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A convenient route to conjugates of 1,2-diglycerides with functionalized oligoethylene glycol spacer arms

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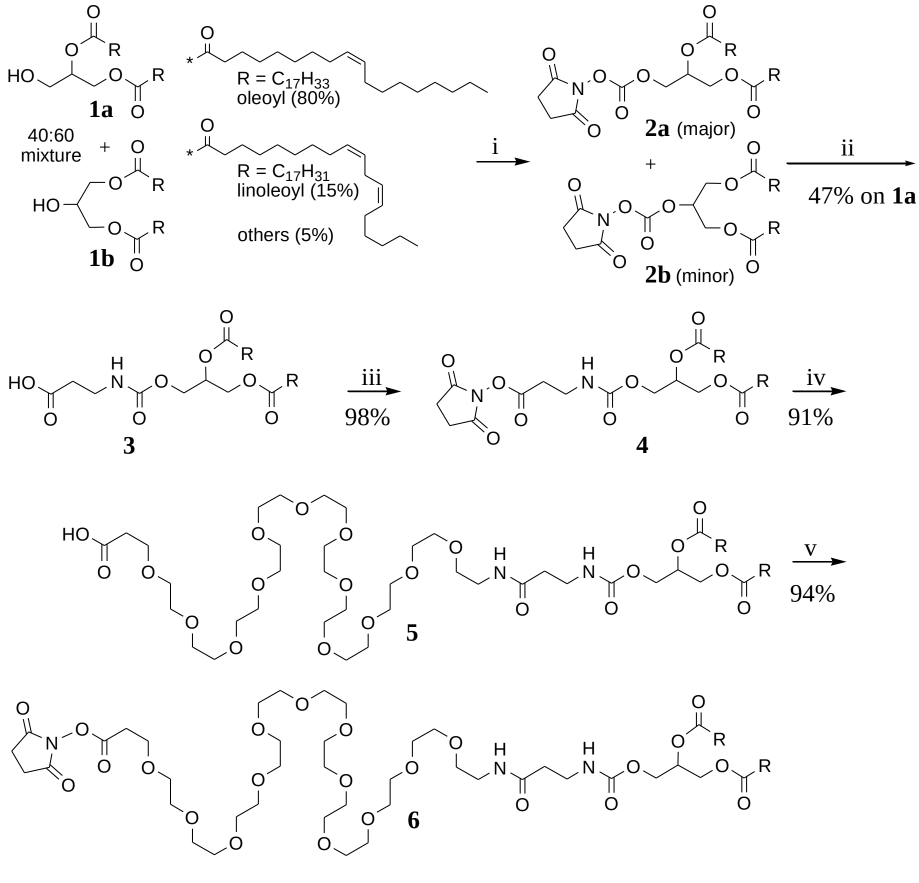
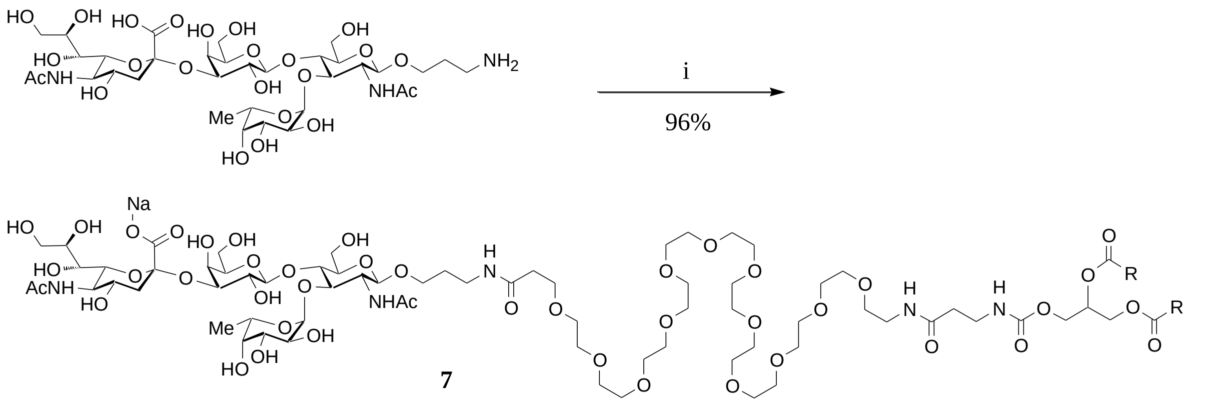
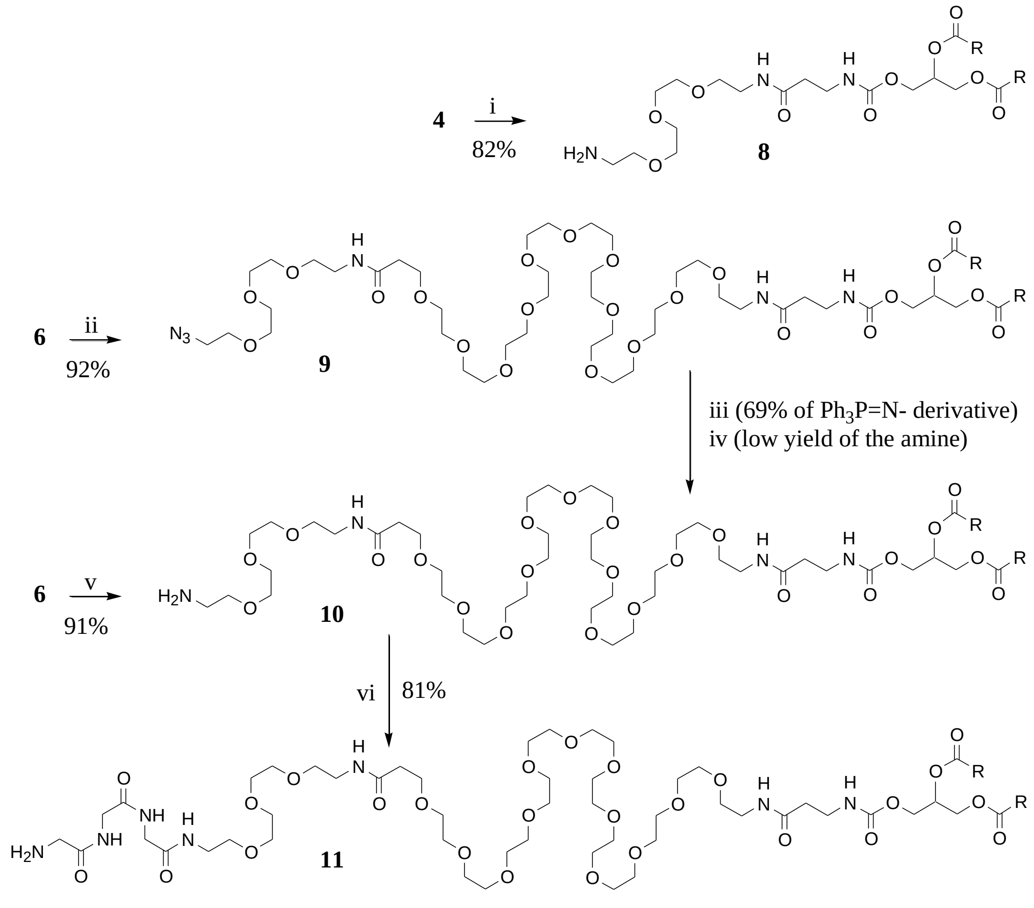


Scheme S1. Synthesis of diglyceride acids 1,2-DAG-βAla-COOH (**3**) and 1,2-DAG-βAla-PEG(12)-COOH (**5**) and their active esters (**4**) and (**6**). Acid **3** was prepared from a mixture of 1,2-DAG (**1**) and 1,3-DAG (**2**). *Reagents and conditions:* i, DSC (2 eq.), Et_3N (1 eq.), $MeCN/CH_2Cl_2$ 3:1, 1.7 h/20°C; ii, β-alanine (powder, excess), Et_3N, $MeCN/CH_2Cl_2$ 3:1, 1 h/20°C, separation on silica gel; iii, DSC (2 eq.), Et_3N (1.5 eq.), $MeCN/CH_2Cl_2$ 2:1, 40 min/20°C; iv, H_2N-PEG(12)-COOH (1 eq.), Et_3N (1 eq.), CH_2Cl_2, 2 h/20°C; v, DSC (3 eq.), Et_3N (1.5 eq.), $MeCN/CH_2Cl_2$ 2:1, 40 min/20°C.



Scheme S2. Synthesis of neoglycolipid SiaLe^X-sp-PEG(12)-βAla-1,2-DAG (**7**). *Reagents and conditions:* i, **6**, Et_3N (2 eq.), DMSO/dichloroethane 1:1, 2 h/20°C; + aqueous NaOAc (20 eq.), Sephadex LH-20 column in Pr^{i}OH/water 3:5.



Scheme S3. Synthesis of diglyceride amines 1,2-DAG-βAla-PEG(3)-NH_2 (**8**), 1,2-DAG-βAla-PEG(12)-PEG(3)-NH_2 (**10**), 1,2-DAG-βAla-PEG(12)-PEG(3)-Gly_3-NH_2 (**11**) and azide 1,2-DAG-βAla-PEG(12)-PEG(3)-N_3 (**9**). Reagents and conditions: i, H_2N-PEG(3)-NH_2 diamine (8 eq.), dichloroethane, 2 min/20°C, addition of AcOH; ii, N_3-PEG(3)-NH_2 amine (1.5 eq.), $MeCN/CH_2Cl_2$/dichloroethane 2:2:2, 15 min/20°C; iii, PPh_3 (8 eq.), 1,4-dioxane/water 4:1, 3 h/20°C; iv, treatment of iminophosphorane in 1,4-dioxane/water pH ~8, 15 h/20°C resulted in amine **10** but with noticeable deacylation of diglyceride; v, H_2N-PEG(3)-NH_2 diamine (8 eq.), dichloroethane, 2 min/20°C, addition of AcOH; vi, Fmoc-Gly_3-ONSu (2 eq.), DMF, 1 h/20°C, then piperidine (5% by volume), 15 min/20°C, addition of AcOH.

Experimental

A mixture of 1,2- (**1a**) and 1,3- (**1b**) diglycerides (the ratio of 1,2 and 1,3-diglycerides is about 2:3) was obtained by partial hydrolysis of olive oil followed by purification of products on a silica gel; the acyl groups in the diglycerides ~ 80% are oleoyls and ~ 15% — linoleoyls (NMR data, see below). This is in good agreement with the literature [S1]. Further, the obtained derivatives of 1,2-diglycerides are designated as derivatives of diacylglycerol (DAG), but the NMR and mass spectra are described for the major component, dioleoylglycerol (DOG), with the exception of the initial mixture of diglycerides. *N,N'*-Disuccinimidyl carbonate (DSC) and *N*-hydroxysuccinimide were purchased from Sigma-Aldrich. H_2N-PEG(12)-COOH (1-amino-3,6,9,12,15,18,21,24,27,30,33,36-dodecaoxanonatriacontan-39-oic acid) and H_2N-PEG(3)-N_3 (1-amino-11-azido-3,6,9-trioxaundecane) was purchased from Iris Biotech GmbH. Spacered tetrasaccharide SiaLe^X was prepared as described [S2]. All evaporations were performed at 30 °C; drying or freeze-drying was performed at 0.01 mBar.

NMR spectra were recorded on NMR spectrometer Bruker Avance 700 (Bruker BioSpin MRI GmbH). High resolution mass spectra (HR MS) were measured on a Bruker microTOF II instrument using electrospray ionization (ESI) [S3].

TLC was performed using precoated aluminum sheets Kieselgel 60 F_{254} (Merck, Darmstadt, Germany); detection under UV-light, visualization by charring with 7% phosphoric acid or treatment with 2% solution of ninhydrin (for amines).

1,2-DAG + 1,3-DAG mixture 1a,b TLC: 1,3-DAG R_f = 0.59, 1,2-DAG R_f = 0.51 (hexane/ethyl acetate 2:1 by volume + 1% AcOH). ^{1}H NMR of 1,2-DAG + 1,3-DAG 40:60 mixture (700 MHz, $CDCl_3$, 30°C): δ 7.258 (s, $CHCl_3$), 5.339 (m, ~4.3H; CH=CH of *oleic* and *linoleic* acids), 5.076 (m, ~0.4H; CH-O(CO) of 1,2-DAG), 4.314 (dd, ~0.4H, J = 11.9, 4.5 Hz; CH-O(CO) of 1,2-DAG), 4.229 (dd, ~0.4H, J = 11.9, 5.7 Hz; CH-O(CO) of 1,2-DAG), 4.178 (dd, ~1.2 H, J = 11.6, 4.4 Hz; CH-O(CO) of 1,3-DAG), 4.129 (dd, ~1.2 H, J = 11.6, 5.9 Hz; CH-O(CO) of 1,3-DAG), 4.072 (m, ~0.6H; C$\underline{H}$-OH of 1,3-DAG), 3.724 (m, ~0.8H; C$\underline{H_2}$-OH of 1,2-DAG), 2.766 (t, ~0.3H, J = 7.0 Hz; C=C-C$\underline{H_2}$-C=C of *linoleic* acid), 2.453 (d, ~0.6H, J = 4.8 Hz; OH of 1,3-DAG), 2.335 (m, 4H, 2 CH_2(CO)), 2.008 (m, ~7.5H, CH_2-C=C-CH_2 of *oleic* and CH_2-C=C-C-C=C-CH_2 of *linoleic* acids), 1.619 (m, ~4.4H, 2 CH_2-C-(CO) and OH of 1,3-DAG), 1.294 (m, ~38H, ~19 CH_2), 0.876 (t, 6H, J = 7.0 Hz, 2 CH_3) ppm.

1,2-DAG-βAla-COOH (3) To a stirred solution of 1,2-DAG (**1a**) and 1,3-DAG (**1b**) mixture (40:60, 1110 mg, 1.787 mmol) in MeCN (16 mL) and CH_2Cl_2 (5 mL), DSC (916 mg, 3.575 mmol) and Et_3N (250 μL, 1.787 mmol) were added. After 15 min of stirring at room temperature (rt), DSC dissolved completely, and after 100 min, 1,2-DAG conversion was more than 90%; the reaction mixture contained (TLC data) mainly 1,3-DAG, *N*-succinimidyl carbonate 1,2-DAG-(CO)-ONSu (**2a**, major) and *N*-succinimidyl carbonate 1,3-DAG-(CO)-ONSu (**2b**, minor). The presence of *N*-oxysuccinimide residue in the structure of **2a,b** was indicated by the intense absorption under UV after heating of the TLC plates (*N*-hydroxysuccinimide absorbs).To the stirred mixture, a solution of AcOH (164 μL, 2.86 mmol) and Et_3N (250 μL, 1.79 mmol) in MeCN (3 mL) was added, and then, 20 min later, dry β-alanine as fine powder (1274 mg, 14.3 mmol) and Et_3N (498 μL, 3.58 mmol) were added. The mixture was stirred for 1 h at rt, and then, after addition of AcOH (818 μL, 14.3 mmol), was evaporated to dryness. The lipid derivatives were extracted into $CHCl_3$ (40 mL) with $CHCl_3/H_2O$ (20 mL) mixture. The chloroform extract was washed with 20 ml 0.1 M HCl, 40 ml H_2O, filtered through cotton wool and evaporated to a minimal volume. The residue consisted of unreacted 1,3-DAG **1b** with a small amount of 1,2-DAG **1a** and a mixture of 1,2-DAG-βAla-COOH with 1,3-DAG-βAla-COOH ~ 6:1. The mixture was separated on a silica gel column (400 ml of silica gel in chloroform/hexane 1:1) in eluent gradient from chloroform/hexane/ethyl acetate 4:4:1 (by volume) to chloroform/hexane/ethyl acetate 4:4:1+ 2% AcOH. The selected fractions were evaporated; the residues were co-evaporated with heptane and dried in vacuum.

According to the order of elution from the column, 641 mg (58% from loaded in the reaction) of a mixture of 1,3-DAG with a small amount of 1,2-DAG, 189 mg of 1,2-DAG-βAla-COOH/1,3-DAG-βAla-COOH mixture (~2:1, contains ~ 126 mg of target 1,2-DAG-βAla-COOH, 24% on initial 1,2-DAG) and 248 mg (yield 47% on initial 1,2-DAG) of target 1,2-DAG-βAla-COOH were obtained.

For the unambiguous identification of 1,2-DAG-βAla-COOH and 1,3-DAG-βAla-COOH, they were also obtained independently from individual 1,2-DAG **1a** and 1,3-DAG **1b**.

TLC: 1,3-DAG R_f = 0.53, 1,2-DAG R_f = 0.45, 1,3-DAG-(CO)-ONSu R_f = 0.38, 1,2-DAG-(CO)-ONSu R_f = 0.32 (hexane/ethyl acetate 2:1 by volume). TLC: 1,3-DAG R_f = 0.59, 1,2-DAG R_f = 0.51, 1,3-DAG-βAla-COOH R_f = 0.32, 1,2-DAG-βAla-COOH R_f = 0.25 (hexane/ethyl acetate 2:1 by volume + 1% AcOH).

^{1}H NMR for 1,2-DOG-βAla-COOH (700 MHz, $[D]CHCl_3/[D_4]CH_3OH$ 1:1, 30°C):

δ 5.344 (m, 4H, 2 CH=CH), 5.247 (m, 1H, CH-O(CO)), 4.352 (dd, 1H, J = 12.0, 3.8 Hz, CH-O(CO)), 4.249 (dd, 1H, J = 11.8, 4.5 Hz, CH-O(CO)), 4.165 (m, 2H, 2 CH-O(CO)), 3.396 (t, 2H, J = 6.7 Hz, CH_2N), 3.344 (p, J = 1.7 Hz, residual H of $[D4]CH_3OH$), 2.525 (t, 2H, J = 6.5 Hz, $C\underline{H}_2$(CO)OH), 2.339 (m, 4H, 2 CH2(CO)), 2.027 (m, 8H, 4 CH_2-C=C), 1.622 (m, 4H, 2 CH_2-C-(CO)), 1.310 (m, 40H, 20 CH_2), 0.892 (t, 6H, J = 7.2 Hz, 2 CH_3) ppm.

[13]C NMR for 1,2-DOG-βAla-COOH (176 MHz, [D]$CHCl_3$/[D_4]CH_3OH 1:1, 30°C) δ 174.00 (COOH), 173.50 and 173.11 (O<u>C</u>OCH_2), 156.23 (NHCOO), 129.55 and 129.29 (2 C=C), 77.23 ($CDCl_3$), 69.18 (C-2 of glycerol), 62.32 and 61.94 (C-1 and C-3 of glycerol), 48.05 (CD_3OD), 36.27, 33.78, 33.75 and 33.63 (CO-<u>C</u>H_2<u>C</u>H_2-NH and 2 CO<u>C</u>H_2), 31.52-22.24 (26 CH_2), 13.36 (2 CH_3) ppm.

HRESIMS: found m/z 758.5532; calc. for $C_{43}H_{77}NO_8Na$ $[M+Na]^+$ 758.5541.

[1]H NMR of 1,3-isomer for 1,3-DOG-βAla-COOH (700 MHz, [D]$CHCl_3$/[D_4]CH_3OH 1:1, 30°C): δ 5.344 (m, 4H, 2 CH=CH), 5.122 (p, 1H, J = 5.3 Hz, CH-OH)), 4.270 (dd, 2H, J = 11.8, 4.5 Hz, 2 CH-O(CO)), 4.196 (dd, 2H, J = 11.8, 5.8 Hz, 2 CH-O(CO)), 3.403 (t, 2H, J = 6.5 Hz, CH_2N), 3.344 (p, J = 1.7 Hz, residual H of [D4]CH_3OH), 2.519 (t, 2H, J = 6.5 Hz, C<u>H</u>$_2$(CO)OH), 2.343 (t, 4H, *J* = 7.5 Hz, 2 CH2(CO)), 2.025 (m, 8H, 4 CH_2-C=C), 1.626 (m, 4H, 2 CH_2-C-(CO)), 1.308 (m, 40H, 20 CH_2), 0.892 (t, 6H, J = 7.2 Hz, 2 CH_3) ppm.

1,2-DAG-βAla-ONSu (4) To a stirred solution of 1,2-DAG-βAla-COOH (**3**, 224 mg, 0.304 mmol) in a mixture of MeCN (3 ml) and CH_2Cl_2 (1.5 ml), DSC (155 mg, 0.608 mmol) and Et_3N (63 μl, 0.456 mmol) were added. After 40 min of stirring at rt, AcOH (255 μl) was added, and the solution was applied on a Sephadex LH-20 column (150 ml in $CHCl_3$/Pr^iOH 2:1 + 0.5% AcOH). Fractions containing 1,2-DAG-βAla-ONSu were combined, evaporated, and the residue was dried. Yield of 1,2-DAG-βAla-ONSu was 249 mg (98%) with an active ester content >95%.

TLC: 1,2-DAG-βAla-ONSu R_f = 0.44 (hexane/ethyl acetate 2:1 by volume + 1.5% AcOH).

1,2-DAG-βAla-PEG(12)-COOH (5) To a stirred solution of 1,2-DAG-βAla-ONSu (**4**) (196 mg, 0.235 mmol) in CH_2Cl_2 (2 ml), a solution of H_2N-PEG(12)-OH (146 mg, 0.235 mmol) in CH_2Cl_2 (2 ml) and Et_3N (33 μl, 0.237 mmol) were added. After 2 h at rt, the solution was evaporated, the residue was dissolved in a mixture of ethyl acetate/MeCN (1:1), applied onto a column with silica gel (80 ml in ethyl acetate), and the components were eluted with ethyl acetate/MeCN/water 40:40:4 + 2% AcOH, 40:40:7 + 2% AcOH and 40:40:10 + 2% AcOH. The selected fractions were evaporated, co-evaporated with heptane and dried in vacuum. Yield of 1,2-DAG-βAla-PEG(12)-COOH was 286 mg (91%).

TLC: 1,2-DAG-βAla-PEG(12)-COOH R_f = 0.27 (ethyl acetate/MeCN/water/AcOH 20:20:5:1), R_f = 0.50 (ethyl acetate/MeCN/water/AcOH 10:20:5:2).

[1]H NMR for 1,2-DOG-βAla-PEG(12)-COOH (700 MHz, [D]$CHCl_3$/[D_4]CH_3OH 1:1, 30°C):

δ 5.346 (m, 4H, 2 CH=CH), 5.251 (m, 1H, CH-O(CO)), 4.353 (dd, 1H, J = 11.9, 3.8 Hz, CH-O(CO)), 4.243 (dd, 1H, J = 11.7, 4.5 Hz, CH-O(CO)), 4.157 (m, 2H, 2 CH-O(CO)), 3.751 (t, 2H, J = 6.3 Hz, CH_2O), 3.668 (m, 44H, CH_2O), 3.562 (t, 2H, J = 5.4 Hz, CH_2O), 3.397 (m, 4H, 2

CH_2N), 3.344 (p, J = 1.7 Hz, residual H of [D4]CH_3OH), 2.549 (t, 2H, J = 6.3 Hz, C<u>H</u>$_2$(CO)OH), 2.418 (t, 2H, J = 6.6 Hz, CH_2(CO)N), 2.339 (m, 4H, 2 CH2(CO)), 2.031 (m, 8H, 4 CH_2-C=C), 1.627 (m, 4H, 2 CH_2-C-(CO)), 1.304 (m, 40H, 20 CH_2), 0.894 (t, 6H, J = 7.15 Hz, 2 CH_3) ppm.

^{13}C NMR for 1,2-DOG-βAla-PEG(12)-COOH (176 MHz, [D]$CHCl_3$/[D_4]CH_3OH 1:1, 30°C) δ 175.09 (COOH), 173.45, 173.04 and 172.05 (NH<u>C</u>OCH_2 and 2 O<u>C</u>OCH_2), 156.21 (NHCOO), 129.54 and 129.26 (2 C=C), 77.23 ($CDCl_3$), 70.08-69.57 (24 OCH_2 of PEG), 69.17 (C-2 of glycerol), 66.83 (O-<u>C</u>$H_2$$CH_2$-COO), 62.29 and 61.93 (C-1 and C-3 of glycerol), 48.04 (CD_3OD), 38.85 (O-CH_2<u>C</u>H_2-N), 36.94, 35.41, 35.34, 33.77 and 33.61 (CO-<u>C</u>H_2<u>C</u>H_2-NH and 3 CO<u>C</u>H_2), 31.50-22.23 (26 CH_2), 13.35 (2 CH_3) ppm.

HRESIMS: found m/z 1357.9126; calc. for $C_{70}H_{130}N_2O_{21}Na$ $[M+Na]^+$ 1357.9058.

1,2-DAG-βAla-PEG(12)-ONSu (6) To a stirred solution of 1,2-DAG-βAla-PEG(12)-COOH (**5**) (181 mg, 0.1355 mmol) in a mixture of MeCN (3 ml) and CH_2Cl_2 (1.5 ml), DSC (104 mg, 0.407 mmol) and Et_3N (28 μl, 0.201 mmol) were added. After 40 min at rt, AcOH (300 μl) was added, and the solution was applied onto a Sephadex LH-20 column (150 ml in $CHCl_3$/Pr^{i}OH 2:1 + 0.5% AcOH). Fractions containing 1,2-DAG-βAla-PEG(12)-ONSu were combined, evaporated, and the residue was dried. Yield of 1,2-DAG-βAla-PEG(12)-ONSu was 183 mg (94%) with an active ester content >95%.

TLC: 1,2-DAG-βAla-PEG(12)-ONSu R_f = 0.52, 1,2-DAG-βAla-PEG(12)-COOH R_f = 0.45 (ethyl acetate/MeCN/water/AcOH 10:20:5:1).

SiaLe^X-sp-PEG(12)-βAla-1,2-DAG (7) To a stirred solution of SiaLe^X-sp-NH_2 (14.4 mg, 0.0164 mmol) in DMSO (1 ml), a solution of 1,2-DAG-βAla-PEG(12)-ONSu (**6**) (23.2 mg, 0.0162 mmol) in dichloroethane (1 ml) and Et_3N (4.5 μl, 0.0324 mmol) were added. After 2 h at rt, AcOH (1.85 μl, 0.0324 mmol) and Pr^{i}OH (3 ml) were added, and the solution was evaporated without heating to remove dichloroethane. The residue was diluted with water (0.3 ml) and 81 μl of 4 M aqueous NaOAc solution (4 M NaOAc was acidified with AcOH, ~0.05 mol/mol NaOAc, to pH 6.4). The resulting solution was immediately applied onto a Sephadex LH-20 column (100 ml in Pr^{i}OH/water 3:5). Fractions containing SiaLe^X-sp-PEG(12)-βAla-1,2-DAG were combined, evaporated, and the residue was freeze-dried from water (0.01 mBar). Yield of SiaLe^X-sp-PEG(12)-βAla-1,2-DAG was 34.6 mg (96%).

TLC: SiaLe^X-sp-PEG(12)-βAla-1,2-DAG R_f = 0.54 (MeCN/EtOH/water/ 4:2:1).

^{1}H NMR for SiaLe^X-sp-PEG(12)-βAla-1,2-DOG (700 MHz, [D_4]CH_3OH, 30°C):

δ 5.345 (m, 4H, 2 CH=CH), 5.235 (m, 1H, CH-O(CO)), 5.035 (d, 1H, J = 4 Hz, H-1 of Fucα), 4.817 (broad.q, 1H, J = 6.6 Hz, H-5 of Fucα), 4.499 (d, 1H, J = 7.8 Hz, H-1 of Galβ), 4.414 (d,

1H, J = 8.2 Hz, H-1 of GlcNAcβ), 4.364 (dd, 1H, J = 12.0, 3.5 Hz, CH-O(CO)), 4.238 (dd, 1H, J = 11.8, 4.4 Hz, CH-O(CO)), 4.144 (m, 2H, 2 CH-O(CO)), 4.06- 3.41 (74H, main part of PEG(12) and SiaLe^{X}), 3.367 (t, 2H, J = 5.3 Hz, CH_2N), 3.306 (p, J = 1.7 Hz, residual H of [D4]CH_3OH), 3.167 (m, 1H, CHN), 2.877 (dd, 1H, J = 12.4 Hz, J = 4.1 Hz, H-3eq of Neu5Acα), 2.448 (m, 2H, CH_2(CO)), 2.397 (t, 2H, J = 6.8 Hz, CH_2(CO)), 2.321 (t, 4H, J = 7.1 Hz, 2 CH_2(CO)), 2.033 (m, 8H, 4 CH_2-C=C), 2.005 (s, 3H, N(CO)CH_3), 1.968 (s, 3H, N(CO)CH_3), 1.721 (m, 3H, H-3ax of Neu5Acα and O-$CH_2C\underline{H}_2CH_2$-N), 1.607 (m, 4H, 2 CH_2-C-(CO)), 1.314 (m, 40H, 20 CH_2), 1.156 (d, 3H, J = 6.5 Hz, CH_3 of Fucα), 0.900 (t, 6H, J = 7.1 Hz, 2 CH_3) ppm.
^{13}C NMR for SiaLe^{X}-sp-PEG(12)-βAla-1,2-DOG (176 MHz, [D_4]CH_3OH, 30°C) δ 175.63 (COO of Neu5Acα), 174.98, 174.95, 174.59 and 174.08 (4 $\underline{C}OCH_2$), 173.95 and 173.92 (2 N$\underline{C}OCH_3$), 158.25 (NHCOO), 131.09 and 130.93 (2 C=C), 104.12 (C-2 of Neu5Acα), 102.65, 101.04 and 100.32 (C-1 of GclNAcβ, C-1 of Galβ, C-1 of Fucα) 78.17-54.15, 49.15 (CD_3OD), 42.52 (C-3 of Neu5Acα), 40.54-22.72, 16.73 (C-6 of Fucα), 14.61 (2 CH_3) ppm.
HRESIMS: found m/z 2217.2444; calc. for $C_{104}H_{187}N_5O_{43}Na$ $[M+Na]^+$ 2217.2492.

1,2-DAG-βAla-PEG(3)-NH_2 AcOH salt (8) To a stirred solution of H_2N-PEG(3)-NH_2 diamine (95 mg, 0.494 mmol; obtained by hydrogenation of N_3-PEG(3)-NH_2 in MeOH in the presence of Pd/C catalyst) in dichloroethane (2 ml), a solution of 1,2-DAG-βAla-ONSu (**4**, 51.1 mg, 0.0613 mmol) in dichloroethane (2 ml) was added quickly dropwise and, after 2 min, the mixture was acidified with AcOH (229 ml, 4 mmol). The solution was evaporated, the resudue was dissolved in ethyl acetate (4 ml) and applied on a silica gel column (20 ml in ethyl acetate). Elution with ethyl acetate/acetonitrile/water/AcOH 20:20:5:1 and then with 10:20:5:1 gave chromatographically individual product which could contain solubilized silica gel. The product was dissolved in $CHCl_3$/MeOH (2:1) mixture and was passed through Sephadex LH-20 column (100 ml in $CHCl_3$/MeOH 2:1 +0.4% AcOH). The selected fractions were evaporated, co-evaporated with heptane and dried in vacuum. Yield of 1,2-DAG-βAla-PEG(3)-NH_2 AcOH salt was 49 mg (82%).
TLC: 1,2-DAG-βAla-PEG(3)-NH_2 AcOH salt R_f = 0.48 (EtOAc/MeCN/water/AcOH 10:20:5:1).
^{1}H NMR for 1,2-DOG-βAla-PEG(3)-NH_2 AcOH salt (700 MHz, [D]$CHCl_3$/[D_4]CH_3OH 1:1, 30°C): δ 5.344 (m, 4H, 2 CH=CH), 5.249 (m, 1H, CH-O(CO)), 4.351 (dd, 1H, J = 12.0, 3.7 Hz, CH-O(CO)), 4.235 (dd, 1H, J = 11.8, 4.7 Hz, CH-O(CO)), 4.152 (m, 2H, 2 CH-O(CO)), 3.727 (m, 2H, CH_2O), 3.684 and 3.646 (m, 8H, 5 CH_2O), 3.560 (t, 2H, *J* = 5.5 Hz, CH_2O),3.397 (m, 4H, 2 CH_2NCO), 3.344 (p, J = 1.7 Hz, residual H of [D4]CH_3OH), 3.127 (m, 2H, $OCH_2C\underline{H}_2NH_2$), 2.409

(t, 2H, J = 6.7 Hz, C<u>H</u>$_2$(CO)OH), 2.339 (m, 4H, 2 CH2(CO)), 2.027 (m, 8H, 4 CH_2-C=C), 2.022 (s, C<u>H</u>$_3$(CO)OH), 1.627 (m, 4H, 2 CH_2-C-(CO)), 1.311 (m, 40H, 20 CH_2), 0.891 (t, 6H, *J* = 7.2 Hz, 2 CH_3) ppm.

^{13}C NMR for 1,2-DOG-βAla-PEG(3)-NH_2 AcOH salt (176 MHz, [D]$CHCl_3$/[D_4]CH_3OH 1:1, 30°C) δ 173.49, 173.09 and 172.08 (<u>C</u>OCH_2), 155.92 (NHCOO), 129.58 and 129.29 (2 C=C), 77.23 ($CDCl_3$), 70.06-69.59 (6 OCH_2 of PEG), 69.21 (C-2 of glycerol), 62.32 and 61.96 (C-1 and C-3 of glycerol), 48.10 (CD_3OD), 39.92, 38.82 and 36.97 (3 CH_2N), 35.47, 33.81 and 33.65 (3 CO<u>C</u>H_2), 31.53-22.25 (26 CH_2), 13.40 (2 CH_3) ppm.

HRESIMS: found m/z 910.7091; calc. for $C_{51}H_{96}N_3O_{10}$ $[M+H]^+$ 910.7090.

1,2-DAG-βAla-PEG(12)-PEG(3)-N_3 (9) To a stirred solution of 1,2-DAG-βAla-PEG(12)-ONSu (**6**, 68 mg, 0.0475 mmol) in dichloroetane (1.6 ml), N_3-PEG(3)-NH_2 amine (15.5 mg, 0.071 mmol) in dichloroethane (0.4 ml) was added. After 15 min, the mixture was acidified with AcOH (4 ml) and was passed through Sephadex LH-20 column (100 ml in $CHCl_3$/MeOH 2:1 +0.1% AcOH). The selected fractions were evaporated, co-evaporated with heptane and dried in vacuum. Yield of 1,2-DAG-βAla-PEG(12)-PEG(3)-N_3 was 67 mg (92%).

TLC: 1,2-DAG-βAla-PEG(12)-PEG(3)-N_3 R_f = 0.43 (ethyl acetate/MeCN/water/AcOH 10:20:5:1).

^{1}H NMR for 1,2-DOG-βAla-PEG(12)-PEG(3)-N_3 (700 MHz, [D]$CHCl_3$/[D_4]CH_3OH 1:1, 30°C): δ 5.346 (m, 4H, 2 CH=CH), 5.246 (m, 1H, CH-O(CO)), 4.354 (dd, 1H, J = 12.0, 3.7 Hz, CH-O(CO)), 4.244 (dd, 1H, J = 11.7, 4.5 Hz, CH-O(CO)), 4.158 (m, 2H, 2 CH-O(CO)), 3.748 (t, 2H, J = 6.2 Hz, OCH_2), 3.702-3.627 (m, 54H, 27 CH_2O of PEGs), 3.565 (m, 4H, 2 OCH_2), 3.401 (m, 8H, 3CH_2NCO and CH_2N_3), 3.344 (p, J = 1.7 Hz, residual H of [D4]CH_3OH), 2.482 (t, 2H, *J* = 6.2 Hz, OCH_2C<u>H</u>$_2$CO), 2.419 (t, 2H, J = 6.6 Hz, C<u>H</u>$_2$(CO)N), 2.339 (m, 4H, 2 CH2(CO)), 2.027 (m, 8H, 4 CH_2-C=C), 1.630 (m, 4H, 2 CH_2-C-(CO)), 1.314 (m, 40H, 20 CH_2), 0.894 (t, 6H, *J* = 7.2 Hz, 2 CH_3) ppm.

^{13}C NMR for 1,2-DOG-βAla-PEG(12)-PEG(3)-N_3 (176 MHz, [D]$CHCl_3$/[D_4]CH_3OH 1:1, 30°C) 173.44, 173.03, 172.15 and 172.03 (2 O<u>C</u>OCH_2 and 2 NH<u>C</u>OCH_2), 156.21 (NHCOO), 129.53 and 129.26 (2 C=C), 77.23 ($CDCl_3$), 70.07-69.15 (29 CH_2O of PEG), 69.10 (C-2 of glycerol), 66.79 (O-<u>C</u>H_2CH_2-COO), 62.28, 61.92 (C-1 and C-3 of glycerol), 50.26 (CH_2-N_3), 48.02 (CD_3OD), 38.86 (O-CH_2<u>C</u>H_2-N), 36.94, 36.19, 35.40, 33.76, 33.60 (CO-<u>C</u>H_2<u>C</u>H_2-NH and 3 CO<u>C</u>H_2), 31.50-22.22 (26 CH_2), 13.35 (2 CH_3) ppm.

HRESIMS: found m/z 1553.0743; calc. for $C_{78}H_{146}N_6O_{23}NH_4$ $[M+NH_4]^+$ 1553.0778.

HRESIMS: found m/z 1558.0332; calc. for $C_{78}H_{146}N_6O_{23}Na$ $[M+Na]^+$ 1558.0332.

Iminophosphorane 1,2-DAG-βAla-PEG(12)-PEG(3)-N=PPh₃ To a stirred solution of 1,2-DAG-βAla-PEG(12)-PEG(3)-N_3 (**9**) (65 mg, 0.0423 mmol) in 1,4-dioxane/water 9:1 (2 ml) triphenylphosphine (88 mg, 0.336 mmol) was added. One hour later, water (0.25 ml) was added, the mixture was kept at room temperature for 3 h, acidified with AcOH to pH 6 and evaporated. The major ninhydrin-positive product (it was supposed this product was 1,2-DAG-βAla-PEG(12)-PEG(3)-NH_2 amine) was isolated on silica gel column (30 ml in ethyl acetate), elution with ethyl acetate/acetonitrile/water/AcOH 10:20:5:1 gave 52 mg (69%) of the title 1,2-DAG-βAla-PEG(12)-PEG(3)-N=PPh_3 iminophosphorane.

TLC: 1,2-DAG-βAla-PEG(12)-PEG(3)-N=PPh_3 R_f = 0.24 (ethyl acetate/MeCN/water/AcOH 10:20:5:1), ninhydrin-positive.

^{1}H NMR for 1,2-DOG-βAla-PEG(12)-PEG(3)-N=PPh_3 (700 MHz, [D_4]CH_3OH, 30°C): δ 7.885, 7.840 and 7.751 (m, total 15H, PPh_3), 5.342 (m, 4H, 2 CH=CH), 5.232 (m, 1H, CH-O(CO)), 4.359 (dd, 1H, J = 12.1, 3.5 Hz, CH-O(CO)), 4.235 (dd, 1H, J = 11.8, 4.3 Hz, CH-O(CO)), 4.140 (m, 2H, 2 CH-O(CO)), 3.73-3.32 (~64H, 32 CH_2 of PEGs), 3.306 (p, J = 1.7 Hz, residual H of [D4]CH_3OH), 3.205 (m, 2H), 2.416 (t, 2H, *J* = 6 Hz, CH_2CO), 2.391 (t, 2H, J = 6.8 Hz, CH_2CO), 2.316 (m, 4H, 2 CH2(CO)), 2.032 (m, 8H, 4 CH_2-C=C), 1.605 (m, 4H, 2 CH_2-C-(CO)), 1.306 (m, 40H, 20 CH_2), 0.898 (t, 6H, *J* = 7.1 Hz, 2 CH_3) ppm.

At pH ~8 in 1,4-dioxane/water (2:1) 1,2-DAG-βAla-PEG(12)-PEG(3)-N=PPh_3 iminophosphorane slowly hydrolyzed by stable in mild conditions phospho-imino bond to 1,2-DAG-βAla-PEG(12)-PEG(3)-NH_2 amine (**10**), but it is accompanied by a noticeable deacylation of diglyceride.

1,2-DAG-βAla-PEG(12)-PEG(3)-NH₂ AcOH salt (10) To a stirred solution of H_2N-PEG(3)-NH_2 diamine (95 mg, 0.494 mmol; obtained by hydrogenolysis of N_3-PEG(3)-NH_2 in MeOH in the presence of Pd/C catalyst) in dichloroethane (1 ml) a solution of 1,2-DAG-βAla-PEG(12)-ONSu (**6**, 89 mg, 0.0621 mmol) in dichloroethane (3 ml) was added quickly dropwise and, in 2 min, the mixture was acidified with AcOH (229 ml, 4 mmol). The solution was evaporated, the resudue was dissolved in ethyl acetate (4 ml) and applied on silica gel column (20 ml in ethyl acetate). Elution with EtOAc/$CHCl_3$/MeOH/water/AcOH (50:20:60:20:3) gave chromatographically individual substance which could contain solubilized silica gel. The product was dissolved in $CHCl_3$/MeOH (2:1) mixture and was passed through Sephadex LH-20 column (100 ml in $CHCl_3$/MeOH, 2:1, +0.4% AcOH). The selected fractions were evaporated, coevaporated with heptane and dried in vacuum. Yield of 1,2-DAG-βAla-PEG(12)-PEG(3)-NH_2 AcOH salt was 88.4 mg (91%).

TLC: 1,2-DAG-βAla-PEG(12)-PEG(3)-NH_2 AcOH salt R_f = 0.45 (ethyl acetate/chloroform/methanol/water/AcOH 50:20:60:20:3).

^{1}H NMR for 1,2-DOG-βAla-PEG(12)-PEG(3)-NH_2 AcOH salt (700 MHz, [D]$CHCl_3$/[D_4]CH_3OH 1:1, 30°C): δ 5.345 (m, 4H, 2 CH=CH), 5.248 (m, 1H, CH-O(CO)), 4.352 (dd, 1H, J = 12.0, 3.7 Hz, CH-O(CO)), 4.242 (dd, 1H, J = 11.8, 4.6 Hz, CH-O(CO)), 4.156 (m, 2H, 2 CH-O(CO)), 3.746, 3.698 and 3.668 (m, 56H, 28 CH_2O of PEGs), 3.577 (t, 2H, *J* = 5.5 Hz, OCH_2), 3.561 (t, 2H, *J* = 5.5 Hz, OCH_2), 3.404 (m, 6H, 3CH_2NCO), 3.344 (p, J = 1.7 Hz, residual H of [D4]CH_3OH), 3.168 (m, 2H, $OCH_2C\underline{H}_2NH_2$), 2.483 (t, 2H, *J* = 6 Hz, $OCH_2C\underline{H}_2CO$), 2.416 (t, 2H, J = 6.6 Hz, $C\underline{H}_2$(CO)N), 2.339 (m, 4H, 2 CH2(CO)), 2.028 (m, 8H, 4 CH_2-C=C), 2.023 (s, $C\underline{H}_3$(CO)OH), 1.627 (m, 4H, 2 CH_2-C-(CO)), 1.312 (m, 40H, 20 CH_2), 0.892 (t, 6H, *J* = 7.2 Hz, 2 CH_3) ppm.

^{13}C NMR for 1,2-DOG-βAla-PEG(12)-PEG(3)-NH_2 AcOH salt (176 MHz, [D]$CHCl_3$/[D_4]CH_3OH 1:1, 30°C) δ 173.47, 173.06, 172.16 and 172.03 (2 $O\underline{C}OCH_2$ and 2 $NH\underline{C}OCH_2$), 156.21 (NHCOO), 129.58 and 129.29 (2 C=C), 77.23 ($CDCl_3$), 70.08-69.19 (29 CH_2O of PEG), 69.14 (C-2 of glycerol), 66.80 (O-$\underline{C}H_2CH_2$-COO), 62.30, 61.95 (C-1 and C-3 of glycerol), 48.09 (CD_3OD), 40.02 and 38.85 (O-$CH_2\underline{C}H_2$-N), 36.95, 36.18, 35.42, 33.80, 33.65 (CO-$\underline{C}H_2\underline{C}H_2$-NH and 3 $CO\underline{C}H_2$), 31.52-22.25 (26 CH_2), 13.40 (2 CH_3) ppm.

HRESIMS: found m/z 1510.0616; calc. for $C_{78}H_{148}N_4O_{23}H$ $[M+H]^+$ 1510.0607.

1,2-DAG-βAla-PEG(12)-PEG(3)-Gly₃-NH₂ AcOH salt (11)

To a stirred solution of 1,2-DAG-βAla-PEG(12)-PEG(3)-NH_2 AcOH salt (**10**) (30.3 mg, 0.0193 mmol) in DMF (1 ml), a solution of Fmoc-Gly_3-ONSu (0.0386 mmol in 0.4 ml of DMF; prepared from Fmoc-Gly_3-OH, *N*,*N′*-dicyclohexylcarbodiimide and *N*-hydroxysuccinimide without isolation) was added. In 1 h at rt, piperidine (70 µl) was added and, 15 min later, the mixture was acidified with AcOH (162 µl). The solution was passed through Sephadex LH-20 column (100 ml in $CHCl_3$/MeOH, 2:1, +0.4% AcOH), selected fractions were combined and evaporated. The residue (contains small impurities) was dissolved in ethyl acetate (4 ml) and applied on silica gel column (20 ml in ethyl acetate). Elution with ethyl acetate/chloroform/methanol/water/AcOH 50:20:60:20:3 gave chromatographically individual substance which could contain solubilized silica gel. The product was dissolved in chloroform/methanol 2:1 mixture and was passed through Sephadex LH-20 column (100 ml in chloroform/methanol 2:1 +0.4% AcOH). The selected fractions were evaporated, co-evaporated with heptane and dried in vacuum. Yield of 1,2-DAG-βAla-PEG(12)-PEG(3)-Gly_3-NH_2 AcOH salt was 27.3 mg (81%).

TLC: 1,2-DAG-βAla-PEG(12)-PEG(3)-Gly_3-NH_2 AcOH salt R_f = 0.38 (ethyl acetate/chloroform/methanol/water/AcOH 50:20:60:20:3).

^{1}H NMR for 1,2-DOG-βAla-PEG(12)-PEG(3)-Gly_3-NH_2 AcOH salt (700 MHz, [D]$CHCl_3$/[D_4]CH_3OH 1:1, 30°C): δ 5.346 (m, 4H, 2 CH=CH), 5.250 (m, 1H, CH-O(CO)), 4.355 (dd, 1H, J = 12.0, 3.7 Hz, CH-O(CO)), 4.243 (dd, 1H, J = 11.8, 4.6 Hz, CH-O(CO)), 4.158 (m, 2H, 2 CH-O(CO)), 3.982 (s, 2H, CH_2NHCO of Gly), 3.911 (s, 2H, CH_2NHCO of Gly), 3.766 (s, 2H, $C\underline{H}_2NH_2$ of Gly), 3.750 (t, 2H, *J* = 6.2 Hz, CH_2O), 3.670 and 3.644 (m, 52H, 26 CH_2O of PEGs), 3.578 (m, 6H, 3 CH_2O of PEGs), 3.429 (t, 2H, *J* = 5.4 Hz, CH_2NCO), 3.400 (m, 6H, 3 CH_2NCO), 3.344 (p, J = 1.7 Hz, residual H of [D4]CH_3OH), 2.489 (t, 2H, *J* = 6.2 Hz, $OCH_2C\underline{H}_2CO$), 2.419 (t, 2H, J = 6.6 Hz, $C\underline{H}_2$(CO)OH), 2.340 (m, 4H, 2 CH2(CO)), 2.030 (m, 8H, 4 CH_2-C=C), 2.023 (s, $C\underline{H}_3$(CO)OH), 1.628 (m, 4H, 2 CH_2-C-(CO)), 1.309 (m, 40H, 20 CH_2), 0.894 (t, 6H, *J* = 7.2 Hz, 2 CH_3) ppm.

^{13}C NMR for 1,2-DOG-βAla-PEG(12)-PEG(3)-Gly_3-NH_2 AcOH salt (176 MHz, [D]$CHCl_3$/[D_4]CH_3OH 1:1, 30°C) δ 173.44, 173.03, 172.15 and 172.05 (2 $O\underline{C}OCH_2$ and 2 $NH\underline{C}OCH_2$), 169.72, 169.56 and 167.39 (3 $\underline{C}OCH_2N$), 156.22 (NHCOO), 129.53 and 129.24 (2 C=C), 77.23 ($CDCl_3$), 70.06-69.10 (29 CH_2O of PEG), 68.92 (C-2 of glycerol), 66.76 (O-$\underline{C}H_2CH_2$-COO), 62.27 and 61.93 (C-1 and C-3 of glycerol), 48.00 (CD_3OD), 42.29, 42.04 and 40.80 (3 $CO\underline{C}H_2N$), 40.61, 38.82 (O-$CH_2\underline{C}H_2$-N), 36.94-33.60 (CO-$\underline{C}H_2\underline{C}H_2$-NH and 3 $CO\underline{C}H_2$), 31.49-22.21 (26 CH_2), 13.34 (2 CH_3) ppm.

HRESIMS: found m/z 841.0655; calc. for $C_{84}H_{157}N_7O_{26}H_2$ $[M+2H]^{2+}$ 841.0662.

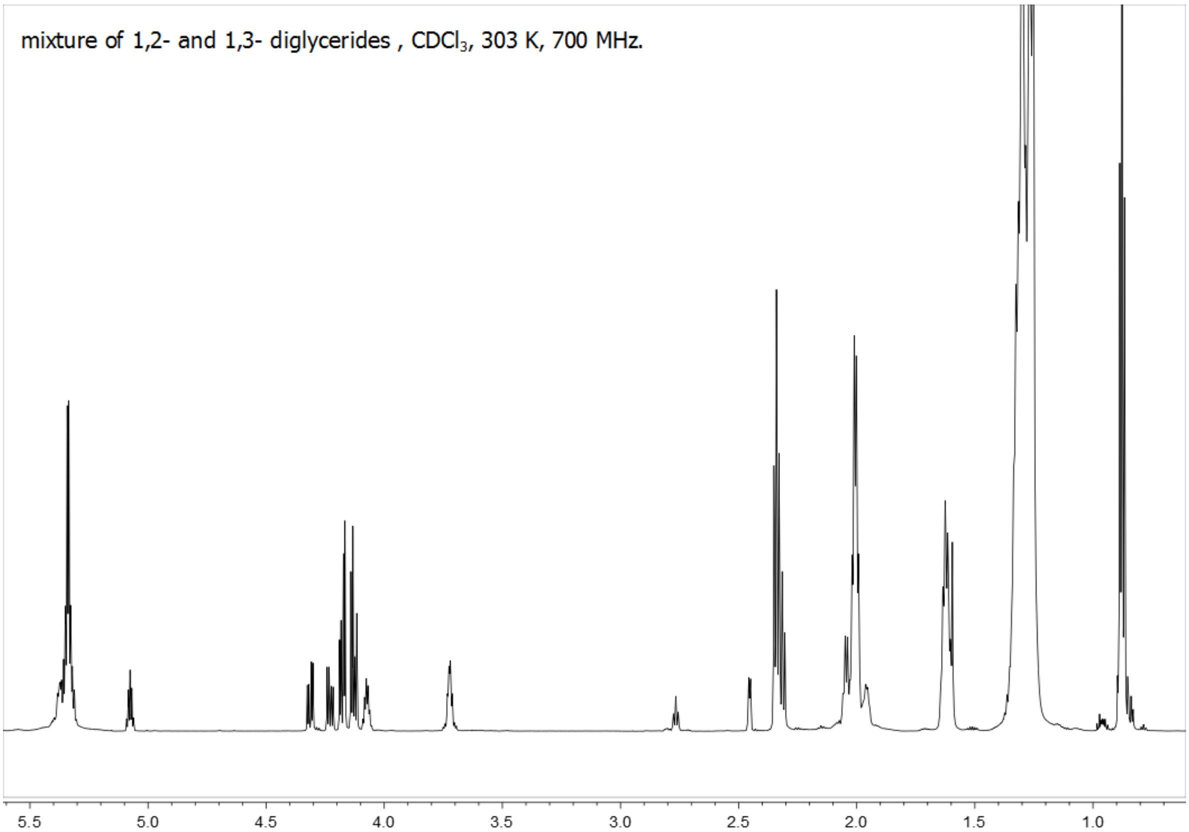
References

S1. M. Bockisch, *Fats and Oils Handbook*, 1st edn., AOCS Press, Hamburg, 1998, pp. 214–215. https://doi.org/10.1016/C2015-0-02417-0

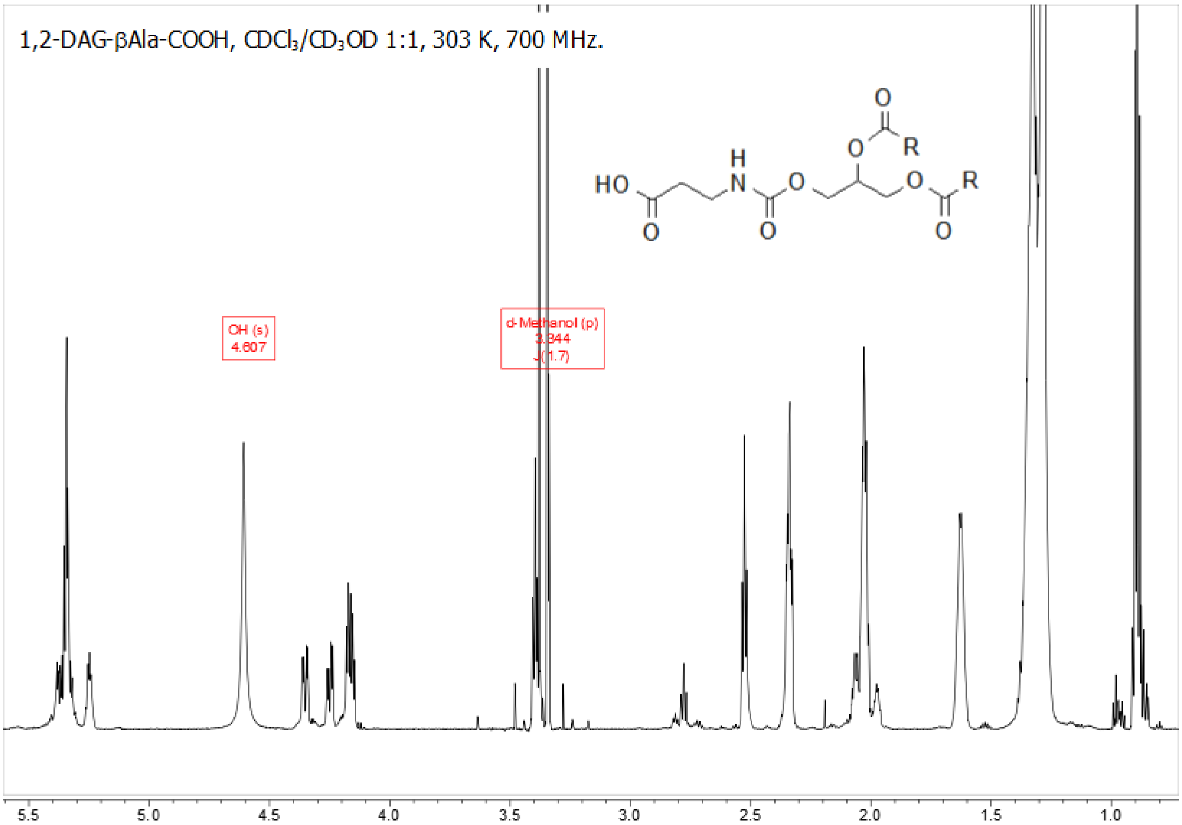
S2. G.V. Pazynina, M.A. Sablina, A.B. Tuzikov, A.A. Chinarev and N.V. Bovin. Synthesis of complex α2-3 sialooligosaccharides, including sulfated and fucosylated ones, using Neu5Acα2-3Gal as a building block. *Mendeleev Commun.*, 2003, **13** (6), 245-248. DOI: 10.1070/MC2003v013n06ABEH001845

S3. A. M. Tsedilin, A. N. Fakhrutdinov, D. B. Eremin, S. S. Zalesskiy, A. O. Chizhov, N. G. Kolotyrkina and V. P. Ananikov. *Mendeleev Commun.*, 2015, **25** (11), 454.

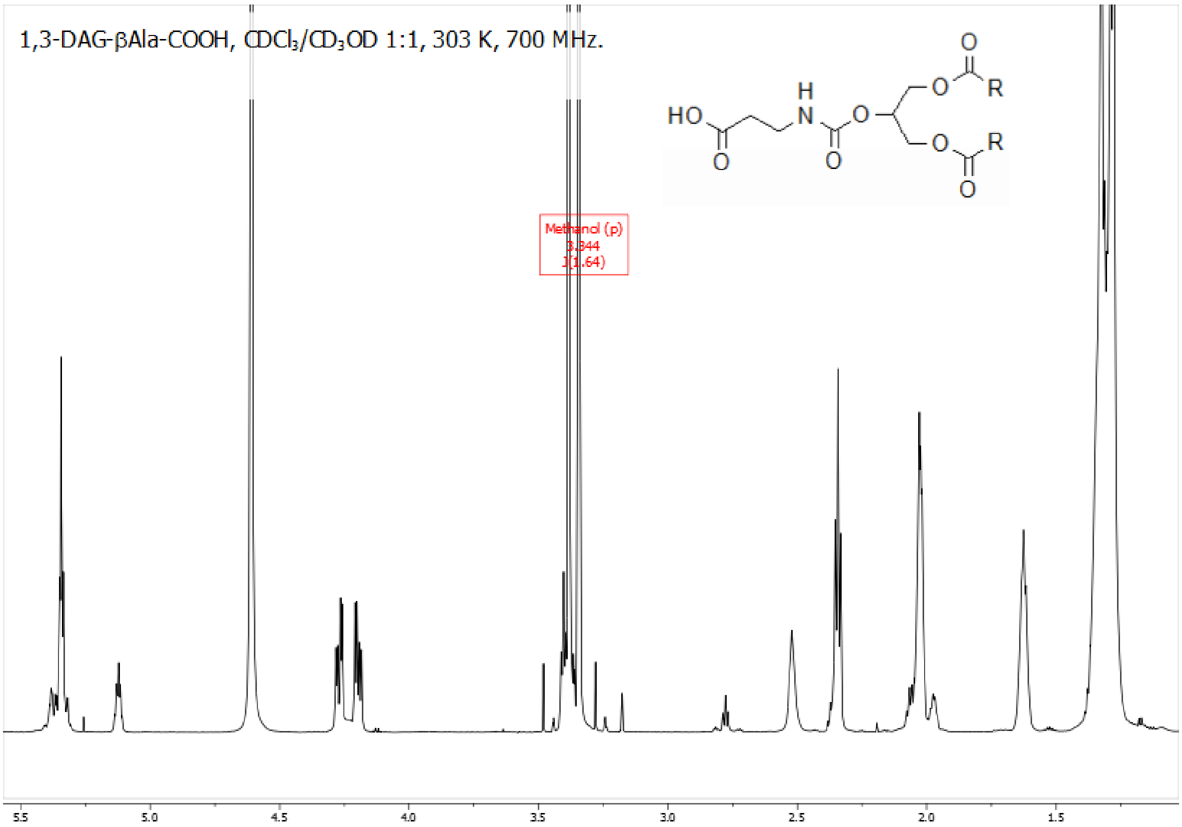
NMR spectra



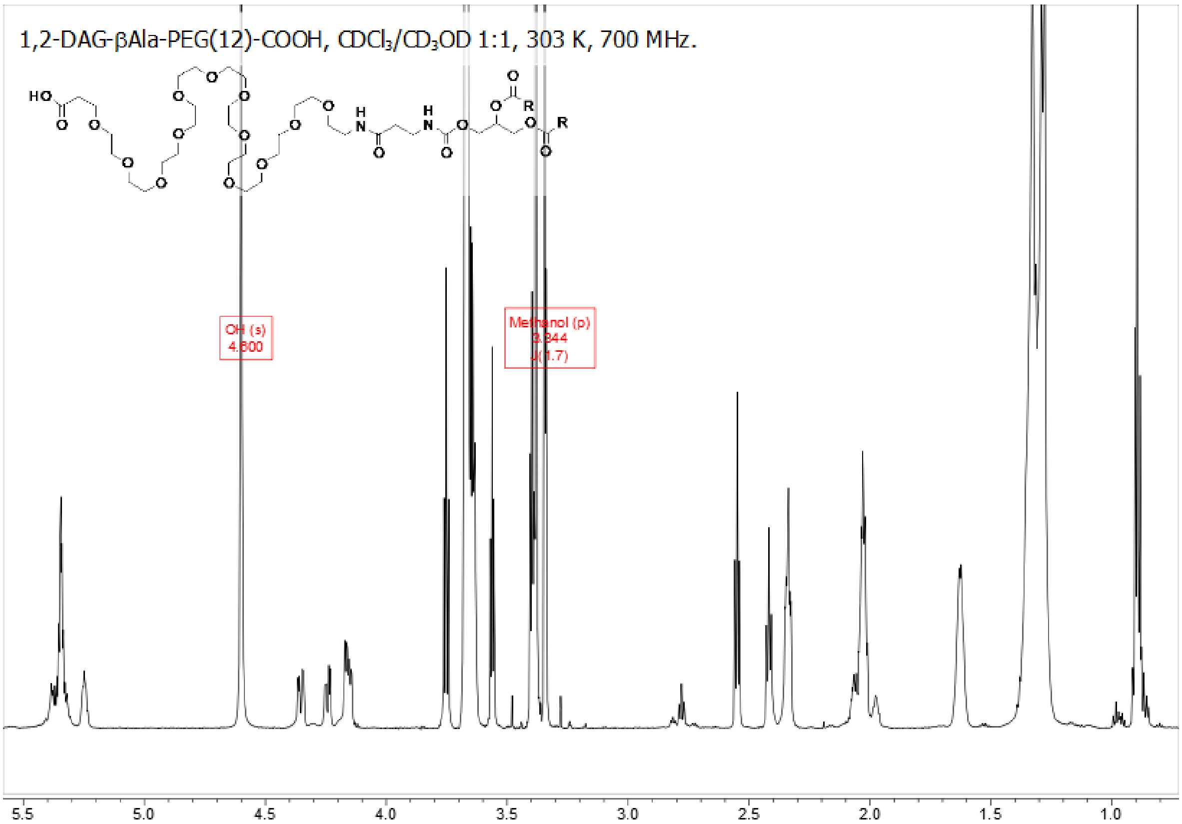
*[1]H NMR spectrum of mixture of 1,2- (**1a**) and 1,3- (**1b**) glycerides (~40:60).*



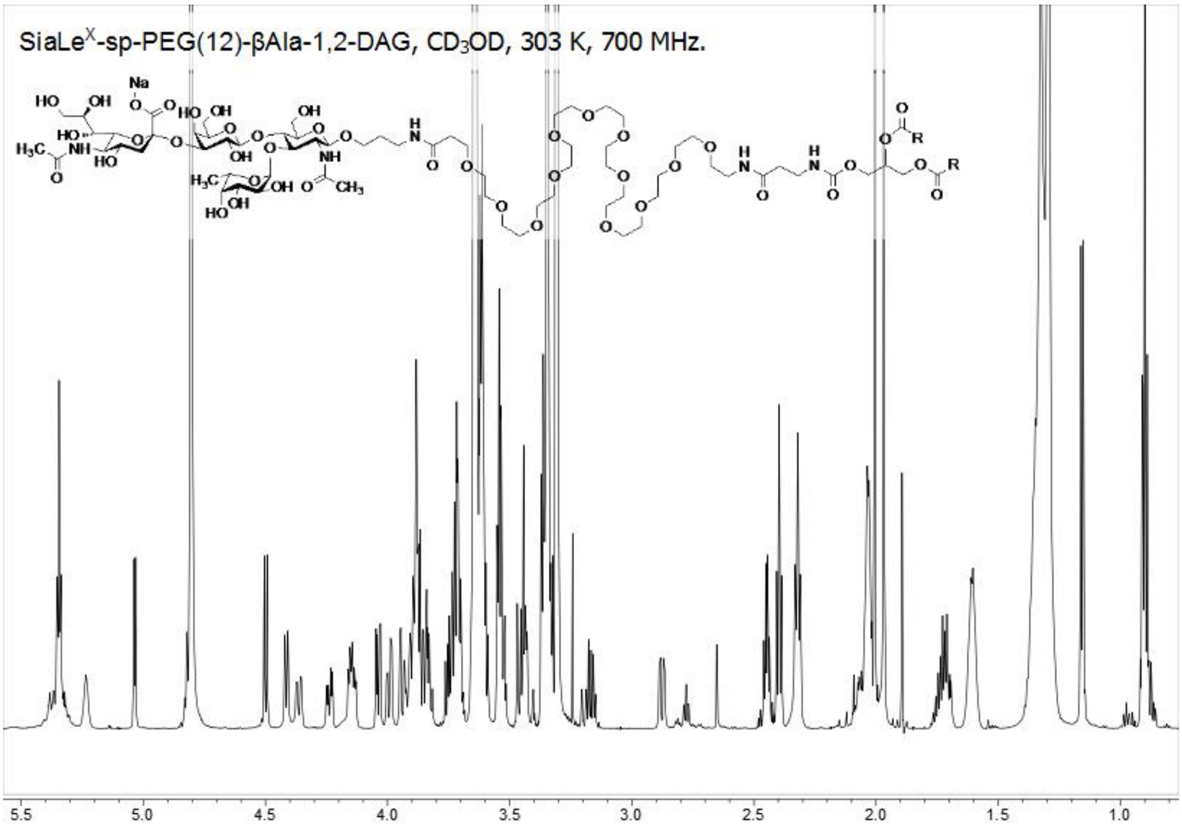
*[1]H NMR spectrum of 1,2-DAG-βAla-COOH (**3**).*



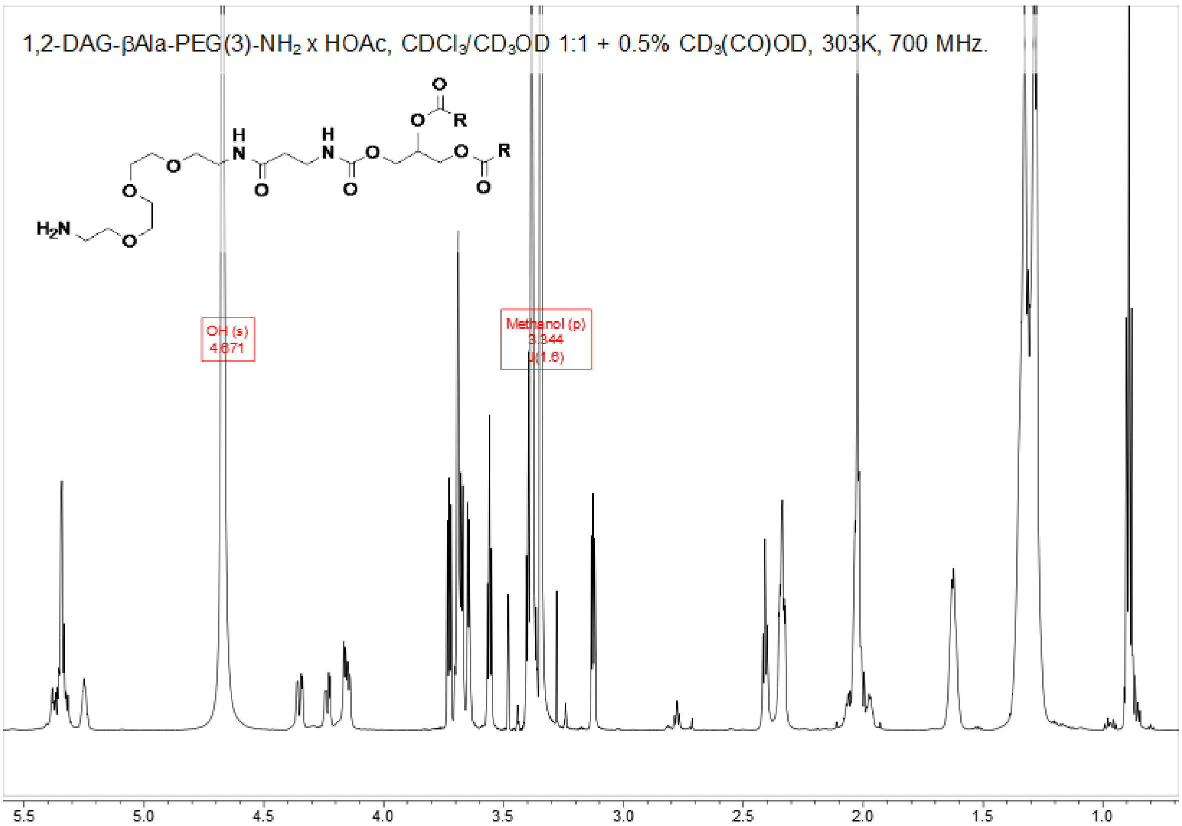
^{1}H NMR spectrum of 1,3-DAG-βAla-COOH.



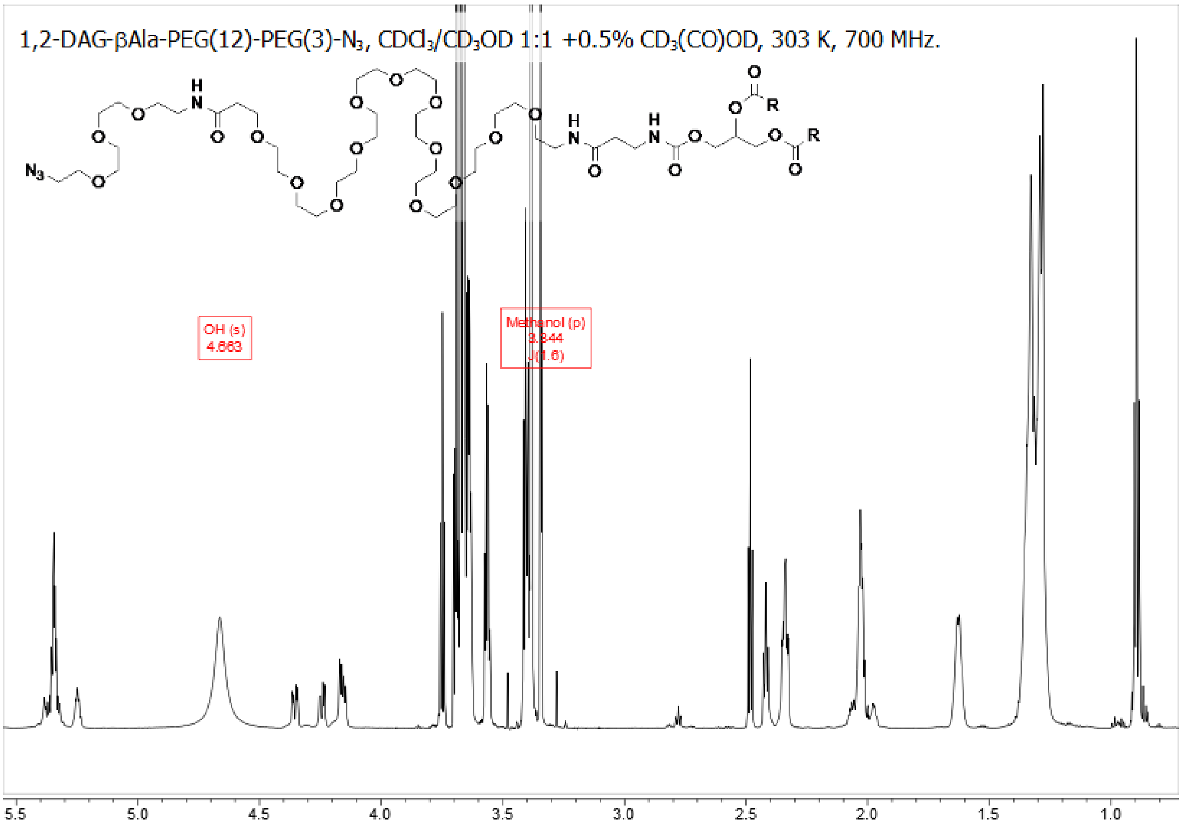
^{1}H NMR spectrum of 1,2-DAG-βAla-PEG(12)-COOH (**5**).



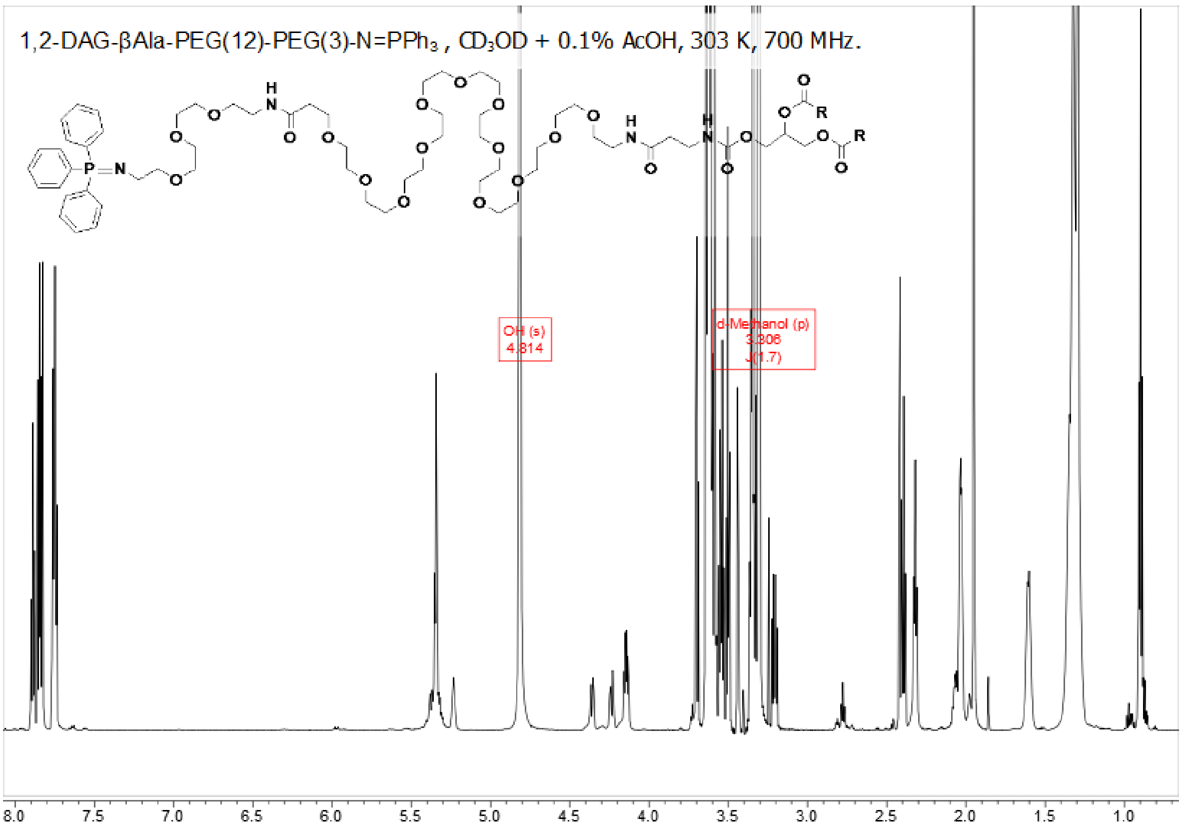
^{1}H NMR spectrum of SiaLe^{X}-sp-PEG(12)-βAla-1,2-DAG (**7**).



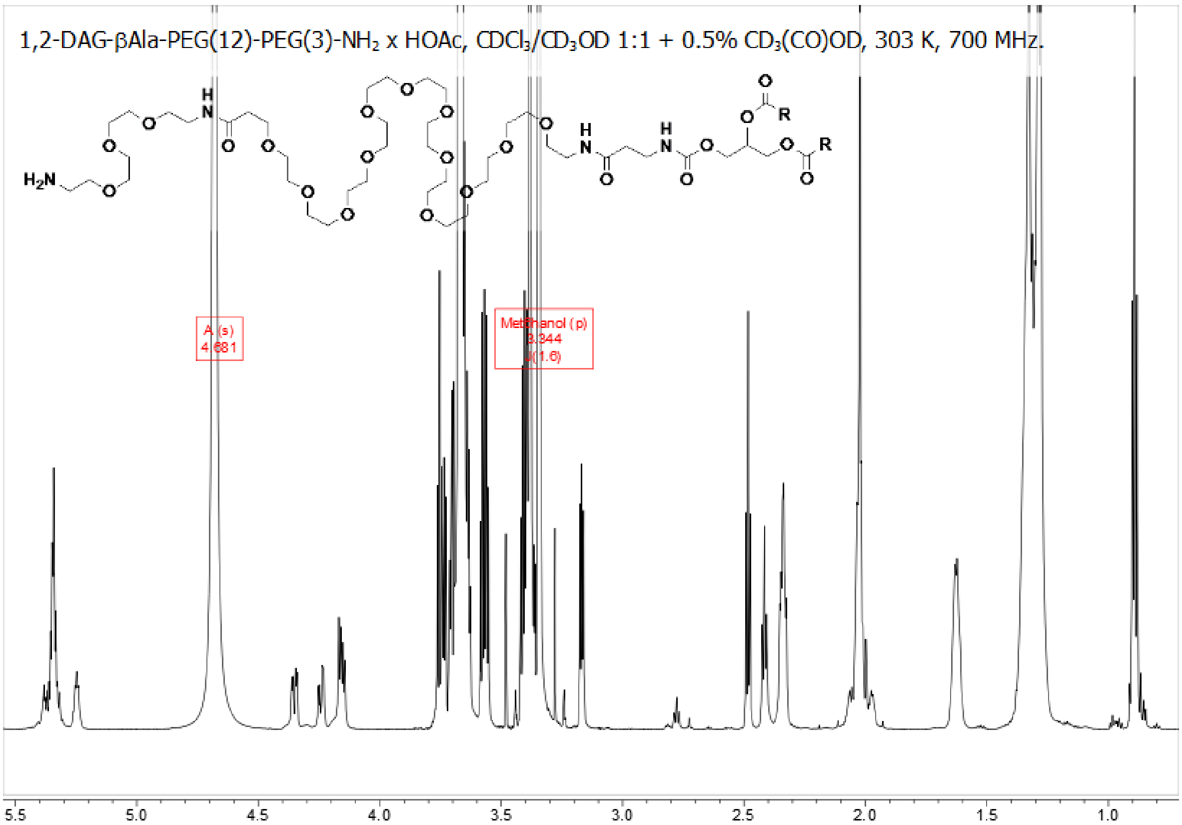
^{1}H NMR spectrum of 1,2-DAG-βAla-PEG(3)-NH_2 AcOH salt (**8**).



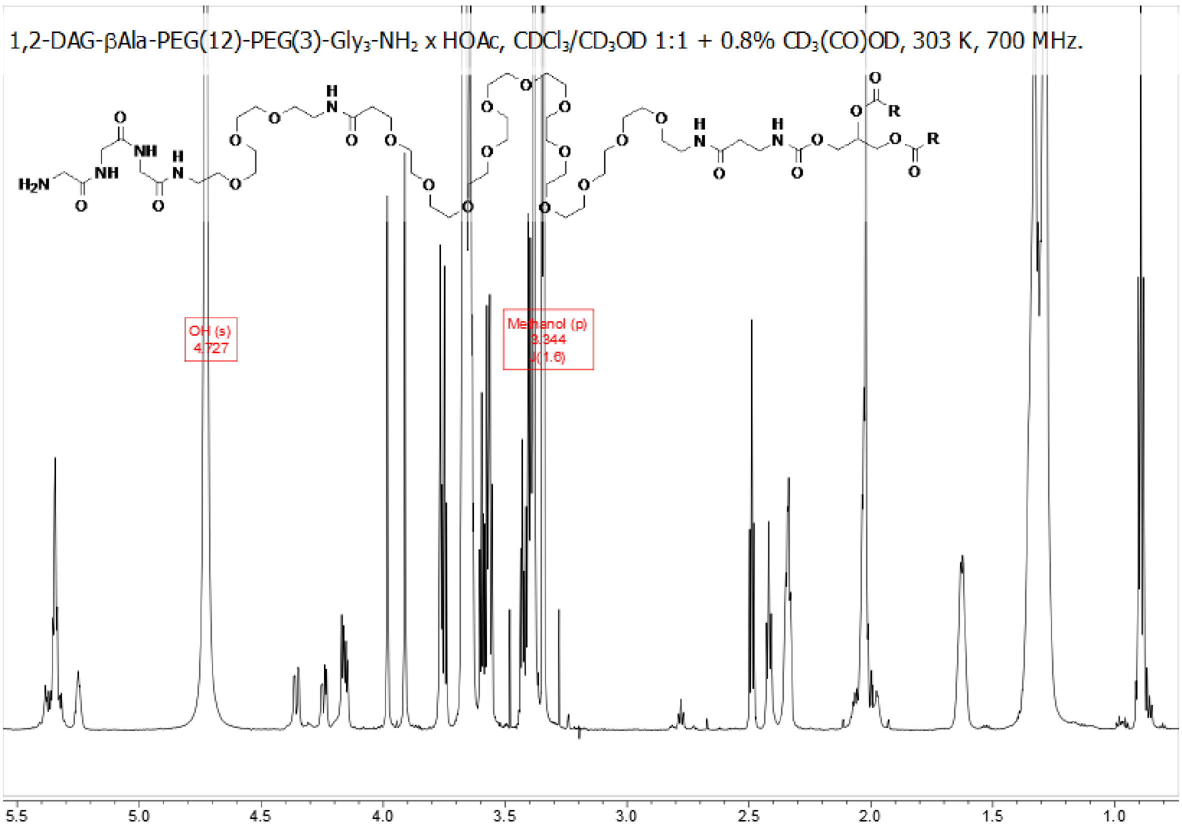
^1H NMR spectrum of 1,2-DAG-βAla-PEG(12)-PEG(3)-N_3 (**9**).



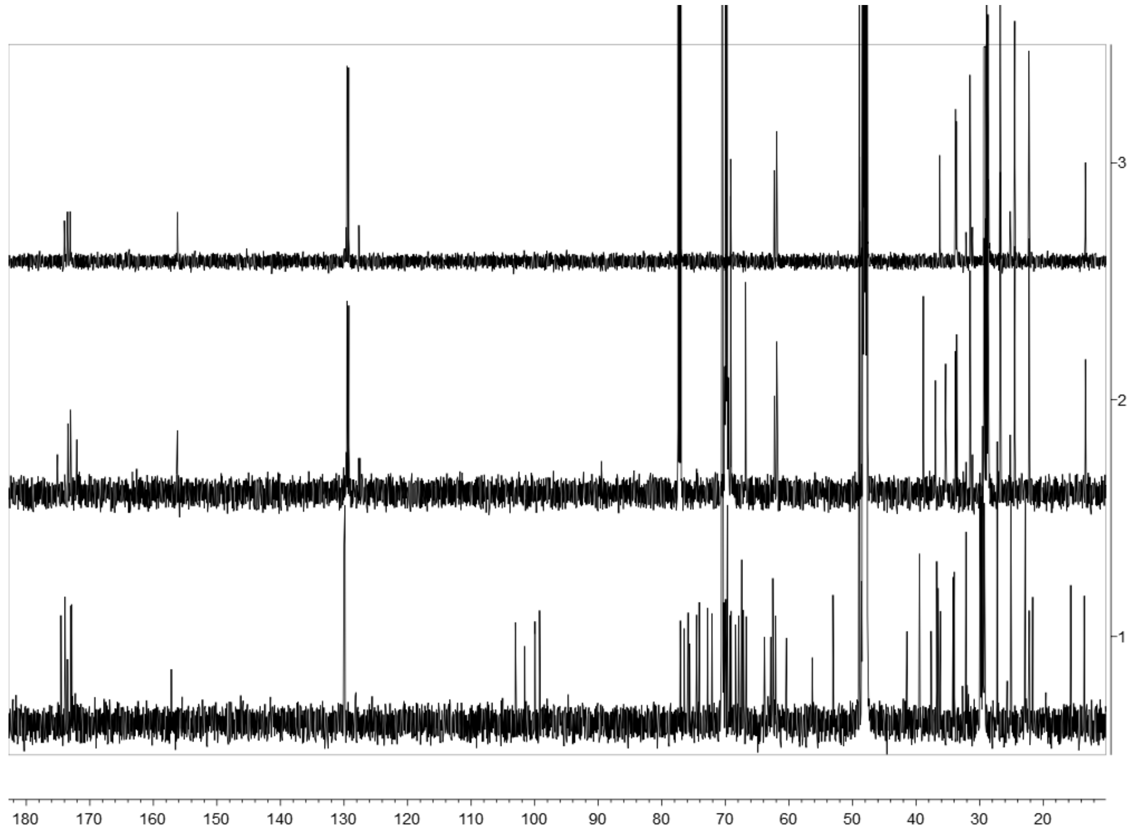
^1H NMR spectrum of iminophosphorane 1,2-DAG-βAla-PEG(12)-PEG(3)-N=PPh_3 .



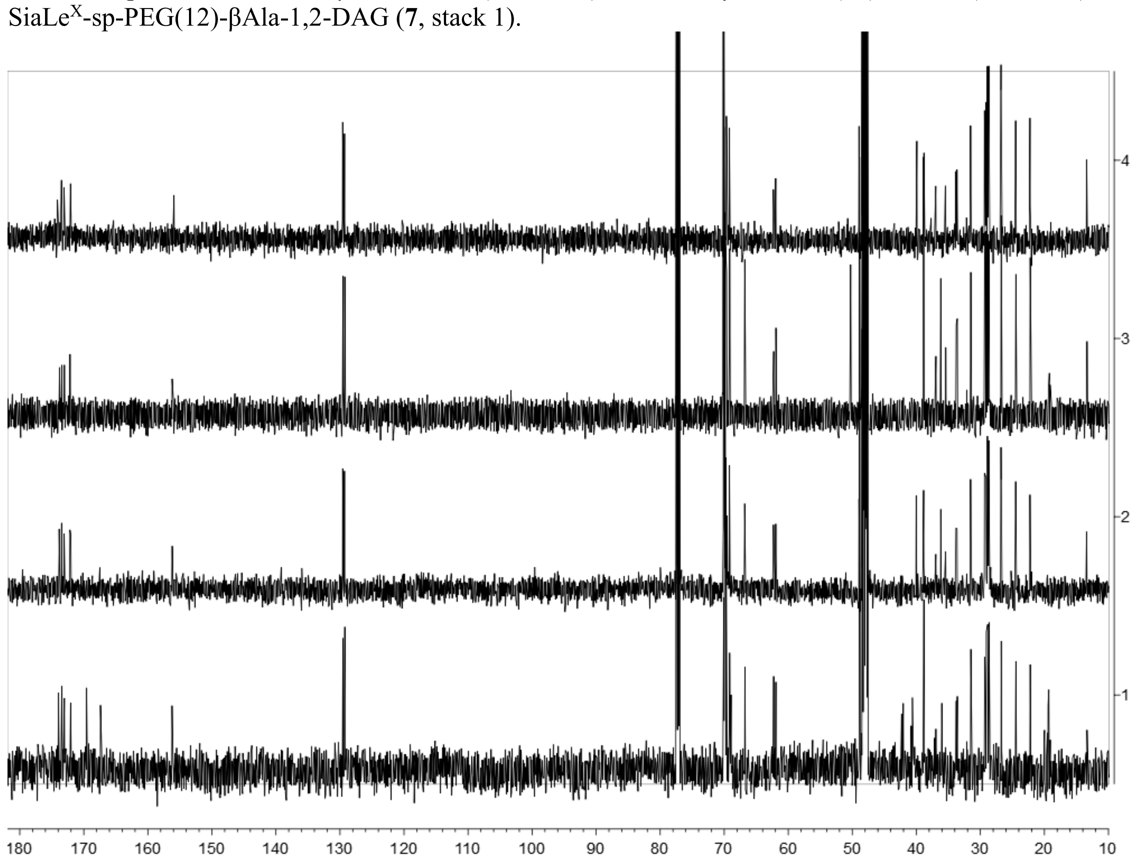
^1H NMR spectrum of 1,2-DAG-βAla-PEG(12)-PEG(3)-NH_2 AcOH salt (**10**).



^1H NMR spectrum of 1,2-DAG-βAla-PEG(12)-PEG(3)-Gly_3-NH_2 AcOH salt (**11**).

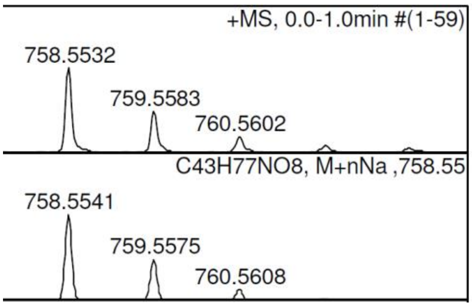


^{13}C NMR spectra of 1,2-DAG-βAla-COOH (**3**, stack 3), 1,2-DAG-βAla-PEG(12)-COOH (**5**, stack 2) and SiaLe^{X}-sp-PEG(12)-βAla-1,2-DAG (**7**, stack 1).

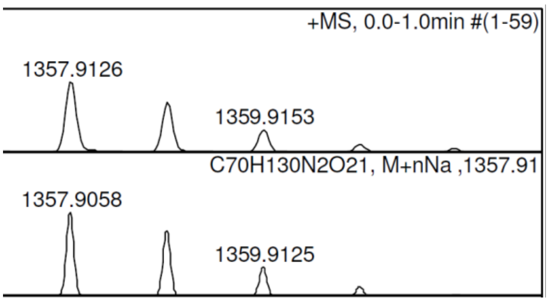


^{13}C NMR spectra of 1,2-DAG-βAla-PEG(3)-NH_2 (**8**, stack 4), 1,2-DAG-βAla-PEG(12)-PEG(3)-N_3 (**9**, stack 3), 1,2-DAG-βAla-PEG(12)-PEG(3)-NH_2 (**10**, stack 2) and 1,2-DAG-βAla-PEG(12)-PEG(3)-Gly_3-NH_2 (**11**, stack 1).

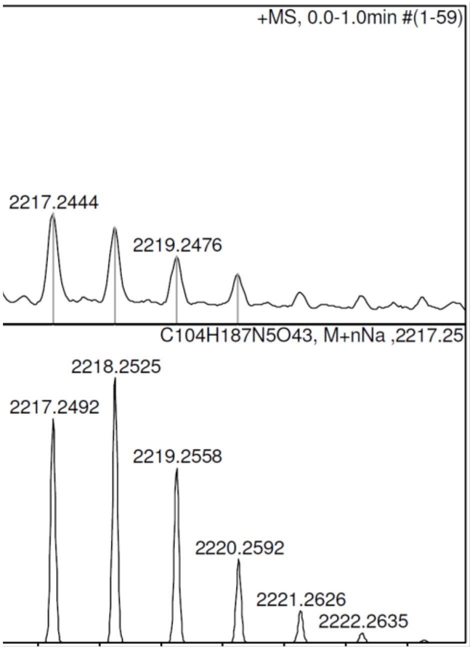
Fragments of mass spectra corresponding to the major component — 1,2-dioleoylglycerol (DOG) derivative



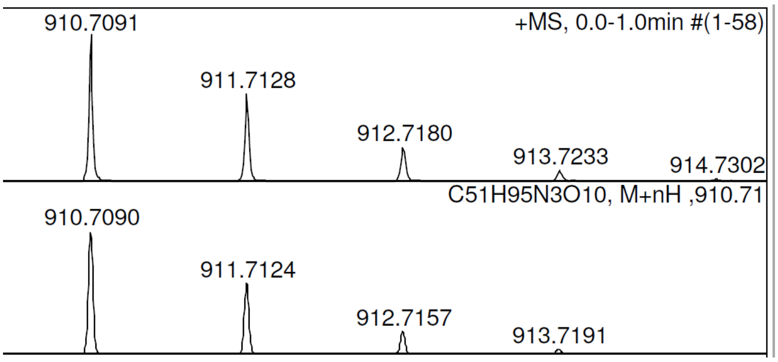
*1,2-DOG-βAla-COOH (**3**)*
HRESIMS: found m/z 758.5532; calc. for $C_{43}H_{77}NO_8Na$ $[M+Na]^+$ 758.5541.



*1,2-DOG-βAla-PEG(12)-COOH (**5**)*
HRESIMS: found m/z 1357.9126; calc. for $C_{70}H_{130}N_2O_{21}Na$ $[M+Na]^+$ 1357.9058.

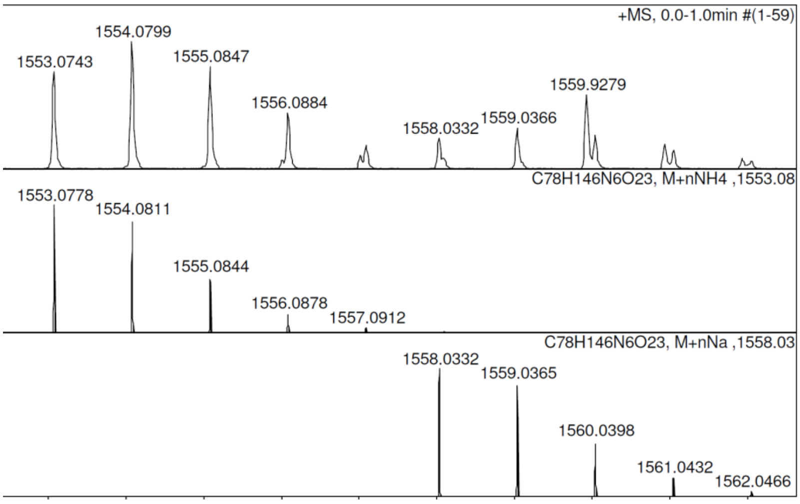


*SiaLe^X-sp-PEG(12)-βAla-1,2-DOG (**7**)*
HRESIMS: found m/z 2217.2444; calc. for $C_{104}H_{187}N_5O_{43}Na$ $[M+Na]^+$ 2217.2492.



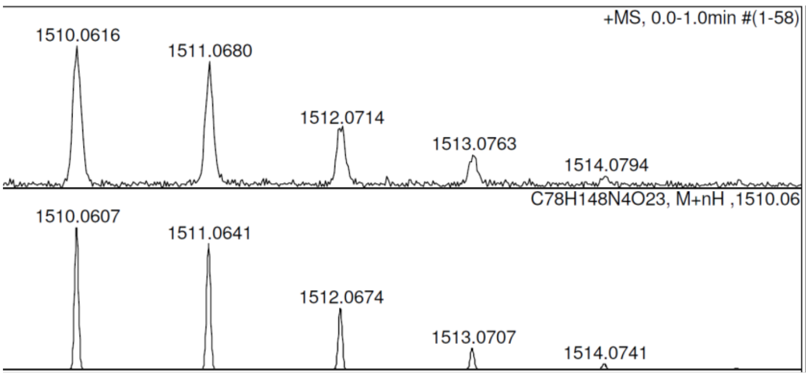
*1,2-DOG-βAla-PEG(3)-NH$_2$ (**8**)*

HRESIMS: found m/z 910.7091; calc. for $C_{51}H_{96}N_3O_{10}$ [M+H]$^+$ 910.7090.



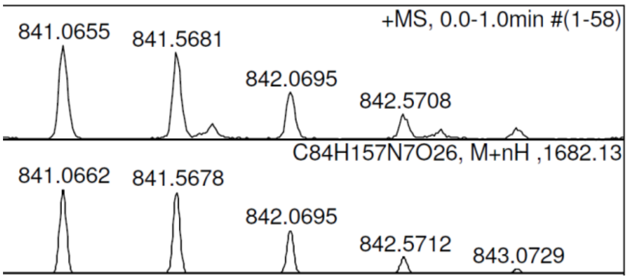
*1,2-DOG-βAla-PEG(12)-PEG(3)-N$_3$ (**9**)*

HRESIMS: found m/z 1553.0743; calc. for $C_{78}H_{146}N_6O_{23}NH_4$ [M+NH$_4$]$^+$ 1553.0778.
HRESIMS: found m/z 1558.0332; calc. for $C_{78}H_{146}N_6O_{23}Na$ [M+Na]$^+$ 1558.0332.



*1,2-DOG-βAla-PEG(12)-PEG(3)-NH$_2$ (**10**)*

HRESIMS: found m/z 1510.0616; calc. for $C_{78}H_{148}N_4O_{23}H$ [M+H]$^+$ 1510.0607.



*1,2-DOG-βAla-PEG(12)-PEG(3)-Gly$_3$-NH$_2$ (**11**)*

HRESIMS: found m/z 841.0655; calc. for $C_{84}H_{157}N_7O_{26}H_2$ $[M+2H]^{2+}$ 841.0662.