Wang Linker Free of Side Reactions

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Supporting Information

Experimental Methods

General: All Fmoc-L-AA-OH were purchased from Iris Biotech. Wang resin and DMAP were purchased from Novabiochem. $Pd(PPh_3)_4$ was purchased from Strem Chemicals. K_2CO_3, HCl were purchased from Acros. ICl, TMSA, CuBr, Sodium ascorbate, DIEA, 2.6-Lutidine, $NaBH_4$, DIPCDI and TIS were purchased from Sigma-Aldrich. Oxyma were purchased from Luxembourg Industries and all were used without purification unless noted. All other reagents were purchased from Carlo Erba and used without further purification.

Chromatography and Spectroscopic Measurements: Melting points were determined in capillary tubes in an Electrothermal 9100 apparatus and are uncorrected. The IR spectral was determined in an FT-IR Nexus (Termo Nicolet 760). Analytical HPLC runs were performed in an HPLC PDA 2695 Alliance using an RP-C18 column (10 mm, 4.6 nm x 100 nm reverse phase column) with a gradient from 100% of the aqueous 0.1% TFA (eluent A) to 5% eluent A- 100% of 0.5% TFA in acetonitrile (eluent B) over 8min, with a flow rate of 1 mL/min. HPLC gradient: Leu-enkephalin (H-Tyr-Gly-Gly-Phe-Leu-OH) **6** ((%B) 20-50 in 8min), RGD pentapetide (H-Arg-Gly-Asp-Gly-Trp-OH) **7** ((%B)

10-40 in 8min) and Indolicidin (H-Ile-Leu-Pro-Trp-Lys-Trp-Pro-Trp-Trp-Pro-Trp-Arg-Arg-OH)) **8** ((%B) 30-50 in 8min).

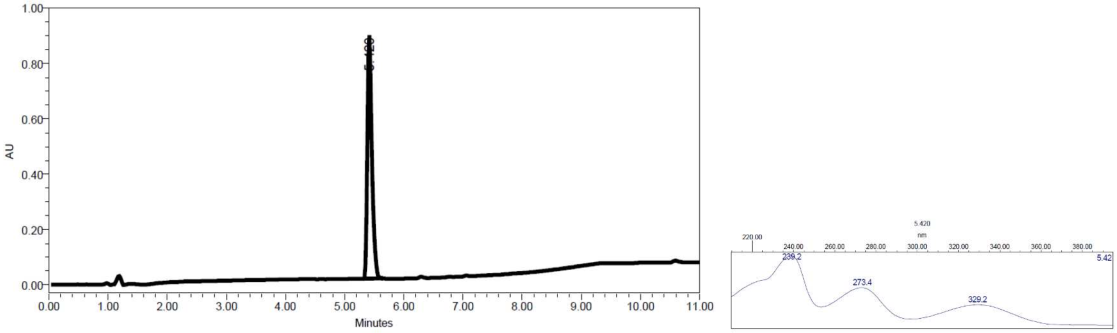
Absorbance was detected at 220 and 254nm. RP-HPLC-ESMS was performed on a Waters Micromass ZQ spectrometer. Linear gradients of ACN (+0.07% formic acid) into H2O (0.1% formic acid) were run at 0.3 mL/min flow rate over 8 min. NMR spectra were recorded on a Bruker DPX 400 (400 MHz-1H, 75.4-13C). Chemical shifts are given as δ values against tetramethylsilane as the internal standard and J values are given in Hz. Mass spectra were recorded using an Electron Spray Ionization (ESI) technique on a Micromass ZQ, Waters SN:MAA 076. AAA was performed for peptide attached to resin and the peptides cleaved with Waters Pico-Tag in duplicate, after hydrolyzing resin samples for 15 h at 130 °C with concentrated HCl-propionic acid (1:1) in the presence of phenol. ESI MS was performed on a Micromass LCT mass spectrometer.

Abbreviations: DMF, N,N'-dimethylformamide; DIEA, diisopropyl ethyl amine; DCM, dichloromethane; Oxyma, ethyl 2-cyano-2-(hydroxyimino)acetate; DIPCDI, N,N'-diisopropylcarbodiimide; DMAP, 4-dimethylaminopyridine; TFA, trifluoroacetic acid; LCMS, liquid chromatography mass spectrometry; PDA, photodiode array.

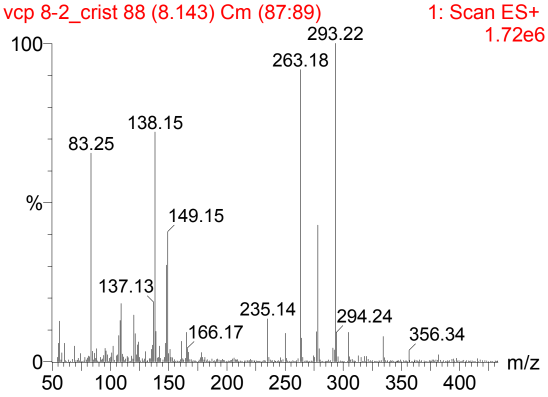
Experimental procedures and characterization

5-Iodo-2,4-dimethoxybenzaldehyde (2). To a solution of 2.4-dimethoxybenzaldehyde (6 eq) in methanol (28 mL) was added iodine monochloride (1.16 g in 3 mL methanol) dropwise over 20 min at RT. The solution was allowed to stir at this temperature. HPLC showed about 74% conversion after over night. The reaction mixture was then poured into a solution of HCl (0.5 M, 30 mL). The precipitate was collected by filtration, washed with water, and dried in vacuo (40 °C) to give a crude product. The crude product was further purified by recrystallization from THF/hexane (1:1) to give the title compound as an off-white solid. Yield: 60 %.

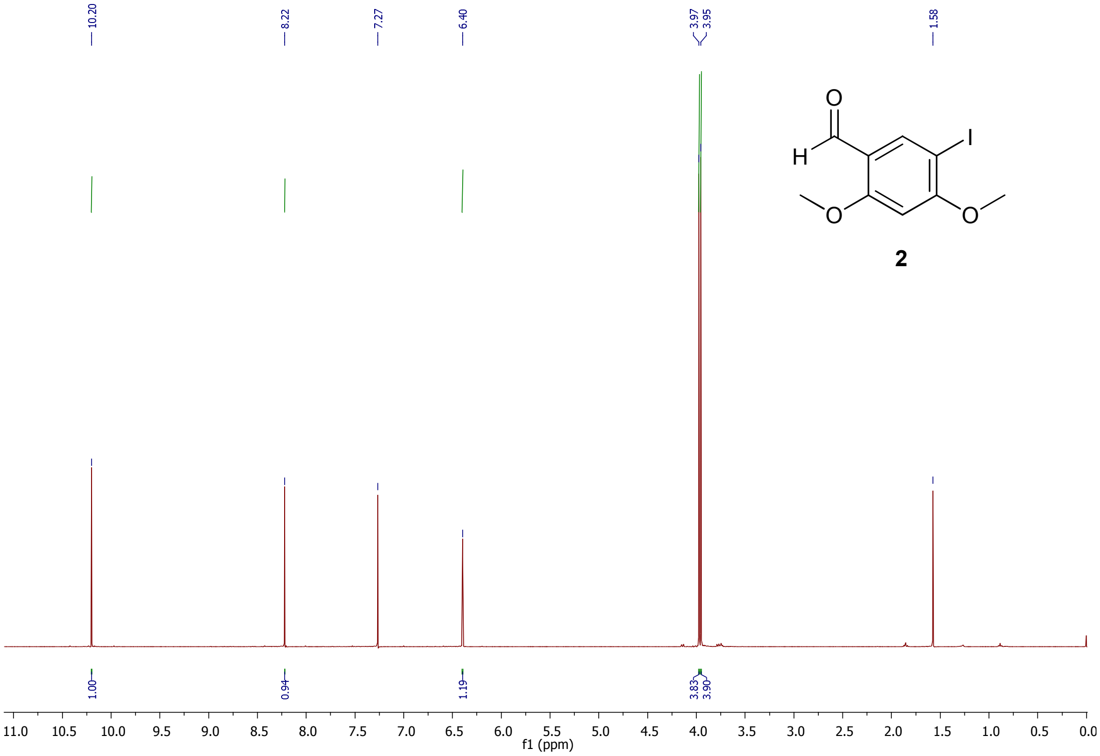
^{1}H NMR (400 MHz, $CDCl_3$): δ 10.20 (s, 1H), 8.22 (s, 1H), 6.39 (s, 1H), 3.97 (s, 3H), 3.95ppm (s, 3H); Calculated for $C_9H_9IO_3$: 291.9596; ESI-MS (M+H): 293.22



HPLC and UV profiles to 5-Iodo-2,4-dimethoxybenzaldehyde **2**



ESI-MS of 5-Iodo-2,4-dimethoxybenzaldehyde **2**

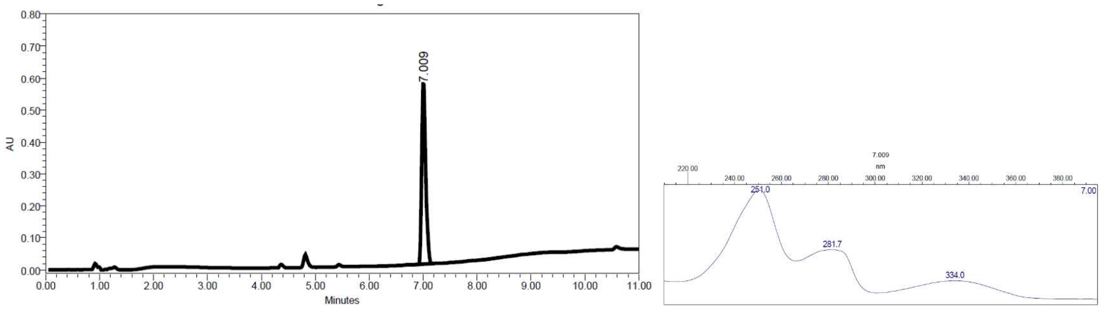


H^1-NMR spectra of 5-Iodo-2,4-dimethoxybenzaldehyde (**2**)

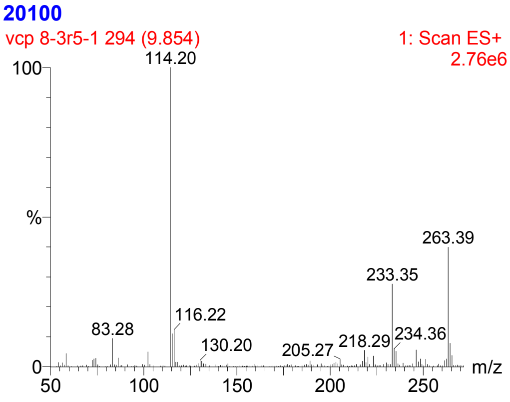
2,4-dimethoxy-5-((trimethylsilyl)ethynyl)benzaldehyde (3)

A solution of 5-iodo-2,4- dimethylbenzaldehyde (**2**) (1.02 eq) in THF (2.1 mL) and *i*Pr_2NH (840 µL) was degassed by bubbling argon through the solution. After cooling to 0ºC, [$Pd(PPh_3)_4$] (0.031 eq) and CuI (0.0021 eq) were added and the mixture was stirred at RT for 10 min. An amount of TMSA (1.27 eq) was slowly added in a dropwise manner, and the reaction mixture was stirred. HPLC showed about 85% conversion after 4 h at RT. After diluting with ethyl acetate (200 mL) the mixture was successively washed with ice cold 2M HCl (3x200 mL) and sat. aq. NH_4Cl (3x100 mL). The organic layer was dried over Na_2SO_4, evaporated to dryness, and the residue was purified by Comby Flash Rf 200 sytem RP-column (C18) medium pressure liquid chromatography on H_2O, ACN giving 2,4-dimethoxy-5-((trimethylsilyl)ethynyl)benzaldehyde (**3**) as a white solid. Yield: 69%.

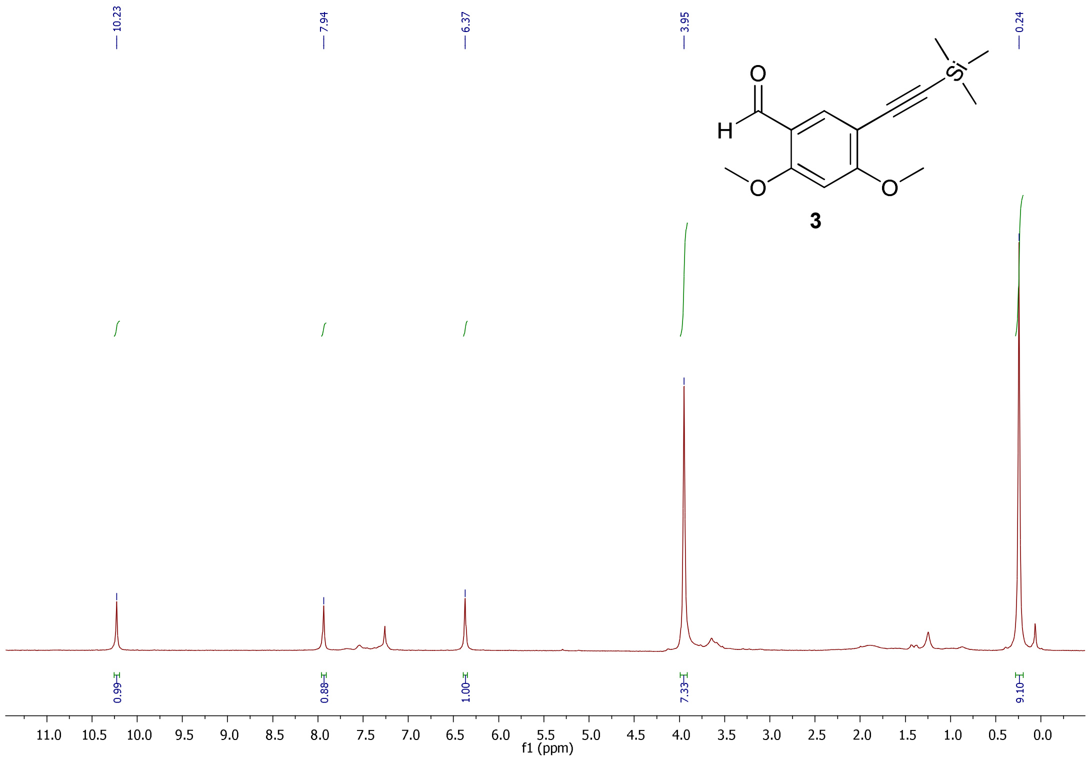
^1H NMR (400 MHz, $CDCl_3$): δ = 10.23 (s, 1H), 7.94 (s, 1H), 6.37 (s, 1H), 3.94 (s, 3H), 3.95ppm (s, 3H); ^{13}C NMR (400 MHz, $CDCl_3$): δ= 0.17, 55.8, 56.5, 94.1, 98.1, 99.7, 105.7, 118.5, 135.2, 163.8, 166.3, 187.7 ppm; Calculated for $C_{14}H_{18}O_3Si$: 262.1025; ESI-MS (M+H): 263.39.



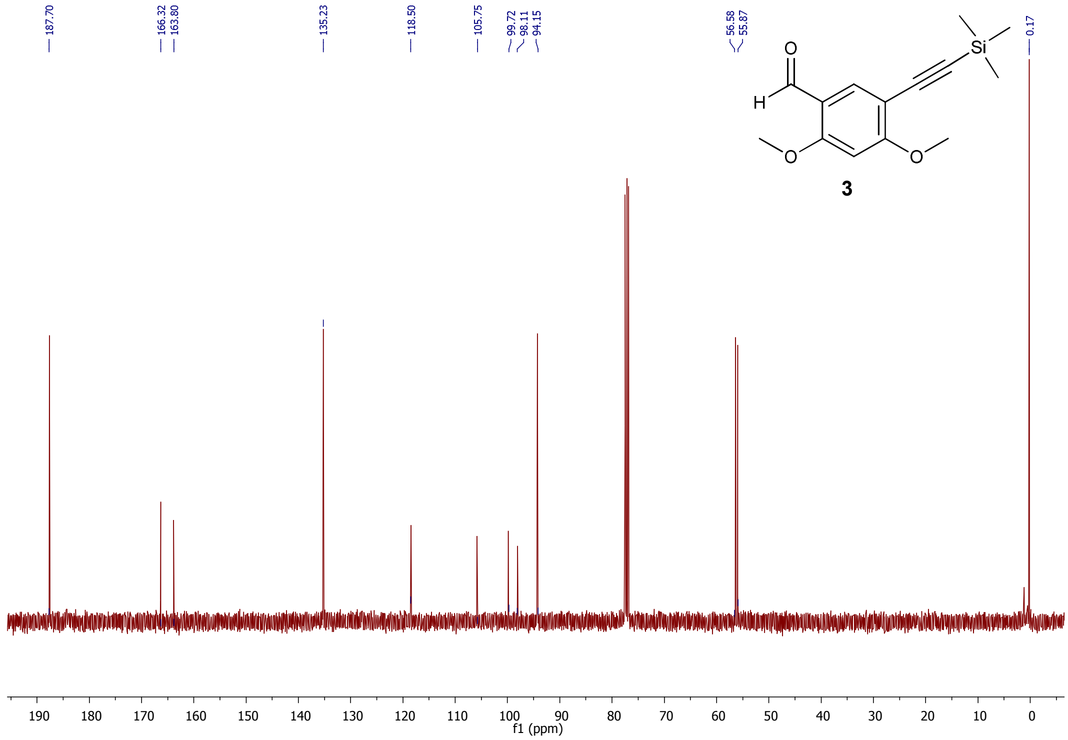
HPLC and UV profiles to 2,4-dimethoxy-5-((trimethylsilyl)ethynyl)benzaldehyde (**3**)



ESI-MS of 2,4-dimethoxy-5-((trimethylsilyl)ethynyl)benzaldehyde (**3**)



H^1-NMR spectra of 2,4-dimethoxy-5-((trimethylsilyl)ethynyl)benzaldehyde (**3**)

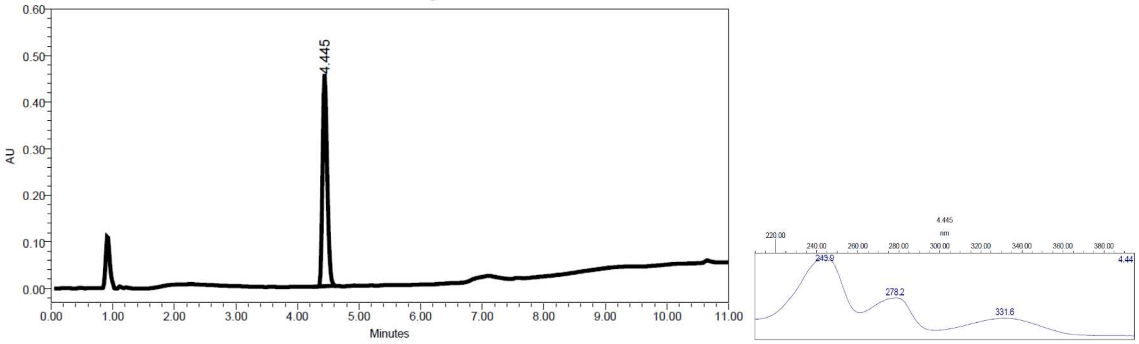


C^{13}-NMR spectra of 2,4-dimethoxy-5-((trimethylsilyl)ethynyl)benzaldehyde (**3**)

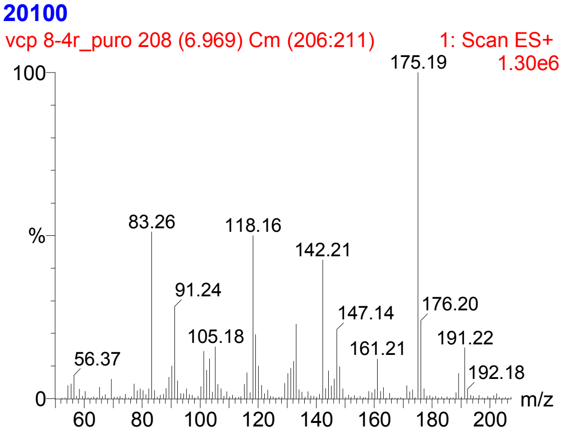
5-ethynyl-2,4-dimethoxybenzaldehyde (4)

To a solution of 2,4-dimethoxy-5-((trimethylsilyl)ethynyl)benzaldehyde (**3**) (0.70 eq) in degassed MeOH (7.2 mL) and DCM (720 µL) was added K_2CO_3 (1.42 eq), and the resulting mixture was stirred HPLC showed about 100% conversion after 1 h at RT under argon atmosphere. After removal of the solvent in vacuo at RT the residue was poured into ice cold water (8 mL) and extracted with dichloromethane (5x2.5 mL). The combined organic extracts were washed with water (2x3 mL) and brine. After drying over Na_2SO_4, the solvent was evaporated in vacuo at RT. The residue was purified by Comby Flash Rf 200 sytem RP-column (C18) medium pressure liquid chromatography on H_2O-ACN yielding 5-ethynyl-2,4-dimethoxybenzaldehyde (**4**) as colorless needles. Yield: 70%; mp=128.2-129.3ºC.

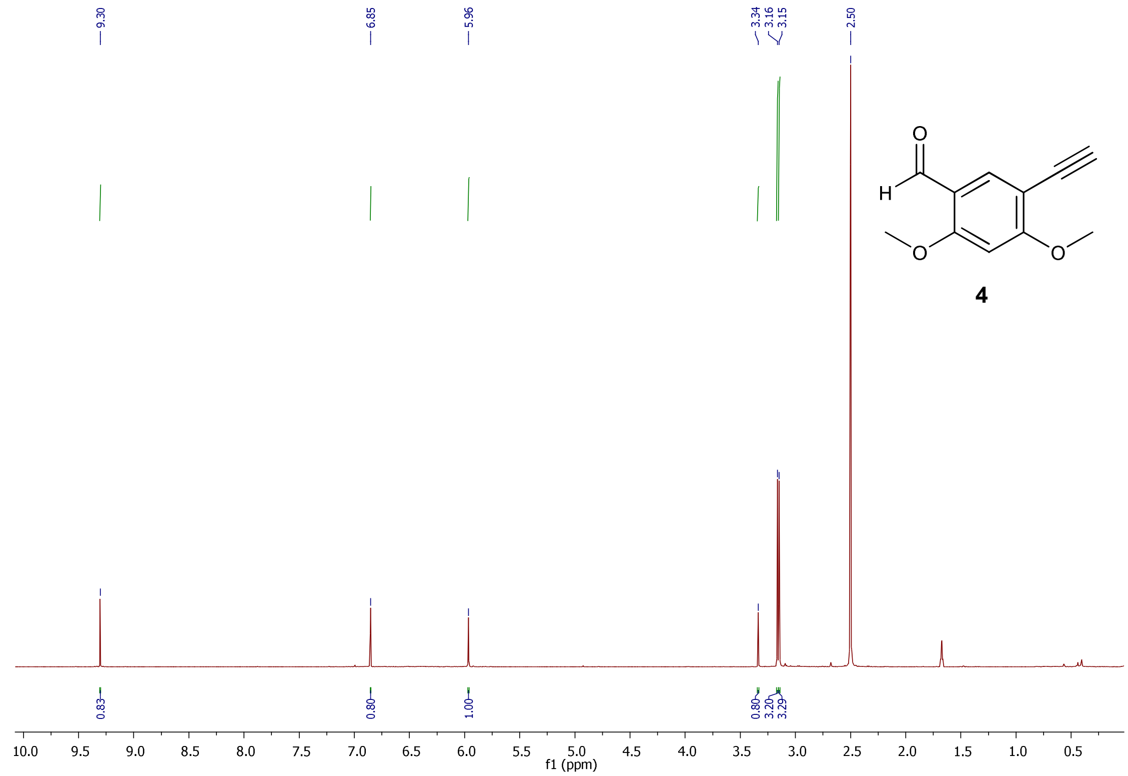
^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.15 (s, 3H), 3.16 (s, 3 H), 3.34 (s, 1H), 5.96 (s, 1H), 6.85 (s, 1H), 9.30 ppm (s, 1H); ^{13}C NMR (400 MHz, $CDCl_3$): d=56.4, 56.5, 78.7, 83.6, 103.7, 117.5 133.1, 163.7, 166.3, 185.6 ppm; Calculated for $C_{11}H_{10}O_3$: 190.0630; ESI-MS (M+H): 191.22; HRMS (ESI, M+H): 191.0704



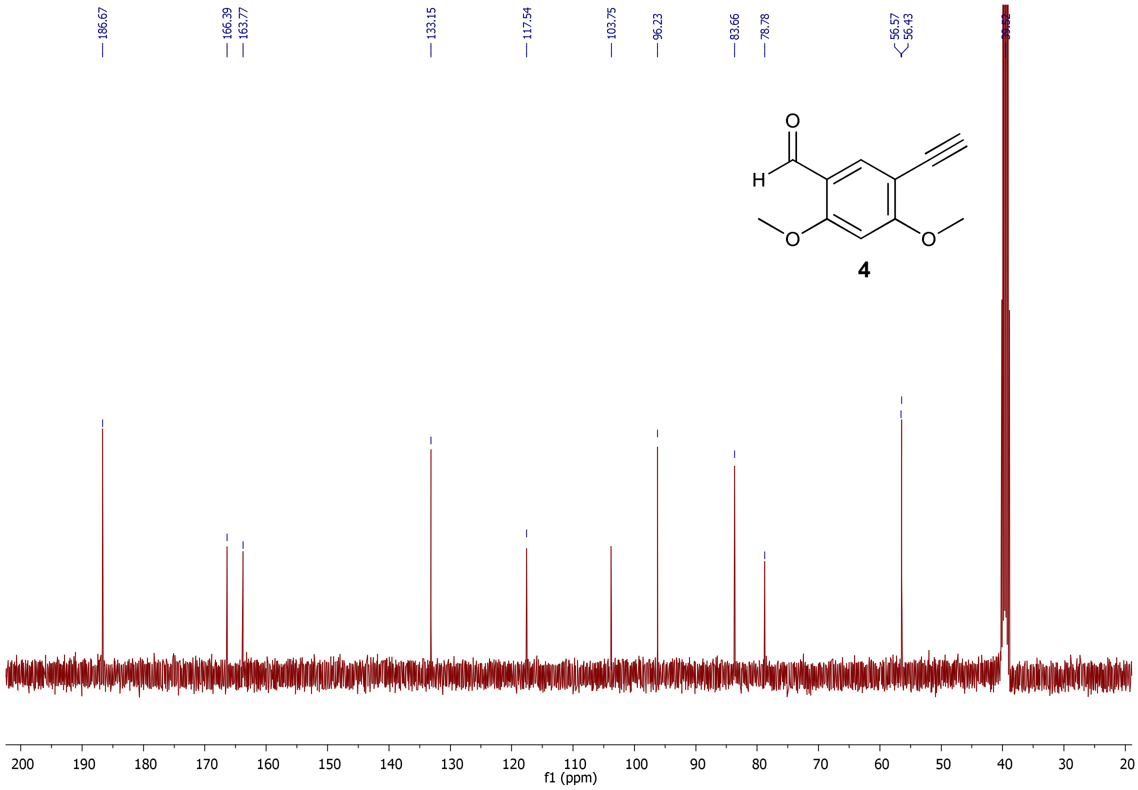
HPLC and UV profiles of 5-ethynyl-2,4-dimethoxybenzaldehyde (**4**)



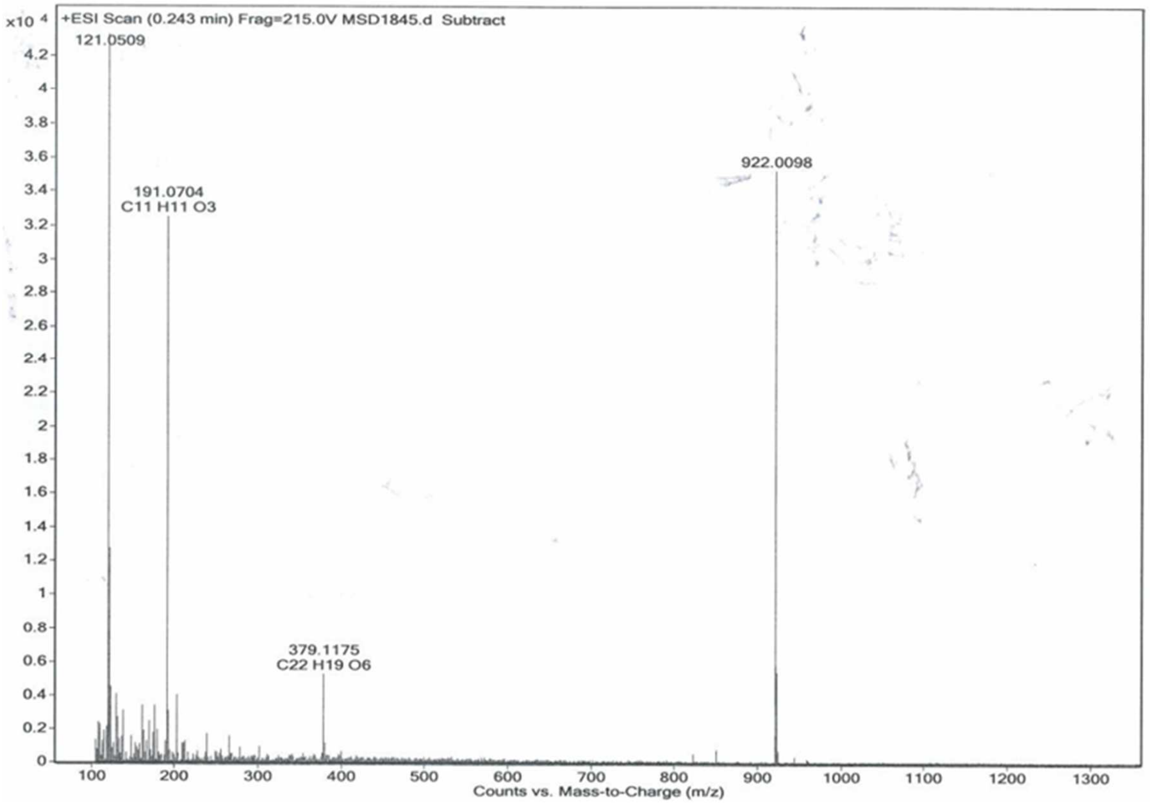
ESI-MS of 5-ethynyl-2,4-dimethoxybenzaldehyde (**4**)



H^1-NMR spectra of 5-ethynyl-2,4-dimethoxybenzaldehyde (**4**)



C^{13}-NMR spectra of 5-ethynyl-2,4-dimethoxybenzaldehyde (**4**)



HRMS spectra of 5-ethynyl-2,4-dimethoxybenzaldehyde (**4**)

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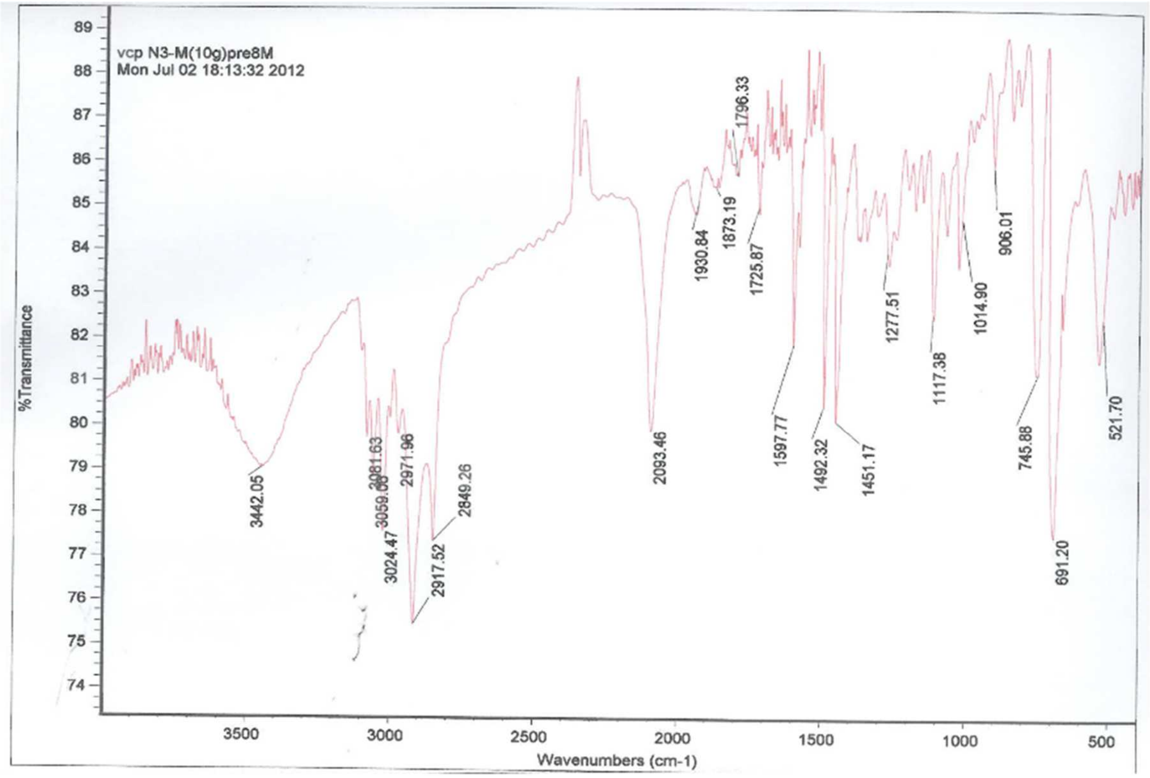
Azidomethylpolystyrene (5)

The azidomethylpolystyrene (**5**) was synthesized following the reported procedure.[i]

The progress of reaction was determinate by IR, following the disappearance of vibration band of vibration band $\nu_{N=N=N} = 2092 cm^{-1}$.

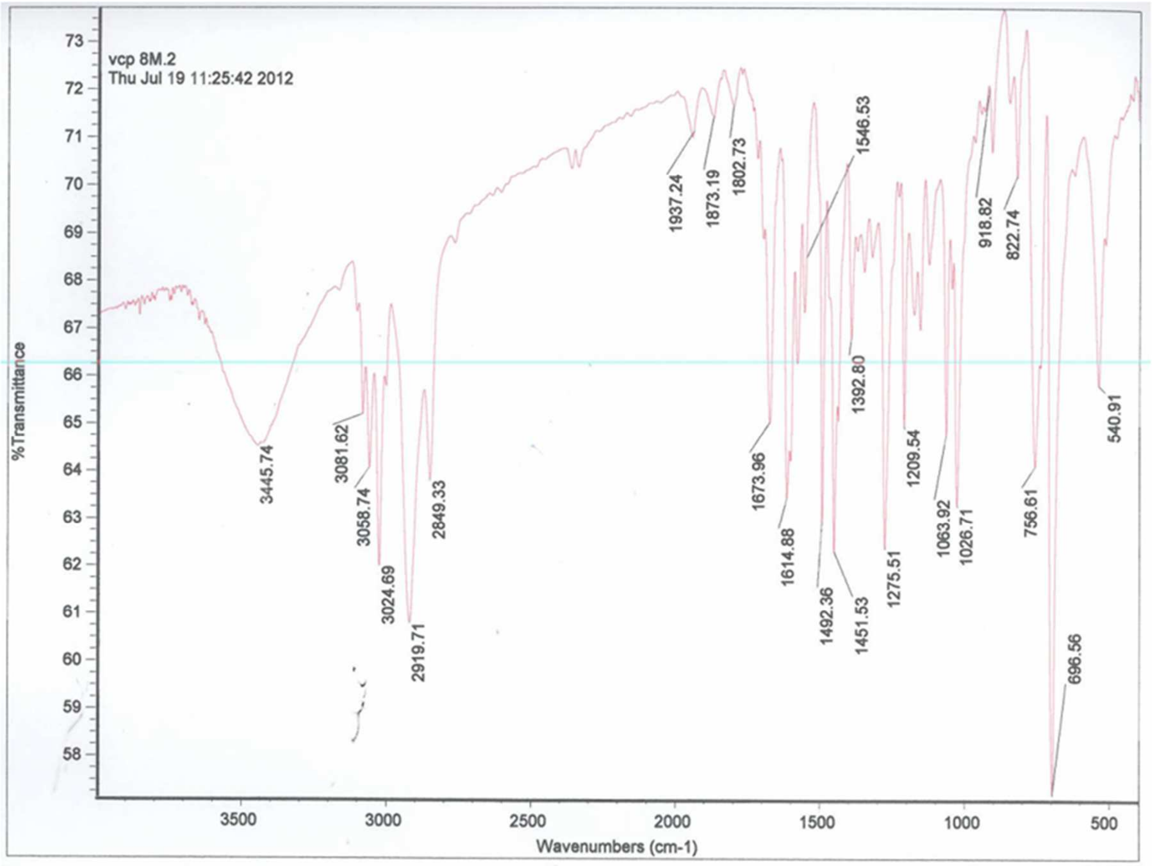


IR spectra of azide polystyrene resin (5)

Synthesis of CHO-BTL resin (Click Chemistry reaction).

The solvents used in the Click Chemistry procedure (DMF and ACN) were purged of molecular oxygen by bubbling with argon for 20 minutes.

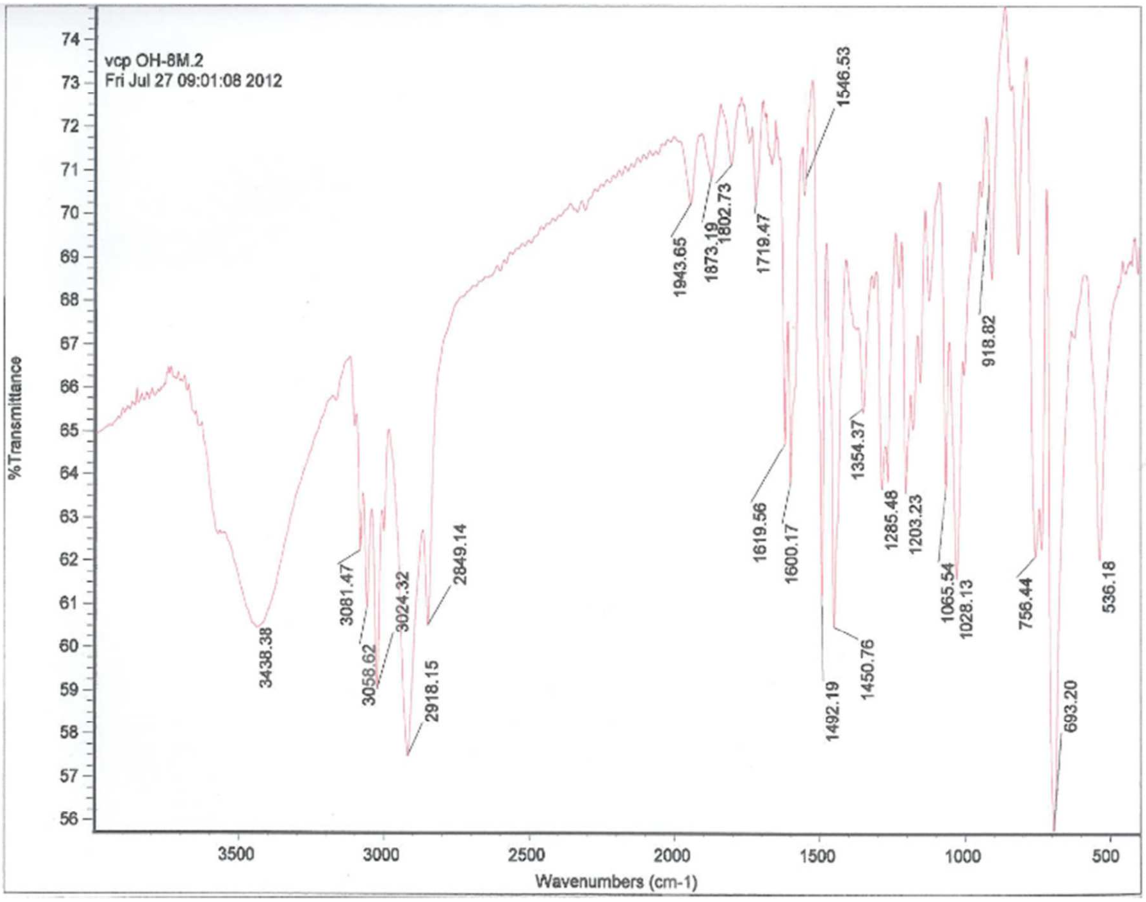
The Azidomethylpolystyrene resin **5** (300mg; 0.6 mmol N_3/g, 1eq) was swelled with DMF and DCM (3x3mL) at 10min, following by the Click chemistry reaction through the addition of 5-ethynyl-2,4-dimethoxybenzaldehyde (**4**) (1.25 eq) diluted in 2.5 mL of purged DMF, DIEA (10 eq), 2,6-lutidine (10 eq), Sodium ascorbate (1% solution in DMF, 1eq) and CuBr (1% solution in acetonitrile, 1 eq). The resin was shaking at room temperature for 16 h. Finally, the resin was washed with different solvents (3 × H_2O, 3 × methanol, 3 × DMF, 3 × DCM). The progress of reaction was determinate by IR, following the disappearance of vibration band $\nu_{N=N=N}$ = 2092cm-1 and the appearance of vibration bands $\nu_{C=O}$ = 1673cm^{-1} and ν_{-C-N-} = 1546cm^{-1}.



IR spectra of CHO-BTL resin.

Synthesis of OH-BTL resin

The CHO-BTL resin was swelled with DMF and DCM (3x3mL) at 10min. $NaBH_4$ (1.3 mmol) in a mixture of MeOH/THF (50:50) was added at 10ºC to the resin. The resin was slow stirred at room temperature for 2 h, and then 20 mL of HCl (1%) was added to reach pH = 4. After, the resin was washed with different solvents (3 × H_2O, 3 × methanol, 3 × DMF, 3 × DCM). The progress of reaction was determinate by IR, following the disappearance of vibration band $\nu_{C=O} = 1673 cm^{-1}$.



IR spectra of OH-BTL resin.

Solid Phase Peptide Synthesis

The following peptides were synthesized by SPPS on OH-BTL resin and Wang resin: Leu-enkephalin (H-Tyr-Gly-Gly-Phe-Leu-OH) **6**, RGD pentapetide (H-Arg-Gly-Asp-Gly-Trp-OH) **7** and Indolicidin (H-Ile-Leu-Pro-Trp-Lys-Trp-Pro-Trp-Trp-Pro-Trp-Arg-Arg-OH)) **8**. And the molecule :H-Trp-OH.

A 20 mL polypropylene tube was charged with 100 mg of **OH-BTL** resin (0.6 mmol/g) or Wang resin (0.9 mmol/g). The suspension was agitated gently, and Fmoc-AA-OH (4eq), DIPCDI (2eq), DMAP (0,4eq) in DCM was added to the tube. The resin was shaken for 16h at room temperature, and then the mixture was filtered and washed with DMF (3 × 5 mL) and CH_2Cl_2 (3 × 5 mL). This procedure was repeated with the same proportions of coupling of regents and the amino acid in DMF for 2h at room temperature, and then the mixture was filtered and washed with DMF (3 × 5 mL) and

CH_2Cl_2 (3 × 5 mL). After the first aminoacid coupling, the resin was acetylated in order to capped all alcohol reactive groups present in the resin (Ac_2O (10eq), DIEA (10eq) in DCM, 30 min, RT). The Fmoc quantification was carried out by measured of UV absorbance of Dibenzo-fulvene to 301nm, after the standard deprotection procedure (piperidine 20% in DMF (1 x 1min, 3 x 5min)).* For the remaining aminoacids, coupling conditions to SPPS was used (Fmoc-AA-OH (3eq), Oxyma (3eq) and DIPCDI (3eq) in DMF for 1h, RT). The peptide was cleavage of the resin by treatment with TFA-TIS-H_2O (95:2.5:2.5) for 1h and precipitation with $(C_2H_5)_2O$.

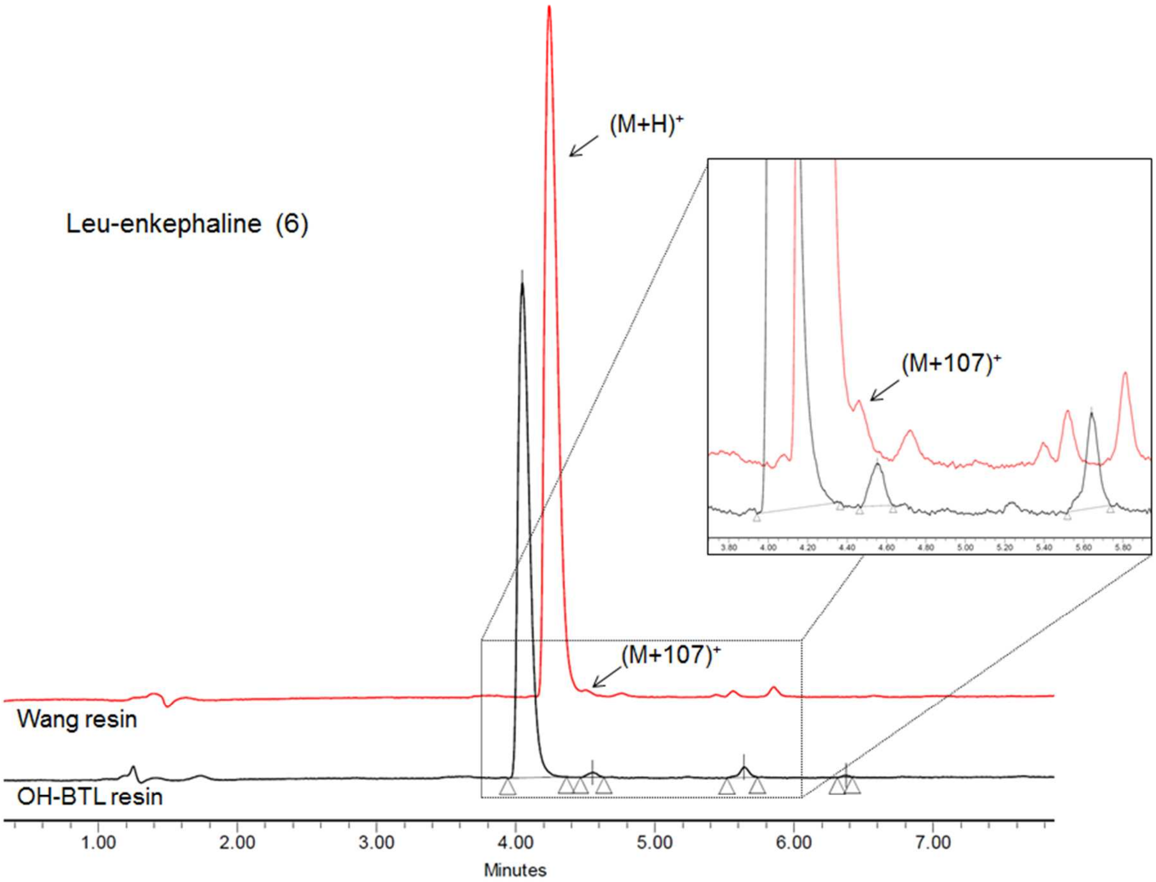
* The filtrate of the Fmoc deprotection reaction together with the two-washing filtrate was collected. The loading capacity was obtained based on the UV absorption of the diluted filtrate solution at 301 nm.

Peptides characterization:

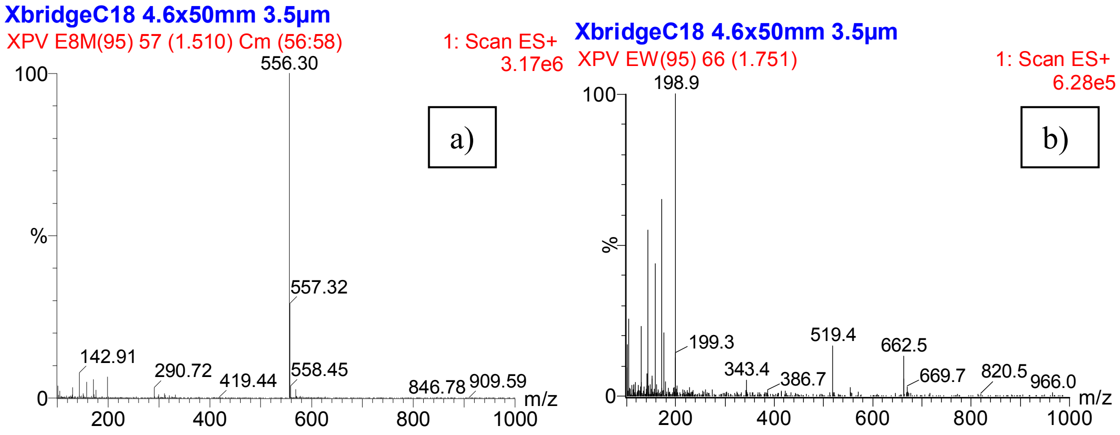
Leu-enkephaline (H-Tyr-Gly-Gly-Phe-Leu-OH) (6)

Calculated for $C_{28}H_{37}N_5O_7$: 555.2693

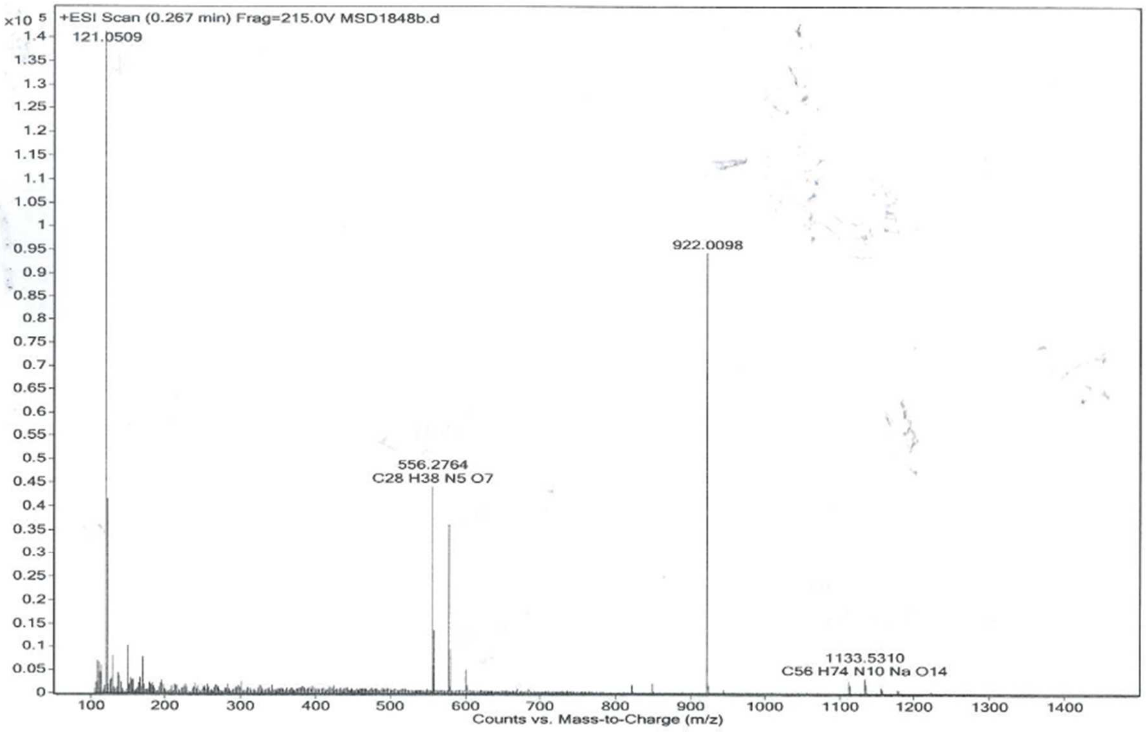
ESI-MS (M+H): 556.30; HRMS (ESI, M+H): 556.2764



HPLC profiles to comparison of Leu-enkephaline (**6**) synthesis on OH-BTL or Wang resin.



ESI-MS to Leu-enkephaline (**6**) a) Mass spectra corresponding to peptide **6,** b) Mass spectra corresponding to peptide alkylated **6** (M+107)$^+$

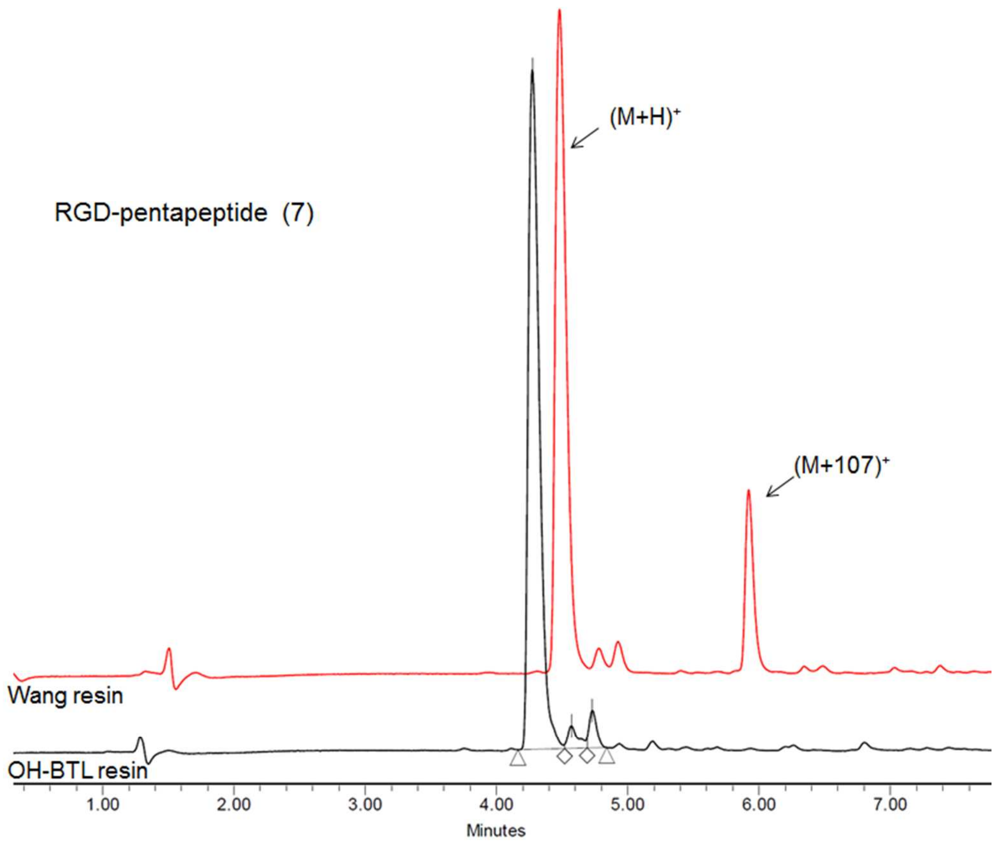


HRMS spectra to Leu-enkephaline (**6**)

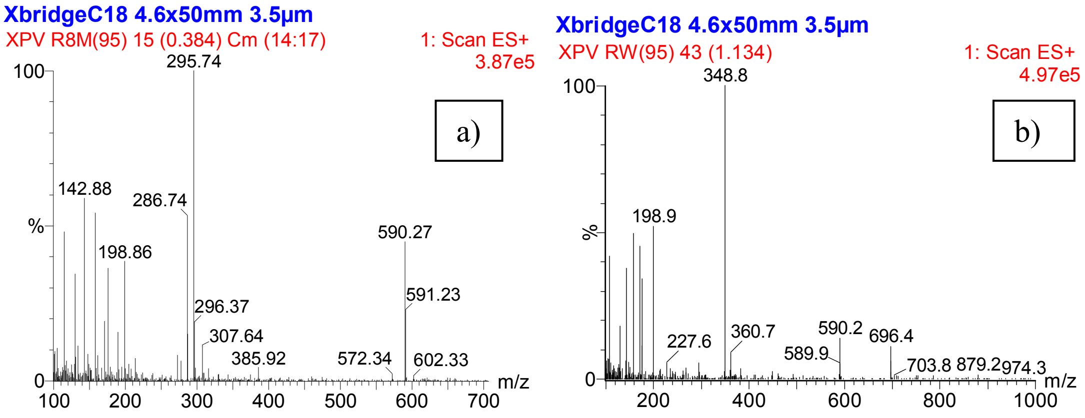
RGD pentapetide (H-Arg-Gly-Asp-Gly-Trp-OH) (7)

Calculated for $C_{25}H_{35}N_9O_8$:589.2609

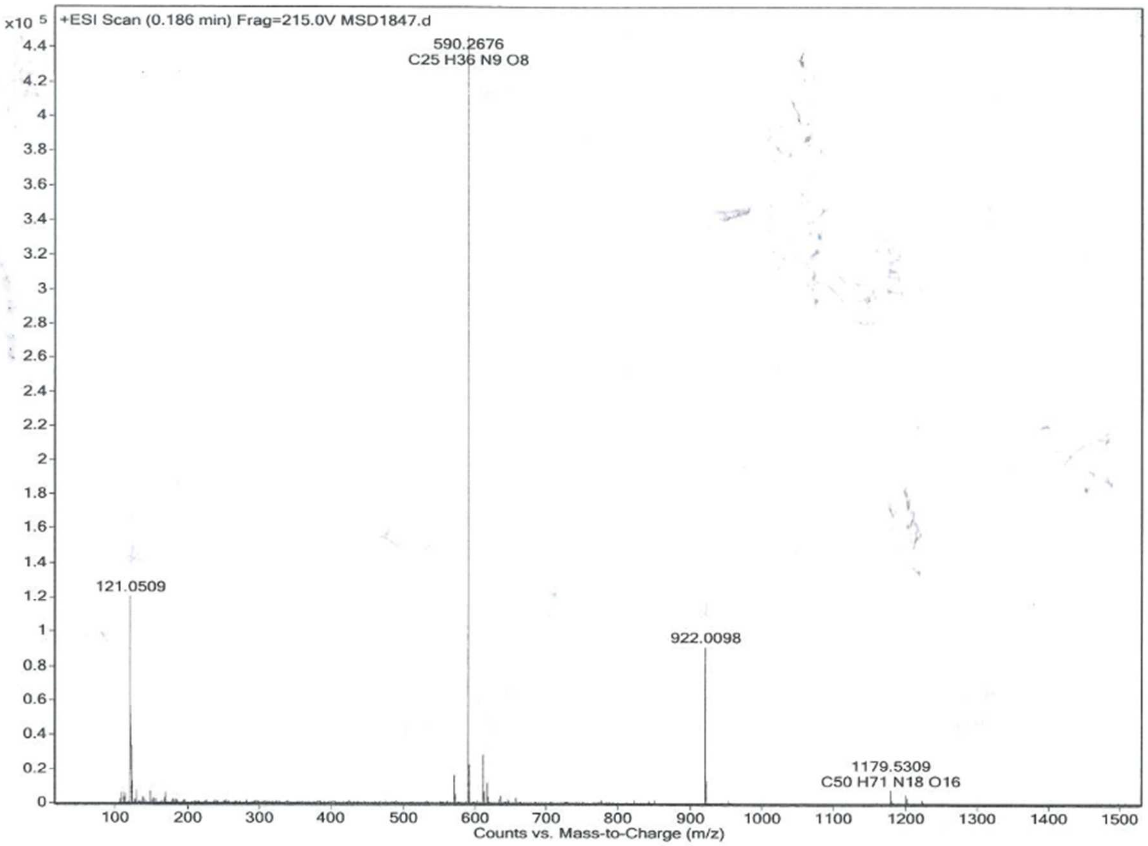
ESI-MS (M+H): 590.27; HRMS (ESI, M+H): 590.2676



HPLC profiles to comparison of RGD pentapeptide 7 synthesis on OH-BTL or Wang resin.



ESI-MS to RGD pentapeptide 7 a) Mass spectra corresponding to peptide **7,** b) Mass spectra corresponding to peptide alkylated **7** (M+107)$^+$

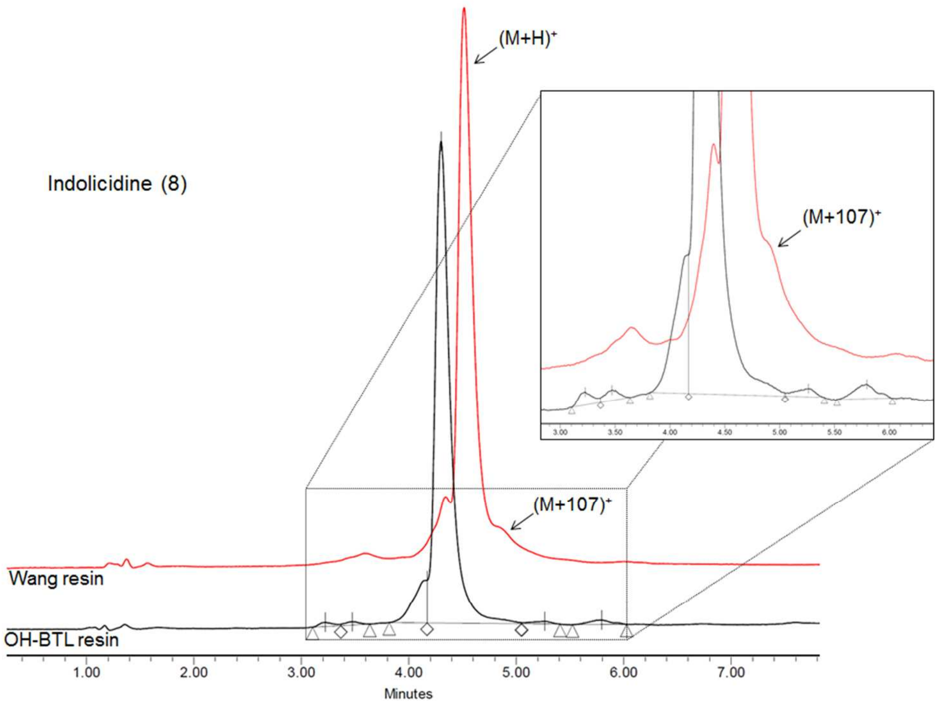


HRMS spectra to RGD pentapeptide 7

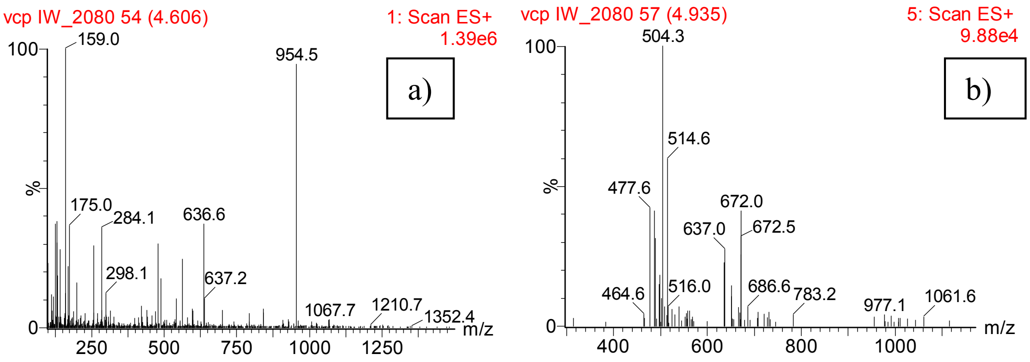
Indolicidin (H-Ile-Leu-Pro-Trp-Lys-Trp-Pro-Trp-Trp-Pro-Trp-Arg-Arg-OH)) (8)

Calculated for $C_{100}H_{131}N_{25}O_{14}$:1906.0307g/mol

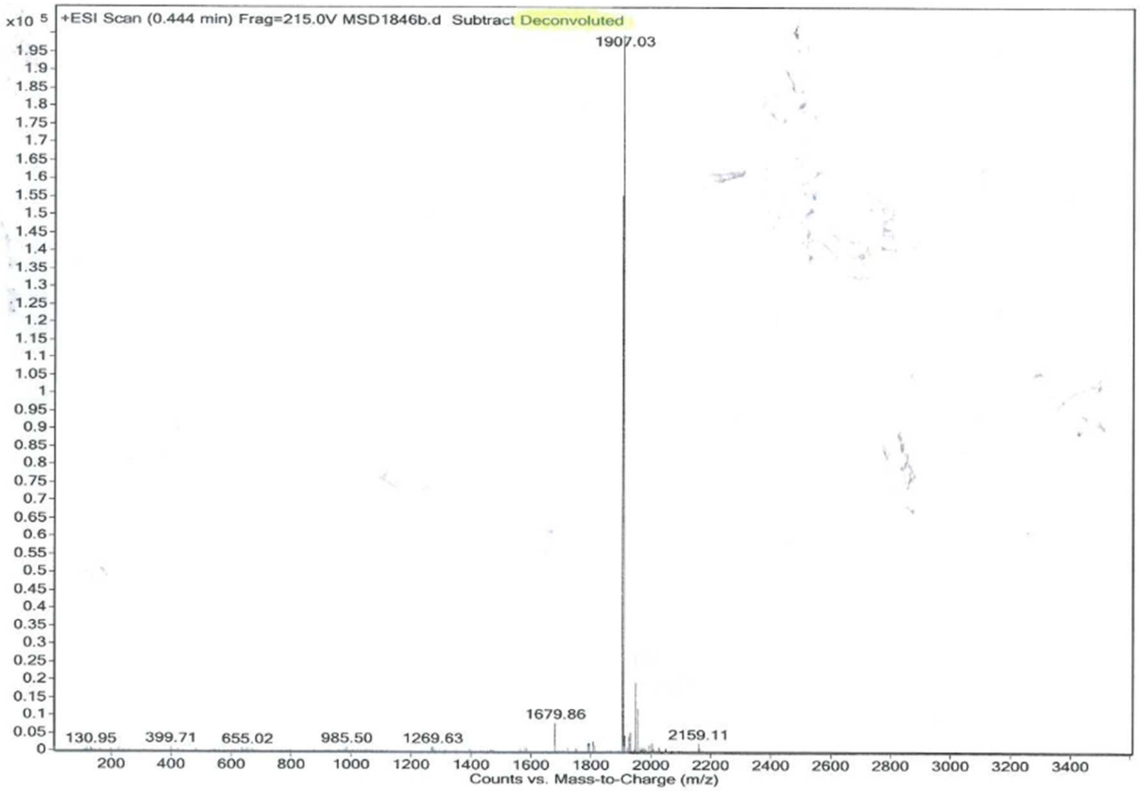
ESI-MS (M+2H/2, M+3H/3): 954.37, 636.72; HRMS (ESI, M+H, deconvoluted): 1907.03.



HPLC profiles to comparison of Indolicidine (**8**) synthesis on OH-BTL or Wang resin.



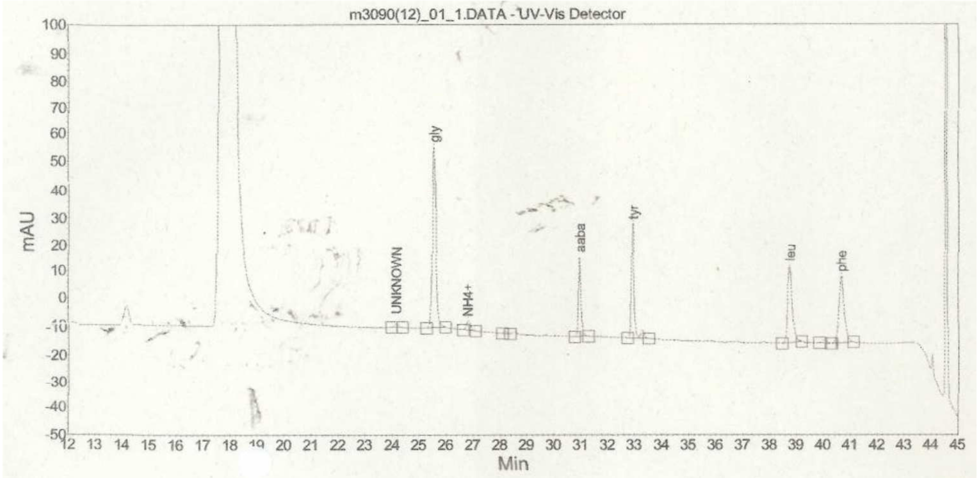
ESI-MS to Indolicidine (**8**) a) Mass spectra corresponding to peptide **8,** b) Mass spectra corresponding to peptide alkylated **8** $(M+107)^+$



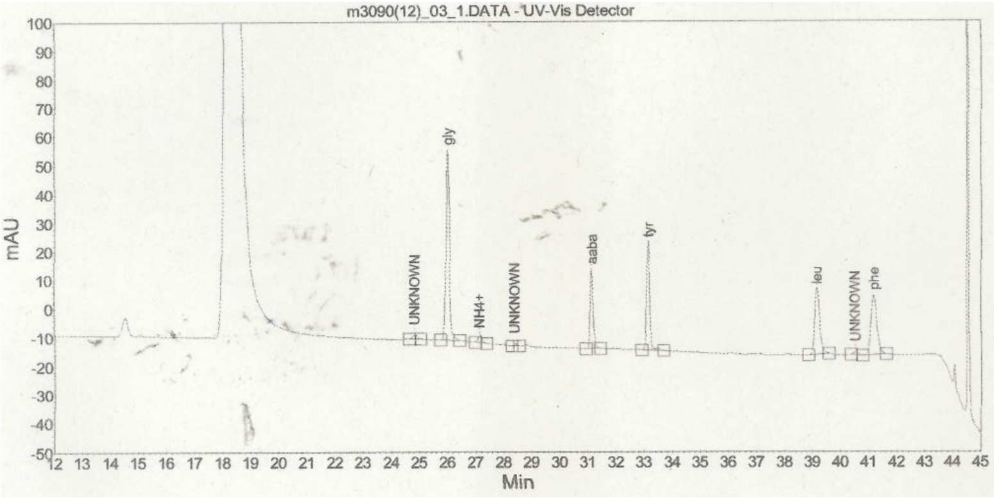
HRMS spectra to Indolicidine (**8**)

Aminoacid analysis (AAA)

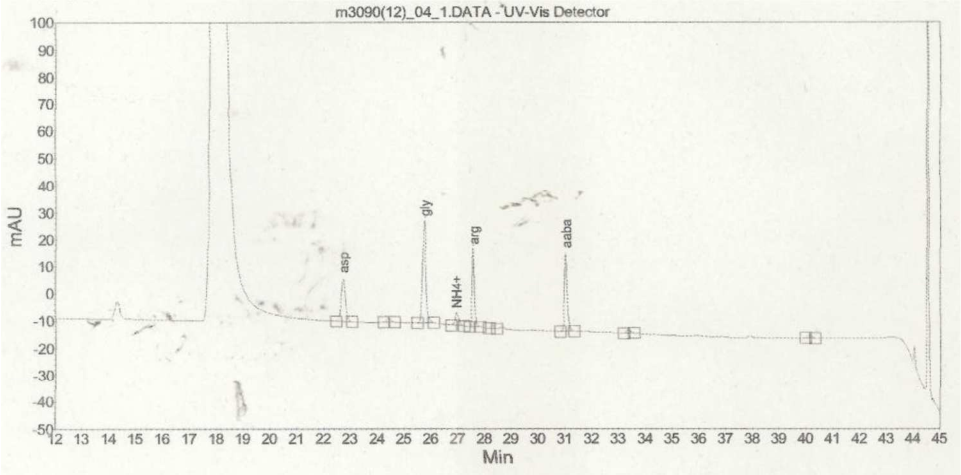
Leu-enkephalin attached to OH-BTL resin



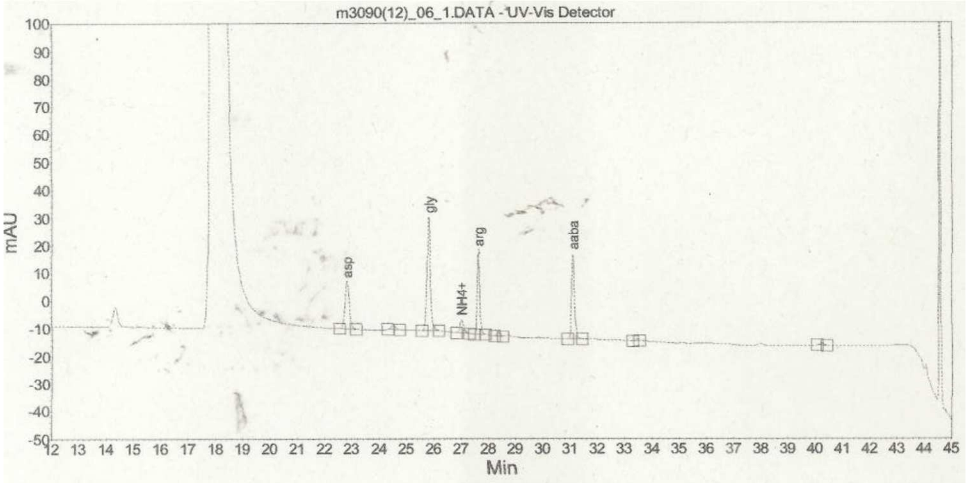
Leu-enkephalin attached to Wang resin



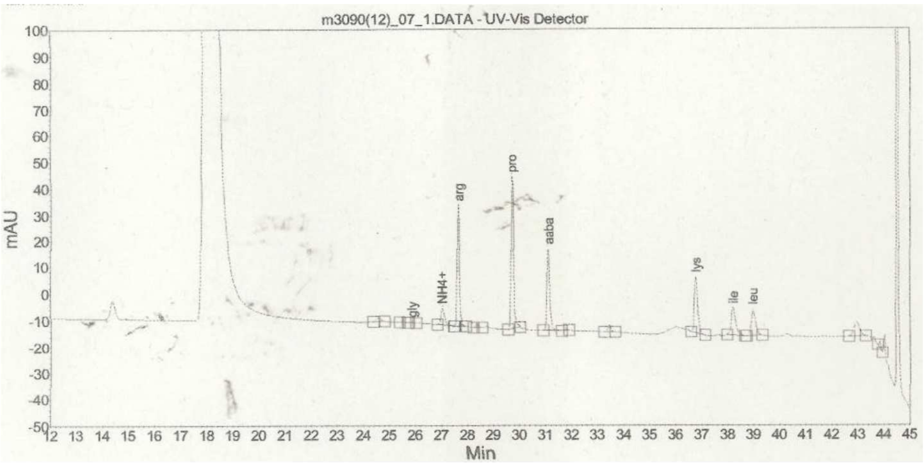
RGD pentapetide attached to OH-BTL resin



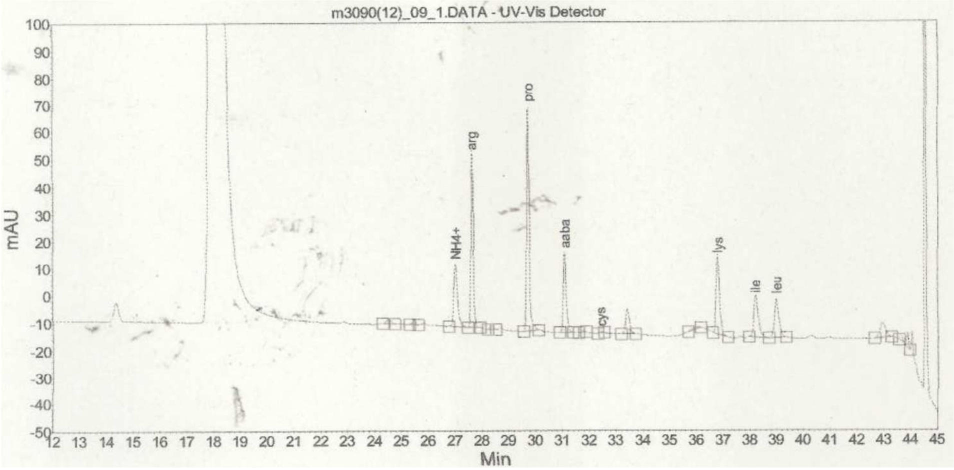
RGD pentapetide attached to Wang resin



Indolicidin attached to OH-BTL resin



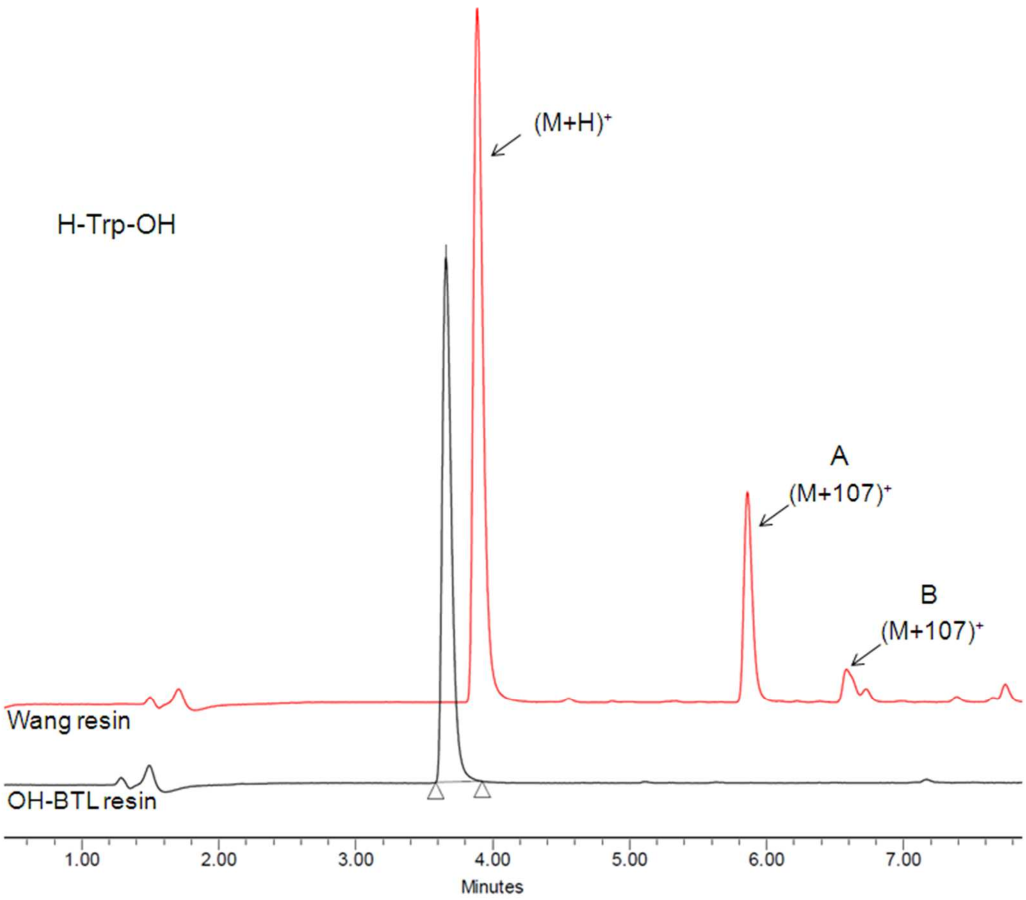
Indolicidin attached to Wang resin



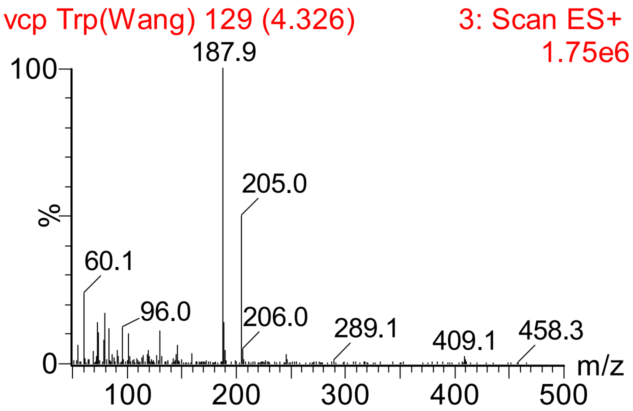
H-Trp-OH

Calculated for $C_{11}H_{12}N_2O_2$: 204,0899 g/mol

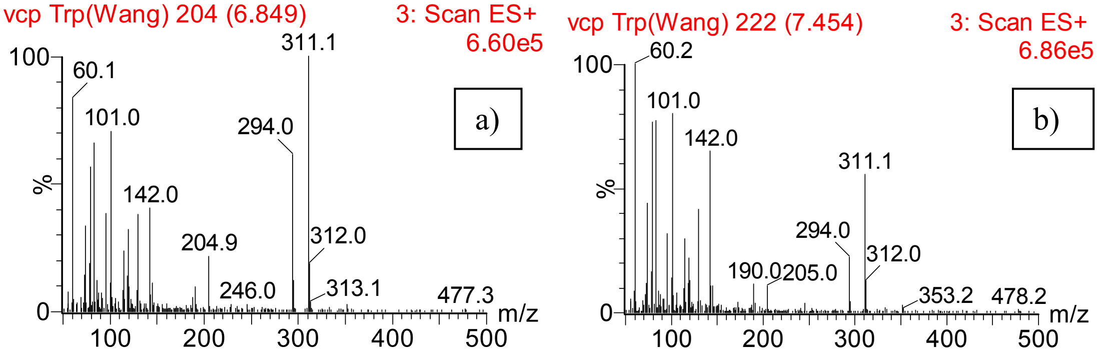
ESI-MS (M+H): 205.0;



HPLC profiles to comparison of H-Trp-OH on OH-BTL or Wang resin.



ESI-MS to H-Trp_OH



ESI-MS to by product on H-Trp-OH crude a) Mass spectra corresponding to alkylated Trp derivative; b) Mass spectra corresponding to undesired cleavage product.

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[i] Turner, R., A.; Oliver, A., G.; Lokey, R., S. *Org. Lett.*2007, 9, 5011.