SUPPLEMENTAL INFORMATION

A search for ceramide binding proteins using bifunctional lipid analogs yields CERT-related protein StarD7

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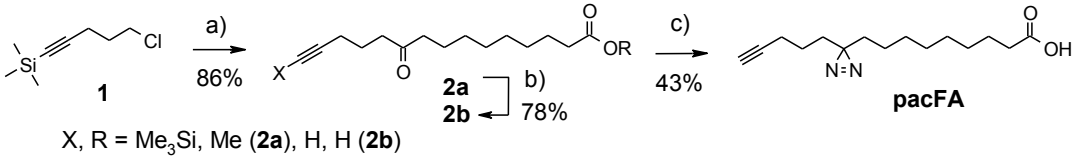
Synthesis of bifunctional lipid analogs

General aspects - Starting compounds and solvents were purchased from the indicated suppliers and used as obtained. Unstabilized solvents CH_2Cl_2 (SeccoSolv, Merck), Et_2O (SeccoSolv, Merck), MeOH (Fisher), DMF (Roth) and THF (Sigma) were dry grade. Reactions were carried out under Ar in dry Schlenk flasks with protection from light, where necessary. Column chromatography (CC): silica gel 60 (SiO_2; Merck, Germany). TLC: aluminum plates, SiO_2 60 F_{254}, 0.2-mm layer (Merck, Germany). NMR spectra were obtained using an AMX-500 spectrometer (Bruker, DHelvetica Rheinstetten). ^{1}H: 500.14; ^{13}C: 125.76; ^{31}P: 101.3 MHz; δ in ppm relative to the signals of the residual protons of $CHCl_3$ (δ =7.26 ppm) in $CDCl_3$, carbons of $CDCl_3$ (δ =77.00 ppm) and external 85% H_3PO_4 for ^{31}P; *J* in Hz. ESI-MS analysis was performed using a Bruker Daltronics Esquire HCT instrument (Bruker Daltronics, D-Leipzig); ionization was performed with a 2% aq. HCOOH solution. Elemental analyses (C, H, N) were performed on a VarioMICRO instrument (Fa. Elementar, D-Hanau). Abbreviations of commonly used reagents: DMAP (4-(dimethylamino)-pyridin), DCC (N,N'-dicyclohexylcarbodiimide), HOBT (1-hydroxybenzotriazol), EDCI (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide), HBTU (2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium-hexafluorophosphat), Boc_2O (di-*tert*-butyldicarbonat).

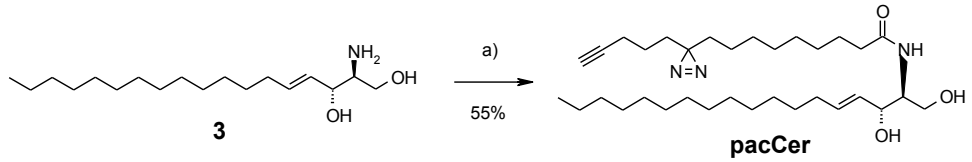


Scheme 1. Synthesis of pacFA. *Reaction conditions*: a) 1. Mg, THF; 2. $ClCO(CH_2)_8COOMe$, THF; b) KOH, MeOH; c) 1. NH_3, H_2NOSO_3H, -60°C, 6h; 2. Et_3N, I_2, MeOH, rt, 30 min.

9-(3-[Pent-4-ynyl]-3H-diazirin-3-yl)-nonanoic acid / pacFA – A solution of hydroxylamine-O-sulfonic acid (93 mg, 0.82 mmol) in MeOH (0.5 ml) was added to a pre-cooled (-60°C) suspension of 10-oxo-pentadec-14-ynoic acid **2b** (101 mg, 0.4 mmol, prepared in 2 steps from 1-chloro-5-(trimethylsilyl)-4-pentyne **1** (Alfa Aesar) and 9-methoxycarbonylnonanoyl chloride according to [1]) in liquid ammonia (8 ml) and the resulting mixture was stirred at this temperature over 6 h. Cooling bath (acetone-CO_2) was removed and the reaction mixture was allowed to warm to room temperature overnight (Attention: work in a hood. Strong ammonia evaporation). Light beige powdery residue was suspended in MeOH (5 ml) and concentrated in vacuum to remove traces of ammonia. It was repeatedly suspended in MeOH (5 ml) and the precipitate of $(NH_4)_2SO_4$ was separated, washed with MeOH (4 x 1 ml) and the filtrate was alkalized with Et_3N (0.5 ml).

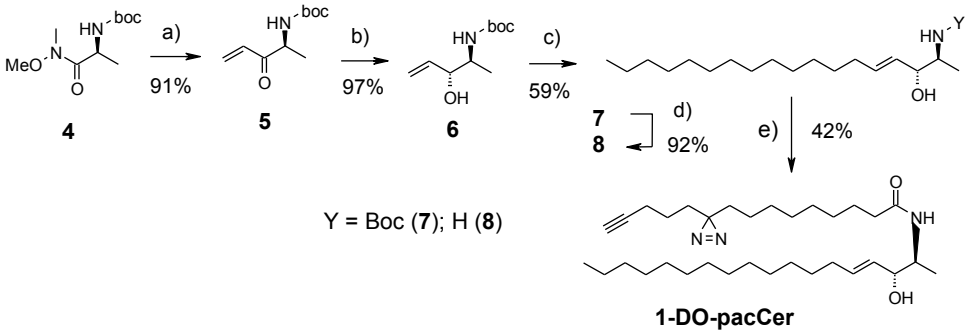
The solution obtained was protected from light and a solution of iodine (102 mg, 0.4 mmol) in MeOH (4 ml) was added dropwise until iodine stain no longer disappeared. The reaction mixture was

concentrated in vacuum, the residue was taken into AcOEt (15 ml) and washed with H_2O, dried (Na_2SO_4) and concentrated. **pacFA** was isolated from apricot residue by chromatography on silica gel, eluted with a mixture pentane:Et_2O:AcOH 75:24:1 v/v/v, to give 45.2 mg (43%) of colorless oil. TLC (pentane:Et_2O:AcOH 50:50:1 v/v/v): Rf 0.60. ^{1}H NMR ($CDCl_3$): 2.36 (2H, t, *J* 7.5, H_2CCO), 2.17 (2H, dt, *J* 7.1, 2.5, $H_2CC{\equiv}$), 1.96 (1H, t, *J* 2.5, $HC{\equiv}$), 1.64 (2H, q, *J* 7.1, $H_2CCC{\equiv}$), 1.52-1.49 (2H, m, H_2CCN_2), 1.40-1.25 (12H, m), 1.13-1.07 (2H, m). ^{13}C NMR ($CDCl_3$): 179.95 (C=O), 83.42 (-C≡), 68.82 (HC≡), 53.37 (CN_2), 33.97 (CC=O), 32.83 (CCN_2), 31.83 (CCN_2), 29.09, 29.06, 28.99, 28.92, 24.58, 23.73, 22.73, 17.93 (CC≡). Spectral data are in good agreement with those reported [1]. ESI MS (positive mood): 237.2 $[M-28+1]^+$, 265.2 $[M+1]^+$, 287.2 $[M+23]^+$, 529.3 $[2M+1]^+$.



Scheme 2. Synthesis of pacCer. *Reaction conditions*: a) pacFA, EDCI*HCl, HOBT, CH_2Cl_2, 0°C, 15 h.

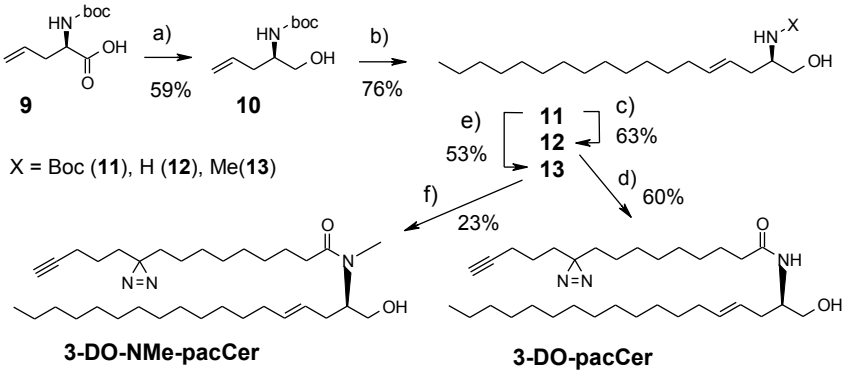
N-9-(3-[Pent-4-ynyl]-3*H*-diazirin-3-yl)-nonanoyl-D-*erythro*-sphingosine / pacCer – A solution of EDCI*HCl (5.74 mg, 0.03 mmol) in CH_2Cl_2 (0.25 ml) was added dropwise and with protection from light to a pre-cooled (ice-bath) solution of pacFA (7.23 mg, 0.027 mmol), D-*erythro*-Sphingosine **3** (7.8 mg, 0.26 mmol, Enzo Biochem Inc.) and HOBT (4.0 mg, 0.03 mmol) in CH_2Cl_2 (0.5 ml) and the resulting mixture was stirred overnight. The reaction mixture was concentrated in vacuum and the resulting **pacCer** was isolated by chromatography on silica gel, eluted with CH_2Cl_2-AcOEt, 1:2 v/v mixture to give 7.7 mg (55%) of colorless oil. TLC (AcOEt): R_f 0.68. ^{1}H NMR ($CDCl_3$): 6.27 (1H, d, *J* 7.3, NH), 5,83-5.77 (1H, m, HC=), 5.54 (1H, dd, *J* 15.5, 6.5, HC=), 4.34-4.33 (1H, m, HCOH), 3.98-3.95 (1H, dd, *J* 11.2, 3.8, HCH-O), 3.94-3.91 (1H, m, HCN), 3.72 (1H, dd, *J* 11.2, 3.2, HCH-O), 2.24 (2H, t, *J* 7.5, H_2CCO), 2.17 (2H, dt, *J* 6.9, 2.5, $H_2CC{\equiv}$), 2.09-2.05 (2H, m, H_2CCO), 1.96 (1H, t, *J* 2.5, $HC{\equiv}$), 1.64 (2H, q, *J* 7.3, $H_2CCC{\equiv}$), 1.52-1.49 (2H, m, H_2CCN_2), 1.40-1.25 (12H, m), 1.12-1.08 (2H, m, CH_2), 0.90 (3H, t, *J* 7.0, CH_3). ^{13}C NMR ($CDCl_3$): 173.79 (C=O), 134.24 (C=), 128.85 (C=), 83.44 (-C≡), 74.65 (HCOH), 68.87 (HC≡), 62.49 (H_2COH), 60.37 (CN_2), 54.52 (CN), 36.78 (CC=O), 32.84 (CC=), 32.28, 31.92 (CCN_2), 31.85 (CCN_2), 29.68, 29.49, 29.34, 29.20, 29.18, 29.16, 28.40, 25.67, 23.77 (CCH_3), 22.75, 22.68, 17.93 (CC≡), 14.09 (CH_3). ESI MS (positive mood): 683.6 $[M-28+1]^+$, 711.6 $[M+1]^+$, 733.6 $[M+23]^+$, 1423.2 $[2M+1]^+$.



Scheme 3. Synthesis of 1-DO-pacCer. *Reaction conditions*: a) 1. H_2C=CHMgBr, THF, -45°C, 1.5h; 2. HCl, 0°C; b) 1. $LiAlH(OtBu)_3$, EtOH, -60°C, 4h; 2. HCl, -60°C; c) $C_{13}H_{27}$-CH=CH_2, Grubbs (II) cat, CH_2Cl_2, 65°C, 5h; d) HCl, EtOH, 65°C, 1h; e) pacFA, EDCI, HBTU, iPr_2NEt, CH_2Cl_2, rt, 15 h.

(2*S*,3*R*,4*E*)-2-Aminooctadec-4-en-3-ol / reaction product (8) − Diluted HCl (5M, 0.1 ml) was added dropwise to pre-cooled (ice-bath) solution of *N*-[(1*S*,2*R*,3*E*)-2-hydroxy-1-methyl-3-heptadecen-1-yl]-carbamic acid *tert*-butyl ester **7** (42 mg, 0.11 mmol, prepared in 3 steps from *N*-(*tert*-butoxycarbonyl)-L-alanin-*N*'-methoxy-*N*'-methylamid (**4**), Acros Organics, according to [2]) in EtOH (0.1 ml) and the resulting mixture was stirred at 65°C during 1h. It was diluted with CH_2Cl_2 (4 ml), cooled on an ice-bath and carefully neutralized with $NaHCO_3$ aq. Organic phase was separated, the water phase was extracted with CH_2Cl_2 (2 x 4 ml), combined extracts were washed with brine, dried (Na_2SO_4) and concentrated to give aminoalcohol **8** (28 mg, 91%) as yellowish solid. TLC (CH_2Cl_2-NH_3/MeOH 20:1 v/v): R_f 0.17. ^{1}H NMR ($CDCl_3$): 5.77-5.71 (1H, m, =CH), 5.46 (1H, dd, *J* 15.4, 6.8 Hz, =CH), 3.94-3.92 (1H, m, HC-O), 3.01-2.99 (1H, m, HC-N), 2.07 (2H, q, *J* 7.2, CH_2C=), 1.85 (3H, br s, OH, NH2), 1.40-1.37 (2H, m), 1.28 (24H, br s), 1.08 (3H, d, *J* 6.8, $C\underline{H}_3CH$), 0.90 (3H, t, *J* 7, $C\underline{H}_3CH_2$). In good agreement with that reported [2]. ^{13}C NMR ($CDCl_3$): 134.16 (C=), 128.63 (C=), 76.31 (C-O), 50.94 (C-N), 32.38 ($\underline{C}C$=), 32.33, 31.88, 29.64, 29.63, 29.61, 29.58, 29.45, 29.31, 29.27, 29.17, 22.64, 18.50 ($\underline{C}H_3CH$), 14.04 ($\underline{C}H_3CH_2$).

***N*-[(1*S*,2*R*,3*E*)-2-hydroxy-1-methyl-3-heptadecen-1-yl]-octanamide / 1-DO-pacCer** – A solution of HBTU (8.70 mg, 0.023 mmol) in CH_2Cl_2 (0.1 ml) was added dropwise and with protection from light to a pre-cooled (ice-bath) solution of pacFA (5.80 mg, 0.022 mmol), (2*S*,3*R*,4*E*)-2-aminooctadec-4-en-3-ol **8** (5.61 mg, 0.20 mmol) and *i*Pr_2NEt (7.70 mg, 0.06 mmol) in CH_2Cl_2 (0.2 ml) and the resulting mixture was stirred at room temperature over 15h. The reaction mixture was concentrated in vacuum and the resulting **1-DO-pacCer** was isolated by chromatography on silica gel, eluted with CH_2Cl_2-AcOEt 9:1 v/v mixture to give 4.4 mg (42%) of colorless paste. TLC (CH_2Cl_2-AcOEt 9:1 v/v): R_f 0.38. ^{1}H NMR ($CDCl_3$): 5.77-5.71 (1H, m, =CH), 5.57 (1H, d, *J* 7, NH), 5.40 (1H, dd, *J* 16, 7, =CH), 4.14-4.10 (2H, m; CH-O, CH-N), 2.18 (2H, q, *J* 7), 2.17 (2H, q, *J* 7), 2.07 (2H, q, *J* 7, CH_2COO), 1.96 (1H, t, *J* 2.2, ≡CH), 1.63 (2H, qt, *J* 7, CH_2C=), 1.52-1.49 (2H, m), 1.40-1.25 (42H; m), 1.13 (3H, d, *J* 6.7, CH_3), 0.90 (3H, t, *J* 7, CH_3). ^{13}C NMR ($CDCl_3$): 173.56 (C=O), 134.08 (C=), 128.23 (C=), 83.42 (C≡), 75.63 (C-O), 68.83 (≡CH), 50.01 (C-N), 36.78 ($\underline{C}C$=O), 32.84 ($\underline{C}C$=), 32.33, 31.89 ($\underline{C}CN_2$), 31.85 ($\underline{C}CN_2$), 29.65, 29.63, 29.60, 29.47, 29.32, 29.18, 29.16, 29.13, 29.09, 28.36, 25.68, 23.74, 22.74, 22.65, 17.95 ($\underline{C}C$≡), 15.22 ($\underline{C}H_3CH$), 14.06 ($\underline{C}H_3CH_2$). ESI MS (positive mood): 484.43 [M-18-28+1]$^+$, 512.44 [M-18+1]$^+$, 530.43 [M+1]$^+$, 552.44 [M+23]$^+$, 1059.76 [2M+1]$^+$.



Scheme 4. Synthesis of 3-DO-pacCer and 3-DO-NMe-pacCer. *Reaction conditions*: a) 1.ClCO*i*Bu, 4-Me-morpholine, THF, rt, 5h; 2. $NaBH_4$, THF-H_2O, rt, 13h; b) $C_{13}H_{27}$-CH=CH_2, Grubbs (II) cat, CH_2Cl_2, 75°C, 5h; c) CF_3COOH, CH_2Cl_2, rt, 1h; d) pacFA, EDCI*HCl, HOBT, CH_2Cl_2, rt, 25h; e) $LiAlH_4$, THF, refl. 3h; f) pacFA, EDCI*HCl, HOBT, CH_2Cl_2, rt, 3 d.

***tert*-Butyl ((2*R*)-1-hydroxypent-4-en-2-yl)carbamate / reaction product (10)** – A solution of isobutyl chloroformate (221 mg, 1.62 mmol) in THF (1 ml) was added dropwise to a pre-cooled (ice-bath) mixture of (*R*)-N-Boc-allylglycin (**9**) (322 mg, 1.5 mmol, Acros Organics, 95%, 98% ee) and 4-methylmorpholine (163 mg, 1.62 mmol) in THF (5 ml), after 15 min cooling bath was removed and stirring off-white suspension was continued at room temperature for 5 hours. Abundant white precipitate was separated, washed with THF (10 ml), filtrate was cooled (ice-bath) and fine crystals of $NaBH_4$ (113 mg, 3 mmol) followed by H_2O (0.3 ml) were added successively. Intensive gas evolution completed in 5 min, cooling bath was removed and the mixing was stirred at room temperature overnight. The reaction mixture was diluted with AcOEt (10 ml), washed with $NaHCO_3$ aq, organic layer was separated, water phase extracted with AcOEt (4 ml), extracts were washed with brine, dried (Na_2SO_4) and concentrated in vacuum. The residue was separated on silica gel, eluted with gradient mixture CH_2Cl_2-AcOEt (100:1 – 3:1), resulted in isolation of Boc-amine **10** (176 mg, 59%) as colorless liquid. TLC (CH_2Cl_2-AcOEt 4:1 v/v): R_f 0.32. ^{1}H NMR ($CDCl_3$): 5.83-5.75 (1H, m, =CH), 5.15-5.10 (2H, m, $=CH_2$), 4.66 (1H, br s, NH), 3.69-3.58 (3H, m, CH_2O, CHN), 2.34-2.21 (2H, m, $CH_2C=$), 2.14 (1H, br s, OH), 1.44 (9H, s, Boc). ^{13}C NMR ($CDCl_3$): 156.36 (OC=O), 134.12 (=CH), 118.06 ($H_2C=$), 79.75 ($\underline{C}CH_3$), 65.45 (COH), 52.27 (CN), 35.98 ($\underline{C}C=$), 28.17 (CH_3).

***tert*-Butyl ((2*R*,4*E*) -1-hydroxyoctadec-4-en-2-yl)carbamate / reaction product (11)** – Powder of Grubbs (II) catalyst (27 mg, 0.032 mmol, Sigma-Aldrich) was added to a degassed solution of Boc-amine **10** (83 mg, 0.41 mmol) and pentadecene (871 mg, 4.1 mmol) in CH_2Cl_2 (0.5 ml) at room temperature. Argon was bubbled through the solution during 10 min and the reaction mixture was stirred at 75^{o}C over 5 h under argon atmosphere. The brown solution was cooled to room temperature, concentrated and the resulting Boc-aminoalcohol was isolated by chromatography on silica gel, eluted with a gradient mixture CH_2Cl_2-AcOEt (100:0 – 10:1 v/v) to give 120 mg (76%) of **11** as colorless oil. TLC (CH_2Cl_2-AcOEt 4:1 v/v): R_f 0.43. ^{1}H NMR ($CDCl_3$): 5.57-5.51 (1H, m, =CH), 5.41-5.35 (1H, m, =CH), 4.65 (1H, br s, NH), 3.71-3.67 (2H, m, HCN, HCO), 3.63-3.59 (1H, m, HCO), 2.30-2.15 (2H, m, CH_2CN), 2.04-1.99 (2H, m, $CH_2C=$), 1.51 (9H, s, Boc), 1.37-1.27 (22H, m), 0.90 (3H, t, *J* 7.1, CH_3). ^{13}C NMR ($CDCl_3$): 156.50 (C=O), 134.62 (=C), 125.07 (=C), 79.68 ($\underline{C}CH_3$), 65.71 (COH), 52.68 (CN), 32.54 ($\underline{C}CN$), 31.89, 29.65, 29.62, 29.59, 29.37, 29.32, 29.14, 29.10, 28.36 (CH_3), 22.65 ($\underline{C}CH_3$), 14.05 (CH_3).

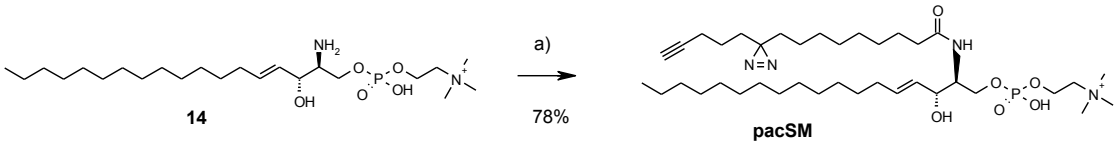
(2*R*, 4*E*)-2-Aminooctadec-4-en-1-ol (12) / reaction product (12) – A solution of CF_3COOH (36 mg, 0.3 mmol) in CH_2Cl_2 (0.1 ml) was added dropwise to a pre-cooled (ice-bath) solution of Boc-amino-alcohol **11** (114.4 mg, 0.3 mmol) in CH_2Cl_2 (0.1 ml). The resulting mixture was stirred at room temperature over 20 h till signal of the Boc-group in the NMR spectrum disappeared completely. The reaction mixture was concentrated in vacuum and crimson residue was separated by chromatography on silica gel, eluted with CH_2Cl_2-MeOH-NH_3/MeOH 95:5:1 v/v/v mixture to give 53.1 mg (63%) of deprotected aminoalcohol **12** as colorless oil. TLC (CH_2Cl_2-MeOH-NH_3/MeOH 95:5:1 v/v/v): R_f 0.25. ^{1}H NMR ($CDCl_3$): 5.55-5.50 (1H, m, =CH), 5.42-5.34 (1H, m, =CH), 3.60 (1H, dd, *J* 11.0, 4.0, $\underline{H}$CH-O), 3.32 (1H, dd, *J* 10.5, 7.6, $\underline{H}$CH-O), 2.91-2.86 (1H, m, HCN), 2.19-2.14 (1H, m, $\underline{H}$CH-C=), 2.08-1.96 (3H, m, $\underline{H}$CH-C=, $H_2CC=$), 1.77 (3H, br s, NH_2, OH), 1.36-1.28 (22H, m), 0.90 (3H, t, *J* 7.1, CH_3). ^{1}H NMR is in good agreement with that reported [3]. ^{13}C NMR ($CDCl_3$): 134.17 (=C), 125.90 (=C), 66.64 (COH), 52.46 (CN), 37.92 ($\underline{C}CN$), 32.60, 31.89, 29.65, 29.62, 29.60, 29.52, 29.48, 29.47, 29.32, 29.17, 22.65 ($\underline{C}CH_3$), 14.05 (CH_3). ESI MS (*m/z*, positive mood): 284.27 $[M+1]^+$.

***N*-[(1*S*,3*E*)-1--(Hydroxymethyl)-3-heptadecen-1-yl]-9-[3-(pent-4-ynyl)-3*H*-diazirin-3-yl]-nonanamide / 3-DO-pacCer** – A solution of EDCI*HCl (5.70 mg, 0.03 mmol) in CH_2Cl_2 (0.2 ml) was added dropwise and with protection from light to a pre-cooled (ice-bath) solution of pacFA (6.60

mg, 0.025 mmol), aminoalcohol **12** (7.1 mg, 0.025 mmol) and HOBT (4.0 mg, 0.03 mmol) in CH_2Cl_2 (0.2 ml), cooling bath was removed and the resulting mixture was stirred at room temperature over 25 h (after 30 min a loose precipitate formed). The reaction mixture was concentrated and the resulting **3-DO-pacCer** was isolated by chromatography on silica gel, eluted with CH_2Cl_2-AcOEt-AcOH 20:20:1 v/v/v mixture to give 7.9 mg (60%) of colorless oil. TLC (CH_2Cl_2-AcOEt-AcOH 20:20:1 v/v/v): R_f 0.4. ^{1}H NMR ($CDCl_3$): 5.60-5.52 (2H, m, NH, =CH), 5.40-5.34 (1H, m, =CH), 3.98-3.92 (1H, m, CH-N), 3.71-3.60 (2H, oct.AB, H_A – 3.69, *J* 11.0, 3.2, H_B – 3.61, *J* 11.0, 6.0, CH_2-O), 2.31-2.26 (1H, m, HCH-CN), 2.22-2.16 (4H, m, $CH_2C{\equiv}$, $CH_2C{=}O$), 2.04-2.00 (2H, m, $CH_2C{=}$), 1.96 (1H, t, *J* 2.6, ≡CH), 1.65-1.57 (2H, m), 1.52-1.49 (1H, m), 1.40-1.25 (34H, m), 1.10 (2H, q, *J* 7.1), 0.90 (3H, t, *J* 7, CH_3). ^{13}C NMR ($CDCl_3$): 174.15 (C=O), 134.86 (=C), 125.08 (=C), 83.42 (≡C), 68.83 (≡CH), 65.90 (COH), 51.81 (HCN), 36.76 (CC=O), 34.43 (CCN), 32.84, 32.52, 31.90 (CCN_2), 31.85 (CCN_2), 29.66, 29.63, 29.49, 29.43, 29.32, 29.19, 29.16, 29.12, 29.09, 28.34, 25.66, 23.75 (CCH_3), 22.74, 22.65, 17.94 (CC≡), 14.06 (CH_3).

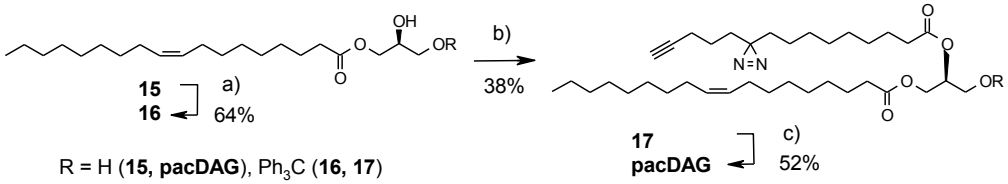
(2*R*, 4*E*)-2-Methylaminooctadec-4-en-1-ol / reaction product (13) – Powder of $LiAlH_4$ (13 mg, 0.35 mmol, Sigma, 95%) was added in portions to a pre-cooled (ice-bath) solution of *tert*-butyl ((2*R*,4*E*)-1-hydroxyoctadec-4-en-2-yl)carbamate **11** (26 mg, 0.068 mmol) in THF (0.1 ml), after 15 min cooling bath was removed and the reaction mixture was stirred under reflux over 3h. It was cooled down in ice-bath, diluted with Et_2O (4 ml), water (2 ml) was carefully added and the reaction mixture was stirred until gas evolution ceased. Organic layer was separated, water phase was extracted with Et_2O (4 ml), extracts were washed with bine, dried (Na_2SO_4) and concentrated. The residue was separated on silica gel, eluted with a mixture CH_2Cl_2-MeOH-NH_3/MeOH 95:5:0.5 v/v/v resulting in isolation of methyl-aminoalcohol **13** (10.6 mg, 53%) as colorless scurf. TLC (CH_2Cl_2-MeOH-NH_3/MeOH 95:5:0.5 v/v/v): R_f 0.1. ^{1}H NMR ($CDCl_3$): 5.55-5.49 (1H, m, =CH), 5.38-5.31 (1H, m, =CH), 3.65 (1H, dd, J 10.7, 4.0, CH-O), 3.35 (1H, dd, *J* 10.7, 6.0, CH_2-O), 2.59-2.54 (1H, m, CH-N), 2.44 (3H, s, NCH_3), 2.23-2.15 (2H, m, CH_2CN), 2.09 (2H, br s, NH, OH), 2.05-1.99 (2H, m, $CH_2C{=}$), 1.37-1.33 (2H, m), 1.28 (20H, br s), 0.90 (3H, t, *J* 7, CH_3). ^{13}C NMR ($CDCl_3$): 134.23 (=C), 125.77 (=C), 62.47 (COH), 60.29 (HCN), 34.36 (CCN), 33.47 (NCH_3), 32.58, 31.89 ($CH_2C{=}$), 29.65, 29.62, 29.61, 29.46, 29.43, 29.32, 29.15, 22.65 (CCH_3), 14.05 (CH_3). ESI MS (*m/z*, positive mood): 298.29 $[M+1]^+$.

***N*-[(1*S*,3*E*)-1-(Hydroxymethyl)-3-heptadecen-1-yl]-*N*-methyl-9-[3-(pent-4-ynyl)-3*H*-diazirin-3-yl]-nonanamide / 3-DO-NMe-pacCer** – A solution of EDCI*HCl (6.80 mg, 0.036 mmol) in CH_2Cl_2 (0.2 ml) was added dropwise and with protection from light to a pre-cooled (ice-bath) suspension of pacFA (8.30 mg, 0.031 mmol), methyl-aminoalcohol **13** (10.6 mg, 0.036 mmol) and HOBT (4.90 mg, 0.036 mmol) in CH_2Cl_2 (0.2 ml), cooling bath was removed and the resulting mixture was stirred at room temperature over 3 d. Clear reaction mixture was concentrated and the resulting **3-DO-NMe-pacCer** was isolated by chromatography on silica gel, eluted with CH_2Cl_2-AcOEt-AcOH 20:20:1 v/v/v mixture to give 3.95 mg (23%) of colorless oil. TLC (CH_2Cl_2-AcOEt-AcOH 20:20:1 v/v/v): R_f 0.4. ^{1}H NMR ($CDCl_3$): 5.55-5.47 (1H, m, =CH), 5.34-5.28 (1H, m, =CH), 4.48 (1H, br s, HCN), 3.74-3.60 (2H, m, CH_2-O), 2.90 (3H, s, NCH_3), 2.34-2.23 (?H, m, HCH-CN), 2.22-2.16 (4H, m, $CH_2C{\equiv}$, $CH_2C{=}O$), 1.96-1.94 (2H, m, $CH_2C{=}$), 1.96 (1H, t, *J* 2.6, ≡CH), 1.65-1.57 (2H, m), 1.52-1.49 (1H, m), 1.40-1.25 (34H, m), 1.10 (2H, q, *J* 7.1), 0.90 (3H, t, *J* 7, CH_3). ^{13}C NMR ($CDCl_3$): 175.35 (C=O), 133.58 (=C), 125.58 (=C), 83.42 (≡C), 68.83 (≡CH), 63.44 (COH), 53.35 (HCN), 34.22 (CCN), 32.84, 32.52, 31.90 (CCN_2), 31.85 (CCN_2), 29.66, 29.63, 29.49, 29.43, 29.32, 29.26, 29.22, 29.16, 29.12, 29.09, 25.10, 23.77 (CCH_3), 22.74, 22.66, 17.94 (CC≡), 14.06 (CH_3). ESI MS (*m/z*, positive mood): 516.47 $[M-28+1]^+$, 526.46 $[M-18+1]^+$, 544.49 $[M+1]^+$, 566.44 $[M+23]^+$, 1087.44 $[2M+1]^+$.



Scheme 5. Synthesis of pacSM. *Reaction conditions*: a) pacFA, EDCI, HOBT, THF, rt, 2 d.

N-9-(3-[Pent-4-ynyl]-3*H*-diazirin-3-yl)-nonanoylsphingomyelin / pacSM – A solution of EDCI (4.05 mg, 0.026 mmol) in THF (0.25 ml) was added dropwise and with protection from light to a pre-cooled (ice-bath) suspension of pacFA (6.30 mg, 0.024 mmol), D-*erythro*-Sphingosylphosphocholine (**14**) (10.6 mg, 0.23 mmol, Avanti-Polar Lipids Inc.) and HOBT (3.52 mg, 0.026 mmol) in THF (0.5 ml) and the resulting mixture was stirred at room temperature over 2d. The mixture was concentrated in vacuum and the resulting **pacSM** was isolated by chromatography on silica gel, eluted with MeOH-AcOH, 100:1 v/v mixture to give 12.6 mg (78%) of colorless paste. TLC (AcOEt): R_f 0.32. ^{1}H NMR (CD_3OD): 5.75-5.70 (1H, m, =CH), 5.50-5.45 (1H, m, =CH), 4.30 (2H, br s, H_2COP), 4.14-4.06 (2H, m; CH-O, CH-N), 4.01-3.95 (2H, m, CH_2OP), 3.66-3.64 (2H, m, CH_2N), 3.24 (9H, s, $N(CH_3)_3$), 2.22-2.15 (5H, m, H_2CCO, $H_2CC{\equiv}$), 2.04 (2H, q, *J* 7, CH_2COO), 1.96 (1H, t, *J* 2.2, ≡CH), 1.91-1.85 (1H, m, $H_2CC{=}$), 1.63-1.58 (2H, m, $H_2CCC{\equiv}$), 1.52-1.49 (2H, m, H_2CCN_2), 1.40-1.37 (4H, m), 1.31 (30H; br s), 1.13-1.10 (2H, m, CH_2), 0.92 (3H, t, *J* 7, CH_3). ^{13}C NMR (CD_3OD): 174.47 (C=O), 133.65 (C=), 129.81 (C=), 82.73 (C≡), 71.23 (C-OH), 68.83 (≡CH), 66.16 (d, $^{1\text{-}2}J_{CP}$ 6, COP), 64.43 (d, $^{1\text{-}3}J_{CP}$ 6, CN), 59.02 (d, $^{1\text{-}3}J_{CP}$ 6, COP), 55.16, 53.94 (d, $^{1\text{-}4}J_{CP}$ 8, HCCO), 53.38 (NCH_3), 53.35 (NCH_3), 53.32 (NCH_3), 35.94 (<u>C</u>C=O), 32.45 (<u>C</u>C=), 32.02, 31.65 (<u>C</u>CN_2), 31.42 (<u>C</u>CN_2), 29.41, 29.38, 29.35, 29.30, 29.04, 28.99, 28.88, 27.79, 25.67, 23.46, 22.59, 22.30, 17.17 (<u>C</u>C≡), 13.01 (<u>C</u>H_3CH_2). ^{31}P NMR ($CDCl_3$): 0.03 ppm (br s, O_2POOH). ESI MS (positive mood): 683.6 $[M\text{-}28+1]^+$, 711.6 $[M+1]^+$, 733.6 $[M+23]^+$, 1423.2 $[2M+1]^+$.



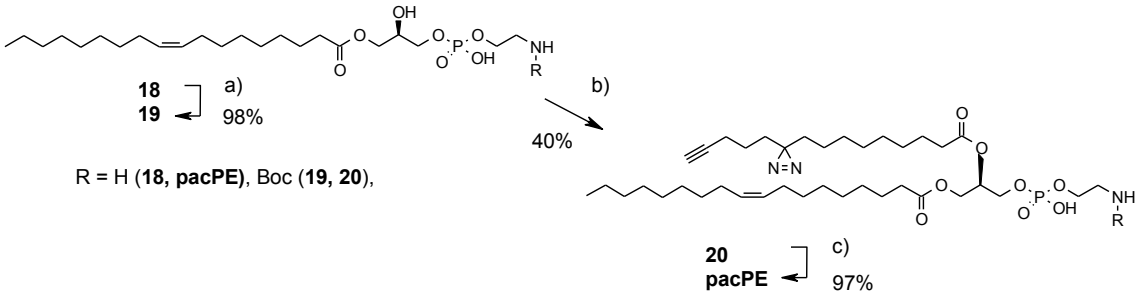
Scheme 6. Synthesis of pacDAG. *Reaction conditions*: a) TrCl, PyH, rt, 2d; b) pacFA, EDCI, DMAP, CH_2Cl_2, rt; c) CF_3COOH, CH_2Cl_2, rt, 1 h.

1-Oleoyl-3-trityl-*sn*-glycerol / reaction product (16) – A solution of trityl chloride (155 mg, 0.55 mmol) in CH_2Cl_2 (0.5 ml) was added to a pre-cooled (ice-bath) solution of 1-oleoyl-*sn*-glycerol (15) (178 mg, 0.5 mmol, Santa Cruz Biotech Inc.) in pyridine (1 ml), cooling bath was removed and the reaction mixture was stirred at room temperature over 2 days. Clear orange solution was concentrated in vacuum and purified on silica gel, eluted with a mixture pentane-CH_2Cl_2-Et_3N 200:40:1 v/v/v, resulted in isolation of trityl glycerol **16** (191 mg, 64%) as colorless solid. TLC (pentane-CH_2Cl_2-Et_3N 200:100:1 v/v/v): R_f 0.33. ^{1}H NMR ($CDCl_3$): 7.45-7.43 (6H, m), 7.34-7.31 (6H, m), 7.27-7.25 (3H, m), 5.38-5.35 (2H, m, HC=CH), 4.24-4.16 (2H, m, CH_2OCO), 4.03-3.97 (1H, m, <u>H</u>COH), 3.26-3.21 (2H, m, H_2CO), 2.38 (1H, d, *J* 5, OH), 2.29 (2H, t, *J* 7.5, $H_2CC{=}O$), 2.05-2.01 (4H, m, $H_2CC{=}CCH_2$), 1.62-1.57 (2H, m), 1.36-1.28 (20H, m), 0.90 (3H, t, *J* 6.9, CH_3). ^{13}C NMR ($CDCl_3$): 173.88 (C=O), 143.64 (C_{Ph}), 130.02 (C=), 129.72 (C=), 128.64 (C_{Ph}), 127.88 (C_{Ph}), 127.16 (C_{Ph}), 86.89 (C-O), 69.36 (COH), 65.66 (C-O), 64.27 (C-O), 34.14 (<u>C</u>C=O), 31.89, 29.76, 29.69, 29.50, 29.31, 29.29, 29.14,

29.09, 27.22 (CC=), 27.17 (CC=), 24.87, 22.65 (CCH_3), 14.06 (CH_3). ESI MS (*m/z*, positive mood): 243.03 $[Ph3C]^+$, 621.4 $[M+23]^+$, 1219.73 $[2M+23]^+$.

1-Oleoyl-2-(9-(3-[pent-4-ynyl]-3*H*-diazirin-3-yl)-nonanoyl)-3-trityl-*sn*-cglycerol / tri-pacDAG (17) – A solution of EDCI (5.6 mg, 0.035 mmol) in CH_2Cl_2 (0.3 ml) was added dropwise and with protection from light to a pre-cooled (ice-bath) solution of pacFA (7.20 mg, 0.027 mmol), trityl glycerol **16** (17.4 mg, 0.29 mmol) and DMAP (4.40 mg, 0.035 mmol) in CH_2Cl_2 (0.5 ml), cooling bath was removed and the resulting mixture was stirred at room temperature overnight. The reaction mixture was concentrated and the resulting **tri-pacDAG 17** was isolated by chromatography on silica gel, eluted with pentane-Et_2O-CH_2Cl_2-Et_3N 400:100:100:1 v/v/v/v mixture to give 8.6 mg (38%) of a colorless paste followed by the starting trityl glycerol **16** (6.0 mg, 29%, TLC: R_f 0.34). TLC (pentane-Et_2O-CH_2Cl_2-Et_3N 400:100:100:1 v/v/v/v): R_f 0.8. ^{1}H NMR ($CDCl_3$): 7.48-7.43 (6H, m, CH_{Ph}), 7.36-7.23 (9H, m, CH_{Ph}), 5.40-5.35 (2H, m, HC=CH), 5.31-5.23 (1H, m, CHOH), 4.40-4.21 (2H, oct.AB, C_A – 4.37, *J* 11.8, 3.8, C_B – 4.25, *J* 11.8, 6.5, H_2COCO), 3.32-3.21 (2H, m, H_2CO), 2.36 (2H, t, *J* 7.5, H_2CC=O), 2.25 (2H, t, *J* 7.5, H_2CC=O), 2.18 (2H, dt, *J* 6.8, 2.8, H_2CC≡), 2.07-2.01 (4H, m , H_2CC=CCH_2), 1.97 (1H, t, *J* 2.8, ≡CH), 1.70-1.47 (8H, m), 1.40-1.24 (30H, m), 1.08 (2H, q, *J* 7.1), 0.91 (3H, t, *J* 7, CH_3). ^{13}C NMR ($CDCl_3$): 173.31 (C=O), 172.92 (C=O), 143.60 (C_{Ph}), 130.02 (=C), 129.72 (=C), 128.63 (C_{Ph}), 127.84 (C_{Ph}), 127.12 (C_{Ph}), 86.69 (CC_{Ph}), 83.43 (≡C), 70.46 (HC-O), 68.83 (≡CH), 62.82 (H_2CO), 62.24 (H_2CO), 34.35 (H_2CC=O), 34.09 (H_2CC=O), 32.86, 31.90 (CCN_2), 31.85 (CCN_2), 30.34, 29.77, 29.73, 29.52, 29.32, 29.17, 29.13, 29.12, 29.11, 28.36, 27.23 (CC=), 27.19 (CC=), 24.90, 24.83, 23.78 (CCH_3), 22.76 ($CCCN_2$), 22.67 ($CCCN_2$), 17.96 (CC≡), 14.08 (CH_3). ESI MS (*m/z*, positive mood): 839.6 $[M-28+23]^+$, 863.6 $[M+18+1]^+$, 867.6 $[M+23]^+$, 883.6 $[M+39]^+$.

1-Oleoyl-2-(9-(3-[pent-4-ynyl]-3*H*-diazirin-3-yl)-nonanoyl)-*sn*-glycerol / pacDAG – A solution of CF_3COOH (12 mg, 0.1 mmol) in CH_2Cl_2 (0.1 ml) was added dropwise and with protection from light to a pre-cooled (ice-bath) solution of **tri-pacDAG 17** (8.6 mg, 0.01 mmol) in CH_2Cl_2 (0.1 ml), cooling bath was removed and the resulting mixture was stirred at room temperature 1 h. The reaction mixture was concentrated in vacuum and colorless residue was separated by chromatography on silica gel, eluted with pentane-Et_2O-CH_2Cl_2 3:1:1 v/v/v mixture to give 3.1 mg (52%) of **pacDAG** as colorless oil. TLC (pentane- Et_2O-CH_2Cl_2 3:1:1 v/v/v): R_f 0.3. ^{1}H NMR ($CDCl_3$): 5.40-5.35 (2H, m, HC=CH), 4.21-4.13 (4H, m, 2x H_2C-O), 4.11-4.08 (2H, m, HC-O), 2.38 (4H, m, 2 x H_2CC=O), 2.18 (2H, dt, *J* 6.8, 2.8, H_2CC≡), 2.05-2.02 (4H, m , H_2CC=CCH_2), 1.96 (1H, t, *J* 2.8, ≡CH), 1.68-1.63 (4H, m, 2 x H_2CCCN_2), 1.58-1.49 (4H, m), 1.390-1.24 (30H, m), 1.10 (2H, q, *J* 7.1), 0.90 (3H, t, *J* 7, CH_3). ^{13}C NMR ($CDCl_3$): 173.81 (C=O), 173.78 (C=O), 130.01 (=C), 129.70 (=C), 83.42 (≡C), 68.83 (≡CH), 68.45 (HC-O), 65.05 (H_2C-O), 34.08 (CC=O), 34.04 (CC=O), 32.84, 31.88 (CCN_2), 31.85 (CCN_2), 30.32, 29.74, 29.66, 29.49, 29.30, 29.27, 29.12, 29.07, 29.01, 29.00, 28.34, 27.21 (CC=), 27.15 (CC=), 24.86, 24.81, 23.73 (CCH_3), 22.74 ($CCCN_2$), 22.64 ($CCCN_2$), 17.96 (CC≡), 14.05 (CH_3). ESI MS (*m/z*, positive mood): 557.5 $[M-28-17+1]^+$, 575.5 $[M-28+1]^+$, 585.5 $[M-17+1]^+$, 603.5 $[M+1]^+$, 625.5 $[M+23]^+$.

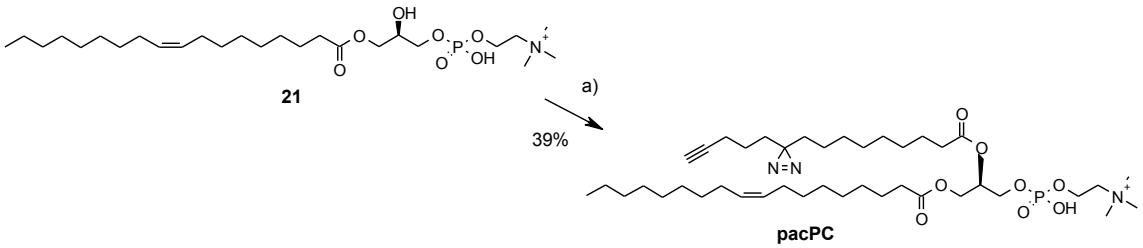


Scheme 7. Synthesis of pacPE. *Reaction conditions*: a) Boc_2O, Et_3N, CH_2Cl_2, rt; b) pacFA, EDCI, DMAP, rt, 5 d; c) CF_3COOH, CH_2Cl_2, rt.

1-Oleoyl-2-hydroxy-*sn*-glycero-3-(N-*t*-butyloxycarbonyl)phosphoethanolamine / reaction product (19) – A suspension of 1-oleoyl-2-hydroxyl-*sn*-glycero-3-phosphoethanolamine (**18**) (43.1 mg, 0.09 mmol, Avanti-Polar Lipids Inc.) in CH_2Cl_2 (1 ml) was treated with Et_3N (10.9 mg, 0.18 mmol) at room temperature, after 5 min a solution of Boc_2O (23.5 mg, 0.11 mmol) in CH_2Cl_2 (0.25 ml) was added and the resulting mixture was stirred overnight. Clear solution was concentrated in vacuum and purified on silica gel, eluted with a mixture CH_2Cl_2-MeOH, 3:1 v/v, resulted in isolation of Boc-phosphoethanolamine **19** (52 mg, 98%) as beige solid. TLC (CH_2Cl_2-MeOH 3:1 v/v): R_f 0.47. ^{1}H NMR ($CDCl_3$): 5.63 (1H, br s, NH), 5.41-5.35 (2H, m, HC=CH), 4.18-3.90 (7H, m, HCO, H_2C-O), 3.38 (2H, br s), 2.34 (2H, t, *J* 7,0 CH_2C=O), 2.08-1.99 (4H, m, CH_2C=CH_2), 1.70-1.58 (2H, m), 1.45 (9H, s, 3 x CH_3), 1.39-1.25 (18H, m), 0.90 (3H, t, *J* 7, CH_3). [4]. ^{13}C NMR ($CDCl_3$): 173.71 (C=O), 156.10 (NC=O), 129.98 (=C), 129.74 (=C), 79.00 (O<u>C</u>CH_3), 69.48 (d, $^{1\text{-}3}J_{CP}$ 6, COP), 67.47 (d, $^{1\text{-}4}J_{CP}$ 8, <u>C</u>COP), 64.90 (d, $^{1\text{-}5}J_{CP}$ 5, CN), 64.82 (d, $^{1\text{-}3}J_{CP}$ 6, COP), 34.42 (d, $^{1\text{-}3}J_{CP}$ 5, <u>C</u>CCOP), 34.15 (<u>C</u>C=O), 31.89, 29.76, 29.71, 29.50, 29.30, 29.20, 29.14, 28.44 (H_3<u>C</u>CO), 27.21 (CC=), 27.18 (CC=), 24.89 (<u>C</u>CC=O), 22.66 (<u>C</u>CH_3), 14.08 (CH_3). ^{31}P NMR ($CDCl_3$): 1.93 ppm (br s, O_2POOH). ESI MS (*m/z*, positive mood): 602.4 $[M+23]^+$, 1181.7 $[2M+23]^+$.

1-Oleoyl-2-(9-(3-[pent-4-ynyl]-3*H*-diazirin-3-yl)-nonanoyl)-*sn*-glycero-3-(N-*t*-butyloxycarbonyl)phosphoethanolamine / Boc-pacPE (20) – A solution of EDCI (4.59 mg, 0.030 mmol) in CH_2Cl_2 (0.25 ml) was added dropwise and with protection from light to a pre-cooled (ice-bath) solution of pacFA (7.50 mg, 0.028 mmol), Boc-phosphoethanolamine **19** (14.3 mg, 0.25 mmol) and DMAP (3.60 mg, 0.03 mmol) in CH_2Cl_2 (0.5 ml), cooling bath was removed and the resulting mixture was stirred at room temperature over 5 days. The resulting **Boc-pacPE 20** was isolated by chromatography on silica gel, eluted with CH_2Cl_2-MeOH-AcOH 82:17:1 v/v/v mixture to give 8.2 mg (40%) of a colorless paste followed by the starting Boc-phosphoethanolamine **19** (7.8 mg, 54%, TLC: R_f 0.47). TLC (CH_2Cl_2-MeOH-AcOH 82:17:1 v/v/v): R_f 0.76. ^{1}H NMR ($CDCl_3$): 5.56 (1H, br s, NH), 5.40-5.32 (2H, m, HC=CH), 5.24 (1H, br s, HCO), 4.40-4.35 (1H, m, <u>H</u>CH-O), 4.20-4.14 (1H, m, <u>H</u>CH-O), 3.96-3.87 (3H, m, H_2CO), 3.79-3.75 (1H, m), 3.31-3.30 (2H, m, H_2CN), 2.33-2.29 (4H, m, 2 x CH_2C=O), 2.18-2.16 (2H, m, CH_2C≡), 2.04-2.01 (4H, m, CH_2C=CCH_2), 1.96 (1H, t, *J* 2.6, ≡CH), 1.64-1.58 (4H, m), 1.52-1.49 (2H, m), 1.43 (9H, s, 3 x CH_3), 1.40-1.25 (32H, m), 1.01 (2H, br s), 0.90 (3H, t, *J* 7, CH_3). ^{13}C NMR ($CDCl_3$): 173.50 (C=O), 173.38 (C=O), 156.34 (NC=O), 130.00 (=C), 129.68 (=C), 83.40 (≡C), 79.00 (O<u>C</u>CH_3), 70.51 (HC-O), 68.84 (≡CH), 65.50 (COP), 64.51 (COP), 62.51 (H_2CO), 35.35 (CH_2N), 34.19 (<u>C</u>C=O), 34.05 (<u>C</u>C=O), 32.85, 31.88 (<u>C</u>CN_2), 31.85 (<u>C</u>CN_2), 29.74, 29.50, 29.30, 29.28, 29.23, 29.14, 29.12, 29.05, 28.38 (3 x CH_3), 28.32, 27.21 (CC=), 27.19 (CC=), 24.82, 24.79, 23.78 (<u>C</u>H_2CH_3), 22.74, 22.64, 17.94 (<u>C</u>C≡), 14.05 (CH_3). ^{31}P NMR ($CDCl_3$): 1.66 ppm (br s, O_2POOH). ESI MS (*m/z*, positive mood): 848.6 $[M+23]^+$.

1-Oleoyl-2-(9-(3-[pent-4-ynyl]-3*H*-diazirin-3-yl)-nonanoyl)-*sn*-glycero-3-phosphoethanolamine / pacPE – A solution of CF_3COOH (12 mg, 0.1 mmol) in CH_2Cl_2 (0.1 ml) was added dropwise and with protection from light to a pre-cooled (ice-bath) solution of Boc-pacPC **20** (8.20 mg, 0.01 mmol) in CH_2Cl_2 (0.1 ml), cooling bath was removed and the resulting mixture was stirred at room temperature overnight. The reaction mixture was concentrated in vacuum and the residue was separated by chromatography on silica gel, eluted with CH_2Cl_2-MeOH 4:1 v/v mixture to give 7.2 mg (97%) of **pacPE** as colorless oil. TLC (CH_2Cl_2-MeOH 4:1 v/v/v): R_f 0.3. ^{1}H NMR ($CDCl_3$): 7.88 (1H, br s, NH_2), 5.36 (1H, br s, OH), 5.32 (2H, br s, HC=CH), 5.11 (1H, br s, HCO), 4.38 (1H, br s, HCH-O), 4.12-3.98 (3H, m, H_2C-O, HC-O), 3.90 (1H, br s, HC-O), 3.76 (1H, br s), 3.12 (2H, br s, H_2CN), 2.30 (4H, br s, 2 x CH_2C=O), 2.17 (2H, br s, CH_2C≡), 2.03 (4H, br s, CH_2C=CCH_2), 1.96 (1H, br s, ≡CH), 1.61-1.55 (4H, m), 1.52-1.49 (2H, m), 1.28 (29H, br s), 1.09 (2H, br s), 0.90 (3H, br s, CH_3). ^{13}C NMR ($CDCl_3$): 174.61 (C=O), 174.43 (C=O), 129.97 (=C), 129.64 (=C), 83.40 (≡C), 71.06 (HC-O), 68.85 (≡CH), 67.20 (COP), 65.61 (COP), 61.91 (H_2CO), 34.46 (CH_2N), 34.12 (CC=O), 34.08 (CC=O), 32.84, 31.89 (CCN_2), 31.83 (CCN_2), 29.76, 29.67, 29.62, 29.52, 29.32, 29.28, 29.23, 29.14, 29.08, 29.05, 28.96, 28.32, 27.21 (CC=), 26.74, 24.67, 23.78 (CH_2CH_3), 22.74, 22.64, 17.94 (CC≡), 14.05 (CH_3). ^{31}P NMR (CDCl3): 0.75 (br s, O_2POOH). ESI MS (positive mood): 726.46 $[M+1]^+$, 748.52 $[M+23]^+$, 1452.52 $[2M+1]^+$.



Scheme 8. Synthesis of pacPC. *Reaction conditions*: a) pacFA, DCC, DMAP, CH_2Cl_2, rt, 3d

1-Oleoyl-2-(9-(3-[pent-4-ynyl]-3*H*-diazirin-3-yl)-nonanoyl)-*sn*-glycero-3-phosphocholine / pacPC – A solution of DCC (5.80 mg, 0.028 mmol) in CH_2Cl_2 (0.25 ml) was added dropwise and with protection from light to a pre-cooled (ice-bath) solution of pacFA (6.30 mg, 0.024 mmol), 1-oleoyl-2-hydroxyl-*sn*-glycero-3-phosphocholine (**21**) (12.4 mg, 0.24 mmol, Avanti-Polar Lipids Inc.) and DMAP (3.40 mg, 0.028 mmol) in CH_2Cl_2 (0.5 ml), cooling bath was removed and the resulting mixture was stirred at room temperature over 3 days. The mixture was separated from the precipitate of dicyclohexyl urea, concentrated in vacuum and the resulting **pacPC** was isolated by chromatography on silica gel, eluted with CH_2Cl_2-MeOH-AcOH-H_2O 25:50:2:2 v/v/v/v mixture to give 7.23 mg (39%) of a colorless paste followed by the starting phosphocholine **21** (7.1 mg, 57%, TLC: R_f 0.15). TLC (CH_2Cl_2-MeOH-AcOH-H_2O 25:50:2:2 v/v/v/v): R_f 0.28. ^{1}H NMR ($CDCl_3$): 5.41-5.33 (2H, m, HC=CH), 5.24 (1H, br s, HCO), 4.55 (2H, br s), 4.37-4.34 (2H, m), 4.16-4.03 (4H, m) 3.44 (9H, br s, $N(CH_3)_3$), 2.31-2.28 (4H, m, 2 x CH_2C=O), 2.17 (2H, dd, *J* 7.1, 2.6, CH_2C≡), 2.04-2.01 (4H, m, 2 x CH_2C=), 1.96 (1H, t, *J* 2.6, ≡CH), 1.64-1.58 (4H, m), 1.52-1.49 (2H, m), 1.40-1.25 (32H, m), 1.01 (2H, q, *J* 7.1), 0.90 (3H, t, *J* 7, CH_3). ^{13}C NMR ($CDCl_3$): 173.51 (C=O), 173.14 (C=O), 130.02 (=C), 129.67 (=C), 83.40 (≡C), 70.51 (d, $^{1\text{-}4}J_{CP}$ 8, HCO), 68.89 (≡CH), 66.57 (d, $^{1\text{-}3}J_{CP}$ 6, COP), 64.61 (d, $^{1\text{-}4}J_{CP}$ 8, CN), 63.61 (d, $^{1\text{-}3}J_{CP}$ 6, COP), 62.92 (d, $^{1\text{-}5}J_{CP}$ 5, HCO), 54.65 (NCH_3), 34.26 (CC=O), 34.10 (CC=O), 32.83, 31.88 (CCN_2), 31.85 (CCN_2), 30.31, 29.74, 29.66, 29.49, 29.30, 29.28, 29.22, 29.20, 29.16, 29.12, 29.02, 28.35, 27.22 (CC=), 27.18 (CC=), 24.86, 23.77 (CCH_3), 22.74, 22.64, 17.94 (CC≡), 14.06 (CH_3). ^{31}P NMR (CDCl3): -1.20 (s, O_2POOH). ESI MS (positive mood): 768.58 $[M+1]^+$, 790.56 $[M+23]^+$.

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