Non-leaching antibacterial cotton fabrics based on lipidated peptides

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Supplementary Information

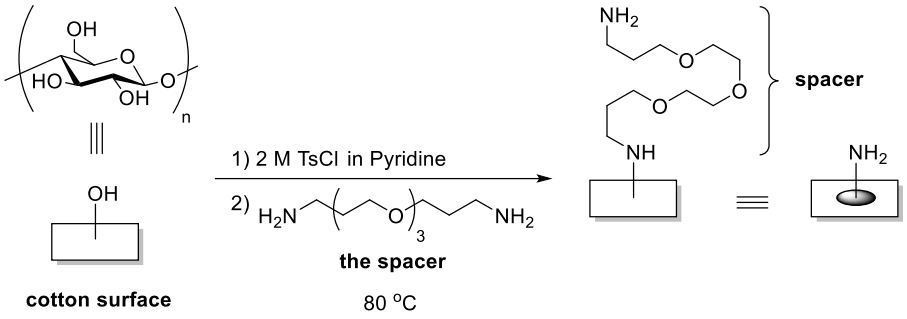
Representative prices of amino acids and fatty acids used in this study

The prices (in USD) listed herein are from Sigma-Aldrich (www.sigmaaldrich.com – accessed 13 June 2017). It is apparent that fatty acids are generally and significantly cheaper than amino acids, especially those amino acids with extra functional groups like arginine.

The prices are shown for educational discussion only – the authors have no conflict of interest with the company.

Reagent (catalog no.)	**Size (g or mL)**	**Price ($)**	**Price per gram ($) assuming 1 g/mL for density**
butyric acid (B103500)	100 mL	25.30	0.25
dodecanoic acid (L4250)	100 g	29.50	0.30
Fmoc-Leu-OH (47633)	50 g	53.60	1.07
Fmoc-D-Leu-OH (8.52145)	25 g	276.00	11.04
Fmoc-Arg(Pbf)-OH (47349)	25 g	302.00	12.08
Fmoc-D-Arg(Pbf)-OH (47348)	1 g	104.00	104.00

Initial modification of cotton fabrics



All cotton surfaces used in this work were pre-modified with 4,7,10-trioxa-1,13-tridecanediamine ("the spacer" as shown above). This serves to increase the length of the active molecular scaffolds and to convert the hydroxyl group on cellulose into the amino group that is more efficient in subsequent chemical reactions. First, cotton fabrics were cut into pieces of 6x6 cm^2. 1x1 cm^2 grids were then drawn on each piece, along with a pencil mark at the center of each grid. Subsequently, cotton sheets were washed with 1:1 DMF:H_2O (2x, 5 min each), MeOH (5 min), and CH_2Cl_2 (5 min), respectively, and this was followed by air drying. Thereafter, cotton sheets were submersed into a solution of 2 M tosyl chloride in pyridine. The container was shaken at 160 rpm for 1.50 h at room temperature. After washing with DMF (2x, 3 min each), MeOH (3 min), CH_2Cl_2 (3 min), and air drying, the tosylated cotton sheets were submersed in 1 M spacer in DMF at room temperature for 18 h (with shaking at 160 rpm). The spacer-attached cotton fabrics were subsequently washed with 5 M NaOH in 7:1 MeOH:H_2O (1x 5 min), MeOH (1x, 5 min), DMF (1x, 5 min), and water (2x, 5 min each), respectively. Final air drying by incubating in an oven at 40 °C for 18 h provided the amino-functionalized cotton fabrics.

Detailed procedure for lipidated peptide synthesis on cotton

Typical peptide coupling cycles.

The addition of each Fmoc-protected amino acid follows the following steps.

1) *Carbodiimide coupling*. An Fmoc-amino acid (0.45 M) and HOBt (0.9 M) were dissolved in DMF. DIC (1.35 M) was then added to this solution, and the resulting mixture was directly spotted (2 μL) on the amino functionalized cotton surface. The fabrics were kept at room temperature for 10 min. These processes (spotting and 10-min incubation) were repeated 2 more times, totaling 3 spotting steps. Subsequently, the fabrics were washed with 1:1 DMF:H_2O (3 min, 2x).
2) *Capping*. Unreacted amino groups from the previous step were capped by immersing cotton surfaces in 5% of propionic anhydride (0.5 mL in 9.5 mL of DMF). The container was shaken at 160 rpm for 15 min. Thereafter, the fabrics were washed with 1:1 DMF:H_2O (3 min, 2x), and MeOH (3 min, 1x). The cotton sheet was left in an open atmosphere for drying.
3) *Fmoc deprotection*. Fmoc group was cleaved off the latest amino acid by a treatment of cotton fabrics with 5% piperidine (0.5 mL) and 2% DBU (0.2 mL) in DMF (9.3 mL) for 15 min with shaking at 160 rpm. The cotton surfaces were washed 1:1 DMF:H_2O (3 min, 2x), and MeOH (3 min, 1x). The cotton sheet was left in an open atmosphere for drying. The resulting fabrics were then ready for the next coupling cycle.

Coupling of the final amino acid (no matter if a fatty acid would subsequently be appended.)

1) *Carbodiimide coupling*. The same condition as outlined above was used.
2) *Capping*. The same condition as outlined above was used.
3) *Fmoc deprotection*. Fmoc group was cleaved off by a treatment of cotton fabrics with 20% piperidine (2 mL) and 2% DBU (0.2 mL) in DMF (7.8 mL) for 15 min with shaking at 160 rpm. The cotton surfaces were washed 1:1 DMF:H_2O (3 min, 2x), and MeOH (3 min, 1x). The cotton sheet was left in an open atmosphere for drying.

Coupling of a fatty acid (whether it was coupled to an amino acid or directly to the amino group of the spacer molecule as in the surfaces **C12**, **C16**, and **C18**.)

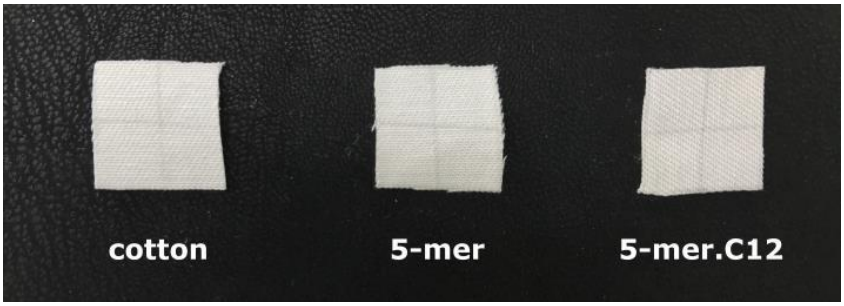
1) *Carbodiimide coupling*. The same condition as outlined above was used.
2) *Capping*. The same condition as outlined above was used.

Final side-chain deprotection

After the last coupling (whether it was a fatty acid or an amino acid), an acid treatment to remove acid-labile side-chain protecting group was performed. First, cotton fabrics were immersed in a solution of 90% TFA: 1% phenol: 2%H_2O: 3% TIPS: 4% CH_2Cl_2 for 30 min without shaking. The second deprotection step was followed with incubation in 50% TFA: 1% phenol: 2%H_2O: 3% TIPS: 44% CH_2Cl_2 for 3 h without shaking. The functionalized cotton fabrics were then rinsed with (in the following order) CH_2Cl_2 (3x, 5 min each), MeOH (3x, 5 min each) and H_2O (multiple times until the pH of the washing solution was not acidic, 5 min each).

Physical appearance of a selected set of modified surfaces

The photos of a selected set of modified surfaces, along with the control, were taken and shown below. It can be seen that no visible change in the appearance occurred in this fabrication method.



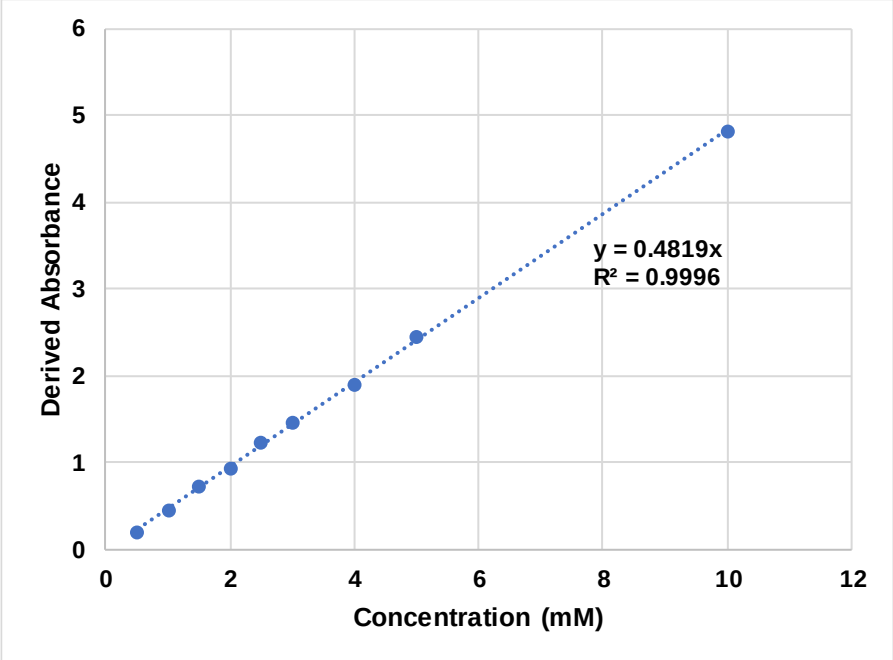
Contact angles of some modified surfaces

The contact angles were determined from a standard goniometer (ramé-hart model 200-F1) using DROPimage software. 4-μL of water was dropped onto each surface. The observation was done immediately although it should be noted that extra repetitions of determination were needed for the hydrophilic amino-terminated surface (prepared as shown in page S-3). This is because the water was absorbed through this surface very rapidly.

surface	water contact angles (°)
unmodified cotton	121.7±1.9
amino-terminated cotton surface	46.5±3.9
5-mer	84.1±6.1
5-mer.C12	123.2±1.8

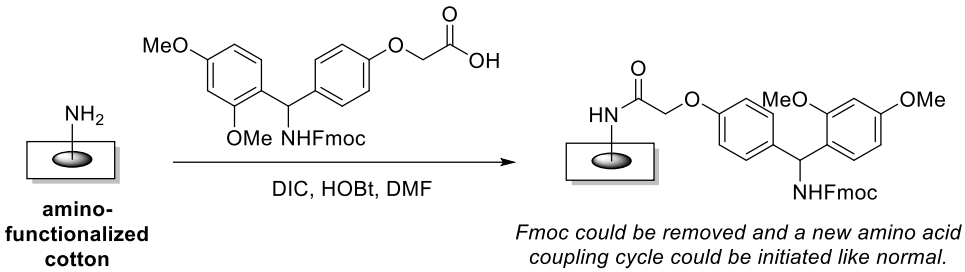
Loading quantification of peptides

The amount of peptide per spot was determined by measuring UV absorbance of dibenzofulvene that was cleaved off during Fmoc deprotection step. With piperidine, dibenzofulvene was trapped and became a fulvene-piperidine adduct, 1-(9*H*-fluoren-9-ylmethyl)-piperidine. A hole-punched fabric (about 0.28 cm^2 in diameter) was immersed in a solution of 20% piperidine (100 µL) and 2% DBU (10 µL) in DMF (390 µL) for 15 min with occasional vortexing and shaking. Thereafter, a 2-µL aliquot was taken for UV measurement by a Thermo Scientific Nanodrop 2000 spectrophotometer. The molar extinction coefficient (ε) was determined to be 4.819 $mM^{-1}cm^{-1}$ at 296 nm using Fmoc-glycine as a model. The loading was calculated from an average of at least three independent measurements.



Sample	1-mer	5-mer	9-mer
loading (µmol/cm^2)	0.40	0.23	0.21

Cleavage strategies for solution-phase characterisation of lipidated peptides

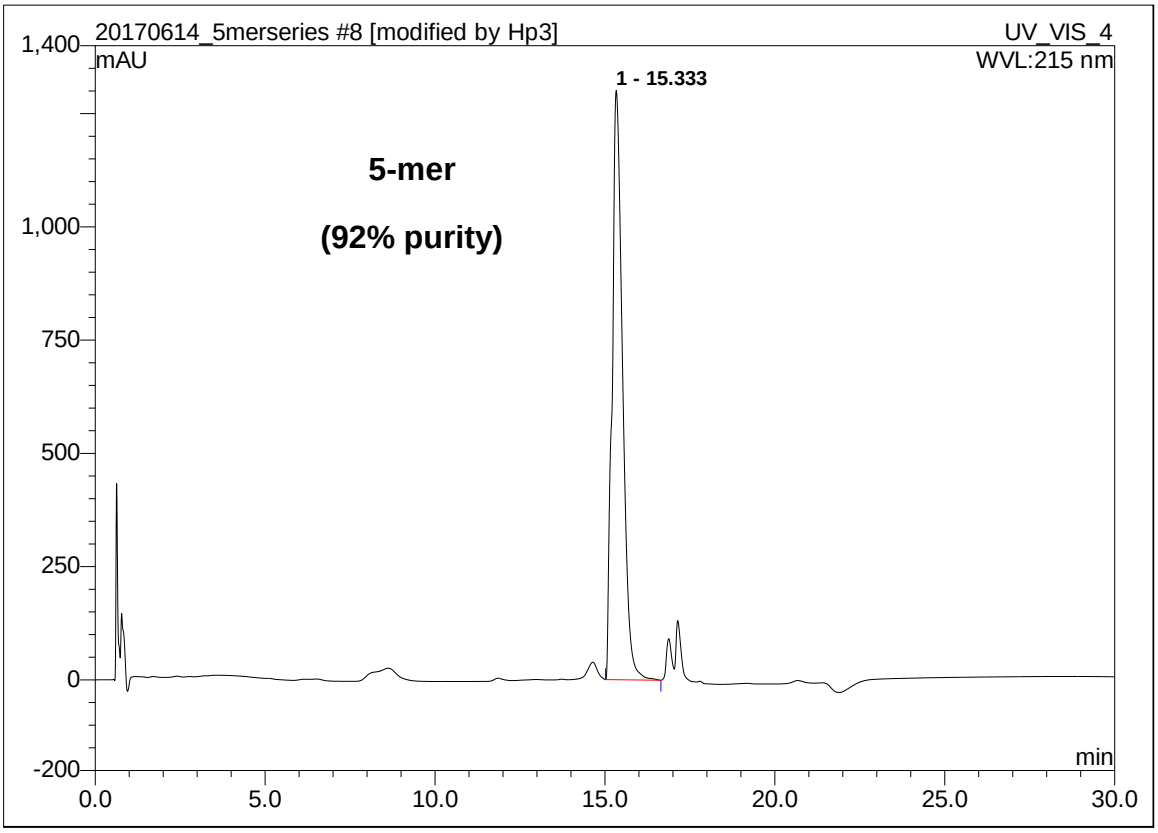


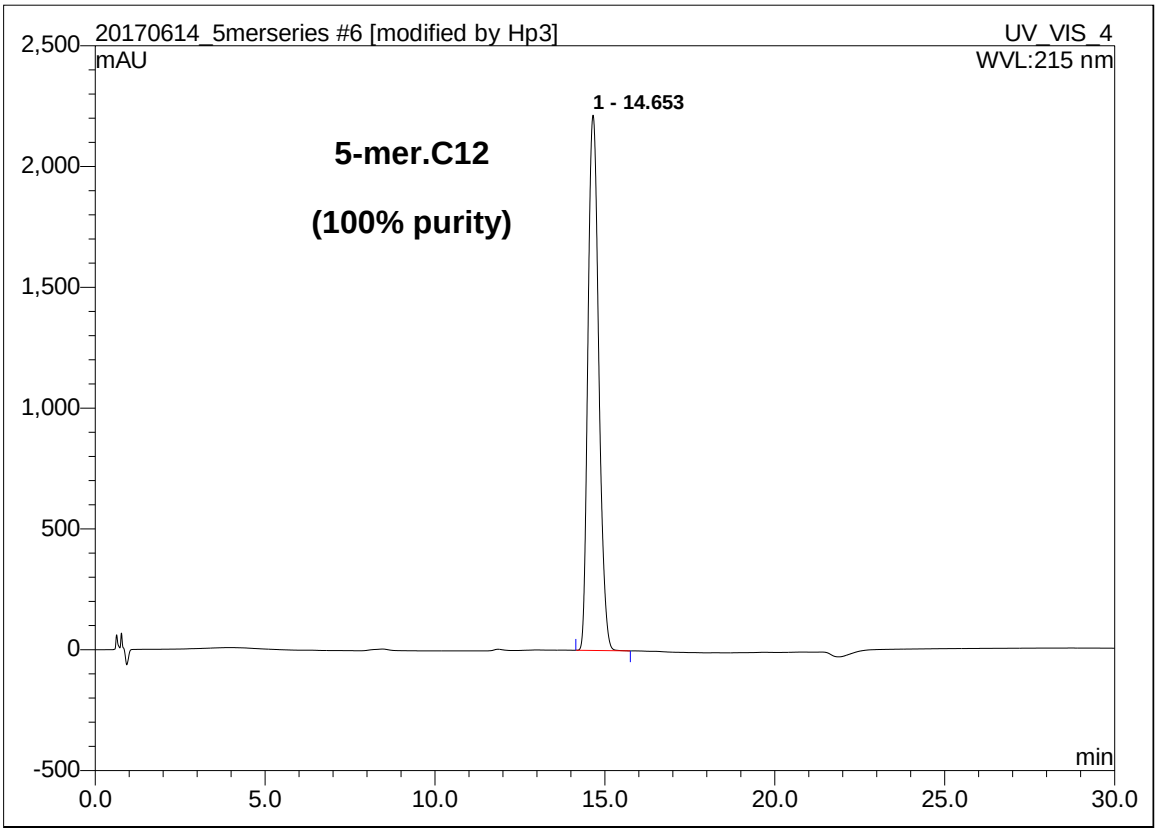
To enable solution-phase characterisations (HPLC and MALDI-TOF MS), a linker needs to be inserted between the spacer and the first amino acid (see scheme above). The chosen linker was 4-[(2,4-dimethoxyphenyl)-(Fmoc-amino)methyl]phenoxyacetic acid (Fmoc-Rink amide linker), which is acid labile. In the final acid deprotection step, this linker would be cleaved off and the entire peptide chain (with the *C*-terminus that was connected to the Rink linker as a primary carboxamide) would then solubilize in the solvent, thereby allowing manipulation like ordinary compounds in solution phase.

Coupling conditions. All sub-steps (carbodiimide coupling, capping, and Fmoc deprotection) were performed exactly as shown in the typical peptide coupling cycles (page S-3), substituting Fmoc-Rink amide linker for the amino acid.

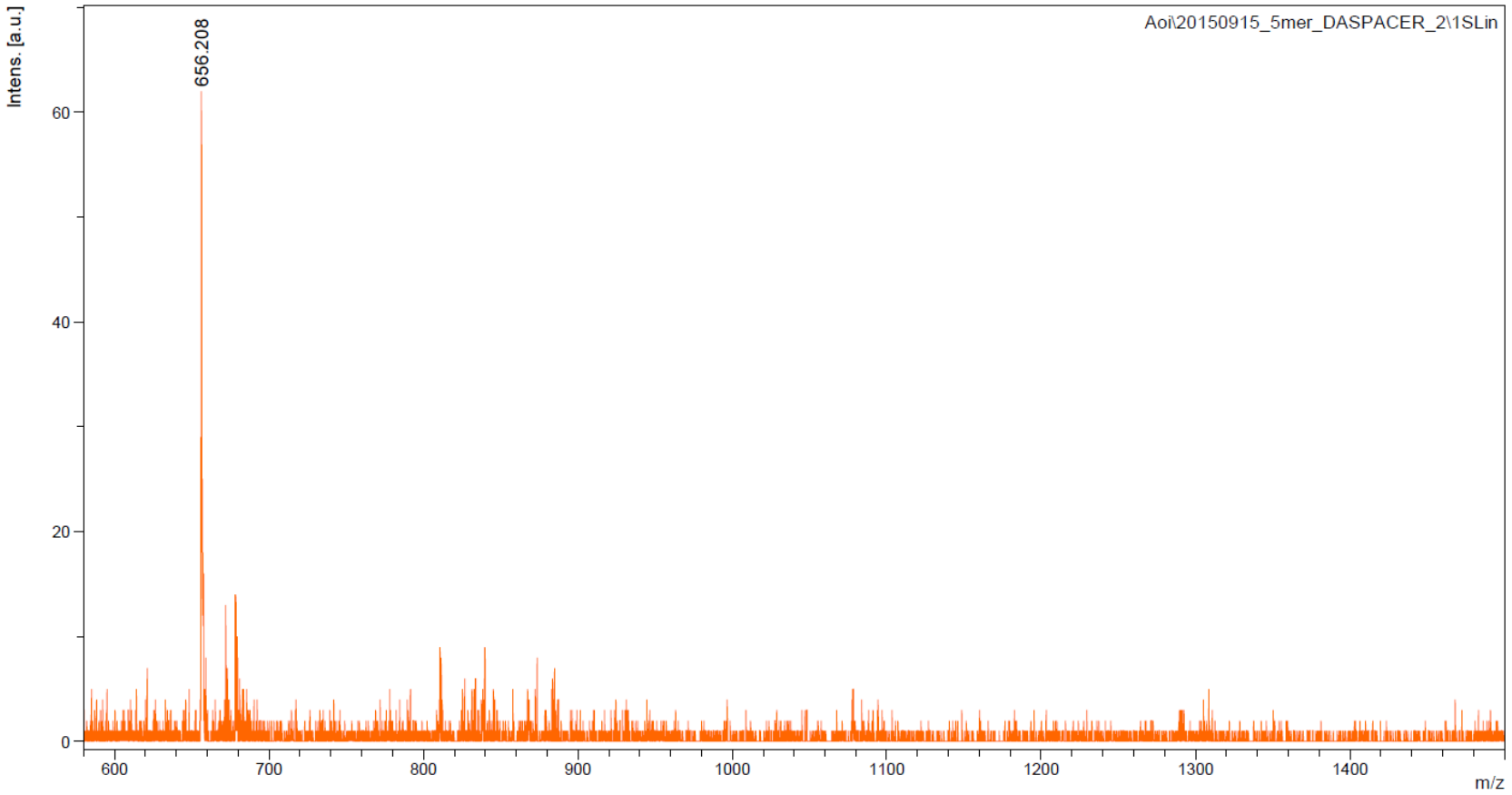
Final deprotection. For the purpose of solution-phase analyses, 6 spots (MALDI-TOF MS) or 24 spots (HPLC) were punched from cotton fabrics by a hole puncher and all spots were added to a vial containing a deprotection cocktail (1 mL containing 88% TFA: 5% phenol: 5% H_2O: 2% TIPS). This solution was incubated without shaking for 2 h. Thereafter, the solvent was removed by gently flushing the solution with air. 2 mL of 1:1 MeOH:H_2O was then added to the cleaved peptides, some of which may still be physically adsorbed on the fabric. After shaking and vortexing, the solution containing the cleaved products was transferred to a new vial, followed by air drying. This process of adding and transferring 2 mL of 1:1 MeOH:H_2O was repeated twice (totaling 6 mL). After the final air drying, 100 μL of 1:1 MeOH:H_2O was added to the crude peptide and the resulting solution was analysed by either HPLC (Ultimate 3000 UPLC, Thermo Scientific) or MALDI-TOF Mass Spectrometer (Microflex, Bruker Daltonics).

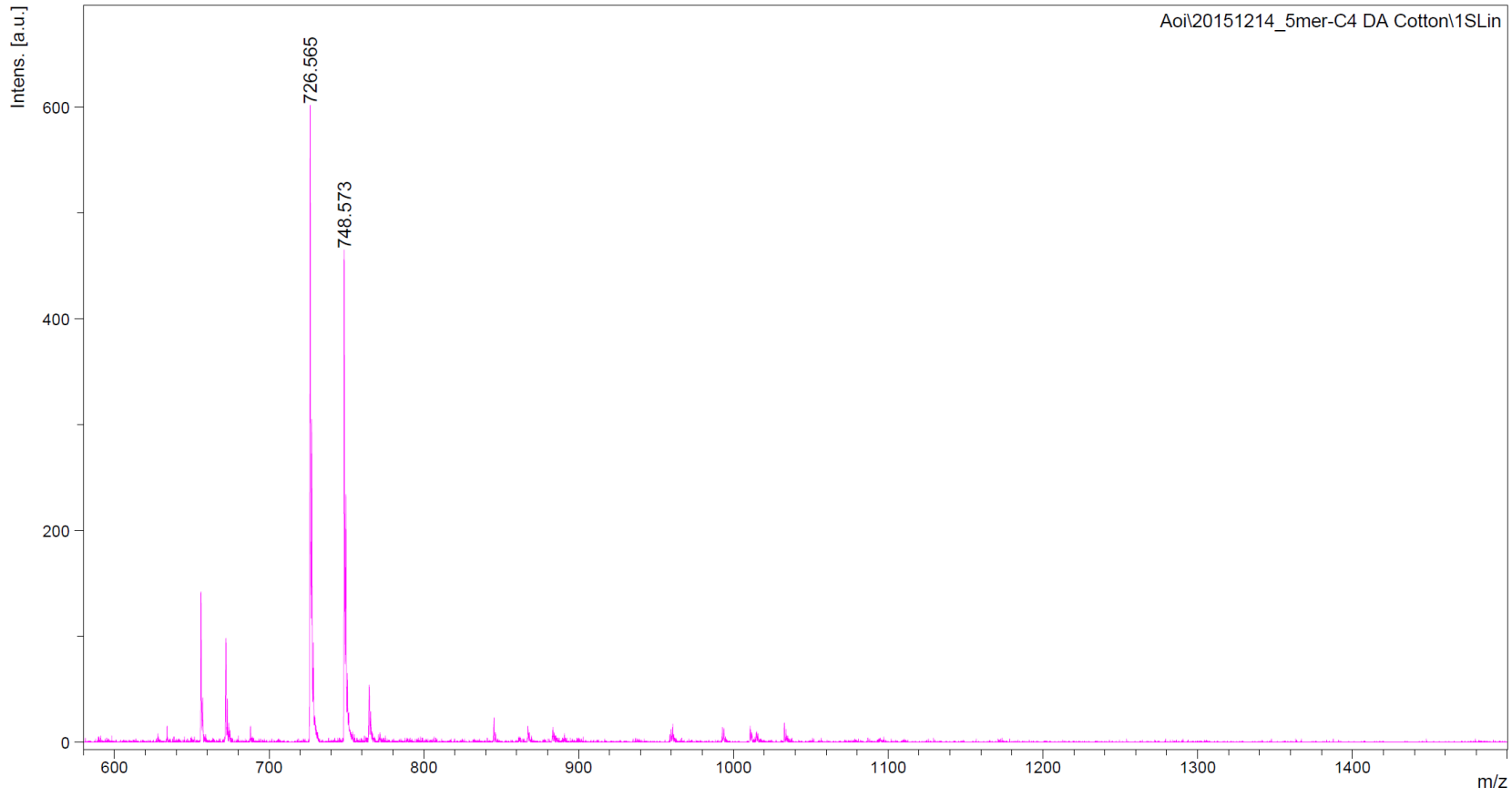
HPLC chromatogram of selected peptides

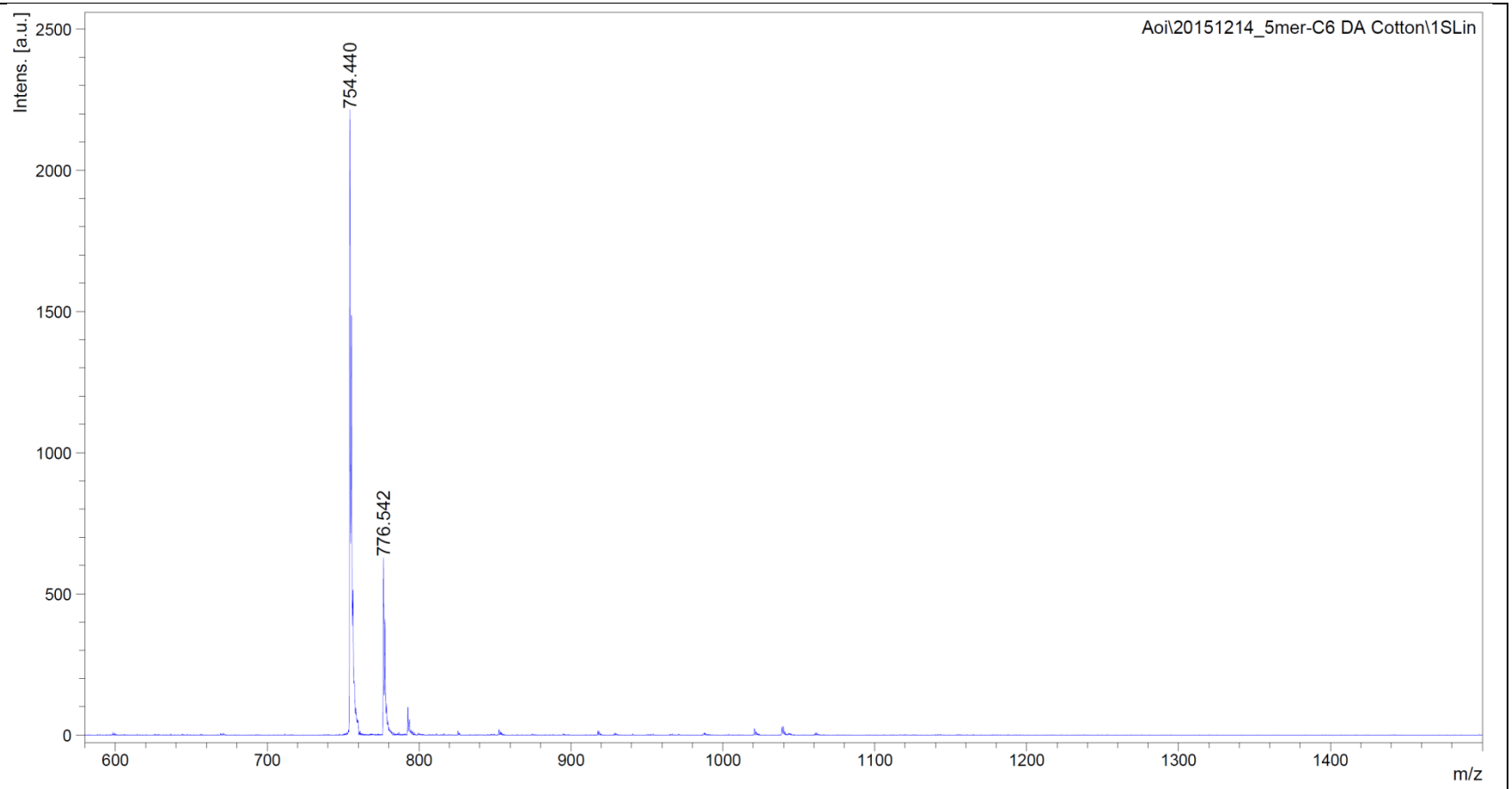


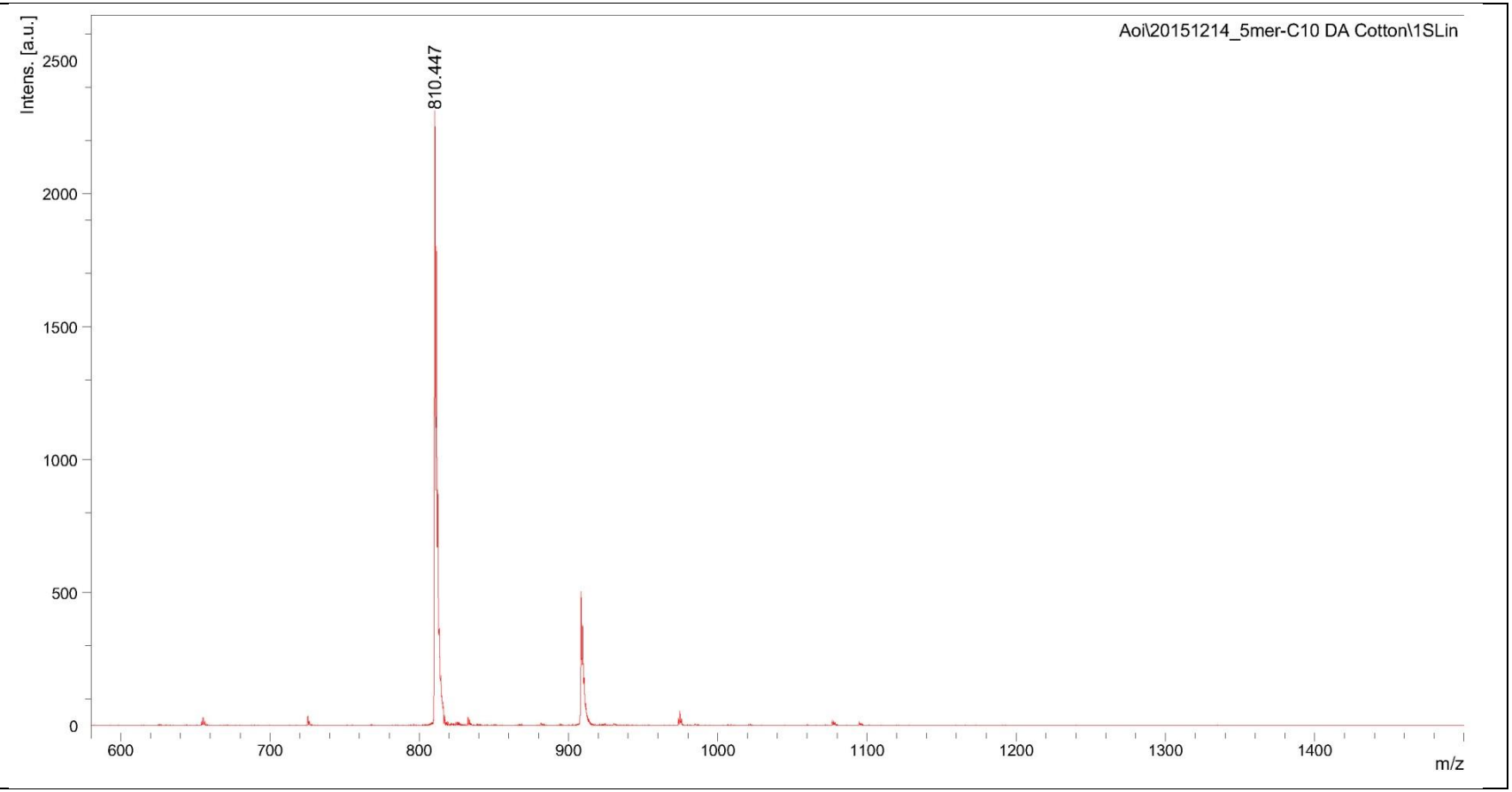


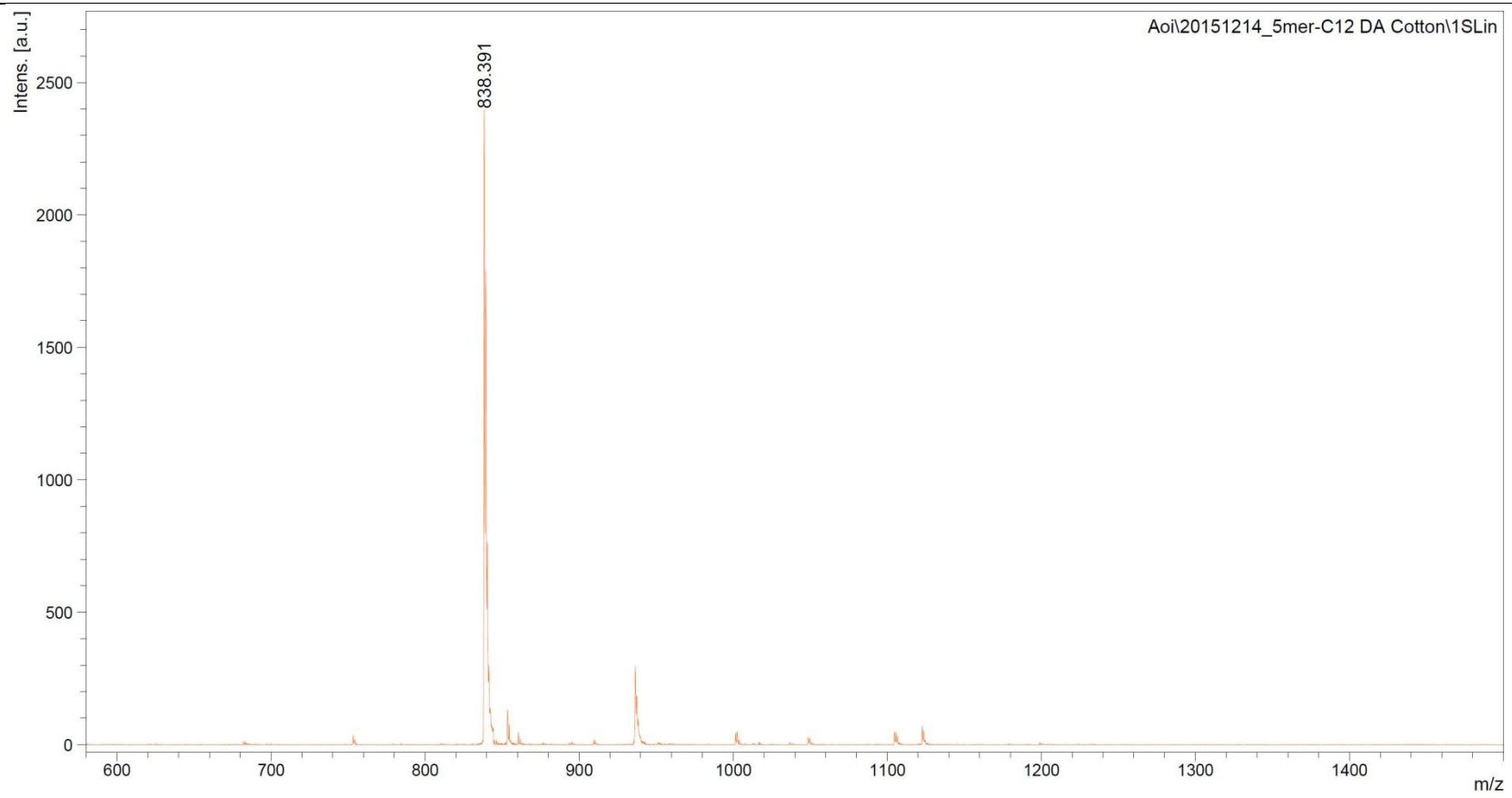
MALDI-TOF MS data of lipidated peptides cleaved off cotton surfaces

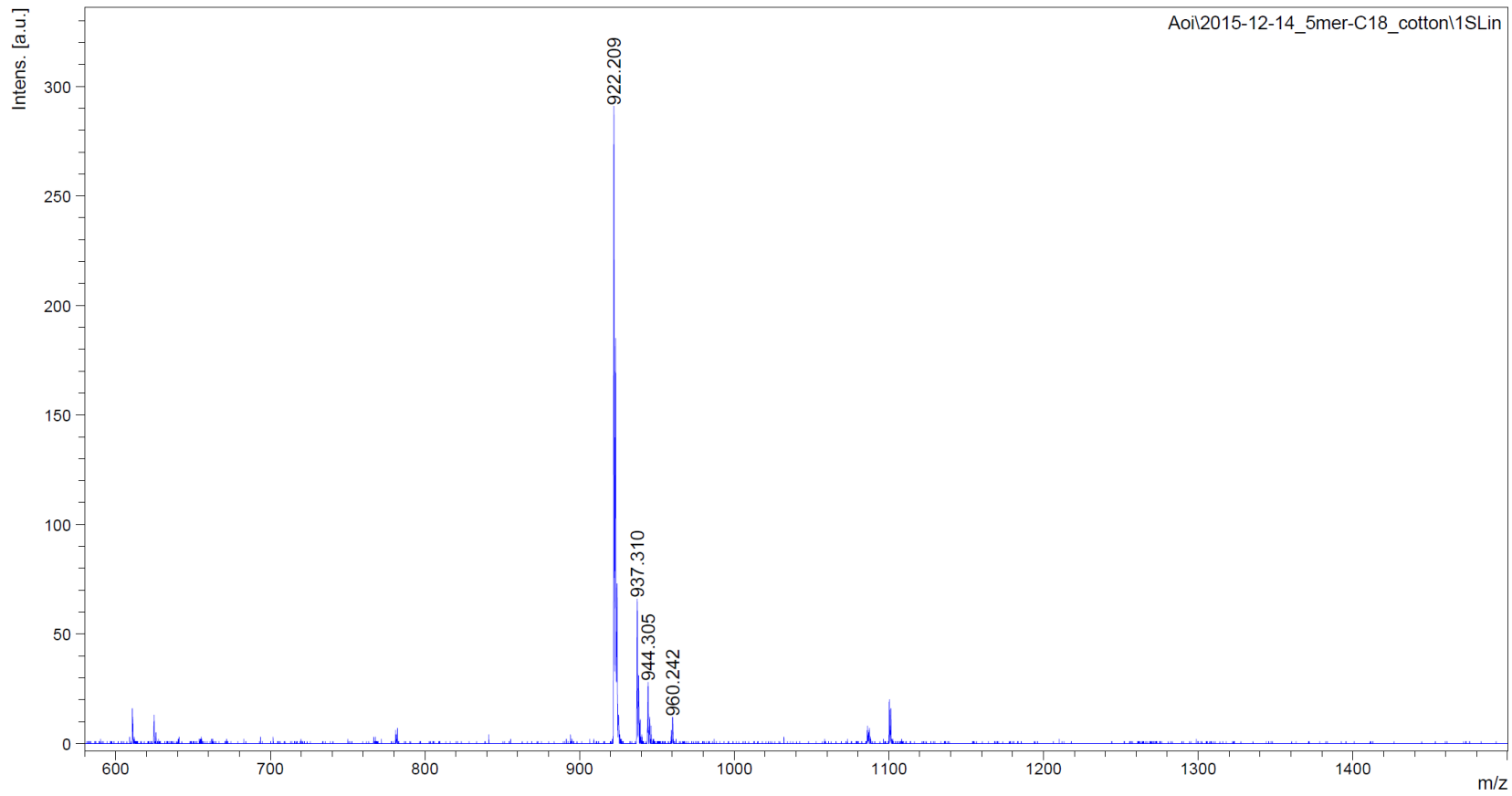
Compound Cal. Mass as $[M+H]^+$ Obs. Mass	Mass Spectrum
5-mer Cal. Mass: 656.80 Obs. Mass: 656.21	

5-mer.C4 Cal. Mass: 726.89 Obs. Mass: 726.57	

5-mer.C6 Cal. Mass: 754.95 Obs. Mass: 754.44	

5-mer.C10 Cal. Mass: 811.05 Obs. Mass: 810.45	

5-mer.C12 Cal. Mass: 839.11 Obs. Mass: 838.391	

5-mer.C18 Cal. Mass: 923.27 Obs. Mass: 922.209	

9-mer Cal. Mass: 1202.48 Obs. Mass: 1202.247