SUPPLEMENTARY INFORMATION

Involvement of lipid rafts in the localization and dysfunction effect of the antitumor ether phospholipid edelfosine in mitochondria

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I - Materials and Methods

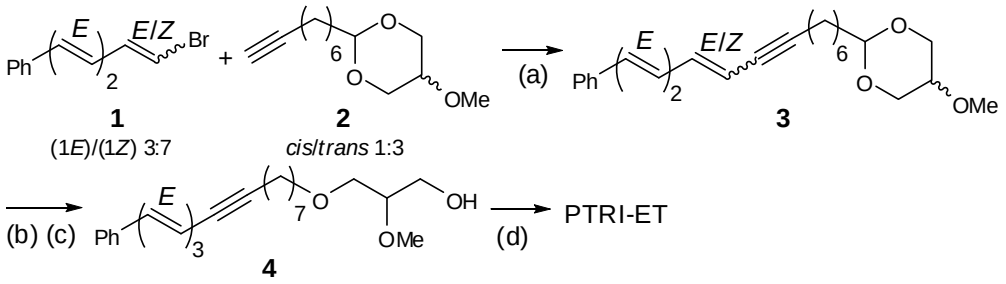
All reactions were carried out under argon atmosphere. Commercial reagents were used as received. Compounds 1-bromo-6-phenylhexa-1,3,5-triene (**1**, 3:7 *all*-(*E*)/(1*Z*,3*E*,5*E*) mixture), 5-methoxy-2-oct-7'-ynyl-[1,3]-dioxane (**2**, 1:3 *cis*/*trans* mixture), 2-(14'-phenyltetradeca-9',11',13'-trien-7'-ynyl)-5-methoxy-[1,3]-dioxane (**3**, corresponding mixture of isomers from the reaction **1** + **2**), *rac*-2-*O*-methylglycerol[1], 3,5-dimethyl-4-ethyl-2-formyl-1*H*-pyrrole[2] and 2-iodo-1,3,5,7-tetramethyl-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene[3] were prepared as described elsewhere. Solvents were purified by standard methods and deoxygenated by argon bubbling prior to use. Acetonitrile, chloroform, dichloromethane, DMF and THF were dried by passing through activated alumina columns in a PureSolv purification system. Yields refer to the isolated pure compound. Analytical thin layer chromatography (TLC) was carried out on precoated silica gel plates, Merck 60F254, 0.25 mm. Compounds were visualized with 254-nm irradiation and phosphomolybdic acid solution followed by heating, or by their fluorescence under 365-nm irradiation. Flash column chromatography was performed on silica gel, Merck 60, 230–400 mesh, 0.040–0.063 mm. High performance liquid chromatography (HPLC) was carried out with an HP Agilent 1100 chromatograph equipped with a 5-μm C18 reverse phase column (4.6×150 mm) and a diode array detector, using as eluent MeOH–H_2O 9:1 v/v with 10 mM H_3PO_4 at a flow rate of 1.2 mL min^{-1}, and detection at 346, 354, 375 and 529 nm. ^1H and ^{13}C NMR spectra were recorded on INOVA-300 or Bruker 300 spectrometers at room temperature, unless otherwise noted. Chemical shifts are reported in parts per million (ppm), using as internal reference the proton signal of the trace of non-deuterated solvent or the carbon signal of the solvent (δ 7.26 and 77.0, respectively, in the case of chloroform). Abbreviations: s (singlet), br. s (broad singlet), d (doublet), t (triplet), q (quartet), m (complex multiplet). Carbon and proton assignments were based on HSQC, HMBC and COSY. IR spectra (in cm^{-1}) were recorded in Perkin-Elmer 681 and FT-Spectrum One spectrophotometers. Low resolution mass spectra were registered by electron impact (70 eV) in a Hewlett-Packard 5973 spectrometer in the direct-injection mode, and by electrospray in the positive mode in a Hewlett-Packard 1100 apparatus. High resolution mass spectra were determined in an AutoSpec Micromass (Waters) instrument, in the L-SIMS mode using Cs^+ (30 kV) in *m*-NBA matrix, with PEG as internal standard. UV-Vis absorption spectra were registered on Varian CARY-3E or Perkin-Elmer Lambda-2 spectrophotometers. Corrected fluorescence spectra were recorded in a PC1 ISS spectrofluorimeter. The fluorescence quantum yield (Φ_f) of the BDP analogues was determined by reference to that of the BDP dye PM567 in methanol ($\Phi_f = 0.91$)[4], using the expression of Parker and Rees[5].

Analogue PTE-ET was synthesized as described elsewhere.[1] Absorption and fluorescence spectra in DMSO solution are shown in Figure S1, panel A.

II - Synthesis of PTRI-ET

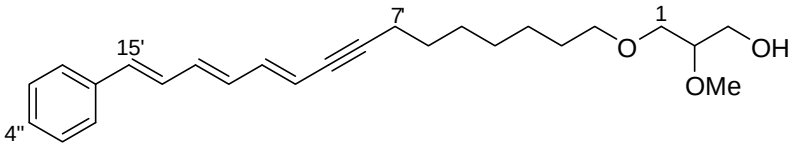
This analogue was obtained from the cyclic acetal **3**, an intermediate product in the PTE-ET synthesis that resulted from the Sonogashira-Hagihara cross-coupling between **1** and **2** (Scheme S1). Acetal ring opening, selective precipitation of the *all*-(*E*) isomer **4** and introduction of the phosphocholine group at the terminal alcohol[6] yielded the target analogue.

Scheme S1[a]



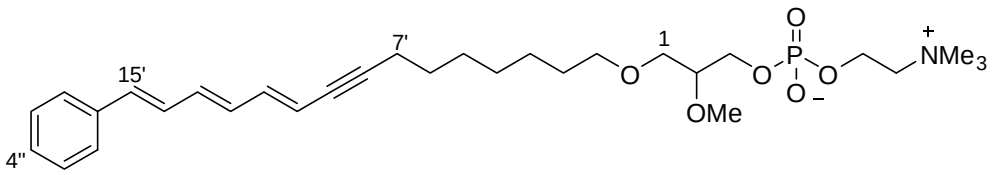
[a] Reagents and conditions: (a) **2**/**1** mole ratio = 1.3, $Pd(PPh_3)_2Cl_2$ (7.5 mol%), CuI (25 mol%), Et_2NH, THF, Ar, room temp., 2 h, 90%; (b) DIBAL-H, MePh, Ar, 0 ºC to room temp., 96%; (c) precipitation from CH_2Cl_2 with *n*-pentane, 25%; (d) 2-chloro-1,3,2-dioxaphospholane-2-oxide, Me_3N, MeCN, Ar, pressure tube, −78 ºC to room temp., 3 h, 80 ºC, 4 h, 35%.

all-(*E*)-3-*O*-(15'-Phenylpentadeca-10',12',14'-trien-8'-ynyl)-2-*O*-methyl-*rac*-glycerol (**4**):



DIBAL-H (3 mL, 1 M in toluene) was added (0 ºC, argon, stirring) to the acetal **3** (isomer mixture) (53 mg, 0.14 mmol). After 4 h at room temperature, a saturated aqueous solution of NH_4Cl was added at 0 ºC, until no gas evolution was observed. Subsequent workup and purification by column chromatography (silica gel, hexane:EtOAc, 2:1 v/v) yielded a yellow waxy solid (yield 50 mg, 96%) from which the *all*-(*E*) alcohol **4** was isolated by precipitation from a saturated solution in CH_2Cl_2 with *n*-pentane. Yield 12.5 mg, 25%. TLC (hexane:EtOAc, 2:1): R_f = 0.27. ^{1}H NMR (300 MHz, $CDCl_3$, 30 °C): δ 1.20–1.45 (m, 6H, H-3', H-4', H-5), 1.46–1.66 (m, 4H, H-2', H-6'), 2.24 (m, 1H, O*H*), 2.35 (td, 2H, *J* = 6.9 and 2.1 Hz, H-7'), 3.40–3.47 (m, 3H, H-1', H-2), 3.47 (s, 3H, OCH_3), 3.54 (m, 2H, H-1), 3.60–3.85 (m, 2H, H-3), 5.63 (dt, 1 H, *J* = 15.6 and 2.1 Hz, H-10'), 6.32 (dd, 1H, *J* = 14.4 and 10.4 Hz, H-12'), 6.39 (dd, 1H, *J* = 14.4 and 10.4 Hz, H-13'), 6.58 (d, 1 H, *J* = 15.3 Hz, H-15'), 6.59 (dd, 1H, *J* = 15.3 and 10.2 Hz, H-11'), 6.80 (dd, 1H, *J* = 15.3 and 9.6 Hz, H-14'), 7.21 (t, 1 H, *J* = 7.5 Hz, H-4''), 7.31 (t, 2H, *J* = 7.5 Hz, H-3'', H-5''), 7.39 (d, 1 H, *J* = 7.5 Hz, H-2'', H-6''); ^{13}C NMR (75 MHz, $CDCl_3$, 30 °C): δ 19.7 (C-7'), 25.9 (C-3'), 28.6 (C-4'), 28.8 (C-5'), 28.9 (C-6'), 29.5 (C-2'), 57.7 (O*C*H_3), 62.6 (C-1), 70.6 (C-3), 71.8 (C-1'), 79.8 (C-2), 80.3 (C-9'), 94.5 (C-8'), 112.0 (C-10'), 126.4 (C-2'', C-6''), 127.7 (C-4''), 128.6 (C-3'', C-5''), 128.7 (C-14'), 132.5 (C-12'), 133.6 (C-15'), 134.2 (C-13'), 137.1 (C-1''), 140.5 (C-11'); FT IR (KBr) ν_{max}: 3414, 3020, 2930, 2852, 1600, 1463, 1002, 747, 689 cm^{-1}; EI MS (70 eV), *m/z* (%): 382 (20) [M^+], 219 (5), 205 (14), 193 (36), 179 (58), 165 (36), 152 (13), 141 (20), 129 (30), 117 (51), 105 (22), 91 (100); UV-Vis (MeOH): λ_{max} (nm): 354, 337 (max.), 322.

all-(*E*)-1-*O*-(15'-Phenylpentadeca-10',12',14'-trien-8'-ynyl)-2-*O*-methyl-*rac*-glycero-3-phosphocholine (PTRI-ET)



Trimethylamine (ca. 2 mL) was condensed into a solution of alcohol **4** (60 mg, 0.16 mmol) in acetonitrile (10 mL) in a pressure tube at –78 ºC under argon. 2-Chloro-1,3,2-dioxaphospholane-2-oxide (120 µL, 1.3 mmol) was then added to the cooled solution and the reactor was closed and left 3 h at room temperature with stirring and 4 h at 80 ºC. After cooling back to room temperature, the reactor was opened, the solvent and the excess trimethylamine were vacuum-evaporated, the residual solid was dissolved in THF:H_2O 9:1 v/v (10 mL), and Amberlite MB-3 was added until change of color of the resin (saturation). The mixture was filtered, the resin was washed with MeOH (3×10 mL), the filtrate and washings were collected, the solvent was vacuum-evaporated, and the residual solid was purified by column chromatography (silica gel), using first $CHCl_3$:MeOH, 9:1 v/v, for separating non-polar products, and then $CHCl_3$:MeOH:H_2O, 65:25:5 v/v/v. The solid isolated from the chromatography was purified by precipitation with *n*-pentane from a saturated solution in CH_2Cl_2. Yellow waxy solid, stable for months at –20 ºC in the dark; yield 20 mg, 35%; TLC (MeOH:H_2O 9:1): R_f = 0.20; ^{1}H NMR (300 MHz, $CDCl_3$, 30 ºC): δ 1.20–1.40 (m, 8H, H-3' to H-6'), 1.52 (m, 2H, H-2'), 2.10 (m, 2H, H-7'), 3.34 (s, 9H, N(CH_3)$_3$), 3.80 (m, 2H, CH_2N), 3.43 (s, 3H, OCH_3), 3.64–4.00 (m, 7H, H-1, H-2, H-3, H-1'), 4.30 (br. s, 2H, POCH_2CH_2N), 5.63 (d, 1H, J = 15.5 Hz, H-10'), 6.34 (dd, 1H, J = 14.7 and 10.1 Hz, H-12'), 6.44 (dd, 1H, J = 14.7 and 9.9 Hz, H-13'), 6.57 (dd, 1H, J = 15.5 and 9.9 Hz, H-11'), 6.58 (d, 1H, J = 15.5 Hz, H-15'), 6.80 (dd, 1H, J = 15.5 and 9.9 Hz, H-14'), 7.20 (t, 1H, J = 7.5 Hz, H-4"), 7.27 (t, 2H, J = 7.5 Hz, H-3", H-5"), 7.38 (d, 2H, J = 7.5 Hz, H-2", H-6"); ^{13}C NMR (75 MHz, $CDCl_3$, 30 °C): δ 19.0 (C-7'), 26.0 (C-3'), 28.7, 28.9, 29.0, 29.7 (C-2', C-4', C-5', C-6'), 54.3 [N(CH_3)$_3$], 57.8 (OCH_3), 59.3 (d, J = 5.5 Hz, C-1), 64.9 (d, J = 4.4 Hz, CH_2N), 66.3 (d, J = 5.5 Hz, OCH_2CH_2N), 71.7 (C-3), 70.3 (C-1'), 79.6 (d, J = 7.7 Hz, C-2), 80.4 (C-9'), 94.6 (C-8'), 112.1 (C-10'), 126.4 (C-2", C-6"), 127.7 (C-4"), 128.6 (C-3", C-5"), 128.7 (C-14'), 132.5 (C-12'), 133.7 (C-15'), 134.3 (C-13'), 137.2 (C-1"), 140.5 (C-11'); ESI$^+$ MS: 548.3 [M + H$^+$]; HR MS (L-SIMS): calc. for ($C_{30}H_{48}NO_6P$)H$^+$ 548.3141. Found 548.3155; FT IR (KBr) ν_{max}: 3391, 3021, 2929, 2855, 2100, 1660, 1464, 1235, 1089, 994, 969, 923, 802, 748, 690 cm^{-1}; UV-Vis (DMSO) λ_{max} (nm) (ε, M^{-1} cm^{-1}): 329 (43000), 345 (62000), 363 (51000); emission (corrected) (DMSO) λ_{max}: 423, 446 nm (Figure S2, panel B).

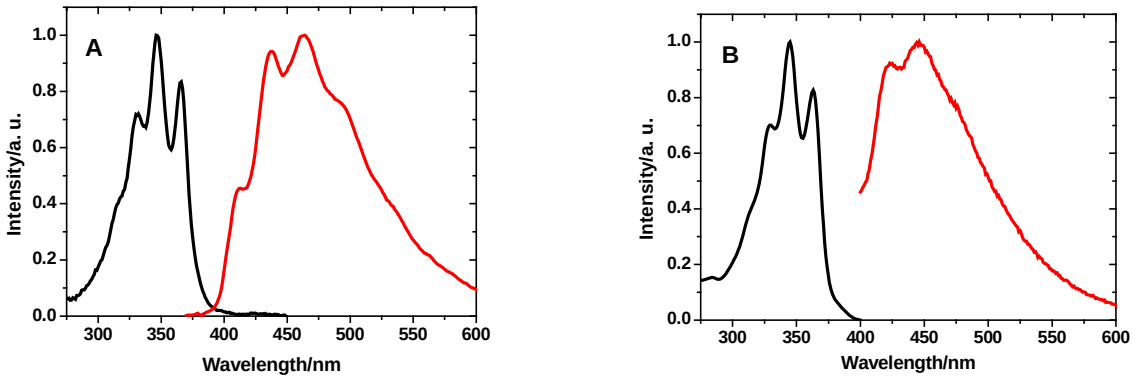
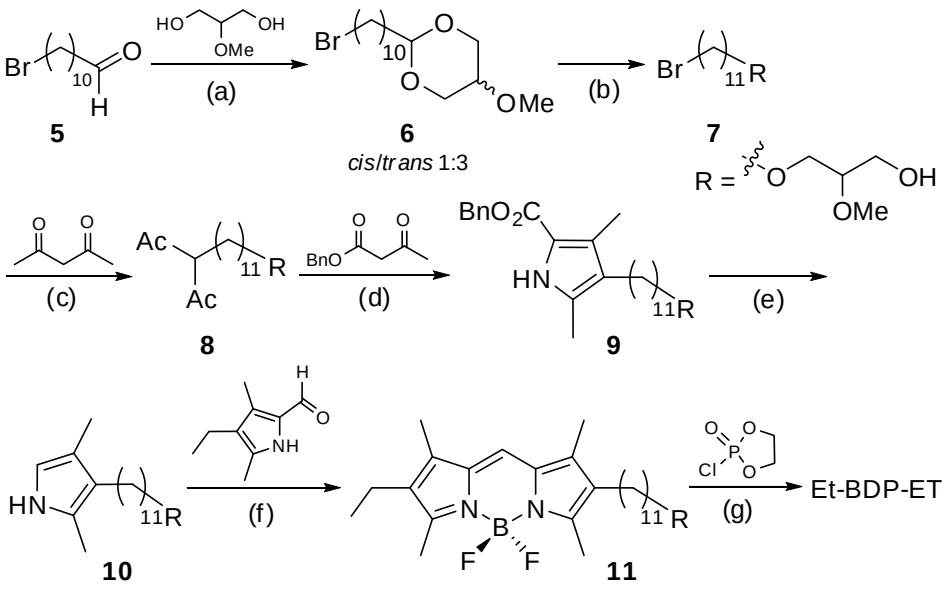


Figure S1. Absorption and corrected fluorescence spectra (normalized) of the phenylpolyene analogues of edelfosine 1–2 × 10^{-6} M in DMSO, λ_{exc} = 365 ± 2 nm. A) PTE-ET; B) PTRI-ET.

III - Synthesis of Et-BDP-ET

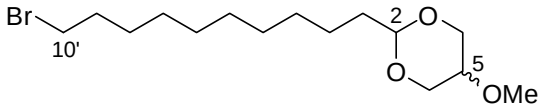
The analogue was prepared in seven steps (Scheme S2): a) the reaction of 10-bromoundecanal (**5**) with *rac*-2-*O*-methylglycerol provided the cyclic acetal **6** as a *cis*/*trans* mixture (yields 25% and 68%, respectively), using conditions described elsewhere for the synthesis of similar acetals[1]; b) the cleavage of **6** with DIBAL-H yielded the disubstituted glycerol **7**[1,7]; c) the reaction of **7** with acetylacetone produced the alkylated 1,3-diketone **8**[8]; d) the α-benzyloxycarbonyl pyrrole **9** was prepared from **8**, benzyl acetoacetate, sodium nitrite and zinc, under Johnson-modified Knorr conditions[9]; e) the elimination of the benzyloxycarbonyl group in **9** by hydrogenolysis/decarboxylation[10] yielded the α-H pyrrole **10**; f) the asymmetric BDP compound **11** was prepared by condensing 3,5-dimethyl-4-ethyl-2-formyl-1*H*-pyrrole with **10**, and subsequent treatment of the dipyrromethene thus obtained with boron trifluoride diethyl etherate[11]; g) finally, the phosphocholine group was introduced by reaction with 2-chloro-1,3,2-dioxaphospholane-2-oxide and trimethylamine[12], to yield analogue Et-BDP-ET.

Scheme S2 [a]



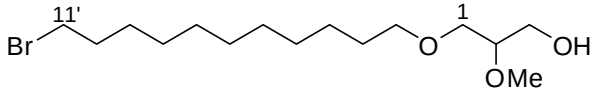
[a] Reagents and conditions: (a) *rac*-2-*O*-methylglycerol/**5** molar ratio = 1.3, *p*TsOH, MePh, reflux, 14 h, 93%; (b) DIBAL-H, MePh, 0 ºC, then room temp., 24 h, 85%; (c) acetylacetone/**7** molar ratio = 10, K_2CO_3, 18-crown-6, acetone, reflux, 24 h, 70%; (d) benzyl acetoacetate, $NaNO_2$, H_2O, AcOH, 5 ºC, 3 h, then room temp., 14 h, add **8** (molar ratio benzyl acetoacetate/**8** = 1.0) and Zn dust, 65 ºC, 1 h, 30%; (e) H_2, Pd/C, EtOH, 40 psi, 4 h, filtration and solvent elimination, then TFA, Ar, 24%; (f) 3,5-dimethyl-4-ethyl-2-formyl-1*H*-pyrrole/**10** molar ratio = 1.0, $POCl_3$, CH_2Cl_2, Ar, room temp., 5 h, then DIPEA, $BF_3.OEt_2$, 20 min, 50%; (g) 2-chloro-1,3,2-dioxaphospholane-2-oxide/**11** molar ratio = 2.0, Me_3N, MeCN, Ar, pressure tube, −78 ºC to room temperature, 3 h, then 80 ºC, 4 h, 65%.

5-Methoxy-2-(10'-bromodecyl)-[1,3]-dioxane (**6**) (1:3 *cis*/*trans* mixture):



A solution of 11-bromoundecanal (**5**) (4.71 g, 18.9 mmol), *rac*-2-*O*-methylglycerol (2.60 g, 24.6 mmol), and *p*-toluenesulfonic acid (108 mg, 0.57 mmol) in toluene (200 mL) was refluxed for 14 h. After workup, isomers *cis*-**6** and *trans*-**6** were separated by column chromatography (silica gel, hexane:EtOAc, 4:1 v/v). Data of *cis*-**6**: colorless oil, yield 1.58 g, 25%; TLC (hexane:EtOAc 4:1 v/v): R_f = 0.40; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.20–1.45 (m, 14H, H-2' to H-8'), 1.63 (m, 2H, H-1'), 1.83 (q, J = 7.0 Hz, 2H, H-9'), 3.05 (m, 1H, H-5), 3.39 (t, J = 6.9 Hz, 2H, H-10'), 3.42 (s, 3H, CH_3), 3.79 (dd, J = 12.6 and 1.4 Hz, 2H, H-4_{ax}, H-6_{ax}), 4.19 (dd, J = 12.6 and 1.4 Hz, 2H, H-4_{eq}, H-6_{eq}), 4.53 (t, J = 5.4 Hz, 1H, H-2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 23.9, 28.1, 28.7, 29.3 (C-2' to C-8'), 32.8 (C-9'), 34.0 (C-10'), 34.8 (C-1'), 56.5 (O*C*H_3), 68.0 (C-4, C-6), 72.4 (C-5), 102.4 (C-2); FT IR (KBr) ν_{max}: 2976, 2922, 2851, 2819, 1469, 1437, 1407, 1347, 1287, 1248, 1153, 1108, 1082, 1001, 956, 942, 904, 777 cm^{-1}; EI MS, *m*/*z* (%): 117 (100) [M − $C_{10}H_{20}$Br] (M: nominal mass). Data of *trans*-**6**: white solid, yield 4.30 g, 68%; TLC (hexane:EtOAc, 4:1 v/v): R_f = 0.90; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.21−1.45 (m, 14H, H-2' to H-8'), 1.58 (m, 2H, H-1'), 1.84 (q, J = 7.0 Hz, 2H, H-9'), 3.30–3.44 (m, 5H, H-4_{ax}, H-5, H-6_{ax}, H-10'), 3.37 (s, 3H, CH_3), 4.24 (dd, J = 10.4 Hz, J = 4.3 Hz, 2H, H-4_{eq}, H-6_{eq}), 4.38 (t, J = 5.1 Hz, 1H, H-2); ^{13}C-RMN (75 MHz, $CDCl_3$): δ 24.1, 28.1, 28.7, 29.4 (C-2' to C-8'), 32.8 (C-9'), 34.0 (C-10'), 34.4 (C-1'), 57.2 (*C*H_3), 69.5 (C-4, C-6), 69.8 (C-5), 102.2 (C-2); FT IR (KBr) ν_{max}: 2979, 2923, 2850, 1633, 1462, 1408, 1281, 1252, 1199, 1149, 1131, 1108, 1035, 960 cm^{-1}; EI MS, *m*/*z* (%): 117 (100) [M − $C_{10}H_{20}$Br] (M: nominal mass).

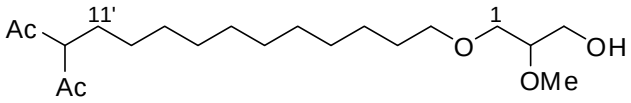
1-*O*-(11'-Bromoundecyl)-2-*O*-methyl-*rac*-glycerol (**7**):



DIBAL-H (42 mL, 1 M in toluene) was added (0 ºC, argon, stirring) to the acetal **6** (isomer *cis*, *trans* or mixtures) (2.83 g, 8.39 mmol). After 24 h at room temperature, a saturated aqueous solution of NH_4Cl

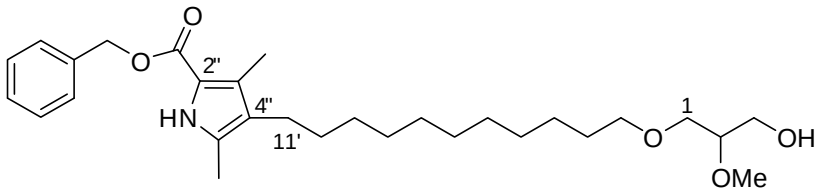
was added at 0 ºC, until no gas evolution was observed. The subsequent workup and purification by column chromatography (silica gel, hexane:EtOAc, 1:1 v/v) yielded alcohol **7** as a colorless oil, yield 2.42 g, 85%; TLC (hexane:EtOAc, 1:1 v/v): R_f = 0.40; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.22–1.36 (m, 12H, H-3' to H-8'), 1.41 (m, 2H, H-9'), 1.56 (m, 2H, H-2'), 1.84 (q, J = 7.1 Hz, 2H, H-10'), 2.25 (dd, J = 6.7 and 5.6 Hz, 1H, O*H*), 3.39 (t, J = 6.8 Hz, 2H, H-11'), 3.41–3.48 (m, 3H, H-2, H-1'), 3.46 (s, 3H, CH_3), 3.53 (m, 2H, H-1), 3.64 (m, 1H, H-3a), 3.74 (m, 1H, H-3b); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.1 (C-9'), 26.0, 28.7, 29.4, 29.4, 29.5, 29.6 (C-2' to C-8'), 32.8 (C-10'), 34.0 (C-11'), 57.7 (*C*H$_3$), 62.6 (C-3), 70.6 (C-1), 71.9 (C-1'), 79.9 (C-2); FT IR (KBr) ν_{max}: 3436, 2928, 2855, 1649, 1464, 1119, 1089 cm^{-1}; ESI$^+$ MS, *m*/*z*: 339.0 [M + H$^+$], 361.0 [M + Na$^+$] (M: nominal mass).

1-*O*-(12',12'-Diacetyldodecyl)-2-*O*-methyl-*rac*-glycerol (**8**):



A mixture of the glycerol **7** (2.42 g, 7.13 mmol), acetylacetone (7.21 g, 71.30 mmol), K_2CO_3 (4.93 g, 35.65 mmol) and 18-crown-6 (57 mg, 0.21 mmol) in acetone (50 mL) was refluxed for 24 h. After filtration of the non-dissolved material, the solvent was removed under reduced pressure and the residue was purified by column chromatography (silica gel, hexane:EtOAc, 1:1 v/v). Colorless oil, yield 1.79 g, 70%; TLC (hexane:EtOAc, 1:4 v/v): R_f = 0.35; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.22–1.41 (m, 16H, H-3' to H-10'), 1.55 (m, 2H, H-2'), 1.69 (m, 2H, H-11'), 2.13 and 2.26 (two s, each 3H, 2×CH_3CO), 3.39–3.48 (m, 3H, H-2, H-1'), 3.45 (s, 3H, CH_3O), 3.52 (m, 2H, H-1), 3.64 (m, 1H, H-3a), 3.74 (m, 1H, H-3b), 3.74 (t, J = 6.5 Hz, 1H, H-12'); ^{13}C NMR (75 MHz, $CDCl_3$): δ 19.8 (*C*H$_3$CO), 25.9 (C-10'), 28.6 (C-11'), 26.0, 29.2, 29.4, 29.4, 29.5, 29.5 (C-2' to C-9'), 31.9 (*C*H$_3$CO), 57.7 (*C*H$_3$O), 62.6 (C-3), 68.2 (C-12'), 70.6 (C-1), 71.9 (C-1'), 79.8 (C-2), 197.0 (2×*C*O); FT IR (neat) ν_{max}: 3436, 2928, 2850, 1679, 1583, 1461, 1402, 1359, 1275, 1169, 1117, 954 cm^{-1}; ESI$^+$ MS, *m*/*z*: 359.2 [M + H$^+$], 381.2 [M + Na$^+$].

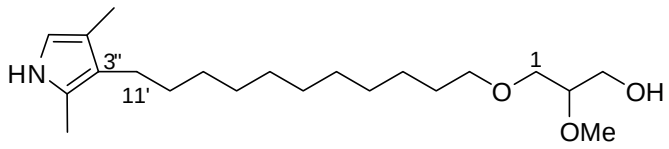
1-*O*-[11'-(2"-Benzyloxycarbonyl-3",5"-dimethyl-1*H*-pyrrol-4"-yl)undecyl]-2-*O*-methyl-*rac*-glycerol (**9**):



A solution of $NaNO_2$ (344 mg, 4.98 mmol) in water (2 mL) was added with stirring to an ice-cooled solution of benzyl acetoacetate (960 mg, 4.98 mmol) in acetic acid (8 mL). After stirring at 5 ºC for 3 h and at room temperature for 14 h, diacetylalcohol **8** was added (1.788 g, 4.98 mmol). Then, Zn dust (648 mg, 9.91 mmol) was slowly added and the mixture was kept at 65 ºC for 1 h. The subsequent workup and purification by column chromatography (silica gel, hexane:EtOAc, 1:1 v/v v/v) allowed the separation of pyrrole **9** as a yellow oil, yield 720 mg, 30%; TLC (hexane:EtOAc, 1:1 v/v): R_f = 0.50; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.19–1.40 (m, 16H, H-3' to H-10'), 1.55 (m, 2H, H-2'), 2.16, 2.26 (two s, each 3H, CH_3-

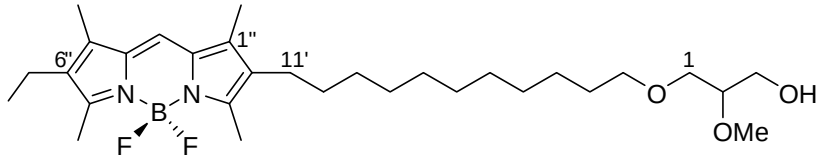
C3", CH_3-C5"), 2.31 (t, J = 7.4 Hz, 2H, H-11'), 3.37–3.49 (m, 3H, H-2, H-1'), 3.44 (s, 3H, CH_3O), 3.52 (m, 2H, H-1), 3.62 (dd, J = 11.4 and 4.9 Hz, 1H, H-3a), 3.74 (dd, J = 11.4 and 3.9 Hz, 1H, H-3b), 5.27 (s, 2H, CH_2Ph), 7.36 (m, 5H, H-Ph), 8.84 (br. s, 1H, N*H*); ^{13}C NMR (75 MHz, $CDCl_3$): δ 10.7 (*C*H_3-pyrrole), 11.4 (*C*H_3-pyrrole), 23.9 (C-11'), 29.3–29.5 and 30.8 (C-2' to C-10'), 57.7 (*C*H_3O), 62.5 (C-3), 65.2 (*C*H_2Ph), 70.5 (C-1), 71.8 (C-1'), 79.9 (C-2), 116.2 (C-2"), 122.5 (C-4"), 127.9, 127.9, 128.4 (C_o, C_m, C_p, C-3"), 129.9 (C-5"), 136.6 (C_i), 161.3 (*C*O_2Bn); FT IR (neat) ν_{max}: 3319, 2928, 2850, 1740, 1682, 1667, 1502, 1440, 1365, 1264, 1116, 1088 cm^{-1}; ESI$^+$ MS, *m/z*: 488.4 [M + H$^+$], 510.3 [M + Na$^+$].

1-*O*-[11'-(2",4"-Dimethyl-1*H*-pyrrol-3"-yl)undecyl-2-*O*-methyl-*rac*-glycerol (**10**):



A stirred mixture of **9** (1.24 g, 2.54 mmol), EtOH (30 mL) and Pd/C 10% (130 mg) was hydrogenated at 40 psi for 4 h. After filtration, the solvent was removed and the residue was treated with TFA (1.5 mL) for 5 min. Then, argon was bubbled until dryness and the residue was dissolved in CH_2Cl_2 (50 mL); the solution thus obtained was washed successively with water, saturated aqueous solution of $NaHCO_3$ and water. After drying with sodium sulfate, the solvent was removed and the residue was purified by column chromatography (silica gel, hexane:EtOAc, 4:1 v/v). The α-H pyrrole **10** was a colorless oil that slowly darkens on standing; yield 201 mg, 24%; TLC (hexane:EtOAc, 1:1 v/v): R_f = 0.55; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.20–1.44 (m, 16H, H-3' to H-10'), 1.55 (m, 2H, H-2'), 2.07, 2.14 (two s, each 3H, CH_3-C3", CH_3-C5"), 2.33 (t, J = 7.4 Hz, 2H, H-11'), 3.39–3.49 (m, 3H, H-2, H-1'), 3.45 (s, 3H, CH_3O), 3.53 (m, 2H, H-1), 3.63 (dd, J = 12.1 and 4.7 Hz, 1H, H-3a), 3.74 (dd, J = 11.5 and 4.1 Hz, 1H, H-3b), 6.36 (s, 1H, H-5"), 7.60 (br. s, 1H, N*H*); ^{13}C NMR (75 MHz, $CDCl_3$): δ 10.4 (*C*H_3-pyrrole), 11.3 (*C*H_3-pyrrole), 24.3 (C-11'), 29.3–29.5, 31.1 (C-2' to C-10'), 57.7 (*C*H_3O), 62.6 (C-3), 70.5 (C-1), 71.9 (C-1'), 80.0 (C-2), 112.6 (C-5"), 117.9, 119.0, 123.5 (C-2" to C-4"); FT IR (neat) ν_{max}: 3400, 2927, 2855, 1741, 1695, 1464, 1367, 1240, 1118, 1047 cm^{-1}; ESI$^+$ MS, *m/z*: 354.2 [M + H$^+$], 392.3 [M + K$^+$].

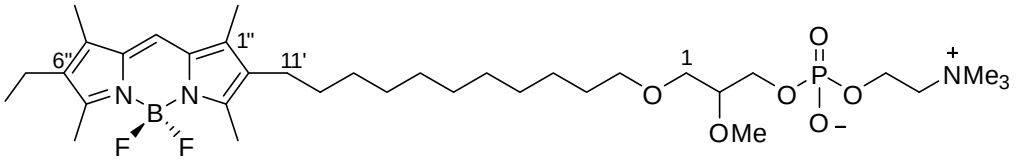
1-*O*-[11'-(6"-Ethyl-1",3",5",7"-tetramethyl-4",4"-difluoro-4"-bora-3a",4a"-diaza-*s*-indacen-2"-yl)undecyl]-2-*O*-methyl-*rac*-glycerol (**11**):



Phosphorus oxychloride (87 mg, 53 µL, 0.57 mmol) was added to a stirred solution of pyrrole **10** (200 mg, 0.57 mmol) and 3,5-dimethyl-4-ethyl-2-formyl-1*H*-pyrrole (86 mg, 0.57 mmol) in CH_2Cl_2 (150 mL) under argon atmosphere. After stirring the mixture at room temperature for 5 h, diisopropylethylamine (DIPEA) (366 mg, 493 µL, 2.83 mmol) was added and 5 min latter $BF_3.OEt_2$ (402 mg, 356 µL, 2.83

mmol). After 5 min, the same amount of DIPEA and $BF_3.OEt_2$ was added and the mixture was stirred for 20 min. The workup allowed the isolation of a crude product that was purified by column chromatography (silica gel, hexane:EtOAc, 7:3 v/v). Red waxy solid, yield 151 mg, 50%; TLC (hexane:EtOAc, 1:1 v/v): R_f = 0.60; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.05 (t, J = 7.6 Hz, 3H, CH_2CH_3), 1.22–1.36 (m, 14H, H-3' to H-9'), 1.40 (m, 2H, H-10'), 1.55 (q, J = 6.7 Hz, 2H, H-2'), 2.14, 2.15 (two s, each 3H, CH_3-C1", CH_3-C7"), 2.35 (m, 4H, H-11', CH_2CH_3), 2.47 (two s, each 3H, CH_3-C3", CH_3-C5"), 3.40–3.48 (m, 3H, H-2, H-1'), 3.45 (s, 3H, CH_3O), 3.53 (m, 2H, H-1), 3.63 (dd, J = 11.6 and 5.2 Hz, 1H, H-3a), 3.74 (dd, J = 11.6 and 4.2 Hz, 1H, H-3b), 6.93 (s, 1H, C*H*-8"); ^{13}C NMR (75 MHz, $CDCl_3$): δ 9.3, 9.5 (CH_3-C1", CH_3-C7"), 12.5, 12.6 (CH_3-C3", CH_3-C5"), 14.5 (CH_2CH_3), 17.2 (CH_2CH_3), 24.0 (C-11'), 26.0, 29.4–29.5 (C-2' to C-9'), 30.1 (C-10'), 57.7 (CH_3O), 62.6 (C-3), 70.5 (C-1), 71.8 (C-1'), 79.8 (C-2), 118.5 (C-8"), 130.2, 131.5, 132.4, 136.5, 136.9 (C-1", C-7", C-7a", C-8a", C-2", C-6"), 154.5, 154.9 (C-3", C-5"); FT IR (neat) ν_{max}: 3394, 2925, 2850, 1604, 1471, 1226, 1191, 1069, 976 cm^{-1}; ESI$^+$ MS, *m/z*: 515.3 [M – F]$^+$, 495.5 [M – 2F + H$^+$]; HPLC (reverse phase C_{18} column, MeOH:H_2O, 9:1 v/v, 1.2 mL min^{-1}, λ_{anal} 375 and 529 nm), R_t = 10.18 min (98% purity).

1-*O*-[11'-(6"-Ethyl-1",3",5",7"-tetramethyl-4",4"-difluoro-4"-bora-3a",4a"-diaza-*s*-indacen-2"-yl)undecyl]-2-*O*-methyl-*rac*-glycero-3-phosphocholine (Et-BDP-ET):



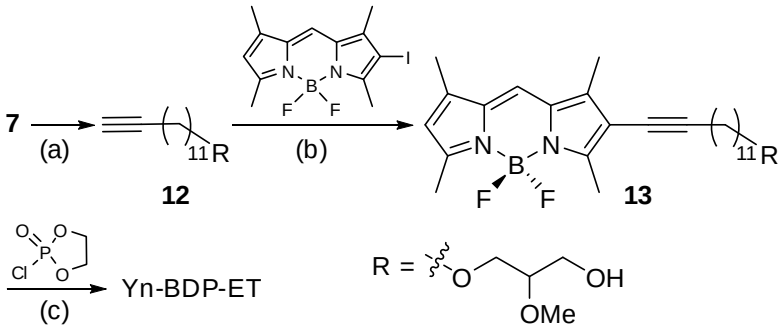
Trimethylamine (ca. 2 mL) was condensed into a solution of alcohol **11** (81 mg, 0.15 mmol) in acetonitrile (10 mL) in a pressure tube at –78 ºC under argon. 2-Chloro-1,3,2-dioxaphospholane-2-oxide (28 μL, 0.30 mmol) was then added to the cooled solution and the reactor was closed and left 3 h at room temperature and 4 h at 80 ºC. After cooling back to room temperature, the reactor was opened, the solvent and the excess trimethylamine were vacuum-evaporated, the residual solid was dissolved in THF:H_2O 9:1 v/v (10 mL), and Amberlite MB-3 was added until change of color of the resin (saturation). The mixture was filtered, the resin was washed with MeOH (3×10 mL), the filtrate and washings were collected, the solvent was vacuum-evaporated, and the residual solid was purified by column chromatography (silica gel), using first $CHCl_3$:MeOH, 9:1 v/v, for separating non-polar products, and then $CHCl_3$:MeOH:H_2O, 65:25:5 v/v/v. Waxy oil, yield 70 mg, 65%; TLC ($CHCl_3$:MeOH:H_2O, 65:25:5 v/v/v): R_f = 0.20; ^{1}H NMR (300 MHz, CD_3OD): δ 1.06 (t, J = 7.6 Hz, 3H, CH_2CH_3), 1.25–1.37 (m, 14H, H-3' to H-9'), 1.41 (m, 2H, H-10'), 1.54 (q, J = 6.7 Hz, 2H, H-2'), 2.15, 2.16 (two s, each 3H, CH_3-C1", CH_3-C7"), 2.39 (m, 4H, H-11', CH_2CH_3), 2.41, 2.42 (two s, each 3H, CH_3-C3", CH_3-C5"), 3.22 (s, 9H, N(CH_3)$_3$), 3.45 (s, 3H, CH_3O), 3.41–3.59 (m, 6H, H-1, H-2, H-1'), 3.63 (m, 2H, CH_2N), 3.85-4.01 (m, 2H, H-3), 4.27 (m, 2H, OCH_2CH_2N), 7.20 (s, 1H, C*H*-8"); ^{13}C NMR (75 MHz, CD_3OD): δ 9.4, 9.6 (CH_3-C1", CH_3-C7"), 12.7, 12.9 (CH_3-C3", CH_3-C5"), 15.0 (CH_2CH_3), 18.1 (CH_2CH_3), 24.8 (C-11'), 27.3, 30.5-30.8 (C-2' to C-9'), 31.3 (C-10'), 54.67, 54.72 and 54.77 (N(CH_3)$_3$), 58.3 (CH_3O), 60.4 (d, J = 5.0 Hz, OCH_2CH_2N), 66.2 (d, J

= 5.8 Hz, C-3), 67.5 (br. s, CH_2N), 71.1 (C-1), 72.7 (C-1'), 80.9 (d, J = 8.0 Hz, C-2), 120.6 (C-8"), 131.4, 132.8, 133.9, 138.6, 138.9 (C-1", C-7", C-7a", C-8a", C-2", C-6"), 155.4, 155.6 (C-3", C-5"); FT IR (neat) ν_{max}: 2922, 2847, 1602, 1469, 1226, 1065, 975; ESI$^+$ MS, m/z: 700.5 [M + H$^+$], 1399.7 [2M + H$^+$]; Analysis (calcd., found for $C_{35}H_{61}BF_2N_3O_6P$ + 4.5 H_2O): C (53.84; 53.81), H (9.04, 8.85) N (5.38, 5.43); HPLC (reverse phase C_{18} column, MeOH:H_2O 9:1 v/v + 10 mM H_3PO_4, 1.2 mL min^{-1}, λ_{anal} 375 and 529 nm), R_t = 5.60 min (100% purity); UV-Vis (EtOH), λ_{max} (ε, M^{-1} cm^{-1}): 380 nm (9000), 503 nm (shoulder) (33500), 529 nm (79000); UV-Vis (DMSO): λ_{max} (ε, M^{-1} cm^{-1}): 529 nm (79000); emission (corrected) (EtOH): λ_{max} 537 nm (Figure S2, panel A), Φ_f (EtOH) 0.60.

IV – Synthesis of Yn-BDP-ET

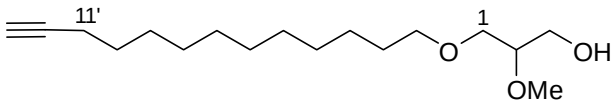
The analogue was prepared from the brominated alcohol **7** in three steps (Scheme S3): a) **7** was alkynylated with lithium acetylenide/ethylenediamine complex[13], yielding the acetylenic alcohol **12**; b) a Sonogashira-Hagihara cross-coupling reaction[14] between **12** and 2-iodo-1,3,5,7-tetramethyl-BDP provided alcohol **13**[3]; c) finally, the phosphocholine group was introduced in **13** by reaction with 2-chloro-1,3,2-dioxaphospholane-2-oxide and trimethylamine, yielding analogue Yn-BDP-ET.

Scheme S3 [a]



[a] Reagents and conditions: (a) lithium acetylenide/ethylenediamine complex, DMSO, 5ºC to room temp., 16 h, 85%; (b) 2-iodo-1,3,5,7-tetramethyl-BDP/**12** molar ratio = 1.0, $PdCl_2(PPh_3)$, CuI, PhOH, nBu$_4$NI, iPr$_2$EtN, DMF, room temp., 3 h, 72%; (c) 2-chloro-1,3,2-dioxaphospholane-2-oxide/**13** molar ratio = 2.1, Me_3N, MeCN, Ar, pressure tube, −78 ºC to room temp, 3 h, then 80 ºC, 4 h, 33%.

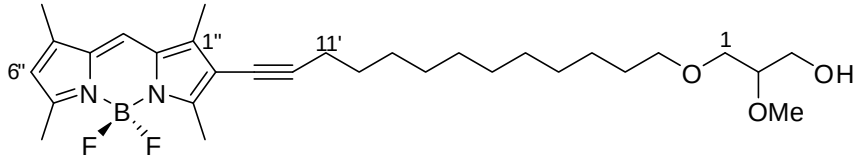
1-*O*-(Tridec-12'-ynyl)-2-*O*-methyl-*rac*-glycerol (**12**):



A degassed solution of lithium acetylenide/ethylenediamine complex (1.34 g, 14.58 mmol) in dry DMSO (5 mL) was added to a stirred solution of alcohol **7** (1.98 g, 5.83 mmol) in dry DMSO (15 mL) at 5 ºC, and the mixture was kept stirred for 16 h at room temp. Water (20 mL) was slowly added and the resulting mixture was diluted further with water (100 mL). The subsequent workup yielded a residue that was purified by column chromatography (silica gel, hexane:EtOAc, 4:1 v/v). Pale yellow oil, yield 1.4 g,

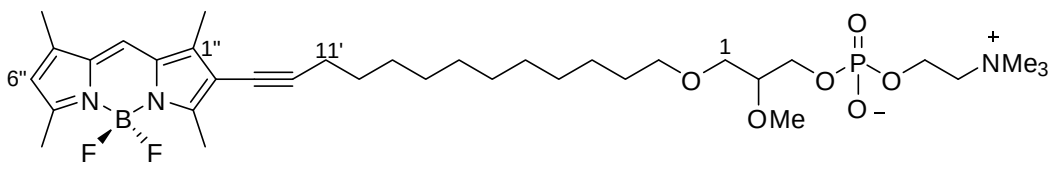
85%; TLC (hexane:EtOAc, 1:1 v/v): R_f = 0.55; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.22–1.42 (m, 14H, H-3' to H-9'), 1.46–1.60 (m, 4H, H-2', H-10'), 1.93 (t, J = 2.6 Hz, 1 H, H-13'), 2.17 (td, J = 7.1 and 2.6 Hz, 2H, H-11'), 3.40–3.49 (m, 3H, H-2, H-1'), 3.46 (s, 3H, CH_3), 3.53 (m, 2H, H-1), 3.63 (dd, J = 11.6 and 4.7 Hz, 1H, H-3a), 3.75 (dd, J = 11.6 and 4.7 Hz, 1H, H-3b); ^{13}C NMR (75 MHz, $CDCl_3$): δ 18.4 (C-11'), 26.0, 28.5, 28.7, 29.1, 29.4 to 29.6 (C-2' to C-10'), 57.7 (*C*H_3), 62.6 (C-3), 68.0 (C-13'), 70.6 (C-1), 71.9 (C-1'), 79.8 (C-2), 84.8 (C-12'); FT IR (KBr) ν_{max}: 3445, 3312, 2928, 2855, 1465, 1119 cm^{-1}; ESI$^+$ MS, *m*/*z*: 285.2 [M + H$^+$], 307.3 [M + Na$^+$].

1-*O*-[13'-(1",3",5",7"-Tetramethyl-4",4"-difluoro-4"-bora-3a",4a"-diaza-*s*-indacen-2"-yl)tridec-12'-ynyl]-2-*O*-methyl-*rac*-glycerol (**13**):



A mixture of 2-iodo-1,3,5,7-tetramethyl-BDP (393 mg, 1.05 mmol), $PdCl_2(PPh_3)_2$ (81 mg, 0.12 mmol), CuI (68 mg, 0.36 mmol), phenol (89 mg, 0.95 mmol), nBu$_4$NI (1.36 g, 3.68 mmol), DMF (10 mL) and iPr$_2$EtN (5 mL) was stirred under argon at room temp. for 5 min. Then, alkyne **12** (300 mg, 1.05 mmol) was added and the mixture was maintained at room temp. for 3 h with stirring. After dilution with EtOAc (40 mL), the work up yielded a residue that was purified by column chromatography (silica gel, hexane:EtOAc, 7:3 v/v). Red waxy solid, yield 401 mg, 72%; TLC (hexane:EtOAc, 1:1 v/v): R_f = 0.45; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.20–1.48 (m, 14H, H-3' to H-9'), 1.50–1.63 (m, 4H, H-2', H-10'), 2.19, 2.21 (two s, each 3H, CH_3-C1", CH_3-C7"), 2.43 (t, J = 6.9 Hz, 2H, H-11'), 2.49, 2.55 (two s, each 3H, CH_3-C3", CH_3-C5"), 3.38–3.48 (m, 3H, H-2, H-1'), 3.44 (s, 3H, CH_3O), 3.51 (m, 2H, H-1), 3.61 (dd, J = 11.5 and 5.2 Hz, 1H, H-3a), 3.72 (dd, J = 11.5 and 3.8 Hz, 1H, H-3b), 6.01 (s, 1H, H-6"), 6.98 (s, 1H, H-8"); ^{13}C NMR (75 MHz, $CDCl_3$): δ 10.4, 11.1 (*C*H_3-C1", *C*H_3-C7"), 13.3, 14.6 (*C*H_3-C3", *C*H_3-C5"), 19.6 (C-11'), 26.0, 28.7, 28.9, 29.0, 29.3–29.5 (C-2' to C-10'), 57.6 (*C*H_3O), 62.5 (C-3), 70.5 (C-1), 71.8 (C-1'), 72.3 (C-13'), 79.8 (C-2), 96.5 (C12'), 114.5 (C-2"), 119.3 (C-6"), 120.2 (C-8"), 131.6, 134.0, 140.9, 141.9 (C-1", C-7", C-7"a, C-8"a), 157.7, 157.8 (C-3", C-5"); FT IR (neat) ν_{max}: 3428, 2927, 2850, 1602, 1574, 1471, 1406, 1240, 1194, 1160, 1079, 1000, 894, 807 cm^{-1}; ESI$^+$ MS, *m*/*z*: 553.3 [M + Na$^+$], 511.3 [M – F]$^+$, 491.3 [M – 2F + H]$^+$; HPLC (reverse phase C_{18} column, MeOH:H_2O, 9/1 v/v, 1.8 mL min^{-1}, λ_{anal} 280, 375 and 529 nm): R_t = 6.32 min (100% purity).

1-*O*-[13'-(1",3",5",7"-Tetramethyl-4",4"-difluoro-4"-bora-3a",4a"-diaza-*s*-indacen-2"-yl)tridec-12'-ynyl)-2-*O*-methyl-*rac*-glycero-3-phosphocholine (Yn-BDP-ET):



Prepared from **13** (56 mg, 0.10 mmol) and purified under the conditions described above for the synthesis

of analog Et-BDP-ET. Red solid, yield 23 mg, 33%; mp: 124-127 ºC; TLC ($CHCl_3$:MeOH:H_2O, 65:25:5 v/v/v): *R*f = 0.20; ^{1}H NMR (300 MHz, CD_3OD): δ 1.26–1.40 (m, 14H, H-3' to H-9'), 1.46–1.66 (m, 4H, H-2', H-10'), 2.25, 2.26 (two s, each 3H, C*H*$_3$-C1", C*H*$_3$-C7"), 2.47 (m, 8H, C*H*$_3$-C3", C*H*$_3$-C5", H-11'), 3.21 (s, 9H, N(C*H*$_3$)$_3$), 3.45 (s, 3H, C*H*$_3$O), 3.41–3.58 (m, 6H, H-1, H-2, H-1'), 3.63 (m, 2H, C*H*$_2$N), 3.85–4.00 (m, 2H, H-3), 4.27 (m, 2H, OC*H*$_2$CH$_2$N), 6.14 (s, 1H, C*H*-6"), 7.36 (s, 1H, C*H*-8"); ^{13}C NMR (75 MHz, CD_3OD): δ 10.6, 11.3 (*C*H$_3$-C1", *C*H$_3$-C7"), 13.5, 14.8 (*C*H$_3$-C3", *C*H$_3$-C5"), 20.3 (C-11'), 27.3, 29.9, 30.1, 30.2, 30.7–30.8 (C-2' to C-10'), 54.66, 54.71, 54.77 (N(*C*H$_3$)$_3$), 58.3 (*C*H$_3$O), 60.4 (d, *J* = 5.0 Hz, OC*H*$_2$CH$_2$N), 66.2 (d, *J* = 5.6 Hz, C-3), 67.5 (br. s, *C*H$_2$N), 71.1 (C-1), 72.7 (C-1'), 73.4 (C-13'), 80.9 (d, *J* = 8.1 Hz, C-2), 97.5 (C-12'), 115.6 (C-2"), 120.7 (C-6"), 122.6 (C-8"), 132.9, 135.8, 142.5, 144.6 (C-1", C-7", C-7"a, C-8"a), 157.9, 159.5 (C-3", C-5"); FT IR (KBr) ν_{max}: 2927, 2854, 1619, 1472, 1406, 1247, 1159, 1068, 974 cm^{-1}; ESI$^+$ MS, *m*/*z*: 696.3 [M + H$^+$], 1391.7 [2M + H+]; Analysis (calcd., found for $C_{35}H_{57}BF_2N_3O_6P$ + 1.5 H_2O): C (58.17; 58.15), H (8.37, 8.86) N (5.81, 5.83); HPLC (reverse phase C_{18} column, MeOH:H_2O 9:1 v/v + 10 mM H_3PO_4, 1.2 mL min^{-1}, λ_{anal} 280, 375, 529 nm): R_t = 4.29 min (100% purity); UV-Vis (EtOH), λ_{max} (ε, M^{-1} cm^{-1}): 390 nm (9009), 533 nm (56620); UV-Vis (DMSO): λ_{max} (ε, M^{-1} cm^{-1}): 530 nm (42255); emission (corrected) (EtOH): λ_{max} 553 nm (Figure S2, panel B), Φ_f (EtOH) 0.50.

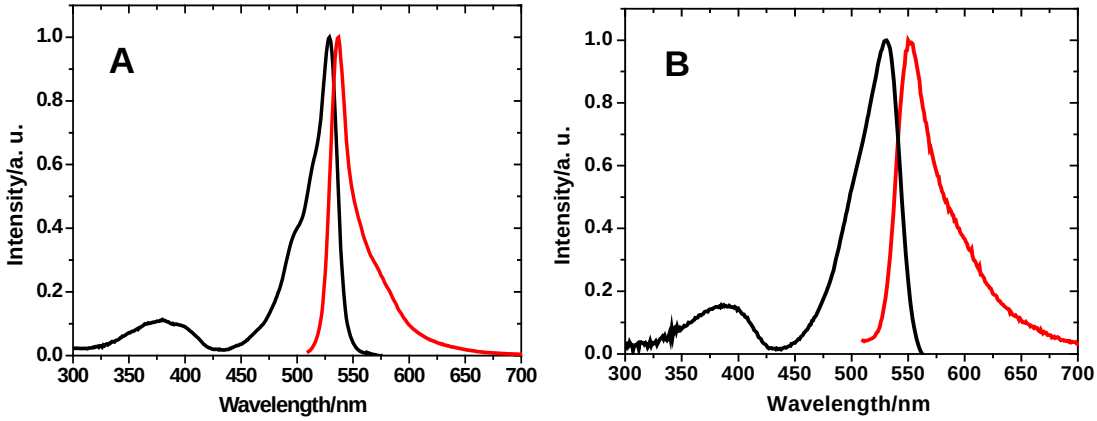


Figure S2. Absorption and corrected fluorescence spectra (normalized) of the BDP fluorescent analogues of edelfosine in ethanol solution, 10^{-5}–10^{-6} M. A) Et-BDP-ET; B) Yn-BDP-ET.

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