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Direct modification and antimicrobial activity of L-lysyl-L-leucine and its analogues

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Materials methods

The experiments used the following commercially available chemicals and reagents without further purification: di-*tert*-butyl dicarbonate (Boc$_2$O), 99 %; trifluoroacetic acid, 99 % (Chemical line); *N*,*N*-dicyclohexylcarbodiimide (DCC), 99 % (Lancaster); n-nonyl alcohol, c.p.; n-decyl alcohol, c.p.; n-undecyl alcohol, c.p.; n-dodecyl alcohol, c.p.; L-lysine monohydrochloride, 98 %; L-leucine, 99,5 %; D-leucine, 99,5 %; D,L-leucine, 99,5%; L-isoleucine 98 %; L-norleucine 98 %; (Sigma-Aldrich); p-toluenesulfonic acid monohydrate, 99 %; 4-dimethylaminopyridine (DMAP), 98 % (Acros); NaOH, analytical grade (Chimmed); acetone, analytical grade; methylene chloride, c.p. (Ecos-1); tetrahydrofuran (THF), analytical grade (Component-Reagent); dioxane hydrochloride, 20% (Chemical Line); boron trifluoride etherate, 36% (Sigma-Aldrich).

The spectra obtained by ^{1}H nuclear magnetic resonance (NMR) spectroscopy were recorded in deuterated chloroform (CDCl$_3$) (Solvex-D, Russia), on a Bruker DPX-300 NMR spectrometer (Bruker BioSpin, Germany) with an operating frequency of 300 MHz. The internal standard was hexamethyldisiloxane (Sigma-Aldrich, Germany). Mass-spectra were obtained on a chromatograph-mass spectrometer LC-MS Agilent Infinity 1260 (USA). Elemental analyses were carried out on a CHNS-analyzer Thermo Finnigan. Thin-layer chromatography (TLC) was performed on Sorbfil plates (IMID, Russia). Substances containing free amino group were detected by 5% ninhydrin solution (Acros Organics, Belgium) followed by heating to 50-80 °C. Preparative thin-layer chromatography was performed using silica gel plates TLC with silica gel 60 F$_{254}$ (Merck, Germany).

Boc$_2$-L-lysyl-L-leucine *n*-decyl ester (Boc$_2$-L-lysyl-L-leucine-C$_{10}$). A solution of 0.31 g (1.48 mmol) of DCC in 5 ml of anhydrous dichloromethane and a catalytic amount of DMAP were added with stirring to a solution of 0.38 g (1.11 mmol) of Boc-protected L-lysine in 5 ml of anhydrous CH$_2$Cl$_2$ at 0 °C. After 10 min, 0.20 g (0.73 mmol) of L-leucine *n*-decyl ester dissolved in 5 ml of anhydrous CH$_2$Cl$_2$ was added to the reaction mixture. The mixture was kept under vigorous stirring for 24 h. After the reaction was complete, the precipitate was filtered off, the solution was washed with distilled water (5x30 ml), and dried with sodium sulfate. The solvent was removed *in vacuo*. The product was isolated by preparative chromatography on a silica gel plate in system toluene-ethyl acetate 3:1. 0.30 g (70%) of the condensation product was obtained.

^{1}H NMR spectrum ($CDCl_3$, δ, ppm): 0.91 (m, 9H, $CH_2\underline{CH_3}$), 1.19 (m, CH_3 (EtOH)), 1.28 (m, 16H, CH_2), 1.45 (s, 18H, $C(CH_3)_3$), 1.67 (m, 4H, β CH_2), 1.71 (m, 1H, $\underline{CH}CH_3$), 1.81 (m, 2H, γ CH_2 (Lys)), 1.95 (m, 2H, β CH_2 (Lys)), 3.12 (m, 2H, $\underline{CH_2}NH$), 3.5 (m, , CH_2 (EtOH)), 3.66 (t, 0.4 H, $CH_2\underline{NH}$), 4.05 (bs, 1H, $CH\underline{NH}$ (Boc)), 4.15 (t, 2H, α CH_2 ($C_{10}H_{21}OH$)), 4.61 (m, 2H, CH (Leu), $\underline{CH}NH$(Boc)), 5.1 (bs, 1H, $CH\underline{NH}$ (Boc)), 6.5 (m, 1H, NH (Lys)). (Fig. S1.)

Boc_2-L-lysyl-D-leucine *n*-decyl ester. The reaction was carried out similarly to the preparation of the compound Boc_2-L-lysyl-L-leucine-C_{10}. From 0.161 g (0.59 mmol) of *n*-decyl ester of D-leucine, 0.21 g (61%) of the condensation product were obtained.

^{1}H NMR spectrum ($CDCl_3$, δ, ppm): 0.91 (m, 9H, $CH_2\underline{CH_3}$), 1.19 (m, CH_3 (EtOH)), 1.29 (m, 16H, CH_2), 1,45 (s, 18H, $C(CH_3)_3$), 1.67 (m, 4H, β CH_2), 1.71 (m, 1H, $\underline{CH}CH_3$), 1.82 (m, 2H, γ CH_2 (Lys)), 1.95 (m, 2H, β CH_2 (Lys)), 3.11 (m, 2H, $\underline{CH_2}NH$), 3.5 (m, CH_2 (EtOH)), 4.05 (bs, 1H, $CH\underline{NH}$ (Boc)), 4.15 (t, 2H, α CH_2 ($C_{10}H_{21}OH$)), 4.60 (m, 2H, CH (Leu), $\underline{CH}NH$(Boc)), 5.1 (bs, 1H, $CH\underline{NH}$ (Boc)), 6.5 (m, 1H, NH (Leu)) (Fig. S2)

Boc_2-L-lysyl-D,L-leucine *n*-decyl ester. The reaction was carried out similarly to the preparation of the compound Boc_2-L-lysyl-L-leucine-C_{10}. From 0.037 g (0.14 mmol) of *n*-decyl ester of D,L-leucine, 0.038 g (45%) of the condensation product were obtained.

^{1}H NMR spectrum ($CDCl_3$, δ, ppm): 0.91 (m, 9H, $CH_2\underline{CH_3}$), 1.19 (m, CH_3 (EtOH)), 1.28 (m, 16H, CH_2), 1.45 (s, 18H, $C(CH_3)_3$), 1.67 (m, 4H, β CH_2), 1.73 (m, 1H, $\underline{CH}CH_3$), 1.83 (m, 2H, γ CH_2 (Lys)), 1.95 (m, 2H, β CH_2 (Lys)), 3.11 (m, 2H, $\underline{CH_2}NH$), 3.5 (m, CH_2 (EtOH)), 3.66 (t, 0.8 H, $CH_2\underline{NH}$), 4.05 (bs, 1H, $CH\underline{NH}$ (Boc)), 4.13 (t, 2H, α CH_2 ($C_{10}H_{21}OH$)), 4.61 (m, 2H, CH (Leu), $\underline{CH}NH$(Boc)), 5.1 (bs, 1H, $CH\underline{NH}$ (Boc)), 6.5 (d, 0.6 H, NH (Leu)) (Fig. S3).

Boc_2-L-lysyl-L-leucine *n*-nonyl ester. The reaction was carried out similarly to the preparation of the compound Boc_2-L-lysyl-L-leucine-C_{10}. From 0.065 (0.73 mmol) of *n*-nonyl ester of L-leucine, 0.089 g (61%) of the condensation product was obtained.

^{1}H NMR spectrum ($CDCl_3$, δ, ppm): 0.90 (m, 3H, $CH_2\underline{CH_3}$), 1.19 (m, CH_3 (EtOH)), 1.30 (m, 14H, CH_2), 1.45 (s, 18H, $C(CH_3)_3$), 1.65 (m, 4H, β CH_2), 1.75 (m, 1H, $\underline{CH}CH_3$), 1.83 (m, 2H, γ CH_2 (Lys)), 1.95 (m, 2H, β CH_2 (Lys)), 3.11 (m, 2H, $\underline{CH_2}NH$), 3.5 (m, CH_2 (EtOH)), 3.66 (t, 0.6H, $CH_2\underline{NH}$), 4.05 (bs, 1H, $CH\underline{NH}$ (Boc)), 4.12 (t, 2H, α CH_2 ($C_9H_{19}OH$)), 4.61 (m, 2H, CH (Leu), $\underline{CH}NH$(Boc)), 5.1 (bs, 1H, $CH\underline{NH}$ (Boc)), 6.45 (d, 0.6H, NH (Leu)) (Fig. S4).

Boc_2-L-lysyl-L-leucine *n*-undecyl ester. The reaction was carried out similarly to the preparation of the compound Boc_2-L-lysyl-L-leucine-C_{10}. From 0.30 g (1.1 mmol) of L-leucine *n*-undecyl ester, 0.49 g (72%) of the condensation product was obtained.

^1H NMR spectrum ($CDCl_3$, δ, ppm): 0.90 (m, 9H, $CH_2\underline{CH_3}$), 1.19 (m, CH_3 (EtOH)), 1.28 (m, 18H, CH_2), 1.45 (s, 18H, $C(CH_3)_3$), 1.65 (m, 4H, β CH_2), 1.72 (m, 1H, $\underline{CH}CH_3$), 1.82 (m, 2H, γ CH_2 (Lys)), 1.95 (m, 2H, β CH_2 (Lys)), 3.11 (m, 2H, $\underline{CH_2}NH$), 3.5 (m, CH_2 (EtOH)), 4.05 (bs, 1H, $CH\underline{NH}$ (Boc)), 4.11 (t, 2H, α CH_2 ($C_{11}H_{23}OH$)), 4.60 (m, 1H, CH (Leu), $\underline{CH}NH$(Boc)), 5.12 (bs, 1H, $CH\underline{NH}$ (Boc)), 6.47 (d, 1H, NH (Leu)) (Fig. S5).

Boc_2-L-lysyl-L-leucine *n*-dodecyl ester. The reaction was carried out similarly to the preparation of the compound Boc_2-L-lysyl-L-leucine-C_{10}. From 0.30 g (1.0 mmol) of L-leucine *n*-dodecyl ester, 0.42 g (67%) of the condensation product were obtained.

^1H NMR spectrum ($CDCl_3$, δ, ppm): 0.91 (m, 9H, $CH_2\underline{CH_3}$), 1.19 (m, CH_3 (EtOH)), 1.28 (m, 20H, CH_2), 1.45 (s, 18H, $C(CH_3)_3$), 1.67 (m, 4H, β CH_2), 1.74 (m, 1H, $\underline{CH}CH_3$), 1.85 (m, 2H, γ CH_2 (Lys)), 1.94 (m, 2H, β CH_2 (Lys)), 3.12 (m, 2H, $\underline{CH_2}NH$), 3.5 (m, CH_2 (EtOH)), 4.07 (bs, 1H, $CH\underline{NH}$ (Boc)), 4.12 (t, 2H, α CH_2 ($C_{12}H_{25}OH$)), 4.60 (m, 2H, CH (Leu), $\underline{CH}NH$(Boc)), 5.09 (bs, 1H, $CH\underline{NH}$ (Boc)), 6.45 (d, 1H, NH (Leu)) (Fig. S6).

Boc_2-L-lysyl-L-isoleucine *n*-decyl ester. The reaction was carried out similarly to the preparation of the compound Boc_2-L-lysyl-L-leucine-C_{10}. From 0.20 g (0.74 mmol) of L-isoleucine *n*-decyl ester, 0.24 g (54%) of the condensation product was obtained.

^1H NMR spectrum ($CDCl_3$, δ, ppm): 0.91 (m, 9H, $CH_2\underline{CH_3}$), 1.19 (m, CH_3 (EtOH)), 1.29 (m, 16H, CH_2), 1.45 (s, 18H, $C(CH_3)_3$), 1.60 (m, 4H, CH_2 (Lys)), 1.81 (m, 4H, β CH_2), 1.95 (m, 2H, β CH_2 ($C_{10}H_{21}OH$))), 3.13 (m, 2H, $\underline{CH_2}NH$), 3.5 (m, CH_2 (EtOH)), 4.07 (bs, 1H, $CH\underline{NH}$ (Boc)), 4.17 (t, 2H, α CH_2 ($C_{10}H_{21}OH$)), 4.59 ((m, 2H, CH (Ile), $\underline{CH}NH$(Boc)), 5.09 (bs, 1H, $CH\underline{NH}$ (Boc)), 6.6 (m, 1H, NH (Ile)) (Fig. S7).

Boc_2-L-lysyl-L-norleucine *n*-decyl ester. The reaction was carried out similarly to the preparation of the compound Boc_2-L-lysyl-L-leucine-C_{10}. From 0.10 g (0.37 mmol) of L-norleucine *n*-decyl ester, 0.14 g (63%) of the condensation product was obtained.

^1H NMR spectrum ($CDCl_3$, δ, ppm): 0.91 (m, 6H, $CH_2\underline{CH_3}$), 1.19 (m, CH_3 (EtOH)), 1.25 (m, 20H, CH_2), 1.45 (c, 18H, $C(CH_3)_3$), 1.65 (m, 4H, β CH_2), 1.83 (m, 2H, β CH_2 (Lys)), 1.92 (m, 2H, β CH_2 (Lys)), 3.15 (m, 2H, $\underline{CH_2}NH$, 3.5 (m, CH_2 (EtOH)), 3.66 (t, 0.9 H, $CH_2\underline{NH}$), 4.05 (bs, 1H, $CH\underline{NH}$ (Boc)), 4.15 (t, 2H, α CH_2 ($C_{10}H_{21}OH$)), 4.59 (m, 2H, CH (Nle), $\underline{CH}NH$(Boc)), 5.1 (bs, 1H, $CH\underline{NH}$ (Boc)), 6.6 (m, 1H, NH (Leu)) (Fig. S8).

L-Lysyl-L-leucine *n*-decyl ester hydrotrifluoroacetate (1a). To a solution of 4.5 mg (8.5 uM) of Boc-protected L-lysyl-L-leucine *n*-decyl ester in 1 ml of anhydrous dichloromethane was added 1 ml of trifluoroacetic acid. The reaction mixture was kept for 2 h at 0 °C. After completion of the reaction, the solvent and excess trifluoroacetic acid were distilled off under vacuum. Product **1a** was obtained in quantitative yield.

Found, %: C 49.82; H 7.54; N 6.67. $C_{26}H_{47}F_6N_3O_7$. Calculated, %: C 49.75; H 7.55; N 6.69.

ESI MS, m/z: $[M+H]^+$ 400.3 (Fig. S9).

L-Lysyl-D-leucine *n*-decyl ester hydrotrifluoroacetate (1b). The reaction was carried out similarly to the preparation of hydrotrifluoroacetate of *n*-decyl ester of L-lysyl-L-leucine. Product **1b** was obtained in quantitative yield.

Found, %: C 49.80; H 7.53; N 6.64. $C_{26}H_{47}F_6N_3O_7$. Calculated, %: C 49.75; H 7.55; N 6.69.

ESI MS, m/z: $[M+H]^+$ 400.3 (Fig. S10).

L-Lysyl-D,L-leucine *n*-decyl ester hydrotrifluoroacetate (1c). The reaction was carried out similarly to the preparation of hydrotrifluoroacetate of *n*-decyl ester of L-lysyl-L-leucine. Product **1c** was obtained in quantitative yield.

Found, %: C 49.81; H 7.53; N 6.65. $C_{26}H_{47}F_6N_3O_7$. Calculated, %: C 49.75; H 7.55; N 6.69.

ESI MS, m/z: $[M+H]^+$ 400.3 (Fig. S11).

L-Lysyl-L-leucine *n*-nonyl ester hydrotrifluoroacetate (1d). The reaction was carried out similarly to the preparation of hydrotrifluoroacetate of *n*-decyl ester of L-lysyl-L-leucine. Product **1d** was obtained in quantitative yield.

Found, %: C 49.97; H 7.38; N 6.80. $C_{25}H_{45}F_6N_3O_7$. Calculated, %: C 49.93; H 7.39; N 6.85.

ESI MS, m/z: $[M+H]^+$ 386.3 (Fig. S12).

L-Lysyl-L-leucine *n*-undecyl ester hydrotrifluoroacetate (1e). The reaction was carried out similarly to the preparation of hydrotrifluoroacetate of *n*-decyl ester of L-lysyl-L-leucine. Product **1e** was obtained in quantitative yield.

Found, %: C 50.60; H 7.68; N 6.62. $C_{26}H_{47}F_6N_3O_7$. Calculated, %: C 50.54; H 7.70; N 6.55.

ESI MS, m/z: $[M+H]^+$ 414.4 (Fig. S13).

L-Lysyl-L-leucine *n*-dodecyl ester hydrotrifluoroacetate (1f). The reaction was carried out similarly to the preparation of hydrotrifluoroacetate of *n*-decyl ester of L-lysyl-L-leucine. Product **1f** was obtained in quantitative yield.

Found, %: C 51.33; H 7.82; N 6.36. $C_{28}H_{51}F_6N_3O_7$. Calculated, %: C 51.29; H 7.84; N 6.41.

ESI MS, m/z: $[M+H]^+$ 428.4 (Fig. S14).

L-Lysyl-L-isoleucine *n*-decyl ester hydrotrifluoroacetate (1g). The reaction was carried out similarly to the preparation of hydrotrifluoroacetate of *n*-decyl ester of L-lysyl-L-leucine. Product **1g** was obtained in quantitative yield.

Found, %: C 49.79; H 7.52; N 6.64. $C_{26}H_{47}F_6N_3O_7$. Calculated, %: C 49.75; H 7.55; N 6.69.

ESI MS, m/z: $[M+H]^+$ 400.3 (Fig. S15).

L-Lysyl-L-norleucine *n*-decyl ester hydrotrifluoroacetate (1h). The reaction was carried out similarly to the preparation of hydrotrifluoroacetate of *n*-decyl ester of L-lysyl-L-leucine. Product **1h** was obtained in quantitative yield.

Found, %: C 49.78; H 7.54; N 6.63. $C_{26}H_{47}F_6N_3O_7$. Calculated, %: C 49.75; H 7.55; N 6.69.

ESI MS, m/z: $[M+H]^+$ 400.4 (Fig. S16).

L-Lysyl-L-leucine *n*-decyl ester hydrochloride (1i). To a solution of 5.0 mg (9.2 uM) of Boc-protected *n*-decyl ester of L-lysyl-L-leucine was added 1 ml of dioxane hydrochloride. The reaction mixture was maintained for 2 h at 0 °C. The solvent was removed *in vacuo*. Product **1i** was obtained in quantitative yield.

Found, %: C 55.87; H 10.04; N 8.85. $C_{22}H_{47}Cl_2N_3O_3$. Calculated, %: C 55.92; H 10.03; N 8.89.

ESI MS, m/z: $[M+H]^+$ 400.3 (Fig. S17).

L-Lysyl-L-leucine *n*-decyl ester trifluoroborane (1j). To a solution of 37.5 mg (69 uM) of Boc-protected L-lysyl-L-leucine *n*-decyl ester in 2 ml of dry dichloromethane was added 2 ml of trifluoroacetic acid. The reaction mixture was kept for 2 h at 0 °C. After completion of the reaction, the solvent and excess trifluoroacetic acid were distilled off under vacuum. The obtained substance was dissolved in methylene chloride, the solution was washed with distilled water (5x30 ml), dried with sodium sulfate, and the solvent was removed in vacuum. The product was purified from impurities using preparative chromatography on a silica gel plate. The obtained L-lysyl-L-leucine decyl ester was dissolved in 3 ml of ethyl acetate, and 20 µl (0.16 mmol) of boron trifluoride etherate were added, and the mixture was stirred for 10 min. The solvent was removed in vacuum. Product **1j** was obtained in quantitative yield.

Found, %: C 49.32; H 8.44; N 7.80. $C_{22}H_{45}B_2F_6N_3O_3$. Calculated, %: C 49.37; H 8.47; N 7.85.

ESI MS, m/z: $[M+H]^+$ 400.3 (Fig. S18).

Evaluation of antibacterial activity in liquid nutrient medium:

Antibacterial activity was studied by determining the minimum inhibitory concentration (MIC) by serial broth microdilution [I. Wiegand, K. Hilpert, R.E. Hancock, *Nat Protoc.* 2008, **3**, 163. doi: 10.1038/nprot.2007.521]. Suspensions of *B. subtilis* 534 and *E. coli* M17 with a concentration of 1.5×10^8 CFU/mL and an optical density of 0.5 McFarland units were used as test microorganisms. The wells of a 96-well plate were filled with 100 µL each of the contaminated broth and appropriate amounts of the test compounds. The plates were incubated at 36 ± 1°C for 16–18 h. The MIC of the substances is taken as the first transparent well in the row when counting from the right-hand side.

Suspensions of *B. subtilis* 534 and *E. coli* M17 with a concentration of 1.5×10^8 CFU/mL and an optical density of 0.5 McFarland units were used as test microorganisms. The final titer of the inoculum in nutrient broth was 10^5 CFU/ml. The wells of a 96-well plate were filled with 50 μL of the contaminated broth. Next, successive twofold dilutions of hazardous substances and positive control were prepared in the wells of the plate. Wells with contaminated broth, not containing the substances, served as controll growth of microorganisms (negative control). The glycopeptide antibiotic vancomycin was used as a positive control. The plates were incubated at 36 ± 1°C for 16–18 h. The MIC of the substances is taken as the first transparent well in the row when counting from the right-hand side.

Formation of flat bilayer lipid membranes (BLMs) and measurement of their electrical conductance: The BLMs were formed on the holes of a copper mesh (SPI-Grids, 200 mesh, 3 mm). 0.4 μl of the lipid mixture in a solution of n-octane:n-decane (1:1 v/v) (Acros Organics, USA) at a concentration of 15 mg/ml was preliminarily applied to the mesh and dried under a stream of argon for 5-10 s until the solvent completely evaporated. Then the mesh was fixed in a Petri dish at a distance of 1-2 mm from the bottom and filled with 4 ml of a working buffer solution of 100 mM KCl (Sigma Aldrich, Saint-Louis, MO, USA) and 10 mM HEPES (Helicon, Russia) at pH 7.5. A Milli-Q water purification system (MilliPore, Direct-Q 3UV system, USA) was used to prepare the buffer. A lipid mixture of the same composition in squalane (Sigma Aldrich, Saint-Louis, MO, USA) at a concentration of 25 mg/ml was used to form BLMs. The membranes were formed by the “brush painting” method. The brush was washed sequentially with Milli-Q water, ethanol (1 ml), and chloroform (1 ml), and then dried under an argon stream. When the lipid mixture was applied to the mesh, BLMs spontaneously formed on the mesh holes, and the process was monitored in a microscope (magnification 40x). Lipid mixtures were prepared using stock solutions of 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine (DOPC), 1,2-dioleoyl-*sn*-glycero-3-phosphatidyl-(1'-*rac*-glycerol) (sodium salt) (DOPG), 1,2-diolenyl-*sn*-glycero-3-phosphatidylethanolamine (DOPE) (all from Avanti Polar Lipids, Alabaster, AL, USA) dissolved in chloroform (Sigma Aldrich, Saint-Louis, MO, USA) at a concentration of 10 mg/ml each. BLMs with a lipid composition of DOPC:DOPG:DOPE 6:2:2 mol% were used in the work. The test substance was dissolved in a working buffer solution to obtain a concentration of 2 nM (corresponding to MIC 1.5625 μg/ml) before the experiment and stored at +4 °C. To add the test substance directly to the lipid bilayer, the solution was injected onto the membrane from a glass patch pipette and the change in membrane conductance was monitored in real time using patch-clamp technology (HEKA EPC 8). Electrical measurements were performed at a fixed voltage (+100 mV) using a pair of Ag/AgCl electrodes.

Estimation of the size of pores in the membrane caused by the test amphiphile. The pore radius was measured according to the formula:

$$R = \left(l + \frac{\pi r}{2}\right) \frac{\rho}{\pi r^2}$$

where R is the electrical resistance of the pore found from experimental data, l = 4 nm is the thickness of the lipid membrane, ρ = 1.264 $\Omega^{-1}m^{-1}$ is the specific resistance of the electrolyte solution (buffer solution, 100 mM KCl), r is the radius of the pore lumen.

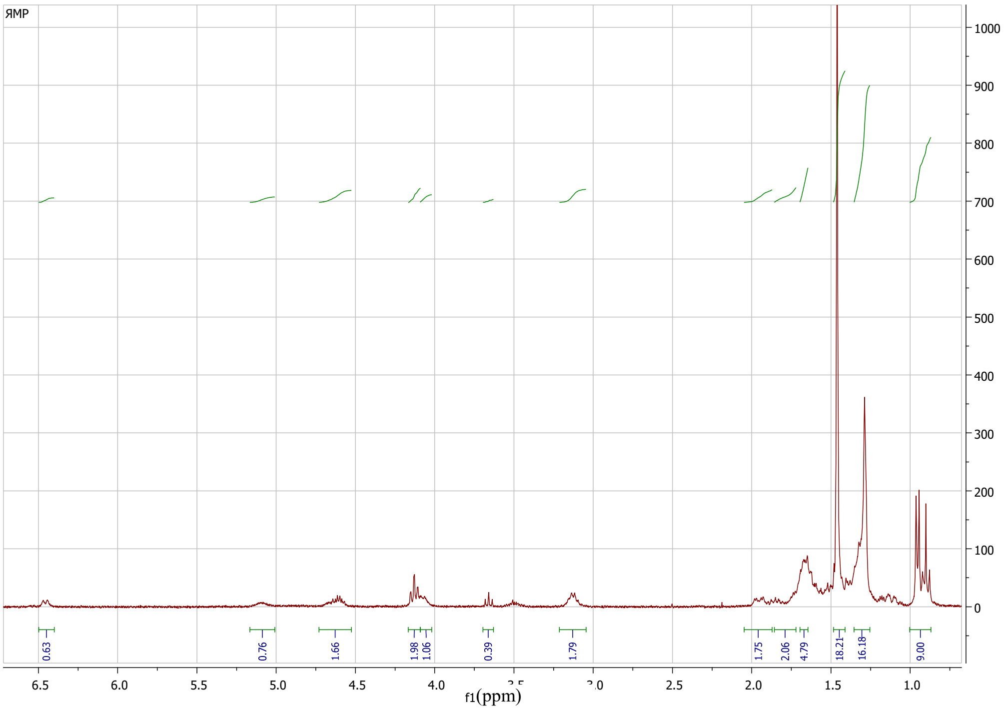

Figure S1 ^{1}H NMR spectrum of Boc$_2$-L-lysyl-L-leucine *n*-decyl ester

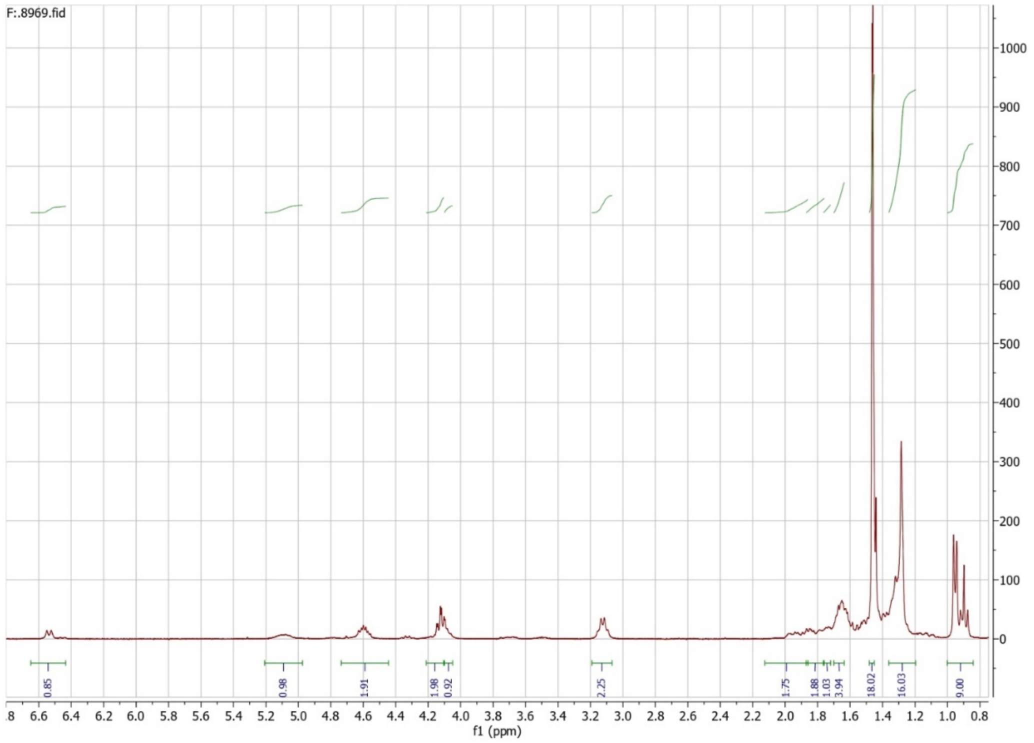

Figure S2 ^{1}H NMR spectrum of Boc$_2$-L-lysyl-D-leucine *n*-decyl ester

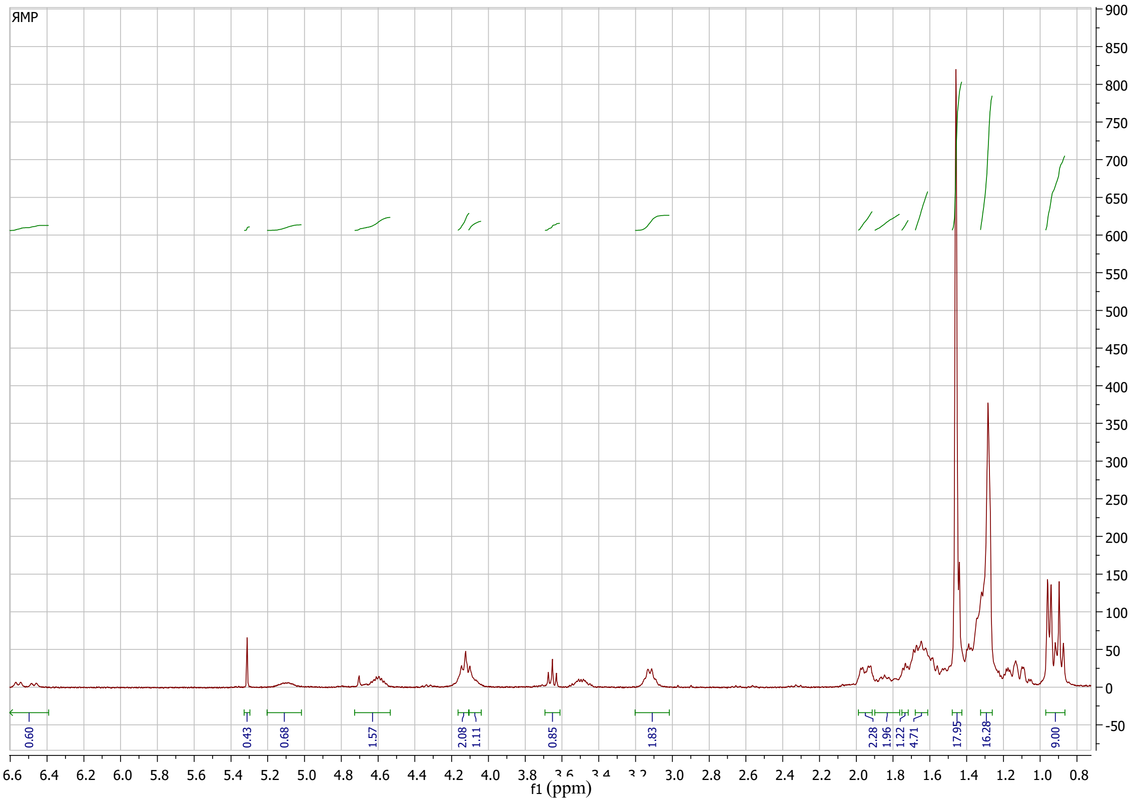

Figure S3 ^{1}H NMR spectrum of Boc$_2$-L-lysyl-D,L-leucine *n*-decyl ester

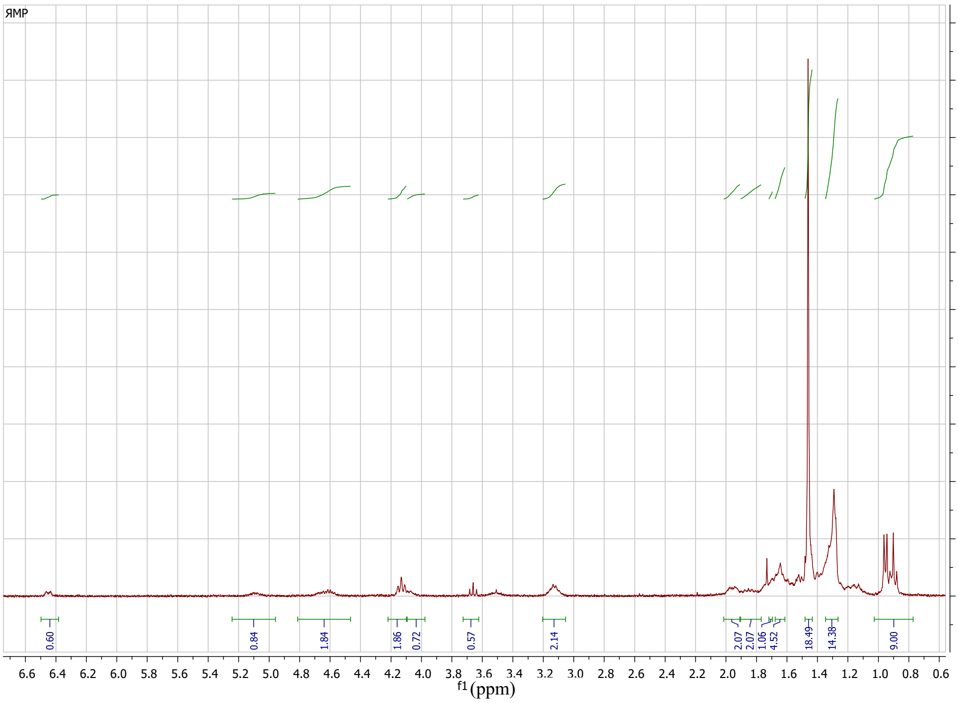

Figure S4 ^{1}H NMR spectrum of Boc$_2$-L-lysyl-L-leucine *n*-nonyl ester

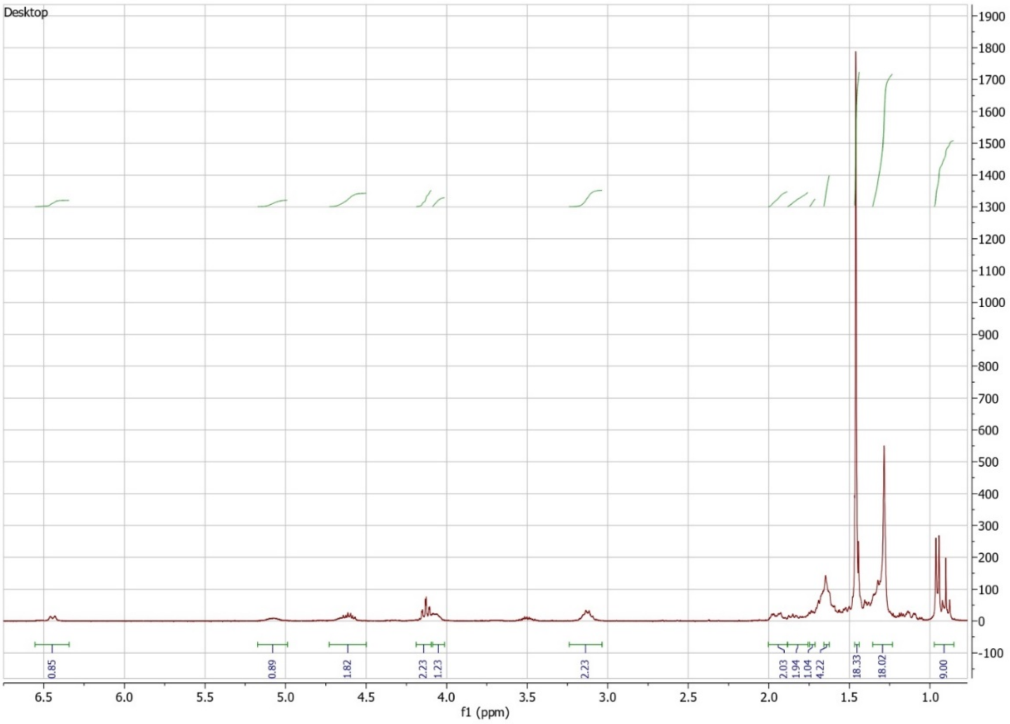

Figure S5 ^{1}H NMR spectrum of Boc$_2$-L-lysyl-L-leucine *n*-undecyl ester

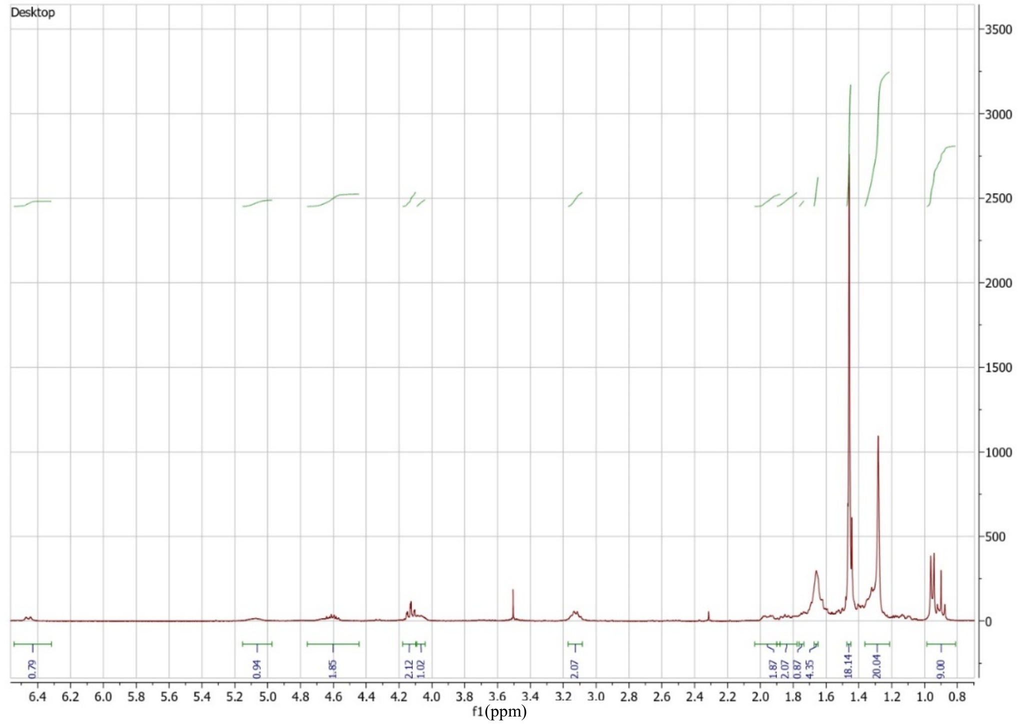

Figure S6 ^{1}H NMR spectrum of Boc$_2$-L-lysyl-L-leucine *n*-dodecyl ester

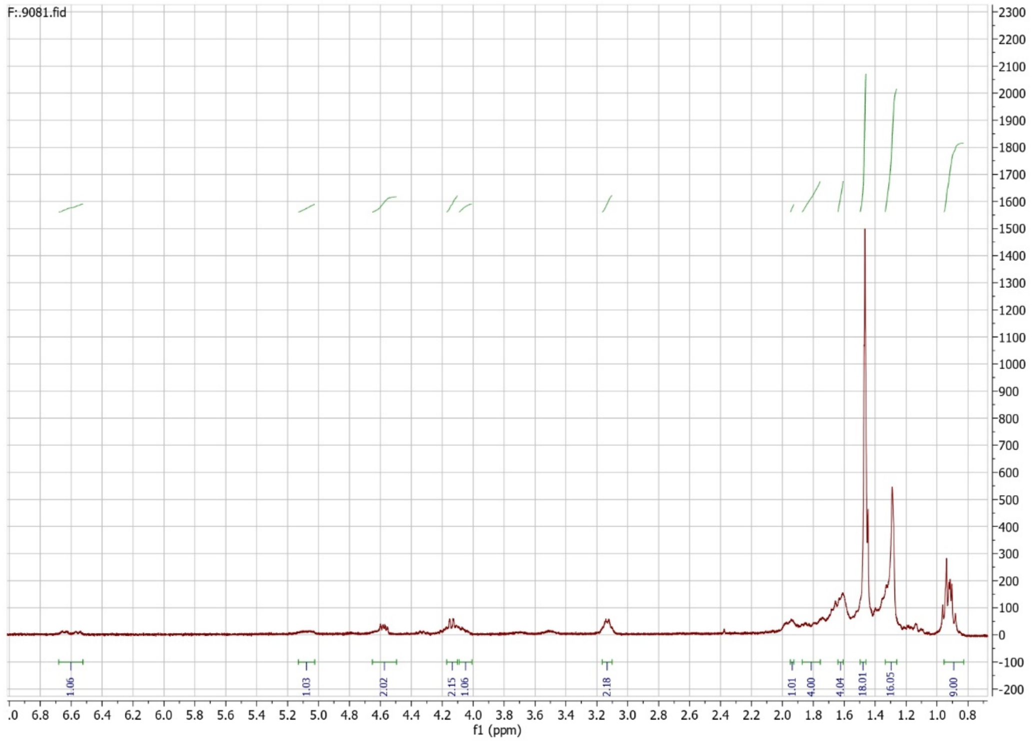

Figure S7 ^{1}H NMR spectrum of Boc$_2$-L-lysyl-L-isoleucine *n*-decyl ester

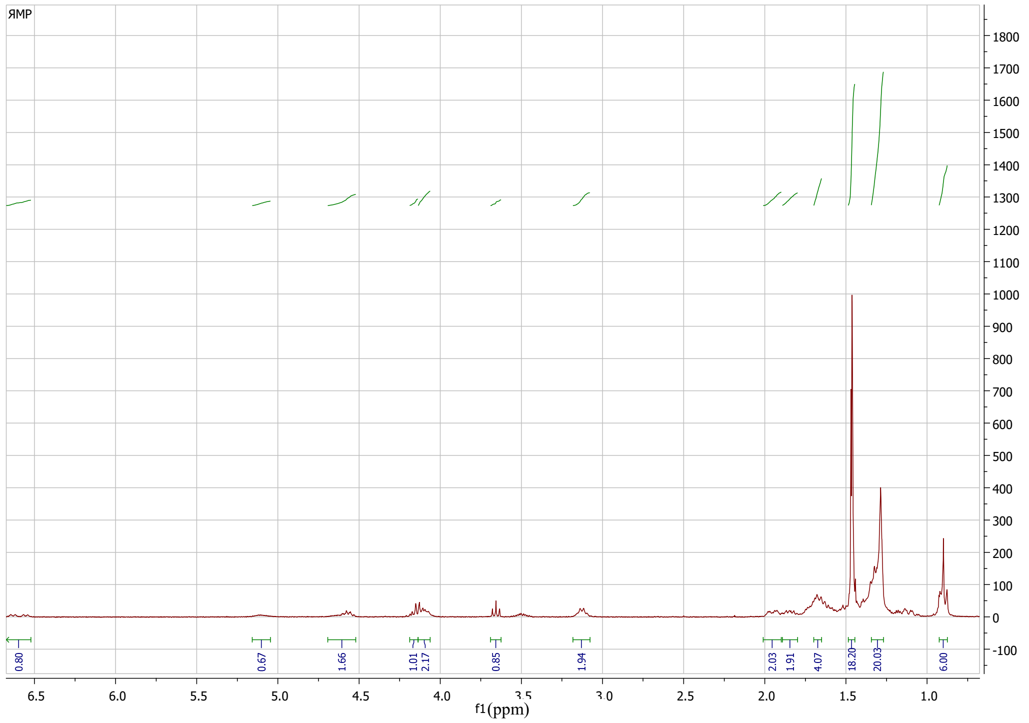

Figure S8 ^{1}H NMR spectrum of Boc$_2$-L-lysyl-L-norleucine *n*-decyl ester

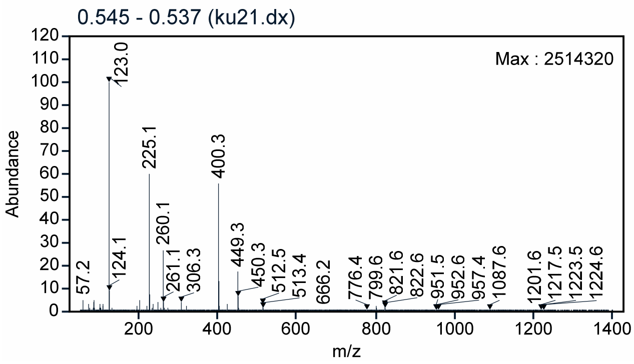

Figure S9 ESI-MS **1a**

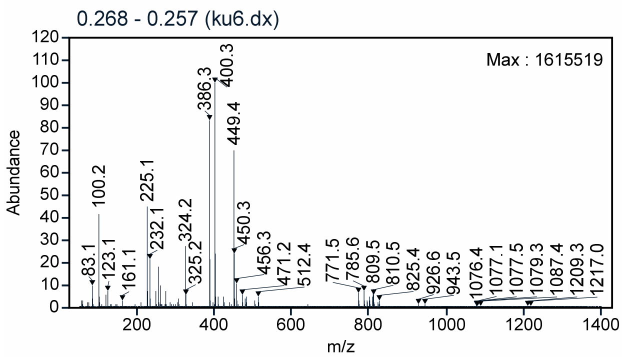

Figure S10 ESI-MS **1b**

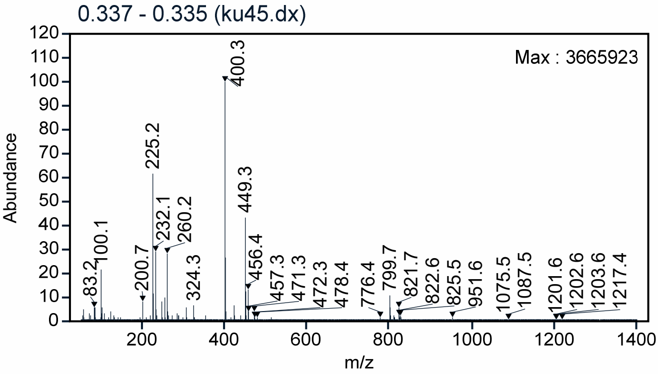

Figure S11 ESI-MS **1c**

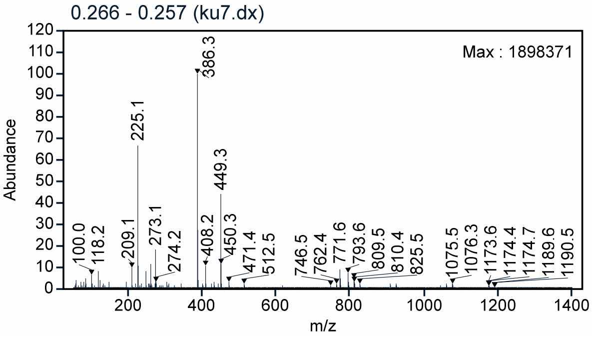

Figure S12 ESI-MS **1d**

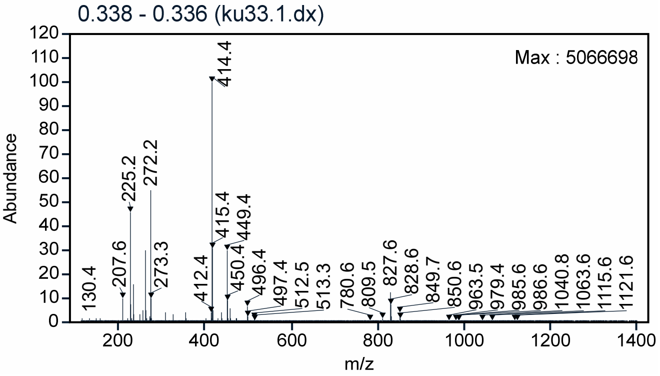

Figure S13 ESI-MS **1e**

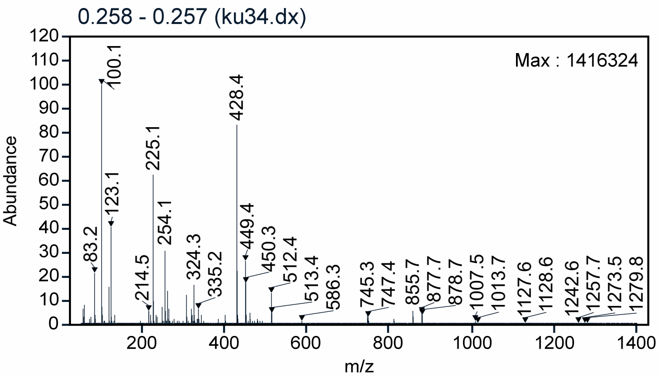

Figure S14 ESI-MS **1f**

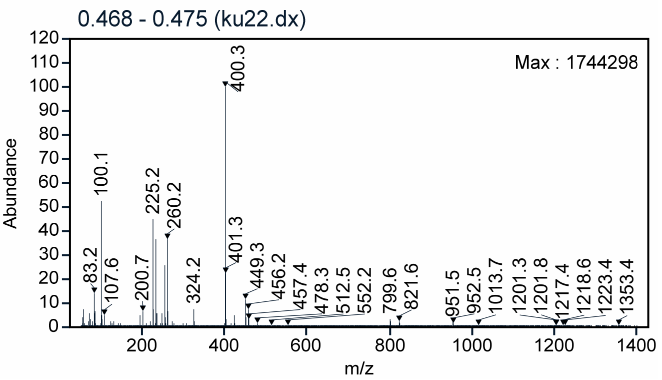

Figure S15 ESI-MS **1g**

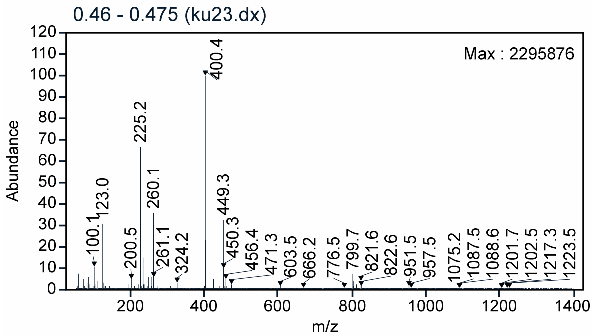

Figure S16 ESI-MS **1h**

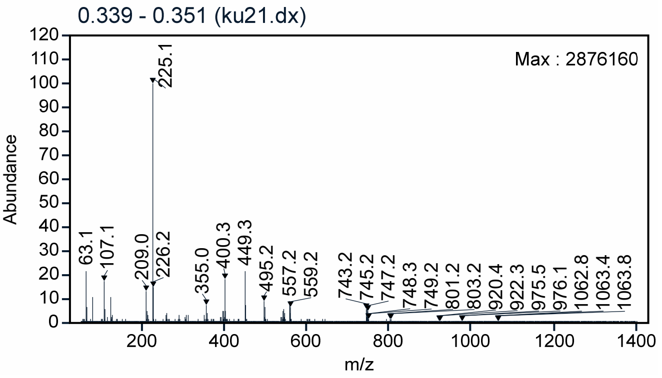

Figure S17 ESI-MS **1i**

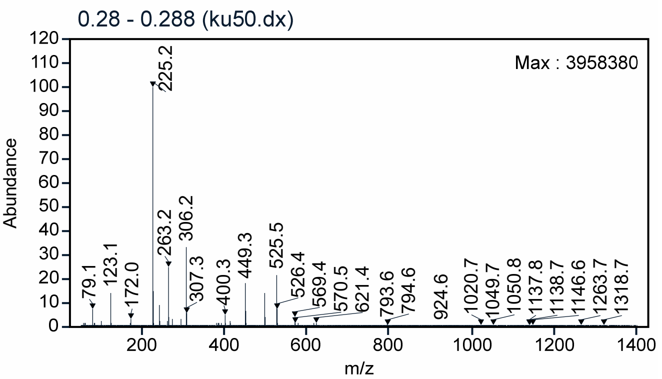

Figure S18 ESI-MS **1j**