Supporting information

Enantioselective ester hydrolysis by an achiral catalyst co-embedded with chiral amphiphiles into a vesicle membrane

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Content

General methods and materials

Solvents were used as p.a. grade or dried and distilled according to common procedures.

NMR-Spectroscopy: NMR-spectra were recorded on a Bruker Avance 400 (^{1}H: 400 MHz, ^{13}C: 101 MHz, T = 300 K) or a Bruker Avance 300 (^{1}H: 300 MHz, ^{13}C: 75 MHz, T = 295 K) using the solvent residual peak as internal reference ($CDCl_3$: δ H 7.26). The chemical shifts are reported in δ [ppm] relative to internal standards (solvent residual peak). The spectra were analyzed by first order, the coupling constants *J* are given in Hertz [Hz]. Characterization of the signals: s = singlet, d = doublet, t = triplet, m = multiplet. Integration is determined as the relative number of atoms. Error of reported values: chemical shift: 0.01 ppm for ^{1}H-NMR, 0.1 ppm for ^{13}C-NMR and 0.1 Hz for coupling constants. The solvent used is reported for each spectrum.

Thin Layer Chromatography: Aluminum plates coated with silica gel (ALUGRAM Xtra SIL G/UV_{254} from Macherey-Nagel) were used. Detection was done by UV light (254 nm, 366 nm) or oxidation, using a $KMnO_4$-solution.

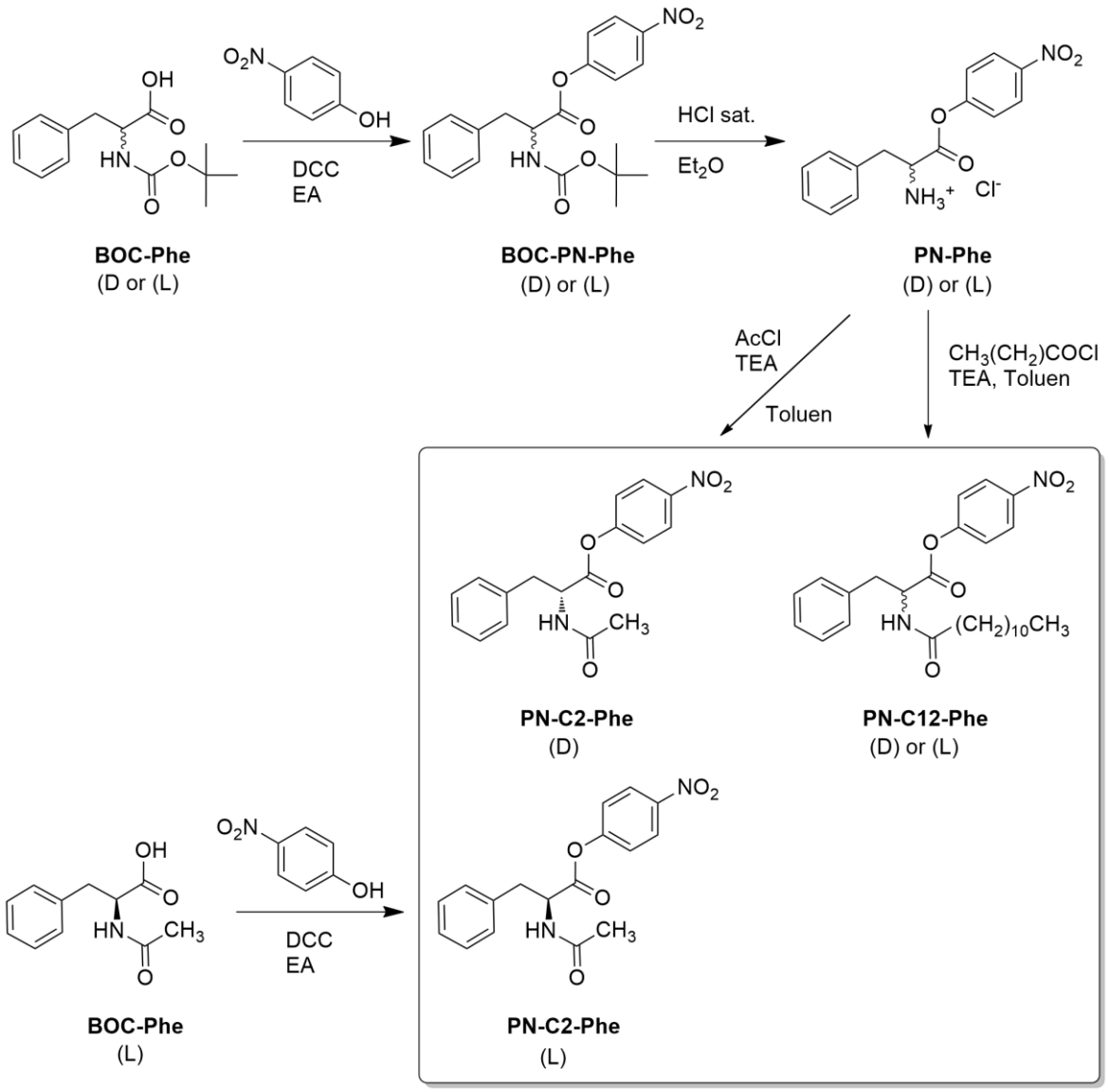
Polarimetry: Polarimetry measured in 1 dm cuvette on a PERKIN-ELMER 241 polarimeter.

UV/Vis-Spectroscopy: UV/Vis Spectra were recorded on a Cary 50 UV/Vis spectrophotometer.

Dynamic Light Scattering: DLS measurements were performed on a Malvern Zetasizer Nano at 25 °C using 1 cm disposable polystyrene cuvettes (VWR).

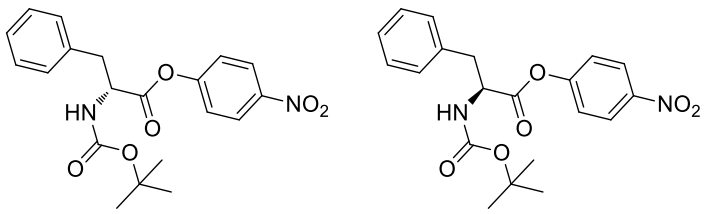
Synthesis

The synthesis of the metal complex **Zn_2Cy** was previously reported and the compound was prepared in the same manner.[1] Substrates **L(D)-PN-C12-Phe** were prepared via DCC coupling of BOC protected L(D)-phenylalanine followed by deprotection with saturated HCL in diethylether and consecutive acylation with octadecanoyl chloride. Acetylated compounds **L(D)-PN-C2-Phe** were prepared either by acylation of amine in 4-nitrophenyl-alnine **PN-Phe** (D enantiomere) or via DCC coupling of 4-nitrophenol and N-acetyl-phenylalanine (L enantiomere) (Scheme S1).



Scheme S1: Synthesis of the substrates.

(L) and (D) 4-Nitrophenyl-Boc-phenylalaninate (Boc-Phe)[2]



(L)-Phenylalanin (2.38 g, 9 mmol), 4-nitrophenol (1.25 g, 9 mmol) and DCC (1.95 g, 9.45 mmol) were dissolved in dry ethylacetate (35 mL) equipped with drying tube and stirred overnight. Solids were filtered off and washed with water (30 ml) and saturated aq. $NaHCO_3$ (30 ml). The aqueous phase was extracted with ethyl acetate (2 x 30 ml). Combined organic layers were washed with brine and dried over $MgSO_4$. Solids were

filtered off and solvent removed in *vacuo*. Crude product was recrystallized from ethanol to obtain 1.8 g (52 %) of **L-Boc-Phe** enantiomer in form of white crystals.

D-Boc-Phe was obtained by the same procedure in 43 % yield.

^{1}H-NMR (300 MHz; $CDCl_3$): δ 8.24 (d, *J* = 9.0 Hz, 2H), 7.41 – 7.27 (m, 3H), 7.25 – 7.20 (m, 2H), 7.14 (d, J = 9.0 Hz, 2H), 5.10 – 5.00 (m, 1H), 4.71 – 4.85 (m, 1H), 3.32 – 2.92 (m, 2H), 1.44 (s, 9H).

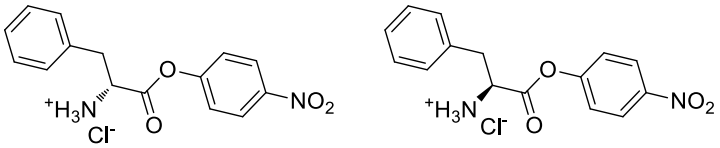
^{13}C-NMR (75 MHz; $CDCl_3$): δ 170.1, 155.2, 145.5, 135.4, 129.4, 128.9, 127.5, 125.3, 122.3, 80.6, 77.2, 54.7, 38.2, 28.3.

MS (ESI(+)):m/z 409.15 [MNa^+].

(L) $[\alpha]_D^{21}$= - 9.2° ($CHCl_3$)

(D) $[\alpha]_D^{21}$= 9.1° ($CHCl_3$)

(L) and (D) 4-Nitrophenyl phenylalaninate (PN-Phe)[3]



Protected L-phenylalaninate **L-Boc-Phe** (387 mg, 1.00 mmol) was suspended in sat. HCl diethylether solution (20 mL) and stirred under the drying tube at room temperature for 3 h. Solvent was removed in *vacuo* and the residue was recrystallized from methanol to obtain 223 mg (69 %) of product **L-PN-Phe** in form of white needles.

The D-enantiomere was obtained by the same procedure in 53 % yield.

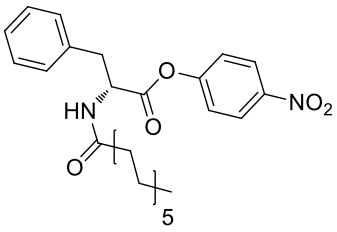
^{1}H-NMR (300 MHz; MeOD): δ 8.38 – 8.17 (m, 2H), 7.50 – 7.20 (m, 7H), 4.70 (dd, *J* = 8.6, 5.7 Hz, 1H), 3.48 – 3.36 (m, 2H).

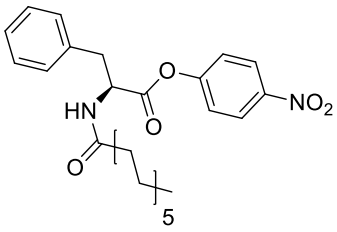
^{13}C-NMR (75 MHz; MeOD): δ 168.5, 155.7, 147.5, 135.2, 130.7, 130.4, 129.3, 126.4, 123.6, 55.4, 37.7.

(L) $[\alpha]_D^{21}$= - 53.8° (DMSO)

(D) $[\alpha]_D^{21}$= 54.6° (DMSO)

(L) and (D) 4-Nitrophenyl *N*-dodecanoylphenylalaninate (PN-C12-Phe)[4]





The ammonium salt of **D-PN-Phe** (200 mg, 0.62 mmol) was suspended in toluene (20 mL) and triethylamine (157 mg, 1.55 mmol) was added under nitrogen atmosphere. Subsequently dodecanoyl chloride (271 mg, 1.24 mmol) was added and mixture was stirred at room temperature for 1h. Solids were filtered off and washed with toluene. Solvent was removed from filtrate in *vacuo* and the residue was purified by flash chromatography (PE → PE:EA, 1:1). 190 mg (65 %) of the product **D-PN-C12-Phe** obtained in form of oil which crystalized at RT. To obtain highest purity, substrates were recrystallized from ethanol.

The L-enantiomer was obtained by the same procedure in 40 % yield.

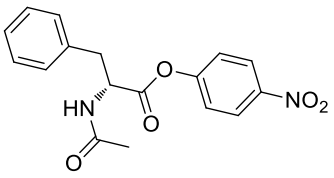
^{1}H-NMR (300 MHz; $CDCl_3$): δ 8.38 – 8.19 (m, 2H), 7.44 – 7.29 (m, 3H), 7.29 – 7.05 (m, 4H), 5.94 (t, *J* = 9.9 Hz, 1H), 5.15 – 4.98 (m, 1H), 3.32 – 3.17 (m, 2H), 2.30 – 2.07 (m, 2H), 1.57 (dd, *J* = 27.4, 20.4 Hz, 2H), 1.43 – 1.13 (m, 16H), 0.87 (t, *J* = 6.7 Hz, 3H).

^{13}C-NMR (75 MHz; $CDCl_3$): NMR resonance data are identical with the literature.[4]

(L) $[\alpha]_D^{21}$= - 15.7° (DMSO)

(D) $[\alpha]_D^{21}$= 17.0° (DMSO)

(D) 4-Nitrophenyl N-acetlyphenylalaninate (D-PN-C2-Phe)[2]



The ammonium salt of 4-nitrophenyl D-phenylalaninate (77 mg, 0.24 mmol) was suspended in toluene (10 mL) and triethylamine (60 mg, 0.60 mmol) was added under nitrogen atmosphere. Subsequently acyl chloride (38 mg, 0.48 mmol) was added and mixture was stirred at room temperature for 1h. Solids were filtered off and washed with

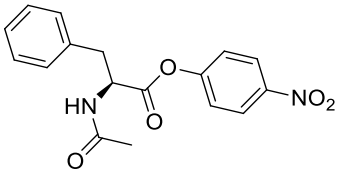
toluene. Solvent was removed from filtrate in *vacuo* and the residue was purified by flash chromatography (PE:EA, 0:1 → 1:1). The residue was recrystallized from ethanol to obtain 46 mg (58 %) of product **L-PN-C2-Phe** in form of white crystals.

^{1}H-NMR (300 MHz; $CDCl_3$): δ8.30 – 8.19 (m, 2H), 7.42 – 7.28 (m, 3H), 7.25 – 7.19 (m, 2H), 7.19 – 7.08 (m, 2H), 5.95 (d, *J* = 7.2 Hz, 1H), 5.07 (dd, *J* = 13.8, 6.5 Hz, 1H), 3.35 – 3.15 (m, 2H), 2.05 (s, 3H).

^{13}C-NMR (75 MHz; $CDCl_3$): NMR resonance data are identical with the literature.

(D) $[\alpha]_D^{21}$= 1.6° (DMSO)

(L) 4-Nitrophenyl *N*-acetylphenylalaninate (L-PN-C2-Phe)



N-Acetly L-phenylalanine (324 mg, 1.56 mmol), 4-nitrophenol (218 mg, 1.56 mmol) and DCC (354 mg, 1.72 mmol) were dissolved in dry ethyl acetate (20 mL), the reaction flask was equipped with drying tube and the reaction mixture was stirred overnight. Solids were filtered off and washed with water (20 ml) and saturated aq. $NaHCO_3$ (15 ml). The aqueous phase was extracted with ethyl acetate (2 x 20 ml). Combined organic layers were washed with brine and dried over $MgSO_4$. Solids were filtered off and solvent removed in *vacuo*. The crude product was recrystallized from ethanol to obtain 300 mg (58 %) of **L-PN-Ac-Phe** in form of white crystals.

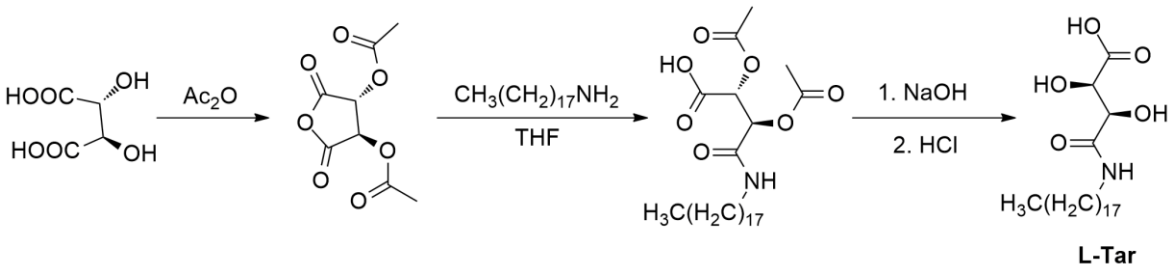
^{1}H-NMR (300 MHz; $CDCl_3$): δ same as for D derivative.

^{13}C-NMR (75 MHz; $CDCl_3$): δ same as for D derivative.

(L) $[\alpha]_D^{21}$= - 1.5° (DMSO)

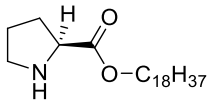
Synthesis of the membrane additives

Glucose **L-Glu** was purchased by Sigma Aldrich. L-Histidine derivatives **L-His-COOH** and **L-His-OH** were prepared according to known procedure.[4] Spartein **(-)-Spa** and proline **L-Pro** derivatives were prepared by alkylation (for **(-)-Spa**) or esterification (for **L-Pro**) with octadecylbromide or alcohol.[5, 6] Amphiphilic tartrate derivative **L-Tar** was prepared from L-tartrate by a series of protection and amide bond formation steps with octadecylamine and anhydride to obtain amphiphilic free acids according to Scheme S2.



Scheme S2: Synthesis of the tartrate derivative **L-Tar**.

L-Proline octadecyl ester (L-Pro)



L-Proline (772 mg, 6.710 mmol) and octadecanole (8 g, 29.57mmol) were heated up to 60 °C producing viscous suspension. Thionyl chloride (880 mg, 7.40 mmol) was added followed by DMAP (4 mg, 0.03 mmol). The reaction mixture was stirred at 60 °C for 5 h. Afterwards DCM (20 ml) was added and the suspension was cooled down to 0 °C. After a dropwise addition of triethylamine (1154 mg, 11.41 mmol), the reaction mixture was stirred for additional 3 h. Et_2O (10 ml) was added and the precipitated crude product was filtered off. The residue was purified by column chromatography (PE:EA; 1:1). The product **L-Pro** 270 mg (11 %) was obtained as a white crystalline solid.

^{1}H NMR (300 MHz, $CDCl_3$): δ 4.10 (t, *J* = 6.7 Hz, 2H), 3.75 (dd, *J* = 8.7, 5.5 Hz, 1H), 3.08 (dt, *J* = 10.2, 6.5 Hz, 1H), 2.90 (dt, *J* = 10.2, 6.7 Hz, 1H), 2.29 (bs, 1H), 2.12 (m, 1H), 1.92 – 1.59 (m, 5H), 1.39 – 1.20 (m, 30H), 0.87 (t, *J* = 6.7 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$): δ 175.6, 65.1, 59.8, 47.1, 31.9, 30.3, 29.7 , 29.7, 29.6, 29.6, 29.5, 29.4, 29.2, 28.6, 25.9, 25.5, 22.7, 14.1.

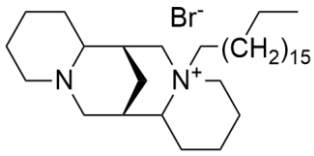
MS (ESI(+)): m/z = 368.35 [MH^+]

MP: 145 – 150 °C

The NMR-data identical to the literature reference.[5]

$[\alpha]_D^{21}$= - 24.8° ($CHCl_3$)

(-)-*N*-Octadecylsparteinium ((-)-Spa)[6]



A mixture of (-)-spartein (611 mg, 2.6 mmol), octadecylbromide (869 mg, 2.6 mmol), potassium carbonate (3g, 21.7 mmol) and sodium iodide (3 g, 20.0 mmol) in MeCN:DMF (40 ml, 1:1) was refluxed under nitrogen atmosphere for 15 hours. After cooling to room temperature the reaction mixture was poured on crushed ice and the formed precipitate was filtered off. After recrystallization from diethyl ether the pure product was obtain in form of a white solid.

Yield: 0.6 g; 41 %

^{1}H NMR (300 MHz, $CDCl_3$): δ 3.39 (t, J = 6.9 Hz, 2H), 2.84 – 2.63 (m, 5H), 2.52 (d, J = 10.9 Hz, 2H), 2.34 (dd, J = 11.2, 3.4 Hz, 2H), 2.13 – 1.77 (m, 12H), 1.78 – 1.63 (m, 4H), 1.63 – 0.96 (m, 33H), 0.86 (t, J = 6.6 Hz, 3H).

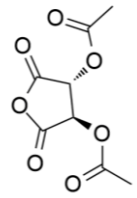
^{13}C NMR (75 MHz, $CDCl_3$): δ 64.2, 63.1, 51.5, 32.2, 31.9, 29.7, 29.6, 29.5, 29.40, 29.2, 26.2, 22.8, 22.7, 14.1.

MS (ESI(+)): m/z = 487.49 [M^+]

MP: 71 – 75 °C

$[\alpha]_D^{21}$= - 5.0° (DMSO)

2,5-Dioxotetrahydrofuran-3,4-diyl diacetate[7]



Sulfuric acid (0.12 ml, 2.25 mmol) was added dropwise to a suspension of L-tartratic acid (4.0 g, 27 mmol) in acetic anhydrid (13.6 g, 133 mmol) and the reaction mixture was stirred at 140 °C for 1 h. The reaction mixture was cooled in an ice bath and the formed crystals were washed with toluene (2 × 3 ml) and Et_2O (10 ml). The white crystalline product was dried under vacuum.

Yield: 4.28 g; 73%

^{1}H NMR (300 MHz, $CDCl_3$): δ 5.68 (s, 2H), 2.23 (s, 6H).

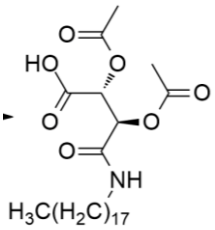
^{13}C NMR (75 MHz, $CDCl_3$): δ 169.8, 163.4, 72.1, 20.1.

MS (APCI EIC(+)): m/z = 217.03 [MH^+]

MP: 130 – 132 °C

$[\alpha]_D^{21}$= 37.9° (DMSO)

L-2,3-Bis(acetyloxy)-3-(octadecylcarbamoyl)propanoic acid[8]



1-Aminooctadecane (624 mg; 2.31 mmol) was added to a solution of 2,5-dioxotetrahydro-furan-3,4-diyl diacetate (500 mg; 2.31 mmol) in THF (20 ml) and the reaction mixture was stirred at room temperature for 16 h; the reaction was monitored by TLC (PE:EA; 1:1). The solvent was removed in *vacuo* to obtain the pure product in form of a white crystalline solid.

Yield: 1.12 g; 99%

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.27 (t, J = 5.7 Hz, 1H), 5.76 (d, *J* = 2.5 Hz, 1H), 5.60 (d, *J* = 2.5 Hz, 1H), 3.33 (m, 1H), 3.19 (m, 1H), 2.17 (s, 3H), 2.11 (s, 3H), 1.48 (tt, *J* = 6.6, 6.0 Hz, 2H), 1.37 – 1.12 (m, 30H), 0.86 (t, *J* = 6.8 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$): δ 169.9, 169.5 , 169.0, 166.0, 72.8, 72.0, 71.2, 67.9, 39.7, 31.9, 29.7, 29.7, 29.6, 29.5, 29.4, 29.4, 29.3, 26.8, 26.6, 25.5, 22.7, 20.6, 20.3, 14.1.

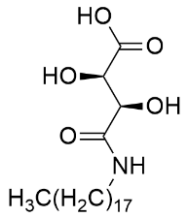
MS (ESI(+)): m/z = 486.34 [MH^+]

MP: 80 – 83 °C

The NMR-data match the literature reference.[8]

$[\alpha]_D^{21}$= - 8.2° (DMSO)

L-2,3-Bis(hydroxy)-3-(octadecylcarbamoyl)propanoic acid (L-Tar)



Diacetate-N-octadecyl-4-amino-L-tartrate (204 mg; 0.420 mmol) and NaOH (52 mg; 1.30 mmol) were dissolved in a mixture of H_2O (10 ml) in MeOH (20 ml) and stirred at room temperature for 4 h. The precipitated crude product was filtered off and washed with HCl_{aq} (1 M; 8 ml). The product, a white crystalline solid, was crystallized from MeOH (10 ml).

Yield: 72 mg; 43%

^{1}H NMR (400 MHz, DMSO): δ 7.63 (t, *J* = 5.9 Hz, 1H), 4.32 (d, *J* = 1.8 Hz, 1H), 4.17 (s, 1H), 3.07 (dd, *J* = 13.9, 6.2 Hz, 2H), 1.40 (m, 2H), 1.24 (m, 30H), 0.86 (t, *J* = 6.8 Hz, 3H).

^{13}C NMR (101 MHz, DMSO): δ 173.8, 170.9, 72.9, 71.5, 38.3, 31.2, 29.1, 28.9, 28.9, 28.7, 28.6, 26.2, 22.0, 13.8.

MS (ESI(+)): m/z = 402.33 [MH^+]

MP: 139 – 146 °C

$[\alpha]_D^{21}$= 30.1° (DMSO)

Preparation and characterization of the vesicles

Micellar Solutions

Appropriate aliquots of a stock solution of **Zn_2Cy** ($CHCl_3$, 1 mM, 150 µl) were added to a solution of the membrane additive (0.5 mM; 600 µl), the mixture of amphiphiles was dried in a thermomixer at 75 °C, residual solvent was removed in *vacuo* for 15 min. HEPES-buffer (25 mM; pH = 7,4; 3 ml) was added and micelles were formed in the ultrasonic bath (70 °C, 20 min). For the blank measurements cyclen **Zn_2Cy** was omitted.

Vesicular Solutions

Out of the stock solutions of membrane additives (0.5 mM, $CHCl_3$) 600 µl was combined with either DOPC ($CHCl_3$, 4.3 mM, 593 µl) or DSPC ($CHCl_3$, 6.36 mM, 401 µl) and a solution of bis-Zn(II)-cyclen **Zn_2Cy** ($CHCl_3$, 1mM, 150 µl) was added, the mixture of amphiphiles

was dried in a thermomixer at 75 °C, the residual solvent was removed in *vacuo* for 15 min. HEPES-buffer (25 mM; pH = 7,4; 3 ml) was added and vesicles were formed in the ultrasonic bath (70 °C, 20 min). Vesicles formed via sonication were approximately 100 nm in size and stable for days (Figure S1).

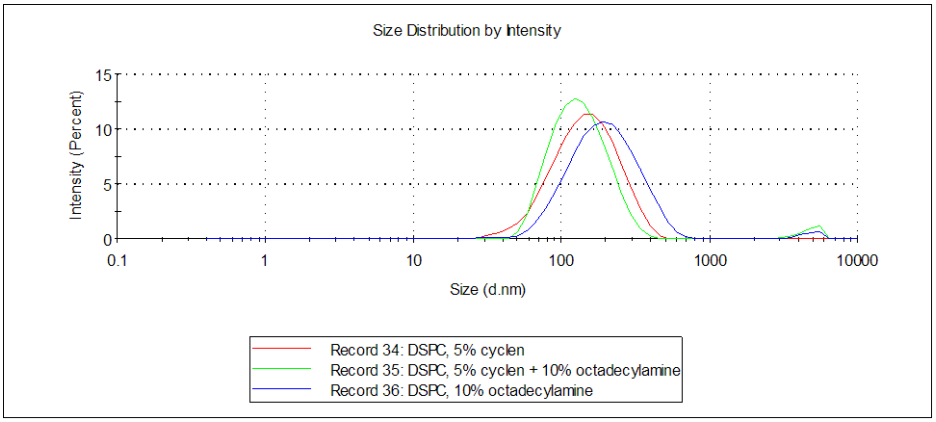


Figure S1: DLS measurement of vesicles with selected amphiphiles.

Kinetic measurements

The kinetics of the hydrolysis was monitored using the two enantiopure substrates (L) and (D). From a freshly prepared stock solution of substrate (1 mM in MeCN) was added 7 µl to a 700 µl of buffered vesicular solution (0.05 mM **Zn_2Cy**, 0.1 mM membrane additive, 0.85 mM phospholipid). The same procedure was used for micellar solutions. The kinetic measurement was started after shaking 5 times (20 sec). The reaction kinetics of the hydrolysis was recorded as the increase of absorbance at the absorption maximum of 4-nitrophenol at pH 7.4 (λ_{max} = 400 nm) in buffered solution (25 mM, HEPES). The extinction coefficient used for 4-nitrophenol at pH 7.4 (ε = 9.8 $mM^{-1}cm^{-1}$) was reported previously.[9] The hydrolysis was carried out under pseudo first order conditions and rate constants were derived from the initial slope plotting changes in absorbance vs reaction time. Measurements were done in triplicates in case of **L-Tar**, **D** and **L-His-COOH**, **L-His-OH** and standard deviations for the slope was calculated to be below 10%. The hydrolysis of the substrates **PN-Phe** and **PN-C2-Phe** was performed at pH 7.4 using tartrate **L-Tar** as membrane additive in DOPC bilayer with cyclen **$ZnCy_2$**.

References

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