Supporting information

Cooperative hydrolysis of aryl esters on functionalised membrane surfaces and micellar solutions

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Content

General methods and materials

Dynamic Light Scattering

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

NMR Spectra

Bruker Avance 300 (1H: 300.1 MHz, 13C: 75.5 MHz, T = 300 K). The chemical shifts are reported in δ [ppm] relative to external standards (solvent residual peak). The spectra were analysed by first order, the coupling constants are given in Hertz [Hz]. Characterization of the signals: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = double doublet, dt = double triplet. Integration is determined as the relative number of atoms. Error of reported values: chemical shift: 0.01 ppm for 1H-NMR, 0.1 ppm for 13C-NMR and 0.1 Hz for coupling constants. The solvent used is reported for each spectrum.

Mass Spectra

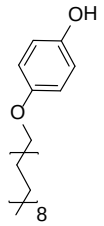
Finnigan MAT TSQ 7000 (ESI).

General

Thin layer chromatography (TLC) analyses were performed on silica gel 60 F-254 with a 0.2 mm layer thickness. Detection via UV light at 254 nm / 326 nm or through staining with $KMnO_4$. Column chromatography was performed on silica gel (70–230 mesh) from Merck, flash chromatography on Biotage Isolera one using silica gel (230–400 mesh) from Merck. Commercially available solvents of standard quality were used. Starting materials were purchased from either Acros or Sigma-Aldrich and used without any further purification. Phospholipids were purchased from Avanti Polar Lipids Inc. Commercially available solvents of standard quality were used. If otherwise stated, purification and drying was done according to accepted general procedures. Enzymes were purchased from Sigma Aldrich.

Synthesis of membrane additives used as membrane additives

Zinc complex **1** was synthesized previously according to the published procedure.[1] Membrane additives **A1**, **A5**, **A6**, **A14**, **9-4**, **A17** were purchased and used as received, compound **A12** was recrystallized from ethanol prior to use. Amphiphiles **A2**[2], **A8**[3], **A7**[4], **A6**[5], **A13**[6], **A9**[7], **A10**[7], **A11**[7], **CF**[8], **TAMRA**[9] were synthesised according to published procedures. Amphiphiles **A3, A16**[10, 11] (Scheme 2) were prepared by modified, published procedures.



4-Octadecyloxyphenol (A3)

Octadecylbromide (1.0 g, 3.0 mmol), hydroquinone (1.651 g, 15 mmol) and potassium carbonate (4.14 g, 30 mmol) were suspended in dry acetonitrile (60 ml), heated to 90 °C under nitrogen atmosphere and stirred for 12 h. After cooling, the solvent was evaporated and the residue suspended in water, which was adjusted with aq. HCl to be acidic in pH. The aqueous mixture was extracted with diethylether (3×60 ml) and the combined organic layers were dried over magnesium sulphate. Solids were filtered off and the solvent was removed *in vacuo*. The residue was purified by flash chromatography (petrolether with a gradient, up to 30%, of ethyl acetate) to obtain 500 mg (46%) of compound **A3** in form of white powder.

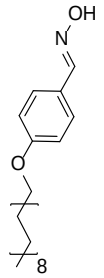
Yield: 46%.

MP: 93 °C (lit 91-92 °C)

^{1}H-NMR (300 MHz; $CDCl_3$): δ 6.89 – 6.57 (m, 4H), 3.89 (t, *J* = 6.6 Hz, 2H), 1.85 – 1.60 (m, 2H), 1.25 (s, 30H), 0.88 (t, *J* = 6.6 Hz, 3H).

^{13}C-NMR (75 MHz; $CDCl_3$): δ 153.35, 149.28, 115.99, 115.58, 68.71, 31.96, 29.73 (7C), 29.69, 29.64, 29.62, 26.45, 29.40 (2C), 26.08, 22.73, 14.17.

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



4-Octadecyloxybenzaldehyde oxime[5] (A6)

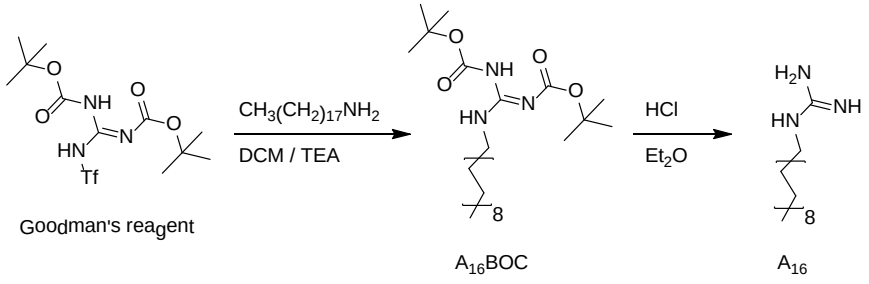
Yield: 76**%.**

MP: 84 °C

^{1}H-NMR (300 MHz; $CDCl_3$): δ 8.08 (s, 1H), 7.50 (d, *J* = 8.8 Hz, 2H), 6.89 (d, *J* = 8.8 Hz, 2H), 3.97 (t, *J* = 6.6 Hz, 2H), 1.86 – 1.71 (m, 2H), 1.51 – 1.16 (m, 30H), 0.88 (t, *J* = 6.8 Hz, 3H).

^{13}C-NMR (75 MHz; $CDCl_3$): δ 160.70, 150.00, 128.48 (2C), 124.35, 114.76 (2C), 68.13, 31.95, 29.73 (7C), 29.69, 29.62, 29.59, 29.39, 29.19, 26.03, 22.72, 14.15.

MS (ESI(+)): m/z 390.34 [MH^+].



Scheme 1: Synthesis of 1-octadecylguanidine (**A16**)

1,3-Bis-(tert-butoxycarbonyl)-2-octadecyl-guanidine (A16-BOC)

Octadecylamine (93 mg, 0.345 mmol) was dissolved in dry DCM (5 ml) and triethylamine (18 mg, 0.172 mmol) was added. The reaction mixture cooled in an ice bath under nitrogen atmosphere. A solution of Goodman's reagent (100 mg, 0.25 mmol) in dry DCM (5 ml) was added drop wise over a period of 5 minutes. The ice bath was removed and the reaction mixture stirred at RT overnight. The solvent was removed under reduced pressure and the residue was purified by column chromatography (ethyl acetate: petroleum ether; 1:10) to obtain 100 mg (56%) of product **A16-BOC** in form of colorless oil.

^{1}H-NMR (300M Hz; $CDCl_3$): δ 3.46 – 3.30 (m, 2H), 1.60 – 1.50 (m, 2H), 1.49 (s, *9H*), 1.50 (s, *9H*), 1.19 – 1.39 (m, 30H), 0.93 – 0.79 (m, 3H).

^{13}C-NMR (75 MHz; $CDCl_3$): δ 156.10, 153.34, 83.01, 41.03, 31.95, 29.85 (9C), 29.60, 29.50, 29.39, 29.29, 28.97, 28.32 (3C), 28.09 (3C), 26.87, 22.72, 14.16.

MS (ESI(+)): m/z 512.44 [MH^+].

1-Octadecylguanidine (A16)

Boc protected guanidine **A16-BOC** (90 mg, 0.16 mmol) was dissolved in dry diethylether (5 ml), a saturated solution of HCl in diethylether (15 ml) was added and reaction mixture was stirred at room temp for 3 days. Solvents were removed *in vacuo* and the residue was purified by crystallisation from THF to obtain 45 mg (73%) of 1-octadecylgudinium (**A16)** in form of white crystals.

MP: 153 °C decomp. (lit[12] 230.5 °C)

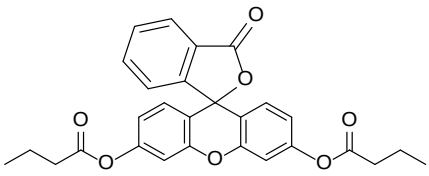
^{1}H-NMR (300 MHz; MeOD): δ 3.16 (t, *J* = 7.1 Hz, 2H), 1.63 – 1.50 (m, 2H), 1.25 – 1.43 (m, *30H*), 0.90 (t, *J* = 6.7 Hz, 3H)

^{13}C-NMR (75 MHz; MeOD): δ 42.52, 33.13, 30.83 (9C), 30.75, 30.71, 30.53, 30.40, 29.95, 27.76, 23.79, 14.50.

MS (ESI(+)):m/z 312.34 [MH^+].

Synthesis of fluorescein esters

Esters **FDA** and **F-C2** were prepared by known procedures from the anhydrides of the parent acids.[13] Esters **F-C3**, **F-C7**, **F-C15**, **F-Ph**, **F-*i*Pr**, **F-*t*Bu** were prepared from chlorides of the acid. Fluorescein (200 mg, 0.602 mmol, 1 eqv) was suspended in dry toluene (15 ml) followed by addition of carboxychloride (1.204, 2 eqv), triethylamine (0.167 ml, 1.204 mmol, 2 eqv) and DMAP (5 mg). The reaction mixture was stirred under nitrogen atmosphere for 24 h. Solids were filtered off and washed with toluene. Solvent was removed under reduced pressure and the residue was purified two times by flash chromatography (petrolether with gradient, up to 30%, of ethyl acetate) or crystallization.

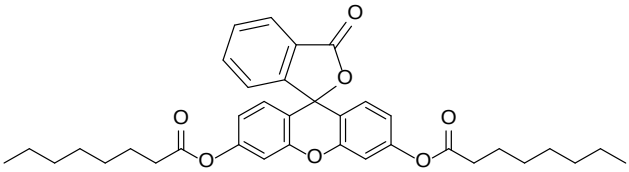


Fluorescein dibutyrate (F-C3)

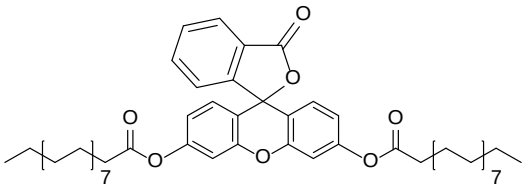
Yield: 65**%.**

MP: 139 °C (lit[13] 123 °C)

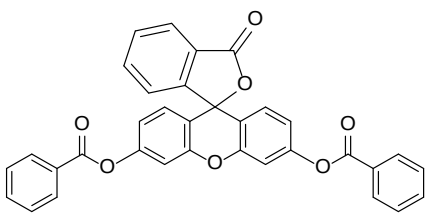
^{1}H-NMR (300 MHz; $CDCl_3$): δ 8.02 (dd, *J* = 6.5, 1.3 Hz, 1H), 7.73 – 7.54 (m, 2H), 7.21 – 7.12 (m, 1H), 7.08 (dd, *J* = 1.8, 0.7 Hz, 2H), 6.89 – 6.72 (m, 4H), 2.54 (t, *J* = 7.4 Hz, 4H), 1.77 (h, *J* = 7.4 Hz, 4H), 1.02 (t, *J* = 7.4 Hz, 6H).
^{13}C-NMR (75 MHz; $CDCl_3$): δ 171.59, 169.18, 152.9, 152.15, 151.58, 135.33, 130.09, 128.96, 126.11, 125.21, 124.09, 117.80, 116.33, 110.40, 81.73, 36.15, 18.36, 13.62.
MS (ESI(+)):m/z 473.16 [MH^+].



Fluorescein dioctanoate (F-C7)
Yield: 77 **%.**
MP: 49.3 °C (lit[13] 49 °C)
^{1}H-NMR (300 MHz; $CDCl_3$): δ 8.03 (dd, *J* = 6.5, 1.3 Hz, 1H), 7.73 – 7.57 (m, 2H), 7.22 – 7.14 (m, 1H), 7.08 (dd, *J* = 1.9, 0.6 Hz, 2H), 6.88 – 6.74 (m, 4H), 2.56 (t, *J* = 7.5 Hz, 4H), 1.83 – 1.68 (m, 4H), 1.51 – 1.22 (m, 16H), 0.71 – 0.91 (m, 6H).
^{13}C-NMR (75 MHz; $CDCl_3$) δ 171.80, 169.22, 152.94, 152.16, 151.58, 135.30, 130.06, 128.96, 126.12, 125.23, 124.10, 117.78, 116.31, 110.39, 81.76, 34.36, 31.65, 29.03, 28.92, 24.85, 22.61, 14.10.
MS (ESI(+)):m/z 585.28 [MH^+].

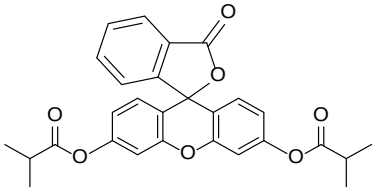


Fluorescein dipalmitoate (F-C15)
Purified by additional crystallisation from toluene. **Yield:** 43**%.**
MP: 78 °C (lit[13] 69 °C)
^{1}H-NMR (300 MHz; $CDCl_3$): δ 8.03 (dd, *J* = 6.5, 1.1 Hz, 1H), 7.74 – 7.55 (m, 2H), 7.18 (d, *J* = 7.0 Hz, 1H), 7.08 (d, *J* = 1.4 Hz, 2H), 6.86 – 6.71 (m, 4H), 2.56 (t, *J* = 7.5 Hz, 4H), 1.84 – 1.66 (m, 4H), 1.50 – 1.13 (m, 48H), 0.88 (t, *J* = 6.7 Hz, 6H).
^{13}C-NMR (75 MHz; $CDCl_3$): δ 171.78, 169.19, 152.96, 152.16, 151.58, 135.28, 130.04, 128.95, 126.13, 125.23, 124.10, 117.78, 116.32, 110.39, 81.74, 34.37, 31.95, 29.75 – 29.66 (bs, 5C), 29.62, 29.48, 29.39, 29.26, 29.09, 24.85, 22.72, 14.16.
MS (ESI(+)):m/z 585.28 [MH^+].

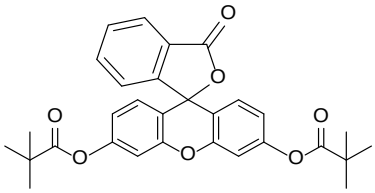


Fluorescein dibenzoate (F-Ph)
Yield: 60**%.**
MP: 217 °C (lit[14] 208-210 °C)

^{1}H-NMR (300 MHz; $CDCl_3$): δ 8.27 – 8.14 (m, 4H), 8.07 (d, *J* = 7.1 Hz, 1H), 7.78 – 7.59 (m, 4H), 7.52 (t, *J* = 7.6 Hz, 4H), 7.31 – 7.16 (m, 3H), 6.95 (dt, *J* = 17.8, 5.4 Hz, 4H).
^{13}C-NMR (75 MHz; $CDCl_3$): δ 169.25, 164.66, 152.99, 152.39, 151.70, 135.40, 134.00, 130.31, 130.14, 129.10, 128.99, 128.72, 126.15, 125.29, 124.17, 117.98, 116.58, 110.66, 81.78.
MS (ESI(+)):m/z 541.13 [MH^+].



Fluorescein diisobutyrate (F-iPr)
Yield: 70**%.**
MP: 155.6 °C
^{1}H-NMR (300 MHz; $CDCl_3$): δ 8.02 (dt, *J* = 9.5, 5.1 Hz, 1H), 7.73 – 7.55 (m, 2H), 7.21 – 7.13 (m, 1H), 7.07 (dd, *J* = 2.0, 0.5 Hz, 2H), 6.87 – 6.74 (m, 4H), 2.80 (hept, *J* = 7.0 Hz, 2H), 1.31 (dd, *J* = 7.0, 0.6 Hz, 12H).
^{13}C-NMR (75 MHz; $CDCl_3$): δ 175.07, 169.23, 152.98, 152.32, 151.59, 135.31, 130.06, 128.94, 126.10, 125.23, 124.08, 117.72, 116.28, 110.33, 81.76, 34.19, 18.87.
MS (ESI(+)): m/z 473.16 [MH^+].



Fluorescein dipivaloate (F-tBu)
Yield: 50**%.**
MP: 99.6 °C
^{1}H-NMR (300 MHz; $CDCl_3$): δ 8.02 (dt, *J* = 9.9, 5.1 Hz, 1H), 7.73 – 7.55 (m, 2H), 7.20 – 7.10 (m, 1H), 7.06 (t, *J* = 4.7 Hz, 2H), 6.79 (dt, *J* = 8.7, 5.4 Hz, 4H), 1.35 (s, 18H).
^{13}C-NMR (75 MHz; $CDCl_3$): δ 176.55, 169.26, 153.05, 152.57, 151.58, 135.32, 130.06, 128.91, 126.05, 125.22, 124.04, 117.72, 116.24, 110.32, 81.76, 39.20, 27.08.
MS (ESI(+)): m/z 501.19 [MH^+].

Preparation and characterization of the membrane

Solutions of vesicles were prepared by a well-established procedure.[15] Membranes were prepared by sonication or treatment in a thermomixer of emulsions of amphiphiles in 25 mM HEPES (pH = 7.4) buffer for 10-20 min at 70 °C and used with or without an extrusion step. Micellar solutions were prepared by the same procedure with the exception of no addition of lipids. For each system the catalytic activity was determined and DLS analysis provided the size distribution of the vesicles.

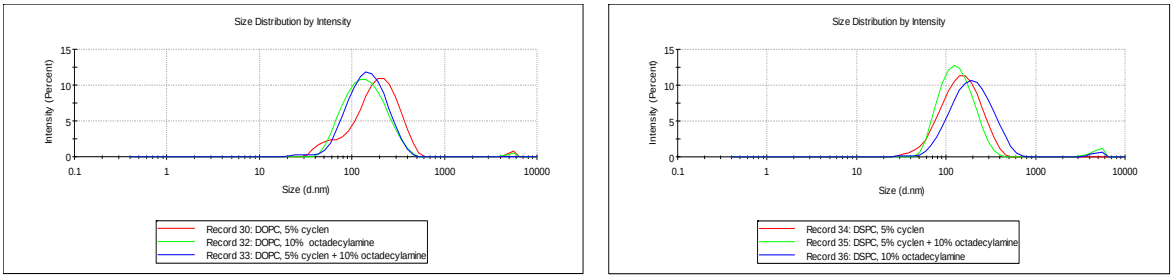


Figure S1: Examples of DLS measurement of mixtures made from lipids DSPC, DOPC (0.475 mM) with 5 % of cyclen **1** (0.025 mM), 5 % of cyclen **1** (0.025 mM) + 10 % octadecylamine **A14** (0.05 mM) and 10 % octadecylamine **A14** (0.05 mM)

Measurements showed that there is only a small difference between the activities of membranes prepared by different procedures. DLS measurements (Figure S1) show that differently produced membranes are mostly in the shape of approximately 150 nm vesicles and do not change with composition. All measurements in this publication refer to vesicles prepared by sonication at a temperature higher than the transition temperature of the lipid used without extrusion step.

Determination of cmc value for cyclen 1

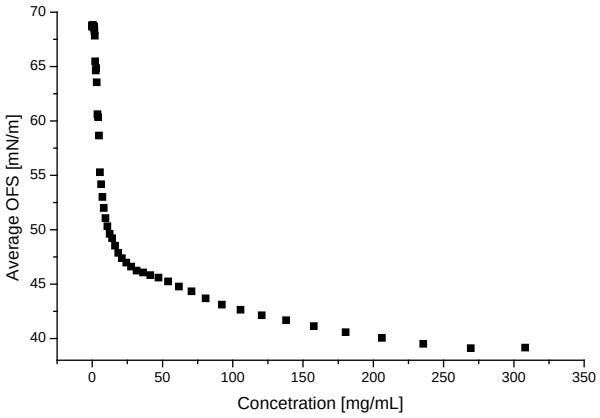


Figure S2: Isotherm of surface tension of the aqueous solution of Zn complex **1**

Calibration curves for fluorescein concentration

The recorded fluorescence corresponds to the fluorescein produced by hydrolysis of FDA. To determine the fluorescein concentration a calibration curve was recorded. In vesicular solutions containing cyclen **1** the intensity of fluorescein is slightly quenched. Linear regression was applied to determine the slope $k_{buffer} = 1.02 \times 10^{10}$ f.u./M and $k_{vesicles} = 8.67 \times 10^{9}$ f.u./M, which was used for calculations of pseudo first order kinetic constants. These rates were derived from the linear region of the calibration curve (up to 50 000 fluorescence unit). Membrane additives and lipids did not change the emission signal in the concentration range of the calibration curve so $k_{vesicles}$ was used in all cases.

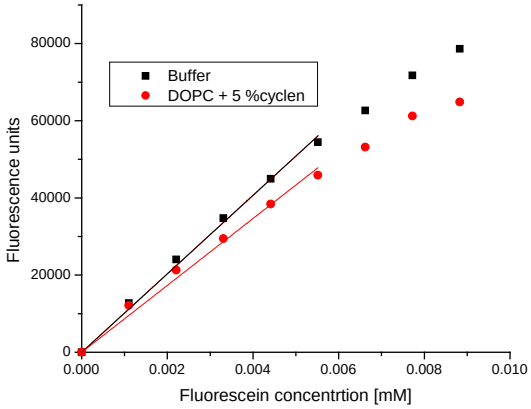


Figure S3: Fluorescein calibration curves

Estimated pKa values of membrane additives

Compounds with short alkyl chains were chosen for the approximate determination of p*K*a values of membrane additive used for their sorting in scheme 1.

Membrane additives	pKa	model compound (lit. ref.)
A1	4.76	acetic acid[16]
A2	8.05	4-acylphenol[16]
A3	10.20	4-methoxyphenol[16]
A4	11.16	4-methoxybenzaldoxim[17]
A5	12.00	methanthiol[16]
A6	15.54	methanol[16]
	pKa of conj. Acid	
A7	0.18	urea[16]
A8	5.14	pyridine[16]
A9	6.95	imidazol[16]
A10	6.95	imidazol[16]
A11	6.95	imidazol[16]
A12	9.76	trimethylamine[16]
A13	9.82	piperazine[16]
A14	10.62	methylamine[16]
A15	10.64	dimethylamine[16]
A16	13.71	guanidin[16]

Table S1: Table of pKa values used as a estimated values for amphiphilic compound

Kinetic measurements

All the fluorescence measurements were performed in the FLUOstar Omega micro-titre plate reader using excitation filter at 485 nm and emission 520 nm. For every measurement 200 scans were performed over 16 hours. The fluorescein concentration was determined from calibration curves. The observed kinetic hydrolysis constant was calculated from the initial slope of the recorded increase of fluorescence. Each well of the micro-titre plate contained 200 µl of the solution in 25 mM HEPES (pH = 7.4) buffer with a total concentration of the amphiphiles of 0.5 mM (different lipids and amounts of membrane additives and cyclen) and 0.02 mM of the substrate (FDA). Stock solutions of the ester were made in DMSO: buffer (32: 1) resulting in the presence of 1.5 vol. % of DMSO in all measured solution. The same procedure was used for all fluorescein esters.
For micellar hydrolysis the same solutions and concentrations were used as for vesicles, but the increase of fluorescence concentration was measured with a Cary 50 UV-Vis spectrophotometer. Measurements were performed in 10 mm cuvette and the change of absorption was monitored at λ_{max} = 505 nm for 3 minutes. For evaluation of k_{obs} the published extinction coefficient for fluorescein of 76 900 $M^{-1}cm^{-1}$ was used.[18] All the measurements were repeated and did not exceed 10 % error.

Optimization of bis-Zn-cyclen 1 concentration in the membrane

The amount of embedded zinc-cyclen **1** in DSPC vesicles membranes was varied and the effect on hydrolysis activity determined (Figure S4).

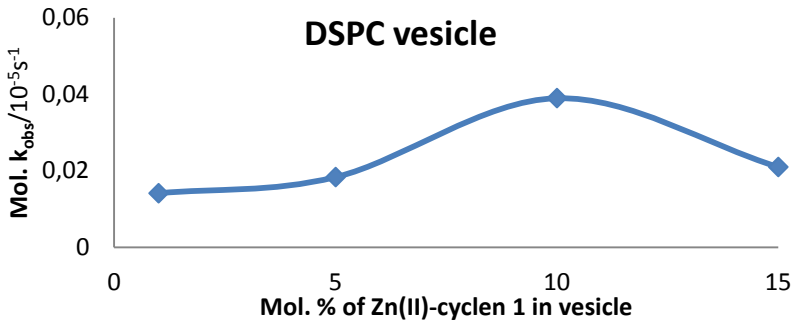


Figure S4: Hydrolytic activity on different cyclen **1** loading (0–) in DSPC vesicles

The rate of the hydrolysis reaction increases with the amount of cyclen **1** embedded in the membrane, but decreases again above 10 % cyclen **1** with respect to the lipid. The hydrolysis rate as a function of the amount of complex **1**, is highest with 5 mol. % of cyclen in the DSPC membrane. This amount of embedded complex **1** was used for all investigations.

Optimization of membrane additives concentration

The optimal ratio of membrane additive and cyclen for hydrolysis was determined for the DOPC membrane with embedded 5 mol% of bis-Zn-cyclen **1**. Addition of more than 10 mol% of the supplement additive does not change the rate of hydrolysis any more (Figure S5). Therefore a ratio of 10 mol% of membrane additive and 5 mol% of cyclen with respect to the lipid was used for all kinetic measurements.

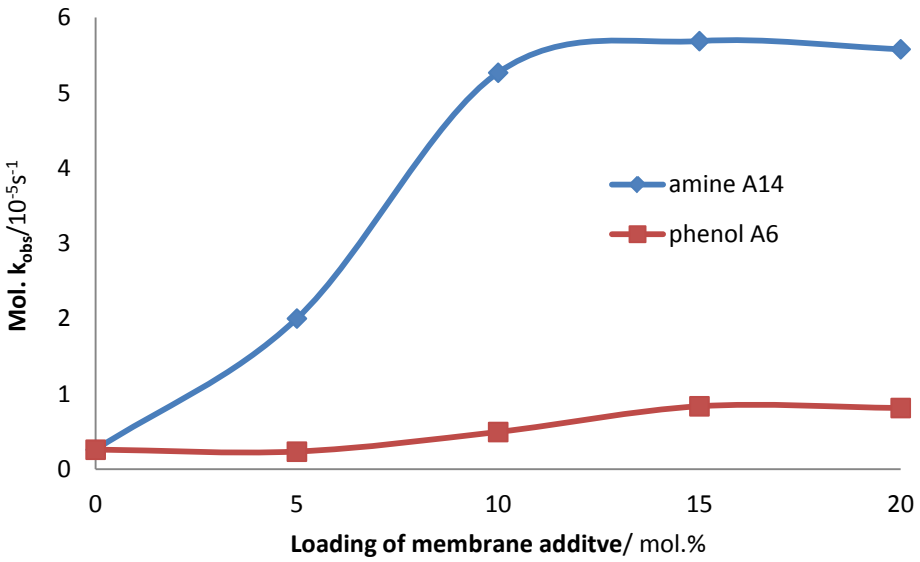


Figure S5: Dependence of the hydrolytic activity of 5 mol% bis-Zn-cyclen **1** (0.025 mM) in DOPC membranes with increasing amount of membrane additive **A14** and **A2** (0–0.1 mM).

Effect of different lipids

Different lipids with different transition temperature were examined for their hydrolytic properties with embedded bis-Zn-cyclen **1** (Figure S6, Table S2) and membrane additives. DOPC and DSPC lipids were used for the investigation of the membrane additives as they have a large difference in their transition temperature.

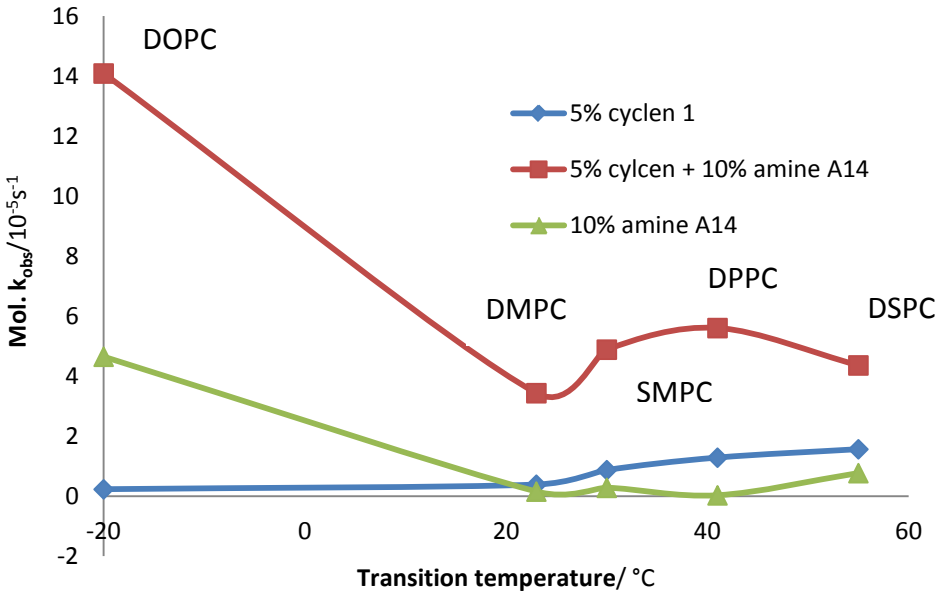


Figure S6: Hydrolytic rates of bis-Zn-cyclen **1** (0.025 mM) and/or amine **A14** (0.05 mM) containing vesicles made from various lipids compared with transition temperature of their membrane

		5 % cyclen **1**	*5 % cyclen* **1** *+ 10 % amine* **A14**	**10 % amine A14**
Lipid	T_m °C	k_{obs} $s^{-1}\ 10^{-5}$	k_{obs} $s^{-1}\ 10^{-5}$	k_{obs}^{0} $s^{-1}\ 10^{-5}$
DOPC	**-20**	0.23	14.09	4.65
DMPC	**23**	0.39	3.44	0.16
SMPC	**30**	0.87	4.89	0.28
DPPC	**41**	1.29	5.61	0.03
DSPC	**55**	1.57	4.36	0.77

Table S2: Hydrolytic rates of bis-Zn-cyclen **1** containing vesicles, T_m – transition temperature

Various substrates

Selected functionalized vesicles were tested for their hydrolytic activity for different fluorescein esters.

		FDA	**F-C2**	**F-Ph**	**F-*i*Pr**	**F-C3**	**F-*t*Bu**	**F-C7**	**F-C15**
	Lipophilicity[a]	3.20	4.22	4.41	4.93	5.24	5.75	9.31	17.47
DOPC	5 % **Cyclen** + 10 % **A11**	10.69	9.07	0.04	4.69	5.54	0.15	0.27	0.48
	5 % **Cyclen** + 10 % **10-2**	17.44	12.55	0.11	2.86	4.29	0.07	0.36	0.55
	5 % **Cyclen**	0.27	0.17	0.00	0.07	0.07	0.02	0.01	0.05
DSPC	5 % **Cyclen** + 10 % **A11**	6.17	5.25	0.10	3.02	2.99	0.28	0.42	0.96
	5 % **Cyclen** + 10 % **10-2**	3.87	3.40	0.17	1.44	2.03	0.64	0.26	0.37
	5 % **Cyclen**	1.57	0.94	0.04	0.48	0.50	0.24	0.10	0.10

Table S3: Hydrolytic rates ($10^{-5}s^{-1}$) of different fluorescein esters using selected membrane systems, [a] Lipophilicity was calculated using software ACDlabs

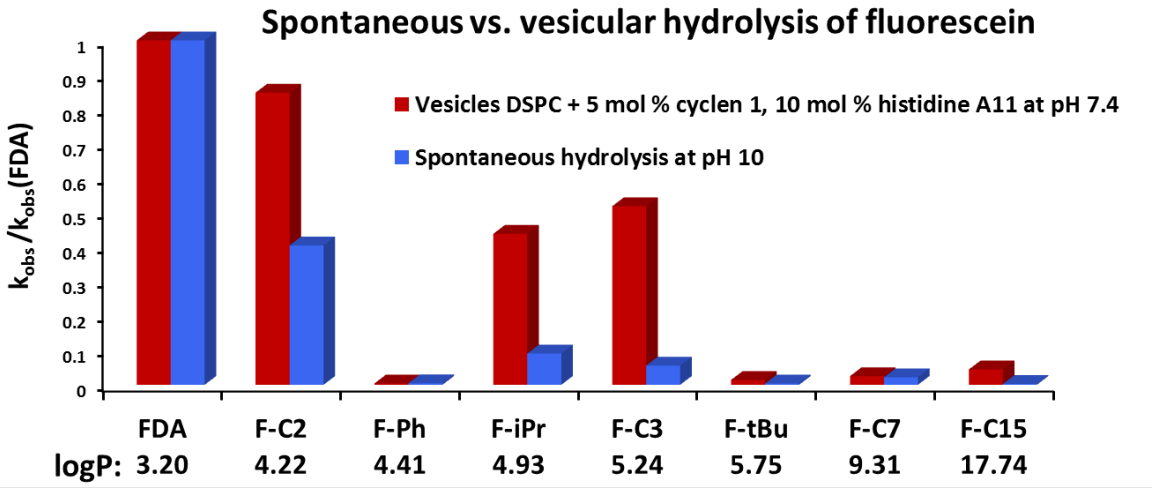


Figure S7: Comparison of the ratios of k_{obs} for different fluorescein esters (0.02 mM) hydrolyzed spontaneously and by vesicles at.

Processing of the kinetic data

Vesicular hydrolysis

For every measurement 200 scans were performed over 16 hours at 25°C. Using pseudo first order conditions the rate constant of hydrolysis (k_{obs}) was derived from the slope of the increasing fluorescein concentration over 20 min (s^{-1}) and the initial concentration of FDA (Figure S8).

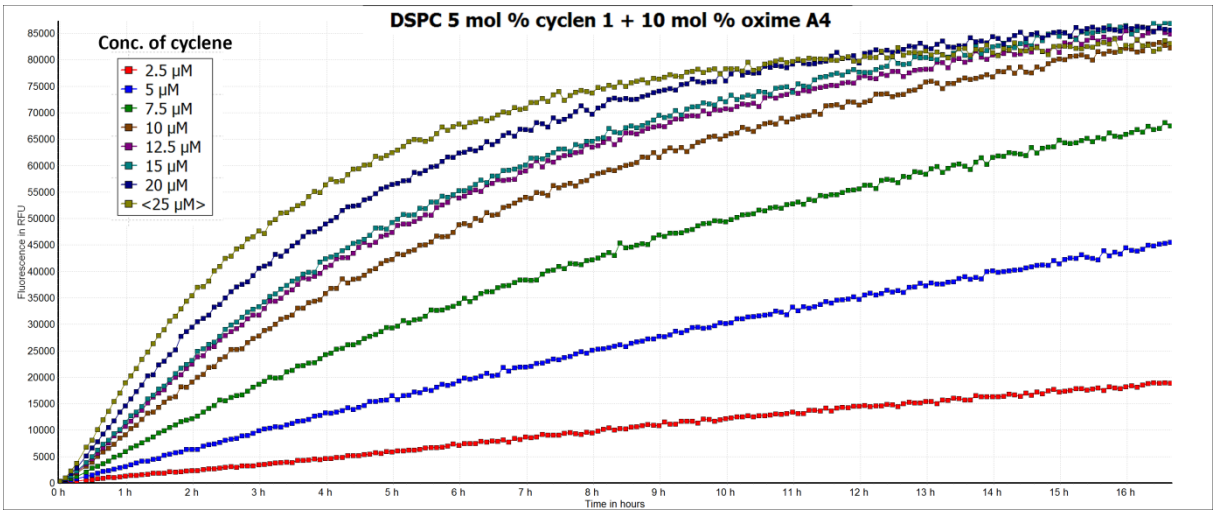
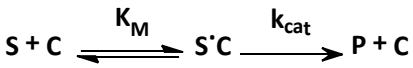


Figure S8: Example of kinetic data used for determination of k_{obs} for DOPC (0.425 mM), 5 mol % cyclen **1** (0.025 mM), 10 mol % oxime **A4** (0.05 mM)



Scheme S1: Model for saturation kinetics

The simple model for saturation kinetics can be described by equations (1) and (2):

$$\frac{d[P]}{dt} = k_{obs}\{[S]_0 + [C \cdot S]\} = k_{cat}[C \cdot S] \quad (1)$$

$$K_M = [C \cdot S]/[C]_0[S]_0 \quad (2)$$

Equation (3) is derived from equations (1) and (2), and was used for non-linear fitting. As concentration of catalyst, the overall cyclen complex **1** concentration in solution was used.

$$k_{obs} = \frac{k_{cat}c_{cyclen}}{K_M + c_{cyclen}} \quad (3)$$

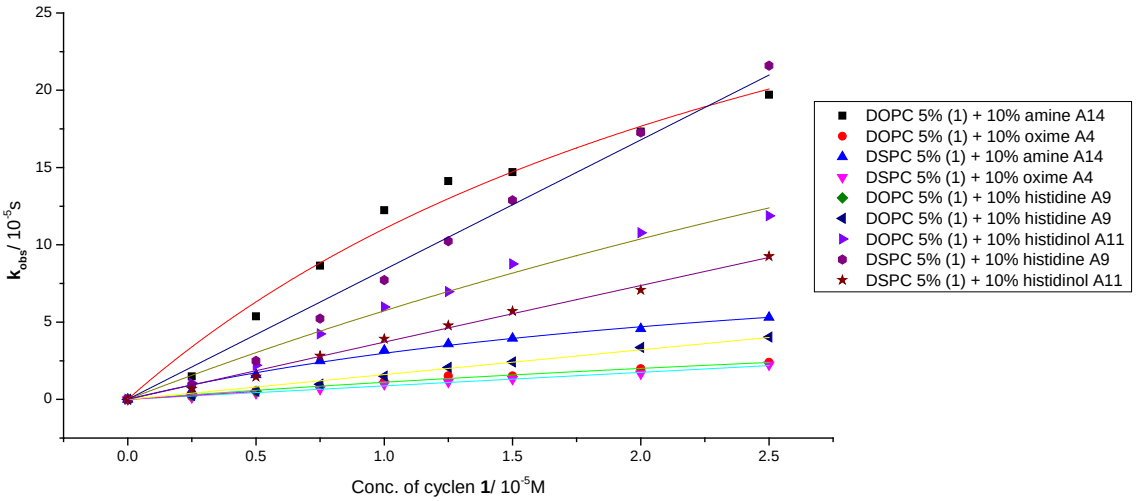


Figure S9: Plot of data and fitted curves of for determining the second rate constants for micellar solutions

The exact molecular reaction mechanism is different from the kinetic model, because two ester groups of FDA need to be cleaved and the substrate·- catalyst complex can consist of several cyclen complexes **1** and membrane additives **A**.

Micellar hydrolysis

Measurements were performed at 25°C under pseudo first order conditions. The hydrolysis rate constants (k_{obs}) were derived from the slope of increasing fluorescein concentration over 3 min (s^{-1}) and the initial concentration of FDA (Figure S10).

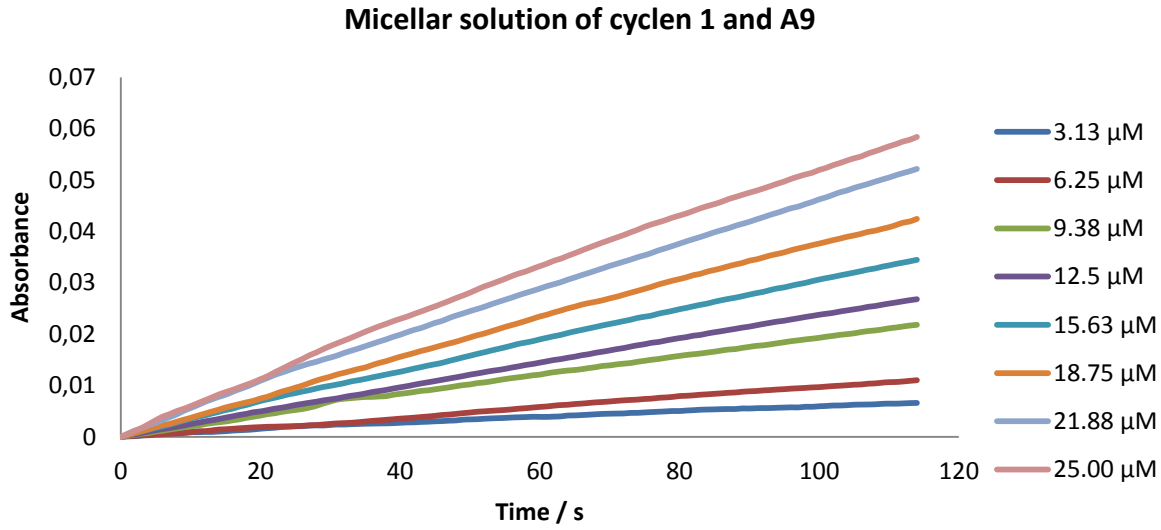


Figure S10: Kinetic data used for determination of k_{obs} in micellar solution of cyclen **1** and histidine **A9**

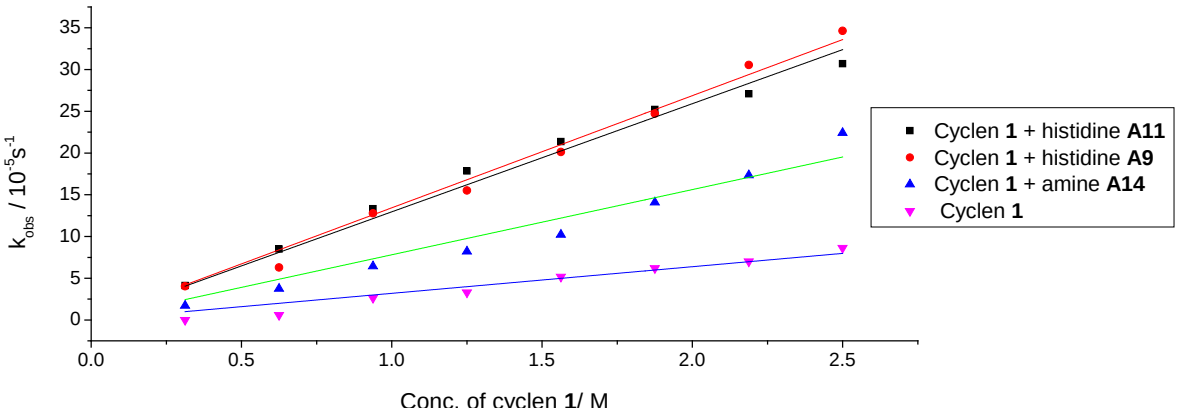


Figure S11: Fitted data of determining the second order rate constants for micellar solutions

Comparison with enzymes

All the enzymes were purchased from Sigma Aldrich and were used fresh as delivered. Stock solutions of enzymes were made in 25 mM HEPES buffer in concentration 0.5 mg/ml and were used in the same manners as vesicular solutions. Kinetic measurements for obtaining k_{obs} were performed similarly as for vesicular solutions (Figure S12). Same conditions and models were applied for processing the data; weight concentrations instead of molarities were used. For comparison with the enzymatic K_M values the data for vesicle solutions were recalculated from molar activity to mass activity considering the composition 85 mol % DOPC, 5 mol % cyclen **1,** 10 mol % **A14** by the equation (4).

$$K_M\left(\left(\frac{mg}{ml}\right)^{-1} s^{-1}\right) = K_M(M^{-1}s^{-1}) \cdot (0.85 \cdot M_{DOPC} + 0.05 \cdot M_{cyclen\,1} + 0.10 \cdot M_{A14}) \qquad (4)$$

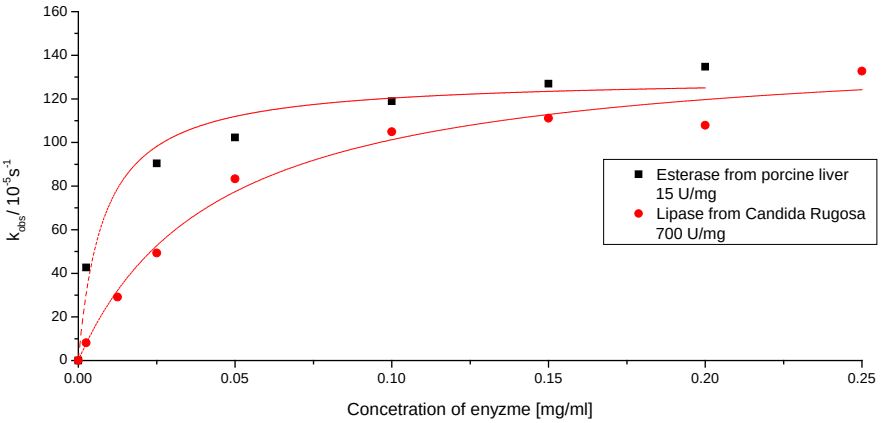
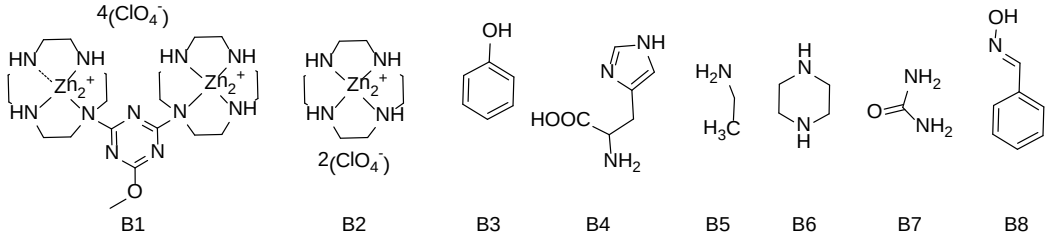


Figure S12: Kinetic data for enzymes

Control experiments

All functional groups of the amphiphilic compounds were tested for their hydrolytic properties in homogeneous solution. For this purpose the corresponding molecules lacking the alkyl chain (Scheme S2) were tested under the same conditions as the amphiphilic samples (Table S4).



Scheme S2: Water soluble non-amphiphilic derivatives of Zn-cyclen complexes and membrane additives

None of these compounds induced noticeable hydrolytic activity in comparison to the spontaneous hydrolysis of the FDA in buffer (Table S4).

Compound	k_{obs} s^{-1} 10^{-5}
B1	0.003
B2	0.006
B3	0.0035
B4	0.004
B5	0.003
B6	0.004
B7	0.003
B8	0.005
5 mol % cyclen*	0.24
DOPC only	0.005
Buffer only	0.003

Table S4: Initial rates of samples prepared using same method, but non amphiphilic molecules of membrane additives and cyclen **1**. For comparison the hydrolysis rates for bis- Zn-cyclen **1** in DOPC membrane (*) and spontaneous hydrolysis in buffer under identical conditions are given.

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