d, *J*=3.3, O<u>H</u>), 6.98-7.33 (50H, m, C$_6$<u>H</u>$_5$).^{13}C NMR ($CDCl_3$) δ: 69.6, 69.7, 69.8, 69.9, 70.0, 70.2, 70.3, 127.9, 128.0, 128.1, 128.2, 128.3, 128.4, 128.5, 128.9. IR (KBr) 3420, 3050, 3000, 1605, 1500, 1455, 1380, 1280, 1215, 880, 740, 700 cm^{-1}. HRMS(FAB) *m/z* calcd for $C_{76}H_{77}O_{21}P_5$ $(M+Na)^+$ 1503.3543 Found:1503.3721. TLC; R*f* 0.31 (CH_2Cl_2:AcOEt=1:5).

DL-2, 3,4,5,6-penta- *O*-(dibenzoyloxy phosphoryl)-*myo*-inositol 1-{[1,2-*O*-diheptanoyl-*sn*-glyceryl](benzyl)phosphate} (34)

To a mixture of **18** (0.17 g, 0.54 mmol) in CH_2Cl_2 (5 ml) was added Benzyl-*N, N, N', N'*-tetraisopropylphosphoramidite (0.20 ml, 0.54 mmol) followed by MS4A, and the resulting mixture was stirred at room temperature under argon for 15 min. To the mixture was added 1*H*-tetrazole (0.038 g, 0.54 mmol), and the resulting mixture was stirred at room temperature under argon for 10

min. To the mixture was added completely dissolved compound **33** (0.17 g, 0.114 mmol) in CH_2Cl_2 (5 ml) with MS4A, followed by adding 1*H*-tetrazole (0.076 g, 1.08 mmol), and the resulting mixture was stirred at room temperature for further 24hrs. To the mixture was added *tert*-butylhydroperoxide (0.082 ml, 0.818 mmol), and stirred at room temperature for further 5min. The mixture was purified by silica gel column chromatography (Hexane:CH_2Cl_2:AcOEt=1:2:1 followed to CH_2Cl_2:MeOH=20:1) to afford compound **34** (0.135 g, 61%) as a colorless oil.

^{1}H NMR ($CDCl_3$) δ: 0.71-0.96 (6H, m, C$\underline{H}_3$ x 2), 1.19-1.29 (12H, m, C$\underline{H}_2$ x 6), 1.48-1.51 (4H, m, C$\underline{H}_2$ x 2), 2.04-2.21 (4H, m, C$\underline{H}_2$ x 2), 3.65-4.08 (1H, m, C$\underline{H}$), 4.10-4.31 (3H, m, C$\underline{H}_2$, C$\underline{H}$), 4.44-4.52 (3H, m, C$\underline{H}_2$, C$\underline{H}$), 4.88-5.26 (25H, m, C$\underline{H}_2$C$_6$H$_5$ x 11, C$\underline{H}$ x3), 5.58-5.60 (1H, d, *J*=6.6, C$\underline{H}$), 6.90-7.37 (55H, m, C$_6\underline{H}_5$). ^{13}C NMR ($CDCl_3$) δ: 14.0, 22.4, 24.7, 28.7, 31.4, 33.9, 61.6, 61.7, 69.6, 69.7, 69.8, 70.0, 70.2, 71.0, 72.1, 73.4, 73.5, 74.5, 74.9, 75.2, 111.7, 127.1, 127.8, 127.9, 128.0, 128.1, 128.2, 128.3, 128.4, 128.5, 135.9, 172.8, 173.1. IR (KBr) 2860, 1740, 1500, 1455, 1380, 1280, 1215, 1020, 890, 740, 700 cm^{-1}. MS(FAB) *m/z* calcd for $C_{100}H_{114}O_{28}P_6$ $(M+Na)^+$ 1971.58 Found:1971.68. Anal. Calcd for $C_{100}H_{114}O_{28}P_6$: C, 61.60; H, 5.89. Found: C, 61.39; H, 5.91. TLC; R*f* 0.34 (CH_2Cl_2:MeOH=20:1).

DL-1-*O*-(1,2-*O*-diheptanoyl-*sn*-glyceryl) hydrogen phosphoryl]-*myo*-inositol 2,3,4,5,6-pentakis(hydrogenphosphate): 35

To a solution of **34** (0.10 g, 0.0513 mmol) in *t*BuOH (8 ml) and H_2O (1.5 ml) was added 10% Pd-C (0.05 g, 0.0469 mmol), and the resulting mixture was stirred at room temperature under hydrogen for 24 h. The mixture was filtered through a pad of celite, and then washed the celite pad with H_2O. The resulting filtrate was lyophilized. The residue was dissolved H_2O (2 ml), and filtered through the cation-exchange resin. To the parts of resulting filtrate (0.0174 g, 0.0182 mmol) was added triethylamine (0.034 ml, 0.245 mmol), and concentrated under reduced pressure. The resulting residue was dissolved in H_2O, and lyophilized to afford **35** (0.0169 g, 80% from compound **34**) as a white solid. The data was identical to that of compound **2**.