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Synthesis of novel amphiphilic spin probes with the paramagnetic doxyl group in the polar region

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ABSTRACT

The use of ESR and specially designed spin probes has led to major breakthroughs in understanding the complexity of biological membranes. Research has been focused mainly on molecular events within the lipid bilayer, and few probes have been designed for studying events in the extracellular space near the membrane surface. We have prepared a series of amphiphilic spin probes in which an ethylene glycol type hydrophilic spacer was introduced between a hydrophobic anchor and the doxyl group, placing the latter above the membrane in the extracellular space. Furthermore, the 2pπ orbital, containing the unpaired electron of the nitroxide group, would be orientated perpendicular to the membrane surface, making it more useful for ESR investigations of structural and dynamic properties close to the membrane surface in different situations of the cell life.

1. Introduction

Development of stable nitroxide free radicals has been intensive in the last 50 years.[1] Originally, they were used in electron spin resonance (ESR) spectroscopy as spin probes and spin labels.[2] Later they became important in chemistry as co-oxidants and antioxidants in materials science,[3] as candidates for organic magnets,[4] as polymerization modulators,[5] as pH sensitive probes[6] and, in biology and pharmacology, as biologically active ligands,[7–10] etc. Furthermore, the combination of two labels in the same molecule, e.g., a paramagnetic nitroxide and a fluorescent dye opens up new fields of use as dual probes.[11]

Nitroxides, with their unique stability of the radical, which is resistant to many mild reaction conditions, and especially with their paramagnetic anisotropy, are becoming more and more important as molecular tools, since their structure can be adapted to particular requirements. The most popular and useful heterocyclic compounds containing a nitroxide group are shown in Figure 1.

These compounds have contributed to the determination of the structure and dynamics of biological membranes.[12] More recently they have been applied in ESR determination of membrane heterogeneity, aggregation of membrane components and membrane domain formation. In the last 30 years, membrane investigations have been focused mostly on molecular events within the lipid bilayer. Consequently the paramagnetic oxazolidine (doxyl) ring containing the nitroxide group (Fig. 2) was located mostly in a lipophilic part of the probe. Only in some cases was the nitroxide moiety (of piperidine or pyrrolidine type) located in the polar head group of phospholipids.[13] Due to the possibility of introducing the doxyl group onto the fatty acid alkyl chain, such probes have a great potential for investigating molecular events in different regions of the membrane bilayer. Recently, molecular dynamics simulations of spin labelled alkyl phosphates in model lipid bilayer have been reported,[14,15] showing that the position of the doxyl group along the fatty acid alkyl chain has an influence on the overall spin probe–membrane interaction. However, the small doxyl group shows only a small perturbation effect on the surrounding alkyl chain motion in a lipid bilayer.

While the structure and dynamics of the lipid part of the membrane have been widely studied, molecular events in the extracellular space in the vicinity of the membrane surface remain to be investigated. The structure of the extracellular matrix is much more complex than that of the lipid bilayer. Also the expected dynamics of oligosaccharide units of glycolipids, glycopeptides and other membrane constituents exposed to the outer membrane space are likely to be important in cell–cell interactions. There is, therefore, a need for spin probes reporting directly from the extracellular membrane region. Recently, the first synthesis and application[16,17] of a spin probe to describe the dynamics close to a biological membrane surface has been reported. The spin probe has a piperidine type of nitroxide (Fig. 3) in the extracellular polar region. However, due to the predominantly parallel orientation to the membrane surface of the 2pπ orbital (along the z axis) containing the unpaired electron of the nitroxide group, the spin probe

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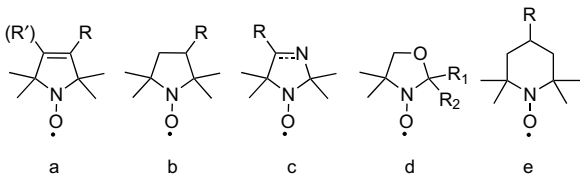


Figure 1. Typical structures of stable nitroxides: pyrroline (a), pyrrolidine (b), imidazoline (c), oxazolidine ('doxyl', d) and piperidine (e). R marks the position where different groups can be attached.

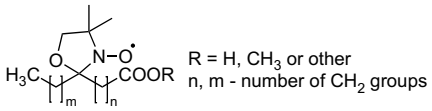


Figure 2. General structure of a fatty acid spin probe.

was not optimal. A probe with predominantly perpendicular 2pπ orbital orientation would be more useful.[18]

Here we present the synthesis of novel amphiphilic spin probes, which (i) would be able to report from the extracellular region close to membrane surface and in which, in its extended conformation, (ii) the nitroxide 2pπ orbital would be perpendicular to the membrane surface. The design of these compounds is based on three requirements: they should comprise (i) a lipophilic part acting as an anchor, enabling its insertion into the lipid bilayer, (ii) a polar spacer group of variable length unable to penetrate into the lipid and (iii) at the opposite end of the polar spacer, a doxyl type of nitroxide with minimal lipophilic character. The latter is of importance due to the possibility that the nitroxide moiety might curl back in the hydrophobic region of the membrane if a more hydrophobic nitroxide was used. According to our experiences from synthesis of doxyl fatty acids the compounds with the doxyl group are more polar than the parent carbonyl compounds. Moreover, Mravljak et al.[14] pointed out that more or less ordered membrane surrounding tries to push the doxyl group out of the hydrophobic region and only the length (i.e., large lipophilicity) of the alkyl chains attached to the doxyl group force it to be located within the lipid bilayer. In our case, the doxyl group is located at the very end of the polar spacer and it has a little chance to penetrate into the hydrophobic membrane region. Furthermore, when the probe conformation is extended, its 2pπ orbital should be perpendicular to the membrane surface (Fig. 3). In the novel amphiphilic spin probes presented, ethylene glycol and amino glycol types of spacer were used. The distance between the nitroxide group and lipophilic part of the probe would vary (Table 1, Fig. 4) from 0.9 nm to 1.7 nm.

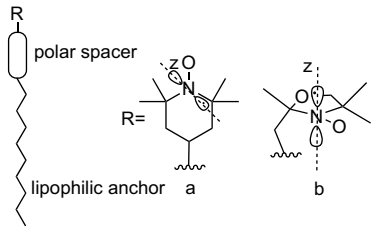


Figure 3. Amphiphilic spin probe after insertion of the lipophilic part into the membrane bilayer has nitroxide group in water surroundings and its 2pπ orbital is perpendicular (a) or parallel (b) to the long molecular axis or parallel (a) and perpendicular (b) to the membrane surface.

Table 1
Distances between the acyl carbonyl and nitrogen of the nitroxide group (Fig. 4)

Compounds	**4**, **5**	**15**, **17**	**14**, **16**	**22**, **23**	**27**, **28**
Distance (*d*)/nm	1.7	1.3	0.9	0.9	1.3

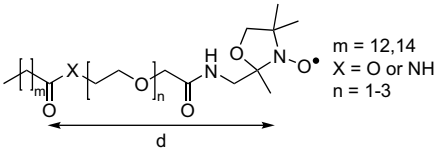
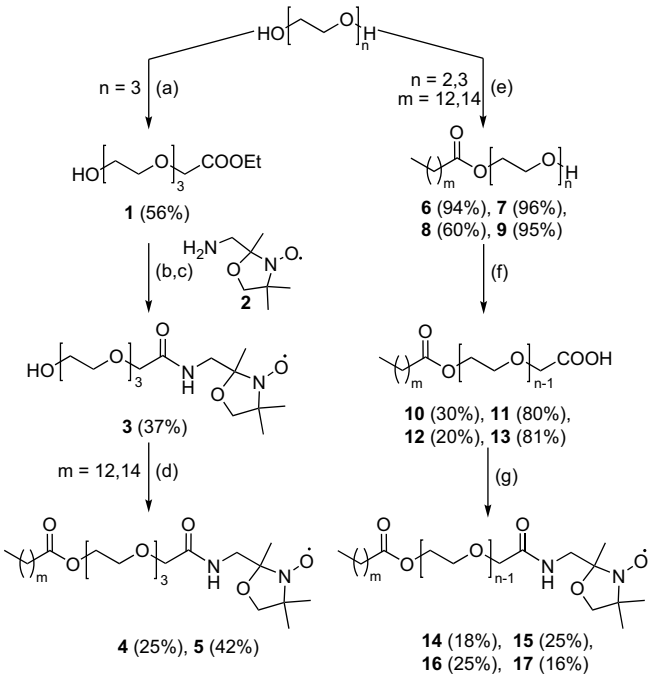


Figure 4. Schematic structure of final compounds.

2. Results and discussion

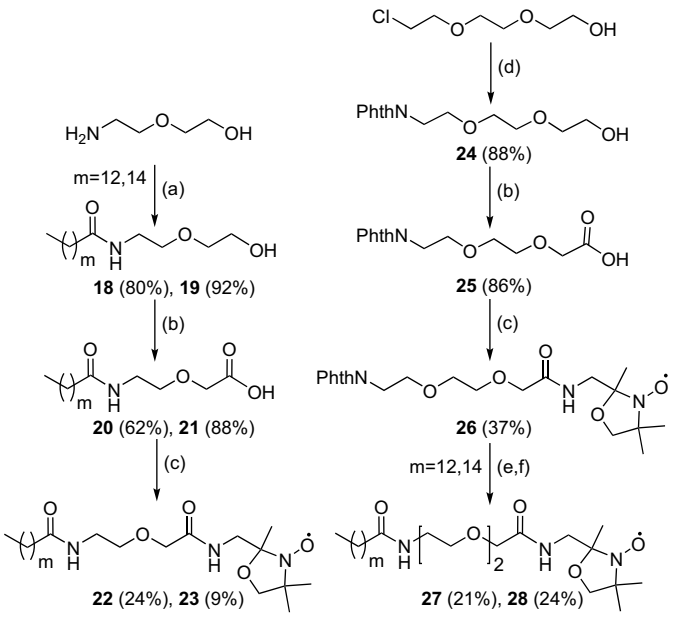
All paramagnetic compounds were characterized by NMR spectroscopy, using in situ nitroxide reduction by phenylhydrazine to the corresponding non-paramagnetic derivatives of hydroxylamine.[19] The final compounds (Scheme 1) have a tetradecanoyl (**4**, **14**, **15**, **22**, **27**) or palmitoyl (**5**, **16**, **17**, **23**, **28**) acyl residue as the lipophilic part of the molecule. Both acyl residues are natural anchors for proteins, which are bound to biological membranes.



Scheme 1. Reagents and conditions: (a) ethyl diazoacetate, $BF_3 \cdot Et_2O$, CH_2Cl_2, 0 °C, 2 h; (b) NaOH, dioxane/H_2O=2:1, rt, 2 h; (c) **2**, DCC, CH_2Cl_2, 0 °C, 3 h; (d) acyl chloride, Et_3N, CH_2Cl_2, 0 °C, 0.5 h; (e) acyl chloride, Et_3N, CH_2Cl_2, 0 °C, 6 h; (f) CrO_3 dissolved in 35% H_2SO_4, acetone, 0 °C→rt, 3 h; (g) **2**, DCC, Et_3N, acetone, 0 °C→rt, 2 h.

The central part of the probes is a polar spacer of variable length (Table 1), for that role mono-, di- and triethylene glycols or the corresponding amino ethylene glycols were chosen. Acyl residues are bound to the polar spacer via an ester bond (**4**, **5**, **14**, **15**, **16** and **17**) (Scheme 1) or an amide bond (**22**, **23**, **27** and **28**) (Scheme 2). Final compounds **4** and **5** have the longest spacer (Table 1), composed of triethylene glycol residues. The synthetic strategy includes the reaction of commercially available triethylene glycol with ethyl

diazoacetate, hydrolysis of the resulting ester **1**, followed by coupling of the acid with amine **2**.[20] The free amine **2** was unstable after removal of the phthalimido protecting group with methylamine. It was necessary to use it immediately after deprotection and without purification. The instability of free amine **2** was probably the main reason for the relatively low yields of compounds **3**, **14**, **15**, **16**, **17**, **22**, **23** and **26**. Final esters **4** and **5** were obtained by acylating the hydroxyl moiety of compound **3** with the corresponding acid chloride (Scheme 1).



Scheme 2. Reagents and conditions: (a) acyl chloride, Et_3N, CH_2Cl_2, 0 °C, 4 h; (b) CrO_3 dissolved in 35% H_2SO_4, acetone, 0 °C→rt, 3 h; (c) **2**, TBTU, Et_3N, CH_2Cl_2, 0 °C, 3 h; (d) potassium phthalimide, KI, DMF, 110 °C, 17 h; (e) NH_2Me, MeOH, rt, 3 h; (f) acyl chloride, Et_3N, CH_2Cl_2, 0 °C, 2 h.

For preparing probes with shorter spacer groups of one or two ethylene glycol units, as in compounds **14**–**17**, the same synthetic pathway was tried. Analogous esters of compound **1** were prepared from ethylene or diethylene glycol and ethyl diazoacetate, but hydrolysis of them under similar conditions did not yield expected products. Therefore a different strategy was chosen. Firstly diethylene and triethylene glycol were acylated with the corresponding acid chloride to produce monoesters **6**–**9**. The monoesters were then oxidized by Jones reagent on the terminal hydroxymethyl group to yield the corresponding carboxylic acids **10**–**13**. By coupling them with amine **2** in the last stage, this approach has enabled preparation of final compounds **14**–**17** (Scheme 1).

To make the spacer group more polar the ester moiety between the acyl residue and spacer was replaced by an amide group in compounds **22**, **23**, **27** and **28**. Amides have the potential for an additional hydrogen bond.

Synthesis of **22** and **23** (Scheme 2) started by selectively acylating the amino group of 2-aminoethoxyethanol to produce amides **18** and **19**. Subsequent oxidation with Jones reagent afforded carboxylic acids **20** and **21**. These products were much less susceptible to oxidative shortening of the spacer moiety by one oxyethyl group, in contrast to compounds **10**–**13** and **25**, most probably because carboxylic acids **20** and **21** were poorly soluble in acetone and started to precipitate in the reaction mixture. During the final reaction step TBTU was used instead of DCC as coupling reagent, since it gave higher yields but most importantly it altered the byproduct profile and made purification of amides **22** and **23** easier.

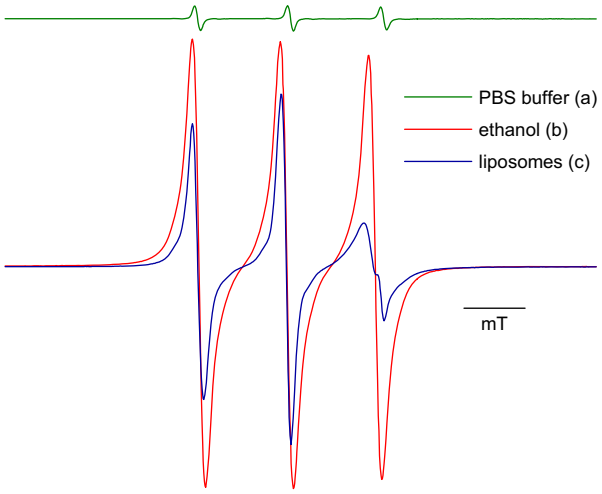


Figure 5. Recorded ESR spectra of **15** dissolved in (a) phosphate buffer saline (pH=7.4), (b) ethanol and (c) liposome suspension.[21]

In order to lengthen the spacer moiety by one oxyethyl segment potassium phthalimide was alkylated with 2-(2-(2-chloroethoxy)-ethoxy)ethanol to yield compound **24** (Scheme 2). Oxidation of compound **24** with Jones reagent produced carboxylic acid **25**, which was coupled with amine **2** to yield phthalimide **26**. TBTU was once more used, since it gave better results than DCC as with the synthesis of amides **22** and **23**. After removal of the phthalimido protection group of compound **26** with methylamine, the resulting amine was directly acylated without purification to produce amides **27** and **28**.

By introducing polar spacer groups of different lengths between the nitroxide moiety and the lipophilic anchor, information about molecular dynamics of oligosaccharide chains of glycolipids and glycoproteins at different distances from the membrane surface should be obtainable. It is expected that in the case of ordered regions of oligosaccharide chains in extracellular space the presented spin probes will provide important and new information. Furthermore, a difference between compounds that have the lipophilic anchor attached by an ester bond (e.g., **15**) and by an amide bond (e.g., **27**) is anticipated. The amide functional group can establish one more hydrogen bond and therefore should position itself higher in the membrane, pushing the nitroxide reporting group further away from the membrane. The ester functionality on the other hand can penetrate deeper into the membrane, bringing the nitroxide moiety closer to it. The ester group allows rotational movement and is, in that sense, much less rigid than the amide.

From the ESR spectra signal intensity only slight solubility of compound **15** in PBS buffer (a) is observed (Fig. 5). The increase of the intensity of the ESR signal after the addition of liposome suspension (c) is due to the incorporation of **15** into the liposome bilayer with its alkyl chain, while the doxyl group remains in water surroundings. For comparison, ESR spectra of compound **15** in ethanol (b) were recorded. In this solvent compound **15** is completely dissolved.

We expect that the novel spin probes presented here would be a new potential tool for ESR study of extracellular space of cells. Their application will give a new insight into structural and dynamic properties close to the membrane surface at different situations of the cell life, and information for further development of spin probes for the extracellular space.

3. Conclusion

To summarize, we have succeeded in synthesizing new amphiphilic spin probes in which a polar spacer group is inserted

between the nitroxide and the lipophilic part. This will enable the complex molecular motion of oligosaccharide chains and other membrane constituents above the biological membrane surface to be studied by ESR. Furthermore, by varying the spacer length and type of bond that connects the spacer and lipophilic parts, we have access to a versatile group of compounds from which to choose, according to the experiment. Further work with synthesis and testing on artificial and cellular membranes is in progress.

4. Experimental

4.1. General

Chemicals from Sigma–Aldrich, Acros, Avanti Polar Lipids and Fluka were used without further purification. Solvents were used without additional purification or drying. Analytical TLC was performed on Merck silica gel (60 F 254) plates (0.25 mm) and components visualized with ultraviolet light and dyed with 20% sulfuric acid in ethanol, Rhodamine G6, ninhydrin and bromocresol green. Silica gel 60 (0.04–0.063 mm, Merck) was used for column chromatography. Melting points were determined on a Reichert hot stage microscope and are uncorrected. IR spectra were obtained on a Perkin–Elmer FT-IR System Spectrum BX. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE DPX$_{300}$ spectrometer in $CDCl_3$ and DMSO-d_6 solution with TMS as internal standard. The nitroxyl groups were reduced to the corresponding hydroxylamines with 2 equiv of phenylhydrazine dissolved in $CDCl_3$, prior to NMR analysis. ESR spectra of ethanolic solutions were recorded at ambient temperature in a glass capillary (1 mm i.d.) using a Bruker X-band CW-ESR spectrometer ESR-300 at 10 mW microwave power; all a_N values of compounds **3**–**5**, **14**–**17**, **22**, **23**, **26**–**28** were approximately 1.45 mT. Microanalyses were performed on a 240 C Perkin–Elmer CHN analyzer. Mass spectra were obtained using Micromass AutospecQ.

4.2. Synthetic procedures

*4.2.1. Ethyl 2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy) acetate (**1**)*

Compound **1** was synthesized according to Ref. 22. Spectral data is in accordance with the literature.[22]

*4.2.2. 2-(Aminomethyl)-2,4,4-trimethyloxazolidin-3-oxyl (**2**)*

Compound **2** was synthesized according to Ref. 20. Spectral data is in accordance with the literature.[20]

*4.2.3. Synthesis of N-((3-oxyl-2,4,4-trimethyloxazolidin-2-yl) methyl)-2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)-acetamide (**3**)*

To compound **1** (200 mg, 0.85 mmol) dissolved in a mixture of dioxane (2 mL) and water (1 mL) was added powdered NaOH (68 mg, 1.7 mmol). The reaction mixture was stirred at room temperature for 2 h. HCl (2 M, 2 mL) was then added and the solvent evaporated under reduced pressure. The residue was washed several times with dichloromethane (100 mL), combined washings were dried with Na_2SO_4 and the solvent evaporated under reduced pressure. To the obtained colourless oil dissolved in ethyl acetate (2 mL) and cooled to 0 °C was added DCC (175 mg, 0.85 mmol). After 10 min, compound **2** (135 mg, 0.85 mmol) dissolved in ethyl acetate (1 mL) was added dropwise. Reaction was continued for 3 h at room temperature. The reaction mixture was then filtered and the solvent evaporated under reduced pressure. The crude product was purified using silica gel chromatography (dichloromethane/methanol=20:1).

Yield 37%; orange oil; R_f=0.35 (dichloromethane/methanol=15:1); IR (NaCl, cm^{-1}): 3326, 2929, 1754, 1667, 1537, 1454, 1350, 1209, 1120, 938; MS (ESI) *m*/*z*: 350 (MH)$^+$; HRMS (ESI) *m*/*z*: calcd for $C_{15}H_{30}N_2O_7$ *m*/*z*: 350.2066 (MH)$^+$, found 350.2061; ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.10 (s, 3H, CH_3), 1.11 (s, 3H, CH_3), 1.16 (s, 3H, CH_3), 3.03 (dd, 1H, J_1=13.2 Hz, J_2=4.5 Hz, NHCH_aH$_b$C), 3.40–3.65 (m, 15H, 6×OCH$_2$, NHCH$_a$$H_bC, OCH_a$$H_b$C(CH$_3$)$_2$), 3.90 (s, 2H, OC$H_2$CONH), 4.27–4.31 (m, 1H, CH$_2$O*H*); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 20.4, 21.0, 27.8, 46.0, 61.4, 62.7, 69.8, 70.0, 70.2, 70.4, 70.6, 72.5, 75.0, 97.3, 170.1.

*4.2.4. General procedure for acylation of compound **3***

Acyl chloride (0.24 mmol) dissolved in dichloromethane (2 mL) was added dropwise to a stirred solution of **3** (55 mg, 0.16 mmol) and Et_3N (32 mg, 0.32 mmol) in dichloromethane (3 mL) at 0 °C. Reaction was continued for 0.5 h at room temperature. The reaction mixture was washed with 0.1 M HCl (5 mL), saturated aqueous solution of $NaHCO_3$ (5 mL), brine (10 mL), dried with Na_2SO_4 and the solvent evaporated under reduced pressure. The crude product was purified using silica gel chromatography (dichloromethane/hexane/methanol=8:8:1).

*4.2.5. 1-(3-Oxyl-2,4,4-trimethyloxazolidin-2-yl)-3-oxo-5,8,11-trioxa-2-azatridecan-13-yl tetradecanoate (**4**)*

Yield 25%; orange oil; R_f=0.75 (dichloromethane/methanol=15:1); IR (NaCl, cm^{-1}): 3352, 2923, 2853, 2360, 1736, 1687, 1530, 1464, 1367, 1248, 1111, 1049; MS (ESI) *m*/*z*: 560 (MH)$^+$; HRMS (ESI) *m*/*z*: calcd for $C_{29}H_{56}N_2O_8$ *m*/*z*: 560.4037 (MH)$^+$, found 560.4017; ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 0.88 (t, 3H, J=6.3 Hz, CH_3), 1.16 (s, 3H, CH_3), 1.22 (s, 3H, CH_3), 1.23–1.31 (m, 20H, CH_2), 1.32 (s, 3H, CH_3), 1.56–1.65 (m, 2H, CH_2CH$_2$COO), 2.31 (t, 2H, J=7.5 Hz, CH$_2$COO), 3.13 (dd, 1H, J_1=13.5 Hz, J_2=4.8 Hz, NHCH_aH$_b$C), 3.57–3.70 (m, 13H, 5×OCH$_2$, OCH_2CONH, NHCH$_a$$H_b$C), 3.92 (d, 1H, J=15.6 Hz, OCH_aH$_b$C(CH$_3$)$_2$), 4.03 (d, 1H, J=15.6 Hz, OCH$_a$$H_b$C(CH$_3$)$_2$), 4.22 (t, 2H, J=4.8 Hz, COOCH$_2$); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 14.1, 20.1, 20.7, 22.6, 24.9, 25.4, 29.1, 29.2, 29.3, 29.4, 29.56, 29.60, 29.63, 31.9, 34.1, 46.3, 62.5, 63.1, 69.1, 70.37, 70.39, 70.44, 70.6, 71.1, 75.2, 97.1, 170.3, 173.8. Anal. Calcd for $C_{29}H_{56}N_2O_8$ (%): C, 62.23; H, 9.90; N, 5.00. Found: C, 62.03; H, 10.23; N, 4.72.

*4.2.6. 1-(3-Oxyl-2,4,4-trimethyloxazolidin-2-yl)-3-oxo-5,8,11-trioxa-2-azatridecan-13-yl palmitate (**5**)*

Yield 42%; orange oil; R_f=0.75 (dichloromethane/methanol=15:1); IR (KBr, cm^{-1}): 3354, 2923, 2853, 1736, 1686, 1529, 1464, 1372, 1248, 1116, 1049, 948, 857, 720; MS (ESI) *m*/*z*: 588 (MH)$^+$; HRMS (ESI) *m*/*z*: calcd for $C_{31}H_{60}N_2O_8$ *m*/*z*: 588.4350 (MH)$^+$, found 588.4370; ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 0.88 (t, 3H, J=6.6 Hz, CH_3), 1.15 (s, 3H, CH_3), 1.22 (s, 3H, CH_3), 1.23–1.32 (m, 24H, CH_2), 1.33 (s, 3H, CH_3), 1.55–1.66 (m, 2H, CH_2CH$_2$COO), 2.28–2.33 (m, 2H, CH$_2$COO), 3.13 (dd, 1H, J_1=13.5 Hz, J_2=4.8 Hz, NHCH_aH$_b$C), 3.57–3.73 (m, 13H, 5×OCH$_2$, NHCH$_a$$H_bC, OCH_2$CONH), 3.92 (d, 1H, J=15.3 Hz, OCH_aH$_b$C(CH$_3$)$_2$), 4.01 (d, 1H, J=15.3 Hz, OCH$_a$$H_b$C(CH$_3$)$_2$), 4.28 (t, 2H, J=4.3 Hz, COOCH$_2$); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 14.1, 20.1, 20.7, 22.6, 24.9, 25.4, 29.1, 29.2, 29.3, 29.4, 29.60, 29.64, 31.9, 34.1, 46.3, 62.4, 63.1, 69.1, 70.36, 70.39, 70.43, 70.6, 71.0, 75.2, 97.1, 170.3, 173.8.

*4.2.7. Synthesis of compounds **6**–**9***

Compounds **6**–**9** were synthesized according to Ref. 23.

*4.2.7.1. 2-(2-Hydroxyethoxy)ethyl tetradecanoate (**6**).* Spectral data is in accordance with the literature.[23]

*4.2.7.2. 2-(2-Hydroxyethoxy)ethyl palmitate (**7**).* Spectral data is in accordance with the literature.[23]

*4.2.7.3. 2-(2-(2-Hydroxyethoxy)ethoxy)ethyl tetradecanoate (**8**).* Spectral data is in accordance with the literature.[23]

*4.2.7.4. 2-(2-(2-Hydroxyethoxy)ethoxy)ethyl palmitate (**9**).* Spectral data is in accordance with the literature.[23]

*4.2.8. General procedure for oxidation of monoacylated derivatives (**10**–**13**)*

To a stirred solution of monoacylated derivative (5 mmol) in acetone (60 mL) at 0 °C, CrO_3 (12 mmol) dissolved in 35% H_2SO_4 (25 mL) was added dropwise. The temperature of the reaction mixture was then allowed to reach room temperature and stirring was continued for 3 h. The reaction mixture was poured into water (80 mL) and the product extracted with ethyl acetate (4×50 mL). The combined organic phases were washed with brine (70 mL), dried with Na_2SO_4 and the solvent evaporated under reduced pressure.

*4.2.8.1. 2-(2-(Tetradecanoyloxy)ethoxy)acetic acid (**10**).* Yield 30%; white solid; R_f=0.3 (dichloromethane/methanol=10:1); mp 43–46 °C; IR (KBr, cm^{-1}): 2917, 2849, 2367, 1733, 1701, 1473, 1420, 1280, 1253, 1228, 1202, 1182, 1149, 1064, 923, 715, 699; MS (ESI) *m*/*z*: 331 $(MH)^+$, 353 $(MNa)^+$; HRMS (ESI) *m*/*z*: calcd for $C_{18}H_{35}O_5$ *m*/*z*: 331.2484 $(MH)^+$, found 331.2482; ^1H NMR ($CDCl_3$, 300 MHz): δ (ppm) 0.88 (t, 3H, *J*=6.6 Hz, CH_3), 1.23–1.31 (m, 20H, CH_2), 1.55–1.70 (m, 2H, CH_2CH_2COO), 2.34 (t, 2H, *J*=7.5 Hz, CH_2COO), 3.80 (t, 2H, *J*=4.2 Hz, OCH_2) 4.18 (s, 2H, CH_2COOH), 4.28 (t, 2H, *J*=4.2 Hz, $COOCH_2$); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 14.0, 22.6, 24.8, 29.0, 29.2, 29.3, 29.4, 29.6, 31.9, 34.1, 63.1, 68.3, 69.6, 173.9, 174.7.

*4.2.8.2. 2-(2-(Palmitoyloxy)ethoxy)acetic acid (**11**).* Yield 80%; white solid; R_f=0.3 (dichloromethane/methanol=10:1); mp 68–69 °C; IR (KBr, cm^{-1}): 2914, 2849, 1731, 1700, 1472, 1420, 1287, 1266, 1244, 1220, 1198, 1181, 1149, 1064, 923, 715, 700; MS (ESI) *m*/*z*: 359 $(MH)^+$, 381 $(MNa)^+$; HRMS (ESI) *m*/*z*: calcd for $C_{20}H_{39}O_5$ *m*/*z*: 359.2797 $(MH)^+$, found 359.2797; ^1H NMR ($CDCl_3$, 300 MHz): δ (ppm) 0.88 (t, 3H, *J*=6.6 Hz, CH_3), 1.20–1.34 (m, 24H, CH_2), 1.56–1.70 (m, 2H, CH_2CH_2COO), 2.34 (t, 2H, *J*=7.5 Hz, CH_2COO), 3.80 (t, 2H, *J*=4.5 Hz, OCH_2) 4.17 (s, 2H, CH_2COOH), 4.23 (t, 2H, *J*=4.2 Hz, $COOCH_2$); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 14.0, 22.6, 24.8, 29.1, 29.2, 29.3, 29.4, 29.55, 29.59, 29.6, 31.9, 34.1, 63.1, 68.0, 69.7, 173.9, 174.6.

*4.2.8.3. 2-(2-(2-(Tetradecanoyloxy)ethoxy)ethoxy)acetic acid (**12**).* Yield 20%; white solid; mp 34–36 °C; R_f=0.3 (dichloromethane/methanol=10:1); IR (KBr, cm^{-1}): 2919, 2850, 1729, 1472, 1249, 1202, 1183, 1152, 1135, 1047, 954, 921, 716; MS (ESI) *m*/*z*: 375 $(MH)^+$, 397 $(MNa)^+$; HRMS (ESI) *m*/*z*: calcd for $C_{20}H_{38}O_6Na$ *m*/*z*: 397.2566 $(MNa)^+$, found 397.2565; ^1H NMR ($CDCl_3$, 300 MHz): δ (ppm) 0.89 (t, 3H, *J*=6.9 Hz, CH_3), 1.23–1.34 (m, 20H, CH_2), 1.56–1.68 (m, 2H, CH_2CH_2COO), 2.34 (t, 2H, *J*=7.5 Hz, CH_2COO), 3.69–3.78 (m, 6H, OCH_2), 4.18 (s, 2H, CH_2COOH), 4.25 (t, 2H, *J*=4.5 Hz, $COOCH_2$); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 14.0, 22.6, 24.8, 29.0, 29.1, 29.2, 29.3, 29.48, 29.52, 29.55, 31.8, 34.0, 63.0, 68.3, 69.1, 70.2, 70.9, 173.8, 173.9.

*4.2.8.4. 2-(2-(2-(Palmitoyloxy)ethoxy)ethoxy)acetic acid (**13**).* Yield 81%; white solid; R_f=0.3 (dichloromethane/methanol=10:1); mp 38–40 °C; IR (KBr, cm^{-1}): 2916, 2849, 1734, 1467, 1245, 1220, 1198, 1180, 1125, 954, 721; MS (ESI) *m*/*z*: 403 $(MH)^+$, 425 $(MNa)^+$; HRMS (ESI) *m*/*z*: calcd for $C_{22}H_{42}O_6Na$ *m*/*z*: 425.2879 $(MNa)^+$, found 425.2885; ^1H NMR ($CDCl_3$, 300 MHz): δ (ppm) 0.88 (t, 3H, *J*=6.3 Hz, CH_3), 1.20–1.36 (m, 24H, CH_2), 1.56–1.68 (m, 2H, CH_2CH_2COO), 2.33 (t, 2H, *J*=7.5 Hz, CH_2COO), 3.68–3.74 (m, 6H, OCH_2), 4.12 (s, 2H, CH_2COOH), 4.25 (t, 2H, *J*=4.5 Hz, $COOCH_2$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ (ppm) 13.7, 22.0, 24.4, 28.4, 28.7, 28.9, 29.1, 31.3, 33.3, 62.9, 67.5, 68.2, 69.7, 171.4, 172.6.

*4.2.9. General procedure for synthesis of **14**–**17***

To a solution of compounds **10**–**13** (1 mmol) and Et_3N (100 mg, 1 mmol) in acetone (2 mL), was added DCC (160 mg, 0.77 mmol) at 0 °C. After 10 min, **2** (0.52 mmol) dissolved in acetone (1 mL) was added. The temperature of the reaction mixture was allowed to reach room temperature and stirring was continued for 2 h. The reaction mixture was filtered and the solvent evaporated under reduced pressure. The residue was dissolved in ethyl acetate (10 mL) and washed with 0.1 M HCl (10 mL), saturated aqueous solution of $NaHCO_3$ (10 mL), brine (30 mL), dried with Na_2SO_4 and the solvent evaporated under reduced pressure. The crude product was purified using silica gel chromatography; compounds **14**, **16** (ethyl acetate/hexane=1:1) and compounds **15**, **17** (ethyl acetate/hexane=2:1).

*4.2.9.1. 2-(2-((3-Oxyl-2,4,4-trimethyloxazolidin-2-yl)methylamino)-2-oxoethoxy)ethyl tetradecanoate (**14**).* Yield 18%; orange oil; R_f=0.6 (ethyl acetate); IR (NaCl, cm^{-1}): 3409, 2924, 2853, 1737, 1691, 1529, 1463, 1372, 1248, 1176, 1114, 1051, 720; MS (ESI) *m*/*z*: 472 $(MH)^+$, 473 $(MH_2)^+$; HRMS (ESI) *m*/*z*: calcd for $C_{25}H_{49}N_2O_6$ *m*/*z*: 473.3591 $(MH_2)^+$, found 473.3570; ^1H NMR ($CDCl_3$, 300 MHz): δ (ppm) 0.88 (t, 3H, *J*=6.3 Hz, CH_3), 1.16 (s, 3H, CH_3), 1.24 (s, 3H, CH_3), 1.25–1.31 (m, 20H, CH_2), 1.33 (s, 3H, CH_3), 1.56–1.68 (m, 2H, CH_2CH_2COO), 2.33 (t, 2H, *J*=7.5 Hz, CH_2COO), 3.19 (dd, 1H, J_1=13.5 Hz, J_2=5.1 Hz, $NHCH_aH_bC$), 3.56 (dd, 1H, J_1=13.8 Hz, J_2=7.5 Hz, $NHCH_aH_bC$), 3.57 (d, 1H, *J*=8.1 Hz, $OCH_aH_bC(CH_3)_2$), 3.67–3.73 (m, 2H, OCH_2), 3.70 (d, 1H, *J*=8.4 Hz, $OCH_aH_bC(CH_3)_2$), 4.00 (s, 2H, OCH_2CONH), 4.27 (t, 2H, *J*=4.5 Hz, $COOCH_2$); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 14.1, 19.9, 21.0, 22.6, 24.9, 25.7, 29.1, 29.2, 29.3, 29.4, 29.57, 29.60, 29.64, 31.9, 34.2, 45.9, 62.3, 62.9, 69.5, 70.6, 75.4, 97.1, 169.9, 174.0.

*4.2.9.2. 2-(2-(2-((3-Oxyl-2,4,4-trimethyloxazolidin-2-yl)methylamino)-2-oxoethoxy)ethoxy)ethyl tetradecanoate (**15**).* Yield 25%; orange oil; R_f=0.45 (ethyl acetate); IR (NaCl, cm^{-1}): 3361, 2924, 2853, 1737, 1688, 1530, 1463, 1367, 1248, 1112, 1049, 516; MS (ESI) *m*/*z*: 515 $(MH)^+$; HRMS (ESI) *m*/*z*: calcd for $C_{27}H_{52}N_2O_7$ *m*/*z*: 516.3775 $(MH)^+$, found 516.3765; ^1H NMR ($CDCl_3$, 300 MHz): δ (ppm) 0.88 (t, 3H, *J*=6.3 Hz, CH_3), 1.15 (s, 3H, CH_3), 1.23 (s, 3H, CH_3), 1.24–1.31 (m, 20H, CH_2), 1.33 (s, 3H, CH_3), 1.56–1.68 (m, 2H, CH_2CH_2COO), 2.32 (t, 2H, *J*=7.2 Hz, CH_2COO), 3.14 (dd, 1H, J_1=13.5 Hz, J_2=5.1 Hz, $NHCH_aH_bC$), 3.56–3.74 (m, 9H, 3×OCH_2, $NHCH_aH_bC$, OCH_2CONH), 3.92 (d, 1H, *J*=15.6 Hz, $OCH_aH_bC(CH_3)_2$), 4.04 (d, 1H, *J*=15.6 Hz, $OCH_aH_bC(CH_3)_2$), 4.22 (t, 2H, *J*=4.8 Hz, $COOCH_2$); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 14.1, 20.0, 20.6, 22.6, 24.9, 25.5, 29.1, 29.2, 29.3, 29.4, 29.56, 29.60, 29.63, 31.9, 34.2, 46.3, 62.5, 62.9, 69.4, 70.3, 70.4, 70.9, 75.2, 97.1, 170.3, 173.9.

*4.2.9.3. 2-(2-((3-Oxyl-2,4,4-trimethyloxazolidin-2-yl)methylamino)-2-oxoethoxy)ethyl palmitate (**16**).* Yield 25%; orange oil; R_f=0.55 (ethyl acetate); IR (NaCl, cm^{-1}): 3411, 2923, 2853, 1737, 1692, 1529, 1464, 1371, 1248, 1176, 1137, 1051, 720; MS (ESI) *m*/*z*: 500 $(MH)^+$; HRMS (ESI) *m*/*z*: calcd for $C_{27}H_{52}N_2O_6$ *m*/*z*: 500.3825 $(MH)^+$, found 500.3839; ^1H NMR ($CDCl_3$, 300 MHz): δ (ppm) 0.88 (t, 3H, *J*=6.6 Hz, CH_3), 1.15 (s, 3H, CH_3), 1.24 (s, 3H, CH_3), 1.25–1.31 (m, 24H, CH_2), 1.33 (s, 3H, CH_3), 1.56–1.68 (m, 2H, CH_2CH_2COO), 2.32 (t, 2H, *J*=7.5 Hz, CH_2COO), 3.17 (dd, 1H, J_1=13.8 Hz, J_2=5.1 Hz, $NHCH_aH_bC$), 3.56 (dd, 1H, J_1=13.8 Hz, J_2=7.5 Hz, $NHCH_aH_bC$), 3.58 (d, 1H, *J*=8.1 Hz, $OCH_aH_bC(CH_3)_2$), 3.68 (t, 2H, *J*=4.8 Hz, OCH_2), 3.70 (d, 1H, *J*=8.4 Hz, $OCH_aH_bC(CH_3)_2$), 3.96 (s, 2H, OCH_2CONH), 4.25 (t, 2H, *J*=4.5 Hz, $COOCH_2$); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 14.0, 19.7, 20.9, 22.6, 24.8, 25.6, 29.1, 29.2, 29.3, 29.4, 29.55, 29.58, 29.62, 31.9, 34.1, 45.9, 62.3, 62.6, 69.6, 70.5, 75.4, 97.1, 169.9, 173.9.

*4.2.9.4. 2-(2-(2-((3-Oxyl-2,4,4-trimethyloxazolidin-2-yl)methylamino)-2-oxoethoxy)ethoxy)ethyl palmitate (**17**).* Yield 16%; orange oil; R_f=0.4 (ethyl acetate); IR (NaCl, cm^{-1}): 3364, 2923, 2853, 1737, 1688, 1530, 1464, 1367, 1249, 1113, 1051, 500; MS (ESI) *m*/*z*: 544 $(MH)^+$, 545 $(MH_2)^+$; HRMS (ESI) *m*/*z*: calcd for $C_{29}H_{57}N_2O_7$ *m*/*z*: 545.4166 $(MH_2)^+$, found 545.4180; ^1H NMR ($CDCl_3$, 300 MHz):

δ (ppm) 0.88 (t, 3H, J=6.6 Hz, CH_3), 1.16 (s, 3H, CH_3), 1.23 (s, 3H, CH_3), 1.24–1.31 (m, 24H, CH_2), 1.33 (s, 3H, CH_3), 1.56–1.68 (m, 2H, CH_2CH_2COO), 2.33 (t, 2H, J=7.5 Hz, CH_2COO), 3.14 (dd, 1H, J_1=13.5 Hz, J_2=5.1 Hz, $NHCH_aH_bC$), 3.57–3.76 (m, 9H, 3×OCH_2, $NHCH_aH_bC$, OCH_2CONH), 3.93 (d, 1H, J=15.6 Hz, $OCH_aH_bC(CH_3)_2$), 4.06 (d, 1H, J=15.9 Hz, $OCH_aH_bC(CH_3)_2$), 4.27 (t, 2H, J=4.8 Hz, $COOCH_2$); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 14.0, 20.0, 20.6, 22.6, 24.8, 25.4, 29.0, 29.17, 29.25, 29.4, 29.50, 29.55, 29.58, 31.8, 34.1, 46.2, 62.4, 62.9, 69.3, 70.2, 70.4, 70.8, 75.1, 97.1, 170.2, 173.8.

*4.2.10. Synthesis of compounds **18** and **19***

Compounds **18** and **19** were synthesized according to Ref. 24.

*4.2.10.1. N-(2-(2-Hydroxyethoxy)ethyl)tetradecanamide (**18**).* Spectral data is in accordance with the literature.[24]

*4.2.10.2. N-(2-(2-Hydroxyethoxy)ethyl)palmitamide (**19**).* Spectral data is in accordance with the literature.[25]

*4.2.11. Synthesis of compounds **20** and **21***

Compounds **20** and **21** were prepared as described for compounds **10**–**13**.

*4.2.11.1. 2-(2-Tetradecanamidoethoxy)acetic acid (**20**).* Yield 62%; white crystals; R_f=0.2 (dichloromethane/methanol=10:1); mp 72–74 °C; IR (KBr, cm^{-1}): 3301, 2916, 2848, 2344, 1718, 1635, 1559, 1472, 1425, 1260, 1149, 883, 719; MS (ESI) m/z: 330 $(MH)^+$, 352 $(MNa)^+$; HRMS (ESI) m/z: calcd for $C_{18}H_{36}NO_4$ m/z: 330.2644 $(MH)^+$, found 330.2629; ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 0.88 (t, 3H, J=6.3 Hz, CH_3), 1.22–1.32 (m, 20H, CH_2), 1.58–1.68 (m, 2H, CH_2CH_2COO), 2.23 (t, 2H, J=7.8 Hz, CH_2COO), 3.46 (q, 2H, J=5.1 Hz, $NHCH_2CH_2$), 3.65 (t, 2H, J=5.1 Hz, OCH_2), 4.12 (s, 2H, OCH_2COOH), 6.60–6.80 (m, 1H, CONH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ (ppm) 13.8, 22.0, 25.2, 28.6, 28.7, 28.8, 28.9, 29.01, 29.04, 31.3, 35.3, 38.3, 67.4, 69.3, 171.6, 172.1.

*4.2.11.2. 2-(2-Palmitamidoethoxy)acetic acid (**21**).* Spectral data is in accordance with the literature.[26]

*4.2.12. General procedure for synthesis of **22** and **23***

To a solution of compound **20** or **21** (0.5 mmol), Et_3N (100 mg, 1 mmol) and **2** (0.5 mmol) in dichloromethane (3 mL) was added TBTU (240 mg, 0.75 mmol) at 0 °C. The temperature of the reaction mixture was then allowed to reach room temperature and stirring was continued for 2 h. After that the reaction mixture was filtered and solvent evaporated under reduced pressure. The residue was dissolved in ethyl acetate (10 mL) and washed with 0.1 M HCl (10 mL), saturated aqueous solution of $NaHCO_3$ (10 mL), brine (30 mL), dried with Na_2SO_4 and the solvent evaporated under reduced pressure. The crude product was purified using silica gel chromatography (ethyl acetate/acetone=5:1).

*4.2.12.1. N-(2-(2-((3-Oxyl-2,4,4-trimethyloxazolidin-2-yl)methylamino)-2-oxoethoxy)ethyl)tetradecanamide (**22**).* Yield 24%; orange oil; R_f=0.5 (dichloromethane/methanol=10:1); IR (KBr, cm^{-1}): 3310, 2924, 2853, 1653, 1541, 1456, 1374, 1249, 1141, 1052, 722; MS (ESI) m/z: 471 $(MH)^+$; HRMS (ESI) m/z: calcd for $C_{25}H_{49}N_3O_5$ m/z: 471.3672 $(MH)^+$, found 471.3687; ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 0.88 (t, 3H, J=6.6 Hz, CH_3), 1.18 (s, 3H, CH_3), 1.22–1.30 (m, 23H, 10×CH_2, CH_3), 1.33 (s, 3H, CH_3), 1.57–1.67 (m, 2H, CH_2CH_2COO), 2.18 (t, 2H, J=7.2 Hz, CH_2COO), 3.16 (dd, 1H, J_1=13.5 Hz, J_2=4.8 Hz, $NHCH_aH_bC$), 3.47–3.62 (m, 5H, OCH_2, $CONHCH_2$, $NHCH_aH_bC$), 3.59 (d, 1H, J=7.8 Hz, $OCH_aH_bC(CH_3)_2$), 3.70 (d, 1H, J=8.1 Hz, $OCH_aH_bC(CH_3)_2$), 3.94 (s, 2H, OCH_2CONH), 5.78–5.84 (m, 1H, CONH); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 14.8, 20.1, 21.1, 22.6, 25.5, 25.7, 29.25, 29.28, 29.31, 29.4, 29.58, 29.61, 31.85, 36.7, 39.1, 46.2, 62.7, 70.4, 70.6, 75.2, 97.3, 169.8, 174.1.

*4.2.12.2. N-(2-(2-((3-Oxyl-2,4,4-trimethyloxazolidin-2-yl)methylamino)-2-oxoethoxy)ethyl)palmitamide (**23**).* Yield 9%; orange solid; R_f=0.5 (dichloromethane/methanol=10:1); mp 40–42 °C; IR (KBr, cm^{-1}): 3315, 2917, 2848, 2370, 1636, 1559, 1458, 1374, 1249, 1127, 1049, 719; MS (ESI) m/z: 499 $(MH)^+$, 500 $(MH_2)^+$; HRMS (ESI) m/z: calcd for $C_{27}H_{54}N_3O_5$ m/z: 500.4063 $(MH_2)^+$, found 500.4080; ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 0.88 (t, 3H, J=6.3 Hz, CH_3), 1.19 (s, 3H, CH_3), 1.22–1.30 (m, 27H, CH_3, 12×CH_2), 1.33 (s, 3H, CH_3), 1.58–1.68 (m, 2H, CH_2CH_2COO), 2.18 (t, 2H, J=7.2 Hz, CH_2COO), 3.16 (dd, 1H, J_1=13.2 Hz, J_2=4.5 Hz, $NHCH_aH_bC$), 3.48–3.62 (m, 5H, OCH_2, $CONHCH_2$, $NHCH_aH_bC$), 3.59 (d, 1H, J=7.8 Hz, $OCH_aH_bC(CH_3)_2$), 3.70 (d, 1H, J=8.1 Hz, $OCH_aH_bC(CH_3)_2$), 3.95 (s, 2H, OCH_2CONH), 5.77–5.84 (m, 1H, CONH); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 14.0, 20.1, 21.1, 22.6, 25.4, 25.7, 29.24, 29.27, 29.3, 29.4, 29.57, 29.61, 31.8, 36.7, 39.1, 46.2, 62.7, 70.4, 70.6, 75.2, 97.3, 169.7, 174.1.

*4.2.13. N-(2-(2-(2-Hydroxyethoxy)ethoxy)ethyl) phthalimide (**24**)*

Compound **24** was synthesized according to Ref. 27. Spectral data is in accordance with the literature.[27]

*4.2.14. 2-(2-(2-(1,3-Dioxoisoindolin-2-yl)ethoxy)ethoxy) acetic acid (**25**)*

Compound **25** was synthesized according to Ref. 28. Spectral data is in accordance with the literature.[28]

*4.2.15. Synthesis of 2-(2-(2-phthalimido ethoxy)ethoxy)-N-((3-oxyl-2,4,4-trimethyloxazolidin-2-yl)methyl) acetamide (**26**)*

To a solution of **25** (150 mg, 0.5 mmol), Et_3N (100 mg, 1 mmol) and **2** (0.5 mmol) in dichloromethane (3 mL) was added TBTU (240 mg, 0.75 mmol) at 0 °C. The temperature of the reaction mixture was allowed to reach room temperature and stirring was continued for 2 h. The reaction mixture was filtered and solvent evaporated under reduced pressure. The residue was dissolved in ethyl acetate (10 mL) and washed with 0.1 M HCl (10 mL), saturated aqueous solution of $NaHCO_3$ (10 mL), brine (30 mL), dried with Na_2SO_4 and the solvent evaporated under reduced pressure. The crude product was then purified using silica gel chromatography (ethyl acetate/hexane=4:1).

Yield 37%; orange oil; R_f=0.75 (dichloromethane/methanol=10:1); IR (NaCl, cm^{-1}): 3372, 2931, 1773, 1712, 1682, 1530, 1466, 1427, 1394, 1249, 1108, 1024, 721, 529; MS (ESI) m/z: 435 $(MH)^+$; HRMS (ESI) m/z: calcd for $C_{21}H_{29}N_3O_7$ m/z: 435.2006 $(MH)^+$, found 435.2019; ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 1.14 (s, 2H, CH_3), 1.17 (s, 2H, CH_3), 1.29 (s, 2H, CH_3), 3.15 (dd, 1H, J_1=13.5 Hz, J_2=5.1 Hz, $NHCH_aH_bC$), 3.49–3.67 (m, 7H, 3×OCH_2, $NHCH_aH_bC$), 3.76 (t, 2H, J=5.4 Hz, NCH_2), 3.76 (d, 1H, J=15.9 Hz, $OCH_aH_bC(CH_3)_2$), 3.91 (s, 2H, OCH_2CONH), 3.93 (d, 1H, J=15.9 Hz, $OCH_aH_bC(CH_3)_2$), 7.69 (dd, 2H, J_1=5.4 Hz, J_2=3.3 Hz, ArH), 7.83 (dd, 2H, J_1=5.4 Hz, J_2=3.3 Hz, ArH); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 20.0, 20.5, 25.3, 36.9, 46.0, 62.3, 68.0, 69.6, 70.3, 70.7, 75.1, 97.0, 123.2, 131.9, 133.9, 168.3, 170.0.

*4.2.16. General procedure for synthesis of compounds **27** and **28***

Methylamine aqueous solution (0.5 mL) was added to a solution of compound **26** (65 mg, 0.15 mmol) in methanol (1 mL) at room temperature. After 3 h of stirring, the solvents were evaporated under reduced pressure. To the residue dissolved in dichloromethane (3 mL) were added Et_3N (26 mg, 0.25 mmol) and acylating reagent (0.23 mmol) in dichloromethane (1 mL) at 0 °C. The temperature of the reaction mixture was then allowed to reach room temperature and stirring was continued for 2 h. To the reaction mixture was added dichloromethane (10 mL) and the obtained solution was washed with 0.1 M HCl (10 mL), saturated aqueous solution of $NaHCO_3$ (10 mL), brine (50 mL), dried with Na_2SO_4 and the solvent evaporated under reduced pressure. The crude product was purified using silica gel chromatography (dichloromethane/methanol=20:1→15:1).

*4.2.16.1. N-(2-(2-(2-((3-Oxyl-2,4,4-trimethyloxazolidin-2-yl)methylamino)-2-oxoethoxy)ethoxy)ethyl) tetradecanamide (**27**).* Yield 21%; orange oil; R_f=0.55 (dichloromethane/methanol=10:1); IR (NaCl, cm^{-1}): 3317, 2923, 2853, 2359, 2340, 1651, 1538, 1463, 1371, 1248, 1112, 1050, 671; MS (ESI) *m*/*z*: 515 $(MH)^+$, 537 $(MNa)^+$; HRMS (ESI) *m*/*z*: calcd for $C_{27}H_{53}N_3O_6$ *m*/*z*: 515.3934 $(MH)^+$, found 515.3959; ^1H NMR ($CDCl_3$, 300 MHz): δ (ppm) 0.88 (t, 3H, *J*=6.3 Hz, CH_3), 1.16 (s, 3H, CH_3), 1.22–1.30 (m, 23H, 10×CH_2, CH_3) 1.34 (s, 3H, CH_3), 1.54–1.68 (m, 2H, CH_2CH_2COO), 2.15 (t, 2H, *J*=7.2 Hz, CH_2COO), 3.17 (dd, 1H, J_1=13.8 Hz, J_2=5.1 Hz, $NHCH_aH_bC$), 3.44–3.72 (m, 11H, 3×OCH_2, $CONHCH_2$, OCH_2-CONH, $NHCH_aH_bC$), 3.92 (d, 1H, *J*=15.9 Hz, $OCH_aH_bC(CH_3)_2$), 4.03 (d, 1H, *J*=15.9 Hz, $OCH_aH_bC(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 14.0, 19.9, 20.7, 22.6, 25.6, 25.7, 29.2, 29.3, 29.4, 29.5, 29.6, 31.8, 36.5, 39.0, 46.0, 62.1, 70.0, 70.3, 70.4, 70.9, 75.4, 97.1, 170.4, 173.5.

*4.2.16.2. N-(2-(2-(2-((3-Oxyl-2,4,4-trimethyloxazolidin-2-yl)methylamino)-2-oxoethoxy)ethoxy)ethyl) palmitamide (**28**).* Yield 24%; orange solid; R_f=0.45 (dichloromethane/methanol=10:1); mp 34–36 °C; IR (NaCl, cm^{-1}): 3305, 2916, 2848, 1673, 1640, 1545, 1462, 1371, 1249, 1136, 1049, 719; MS (ESI) *m*/*z*: 543 $(MH)^+$, 565 $(MNa)^+$; HRMS (ESI) *m*/*z*: calcd for $C_{29}H_{57}N_3O_6$ *m*/*z*: 543.4247 $(MH)^+$, found 543.4226; ^1H NMR ($CDCl_3$, 300 MHz): δ (ppm) 0.88 (t, 3H, *J*=6.3 Hz, CH_3), 1.17 (s, 3H, CH_3), 1.20–1.30 (m, 27H, 12×CH_2, CH_3) 1.35 (s, 3H, CH_3), 1.56–1.68 (m, 2H, CH_2CH_2COO), 2.16 (t, 2H, *J*=7.5 Hz, CH_2COO), 3.17 (dd, 1H, J_1=13.8 Hz, J_2=5.4 Hz $NHCH_aH_bC$), 3.45–3.73 (m, 11H, 3×OCH_2, $CONHCH_2$, OCH_2CONH, $NHCH_aH_bC$), 3.94 (d, 1H, *J*=15.9 Hz, $OCH_aH_bC(CH_3)_2$), 4.06 (d, 1H, *J*=15.9 Hz, $OCH_aH_bC(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 14.0, 19.9, 20.7, 22.6, 25.6, 25.7, 29.2, 29.3, 29.4, 29.5, 29.6, 31.8, 36.5, 39.0, 46.0, 62.1, 70.0, 70.3, 70.4, 70.9, 75.4, 97.1, 170.5, 173.5.

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