Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

Efficient liposome fusion mediated by lipid-nucleic acid conjugates

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Table of contents

Sequences and analysis of lipid-DNA conjugates

Table S1. Synthesis of oligonucleotides was performed as reported earlier.[1]

#	Abbr.	Sequence (5'-)	R_t [min]	mass (calc.)	mass (found)
conjugates without modifications					
01	TX	T**X** TGT GGA AGA AGT TGG TG	10.1[a)]	6265.65	6267.34
02		CAC CAA CTT CTT CCA CA **X**T	15.2[c)]	6264.90	6267.56
03	TY	T**Y** TGT GGA AGA AGT TGG TG	13.5[c)]	6581.12	6583.11
04		CAC CAA CTT CTT CCA CA **Y**T	13.4[c)]	6269.97	6271.27
05	TZ	T**Z** TGT GGA AGA AGT TGG TG	16.2[c)]	6373.83	6373.98
06		CAC CAA CTT CTT CCA CA **Z**T	16.3[c)]	6062.68	6062.31
07	TWW	T**WW** TGT GGA AGA AGT TGG TG	10.0[a)]	6611.99	6615.07
08		CAC CAA CTT CTT CCA CA **WW**T	10.3[a)]	6300.84	6303.32
09	X	**X** TGT GGA AGA AGT TGG TG	15.5[d)]	5960.72	5962.33
10		CAC CAA CTT CTT CCA CA **X**	15.5[d)]	5649.57	5648.97
11	Z	**Z** TGT GGA AGA AGT TGG TG	11.9[a)]	6069.65	6074.84
12		CAC CAA CTT CTT CCA CA **Z**	11.9[a)]	5758.50	5767.29
LNA-modified conjugates					
13	TX(1,3)	T**X** ^{L}**T**G^{L}**T** GGA AGA AGT TGG TG	14.8[e)]	6320.92	6323.24
14	TX(6,9)	CAC CAA CT^{L}**T** CT^{L}**T** CCA CA **X**T	12.2[f)]	6009.77	6012.21
15	TZ(1,3)	T**Z** ^{L}**T**G^{L}**T** GGA AGA AGT TGG TG	10.9[a)]	6429.85	6438.14
16	TZ(6,9)	CAC CAA CT^{L}**T** CT^{L}**T** CCA CA **Z**T	11.1[a)]	6118.70	6128.56
PEG-modified conjugates					
17	TXP3	T**X PEG$_3$** TGT GGA AGA AGT TGG TG	8.7[b)]	6476.94	6478.72
18		CAC CAA CTT CTT CCA CA **PEG$_3$ X**T	8.8[b)]	6165.79	6171.00
19	TXP6	T**X PEG$_6$** TGT GGA AGA AGT TGG TG	8.8[b)]	6609.02	6612.88
20		CAC CAA CTT CTT CCA CA **PEG$_6$ X**T	8.8[b)]	6297.87	6302.11
21	XP3	**X PEG$_3$** TGT GGA AGA AGT TGG TG	11.0[a)]	6172.76	6173.10
22		CAC CAA CTT CTT CCA CA **PEG$_3$ X**	15.5[d)]	5861.61	5862.07
23	P3XP3	**PEG$_3$ X PEG$_3$** TGT GGA AGA AGT TGG TG	14.4[d)]	6384.80	6383.50
24		CAC CAA CTT CTT CCA CA **PEG$_3$ X PEG$_3$**	14.6[d)]	6073.65	6075.01
25	TZP3	T**Z PEG$_3$** TGT GGA AGA AGT TGG TG	11.3[a)]	6585.87	6591.83
26		CAC CAA CTT CTT CCA CA **PEG$_3$ Z**T	11.7[a)]	6274.72	6280.44

27	ZP3	**Z PEG$_3$** TGT GGA AGA AGT TGG TG	11.9[a)]	6281.69	6288.72
28		CAC CAA CTT CTT CCA CA **PEG$_3$ Z**	11.8[a)]	5970.54	5977.60
29	P3ZP3	**PEG$_3$ Z PEG$_3$** TGT GGA AGA AGT TGG TG	11.3[a)]	6493.73	6503.36
30		CAC CAA CTT CTT CCA CA **PEG$_3$ Z PEG$_3$**	11.3[a)]	6182.58	6188.31
31	TWWP3	T**WW PEG$_3$** TGT GGA AGA AGT TGG TG	10.4 [a)]	6868.05	6872.67
32		CAC CAA CTT CTT CCA CA **PEG$_3$ WW**T	10.4[a)]	6556.90	6561.75
Reference oligonucleotides					
33	non-modified	TGT GGA AGA AGT TGG TG	n.a.[g]	5345.5	verified by the supplier [g]
34		CAC CAA CTT CTT CCA CA	n.a.[g]	5034.3	
Reference zipper					
35	Chol-TEG	CholPEG$_3$ TCC GTC GTG CCT TAT TTC TGA TGT CCA	10.1[b,h]	8865.2	8869.2
36		TGG ACA TCA GAA ATA AGG CAC GAC GGA PEG$_4$Chol	10.2[b,h]	9130.5	9135.8
37		CholPEG$_3$ TCC GTC GTG CCT	10.8[b,h]	4269.2	4273.6
38		AGG CAC GAC GGA PEG$_4$Chol	10.9[b,h]	4459.5	4463.4

HPLC/UPLC method (flow, time [min], solvent gradient 0.05M TEAA, pH7.0 / ACN/H_2O 3:1); **a) Method A (UPLC)**: 1 mL/min; 0→1, 90:10; →10, →0:100; →13, 0:100; **b) Method B (UPLC)**: 1 mL/min; 0→1, 90:10; →7.5, 0:100; →15, 0:100; **c) Method C (HPLC)**: 1 mL/min; 0→4, 90:10; →8, →50:50; →16, →0:100, →19, 0:100; **d) Method D (HPLC)**: 1 mL/min; 0→2.5, 90:10; →6.5, 50→50; →15, →0:100; →18, →0:100; **e) Method E (HPLC)**: 1 mL/min; 0→4, 90:10; →8, →50:50; →16, →0:100; →19, 0:100; **f) Method F (HPLC)**: 0.8 mL/min; 0→2, 100:0; →5, →30:70; →15, →0:100; →30, →0:100. **g)** Cartridge purified and mass verified by supplier (Sigma-Aldrich). **h)** RP HPLC purified from the supplier (Sigma-Aldrich), purity and mass verified in our laboratory.

Nanoparticle Tracking Analysis (NTA)

The size distributions of freshly extruded liposomes (DOPC, DOPE, Cholesterol (Chol); 2:1:1, molar ratio) and samples from fusion experiments were analyzed using a NanoSight (Wiltshire, UK) instrument, equipped with the NanoSight LM14 flow-cell and laser assembly (405 nm diode laser), a 20× objective and CCD camera. Data was recorded and analyzed using the NTA v. 2.3 software (Recording: 5 × 30 s with Shutter 387 (25 fps), Gain 200-350, Histogram: 520 lower threshold); Analysis: Detection threshold 10, Blur 'auto', Min. Track Length 10, Min. expected size 'auto'). Liposome preparations were measured at 2 or 5 µM total lipid concentration ([lip] = [DOPC]+[DOPE]+[Chol]).

Liposome size distributions measured by Nanoparticle Tracking Analysis

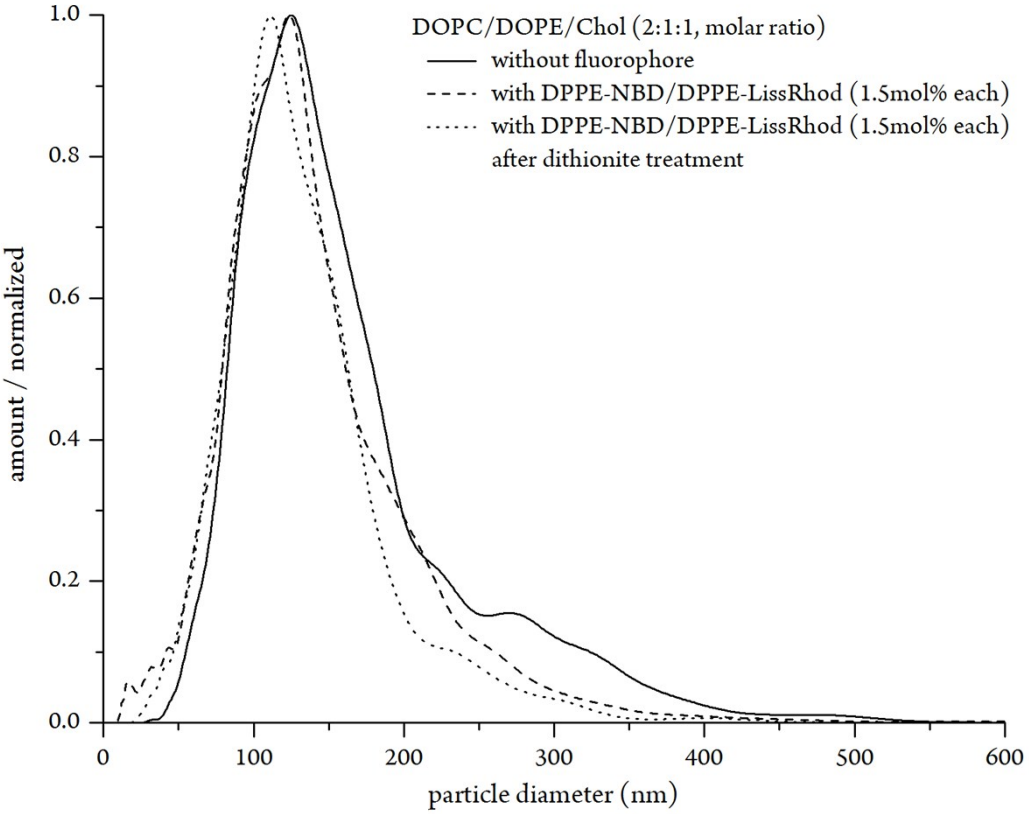


Fig. S1. Liposome mean diameter was determined to be 131 nm by NanoSight®.

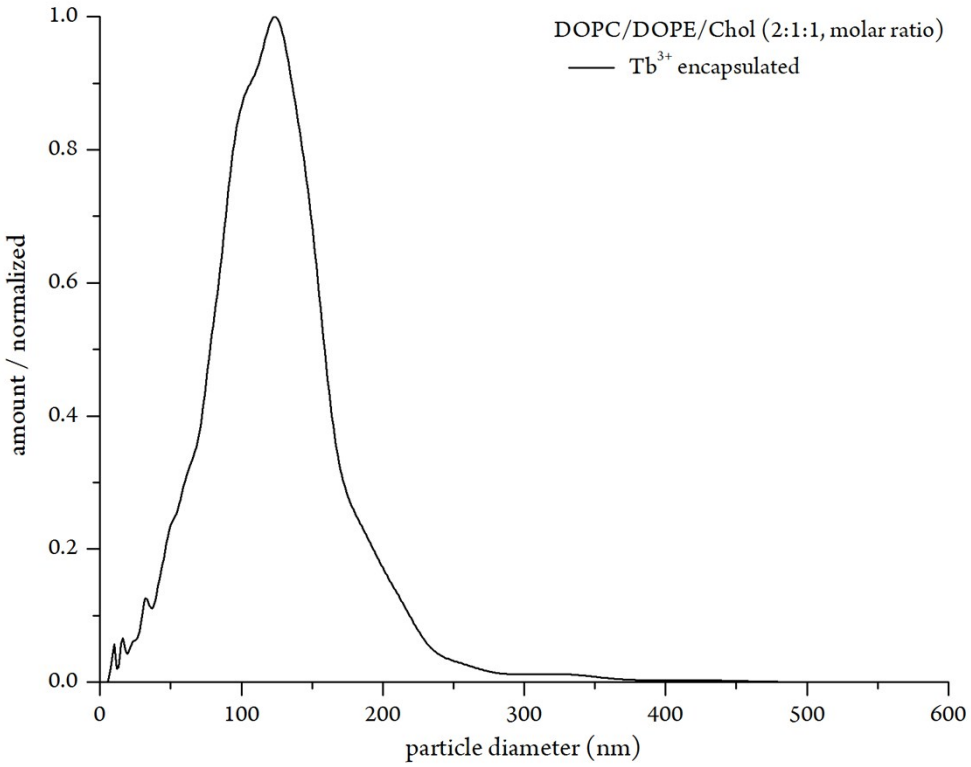


Fig. S2. Liposome (with encapsulated Tb^{3+}) mean diameter was determined to be 148 nm by NanoSight®.

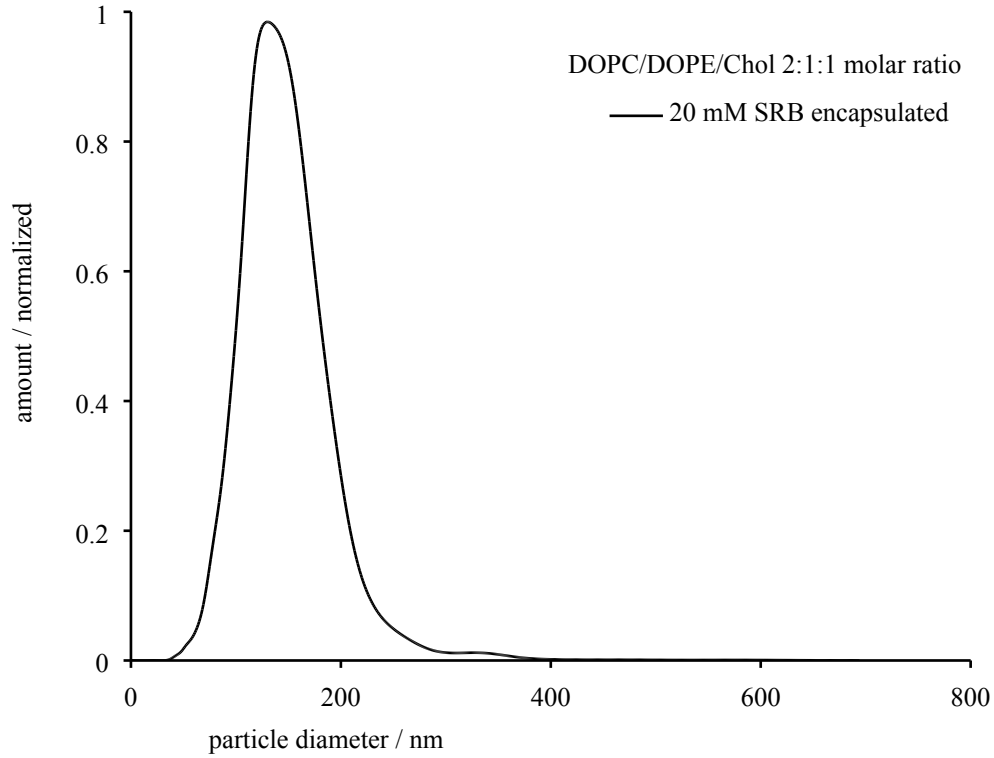


Fig. S3. Liposome (encapsulated with SRB) mean diameter was determined to be 135 nm by NanoSight®.

Additional Fusion and Leakage Data

Lipid Mixing (FRET) and Leakage (terbium citrate) — Example fluorescence time series for total lipid mixing, fusion and terbium leakage at 22 °C (P3ZP3)

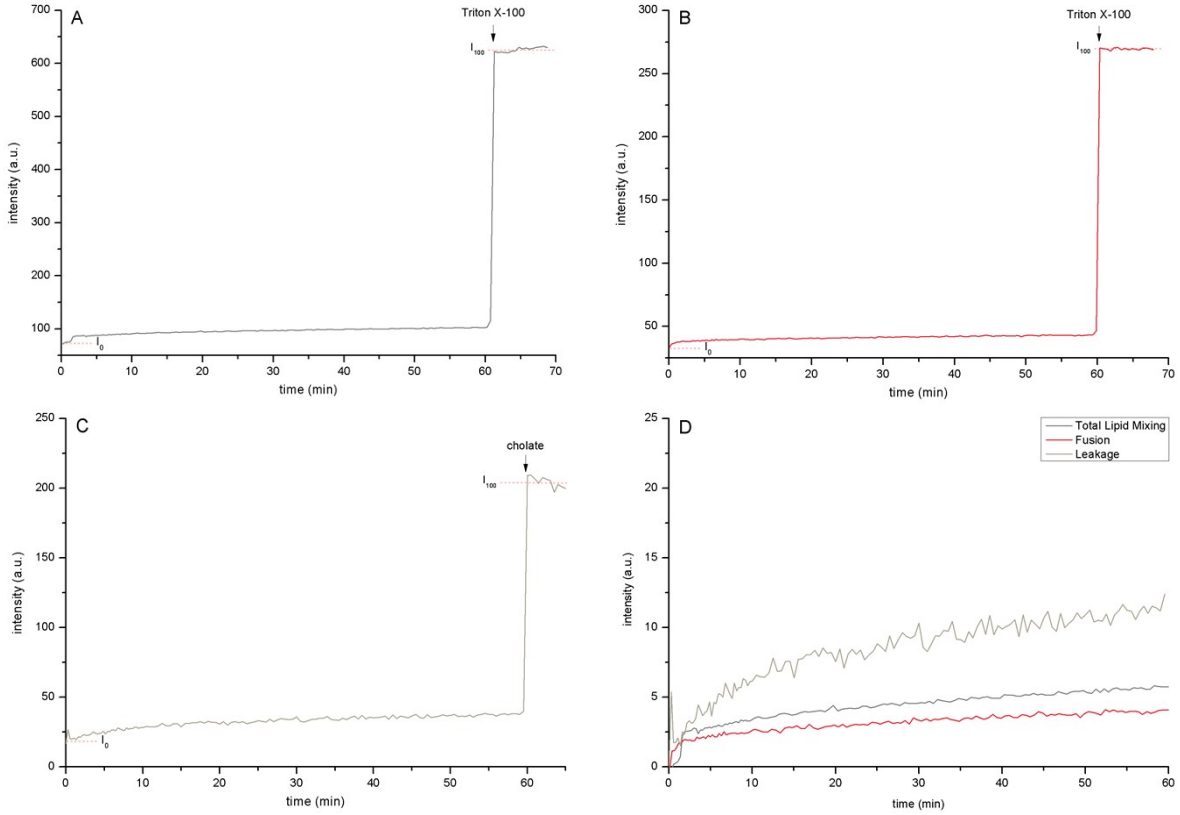


Fig. S4. Fluorescence time scans for the P3**Z**P3 system at 22 °C. A: Total lipid mixing, B: fusion, C: leakage, D: total lipid mixing, fusion and leakage normalized with the obtained value for maximum fluorescence.

Lipid mixing vs. terbium leakage at different temperatures

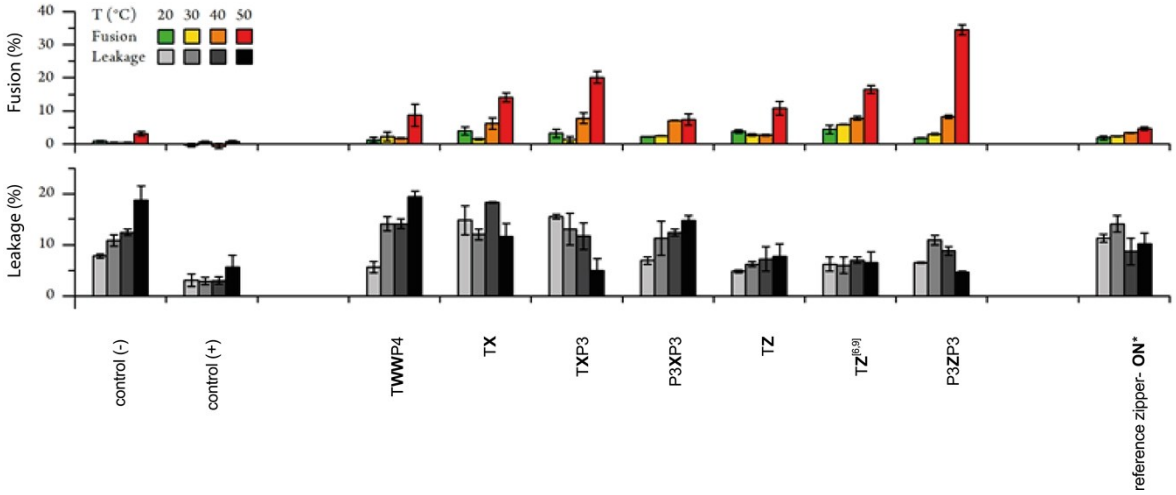


Fig. S5. Temperature dependency of the fusion and leakage process mediated by LiNAs with DOPC/DOPE/CH (2:1:1, molar ratio) liposomes, control experiments without (-) and with (+) non-complementary LiNA as well as a zipper system known from literature reference zipper ON*). Standard LiNA-concentration (ca. 140 and 70 LiNA/liposome, respectively), T_M (unmod. DNA, ON-33/ON-34) = 57.7 °C; values after 30 min.

Additional data for SRB assay – content mixing and leakage

The assay is based on the self-quenching properties of 20 mM SRB entrapped in vesicles and has previously been used to measure content mixing.[2,3] The fluorescence of an SRB-filled vesicle suspension is inversely proportional to the concentration of entrapped SRB. Upon content mixing with unlabeled liposomes - or leakage into the outer medium - the dye is diluted, which leads to an increase in fluorescence. The measured fluorescence increase is thus based on both content mixing and leakage and has to be compared to an experiment where the entrapped [SRB] is the same in all liposomes and any fluorescence increase must stem from leakage. Fig. S6 gives representative the raw data for both content mixing, leakage and control with non-complementary LiNAs

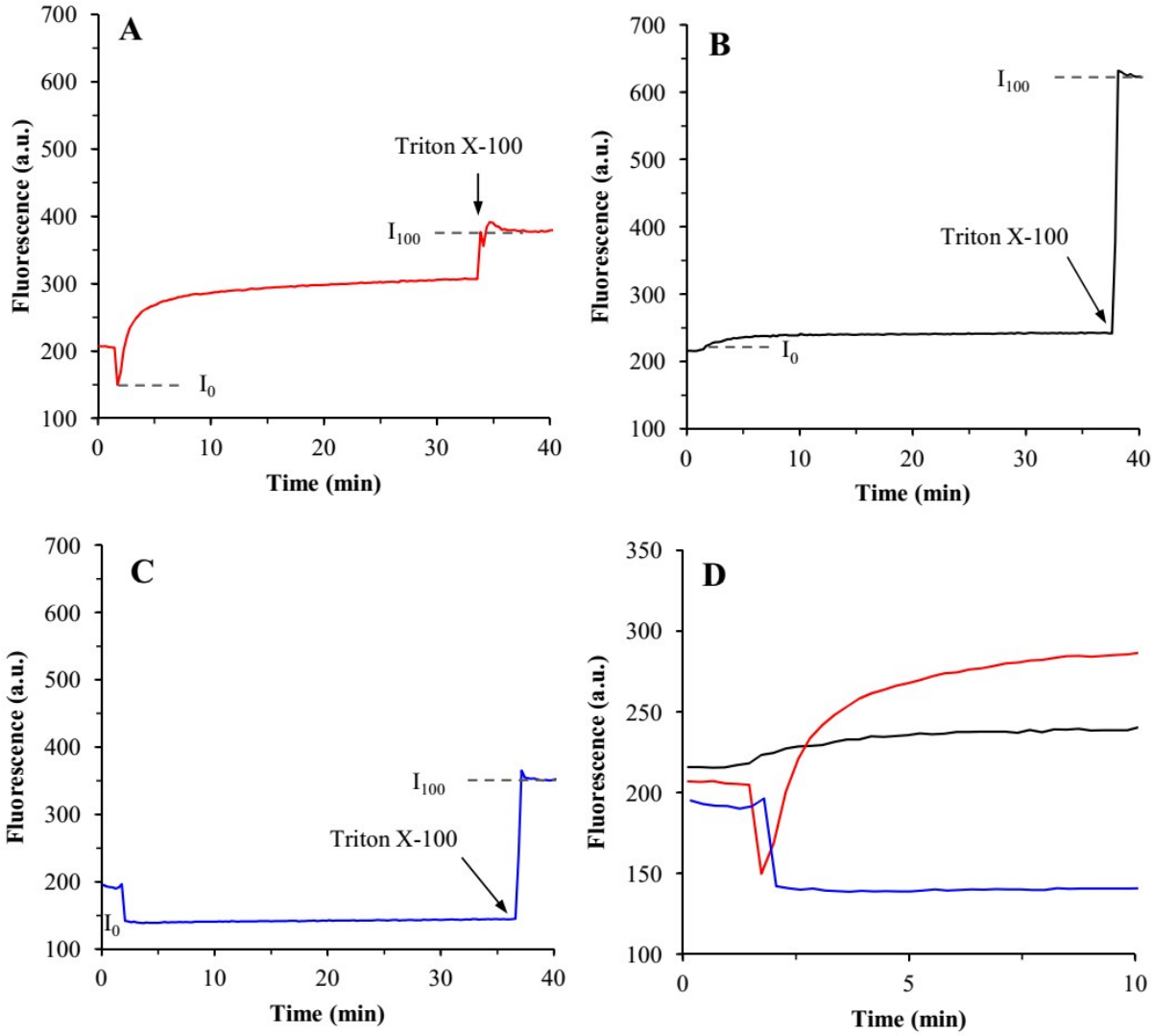


Fig. S6.. Fluorescence time scans for the T**X**P3 system at 50°C. A: Signal increase during content mixing, including leakage, B: leakage only, C: content mixing, control (+), non-complementary LiNAs, D: Overlay and zoom of A, B and C

NTA of fusion experiments

Samples were prepared identically as for content mixing. Plain liposomes were used for both populations at a 1:1 ratio ([lip] = 275 µM, each [LiNA] = 0.105 µM, lipid/LiNA ratio 1312:1). The two different liposome populations were heated to 50 °C, then mixed and incubated for to 30 min at 50 °C. At 1, 5, 10, 15 and 30 min, aliquots of the fusion mixtures were transferred and diluted [lip] = 5 µM (55-fold dilution) in buffer at 20 °C. This dilution effectively slowed down further fusion and aggregation processes. The t_0 sample corresponds a preparation with unpaired LiNA (T**X**P3) after 15 min incubation. A 0.2 min sample was prepared separately by mixing 9 µl of each pre-heated population, brief vortexing, and addition of 982 µl buffer after 12 seconds. An unpaired (T**X**P3) sample was likewise incubated for 30 minutes and analyzed. All samples were analyzed by NTA as described above within 10 minutes.

Similar trends in the size distribution vs. fusion time were observed even if the diluted samples were kept for several hours in a diluted state.

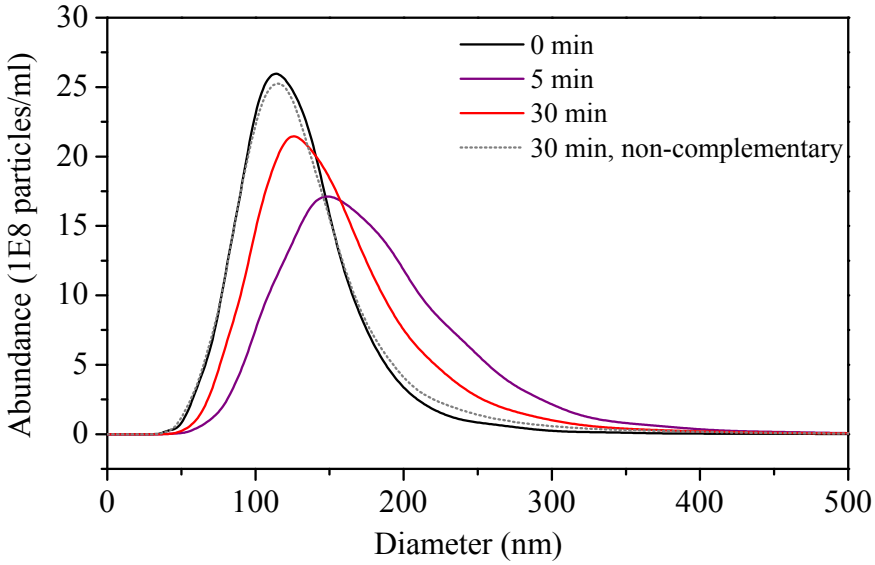


Fig. S7. Size distribution envelopes of TXP3 zipper fusion experiment at 50 °C. Before mixing, the populations (0 min), after 5 and 30 min including a control with non-complementary T**X**P3 likewise heated for 30 min at 50 °C.

To ensure that the particles sizes observed after 30 min at 50 °C were not based on aggregates, samples aliquots were heated to 85 °C for 5 minutes, cooled to RT analyzed at 20 °C. From temperature dependent turbidity measurements (not shown), we know that such a procedure eliminates any excess turbidity formed by vesicle aggregates, because the segregation of complementary LiNAs between the two populations is lost. Table S2 lists the average particles diameters observed before and after fusion at 50 °C compared to the theoretically attainable diameter (D_{fused}) for fusing a pair of liposomes of the inital diameter ($D_{initial}$) measured. Assuming that volume is conserved upon fusion ($V_{fused} = 2 \times V_{initial}$), D_{fused} was calculated as $D_{fused} = \sqrt[3]{2} \times D_{initial}$.

Sample	Comment	Avg. Diameter (nm) [a]
Initial	0 min	134 ± 4
Complementary	30 min at 50 °C	161 ± 4
Complementary, reheated	30 min at 50 °C, 5 min at 85 °C	164 ± 4
Non-complementary	30 min at 50 °C	138 ± 1
Theoretical	Calculated $\sqrt[3]{2} \times 134$ nm	169

[a]Errors given are standard deviations between sample triplicates.

Gel electrophoresis

Agarose gel electrophoresis was performed to assay the spontaneous anchoring of LiNAs into Liposomes (L). A 3% agarose gel (NuSieve 3:1 Agarose, Lonza) was pre-stained using GelRed (Biotium, Inc.) during preparation in 0.1M Tris·HCl, 0.09M borate, 2 mM EDTA (TBE) buffer. Samples containing liposomes (5.4 mM POPC, 100 nm nominal diameter) and LiNAs with palmityl, cholesteryl or phytanyl anchors (X-L, Y-L, Z-L, respectively). Ladder (O'Gene Ruler Ultra Low Range, Thermo Fisher) and samples containing approx. 250 pmol LiNA (lipid/DNA ratio 430:1) were loaded with 6X Blue Loading Dye (Thermo Fisher). Gel electrophoresis was performed for 90 min at 50 mA at 4.5 to 5 V/cm and imaged based on GelRed Fluorescence (see Figure S8).

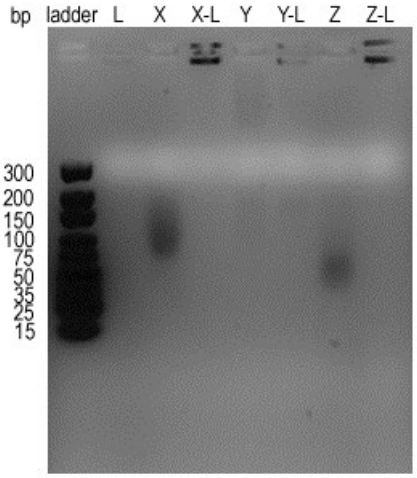
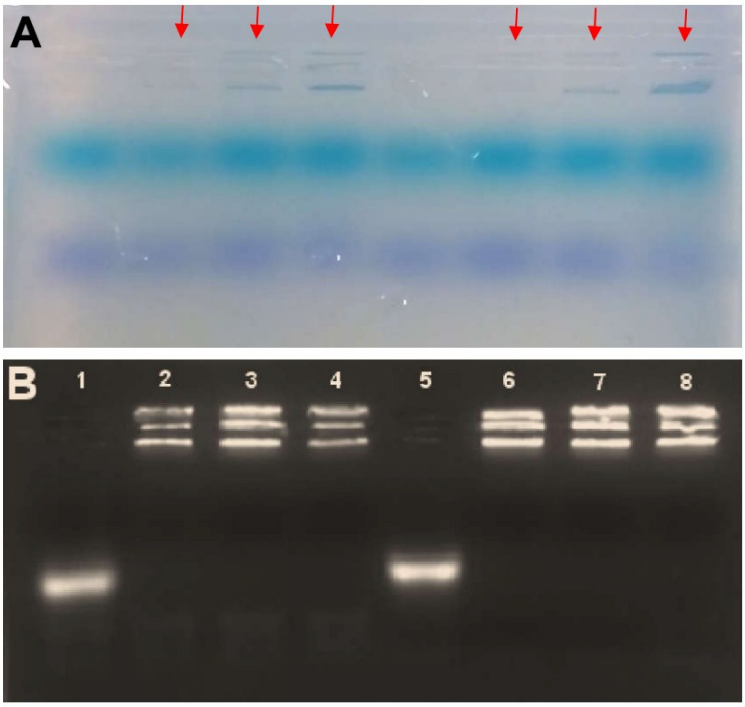


Fig. S8. Ladder: O'Gene Ruler Ultra Low Range; L: liposomes; X: ON02; X-L: liposome-ON02 conjugate; Y: ON04; Y-L: liposome-ON04 conjugate; Z: ON06; Z-L: liposome-ON06 conjugate.

Further tests were performed to interrogate the anchoring behaviour of LiNAs at elevated temperature and with liposomes composed of the DOPC/DOPE/Chol (2:1:1) lipid mixture used in fusion experiments. To suppress diffusion of liposomes out of the wells under these conditions, 1X Blue Loading Dye was encapsulated to match liposome buoyancy to the solution loaded in the wells, which also allowed to confirm liposome-retention in the wells by eye after electrophoresis. Liposomes were thus prepared by re-hydrating the lipid films using a solution in HBS and extrusion to 100 nm as described (see Experimental procedures). T**X**P3-type LiNAs were added to the liposomes at different Lipid/LiNA ratios (440:1 to 1320:1 and 4000:1), supplied as LiNA/DNA duplexes (non-modified reference ONs, see Table S1) and incubated 15 min. at room temperature before sample loading. Samples were prepared in a HEPES-buffered saline (HBS, 10 mM HEPES-NaOH, 100 mM NaCl, pH 7) as used for fusion experiments and an appropriate aliquot of 6X Blue Loading dye to a final volume of 24 µl. To show LiNA anchoring stability up to 50 °C, the gels were run at elevated temperatures: Before loading, the gel was immersed in pre-heated TBE buffer in the electrophoresis chamber and

placed in an oven (set to 55 °C) for 30 min prior to loading. After loading (on benchtop), the chamber was placed in the oven for running the electrophoresis (50 mA, 4.5 to 5 V/cm). The buffer temperature was measured after loading (43 °C), and at the end of the run (51 °C). Images of the gel are shown in Figure S9. Double-stranded LiNA/DNA allowed a much stronger contrast with GelRed and gave a more defined band for the freely migrating duplex in absence of liposomes (see Fig. S9).



C

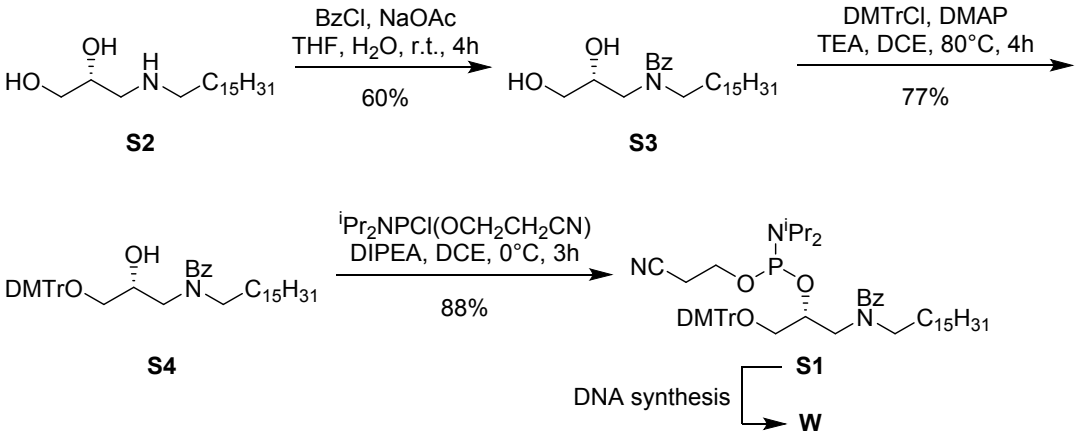
Lane	1	2	3	4	5	6	7	8
Lipid/LiNA ratio	0:1	440:1	1320 :1	4000:1	0:1	440:1	1320 :1	4000:1
ds(LiNA/DNA) (50 pmol)		5'-T**X**P3 type ds(ON-17/ON-34)				3'-T**X**P3 type ds(ON-18/ON-33)		

Fig. S9. Agarose gel for T**XP3**-LiNAs. A) Photograph of gel after run. Red arrows indicate liposome-containing wells (22, 66 and 200 nmol lipid). The entrapped loading dye is only visible for the two higher concentrations. B) Fluorescent image of the gel. C) Sample legend. The ds(LiNA/DNA) (40 pmol in all wells) remained anchored to the liposomes for all tested lipid/LiNA ratios (22, 66 or 200 nmol lipid). Faint bands can be observed in lanes 1-4 at slightly higher mobility compared to the free ds(LiNA/DNA), likely stemming from traces of de-hybridized ssDNA.

Supplemental experimental procedures

400 MHz-^{1}H, 101 MHz-^{13}C, and 126 MHZ-^{31}P NMR spectra were recorded on a Bruker Avance III spectrometer. All ^{13}C and ^{31}P spectra are ^{1}H-decoupled. All spectra were recorded at 25°C and were referenced internally to solvent reference frequencies. Chemical shifts (δ) are quoted in ppm, and coupling constants (*J*) are reported in Hz. Index a and b indicate diastereotopic protons. Assignment of signals was carried out using ^{1}H,^{1}H-COSY, HSQC and HMBC spectra obtained on the spectrometer mentioned above. ESI mass spectrometry was performed on a Bruker microTOF-Q II system.

Synthesis of mono-lipidated membrane anchor phosphoramidite S1



Scheme S1. Synthesis of mono-lipidated membrane anchor phosphoramidite **S1**

(*R*)-*N*-Benzoyl-*N*-hexadecyl-3-aminopropane-1,2-diol (S3). A solution of **S2** (500 mg, 1.58 mmol) in a mixture of THF (25 mL) and aqueous half-saturated sodium acetate solution (25 mL) was treated with benzoyl chloride (0.19 mL, 1.58 mmol) dropwise. The reaction mixture was stirred at room temperature for 4 h. The aqueous layer was extracted with ethyl acetate (3x 50 mL) and the combined organic layers were washed with sodium hydrogencarbonate solution (1x 50 mL) and brine (1x 50 mL) and dried over sodium sulfate. The solvent was removed under reduced pressure and the residue was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate 1:1→0:1) to yield 396 mg (0.94 mmol, 60%) as white solid.

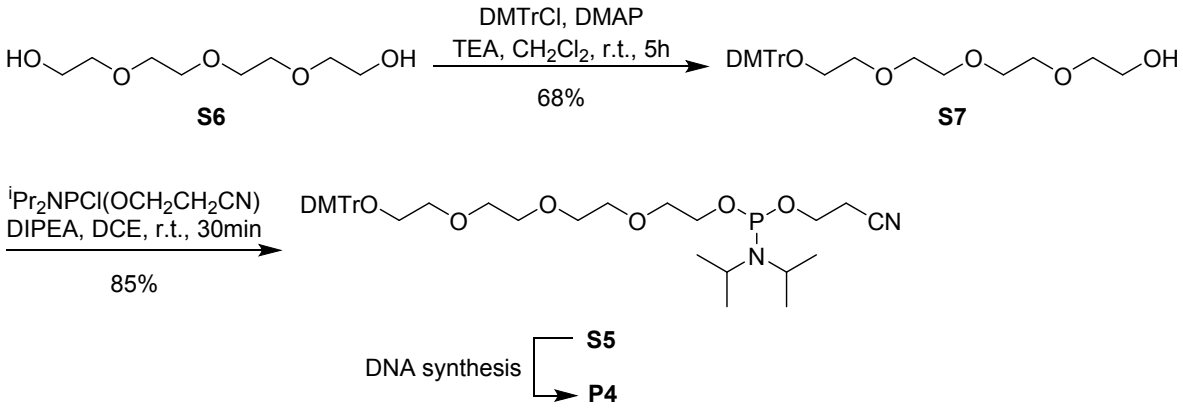
R_f 0.26 (ethyl acetate); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, 3H, J = 6.8, palm-16-H_3), 0.99-1.42 (m, 26H, palm-3-H_2-15-H_2), 1.45-1.62 (m, 2H, palm-2-H_2), 3.14-3.40 (m, 2H, palm-1-H_2), 3.54-3.77 (m, 4H, 1-H_2, 3-H_2), 3.86-3.98 (m, 1H, 2-H), 7.32-7.52 (m, 5H, Bz-CH); ^{13}C (101 MHz, $CDCl_3$): δ 14.25 (palm-C16), 22.83, 26.56, 28.90, 29.13, 29.50, 29.62, 29.72, 29.79, 32.06 (palm-C2-C15), 48.72 (C3), 51.20 (palm-C1), 63.95 (C1), 71.12 (C2), 126.56, 128.67, 129.88 (Bz-CH), 136.05 (Bz-C), 174.22 (Bz-CO); HRMS (ESI): calcd. for $C_{26}H_{46}NO_3$ 420.3471 $[M+H]^+$, found 420.3472.

(*R*)-*N*-Benzoyl-*N*-hexadecyl-3-amino-1-(dimethoxytriphenylmethyloxy)-2-propanol (S4). A solution of DMTr chloride (808 mg, 2.38 mmol) in dry DCE (10 mL) was added dropwise to a solution of **S3** (1.00 g, 2.38 mmol), DMAP (29 mg, 0.24 mmol) and triethylamine (1.0 mL, 7.14 mmol) in dry DCE (20 mL) under nitrogen atmosphere at room temperature. The reaction mixture was stirred at 80°C over night and the solvent was removed under reduced pressure. The residue was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate 4:1→1:1, 0.1% TEA) yielding 1.31 g (1.82 mmol, 77%) as yellow oil.

R_f 0.24 (petroleum ether/ethyl acetate 4:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, 3H, J = 6.9, palm-15-H_3), 1.01-1.74 (m, 28H, palm-2-H_2-15-H_2), 3.06-3.14 (m, 1H, 1-H_a), 3.15-3.26 (m, 2H, palm-1-H_2), 3.28-3.36 (1-H_b), 3.58-3.68 (m, 1H, 3-H_a), 3.73-3.84 (m, 1H, 3-H_b), 3.79 (s, 6H, DMTr-OCH_3), 4.06-4.17 (m, 1H, 2-H), 6.74-6.91 (m, 4H, Bz-CH, DMTr-CH), 7.14-7.50 (m, 14H, Bz-CH, DMTr-CH); ^{13}C (101 MHz, $CDCl_3$): δ 14.26 (palm-C16), 26.64, 28.82, 29.24, 29.50, 29.67, 29.76, 29.83, 32.06 (palm-C2-C15), 50.51 (C3), 51.33 (palm-C1), 55.35 (DMTr-OCH_3), 65.16 (C1), 71.38 (C2), 86.34 (DMTr-CAr_3), 113.29, 113.31, 126.65, 126.97, 127.99, 128.20, 128.53, 129.67, 130.17 (Bz-CH, DMTr-CH), 136.01, 144.98, 158.69 (Bz-C, DMTr-C), 174.35 (Bz-CO); HRMS (ESI): calcd. for $C_{47}H_{63}NNaO_5$ 744.4605 $[M+Na]^+$, found 744.4598.

2-Cyanoethyl (1-(*R*)-(*N*-benzoyl-*N*-hexadecyl-1-(dimethoxytriphenylmethyloxy)-3-amino-2-yl) *N*,*N*-diisopropylphosphoramidite (S1). A solution of **S4** (500 mg, 0.69 mmol) and DIPEA (0.50 mL, 2.84 mmol) in dry DCE (10 mL) under nitrogen atmosphere was treated dropwise with 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (0.25 mL, 0.90 mmol) at 0°C. The reaction mixture was stirred for 3h and was allowed to reach room temperature within that time. The reaction was treated with sat. sodium hydrogencarbonate solution (10 mL) and the aqueous layer was extracted with DCE (2x 10 mL). The combined organic layers were dried over magnesium sulfate and the solvent was removed under reduced pressure. Column chromatography (SiO_2, petroleum ether/ethyl acetate 3:1, 0.1% TEA) yielded 559 mg (0.61 mmol, 88 %) as pale yellow oil. The product was analyzed by ^{31}P NMR and HRMS analysis, and diluted with anhydrous acetonitrile to give a 0.05 M solution.

R_f 0.36 (petroleum ether/ethyl acetate 3:1); ^{31}P (162 MHz, $CDCl_3$): δ 148.87, 149.30, 149.80, 150.44; HRMS (ESI): calcd. for $C_{56}H_{80}N_3NaO_6P$ 944.5657 $[M+Na]^+$, found 944.5677.



Scheme S2. Synthesis of mono-lipidated membrane anchor phosphoramidite **S5**

12-*O*-(Dimethoxytriphenylmethyloxy)-tetraethylene glycol (S7). Tetraethylene glycol (10.0 g, 51.5 mmol, 8.7 eq) was coevaporated with toluene (3x 50 ml), placed under high vacuum overnight and treated with a solution of DMAP (37 mg, 0.30 mmol, 0.05 eq) and TEA (1.3 ml, 9.3 mmol, 1.6 eq) in dry DCM (5 ml) under an atmosphere of N_2 at rom temperature. DMTr chloride (2.0 g, 5.9 mmol, 1.0 eq) in dry DCM (20 ml) was added dropwise. The reaction mixture was stirred for 5 h at room temperature and subsequently poured into 5% aq. Na_2CO_3 solution (100 mL). The organic layer was washed with water (2x 100 mL), dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude residue was coevaporated with toluene (2x 50 mL) and purified by column chromatography (petroleum ether/ethyl acetate 1:1→0:1, 1% TEA) yielding 1.98 g (4.00 mmol, 68%) as pale yellow oil.

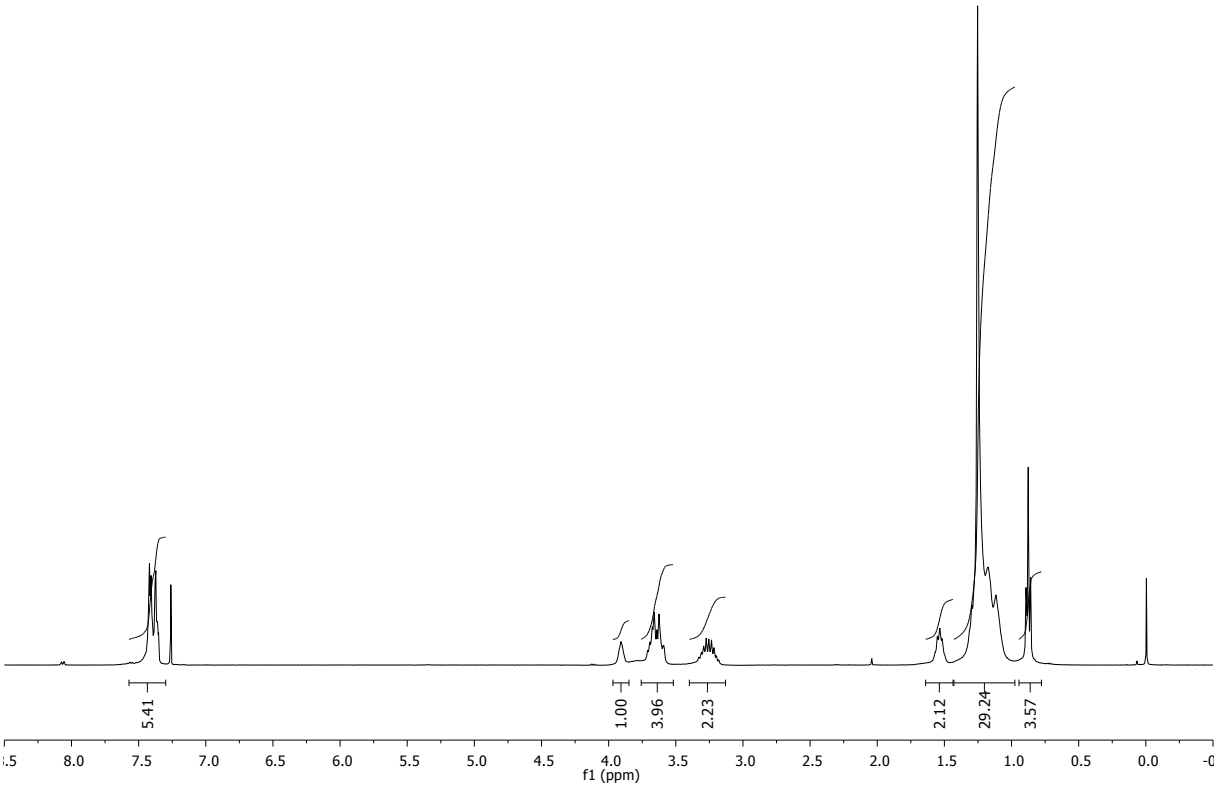
R_f 0.42 (petroleum ether/ethyl acetate 4:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.23 (t, 2H, J = 5.2, CH_2O-DMTr), 3.59 (m, 2H, C*H*$_2$OH), 3.65-3.70 (m, 12H, CH_2O), 3.78 (s, 6H, DMTr-OCH_3), 6.78-6.85 (m, 4H, DMTr-CH), 7.16-7.22 (m, 1H, DMTr-CH), 7.24-7.30 (m, 2H, DMTr-CH), 7.32-7.38 (m, 4H, DMTr-CH), 7.43-7.49 (m, 2H, DMTr-CH); ^{13}C (101 MHz, $CDCl_3$): δ 55.22 (DMTr-OCH_3), 61.78 (CH_2OH), 63.17 (CH_2O-DMTr), 70.45, 70.73, 70.74, 70.79 (CH_2O), 85.97 (DMTr-CAr_3), 113.06 (DMTr-CH), 126.67 (DMTr-CH), 127.75 (DMTr-CH), 128.24 (DMTr-CH), 130.09 (DMTr-CH), 136.36 (DMTr-C), 145.11 (DMTr-C), 158.41 (DMTr-C); HRMS (ESI): calcd. for $C_{29}H_{36}NaO_7$ 519.2353 $[M+NA]^+$, found 519.2334.

2-Cyanoethyl (12-*O*-(dimethoxytriphenylmethyloxy)-tetraethylene glycol-1-yl) *N*,*N*-diisopropyl-phosphoramidite (S5). A solution of **S7** (400 mg, 0.81 mmol, 1.0 eq) in dry DCE (2 mL) was treated with DIPEA (0.28 mL, 1.65 mmol, 2.0 eq) and degassed with a stream of N_2. 2-Cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (0.2 mL, 0.90 mmol, 1.1 eq) was added dropwise at room temperature and the reaction mixture was stirred for 30 min. The solvent was removed under reduced

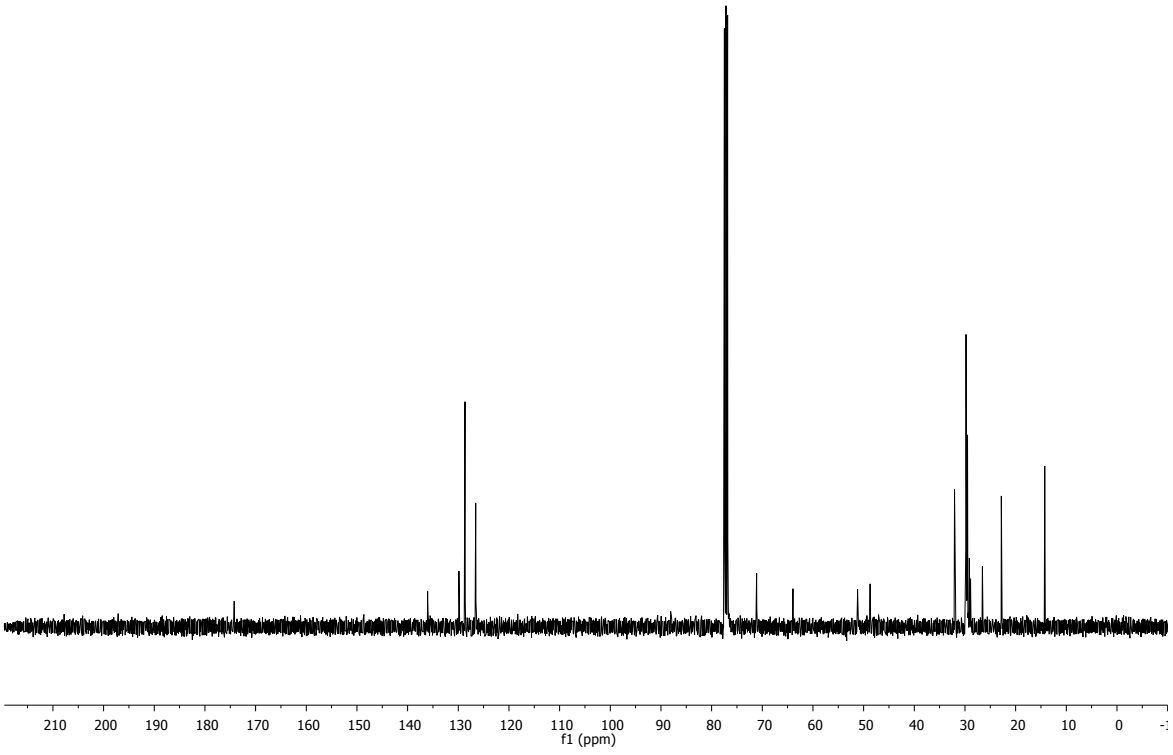
pressure and the crude residue was purified by column chromatography (petroleum ether/ethyl acetate 2:1, 1% TEA) yielding 479 mg (0.69 mmol, 85%) as pale yellow oil.

R_f 0.58 (petroleum ether/ethyl acetate 2:1); ^{31}P (162 MHz, $CDCl_3$): δ 148.52; HRMS (ESI): calcd. for $C_{38}H_{53}N_2NaO_8P$ 719.3432 $[M+Na]^+$, found 719.3397.

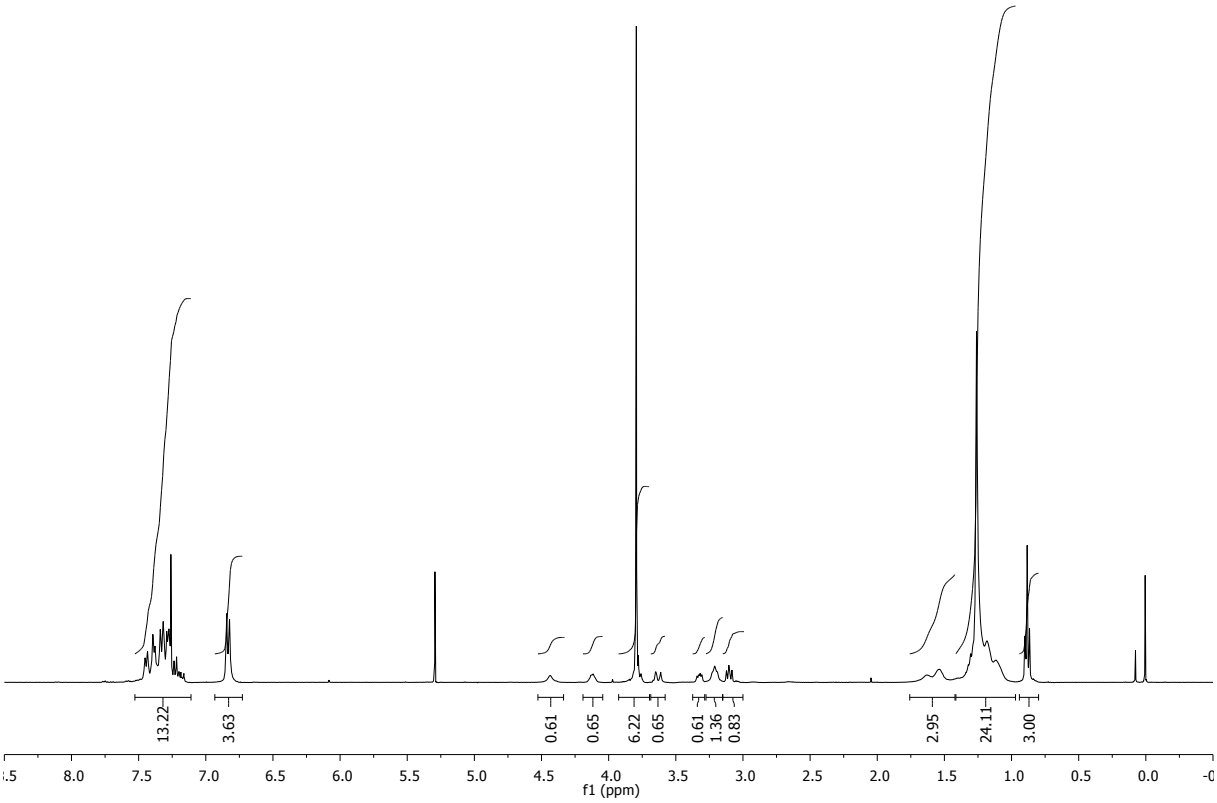
^{1}H, ^{13}C (compounds S3, S4, S7) and ^{31}P spectra (compounds S1, S5)



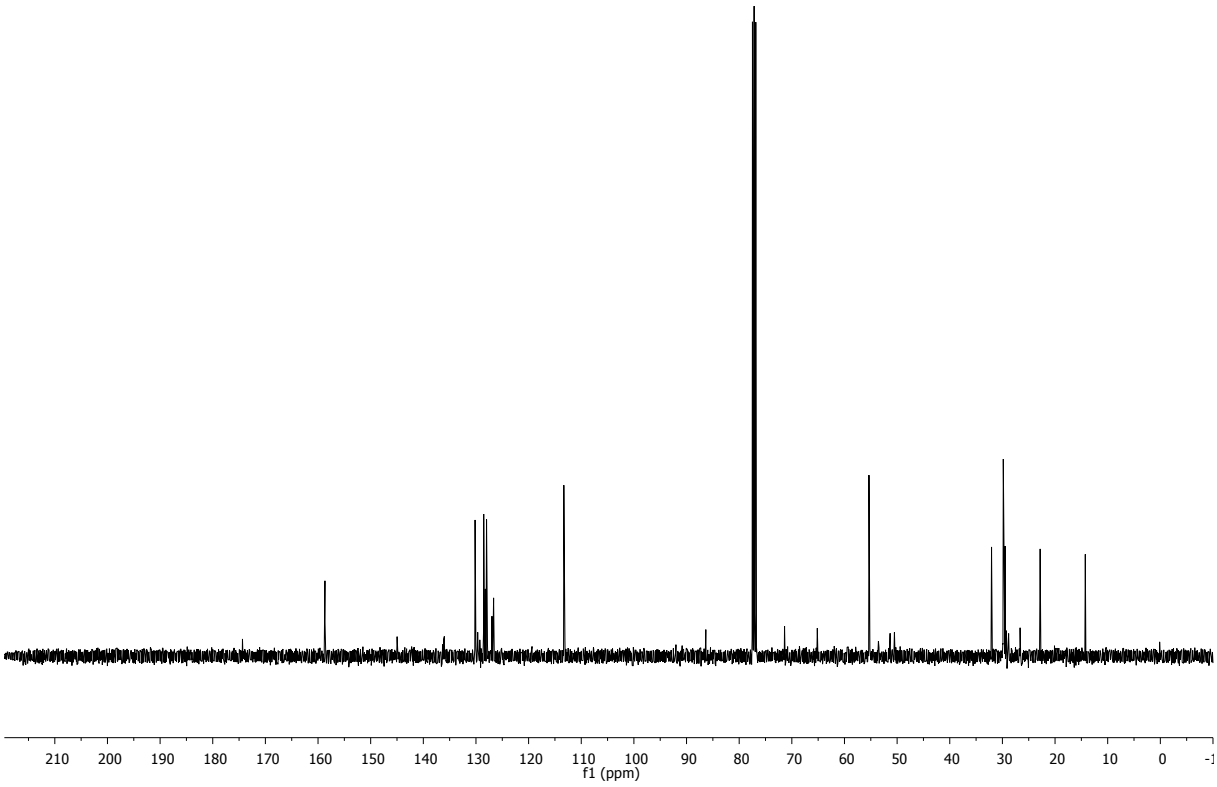
^{1}H-NMR spectrum of **S3** (400 MHz, $CDCl_3$, 25°C)



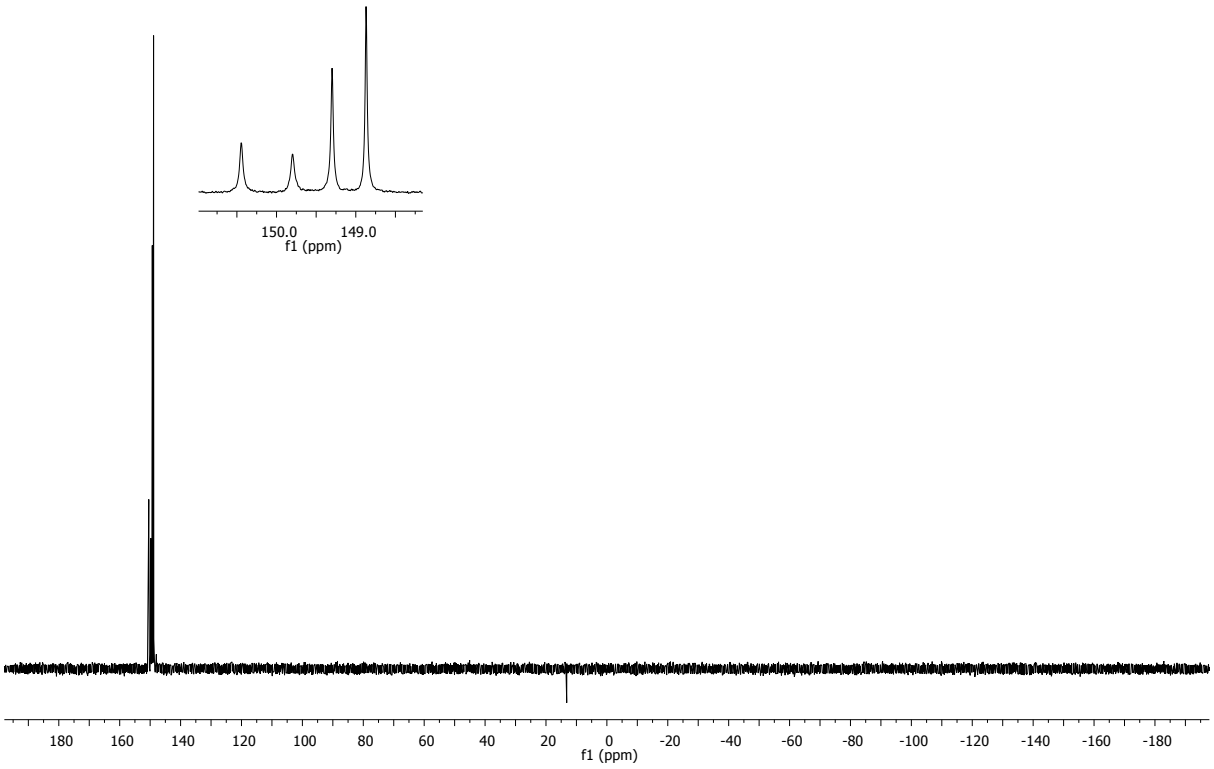
^{13}C-NMR spectrum of **S3** (101 MHz, $CDCl_3$, 25°C)



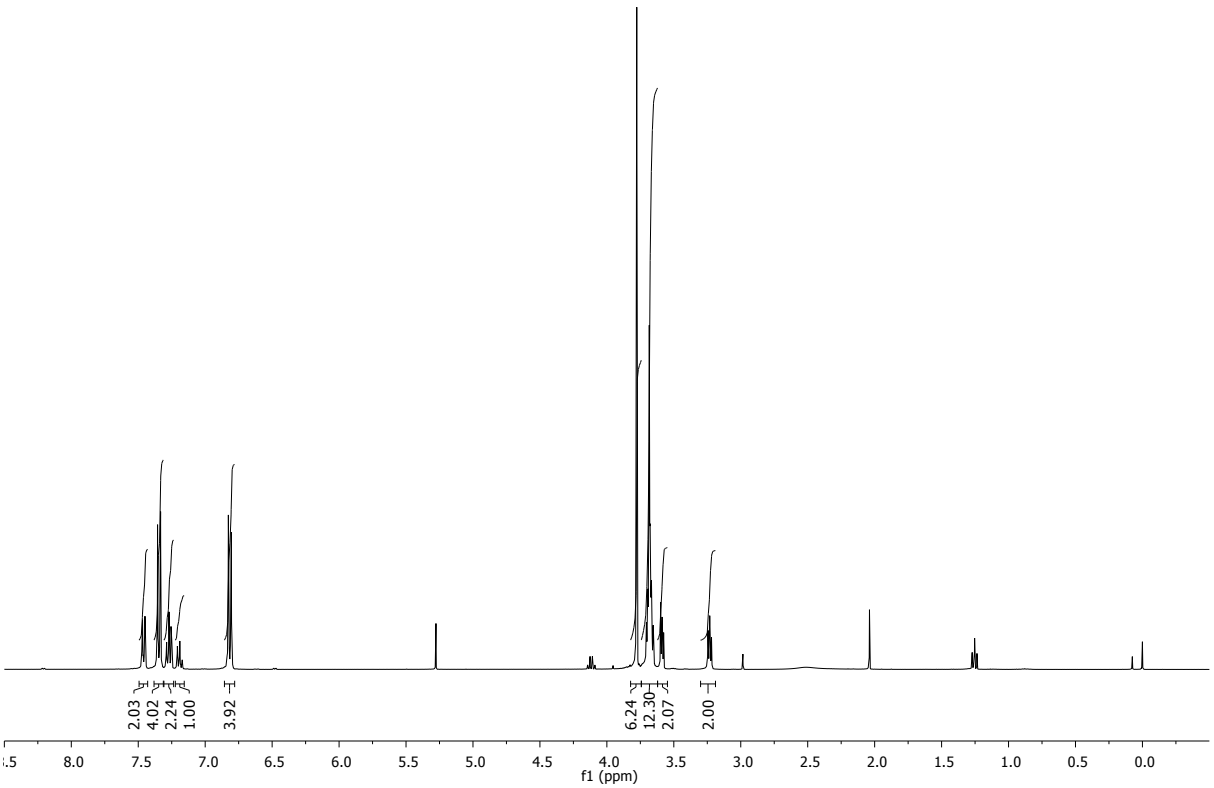
^{1}H-NMR spectrum of **S4** (400 MHz, $CDCl_3$, 25°C)



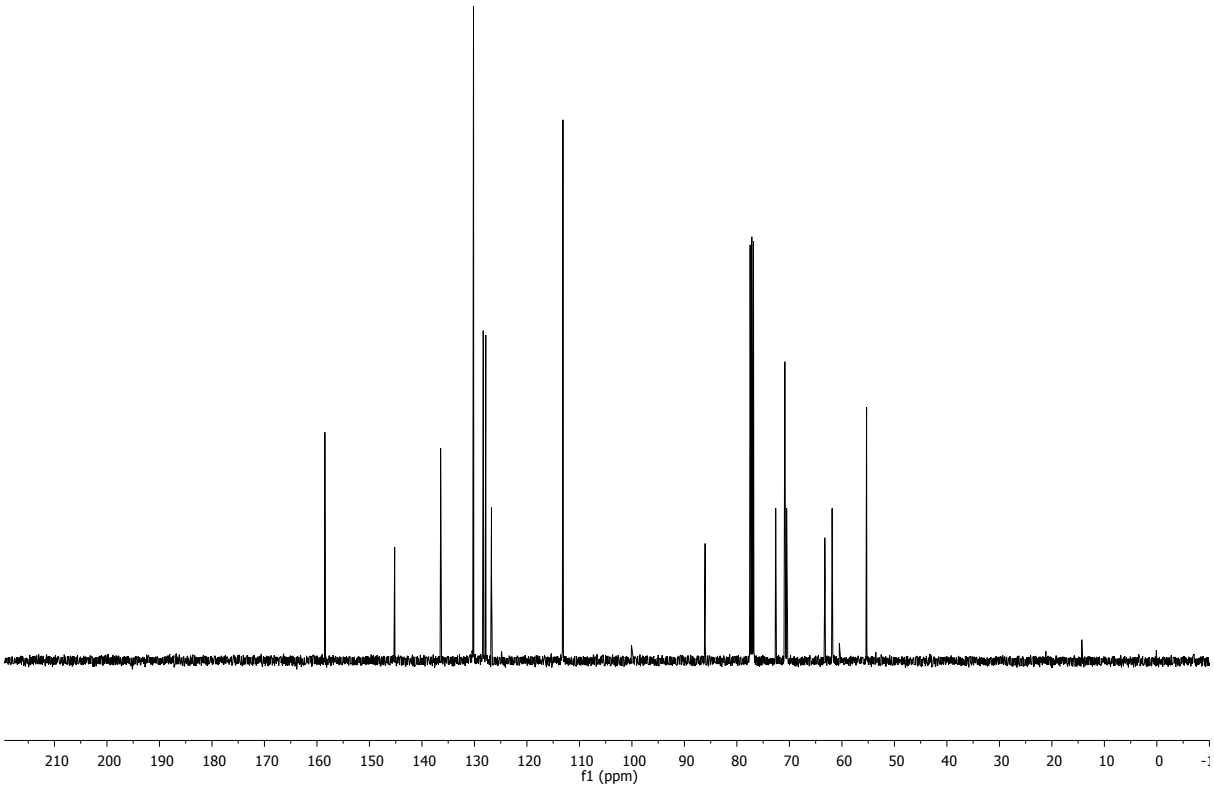
^{13}C-NMR spectrum of **S4** (101 MHz, $CDCl_3$, 25°C)



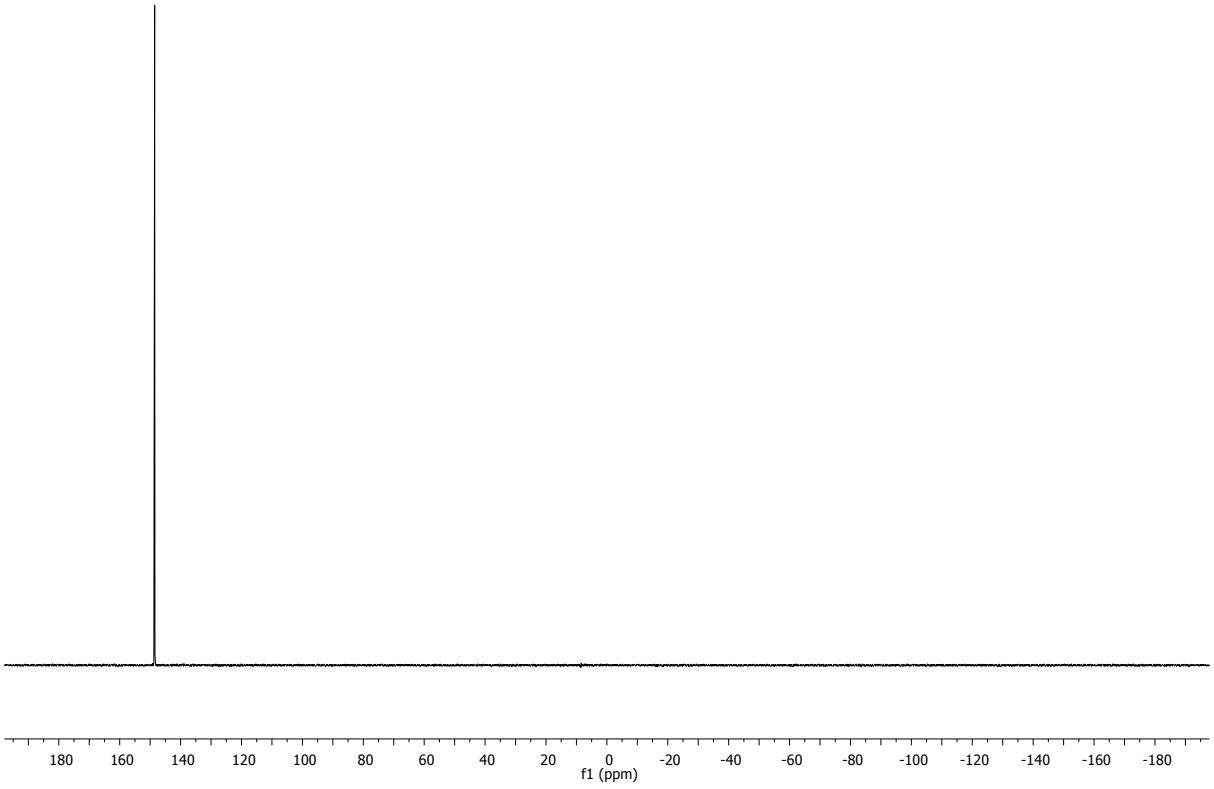
^{31}P-NMR spectrum of **S1** (162 MHz, $CDCl_3$, 25°C)[4]



^{1}H-NMR spectrum of **S7** (400 MHz, $CDCl_3$, 25°C)



^{13}C-NMR spectrum of **S7** (101 MHz, $CDCl_3$, 25°C)



^{31}P-NMR spectrum of **S5** (162 MHz, $CDCl_3$, 25°C)

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