Tetrahedron 65 (2009) 8014–8020

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Synthesis of hydrophobic phase-tagged prolyl peptides featuring rapid reaction/separation

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ARTICLE INFO

Article history:
Received 6 May 2009
Received in revised form 20 July 2009
Accepted 20 July 2009
Available online 24 July 2009

ABSTRACT

Hydrophobically tagged prolyl peptides were synthesized using an electrochemically modified prolyl moiety. The hydrophobic tag served a useful handle for the repetitive reaction/separation steps during solution-phase peptide synthesis. Furthermore, the tag was also effective as an anchor on solid phases for the evaluation of activity of immobilized peptides.

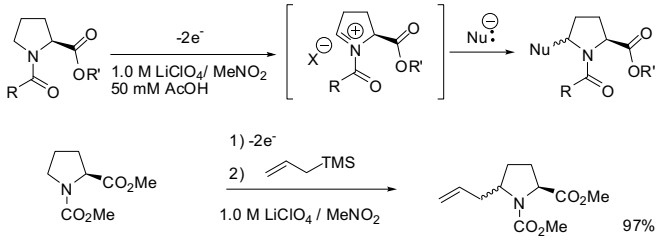
1. Introduction

Over the past few decades, peptide synthesis based on solid-phase techniques has grown in popularity to become essential for automated synthesis and combinatorial chemistry.[1–5] Alternatively, tag-assisted solution-phase organic synthesis has also developed into an important technique for solid-supported and combinatorial methodologies.[6–8] Efforts to design effective phase-tags that can improve the purification of the desired products using the solid-phase extraction (SPE)[9–11] have achieved higher reactivity with ease of separation from solutions. Furthermore, effective phase-tags can provide the possibility of using routine analytical methods to monitor reaction processes, and also help directly determine the structures of products on the liquid-phase-tags. In particular, hydrophobic phase-tagging strategy based on SPE featuring ODS-silica (octadecylsilyl silica) has been recognized as an effective method to effectively generate target products.

Recently, we have developed a cycloalkane-based thermomorphic biphasic reaction system that can readily accomplish the multi-step synthesis of hydrophobic tag-assisted peptides.[12–17] Specifically, benzyl moieties with long alkoxy groups can serve as effective protective groups of C-terminal peptides and as hydrophobic tags that assist selective separation during the on-cooling phase separation of the cycloalkane solution from excessive reagents in polar organic solvents. Additionally, the hydrophobic tags can also function as anchors for retention on the long alkyl chain-modified beads in aqueous phases through hydrophobic interactions. Retention on such hydrophobic beads can also provide for the evaluation of biological and biochemical activities of peptides on the immobilized peptides.

Introduction of the hydrophobic tags at the inert methylene group of prolyl residues, which are typically located at relatively acute positions within a peptide or protein, can be favorable in minimizing conformational changes of the entire molecule. The specific site for connecting the tags with 'hydrophilic' linkers would also effectively repel the peptides from the surface of the beads for the evaluations of the functions of peptides immobilized on the solid phase. Furthermore, the prolyl residues can function as either the N- or C-terminal residues of peptides during fragment syntheses because of its resistance to isomerization during activation of the carboxylate moiety.

Previously, we have developed an efficient method for introducing various functional groups at the *N*-α′-position of prolyl moieties via electrochemical oxidation in a $LiClO_4/CH_3NO_2$ electrolyte solution (Scheme 1).[18–21] In this case, our electrochemical methodology was applied toward the novel synthesis of hydrophobically tagged prolyl peptides. Herein, we report the synthesis of targeted hydrophobic phase-tagged prolyl residues featuring an anodically introduced proline derivative as the key intermediate. Furthermore, our methodology was employed in the solution-phase synthesis and activity-evaluations of prolyl peptides (Fig. 1).



Scheme 1.

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0040-4020/$ – see front matter © 2009 Published by Elsevier Ltd.
doi:10.1016/j.tet.2009.07.045

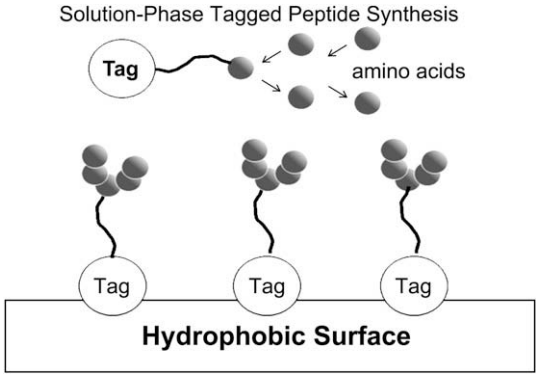
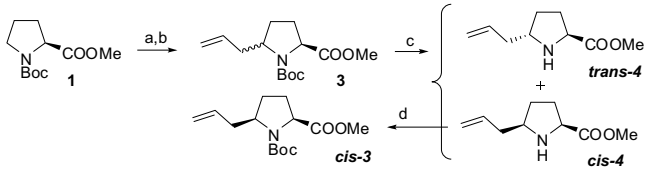


Figure 1. Concept of multi-step solution-phase peptides on a hydrophobic tag with ease of separation and retention of those immobilized products on the hydrophobic beads for the activity evaluation.

2. Results and discussion

2.1. Electrochemical synthesis of *cis*-allyl-proline

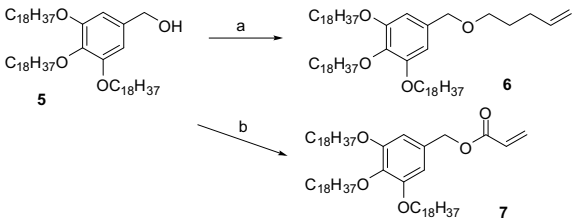
First, Boc-L-Pro-OMe (**1**) was electrolyzed at 1.9 V (vs Ag/AgCl) by direct anodic oxidation using a glassy carbon anode and a platinum cathode in a 1.0 M $LiClO_4/CH_3NO_2$ electrolyte solution in the presence of acetic acid (50 mM) at 0 °C (Scheme 2). Upon completion of the oxidation with the passage of an electrical current of 2.2 F/mol, an excess amount of allyltrimethylsilane **2** was added. The reaction mixture was allowed to stand at rt for 16 h to give Boc-L-(5-allyl)Pro-OMe (**3**) as an inseparable 1:1 mixture of cis- and trans-isomers in 77% yield.[22–27] Subsequently, the isomers were separated using column chromatography after removal of the Boc protection group. The purified cis-isomer of **4** (*cis*-**4**) was re-protected using Boc_2O in CH_3CN to give the cis-isomer of **3** (*cis*-**3**) in 68% yield.



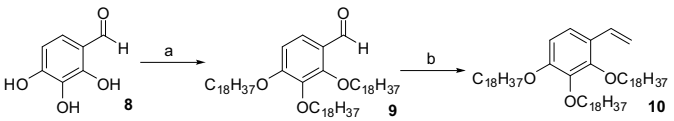
Scheme 2. (a) $-2e^-$, 1 M $LiClO_4/CH_3NO_2$, 50 mM AcOH; (b) allylTMS (**2**); (c) 4 M HCl/EtOAc, rt; (d) Bc_2O, DMAP, CH_3CN, rt.

2.2. Synthesis of hydrophobic phase-tagged proline derivatives

To assess the reactivity of the alkene moiety toward olefin cross-metathesis, pure *cis*-**3** was reacted with three hydrophobic tags: 1,2,3-tris-octadecyloxy-5-pent-4-enyloxymethyl-benzene (**6**), 3,4,5-tris-octadecyloxybenzyl acrylate (**7**) (Scheme 3), and 1,2,3-tris-octadecyloxy-4-vinylbenzene (**10**) (Scheme 4), which possess an aliphatic, an acrylate, and a conjugated (with aromatic ring) alkene as the terminal moieties, respectively. As shown in Schemes 3 and 4, compounds **6** and **7** were synthesized from 1,2,3-tris-octadecyloxybenzyl alcohol

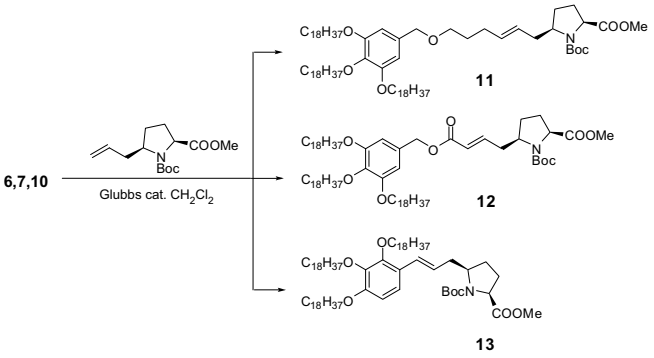


Scheme 3. (a) 5-Bromo-pent-1-ene, NaH, DMF, rt to 80 C, 5 h, 63%; (b) acrylic acid, DIPCI, DMAP(cat.), CH_2Cl_2, reflux, 3 h, 83%.



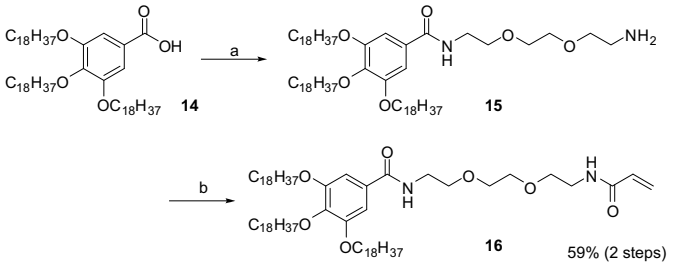
Scheme 4. (a) $C_{18}H_{37}Br$, K_2CO_3, DMI, 70 °C, 20 h (b) Ph_3PCH_3Br, PhLi, toluene, reflux, 6 h, 58% (two steps).

(**5**), whereas **10** was synthesized from 2,3,4-trihydroxybenzaldehyde (**8**) via octadecylation of the phenol groups followed by Wittig reaction. A solution of *cis*-**3** and hydrophobic tags **6**, **7**, or **10** was refluxed in anhydrous CH_2Cl_2 in the presence of Grubbs catalyst (second generation), then purified using column chromatography to afford the corresponding hydrophobic phase-tagged proline derivatives (**11**, 52%; **12**, 86%; **13**, 9%, respectively) (Scheme 5). In the case of **12**, the acrylic moiety was reactive with the allyl group of the proline derivative, even in the presence of highly hydrophobic groups.

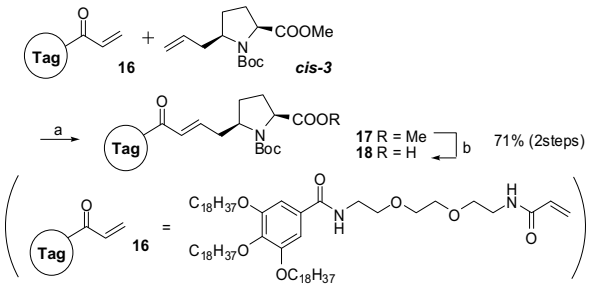


Scheme 5. Synthesis of hydrophobically tagged proline derivatives.

Subsequently, based on these preliminary studies, we undertook the synthesis of a hydrophobic tag that possesses a hydrophilic spacer with a terminal acrylamide group. When connected to an allylprolyl group, which would allow peptide-elongation, the hydrophilic spacer should serve to extending the key peptide units into the aqueous phases, while repelling from the intramolecular tags that are anchored on the solid phases. Accordingly, as shown in Scheme 6, 2-2′-(ethylenedioxy)diethylamine was introduced as a hydrophilic spacer for 3,4,5-tris(octadecyloxy)benzoic acid (**14**), followed by the introduction of an acrylamide moiety to give **16** (59% in TWO steps) (Scheme 6). As shown in Scheme 7, the Grubbs coupling reaction was carried out by refluxing a solution of hydrophobic tag **16** and *cis*-**3** in anhydrous CH_2Cl_2 in the presence of Grubbs catalyst (second generation). Addition of ethyl vinyl ether afforded the hydrophobic phase-tagged Boc-proline methyl ester (**17**), which was precipitated by the addition of CH_3CN. The methyl ester group of **17** was deprotected via hydrolysis using aqueous 1 M LiOH. The residue was purified using flash chromatography to give **18** as white powder in 71% yield (from **16**) (Scheme 7).

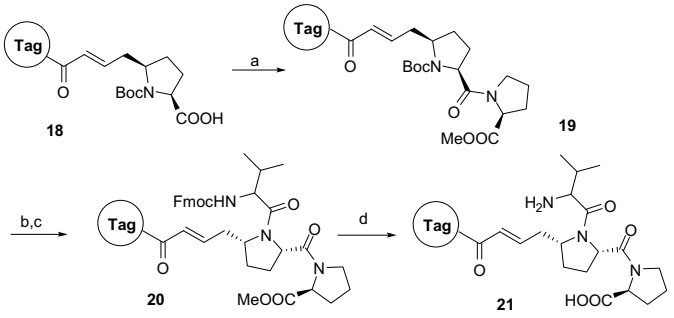


Scheme 6. (a) 2,2′-(Ethane-1,2-diylbis(oxy))diethanamine, **14**, DIPCI, HOBt, THF, rt; (b) acryloyl chloride, TEA, THF, rt.



Scheme 7. (a) Grubbs catalyst second generation, CH_2Cl_2, reflux; (b) LiOH, THF/water, 35 °C.

Using the hydrophobic phase-tagged Boc-proline **18**, the synthesis of a tagged lactotripeptide (VPP) was carried out as a model reaction to illustrate both N- and C-terminal peptide-bond elongations based on the tagged proline moiety. The C-terminal of **18** was activated at rt using diisopropylcarbodiimide (DIPCI) and 1-hydroxy-1*H*-benzotriazole (HOBt) (Scheme 8). The subsequent coupling between activated **18** and NH-Pro-OMe HCl was carried out in anhydrous THF in the presence of *N*,*N*-diisopropylethylamine (DIPEA). Upon stirring the reaction for 1 h at rt, an excess amount of CH_3CN was poured into the reaction mixture to precipitate the protected dipeptide **19** in 96% yield. After deprotection of the Boc group in anhydrous THF using 4 M HCl/EtOAc at rt, and removal of excess HCl in vacuo, deprotected **19** was redissolved in THF, treated with DIPEA, then coupled with Fmoc-Val-OH using DIPCI and HOBt. Upon precipitation by the addition of excess CH_3CN, protected tripeptide **20** was obtained in 91% yield as a white powder. The Fmoc group was removed by treatment with LiOH in THF to afford lactotripeptide **21** in 93% yield after recrystallization from THF/CH_3CN.



Scheme 8. (a) HCl-NH-Pro-OMe, DIPCI, HOBt, DIPEA, rt, 96%; (b) 4 M HCl/EtOAc, THF, rt; (c) Fmoc-Val-OH, DIPCI, HOBt, DIPEA, rt, 91% (two steps); (d) LiOH, THF/water, 35 °C, 93%.

To evaluate the hydrophobic tag as an anchor on hydrophobic beads dispersed in aqueous solutions, 4-(pyren-1-yl)butanoic acid was introduced as a fluorescence marker to the N-terminal of **21** to give **22**.[28–30] Upon completion of the condensation reaction, ODS beads were dispersed within the reaction mixture, then washed with water. Subsequently, the fluorescence of an aqueous dispersion of the ODS beads was measured using UV-irradiation (Fig. 2a). As a control experiment, an aqueous dispersion of ODS beads that was subjected to a reaction mixture of THF without **21** was also measured (Fig. 2b). In the absence of **21**, the fluorescence was scarcely observed (Fig. 2b). The fluorescence was also not observed on the ODS after the reaction of 4-(pyren-1-yl)butanoic acid and corresponding tripeptide (H-Val-Pro-Pro-OH)[31] in the presence of ODS followed by washing. The presence of fluorescence on the surface of ODS (Fig. 2a) suggests that the hydrophobic phase-tagged peptide are retained on the surfaces of hydrophobic ODS beads.

In addition, fragment peptide-elongation reactions were performed to confirm the functions of the hydrophobic tag for separations in each of muti-step reactions. As shown in Scheme 9, after

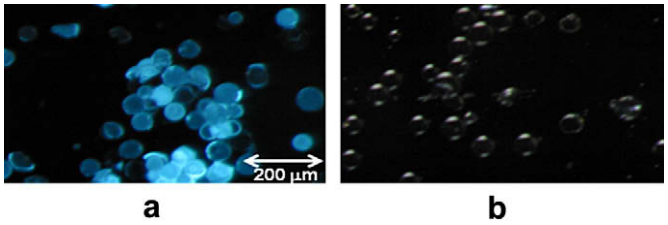
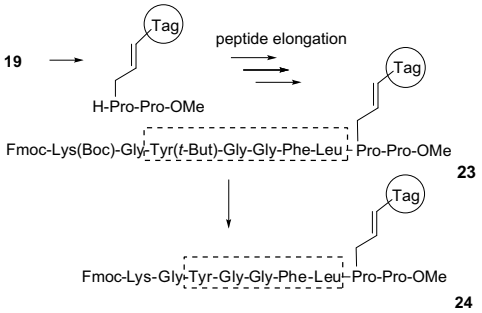


Figure 2. Microscopic observation of a pyrene-modified tagged peptide retained on ODS; (a) in the presence of the reaction mixture of 4-(pyren-1-yl)butanoic acid and **21**; (b) in the presence of the reaction mixture of 4-(pyren-1-yl)butanoic acid without **21** as a control.



Scheme 9. An example of peptide elongation on the hydrophobic phase-tagged prolyl peptides.

removing the Boc group of **19**, Fmoc-Leu-enkephalin moiety (Fmoc-Tyr-Gly-Gly-Phe-Leu-OH) was constructed stepwise on the prolyl NH group. Furthermore, Fmoc-Gly-OH and following Fmoc-Lys(Boc)-OH were attached in turn to introduce as a terminal hydrophilic moiety. Finally, Boc and *t*-Bu groups on the side chains of compound **23** were removed to confirm the completion of typical deprotection reactions of side chains. Each reaction and isolation of the tagged products were also confirmed to be accomplished by TLC analysis ($CHCl_3$/MeOH 9:1) in each step, and the final product **24** detected as a single spot on the TLC was analyzed by TOF MS (ESIMS). These sequential schemes showed that the tagged prolyl peptides could also be used as platforms for the solution-phase multi-step peptide syntheses.

3. Conclusion

Hydrophobic phase-tagged lactotripeptide (VPP) was synthesized as a model compound following the methodology of sequential precipitation. Introduction of a 3,4,5-tris(octadecyloxy)phenyl group as a hydrophobic tag was successfully accomplished via olefin metathesis between the acrylamide moiety of the hydrophobic tag and the electrochemically synthesized 5-allylprolyl residue. In the case of multi-step peptide syntheses, the hydrophobic tag was effective as a phase-tag by assisting the separation/purification of the product via precipitation. Furthermore, the tagged peptide was shown to be effectively anchored on ODS beads, which would allow for evaluating biochemical interactions of the peptide in aqueous dispersions.

4. Experimental section

4.1. Spectroscopic measurements

NMR spectra were measured in $CDCl_3$ using JEOL AL-400 and AL-600 spectrometers at 400, 600 (^{1}H) MHz and at 100, 150 (^{13}C) MHz, with tetramethylsilane as the internal standard. MS-spectra (MALDI, ESI) and infrared spectra were measured on Voyager-DE STR MALDI-TOF MS, JMS-T100LC AccuTOF, and JEOL WINSPEC-50, respectively.

4.2. Microscopic observations

ODS-silica was observed using LEICA MZ 16F equipped with LEICA 10447160 (10x/21B), LEICA 10447243 (PLANAPO 5.0x/0.50 LWD), under UV-irradiation (LEICA Filter 10447287).

4.3. Synthetic procedures

*4.3.1. Boc-L-(5-allyl)Pro-OMe (**3**).* A solution of Boc-L-Pro-OMe (**1**, 1.0 mmol) in 1.0 M $LiClO_4$/anhydrous CH_3NO_2 (20 mL) was electrolyzed under constant current at 0 °C, in the presence of 50 mM acetic acid, using an undivided cell equipped with a glassy carbon plate anode (60 mm×20 mm) and a Pt cathode (10 mm×10 mm) under an Ar atmosphere. Upon passage of 2.2 F/mol, the reaction mixture was treated with allyltrimethylsilane (**2**, 3.0 mmol), then allowed to stand for 16 h at rt. After concentration, the residue was purified using silica-gel column chromatography (*n*-hexane/EtOAc 6:1) to afford **3** as a mixture of cis- and trans-isomers (68% yield, colorless oil). ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.85–5.73 (1H, m), 5.10–5.03 (2H, m), 4.34–4.08 (1H, m), 3.98–3.83 (1H, m), 3.74–3.71 (3H, m), 2.74–2.43 (1H, m), 2.25–2.09 (2H, m), 2.00–1.87 (2H, m), 1.82–1.71 (1H, m), 1.48–1.40 (9H, m); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.9, 173.7, 173.6, 173.3, 154.3, 154.2, 153.6, 153.5, 135.3, 135.0, 135.0, 117.3, 117.2, 116.9, 79.9, 79.9, 79.8, 60.3, 60.1, 59.8, 59.7, 59.4, 58.0, 57.9, 57.5, 57.4, 52.0, 52.0, 51.8, 39.1, 38.9, 38.1, 38.1, 29.5, 28.7, 28.4, 28.2, 28.0.

*4.3.2. cis-NH-L-(5-Allyl)Pro-OMe (cis-**4**).* The mixture of the cis- and trans-isomers of **3** (0.5 mmol) was treated with 4 M HCl in EtOAc (60 mL). After stirring for 30 min, the reaction mixture was concentrated, diluted with 100 ml of EtOAc, then washed with 100 mL of $NaHCO_3$ (satd) and 100 mL of brine. The resulting organic layer was dried over anhydrous Na_2SO_4, then concentrated to give **4** as a mixture of diastereomers (169 mg, 83%, cis/trans 1:1), which were separated using silica-gel column chromatography (Et_2O/MeOH 97:3) to afford pure *cis*-**4** as a colorless oil (45% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.82 (1H, ddd, *J*=17.2, 10.3, 7.0 Hz), 5.14 (1H, dd, *J*=17.2, 1.5 Hz), 5.08 (1H, dd, *J*=10.3, 1.5 Hz), 3.85–3.83 (1H, m), 3.74 (3H, s), 3.22–3.17 (1H, m), 2.36–2.25 (2H, m), 2.17–2.10 (1H, m), 1.97–1.88 (2H, m), 1.41–1.33 (1H, m); ^{13}C NMR (150 MHz, $CDCl_3$) δ 175.0, 134.8, 117.3, 59.1, 58.6, 52.2, 39.5, 30.8, 30.0.

*4.3.3. cis-Boc-L-(5-allyl)Pro-OMe (cis-**3**).* To a solution of *cis*-**4** (43 mg, 2.6 mmol) in anhydrous CH_3CN (50 mL) was added Boc_2O (84 mg, 3.8 mmol) and DMAP (22 mg, 0.18 mmol). After stirring at rt for 1 h, the reaction mixture was diluted with aqueous 5% citric acid (100 mL), then extracted twice with 50 mL of EtOAc. The combined organic layer was washed with 100 mL of $NaHCO_3$ (satd) and brine (100 mL), dried over anhydrous Na_2SO_4, then concentrated in vacuo. The residue was purified using silica-gel column chromatography (*n*-hexane/EtOAc 6:1) to afford *cis*-**3** as colorless oil (68% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.85–5.77 (1H, m), 5.08 (1H, dd, *J*=17.2, 1.5 Hz), 5.04 (1H, d, *J*=10.3 Hz), 4.35–4.19 (1H, m), 3.98–3.83 (1H, m), 3.74 (3H, s), 2.75–2.59 (1H, m), 2.24–2.15 (2H, m), 2.00–1.86 (2H, m), 1.82–1.76 (1H, m), 1.48–1.39 (9H, m); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.8, 173.6, 154.2, 153.5, 135.3, 116.8, 79.9, 60.1, 59.7, 58.0, 57.9, 52.0, 51.8, 39.1, 38.1, 29.5, 28.7, 28.3, 28.2, 28.0.

*4.3.4. 1,2,3-Tris-octadecyloxy-5-pent-4-enyloxymethyl-benzene (**6**).* To a heated solution of **5** (1043.4 mg, 1.14 mmol) in DMF (30 mL) (heated to 80 °C for complete dissolution) were added NaH (137.2 mg, 3.43 mmol) and 5-bromo-pent-1-ene (0.27 mL, 2.28 mmol). After stirring at 80 °C for 5 h, the reaction mixture was quenched with 10 mL of methanol followed by addition of 30 mL of water, then extracted, while warm, with *n*-hexane (100 mL). The *n*-hexane layer was dried over anhydrous Na_2SO_4, then concentrated in vacuo. The residue was purified by recrystallization (20 mL of MeOH), then by column chromatography (*n*-hexane/EtOAc 30:1) to afford **6** as white powder (705.0 mg, 63% yield). Mp 44–45 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.52 (2H, s), 5.82 (1H, ddt, *J*=16.9, 10.3, 6.6 Hz), 5.02 (1H, ddt, *J*=16.9, 1.8, 1.8 Hz), 4.96 (1H, ddt, *J*=10.3, 1.8, 1.0 Hz), 4.39 (2H, s), 3.96 (4H, t, *J*=6.6 Hz), 3.92 (2H, t, *J*=6.6 Hz), 3.47 (2H, t, *J*=6.6 Hz), 2.17–2.12 (2H, m), 1.81–1.76 (4H, m), 1.76–1.69 (4H, m), 1.49–1.43 (6H, m), 1.37–1.21 (84H, m), 0.88 (9H, t, *J*=7.0 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 153.1, 138.3, 137.6, 133.6, 114.7, 106.2, 73.4, 73.1, 69.7, 69.1, 31.9, 30.4, 30.3, 29.8, 29.7, 29.7, 29.6, 29.4, 29.4, 29.0, 26.1, 26.1, 22.7, 14.1; IR (thin film) ν_{max} 3076, 2954, 2848, 1716, 1587, 1502, 1468, 1437, 1335, 1227, 1120 cm^{-1}; MALDI-TOF MS (*m*/*z*): calcd for $C_{66}H_{125}O_4$ 981.9663, found 981.9653.

*4.3.5. 3,4,5-Tris-octadecyloxybenzyl acrylate (**7**).* To a solution of **5** (3.33 g, 3.65 mmol) in CH_2Cl_2 (60 mL) at rt was added a solution of acrylic acid (0.50 mL, 7.3 mmol), DIPCI (2.3 mL, 114.6 mmol), and DMAP (134.4 mg, 1.10 mmol). After refluxing for 3 h, the reaction solution was concentrated in vacuo. The residue was purified by recrystallization (MeOH), then by silica-gel column chromatography (*n*-hexane/EtOAc 30:1) to afford **7** as a white powder (2.93 mg, 83% yield). Mp 54–56 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.56 (2H, s), 6.45 (1H, dd, *J*=17.2, 1.5 Hz), 6.17 (1H, dd, *J*=17.2, 10.6 Hz), 5.85 (1H, dd, *J*=10.6, 1.5 Hz), 5.09 (2H, s), 3.96 (4H, t, *J*=6.6 Hz), 3.94 (2H, t, *J*=6.6 Hz), 1.82–1.76 (4H, m), 1.76–1.70 (2H, m), 1.49–1.43 (6H, m), 1.37–1.22 (84H, m), 0.88 (9H, t, *J*=7.0 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 153.2, 138.3, 131.0, 130.7, 128.4, 117.4, 107.0, 73.4, 69.2, 66.7, 31.9, 30.3, 29.8, 29.7, 29.7, 29.7, 29.6, 29.4, 29.4, 29.4, 26.1, 26.1, 22.7, 14.1; IR (thin film) ν_{max} 3064, 2956, 2918, 2848, 1726, 1593, 1512, 1468, 1439, 1336, 1124 cm^{-1}; MALDI-TOF MS (*m*/*z*): calcd for $C_{64}H_{119}O_5$ 967.9057, found 967.9042.

*4.3.6. 1,2,3-Tris-octadecyloxy-4-vinylbenzene (**10**).* To a solution of stearyl bromide (6.04 g, 18.1 mmol) in DMI (30 mL) at 70 °C was added 2,3,4-trihydroxybenzaldehyde (807.6 mg, 5.24 mmol), followed by the addition of K_2CO_3 (3.0 g). After stirring at 70 °C for 20 h, the reaction mixture was treated with water (50 mL), then extracted, while warm, with *n*-hexane (100 mL). The *n*-hexane layer was dried over anhydrous Na_2SO_4, then concentrated in vacuo. The residue was purified by recrystallization (EtOAc/MeOH) to afford the precursor of **10**.

Separately, to a solution of Ph_3PCH_3Br (1.57 g, 4.41 mmol) in anhydrous toluene (80 mL) was added PhLi (2.1 mL, 4.41 mmol) at 0 °C, then allowed to warm to rt while stirring for 1 h. To this solution was added a solution of the precursor of **10** (2.0 g, 2.2 mmol) in anhydrous toluene (80 mL). After refluxing for 6 h, the reaction mixture was concentrated in vacuo. The residue was purified by recystallization (EtOAc/MeOH), then by silica-gel column chromatography (*n*-hexane/EtOAc 30:1) to afford **10** as a white powder (2.76 mg, 58% yield). Mp 53–54 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.16 (1H, d, *J*=8.8 Hz), 6.94 (1H, dd, *J*=18.0, 11.0 Hz), 6.63 (1H, d, *J*=8.8 Hz), 5.61 (1H, dd, *J*=18.0, 1.5 Hz), 5.15 (1H, dd, *J*=11.0, 1.5 Hz), 3.99–3.94 (6H, m), 1.84–1.78 (2H, m), 1.78–1.72 (4H, m), 1.50–1.42 (6H, m), 1.37–1.22 (84H, m), 0.88 (9H, t, *J*=7.0 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 153.2, 151.1, 141.8, 131.4, 124.8, 120.1, 112.4, 108.5, 74.0, 73.6, 68.7, 31.9, 30.4, 30.3, 29.7, 29.7, 29.6, 29.6, 29.5, 29.4, 29.4, 26.2, 26.1, 22.7, 14.1; IR (thin film) ν_{max} 3080, 2956, 2916, 2848, 1626, 1595, 1496, 1466, 1375, 1294, 1093 cm^{-1}; MALDI-TOF MS (*m*/*z*): calcd for $C_{62}H_{117}O_3$ 909.9003, found 909.9009.

4.4. General procedure for hydrophobic phase-tagged proline derivatives by cross-metathesis

To a solution of hydrophobic tag **6**, **7**, or **10** (0.1 mmol) and *cis*-**3** (0.1 mmol) in anhydrous CH_2Cl_2 (30 mL) was added Grubbs catalyst (second generation) **4** (0.005 mmol), then refluxed for 12 h under

an Ar atmosphere. To this solution was added ethyl vinyl ether (0.1 mL, 1.0 mmol), then stirred for 1 h to quench the catalyst. The hydrophobic proline derivative was crystallized with CH_3CN. The resulting crystals were isolated by filtration, rinsed with CH_3CN to remove unreacted *cis*-**3**″, then purified using silica-gel column chromatography (*n*-hexane/EtOAc 20:1) to afford the corresponding hydrophobic phase-tagged proline derivatives (**11**, 52%; **12**, 86%; **13**, 9%, respectively).

*4.4.1. cis-Boc-L-Pro{5-[6-(3,4,5-tris-octadecyloxy-benzyloxy)-hex-2-enyl]}-OMe (**11**).* White powder. Mp 42–44 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.52 (2H, s), 5.55–5.37 (2H, m), 4.41–4.37 (2H, m), 4.33–4.18 (1H, m), 4.12–3.90 (7H, m), 3.72 (3H, s), 3.48–3.43 (2H, m), 2.69–2.53 (1H, m), 2.19–2.06 (4H, m), 1.98–1.89 (2H, m), 1.84–1.70 (9H, m), 1.48–1.40 (15H, m), 1.36–1.23 (84H, m), 0.88 (9H, t, J=7.0 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.8, 173.5, 154.1, 153.4, 153.0, 137.4, 133.5, 132.2, 127.0, 106.0, 79.7, 73.2, 73.1, 69.7, 69.0, 68.9, 60.0, 59.6, 58.3, 51.9, 51.7, 37.8, 36.7, 31.9, 30.3, 29.7, 29.7, 29.6, 29.4, 29.3, 28.7, 28.3, 28.2, 28.0, 26.1, 26.1, 22.6, 14.0; IR (thin film) ν_{max} 2956, 2916, 2850, 1753, 1701, 1591, 1504, 1468, 1437, 1389, 1115 cm^{-1}; ESIMS (*m*/*z*): 1223 (M+H$^+$), 1245 (M+Na$^+$). HR-ESIMS (*m*/*z*) calcd for $C_{78}H_{143}NNaO_8$ 1245.0711, found 1245.0731.

*4.4.2. cis-Boc-L-Pro{5-[3-(3,4,5-tris-octadecyloxy-benzyloxy-carbonyl)-allyl]}-OMe (**12**).* White powder. Mp 49–51 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.03–6.96 (1H, m), 6.57–6.54 (2H, m), 5.94 (1H, d, J=15.8 Hz), 5.05 (2H, s), 4.36–4.20 (1H, m), 4.06–3.97 (1H, m), 3.96 (4H, t, J=6.6 Hz), 3.93 (2H, t, J=6.6 Hz), 3.75––3.71 (3H, m), 2.91–2.72 (1H, m), 2.44–2.34 (1H, m), 2.25–2.18 (1H, m), 2.04–1.91 (2H, m), 1.82–1.70 (7H, m), 1.49–1.39 (15H, m), 1.36–1.22 (84H, m), 0.88 (9H, t, J=7.0 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.7, 173.6, 166.2, 153.1, 146.2, 146.1, 138.2, 138.0, 130.9, 130.8, 123.1, 122.9, 107.1, 106.8, 80.4, 80.2, 73.4, 69.1, 66.5, 66.4, 60.0, 59.6, 57.3, 57.1, 52.2, 52.0, 37.9, 36.6, 31.9, 30.3, 30.2, 29.7, 29.7, 29.6, 29.6, 29.4, 29.4, 29.3, 28.9, 28.7, 28.3, 28.2, 28.0, 26.1, 26.1, 22.7, 14.1; IR (thin film) ν_{max} 2954, 2916, 2850, 1751, 1699, 1589, 1468, 1439, 1390, 1333, 1174, 1119 cm^{-1}; ESIMS (*m*/*z*): 1231 (M+Na$^+$). HR-ESIMS (*m*/*z*) calcd for $C_{68}H_{137}NNaO_9$ 1231.0191, found 1231.0196.

*4.4.3. cis-Boc-L-Pro{5-[3-(2,3,4-tris-octadecyloxy-phenyl)-allyl]}-OMe (**13**).* White powder. Mp 47.0–47.5 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.13–7.09 (1H, m), 6.66–6.59 (2H, m), 6.13–6.06 (1H, m), 4.36–4.20 (1H, m), 4.06–3.97 (1H, m), 3.97–3.92 (6H, t, J=6.2 Hz), 3.74 (3H, s), 2.85–2.71 (1H, m), 2.41–2.35 (1H, m), 2.21–2.15 (1H, m), 2.02–1.95 (1H, m), 1.92–1.71 (8H, m), 1.49–1.40 (15H, m), 1.36–1.24 (84H, m), 0.88 (9H, t, J=7.0 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.9, 173.6, 154.3, 153.6, 152.6, 152.5, 150.6, 141.9, 126.5, 126.4, 125.9, 125.7, 124.9, 124.7, 120.2, 120.1, 108.6, 79.9, 74.0, 73.6, 68.7, 60.3, 59.8, 58.6, 58.2, 52.1, 51.9, 38.5, 37.7, 31.9, 30.4, 29.7, 29.7, 29.6, 29.6, 29.6, 29.4, 29.4, 29.4, 28.8, 28.5, 28.4, 28.3, 28.1, 26.2, 26.1, 22.7, 14.1; IR (thin film) ν_{max} 2956, 2916, 2850, 1753, 1701, 1470, 1394, 1296, 1173, 1090 cm^{-1}; ESIMS (*m*/*z*): 1151 (M+H$^+$). HR-ESIMS (*m*/*z*) calcd for $C_{74}H_{136}NO_7$ 1151.0317, found 1151.0311.

*4.4.4. N-{2-[2-(2-Acryloylamino-ethoxy)-ethoxy]-ethyl}-3,4,5-tris-octadecyloxybenzamide (**16**).* To a solution of **14** (1.82 g, 1.96 mmol) in anhydrous THF (100 mL) was added a mixture of DIPCI (0.61 mL, 3.93 mmol) and HOBt (531.0 mg, 3.93 mmol). After stirring at rt for 90 min, the reaction mixture was slowly added dropwise to a solution of 2-2′-(ethylenedioxy)diethylamine (0.86 mL, 5.89 mmol) in anhydrous THF (50 mL), then stirred at rt for 1 h. The resulting intermediate was purified by recrystallization (CH_3CN), then redissolved in anhydrous THF (200 mL). To this solution was added a mixture of TEA (0.27 mL, 1.96 mmol) and acryloyl chloride (0.48 mL, 5.89 mmol), then stirred at rt for 30 min. After concentration, the residue was purified by recrystallization (THF/CH_3CN), followed by silica-gel flash chromatography ($CHCl_3$/MeOH 98:2) to afford **16** as a white powder (1.28 mg, 59% yield). Mp 88–90 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.00 (2H, s), 6.59 (1H, t, J=5.5 Hz), 6.28 (1H, dd, J=16.9, 1.5 Hz), 6.14 (1H, br), 6.10 (1H, dd, J=16.9, 10.3 Hz), 5.62 (1H, dd, J=10.3, 1.5 Hz), 4.02–3.96 (6H, m), 3.68 (2H, t, J=5.1 Hz), 3.67–3.62 (6H, m), 3.58 (2H, t, J=5.1 Hz), 3.52–3.49 (2H, m), 1.83–1.77 (4H, m), 1.76–1.71 (2H, m), 1.50–1.42 (6H, m), 1.36–1.23 (84H, m), 0.88 (9H, t, J=7.0 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.5, 165.5, 153.0, 141.3, 130.8, 129.4, 126.4, 106.0, 73.5, 70.3, 70.1, 69.9, 69.7, 69.4, 39.9, 39.2, 31.9, 30.3, 29.7, 29.6, 29.4, 29.4, 29.3, 26.1, 22.7, 14.1; IR (thin film) ν_{max} 3307, 3253, 3080, 3034, 2954, 2916, 2848, 1659, 1626, 1581, 1551, 1504, 1470, 1425, 1382, 1351, 1236, 1124 cm^{-1}; ESIMS (*m*/*z*): 1112 (M+H$^+$), 1114 (M+Na$^+$). HR-ESIMS (*m*/*z*) calcd for $C_{70}H_{131}N_2O_7$ 1111.9956, found 1111.9956.

*4.4.5. cis-Boc-L-Pro(5-{5-[3-(2-{2-[2-(3,4,5-tris-octadecyloxy-benzoylamino)-ethoxy]-ethoxy}-ethylcarbamoyl)-allyl]})-OH (**18**).* To a solution of hydrophobic tag **16** (0.1 mmol) and *cis*-**3** (0.1 mmol) in anhydrous CH_2Cl_2 (30 mL) was added Grubbs (catalyst second generation) (0.005 mmol), then refluxed for 12 h under an Ar atmosphere. To this solution was added ethyl vinyl ether (0.1 mL, 1.0 mmol), then stirred for 1 h to quench this catalyst. The tagged proline derivative was crystallized from CH_3CN. The crystals were isolated by filtration, rinsed with CH_3CN to remove unreacted *cis*-**3**, dissolved in THF (8 mL), then treated with aqueous 1 M LiOH (2 mL). After stirring at 35 °C for 24 h, the organic layer was separated, then dried over anhydrous Na_2SO_4. The crude product was purified by recrystallization (CH_3CN), followed by silica-gel flash chromatography ($CHCl_3$/MeOH 98:2) to afford **18** as a white powder (95.1 mg, 71% yield). Mp 74–78 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.11–7.01 (3H, m), 6.78–6.70 (1H, m), 6.46–6.39 (1H, m), 5.87 (1H, d, J=15.4 Hz), 4.32–4.18 (1H, m), 4.14–4.05 (1H, m), 4.00 (4H, t, J=6.6 Hz), 3.98 (2H, t, J=6.6 Hz), 3.79–3.73 (2H, m), 3.73–3.62 (4H, m), 3.62–3.57 (2H, m), 3.57–3.50 (2H, m), 3.50–3.41 (2H, m), 3.39–3.31 (1H, br), 2.89–2.56 (1H, m), 2.38–2.24 (1H, m), 2.20–2.09 (1H, m), 2.07–1.94 (2H, m), 1.84–1.69 (8H, m), 1.49–1.42 (15H, m), 1.36–1.22 (84H, m), 0.88 (9H, t, J=7.0 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.9, 167.8, 166.1, 155.5, 153.0, 141.2, 138.6, 129.1, 127.2, 106.0, 73.5, 70.2, 70.0, 69.9, 69.3, 68.1, 60.3, 57.4, 39.8, 39.2, 37.2, 31.9, 30.3, 29.7, 29.7, 29.7, 29.6, 29.4, 29.4, 29.3, 28.3, 26.1, 26.1, 22.7, 14.1; IR (thin film) ν_{max} 3261, 3088, 2954, 2916, 2848, 1736, 1697, 1670, 1635, 1581, 1545, 1502, 1470, 1425, 1389, 1350, 1238, 1122 cm^{-1}; ESIMS (*m*/*z*): 1339 (M+H$^+$), 1345 (M+Li$^+$), 1361 (M+Na$^+$). HR-ESIMS (*m*/*z*) calcd for $C_{81}H_{148}N_3O_{11}$ 1339.1114, found 1339.1128.

*4.4.6. cis-Boc-L-Pro(5-{5-[3-(2-{2-[2-(3,4,5-tris-octadecyloxy-benzoylamino)-ethoxy]-ethoxy}-ethylcarbamoyl)-allyl]})-Pro-OMe (**19**).* To a solution of **18** (446.8 mg, 0.33 mmol) in THF (30 mL) was added a mixture of DIPCI (0.10 mL, 0.67 mmol) and HOBt (90.5 mg, 0.67 mmol), then stirred at rt for 90 min. To this reaction mixture was added a solution of H-Pro-OMe/HCl (82.0 mg, 0.5 mmol) and DIPEA (0.17 mL, 0.99 mmol) in anhydrous THF (6 mL), then stirred at rt for 1 h. The product was precipitated by the addition of CH_3CN (30 mL) to afford **19** as a white powder (459.8 mg, 96% yield). Mp 67–69 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.06–7.00 (2H, m), 6.95–6.91 (1H, br), 6.83–6.73 (1H, m), 6.53–6.26 (1H, br), 5.92–5.85 (1H, m), 4.57–4.39 (2H, m), 4.03–3.95 (7H, m), 3.76–3.42 (16H, m), 2.85–2.63 (1H, m), 2.52–2.43 (1H, m), 2.21–2.10 (2H, m), 2.03–1.90 (5H, m), 1.84–1.70 (7H, m), 1.49–1.37 (15H, m), 1.36–1.22 (84H, m), 0.88 (9H, t, J=7.0 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.7, 171.3, 167.4, 166.4, 154.3, 153.3, 153.0, 141.0, 140.8, 129.4, 125.8, 125.7, 105.8, 80.1, 79.8, 73.4, 70.3, 70.2, 70.2, 69.9, 69.9, 69.8, 69.3, 58.8, 58.8, 58.7, 57.7, 57.7, 52.2, 52.1, 46.5, 39.8, 39.1, 37.3, 36.0, 31.9, 30.3, 29.7, 29.6, 29.6, 29.4, 29.4, 29.3, 28.9, 28.8, 28.7, 28.4, 28.3, 28.0, 27.5, 26.1, 24.9, 24.8, 22.7, 14.1; IR (thin film) ν_{max} 3500, 3263, 3078, 2953, 2914, 2848, 1747, 1695, 1668, 1633, 1581, 1543, 1504, 1470, 1394,

1394, 1338, 1238, 1122 cm^{-1}; ESIMS (m/z): 1450 ($M+H^+$), 1456 ($M+Li^+$), 1472 ($M+Na^+$). HR-ESIMS (m/z) calcd for $C_{87}H_{156}N_4LiO_{12}$ 1456.1880, found 1456.1870.

*4.4.7. Fmoc-Val-cis-L-Pro(5-{5-[3-(2-{2-[2-(3,4,5-tris-octadecyloxy-benzoylamino)-ethoxy]-ethoxy}-ethylcarbamoyl)-allyl]})-Pro-OMe (**20**).* To a solution of **19** (272.6 mg, 0.19 mmol) in anhydrous THF (10 mL) was added 4 M HCl/EtOAc (10 mL). After stirring at rt for 1.5 h, the reaction mixture was concentrated in vacuo to remove excess HCl. The residue was dissolved in anhydrous THF (20 mL), treated with DIPEA (0.10 mL, 0.56 mmol), then stirred at rt 3 h. To this reaction mixture was added a solution of Fmoc-Val-OH (129.0 mg, 0.38 mmol), DIPCI (0.06 mL, 0.38 mmol), and HOBt (51.3 mg, 0.38 mmol) in anhydrous THF (5 mL). After stirring at rt for 1 h, the crude product was precipitated by the addition of CH_3CN (30 mL) to afford **20** as a white powder (288.9 mg, 91% yield). Mp 75–78 °C; ^1H NMR (600 MHz, $CDCl_3$) δ 7.76 (2H, d, J=7.3 Hz), 7.58 (2H, d, J=7.3 Hz), 7.40 (2H, t, J=7.3 Hz), 7.31 (2H, t, J=7.3 Hz), 6.99 (2H, s), 6.83 (1H, dt, J=15.0, 7.3 Hz), 6.66 (1H, d, J=5.1 Hz), 6.25–6.15 (1H, m), 5.91 (1H, d, J=15.0 Hz), 5.21 (1H, dd, J=9.2, 4.4 Hz), 4.65–4.60 (2H, m), 4.58–4.53 (1H, m), 4.44 (1H, dd, J=10.6, 7.0 Hz), 4.29 (1H, dd, J=10.6, 7.0 Hz), 4.20 (1H, t, J=7.0 Hz), 4.16 (1H, t, J=9.2 Hz), 3.99 (4H, t, J=6.2 Hz), 3.97 (2H, t, J=6.2 Hz), 3.89–3.83 (1H, m), 3.70 (3H, s), 3.67–3.60 (10H, m), 3.59–3.54 (2H, m), 3.53–3.45 (2H, m), 2.74–2.60 (1H, m), 2.30–2.19 (1H, m), 2.07–1.91 (6H, m), 1.82–1.70 (8H, m), 1.49–1.42 (6H, m), 1.36–1.23 (84H, m), 0.98 (6H, t, J=6.6 Hz), 0.88 (9H, t, J=7.0 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.5, 171.1, 170.3, 167.4, 165.5, 156.1, 153.0, 143.7, 143.6, 141.2, 141.1, 139.4, 129.3, 127.6, 127.0, 126.9, 126.5, 125.1, 124.9, 119.9, 119.8, 105.8, 73.4, 70.2, 70.2, 69.9, 69.8, 69.3, 66.9, 58.9, 58.6, 57.7, 57.6, 52.1, 47.1, 46.6, 39.8, 39.1, 37.2, 31.8, 31.5, 30.3, 29.7, 29.7, 29.6, 29.5, 29.4, 29.3, 29.3, 28.7, 26.3, 26.0, 26.0, 24.8, 22.6, 19.9, 18.2, 14.0; IR (thin film) ν_{max} 3525, 3444, 3277, 3070, 2954, 2916, 2848, 1747, 1714, 1659, 1633, 1581, 1539, 1470, 1338, 1238, 1122 cm^{-1}; ESIMS (m/z): 1671 ($M+H^+$), 1693 ($M+Na^+$). HR-ESIMS (m/z) calcd for $C_{102}H_{167}N_5NaO_{13}$ 1671.2639, found 1671.2647.

*4.4.8. NH_2-Val-cis-L-Pro(5-{5-[3-(2-{2-[2-(3,4,5-tris-octadecyloxy-benzoylamino)-ethoxy]-ethoxy}-ethylcarbamoyl)-allyl]})-Pro-OH (**21**).* To a solution of **20** (152.3 mg, 0.11 mmol) in THF (6 mL) was added aqueous 1 M LiOH (6 mL), then stirred at 35 °C for 24 h. The organic layer was separated, then dried over Na_2SO_4. The product was precipitated by the addition of CH_3CN (20 mL) to give **21** as a white powder (140.2 mg, 93% yield). Mp 186–188 °C; ^1H NMR (600 MHz, $CDCl_3$) δ 7.01 (2H, s), 6.82 (1H, t, J=5.1 Hz), 6.72–6.65 (1H, m), 6.18 (1H, t, J=5.1 Hz), 5.90 (1H, s), 5.83 (1H, d, J=15.4 Hz), 4.21–4.15 (1H, m), 4.05 (1H, dd, J=11.0, 6.6 Hz), 4.03–3.95 (6H, m), 3.83 (1H, s), 3.71–3.60 (9H, m), 3.59–3.55 (2H, m), 3.51–3.46 (2H, m), 2.77–2.71 (1H, m), 2.64–2.58 (1H, m), 2.28–2.20 (2H, m), 2.12–1.85 (7H, m), 1.84–1.76 (4H, m), 1.76–1.70 (2H, m), 1.52–1.42 (6H, m), 1.39–1.20 (84H, m), 1.06 (3H, d, J=6.6 Hz), 0.91 (3H, d, J=6.6 Hz), 0.88 (9H, t, J=6.6 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.5, 167.5, 166.2, 165.5, 153.0, 141.2, 139.3, 129.4, 126.7, 105.9, 73.5, 70.2, 70.2, 70.0, 69.8, 69.4, 60.4, 59.7, 56.4, 39.8, 39.2, 35.3, 31.9, 30.3, 29.7, 29.7, 29.6, 29.6, 29.4, 29.4, 29.3, 28.0, 27.8, 26.1, 26.1, 25.5, 22.7, 19.3, 16.0, 14.1; IR (thin film) ν_{max} 3259, 3078, 2954, 2916, 2850, 1718, 1637, 1581, 1543, 1468, 1338, 1238, 1122 cm^{-1}; ESIMS (m/z): 1435 ($M+H^+$), 1441 ($M+Li^+$), 1457 ($M+Na^+$). HR-ESIMS (m/z) calcd for $C_{86}H_{156}N_5O_{11}$ 1435.1801, found 1435.1815.

*4.4.9. Introduction of 4-(pyren-1-yl)butanoic acid to compound **21**.* To a solution of 1-pyrenebutyric acid (3.5 mg, 0.012 mmol) DIPCI (1.8 μl, 0.012 mmol), HOBt (1.6 mg, 0.012 mmol), DIPEA (2 μl, 0.0012 mmol) in 4 ml of THF, compound **21** (0.00407 mg) was added and stirred for 8 h at rt. After the completion of condensation, water (4 ml) and cyclohexane (4 ml) were added and the product **22** was recovered from the cyclohexane layer (90%). Compound **22**: ESIMS (m/z) calcd for $C_{106}H_{169}N_5O_{12}Na$ 1727.27, found 1727.2.

4.4.10. Elongation of peptide chains on the hydrophobic tag. To a solution of compound **19** (26.2 mg, 0.018 mmol) in 2 ml of CH_2Cl_2, 4 M HCl/EtOAc (2 ml) was added and stirred for 90 min at rt. After the completion of the deprotection of Boc group, the solution was concentrated in vacuo. The residue was then dissolved in dry THF (2 ml) with DIPEA (13.6 μl, 0.072 mmol), stirred for 10 min at rt. The reaction mixture was poured into the THF solution of Fmoc-Leu-OH (0.036 mmol), HBTU (0.036 mmol), HOBt (0.036 mmol) and stirred for 2 h at rt. Acetonitrile was then poured into the reaction mixture to give the product as a precipitation. After separation of the precipitation by filtration, the product was then dissolved in 3 ml of THF. DBU (0.045 ml) was added to the solution, stirred for 5 min at rt to complete the deprotection of Fmoc group. The deprotected product was then isolated as the precipitation by the addition of acetonitrile and following filtration. According to the condensation and deprotection, Fmoc-Phe-OH, Fmoc-Gly-OH, Fmoc-Gly-OH, Fmoc-Tyr(*t*-Bu)-OH, Fmoc-Gly-OH, and Fmoc-Lys (Boc)-OH were introduced stepwise. Completion of the reactions and purification of every reaction steps were confirmed by TLC (silica-gel, $CHCl_3$/MeOH 9:1, yield 67% from compound **19**). Boc and *t*-Bu groups on Lys and Tyr residues of compound **23** were removed by the acid treatment with 4 M HCl/EtOAc (4 ml) in CH_2Cl_2 (2 ml) for 3 h at rt to give **24**. The product was isolated by the precipitation with CH_3CN followed by filtration. The structure of the isolated model compound **24** was confirmed by ESIMS. *Fmoc-L-Lys-Gly-L-Tyr-Gly-Gly-L-Phe-L-Leu-L-Pro(5-{5-[3-(2-{2-[2-(3,4,5-Tris-octadecyloxy-benzoyl-amino)-ethoxy]-ethoxy}-ethylcarbamoyl)-allyl]})-Pro-OMe* (**24**): ESIMS (m/z) calcd for $C_{133}H_{209}N_{12}O_{20}$ 2294.57, found 2294.6 ($M+H^+$).

Acknowledgements

This work was partially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology.

Supplementary data

^1H and ^{13}C NMR spectra of compound **4**, *cis*-**4**, *trans*-**4**, **6**, **7**, **10**, **11**, **12**, **13**, **16**, **18**, **19**, **20**, and **21** and ESIMS spectra of compound **22** and **24** are provided. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2009.07.045

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